Highly Efficient Syntheses of the Phytoalexin-Elicitor Active β-(1→3)-
Branched β-(1→6)-Linked Heptaglucose and Its Dodecyl Glycoside

Yuetao Yi, a Zhongxuan Zhou, a Jun Ning, a Fanzuo Kong, a Jianqiang Li b

a Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, P. O. Box 2871, Beijing 100085, China
b Department of Plant Pathology, China Agricultural University, Beijing 100094, China
Fax +86(10)62923563; E-mail: jning@mail.reees.ac.cn
Received 31 October 2002; revised 17 December 2002

Abstract: A highly efficient method for the synthesis of 3,6-branched gluco-oligosaccharides was developed by using 1,2:5,6-di-O-isopropylidene-α-D-glucopyranose, 2,3,4,6-tetra-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate, and 6-O-acetyl-2,3,4-tri-O-benzoyl-α-D-glucopyranosyl trichloroacetimidate through a regio- and stereoselective manner. The β-(1→3)-branched β-(1→6)-linked heptaglucose 1 and its dodecyl glycoside 2 having phytoalexin-elicitor activity were prepared using the developed strategy. Bioassays showed that the phytoalexin-elicitor activity of the do-
decyl glycoside 2 is slightly more than that of the corresponding end free heptaglucose 1.

Key words: oligosaccharides, phytoalexin-elicitor, synthesis, gly-
cosides, glycosylations

A central problem in carbohydrate chemistry is how to prepare oligosaccharides efficiently and simply. In the last decades, much effort has been paid to the oligosaccharide synthesis. However, up to date, there are no general applicable methods or strategies for oligosaccharide synthesis, and consequently the preparation of oligosaccharides is very time consuming compared with the synthesis of other biopolymers such as peptides and nucleic acids. Generally speaking, production of a complex oligosaccharide on an industrial scale is very difficult if not impossible so far. We always ask the question as to which method is the most suitable in carbohydrate synthesis. Does a single powerful method or strategy in the synthesis of oligosaccharides really exist? Maybe, owing to this structural complexity, the preparation of oligosaccharides will never achieve the same levels as the preparation of peptides and nucleic acids, but we can create relatively general procedures which are peculiarly effective for certain types of oligosaccharides.

3,6-Branching gluco-oligosaccharides are a common structure characteristic of many biologically active polysaccharides such as the phytoalexin-elicitor β-glucan and antitumor polysaccharides from schizophyllan, sceroglucan, and lentinan. The β-(1→3)-branched β-(1→6)-linked glucose oligomers isolated from mycelial walls of the fungus Phytophthora megasperma f. sp. Glycinea can induce the formation of phytoalexins in soybean. The most active heptasaccharide 1 (Figure 1) is effective in very low doses, approximately 0.1 pmol per cotyledon. It should be noted that, although much of this work was done with soybean cotyledons, it was established that the glucan-elicitor also elicited the synthesis of different phytoalexins in a wide range of other plant species. These important discoveries stimulated the interest of scientists. Since their isolation and identification, the glucan-elicitors have been prepared by different groups, and various methods and strategies have been used including the very elegant solid-phase strategies. Recently, in a preliminary communication, we have disclosed a newly efficient method suitable for large scale synthesis of 3,6-branched β-linked gluco-oligosaccharides using 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose as the starting material, and the β-(1→3)-branched β-(1→6)-linked glucohexaose phytoalexin-elicitor on a 100 g scale was achieved in our laboratory.

Some research reports show that modification of oligosaccharides with hydrophobic long alkyls at the reducing terminal can increase their biological activity. The reason is that the newly formed compounds are composed both of a hydrophilic oligosaccharide portion and a hydrophobic long alkyl portion, and have the characteristic of a surface-active agent able to coagulate together. In search of higher phytoalexin-elicitor active oligosaccharides, a dodecyl β-(1→3)-branched β-(1→6)-linked heptaglucoside 2 was synthesized in our laboratory. In this paper, we present in detail the syntheses of the heptaglucose 1 and its dodecyl glycoside 2. Also the bioassay results of the compounds 1 and 2 as the phytoalexin-elicitors are given.

