Biological Properties of D- and L-1-Deoxyazasugars

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L-Enantiomers of 1-deoxynojirimycin (DNJ), 1-deoxymannojirimycin (*manno*-DNJ), 1-deoxyallonojirimycin (*allo*-DNJ), 1-deoxyaltronojirimycin (*altro*-DNJ), 1-deoxygalactonojirimycin (*galacto*-DNJ), 1-deoxygulonojirimycin (*gulo*-DNJ), and 1-deoxyidonojirimycin (*ido*-DNJ) were prepared according to prior methods for the D-enantiomers. These enantiospecific syntheses established unambiguously the absolute configuration of naturally occurring DNJ, *manno*-DNJ, *allo*-DNJ, *altro*-DNJ, and *gulo*-DNJ. Although D-DNJ and D-*galacto*-DNJ are known to be powerful competitive inhibitors of α-glucosidase and α-galactosidase, respectively, with K_i values in the nM range, L-DNJ and L-*galacto*-DNJ were noncompetitive inhibitors of α-glucosidase and α-galactosidase, respectively, with K_i values in the μM range. However, the azasugar mimicking the structure of the terminal sugar moiety of the natural substrate is not always an inhibitor of the glycosidase responsible for the hydrolysis. D-*manno*-DNJ is known as a much better inhibitor of α-L-fucosidase than α-mannosidase, while L-*allo*-DNJ was a better inhibitor than D-*manno*-DNJ of α-mannosidase. L-*galacto*-DNJ can be regarded as the 6-hydroxylated derivative of deoxyfuconojirimycin (DFJ), which is a powerful inhibitor of α-L-fucosidase with a K_i value in the nM range. However, this replacement of the methyl group in DFJ by a hydroxymethyl group reduced its affinity by about 50-fold. This suggests that there is a hydrophobic region in or around the active site of α-L-fucosidase**.** It has been found that inhibitors of human lysosomal glycosidases have therapeutic potential for the corresponding lysosomal storage diseases (*Nat. Med.* **1999**, *5*, 112; *Proc. Natl. Acad. Sci. USA*, **2002**, *99*, 15428). Inhibition of human lysosomal glycosidases by the 1-deoxyazasugars synthesized was investigated. D-*galacto*-DNJ is a potent inhibitor of lysosomal α-galactosidase (IC_{50} = 90 nM) and is now being evaluated preclinically for its potential use in Fabry disease, while D-DNJ inhibiting α-glucosidase (IC_{50} = 40 nM) potently does not appear to become a potential therapeutic agent because of additional inhibitory activity toward glycoprotein processing α-glucosidases. On the other hand, although L-*allo*-DNJ is a moderate inhibitor of α-mannosidase (IC_{50} = 64 μM), it may become a key compound for the drug design of potential therapeutic agents for α-mannosidosis.

Introduction

Glycosidases are involved in a wide range of anabolic and catabolic process, such as digestion, lysosomal catabolism of glycoconjugates, biosynthesis of glycoproteins, and the endoplasmic reticulum (ER) quality control and ER-associated degradation of glycoproteins. Hence, modifying or blocking these processes in vivo by inhibitors is of great interest from a therapeutic point of view. Azasugars (or iminosugars) are an important class of glycosidase inhibitors and are arousing great interest as potential therapeutic agents such as antidiabetics, antiobesities, antivirals, and therapeutic agents for some genetic disorders.[1−5] Miglitol (Glyset)[6] has been approved as the second-generation α-glucosidase inhibitor to treat type 2 diabetes and *N*-butyl-1-deoxynojirimycin (Zavesca)[7−9] has also been approved for use in patients with type 1 Gaucher disease in the European Union in 2002 and in the U.S. in 2003. Recently, it has been demonstrated that competitive inhibitors of lysosomal glycosidases have therapeutic potential for lysosomal storage diseases caused by genetic defects in lysosomal enzymes.[10−13] 1-Deoxygalactonojirimycin, a powerful inhibitor of lysosomal α-galactosidase, was found to serve as an enhancer of mutant enzyme activity in cells derived from Fabry patients at subinhibitory intracellular concentrations[10] and is currently in preclinical trials for practical use. On the other hand, the addition of *N*-nonyl-1-deoxynojirimycin to the Gaucher cell culture medium at subinhibitory concentrations leads to a 2-fold increase in the intracellular activity of lysosomal β-glucosidase (β-glucocerebrosidase).[12] These enhancers of intracellular mutant enzyme activity are called 'chemical chaperones'.[10,14,15] Competitive inhibi-

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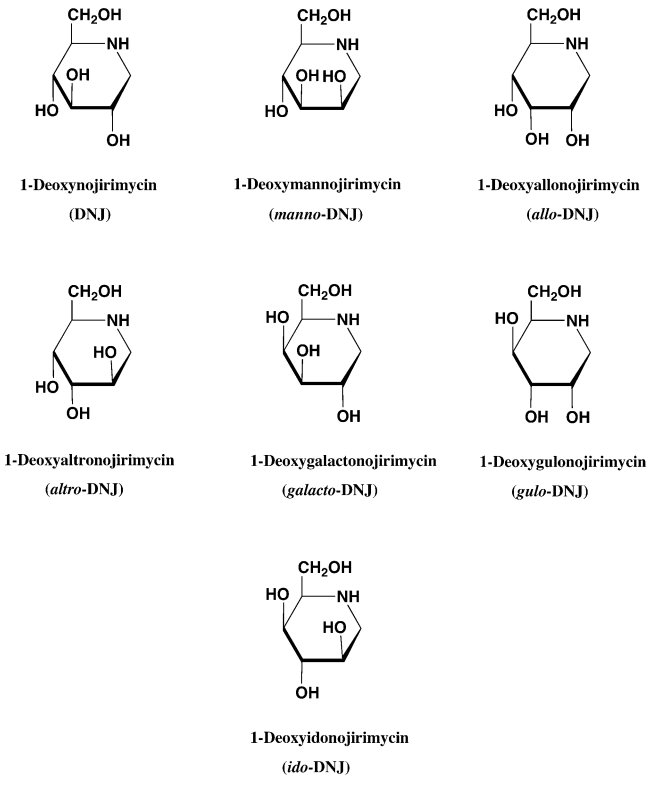
tors of lysosomal glycosidases have therapeutic potential as 'chemical chaperones' for lysosomal storage diseases.

