Supporting information

Identification of a novel mycobacterial arabinosyltransferase activity which adds an arabinosyl residue to α-D-mannosyl residues

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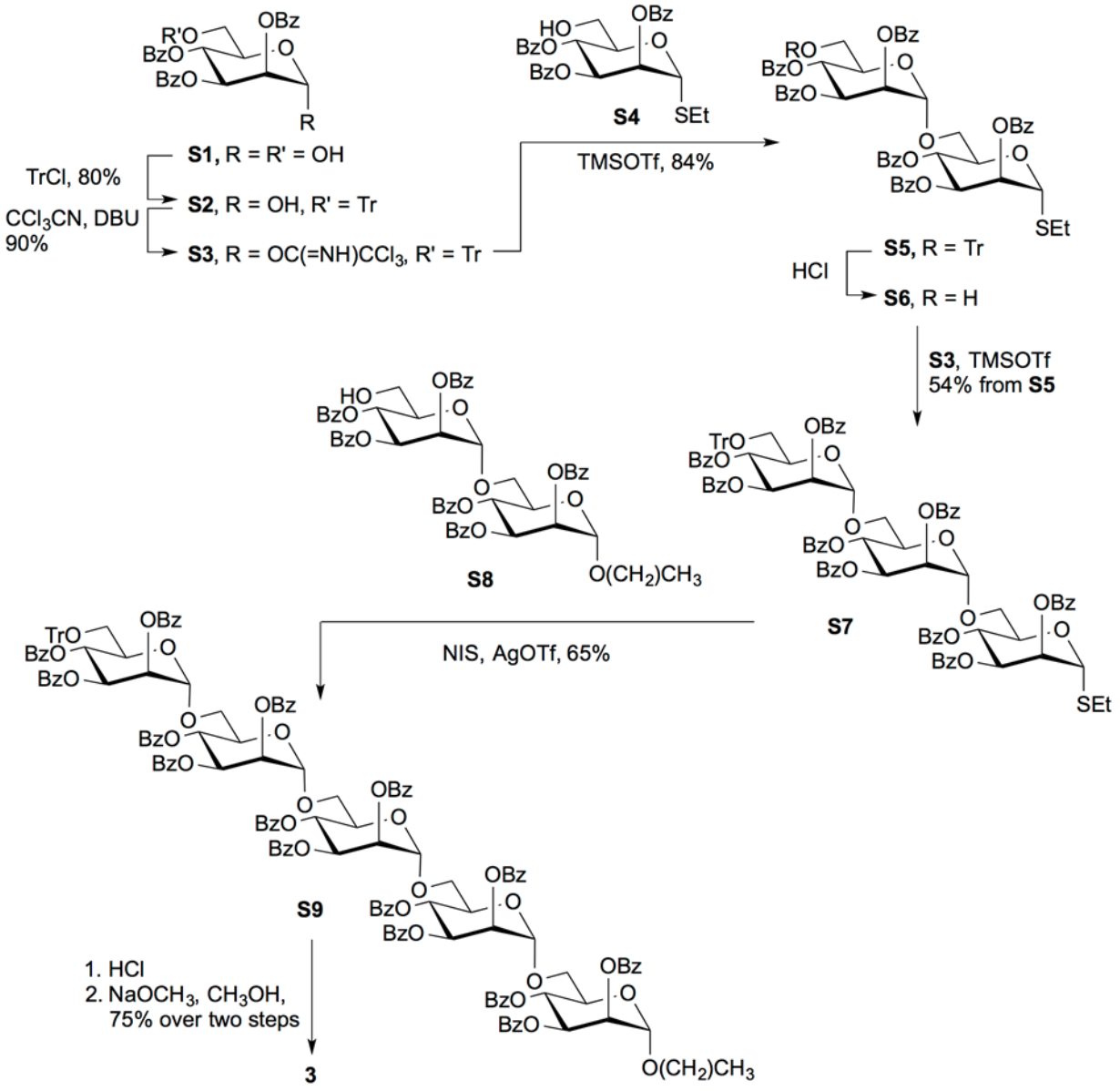
Supplementary Methods

Preparation of synthetic mannoside acceptors - Compounds **1**, **2**, and **4** were synthesized as described (*1, 2*). Scheme S1 shows the synthesis of compound **3**. Starting from the (*3*) diol **S1**, the compound was tritylated on O-6 to give **S2** and then converted to the corresponding trichloroacetimidate **S3** in 72% overall yield. Reaction of **S3** with the previously (*4*) thioglycoside **S4** and TMSOTf led to the formation of, in 84% yield, disaccharide **S5**. The trityl group in **S5** was removed and then the resulting alcohol **S6** was glycosylated with **S3** and TMSOTf to afford trisasccharide **S7** in 54% overall yield over the two steps. Once in hand, coupling of **S7** and the (*5*) disaccharide alcohol **S8** in the presence of NIS and silver triflate gave a 65% yield of pentasaccharide **S9**. Deprotection of **S9** was achieved in two steps: cleavage of the trityl ether under acidic conditions and removal of the benzoate esters under basic conditions to afford **3** (75% yield from **S9**).

