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The Use of Pyranoside-into-Furanoside Rearrangement and Controlled O(5) → O(6) Benzoyl Migration as the Basis of a Synthetic Strategy To Assemble (1→5)- and (1→6)-Linked Galactofuranosyl Chains

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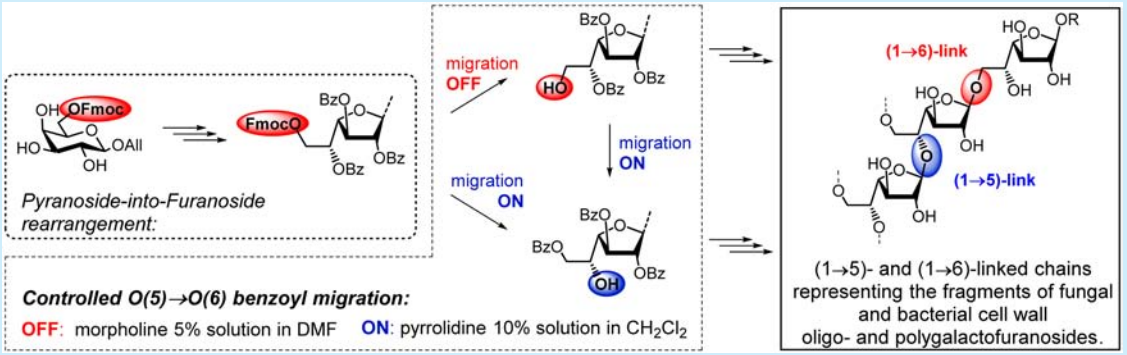
Supporting Information



ABSTRACT: A new pyranoside-into-furanoside (PIF) rearrangement of selectively protected galactopyranosides, followed by controlled O(5) → O(6) benzoate migration, gives either 5-OH or 6-OH products. It has been applied for the synthesis of four oligosaccharides related to the galactomannan from *Aspergillus fumigatus*. The assembly of target oligosaccharides containing both (1→5) and (1→6) linkages between galactofuranosyl residues was performed by applying terminal mannoside and digalactofuranoside blocks, forming a versatile approach toward fungal and bacterial carbohydrate antigens containing both 5-O- and 6-O-substituted galactofuranoside residues.

Aspergillus fumigatus is a dangerous fungal pathogen that causes severe and often fatal invasive aspergillosis (IA) in immunocompromised patients.[1] At risk are patients with cancer and those undergoing intensive immunosuppressive therapy after receiving organ transplants.[2] Galactomannan is a specific carbohydrate antigen of *A. fumigatus*,[3] and therefore, its detection in the patient serum is used for diagnosis of IA.[1] Oligosaccharide ligands structurally related to galactomannan could potentially help with the development of vaccines against this dangerous pathogen.

Galactomannan represents a structurally diverse heteropolysaccharide build up from a poly-D-mannose backbone with oligogalactofuranoside side chains attached to some of the mannose units via (1→3) or (1→6) bonds.[3] The galactofuranoside residues in the side chains are mainly β-(1→5)-linked, although the presence of β-(1→6) linkages has also been reported (Figure 1A).[4] It is noteworthy that alternating (1→5)-/(1→6)-linked galactofuranoside chains are also produced by some other microorganisms, including the dangerous pathogen *Mycobacterium tuberculosis* as well as other *Mycobacterium* species (Figure 1B).[5] Polysaccharides of the bacteria *Actinobacillus pleuropneumoniae*[6] and *Bifidobacterium catenulatum*[7] also contain (1→5)-/(1→6)-linked galactofuranoside backbones bearing α-galactopyranoside branches (Figure 1C).

The need for oligosaccharide ligands related to galactomannan continues to challenge the development of synthetic approaches for their acquisition. Previously, the preparations of different oligosaccharide fragments of galactomannan corresponding to the homogalactofuranosyl or heterosaccharide chains were described.[8] In all synthesized oligosaccharides, the galactofuranoside units were interconnected with (1→5) linkages. However, for detailed immunological investigations, a more representative series of shorter and longer spacer armed oligosaccharide antigens with both (1→5) and (1→6) linkages between galactofuranosyl units was required. Herein we describe the synthesis of tri-, penta-, and heptasaccharides **1**–**3** related to the galactomannan of *A. fumigatus* as as well as pentasaccharide **4** containing one (1→6) linkage between galactofuranoside residues, which is isomeric to compound **2** (Figure 1).

