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Carbohydrate Research 340 (2005) 2345–2351
Carbohydrate RESEARCH

Synthesis of β-D-Glc*p*-(1→3)-[β-D-Glc*p*-(1→6)]-β-D-Glc*p*-(1→3)-β-D-Glc*p*-(1→6)-[β-D-Gal*p*-(1→4)-β-D-Glc*p*-(1→3)]-β-D-Glc*p*OLauryl, an oligosaccharide with anti-tumor activity

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Received 1 August 2005; received in revised form 21 August 2005; accepted 24 August 2005

Abstract—A concise and effective synthesis of lauryl heptasaccharide **17** was achieved from the key intermediates lauryl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-glucopyranoside (**10**) and isopropyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-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)-2,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (**15**). The key trisaccharide glycosyl acceptor **10** was constructed by coupling 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**3**) with lauryl 6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranoside (**9**), followed by deacetylation. The thioglycoside donor **15** was obtained by condensation of 2,4,6-tri-*O*-acetyl-3-*O*-allyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**11**) with isopropyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**12**), followed by debenzylidenation and acetylation. A bioassay of the inhibition of S_{180} noumenal tumors showed that lauryl heptasaccharide **17** could be employed as a potential agent for cancer treatment.

Keywords: Oligosaccharide; Glycosylation; Anti-tumor

1. Introduction

Evidence has accumulated proving that simple sugars can be employed to inhibit the aggregation of certain types of tumor cells.[1,2] For example, Raz and Lotan[3,4] found that the lectin-like activity of several tumor cell lines, including five human melanomas (A375, SH4, Hs294, Hs852, and Hs939), human cervical adenocarcinoma (HeLa-S3), murine melanoma (B16-F1), and murine fibrosarcoma (UV-2237P) could be inhibited by lactose (**1**, Fig. 1). Accordingly, it is reasonable to believe that the structure of lactose (4-*O*-β-D-galactopyranosyl-D-glucopyranose) may be useful for finding new drugs to inhibit the lectin-like ability of tumor cells that play an important role in tumor growth at the primary site, invasion into surrounding host tissue,

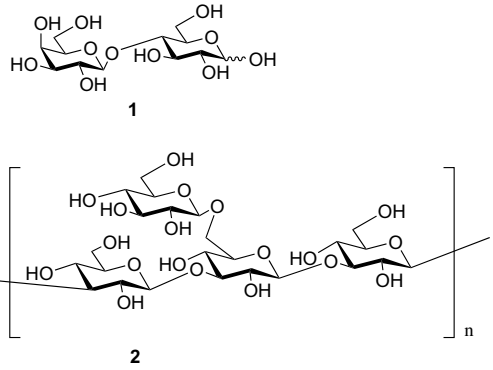

Figure 1. Structures of lactose **1** and schizophyllan **2**.

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doi:10.1016/j.carres.2005.08.010

dissemination, embolization, and implantation at distant secondary sites to form metastases.

Schizophyllan (**2**), produced by *Schizophyllum commune* Fries, consists of a main chain of (1→3)-β-linked D-glucose residues with one (1→6)-β-linked D-glucopyranosyl group for every three glucopyranose residues (Fig. 1).[5,6] Investigations on the application of schizophyllan as an anti-tumor or anti-infective agent showed that schizophyllan appears to have a curative effect against Sarcoma-180 ascites, lung cancer, stomach cancer, and ovarian cancer.[7–14]

