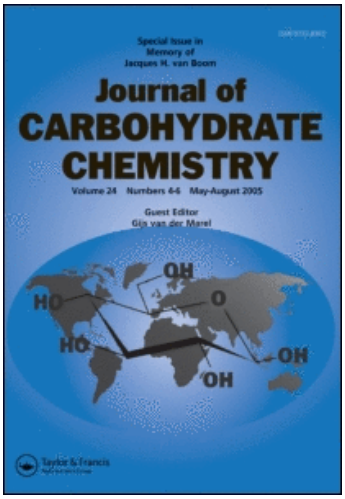

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Synthesis of Oligosaccharide Fragments of the Lipoarabinomannan from *Rhodococcus ruber*

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Synthesis of Oligosaccharide Fragments of the Lipoarabinomannan from *Rhodococcus ruber*

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The synthesis of two oligosaccharide fragments (**1** and **2**) of the lipoarabinomannan from *Rhodococcus ruber* is reported. Thioglycoside donors were used to assemble these glycans, which were prepared as their 8-(methoxycarbonyl)octyl glycosides for potential incorporation into neoglycoconjugates.

Keywords Arabinofuranosides, Glycosylation, Lipoarabinomannan, Thioglycosides

INTRODUCTION

A major immunomodulatory molecule in mycobacteria, including the human pathogen *Mycobacterium tuberculosis*, is lipoarabinomannan (LAM), a cell wall polysaccharide composed of mannopyranose and arabinofuranose residues bound to a phosphatidylinositol moiety.[1,2] Structural differences in LAM molecules are observed among different mycobacterial species,[3–6] and an increasing number of recent papers have reported the structures of LAM molecules from other actinomycetes.[7–14] Among these[9] is the LAM from *Rhodococcus ruber* (RruLAM), a species closely related to the opportunistic human pathogen *Rhodococcus equi*.

The structure of RruLAM is similar to that of mycobacterial LAM in that the mannan domain consists of an α-(1 $\rightarrow$ 6)-linked chain of mannopyranose residues. However, unlike mycobacterial LAM, which has a large arabinan

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Dedicated to the memory of Jacques H. van Boom.
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motif attached to this mannan backbone, RruLAM has no separate arabinan domain per se. Instead, the arabinofuranose residues are found as single "capping" units attached α-(1 $\rightarrow$ 2) to the mannan backbone. Approximately 45% of the residues in the mannan core are capped at O-2, and the majority of these capping motifs are α-arabinofuranose residues, although in a small percentage of cases the α-arabinofuranose cap is replaced with an α-mannopyranose moiety. The proposed structure of RruLAM is shown in Figure 1.

We have a long-standing interest in the synthesis of oligosaccharide fragments of cell wall polysaccharides from mycobacteria and related organisms.[15–19] As part of this program, we were interested in the synthesis of RruLAM fragments, functionalized to allow future preparation of neoglycoconjugates. Reported here is the preparation of disaccharide **1** and trisaccharide **2** (Fig. 2), as their 8-(methoxycarbonyl)octyl glycosides. Another similarly functionalized fragment of RruLAM, disaccharide **3**, has previously been synthesized.[20]

RESULTS AND DISCUSSION

The synthesis of **1** and **2** relied on the use of the previously reported protected 8-methoxycarbonyloctyl glycosides **4**[20] and **5**[21] and the known thioglycosides **6**[22] and **7**.[23] With these building blocks in hand, the preparation of the two targets was straightforward. The synthesis of **1** (Sch. 1) involved first the

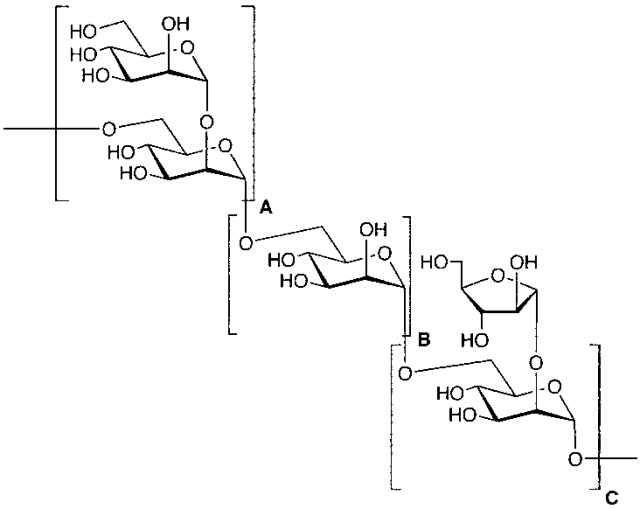


Figure 1: Proposed structure of the lipoarabinomannan from *Rhodococcus ruber* (RruLAM). Approximately 45% of the α-(1 $\rightarrow$ 6)-linked D-Man*p* residues are capped. The values for A, B, and C are approximately $\leq$1, 15, and 11, respectively.