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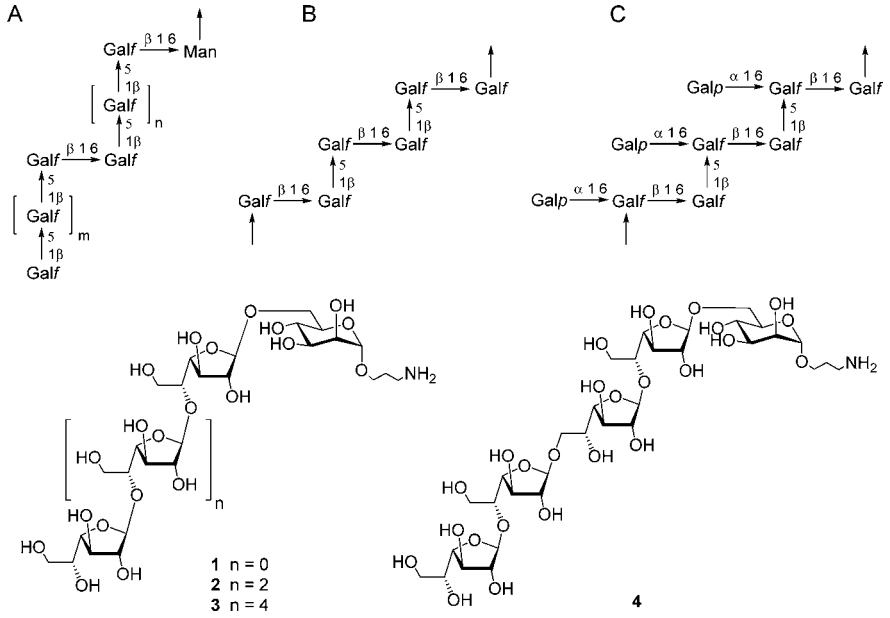


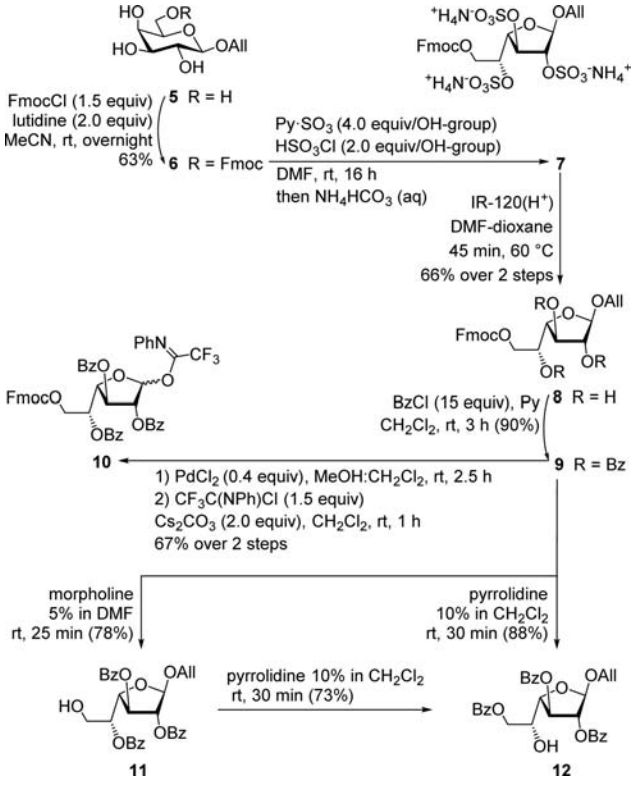
Figure 1. Structures of (A) galactomannan from *A. fumigatus*,[4] (B) arabinogalactan from *M. tuberculosis*,[5] (C) galactan from *A. pleuropneumoniae*[6] and *B. catenulatum*,[7] and target spacered oligosaccharides **1**–**4** related to galactomannan from *A. fumigatus*.

The synthesis of pentasaccharide **2** was previously performed by us with the use of a temporarily 5′-O-chloroacetylated galactofuranoside block suitable for homo-(1→5)-chain elongation.[8e] Herein we report syntheses based on the use of selectively protected allyl galactofuranoside **9** bearing a selectively removable 9-fluorenylmethoxycarbonate (Fmoc) group at O(6). Thus, O(6) deprotection permits subsequent chain elongation via (1→6) glycosylation. The choice of the Fmoc group as a temporary protection was argued by its stability under many reaction conditions, including ones in automated solid-phase synthesis.[9]

In order to prepare galactofuranoside **9**, regioselective introduction of the Fmoc group into allyl *β*-galactopyranoside **5**[10] in the presence of 2,6-lutidine was performed to give 6-O-acylated derivative **6** in 63% yield (Scheme 1).[11] Then **6** was transformed into isomeric furanoside **8** using the recently discovered pyranoside-into-furanoside (PIF) rearrangement[12] via totally sulfated intermediate **7**. Further per-O-benzoylation of **8** gave the desired bifunctional block **9**, which was transformed into glycosyl donor **10** via deallylation and imidation (**9** → **10**) or into 6-hydroxyglycosyl acceptor **11** by removal of the Fmoc group (Scheme 1).

