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Synthesis of L-arabinose-containing fragments of the oat root saponin Avenacin A-1

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Dedicated to Prof. Nirmolendu Roy, Indian Association for the Cultivation of Science, Calcutta, on the occasion of his retirement

Abstract—Chemical syntheses of two disaccharides, benzyl β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside (**1**) and benzyl β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranoside (**2**), and a trisaccharide, benzyl β-D-glucopyranosyl-(1 → 2)-3-*O*-acetyl-4-*O*-(β-D-glucopyranosyl)-α-L-arabinopyranoside (**3**), related to oat root triterpenoid saponin Avenacin A-1 are reported.

Keywords: Saponin; Aglycone; Di- and tri-saccharides

1. Introduction

Saponins are glycosylated plant secondary metabolites that are found in many major food crops.[1] Numerous plant species synthesize triterpenoid saponins as part of their normal programme of growth and development; examples include plants that are exploited as sources of drugs, such as ginseng and liquorice, and also crop plants, such as legumes and oats.[2] Because many saponins have potent antifungal properties and are present in healthy plants in high concentration, these molecules may act as preformed chemical barriers to fungal attack.[3] Despite commercial interest in this group of natural products, the genetic machinery required for the elaboration of this important family of plant secondary metabolites is as yet largely uncharacterized. One common feature shared by all saponins is the presence of a sugar chain attached to the aglycone at the C-3 hydroxyl position. The sugar chains differ substantially between saponins, but are often branched and may consist of up to five sugar units (usually selected from glucose, arabinose, glucuronic acid, xylose or rhamnose).[4] An understanding of the glycosylation process, which is believed to be the terminal stage in the saponin biosynthesis, is important since the presence of the C-3 sugar chain is critical for saponin biological activity.[5] To obtain a better understanding of the glycosyltransferases involved and in order to establish the order of events in saponin biosynthesis, synthetic saccharide fragments would be very useful. Herein we describe the syntheses of two disaccharides, **1** and **2**, and a trisaccharide, **3**, related to the oat root triterpenoid saponin Avenacin A-1 (Fig. 1).[6]

2. Results and discussion

With appropriate use of protecting groups, benzyl α-L-arabinopyranoside **4**[7] provides a convenient starting point for the preparation of the requisite 1,2- and 1,4-linked disaccharides, **1** and **2**, respectively, as well as the related trisaccharide **3**. Acetonide protection of the 3,4-*cis*-diol of **4** provides direct access to the 2-OH group for glycosylation in connection with synthesis of **1**. On the other hand, use of tin acetal chemistry potentially enables direct protection of the 3-OH group of **4** leaving the selective mono-O-acylation of the equatorial 2-OH

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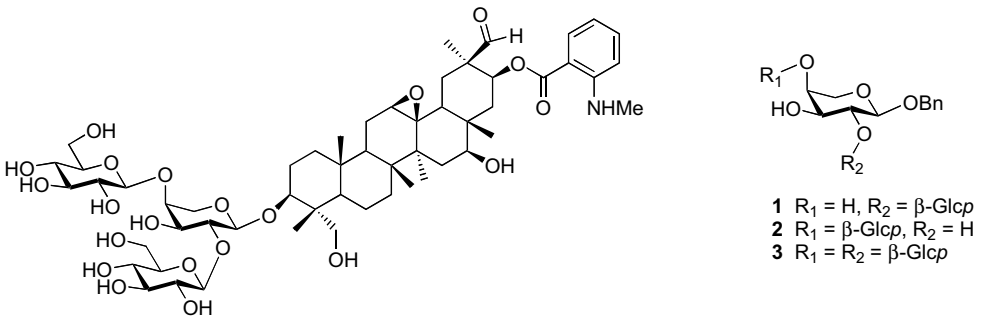
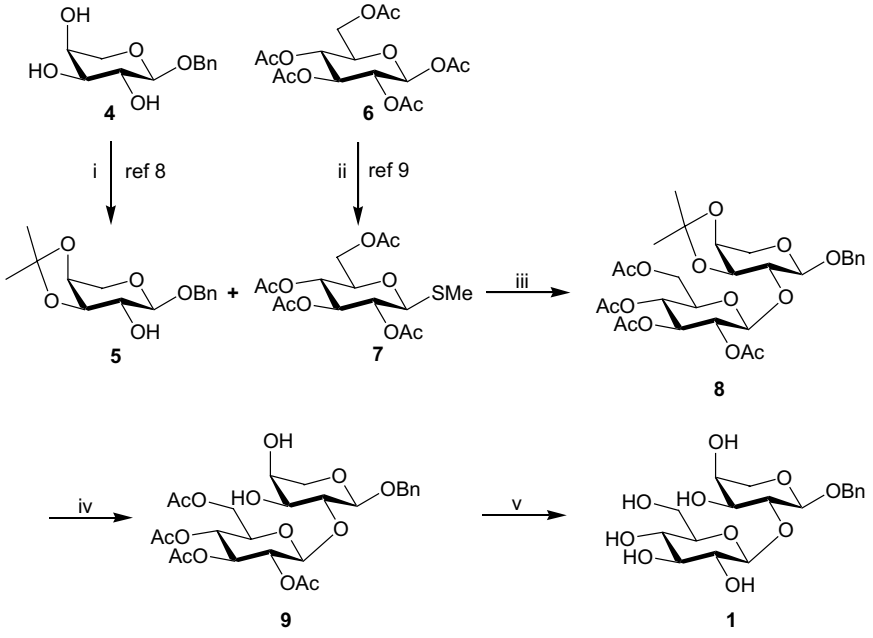


Figure 1. Structures of Avenacin A-1, target disaccharides, **1** and **2**, and trisaccharide, **3**.

over the axial 4-OH group, and subsequently glycosylation of the latter, to provide access to 1,4-linked disaccharide **2**. Alternatively, di-O-glycosylation of the same 3-O-protected diol intermediate furnishes the protected form of branched trisaccharide **3** directly.

