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Synthesis and Enzymatic Evaluation of Five-Membered Iminocyclitols and a Pseudodisaccharide

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Abstract—Described here are the synthesis of five-membered iminocyclitols with *galacto*-configuration and a pseudodisaccharide, and their inhibitory activities against β-galactosyltransferase, β-galactosidase and α-mannosidase. © 2000 Published by Elsevier Science Ltd.

Introduction

Glycosyltransferases and glycosidases are important classes of enzymes responsible for the synthesis and degradation of oligosaccharides.[1] Development of specific inhibitors of such enzymes is of current interest as these inhibitors may be used as modulators to control cellular functions. Enzymatic hydrolysis of a glycosidic bond generally takes place via general acid and base catalysis that requires two critical residues, a proton donor and a nucleophile (Fig. 1(A)).[2] A distorted half-chair-like transition state leading to a carboxonium ion is considered to be involved in the reaction. A similar mechanism is expected for the glycosyltransferase catalyzed reaction where a base (designated as B in Figure 1(B)) is thought to be involved in the abstraction of the acceptor hydroxyl proton. Five-membered iminocyclitols carrying hydroxyl groups with specific orientation to mimic the shape and charge of the transition state of the reacting sugar moiety have been shown to be potent inhibitors of such enzymes.[1–4] Since a cation-like transition state is expected to be involved in both the glycosyltransferase and glycosidase catalyzed reactions, iminocyclitols can be used as core components for the development of transition-state analogue inhibitors of both families of enzymes.

In order 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 a general and straightforward synthetic process using pentofuranoses as the starting material.[3h,5] In addition, capillary zone electrophoresis (CZE) has been used and proven extremely useful for the kinetic evaluation of glycoenzymes.[3h,6]

We report here the chemical synthesis of several five-membered iminocyclitols with *galacto* configuration (Fig. 2) and a pseudodisaccharide consisting of an iminocyclitol and *N*-acetylglucosamine, and evaluations of their inhibitory activities against β-galactosidase (EC 3.2.1.23), α-mannosidase (EC 3.2.1.24) and β-galactosyltransferase (EC 2.4.1.22) using capillary zone electrophoresis.

Results and Discussions

In order to study the mechanism of enzymes associated with processing of oligosaccharides and to develop inhibitors of such enzymes, we have selected a series of five-membered iminocyclitols **1**–**5** (Fig. 2). Since it appears that compound **4** exists as an equilibrium mixture of conformations including those mimic galactose (**4a**) and mannose (**4b**), **4** may inhibit the enzymes associated with the processing of these carbohydrates, where six-membered iminocyclitols such as deoxygalactonojirimycin and deoxymannojirimycin have been shown to be inhibitors of these enzymes. In addition, an extended carbon chain at *C*-2 was incorporated for attachment of another group to mimic the aglycon moiety of glycosides. Also, the ring nitrogen is alkylated in order to

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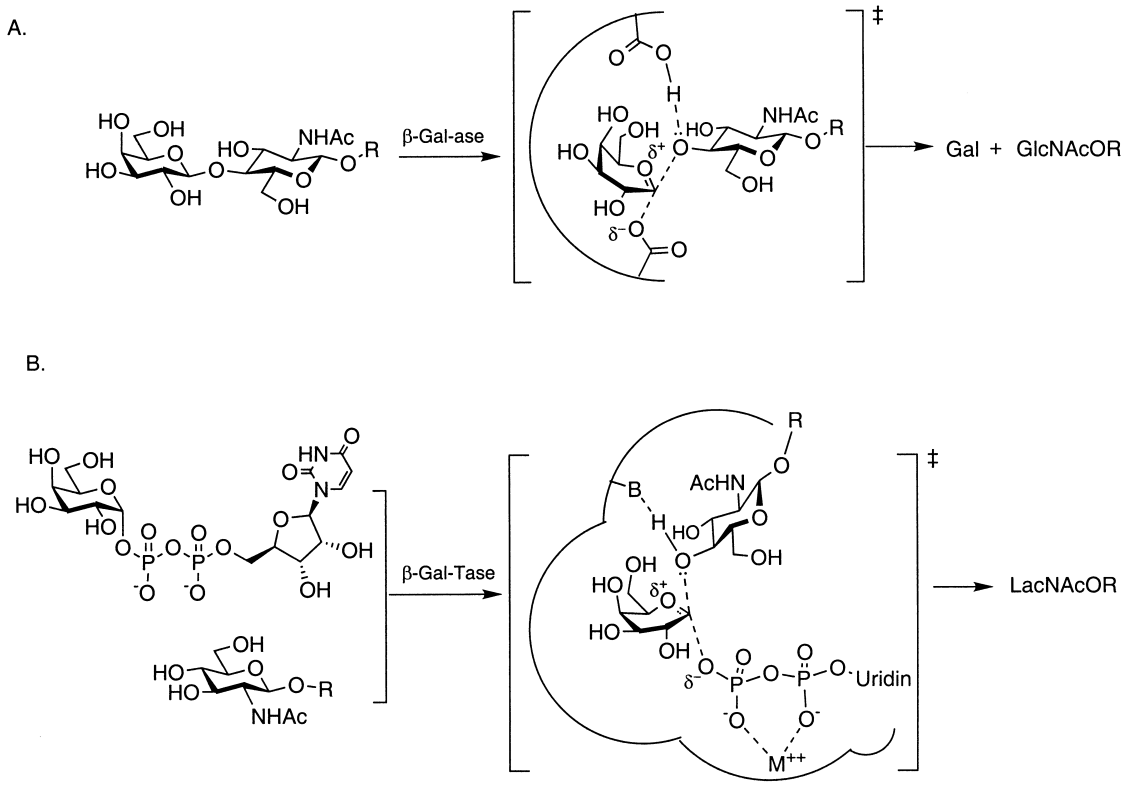


Figure 1. A. Proposed β-Gal-ase reaction. A carboxylic acid and a carboxylate residue are involved in the hydrolytic reaction. B. Schematic drawing of β-GalT-ase reaction.

investigate the effect of *N*-substitution on the inhibition activity.

Synthesis of compounds 2 and 3 (Scheme 1)

Wittig reaction of 2,3,5-tri-*O*-benzyl-*lyxo*-furanose[7] with methyl (triphenylphosphoranylidene)acetate afforded a 1:1 mixture of *E*- and *Z*-**6**, which yielded **6** (*E*) (66%) after irradiation with a 200 W lamp. The methoxycarbonyl group was converted to the TBDMS protected alcohol (**8**) via di-*iso*-butylaluminum hydride (DIBAL) reduction followed by silylation. In order to introduce the azide function in *R*-configuration at the C-6 position, a double inversion was carried out. The 6-OH group was first chloromesylated[5,8] (**9**, 97%) and the product was treated with CsOAc to give **10** (75%). After removal of the acetyl group, the OH group of **11** was again chloromesylated for the second inversion (**12**) and the TBDMS group was deprotected to give the allylic alcohol **13** for Sharpless epoxidation in the presence of D-(−)-diethyl tartrate to afford **14** (86%). Compound **14** was treated with NaN_3 to give azide **15** (93%), which was subjected to a reduction condition to give **16** (76%). The benzyl groups were hydrogenolyzed to give the target compound **2** (93%). Compound **3** was obtained via methylation of the amino function in **16** and hydrogenolysis.

Synthesis of compounds 4 and 5 (Scheme 2)

In order to obtain compounds **4**[9] and **5**, the secondary amino function of **16** was first protected with the Boc group to give **18** (72%). The C-1′–C-2′ bond of **18** was then cleaved using $Pb(OAc)_4$ to give the aldehyde **19** (90%). Compound **20** obtained by reduction of the aldehyde using DIBAL was hydrogenated to give **4** (83%). On the other hand, the hydroxyl group of **20** was benzylated (**21**, 90%) and the Boc group was deprotected to give the amino compound **22** (92%) for the *N*-alkylation reaction. Compound **5** was obtained after methylation of **22** using formaldehyde under a reductive condition to give **23**, followed by hydrogenolysis.

Synthesis of pseudodisaccharide 1 (Scheme 3)

Adding a leaving group, or its mimic, to the leaving group position of these mimics is expected to increase its specificity and/or affinity against glycoenzymes.[4e,f,10] Therefore, we have conducted the synthesis of pseudodisaccharide **1**, using the synthesized five-membered iminocyclitol, as potential inhibitor of β-galactosyltransferase (GalT-ase), β-galactosidase (Gal-ase) and α-mannosidase (Man-ase). Compound **1** was designed to mimic the expected transition-state complex in the enzymatic transformation (Figures 1 and 3), since compound **1** can be considered as a mimic of β-D-Gal-(1→4)-β-D-GlcNAc-OR, or α-D-Man-(1→6)-β-D-GlcNAc-OR, both representing common mammalian disaccharides. Among these enzymes, it is known that GalT-ase has the most strict substrate specificity especially on the GlcNAc moiety. For this reason, we have decided, based on the mapping studies on the substrate specificity of GalT-ase,[11] that the acceptor C-6 of the GlcNAc residue to be used to connect with an iminocyclitol through a two-carbon spacer, which would permit the formation of a hydrogen bond between the ammonium hydrogen and the *O*-4 of GlcNAc.

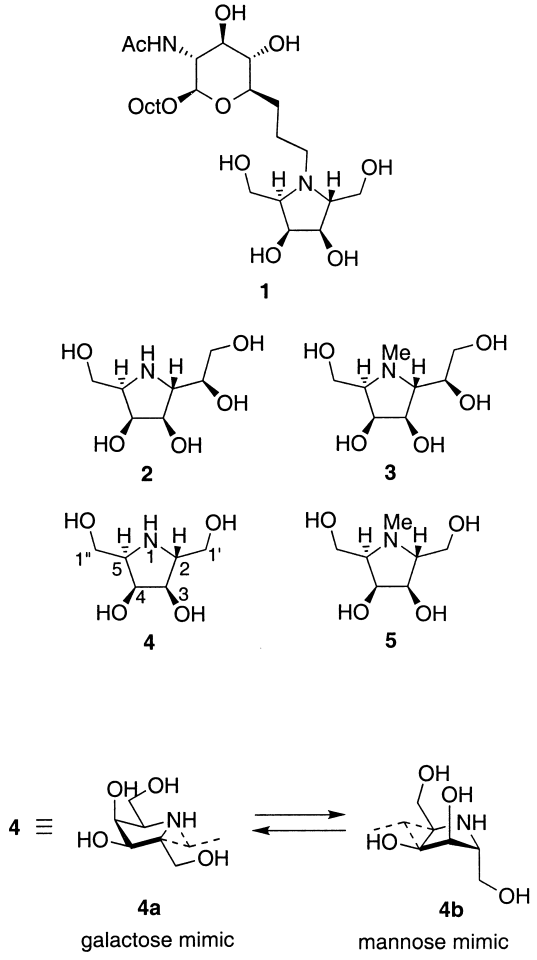
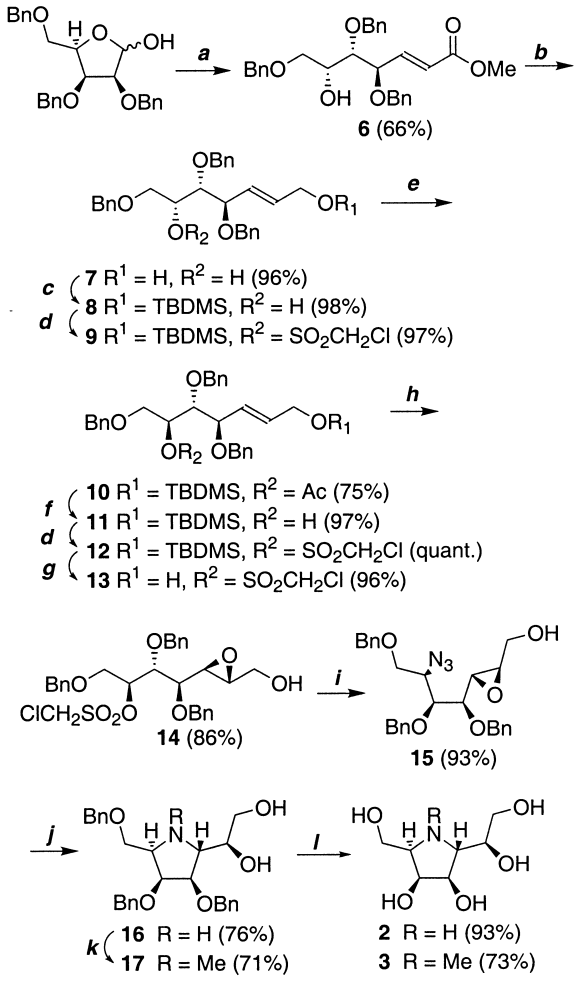


Figure 2. Structures of compounds **1–5** and their similarity to the transition state analogues of galactopyranoside and mannopyranoside hydrolysis.



