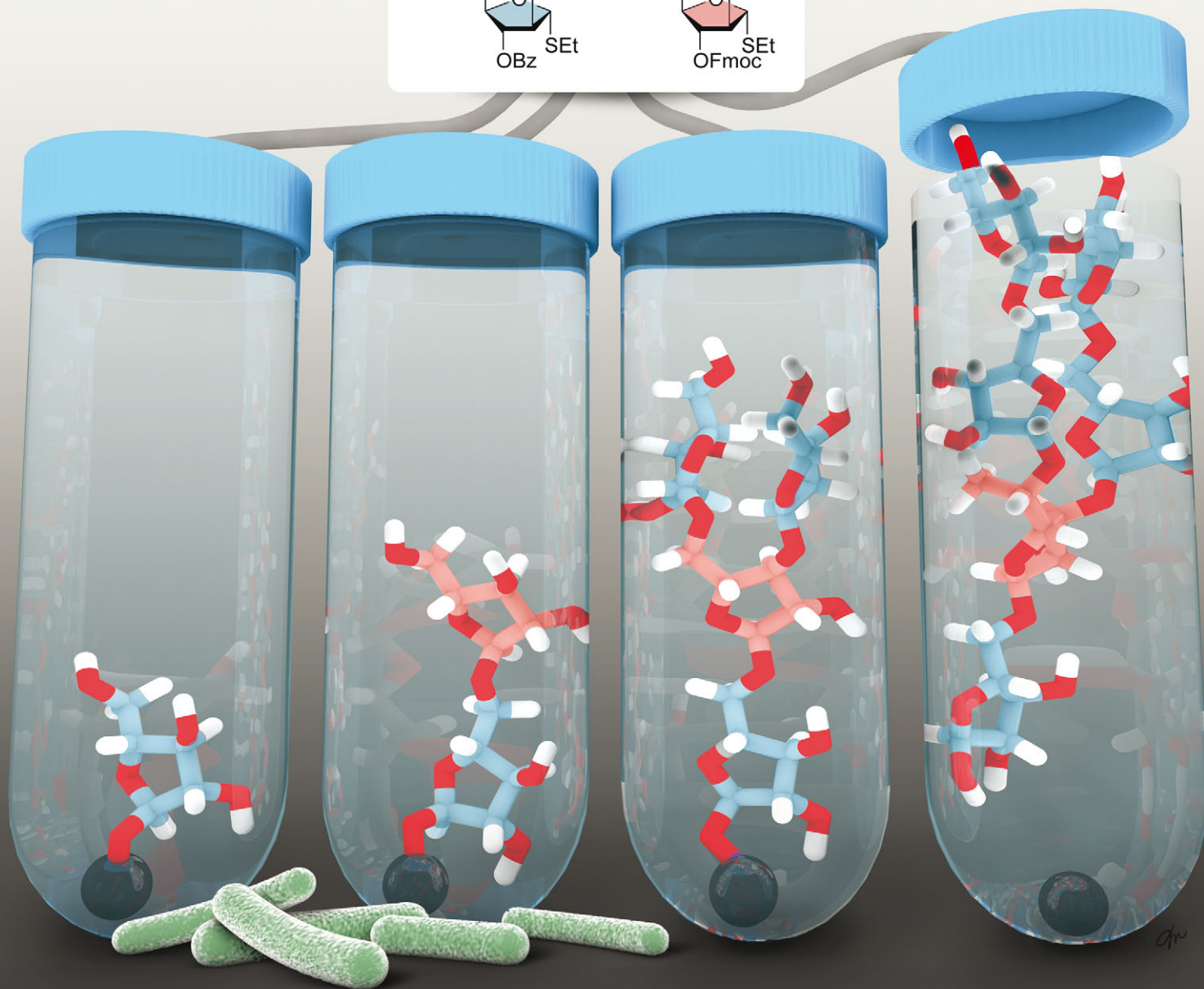
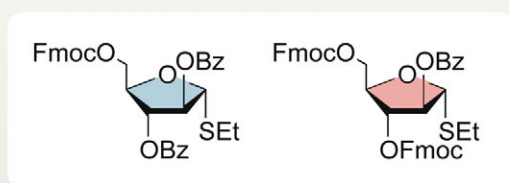


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Automated solid phase synthesis of oligoarabinofuranosides



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Automated solid phase synthesis enables rapid access to the linear and branched arabinofuranoside oligosaccharides. A simple purification step is sufficient to provide the conjugation ready oligosaccharides in good yield.

