1-*N*-Iminosugars: Potent and Selective Inhibitors of β-Glycosidases

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Abstract: A series of 1-*N*-iminosugars were synthesized to supply the need for glycosidase inhibitors that are both highly potent and selective for β-glycosidases. Designed on the basis of the transition-state model of the β-glucosidase reaction, these iminosugar inhibitors differ from the currently available inhibitors in possessing a nitrogen atom at the anomeric position of the pyranose ring, thereby generating a positive charge on the anomeric position rather than on the ring oxygen of the sugar. Their syntheses, starting with a readily available carbohydrate derivative, involve (i) introduction of an amino functionality as an azido group, (ii) formation of a 1-*N*-iminopyranose ring with reductive amination, and (iii) stereoselective introduction of a hydroxymethyl or methyl group and were accomplished in a highly stereoselective and efficient manner. The inhibitory potencies of the 1-*N*-iminosugars were evaluated against several α- and β-glycosidases, and they were found to be extremely potent and highly specific against the corresponding β-glycosidases, with K_i values in the nanomolar range.

Inhibitors of glycosidases have been attractive target compounds for synthetic chemists and biochemists, not only because they serve as useful biological tools for studying the biological functions of oligosaccharides[1] but also because they may have great potential as drugs to treat a variety of carbohydrate-mediated diseases.[2] This group of inhibitors, which are derivatives of naturally occurring azasugars, are now finding clinical application as anti-HIV,[3] anticancer,[4] and antidiabetic agents,[5] and their effectiveness has made the development of additional glycosidase inhibitors a matter of considerable interest to synthetic chemists.[6,7]

α-Glycosidases are generally believed to go through an E2-type elimination mechanism (**2** → **3** in Figure 1) during which a positively charged aglycon (leaving group) and the lone pair of the ring oxygen are positioned antiperiplanar and cooperatively facilitate the glycosidic bond-cleaving reaction.[8] In the case of the β-glycosidase reaction, if the enzyme proceeds via an E2-type mechanism similar to that of the α-glycosidases, the protonated substrate **5** has to go through a highly strained intermediate **6**[8] that may not favor further reaction. It is, therefore, quite natural to assume that the β-glycosidase reaction may follow different reaction steps than those of the α-glycosidase reaction in order to reach the presumed reaction intermediate **3**.

When we used a molecular mechanics program to study the reaction intermediate of the β-glucosidase reaction in terms of conformation and charge distribution, we found that the positive charge was generated at the anomeric position rather than at the position of the ring oxygen.[9] In addition, extensive work by the Withers group using 2-fluoro[10] or 5-fluoro[11] carbohydrate derivatives has made it clear that retaining β-glycosidases carry out the glycoside-cleaving reaction via a covalent glycosyl-enzyme intermediate.[12] On the basis of these two findings, we have designed a new class of iminosugars, 1-*N*-iminosugars **9**,

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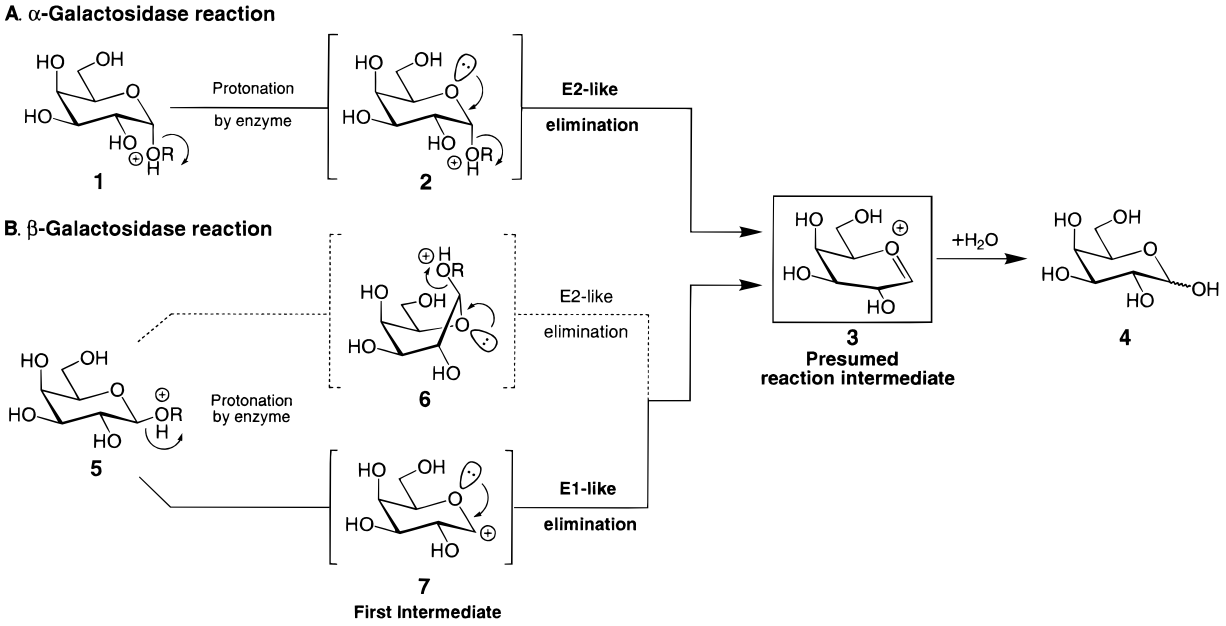

Figure 1. Well-accepted α- and *β*-galactosidase reaction mechanism.

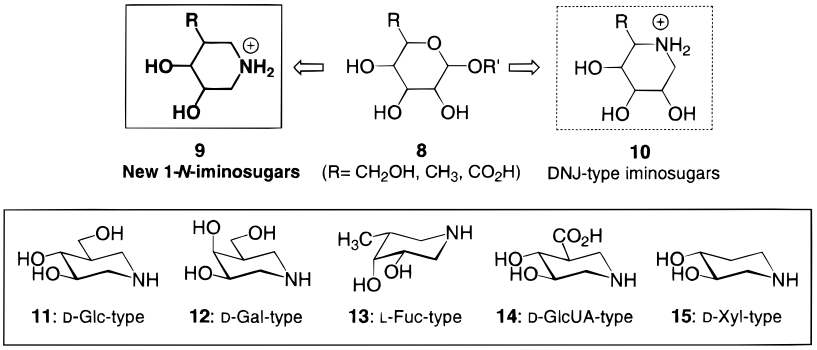

Figure 2. General design strategy of glycosidase inhibitor.

in which a nitrogen atom is at the anomeric position of a monosaccharide; these new iminosugars are different from the conventional deoxynojirimycin (DNJ)-type iminosugars **10** where a nitrogen atom replaces the ring oxygen of a monosaccharide. We have synthesized a variety of 1-*N*-iminosugars, including D-glucose-type **11**,[13,14] D-galactose-type **12**,[15] L-fucose-type **13**,[16,17] D-glucuronic acid-type **14**,[18] and D-xylose-type **15** (Figure 2). We describe herein the detailed synthesis of these 1-*N*-iminosugars and the evaluation of their inhibitory activities against a variety of glycosidases.

Synthesis of D-Glucose-Type 1-*N*-Iminosugar 11 (Scheme 1). The synthesis of D-glucose-type 1-*N*-iminosugar **11** was based on our previous synthesis of another D-glucose-type 1-*N*-iminosugar with a 5-OH group.[13] Bols et al. have prepared D-glucose-type 1-*N*-iminosugar **11**, but their synthesis was rather complicated.[14] We therefore sought to synthesize **11** in a simple and practical manner. D-Lyxose was sequentially treated with (1) concentrated H_2SO_4/acetone;[19] (2) TsCl/pyr; and (3) BzCl/pyr to give a 5-tosyl-1-*O*-benzoate **17** in high yield. Azido substitution of the 5-tosyloxy group of **17** gave a 5-azido derivative **18**. Treatment of **18** with methanolic sodium methoxide followed by Ho's aldol reaction[20] (K_2CO_3/HCHO/aqueous MeOH) afforded **19** with a hydroxymethyl group at the C-2 position which subsequently became the C-5 position of the iminosugar. Hydrogenation of **19** over $Pd(OH)_2$ underwent an intramolecular cyclization to give a free glucose-type 1-*N*-iminosugar **20** with an additional OH group at the C-5 position. Sequential treatment of **20** with Boc_2O in MeOH and BzCl in pyridine gave **21** in high yield and left only the tertiary 5-OH free. Attempts (thiocarbonyldiimidazole, phenoxythiocarbonyl chloride−DMAP) to remove the 5-OH group at the C-5 failed because of its low reactivity.

Methyloxalyl chloride−tributyltin hydride has been used to deoxygenate a tertiary OH group in steroids[21] and nucleoside[22] derivatives, and we employed this system for our synthesis. When **21** was treated with methyloxalyl chloride in acetonitrile in the presence of DMAP,[22] the reaction proceeded quickly (∼30 min) to convert **21** to a faster moving compound in TLC.

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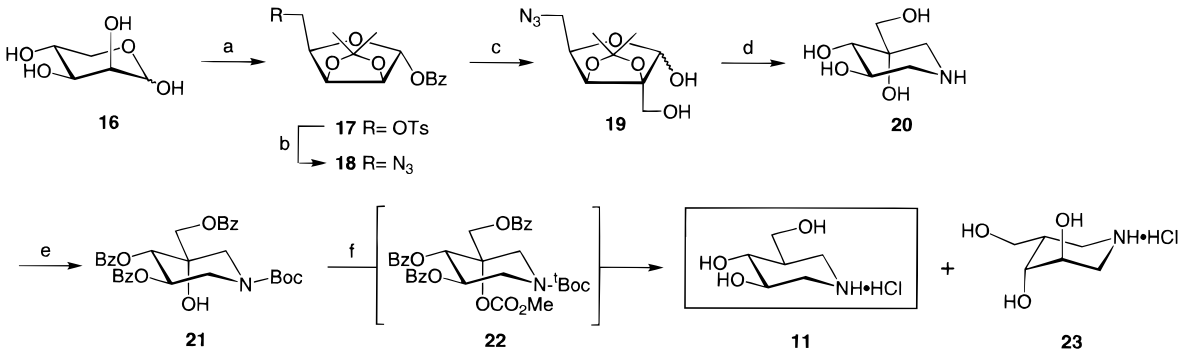
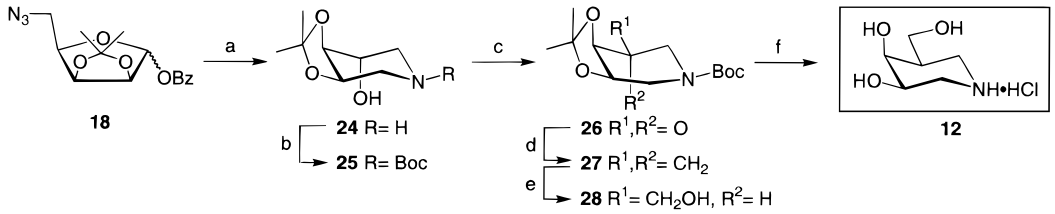
Scheme 1[a]



[a] Reagents and conditions: (a) (i) acetone-concentrated H_2SO_4/rt/overnight, (ii) TsCl/pyridine/0 °C to r.t./overnight, (iii) BzCl/pyridine/0 °C to r.t./overnight (41% overall yield); (b) NaN_3/DMSO/100 °C/overnight (74%); (c) (i) NaOMe/MeOH/r.t. (ii) K_2CO_3/30% HCHO−MeOH/85 °C/overnight (80−90%); (d) (i) H_2/Pd(OH)$_2$/MeOH/r.t./overnight, (ii) 1 N HCl; (e) (i) Boc_2O/Et_3N/MeOH/r.t./overnight, (ii) BzCl/pyridine/0 °C to r.t./overnight; (f) (i) MeOCOCOCl/DMAP/CH_3CN, (ii) Bu_3SnH/VAZO/toluene/100 °C, (iii) SiO_2 chromatography with iPrOH/H_2O/NH_4OH (7:2:1).