Scheme 1. Reagents and conditions: (a) (1) $Ph_3P=CHCO_2Me$/benzene; (2) PhSSPh, hγ, cyclohexane; (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) M-HCl/THF; (h) *t*-BuOOH-Ti(O-*i*-Pr)$_4$-D-(−)-diethyltartrate-MS 4Å/CH_2Cl_2; (i) NaN_3/DMF; (j) Ph_3P/THF; (k) HCHO-$NaBH_3CN$/MeOH; (l) H_2-Pd(OH)$_2$-C/M HCl:MeOH (1:1).

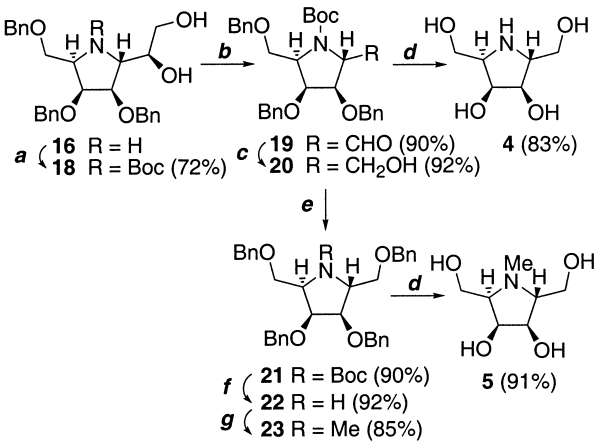
At first, the *N*-phthaloyl protected octyl β-D-glucosaminide[12] was treated with 1,1,2,2-tetramethoxycyclohexane[13] in the presence of acid catalysis in order to selectively protect the 1,2-*trans* equatorial diol system to afford **24** (72%). The aldehyde obtained by Swern oxidation of the remaining hydroxyl group was then coupled with methyl (triphenylphosphoranylidene)acetate to give **25** (93%). Reduction of the methoxycarbonyl group using DIBAL afforded **26** (74%), which was then treated with hydrazine hydrate in order to reduce the double bond and to deprotect the phthaloyl group at once. Selective *N*-acetylation of the obtained amine gave compound **27** (93%, two-steps). Swern oxidation of the product afforded the aldehyde **28** (90%) which was used for coupling with the protected iminocyclitol **22** in the presence of $NaBH_3CN$ to afford the conjugate **29** (77%). Compound **29** was finally deprotected via hydrogenolysis of the benzyl groups and acid hydrolysis of the cyclohexane-1,2-diacetal group to give compound **1** (63%).

Inhibition of glycosidases

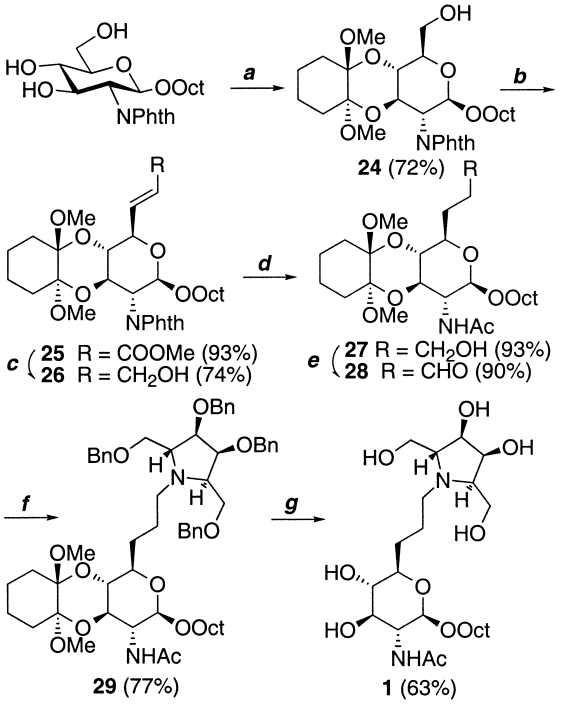
All compounds synthesized were found to be competitive inhibitors of both β-galactosidase (Gal-ase) and α-mannosidase (Man-ase) as shown in Table 1. Of these synthetic compounds, pseudodisaccharide **1** was found to be the most potent inhibitor of Gal-ase with $K_i = 3.3\,\mu M$. Iminocyclitols without an aglycon also showed a potent inhibitory activity in general. Indicating that the five-membered iminocyclitols may mimic both the *galacto* and *manno* configurations (see Fig. 2). Interestingly, the best inhibitor against Gal-ase was compound **1** and the worst was **4**, but the result was opposite for the inhibition of Man-ase. Together with this observation, the result shown in Table 1 indicates that Gal-ase tolerates and accepts *N*-alkylation and additional side-chain at C-2. On the other hand, Man-ase tolerates neither of these modifications. Compound **4** was found to be a strong inhibitor of Man-ase with apparent $K_i = 27\,\mu M$, compared to 43 μM for deoxymannojirimycin.[14]

Inhibition of β-galactosyltransferase (GalT-ase)

Iminocyclitols have been considered to mimic the transition state of the donor sugar moiety of glycosyltransferase reactions and have been shown to inhibit such enzymes.[4] Compounds **1–5** were examined as inhibitors of GalT-ase.



Scheme 2. Reagents and conditions: (a) $(Boc)_2O$-Et_3N/CH_2Cl_2; (b) $Pb(OAc)_4$/toluene; (c) DIBAL/CH_2Cl_2; (d) H_2-$Pd(OH)_2$-C/HCl-MeOH; (e) BnBr-Ag_2O-KI/DMF; (f) TFA/CH_2Cl_2; (g) HCHO-$NaBH_3CN$/MeOH.



Scheme 3. Reagents and conditions: (a) 1,1,2,2,-tetramethoxycyclohexane-CSA/$CH(OMe)_3$, MeOH; (b) (1) DMSO-$(COCl)_2/CH_2Cl_2$ then Et_3N; (2) $Ph_3P{=}CHCO_2Me$/benzene; (c) DIBAL/CH_2Cl_2; (d) (1) $H_2NNH_2{\cdot}H_2O$/EtOH; (2) Ac_2O/MeOH; (e) DMSO-$(COCl)_2/CH_2Cl_2$ then Et_3N; (f) **22**, $NaBH_3CN$/MeOH; (g) (1) H_2-$Pd(OH)_2$-C/THF-M HCl (2:1); (2) TFA-H_2O.

Among them, compounds **3**–**5** showed some inhibitory activity, and **4** was as potent as UDP ($IC_{50} = \sim 0.1$ mM, Table 2). The activities of these compounds were affected by pH of the reaction medium where stronger activities were observed at lower pH in general, despite that the inhibition of UDP was unaffected (Table 2). It was suggested that the protonated form of the iminocyclitol has high affinity to the enzyme with optimal pH around 8.[15] The pK_a values of some inhibitors (**4**, $pK_a = 8.0$; **5**, $pK_a = 7.0$) also support the assumption. The substrate inhibition of GalT-ase, usually occurring at concentrations over 0.2 mM for 4-methylumberiferyl

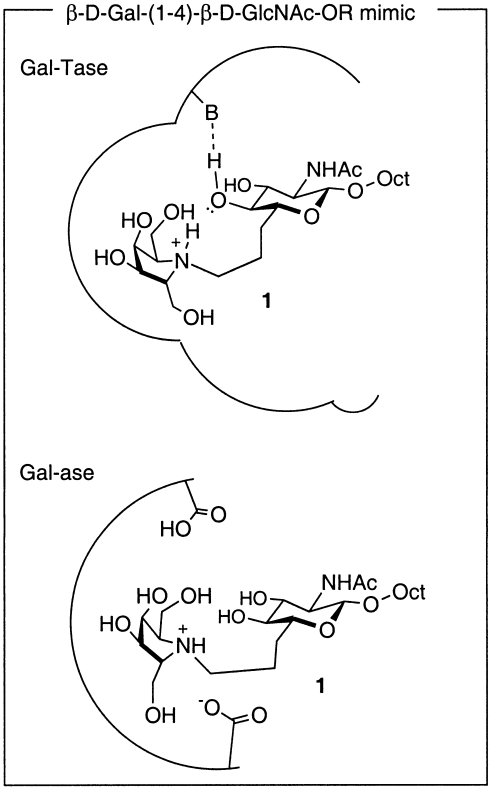


Figure 3. Pseudodisaccharide **1** may mimic the transition states of both β-galactosyltransferase and β-galactosidase.

Table 1. Inhibition assay results of **1**–**5** against β-galactosidase and α-mannosidase

Compound	K_i (M)	
	β-Galactosidase[a] *Aspergillus oryzae*	α-Mannosidase[b] Jack bean
1	3.3×10^{-6}	1.7×10^{-3}
2	8.5×10^{-4}	2.1×10^{-4}
3	3.1×10^{-4}	($IC_{50} > 4\times10^{-3}$)
4	1.0×10^{-3}	2.7×10^{-5}
5	7.6×10^{-5}	1.9×10^{-4}
Deoxy-Gal-NJ	4.3×10^{-6}	—
Deoxy-Man-NJ	—[c]	4.3×10^{-5}

[a] $K_m = 0.67 > \text{mM}$, $V_{max} = 0.6\,\mu\text{M/s/mg}$.
[b] $K_m = 1.02\,\text{mM}$, $V_{max} = 0.6\,\mu\text{M/s/mg}$.

Table 2. IC_{50} values of **1**–**5** against β-1,4-galactosyltransferase at different pH.[a]

Compound	IC_{50} (M)		
	pH 6.5	pH 7.5	pH 8.5
1	—[a]	—[a]	—[a]
2	—[a]	—[a]	—[a]
3	$> 10^{-2}$	Very weak	—[a]
4	1.0×10^{-4}	2.2×10^{-4}	7.5×10^{-4}
5	1.5×10^{-3}	$> 10^{-2}$	Very weak
UDP	1.1×10^{-4}	7.0×10^{-5}	1.3×10^{-4}

[a]Practically no inhibition.

