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Chemical synthesis of 6′-α-maltosyl-maltotriose, a branched oligosaccharide representing the branch point of starch

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Abstract

Chemical synthesis of the branched pentasaccharide 6′-α-maltosyl-maltotriose (**15**) is reported, based on the use of one synthon as a glycosyl acceptor and another synthon as a glycosyl donor. The synthon used as glycosyl acceptor was phenyl 2,3,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**7**) and was synthesized from D-glucose with phenyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside and phenyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside as key intermediates. The synthon used as glycosyl donor was *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 6)-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)]-2,3-di-*O*-benzyl-α,β-D-glucopyranosyl trichloroacetimidate (**12**) and was synthesized from phenyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 6)-*O*-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3-di-*O*-acetyl-1-thio-β-D-glucopyranoside with *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 6)-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)]-2,3-di-*O*-benzyl-D-glucopyranose as an intermediate. Condensation of compounds **7** and **12** followed by removal of the phenylthio group and debenzylation provided the branched pentasaccharide **15**. Alternatively, the branched pentasaccharide was produced from amylopectin by consecutive alpha- and beta-amylase treatments and purified by chromatography.

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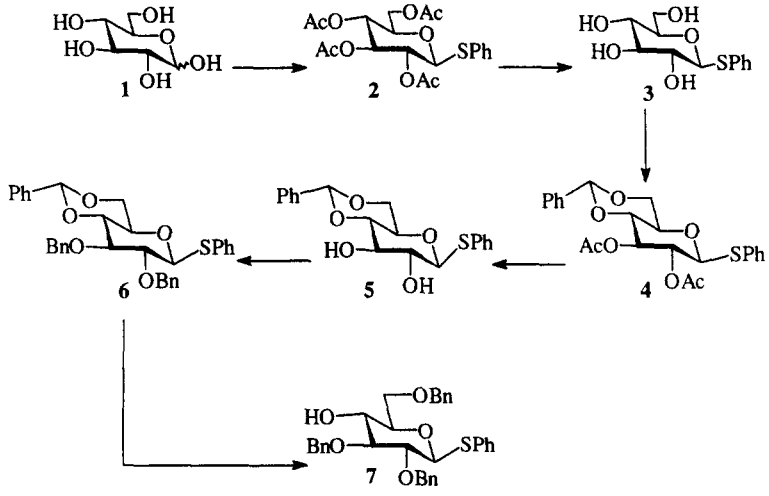
The identity of the products obtained by chemical synthesis and enzymatic hydrolysis is documented by ^{1}H and ^{13}C NMR spectra.

Keywords: Branch point of starch; Thiophenolysis; *trans*-Glycosidation; Enzymatic preparation of oligosaccharides

1. Introduction

In plants, starch in the chloroplasts of photosynthetic tissues represents a transient polysaccharide reserve material whereas starch deposited in the amyloplasts of seeds, swollen stems, and roots constitutes more permanent storage material. The starch is deposited as granules composed of a mixture of amylose which is a predominantly linear (1 → 4)-α-D-glucan, and of amylopectin which is a higher molecular mass (1 → 4)-α-D-glucan with frequent α-(1 → 6)branch points [1,2]. Three committed enzymes are involved in starch biosynthesis: ADPG pyrophosphorylase, starch synthases, and starch branching enzyme. ADPG pyrophosphorylase provides activated glucosyl residues in the form of ADP-glucose which are used by the synthases for transfer to maltodextrin primers or for extension of (1 → 4)-α-D-glucosyl chains from the non-reducing end of the polymer. The synthesis of amylose is catalyzed by isoforms of granule-bound starch synthases [3]. The synthesis of amylopectin, the major polysaccharide component of the granule, is catalyzed by isoforms of soluble starch synthase and branching enzyme. The isoforms of the synthases and branching enzymes exhibit different substrate specificity with respect to the structure of the preferred primers and length of glycosyl side chains to be transferred [4–7]. The lack of appropriate, chemically defined, oligosaccharide substrates has prevented detailed analyses of the substrate specificity of the different isoforms. Consequently, the biological importance of different isoforms in controlling and defining the chemical structure of starch during its synthesis remains largely unresolved. Starch structure has been studied by characterizing the oligosaccharides formed by the action of starch-degrading enzymes [8–10]. If complex oligosaccharides with a defined number of glucose residues between the branch points were available, these could be tested as specific substrates for the degradative enzymes and a more precise analysis obtained.

Accordingly, we have initiated a research programme based on the chemical synthesis of complex oligosaccharides of interest for studies of starch biosynthesis and degradation. The approach is based upon the use of a limited number of synthons for efficient chemical syntheses of a range of complex oligosaccharides with defined chemical structures. In this context, we have previously [11] reported the chemical synthesis of a protected branched tetrasaccharide thioglycoside, phenyl *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 6)-*O*-[(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl)-(1 → 4)]-2,3-di-*O*-acetyl-1-thio-β-D-glucopyranside (**8**). The chemical structure of **8** was ascertained by chemical and physical analysis and by a complete assignment of NMR spectra of **8** based on two-dimensional homonuclear and heteronuclear chemical shift correlation [11]. In the present paper we report the chemical synthesis of 6′-α-maltosyl-maltotriose (**15**),



Scheme 1. Synthesis of the glycosyl acceptor **7**.

comprising the branch point of starch, using **8** as a precursor. Synthesis of **15** by controlled enzymatic degradation of amylopectin is presented as an alternative route.

