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Rhamnogalacturonan II: Chemical Synthesis of a Substructure Including α -2,3-Linked Kdo

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Abstract: The synthesis of a fully deprotected Kdo-containing rhamnogalacturonan II pentasaccharide is described. The strategy relies on the preparation of a suitably protected homogalacturonan tetrasaccharide backbone through a post-glycosylation oxidation approach, and its stereoselective glycosylation with a Kdo fluoride donor.

Rhamnogalacturonan II (RG-II) is a complex polysaccharide representing around 10% of pectin in the cell wall of higher plants.^[1] Its structure is highly conserved among plant species and it is constituted by a homogalacturonan (HG, GalA- α -(1 \rightarrow 4)-GalA) backbone decorated with side branches, named A–F, consisting of 13 different types of monosaccharides interconnected by over 20 different glycosidic linkages (Figure 1).^[2,3] Interestingly, the monosaccharides composing the side-chains include a number of rare sugars such as 3-deoxy-D-manno-oct-2-ulosonic acid (Kdo), L-aceric acid (Ace), D-apiose (Api), 3-deoxy-D-lyxo-hept-2-ulosaric acid (Dha), L-galactose, 2-O-methyl-D-xylose, and 2-O-methyl-L-fucose. Unlike rhamnogalacturonan I (RG-I), the RG-II structure has been found to be evolutionarily conserved in all vascular plants, with minor variations limited to methylation and/or acetylation of the side chains.^[3] RG-II exists predominantly as a dimer that is covalently cross-linked by a 1:2 borate-diol diester between apiofuranosyl residues belonging to side chain A of two different RG-II monomers.^[4,5] This important structural characteristic contributes to the generation of networks of pectic polysaccharides by favouring cell adhesion and wall mechanical strength. The fundamental role of RG-II in the life of plants is further supported by the observation that genetic modifications affecting RG-II structure dramatically decrease the formation of the dimer, in turn affecting plant growth and development.^[6,7] Furthermore, mutations preventing the synthesis of sugar nucleotides UDP-Api and CMP-Kdo are

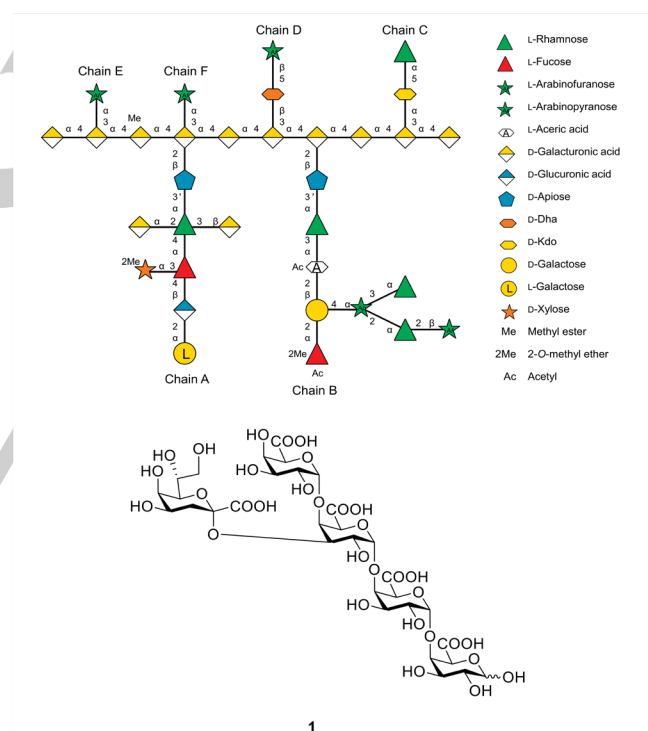
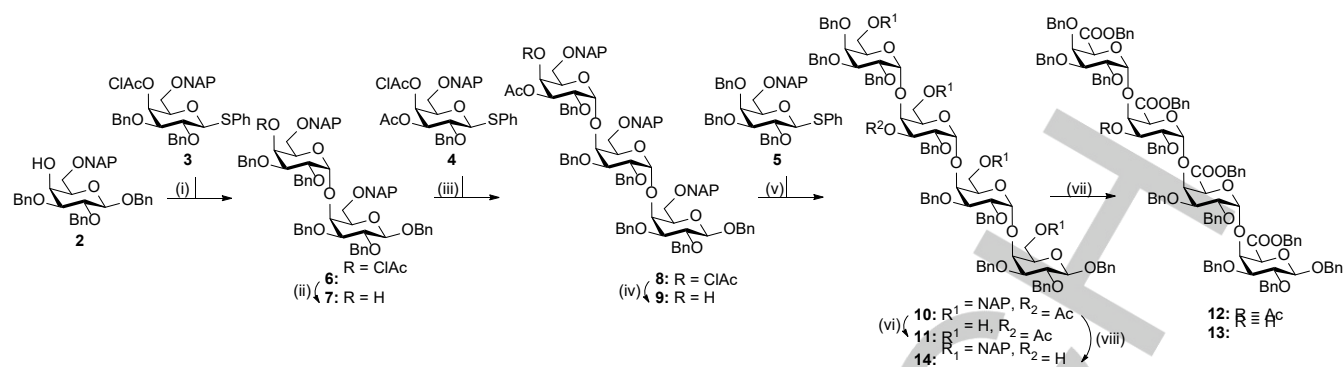


