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Carbohydrate Research 307 (1998) 69–76

CARBOHYDRATE RESEARCH

New enzymatic synthesis of 6^3-modified maltooligosaccharides and their inhibitory activities for human α-amylases

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Received 30 June 1997; accepted 20 December 1997

Abstract

Ten new 6^3-modified maltopentaoses and tetraoses were synthesized by enzymatic reactions utilizing cyclodextrin glycosyltransferase (EC 2.4.1.19) and subsequent human salivary α-amylase (HSA) (EC 3.2.1.1). Among these compounds, α-D-glucopyranosyl-(1→4)-α-D-glucopyranosyl-(1→4)-(6-deoxy-α-D-glucopyranosyl)-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose (**11**) and α-D-glucopyranosyl-(1→4)-(6-deoxy-α-D-glucopyranosyl)-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose (**12**) showed strong inhibitory activities for human pancreatic α-amylase (HPA) and HSA. The IC_{50} of 6^3-deoxymaltopentaose **11** (8.0×10^{-5} M for HPA, 1.0×10^{-4} M for HSA) and 6^3-deoxymaltotetraose **12** (2.0×10^{-3} M for HPA, 2.0×10^{-3} M for HSA) were lower than that of 6^3-deoxymaltotriose [(6-deoxy-α-D-glucopyranosyl)-(1→4)-α-D-glucopyranosyl-(1→4)-D-glucopyranose **13**; 2.0×10^{-3} M for HPA, 4.2×10^{-2} M for HSA]. © 1998 Elsevier Science Ltd. All rights reserved

Keywords: α-Amylase; Inhibitor; Deoxymaltooligosaccharide; Cyclodextrin glycosyltransferase; Enzymatic synthesis

1. Introduction

Many α-amylase (EC 3.2.1.1) inhibitors have been obtained from microbial [1,2] and plant origins [3–5], and have also been found in synthetic substances [6–12]. They have been proved of great value because of their various uses as tools for the investigation of active sites of α-amylases [10–13], as reagents for measurement of α-amylase isoenzymes activities by selective inhibition [3,6,14], or as oral agents for treatment of diabetes [4,15]. We recently reported that deoxymaltooligosaccharides having a 6-deoxy-D-glucosyl group at the non-reducing end were highly selective human pancreatic α-amylase (HPA) inhibitors [6], and that 6^3-deoxymaltotriose (**13**) was the strongest one. Omichi et al. [8] reported inhibition of 6^3-deoxy-6^3-iodomaltotriose (**7**) for human α-amylases, and Lehmann et al. [10] indicated inhibition of 6^3-amino-6^3-deoxymaltotriose (**16**) for porcine pancreatic α-amylase. Saito [16] showed that, with regard to the hydrolysis rate of maltooligosaccharides by human α-amylases, that of maltotriose

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PII S0008-6215(98)00029-9

(G3) was extremely low, that of maltotetraose (G4) and maltoheptaose (G7) were moderate, and that of maltopentaose (G5) and maltohexaose (G6) were high. The report also showed that G4, G5 and G6 had smaller K_m values than G7. It is thus presumed that maltooligosaccharides having four to six glucosyl chain lengths would be the best match to human α-amylases. Therefore, 6^3-modified maltotrioses having some α-glucosyl units at 4^3-positions were expected to be more potent human α-amylases inhibitors (Fig. 1).

In this report, we describe the enzymatic syntheses of 10 new 6^3-modified maltooligosaccharides using transglycosylation with cyclodextrin glycosyltransferase (CGTase) (EC 2.4.1.19) followed by unique hydrolysis with human salivary α-amylase (HSA). We discuss the effects of various substituents and glucosyl chain lengths on the inhibitory activity of human α-amylases.

2. Results and discussion

Synthesis.—The chemical synthesis for the introduction of a glucosyl moiety having an α-(1→4) linkage to the non-reducing end of 6^3-modified maltotrioses is an exciting but difficult challenge. There is no really efficient method available to a carbohydrate chemist because of the great number of steps necessary for such a synthesis and the low yield. We therefore decided to focus on enzymatic syntheses and mono-6-*O*-*p*-toluenesulfonylcyclomaltohexaose (**1**) [17] was chosen as a readily available starting material. Only the cleavage of a glucosyl linkage of **1** is required to obtain linear modified maltooligosaccharides. Moreover, the *p*-toluenesulfonyloxy group is easily changed to various functional groups. First attempts to selectively cleave **1** with cyclomaltodextrinase (EC 3.2.1.54) [18] or taka-amylase A (EC 3.2.1.1) [19] were unsuccessful and gave complex mixtures. We then examined the transglycosylation method using CGTase [20–22]. The CGTase transglycosylation of maltose as the glucosyl donor with **1** was first carried out at 40 °C (Scheme 1). Similar to the CGTase transglycosylation pattern of mono-3,6-anhydrocyclomaltooligosaccharides with various donors reported by Simiand et al. [20], 6-*O*-*p*-toluenesulfonylmaltooctaose was mainly produced at the early stage of the CGTase reaction. A complex mixture was then concomitantly produced as the reaction progressed. Because of complexity at the later stage, it seemed that the enzyme had not only coupling activity of maltose to **1** but also disproportional and hydrolytic activity for accumulated decycling products.

