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Synthesis of two repeat units corresponding to the backbone of the pectic polysaccharide rhamnogalacturonan I

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Abstract—A tetrasaccharide corresponding to a sequence of the rhamnogalacturonan I backbone has been synthesized. This synthesis relies on only two protected monosaccharides and proceeds through a common disaccharide intermediate. Synthesis of this tetrasaccharide has been designed to allow for the addition of branching elements at the 4-positions of the rhamnosyl units, or further chain elongation at the 2-position.

Keywords: Rhamnogalacturonan I; Galacturonic acid; *cis*-Glycoside

1. Introduction

Molecular mechanisms in plant glycobiology are often studied using immunofluorescent labeling techniques, requiring the use of monoclonal antibodies.[1–3] The binding specificity of the antibody is most highly defined when the antibody has been raised against a well-characterized epitope. Chemical synthesis is thus necessary to ensure that the binding specificities of the monoclonal antibodies that are generated are unambiguous.[4,5]

Rhamnogalacturonan I (RG-I) is a complex polysaccharide found in plant tissues. The backbone of RG-I consists of alternating repeat units of L-rhamnose and D-galacturonic acid: [-α-L-Rha*p*-(1→4)-α-D-GalA*p*-(1→2)-]. RG-I is a highly substituted polysaccharide, with a high degree of substitution occurring at the C-4 position of the rhamnosyl residue,[6] forming branched chains. The chains consist mainly of galactose and arabinose,[7] although substitution with glucuronic acid occasionally occurs at O-3 of the galacturonic acid residue.

Many of the structural features of RG-I have been identified, but little is known about how its structure relates to its function.[8] Currently, immunofluorescent studies of RG-I investigating the role of RG-I in plant tissue are usually limited to using antibodies that have been raised against the galactan and arabinan side chains (LM5[3] and LM6[9] antibodies, respectively). No antibody is currently in use that demonstrates specific binding to the RG-I backbone.

There are a few reports in the literature of chemical synthesis of oligosaccharide fragments found within the RG-I polysaccharide.[10–12] We report here the synthesis and characterization of a versatile tetrasaccharide corresponding to two repeat units of the RG-I backbone. A block synthesis approach has been used which allows for the coupling of two disaccharide units derived from a single disaccharide to form the desired tetrasaccharide **14**. The 4-position of the rhamnosyl residue has been orthogonally protected to allow for the possible substitution of RG-I side chains.

Pozsgay mentions the difficulties of late stage oxidation in terms of achieving yields that are often not satisfactory.[13] Indeed, difficulties were experienced in our lab with initial attempts at building an RG-I tetrasaccharide using galactose, and thus a new synthetic strategy was devised based on commercially available D-galacturonic acid as one of the monosaccharide building blocks (compounds **2** and **3**); this avoided the difficulties of late stage

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oxidation at C-6 of galactose to galacturonic acid. The resulting end product was easily isolated both in the methyl ester form **16** and as the free carboxylic acid **17**.

2. Results and discussion

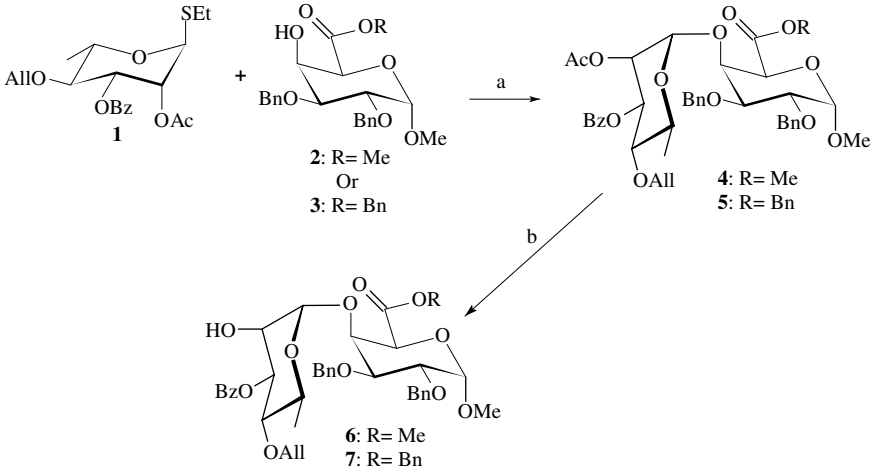
This synthesis utilizes two protected monosaccharide building blocks, an α-L-rhamnose derivative **1** and an α-D-galacturonic acid derivative **2**. Synthesis of monosaccharide **1** had been previously published.[10] Monosaccharide **2** was prepared from methyl (methyl 2-*O*-benzyl-α-D-galactopyranosid)uronate[14] by selective benzylation at C-3. Addition of the benzyl group at C-3 was performed by stannylene acetal formation under the conditions similar to those cited by Nolting and co-workers.[15] In addition to benzylation at C-3 (compound **2**), benzylation was also observed at the C-6 position to give compound **3**. Production of this dibenzylated compound could not be avoided, so we decided to carry both monosaccharides forward to the disaccharide stage in parallel syntheses. The strategy was to transform both these disaccharides into glycosyl donors that could be then used to prepare a tetrasaccharide. This would allow us to investigate the differences in donor reactivity due to the protection of the carboxylic acid as either the benzyl ester or the methyl ester.

Glycosylation of **2** or **3** using **1**[10] with catalytic amounts of triflic acid afforded disaccharides **4** and **5** in yields of 78% and 80%, respectively (see Scheme 1). NMR spectroscopy was used to determine whether the newly formed glycosidic linkages for **4** and **5** were α or β. The one-bond ^{13}C–^{1}H coupling constants ($^{1}J_{13C,1H}$) for **4** and **5** at C-1 of the rhamnosyl residues, as determined by analysis of the HMQC (inverse) spectra, were found to be 173.7 Hz and 173.3 Hz, respectively, indicative of an α-linkage.[16]

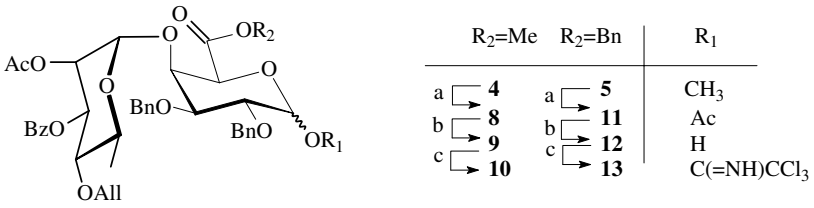
In principle, both **4** and **5** can lead to either disaccharide acceptors (removal of the 2-*O*-acetate group) or disaccharide donors (removal of the anomeric methyl groups and subsequent activation); this flexibility provides considerable synthetic economy. Removal of the 2-*O*-acetate on the rhamnosyl portion of disaccharide **4** was accomplished by using methanolic HCl[17] to give acceptor **6** in a yield of 80%. We expected that reaction of **5** under these conditions would result in the exchange of the benzyl group as well as the loss of the 2-*O*-acetate. Indeed, when **5** was let stand for an extended period (77 h), a mixture of products was formed (**4**, 7%; **5**, 5%; **6**, 44%, and **7**, 36%). We did not investigate this avenue any further, although it is possible that even longer reaction times would produce an even larger amount of **6**, providing a viable route to convert the benzyl ester **3** into usable disaccharide product **6**.

With disaccharides **4** and **5** in hand, we wished to determine which of these would serve as the most effective donor in subsequent glycosylation reactions. Accordingly, both **4** and **5** were, in three steps, transformed into their glycosyl imidates **10** and **13**, respectively (see Scheme 2). Acetolysis of **4** and **5** afforded the anomeric acetates **8** and **9** in 81% and 91%, respectively.[18] The reaction to form **8** was complete after about 2 h, and further reaction times lead to no change in yield. Conversely, if the acetolysis reaction to form **9** was left longer than about 2 h, a decrease in the yield was observed; after 4–5 h the yields had dropped to about 25%.