Scheme 2[a]



[a] Reagents and conditions: (a) (i) NaOMe/MeOH, (ii) H_2/Pd(OH)$_2$/MeOH/r.t./overnight, (b) Boc_2O/Et_3N/MeOH; (c) DMSO/(COCl)$_2$/CH_2Cl_2/−78 °C then Et_3N/−78 to 0 °C; (d) *n*-BuLi/$CH_3PPh_3^+Br^-$/DME/0 °C to r.t./overnight; (e) (i) 9-BBN/THF/0 °C to r.t./3 h, (ii) NaOH/30% H_2O_2/r.t./overnight; (f) 1 N HCl/r.t./overnight.

Purification of the product was troublesome because of the instability of the product which was easily hydrolyzed back to the starting material as has been noted by the Matsuda group.[22] For the workup, we passed the ethyl acetate extract through a short column of dry silica gel and eluted the column with ethyl acetate in order to dry the oxalate **22**. Reduction of **22** with tributyltin hydride in toluene in the presence of 1,1′-azobis-(cyclohexanecarbonitrile) (VAZO) gave a moderate conversion of the oxalate **22** into a mixture of the deoxygenated products, D-glucose and L-idose derivatives. After stepwise removal of the protective groups (i) NaOMe and (ii) 1 N HCl, each of the isomers was completely separated by silica gel chromatography with 2-propanol−water−NH_4OH (7:2:1, v/v) to afford the faster moving D-glucose-type **11** and the slower moving L-idose-type **23** iminosugars in a ratio of 2:1. These structures were confirmed with their ^{1}H NMR spectra.

Synthesis of D-Galactose-Type 1-*N*-Iminosugar 12 (Scheme 2). For the synthesis of D-galactose-type 1-*N*-iminosugar, we decided to introduce the 5-hydroxymethyl group in a stepwise manner: (i) introduction of a C-5 exo-methylene group and (ii) subsequent hydroboration reaction.[15] In this strategy, we anticipated that the stereoselectivity of the hydroboration could be controlled by the bicyclic structure of the substrate in favor of the formation of the desired D-galacto configuration.

O-Debenzoylation of the synthetic intermediate of the D-glucose-type iminosugar **18** followed by hydrogenation led to intramolecular reductive amination, giving a cyclic imino derivative **24**. This step was followed by the *N*-protection with a Boc group, giving a 3,4-*O*-isopropylidenepiperidine derivative **25** with a free OH group at the *C*-5 position. Swern oxidation[23] of **25** with DMSO−$(COCl)_2$ afforded a 5-keto derivative **26**, and the subsequent Witting methylenation gave an exo-methylene derivative **27**. As expected, hydroboration of **27** with a sterically bulky 9-BBN followed by oxidative treatment gave a single isomer **28**, with a desired galacto configuration. Acid treatment of **28** gave the galactose-type 1-*N*-iminosugar **12**.

Synthesis of L-Fucose-Type 1-*N*-Iminosugar 13 (Scheme 3). Synthesis of L-fucose-type 1-*N*-iminosugar was carried out using a strategy similar to that used for synthesis of the D-galactose-type iminosugar.[13] D-Ribose **29** was converted to a 5-azido-1-*O*-benzoyl-2,3-*O*-isopropylidene derivative **31** in a manner similar to that for synthesizing **18** from **16**. Removal of the 1-*O*-benzoate group of **31** followed by reductive amination afforded a piperidine derivative **32**, which was then treated with Boc_2O to give **33**. By a strategy similar to that used for preparing the galactose-type iminosugar **12**, the piperidine derivative **33** was oxidized, giving a 5-keto derivative **34**, which was subsequently methylenated to give **35**. Hydrogenation of **35** on Pd−C occurred from the less hindered side (convex side) to give only a single isomer **36** with a desired L-fuco configuration. Acidic treatment of **36** furnished the synthesis of L-fucose-type 1-*N*-iminosugar **13**.

Synthesis of D-Glucuronic Acid-Type 1-*N*-Iminosugar 14 (Scheme 4). D-Glucuronic acid-type 1-*N*-iminosugar **14** was synthesized in a strategy similar to that for the D-galactose-type iminosugar:[18] (i) introduction of a *C*-5 exo-methylene group, (ii) hydroboration, and (iii) subsequent oxidation of the hydroxymethyl group to the corresponding carboxylate. D-Arabinose **37** was converted to a 5-azidofuranose derivative **38** according to the published procedure of Legler et al.[24] Treatment of **38** with 4-methoxyphenol in the presence of TMSOTf gave a single isomer of **39**. Exchange of the *O*-protective groups

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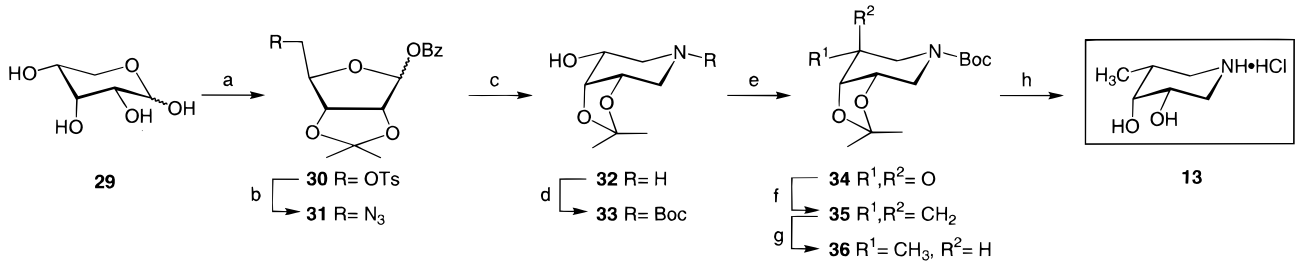
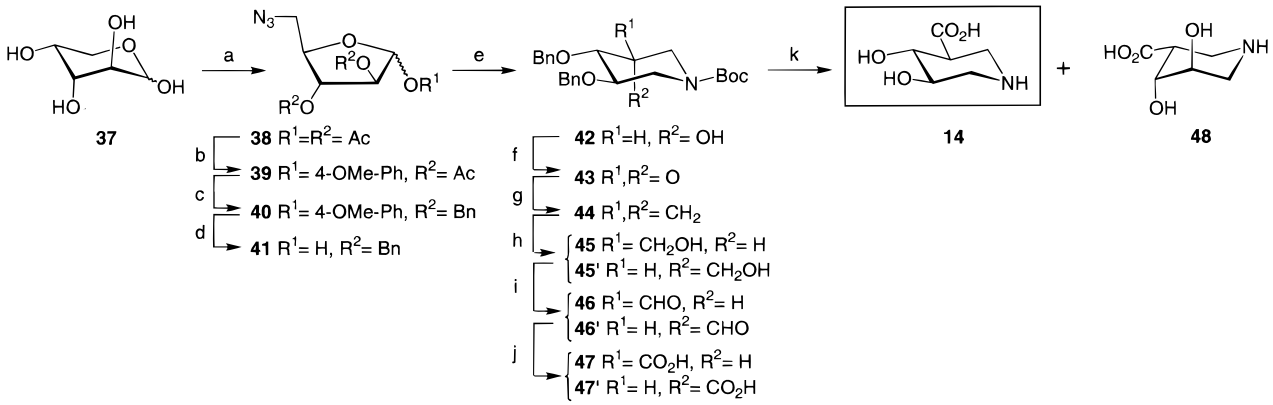
Scheme 3[a]



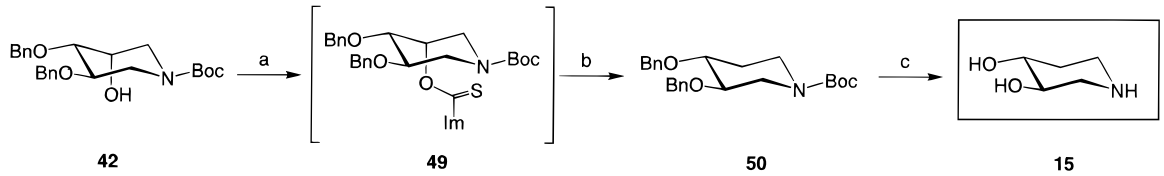
[a] Reagents and conditions: (a) (i) acetone-concentrated H_2SO_4/r.t./overnight, (ii) TsCl/pyridine/0 °C to r.t./overnight, (iii) BzCl/pyridine/0 °C to r.t./3 h (48% overall); (b) NaN_3/DMSO/60−65 °C/overnight (91%); (c) (i) NaOMe/MeOH/r.t. (ii) H_2/$Pd(OH)_2$/MeOH/r.t./overnight (75% overall); (d) Boc_2O/Et_3N/MeOH/r.t./overnight (73%); (e) (i) DMSO/$(COCl)_2$/CH_2Cl_2/−60 °C then Et_3N/−60 to 0 °C (80%); (f) *n*-BuLi/$CH_3PPh_3^+Br^-$/DME/0 °C to r.t./overnight (77%); (g) H_2/Pd−C/MeOH/r.t./5 h (82%); (h) 1 N HCl/r.t./overnight (90−95%).

Scheme 4[a]



[a] Reagents and conditions: (a) ref; (b) 4-MeO-phenol/TMSOTf/CH_2Cl_2/r.t./4 h; (c) (i) NaOMe/MeOH, (ii) NaH/BnBr/DMF/0 °C to r.t./overnight; (d) $(NH_4)_2Ce(NO_3)_6$/CH_3CN-H_2O/0 °C/5 min; (e) (i) H_2/Lindlar catalyst/MeOH/r.t./overnight, (ii) Boc_2O/Et_3N/MeOH/r.t./4 h; (f) DMSO/$(COCl)_2$/CH_2Cl_2/−78 °C then Et_3N/−78 to 0 °C; (g) $LiN(TMS)_2$/$CH_3PPH_3^+Br^-$/THF/0 °C to r.t./overnight; (h) 9-BBN/THF/0 °C to r.t./overnight then NaOH/35% H_2O_2/0 °C to r.t./overnight; (i) DMSO/$(COCl)_2$/CH_2Cl_2/−78 °C then Et_3N/−78 °C to r.t./overnight; (j) $NaClO_2$/35% H_2O_2/CH_3CN−phosphate buffer/0 °C to r.t./1 h; (k) (i) H_2/$Pd(OH)_2$/MeOH/r.t./overnight, (ii) 1 N HCl, (iii) SiO_2 chromatography.

Scheme 5[a]



[a] Reagents and conditions: (a) thiocarbonyldiimidazole/$(CH_2Cl)_2$/80 °C/overnight; (b) Bu_3SnH/AlCN/toluene/100 °C/overnight; (c) (i) H_2/$Pd(OH)_2$/MeOH/room temperature/overnight, (ii) 1 N HCl.

of **39** from the acetyl to the more stable benzyl groups afforded **40**, which was then treated with $(NH_4)_2Ce(NO_3)_6$ to remove the C-1 4-methoxyphenol group and give an anomeric free **41**. Reductive amination of **41** over a Lindlar catalysis[25] (Pd−$BaSO_4$) afforded a piperidine derivative **42** after *N*-butoxycarbonylation. Oxidation of **42** with DMSO−$(COCl)_2$[23] to give a 5-keto derivative **43** followed by a Wittig methylenation gave **44**. Hydroboration of **44** with 9-BBN gave a mixture of **45** with a D-gluco configuration ($R^1 = CH_2OH$, $R^2 = H$) and the other isomer of L-idose **45′**. Conversion of **45** to the corresponding uronic acid derivative was performed in a stepwise manner. First, oxidation of **45** with DMSO−$(COCl)_2$[23] gave an aldehyde **46**, which was subsequently further oxidized by H_2O_2 and $NaClO_2$ in a CH_3CN−phosphate buffer system[26] to produce a D-glucuronic acid derivative **47**. Hydrogenolytic removal of the benzyl group of **47** followed by acidic treatment furnished the D-glucuronic acid-type 1-*N*-iminosugar **14** and an easily separable L-iduronic acid derivative **48**.