In our synthesis, 1,2:5,6-di-O-isopropylidene-α-D-glucofuranose (4), 3-O-benzoyl-1,2,6-isopropylidene-α-D-glucofuranose (5), 2,3,4,6-tetra-O-benzoyl-α-D-glucopy-
ranosyl trichloroacetimidate (6), 6-O-acetyl-2,3,4-tri-O-benzoyl-O-D-glucopyranosyl trichloroacetimidate (8) and dodecyl 2,3,4-tri-O-benzoyl-β-D-glucopyranoside (10) were the basic building materials. Compound 4 was obtained according to the standard method. Compound 5 was prepared from 4. Compound 6 was obtained as fine crystals from benzoylation of D-glucose, followed by 1-O-debenzoylation with ammonium in THF–MeOH and trichloroacetimidation (Scheme 1). Compound 8 was prepared as crystals by the benzoylation of 1,6-anhydro-β-D-glucopyranose (7, levoglucosan), a cheap material obtained from the pyrolysis of cellulose, followed by acetolysis, 1-O-deacetylation, and trichloroacetimidation. Coupling of 8 with dodecyl alcohol gave compound 9 which was selectively converted to 10 using MeOH containing 0.3% HCl. The solution of MeOH containing HCl was formed in situ by adding acetyl chloride to MeOH.

**Scheme 1** Preparation of building materials 4, 5, 6, 8, and 10

Coupling of 1,2,5,6-di-O-isopropylidene-α-D-glucofuranose (4) with perbenzoylglucosyl trichloroacetimidate 6 in the presence of TMSOTf as catalyst, followed by selective 5,6-O-deacetonation afforded β-(1→3)-linked disaccharide 11 as crystals in a high yield (76% for two steps) (Scheme 2). Condensation of 11 with 6 catalyzed by TM-SOTf gave regio- and stereoselectively the 3,6-branched trisaccharide 12 in 87% yield. Removal of the 1,2-O-isopropylidene group of 12 in 80% HOAc followed by acetylation with acetic anhydride in pyridine, selective 1-O-deacetylation with ammonia in THF–MeOH, and subsequent treatment with trichloroacetonitrile in the presence of K₂CO₃ afforded the desired trisaccharide glycosyl donor 13 in 71% yield (for four steps). Condensation of 11 with 8 afforded the 3,6-branched trisaccharide 14 in 85% yield. Selective 6-O-deacetylation of 14 in CH₂Cl₂–MeOH containing 0.3% HCl gave the trisaccharide acceptor 15 in 90% yield.

**Scheme 2** Preparation of the trisaccharide donor 13 and acceptor 15

Coupling of 13 with 15 with TMSOTf as the catalyst afforded regio- and stereoselectively the blocked hexasaccharide 16 in a high yield (84%) (Scheme 3). Using the same operations for the preparation of the trisaccharide glycosyl donor 13, the hexasaccharide glycosyl donor 18 was obtained from 17. Coupling of 17 with 5 and 10 afforded the blocked heptasaccharides 18 and 19, respectively (Scheme 4). Deiso-propylidenation of 18 in 80% HOAc, followed by deacetylation in an ammonia-saturated solution of 1:9 CH₂Cl₂–MeOH, furnished the free heptasaccharide 1 as an amorphous white solid in 90% yield. Deprotection of 19 gave 2.

In all of the synthesis, very easily accessible materials and cheap reagents were used and the reactions were carried out smoothly in high yields and in large scales. In the synthesis, several intermediates were not separated and used directly for the further reaction simplifying the operation substantially. The sole use of acyl groups in the synthesis further simplified the procedure.

The bioassay was carried out according to the method devised by Ayers et al. The phytoalexin-elicitor sugars can
stimulate glyceollin accumulation in cotyledons of soybean seedlings. The activities of the synthesized oligosaccharides were determined by their concentrations required to elicit half-maximal accumulation of glyceollin (C\textsubscript{50}). The test results showed that C\textsubscript{50} of the dodecyl \(\beta-(1\rightarrow6)\)-linked hexaglucoside 2 and its underivatized 1 are about 6 nM and 9 nM respectively, indicating that modification of the reducing-end of this oligosaccharide does not have a significant effect on its biological activity. Similar conclusion was reported by others.\textsuperscript{5h}

Optical rotations were determined at 20 °C with a Perkin-Elmer Model 241-Mc automatic polarimeter. Melting points were determined with a Mel-Temp apparatus. \(^1\)H NMR and \(^1\)C NMR spectra were recorded with Bruker ARX 400 spectrometers (400 MHz for \(^1\)H, 100 MHz for \(^1\)C) for solutions in CDCl\(_3\), or D\(_2\)O as indicated. Chemical shifts are given in ppm downfield from internal Me\(_4\)Si. TLC was performed on silica gel HF\(_254\) with detection by charring with 30% (v/v) H\(_2\)SO\(_4\) in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (3 \(\times\) 30 cm, 6.5 \(\times\) 50 cm, 9 \(\times\) 60 cm, 12 \(\times\) 80 cm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (bp 60–90 °C) as eluent. Solutions were concentrated at <60 °C under reduced pressure.