Removal of the anomeric acetates from **8** and **9** using hydrazine acetate[19] did not proceed smoothly, with yields in the 50% range. This difficulty was overcome by using a procedure provided by Kartha et al. where powdered 4 Å molecular sieves were added to methanolic solutions of anomeric acetates.[20] Accordingly, anomeric acetates **8** and **9** were dissolved in methanol



Scheme 1. Synthesis of the disaccharides and glycosyl acceptors. Reagents and conditions: (a) *N*-iodosuccinimide, triflic acid (cat.), in CH_2Cl_2 −40 °C (78% **4** and 80% **5**); (b) methanolic HCl in CH_2Cl_2 (80% **6** and 37% **7**).

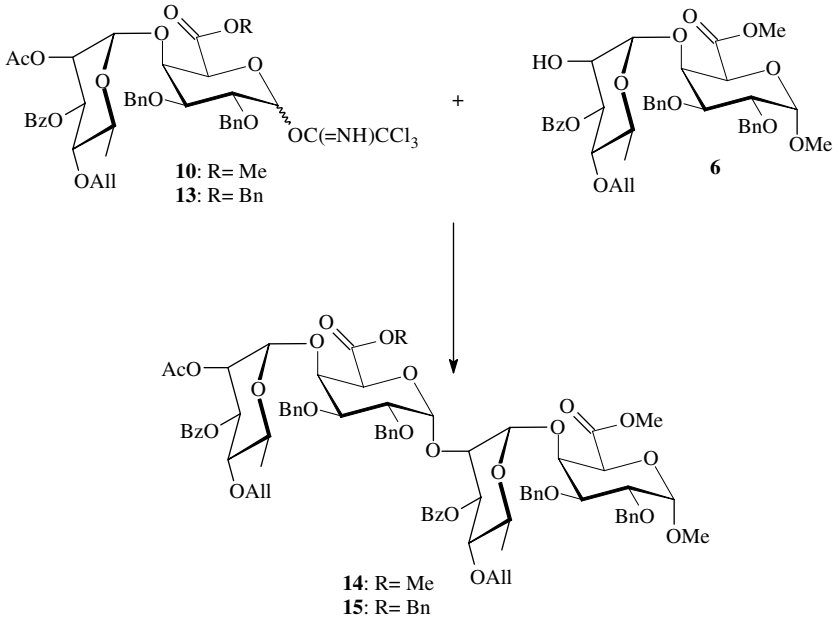


Scheme 2. Synthesis of disaccharide glycosyl donors. Reagents and conditions: (a) 1% H_2SO_4 in AcOH and Ac_2O (81% **8** and 91% **11**); (b) powdered molecular sieves in CH_2Cl_2/MeOH (89% **9** and 76% **12**); (c) trichloroacetonitrile, DBU, in CH_2Cl_2 (81% **10** and 82% **13**).

and powdered 4 Å molecular sieves were added to the solution. The solutions were let stand at room temperature and the reactions proceeded slowly, but cleanly to provide hemiacetals **10** and **11** in 81% and 82%, respectively. Transformation of hemiacetals **9** and **12** into the corresponding imidates **10** and **13** was accomplished by the treatment with trichloroacetonitrile and DBU.[21] The methyl ester **10** was isolated as the α-anomer, whereas the benzyl ester **13** was isolated as an anomeric mixture.

Glycosylation of the disaccharide acceptor **6** to form the tetrasaccharide structure proved to be problematic. Glycosylation of **6**, using silver triflate (AgOTf) as promoter, with either the methyl ester **10** or the benzyl ester **13** imidates as donor, led to formation of the desired tetrasaccharide product (see Scheme 3); however only low yields were obtained (39% for **14** and an impure sample for **15**). The reaction mixture containing **15** was purified by chromatography multiple times using various eluting solvents in an attempt to isolate pure tetrasaccharide but no attempts were successful. Although the ^1H, ^{13}C, and COSY spectra could be clearly assigned for **15**, the sample failed both elemental and MS analyses, and is thus not included here. The low yield for tetrasaccharide **14** is partially a result of multiple chromatographic purification steps required to separate the desired product from unreacted glycosyl acceptor **6**. No glycosylation attempts, despite the addition of more equivalents of donor and promoter, were successful in eliminating the presence of unreacted acceptor **6**.

A number of methods were tried in an effort to increase the conversion of glycosyl acceptor into tetrasaccharide product. Activation of the imidate donors **10** and **13** using trimethylsilyl trifluoromethanesulfonate (TMSOTf)[22] or *tert*-butyldimethylsilyl trifluoromethanesulfonate (*t*-BDMSOTf)[23] both proved unsuccessful. Attempts to generate thioglycoside donors from the 1-*O*-acetate **8** using *p*-thiocresol and either boron trifluoride etherate ($BF_3{\cdot}Et_2O$) or tin(IV) chloride ($SnCl_4$) did not result in the formation of significant amounts of thioglycoside. Attempts were also made to generate an α-bromide donor in situ from hemiacetal **12** using triphenyl phosphine ($P(Ph)_3$) and carbon tetrabromide (CBr_4),[24,25] followed by the addition of the disaccharide



Scheme 3. Synthesis of the fully protected tetrasaccharides. Reagents and conditions: AgOTf, molecular sieves in CH_2Cl_2 (39% **14** and undetermined yield **13**).

acceptor **6**; TLC analysis indicated that no reaction had taken place.

This particular glycosylation is one that could benefit by the use of solid support during solution phase synthesis to circumvent some of the purification difficulties in obtaining both tetrasaccharides **14** and **15**.[26]

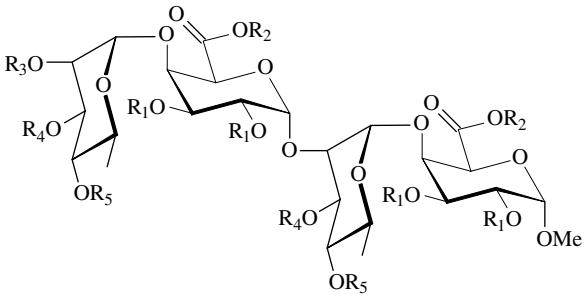
Complete NMR analysis was performed on tetrasaccharide **14**. Hydrogen and carbon assignments were performed through a combined analysis of ^{1}H NMR, ^{13}C NMR, COSY, and HMQC (inverse) spectra. All signals corresponding to the ring protons and carbons could be successfully accounted for, as well as all protecting groups expected to be present.

Four signals corresponding to C-1 of each of the four monosaccharide residues could be seen in the 95 ppm to 100 ppm region of the ^{13}C spectrum. HMQC (inverse) analysis was used to determine the one-bond ^{13}C–^{1}H coupling constants ($^{1}J_{13C,1H}$) for C-1″ to H-1″ on the donor galacturonic acid residue to verify whether or not the newly formed linkage was indeed in the desired α-configuration. The observed coupling constant fell within the expected range[16] at 171.5 Hz, indicating that the α-linkage had been successfully formed.

A fully deprotected sample of tetrasaccharide was obtained from **14** in four steps (see Scheme 4). The allyl groups were removed by treatment with Wilkinson's catalyst ($[(C_6H_5)_3P]_3RhCl$), followed by the cleavage of the resulting vinyl ether using a combination of mercury(II) oxide and mercury(II) chloride.[27] The yellow-brown foam was treated with sodium methoxide to remove the benzoate and the acetate groups. The crude material was purified by chromatography before being submitted to hydrogenation conditions.[28] Removal of the benzyl groups present on the partially deprotected tetrasaccharide was successfully accomplished by placing the purified compound under $H_{2(g)}$ (40 psi) in the presence of a palladium acetate catalyst, giving **16** (33% yield). ^{1}H NMR, ^{13}C NMR, HMQC (inverse), and COSY analyses were performed on compound**16**; the spectra thus obtained were consistent with the proposed structure.

