Carbohydrate Synthesis

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A Highly Convergent Chemical Synthesis of Conformational Epitopes of Rhamnogalacturonan II**

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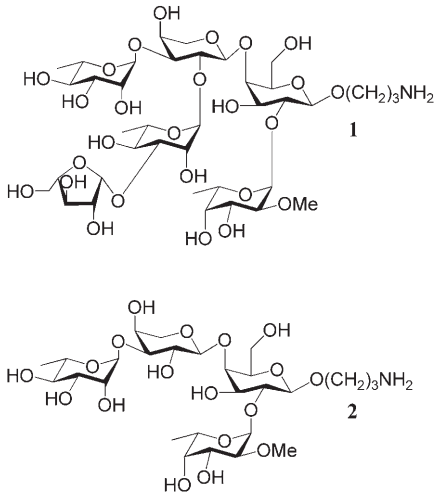
Recent estimates indicate that more than 2000 genes encode proteins involved in the biosynthesis and remodeling of the polysaccharide-rich primary cell wall of plants.[1] The functions of only a handful of these gene products are known.[2,3] Furthermore, there is data to support the hypothesis that carbohydrate epitopes change during plant development; however, detailed knowledge about these processes is lacking. It is critical that these deficiencies in knowledge be addressed urgently because advances in plant sciences and genetics will provide researchers with tools to develop the next generation of agroenergy and agromaterial crops, which will be higher-yielding and tailored for modern biorefinery operations.[4]

Monoclonal antibodies are attractive tools for monitoring changes in the composition and organization of the primary plant wall at the cellular and subcellular level.[5] To date, only a small number of such monoclonal antibodies have been generated for which the binding specificities have been characterized. Synthetic chemistry can provide well-defined plant-derived oligosaccharides,[6–8] which can be employed for the production of monoclonal antibodies. In particular, the highly complex pectins are prime targets for this purpose. For example, rhamnogalacturonan II (RGII) is a structurally highly complex pectic oligosaccharide of the primary cell wall of higher plants[9] and is composed of four structurally different oligosaccharides referred to as side chains A–D, which are attached to a linear α(1→4)-linked D-GalpA polysaccharide backbone. These polysaccharides are cross-linked covalently through borate diesters (1:2) between two apiofuranosyl residues of the A side chain. The resulting three-dimensional pectic network contributes to the mechanical properties of the primary cell wall and is required for normal plant growth and development. Indeed, changes in cell-wall properties that decrease the borate cross-linking of pectin lead to symptoms such as dwarfism.[10]

As part of a program to generate well-defined monoclonal antibodies against fragments of primary plant cell walls, we report herein the chemical synthesis of hexasaccharide **1** (Scheme 1), which is derived from the B side chain of RGII. The simpler tetrasaccharide **2** was also prepared, as several plant species, such as *Arabidopsis*, lack the β-L-Ara*f*-(1→3)-L-Rham*p* moiety of **1**.[9] In particular, we were interested in whether the presence of this disaccharide moiety would affect the ring conformation of the crowded α-L-Ara*p* residue.[11] The oligosaccharides were substituted with an artificial aminopropyl spacer to facilitate conjugation to carrier proteins, as required for immunization and ELISA.



Scheme 1. Targeted hexasaccharide **1** and tetrasaccharide **2**.

The chemical synthesis of **1** was challenging owing to the presence of a number of unusual monosaccharide moieties, the crowdedness around the central arabinopyranoside moiety, and the presence of 1,2-*cis*-linked 2-*O*-methyl-α-L-fucoside and β-L-arabinofuranoside residues, which are difficult to introduce in a stereoselective manner.[12–15] Initially, we envisaged that hexasaccharide **1** could be prepared in a highly convergent manner from disaccharides **13**, **14**, and **15** (see Scheme 3), which in turn could be prepared from monosaccharides **3**–**8** (Scheme 2).[16–20]