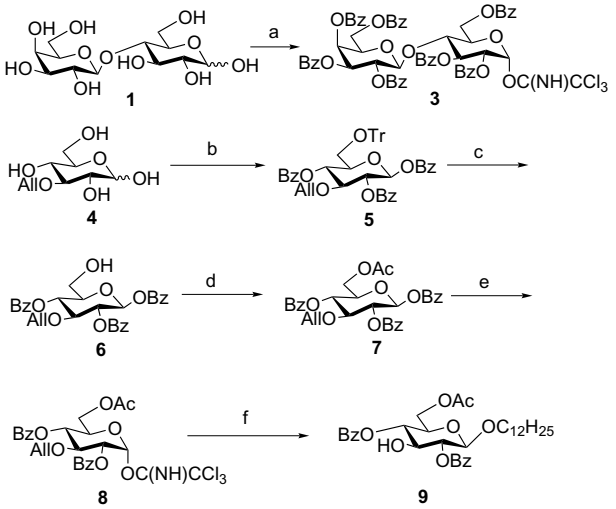
Although numerous carbohydrate structures occur in nature, in general, the role of oligosaccharide structures has not been widely studied. This can be attributed mainly to difficulties in synthesizing oligosaccharides. Unlike proteins and nucleic acids, oligosaccharides are more difficult to synthesize because (i) the molecules are typically branched rather than linear, (ii) the monosaccharide units can be connected by α or β linkages, and (iii) oligosaccharide synthesis requires multiple selective protection and deprotection steps. Although over the past few decades, considerable progress has been made in this field,[15] there is still no general route for oligosaccharide synthesis and glycosylation chemistry is often not predictable. To facilitate the synthesis of target oligosaccharides, regio- and stereoselective glycosylation of glycosyl donors with unprotected or partially protected sugar acceptors has been extensively studied. Employing this strategy, in the last few years, we have prepared many oligosaccharides with various structures such as 3,6-branched gluco-oligosaccharides,[16] 2,6-branched manno-oligosaccharides,[17] as well as 2,6-branched,[18] 3,6-branched, and 5,6-branched galacto-oligosaccharides.[19]

Several approaches have been taken with success for the chemical synthesis of oligosaccharides.[2] Most involve the activation of the anomeric leaving group with a Lewis acid and then displacement of that leaving group by the free hydroxyl of the acceptor sugar. The Koenigs–Knorr method for coupling glycosyl halides, one of the first techniques to gain widespread usage, is still in common use. Trichloroacetimidates,[20] prepared by the reaction of reducing sugars with trichloroacetonitrile and a base are used frequently for coupling, as are glycosyl sulfoxides,[21] phosphates[22] and phosphates,[23] and thio-[24] and pentenyl glycosides.[25]

Providing sufficient quantities of a sample is a basic prerequisite for detailed studies on a compound's fundamental biochemical properties and possible biological functions. To further study the structure–activity relationships of lactose and schizophyllan, we report here the synthesis of compound **17**, which contains a glucotetraose moiety, the key fraction of schizophyllan, and lactose. We anticipated that these two structural motifs would be able to amplify anti-tumor activity.