Data and Procedures for the Synthesis of acceptor 3 -

2,3,4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannopyranose (**S2**)

Diol **S1** (*3*) (115 mg, 0.23 mmol) was dissolved in pyridine (2.0 mL) and then chlorotriphenylmethane (71.6 mg, 0.26 mmol) was added. The reaction mixture was stirred at 40 °C overnight and then cooled and evaporated. The resulting residue was purified by chromatography (4:1 hexane–ethyl acetate) to give **S2** (137 mg, 80%) as a pale yellow foam. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.22 – 8.17 (m, 2H), 7.89 – 7.85 (m, 2H), 7.78 – 7.74 (m, 2H), 7.65 – 7.60 (m, 1H), 7.52 – 7.39 (m, 10H), 7.34 – 7.25 (m, 4H), 7.17 – 7.06 (m, 9H), 6.17 (t, *J* = 10.2 Hz, 1H), 5.89 (dd, *J* = 10.2, 3.3 Hz, 1H), 5.75 (dd, *J* = 3.3, 1.9 Hz, 1H), 5.58 (d, *J* = 1.5 Hz, 1H), 4.48 (ddd, *J* = 10.1, 3.8, 2.2 Hz, 1H), 3.45 (dd, *J* = 10.5, 2.2 Hz, 1H), 3.26 (dd, *J* = 10.5, 4.0 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 165.7, 165.6, 165.0, 149.6, 143.7, 136.2, 133.4, 133.0, 132.9, 130.0, 129.8, 129.7, 129.6, 129.5, 129.3, 128.6, 128.23, 128.16, 127.7, 126.8, 123.8, 92.4, 86.6, 71.3, 70.4, 70.3, 66.9, 62.1; HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{46}H_{38}NaO_9$: 757.2408, found: 757.2403.



Scheme S1. Synthesis of Pentasaccharide **3**

2,3,4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannopyranosyl trichloroacetimidate (**S3**). Monosaccharide **S2** (41 mg, 0.056 mmol) was dissolved in dichloromethane (2 mL) and then cooled to 0 °C before trichloroacetonitrile (23 μL, 0.23 mmol) and DBU (1 μL, 6.68 μmmol) were added. The mixture was stirred while warming to room temperature and being monitored by TLC. After the reaction was complete, the solution was concentrated and the resulting residue

was purified by chromatography (6:1 hexane–ethyl acetate) to give **S3** (35 mg, 90%) as a pale yellow form. ^{1}H NMR (600 MHz, $CDCl_3$): δ 8.89 (s, 1H), 8.25–8.19 (m, 2H), 7.92–7.86 (m, 2H), 7.79–7.75 (m, 2H), 7.69–7.63 (m, 1H), 7.54–7.49 (m, 3H), 7.48–7.43 (m, 7H), 7.35–7.26 (m, 4H), 7.18–7.14 (m, 6H), 7.14–7.09 (m, 3H), 6.69 (d, *J* = 1.9 Hz, 1H), 6.32 (t, *J* = 10.3 Hz, 1H), 5.98 (dd, *J* = 3.3, 2.0 Hz, 1H), 5.92–5.85 (m, 1H), 4.48 (ddd, *J* = 10.3, 3.7, 2.2 Hz, 1H), 3.52 (dd, *J* = 10.6, 2.2 Hz, 1H), 3.26 (dd, *J* = 10.6, 3.9 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$): δ 165.6, 165.4, 164.9, 159.9, 143.6, 133.6, 133.2, 133.1, 130.1, 129.8, 129.7, 129.23, 129.21, 129.0, 128.8, 128.6, 128.3, 128.2, 127.7, 126.9, 95.1, 90.8, 86.5, 73.2, 70.5, 69.1, 66.0, 61.6.

Ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-α-D-mannopyranoside (**S5**). Monosaccharide **S4 (*4*)**(53.3 mg, 0.099 mmol) and trichloroacetimidate **S3** (92.6 mg, 0.13 mmol) were dissolved in dichloromethane (4 mL), 4 Å molecular sieves (20 mg) were added and the mixture was cooled to 0 °C. Next, 10% TMSOTf (29 μL, 0.016 mmol) was added and the reaction was stirred while warming to room temperature and being monitored by TLC. After the reaction was complete, the solution was neutralized with two drops of triethylamine, filtered and concentrated. The resulting residue was purified by chromatography (6:1→1:1 hexane–ethyl acetate) to give **S5** (87 mg, 84%) as a pale yellow solid. ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.19–8.16 (m, 2H), 8.09–8.06 (m, 2H), 8.03–7.98 (m, 4H), 7.88–7.83 (m, 4H), 7.62–7.52 (m, 5H), 7.50–7.39 (m, 7H), 7.34–7.26 (m, 6H), 6.07–6.01 (m, 2H), 5.88 (dd, *J* = 9.9, 3.4 Hz, 1H), 5.85 (dd, *J* = 3.4, 1.5 Hz, 1H), 5.82 (t, *J* = 10.1 Hz, 1H), 5.74 (dd, *J* = 3.4, 1.7 Hz, 1H), 5.62 (d, *J* = 1.2 Hz, 1H), 5.15 (d, *J* = 1.6 Hz, 1H), 4.82 (ddd, *J* = 10.2, 5.9, 1.9 Hz, 1H), 4.12–4.09 (m, 1H), 4.05–4.00 (m, 1H), 3.77 (dd, *J* = 10.9, 2.0 Hz, 1H), 3.68–3.62 (m, 1H), 3.60–3.54 (m, 1H), 2.93–2.79 (m, 2H), 2.60–2.53 (m, 1H), 1.47 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.6, 165.6, 165.5, 165.44, 165.38, 165.2, 133.7, 133.6, 133.52, 133.48, 133.2, 133.1, 130.0, 129.93, 129.91, 129.83, 129.79, 129.7, 129.4, 129.3, 129.2, 129.0, 128.9, 128.8, 128.7, 128.6, 128.51, 128.49, 128.3, 97.7, 82.1, 72.2, 71.0, 70.7, 70.4, 69.9, 69.5, 67.3, 67.2, 66.8, 61.1, 60.4, 25.5, 21.0, 14.8, 14.2; HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{56}H_{50}NaO_{16}S$: 1033.2712, found: 1033.2705.

Ethyl 2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-1-thio-α-D-mannopyranoside (**S6**). Disaccharide **S5** (87 mg, 0.07 mmol) was dissolved in 1:1 dichloromethane–methanol (12 mL) and HCl (2 mL of a solution prepared by adding 0.2 mL of conc HCl to 1.8 mL methanol) was added. The reaction was stirred for 3 hours and then neutralized by the addition of saturated sodium bicarbonate aqueous solution. The solution was

concentrated and the residue was purified by chromatography (4:1 hexane–ethyl acetate) to give the product (**S6**), which was used immediately in the next step.

