MOLECULAR BASIS OF ARABINOBIO-HYDROLASE ACTIVITY IN PHYTOPATHOGENIC FUNGI. CRYSTAL STRUCTURE AND CATALYTIC MECHANISM OF *FUSARIUM GRAMINEARUM* GH93 α-L-ARABINOFURANOSIDASE

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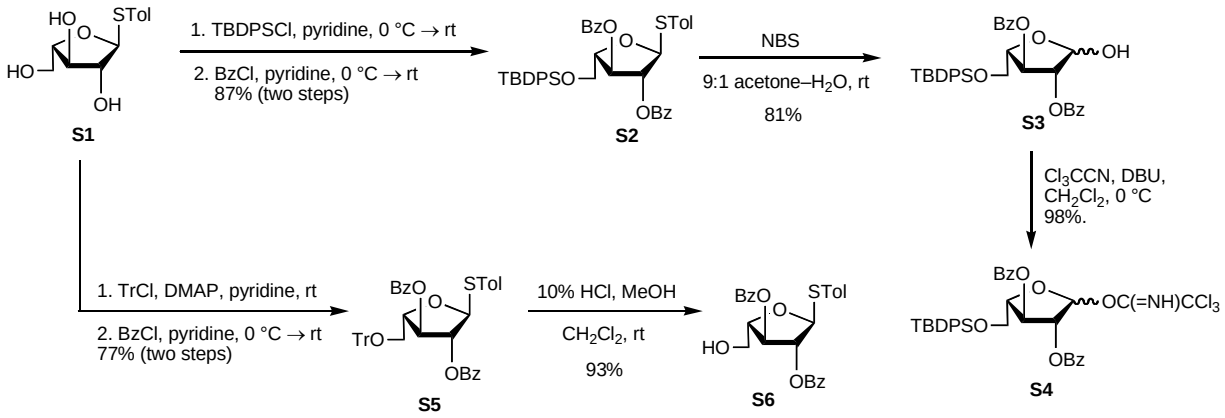
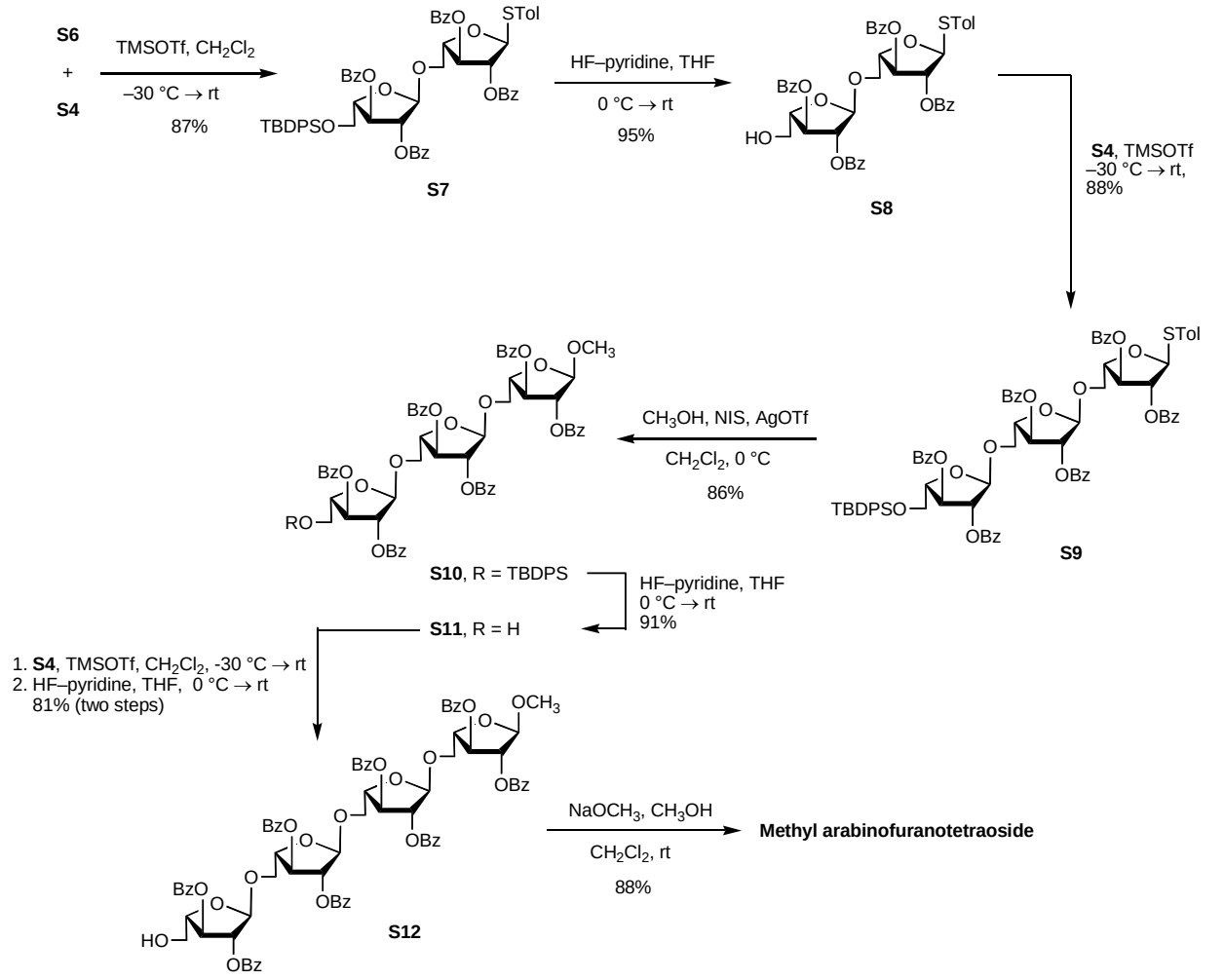
SUPPLEMENTAL MATERIAL

