

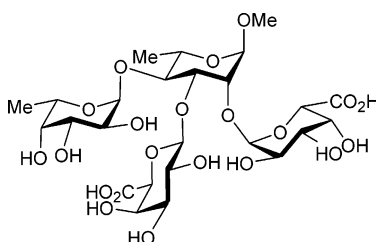
Synthesis of a 2,3,4-Triglycosylated Rhamnoside Fragment of Rhamnogalacturonan-II Side Chain A Using a Late Stage Oxidation Approach

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Pectic polysaccharide RG-II, a key component of plant primary cell walls, is known to exist as a dimer formed by means of borate diester cross-links between apiosyl residues of one of its constituent side-chain oligosaccharides. Described herein is the strategy for the synthesis of the branched tetrasaccharide α -D-GalA-(1 \rightarrow 2)-[β -D-GalA-(1 \rightarrow 3)]-[α -L-Fuc-(1 \rightarrow 4)]- α -L-Rha-OMe, an RG-II fragment that is linked to the apiosyl residue that is thought to be responsible for the borate complexation in RG-II dimer. Iterative glycosylation of the rhamnoside acceptors derived from the key 2,3-orthoacetate of methyl 4-*O*-methoxybenzyl- α -D-rhamnopyranoside afforded the protected tetrasaccharide. The target dicarboxylic acid saccharide was subsequently prepared by removal of protecting groups followed by TEMPO-mediated oxidation of galactopyranosyl residues to galactopyranosyluronic acids.

Introduction

Primary cell walls of higher plants consist of a complex network of polysaccharides,¹ among which rhamnogalacturonan-II (RG-II) is of particular interest as a result of its uniquely complex “mega-oligosaccharide” structure² and demonstrated ability to form dimers as a result of borate complexation.³ RG-II is composed of four structurally different oligosaccharides attached to a backbone made up of α -(1 \rightarrow 4)-linked D-GalpA residues. Primary structures of each RG-II side chain fragment have been elucidated,^{4–8} though some of the detail has been revised

in more recent studies.^{9–11} Models of specific attachment points for each side chain to the backbone, as well as three-dimensional models of RG-II, have been proposed,^{12,13} but their validity remains to be confirmed. Structurally conserved in plants, RG-II predominantly

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(1) Carpita, N.; McCann, M. In *Biochemistry & Molecular Biology of Plants*; Buchanan, B., Gruissem, W., Jones, R. L., Eds.; American Society of Plant Physiologists: Rockville, MD, 2000; pp 52–108.

(2) Ridley, B. L.; O'Neill, M. A.; Mohnen, D. A. *Phytochemistry* **2001**, *57*, 929–967.

(3) Ishii, T.; Matsunaga, T.; Pellerin, P.; O'Neill, M. A.; Darvill, A.; Albersheim, P. *J. Biol. Chem.* **1999**, *274*, 13098–13104.

(4) Darvill, A. G.; McNeil, M.; Albersheim, P. *Plant Physiol.* **1978**, *62*, 418–422.

(5) Spellman, M. W.; McNeil, M.; Darvill, A. G.; Albersheim, P.; Henrick, K. *Carbohydr. Res.* **1983**, *122*, 115–129.

(6) Spellman, M. W.; McNeil, M.; Darvill, A. G.; Albersheim, P.; Dell, A. *Carbohydr. Res.* **1983**, *122*, 131–153.

(7) York, W. S.; Darvill, A. G.; McNeil, M.; Albersheim, P. *Carbohydr. Res.* **1985**, *138*, 109–126.

(8) Melton, L. D.; McNeil, M.; Darvill, A. G.; Albersheim, P.; Dell, A. *Carbohydr. Res.* **1986**, *146*, 279–305.

(9) Baydoun, E. A. H.; Fry, S. C. *J. Plant Physiol.* **1988**, *132*, 484–490.

(10) Vidal, S.; Doco, T.; Williams, P.; Pellerin, P.; York, W. S.; O'Neill, M. A.; Glushka, J.; Darvill, A. G.; Albersheim, P. *Carbohydr. Res.* **2000**, *326*, 277–294.

(11) Glushka, J. N.; Terrell, M.; York, W. S.; O'Neill, M. A.; Gucwa, A.; Darvill, A. G.; Albersheim, P.; Prestegard, J. H. *Carbohydr. Res.* **2003**, *338*, 341–352.

(12) Hervé du Penhoat, C.; Gey, C.; Pellerin, P.; Pérez, S. *J. Biomol. NMR* **1999**, *14*, 253–271.

(13) Rodríguez-Carvajal, M. A.; Hervé du Penhoat, C.; Mazeau, K.; Doco, T.; Pérez, S. *Carbohydr. Res.* **2003**, *338*, 651–671.

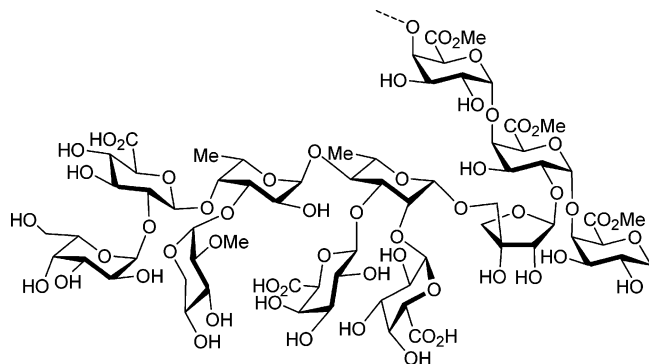


FIGURE 1. Structure of the side-chain A octasaccharide of rhamnogalacturonan-II, shown attached to a polygalacturonan backbone.

exists in the form of a dimer^{3,14} that is cross-linked through a 1:2 borate-diol diester between two apiofuranosyl residues belonging to side chain A (Figure 1) of two different RG-II molecules.

Recent studies revealed an essential role played by borate diester cross-linking of RG-II in plant cell wall organization.^{15,16} It has been shown that small changes in RG-II structure, in particular removal of the L-fucopyranose residue,¹⁵ resulting from genetic modifications can dramatically decrease formation of RG-II dimer and, as a consequence, severely affect plant growth and development. In addition, it has been also demonstrated^{14,17} that Ca^{2+} and other divalent cations stabilize RG-II dimer, suggesting that calcium may have a specific but as yet unidentified binding site close to the borate diester cross-link in dimeric RG-II. To understand the structural importance of borate cross-linking of RG-II and to gain insight into its biosynthesis, well-defined fragments of this oligosaccharide are required. In a previous paper¹⁸ we reported the preparation of a β -L-Rhap-(1 \rightarrow 3')- β -D-Apif-OMe disaccharide fragment of the side chains A and B of RG-II. Here we describe the synthesis of another fragment of RG-II side chain A, tetrasaccharide **1** (Figure 2), incorporating two galactopyranosyluronic acid residues and a fucopyranosyl residue. Considering the importance of fucosylation in RG-II,¹⁵ tetrasaccharide **1** and its smaller fragments will be useful probes for studying the fucosyltransferase activity involved in biosynthesis of RG-II.

Results and Discussion

A notable feature of tetrasaccharide **1** is the presence of two uronic acid residues and two 6-deoxy sugar residues within its structure. This combination of functionalities makes it possible to introduce carboxylic groups through an oxidation step at the late stage of the oligosaccharide preparation, a route that is generally

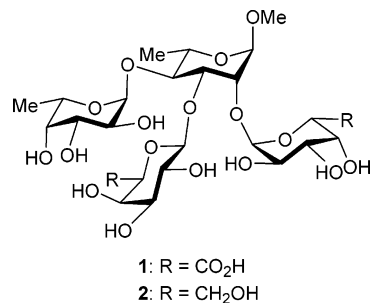
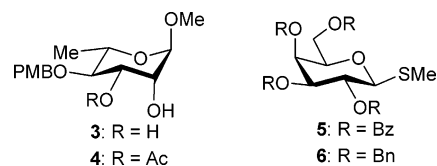


FIGURE 2. Target structure **1** of the trisubstituted methyl rhamnoside fragment of RG-II and its neutral analogue **2**, which serves as a precursor of **1**.

employed^{19,20} to avoid problematic glycosylation with galactopyranosyluronic acid donors. Therefore, neutral tetrasaccharide **2** (Figure 2) can be considered as a precursor to diuronic acid tetrasaccharide **1**. Our synthetic strategy for the assembly of oligosaccharide **2**, consisting of three consecutive glycosylations of a rhamnopyranoside core, is defined by the availability of a suitable rhamnopyranoside acceptor.