2,3,4,6-Tetra-O-benzyl-\(\alpha\)-D-glucopyranosyl Trichloroacetimidate (6) BzCl (680 mL, 5.83 mol) was added under vigorous stirring to a solution of D-glucose (3; 200 g, 1.11 mmol) in toluene (2500 mL) and pyridine (473 mL, 5.85 mol) over 1 h at 70 °C, and then the mixture was stirred at 70 °C for further 7 h. Filtration of pyridine hydrochloride salt and concentration of the filtrate gave a residue which was directly dissolved in a solution of NH\(_3\) (1.5 N) in 2:1 THF–MeOH (5600 mL). The solution was kept at r.t. for 12 h, at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in CH\(_2\)Cl\(_2\) (1000 mL), and then CC\(_1\)CN (120 mL, 1.2 mol) and KC\(_2\)O\(_3\) (300 g, 2.17 mol) were added. The reaction mixture was stirred for 24 h at r.t., at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was decolorized by passing through a short column (12 \(\times\) 80 cm) packed with silica gel (2500 mL) using petroleum ether–EtOAc (3:1) as eluent. The product 6 was further purified by crystallization from petroleum ether–EtOAc, 3:1; white crystals; yield: 461 g (56% for three steps).\textsuperscript{12}

6-O-Acetyl-2,3,4-tri-O-benzyl-\(\alpha\)-D-glucopyranosyl Trichloroacetimidate (8) BzCl (223 mL, 1.92 mol) was added under vigorous stirring to a solution of 7 (100 g, 0.62 mmol) in toluene (700 mL) and pyridine (158 mL, 1.95 mmol) over 1 h. The mixture was warmed to 70 °C, and then stirred at 70 °C for 7 h. Filtration of pyridine hydrochloride salt and concentration of the filtrate gave a residue which was directly dissolved in a mixture of CH\(_2\)Cl\(_2\) (230 mL), Ac\(_2\)O (230 mL, 2.44 mol), and AcOH (230 mL). Then H\(_2\)SO\(_4\) (23 mL) was added dropwise. This reaction mixture was kept for 20 h at r.t., then poured into ice water. After stirring for 15 min, the mixture was extracted with CH\(_2\)Cl\(_2\). The combined CH\(_2\)Cl\(_2\) extracts were washed with 10% aq NaHCO\(_3\), and then concentrated to a syrup. The resulting residue was added to a solution of NH\(_3\) (1.5 N) in THF–MeOH, 2:1 (2400 mL). The solution was kept at room temperature for 3 h, at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was concentrated under reduced pressure, and the residue was dissolved in CH\(_2\)Cl\(_2\) (500 mL), and then CC\(_1\)CN (86 mL, 0.85 mol) and K\(_2\)CO\(_3\) (200 g, 1.45 mol) were added. The reaction mixture was stirred for 24 h at r.t., at the end of which time TLC (petroleum ether–EtOAc, 3:1) indicated that the reaction was complete. The mixture was filtered, the solution was concentrated under reduced pressure, and the residue was purified on a column (9 \(\times\) 30 cm, 6.5 \(\times\) 50 cm, 9 \(\times\) 60 cm, 12 \(\times\) 80 cm) using petroleum ether–EtOAc (3:1) as eluent to give 8 as white crystals; yield: 226.3 g (54% for four steps); mp 81–83 °C; [\(\alpha\)]\textsubscript{D}\textsuperscript{+}9.0 (c = 1.3, CHCl\(_3\)).

\(^1\)H NMR (400 MHz, CDCl\(_3\)): 5 = 8.65 [s, 1 H, OC(NH)(CCl\(_3\))], 7.96–7.26 (m, 15 H, 3 PhH), 6.82 (d, 1 H, \(J\textsubscript{1,2} = 3.6\) Hz, H-1), 6.24 (dd, 1 H, \(J\textsubscript{1,3} = J\textsubscript{3,4} = 9.8\) Hz, H-3), 5.74 (dd, 1 H, \(J\textsubscript{2,3} = J\textsubscript{4,5} = 9.8\) Hz, H-4), 3.61–3.50 (m, 3 H, 3 OAc) ppm.