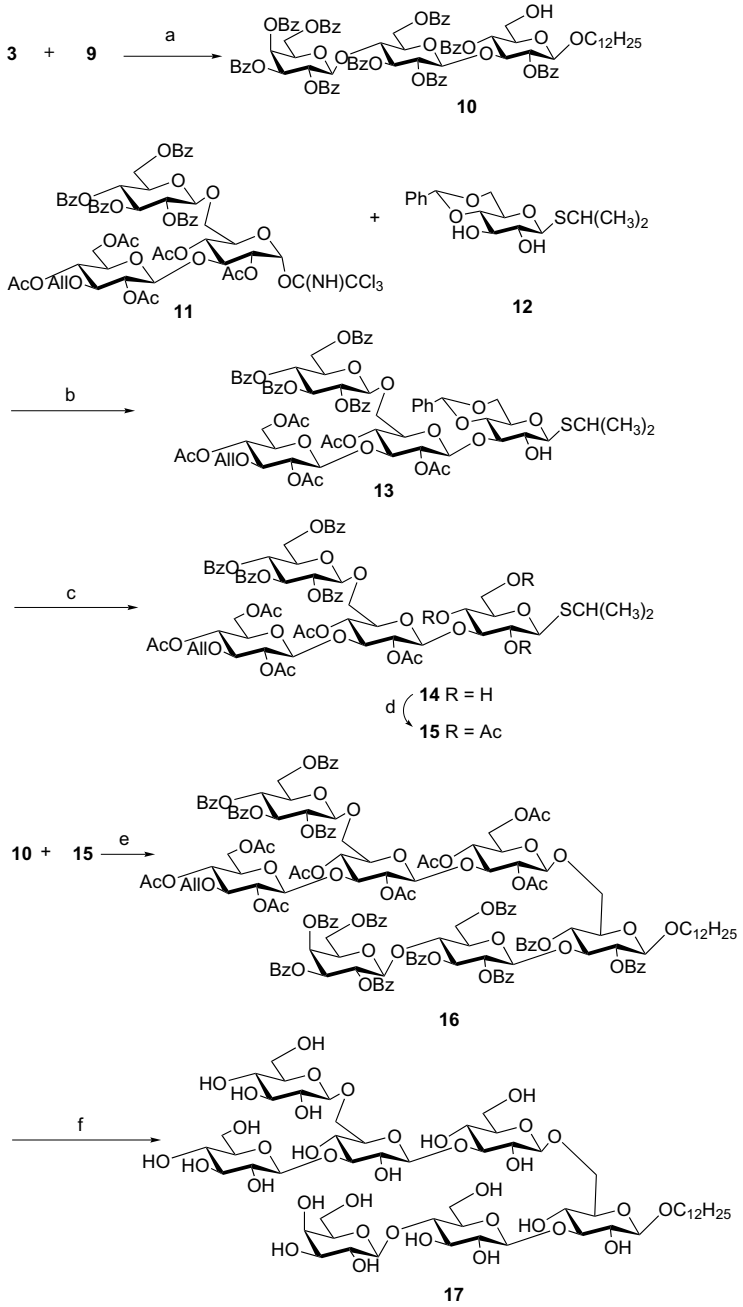
2. Results and discussion

In our synthesis, 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**3**) and lauryl 6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranoside (**9**) were the starting materials; trisaccharide **10** and the tetrasaccharide **15** were the key intermediates. Compound **3** was prepared as fine crystals via benzoylation of D-lactose (**1**) followed by 1-*O*-debenzoylation with ammonia in tetrahydrofuran–methanol and subsequent treatment with trichloroacetonitrile in the presence of potassium carbonate[26] (Scheme 1). Tritylation of 3-*O*-allyl-D-glucopyranose (**4**)[27] followed by benzoylation in a one-pot manner gave 3-*O*-allyl-1,2,4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-β-D-glucopyranose (**5**, 71%), detritylation of which afforded 3-*O*-allyl-1,2,4-tri-*O*-benzoyl-β-D-glucopyranose (**6**) in 90% yield. Acetylation of **6** with acetic anhydride in pyridine, then selective 1-*O*-debenzoylation with ammonia in tetrahydrofuran–methanol followed by treatment with trichloroacetonitrile in the presence of potassium carbonate in dichloromethane, afforded 6-*O*-acetyl-3-*O*-allyl-2,4-di-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (**8**) in 77% yield (over the three steps). Coupling of **8** with lauryl alcohol catalyzed by trimethylsilyl trifluoromethanesulfonate (TMSOTf) in dry dichloromethane, followed by 3-*O*-deallylation using palladium chloride in methanol–dichloromethane, afforded the glycosyl acceptor lauryl 6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranoside (**9**) in 78% overall yield (over two steps).



Scheme 1. Reagents and conditions: (a) (i) BzCl, pyridine, rt, 12 h; (ii) 3:2 (v/v), THF/CH_3OH saturated dry NH_3, rt, 3 h; (iii) CH_2Cl_2, CCl_3CN and K_2CO_3, rt, 12 h, 56% (over three steps); (b) (i) chlorotriphenylmethane (1.2 equiv), pyridine, 40 °C, 48 h; (ii) BzCl (3.3 equiv), rt, 24 h, 71% (for two steps); (c) CH_2Cl_2/CH_3OH/0.1% AcCl, rt, 1 h, 90%; (d) Ac_2O/pyridine, rt, 5 h; (e) (i) 3:2 (v/v), THF/CH_3OH saturated dry NH_3, rt, 3 h; (ii) CH_2Cl_2, CCl_3CN and K_2CO_3, rt, 12 h, 77% from **6**; (f) (i) lauryl alcohol, TMSOTf, CH_2Cl_2, rt, 2 h; (ii) 2:1 (v/v), CH_3OH/CH_2Cl_2, $PdCl_2$, rt, 6 h, 78% (for two steps).

With the building blocks **3**, **9**, 2,4,6-tri-*O*-acetyl-3-*O*-allyl-β-D-glucopyranosyl-(1→3)-[2,3,4,6-tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→6)]-2,4-di-*O*-acetyl-α-D-glucopyranosyl trichloroacetimidate (**11**),[27] and isopropyl 4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (**12**)[28] in hand, construction of the target compound was readily carried out. As shown in Scheme 2, condensation of **3** and **9** catalyzed by trimethylsilyl trifluoromethanesulfonate in dry dichloromethane, followed by deacetylation with acetyl chloride in methanol–dichloromethane[29] gave the corresponding trisaccharide **10**. Regio- and stereoselective coupling[27,28,30] of **11** with **12** followed by debenzylidenation in 90% acetic acid and acetylation with acetic anhydride in pyridine afforded the desired tetrasaccharide thioglycoside donor **15** in 92% yield (over three steps). Using **10** as the glycosyl acceptor and **15** as the glycosyl donor, the fully protected lauryl heptasaccharide **16** was easily obtained using *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate as catalysts in dichloromethane. Deallylation of **16** using palladium



