N-Alkylated Nitrogen-in-the-Ring Sugars: Conformational Basis of Inhibition of Glycosidases and HIV-1 Replication

Naoki Asano,* Haruhisa Kizu, Kengo Oseki, Emiko Tomioka, Katsuhiko Matsui, Mika Okamoto,† and Masanori Baba†

Faculty of Pharmaceutical Sciences, Hokuriku University, Kanagawa-machi, Kanazawa 920-11, Japan, and Division of Human Retroviruses, Center for Chronic Viral Diseases, Faculty of Medicine, Kagoshima University, Kagoshima 890, Japan

Received February 24, 1995®

The conformations of nitrogen-in-the-ring sugars and their *N*-alkyl derivatives were studied from ^{1}H NMR analyses, mainly using ^3J(H,H) coupling constants and quantitative NOE experiments. No significant difference was seen in the ring conformation of 1-deoxynojirimycin (**1**), *N*-methyl-1-doexynojirimycin (**2**), and *N*-butyl-1-deoxynojirimycin (**3**). However, it was shown that the C6 OH group in **1** is predominantly equatorial to the piperidine ring, while that in **2** or **3** is predominantly axial, and its *N*-alkyl group is oriented equatorially. In the furanose analogues 1,4-dideoxy-1,4-imino-D-arabinitol (**4**) and its *N*-methyl (**5**) and *N*-butyl (**6**) derivatives, the five-membered ring conformation differed significantly by the presence or absence of the N-substituted group and the length of the *N*-alkyl chain. Compound **3** reduced its inhibitory effect on almost all glycosidases, resulting in an extremely specific inhibitor for processing α-glucosidase I since N-alkylation of **1** is known to enhance both the potency and specificity of this enzyme *in vitro* and *in vivo*. This preferred (C6 OH axial) conformation in **2** and **3** appears to be responsible for their strong α-glucosidase I activity. Compound **4** is a good inhibitor of intestinal α-glucohydrolases, α-glucosidase II, and Golgi α-mannosidases I and II, but its *N*-alkyl derivatives **5** and **6** markedly decreased inhibitory potential for all enzymes tested. In the case of 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **7**), which is a potent β-galactosidase inhibitor, its *N*-methyl (**8**) and *N*-butyl (**9**) derivatives completely lost potency toward β-galactosidase as well. N-Alkylation of compounds **4** and **7**, known well as potent yeast α-glucosidase inhibitors, resulted in a serious loss of inhibitory activity toward yeast α-glucohydrolases. Activity of these nine sugar analogues against HIV-1 replication was determined, based on the inhibition of virus-induced cytopathogenicity in MT-4 and MOLT-4 cells. Compounds **2** and **3**, which are better inhibitors of α-glucosidase I than **1**, proved active with EC_{50} values of 69 and 49 μg/mL in MT-4 cells and 100 and 37 μg/mL in MOLT-4 cells, respectively, while none of the furanose analogues exhibited any inhibitory effects on HIV-1. The change in potency and specificity of bioactivity by N-alkylation of nitrogen-in-the-ring sugars appears to be correlated with their conformational change.

Introduction

Glycosidases are involved in several important biological processes, such as digestion, the biosynthesis of glycoproteins, and the lysosomal catabolism of glycoconjugates. Therefore, glycosidase inhibitors have potentials, for example, diabetes type 2,[1,2] cancers,[3–5] viral infection,[6–8] and hereditary lysosomal storage diseases.[9–11] Such inhibitors are also being used to study the mechanism of action, topography of the active site, and purification of glycosidases.

The biosynthesis of oligosaccharide chains in N-linked glycoprotein involves the cotranslational transfer of a $Glc_3Man_9(GlcNAc)_2$ precursor from a dolichol carrier onto the asparagine residues in the proper sequon of the protein. Initial processing of the oligosaccharides occurs in the endoplasmic reticulum (ER) and starts with removal of the outermost α1,2-linked glucose residue by α-glucosidase I. α-Glucosidase II removes the two remaining α1,3-linked glucose residues. Further trimming of the oligosaccharides by ER (or soluble) α-mannosidase and Golgi α-mannosidases I and II permits subsequent processing to complex and hybrid type structures through the action of Golgi resident transferases. These topics have been reviewed by Fuhrmann et al.[12] and Elbein.[13,14]

1-Deoxynojirimycin (**1**) is a better inhibitor of α-glucosidase II than of 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 from *S. cerevisiae*.[15] N-Alkylation of **1** induces a shift in specific inhibition of purified glucosidases from α-glucosidase II to α-glucosidase I.[16,17] In cell culture, *N*-alkyl derivatives are also more effective inhibitors of glucosidase I than is the parent compound **1**, since a much larger proportion of the oligosaccharides formed in the presence of inhibitors contains three glucose residues.[18–20] 1,4-Dideoxy-1,4-imino-D-arabinitol (**4**) is a potent inhibitor of yeast α-glucosidase (IC_{50} = 0.18 μM).[21] Recently we have reported that compound **4** is a good inhibitor with a broad inhibitory spectrum toward mammalian glycosidases, such as ER α-glucosidase II, Golgi α-mannosidases I and II, intestinal isomaltase, and trehalase.[22] 2,5-Dideoxy-2,5-imino-D-mannitol (**7**), known as DMDP, is a potent inhibitor of yeast α-glucosidase (IC_{50} = 3.3 μM), almond β-glucosidase (IC_{50} = 7.8 μM),[21] and mouse intestinal α-glucosidases, β-glucosidases,

* To whom correspondence should be addressed at Faculty of Pharmaceutical Sciences, Hokuriku University, Kanagawa-machi, Kanazawa 920-11 Japan. FAX: +81 762-29-2781.
† Kagoshima University.
® Abstract published in *Advance ACS Abstracts*, June 1, 1995.

0022-2623/95/1838-2349$09.00/0

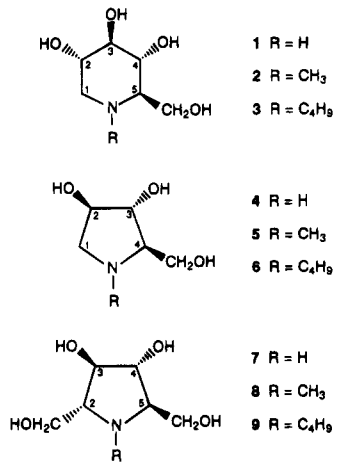

Figure 1.

and β-galactosidase.[23] In influenza virus-infected MDCK cells, DMDP (**7**) at concentrations of 250 μg/mL inhibited α-glucosidase I to accumulate glycopeptides having $Glc_3Man_9(GlcNAc)_2$ as the major oligosaccharides.[24]

Human immunodeficiency virus (HIV) is the causative agent of acquired immune deficiency syndrome (AIDS). The envelope glycoprotein gp120 of HIV is heavily N-glycosylated, and it is the major surface component by which HIV attaches to the CD4 receptor on helper T-cells. HIV-1 gp120 has 20–25 potential sites for N-linked glycosylation with the carbohydrate contributing 50% of its apparent molecular weight.[25,26] Therefore, interference with processing of viral envelope glycoprotein could be an attractive target for chemotherapy of HIV infections. A range of sugar analogues have been screened to date for anti-HIV activity *in vitro*, and the α-glucosidase inhibitors, especially processing α-glucosidase I inhibitors, have been found to inhibit HIV-induced syncytium formation and viral infectivity.[27–29] Drugs that act on the enzymes involved in later stages of the oligosaccharide processing pathway have no observed effect, that is, processing α-mannosidase inhibitors had no effect against HIV.[6,29,30] In the course of screening, *N*-butyl-1-deoxynojirimycin (**3**) was identified as the most active anti-HIV agent without significant cytotoxity.[30] It is presumed, although not proven, that the anti-HIV activity of **3** results from the inhibition of processing α-glucosidase I.