13 + 15

\[
\begin{align*}
\text{CH}_2\text{Cl}_2, \text{TMSOTf, r.t.} \\
\text{Scheme 3} \quad \text{Preparation of the hexasaccharide donor 17}
\end{align*}
\]

5 + 17

\[
\begin{align*}
\text{CH}_2\text{Cl}_2, \text{TMSOTf, r.t.} \\
\text{Scheme 4} \quad \text{Preparation of heptasaccharide 1 and its dodecyl glycoside 2}
\end{align*}
\]
Dodecyl 6-O-Acetyl-2,3,4-tri-O-benzyl-β-D-glucopyranoside (9)

To a stirred solution of 8 (4 g, 5.89 mmol) and dodecyl alcohol (1.9 g, 10.2 mmol) in anhyd CH₂Cl₂ (80 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 40 µL) at r.t. After 3 h, Et₃N was added to the solution to quench the reaction. The solution was concentrated and the residue was subjected to column chromatography on silica gel (3 × 30 cm, 150 mL) using petroleum ether–EtOAc (3:1) as eluent to give the trisaccharide 9; 3.8 g (92%); [α]D +34.2 (c = 1.0, CHCl₃).

1H NMR (400 MHz, CDCl₃): δ = 7.88–7.24 (m, 15 H, 3 PhH), 5.91 (dd, 1 H, J₁₂ = 9.7 Hz, H-3'), 5.63 (dd, 1 H, J₁₁ = J₁₂ = 9.7 Hz, H-3), 5.50 (dd, 1 H, J₁₁ = J₁₂ = 9.7 Hz, H-2'), 4.82 (dd, 1 H, J₁₁ = J₁₂ = 7.9 Hz, H-1'), 4.69 (dd, 1 H, J₁₁ = J₁₂ = 7.9 Hz, H-1), 4.36 (dd, 1 H, J₁₁ = J₁₂ = 12.2 Hz, H-6), 4.29 (dd, 1 H, J₁₁ = J₁₂ = 3.5, J₆₆ = 12.2 Hz, H-6'), 4.06 (m, 1 H, H-5), 3.95–3.55 (m, 2 H, CH₂C₆H₅), 2.03 (s, 3 H, COCH₃), 1.54–0.86 (m, 23 H, CH₃C₆H₅).

Anal. Calc'd for C₃₉H₄₈O₉: C, 70.89; H, 7.32. Found: C, 70.51; H, 3.83.

3.6-Di-O-(2,3,4,6-tetra-O-benzoyl-β-D-glucopyranosyl)-1,2-O-isopropylidene-α-D-glucopyranosyl (12); Typical Procedure

To a stirred solution of 11 (80 g, 0.1 mol) and 6 (80 g, 0.108 mol) in anhyd CH₂Cl₂ (400 mL) was added TMSOTf (200 µL, 1.0 mmol) at r.t. After 3 h, Et₃N was added to the solution to quench the reaction. The solution was concentrated and the residue was subjected to column chromatography on silica gel (9 × 60 cm, 3000 mL) using petroleum ether–EtOAc (1:5) as eluent to give the trisaccharide 12 as a white amorphous solid; yield: 119.8 g (87%); [α]D +25.3 (c = 1.0, CHCl₃).

1H NMR (400 MHz, CDCl₃): δ = 8.06–7.28 (m, 40 H, 8 PhH), 5.88 (dd, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-3'), 5.87 (dd, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-3), 5.69 (dd, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-4'), 5.64 (dd, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-4), 5.53 (dd, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-2'), 5.43 (dd, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-2), 5.41 (dd, 1 H, J₁₂ = J₁₃ = 3.5 Hz, H-1'), 4.96 (d, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-1), 4.93 (d, 1 H, J₁₂ = J₁₃ = 9.7 Hz, H-1'), 4.68 (dd, 1 H, J₁₂ = J₁₃ = 3.4, J₆₆ = 12.2 Hz, H-6), 4.48 (dd, 1 H, J₁₂ = J₁₃ = 4.9, J₆₆ = 12.2 Hz, H-6'), 4.67 (dd, 1 H, J₁₂ = J₁₃ = 3.4, J₆₆ = 12.2 Hz, H-6'), 4.35 (dd, 1 H, J₁₂ = J₁₃ = 4.9, J₆₆ = 12.2 Hz, H-6'), 4.34–3.65 (m, 8 H, 10 ΦH), 1.26, 1.03 [2 s, 6 H, (CH₃)₂O].