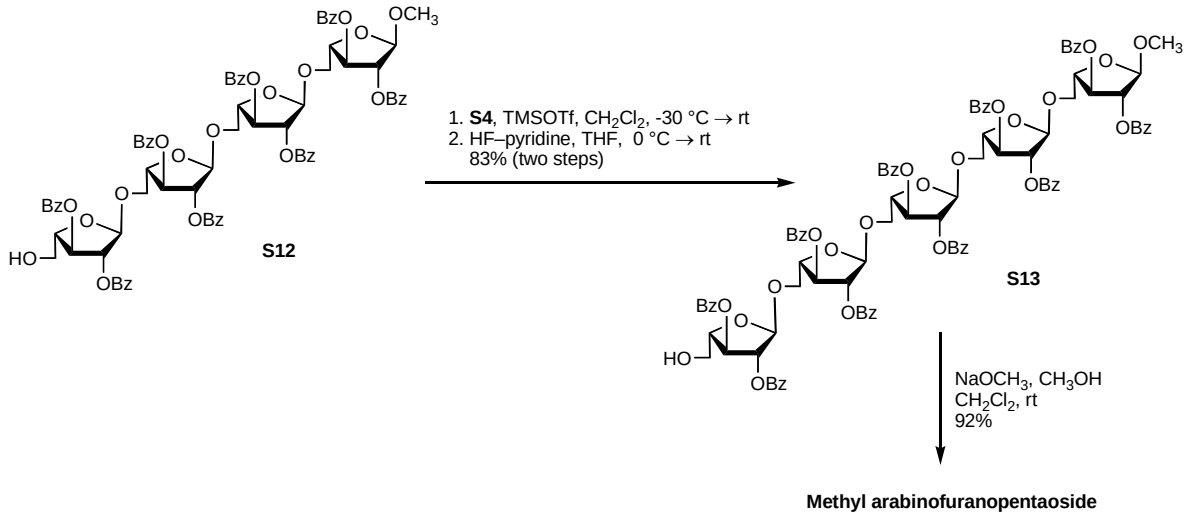
MATERIAL AND METHODS

Synthesis of Tetrasaccharide





Synthesis of Pentasaccharide



1. General Methods

Unless stated otherwise, all reactions were carried at rt under anhydrous conditions and a positive pressure of argon. The solvents used in reactions were purified by successive passage through columns of alumina and copper under nitrogen. Reactions were monitored by TLC on Silica Gel 60 F_{254} (0.25 mm, E. Merck). TLC spots were detected under UV light or by charring with acidified *p*-anisaldehyde in EtOH. All column chromatography was performed on silica gel. Optical rotations were measured at 22 ± 2°C. ^{1}H NMR spectra were recorded at 400, 500 or 600 MHz and chemical shifts were referenced to $CDCl_3$ (7.26, $CDCl_3$), CD_2Cl_2 (5.32, CD_2Cl_2) or HOD (4.78, D_2O and CD_3OD). ^{13}C spectra were recorded at 125 MHz, with chemical shifts referenced to internal $CDCl_3$ (77.23, $CDCl_3$), CD_2Cl_2 (53.8, CD_2Cl_2) or CD_3OD (48.9, CD_3OD). During reaction workup, organic solvents were washed with equal amounts of aqueous solutions during separations and concentrated under vacuum at <40 °C. ESI-MS spectra were carried out on samples suspended in THF or CH_3OH and added NaCl.

