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Chemoenzymatic synthesis of 6$^{\omega}$-*S*-α-D-glucopyranosyl-6$^{\omega}$-thiomaltooligosaccharides: their binding to *Aspergillus niger* glucoamylase G1 and its starch-binding domain ☆

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Abstract

A coupling reaction of cyclodextrin glucosyltransferase (CGTase) with glucose and 6-deoxy-6-iodo-cyclomaltoheptaose (**1**), in the presence of glucoamylase, followed by acetylation, led to a convenient synthesis of acetylated 6^{III}-deoxy-6^{III}-iodo-maltotriose (**2**) and 6^{IV}-deoxy-6^{IV}-iodo-maltotetraose (**3**). Nucleophilic displacement of the iodine atom of these protected maltotriose and maltotetraose analogs by the activated form of 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucose (**4**) afforded peracetylated 6^{III}-*S*-α-D-glucopyranosyl-6^{III}-thiomaltotriose (**5**) and 6^{IV}-*S*-α-D-glucopyranosyl-6^{IV}-thiomaltotetraose (**6**) in high yield. The interaction of OH-free tetra- and penta-saccharides (**7** and **8**) with both glucoamylase G1 from *Aspergillus niger* as well as its isolated starch-binding domain fragment were studied by UV difference spectroscopy. It was found that the starch-binding domain has higher affinity for **7** and **8** than for maltotetraose and maltopentaose.

☆ Note from the editor: Superscript ω is used as locant to indicate a terminal nonreducing glucopyranosyl unit in maltooligosaccharides.

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1. Introduction

Most fungal glucoamylases possess a separate starch-binding domain [1] located either C- or N-terminal of the catalytic domain [2,3]. Glucoamylase catalyses the hydrolytic release of β-D-glucose from non-reducing ends of starch and related oligo- and poly-saccharides. Although several glucoamylases with high activity on raw starch also have strong activity on α-(1 → 6) linkages at non-reducing ends [4,5], it remains to be demonstrated whether the starch-binding domain plays a role in hydrolytic or reversion reactions. This domain was obtained previously after proteolytic cleavage of the *Aspergillus niger* glucoamylase G1 [6]. It was characterized with respect to binding affinity of maltodextrins and an increasing binding constant was observed with increasing chain length up to maltononaose [7].

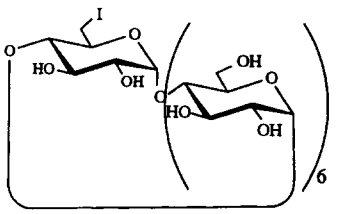
We have also demonstrated that 6^{II}-thiopanose exerts mild stimulation of the activity of G1 towards soluble starch and weak inhibition during hydrolysis of panose. However, these properties were not found in G2, a smaller form of G1, in which the starch-binding domain is absent [8].

For a better understanding of the role of this domain during starch hydrolysis, it was emphasized that higher oligomers of 6^{II}-thiopanose should be useful tools. In the present paper, we report the chemoenzymatic synthesis of 6^{ω}-*S*-α-D-glucopyranosyl-6^{ω}-thiomaltodextrins and the results of our binding studies of these compounds for the isolated starch-binding domain of G1 glucoamylase from *A. niger*. Since these analogues are not cleaved by glucoamylase, the binding was studied also using the intact enzymatically active protein G1, containing the catalytic domain, a highly *O*-glycosylated peptide linker and the C-terminal starch-binding domain [9], and the G2 form that has the same enzymatic activity, but lacks the starch-binding domain and hence has no capacity for adsorption onto starch granules [2].

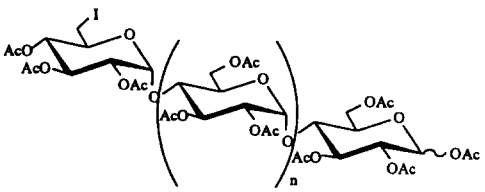
2. Results and discussion

Synthesis.—In the context of our studies on the utilization of cyclodextrin glucosyltransferase (EC 3.2.1.19) as biocatalyst, we have reported the successful conversion of cyclodextrin derivatives into modified maltooligosaccharides [10] and the synthesis of regioselectively modified cyclodextrins [11]. We thought that, starting from 6-deoxy-6-iodo-β-cyclodextrin (**1**), this enzyme should provide the most direct approach to 6^{ω}-deoxy-6^{ω}-iodomaltodextrins. In fact, the coupling reaction of glucose with **1** [12] catalysed by CGTase with a concomitant treatment with glucoamylase led preponderantly to two new compounds. After acetylation, they were identified as peracetylated 6^{III}-deoxy-6^{III}-iodo-maltotriose (**2**) and 6^{IV}-deoxy-6^{IV}-iodo-maltotetraose (**3**) by mass spectroscopy. Since glucoamylase, an exo-enzyme which acts on maltooligosaccharides and liberates glucose from the non-reducing end, cannot catalyse the release of C-6 modified glucose units [13,14], it was assumed that the 6-deoxy-6-iodo-glucosyl unit