Tetrasaccharide **16** was dissolved in H_2O and saponified by the addition of 1.0 M NaOH until pH 11–12, and then acidified using 1.0 M HCl until pH 2–3. The product was placed on a P2 gel column to remove any salts present after saponification. The purified fractions were pooled and lyophilized, producing the final target compound **17** as a white solid (77% yield).

This synthesis has been planned to allow the fully protected tetrasaccharide to be further elongated by the removal of the 2-*O*-acetyl group of the non-reducing terminus and thus generating a glycosyl acceptor, or alternatively, removal of the anomeric methyl group and the generation of a glycosyl donor. In addition, the removal of the 4-*O*-allyl groups from the rhamnosyl residues allows for the addition of branching monosaccharide residues leading to branched segments found within the RG-I polysaccharide structure.



		R_1	R_2	R_3	R_4	R_5
a, b, c →	**14**	Bn	CH_3	Ac	Bz	All
	16	H	CH_3	H	H	H
d →	**17**	H	H	H	H	H

Scheme 4. Deprotection of tetrasaccharide **14**. Reagents and conditions: (a) (i) $[(C_6H_5)_3P]_3RhCl$, DABCO in EtOH/toluene/H_2O; (ii) HgO, $HgCl_2$ in acetone/H_2O; (b) NaOMe in MeOH (pH 11–12) (c) H_2 (40 psi) over $Pd(OAc)_2$; (d) (i) $NaOH_{aq}$ (pH 11–12); (ii) HCl_{aq} (pH 2–3).

3. Experimental

3.1. General methods

^{1}H NMR (300.13 MHz) and ^{13}C NMR (75.03 MHz) spectra were compiled with a Bruker AMX 300 spectrometer. Chemical shifts are relative to external tetramethyl silane (TMS). Electrospray ionization mass spectra were measured on Water/Micromass LCT. The samples were dissolved in MeOH. The solution concentration was about 25–50 μM. They were infused to LCT at a flow rate of 20 μL/min. Optical rotations were measured on an Autopol III polarimeter. Melting points were performed on an Electrothermal Digital Melting Point Apparatus (IA9100). All reactions were monitored by thin-layer chromatography (TLC) using silica gel on an aluminum support (Silicycle 250 μm, F-254 indicator), with detection by charring with 5% sulfuric acid (v/v) in EtOH. TLCs were performed with solvents of appropriately adjusted polarity consisting of A, 1:1 hexane–EtOAc; B, 3:2 hexanes–EtOAc; C, 2:1 hexanes–EtOAc; D, 3:1 hexanes–EtOAc; E, 2:1:1 hexanes–EtOAc–$CHCl_3$; F, 4:1 hexanes–EtOAc; G, 8:1 toluene–acetone; H, 3:1:1 hexanes–EtOAc–$CHCl_3$; I, 2:1:1 hexanes–EtOAc–CH_2Cl_2; J, 4:1:1 hexanes–EtOAc–CH_2Cl_2; K, 4:3:3 hexanes–EtOAc–$CHCl_3$; L, 6:6:1 hexanes–EtOAc–MeOH; M, 12:2:1 EtOAc– hexanes–MeOH; N, 16:2:1 EtOAc–hexanes–MeOH; O, 10:3:1 EtOAc–MeOH–AcOH; P, 20:20:1 EtOAc–MeOH–AcOH; Q, 7:3 CH_3CN–H_2O. All chromatography was

performed using low pressure liquid chromatography columns packed with silica gel (Silicycle 230–400 mesh (40–63 μm) 60 Å).

3.2. Methyl (methyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate (2) and benzyl (methyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate (3)

Methyl (methyl 2-*O*-benzyl-α-D-galactopyranosid)uronate[14] (6.276 g, 20.08 mmol), Bu_2SnO (6.000 g, 24.10 mmol), and toluene (240 mL) were heated at reflux for 5 h 40 min; water was removed by 4 Å molecular sieves placed in a Soxhlet extractor. The reaction mixture was then heated in a 70 °C oil bath and Bu_4NBr (7.8 g, 24 mmol) was added, followed by BnBr (5.9 mL). The reaction was monitored by TLC (solvent A) and stopped after 6.5 h and allowed to cool to rt; MeOH was added and the mixture was evaporated to dryness. The resulting syrup was purified by chromatography (eluting solvent B) to give **2** (5.376 g, 67%) and **3** (2.499 g, 26%). Anal. for **2**: $[\alpha]_D^{22}$ +33.3 (*c* 0.6, $CHCl_3$), lit.[29] $[\alpha]_D^{25}$ +33.3; ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 7.41–7.26 (10H, aromatic), 4.83, 4.66 (2H, OCH_2Ph), 4.81, 4.74 (2H, OCH_2Ph), 4.76 (d, 1H, $J_{2,1} = 3.3$ Hz, H-1), 4.39 (2H, H-4, H-5), 3.95 (dd, 1H, $J_{3,4} = 3.1$ Hz, H-3), 3.89 (dd, 1H, $J_{2,3} = 9.8$ Hz, H-2), 3.82 (s, 3H, CO_2CH_3), 3.41 (s, 3H, OCH_3). Anal. Calcd for $C_{22}H_{26}O_7$: C, 65.66; H, 6.51. Found: C, 65.24; H, 6.40.

Anal. for **3**: mp 101.2–102.0 °C; $[\alpha]_D^{22}$ +24.4 (*c* 0.6, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 7.45–7.26 (15H, aromatic), 5.27 (2H, CO_2CH_2Ph), 4.83, 4.66 (2H, OCH_2Ph), 4.82, 4.72 (2H, OCH_2Ph), 4.77 (d, 1H, $J_{1,2} = 3.2$ Hz, H-1), 4.41 (1H, H-5), 4.40–4.36 (1H, H-4), 3.95 (dd, 1H, $J_{3,4} = 3.0$ Hz, H-3), 3.90 (dd, 1H, $J_{2,3} = 9.8$ Hz, H-2), 3.83 (s, 3H, CO_2CH_3), 3.38 (s, 3H, OCH_3). Anal. Calcd for $C_{28}H_{30}O_7$: C, 70.28; H, 6.32. Found: C, 69.98; H, 6.50.

