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Synthesis of Laminarin Oligosaccharide Derivatives Having D-Arabinofuranosyl Side-Chains

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ABSTRACT

An efficient glycosylation strategy was applied in the synthesis of β -D-glucopyranosyl- $(1\rightarrow 3)$ - $[\alpha$ -D-arabinopyranosyl- $(1\rightarrow 4)$]- β -D-glucopyranosyl- $(1\rightarrow 3)$ - β -D-glucopyranosyl- $(1\rightarrow 3)$ - β -D-glucopyranosyl- $(1\rightarrow 3)$ -glycosidic bond formation between glucopyranosyl residues. The new strategy using a 4,6-*O*-benzylidenated acceptor avoided generation of the α major isomer in the attempted β -D- $(1\rightarrow 3)$ glycosylation under standard glycosylation conditions. The hexasaccharide we prepared showed about 30% tumor growth inhibition towards S180 model study.

Key Words: Oligosaccharide; Laminarin; Glycosylation; Antitumor agent.

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INTRODUCTION

Many natural polysaccharides, such as lentinan,^[1,2] schizophyllan,^[3] pestalosan^[4] and other fungal $(1\rightarrow 6)$ -branched $(1\rightarrow 3)$ - β -D-glucans,^[5-7] exhibit high inhibition to the growth of implanted tumors in mice. It is believed that the activity is closely related to the organization of the $(1\rightarrow 3)$ - β -D-linked backbone into a triple helix, the frequency and complexity of the side-branching, and to the polymer molecular weight.^[8,9] To investigate the structure-activity relationship, we have synthesized a series of β -D-glucosyl oligosaccharides to mimic the repeating units of natural β -glucan chains.^[10] The mice tumor tests revealed that our synthetic glucosyl oligosaccharides showed weaker antitumor activities compared to their natural polysaccharides. However, a literature survey found that some synthetic branching-oligosaccharides with arabinose side-chains exhibited antitumor activity as high as natural polysaccharides.^[11] We thus focused our attention on the synthesis of β -D-glucopyranosyl- $(1\rightarrow 3)$ - $[\alpha$ -Darabinopyranosyl- $(1\rightarrow 4)$]- β -D-glucopyranosyl- $(1\rightarrow 3)$ - β -D-glucopyranosyl- $(1\rightarrow 3)$ - $[\alpha$ -Darabinopyranosyl- $(1\rightarrow 6)$]-D-glucopyranose to mimic the bioactive arabinosyl curdlan.

To prepare this hexasaccharide, one would think that a 3+3 strategy is more efficient. However, the unexpected α glycosides were predominantly formed in this case using fully acylated imidates or thioglycosides as glycosyl donors under standard glycosylation conditions (see Scheme 1 for examples).^[12] However, we found that a 4,6-*O*-benzylidenated acceptor was helpful in β -D-(1 \rightarrow 3) bond formation. Here we report the synthesis of β -D-glucopyranosyl-(1 \rightarrow 3)-[α -D-arabinopyranosyl-(1 \rightarrow 4)]- β -D-



Scheme 1. a) NIS, TMSOTf, CH₂Cl₂, 56%; b) TMSOTf, CH₂Cl₂, 77%.

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glucopyranosyl- $(1\rightarrow 3)$ - β -D-glucopyranosyl- $(1\rightarrow 3)$ - $[\alpha$ -D-arabinopyranosyl- $(1\rightarrow 6)$]-D-glucopyranose based on a sequential glycosylation strategy, and antitumor activities of the hexasaccharide.