Ethyl 2,3,4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (S7). Disaccharide **S6** (40.6 mg, 0.04 mmol) and trichloroacetimidate **S3** (36 mg, 0.052 mmol) were dissolved in dichloromethane (4.5 mL), 4 Å molecular sieves (20 mg) were added and the mixture was cooled to 0 °C. Next, 10% TMSOTf (8 μL, 0.004 mmol) was added and the reaction was stirred while warming to room temperature and being monitored by TLC. After the reaction was complete, the solution was neutralized with two drops of triethylamine, filtered and concentrated. The resulting residue was purified by chromatography (6:1→1:1 hexane–ethyl acetate) to give **S7** (38 mg, 54%) as a pale yellow foam. 13 mg disaccharide **S6** was also recovered. ^{1}H NMR (498 MHz, $CDCl_3$): δ 8.24–8.20 (m, 2H), 8.15–8.11 (m, 2H), 8.07–7.97 (m, 6H), 7.93–7.85 (m, 6H), 7.79–7.74 (m, 2H), 7.64–7.58 (m, 1H), 7.54–7.50 (m, 3H), 7.49–7.41 (m, 8H), 7.39–7.33 (m, 9H), 7.32–7.26 (m, 9H), 7.25–7.19 (m, 3H), 7.13–7.07 (m, 6H), 7.07–7.01 (m, 3H), 6.30 (t, *J* = 9.8 Hz, 1H), 6.11–6.02 (m, 3H), 5.94–5.87 (m, 3H), 5.85 (dd, *J* = 10.2, 3.3 Hz, 1H), 5.65 (s, 1H), 5.61 (dd, *J* = 3.2, 1.7 Hz, 1H), 5.19 (d, *J* = 1.4 Hz, 1H), 4.95 (ddd, *J* = 10.4, 4.6, 1.7 Hz, 1H), 4.91 (d, *J* = 1.5 Hz, 1H), 4.32 (dd, *J* = 11.0, 4.7 Hz, 1H), 4.30–4.26 (m, 1H), 4.19–4.14 (m, 1H), 3.98 (dd, *J* = 10.7, 5.9 Hz, 1H), 3.80 (dd, *J* = 11.0, 1.8 Hz, 1H), 3.43 (dd, *J* = 10.6, 1.7 Hz, 1H), 3.18 (dd, *J* = 10.5, 2.0 Hz, 1H), 2.99 (dd, *J* = 10.4, 4.4 Hz, 1H), 2.88–2.69 (m, 2H), 1.38 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$): δ 165.7, 165.53, 165.46, 165.34, 165.26, 165.2, 165.0, 143.7, 133.4, 133.33, 133.28, 133.2, 133.1, 133.0, 132.90, 132.86, 130.1, 130.0, 129.94, 129.91, 129.86, 129.82, 129.80, 129.78, 129.7, 129.60, 129.56, 129.5, 129.4, 129.2, 129.12, 129.08, 128.8, 128.63, 128.57, 128.40, 128.37, 128.3, 128.2, 128.1, 127.6, 126.7, 98.00, 97.3, 86.3, 82.2, 72.2, 71.0, 70.7, 70.6, 70.4, 70.3, 69.9, 69.5, 66.9, 66.8, 66.6, 66.5, 65.6, 61.8, 25.4, 14.8; HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{102}H_{86}NaO_{24}S$: 1749.5122, found: 1749.5097.

Octyl 2,3,4-tri-*O*-benzoyl-6-*O*-triphenylmethyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranosyl-(1→6)-2,3,4-tri-*O*-benzoyl-α-D-mannopyranoside (S9). Trisaccharide thioglycoside **S7** (27 mg, 15 μmol) and disaccharide **S8** (*5*) (17 mg, 15 μmol) were dissolved in dichloromethane (4 mL) and 4Å molecular sieves (10 mg) were added. The solution was cooled to 0 °C and then NIS (8 mg, 35 μmol) and AgOTf (3 mg, 12 μmol) were

