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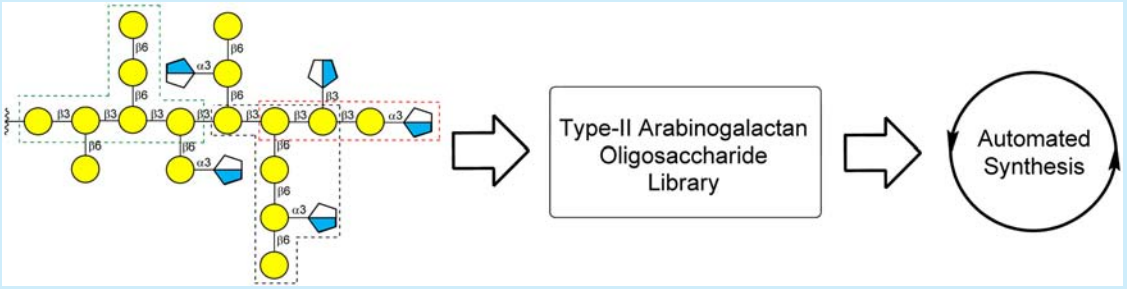
Automated Glycan Assembly of Oligosaccharides Related to Arabinogalactan Proteins

Max P. Bartetzko, Frank Schuhmacher, Heung Sik Hahm, Peter H. Seeberger, and Fabian Pfrengle*

Department of Biomolecular Systems, Max-Planck-Institute of Colloids and Interfaces, Am Mühlenberg 1, 14476 Potsdam, Germany
Institute of Chemistry and Biochemistry, Freie Universität Berlin, Arnimallee 22, 14195 Berlin, Germany

Supporting Information



ABSTRACT: Arabinogalactan proteins are heavily glycosylated proteoglycans in plants. Their glycan portion consists of type-II arabinogalactan polysaccharides whose heterogeneity hampers the assignment of the arabinogalactan protein function. Synthetic chemistry is key to the procurement of molecular probes for plant biologists. Described is the automated glycan assembly of 14 oligosaccharides from four monosaccharide building blocks. These linear and branched glycans represent key structural features of natural type-II arabinogalactans and will serve as tools for arabinogalactan biology.

A large number of the proteins embedded in the plant cell wall are glycoproteins and proteoglycans.[1] Arabinogalactan proteins (AGPs) are one major proteoglycan family[2] that consists of arabinogalactan (AG) polysaccharides attached to a hydroxyproline-rich peptide backbone (Figure 1).[3] The AG-moieties are classified as type II, characterized by a β-(1,3)-D-galactan backbone heavily branched with β-(1,6)-D-galactan side chains. The galactan core may be further substituted with α-(1,3)-linked L-arabinofuranoses, short arabinan oligosaccharides, or less frequently, rhamnose, fucose, and glucuronic acid residues.[4]

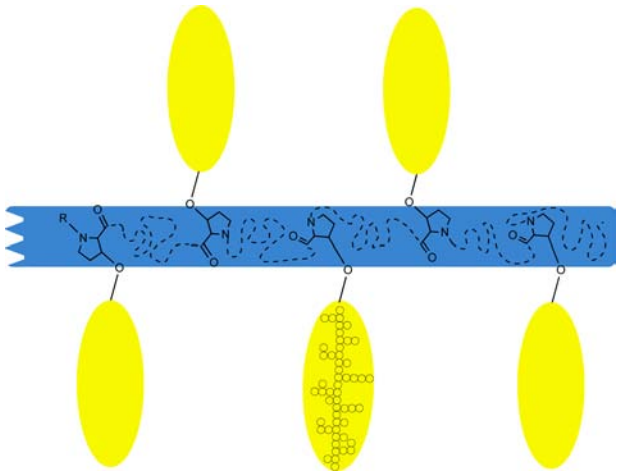


Figure 1. "Wattle-blossom" model of an AGP.[3] Highly branched type-II arabinogalactan (yellow ovals with black circles) is attached to a hydroxyproline rich protein backbone (blue).