Dodecyl 2,3,4-Tri-O-benzyl-β-D-glucopyranoside (10)

Acetyl chloride (0.6 mL) was added to a solution of Dodecyl 2,3,4-Tri-O-benzyl-β-D-glucopyranoside (9) in anhyd CH₂Cl₂ (80 mL) was added trimethylsilyl trifluoromethanesulfonate (TMSOTf, 40 µL) at r.t. After 3 h, Et₃N was added to the solution to quench the reaction. The solution was concentrated, and the residue was added to a solution of ammonia (1.5 N) in THF–MeOH, 3:1 (500 mL) and the solution was stirred at r.t. for 3 h. The solution was concentrated and the residue was dissolved in CH₂Cl₂ (200 mL). To the solution were added K₂CO₃ (10 g, 0.072) and CCl₃CN (8 mL, 0.072 mol), and the mixture was stirred at r.t. for 12 h. The mixture was filtered and the solvents were washed with CH₂Cl₂. The filtrate and the washings were concentrated, and the residue was subjected to column chromatography on silica gel (6.5 × 50 cm, 1300 mL) using petroleum ether–EtOAc (2:1) as eluent to give the trisaccharide donor 13 as a white amorphous solid; yield: 40.4 g (71% for four steps); [α]D +23.3 (c = 1.0, CHCl₃).

1H NMR (400 MHz, CDCl₃): δ = 8.33 (s, 1 H, O(=NH)CCl), 8.07–7.19 (m, 40 H, 8 PhH), 6.19 (d, 1 H, J₁₁ = J₁₂ = 3.6 Hz, H-1'), 5.91 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-3'), 5.58 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-3), 5.62 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-4'), 5.61 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-4), 5.46 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-2'), 5.42 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-2), 4.97 (d, 1 H, J₁₁ = J₁₂ = 7.9 Hz, H-1'), 4.96 (d, 1 H, J₁₁ = J₁₂ = 7.9 Hz, H-1'), 4.85 (dd, 1 H, J₁₁ = J₁₂ = 9.5 Hz, H-4), 4.67–5.49 (m, 3 H, 3 ΦH), 4.19–4.02 (m, 4 H, 3.91 (dd, 1 H, J₆₆ = 1.9 Hz, 1.9 Hz), 1.94, 1.78 [2 s, 6 H, 2 CH₂CO].


6-O-Acetyl-2,3,4-tri-O-benzyl-β-D-glucopyranosyl-(1→6)-[2,3,4-Tetra-O-benzyl-β-D-glucopyranosyl-(1→3)]-1,2-O-isopropylidene-α-D-glucopyranosyl (14)

Using the same procedure as described for the preparation of 12 from 6 and 11, the trisaccharide 14 was prepared from 8 (73.3 g, 0.108 mol) and 11 (80 g, 0.1 mol); white amorphous solid; yield: 118.9 g (85%); [α]D +18.6 (c = 1.1, CHCl₃).

1H NMR (400 MHz, CDCl₃): δ = 8.05–7.26 (m, 35 H, 7 PhH), 5.87 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-3'), 5.84 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-3), 5.65 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-4'), 5.59 (dd, 1 H, J₁₁ = J₁₂ = 9.6 Hz, H-4'), 5.51 (dd, 1 H, J₁₁ = J₁₂ = 7.9 Hz, J₁₃ = 9.6 Hz, H-
2H), 5.43 (dd, 1 H, \( J_{1,2} = 7.9 \), \( J_{1,3} = 9.6 \) Hz, H-2\( ^{\alpha} \)), 5.42 (dd, 1 H, \( J_{1,2} = 3.6 \), H-2\( ^{\alpha} \)), 4.96 (dd, 1 H, \( J_{1,2} = 9.6 \) Hz, H-3\( ^{\alpha} \)), 4.93 (dd, 1 H, \( J_{1,2} = 9.6 \) Hz, H-1\( ^{\alpha} \)), 4.71–3.79 (m, 12 H), 2.05 (s, 3 H, CH(C)=O), 1.33, 1.05 (2 s, 6 H, C\( (CH_{3})_{2} \)).