2. *p*-Tolyl 2,3-di-*O*-benzoyl-5-*O*-*t*-butyldiphenylsilyl-1-thio-α-L-arabinofuranoside (S2)

To a stirred solution of **S1**[1] (1.02 g, 3.9 mmol) in pyridine (10 mL) was added *t*-butylchlorodiphenylsilane (1.07 g, 3.9 mmol) at 0 °C. The reaction mixture was stirred overnight while warming to rt. The solution was subsequently cooled to 0 °C, and benzoyl chloride (1.2 mL, 10.2 mmol) was added. The reaction mixture was stirred overnight while warming to rt, prior to quenching the reaction by the addition of excess CH_3OH. The mixture was diluted with CH_2Cl_2 and washed successively with a satd aq soln of $NaHCO_3$ and water. The organic layer was dried (Na_2SO_4), filtered, and concentrated to a pale yellow syrup that was purified by column chromatography to give **S2** (2.43 g, 87%) as a colorless syrup: R_f 0.47 (6:1, hexanes–EtOAc). $[\alpha]_D$ = –48.7 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.12–8.10 (m, 2 H), 8.07–8.04 (m, 1 H), 7.99–7.96 (m, 2 H), 7.73–7.69 (m, 4 H), 7.63–7.54 (m, 2 H), 7.51–7.30 (m, 11 H), 7.14–7.12 (m, 2 H), 5.72–5.70 (m, 2H), 5.65 (app. q, 1H, *J* = 2.1 Hz), 4.61 (app. q, 1 H, *J* = 4.6 Hz), 4.04–4.03 (m, 2 H), 2.33 (s, 3 H), 1.05 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.5, 165.3, 137.9, 135.7, 135.6, 133.5, 133.4, 133.2, 133.1, 133.0, 132.9, 132.8, 130.0, 129.9, 129.8, 129.7, 129.6, 129.4, 129.1, 128.5, 128.4, 128.3, 127.7, 91.4 (C-1), 83.2, 82.4, 77.7, 63.6, 26.8, 21.1, 19.3. ESIMS: *m/z* calcd for (M + Na) $C_{42}H_{42}O_6SiS$: 725.2361. Found: 725.2363.

3. 2,3-Di-*O*-benzoyl-5-*O*-*t*-butyldiphenylsilyl-L-arabinofuranose (S3)

An excess of *N*-bromosuccimide (2.46 g, 13.8 mmol) was added to a solution of **S2** (2.43 g, 3.46 mmol) in acetone–water (9:1, 60 mL) and the mixture was stirred at rt for 45 min. The solvent was evaporated to a crude solid residue and redissolved in EtOAc (200 mL) before washing with a satd aq soln of $NaHCO_3$ (3 x 50 mL) and water (3 x 50 mL). The organic phase was

subsequently dried (Na_2SO_4), filtered, and concentrated to a pale yellow syrup that was purified by column chromatography (6:1 hexanes–EtOAc) to afford **S3** (1.67 g, 1.1:1 α:β mixture 81%) as a white foam: R_f 0.28 (4:1 hexanes–EtOAc). ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.13–8.00 (m, 4 H), 7.86–7.27 (m, 16 H), 6.07 (dd, 0.5 H, *J* = 4.0, 5.8 Hz), 5.76 (m, 0.5 H), 5.71–5.69 (m, 0.5 H), 5.65 (s, 0.5 H), 5.60 (dd, 0.5 H, *J* = 4.8, 5.7 Hz), 5.53 (dd, 0.5 H, *J* = 1.1 Hz), 4.61 (app. q, 0.5 H, *J* = 4.7 Hz), 4.31–4.28 (m, 0.5 H), 4.11 (dd, 0.5 H, *J* = 3.1, 11.1 Hz), 3.97 (dd, 0.5 H, *J* = 2.6, 11.1 Hz), 1.16 (s, 4.5 H, $C(CH_3)_3$), 1.08 (s, 4.5 H, $C(CH_3)_3$); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1, 165.8, 165.7, 165.6, 135.9, 135.7, 135.6, 135.5, 133.7, 133.5, 133.4, 133.2, 132.1, 131.8, 130.2, 130.1, 130.0, 129.9, 129.7, 129.4, 129.3, 129.2, 129.1, 128.6, 128.5, 128.4, 128.0, 127.8, 127.7, 126.6, 101.1 (C-1), 95.5 (C-1), 83.4, 83.0, 82.6, 82.2, 80.4, 79.3, 77.6, 76.6, 76.4, 65.1, 63.7, 26.9, 26.8, 19.3. ESIMS: *m/z* calcd for (M + Na) $C_{35}H_{36}O_7Si$: 619.2123. Found: 619.2122.