Starting from benzyl α-L-arabinopyranoside **4**,[7] benzyl (3,4-*O*-isopropylidene)-α-L-arabinopyranoside **5** was prepared in 95% yield by treatment with iodine in acetone.[8] In a separate experiment, commercially available β-D-glucose penta-O-acetate **6** was converted to the methyl thioglycoside donor, methyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside **7**, in 90% yield using a convenient iodine-hexamethyldisilane-dimethyldisulfide activation procedure developed in this laboratory.[9] In keeping with the use of a participating ester group at C-2 of the donor, glycosylation of acceptor **5** with donor **7** using *N*-iodosuccinimide and triflic acid[10] as an iodonium ion source gave the beta-linked disaccharide benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 2)-(3,4-*O*-isopropylidene)-β-L-arabinopyranoside **8** in 82% yield. Hydrolysis of the isopropylidene ketal using fluoroboric acid in methanol[11] afforded benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside **9** in 98% yield, which on subsequent de-O-acetylation under Zemplén conditions[12] gave the target disaccharide benzyl β-D-glucopyranosyl-(1 → 2)-α-L-arabinopyranoside **1** in 97% yield (Scheme 1).

In a separate experiment, benzyl α-L-arabinopyranoside **4** was treated with dibutyltin oxide in methanol to give the corresponding 3,4-*O*-stannylidene derivative,[13] which was subsequently treated with *p*-methoxybenzyl chloride in the presence of tetrabutylammonium bromide in toluene to afford benzyl 3-*O*-(4-methoxybenzyl)-α-L-arabinopyranoside **10** in 70% overall yield (Scheme 2). By analogy with the corresponding β-D-galactopyranosides where the 2-OH group is inherently more

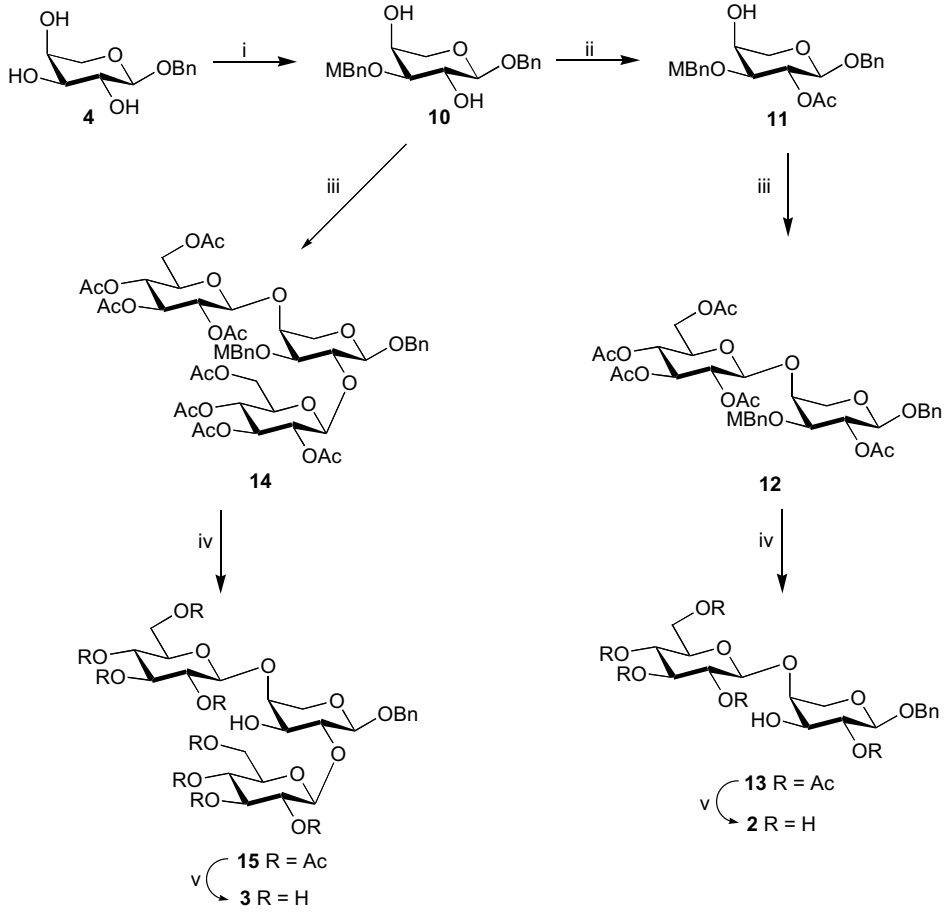


Scheme 1. Synthesis of 1,2-linked disaccharide. Reagents and conditions: (i) acetone, I_2; (ii) Me_2S_2, HMDS, I_2, CH_2Cl_2; (iii) donor **7**, NIS, TfOH, CH_2Cl_2, MS 4 Å; (iv) HBF_4, MeOH; (v) NaOMe, MeOH.

reactive than the 4-OH group,[14] arabinoside **10** was treated with acetyl chloride in CH_2Cl_2 and pyridine at 0 °C to afford 2-O-protected benzyl 2-*O*-acetyl-3-*O*-(4-methoxybenzyl)-α-L-arabinopyranoside **11** in 77% yield. The success of the reaction was evident from the downfield shift in the ^1H NMR signal of the H-2 proton (δ_H 3.85 ppm in **10** to δ_H 5.18 ppm in **11**). Subsequent glycosylation of arabinoside acceptor **11** with thioglucoside donor **7** using *N*-iodosuccinimide and triflic acid gave the disaccharide benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-2-*O*-acetyl-3-*O*-(4-methoxybenzyl)-α-L-arabinopyranoside **12** in 78% yield. Deprotection of the *p*-methoxybenzyl group with ceric ammonium nitrate in acetonitrile–water,[15] giving **13**, and subsequent de-O-acetylation with sodium methoxide in methanol gave the target disaccharide, benzyl β-D-glucopyranosyl-(1 → 4)-α-L-arabinopyranoside **2**, in 65% overall yield.