Figure 1 RG-II polysaccharide structure and target pentasaccharide 1

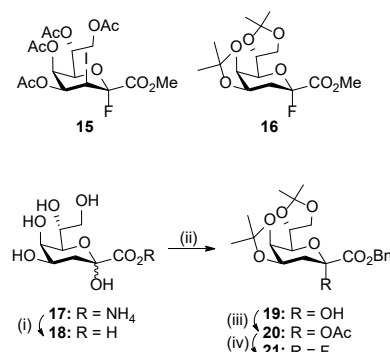
lethal and provide further evidence for the essential role of RG-II in plant growth.^[8–10] Though the complexity of the RG-II structure requires the involvement of multiple glycosyltransferases (GTs), activated donors, and additional methylating/acetylating enzymes for its biosynthesis, to date only rhamnogalacturonan xylosyl transferases RGXT1–4 (CAZy family GT77) have been characterized for the synthesis of the A side chain (transferring α -(1 \rightarrow 3)-D-xylose on the internal L-fucose).^[11–13] Additional 26 putative GTs have been listed by a bioinformatic approach.^[14]



Scheme 1: Reagents and conditions: (i) NIS, TESOTf, 4 Å MS, CH₂Cl₂, -40 °C, 1 h, 72%; (ii) MeONa, MeOH, THF, RT, 1.5 h, 96%; (iii) NIS, TESOTf, 4 Å MS, CH₂Cl₂, -40 °C, 2 h, 85%; (iv) thiourea, NaHCO₃, TBAI, THF, 55 °C, 33 h, 79%; (v) NIS, TESOTf, 4 Å MS, Et₂O, 0 °C, 1 h, 68% (α : β =5:1); (vi) DDQ, CH₂Cl₂/MeOH/H₂O, RT, 12 h, 52%; (vii) for **12**: a. Dess-Martin periodinane, CH₂Cl₂, RT, 12 h, b. NaClO₂, NaH₂PO₄, H₂O, THF, *t*-BuOH, RT, 4 h, c. PhCHN₂, AcOEt, RT, 3 h, 72% (over three steps), for **13**: a. Dess-Martin periodinane, CH₂Cl₂, RT, 12 h, b. NaClO₂, NaH₂PO₄, H₂O, THF, *t*-BuOH, RT, 4 h, c. MeONa, MeOH, THF, RT, on, d. PhCHN₂, AcOEt, RT, 1.5 h, 68% (over four steps); (viii) MeONa, MeOH, THF, RT, on, 81%.