4. *p*-Tolyl 2,3-di-*O*-benzoyl-5-*O*-triphenylmethyl-1-thio-α-L-arabinofuranoside (S5)

Triol **S1**[1] (3.12 g, 12.2 mmol) was dissolved in pyridine (25 mL) and CH_2Cl_2 (10 mL) before adding chlorotriphenylmethane (3.37 g, 12.1 mmol) and DMAP (0.15 g, 1.2 mmol). The reaction mixture was warmed to 40 °C and stirred overnight. Analysis by TLC indicated the presence of a new single product, R_f 0.75 (6:1 CH_2Cl_2–CH_3OH), presumably corresponding to the trityl protected thioglycoside. The solution was then cooled to 0 °C and benzoyl chloride (6.3 mL, 54.9 mmol) was added dropwise over 15 min before the reaction warmed to rt while stirring overnight. After diluting the reaction mixture with CH_2Cl_2 (50 mL), the organic phase was washed successively with dilute 0.1 M HCl (30 mL) and water (2 x 20 mL) before drying (Na_2SO_4). After filtration of the drying agent, the solvent was evaporated to a dark syrup that was purified by column chromatography (6:1 hexane–EtOAc) to give **S5** (6.62 g, 77% over two

steps) as a white foam: R_f 0.43 (4:1 hexanes–EtOAc). $[\alpha]_D$ = –41.8 (*c* 3.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.13–8.10 (m, 2 H), 7.97–7.94 (m, 2 H), 7.63–7.14 (m, 25 H), 5.73 (d, 1 H, *J* = 1.8 Hz), 5.68–5.65 (m, 2 H), 4.71 (app. q, 1 H, *J* = 4.9 Hz), 3.57–3.48 (m, 2 H), 2.35 (s, 1 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.4, 165.3, 146.9, 143.8, 138.0, 133.4, 132.9, 130.0, 129.9, 129.8, 129.4, 129.1, 128.8, 128.5, 127.9, 127.8, 127.3, 127.0, 91.6, 82.4, 82.0, 78.1, 63.5, 21.2. ESIMS: *m/z* calcd for (M + Na) $C_{45}H_{38}O_6S$: 729.2281. Found: 729.2284.

5. *p*-Tolyl 2,3-di-*O*-benzoyl-1-thio-α-L-arabinofuranoside (S6)

To a solution of **S5** (2.51 g, 3.55 mmol) in CH_2Cl_2 (10 mL) was added a 10% HCl in MeOH solution (30 mL) and the reaction was stirred for 5 h before quenching by the addition of Et_3N. The solution was concentrated to a colourless syrup that was purified by column chromatography (6:1 hexanes–EtOAc) to afford **S6** as a colorless oil: R_f 0.39 (3:1, hexane–EtOAc); $[\alpha]_D$ = –68.4 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.09–8.16 (m, 2 H), 8.02–8.08 (m, 2 H), 7.40–7.66 (m, 8 H), 7.15 (app. d, 2 H, *J* = 8.0 Hz), 5.70–5.75 (m, 2 H), 5.55 (br. d, 1H, *J* = 4.5 Hz), 4.59 (app. q, 1 H, *J* = 4.5 Hz), 3.98–4.08 (m, 2 H), 2.33 (s, 3 H); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.0, 165.2, 138.2, 133.7, 133.6, 132.9, 130.1, 130.0, 130.0, 129.9, 129.9, 129.8, 129.0, 129.0, 128.6, 91.8 (C-1), 83.7, 82.2, 77.9, 62.0, 21.2. ESIMS: *m/z* calcd. for (M + Na) $C_{26}H_{24}O_6S$: 487.1186. Found: 487.1190.

6. *p*-Tolyl 2,3-Di-*O*-benzoyl-5-*O*-*t*-butyldiphenylsilyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-1-thio-α-L-arabinofuranoside (S7)

To a solution of alcohol **S3** (5.93 g, 9.9 mmol) and trichloroacetonitrile (5.0 mL, 49.9 mmol) in CH_2Cl_2 (90 mL) at 0 °C was added DBU (150 μL, 1.0 mmol). The reaction mixture was stirred

at 0 °C for 30 min and then warmed to rt over 30 min. The solvent was removed to yield a dark syrup that was purified on a short silica column (4:1 hexanes–EtOAc) to yield the trichloroacetimidate derivative **S4** (7.22 g, 98%), which was used immediately in the following glycosylation. Alcohol **S6** (1.09 g, 2.38 mmol) and donor **S4** (1.96 g, 2.53 mmol) were dissolved in CH_2Cl_2 (30 mL) containing crushed 4 Å molecular sieves (~1.5 g) and stirred at rt for 30 min before cooling to –30 °C. A solution of 1% TMSOTf in CH_2Cl_2 (4.60 mL, 0.24 mmol) was added dropwise over 5 min. The reaction mixture was warmed to –5 °C over 30 min and quenched by the addition of Et_3N (0.10 mL). The solution was diluted with CH_2Cl_2 (100 mL), filtered, and the filtrate was concentrated to a syrup that was purified by column chromatography (6:1 hexanes–EtOAc) to afford **S7** (2.16 g, 87%) as a white foam: R_f 0.44 (4:1 hexanes–EtOAc). $[\alpha]_D$ = –39.3 (*c* 1.1, CH_2Cl_2); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.14–7.91 (m, 8 H), 7.75–7.70 (m, 4 H), 7.63–7.53 (m, 2 H), 7.49–7.22 (m, 18 H), 7.15–7.02 (m, 2 H), 5.75–5.71 (m, 3 H), 5.66 (d, 1 H, *J* = 4.7 Hz), 5.57 (d, 1 H, *J* = 1.3 Hz), 5.38 (s, 1 H), 4.74 (app. q, 1 H, *J* = 7.5), 4.52 (app. q, 1 H, *J* = 4.7 Hz), 4.23 (app. q, 1 H, *J* = 4.1 Hz), 4.22–3.94 (m, 3 H), 2.30 (s, 3 H), 1.04 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.5, 165.3, 165.1, 137.7, 135.6, 135.6, 133.5, 133.4, 133.3, 133.2, 133.1, 133.0, 132.6, 130.0, 129.9, 129.8, 129.6, 129.2, 129.2, 128.9, 128.5, 128.4, 128.3, 128.2, 127.6, 106.0 (C-1'), 91.5 (C-1), 83.2, 82.0, 77.5, 77.3, 65.8, 63.4, 26.8, 21.1, 19.3. ESIMS: *m/z* calcd for (M + Na) $C_{61}H_{58}O_{12}SiS$: 1065.3311. Found: 1065.3309.