Synthesis of D-Xylose-Type Iminosugar 15 (Scheme 5). The D-xylose-type 1-*N*-iminosugar **15** was easily prepared from a synthetic intermediate **42** during the synthesis of the D-glucuronic acid-type iminosugar **14**. Treatment of **43** with thiocarbonyldiimidazole gave a thiocarbonylimidate derivative **49**, which was subsequently subjected to radical deoxygenation with Bu_3SnH to give a deoxygenated derivative **50**. Hydrogenolysis of **50** followed by acidic treatment afforded the D-xylose-type 1-*N*-iminosugar **15**.

***N*-Alkylation of 1-*N*-Iminosugars.** *N*-Alkylation of 1-*N*-iminosugars was performed by reductive amination of *n*-butanal and borane−pyridine complex in MeOH−phosphate buffer[27] to give the corresponding *N*-*n*-butyl 1-*N*-iminosugars almost quantitatively.

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Evaluation of the Inhibitory Potencies of the 1-*N*-Iminosugar Derivatives. We evaluated[28] the ability of each of the synthesized 1-*N*-iminosugars to inhibit a series commercially available glycosidases: α-glucosidase (yeast), β-glucosidase (almond), α-galactosidase (green coffee beans), β-galactosidase (*Aspergillus orizae*), α-mannosidase (jack bean), α-fucosidase (bovine kidney), and β-glucuronidase (bovine liver), all obtained from Sigma Chemical Co., (St. Louis, MO). The IC_{50} values obtained are summarized in Table 1.

The conventional type iminosugar deoxynojirimycin (DNJ) inhibits α-glucosidase more potently (K_i = 12.6 μM) than β-glucosidase (K_i = 47 μM).[8c] As expected, the glucose-type 1-*N*-iminosugar **11** (entry 1) was found to be a very potent inhibitor of β-glucosidase with an IC_{50} value of 0.1 μM; in contrast, it did not effectively inhibit α-glucosidase (IC_{50}, 150 μM). The structure of the 1-*N*-iminosugar does not include a C-2 OH group; however, the IC_{50} value of this iminosugar against α-mannosidase was determined to be 250 μM, which is similar to that for α-glucosidase, and it may be concluded that the glucose-type 1-*N*-iminosugar **11** did not discriminate between α-glucosidase and α-mannosidase. Even though **11** did not inhibit either α-galactosidase and α-fucosidase, it showed a modest inhibition of β-galactosidase. When the nitrogen group was butylated, the *N*-butylglucose-type 1-*N*-iminosugar **51** (entry 2) inhibited β-glucosidase 380-fold less potent than did the *N*-unmodified iminosugar **11**. *N*-Modification (alkylation) has been reported to not alter the inhibitory activity of the DNJ-type iminosugar very much;[29] however, the inhibitory potencies of 1-*N*-iminosugars were found to be significantly affected. It is interesting that the inhibitory potency against α-glucosidase remained the same upon *N*-alkylation. With an additional OH group at the C-5 position of **11**, compound **20**[11] (entry 3) was also a potent inhibitor of β-glucosidase but was not as potent as **11**, which lacks this OH group. The *N*-butyl derivative **52** showed an inhibition pattern similar to that of **51**.

DNJ-type iminosugar galactonojirimycin is a very potent inhibitor of α-galactosidase with a Ki of 1.6 nM but is not very effective for α-galactosidase (K_i = 160 nM).[8c,31] The galactose-type 1-*N*-iminosugar **12** (entry 5) was found to be an extremely potent inhibitor of β-galactosidase, with an IC_{50} value of 12 nM (K_i = 4 nM).[15] It was also a potent inhibitor of β-glucosidase (IC_{50} of 190 nM), although the stereochemistry at the C-4 OH group is different. These data suggested that the most critical factor required for potent inhibition may be the charge distribution. *N*-Butylation of **12** (entry 6) dramatically weakened the inhibitory potency against β-galactosidase (by ~400-fold); the IC_{50} of **53** was determined to be 5 μM. However, **53** showed a slightly higher (about 4-fold) inhibitory activity against α-galactosidase. The *N*-oxide derivative **54** (entry 7) showed moderate inhibitory activity similar to that of **53**, and it may indicate that the oxidation of the nitrogen atom did not help produce a clear positive charge on the nitrogen atom. The presence of the an additional OH group at the C-5 position, as in **55**[30] and **56**,[30] significantly abolished the inhibitory activity of the iminosugar (entries 8 and 9).

Fuconojirimycin, a DNJ-type iminosugar, is a potent inhibitor of α-fucosidase, with a Ki of 4.8 nM;[32] however, L-fucose-type 1-*N*-iminosugar **13** (entry 10) was, as expected, a moderate inhibitor against α-fucosidase, with an IC_{50} of 26 μM, because the 1-*N*-iminosugar was designed as an inhibitor for β-glycosidase and not for α-glycosidase.[16] It is noteworthy that the *N*-butyl derivative **57** (Entry 11) was found to inhibit β-glucosidase effectively, with an IC_{50} of 80 μM.

A conventional glucuronic acid-type iminosugar is a weak inhibitor of β-D-glucuronidase, with a Ki of 80 μM,[33] whereas, as expected, the glucuronic acid-type 1-*N*-iminosugar **15** (Entry 13) was a very potent inhibitor of β-D-glucuronidase with a Ki of 79 nM, and this iminosugar did not inhibit other glycosidases at concentrations below 1 mM.[18] L-Iduronic acid-type iminosugar **48** (Entry 14) inhibits β-D-glucuronidase only modestly (IC_{50} = 1.3 μM).

We have also examined the inhibitory potencies of other 1-*N*-iminosugars that were easily prepared from the synthetic intermediates of the syntheses of **11**−**14**. A C-2 symmetrical iminosugar **58** (Entry 15) which was prepared from **32** by acidic treatment was found to be a potent inhibitor of β-glucosidase, with an IC_{50} of 8.8 μM. It also inhibited β-galactosidase only weakly (IC_{50} = 40 μM). The *N*-butyl derivative **59** (Entry 16) had a 60-fold lower inhibitory activity against β-glucosidase.

The L-galactose-type 1-*N*-iminosugar **60**, which was prepared by hydroboration of **36**, and its *N*-butyl derivative **61** inhibited β-glucosidase and β-galactosidase only weakly (entries 17 and 18). Another iminosugar **62**[24,34a] (Entry 19), prepared from the synthetic intermediate **24**, was found to inhibit α- and β-galactosidases equally, with IC_{50} values of 50−70 μM. It is interesting that the L-idose-type 1-*N*-iminosugar **23** (Entry 21) inhibited β-glucosidase quite well, with an IC_{50} of 8 μM.

Discussion. We have designed and synthesized a new class of glycosidase inhibitors, 1-*N*-iminosugars. All the syntheses were straightforward and highly stereoselective and were carried out using readily available monosaccharides. There was a remarkable difference between our 1-*N*-iminosugars **9** and the conventional DNJ-type iminosugars **10**. Iminosugars with a nitrogen atom replacing the ring oxygen position **10** tend to inhibit the corresponding α-glycosidases more potently than the β-glycosidases.[8c] In contrast, we have found that our 1-*N*-iminosugars **9** with a nitrogen atom at the anomeric position are extremely potent inhibitors of the corresponding β-glycosidases, and show only modest inhibitory potencies against α-glycosidases.

In the case of the α-galactosidase reaction (A in Figure 3), (1) a substrate α-galactoside as a ground-state structure is bound by the enzyme active site, (2) the aglycon of the enzyme-bound substrate is protonated by a general acid, and (3) an E2-like elimination reaction takes place between the lone electron pair of the ring oxygen and the protonated aglycon shown in **64**.[8] Because these two components in the E2 elimination are positioned antiperiplanar to each other, this elimination process proceeds rapidly without a noticeable intermediate to lead to a

(28) (a) Halvorson, H. *Methods Enzymol*. **1966**, *8*, 559−562. (b) Dale, M. P.; Ensley, H. E.; Kern, K.; Sastry, K. A. R.; Byers, L. D. *Biochemistry* **1985**, *24*, 3530−3539.

(29) (a) For study of *N*-alkylated iminosugars with isolated enzymes, see: Al Daher, S.; Fleet, G.; Namgoong, S. K.; Winchester, B. *Biochem. J.* **1989**, *258*, 613−615. (b) For study of *N*-alkylated iminosugars with cells, see: Tan, A.; van den Broek, L.; van Boeckel, S.; Ploegh, H.; Bolscher, J. *J. Biol. Chem.* **1991**, *266*, 14504−14510.

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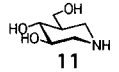
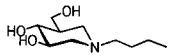
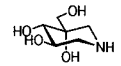
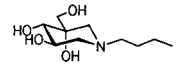
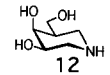
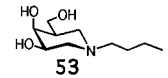
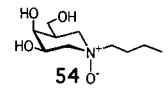
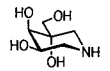
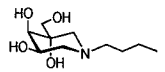
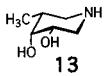
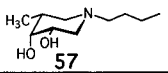
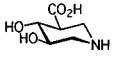
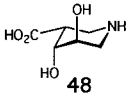
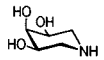
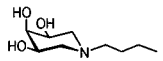
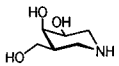
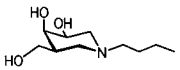
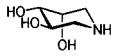
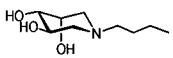
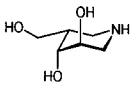
(33) Cenci di Bello, I.; Dorling, P.; Fellows, L.; Winchester, B. *FEBS Lett*. **1984**, *176*, 61−64.

(34) We synthesized the iminosugars **62** and **63** from **24** which was a synthetic intermediate for the galactose-type 1-*N*-iminosugar **12**. Their syntheses have been reported. For **62**: (a) Bernotas, R. C.; Papandreous, G.; Urbach, J.; Ganem, B. *Tetrahedron Lett*. **1990**, *31*, 3393−3396. (b) See ref 24. For **63**: McCaig, A. E.; Chomier, B.; Wightman, R. H. *J. Carbohydr. Chem*. **1994**, *13*, 397−407.