Thus, we planned to obtain the core 2-*O*-methyl-α-L-Fuc*p*-(1→2)-β-D-Gal*p* unit by glycosylation of the fucosyl donor **3** with the spacer-containing galactoside **4a** or **4b**, followed by regioselective opening of the benzylidene ring to give the glycosyl acceptors **12** and **13**. Compounds **12** and **13** would then be coupled with disaccharide **14** (see Scheme 3), which we intended to prepare by the chemoselective glycosylation of trichloroacetimidate **5** with thioglycoside **6**. The attractiveness of the latter glycosylation approach is that the thioglycoside functionality of **6** is stable under the conditions used for the activation of trichloroacetimidate **5**. However, the resulting thioglycosyl disaccharide **14** can be employed as a glycosyl donor by activating the anomeric thiophenyl

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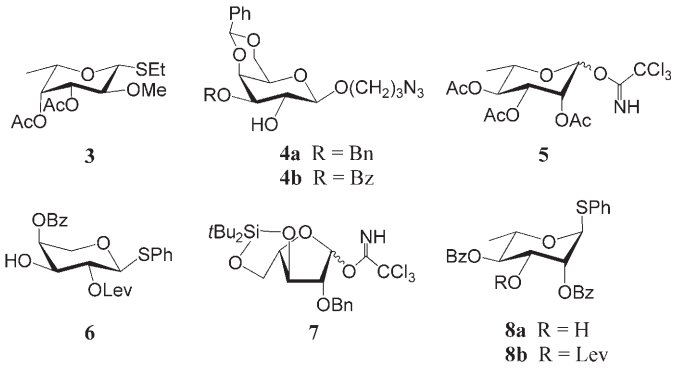
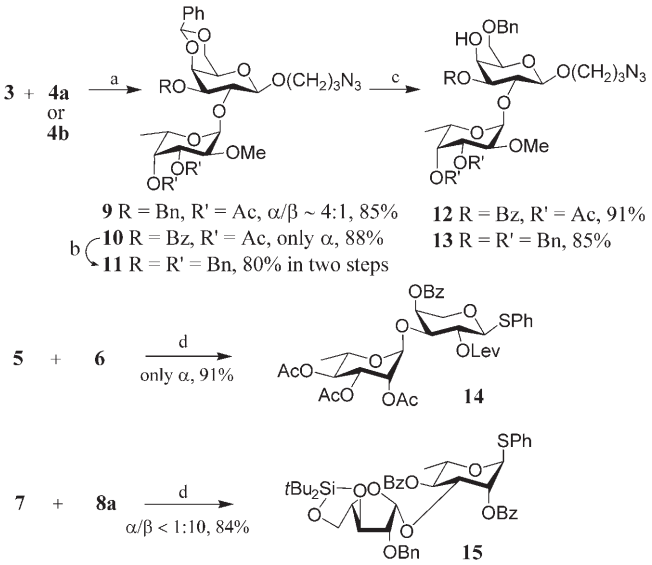
Supporting information for this article, including synthetic procedures and ^{1}H and ^{13}C NMR spectra for all compounds is available on the WWW under http://www.angewandte.org or from the author.



Scheme 2. Building blocks for the synthesis of oligosaccharides **1** and **2**. Bn = benzyl, Bz = benzoyl, Lev = levulinoyl.



Scheme 3. Reagents and conditions: a) NIS, TfOH, 4-Å MS, CH_2Cl_2/Et_2O (1:4), −20 °C; b) 1. NaOMe, MeOH, RT; 2. NaH, BnBr, TBAI, DMF, 0 °C; c) $NaCNBH_3$, THF, 2 M HCl, Et_2O, 4-Å MS, RT; d) TMSOTf, 4-Å MS, CH_2Cl_2, −70 °C. DMF = *N*,*N*-dimethylformamide, MS = molecular sieves, NIS = *N*-iodosuccinimide, TBAI = tetrabutylammonium iodide, Tf = trifluoromethanesulfonyl, TMS = trimethylsilyl.

moiety with a thiophilic promoter.[21] The Lev ester of the Ara*p* moiety of the tetrasaccharide could then be removed and the resulting alcohol coupled with disaccharide **15** to give a fully protected hexasaccharide. The preparation of disaccharide **15** also exploits the selective activation of a trichloroactimidate functionality in the presence of a thioglycoside.

