A Versatile Synthetic Strategy for the Preparation and Discovery of New Iminocyclitols as Inhibitors of Glycosidases

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A series of iminocyclitols was prepared using a versatile synthetic strategy, and their inhibition of glycosidases was evaluated using capillary electrophoresis. The study has demonstrated that remarkable specificities in enzyme inhibition can be achieved with small modifications on the aglycon side chain and the ring nitrogen. Among the compounds synthesized, (2*R*,3*R*,4*R*,5*R*)-*N*-methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine was found to be very potent against *β*-*N*-acetylhexosaminidase P with the K_i value of 80 nM.

Introduction

Glycosyltransferases and glycosidases are important classes of enzymes involved in the biosynthesis of oligosaccharides with diverse structures.[1] Development of specific inhibitors of such enzymes has been considered to be a useful strategy for the control of cellular functions, especially those related to metabolic disorders and diseases. Enzymatic hydrolysis of the glycosidic bonds generally takes place via general acid−base catalyses that require two critical residues, a proton donor and a nucleophile (Figure 1).[2] Five- or six-membered iminocyclitols carrying hydroxyl groups with specific orientation and a secondary amine have been used to mimic the shape and charge of the transition state of the reaction and have been shown to be potent inhibitors of such enzymes.[1a,3,4]

To study the inhibition of glycoenzymes, generation of such compounds and evaluation of their activities are necessary. Regarding the synthesis of iminocyclitols, we have developed two distinct methods and both are proven equally useful. One is based on aldolase-catalyzed reactions[5] and the other is based on multistep chemical transformations.[6] Having the synthetic method available

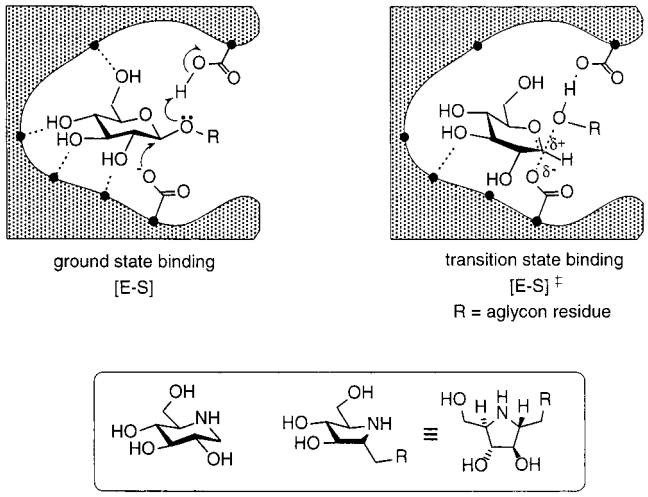

Figure 1. Proposed mechanism and transition state of *β*-glucosidase-catalyzed reaction and representative structures of transition-state analogue inhibitors.

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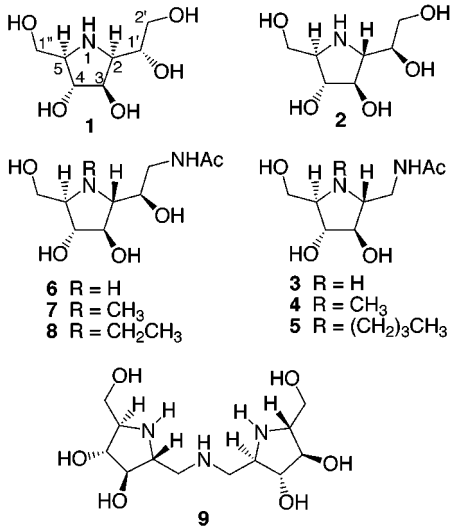
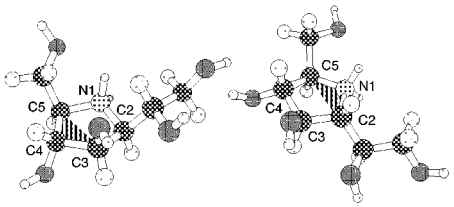


Figure 2. Structures of target compounds.

Table 1. Conformations of Compounds 1 and 2 Suggested by ^{1}H NMR

	Compound **1**		Compound **2**	
	J (Hz)	θ(°)	*J* (Hz)	θ(°)
H2-C2-C3-H3	3.9	44.8	7.3	154.2
H3-C3-C4-H4	1.2	110.5	6.5	148.2
H4-C4-C5-H5	3.5	127.6	7.5	155.5
Suggested conformation	$^1T_2$		$^4T_3$	
Structure				

for these compounds, we are able to evaluate the inhibitory activities of a wide range of structures related to iminocyclitols. In addition, as part of our efforts to develop rapid and reliable assay methods without using radioactive isotopes, we have reported methods based on electrospray mass spectrometry[7] and capillary zone electrophoresis (CZE).[8]

We describe here the chemical syntheses of several five-membered iminocyclitols (Figure 2) and evaluation of their inhibitory activities against several glycosidases[9] using capillary zone electrophoresis.

Results and Discussion

Synthesis of Compounds 1 and 2 (Scheme 1).[6a] Wittig reaction of 2,3,5-benzyl-protected D-arabinofuranose (**10**) with methyl (triphenylphosphoranylidene)-acetate afforded *E*-**11**, of which the methoxycarbonyl group was converted to the TBDMS-protected alcohol (**13**) via diisobutylaluminum hydride (DIBAL) reduction followed by silylation. To introduce the azide function to the *R*-configuration at the C-6 position, double inversion reactions were carried out. The 6-OH group was chloromesylated[10] (**14**) and treated with CsOAc to give **15**. The leaving group was selected after several attempts using methanesulfonyl and trifluoromethanesulfonyl groups, which did not give satisfactory results. After removal of the acetate (**16**), the OH group was again chloromesylated for the second inversion (**17**) and the TBDMS group was deprotected to unmask the allylic alcohol for Sharpless epoxidation (**18**). The reason for the introduction of an azide group after epoxidation is to avoid the undesired 1,3-dipole addition reaction of the introduced azide with the present double bond, which would further undergo thermolysis under the reaction conditions. The allyl alcohol **18** was epoxidized in the presence of (+)- and (−)-diethyl tartrates to afford **19a** and **19b**, respectively. No diastereoisomer was observed for either reaction according to ^{1}H NMR. Compounds **19a** and **19b** were treated with NaN_3 to give azides **20a** and **20b**, both of which were subjected to a reduction condition to give **21a** and **21b**. Finally, benzyl groups were hydrogenolyzed to give the target compounds **1** and **2**, respectively.[11] It is noted that the chloromesyl group served as a very good leaving group as well as a protecting group in these transformations.

The ^{1}H NMR analysis of compounds **1** and **2** suggested that they adopted different ring conformations. The coupling constants for the ring protons of **1** were suggestive of a $^1T_2$ conformation, whereas **2** adopted the $^4T_3$ confomation (Table 1). The detailed conformations of these compounds, however, cannot be discussed only by ^{1}H NMR, as five-member-ring compounds are known to be flexible[12] and may exist as equilibrium mixtures. It is assumed that **2** probably well mimics the transition state of the glycosidic cleavage.

Synthesis of Compounds 3−9 (Schemes 2 and 3). We selected the 2(*R*),5(*R*)-configured structures related to **2** for further investigation. Our objectives are (1) to probe the critical functional groups in inhibition, (2) to seek the possibility of introducing a functional group to the side chain for connection to an aglycon group to create a library,[7a] and (3) to examine the potency of such compounds, including those having the NHAc group as inhibitors of β-*N*-acetylglucosaminidases, as **3** is known to be a potent inhibitor of the enzyme.[13,14] In addition to the imimocyclitol framework with modified side chains, compounds with an alkyl group on the ring nitrogen were included because such a modification would enhance the basicity of the nitrogen atom and increase the hydropho-

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(11) Compound **2**, while its configuration of C-1′ was not determined, was isolated from *Hyacintoides nonscripta* (Watson, A. A.; Nash, R. J.; Wormald, M. R.; Harvey, D. J.; Dealler, S.; Lees, E.; Asano, N.; Kizu, H.; Kato, A.; Griffiths, R. C.; Cairns, A. J.; Fleet, G. W. J. *Phytochem.* **1997**, *46*, 255−259) and was reported to have K_i = 4 mM and 85 mM against β-glucosidase from almond and α-glucosidase from baker's yeast, respectively. Detailed comparison of ^{1}H and ^{13}C NMR data of these compounds revealed that they were identical.

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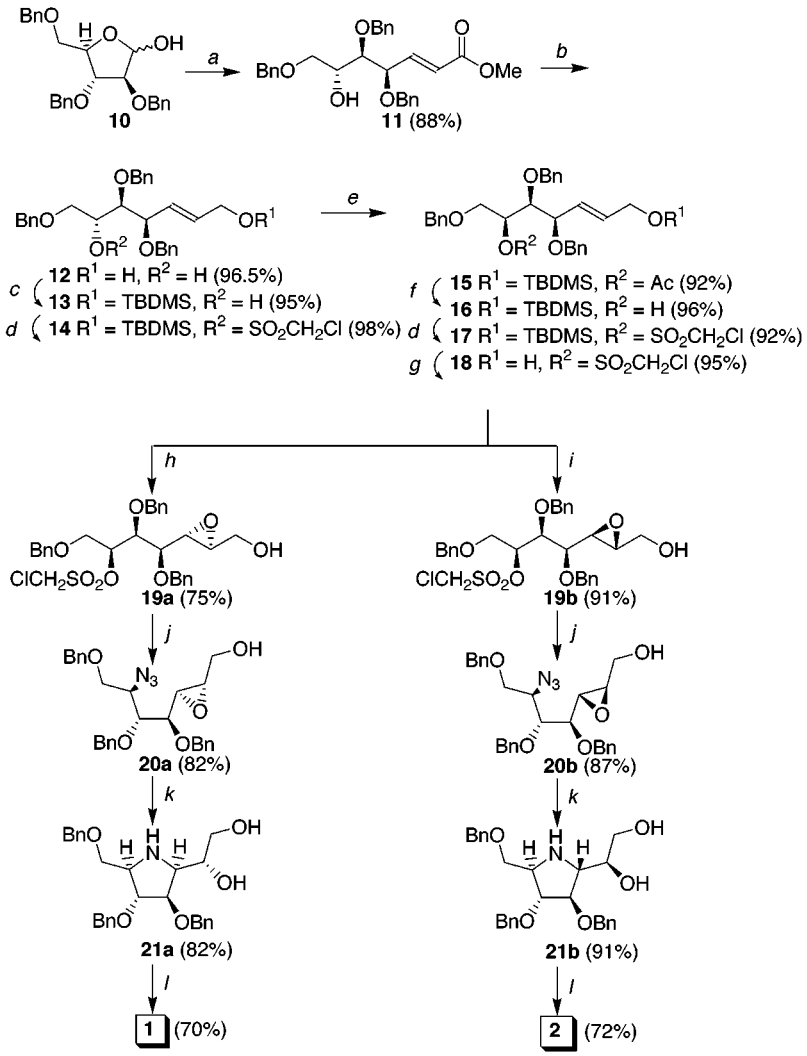
Scheme 1



a $Ph_3P{=}CHCO_2Me$ / benzene; *b* DIBAL / CH_2Cl_2; *c* TBDMSCl - Et_3N -DMAP / DMF; *d* $ClCH_2SO_2Cl$ - Pyr.; *e* CsOAc - 18-crown-6 / toluene; *f* NaOMe; *g* 1N-HCl / THF; *h* *t*-BuOOH - Ti(O-*i*-Pr)$_4$ - L-(+)-diethyltartrate - MS 4A / CH_2Cl_2; *i* *t*-BuOOH - Ti(O-*i*-Pr)$_4$ - D-(-)-diethyltartrate - MS 4A / CH_2Cl_2; *j* NaN_3 / DMF; *k* Ph_3P / THF; *l* H_2 - Pd/C / MeOH.

bicity, thereby affecting the binding affinity to the target enzyme.[12a,15]

Compound **21b** was used as a starting material for the syntheses of compounds **3**−**9**. The secondary amine function of **21b** was protected with the Boc group (**22**), of which the C-1′−C 2′ bond was cleaved using $Pb(OAc)_4$ to give the aldehyde **23**. Compound **24** obtained by reduction of the aldehyde function using DIBAL was mesylated (**25**) and treated with NaN_3 to afford **26**. During the substitution reaction, **27**[6b] was obtained as a byproduct (28%), which could be converted back to **24**. The azide group was then reduced selectively in the presence of benzyl groups and the amine was acetylated to give **29**. Protecting groups were finally removed sequentially by hydrogenolysis and acid hydrolysis to give compound **3**. When the Boc group was removed first followed by hydrogenolysis, the process took a longer reaction time (1 week) and gave a mixture of **3** and **4**.