3.3. Methyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate (4)

A sample of compound **2** (0.356 g, 0.885 mmol) was dissolved in freshly distilled CH_2Cl_2 (15.8 mL); 3 Å molecular sieves were added, and the solution was stirred under $N_{2(g)}$ in a pyr/$N_{2(l)}$ bath for 10 min (the pyr/$N_{2(l)}$ bath (approximately −35 °C to −40 °C) was maintained for the duration of the reaction). The thioglycoside donor **1**[10] (0.489 g, 1.24 mmol, 1.4 equiv to acceptor) was then added and the mixture was stirred for an additional 10 min. NIS (0.298 g, 1.32 mmol, 1.06 equiv to donor) was added and the mixture was stirred for another 10 min followed by the addition of 49 μL of TfOH (0.554 mmol, 0.45 equiv to donor). The reaction was monitored by TLC (solvent C) and halted after 15 min by neutralization using Et_3N. The reaction mixture was washed sequentially with satd $NaHCO_3$ and 10% (w/v) $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4, filtered, evaporated to dryness, and purified by column chromatography (eluting solvent C), giving **4** (0.504 g, 78%). $[\alpha]_D^{22}$ +3.3 (*c* 0.6, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 8.05–7.95 (m, 2H, aromatic), 7.61–7.24 (13H, aromatic), 5.78 (1H, OCH_2-CH=CH_2), 5.61 (dd, 1H, $J_{2,3} = 3.1$ Hz, H-2 Rha), 5.49 (dd, 1H, $J_{3,4} = 9.4$ Hz, H-3 Rha), 5.20–5.04 (2H, OCH_2CH=CH_2), 5.14 (d, $J_{1,2} = 2.2$ Hz, H-1 Rha), 4.90, 4.70 (2H, OCH_2Ph), 4.84, 4.72 (2H, OCH_2Ph), 4.71 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1 GalA), 4.44 (1H, H-4 GalA), 4.39 (1-H, H-5 GalA), 4.12 (2H, OCH_2CH=CH_2), 4.09 (dd, 1H, $J_{2,3} = 10.0$ Hz, H-2 GalA), 3.95 (dd, 1H, $J_{3,4} = 2.7$ Hz, H-3 GalA), 3.83 (s, 3H, CO_2CH_3), 3.79 (dd, 1H, $J_{5,6} = 6.2$ Hz, H-5 Rha), 3.48 (dd, 1H, $J_{4,5} = 9.5$ Hz, H-4 Rha), 3.39 (s, 3H, OCH_3), 2.00 (s, 3H, $OCOCH_3$), 1.35 (3H, H-6 Rha); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 169.5, 169.1 (*C*=O), 165.2 (*C*=O, benzoate), 138.4, 133.1, 130.4, 129.7, 128.8, 128.6, 128.5, 128.1, 127.8, 127.7 (C aromatic), 134.9 (OCH_2CH=CH_2), 116.8 (OCH_2-CH=CH_2), 99.6 (C-1 GalA, $^1J_{13C,1H} = 170.8$ Hz), 99.4 (C-1 Rha, $^1J_{13C,1H} = 173.7$ Hz), 78.5 (C-4 Rha), 77.5 (C-3 GalA), 76.9 (C-4 GalA), 75.8 (C-2 GalA), 74.5 (CH_2Ph), 73.6 (OCH_2CH=CH_2, CH_2Ph), 72.0 (C-3 Rha), 70.5 (C-2 Rha), 70.3 (C-5, GalA), 68.4 (C-5 Rha), 56.3 (OCH_3), 52.7 (CO_2CH_3), 21.0 ($OCOCH_3$), 18.2 (C-6 Rha); ESI-MS found *m*/*z* 757.2839 $[M+Na]^+$. Calcd for $C_{40}H_{46}O_{13}Na$: 757.2836.

3.4. Benzyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate (5)

A sample of compound **3** (0.432 g, 0.903 mmol) was dissolved in freshly distilled CH_2Cl_2 (17.0 mL); 3 Å molecular sieves were added, and the solution was stirred under $N_{2(g)}$ in a pyr/$N_{2(l)}$ bath for 10 min (the pyr/$N_{2(l)}$ bath (approximately −35 °C to −40 °C) was maintained for the duration of the reaction). The thioglycoside donor **1**[10] (0.499 g, 1.26 mmol, 1.4 equiv to acceptor) was then added and the mixture was stirred for an additional 10 min, followed by the addition of NIS (0.304 g, 1.35 mmol, 1.07 equiv to donor) and stirring for another 10 min 50 μL of TfOH (0.565 mmol, 0.45 equiv to donor) was added and the progress of the reaction was followed by TLC (solvent C). The reaction mixture turned a deep purple almost immediately and was neutralized thereafter with Et_3N, washed sequentially with satd $NaHCO_3$, and 10% (w/v) $Na_2S_2O_3$. The organic layer was dried over Na_2SO_4, filtered, evaporated to dryness, and purified by column chromatography (eluting solvent D), giving **5** (0.586 g, 80%). $[\alpha]_D^{22}$ +0.4 (*c* 0.5, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 8.06–7.97 (2H, aromatic),

7.63–7.19 (18H, aromatic), 5.80 (1H, $OCH_2CH{=}CH_2$), 5.65 (dd, 1H, $J_{2,3} = 3.3$ Hz, H-2 Rha), 5.54 (dd, 1H, $J_{3,4} = 9.5$ Hz, H-3 Rha), 5.32, 5.14 (2H, CO_2CH_2Ph), 5.13 (2H, $OCH_2CH{=}CH_2$), 5.10 (d, 1H, $J_{1,2} = 2.1$ Hz, H-1 Rha), 4.91, 4.74 (2H, OCH_2Ph), 4.84, 4.68 (2H, OCH_2Ph), 4.72 (d, 1H, $J_{1,2} = 3.5$ Hz, H-1 GalA), 4.45–4.39 (2H, H-4, H-5, GalA), 4.23–4.06 (2H, $OCH_2CH{=}CH_2$), 4.23–4.06 (1H, H-2, GalA), 3.98–3.88 (1H, $J_{3,4} = 2.8$ Hz, H-3 GalA), 3.98–3.88 (1H, $J_{6,5} = 6.2$ Hz, H-5 Rha), 3.50 (dd, 1H, $J_{4,5} = 9.5$ Hz, H-4 Rha), 3.38 (s, 3H, OCH_3), 2.01 (s, 3H, $OCOCH_3$), 1.35 (3H, H-6 Rha); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 169.5, 168.3 (*C*=O), 165.2 (*C*=O, benzoate), 138.5, 135.2, 133.1, 130.5, 129.7, 128.8, 128.7, 128.6, 128.5, 128.1, 127.9, 127.7 (C aromatic), 134.9 ($OCH_2CH{=}CH_2$), 116.8 ($OCH_2CH{=}CH_2$), 99.7 (C-1 Rha, $^1J_{13C,1H} = 173.3$ Hz), 99.6 (C-1 GalA, $^1J_{13C,1H} = 172.5$ Hz), 78.6 (C-4 Rha), 77.7 (C-4 GalA), 77.5 (C-3 GalA), 75.9 (C-2 GalA), 74.6, 73.7 (CH_2Ph), 73.6 ($OCH_2CH{=}CH_2$), 72.1 (C-3 Rha), 70.6 (C-2 Rha), 70.2 (C-5, GalA), 68.6 (C-5 Rha), 67.5 (CO_2CH_2Ph), 56.3 (OCH_3), 21.0 ($OCOCH_3$), 18.4 (C-6 Rha); ESI-MS found *m*/*z* 833.3148 $[M+Na]^+$. Calcd for $C_{40}H_{46}O_{13}Na$: 833.3149.

3.5. Methyl (4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate (6)

A sample of compound **4** (0.382 g, 0.520 mmol) was dissolved in freshly distilled CH_2Cl_2 (8.0 mL), to which methanolic HCl (8.0 mL + 1.5 mL after approximately 49 h; prepared by the addition of 1.0 mL of acetyl chloride to 35 mL distilled MeOH) was added. The reaction mixture was allowed to sit at rt for approximately 4 days, after which the reaction mixture was diluted with CH_2Cl_2, washed with satd $NaHCO_3$, filtered, dried over Na_2SO_4, and evaporated to dryness (TLC solvent A). The resulting syrup was purified by chromatography (eluting solvent C) to give pure **6** (0.287 g, 80%). $[\alpha]_D^{22}$ +13 (*c* 0.5, $CHCl_3$);^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 8.13–8.06 (m, 2H, aromatic), 7.64–7.21 (13H, aromatic), 5.80 (1H, $OCH_2CH{=}CH_2$), 5.49 (dd, 1H, $J_{3,4} = 8.2$ Hz, H-3 Rha), 5.13 (2H, $OCH_2CH{=}CH_2$), 5.13 (d, 1H, $J_{1,2} = 2.8$ Hz, H-1 Rha), 4.86, 4.69 (2H, OCH_2Ph), 4.84, 4.71 (2H, OCH_2Ph), 4.75 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1 GalA), 4.48–4.44 (1H, H-4, GalA), 4.43–4.39 (1H, H-5, GalA), 4.32 (dd, 1H, $J_{3,2} = 3.1$ Hz, H-2 Rha), 4.24–4.05 (2H, $OCH_2CH{=}CH_2$), 4.05 (dd, 1H, $J_{2,3} = 10.1$ Hz, H-2 GalA), 3.97 (dd, 1H, $J_{3,4} = 2.7$ Hz, H-3 GalA), 3.84 (s, 3H, CO_2CH_3), 3.77 (dd, 1H, $J_{5,6} = 6.2$ Hz, H-5 Rha), 3.51 (dd, 1H, $J_{4,5} = 9.3$ Hz, H-4 Rha), 3.40 (s, 3H, OCH_3), 1.33 (3H, H-6 Rha); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 168.9 (CO_2CH_3), 165.5 (*C*=O, benzoate), 138.4, 138.2, 133.3, 130.4, 129.9, 128.7, 128.1, 128.0, 127.9 (C aromatic), 134.8 ($OCH_2CH{=}CH_2$), 117.0 ($OCH_2CH{=}CH_2$), 102.0 (C-1 Rha, $^1J_{13C,1H} = 170.6$ Hz), 99.4 (C-1 GalA, $^1J_{13C,1H} = 170.6$ Hz), 78.9 (C-4 Rha), 77.7 (C-3 GalA), 77.1 (C-4 GalA), 75.9 (C-2 GalA), 74.3 (CH_2Ph), 74.1 (C-3 Rha), 73.9 (CH_2Ph), 73.1 ($OCH_2CH{=}CH_2$), 70.4 (C-5, GalA), 69.9 (C-2 Rha), 68.4 (C-5 Rha), 56.3 (OCH_3), 52.7 (CO_2CH_3), 18.5 (C-6 Rha). Anal. Calcd for $C_{38}H_{44}O_{12}$: C, 65.88; H, 6.40. Found: C, 65.52; H, 6.32.