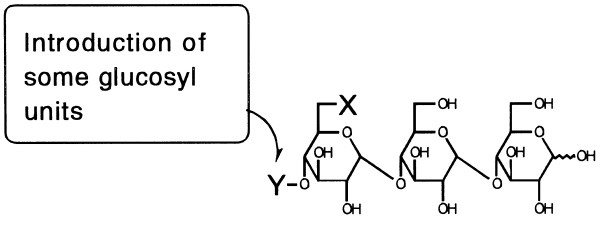
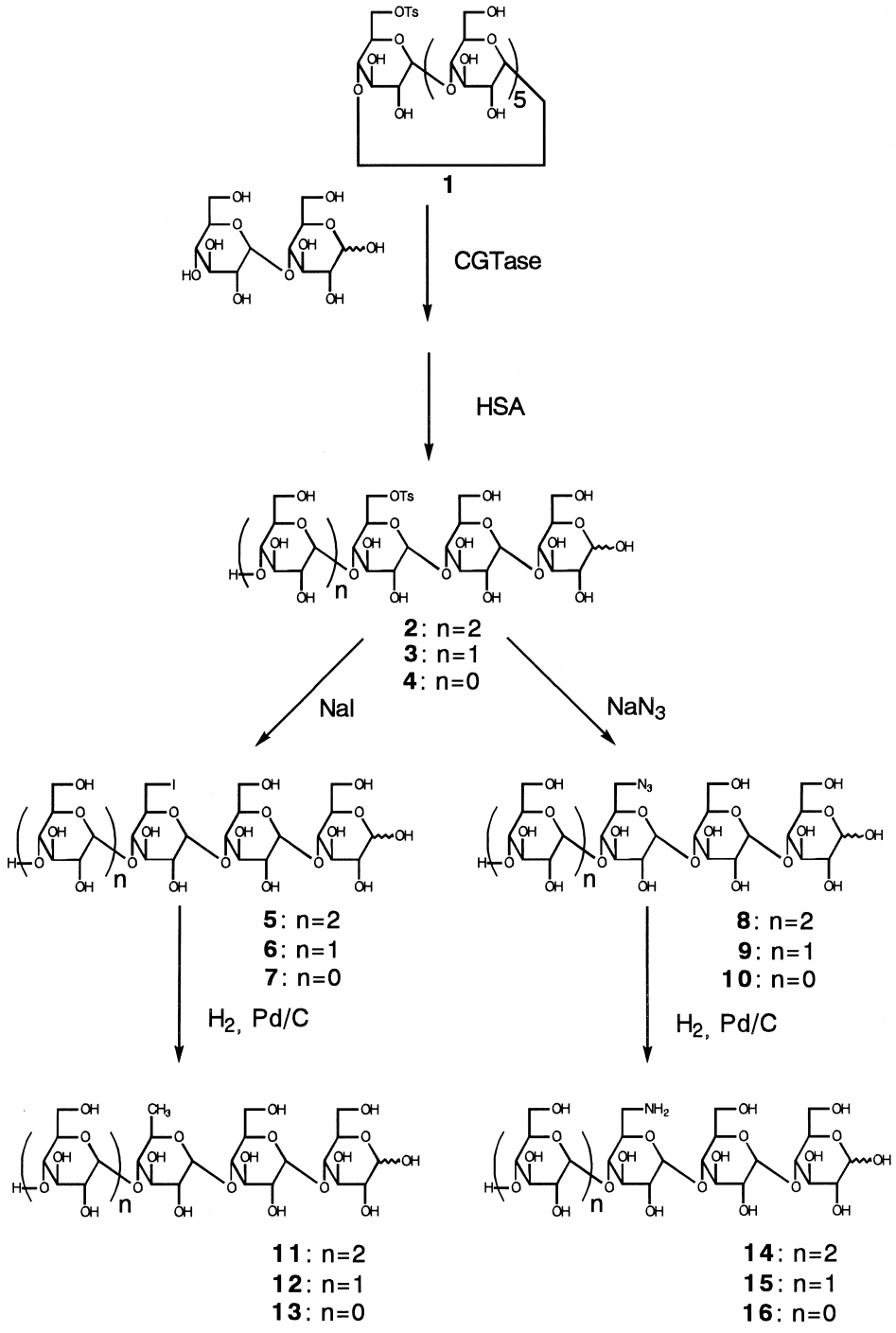


Fig. 1. Design of modified maltooligosaccharide for a human α-amylase inhibitor. Abbreviation: X, various functional groups; Y, some glucosyl units.

We reported that the hydrolysis of 2-chloro-4-nitrophenyl 6^5-*O*-*p*-toluenesulfonylmaltopentaoside by HSA was very limited and the products were almost 6^3-*O*-*p*-toluenesulfonylmaltotriose and 2-chloro-4-nitrophenyl maltoside [23]. This finding led us to expect that HSA could hydrolyze selectively between the second and third glucose residue from the 6-*O*-*p*-toluenesulfonylglucose residue, when the CGTase transglycosylation products and HSA are incubated, and then the 4^3-*O*-maltooligosyl-6^3-*O*-*p*-toluenesulfonylmaltotrioses could be obtained. As expected, the subsequent hydrolyses by HSA gave 6^3-*O*-*p*-toluenesulfonylmaltopentaose (**2**, 17%), 6^3-*O*-*p*-toluenesulfonylmaltotetraose (**3**, 23%), and 6^3-*O*-*p*-toluenesulfonylmaltotriose (**4**, 6%), but 6^3-*O*-*p*-toluenesulfonylmaltohexaose was not available, also as anticipated.