To obtain compounds **4** and **5**, the Boc group of **29** was deprotected (**30**) followed by *N*-methylation via reductive alkylation, and the benzyl groups were hydrogenolyzed to give **4** or **5**.

The intermediate **22** was also utilized to obtain **6**−**8**. Compound **22** was converted to the 2′-acetamido compound (**37**) via tosylation (**33**), substitution reaction with NaN_3 (**34**), benzylation (**35**), and selective reduction of the azide function to an amine followed by acetylation (**37**). Sequential deprotection of the benzyl group and the Boc group resulted in **6**.

Compound **37** was treated with TFA to give **38**, which was alkylated in the same manner as for the syntheses of **4** and **5** and finally hydrogenolyzed to afford **7** and **8**. The dimer **9** was obtained as the result of self-quenching under reductive amination in the presence of ammonium acetate followed by deprotection.

Inhibition Analysis with CZE (Figure 3). The commonly used method for the assay of glycosidase reactions is based on spectrophotometric analysis using chromogenic aglycons to detect the released chromophore

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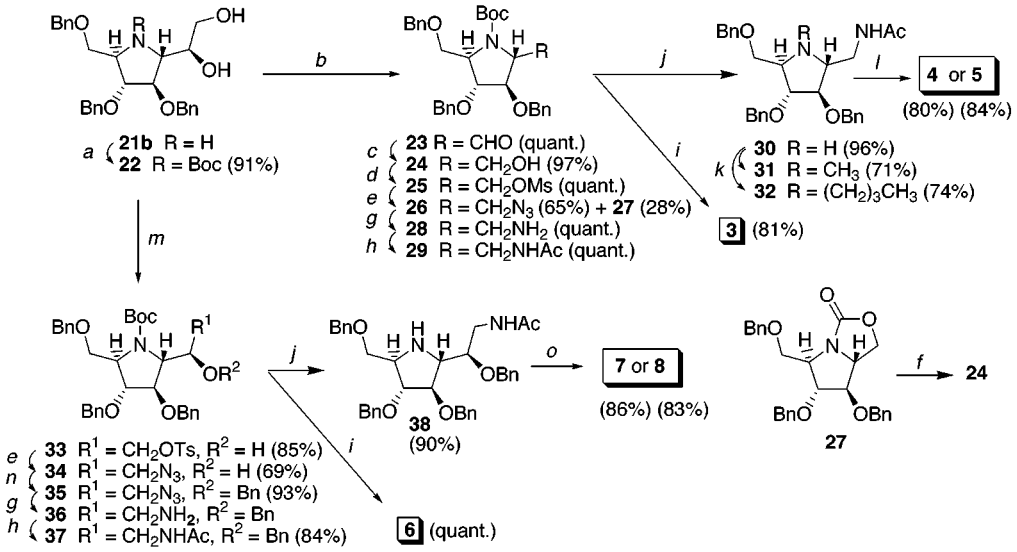
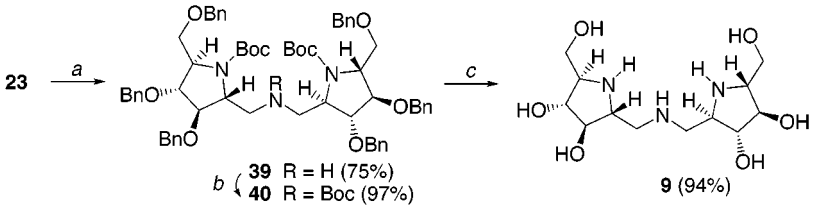
Scheme 2



a $(Boc)_2O$ - Et_3N / CH_2Cl_2; *b* $Pb(OAc)_4$ / toluene; *c* DIBAL / CH_2Cl_2; *d* MsCl - Et_3N / CH_2Cl_2; *e* NaN_3 / DMF; *f* 1) $LiAlH_4$ / THF, 2) $(Boc)_2O$ - Et_3N / CH_2Cl_2; *g* H_2 - Pd/C / MeOH; *h* Ac_2O - Pyr.; *i* 1) H_2 - Pd/C / MeOH - HCl, 2) TFA; *j* TFA; *k* CH_2O or $CH_3(CH_2)_2CHO$ - $NaBH_3CN$ / MeOH; *l* H_2 - Pd/C / MeOH - HCl; *m* TsCl - Pyr.; *n* BnBr - Ag_2O - KI / DMF; *o* 1) CH_2O or CH_3CHO - $NaBH_3CN$ / MeOH, 2) H_2 - Pd/C / MeOH - HCl.

Scheme 3



a NH_4OAc - $NaBH_3CN$ / MeOH; *b* $(Boc)_2O$ - Et_3N / CH_2Cl_2; *c* 1) Pd/C / MeOH - HCl, 2) TFA.

directly[16] or using a substrate so the product is detected with an NADH-coupled reaction[17] Recently, we have described the use of CZE in the assay of the galactosyltransferase reaction as an alternative to the radioactive analytical method.[8a] Our aim is to use the technique as a general analytical method not only for glycosyltransferases but also for glycosidases. In addition, since the method relies on the peak separation, potential ambiguity arising from the possibilities of formation of byproducts can be eliminated. The analysis of transferase reactions requires peak separation of the substrate and the product before the actual kinetic analysis; however, the analysis of glycosidase reactions should be much more straightforward because the cleaved chromophore is usually acidic and can easily be distinguished from neutral carbohydrates. To make the CZE analysis a general method, however, one has to find a condition (usually the buffer system) that gives different migration times for the substrate and the released aglycon. This is especially necessary in the case where the released aglycon does not have a specific absorbance or fluorescence.

Initially, we tried to reduce the total volume of assay solution because this is the only way to reduce the amount of enzyme and substrate used in the assay. The 96-well microtiter plate with round bottom was used for the assay, and each well was sealed with tape. Thus, the assay was examined and carried out in a total volume of as little as 20 μL containing 1.76 mU of a glycosidase such as β-*N*-acetylhexosaminidase P. The electrophoresis was carried out using 50 mM borate buffer (pH 9.2−10.2) as the electrolyte, and the progress was monitored at 37 °C. The injected volume of approximately 38.4 nL of a reaction mixture contained as little as 3.8 pmol of the substrate *p*-nitrophenyl (PNP) glycoside; thus the amount of PNP detected was less than 10^{-12} mol. The peak corresponding to the *p*-nitrophenol, which appeared at around 6.5 min in these conditions, was monitored at 405 nm.

The inhibition studies of compounds **1**−**9** against α- and β-glucosidases (from *Saccharomyces sp.* and sweet almond, respectively) were carried out. PNP glycosides of the parent sugars were used as the substrates throughout the assay. The apparent K_m and V_{max} values for each substrate were calculated from the double-reciprocal plot of standard $[1/(v - 1)]/[\text{substrate}]$ curve.[18] The K_i values were determined from a replot of the slopes of

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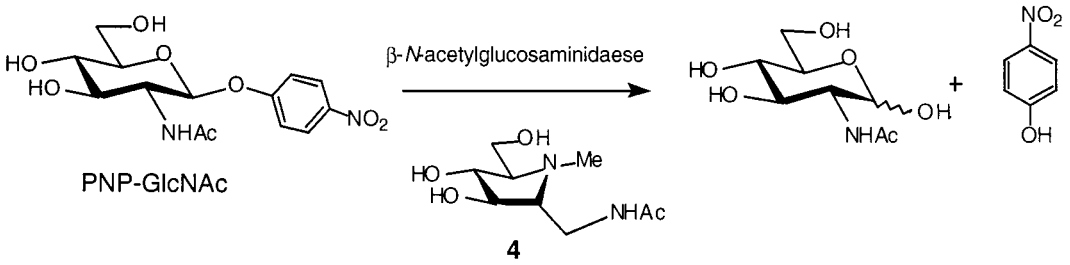

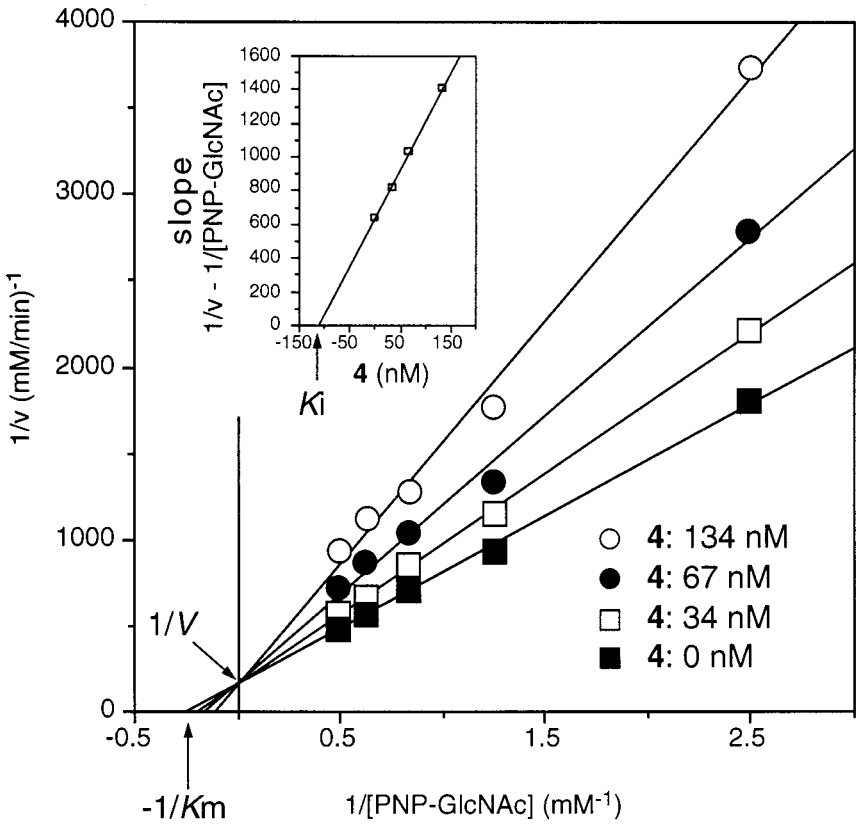

Figure 3. Double-reciprocal plots were carried out to obtain K_m and V_{max} values. Also K_i values were obtained from a replot of the slopes obtained from double-reciprocal plot. As representative of such plots, a double-reciprocal plot of $1/v$ vs 1/[S] in the β-*N*-acetylglucosaminidase reaction was shown. The concentrations of **4** were (■) 0 nM, (□) 34 nM, (●) 67 nM, and (○) 134 nM. The K_m value caluculated form the plot (■) was 4.1 mM. (Inset) Replot of slopes {[1/(v − 1)]/[PNP-GlcNAc]} vs **4**. K_i for **4** = 0.11 μM.

Lineweaver−Burk plots vs the inhibitor concentrations. The analysis of inhibition of β-*N*-acetylglucosaminidase from bovine kidney by **4** was shown in Figure 3, where the apparent K_m and V_{max} values for PNP-GlcNAc were determined to be 4.1 mM and 6.4 (μM/s)/mg, and the K_i value of the competitive inhibitor **4** was determined to be 0.11 μM. Kinetic parameters thus obtained for the enzymes examined with other inhibitors were shown in Table 2.

As shown in Table 2, remarkable inhibitory specificities were observed for the compounds synthesized against glycosidases. Compounds **1** and **2** were designed and synthesized previously to inhibit β- and α-glucosidase, respectively. In our design, the side chain at position 2 in each compound is modified to mimic the aglycon part of the substrate for each enzyme. However, compound **2** showed potent inhibitory activities against both α- and β-glucosidases, whereas **1** was shown to be a weak inhibitor against both enzymes.[19] The differences in the inhibitory activities may be explained by their conformational differences as suggested by the ^{1}H NMR analysis (Table 1). The *N*-methylated derivatives of **1** and **2** were also prepared but exhibited weaker inhibitory activities against both enzymes (IC_{50} > 1 mM).

Replacement of the 2′-hydroxyl group of **6** with the NHAc group had no impact on the activity toward glucosidases. It showed an inhibitory activity identical to that of **2**, but alkylation of the ring nitrogen had negative effects.

Compounds **3**−**5**, which lack one carbon and one hydroxyl group, showed no inhibitory activity against β-glucosidase up to 500 μM and only very weak inhibition against α-glucosidase. Instead, these compounds were found to be extremely potent inhibitors of *N*-acetylglucosaminidase from bovine kidney[13] and human placenta (A and P). Also, methylation of the ring nitrogen im-

(19) Previously, inhibition assays of aza sugars **1** and **2** using a photometric assay system were carried out, which showed good agreement with the results obtained by CZE.

