Synthesis of New *N*-Containing Maltooligosaccharides, α-Amylase Inhibitors, and Their Biological Activities

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Fifteen new *N*-containing maltooligosaccharides were obtained using the chemoenzymatic method. Among these compounds, maltooligosaccharides having 6-amino-6-deoxy-D-sorbitol residue, (3*R*,4*R*,5*R*,6*S*)-hexahydro-3,4,5,6-tetrahydroxy-1*H*-azepine residue, and (3*R*,5*R*)-3,4,5-trihydroxypiperidine residue at the reducing end showed strong inhibitory activities for human pancreatic α-amylase (HPA) (EC 3.2.1.1) and human salivary α-amylase (HSA). The administration of (3*R*,4*R*,5*R*,6*S*)-hexahydro-3,5,6-trihydroxy-1*H*-azepine-4-yl *O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (13, $IC_{50}=4.3\times10^{-5}$ M for HPA, $IC_{50}=8.2\times10^{-5}$ M for HSA) and (3*R*,5*R*)-3,5-dihydroxypiperidine-4-yl *O*-α-D-glucopyranosyl-(1→4)-α-D-glucopyranoside (18, $IC_{50}=3.4\times10^{-5}$ M for HPA, $IC_{50}=4.6\times10^{-5}$ M for HSA) to ICR mice suppressed postprandial hyperglycemia.

Key words α-amylase; inhibitor; postprandial hyperglycemia; chemoenzymatic synthesis

When carbohydrate is administered to a healthy human as diet, blood glucose level rises temporally, then slowly lowers to about 100 mg/dl where it remains constant. In the case of a patient with diabetes, the glucose level rises steeply and hyperglycemia (>200 mg/dl) occurs. Hyperglycemia is known to cause many complications such as cataract, arteriosclerosis, neuralgia, *etc*.[1—4] To suppress hyperglycemia, α-glucosidase (EC 3.2.1.20) inhibitors, acarbose[5,6] and AO-128,[7—11] have been used as oral agents for treatment of diabetes. Although these drugs show potent pharmacological action, they have such side effects as abdominal pain, flatus, diarrhea, and soft feces in the colon.[12,13] The abdominal distress occurs because of the fermentation of maltose which accumulates as a result of α-glucosidase inhibition.

An inhibitor of α-amylase (EC 3.2.1.1) which acts in the first step of carbohydrate digestion, is expected to be a better suppressor of postprandial hyperglycemia, since it is assumed that the inhibitor would not result in an abnormal accumulation of maltose.

We investigated the influence of various substituents at 6^3-position and the glucosyl chain length of 6^3-modified maltooligosaccharides on human α-amylase inhibition.[14—16] Other studies of synthetic α-amylase inhibitors are the following. Lehmann *et al*.[17] reported inhibition of 6^1-amino-6^1-deoxymaltotriose for porcine pancreatic α-amylase, and Arai *et al*.[18] indicated inhibition of modified maltooligosaccharides having deoxynojirimycin residue at the reducing end for microbial and animal α-amylases. Many iminocyclitols showed strong inhibitory activities for α-glucosidases.[19—21]

We therefore designed fifteen new modified maltooligosaccharides having *N*-containing pseudo sugar residues; these were three kinds of iminocyclitols[19—21] with 6-amino-6-deoxysorbitol[22] at the reducing end (Fig. 1). In this paper we describe the synthesis of these newly modified maltooligosaccharides and discuss the effects of various *N*-containing pseudo sugars and glucosyl chain length on the inhibitory activities of human α-amylases and rat maltase. Suppression activities of postprandial hyperglycemia for ICR mice are also reported.

Results and Discussion

As shown in Chart 1, 6^1-azido-6^1-deoxymaltooligosaccharides (**1**—**5**) as beneficial intermediates were selected for synthesis of sorbitol (A)-, azepine (B)-, and 4-piperidine (C)-type compounds. Transglycosylation of 6-azido-6-deoxyglucose (**1**) with α-cyclodextrin (α-CD) using cyclodextrin glycosyltransferase (CGTase)[17,23] (EC 2.4.1.19) and subsequent β-amylase (EC 3.2.1.2) hydrolysis gave **2** (yield 16%), **3** (yield 22%), **4** (yield 9%), and **5** (yield 5%), respectively. Reduction of **1**—**5** with $NaBH_4$ in *N*,*N*-dimethylformamide (DMF) at 80 °C gave corresponding A-type derivatives: **6** from **1** (yield 52%), **7** from **2** (yield 37%), **8** from **3** (yield 52%), **9** from **4** (yield 45%), or **10** from **5** (yield 67%).

Lehmann *et al*.[17] reported that catalytic hydrogenation of **2** or **3** over palladium-on-charcoal (2 h) gave 6^1-amino-6^1-deoxymaltose or 6^1-amino-6^1-deoxymaltotriose. In our experiments, differing from their report, **1**—**5** underwent ring expansion *via* Pd-mediated reductive amination (16 h) to give B-type compounds: **11** from **1** (yield 51%), **12** from **2** (yield 81%), **13** from **3** (yield 37%), **14** from **4** (yield 61%), or **15** from **5** (yield 83%). Since the reaction time was longer than Lehmann's condition, the B-type derivative would be generated (see Chart 1).

C-Type compounds were synthesized in the following sequence. Oxidation of **1**—**5** with Br_2 in H_2O in the presence of $BaCO_3$ at room temperature gave maltooligosaccharides