Table 1. Inhibitory Potencies of 1-*N*-Iminosugars (IC_{50}, μM)

entry	inhibitor	Glycosidases					
		β-Glc'ase	α-Glc'ase	β-Gal'ase	α-Gal'ase	α-Man'ase	α-Fuc'ase
1	 **11**	0.1	150	270	>2000	250	>1000
2	 **51**	38	140	50	nd	nd	nd
3	 **20**	6.0	380	>5000	>5000	>2000	900
4	 **52**	850	1500 (pH 5)	nd	nd	nd	>1000
5	 **12**	0.19	>2000	0.012	200	>2000	>1000
6	 **53**	95	>2000	5	45	nd	>1000
7	 **54**	160	nd	9.5	90	nd	>1000
8	 **55**	420	>2000	17.5	610	>2000	>1000
9	 **56**	400	nd	nd	nd	nd	>1000
10	 **13**	500	>1000	>1000	>1000	>1000	26
11	 **57**	80	>1000	>1000	>1000	>1000	270

entry	inhibitor	Glycosidases					
		β-Glc'ase	α-Glc'ase	β-Gal'ase	α-Gal'ase	α-Man'ase	α-Fuc'ase
13	 **15**	>1000	>1000	>1000	>1000	>1000	>1000
	(Ki = 79 nM for β-glucuronidase from bovine liver)						
14	 **48**	>1000	>1000	>1000	>1000	>1000	>1000
	(IC_{50} = 1300 nM for β-glucuronidase from bovine liver)						
15	 **58**	8.8	>1000	40	>1000	360	>1000
16	 **59**	530	>2000	43	725	nd	>1000
17	 **60**	500	1000	300	>1000	>1000	370
18	 **61**	150	nd	300	>1000	>1000	>1000
19	 **62**	100	>2000	50	70	>1000	450
20	 **63**	200	>2000	>1000	>1000	>1000	80
21	 **23**	8.0	>1000	>1000	>1000	>1000	>1000

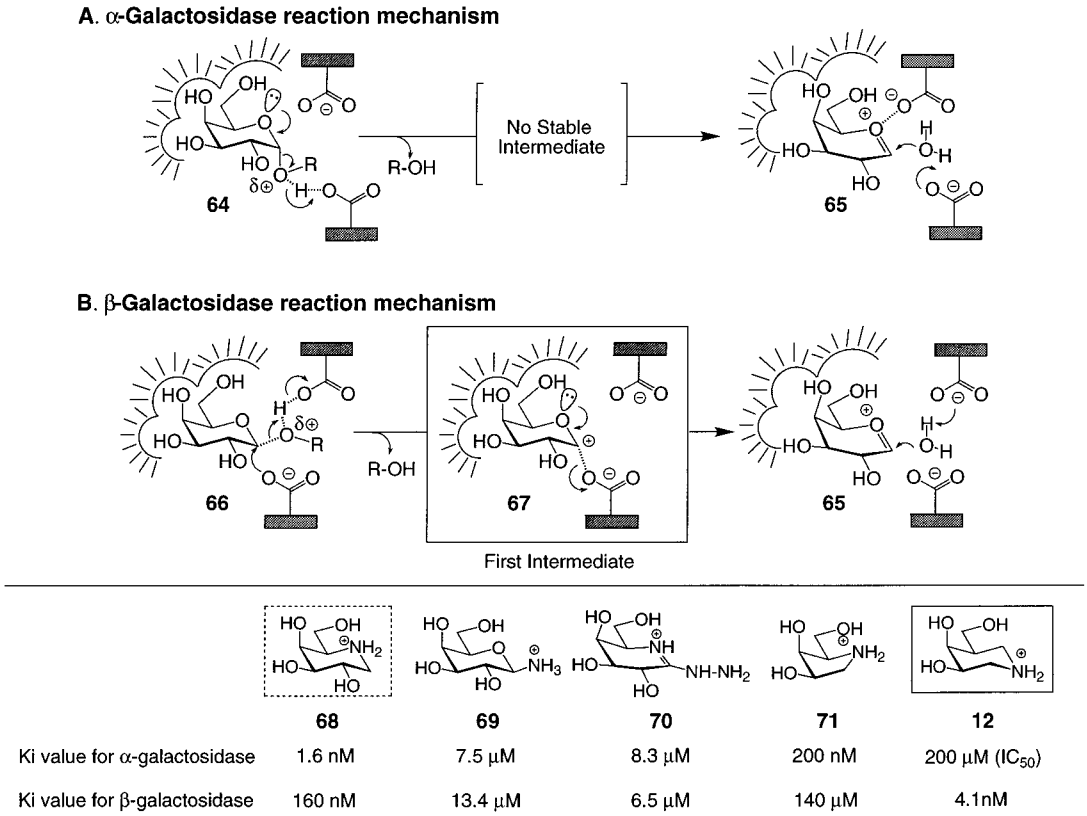


Figure 3. Proposed reaction mechanism of α- and β-galactosidases.

well-accepted transition-state structure **65** in which the galactose has a half-chair conformation and a positive charge developing at the position of the ring oxygen. A naturally occurring deoxygalactonojirimycin (DGJ) **68**, a conventional DNJ-type iminosugar, is an extremely potent inhibitor of α-galactosidase (K_i = 1.6 nM)[8c,31] presumably because it has a ground-state conformation and has a positive charge that is close enough to be able to interact with the negative charge of the general base. A galactosylamine derivative **69** has a positive charge on the glycosyl amino group that is a little too far to interact with the general base, and therefore it shows a modest inhibitory potency against α-galactosidase.[8c] Ganem's amidine derivative **70**[35] has both characteristics, making it a potential potent inhibitor of glycosidase: a half-chair conformation and a positive charge at the ring oxygen position mimicking the transition-state structure **65**; however, its Ki value was reported to be 8.3 μM for α-galactosidase.[6c,35] This result was rather unexpected because it has been thought that a transition-state mimetics would be a more potent inhibitor than would a compound that is a ground-state analogue. It is possible to assume that a conformational difference between the ground-state **64** and the amidine derivative **70** could disrupt the binding of the inhibitor to an enzyme active site that binds to a substrate with a ground-state conformation. A five-membered iminosugar **71** prepared by the Wong group,[24] which shares some of the characteristics of both DGJ and Ganem's amidine-type compound, also showed an inhibitory potency that was similar to that of the amidine derivative **70**, probably for the reasons already cited for the amidine **70**. Our 1-*N*-iminosugar inhibitor **12** showed only a weak inhibition of α-galactosidase (IC_{50} = 200 μM) because the positive charge on the C-1 nitrogen atom is too far away for interacting with the general base.

As in the case of the β-galactosidase reaction mechanism (B in Figure 3), a protonated aglycon of the enzyme-bound substrate β-galactoside **66** departs from the sugar ring with the aid of the general base. This leaves (albeit transiently) a galactose derivative having a positive charge at the anomeric position **67**. We gave the name "First Intermediate" to this derivative **67**, a relatively stable reaction intermediate of the β-galactosidase reaction that is stabilized by the general base group. The complex of First Intermediate−general base is similar in structure to the glycosyl enzyme complex described by the Withers group.[12,37] First Intermediate is then collapsed, with the aid of the lone electron pair of the ring oxygen, shown as **67**, to yield the common reaction intermediate **65**. DGJ **68** was found to be a less potent inhibitor of β-galactosidase than of α-galactosidase[8c] presumably because positive charge may be located far from the general base, even though it has a ground-state conformation which is favorable for binding to the enzyme active site. The galactosylamine derivative **69** is still a moderate inhibitor of β-galactosidase,[8c] again because its positive charge is located far from the general base. Because Ganem's amidine **70** was prepared to target, with respect to conformational and charge characters, the well-accepted transition-state structure **65**, which is common to both α- and β-galactosidases, the compound shows similar inhibitory potencies against both α- (K_i = 8.3 μM) and β-galactosidases (K_i = 6.5 μM).[6c,35] This finding may indicate that a compound which mimics the transition state of the glycosidase reaction could be a potent inhibitor but rather nonspecific. Wong's five-membered iminosugar **71** shows a similar decrease in inhibitory potency when compared to that

(35) (a) Tong, M. K.; Papandreou, G.; Ganem. B *J. Am. Chem. Soc.* **1990**, *112*, 6137−6139. (b) An unsuccessful approach to a similar amidine derivative was reported: Bird, P.; Dolphin, D. H.; Withers, S. G. *Can. J. Chem.* **1990**, *68*, 317−322.

(36) Wang, Y.-F.; Takaoka, Y.; Wong, C.-H. *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 1242−1244.

(37) Withers, S. G.; Street, I. P. *J. Am. Chem. Soc.* **1988**, *110*, 8551−8553.

of DGJ.[36] Our 1-*N*-iminosugar **12** has both characteristics required for targeting First Intermediate **67**: a ground-state-like conformation and correct positioning of the positive charge to allow it to interact with the general base; consistent with these features, it was found to be an extremely potent inhibitor of β-galactosidase, with a Ki value of 4.1 nM. Further study is necessary to determine whether First Intermediate is formed via an S_N1-type process followed by subsequent charge stabilization or whether it is formed via an assisted internal concerted process involving the carboxylate ion as a nucleophile as suggested by the Wither's group.[10−12] In any cases, our work has not only further confirmed the structure of the intermediate of glycosidase reaction by the Wither's group but has also suggested a strategy for designing a potent and specific inhibitor of β-glycosidase: (i) a proper charge distribution (positive charge at the anomeric position) and (ii) a ground-state-like conformation (a chair conformation).

Summary. We have described the synthesis, from readily available monosaccharides, of a new class of 1-*N*-iminosugars with a nitrogen atom at the anomeric position. These syntheses were carried out in a highly stereoselective and efficient manner and produced 1-*N*-iminosugars that are highly potent and specific inhibitors of β-glycosidases. The concept of iminosugars that we demonstrated herein clearly indicates a design strategy for development of selective inhibitors of glycosidases: We could develop a potent and specific inhibitor of a β-glycosidase by synthesizing an analogue of our 1-*N*-iminosugars **9**; in contrast, an analogue of the conventional DNJ-type iminosugars **10** would be expected to be a potent inhibitor of α-glycosidases.

Experimental Section

General Methods. All melting points are uncorrected. The reagents used were purchased from Aldrich, Sigma, or Acros, and the solvents were reagent grade and used as supplied. Organic extracts were dried over anhydrous $MgSO_4$ and concentrated in vacuo. ^{1}H NMR spectra were recorded at 300 MHz, and ^{13}C NMR spectra were recorded at 75 MHz on a Bruker AXM 300 spectrometer. Internal standards used in ^{1}H NMR spectra were TMS (δ 0.00) for $CDCl_3$, HOD (δ 4.78) for D_2O, and in ^{13}C NMR were $CDCl_3$ (δ 75.0) for $CDCl_3$ and CH_3CN (δ 1.30) for D_2O. Elemental analysis were performed by Galbraith Laboratories (Knoxville, TN), and mass spectral data were analyzed by Mass Spectrometry Laboratory at University of Illinois (Urbana−Champaign, IL).

Synthesis of D-Glucose-Type 1-*N*-Iminosugar. 1-*O*-Benzoyl-2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-α-D-lyxofuranose (17). A mixture of D-lyxose (12.6 g, 83.9 mmol) and concentrated H_2SO_4 (0.74 mL) in dry acetone (300 mL) was stirred overnight at room temperature, and the reaction mixture was neutralized by stirring with solid anhydrous Na_2CO_3 (30 g) for 3 h. The suspension was filtered and concentrated to give crude 2,3-*O*-isopropylidene-D-lyxofuranose as a colorless syrup. This was employed for the next step without further purification.

p-Toluenesulfonyl chloride (17.6 g, 92.3 mmol) was added portionwise to a cooled solution of the above isopropylidene derivative in pyridine (300 mL) at 0−5 °C, and the mixture was stirred overnight at room temperature. The reaction mixture was then cooled to 0−5 °C, and benzoyl chloride (14.2 g, 101 mmol) was added dropwise to the mixture. After 1 h, the reaction mixture was poured into ice−water and extracted with EtOAc. The combined extracts were successively washed with diluted HCl solution, aqueous $NaHCO_3$ solution, and brine, dried, and concentrated to give crude **17** (29.3 g, 78% from D-lyxose) as a colorless solid. A small portion was recrystallized from hexanes−EtOAc to give an analytical sample as colorless needles: mp 115−118 °C (hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.30 and 1.39 (each s, each 3H, CH_3 of isopropylidene group), 2.42 (s, 3H, CH_3 of tosyl group), 4.21 (ddd, 1H, J = 3.2 and 8.9 Hz, H-4), 4.36−4.44 (m, 2H, H-5), 4.83 (d, 1H, J = 5.8 Hz, H-2), 4.87 (dd, 1H, J = 3.2 and 5.8 Hz, H-3), 6.35 (s, 1H, H-1), 7.32 (d, 2H, J = 8.0 Hz, *o*-aromatic H of benzoyl group), 7.45 (t, 2H, J = 7.7 Hz, *m*-aromatic H of benzoyl group), 7.59 (tt, 1H, J = 1.4 and 7.4 Hz, *p*-aromatic H of benzoyl group), 7.81 (d, 2H, J = 8.3 Hz, aromatic H of tosyl group), 7.98 (d, 2H, J = 8.4 Hz, aromatic H of tosyl group). Anal. Calcd for $C_{22}H_{24}SO_8$: C, 58.92; H, 5.39. Found: C, 58.52; H, 5.52.