Table 2. Inhibition Assay Results of Compounds 1−9

	K_i (μM)				
compd	α-glucosidase[a] *Saccaromyces sp*	β-glucosidase[b] sweet almond	β-*N*-acetylglucosaminidase bovine kidney[c]	β-*N*-acetylhexosaminidase human placenta A[d]	β-*N*-acetylhexosaminidase p[e]
1[f]	330	50	−[h]	−	−
2[f]	28	2.6	−	−	−
3	380	*[g]	2.9×10^{-1}	2.2×10^{-1}	2.6×10^{-1}
4	ni	ni	1.1×10^{-1}	1.4×10^{-1}	8.0×10^{-2}
5	ni	ni	1.3	5.1×10^{-1}	2.4×10^{-1}
6	*	2.2	*	−	−
7	*	45	*	−	−
8	ni	120	ni[i]	−	−
9	53	37	−	−	−

[a] $K_m = 0.30$ mM, $V_{max} = 0.7$ (μM/s)/mg. [b] $K_m = 3.2$ mM, $V_{max} = 3.2$ (μM/s)/mg. [c] $K_m = 4.1$ mM, $V_{max} = 6.4$ (μM/s)/mg. [d] $K_m = 2.5$ mM, $V_{max} = 2.1$ (μM/s)/mg. [e] $K_m = 2.8$ mM, $V_{max} = 2.3$ (μM/s)/mg. [f] Preliminary assay result using photometric assay gave K_i values: 430 and 18 μM for compound **1** and 7.2 and 7.6 μM for compound **2** toward α-glucosidase and β-glucosidase, respectively. See also refs 6a and 19. [g] *: poor inhibitor with IC_{50} above 0.5 mM. [h] −: not tested. [i] ni: not inhibitor.

proved the activity; however, a decrease in activity was observed with a longer *N*-alkyl substituent.

It was revealed that an acetamido group is necessary at the C-1′ position of the five membered iminocyclitols in order to inhibit *N*-acetylglucosaminidase. It was also revealed that, in the case of inhibition of glucosidases, an OH group at C-1′ is required, perhaps to mimic the OH-2 group of glucose. The dimeric derivative **9**, however, has an inhibitory activity at the same level as **1** and **7** toward the β-glucosidase and also is as effective as **2** toward the α-glucosidase despite the absence of an OH group to mimic the OH-2 group of glucose. The additional moieties may have been used to circumvent the lack of OH group or to give additional binding affinity.[20]

Conclusion

We have synthesized a series of five-membered iminocyclitols starting from a single starting material through the Wittig reaction, Sharpless epoxidation, and double inversion reactions using the (chloromethyl)sulfonyl group as a leaving group. This versatile synthetic strategy provides a useful route to a number of heterocycles as potential inhibitors of glycosidases.

Through this study, it was suggested that the differences in the conformation and the orientation of side chains and OH groups might be the main reason for the observed higher inhibitory activity of the 2(*R*),5(*R*)-isomer **2** compared to the 2(*S*),5(*R*)-isomer **1**. Using **2** as a starting material, a number of iminocyclitols were synthesized and tested as glycosidase inhibitors using capillary electrophoresis, and the results showed remarkable specificities toward several glycosidases. Among such compounds, **6** and **3**−**5** were shown to be potent inhibitors of β-glucosidase and β-*N*-acetylglucosaminidase, respectively.

This study suggests that the amine function of **6** or the OH function of **24** may be used to make conjugates with various aglycon groups to prepare inhibitors with improved specificities. In addition, the observation that *N*-methylation of **3** enhances the inhibition of a specific enzyme provides a new direction in the field.

Experimental Section

General Methods for the Synthesis. Dried solvents were used for all reactions. Solutions were evaporated under reduced pressure at a bath temperature not exceeding 50 °C. Column chromatography was performed on silica gel or Iatro Beads (60 μm). Gel permeation chromatography was performed using Bio Gel P-2. Melting points were measured with a melting point apparatus and are uncorrected. Optical rotations were measured in a 1.0 dm tube with a polarimeter at 24 ± 1 °C. ^{1}H NMR (270 MHz) spectra were recorded on solutions in $CDCl_3$ or D_2O using Me_4Si (δ 0.00) or DOH (δ 4.80) as the internal standard. ^{13}C NMR (67.5 MHz) spectra were recorded on solutions in $CDCl_3$ or D_2O using Me_4Si (δ 0.00), $CDCl_3$ (77.00), CD_3CN (δ 118.20), or CD_3OD (δ 49.80) as the internal standard. Some key compounds were measured with a 400 MHz spectrometer as indicated. Only partial assignments were reported. The FAB and HR FAB mass spectra were obtained with glycerol and 3-nitrobenzyl alcohol as the matrix. MALDITOF mass spectra were recorded with 2,5-dihydroxybenzoic acid as the matrix.

Methyl (4*R*,5*R*,6*R*)-6-Hydroxy-4,5,7-tris(benzyloxy)-2(*E*)-heptenoate (11). A solution of 2,3,5-tri-*O*-benzylarabinofuranose (**10**) (420 mg, 1.0 mmol) and methyl (triphenylphosphoranylidene)acetate (435 mg, 1.3 mmol) in benzene (10 mL) was heated under reflux for 12 h. After cooling, the solvent was removed in vacuo and the crude mixture was purified by flash column chromatography (3:1 hexane−EtOAc). The *E*-isomer (**11**) was obtained as a major product (419 mg, 88%), $[\alpha]_D$ −4.1° (*c* 2.2, $CHCl_3$) along with the *Z*-isomer (47 mg, 10%).

^{1}H NMR for **11** ($CDCl_3$): δ 7.40−7.20 (15H, m), 7.01 (1H, dd, J = 5.6, 15.8 Hz), 6.11 (1H, dd, J = 1.3, 15.8 Hz), 4.60−4.40 (6H, m), 4.29 (1H, m), 3.95 (1H, m), 3.74 (3H, s), 3.60 (1H, m), 3.60 (2H, m), and 2.67 (1H, d, J = 4.3 Hz). ^{13}C NMR ($CDCl_3$): δ 166.27, 145.48, 122.75, 79.84, 77.81, 74.14, 73.35, 71.86, 70.64, 70.05, and 51.57. MALDITOF MS, *m*/*z*: 499 [M + Na]$^+$. Anal. Calcd for $C_{29}H_{32}O_6$: C, 73.09; H, 6.77. Found: C, 72.93; H, 6.85.

(4*R*,5*R*,6*R*)-4,5,7-Tris(benzyloxy)-2(*E*)-heptene-1,6-diol (12). To a solution of compound **11** (3.9 g, 8.19 mmol) in dry CH_2Cl_2, was added a 1 M solution of DIBAL (24.6 mL, 3 equiv) at 0 °C. The reaction mixture was stirred at the temperature for 1.5 h. MeOH (4 mL) was added at 0 °C and the temperature was raised to room temperature (rt). Saturated NaCl (8 mL) was added and the mixture was diluted with Et_2O (200 mL). $MgSO_4$ (21 g) was added, and the whole mixture was stirred for 1 h and then filtered through a Celite pad. The solvent was removed in vacuo, and the crude mixture was purified by column chromatography (1:1 hexane−EtOAc) to give the alcohol **12** (3.54 g, 96.5%), $[\alpha]_D$ −22.6° (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.83 (1H, ddd, J = 4.6, 4.8, 15.8 Hz), 5.72 (1H, dd, J = 6.9, 15.8 Hz), 4.60−4.40 (6H, m), 4.06 (2H, m), 4.06 (1H, m), 3.98 (1H, m), and 3.60 (2H, m). ^{13}C NMR ($CDCl_3$): δ 133.48, 127.87, 80.38, 79.12, 73.91, 73.33, 70.87, 70.73, 70.33, and 62.66. MALDITOF MS, *m*/*z*: 471 [M + Na]$^+$. Anal. Calcd for $C_{28}H_{32}O_5$: C, 74.98; H, 7.19. Found: C, 74.56; H, 7.23.

(20) MacGregor, E. A.; Svensson, B. *Biochem. J.* **1989**, *259*, 145−152.

(4*R*,5*R*,6*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-4,5,7-tris-(benzyloxy)-2(*E*)-hepten-6-ol (13). Compound **12** (5.17 g, 11.54 mmol) was dissolved in DMF (100 mL); to this solution was added TBDMSCl (2.09 g, 13.9 mmol), Et_3N (4 mL, 28.85 mmol), and DMAP (50 mg). The reaction mixture was stirred at rt for 1 h. The mixture was diluted with EtOAc, and the organic layer was washed with H_2O and brine and dried with Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (10:1 hexane−EtOAc) to give **13** (6.15 g, 95%) as a colorless oil, $[\alpha]_D$ −18.2° (*c* 1.05, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.75 (2H, bs, H-2), 4.60−4.40 (6H, bs), 4.12 (2H, m), 4.04 (1H, m), 3.93 (1H, m), 3.55 (1H, m), 3.55 (2H, m), 2.74 (1H, d, J = 4.9 Hz), 0.89 (9H, s), and 0.08 (6H, s). ^{13}C NMR ($CDCl_3$): δ 134.07, 126.38, 80.70, 79.16, 74.05, 73.32, 70.91, 70.53, 70.32, 62.98, 25.09, 18.35, and −5.23.

Anal. Calcd for $C_{34}H_{46}O_5Si$: C, 72.56; H, 8.24. Found: C, 72.47; H, 8.35.

(4*R*,5*R*,6*R*)-1-[(*tert*-Butyldimethylsilyl)oxy]-6-[((chloromethyl)sulfonyl)oxy]-4,5,7-tris(benzyloxy)-2(*E*)-heptene (14). A solution of compound **13** (480 mg, 0.85 mmol) and chloromethanesulfonyl chloride (91 mL, 1.0 mmol) in pyridine (2 mL) was stirred at rt for 0.5 h; then the mixture was diluted with EtOAc, washed with H_2O and brine, and dried over Mg_2SO_4. After removal of the solvent, the residue was purified by column chromatography (10:1 hexane−EtOAc) to give **14** (564 mg, 98%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.83 (1H, ddd, J = 3.3, 4.0, 15.7 Hz), 5.73 (1H, dd, J = 7.3, 15.7 Hz), 5.07 (1H, dd, J = 3.0, 7.6 Hz), 4.69 (2H, s), 4.61 (1H, AB, J = 12.2 Hz), 4.58 (1H, AB, J = 11.5 Hz), 4.49 (2H, bs), 4.49 (1H, AB, J = 12.2 Hz), 4.36 (1H, AB, J = 11.5 Hz), 4.18 (2H, d, J = 3.3 Hz), 4.00 (1H, dd, J = 5.3, 7.3 Hz), 3.89 (1H, dd, J = 3.3, 5.3 Hz), 3.86 (1H, dd, J = 3.0, 11.6 Hz), 3.78 (1H, dd, J = 7.6, 11.6 Hz), 0.92 (9H, s), and 0.09 (6H, s). ^{13}C NMR ($CDCl_3$): δ 135.16, 125.48, 84.96, 81.71, 79.64, 75.02, 73.42, 70.64, 68.88, 62.73, 54.05, 25.91, 18.36, and −5.27. Anal. Calcd for $C_{35}H_{47}O_7ClSSi$: C, 62.25; H, 7.02. Found: C, 62.22; H, 7.04.

(4*R*,5*R*,6*S*)-6-Acetoxy-1-[(*tert*-butyldimethylsilyl)oxy]-4,5,7-tris(benzyloxy)-2(*E*)-heptene (15). The stirred mixture of compound **14** (2.40 g, 3.56 mmol), CsOAc (3.40 g, 5 equiv), and 18-crown-6 (950 mg, 1 equiv) was heated under reflux in toluene (80 mL) for 12 h. After cooling to rt, the reaction mixture was washed with H_2O and brine and dried over Na_2SO_4, and the solvent was removed in vacuo. The crude material was purified by column chromatography (10:1 hexane−EtOAc) to afford **15** (1.98 g, 92%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.78 (1H, ddd, J = 3.6, 3.6, 15.5 Hz), 5.68 (1H, dd, J = 6.9, 15.5 Hz), 5.20 (1H, ddd, J = 4.6, 5.3, 5.6, Hz), 4.82 (1H, AB, J = 11.6 Hz), 4.62 (1H, AB, J = 11.6 Hz), 4.60 (1H, AB, J = 11.9 Hz), 4.42 (1H, AB, J = 11.9 Hz), 4.33 (1H, AB, J = 11.9 Hz), 4.31 (1H, AB, J = 11.9 Hz), 4.20 (2H, d, J = 3.6 Hz), 4.00 (1H, dd, J = 5.9, 6.9 Hz), 3.77 (1H, dd, J = 4.6, 5.9 Hz), 3.57 (1H, dd, J = 5.3, 9.9 Hz), 3.45 (1H, dd, J = 5.6, 9.9 Hz), 2.00, (3H, s), 0 92 (9H, s), and 0.09 (6H, s). ^{13}C NMR ($CDCl_3$): δ 170.13, 134.63, 126.09, 80.34, 79.89, 75.31, 72.99, 72.22, 70.46, 67.93, 62.97, 25.90, 21.06, 18.35, and −5.23. Anal. Calcd for $C_{36}H_{48}O_6Si$: C, 71.49; H, 8.00. Found: C, 71.44; H, 8.09.