For the protection of position 4 of the rhamnose residue, a methoxybenzyl group was chosen because of its orthogonality to benzyl groups that were to be used as galactosyl donor protecting groups. The possibility of regioselective 3-*O*- β -galactosylation of readily available diol **3**²¹ with benzoylated thiogalactosides **5**²² was first examined, but the desired (1 \rightarrow 3)-galactoside was not found in a complex mixture of reaction products. In contrast to glycosylation, partial acetylation of **3** with AcCl in pyridine, as described for a similar rhamnose derivative,²³ led regioselectively to 3-acetate **4** in 88% yield. Glycosylation of **4** with benzylated thiogalactoside **6**²⁴ in MeCN in the presence of TMSOTf-TfOH afforded a 1:1 mixture of anomeric disaccharides that was difficult to separate, thus making this route unsuitable for the construction of **2**.



In a recent study we demonstrated²⁵ that one-pot ortho esterification–benzylation–ortho ester rearrangement can give easy access to useful glycosyl acceptors. Application of this reaction sequence to methyl α -L-rhamnopyranoside (**7**) afforded, through intermediate 2,3-orthoacetate **8** and 4-*O*-*p*-methoxybenzyl derivative **9**, the alcohol **10** possessing orthogonal protecting groups at positions 2 and 4 (Scheme 1).

(14) O'Neill, M. A.; Warrenfeltz, D.; Kates, K.; Pellerin, P.; Doco, T.; Darvill, A. G.; Albersheim, P. *J. Biol. Chem.* **1996**, *271*, 22923–22930.

(15) O'Neill, M. A.; Eberhard, S.; Albersheim, P.; Darvill, A. G. *Science* **2001**, *294*, 846–849.

(16) Iwai, H.; Masaoka, N.; Ishii, T.; Satoh, S. *Proc. Nat. Acad. Sci. U.S.A.* **2002**, *99*, 16319–16324.

(17) Matoh, T.; Takasaki, M.; Kobayashi, M.; Takabe, K. *Plant Cell Physiol.* **2000**, *41*, 363–366.

(18) Chauvin, A. L.; Nepogodiev, S. A.; Field, R. A. *Carbohydr. Res.* **2004**, *339*, 21–27.

(19) Clausen, M. H.; Jorgensen, M. R.; Thorsen, J.; Madsen, R. *J. Chem. Soc., Perkin Trans. 1* **2001**, 543–551.

(20) Clausen, M. H.; Madsen, R. *Chem. Eur. J.* **2003**, *9*, 3821–3832.

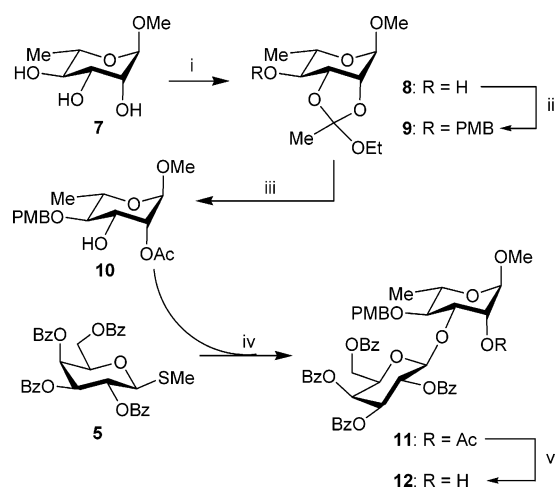
(21) Capozzi, G.; Ciampi, C.; Delogu, G.; Menichetti, S.; Nativi, C. *J. Org. Chem.* **2001**, *66*, 8787–8792.

(22) Ito, Y.; Nunomura, S.; Shibayama, S.; Ogawa, T. *J. Org. Chem.* **1992**, *57*, 1821–1831.

(23) Zhang, J.; Kong, F. *Tetrahedron* **2003**, *59*, 1429–1441.

(24) Koike, K.; Sugimoto, M.; Sato, S.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *163*, 189–208.

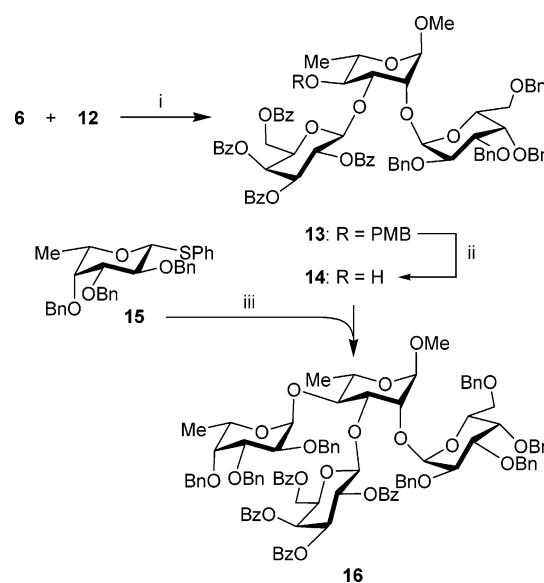
(25) Mukhopadhyay, B.; Field, R. A. *Carbohydr. Res.* **2003**, 338.

SCHEME 1^a

^a Reagents and conditions: (i) MeC(OEt)₃, TsOH, MeCN; (ii) *p*-MeOC₆H₄CH₂Cl, NaH, MeCN; (iii) 80% aq AcOH, overall yield (i–iii) 70%; (iv) NIS, TfOH, MS 4 Å, CH₂Cl₂; 70%; (v) 0.4 M HCl in MeOH, 45%.

It is known that perbenzylated glycosyl donors can be used both for preparation of α - and β -glycosides.²⁶ In the latter case reactions are carried out in acetonitrile or propionitrile and proceed via kinetic α -nitrium ion intermediates.^{27,28} However, this approach was inefficient for β -galactosylation of rhamnoside **10**; essentially no stereoselectivity was observed in reaction with thiogalactoside donor **6**. Successful 1,2-*trans*-galactosylation of **10** was achieved with perbenzoylated thiogalactoside donor **5** in the presence of NIS-TfOH²⁹ as a promoter, yielding disaccharide **11** in 70% yield. The ³J_{1,2'} coupling constant (7.8 Hz) and the position of the C-1' signal (101.8 ppm) in NMR spectra of **11** clearly indicated the β -D-configuration of the galactopyranosyl residue. This disaccharide was subjected to mild acid methanolysis,³⁰ which allowed selective removal of the 2-*O*-acetyl group to give alcohol **12** in 45%. Attempts to optimize reaction conditions in order to improve the modest yield were not successful.

The second glycosylation required formation of a 1,2-*cis*-galactosidic linkage; the reaction was carried out between disaccharide **12** and benzylated thiogalactoside **6** (Scheme 2) in the presence of NIS-TMSOTf.³¹ This reaction afforded an 87% yield of trisaccharide **13**, having the desired α -configuration of the 2-*O*-galactopyranosyl residue, which was confirmed by the chemical shift of its anomeric carbon at 98.1 ppm (the C-1 resonance for the perbenzylated β -galactopyranoside is expected at 102–104 ppm, cf. ref 32). Removal of the 4-*O*-*p*-methoxybenzyl group using CAN in MeCN–H₂O³³ led to alcohol **14** in 67% yield. The latter was glycosylated with known

SCHEME 2^a

^a Reagents and conditions: (i) NIS, TMSOTf, CH₂Cl₂, 87%; (ii) CAN, MeCN–H₂O (9:1), 93%; (iii) 2 equiv of NIS, 2 equiv of TfOH, Et₂O–CH₂Cl₂ (5:1), 87%.

phenyl thiofucopyranoside donor **15**^{34,35} activated with NIS-TfOH in CH₂Cl₂–Et₂O solution to form tetrasaccharide **16** in 61% yield. The structure of **16** was confirmed by its NMR spectra, which were assigned using COSY and gHSQC experiments. The ¹H NMR spectrum of **16** showed the expected doublets for anomeric signals of an α -fucopyranosyl residue (5.22 ppm, *J*_{1,2} = 3.4 Hz) and an α -galactopyranosyl residue (5.33 ppm, *J*_{1,2} = 3.5 Hz), whereas resonances of the anomeric protons of α -rhamnopyranosyl and β -galactopyranosyl residues were part of multiplets at 4.47–4.79 and 4.82 ppm, respectively. The ¹³C spectrum showed four anomeric carbons at 97.1, 99.2, 99.5, and 100.6 ppm for α -Galp, α -Rhap, α -Fucp, and β -Galp, respectively. Electrospray mass spectrometry confirmed the tetrasaccharide structure of **16** by the presence of the [M + NH₄]⁺ ion (1712.8).