To access branched trisaccharide **3**, direct glycosylation of arabinoside diol acceptor **10** with 2.2 mol equiv of thioglucoside donor **7** using *N*-iodosuccinimide and triflic acid gave the required trisaccharide, benzyl 2,3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl-(1 → 4)-3-*O*-(4-methoxybenzyl)-2-*O*-(2,3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-α-L-arabinopyranoside **14**, in 74% yield (Scheme 2). The stoichiometry of this reaction is key; use of a greater excess of donor (e.g., 3 mol equiv of **7**) gave trisaccharide **14** in only 30% yield. This compound was accompanied by 2-O-acetylated 1,4-linked disaccharide **12** as the major product (35%), the latter arising from initial acetyl transfer from the donor to the 2-OH group of the acceptor followed by glycosylation of the 4-OH group. Deprotection as for disaccharide **12** gave the target trisaccharide, benzyl β-D-glucopyranosyl-(1 → 2)-3-*O*-acetyl-4-*O*-(β-D-glucopyranosyl)-α-L-arabinopyranoside **3**, in 65% overall yield.

NMR chemical shift data for the three synthetic compounds, **1**–**3** (Table 1) are in accord with those reported previously for the natural branched trisaccharide derived from Avenacin A-1.[6] Comparison of deuterated pyridine and deuterium oxide as NMR solvents shows no selective effects arising from pyridine complexation to one or other structure. Coupling constant data confirm the anomeric configurations (Glc $J_{1,2}$ values both 7.5 Hz; Ara $J_{1,2}$ 6.0 Hz) of the linkages synthesized and also confirm that 2,4-di-O-glycosylation of



Scheme 2. Synthesis of 1,4-linked disaccharide, and trisaccharide. Reagents and conditions: (i) Bu_2SnO, toluene, *p*-methoxybenzyl chloride, Bu_4NBr, toluene; (ii) AcCl, Py, CH_2Cl_2, 0 °C; (iii) donor **7**, NIS, TfOH, CH_2Cl_2; (iv) CAN, CH_3CN–H_2O (9:1); (v) NaOMe, MeOH.

Table 1. Comparison of ^1H and ^{13}C NMR chemical shift data of disaccharides (**1**) and (**2**) and trisaccharide (**3**) in C_5D_5N and D_2O (values in ppm)

	Solvent	Ara H-1	Glc-(1→2) H-1	Glc-(1→4) H-1	Ara C-1	Glc-(1→2) C-1	Glc-(1→4) C-1
(1→2)-Linked disaccharide (**1**)	C_5D_5N	4.95	5.28		103.2	105.4	
	D_2O	4.43	4.47		101.1	103.8	
(1→4)-Linked disaccharide (**2**)	C_5D_5N	5.05		5.23	103.3		105.1
	D_2O	4.43		4.47	100.3		103.0
Trisaccharide (**3**)	C_5D_5N	4.98	5.29	5.13	103.2	105.7	105.2
	D_2O	4.46	4.48	4.42	100.1	103.7	102.8
Natural trisaccharide[6]	C_5D_5N		5.31	5.08	103.5	105.6	105.5

the beta-linked L-arabinopyranose skeleton does not induce any significant change in the preferred $^4C_1$ chair conformation of this ring (Ara $J_{2,3}$ 8.4 Hz, $J_{3,4}$ 3.2 Hz).

In summary, we have established short scalable syntheses of the glycone, and fragments thereof, of the oat root saponin Avenacin A-1. Studies exploring the use of these compounds as potential glucosyltransferase substrates, and products, will be reported in due course.

3. Experimental

3.1. General methods

All reagents and solvents were dried prior to use according to standard methods.[16] Commercial reagents were used without further purification unless otherwise stated. Analytical TLC was performed on silica gel 60-F_{254} (Merck or Whatman) with detection by fluorescence and/or by charring following immersion in a 10% ethanolic solution of sulfuric acid. An orcinol dip, prepared by the careful addition of concentrated sulfuric acid (20 cm^3) to an ice-cold solution of 3,5-dihydroxytoluene (360 mg) in EtOH (150 cm^3) and water (10 cm^3), was used to detect deprotected compounds by charring. Flash chromatography was performed with silica gel 60 (Fluka). Optical rotations were measured at the sodium D-line at ambient temperature, with a Perkin Elmer 141 polarimeter. ^1H NMR and ^{13}C NMR spectra were recorded on a Varian Unity plus spectrometer at 400 and 100 MHz, respectively, using Me_4Si or CH_3OH as internal standards, as appropriate.

The following compounds were prepared essentially as described in the literature: benzyl α-L-arabinopyranoside **4**,[7] benzyl (3,4-*O*-isopropylidene)-α-L-arabinopyranoside **5**[8] and methyl 2,3,4,6-tri-*O*-acetyl-1-thio-β-D-glucopyranoside **7**.[9]

3.1.1. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-(3,4-*O*-isopropylidene)-α-L-arabinopyranoside (8). A suspension of isopropylidenated arabinoside **5** (500 mg, 1.8 mmol), thioglucoside **7** (880 mg, 2.3 mmol) and 4 Å molecular sieves (2 g) in 20 mL dry CH_2Cl_2 was stirred overnight under nitrogen. The mixture was cooled to −20 °C and NIS (1.45 g, 3.0 mmol) was added, followed by TfOH (57 μL, 0.3 mmol), and the mixture was stirred for 45 min. The mixture was then filtered through Celite® and the filtrate was diluted with CH_2Cl_2 (25 mL), washed with 10% aq $Na_2S_2O_3$ solution (30 mL × 2), aq $NaHCO_3$ solution (30 mL × 2) and water (30 mL × 2). The organic extract was dried (Na_2SO_4) and concentrated to a syrup. Flash chromatography (silica gel, 80 g; *n*-hexane–EtOAc 2:1) gave protected disaccharide **8** as an amorphous white solid (900 mg, 82%). $[\alpha]_D$ +49.3 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.34–7.24 (m, 5H, aromatic protons), 5.16 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.6 Hz, H-3′), 5.06 (t, 1H, $J_{3',4'}$, H-4′), 4.98 (dd, 1H, $J_{1',2'}$, 8.4 Hz, $J_{2',3'}$, H-2′), 4.85 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.80 (d, 1H, $J_{1',2'}$, H-1′), 4.57 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.47 (d, 1H, $J_{1,2}$ 6.4 Hz, H-1), 4.22 (m, 1H, H-4), 4.10 (dd, 1H, $J_{5',6'}$ 4.4 Hz, $J_{6a',b'}$ 12.4, H-6a′), 4.03 (dd, 1H, $J_{2,3}$ 7.6 Hz, H-3), 3.98 (dd, 1H, $J_{4,5a}$ 4.8 Hz, $J_{5a,5b}$ 12.8 Hz, H-5a), 3.88 (dd, $J_{5',b'}$, $J_{6a',b'}$, H-6b′), 3.74 (t, 1H, $J_{1,2}$, $J_{2,3}$, H-2), 3.71 (dd, 1H, $J_{4,5b}$ 4.4 Hz, $J_{5a,5b}$, H-5b), 3.50 (m, 1H, H-5′), 1.98, 1.97, 1.96, 1.95 (4s, 12H, 4 × COCH_3), 1.47, 1.30 (2s, 6H, 2 × isopropylidene–CH_3); ^{13}C NMR ($CDCl_3$): 170.5, 170.2, 169.3, 169.2 (4 × $COCH_3$), 137.4, 128.2, 127.5, 127.2 (aromatic carbons), 109.9 [$C(CH_3)_2$], 100.9 (C-1′), 99.8 (C-1), 81.5, 77.3, 72.7, 72.3, 71.8, 71.5, 69.8 (CH_2Ph), 68.1, 62.1 (C-6′), 61.7 (C-5), 27.7, 25.7 (2 × isopropylidene–CH_3), 20.6, 20.5, 20.4, 20.3 (4 × COCH_3). HRMS: calcd for $C_{29}H_{42}O_{14}N$ (M+NH_4): 628.2600; found: *m*/*z* 628.2606.