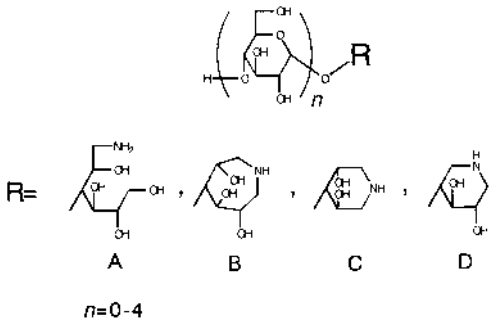

Fig. 1. Design of Modified Maltooligosaccharide for Human α-Amylase Inhibitor

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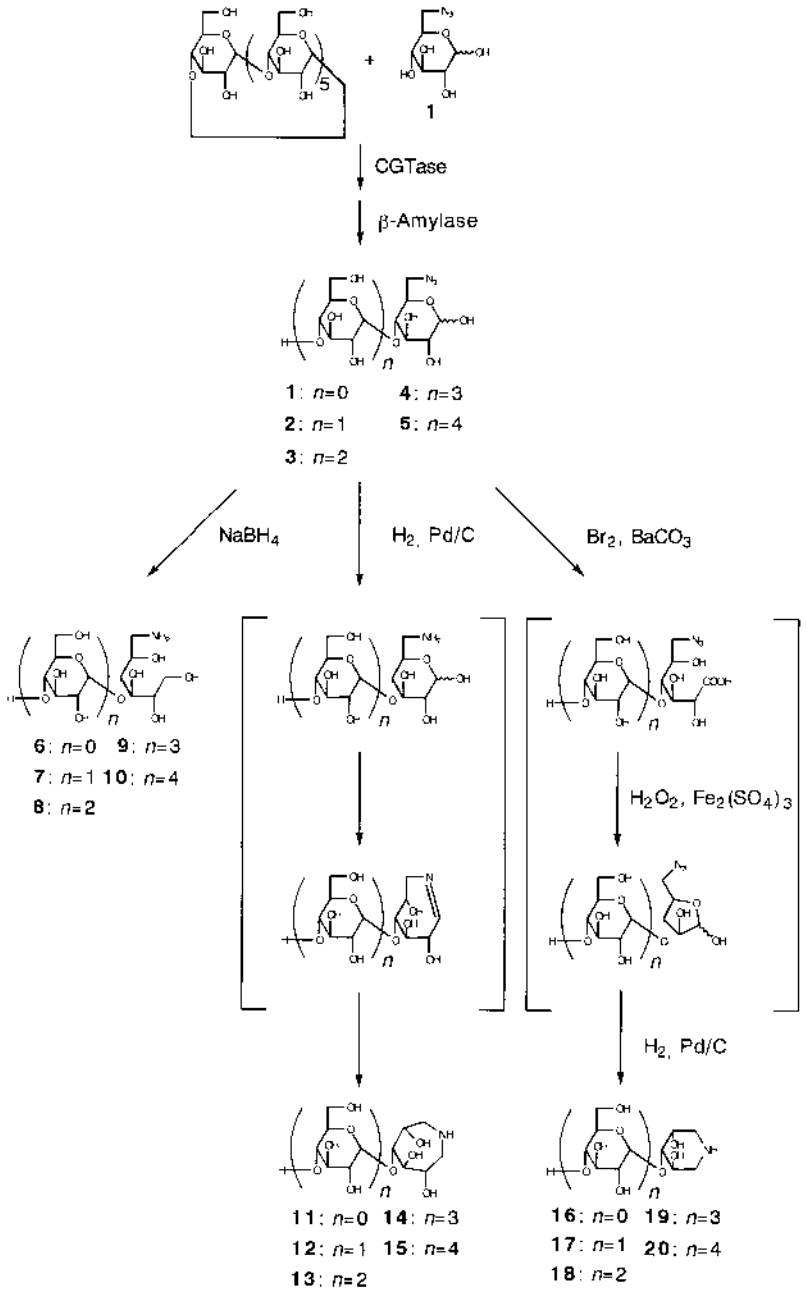

Chart 1

having 6^1-azido-6^1-deoxygluconic acid residue at the reducing end (not purified). After removal of the excess Br_2 under reduced pressure, oxidative decarboxylation[24] with $Fe_2(SO_4)_3$ and H_2O_2 at 40 °C gave 5-azido-5-deoxyarabinosyl derivatives (not purified). They were then reduced by catalytic hydrogenation over palladium-on-charcoal in H_2O to yield the corresponding iminocyclitol: **16** from **1** (overall yield 14%), **17** from **2** (overall yield 11%), **18** from **3** (overall yield 23%), **19** from **4** (overall yield 12%), or **20** from **5** (overall yield 11%).

Kitahata *et al.*[25] reported that D-xylose is a good acceptor for transglycosylation with soluble starch using CGTase. Transglycosylation of deoxynojirimycin (a glucose type iminocyclitol) with soluble starch using CGTase was also reported.[18] These results led us to expect that the CGTase could catalyze the transglycosylation of (3*S*,5*R*)-3,4,5-trihydroxypiperidine[21] (**21**, a xylose type iminocyclitol) with α-CD to give 3-piperidine (D)-type derivatives (Chart 2). As we expected, incubation of **21** with α-CD and CGTase, and

Chart 2

Table 1. ^{13}C-NMR Chemical Shift Data

	Carbon	Compound	
		21	**22**
Piperidine moiety	2^1	52.14	51.03
	3^1	73.87	82.66
	4^1	81.26	79.96
	5^1	73.87	72.49
	6^1	52.14	51.76
Glucosyl moiety	1^2		102.73
	2^2		73.66
	3^2		75.77[a]
	4^2		72.49
	5^2		74.48[a]
	6^2		63.49

Spectra were obtained at 125 MHz with a Bruker AVANCE 500 spectrometer. *a*) May be exchangeable.

subsequent β-amylase hydrolysis gave **22** (yield 3%), **23** (yield 27%), and **24** (yield 16%), respectively. Additionally, (3*R*,4*R*,5*S*)-4,5-dihydroxypiperidine-3-yl tris[*O*-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside was produced in a trace amount.

Structural Elucidation of Modified Oligosaccharides Structures of the synthesized compounds were established by spectral data and elemental analyses.

We determined the numbers of glucosyl residue in the compounds (A-, B-, C-, D-) from the integration of ^{1}H-NMR signals at δ 5.4 (J=3.9 Hz) assigned to each glucosyl H-1. The ^{13}C-NMR spectra showed characteristic substituents: about δ 53 (–CH_2N_3, **1**—**5**), about δ 45 (–CH_2NH_2, **6**—**10**), about δ 66 (–CH_2OH on sorbitol moiety, **6**—**10**), about δ 51 (–$\underline{C}H_2NH\underline{C}H_2$–, **11**—**24**), and about δ 63 (–CH_2OH on glucosyl moiety, **2**—**5**, **7**—**10**, **12**—**15**, **17**—**20**, **22**—**24**).

In the case of D-type compounds, the linkage between glucose and piperidine residue was elucidated by ^{1}H- and ^{13}C-NMR. The anomeric type of the linkage was determined to be α by the doublet signal assigned to H-1 δ 5.21 (J=3.9 Hz). Although the ^{13}C-NMR spectrum of **21** showed only three signals because of its symmetry, compound **22** showed eleven signals and a downfield shift (+8.79 ppm) of the C-3 signal of the piperidine residue (Table 1). Arai *et al.*[18] also reported that downfield shift (+7.62 ppm) was observed at the C-4 signal on deoxynojirimycin moiety of α-D-glucopyranosyl-(1→4)-deoxynojirimycin, compared with the chemical shift of deoxynojirimycin. Thus the linkage between glucose residue and piperidine residue was elucidated to be α-(1→3), not to be α-(1→4). Linkage of the other compounds (**23**, **24**) was determined in a similar manner.