2. Results and discussion

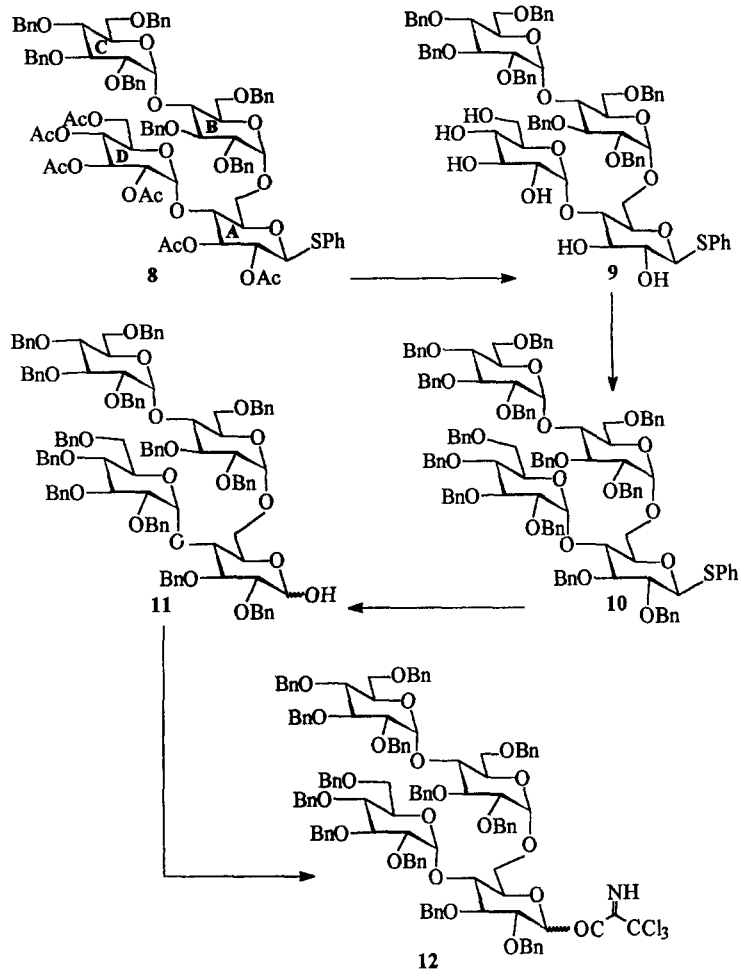
The chemical synthesis.—The synthetic target **15** may be retrosynthesized into two synthons, the glycosyl acceptor **7** and the glycosyl donor **12**. Phenyl thioglycosides are stable under a variety of reaction conditions [12] (acylation, alkylation, acetalation). The phenylthio group is efficiently removed using *N*-bromosuccinimide under mild conditions [11] and consequently was chosen as the blocking group for the anomeric center of **7**.

The synthesis of the desired glycosyl acceptor **7** from D-glucose (**1**) is outlined in Scheme 1. D-Glucose (**1**) was converted in a one-pot synthesis into tetra-*O*-acetyl-α-D-glucopyranosyl bromide using acetic anhydride and hydrogen bromide in acetic acid [13]. Thiophenolysis at room temperature under phase-transfer catalysis conditions using tetrabutylammonium hydrogen sulfate (TBAHS) as catalyst [14] afforded the phenyl thioglycoside derivative **2** in 88% overall yield. A doublet at δ 4.71 with $J_{1,2}$ 10.0 Hz in the ^{1}H NMR spectrum of **2** clearly demonstrated that the glycosidic linkage had the β configuration. Quantitative conversion of **2** into phenyl 1-thio-β-D-glucopyranoside (**3**) was obtained by deacetylation using sodium methoxide in methanol.

Cyclic acetals constitute one of the most useful derivatives for regioselective O-protection in carbohydrate chemistry [15–18] and were used in the present study. Optimal results were obtained using α,α-dibromotoluene in pyridine [17,18]. Reaction of **3** with this reagent under reflux and subsequent in situ acetylation to facilitate the work-up afforded the 4,6-*O*-benzylidene derivative **4** in 82% yield without chromatographic

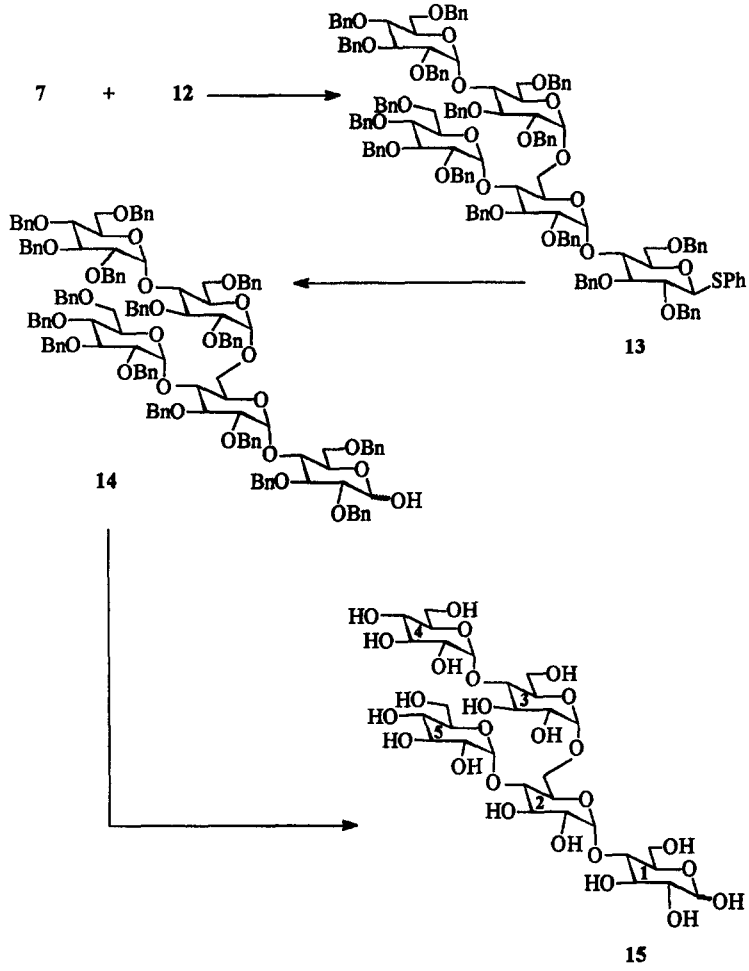
purification. The ^{1}H NMR spectrum of **4** (see Experimental) revealed a 1-proton-singlet signal at δ 5.49 assignable to the methine proton of the benzylidene group. The ^{13}C NMR spectrum showed the corresponding methine carbon resonating at δ 101.4. Standard deacetylation of **4** by treatment with sodium methoxide in methanol provided phenyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**5**) in 96% yield. Di-*O*-benzylation of **5** with benzyl bromide under phase-transfer catalysis conditions using TBAHS [19,20] provided **6** in quantitatve yield. Reductive cleavage of the benzylidene acetal function of **6** was performed regioselectively using sodium cyanoborohydride–HCl(gas)–diethyl ether in tetrahydrofuran [21], resulting in the desired glycosyl acceptor **7** as a 6-benzyl ether with a single free hydroxy group at carbon-4. After purification using silica gel chromatography, **7** was obtained in 94% yield. Compound **7** had previously been synthesized in four steps from β-D-glucose pentaacetate [22] but the experimental details were not documented.