3.1.2. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside (9). To a solution of protected disaccharide **8** (500 mg, 0.8 mmol) in MeOH (15 mL) was added aq HBF_4 (50 μL, 48% w/v) and the solution was stirred at room temperature for 30 min. Solvents were removed in vacuo, the residue was dissolved in CH_2Cl_2 (30 mL), washed with aq $NaHCO_3$ solution (25 mL × 2) and water (25 mL × 2). The organic extract was dried (Na_2SO_4) and concentrated to give the title compound **9** as colourless syrup (460 mg, 98%). $[\alpha]_D$ +31.9 (*c* 1.1, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.35–7.24 (m, 5H, aromatic protons), 5.14 (t, 1H, $J_{2',3'} = J_{3',4'}$ 9.6 Hz,

H-3′), 5.03 (t, 1H, $J_{3',4'}$, H-4′), 4.96 (dd, 1H, $J_{1',2'}$, 7.6 Hz, $J_{2',3'}$, H-2′), 4.84 (d, 1H, J 11.6 Hz, CH_2Ph), 4.70 (d, 1H, $J_{1',2'}$, H-1′), 4.68 (d, 1H, $J_{1,2}$ 6.8 Hz, H-1), 4.51 (d, 1H, J 12.0 Hz, CH_2Ph), 4.09 (m, 1H, H-6a′), 3.89 (dd, 1H, $J_{5,6b}$ 2.4 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b′), 3.83–3.79 (m, 2H, H-2, H-4), 3.78–3.73 (m, 2H, H-3, H-5a), 3.57 (dd, 1H, $J_{4,5}$ 3.6 Hz, $J_{5a,5b}$ 12.0 Hz, H-5b), 3.48 (m, 1H, H-5′), 2.02, 2.00, 1.97, 1.96 (4s, 12H, 4×COCH_3); ^{13}C NMR ($CDCl_3$): 170.5, 170.1, 169.8, 169.3 (4×*C*OCH$_3$), 136.7, 128.5, 128.0, 127.6 (aromatic carbons), 101.1 (C-1′), 99.2 (C-1), 78.3, 72.5, 71.9, 71.6, 70.2 (*C*H$_2$Ph), 70.1, 67.9, 65.6, 61.8 (C-5), 61.5 (C-6′), 20.6, 20.5, 20.4, 20.3 (4×CO*C*H$_3$). HRMS: calcd for $C_{26}H_{38}O_{14}N$ (M+NH_4): 588.2292; found: m/z 588.2289.

3.1.3. Benzyl β-D-glucopyranosyl-(1→2)-α-L-arabinopyranoside (1). Methanolic NaOMe solution (0.5 M, 1 mL) was added to a solution of protected disaccharide **9** (400 mg, 0.7 mmol) in MeOH (10 mL) and the solution was allowed to stir for 3 h at room temperature. The solution was then neutralized with Dowex® 50W (H^+) resin, filtered and concentrated to get the title disaccharide **1** as amorphous white solid (270 mg, 97%). $[\alpha]_D$ +13.6 (*c* 0.8, H_2O); ^{1}H NMR (D_2O): 7.29–7.20 (m, 5H, aromatic protons), 4.69 (d, 1H, J 11.6 Hz, CH_2Ph), 4.50 (d, 1H, J 11.6 Hz, CH_2Ph), 4.47 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.43 (d, 1H, $J_{1,2}$ 6.0 Hz, H-1), 3.76 (m, 1H, H-4), 3.72 (dd, 1H, $J_{4,5a}$ 3.6 Hz, $J_{5a,5b}$ 12.4 Hz, H-5a), 3.69 (dd, 1H, $J_{2,3}$ 8.0 Hz, $J_{3,4}$ 3.2 Hz, H-3), 3.64 (dd, 1H, $J_{1,2}$, $J_{2,3}$, H-2), 3.51 (dd, 1H, $J_{5',6a'}$ 2.0 Hz, $J_{6a',b'}$ 12.4, H-6a′), 3.46–3.42 (m, 2H, H-5b, H-6b′), 3.27 (t, 1H, $J_{2',3'} = J_{3',4'}$ 9.2 Hz, H-3′), 3.19 (t, 1H, $J_{3',4'}$, H-4′), 3.11 (m, 1H, H-5′), 3.08 (dd, 1H, $J_{1',2'}$, $J_{2',3'}$, H-2′); ^{13}C NMR (D_2O): 137.3, 129.2, 129.1, 128.8 (aromatic carbons), 103.8 (C-1′), 100.1 (C-1), 78.1, 76.3, 76.1, 73.8, 70.2 (*C*H$_2$Ph), 70.1, 69.5, 68.1, 63.2 (C-5), 61.4 (C-6′). HRMS: calcd for $C_{18}H_{30}O_{10}N$ (M+NH_4): 420.1864; found: m/z 420.1865.