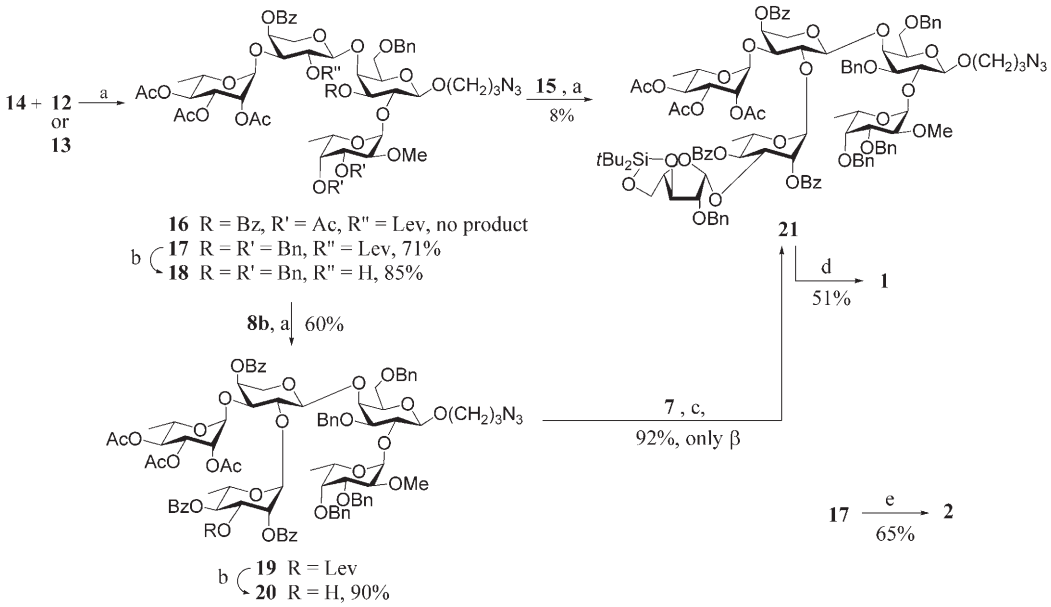
Finally, we planned to introduce the β-arabinofuranoside residue stereoselectively by employing the conformationally constrained glycosyl donor **7**. The 3,5-*O*-(di-*tert*-butylsilyl) protecting group of this compound locks the five-membered ring in an E_3 conformation, in which the approach of a reagent from the α face is blocked as a result of steric hindrance by H2.[22]

Thus, NIS/TfOH-promoted glycosylation[23] of **3** with **4a** in a mixture of dichloromethane and diethyl ether gave disaccharide **9** in an excellent yield of 85% but in a disappointing α/β ratio of approximately 4:1 (Scheme 3). Fortunately, the use of the less reactive glycosyl acceptor **4b**, which has an electron-withdrawing benzoyl ester group at C2 rather than an activating benzyl ether, led to disaccharide **10** in excellent yield almost exclusively as the α anomer. Furthermore, this compound could be converted readily into **11** by treatment with sodium methoxide in methanol followed by benzylation with benzyl bromide and sodium hydride in DMF. The benzylidene acetals of **10** and **11** were opened regioselectively by using $NaCNBH_3$ and HCl in diethyl ether to give the glycosyl acceptors **12** and **13**, respectively.

Disaccharide **14** was prepared in 91% yield by the TMSOTf-mediated glycosylation[24] of trichloroacetimidate **5** with thioglycoside **6**. The α anomer of **14** was formed exclusively as a result of neighboring-group participation by the acetyl ester functionality at C2 in **5**. Disaccharide **15** was obtained mainly as the β anomer in 84% yield by coupling **7** with **8a** in the presence of a catalytic amount of TMSOTf. The β-anomeric configuration of **15** was confirmed by the observed chemical shifts for H1 ($\delta_H = 5.15$ ppm, $J_{1,2} = 5.4$ Hz) and C1 ($\delta_C = 97.8$ ppm). Fortunately, no aglycon transfer was observed in either glycosylation. (Aglycon transfer occasionally occurs when trichloroacetimidates are coupled with thioglycosyl acceptors.)