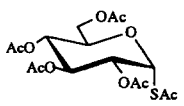
was the ultimate residue. Compound **2** was converted into pure β anomer by a two-step procedure and the ^{1}H NMR spectrum showed signals in agreement with the given structure. 6^{III}-Deoxy-6^{III}-iodo-maltotriose has been synthesized before either by chemical modification of maltotriose [15] or by amylolysis of **1** by Taka-amylase followed by glucoamylase treatment [13]. The first approach led to a low overall yield, whereas a precise yield was not given for the latter one. We therefore consider the alternative approach developed in this work as a considerable improvement. The high yields obtained during the preparation of **2** and **3** confirm the preferential binding of monomodified cyclodextrin in the active site of CGTase [10].



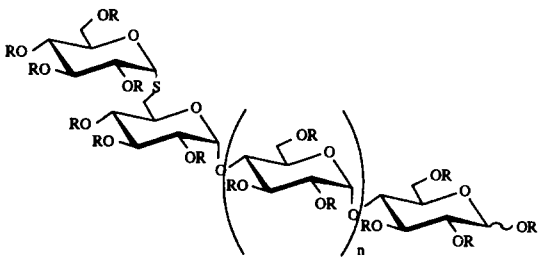
1



2 $n = 1$
3 $n = 2$



4



5 $n = 1$, R = Ac
6 $n = 2$, R = Ac
7 $n = 1$, R = H
8 $n = 2$, R = H
9 $n = 0$, R = H

Compounds **2** and **3** constituted the ideal precursors for the preparation of the target molecules. Displacement in high yield of iodine at primary positions of maltose with 2,3,4-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose (**4**) has been described [8,16]. The same procedure, applied to compounds **2** and **3**, afforded the expected 6^{III}- and 6^{IV}-α-D-glucopyranosyl-6^{III}-thiomaltotriose and 6^{IV}-thiomaltotetraose (**5** and **6**) in 86 and 80% yields respectively. Conventional *O*-deacetylation led to **7** and **8** in quantitative yield.

Binding studies.—The dissociation constant K_d was determined by UV difference spectroscopy essentially as described previously [17]. With increasing degree of polymerization from 6^{II}-thiopanose (**9**) to the 6^{IV}-thiocompound (**8**), decreasing values of K_d from about ~ 1 mM to 0.2 mM were obtained (Table 1). Moreover, there was no

Table 1
Dissociation constants for 6-thiopanose homologs **7–9** and glucoamylase G1 or its starch-binding domain. Comparison to the dissociation constants found for maltodextrins and the starch-binding domain

Protein	dp	Ligand		
		Thiopanose homologs [a]		Maltodextrins [b]
		K_d (mM)	K_d (mM)	K_d (mM)
SBD	3	0.96 ± 0.05	(1.6 ± 0.11)	3.8
	4	0.30 ± 0.03	(0.56 ± 0.06)	3.8
	5	0.22 ± 0.02	(0.32 ± 0.01)	1.3
G1	3	0.84 ± 0.04	(1.7 ± 0.04)	-
	4	0.31 ± 0.02	(0.53 ± 0.02)	-
	5	0.17 ± 0.02	(0.15 ± 0.01)	-

[a] Values in parentheses, determined by titration calorimetry are for the site of highest affinity.
[b] From ref. [7].

significant difference in affinity between the isolated starch-binding fragment and the intact *A. niger* glucoamylase G1. Since the G2 form of glucoamylase has the same substrate affinity as G1 [18] and did not undergo any spectral perturbation when 6^{II}-thiopanose (**9**) was added up to a concentration of 2.2 mM; obviously the difference spectra obtained with G1 in the present study were due to ligand binding at the starch-binding domain.