RESULTS AND DISCUSSION

Glycosylation of 2,3,5-tri-O-benzoyl- α -D-arabinofuranosyl trichloroacetimidate (1), Scheme 2, and allyl 2,6-di-O-benzoyl-3-O-tert-butyldimethylsilyl- α -D-glucopyranoside (2)^a in dry CH₂Cl₂ with TMSOTf as promoter afforded allyl 2,3,5-tri-O-benzoyl- α -Darabinofuranosyl- $(1 \rightarrow 4)$ -2,6-di-O-benzoyl-3-O-tert-butyldimethylsilyl- α -D-glycopyranoside (3, 78.4%). Removal of the *tert*-butyldimethylsilyl (TBS) group from 3 with tetrabutylammonium fluoride (TBAF) was not straightforward. Thus, disaccharide 3 was treated with 90% aqueous trifluoroacetic acid (TFA) under reflux for 3 h to generate 4 in 95% yield. The α -(1 \rightarrow 4) glycosidic bond of 4 was very stable under these acidic conditions. Condensation of 4 with 2,3,4,6-tetra-O-benzoyl- α -D-glucopyranosyl trichloroacetimidate (5) under standard glycosylation conditions gave trisaccharide 6 in good yield (80.4%). Treatment of **6** with PdCl₂ in MeOH yielded **7**, which was further transformed into imidate 8 by reacting with trichloroacetonitrile and DBU in methylene chloride; yield of 68.3% for two steps. Critical glycosylation of trisaccharide imidate 8 and disaccharide diol $9^{[10]}$ using the method as described in the preparation of 3 gave desired pentasaccharide 10 as the predominant product.^b Acetylation of 10 with acetic anhydride in pyridine followed by CeCl₃·7H₂O catalyzed regioselective removal of the di-O-isopropylidene group^[13] gave pentasaccharide diol **11** in 50% yield (from **9**). H- 1^{III} and H- 2^{II} of **11** in its ¹H NMR spectrum appear at δ 4.45 ppm (J 7.3 Hz) and 4.72 ppm, respectively, confirming the β -(1 \rightarrow 3) linkage between sugar units II and III. Primary hydroxyl group favored glycosylation of 11 and 1 in CH_2Cl_2 at $-15^{\circ}C$ furnished the hexasaccharide derivative 12 in 65% yield. HMQC experiments showed C-1^{III} at 100.96 ppm (¹³C NMR), while the corresponding H-1^{III} appeared at 4.47 ppm (¹H NMR), indicating a β linkage between sugar residue II and III in **12**. Full deprotection of 12 with 90% TFA, followed by deacetylation with NaOMe in MeOH, afforded target hexasaccharide 13 in 43% isolated yield.

^aTo a solution of allyl 2,6-di-*O*-benzoyl-3-*O*- α -D-glucopyranoside (1.0 g, 2.33 mmol) in dry DMF (8 mL) was added imidazole (380 mg) and TBSCl (385 mg, 2.56 mmol) at rt. After 4 h, the mixture was worked up as usual to give syrupy allyl 2,6-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl- α -D-glucopyranoside (872 mg, 69%). To prove the structure, a small amount of the above compound was acetylated with acetic anhydride in pyridine to give the following ¹H NMR (400 MHz, CDCl₃): -0.14, 0.17 (2 s, 6 H, 2 SiCH₃), 0.73 (s, 9 H, C(CH₃)₃), 3.99 (dd, 1 H, *J* 7.3, 13.2 Hz, OCH₂-CH=CH₂), 4.16–4.20 (m, 2 H, H-5, OCH₂-CH=CH₂), 4.31 (dd, 1 H, *J* 5.5, 12.5 Hz, H-6a), 4.36 (t, 1 H, *J* 9.1 Hz, H-3), 4.49 (dd, 1 H, *J* 2.6, 12.5 Hz, H-6b), 5.05 (dd, 1 H, OCH₂-CH=CH₂), 5.09 (m, 2 H, H-1, H-4), 5.14 (dd, 1 H, *J* 3.7, 9.1 Hz, H-2), 5.25 (dd, 1 H, *J* 1.6, 12.7 Hz, OCH₂-CH=CH₂), 5.80–5.84 (m, 1 H, OCH₂-CH=CH₂), 7.43–8.25 (m, 10 H, Ph). These spectra assignments are consistent with the proposed structure for **2**.

^bCompound **10** was contaminated by an inseparable side product, presumably the decomposed donor based on mass analysis. Thus it was hydrolyzed directly to give pure **11**.



Scheme 2. a) TMSOTf, CH₂Cl₂, 78.4% for **3**; 80.4% for **6**; 65% for **12**; b) 90% TFA, 95%. c) Pd₂Cl₂, MeOH; d) CCl₃CN, DBU, CH₂Cl₂, 68.3% for two steps; e) Ac₂O, Pyr; CH₃CN, CeCl₃·6H₂O, H₂O, 50% from **9**; f) 90% TFA; NaOMe. MeOH, 43% for two steps.

