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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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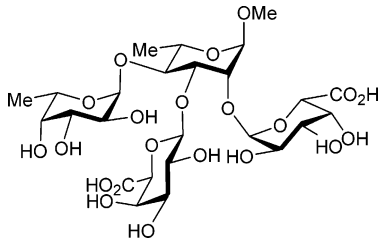

Pectic polysaccharide RG-II, a key component of plant primary cell walls, is known to exist as a dimer formed by means of borate diester cross-links between apiosyl residues of one of its constituent side-chain oligosaccharides. Described herein is the strategy for the synthesis of the branched tetrasaccharide α-D-GalA-(1→2)-[β-D-GalA-(1→3)]-[α-L-Fuc-(1→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" structure[2] 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→ 4)-linked D-Gal*p*A 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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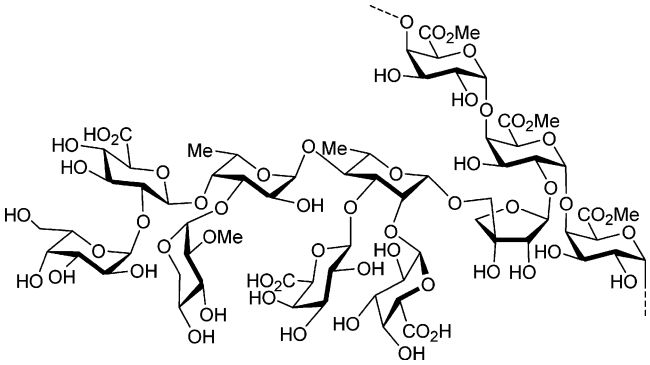


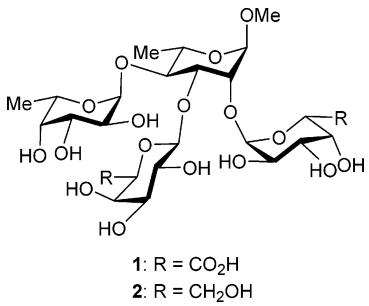
FIGURE 1. Structure of the side-chain A octasaccharide of rhamnogalacturonan-II, shown attached to a polygalacturonan backbone.

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 paper[18] we reported the preparation of a β-L-Rha*p*-(1→3′)-β-D-Api*f*-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

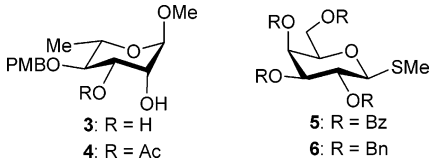


1: R = CO_2H
2: R = CH_2OH

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→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**.



3: R = H
4: R = Ac
5: R = Bz
6: R = Bn

In a recent study we demonstrated[25] 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).

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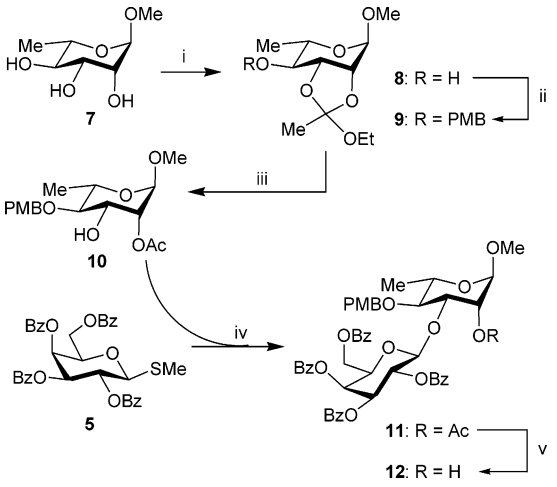
SCHEME 1[a]

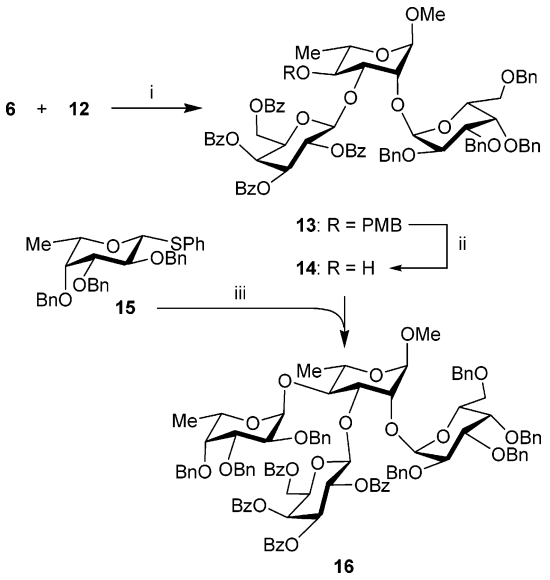


[a] Reagents and conditions: (i) $MeC(OEt)_3$, TsOH, MeCN; (ii) *p*-$MeOC_6H_4CH_2Cl$, NaH, MeCN; (iii) 80% aq AcOH, overall yield (i–iii) 70%; (iv) NIS, TfOH, MS 4 Å, CH_2Cl_2; 70%; (v) 0.4 M HCl in MeOH, 45%.