Deprotection of protected saccharides **11**, **13**, and **16** was carried out in the following order (Scheme 3): removal of the *p*-methoxybenzyl group using CAN (for **11** and **13**), Zemplen deacylation, and finally catalytic hydrogenolysis (for **13** and **16**). Complete removal of benzyl protecting groups in the tetrasaccharide required noticeably longer reaction time (7 days) than in the case of trisaccharide (24 h). Purification of deprotected oligosaccharides was achieved using chromatography on silica gel with CH₂Cl₂–MeOH (9:1) as eluent to give disaccharide **17** (67%), trisaccharide **19** (74%), and tetrasaccharide **2** (61%). Their successful deprotection was confirmed by ¹H and ¹³C NMR spectroscopy and mass spectrometry. Primary OH groups of compounds **17**, **19**, and **2** were selectively oxidized to carboxylic acids using

(26) Demchenko, A. V. *Curr. Org. Chem.* **2003**, *7*, 35–79.

(27) Braccini, I.; Derouet, C.; Esnault, J.; Hervé du Penhoat, C.; Mallet, J. M.; Michon, V.; Sinay, P. *Carbohydr. Res.* **1993**, *246*, 23–41.

(28) Fukase, K.; Hasuoka, A.; Kinoshita, I.; Aoki, Y.; Kusumoto, S. *Tetrahedron* **1995**, *51*, 4923–4932.

(29) Veeneman, G. H.; van Leeuwen, S. H.; van Boom, J. H. *Tetrahedron Lett.* **1990**, *31*, 1331–1334.

(30) Byramova, N. E.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1983**, *124*, C8–C11.

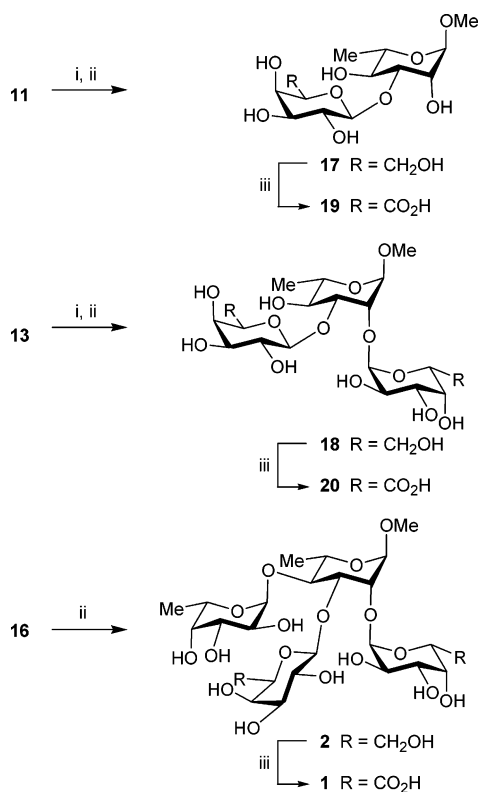
(31) Boons, G. J.; Bowers, S.; Coe, D. M. *Tetrahedron Lett.* **1997**, *38*, 3773–3776.

(32) Hada, N.; Kuroda, M.; Takeda, T. *Chem. Pharm. Bull.* **2000**, *48*, 1160–1165.

(33) Classon, B.; Garegg, P. J.; Samuelsson, B. *Acta Chem. Scand. B* **1984**, *38*, 419–422.

(34) Komba, S.; Ishida, H.; Kiso, M.; Hasegawa, A. *Bioorg. Med. Chem.* **1996**, *4*, 1833–1847.

(35) Chervin, S. M.; Lowe, J. B.; Koreeda, M. *J. Org. Chem.* **2002**, *67*, 5654–5662.

SCHEME 3^a

^a Reagents and conditions: (i) CAN, MeCN–H₂O (9:1); (ii) 0.05% NaOMe in MeOH then H₂, Pd/C, EtOH; 67% for (**17**), 74% for (**19**), 61% for (**2**); (iii) TEMPO, NaOCl, KBr, H₂O; 73% for (**19**), 64% for (**20**), 47% for (**1**).

a TEMPO–NaOCl–KBr system in water^{36–38} to give uronic acid **18**, diuronic acid **20**, and target tetrasaccharide **1**. These reactions were complete in less than 2 h though some decrease of reaction rate with increasing saccharide complexity was observed. All oxidized oligosaccharides were successfully purified using silica gel column chromatography with CH₂Cl₂–MeOH–AcOH (20:20:1) as eluent.

¹³C NMR spectra of **1**, **19**, and **20** clearly showed the presence of the expected number of anomeric signals (Table 1) and peaks at lower field corresponding to resonances of carbonyl groups of galactopyranosyluronic acid residues. The peaks of the OMe group, C-6 of fucopyranose, and C-6 of rhamnopyranose residues were also clearly identified. gHSQC experiments produced well-resolved 2D spectra for compounds **1**, **19**, and **20** as illustrated for tetrasaccharide **1** (Figure 3). However because of a considerable overlap of resonances of H-2–H-5 of pyranose rings, a complete assignment of all signals in the ¹H NMR spectra proved impractical. Taking into account that the additional sugar residues in the full RG-II structure can make a significant impact on three-dimensional structure, the observed chemical shifts of anomeric signals in both ¹H and ¹³C NMR spectra of tetrasaccharide **1** were in a good agreement

TABLE 1. ¹H NMR and ¹³C NMR Data for Anomeric Signals of Oligosaccharides **19**, **20**, and **1** in D₂O at 20 °C

compound		α-L-Rhap	β-D-GalpA	α-D-GalpA	α-L-Fucp
19 ^a	δ _{H-1}	4.69	4.58		
	δ _{C-1}	101.3	104.3		
20 ^a	δ _{H-1}	4.60 ^b	4.45	4.89	
	δ _{C-1}	98.4	105.4	97.3	
1	δ _{H-1}	4.67 ^c	4.57	5.11	5.43
	δ _{C-1}	99.9	105.2	100.8	99.6
RG-II ^d	δ _{H-1}	4.65–4.67	4.47	4.865	5.23
	δ _{C-1}	100	105.0	99	99.7

^a ¹H NMR (400 MHz) and ¹³C NMR (100 MHz). ^b ¹H NMR (600 MHz) and ¹³C NMR (150 MHz). ^c The signal overlaps with the resonance of HOD. ^d NMR data¹³ for tetrasaccharide fragment of RG-II corresponding to oligosaccharide **1**.

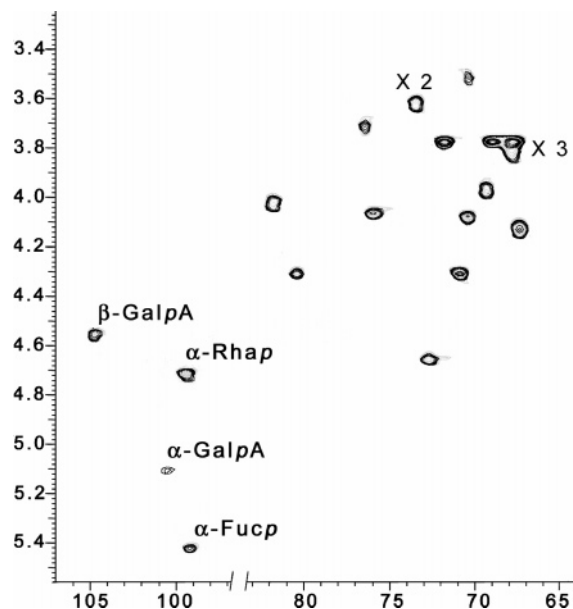


FIGURE 3. Pyranose ring protons region of the 2D-transformed data matrix from the gradient-enhanced natural abundance HSQC experiment conducted on the tetrasaccharide **1** (600 MHz, D₂O).

with corresponding signals¹³ of the same tetrasaccharide fragment of natural RG-II (Table 1).