Four naturally occurring 1-deoxyazasugars have been isolated to date. 1-Deoxynojirimycin (DNJ) and 1-deoxymannojirimycin (*manno*-DNJ) have been isolated from many plants and microorganisms.[3] 1-Deoxyaltronojirimycin (*altro*-DNJ) and 1-deoxygulonojirimycin (*gulo*-DNJ) recently have been discovered from *Scilla sibirica* (Hyacinthaceae)[16] and *Angylocalyx pynaertii* (Leguminosae).[17] Many efforts have been devoted in recent years to develop methodologies for the asymmetric syntheses of azasugars owing to their promising therapeutic potential in a wide range of diseases including diabetes, viral infection, cancer, and lysosomal storage disorders.[1−5] However, many syntheses of azasugars have been focused on derivatives of D-*gluco*, D-*manno*, or D-*galacto* configurations because their targeting enzymes are essential for survival and existence of all living organisms.[1,18,19] There are a number of reports and reviews on in vitro and in vivo glycosidase inhibition by DNJ, *manno*-DNJ, *galacto*-DNJ, and their derivatives.[1−5,7,8,20−22] On the other hand, there are few reports that systematically studied biological property of the enantiomers of DNJ, *manno*-DNJ, and *galacto*-DNJ, and other diastereomers of DNJ. Herein, we report the syntheses of both enantiomers of DNJ, *manno*-DNJ, *allo*-DNJ, *galacto*-DNJ, *altro*-DNJ, *gulo*-DNJ, and *ido*-DNJ and systematic studies of their glycosidase inhibitory activities.



Results and Discussion

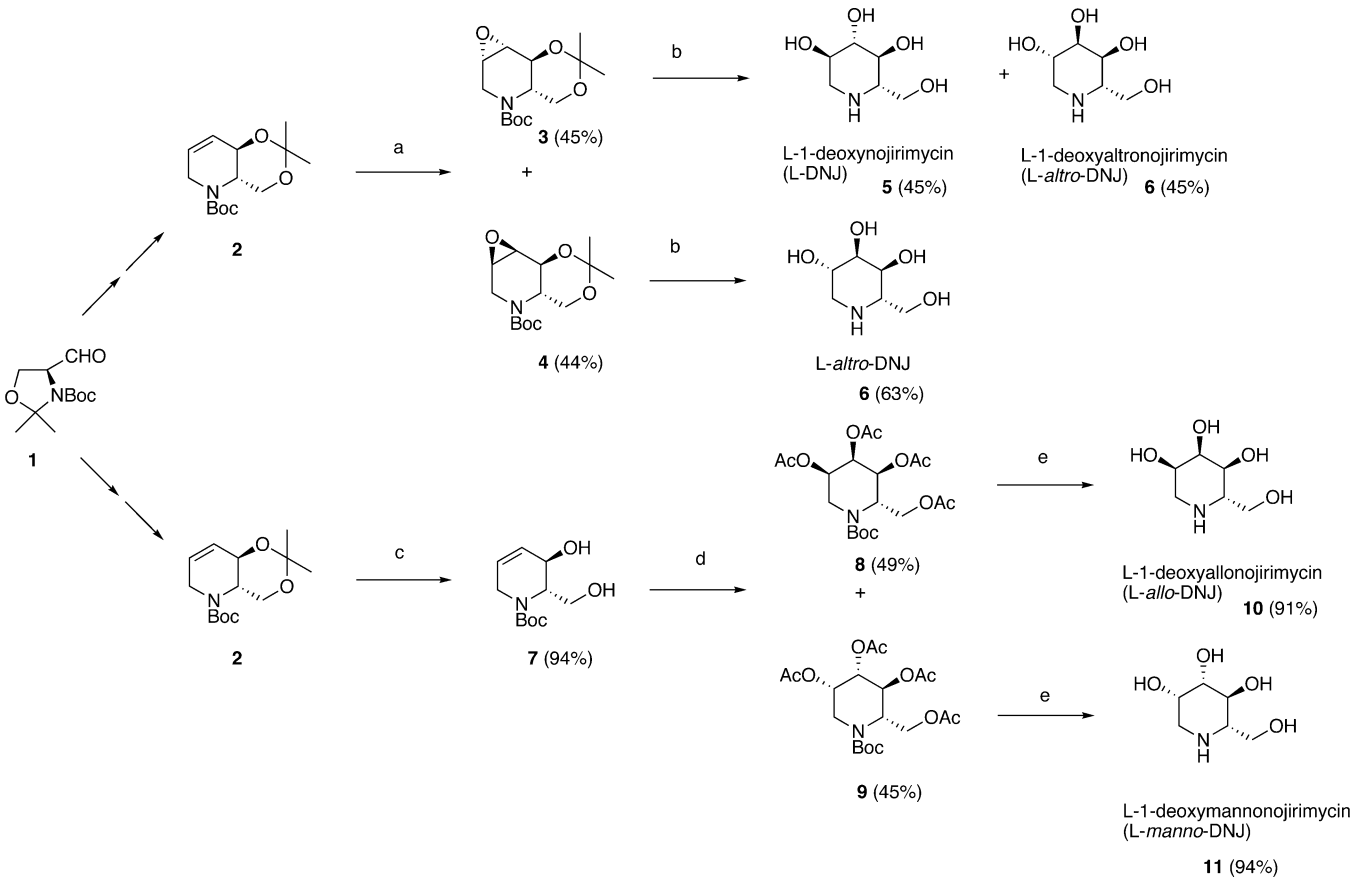
Chemistry. The D-enantiomers of *galacto*-DNJ, *gulo*-DNJ, and *ido*-DNJ were synthesized in a highly stereocontrolled mode from cis-4,5-oriented dioxanylpiperidene as a common chiral building block according to the literature.[23] This *cis*-4,5-oriented dioxanylpiperidene is prepared via the *syn*-vinyl alcohol starting from the D-serine-derived Garner aldehyde,[24] which reacts with organometallic reagents with a high degree of diastereoselectivity and little racemization,[25,26] using catalytic ring closing metathesis for the construction of the piperidine ring.[27] The D-enantiomers of DNJ, *manno*-DNJ, *allo*-DNJ, and *altro*-DNJ were also similarly synthesized from trans-4,5-oriented dioxanylpiperidene via the *anti*-vinyl alcohol from the D-serine-derived Garner aldehyde according to the literature.[28]

The L-enantiomers of DNJ, *manno*-DNJ, *allo*-DNJ, *altro*-DNJ, *galacto*-DNJ, *gulo*-DNJ, and *ido*-DNJ were enantiospecifically synthesized from dioxanylpiperidenes **2** and **12** starting from the L-serine-derived Garner aldehyde **1** (Schemes 1 and 2). Epoxidation of **2** with the dioxirane gave *anti*-epoxide **3** and *syn*-epoxide **4**. Acidic hydrolysis of **3** provided a diastereomeric mixture of L-DNJ (**5**) and L-*altro*-DNJ (**6**). The hydrolysis of **4** afforded only **6**. Deprotection of the acetonide in **2**, followed by a sequence of dihydroxylation and acetylation of the resulting diol **7**, yielded a separable mixture of **8** and **9**, which were converted with hydrolysis to L-*allo*-DNJ (**10**) and L-*manno*-DNJ (**11**), respectively (Scheme 1). Treatment of **12** with the oxirane provided the epoxide **13**, which was subjected to cleavage of epoxide and hydrolysis to give L-*ido*-DNJ (**16**). The acetonide of **12** was deprotected to afford **14**, which was subjected to syn epoxidation, followed by acetalization to afford **15**. The acidic hydrolysis of **15** gave L-*galacto*-DNJ (**17**). The stereoselective dihydroxylation of **12** provided the diol **18**, which was deprotected with acidic hydrolysis to afford L-*gulo*-DNJ (**19**) (Scheme 2).