Basic reaction conditions commonly used for Fmoc cleavage (e.g., treatment with piperidine in DMF[13]) could be accompanied by side benzoate migration. The O(5) → O(6) migration of the benzoyl group was also observed during acidic detritylation.[14] Thus, the search for a reliable Fmoc deprotection protocol was performed, first with the use of **9** as a model substrate. In particular, it was found that removal of the Fmoc group could be efficiently performed by treatment with more accessible pyrrolidine[15] (20% solution in DMF). The reaction was complete in less than 1 min. However, the formation of a mixture of the desired monosaccharide **11** (70%) and the benzoate migration product **12** (10%) was observed. The structure of product **12** was evident from proton chemical shifts (5.65 ppm for H(5) and 4.05–4.10 ppm for both H(6) in **11** vs 4.47 ppm for H(5) and 4.61 and 4.48 ppm for H(6) in **12**). To improve the reaction selectivity, we applied

Scheme 1. Synthesis of Galactofuranoside Products 8–12 Employing PIF Rearrangement and Controlled O(5) → O(6) Benzoyl Migration



morpholine (5% solution in DMF) as a milder base. The reaction was complete in 25 min and gave furanoside **11** (78%) with only a trace (4%) of the migration product **12**, which was separated by SiO_2 column chromatography.

Product **12** with a free OH group at O(5) can be regarded as a convenient precursor for the synthesis of 5-O-substituted galactofuranoside derivatives, including the target (1→5)-linked

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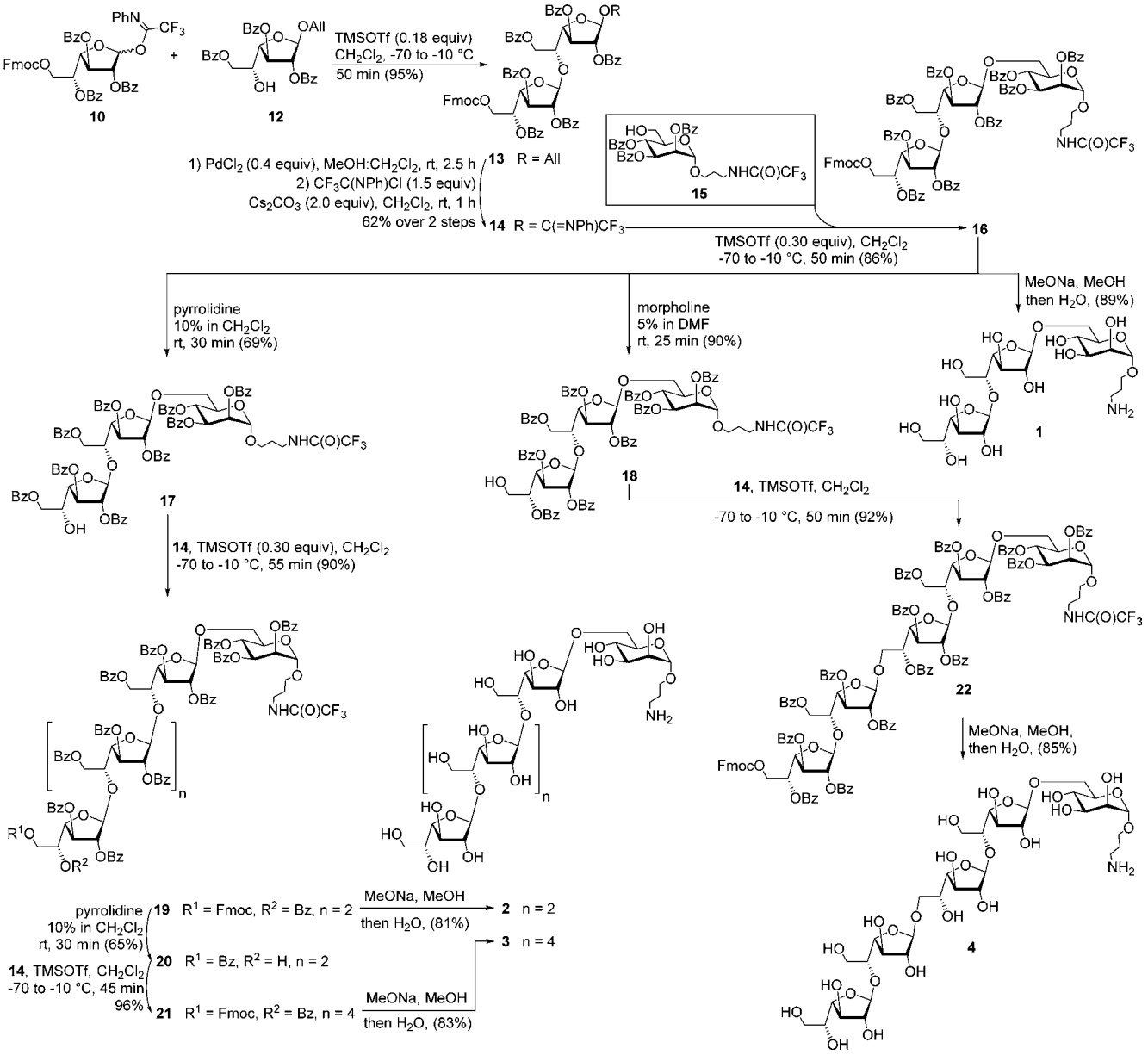
Scheme 2. Assembly of Target Structures 1−4