The negative ion mode electrospray mass spectrum of the tetrasaccharide **1** contained two major peaks corresponding to ions formed as a result of the ionization of one carboxylic group in the diuronic acid **1** (m/z 675.7, $[M - H]^-$) and the monosodium salt of **1** (m/z 697.2, $[M + Na - 2H]^-$). In addition, a fragmentation peak corresponding to the loss of one galactopyranosyluronic acid residue from **1** (m/z 499.0, $[M - GalpA]^-$) was observed. The negative ion mode mass spectrum of trisaccharide **20** showed a similar pattern of ionization/fragmentation having peaks m/z 529.6 $[M - H]^-$ and 353.5 $[M - GalpA]^-$ that are typical for negative ion mode mass spectra of oligogalacturonates.³⁹

In summary, the synthesis of trisubstituted rhamnoside **1**, a tetrasaccharide fragment of rhamnogalacturonan-II, was successfully accomplished by iterative β-galactosylation, α-galactosylation, and α-fucosylation of a methyl rhamnoside acceptor, followed by TEMPO-

(36) de Nooy, A. E. J.; Besemer, A. C.; van Bekkum, H. *Synthesis* **1996**, 1153–1174.

(37) Baisch, G.; Ohrlein, R. *Carbohydr. Res.* **1998**, *312*, 61–72.

(38) Li, K. C.; Helm, R. F. *Carbohydr. Res.* **1995**, *273*, 249–253.

(39) Körner, R.; Limberg, G.; Christensen, T. M. I. E.; Mikkelsen, J. D.; Roepstorff, P. *Anal. Chem.* **1999**, *71*, 1421–1427.

catalyzed oxidation. Regioselective oxidation of two primary OH groups in tetrasaccharide **2** represents the first example of the successful application of a TEMPO–NaOCl oxidation method to highly branched oligosaccharide structures. The synthetic route described also gave access to β -D-GalA-(1 \rightarrow 3)- α -L-Rha-OMe (**19**) and α -D-GalA-(1 \rightarrow 2)-[β -D-GalA-(1 \rightarrow 3)]- α -L-Rha-OMe (**20**). The strategy developed for the synthesis of **1** will be useful for assembling larger fragments of chain A of RG-II, extended at both the rhamnose and fucose ends of the tetrasaccharide. In addition, trisaccharide **20** and tetrasaccharide **1** represent potential acceptor and product for the plant fucosyltransferase, which has been shown¹⁵ to be essential for construction of structurally and functionally competent RG-II.

Experimental Section

Methyl 4-O-(4-Methoxybenzyl)- α -L-rhamnopyranoside (3). A solution of methyl 2,3-*O*-isopropylidene- α -L-rhamnopyranoside (0.51 g, 2.3 mmol) in DMF (4.3 mL) was added dropwise to a stirred, cooled (0 °C) mixture of NaH (60% dispersion in mineral oil, 0.53 g, 10.0 mmol) in DMF (6.3 mL). 4-Methoxybenzyl chloride (0.47 mL, 3.4 mmol) was then added slowly, and the mixture was allowed to warm slowly to room temperature (3 h). After 1 h of stirring at room temperature MeOH (1 mL) was added carefully, the mixture was concentrated, and the resulting residue was dissolved in CH₂Cl₂, washed with water, dried, and concentrated. Column chromatography (toluene–EtOAc, 8:1) gave methyl 2,3-*O*-isopropylidene-4-*O*-(4-methoxybenzyl)- α -L-rhamnopyranoside (0.74 g, 99%), which was stirred with Amberlite IRA-120 (H⁺, 2 g) in MeOH (6.5 mL) for 18 h at 20 °C. The resin was filtered off, the solution was concentrated, and the product was purified by chromatography (toluene–EtOAc, 8:1 \rightarrow 3:1) to afford **3** (0.22 g, 34%): R_f = 0.10 (toluene–EtOAc, 3:2); [α]_D –43 (c 0.9, CHCl₃). δ_H (400 MHz, CDCl₃): 1.34 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6), 2.45–2.56 (2 H, broad s, OH), 3.30 (1 H, dd, $J_{3,4}$ = $J_{4,5}$ 10 Hz, H-4), 3.34 (3 H, s, OCH₃), 3.70 (1 H, m, H-5), 3.79 (3 H, s, CH₃OC₆H₄CH₂O), 3.85 (1 H, dd, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 10.1 Hz, H-3), 3.90 (1 H, dd, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.5 Hz, H-2), 4.63 (1 H, d, $J_{1,2}$ 1.5 Hz, H-1), 4.62–4.69 (2 H, m, CH₃OC₆H₄CH₂O), 7.25–7.29 (4 H, CH₃OC₆H₄CH₂O). δ_C (100 MHz, CDCl₃): 17.8 (C-6), 54.7 (OCH₃), 55.2 (CH₃OC₆H₄CH₂O), 67.0 (C-5), 70.0 (C-2), 71.4 (C-3), 74.6 (CH₃OC₆H₄CH₂O), 81.2 (C-4), 100.4 (C-1), 114.1, 129.7, 130.5, 159.6 (CH₃OC₆H₄CH₂O). ESI-MS found m/z 316.1755 [M + NH₄]⁺, calcd for C₁₅H₂₂O₆·NH₄ 316.1759.

Methyl 3-O-Acetyl-4-O-(4-methoxybenzyl)- α -L-rhamnopyranoside (4). A solution of AcCl (0.05 mL, 0.70 mmol) in CH₂Cl₂ (2 mL) was added dropwise to the solution of **3** (0.17 g, 0.57 mmol) in CH₂Cl₂ (5 mL) containing pyridine (4 mL) under N₂, at 0 °C. The mixture was allowed to warm to room temperature over 2 h and diluted with CH₂Cl₂, and the organic solution was washed with water and 1 M aqueous HCl, dried, and concentrated. Purification of the residue by chromatography (toluene–EtOAc, 3:1) gave the title compound (0.13 g, 88%): R_f = 0.17 (toluene–EtOAc, 3:2); [α]_D –24 (c 0.8, CHCl₃). δ_H (400 MHz, CDCl₃): 1.32 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6), 2.16 (3 H, s, OCOCH₃), 2.16 (1 H, broad s, OH), 3.37 (3 H, s, OCH₃), 3.52 (1 H, dd, $J_{3,4}$ = $J_{4,5}$ 9.5 Hz, H-4), 3.75–3.80 (m, CH₃OC₆H₄CH₂O and H-5), 4.04 (1 H, dd, $J_{1,2}$ 2.0, $J_{2,3}$ 3.3 Hz, H-2), 4.57 (2 H, m, CH₃OC₆H₄CH₂O), 4.63 (1 H, d, $J_{1,2}$ 2.0 Hz H-1), 5.20 (1 H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.5 Hz, H-3), 6.86–7.27 (4 H, CH₃OC₆H₄CH₂O). δ_C (100 MHz, CDCl₃): 18.2 (C-6), 21.4 (CH₃CO), 55.1 (OCH₃), 55.5 (CH₃OC₆H₄CH₂O), 67.7 (C-5), 69.9 (C-2), 74.4 (C-3), 74.8 (CH₃OC₆H₄CH₂O), 78.5 (C-4), 100.6 (C-1), 114.0, 129.6, 130.4, 159.5 (CH₃OC₆H₄CH₂O), 170.2 (CH₃CO). ESI-MS found m/z 341.1595 [M + NH₄]⁺, calcd for C₁₇H₂₄O₇·NH₄ 341.1599.