Anal. Calcd for C\(_{151}\)H\(_{130}\)Cl\(_{3}\)NO\(_{50}\): C, 63.30; H, 4.57. Found: C, 63.29; H, 4.53.

**2,3,4-Tri-O-benzyl-β-D-glucopyranosyl(1→6)-[2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl-(1→3)]-I,2-O-isopropylidene-α-D-glucofuranose (15)**

AcCl (6 mL) was added to a solution of 14 (100 g, 0.076 mol) in MeOH–CH\(_{2}\)Cl\(_{2}\) (1:1, 1000 mL), and the mixture was kept under r.t. for 20 h. After neutralization and concentration, the residue was subjected to column chromatography on silical gel (9 x 60 cm, 3000 mL) using petroleum ether–EtOAc (1:5:1) as eluent to give compound 15 as a white amorphous solid; yield: 87.1 g (90%); \( [\alpha]_{D}^{25} +34.3 \) (c = 1.0, CHCl\(_{3}\)).

**1H NMR (400 MHz, CDCl\(_{3}\)):** δ = 8.05–7.26 (m, 35 H, 7 PhH), 5.91 (dd, 1 H, \( J_{1,2} = J_{1,3} = 9.8 \) Hz, H-3\( ^{\alpha} \)), 5.90 (dd, 1 H, \( J_{1,2} = J_{1,3} = 9.8 \) Hz, H-3\( ^{\beta} \)), 5.73 (dd, 1 H, \( J_{1,2} = J_{1,3} = 9.8 \) Hz, H-4\( ^{\alpha} \)), 5.56 (dd, 1 H, \( J_{1,2} = J_{1,3} = 9.8 \) Hz, H-4\( ^{\beta} \)), 5.54–5.42 (m, 3 H), 4.99 (d, 1 H, \( J_{1,2} = 7.9 \) Hz, H-1\( ^{\alpha} \)), 4.95 (d, 1 H, \( J_{1,2} = 7.9 \) Hz, H-1\( ^{\beta} \)), 4.75–3.77 (m, 12 H), 1.33, 1.05 (2 s, 6 H, C\( (CH_{3})_{2} \)).


**β-D-Glucopyranosyl(1→6)-[β-D-glucofuranose (16)]**

The heptasaccharide 16 was prepared by coupling 13 (50 g, 0.032 mol) with 3-O-benzyl-1,2-di-O-isopropylidene-α-D-glucopyranose (0.69 g, 3.09 mmol) under the same conditions as described for the preparation of 12 from 6 and 11; yield: 4.6 g (86%); \( [\alpha]_{D}^{25} +4.3 \) (c = 1.0, CHCl\(_{3}\)).

**13C NMR (100 MHz, CDCl\(_{3}\)):** δ = 169.58, 169.29 168.23, 168.15 (4 C\( (O) \)), 165.62, 165.55, 165.50, 165.28, 165.25, 165.10, 165.07, 165.04, 165.01, 164.93 (16 PhC\( O \)), 112.05 (C\( (CH_{3})_{2} \)), 105.04, 101.23, 101.05, 100.96, 100.95, 100.58, 100.38 (7 C\( (CH_{3})_{2} \)), 83.38, 82.20 (2 C\( 3 \)), 26.57, 26.11 (C\( (CH_{3})_{2} \)), 20.78, 20.60, 20.50, 20.42 (4 C\( (CH_{3})_{2} \)).

Anal. Calcd for C\(_{188}\)H\(_{176}\)O\(_{58}\): C, 67.14; H, 5.27. Found: C, 67.36; H, 5.35.

**ESMS for C\(_{42}\)H\(_{72}\)O\(_{36}\) (1153.01):** 1152.00 [M – 1]+.

Compounds were prepared and purified by column chromatography on silica gel in various solvents.

**Synthesis 2003, No. 4, 491–496 ISSN 0039-7881 © Thieme Stuttgart · New York**
13C NMR (100 MHz, D2O): $\delta = 105.67, 105.64, 105.45, 105.41, 105.31, 105.21, 104.94$ (7 C-1), $87.17, 87.03$ (2 C-3), $34.20, 32.72, 32.34, 31.96, 31.68, 28.05, 24.94, 16.49$.

ESMS for C54H96O36 (1321.33): $1320.2 [M - 1]^+$. 

Acknowledgment

This work was supported by the Beijing Natural Science Foundation (6021004) and National Natural Science Foundation of China (30070864), and by The Ministry of Science and Technology (2001AA246014).

References