(4*R*,5*R*,6*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]-4,5,7-tris-(benzyloxy)-2(*E*)-hepten-6-ol (16). Compound **15** (120 mg, 0.2 mmol) was dissolved with MeOH (2 mL) and treated with a 1 M solution of NaOMe (600 mL, 3 equiv) at rt for 1 h. The solvent was removed, and the residue was diluted with EtOAc, washed with H_2O and brine, and dried over Na_2SO_4. Purification by column chromatography using 5:1 hexane−EtOAc as an eluent gave **16** (107 mg, 96%), $[\alpha]_D$ −5.4° (*c* 1.13, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.88 (1H, dd, J = 4.3, 15.5 Hz), 5.72 (1H, ddd, J = 1.7, 7.6, 15.5 Hz), 4.87 (1H, AB, J = 11.2 Hz), 4.63 (1H, AB, J = 11.9 H), 4.55 (1H, AB, J = 11.2 Hz), 4.46 (1H, AB, J = 11.9 Hz), 4.41 (1H, AB, J = 11.9 Hz), 4.37 (1H, AB, J = 11.9 Hz), 4.22 (2H, dd, J = 1.7, 4.3 Hz), 4.14 (1H, dd, J = 6.3 7.6 Hz), 3.91 (1H, dddd, J = 2.6, 5.9, 6.3 6.9 Hz), 3.60 (1H, dd, J = 2.6, 6.3 Hz), 3.45 (1H, dd, J = 5.9, 9.6 Hz), 3.40 (1H, dd, J = 6.3, 9.6 Hz), 2.49 (1H, d, J = 6.9 Hz), 0 92 (9H, s), and 0.09 (6H, s). ^{13}C NMR ($CDCl_3$): δ 134.68, 126.47, 81.26, 80.45, 75.06, 73.21, 71.16, 70.55, 70.03, 62.97, 25.90, 18.37, and −5.23. Anal. Calcd for $C_{34}H_{46}O_5Si$: C, 72.56; H, 8.24. Found: C, 72.56; H, 8.34.

(4*R*,5*R*,6*S*)-1-[(*tert*-Butyldimethylsilyl)oxy]-6-[((chloromethyl)sulfonyl)oxy]-4,5,7-tris(benzyloxy)-2(*E*)-heptene (17). A mixture of compound **16** (1.82 g, 3.23 mmol) and chloromethanesulfonyl chloride (342 mL, 3.83 mmol) in pyridine (14 mL) was stirred at rt for 0.5 h. The mixture was diluted with EtOAc, washed with H_2O and brine, and dried over Na_2SO_4. After removal of the solvent, the residue was purified by column chromatography (15:1 hexane−EtOAc) to give **17** (1.99 g, 92%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.92 (1H, dd, J = 4.3, 15.5 Hz), 5.75 (1H, dd, J = 7.6, 15.5 Hz), 4.97 (1H, m), 4.70−4.20 (8H, m), 4.20 (2H, m), 4.04 (1H, dd, J = 4.6, 7.6 Hz), 3.70 (1H, dd, J = 4.6, 5.9 Hz), 3.61 (2H, m), 0 92 (9H, s), and 0.09 (6H, s). ^{13}C NMR ($CDCl_3$): δ 134.66, 125.07, 84.06, 79.93, 78.65, 75.13, 73.40, 70.42, 69.24, 62.84, 54.18, 25.93, 18.38, and −5.23. Anal. Calcd for $C_{35}H_{47}O_7ClSSi$: C, 62.25; H, 7.02. Found: C, 62.13; H, 7.04.

(4*R*,5*R*,6*S*)-6-[((Chloromethyl)sulfonyl)oxy]-4,5,7-tris-(benzyloxy)-2(*E*)-hepten-1-ol (18). A solution of compound **17** (1.0 g, 1.48 mmol) in THF (5 mL) was treated with 1N HCl (5 mL) at rt for 8 h. The THF was removed, the mixture was diluted with EtOAc, and the organic layer was washed with aqueous Na_2CO_3, H_2O, and brine. After concentration, the residue was purified by column chromatography (3:1 hexane−EtOAc) to aford **18** (790 mg, 95%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.95 (1H, ddd, J = 5.0, 5.0, 15.8 Hz), 5.65 (1H, dd, J = 7.6, 15.8 Hz), 4.99 (1H, ddd, J = 3.0, 5.0, 6.9 Hz), 4.71 (1H, AB, J = 11.6 Hz), 4.67 (1H, AB, J = 11.9 Hz), 4.66 (1H, AB, J = 11.6 Hz), 4.56 (1H, AB, J = 11.6 Hz), 4.50 (1H, AB, J = 11.9 Hz), 4.44 (1H, AB, J = 11.6 Hz), 4.37 (1H, AB, J = 11.6 Hz), 4.29 (1H, AB, J = 11.6 Hz), 4.12 (2H, bs), 4.04 (1H, dd, J = 5.0, 7.6 Hz), 3.68 (1H, dd, J = 5.0, 5.0 Hz), 3.66 (1H, dd, J = 7.0, 11.6 Hz), 3.56 (1H, dd, J = 3.0, 11.6 Hz), and 1.70 (1H, bd). ^{13}C NMR ($CDCl_3$): δ 135.29, 126.25, 83.88, 79.32, 78.82, 74.84, 73.39, 70.60, 69.47, 62.63, and 54.25. Anal. Calcd for $C_{29}H_{33}O_7ClS$: C, 62.08; H, 5.93. Found: C, 61.83; H, 5.96.

(2*S*,3*R*,4*S*,5*R*,6*S*)-6-[((Chloromethyl)sulfonyl)oxy]-2,3-epoxy-4,5,7-tris(benzyloxy)-2(*E*)-hepten-1-ol (19a). A solution of Ti(O-*i*-Pr)$_4$ (365 μL, 1.26 mmol) and L-(+)-diethyltartrate (210 μL, 1.26 mmol) in CH_2Cl_2 (4 mL) was stirred at −25 °C for 0.5 h in the presence of MS 4A (activated at 150 °C by microwave oven). To this mixture was added a solution of compound **18** (350 mg, 0.63 mmol) in CH_2Cl_2 (1 mL), and the mixture was stirred at the temperature for 0.5 h. A solution of *t*-BuOOH (5 M, 365 μL, 1.89 mmol) was added, and the mixture was stirred at the same temperature for 48 h. A solution of 10% tartaric acid was added at −25 °C and stirred for 0.5 h at the temperature and for 0.5 h at rt. The solution was filtered through a Celite pad, the filtrate was washed with H_2O and brine, and the solvent was removed. The residue was dissolved in Et_2O (20 mL) and stirred with 10% NaOH solution at 0 °C for 0.5 h. The organic layer was washed with H_2O and brine, dried, and concentrated. The crude mixture was purified by column chromatography (1:1 hexane−EtOAc) to afford **19a** (270 mg, 75%) as a colorless oil.

^{1}H NMR ($CDCl_3$): δ 7.35−7.20 (15H, m), 5.03 (1H, ddd, J = 2.6, 6.6, 6.6 Hz), 4.75−4.35 (8H, m), 3.90 (1H, dd, J = 3.3, 6.6 Hz), 3.72 (1H, dd, J = 2.3, 11.5 Hz), 3.69 (1H, dd, J = 2.6, 12.9 Hz), 3.57 (1H, dd, J = 2.3, 11.5 Hz), 3.44 (1H, dd, J = 2.6, 12.9 Hz), 3.37 (1H, dd, J = 3.3, 5.6 Hz), 3.11 (1H, dd, J = 2.3, 5.6 Hz), and 3.00 (1H, m). ^{13}C NMR ($CDCl_3$): δ 83.69, 77.77, 76.62, 75.01, 73.35, 72.98, 68.63, 60.97, 58.06, 54.07, and 53.87. MALDITOF MS, *m*/*z*: 600 $[M + Na]^+$.

(2*S*,3*R*,4*S*,5*R*,6*R*)-6-Azido-2,3-epoxy-4,5,7-tris(benzyloxy)-2(*E*)-hepten-1-ol (20a). Compound **19a** (225 mg, 0.39 mmol) was dissolved with DMF (3 mL) and treated with NaN_3 (51 mg, 0.78 mmol) at 70 °C for 0.5 h. After cooling to rt, the mixture was diluted with EtOAc, washed with H_2O and brine, and dried and the solvent was removed. The residue was

purified by column chromatography (2:1 hexane−EtOAc) to give **20a** (157 mg, 82%) as a colorless oil.

IR 2200 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 4.70−4.40 (6H, m), 3.80 (2H, m), 3.70 (3H, m), 3.60 (1H, m), 3.46 (1H, dd, J = 2.6, 6.3 Hz), 3.08 (1H, dd, J = 2.0, 6.3 Hz), and 2.97 (1H, m). ^{13}C NMR ($CDCl_3$): δ 78.20, 77.61, 74.79, 73.46, 73.33, 69.54, 61.10, 60.79, 58.58, and 54.20.

(1′*R*,2*S*,3*R*,4*R*,5*R*)-3,4-Bis(benzyloxy)-5-[(benzyloxy)-methyl]-2-[1′,2′-dihydroxyethyl]pyrrolidine (21a). A solution of compound **20a** (175 mg, 0.36 mmol) and triphenylphosphine (113 mg, 0.43 mmol) in THF (5 mL) containing ca. 0.5% H_2O was stirred at rt for 48 h. After removal of the solvent, the residual mixture was purified by column chromatography (20:1 $CHCl_3$−MeOH) to give **21a** (137 mg, 82%).

^1H NMR ($CDCl_3$): δ 7.35−7.20 (15H, m), 4.65−4.30 (6H, m), 4.10 (1H, bd, J = 4.6 Hz), 3.90 (1H, m), 3.90 (1H, m), 3.70 (2H, m), 3.50 (2H, m), 3.30 (1H, m), 3.26 (1H, dd, J = 4.6, 6.9 Hz). ^{13}C NMR ($CDCl_3$): δ 84.33, 83.94, 73.14, 71.63, 71.25, 70.82, 70.80, 65.34, 63.87, and 62.86.

(2*R*,3*S*,4*R*,5*R*,1′*R*)-5-(Hydroxymethyl)-3,4-dihydroxy-2-(1,2-dihydroxyethyl)pyrrolidine (1). A solution of **21a** (58 mg, 0.13 mmol) in MeOH (1 mL) was stirred with Pd/C under a H_2 atmosphere at rt for 48 h. The crude material, obtained after removal of the catalyt and solvent, was purified by column chromatography (6:4:1 $CHCl_3$−MeOH−H_2O) to afford **1** (17 mg, 70%); $[\alpha]_D$ +11° (*c* 0.1, D_2O).

^1H NMR (D_2O): δ 4.03 (1H, dd, J = 1.2, 3.9 Hz), 3.80 (1H, dd, J = 1.2, 3.5 Hz), 3.77 (1H, ddd, J = 3.0, 6.5, 9.3 Hz), 3.65 (1H, dd, J = 3.0, 12.1 Hz), 3.61 (1H, dd, J = 5.5, 11.6 Hz), 3.56 (1H, dd, J = 6.4, 11.6 Hz), 3.49 (1H, dd, J = 6.5, 12.1 Hz), 3.09 (1H, dd, J = 3.9, 9.3 Hz), and 2.97 (1H, ddd, J = 3.5, 5.5, 6.4 Hz). ^{13}C NMR (D_2O): δ 79.24, 77.47, 69.84, 67.10, 64.36, 62.44, and 62.07. MALDITOF MS, *m*/*z*: 194 $[M + H]^+$, 216 $[M + Na]^+$.