The growing interest towards the plant biomass as an abundant reservoir for the production of next generation biofuels and value-added products requires in-deep understanding of the structure of plant cell wall components, specifically their biosynthesis and degradation. In light of this, synthetic chemistry can help by furnishing well-defined oligosaccharide fragments that can be used for probing the activity of newly isolated hydrolytic enzymes, GTs involved in the biosynthesis of plant cell wall components, or in the production of novel monoclonal antibodies (mAbs) for elucidation of differences in plant cell wall components in mutants, organ and tissue types, and different developmental stages.^[15,16] Notably, the plant/algal glycome differs in its composition from the mammalian and bacterial ones, with a higher incidence of uronic acids (D-GalpA, D-GlcpA), furanoses (D/L-Araf), and rare sugars such as D-Api and L-Gal.^[17,18] The essential role of RG-II in pectin structure has prompted several synthetic efforts towards the preparation of small oligosaccharide fragments of this complex structure. In particular, much attention has been focused to the preparation of portions of side chains A and B,^[19–24] especially containing the rare sugar D-Api. Conversely, no synthesis has been reported of side chain B fragments containing L-Ace residues, possibly due to its tedious synthesis from L-xylose or D-arabinose.^[25,26] Additionally, to date no RG-II fragments containing the smaller side chains C and D have been synthetically targeted, although several procedures have been described to access Kdo,^[27–29] a common monosaccharide found in bacterial polysaccharides, and Dha.^[30] In this context, we have been interested in the development of a synthetic strategy to access the HG backbone of RG-II and its further functionalization with a branching Kdo moiety, in order to obtain a unique RG-II pentasaccharide fragment including the unexplored C side chain (Figure 1, compound **1**). The synthesis of target pentasaccharide **1** was envisioned to proceed *via* the preparation of the linear tetrasaccharide HG backbone utilizing four orthogonally protected D-galactose building blocks and a post-glycosylation oxidation approach (Scheme 1). Following, a [4+1] glycosylation was planned to

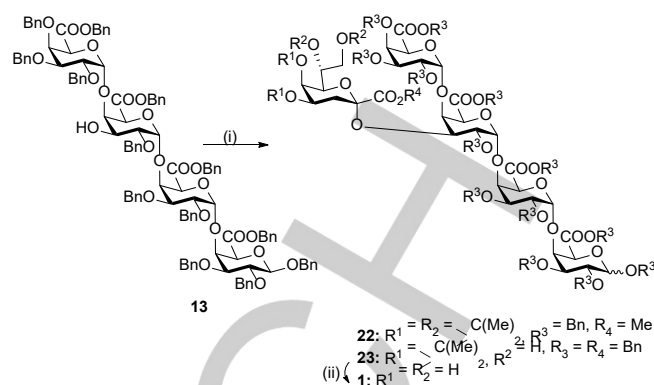
insert the branching Kdo pyranose moiety. After preparation of the planned building blocks **2–5** (Supporting Information), the assembly started with a NIS/TESOTf promoted glycosylation with glycosyl acceptor **2** and donor **3** yielding disaccharide **6** in 72% yield. Careful temperature control (-40 °C) led to the exclusive formation of the desired α -product. Subsequently, removal of the chloroacetyl ester by Zemplén conditions (\rightarrow **7**, 96%) and glycosylation of the newly formed disaccharide acceptor **7** with glycosyl donor **4**, under the established conditions of the [1+1] glycosylation, yielded trisaccharide **8** in 85% yield and as a single α -anomer. Chemoselective removal of the chloroacetyl ester was then achieved in 79% yield by treatment of **8** with thiourea together with NaHCO₃ and tetrabutylammonium iodide (TBAI) at 55 °C in THF. Finally, the same glycosylation protocol was applied for the reaction of trisaccharide acceptor **9** and donor **5** to discover that tetrasaccharide **10** was only synthesized in a poor 34% yield and as a 1:1 α : β mixture, possibly a consequence of the decreased reactivity of the two coupling partners. Thus, glycosylation yield and selectivity were improved by performing the reaction in Et₂O and by increasing the temperature to 0 °C, leading to the formation of tetrasaccharide **10** in 68% yield and as a separable 5:1 α : β mixture. Removal of the 2-naphthylmethyl