Scheme 2. Reagents and conditions: (a) (i) TMSOTf, CH_2Cl_2, rt, 2 h; (ii) CH_3OH/CH_2Cl_2, AcCl, rt, 7 h, 85% (for two steps); (b) TMSOTf, CH_2Cl_2, rt, 2 h; (c) 90% $HOAc/H_2O$, reflux, 2 h; (d) Ac_2O/pyridine, rt, 5 h, 92% from **11** and **12**; (e) CH_2Cl_2, NIS, TMSOTf, rt, 2 h, 87%; (f) (i) CH_3OH/CH_2Cl_2, $PdCl_2$, rt, 6 h; (ii) CH_3OH/CH_2Cl_2 saturated with NH_3, rt, 96 h, 82% (for two steps).

Table 1. Inhibition of S_{180} tumor by **17** and CPA

Groups	Number of mice	Weight (g)	Tumor weight (g)	Inhibition (%)
S_{180} Cell line group	20	28.59 ± 0.95	1.69 ± 0.64	
Group 1: treatment with **17** (1 mg/kg)	10	29.73 ± 1.00	1.03 ± 0.63*	39.1
Group 2: treatment with **17** (2 mg/kg)	10	29.83 ± 0.90	1.02 ± 0.52*	39.9
Group 3: treatment with **17** (4 mg/kg)	10	29.41 ± 0.82	1.05 ± 0.32*	37.9
Group 4: treatment with CPA (40 mg/kg)	10	27.30 ± 0.69	0.29 ± 0.13**	82.8

Compared to S_{180} cell line group, *$p < 0.01$, **$p < 0.001$.

chloride in methanol–dichloromethane, followed by deprotection with ammonia in methanol, gave the target compound **17** in excellent (82%) yield.

Inhibition of S_{180} noumenal tumors by the synthetic oligosaccharide was investigated. The tumor inhibition ratios for **17** were 39.1%, 39.9%, and 37.9% at a dose of 1, 2, and 4 mg/kg/day, respectively (Table 1). These activities can be compared with cyclophosphamide (CPA), which gave 82.8% tumor inhibition at a concentration of 40 mg/kg/day. These results indicate that **17** is a potent inhibitor of tumor growth and thus potential agent for cancer treatment.

In conclusion, a highly efficient and concise synthesis of **17**, a heptasaccharide with anti-tumor activity, was achieved through the regio- and stereoselective glycosylations. All new compounds involved in this study were characterized by optical rotations, ^{1}H NMR and ^{13}C NMR spectroscopy, and elemental analyses or electrospray-ionization MS. In all reactions, easily accessible materials and inexpensive reagents were used and the reactions were carried out in high yields and in large scale. In executing the synthesis, several intermediates were not separated but instead were used directly in the next reaction thus substantially streamlining the synthesis.

3. Experimental

3.1. General methods

Melting points were determined with a Mel-Temp apparatus. Optical rotations were measured at 25 °C in the stated solvent and are in units of degrees mL/g dm. ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded in $CDCl_3$ solutions at room temperature unless otherwise specified. Chemical shift (δ) values are given in ppm; coupling constants (J) are in hertz. Mass spectra were recorded on an Autospec mass spectrometer using the electrospray-ionization technique. Thin-layer chromatography (TLC) was performed on silica gel HF_{254} with detection by charring with 30% (v/v) H_2SO_4 in CH_3OH or in some cases with a UV detector. Column chromatography was conducted by elution of a column (10×240 mm, 18×300 mm od 35×400 mm) of silica gel (100–200 mesh) with EtOAc–petroleum ether (60–90 °C) as the eluent. Solutions were concentrated at <60 °C under reduced pressure. Dry reaction solvents were distilled over CaH_2 and stored over molecular sieves.