3.1.4. Benzyl 3-*O*-(4-methoxybenzyl)-α-L-arabinopyranoside (10). A suspension of benzyl α-L-arabinopyranoside **4** (2.4 g, 10 mmol) and Bu_2SnO (2.7 g, 11 mmol) in dry MeOH (80 mL) was refluxed for 3 h (until the solution became clear). Solvents were evaporated under reduced pressure and residual MeOH was co-evaporated with toluene. The residue was dissolved in dry toluene, *p*-methoxybenzyl chloride (25 mmol) and Bu_4NBr (3.5 g, 11 mmol) were added and the mixture was stirred at 60 °C for 16 h. Solvents were evaporated and the residue was fractionated by column chromatography using *n*-hexane–EtOAc (1:1) to afford compound **10** (2.5 g, 70%) as colourless glass. $[\alpha]_D$ +47.9 (*c* 0.9, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 7.38–7.25 (m, 9H, aromatic protons), 4.91 (d, 1H, J 11.7 Hz, CH_2Ph), 4.69, 4.63 (2d, 2H, J 11.4 Hz, CH_2C$_6$H$_4$OMe), 4.59 (d, 1H, J 11.7 Hz, CH_2Ph), 4.25 (d, 1H, $J_{1,2}$ 7.2 Hz, H-1), 4.60 (dd, 1H, $J_{4,5a}$ 2.1 Hz, $J_{5a,5b}$ 12.9 Hz, H-5a), 3.87 (m, 1H, H-4), 3.85 (dd, 1H, $J_{1,2}$, $J_{2,3}$ 9.0 Hz, H-2), 3.79 (s, 3H, CH$_2$–C$_6$H$_4$–OCH_3), 3.43 (dd, 1H, $J_{4,5b}$ 1.5 Hz, $J_{5a,5b}$, H-5b), 3.42 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 3.6 Hz, H-3); ^{13}C NMR ($CDCl_3$): 159.6, 137.2, 129.8, 129.6, 128.5, 128.2, 127.9 114.0 (aromatic carbons), 101.9 (C-1), 79.4, 71.8 (*C*H$_2$–C$_6$H$_4$–OCH$_3$), 70.9 (*C*H$_2$Ph), 70.5, 66.3, 65.6 (C-5), 55.2 (CH$_2$–C$_6$H$_4$–O*C*H$_3$). HRMS: calcd for $C_{20}H_{28}O_6N$ (M+NH_4): 378.1917; found: m/z 378.1915.

3.1.5. Benzyl 2-*O*-acetyl-3-*O*-(4-methoxybenzyl)-α-L-arabinopyranoside (11). To an ice-cold solution of compound **10** (1.0 g, 2.8 mmol) in dry CH_2Cl_2 (15 mL) and pyridine (3 mL) was added acetyl chloride (215 μL, 3.0 mmol) and the mixture was allowed to stir for 1 h. When TLC (hexane–EtOAc 3:1) showed complete conversion, 1 mL of water was added and solvent was removed in vacuo. The residue was then purified by flash chromatography using hexane–EtOAc 3:1 to give partially protected arabinoside **11** (870 mg, 77%) as syrup. $[\alpha]_D$ +43.7 (*c* 0.7, $CHCl_3$); ^{1}H NMR ($CDCl_3$): 7.39–7.24 (m, 9H, aromatic protons), 5.18 (dd, 1H, $J_{1,2}$ 7.2 Hz, $J_{2,3}$ 9.0 Hz, H-2), 4.89 (d, 1H, J 11.7 Hz, CH_2Ph), 4.67, 4.61 (2d, 2H, J 11.4 Hz, CH_2C$_6$H$_4$OMe), 4.51 (d, 1H, J 11.7 Hz, CH_2Ph), 4.25 (d, 1H, $J_{1,2}$, H-1), 4.60 (dd, 1H, $J_{4,5a}$ 2.1 Hz, $J_{5a,5b}$ 12.9 Hz, H-5a), 3.84 (m, 1H, H-4), 3.77 (s, 3H, CH$_2$–C$_6$H$_4$–OCH_3), 3.41 (dd, 1H, $J_{4,5b}$ 1.5 Hz, $J_{5a,5b}$ 12.9 Hz, H-5b), 3.38 (dd, 1H, $J_{2,3}$, $J_{3,4}$, H-3), 2.02 (s, 3H, COCH_3). ^{13}C NMR ($CDCl_3$): 170.5 (*C*OCH$_3$), 159.1, 137.7, 129.4, 129.2, 128.3, 128.1, 127.9 114.2 (aromatic carbons), 101.7 (C-1), 79.8, 71.8 (*C*H$_2$–C$_6$H$_4$–OCH$_3$), 70.5 (*C*H$_2$Ph), 70.1, 66.1, 65.3, 55.1 (CH$_2$–C$_6$H$_4$–O*C*H$_3$), 20.1 (CO*C*H$_3$). HRMS: calcd for $C_{22}H_{30}O_7N$ (M+NH_4): 420.2022; found: m/z 420.2018.

3.1.6. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-3-*O*-(4-methoxybenzyl)-α-L-arabinopyranoside (12). A suspension of partially protected arabinoside **11** (500 mg, 1.2 mmol), thioglucoside **7** (610 mg, 1.6 mmol) and 4 Å molecular sieves (1 g) in 15 mL dry CH_2Cl_2 was stirred overnight under nitrogen atmosphere. The mixture was cooled to −20 °C, NIS (250 mg, 2.1 mmol) was added followed by TfOH (10 μL, 0.21 mmol), the mixture was stirred for 40 min, filtered through Celite® and the filtrate was diluted with CH_2Cl_2 (25 mL). The solution was washed with 10% aq $Na_2S_2O_3$ solution (30 mL×2), aq $NaHCO_3$ solution (30 mL×2) and water (30 mL×2), the organic layer was separated, dried (Na_2SO_4) and concentrated to a syrup. Flash chromatography (silica gel, 40 g; *n*-hexane–EtOAc 1:1) gave protected 1,2-linked disaccharide **12** as an amorphous white solid (685 mg, 78%). $[\alpha]_D$ +32.9 (*c* 0.6, $CHCl_3$); ^{1}H NMR: 7.38–7.25 (m, 5H, CH$_2$C$_6$*H*$_5$), 7.23, 6.86 (2d, 4H, CH$_2$C$_6$*H*$_4$OMe), 5.17 (dd, 1H, $J_{1,2}$ 7.6 Hz, $J_{2,3}$ 9.2 Hz, H-2), 5.11–5.08 (m, 2H, H-3′, H-4′), 4.99 (dd, 1H, $J_{1',2'}$ 8.0 Hz, $J_{2',3'}$ 9.6 Hz, H-2′), 4.92 (d, 1H, J 12.0 Hz, CH_2Ph), 4.89 (d, 1H, $J_{1',2'}$, H-1′), 4.56 (d, 1H, J