Naturally occurring DNJ was prepared from *Morus alba* (Moraceae),[29] and *manno*-DNJ, *altro*-DNJ, and *gulo*-DNJ from *A. pynaertii* (Leguminosae),[17,30] according to the literature. We isolated *allo*-DNJ from a Thai traditional crude drug "Thob-taeb", whose biological origin is *Connarus ferruginens* (Combretaceae). The ^{1}H and ^{13}C NMR spectra of DNJ, *manno*-DNJ, *allo*-DNJ, *altro*-DNJ, and *gulo*-DNJ were completely identical with those of the corresponding synthetic 1-deoxyazasugar. The absolute configurations of natural DNJ, *manno*-DNJ, *allo*-DNJ, *altro*-DNJ, and *gulo*-DNJ were determined to be D-, D-, D-, D-, and L-enantiomers, respectively, from the value and sign of the optical rotation, as shown in Table 1.

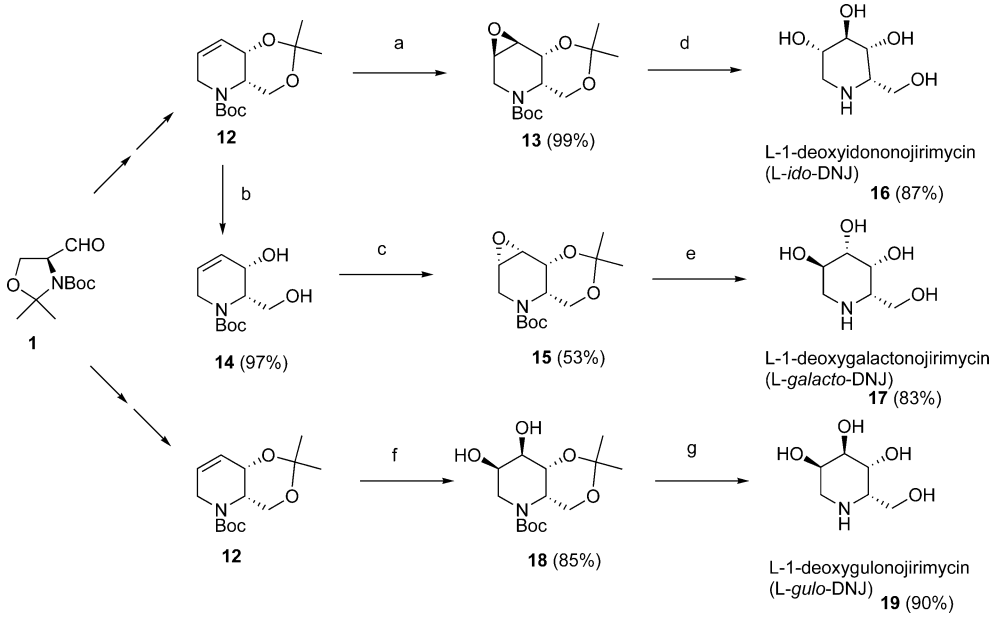
Biology. The IC_{50} values of the D- and L-enantiomers of 1-deoxyazasugars toward a variety of glycosidases are shown in Table 2. D-DNJ has been known to be a potent inhibitor of all types of α-glucosidases other than yeast α-glucosidase.[21,31−35] Interestingly, its enantiomer L-DNJ was also a fairly potent inhibitor of rice α-glucosidase, with an IC_{50} value of 4.3 μM. While D-*galacto*-DNJ is known as an extremely powerful inhibitor of coffee bean α-galactosidase,[36] its L-enantiomer was a 4000-fold weaker inhibitor than the D-*galacto*-DNJ of the enzyme. The epimerization at C-3 in D-*galacto*-DNJ to give D-*ido*-DNJ drastically reduced its inhibitory potency over 4 orders of magnitude. D-*manno*-DNJ mimicking D-mannose is an inhibitor of Golgi α-mannosidases I and II with IC_{50} values of 25 and 410 μM, respectively,[35] but is a very poor inhibitor of plant and lysosomal α-mannosidases. It is of much more interest that L-*allo*-DNJ is a better inhibitor than D-*manno*-DNJ of both α-mannosidases. The inhibition of D-*manno*-DNJ, D-*allo*-DNJ,

Scheme 1[a]



[a] Reagents and conditions: (a) Na_2EDTA, CF_3COCF_3, $NaHCO_3$, Oxone, CH_3CN, 0 °C, 1 h; (b) (i) concd H_2SO_4, 1,4-dioxane, H_2O, reflux, 3 h; (ii) Dowex 50W-X8 (H^+ form) (c) *p*-TsOH·H_2O, MeOH, rt, 2 h; (d) (i) cat. K_2OsO_4·$2H_2O$, NMO, acetone, rt, overnight; (ii) Ac_2O, pyridine, rt, overnight; (e) (i) 6 N HCl, MeOH, reflux, 8 h; (ii) Dowex 50W-X8 (H^+ form).