To evaluate the influence of various substituents at the 6^3-position and the glucosyl chain length of 6^3-modified maltooligosaccharides on human α-amylase inhibition, we converted 6^3-*O*-*p*-toluenesulfonylmaltooligosaccharides (**2**–**4**) to 6^3-deoxy-6^3-iodo- (**5**–**7**), 6^3-azido-6^3-deoxy- (**8**–**10**), 6^3-deoxy- (**11**–**13**), or 6^3-amino-6^3-deoxy- (**14**–**16**) derivatives in the usual way as shown in Scheme 1. Reaction of the tosylates (**2**–**4**) with NaI or NaN_3 in *N*,*N*-dimethylformamide (DMF) at 80 °C gave 6^3-deoxy-6^3-iodomaltopentaose (**5** from **2**, 79%), 6^3-deoxy-6^3-iodomaltotetraose (**6** from **3**, 86%), 6^3-deoxy-6^3-iodomaltotriose (**7** from **4**, 82%), 6^3-azido-6^3-deoxymaltopentaose (**8** from **2**, 62%), 6^3-azido-6^3-deoxymaltotetraose (**9** from **3**, 77%), or 6^3-azido-6^3-deoxymaltotriose (**10** from **4**, 73%), respectively. Reductive dehalogenation of the iodo derivatives (**5**–**7**) with hydrogen in the presence of



Scheme 1. Synthesis of 6^3-modified maltooligosaccharides.

palladium-charcoal gave 6^3-deoxymaltopentaose (**11** from **5**, 81%), 6^3-deoxymaltotetraose (**12** from **6**, 72%), or 6^3-deoxymaltotriose (**13** from **7**, 78%). Reduction of the azido derivatives (**8**–**10**) afforded 6^3-amino-6^3-deoxymaltopentaose (**14** from **8**, 82%), 6^3-amino-6^3-deoxymaltotetraose (**15** from **9**, 69%), or 6^3-amino-6^3-deoxymaltotriose (**16** from **10**, 77%).

Confirmation of structures of the modified oligosaccharides.—Structures of the synthesized compounds were established by spectral data, elemental analyses, and hydrolysis tests using glucoamylase (GA) (EC 3.2.1.3). In the case of iodomaltopentaose **5**, we determined the glucosyl chain length and the α-(1→4) linkages by ^{1}H NMR. Signals at δ 4.65 (0.5 H, d, J 7.8 Hz) and δ 5.23 (0.5 H,

d, J 4.2 Hz) were assigned to the reducing end H-1 (α and β, respectively) and signals at δ 5.31–5.42 (4 H, each d, J 3.9–4.2 Hz) were assigned to four other glucosyl H-1 protons, indicating the presence of four α-glucosyl bonds and one reducing end. With regard to length and linkage, the other compounds (**2**–**4** and **6**–**16**) were determined in a similar way. The ^{13}C NMR spectra showed characteristic substituents: δ 133 and 131 (-SO_2*Ph*CH_3), δ 24 (-SO_2Ph*C*H_3), δ 11 (-CH_2I), δ 54 (-CH_2N_3), δ 20 (-CH_3), and δ 44 (-CH_2NH_2).

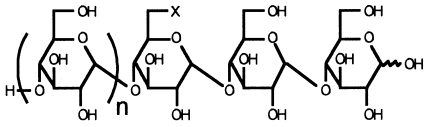
The position of the substituents was established by the hydrolysis test using GA. Because GA is an exo-type enzyme and hydrolyzes α-(1→4) glucosidic bonds, glucose residues are liberated one by one from the non-reducing end. It is also known that GA hydrolyzes 6^1-deoxy-6^1-iodomaltose to liberate glucose, but does not split 6^2-deoxy-6^2-iodomaltose [24]. When isolated **5**–**7** and GA were incubated, **7** was resistant to the hydrolysis, and **5** and **6** were hydrolyzed to give **7** with glucose in a molar ratio of 1:2 and 1:1, respectively. Thus the 6-deoxy-6-iodo group must exist at the 6^3-position in the oligosaccharides (**5**–**7**). The results of the hydrolysis tests on iodo derivatives suggested that the *O*-*p*-toluenesulfonyl group (**2**–**4**), the precursor of iodo, azido (**8**–**10**), deoxy (**11**–**13**), and amino (**14**–**16**) groups which are derived from the *O*-*p*-toluenesulfonyl group also exists at the 6^3-position.

Inhibition of α-amylases by the modified maltooligosaccharides.—In the previous report [6], we used 2-chloro-4-nitrophenyl β-maltopentaoside as a substrate to measure the activity of the human α-amylases inhibited. In that case, coupling enzymes of α-glucosidase and β-glucosidase were required to assay. To exclude interaction between coupling enzymes and inhibitors, we chose blue starch as a substrate in the present study. This method allows the determination of the activity of inhibited α-amylases without coupling enzymes.