(2*R*,3*S*,4*S*,5*R*,6*S*)-6-[((Chloromethyl)sulfonyl)oxy]-2,3-epoxy-4,5,7-tris(benzyloxy)-2(*E*)-hepten-1-ol (19b). Compound **19b** was synthesized using **18** (250 mg, 0.43 mmol), a solution of Ti(O-*i*-Pr)$_4$ (260 μL, 0.86 mmol), D-(−)-diethyltartrate (150 μL, 0.86 mmol), a solution of *t*-BuOOH (5 M, 260 μL, 1.29 mmol), a solution of 10% tartaric acid, MS 4A, and CH_2Cl_2 (4 mL, total volume) as described for the synthesis of compound **19a**. Yield of **19b**: 235 mg, 91%, a colorless oil.

^1H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 5.09 (1H, ddd, J = 2.3, 6.3, 6.3,Hz), 4.80−4.30 (8H, m), 3.78 (1H, dd, J = 3.3, 6.3 Hz), 3.50 (4H, m), 3.30 (2H, m), and 2.98 (1H, m).

(2*R*,3*S*,4*S*,5*R*,6*R*)-6-Azido-2,3-epoxy-4,5,7-tris(benzyloxy)-2(*E*)-hepten-1-ol (20b). Compound **20b** was synthesized using **19b** (195 mg, 0.34 mmol), NaN_3 (44 mg, 0.68 mmol) and DMF (3 mL) as described for the synthesis of **20a**. Yield of **20b**: 144 mg, 87%, a colorless oil; $[\alpha]_D$ −29.3° (*c* 1, $CHCl_3$).

IR 2170 cm^{-1}. ^1H NMR ($CDCl_3$): δ 7.40−7.20 (15H, m), 4.80−4.40 (6H, m), 3.80 (1H, m), 3.70 (3H, m), 3.66 (1H, dd, J = 2.6, 7.3 Hz), 3.50 (1H, m), 3.29 (2H, m), and 2.92 (1H, m). ^{13}C NMR ($CDCl_3$): δ 79.46, 78.74, 74.18, 73.35, 72.27, 69.20, 61.06, 60.94, 56.50, and 54.22. FAB MS, *m*/*z*: 490 $[M + H]^+$.

(2*R*,3*S*,4*R*,5*R*,1′*R*)-5-[(Benzyloxy)methyl]-3,4-bis(benzyloxy)-2-(1,2-dihydroxyethyl)pyrrolidine (21b). Compound **21b** was synthesized using **20b** (3.44 g, 7.03 mmol), triphenylphosphine (2.21 g, 8.44 mmol) and THF (36 mL), as described for the synthesis of **21a**. Yield of **21b**: 2.98 g, 91%.

^1H NMR ($CDCl_3$): δ 7.36−7.25 (15H, m), 4.59−4.43 (6H, m), 4.10 (1H, dd, J = 3.6, 4.6 Hz), 3.93 (1H, dd, J = 3.6, 4.0 Hz), 3.70 (1H, 2H, m), 3.37 (1H, m), 3.49 (2H, m), and 3.26 (1H, dd, J = 4.6, 5.0 Hz). ^{13}C NMR ($CDCl_3$): δ 85.50, 85.39, 73.17, 71.88, 71.77, 70.44, 69.47, 65.28, 64.93, and 61.78. MALDITOF MS, *m*/*z*: 464 $[M + H]^+$. Anal. Calcd for $C_{28}H_{33}NO_5$: C, 72.55; H, 7.17; N, 3.02. Found: C, 72.48; H, 7.17; N, 3.03.

(1′*R*,2*S*,3*R*,4*R*,5*R*)-3,4-Dihydroxy-2-(1,2-dihydroxyethyl)-5-(hydroxymethyl)pyrrolidine (2). Compound **2** was obtained by hydrogenolysis of **21b** (100 mg, 0.22 mmol), which was carried out as described for the synthesis of **1** in MeOH (1 mL), to afford **2** (30 mg, 72%); $[\alpha]_D$ +25.6° (*c* 0.3, D_2O).

^1H NMR (D_2O): δ 3.94 (1H, dd, J = 6.5, 7.3 Hz), 3.73 (1H, dd, J = 6.5, 7.5 Hz), 3.64 (1H, ddd, J = 3.3, 5.8, 6.9 Hz), 3.58 [(1H, dd, J = 3.3, 11.8 Hz), 3.55 (1H, dd, J = 5.8, 11.8 Hz)], 3.50 (1H, dd, J = 6.2, 11.8 Hz), 3.47 (1H, dd, J = 6.9, 11.8 Hz), 2.92 (1H, ddd, J = 5.8, 6.2, 7.5 Hz), and 2.89 (1H, dd, J = 5.8, 7.3 Hz). ^{13}C NMR (D_2O): δ 78.27, 78.09, 73.14, 63.77, 62.35, 61.97, and 61.93. MALDITOF MS, *m*/*z*: 194 $[M + H]^+$, 216 $[M + Na]^+$.

(1″*R*,2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]-2-(1,2-dihydroxyethyl)pyrrolidine (22). To a solution of **21** (336 mg, 0.73 mmol) in CH_2Cl_2 (7 mL) and Et_3N (121 μL, 0.87 mmol) was added $(Boc)_2O$ (412 μL, 1.74 mmol) at 0 °C, and the mixture was stirred at rt for 20 h. The reaction mixture was diluted with CH_2Cl_2 and washed with 10% citric acid, saturated $NaHCO_3$, and water, dried over $MgSO_4$, and concentrated. The resulting material was purified on a column of silica gel eluted with hexane−EtOAc (3:1) to afford **22** (374 mg, 91%); $[\alpha]_D$ = −31.3° (*c* 1.0, $CHCl_3$).

^1H NMR ($CDCl_3$): δ 7.25−7.31 (15H), 4.54 (2H, s), 4.49 (2H, s), 4.76 (1H, AB, J = 10.2 Hz), 4.26 (1H, s), 4.21 (1H, s), 4.18 (1H, AB, J = 10.2 Hz), 4.03 (1H, dd, J = 4.10, 10.6 Hz), 3.96 (1H, d, J = 7.9 Hz), 3.83 (1H, dd, J = 4.0, 8.6 Hz), 3.78−3.86 (1H, m), 3.74 (1H, dd, J = 5.9, 8.2 Hz), 3.54−3.60 (2H, m), 3.51 (1H, dd, J = 8.6, 10.6 Hz), 3.25 (1H, d, J = 9.6 Hz), and 1.41 (9H, s). ^{13}C NMR ($CDCl_3$): δ 156.08, 83.25, 83.02, 81.47, 73.37, 71.59, 71.47, 71.59, 68.36, 65.73, 63.92, 62.89, and 28.59. Anal. Calcd for $C_{33}H_{41}NO_7$: C, 70.32; H, 7.33; N, 2.48. Found: C, 70.36; H, 7.36; N, 2.48.

(2*S*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine-2-carbaldehyde (23). To a solution of compound **22** (740 mg, 1.3 mmol) in toluene (13 mL) was added $Pb(OAc)_4$ (959 mg, 2.0 mmol). The reaction mixture was stirred for 1.5 h at rt, then diluted with Et_2O, filtered through a Celite pad, and concentrated. The resulting residue was purified on a column of silica gel eluted with 8:1 hexane−EtOAc to afford **23** (684.8 mg, ∼quantitative); $[\alpha]_D$ −56.6° (*c* 1.0, $CHCl_3$).

NMR ($CDCl_3$) analysis showed that **23** existed as two conformational isomers designated to be "major" and "minor". Major/minor = 10/7. ^1H NMR: (for a major component) δ 9.39 (1H, d, J = 2.6 Hz), 7.19−7.31 (15H, m), 4.42−4.65 (6H, m), 4.34 (1H, t, J = 4.6 Hz), 4.20 (1H, d, J = 2.6 Hz), 4.18 (1H, s), 4.03 (1H, s), 3.94 (1H, dd, J = 4.6, 8.9 Hz), 3.62 (1H, dd, J = 4.6, 8.9 Hz), and 1.42 (9H, s); (for a minor component) δ 9.45 (1H, d, J = 2.0 Hz), 7.19−7.31 (15H, m), 4.42−4.65 (6H, m), 4.34 (1H, d, J = 2.0 Hz), 4.19 (1H, s), 4.12−4.18 (1H, m), 4.07 (1H, s), 3.77 (1H, dd, J = 4.3, 8.9 Hz), 3.58 (1H, dd, J = 4.6, 8.9 Hz), and 1.44 (9H, s). ^{13}C NMR ($CDCl_3$): (for a major component) δ 200.75, 153.74, 84.65, 81.15, 81.02, 73.06, 71.72, 71.64, 71.09, and 70.87, 70.42, 67.69, 62.84, and 28.26; (for a minor component) δ 201.07, 153.74, 83.20, 81.33, 79.75, 73.06, 71.72, 71.64, 71.09, 70.87, 71.01, 68.16, 63.13, and 28.30.

(2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]-2-(hydroxymethyl)pyrrolidine (24). To a solution of **23** (1.17 g, 2.2 mmol) in CH_2Cl_2 (15 mL) cooled to 0 °C was added 0.98 M diisobutylaluminum hydride (DIBAL) in hexane (2.7 mL, 2.6 mmol), and the resulting mixture was stirred for 0.5 h until completion. MeOH (1 mL) was added to the mixture and was stirred at rt for 0.5 h. The mixture was diluted with Et_2O, washed with brine, dried over $MgSO_4$, and concentrated to give a syrup, which was purified by flash column chromatography using 4:1 hexane−EtOAc as the eluent to give **24** (1.14 g, 97%); $[\alpha]_D$ −42° (*c* 1.0, $CHCl_3$). Major/minor = 20/7.

^1H NMR ($CDCl_3$): (for a major component) δ 7.19−7.35 (15H, m), 4.38−4.67 (6H, m), 4.14 (1H, s), 4.11 (1H, dd, J = 3.3, 4.6 Hz), 4.04−4.08 (1H, m), 4.07 (1H, dd, J = 4.0, 10.6 Hz), 3.82 (1H, s), 3.79−3.83 (2H, m), 3.75 (1H, dd, J = 8.9, 4.0 Hz), 3.51 (1H, dd, J = 8.9, 10.56 Hz), and 1.42 (9H, s); (for a minor component) δ 7.19−7.35 (15H, m), 4.38−4.67 (6H, m), 4.28 (1H, dd, J = 4.0, 10.6 Hz), 4.17 (1H, s), 3.99−4.04 (1H, m), 3.96 (1H, s), 3.87−3.93 (1H, m), 3.79−3.83 (2H, m), 3.43−3.51 (1H, m), 2.78 (1H, broad dd, J = 3.3, 4.93 Hz), and 1.47 (9H, s). ^{13}C NMR ($CDCl_3$): (for a major component) δ 155.49, 84.15, 81.65, 80.72, 73.03,71.27, 68.30, 66.45, 64.58, 63.36, and 28.34; (for a minor component) δ 155.49, 85.68, 80.72, 80.20, 73.03, 71.27, 67.76, 65.41, 63.02, 62.23, and 28.34. Anal. Calcd

for $C_{32}H_{39}NO_6$: C, 72.02; H, 7.37; N, 2.62. Found: C, 72.19; H, 7.54; N, 2.52.

(2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-2-(azidomethyl)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (26) and (2*R*,3*R*,4*R*,5*R*)-(3,4-Bis(benzyloxy)-5-[(benzyloxy)-methyl]pyrrolidino[1,2-*c*]oxazole-3-one (27). To a solution of compound **24** (371 mg, 0.70 mmol) in CH_2Cl_2 (7 mL) was added MsCl (81 μL, 1.04 mmol) and Et_3N (145 μL, 1.04 mmol) at 0 °C. The mixture was stirred at rt for 2 h, diluted with EtOAc, washed successively with 1 N HCl, saturated $NaHCO_3$, water, and brine, then dried over $MgSO_4$, and concentrated. The resulting syrup was purified on a column of silica gel eluted with 5:1 hexane−EtOAc to give mesyl ester **25** [R_f 0.48 (2:1 hexane−EtOAc); 424 mg, quantitative] which was then dissolved in DMF (9 mL). To this solution was added NaN_3 (451 mg, 6.9 mmol), and the mixture was stirred at 70 °C for 35 h. The mixture was concentrated to about half-volume and diluted with EtOAc, washed with water, dried over $MgSO_4$, and concentrated. The residue was purified on a column of silica gel using 9:1 and 3:1 hexane−EtOAc as eluent. The former eluent afforded the desired **26** [R_f 0.76 (2:1 hexane−EtOAc); 253 mg, 65%] (major/minor = 5/4) and the latter gave oxazolone **27** [R_f 0.32 (2:1 hexane−EtOAc); 94 mg, 28%].