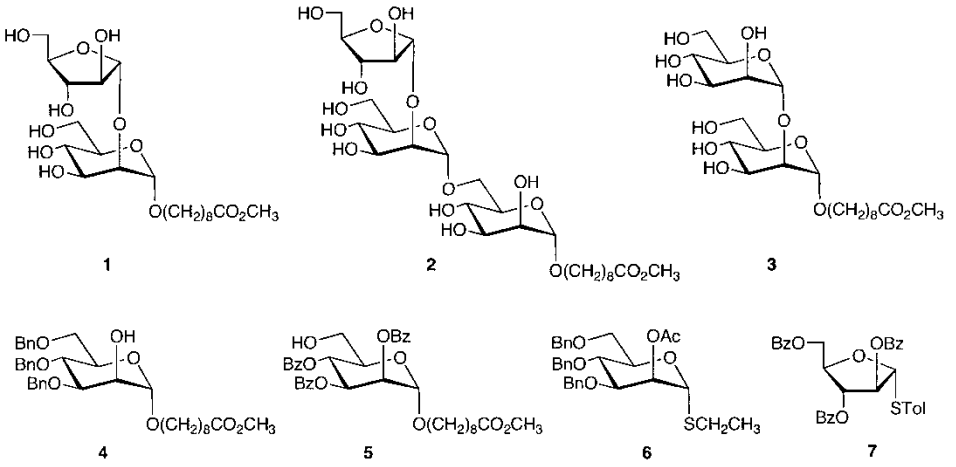
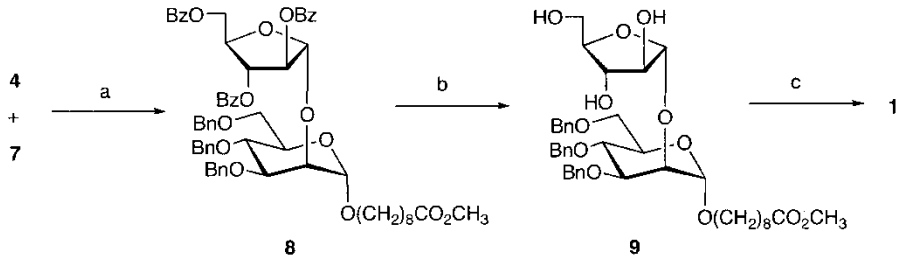


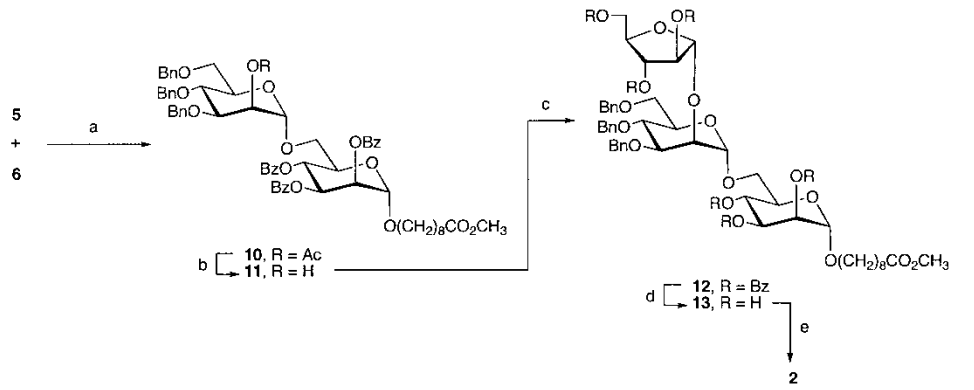
Figure 2: Synthetic targets **1** and **2**, related structure **3**, and monosaccharide building blocks (**4**–**7**) required for synthesis of **1** and **2**.

coupling of alcohol **4**, with thioglycoside **7** promoted by *N*-iodosuccinimide and silver triflate.[24] The expected disaccharide, **8**, was obtained in 75% yield. The anomeric stereochemistry of the arabinofuranosyl residue could be clearly established by NMR spectroscopy. In the ^{1}H NMR spectrum of **8**, the resonance for the anomeric hydrogen appeared as a singlet, which is consistent with the α-arabinofuranose stereochemistry.[25] Had the β-isomer been formed, this signal would have appeared as a doublet with a 4–5 Hz coupling constant. Similarly, in the ^{13}C NMR spectrum of **8**, the chemical shift of the anomeric carbon was 106.8 ppm as would be expected for an α-arabinofuranoside. This disaccharide was deprotected in two steps. Treatment of **8** with sodium methoxide removed the benzoyl groups and gave **9** in 95% yield. Subsequent hydrogenation of the benzyl ethers afforded a 92% yield of the final target, **1**.