7. *p*-Tolyl 2,3-Di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-1-thio-α-L-arabinofuranoside (S8)

The silyl protected disaccharide thioglycoside **S7** (1.0 g, 0.96 mmol) was dissolved in pyridine–THF (1:5, 30 mL) and cooled to 0 °C before adding HF–pyridine (0.95 mL) dropwise. The

reaction was warmed to rt and stirred overnight before being diluted with EtOAc (50 mL) and poured into a satd aq soln of $NaHCO_3$ (150 mL). The crude product was extracted with EtOAc (70 mL × 2) and the combined organic phase was washed with water (75 mL × 2) and dried (Na_2SO_4). After filtration of the desiccant, the filtrate was concentrated and the resulting crude syrup was purified by column chromatography (2:1 hexanes–EtOAc) to afford **S8** (0.74 g, 95%) as a white foam. R_f 0.54 (2:1 hexanes–EtOAc). $[\alpha]_D$ = –44.9 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (600 MHz, $CDCl_3$) δ_H 8.12–8.00 (m, 6 H), 7.96–7.92 (m, 2 H), 7.63–7.56 (m, 2 H), 7.54–7.37 (m, 10 H), 7.29 (d, 2 H, *J* = 7.8 Hz), 7.10 (d, 2 H, *J* = 8.4 Hz), 5.76–5.70 (m, 3 H), 5.64 (d, 1 H, *J* = 1.3 Hz), 5.45 (d, 1 H, *J* = 4.2 Hz), 5.40 (s, 1 H, H-1'), 4.72 (q, 1 H, *J* = 4.4 Hz), 4.49 (app. q, 1 H, *J* = 4.2 Hz), 4.24 (app. q, 1 H, *J* = 4.4 Hz), 4.03–3.97 (m, 2 H), 3.95 (dd, 1 H, *J* = 12.1, 4.2 Hz), 2.31 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.1, 165.5, 165.1, 137.9, 133.5, 133.3, 132.6, 130.0, 129.9, 129.8, 129.7, 129.1, 129.0, 128.9, 129.0, 128.9, 128.5, 128.3, 105.8 (C-1'), 91.5 (C-1), 83.7, 82.1, 82.0, 81.7, 77.7, 77.4, 66.0, 62.3, 21.4. ESIMS: *m/z* calcd for (M + Na) $C_{45}H_{40}O_{12}S$: 827.2133. Found: 827.2131.

8. *p*-Tolyl 2,3-Di-*O*-benzoyl-5-*O*-*t*-butyldiphenylsilyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-1-thio-α-L-arabinofuranoside (S9)

Compound **S9** was prepared as a white foam (1.21 g, 96%) from trichloroacetimidate derivative **S4** (0.74 g, 1.0 mmol), alcohol **S8** (0.74 g, 0.91 mmol), crushed 4 Å molecular sieves (~0.75 g), and 1% TMSOTf (2 mL, 0.10 mmol, CH_2Cl_2) in CH_2Cl_2 (30 mL) as described for the synthesis of **S7**. R_f 0.43 (4:1 hexanes–EtOAc); $[\alpha]_D$ +11.2 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.11–7.91 (m, 12 H), 7.72–7.68 (m, 4 H), 7.61–7.28 (m, 26 H), 7.10–7.07 (m, 2 H), 5.76–5.71 (m, 3 H), 5.67–5.62 (m, 3 H), 5.58 (br. s, 1 H), 5.39 (s, 2 H), 4.70 (app. q, 1 H, *J* = 4.3 Hz), 4.63