The construction of the glycosyl donor **12** is shown in Scheme 2. The acetyl groups of the protected branched-tetrasaccharide thioglycoside **8** [11] were quantitatively removed, using standard procedures, by sodium methoxide in methanol to provide **9**. Treatment of **9** with benzyl bromide–sodium hydride in DMF afforded the fully *O*-benzylated branched-tetrasaccharide thioglycoside **10** in 75% yield after chromatographic purification. The phenylthio group of **10** was efficiently converted into a free HO-group by the action of *N*-bromosuccinimide in aqueous acetone [11] to give **11** in 94% yield after purification by column chromatography on silica gel. Reaction of **11** with trichloroacetonitrile in the presence of anhydrous potassium carbonate as catalyst [23,24] afforded a 96% yield of an anomeric mixture of the glycosyl trichloroacetimidate derivative **12** as the desired glycosyl acceptor. Chromatography revealed no impurities. The $\alpha:\beta$ ratio of the anomeric mixture was 2:3 as estimated from the ^{1}H and ^{13}C NMR spectra. Separation of the anomeric mixture of **12** was performed by column chromatography on silica gel with diethyl ether–pentane (2:3 v/v) as eluent. Pure α and β anomers were obtained in yields of 26 and 45%, respectively. The two anomeric trichloroacetimidates were investigated with respect to their reactivity and the identity of the product formed upon reaction with the glycosyl donor **7**. The reactivity of the two anomers was different, but both gave rise to predominant formation of the α-glycoside, probably due to the participation of the solvent diethyl ether [24]. Consequently, the anomeric mixture of **12** was used for the glycosylation reaction without prior chromatographic separation. Reaction of **12** with the glycosyl acceptor **7** (Scheme 3) in diethyl ether was achieved using trimethylsilyl triflate as catalyst and provided the desired branched-pentasaccharide thioglycoside derivative **13** in 65% yield after chromatographic purification. Minor amounts (~5%) of the β isomer were removed by chromatography (see Experimental). The ^{1}H NMR spectrum of **13** showed a doublet at δ 4.59 with $J_{1,2}$ 10.2 Hz assignable to the anomeric proton of glucose unit 1 (for nomenclature see Scheme 3 compound **15**) reflecting the known β-linkage position of the phenylthio group. The four doublets at δ 5.37 ($J_{1,2}$ 3.7 Hz), 5.53 ($J_{1,2}$ 3.8 Hz), 5.73 ($J_{1,2}$ 3.7 Hz), and 5.77 ($J_{1,2}$ 3.8 Hz) correspond to the four remaining anomeric protons. The chemical shifts and the characteristic small $J_{1,2}$-couplings for these anomeric protons clearly indicate that the newly formed glycosidic linkage has the α configuration and confirm the stereochemistry of **13**. Also, the ^{1}H-decoupled ^{13}C NMR spectrum



Scheme 2. Synthesis of the glycosyl donor **12**.

of **13** contains a diagnostic signal for the anomeric carbon of glucose unit 1, resonating at δ 87.3 due to the β linkage of the phenylthio group. The four additional anomeric carbon atoms, resonating at δ 96.2, 96.5, 96.8, and 97.0, are in good agreement with an α configuration [11]. By treating the thioglycoside **13** with *N*-bromosuccinimide–acetone–water [11] the phenylthio group was replaced with a free HO-group to afford *O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopranosyl)-(1 → 4)-*O*-(2,3,6-tri-*O*-benzyl-α-D-glucopyranosyl)-(1 → 6)-*O*-[(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)]-*O*-2,3-di-*O*-benzyl-α-D-glucopyranosyl)-(1 → 4)-2,3,6-tri-*O*-benzyl-D-glucopyranose (**14**) in 97% yield after chromatographic purification. Hydrogenolysis of **14** for removal of the benzyl ether protecting groups was performed under hydrogen in the presence of 20% palladium hydroxide on carbon in ethanol [25] and gave the desired branched pentasaccharide **15**.



Scheme 3. Synthesis of the branched pentasaccharide **15**.

The enzymatic preparation.—Alpha-amylases are endo-glycosidases which hydrolyze α-(1 → 4) linkages in the starch chains by random attack at points away from the non-reducing ends. Beta-amylases are exo-glycosidases, also acting upon α-(1 → 4) linkages in the starch chains, but performing the attack from the non-reducing terminal end, liberating maltose. Neither alpha- nor beta-amylases can cleave the α-(1 → 6) linkages in starch [8]. The α-(1 → 4) hydrolyzing activity of the two enzymes is restricted in the vicinity of an α-(1 → 6) linkage. A complete alpha- and beta-amylase digestion of starch will therefore result in the production of glucose, maltose, lower linear dextrins, and the desired branched-pentasaccharide **15**. To obtain **15** according to this approach, gelatinized amylopectin was subjected to an initial alpha-amylase treatment. The pentaose and higher oligosaccharides formed were recovered as the ethanol eluate from a charcoal column. After a second alpha-amylase treatment, pentaose and

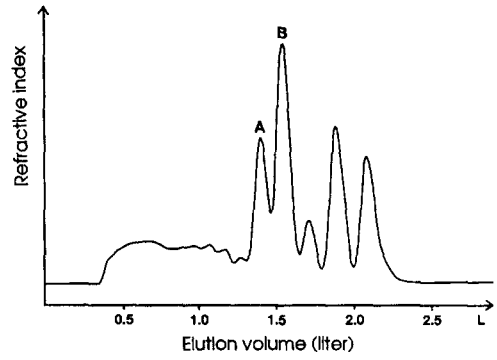


Fig. 1. Fractionation on Bio-Gel P-2 of hydrolytic products obtained from amylopectin upon two consecutive digestions with Termamyl 120 L. The components eluting in peaks A and B were combined and subjected to beta-amylase digestion.

higher oligosaccharides were recovered by gel filtration (Fig. 1) and subjected to treatment with beta-amylase. The pentaose-containing fractions were recovered by gel filtration and analyzed by Dionex PA-100 chromatography. Essentially one component clearly separated from authentic standards of unbranched maltodextrins, including

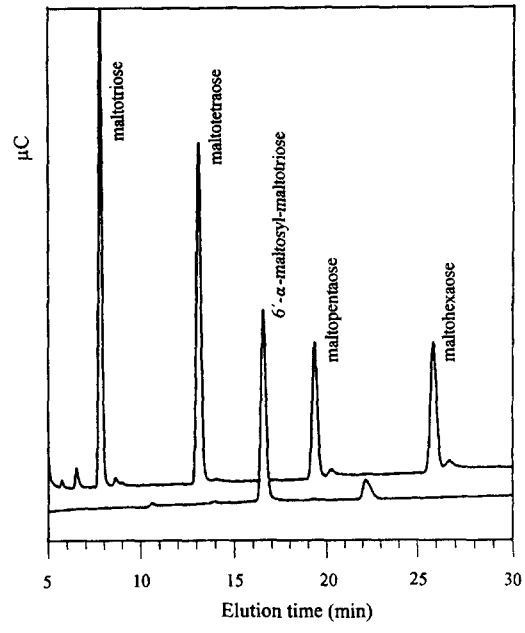


Fig. 2. Analysis of enzymatically produced 6′-α-maltosyl-maltotriose (**15**) by Dionex PA-100 chromatography. The column was eluted with 0.1 M NaOH and an NaOAc gradient from 0.05 to 0.13 M run for 39 min. The unit μC indicates the electrical output from the HPLC detection system. The elution profiles of authentic standards are shown on a separate profile.

