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Synthesis of natural β-D-(1→3)-glucopyranosyl oligosaccharides

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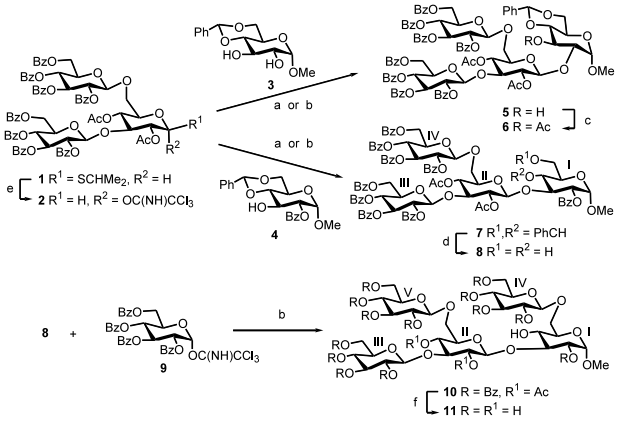
Abstract

β-D-(1→3)-Glucan core structure derivatives corresponding to schizophyllan, epiglucan and lentinan were synthesized efficiently. 4,6-*O*-Benzylidenated glucopyranosyl acceptors were found to be helpful in the attempted β-D-(1→3) bond formation. The epiglucan pentasaccharide showed a weak anti-tumor activity in preliminary mice tests. © 2002 Elsevier Science Ltd. All rights reserved.

Keywords: Glycosylation; Oligosaccharides; Regioselectivity

1. Introduction

A family of glucans which contain a main chain of β-D-(1→3)-glucopyranosyl units, and are substituted at O-6 by a single unit of β-D-glucopyranosyl side chains have received considerable attention because of their antitumor activity (immunomodulating action).[1]



Scheme 1. *Reagents and conditions*: (a) NIS, TMSOTf, CH_2Cl_2; 45% for **5**; 39% for **7**; (b) TMSOTf, CH_2Cl_2; 68% for **5**; 85% for **7**; 84% for **10**; (c) Ac_2O, Pyr; (d) 50% TFA, 2:1 CH_3CN–CH_2Cl_2, 35 °C; 88%; (e) NBS, CH_2Cl_2, H_2O; Cl_3CCN, DBU; 73% for two steps; (f) NaOMe, MeOH; 79%.

Schizophyllan,[2] scleroglucan,[3] epiglucan[4] and lentinan[5] are the most well-known members of this group of polysaccharides. It has been suggested that the immunopharmacological activity of soluble (1→3)-β-D-glucans are related to the organization of the (1→3)-β-linked backbone into a triple helix, the frequency and the complexity of side-branching and to their molecular weight.[6] However, Tsuzuki and coworkers[7] found that the conformation of the glucans, either single or triple helix, is independent of the hematopoietic response. These results encourage chemists to prepare the structures of the minimum size of the natural polysaccharides and investigate their structure–activity relationships.[8]

In our previous synthesis of the sanqi hexasaccharide via a 3 + 3 strategy,[9] we obtained an unexpected α-product even using an O-2 acetylated donor under standard glycosylation conditions. Further investigation[10] showed that this α priority is especially general in complex carbohydrate coupling reactions for attempted 1→3 glycosidic bond formation. To find an easy way of preparing branched β-D-(1→3)-glucan core structures, we tried to use a 4,6-*O*-benzylidenated glucopyranosyl acceptor in the glycosylation and have gotten some positive results. We now report the synthesis of schizopyllan, epiglucan and lentinan core structure derivatives, which contain a β-D-(1→3)-glucopyranosyl backbone, using 4,6-*O*-benzylidene-protected glucopyranosyl acceptors under usual glycosylation conditions.

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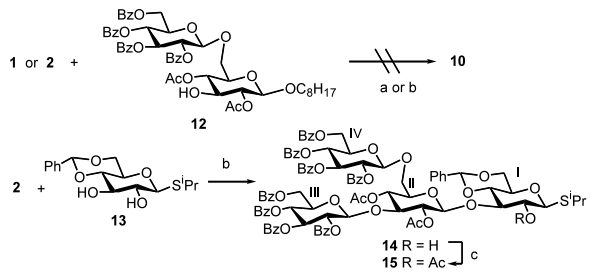
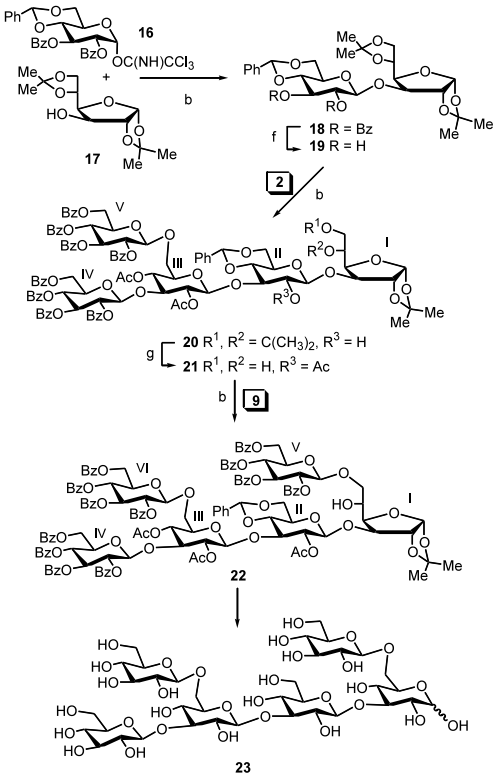