Methyl 2-O-Acetyl-4-O-(4-methoxybenzyl)- α -L-rhamnopyranoside (10). A solution of methyl α -L-rhamnopyranoside

(2.00 g, 11.2 mmol) and triethylorthoacetate (3.7 mL, 20.2 mmol) in MeCN (20 mL) was stirred for 15 min at room temperature, TsOH·H₂O (20 mg, 0.10 mmol) was added, the mixture was stirred until TLC (hexanes–EtOAc, 2:1) showed disappearance of starting material (~45 min), and the solution was neutralized with Et₃N. NaH (60% dispersion in mineral oil, 0.86 g, 22.4 mmol) was added to the mixture and after 10 min of stirring 4-methoxybenzyl chloride (3.0 mL, 22.4 mmol) was added carefully. Stirring continued until TLC (hexanes–EtOAc, 3:1) indicated that the reaction was complete (~1 h), MeOH (2 mL) was added, and the mixture was concentrated. The residue was dissolved in 80% aqueous AcOH (20 mL) and stirred for 30 min. The solution was diluted with CH₂Cl₂, washed with H₂O and aqueous NaHCO₃ solution, dried, and concentrated. Column chromatography in hexanes–EtOAc (4:1 \rightarrow 3:1) gave **10** (2.80 g, 77%): R_f = 0.33 (toluene–EtOAc, 4:1); [α]_D –32 (c 1.8, CHCl₃). δ_H (400 MHz, CDCl₃): 1.34 (3 H, d, $J_{5,6}$ 6.4 Hz, H-6), 2.15 (3 H, s, CH₃CO), 2.23 (1 H, d, $J_{3,OH}$ 4.2 Hz, OH), 3.33 (4 H, m, H-4, OCH₃), 3.66–3.72 (1 H, m, H-5), 3.79 (3 H, s, CH₃OC₆H₄CH₂O), 4.05 (1 H, m, H-3), 4.60 (1 H, s, H-1), 4.62–4.76 (2 H, m, CH₃OC₆H₄CH₂O), 5.08 (1 H, m, H-2), 6.87–7.30 (4 H, m, CH₃OC₆H₄CH₂O). δ_C (100 MHz, CDCl₃): 17.9 (C-6), 20.9 (CH₃CO), 54.8 (OCH₃), 55.2 (CH₃OC₆H₄CH₂O), 67.2 (C-5), 70.1 (C-3), 72.7 (C-2), 74.8 (CH₃OC₆H₄CH₂O), 81.3 (C-4), 98.4 (C-1), 113.9, 129.6, 130.5, 159.5 (CH₃OC₆H₄CH₂O), 170.9 (CH₃CO). ESI-MS found m/z 358.1860 [M + NH₄]⁺, calcd for C₁₇H₂₄O₇·NH₄ 358.1860.

Methyl 2-O-Acetyl-4-O-(4-methoxybenzyl)-3-O-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)- α -L-rhamnopyranoside (11). A solution of compound **10** (0.54 g, 1.5 mmol) and benzoylated thiogalactopyranoside **5**²² (1.23 g, 1.8 mmol) in dry CH₂Cl₂ (4.5 mL) was stirred in the presence of molecular sieves 4 Å (0.6 g) for 2 h at room temperature, NIS (0.40 g, 1.8 mmol) was added, and the mixture was cooled to 0 °C. A solution of TfOH (0.016 mL, 0.18 mmol) in CH₂Cl₂–Et₂O (1:1, 13.5 mL) was added to the mixture, and stirring was continued for 1 h. The mixture was diluted with CH₂Cl₂, washed with 10% aqueous Na₂S₂O₃ solution until decolorized, then washed successively with water, aqueous NaHCO₃ solution, and brine, dried, and concentrated. The resulting residue was purified by chromatography (toluene–EtOAc, 8:1) to give **11** (1.07 g, 70%): R_f = 0.33 (toluene–EtOAc, 8:1); [α]_D +43 (c 1.5, CHCl₃). δ_H (400 MHz, CDCl₃): 1.20 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 2.22 (3 H, s, CH₃CO), 3.27 (3 H, s, OCH₃), 3.42 (1 H, dd, $J_{3,4}$ = $J_{4,5}$ 9.3 Hz, H-4 Rha), 3.64–3.67 (1 H, m, H-5 Rha), 3.77 (3 H, s, CH₃OC₆H₄CH₂O), 4.22 (1 H, dd, $J_{2,3}$ 3.6 Hz, $J_{3,4}$ 9.3 Hz, H-3 Rha), 4.25 (1 H, d, J 10.8 Hz, CH₃OC₆H₄CH₂O), 4.31 (1 H, t, $J_{5,6a}$ = $J_{5,6b}$ 6.4 Hz, H-5 Gal), 4.43 (1 H, dd, $J_{5,6a}$ 6.4 Hz, $J_{6a,6b}$ 11.3 Hz, H-6a Gal), 4.50 (1 H, d, J 10.8 Hz, CH₃OC₆H₄CH₂O), 4.58 (1 H, d, $J_{1,2}$ 1.8 Hz, H-1 Rha), 4.61 (1 H, dd, $J_{5,6b}$ 6.6 Hz, $J_{6a,6b}$ 11.3 Hz, H-6b Gal), 5.16 (1 H, d, $J_{1,2}$ 7.8 Hz, H-1 Gal), 5.42 (1 H, dd, $J_{1,2}$ 1.8 Hz, $J_{2,3}$ 3.7 Hz, H-2 Rha), 5.59 (1 H, dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.3 Hz, H-3 Gal), 5.84 (1 H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.4 Hz, H-2 Gal), 5.96 (1 H, d, $J_{3,4}$ 3.3 Hz, H-4 Gal), 6.71–8.14 (24 H, Ar). δ_C (100 MHz, CDCl₃): 18.1 (C-6 Rha), 21.3 (CH₃CO), 55.0 (OCH₃), 55.4 (CH₃OC₆H₄CH₂O), 62.2 (C-6 Gal), 67.5 (C-5 Rha), 68.2 (C-4 Gal), 70.4 (C-2 Gal), 71.4 (C-5 Gal), 72.0 (C-3 Gal), 72.4 (C-2 Rha), 74.9 (CH₃OC₆H₄CH₂O), 78.3 (C-3 Rha), 79.7 (C-4 Rha), 98.5 (C-1 Rha), 101.8 (C-1 Gal), 113.8–159.2 (Ar), 165.4, 165.7, 165.8, 166.3 (C₆H₅CO), 170.4 (OCOCH₃). ESI-MS found m/z 336.3436 [M + NH₄]⁺, calcd for C₅₁H₅₀O₁₆·NH₄ 336.3437.

Methyl 4-O-(4-Methoxybenzyl)-3-O-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)- α -L-rhamnopyranoside (12). AcCl (0.27 mL, 3.8 mmol) was added to a solution of **11** (0.83 g, 0.90 mmol) in MeOH (9 mL) at 0 °C, and the mixture was allowed to warm to room temperature. When the starting material was no longer present (~24 h) the mixture was neutralized with Et₃N and concentrated to dryness. Column chromatography (toluene–EtOAc, 8:1) afforded **12** (0.36 g, 45%): R_f = 0.17 (toluene–EtOAc, 8:1); [α]_D +77 (c 1.0, CHCl₃). δ_H (400 MHz, CDCl₃): 1.19 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 3.01