Inhibition of Human α-Amylases by the *N*-Containing Sugars Inhibitory activities of the compounds were determined against human pancreatic α-amylase (HPA) and human salivary α-amylase (HSA) and are shown in Table 2.

We earlier reported that 6^3-deoxymaltotriose (Fig. 2) showed a large difference in inhibitory activity between HPA (IC_{50}=2.0 mM) and HSA (IC_{50}=42 mM).[14—16] A-type derivatives (**8**—**9**), B-type derivatives (**13**—**15**), and C-type derivatives (**18**—**20**) showed stronger activity than 6^3-deoxymaltotriose, and these compounds did not show remarkable difference in inhibitory activity between the two α-amylases.

In the same chain length, the activity of B-type derivatives (**12**—**15**) and C-type derivatives (**17**—**20**) was stronger than that of A-type derivatives (**7**—**10**). Optimal chain length for strong α-amylase inhibition was three or four.

With regard to the specificity of the structure of *N*-containing sugar, D-type derivatives (**22**—**24**) have similar piperidine ring to C-type derivatives (**17**—**19**), but did not show inhibitory activity (under 5 mM). It is presumed that an important role in α-amylase inhibition is played not only by amino groups but also by hydroxyl groups located on either side of the glucosyl position.

HPA and HSA have been shown to have five to seven binding sites, called subsites, for glucose residues of substrate.[26—29] From our results, glucose residues of a strong inhibitor may be bound to subsites-2 to -3 or -2 to -4, and the amino groups of *N*-containing sugar moiety at subsite-1 seem to cause ionic interaction with the enzymes at the active site (Fig. 3).

Inhibition of Rat Intestinal Maltase by the *N*-Containing Sugars The IC_{50} values of the compounds **6**—**24** are shown in Table 1. All tested compounds showed weak or no inhibition against the maltase.

Suppression Test of Postprandial Hyperglycemia Since **13** and **18** showed strong inhibitory activity for the two α-amylases but had no inhibitory activity for maltase, the two compounds were selected for use in the suppression test. When cornstarch and **13** or **18** were orally administered to ICR mice, the rise in blood glucose concentration was significantly suppressed compared to the control (Fig. 4). After administration, neither death nor a side effect such as diarrhea

Table 2. Inhibitory Activities of Modified Maltooligosaccharide against HPA, HSA, and Rat Maltase

Compd.	R	*n*	IC_{50}[a] (mM)		
			HPA	HSA	Maltase
6	A	0	NI[b]	NI[b]	NI[c]
7	A	1	NI[b]	NI[b]	NI[c]
8	A	2	0.13	0.25	NI[c]
9	A	3	0.27	0.46	NI[c]
10	A	4	NI[b]	NI[b]	NI[c]
11	B	0	NI[b]	NI[b]	5.5
12	B	1	2.5	2.0	NI[c]
13	B	2	0.043	0.082	NI[c]
14	B	3	0.030	0.049	NI[c]
15	B	4	0.23	0.36	NI[c]
16	C	0	NI[b]	NI[b]	7.5
17	C	1	3.0	2.0	1.0
18	C	2	0.034	0.046	NI[c]
19	C	3	0.025	0.042	NI[c]
20	C	4	0.070	0.070	NI[c]
21	D	0	NI[b]	NI[b]	NI[c]
22	D	1	NI[b]	NI[b]	NI[c]
23	D	2	NI[b]	NI[b]	NI[c]
24	D	3	NI[b]	NI[b]	NI[c]

a) IC_{50}: molar concentration required to give 50% inhibition. *b*) Less than 50% inhibition at 5.0 mM. *c*) Less than 50% inhibition at 10 mM.

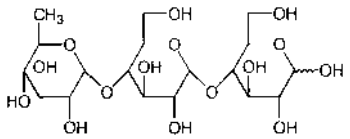

Fig. 2. Chemical Structure of 6^3-Deoxymaltotriose

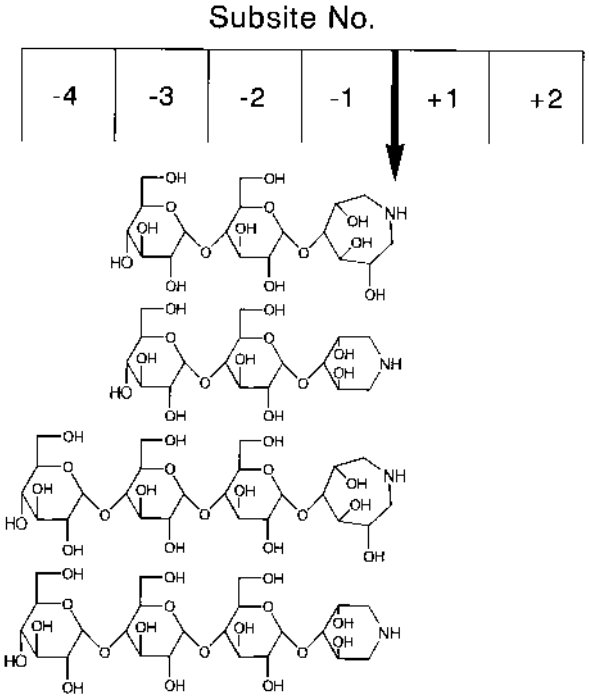

Fig. 3. Binding Model of Synthesized Inhibitors on the Subsites of Human α-Amylases

The arrowhead indicates the catalytic site and each sequentially numbered box represents a subsite.

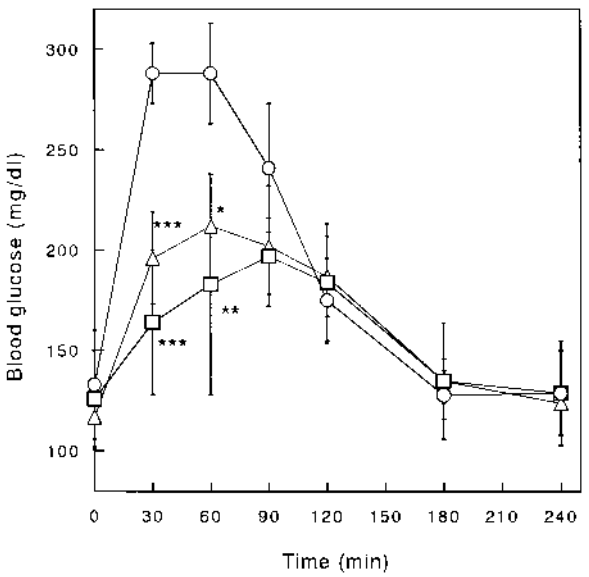

Fig. 4. Effect of **13** and **18** on Blood Glucose Levels in Cornstarch (2000 mg/kg) Loaded ICR Mice

○, control; □, **13** (60 mg/kg); △, **18** (60 mg/kg). Each datum represents mean±S.D. (n=5). *,**,***: significantly different from control group with $p<0.05$, $p<0.01$, and $p<0.001$, respectively.

or soft feces was observed. IC_{50} value of **13** (0.060 mM) or **18** (0.041 mM) for pancreatic α-amylase of ICR mice was almost equal to that of HPA. Result of the suppression test for mouse hyperglycemia was thus believed to reflect suppression in the case of human.

