ORGANIC LETTERS
2013
Vol. 15, No. 8
1826–1829

Synthesis of a Backbone Hexasaccharide Fragment of the Pectic Polysaccharide Rhamnogalacturonan I

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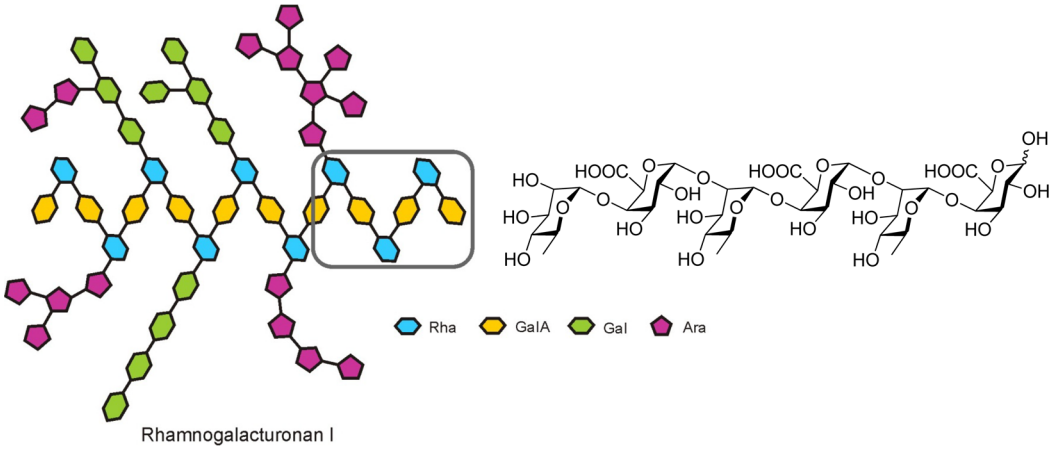
Received February 14, 2013

ABSTRACT



Synthesis of the fully unprotected hexasaccharide backbone of the pectic polysaccharide rhamnogalacturonan I is described. The strategy relies on iterative coupling of a common pentenyl disaccharide glycosyl donor followed by a late-stage oxidation of the C-6 positions of the galactose residues. The disaccharide donor is prepared by an efficient chemoselective armed-disarmed coupling of a thiophenyl rhamnoside donor with a pentenyl galactoside acceptor bearing the strongly electron-withdrawing pentafluorobenzoyl ester (PFBz) protective group.

Pectins are highly heterogeneous polysaccharides of plant origin. They are found in the primary cell wall and contribute to various cell functions, including support, defense, signaling, and cell adhesion.[1] Pectins also play important roles as food additives, serving as stabilizing and thickening agents in products such as jams, yogurts, and jellies.[2] Rhamnogalacturonan I (RG-I) is one of the structural classes of pectic polysaccharides, along with homogalacturonan, rhamnogalacturonan II, and xylogalacturonan.[3] The chemical structure of RG-I is complex having a backbone consisting of alternating α-linked L-rhamnose and D-galacturonic acid units with numerous branches of arabinans, galactans, or arabinogalactans positioned at C-4 of the rhamnose residues.

The structural complexity of pectin together with the wide range of its practical applications and desire to understand its structure and functions in details have inspired many researchers to pursue chemical syntheses of pectic oligosaccharides. Herein, we report the synthesis of a hexasaccharide fragment of the RG-I rhamnogalacturonan backbone (**1**, Figure 1).

Synthesis of the fully unprotected hexasaccharide fragment of RG-I has not been previously reported. However, smaller fully and partially unprotected RG-I oligosaccharides, as well as fully protected oligosaccharides up to

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10.1021/ol400430p
Published on Web 04/02/2013

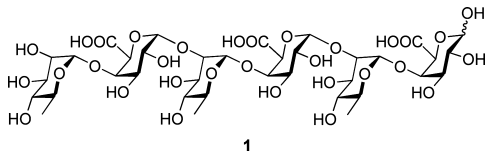

Figure 1. Structure of the hexasaccharide fragment of RG-I.