The preparation of **2** followed a similar route, as illustrated in Scheme 2. Reaction of alcohol **5**, with thioglycoside **6**, in the presence of *N*-iodosuccinimide



Scheme 1: (a) NIS, AgOTf, CH_2Cl_2, 0°C, 75%; (b) $NaOCH_3$, CH_3OH, rt, 95%; (c) H_2, Pd/C, CH_3OH, rt, 92%.



Scheme 2: (a) NIS, AgOTf, CH_2Cl_2, 0°C, 65%; (b) AcCl, CH_3OH, 0°C → rt, 60%; (c) **7**, NIS, AgOTf, CH_2Cl_2, 0°C, 68%; (d) $NaOCH_3$, CH_3OH, rt, 85%; (e) H_2, $Pd(OH)_2$, CH_3OH, rt, 82%.

and silver triflate afforded the product disaccharide **10**, in 65% yield. The acetyl group was then selectively cleaved in the presence of the benzoate esters upon treatment of **10** with methanolic HCl.[26] The product, disaccharide alcohol **11**, was obtained in 60% yield. The arabinofuranose residue was introduced by way of thioglycoside **7** under conditions identical to those used for the other glycosylations and the protected trisaccharide **12** was obtained in 68% yield. The α-stereochemistry of the arabinofuranose moiety could be established from the chemical shift of the anomeric carbon of this residue, which was 106.6 ppm.[25] Deprotection of the product proceeded without incident. Compound **12** was first treated with sodium methoxide, which provided, in 85% yield, a product, **13**, in which all of the acyl groups had been cleaved. Trisaccharide **2** was obtained in 82% yield by hydrogenation of the benzyl ethers in **13**.

In summary, we have completed the synthesis of two fragments of LAM from *Rhodoccus ruber*. LAM fragments of known structure are expected to be useful tools in elucidating the biologic role of these glycans. The ester functionality present in the aglycone of these oligosaccharides will facilitate the preparation of neoglycoconjugates for use in, for example, assays requiring microtiter plates.

EXPERIMENTAL

Solvents were distilled from the appropriate drying agents before use. Unless stated otherwise, all reactions were carried out at rt and under a positive pressure of argon and were monitored by TLC on silica gel 60 F_{254} (0.25 mm, E. Merck). Spots were detected under UV light by charring with 10% H_2SO_4 in ethanol or by charring with anisaldehyde in ethanol. During the work-up of reaction mixtures, crude products in organic solvents were washed with

equal volumes of aqueous solutions. Solvents were evaporated under reduced pressure and below 40°C (bath). Column chromatography was performed on silica gel 60 (40–60 μM) or Iatrobeads, which refers to a beaded silica gel 6RS–8060 manufactured by Iatron Laboratories (Tokyo). The ratio between silica gel and crude product ranged from 100 to 50 : 1 (w/w). Optical rotations were measured at 21 ± 2°C and are in units of degrees · mL/g · dm. ^{1}H NMR spectra were recorded at 400, 500, or 600 MHz, and chemical shifts are referenced to TMS (0.0 ppm, $CDCl_3$), CH_3OH (4.78 ppm, CD_3OD), or HOD (4.78 ppm, D_2O). ^{13}C NMR spectra were recorded at 100 MHz, and ^{13}C chemical shifts are referenced to $CDCl_3$ (77.00 ppm, $CDCl_3$), CD_3OD (49.15 ppm, CD_3OD), or external dioxane (68.11 ppm, D_2O). Electrospray mass spectra were recorded on samples suspended in THF or CH_3OH with added NaCl.

