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Synthesis of a disaccharide fragment of rhamnogalacturonan II

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Abstract—A disaccharide portion of the A-side chain of the rhamnogalacturonan II oligosaccharide has been prepared. Glycosylation of methyl (methyl 3,4-*O*-isopropylidene-α-D-galactopyranosid)uronate with *p*-tolyl 2,3-di-*O*-acetyl-3-*C*-(benzyloxymethyl)-1-thio-α/β-D-erythrofuranoside was carried out using *N*-iodosuccinimide as promoter and silver trifluoromethanesulfonate as catalyst. Removal of the protecting groups gave the β-D-Api*f*-(1→2)-α-D-Gal*p*A-OMe disaccharide.

Keywords: Apiose; Galacturonic acid; Rhamnogalacturonan II

1. Introduction

Rhamnogalacturonan II (RG II) is a complex oligosaccharide found in the primary cell walls of higher plants.[1] The structure of RG II has been determined by methylation analysis and mass-spectrometry analysis of fragments obtained by enzymatic and chemical hydrolysis. These studies have shown that RG II has a backbone comprised of 1,4-linked α-D-galactopyranosiduronic acid residues. Five oligosaccharide side chains are attached to the 2-positions of the homogalacturonan backbone; two of these side chains are structurally complex.[2] These side chains contain several unusual sugars and linkages, such as aceric acid (3-*C*-(carboxy)-5-deoxy-L-xylose), Kdo (3-deoxy-D-*manno*-oct-2-ulosonic acid), D-Dha (3-deoxy-D-*lyxo*-hept-2-ulosaric acid), β-L-rhamnose, and D-apiose (3-*C*-(hydroxymethyl)-D-erythrofuranose). Two of the side chains in RG II are linked to the homogalacturonan backbone via a D-apiose residue. More recently, NMR analyses and computational studies have been performed to further elucidate the three-dimensional structure of RG II.[3,4]

The structure of RG II is complex, and its biological function is still not fully understood. It is thought to be important in the mechanical stability of the primary cell wall of higher plants. RG II can form even more complex structures by forming dimers. These dimers are the result of a 1:2 borate diol ester that forms between boron and the apiose residues of separate RG II oligosaccharides.[5,6] These dimers have been implicated in plant growth and health.[7] To more fully understand the structure and function of RG II oligosaccharides it would be useful to have fragments of its structure available for NMR analysis and boron complexation studies. Towards this end, the synthesis of a β-rhamnosyl-apiose disaccharide has recently been published.[8] In this paper we describe the synthesis of a β-apiosyl-galacturonic acid disaccharide, **1** (Fig. 1).

2. Results and discussion

Our first approach for the preparation of disaccharide **1** was to form the glycosidic linkage using galactose in the place of galacturonic acid. Partial deprotection and selective oxidation of the primary hydroxyl group of galactose would provide the target disaccharide **1**. Because D-apiose in not commercially available, this work started with the preparation of the 2,3-*O*-isopropylidene

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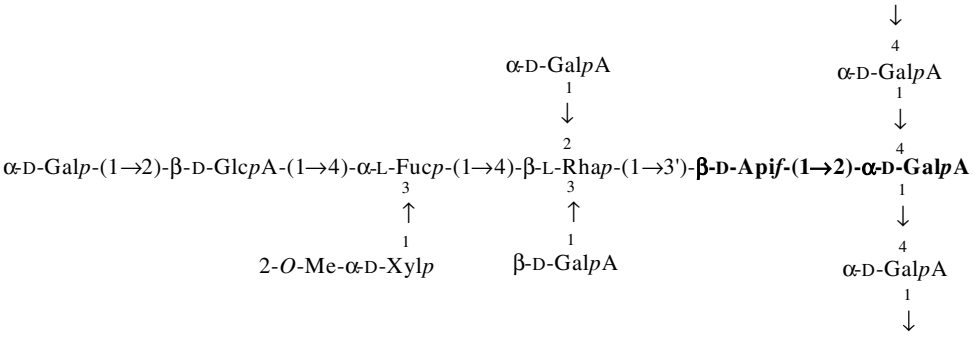