hexamers, have been prepared by different approaches. Some of the strategies used galacturonic acid as the starting material, while others favored the oxidation of galactose to galacturonic acid at a late stage, i.e., pre- and postglycosylation–oxidation strategies, respectively. Reimer and co-workers[4] reported the synthesis of a protected tetrasaccharide containing galactose instead of galacturonic acid as an intermediate for the preparation of RG-I fragments. The protective group pattern was designed to allow for further chain elongation and introduction of branching. It was envisioned that the global deprotection and oxidation of the primary hydroxyl groups of the galactose units would furnish the native oligosaccharides. In later work, the group synthesized the fully unprotected methyl glycoside of an RG-I tetrasaccharide, both in the methyl ester and the free carboxylic acid forms.[5] In this case, a similar protective group pattern was used, but galacturonic acid was employed from the early stages. This lowered the overall number of synthetic steps by avoiding the late stage oxidation. Unfortunately, the key glycosylation reaction proved to be problematic and only low yields of the protected tetrasaccharide product could be obtained. Vogel and co-workers[6] prepared a partially deprotected RG-I trisaccharide bearing a benzoyl group at C-4 of the rhamnose residue where galacturonic acid was used as a starting material. Later, the same group reported the synthesis of the fully unprotected propyl glycoside of an RG-I tetrasaccharide, as well as synthesis of its protected hexasaccharide fragment and protected tri- and tetrasaccharides suitable for the assembly of the branched RG-I fragments.[7] The synthesis was based on a modular design principle and used galacturonic acid as the starting material. Takeda and co-workers prepared[8] the unprotected propyl glycoside of an RG-I tetrasaccharide using a late-stage oxidation approach. All the mentioned work employed the generation of glycosyl donors before each glycosylation step. In a recent report by Davis and co-workers,[9] a latent-active approach was utilized and combined with the late-stage oxidation strategy to synthesize the fully unprotected RG-I tetrasaccharide and its dimethyl ester. Interestingly, the initial attempt to couple a galactorhamnosyl disaccharide donor to the galactose of a disaccharide acceptor failed due to a lack of reactivity, forcing the authors to change the strategy and assemble the RG-I tetrasaccharide through galactosylation instead of rhamnosylation. The potential of this methodology for iterative elongation of the oligosaccharide chain was demonstrated by preparation of a fully protected analog of the native hexasaccharide, containing both galactose and galacturonic acid residues.

Retrosynthetic analysis of the target RG-I hexasaccharide **1** is depicted in Figure 2.

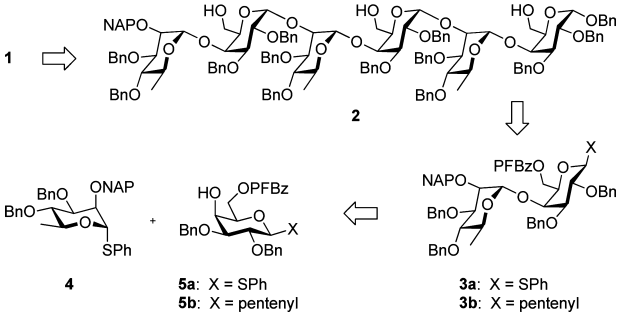

Figure 2. Retrosynthesis of the RG-I hexasaccharide **1**.

Choosing between the two possible approaches[10] for synthesis of oligosaccharides containing uronic acids (that is, oxidation prior to or after glycosylation), we adopted the postglycosylation strategy, which we had previously successfully employed[11] in the synthesis of homogalacturonans. Although it requires additional protective group manipulations, the nonoxidized carbohydrates are generally more reactive glycosyl donors than their oxidized counterparts,[12] where the reactivity is decreased by the presence of the electron-withdrawing carboxyl groups. Moreover, introduction of the carboxylic acid functionality at a late stage of the synthesis reduces the risk of possible side reactions, such as epimerization to L-altruronic acid and β-elimination leading to the formation of 4-deoxy-L-*threo*-hex-4-enopyranuronic acid. According to this reasoning, we envisioned that the target hexasaccharide **1** could be obtained from the partially deprotected hexasaccharide **2** by oxidation of the primary hydroxyl groups to the carboxylic acids followed by a global deprotection.