8-(Methoxycarbonyl)octyl 2-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (8). Alcohol **4** (200 mg, 0.322 mmol), thioglycoside **7** (237 mg, 0.418 mmol), and powdered 4Å molecular sieves (0.50 g) were dried overnight under vacuum with P_2O_5. Freshly distilled CH_2Cl_2 (5 mL) was added and the mixture was cooled to 0°C before *N*-iodosuccinimide (108 mg, 0.483 mmol) and AgOTf (17 mg, 0.066) were added. The reaction mixture was stirred for 30 min and then neutralized with triethylamine, before being filtered through Celite and concentrated. The resulting residue was purified by column chromatography (6 : 1, hexanes : EtOAc) to yield **8** (257 mg, 75%) as a clear oil. R_f 0.44 (3 : 1, hexanes : EtOAc); $[\alpha]_D$ + 13.1 (*c* 0.9, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) : δ_H, 8.10–8.00 (m, 6 H, aromatic H), 7.70–7.48 (m, 3 H, aromatic H), 7.44–7.15 (m, 23 H, aromatic H), 5.73 (d, 1 H, J = 1.2 Hz, H-2′), 5.60 (d, 1 H, J = 1.2, 4.5 Hz, H-3′), 5.58 (s, 1 H, H-1′), 4.99 (d, 1 H, J = 1.8 Hz, H-1), 4.87 (d, 1 H, J = 10.8 Hz, PhCH_2), 4.84 (dd, 1 H, J = 5.1, 11.6 Hz, H-5a′), 4.76 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.73 (d, 1 H, J = 12.0 Hz, PhCH_2), 4.70–4.68 (m, 2 H, H-4′, H-5b′), 4.58–4.53 (m, 3 H, PhCH_2), 4.16–4.12 (s, 1 H, H-2′), 3.98 (dd, 1 H, J = 2.9, 9.3 Hz, H-3), 3.95 (dd, 1 H, J = 9.6, 9.6 Hz, H-4), 3.84–3.81 (ddd, 1 H, J = 1.7, 5.6, 9.6 Hz, H-5), 3.73 (dd, 1 H, J = 1.8, 10.8 Hz, H-6a), 3.71–3.68 (m, 2 H, H-6b, aglycone OCH_2), 3.67 (s, 3 H, OCH_3), 3.39 (dt, 1 H, J = 6.6, 9.5 Hz, aglycone OCH_2), 2.31 (t, 2 H, J = 7.5 Hz, CH_2C═O), 1.65–1.55 (m, 4 H, aglycone CH_2), 1.35–1.31 (m, 8 H, aglycone CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) : δ_C, 175.1, 166.2, 165.8, 165.2, 138.5, 138.4, 133.4, 133.0, 130.1, 129.9, 129.8, 129.8, 129.3, 129.3, 128.5, 128.4 (2), 128.3 (3), 128.2 (2), 128.1, 127.6 (2), 127.5 (2), 106.8, 99.1, 82.0, 81.2, 80.0, 77.7, 75.2, 75.1, 74.4, 73.2, 72.2, 71.8, 69.5, 67.8, 63.7, 51.4, 34.1, 29.5, 29.3, 29.2, 29.1, 26.1, 24.9. HRMS (ESI) calcd. for (M + Na) $C_{63}H_{68}O_{15}$: 1087.4450; found 1087.4448.

8-(Methoxycarbonyl)octyl 2-*O*-(D-arabinofuranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranoside (9). Disaccharide **8** (219 mg, 0.21 mmol) was

dissolved in CH_3OH (10 mL) and the solution was stirred for 10 min before solid $NaOCH_3$ was added in small portions until the pH reached 9. The reaction mixture was stirred for 2 hr and then neutralized with acetic acid. The crude product was purified by column chromatography (17 : 1, CH_2Cl_2 : CH_3OH) to yield **9** as a colorless oil (150 mg, 95%). R_f 0.37 (17 : 1 CH_2Cl_2 : CH_3OH); $[\alpha]_D + 63.6$ (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ_H, 7.37–7.25 (m, 15 H, aromatic H), 7.15–7.13 (m, 2 H, aromatic H), 5.14 (s, 1 H, H-1′), 4.90 (d, 1 H, $J = 2.2$ Hz, H-1), 4.80 (d, 1 H, $J = 10.7$ Hz, PhCH_2), 4.72 (d, 1 H, $J = 11.7$ Hz, PhCH_2), 4.67 (d, 1 H, $J = 11.7$ Hz, PhCH_2), 4.62 (d, 1 H, $J = 12.4$ Hz, PhCH_2), 4.48 (d, 1 H, $J = 12.4$ Hz, PhCH_2), 4.39 (d, 1 H, $J = 10.7$ Hz, PhCH_2), 4.22–4.21 (m, 1 H, H-4′), 4.19 (s, 1 H, H-2′), 4.01 (s, 1 H, H-3′), 3.93 (dd, 1 H, $J = 2.8$, 9.3 Hz, H-3), 3.89–3.80 (m, 4 H, H-5a′, H-5b′, H-5, H-2), 3.69–3.61 (m, 7 H, aglycone OCH_2, H-4, C-6a, C-6b, OCH_3), 3.39 (dt, 1 H, $J = 6.6$, 9.5 Hz, aglycone OCH_2), 2.31 (t, 2 H, $J = 7.5$ Hz, CH_2C═O), 1.65–1.55 (m, 4 H, aglycone CH_2), 1.35–1.31 (m, 8 H, aglycone CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C, 174.4, 138.3, 138.2, 138.1, 128.5, 128.4, 128.3, 128.0 (2), 127.9, 127.6 (2), 109.4, 98. 8, 87.6, 79.2, 78.8, 78.2, 75.2, 74.8, 74.5, 73.3, 72.4, 71.4, 68.5, 67.9, 62.1, 51.5, 34.1, 29.4, 29.3, 29.2, 29.1, 26.0, 24.9. HRMS (ESI) calcd. for (M + Na) $C_{42}H_{56}O_{12}$: 775.3664; found 775.3664.