Compounds were tested to determine the inhibitory activity of the two human α-amylases, and the enzyme inhibition data *in vitro* (IC_{50}: molar concentrations required for a 50% inhibition) against HPA and HSA are shown in Table 1. The tested concentration of inhibitors was under 5.0 mM except for **13** which was the strongest inhibitor in the previous report [6]. All of the tested compounds were only cleaved to a minor extent by the two human α-amylases under the conditions used (by HPLC analysis). Among ten modified maltopentaoses and maltotetraoses, the IC_{50} of 6^3-deoxymaltopentaose (**11**) and 6^3-deoxymaltotetraose (**12**) was lower than that of 6^3-deoxymaltotriose (**13**). The strongest inhibitor of **11** has a 25-fold stronger activity for HPA and 420-fold stronger activity for HSA than that of **13**. Although 6^3-deoxy-6^3-iodomaltopentaose (**5**) showed a moderate inhibitory activity, 6^3-*O*-*p*-toluenesulfonyl- (**2**–**4**), 6^3-deoxy-6^3-iodo- (**6**–**7**), 6^3-azido-6^3-deoxy- (**8**–**10**), and 6^3-amino-6^3-deoxy- (**14**–**16**) maltooligosaccharides exhibited no appreciable inhibition for the two α-amylases. It has been reported that **7** and **16** are inhibitors for human α-amylases [8] and porcine pancreatic α-amylase [10], respectively, but neither compound showed any apparent inhibition for the two human α-amylases under the conditions used. For 50% inhibition, a concentration of more than 5.0 mM may be required in the cases of **7** and **16**.

In conclusion, we have developed a simple synthetic method for 6^3-modified maltooligosaccharides using CGTase and HSA, and 10 6^3-modified maltopentaoses and tetraoses were newly synthesized along with five known maltotrioses modified at the 6^3-position. Among these compounds, we

Table 1
Inhibitory activity for HPA and HSA by 6^3-modified maltooligosaccharides



Compound no.	X	*n*	IC_{50} [a](M)	
			HPA	HSA
2	OTs	2	NI [b]	NI
3	OTs	1	NI	NI
4	OTs	0	NI	NI
5	I	2	4.5×10^{-3}	NI
6	I	1	NI	NI
7	I	0	NI	NI
8	N_3	2	NI	NI
9	N_3	1	NI	NI
10	N_3	0	NI	NI
11	H	2	8.0×10^{-5}	1.0×10^{-4}
12	H	1	2.0×10^{-3}	2.0×10^{-3}
13	H	0	2.0×10^{-3}	4.2×10^{-2}
14	NH_2	2	NI	NI
15	NH_2	1	NI	NI
16	NH_2	0	NI	NI

[a] IC_{50}: Molar concentration required to give 50% inhibition.
[b] Less than 50% inhibition at 5.0×10^{-3} M.

found that 6^3-deoxymaltopentaose (**11**) was the strongest human α-amylases inhibitor. For inhibition of the two human α-amylases, we showed that the most effective group on the 6^3-position of maltooligosaccharides is the deoxy group and the most suitable chain length is five.

3. 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., Tokyo, Japan. Standard HPA and HSA were obtained from International Reagents Corp., Kobe, Japan. CGTase (from *Bacillus macerans*) was purchased from Amano Co., Ltd., Japan, and glucoamylase (from *Rhizopus* sp.) was purchased 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 MHz and ^{13}C NMR spectra were taken at 50.10 MHz with a JEOL JNM-FX200 spectrometer using sodium 3-(trimethylsilyl)propionate as an internal standard and D_2O as a solvent. HPLC was performed on a TSK gel Amide-80 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. Column chromatography was performed on YMC-GEL ODS-AQ (120-S50).

α-D-*Glucopyranosyl-(1→4)*-α-D-*glucopyranosyl-(1→4)-(6*-O-p-*toluenesulfonyl*-α-D-*glucopyranosyl)-(1→4)*-α-D-*glucopyranosyl-(1→4)*-D-*glucopyranose* (**2**), α-D-*glucopyranosyl-(1→4)-(6*-O-p-*toluenesulfonyl*-α-D-*glucopyranosyl)-(1→4)*-α-D-*glucopyranosyl-(1→4)*-D-*glucopyranose* (**3**), *(6*-O-p-*toluenesulfonyl*-α-D-*glucopyranosyl)-(1→4)*-α-D-*glucopyranosyl-(1→4)*-D-*glucopyranose* (**4**).—CGTase (270 mL, 162,000 U) [23] was added to a stirred solution of mono-6-*O*-*p*-toluenesulfonylcyclomaltohexaose (**1**; 270 g, 0.24 mol) [17] and maltose (864 g, 2.4 mol) in 10 mM PIPES buffer (pH 5.2, 6.4 L), and the mixture was stirred at 40 °C for 1 h. The solution was boiled for 1 h to inactivate the enzyme. After cooling, the solution was adjusted to pH 7.0 with 1.0 M NaOH. Then, 150 mL (~250,000 Somogyi U) of human salivary amylase was added and the mixture was stirred at 37 °C for 48 h. The solution was boiled for 1 h to stop the reaction. After cooling, precipitated enzymes were removed by filtration through a short pad of Celite®. The filtrate was then passed through a column of ODS gel eluted using a CH_3CN–H_2O gradient of 0 to 20%. Freeze-dried appropriate fractions gave **2** (40.7 g, 41.4 mmol, 17%), **3** (45.0 g, 54.8 mmol, 23%), and **4** (9.3 g, 14.1 mmol, 6%), respectively.