With disaccharides **12**–**15** in hand, we focused our attention on the assembly of the hexasaccharide **1** (Scheme 4). Unfortunately, NIS/TfOH-promoted glycosylation of **12** with **14** in dichloromethane at 0 °C did not lead to the formation of tetrasaccharide **16**, and the reaction was not improved by varying the solvent, temperature, and promoter. The reactivity of the hydroxy group at C4 of **12** is probably too low as a result of its axial orientation and the presence of the neighboring deactivating electron-withdrawing benzoyl ester functionality. We envisaged that the reactivity of the glycosyl acceptor could be improved by replacing the electron-withdrawing benzoyl ester group with a benzyl ether group. Indeed, the coupling of **13** with **14** in the presence of NIS/TfOH as the thiophilic activator gave tetrasaccharide **17** in 71% yield. The Lev ester group of **17** was removed selectively by treatment with hydrazinium acetate in a mixture of dichloromethane and methanol to give the glycosyl acceptor **18**, the coupling of which with the thioglycosyl donor **15** led to the formation of hexasaccharide **21** in an unacceptable yield of 8%. The glycosylation is probably poor-yielding as a result of steric crowding around the C2 hydroxy group of the arabinopyranosyl moiety.

We expected the stepwise introduction of the monosaccharide units of the β-L-Ara*f*(1→3)-α-L-Rha*p* moiety to be more efficient. Thus, NIS/TfOH-promoted glycosylation of **18** with **8b** gave pentasaccharide **19** in 60% yield. Next, the Lev ester group in **19** was removed under standard conditions, and the resulting glycosyl acceptor **20** was coupled with the conformationally constrained arabinofuranoyl donor **7** to give hexasaccharide **21** in 92% yield. Careful examination of the NMR spectroscopic data for this compound revealed that only the β anomer had been formed. An NIS/TfOH-promoted glycosylation of a 3,5-*O*-(di-*tert*-butylsilyl)-protected 1-thioarabinofuranoside with **20** gave **21** in good yield but



Scheme 4. Reagents and conditions: a) NIS, TfOH, 4-Å MS, CH_2Cl_2, −20 °C; b) NH_2NH_2·AcOH, CH_2Cl_2/MeOH, RT; c) TMSOTf, 4-Å MS, CH_2Cl_2, −70 °C; d) 1. TBAF, THF, RT; 2. NaOMe/MeOH, RT; 3. Pd/C, *t*BuOH/AcOH/H_2O, RT; e) 1. NaOMe/MeOH, RT; 2. Pd/C, *t*BuOH/AcOH/H_2O, RT. TBAF = tetrabutylammonium fluoride.

with slightly reduced anomeric selectivity.[22] Preactivation of the 1-thioarabinofuranoside with Ph_2SO/Tf_2O followed by the addition of **20** led to the formation of **21** with decreased anomeric selectivity.[25]

Finally, **21** was deprotected to give **1** by a three-step procedure that involved the removal of the di-*tert*-butylsilyl protecting group by treatment with TBAF in THF, saponification of the acetyl and benzoyl ester groups with sodium methoxide in methanol, and subsequent catalytic hydrogenolysis over Pd/C to remove the benzyl ether groups and convert the azido moiety into an amine. Tetrasaccharide **2** was obtained readily from **17** by a standard three-step deprotection sequence.