8-(Methoxycarbonyl)octyl 2-*O*-(α-D-arabinofuranosyl)-α-D-mannopyranoside (1). Disaccharide **9** (100 mg, 0.13 mmol) was dissolved in CH_3OH (5 mL) and 10% Pd/C (20 mg) was added. The solution was stirred under a H_2 atmosphere for 8 hr and the reaction mixture was then filtered through Celite and concentrated. The crude product was purified by column chromatography (10 : 1, CH_2Cl_2 : CH_3OH) to yield **10** as a colorless oil (58 mg, 92%). R_f 0.30 (9 : 1, CH_2Cl_2 : CH_3OH); $[\alpha]_D + 56.0$ (*c* 0.4, CH_3OH); ^{1}H NMR (500 MHz, CD_3OD): δ_H, 5.04 (d, 1 H, $J = 1.8$ Hz, H-1′), 4.91 (s, 1 H, H-1), 4.06 (dd, 1 H, $J = 1.8$, 5.9 Hz, H-2′), 3.98–3.94 (m, 1 H, H-3), 3.82–3.57 (m, 11 H, H-2′, H3′, H-4′, H-4, H5, H-6a, H-6b, aglycone OCH_2, OCH_3), 3.51–3.48 (m, 2 H, H-5a′, H-5b′), 3.39 (dt, 1 H, $J = 6.6$, 9.5 Hz, aglycone OCH_2), 2.30 (t, 2 H, $J = 7.4$ Hz, CH_2C═O), 1.61–1.56 (m, 4 H, aglycone CH_2), 1.39–1.31 (m, 8 H, aglycone CH_2); ^{13}C NMR (125 MHz, CD_3OD): δ_C, 176.1 (C═O), 111.4 (C-1′), 100.7 (C-1), 85.5 (C-4′), 83.2 (C-2′), 79.4 (C-3′), 78.4 (C-2), 74.6 (C-4), 72.3 (C-3), 69.0 (C-5), 68.6 (aglycone O*C*H_2), 63.0 (C-5′), 62.9 (C-6), 52.0 (O*C*H_3), 34.8 (aglycone *C*H_2), 30.6 (aglycone *C*H_2), 30.4 (aglycone *C*H_2), 30.3 (aglycone *C*H_2), 30.1 (aglycone *C*H_2), 27.3 (aglycone *C*H_2), 26.0 (aglycone *C*H_2). HRMS (ESI) calcd. for (M + Na) $C_{21}H_{38}O_{12}$: 505.2256; found 505.2256.

8-(Methoxycarbonyl)octyl 6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (10). Alcohol **5** (680 mg, 1.03 mmol) was glycosylated with thioglycoside **6** (459 mg, 0.86 mmol) in CH_2Cl_2 (30 mL) using *N*-iodosuccinimide (289 mg, 1.28 mmol) and silver triflate (5 mg, 0.21 mmol) in the presence of powdered 4Å molecular