galactofuranoside chains related to *A. fumigatus* galactomannan. Thus, having encountered this side O(5) → O(6) benzoyl migration, we decided to perform this reaction in a preparative way in CH_2Cl_2 because it is known that the use of nonpolar solvents increases the migration rate.[16] Indeed, the O(5) → O(6) benzoyl migration rate under the treatment with pyrrolidine (10%) was significantly greater in CH_2Cl_2 than in DMF. Even initially the reaction migration product **12** was observed (TLC control), and after 20−30 min, only the product of benzoate migration, **12**, was detected in the reaction mixture. The 6-OH product **11** could be converted into the 5-OH derivative **12** in 73% yield by treatment with pyrrolidine in CH_2Cl_2, but the reverse reaction (**12** → **11**) in DMF does not take place.

For the synthesis of digalactoside building block **14**, TMSOTf-promoted coupling of donor **10** and acceptor **12** was performed to give the β-(1→5)-linked disaccharide precursor **13** (Scheme 2). Its structure, particularly the β configuration of the newly formed bond, was determined on the basis of the singlet shape of the H(1)′ signal ($J_{H(1)',H(2)'}$ < 1.0 Hz) and the characteristic low-field chemical shift of C(1)′ (105.5 ppm) in NMR spectra.[17] Removal of the allyl aglycon in **13** and the further introduction of the imidate group gave disaccharide donor **14**. Its coupling with known[8e] mannose acceptor **15** proceeded with stereoselective β-(1→6) bond formation to give the trisaccharide product **16**. The characteristic for β-galactofuranosides chemical shift of C(1)′ (106.4 ppm) and singlet shape of the H(1)′ signal as well as the low-field chemical shift of C(6) (66.1 ppm for **16** vs 61.4 ppm for **15**) confirmed the structure of the formed product. Deblocking of **16** by treatment with MeONa in MeOH afforded the desired trisaccharide **1**.

The conditions developed for the controlled Fmoc deprotection with or without Bz migration toward either 5-OH or 6-OH derivatives were applied for the transformation of trisaccharide **16**. Thus, its treatment with pyrrolidine in CH_2Cl_2 resulted in the formation of 5-hydroxy acceptor **17** in 69% yield. The structure of product **17** was confirmed by the low-

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field chemical shift of H(6)″ (4.48 ppm) and the high-field chemical shift of H(5)″ (4.35 ppm). On the other hand, removal of the Fmoc protection under "no migration" conditions by treatment with morpholine in DMF gave 6-OH acceptor **18** in 90% isolated yield. The structure of **18** was confirmed by the high-field shift of H(6)″ (3.95 ppm) and the low-field shift of H(5)″ (5.52 ppm).

Glycosylation of acceptors **17** and **18** by donor **14** gave protected oligosaccharides **19** and **22** containing β-(1→5)- and β-(1→6)-glycosyl bonds, respectively. Their deblocking afforded target pentasaccharides **2** and **4**. Removal of the Fmoc group in pentasaccharide **19** under "Bz migration" conditions (**19** → **20**) and subsequent glycosylation with donor **14** (**20** → **21**) afforded heptasaccharide **21**. Its deblocking gave the target heptasaccharide **3**.

In summary, a new approach for the synthesis of oligogalactofuranosyl chains built from β-(1→5)- and β-(1→6)-linked units was developed using PIF rearrangement in combination with controlled Bz migration under Fmoc removal conditions. The efficiency of the developed strategy was illustrated by the synthesis of tri-, penta-, and heptasaccharides related to the galactomannan of the *A. fumigatus* cell wall.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02735.

Experimental procedures, complete characterization data, and copies of ^{1}H and ^{13}C NMR and mass spectra of all new compounds (PDF)

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Notes

The authors declare no competing financial interest.

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