In conclusion, we synthesized four new types of *N*-containing maltooligosaccharides by an elegant chemoenzymatic method. For inhibition of two human α-amylases, effective units at the reducing end of maltooligosaccharides were B-type or C-type, and the optimal chain length of glucose residue was three or four. We also showed the possibility that **13** or **18** might be used as an oral agent for treatment of diabetes.

Experimental

Reagents and Materials All chemicals were reagent grade unless otherwise noted. An α-amylase activity determination kit (Neo·Amylase Test Daiichi) using blue starch as a substrate was obtained from Daiichi Pure Chemicals Co., Ltd., Japan. A glucose quantitative kit (Glucose B-test Wako) and β-amylase (from wheat) were obtained from Wako Pure Chemical Industries, Ltd., Japan. Standard HPA and HSA were obtained from International Reagents Corp., Japan. CGTase (from *Bacillus macerans*) was purchased from Amano Co., Ltd., Japan, and glucoamylase (from *Rhizopus* sp.) from Toyobo Co., Ltd., Japan.

Apparatus All melting points were determined on a Yanagimoto micro-melting point apparatus and are uncorrected. Optical rotations were determined with a JASCO DIP-360 digital polarimeter at 25 °C. IR spectra were recorded with a JASCO FT/IR-7300 spectrometer. ^{1}H-NMR spectra were recorded at 199.5 or 500.1 MHz and ^{13}C-NMR spectra were taken at 50.10 or 125.7 MHz with a JEOL JNM-FX200 or Bruker AVANCE 500 spectrometer using sodium 3-(trimethylsilyl)propionate as an internal standard and D_2O as a solvent. HPLC was performed on a (A) TSK gel Amide-80 column (4.6 mm i.d.×250 mm) or (B) TSK gel Amino-60 column (4.6 mm i.d.×250 mm) with a flow rate of 1.0 ml/min using a JASCO pump (880-PU) and a refractive index detector (Shodex RI SE-71) at room temperature. Visible absorption (620 nm) was recorded with a Hitachi 557 spectrometer. Blood glucose concentration was measured with an ANTSENSE (Bayer-Sankyo).

Preparation of Compounds 2—5 CGTase (1.4 ml, 840 U) was added to a stirred solution of α-CD (180 g, 185 mmol) and **1**[23] (10 g, 49 mmol) in 10 mM piperazine-*N*,*N*′-bis(2-ethanesulfonic acid) (PIPES) buffer (pH 5.2, 1.0 l), and the mixture was stirred at 37 °C for 50 min. The solution was boiled for 30 min to inactivate the enzyme. After cooling, 1.1 g (367 U) of β-amylase was added and the mixture was stirred at 37 °C for 5.5 h, then boiled for 1 h to stop the reaction. After cooling, toluene (100 ml) was added and the mixture was stirred at room temperature for 5 h; the insoluble materials were removed by filtration with a short pad of Celite®. The filtrate was then evaporated under reduced pressure to about 100 ml and the solution was passed through an active carbon column. 6^1-Azido derivatives were eluted using an EtOH–H_2O gradient of 0 to 50% (v/v), and the appropriate fractions were freeze-dried to give **2** (2.79 g, 7.60 mmol, 16%), **3** (5.58 g, 10.5 mmol, 22%), **4** (2.77 g, 4.01 mmol, 9%), and **5** (2.02 g, 2.37 mmol, 5%).

O-α-D-Glucopyranosyl-(1→4)-6-azido-6-deoxy-D-glucopyranose (**2**): Colorless powder; mp 97—99 °C; $[\alpha]_D$ +150° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3320, 2930, 2110, 1350, 1280, 1150, 1020; ^{1}H-NMR δ: 3.25—4.10 (m), 4.67 (d, 0.5H, J=8.1 Hz, αH-1a), 5.24 (d, 0.5H, J=3.7 Hz, βH-1a), 5.38 (d, 1H, J=3.4 Hz, H-1b); ^{13}C-NMR δ: 53.78 (C-6a), 63.42 (C-6b), 94.75, 98.66 (C-1a), 102.60 (C-1b); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 4.5, 4.7 min. *Anal*. Calcd for $C_{12}H_{21}N_3O_{10}\cdot 2H_2O$: C, 35.73; H, 6.25; N, 10.42. Found: C, 35.44; H, 6.12; N, 10.15.

Bis[*O*-α-D-glucopyranosyl-(1→4)]-6-azido-6-deoxy-D-glucopyranose (**3**): Colorless powder; mp 128—130 °C; $[\alpha]_D$ +160° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3320, 2920, 2110, 1350, 1290, 1150, 1020; ^{1}H-NMR δ: 3.25—4.10 (m), 4.67 (d, 0.5H, J=7.8 Hz, αH-1a), 5.24 (d, 0.5H, J=3.7 Hz, βH-1a), 5.37, 5.40 (each d, each 1H, J=3.7, 3.9 Hz, H-1b—c); ^{13}C-NMR δ: 53.73 (C-6a), 63.42 (C-6b—c), 94.75, 98.66 (C-1a), 102.31, 102.39 (C-1b—c); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 5.3, 5.6 min. *Anal*. Calcd for $C_{18}H_{31}N_3O_{15}\cdot 0.66H_2O$: C, 39.93; H, 6.02; N, 7.76. Found: C, 39.88; H, 5.94; N, 7.58.

Tris[*O*-α-D-glucopyranosyl-(1→4)]-6-azido-6-deoxy-D-glucopyranose (**4**): Colorless powder; mp 147—149 °C; $[\alpha]_D$ +182° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3310, 2930, 2110, 1350, 1290, 1150, 1020; ^{1}H-NMR δ: 3.25—4.10 (m), 4.67 (d, 0.5H, J=7.8 Hz, αH-1a), 5.24 (d, 0.5H, J=3.9 Hz, βH-1a), 5.37, 5.37, 5.40 (each d, each 1H, J=2.7, 2.7, 3.7 Hz, H-1b—d); ^{13}C-NMR δ: 53.83 (C-6a), 63.39 (C-6b—d), 94.77, 98.69 (C-1a), 102.23, 102.34, 102.58 (C-1b—d); t_R (column: A, eluent: 6:4 (v/v) CH_3CN–H_2O): 6.4, 6.7 min. *Anal*. Calcd for $C_{24}H_{41}N_3O_{20}\cdot 0.66H_2O$: C, 40.97; H, 6.06; N, 5.97. Found: C, 40.97; H, 6.02; N, 5.95.