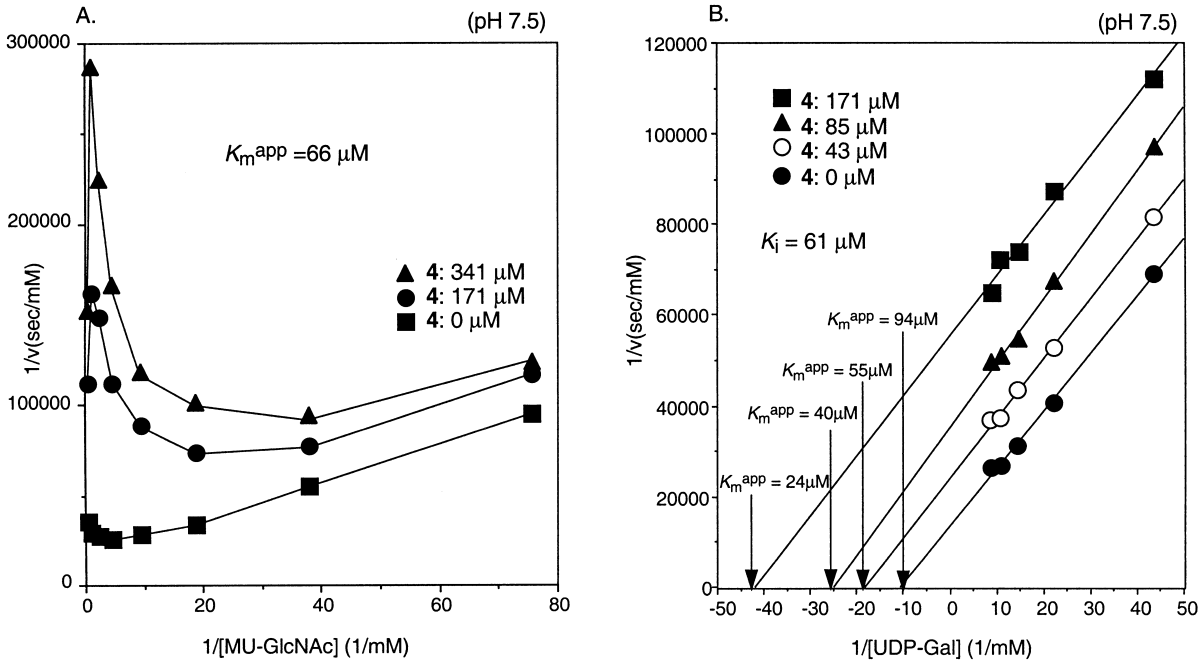


Figure 4. A. Effect of inhibitor **4** on the substrate inhibition of β-1,4-GalT-ase by MU-GlcNAc. B. Lineweaver–Burk plot of β-1,4-GalT-ase reaction using UDP-Gal in the presence of compound **4** . K_i of **4** toward UDP-Gal was estimated using the equation $K_m^{app} = K_m/(1+i/K_i)$.

β-*N*-acetylglucosaminide (MU-GlcNAc) as acceptor (Fig. 4(A)), was also affected by **4**. At lower concentrations, however, an uncompetitive type of inhibition was observed. In addition, compound **4** was shown to be an uncompetitive inhibitor versus UDP-Gal ($K_m = 94\,\mu M$) with apparent $K_i = 61\,\mu M$ (Fig. 4B). Synergistic effect with nucleoside, which is observed for inhibition of α-L-fucosyltransferase with azasugars,[4a,f,g] was not observed in this case.

Compound **1** designed to mimic the transition-state complex was shown not to inhibit GalT-ase at all. This result is somewhat puzzling because the previous data suggest that modification on acceptor GlcNAc is tolerated by the enzyme at position *O*-6.[10b,11] A possible reason would be the requirement of the hydrogen bond donor at the *O*-6 position of the acceptor by the enzyme. In this case, an ether-linked derivative would have potential. Another possibility would be due to the misorientation of **1** in the binding site or the sugar moieties contribute little to the overall binding energy.

Conclusion

A series of *galacto*-iminocyclitols and a pseudodisaccharide have been synthesized and evaluated as inhibitors of glycoenzymes using capillary electrophoresis. The results indicated that alkylation of the ring nitrogen of the iminocyclitols has no significant effect on β-galactosidase. Further, the pseudodisaccharide **1** exhibited a strong inhibition activity against β-galactosidase. The five-membered iminocyclitol **4** was shown to inhibit α-mannosidase ($K_i = 27\,\mu M$) and β-galactosyltransferase ($K_i = 61\,\mu M$ against UDP-Gal).

Experimental

General methods for 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 (Merck Kieselgel 60) or Iatro Beads (60 μ) (Dia-Iatron Laboratories Inc.) when specified. Gel permeation chromatography was performed using Bio Gel P-2. Melting points (mp) were measured with Yanaco MP-S3 micro melting point apparatus. Optical rotations were measured in a 1.0 dm tube with a Horiba SEPA-200 polarimeter at 25±1 °C. JEOL EX-270 spectrometer was used to obtain NMR spectra at 25 °C. ^{1}H NMR (270 MHz) were recorded 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 in $CDCl_3$ or D_2O using Me_4Si (δ 0.00), $CDCl_3$ (δ 77.00), or CD_3CN (δ 118.2) as the internal standard. Some key compounds were measured with JEOL 500 MHz spectrometer as indicated. Only partial assignments were reported. The FAB mass and HR FAB mass spectra were obtained on JEOL JMS HX-110 with glycerol and 3-nitrobenzylalcohol as the matrix. MALDITOF mass spectra were recorded on Kratos KOMPACT MALDI III with 2,5-dihydroxybenzoic acid as matrix. ESI mass spectra were measured with a triple stage quadrupole mass spectrometer (Finnigan MAT TSO 700) equipped with the ESI ion source.

Materials. 2,3,5-tri-*O*-Benzyllyxofuranose was prepared according to the described procedure.[7] Octyl 2-deoxy-2-phthalimido-β-D-glucopyranoside was synthesized according to the literature method.[12]

Methyl (4*R*,5*S*,6*R*)-6-hydroxy-4,5,7-tribenzyloxy-2(*E*)-heptenoate (6*E*). A solution of 2,3,5-tri-*O*-benzyllyxofuranose (2.29 g, 5.44 mmol) and methyl (triphenylphosphoranylidene)acetate (2.73 g, 8.16 mmol) in benzene (45 mL) was heated under reflux overnight. After cooling, the solvent was removed in vacuo and the crude mixture was purified by column chromatography (5:1 *n*-hexane:EtOAc). A mixture of **6** (*E*) and **6** (*Z*) was obtained (2.40 g, 93%, *E*:*Z* = 1:1). A solution of **6** (2.40 g, 5.04 mmol) and diphenyl disulfide (1.32 g, 6.05 mmol) in cyclohexane (50 mL) was irradiated with a 200 W lamp at rt for 5 days After removal of the solvent, the residue was purified by column chromatography (5:1 *n*-hexane:EtOAc) to give **6** (*E*) (1.58 g, 66% in two steps): $[\alpha]_D$ −16.6° (*c* 1.2, $CHCl_3$); ^{1}H NMR for **6** (*E*) ($CDCl_3$) δ 7.38–7.20 (m, 15H, aromatic), 7.00 (dd, 1H, *J* = 6.3, 15.8 Hz, H-3), 6.13 (dd, 1H, *J* = 1.3, 15.8 Hz, H-2), 4.63–4.39 (m, 6H, benzyl methylene), 4.28 (dt, 1H, *J* = 1.3, 6.3 Hz, H-4), 4.06–3.98 (m, 1H, H-6), 3.76 (s, 3H, OCH_3), 3.64 (dd, 1H, *J* = 2.6, 6.3 Hz, H-5), 3.52 (dd, 1H, *J* = 5.9, 9.8 Hz, H-7), 3.46 (dd, 1H, *J* = 6.3, 9.8 Hz, H-7), 2.65 (d, 1H, *J* = 6.6 Hz, OH); ^{13}C NMR ($CDCl_3$) δ 166.22 (C-1), 145.64 (C-3), 137.74, 137.34, 136.62, 128.48, 128.34, 128.27, 128.23, 128.09, 127.94, 127.73, 127.62, 127.54 and 127.48 (aromatic), 123.13 (C-2), 79.91 (C-5), 78.20 (C-4), 74.13, 73.24 and 71.68 (benzyl methylene), 70.73 (C-7), 69.42 (C-6), 51.54 (OCH_3); MALDITOF MS *m*/*z*: 499 $[M + Na]^+$. ^{1}H NMR for **6** (*Z*) ($CDCl_3$) δ 7.35–7.23 (m, 15H, aromatic), 6.32 (dd, 1H, *J* = 8.6, 11.7 Hz, H-3), 5.99 (dd, 1H, *J* = 1.2, 11.7 Hz, H-2), 5.51 (ddd, 1H, *J* = 1.2, 3.6, 8.6 Hz, H-4), 4.78 (d, 1H, *J* = 11.6 Hz, benzyl methylene), 4.63–4.38 (m, 5H, benzyl methylene), 3.99 (dt, 1H, *J* = 2.9, 5.4 Hz, H-6), 3.74 (dd, 1H, *J* = 2.9, 3.6 Hz, H-5), 3.69 (s, 3H, OCH_3), 3.53 (dd, 1H, *J* = 5.6, 9.6 Hz, H-7), 3.48 (dd, 1H, *J* = 6.1, 9.5 Hz, H-7), 3.25 (d, 1H, *J* = 4.3 Hz, OH); ^{13}C NMR ($CDCl_3$) δ 166.04 (C-1), 147.01 (C-3), 138.17, 137.84, 137.75, 128.44, 128.34, 128.26, 128.21, 127.89, 127.81, 127.74, 127.65 and 127.53 (aromatic), 122.62 (C-2), 79.41 (C-5), 75.81 (C-4), 73.21, 72.96 and 71.95 (benzyl methylene), 70.82 (C-7), 70.48 (C-6), 51.43 (OCH_3); MALDITOF MS *m*/*z*: 499 $[M + Na]^+$.

(4*R*,5*S*,6*R*)-4,5,7-Tribenzyloxy-2(*E*)-hepten-1,6-diol (7). To a solution of compound **6** (*E*) (133 mg, 0.28 mmol) in CH_2Cl_2 was added 0.98 M solution of DIBAL in *n*-hexane (0.86 mL, 3 equiv) at 0 °C. The reaction mixture was stirred at the temperature for 0.5 h. MeOH (0.14 mL) was added at 0 °C and the temperature was raised to rt. Saturated NaCl (0.3 mL) was added and the mixture was diluted with Et_2O (7 mL). $MgSO_4$ (0.72 g) was added and the whole mixture was stirred for 1 h, then filtered through a celite pad. The solvent was removed in vacuo and the crude mixture was purified by column chromatography (1:1 *n*-hexane:EtOAc) to give **7** (120 mg, 96%): $[\alpha]_D$ −31.6° (*c* 1.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.34–7.24 (m, 15H, aromatic), 5.88 (dt, 1H, *J* = 4.9, 15.5 Hz, H-2), 5.69 (dd, 1H, *J* = 7.4, 15.5 Hz, H-3), 4.66–4.35 (m, 6H, benzyl methylene), 4.15–4.00 (m, 4H, H-1, H-4, and H-6), 3.60 (dd, 1H, *J* = 3.0, 5.6 Hz, H-5), 3.51 (d, 2H, *J* = 5.9 Hz, H-7), 2.98 (dd, 1H, *J* = 2.3, 5.6 Hz, OH), 1.72 (brs, 1H, OH); ^{13}C NMR ($CDCl_3$) δ 137.95, 137.90 and 137.84 (aromatic), 134.18 (C-2), 128.25, 128.12, 127.87, 127.78, 127.71, 127.62 and 127.51 (C-3 and aromatic), 79.97 (C-5), 79.70 (C-4), 73.82, 73.23 and 70.57 (benzyl methylene), 70.91 (C-7), 69.62 (C-6), 62.46 (C-1); MALDITOF MS *m*/*z*: 471 $[M + Na]^+$.

(4*R*,5*S*,6*R*)-1-*tert*-Butyldimethylsilyloxy-4,5,7-tribenzyloxy-2(*E*)-hepten-6-ol (8). Compound **7** (2.72 g, 6.06 mmol) was dissolved in DMF (55 mL), to this solution was added TBDMSCl (1.37 g, 9.1 mmol), Et_3N (2.5 mL, 18 mmol) and DMAP (0.37 g, 3.0 mmol). 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 $MgSO_4$. After removal of solvent, the residue was purified by column chromatography (9:1 *n*-hexane:EtOAc) to give **8** (3.34 g, 98%) as colorless oil: $[\alpha]_D$ −29.6° (*c* 1.3, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.31–7.24 (m, 15H, aromatic), 5.85 (dt, 1H, *J* = 3.6, 15.5 Hz, H-2), 5.75 (dd, 1H, *J* = 7.1, 15.5 Hz, H-3), 4.69–4.34 (m, 6H, benzyl methylene), 4.22 (d, 2H, *J* = 3.6 Hz, H-1), 4.13–4.03 (m, 2H, H-4 and H-6), 3.58 (dd, 1H, *J* = 2.8, 5.8 Hz, H-5), 3.51 (d, 2H, *J* = 5.9 Hz, H-7), 2.88 (d, 1H, *J* = 5.9 Hz, OH), 0.91 (s, 9H, $OSiC(CH_3)_3$), 0.08 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 134.64 (C-2), 138.02, 128.30, 128.09, 127.78, 127.75, 127.67 and 127.57 (aromatic), 126.79 (C-3), 80.07 (C-5), 79.62 (C-4), 73.80, 73.24 and 70.51 (benzyl methylene), 70.98 (C-7), 69.67 (C-6), 62.93 (C-1), 25.86 ($C(CH_3)_3$), 18.31 ($C(CH_3)_3$), −5.28 ($Si(CH_3)_2$). Anal. calcd for $C_{34}H_{46}O_5Si$: C, 72.56; H, 8.24. Found: C, 72.29; H, 8.31.