12.0 Hz, CH_2Ph), 4.48 (d, 1H, J 10.8 Hz, CH_2C$_6$H$_4$OMe), 4.42 (d, 1H, $J_{1,2}$, H-1), 4.40 (d, 1H, J 10.8 Hz, CH_2C$_6$H$_4$OMe), 4.09 (dd, 1H, $J_{4,5a}$ 4.0 Hz, $J_{5a,5b}$ 12.8 Hz, H-5a), 3.95 (dd, 1H, $J_{5',6a'}$ 2.8 Hz, $J_{6a',b'}$ 13.2 Hz, H-6a′), 3.88 (m, 1H, H-4), 3.87 (m, 1H, H-6b′), 3.78 (s, 3H, CH$_2$C$_6$H$_4$OCH_3), 3.49 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 3.6 Hz, H-3), 3.44–3.41 (m, 2H, H-5b, H-5′), 2.10, 2.09, 2.05, 2.00, 1.98 (5s, 15H, 5×COCH_3); ^{13}C NMR (CDCl$_3$): 170.7, 170.5, 170.4, 170.0, 169.4 (5×COCH$_3$); 159.6, 137.5, 130.5, 129.3, 128.4, 127.7, 127.3, 113.9 (aromatic carbons); 101.7 (C-1′), 100.7 (C-1), 77.6, 76.8, 73.0, 72.1, 71.8, 71.7 (CH$_2$C$_6$H$_4$OCH$_3$), 70.8 (CH$_2$Ph), 67.9, 67.3, 63.4 (C-5), 61.7 (C-6′), 55.1 (CH$_2$C$_6$H$_4$OCH$_3$), 20.9, 20.5, 20.4, 20.3, 20.2 (5×COCH$_3$). HRMS: calcd for C$_{36}$H$_{48}$O$_{16}$N (M+NH$_4$): 750.2973; found: m/z 750.2969.

3.1.7. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→4)-2-*O*-acetyl-α-L-arabinopyranoside (13). To a solution of protected disaccharide **12** (500 mg, 0.7 mmol) in CH$_3$CN–H$_2$O (9:1, 25 mL) was added CAN (770 mg, 1.4 mmol) and the mixture was stirred at rt for 30 min. Solvents were removed in vacuo, the resulting residue dissolved in CH$_2$Cl$_2$ (20 mL) and washed successively with NaHCO$_3$ (2×15 mL) and then water (2×15 mL), organic layer was separated, dried (Na$_2$SO$_4$) and concentrated, and the residue was purified by flash chromatography using 1:1 n-hexane–EtOAc to give partially protected disaccharide **13** as a colourless glass (310 mg, 70%). [α]$_D$ +23.2 (c 1.0, CHCl$_3$); ^{1}H NMR (CDCl$_3$): 7.31–7.26 (m, 5H, aromatic protons), 5.14 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.3 Hz, H-3′), 5.03 (t, 1H, $J_{3',4'}$, H-4′), 5.00 (dd, 1H, $J_{1,2}$ 6.3 Hz, $J_{2,3}$ 8.4 Hz, H-2), 4.96 (dd, 1H, $J_{1',2'}$ 7.8 Hz, $J_{2',3'}$, H-2′), 4.85 (d, 1H, J 11.7 Hz, CH$_2$Ph), 4.73 (d, 1H, $J_{1',2'}$, H-1′), 4.64 (d, 1H, $J_{1,2}$, H-1), 4.54 (d, 1H, J 11.7 Hz, CH_2Ph), 4.09 (dd, 1H, $J_{4,5a}$ 3.9 Hz, $J_{5a,5b}$ 12.3 Hz, H-5a), 3.95–3.88 (m, 3H, H-3, H-5b, H-6a′), 3.80 (m, 1H, H-4), 3.58–3.48 (m, 2H, H-5′, H-6b′), 3.01 (br s, 1H, OH), 2.06, 2.03, 1.99, 1.96, 1.95 (5×COCH_3); ^{13}C NMR (CDCl$_3$): 170.6, 170.3, 170.2, 170.0, 169.4 (5×COCH$_3$); 136.8, 128.5, 128.0, 127.6 (aromatic carbons); 100.8 (C-1′), 99.8 (C-1), 78.2, 72.4, 71.9, 71.5, 70.5 (CH$_2$Ph), 68.6, 68.4, 67.9, 61.4 (C-5), 60.0 (C-6′), 20.8, 20.5, 20.4, 20.3, 20.2 (5×COC_3). HRMS: calcd for C$_{28}$H$_{40}$O$_{15}$N (M+NH$_4$): 630.2398; found: m/z 630.2394.