Scheme 2. *Reagents and conditions*: (a) NIS, TMSOTf, CH_2Cl_2; (b) TMSOTf, CH_2Cl_2; 85% for **14**; (c) Ac_2O, Pyr.



Scheme 3. *Reagents and conditions*: (a) TMSOTf, CH_2Cl_2; 56% for **18**; 78% for **20**; 87% for **22**; (b) NaOMe, MeOH; (c) Ac_2O, Pyr; then $CeCl_3{\cdot}7H_2O$; 78% for two steps; (d) 90% TFA, rt, 30 min; then NaOMe, MeOH; 53%.

2. Results and discussion

We have shown that the coupling reaction of trisaccharide thioglycoside **1** or trichloroacetimidate donor **2** and octyl 2,4,6-tri-*O*-acetyl-β-D-glucopyranoside gave α,β-mixed tetrasaccharide.[10] To take the advantage of our easy entry[11] to the trisaccharide donor **1** in the preparation of β-D-(1→3)-glucan core structures, we tried the glycosylation of thioglycoside **1** and methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**3**) in the presence of NIS (2.5 equiv) and TMSOTf (0.3 equiv), and obtained the undesired β-(1→2) product **5** in a modest yield (Scheme 1). The β-(1→2) linkage in **5** was confirmed by the peaks at δ 3.68 ppm (dd, H-2^{I}, $J_{1,2}$ 3.5 Hz, $J_{2,3}$ 9.8 Hz) and δ 4.40 ppm (d, H-1^{II}, J 8.0 Hz) in the ^{1}H NMR spectrum of its acetylated derivative, **6**. When methyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**4**) was used as acceptor in the above reaction, β-(1→3)-linked schizophyllan tetrasaccharide **7** was obtained in 39%. The yield of this reaction improved significantly when using the corresponding trisaccharide imidate donor **2** (85%). Hydrolysis of 4,6-*O*-benzylidene of **7** with 50% trifluoroacetic acid in 2:1 CH_3CN–CH_2Cl_2 at 35 °C gave tetrasaccharide diol **8**, which was further subjected to the regioselective glycosylation with **9**, furnished fully protected pentasaccharide **10** in a total yield of 74%. Removal of the acyl groups from **10** with NaOMe in MeOH accomplished the synthesis of the epiglucan core structure derivative **11**. ^{13}C NMR spectroscopy of **11** showed the reducing end α C-1^{I} at 99.94 ppm, and all other β C-1s (C-1$^{II\text{-}V}$) appeared at 103.48 ppm. It is worth noting that direct condensation of **1** or **2** with disaccharide acceptor **12** under standard glycosylation conditions gave no coupled products. Interestingly, when trichloroacetimidate **2** was coupled with isopropyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**13**) in CH_2Cl_2 with TMSOTf as catalyst, the β-(1→3)-linked tetrasaccharide **14** was obtained in high yield (Scheme 2). The expected regio- and stereoselectivity of this reaction was further confirmed by its acetylated analog **15** showing H-2^{I} at δ 4.88 ppm (J 9.6, 10.4 Hz), while H-1^{II} appeared at δ 4.32 ppm ($J_{1,2}$ 8.0 Hz).

Encouraged by this model study, we envisaged a facile synthesis of the lentinan hexsaccharide derivative as shown in Scheme 3. TMSOTf-catalyzed condensation of 4,6-*O*-benzylidene-2,3-di-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**16**) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucopyranose (**17**) in CH_2Cl_2 at 0 °C gave disaccharide **18**, which was deacylated with NaOMe in MeOH to furnish the diol **19** in 56% yield. Critical glycosylation of trisaccharide imidate **2** and disaccharide diol **19** using the method as described in the model study gave desired pentasaccharide **20** as the predominant products. Acetylation of **20** with acetic anhydride in pyridine followed by $CeCl_3{\cdot}7H_2O$ catalyzed regioselective *O*-deisopropylidenation[12] gave pentasaccharide diol **21** in a good yield. The primary hydroxyl group favored the glycosylation of **21** with **9** in CH_2Cl_2 at −15 °C to furnish the lentinan hexasaccharide derivative **22** in excellent yield. 2D COSY experiments showed C-1^{III} at δ 100.11 ppm in the ^{13}C NMR, while the corresponding H-1^{III} appeared at δ 4.33 ppm (d, J 8.0 Hz) in the ^{1}H NMR, indicating a β linkage between sugar residue II and III in **22**. Full deprotection of **22** with 90% TFA, followed by deacylation with MeONa in MeOH, afforded hexasaccharide **23** in 53% yield.