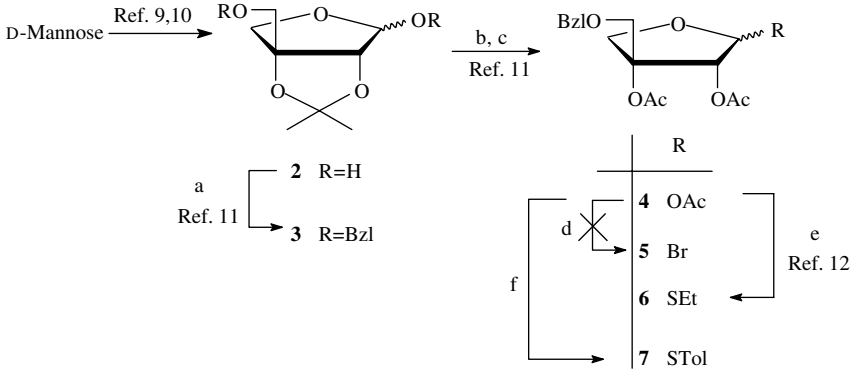
Figure 1. Structure of the A-side chain of rhamnogalacturonan II with the sequence of target disaccharide **1** in bold.

apiofuranose **2** from D-mannose in six steps.[9,10] To determine the most effective glycosyl donor for this particular glycosylation we prepared three apiose derivatives (see Scheme 1). 1,2,3-Tri-*O*-acetyl-3-*C*-(benzyloxymethyl)-α/β-D-erythrofuranose **4** was prepared from **2** by benzylation, followed by acetolysis and acetylation as described by Tapiéro.[11] Preparation of glycosyl bromide **5** by the treatment of **4** with hydrogen bromide in acetic acid was unsuccessful, proceeding with significant decomposition. Reaction of **4** with ethanethiol and a catalytic amount of $SnCl_4$ gave ethyl 2,3-di-*O*-acetyl-3-*C*-(benzyloxymethyl)-1-thio-α/β-D-erythrofuranoside **6**, as described by van Boom and co-workers.[12] Based on the utility of thiocresyl derivatives in the synthesis of arabinofuranosides, we decided to prepare the thiocresyl apiofuranoside.[13] Treatment of **4** with *p*-thiocresol and $BF_3{\cdot}Et_2O$ gave *p*-tolyl 2,3-di-*O*-acetyl-3-*C*-(benzyloxymethyl)-1-thio-α/β-D-erythrofuranoside **7** in 95% yield.

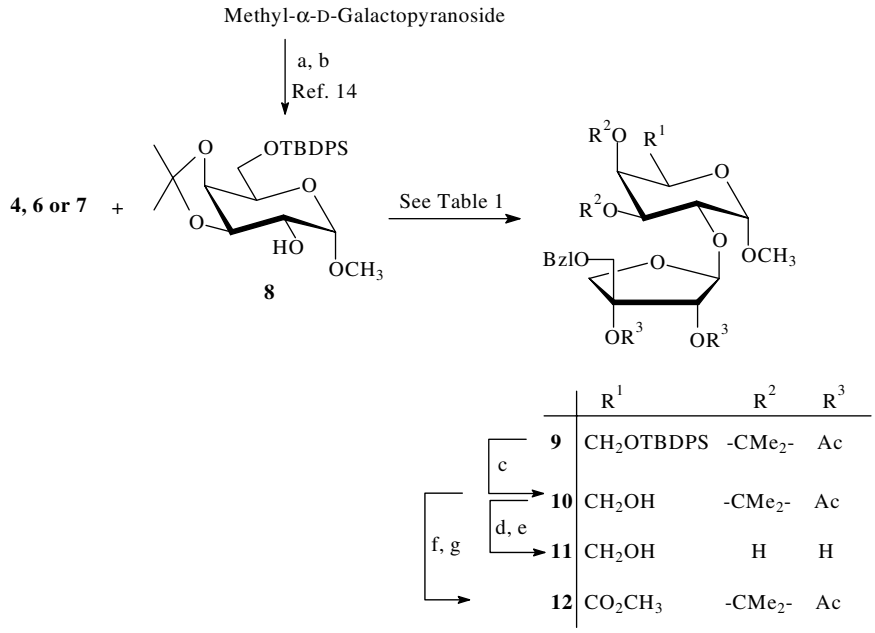
The apiofuranosyl donors **4**, **6**, and **7** were used together with the known galactose derivative **8**[14] to prepare disaccharide **9** (see Scheme 2). A summary of these reactions is given in Table 1. The best results were obtained using the thiocresyl derivative **7** with *N*-iodosuccinimide and a catalytic amount of silver triflate. Under these conditions, disaccharide **9** was obtained in 77% yield. In our experience, we obtained better results using the thiocresyl derivative **7** rather than the thioethyl derivative **6**. We found its preparation easier and we obtained higher yields in the subsequent glycosylation reaction.