(app. q, 1 H, J = 4.2 Hz), 4.51 (app. q, 1 H, J = 4.7 Hz), 4.25 (dd, 1 H, J = 4.3, 11.2 Hz), 4.18 (dd, 1 H, J = 4.2, 11.3 Hz), 4.02–3.90 (m, 4 H), 2.30 (s, 3 H), 1.03 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.6, 165.5, 165.3, 165.2, 165.1, 137.9, 133.5, 133.3, 133.2, 133.1, 133.0, 132.5, 130.0, 129.8, 129.6, 129.3, 129.1, 129.0, 128.9, 128.5, 128.4, 128.3, 128.2, 127.7, 106.0 (2 C, C-1', C-1"), 91.5 (C-1), 83.2, 82.1, 82.0, 81.6, 77.5, 77.0, 76.8, 65.8, 63.4, 26.8, 21.1, 19.3. ESIMS m/z calcd for (M + Na) $C_{80}H_{74}O_{18}SiS$: 1405.4257. Found: 1405.4256.

9. Methyl 2,3-di-*O*-benzoyl-5-*O*-*t*-butyldiphenylsilyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranoside (S10)

Thioglycoside **S9** (0.50 g, 0.36 mmol) was dried over P_2O_5 under vacuum for 5 h before being dissolved in CH_2Cl_2 (5 mL). The solution was cooled to 0 °C following the addition of dry CH_3OH (0.20 mL, 4.9 mmol). *N*-iodosuccinimide (0.10 g, 0.44 mmol) and silver triflate (22 mg, 0.086 mmol) were added and the solution was stirred for 30 min before being neutralized with Et_3N. The reaction mixture was diluted with CH_2Cl_2 (20 mL) and filtered through Celite. The filtrate was washed with a satd aq soln of sodium thiosulfate (3 × 15 mL), water (15 mL) and dried (Na_2SO_4) before being filtered and concentrated to a colourless syrup that was purified by column chromatography (4:1 hexanes–EtOAc) to obtain **S10** as a white foam (0.402 g, 86%). R_f 0.31 (4:1 hexanes–EtOAc); $[\alpha]_D$ +6.3 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.09–7.89 (m, 12 H), 7.73–7.67 (m, 4 H), 7.59–7.27 (m, 24 H), 5.66–5.61 (m, 4 H), 5.57 (d, 1 H, J = 1.4 Hz), 5.51 (d, 1 H, J = 1.5 Hz), 5.40 (s, 1 H), 5.39 (s, 1 H), 5.12 (s, 1 H), 4.64 (app. q, 1 H, J = 4.5 Hz), 4.50 (app. q, 1 H, J = 4.7 Hz), 4.44 (app. q, J = 4.8 Hz), 4.23–4.17 (m, 2 H), 4.01–3.90 (m, 4 H), 3.47 (s, 3 H), 1.02 (s, 9 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 165.7, 165.6, 165.4, 165.2, 165.1, 135.6, 133.4, 133.3, 133.2, 133.1, 133.0, 129.9, 128.9, 129.8, 129.6, 129.3, 129.2, 129.0,

128.5, 128.4, 128.3, 128.2, 127.6, 106.8 (C-1), 106.0 (C-1'), 105.9 (C-1''), 83.2, 82.1, 82.0, 81.7, 77.3, 66.0, 65.9, 26.8, 19.3. ESIMS *m*/*z* calcd for (M + H) $C_{74}H_{70}O_{19}Si$: 1313.4172. Found: 1313.4177.

10. Methyl 2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranoside (S11)

To a solution of compound **S10** (0.252 g, 0.2 mmol) in pyridine–THF (1:5, 6.0 mL) at 0 °C was added HF–pyridine (0.20 mL) dropwise. The reaction was stirred for 5 min before warming to rt and being stirred overnight. The reaction was diluted with EtOAc (20 mL) and poured into a satd aq soln of $NaHCO_3$ (75 mL) before extracting with EtOAc (2 × 30 mL). The combined organic layer was washed with water (50 mL) and dried (Na_2SO_4) before filtering and concentrating to a pale yellow syrup that was purified by column chromatography (4:1 hexanes–EtOAc) to afford **S11** (0.19 g, 91%) as a white foam: R_f 0.17 (4:1 hexanes–EtOAc); $[\alpha]_D$ +9.8 (*c* 0.4, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.08–8.01 (m, 8 H), 7.94–7.89 (m, 4 H), 7.61–7.49 (m, 4 H), 7.48–7.37 (m, 10 H), 7.31–7.25 (m, 4 H), 5.67–5.62 (m, 4 H), 5.51 (d, 1 H, *J* = 1.5 Hz), 5.44–5.41 (m, 3 H), 5.12 (s, 1 H), 4.64 (app. q, 1 H, *J* = 4.4 Hz), 4.48 (app. q, 1 H, *J* = 4.1 Hz), 4.46–4.42 (m, 1 H), 4.22–4.17 (m, 2 H), 4.03–3.90 (m, 4 H), 3.45 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0, 165.7, 165.4, 165.2, 165.0, 133.5, 133.4, 133.3, 133.2, 129.9, 129.8, 129.2, 129.1, 129.0, 128.5, 128.4, 128.3, 106.8 (C-1), 105.8 (2 C, C-1', C-1''), 83.7, 82.0, 81.9, 81.7, 81.6, 77.7, 77.4, 77.3, 66.1, 65.9, 54.9. ESIMS *m*/*z* calcd for (M + Na) $C_{58}H_{52}O_{19}$: 1075.2995. Found: 1075.2987.