The K_d values found here are similar to those recently determined by titration calorimetry [19]. Significantly poorer affinity corresponding to approximately one order of magnitude higher K_d values were reported for binding to the isolated starch-binding domain of maltotriose, maltotetraose and maltopentaose [7]. The effect of the sulfur atom in the binding to the starch-binding domain of compounds **7–9** presumably accounts for a tighter binding, since: (i) K_d for panose of SBD is 3.8 ± 0.6 mM; (ii) cockchafer trehalase exhibits less affinity for the competitive inhibitor α-D-glucopyranosyl 1-thio-α-D-mannopyranoside than for its *O*-glycosyl analogue [20]; (iii) methyl α-thiomaltoside binds to the active site of glucoamylase G2 with the same affinity as the parent substrate [21].

However, before it can be definitively concluded that the starch-binding site has preferential affinity for compounds containing *S*-linked glucosyl residue at their non-reducing end, the affinity of the 4^{ω}-*S*-α-D-glucopyranosyl-4^{ω}-thiomaltodextrins has to be determined in similar ligand binding measurements. The synthesis and binding properties of such compounds will be reported in due course.

3. Experimental

Material and general methods.—Glucoamylase G1 form from *A. niger* was purified as reported earlier from AMG 200 L (Novo Nordisk) [2]; the starch-binding domain was produced from G1 by proteolysis and purified as described previously [6]. Glucoamylase for chemoenzymatic synthesis (amyloglucosidase, *A. niger*) was from Boehringer,

Meylan, France; CGTase (*Bacillus macerans*) was a gift from Amano Co. Ltd., Japan; cyclomaltoheptaose was a gift from Roquette Frères, Lestrem, France. Optical rotations were measured with a Perkin–Elmer 241 polarimeter. NMR spectra were recorded on a Bruker AC 300 instrument. Chemical shifts are given in ppm and J values are in Hz. Low-resolution mass spectra were recorded on a Nermag R.1010 C spectrometer using desorption chemical ionization (DCI) or fast-atom bombardment (FAB) modes. TLC was done on Silica Gel F 254 (Riedel de Haën) with detection by UV light and/or charring with a 3:45:45 H_2SO_4–MeOH–water solution.

Solvents as pyridine and CH_2Cl_2 were distilled and kept over calcium hydride. DMF was dried over 4 Å molecular sieves. Melting points were taken on a Büchi-535 apparatus in open capillaries.

6^{III}-*S*-α-D-Glucopyranosyl-6^{III}-thiomaltotriose (**7**) and 6^{IV}-*S*-α-D-glucopyranosyl-6^{IV}-thiomaltotetraose (**8**) were purified by HPLC on a μ-Bondapak NH_2 column (Waters, Milford, 125 Å, 10 μm, 12 × 150 mm with 7:3 MeCN–water as eluent. All solvents were evaporated under reduced pressure. When CH_2Cl_2 solutions were extracted with H_2O, the aqueous phases were re-extracted with CH_2Cl_2, and the collected organic phases were combined and dried on Na_2SO_4. For flash chromatography, Merck Silica Gel 60 (230–400 mesh) was used.

O-(2,3,4-Tri-O-acetyl-6-deoxy-6-iodo-α-D-glucopyranosyl)-(1 → 4)-l-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-O-1,2,3,6-tetra-O-acetyl-D-glucopyranose (**2**) *and O-(2,3,4-tri-O-acetyl-6-deoxy-6-iodo-α-D-glucopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-O-(2,3,6-tri-O-acetyl-α-D-glucopyranosyl)-(1 → 4)-1,2,3,6-6-tetra-O-acetyl-D-glucopyranose* (**3**).—To a solution of 6-deoxy-6-iodo-cyclomaltoheptaose (**1**, 500 mg, 0.40 mmol, [12]) in water (50 mL) were added CGTase (160 μL, 635 U/mL), glucoamylase (100 mg, 600 U) and D-glucose (500 mg, 2.77 mmol). The mixture was stirred at 40°C for 24 h, heated for 2 min at 100°C, and freeze-dried. The residue was acetylated in 1:1 pyridine–Ac_2O (40 mL) in the presence of 4-dimethylaminopyridine (10 mg). The mixture was stirred at room temperature for 12 h. The solvents were evaporated, the residue diluted in CH_2Cl_2 and the resulting mixture washed with ice-cold aq $KHSO_4$ (10%), ice-cold saturated $NaHCO_3$ and cold water. Column chromatography of the residue (1:3 acetone–hexane) afforded a mixture of anomeric compounds **2** (245 mg, 59%) and **3** (116 mg, 22%) in an α:β ratio: 1:9 (^{1}H NMR).