added. The mixture was stirred while warming to room temperature with the reaction being monitored by TLC. After the reaction was complete, the solution was neutralized with saturated sodium bicarbonate aqueous solution, filtered and concentrated. The resulting residue was purified by chromatography (6:1 Hexane–Ethyl acetate) to give **S7** (28 mg, 65%) as a pale yellow foam. ^{1}H NMR (500 MHz, $CDCl_3$): δ 8.25–8.19 (m, 4H), 8.17–8.12 (m, 6H), 8.10–8.03 (m, 6H), 8.00–7.87 (m, 12H), 7.84–7.80 (m, 2H), 7.66–7.60 (m, 1H), 7.59–7.36 (m, 25H), 7.36– 7.29 (m, 16H), 7.28–7.19 (m, 7H), 7.19–7.14 (m, 2H), 7.13–7.07 (m, 6H), 7.07–7.01 (m, 3H), 6.28 (t, *J* = 10.1 Hz, 1H), 6.24 (t, *J* = 10.0, Hz, 1H), 6.21 (t, *J* = 10.0 Hz, 1H), 6.13–6.06 (m, 3H), 6.03–5.96 (m, 3H), 5.96 (dd, *J* = 2.9, 1.6 Hz, 1H), 5.89 (dd, *J* = 10.2, 3.3 Hz, 1H), 5.86 (dd, *J* = 3.3, 1.6 Hz, 1H), 5.81 (dd, *J* = 3.3, 1.7 Hz, 1H), 5.77 (dd, *J* = 3.2, 1.7 Hz, 1H), 5.73 (dd, *J* = 3.2, 1.7 Hz, 1H), 5.27 (d, *J* = 1.4 Hz, 1H), 5.17 (d, *J* = 1.5 Hz, 1H), 5.02 (d, *J* = 1.4 Hz, 1H), 4.97 (d, *J* = 1.5 Hz, 1H), 4.92 (d, *J* = 1.4 Hz, 1H), 4.55 (ddd, *J* = 10.0, 4.2, 1.7 Hz, 1H), 4.39–4.33 (m, 1H), 4.33–4.31 (m, 1H), 4.30 (d, *J* = 4.0 Hz, 1H), 4.24–4.19 (m, 1H), 4.04–3.98 (m, 2H), 3.98–3.89 (m, 3H), 3.85 (dd, *J* = 11.3, 3.7 Hz, 1H), 3.62 (dt, *J* = 9.6, 6.7 Hz, 1H), 3.57–3.49 (m, 2H), 3.35 (dd, *J* = 11.1, 1.5 Hz, 1H), 3.03 (dd, *J* = 10.5, 1.7 Hz, 1H), 2.95 (dd, *J* = 10.4, 4.2 Hz, 1H), 1.77–1.70 (m, 2H), 1.46–1.37 (m, 2H), 1.36–1.20 (m, 8H), 0.87 (t, *J* = 7.4 Hz, 3H); ^{13}C NMR (126 MHz, $CDCl_3$): δ 165.8, 165.6, 165.53, 165.51, 165.50, 165.47, 165.4, 165.32, 165.28, 165.21, 165.16, 165.1, 143.7, 133.5, 133.43, 133.36, 133.34, 133.30, 133.26, 133.1, 133.0, 132.92, 132.91, 132.8, 130.08, 130.06, 130.0, 129.94, 129.88, 129.83, 129.76, 129.65, 129.59, 129.56, 129.50, 129.47, 129.45, 129.42, 129.38, 129.33, 129.32, 129.29, 129.24, 129.21, 129.20, 128.82, 128.78, 128.7, 128.64, 128.59, 128.50, 128.45, 128.43, 128.3, 128.2, 128.1, 127.6, 126.7, 98.3, 98.1, 97.8 (3C), 86.3, 76.5, 70.8, 70.7, 70.59, 70.56, 70.53, 70.47, 70.4, 70.30, 70.25, 70.2, 69.6, 69.50, 69.45, 69.4, 68.7, 66.9, 66.84, 66.79, 66.5, 66.4, 66.3, 66.0, 65.5, 61.6, 31.9, 29.5, 29.3, 26.2, 22.7, 14.1; HRMS-ESI *m/z* $[M + Na]^+$ calcd for $C_{162}H_{142}NaO_{41}$: 2765.8919, found: 2765.8892.

Octyl α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranosyl-(1→6)-α-D-mannopyranoside (**3**). Pentasaccharide **S9** (27 mg, 9.8 μmol) was dissolved in 1:1 dichloromethane–methanol (4 mL) and HCl (1 mL of a solution prepared by adding 0.1 mL of conc HCl to 0.9 mL methanol) was added. The reaction was stirred for five hours and then neutralized by the addition of saturated sodium bicarbonate aqueous solution. The solution was then concentrated and re-dissolved in methanol (5 mL) and then a small piece of sodium was added. The reaction mixture was stirred at room temperature overnight and then neutralized by the addition of washed Amberlite IR-120H+ resin. The

solution was filtered and the filtrate was concentrated to give a residue that was purified by chromatography (5:1 dichloromethane–methanol) to give **3** (7 mg, 75%) as an off-white solid. ^{1}H NMR (500 MHz, CD_3OH): δ 4.85 (d, *J* = 1.5 Hz, 1H), 4.84–4.81 (m, 2H), 4.79 (d, *J* = 1.3 Hz, 1H), 4.73 (d, *J* = 1.2 Hz, 1H), 3.89–3.76 (m, 15H), 3.76–3.67 (m, 8H), 3.67–3.56 (m, 8H), 3.45–3.38 (m, 1H), 1.63–1.53 (m, 2H), 1.43–1.35 (m, 2H), 1.35–1.24 (m, 8H), 0.90 (t, *J* = 6.9 Hz, 3H); ^{13}C NMR (126 MHz, CD_3OH): δ 101.5, 101.01, 100.96, 100.8, 100.5, 74.4, 73.0, 72.93, 72.87, 72.79, 72.76, 72.7, 72.54, 72.51, 72.4, 72.3, 72.1, 72.04, 71.99, 68.80, 68.76, 68.73, 68.70, 68.6, 67.4, 67.3, 67.1, 67.0, 62.9, 33.0, 30.7, 30.6, 30.4, 27.5, 23.8, 14.5; HRMS-ESI *m/z* [M + Na]$^{+}$ calcd for $C_{38}H_{68}NaO_{26}$: 963.3891, found: 963.3883.