The antitumor activity of epiglucan pentasaccharide **11** was preliminarily studied according to the method

described by Sasaki and co-workers.[5b] Kun-min mice weighing about 20 g and seven days Sarcoma-180 ascites (5×10^6 cells) were used for the bioassay. Lentinan (imported from Japan for medical usage) and Cisplatin® (CDDP) were selected as the positive controls in the parallel tests. The epiglucan pentasaccharide and lentinan were injected daily for 14 days, while CDDP was given every other day. The tumor inhibition ratios for **11**, lentinan and CDDP were 21–25% (dosage: 1.0–10 mg/kg/day), 31–36% (dosage: 1 mg/Kg/day) and 55–57% (dosage: 2 mg/kg/every other day), respectively.

In conclusion, we have described the synthesis of the core structure derivatives of schizophyllan, epiglucan and lentinan. From our experiments, 4,6-*O*-benzylidenated glucopyranosyl acceptors seem to be helpful for β bond formation in complex oligosaccharide synthesis. It would appear that the 3-OH groups in the current acceptors are less hindered compared to those in their 4,6-diacylated counterparts. Apparently the orthoester intermediate is formed and rearranges smoothly under standard glycosylation conditions to give a high yield of the product.

3. Experimental

General methods.—Optical rotations were determined at 25 °C with a Perkin–Elmer Model 241-Mc automatic polarimeter. ^{1}H, ^{13}C NMR and ^{1}H–^{1}H, ^{1}H–^{13}C COSY spectra were recorded with a Bruker ARX 400 spectrometer for solutions in $CDCl_3$ or D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si. Mass spectra were measured using either MALDITOF-MS with α-cyano-4-hydroxycinnamic acid (CCA) as the matrix or a VG PLATFORM mass spectrometer using the ESI technique. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in MeOH, or in some cases by a UV lamp.

Isopropyl 2,3,4,6-tetra-O-*benzoyl-β*-D-*glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl-(1→6)]-2,4-di*-O-*acetyl-1-thio-β*-D-*glucopyranoside* (**1**).—Compound **1** was prepared according to our previous general method.[11] $[\alpha]_D$ −5° (*c* 4, $CHCl_3$); ^{1}H NMR: 0.92, 1.02 (2 d, 6 H, 2 CH_3), 1.90, 1.92 (2 s, 6 H, 2 CH_3CO), 2.73–2.81 (m, 1 H, SCH), 3.50 (br t, 1 H, H-5^I), 3.66 (dd, 1 H, *J* 8.0, 11.2 Hz, H-6a^I), 3.86–3.90 (m, 2 H, H-3^I, H-6b^I), 4.10–4.15 (m, 2 H, H-5^{III}, H-5^{II}), 4.25 (d, 1 H, *J* 9.6 Hz, H-1^I), 4.45, 4.50, 4.60, 4.65 (4 dd, 4 H, 2 H-6^{II}, 2 H-6^{III}), 4.76 (t, 1 H, H-2^I), 4.81 (t, 1 H, *J* 9.6 Hz, H-4^I), 4.92 (d, 1 H, *J* 8.7 Hz, H-1^{II}), 4.95 (d, 1 H, *J* 8.0 Hz, H-1^{III}), 5.39 (dd, 1 H, *J* 8.0, 9.7 Hz, H-2^{III}), 5.51 (dd, 1 H, *J* 8.7, 9.4 Hz, H-2^{II}), 5.64 (t, 1 H, *J* 9.7 Hz, H-4^{III}), 5.68 (t, 1 H, *J* 9.4 Hz, H-4^{II}), 5.85 (t, 1 H, *J* 9.4 Hz, H-3^{II}), 5.90 (t, 1 H, *J* 9.7 Hz, H-3^{III}), 7.25–8.03 (m, 40 H, Ph). Anal. Calcd for $C_{81}H_{74}O_{25}S$: C, 65.76; H, 5.04. Found: C, 65.42; H, 5.09.