Castanospermine has been found to be more effective against processing α-glucosidase I and HIV than 1-deoxynojirimycin (**1**), but N-alkylation of **1** resulted in analogues with increased potency and specificity on both bioactivities.[20,31] Currently, there are some attempts to investigate stereochemical correlations of activity of glucosidase inhibitors from their three-dimensional structures.[20,32,33] These works suggest that the greater activity of castanospermine than **1** may be due to the fixed positioning of the C1 OH group (corresponding to the C6 OH group of **1**) oriented axially to the six-membered ring and that the increased potency and specificity of the *N*-alkyl derivatives of **1** may be due to the axial orientation of the C6 OH group to the piperidine ring. We focused on three glycosidase inhibitors, 1-deoxynojirimycin (**1**), 1,4-dideoxy-1,4-imino-D-arabinitol (**4**), and 2,5-dideoxy-2,5-imino-D-mannitol (**7**), and studied their conformational change upon N-alkylation using ^3J(H,H) coupling constants and quantitative NOE experiments. In the paper, we describe changes in conformation and bioactivities (glycosidase inhibitory activity and anti-HIV-1 activity) by N-alkylation of nitrogen-in-the-ring sugars.

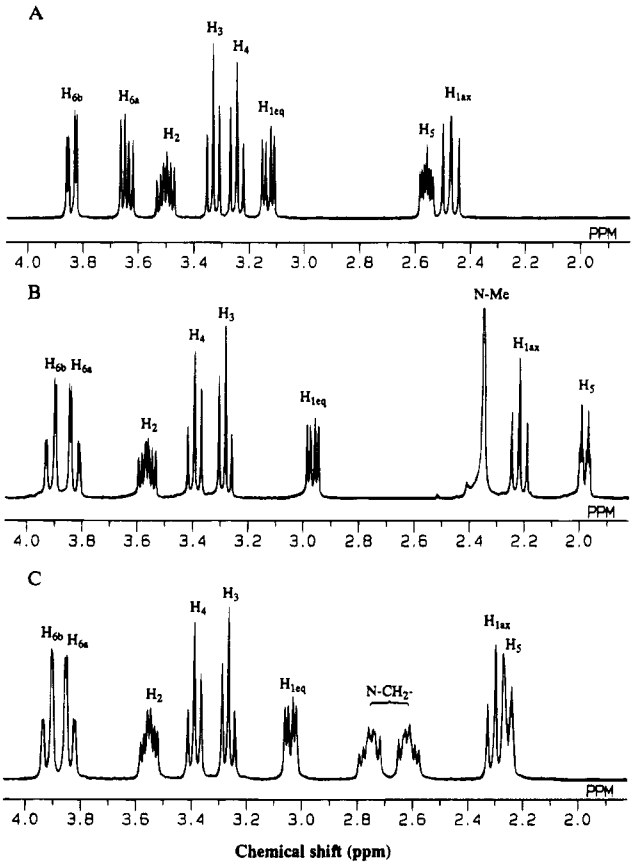

Figure 2.

Results and Discussion

Conformational Studies. The coupling constants measured from the 400 MHz ^1H NMR spectra in D_2O of 1-deoxynojirimycin (**1**) and the *N*-methyl (**2**) and *N*-butyl (**3**) derivatives are shown in Table 1. These three compounds exist in aqueous medium in the chair conformation because they have four large vicinal coupling constants, $^3J_{1ax,2}$, $^3J_{2,3}$, $^3J_{3,4}$, and $^3J_{4,5}$. A comparison of the magnitudes in these four coupling constants revealed that there was no significant difference in the ring conformation among these three compounds. The major differences in coupling constants listed in Table 1 and in signals seen in Figure 2 center on the averaged $^3J_{5,6a}$ and $^3J_{5,6b}$ coupling constants, which relate to various rotamer populations about the C5–C6 bond. Glaser and Perlin[34] have estimated the rotamer population of the C5 hydroxymethyl group by the two averaged $^3J_{5,6a}$ and $^3J_{5,6b}$ coupling constants. We estimated its population using the two averaged $^3J_{5,6a}$ and $^3J_{5,6b}$ coupling constants and quantitative NOE experiments. The coupling constants $^3J_{5,6b}$ (2.9 Hz) and $^3J_{5,6a}$ (6.2 Hz) for **1** suggest a large amount of the gauche-trans rotamer. On the other hand, the coupling constants $^3J_{5,6a} = {}^3J_{5,6a} = 2.6$ Hz for **2** or $^3J_{5,6a} = 2.9$ Hz and $^3J_{5,6b} = 2.5$ Hz for **3** suggest a large population of the gauche-gauche rotamer. Furthermore, as seen in Table 2, irradiation of H5 in **2** enhanced the NOE intensity of H6a (3.4%) and H6b (3.7%), and definite NOE effects were observed between H4 and H6a and between N-CH_3 and H6b. A similar result was

Table 1. Coupling Constants for 1-Deoxynojirimycin (**1**), 1,4-Dideoxy-1,4-imino-D-arabinitol (**4**), and Their Methyl (**2**, **5**) and Butyl (**3**, **6**) Derivatives in D_2O

	coupling constants (Hz)										
compd	$J_{1a(ax),1b(eq)}$	$J_{1a(ax),2}$	$J_{1b(eq),2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5a}$	$J_{4,5b}$	$J_{5a,5b}$	$J_{4,5}$	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
1	12.1	10.9	5.1	9.1	9.1				9.1	6.2	2.9	11.3
2	11.6	10.8	4.9	9.5	9.5				9.5	2.6	2.6	12.8
3	11.6	10.9	5.1	9.2	9.2				9.9	2.9	2.5	12.5
4	12.1	4.0	5.8	3.7	5.5	6.3	4.8	11.7				
5[a]	11.3	5.9	1.8[b]	2.6	5.1	5.1	5.1	11.7				
6[a]	11.3	5.9	2.4[b]	4.8	7.7	5.9	4.7	11.3				

[a] A W-shaped long-range coupling (~1 Hz) was observed between H1b and H3 in compounds **5** and **6**. [b] This value was determined by decoupling experiments.

Table 2. Results of NOE Experiments on *N*-Methyl-1-deoxynojirimycin (**2**) and *N*-Methyl-1-deoxymannojirimycin (**11**)[a]

	NOE intensity enhancements (%)[b]	
H irradiated	**2**	**11**
H1ax	H3 (9.2), H5 (7.8)	H2 (8.6), H3 (7.3)
H1eq	H2 (12.2), N−CH_3 (4.7)	H2 (7.4), N−CH_3 (4.3)
H5	H1ax (4.2), H3 (9.3), H6a (3.4) H6b (3.7), N−CH_3 (2.4)	H1ax (3.9), H3 (7.3), N−CH_3 (2.8)
H6a	H4 (3.2), H5 (5.5)	H4 (2.7), H5 (5.1)
H6b	H5 (7.3), N−CH_3 (4.7)	H5 (5.0), N−CH_3 (3.3)
N−CH_3	H1eq (7.9), H5 (7.0), H6b (7.3)	H1eq (6.9), H5 (7.8), H6b (6.5)

[a] Data were obtained using experimental mode "DIFNOE2" supplied by Jeol for the difference NOE measurement. Measurement conditions are as follows: acquisition time, 2.73 s; irradiation time, 5.27 s; irradiation power, 20−33 dB; temperature, ambient. [b] NOE intensities, shown as percentages, were estimated by comparing the enhanced signal area with signal intensity (−100%) of the irradiated proton.

obtained with *N*-methyl-1-deoxymannojirimycin (**11**). Thus, the predominant C6 OH axial stereoconfiguration in **2** was corroborated by the two averaged $^3J_{5,6a}$ and $^3J_{5,6b}$ coupling constants and the NOE experiments. Additionally, the definite NOE effect between N-CH_3 and H5 indicates the equatorial orientation of the *N*-methyl group in **2**.