Scheme 2: Kdo donors **15**, **16**, **21**. Reagents and conditions: (i) Dowex 50WX8 H⁺, 94%; (ii) a. PhCHN₂, CH₃CN, H₂O, RT, 1 h, b. acetone, *p*-TsOH, RT, on, 48% (over two steps), (iii) Ac₂O, DMAP, pyridine, 2 h, 64%, (iv) 70% HF-pyridine, pyridine, -70 °C to RT, 2 h, 42%

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(NAP) groups protecting the primary C-6 alcohols also proved challenging, affording, at its best, the desired tetraol **11** in 52% yield after treatment with DDQ in $\text{CH}_2\text{Cl}_2:\text{MeOH}:\text{H}_2\text{O}$ at room temperature. Finally, the newly formed hydroxyl groups were oxidized to the corresponding carboxylic acids *via* a two-step protocol involving treatment with Dess-Martin periodinane followed by sodium chlorite oxidation, and subsequently protected as benzyl esters after reaction with phenyldiazomethane (\rightarrow **12**, 72% over three steps). The obtained HG backbone **12** was then reacted under standard Zemplén conditions to remove the acetyl ester protecting the 3''-OH. However, the reaction produced a number of byproducts that were identified by LC-MS as a mixture of tetrasaccharides without the 3''-OAc moiety and transesterified to the corresponding methyl esters. Further investigations into a viable deacetylation protocol were carried out (e.g. change in equivalents of MeONa, DBU/MeOH, KCN/MeOH, AcCl/MeOH) with noticeably poor results in the tested conditions. The disappointing results prompted the search of a novel approach towards the preparation of tetrasaccharide acceptor **13**. Gratifyingly, when Zemplén removal of the acetyl ester was performed after the two-step oxidation and before the benzylation of the formed carboxylic acids, desired product **13** could be obtained in 68% yield over four reaction steps. The key glycosylation reaction for the introduction of the desired Kdo side-chain involved the use of Kdo donors **15**, **16**, and **21** (Scheme 2). In particular, donor **21** represents the ideal glycosylation candidate as its COOBn functionality can be removed at a late stage of the synthesis during global deprotection and without any additional saponification steps. While glycosyl donors **15**^[31] and **16**^[32] were prepared according to published procedures, Kdo fluoride donor **21** was prepared in five steps from Kdo ammonium salt, previously obtained from D-arabinose and oxaloacetic acid following the Cornforth reaction.^[33,34] Namely, Kdo ammonium salt **17**^[34] was first converted into the corresponding carboxylic acid by stirring in water in the presence of Dowex H⁺ resin (\rightarrow **18**, 94%), then the acid was treated with an ethereal solution of phenyldiazomethane, followed by the introduction of the 4,5-O- and 7,8-O-isopropylidene rings (\rightarrow **19**, 48% over two steps). Compound **19** was then acetylated (\rightarrow **20**, 64%) and reacted with 70% HF-pyridine at low temperature (-70 °C to RT) to afford the corresponding fluoride donor **21** in 42% yield. To the best of our knowledge, this route developed to access Kdo donor **21** represents a shorter and higher-yielding protocol compared to other synthetic pathways that have been previously reported. With glycosyl donors **15**, **16**, and **21** in hand the glycosylation reaction was first screened with model tetrasaccharide acceptor **14**, obtained from the deacetylation of tetrasaccharide **10** (\rightarrow **14**, 81%). While arguably the reactivity of model acceptor **14** can be assumed to be different from the GlcA-containing glycosyl acceptor **13**, testing several glycosylation conditions with the less precious material **13** allowed for a more systematic search of optimal glycosylation conditions (Table S1 in Supporting



Scheme 3: Reagents and conditions: (i) for **22**: **16**, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 4 Å MS, 0 °C, 1 h, 26 %, for **23**: **21**, $\text{BF}_3 \cdot \text{Et}_2\text{O}$, 4 Å MS, -40 °C, 9 h, 31%; (ii) a. 80% aq. AcOH, 2 h, b. H_2 , 5% Pd/C, AcOEt/EtOH/ H_2O , o/n, 85% (over two steps).