Analytical Methods

LC/MS analysis of per-O-acetylated enzymatic products - The enzymatic products (1-butanol extracts) were *per-O*-acetylated as described previously (*6*) and analyzed by LC/MS on an Agilent 6220A time-of-flight (TOF) mass spectrometer equipped with an electrospray ionization/atmospheric pressure chemical ionization (ESI/APCI), and a multimode source operated in the positive ion mode. An HPLC equipped with an Agilent 1200 binary pump (Agilent technologies; Palo Alto, CA) and a 2.1 inner diameter x 150 mm, 3.5 µm XBridge reverse phase C18 column (Waters) heated to 45°C was used for separation. Separation was done with a flow rate of 0.32 mL/min using solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol). Typically, 2 µL of sample were injected in 25% solvent B (75% solvent A) followed by a gradient over 30 min to 100% solvent B which was held for an additional 10 min. Mass spectra were acquired at a rate of 1.02 spectra / seconds from *m/z* 250 to 3,200 Da. The data collected was analyzed using the Mass Hunter software (Agilent).

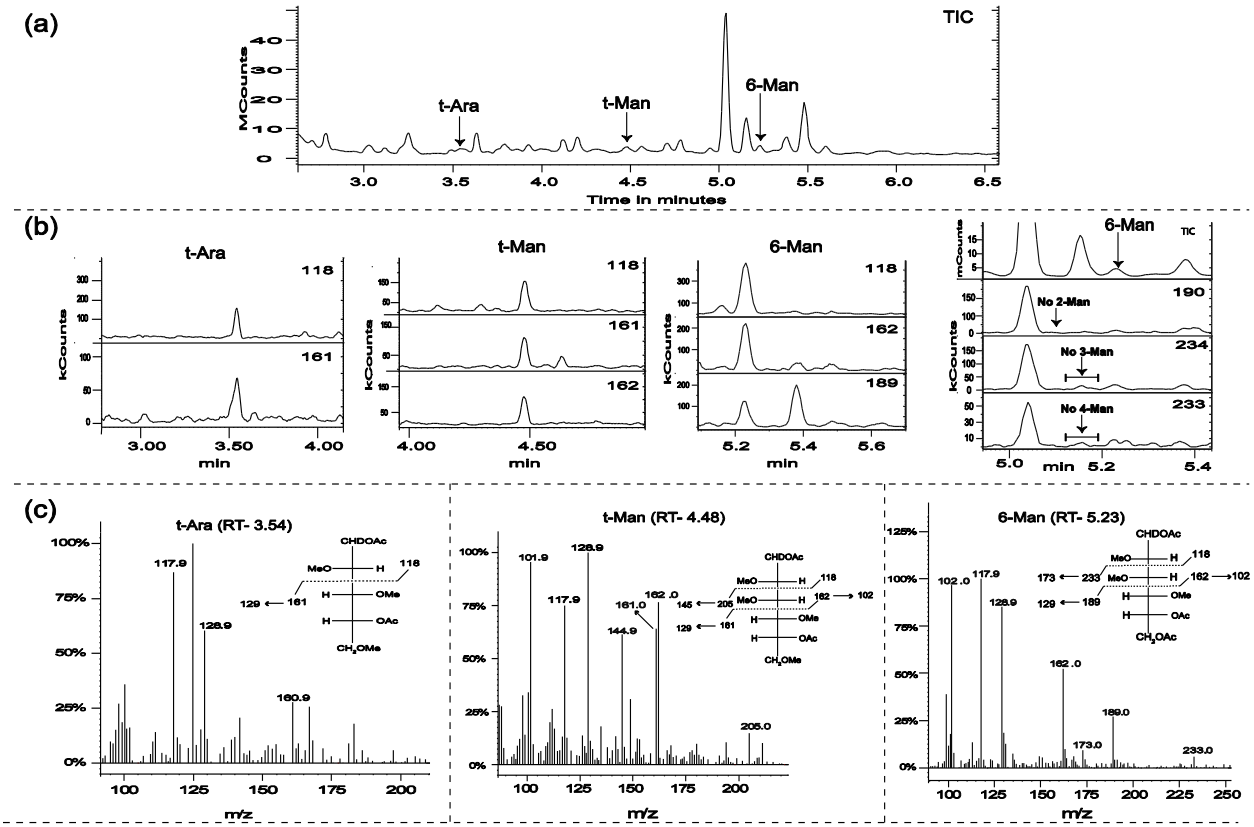
Per-O-methylation and LC/MS/MS analysis of the methylated enzymatic products - 1-butanol extracted enzymatic products were methylated as described by Ciucanu *et al*. (*7*) and analyzed by LC/MS using a Waters Acquity UPLC system equipped with a Waters Acquity UPLC HSS T3 column (1.8 µM, 1.0 x 100 mm). Injections were made in 100% solvent A (water, 0.1% formic acid), held in solvent A for 1 min, ramped to 98% solvent B (acetonitrile, 0.1% formic acid) over 12 min, held in 98% solvent B for 3 min, and then returned to starting conditions over 0.05 min and allowed to re-equilibrate for 3.95 min, with a 200 µL/min constant flow rate. The column and samples were held at 50 °C and 5 °C, respectively. The column eluent was infused into a Waters Xevo G2 Q-TOF-MS with an electrospray source in positive mode, scanning 50-1,200 *m/z* at 0.2 seconds per scan. MS/MS data was acquired using 0.2 second scan via collisional-