Glaser and Perlin[34] have determined from ^{13}C NMR studies that the two isomers differing in stereochemistry at the *N*-methyl group of **2**, equatorial and axial *N*-methyl derivatives, exist in an ~11:1 ratio in acidic D_2O with trifluoroacetic acid. From ^1H NMR studies, they have further found that the C5 hydroxymethyl gauche-gauche rotamer of the cationic equatorial *N*-methyl diastereomer is strongly preponderant (≥90%) in aqueous solution. Hempel et al.[33] have determined the high-resolution structures of **1** and castanospermine from X-ray diffraction. They report that the C1 OH group of castanospermine (corresponding to the C6 OH group of **1**) is axial to the six-membered ring and fixed in position by the five-membered ring closure, while the C6 OH group of **1** is equatorial in the crystal structure and the NH···O6 intramolecular interaction stabilizes the C6−O6 conformation of **1**.

In the case of furanose analogues, the magnitudes in vicinal coupling constants within the five-membered ring of 1,4-dideoxy-1,4-imino-D-arabinitol (**4**) were significantly different from those of the *N*-methyl (**5**) and *N*-butyl (**6**) derivatives, seen in Table 1 and Figure 3. This suggests that a substantial change occurred in a ring conformation by N-alkylation of **4**. Especially the H1a and H1b signals of *N*-alkyl derivatives apparently differed from those of the parent compound **4** (Figure 3). We undertook a quantitative NOE experiment to determine a preference for conformer population in furanose analogues. In compound **4**, irradiation of H4, H5a, and H5b enhanced the NOE intensity of H2 (1.5%), H3 (3.8%), and H1b (4%), respectively (Table 3). These NOE enhancements suggest a high preference for the

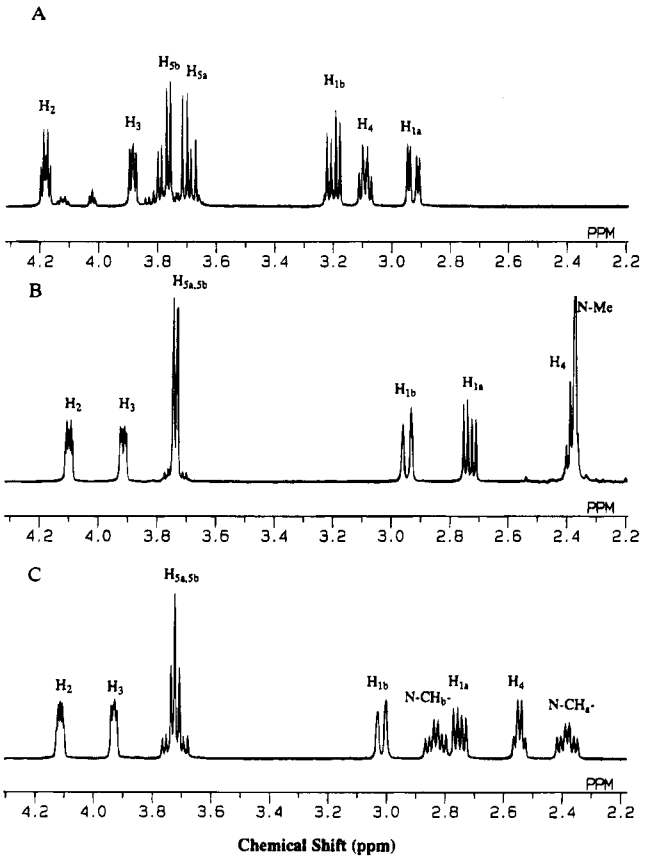

Figure 3.

C1-endo conformer (Figure 4A) of **4**. The introduction of an *N*-methyl group of **4** abolished the NOE effects between H1b and H5b and between H2 and H4, whereas irradiation of H1a enhanced the NOE intensity of H4 (3.6%) and *N*-methyl (2.5%) signals. These observations suggest the strong C1-exo preference of **5** (Figure 4B). The C1-exo conformation increases the distance between H1b and the C5 hydroxymethyl and between H2 and

Table 3. Results of NOE Experiments on 1,4-Dideoxy-1,4-imino-D-arabinitol (**4**) and Its Methyl (**5**) and Butyl (**6**) Derivatives

H irradiated	NOE intensity enhancements (%)		
	4	**5**	**6**
H1a		H4 (3.6), N−CH_3 (2.5)	H4 (2.5), N−CH_2− (6.6)
H1b	H5b (2.1)		
H2	H4 (1.3)		H4 (1.3)
H3	H5a (1.4)	H5a/5b (3.0)	H5a/5b (3.2)
H4	H2 (1.5)	H1a (2.1)	H1a (1.4), H2 (2.0), N−CH_2− (3.5, 2.3), N−CH_2−CH_2− (2.5)
H5a	H3 (3.8)	H3 (8.2)[a]	H3 (10.7),[a] H4 (10.6)[a]
H5b	H1b (4.0)		N−CH_2− (3.6)[a]

[a] Both H5a and H5b were irradiated.

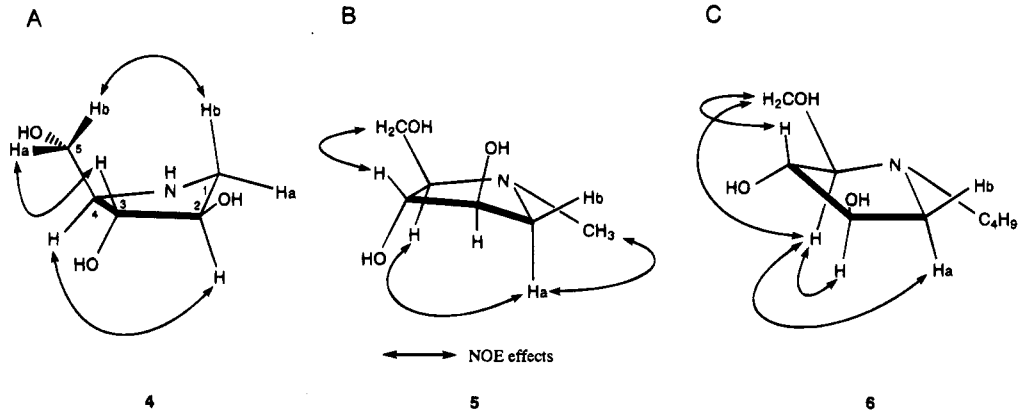

Figure 4.

H4, resulting in abolishment of the NOE enhancements between H1b and H5b and between H2 and H4. Furthermore, the C1-exo conformation provides a W-shaped long-range coupling (~1 Hz) between H1b and H3 and a dihedral angle close to 90° between C1−H1b and C2−H2 bonds, which is supported by a small coupling of 1.8 Hz for $^3J_{1b,2}$. On the other hand, the introduction of an *N*-butyl group to **4** produced NOE effects between H4 and H1a and between H4 and H2. This suggests the conformational shift toward C4-exo (Figure 4C). As seen in Table 1, a significant difference in coupling constants between **6** and **4** or **5** is the magnitude for $^3J_{3,4}$. The C4-exo conformation of **6** permits pseudotrans diaxial orientations for H3 and H4, resulting in a large magnitude of $^3J_{3,4}$ (7.7 Hz). Thus, in furanose analogues, the ring conformation differed significantly by the presence or absence of the N-substituted group and the length of the *N*-alkyl chain.

As to another furanose analogue, DMDP (**7**), it is impossible to obtain conformational information from NMR experiments because of the simplicity of the NMR spectra (three peaks in the ^{13}C NMR spectrum, four spin systems in the ^1H NMR spectrum) caused by its symmetrical structure.