Kun min mice weighing 20-22 g were used for the bioassay. Seven-day-old S180 ascites (0.2 mL, about 2×10^6 cells) were transplanted into the right groins of mice. The test samples, dissolved in distilled water, were injected daily for 7 days starting 24 h after tumor implantation. At the end of the tenth day, the mice were killed, and the tumors were extirpated and weighted. The results are summarized in Table 1.

In conclusion, we have described the synthesis of the hexasaccharide 13 having a $(1\rightarrow 3)$ - β -D-glucan backbone and two arabinofuranosyl side chains. We showed here that a predominant β product could be formed using a 4,6-O-benzylidenated

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	Dose (mg/Kg)	Tumor growth inhibition (%)	Body weight (g)		Tumor		
Sample			Day 1	Day 10	weigth (g)	р	
Control	0		20.0	29.6	1.68 ± 0.63	< 0.001	
CTX	80×1	89.3	21.3	27.4	0.19 ± 0.11	< 0.01	
13	5.0×7	35.1	20.1	29.7	1.09 ± 0.63	< 0.01	
13	1.5×7	33.9	20.0	26.9	1.11 ± 0.60	< 0.01	
13	0.5×7	31.5	20.0	29.6	0.15 ± 0.41	< 0.01	

Table 1. Preliminary studies on antitumor activity of hexasaccharide 13.

glucopyranosyl acceptor in complex oligosaccharide synthesis, while the α major was generated using 4,6-diacylated acceptor under the same reaction conditions. The hexasaccharide 13 showed a mild antitumor activity towards S180 model study.

EXPERIMENTAL

General methods. Optical rotations were determined at 20°C with a Perkin– Elmer Model 241-Mc automatic polarimeter. ¹H NMR, ¹³C NMR, ¹H–¹H COSY and HMQC spectra were recorded with ARX 400 spectrometers for solutions in CDCl₃ and D₂O. Chemical shifts are given in ppm downfield from internal Me₄Si, or DSS in case of D₂O. Mass spectra were measured using MALDI-TOF-MS with (α -cyano-4hydroxycinnamic acid (CCA) as matrix. Thin-layer chromatography (TLC) was performed on silica gel HF₂₅₄ with detection by charring with 30% (v/v) H₂SO₄ in MeOH or in some cases by a UV detector. Column chromatography was conducted by elution of a column (16×240 mm, 18×300 mm, 35×400 mm) of silica gel (100–200 mesh) with EtOAc-petroleum ether (bp 60–90°C) as the eluent. Solutions were concentrated at <60°C under diminished pressure.

Allyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl-(1→4)-2,6-di-*O*-benzoyl-3-*O*tert-butyldimethylsilyl-β-D-glucopyranoside (3). Compound 1 (1.33 g, 2.2 mmol) and 2 (1.09 g, 2.0 mmol) were pre-dried in one flask under vacuum for 4 h. The mixture was then dissolved in CH₂Cl₂ (15 mL). To the solution was added Me₃SiOTf (35 µL, 0.19 mmol) under an N₂ atmosphere at 0°C. The mixture was stirred at these conditions for 1 h, then neutralized with triethylamine, concentrated under reduced pressure, and purified on a silica gel column with petroleum ether–EtOAc (6:1) as the eluent to give 3 (1.54 g, 78.4%) as a syrup; $[α_D]^{20}$ + 31° (*c* 1, CHCl₃); ¹H NMR (CDCl₃) δ −0.01 (s, 3 H, SiCH₃), 0.16 (s, 3 H, SiCH₃), 0.68 (s, 9 H, C(CH₃)₃), 3.98– 4.40 (m, 2 H, H-3^I, H-5^I), 4.13 (dd, 1 H, J 5.1, 11.9 Hz, OCH₂–CH=CH₂), 4.22 (dd, 1 H, J 5.1, 13.0 Hz, OCH₂–CH=CH₂), 4.45–4.53 (m, 4 H, H-6a^I, H-6b^I, H-4^{II}, H-4^{II}, 4.69–4.75 (m, 2 H, H-5a^{II}, H-5b^{II}), 5.05 (d, 1 H, J 3.7 Hz, H-1^I), 5.11–5.13 (m, 1 H, OCH₂–CH=CH₂), 5.15 (dd, 1 H, J 3.7, 9.5 Hz, H-2^I), 5.26–5.28 (m, 1 H, OCH₂– CH=CH₂), 5.51 (d, 1 H, J 3.3 Hz, H-3^{II}), 5.68 (s, 1 H, H-1^{II}), 5.85 (m, 1 H, OCH₂– CH=CH₂), 5.87 (s, 1 H, H-2^{II}), 7.37–8.29 (m, 25 H, Ph).