Tetrakis[*O*-α-D-glucopyranosyl-(1→4)]-6-azido-6-deoxy-D-glucopyranose (**5**): Colorless powder; mp 155—157 °C (dec.); $[\alpha]_D$ +185° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3310, 2930, 2110, 1360, 1150, 1030; ^{1}H-NMR δ: 3.25—4.10 (m), 4.67 (d, 0.5H, J=8.8 Hz, αH-1a), 5.24 (d, 0.5H, J=3.9 Hz,

βH-1a), 5.30—5.45(m, 4H); ^{13}C-NMR δ: 53.73 (C-6a), 63.37 (C-6b—e), 94.77, 98.66 (C-1a), 102.22, 102.29, 102.53, 102.65 (C-1b—e); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 7.7, 8.2 min. *Anal*. Calcd for $C_{30}H_{51}N_3O_{25}\cdot 2.66H_2O$: C, 39.96; H, 6.30; N, 4.66. Found: C, 39.59; H, 6.07; N, 4.54.

General Procedure for the Preparation of Compounds 6—10 Sodium borohydride (5—10 mol eq for **1**—**5**) was added to a solution of **1** (1.50 g, 7.31 mmol), **2** (1.50 g, 4.08 mmol), **3** (1.0 g, 1.89 mmol), **4** (1.2 g, 1.74 mmol), or **5** (1.0 g, 1.17 mmol) in DMF (80—150 ml), and the mixture was stirred at 80 °C for 6 h. The mixture was neutralized with HCl (1 M), and then evaporated under reduced pressure to leave a syrupy residue. This residue was chromatographed on ion-exchange gel (Dowex 1×4, OH^- form) with H_2O, and the appropriate fractions were freeze-dried to give **6** (0.69 g, 3.81 mmol, 52%), **7** (0.51 g, 1.49 mmol, 37%), **8** (0.50 g, 0.99 mmol, 52%), **9** (0.52 g, 0.78 mmol, 45%), or **10** (0.65 g, 0.78 mol, 67%), respectively.

6-Amino-6-deoxy-D-sorbitol (**6**): ^{1}H-NMR δ: 2.81 (dd, 1H, *J*=8.06, 13.4 Hz, H-6), 3.05 (dd, 1H, *J*=3.66, 13.4 Hz, H-6), 3.52—3.89 (m, 6 H, H-1—5); ^{13}C-NMR δ: 45.68 (C-6), 65.24 (C-1), ; t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 11.0 min.

O-α-D-Glucopyranosyl-(1→4)-6-amino-6-deoxy-D-sorbitol (**7**): Colorless powder; mp 75—77 °C; $[\alpha]_D$ +103° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3290, 2930, 1590, 1420, 1340, 1150, 1080, 1030; ^{1}H-NMR δ: 2.80—2.95 (m, 2H, H-6a), 3.35—4.05 (m), 5.12 (d, 1H, *J*=3.7 Hz, H-1b); ^{13}C-NMR δ: 45.39 (C-6a), 63.34 (C-6b), 65.63 (C-1a), 103.31 (C-1b); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 14.5 min. *Anal*. Calcd for $C_{12}H_{25}NO_{10}\cdot 0.33H_2O$: C, 41.26; H, 7.41; N, 4.01. Found: C, 41.41; H, 7.43; N, 3.96.

Bis[*O*-α-D-glucopyranosyl-(1→4)]-6-amino-6-deoxy-D-sorbitol (**8**): Colorless powder; mp 108—110 °C; $[\alpha]_D$ +143° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3280, 2900, 1560, 1420, 1340, 1150, 1080, 1030; ^{1}H-NMR δ: 2.80—2.95 (m, 2H, H-6a), 3.35—4.05 (m), 5.14, 5.40 (each d, each 1H, *J*=2.7, 2.2 Hz, H-1b—c); ^{13}C-NMR δ: 45.29 (C-6a), 63.44 (C-6b—c), 65.65 (C-1a), 102.60, 103.04 (C-1b—c); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 19.9 min. *Anal*. Calcd for $C_{18}H_{35}NO_{15}\cdot 0.5H_2O$: C, 42.02; H, 7.05; N, 2.72. Found: C, 42.19; H, 7.13; N, 2.87.

Tris[*O*-α-D-glucopyranosyl-(1→4)]-6-amino-6-deoxy-D-sorbitol (**9**): Colorless powder; mp 130—132 °C (dec.); $[\alpha]_D$ +151° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3330, 2930, 1560, 1420, 1340, 1150, 1080, 1030; ^{1}H-NMR δ: 2.80—2.95 (m, 2H, H-6a), 3.35—4.05 (m), 5.14, 5.40 (each d, 1H, 2H, *J*=2.7, 2.2 Hz, H-1b—c); ^{13}C-NMR δ: 45.24 (C-6a), 63.51 (C-6b—d), 65.75 (C-1a), 102.46, 102.68, 102.94 (C-1b—d); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 23.8 min. *Anal*. Calcd for $C_{24}H_{45}NO_{20}\cdot 2H_2O$: C, 40.97; H, 7.02; N, 1.99. Found: C, 40.95; H, 6.70; N, 1.93.

Tetrakis[*O*-α-D-glucopyranosyl-(1→4)]-6-amino-6-deoxy-D-sorbitol (**10**): Colorless powder; mp 135—137 °C (dec.); $[\alpha]_D$ +165° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3320, 2930, 1560, 1420, 1340, 1150, 1080, 1030; ^{1}H-NMR δ: 2.80—2.95 (m, 2H, H-6a), 3.30—4.05 (m), 5.05, 5.35 (each d, 1H, 3H, *J*=2.7, 3.7 Hz, H-1b—e); ^{13}C-NMR δ: 45.23 (C-6a), 63.41 (C-6b—e), 66.68 (C-1a), 102.44, 102.67, 102.78, 102.94 (C-1b—e); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 26.5 min. *Anal*. Calcd for $C_{30}H_{55}NO_{25}\cdot 0.5H_2O$: C, 42.96; H, 6.73; N, 1.67. Found: C, 42.88; H, 6.65; N, 1.52.