3.2. Bioassay for inhibition of S_{180}

CPA was purchased from Shanghai Hualian Pharmaceutical Co. Ltd (No. 030907). Sixty female and male Kunming mice (weight 18–22 g, grade SPF) were obtained from the Chongqing Academy of Chinese Materia Medica. The S_{180} cell line was provided by the Department of Tumorology, Institute of Pharmacy, the Chinese Academy of Medicine. The mice were randomly divided into five groups according to weight, and the S_{180} cell line of ascites cancer 0.2 mL (amount of cell 2×10^6) was planted intraperitoneally into the euterocelia. At 24 h after the planting, the mice were treated with **17** or CPA. The oligosaccharide **17** was dissolved in saline at 0.1 mg/mL, given at a dose of 1, 2, or 4 mg/kg/day to the mice for nine consecutive days. CPA was dissolved in saline at 4 mg/mL and administered intraperitoneally to the mice at a dose of 40 mg/kg/day over nine consecutive days.

3.3. 2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (3)

D-Lactose (**1**, 3.6 g, 10.0 mmol) was treated with BzCl (11.2 mL, 96.0 mmol) and pyridine (40 mL) for 12 h at rt. The benzoylated sugar was dissolved in a solution of 3:2 THF–CH_3OH (120 mL) and saturated dry NH_3. The solution was kept at rt for 3 h at the end of which time TLC (1:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was concentrated and the residue was dissolved in a solution of CH_2Cl_2 (60 mL) and CCl_3CN (1.5 mL, 15.0 mmol) containing K_2CO_3 (4.1 g, 30.0 mmol). The reaction mixture was stirred for 12 h at rt. The mixture was filtered and the combined filtrate and washings were concentrated, and the residue was purified by column chromatography (3:1, petroleum ether–EtOAc) to give **3** (6.8 g, 56% for three steps) as a white solid: $[\alpha]_D$ +54.5 (c 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$, 400 MHz): δ 8.56 (s, 1H, OC(N*H*)CCl_3), 7.99–7.19 (m, 35H, Bz*H*), 6.71 (d, 1H, $J = 3.6$ Hz, H-1), 6.16 (t, 1H, $J = 9.9$ Hz, H-3), 5.77–5.72 (m, 2H, H-2′, H-4′), 5.54 (dd, 1H, $J = 3.6$,

10.2 Hz, H-2), 5.38 (dd, 1H, $J = 3.3$, 10.3 Hz, H-3′), 4.93 (d, 1H, $J = 7.9$ Hz, H-1′), 4.58–4.50 (m, 2H, H-6a, 6b), 4.36–4.32 (m, 2H, H-4, H-5), 3.90 (m, 1H, H-5′), 3.74–3.69 (m, 2H, H-6a′, 6b′). Anal. Calcd for $C_{63}H_{50}Cl_3NO_{18}$: C, 62.26; H, 4.15. Found: C, 62.15; H, 4.32.

3.4. 6-*O*-Acetyl-3-*O*-allyl-2,4-di-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate (8)