(4*R*,5*S*,6*R*)-1-*tert*-Butyldimethylsilyloxy-6-[(chloromethylsulfonyl)-oxy]-4,5,7-tribenzyloxy-2(*E*)-heptene (9). A solution of compound **8** (5.97 g, 10.60 mmol) and chloromethylsulfonyl chloride (1.15 mL, 13 mmol) in pyridine (6 mL) was stirred at rt for 0.5 h, then the mixture was diluted with EtOAc and washed with H_2O and brine, dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (9:1 *n*-hexane:EtOAc) to give **9** (6.93 g, 97%) as a colorless oil: $[\alpha]_D$ +8.9° (*c* 2.1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 7.35–7.25 (m, 15H, aromatic), 5.92 (dt, 1H, *J* = 3.3, 15.5 Hz, H-2), 5.79 (dd, 1H, *J* = 7.9, 15.5 Hz, H-3), 5.20–5.12 (m, 1H, H-6), 4.75–4.39 (m, 8H, benzyl methylene and OSO_2CH_2Cl), 4.23 (brs, 2H, H-1), 4.10 (t, 1H, *J* = 8.0 Hz, H-4), 3.78–3.69 (m, 2H, H-5 and H-7), 3.50 (dd, 1H, *J* = 3.3, 10.9 Hz, H-7), 0.92 (s, 9H, $OSiC(CH_3)_3$), 0.08 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 138.02, 137.45 and 137.00 (aromatic), 135.95 (C-2), 128.52, 128.37, 128.17, 128.05, 127.99 and 127.65 (aromatic), 126.09 (C-3), 83.11 (C-6), 79.37 (C-5), 78.83 (C-4), 74.61, 73.44 and 70.35 (benzyl methylene), 69.52 (C-7), 62.77 (C-1), 54.25 (OSO_2CH_2Cl), 25.91 ($C(CH_3)_3$), 18.35 ($C(CH_3)_3$), −5.25 ($Si(CH_3)_2$). Anal. calcd for $C_{35}H_{47}O_7ClSSi$: C, 62.25; H, 7.02. Found: C, 61.77; H, 7.02.

(4*R*,5*S*,6*S*)-6-Acetoxy-1-*tert*-butyldimethylsilyloxy-4,5,7-tribenzyloxy-2(*E*)-heptene (10). A stirred mixture of compound **9** (1.88 g, 2.78 mmol), CsOAc (2.67 g, 14 mmol), and 18-Crown-6 (0.74 g, 2.8 mmol) in toluene (60 mL) was heated under reflux overnight. After cooling to rt, the reaction mixture was washed with H_2O and brine, dried over $MgSO_4$ and the solvent was removed in vacuo. The crude material was purified by column

chromatography (19:1 *n*-hexane:EtOAc) to afford **10** (1.26 g, 75%) as a colorless oil: $[\alpha]_D$ −38.6° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.32–7.24 (m, 15H, aromatic), 5.77–5.74 (m, 2H, H-2 and H-3), 5.24 (dt, 1H, *J* = 3.3, 5.6 Hz, H-6), 4.73–4.34 (m, 6H, benzyl methylene), 4.21 (br. s, 2H, H-1), 3.93 (dd, 1H, *J* = 4.3, 7.3 Hz, H-4), 3.86, (t, 1H, *J* = 5.1 Hz, H-5), 3.73 (dd, 1H, *J* = 5.9, 11.0 Hz, H-7), 3.66 (dd, 1H, *J* = 3.3, 11.0 Hz, H-7), 2.00 (s, 3H, $OCOCH_3$), 0.91 (s, 9H, $OSiC(CH_3)_3$), 0.07 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 170.00 (CO), 138.36 and 138.17 (aromatic), 135.16 (C-2), 128.28, 128.21, 127.94, 127.69, 127.62, 127.53 and 127.40 (aromatic) 126.23 (C-3), 80.07 (C-5), 79.78 (C-4), 74.05, 72.96 and 70.13 (benzyl methylene), 72.25 (C-6), 68.48 (C-7), 63.04 (C-1), 25.93 ($SiC(CH_3)_3$), 21.15 ($COCH_3$), 18.36 ($SiC(CH_3)_3$), −5.23 ($Si(CH_3)_2$). Anal. calcd for $C_{36}H_{48}O_6Si$: C, 71.49; H, 8.00. Found: C, 71.11; H, 8.04.

(4*R*,5*S*,6*S*)-1-*tert*-Butyldimethylsilyloxy-4,5,7-tribenzyloxy-2(*E*)-hepten-6-ol (11). Compound **10** (4.46 g, 7.33 mmol) was dissolved in MeOH (90 mL) and treated with 1 M solution of NaOMe (22 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 $MgSO_4$. The crude material was purified by column chromatography (19:1 *n*-hexane:EtOAc) to give **11** (4.00 g, 97%): $[\alpha]_D$ −43.1° (*c* 1.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.33–7.24 (m, 15H, aromatic), 5.92–5.76 (m, 2H, H-2 and H-3), 4.77–4.36 (m, 6H, benzyl methylene), 4.24–4.22 (m, 2H, H-1), 4.16 (dd, 1H, *J* = 4.3, 6.9 Hz, H-4), 3.88–3.80 (m, 1H, H-6), 3.69 (dd, 1H, *J* = 4.5, 7.8 Hz, H-7), 3.63–3.61 (m, 2H, H-5 and H-7), 2.77 (d, 1H, *J* = 4.6 Hz, OH), 0.91 (s, 9H, $OSiC(CH_3)_3$), 0.07 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 138.52, 138.43 and 138.06 (aromatic), 135.13 (C-2), 128.41, 128.34, 128.23, 128.01, 127.85, 127.71, 127.64, 127.53 and 127.46 (aromatic), 126.61 (C-3), 81.22 (C-4), 81.11 (C-5), 74.07, 73.39 and 70.31 (benzyl methylene), 71.09 (C-7), 71.00 (C-6), 63.16 (C-1), 25.93 ($C(CH_3)_3$), 18.38 ($C(CH_3)_3$), −5.17 ($Si(CH_3)_2$); MALDITOF MS *m*/*z*: 585 $[M + Na]^+$.

(4*R*,5*S*,6*S*)-1-*tert*-Butyldimethylsilyloxy-6-[(chloro-methylsulfonyl)-oxy]-4,5,7-tribenzyloxy-2(*E*)-heptene (12). A mixture of compound **11** (2.28 g, 4.06 mmol) and chloromethylsulfonyl chloride (440 μL, 4.9 mmol) in pyridine (5 mL) was stirred at rt for 0.5 h. The mixture was diluted with EtOAc, washed with H_2O and brine, and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (19:1 *n*-hexane:EtOAc) to give **12** (2.74 g, quant) as colorless oil: $[\alpha]_D$ −42.3° (*c* 1.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.35–7.24 (m, 15H, aromatic), 5.89 (dt, 1H, *J* = 4.3, 15.5 Hz, H-2), 5.68 (dd, 1H, *J* = 7.6, 15.5 Hz, H-3), 5.22 (dt, 1H, *J* = 2.3, 8.3 Hz, H-6), 4.71–4.52 (m, 7H, benzyl methylene and OSO_2CH_2Cl), 4.30 (d, 1H, *J* = 11.6 Hz, benzyl methylene), 4.23 (brm, 2H, H-1), 3.92 (t, 1H, *J* = 7.3 Hz, H-4), 3.81 (dd, 1H, *J* = 2.3, 7.3 Hz, H-5), 3.79 (dd, 1H, *J* = 7.3, 11.6 Hz, H-7), 3.65 (dd, 1H, *J* = 2.3, 11.6 Hz, H-7), 0.92 (s, 9H, $OSiC(CH_3)_3$), 0.09 (s, 6H, $OSi(CH_3)_2$); ^{13}C NMR ($CDCl_3$) δ 137.75, 137.48 and 137.29 (aromatic), 135.98 (C-2), 128.45, 128.36, 128.28, 128.12, 127.94, 127.89, 127.80 and 127.64 (aromatic), 125.82 (C-3), 84.85 (C-6), 81.17 (C-5), 78.53 (C-4), 74.29, 73.32, and 70.08 (benzyl methylene), 68.75 (C-7), 62.80 (C-1), 54.07 (OSO_2CH_2Cl), 25.88 ($C(CH_3)_3$), 18.33 ($C(CH_3)_3$), −5.28 ($Si(CH_3)_2$). Anal. calcd for $C_{35}H_{47}O_7ClSSi$: C, 62.25; H, 7.02. Found: C, 62.00; H, 7.02.

(4*R*,5*S*,6*S*)-6-[(Chloromethylsulfonyl)oxy]-4,5,7-tribenzyloxy-2(*E*)-hepten-1-ol (13). A solution of compound **12** (2.14 g, 3.16 mmol) in THF (15 mL) was treated with *N* HCl (15 mL) at rt overnight. The mixture was diluted with EtOAc and the organic layer was washed with aq $NaHCO_3$ and brine. After concentration the residue was purified by column chromatography (4:1 *n*-hexane: EtOAc) to afford **13** (1.70 g, 96%) as colorless oil: $[\alpha]_D$ −42.9° (*c* 1.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.35–7.24 (m, 15H, aromatic), 5.94 (dt, 1H, *J* = 5.1, 15.5 Hz, H-2), 5.64 (ddd, 1H, *J* = 1.0, 7.6, 15.5 Hz, H-3), 5.18 (dt, 1H, *J* = 3.4, 7.2 Hz, H-6), 4.72–4.30 (m, 8H, benzyl methylene and OSO_2CH_2Cl), 4.16 (brs, 2H, H-1), 3.96 (t, 1H, *J* = 6.8 Hz, H-4), 3.82 (dd, 1H, *J* = 3.6, 5.9 Hz, H-5), 3.78 (dd, 1H, *J* = 7.9, 11.6 Hz, H-7), 3.70 (dd, 1H, *J* = 2.0, 11.6 Hz, H-7), 1.68 (brs, 1H, OH); ^{13}C NMR ($CDCl_3$) δ 137.79, 137.45, and 137.23 (aromatic), 135.43 (C-2), 128.52, 128.37, 128.26, 127.96, 127.87 and 127.71 (aromatic), 127.20 (C-3), 84.62 (C-6), 80.93 (C-5), 78.54 (C-4), 74.16, 73.42 and 70.42 (benzyl methylene), 68.93 (C-7), 62.68 (C-1), 54.16 (OSO_2CH_2Cl); MALDITOF MS *m*/*z*: 583 $[M + Na]^+$.