General Procedure for the Preparation of Compounds 11—15 A solution of **1** (5.0 g, 24.4 mmol), **2** (1.0 g, 2.72 mmol), **3** (7.2 g, 13.7 mmol), **4** (1.2 g, 1.74 mmol), or **5** (1.1 g, 1.29 mmol) in distilled water (50—200 ml) was stirred vigorously over 10% Pd/C (0.45—1.0 g) under H_2 at ordinary pressure for 16 h, and the Pd/C was removed by filtration with a short pad of Celite®. The filtrate was then evaporated under reduced pressure to leave a syrupy residue. The residue was chromatographed on ion-exchange gel (Dowex 1×4, OH^- form) with H_2O, and the appropriate fractions were freeze-dried to give **11** (2.04 g, 12.5 mmol, 51%), **12** (0.72 g, 2.21 mmol, 81%), **13** (2.48 g, 5.10 mmol, 37%), **14** (0.69 g, 1.06 mmol, 61%), or **15** (0.87 g, 1.07 mmol, 83%), respectively.

(3*R*,4*R*,5*R*,6*S*)-Hexahydro-3,4,5,6-tetrahydroxy-1*H*-azepine (**11**): ^{1}H-NMR δ: 2.85—2.95 (m, 4H, H-2, H-7), 3.60—3.85 (m, 3H), 3.95—4.08 (m, 1H); ^{13}C-NMR δ: 51.86, 51.95 (C-2, C-7); t_R (column: B, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 5.2 min.

(3*R*,4*R*,5*R*,6*S*)-Hexahydro-3,5,6-trihydroxy-1*H*-azepine-4-yl α-D-Glucopyranoside (**12**): Colorless powder; mp 89—91 °C (dec.); $[\alpha]_D$ +85° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3290, 2910, 1670, 1560, 1420, 1140, 1080, 1030; ^{1}H-NMR δ: 2.85—3.12 (m, 4H, H-2a, H-7a), 3.37—4.17 (m), 5.16 (d, 1H, *J*=3.9 Hz, H-1b); ^{13}C-NMR δ: 51.76, 52.13 (C-2a, C-7a), 63.39 (C-6b), 102.14 (C-1b); t_R (column: B, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 7.7 min. *Anal*. Calcd for $C_{12}H_{23}NO_9\cdot 1.33H_2O$: C, 41.26; H, 7.41; N, 4.01. Found: C, 41.16; H, 7.25; N, 3.78.

(3*R*,4*R*,5*R*,6*S*)-Hexahydro-3,5,6-trihydroxy-1*H*-azepine-4-yl *O*-α-D-Glucopyranosyl-(1→4)-α-D-glucopyranoside (**13**): Colorless powder; mp 130—132 °C (dec.); $[\alpha]_D$ +134° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3300, 2920, 1540, 1420, 1150, 1080, 1030; ^{1}H-NMR δ: 2.85—3.12 (m, 4H, H-2a, H-7a), 3.37—4.17 (m), 5.17, 5.37 (each d, each 1H, *J*=3.9, 3.4 Hz, H-1b—c); ^{13}C-NMR δ: 51.72, 52.08 (C-2a, C-7a), 63.40 (C-6b—c), 102.41, 102.61 (C-1b—c); t_R (column: B, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 9.4 min. *Anal*. Calcd for $C_{18}H_{33}NO_{14}\cdot 2H_2O$: C, 41.30; H, 7.12; N, 2.68. Found: C, 41.02; H, 6.98; N, 2.51.

(3*R*,4*R*,5*R*,6*S*)-Hexahydro-3,5,6-trihydroxy-1*H*-azepine-4-yl Bis[*O*-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside (**14**): Colorless powder; mp 158—160 °C (dec.); $[\alpha]_D$ +152° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3310, 2930, 1540, 1420, 1150, 1080, 1030; ^{1}H-NMR δ: 2.85—3.12 (m, 4H, H-2a, H-7a), 3.35—4.18 (m), 5.17, 5.38 (each d, 1H, 2H, *J*=3.9, 3.2 Hz, H-1b—d); ^{13}C-NMR δ: 51.45, 51.67 (C-2a, C-7a), 63.44 (C-6b—d), 101.73, 102.46, 102.65 (C-1b—d); t_R (column: B, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 11.4 min. *Anal*. Calcd for $C_{24}H_{43}NO_{19}\cdot H_2O$: C, 43.18; H, 6.79; N, 2.10. Found: C, 43.09; H, 6.69; N, 2.11.

(3*R*,4*R*,5*R*,6*S*)-Hexahydro-3,5,6-trihydroxy-1*H*-azepine-4-yl Tris[*O*-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside (**15**): Colorless powder; mp 160—162 °C (dec.); $[\alpha]_D$ +161° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3280, 2930, 1650, 1420, 1150, 1080, 1030; ^{1}H-NMR δ: 2.85—3.04 (m, 4H, H-2a, H-7a), 3.35—4.18 (m), 5.17, 5.35—5.47 (d, m, 1H, 3H, *J*=3.7 Hz, H-1b—e); ^{13}C-NMR δ: 51.06, 51.47 (C-2a, C-7a), 63.41 (C-6b—e), 101.73, 102.43, 102.51, 102.65 (C-1b—e); t_R (column: B, eluent: 6 : 4 (v/v) CH_3CN–H_2O): 13.2 min. *Anal*. Calcd for $C_{30}H_{53}NO_{24}\cdot 1.5H_2O$: C, 42.96; H, 6.73; N, 1.67. Found: C, 42.90; H, 6.75; N, 1.57.

General Procedure for the Preparation of Compounds 16—20 Barium carbonate (1.4 mol eq for **1**—**5**) and Br_2 (1.2 mol eq for **1**—**5**) were added to a stirred solution of **1** (5.0 g, 24.4 mmol), **2** (2.0 g, 5.44 mmol), **3** (3.0 g, 5.67 mmol), **4** (1.5 g, 2.17 mmol), or **5** (3.5 g, 4.10 mmol) in H_2O (50—200 ml), and the mixture was stirred at room temperature for 16 h. After removal of the excess Br_2 under reduced pressure, $Fe_2(SO_4)_3\cdot xH_2O$ (100—300 mg) and H_2O_2 (600—1000 μl) were added to the stirred reaction mixture, and the mixture was stirred at 40 °C for 8 h. It was then passed through an active carbon column using an EtOH–H_2O gradient of 0 to 50% and the appropriate fractions were freeze-dried. Each semipurified intermediate in distilled water (30—60 ml) was stirred vigorously over 10% Pd/C (0.45—1.0 g) under H_2 at ordinary pressure for 16 h. The Pd/C was removed by filtration with a short pad of Celite®. The filtrate was then evaporated under reduced pressure to leave a syrupy residue. The residue was chromatographed on ion-exchange gel (Dowex 1×4, OH^- form) with H_2O, and the appropriate fractions were freeze-dried to give **16** (0.45 g, 3.4 mmol, 14%), **17** (0.18 g, 0.61 mmol, 11%), **18** (0.61 g, 1.32 mmol, 23%), **19** (0.16 g, 0.26 mmol, 12%), or **20** (0.34 g, 0.43 mmol, 11%), respectively.