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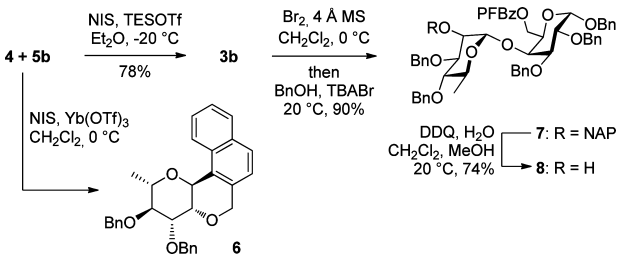
Hexasaccharide **2** in turn was planned to be assembled by two iterative glycosylations using disaccharide **3**. Employing the common disaccharide **3** would minimize the number of monosaccharide building blocks required for the synthesis. Donor **3** was designed to possess a nonparticipating benzyl (Bn) ether at C-2 promoting the formation of the α-glycosidic linkage and was intended to be produced through a chemoselective coupling between rhamnose donor **4** and galactose acceptor **5**. Donor **4** was designed to carry a nonparticipating 2-naphthylmethyl (NAP) ether at C-2 ensuring the formation of the α-glycosidic linkage and later on allowing for selective deprotection and elongation of the oligosaccharide chain at this position. The C-6 moiety in acceptor **5** was capped with a pentafluorobenzoyl ester (PFBz) that could be selectively removed to release this position for oxidation. Apart from functioning as a temporary protective group, the PFBz ester was also envisioned to tune the reactivity of **5**. It is known that electron-withdrawing protective groups decrease the reactivity of glycosyl donors,[13] and the donors protected with electron-donating (ether) groups can be selectively activated in a glycosylation reaction over the donors protected with electron-withdrawing (ester) groups. This phenomenon, first formulated by Fraser-Reid,[14] is known as the "armed-disarmed effect". In the present synthesis, the "armed" rhamnose donor **4** fully protected with ether groups was planned to be selectively activated over the "disarmed" galactose acceptor **5** bearing an electron-withdrawing PFBz group. In addition to the electronic effects of the protective groups, rhamnose was expected to have a higher reactivity, because it is a deoxy sugar and lacks the electron-withdrawing group at C-6 compared to galactose.[13]

Protected monosaccharide building blocks **4**,[15] **5a**,[16] and **5b**[11b] were synthesized from L-rhamnose and D-galactose (see the Supporting Information). Initially, the use of galactose thiophenyl acceptor **5a** in a chemoselective coupling with rhamnose thiophenyl donor **4** was explored. When *N*-iodosuccinimide (NIS) in the presence of a catalytic amount of triethylsilyl trifluoromethanesulfonate (TESOTf) was applied as the promoter it was possible to obtain the target disaccharide **3a** but, unfortunately, only as an inseparable mixture in almost equal amounts with a trisaccharide by-product derived from a reaction of **3a** with **4**. Attempts to conduct this glycosylation under different reaction conditions (applying I_2 as the promoter, converting **4** into the corresponding glycosyl bromide and subsequent activation with silver triflate, or applying in situ anomerisation conditions) did not improve the reaction outcome. In some cases, most of donor **4** was converted into a *C*-glycoside through an intramolecular reaction (vide infra). Given the lack of success in synthesizing thiophenyl disaccharide **3a**, we turned to pentenyl glycosides as an alternative (Scheme 1). The NIS/TESOTf-mediated coupling of galactose pentenyl acceptor **5b** with the identical rhamnose donor **4** produced the desired disaccharide **3b** as the sole product, and we isolated the α-anomer in 78% yield after flash chromatography. As an alternative to the armed−disarmed approach that we describe here, we also explored selective activation of the thiophenyl glycoside with other promotors: MeOTf[17] resulted in a low yielding glycosylation with many byproducts while activation with NIS/$Yb(OTf)_3$[18] mainly led to the formation of the *C*-glycoside **6** (Scheme 1). Attempts to preactivate the glycosyl donor with diphenyl sulfoxide and triflic anhydride[19] also resulted in the formation of **6** as the major product. This could be circumvented by replacing the *O*-2 NAP protective group with chloroacetyl and with that thioglycoside donor the preactivation conditions gave a coupling yield of 45%. Nonetheless, the armed−disarmed coupling of **4** and **5b** resulted in the highest yield; the reactivity difference between the thiophenyl glycoside and the corresponding pentenyl glycosides was somewhat surprising, and we are currently investigating whether this is a general trend.