(2*R*,3*S*,4*S*,5*S*,6*S*)-6-[(Chloromethylsulfonyl)oxy]-2,3-epoxy-4,5,7-tribenzyloxy-heptan-1-ol (14). A solution of Ti(O-*i*-Pr)$_4$ (2.9 mL, 9.8 mmol) and D-(−)-diethyltartrate (2.33 g, 11 mmol) in CH_2Cl_2 (50 mL) was stirred at −25 °C for 0.5 h in the presence of MS 4 Å. To this mixture was added a solution of compound **13** (2.71 g, 4.83 mmol) in CH_2Cl_2 (5 mL), and the mixture was stirred at the temperature for 0.5 h. 5 M solution of *t*-BuOOH (2.9 mL, 15 mmol) was added and the mixture was stirred at the same temperature for 90 h. Dimethyl sulfide (1.3 mL) was added at −25 °C and stirred for 1.5 h at the temperature. 10% Solution of tartaric acid (15 mL) and Et_2O (30 mL) were 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, and the solvent was removed. The residue was dissolved in Et_2O (30 mL) and stirred with 10% NaOH solution (25 mL) 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 (2:1 *n*-hexane:EtOAc) to afford **14** (2.25 g, 86%) as colorless oil: $[\alpha]_D$ −19.0° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.37–7.26 (m, 15H, aromatic), 5.24 (dt, 1H, *J* = 2.5, 8.0 Hz, H-6), 4.83–4.43 (m, 8H, benzyl methylene and OSO_2CH_2Cl), 3.87 (dd, 1H, *J* = 2.5, 7.0 Hz, H-5), 3.81 (dd, 1H, *J* = 2.6, 12.9 Hz, H-1), 3.75 (dd, 1H, *J* = 8.0, 11.0 Hz, H-7), 3.60 (dd, 1H, *J* = 2.5, 11.0 Hz, H-7), 3.55 (dd, 1H, *J* = 3.5, 12.5 Hz, H-1), 3.36 (t, 1H, *J* = 7.0 Hz, H-4), 3.23 (dd, 1H, *J* = 2.3, 7.0 Hz, H-3), 2.99 (dt, 1H, *J* = 2.3, 3.5 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 137.27, 137.07, 136.95, 128.48, 128.39, 128.23, 128.14, 127.98 and 127.84 (aromatic), 84.55 (C-6), 79.21 (C-5), 78.06 (C-4), 73.80, 73.41 and 72.31 (benzyl methylene), 68.70 (C-7), 60.72 (C-1), 56.32 (C-3), 55.33 (C-2), 54.05 (OSO_2CH_2Cl).

(2*R*,3*S*,4*S*,5*S*,6*R*)-6-Azide-2,3 epoxy-4,5,7-tribenzyloxy-heptan-1-ol (15). Compound **14** (2.23 g, 3.86 mmol) was dissolved with DMF (50 mL) and treated with NaN_3 (0.64 g, 9.8 mmol) at 70 °C for 1 h. The mixture was diluted with EtOAc at rt and washed with H_2O and brine, dried and the solvent was removed. The residue was purified by column chromatography (2:1 *n*-hexane: EtOAc) to give **15** (1.76 g, 93%) as colorless oil: $[\alpha]_D$ −18.5° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.34–7.23 (m, 15H, aromatic), 4.83–4.41 (m, 6H, benzyl methylene), 3.84–3.78 (m, 2H, H-1 and H-6), 3.72 (dd, 1H, *J* = 2.3, 8.2 Hz, H-5), 3.65 (dd, 1H, *J* = 7.2, 9.2 Hz, H-7), 3.57–3.52 (m, 3H, H-1, H-4 and H-7), 3.26 (dd, 1H, *J* = 2.3, 6.6 Hz, H-3), 3.09 (dt, 1H, *J* = 2.3, 3.6 Hz, H-2); ^{13}C NMR ($CDCl_3$) δ 137.56, 137.38, 128.52, 128.41, 128.18, 128.05, 127.84, 127.71, 127.55 and 127.42 (aromatic), 78.73 (C-4), 78.46 (C-5), 74.66, 73.32 and 72.90 (benzyl methylene), 69.17 (C-7), 60.68 (C-1), 60.63 (C-6), 56.17 (C-3), 55.62 (C-2); MALDITOF MS *m*/*z*: 512 $[M+Na]^+$.

(1′*R*,2*R*,3*R*,4*S*,5*R*)-[3,4-Dibenzyloxy-5-benzyloxymethyl-2-(1′,2′-dihydroxy-ethyl)]-pyrrolidine (16). A solution of compound **15** (510 mg, 1.04 mmol) and triphenylphosphine (0.42 g, 1.6 mmol) in THF (10 mL) was stirred at rt for 4 days After removal of the solvent, the residual mixture was purified by column chromatography (98:1:1 $CHCl_3$:MeOH:Et_3N) to afford **16** (367 mg, 76%), as colorless powder: mp 95 °C; [α]D +36.2° (*c* 0.5, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.33–7.28 (m, 15H, aromatic), 4.69–4.42 (m, 6H, benzyl methylene), 4.07 (t, 1H, *J* = 4.0 Hz, H-4), 3.96 (dd, 1H, *J* = 4.0, 7.3 Hz, H-3), 3.71–3.59 (m, 5H, H-1′, H-2′ and H-1″), 3.51–3.40 (m, 2H, H-2 and H-5), 3.13 (brs, 2H, OH); ^{13}C NMR ($CDCl_3$) δ 138.20, 137.88, 137.41, 128.41, 128.37, 128.28, 127.82 and 127.69 (aromatic), 80.43 (C-3), 77.70 (C-4), 73.32, 73.23 and 72.40 (benzyl methylene), 71.91 (C-1′) 69.00 (C-1″), 64.26 (C-2′), 62.23 (C-2 or C-5), 59.10 (C-2 or C-5). Anal. calcd for $C_{28}H_{33}NO_5$: C, 72.55; H, 7.17; N, 3.02. Found: C, 72.03; H, 7.21; N, 2.89.

(1′*R*,2*R*,3*R*,4*S*,5*R*)-[3,4-Dihydroxy-5-hydroxymethyl-2-(1′,2′-dihydroxy-ethyl)]-pyrrolidine (2). A solution of **16** (15 mg, 0.03 mmol) in MeOH (0.5 mL) and M HCl (0.5 mL) was stirred with a catalytic amount of 20% $Pd(OH)_2$ on C under H_2 atmosphere at rt overnight. The crude material, obtained after removal of the catalyst and the solvent, was treated with Dowex 1X8 (OH^-) eluted with water to give **2** (5.7 mg, 93%); mp 134 °C; $[\alpha]_D$ +24.6° (*c* 1.2, H_2O). Compound **2** was further purified for inhibition assay using Sep-Pak PLUS CM, regenerated with M HCl (10 mL) and water (20 mL), eluted with water (20 mL) and 10% NH_3 (10 mL). The latter eluent containing **2** was filtered through a Millex GV filter and lyophilized. ^1H NMR (D_2O) δ 4.23 (dd, 1H, *J* = 4.3, 8.1 Hz, H-3), 4.19 (t, 1H, *J* = 4.3 Hz, H-4), 3.84–3.80 (m, 1H, H-1′), 3.80 (dd, 1H, *J* = 6.5, 11.1 Hz, H-1″), 3.73 (dd, 1H, *J* = 3.6, 11.9 Hz, H-2′), 3.66 (dd, 1H, *J* = 6.5, 11.1 Hz, H-1″), 3.63 (dd, 1H, *J* = 7.3, 11.9 Hz, H-2′), 3.34 (dt, 1H, *J* = 3.5, 6.5 Hz, H-5), 3.13 (dd, 1H, *J* = 5.2, 8.1 Hz, H-2); ^{13}C NMR (D_2O) δ 72.15 (C-3), 71.49 (C-1′), 71.27 (C-4), 62.49 (C-2′), 60.48 (C-2), 59.29 (C-5 or C-1″), 59.20 (C-5 or C-1″). Anal. calcd for $C_7H_{15}NO_5$: C, 43.52; H, 7.83; N, 7.25. Found: C, 43.18; H, 7.68; N, 6.95.

(1′*R*,2*R*,3*R*,4*S*,5*R*)-*N*-Methyl-[3,4-dibenzyloxy-5-benzyloxymethyl-2-(1′,2′-dihydroxy-ethyl)]-pyrrolidine (17). To a solution of **16** (46 mg, 0.12 mmol) dissolved in MeOH (1 mL) at 0 °C was added a 37% formaldehyde solution (0.1 mL, 1.2 mmol) and $NaBH_3CN$ (22 mg, 0.33 mmol). The mixture was stirred at rt overnight. The reaction mixture was added H_2O, extracted with $CHCl_3$ and dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (99:2:1 $CHCl_3$:MeOH:Et_3N) to yield **17** (33 mg, 71%) as colorless oil: $[\alpha]_D$ +32.9° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.31–7.24 (m, 15H, aromatic), 4.72–4.45 (m, 6H, benzyl methylene), 3.97–3.91 (m, 2H, H-3 and H-4), 3.84–3.77 (m, 3H, H-1′ and H-1″), 3.57–3.50 (m, 3H, H-2′ and H-5), 2.82 (t, 1H, *J* = 3.6 Hz, H-2), 2.75 (brs, 2H, OH), 2.51 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.29, 138.20, 138.13, 128.39, 127.85, 127.62 and 127.44 (aromatic), 79.05 (C-3 or C-4), 77.52 (C-3 or C-4), 73.33, 72.76 and 72.69 (benzyl methylene), 70.53 (C-2), 68.88 (C-1′), 66.40 (C-1″), 64.17 (C-2′), 63.92 (C-5), 36.95 (CH_3).

(1′*R*,2*R*,3*R*,4*S*,5*R*)-*N*-Methyl-[3,4-dihydroxy-5-hydroxymethyl-2-(1′,2′-dihydroxy-ethyl)ethyl]-pyrrolidine (3). To compound **17** (22 mg, 0.045 mmol) dissolved in MeOH (0.5 mL) and 1 M HCl (0.5 mL) was added a catalytic amount of 20% $Pd(OH)_2$ on C. The reaction mixture was stirred under H_2 atmosphere at rt for 3 days After filtration and evaporation of the solvent, the residue was purified on a column of Iatro Beads (50:50:1 $CHCl_3$: MeOH:28% NH_3) to afford **3** (7 mg, 73%); mp 147 °C; $[\alpha]_D$ +20.9° (*c* 0.6, H_2O). Compound **3** was further purified for inhibition assay using Sep-Pak PLUS CM, regenerated with M HCl (10 mL) and water (20 mL), eluted with water (20 mL) and 10% NH_3 (10 mL). The latter eluent containing **3** was filtered through a Millex GV filter and lyophilized. ^1H NMR (D_2O) δ 4.21–4.17 (m, 2H, H-3 and H-4), 3.97–3.92 (m, 2H, H-1′ and H-1″), 3.80 (dd, 1H, *J* = 4.4, 12.2 Hz, H-1″), 3.69 (dd, 1H, *J* = 4.4, 11.7 Hz, H-2′), 3.63 (dd, 1H, *J* = 7.5, 11.7 Hz, H-2′), 3.18 (brd, 1H, *J* = 4.4 Hz, H-5), 2.87 (s, 1H, H-2), 2.49 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 70.57 (C-2), 70.13 (C-3 or C-4), 69.35 (C-3 or C-4), 68.56 (C-1′), 64.87 (C-5), 62.48 (C-2′), 56.15 (C-1″), 34.19 (CH_3); HRFAB MS calcd for $C_8H_{17}NO_5$; $[M+H]^+$ 208.1185; found: *m*/*z* 208.1185.

(1′*R*,2*R*,3*R*,4*S*,5*R*)-*N*-Butyloxycarbonyl-[3,4-dibenzyloxy-5-benzyloxymethyl-2-(1′,2′-dihydroxy-ethyl)]-pyrrolidine (18). To a solution of **16** (183 mg, 0.39 mmol) in CH_2Cl_2 (4 mL) and Et_3N (66 μL, 0.47 mmol), $(Boc)_2O$ (220 μL, 0.96 mmol) was added at 0 °C and the mixture was stirred at rt overnight. 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 by column chromatography (1:1 *n*-hexane:EtOAc) to afford **18** (160 mg, 72%) as colorless oil: $[\alpha]_D$ +8.6° (*c* 2.7, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.32–7.23 (m, 15H, aromatic), 4.74–4.43 (m, 6H, benzyl methylene), 4.26 (dd, 1H, *J* = 6.3, 8.9 Hz, H-2′ or H-1″), 4.18–4.08 (m,

3H, H-3, H-4 and H-5), 3.85–3.80 (m, 2H, H-2 and OH), 3.55–3.46 (m, 3H, H-2′ and H-1″), 3.25 (brs, 1H, H-1′), 3.11 (brt, 1H, $J=8.6$ Hz, OH), 1.43 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 156.10 (NCOO), 138.37, 138.28, 138.08, 128.28, 128.23, 128.18, 127.82, 127.58, 127.51, 127.46, 127.39 and 127.28 (aromatic), 81.12 ($C(CH_3)_3$), 79.08 (C-3 or C-4), 77.32 (C-3 or C-4), 73.14 and 72.13 (benzyl methylene), 71.93 (C-1′ and benzyl methylene), 70.10 (C-2′ or C-1″), 62.95 (C-2), 62.33 (C-2′ or C-1″), 58.91 (C-5), 28.26 ($C(CH_3)_3$); MALDITOF MS m/z: 586 $[M+Na]^+$.