Arabinogalactan (AG) and lipoarabinomannan (LAM) polysaccharides are the major components of mycobacterial cell envelopes and contain multiple arabinose residues.¹ Arabinogalactan consists of three identical oligoarabinosides attached to a galactofuranose.^{2–4} Each oligoarabinoside contains arabinofuranose (Araf) residues, linked together *via* α -(1–5), α -(1–3) and β -(1–2) glycosidic linkages.

Synthetic oligoarabinofuranosides are useful tools for studying the biosynthetic enzymes involved in the mycobacterial cell wall synthesis.^{5–9} Access to various arabinofuranose fragments and analogues that can be immobilized on surfaces of glycan arrays and particles is instrumental in identifying potential ligands for these enzymes and developing biological assays to study their substrate specificities.¹⁰ Several solution phase strategies for synthesis of arabinofuranosidic oligomers were established.^{11–17} The groups of Lowary and Ito have accomplished the synthesis of the arabinan dodecasaccharide *via* a convergent fragment approach.^{18,19} These syntheses are challenging because they require many discrete operations and multiple purification steps.

Automated solid phase synthesis significantly reduces the time-requirements and enables rapid access to structurally-defined oligosaccharides,^{20–24} as well as conjugation ready oligosaccharide analogues.^{25,26} A combination of new technologies and synthetic methods was developed and adjusted to keep the automation technique up to date with the most recent developments in carbohydrate chemistry.^{20–26} The synthesis of

oligosaccharides such as Lewis X and Globo H demonstrated that complex carbohydrates as well as challenging glycosidic linkages can be accessed using an oligosaccharide synthesizer.^{21,22} To date, no automated oligofuranoside syntheses have been reported. Here, we describe the first automated synthesis of linear and branched oligoarabinofuranosides (Fig. 1).

The simple linear (1, 2) and branched (3, 4) oligoarabinofuranoside targets were pursued in order to test the ability of the automated route to produce both linear and branched structures. Linear α -D-(1–5)-Araf 1 and 2 have been prepared *via* iterative glycosylations using arabinose building block 7. Thioglycoside 7 was designed according to the requirements of the automated approach, with 9-fluorenylmethoxycarbonyl (Fmoc) as a temporary protecting group at C-5 and benzoates at C-2 and C-3 as participating protecting groups that are readily removed during cleavage from the solid support. The synthesis of thioglycoside 7 was accomplished in three steps starting from thioglycoside 5.^{27,28} Regioselective trityl protection of the primary hydroxyl in 5 followed by the benzoyl protection of remaining hydroxyls and the removal of the trityl group furnished thioglycoside 6 (Scheme 1). Fmoc protection of the C-5 hydroxyl produced the desired thioglycoside building block 7 in high yield.

Linear α -D-(1–5)-Araf oligosaccharides 1 and 2 were assembled using an oligosaccharide synthesizer (Scheme 2).²⁵ Glycosylations were carried out at temperatures between -40 °C and -20 °C using thioglycoside 7 and a mixture of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH). Each cycle involved two additions of a glycosylating agent (double coupling), using five equivalents of the building block. Removal of the Fmoc group with piperidine uncovered the hydroxyl group ready for elongation. The glycosylation-deprotection cycle was repeated twice for the synthesis of disaccharide 1 and six times for the preparation of hexasaccharide 2. Following oligosaccharide assembly on the solid support, cleavage from the resin using sodium methoxide solution furnished disaccharide 8 and hexasaccharide 9 that were purified by reverse phase preparative HPLC. Hydrogenolysis of 8 and 9 followed by a simple Sep-Pak purification afforded disaccharide 1 and hexasaccharide 2 in overall yield of 85% and 65%, respectively, based on resin loading.