A mixture of **4** (12 g, 54.5 mmol) and chlorotriphenylmethane (18.2 g, 65.6 mmol) in pyridine (80 mL) was stirred vigorously for 48 h at rt, at the end of which time TLC (3:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was cooled to 0 °C, then BzCl (21 mL, 180 mmol) was added dropwise over 30 min before the reaction was warmed to rt. The mixture was stirred for 24 h and then water (200 mL) was added and stirring was continued for 30 min. The mixture was extracted with CH_2Cl_2 and the combined extracts were washed with 1 N HCl and saturated aq $NaHCO_3$, dried (Na_2SO_4), and concentrated to a syrup that was purified by column chromatography (7:1, petroleum ether–EtOAc) to give **5** (29.8 g, 71%) as a white solid. A mixture of compound **5** (15.5 g, 20 mmol) and 150 mL 0.1% AcCl solution of 2:1 CH_2Cl_2–CH_3OH (v/v) was left at rt about 1 h, at the end of which time TLC (3:1, petroleum ether–EtOAc) indicated the reaction was complete. The solution was neutralized with Et_3N and concentrated. The resultant residue was purified by column chromatography (3:1, petroleum ether–EtOAc) to give crystalline **6** (9.6 g, 90%). Compound **6** (8 g, 15 mmol) was acetylated with Ac_2O (20 mL) in pyridine (20 mL) for 5 h at rt. The mixture was concentrated and the residue was dissolved in a solution of 3:2 THF–CH_3OH (150 mL) and saturated dry NH_3. The solution was kept at rt for 3 h, at the end of which time TLC (3:1, petroleum ether–EtOAc) indicated that the reaction was complete. The solution was then concentrated and the residue was dissolved in CH_2Cl_2 (40 mL). To the solution were added K_2CO_3 (2 g), CCl_3CN (2 mL) and the mixture was stirred at rt for 12 h. The mixture was filtered, the filtrate concentrated, and the residue was purified by column chromatography (3:1, petroleum ether–EtOAc) to afford **8** (5.7 g, 77% for three steps) as a white solid: $[\alpha]_D$ +62.4 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.60 (s, 1H, N*H*), 8.09–7.42 (m, 10H, Bz*H*), 6.69 (d, 1H, $J = 3.6$ Hz, H-1), 5.62 (m, 1H, CH_2–C*H*–CH_2), 5.51 (t, 1H, $J = 10.0$ Hz, H-4), 5.42 (dd, 1H, $J = 3.6$, 10.0 Hz, H-2), 5.06 (dd, 1H, $J = 1.6$, 17.6 Hz, CH_2–CH–C*H*$_2$), 4.96 (dd, 1H, $J = 1.6$, 10.4 Hz, CH_2–CH–C*H*$_2$), 4.35–4.21 (m, 4H, H-3, H-5, C*H*$_2$–CH–CH_2), 4.19–4.07 (m, 2H, H-6), 2.06 (s, 3H, COC*H*$_3$). Anal. Calcd for $C_{27}H_{26}Cl_3NO_9$: C, 52.74; H, 4.26. Found: C, 52.24; H, 4.64.

3.5. Lauryl 6-*O*-acetyl-2,4-di-*O*-benzoyl-β-D-glucopyranoside (9)

Trimethylsilyl trifluoromethanesulfonate (50 μL) was added to a solution of **8** (3.07 g, 5.0 mmol) and lauryl alcohol (1.12 g, 6.0 mmol) in CH_2Cl_2 (50 mL) at rt. The reaction mixture was stirred for 2 h, at the end of which time TLC (5:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was neutralized with Et_3N and concentrated. The resulting residue was dissolved in a solution of CH_3OH (40 mL) and CH_2Cl_2 (20 mL). To this solution was added $PdCl_2$ (60 mg), and the mixture was stirred for 6 h at rt, at the end of the which time TLC (4:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered and the filtrate was concentrated. The resulting residue was purified by column chromatography (7:1, petroleum ether–EtOAc) to give **9** (2.33 g, 78% for two steps) as a syrup: $[\alpha]_D$ −15.6 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.09–7.45 (m, 10H, Bz*H*), 5.32 (t, 1H, $J = 9.5$ Hz, H-4), 5.19 (dd, 1H, $J = 7.8$, 9.3 Hz, H-2), 4.70 (d, 1H, $J = 7.8$ Hz, H-1), 4.30 (d, 2H, $J = 4.0$ Hz), 4.10 (t, 1H, $J = 9.3$ Hz, H-3), 3.96–3.88 (m, 2H), 3.54 (m, 1H), 2.06 (s, 3H, COC*H*$_3$), 1.30–1.11 (m, 20H, $CH_2C_{10}H_{20}CH_3$), 0.91 (t, 3H, $J = 6.9$ Hz, CH_2CH_3). Anal. Calcd for $C_{34}H_{46}O_9$: C, 68.21; H, 7.74. Found: C, 68.12; H, 7.85.