(2*S*,3*R*,4*S*,5*R*)-*N*-Butyloxycarbonyl-(3,4-dibenzyloxy-5-benzyloxymethyl)-pyrrolidine-2-carbaldehyde (19). To a solution of compound **18** (1.02 g, 1.80 mmol) in toluene (20 mL), $Pb(OAc)_4$ (1.34 g, 2.7 mmol) was added. 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 by column chromatography (9:1 *n*-hexane:EtOAc) to afford **19** (0.86 g, 90%): $[\alpha]_D$ +1.7° (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$) showed **19** exist as 1:1 mixture of two conformational isomers. ^1H NMR: δ 9.48 (d, 1H, $J=1.7$ Hz, CHO), 9.40 (d, 1H, $J=2.6$ Hz, CHO), 7.32–7.24 (m, 30H), 4.75–4.36 (m, 12H), 4.34–4.26 (m, 4H), 4.22–4.05 (m, 2H), 4.02–3.92 (m, 4H), 3.73–3.68 (m, 2H), 1.40, 1.43 (s, each 3H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 198.42 and 198.06 (CHO), 154.50 and 153.06 (NCOO),137.2–138.6, 127.3–128.8 (aromatic), 81.21 and 81.13 ($C(CH_3)_3$), 78.64, 78.19, 77.31 and 77.20 (C-3 and C-4), 73.17, 72.96, 72.89, 72.60, 72.54 and 72.42 (benzyl methylene), 69.60, 69.15, 68.47, 58.01, 57.79, 28.21 and 28.12 ($C(CH_3)_3$).

(2*R*,3*R*,4*S*,5*R*)-*N*-Butyloxycarbonyl-(3,4-dibenzyloxy-5-benzyloxymethyl-2-hydroxymethyl)-pyrrolidine (20). To a solution of **19** (0.86 g, 1.62 mmol) in CH_2Cl_2 (20 mL) cooled to 0 °C was added a 0.98 M solution of DIBAL in *n*-hexane (3.3 mL, 2 equiv) and the resulting mixture was stirred for 1.5 h. MeOH (0.5 mL) was added at 0 °C and the temperature was raised to rt. Saturated NaCl (1 mL) was added and the mixture was diluted with Et_2O (27 mL). $MgSO_4$ (2.80 g) was added and the whole mixture was stirred for 1 h, then filtered through a Celite pad. The solvent was removed in vacuo and the crude mixture was purified by column chromatography (2:1 *n*-hexane:EtOAc) to give **20** (0.79 g, 92%) as colorless oil: $[\alpha]_D$ +7.8° (*c* 1.4, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.32–7.23 (m, 15H, aromatic), 4.77–4.44 (m, 6H, benzyl methylene), 4.16–4.11 (m, 3H), 3.98 (brs, 1H), 3.83 (brs, 1H), 3.64 (brs, 3H), 1.43 (s, 9H, $C(CH_3)_3$); ^{13}C NMR ($CDCl_3$) δ 155.65 (NCOO), 138.40, 138.20, 138.02, 128.25, 128.16, 127.75, 127.62, 127.53, 127.48 and 127.31 (aromatic), 80.68 ($C(CH_3)_3$), 79.41 (C-3 or C-4), 77.20 (C-3 or C-4), 73.14, 72.45 and 72.17 (benzyle methylene), 69.74 (C-1′ or C-1″), 64.17 (C-2 or C-5), 64.06 (C-1′ or C-1″), 58.71 (C-2 or C-5), 28.3 ($C(CH_3)$). Anal. calcd for $C_{32}H_{39}NO_6$: C, 72.02; H, 7.37; N, 2.62. Found: C, 71.54; H, 7.44; N, 2.56.

(2*R*,3*R*,4*S*,5*R*)-3,4-Dihydroxy-2,5-dihydroxymethyl-pyrrolidine (4). Hydrogenolysis of **20** (29 mg, 0.05 mmol) was carried out as described for the synthesis of **2** to give **4** (7 mg, 83%); $[\alpha]_D$ +34.8° (*c* 0.7, H_2O). Compound **4** was further purified for inhibition assay using Sep-Pak PLUS CM, regenerated with M HCl (10 mL) and water (20 mL), eluted with water (20 mL) and 10% NH_3 (10 mL). The latter eluent containing **4** was filtered through a Millex GV filter and lyophilized. ^1H NMR (D_2O) δ 4.19 (t, 1H, $J=4.2$ Hz, H-4), 4.00 (dd, 1H, $J=4.2$, 8.4 Hz, H-3), 3.80 (dd, 1H, $J=6.7$, 11.0 Hz, H-1″), 3.76 (dd, 1H, $J=3.8$, 11.6 Hz, H-1′), 3.65 (dd, 1H, $J=5.9$, 11.6 Hz, H-1′), 3.65 (dd, 1H, $J=6.7$, 11.0 Hz, H-1″), 3.33 (dt, 1H, $J=3.8$, 6.7 Hz, H-5), 3.15 (ddd, 1H, $J=3.8$, 5.9, 8.4 Hz, H-2); ^{13}C NMR (D_2O) δ 71.99 (C-3), 70.53 (C-4), 60.53 (C1′ or C-1″), 60.01 (C-2), 59.43 (C-1′ or C-1″), 58.75 (C-5); HRFAB MS calcd for $C_6H_{13}NO_4$: $[M+H]^+$ 164.0923; found: m/z 164.0920. Other physical data are also in good agreement to those of reported values.[8]

(2*R*,3*R*,4*S*,5*R*)-*N*-Butyloxycarbonyl-(3,4-dibenzyloxy-2,5-dibenzyloxymethyl)-pyrrolidine (21). To a solution of **20** (63 mg, 0.12 mmol) in DMF (1 mL) was successively added Ag_2O (160 mg, 0.69 mmol), BnBr (86 μL, 0.69 mmol), and KI (62 mg, 0.37 mmol) at 0 °C. The reaction mixture was stirred at rt for 1 h, then diluted with EtOAc. After filtration through a Celite pad, the mixture was extracted with EtOAc, the organic layer was washed with H_2O and dried over $MgSO_4$, and concentrated. The residue was purified by column chromatography (9:1 *n*-hexane:EtOAc) to yield **21** (66 mg, 90%): $[\alpha]_D$ −0.7° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) showed **21** exists as a 2:1 mixture of two conformational isomers. Due to the complex pattern of the spectrum, the assignment was not made. δ 7.57–7.18 (m, 20H), 4.77–4.38 (m), 4.13–4.29 (m), 4.07–3.98 (m), 3.85 (d, minor, $J=4.3$ Hz), 3.75 (d, minor, $J=8.6$ Hz), 3.61–3.51 (m), 3.35 (dd, minor, $J=6.9$, 9.6 Hz), 1.43 (s), 1.39 (s); ^{13}C NMR ($CDCl_3$) δ 154.05, 153.46, 137.9–139.0, 126.9–128.4, 80.50, 79.88, 79.80, 79.40, 77.22, 76.73, 73.16, 72.80, 72.71, 72.44, 72.33, 72.18, 71.93, 70.40, 69.13, 68.99, 68.18, 61.57, 61.49, 58.13, 57.63, 28.4; MALDITOF MS m/z: 646 $[M+Na]^+$.

(2*R*,3*R*,4*S*,5*R*)-3,4-Dibenzyloxy-2,5-dibenzyloxymethyl-pyrrolidine (22). Compound **21** (219 mg, 0.35 mmol) was treated with TFA:CH_2Cl_2 (1:2 v/v, 6 mL) at rt for 1 h. The resulting solution was added saturated $NaHCO_3$ and extracted with CH_2Cl_2. The combined organic layers were dried over $MgSO_4$, filtered, evaporated, and purified by column chromatography (50:50:1 *n*-hexane: EtOAc-Et_3N) to afford **22** (170 mg, 92%): $[\alpha]_D$ +40.2° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.32–7.24 (m, 20H, aromatic), 4.72 (d, 1H, $J=11.9$ Hz, benzyl methylene), 4.61–4.41 (m, 7H, benzyl methylene), 4.05 (t, 1H, $J=4.4$ Hz, H-4) 3.83 (dd, 1H, $J=4.4$, 6.3 Hz, H-3), 3.70 (dd, 1H, $J=6.3$, 9.0 Hz, H-1′ or H-1″), 3.62 (dd, 1H, $J=7.3$, 9.0 Hz, H-1′ or H-1″), 3.54–3.44 (m, 4H, H-2, H-5 and H-1′ or H-1″), 2.19 (brs, 1H, NH); ^{13}C NMR ($CDCl_3$) δ 138.53, 138.20, 138.10, 128.16, 128.09, 127.66, 127.60, 127.49, 127.46, 127.42 and 127.33 (aromatic), 80.59 (C-3), 77.72 (C-4), 73.16, 72.99 and 72.02 (benzyl methylene), 70.63 (C-1′ or C-1″), 69.83 (C-1′ or C-1″), 59.77 (C-2 or C-5), 58.76 (C-2 or C-5); MALDITOF MS m/z: 524 $[M+H]^+$.

(2*R*,3*R*,4*S*,5*R*)-*N*-Methyl-(3,4-dibenzyloxy-2,5-benzyloxymethyl)-pyrrolidine (23). To a solution of **22** (41 mg, 0.08 mmol) in MeOH (1 mL) at 0 °C was added a 37% formaldehyde solution (0.1 mL, 1.2 mmol) and $NaBH_3CN$ (10 mg, 0.15 mmol). The mixture was stirred at rt overnight. The reaction mixture was added H_2O, extracted with $CHCl_3$ and dried with $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (1:1 *n*-hexane:EtOAc) to yield **23** (36 mg, 85%): $[\alpha]_D$ +10.8° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.32–7.23 (m, 20H, aromatic), 4.64–4.42 (m, 8H, benzyl methylene), 4.01 (t, 1H, *J* = 5.6 Hz, H-4), 3.86–3.81 (m, 3H, H-3 and H-1″), 3.49–3.32 (m, 3H, H-1′ and H-5), 3.01–2.96 (m, 1H, H-2), 2.53 (s, 3H, CH_3); ^{13}C NMR ($CDCl_3$) δ 138.67, 138.43, 138.24, 128.32, 128.28, 128.19, 127.73, 127.67, 127.64, 127.58, 127.49, 127.42 and 127.37 (aromatic), 79.62 (C-3), 77.91 (C-4), 73.28, 73.22, 72.31 and 71.75 (benzyl methylene), 69.67 (C-1′), 68.09 (C-2), 67.65 (C-1″), 64.83 (C-5), 37.11 (CH_3). Anal. calcd for $C_{35}H_{39}NO_4$: C, 78.18; H, 7.31; N, 2.60. Found: C, 77.75; H, 7.42; N, 2.58.