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-nitrilium 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-TfOH[29] 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 dissacharide **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 $MeCN-H_2O$[33] led to alcohol **14** in 67% yield. The latter was glycosylated with known

SCHEME 2[a]



[a] Reagents and conditions: (i) NIS, TMSOTf, CH_2Cl_2, 87%; (ii) CAN, $MeCN-H_2O$ (9:1), 93%; (iii) 2 equiv of NIS, 2 equiv of TfOH, $Et_2O-CH_2Cl_2$ (5:1), 87%.

phenyl thiofucopyranoside donor **15**[34,35] activated with NIS-TfOH in $CH_2Cl_2-Et_2O$ 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 ^{13}C spectrum showed four anomeric carbons at 97.1, 99.2, 99.5, and 100.6 ppm for α-Gal*p*, α-Rha*p*, α-Fuc*p*, and *β*-Gal*p*, respectively. Electrospray mass spectrometry confirmed the tetrasaccharide structure of **16** by the presence of the $[M + NH_4]^+$ 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 CH_2Cl_2-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 ^{13}C NMR spectroscopy and mass spectrometry. Primary OH groups of compounds **17**, **19**, and **2** were selectively oxidized to carboxylic acids using

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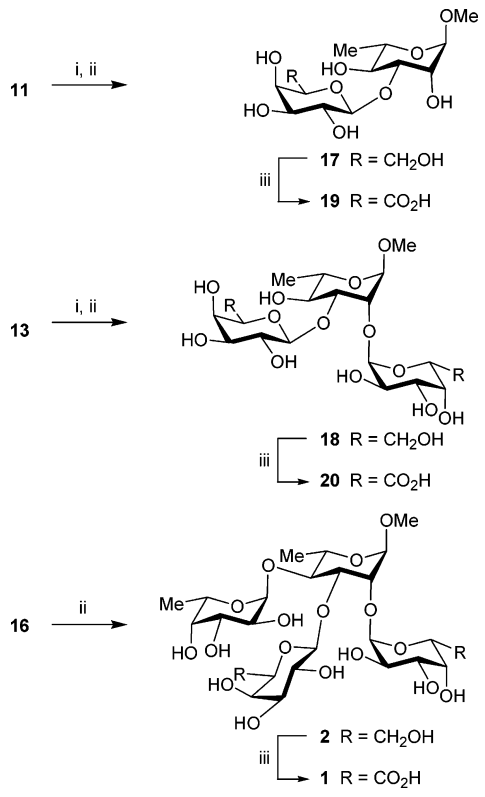
SCHEME 3[a]



[a] Reagents and conditions: (i) CAN, MeCN−H_2O (9:1); (ii) 0.05% NaOMe in MeOH then H_2, Pd/C, EtOH; 67% for **(17)**, 74% for **(19)**, 61% for **(2)**; (iii) TEMPO, NaOCl, KBr, H_2O; 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 CH_2Cl_2−MeOH−AcOH (20:20:1) as eluent.

^{13}C 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 ^{13}C NMR spectra of tetrasaccharide **1** were in a good agreement

TABLE 1. ^1H NMR and ^{13}C NMR Data for Anomeric Signals of Oligosaccharides 19, 20, and 1 in D_2O at 20 °C

compound		α-L-Rha*p*	β-D-Gal*p*A	α-D-Gal*p*A	α-L-Fuc*p*
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 ^{13}C NMR (100 MHz). [b] ^1H NMR (600 MHz) and ^{13}C NMR (150 MHz). [c] The signal overlaps with the resonance of HOD. [d] NMR data[13] for tetrasaccharide fragment of RG-II corresponding to oligosaccharide **1**.