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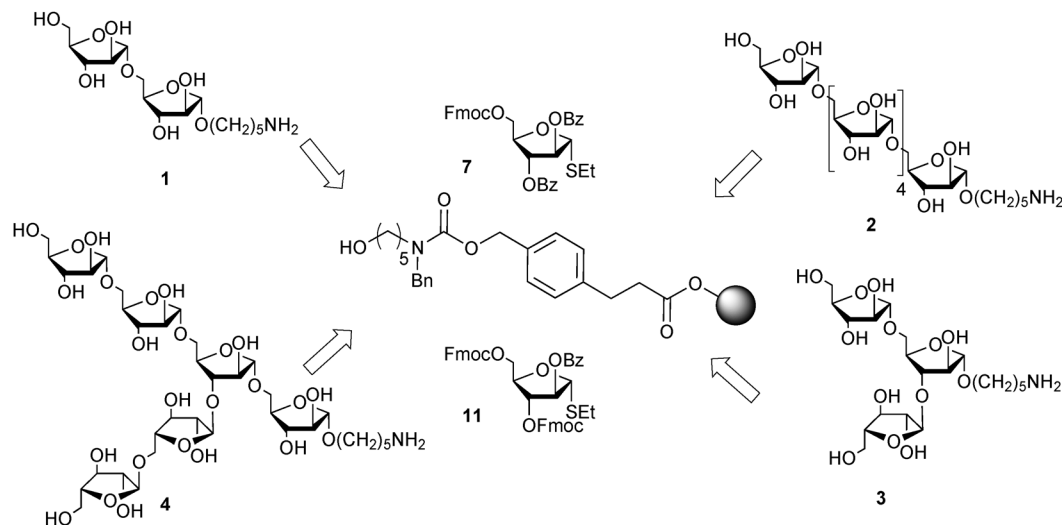
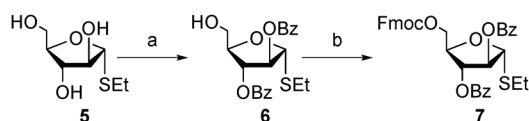
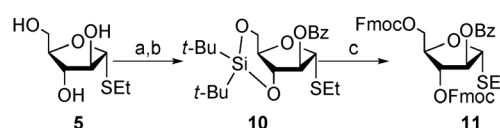


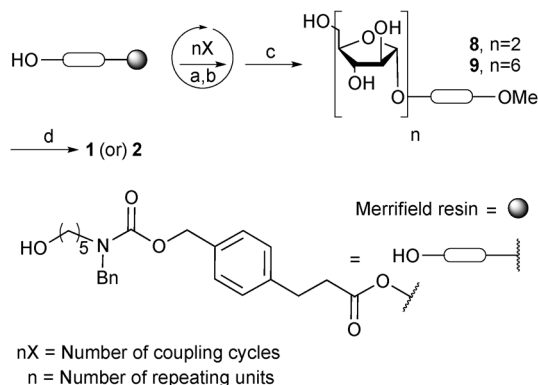
Fig. 1 Structures and retrosynthetic analysis of α -D-(1→5), α -D-(1→3) oligoarabinofuranosides 1–4.



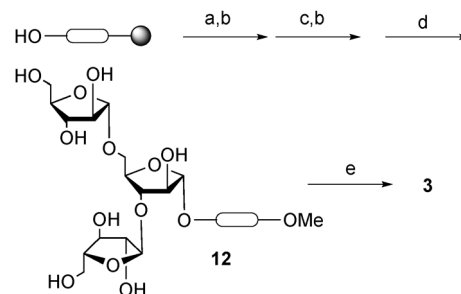
Scheme 1 Synthesis of 7. Reagents and conditions: (a) (i) TrCl, Py; then BzCl; (ii) PTSA, MeOH, 77% (two steps); (b) FmocCl, Py, 96%.



Scheme 3 Synthesis of building block 11. Reagents and conditions: (a) t -Bu₂Si-(OTf)₂, 2,6-lutidine, 60%; (b) BzCl, Py, 89%; (c) (i) HF-Py, THF; (ii) FmocCl, Py, 85% (two steps).



Scheme 2 Automated synthesis of oligosaccharides 1 and 2. Reagents and conditions: (a) 7, NIS/TfOH, -40 °C to -20 °C; (b) 20% piperidine in DMF; (c) NaOMe in MeOH; (d) Pd/C, H₂, MeOH/H₂O/EtOAc/AcOH.



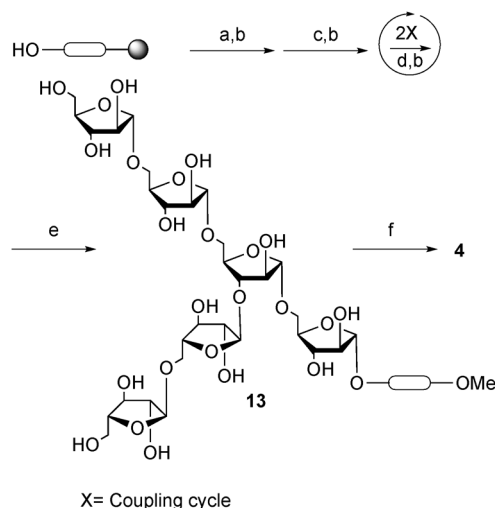
Scheme 4 Automated synthesis of branched trisaccharide 3. Reagents and conditions: (a) 11, NIS/TfOH, -40 °C to -20 °C, repeated twice; (b) 20% piperidine in DMF; (c) 7, NIS/TfOH, -40 °C to -20 °C, repeated four times; (d) NaOMe in MeOH; (e) Pd/C, H₂, MeOH/H₂O/EtOAc/AcOH.

