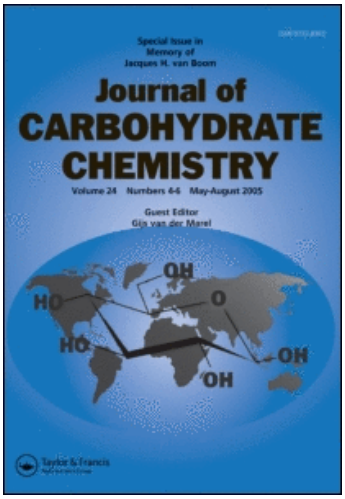

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Synthesis of Laminarin Oligosaccharide Derivatives Having d-Arabinofuranosyl Side-Chains

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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→3)-[α-D-arabinopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[α-D-arabinopyranosyl-(1→6)]-D-glucopyranose to secure β-D-(1→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→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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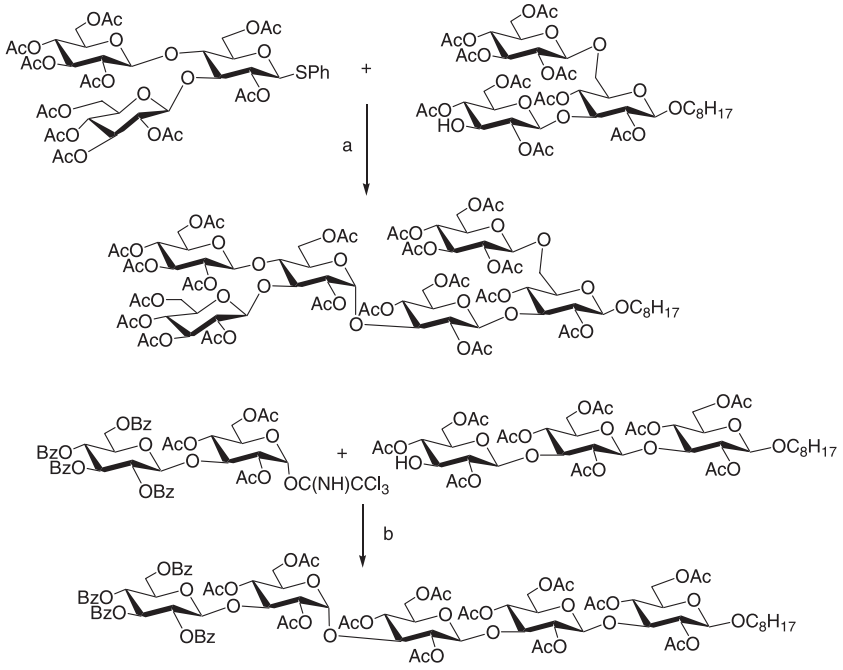
INTRODUCTION

Many natural polysaccharides, such as lentinan,[1,2] schizophyllan,[3] pestalosan[4] and other fungal (1→6)-branched (1→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→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→3)-[α-D-arabinopyranosyl-(1→4)]-β-D-glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[α-D-arabinopyranosyl-(1→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→3) bond formation. Here we report the synthesis of β-D-glucopyranosyl-(1→3)-[α-D-arabinopyranosyl-(1→4)]-β-D-



Scheme 1. a) NIS, TMSOTf, CH_2Cl_2, 56%; b) TMSOTf, CH_2Cl_2, 77%.

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glucopyranosyl-(1→3)-β-D-glucopyranosyl-(1→3)-[α-D-arabinopyranosyl-(1→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 CH_2Cl_2 with TMSOTf as promoter afforded allyl 2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl-(1→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→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 $PdCl_2$ 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 $CeCl_3 \cdot 7H_2O$ 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 ^{1}H NMR spectrum appear at δ 4.45 ppm (J 7.3 Hz) and 4.72 ppm, respectively, confirming the β-(1→3) linkage between sugar units II and III. Primary hydroxyl group favored glycosylation of **11** and **1** in CH_2Cl_2 at -15°C furnished the hexasaccharide derivative **12** in 65% yield. HMQC experiments showed C-1^{III} at 100.96 ppm (^{13}C NMR), while the corresponding H-1^{III} appeared at 4.47 ppm (^{1}H 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.