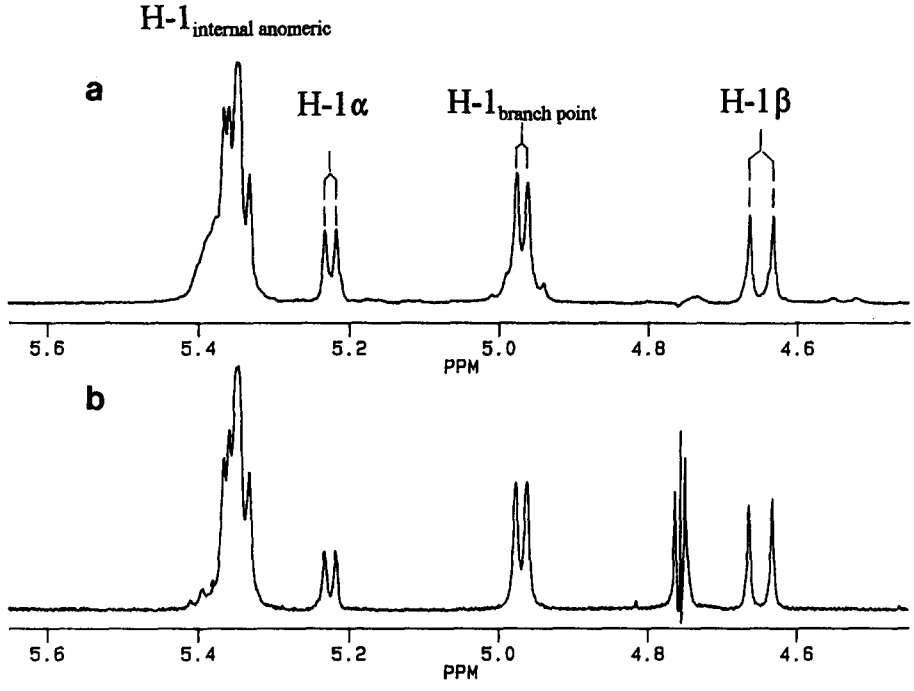


Fig. 3. 250-MHz ^{1}H NMR spectrum of **15** in D_2O: (a) chemically synthesized; (b) prepared enzymatically.

pentaose, was obtained (Fig. 2). This component coelutes with synthetic **15** and the ^{1}H and ^{13}C NMR spectra of the anomeric region of the component (Figs 3 and 4) are virtually identical to those of synthetically prepared **15**, the small differences being ascribed to low amounts of impurities in the two preparations. In these figures the doublet at δ 4.99 with $J_{1,2}$ 3.6 Hz (Fig. 3), assignable to the anomeric proton at the branch point, as well as the corresponding anomeric carbon resonance at δ 99.4 (Fig. 4) are in good agreement with those values (δ 4.96 with $J_{1,2}$ 3.6 Hz and δ 99.6, respectively) reported [26] for the methyl β-glycoside derivative of **15**. Characteristic of the enzymatic approach is the low yield obtained, compared with the chemical synthesis. Another advantage of the latter approach is the possibility of obtaining derivatives where specific hydroxyl groups are either activated or protected as required for the chemical synthesis of more complex branched oligosaccharides.

3. Experimental

General methods.—Melting points were determined using a Mettler FP81 MBC Cell connected to a Mettler FP80 Central Processor unit. Optical rotations were measured at 21 ± 2°C with an Optical Activity Ltd AA-1000 Polarimeter.

All reactions were monitored by TLC on aluminum sheets coated with Silica Gel $60F_{254}$ (0.2-mm thickness, E. Merck, Darmstadt, Germany) and the spots were detected

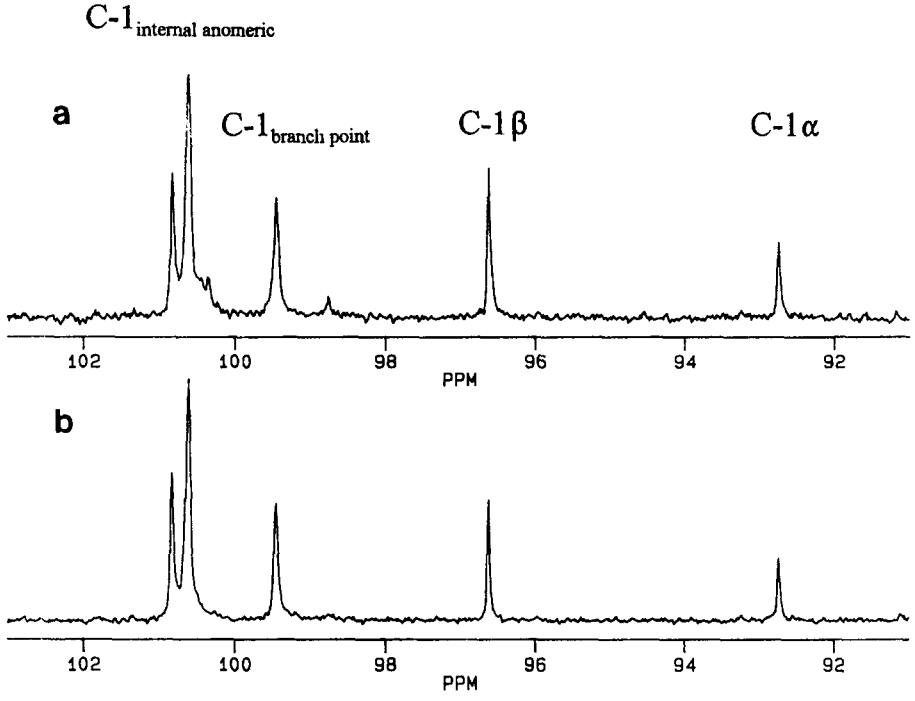


Fig. 4. 63.0-MHz ^{13}C NMR spectrum of **15** in D_2O: (a) chemically synthesized; (b) prepared enzymatically.

by charring with 10% H_2SO_4 in MeOH. Column chromatography was caried out using Silica Gel 60 (particle size 0.040–0.063 mm, 230–400 mesh ASTM, E. Merck). Solvent extracts were dried with anhydrous Na_2SO_4 unless otherwise specified. Microanalyses were performed at the Chemistry Department, Leo Pharmaceutical Company, Copenhagen, Denmark. The purity of **15** was verified by analytical Dionex PA-100 chromatography.