sieves (1.2 g) as described for the preparation of **8**. The product was purified by column chromatography (4 : 1, hexanes : EtOAc) to yield **10** (636 mg, 65%) as an oil. R_f 0.33 (3 : 1, hexanes : EtOAc); $[\alpha]_D$ −26.9 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) : δ_H, 8.11–7.82 (m, 6 H, aromatic H), 7.61–7.14 (m, 24 H, aromatic H), 5.93 (dd, 1 H, $J = 10.0$, 10.0 Hz, H-4), 5.87 (dd, 1 H, $J = 3.3$, 10.2 Hz, H-3), 5.64 (dd, 1 H, $J = 1.8$, 3.3 Hz, H-2), 5.38 (dd, 1 H, $J = 1.8$, 3.4 Hz, H-2′), 5.04 (d, 1 H, $J = 1.7$ Hz, H-1), 4.89 (d, 1 H, $J = 1.7$ Hz, H-1′), 4.83 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.57 (d, 1 H, $J = 12.1$ Hz, PhCH_2), 4.51 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.44 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.37 (d, 1 H, $J = 12.1$ Hz, PhCH_2), 4.34 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.26–4.20 (m, 1 H, H-5), 3.98–3.92 (m, 2 H, H-5′, H-3′), 3.86 (dd 1 H, $J = 9.7$, 9.7 Hz, H-4′), 3.78–3.72 (m, 2 H, aglycone OCH_2), 3.72–3.65 (m, 6 H, OCH_3, H-6a, H-6a′, H-6b), 3.56–3.49 (m, 2 H, H-2, H-6b′), 2.31 (dd, 2 H, $J = 7.5$, 7.5 Hz, CH_2C═O), 2.12 (s, 3 H C(O)CH_3), 1.70–1.60 (m, 4 H, aglycone CH_2), 1.45–1.30 (m, 8 H, aglycone CH_2); ^{13}C NMR (125 MHz, $CDCl_3$) : δ_C, 170.2, 166.2, 165.6, 165.5, 165.4, 138.6, 138.2, 138.0, 133.4, 133.3, 133.1, 129.9, 128.9, 129.7 (2), 129.5, 129.2, 128.4, 128.3 (2), 128.2, 127.8, 127.5, 127.3, 98.0, 97.5, 78.5, 75.0, 74.2, 73.3, 71.8, 71.5, 70.8, 70.2, 69.2, 68.6 (2), 68.5, 67.5, 66.8, 51.4, 34.1, 29.4, 29.3, 29.2, 29.1, 26.1, 25.0, 21.1. HRMS (ESI) calcd. for (M + Na) $C_{66}H_{72}O_{17}$: 1159.4667; found 1159.4681.

8-(Methoxycarbonyl)octyl 6-*O*-(3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (11). A solution of methanolic HCl was prepared by dissolving acetyl chloride (1.5 mL) in CH_3OH (48.5 mL) at 0°C. The entirety of this solution was used to dissolve disaccharide **10** (614 mg, 0.54 mmol) and the reaction mixture was stirred for 6 hr. The solution was partially concentrated, diluted with CH_2Cl_2, and washed successively with a saturated aqueous solution of $NaHCO_3$, water, and brine. The organic phase was dried with $MgSO_4$, filtered through cotton, and concentrated, and the resulting residue was purified by column chromatography (4 : 1, hexanes : EtOAc) to afford **11** (281 mg, 60%) as an oil. R_f 0.25 (3 : 1, hexanes : EtOAc); $[\alpha]_D$ −15.1 (c 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) : δ_H, 8.10–7.83 (m, 6 H, aromatic H), 7.56–7.14 (m, 24 H, aromatic H), 5.97 (dd, 1 H, $J = 10.1$, 10.1 Hz, H-4), 5.88 (dd, 1 H, $J = 3.4$, 10.1 Hz, H-3), 5.65 (dd, 1 H, $J = 1.8$, 3.3 Hz, H-2), 5.05 (d, 1 H, $J = 1.7$ Hz, H-1), 5.01 (d, 1 H, $J = 1.5$ Hz, H-1′), 4.80 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.54 (d, 1 H, $J = 12.2$ Hz, PhCH_2), 4.52 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.49 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.54 (d, 1 H, $J = 12.2$ Hz PhCH_2), 4.39 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.23–4.20 (m, 2 H, H-5, H-5′), 4.02 (dd, 1 H, $J = 1.2$, 2.6 Hz, H-2′), 3.94 (dd, 1 H, $J = 4.6$, 11.2 Hz, H-6a), 3.85–3.70 (m, 5 H, aglycone OCH_2, H-3′, H-4′, H-6a′), 3.66 (s, 3 H, OCH_3), 3.62 (dd, 1 H, $J = 4.4$, 10.8 Hz, H-6b′), 3.50–3.53 (m, 2 H, H-6b, H-6a), 2.32 (dd, 2 H, $J = 7.5$, 7.5 Hz, CH_2C═O), 1.59–1.72 (m, 4 H, aglycone CH_2), 1.30–1.42 (m, 8 H, aglycone CH_2); ^{13}C NMR (125 MHz,