Disaccharide **9** was partially deprotected by treatment with tetrabutyl ammonium fluoride to remove the *tert*-butyl diphenylsilyl group to give **10**. Transesterification of the acetate groups using sodium methoxide, and removal of the isopropylidene group under acidic conditions gave disaccharide **11** with a benzyl group on the primary hydroxyl of the apiose residue, and the primary hydroxyl of the galactose residue unprotected. We then attempted a TEMPO-mediated selective oxidation of the primary hydroxyl of the galactose residue to give the corresponding galacturonic acid. The high water solubility of **11** allowed us to employ variations of typical conditions (aq $NaHCO_3$, NaBr, 5% NaOCl, TEMPO, MeCN, 0 °C or aq NaOH, NaBr, 12% NaOCl, TEMPO, 0 °C).[15–17] The reaction mixtures were pre-purified using C-18 solid-phase extraction cartridges, and ^{1}H NMR spectroscopic analysis of the partially purified material indicated that the glycosidic linkage had been cleaved.

An alternate route for TEMPO oxidation of the galactose residue was then attempted by oxidizing the partially deprotected disaccharide **10** using biphasic



Scheme 1. Synthesis of apiofuranosyl donors. Reagents and conditions: (a) BzlBr, NaH, DMF; (b) 80% formic acid; (c) Ac_2O, pyridine; (d) 30% HBr in HOAc; (e) EtSH, $SnCl_4$ (cat.); (f) TolSH, $BF_3{\cdot}Et_2O$.



Scheme 2. Synthesis of a galactose-based disaccharide. Reagents and conditions: (a) TBDPSCl, imidazole; (b) $(CH_3)_2C(OCH_3)_2$, acetone, camphorsulfonic acid; (c) *n*-Bu_4F, THF; (d) $NaOCH_3$, CH_3OH; (e) EtOH, HCl; (f) TEMPO, CH_2Cl_2, KBr, *n*-Bu_4Cl, satd aq $NaHCO_3$, NaOCl soln; (g) CH_2N_2 in Et_2O.

Table 1. Results of glycosylation reactions to form disaccharide **9**

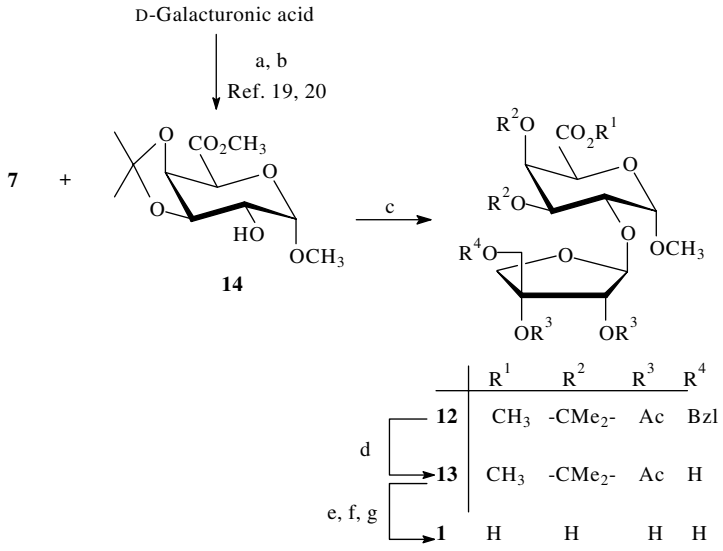
Entry	Donor	Promoter	Yield (%)
1	**4**	$BF_3{\cdot}Et_2O$	41
2	**4**	cat. TSMOTf	58
3	**6**	NIS/cat. AgOTf	65
4	**7**	NIS/cat. AgOTf	77

TEMPO oxidation conditions.[18] With only a single hydroxyl group present there would be no competing oxidation of secondary hydroxyl groups as may have occurred in our attempted oxidation of **11**. Oxidation of **10** proceeded smoothly providing the desired galacturonic acid residue. To aid in purification and characterization, the carboxylic acid group was esterified by treatment with diazomethane to give the methyl ester derivative **12** in 72% yield.