11. Methyl 2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranoside (S12)

Alcohol **S11** (0.301 g, 0.29 mmol) and donor **S4** (0.240 g, 0.32 mmol) were dissolved in CH_2Cl_2 (10 mL) containing 4 Å molecular sieves (~0.3 g) and stirred at rt for 15 min before cooling to –30 °C. A solution of 1% TMSOTf in CH_2Cl_2 (0.60 mL, 0.03 mmol) was added dropwise over 5 min. The reaction mixture was warmed to –5 °C over 30 min, diluted with CH_2Cl_2 (50 mL), and filtered through Celite. The organic phase was poured into a satd aq soln of $NaHCO_3$ (25 mL) before being washed with a satd aq soln of $NaHCO_3$ (20 mL) and water (20 mL). After drying (Na_2SO_4), the organic layer was filtered and concentrated to a colourless syrup, which contained the *t*-butyldiphenylsilyl protected tetrasaccharide intermediate. This material was subsequently dissolved in pyridine–THF (2:5, 8.0 mL) and cooled to 0 °C before adding HF–pyridine (0.30 mL) dropwise. The reaction was stirred for 5 min before warming to rt and being stirring overnight. The reaction was diluted with EtOAc (20 mL) and poured into a satd aq $NaHCO_3$ soln (75 mL) before extracting with EtOAc (2 × 30 mL). The combined organic layer was washed with water (50 mL), dried (Na_2SO_4), filtered and concentrated to a pale yellow syrup, which was purified by column chromatography (2:1 hexanes–EtOAc) to afford **S12** (0.33 g, 81% two steps) as a white foam: R_f 0.44 (2:1 hexanes–EtOAc); $[\alpha]_D$ +1.1 (*c* 0.5, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.06–8.00 (m, 10 H), 7.93–7.86 (m, 6 H), 7.60–7.37 (m, 19 H), 7.29–7.22 (m, 5 H), 5.66–5.61 (m, 6 H), 5.51 (d, 1 H, *J* = 1.5 Hz), 5.43–5.39 (m, 4 H), 5.12 (s, 1 H, H-1), 4.63–4.58 (m, 2 H), 4.48–4.41 (m, 2 H), 4.22–4.14 (m, 3 H), 4.02–3.89 (m, 6 H), 3.45 (s, 3 H); NMR (125 MHz, $CDCl_3$) δ_C 166.1, 165.7, 165.6, 165.5, 165.2, 165.1, 133.5, 133.4, 133.3, 133.2, 129.9, 129.8, 129.2, 129.1, 129.0, 128.5, 128.4, 128.3, 106.8 (C-1), 105.8 (3 C, C-1', C-1", C-

1'''), 83.6, 82.0, 81.7, 81.6, 77.7, 77.3, 77.2, 66.1, 65.9, 62.3, 54.9. ESIMS *m*/*z* calcd for (M + Na) $C_{77}H_{68}O_{25}$: 1415.3942. Found: 1415.3950.

12. Methyl α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranoside

Alcohol **S12** (0.20 g, 0.15 mmol) was dissolved in a solution of 1:1 CH_2Cl_2–CH_3OH (1:1, 5 mL) to which 1M $NaOCH_3$ (0.10 mL) was added. The solution was stirred overnight before diluting with CH_2Cl_2 (10 mL) and neutralizing with Amberlite IR120 (H^+) cation exchange resin. The solution was filtered through Celite and concentrated to a pale yellow syrup that was purified by column chromatography (4:1 CH_2Cl_2–MeOH) to yield the product (72 mg, 88%) as a white foam: R_f 0.42 (3:1 CH_2Cl_2–MeOH); $[\alpha]_D$ –88.5 (*c* 0.8, H_2O); ^{1}H NMR (500 MHz, D_2O) δ_H 5.09–5.07 (m, 4 H), 4.93 (d, 1 H, *J* = 1.6 Hz), 4.23–4.19 (m, 3 H), 4.18–4.14 (m, 1 H), 4.13–4.11 (m, 4 H), 4.10–4.07 (m, 2 H), 4.05 (dd, 1 H, *J* = 1.7, 3.2 Hz), 4.02–3.98 (m, 4 H), 3.95 (dd, 1 H, *J* = 3.2, 6.0), 3.91–3.85 (m, 4 H), 3.84 (d, 1 H, *J* = 3.4 Hz), 3.82–3.77 (m, 4 H), 3.76 (d, 1 H, *J* = 3.3 Hz), 3.72 (d, 1 H, *J* = 5.8 Hz), 3.70 (d, 1 H, *J* = 5.8 Hz), 3.41 (s, 3 H); ^{13}C NMR (125 MHz, D_2O) δ_C 109.3 (C-1), 108.4 (2 C, C-1', C-1''), 108.3 (C-1'''), 84.8, 83.2, 83.1, 81.8, 81.7, 81.5, 77.6, 77.4, 67.8, 67.6, 62.1, 55.9. ESIMS *m*/*z* calcd for (M + Na) $C_{21}H_{36}O_{17}$: 583.1845. Found: 583.1843.