Scheme 1. Synthesis of the Disaccharide Building Blocks



To assemble the hexasaccharide from the disaccharide **3b**, it was first converted to the glycosyl bromide and then, by glycosylation of benzyl alcohol, to benzyl glycoside **7**. This two-step sequence ensured the formation of the α-glycoside, where direct activation with NIS/TESOTf resulted in an α/β-mixture. This was followed by removal of the NAP-group at C-2′ by oxidation with DDQ in CH_2Cl_2 in the presence of water furnishing disaccharide acceptor **8**. Pentenyl disaccharide **3b** was used as the key disaccharide donor in the further iterative assembly of hexasaccharide **2** (Scheme 2). Glycosylation of **8** with **3b** using the aforementioned conditions led to the formation of tetrasaccharide **9** as a single α-isomer in 71% yield. Tetrasaccharide **9** was subjected to the same procedure for removal of the NAP-group with DDQ to furnish tetrasaccharide acceptor **10**, which was coupled again with donor **3b** and the crude product was directly subjected to the Zemplén conditions, and after the selective removal of

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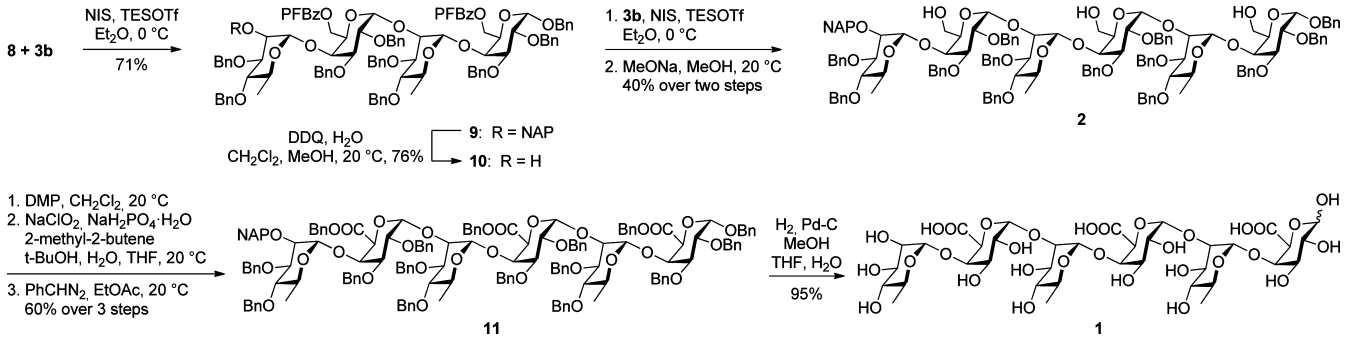
Scheme 2. Assembly of the Hexasaccharide



the PFBz-groups at C-6 of galactose, the hexasaccharide **2** was isolated in a pure form in 40% yield over two steps. The liberated primary alcohols were oxidized with Dess–Martin periodinane to aldehydes and then with $NaClO_2$ to carboxylic acids. The carboxylic acid functionalities were protected as benzyl esters by reaction with $PhCHN_2$ to facilitate purification. Finally, treatment of **11** under standard conditions for catalytic hydrogenolysis allowed removal of all the benzyl groups as well as the NAP group, furnishing the fully unprotected hexasaccharide **1**.

In summary, we have presented the first successful synthesis of a fully unprotected hexasaccharide RG-I fragment employing a highly modular synthesis that takes advantage of the armed–disarmed effect to generate the key disaccharide donor in a chemoselective fashion. We envision that this flexible strategy allows for easy introduction of side chains with galactan and arabinan, which will be the focus of future efforts, in addition to using hexasaccharide **1** in characterization of enzymes acting on RG-I.

Acknowledgment. This work was partially financed by the EU 7th Framework Programme via the Marie Curie Initial Training Network, LeanGreenFood. We thank Mr. Clive Phipps Walter, Novozymes, and Dr. Kok-Phen Yan, University of Southern Denmark, for MALDI-MS analysis.

Supporting Information Available. Experimental procedures and analytical and spectral data, including copies of the NMR spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

The authors declare no competing financial interest.