Information; summary of [4+1] glycosylation attempts). Acceptor **14** was initially tested in glycosylations with Kdo fluoride donor **15**. The glycosylation was first carried out with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in CH_2Cl_2 . The role of the temperature was studied by performing experiments at -40 °C, 0 °C and room temperature but in all three cases, a complex mixture of degradation products was obtained. Donor elimination was observed when the solvent was changed to CH_3CN or different promoting systems, such as TESOTf, TMSOTf, TfOH were tested in CH_2Cl_2 and at low temperatures. These results prompted the testing of a new set of glycosylations employing di-O-isopropylidene Kdo fluoride donor **16**. Product formation was observed when the reaction was first performed with $\text{BF}_3 \cdot \text{Et}_2\text{O}$ in CH_2Cl_2 at -40 °C, although in a complex mixture with hydrolysed donor and the glycal ester byproduct. Repeating the reaction at a higher temperature showed a decrease in product formation, while increasing the amount of promoter was beneficial. Other promoting systems (TMSOTf or TfOH) were also tested, without observing any product formation. The screened conditions with model acceptor **14** and donors **15**–**16** indicated that fluoride donor **16** tended to be more reactive, although more labile in comparison to the acetylated 3-iodo-donor **15**. In fact, possibly due to the di-O-isopropylidene protecting moieties, fluoride **16** seemed more prone to decomposition and/or elimination side-reactions, especially with little variations in terms of equivalents of promoter or reaction temperature. In light of these preliminary results, glycosylation of HG acceptor **13** was tested with donor **16** in the presence of $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (2 equiv.) at 0 °C. The reaction led to the formation of the corresponding pentasaccharide product **22** in 26% yield and as a single α -isomer. This result prompted the design of a protocol in which 1 equiv. of promoter ($\text{BF}_3 \cdot \text{Et}_2\text{O}$) would be added every 3 hours at low temperature to minimize the side-reactions leading to acetal cleavage and to suppress donor elimination. Gratifyingly, coupling between Kdo donor **21** and acceptor **13** following the newly designed protocol afforded the desired pentasaccharide product **23**, although with loss of the 7,8-O-isopropylidene acetal, in 31% yield and with complete α -

stereoselectivity. While not high yielding, the coupling proved successful and enough material was produced to continue towards the preparation of the desired final compound **1**. To this end, hydrolysis of the 4,5-*O*-isopropylidene acetal with 80% aq. AcOH and global deprotection *via* hydrogenolysis produced the desired RG-II fragment **1** in 61% yield over 2 steps (Scheme 3).

In conclusion, the synthesis of the first RG-II side-chain C fragment was accomplished in 10 steps starting from four D-Gal building blocks and a suitably protected Kdo fluoride donor. The [4+1] synthetic strategy here presented allows for the easy assembly of a suitably protected HG backbone and its subsequent branching. In particular, the modularity of the approach makes it easily translatable to the synthesis of RG-II fragments carrying other short side-chains such as D, E, and F.

In addition, while developing the synthesis of the RG-II side-chain C fragment, a novel synthetic approach for the preparation of Kdo donor **21** was also developed with a shorter and higher-yielding protocol (5 steps, 12% global yield).

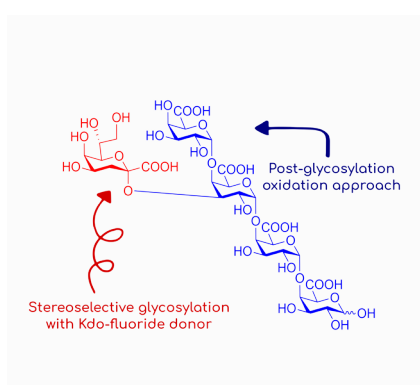
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Chemical synthesis of a Kdo-containing rhamnogalacturonan II pentasaccharide was achieved by a [4+1] post-glycosylation oxidation approach