β-Anomer of **2**: To an ice-cold solution of anomeric mixture of **2** (245 mg, 0.24 mmol) in CH_2Cl_2 (5 mL) was added HBr/AcOH (1 mL). The mixture was stirred for 2 h at 0°C, then diluted with CH_2Cl_2, and the organic solution was washed successively with ice-cold water, ice-cold saturated $NaHCO_3$.

To a solution of the residue in 1:1 AcOH–Ac_2O (5 mL) was added AgOAc (250 mg). The mixture was stirred in the dark for 12 h at room temperature, then filtered through Celite, diluted with CH_2Cl_2, washed as described above. Chromatography (1:3 acetone–hexane) gave **2** (β-anomer) (200 mg, 82%); mp 189°C (Et_2O); $[\alpha]_D^{20}$ + 82° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.72 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.37 (d, 1 H, $J_{1,2}$ 4.1 Hz, H-1^{III}), 5.35 (t, 1 H, $J_{3,2} = J_{3,4} = 10.3$ Hz, H-3^{II}), 5.33 (t, 1 H, $J_{3,2} = J_{3,4} = 10.5$ Hz, H-3^{III}), 5.27 (t, 1 H, $J_{3,2} = J_{3,4} = 9.4$ Hz, H-3), 5.24 (d, 1 H, $J_{1,2}$ 4.2 Hz, H-1^{II}), 4.94 (dd, 1 H, H-2), 4.87 (t, 1 H, $J_{4,5}$ 10.5 Hz, H-4^{III}), 4.79 (dd, 1 H, H-2^{III}), 4.73 (dd,

1 H, H-2^{II}), 4.46 (dd, 1 H, $J_{6a,5}$ 2.6, $J_{6a,6b}$ 12.3 Hz, H-6^{II}a), 4.43 (dd, 1 H, $J_{6a,5}$ 2.6, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.28 (dd, 1 H, $J_{6b,5}$ 4.4 Hz, H-6b), 4.25 (dd, 1 H, $J_{6b,5}$ 3.7 Hz, H-6^{II}b), 3.97 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 3.93 (m, 1 H, H-5^{II}), 3.89 (t, 1 H, $J_{4,5}$ 10.3 Hz, H-4^{II}), 3.84 (m, 1 H, H-5), 3.64 (m, 1 H, H-5^{III}), 3.28 (dd, 1 H, $J_{6a,5}$ 2.9, $J_{6a,6b}$ 11.2 Hz, H-6^{III}a), 3.11 (dd, 1 H, $J_{6b,5}$ 6.0 Hz, H-6^{III}b); ^{13}C NMR ($CDCl_3$): δ 95.5, 95.0 (C-1^{II}, C-1^{III}), 91.0 (C-1), 62.4 (C-6, C-6^{II}), 3.7 (C-6^{III}); DCIMS (NH_3 + isobutane): m/z 1052 $[M + NH_4]^+$. Anal. Calcd for $C_{38}H_{51}IO_{25}$: C, 44.00; H, 4.93; I, 12.20. Found: C, 43.98; H, 4.97; I, 12.23.

3: ^{1}H NMR ($CDCl_3$): δ 6.25 (d, $J_{1,2}$ 3.5 Hz, H-1), 5.76 (d, $J_{1,2}$ 8.1 Hz, H-1), 5.39 (d, 1 H, $J_{1,2}$ 4.9 Hz, H-1^{IV}), 5.34 (t, 1 H, $J_{2,3} = J_{3,4} = 9.5$ Hz, H-3^{IV}), 5.40–5.24 (m, 5 H, H-1^{II}, -1^{III}, H-3, -3^{II}, -3^{III}), 4.94 (dd, 1 H, $J_{2,3}$ 8.3 Hz, H-2), 4.87 (t, 1 H, $J_{4,5}$ 9.5 Hz, H-4^{IV}), 4.79 (dd, 1 H, H-2^{IV}), 4.75–4.70 (m, 2 H, H-2^{II}, -2^{III}), 4.48–4.22 (m, 6 H, H-6a, -6a^{II}, -6a^{III}, H-6b, -6b^{II}, -6b^{III}), 4.01–3.83 (m, 6 H, H-4, -4^{II}, -4^{III}, H-5, -5^{II}, -5^{III}), 3.64 (m, 1 H, H-5^{IV}), 3.28 (dd, 1 H, $J_{6a,5}$ 2.8, $J_{6a,6b}$ 11.2 Hz, H-6^{IV}a), 3.11 (dd, 1 H, $J_{6b,5}$ 6.1 Hz, H-6^{IV}b);^{13}C NMR ($CDCl_3$): δ 95.8–95.3 (C-1^{II}, -1^{III}, -1^{IV}) 92.1 (C-1β), 88.8 (C-1α); 62.5 (C-6, -6^{II}, -6^{III}); 4.0 (C-6^{IV}); DCIMS (NH_3 + isobutane): m/z 1340 $[M + NH_4]^+$.