5-Azido-5-deoxy-2-(hydroxymethyl)-2,3-*O*-isopropylidene-α/*β*-D-lyxofuranose (19). A mixture of **17** (21.0 g, 46.8 mmol) and NaN_3 (13.2 g, 203 mmol) in DMF (150 mL) was heated overnight at 120 °C, and the reaction mixture was poured into ice−water and extracted with EtOAc. The combined extracts were successively washed with water, aqueous $NaHCO_3$ solution, and brine, dried, and concentrated to give an azido derivative **18** as a colorless solid. This was employed for the next step without further purification.

A solution of the above **18** in MeOH (250 mL) and 25% methanolic NaOMe (2.5 mL) was stirred for 30 min at room temperature, and the solution was neutralized by the addition of AcOH. The mixture was concentrated, and the residue was passed through silica gel with toluene−EtOAc (20:1) to give the crude *O*-debenzoylated derivative as a colorless oil. This was employed for the next step without further purification.

A solution of the above anomeric free derivative, 30% HCHO solution (125 mL), and K_2CO_3 (12.5 g, 90.4 mmol) in MeOH (200 mL) was heated for 24 h at 85 °C. After cooling, the reaction mixture was neutralized by the addition of 10% H_2SO_4 solution, concentrated, diluted with water, and extracted with EtOAc. The combined extracts were successively washed with water, aqueous $NaHCO_3$ solution, and brine, dried, and concentrated. The residue was chromatographed on silica gel with hexanes−EtOAc (20:1 to 2:1) to give **19** (9.2 g, 80%) as a colorless oil, which crystallized on standing. A small portion was recrystallized from hexanes−EtOAc to give an analytical sample as colorless needles: mp 79−81 °C (hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.48 and 1.57 (each s, each 3H, CH_3 of the isopropylidene group), 3.56 (dd, 1H, J = 6.43 and 13.04 Hz, H-5a), 3.59 (dd, 1H, J = 6.47 and 13.04 Hz, H-5b), 3.74 (ddd, 1H, J = 2.86 and 6.45 Hz, H-4), 3.80 (d, 1H, J = 2.89 Hz, H-3), 3.85 (d, 1H, J = 11.7 Hz, H-2′a), 4.60 (d, 1H, J = 2.97 Hz, H-1), 4.92 (d, 1H, J = 11.7 Hz, H-2′b).

***tert*-Butyl (3*S*,4*S*,5*R*)-4,5-Di-*O*-benzoyl-3-((benzoyloxy)methyl)-3,4,5-trihydroxypiperidine-1-carboxylate (21).** A mixture of **19** (2.0 g, 8.16 mmol) and $Pd(OH)_2$ (450 mg) in MeOH (100 mL) was vigorously stirred under an atmosphere of H_2 at room temperature for 12 h, the reaction mixture was filtered through a Celite pad, and the filter cake was washed with aqueous 50% MeOH. The combined filtrates were concentrated to give a crude piperidine derivative. This was employed for the next step without further purification.

A solution of the above piperidine derivative in 1 N HCl (30 mL) was stirred overnight at room temperature, and the mixture was concentrated. The residue was coevaporated with MeOH and 1,4-dioxane several times to give crude **20**. This was employed for the next step without further purification.

A mixture of the above **20**, Et_3N (1.79 mL, 12.9 mmol), and $(Boc)_2O$ (3.74 g, 17.2 mmol) in MeOH (100 mL) was stirred overnight at room temperature, and the reaction mixture was concentrated to give an *N*-Boc derivative. This was employed for the next step without further purification.

Benzoyl chloride (6.03 g, 42.9 mmol) was added dropwise to a solution of the above *N*-Boc derivative in pyridine (50 mL) at 0−5 °C, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was poured into ice−water and extracted with EtOAc. The combined extracts were successively washed with water, diluted $CuSO_4$ solution, aqueous $NaHCO_3$ solution, and brine, dried, and concentrated. The residue was chromatographed on silica gel with hexanes−EtOAc (20:1 to 2:1) to give **21** (2.3 g, 49% overall) as a colorless solid. A small portion was recrystallized from hexanes−EtOAc to give an analytical sample as colorless cubes: mp 174−176 °C (hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H, tBu of Boc group), 3.35 (m, 2H), 4.18 (m, 1H), 4.38 (m, 1H), 4.40 (d, 1H, J = 11.7 Hz), 4.50 (d, 1H, J = 11.7 Hz), 5.52 (m, 1H, H-3), 5.68 (d, 1H, J = 8.2 Hz, H-4), 7.32−7.58 (m, 9H, aromatic H of benzoyl group), 7.91−8.02 (m, 6H, aromatic H of benzoyl group).

D-Glucose-Type 1-*N*-Iminosugar: (3*R*,4*R*,5*R*)-5-(Hydroxymethyl)-piperidine-3,4-diol Hydrochloride Salt (11). MeOCOCOCl (0.19 mL, 255 mg, 2.08 mmol) was added dropwise to a cooled solution of **21** (700 mg, 1.22 mmol) and DMAP (340 mg, 2.78 mmol) in CH_3CN (10 mL) at 0−5 °C, and the reaction mixture was stirred for 1 h at room temperature. The reaction mixture was poured into ice−water and extracted with EtOAc, and the combined extracts were successively washed with water, aqueous $NaHCO_3$ solution, and brine, dried, and passed through a short column of dry silica gel with EtOAc. Due to the liability of the compound, the combined filtrates were concentrated and the compound was employed for the next step immediately.

A solution of Bu_3SnH (809 mg, 2.78 mmol) and VAZO (30 mg, catalytic amount) in toluene (30 mL) was added dropwise to a solution of the above oxalyl ester in toluene (10 mL), and the mixture was heated at 100 °C for 1 h. The reaction mixture was concentrated, and the residue was passed through silica gel with toluene−EtOAc (40:1) to give crude deoxygenated derivatives.

A solution of the above deoxygenated derivatives and 25% methanolic NaOMe (0.20 mL) in MeOH (5 mL) was stirred for 2 h at room temperature. Next, 1 N HCl (5 mL) was added to the mixture, and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with water (20 mL) and applied onto a column of Dowex 50W-X8 [H^+] resin (20 mL). The column was washed with water (30 mL), and the product was eluted out with 3% NH_4OH (50 mL). The fractions containing the mixture of **11** and **23** were pooled and concentrated. The residue was chromatographed on silica gel with 2-propanol−H_2O−NH_4OH (7:2:1) to give chromatographically pure **11** (R_f 0.37 in 2-propanol−H_2O−NH_4OH, 7:2:1) and **23** (R_f 0.26 in 2-propanol−H_2O−NH_4OH, 7:2:1). Water (10 mL) was added to the residue, and the solution was concentrated in order to remove the residual NH_4OH. Water (10 mL) and 1 N HCl (5 mL) were added to residue, and the solution was concentrated in order to form a hydrochloride salt of the iminosugar. The residue was applied onto a column of Sephadex G-25 (1.0 × 25 cm) and eluted with water. The fractions containing the product were pooled and concentrated. The residue was lyophilized from water (5 mL) to afford **11** (80 mg, 36%) as a colorless amorphous powder (HCl salt). In a similar manner, **23** (40 mg, 18%) was also obtained as a colorless amorphous powder (HCl salt). For **11** (D-glucose-type): ^{1}H NMR (D_2O) δ 1.91−2.02 (m, 1H, H-5), 2.88 (t, 1H, J = 11.92 Hz), 2.97 (t, 1H, J = 12.68 Hz), 3.48−3.56 (m, 2H), 3.71−3.85 (m, 3H). The data were in good agreement with those reported by Bols et al.[14] For L-idose-type 1-*N*-iminosugar **(3*R*,4*R*,5*S*)-5-hydroxymethyl-piperidine-3,4-diol hydrochloride salt** (**23**): ^{1}H NMR (D_2O) δ 2.37−2.45 (m, 1H, H-5), 3.00 (t, 1H, J = 12.52 Hz), 3.22−3.38 (m, 3H), 3.59 (dd, 1H, J = 7.19, 11.29 Hz, H-7a), 3.70 (dd, 1H, J = 6.56, 11.29 Hz, H-7b), 3.98−4.01 (m, 1H), 4.04−4.07 (m, 1H).

***N*-Butyl D-Glucose-Type 1-*N*-Iminosugar: (3*R*,4*R*,5*R*)-*N*-Butyl-5-(hydroxymethyl)piperidine-3,4-diol Hydrochloride Salt (51).** BH_3·pyridine (8 M; 50 mL, 0.40 mmol) was added to a cooled mixture of **11** (24.7 mg, 0.13 mmol) and *n*-PrCHO (19 mg, 0.27 mmol; 25 mL) in MeOH (2 mL) and phosphate buffer (100 mM, pH 6.8; 5 mL) at 0−5 °C, and the reaction mixture was stirred overnight at room temperature. The mixture was diluted with water (30 mL), and Dowex 50W-X8 [H^+] resin (30 mL) was carefully added to the mixture (hydrogen bubble was evolved). After the bubbling ceased, the resin was packed in a column and washed with water (50 mL). The product was eluted out with 3% NH_4OH, and the fractions containing the product were pooled and concentrated. The residue was diluted with water and 1 N HCl (until the solution became acidic), and the resulting mixture was concentrated. The residue was purified with Sephadex G-25, with water, and the fractions were concentrated. The product was lyophilized from water to give **51** (25 mg, 80%): ^{1}H NMR (D_2O) δ 0.94 (t, 3H, J = 7.38 Hz, CH_3 of Bu), 1.39 (q, 2H, J = 7.43 Hz, $CH_3CH_2CH_2CH_2N$), 1.68−1.79 (m, 2H, $CH_3CH_2CH_2CH_2N$), 1.92−2.07 (m, 1H, H-5), 2.85−3.04 (m, 1H), 3.18−3.25 (m, 2H), 3.53 (t, 1H, J = 10.13 Hz), 3.61−3.66 (m, 1H), 3.72−3.86 (m, 3H).

Synthesis of D-Galactose-Type 1-*N*-iminosugar. *tert*-Butyl (4*R*,5*R*)-4,5-*O*-Isopropylidene-4,5-dihydroxy-3-piperidone-1-carboxylate (26). A solution of **18** (1.0 g, 3.13 mmol) and 25% methanolic NaOMe (0.2 mL) in MeOH (20 mL) was stirred for 30 min at room temperature, and the reaction mixture was neutralized by the addition of AcOH. The mixture was concentrated, and the residue was passed through silica gel with toluene−EtOAc (20:1) to give crude anomeric free product. This was employed for the next step without further purification.

A mixture of the above product and $Pd(OH)_2$ (250 mg) in MeOH (200 mL) was vigorously stirred under 1 atm of H_2 for 12 h at room temperature, the reaction mixture was filtered through a Celite pad, and the filter cake was washed with aqueous MeOH. The combined filtrates were concentrated to give a piperidine derivative **24**. This was employed for the next step without further purification.

A solution of the above **24**, $(Boc)_2O$ (1.02 g, 4.70 mmol), and Et_3N (0.48 g, 4.70 mmol) in MeOH (20 mL) was stirred overnight at room temperature, and the reaction mixture was concentrated. The residue was diluted with EtOAc, and the organic layer was successively washed with water and brine, dried, and concentrated to give **25**. This was employed for the next step without further purification.