(2*R*,3*R*,4*S*,5*R*)-*N*-Methyl-(3,4-dihydroxy-2,5-hydroxymethyl)-pyrrolidine (5). Compound **5** was synthesized from **23** (30 mg, 0.055 mmol) according to the procedure described for the synthesis of compound **2** to afford **5** (9 mg, 91%): $[\alpha]_D$-16.6° (*c* 0.4, H_2O); ^1H NMR (D_2O) δ 4.25 (t, 1H, *J* = 5.6 Hz, H-4), 4.07 (t, 1H, *J* = 5.1 Hz, H-3), 3.92 (dd, 1H, *J* = 5.1, 11.8 Hz, H-1″), 3.79 (dd, 1H, *J* = 4.4, 11.8 Hz, H-1″), 3.76 (dd, 1H, *J* = 5.1, 11.8 Hz, H-1′), 3.68 (dd, 1H, *J* = 5.1, 11.8 Hz, H-1′), 3.12 (q, 1H, *J* = 5.1 Hz, H-5), 2.95 (q, 1H, *J* = 5.1 Hz, H-2), 2.50 (s, 3H, CH_3); ^{13}C NMR (D_2O) δ 71.58 (C-3), 69.78 (C-4), 69.23 (C-2), 65.21 (C-5), 59.35 (C-1′), 56.53 (C-1″), 34.59 (CH_3); HRFAB MS calcd for $C_7H_{15}NO_4$ $[M+H]^+$ 178.1079; found: *m*/*z* 178.1096

Octyl 2-deoxy-3,4-*O*-[(1′*S*,2′*S*)-dimethoxycyclohexylidene]-2-phthalimido-β-D-glucopyranoside (24). A catalytic amount of CSA was added to a solution of octyl 2-deoxy-2-phthalimido-β-D-glucopyranoside (115 mg, 0.27 mmol), 1,1,2,2-tetramethoxy cyclohexane (92 mg, 0.45 mmol) and trimethyl orthoformate (0.5 mL) in MeOH (3 mL), and the mixture was heated under reflux overnight. After neutralization with $NaHCO_3$, the solvent was removed in vacuo and the crude material was purified by column chromatography (9:1 *n*-hexane:EtOAc) to give **24** (111 mg, 72%) as white powder and the unreacted starting material (11 mg, 9%): mp 119 °C; $[\alpha]_D$ +113.7° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.91–7.69 (m, 4H, aromatic), 5.18 (d, 1H, *J* = 8.3 Hz, H-1), 4.86 (dd, 1H, *J* = 9.9, 11.6 Hz, H-3), 4.28 (dd, 1H, *J* = 8.3, 11.6 Hz, H-2), 3.94 (t, 2H, *J* = 9.9 Hz, H-4 and H-6), 3.85–3.70 (m, 3H, H-5, H-6 and $CH_2(CH_2)_6CH_3$), 3.39 (dt, 1H, *J* = 6.6, 9.6 Hz, $CH_2(CH_2)_6CH_3$), 3.23 and 3.05 (s, each 3H, OCH_3), 2.02 (brs, 1H, OH) 1.81–0.89, (m, 20H, $CH_2(CH_2)_6CH_3$ and cyclohexyl-H), 0.81 (t, 3H, *J* = 7.1 Hz, $((CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$) δ 168.39 and 167.44 (CO), 134.03, 133.94, 131.79, 131.59, 123.65 and 123.0 (aromatic), 99.07, 98.78 and 98.53 (C-1 and $COCH_3$), 73.95 (C-5), 69.96 ($CH_2(CH_2)_6CH_3$), 68.27 (C-4), 66.51 (C-3), 61.33 (C-6), 54.09 (C-2), 46.92 and 46.70 ($COCH_3$), 31.59, 29.26, 29.06, 26.90, 25.72, 22.52, 21.30 and 21.22 (cyclohexyl-C and $CH_2(CH_2)_6CH_3$), 14.00 ($(CH_2)_7CH_3$). Anal. calcd for $C_{30}H_{43}NO_9$: C, 64.15; H, 7.72; N, 2.49. Found: C, 63.96; H, 7.76; N, 2.47.

Octyl 2,6,7-trideoxy-7-methoxycarbonyl-3,4-*O*-[(1′*S*,2′*S*)-dimethoxycyclohexylidene]-2-phthalimido-β-D-glucooct-6(*E*)-enopyranoside (25). To a solution of DMSO (220 μL, 3.5 mmol) in CH_2Cl_2 (6 mL) was added a 2 M solution of oxalyl chloride in CH_2Cl_2 (0.9 mL, 1.8 mmol) at −78 °C and the mixture was stirred at the temperature for 0.5 h. To this mixture was added a solution of compound **24** (507 mg, 0.90 mmol) in CH_2Cl_2 (4 mL) and the mixture was stirred at the temperature for 0.5 h. Et_3N (1.0 mL, 7.2 mmol) was added and the mixture was stirred at rt for 15 min, then the mixture was diluted with CH_2Cl_2 and washed with H_2O, dried over $MgSO_4$, and evaporated in vacuo. The resulting crude aldehyde was used for the next step without further purification. The solution of crude aldehyde and methyl (triphenylphosphoranylidene)acetate (0.45 g, 1.3 mmol) in benzene (10 mL) was heated under reflux overnight. After cooling, the solvent was removed in vacuo and the crude mixture was purified by column chromatography (5:1 *n*-hexane-EtOAc). *E*-isomer **25** (492 mg, 93% in two steps) was obtained as a sole product: mp 79 °C; $[\alpha]_D$ +104.3° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.91–7.72 (m, 4H, aromatic), 7.11 (dd, 1H, *J* = 4.0, 15.8 Hz, H-6), 6.24 (dd, 1H, *J* = 1.7, 15.8 Hz, H-7), 5.20 (d, 1H, *J* = 8.3 Hz, H-1), 4.88 (dd, 1H, *J* = 9.9, 11.2 Hz, H-3), 4.34–4.27 (m, 2H, H-2 and H-5), 3.82 (dt, 1H, *J* = 6.3, 9.6 Hz, $CH_2(CH_2)_6CH_3$), 3.76 (s, 3H, $COOCH_3$), 3.66 (t, 1H, *J* = 9.9 Hz, H-4), 3.41 (dt, 1H, *J* = 6.6, 9.6 Hz, $CH_2(CH_2)_6CH_3$), 3.13 and 3.06 (s, each 3H, OCH_3), 1.80–0.97, (m, 20H, $CH_2(CH_2)_6CH_3$ and cyclohexyl-H), 0.81 (t, 3H, *J* = 7.1 Hz, $(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$) δ 168.37, 167.37 and 166.63 (CO), 142.41 (C-6), 134.05, 133.96, 131.79, 131.57, 123.67 and 123.00 (aromatic), 121.78 (C-7), 98.80 (C-1 and $COCH_3$), 72.09 (C-4 and C-5), 69.78 ($CH_2(CH_2)_6CH_3$), 66.78 (C-3), 53.89 (C-2), 51.61 ($COOCH_3$), 46.96 and 46.76 (OCH_3), 31.61, 29.26, 29.06, 26.88, 25.73, 22.54 and 21.26 (cyclohexyl-H and $CH_2(CH_2)_7CH_3$), 14.00 ($(CH_2)_7CH_3$). Anal. calcd for $C_{33}H_{45}NO_{10}$: C, 64.37; H, 7.37; N, 2.27. Found: C, 64.36; H, 7.43; N, 2.38.

Octyl 2,6,7-trideoxy-3,4-*O*-[(1′*S*,2′*S*)-dimethoxycyclohexylidene]-2-phthalimido-β-D-glucooct-6(*E*)-enopyranoside (26). To a solution of compound **25** (483 mg, 0.78 mmol) in CH_2Cl_2 (10 mL) was slowly added a 0.98 M solution of DIBAL (5.6 mL, 7 equiv) at −78 °C. The reaction mixture was stirred at the temperature for 3 h. MeOH (0.9 mL) was added at −78 °C and the temperature was raised to rt. Saturated NaCl (1.8 mL) was added and the mixture was diluted with Et_2O (45 mL). $MgSO_4$ (4.7 g) was added and the whole mixture was stirred for 1.5 h, then filtered through a Celite pad. The solvent was removed in vacuo and the crude mixture was purified by column chromatography (1:1 *n*-hexane: EtOAc) to give **26** (344 mg, 74%) and the starting material **25** (123 mg, 26%): mp 156 °C; $[\alpha]_D$ +106.9° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.90–7.70 (m, 4H, aromatic), 6.11 (dt, 1H, *J* = 5.4, 15.7 Hz, H-7), 5.86 (dd, 1H, *J* = 5.4, 15.7 Hz, H-6), 5.18 (d, 1H, *J* = 8.3 Hz, H-1), 4.85 (dd, 1H, *J* = 9.9,

11.2 Hz, H-3), 4.30 (dd, 1H, $J=8.3$, 11.5 Hz, H-2), 4.22-4.08 (m, 3H, H-5 and H-8), 3.82 (dt, 1H, $J=6.1$, 9.9 Hz, $CH_2(CH_2)_6CH_3$), 3.65 (t, 1H, $J=9.9$ Hz, H-4), 3.39 (dt, 1H, $J=6.6$, 9.9 Hz, $CH_2(CH_2)_6CH_3$), 3.16 and 3.05 (s, each 3H, OCH_3), 1.74–0.88 (m, 20H, $CH_2(CH_2)_6CH_3$ and cyclohexyl-H), 0.81 (t, 3H, $J=7.1$ Hz, $(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$) δ 168.30 and 167.35 (CO), 133.93 (C-7), 133.84, 132.76, 131.64, 131.46, 123.49 and 122.86 (aromatic), 125.88 (C-6), 98.56 (C-1 and $COCH_3$), 73.13 (C-5), 72.24 (C-4), 69.53 ($CH_2(CH_2)_7CH_3$), 66.61 (C-3), 62.70 (C-8), 53.98 (C-2), 46.74 and 46.51 (OCH_3), 31.47, 29.11, 28.93, 26.78, 25.63, 22.39, 21.17 and 21.12 (cyclohexyl-C and $CH_2(CH_2)_6CH_3$), 13.87 ($(CH_2)_7CH_3$). Anal. calcd for $C_{32}H_{45}NO_9$: C, 65.40; H, 7.72; N, 2.38. Found: C, 64.93; H, 7.70; N, 2.36.

Octyl 2-acetamido-2,6,7-trideoxy-3,4-*O*-[(1′*S*,2′*S*)-dimethoxycyclohexylidene]-β-D-glucooctpyranoside (27). A solution of **26** (116 mg, 0.20 mmol) and hydrazine monohydrate (0.3 mL) in EtOH (3 mL) was heated under reflux overnight. After cooling, the solvent was removed in vacuo. To the solution of the residue in MeOH (3 mL) was added Ac_2O (0.3 mL) and the mixture was stirred at rt overnight. After removal of the solvent, the residue was purified by column chromatography (1:3 *n*-hexane: EtOAc) to give **27** (92 mg, 93%): $[\alpha]_D$ +83.8° (*c* 0.8, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 5.71 (d, 1H, $J=6.9$ Hz, NH), 5.13 (d, 1H, $J=7.9$ Hz, H-1), 4.64 (t, 1H, $J=10.2$ Hz, H-3), 3.84 (dt, 1H, $J=6.6$, 9.6 Hz, $CH_2(CH_2)_6CH_3$), 3.66 (t, 2H, $J=6.1$ Hz, H-8), 3.57–3.41 (m, 3H, H-4, H-5, and $CH_2(CH_2)_6CH_3$), 3.18 and 3.17 (s, each 3H, OCH_3), 3.09 (ddd, 1H, $J=6.9$, 7.9, 10.2 Hz, H-2), 1.97 (s, 3H, $NHCOCH_3$), 2.10–2.00, 1.90–1.23 (m, 24H, H-6, H-7, $CH_2(CH_2)_6CH_3$ and cyclohexyl-H), 0.87 (t, 3H, $J=6.4$ Hz, $(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$) δ 170.76 (NHCO), 99.82 (C-1), 98.85 and 98.71 ($COCH_3$), 74.00 (C-4 or C-5), 72.27 (C-4 or C-5), 70.37 ($CH_2(CH_2)_6CH_3$), 67.87 (C-3), 62.79 (C-8), 57.09 (C-2), 47.08 and 46.94 (OCH_3), 23.79 ($NHCOCH_3$), 32.06, 29.81, 29.58, 29.54, 28.99, 27.24, 27.19, 26.17, 22.90 and 21.60 (C-6, C-7, cyclohexyl-C and $CH_2(CH_2)_6CH_3$), 14.34 ($(CH_2)_7CH_3$).