We then investigated the preparation of the target disaccharide **1** using a protected galacturonate ester derivative directly, rather than relying on oxidation of galactose to provide the desired galacturonic acid residues (see Scheme 3). Accordingly, glycosyl acceptor **14**[19,20] was prepared by dissolving D-galacturonic acid in methanol and refluxing with acidic resin beads until an equilibrium mixture was obtained. Methyl (methyl α/β-D-galactopyranosid)uronate was precipitated from the filtrate. Treatment of the precipitate with dimethoxypropane and camphorsulfonic acid afforded the selectively protected galacturonate ester derivative **14**. Reaction of the thiocresyl derivative **7** and the acceptor **14**, in the presence of *N*-iodosuccinimide and a catalytic amount of silver triflate, proceeded smoothly to give disaccharide **12** in 83% yield. Removal of the benzyl group from the 3′-position of the apiose residue gave disaccharide **13**. This compound, with a free hydroxyl at the 3′-position, is suitable for construction of higher order RG-II oligosaccharides. The acetate groups of **13** were removed by mild treatment with sodium methoxide, followed by removal of the isopropylidene group by hydrolysis using acidic resin beads, and finally saponification of the methyl ester to give the fully deprotected disaccharide **1**.

Assignment of signals in the NMR spectra was made based on analysis of two-dimensional homonuclear and heteronuclear shift correlation experiments. Assignment of the anomeric configuration of the newly formed glycosidic linkage in **9** and **12** was made based on the values of ^1H–^1H scalar coupling constants ($^3J_{1,2}$) for the apiose residue. The values typically observed for apiose sugars are about 1 Hz for 1,2-*trans* linkages, and about 4–5 Hz for 1,2-*cis* linkages.[11,21] The observed $^3J_{1,2}$ values (signals appearing as broad singlets) for **9** and **12** are consistent with formation of a 1,2-*trans*, or β-linkage. In addition, the ^{13}C-chemical shift values (about 111 ppm) for the anomeric carbons of the apiose residues of compounds **1** and **11** are in agreement with those found for 1,2-*trans* linkages in model compounds.[21]

In summary, we have prepared the target disaccharide **1** using an indirect approach, where galactose takes the place of galacturonic acid followed by subsequent oxidation, and we have also used a direct approach where a protected galacturonic acid residue is incorporated



Scheme 3. Synthesis of target disaccharide **1**. Reagents and conditions: (a) CH_3OH, Amberlyst 15 H^+ resin; (b) $(CH_3)_2C(OCH_3)_2$, camphorsulfonic acid (cat.); (c) NIS, AgOTf (cat.); (d) $Pd(OAc)_2$, H_2(g); (e) $NaOCH_3$, CH_3OH; (f) H_2O, Amberlyst 15 H^+ resin; (g) NaOH (aq).

into the synthesis at the very beginning. In this particular synthesis it is more efficient to use the direct approach. The glycosylation of the galacturonic acid derivative **14** proceeds with good yield and no further manipulation is required at a later stage in the synthesis, as is required using the indirect approach.

3. Experimental

3.1. General methods

^{1}H NMR and ^{13}C NMR spectra were obtained with a Bruker AMX 300 (75 MHz for ^{13}C) spectrometer or Varian Inova 500 (125 MHz for ^{13}C) and 600 MHz spectrometers. Optical rotation of the final product was conducted using a Perkin–Elmer model 241 polarimeter. Electrospray ionization mass spectra were recorded on a Micromass Zabspec TOF Mass Spectrometer. Thin-layer chromatography (TLC) utilized Silica Gel-60 F_{254} (E. Merck), with detection by UV light and by charring with 5% sulfuric acid in ethanol. Medium-pressure column chromatography was performed with silica gel (E. Merck, 230–400 mesh).

3.2. *p*-Tolyl 2,3-di-*O*-acetyl-3-*C*-(benzyloxymethyl)-1-thio-α/β-D-erythrofuranoside (7)