13. Methyl 2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl-(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranosyl -(1→5)-2,3-di-*O*-benzoyl-α-L-arabinofuranoside (S13)

Compound **S13** was prepared as a white foam (0.20 g, 83%) from trichloroacetimidate donor **9** (0.11 g, 0.15 mmol), alcohol **19** (0.19 g, 0.14 mmol), 4 Å molecular sieves (~0.1 g), 1% TMSOTf (0.30 mL, 0.02 mmol, CH_2Cl_2) in CH_2Cl_2 (5.0 mL), and subsequently deprotected with HF–pyridine (0.30 mL) in pyridine–THF (2:5, 8.0 mL) and as described for **S12**. Data for **S13**: R_f 0.38 (2:1 hexanes–EtOAc); $[\alpha]_D$ –3.5 (*c* 0.6, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$) δ_H 8.05–7.99 (m, 12 H), 7.93–7.84 (m, 8 H), 7.60–7.36 (m, 24 H), 7.29–7.21 (m, 6 H), 5.66–5.60 (m, 8 H), 5.51 (d, 1 H, *J* = 1.5 Hz), 5.42–5.38 (m, 5 H), 5.11 (s, 1 H), 4.59 (m, 3 H), 4.44 (m, 2 H), 4.18 (m, 4 H), 4.02–3.87 (m, 6 H), 3.44 (s, 3 H); ^{13}C NMR (125 MHz, $CDCl_3$) δ_C 166.0 (C=O), 165.7 (C=O), 165.6(3) (C=O), 165.4 (C=O), 165.1(4) (C=O), 133.4, 133.3, 133.2, 133.1, 129.9, 129.8, 129.2, 129.1, 129.0, 128.5, 128.4, 128.3, 128.2, 106.8 (C-1), 105.9 (2 C, 2 × C-1), 105.8 (2 C, 2 × C-1), 83.6, 82.1, 81.7, 81.6, 81.5, 77.7, 77.3, 77.2, 66.1, 65.9, 65.8, 64.4, 62.3, 60.4, 54.9. ESIMS *m*/*z* calcd for (M + Na) $C_{96}H_{84}O_{31}$: 1755.4889. Found: 1755.4890.

14. Methyl α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranosyl-(1→5)-α-L-arabinofuranoside.

The product was obtained as a white foam (55 mg, 92%) from alcohol **S13** (0.15 g, 0.087 mmol) and $NaOCH_3$ (0.10 mL, 1 M) in 1:1 CH_2Cl_2:CH_3OH (5 mL) as described for methyl arabinofuranotetraoside. Data for pentasaccharide: R_f 0.34 (7:3 CH_2Cl_2–MeOH); $[\alpha]_D$ –134.7 (*c* 0.7, H_2O); ^{1}H NMR (500 MHz, D_2O) δ_H 5.09–5.06 (m, 5 H), 4.93 (d, 1 H, *J* = 1.6 Hz), 4.22–4.18 (m, 4 H), 4.17–4.13 (m, 1 H), 4.13–4.11 (m, 5 H), 4.10–4.06 (m, 2 H), 4.05 (dd, 1 H, *J* = 1.7, 3.2 Hz), 4.01–3.98 (m, 5 H), 3.94 (dd, 2 H, *J* = 3.3, 6.0 Hz), 3.90–3.85 (m, 5 H), 3.83 (d, 1 H, *J* = 3.4 Hz), 3.81–3.77 (m, 5 H), 3.76 (d, 1 H, *J* = 3.3 Hz), 3.72 (d, 2 H, *J* = 5.8 Hz), 3.69 (d, 1 H, *J* = 5.8 Hz), 3.41 (s, 3 H); ^{13}C NMR (125 MHz, D_2O) δ_C 109.3 (C-1), 108.4 (3 C, 3 × C-1), 108.3 (C-1),

84.8, 83.2, 83.1, 81.8, 81.7, 81.5, 77.6, 77.5, 77.4, 67.9, 67.8, 67.7, 67.6, 62.1, 55.9. ESIMS *m*/*z* calcd for (M + Na) $C_{26}H_{44}O_{21}$: 715.2267. Found: 715.2269.

References:

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