(3*R*,5*R*)-3,4,5-Trihydroxypiperidine (**16**): ^{1}H-NMR δ: 2.37 (dd, 1H, *J*=9.3, 13.2 Hz, H-2), 2.67 (dd, 1H, *J*=2.5, 14.2 Hz, H-6), 2.87 (dd, 1H, *J*=4.2, 14.2 Hz, H-6), 3.04 (dd, 1H, *J*=9.6, 13.2 Hz, H-2), 3.56 (dd, 1H, *J*=3.2, 8.6 Hz, H-4), 3.70-3.85 (m, 1H, H-3), 3.92—4.00 (m, 1H, H-5); ^{13}C-NMR δ: 51.01, 51.42 (C-2, C-6); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 9.5 min.

(3*R*,5*R*)-3,5-Dihydroxypiperidine-4-yl α-D-Glucopyranoside (**17**): Colorless powder; mp 121—123 °C (dec.); $[\alpha]_D$ +53° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3280, 2910, 1560, 1340, 1150, 1020; ^{1}H-NMR δ: 2.36 (dd, 1H, *J*=9.3, 13.2 Hz, H-2a), 2.68 (dd, 1H, *J*=2.5, 14.2 Hz, H-6a), 2.90 (dd, 1H, *J*=4.1, 14.2 Hz, H-6a), 3.04 (dd, 1H, *J*=4.6, 13.2 Hz, H-2a), 3.40—4.15 (m), 5.20 (d, 1H, *J*=3.9 Hz, H-1b); ^{13}C-NMR δ: 51.30, 51.37 (C-2a, C-6a), 63.49 (C-6b), 102.75 (C-1b); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 11.8 min. *Anal*. Calcd for $C_{11}H_{21}NO_8\cdot H_2O$: C, 42.17; H, 7.40; N, 4.47. Found: C, 42.53; H, 7.25; N, 4.39.

(3*R*,5*R*)-3,5-Dihydroxypiperidine-4-yl *O*-α-D-Glucopyranosyl-(1→4)-α-D-glucopyranoside (**18**): Colorless powder; mp 157—159 °C (dec.); $[\alpha]_D$ +117° (*c*=0.25, H_2O); IR (KBr) cm^{-1}: 3310, 2930, 1560, 1340, 1150, 1040; ^{1}H-NMR δ: 2.41 (dd, 1H, *J*=9.5, 13.2 Hz, H-2a), 2.70 (dd, 1H, *J*=2.2, 14.2 Hz, H-6a), 2.93 (dd, 1H, *J*=3.4, 14.2 Hz, H-6a), 3.10 (dd, 1H, *J*=3.9, 13.2 Hz, H-2a), 3.40—4.15 (m), 5.20, 5.37 (each d, each 1H, *J*=3.9 Hz, H-1b—c); ^{13}C-NMR δ: 51.22, 51.57 (C-2a, C-6a), 63.59 (C-6b—c), 102.24, 102.56 (C-1b—c); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 13.1 min. *Anal*. Calcd for $C_{17}H_{31}NO_{13}$: C, 44.64; H, 6.83; N, 3.06. Found: C, 44.21; H, 6.85; N, 2.96.

(3*R*,5*R*)-3,5-Dihydroxypiperidine-4-yl Bis[*O*-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside (**19**): Colorless powder; mp 160—163 °C (dec.); $[\alpha]_D$

+135° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3290, 2930, 1560, 1400, 1150, 1030; ^{1}H-NMR δ: 2.52 (dd, 1H, J=9.0, 12.9 Hz, H-2a), 2.80 (dd, 1H, J=2.2, 13.9 Hz, H-6a), 3.00 (dd, 1H, J=4.4, 13.9 Hz, H-6a), 3.14 (dd, 1H, J=4.0, 12.9 Hz, H-2a), 3.40—4.15 (m), 5.20, 5.35, 5.37 (each d, each 1H, J=3.9, 3.7, 4.2 Hz, H-1b—d); ^{13}C-NMR δ: 51.03, 51.32 (C-2a, C-6a), 63.56 (C-6b—d), 102.23, 102.39, 102.70 (C-1b—d); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 16.0 min. *Anal*. Calcd for $C_{23}H_{41}NO_{18}$: C, 44.59; H, 6.67; N, 2.26. Found: C, 44.20; H, 6.42; N, 2.03.

(3*R*,5*R*)-3,5-Dihydroxypiperidine-4-yl Tris[*O*-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside (**20**): Colorless powder; mp 168—170 °C (dec.); $[\alpha]_D$ +169° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3270, 2920, 1550, 1400, 1150, 1030; ^{1}H-NMR δ: 2.48 (dd, 1H, J=9.3, 13.0 Hz, H-2a), 2.78 (dd, 1H, J=2.2, 14.1 Hz, H-6a), 3.99 (dd, 1H, J=3.4, 14.1 Hz, H-6a), 3.15 (dd, 1H, J=4.0, 13.0 Hz, H-2a), 3.40—4.15 (m), 5.20, 5.35 (d, m, 1H, 3H, J=3.9 Hz, H-1b—e); ^{13}C-NMR δ: 51.01, 51.25 (C-2a, C-6a), 63.51 (C-6b—e), 102.21, 102.36, 102.50, 102.70 (C-1b—e); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 22.4 min. *Anal*. Calcd for $C_{29}H_{51}NO_{23}\cdot 0.5H_2O$: C, 44.05; H, 6.63; N, 1.77. Found: C, 44.23; H, 6.35; N, 1.58.

Preparation of Compounds 21—24 Compound **21** was prepared by the procedure of Bernotas *et al*.[21]

CGTase (5.5 ml, 3,300 U)[23] was added to a stirred solution of α-CD (12 g, 12.3 mmol) and **21** (0.5 g, 3.76 mmol) in 10 mM PIPES buffer (pH 5.2, 150 ml), and the mixture was stirred at 37 °C for 16 h. The solution was boiled for 1 h to inactivate the enzyme. After cooling, 650 mg (217 U) of β-amylase was added and the mixture was stirred at 37 °C for 3 h. The solution was boiled for 1 h to stop the reaction. After cooling, precipitated enzymes were removed by filtration with a short pad of Celite®. The mixture was chromatographed on ion-exchange gel (Dowex 1×4, OH^- form) with H_2O, and the appropriate fractions were freeze-dried to give **22** (0.03 g, 0.10 mmol, 2.7%), **23** (0.46 g, 1.01 mmol, 27%), and **24** (0.37 g, 0.60 mmol, 16%), respectively.