Methyl 2,3,4,6-tetra-O-*benzoyl-β*-D-*glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl-(1→6)]-2,4-di*-O-*acetyl-β*-D-*glucopyranosyl-(1→2)-3*-O-*acetyl-4,6*-O-*benzylidene-α*-D-*glucopyranoside* (**6**).—(a) Method A: To a solution of **1** (300 mg, 0.203 mmol) and **3** (56 mg, 0.2 mmol) in anhyd CH_2Cl_2 (6 mL) was added NIS (114 mg, 0.507 mmol) and TMSOTf (11 μL, 0.06 mmol) at −15 °C. The mixture was stirred under these conditions for 2 h and neutralized with Et_3N. The solution was concentrated and subjected to the column chromatography (2:3 EtOAc–petroleum ether) to give syrupy **5** (153 mg, 45%). (b) Method B: To a solution of **2** (314 mg, 0.2 mmol) and **3** (51 mg, 0.18 mmol) in anhyd CH_2Cl_2 (4 mL) was added TMSOTf (6 μL, 0.03 mmol) in the presence of 4 Å molecular sieves under an N_2 atmosphere at −15 °C. The mixture was stirred under these conditions for 1.5 h, then neutralized with Et_3N. The solution was concentrated and subjected to column chromatography (2:3 EtOAc–petroleum ether) to give syrupy **5** (207 mg, 68%), which was further acetylated with AC_2O in pyridine for 4 h, to give **6** (267 mg, 99%) as a syrup: $[\alpha]_D$ +45° (*c* 2.4, $CHCl_3$); ^{1}H NMR: 1.89, 1.90, 2.04 (3 s, 9 H, 3 CH_3CO), 3.21 (s, 3 H, CH_3O), 3.58–3.63 (m, 2 H, H-5^I, H-5^{II}), 3.68 (dd, 1 H, *J* 3.5, 9.8 Hz, H-2^I), 3.78–3.90 (m, 5 H, 2 H-6^I, 2 H-6^{II}, H-3^{II}), 4.12–4.20 (m, 3 H, H-5^{IV}, H-5^{III}, H-4^I), 4.40 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.48, 4.50 (2 d, 2 H, *J* 5.2 Hz, 2 H-6), 4.60, 4.64 (2 dd, 2 H, *J* 3.0, 12.1 Hz, 2 H-6), 4.71 (d, 1 H, *J* 3.5 Hz, H-1^I), 4.76 (t, 1 H, *J* 9.5 Hz, H-4^{II}), 4.81 (dd, 1 H, *J* 8.0, 9.2 Hz, H-2^{II}), 4.86 (d, 1 H, *J* 7.8 Hz, H-1^{III}), 5.03 (d, 1 H, *J* 7.9 Hz, H-1^{IV}), 5.39 (dd, 1 H, *J* 8.9, 9.2 Hz, H-2^{IV}), 5.43 (t, 1 H, *J* 9.8 Hz, H-3^I), 5.49 (dd, 1 H, *J* 8.0, 9.5 Hz, H-2^{III}), 5.50 (s, 1 H, PhCH), 5.65 (dd, 1 H, *J* 3.2, 9.7 Hz, H-4^{IV}), 5.69 (dd, 1 H, *J* 3.0, 9.6 Hz, H-4^{III}), 5.88 (t, 1 H, *J* 9.6 Hz, H-3^{III}), 5.91 (t, 1 H, *J* 9.6 Hz, H-3^{IV}). ^{13}C NMR: 20.44, 20.50, 20.96, 55.46, 62.21, 62.99, 63.12, 68.04, 68.75, 68.94, 69.45, 69.54, 70.48, 71.92 (2 C), 72.00, 72.49, 72.55, 72.91, 74.30, 77.55, 78.42, 79.32, 99.92 (C-1^I), 100.77 (PhCH), 101.02, 101.16, 101.30 (C-1^{II,III,IV}), 164.95, 165.08, 165.14, 165.65, 165.82, 166.02 (2 C), 168.11, 169.19, 169.44 (CO). MALDITOF-MS Calcd for $C_{94}H_{86}O_{32}$: 1726 [M]. Found 1749.5 $[M + Na]^+$.

Methyl 2,3,4,6-tetra-O-*benzoyl-β*-D-*glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzoyl-β*-D-*glucopyranosyl-(1→6)]-2,4-di*-O-*acetyl-β*-D-*glucopyranosyl-(1→3)-2*-O-*benzoyl-4,6*-O-*benzylidene-α*-D-*glucopyranoside* (**7**).—To a solution of **2** (1.1 g, 0.7 mmol) and **4** (258 mg, 0.67 mmol) in anhyd CH_2Cl_2 (10 mL) was added TMSOTf (15 μL, 0.08 mmol) in the presence of 4 Å molecular sieves under an N_2 atmosphere at 0 °C. The mixture was stirred under these conditions for 1.5 h, then neutralized with Et_3N and concentrated. The residue was subjected to column chromatography (2:3 EtOAc–