3.6. Benzyl (4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate (7)

A solution of **5** (0.500 g, 0.617 mmol) in CH_2Cl_2 (11.7 mL) was treated with 11.3 mL methanolic HCl (prepared by the addition of 1.0 mL of acetyl chloride to 35 mL distilled MeOH). The reaction was stirred under $N_{2(g)}$ at rt for approximately 77.5 h, after which time the mixture was worked up by diluting with CH_2Cl_2, washing with satd $NaHCO_3$, drying over Na_2SO_4, filtering, and evaporating to dryness (TLC solvent B). The resulting syrup was purified by chromatography (eluting solvent B); two samples were collected, one of which consisted of the compounds corresponding to the two upper spots that appeared on TLC, and a second sample that consisted of the compounds corresponding to the two lower spots. Both of these samples were then individually purified by chromatography again (eluting solvent C) and yielded the following: **5**, 0.027 g, 5%; **7**, 0.174 g, 37%; **4**, 0.033 g, 7%; **6**, 0.189 g, 44%. Anal. for **7**: $[\alpha]_D^{22}$ +6.9 (*c* 0.4, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 8.14–8.07 (2H, aromatic), 7.65–7.22 (18H, aromatic), 5.81 (1H, $OCH_2CH{=}CH_2$), 5.44 (dd, 1H, $J_{3,4} = 8.2$ Hz, H-3 Rha), 5.30, 5.15 (2H, CO_2CH_2Ph), 5.15 (2H, $OCH_2CH{=}CH_2$), 5.13 (d, 1H $J_{1,2} = 2.9$ Hz, H-1 Rha), 4.87, 4.70 (2H, OCH_2Ph), 4.85, 4.68 (2H, OCH_2Ph), 4.75 (d, 1H, $J_{1,2} = 3.4$ Hz, H-1 GalA), 4.45–4.40 (2H, H-4, H-5, GalA), 4.38 (d, 1H, $J_{3,2} = 3.0$ Hz, H-2 Rha), 4.28–4.03 (2H, $OCH_2CH{=}CH_2$), 4.07 (dd, 1H, $J_{2,3} = 10.1$ Hz, H-2 GalA), 3.95 (dd, 1H, $J_{3,4} = 2.7$ Hz, H-3 GalA), 3.90 (dd, 1H, $J_{5,6} = 6.2$ Hz, H-5 Rha), 3.54 (dd, 1H, $J_{5,4} = 9.3$ Hz, H-4 Rha), 3.39 (s, 3H, OCH_3), 1.34 (3H, H-6 Rha); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 168.2 (CO_2CH_3), 165.5 (*C*=O, benzoate), 138.4, 138.3, 135.3, 133.3, 129.9, 129.0 128.9, 128.8, 128.7, 128.1, 128.0, 128.0, 127.8, 127.2 (C aromatic), 134.8 ($OCH_2CH{=}CH_2$), 117.0 ($OCH_2CH{=}CH_2$), 102.5 (C-1 Rha, $^1J_{13C,1H} = 170.4$ Hz), 99.4 (C-1 GalA, $^1J_{13C,1H} = 170.9$ Hz), 79.5 (C-4 Rha), 78.2 (C-4 GalA), 77.5 (C-3 GalA), 76.0 (C-2 GalA), 74.0 (CH_2Ph), 74.2 (C-3 Rha), 74.0 (CH_2Ph), 73.2 ($OCH_2CH{=}CH_2$), 70.3 (C-5, GalA), 69.8 (C-2 Rha), 68.6 (C-5 Rha), 67.6 (CO_2CH_2Ph), 56.3 (OCH_3), 18.6 (C-6 Rha); ESI-MS found *m*/*z* 791.3041 $[M+Na]^+$. Calcd for $C_{44}H_{48}O_{12}Na$: 791.3043.

3.7. Methyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(1-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosid)uronate (8)

A sample of compound **4** (1.860 g, 2.514 mmol) was dissolved in AcOH (41.7 mL) and Ac_2O (41.7 mL). The reaction mixture was then cooled in an ice bath for 10 min, after which H_2SO_4 (417 μL) was slowly added. The reaction was followed by TLC (solvent E) and showed completion after approximately 2 h 15 min. The reaction mixture was allowed to warm to rt during the progress of the reaction. $NaOAc{\cdot}3H_2O$ (4.38 g) was then added and the mixture was stirred until all $NaOAc{\cdot}3H_2O$ had dissolved. The mixture was then evaporated to dryness, taken up in EtOAc, washed with H_2O (extracted 4× with EtOAc), dried over Na_2SO_4, filtered, evaporated to dryness, and finally co-evaporated three times with EtOH. The resulting syrup was purified by chromatography (eluting solvent A) to obtain pure **8** (1.555 g, 81%). NMR data are given only for the major component, the α-anomer. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 2.12 (s, 3H, GalA –OCOCH_3), 2.01 (s, 3 H, Rha –OCOCH_3); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 169.7, 169.5, 169.1, 168.8 (*C*=O, Rha O*C*(O)CH_3, GalA O*C*(O)CH_3 and *C*O_2CH_3), 165.2 (*C*=O, benzoate), 138.2–127.8 (C aromatic), 134.8 ($OCH_2CH{=}CH_2$), 116.9 ($OCH_2CH{=}CH_2$), 99.3 (C-1 Rha, $^1J_{13C,1H}$ = 174.8 Hz), 90.6 (C-1 GalA, $^1J_{13C,1H}$ = 177.9 Hz), 78.5 (C-4 Rha), 77.2 (C-3 GalA), 75.8 (C-4 GalA), 74.9 (C-2 GalA), 74.1 (*C*H_2Ph), 73.7 ($OCH_2CH{=}CH_2$), 73.4 (*C*H_2Ph), 72.6 (C-5 GalA), 72.0 (C-3 Rha), 70.5 (C-2 Rha), 68.5 (C-5 Rha), 52.9 (CO_2CH_3), 21.2 (GalA OCO*C*H_3), 21.0 (Rha OCO*C*H_3), 18.2 (C-6 Rha). Anal. Calcd for $C_{41}H_{46}O_{14}$: C, 64.56; H, 6.08. Found: C, 64.70; H, 5.79.