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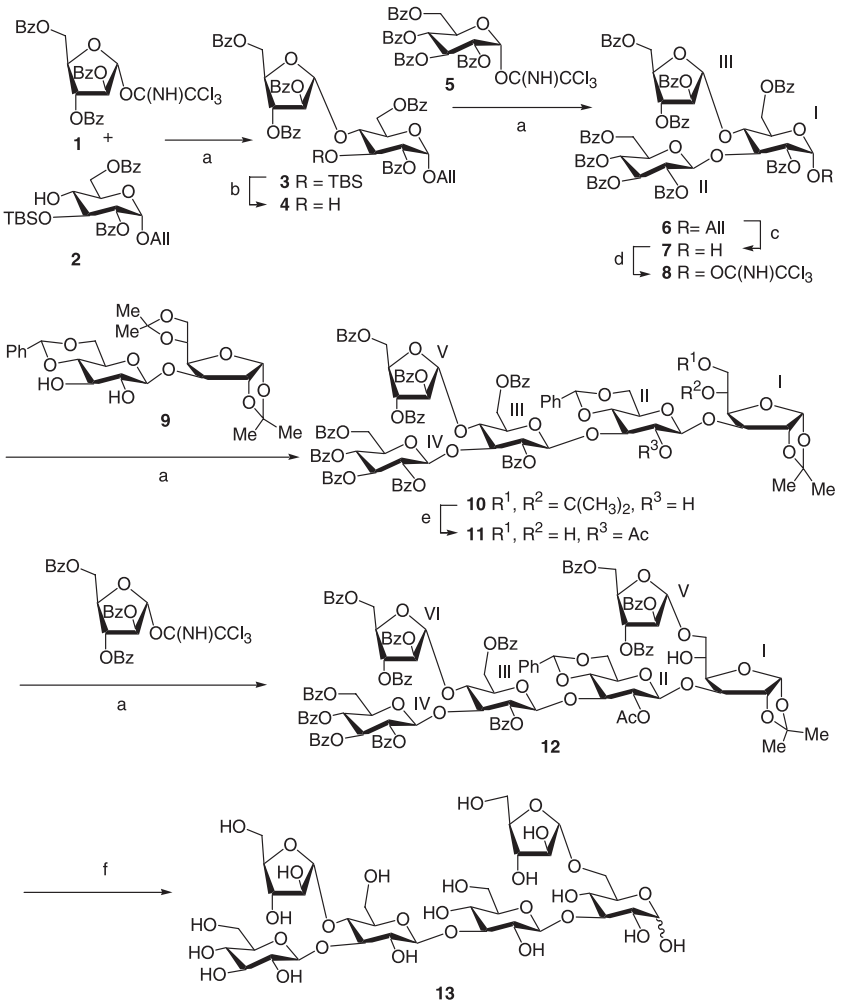
[a]To a solution of allyl 2,6-di-*O*-benzoyl-3-*O*-α-D-glucopyranoside (1.0 g, 2.33 mmol) in dry DMF (8 mL) was added imidazole (380 mg) and TBSCl (385 mg, 2.56 mmol) at rt. After 4 h, the mixture was worked up as usual to give syrupy allyl 2,6-di-*O*-benzoyl-3-*O*-*tert*-butyldimethylsilyl-α-D-glucopyranoside (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 ^{1}H NMR (400 MHz, $CDCl_3$): −0.14, 0.17 (2 s, 6 H, 2 SiCH_3), 0.73 (s, 9 H, C(CH_3)$_3$), 3.99 (dd, 1 H, J 7.3, 13.2 Hz, OCH_2–CH═CH_2), 4.16–4.20 (m, 2 H, H-5, OCH_2–CH═CH_2), 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, OCH$_2$–CH═CH_2), 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, OCH$_2$–CH═CH_2), 5.80–5.84 (m, 1 H, OCH$_2$–CH═CH$_2$), 7.43–8.25 (m, 10 H, Ph). These spectra assignments are consistent with the proposed structure for **2**.

[b]Compound **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, CH_2Cl_2, 78.4% for **3**; 80.4% for **6**; 65% for **12**; b) 90% TFA, 95%. c) Pd_2Cl_2, MeOH; d) CCl_3CN, DBU, CH_2Cl_2, 68.3% for two steps; e) Ac_2O, Pyr; CH_3CN, $CeCl_3{\cdot}6H_2O$, H_2O, 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→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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Table 1. Preliminary studies on antitumor activity of hexasaccharide **13**.

Sample	Dose (mg/Kg)	Tumor growth inhibition (%)	Body weight (g)		Tumor weigth (g)	p
			Day 1	Day 10		
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

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. ^{1}H NMR, ^{13}C NMR, ^{1}H–^{1}H COSY and HMQC spectra were recorded with ARX 400 spectrometers for solutions in $CDCl_3$ and D_2O. Chemical shifts are given in ppm downfield from internal Me_4Si, or DSS in case of D_2O. Mass spectra were measured using MALDI-TOF-MS with (α-cyano-4-hydroxycinnamic acid (CCA) as matrix. 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 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 CH_2Cl_2 (15 mL). To the solution was added Me_3SiOTf (35 μL, 0.19 mmol) under an N_2 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; $[\alpha_D]^{20}$ + 31° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ −0.01 (s, 3 H, SiC*H*$_3$), 0.16 (s, 3 H, SiC*H*$_3$), 0.68 (s, 9 H, C(C*H*$_3$)$_3$), 3.98–4.40 (m, 2 H, H-3^I, H-5^I), 4.13 (dd, 1 H, *J* 5.1, 11.9 Hz, OC*H*$_2$–CH=CH$_2$), 4.22 (dd, 1 H, *J* 5.1, 13.0 Hz, OC*H*$_2$–CH=CH$_2$), 4.45–4.53 (m, 4 H, H-6a^I, H-6b^I, H-4^{II}, H-4^I), 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, OCH$_2$–CH=C*H*$_2$), 5.15 (dd, 1 H, *J* 3.7, 9.5 Hz, H-2^I), 5.26–5.28 (m, 1 H, OCH$_2$–CH=C*H*$_2$), 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, OCH$_2$–C*H*=CH$_2$), 5.87 (s, 1 H, H-2^{II}), 7.37–8.29 (m, 25 H, Ph).