petroleum ether) to give **7** (1.068 g, 85%) as a syrup: $[\alpha]_D$ −16° (*c* 4, $CHCl_3$); ^{1}H NMR: 1.49, 1.93 (2 s, 6 H, 2 CH_3CO), 2.91 (dt, 1 H, *J* 9.9, 2.5 Hz), 3.38 (s, 3 H, OCH_3), 3.42–3.55 (m, 3 H), 3.64 (dd, 1 H, *J* 2.0, 11.9 Hz), 3.70–3.75 (m, 2 H), 3.80–3.95 (m, 3 H), 4.40 (dd, 1 H, *J* 10.8, 4.7 Hz), 4.47 (d, 1 H, *J* 8.0 Hz, H-1^{II}), 4.52 (d, 1 H, *J* 8.0, 9.4 Hz, H-1^{III}), 4.56–4.65 (m, 4 H), 4.84 (t, 1 H, *J* 9.4 Hz, H-2^{II}), 4.95 (t, 1 H, *J* 9.5 Hz, H-4^{II}), 4.92–4.96 (m, 2 H, H-1^{I}, H-2^{I}), 5.30 (d, 1 H, *J* 8.1 Hz, H-1^{IV}), 5.31 (dd, 1 H, *J* 8.0, 9.4 Hz, H-2^{III}), 5.45 (dd, 1 H, *J* 8.0, 9.6 Hz, H-2^{IV}), 5.54 (t, 1 H, *J* 9.5 Hz, H-4^{III}), 5.59 (t, 1 H, *J* 9.6 Hz, H-4^{IV}), 5.64 (s, 1 H, PhCH), 5.75 (t, 1 H, *J* 9.6 Hz, H-3^{IV}), 5.77 (t, 1 H, *J* 9.6 Hz, H-3^{III}), 7.18–8.24 (m, 50 H, Ph). Anal. Calcd for $C_{99}H_{88}O_{32}$: C, 66.44; H, 4.96. Found: C, 66.79; H, 5.05.

Methyl 2,3,4,6-tetra-O-*benzoyl*-β-D-*glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl-(1→6)]-2,4-di*-O-*acetyl*-β-D-*glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl-(1→6)]-2*-O-*benzoyl*-α-D-*glucopyranoside* (**10**). Compound **7** (900 mg, 0.5 mmol) was treated with 50% TFA (3 mL) in 2:1 CH_3CN–CH_2Cl_2 (15 mL) at 35 °C for 3 h, then concentrated and subjected to column chromatography (3:2 EtOAc–petroleum ether) to give **8** (753 mg, 88%). Compounds **8** (790 mg, 0.46 mmol) and **9** (363 mg, 0.49 mmol) were reacted as described in the preparation of **7** to give syrupy **10** (889 mg, 84%): $[\alpha]_D$ −4° (*c* 12, $CHCl_3$); ^{1}H NMR: 1.33, 1.89 (2 s, 6 H, 2 CH_3CO), 3.19 (s, 3 H, CH_3CO), 3.40–3.45 (m, 2 H, H-3, H-5), 3.60 (t, 1 H, *J* 9.4 Hz, H-3), 3.65–3.72 (m, 2 H, 2 H-6), 3.90–4.00 (m, 3 H, H-4^{I},5,6), 4.04–4.10 (m, 2 H, 2 H-5), 4.20–4.23 (m, 1 H, H-5), 4.24 (d, 1 H, *J* 8.0 Hz, H-1), 4.36 (d, 1 H, *J* 10.0 Hz, H-1), 4.40–4.55 (m, 5 H, 5 H-6), 4.58 (d, 1 H, *J* 7.8 Hz, H-1), 4.60–4.78 (m, 4 H, H-2^{II}, H-4^{II}, 2 H-6), 4.91–4.94 (m, 2 H, *J* 3.6, 7.8 Hz, H-1^{I}, H-2^{I}), 5.31 (d, 1 H, *J* 8.0 Hz, H-1), 5.33 (dd, 1 H, *J* 8.0, 9.6 Hz, H-2), 5.50 (dd, 1 H, *J* 8.0, 9.8 Hz, H-2), 5.55–5.63 (m, 3 H, H-2, 2 H-4), 5.68 (t, 1 H, *J* 10.0 Hz, H-4), 5.75 (t, 1 H, *J* 9.6 Hz, H-3), 5.87 (t, 1 H, *J* 9.6 Hz, H-3), 5.93 (t, 1 H, *J* 10.0 Hz, H-3), 7.22-8.10 (m, 65 H, Ph); ^{13}C NMR: 19.71, 20.93, 54.62, 62.84, 62.97, 63.29, 66.81, 68.12, 69.41, 69.49, 69.74, 70.07, 71.39, 71.79, 71.80, 71.95, 71.96, 72.00, 72.52, 72.62, 72.67, 72.98, 75.01, 77.20, 78.30, 81.19, 96.61 (C-1^{I}), 99.93, 100.79, 101.01, 102.38 (C-1^{II-V}), 164.89, 164.96, 164.98, 165.05 (2C), 165.14, 165.17, 165.55, 165.65, 165.76, 165.94, 166.06, 166.12, 167.50, 169.27. Anal. Calcd for $C_{126}H_{110}O_{41}$: C, 66.37; H, 4.86. Found: C, 66.11; H, 4.92.