The ^1H NMR and ^{13}C NMR spectra were recorded on a Bruker AC250P spectrometer at 250 and 63 MHz, respectively. In water, dioxane was used as internal reference [δ_H(dioxane) = 3.75; δ_C(dioxane) = 67.4]. In other solvents, δ_H-values are relative to internal Me_4Si and δ_C-values are referenced to the solvent [$\delta_C(CDCl_3) = 77.0$; $\delta_C(Me_2SO\text{-}d_6) = 39.4$]. A complete interpretation of the ^1H and ^{13}C NMR spectra of **8** has already been presented, based on two-dimensional homonuclear and heteronuclear chemical shift correlations [11]. Because the structure of compound **8** is contained within compound **15**, only partial NMR data are shown for **15**. All additional data are in agreement with the structure presented for **15**. The glucopyranosyl units of the branched tetrasaccharides are labelled **A** to **D** as indicated for compound **8** (Scheme 2) and those of the branched pentasaccharides are **1** to **5** as indicated for compound **15** (Scheme 3).

*Phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-*D*-glucopyranoside* (**2**).—To a solution of tetra-*O*-acetyl-α-D-glucopyranosyl bromide {prepared in situ from hydrated D-glucose (**1**; 10 g, 55.51 mmol) by the method of Kartha and Jennings [13]} and Bu_4NHSO_4 (18.85

g, 55.51 mmol) in CH_2Cl_2 (230 mL) was added 1 M aq Na_2CO_3 (230 mL) and thiophenol (17.5 mL, 170.43 mmol). The two-phase mixture was vigorously stirred for 30 min at room temperature. The mixture was diluted with CH_2Cl_2 (100 mL) and the organic phase was separated, successively washed with 1 M aq NaOH (3 × 50 mL), water (3 × 50 mL), and brine (50 mL), and dried. The CH_2Cl_2 was removed in vacuo and the residue crystallized from 96% EtOH to give **2** (21.56 g, 88%, colorless needles); $[\alpha]_D$ −15.5° (*c* 0.36, $CHCl_3$) {lit. [27] $[\alpha]_D$ −16° ($CHCl_3$)}; mp 117–118°C (lit. [27] mp 117–118°C); ^{1}H NMR data ($CDCl_3$): δ 1.99 (s, 3 H, $COCH_3$), 2.02 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 2.09 (s, 3 H, $COCH_3$), 3.73 (ddd, 1 H, $J_{5,6a}$ 2.9, $J_{5,6b}$ 4.8, $J_{4,5}$ 10.0 Hz, H-5), 4.17 (dd, 1 H, $J_{5,6a}$ 2.7, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.24 (dd, 1 H, H-6b), 4.71 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 4.97 (dd, 1 H, $J_{2,3}$ 9.2 Hz, H-2), 5.04 (dd, 1 H, $J_{3,4}$ 9.6, $J_{4,5}$ 9.8 Hz, H-4), 5.23 (t, 1 H, H-3), 7.32 (m, 3 H, H-arom), 7.50 (m, 2 H, H-arom); ^{13}C NMR data ($CDCl_3$): δ 20.5, 20.5, 20.7, 20.7 (4 $COCH_3$), 62.1 (C-6), 68.1 (C-4), 69.9 (C-2), 73.9 (C-3), 75.7 (C-5), 85.6 (C-1), 128.3 (*p*-C_{arom}), 128.9 (*m*-C_{arom}), 131.6 (*ipso*-C_{arom}), 133.0 (*o*-C_{arom}), 169.2, 169.3, 170.1, 170.5 (4 $COCH_3$).

*Phenyl 1-thio-β-*D*-glucopyranoside* (**3**).—MeONa (30%) in MeOH (25 mL) was added gradually to a stirred suspension of **2** (20 g, 45.41 mmol) in dry MeOH (300 mL) at 0°C. Stirring was continued for 10 min at 0°C and the mixture neutralized by addition of Dowex 50W-X8 (H^+ form, 200–400 mesh) resin. The resin was filtered off and washed with MeOH (3 × 50 mL). The residue obtained upon evaporation of the combined filtrates was coevaporated with toluene (3 × 50 mL). The white precipitate formed upon toluene treatment was collected by filtration and air-dried to give **3** (12.3 g, 100%); $[\alpha]_D$ −70.5° (*c* 0.8, H_2O) {lit. [28] $[\alpha]_D$ −70.5° (*c* 2, H_2O)}; mp 132–133°C (lit. [28] mp 133°C); ^{1}H NMR data (Me_2SO-d_6): δ 3.09 (m, 2 H, H-2,5), 3.23 (m, 2 H, H-3,4), 3.47 (m, 1 H, H-6), 3.70 (m, 1 H, H-6), 4.56 (d, 1 H, *J* 5.7 Hz, OH), 4.62 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 4.99 (d, 1 H, *J* 5.1 Hz, OH), 5.10 (d, 1 H, *J* 4.6 Hz, OH), 5.29 (d, 1 H, *J* 6.0 Hz, OH), 7.27 (m, 3 H, H-arom), 7.47 (m, 2 H, H-arom); ^{13}C NMR data (Me_2SO-d_6): δ 61.0 (C-6), 69.7 (C-4), 72.3 (C-3), 78.1 (C-5), 80.8 (C-2), 87.0 (C-1), 126.3, 128.8, 129.5, 134.9 (C-arom, SPh).

*Phenyl 2,3-di-*O*-acetyl-4,6-*O*-benzylidene-1-thio-β-*D*-glucopyranoside* (**4**).—A solution of **3** (10 g, 36.72 mmol) and α,α-dibromotoluene (7.77 mL) in dry pyridine (300 mL) was refluxed for 2.5 h at 140°C. The mixture was cooled to room temperature and Ac_2O (80 mL) was added gradually. The mixture was stirred for 24 h at room temperature, poured into ice–water, and stirred for an additional 1 h. The precipitate formed was filtered off, washed several times with water, air-dried, and crystallized from 96% EtOH to give **4** (8.25 g, 82%, colorless needles); $[\alpha]_D$ −53.4° (*c* 0.59, $CHCl_3$); mp 205–206°C; ^{1}H NMR data ($CDCl_3$): δ 2.03 (s, 3 H, $COCH_3$), 2.10 (s, 3 H, $COCH_3$), 3.56 (m, 1 H, H-5), 3.66 (t, 1 H, $J_{3,4} = J_{4,5} = 9.4$ Hz, H-4), 3.78 (dd, 1 H, $J_{5,6a}$ 9.8, $J_{6a,6e}$ 10.3 Hz, H-6*a*), 4.38 (dd, 1 H, $J_{5,6e}$ 4.7 Hz, H-6*e*), 4.80 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1), 5.01 (dd, 1 H, $J_{2,3}$ 8.9 Hz, H-2), 5.34 (t, 1 H, H-3), 5.49 (s, 1 H, PhC*H*), 7.34 (m, 6 H, H-arom, SPh and PhCH), 7.44 (m, 4 H, H-arom, SPh and PhCH); ^{13}C NMR data ($CDCl_3$): δ 20.7, 20.7 (2 $COCH_3$), 68.4 (C-6), 70.6, 70.7 (C-2,4), 72.7 (C-3), 78.0 (C-5), 86.5 (C-1), 101.4 (Ph*C*H), 126.1, 128.2, 128.4, 129.0, 129.1, 131.7, 132.9, 136.7 (C-arom, SPh and PhCH), 169.4, 170.0 (2 $COCH_3$). Anal. Calcd for $C_{23}H_{24}O_7S$: C, 62.15; H, 5.44; S, 7.21. Found: C, 62.04; H, 5.54; S, 7.17.