Inhibition of Glycosidases. The IC_{50} values of nitrogen-in-the-ring sugars and their *N*-alkyl derivatives against glycosidases in rat intestinal brush border membranes are shown in Table 4. 1-Deoxynojirimycin (**1**) is a potent inhibitor of rat intestinal maltase, sucrase, and isomaltase. N-Methylation of **1** enhanced inhibitory potential toward all intestinal glycosidases except isomaltase, whereas N-butylation of **1** reduced its inhibition of all intestinal enzymes except trehalase. Furanose analogues 1,4-dideoxy-1,4-imino-D-arabinitol (**4**) and 2,5-dideoxy-2,5-imino-D-mannitol (**7**) are potent inhibitors of intestinal isomaltase and lactase, respectively. As seen in Table 4, the loss of one hydroxymethyl

Table 4. Concentration of Nitrogen-in-the-Ring Sugars and Their *N*-Alkyl Derivatives (μM) Giving 50% Inhibition of Glycosidases in Rat Intestinal Brush Border Membranes[a]

compd	IC_{50} (μM)					
	maltase	sucrase	isomaltase	trehalase	lactase	cellobiase
1	0.36	0.21	0.3	42	34	520
2	0.12	0.04	4.4	28	4.4	120
3	2.1	0.58	2.7	13	1000	NI
4	55	16	5.8	25	260	NI
5	500	70	100	NI	NI	NI
6	NI[b]	270	340	NI	NI	NI
7	NI	44	91	360	3.6	34
8	NI	620	NI	NI	NI	NI
9	NI	NI	NI	NI	NI	NI

[a] Glycosidase activities were colorimetrically assayed by the D-glucose oxidase−peroxidase method using the corresponding disaccharide. [b] NI = less than 50% inhibition at 1000 μM.

group from **7** enhances inhibition of intestinal α-glucohydrolases and trehalase but almost eliminates inhibition of β-glucosidase and β-galactosidase. Interestingly, the synthetic enantiomer of **4**, 1,4-dideoxy-1,4-imino-L-arabinitol, has been known to be a more effective inhibitor of mouse intestinal α-glucosidases.[23] The N-alkylation of furanose analogues **4** and **7** markedly lowered or abolished their inhibition toward all intestinal enzymes tested. Intestinal glycosidase inhibitors have potential use as antidiabetic drugs. Extensive programs aimed at providing products with superior inhibitory efficacy have been completed by Bayer AG, and two derivatives, *N*-(hydroxyethyl)-1-deoxynojirimycin (BAY m 1099) and *N*-[β-[4-(ethoxycarbonyl)phenoxy]-ethyl]-1-deoxynojirimycin (BAY 0 1248), were selected for further development as antihyperglycemic drugs.[35]

The IC_{50} and K_i values of nitrogen-in-the-ring sugars and their *N*-alkyl derivatives against glycosidases in rat liver are summarized in Tables 5 and 6. We previously reported that compounds **1** and **4** are good inhibitors of processing ER α-glucosidase II, but DMDP (**7**) has no

Table 5. Concentration of Nitrogen-in-the-Ring Sugars and Their *N*-Alkyl Derivatives (μM) Giving 50% Inhibition of Glycosidases in Rat Liver[a]

	IC_{50} (μM)				
	α-glucosidase		α-mannosidase		
compd	ER II[b]	lysosomal	Golgi II[c]	soluble	lysosomal
1	4.6	0.4	NI	NI	1000
2	20	1.5	NI	500	1000
3	15	5.3	NI	NI	NI
4	20	130	46	NI	110
5	310	180	64	NI	360
6	200	750	130	NI	NI
7	NI[d]	92	NI	260	NI
8	NI	470	NI	190	NI
9	NI	NI	NI	850	NI

[a] α-Glucosidase and α-mannosidase activities were colorimetrically determined using *p*-nitrophenyl α-D-glucopyranoside and *p*-nitrophenyl α-D-mannopyranoside, respectively. [b] ER II = endoplasmic reticulum α-glucosidase II. [c] Golgi II = Golgi α-mannosidase II. [d] NI = less than 50% inhibition at 1000 μM.

Table 6. Measured K_i Values and Modes of Inhibition[a]

	K_i (μM)		
inhibitor	ER α-glucosidase II	Golgi α-mannosidase II	inhibition
1	1.3	NI	competitive
2	5.8	NI	competitive
3	3.7	NI	competitive
4	9.7	35	competitive
5	150	51	competitive
6	95	120	competitive

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

effect on this enzyme even at 1 mM. N-Alkylation of **1** and **4** did not improve, but rather reduced, inhibition for ER α-glucosidase II as did N-alkylation of **1** for ER α-glucosidase I inhibition.[16,17] Additionally, the *N*-alkyl derivatives of **1**, **4**, and **7** decreased their inhibitory action toward lysosomal α-glucosidase. Compound **4** has a relatively broad specificity of inhibition of glycosidases, such as intestinal isomaltase, ER α-glucosidase II, trehalase, Golgi α-mannosidases I and II, and lysosomal α-mannosidase.[22] The *N*-alkyl derivatives of **4** reduced inhibitory potential toward α-mannosidases, and *N*-alkylation of **4** and **7**, which are potent inhibitors of yeast α-glucosidase,[21] did not enhance inhibition for yeast glycosidases as well (data not shown). Thus, in the present work, N-alkylation of nitrogen-in-the-ring sugars could not improve their inhibition for all glycosidases tested with the exception of inhibition of **2** for intestinal glycosidases. Compound **3** resulted in enhancing markedly the specificity for ER α-glucosidase I.

Castanospermine has been found to be more effective against ER α-glucosidase I (in both purified enzyme[36] and cell culture system[20]) than **1**. Tan et al.[20] and Hempel et al.[33] suggest that this greater activity of castanospermine than **1** may be due to the fixed positioning of the C1 OH group (corresponding to the C6 OH group in **1**) oriented axially to the piperidine ring by the five-membered ring closure. From an X-ray diffraction study, Hempel et al.[33] have shown that the C6 OH group of **1** is equatorial to the piperidine ring in the crystal structure and the NH···O6 intramolecular interaction stabilizes the C6–O6 conformation of **1**. They also suggest that substitution at the nitrogen atom in **1**, which would disrupt this intramolecular interaction, might be expected to increase the potency of the *N*-alkyl derivatives, by favoring the C6 OH axial conformation. A recent structure determination of the complex of **1** with glucoamylase from *Aspergillus awamori* var. X100[32] indicates that compound **1** best fits the active site of this enzyme with the C6 OH group oriented axially to the piperidine ring. Our present NMR studies exposed that the C6 OH group of the *N*-alkyl derivatives of **1** is in a position perpendicular to the six-membered ring with a chair conformation. These results suggest that this preferred (C6 OH axial) conformation of **2** or **3** best fits the active site of ER α-glucosidase I or glucoamylase from *A. awamori* and is responsible for its strong inhibitory activity. However, it is difficult to predict the effect of N-alkylation on efficacy of inhibition. N-Alkylation of 1-deoxymannojirimycin (**10**) did not improve, but rather reduced, its inhibition for (Man_9) mannosidase,[37] although the C6 OH group of *N*-methyl-1-deoxymannojirimycin (**11**) is in a position perpendicula to the six-membered ring as shown in Table 2. In the case of furanose analogues, *N*-alkylation of **4** and **7** markedly reduced or abolished the potency for all glycosidases. A similar effect has been observed for other furanose analogues, 1,4-dideoxy-1,4-imino-L-allitol and 1,4-dideoxy-1,4-imino-D-mannitol.[38] The present work revealed that N-alkylation of **4** causes a significant ring conformational change. This conformational change may be responsible for reduction of activities.

Table 7. Inhibitory Effects of Nitrogen-in-the-Ring Sugars and Their *N*-Alkyl Derivatives on HIV-1 Replication in MT-4 and MOLT-4 Cells[a]

	MT-4 cells		MOLT-4 cells	
compd	EC_{50}[b] (mg/mL)	CC_{50}[c] (mg/mL)	EC_{50} (mg/mL)	CC_{50} (mg/mL)
1	>100	>100	>100	>100
2	69	>100	100	>100
3	49	>100	37	>100
4	>100	>100	>100	>100
5	>100	>100	>100	>100
6	>100	>100	>100	>100
7	>100	>100	>100	>100
8	>100	>100	>100	>100
9	>100	>100	>100	>100

[a] All data represent mean values for two separate experiments. [b] Effective concentration giving 50% inhibition of HIV-1-induced cytopathogenicity. [c] Cytotoxic concentration giving 50% inhibition of growth of mock-infected cells.