Compound **2**: colorless amorphous; mp 158–161 °C (dec.); $[\alpha]_D$ +140° (*c* 0.25, H_2O); ν_{max} 3310, 2930, 1650, 1600, 1360, and 1020 cm^{-1}; ^{1}H NMR (D_2O): δ 7.85 (d, 2 H, *J* 8.4 Hz, H-2 and H-6 of SO_2PhCH_3), 7.54 (d, 2 H, *J* 8.4 Hz, H-3 and H-5 of SO_2PhCH_3), 5.24 (d, 0.5 H, *J* 3.7 Hz, β H-1a), 5.35, 5.33, 5.31, and 5.19 (d, each 1 H, *J* 3.9–4.2 Hz, H-1b–e), 4.65 (d, 0.5 H, *J* 8.1 Hz, α H-1a), 4.45–4.38 (m, 2 H, H-6c), 4.00–3.22 (m, ~28 H, H-2–6), 2.42 (s, 3 H, SO_2PhCH_3); ^{13}C NMR: δ 132.99 and 130.51 (SO_2PhCH_3), 102.94, 102.71, 102.70, and 102.38 (C-1b–e), 98.69 and 94.75 (C-1a), 23.57 (SO_2PhCH_3); t_R (6:4 CH_3CN–H_2O): 5.5 min. Anal. Calcd for $C_{43}H_{66}O_{32}S·0.25H_2O$: C, 44.40; H, 6.04; S, 3.20. Found: C, 44.47; H, 5.97; S, 3.30.

Compound **3**: colorless amorphous; mp 152–154 °C (dec.); $[\alpha]_D$ +135° (*c* 0.25, H_2O); ν_{max} 3300, 2930, 1640, 1600, 1360, and 1020 cm^{-1}; ^{1}H NMR (D_2O): δ 7.85 (d, 2 H, *J* 8.5 Hz, H-2 and H-6 of SO_2PhCH_3), 7.53 (d, 2 H, *J* 8.5 Hz, H-3 and H-5 of SO_2PhCH_3), 5.24 (d, 0.5 H, *J* 3.9 Hz, β H-1a), 5.35, 5.28, and 5.18 (d, each 1 H, *J* 3.4–3.9 Hz, H-1b–d), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.42–4.35 (m, 2 H, H-6c), 4.01–3.25 (m, ~22 H, H-2–6), 2.48 (s, 3 H, SO_2PhCH_3); ^{13}C NMR: δ 132.9 and 130.5 (SO_2PhCH_3), 102.80, 102.41, and 102.31 (C-1b–d), 98.66 and 94.72 (C-1a), 23.52 (SO_2PhCH_3); t_R (6:4 CH_3CN–H_2O): 4.5 min. Anal. Calcd for $C_{31}H_{48}O_{23}S·0.66H_2O$: C, 44.71; H, 5.97; S, 3.85. Found: C, 44.75; H, 6.01; S, 3.67.

Compound **4**: colorless amorphous; mp 146–148 °C (dec.); $[\alpha]_D$ +118° (*c* 0.25, H_2O); ν_{max} 3330, 2930, 1640, 1600, 1360, and 1030 cm^{-1}; ^{1}H NMR (D_2O): δ 7.86 (d, 2 H, *J* 8.5 Hz, H-2 and H-6 of SO_2PhCH_3), 7.52 (d, 2 H, *J* 8.5 Hz, H-3 and H-5 of SO_2PhCH_3), 5.23 (d, 0.5 H, *J* 3.9 Hz, β H-1a), 5.34 and 5.20 (d, each 1 H, *J* 3.9 Hz, H-1b–c), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.45–4.35 (m, 2 H, H-6c),

4.01–3.23 (m, ~16 H, H-2–6), 2.47 (s, 3 H, SO_2PhCH_3); ^{13}C NMR: δ 132.94 and 130.55 (SO_2PhCH_3), 102.41 and 102.33 (C-1b–c), 98.69 and 94.74 (C-1a), 23.52 (SO_2PhCH_3); t_R (6:4 CH_3CN–H_2O): 3.8 min. Anal. Calcd for $C_{25}H_{38}O_{18}S\cdot0.33H_2O$: C, 45.18; H, 5.86; S, 4.82. Found: C, 45.04; H, 5.86; S, 4.72.

General procedure for the preparation of α-D-*glucopyranosyl-(1→4)-*α-D-*glucopyranosyl-(1→4)-(6-deoxy-6-iodo-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**5**)*,* α-D-*glucopyranosyl-(1→4)-(6-deoxy-6-iodo-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**6**)*, and (6-deoxy-6-iodo-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**7**).—Sodium iodide (10 mol equiv for **2**–**4**) was added to a stirred solution of **2** (6.00 g, 6.1 mmol), **3** (6.00 g, 7.3 mmol), or **4** (1.50 g, 2.28 mol) in DMF (150 mL, 150 mL, or 50 mL for **2**, **3**, or **4**, respectively), and the mixture was stirred at 80 °C for 3 h. Then, the mixture was concentrated under reduced pressure to leave a syrupy residue, which was chromatographed on ODS gel with 3:97 CH_3CN–H_2O, and the appropriate fractions were freeze-dried to give **5** (4.55 g, 4.84 mmol, 79%), **6** (4.90 g, 6.31 mmol, 86%), or **7** (1.15 g, 1.87 mmol, 82%), respectively.