Scheme 2[a]



[a] Reagents and conditions: (a) Oxone, CF_3COCH_3, $NaHCO_3$, aq Na_2·EDTA, CH_3CN, 0 °C; (b) *p*-TsOH·H_2O, MeOH, rt; (c) (i) *m*-CPBA, NaH_2PO_4, CH_2Cl_2, 0 °C to room temperature; ii) 2,2-dimethoxypropane, cat. PPTS, acetone, rt; (d) (i) 0.3 M KOH, 1,4-dioxane, H_2O, reflux; (ii) 6 N HCl, MeOH, rt; (iii) Amberlite IRA-410 (OH^- form) (e) (i) H_2SO_4, 1,4-dioxane, H_2O, reflux; (ii) Amberlite IRA-410 (OH^- form); (iii) Dowex 1-X2 (OH^- form); (f) K_2OsO_4·$2H_2O$, NMO, acetone, H_2O, rt; (g) (i) 6 N HCl, MeOH, rt; (ii) Dowex 50W-X8 (H^+ form).

and L-*gulo*-DNJ toward α-L-fucosidase were moderate, whereas L-*galacto*-DNJ was a potent inhibitor of the enzyme, with an IC_{50} value of 0.63 μM. Zhou et al. have reported that L-*manno*-DNJ is a fairly good inhibitor of *Aspergillus niger* α-galactosidase, with a K_i value of 8.2 μM.[37] However, in our investigation L-*manno*-DNJ

Table 1. Specific Rotation Values of Natural and Synthetic 1-Deoxyazasugars

	$[\alpha]_D$ (H_2O)		
1-deoxyazasugar	natural	D-enantiomer	L-enantiomer
DNJ	+42.1	+40.3	−40.1
manno-DNJ	−41.4	−40.2	+40.2
allo-DNJ	+35.5*	+36.2*	−37.0*
altro-DNJ	+19.1	+19.1	−21.0
galacto-DNJ	none	+50.2	−50.6
gulo-DNJ	+14.3	−15.0	+14.9
ido-DNJ	none	−10.5	+8.7

*Measurement in MeOH.

showed no significant inhibitory activity toward this enzyme even at the high concentration of 1 mM.

Fleet et al.[38] synthesized 1-deoxyfuconojirimycin (DFJ, 1,5-dideoxy-1,5-imino-L-fucitol) and reported that it is a very powerful competitive inhibitor of bovine epididymis α-L-fucosidase with a K_i value of 4.8 nM. The K_i values of DFJ analogues toward bovine epididymis α-L-fucosidase were shown in Figure 1. We previously reported that the K_i value of DFJ toward this enzyme is 1.7 nM.[17] The K_i for L-*galacto*-DNJ, which can be regarded as the C-6 hydroxylated derivative of DFJ, was calculated to be 80 nM. This means that the alteration of the methyl group in DFJ to a hydroxymethyl group decreased its affinity by about 50-fold. In addition, the difference in affinity by about 2 orders of magnitude was observed between β-L-homofuconojirimycin[39] and β-homomannojirimycin,[17] which differ by methyl and hydroxymethyl functionality. These results support the hypothesis that there might be a hydrophobic region in the active site close to where the methyl group of the inhibitor or substrate binds.[17,38,40,41] Introduction of a hydroxymethyl group into the β-anomeric position in DFJ and L-*galacto*-DNJ to give β-L-homofuconojirimycin and β-homomannojirimycin, respectively, resulted in 4- to 5-fold lower affinities. Winchester et al.[37] have suggested that the common structural feature to all inhibitors of α-L-fucosidase is the correct stereochemistry of the three hydroxyl groups on the piperidine ring corresponding to C-2, C-3, and C-4 of L-fucose. D-*manno*-DNJ is a moderate inhibitor with this minimum structural feature for inhibition of α-L-fucosidase. On the other hand, inversion of the configuration at C-3 in L-*galacto*-DNJ having the minimum structural feature to give L-*gulo*-DNJ lowered its affinity by about 200-fold.

The enzyme can distinguish between the desired substrate and its mirror image, also called the enantiomer. This is because the enzyme has an active site that will only accept one of the enantiomers but reject the other. For instance, α-glucosidase cleaves the terminal D-glucosyl residue of the substrate but does not cleave the L-glucosyl residue. In the present study, it was truly an amazing result that L-DNJ and L-*galacto*-DNJ inhibited α-glucosidase and α-galactosidase, respectively. These inhibitory activities are not due to the presence of their antipode impurities because it is impossible to take place with racemization in all reaction steps except for addition of vinylmetals to Garner aldehyde and it has been known that this addition reactions are accompanied by little racemization.[25,26] Furthermore, their reasonable optical rotation values (Table 1) deny the presence of their antipode impurities. Whether they dominate the active sites and show competitive inhibition is a very important problem. Hence, we examined the nature of their inhibition by Lineweaver−Burk plots. The Lineweaver−Burk plots of D-DNJ and L-DNJ inhibition of rice α-glucosidase are shown in Figure 2. D-DNJ mimicking D-glucose inhibited the enzyme in a competitive manner, with a K_i value of 5.7 nM, whereas L-DNJ was a noncompetitive inhibitor of the enzyme, with a K_i value of 4.5 μM. Similarly, D-*galacto*-DNJ and L-*galacto*-DNJ were competitive (K_i = 3.5 nM) and noncompetitive (K_i = 7.3 μM) inhibitors of coffee bean α-galactosidase, respectively. It is thought that L-DNJ is bound to the site (regulatory site) other than the active site and the shape of the active site consequently changes so that substrate can no longer fit there.

The endoplasmic reticulum (ER) possesses efficient quality control mechanisms to ensure that transport is limited to properly folded and assembled proteins.[42−44] Recent experimental data show that some human genetic diseases are due to mutations in proteins that influence their folding and lead to retaining of mutant proteins in the ER and successive degradation.[45,46] Genetically inherited diseases are often characterized by specific point mutations or deletions that give rise to proteins that fail to achieve their properly folded state. The major problem in lysosomal storage diseases appears to be a failure of the newly synthesized mutant enzyme proteins to exit from the ER and to move to the lysosomes via the Golgi apparatus. Studies on residual mutant α-galactosidase A activity in many Fabry patients have revealed that some have kinetic properties similar to those of the normal enzyme but are significantly less stable.[47] Furthermore, the intracellular mutant α-galactosidase A formed aggregates in the ER and was quickly degraded.[48] These observations raise the possibility that a functional compound that can elicit the proper folding and trafficking of the mutant protein might prove to be effective strategy for the treatment of the genetic disorders. In fact, it has been demonstrated that D-*galacto*-DNJ and the *N*-nonyl derivative of D-DNJ serve as chemical chaperones that facilitate the correct folding of mutant enzymes and promote the smooth escape from the ER quality control system in the cells derived from Fabry and Gaucher patients, respectively.[10−12] These results suggest that competitive inhibitors of lysosomal glycosidases have therapeutic potential for the treatment of lysosomal storage diseases.