Carbohydrate decoration is the basis for AGP heterogeneity in different tissues and plants,[5] impeding the assignment of AGP function in essential biological processes such as cell differentiation[6] and organ development.[7] Monoclonal antibodies (mAbs) are powerful tools to locate AG polysaccharides in immunolabeling studies of plant cell walls.[8] However, since the precise molecular structures bound by these antibodies are mostly unknown,[9] it remains uncertain how the molecular structure of the AG influences the localization and function of particular AGPs.[10]

A better understanding of AGP glycan structure and function requires well-defined, homogeneous oligosaccharides that are accessible exclusively by chemical synthesis. While several chemical syntheses of type-II arabinogalactan oligosaccharides have been reported,[11] a general approach providing convenient access to AGP-derived oligosaccharides is missing. Key to such an approach is the ability to produce many complex oligosaccharides from a limited set of building blocks. Automated glycan assembly[12] offers the possibility to construct carbohydrate libraries in a timely manner provided that fully orthogonal protecting groups and capping steps are used.[13] Here, we report the automated glycan assembly of 14 type-II

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arabinogalactan glycans of different size and complexity based on four monosaccharide building blocks (Figure 2).

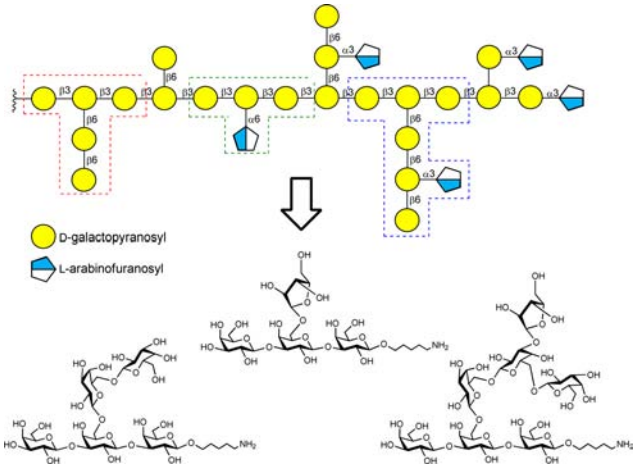
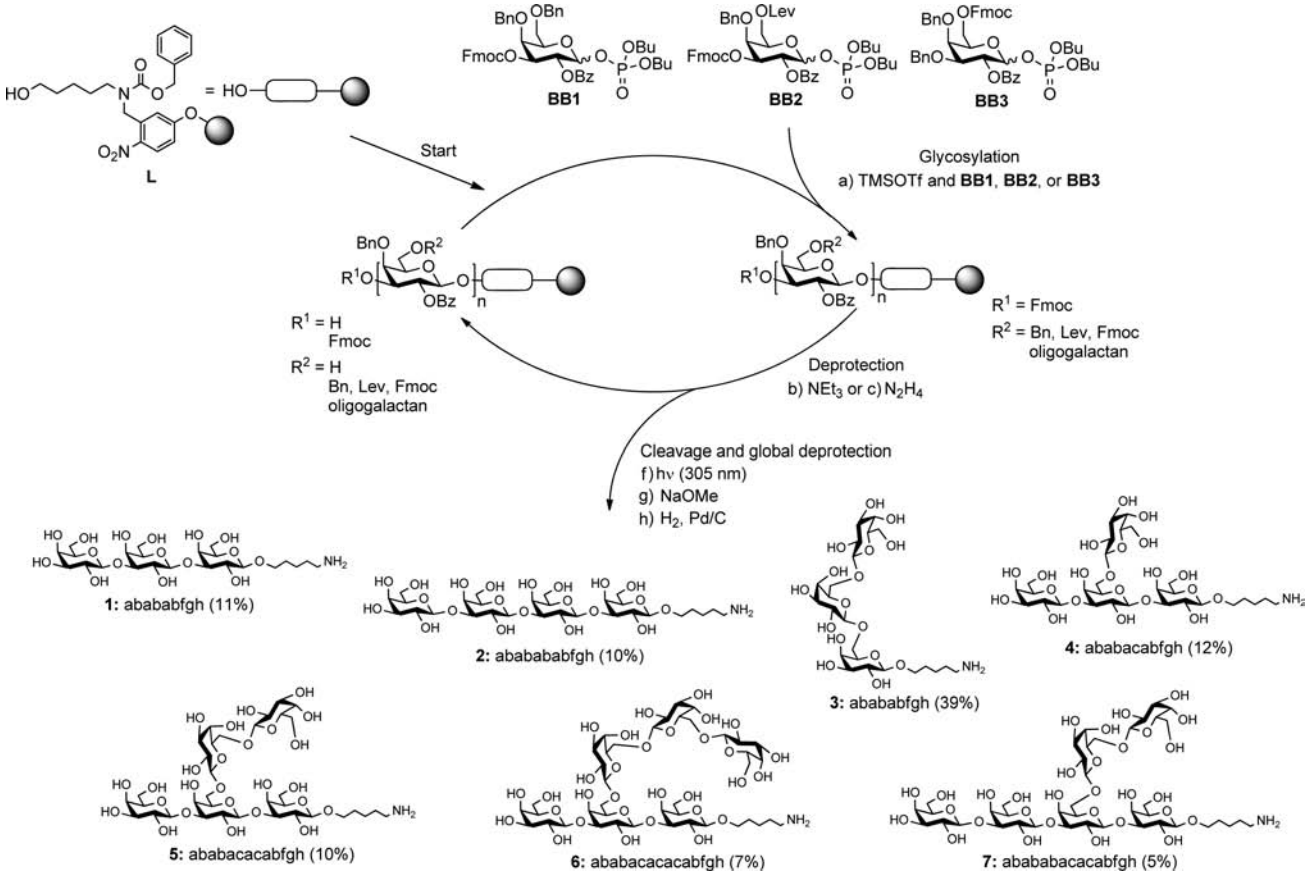