3.1.8. Benzyl β-D-glucopyranosyl-(1→4)-α-L-arabinopyranoside (2). Methanolic NaOMe solution (0.5 M, 2 mL) was added to a solution of partially protected 1,4-linked disaccharide **13** (200 mg) in MeOH (20 mL) and the solution was stirred for 3 h at room temperature. The solution was then neutralized with Dowex® 50W (H$^+$) resin, filtered and concentrated to get the title disaccharide **2** as amorphous white solid (120 mg, 95%). [α]$_D$ +19.4 (c 0.9, H$_2$O); ^{1}H NMR (D$_2$O): 7.28–7.19 (m, 5H, aromatic protons); 4.69 (d, 1H, J 12.0 Hz, CH_2Ph), 4.50 (d, 1H, J 12.0 Hz, CH_2Ph), 4.47 (d, 1H, $J_{1',2'}$ 8 Hz, H-1′), 4.43 (d, 1H, $J_{1,2}$ 6.4 Hz, H-1), 3.76 (m, 1H, H-4), 3.71 (dd, 1H, $J_{4,5a}$ 3.6 Hz, $J_{5a,5b}$ 12.0 Hz, H-5a), 3.68 (dd, 1H, $J_{2,3}$ 8.0 Hz, $J_{3,4}$ 3.2 Hz, H-3), 3.64 (dd, 1H, $J_{1,2}$, $J_{2,3}$, H-2), 3.52 (dd, 1H, $J_{5',6a'}$ 2.0 Hz, $J_{6a',b'}$ 12.4 Hz, H-6a′), 3.47–3.41 (m, 2H, H-5b, H-6b′), 3.26 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.2 Hz, H-3′), 3.18 (t, 1H, $J_{3',4'}$, H-4′), 3.12 (m, 1H, H-5′), 3.08 (dd, 1H, $J_{1',2'}$, $J_{2',3'}$, H-2′); ^{13}C NMR (D$_2$O): 136.8, 128.8, 128.5 (aromatic carbons); 103.0 (C-1′); 100.3 (C-1), 78.7, 76.0, 75.6, 73.8, 71.7, 71.2 (CH$_2$Ph), 69.5, 67.6, 64.8 (C-5), 60.7 (C-6′). HRMS: calcd for C$_{18}$H$_{30}$O$_{10}$N (M+NH$_4$): 420.1864; found: m/z 420.1865.

3.1.9. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-3-*O*-(4-methoxybenzyl)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-arabinopyranoside (14). A suspension of methoxybenzylated arabinoside **10** (500 mg, 1.4 mmol), thioglucoside **7** (1.6 g, 4.2 mmol) and 4 Å molecular sieves (2 g) in 20 mL dry CH$_2$Cl$_2$ was stirred overnight under nitrogen. The mixture was cooled to −20 °C and NIS (661 mg, 5.5 mmol) was added followed by TfOH (26 μL, 0.55 mmol), the mixture was stirred for 40 min, filtered through Celite® and diluted with CH$_2$Cl$_2$ (25 mL). The resulting solution was washed with 10% aq Na$_2$S$_2$O$_3$ solution (30 mL×2), aq NaHCO$_3$ solution (30 mL×2) and water (30 mL×2). The organic extract was dried (Na$_2$SO$_4$) and concentrated to a syrup. Flash chromatography (silica gel, 40 g; n-hexane–EtOAc 1:1) gave protected trisaccharide **14** as an amorphous white solid (1.1 g, 74%). [α]$_D$ +27.9 (c 0.6, CHCl$_3$); ^{1}H NMR (CDCl$_3$): 7.27–7.15 (m, 5H, CH$_2$C$_6$$H_5$); 7.17, 6.81 (2d, 4H, CH$_2$C$_6$$H_4$OMe), 5.08 (t, 1H, $J_{2',3'}$, $J_{3',4'}$ 9.2 Hz, H-3′), 5.06 (t, 1H, $J_{2'',3''}$, $J_{3'',4''}$ 9.2 Hz, H-3″), 5.02 (t, 1H, $J_{3',4'}$, H-4′), 4.99 (t, 1H, $J_{3'',4''}$, H-4″), 4.93 (dd, $J_{1',2'}$ 8.0 Hz, $J_{2',3'}$, H-2′), 4.91 (dd, $J_{1'',2''}$ 8.0 Hz, $J_{2'',3''}$, H-2″), 4.83 (d, 1H, J 11.6 Hz, CH_2Ph), 4.68 (d, 1H, $J_{1',2'}$, H-1′), 4.54 (d, 1H, $J_{1'',2''}$, H-1″), 4.44 (2d, 2H, J 11.6 Hz, CH_2C$_6$H$_4$OMe), 4.38 (d, $J_{1,2}$ 6.4 Hz, H-1), 4.35 (d, 1H, J 11.6 Hz, CH_2Ph), 4.12–4.17 (m, 1H, H-6a′), 4.03–3.99 (m, 2H, H-6b′, H-6a″), 3.96 (dd, 1H, $J_{4,5a}$ 4.0 Hz, $J_{5a,5b}$ 11.2 Hz, H-5a), 3.89 (m, 1H, H-4), 3.77 (dd, 1H, $J_{5'',6b''}$ 2.0 Hz, $J_{6a'',6b''}$ 10.0 Hz, H-6b″), 3.75 (dd, 1H, $J_{1,2}$, $J_{2,3}$ 8.8 Hz, H-2), 3.71 (s, 3H, CH$_2$C$_6$H$_4$OCH_3), 3.51 (m, 1H, H-5′), 3.36 (dd, 1H, $J_{2,3}$, $J_{3,4}$ 2.8 Hz, H-3), 3.32–3.29 (m, 2H, H-5b, H-5″), 2.02, 1.99(2), 1.98(2), 1.97, 1.96, 1.95 (8s, 24H, 8×COCH_3); ^{13}C NMR (CDCl$_3$): 170.5(2), 170.2, 170.1, 169.8 (2), 169.8, 169.5 (8×COCH$_3$), 159.3, 137.6, 129.7, 129.4, 128.0, 127.3, 127.0, 113.7 (aromatic carbons), 101.4 (C-1′), 100.7 (C-1″), 100.5 (C-1), 78.3, 77.7, 74.7, 74.6, 72.7, 72.6, 72.3, 71.5, 71.4 (CH$_2$C$_6$H$_4$OCH$_3$), 71.1 (CH$_2$Ph), 69.9, 69.5, 68.2, 67.8, 67.5, 67.2, 62.7 (C-5), 61.6 (C-6′), 61.4 (C-6″), 54.9 (CH$_2$C$_6$H$_4$OCH$_3$), 20.6(2), 20.1(2), 20.0(2), 19.9(2) (8×COCH$_3$). HRMS: calcd for C$_{48}$H$_{64}$O$_{24}$N (M+NH$_4$): 1038.3818; found: m/z 1038.3824.