A sample of 1,2,3-tri-*O*-acetyl-3-*C*-(benzyloxymethyl)-α/β-D-erythrofuranoside **4**[11] (1.829 g, 4.992 mmol) was dissolved in CH_2Cl_2 (10.0 mL). *p*-Thiocresol (0.750 g, 6.039 mmol) in CH_2Cl_2 (10.0 mL) was then added, followed by addition of $BF_3{\cdot}Et_2O$ (0.90 mL, 7.3 mmol). The reaction mixture was stirred under N_2 at room temperature for 25 min and then neutralized by the addition of Et_3N. The mixture was diluted with CH_2Cl_2 and washed successively with H_2O and brine. The organic layer was dried over Na_2SO_4, filtered, and concentrated to dryness. The syrupy residue (17:1 α/β mixture) was purified by chromatography using 5:1 hexane–ethyl acetate as eluant. The title compound **7** was obtained as a syrup (α-anomer, R_f 0.23: 1.927 g, 89.7%; β-anomer, R_f 0.39: 0.113 g, 5.3%). The following data are given for the α-anomer. $[\alpha]_D^{25}$ −58.0 (*c* 0.56, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H, 7.4–7.1 (m, 9H, aromatic), 5.39 (d, 1H, $J_{2,1}$ 5.1 Hz, H-2), 5.26 (d, 1H, H-1), 4.50 (s, 2H, OCH_2Ph), 4.30, 4.18 (2d, 2H, $J_{4a,4b}$ 10.5 Hz, H-4a, H-4b), 4.03, 3.74 (2d, 2H, $J_{3'a,3'b}$ 10.2 Hz, H-3′a, H-3′b), 2.32 (s, 3H, $SC_6H_4CH_3$), 2.09, 2.07 (2s, 6H, OCOCH_3); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C, 170.0, 169.5 (*C*=O acetate), 138.4, 137.9, 133.5, 130.0, 129.2, 128.6, 128.0, 127.9 (aromatic), 89.8 (C-1), 85.1 (C-3), 76.2 (C-2), 73.7 (OCH_2Ph), 73.2 (C-4), 68.9 (C-3′), 21.7, 20.9 (OCOCH_3), 21.3 ($SC_6H_4CH_3$). Anal. Calcd for $C_{23}H_{26}O_6S$: C, 64.17; H, 6.09. Found: C, 63.88; H, 6.38.

3.3. Methyl (2,3-di-*O*-acetyl-3-*C*-(benzyloxymethyl)-β-D-erythrofuranosyl)-(1→2)-6-*O*-(*tert*-butyldiphenyl)silyl-3,4-*O*-isopropylidene-α-D-galactopyranoside (9)

Compound **9** was prepared using four different procedures, as summarized in Table 1.

3.3.1. Table 1, entry 1. 1,2,3-Tri-*O*-acetyl-3-*C*-(benzyloxymethyl)-α/β-D-erythrofuranoside **4**[11] (0.180 g, 0.491 mmol) and galactose derivative **8**[14] (0.255 g, 0.540 mmol) were dissolved in CH_2Cl_2 (7.5 mL). Pow-

dered 4 Å molecular sieves (0.4 g) were added and after the mixture was stirred under N_2 at 0 °C for 1.5 h. $BF_3 \cdot Et_2O$ (0.10 mL, 0.77 mmol) was added. After being stirred for an additional 10 min the reaction mixture was neutralized with Et_3N, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was washed successively with H_2O and brine. The filtrate was dried over Na_2SO_4, filtered, and concentrated to dryness and purified by chromatography using 5:1 hexane–ethyl acetate as eluant. Compound **9** was obtained as a syrup (0.158 g, 41.4%).

3.3.2. Table 1, entry 2. Samples of **4**[11] (0.0695 g, 0.190 mmol) and **8**[14] (0.0767 g, 0.162 mmol) were dissolved in CH_2Cl_2 (10.0 mL). Powdered 4 Å molecular sieves (0.1 g) were added and after the mixture was stirred under N_2 at 25 °C for 45 min trimethylsilyl trifluoromethanesulfonate (5 μL, 0.03 mmol) was added to the mixture. After being stirred for an additional 10 min, the reaction mixture was neutralized with Et_3N, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was washed successively with satd aq $NaHCO_3$ and H_2O. The filtrate was dried over Na_2SO_4, filtered, and concentrated to dryness and purified by chromatography using 2:1 hexane–ethyl acetate as eluant. Compound **9** was obtained as a syrup (0.0742 g, 58.7%).