S-*(2,3,4,6-Tetra*-O-*acetyl*-α-D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*acetyl-6-thio*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*acetyl*-α-D-*glucopyranosyl)-(1 → 4)-1,2,3,6-tetra*-O-*acetyl*-β-D-*glucopyranose* (**5**).—To a solution of β-**2** (250 mg, 0.24 mmol), 2,3,4,6-tetra-*O*-acetyl-1-*S*-acetyl-1-thio-α-D-glucopyranose (**4**, 200 mg, 0.49 mmol, 2 eq) and dithioerythritol (50 mg) in HMPA (4 mL) was added cysteamine (40 mg). After 5 h at room temperature, ice and water (20 mL) were added to the mixture, the precipitate was filtered on Celite, then dissolved in CH_2Cl_2 and washed with water. The solvent was evaporated and the residue was purified by chromatography (1:1 $CHCl_3$–EtOAc). Compound **5** was isolated in 86% yield (264 mg): mp 129°C (Et_2O–hexane); $[\alpha]_D^{20}$ + 143° (*c* 0.5, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.82 (d, 1 H, $J_{1,2}$ 5.8 Hz, H-1^{IV}), 5.72 (d, 1 H, $J_{1,2}$ 8.0 Hz, H-1), 5.39–5.23 (m, 6 H, H-1^{II}, -1^{III}, H-3, -3^{II}, -3^{III}, -3^{IV}), 5.08–4.92 (m, 4 H, H-2, -2^{IV}, H-4^{III}, -4^{IV}), 4.74 (dd, 2 H, $J_{2,1}$ 4.0, $J_{2,3}$ 10.5 Hz, H-2^{II}, -2^{III}), 4.44–3.91 (m, 11 H, H-4, -4^{II}, H-5^{II}, -5^{III}, -5^{IV}, H-6ab, -6^{II}ab, -6^{IV}ab), 3.86 (m, 1 H, H-5), 2.80 (dd, 1 H, $J_{6a,5}$ 3.2, $J_{6a,6b}$ 14.4 Hz, H-6^{III}a), 2.52 (dd, 1 H, $J_{6b,5}$ 4.5 Hz, H-6^{III}b); ^{13}C NMR ($CDCl_3$): δ 95.8, 95.2 (C-1^{II}, -1^{III}), 91.2 (C-1), 82.6 (C-1^{IV}); 74.9, 73.6, 72.9, 72.3, 71.7, 70.8, 70.5, 70.4, 70.1, 69.9, 69.8 (3), 68.8, 68.7, 68.3, 67.8 (C-2, -2^{II}, -2^{III}, -2^{IV}, -3, -3^{II}, -3^{III}, -3^{IV}, -4, -4^{II}, -4^{III}, -4^{IV}, -5, -5^{II}, -5^{III}, -5^{IV}), 62.7 (C-6, -6^{II}), 61.7 (C-6^{IV}), 29.7 (C-6^{III}); DCIMS (NH_3 + isobutane): m/z 1287 $[M + NH_4]^+$. Anal. Calcd for $C_{52}H_{70}O_{34}S$: C, 49.13; H, 5.55; S, 2.52. Found: C, 48.51; H, 5.61; S, 2.56.