3.1.10. Benzyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl-(1→2)-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-arabinopyranoside (15). To a solution of protected trisaccharide **14** (200 mg, 0.3 mmol) in CH_3CN–H_2O (9:1, 10 mL) was added CAN (330 mg, 0.6 mmol) and the mixture was stirred at rt for 30 min. Solvents were removed in vacuo, the residue was dissolved in CH_2Cl_2 (20 mL) and washed successively with $NaHCO_3$ (2×15 mL) and then water (15 mL), organic extract was separated and dried (Na_2SO_4) and concentrated, and the residue was purified by flash chromatography using 1:1 *n*-hexane–EtOAc to give partially protected trisaccharide **15** as a white powder (120 mg, 70%). $[\alpha]_D$ +36.8 (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): 7.28–7.22 (m, 5H, aromatic protons); 5.15 (t, 1H, $J_{2',3'} = J_{3',4'}$ 9.6 Hz, H-3′), 5.10 (t, 1H, $J_{2'',3''} = J_{3'',4''}$ 9.6 Hz, H-3″), 4.99 (t, 1H, $J_{3',4'}$, H-4′), 4.97 (t, 1H, $J_{3'',4''}$, H-4″), 4.90 (dd, $J_{1',2'}$ 6.9 Hz, $J_{2',3'}$, H-2′), 4.89 (dd, $J_{1'',2''}$ 7.5 Hz, $J_{2'',3''}$, H-2″), 4.81 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.64 (d, 1H, $J_{1',2'}$, H-1′), 4.62 (d, 1H, $J_{1'',2''}$, H-1″), 4.60 (d, $J_{1,2}$ 6.9 Hz, H-1), 4.45 (d, 1H, *J* 12.0 Hz, CH_2Ph), 4.17 (dd, 1H, $J_{5',6a'}$ 3.6 Hz, $J_{6a',b'}$ 12.3 Hz, H-6a′), 4.10–4.05 (m, 2H, H-6b′, H-6a″), 4.03 (dd, 1H, $J_{4,5a}$ 4.2 Hz, $J_{5a,5b}$ 12.6 Hz, H-5a), 3.94 (m, 1H, H-3), 3.85 (m, 1H, H-4), 3.79 (dd, 1H, $J_{5'',6b''}$ 2.1 Hz, $J_{6a'',6b''}$ 12.3 Hz, H-6b″), 3.69–3.62 (m, 2H, H-2, H-5′), 3.54 (dd, 1H, $J_{4,5b}$ 2.7 Hz, $J_{5a,5b}$, H-5b), 3.42 (m, 1H, H-5″), 2.87 (br s, 1H, O*H*), 2.09, 2.08, 2.06, 2.04, 2.02, 2.01, 1.99, 1.97 (8s, 24H, 8×COCH_3); ^{13}C NMR ($CDCl_3$): 170.6, 170.5, 170.2, 170.1, 169.7 (2), 169.6, 169.3 (8×*C*OCH$_3$), 136.9, 128.3, 127.8, 127.5 (aromatic carbons), 101.4 (C-1′), 101.1 (C-1″), 99.1 (C-1), 79.3, 74.9, 72.4, 72.3, 71.7, 71.6, 71.5, 71.3 (*C*H_2Ph), 69.9, 69.7, 68.1, 67.8, 61.7 (C-5), 61.3 (C-6′), 60.1 (C-6″), 20.4(2), 20.3(2), 20.2(2), 20.0(2) (8×*C*OCH$_3$). HRMS: calcd for $C_{40}H_{56}OH_{23}N$ (M+NH_4): 918.3238; found: *m*/*z* 918.3232.

3.1.11. Benzyl β-D-glucopyranosyl-(1→2)-4-*O*-(β-D-glucopyranosyl)-α-L-arabinopyranoside (3). To a solution of partially protected trisaccharide **15** (100 mg, 0.18 mmol) in dry MeOH (10 mL), NaOMe was added and the solution was stirred at rt for 3 h. The solution was neutralized with Dowex® 50W (H^+) resin and filtered. Deprotected trisaccharide **3** was obtained as white powder upon evaporation of solvents (70 mg, 95%). $[\alpha]_D$ +13.1 (*c* 0.9, H_2O); ^1H NMR (D_2O): 7.29–7.19 (m, 5H, aromatic protons), 4.69 (d, 1H, *J* 11.6 Hz, CH_2Ph), 4.50 (d, 1H, *J* 11.6 Hz, CH_2Ph, 4.48 (d, 1H, $J_{1',2'}$ 8.0 Hz, H-1′), 4.46 (d, 1H, $J_{1,2}$ 6.4 Hz, H-1), 4.42 (d, 1H, $J_{1'',2''}$ 8.0 Hz, H-1″), 3.98 (dd, 1H, $J_{4,5a}$ 4.2 Hz, $J_{5a,5b}$ 12.6 Hz, H-5a), 3.90 (m, 1H, H-4), 3.79 (dd, 1H, $J_{2,3}$ 8.4 Hz, $J_{3,4}$ 3.2 Hz, H-3), 3.70 (dd, 1H, $J_{1,2}$, $J_{2,3}$, H-2), 3.69 (m, 1H, H-6a′), 3.56–3.48 (m, 2H, H-6b′, H-6a″), 3.47–3.42 (m, 2H, H-5b, H-6b″), 3.32–3.22 (m, 5H, H-3′, H-3″, H-4′, H-4″, H-5′), 3.20 (t, $J_{1',2'}$, $J_{2',3'}$, H-2′), 3.12–3.08 (m, 2H, H-2″, H-5″); ^{13}C NMR (D_2O): 136.7, 128.8, 128.5 (aromatic carbons), 103.7 (C-1′), 102.8 (C-1″), 100.1 (C-1), 78.3, 76.0, 75.9, 75.7, 75.6, 73.7(2), 73.4, 71.2, 71.1 (*C*H_2Ph), 69.6, 69.5, 60.7 (3 carbons, C-5, C-6′, C-6″). HRMS: calcd for $C_{24}H_{40}O_{15}N$ (M+NH_4): 582.2392; found: *m*/*z* 582.2389.

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