Compound **5**: colorless and amorphous; mp 168–170 °C (dec.); $[\alpha]_D$ +147° (*c* 0.25, H_2O); ν_{max} 3330, 2930, 1650, 1360, 1150, and 1030 cm^{-1}; ^1H NMR (D_2O): δ 5.42, 5.35, 5.33, and 5.31 (d, each 1 H, *J* 3.9–4.2 Hz, H-1b–e), 5.23 (d, 0.5 H, *J* 4.2 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.01–3.23 (m, ~28 H, H-2–6); ^{13}C NMR: δ 102.86, 102.64, 102.52, and 102.40 (C-1b–e), 98.80 and 94.86 (C-1a), 10.50 (CH_2I); t_R (6:4 CH_3CN–H_2O): 8.0 min. Anal. Calcd for $C_{30}H_{51}IO_{25}\cdot H_2O$: C, 37.67; H, 5.58. Found: C, 37.55; H, 5.62.

Compound **6**: colorless and amorphous; mp 165–167 °C (dec.); $[\alpha]_D$ +137° (*c* 0.25, H_2O); ν_{max} 3330, 2930, 1650, 1420, 1150, and 1030 cm^{-1}; ^1H NMR (D_2O): δ 5.39, 5.37, and 5.23 (d, each 1 H, *J* 3.7–4.2 Hz, H-1b–d), 5.23 (d, 0.5 H, *J* 3.7 Hz, β H-1a), 4.64 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.01–3.23 (m, ~24 H, H-2–6); ^{13}C NMR: δ 102.41, 102.33, and 102.29 (C-1b–d), 98.66 and 94.75 (C-1a), 10.46 (CH_2I); t_R (6:4 CH_3CN–H_2O): 6.6 min. Anal. Calcd for $C_{24}H_{41}IO_{20}\cdot H_{20}$: C, 36.28; H, 5.45. Found: C, 36.28; H, 5.48.

Compound **7**: colorless and amorphous; mp 158–160 °C (dec.); $[\alpha]_D$ +111° (*c* 0.25, H_2O); ν_{max} 3330, 2930, 1650, 1420, 1150, and 1030 cm^{-1}; ^1H NMR (D_2O): δ 5.37, and 5.36 (d, each 1 H, *J* 3.7–3.9 Hz, H-1b–c), 5.23 (d, 0.5 H, *J* 4.1 Hz, β H-1a), 4.64 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.01–3.23 (m, ~18 H, H-2–6); ^{13}C NMR: δ 103.30 and 102.96 (C-1b–c), 99.34 and 95.42 (C-1a), 10.50 (CH_2I); t_R (6:4 CH_3CN–H_2O): 5.2 min. Anal. Calcd for $C_{18}H_{31}IO_{15}\cdot0.5H_2O$: C, 34.68; H, 5.17. Found: C, 34.61; H, 5.15.

General procedure for the preparation of α-D-*glucopyranosyl-(1→4)-*α-D-*glucopyranosyl-(1→4)-(6-azido-6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**8**)*,* α-D-*glucopyranosyl-(1→4)-(6-azido-6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**9**)*, and (6-azido-6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**10**).—Sodium azide (10 mol equiv for **2**–**4**) was added to a stirred solution of **2** (6.00 g, 6.10 mmol), **3** (6.00 g, 7.31 mmol), or **4** (6.00 g, 9.11 mmol) in DMF (150 mL), and the mixture was stirred at 80 °C for 2 h. The solvent of the mixture was then evaporated under reduced pressure to leave a syrupy residue, which was chromatographed on ODS gel with 3:97 CH_3CN–H_2O, and the appropriate fractions were freeze-dried to give **8** (3.23 g, 3.78 mmol, 62%), **9** (3.87 g, 5.60 mmol, 77%), or **10** (3.52 g, 6.65 mmol, 73%), respectively.

Compound **8**: colorless and amorphous; mp 167–169 °C (dec.); $[\alpha]_D$ +175° (*c* 0.25, H_2O); ν_{max} 3320, 2930, 2210, 1640, 1150, and 1020 cm^{-1}; ^1H NMR (D_2O): δ 5.42–5.31 (m, 4 H, H-1b–e), 5.23 (d, 0.5 H, *J* 3.9 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.00–3.23 (m, ~30 H, H-2–6); ^{13}C NMR: δ 102.73, 102.48, 102.36, and 102.29 (C-1b–e), 98.67 and 94.72 (C-1a), 54.05 (CH_2N_3); t_R (6:4 CH_3CN–H_2O): 8.1 min. Anal. Calcd for $C_{30}H_{51}N_3O_{25}\cdot1.33H_2O$: C, 41.05; H, 6.16; N, 4.79. Found: C, 41.03; H, 6.01; N, 4.78.