(1 H, broad s, OH), 3.20 (3 H, s, OCH₃), 3.47 (1 H, dd, $J_{3,4} = J_{4,5}$ 8.9 Hz, H-4 Rha), 3.58–3.62 (1 H, m, H-5 Rha), 3.72 (3 H, s, CH₃OC₆H₄CH₂O), 4.03 (1 H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 8.9 Hz, H-3 Rha), 4.07 (1 H, broad s, H-2 Rha), 4.27 (1 H, d, J 10.8 Hz, CH₃OC₆H₄CH₂O), 4.40 (1 H, dd, $J_{5,6a}$ 4.4 Hz, $J_{5,6b}$ 8.2 Hz, H-5 Gal), 4.41 (1 H, s, H-1 Gal), 4.50 (1 H, d, J 10.8 Hz, CH₃OC₆H₄CH₂O), 4.54 (1 H, dd, $J_{5,6a}$ 4.4 Hz, $J_{6a,6b}$ 11.6 Hz, H-6a Gal), 4.62 (1 H, dd, $J_{5,6b}$ 8.2 Hz, $J_{6a,6b}$ 11.6 Hz, H-6b Gal), 5.11 (1 H, d, $J_{1,2}$ 8.1 Hz, H-1 Gal), 5.62 (1 H, dd, $J_{2,3}$ 10.2 Hz, $J_{3,4}$ 3.5 Hz, H-3 Gal), 5.96 (1 H, dd, $J_{1,2}$ 8.1, $J_{2,3}$ 10.2 Hz, H-2 Gal), 6.00 (1 H, d, $J_{3,4}$ 3.5 Hz, H-4 Gal), 6.63–8.12 (24 H, m, Ar). δ_C (100 MHz, CDCl₃): 18.1 (C-6 Rha), 54.8 (OCH₃), 55.4 (CH₃OC₆H₄CH₂O), 62.7 (C-6 Gal), 67.3 (C-5 Rha), 68.4 (C-4 Gal), 69.8 (C-2 Gal), 70.3 (C-5 Gal), 72.1 (C-3 Gal), 72.2 (C-2 Rha), 74.9 (CH₃OC₆H₄CH₂O), 78.9 (C-3 Rha), 83.3 (C-4 Rha), 100.1 (C-1 Rha), 101.9 (C-1 Gal), 113.7–159.1 (Ar), 165.6, 165.7, 165.9, 166.3 (PhCO). ESI-MS found m/z 894.3338 [M + NH₄]⁺, calcd for C₄₉H₄₈O₁₅·NH₄ 894.3331.

Methyl 4-O-(4-Methoxybenzyl)-2-O-(2,3,4,6-tetra-O-benzyl- α -D-galactopyranosyl)-3-O-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)- α -L-rhamnopyranoside (13). A solution of disaccharide **12** (0.36 g, 0.41 mmol) and methyl 2,3,4,6-tetra-O-benzyl-1-thio- β -D-galactopyranoside **6²⁴** (0.47 g, 0.82 mmol) in 15 mL of dry CH₂Cl₂–Et₂O (2:3) was stirred with molecular sieves 4 Å (0.5 g) for 2 h at room temperature. After addition of NIS (0.18 g, 0.8 mmol) the mixture was cooled to –55 °C and TMSOTf (0.015 mL, 0.07 mmol) was added. The mixture was stirred for 1 h at –55 °C, treated with Et₃N (0.27 mL), then allowed to warm to room temperature, and diluted with CH₂Cl₂. The organic solution was washed with 10% aqueous Na₂S₂O₃ solution, water, aqueous NaHCO₃ solution, and brine, dried, and concentrated. Purification of the residue by column chromatography (toluene–EtOAc, 9:1) gave **13** (0.49 g, 87%): $R_f = 0.29$ (toluene–EtOAc, 8:1); [α]_D +77 (c 0.8, CHCl₃). δ_H (400 MHz, CDCl₃): 1.10 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 3.12 (3 H, s, OCH₃), 3.42 (1 H, dd, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4 Rha), 3.57–3.60 (1 H, m, H-5 Rha), 3.73 (3 H, s, CH₃OC₆H₄CH₂O), 3.91–4.04 (2 H, m, H-6a and H-6b α -Gal), 4.09 (2 H, s, H-2 Rha and H-4 α -Gal), 4.13 (1 H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.2 Hz, H-3 Rha), 4.19–4.71 (14 H, m, PhCH₂, H-2, H-3, H-5 α -Gal, H-5, H-6a and H-6b β -Gal, H-1 Rha), 4.80 (2 H, m, PhCH₂), 5.04 (1 H, d, J 10.7 Hz, PhCH₂), 5.25 (2 H, m, H-1 β -Gal, H-1 α -Gal), 5.57 (1 H, dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.2 Hz, H-3 β -Gal), 5.92 (1 H, d, $J_{3,4}$ 3.2 Hz H-4 β -Gal), 5.96 (1 H, dd, $J_{1,2}$ 7.9, $J_{2,3}$ 10.4 Hz, H-2 β -Gal), 6.71–8.10 (44 H, m, Ar). δ_C (100 MHz, CDCl₃): 17.8 (C-6 Rha), 54.8 (OCH₃), 55.4 (CH₃OC₆H₄CH₂O), 62.4 (C-6 β -Gal), 67.8 (C-5 Rha), 68.4 (C-4 β -Gal), 69.3 (C-6 α -Gal), 70.2, 71.5 (C-5 α -Gal, C-5 β -Gal), 70.4 (C-2 β -Gal), 72.9 (C-3 β -Gal), 72.7, 73.2, 73.7 (PhCH₂), 74.5 (CH₃OC₆H₄CH₂O), 75.5 (PhCH₂), 76.1, 78.3 (C-2 and C-3 α -Gal), 77.6 (C-4 α -Gal), 78.3, 79.3 (C-2 and C-4 Rha), 79.7 (C-3 Rha), 98.1 (C-1 α -Gal), 98.7 (C-1 Rha), 102.5 (C-1 β -Gal), 113.6–158.9 (Ar), 164.9, 165.5, 165.6, 165.9 (C₆H₅CO). ESI-MS found m/z 1416.8 [M + NH₄]⁺, calcd for C₈₃H₈₂O₂₀·NH₄ 1416.6.

Methyl 2-O-(2,3,4,6-Tetra-O-benzyl- α -D-galactopyranosyl)-3-O-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)- α -L-rhamnopyranoside (14). A solution of protected trisaccharide **13** (0.26 g, 0.18 mmol) and (NH₄)₂Ce(NO₃)₆ (0.58 g, 1.06 mmol) in CH₃CN–H₂O (9:1, 3 mL) was stirred at room temperature for 0.5 h, diluted with CH₂Cl₂, washed with water, aqueous NaHCO₃ solution and brine, dried, and concentrated. The residue was purified by column chromatography (toluene–EtOAc, 4:1) to afford **14** (0.24 g, 93%): $R_f = 0.11$ (toluene–EtOAc, 8:1); [α]_D +74 (c 2.7, CHCl₃). δ_H (400 MHz, CDCl₃): 1.15 (3 H, d, $J_{5,6}$ 5.5 Hz, H-6 Rha), 3.14 (3 H, s, OCH₃), 3.59–3.65 (2 H, m, H-5 Rha and H-3 Rha), 3.75 (1 H, dd, $J_{5,6a}$ 7.7 Hz, $J_{6a,6b}$ 9.2 Hz, H-6a α -Gal), 3.84 (1 H, dd, $J_{5,6b}$ 5.8 Hz, $J_{6a,6b}$ 9.2 Hz, H-6b α -Gal), 3.90–3.93 (1 H, m, H-4 Rha), 4.19–4.22 (4 H, H-2 Rha, H-2, H-3 and H-4 α -Gal), 4.25–4.29 (3 H, m, H-5 α -Gal, H-5 and H-6a β -Gal), 4.44–4.68 (6 H, m, H-6a β -Gal, PhCH₂), 4.72 (1 H, d, $J_{1,2}$ 1.3 Hz, H-1 Rha), 4.80 (2 H, m, PhCH₂), 5.02 (1 H, d, J 10.9 Hz, PhCH₂), 5.21 (1 H, d, $J_{1,2}$

2.6 Hz, H-1 α -Gal), 5.29 (1 H, d, $J_{1,2}$ 8.1 Hz, H-1 β -Gal), 5.57 (1 H, dd, $J_{2,3}$ 10.4 Hz, $J_{3,4}$ 3.3 Hz, H-3 β -Gal), 5.82–5.86 (2 H, m, H-2 and H-4 β -Gal). δ_C (100 MHz, CDCl₃): 17.8 (C-6 Rha), 54.6 (OCH₃), 62.4 (C-6 β -Gal), 68.1 (C-4 β -Gal), 68.2 (C-5 Rha), 69.3 (C-6 α -Gal), 70.1 (C-2 β -Gal), 70.3 (C-5 α -Gal), 71.2, 71.3 (C-5 β -Gal, C-3 Rha), 71.8 (C-3 β -Gal), 72.8, 72.9, 73.4 (PhCH₂), 75.0 (PhCH₂), 75.6, 77.3, 78.1, 78.9 (C-2 Rha, C-2, C-3 and C-4 α -Gal), 80.6 (C-4 Rha), 98.1 (C-1 α -Gal), 99.1 (C-1 Rha), 101.6 (C-1 β -Gal), 114.3–139.1 (Ar), 165.1, 165.5, 165.6, 166.0 (PhCO). ESI-MS found m/z 1296.5 [M + NH₄]⁺, calcd for C₇₅H₇₄O₁₉·NH₄ 1296.4.