3.8. Methyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-α/β-D-galactopyranosid)uronate (9)

A sample of compound **8** (0.794 g, 1.04 mmol) was dissolved in distilled MeOH (25.8 mL) and CH_2Cl_2 (14.7 mL). Powdered 4 Å MS (0.529 g, 0.667 equiv by weight to **8**) were then added. The reaction was stirred at rt for approximately 4 days, after which the solids were removed by filtration (TLC solvent A). The filtrate was evaporated to dryness and the resulting syrup was purified by chromatography (eluting solvent A) to give pure **9** (0.670 g, 90%). ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 134.8 ($OCH_2CH{=}CH_2$), 116.9 ($OCH_2CH{=}CH_2$), 99.9, 99.3 (C-1$^{\alpha/\beta}$ Rha, $^1J_{13C,1H}$ = 173.4 Hz), 92.4, 92.3 (C-1$^{\alpha/\beta}$ GalA, $^1J_{13C,1H}$ = 171.5 Hz, 172.3 Hz), 52.7 (CO_2CH_3), 21.0 (Rha OCO*C*H_3), 18.4, 18.2 (C-6$^{\alpha/\beta}$ Rha); ESI-MS found *m*/*z* 743.2676 $[M+Na]^+$. Calcd for $C_{39}H_{44}O_{13}Na$: 743.2680.

3.9. Methyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-2,3-di-*O*-benzyl-α-D-galacto-pyranosyluronate trichloroacetimidate (10)

A sample of hemiacetal **9** (0.592 g, 0.821 mmol) was dissolved in freshly distilled CH_2Cl_2 (13.5 mL) and stirred under $N_{2(g)}$ in the presence of 4 Å MS (0.590 g) at rt for approximately 10 min, after which the mixture was placed in an ice bath and stirred for an additional 15 min. Trichloroacetonitrile (837 μL, 8.35 mmol, 10.2 equiv) was then added, followed by DBU (147 μL, 0.983 mmol, 1.20 equiv). The reaction was followed by TLC (TLC solvent A) and stopped after 10 min. The mixture was immediately placed on a vacuum column (eluting solvent F); fractions containing **10** were combined and evaporated to dryness. Impure **10** was then purified by chromatography twice; **10** was first purified by chromatography using eluting solvent C (0.498 g pure **10**); impure fractions were collected and purified by chromatography again using eluting solvent G (0.079 g). The overall yield of **10** was 0.577 g (81%). ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 8.63 (s, 1H, C=N*H*), 8.05–7.97 (2H, aromatic), 7.63–7.20 (13H, aromatic), 6.67 (d, 1H, $J_{1,2}$ = 3.3 Hz, H-1 GalA), 5.79 (1H, $OCH_2CH{=}CH_2$), 5.61 (dd, 1H, $J_{2,1}$ = 2.2 Hz, $J_{2,3}$ = 3.1 Hz, H-2 Rha), 5.48 (dd, 1H, $J_{3,4}$ = 9.4 Hz, H-3 Rha), 5.25–5.03 (3H, H-1 Rha, $OCH_2CH{=}CH_2$), 4.85–4.70 (4H, 2 O*C*H_2Ph), 4.65–4.61 (1H, H-5, GalA), 4.57–4.52 (1H, H-4, GalA), 4.30 (dd, 1H, $J_{2,3}$ = 10.0 Hz, H-2 GalA), 4.22–4.05 (2H, $OCH_2CH{=}CH_2$), 4.05 (dd, 1H, $J_{3,4}$ = 2.7 Hz, H-3 GalA), 3.85 (s, 3H, CO_2CH_3), 3.78 (dd, 1H, $J_{5,6}$ = 6.2 Hz, H-5 Rha), 3.50 (dd, 1H, $J_{4,5}$ = 9.5 Hz, H-4 Rha), 2.04 (s, 3H, OCOCH_3), 1.37 (3H, H-6 Rha); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 169.6, 168.4 (*C*=O), 165.3 (*C*=O, benzoate), 160.7 (O*C*(NH)CCl_3), 138.4, 138.0, 133.2, 130.3, 129.7, 128.6, 128.5, 128.5, 128.1, 127.9, 127.8, 127.7 (C aromatic), 134.8 ($OCH_2CH{=}CH_2$), 116.9 ($OCH_2CH{=}CH_2$), 99.3 (C-1 Rha, $^1J_{13C,1H}$ = 174.7 Hz), 94.9 (C-1 GalA, $^1J_{13C,1H}$ = 180.4 Hz), 78.5 (C-4 Rha), 76.3 (C-3 GalA), 75.7 (C-4 GalA), 75.5 (C-2 GalA), 73.7 ($OCH_2CH{=}CH_2$), 73.7, 73.3 (*C*H_2Ph), 72.8 (C-5, GalA), 72.0 (C-3 Rha), 70.5 (C-2 Rha), 68.6 (C-5 Rha), 52.9 (CO_2CH_3), 21.0 (OCO*C*H_3), 18.2 (C-6 Rha); ESI-MS found *m*/*z* 886.1777 $[M+Na]^+$. Calcd for $C_{41}H_{44}NO_{13}Cl_3Na$: 886.1776.

3.10. Benzyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(1-*O*-acetyl-2,3-di-*O*-benzyl-α/β-D-galactopyranosid)uronate (11)

A sample of **11** was prepared by dissolving dissacharide **5** (1.830 g, 2.257 mmol) in AcOH (37.3 mL) and Ac_2O (37.3 mL). The reaction mixture was then cooled in an ice bath for 10 min, after which H_2SO_4 (370 μL) was slowly added. The reaction was followed by TLC

(TLC solvent H) and showed completion after approximately 2 h 05 min. The reaction mixture was allowed to warm to rt during the progress of the reaction. NaOAc·3H_2O (3.911 g) was then added and the mixture was stirred until all NaOAc·3H_2O had dissolved. The mixture was then evaporated to dryness, taken up in EtOAc, washed with H_2O (extracted 4× with EtOAc), dried over Na_2SO_4, filtered, evaporated to dryness, and finally co-evaporated three times with EtOH. The resulting syrup was purified by chromatography (eluting solvent C) to obtain pure **11** (1.730 g, 91%). NMR data are given only for the major component, the α-anomer. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 2.12 (s, 3H, GalA –OCOCH_3), 2.01 (s, 3H, Rha –OCOCH_3); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 169.7, 169.5, 169.0, 167.6 (*C*=O, Rha O*C*(O)CH_3, GalA O*C*(O)CH_3 and *C*O_2CH_2Ph), 165.2 (*C*=O, benzoate), 138.2–127.8 (C aromatic), 134.8 (OCH_2CH=CH_2), 116.9 (OCH_2-CH=*C*H_2), 99.6 (C-1 Rha, $^1J_{13C,1H}$ = 173.9 Hz), 90.6 (C-1 GalA, $^1J_{13C,1H}$ = 177.5 Hz), 78.5 (C-4 Rha), 77.2 (C-3 GalA), 76.6 (C-4 GalA), 75.0 (C-2 GalA), 73.8 (OCH_2CH=CH_2), 73.5, 73.3 (*C*H_2Ph), 72.5 (C-5 GalA), 72.1 (C-3 Rha), 70.5 (C-2 Rha), 68.7 (C-5 Rha), 67.8 (*C*O_2CH_2Ph), 21.3 (GalA OCO*C*H_3), 21.0 (Rha OCO*C*H_3), 18.4 (C-6 Rha). Anal. Calcd for $C_{47}H_{50}O_{14}$: C, 67.29; H, 6.01. Found: C, 67.08; H, 6.05.

3.11. Benzyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(2,3-di-*O*-benzyl-α/β-D-galactopyranosid)uronate (12)

A sample of compound **11** (0.277 g, 0.330 mmol) was dissolved in distilled MeOH (8.2 mL) and CH_2Cl_2 (4.7 mL). Powdered 4 Å MS (0.185 g, 0.667 equiv by weight to **11**) were then added. An additional 0.055 g of 4 Å molecular sieves was added on day 3. The reaction mixture was stirred at rt for approximately 4 days in total, after which the solids were removed by filtration. The filtrate was evaporated to dryness and the resulting syrup was purified by chromatography (eluting solvent I) to give pure **12** (0.200 g, 76%). ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 134.9 (OCH_2CH=CH_2), 116.9 (OCH_2CH=*C*H_2), 99.5 (C-1$^{\alpha/\beta}$ Rha, $^1J_{13C,1H}$ = 172.6 Hz), 92.4, (C-1$^{\alpha/\beta}$ GalA, $^1J_{13C,1H}$ = 173.2 Hz), 21.0 (Rha OCO*C*H_3), 18.3 (C-6$^{\alpha/\beta}$ Rha). Anal. Calcd for $C_{45}H_{48}O_{13}$: C, 67.83; H, 6.07. Found: C, 67.96; H, 6.16.