Anal. Calcd for $C_{55}H_{58}O_{15}Si$: 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→3)-[2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl-(1→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 μL, 0.19 mmol) under an N_2 atmosphere at 0°C. The mixture was stirred under this condition for 1.5 h, neutralized with Et_3N 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° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 3.90 (dd, 1 H, *J* 6.3, 14.3 Hz, OCH_2), 3.96–3.98 (m, 2 H, H-5^I, H-4^I), 4.25 (dd, 1 H, *J* 5.2, 14.3 Hz, OCH_2), 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, =C*H*), 5.90–5.92 (m, 2 H, H-1^{III}, H-2^{III}), 7.07–8.26 (m, 45 H, Ph). ^{13}C NMR (100 MHz, $CDCl_3$) δ 63.05 (C-6^{II}), 63.50 (C-6^I), 63.97 (C-5^{III}), 68.66 (OCH_2), 69.05 (C-4^I), 70.45 (C-4^{II}), 71.84 (C-2^{II}), 72.13 (C-5^{II}), 73.24 (C-3^{II}), 73.86 (C-2^I), 74.45 (C-5^I), 77.23 (C-3^I), 78.27 (C-3^{III}), 81.76 (C-2^{III}), 82.70 (C-4^{III}), 94.92 (C-1^I), 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 $C_{83}H_{70}O_{24}$: 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-D-arabinofuranosyl-(1→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 $PdCl_2$ (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 CH_2Cl_2 (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° (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) 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, N*H*).

Anal. Calcd for $C_{82}H_{66}Cl_3NO_{24}$: C, 63.31; H, 4.28. Found: C, 63.03; H, 4.31.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl-(1→4)]-2,6-di-*O*-benzoyl-β-D-glucopyranosyl(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→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 N_2 atmosphere at 0°C. The mixture was stirred under these conditions for 1.5 h, then neutralized with Et_3N, 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 CH_3CN, then $CeCl_3 \cdot 6H_2O$ (100 mg, 0.26 mmol) and H_2O (0.1 mL) were added. The mixture was stirred under reflux for 3 h, then diluted with water, extracted with methylene chloride (3×15 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; $[\alpha]_D^{20} - 4°$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.26 (s, 3 H, C*H*$_3$), 1.44 (s, 3 H, C*H*$_3$), 1.78 (s, 3 H, C*H*$_3$CO), 2.99 (d, 1 H, *J* 4.1 Hz, O*H*), 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, PhC*H*), 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); ^{13}C NMR (100 MHz, $CDCl_3$) 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 (CMe_2), 164.30, 164.86, 164.88, 165.16, 165.49, 165.52, 165.90, 166.11, 166.25, 168.31 (CO).

Anal. Calcd for $C_{104}H_{96}O_{35}$: C, 65.54; H, 5.08. Found: C, 65.27; H, 5.14.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-[2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl-(1→4)]-2,6-di-*O*-benzoyl-β-D-glucopyranosyl(1→3)-2-*O*-acetyl-4,6-*O*-benzylidene-β-D-glucopyranosyl-(1→3)-[2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl-(1→6)]-1,2-*O*-isopropylidene-α-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 μL, 0.05 mmol) under an N_2 atmosphere at −15°C. The mixture was stirred under these conditions for 1.5 h, then neutralized with Et_3N, 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; $[\alpha]_D^{20} - 6°$ (*c* 1, $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.16 (s, 3 H, C*H*$_3$), 1.65 (s, 3 H, C*H*$_3$), 2.35 (s, 3 H, C*H*$_3$CO), 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-6b^{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}, PhC*H*), 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). ^{13}C NMR (100 MHz, $CDCl_3$) 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 (PH*C*H), 111.82 (*C*Me_2).

Anal. Calcd for $C_{130}H_{116}O_{42}$: 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, H_2O); Selected ^{1}H NMR (D_2O) δ 4.41–4.72 (m, 3.3 H, H-$1^{I,II,III,IV}$ 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-1^{I} of α isomer). Selected ^{13}C NMR (100 MHz, D_2O) δ 103.02, 103.70 (2C), 103.99, 110.23, 110.87 (C-1^{I-VI}). MALDI-TOF-MS: Calcd for $C_{34}H_{58}O_{29}$: 930.3 [M]; Found 953.8 $[M+Na]^+$.

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