Figure 2. Oligosaccharide fragments of type-II arabinogalactan as potential targets for automated glycan assembly.

We initially focused on the (3,6)-galactan backbone without arabinose substitution and designed a set of three galactose building blocks (**BB1**−**BB3**) that allowed for the assembly of a variety of oligogalactans on solid support (Scheme 1). Hydroxyl groups that did not need to be manipulated during the solid-phase synthesis were permanently protected. In the C-2 position, benzoyl esters (Bz) provided neighboring group participation in the glycosylation reactions. All remaining positions were protected with benzyl ethers (Bn, Scheme 1). A temporary fluorenylmethoxycarbonyl (Fmoc) protecting group was installed in either the C-3- (**BB1**) or C-6-position (**BB3**) depending on which position needed to be elongated. To enable branching, a third building block (**BB2**) was furnished with both Fmoc and levulinoyl (Lev) protecting groups. Lev-esters are fully orthogonal to Fmoc groups and have been successfully used in automated glycan assembly.[12] By using dibutyl phosphate as the anomeric leaving group in all the galactose building blocks, we hoped to overcome the poor stereoselectivities and low yields frequently observed in the formation of Gal-β1,3-Gal linkages with other anomeric leaving groups, even in the presence of participating 2-*O*-protecting groups.[11,14]

With the required building blocks in hand, we first tested whether the challenging Gal-β1,3-Gal linkage could be constructed on the solid phase. Three glycosylations on linker-functionalized resin **L** with **BB1** provided the protected (β1,3)-trigalactoside with complete stereoselectivity and full conversion after cleavage of the photolabile linker in a

Scheme 1. Automated Glycan Assembly of Type-II Galactan Fragments 1−7[a]



[a]The letter code below the structures represents the synthesizer modules and deprotection steps applied in the respective synthesis. Reagents and conditions: (a) 2 × 3.75 equiv of **BB1**, **BB2**, or **BB3**, TMSOTf, DCM, −35 °C (5 min) → −20 °C (30 min); (b) three cycles of 20% NEt_3 in DMF, 25 °C (5 min); (c) three cycles of 0.15 M hydrazine in Py/AcOH/H_2O (4:1:0.25), 25 °C (30 min); (f) *hν* (305 nm); (g) NaOMe,THF, 16 h; (h) H_2, Pd/C, EtOAc/MeOH/H_2O/AcOH, 16 h. Yields are based on resin loading.