Methyl β-D-*glucopyranosyl-(1→3)-[*β-D-*glucopyranosyl-(1→6)]-*β-D-*glucopyranosyl-(1→3)-[*β-D-*glucopyranosyl-(1→6)]-*α-D-*glucopyranoside* (**11**).—To a solution of **10** (1.15 g, 0.504 mmol) in MeOH (50 mL) was added NaOMe (5 mL, 0.5 M in MeOH). The mixture was stirred at rt overnight, neutralized with ion exchange resin [Amberlite IR120 (H^+)] and filtered, and the filtrate was then concentrated. The residue was purified on an LH-20 column to give **11** (336 mg, 79%) as an amorphous solid: $[\alpha]_D$ +19° (*c* 1, H_2O); ^{13}C NMR (100 MHz, D_2O): 55.95 (C-6), 61.43 (2 C-6), 68.57 (C-6^{I}), 68.75 (C-6^{II}), 70.32 (3 C-4), 71.16 (C-5), 75.14 (C-5), 76.28 (2 C-5), 76.35 (C-5), 76.57 (C-3^{IV}, C-3^{V}), 76.70 (C-3^{III}), 83.62 (C-3^{I}), 84.69 (C-3^{II}), 99.94 (C-1^{I}), 103.48 (4 C-1). ESIMS Calcd for $C_{31}H_{54}O_{26}$: 842 [M]. Found 841.3 $[M-H]^+$.

Isopropyl 2,3,4,6-tetra-O-*benzoyl*-β-D-*glucopyranosyl-(1→3)-[2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl-(1→6)]-2,4-di*-O-*acetyl*-β-D-*glucopyranosyl-(1→3)-2*-O-*benzoyl-4,6*-O-*benzylidene-1-thio*-β-D-*glucopyranoside* (**15**).—Compounds **2** (150 mg, 0.096 mmol) and **13** (31 mg, 0.095 mmol) were coupled as described in the preparation of **5** (Method B) to afford **14**, which was further acetylated with acetic anhydride in pyridine to give **15** as a syrup (143 mg, 85% for two steps): $[\alpha]_D$ −18° (*c* 0.3, $CHCl_3$); ^{1}H NMR: 1.32, 1.38 (2 d, 6 H, *J* 6.6 Hz, CH_3), 1.90, 1.95, 1.96 (3 s, 9 H, 3 CH_3CO), 2.97 (dt, 1 H, *J* 9.8, 2.5 Hz, H-5^{I}), 3.24–3.31 (m, 1 H, SCH), 3.43 (br t, 1 H, H-5^{II}), 3.49, 3.57 (2 dd, 2 H, *J* 2.5, 9.8 Hz, H-6^{I}), 3.68 (t, 1 H, *J* 9.8, H-4^{I}), 3.73 (br d, 1 H, *J* 12.4, <1 Hz, H-6), 3.82 (d, 1 H, *J* 9.6 Hz, H-1^{I}), 3.85 (dd, 1 H, *J* 3.8, 12.9 Hz, H-6), 3.89 (t, 1 H, *J* 10.4 Hz, H-3^{II}), 4.08–4.24 (m, 3 H, H-5^{III}, 3^{I}, 5^{IV}), 4.32 (d, 1 H, *J* 8.0, H-1^{II}), 4.45–4.61 (m, 3 H, H-6), 4.81 (d, 1 H, *J* 7.9 Hz, H-1^{IV}), 4.88 (dd, 1 H, *J* 9.6, 10.4 Hz, H-2^{I}), 4.98 (dd, 1 H, *J* 8.0, 9.8 Hz, H-2^{II}), 5.02 (d, 1 H, *J* 8.0 Hz, H-1^{III}), 5.08 (d, 1 H, *J* 10.4, H-6), 5.30–5.35 (m, 2 H, H-4^{II}, 2^{III}), 5.41 (dd, 1 H, *J* 8.0, 9.7 Hz, H-2^{IV}), 5.56–5.73 (m, 4 H, H-4^{III}, 4^{IV}, 3^{III}, PhCH), 5.87 (t, 1 H, *J* 9.7 Hz, H-3^{IV}), 7.20–8.24 (m, 45 H, Ph). MALDI-TOF-MS Calcd for $C_{96}H_{90}O_{31}S$: 1770.5 [M]. Found 1793.3 $[M+Na]^+$.

4,6-O-*Benzylidene*-β-D-*glucopyranosyl-(1→3)-1,2:5,6-di*-O-*isopropylidene*-α-D-*glucopyranose* (**19**).—Compounds **16** (768 mg, 1.2 mmol) and **17** (260 mg, 1 mmol) were reacted as described in the synthesis of **7** to give **18**, along with some contaminants. The above residue was treated with NaOMe (2 mL, 0.5 M in MeOH) in MeOH (20 mL) for 4 h and neutralized with Dowex-50W (H^+) resin. After filtration, the filtrate was concentrated and subjected to column chromatography (1:1 EtOAc–petroleum ether) to give **19** as a syrup (286 mg, 56% for two steps): $[\alpha]_D$ −22° (*c* 1, $CHCl_3$); ^{1}H NMR: 1.33, 1.38, 1.46, 1.51 (4 s, 12 H, 2 $C(CH_3)_2$), 3.51 (ddd, 1 H, *J* 9.3, 4.7, 6.1 Hz, H-5^{II}), 3.56 (t, 1 H, *J* 9.3 Hz, H-4^{II}), 3.67 (dd, 1 H, *J* 9.3, 8.2 Hz, H-2^{II}), 3.82 (dd, 1 H, *J* 10.1, 6.1 Hz, H-6a^{II}), 3.84 (dd, 1 H, *J* 10.1, 4.7 Hz, H-6b^{II}), 4.07 (dd, 1 H, *J* 4.4, 9.0 Hz, H-4^{I}), 4.11–4.18 (m, 2 H, H-3^{II}, H-6a^{I}), 4.34–4.40 (m, 3 H, H-3^{I}, H-5^{I}, H-6b^{I}), 4.64 (d, 1 H, *J* 3.6 Hz, H-2^{I}), 4.67 (d, 1 H, *J* 8.2 Hz, H-1^{II}), 5.54 (s, 1 H, PhCH), 5.90 (d, 1 H, *J* 3.6 Hz, H-1^{I}), 7.36–7.51 (m, 5 H, Ph). Anal.