3.12. Benzyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-2,3-di-*O*-benzyl-α/β-D-galactopyranosyluronate trichloroacetimidate (13)

A sample of hemiacetal **12** (0.480 g, 0.602 mmol) was dissolved in freshly distilled CH_2Cl_2 (12.9 mL) and stirred under $N_{2(g)}$ in the presence of 4 Å MS (0.476 g) at rt for approximately 10 min. Trichloroacetonitrile (605 μL, 6.03 mmol, 10.0 equiv) was then added, followed by DBU (108 μL, 0.725 mmol, 1.20 equiv). The reaction was followed by TLC (TLC solvent I) and stopped after 20 min. The mixture was immediately placed on a vacuum column (eluting solvent F); fractions containing **13** were combined and evaporated to dryness. Impure **13** was then purified by chromatography twice (eluting solvent J, followed by chromatography with eluting solvent G), giving pure **13** (0.464 g, 82%). NMR data are given only for the major component, the α-anomer. ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 8.61 (s, C=N*H*); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 169.5, 167.5 (*C*=O), 165.2 (*C*=O, benzoate), 160.7 (O*C*(NH)CCl_3), 138.4–127.7 (C aromatic), 134.8 (OCH_2CH=CH_2), 116.9 (OCH_2CH=*C*H_2), 99.6 (C-1 Rha, $^1J_{13C,1H}$ = 173.2 Hz), 95.0 (C-1 GalA, $^1J_{13C,1H}$ = 180.1 Hz), 78.5 (C-4 Rha), 76.7 (C-4 GalA), 76.3 (C-3 GalA), 75.6 (C-2 GalA), 73.7 (O*C*H_2CH=CH_2, *C*H_2Ph), 73.4 (*C*H_2Ph), 72.8 (C-5, GalA), 72.1 (C-3 Rha), 70.6 (C-2 Rha), 68.7 (C-5 Rha), 67.8 (CO_2CH_2Ph), 21.0 (OCO*C*H_3), 18.4 (C-6 Rha); ESI-MS found *m*/*z* 962.2087 [M+Na]$^+$. Calcd for $C_{47}H_{48}NO_{13}Cl_3Na$: 962.2089.

3.13. Methyl (2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosyluronate)-(1→2)-(2-*O*-acetyl-4-*O*-allyl-3-*O*-benzoyl-α-L-rhamnopyranosyl)-(1→4)-(methyl 2,3-di-*O*-benzyl-α-D-galactopyranosid)uronate (14)

Acceptor **6** (0.243 g, 0.351 mmol, 1.00 equiv) and donor **10** (0.426 g, 0.492 mmol, 1.40 equiv) were placed together in a flask along with 4 Å MS (~0.530 g). Freshly distilled CH_2Cl_2 (5.7 mL) was added; the flask was then purged with $N_{2(g)}$ and allowed to stir at rt and in the dark for 1 h, after which AgOTf was added (0.0483 g, 0.382 equiv to donor). The reaction was followed by TLC (TLC solvent K) and worked up after 17 h 20 min. Workup was performed by diluting the reaction mixture with CH_2Cl_2, removing the solids by filtration, washing the filtrate sequentially with H_2O and satd $NaHCO_3$, and drying the organic layer over Na_2SO_4. The drying agent was removed by filtration and the filtrate evaporated to dryness to afford **14** as a syrup. The crude syrup was purified by chromatography using eluting solvent K; impure fractions were combined and again purified by chromatography with the same solvent. The crude mixture was purified by chromatography a total of four times to give pure **14** (0.190 g, 39%). $[\alpha]_D^{22}$ +63.3 (*c* 0.2, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H 8.03–7.94 (4H, aromatic), 7.64–7.21 (26H, aromatic), 5.86–5.68 (2H, OCH_2CH=CH_2), 5.55 (dd, 1H, $J_{3,2}$ = 3.3 Hz, H-2‴), 5.44 (1H, H-3‴), 5.41 (1H, H-3′), 5.31 (1H, H-1′), 5.19–5.00 (4H, OCH_2-CH=CH_2), 5.11 (1H, H-1‴), 4.94, 4.75 (2H, OCH_2^aPh), 4.85, 4.61 (2H, OCH_2^bPh), 4.78 (1H, H-1″), 4.76 (1H,

H-1), 4.72, 4.66 (2H, OCH_2^dPh), 4.58, 4.42 (2H, OCH_2^cPh), 4.47 (2H, H-4, H-5″), 4.40 (1H, H-2′), 4.31 (1H, H-4″), 4.20–4.10 (4H, OCH_2CH=CH_2), 4.14 (1H, H-2), 3.94 (1H, H-3), 3.94 (1H, H-3″), 3.85 (s, 3H, CO_2CH_3), 3.84 (1H, H-2″), 3.71 (1H, H-5′), 3.70 (1H, H-5‴), 3.50 (1H, H-4′), 3.46 (1H, H-4‴), 3.83 (s, 3H, CO_2CH_3''), 3.30 (s, 3H, OCH_3), 2.02 (s, 3H, OCOCH_3'''), 1.36 (d, 3H, $J_{6,5} = 6.1$ Hz, H-6′), 1.29 (d, 3H, $J_{6,5} = 6.3$ Hz, H-6‴); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C 169.7, 169.2, 168.4 (*C*=O), 165.3, 165.2 (*C*=O, benzoate), 139.0–127.7 (C aromatic), 135.0, 134.8 (OCH$_2$*C*H=CH$_2$), 116.9, 116.7 (OCH$_2$CH=*C*H$_2$), 99.4 (C-1, $^1J_{13C,1H} = 173.2$ Hz), 99.0 (C-1‴, $^1J_{13C,1H} = 174.4$ Hz), 98.4 (C-1′, $^1J_{13C,1H} = 172.6$ Hz), 96.7 (C-1″, $^1J_{13C,1H} = 171.5$ Hz), 78.5 (C-4′, C-4‴), 77.6 (C-3), 76.7 (C-3″), 76.5 (C-2), 76.0 (C-4, C-4″), 75.5 (C-2″), 75.3 (C-2′), 74.4 (OCH_2^aPh), 74.1 (OCH_2^bPh), 73.7, 73.5 (OCH$_2$CH=CH$_2$), 73.5 (C-3′), 72.7 (OCH_2^cPh, OCH_2^dPh), 72.1 (C-3‴), 70.6 (C-5″, C-2‴), 70.5 (C-5), 68.6 (C-5′), 68.3 (C-5‴), 56.3 (OCH_3), 52.7 (CO_2CH_3''), 52.1 (CO_2CH_3), 21.0 (OCOCH_3'''), 18.4 (C-6′), 18.2 (C-6‴). Anal. Calcd for $C_{77}H_{86}O_{24}$: C, 66.27; H, 6.21. Found: C, 66.60; H, 6.34.