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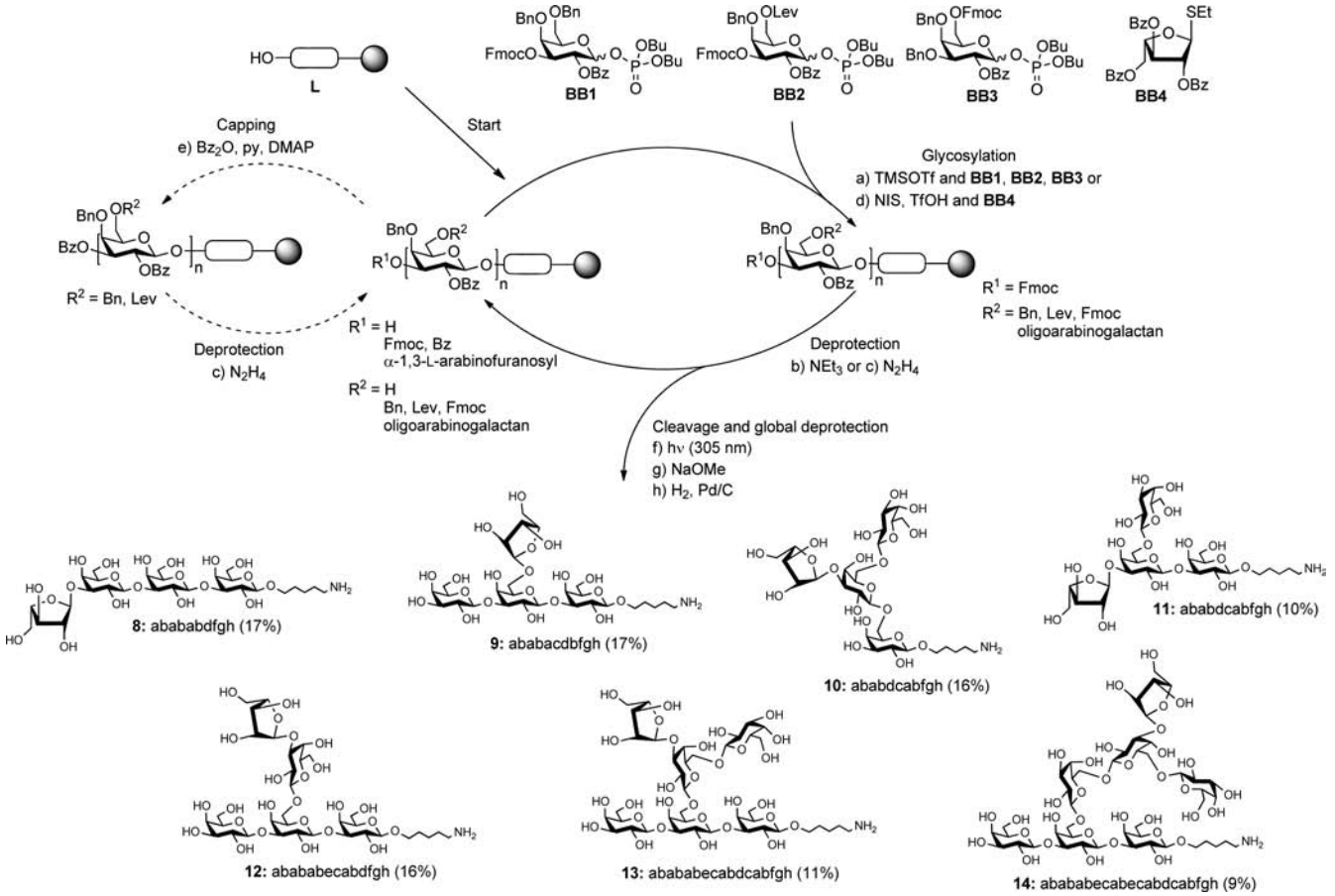
Scheme 2. Automated Glycan Assembly of Type-II Arabinogalactan Fragments 8−14[a]