$CDCl_3$): δ_C, 170.3, 165.6, 165.5, 165.4, 138.5, 138.2, 137.9, 133.4, 133.3, 133.1, 129.9, 129.8, 129.8, 129.5, 129.3, 129.2, 128.6, 128.5, 128.4, 128.3, 127.9, 127.8, 127.7, 127.6, 127.5, 99.4, 97.6, 80.3, 75.0, 74.1, 73.3, 71.8, 71.2, 70.8, 70.3, 69.4, 68.7, 68.6, 68.1, 67.6, 66.4, 51.4, 34.1, 29.4, 29.3, 29.2, 29.1, 26.1, 25.0. HRMS (ESI) calcd. for (M + Na) $C_{64}H_{70}O_{16}$: 1117.4561; found 1117.4576.

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-(2,3,5-tri-*O*-benzoyl-α-D-arabinofuranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (12). Alcohol **11** (261 mg, 0.24 mmol) was glycosylated with thioglycoside **7** (163 mg, 0.29 mmol) in CH_2Cl_2 (20 mL) using *N*-iodosuccinimide (80 mg, 0.36 mmol) and silver triflate (2 mg, 0.06 mmol) in the presence of powdered 4Å molecular sieves (500 mg) as described for the preparation of **8**. The product was purified by column chromatography (4 : 1, hexanes : EtOAc) to yield **12** (250 mg, 68%) as an oil. R_f 0.46 (2 : 1, hexanes : EtOAc); $[\alpha]_D$ −19.8 (*c* 0.6, $CHCl_3$); $_1$H NMR (500 MHz, $CDCl_3$): δ_H, 8.12–7.81 (m, 12 H, aromatic H), 7.62–7.11 (m, 33 H, aromatic H), 5.98 (dd, 1 H, $J = 10.1$, 10.1 Hz, H-4), 5.87 (dd, 1 H, $J = 3.3$, 10.1 Hz, H-3), 5.66–5.62 (m, 2 H, H-2″, H-2), 5.56 (dd, 1 H, $J = 1.0$, 3.4 Hz, H-3″), 5.43 (s, 1 H, H-1), 5.05 (s, 2 H, H-1′, H-1″), 4.80 (d, 1 H, $J = 11.0$ Hz, PhCH_2), 4.80–4.75 (m, 1 H, PhCH_2), 4.64–4.60 (m, 2 H, PhCH_2), 4.52 (d, 1 H, $J =$ 11.5 Hz, PhCH_2), 4.50 (d, 1 H, $J = 11.5$ Hz, PhCH_2), 4.47 (d, 1 H, $J = 11.0$ Hz, PhCH_2), 4.43 (d, 1 H, $J = 12.0$ Hz, PhCH_2), 4.22 (d, 1 H, $J = 12.0$ Hz, PhCH_2), 4.24–4.20 (m, 1 H, H-5), 4.10–4.13 (m, 1 H, H-5′), 4.00–3.88 (m, 5 H, H-4″, H-3′, H-4′, H-2′, H-6b′), 3.80–3.66 (m, 3 H, aglycone OCH_2, H-5a″, H′5b″), 3.64 (s, 3 H, OCH_3), 3.58–3.48 (m, 4 H, H-6a, H-6a′, H-6b, aglycone OCH_2), 2.31 (dd, 2 H, $J = 7.5$, 7.5 Hz, CH_2C═O), 1.60–1.70 (m, 4 H, aglycone CH_2), 1.20–1.44 (m, 8 H aglycone CH_2); ^{13}C NMR (125 MHz, $CDCl_3$): δ_C, 170.2, 166.2, 165.7, 165.6, 165.5, 165.3, 165.1, 138.6, 138.4, 138.3, 133.4, 133.3, 133.2, 133.1, 132.9, 130.0, 130.0, 129.9 (2), 129.8 (2), 129.8, 129.7, 129.5, 129.3, 129.2, 128.6, 128.5, 128.4 (2), 128.3 (2), 128.2 (2), 128.0, 127.6, 127.4 (3), 106.6, 99.3, 97.5, 82.0, 81.2, 80.1, 77.6, 75.0, 74.8, 73.7, 73.0, 72.0, 71.9, 70.9, 69.4, 69.2, 68.6, 67.6, 66.5, 66.5, 63.6, 51.1, 34.1, 29.4, 29.3, 29.2, 29.1, 26.1, 25.0. HRMS (ESI) calcd. for (M + Na) $C_{90}H_{90}O_{23}$; 1561.5770, found 1561.5774.