Anal. Calcd for C55H58O15Si: C, 66.92; H, 5.92. Found: C, 67.18; H, 5.85.

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Allyl 2,3,4,5-tetra-O-benzoyl- β -D-glucopyranosyl- $(1 \rightarrow 3)$ -[2,3,5-tri-O-benzoyl- α -**D-arabinofuranosyl-** $(1 \rightarrow 4)$]-2,6-di-*O*-benzoyl- α -D-glucopyranoside (6). A solution of compound 3 (1.283 g, 1.3 mmol) in 90% aqueous trifluoroacetic acid (10 mL) was stirred at 60°C for about 3 h, then co-evaporated with toluene under diminished pressure to give a residue. Purification of the residue by column chromatography (3:1 petroleum ether-EtOAc) gave 4 (1.079 g, 95.1%) as a syrup. To a solution of compound 4 (1.05 g, 1.2 mmol) and 5 (1.00 g, 1.35 mmol) in anhydrous CH_2Cl_2 (12 mL) was added TMSOTf (35 μ L, 0.19 mmol) under an N₂ atmosphere at 0°C. The mixture was stirred under this condition for 1.5 h, neutralized with Et₃N and concentrated under reduced pressure. The residue was purified on a silica gel column with 3:1 petroleum ether–EtOAc as the eluent to give 6 (1.40 g, 80.4%) as a syrup; $[\alpha_D]^{20} + 30^\circ$ (c 1, CHCl₃); ¹H NMR (CDCl₃) δ 3.90 (dd, 1 H, J 6.3, 14.3 Hz, OCH₂), 3.96-3.98 (m, 2 H, H-5^I, H-4^I), 4.25 (dd, 1 H, J 5.2, 14.3 Hz, OCH₂), 4.19-4.21 (m, 1 H, H-5^{II}), 4.49–4.69 (m, 7 H, H-6a^I, H-6b^I, H-6a^{II}, H-6b^{II}, H-3^I, H-4^{III}, H-5a^{III}), 4.75 (dd, 1 H, J 3.9, 12.0 Hz, H-5b^{III}), 4.94 (dd, 1 H, J 3.7, 10.1 Hz, H-2^I), 4.99 (dd, 1 H, J 1.3, 10.4 Hz, $=CH_2$), 5.09 (d, 1 H, J 3.7 Hz, H-1^I), 5.11 (dd, 1 H, J 1.6, 10.4 Hz, = CH_2), 5.13 (d, 1 H, J 8.0 Hz, H-1^{II}), 5.42 (d, 1 H, J 4.4 Hz, H-3^{III}), 5.45 (dd, 1 H, J 9.6, 8.0 Hz, H-2^{II}), 5.54 (t, 1 H, J 9.6 Hz, H-4^{II}), 5.63 (t, 1 H, J 9.6 Hz, H-3^{II}), 5.64– 5.67 (m, 1 H, =*CH*), 5.90–5.92 (m, 2 H, H-1^{III}, H-2^{III}), 7.07–8.26 (m, 45 H, Ph). ¹³C NMR (100 MHz, CDCl₃) δ 63.05 (C-6^{II}), 63.50 (C-6^I), 63.97 (C-5^{III}), 68.66 (OCH₂), 101.12 (C-1^{II}), 108.35 (C-1^{III}), 111.85 (= CH_2), 164.89, 164.96, 165.23, 165.26, 165.55, 165.62, 166.13, 166.20, 166.30 (CO).

Anal. Calcd for C₈₃H₇₀O₂₄: C, 68.68; H, 4.86. Found: C, 68.92; H, 4.73.