3.3.3. Table 1, entry 3. A mixture of thioglycoside **6**[12] (0.0836 g, 0.226 mmol) and galactose derivative **8**[14] (0.0840 g, 0.178 mmol) was dissolved in CH_2Cl_2 (10.0 mL). Powdered 4 Å molecular sieves (0.1 g) were added and after the mixture was stirred under N_2 at 0 °C for 45 min. *N*-iodosuccinimide (0.0521 g, 0.232 mmol) and silver triflate (0.0062 g, 0.024 mmol) were added. After being stirred for an additional 10 min the reaction mixture was neutralized with Et_3N, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was washed successively with satd aq $Na_2S_2O_3$ and H_2O. The filtrate was dried over Na_2SO_4, filtered, and concentrated to dryness and purified by chromatography using 2:1 hexane–ethyl acetate as eluant. Compound **9** was obtained as a syrup (0.0911 g, 65.8%).

3.3.4. Table 1, entry 4. A mixture of thioglycoside **7** (α-anomer, 0.508 g, 1.18 mmol) and galactose derivative **8**[14] (0.483 g, 1.02 mmol) was dissolved in CH_2Cl_2 (10.0 mL). Powdered 4 Å molecular sieves (1.0 g) were added and after the mixture was stirred under N_2 at 0 °C for 1 h. *N*-iodosuccinimide (0.347 g, 1.54 mmol) and silver triflate (0.108 g, 0.420 mmol) were added. After being stirred for 10 min the reaction mixture was neutralized with Et_3N, diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed successively with satd aq $Na_2S_2O_3$, H_2O, and brine. The filtrate was dried over Na_2SO_4, filtered, concentrated to dryness, and purified by chromatography using 5:1 hexane–ethyl acetate as eluant. Compound **9** was obtained as a syrup (0.616 g, 77.4%); $[\alpha]_D^{25}$ +19.5 (*c* 0.78, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H, 7.8–7.2 (m, 15H, aromatic), 5.48 (br s, 1H, H-2 Api), 5.24 (br s, 1H, H-1 Api), 4.66 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1 Gal), 4.60, 4.51 (2d, 2H, $J_{Ha,Hb}$ 12.2 Hz, OCH_2Ph), 4.23 (1H, H-4 Gal), 4.18, 3.96 (2d, 2H, $J_{4a,4b}$ 11.1 Hz, H-4a, H-4b Api), 4.18 (s, 2H, H-3′a, H-3′b Api), 4.17 (1H, H-3 Gal), 3.99 (1H, H-5 Gal), 3.92, 3.84 (2H, H-6a, H-6b Gal), 3.68 (dd, 1H, $J_{2,3}$ 7.9 Hz, H-2 Gal), 3.28 (s, 3H, OCH_3), 2.09, 2.06 (2s, 6H, OCOCH_3), 1.50, 1.33 (2s, 6H, C(CH_3)$_2$), 1.06 (s, 9H, C(CH_3)$_3$); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C, 170.2, 169.2 (*C*=O acetate), 138.2, 135.8, 133.7, 129.9, 128.6, 127.9, 127.8, 127.7 (aromatic), 109.3 (*C*(CH$_3$)$_2$), 106.4 (C-1 Api), 99.2 (C-1 Gal), 85.9 (C-3 Api), 77.2 (C-2 Api), 76.3 (C-2 Gal), 75.4 (C-3 Gal), 73.6 (O*C*H$_2$Ph, C-3′ Api), 73.4 (C-4 Gal), 70.1 (C-4 Api), 67.7 (C-5 Gal), 63.1 (C-6 Gal), 55.5 (O*C*H$_3$), 28.5, 26.6 (C(*C*H$_3$)$_2$), 27.0 (C(*C*H$_3$)$_3$), 21.9, 21.5 (OCO*C*$_3$). Anal. Calcd for $C_{42}H_{54}O_{12}Si$: C, 64.76; H, 6.99. Found: C, 64.40; H, 7.01.

3.4. Methyl (2,3-di-*O*-acetyl-3-*C*-(benzyloxymethyl)-β-D-erythrofuranosyl)-(1→2)-3,4-*O*-isopropylidene-α-D-galactopyranoside (10)