Inhibitory activity of the D- and L-enantiomers of 1-deoxyazasugars toward human lysosomal glycosidases is shown in Table 3. D-DNJ is a powerful inhibitor of human lysosomal α-glucosidase and may serve as a chemical chaperone in the cells derived from Pompe disease patients. Pompe disease is a rare genetic disorder caused by a deficiency in lysosomal α-glucosidase, which is needed to break down glycogen. However, D-DNJ is also a potent inhibitor of all types of mammalian α-glucosidases, such as digestive α-glucosidases, glycoprotein processing α-glucosidases I and II, and lysosomal α-glucosidase.[21,31−35] The inability to remove D-glucose from N-linked oligosaccharides can have profound effects on synthesis, transport, and/or secretion of the given glycoproteins. The rate of secretion of α_1-antitripsin fell in HepG-2 cells grown in the presence

Table 2. Inhibition of Various Glycosidases by D- and L-1-Deoxyazasugars

	IC_{50} (μM)[a]													
	DNJ		*manno*-DNJ		*allo*-DNJ		*altro*-DNJ		*galacto*-DNJ		*gulo*-DNJ		*ido*-DNJ	
enzyme	D	L	D	L	D	L	D	L	D	L	D	L	D	L
α-glucosidase														
rice	0.03	4.3	–	–	–	–	–	450	–	–	–	–	–	–
rat intestinal maltase	0.65	28	110	–	–	–	–	–	–	–	–	–	–	–
β-glucosidase														
almond	80	980	–	–	–	–	–	–	–	–	–	–	60	–
α-mannosidase														
jack bean	–	–	840	–	–	30	–	–	–	–	–	–	–	–
rat epididymis	–	–	560	–	–	59	–	–	–	–	–	–	–	–
β-mannosidase														
rat epididymis	–	–	–	–	–	–	–	–	–	–	–	–	–	–
α-galactosidase														
coffee bean	880	–	–	–	260	290	–	–	0.003	13	160	–	67	–
Aspergillus niger	–	–	–	–	–	–	–	–	1.8	–	–	–	–	–
β-galactosidase														
bovine liver	–	560	–	–	–	–	–	–	–	560	–	–	850	–
rat epididymis	–	–	–	–	–	–	–	–	24	–	–	–	–	–
α-L-fucosidase														
bovine epididymis	–	–	39	–	194	–	–	–	–	0.63	–	156	–	–

[a] –: less than 50% inhibition at 1000 μM.

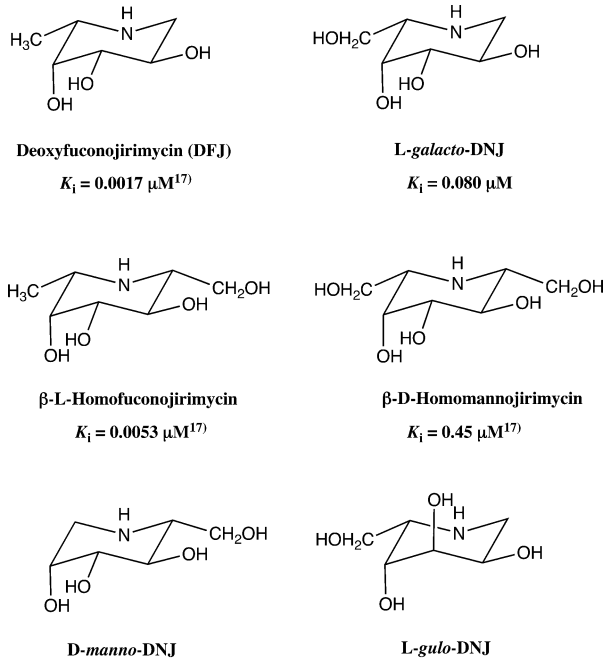


Figure 1. The structures of 1-deoxyfuconojirimycin (DFJ) analogues and their K_i values for bovine epididymis α-L-fucosidase.

of D-DNJ, suggesting that the presence of D-glucose on the oligosaccharide might retard transport of the protein.[49] In fact, it was shown that α_1-antitripsin has accumulated in the ER. When processing α-glucosidase inhibitors are used as therapeutic agents, it remains to be determined what effects occur on in vivo glycoprotein synthesis and/or glycoprotein transport. D-*galacto*-DNJ is known to be a potent inhibitor of human α-galactosidase A and to act as an effective chemical chaperone in the cells derived from Fabry patients.[10,11] This compound (AT1001) is now being evaluated preclinically for its potential use in Fabry disease. Although D-*galacto*-DNJ also shows a moderate inhibitory activity (IC_{50} = 90 μM) toward lysosomal β-galactosidase, it would be less effective as a chemical chaperone in β-galactosidosis cells because relatively high concentrations (0.5−1 mM) were required for mutant enzyme activation.[50] L-*galacto*-DNJ showed a fairy potent inhibitory activity (IC_{50} = 3.5 μM) toward human α-L-fucosidase, but more potent inhibitors such as DFJ and β-L-homofuconojirimycin would be suitable as candidates for the treatment of fucosidosis.[17] D-*manno*-DNJ is known to be an inhibitor of class I α-mannosidases such as ER α-mannosidase I and Golgi α-mannosidase I but not of class II α-mannosidases including ER α-mannosidase II, Golgi α-mannosidase II, and lysosomal α-mannosidase.[51−53] In this study, D-*manno*-DNJ showed likewise no inhibition toward human lysosomal α-mannosidase and only L-*allo*-DNJ inhibited this enzyme with an IC_{50} value of 64 μM. Although swainsonine is well-known as a strong competitive inhibitor of lysosomal α-mannosidase, it has been shown that swainsonine is a lysosomotropic compound and accumulates rapidly in lysosomes of normal cells in culture to produce intracellular inhibition of this enzyme, resulting in the induction of a phenocopy of the genetic α-mannosidosis of humans.[54] When the human cells were placed in contact with medium containing only 0.1 μM swainsonine, ~60% of intracellular α-mannosidase activity was inhibited.[55] α-Mannosidase inhibitors that are suitable for the treatment of α-mannosidosis remain to be discovered.