Physical data for compound **26**: $[\alpha]_D$ −47.5° (*c* 1.0, $CHCl_3$); complex signals were obtained due to the existence of two conformational isomers at an almost 1:1 ratio, and only chemical shifts were reported; ^{1}H NMR ($CDCl_3$) δ 7.39−7.17, 4.66−4.38, 4.22−4.10, 4.04−3.93, 3.85, 3.74, 3.68, 3.49, 3.46, 3.33, 3.29, 1.41, and 1.47; ^{13}C NMR ($CDCl_3$) δ 153.69, 153.41, 83.43, 82.09, 82.75, 81.26, 80.54, 80.38, 73.01, 71.02, 71.07, 68.29, 67.53, 62.98, 62.73, 63.02, 61.94, 50.93, 49.62, 28.39, and 28.34. Anal. Calcd for $C_{32}H_{39}N_4O_5$: C, 68.67; H, 7.02; N, 10.01. Found: C, 68.62; H, 6.85; N, 9.88.

Physical data for compound **27**: $[\alpha]_D$ = +4° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.23−7.38 (15H), 4.62 (1H, AB, *J* = 11.9 Hz), 4.60 (1H, AB, *J* = 11.9 Hz), 4.57 (1H, AB, *J* = 11.9 Hz), 4.53 (1H, AB, *J* = 11.9 Hz), 4.49 (1H, AB, *J* = 11.9 Hz), 4.46 (1H, AB, *J* = 11.9 Hz), 4.36 (1H, ABd, *J* = 9.1, 7.8 Hz), 4.26 (1H, t, *J* = 3.6 Hz), 4.12 (1H, td, *J* = 3.5, 5.3 Hz), 4.07 (1H, ABd, *J* = 9.1, 3.5 Hz), 3.86−3.96 (2H, m), and 3.57 (2H, d, *J* = 5.3 Hz); ^{13}C NMR ($CDCl_3$) δ 160.68, 88.09, 85.84, 73.26, 72.60, 72.06, 69.81, 67.01, 62.37, and 61.94; FAB MS, *m*/*z* 460 $[M + H]^+$. Anal. Calcd for $C_{28}H_{29}NO_5$: C, 73.18; H, 6.36; N, 3.05. Found: C, 72.90; H, 6.42; N, 2.96.

(2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-2-(aminomethyl)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (28). A mixture of compound **26** (65 mg, 0.12 mmol) and 5% Pd on C (ca. 20 mg) in MeOH (2.5 mL) was stirred under a H_2 atmosphere at rt for 1.5 h until completion [R_f 0.49 (9:1 $CHCl_3$−MeOH)]. The reaction mixture was filtered to remove the catalyst and then concentrated to dryness to afford **28** (59 mg, major/minor = 4/3); $[\alpha]_D$ −48.1° (*c* 1.6, $CHCl_3$).

^{1}H NMR ($CDCl_3$): (for major component) δ 7.19−7.34 (15H, m), 4.40−4.68 (6H, m), 4.17 (1H, s), 4.02 (1H, d, *J* = 4.0 Hz), 3.96 (1H, s), 3.80 (1H, dd, *J* = 3.6, 8.9 Hz), 3.77 (dd, 1H, *J* = 4.0, 8.9 Hz), 3.48 (1H, dd, *J* = 4.0, 8.9 Hz), 3.17 (1H, dd, *J* = 3.6, 12.7 Hz), 2.85 (1H, dd, *J* = 8.9, 12.7 Hz), 1.41 (9H, s), and 1.36 (2H, s); (for minor component) δ 7.19−7.34 (15H, m), 4.40−4.68 (6H, m), 4.22 (1H, dd, *J* = 3.9, 10.6 Hz), 4.16 (1H, s), 4.22 (1H, dd, *J* = 4.0, 10.1 Hz), 3.93 (1H, s), 3.68 (1H, dd, *J* = 3.6, 8.6 Hz), 3.48 (1H, dd, *J* = 10.6, 16.4 Hz), 3.00 (1H, dd, *J* = 3.6, 12.2 Hz), 2.90 (1H, dd, *J* = 8.6, 12.2 Hz), 1.46 (9H, s), and 1.36 (2H, s). ^{13}C NMR ($CDCl_3$): (for major component) δ 154.02, 83.56, 82.77, 79.89, 72.98, 71.20, 70.96, 68.56, 66.54, 63.02, 42.03, and 28.39; (for minor component) δ 154.02, 84.85, 81.24, 79.89, 72.98, 71.20, 70.96, 67.78, 66.90, 62.84, 42.88, and 28.39. HRFAB MS: calcd for $C_{32}H_{41}N_2O_4$ $[M + H]^+$ *m*/*z* 533.3015; found *m*/*z* 533.3018.

(2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-2-(acetamidomethyl)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (29). The amine **28** was acetylated using Ac_2O (30 μL) and pyridine (2 mL) to yield **29** (55.7 mg, 83%, major/minor = 10/7) after purification on a column of silica gel using a 20:1 mixture of $CHCl_3$−MeOH as eluent; $[\alpha]_D$ −4.6° (*c* 0.5, $CHCl_3$).

^{1}H NMR ($CDCl_3$): (for major component) δ 7.20−7.34 (15H, m), 6.87 (1H, broad s), 4.36−4.68 (6H, m), 4.16 (1H, s), 4.06 (1H, dd, *J* = 4.0, 10.6 Hz), 3.93 (1H, s), 3.81−4.01 (2H, m), 3.76 (dd, 1H, *J* = 4.0, 8.9 Hz), 3.49 (1H, dd, *J* = 8.9, 10.6 Hz), 3.26−3.35 (1H, m), 1.83 (3H, s), and 1.41 (9H, s); (for minor component) δ 7.20−7.34 (15H, m), 6.30 (1H, broad s), 4.36−4.68 (6H, m), 4.30 (1H, dd, *J* = 4.0, 10.6 Hz), 4.18 (1H, s), 3.87 (1H, s), 3.81−4.01 (3H, m), 3.49 (1H, dd, *J* = 8.9, 10.6 Hz), 3.17−3.22 (1H, m), 1.54 (3H, s), and 1.46 (9H, s). ^{13}C NMR ($CDCl_3$): (for major component) δ 170.17, 153.84, 84.12, 81.80, 80.58, 73.03, 71.61, and 71.34, 67.49, 63.97, 63.54, 42.57, 28.30, and 23.22; (for minor component) δ 170.17, 155.13, 86.15, 84.40, 80.76, 73.03, 71.61, 71.34, 68.25, 63.41, 62.73, 40.7, 28.30, and 22.79. MALDITOF MS, *m*/*z*: 597 $[M + Na]^+$.

(2*R*,3*R*,4*R*,5*R*)-2-(Acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine (3). A solution containing compound **29** (11.7 mg, 0.038 mmol) in MeOH (1.5 mL) was acidified with 0.1 N HCl to pH 4−5, and to it was added a catalytic amount of 5% Pd on C. The reaction mixture was stirred under a H_2 atmosphere at rt overnight. Filtration and evaporation of the solvent afforded a syrup quantitatively, which was then treated with TFA−H_2O (9:1 v/v, 300 μL) and the solution was kept at rt for 1 h. The mixture was neutralized to pH ≈ 8 using 28% NH_3 and concentrated. The resulting residue was purified on a column of Iatro Beads using a 9:2:1 mixture of *i*-PrOH−28% NH_3−H_2O to afford 15 mg of the salt form, which was treated with Dowex 1 × 8 (OH^-) to give **3** (7 mg, 81%). **3** was further purified for the inhibition assay using Sep-Pak PLUS CM, regenerated with 1 M HCl (10 mL) and water (20 mL), and eluted with water (20 mL) and 10% NH_3−H_2O (10 mL). The latter eluent containing **3** was filtered through a Millex GV filter and lyophilized.

^{1}H NMR (D_2O): δ 3.83 (1H, t, *J* = 6.6 Hz), 3.78 (1H, t, *J* = 6.6 Hz), 3.70 (1H, ABd, *J* = 4.5, 11.7 Hz), 3.62 (1H, ABd, *J* = 6.1, 11.7 Hz), 3.41 (1H, ABd, *J* = 5.3, 13.9 Hz), 3.30 (1H, ABd, *J* = 7.3, 13.9 Hz), 3.07 (1H, ddd, *J* = 5.3, 6.6, 7.3 Hz), 3.03 (1H, ddd, *J* = 4.5, 6.1, 6.6 Hz), and 2.01 (3H, s). ^{13}C NMR (D_2O): δ 175.51, 80.31, 78.66, 62.83, 62.54, 60.62, 42.74, and 22.83. HRFAB MS: calcd for $C_8H_{17}N_2O_4$ $[M + H]^+$ *m*/*z* 205.1188; found: *m*/*z* 205.1181.

(2*R*,3*R*,4*R*,5*R*)-2-(Acetamidomethyl)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (30). Compound **29** (142 mg, 0.25 mmol) was treated with TFA−H_2O (95:5 v/v, 1.2 mL) at rt for 2 h. The resulting solution was neutralized with saturated $NaHCO_3$ to pH ≈ 7 and extracted with EtOAc. The combined organic layers were dried over $MgSO_4$, filtered, evaporated, and purified on a column of silica gel eluted with $CHCl_3$−MeOH (20:1) to afford **30** (113 mg, 96%); $[\alpha]_D$ +32.3° (*c* 1.3, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.26−7.36 (15H, m), 6.08 (1H, broad s), 4.52 (6H, s), 3.88 (1H, t, *J* = 3.3 Hz), 3.77 (1H, t, *J* = 3.3 Hz), 3.52 (2H, d, *J* = 5.3 Hz), 3.38 (1H, dd, *J* = 3.3, 5.3 Hz), 3.21−3.47 (3H, m), 2.16 (1H, s), and 1.89 (3H, s). ^{13}C NMR ($CDCl_3$): δ 170.31, 86.88, 85.53, 73.26, 72.04, 71.89, 69.81, 61.98, 61.11, 41.42, and 23.22. HRFAB MS: calcd for $C_{29}H_{35}N_2O_4$ $[M + H]^+$ *m*/*z* 475.2597; found *m*/*z* 475.2590.

(2*R*,3*R*,4*R*,5*R*)-*N*-Methyl-2-(acetamidomethyl)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (31). To a solution of **30** (16 mg, 0.034 mmol) in MeOH (0.5 mL) at 0 °C was added 37% folmaldehyde solution (5.1 mL, 0.068 mmol) and $NaBH_3CN$ (4.2 mg, 0.068 mmol). The mixture was stirred at rt overnight. To the reaction mixture was added H_2O, and the mixture was extracted with $CHCl_3$ and dried with $MgSO_4$. After removal of the solvent, the residue was purified by preparative TLC ($CHCl_3$−MeOH 9:1) to yield **31** (12 mg, 71%); $[\alpha]_D$ −10.8° (*c* 0.3, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.23−7.35 (15H, m), 6.15 (1H, broad s), 4.58, 4.46 (2H, AB, *J* = 11.6 Hz), 4.51 (2H, s), 4.44 (2H, s), 3.94 (1H, s), 3.78 (1H, d, *J* = 4.0 Hz), 3.69 (1H, dd, *J* = 9.1, 4.8 Hz), 3.60 (1H, broad dd, *J* = 14.0, 7.2 Hz), 3.41−3.55 (2H, m), 3.19 (1H, broad d, *J* = 14.0 Hz), 2.98−3.04 (1H, broad m), 2.40 (3H, s), and 1.76 (3H, s). ^{13}C NMR ($CDCl_3$): δ 170.64, 86.36, 83.65, 73.31, 71.82, 71.43, 67.40, 67.17, 66.47, 38.47, 34.59, and 23.04.

(2*R*,3*R*,4*R*,5*R*)-*N*-Butyl-2-(acetamidomethyl)-3,4-bis-(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (32). Compound **32** was synthesized according to the procedure described for the synthesis of **31** using *n*-butanal instead of formaldehyde. Yield: 74%, $[\alpha]_D$ −35° (*c* 0.56, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.22−7.32 (15H, m), 5.28 (1H, broad d, J = 6.6 Hz), 4.58, 4.48 (2H, AB, J = 12.04 Hz), 4.49 (2H, s), 4.45 (2H, s), 3.99 (1H, s), 3.75 (1H, d, J = 3.3 Hz), 3.68 (1H, dd, J = 7.9, 3.6 Hz), 3.46−3.58 (3H, m), 3.19 (1H, broad d, J = 14.5 Hz), 3.03−3.09 (1H, broad m), 2.45−2.65 (2H, m), 1.18−1.54 (4H, m), 1.69 (3H, s), and 0.90 (3H, t, J = 7.1 Hz). ^{13}C NMR ($CDCl_3$): δ 170.64, 86.49, 83.00, 73.39, 71.75, 71.27, 66.38, 65.23, 63.45, 45.90, 38.96, 30.14, 22.86, 20.45, and 13.91. HRFAB MS: calcd for $C_{33}H_{243}N_2O_4$ $[M + H]^+$ m/z 531.3223; found m/z 531.3260.