Compound **9**: colorless and amorphous; mp 153–155 °C (dec.); $[\alpha]_D$ +165° (*c* 0.25, H_2O); ν_{max} 3350, 2930, 2210, 1640, 1150, and 1040 cm^{-1}; ^1H NMR (D_2O): δ 5.40–5.30 (m, 3 H, H-1b–d), 5.23 (d, 0.5 H, *J* 3.7 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 8.1 Hz, α H-1a), 4.00–3.25 (m, ~24 H, H-2–6); ^{13}C NMR: δ 102.82, 102.51, and 102.39 (C-1b–d), 98.69 and 94.75 (C-1a), 54.10 (CH_2N_3); t_R (6:4 CH_3CN–H_2O): 6.6 min. Anal. Calcd for $C_{24}H_{41}N_3O_{20}\cdot1.5H_2O$: C, 40.11; H, 6.17; N, 5.85. Found: C, 40.04; H, 6.03; N, 5.87.

Compound **10**: colorless and amorphous; mp 140–142 °C (dec.); $[\alpha]_D$ +150° (*c* 0.25, H_2O); ν_{max} 3290, 2930, 1660, 1360, 1150, and 1030 cm^{-1}; ^1H

NMR (D_2O): δ 5.36 (d, 2 H, *J* 3.2 Hz, H-1b–c), 5.23 (d, 0.5 H, *J* 3.9 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 8.1 Hz, α H-1a), 4.01–3.25 (m, ~18 H, H-2–6); ^{13}C NMR: δ 102.73 and 102.31 (C-1b–c), 98.80 and 94.72 (C-1a), 54.17 (CH_2N_3); t_R (6:4 CH_3CN–H_2O): 5.0 min. Anal. Calcd for $C_{18}H_{31}N_3O_{15}·0.5H_2O$: C, 40.15; H, 5.99. Found: C, 40.02; H, 5.86.

General procedure for the preparation of α-D-*glucopyranosyl-(1→4)-*α-D-*glucopyranosyl-(1→4)-(6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**11**)*,* α-D-*glucopyranosyl-(1→4)-(6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**12**)*, and (6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**13**).—A solution of **5** (2.50 g, 2.66 mmol), **6** (2.50 g, 3.84 mmol), or **7** (2.50 g, 3.84 mmol) in distilled water (50 mL) was stirred vigorously over 10% Pd-C (1.25–1.5 g) under H_2 at ordinary pressure for 4 h. The catalyst was removed by filtration through a short pad of Celite®. The filtrate was concentrated under reduced pressure to leave a syrupy residue, which was chromatographed on ODS gel with 2:98 CH_3CN–H_2O, and the appropriate fractions were freeze-dried to give **11** (1.74 g, 2.14 mmol, 81%), **12** (1.80 g, 2.77 mmol, 72%), or **13** (1.85 g, 3.78 mol, 78%), respectively.

Compound **11**: colorless and amorphous; mp 218–220 °C (dec.); $[\alpha]_D$ +176° (*c* 0.25, H_2O); ν_{max} 3300, 2930, 1640, 1360, 1150, and 1030 cm^{-1}; ^1H NMR (D_2O): δ 5.30–5.39 (m, 3 H, H-1), 5.27 (d, 1 H, *J* 4.1 Hz, H-1), 5.23 (d, 0.5 H, *J* 3.7 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 3.23–4.01 (m, ~28 H, H-2–6), 1.33 (d, 3 H, *J* 6.1 Hz, H-6c); ^{13}C NMR: δ 102.58, 102.46, 102.39, and 102.31 (C-1b–e), 98.66 and 94.75 (C-1a), 19.95 (CH_3); t_R (6:4 CH_3CN–H_2O): 9.2 min. Anal. Calcd for $C_{30}H_{52}O_{25}·1.33H_2O$: C, 43.06; H, 6.58. Found: C, 42.96; H, 6.48.

Compound **12**: colorless and amorphous; mp 172–174 °C (dec.); $[\alpha]_D$ +171° (*c* 0.25, H_2O); ν_{max} 3300, 2930, 1650, 1420, 1150, and 1040 cm^{-1}; ^1H NMR (D_2O): δ 5.36 (d, 2 H, *J* 3.7 Hz, H-1), 5.25 (d, 1 H, *J* 3.9 Hz, H-1), 5.23 (d, 0.5 H, *J* 3.7 Hz β H-1a), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.01–3.23 (m, ~22 H, H-2–6), 1.33 (d, 3 H, *J* 6.4 Hz, H-6c); ^{13}C NMR: δ 102.60, 102.39, and 102.34 (C-1b–d), 98.66 and 94.75 (C-1a), 19.97 (CH_3); t_R (6:4 CH_3CN–H_2O): 7.2 min. Anal. Calcd for $C_{24}H_{42}O_{20}·H_2O$: C, 43.11; H, 6.63. Found: C, 42.93; H, 6.42.

Compound **13**: colorless and amorphous; mp 153–155 °C; $[\alpha]_D$ +158° (*c* 0.51, H_2O); ν_{max} 3400, 2950, 1360, 1150, and 1040 cm^{-1}; ^1H NMR (D_2O): δ 5.36 and 5.27 (d, each 1 H, *J* 2.9–3.9 Hz, H-1b–c), 5.24 (d, 0.5 H, *J* 3.7 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.01–3.23 (m, ~16 H, H-2–6), 1.28 (d, 3 H, *J* 6.1 Hz, H-6c); ^{13}C NMR: δ 102.68 and 102.28 (C-1b–c), 98.59 and 94.70 (C-1a), 19.34 (CH_3); t_R (6:4 CH_3CN–H_2O): 5.9 min. Anal. Calcd for $C_{18}H_{32}O_{15}·H_2O$: C, 42.69; H, 6.77. Found: C, 42.79; H, 6.70.