3.14. Methyl (α-L-rhamnopyranosyl)-(1→4)-(methyl α-D-galactopyranosyluronate)-(1→2)-(α-L-rhamnopyranosyl)-(1→4)-(methyl α-D-galactopyranosid)uronate (16)

A sample of **14** (0.190 g, 0.136 mmol) was dissolved in EtOH–toluene–H_2O 10:3:1 (28.0 mL) and the reaction flask was purged with $N_{2(g)}$. Wilkinson's catalyst ($[(C_6H_5)_3P]_3RhCl$; 0.0502 g, 0.0543 mmol, 0.4 equiv) was added, followed by DABCO (0.0153 g, 0.136 mmol, 1.00 equiv). The reaction was set to reflux and monitored by TLC (TLC solvent K). After approximately 22 h an additional 0.2 equiv of Wilkinson's catalyst was added, followed by 0.5 equiv of DABCO; the reaction was stopped after 46 h. The mixture was evaporated to dryness, taken up in CH_2Cl_2, and sequentially washed with 0.1 M HCl, satd $NaHCO_3$, and H_2O. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The resulting syrup was dissolved in 9:1 acetone–H_2O (20 mL). $HgCl_2$ and HgO were then added and the reaction mixture was allowed to stir at rt. TLC (TLC solvent K) indicated that the reaction was not complete, so additional portions of $HgCl_2$ (0.0369 g) and HgO (0.0290 g) were added after 26 h 20 min and again after 45 h. No significant change was observed, so the reaction mixture was filtered after 50 h 50 min through Celite and evaporated to dryness. The residue was taken up in EtOAc and sequentially washed with satd KI, satd $Na_2S_2O_3$, and H_2O. The organic layer was dried over Na_2SO_4, filtered, and evaporated to dryness. The residue was purified by chromatography (TLC and eluting solvent L) and samples containing the target compound were pooled to yield a yellow-brown foam.

This residue was taken up in freshly distilled MeOH (12.0 mL) and 1.0 M NaOMe was added until litmus paper indicated a pH between 11 and 12. TLC (TLC solvent M) taken approximately 24 h after the start of the reaction indicated that the reaction had gone to completion. The reaction was neutralized by the addition of Amberlyst® 15H$^+$ resin beads, filtered, and evaporated to dryness to afford a yellowish syrup. The syrup was purified by chromatography using eluting solvent N, giving a clear, colorless syrup.

This syrup was dissolved in EtOAc–MeOH–AcOH 5:5:1 (11.0 mL), to which $Pd(OAc)_2$ (0.209 g, 0.931 mmol) was added. The mixture was placed under $H_{2(g)}$ at 40 psi for 1 h. TLC (solvent N) indicated no starting material, with all material present on the baseline. The mixture was worked up by filtering through Celite (rinsed with EtOAc and MeOH), followed by evaporation and drying under high vacuum; a white powder (0.032 g, 33%) was collected. TLC showed (TLC solvent O) one spot without any visible trace of impurities. $[\alpha]_D^{22}$ +59.7 (*c* 0.2, MeOH); ^{1}H NMR (300.13 MHz, CH_3OD): δ_H 5.21 (1H, H-1′), 5.14 (d, 1H, $J_{1,2} = 1.5$ Hz, H-1‴), 5.11 (1H, H-5), 5.00 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1), 4.81 (d, 1H, $J_{1,2} = 3.7$ Hz, H-1″), 4.52 (1H, H-5″), 4.39–4.32 (2H, H-4″, H-4), 4.11–4.06 (1H, H-2′), 4.01–3.90 (3H, H-2‴, H-3, H-3″), 3.83–3.69 (3H, H-2″, H-2, H-3′), 3.81 (s, 3H, CO_2CH_3), 3.78 (s, 3 H, CO_2CH_3), 3.63 (dd, 1H, $J_{3,2} = 3.2$ Hz, $J_{3,4} = 9.4$ Hz, H-3‴), 3.54–3.39 (2H, H-5′, H-5‴), 3.41 (s, 3H, OCH_3), 3.38–3.29 (2H, H-4‴, H-4′), 1.35–1.20 (6H, H-6′, H-6‴); ^{13}C NMR (75.03 MHz, CH_3OD): δ_C 170.1, 169.7 (*C*O_2CH_3), 102.2 (C-1‴, $^1J_{13C,1H} = 171.5$ Hz), 100.6 (C-1″, $^1J_{13C,1H} = 171.0$ Hz), 99.8 (C-1′, $^1J_{13C,1H} = 171.5$ Hz), 98.3 (C-1, $^1J_{13C,1H} = 171.5$ Hz), 78.4 (C-4″), 77.9 (C-4), 76.9 (C-2′), 72.6 (C-4‴), 72.5 (C-4′), 70.9 (C-2‴), 70.8 (C-3), 70.6 (C-5), 70.3 (C-3‴), 70.1 (C-3″), 69.9 (C-5″), 69.8 (C-3′), 69.4 (C-5′), 69.1 (C-5‴), 68.6 (C-2″, C-2), 55.1 (OCH_3), 55.1 (OCH_3), 51.7, 51.6 (CO_2CH_3), 17.0 (C-6‴), 16.9 (C-6′). Anal. Calcd for $C_{27}H_{44}O_{21}$: C, 46.02; H, 6.29. Found: C, 45.88; H, 6.39.

3.15. Methyl α-L-rhamnopyranosyl-(1→4)-α-D-galactopyranosyluronate-(1→2)-α-L-rhamnopyranosyl-(1→4)-(α-D-galactopyranosid)uronate (17)

Saponification of the methyl-esterified tetrasaccharide was achieved by dissolving **16** (0.024 g, 0.034 mmol) in deionized water (2.0 mL). NaOH (1.0 M) was added until pH 11–12; TLC (TLC solvent P) was used to verify the de-esterification and the presence of the highly polar product. HCl (1.0 M) was then added until pH 2–3. The reaction mixture was evaporated to dryness, dissolved in a minimal amount of deionized water, and purified on a

P2 gel column with deionized water as the eluent (TLC solvent Q). Fractions containing the product were pooled and dried via lyophilization to give pure **17** (17.6 mg, 77%). ^{1}H NMR (300.13 MHz, D_2O): δ_H 5.11 (1H, H-1′), 5.09 (1H, H-1‴), 4.88 (d, 1H, $J_{1,2}$ = 3.5 Hz, H-1), 4.72 (d, 1H, $J_{1,2}$ = 3.7 Hz, H-1″), 4.57 (1H, H-5″), 4.31 (1H, H-4″), 4.28 (1H, H-4), 4.16 (1H, H-5), 4.01 (1H, H-3″), 4.00 (1H, H-2′), 3.95 (1H, H-2‴), 3.91 (dd, 1H, $J_{3,4}$ = 2.7 Hz, H-3), 3.81 (1H, H-2″), 3.80 (1H, H-2), 3.79 (1H, H-3′), 3.68 (1H, H-3″), 3.67 (1H, H-5′), 3.66 (1H, H-5‴), 3.30 (1H, H-4′), 3.26 (s, 3H, OCH_3), 3.30 (1H, H-4′), 3.34 (1H, H-4‴), 1.14 (3H, H-6′), 1.13 (3H, H-6‴);^{13}C NMR (75.03 MHz, D_2O): δ_C 175.9 (COOH, COOH″), 102.2 (C-1‴, $^1J_{13C,1H}$ = 173.6 Hz), 100.9 (C-1″, $^1J_{13C,1H}$ = 171.5 Hz), 100.1 (C-1′, $^1J_{13C,1H}$ = 172.4 Hz), 98.9 (C-1, $^1J_{13C,1H}$ = 171.5 Hz), 78.8 (C-4), 77.9 (C-4″), 77.5 (C-2′), 73.7 (C-4‴), 73.4 (C-4′), 72.7 (C-5″), 72.0 (C-5), 71.9 (C-2‴, C-3), 71.8 (C-3″), 71.5 (C-3‴), 70.8 (C-3′), 78.4 (C-4″), 70.6 (C-5′), 70.3 (C-5‴), 69.4 (C-2″), 69.3 (C-2), 56.8 (O*C*H$_3$), 18.2, 18.1 (C-6′, C-6‴); ESI-MS found *m*/*z* 699.1958 $[M+Na]^+$. Calcd for $C_{25}H_{40}O_{21}Na$: 699.1960.

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Supplementary data

^{1}H and ^{13}C NMR spectra of compounds **4**, **5**, **7**, **9**, **10**, **13** and **17**. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.carres.2007.10.030.

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