A solution of DMSO (1.18 g, 15.1 mmol) in CH_2Cl_2 (5 mL) was added dropwise to a cooled solution of $(COCl)_2$ (3.77 mL, 7.6 mmol, 2.0 M solution) in CH_2Cl_2 (15 mL) at −78 °C. After 30 min, to the solution was added dropwise a solution of the above **25** in CH_2Cl_2 (7 mL) at −78 °C, and the mixture was stirred for another 40 min at −78 °C. A solution of Et_3N (1.90 g, 18.8 mmol) in CH_2Cl_2 (5 mL) was then added dropwise to the reaction mixture at −78 °C, and the reaction temperature was gradually raised to 0−5 °C in 30 min. The reaction mixture was poured into ice−water and extracted with EtOAc. The combined extracts were successively washed with water, aqueous $NaHCO_3$ solution, and brine, dried, and concentrated. The residue was chromatographed on silica gel with toluene−EtOAc (10:1 to 4:1) to give **26** (365 mg, 43% overall) as a colorless needle: mp 116.0−116.5 °C (hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3H, CH_3 of isopropylidene group), 1.45 (s, 9H, t-Bu of Boc group), 1.47 (s, 3H, CH_3 of isopropylidene group), 3.51 (m, 1H, H-6), 3.60 (m, 1H, H-6), 4.65 (m, 1H, H-2), 4.83 (m, 2H, H-4 and -5), 4.95 (m, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 24.9, 26.3, 80.5, 111.2, 154.2, 202.8.

***tert*-Butyl (3*R*,4*S*)-3,4-*O*-Isopropylidene-5-methylene-3,4-dihydroxypiperidine-1-carboxylate (27).** A solution of 1.6 M *n*-BuLi (3.47 mL, 5.17 mmol) in hexane was added dropwise to a cooled suspension of methyltriphenylphosphonium bromide (2.19 g, 5.77 mmol) in DME (35 mL) at 0−5 °C, and the mixture was stirred for 1 h at 0−5 °C. A solution of **26** (320 mg, 1.18 mmol) in DME (2 mL) was added dropwise to the solution at 0−5 °C, and the mixture was stirred for 2 h at 0−5 °C. The reaction mixture was then stirred for another 8 h at the ambient temperature. The reaction mixture was poured into ice−water and extracted with EtOAc. The combined extracts were successively washed with water, aqueous $NaHCO_3$ solution, and brine, dried, and concentrated. The residue was chromatographed on silica gel with toluene−EtOAc (30:1 to 10:1) to give **27** (246 mg, 77%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.34 (s, 3H, CH_3 of isopropylidene group), 1.40 (s, 3H, CH_3 of isopropylidene group), 1.46 (s, 9H, tBu of Boc group), 2.94 (m, 1H, H-2), 3.74 (m, 1H, H-6), 3.89 (dd, J = 2.4 and 14.4 Hz, H-2), 4.24−4.36 (m, 2H, H-3 and H-6), 4.71 (d, J = 7.5 Hz, 1H, H-4), 5.20 (s, 1H, C=CH_2), 5.27 (s, 1H, C=CH_2); ^{13}C NMR (CD_3OD) δ 24.94, 27.05, 28.76, 76.05, 77.33, 80.87, 110.25, 141.24, 156.91.

***tert*-Butyl (3*R*,4*S*,5*R*)-3-(Hydroxymethyl)-4,5-*O*-isopropylidene-4,5-dihydroxypiperidine-1-carboxylate (28).** To a stirred solution of **27** (200 mg, 0.743 mmol) in THF (6 mL) was added dropwise a 0.5 M solution of 9-BBN (7.43 mL, 3.72 mmol) in THF at 0−5 °C, and the reaction mixture was stirred for 15 h at room temperature and then cooled to 0−5 °C. To the cooled mixture were successively added water (0.5 mL), 1 N NaOH solution (0.5 mL), and 30% H_2O_2 solution (0.5 mL) at 0−5 °C, and the reaction mixture was stirred for 12 h at room temperature. The reaction mixture was diluted with water and extracted with EtOAc. The combined extracts were successively washed with water and brine, dried, and concentrated. The residue was chromatographed on silica gel with toluene−EtOAc (20:1 to 3:1) to give **28** (121 mg, 56%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.35 and 1.45 (each s, each 3H, CH_3 of isopropylidene group), 1.46 (s, 9H, tBu of Boc group), 2.01 (s, 1H), 3.22 (m, 2H), 3.50 (m, 1H), 3.75 (m, 4H), 4.46 (dd, 1H, J = 2.67 and 7.02 Hz); HRMS (CI) calcd for $C_{14}H_{25}NO_5$ (M^+) 287.1733, found 287.1738.

D-Galactose-Type 1-*N*-Iminosugar: (3*R*,4*S*,5*R*)-5-(Hydroxymethyl)piperidine-3,4-diol Hydrochloride Salt (12). A solution of **28** (103 mg, 0.36 mmol) in 1 N HCl (10 mL) was stirred overnight at room temperature, and the reaction mixture was concentrated. The residue was coevaporated with water (10 mL) and purified with a column of Sephadex G-25 with water. The fractions containing iminosugar were pooled and concentrated, and the residue was lyophilized from water (5 mL) to give **12** (62 mg, 94%) as a colorless amorphous powder: ^{1}H NMR (D_2O) δ 1.89−1.99 (m, 1H, H-5), 2.73 (t, 1H, J = 12.6 Hz, H-2ax), 2.86 (t, 1H, J = 11.9 Hz, H-6ax), 3.05 (dd, 1H, J = 4.29 and 12.21 Hz, H-6eq), 3.09 (dd, 1H, J = 7.07 and 11.89 Hz, H-2eq), 3.40 (dd, 1H, J = 7.24 and 11.29 Hz, H-7a), 3.40 (dd, 1H, J = 6.70 and 11.26 Hz, H-7b), 3.77 (ddd, 1H, J = 2.69, 7.05, and 11.55 Hz, H-3), 3.93 (br s, 1H, H-4); ^{13}C NMR (D_2O) δ 41.54, 42.31, 44.65, 62.71, 68.32, 68.70; HRMS (CI) calcd for $C_6H_{13}NO_3$ (M + H^+) 148.0974, found 148.0976.

Synthesis of L-Fucose-Type 1-*N*-Iminosugar. 1-*O*-Benzoyl-2,3-*O*-isopropylidene-5-*O*-*p*-toluenesulfonyl-β-D-ribose (30). In a manner similar to the preparation of the tosyl derivative **17** from D-lyxose **16**, D-ribose **29** (10 g) was converted to the tosyl derivative **30** (20.0 g, 48% from D-ribose) as colorless needles: mp 82.5−83.0 °C (hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.35 (s, 3H, CH_3), 1.57 (s, 3H, CH_3), 2.39 (s, 3H, $ArCH_3$), 4.05 (dd, 1H, J = 6.9, 10.3 Hz, H-5), 4.10 (dd, 1H, J = 5.1, 10.3 Hz, H-5), 4.48 (dd, 1H, J = 5.1, 6.9 Hz, H-4), 4.87 (s, 2H, H-2,3), 6.43 (s, 1H, H-1), 7.22 (d, 2H, J = 8.2 Hz, aromatic H of the tosyl group), 7.48 (dd, 2H, J = 7.7 Hz, aromatic H of the benzoyl group), 7.62 (dd, 1H, J = 7.3 Hz, aromatic H of the benzoyl group), 7.66 (d, 2H, J = 8.2 Hz, aromatic H of the tosyl group), 8.00 (d, 2H, J = 7.3 Hz, aromatic H of the benzoyl group); ^{13}C NMR ($CDCl_3$) δ 21.47, 24.87, 26.33, 68.84, 81.09, 84.92, 85.09, 102.73, 113.32, 127.78, 128.45, 129.73, 129.80, 133.48, 145.04, 164.61. Anal. Calcd for $C_{22}H_{22}O_8S$: C, 59.19; H, 4.96. Found: C, 55.53; H, 5.50.

5-Azido-1-*O*-benzoyl-5-deoxy-2,3-*O*-isopropylidene-β-D-ribose (31). In a manner similar to the preparation of the azido derivative **18** from the tosyl derivative **17**, **30** (18.7 g, 41.7 mmol) was converted to **31** (12.2 g, 92%) as colorless needles: mp 80.0−80.5 °C (EtOH−hexane); ^{1}H NMR ($CDCl_3$) δ 1.37 (s, 3H, CH_3), 1.59 (s, 3H, CH_3), 3.31 (dd, 1H, J = 6.8 and 12.7 Hz, H-5), 3.55 (dd, 1H, J = 7.0 and 12.7 Hz, H-5), 4.48 (dt, 1H, J = 0.93 and 6.9 Hz, H-4), 4.76 (dd, 1H, J = 0.93 and 6.0 Hz, H-3), 4.90 (d, 1H, J = 6.0 Hz, H-2), 7.46 (dd, 2H, J = 7.4 and 7.9 Hz, *m*-aromatic H), 7.60 (tt, 1H, J = 1.3 and 7.4 Hz, *p*-aromatic H), 8.01 (dd, 2H, J = 1.3 and 7.9 Hz, *o*-aromatic H); ^{13}C NMR ($CDCl_3$) δ 24.9, 26.4, 53.0, 81.7, 85.2, 86.6, 103.1, 113.4, 128.5, 129.6, 133.5, 164.7. Anal. Calcd for $C_{15}H_{17}N_3O_5$: C, 56.42; H, 5.36; N, 13.16. Found: C, 56.76; H, 5.43; N, 12.98.

(3*S*,4*R*,5*R*)-3,4-*O*-Isopropylidenepiperidine-3,4,5-triol (32). In a manner similar to the preparation of the piperidine derivative **24** from the azido derivative **18**, **31** (4.62 g, 14.5 mmol) was converted to **32** (1.88 g, 95% overall) as a colorless amorphous powder: mp 92−95 °C; ^{1}H NMR ($CDCl_3$) δ 1.38 and 1.55 (each s, each 3H, CH_3 of isopropylidene group), 2.74−3.15 (m, 6H, H-2, -6, O*H* and N*H*), 3.79 (m, 1H), 4.15 (m, 1H), 4.27 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 25.5, 26.9, 46.6, 47.2, 64.9, 71.8, 74.2, 108.7; HRMS (CI) calcd for $C_8H_{16}NO_3$ (M + $H)^+$ 174.1130, found 174.1129.

***tert*-Butyl (3*S*,4*R*,5*R*)-2,3-*O*-Isopropylidene-3,4,5-trihydroxypiperidine-1-carboxylate (33).** In a manner similar to the preparation of the *N*-Boc derivative **25** from the piperidine derivative **24**, **32** (4.50 g, 26.0 mmol) was converted to **33** (5.10 g, 73%) as colorless needles: mp 82.5−83.5 °C (from hexanes−EtOAc); ^{1}H NMR ($CDCl_3$) δ 1.38 and 1.51 (each s, each 3H, CH_3 of isopropylidene group), 1.46 (s, 9H, tBu of Boc group), 2.79 (m, 1H), 3.18 (dd, 1H, J = 9.9 and 12.2 Hz), 3.45 (dd, 1H, J = 4.0 and 14.0 Hz), 3.56 (m, 2H), 3.90 (m, 1H), 4.38 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 24.7, 26.6, 28.3, 65.6, 72.2, 79.8, 109.3. Anal. Calcd for $C_{13}H_{23}NO_5$: C, 57.13; H, 8.48; N, 5.12. Found: C, 57.08; H, 8.74; N, 5.21.

***tert*-Butyl (4*S*,5*S*)-4,5-*O*-Isopropylidene-4,5-dihydroxy-3-piperidone-1-carboxylate (34).** In a manner similar to the preparation of the ketone derivative **26** from the hydroxyl derivative **25**, **33** (2.06 g, 7.54 mmol) was converted to **34** (1.63 g, 80%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.40 (s, 3H, CH_3 of isopropylidene group), 1.45 (s, 9H, t-Bu of Boc group), 1.47 (s, 3H, CH_3 of isopropylidene group), 3.51 (m, 1H, H-6), 3.60 (m, 1H, H-6), 4.65 (m, 1H, H-2), 4.83 (m, 2H, H-4 and -5), 4.95 (m, 1H, H-2); ^{13}C NMR ($CDCl_3$) δ 24.9, 26.3, 80.5, 111.2, 154.2, 202.8; FABMS calcd for $C_{13}H_{24}N_2O_5$ (M + $NH_3)^+$ 288.1, found 288.1.