2,3,4,6-Tetra-O-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,5-tri-O-benzoyl-α-Darabinofuranosyl- $(1 \rightarrow 4)$]-2,6-di-O-benzoyl- α -D-glucopyranosyl trichloroacetimidate (8). To a solution of compound 6 (1.435 g, 0.99 mmol) in methanol (20 mL) was added PdCl₂ (0.17 g, 0.50 mmol) at rt. The mixture was stirred under these conditions for 3 h, then filtered, and the filtrate was concentrated. Purification of the residue by column chromatography (3:1 petroleum ether-EtOAc) gave 7 (1.19 g, 85.3%) as a syrup. This syrup was dissolved in anhydrous CH₂Cl₂ (6 mL), and trichloroacetonitrile (0.3 mL, 3 mmol) and DBU (0.05 mL, 0.33 mmol) were added subsequently. The mixture was stirred at rt for 2 h, and then concentrated. Purification of the residue by column chromatography (3:1 petroleum ether-EtOAc) gave 8 (1.052 g, 80.1%) as an amorphous solid; $[\alpha]_D^{20} + 44^{\circ}$ (c 1, CHCl₃); ¹H NMR (CDCl₃) δ 4.14 (m, 3 H, H-5^I H-5^{II}, H-4^I), 4.55–4.80 (m, 8 H, H-6a^I, H-6b^I, H-6a^{II}, H-6b^{II}, H-5a^{III}, H-5b^{III}, H-4^{III}, H-3^I), 5.09 (d, 1 H, J 7.9 Hz, H-1^{II}), 5.21 (q, 1 H, J 3.5, 10.2 Hz, H-2^I), 5.45–5.47 (m, 2 H, H-2^{II}, H-3^{III}), 5.51 (t, 1 H, J 9.5 Hz, H-4^{II}), 5.64 (t, 1 H, J 9.5 Hz, H-3^{II}), 5.95 (s, 1 H, H-1^{III}), 5.97 (s, 1 H, H-2^{III}), 6.53 (d, 1 H, J 3.4 Hz, H-1^I), 7.11-8.26 (m, 45 H, Ph), 8.27 (s,1 H, NH).

Anal. Calcd for C₈₂H₆₆Cl₃NO₂₄: C, 63.31; H, 4.28. Found: C, 63.03; H, 4.31.

2,3,4,6-Tetra-O-benzoyl- β -D-glucopyranosyl- $(1 \rightarrow 3)$ -[2,3,5-tri-O-benzoyl- α -D-arabinofuranosyl- $(1 \rightarrow 4)$]-2,6-di-O-benzoyl- β -D-glucopyranosyl $(1 \rightarrow 3)$ -2-O-acetyl-4,6-O-benzylidene- β -D-glucopyranosyl- $(1 \rightarrow 3)$ -1,2-O-isopropylidene- α -D-glucofuranose (10). To a solution of 8 (1.17 g, 0.75 mmol) and 9 (0.34 g, 0.67mmol) in dry