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-(α-D-arabinofuranosyl)-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl]-α-D-mannopyranoside (13). Trisaccharide **12** (249 mg, 0.16 mmol) was deacylated in CH_3OH (30 mL) with sodium methoxide as described for the preparation of **9**. The product was purified by column chromatography (2 : 1, EtOAc : hexanes) to yield **13** (125 mg, 85%) as an oil. R_f 0.20 (2 : 1, EtOAc : hexanes). The product was characterized by ^{1}H NMR spectroscopy and mass spectrometry and used in the subsequent reaction. ^{1}H NMR (400 MHz, CD_3OD): δ_H, 7.36–7.04 (m, 15 H, aromatic H), 5.19 (s, 1 H, H-1), 5.09 (s, 1 H, H-1′), 4.72–4.58 (m, 5 H,

H-1″, 4 × PhCH_2), 4.44 (d, 1 H, $J = 12.2$ Hz, PhCH_2), 4.32 (d, 1 H, $J = 10.9$ Hz, PhCH_2), 4.18–4.10 (m, 3 H, H-3′, H-5′, H-4″), 3.98–3.50 (m, 18 H, H-2, H-3, H-4, H-6a, H-6b, H-2′, H-4′, H-6a′, H-6b, aglycone OCH_2, OCH_3, H-2″, H-3″, H-5a″, H-5b″), 3.28 (1 H, ddd, $J = 7.5$, 7.5, 9.6 Hz, H-5), 2.28 (dd, 2 H, $J = 7.5$, 7.5 Hz, CH_2C=O), 1.62–1.58 (m, 4 H, aglycone CH_2), 1.32–1.20 (m, 8 H, aglycone CH_2). HRMS (ESI) calcd. for (M + Na) $C_{48}H_{66}O_{17}$: 937.4192; found 937.4198.

8-(Methoxycarbonyl)octyl 6-*O*-[2-*O*-(α-D-arabinofuranosyl)-α-D-mannopyranosyl]-α-D-mannopyranoside (2). Trisaccharide **13** (96 mg, 0.11 mmol) was dissolved in CH_3OH (5 mL) and $Pd(OH)_2$ (60 mg) was added. The solution was stirred under H_2 for 4 hr and then filtered through Celite and concentrated. The product was purified on Iatrobeads (7 : 3, CH_2Cl_2 : CH_3OH) to yield **2** (55 mg, 82%) as an oil. R_f 0.19 (4 : 1, CH_2Cl_2 : CH_3OH); $[\alpha]_D$+29.5 (*c* 1.0, CH_3OH); $_1$H NMR (600 MHz, D_2O): δ_H, 5.23 (d, 1 H, $J = 1.8$ Hz, H-1″), 5.10 (d, 1 H, $J = 1.8$ Hz, H-1′), 4.92 (d, 1 H, $J = 1.8$ Hz, H-1), 4.26 (dd, 1 H, $J = 1.8$, 3.6 Hz, H-2″), 4.17 (ddd, 1 H, $J = 3.6$, 5.4, 9.0 Hz, H-4″), 4.05–3.76 (m, 19, H-2, H-3, H-4, H-5, H-6a, H-6b, H-2′, H-3′, H-4′, H-6a′, H-6b′, H-3″, H-5a″, H-5b″, OCH_2 aglycone, OCH_3), 3.58 (ddd, 1 H, $J = 7.5$, 7.5, 9.6 Hz, H-5′), 2.39 (dd, 2 H, $J = 7.5$, 7.5 Hz, CH_2C=O), 1.67–1.54 (m, 4 H, aglycone CH_2), 1.39–1.25 (m, 8 H, aglycone CH_2); ^{13}C NMR (125 MHz, D_2O): δ_C, 178.8 (C=O), 110.3 (C–1″), 100.4 (C-1′), 99.6 (C-1), 84.5 (C-2″), 82.1 (C-4″), 78.4, 77.4, 73.5, 71.9, 71.7, 71.2, 71.0, 68.9, 67.8, 67.5, 66.9 (C-1), 62.0 (C-5″), 61.6 (C-1′), 52.9 (O*C*H_3), 34.6 (aglycone *C*H_2), 29.3 (aglycone *C*H_2), 29.2 (aglycone *C*H_2), 29.1 (aglycone *C*H_2), 29.0 (aglycone *C*H_2), 26.2 (aglycone *C*H_2), 25.2 (aglycone *C*H_2). HRMS (ESI) calcd. for (M + Na) $C_{27}H_{48}O_{17}$: 667.2784, found 667.2788.

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