Inhibition of HIV-1 Replication. The activity of nitrogen-in-the-ring sugars and their *N*-alkyl derivatives against HIV-1 replication was investigated, based on the inhibition of virus-induced cytopathicity in MT-4 or MOLT-4 cells. As shown in Table 7, 1-deoxynojirimycin (**1**), previously reported to be inhibitory,[6,27–29] was found to have weak inhibitory activity in both cell lines (30% inhibition for MT-4 cells and 26% inhibition for MOLT-4 cells at 100 μM/mL). However, the activity was remarkably increased by N-alkylation, as seen in the literature.[28,29] Thus the *N*-butyl derivative **3** was a more potent inhibitor of cytopathicity than was the *N*-methyl derivative **2** at concentrations which were not cytotoxic, the 50% effective concentration (EC_{50}) values being 49 μg/mL in MT-4 cells and 37 μg/mL in MOLT-4 cells. On the other hand, furanose analogues **4** and **7** and their *N*-alkyl derivatives exhibited no inhibitory activity even at 100 μg/mL in both cell lines. In the literature, DMDP (**7**) has been reported to have a

striking effect on syncytia formation and to reduce viral infectivity at 2 mM (326 μg/mL) in C8166 cells[27] and to show weak inhibition of virus-induced cytopathicity at 320 μg/mL in T-45 cells.[28]

A number of sugar analogues have been screened for anti-HIV activity *in vitro*, and some sugar analogues capable of inhibiting glycoprotein processing have been found to inhibit HIV replication and cellular cytopathicity *in vitro*. These compounds include castanospermine[6,7,27] and its derivatives,[31,39] 1-deoxynojirimycin (**1**)[6,7,27] and its *N*-alkyl derivatives,[28,29] and 7,7a-diepialexine,[40] which all have the common property that they are potent inhibitors of ER α-glucosidase I involved in glycoprotein processing. It is presumed, although not proven, that the anti-HIV activity of these compounds 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.[31,39,40] Recently, Karlsson et al.[41] have shown that ER α-glucosidase I inhibition occurs at the antiviral concentration of **3** (0.5 mM) in a cell culture system. The sequential digestion with purified ER α-glucosidases I and II reveals that the three major oligosaccharides on compound **3**-treated gp120 are not Glc_3Man_9, Glc_2Man_9, and Glc_1Man_9 but Glc_3Man_9, Glc_3Man_8, and Glc_3Man_7. Consequently, they conclude that the effect of **3** on gp120 is nearly complete at the 0.5 mM antiviral concentration and ER α-glucosidase I inhibition is a candidate mechanism for the antiviral activity of this compound.

In the present study, we showed that N-alkylation of **1** led to conformational change (the C6 OH axial conformation) and the *N*-alkyl derivatives, especially *N*-butyl derivative **3**, significantly enhanced the specificity of inhibition of ER α-glucosidase I followed by the significant decrease of almost all glycosidase inhibitory activities. In addition, they further exhibited a more potent anti-HIV-1 activity than did the parent compound **1**. The potencies of inhibition for purified calf liver α-glucosidase I in **2** and **3** are almost same, the K_i values being 0.07 and 0.09 μM, respectively.[17] However, the anti-HIV-1 activity of **3** is obviously more potent than that of **2**, as seen in this work and the literature.[28,29] A possible explanation for this difference in potency is the existence of a permeability barrier for **2** as well as for **1**. N-Butylation of **1** increases its lipophilicity much more than does N-methylation of **1** and can enhance permeability into the host cells. But this possibility may be ruled out because compounds **2** and **3** exhibit the same efficacy for ER α-glucosidase I in a cell culture system.[20] The unknown mechanism of **3** in addition to its potent inhibitory activity toward ER α-glucosidase I appears to be involved in the inhibition of HIV-1. More recent findings of Platt et al.[11,42] noted that compound **3**, but not **1** and **2**, is a novel inhibitor of the glucosyltransferase-catalyzed biosynthesis of glucosylceramide.

Experimental Section

General Methods. Nitrogen-in-the-ring sugars and their *N*-alkyl derivatives were chromatographed by HPTLC silica gel-60F_{254} (E. Merck) using the 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.

Materials. Various *p*-nitrophenyl glycosides and palatinose were purchased from Sigma Chemical Co. Other disaccharides were purchased from Wako Pure Chemical Industries.

Preparation of Nitrogen-in-the-ring Sugars and Their *N*-Alkyl Derivatives. 1-Deoxynojirimycin (**1**), 1,4-dideoxy-1,4-imino-D-arabinitol (**4**), 2,5-dideoxy-2,5-imino-D-mannitol (DMDP, **7**), and 1-deoxymannojirimycin (**10**) were prepared by the methods described in our previous paper.[22] The N-methylated derivatives of nitrogen-in-the-ring sugars were synthesized according to the method of Kato et al.[43] as follows. A solution of a sugar analogue, 37% HCHO, and 80% formic acid was stirred at 80 °C for 3 h and evaporated. The residue was dissolved methanol, applied to an Amberlyst 15 column, washed with methanol, eluted with 0.5 M NH_4OH, and concentrated. N-Methylated derivatives were purified by Dowex 1-X2 (OH^- form) chromatography with water and lyophilized. The N-butylated derivatives were prepared as follows. 1-Bromobutane and $NaHCO_3$ were added to a solution of a sugar analogue in methanol–dioxane (3:2). The reaction mixture was heated under reflux for 48 h and filtered through Celite. The filtrate was applied to an Amberlyst 15 column, washed with methanol, eluted with 0.5 M NH_4OH, and concentrated. N-Butylated derivatives were purified by Dowex 1-X2 (OH^- form) chromatography with water and lyophilized.

1-Deoxynojirimycin (1): R_f 0.44; $[\alpha]_D$ +42.1° (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.47 (dd, 1H, coupling constants are shown in Table 1, H1ax), 2.55 (ddd, 1H, H5), 3.13 (dd, 1H, H1eq), 3.24 (t, 1H, H4), 3.33 (t, 1H, H3), 3.50 (ddd, 1H, H2), 3.64 (dd, 1H, H6a), 3.84 (dd, 1H, H6b); ^{13}C NMR (100 MHz, D_2O) δ 51.5 (C1), 63.3 (C5), 64.2 (C6), 73.7 (C2), 74.3 (C4), 81.2 (C3); FABMS (glycerol matrix) *m/z* 164 (M + 1, 43). Anal. ($C_6H_{13}NO_4$) C, H, N.

***N*-Methyl-1-deoxynojirimycin (2):** R_f 0.30; $[\alpha]_D$ +12.6° (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O) δ 1.99 (dt, 1H, H5), 2.23 (dd, 1H, H1ax), 2.35 (s, 1H, N-CH_3), 2.97 (dd, 1H, H1eq), 3.29 (t, 1H, H3), 3.40 (t, H, H4), 3.57 (ddd, 1H, H2), 3.83 (dd, 1H, H6a), 3.91 (dd, 1H, H6b); ^{13}C NMR (100 MHz, D_2O) δ 43.8 (N-CH_3), 60.3 (C6), 62.3 (C1), 70.9 (C5), 71.5 (C2), 72.6 (C4), 81.1 (C3); FABMS (glycerol matrix) *m/z* 178 (M + 1, 82). Anal. ($C_7H_{15}NO_4$) C, H, N.

***N*-Butyl-1-deoxynojirimycin (3):** R_f 0.57; $[\alpha]_D$ −15.9° (*c* 0.77, H_2O); ^1H NMR (400 MHz, D_2O) δ 0.91 (t, 3H, CH_3), 1.29 (m, 2H, N-CH_2CH_2**CH_2**CH_3), 1.47 (m, 2H, N-CH_2**CH_2**CH_2CH_3), 2.25 (m, 1H, H5), 2.28 (dd, 1H, H1ax), 2.61 (m, 1H, N-CH_2-), 2.75 (m, 1H, N-CH_2-), 3.04 (dd, 1H, H1eq), 3.26 (t, 1H, H3), 3.38 (dd, 1H, H4), 3.55 (ddd, 1H, H2), 3.83 (dd, 1H, H6a), 3.92 (dd, 1H, H6b); ^{13}C NMR (100 MHz, D_2O) δ 16.1, 23.0, 27.9, 54.7 (*N*-butyl), 58.1 (C1), 60.4 (C6), 68.0 (C5), 71.7 (C2), 73.0 (C4), 81.2 (C3); FABMS (glycerol matrix) *m/z* 220 (M + 1, 100). Anal. ($C_{10}H_{21}NO_4$) C, H, N.