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 CH_2Cl_2 was added TMSOTf (20 µL, 0.11 mmol) under an N₂ atmosphere at 0°C. The mixture was stirred under these conditions for 1.5 h, then neutralized with Et₃N, concentrated under reduced pressure, and purified on a silica gel column with 3:2 petroleum ether-EtOAc as the eluent to give crude 10 (1.02 g) as a syrup. This syrup was treated with acetic anhydride (1 mL) in pyridine (2.0 mL) at rt for 4 h and concentrated with the help of toluene. The above crude product was dissolved in CH₃CN, then CeCl₃·6H₂O (100 mg, 0.26 mmol) and H₂O (0.1 mL) were added. The mixture was stirred under reflux for 3 h, then diluted with water, extracted with methylene chloride $(3 \times 15 \text{ mL})$. The organic phases were combined, dried over sodium sulfate and concentrated. Purification of the residue by column chromatography (1:2 petroleum ether-EtOAc) gave 11 (653 mg, 50.1% from 9) as a solid; $[\alpha]_D^{2\bar{0}} - 4^\circ$ (c 1, CHCl₃); ¹H NMR (CDCl₃) δ 1.26 (s, 3 H, CH₃), 1.44 (s, 3 H, CH₃), 1.78 (s, 3 H, $CH_{3}CO$, 2.99 (d, 1 H, J 4.1 Hz, OH), 3.36–3.38 (m, 1 H, H-5^{II}), 3.51–3.53 (m, 1 H, H-5), 3.59 (dd, 1 H, J 5.4, 8.7 Hz, H-4^{II}), 3.67-3.71 (m, 2 H, H-6a, H-6b), 3.77-3.80 (m, 2 H, H-6a, H-6b), 3.88 (t, 1 H, J 8.5 Hz, H-4^{III}), 3.98 (m, 1 H, H-5^I), 4.04 (t, 1 H, J 8.7 Hz), 4.09-4.12 (m, 1 H), 4.19-4.25 (m, 3 H), 4.29 (t, 1 H, J 8.9 Hz, H-3^{II}), 4.31 (q, 1 H, J 5.4, 14.6 Hz, H-5a^V), 4.45 (d, 1 H, J 7.3 Hz, H-1^{III}), 4.46-4.54 (m, 5 H), 4.68 (dd, 1 H, J 2.3, 14.6 Hz, H-5b^V), 4.71–4.73 (m, 2 H, J 7.9, 8.9 Hz, H-1^{II}, H-2^{II}), 4.99 (d, 1 H, J 7.7 Hz, H-1^{IV}), 5.18 (dd, 1 H, J 7.0, 8.2 Hz, H-2^{III}), 5.34 (d, 1 H, J 3.7 Hz, H-3^V), 5.45 (dd, 1 H, J 9.4, 11.0 Hz, H-2^{IV}), 5.48 (s, 1 H, PhCH), 5.54 (t, 1 H, J 9.4 Hz, H-4^{IV}), 5.61 (d, 1 H, J 3.7 Hz, H-1^I), 5.66 (t, 1 H, J 9.4 Hz, H-3^{IV}), 5.70 (d, 1 H, J 0.7 Hz, H-2^V), 5.74 (s, 1 H, H-1^V), 7.13-8.21 (m, 50 H, Ph); ¹³C NMR (100 MHz, CDCl₃) δ 26.79, 26.29, 29.30, 62.89, 63.23, 63.79, 64.19, 66.50, 68.05, 68.61, 70.08, 71.71, 72.04, 72.31, 72.63, 73.10, 73.78, 73.95, 77.19, 78.02, 78.47, 78.70, 79.51, 81.07, 81.55, 82.34, 82.70, 99.52, 99.68, 99.79, 101.364, 104.11 (C-1^V), 107.65 (PhCH), 112.20 (CMe₂), 164.30, 164.86, 164.88, 165.16, 165.49, 165.52, 165.90, 166.11, 166.25, 168.31 (CO).

Anal. Calcd for C₁₀₄H₉₆O₃₅: C, 65.54; H, 5.08. Found: C, 65.27; H, 5.14.