Calcd for $C_{25}H_{34}O_{11}$: C, 58.81; H, 6.71. Found: C, 58.59; H, 6.80.

2,3,4,6-Tetra-O-*benzoyl*-β-D-*glucopyranosyl*-(1→3)-[*2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl*-(1→6)]-*2,4-di*-O-*acetyl*-β-D-*glucopyranosyl*-(1→3)-*2*-O-*acetyl*-*4,6*-O-*benzylidene*-β-D-*glucopyranosyl*-(1→3)-*1,2*-O-*isopropylidene*-α-D-*glucofuranose* (**21**).—Compounds **2** (595 mg, 0.38 mmol) and **19** (180 mg, 0.35 mmol) were glycosylated under the conditions described in the preparation of **14** to give the crude product (**20**, 570 mg), which was acetylated with Ac_2O (1 mL) in pyridine (5 mL) at 40 °C for 2 h. The mixture was concentrated to dryness with the help of toluene. To the above residue (560 mg, 0.286 mmol) in CH_3CN (10 mL) was added $CeCl_3{\cdot}7H_2O$ (115 mg, 0.5 mmol) and two drops of water. The mixture was refluxed for 6 h, at the end of which time TLC showed the complete consumption of the starting material. Concentration and purification of the residue by silica gel column chromatography (2:1 EtOAc–petroleum ether) gave compound **21** (428 mg, 78%): $[\alpha]_D$ −30° (*c* 1, $CHCl_3$); ^{1}H NMR: 1.24, 1.30 (2 s, 6 H, 2 CH_3), 1.90, 1.94, 2.00 (3 s, 9 H, 3 CH_3CO), 3.04–3.08 (m, 1 H, H-5^{IV}), 3.22 (br s, 2 H, OH), 3.46–3.49 (m, 1 H, H-5^{III}), 3.63–3.76 (m, 4 H, H-6a^{II}, H-6b^{II}, H-6a^{IV}, H-6a^{I}), 3.77–3.87 (m, 5 H, H-3^{II}, H-3^{III}, H-6a^{III}, H-6b^{III}, H-6b^{I}), 3.90–3.93 (m, 1 H, H-5^{I}), 4.02–4.15 (m, 3 H, H-4^{III}, H-6b^{IV}, H-5^{V}), 4.20 (dd, *J* 3.3, 7.8 Hz, H-4^{I}), 4.34 (d, 1 H, *J* 7.9 Hz, H-1^{II}), 4.39 (d, 1 H, *J* 4.5 Hz, H-2^{I}), 4.43 (d, 1 H, *J* 3.3 Hz, H-3^{I}), 4.44–4.47 (m, 2 H, H-5^{II}, H-6a^{V}), 4.60 (dd, 1 H, *J* 2.7, 12.0 Hz, H-6b^{V}), 4.83 (d, 1 H, *J* 8.1 Hz, H-1^{V}), 4.87 (t, 1 H, *J* 9.9 Hz, H-4^{III}), 4.92–4.97 (m, 3 H, H-1^{III}, H-2^{II}, H-2^{III}), 5.21 (d, 1 H, *J* 8.1 Hz, H-1^{IV}), 5.28 (dd, 1 H, *J* 8.1, 9.3 Hz, H-2^{IV}), 5.41 (dd, 1 H, *J* 8.1, 9.2 Hz, H-2^{V}), 5.58–5.69 (m, 4 H, *J* 9.6, 9.3 Hz, H-4^{IV}, PhCH, H-4^{V}, H-3^{IV}), 5.85–5.88 (m, 2 H, H-1^{I}, H-3^{V}), 7.23–8.06 (m, 45 H, Ph). Anal. Calcd for $C_{102}H_{98}O_{37}$: C, 63.95; H, 5.16. Found: C, 64.22; H, 5.17.