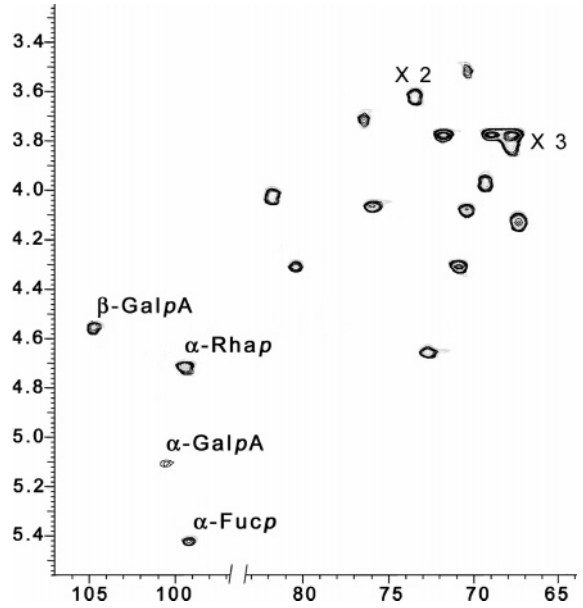


FIGURE 3. Pyranose ring protons region of the 2D-transformed data matrix from the gradient-enhanced natural abundance HSQC experiment conducted on the tetrasaccharide **1** (600 MHz, D_2O).

with corresponding signals[13] 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-