Phenyl 4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**5**).—Compound **4** (8 g, 18 mmol) was deacetylated, as described for **3**, to give **5** (6.23 g, 96%, white powder); $[\alpha]_D$ −69.2° (*c* 0.36, MeOH) {lit. [29] $[\alpha]_D$ −56° (*c* 0.2, $CHCl_3$)}; mp 183.5°C (lit. [29] mp 188–188.5°C); ^{1}H NMR data (Me_2SO-d_6): δ 3.20 (m, 1 H, H-5), 3.42 (m, 1 H, H-2), 3.54 (m, 2 H, H-3,4), 3.68 (t, 1 H, $J_{5,6a} = J_{6a,6e} = 9.9$ Hz, H-6*a*), 4.20 (dd, 1 H, $J_{5,6e}$ 4.5 Hz, H-6*e*), 4.86 (d, 1 H, $J_{1,2}$ 9.7 Hz, H-1), 5.47 (d, 1 H, *J* 5.0 Hz, OH), 5.58 (s, 1 H, PhC*H*), 5.62 (d, 1 H, *J* 6.1 Hz, OH), 7.36 (m, 10 H, H-arom, SPh and PhCH); ^{13}C NMR data (Me_2SO-d_6): δ 67.7 (C-6), 69.6 (C-4), 72.9 (C-3), 74.2 (C-5), 87.0 (C-1), 100.6 (Ph*C*H), 126.3, 126.7, 127.9, 128.8, 128.9, 130.2, 133.8, 137.6 (C-arom, SPh and PhCH).

Phenyl 2,3-di-O-benzyl-4,6-O-benzylidene-1-thio-β-D-glucopyranoside (**6**).—A solution of **5** (5 g, 11.25 mmol) in EtOAc (150 mL) was added to a stirred mixture of Bu_4NHSO_4 (6.18 g, 18.20 mmol), benzyl bromide (62 mL), and aq 50% NaOH (100 mL) at room temperature. The mixture was stirred for 2 h at room temperature and then diluted with EtOAc (200 mL). The organic phase was washed with water (3 × 100 mL), dried, and evaporated to dryness. The residue was crystallized from 96% EtOH to give **6** (5.5 g, 90%, fiber-shaped crystals); $[\alpha]_D$ −21.9° (*c* 0.75, $CHCl_3$) {lit. [29] $[\alpha]_D$ −31.6° (*c* 0.5, pyridine)}; mp 155–156°C (lit. [29] mp 155–157°C); ^{1}H NMR data ($CDCl_3$): δ 3.48 (m, 1 H, H-5), 3.51 (dd, 1 H, $J_{2,3}$ 8.4, $J_{1,2}$ 9.7 Hz, H-2), 3.70 (t, 1 H, $J_{3,4} = J_{4,5} = 9.3$ Hz, H-4), 3.79 (dd, 1 H, $J_{5,6a}$ 9.3, $J_{6a,6e}$ 10.4 Hz, H-6*a*), 3.83 (dd, 1 H, H-3), 4.38 (dd, 1 H, $J_{5,6e}$ 5.0 Hz, H-6*e*), 4.75 (d, 1 H, H-1), 4.77, 4.94 (2 d, 2 H, *J* 11.2 Hz, PhCH_2), 4.81, 4.87 (2 d, 2 H, *J* 10.3 Hz, PhCH_2), 5.58 (s, 1 H, PhC*H*), 7.39 (m, 20 H, H-arom, SPh, $PhCH_2$, and PhCH); ^{13}C NMR data ($CDCl_3$): δ 68.8 (C-6), 70.3 (C-4), 75.4, 75.9 (2 Ph*C*H_2), 80.5, 81.5 (C-2 and C-5), 83.1 (C-3), 88.3 (C-1), 101.2 (Ph*C*H), 125.9, 127.7, 127.8, 128.1, 128.2, 128.2, 128.3, 128.9, 129.0, 132.3, 133.1, 137.2, 138.0, 138.2 (C-arom, SPh, $PhCH_2$, and PhCH).

Phenyl 2,3,6-tri-O-benzyl-1-thio-β-D-glucopyranoside (**7**).—A stirred solution of **6** (5 g, 9.25 mmol) and $NaBH_3CN$ (5.23 g, 83.23 mmol) in dry THF (150 mL) containing powdered 4 Å molecular sieves was cooled to 0°C. A solution of HCl in diethyl ether was added gradually until the solution became acidic (pH region 3–2, gas evolution). After additional stirring for 15 min at 0°C and diluting with EtOAc (200 mL) the reaction mixture was filtered through Celite. The filtrate was washed successively with water (3 × 250 mL), satd aq $NaHCO_3$ (3 × 100 mL), water (3 × 100 mL), and brine (50 mL), and dried. The solvent was evaporated and the residue chromatographed on silica gel (130 g) with 30% diethyl ether in *n*-pentane to give **7** (4.72 g, 94%, white powder crystallized from diethyl ether–*n*-pentane); $[\alpha]_D$ −27.3° (*c* 0.44, $CHCl_3$); mp 71–72°C; ^{1}H NMR data ($CDCl_3$): δ 2.62 (d, 1 H, $J_{4,OH}$ 2.4 Hz, OH-4), 3.47 (m, 1 H, H-5), 3.50 (dd, 1 H, $J_{2,3}$ 8.5, $J_{1,2}$ 9.7 Hz, H-2), 3.53 (t, 1 H, $J_{3,4}$ 8.5 Hz, H-3), 3.65 (td, 1 H, $J_{4,OH}$ 2.3, $J_{4,5}$ 9.0 Hz, H-4), 3.73 (dd, 1 H, $J_{5,6b}$ 5.0, $J_{6a,6b}$ 10.5 Hz, H-6b), 3.79 (dd, 1 H, $J_{5,6a}$ 4.2, $J_{6a,6b}$ 10.4 Hz, H-6a), 4.54, 4.59 (2 d, 2 H, *J* 11.9 Hz, PhCH_2), 4.69 (d, 1 H, H-1), 4.70, 4.91 (2 d, 2 H, *J* 11.4 Hz, PhCH_2), 4.77, 4.91 (2 d, 2 H, *J* 11.1 Hz, PhCH_2), 7.42 (m, 20 H, H-arom, SPh and $PhCH_2$); ^{13}C NMR data ($CDCl_3$): δ 70.4 (C-6), 71.7 (C-4), 73.7, 75.4, 75.5 (3 Ph*C*H_2), 78.2 (C-5), 80.5 (C-2), 86.2 (C-3), 87.7 (C-1), 127.5, 127.6, 127.7, 127.9, 127.9, 128.0, 128.2, 128.4, 128.4, 128.6, 128.9, 131.8, 133.8, 137.9, 137.9, 138.4 (C-arom, SPh and $PhCH_2$). Anal. Calcd for $C_{33}H_{34}O_5S \cdot 0.5H_2O$: C, 71.84; H, 6.39; S, 5.81. Found: C, 71.59; H, 6.44; S, 5.83.