2,3,4,6-Tetra-O-benzoyl- β -D-glucopyranosyl- $(1 \rightarrow 3)$ -[2,3,5-tri-O-benzoyl- α -Darabinofuranosyl- $(1 \rightarrow 4)$]-2,6-di-O-benzoyl- β -D-glucopyranosyl $(1 \rightarrow 3)$ -2-O-acetyl-4,6-O-benzylidene-β-D-glucopyranosyl-(1→3)-[2,3,5-tri-O-benzoyl-α-D-arabinofura**nosyl-(1\rightarrow6)]-1,2-***O***-isopropylidene-\alpha-D-glucofuranose (12). To a solution of 1 (170)** mg, 0.28 mmol) and 11 (533 mg, 0.28 mmol) in dry CH_2Cl_2 (5 mL) was added TMSOTf (10 μ L, 0.05 mmol) under an N₂ atmosphere at -15° C. The mixture was stirred under these conditions for 1.5 h, then neutralized with Et₃N, concentrated under reduced pressure, and purified on a silica gel column with 2:3 petroleum ether-EtOAc as the eluent to give 12 (428 mg, 65%) as a solid; $[\alpha]_D^{20} - 6^\circ$ (c 1, CHCl₃); ¹H NMR (CDCl₃) δ 1.16 (s, 3 H, CH₃), 1.65 (s, 3 H, CH₃), 2.35 (s, 3 H, CH₃CO), 3.09 (m, 1 H, H-5^{II}), 3.14–3.17 (m, 1 H, H-5^I), 3.52 (t, 1 H, J 9.1 Hz, H-4^{II}), 3.38–3.40 (m, 1 H, H-5^{III}), 3.77 (t, 1 H, J 9.1 Hz, H-3^{II}), 3.91-4.10 (m, 6 H, H-6a^I, H-6b^I, H-3^I, H-4^I, H-4^{III}, H-5^{IV}), 4.27-4.29 (m, 3 H, H-4^{IV}, H-3^{III}, H-2^I), 4.37 (d, 1 H, J 7.9Hz, H-1^{II}), 4.47–4.76 (m, 13 H, H-6a^{II}, H-6b^{II}, H-6a^{III}, H-6a^{III}, H-6a^{IV}, H-6b^{IV}, H-5a^I, H-5b^I, H-5a^{VI}, H-5b^{VI}, H-1^{III}, H-2^{II}, H-4^{VI}), 4.96 (d, 1 H, J 7.5Hz, H-1^{IV}), 5.17 (dd, 1 H, J 7.7, 8.2 Hz, H-2^{III}), 5.31-5.34 (m, 2 H, H-3^{VI}, H-1^{VI}), 5.35 (d, 1 H, J 1.4 Hz, H-3^V), 5.44 (dd, 1 H, J 7.6, 9.4 Hz, H-2^{IV}), 5.52–5.56 (m, 3 H, H-2^{VI}, H-3^{VI}, PhCH), 5.59 (d, 1 H, 3.7 Hz, H-3^{IV}), 5.65 (t, 1 H, J 9.4 Hz, H-4^{IV}), 5.69 (s, 1 H, H-2^V), 5.75 (s, 1 H, H-1^V), 7.16-8.02 (m, 65 H, Ph). ¹³C NMR (100 MHz, CDCl₃) δ 20.31,

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21.46, 26.50, 62.97, 63.29, 63.52, 63.76, 63.90, 66.63, 67.06, 67.83, 70.12, 71.78, 72.10, 72.24, 72.65, 73.17, 73.88, 74.06, 74.24, 77.24, 77.65, 77.77, 78.16, 78.57, 78.83, 79.26, 80.20, 81.23, 81.61, 81.69, 82.12, 82.40, 82.78, 99.91 (C-1^{II}), 100.21 (C-1^{IV}), 100.96 (C-1^{III}), 104.65 (C-5^V), 105.22 (C-1^I), 106.58 (C-1^{IV}), 107.75 (PHCH), 111.82 (CMe₂).

Anal. Calcd for C₁₃₀H₁₁₆O₄₂: C, 66.43; H, 4.97. Found: C, 66.79; H, 4.83.

β-D-Glucopyranosyl-(1→3)-[α-D-arabinopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[α-D-arabinopyranosyl-(1→6)]-D-glucopyranose (13). Compound 12 (310 mg, 0.132 mmol) was treated with 90% trifluoroacetic acid (6 mL) for 60 min, concentrated and then co-evaporated with toluene. The syrup was dissolved in methanol (15 mL), deacylated with sodium methoxide (0.5 M, kept pH at 9 to 10), neutralized with Amberlite 120 (H⁺), filtered and concentrated. The residue was chromatographically purified on Sephadex LH-20 (EtOAc, then methanol) to yield 13 (53 mg, 43%) as a solid; $[\alpha]_D$ + 19° (*c* 1, H₂O); Selected ¹H NMR (D₂O) δ 4.41– 4.72 (m, 3.3 H, H-1^{1,II,III,IV} of β isomer), 5.20, 5.25 (2 s, 2 H, H-1^{V,VI}), 5.30 (d, 0.7 H, *J* 2.8 Hz, H-1¹ of α isomer). Selected ¹³C NMR (100 MHz, D₂O) δ 103.02, 103.70 (2C), 103.99, 110.23, 110.87 (C-1^{1-VI}). MALDI-TOF-MS: Calcd for C₃₄H₅₈O₂₉: 930.3 [M]; Found 953.8 [M+Na]⁺.

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