Disaccharide **9** (0.446 g, 0.573 mmol) was dissolved in THF (25 mL) and cooled to 0 °C. To this solution was added 1.0 M tetrabutyl ammonium fluoride solution (0.6 mL). After 4.5 h satd aq NH_4Cl solution (10.0 mL) was added. The reaction mixture was extracted with EtOAc and the combined extracts were dried over Na_2SO_4, filtered, concentrated to dryness, and purified by chromatography using 1:1 hexane–ethyl acetate as eluant. Compound **10** was obtained as a syrup (0.254 g, 82.1%); $[\alpha]_D^{25}$ +42.1 (*c* 0.70, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H, 7.4–7.2 (m, 5H, aromatic), 5.44 (br s, 1H, H-2 Api), 5.21 (br s, 1H, H-1 Api), 4.71 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1 Gal), 4.56, 4.49 (2d, 2H, $J_{Ha,Hb}$ 12.2 Hz, OCH_2Ph), 4.18, 4.13 (2d, 2H, $J_{3a',3b'}$ 8.5 Hz, H-3′a, H-3′b Api), 4.17 (1H, H-3 Gal), 4.16 (1H, H-4 Gal), 4.14, 3.93 (2d, 2H, $J_{4a,4b}$ 10.5 Hz, H-4a, H-4b Api), 3.96 (1H, H-5 Gal), 3.89, 3.78 (2H, H-6a, H-6b Gal), 3.66 (dd, 1H $J_{2,3}$ 7.3 Hz, H-2 Gal), 3.30 (s, 3H, OCH_3), 2.06, 2.03 (2s, 6H, OCOCH_3), 1.48, 1.30 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C, 170.2, 169.3 (*C*=O acetate), 138.1, 128.5, 127.8, 127.7 (aromatic), 109.7 (O*C*(CH$_3$)$_2$), 106.4 (C-1 Api), 99.3 (C-1 Gal), 85.7 (C-3 Api), 76.8 (C-2 Api), 76.1 (C-2 Gal), 75.4 (C-3 Gal), 74.4 (C-4 Gal), 73.6 (C-3′ Api, O*C*H$_2$OPh), 69.9 (C-4 Api), 67.3 (C-5 Gal), 62.7 (C-6 Gal), 55.6 (O*C*H$_3$), 28.4, 26.6 (OC(*C*H$_3$)$_2$), 21.5, 20.8 (OCO*C*H$_3$). Anal. Calcd for $C_{26}H_{36}O_{12}$: C, 57.77; H, 6.71. Found: C, 57.43; H, 6.73.

3.5. Methyl (3-*C*-(benzyloxymethyl)-β-D-erythrofuranosyl)-(1→2)-α-D-galactopyranoside (11)

Disaccharide **10** (0.403 g, 0.745 mmol) was dissolved in CH_3OH and the solution was made basic (pH 11.0) by the addition of $NaOCH_3$ (1.0 M). After 45 min, the reaction mixture was neutralized by the addition of Amberlyst 15 acid resin beads. The resin was removed by filtration and the filtrate concentrated to dryness. The syrup was dissolved in EtOH (2.5 mL) and the solution was made acidic by the addition of 0.5 M HCl (2.5 mL). The reaction mixture was stirred at 35 °C for 1 h and then neutralized by the addition of Amberlyst A-26(OH) resin beads. The resin was removed by filtration and the filtrate concentrated to dryness. The crude syrup was purified by chromatography using ethyl acetate–hexane–methanol–water (7:2:2:0.5) as eluant. Compound **11** was obtained as a syrup (0.245 g, 79.0%); $[\alpha]_D^{25}$ +28.1 (*c* 1.0, H_2O); ^1H NMR (600 MHz, D_2O): δ_H, 7.5–7.4 (m, 5H, aromatic), 5.19 (d, 1H, $J_{1,2}$ 2.7 Hz, H-1 Api), 4.92 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1 Gal), 4.66, 4.62 (2d, 2H, $J_{Ha,Hb}$ 11.9 Hz, OCH_2Ph), 4.04 (d, 1H, H-2 Api), 4.02, 3.92 (2d, 2H, $J_{4a,4b}$ 10.3 Hz, H-4a, H-4b Api), 3.99 (br d, 1H, H-4 Gal), 3.88 (m, 1H, H-5 Gal), 3.86 (dd, 1H $J_{3,2}$ 9.9 Hz, $J_{3,4}$ 3.4 Hz, H-3 Gal), 3.78 (dd, 1H, H-2 Gal), 3.76 (dd, 1H, $J_{6a,5}$ 6.1 Hz, $J_{6a,6b}$ 11.6 Hz, H-6a Gal), 3.73 (dd, 1H, $J_{6b,5}$ 4.6 Hz, H-6b Gal), 3.70, 3.66 (2d, 2H, $J_{3a',3b'}$ 10.5 Hz, H-3′a, H-3′b Api), 3.38 (s, 3H, OCH_3); ^{13}C NMR (125 MHz, D_2O): δ_C, 138.2, 129.6, 129.3, 129.2 (aromatic), 111.0 (C-1 Api), 99.9 (C-1 Gal), 79.7 (C-3 Api), 78.4 (C-2 Api), 77.2 (C-2 Gal), 74.8 (C-4 Api), 74.3 (OCH$_2$Ph), 73.0 (C-3′ Api), 71.7 (C-5 Gal), 70.0 (C-4 Gal), 69.4 (C-3 Gal), 62.0 (C-6 Gal), 55.7 (OCH$_3$). HRMS found *m*/*z* 439.1572 [M+Na$^+$]. Calcd for $C_{19}H_{28}O_{10}Na$ 439.1575.