Phenyl O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 6)*-O-*[*α-D-*glucopyranosyl-(1 → 4)]-1-thio*-β-D-*glucopyranoside* (**9**).—Compound **8** [11] (5 g, 3.05 mmol) was deacetylated, as described for **3**, to give chromatographically pure **9** (4.23 g, quantitative). An analytical sample was crystallized from EtOH–water as a white solid; $[\alpha]_D$ +57.1° (*c* 0.31, $CHCl_3$); mp 147–148°C; ^{1}H NMR data (Me_2SO-d_6): δ 4.71 (d, 1 H, $J_{1,2}$ 10.2 Hz, H-1^A), 5.16 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^B), 5.53 (d, 1 H, $J_{1,2}$ 4.0 Hz, H-1^C), 5.79 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^D), 7.36 (m, 40 H, H-arom, SPh and $PhCH_2$); ^{13}C NMR data (Me_2SO-d_6): δ 87.0 (C-1^A), 95.6 (C-1^B), 96.0 (C-1^C), 101.6 (C-1^D). Anal. Calcd for $C_{79}H_{88}O_{20}S \cdot 0.5H_2O$: C, 67.84; H, 6.41; S, 2.29. Found: C, 67.89; H, 6.41; S, 2.23.

Phenyl O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl-(1 → 6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl-(1 → 4)]-2,3-di*-O-*benzyl-1-thio*-β-D-*glucopyranoside* (**10**).—A solution of **9** (4 g, 2.88 mmol) in dry DMF (50 mL) was stirred with NaH (2.15 g, 60% in mineral oil) for 2 h at room temperature, and the mixture cooled to 0°C. Benzyl bromide (5 mL) was added dropwise to the mixture and stirring was continued overnight at room temperature. The mixture was cooled to 0°C and excess of NaH decomposed by dropwise addition of MeOH. The solution was concentrated in vacuo and diluted with EtOAc (150 mL) and water (50 mL). The organic phase was separated, washed with water (4 × 25 mL) and brine (25 mL), and dried. The solvent was evaporated and the residue chromatographed on silica gel (130 g) with *n*-pentane–diethyl ether (3:2 v/v) as eluent to give **10** (4.15 g, colorless syrup, 75%); $[\alpha]_D$ +53.2° (*c* 0.22; $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 4.52 (d, 1 H, $J_{1,2}$ 10.0 Hz, H-1^A), 5.19 (d, 1 H, $J_{1,2}$ 3.8 Hz, H-1^B), 5.61 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^D), 5.64 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1^C), 7.33 (m, 70 H, H-arom, SPh and $PhCH_2$); ^{13}C NMR data ($CDCl_3$): δ 88.0 (C-1^A), 96.6 (C-1^B), 97.1 (C-1^D), 97.3 (C-1^C).

O-*(2,3,4,6-Tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)]-2,3-di*-O-*benzyl*-α,β-D-*glucopyranose* (**11**).—*N*-Bromosuccinimide (1 g, 5.62 mmol) was added to a stirred solution of **10** (3.87 g, 2.00 mmol) in acetone–water (9:1 v/v) at room temperature and stirring was continued for 10 min at room temperature. The solvent was evaporated in vacuo at room temperature until a turbidity arose. EtOAc (150 mL) was then added and the organic phase washed with satd aq $NaHCO_3$ (3 × 50 mL) and water (3 × 50 mL), dried, and finally evaporated to dryness. The residue was chromatographed on silica gel (130 g) with diethyl ether–*n*-pentane (4:1 v/v) as eluent to give **11** (3.47 g, colorless syrup, 94%); $[\alpha]_D$ +56.8° (*c* 0.59, $CHCl_3$); ^{13}C NMR data ($CDCl_3$): δ 90.4 (C-1^A, α anomer), 95.7 (C-1^D, α anomer), 95.9 (C-1^B, α anomer), 96.2 (C-1^C, α anomer), 96.8 (C-1^D, β anomer), 97.0 (C-1^B, β anomer), 97.0 (C-1^C, β anomer), 97.1 (C-1^A, β anomer).

O-*(2,3,4,6-Tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)]-2,3-di*-O-*benzyl*-α,β-D-*glucopyranosyl trichloroacetimidate* (**12**).—A solution of **11** (4 g, 2.18 mmol) and trichloroacetonitrile (2.5 mL) in dry CH_2Cl_2 (30 mL) was stirred vigorously with anhydrous K_2CO_3 (1.25 g) for 24 h at room temperature under N_2. The mixture was diluted with dry CH_2Cl_2 (20 mL) and filtered through a Celite pad and a silica gel layer. After thorough washing with CH_2Cl_2 (5 × 20 mL), the combined

filtrates were evaporated to obtain chromatographically pure **12** (4.13 g, syrup, 96% yield) which was used directly for the next step without further purification; ^{1}H NMR data ($CDCl_3$) for the α anomer: δ 5.09 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1^B), 5.70 (d, 1 H, $J_{1,2}$ 3.0 Hz, H-1^D), 5.74 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1^C), 6.43 (d, 1 H, $J_{1,2}$ 3.2 Hz, H-1^A), 7.19 (m, 65 H, H-arom), 8.74 (s, 1 H, NH); ^{13}C NMR data ($CDCl_3$): δ 91.0 (CCl_3), 93.3 (C-1^A), 95.7 (C-1^B), 96.6 (C-1^C), 96.7 (C-1^D), 160.7 (C=NH). For the β anomer, ^{1}H NMR data ($CDCl_3$): δ 5.20 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1^B), 5.65 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1^D), 5.73 (d, 1 H, $J_{1,2}$ 3.5 Hz, H-1^C), 5.81 (d, 1 H, $J_{1,2}$ 7.9 Hz, H-1^A), 7.23 (m, 65 H, H-arom), 8.73 (s, 1 H, NH); ^{13}C NMR data ($CDCl_3$): δ 90.8 (CCl_3), 96.5 (C-1^B), 96.7 (C-1^D), 96.8 (C-1^C), 97.7 (C-1^A), 160.5 (C=NH).