S-*(2,3,4,6-Tetra*-O-*acetyl*-α-D-*glucopyranosyl)-(1 → 6)*-O-*(2,3,4-tri*-O-*acetyl-6-thio*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*acetyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*acetyl*-α-D-*glucopyranosyl)-(1 → 4)-1,2,3,6-tetra*-O-*acetyl*-D-*glucopyranose* (**6**). —A solution of **3** (100 mg, 0.07 mmol) was treated with **4** as described for the preparation of **5**. The expected compound **6** (94 mg, 80%) was obtained. ^{1}H NMR ($CDCl_3$): δ 6.25 (d, $J_{1,2}$ 3.7 Hz, H-1) 5.84 (d, 1 H, $J_{1,2}$ 5.7 Hz, H-1^V), 5.79 (d, $J_{1,2}$ 8.1 Hz, H-1), 2.80 (dd, 1 H, $J_{6a,5}$ 3.2, $J_{6a,6b}$ 14.3 Hz, H-6^{IV}a), 2.52 (dd, 1 H, $J_{6b,5}$ 4.4 Hz, H-6^{IV}b); ^{13}C NMR ($CDCl_3$): δ 95.8, 95.3(2) (C-1^{II}, -1^{III}, -1^{IV}), 91.2 (C-1β), 88.8

(C-1α), 82.7 (C-1^{V}), 62.6(2), 61.7(2) (C-6, -6^{II}, -6^{III}, -6^{V}), 29.8 (C-6^{IV}); FAB$^+$MS (*m*-nitrobenzyl alcohol + KCl): *m/z* 1597 $[M + K]^+$.

S-α-D-*Glucopyranosyl-(1 → 6)*-O-*6-thio*-α-D-*glucopyranosyl-(1 → 4)*-O-α-D-*glucopyranosyl-(1 → 4)*-D-*glucopyranose* (**7**).—To a stirred solution of **5** (300 mg, 0.23 mmol) in MeOH (23 mL) was added M methanolic NaOMe (0.2 mL). The mixture was stirred for 12 h at room temperature, neutralized with Amberlite IRN 77 (H^+) resin, and water was added. Methanol was evaporated and then the solution was freeze dried. The expected compound **7** was obtained quantitatively (157 mg, 98%). An aliquot was subject to HPLC on μ-Bondapak NH_2-column (10 μm, 19 × 50 mm, Waters Assoc.) using 75:25 MeCN–water. $[\alpha]_D^{20}$ +193° (*c* 0.75, H_2O); ^{13}C NMR (D_2O): δ 101.3 (C-1^{III}), 101.0, 100.9 (C-1^{II}), 97.3 (C-1β), 93.4 (C-1α), 86.6 (C-1^{IV}), 62.4–62.1 (C-6, -6^{II}, -6^{IV}), 32.0 (C-6^{III}); FAB$^+$MS (glycerol): *m/z* 683 $[M + H]^+$, 705 $[M + Na]^+$. Anal. Calcd for $C_{24}H_{42}O_{20}S \cdot 2H_2O$: C, 40.10; H, 6.45; S, 4.46. Found: C, 40.21; H, 6.35; S, 4.30.

S-α-D-*Glucopyranosyl-(1 → 6)*-O-*6-thio*-α-D-*glucopyranosyl-(1 → 4)*-O-α-D-*glucopyranosyl-(1 → 4)*-O-α-D-*glucopyranosyl-(1 → 4)*-D-*glucopyranose* (**8**).—A solution of **6** (76 mg, 0.05 mmol) was deacetylated and treated as described for the preparation of **7**, to give **8** (40 mg, 98%); $[\alpha]_D^{20}$ + 206° (*c* 0.5, H_2O); ^{13}C NMR (D_2O): δ 101.3–100.7 (C-1^{II}, -1^{III}, -1^{IV}), 97.3 (C-1β), 93.4 (C-1α), 86.5 (C-1^{V}), 62.4-62.0 (C-6, -6^{II}, -6^{III}, -6^{V}), 32.0 (C-6^{IV}); FAB$^+$MS (glycerol): *m/z* 845 $[M + H]^+$. Anal. Calcd for $C_{30}H_{52}O_{20}S \cdot 2H_2O$: C, 40.90; H, 6.40; S, 3.63. Found: C, 39.98; H, 6.41; S, 3.79.

Binding studies.—The binding of compounds **7–9** and panose (not shown) was monitored by UV difference spectroscopy. The measurements were performed using double-chamber cuvettes (individual light path, 0.4375 cm) in 0.05 M sodium acetate pH 5.0 at room temperature essentially as described earlier [17]. Concentrations of starch binding domain or intact glucoamylase G1 were in the range 10–12 μM, respectively, and determined using amino acid analysis [9]. Ligand concentrations were 0.10 to 10 mM dependent on the size. K_d was calculated from ΔA values (292–289 mm) as $(\Delta\epsilon_{max} - \Delta\epsilon_x) \times L_x / \Delta\epsilon_x$ where $\Delta\epsilon$ is the difference in molar extinction at saturating (max) and other (x) ligand concentrations.

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