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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→3)-α-L-Rha-OMe (**19**) and α-D-GalA-(1→2)-[β-D-GalA-(1→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 shown[15] 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 CH_2Cl_2, 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 → 3:1) to afford **3** (0.22 g, 34%): $R_f = 0.10$ (toluene–EtOAc, 3:2); $[\alpha]_D$ −43 (*c* 0.9, $CHCl_3$). δ_H (400 MHz, $CDCl_3$): 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, OCH_3), 3.70 (1 H, m, H-5), 3.79 (3 H, s, C*H*$_3$OC$_6$H$_4$CH$_2$O), 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, CH$_3$OC$_6$H$_4$C*H*$_2$O), 7.25–7.29 (4 H, CH$_3$OC$_6$*H*$_4$CH$_2$O). δ_C (100 MHz, $CDCl_3$): 17.8 (C-6), 54.7 (OCH_3), 55.2 (*C*H$_3$OC$_6$H$_4$CH$_2$O), 67.0 (C-5), 70.0 (C-2), 71.4 (C-3), 74.6 (CH$_3$OC$_6$H$_4$*C*H$_2$O), 81.2 (C-4), 100.4 (C-1), 114.1, 129.7, 130.5, 159.6 (CH$_3$O*C*$_6$H$_4$CH$_2$O). ESI-MS found *m/z* 316.1755 $[M + NH_4]^+$, calcd for $C_{15}H_{22}O_6 \cdot NH_4$ 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 CH_2Cl_2 (2 mL) was added dropwise to the solution of **3** (0.17 g, 0.57 mmol) in CH_2Cl_2 (5 mL) containing pyridine (4 mL) under N_2, at 0 °C. The mixture was allowed to warm to room temperature over 2 h and diluted with CH_2Cl_2, 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); $[\alpha]_D$ −24 (*c* 0.8, $CHCl_3$). δ_H (400 MHz, $CDCl_3$): 1.32 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6), 2.16 (3 H, s, $OCOCH_3$), 2.16 (1 H, broad s, OH), 3.37 (3 H, s, OCH_3), 3.52 (1 H, dd, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.75–3.80 (m, C*H*$_3$OC$_6$H$_4$CH$_2$O 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, CH$_3$OC$_6$H$_4$C*H*$_2$O), 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, CH$_3$OC$_6$*H*$_4$CH$_2$O). δ_C (100 MHz, $CDCl_3$): 18.2 (C-6), 21.4 (*C*H$_3$CO), 55.1 (OCH_3), 55.5 (*C*H$_3$OC$_6$H$_4$CH$_2$O), 67.7 (C-5), 69.9 (C-2), 74.4 (C-3), 74.8 (CH$_3$OC$_6$H$_4$*C*H$_2$O), 78.5 (C-4), 100.6 (C-1), 114.0, 129.6, 130.4, 159.5 (CH$_3$O*C*$_6$H$_4$CH$_2$O), 170.2 (CH$_3$*C*O). ESI-MS found *m/z* 341.1595 $[M + NH_4]^+$, calcd for $C_{17}H_{24}O_7 \cdot NH_4$ 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·H_2O (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 Et_3N. 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 CH_2Cl_2, washed with H_2O and aqueous $NaHCO_3$ solution, dried, and concentrated. Column chromatography in hexanes–EtOAc (4:1 → 3:1) gave **10** (2.80 g, 77%): $R_f = 0.33$ (toluene–EtOAc, 4:1); $[\alpha]_D$ −32 (*c* 1.8, $CHCl_3$). δ_H (400 MHz, $CDCl_3$): 1.34 (3 H, d, $J_{5,6}$ 6.4 Hz, H-6), 2.15 (3 H, s, CH_3CO), 2.23 (1 H, d, $J_{3,OH}$ 4.2 Hz, OH), 3.33 (4 H, m, H-4, OCH_3), 3.66–3.72 (1 H, m, H-5), 3.79 (3 H, s, C*H*$_3$OC$_6$H$_4$CH$_2$O), 4.05 (1 H, m, H-3), 4.60 (1 H, s, H-1), 4.62–4.76 (2 H, m, CH$_3$OC$_6$H$_4$C*H*$_2$O), 5.08 (1 H, m, H-2), 6.87–7.30 (4 H, m, CH$_3$OC$_6$*H*$_4$CH$_2$O). δ_C (100 MHz, $CDCl_3$): 17.9 (C-6), 20.9 (*C*H$_3$CO), 54.8 (OCH_3), 55.2 (*C*H$_3$OC$_6$H$_4$CH$_2$O), 67.2 (C-5), 70.1 (C-3), 72.7 (C-2), 74.8 (CH$_3$OC$_6$H$_4$*C*H$_2$O), 81.3 (C-4), 98.4 (C-1), 113.9, 129.6, 130.5, 159.5 (CH$_3$O*C*$_6$H$_4$CH$_2$O), 170.9 (CH$_3$*C*O). ESI-MS found *m/z* 358.1860 $[M + NH_4]^+$, calcd for $C_{17}H_{24}O_7 \cdot NH_4$ 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 CH_2Cl_2 (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 CH_2Cl_2–Et_2O (1:1, 13.5 mL) was added to the mixture, and stirring was continued for 1 h. The mixture was diluted with CH_2Cl_2, washed with 10% aqueous $Na_2S_2O_3$ solution until decolorized, then washed successively with water, aqueous $NaHCO_3$ 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); $[\alpha]_D$ +43 (*c* 1.5, $CHCl_3$). δ_H (400 MHz, $CDCl_3$): 1.20 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 2.22 (3 H, s, CH_3CO), 3.27 (3 H, s, OCH_3), 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, C*H*$_3$OC$_6$H$_4$CH$_2$O), 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, CH$_3$OC$_6$H$_4$C*H*$_2$O), 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, CH$_3$OC$_6$H$_4$C*H*$_2$O), 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, $CDCl_3$): 18.1 (C-6 Rha), 21.3 (*C*H$_3$CO), 55.0 (OCH_3), 55.4 (*C*H$_3$OC$_6$H$_4$CH$_2$O), 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 (CH$_3$OC$_6$H$_4$*C*H$_2$O), 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 (C$_6$H$_5$*C*O), 170.4 (O*C*OCH$_3$). ESI-MS found *m/z* 336.3436 $[M + NH_4]^+$, calcd for $C_{51}H_{50}O_{16} \cdot NH_4$ 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 Et_3N and concentrated to dryness. Column chromatography (toluene–EtOAc, 8:1) afforded **12** (0.36 g, 45%): $R_f = 0.17$ (toluene–EtOAc, 8:1); $[\alpha]_D$ +77 (*c* 1.0, $CHCl_3$). δ_H (400 MHz, $CDCl_3$): 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, OCH_3), 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, $CH_3OC_6H_4CH_2O$), 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, $CH_3OC_6H_4CH_2O$), 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, $CH_3OC_6H_4CH_2O$), 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, $CDCl_3$): 18.1 (C-6 Rha), 54.8 (OCH_3), 55.4 ($CH_3OC_6H_4CH_2O$), 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 ($CH_3OC_6H_4CH_2O$), 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 (Ph*C*O). ESI-MS found *m/z* 894.3338 $[M + NH_4]^+$, calcd for $C_{49}H_{48}O_{15}{\cdot}NH_4$ 894.3331.