3.6. Lauryl 2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-2,4-di-*O*-benzoyl-β-D-glucopyranoside (10)

Trimethylsilyl trifluoromethanesulfonate (30 μL) was added to a stirred solution of **3** (4.0 g, 3.3 mmol) and **9** (1.8 g, 3.0 mmol) in dry CH_2Cl_2 (40 mL) at rt. The reaction mixture was stirred for 2 h, at the end of which time TLC (3:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was neutralized with Et_3N and concentrated. The resulting residue was dissolved in a solution of CH_3OH (50 mL) and CH_2Cl_2 (10 mL). To the solution was added AcCl (0.3 mL) and the mixture was stirred for 7 h at rt, at the end of the which time TLC (2:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was neutralized with Et_3N, concentrated, and the residue was purified by column chromatography (3:1, petroleum ether–EtOAc) to give **10** (4.1 g, 85% for two steps) as a white amorphous solid: $[\alpha]_D$ +9.2 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.99–7.09 (m, 45H, Bz*H*), 5.62–5.58 (m, 2H), 5.54 (t, 1H, $J = 9.7$ Hz), 5.38 (dd, 1H, $J = 7.8$, 9.5 Hz), 5.32 (t, 1H, $J = 9.3$ Hz), 5.26 (t, 1H, $J = 9.0$ Hz), 5.18 (dd, 1H, $J = 3.3$, 10.2 Hz), 4.90 (d, 1H, $J = 7.8$ Hz), 4.55 (d, 2H, $J = 7.8$ Hz), 4.36 (t, 1H, $J = 9.0$ Hz), 4.20–4.19 (m, 2H), 4.03 (t, 1H, $J = 9.5$ Hz), 3.84–3.73 (m, 2H), 3.67–3.58 (m, 5H), 3.48–3.33 (m, 2H), 1.31–1.00 (m, 20H, $CH_2C_{10}H_{20}CH_3$),

0.91 (t, 3H, $J = 6.8$ Hz, CH_2CH_3); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.45, 165.35, 165.25, 165.20, 165.13, 165.00, 164.84, 164.58, 164.18 (9C, *C*(=O)Ph), 133.10–127.93 (Ph), 100.91, 100.78, 100.51 (3C, C-1_A, 1_B, 1_C). Anal. Calcd for $C_{93}H_{92}O_{25}$: C, 69.39; H, 5.76. Found: C, 69.26; H, 5.87.

3.7. Isopropyl 2,4,6-tri-*O*-acetyl-3-*O*-allyl-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)-2,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside (15)

Trimethylsilyl trifluoromethanesulfonate (30 μL) was added to a stirred solution of **11** (6.55 g, 5.0 mmol) and **12** (1.65 g, 5.0 mmol) in dry CH_2Cl_2 (40 mL) at rt. The reaction mixture was stirred for 2 h, at the end of which time TLC (3:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was neutralized with Et_3N and concentrated. The resultant residue was added to 90% $HOAc/H_2O$ (40 mL), the mixture was heated at reflux for 2 h. The mixture was concentrated and the residue was acetylated with Ac_2O (20 mL) in pyridine (20 mL) for 5 h at rt. The solvent was evaporated and the resulting residue was purified by column chromatography (2:1, petroleum ether–EtOAc) to afford **15** (4.9 g, 92% for three steps) as a white solid: $[\alpha]_D$ −29.5 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 8.01–7.26 (m, 20H, Bz*H*), 5.87–5.68 (m, 3H), 5.58–5.46 (m, 3H), 5.62 (m, 2H), 5.18–5.10 (m, 2H), 5.03–4.94 (m, 3H), 4.85 (m, 1H), 4.66–4.45 (m, 4H), 4.39–4.20 (m, 5H), 4.03–3.88 (m, 6H), 3.83–3.70 (m, 4H), 3.67–3.45 (m, 3H), 3.18 (m, 1H, SC*H*$(CH_3)_2$), 2.11–1.91 (8s, 24H, COCH_3), 1.33–1.25 (m, 6H, SCH(CH_3)$_2$); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.67, 170.60, 169.57, 169.30, 169.17, 168.96, 168.84, 168.60 (8C, *C*(=O)CH_3), 166.05, 165.84, 165.16, 164.99 (4C, *C*(=O)Ph), 134.09–128.32 (Ph), 116.97 (CH_2CH=*C*H_2), 101.25, 100.48, 100.27 (3C, C-1_B, 1_C, 1_D), 83.10 (C-1_A), 35.36 (1C, S*C*H$(CH_3)_2$), 24.44, 24.16 (2C, SCH(*C*H_3)$_2$). Anal. Calcd for $C_{74}H_{84}O_{32}S$: C, 58.57; H, 5.58. Found: C, 58.44; H, 5.71.