1,4-Dideoxy-1,4-imino-D-arabinitol (4): R_f 0.51; $[\alpha]_D$ +6.3° (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.86 (dd, 1H, H1a), 3.01 (ddd, 1H, H4), 3.14 (dd, 1H, H1b), 3.67 (dd, 1H, H5a), 3.75 (dd, 1H, H5b), 3.85 (dd, 1H, H3), 4.16 (ddd, 1H, H2); ^{13}C NMR (100 MHz, D_2O) δ 53.4 (C1), 64.8 (C5), 68.0 (C4), 80.2 (C2), 81.8 C3); FABMS (glycerol matrix) *m/z* 134 (M + 1, 48). 1,4-Dideoxy-1,4-imino-D-arabinitol hydrochloride (**4**·HCl): Anal. ($C_5H_{11}NO_3$·HCl) C, H, N.

1,4-Dideoxy-1,4-(methyliminiumyl)-D-arabinitol (5): R_f 0.40; $[\alpha]_D$ −21.9° (*c* 1, H_2O); ^1H NMR (400 MHz, D_2O) δ 2.37 (s, 3H, N-CH_3), 2.38 (dt, 1H, H4), 2.73 (dd, 1H, H1a), 2.95 (br d, 1H, H1b), 3.72 (dd, 1H, H5a), 3.75 (dd, 1H, H5b), 3.92 (br dd, 1H, H3), 4.10 (ddd, 1H, H2); ^{13}C NMR (100 MHz, D_2O) δ 43.6 (N-CH_3), 63.6 (C1), 64.1 (C5), 76.0 (C4), 78.6 (C2), 82.7 (C3); FABMS (glycerol matrix) *m/z* 148 (M + 1, 100). Anal. ($C_6H_{13}NO_3$) C, H, N.

1,4-Dideoxy-1,4-(butyliminiumyl)-D-arabinitol (6): R_f 0.63; $[\alpha]_D$ −56.3° (*c* 0.6, H_2O); ^1H NMR (400 MHz, D_2O) δ 0.91 (t, 3H, CH_3), 1.31 (m, 2H, N-CH_2CH_2**CH_2**CH_3), 1.47 (m, 2H, N-CH_2**CH_2**CH_2CH_3), 2.38 (m, 1H, N-CH_2-), 2.55 (ddd, 1H, H4), 2.75 (dd, 1H, H1a), 2.83 (m, 1H, N-CH_2-), 3.02 (br d, 1H, H1b), 3.70 (dd, 1H, H5a), 3.75 (dd, 1H, H5b), 3.93 (dd, 1H, H3), 4.11

(dt, 1H, H2); ^{13}C NMR (100 MHz D_2O) δ 16.1, 23.1, 31.9, 58.1 (*N*-butyl), 61.2 (C1), 64.2 (C5), 74.8 (C4), 78.3 (C2), 82.1 (C3); FABMS (glycerol matrix) *m/z* 190 (M + 1, 100). Anal. ($C_9H_{19}NO_3$) C, H, N.

2,5-Dideoxy-2,5-imino-D-mannitol (7): R_f 0.52; $[\alpha]_D$ +56.9° (*c* 0.54, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 3.04 (m, 2H, H2,5), 3.65 (dd, 2H, *J* = 6.2, 11.7 Hz, H1a,6a), 3.73 (dd, 2H, *J* = 4.4, 11.7 Hz, H1b,6b), 3.86 (m, 2H, H3,4); ^{13}C NMR (100 MHz, D_2O) δ 64.4 (C2,5), 64.9 (C1,6), 80.7 (C3,4); FABMS (glycerol matrix) *m/z* 164 (M + 1, 100). Anal. ($C_6H_{13}NO_4$) C, H, N.

2,5-Dideoxy-2,5-(methyliminiumyl)-D-mannitol (8): R_f 0.46; $[\alpha]_D$ −8.5° (*c* 1, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.47 (s, 3H, N-CH_3), 2.91 (m, 2H, H2,5), 3.80 (d, 4H, H1a,1b,6a,6b), 3.96 (m, 2H, H3,4); ^{13}C NMR (100 MHz, D_2O) δ 37.3 (N-CH_3), 62.2 (C1,6), 72.0 (C2,5), 80.7 (C3,4); FABMS (glycerol matrix) *m/z* 178 (M + 1, 75). Anal. ($C_7H_{15}NO_4$) C, H, N.

2,5-Dideoxy-2,5-(butyliminiumyl)-D-mannitol (9): R_f 0.64; $[\alpha]_D$ −45.4° (*c* 0.74, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 0.92 (t, 3H, CH_3), 1.33 (m, 2H, N-CH_2CH_2**CH_2**CH_3), 1.49 (m, 2H, N-CH_2**CH_2**CH_2CH_3), 2.72 (m, 2H, N-CH_2-), 3.01 (m, 2H, H2,5), 3.76 (d, 2H, *J* = 5.5, 11.7 Hz, H1a,6a), 3.81 (dd, 2H, *J* = 4.4, 11.7 Hz, H1b,6b), 4.00 (m, 2H, H3,4); ^{13}C NMR (100 MHz, D_2O) δ 16.1, 23.1, 32.0, 50.3 (*N*-butyl), 62.3 (C1,6), 70.9 (C2,5), 81.6 (C3,4); FABMS (glycerol matrix) *m/z* 220 (M + 1, 100). Anal. ($C_{10}H_{21}NO_4$) C, H, N.

1,5-Dideoxy-1,5-imino-D-mannitol (10): R_f 0.33; $[\alpha]_D$ −41.4° (*c* 0.74, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 2.48 (dt, 1H, *J* = 3.9, 9.5 Hz, H5), 2.77 (dd, 1H, *J* = 1.8, 14.3 Hz, H1ax), 3.01 (dd, 1H, *J* = 2.6, 14.3 Hz, H1eq), 3.57 (dd, 1H, *J* = 3.3, 9.5 Hz, H3), 3.62 (t, 1H, *J* = 9.5 Hz, H4), 3.78 (d, 2H, H6a,6b), 4.00 (ddd, 1H, *J* = 1.8, 2.6, 3.3 Hz, H2); ^{13}C NMR (100 MHz, D_2O) δ 51.1 (C1), 63.3 (C5), 63.5 (C6), 71.1 (C4), 72.0 (C2), 77.4 (C3); FABMS (glycerol matrix) *m/z* 164 (M + 1, 60). Anal. ($C_6H_{13}NO_4$) C, H, N.

1,5-Dideoxy-1,5-(methyliminiumyl)-D-mannitol (11): R_f 0.25; $[\alpha]_D$ −54.0° (*c* 1.4, H_2O); ^{1}H NMR (400 MHz, D_2O) δ 1.95 (ddd, 1H, *J* = 2.5, 2.9, 9.5 Hz, H5), 2.33 (s, 3H, N-CH_3), 2.46 (dd, 1H, *J* = 1.8, 13.2 Hz, H1ax), 2.94 (dd, 1H, *J* = 3.3, 13.2 Hz, H1eq), 3.51 (dd, 1H, *J* = 3.3, 9.9 Hz, H3), 3.70 (dd, 1H, *J* = 9.5, 9.9 Hz, H4), 3.85 (dd, 1H, *J* = 2.9, 12.5 Hz, H6a), 3.94 (dd, 1H, *J* = 2.5, 12.5 Hz, H6b), 3.98 (ddd, 1H, *J* = 1.8, 3.3, 3.3 Hz, H2); ^{13}C NMR (100 MHz, D_2O) δ 44.1 (N-CH_3), 60.9 (C6), 62.0 (C1), 70.3 (C4), 70.7 (C2), 71.4 (C5), 77.2 (C3); FABMS (glycerol matrix) *m/z* 178 (M + 1, 100). Anal. ($C_7H_{15}NO_4$) C, H, N.