Conclusions

The D- and L-enantiomers of DNJ, *manno*-DNJ, *allo*-DNJ, *altro*-DNJ, *galacto*-DNJ, *gulo*-DNJ, and *ido*-DNJ were enantiospecifically synthesized. Naturally occurring DNJ, *manno*-DNJ, *allo*-DNJ, *altro*-DNJ, and *gulo*-DNJ were found to be D-, D-, D-, D-, and L-enantiomers, respectively, from specific rotation values of their both D- and L-enantiomes. D-DNJ and D-*galacto*-DNJ are very potent competitive inhibitors of α-D-glucosidase and α-D-galactosidase, respectively, whereas the corresponding L-enantiomers were noncompetitive inhibitors of the D-glycosidases. From the structure−activity relationships of α-L-fucosidase inhibitors, it was strongly suggested there is a hydrophobic region in the active site close to where the methyl group of the inhibitor or substrate binds. The azasugar mimicking the structure

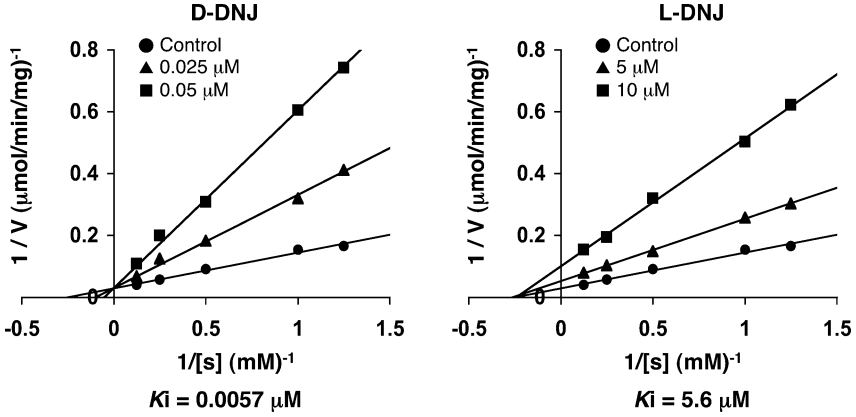


Figure 2. The Lineweaver−Burk plots of D-1-deoxynojirimycin (D-DNJ) and L-1-deoxynojirimycin (L-DNJ) inhibition of rice α-glucosidase.

Table 3. Inhibition of Human Lysosomal Glycosidases by D- and L-1-Deoxyazasugars

	IC_{50} (μM)[a]											
	DNJ		*manno*-DNJ		*allo*-DNJ		*galacto*-DNJ		*gulo*-DNJ		*ido*-DNJ	
	D	L	D	L	D	L	D	L	D	L	D	L
α-glucosidase	0.04	–	-	–	–	–	–	–	–	–	–	–
β-glucosidase	240	–	–	–	–	–	–	–	–	–	66	–
α-mannosidase	–	–	–	–	–	64	–	–	–	–	–	–
β-mannosidase	–	–	–	–	–	–	–	–	–	–	–	–
α-galactosidase	–	–	–	–	–	–	0.07	45	920	–	390	–
β-galactosidase	–	–	–	–	–	–	90	–	–	–	–	–
α-fucosidase	–	–	221	–	–	–	–	3.5	–	150	–	–

[a] –: less than 50% inhibition at 1000 μM.

of the terminal sugar moiety of the natural substrate are not always inhibitors of the glycosidase responsible for the hydrolysis. L-*allo*-DNJ is a much better inhibitor of α-mannosidase than D-*manno*-DNJ. Although inhibitors of human lysosomal glycosidases have therapeutic potential for the corresponding lysosomal storage diseases, inhibitors having additional inhibitory activity toward glycoprotein processing α-glucosidases or showing lysosomotropic behavior do not appear to be candidates in 'chemical chaperone therapy' for genetic lysosomal storage disorders. Azasugars have not only the role as versatile tools for various biochemical problems but also many potential applications as therapeutic agents.

Experimental Section

General Experimental Procedures. The purity of free base azasugars 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 (Tokyo, Japan). ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a JEOL ECP-500 spectrometer (Tokyo, Japan). Chemical shifts are expressed in ppm downfield from sodium 3-(trimethylsilyl)propionate (TSP) in D_2O as an internal standard for the free base samples. The proton and carbon signal assignments were determined from decoupling experiments and COSY and HMBC spectra. FABMS were measured using glycerol as a matrix on a JEOL JMS-700 spectrometer.

Preparation of 1-Deoxyazasugars. D-DNJ, D-*manno*-DNJ, D-*allo*-DNJ, and D-*altro*-DNJ were synthesized from *trans*-4,5-oriented dioxanylpiperidene via the *anti*-vinyl alcohol from D-serine-derived Garner aldehyde according to the literature[28] and D-*galacto*-DNJ, D-*gulo*-DNJ, and D-*ido*-DNJ from cis-4,5-oriented dioxanylpiperidene via the *syn*-vinyl alcohol from the same Garner aldehyde according to the literature.[23] Their L-enantiomers were synthesized by an analogous procedure to that of D-enantiomers, as shown in Schemes 1 and 2. Natural DNJ was isolated from the root bark of *Morus alba*,[29] and *manno*-DNJ, *altro*-DNJ, and *gulo*-DNJ were isolated from the bark of *Angylocalyx pynaertii*[17,30] according to the literature. Natural *allo*-DNJ was isolated from a Thai traditional crude drug "Thob-taeb" as follows. Thob-taeb is the leaves and twigs of *Connarus ferruginens* (Combretaceae) and commercially available in Thailand. This crude drug (3 kg, dry weight) was extracted with 50% aqueous MeOH. The extract was applied to a column of Amberlite IR-120B (800 mL, H^+ form). The 0.5 M NH_4OH eluate was concentrated to give a brown syrup (12 g), which was further chromatographed over a Dowex 1-X2 (400 mL, OH^- form) column with H_2O as eluant to give a colorless syrup (6 g). This syrup was applied to a 90 cm × 2.8 cm Amberlite CG-50 column (NH_4^+ form) with H_2O as eluant (fraction size 15 mL) and fractions 25−30 were collected and concentrated to give a colorless syrup (610 mg). This colorless syrup was further chromatographed with Dowex 1-X2 (87 cm × 1.7 cm, OH^- form) and Amberlite CG-50 column (87 cm × 1.7 cm, NH_4^+ form) columns with H_2O as eluant to give *allo*-DNJ (9.1 mg). This is the first report of its natural occurrence.

D-1-Deoxynojirimycin (D-DNJ): $[\alpha]_D$ +40.3° (*c* 1.47, H_2O). HRMS (FAB): *m/z* 164.0923 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

D-1-Deoxymannojirimycin (D-*manno*-DNJ): $[\alpha]_D$ −40.2° (*c* 0.33, H_2O). HRMS (FAB): *m/z* 164.0923 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

D-1-Deoxyallonojirimycin (D-*allo*-DNJ): $[\alpha]_D$ +36.2° (*c* 0.83, MeOH). HRMS (FAB): *m/z* 164.0922 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

D-1-Deoxyaltronojirimycin (D-*altro*-DNJ): $[\alpha]_D$ +19.1° (*c* 0.80, H_2O). HRMS (FAB): *m/z* 164.0923 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

D-1-Deoxygalactonojirimycin (D-*galacto*-DNJ): $[\alpha]_D$ +50.2° (*c* 1.02, H_2O). HRMS (FAB): *m/z* 164.0921 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