(3*S*,5*R*)-3,4,5-Trihydroxypiperidine (**21**): ^{1}H-NMR δ: 2.38 (dd, 2H, J=10.5, 10.8 Hz, H-2, H-6a), 3.05 (dd, 2H, J=5.1, 10.05 Hz, H-2, H-6), 3.25 (t, 1H, J=8.1 Hz, H-4), 3.40—3.55 (m, 2H, H-3, H-5); ^{13}C-NMR δ: 52.14 (C-2, C-6), 73.87 (C-3, C-5), 81.26 (C-4); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 5.6 min.

(3*R*,4*R*,5*S*)-4,5-Dihydroxypiperidine-3-yl α-D-Glucopyranoside (**22**): Colorless powder; mp 127—129 °C (dec.); $[\alpha]_D$ +97° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3280, 2920, 1440, 1340, 1150, 1040; ^{1}H-NMR δ: 2.42 (dd, 1H, J=9.8, 13.2 Hz, H-2a or H-6a), 2.71 (dd, 1H, J=2.0, 19.5 Hz, H-2a or H-6a), 2.94 (dd, 1H, J=3.9, 14.2 Hz, H-2a or H-6a), 3.09 (dd, 1H, J=6.6, 13.2 Hz, H-2a or H-6a), 3.40—4.17 (m), 5.21 (d, 1H, J=3.9 Hz, H-1b); ^{13}C-NMR δ: 51.03, 51.76 (C-2a, C-6a), 63.49 (C-6b), 82.66 (C-3a), 102.73 (C-1b); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 7.9 min. *Anal*. Calcd for $C_{11}H_{21}NO_8\cdot 0.5H_2O$: C, 43.42; H, 7.29; N, 4.60. Found: C, 43.42; H, 7.20; N, 4.59.

(3*R*,4*R*,5*S*)-4,5-Dihydroxypiperidine-3-yl *O*-α-D-Glucopyranosyl-(1→4)-α-D-glucopyranoside (**23**): Colorless powder; mp 150—153 °C (dec.); $[\alpha]_D$ +138° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3290, 2920, 1440, 1340, 1150, 1030; ^{1}H-NMR δ: 2.33—2.60 (m, 2H, H-2a, H-6a), 3.02—3.33 (m, 2H, H-2a, H-6a), 3.40—4.02 (m), 5.16, 5.35 (each d, each 1H, J=3.9, 3.7 Hz, H-1b—c); ^{13}C-NMR δ: 51.03, 51.81 (C-2a, C-6a), 63.59 (C-6b—c), 82.66 (C-3a), 102.41, 102.65 (C-1b—c); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 9.1 min. *Anal*. Calcd for $C_{17}H_{31}NO_{13}\cdot 0.5H_2O$: C, 43.78; H, 6.91; N, 3.00. Found: C, 43.79; H, 6.79; N, 2.70.

(3*R*,4*R*,5*S*)-4,5-Dihydroxypiperidine-3-yl Bis[*O*-α-D-glucopyranosyl-(1→4)]-α-D-glucopyranoside (**24**): Colorless powder; mp 178—181 °C (dec.); $[\alpha]_D$ +138° (c=0.25, H_2O); IR (KBr) cm^{-1}: 3280, 2930, 1440, 1340, 1150, 1030; ^{1}H-NMR δ: 2.33—2.60 (m, 2H, H-2a, H-6a), 3.02—3.33 (m, 2H, H-2a, H-6a), 3.40—4.02 (m), 5.16, 5.35 (each d, 1H, 2H, J=3.9, 3.7 Hz, H-1b—d); ^{13}C-NMR δ: 50.00, 51.76 (C-2a, C-6a), 63.51 (C-6b—d), 82.58 (C-3a), 102.41, 102.41, 102.68 (C-1b—d); t_R (column: A, eluent: 6 : 4 (v/v) CH_3CN–ammonium formate buffer 25 mM pH 8.5): 10.8 min. *Anal*. Calcd for $C_{23}H_{41}NO_{18}\cdot 0.5H_2O$: C, 43.95; H, 6.73; N, 2.23. Found: C, 43.99; H, 6.66; N, 2.19.

Bioassay Methods. Measurement of IC_{50} for HPA and HSA α-Amylase inhibitory activity was determined using an α-amylase activity determination kit (Neo·Amylase Test Daiichi) utilizing blue starch as a substrate. Four ml of different concentrations of test compound was preincubated at 37 °C for 5 min. Then, 0.1 ml of α-amylase solution containing 300 Somogyi units/dl and one tablet (0.20 g) of blue starch containing buffer reagents were added to the solution. After incubation for 30 min at 37 °C, 1.0 ml of 0.5 M NaOH was added to the reaction mixture to stop the reaction. The mixture was then centrifuged at 1500×***g*** for 5 min, and absorbance of the supernatant solution was measured at 620 nm. For the blank, H_2O was added instead of the α-amylase solution. The concentration producing 50% inhibition (IC_{50}) was determined from a plot of inhibition percent *vs*. the concentration.

Measurement of IC_{50} for Mice Pancreatic α-Amylase Pancreas of two male ICR mice (7 weeks old) were homogenized with 6 ml of 50 mM phosphate buffer (pH 7.0). The homogenate was centrifuged at 100000×***g*** for 1 h and the pancreatic α-amylase activity in the supernatant was determined using blue starch as a substrate; then the supernatant was diluted to adjust to 300 Somogyi units/dl by 50 mM phosphate buffer (pH 7.0). IC_{50} of **13** or **18** was measured using the same method for HPA and HSA.

Measurement of IC_{50} for Rat Maltase Rat small intestinal brush border membrane[30] vesicles were used in the preparation of small intestinal maltase. Reaction was performed by the procedure of Dahlqvist[31] with slight modifications. The substrate (maltose: 37 mM), test compound, and the enzyme in 0.1 M phosphate buffer (pH 7.0, 3.1 ml) were incubated together at 37 °C. After 30 min of incubation, the reaction mixture was heated for 2 min in a boiling water bath to stop the reaction. The amount of liberated glucose was measured by the glucose oxidase method using Glucose B-test Wako.

Suppression Test of Postprandial Hyperglycemia After having been fasted for 20 h, the test compound (**13** or **18**, 60 mg/kg) and cornstarch (2000 mg/kg) were simultaneously given orally to male ICR (Jcl) mice (5 weeks old). Blood glucose concentration was measured before and after (30, 60, 90, 120, 180, 240 min) administration. The control experiment was carried out with distilled water instead of **13** and **18**. Sample blood was collected from the supraorbital vein, and the blood glucose concentration was measured with an ANTSENSE.

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