Octyl 2-acetamido-2,6,7-trideoxy-3,4-*O*-[(1′*S*,2′*S*)-dimethoxycyclohexylidene]-β-D-glucooctdialdo-1,5-pyranoside (28). To a solution of DMSO (16 μL, 0.26 mmol) in CH_2Cl_2 (0.5 mL) was added 2 M solution of oxalyl chloride in CH_2Cl_2 (66 μL, 0.13 mmol) at −78 °C and the mixture was stirred at the temperature for 0.5 h. To this mixture was added a solution of compound **27** (33 mg, 0.07 mmol) in CH_2Cl_2 (1 mL) and the mixture was stirred at the temperature for 0.5 h. Et_3N (74 μL, 0.53 mmol) was added and the mixture was stirred at rt for 15 min, then the mixture was diluted with CH_2Cl_2 and washed with H_2O, dried over $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (1:2 *n*-hexane:EtOAc) to give **28** (30 mg, 90%): $[\alpha]_D$ +100.9° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 9.77 (s, 1H, CHO), 5.88 (brd, 1H, $J=5.6$ Hz, NH), 5.11 (d, 1H, $J=7.9$ Hz, H-1), 4.63 (t, 1H, $J=10.2$ Hz, H-3), 3.79 (dt, 1H, $J=6.6$, 9.6 Hz, $CH_2(CH_2)_6CH_3$), 3.54–3.42 (m, 3H, H-4, H-5 and $CH_2(CH_2)_6CH_3$), 3.19 and 3.16 (s, each 3H, OCH_3), 3.05 (ddd, 1H, $J=5.6$, 7.9, 10.2 Hz, H-2), 2.70–2.45 (m, 2H, H-7), 2.31–2.20 (m, 1H, H-6), 1.96 (s, 3H, $COCH_3$), 1.80–1.23 (m, 21H, H-6, $CH_2(CH_2)_6CH_3$ and cyclohexyl-H), 0.87 (t, 3H, $J=6.6$ Hz, $(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$) δ 201.92 (CHO), 170.28 ($NHCOCH_3$), 99.44 (C-1), 98.56 and 98.40 ($COCH_3$), 72.90 (C-4 or C-5), 71.99 (C-4 or C-5), 70.05 ($CH_2(CH_2)_6CH_3$), 67.33 (C-3), 56.84 (C-2), 46.79 and 46.65 (OCH_3), 40.06 (C-7), 31.74, 29.45, 29.24, 26.92, 26.85, 25.82, 23.63, 23.51, 22.57, 21.28 and 20.97 (C-6, $CH_2(CH_2)_6CH_3$ and cyclohexyl-C), 14.02 ($(CH_2)_7CH_3$). Anal. calcd for $C_{26}H_{45}NO_8$: C, 62.50; H, 9.08; N, 2.80. Found: C, 62.02; H, 9.10; N, 2.94.

Octyl 2-acetamido-2,6,7,8-tetradeoxy-3,4-*O*-[(1′*S*,2′*S*)-dimethoxycyclohexylidene]-8-[(2″*R*,3″*R*,4″*S*,5″*R*)-3″,4″-dibenzyloxy - 2″,5″ - dibenzyloxymethylpyrrolidinyl] - β - D - glucooctpyranoside (29). A solution of **22** (21 mg, 0.04 mmol) and **28** (14 mg, 0.03 mmol) in MeOH (0.9 mL) was added $NaBH_3CN$ (4.5 mg, 0.07 mmol) at 0 °C and stirred at rt overnight. To the mixture was added H_2O, extracted with $CHCl_3$ and dried with $MgSO_4$. After removal of the solvent, the residue was purified by column chromatography (66:33:1 $CHCl_3$: MeOH:Et_3N) to give azasugar **22** (8 mg, 37%) and condensate **29** (22 mg, 77% based on **28**, 54% based on **22**): $[\alpha]_D$ +41.0° (*c* 1.3, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 7.36–7.23 (m 20H, aromatic), 5.59 (d, 1H, $J=6.9$ Hz, NH), 5.05 (d, 1H, $J=7.9$ Hz, H-1), 4.63–4.46 (m, 10H), 3.96 (t, 1H, $J=5.9$ Hz), 3.86–3.76 (m, 3H), 3.64–3.58 (m, 1H), 3.50–3.37 (m, 4H), 3.23–3.05 (m, 2H), 3.19 and 3.17 (s, each 3H, OCH_3), 3.02–2.91 (m, 2H), 2.61–2.50 (m, 1H), 1.96 (s, 3H, $NHCOCH_3$), 1.88–1.68 (m, 8H), 1.53–1.50 (m, 6H), 1.39–1.23 (m, 16H), 0.86 (t, 3H, $J=6.4$ Hz, $(CH_2)_7CH_3$); ^{13}C NMR ($CDCl_3$) δ 170.02 (NHCO), 138.85, 138.81, 138.29, 128.32, 128.19, 128.10, 127.62, 127.53, 127.46, 127.40 and 127.21 (aromatic), 99.41 (C-1), 98.51 and 98.3 ($COCH_3$), 79.43, 78.11, 73.73, 73.24, 73.05, 72.29, 71.93, 71.43, 70.78, 69.82, 67.93, 67.33, 61.55, 57.04, 49.83, 46.79 and 46.58 (OCH_3), 31.79, 29.53, 29.35, 29.25, 27.01, 26.92, 25.95, 24.35, 23.69 ($NHCOCH_3$), 22.63, 21.33, 14.07 ($(CH_2)_7CH_3$); ESI MS *m/z*: 1007.8 $[M+H]^+$.

Octyl 2-acetamido-2,6,7,8-tetradeoxy-8-[(2′*R*,3′*R*,4′*S*,5′*R*)-3′,4′-dihydroxy-2′,5′-dihydroxymethylpyrrolidinyl]-β-D-glucooctpyranoside (1). A solution of **29** (16 mg, 0.016 mmol) in THF (1 mL) and M HCl (0.5 mL) was stirred with 20% $Pd(OH)_2$ on C under H_2 atmosphere at rt for 3 days After filtration, removal of the solvent afforded a crude debenzylated compound. The resulting compound was used for the next step without further purification. The residue was treated with TFA:H_2O (3:2 v/v, 1 mL) at rt overnight. After removal of the solvent, the residue was purified by a column of Iatro Beads (3:1 $CHCl_3$:MeOH) to yield **1** (5.2 mg, 63% in two steps); $[\alpha]_D$ +47.8° (*c* 0.7, H_2O). Compound **1** was further purified for inhibition assay using Sep-Pak PLUS C18, washed with MeOH (20 mL) and water (20 mL), eluted with water (20 mL) and MeOH (20 mL). The latter eluent containing **1** was filtered through a Millex GV filter and lyophilized. ^1H NMR (D_2O) δ 4.50 (d, 1H, $J=8.4$ Hz, H-1), 4.36 (t, 1H, $J=3.8$ Hz, H-4′), 4.28 (dd, 1H, $J=3.8$, 9.3 Hz, H-3′), 4.01 (dd, 1H, $J=5.3$,

12.1 Hz, H-1‴), 3.99 (dd, 1H, $J=3.4$, 12.5 Hz, H-1″), 3.92 (dd, 1H, $J=5.3$, 12.1 Hz, H-1‴), 3.87–3.82 (m, 2H, H-8 and CH_2 $(CH_2)_6CH_3$), 3.78 (ddd, 1H, $J=3.5$, 5.2, 8.0 Hz, H-5′), 3.70–3.64 (m, 4H, H-2, H-8, H-2′ and H-1″), 3.59 (dt, 1H, $J=6.4$, 10.3 Hz, C$H_2(CH_2)_6CH_3$), 3.49, (dd, 1H, $J=8.9$, 10.3 Hz, H-3), 3.40–3.35 (m, 1H, H-5), 3.27 (t, 1H, $J=9.2$ Hz, H-4), 2.03 (s, 3H, $NHCOCH_3$), 1.55–1.52, 1.28–1.27 (m, 16H), 0.86 (t, 3H, $J=6.9$ Hz, $(CH_2)_7CH_3$); ^{13}C NMR (D_2O) δ 174.70 (NHCO), 101.27 (C-1), 74.66 (C-5), 73.95 (C-3), 73.70 (C-4), 71.47, 70.98, 70.73, 70.35, 62.48, 61.99, 58.35, 57.76, 56.04, 31.34, 28.83, 28.74, 28.59, 25.35, 22.45, 22.25, 13.63 ($(CH_2)_7CH_3$); HRFAB MS calcd for $C_{24}H_{46}N_2O_9$: $[M+H]^+$ 507.3281; found: m/z 507.3286.

Glycosidase assay

Materials. The sources of enzymes and substrates are as follows: β-galactosidase (EC 3.2.1.23) from *Aspergillus oryzae*, α-mannosidase (EC 3.2.1.24) from jack bean and *p*-nitrophenyl-β-D-galactopyranoside (PNP-Gal) were from Sigma Chemical Co. (St. Louis, MO, USA); *p*-nitrophenyl-α-D-mannopyranoside (PNP-Man) was from Nacalai Tesque. Inc. (Kyoto, Japan). 1-Deoxygalactonojirimycin was purchased from Toronto Research Chemicals Inc. (North York, ON, Canada) and 1-deoxymannojirimycin was purchased from Sigma Chemical Co. (St Louis, MO, USA). Double deionized water was prepared from a Milli-Q system from Millipore Corp. (Milford, MA, USA).

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 i.d. fused 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 the separation 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 9.4) as electrolyte at a constant temperature of 37 °C. Electropherograms were recorded on a Millennium 2010 system from Millipore Corp.

Kinetic analysis of β-galactosidase. Incubations were performed in a total volume of 20 μL. Unless otherwise stated, reaction mixtures contained 50 mM phosphate buffer (pH 7.0), various amounts of PNP–Gal (0.5–3.0 mM), various amounts of inhibitors and β-galactosidase. After incubation for 10 min at 37 °C, the reaction was terminated by addition of 20 μL of 0.2 M sodium carbonate.

Kinetic analysis of α-mannosidase. Incubations were performed in a total volume of 20 μL. Unless otherwise stated, reaction mixtures contained 50 mM acetate buffer (pH 5.0), various amounts of PNP–Man (0.7–2.6 mM), various amounts of inhibitors and 1.05 mU of α-mannosidase. After incubation for 10 min at 25 °C, the reaction was terminated by addition of 20 μL of 0.2 M sodium carbonate.

Galactosyltransferase assay

Materials. β-Galactosyltransferase (EC 2.4.1.22 from bovine, recombinant, *Spodoptera frugiperda*; Calbiochem-Novabiochem Co. LaJolla, CA), UDP-galactose (UDP-Gal), and uridine 5′-diphosphonate (UDP) were from Sigma Chemical Co. (St Louis, MO, USA). Cacodylic acid sodium salt was from Nacalai Tesque Inc. (Kyoto, Japan). 4-Methyl umbelliferyl 2-acetamido-2-deoxy-β-D-glucopyranoside (MU-GlcNAc) and cacodylic acid were from Wako Pure Chemical Ltd. (Osaka, Japan).

Condition of capillary zone electrophoresis. Assays were performed on a Beckman P/ACE System 5010 (USA), which was equipped with a 57 cm×75 μm i.d. fused silica capillary. Detection was carried out by on-column measurement at 7.0 cm from the cathode. The laser-induced fluorescence (LIF) detector equipped with He-Cd Laser (IK series) from Kimmon Electro Co., Ltd. (Japan) was used. A band-pass filter for excitation at 325 nm was used. The capillary was pretreated or regenerated with 0.1 M KOH, washed with double deionized water and separation buffer prior to each injection. Samples were loaded automatically by pressure injection for 5 s (30.0 nL). Electrophoresis was performed at 15 kV using 50 mM sodium borate as electrolyte at a constant temperature of 35 °C and was detected at 375 nm (em).

Kinetic analysis of galactosyltransferase. Incubations were performed in a total volume of 30 μL. Unless otherwise stated, reaction mixtures contained 0.1 M Cacodylate buffer (pH as supecified in Table 2 and Figure 4(A,B), 10 mM $MnCl_2$, various amounts of UDP–Gal (20–100 μM), various amounts of MU–GlcNAc (10–1700 μM), various amounts of inhibitors and 0.8 mU GalTase. After incubation for 10 min at 37 °C, the reaction was terminated by the addition of 10 μL of 0.12 M EDTA in 30 mM sodium borate.

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