Methyl 4-*O*-(4-Methoxylbenzyl)-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 **6**[24] (0.47 g, 0.82 mmol) in 15 mL of dry $CH_2Cl_2-Et_2O$ (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 Et_3N (0.27 mL), then allowed to warm to room temperature, and diluted with CH_2Cl_2. The organic solution was washed with 10% aqueous $Na_2S_2O_3$ solution, water, aqueous $NaHCO_3$ 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); $[\alpha]_D$ +77 (*c* 0.8, $CHCl_3$). δ_H (400 MHz, $CDCl_3$): 1.10 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 3.12 (3 H, s, OCH_3), 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, $CH_3OC_6H_4CH_2$), 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, PhCH_2, 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, PhCH_2), 5.04 (1 H, d, J 10.7 Hz, PhCH_2), 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, $CDCl_3$): 17.8 (C-6 Rha), 54.8 (OCH_3), 55.4 ($CH_3OC_6H_4CH_2O$), 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 (PhCH_2), 74.5 ($CH_3OC_6H_4CH_2O$), 75.5 (PhCH_2), 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 (C_6H_5CO). ESI-MS found *m/z* 1416.8 $[M + NH_4]^+$, calcd for $C_{83}H_{82}O_{20}{\cdot}NH_4$ 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 $(NH_4)_2Ce(NO_3)_6$ (0.58 g, 1.06 mmol) in CH_3CN-H_2O (9:1, 3 mL) was stirred at room temperature for 0.5 h, diluted with CH_2Cl_2, washed with water, aqeous $NaHCO_3$ 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); $[\alpha]_D$ +74 (*c* 2.7, $CHCl_3$). δ_H (400 MHz, $CDCl_3$): 1.15 (3 H, d, $J_{5,6}$ 5.5 Hz, H-6 Rha), 3.14 (3 H, s, OCH_3), 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, PhCH_2), 4.72 (1 H, d, $J_{1,2}$ 1.3 Hz, H-1 Rha), 4.80 (2 H, m, PhCH_2), 5.02 (1 H, d, J 10.9 Hz, PhCH_2), 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, $CDCl_3$): 17.8 (C-6 Rha), 54.6 (OCH_3), 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 (PhCH_2), 75.0 (PhCH_2), 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 (Ph*C*O). ESI-MS found *m/z* 1296.5 $[M + NH_4]^+$, calcd for $C_{75}H_{74}O_{19}{\cdot}NH_4$ 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 $CH_2Cl_2-Et_2O$ (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 CH_2Cl_2, washed with water, aqueous $NaHCO_3$ 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); $[\alpha]_D$ +42 (*c* 1.02, $CHCl_3$). δ_H (400 MHz, $CHCl_3$): 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, OCH_3), 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, PhCH_2), 4.46−4.84 (12 H, m, H-1 Rha and 11 H PhCH_2), 4.88 (1 H, d, J 10.6 Hz, PhCH_2), 4.91 (1 H, d, $J_{1,2}$ 8 Hz, H-1 *β*-Gal), 5.00 (1 H, d, J 10.6 Hz, PhCH_2), 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, $CHCl_3$): 16.6 (C-6 Fuc), 18.8 (C-6 Rha), 54.5 (OCH_3), 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 (PhCH_2), 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 (C_6H_5CO). ESI-MS found *m/z* 1712.8 $[M + NH_4]^+$, calcd for $C_{102}H_{102}O_{23}{\cdot}NH_4$ 1712.7

Methyl 3-*O*-(*β*-D-Galactopyranosyl)-α-L-rhamnopyranoside (17). Protected disaccharide **11** (23.2 mg, 68.1 *μ*mol) and $(NH_4)_2Ce(NO_3)_6$ (3.61 mmol) were stirred in a mixture of CH_3CN-H_2O (9:1, 5 mL) for 0.5 h at room temperature. The mixture was diluted with CH_2Cl_2, washed with water, aqueous $NaHCO_3$ 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 CH_2Cl_2-MeOH, 9:1) afforded **17** (16.2 mg; 67%): R_f = 0.40 (CH_2Cl_2–MeOH, 2:1); $[\alpha]_D$ −36 (*c* 1.01, MeOH). δ_H (400 MHz, D_2O): 1.29 (3 H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 3.38 (3 H, s, OCH_3), 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, D_2O): 16.8 (C-6 Rha), 54.8 (OCH_3), 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 μmol) 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 → 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, OCH_3), 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 (CCH_3), 55.2 (OCH_3), 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 μmol) 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 → 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, OCH_3), 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 (OCH_3), 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 μmol), TEMPO (7.5 mg, 48 μmol), 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, OCH_3), 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 (OCH_3), 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, OCH_3), 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 (OCH_3), 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, OCH_3), 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 (OCH_3), 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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