Methyl 2-O-(2,3,4,6-Tetra-O-benzyl- α -D-galactopyranosyl)-3-O-(2,3,4,6-tetra-O-benzoyl- β -D-galactopyranosyl)-4-O-(2,3,4-tri-O-benzyl- α -L-fucopyranosyl)- α -L-rhamnopyranoside (16). A mixture of trisaccharide acceptor **14** (110 mg, 86 μ mol), thiofucoside donor **15^{34,35}** (70 mg, 130 μ mol), and 4 Å molecular sieves (0.35 g) in CH₂Cl₂–Et₂O (1:5, 6 mL) was stirred for 17 h at 20 °C, and NIS (90 mg, 400 μ mol) was added. The mixture was cooled to –30 °C, TfOH (10 μ L, 0.04 mmol) was added, and stirring was continued for 0.5 h at –30 °C. The mixture was allowed to warm to room temperature, diluted with CH₂Cl₂, washed with water, aqueous NaHCO₃ solution, and brine, dried, and concentrated. Column chromatography (toluene–EtOAc, 4:1) of the residue afforded protected tetrasaccharide **16** (0.13 g, 87%): $R_f = 0.54$ (toluene–EtOAc, 8:1); [α]_D +42 (c 1.02, CHCl₃). δ_H (400 MHz, CHCl₃): 1.06 (3 H, d, $J_{5,6}$ 6.4 Hz, H-6 Fuc), 1.23 (3 H, br s, H-6 Rha), 3.09 (3 H, s, OCH₃), 3.61–3.77 (4 H, m, H-3, H-4, H-5 Rha, H-2 α -Gal), 3.84–3.94 (4 H, m, H-3, H-6a, H-6b α -Gal, H-5 Fuc), 4.00–4.10 (4 H, m, H-2 Rha, H-2, H-3, H-4 Fuc), 4.17–4.21 (2 H, m, H-5, H-6a β -Gal), 4.27–4.46 (3 H, m, H-5 α -Gal, H-6b β -Gal, PhCH₂), 4.46–4.84 (12 H, m, H-1 Rha and 11 H PhCH₂), 4.88 (1 H, d, J 10.6 Hz, PhCH₂), 4.91 (1 H, d, $J_{1,2}$ 8 Hz, H-1 β -Gal), 5.00 (1 H, d, J 10.6 Hz, PhCH₂), 5.17–5.26 (1 H, m, H-3 β -Gal), 5.22 (1 H, d, $J_{1,2}$ 3.1 Hz, H-1 Fuc), 5.33 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1 α -Gal), 5.55 (1 H, broad s, H-4 β -Gal), 5.75 (1 H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 10.4 Hz, H-2 β -Gal). δ_C (100 MHz, CHCl₃): 16.6 (C-6 Fuc), 18.8 (C-6 Rha), 54.5 (OCH₃), 62.2 (C-6 β -Gal), 66.2 (C-5 Rha), 66.9 (C-5 Fuc), 68.6 (C-4 β -Gal), 68.9 (C-6 α -Gal), 70.1 (C-5 α -Gal), 70.5 (C-2 β -Gal), 71.1 (C-3 β -Gal), 71.9, 72.6, 72.9, 73.0, 73.7, 75.2, 75.6 (PhCH₂), 75.9 (C-2 α -Gal, C-5 β -Gal), 77.6 (C-3 Rha), 77.6, 78.3, 79.3, 79.6, 79.7 (2C) (C-2, C-4 Rha, C-2, C-3, C-4 Fuc, C-4 α -Gal), 80.4 (C-3 α -Gal), 97.1 (C-1 α -Gal), 99.2 (C-1 Rha), 99.5 (C-1 Fuc), 100.6 (C-1 β -Gal), 127.1–139.6 (Ar), 165.1, 165.5, 165.6, 165.7 (C₆H₅CO). ESI-MS found m/z 1712.8 [M + NH₄]⁺, calcd for C₁₀₂H₁₀₂O₂₃·NH₄ 1712.7.

Methyl 3-O-(β -D-Galactopyranosyl)- α -L-rhamnopyranoside (17). Protected disaccharide **11** (23.2 mg, 68.1 μ mol) and (NH₄)₂Ce(NO₃)₆ (3.61 mmol) were stirred in a mixture of CH₃CN–H₂O (9:1, 5 mL) for 0.5 h at room temperature. The mixture was diluted with CH₂Cl₂, washed with water, aqueous NaHCO₃ solution, and brine, dried, and concentrated. Products were purified by column chromatography (toluene–EtOAc). The resulting saccharide was treated with 0.05 M NaOMe in MeOH (10 mL) at 20 °C until complete conversion of starting material into a single product (~1 h). The solution was then neutralized with Amberlite IRA-120 (H⁺) resin, the resin was filtered off and washed with MeOH, and the methanolic solution was concentrated to dryness. Purification of the residue by column chromatography (toluene–EtOAc, 4:1 → 2:1 then CH₂Cl₂–MeOH, 9:1) afforded **17** (16.2 mg; 67%): $R_f = 0.40$ (CH₂Cl₂–MeOH, 2:1); [α]_D –36 (c 1.01, MeOH). δ_H (400 MHz, D₂O): 1.29 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 3.38 (3 H, s, OCH₃), 3.55–3.75 (7 H, H-2, H-3, H-4 H-6a,b Gal and H-4, H-5 Rha), 3.83 (1 H, dd, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.5 Hz, H-3 Rha), 3.89 (1 H, d, $J_{5,6a}$ 3.3 Hz, H-5 Gal), 4.16 (1 H, dd, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.3 Hz, H-2 Rha), 4.58 (1 H, d, $J_{1,2}$ 7.7 Hz, H-1 Gal), 4.68 (1 H, d, $J_{1,2}$ 1.6 Hz, H-1 Rha). δ_C (100 MHz, D₂O): 16.8 (C-6 Rha), 54.8 (OCH₃), 61.1 (C-6 β -Gal), 68.4 (C-5 Rha), 68.7 (C-5 Gal), 70.0 (C-2 Rha), 71.2 (2 C), 72.6, 75.2 (C-4 Rha, C-2, C-3, and C-4 Gal), 80.1 (C-3 Rha), 100.6 (C-1 Rha), 104.5 (C-1 β -Gal).

ESI-MS found m/z 358.1708 $[M + NH_4]^+$, calcd for $C_{13}H_{24}O_{10} \cdot NH_4$ 358.1711.

Methyl 2-O-(α -D-Galactopyranosyl)-3-O-(β -D-galactopyranosyl)- α -L-rhamnopyranoside (18). Protected trisaccharide **13** (85.1 mg; 60.8 μ mol) was treated as described for deprotection of disaccharide **11**, and then the product was dissolved in EtOH (10 mL) and hydrogenated over a catalytic amount of Pd/C for 24 h at room temperature. The mixture was then filtered through a pad of Celite, which was washed with EtOH, and the filtrate was concentrated. Purification of the residue by column chromatography (toluene–EtOAc, 4:1 \rightarrow 2:1 then CH_2Cl_2 –MeOH, 9:1) gave **18** (22.7 mg; 74%): R_f = 0.28 (CH_2Cl_2 –MeOH, 25: 1); $[\alpha]_D$ –62.5 (c 1.1, MeOH). δ_H (400 MHz, D_2O): 1.15 (3 H, d, $J_{1,2}$ 5.6 Hz, H-6 Rha), 3.24 (3 H, s, OCH₃), 3.36 (1 H, dd, $J_{1,2}$ 7.8 Hz, $J_{2,3}$ 9.9 Hz, H-2 β -Gal), 3.45–4.22 (15 H, m), 4.45 (1 H, d, $J_{1,2}$ 7.8 Hz, H-1 β -Gal), 4.65 (1 H, broad s, H-1 Rha), 4.88 (1 H, d, $J_{1,2}$ 3.5 Hz, H-1 α -Gal). δ_C (100 MHz, D_2O): 16.7 (CCH₃), 55.2 (OCH₃), 60.6 (C-6 β -Gal), 61.4 (C-6 α -Gal), 68.7, 69.1, 69.2, 69.5, 69.6, 70.8 (2 C), 71.8, 73.0, 74.9, 75.5, 78.4, 96.8 (C-1 α -Gal), 97.9 (C-1 Rha), 104.9 (C-1 β -Gal). ESI-MS found m/z 520.2233 $[M + NH_4]^+$, calcd for $C_{19}H_{34}O_{15} \cdot NH_4$ 520.2236