General procedure for the preparation of α-D-*glucopyranosyl-(1→4)-*α-D-*glucopyranosyl-(1→4)-(6-amino-6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**14**)*,* α-D-*glucopyranosyl-(1→4)-(6-amino-6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**15**)*, and (6-amino-6-deoxy-*α-D-*glucopyranosyl)-(1→4)-*α-D-*glucopyranosyl-(1→4)-*D-*glucopyranose* (**16**).—A solution of **8** (1.50 g, 1.76 mmol), **9** (1.50 g, 2.17 mmol), or **10** (3.00 g, 5.67 mmol) in distilled water (100 mL) was stirred vigorously over 10% Pd-C (0.45–1.0 g) under H_2 at ordinary pressure for 6 h, when HPLC indicated the absence of **8**, **9**, or **10**. The catalyst was removed by filtration through a short pad of Celite®. Then the filtrate was concentrated under reduced pressure and freeze-dried to give **14** (1.20 g, 1.45 mmol, 82%), **15** (0.99 g, 1.49 mmol, 69%), or **16** (2.20 g, 4.37 mmol, 77%), respectively.

Compound **14**: colorless and amorphous; mp 129–131 °C (dec.); $[\alpha]_D$ +160° (*c* 0.25, H_2O); ν_{max} 3350, 2930, 1650, 1420, 1150, and 1020 cm^{-1}; ^1H NMR (D_2O): δ 5.41 and 5.35 (d, 3 H and 1 H, *J* 3.4–3.9 Hz, H-1b–e), 5.23 (d, 0.5 H, *J* 3.7 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.01–2.83 (m, ~30 H, H-2–6); ^{13}C NMR: δ 102.68, 102.63, 102.63, and 102.36 (C-1b–e), 98.69 and 94.75 (C-1a), 43.88 (CH_2NH_2); t_R (6:4:0.01 CH_3CN–H_2O–H_3PO_4): 18.0 min. Anal. Calcd for $C_{30}H_{53}NO_{25}·H_2O$: C, 42.60; H, 6.55; N, 1.66. Found: C, 42.11; H, 6.27; N, 1.52.

Compound **15**: colorless and amorphous; mp 120–122 °C (dec.); $[\alpha]_D$ +154° (*c* 0.25, H_2O); ν_{max} 3350, 2930, 1650, 1420, 1150, and 1020 cm^{-1}; ^1H NMR (D_2O): δ 5.41, 5.36, and 5.35 (d, each 1 H, *J* 3.7–3.9 Hz, H-1b–d), 5.23 (d, 0.5 H, *J* 3.9 Hz, β H-1a), 4.65 (d, 0.5 H, *J* 7.8 Hz, α H-1a), 4.03–2.80 (m, ~24 H, H-2–6); ^{13}C NMR: δ 102.80, 102.80, and 102.39 (C-1b–d), 98.66 and 94.75 (C-1a), 43.54 (CH_2NH_2); t_R (6:4:0.01 CH_3CN–H_2O–H_3PO_4): 13.0 min. Anal. Calcd for $C_{24}H_{43}NO_{20}·1.33H_2O$:

C, 41.80; H, 6.67; N, 2.03. Found: C, 41.69; H, 6.37; N, 1.94.

Compound **16**: colorless and amorphous; mp 118–120 °C (dec.); $[\alpha]_D$ +150° (*c* 0.51, H_2O); ν_{max} 3290, 2900, 1600, 1360, 1150, and 1030 cm^{-1}; ^1H NMR (D_2O): δ 5.43 and 5.38 (d, each 1 H, *J* 3.4–3.9 Hz, H-1b–c), 5.23 (d, 0.5 H, *J* 3.7 Hz, β H-1a), 4.66 (d, 0.5 H, *J* 8.1 Hz, α H-1a), 4.01–2.80 (m, ~18 H, H-2–6); ^{13}C NMR: δ 102.41 and 102.34 (C-1b–c), 98.61 and 94.72 (C-1a), 44.03 (CH_2NH_2); t_R (6:4:0.01 CH_3CN–H_2O–H_3PO_4): 10.2 min. Anal. Calcd for $C_{18}H_{33}NO_{15}\cdot 0.33H_2O$: C, 42.44; H, 6.66; N, 2.75. Found: C, 42.53; H, 6.72; N, 2.61.

Measurement of IC_{50} for HPA and HSA.— Determination of α-amylase inhibitory activity was carried out using an α-amylase activity determination kit (Neo·Amylase Test Daiichi) utilizing blue starch as a substrate. Four mL of different concentrations of test compounds were preincubated at 37 °C for 5 min. Then, 0.1 mL of an 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 0.5 M NaOH was added to the mixture to stop the reaction. Then, the mixture was centrifuged at 1500×*g* for 5 min, and the 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 per cent versus the concentration.

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