Preparation and Assay Method of Glycosidases in Rat Liver and Brush Border Membranes from Rat Intestine. Rat liver glycosidases were prepared and assayed by the methods described in our previous paper.[22] Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[44] 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 Industries).

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

Cell and Virus. MT-4 cells[45] and MOLT-4 cells[46] were used in the anti-HIV-1 assays. The cells were grown and maintained in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum, 100 U/mL penicillin G, and 100 μg/mL streptomycin. HTLV-III$_B$ strain was used in the anti-HIV-1 assays. The virus was propagated in MT-4 cells. Titer of virus stocks was determined in MT-4 cells, and the virus stocks were stored at −80 °C until use.

Antiviral Assays. Determination of the activities of the compounds against HIV-1 replication was based on the inhibition of virus-induced cytopathicity in MT-4 or MOLT-4 cells, as previously described.[47] The numbers of viable MT-4 and MOLT-4 cells were determined by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) method on days 4 and 7 after virus infection, respectively.[48] 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.

References

(1) Junge, B.; Heiker, F.-R.; Kurz, J.; Muller, L.; Schmidt, D. D.; Wunshe, C. Untersuchungen zur Struktur des α-D-Glucosidase Inhibitors Acarbose. (Structure of an α-D-Glucosidase Inhibitor Acarbose.) *Carbohydr. Res.* **1984**, *128*, 235–268.

(2) Horii, S.; Fukase, H.; Matsuo, T.; Kameda, Y.; Asano, N.; Matsui, K. Synthesis of α-D-Glucosidase Inhibitory Activity of N-Substituted Valiolamine Derivatives as Potential Oral Antidiabetic Agents. *J. Med. Chem.* **1986**, *29*, 1038–1046.

(3) Dennis, J. W. Effects of Swainsonine and Polyinosinic: Polycytidinic Acid on Murine Tumor Cell Growth and Metastasis. *Cancer Res.* **1986**, *46*, 5131–5136.

(4) Humphries, K. J.; Matsumoto, K.; White, S.; Olden, K. Inhibition of Experimental Metastasis by Castanospermine in Mice. *Cancer Res.* **1986**, *46*, 5215–5222.

(5) Ostrander, G. K.; Scribner, N. K.; Rohrschneider, L. R. Inhibition of V-fms-induced Tumor Growth in Nude Mice by Castanospermine. *Cancer Res.* **1988**, *48*, 1091–1094.

(6) Gruters, R. A.; Neefjes, J. J.; Tersmette, M.; de Goede, R. E. Y.; Tulp, A.; Huisman, H. G.; Miedema, F.; Ploegh, H. L. Interference with HIV-induced Syncytium Formation and Viral Infectivity by Inhibitors of Trimming Glucosidase. *Nature* **1987**, *330*, 74–77.

(7) Walker, B. D.; Kowalski, M.; Goh, W. C.; Kozarsky, K.; Sodroski, J. Inhibition of Human Immunodeficiency Virus Syncytium Formation and Virus Replication by Castanospermine. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 8210–8124.

(8) Ratner, L. Glucosidase Inhibitors for Treatment of HIV-1 Infection. *AIDS Res. Hum. Retroviruses* **1992**, *8*, 165–173.

(9) Cenci de Bello, I.; Dorling, P.; Winchester, B. The Storage Products Ingenetic and Swainsonine-induced Human Mannosidoses. *Biochem. J.* **1983**, *215*, 693–696.

(10) Cenci de Bello, I.; Fleet, G.; Namgoong, S. K.; Tadano, K.; Winchester, B. Inhibition of Human α-Mannosidases *in vitro* and *in vivo* by Swainsonine Analogues. *Biochem. J.* **1989**, *259*, 855–861.

(11) Platt, F. M., Neises, G. R.; Dwek, R. A.; Butters, T. D. N-Butyldeoxynojirimycin Is a Novel Inhibitor of Glycolipid Biosynthesis. *J. Biol. Chem.* **1994**, *269*, 8362–8365.

(12) Fuhrmann, U.; Bause, E.; Ploegh, H. Inhibitors of Oligosaccharide Processing. *Biochem. Biophys. Acta* **1985**, *825*, 95–110.

(13) Elbein, A. D. Inhibitors of the Biosynthesis and Processing of N-Linked Oligosaccharide Chains. *Annu. Rev. Biochem.* **1987**, *56*, 497–534.

(14) Elbein, A. D. Glycosidase Inhibitors: Inhibitors of N-Linked Oligosaccharide Processing. *FASEB J.* **1991**, *5*, 3055–3063.

(15) Saunier, B.; Kilker, R. D., Jr.; Tkacz, J. S.; Quaroni, A.; Herscovics, A. Inhibition of N-Linked Complex Oligosaccharide Formation by 1-Deoxynojirimycin, an Inhibitor of Processing Glucosidases. *J. Biol. Chem.* **1982**, *257*, 14155–14161.

(16) Hettkamp, H.; Legler, G.; Bause, E. Purification by Affinity Chromatography of Glucosidase I, an Endoplasmic Reticulum Hydrolase Involved in the Processing of Asparagine-linked Oligosaccharides. *Eur. J. Biochem.* **1984**, *142*, 85–90.

(17) Schweden, J.; Borgmann, C.; Legler, G.; Bause, E. Characterization of Calf Liver Glucosidase I and Its Inhibition by Basic Sugar Analogs. *Arch. Biochem. Biophys.* **1986**, *248*, 335–340.

(18) Romero, P. A.; Saunier, B.; Herscovics, A. Comparison between 1-Deoxynojirimycin and N-Methyl-1-deoxynojirimycin as Inhibitors of Oligosaccharide Processing in Intestinal Epithelial Cells. *Biochem. J.* **1985**, *226*, 733–740.

(19) Datema, R.; Olofsson, S.; Romero, P. A. Inhibitors of Protein Glycosylation and Glycoprotein Processing in *Viral* System. *Pharmacol. Ther.* **1987**, *33*, 221–286.

(20) Tan, A.; van den Broek, L.; van Boeckel, S.; Ploegh, H.; Bolscher, J. Chemical Modification of the Glucosidase Inhibitor 1-Deoxynojirimycin. *J. Biol. Chem.* **1991**, *266*, 14504–14510.

(21) Fleet, G. W. J.; Nicolas, S. J.; Smith, P. W.; Evans, S. V.; Fellows, L. E.; Nash, R. J. Potent Competitive Inhibition of α-Galactosidase and α-Glucosidase Activity by 1,4-Dideoxy-1,4-imino-pentitols: Synthesis of 1,4-Dideoxy-1,4-imino-D-lyxitol and of Both Enantiomers of 1,4-Dideoxy-1,4-iminoarabinitol. *Tetrahedron Lett.* **1985**, *26*, 3127–3130.

(22) Asano, N.; Oseki, K.; Kizu, H.; Matsui, K. Nitrogen-in-the-Ring Pyranoses and Furanoses: Structural Basis of Inhibition of Mammalian Glycosidases. *J. Med. Chem.* **1994**, *37*, 3701–3706.

(23) Scofield, A. M.; Fellows, L. E.; Nash, R. J.; Fleet, G. W. J. Inhibition of Mammalian Digestive Disaccharidases by Polyhydroxy Alkaloids. *Life Sci.* **1986**, *39*, 645–650.

(24) Elbein, A. D.; Mitchell, M.; Sanford, B. A.; Fellows, L. E.; Evans, S. V. The Pyrrolidine Alkaloid, 2,5-Dihyhdroxymethyl-3,4-dihydroxypyrrolidine, Inhibits Glycoprotein Processing. *J. Biol. Chem.* **1984**, *259*, 12409–12413.