Methyl 4-O-(α -L-Fucopyranosyl)-2-O-(α -L-galactopyranosyl)-3-O-(β -D-galactopyranosyl)- α -L-rhamnopyranoside (2). Tetrasaccharide **16** (189.8 mg, 112.0 μ mol) was deacylated and then hydrogenated for 7 days at 30 °C as described for trisaccharide **13**. Purification of the residue by column chromatography (toluene–EtOAc, 4:1 \rightarrow 2:1, then CH_2Cl_2 –MeOH, 9:1) afforded the target compound **2** (27.4 mg, 61%): R_f = 0.21 (CH_2Cl_2 –MeOH, 1:1); $[\alpha]_D$ –5.5 (c 1.3, MeOH). δ_H (400 MHz, D_2O): 1.02 (3 H, d, $J_{5,6}$ 6.6 Hz, H-6 Fuc), 1.18 (3 H, d, $J_{5,6}$ 5.3 Hz, H-6 Rha), 3.41 (3 H, s, OCH₃), 3.52–4.26 (24 H, m), 4.73 (1 H, d, $J_{1,2}$ 7.8 Hz, H-1 β -Gal), 4.70 (1 H, s, H-1 Rha), 4.95 (1 H, s, H-1 α -Gal), 5.24 (1 H, broad s, H-1 Fuc). δ_C (100 MHz, D_2O): 15.6 (C-6 Fuc), 17.9 (C-6 Rha), 55.4 (OCH₃), 60.6, 61.4 (C-6 α -Gal and β -Gal), 67.7, 68.1, 68.2, 68.9, 69.2, 69.5, 58.7, 71.0, 71.5, 72.2, 73.3, 75.0, 75.7, 77.1, 79.9, 84.6, 97.4 (C-1 α -Gal), 98.2 (C-1 Rha), 99.4 (C-1 Fuc), 104.8 (C-1 β -Gal). ESI-MS found m/z 666.2818 $[M + NH_4]^+$, calcd for $C_{25}H_{44}O_{19} \cdot NH_4$ 666.2815.

General Method for the Oxidation of Saccharides 17, 18, and 2. In a typical experiment a solution of a saccharide (800 μ mol), TEMPO (7.5 mg, 48 μ mol), KBr (179 mg, 2.4 mmol), and deionized water (15 mL) was cooled to 0 °C, and NaOCl (5% aqueous solution, 0.72 mL, 10.6 mmol) was slowly added. After TLC showed disappearance of starting material and intermediate products (1–2 h), MeOH (3 mL) was added to quench the reaction. The mixture was concentrated, the solid residue was extracted with MeOH, and the extract was evaporated to dryness. The title compounds were obtained after purification by column chromatography (CH_2Cl_2 –MeOH–AcOH, 25:25:1).

Methyl 3-O-(β -D-Galactopyranosyluronic acid)- α -L-rhamnopyranoside (19) was prepared from disaccharide **17**

(116.7 mg), yield 88.4 mg, 73%: R_f = 0.43 (CH_2Cl_2 –MeOH, 5:1); $[\alpha]_{546}$ –9 (c 1.3, MeOH). δ_H (400 MHz, D_2O): 1.28 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 3.36 (3 H, s, OCH₃), 3.57–4.20 (8 H, m), 4.58 (1 H, d, $J_{1,2}$ 7.8 Hz, H-1 GalA), 4.69 (1 H, d, $J_{1,2}$ 1.6 Hz, H-1 Rha). δ_C (100 MHz, D_2O): 17.4 (C-6 Rha), 55.5 (OCH₃), 58.9, 59.9, 70.9, 71.3, 71.6, 73.2, 76.2, 81.2, 101.3 (C-1 Rha), 104.3 (C-1 GalA), 176.0 (C-6 GalA). ESI-MS found m/z 353.1094 $[M - H]^-$, calcd for $C_{13}H_{21}O_{11}$ 353.1089.

Methyl 2-O-(α -D-Galactopyranosyluronic acid)-3-O-(β -D-galactopyranosyluronic acid)- α -L-rhamnopyranoside (20) was prepared from trisaccharide **18** (17.6 mg), yield 12.6 mg, 64%: R_f = 0.42 (CH_2Cl_2 –MeOH–AcOH, 20:20:1); $[\alpha]_{546}$ –6 (c 1.6, MeOH). δ_H (400 MHz, D_2O): 1.17 (1 H, d, $J_{5,6}$ 5.3 Hz, H-6 Rha), 3.26 (1 H, s, OCH₃), 3.26–4.24 (12 H, m), 4.45 (1 H, d, $J_{1,2}$ 7.9 Hz, H-1 β -GalA), 4.62 (1 H, H-1 Rha), 4.89 (H-1, d, $J_{1,2}$ 3.8 Hz, α -GalA). δ_C (100 MHz, D_2O): 16.9 (C-6 Rha), 55.3 (OCH₃), 60.8, 61.5, 68.9, 69.2, 69.7, 70.9, 72.0, 73.2, 75.1, 75.6, 78.6, 97.3 (C-1 α -GalA), 98.4 (C-1 Rha), 105.4 (C-1 β -GalA), 171.8 (C-6 α -GalA and β -GalA). ESI-MS found m/z 529.0 $[M - H]^-$ 353.4 $[M - GalA]^-$, calcd for $C_{19}H_{29}O_{17}$ 529.14 ($[M - H]^-$).

Methyl 4-O-(α -L-Fucopyranosyl)-2-O-(α -L-galactopyranosyluronic acid)-3-O-(β -D-galactopyranosyluronic acid)- α -L-rhamnopyranoside (1) was prepared from tetrasaccharide **2** (23.6 mg), yield 11.6 mg, 47%: R_f = 0.07 (CH_2Cl_2 –MeOH–AcOH, 20:20:1); $[\alpha]_{546}$ –2 (c 1.06, MeOH). δ_H (600 MHz, D_2O): 1.20 (3 H, d, $J_{5,6}$ 6.6 Hz, H-6 Fuc), 1.36 (3 H, d, $J_{5,6}$ 6.1 Hz, H-6 Rha), 3.39 (3 H, s, OCH₃), 3.52–4.32 (16 H, m), 4.57 (1 H, d, $J_{1,2}$ 5.1 Hz, H-1 β -GalA), 4.67 (1 H, s, H-1 Rha), 5.11 (1 H, s, H-1 α -GalA), 5.43 (1 H, s, H-1 Fuc). δ_C (150 MHz, D_2O): 15.6 (C-6 Fuc), 17.8 (C-6 Rha), 55.2 (OCH₃), 62.9, 67.7, 68.1, 68.2, 68.5, 69.3, 69.7, 70.7, 71.2, 72.1, 73.1, 73.9, 76.9, 80.7, 82.1, 99.6 (C-1 Fuc), 99.9 (C-1 Rha), 100.8 (C-1 α -GalA), 105.2 (C-1 β -GalA), 178.9 (C-6 α -GalA and β -GalA). ESI-MS found m/z 697.2 $[M + Na - 2H]^-$, 675.7 $[M - H]^-$, 499.0 $[M - GalA]^-$, calcd for $C_{25}H_{39}O_{21}$ *m/e* 675.20 ($[M - H]^-$).

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Supporting Information Available: General experimental section and characterization data for new compounds. This material is available free of charge via the Internet at <http://pubs.acs.org>.

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