Phenyl O-*(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)]*-O-*(2,3-di*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)-2,3,6-tri*-O-*benzyl-1-thio*-β-D-*glucopyranoside* (**13**).—A solution of **7** (1 g, 1.84 mmol) and **12** (3 g, 1.51 mmol) in dry diethyl ether (125 mL) was stirred for 30 min at room temperature in the presence of 4 Å molecular sieves (1.5 g, activated powder) under Ar. The stirred mixture was then cooled to −20°C and a solution of trimethylsilyl trifluoromethanesulfonate (50 μL, 0.28 mmol) in dry diethyl ether (5 mL) was added dropwise. Stirring was continued and the temperature was raised to room temperature over a period of 2.5 h. After dilution with diethyl ether (150 mL), solid $NaHCO_3$ (2 g) was added and stirring was continued for 10 min. The mixture was filtered through Celite. The filtrate was washed thoroughly with satd aq $NaHCO_3$ (2 × 50 mL), water (3 × 50 mL), and brine (25 mL), dried, and evaporated to dryness. The residue was chromatographed on silica gel (120 g) with diethyl ether–*n*-pentane (2:3 v/v) as eluent to give pure **13** (2.32 g, gum, 65%); $[\alpha]_D$ +62.3° (*c* 0.42, $CHCl_3$); ^{1}H NMR data ($CDCl_3$): δ 4.59 (d, 1 H, *J* 10.2 Hz, H-1^1), 5.37 (d, 1 H, *J* 3.7 Hz, H-1^3), 5.53 (d, 1 H, *J* 3.8 Hz, H-1^2), 5.73 (d, 1 H, *J* 3.7 Hz, H-1^4), 5.77 (d, 1 H, *J* 3.8 Hz, H-1^5), 7.30 (m, 85 H, H-arom, SPh and $PhCH_2$); ^{13}C NMR data ($CDCl_3$): δ 87.3 (C-1^1), 96.2 (C-1^2), 96.5 (C-1^3), 96.8 (C-1^5), 97.0 (C-1^4).

O-*(2,3,4,6-Tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)*-O-*(2,3,6-tri*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 6)*-O-*[(2,3,4,6-tetra*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)]*-O-*(2,3-di*-O-*benzyl*-α-D-*glucopyranosyl)-(1 → 4)-2,3,6-tri*-O-*benzyl*-D-*glucopyranose* (**14**).—Compound **13** (2 g, 0.85 mmol) was treated with NBS–acetone–water and worked-up as described for **11**. The residue was chromatographed on silica gel (120 g) with diethyl ether–*n*-pentane (3:2 v/v) as eluent to give **14** (1.86 g, white powder, 97%) in an α to β ratio of 1.5; $[\alpha]_D$ +71.0° (*c* 0.1, $CHCl_3$); mp 55–56°C; ^{13}C NMR data ($CDCl_3$): δ 90.9 (C-1^1, α anomer), 95.8 (C-1^2, β anomer), 95.9 (C-1^2, α anomer), 96.4 (C-1^3, α anomer), 96.5 (C-1^3, β anomer), 96.7 (C-1^5, α anomer), 96.8 (C-1^5, β anomer), 96.9 (C-1^4, β anomer), 97.1 (C-1^4, α anomer), 97.2 (C-1^1, β-anomer). Anal. Calcd for $C_{142}H_{148}O_{26}$: C, 75.11; H, 6.57. Found: C, 74.98; H, 6.60.

6′-α*-Maltosyl-maltotriose* (**15**).—To a solution of **14** (0.22 g, 0.1 mmol) in EtOH (20 mL) was added 20% $Pd(OH)_2$ on carbon (125 mg) and the mixture was stirred under hydrogen for 48 h at room temperature. The catalyst was removed by filtration, and the filtrate was evaporated to dryness. The residue was purified by HPLC and lyophilized to give **15** (70 mg, white fibers, 87%); $[\alpha]_D$ +213.1° (*c* 0.16, H_2O); mp 209–210°C; ^{1}H NMR data (D_2O): δ 4.67 (d, 0.6 H, $J_{1,2}$ 7.9 Hz, H-1^1, β anomer), 4.99 (d, 1 H, $J_{1,2}$ 3.6

Hz, H-1^3), 5.24 (d, 0.4 H, $J_{1,2}$ 3.8 Hz, H-1^1, α anomer), 5.37 (m, 3 H, H-1^2, H-1^4, and H-1^5); ^{13}C NMR data (D_2O): δ 92.7 (C-1^1, α anomer), 96.6 (C-1^1, β anomer), 99.4 (C-1^3), 100.6, 100.6, 100.8 (C-1^2, C-1^4, and C-1^5).

Enzymatic preparation of 6′-maltosyl-maltotriose (**15**).—Amylopectin (50 g, Sigma A-7780) was suspended in 167 mL of 0.1 M Ca glycerophosphate/H_3PO_4 (pH 6.0). The temperature was increased to 100°C under stirring and 1.7 mL of Termamyl 120 L (Novo Nordisk) was added. After gelatinization, the temperature was lowered to 90°C and kept constant for 2 h. After cooling to ambient temperature and immediate filtration, the filtrate was applied to a charcoal column [100 g, granules (Merck 2514) carefully rinsed with water, EtOH, and water]. After washing the column with water (3 L) followed by 15% EtOH (2 L), elution was carried out using 50% EtOH (2 L) followed by EtOH (1 L). The two EtOH eluates were combined, concentrated in a rotary evaporator, and freeze-dried (dry matter content: 4.5 g). The freeze-dried material was treated with a new batch of Termamyl 120 L (0.150 mL) as above. The freeze-dried material thus obtained was divided into 1-g portions each of which was applied to a Bio-Gel P-2 column (90 × 5 cm) operated at 60°C with water as the eluent. Material eluted in peaks A and B was combined (total volume: 300 mL), adjusted to pH 4.8 with NaOAc–AcOH (final concentration: 30 mM), and treated with beta-amylase (10 U/mL, Boehringer Mannheim GmbH, 102 822) for 4 h at 37°C with continuous stirring. Enzyme digestion was terminated by boiling for 10 min after which the reaction mixture was frozen and freeze-dried. The freeze-dried sample thus obtained was subjected to gel filtration on Bio-Gel P-2 as reported above. Fractions with a mobility corresponding to pentaose were combined (dry matter content: 120 mg).

Throughout the preparation procedure, the content of the different fractions was monitored by TLC analysis (70:29:1 *n*-PrOH–H_2O–35% NH_4OH) and by HPLC on an Aminex HPX-42A column (300 × 7.8 mm, Bio Rad) run at 80°C using authentic standards.

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