(2*R*,3*R*,4*R*,5*R*)-*N*-Methyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine (4). Compound **31** (18.7 mg, 0.038 mmol) was dissolved in MeOH (1.5 mL), this solution was acidified with 0.1 N HCl to pH 4−5, and a catalytic amount of 5% Pd on C was added. The reaction mixture was stirred under a H_2 atmosphere at rt overnight. The catalyst was removed by filtration, and the solvent was concentrated under vaccum. The residue was purified on a column of Iatro Beads using a 9:2:1 mixture of *i*-PrOH−28% NH_3−H_2O and treated with Dowex 1 × 8 (OH^-) to give **4** (6.6 mg, 80%); $[\alpha]_D$ −26.6° (*c* 0.5, MeOH).

^{1}H NMR (D_2O): δ 3.95 (1H, t, J = 4.6 Hz), 3.83 (1H, t, J = 4.6 Hz), 3.79 (2H, d, J = 4.6 Hz), 3.55 (1H, ABd, J = 4.6, 14.2 Hz), 3.31 (1H, ABd, J = 6.6, 14.2 Hz), 2.94 (1H, ddd, J = 4.6, 6.6, 4.6 Hz), 2.88 (1H, dt, J = 4.6, 4.6 Hz), 2.42 (3H, s), and 2.01 (3H, s). ^{13}C NMR (D_2O): δ 175.34, 80.28, 79.29, 70.00, 68.42, 60.28, 38.99, 35.25, and 22.92. HRFAB MS: calcd for $C_9H_{19}N_2O_4$ $[M + H]^+$ m/z 219.1345; found m/z 219.1346.

(2*R*,3*R*,4*R*,5*R*)-*N*-Butyl-2-(acetamidomethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine (5). Compound **5** was synthesized in 85% from **30** according to the procedure described for the synthesis of compound **4**.

^{1}H NMR (CD_3OD): δ 3.96 (1H, t, J = 2.0 Hz), 3.77 (1H, t, J = 2.0 Hz), 3.73 (1H, ABd, J = 11.2, 5.3 Hz), 3.67 (1H, ABd, J = 11.2, 3.3 Hz), 3.49 (1H, ABd, J = 13.5, 3.3 Hz), 3.18 (1H, ABd, J = 13.5, 6.9 Hz), 2.98−3.04 (2H, m), 2.59−2.77 (2H, m), 1.94 (3H, s), 1.47−1.59 (2H, m), 1.29−1.45 (2H, m), and 0.94 (3H, t, J = 7.3 Hz). ^{13}C NMR (D_2O): δ 173.30, 78.70, 78.06, 66.86, 65.10, 58.40, 46.14, 36.94, 28.25, 21.04, 19.30, and 12.33. HRFAB MS: calcd for $C_{12}H_{25}N_2O_4$ $[M + H]^+$ m/z 261.1814. Found m/z 261.1811.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]-2-(2-azido-1-hydroxyethyl)pyrrolidine (34). To a solution of compound **22** (383 mg, 0.68 mmol) dissolved in pyridine (7 mL) was added TsCl (194 mg, 1.02 mmol). The reaction mixture was stirred at rt for 37 h. H_2O was added to the mixture, and the resulting solution was stirred for 5 min. The mixture was diluted with EtOAc, washed with 1 N HCl, water, saturated $NaHCO_3$, and brine, dried over $MgSO_4$, and concentrated. The residue was purified on a column of silica gel (4:1 hexane−EtOAc) to give **33** [R_f 0.49 (2:1 hexane−EtOAc); 417 mg, 85%], which was then dissolved in DMF (4 mL). To the solution was added NaN_3 (151 mg, 2.33 mmol). The mixture was stirred at 70 °C for 4 h, and H_2O was added. After stirring for 5 min, the mixture was diluted with EtOAc, washed with brine, dried over $MgSO_4$, and concentrated. The residue was purified on a column of silica gel using 8:1 hexane−EtOAc as an eluent to afford **34** (235 mg, 69%, major/minor = 5/2); $[\alpha]_D$ −23.4° (*c* 1.0, $CHCl_3$).

^{1}H NMR ($CDCl_3$): (for major component) δ 7.21−7.76 (15H, m), 4.41−4.62 (6H, m), 4.38 (1H, s), 4.20−4.24 (1H, m), 4.16 (1H, s), 4.05 (1H, s), 4.01−4.11 (1H, m), 4.01−4.05 (1H, m), 3.74 (1H, dd, J = 4.1, 9.2 Hz), 3.47 (1H, dd, J = 9.2, 9.2 Hz), 3.36 (1H, dd, J = 4.3, 12.7 Hz), 3.22 (1H, dd, J = 6.6, 12.7 Hz), and 1.42 (9H, s); (for minor component) δ 7.21−7.76 (15H, m), 4.41−4.62 (6H, m), 4.24−4.34 (1H, m), 4.20−4.24 (1H, m), 4.16 (1H, s), 4.05 (1H, s), 3.92 (1H, broad s), 3.89 (1H, d, J = 4.6 Hz), 3.44−3.51 (2H, m), 3.11−3.25 (2H, m), and 1.47 (9H, s). ^{13}C NMR ($CDCl_3$): (for major component) δ 154.73, 82.25, 81.44, 80.90, 73.07, 71.43, 71.34, 70.57, 68.12, 67.87, 63.24, 54.02, and 28.30; (for minor component) δ 154.73, 81.44, 80.72, 79.71, 73.07, 71.43, 71.34, 69.97, 67.06, 67.06, 62.57, 54.72, and 28.30. Anal. Calcd for $C_{33}H_{40}N_4O_6$: C, 67.33; H, 6.85; N, 9.52. Found: C, 67.19; H, 6.88; N, 9.24.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-2-[2-azido-1-(benzyloxy)ethyl]-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (35). To a solution of **34** (132 mg, 0.22 mmol) in DMF (3 mL) was successively added Ag_2O (208 mg, 0.9 mmol), BnBr (107 μL, 0.9 mmol), and KI (74 mg, 0.45 mmol) at 0 °C. The reaction mixture was stirred at rt for 7 h, Et_2O−water was added, and the mixture was stirred for 10 min. After filtration through a Celite pad, the mixture was extracted with Et_2O, the organic layer was separated, dried over $MgSO_4$, and concentrated. The residue was purified on a column of silica gel using 10:1 hexane−EtOAc as an eluent to yield **35** (139 mg, 93%); $[\alpha]_D$ −30.7° (*c* 1.06, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.18−7.29 (20H, m), 4.39−4.64 (8H, m), 4.18−4.31 (3H, m), 4.03−4.06 (2H, m), 3.80−3.92 (1H, m), 3.45−3.52 (1H, m), 3.36 (2H, broad d, J = 5.0 Hz), and 1.41 (9H, s). ^{13}C NMR ($CDCl_3$): δ 154.73, 83.11, 81.71, 80.27, 77.21, 73.30, 71.05, 71.05, 70.98, 68.32, 65.75, 63.40, 53.41, and 28.36. Anal. Calcd for $C_{40}H_{46}N_4O_6$: C, 70.77; H, 6.83; N, 8.25. Found: C, 70.74; H, 6.85; N, 8.12.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-2-[2-amino-1-(benzyloxy)ethyl]-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (36). To a solution of compound **35** (29 mg, 0.043 mmol) in MeOH (2 mL) was added 5% Pd on C (ca. 20 mg). The mixture was stirred under a H_2 atmosphere at rt for 1 h. Filtration and concentration afforded **36** (25 mg); $[\alpha]_D$ −30.3° (*c* 0.8, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.22−7.29 (20H, m), 4.35−4.62 (8H, m), 4.17 (2H, s), 4.00−4.05 (2H, m), 3.79−3.87 (2H, m), 3.46 (1H, dd, J = 9.2, 9.9 Hz), 2.87 (1H, dd, J = 4.0, 14.5 Hz), 2.58 (1H, dd, J = 3.6, 14.5 Hz), 2.39 (2H, broad s), and 1.41(9H, s). ^{13}C NMR ($CDCl_3$): δ 154.86, 83.20, 82.00, 80.47, 77.79, 72.98, 71.61, 70.86, 70.77, 68.30, 63.67, 63.43, 40.76 and 28.30. HRFAB MS: calcd for $C_{40}H_{49}N_2O_6$ $[M + H]^+$ m/z 653.3591; found m/z 653.3590.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-*N*-Butyloxycarbonyl-2-[2-acetamido-1-(benzyloxy)ethyl]-3,4-bis(benzyloxy)-5-[(benzyloxy)-methyl]pyrrolidine (37). The amine **36** was redissolved in pyridine (1.5 mL), and Ac_2O (0.5 mL) was added to the solution. The mixture was kept at rt overnight and concentrated to dryness. The residue was purified on preparative TLC using a 10:1 mixture of $CHCl_3$−MeOH as the mobile phase to afford **37** (25 mg, 84%); $[\alpha]_D$ +2.6° (*c* 0.4, $CHCl_3$).

^{1}H NMR ($CDCl_3$): δ 7.17−7.34 (20H, m), 6.91(1H, broad d), 4.76 (1H, AB, J = 10.2 Hz), 4.48 (2H, s), 4.48 (1H, AB, J = 12.0 Hz), 4.36 (1H, AB, J = 12.0 Hz), 4.31 (1H, AB, J = 10.2 Hz), 4.27−4.33 (2H, m), 4.14 (2H, s), 3.98 (1H, d, J = 9.6 Hz), 3.97 (1H, dd, J = 4.0, 9.6 Hz), 3.87 (1H, dd, J = 4.0, 8.6 Hz), 3.71(1H, broad d, J = 9.6 Hz), 3.46 (1H, dd, J = 9.6, 8.6 Hz), 2.62 (2H, broad d), 1.98 (3H, s), and 1.40 (9H, s). ^{13}C NMR ($CDCl_3$): δ 170.42 NHCO), 155.36, 83.14, 82.12, 80.74, 75.79, 73.04, 71.27, 70.85, 68.34, 63.99, 63.70, 36.46, 28.30, and 23.74. MALDITOF MS, m/z: 717 $[M + Na]^+$.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-2-(2-Acetamido-1-hydroxyethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine (6). To a solution of compound **37** (41 mg, 0.059 mmol) in MeOH (1.5 mL) were added 0.1 N HCl (0.2 mL) and 5% Pd on C (25 mg), and the mixture was stirred under a H_2 atmosphere at rt overnight. Filtration and evaporation of the solvent afforded a syrup quantitatively, which was then treated with TFA−H_2O (9:1) at rt for 2 h. The mixture was adjusted to pH 8 using NH_3 (28%) and concentrated to give a syrup, which was purified on a column of Iatoro Beads using a 9:1:2 mixture of *i*-PrOH−NH_3(28%)−H_2O to yield a TFA salt (14.5 mg). The residue was treated with Dowex 1 × 8 (OH^-) and eluted with water to give **6** (13.7 mg, quantitative); $[\alpha]_D$ +30.6° (*c* 0.69, MeOH).

^{1}H NMR (D_2O): δ 4.06 (1H, t, J = 7.3 Hz), 3.84 (1H, t, J = 7.3 Hz), 3.78 (1H, ddd, J = 3.6, 6.3, 7.9 Hz), 3.74 (1H, dd, J = 4.3, 11.9 Hz), 3.59 (1H, dd, J = 5.9, 11.9 Hz), 3.47 (1H, dd, J = 3.6, 14.2 Hz), 3.21 (1H, dd, J = 7.9, 14.2 Hz), 3.00 (1H, ddd, J = 4.3, 5.9, 7.3 Hz), 2.95 (1H, dd, J = 6.3, 7.3 Hz), and 2.01 (3H, s). ^{13}C NMR (D_2O): δ 173.60, 77.05, 77.01, 70.44, 60.99,

60.86, 60.63, 41.83, and 20.95. HRFAB MS: calcd for $C_9H_{19}N_2O_5$ $[M + H]^+$ *m*/*z* 235.1294; found *m*/*z* 235.1289.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-2-[2-Acetamido-1-(benzyloxy)ethyl]-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidine (38). Compound **38** was synthesized from **37** according to the procedure described for the synthesis of compound **30**.