(25) Lasky, L. A.; Groopman, J. E.; Fennie, C. W.; Benz, P. M.; Capon, D. J.; Dowbenko, D. J.; Nakamura, G. R.; Nunes, W. M.; Renz, M. E.; Berman, P. W. Neutralization of the AIDS Retrovirus by Antibodies to a Recombinant Envelope Glycoprotein. *Science* **1986**, *233*, 209–212.
(26) Matthews, T. J.; Weinhold, K. J.; Lyerly, H. K.; Langlois, A. J.; Wigzel, H.; Bolognesi, D. Interaction between the Human T-Cell Lymphotropic Virus Type IIIB Envelope Glycoprotein gp 120 and the Surface Antigen CD4: Role of Carbohydrate in Binding and Cell Fusion. *Proc. Natl. Acad. Sci. U.S.A.* **1987**, *84*, 5424–5428.
(27) Tyms, A. S.; Berrie, E. M.; Ryder, T. A.; Nash, R. J.; Hegarty, M. P.; Taylor, D. L.; Mobberley, M. A.; Davis, J. M.; Bell, E. A.; Jeffries, D. J.; Talor-Robinson, D.; Fellows, L. E. Castanospermine and Other Plant Alkaloid Inhibitors of Glucosidase Activity Block the Growth of HIV. *Lancet* **1987**, 1025–1026.
(28) Fleet, G. W. J.; Karpas, A.; Dwek, R. A.; Fellows, L. E.; Tyms, A. S.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Smith, P. W.; Son, J. C.; Wilson, F.; Witty, D. R.; Jacob, G. S.; Rademacher, T. W. Inhibition of HIV Replication by Amino-sugar Derivatives. *FEBS Lett.* **1988**, *237*, 128–132.
(29) Karpas, A.; Fleet, G. W. J.; Dwek, R. A.; Petursson, S.; Namgoong, S. K.; Ramsden, N. G.; Jacob, G. S.; Rademacher, T. W. Aminosugar Derivatives as Potential Anti-human Immunodeficiency Virus Agents. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9229–9233.
(30) Montefiori, D. C.; Robinson, W. E.; Mitchell, W. M. Role of Protein N-Glycosylation in Pathogenesis of Human Immunodeficiency Virus Type 1. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 9248–9252.
(31) Taylor, D. L.; Sunkara, P. S.; Liu, P. S.; Kang, M. S.; Bowlin, T. L.; Tyms, A. S. 6-O-Butanoylcastanospermine (MDL 28,574) Inhibits Glycoprotein Processing and the Growth of HIVs. *AIDS* **1991**, *5*, 693–698.
(32) Harris, E. M. S.; Aleshin, A. E.; Firsov, L. M.; Hanzatko, R. B. Refined Structure for the Complex of 1-Deoxynojirimycin with Glucoamylase from *Aspergillus awamori* var. X 100 to 2.4-Å Resolution. *Biochemistry* **1993**, *32*, 1618–1626.
(33) Hempel, A.; Camerman, N.; Mastropaolo, D.; Camerman, A. Glucosidase Inhibitors: Structures of Deoxynojirimycin and Castanospermine. *J. Med. Chem.* **1993**, *36*, 4082–4086.
(34) Glaser, R.; Perlin, A. S. ^{1}H- And ^{13}C-N.M.R. Studies on N-Methyl-1-deoxynojirimycin, an α-D-Glucosidase Inhibitor. *Carbohydr. Res.* **1988**, *182*, 169–177.
(35) Lembcke, B.; Folsch, U. R.; Creutzfeldt. Effect of 1-Desoxynojirimycin Derivatives on Small Intestinal Disaccharidase Activities and on Active Transport *in vitro*. *Digestion* **1985**, *31*, 120–127.
(36) Szumilo, T.; Kaushal, G. P.; Elbein, A. D. Purification and Properties of Glucosidase I from Mung Bean Seedling. *Arch. Biochem. Biophys.* **1986**, *247*, 261–271.
(37) Schweden, J.; Legler, G.; Bause, E. Purification and Characterization of a Neutral Mannosidase from Calf Liver Acting on $(Man)_9(GlcNAc)_2$ Oligosaccharides. *Eur. J. Biochem.* **1986**, *157*, 563–570.
(38) AL Daher, S.; Fleet, G.; Namgoong, S. K.; Winchester, B. Change in Specificity of Glycosidase Inhibition by N-Alkylation of Amino Sugars. *Biochem. J.* **1989**, *258*, 613–615.
(39) Sunkara, P. S.; Taylor, D. L.; Kang, M. S.; Bowlin, T. L.; Liu, P. S.; Tyms, A. S.; Sjoerdsma, A. Anti-HIV Activity of Castanospermine Analogues. *Lancet* **1989**, 1206.
(40) Taylor, D. L.; Nash, R.; Fellows, L. E.; Kang, M. S.; Tyms, A. S. Naturally Occurring Pyrrolizidines: Inhibition of α-Glucosidase 1 and Anti-HIV Activity of One Stereoisomer. *Antiviral Chem. Chemother.* **1992**, *3*, 273–277.
(41) Karlsson, G. B.; Butters, T. D.; Dwek, R. A.; Platt, F. M. Effects of the Imino Sugar N-Butyldeoxynojirimycin on the N-Glycosylation of Recombinant gp120. *J. Biol. Chem.* **1993**, *268*, 570–576.
(42) Platt, F. M.; Neises, G. R.; Karlsson, G. B.; Dwek, R. A.; Butters, T. D. N-Butylgalactonojirimycin Inhibits Glycolipid Biosynthesis but Does not Affect N-Linked Oligosaccharide Processing. *J. Biol. Chem.* **1994**, *269*, 27108–27114.
(43) Kato, H.; Koshinaka, E.; Arata, Y.; Hanaoka, M. Studies on 1-Azabicyclo Compounds. XVI. Synthesis of 1′-Methylindan-2-spiro-2′-piperazine and Related Compounds. *Chem. Pharm. Bull.* **1973**, *21*, 2661–2666.
(44) Kessler, M; Acuto, O.; Strelli, C.; Murer, H.; Semenza, G. Modified Procedure for the Rapid Preparation of Efficiently Transporting Vesicles from Small Intestinal Brush Border Membranes. Their Use in Investigating Some Properties of D-Glucose and Choline Transport System. *Biochem. Biophys. Acta* **1978**, *506*, 136–154.
(45) Miyoshi, I.; Taguchi, H.; Kubonishi, I.; Yoshimoto, S.; Ohtsuki, Y.; Shiraishi, Y.; Akagi, T. Type C Virus-producing Cell Lines Derived from Adult T Cell Leukemia. *Gann Monogr.* **1982**, *28*, 219–228.
(46) Kikukawa, R.; Koyanagi, Y.; Harada, S.; Kobayashi, N.; Hatanaka, M.; Yamamoto, N. Differential Susceptibility to the Acquired Immunodeficiency Syndrome Retrovirus in Clone Cells of Human Leukemic T-Cell Line, Molt-4. *J. Virol.* **1986**, *57*, 1159–1162.
(47) Baba, M.; De Clercq, E.; Tanaka, H.; Ubasawa, M.; Takashima, H.; Sekiya, K.; Nitta, I.; Umezu, K.; Nakashima, H.; Mori, S.; Shigeta, S.; Walker, R. T.; Miyasaka, T. Potent and Selective Inhibition of Human Immunodeficiency Virus Type-1 (HIV-1) by 5-Ethyl-6-phenylthiouracil Derivatives through their interaction with the HIV-1 Reverse Transcriptase. *Proc. Natl. Acad. Sci. U.S.A.* **1991**, *88*, 2356–2360.
(48) Pauwels, R.; Balzarini, J.; Baba, M.; Snoeck, R.; Schols, D.; Herdewijn, P.; Desmyter, J.; De Clercq, E. Rapid and Automated Tetrazolium-based Colorimetric Assay for the Detection of Anti-HIV Compounds. *J. Virol. Methods* **1988**, *20*, 309–321.

JM950140X