induced dissociation at 60 V collision energy. Calibration was performed using sodium formate with 1 ppm mass accuracy. The capillary voltage was held at 2,200 V, source temperature at 150 °C, and nitrogen desolvation temperature at 350 °C with a flow rate of 800 L/h.

GC/MS linkage analysis - For linkage analysis, partially methylated alditol acetate derivatives of *per-O*-methylated enzymatic products were prepared as described (*8*) and further analyzed by GC/MS using a CP 3800 gas chromatograph (Varian Inc., Palo Alto, CA) equipped with a MS320 mass spectrometer. Helium was used as the carrier gas with a flow rate of 1 mL/min. The samples were run on a DB 5 column (30 m x 0.20 mm inner diameter). The oven temperature was held at 50 ^{o}C for 1 min and programmed at 30 °C/min to 150 °C, then programmed at 5 °C/min to 275 °C. The mass spectrometer was scanned from *m/z* 50-450. The data analysis was carried out on a Varian WS data station.

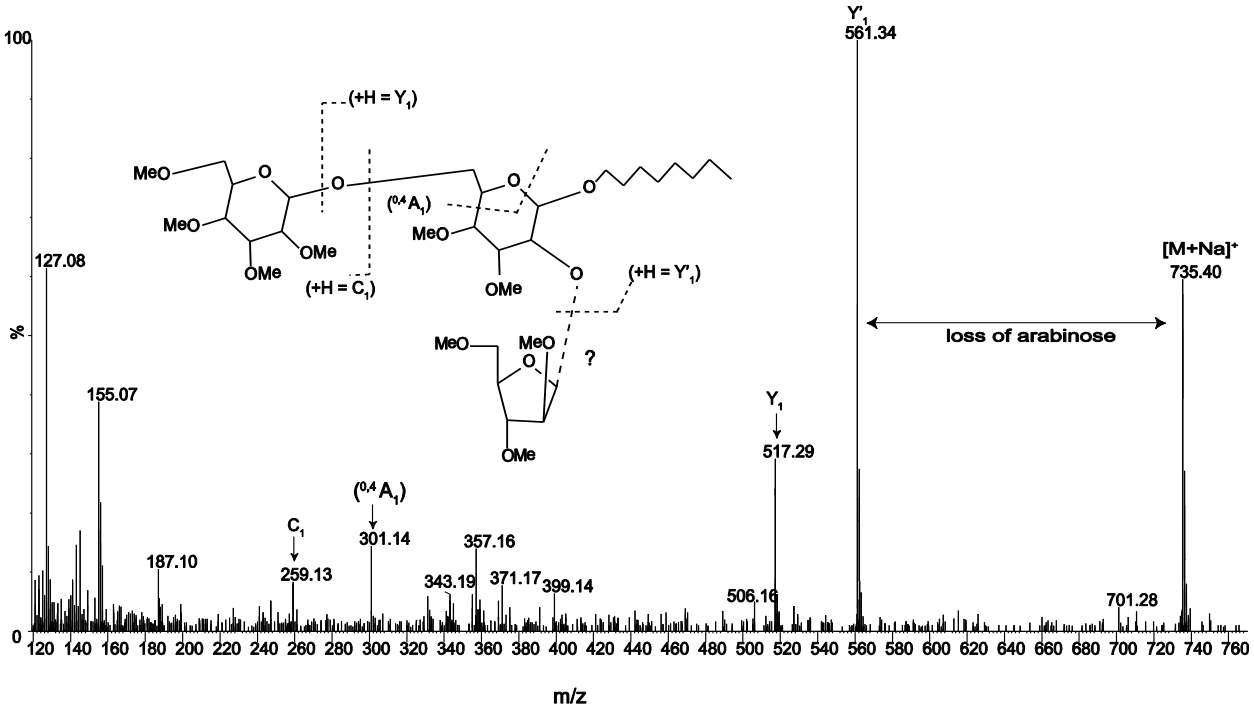
<u>Supplementary Figure 1</u>: GC/MS analysis of the partially acetylated partially methylated alditols from a purified fraction of the major Ara*f*-(1→6)-Man*p*-(1→6)-Man*p*-(1→octyl product (purified peak I on Fig. 4).