***tert*-Butyl (3*S*,4*R*)-3,4-*O*-Isopropylidene-5-methylene-3,4-dihydroxypiperidine-1-carboxylate (35).** In a manner similar to the preparation of the methylene derivative **27** from the ketone derivative **26**, **34** (935 mg, 3.45 mmol) was converted to **35** (610 mg, 66%) as a colorless oil: ^{1}H NMR (CD_3OD) δ 1.34 (s, 3H, CH_3 of isopropylidene group), 1.40 (s, 3H, CH_3 of isopropylidene group), 1.46 (s, 9H, tBu of Boc group), 2.94 (m, 1H, H-2), 3.74 (m, 1H, H-6), 3.89 (dd, J = 2.4 and 14.4 Hz, H-2), 4.24−4.36 (m, 2H, H-3 and H-6), 4.71 (d, J = 7.5 Hz, 1H, H-4), 5.20 (s, 1H, C=CH_2), 5.27 (s, 1H, C=CH_2); ^{13}C NMR (CD_3OD) δ 24.94, 27.05, 28.76, 76.05, 77.33, 80.87, 110.25, 141.24, 156.91; HRMS (CI) calcd for $C_{14}H_{23}NO_4$ (M + H^+) 270.1705, found 270.1710.

***tert*-Butyl (3*S*,4*R*,5*R*)-3,4-*O*-Isopropylidene-5-methyl-3,4-dihydroxypiperidine-1-carboxylate (36).** A suspension of **35** (493 mg, 1.83 mmol) and 10% Pd/C (50 mg) in EtOAc−EtOH (30 mL-20 mL) was vigorously stirred under an atmosphere of H_2 for 10 h at room temperature, the reaction mixture was filtered through a Celite pad, and the filter cake was washed with EtOAc. The combined filtrates were concentrated, and the residue was chromatographed on silica gel with hexanes−EtOAc (30:1 to 10:1) to give **36** (408 mg, 82%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.05 (d, 3H, J = 6.9 Hz, CH_3 at C-5), 1.34 and 1.44 (each s, each 3H, CH_3 of isopropylidene group), 1.46 (s, 9H, tBu of Boc group), 1.90 (m, 1H, H-5), 2.99 (t, 1H, J = 12.3 Hz), 3.34 (m, 2H), 3.68 (m, 1H), 4.20 (dd, 1H, J = 2.5 and 6.9 Hz), 4.27 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 24.7, 26.5, 28.4, 30.7, 72.7, 74.9, 108.2, 164.1; HRMS (CI) calcd for $C_{14}H_{25}NO_4$ (M + H^+) 272.1862, found 272.1860.

L-Fucose-Type 1-*N*-Iminosugar: (3*S*,4*R*,5*R*)-5-methylpiperidine-3,4-diol Hydrochloride Salt (13). In a manner similar to the preparation of **12** from the protected derivative **28**, **36** (278 mg, 1.02 mmol) was converted to **13** (154 mg, 90%) as a colorless amorphous powder (HCl salt): ^{1}H NMR (D_2O) δ 0.93 (d, 3H, J = 6.75 Hz, C-5 CH_3), 1.96 (m, 1H, H-5), 2.73 (dd, 1H, J = 12.6 Hz, H-2ax or -6ax), 2.91 (dd, 1H, J = 11.7 Hz, H-6ax or -2ax), 3.01 (dd, 1H, J = 3.65 and 12.6 Hz, H-2eq or -6eq), 3.17 (dd, 1H, J = 4.22 and 11.3 Hz, H-6eq or -2eq), 3.85 (s, 1H, H-4), 3.87 (dm, 1H, J = 12 Hz, H-3); ^{13}C NMR (D_2O) δ 13.89, 31.88, 41.85, 43.22, 66.25, 69.70; HRMS (CI) calcd for $C_6H_{13}NO_2$ (M + $H)^+$ 132.1025, found 132.1023.

Synthesis of D-Glucuronic Acid-Type 1-*N*-Iminosugar 14. *p*-Methoxyphenyl 5-Azido-5-deoxy-2,3-di-*O*-acetyl-α-D-arabinofuranoside (39). TMSOTf (2.06 g, 1.79 mL, 9.27 mmol) was added dropwise to a cooled solution of **38** (9.3 g, 30.9 mmol) and *p*-methoxyphenol (5.75 g, 46.4 mmol) in CH_2Cl_2 (200 mL) at 0−5 °C, and the mixture was stirred for 3 h at room temperature. The reaction mixture was diluted with CH_2Cl_2, washed successively with saturated $NaHCO_3$ solution and brine, dried, and concentrated. The residue was chromatographed on silica gel with hexanes−EtOAc (20:1 to 4:1) to give **39** (9.5 g, 84%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.14 (s, 3H, CH_3 of acetyl group), 2.15 (s, 3H, CH_3 of acetyl group), 3.47 (dd, 1H, J = 4.8 and 13.3 Hz, H-5a), 3.69 (dd, 1H, J = 3.0 and 13.3 Hz, H-5b), 3.77 (s, 3H, OCH_3), 4.35 (ddd, 1H, J = 3.0, 4.8, and 4.9 Hz, H-4), 5.08 (dd, 1H, J = 1.4 and 4.9 Hz, H-3), 5.36 (d, 1H, J = 1.4 Hz, H-2), 5.61 (s, 1H, H-1), 6.83 (dm, 2H, J = 9.1 Hz, aromatic H), 7.01 (dm, 2H, J = 9.1 Hz, aromatic H); ^{13}C NMR ($CDCl_3$) δ 20.76, 51.34, 55.65, 77.68, 81.45, 82.86, 104.91, 114.63, 118.34, 149.93, 155.33, 169.75, 170.40. Anal. Calcd for $C_{16}H_{19}N_3O_7$: C, 52.60; H, 5.24; N, 11.50. Found: C, 52.80; H, 5.32; N, 11.29.

***p*-Methoxyphenyl 5-Azido-5-deoxy-2,3-di-*O*-benzyl-α-D-arabinofuranoside (40).** A mixture of **39** (8.0 g, 21.9 mmol) and 25% methanolic NaOMe (1.6 mL) in MeOH (160 mL) was stirred for 30 min at room temperature, and the reaction mixture was neutralized with Dowex 50W-X8 [H^+], filtered to remove the resins, and concentrated. The residual oil was diluted with Et_2O, dried, and concentrated.

NaH (60% mineral oil dispersion; 2.10 g, 52.6 mmol) was added portionwise to a cooled solution of the above residue in dry DMF (150 mL) at 0−5 °C, and the suspension was stirred for 30 min at room temperature. The mixture was cooled again at 0−5 °C, benzyl bromide

(8.99 g, 6.25 mL, 52.6 mmol) was added dropwise to this solution, and the reaction mixture was stirred overnight at room temperature. The reaction mixture was poured onto ice−water and extracted with EtOAc. The combined extracts were successively washed with water and brine, dried, and concentrated. The residue was chromatographed on silica gel with hexanes−EtOAc (1:0 to 10:1) to give **40** (9.5 g, 99%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 3.31 (dd, 1H, J = 5.0 and 13.2 Hz, H-5a), 3.51 (dd, 1H, J = 2.8 and 13.2 Hz, H-5b), 3.77 (s, 3H, OCH_3), 4.00 (dd, 1H, J = 3.3 and 6.7 Hz, H-3), 4.30 (dd, 1H, J = 1.1 and 3.5 Hz, H-2), 4.32 (m, 1H, H-4), 4.54 (d, 2H, J = 11.8 Hz, benzylic H), 4.64 (d, 2H, J = 11.8 Hz, benzylic H), 5.60 (s, 1H, H-1), 6.83 (d, 2H, J = 9.1 Hz, aromatic H of *p*-MeOPh group), 6.98 (d, 2H, J = 9.1 Hz, aromatic H of *p*-MeOPh group), 7.31−7.35 (m, 10H, aromatic H of benzyl groups); ^{13}C NMR ($CDCl_3$) δ 51.64, 55.68, 72.34, 72.50, 80.77, 83.61, 88.21, 105.37, 114.61, 118.04, 127.88, 128.01, 128.04, 128.09, 128.51, 128.55, 137.17, 137.55, 150.46, 155.03. Anal. Calcd for $C_{26}H_{27}N_3O_5$: C, 67.67; H, 5.89; N, 9.10. Found: C, 68.00; H, 6.00; N, 8.87.

(3*R*,4*R*,5*R*)-3,4-Di-*O*-benzyl-3,4,5-trihydroxypiperidine (42). $(NH_4)_2Ce(NO_3)_6$ (18.8 g, 34.3 mmol) was added to a cooled solution of **40** (5.0 g, 11.4 mmol) in CH_3CN (90 mL) and H_2O (30 mL) at 0−5 °C, and the mixture was stirred for 5 min at this temperature. The reaction mixture was diluted with Et_2O, washed successively with water, diluted Na_2SO_3 solution, saturated $NaHCO_3$ solution and brine, dried, and concentrated. The residue was chromatographed on silica gel with hexanes−EtOAc (20:1 to 4:1) to give crude **41** as a colorless oil.

A suspended mixture of the above **41** and Pd−$CaCO_3$ (1.50 g) in MeOH (600 mL) was vigorously stirred under the atmosphere of H_2 for 24 h at room temperature. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated. The residue was chromatographed on silica gel with $CHCl_3$−MeOH (1:0 to 10:1) to give the imine (2.14 g, 60%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 2.49 (dd, 1H, J = 8.7 and 13.4 Hz, H-2ax), 2.65 (dd, 1H, J = 2.4 and 13.8 Hz, H-6ax), 3.00 (dd, 1H, J = 4.6 and 13.8 Hz, H-6eq), 3.14 (dd, 1H, J = 4.3 and 13.4 Hz, H-2eq), 3.51 (dd, 1H, J = 3.1 and 7.8 Hz, H-4), 3.65 (ddd, 1H, J = 4.4, 7.8, and 8.7 Hz, H-3), 3.95 (ddd, 1H, J = 2.4, 3.1, and 4.6 Hz, H-5), 4.63 (d, 1H, J = 11.8 Hz, benzylic H), 4.672 (d, 1H, J = 11.7 Hz, benzylic H), 4.674 (d, 1H, J = 11.8 Hz, benzylic H), 4.73 (d, 1H, J = 11.7 Hz, benzylic H), 7.28−7.36 (m, 10H, aromatic H); ^{13}C NMR ($CDCl_3$) δ 48.00, 49.19, 67.20, 72.35, 72.54, 76.02, 81.28, 127.66, 127.79, 127.88, 128.40, 128.50, 138.18, 138.56; HRMS calcd for $C_{19}H_{24}NO_3$ $(M + H)^+$ 314.1756, found 314.1755.

***tert*-Butyl (3*R*,4*R*,5*R*)-3,4-Di-*O*-benzyl-3,4,5-trihydroxypiperidine-1-carboxylate (42).** A solution of **41** (1.61 g, 5.14 mmol), $(Boc)_2O$ (1.68 g, 7.71 mmol), and Et_3N (0.78 g, 7.71 mmol) in MeOH (30 mL) was stirred for 4 h at room temperature, and the reaction mixture was concentrated. The residue was chromatographed on silica gel with hexanes−EtOAc (20:1 to 4:1) to give **42** (1.55 g, 75%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, tBu of Boc group), 3.40−3.50 (m, 3H), 3.60 (dd, 1H, J = 3.2 and 6.0 Hz, H-4), 3.70 (m, 2H), 3.96 (br s, 1H, H-5), 4.53 (d, 1H, J = 11.6 Hz, benzylic H), 4.62 (d, 1H, J = 11.8 Hz, benzylic H), 4.68 (d, 1H, J = 11.8 Hz, benzylic H), 4.70 (d, 1H, J = 11.6 Hz, benzylic H), 7.30−7.36 (m, 10H, aromatic H); FABMS calcd for $C_{24}H_{31}NO_5$ 412.2, found 412.2.