^{1}H NMR ($CDCl_3$): δ 7.24−7.31 (20H, m), 6.55 (1H, broad t), 4.66 (1H, AB, J = 11.5 Hz), 4.48 (1H, AB, J = 11.5 Hz), 4.40−4.51 (6H, m), 4.02 (1H, t, J = 2.6 Hz), 3.89 (1H, dd, J = 2.6, 4.3 Hz), 3.75−3.84 (1H, m), 3.47−3.56 (3H, m), 3.23−3.32 (3H, m, H-2), 2.08 (1H, broad s), and 1.84 (3H, s). ^{13}C NMR ($CDCl_3$): δ 170.01, 86.16, 76.53, 73.21, 71.86, 71.77, 71.47, 70.03, 65.19, 62.46, 39.79, and 23.29.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-*N*-Methyl-2-(2-acetamido-1-hydroxyethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine (7). Compound **7** was synthesized in 86% from **38** according to the procedure described for the synthesis of compound **4**.

^{1}H NMR (D_2O): δ 4.15 (1H, t, J = 4.6 Hz), 3.93 (1H, dd, J = 4.6, 5.6 Hz), 4.09−4.12 (1H, m), 3.79 (2H, d, J = 4.6 Hz), 3.23−3.42 (2H, m), 2.97 (1H, td, J = 4.6, 5.6 Hz), 2.83 (1H, dd, J = 2.3, 4.6 Hz), 2.42 (3H, s), and 2.03 (3H, s). ^{13}C NMR (D_2O): δ 175.41, 79.10, 77.08, 70.76, 70.09, 68.15, 60.09, 43.75, 34.78, and 22.85. HRFAB MS: calcd for $C_{10}H_{21}N_2O_5$ $[M + H]^+$ *m*/*z* 249.1450; found *m*/*z* 249.1445.

(1′*R*,2*R*,3*R*,4*R*,5*R*)-*N*-Ethyl-2-(2-acetamido-1-hydroxyethyl)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidine (8). Compound **8** was synthesized in 83% from **38** according to the procedure described for the synthesis of compound **4**.

^{1}H NMR (D_2O): δ 4.16 (1H, t, J = 4.0 Hz), 3.95 (1H, dd, J = 4.0, 5.0 Hz), 4.01−4.14 (1H, m), 3.79 (1H, ABd, J = 9.9, 4.0 Hz), 3.75 (1H, ABd, J = 9.9, 5.0 Hz), 3.28−3.45 (2H, m), 3.10 (1H, td, J = 5.0, 4.0 Hz), 2.93 (1H, dd, J = 2.0, 4.0 Hz), 2.68−2.87 (2H, m), 2.02 (3H, s), and 1.08 (3H, t, J = 7.3 Hz). HRFAB MS: calcd for $C_{11}H_{23}N_2O_5$ $[M + H]^+$ *m*/*z* 263.1607; found *m*/*z* 263.1647.

***N*,*N*-Bis{[(2*R*,3*R*,4*R*,5*R*)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidinyl]methyl}amine (39).** Ammmonium acetate (59 mg, 0.77 mmol) was added to a solution of aldehyde **23** (41 mg, 0.077 mmol) in MeOH (1 mL), and then sodium cyanoborohydride (5.3 mg, 0.085 mmol) was added to the solution at rt. The mixture was stirred for 22 h, then concentrated, and extracted with $CHCl_3$. The organic layer was washed with saturated $NaHCO_3$ and H_2O and dried over $MgSO_4$. The solvent was evaporated in vacuo, and the residue was purified by preparative TLC (2:1 hexane−EtOAc) to give **39** [R_f 0.46 (2:1 hexane−EtOAc); 30.4 mg, 75%]; $[\alpha]_D$ −58° (*c* 0.75, $CHCl_3$).

^{13}C NMR ($CDCl_3$): δ 153.93, 84.96, 83.65, 82.95, 81.67, 79.75, 79.62, 72.90, 70.82, 68.59, 67.84, 63.97, 62.66, 62.50, 49.65, 48.74, and 28.36. FAB MS, *m*/*z*: 1048 $[M + H]^+$. Anal. Calcd for $C_{64}H_{76}N_3O_{10}$: C, 73.40; H, 7.31; N, 4.01. Found: C, 73.38; H, 7.23; N, 3.95.

***N*-Butyloxycarbonyl-*N*,*N*-bis{[(2*R*,3*R*,4*R*,5*R*)-3,4-bis(benzyloxy)-5-[(benzyloxy)methyl]pyrrolidinyl]methyl}amine (40) and *N*,*N*-Bis{[(2*R*,3*R*,4*R*,5*R*)-3,4-dihydroxy-5-(hydroxymethyl)pyrrolidinyl]methyl}amine (9).** To a solution of **39** (32 mg, 0.03 mmol) in CH_2Cl_2 (2 mL) and Et_3N (5 μL, 0.036 mmol) was added $(Boc)_2O$ (17 μL, 0.073 mmol) at 0 °C, and the mixture was stirred at rt overnight. The reaction mixture was diluted with CH_2Cl_2 and was washed with 10% citric acid, saturated $NaHCO_3$, and water, dried over $MgSO_4$, and concentrated. The resulting material was purified by preparative TLC (2:1 hexanes−EtOA) to afford **40** [R_f 0.69 (2:1 hexane−EtOAc), 33 mg, 97%].

To the solution of compound **40** (18 mg, 0.016 mmol) dissolved in MeOH (1.5 mL), acidified to pH 4−5 with 0.1 N HCl, was added 5% Pd on C (ca. 10 mg). The mixture was stirred under a H_2 atmosphere at rt overnight. The catalyst was filtered off, and the filtrate was concentrated. The residue was treated with TFA−H_2O (9:1, 300 μL) at rt for 3 h. The mixture was adjusted to pH 8 using NH_3 (28%) and concentrated to leave a syrup, which was purified on a column of Iatro Beads using 9:1:2 *i*-PrOH−NH_3(28%)−H_2O. The obtained material was finally treated with Dowex 1 × 8 (OH^-) eluted with water to give **9** after concentration (4.6 mg, 94%); $[\alpha]_D$ +55° (*c* 0.42, MeOH).

^{1}H NMR (D_2O): δ 3.87 (2H, t, J = 7.6 Hz), 3.79 (2H, t, J = 7.6 Hz), 3.77 (2H, ABd, J = 11.9, 4.29 Hz), 3.64 (2H, ABd, J = 11.9, 5.9 Hz), 3.14 (2H, td, J = 4.3, 7.6 Hz), 3.04 (2H, ddd, J = 4.3, 5.9, 7.6 Hz), 2.92 (2H, ABd, J = 12.4, 4.3 Hz), and 2.71 (2H, ABd, J = 12.4, 7.6 Hz). ^{13}C NMR (D_2O): δ 79.03, 76.59, 60.81, 60.63, 58.22, and 51.05. HRFAB MS: calcd for $C_{12}H_{26}N_3O_6$ $[M + H]^+$ *m*/*z* 308.1822; found: *m*/*z* 308.1803.

Enzymatic Assay. Materials. The source of enzymes and substrates are as follows: α-glucosidase (EC 3.2.1.20) from *Saccharomyces sp*. and β-glucosidase (EC 3.2.1.21) from Sweet almond, Toyobo Co., Ltd. (Osaka, Japan); β-*N*-acetylglucosaminidase (EC 3.2.1.30) from bovine kidney and β-*N*-acetylhexosaminidase A and P (EC 3.2.1.52) from human placenta, Sigma Chemical Co. (St. Louis, MO); *p*-nitrophenyl α-D-glucopyranoside and *p*-nitrophenyl β-D-glucopyranoside; *p*-Nitrophenyl 2-*N*-acetyl-2-deoxy-β-D-glucopyranoside (*p*-nitrophenyl *N*-acetyl-β-glucosaminide or *p*-NP-GlcNAc), Seikagaku Kogyo Co., Ltd. (Tokyo, Japan); sodium acetate, sodium dihydrogen phosphate, and sodium hydrogen phosphate, Nacalai Tesque. Inc. (Kyoto, Japan). Double deionized water was prepared from a Milli-Q system from Millipore Corp. (Milford, MA). Millex-GV syringe filters (0.22 μm × 4 mm i.d.) were purchased from Nihon Millipore Ltd. (Yonezawa, Japan).

Kinetic Analysis of α-Glucosidase. To a 1 mL disposable cuvette was added 950 μL of 0.1 M phosphate buffer (pH 7.0) solution, 20 μL of inhibitor solution, and 20 μL of 20 mM *p*-nitrophenyl α-D-glucopyranoside solution. The solution was well mixed and warmed at 37 °C for 5 min, and then 20 μL of the enzyme solution in 10 mM phosphate buffer (pH 7.0) containing 0.2% of BSA was added. The reaction was monitored at 400 nm on Beckmann DU-70 spectrophotometer for 15 s, and the initial rate of hydrolysis was calculated. The same procedure was repeated with three other substrate concentrations. After the initial rates were accumulated, the corresponding Lineweaver−Burk plot at that inhibitor concentration was constructed.

Kinetic Analyssis of β-Glucosidase. To a 1-mL disposable cuvette was added 950 μL of 0.1 M acetate buffer (pH 5.0) solution, 20 μL of inhibitor solution, and 20 μL of 20 mM *p*-nitrophenyl β-D-glucopyranoside solution. The solution was well mixed and warmed at 37 °C for 5 min, and then 20 μL of the enzyme solution, which was dissolved in ice-cold Tris−HCl buffer (pH 7.8) and diluted with 10 mM phosphate buffer (pH 7.0) containing 0.2% of BSA, was added. The reaction was monitored at 400 nm on a Beckmann DU-70 spectrophotometer for 15 s, and the initial rate of hydrolysis was calculated. The same procedure was repeated with three other substrate concentrations. After the initial rates were accumulated, the corresponding Lineweaver−Burk plot at that inhibitor concentration was constructed.

Capilllary Zone Electrophoresis. Condition of Capillary Zone Electrophoresis. Assays were performed on a Waters Quanta 4000E capillary electrophoresis system, which was equipped with a 53 cm × 75 μm fused i.d. silica capillary. Detection was carried out by on-column measurement of UV absorption at 405 nm at 7.5 cm from the cathode. The capillary used was pretreated or regenerated with 0.1 M KOH (2 min) and elution buffer before each injection. Samples were loaded by means of hydrostatic pressure at 10 cm height for 30 s (ca. 38.4 nL). Electrophoresis was performed at 20 kV using 50 mM sodium borate (pH 9.2 for β-*N*-Acetylglucosaminidase assays, pH 9.4 for β-glucosidase assays, pH 10.2 for α-glucosidase and β-*N*-acetylhexosaminidase assays) as electrolyte at a constant temperature of 37 °C. Pherograms were recorded on Millennium 2010 system from Millipore Corp.

Kinetic Analysis of β-*N*-Acetylglucosaminidase. Incubations were performed in a total volume of 50 μL. Unless otherwise stated, reaction mixtures contained 25 mM citrate buffer (pH 4.4), various amount of *p*-NP-GlcNAc (0.5−2.0 mM), and various amounts of inhibitors with 6.25 mU of β-*N*-acetylglucosaminidase. After preincubation for 10 min at 37 °C, the reaction was started by the addition of β-*N*-acetylglucosaminidase and the reaction mixture was incubated for 10

min at 37 °C. Then the reaction was terminated by addition of 100 μL of 50 mM sodium borate.

Kinetic Analysis of *β*-Glucosidase. The procedure is same as that described for the analysis of *β*-*N*-acetylglucosaminidase except for the pH of the reaction mixtures (pH 5.5), the substrate *p*-NP-Glc (0.5−4.0 mM), and the enzyme *β*-glucosidase (12.8 mU).

Kinetic Analysis of α-Glucosidase. The procedure is same as that described for the assay of *β*-Gnase except for the pH of the reaction mixture (phosphate buffer pH 7.0), the substrate [*p*-NP-α-Glc (0.2−1.1 mM)], and the enzyme [α-glucosidase (5 mU)]. The termination of the reaction was carried out by addition of 50 μL of 200 mM Na_2CO_3.

Kinetic Analysis of *β*-*N*-Acetylhexosaminidase from Human Placenta. Incubations were performed in a total volume of 20 μL. Unless otherwise stated, reaction mixtures contained 100 mM citrate buffer (pH 4.5), various amounts of *p*-NP-GlcNAc (0.1−1.1 mM), and various amounts of inhibitors with 3.35 mU of *β*-*N*-acetylhexosaminidase A and 1.76 μU of *β*-*N*-acetylhexosaminidase P. After preincubation for 10 min at 37 °C, the reaction was started by the addition of *β*-*N*-Acetylglucosaminidase and the reaction mixture was incubated for 15 min at 37 °C. Then the reaction was terminated by addition of 20 μL of 0.2 M sodium carbonate.

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Supporting Information Available: ^{1}H NMR spectra of **19**−**21**, **23**, **28**−**32**, and **36**−**38**. This material is available free of charge via the Internet at http://pubs.acs.org.

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