2,3,4,6-Tetra-O-*benzoyl*-β-D-*glucopyranosyl*-(1→3)-[*2,3,4,6-tetra*-O-*benzoyl*-β-D-*glucopyranosyl*-(1→6)]-*2,4-di*-O-*acetyl*-β-D-*glucopyranosyl*-(1→3)-*2*-O-*acetyl*-*4,6*-O-*benzylidene*-β-D-*glucopyranosyl*-(1→3)-[*2,3,4,6-tetra*-O-*benzoyl*-β-D-*gluco pyranosyl*-(1→6)]-*1,2*-O-*isopropylidene*-α-D-*glucofuranose* (**22**).—Compounds **21** (360 mg, 0.188 mmol) and **9** (148 mg, 0.2 mmol) were coupled as described in the preparation of **5** (Method B) to give **22** (430 mg, 87%) as a white solid: $[\alpha]_D$ −12° (*c* 1, $CHCl_3$); ^{1}H NMR: 1.19, 1.33 (2 s, 6 H, $C(CH_3)_2$), 1.89, 1.93, 1.98 (3 s, 9 H, CH_3CO), 3.01 (br s, 1 H, OH), 3.43–3.49 (m, 1 H, H-5^{IV}), 3.52 (t, 1 H, *J* 9.8 Hz, H-4^{II}), 3.60–3.65 (m, 2 H, H-6a^{I},6a^{IV}), 3.72 (t, 1 H, *J* 9.6 Hz, H-3^{III}), 3.76–3.85 (m, 4 H, H-5^{II}, H-6b^{I}, H-6a^{III}, H-5^{V}), 3.90–4.05 (m, 3 H, H-6b^{III}, H-6b^{IV}, H-3^{II}), 4.10–4.20 (m, 4 H, H-5^{I}, H-6a^{II}, H-4^{I}, H-5^{VI}), 4.33 (d, 1 H, *J* 8.0 Hz, H-1^{III}), 4.35 (d, 1 H, *J* 3.3 Hz, H-2^{I}), 4.38 (d, 1 H, *J* 3.3 Hz, H-3^{I}), 4.39 (dd, 1 H, *J* 5.6, 10.9 Hz, H-6b^{II}), 4.48 (dd, 1 H, *J* 4.3, 11.7 Hz, H-6a^{V}), 4.50 (dd, 1 H, *J* 5.1, 12.0 Hz, H-6a^{VI}), 4.60 (dd, 1 H, *J* 2.6, 12.2 Hz, H-6b^{V}), 4.67 (dd, 1 H, *J* 3.9, 11.7 Hz, H-6b^{VI}), 4.84 (d, 1 H, *J* 7.5 Hz, H-1^{IV}), 4.85–4.90 (m, 3 H, *J* 8.8 Hz, H-1^{II}, H-2^{II}, H-4^{III}), 4.95 (t, 1 H, *J* 9.5 Hz, H-2^{III}), 5.02 (d, 1 H, *J* 7.9 Hz, H-1^{VI}), 5.21 (d, 1 H, *J* 8.0 Hz, H-1^{V}), 5.30 (t, 1 H, H-2^{V}), 5.42 (dd, 1 H, *J* 7.5, 9.6 Hz, H-2^{IV}), 5.52 (s, 1 H, PhCH), 5.55–5.72 (m, 5 H, H-2^{VI}, H-4^{IV}, H-3^{V}, H-4^{V}, H-4^{VI}), 5.79 (d, 1 H, *J* 3.3 Hz, H-1^{I}), 5.89 (t, 1 H, *J* 9.6 Hz, H-3^{IV}), 5.91 (t, 1 H, *J* 9.2 Hz, H-3^{VI}), 7.25–8.00 (m, 65 H, Ph). Selected ^{13}C NMR: 99.71 (C-1^{II}), 100.11 (C-1^{III}), 100.31 (C-1^{V}), 101.29 (C-1^{IV}), 101.54 (C-1^{VI}), 105.16(C-1^{I}). Anal. Calcd for $C_{136}H_{124}O_{46}$: C, 65.48; H, 5.01. Found: C, 65.80; H, 5.07.

β-D-Glucopyranosyl-(1→3)-[β-D-*glucopyranosyl*-(1→6)]-β-D-*glucopyranosyl*-(1→3)-β-D-*glucopyranosyl*-(1→3)-[β-D-*glucopyranosyl*-(1→6)]-D-*glucopyranose* (**23**).—Compounds **22** (230 mg, 0.092 mmol) was treated with 90% trifluoroacetic acid for 30 min, concentrated and then co-concentrated with toluene, dissolved in methanol (40 mL), deacylated with sodium methoxide (0.5 M, 1.2 mL), neutralized with Amberlite IR-120 (H^+), filtered and concentrated. The residue was purified on a Sephadex LH-20 column (EtOAc, then MeOH) to yield **23** (48 mg, 53%): $[\alpha]_D$ +11° (*c* 1, H_2O). Selected ^{1}H NMR (D_2O): 5.21 (d, 0.7 H, *J* 3.2 Hz, H-1^{I} of α isomer), 4.61–4.70 (m, 5.3 H, H-1^{I} of β isomer and H-1^{II,III,IV,V,VI}). Selected ^{13}C NMR (100 MHz, D_2O): 96.35, 103.22, 103.38 (C-1$^{I\text{-}VI}$). ESIMS Calcd for $C_{36}H_{62}O_{31}$: 990 [M]. Found 989.2 $[M-H]^+$.

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