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commercial flow photoreactor.[15] As expected, synthesis of a *β*-(1,6)-linked trigalactoside proceeded equally well when using **BB3**. Hence, the stage was set to assemble linear and branched galactan oligosaccharides **1**−**7** by iterative glycosylation and deprotection reactions. Glycosylations were performed twice using 3.75 equiv of galactose building blocks **BB1**−**3** and equimolar amounts of TMSOTf. Notably, we did not observe reduced conversion resulting from the use of slightly less excess building block than the two times 5 equiv that are standard for automated glycan assembly.[12] Every glycosylation was followed by either Fmoc or Lev removal to free the position on galactose for subsequent elongation. All branched structures were synthesized by assembling the linear *β*-(1,3)-linked backbone first and subsequently attaching the *β*-(1,6)-linked side chain. After completion of the automated synthesis, the protected oligosaccharides were cleaved from the solid support and treated with sodium methoxide in methanol. The resulting semiprotected products were subjected to hydrogenolysis, and the fully deprotected oligogalactans **1**−**7** were obtained in 5−39% overall yield, based on the calculated loading of linker-functionalized resin **L** (Scheme 2). The products were purified via preparative reversed-phase HPLC at both the fully and semiprotected stage. The purity of all products was ensured by characterization with analytical HPLC, NMR spectroscopy, and high-resolution mass spectrometry.

The synthesis of galactan fragments containing exclusively galactose proceeded smoothly, and the attachment of L-arabinofuranose substituents to this core structure was expected to be straightforward. Addition of L-arabinose building block **BB4**[13] to the previously used set of galactose building blocks **BB1**−**3** enabled us to produce seven additional type-II arabinogalactan substructures (Scheme 2). In the synthesized AG oligosaccharides, the arabinose unit is either attached to the *β*-(1,3)-linked galactan backbone (**8**, **9**, **11**) or to the *β*-(1,6)-linked galactan side chain (**10**, **12**−**14**). The synthesis of the more elaborate oligosaccharides **12**−**14** required capping steps during the automated synthesis to ensure the selective attachment of the arabinose to the desired galactose unit. For that purpose, the terminal Fmoc group was removed after assembly of the (1,3)-galactan backbone, and the resulting free hydroxyl group was masked as an ester. We found that the standard capping protocol previously applied in automated glycan assembly,[12] employing acetic anhydride in pyridine, was not suitable for our purposes; the resulting acetyl groups do not withstand the conditions required for the removal of Lev groups. Thus, we decided to modify the established protocol in order to obtain benzoyl esters as capping moieties due to their

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stability toward the hydrazine used for Lev deprotection.[16] Quantitative capping was achieved by incubating the resin-bound oligogalactan for 30 min at 40 °C with a solution of benzoic anhydride and DMAP in DCE. After capping the backbone, the side chain was introduced by iterative Lev deprotection and glycosylation reactions. The arabinose building block **BB4** was selectively attached to the side chain after Fmoc deprotection.

The glycosylation conditions established for the synthesis of oligogalactans **1**−**7** were also applied for oligosaccharides **8**−**14**, except that thioglycoside **BB4** was activated with *N*-iodosuccinimide and catalytic amounts of TfOH in DCM. After cleavage of the products from the solid support, global deprotection, and purification, AG oligosaccharides **8**−**14** were obtained in yields between 9 and 17% based on the calculated resin loading (Scheme 2).

In conclusion, we have established a general approach to synthesize glycans found on AGPs. Automated glycan assembly provided 14 type-II AG glycans that are valuable probes for plant research. All products are equipped with an aminopentyl linker at the reducing end and can be directly printed as microarrays to determine the binding specificities of antibodies that recognize plant cell wall glycans.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b02185.

Experimental procedures for solution- and automated solid-phase reactions and characterization data for all compounds (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*E-mail: Fabian.Pfrengle@mpikg.mpg.de

Notes

The authors declare no competing financial interest.

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