Careful examination of the chemical shifts and coupling constants in the ^1H and ^{13}C NMR spectra of compounds **1** and **2** revealed conformational epitopes of the arabinopyranosyl moiety (Table 1 and Figure 1). Thus, the large coupling constants $J_{H1,H2}$ and $J_{H2,H3}$ between vicinal hydrogen atoms and the relatively small values for $J_{H4,H5_a}$ and $J_{H4,H5_b}$ of the arabinopyranosyl moiety of tetrasaccharide **2** are in agreement with a regular $^4C_1$ conformation. However, the values $J_{H1,H2}$ and $J_{H2,H3}$ for compound **1** were found to be much smaller and $J_{H4,H5_b}$ larger than the equivalent values for **2**. These data indicate that the arabinopyranoside ring in **1** adopts a distorted $^1C_4$ conformation. Unfavorable steric interactions of the trisubstituted arabinopyranosyl moiety are probably alleviated somewhat in this conformation.

The difference in the conformation of a disubstituted (as in compound **2**) and a trisubstituted (as in compound **1**) arabinopyranosyl moiety of the B side chain of RGII may be biologically significant, as available data suggest that there is considerable structural variability among the RGII B side chains of different plants as a result of the presence or absence of substituents linked to C2 and/or C3 of the Ara*p* moiety.[9] It may well be possible that the conformation of the Ara*p* moiety affects the cross-linking of

Table 1: Comparison of the ^{13}C and ^1H NMR spectroscopic data for the Ara*p* rings of **1** and **2**.[a]

	1	**2**
C1 [ppm]	104.5	101.3
H1 [ppm]	4.90	4.49
$J_{H1,H2}$ [Hz]	3.0	8.0
$J_{H2,H3}$ [Hz]	4.5	10.0
$J_{H3,H4}$ [Hz]	5.0	3.0
$J_{H4,H5_a}$ [Hz]	5.0	4.0
$J_{H4,H5_b}$ [Hz]	11.0	5.0

[a] The spectra were recorded in D_2O at 25 °C.

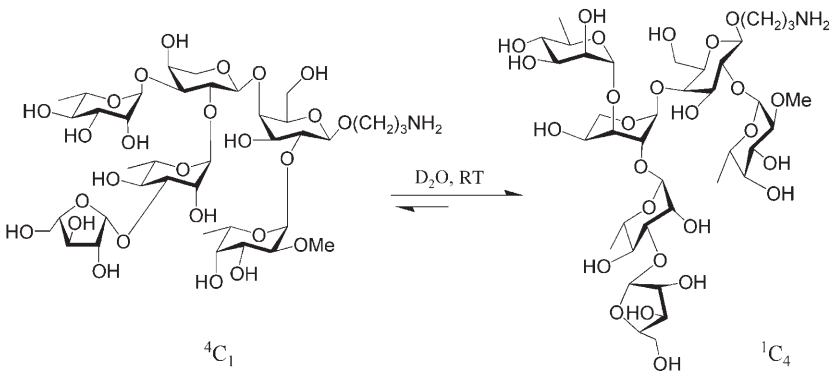


Figure 1. Proposed conformations of hexasaccharide **1**.

RGII by borate esters, which is an essential process for normal plant growth. Additional research will be required to determine the importance of the conformational flexibility of the RGII side chain to the cross-linking process. It is to be expected that antibodies that recognize conformational epitopes of the side chain of RGII will be valuable tools for such studies.

In conclusion, we have shown that an oligosaccharide with a highly dense architecture that contains a number of unusual 1,2-*cis* glycoside residues can be prepared in a highly convergent and stereoselective manner by careful tuning of the reactivity of the glycosyl donors and acceptors of the saccharide building blocks. For the stereoselective installment of the α-linked 2-*O*-methylfucoside unit, it was essential to employ a glycosyl acceptor of reduced reactivity. The β-linked arabino-L-furanoside unit could be introduced by employing a conformationally constrained arabinosyl donor locked in a conformation that blocks its α face. Furthermore, it was necessary to maximize the reactivity of the galactoside residue as a glycosyl acceptor by benzylation of the hydroxy group at C3. A stepwise approach to the installation of the β-L-Ara*f*-(1→3)-α-L-Rha unit at the crowded C2 hydroxy group of Ara*p* was more efficient than the use of a disaccharide building block. Finally, the use of quasiorthogonal trichloroacetimidates and thioglycosides minimized protecting-group manipulations during the assembly of the hexasaccharide.

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