In addition to the formation of α -(1→5) linkages, the synthesis of branched oligoarabinofuranosides 3 and 4 requires introduction of α -(1→3) branching. For this purpose building block 11 was used. Featuring two Fmoc groups, building block 11 enables the removal of temporary protection and glycosylation of both C-3 and C-5 hydroxyls in a single deprotection-glycosylation cycle. *En route* to thioglycoside 11, thioglycoside 5 was converted to silyl acetal 10 in two steps (Scheme 3). Selective removal of the silyl acetal followed by the Fmoc protection of the resulting diol provided building block 11 in 85% yield.

Upon loading the resin with building block 11 using a glycosylation-deprotection cycle, the first elongation in the

synthesis of oligoarabinofuranoside 3 was carried out with thioglycoside 7 using conditions established for the synthesis of 1 and 2 (Scheme 4). The following glycosylation to create the α -(1→5) linkage and install α -(1→3) branching was performed using thioglycoside 7. Removal of Fmoc groups and cleavage from the resin yielded crude trisaccharide 12. A simple purification protocol involving adsorption on a pad of silica gel followed by a hexane and CH₂Cl₂ wash and elution with a mixture of CH₂Cl₂ : MeOH was sufficient to obtain pure trisaccharide 12. Hydrogenolysis of partially protected trisaccharide 12 gave deprotected trisaccharide 3 in 78% overall yield based on resin loading, without the need for further purification.

The automated synthesis of branched hexasaccharide 4 was accomplished *via* four glycosylation cycles using building blocks



Scheme 5 Automated synthesis of branched hexasaccharide **4**. Reagents and conditions: (a) **7**, NIS/TfOH, $-40\text{ }^{\circ}\text{C}$ to $-20\text{ }^{\circ}\text{C}$, repeated twice; (b) 20% piperidine in DMF; (c) **11**, NIS/TfOH, $-40\text{ }^{\circ}\text{C}$ to $-20\text{ }^{\circ}\text{C}$, repeated twice; (d) **7**, NIS/TfOH, $-40\text{ }^{\circ}\text{C}$ to $-20\text{ }^{\circ}\text{C}$, repeated four times; (e) NaOMe in MeOH; (f) Pd/C, H_2 , MeOH/ H_2O /EtOAc/AcOH.

Table 1 Time required for the synthesis of oligosaccharides **1–4**^a

Compound	Steps					Total time (h)	Overall yield (%)
	I	II	III	IV	V		
1	6	6	6	16	3	37	85
2	16	6	6	16	3	47	65
3	8	6	3	16	3	36	78
4	14	6	3	16	3	42	63

^a Time given in hours for all steps, (I) automated synthesis (glycosylation-deprotection); (II) cleavage from resin (NaOMe); (III) first purification step (HPLC or silica pad); (IV) global deprotection (Pd/C, H_2); (V) final purification (Sep-Pak, optional).

7 and **11** (Scheme 5). The automated synthesis of hexasaccharide **13** required about 14 hours. Partially protected hexasaccharide **13** was purified by passing the crude mixture over a silica pad. Hydrogenolysis provided the amine functionalized hexasaccharide **4** in 63% overall yield based on resin loading.

Automated solid phase oligosaccharide synthesis drastically reduces the overall synthesis time from the building block to the final compound. Table 1 provides an overview of the time required for the synthesis of oligosaccharides **1–4**. The overall time to reach branched hexamer **4** was slightly shorter than that for hexamer **2** owing to the simplified purification protocol (Table 1, step III).

In conclusion, linear and branched conjugation-ready oligoarabinofuranosides **1–4** were assembled *via* automated solid phase synthesis. This robust and reproducible route constitutes the first automated solid phase synthesis of oligofuranosides. This method will provide straightforward access to a large number of different arabinogalactan fragments for biological studies.

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