***tert*-Butyl (4*S*,5*R*)-4,5-*O*-Dibenzyl-4,5-dihydroxy-3-piperidone-1-carboxylate (43).** In a manner similar to the preparation of the ketone derivative **26** from the hydroxyl derivative **25**, **42** (1.36 g, 3.39 mmol) was converted to **43** (1.06 g, 78%) as a colorless oil; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, tBu of Boc group), 3.67 (dd, 1H, J = 6.0 and 13.8 Hz), 3.83 (m, 1H), 3.93−4.00 (m, 3H), 4.14 (d, 1H, J = 17.0 Hz), 4.56 (d, 1H, J = 11.6 Hz, benzylic H), 4.62 (d, 1H, J = 11.9 Hz, benzylic H), 4.68 (d, 1H, J = 11.9 Hz, benzylic H), 4.79 (d, 1H, J = 11.6 Hz, benzylic H), 7.28−7.37 (m, 10H, aromatic H). Anal. Calcd for $C_{23}H_{29}NO_5$: C, 69.16; H, 7.31; N, 3.51. Found: C, 69.22; H, 6.95; N, 3.27.

***tert*-Butyl (3*R*,4*R*)-3,4-Di-*O*-benzyl-5-methylene-3,4-dihydroxypiperidine-1-carboxylate (44).** $(TMS)_2NLi$ (1M solution; 15.7 mL, 15.7 mmol) was added dropwise to a cooled suspension of CH_3PPh_3Br (6.68 g, 18.7 mmol) in DME (80 mL), and the mixture was stirred for 1 h at 0−5 °C. A solution of **43** (740 mg, 1.85 mmol) in DME (10 mL) was then added dropwise to the mixture at 0−5 °C, and the reaction mixture was stirred for 18 h at ambient temperature. The reaction mixture was evaporated, and the residue was dissolved in water and EtOAc. The organic layer was successively washed with water and brine, dried, and concentrated. The residue was chromatographed on silica gel with hexanes−EtOAc (30:1 to 10:1) to give **44** (596 mg, 81%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H, t-Bu of Boc group), 3.56 (br s, 2H), 3.75 (dd, 1H, J = 5.3 and 14.2 Hz), 3.91 (m, 2H), 4.15 (m, 1H), 4.45 (d, 1H, J = 11.8 Hz, benzylic H), 4.57 (d, 1H, J = 11.5 Hz, benzylic H), 4.61 (d, 1H, J = 11.5 Hz, benzylic H), 4.71 (d, 1H, J = 11.8 Hz), 5.13 (s, 1H, C=CH), 5.23 (br s, 1H, C=CH), 7.28−7.36 (m, 10H, aromatic H). Anal. Calcd for $C_{24}H_{31}NO_4$: C, 72.52; H, 7.86; N, 3.52. Found: C, 72.60; H, 8.14; N, 3.48.

***tert*-Butyl (3*R*,4*R*,5*RS*)-3,4-Di-*O*-benzyl-3,4-dihydroxy-5-(hydroxymethyl)piperidine-1-carboxylate (45).** In a manner similar to the hydroboration reaction of the methylene derivative **27** to the hydroxyl derivative **28**, **44** (339 mg, 0.853 mmol) was converted to **45** (concomitant with **45′**) (404 mg, quantitative) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H, t-Bu of Boc group), 1.84 (m, 1H, H-5), 3.47−3.84 (m, 8H), 4.58 (d, 1H, J = 11.6 Hz), 4.66 (d, 1H, J = 11.4 Hz), 4.72 (dm, 1H, J = 11.6 Hz), 4.84 (m, 1H), 7.30−7.35 (m, 10H, aromatic H of benzyl group); FABMS calcd for $C_{25}H_{34}NO_5$ $(M + H)^+$ 427.2, found 427.3.

***tert*-Butyl (3*R*,4*R*,5*RS*)-3,4-Di-*O*-benzyl-5-formyl-3,4-dihydroxypiperidine-1-carboxylate (46).** In a manner similar to the oxidation of the hydroxyl derivative **25** to the ketone derivative **26**, **45** (300 mg, 0.724 mmol) was converted to **46** (251 mg, 84%) as a colorless oil: ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H, tBu of Boc group), 2.90−3.12 (m, 2H), 3.60−4.66 (m, 9H), 7.20−7.36 (m, 10H, aromatic H of benzyl group); FABMS calcd for $C_{25}H_{32}NO_5$ $(M + H)^+$ 426.2, found 426.3.

D-Glucuronic Acid-Type 1-*N*-Iminosugar: (3*R*,4*R*,5*S*)-5-Carboxylpiperidine-3,4-diol Hydrochloride Salt (14). To a mixture of **46** (220 mg, 0.53 mmol) and $NaH_2PO_4 \cdot H_2O$ (736 mg, 5.3 mmol) in CH_3CN−H_2O (10 mL-2 mL) were successively added a 35% H_2O_2 solution (0.062 mL, 0.64 mmol) and a solution of $NaClO_2$ (80%, 72 mg, 0.64 mmol) in water (10 mL) at 0−5 °C, and the reaction mixture was stirred for 1 h at room temperature. Na_2SO_3 (100 mg) was added to the mixture to quench the oxidants, and the resulting mixture was extracted with EtOAc. The combined extracts were successively washed with water and brine, dried, and concentrated. The residue was passed through silica gel with $CHCl_3$−MeOH (9:1) to give crude **47** as a colorless oil. This was employed for the next step without further purification.

A mixture of the above **47** and $Pd(OH)_2$ (50 mg) in MeOH (12 mL) was vigorously stirred under the atmosphere of H_2 for 18 h at room temperature. The mixture was filtered through a Celite pad, and the filtrate was concentrated. A mixture of the residue in 1 N HCl (3 mL) was stirred for 3 h at room temperature, and the mixture was concentrated. The residue was chromatographed on silica gel with 2-propanol−H_2O−30% NH_4OH (7:2:1; v/v) to give a chromatographically pure **14**, which was dissolved in 0.1 N HCl and concentrated. The residue was diluted with water (1 mL) and applied onto a column of Sephadex G-25 (1.0 × 25 cm) and eluted with water. The fractions containing the product were pooled and concentrated. The residue was lyophilized from water to give **14** (27.5 mg, 26% overall) as a colorless amorphous powder (HCl salt): ^{1}H NMR (300 MHz, D_2O) δ 2.90 (ddd, 1H, J = 4.3, 7.4, 7.8 Hz, H-5), 3.07 (dd, 1H, J = 7.7, 12.9 Hz, H-2ax), 3.38 (dd, 1H, J = 7.8, 13.2 Hz, H-6ax), 3.50 (dd, 1H, J = 3.6, 12.9 Hz, H-2eq), 3.51 (dd, 1H, J = 4.3, 13.2 Hz, H-6eq), 3.90 (ddd, 1H, J = 3.6, 7.2, 7.7 Hz, H-3), 4.08 (t, 1H, J = 7.0 Hz, H-4); HRMS calcd for $C_6H_{12}NO_4$ $(M + H)^+$ 162.0766, found 162.0766.

D-Xylose-Type 1-*N*-Iminosugar: (3*R*,4*R*)-Piperidine-3,4-diol Hydrochloride Salt (15). A mixture of **42** (900 mg, 2.18 mmol) and thiocarbonyldiimidazole (1.55 g, 8.7 mmol) in $ClCH_2CH_2Cl$ (50 mL) was heated under gentle reflux overnight, and the reaction mixture was concentrated. The residue was chromatographed on silica gel, with hexanes−EtOAc (20:1), to give **49** (940 mg, 83%). Its ^{1}H NMR spectrum (in $CDCl_3$) showed the presence of two components probably due to the isomers around the *N*-Boc group: ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.31 and 1.39 (br s each, Boc group), 5.72 and 5.84 (br s each, H-5), 7.03 (br s, 1H, imidazole), 7.54 (br s, 1H, imidazole).

A solution of **49** (940 mg, 1.80 mmol) and 1,1′-azobis(cyclohexanecarbonitrile) (30 mg) in toluene (20 mL) was added dropwise to a preheated mixture of *n*-Bu_3SnH (1.31 g, 4.49 mmol; 1.21 mL) in toluene (50 mL) at 90 °C, and the reaction mixture was heated overnight at 90 °C. The mixture was concentrated, and the residue was chromatographed on silica gel, with hexanes−EtOAc (50:1), to give **50** (213 mg, 36%) contaminated with a trace amount of tin compound.

A mixture of **50** (213 mg, 0.64 mmol) and 20% $Pd(OH)_2$ (220 mg) in MeOH (5 mL) and 1 N HCl (20 mL) was stirred at 1 atm of H_2 for 18 h at room temperature. The reaction mixture was filtered through a Celite pad, and the filtrate was concentrated. The residue was dissolved in water (30 mL), applied on a column of Dowex 50W-X8 [H^+] resin, and eluted with 3% NH_4OH. The fractions containing the product were pooled, concentrated, and sequentially coevaporated with 1 N HCl and water. The residue was chromatographed on Sephadex G-20, with water, and lyophilized from water to give **15** (65 mg, 66%): ^{1}H NMR (300 MHz, D_2O) δ 1.69−1.82 (m, 1H, H-5ax), 2.24 (tdd, 1H, J = 3.70,3.80,14.84 Hz, H-5eq), 2.97−3.05 (m, 1H), 3.13 (ddd, 1H, J = 3.94,8.49, 12.96 Hz), 3.36 (ddd, 1H, J = 3.96,7.61, 13.20 Hz), 3.45 (dd, 1H, J = 2.45,12.60 Hz, H-2ax), 3.78−3.87 (m, 2H, H-3,4); HRMS calcd for $C_5H_{12}NO_2$ $(M + H)^+$ 118.0868, found 118.0866.

General Procedure for Inhibition Assay. Inhibitory potencies of the 1-*N*-iminosugars were determined by measuring the residual hydrolytic activities of the glycosidases of the corresponding *p*- or *o*-nitrophenyl glycosides in the presence of iminosugars spectrophotometrically on a Beckman Model DU 650. Glycosidases used in this study were purchased from Sigma (St. Louis), and they were α-glucosidase (yeast; G 5003), β-glucosidase (almond; G 4511), α-galactosidase (green coffee beans; G8507), β-galactosidase (*Aspergillus orizae*; G7256), α-mannosidase (Jack bean; M 7257), α-fucosidase (bovine kidney; F 5884), and β-glucuronidase (bovine liver; G 0501).

Each assay was performed in either phosphate buffer (25 mM; pH 6.8) or acetate buffer (25 mM; pH 5.0) with 4-nitrophenyl (*p*-nitrophenyl) glycoside derivatives of the sugars except for β-galactosidase assay in which 2-nitrophenyl (*o*-nitrophenyl) β-galactoside was used. The reactions were initiated by addition of enzyme to a cooled solution of the substrate (0.4−5 mM) at 0−5 °C in the presence or absence of a various concentration of iminosugars in buffer which had been thermodynamically equilibrated. After the mixture was incubated for 10−15 min at 37 °C, the reaction was quenched by addition of 200 mM Na_2CO_3 solution. The absorbance of the resulting mixture was read at 400 nm (for 4-nitrophenol) or 420 nm (for 2-nitrophenol). IC_{50} values were determined as a concentration of the iminosugars that inhibits 50% of the enzyme activity. Ki values were determined with a Sigma Plot program (version 4.1, Jandel Scientific).

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Supporting Information Available: Experimental data for compounds **53** and **57**−**63** (3 pages, print/PDF). See any current masthead page for ordering information and Web access instructions.

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