D-1-Deoxygulonojirimycin (D-*gulo*-DNJ): $[\alpha]_D$ −15.0° (*c* 1.55, H_2O). HRMS (FAB): *m/z* 164.0923 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

D-1-Deoxyidonojirimycin (D-*ido*-DNJ): $[\alpha]_D$ −10.5° (*c* 1.85, MeOH). HRMS (FAB): *m/z* 164.0921 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

L-1-Deoxynojirimycin (L-DNJ): $[\alpha]_D$ −40.1° (*c* 1.42, H_2O). ^1H NMR (D_2O): δ 2.42 (dd, 1H, *J* = 11.0, 12.3 Hz, H-1*ax*), 2.56 (ddd, 1H, *J* = 3.2, 6.4, 9.2 Hz, H-5), 3.13 (dd, 1H, *J* = 5.0, 12.3 Hz, H-1*eq*), 3.25 (t, 1H, *J* = 9.2 Hz, H-4), 3.34 (t, 1H, *J* = 9.2 Hz, H-3), 3.51 (ddd, *J* = 5.0, 9.2, 11.0 Hz, H-2), 3.64 (dd, 1H, *J* = 6.4, 11.4 Hz, H-6a), 3.85 (dd, 1H, *J* = 3.2, 11.4 Hz, H-6b). ^{13}C NMR (D_2O): δ 51.4 (C-1), 63.2 (C-5), 64.1 (C-6), 73.6 (C-2), 74.3 (C-4), 81.1 (C-3). HRMS (FAB): *m/z* 164.0922 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

L-1-Deoxymannojirimycin (L-*manno*-DNJ): $[\alpha]_D$ +40.2° (*c* 0.65, H_2O). ^1H NMR (D_2O): δ 2.49 (dt, 1H, *J* = 4.1, 9.6 Hz, H-5), 2.77 (dd, 1H, *J* = 1.4, 14.2 Hz, H-1*ax*), 3.01 (dd, 1H, *J* = 2.7, 14.2 Hz, H-1*eq*), 3.58 (dd, 1H, *J* = 3.2, 9.6 Hz, H-3), 3.62 (t, 1H, *J* = 9.6 Hz, H-4), 3.78 (d, 2H, H-6a, H-6b), 4.01 (ddd, 1H, *J* = 1.4, 2.7, 3.2 Hz, H-2). ^{13}C NMR (D_2O): δ 51.0 (C-1), 63.2 (C-5), 63.5 (C-6), 71.1 (C-4), 72.0 (C-2), 77.4 (C-3). HRMS (FAB): *m/z* 164.0920 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

L-1-Deoxyallonojirimycin (L-*allo*-DNJ): $[\alpha]_D$ −37.0° (*c* 1.04, MeOH). ^1H NMR (D_2O): δ 2.70 (dd, 1H, *J* = 11.4, 12.4 Hz, H-1*ax*), 2.75 (ddd, 1H, *J* = 3.2, 6.0, 10.1 Hz, H-5), 2.86 (dd, 1H, *J* = 5.0, 12.4 Hz, H-1*eq*), 3.49 (dd, 1H, *J* = 2.7, 10.1 Hz, H-4), 3.66 (dd, 1H, *J* = 6.0, 11.4 Hz, H-6a), 3.71 (ddd, 1H, *J* = 2.7, 5.0, 11.4 Hz, H-2), 3.82 (dd, 1H, *J* = 3.2, 11.4 Hz, H-6b), 4.11 (t, 1H, *J* = 2.7 Hz, H-3). ^{13}C NMR (D_2O): δ 46.4 (C-1), 57.3 (C-5), 64.2 (C-6), 71.0 (C-2), 71.5 (C-4), 74.3 (C-3). HRMS (FAB): *m/z* 164.0925 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

L-1-Deoxyaltronojirimycin (L-*altro*-DNJ): $[\alpha]_D$ −21.0° (*c* 1.24, H_2O). ^1H NMR (D_2O): δ 2.78 (ddd, 1H, *J* = 1.0, 3.2, 14.2 Hz, H-1*eq*), 2.83 (ddd, 1H, *J* = 3.7, 4.6, 9.6 Hz, H-5), 2.97 (dd, 1H, *J* = 2.3, 14.2 Hz, H-1*ax*), 3.74 (dd, 1H, *J* = 3.7, 11.9 Hz, H-6a), 3.78 (dd, 1H, *J* = 4.6, 11.9 Hz, H-6a), 3.83 (dd, 1H, *J* = 3.2, 9.6 Hz, H-4), 3.90 (m, 1H, H-2), 3.95 (m, 1H, H-3). ^{13}C NMR (D_2O): δ 47.3 (C-1), 58.4 (C-5), 63.6 (C-6), 69.0 (C-4), 72.1 (C-2), 73.4 (C-3). HRMS (FAB): *m/z* 164.0924 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

L-1-Deoxygalactonojirimycin (L-*galacto*-DNJ): $[\alpha]_D$ −50.6° (*c* 0.48, H_2O). ^1H NMR (D_2O): δ 2.24 (dd, 1H, *J* = 10.8, 12.4 Hz, H-1*ax*), 2.26 (dt, 1H, *J* = 1.5, 6.6 Hz, H-5), 2.99 (dd, 1H, *J* = 5.2, 12.4 Hz, H-1*eq*), 3.34 (dd, 1H, *J* = 3.1, 9.7 Hz, H-3), 3.43−3.50 (m, 2H, H-6a, H-6b), 3.66 (ddd, 1H, *J* = 5.2, 9.7, 10.8 Hz, H-2), 3.87 (dd, 1H, *J* = 1.5, 3.1 Hz, H-4). ^{13}C NMR (D_2O): δ 51.9 (C-1), 61.7 (C-5), 64.2 (C-6), 70.9 (C-2), 72.1 (C-4), 77.9 (C-3). HRMS (FAB): *m/z* 164.0922 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

L-1-Deoxygulonojirimycin (L-*gulo*-DNJ): $[\alpha]_D$ +14.9° (*c* 0.91, H_2O). ^1H NMR (D_2O): δ 2.73 (dd, 1H, *J* = 10.5, 12.4 Hz, H-1*ax*), 2.93 (ddd, 1H, *J* = 1.0, 5.9, 12.4 Hz, H-1*eq*), 2.98 (ddd, 1H, *J* = 2.2, 6.4, 7.0 Hz, H-5), 3.63 (dd, 1H, *J* = 7.1, 11.2 Hz, H-6a), 3.66 (dd, 1H, *J* = 6.4, 11.2 Hz, H-6b), 3.94 (dd, 1H, *J* = 2.2, 3.9 Hz, H-4), 3.95−3.99 (m, 2H, H-2, H-3). ^{13}C NMR (D_2O): δ 47.0 (C-1), 56.7 (C-5), 63.9 (C-6), 68.5 (C-2), 72.1 (C-4), 73.1 (C-3). HRMS (FAB): *m/z* 164.0921 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