3.8. Lauryl 2,4,6-tri-*O*-acetyl-3-allyl-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)-2,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1→6)-[2,3,4,6-tetra-*O*-benzoyl-β-D-galactopyranosyl-(1→4)-2,3,6-tri-*O*-benzoyl-β-D-glucopyranosyl-(1→3)]-2,4-di-*O*-benzoyl-β-D-glucopyranoside (16)

To a stirred solution of **10** (3.2 g, 2.0 mmol) and **15** (3.3 g, 2.2 mmol) in dry CH_2Cl_2 (50 mL) were added *N*-iodosuccinimide (0.5 g, 2.2 mmol) and TMSOTf (15 μL) at rt. After 2 h, Et_3N was added and the mixture was filtered and the filtrate concentrated. The residue was purified by column chromatography (2:1, petroleum ether–EtOAc) to give **16** (5.3 g, 87%) as a white amorphous solid: $[\alpha]_D$ −12.0 (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$, 400 MHz): δ 7.93–7.01 (m, 65H, Bz*H*), 5.84 (t, 1H, $J = 9.6$ Hz), 5.69–5.64 (m, 2H), 5.58–5.46 (m, 3H), 5.34 (m, 1H), 5.23–5.08 (m, 5H), 5.02–4.98 (m, 2H), 4.85–4.71 (m, 5H), 4.64–4.59 (m, 2H), 4.50–4.43 (m, 3H), 4.35–4.32 (m, 2H), 4.26–4.22 (m, 3H), 4.15–4.12 (m, 2H), 3.99–3.96 (m, 5H), 3.93–3.66 (m, 7H), 3.64–3.58 (m, 5H), 3.55–3.44 (m, 6H), 3.31 (m, 1H), 3.23 (m, 1H), 2.07–2.02 (m, 15H), 1.90 (s, 3H), 1.84 (s, 3H), 1.82 (s, 3H), 1.30–0.95 (m, 20H), 0.89 (t, 3H, $J = 6.9$ Hz); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 170.44, 170.39, 169.46, 169.11, 169.04, 168.94, 168.75, 168.47 (8C, *C*(=O)CH_3), 165.90, 165.67, 165.33, 165.17, 165.11, 165.07, 165.03, 165.00, 164.82, 164.75, 164.59, 164.44, 164.18 (13C, *C*(=O)Ph), 133.99–127.93 (Ph, CH_2CH=CH_2), 116.81 (CH_2CH=*C*H_2), 101.07, 100.85, 100.73, 100.53, 100.45, 100.43, 100.10 (7C, C-1_A, 1_B, 1_C, 1_D, 1_E, 1_F, 1_G), 45.65 (1C, CH_2CH=CH_2). Anal. Calcd for $C_{164}H_{168}O_{57}$: C, 64.56; H, 5.55. Found: C, 64.49; H, 5.74.

3.9. Lauryl β-D-glucopyranosyl-(1→3)-[β-D-glucopyranosyl-(1→6)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→6)-[β-D-galactopyranosyl-(1→4)-β-D-glucopyranosyl-(1→3)]-β-D-glucopyranoside (17)

Compound **16** (3.7 g, 1.2 mmol) was dissolved in a solution of CH_3OH (40 mL) and CH_2Cl_2 (20 mL). To this solution was added $PdCl_2$ (40 mg) and the mixture was stirred for 6 h at rt, at the end of the which time TLC (1:1, petroleum ether–EtOAc) indicated that the reaction was complete. The mixture was filtered, the filtrate concentrated and the residue was then dissolved in a saturated solution of NH_3 in CH_2Cl_2 (30 mL) and CH_3OH (300 mL) at rt. After 96 h, the reaction mixture was concentrated, and the residue was washed four times with CH_2Cl_2 to afford **17** (1.3 g, 82% for two steps) as a white solid: $[\alpha]_D$ −22.6 (*c* 1.0, H_2O). ^1H NMR (D_2O, 400 MHz): δ 4.84–4.62 (m, 4H), 4.38–4.31 (m, 3H), 4.07–3.77 (m, 9H), 3.60–3.34 (m, 18H), 3.29–3.05 (m, 17H), 1.47–1.12 (m, 20H, $CH_2C_{10}H_{20}CH_3$), 0.73 (t, 3H, $J = 6.6$ Hz, CH_2CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 103.14, 102.08, 102.44, 102.04, 101.85, 101.24, 101.09 (C-$1_{A–G}$). Anal. Calcd for $C_{54}H_{96}O_{36}$: C, 49.09; H, 7.32. Found: C, 48.57; H, 7.69. ESIMS for $C_{54}H_{96}O_{36}$ (1321.33): 1320.32 $[M-1]^+$.

Acknowledgements

This work was supported by The Natural Science Fund from the Educational Committee of Sichuan Province (2003407) and The Natural Science Fund from Chongqing University of Post and Telecommunications (A2004–45).

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