The major peak (I) (see Fig. 4) product oligosaccharide was purified by LC using two C-18 columns in tandem. The purified fraction contained signficant amounts of the acceptor dimannoside which "tailed" into the much smaller amounts of the Ara*f*-(1→6)-Man*p*-(1→6)-Man*p*-(1→octyl product in the ratio of 2:1 of disaccharide compared to the trisaccharide based on the intensities of their M+Na ions in the purfied fraction. As shown in the TIC (a), the desired partially acetylated partially methylated alditols were relatively minor components compared to contaminants of unknown origin. The selected ion trace of *m/z* 118 (b) showed the presence of derivatized alditols resulting from *t*-Ara, *t*-Man, and 6-Man as confirmed by the co-elution of the selected ions at *m/z* 161 for *t*-Ara and *t*-Man, *m/z* 162 for *t*-Man and 6-Man, and *m/z* 189 for 6-Man. The lack of significant *m/z* 190 [see the far right panel in (b)] running with the retention time of the derivative formed from 2-Man shows the lack of this compound. Similarly, the lack of significant amounts of *m/z*'s 234 and 233 show the lack of the derviatives resulting from 3-Man and 4-Man as well. The mass spectra of the partially acetylated partially methylated alditols located by the selected ion chromatographs (b) are shown in (c) for the derivatives of *t*-Ara, *t*-Man, and 6-Man.


(a)
TIC
t-Ara
t-Man
6-Man
MCounts
Time in minutes
(b)
t-Ara
t-Man
6-Man
118
161
162
189
190
234
233
No 2-Man
No 3-Man
No 4-Man
kCounts
min
(c)
t-Ara (RT- 3.54)
t-Man (RT- 4.48)
6-Man (RT- 5.23)
m/z

<u>Supplementary Figure 2</u>: The collision induced fragmentation of peak (II) (see Fig. 4).

Collision induced fragmentation of the ion at *m/z* 735.41 at 9.41 minutes (peak II) was performed. The Y and Y' ions at *m/z*'s 517.29 and 561.34, respectively, suggest arabinosylation of the interior mannosyl residues of the Man-(1→6)-Man-(1→octyl substrate. An ion at *m/z* 357.16 at first glance suggests a conflicting Y ion of a linear oligosaccharide (see Fig. 4) but the accurate mass differs from the predicted mass of *m/z* 357.22 and, in fact, *m/z* 357.22 was found for peak (I) but 357.16 for peak (II) in the same LC/MS/MS run. The only other ions assigned were *m/z* 259.13 pointing to a terminal mannosyl residue and *m/z* 301.14 point towards the ($^{0,4}A_1$) cleavage containing the terminal Man*p* unit. The structure is drawn with the *t*-Man residue attached to O-6 of the internal mannosyl mostly on the basis of the known structure of the acceptor oligosaccharide. The arabinosyl residue is shown attached to O-2 of the internal mannosyl residues given the results of the earlier study by Chatterjee *et al*. *(9)*, but there is no direct evidence for this from the collision inducted fragmentation spectrum. Further, this component (peak II, Fig. 4) was not present in a high enough yield to purify and attempt to form the corresponding partially acetylated partially methylated alditols for a linkage analysis. Thus, we conclude that a second product is formed and that the collision induced fragmentation spectrum is quite different from that of the major component (Fig. 4b) and consistent with arabinosylation of the internal mannosyl residue but this conclusion must be considered tentative.


100
%
0
127.08
155.07
187.10
259.13
301.14
357.16
343.19
371.17
399.14
506.16
517.29
561.34
701.28
735.40
[M+Na]+
loss of arabinose
(+H = Y1)
(+H = C1)
(0,4A1)
(+H = Y'1)
MeO
OMe
O
?
120 140 160 180 200 220 240 260 280 300 320 340 360 380 400 420 440 460 480 500 520 540 560 580 600 620 640 660 680 700 720 740 760
m/z

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