L-1-Deoxyidonojirimycin (L-*ido*-DNJ): $[\alpha]_D$ +8.7° (*c* 0.56, MeOH). ^1H NMR (D_2O): δ 2.77 (dd, 1H, *J* = 8.2, 12.8 Hz, H-1*ax*), 2.99 (dd, 1H, *J* = 4.1, 12.8 Hz, H-1*eq*), 3.18 (ddd, 1H, *J* = 4.6, 5.5, 8.3 Hz, H-5), 3.58−3.63 (m, 2H, H-2, H-3), 3.75 (dd, 1H, *J* = 4.6, 8.3 Hz, H-4), 3.77 (dd, 1H, *J* = 5.5, 11.9 Hz, H-6a), 3.79 (dd, 1H, *J* = 8.3, 11.9 Hz, H-6b). ^{13}C NMR (D_2O): δ 46.7 (C-1), 59.1 (C-5), 60.4 (C-6), 72.9 (C-2), 73.4 (C-4), 75.2 (C-3). HRMS (FAB): *m/z* 164.0925 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

Natural 1-deoxynojirimycin: $[\alpha]_D$ +42.1° (*c* 1.00, H_2O). HRMS (FAB): *m/z* 164.0923 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

Natural 1-deoxymannojirimycin: $[\alpha]_D$ −41.4° (*c* 0.74, H_2O). HRMS (FAB): *m/z* 164.0922 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

Natural 1-deoxyallonojirimycin: $[\alpha]_D$ +35.5° (*c* 0.48, H_2O). ^1H NMR (D_2O): δ 2.71 (dd, 1H, *J* = 11.5, 11.9 Hz, H-1*ax*), 2.76 (ddd, 1H, *J* = 3.2, 6.0, 10.1 Hz, H-5), 2.87 (dd, 1H, *J* = 5.0, 11.9 Hz, H-1*eq*), 3.49 (dd, 1H, *J* = 2.7, 10.1 Hz, H-4), 3.65 (dd, 1H, *J* = 6.0, 12.0 Hz, H-6a), 3.71 (ddd, *J* = 3.2, 5.0, 11.5 Hz, H-2), 3.81 (dd, 1H, *J* = 3.2, 12.0 Hz, H-6b), 4.10 (dd, 1H, *J* = 2.3, 2.7 Hz, H-3). ^{13}C NMR (D_2O): δ 46.5 (C-1), 57.3 (C-5), 64.2 (C-6), 71.0 (C-2), 71.5 (C-4), 74.3 (C-3). HRMS (FAB): *m/z* 164.0923 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

Natural 1-deoxyaltronojirimycin: $[\alpha]_D$ +19.1° (*c* 0.56, H_2O). HRMS (FAB): *m/z* 164.0923 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

Natural 1-deoxygulonojirimycin: $[\alpha]_D$ +14.3° (*c* 0.29, H_2O). HRMS (FAB): *m/z* 164.0922 $[M + H]^+$ ($C_6H_{14}NO_4$ requires 164.0923). Anal. ($C_6H_{13}NO_4$) C, H, N.

Biological Assay Methods. The enzymes α-glucosidases (from rice, assayed at pH 5.0), β-glucosidase (from almond, pH 5.0), α-mannosidase (from jack bean, pH 4.5), α-galactosidases (from coffee bean, pH 6.5; from *A. niger*, pH 4.0), β-galactosidase (from bovine liver, pH 6.8; from *A. niger*, pH 4.0), α-L-fucosidase (from bovine epididymis, pH 5.5), *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.[56] and assayed at pH 5.2 for α-mannosidase, β-mannosidase, and β-galactosidase using *p*-nitrophenyl-glycosides. Brush border membranes prepared from rat small intestine according to the method of Kessler et al.[57] were assayed at pH 5.8 for rat intestinal maltase using maltose. For the activity of rice α-glucosidase and rat intestinal maltase, the reaction mixture (0.2 mL) contained 25 mM maltose and the appropriate amount of enzyme, and the incubations were performed for 10−30 min at 37 °C. The reaction was stopped by heating 100 °C for 3 min. After centrifugation (600 g; 10 min), 0.05 mL of resulting reaction mixture was added to 3 mL of Glucose B-test Wako (Wako Pure Chemical Ind.). The absorbance at 505 nm was measured to determine the amount of the released D-glucose. Other glycosidase activities were determined using an appropriate *p*-nitrophenyl glycoside as substrate at the optimum pH of each enzyme. The reaction mixture (1 mL) contained 2 mM of the substrate and the appropriate amount of enzyme. The reaction was stopped by adding 2 mL of 400 mM Na_2CO_3. The released *p*-nitrophenol was measured spectrometrically at 400 nm.

Human placenta α-L-fucosidase and β-glucocerebrosidase (Ceredase) were purchased from Sigma Chemical Co. and Genzyme (Boston, MA), respectively, and assayed at pH 5.5. Human lysosomal α-galactosidase was prepared as described previously.[58] The cell lysate of normal human fibroblasts (GM00498B), which were cultured in MEM medium (Gibco) supplemented with 15% fetal bovine serum and antibiotics at 37 °C under 5% CO_2, was used as the source of lysosomal α-glucosidase, α-mannosidase, β-mannosidase, and β-galactosidase. The reaction mixture consists of 50 μL of 0.15 M sodium phosphate-citrate buffer (pH 4.5), 50 μL of 2% Triton X-100 (Sigma Chemical Co.), 30 μL of the enzyme solution, and 20 μL of an inhibitor solution or H_2O. The reaction mixture was preincubated at 0 °C for 10 min and started by the addition of 50 μL of 6 mM 4-methylumbelliferyl glycoside (Sigma Chemical Co.) (1 mM in the case of β-galactoside), followed by incubation at 37 °C. The reaction was stopped by the addition of 2 mL of 0.1 M glycine buffer (pH 10.6). Liberated 4-methylumbelliferone was measured (excitation 362 nm, emission 450 nm) with a F-4500 fluorescence spectrophotometer (Hitachi, Tokyo, Japan).

Supporting Information Available: Elemental analyses. This material is available free of charge via the Internet at http://pubs.acs.org.

Note Added after ASAP Posting. This manuscript was released ASAP on 9/18/2004 with errors in Table 2 and with an incomplete citation for ref 28. The correct version was posted on 11/9/2004.

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