3.6. Methyl (2,3-di-*O*-acetyl-3-*C*-(benzyloxymethyl)-β-D-erythrofuranosyl)-(1→2) (methyl 3,4-*O*-isopropylidene-α-D-galactopyranosid)uronate (12)

Compound **12** was prepared by two methods.

3.6.1. Preparation of 12 by oxidation and esterification of 10. Disaccharide **10** (0.124 g, 0.229 mmol) was dissolved in CH_2Cl_2 (5 mL) and TEMPO (3 mg) was added. Five milliliters of a basic KBr/*n*-Bu_4Cl solution (12 mL satd aq $NaHCO_3$, 7.5 mg KBr, 10 mg *n*-Bu_4Cl) was added and the reaction mixture was cooled in an ice bath. An NaOCl solution was prepared (3.0 mL 10–13% NaOCl, 3.0 mL satd aq NaCl, 1.5 mL satd aq $NaHCO_3$) and 1.5 mL was added, dropwise over 10 min to the reaction mixture. After 60 min, all of the starting material was consumed and a slower moving spot was observed on TLC (8:1.5:0.5 EtOAc–CH_3OH–H_2O) that migrated (R_f approx. 0.5) with considerable streaking. The reaction mixture was transferred to a separatory funnel and the organic layer was washed with H_2O (3 × 15 mL). The combined aqueous fractions were acidified by the addition of 1.0 M HCl. The acidified aqueous layer was then extracted with EtOAc (5 × 15 mL). The combined EtOAc extracts were dried over Na_2SO_4, filtered, and concentrated to dryness. The residue was taken up in CH_3Cl (10 mL) and ethereal solution of CH_2N_2 was added until the yellow color of the CH_2N_2 persisted. The reaction mixture was diluted with CH_2Cl_2 and washed successively with satd aq $NaHCO_3$ and H_2O. The organic layer was dried over Na_2SO_4, filtered, concentrated to dryness, and purified by chromatography using 2:1 hexane–ethyl acetate as eluant. Compound **12** was obtained as a syrup (0.0947 g, 72.7%). Analytical data are given below in Section 3.6.2.

3.6.2. Preparation of 12 by glycosylation of galacturonate ester derivative (14). Thioglycoside **7** (0.193 g, 0.449 mmol) and galacturonate ester derivative **14**[19,20] (0.131 g, 0.501 mmol) were dissolved in CH_2Cl_2 (18 mL). This solution was stirred with 4 Å molecular sieves (1.2 g) under N_2 for 2 h. The mixture was cooled to 0 °C and silver triflate (0.0267 g, 0.104 mmol) was added followed by the addition of *N*-iodosuccinimide (0.151 g, 0.671 mmol). After being stirred for 10 min, the reaction was quenched with Et_3N, diluted with CH_2Cl_2, and filtered through Celite. The filtrate was washed successively with satd aq $Na_2S_2O_3$, H_2O, and brine. The filtrate was dried over Na_2SO_4, filtered, concentrated to dryness, and purified by chromatography using 2:1 hexane–ethyl acetate as eluant. Compound **12** was obtained as a syrup (0.213 g, 83.5%); $[\alpha]_D^{25}$ +22.4 (*c* 0.68, $CHCl_3$); ^1H NMR (300.13 MHz, $CDCl_3$): δ_H, 7.35–7.20 (m, 5H, aromatic), 5.43 (s, 1H, H-2 Api), 5.19 (s, 1H, H-1 Api), 4.82 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1 GalA), 4.55, 4.48 (2d, 2H, OCH_2Ph), 4.54, (1H, H-5 GalA), 4.43 (dd, 1H, $J_{4,3}$ 5.5 Hz, $J_{4,5}$ 3.0 Hz, H-4 GalA), 4.20 (dd, 1H, $J_{3,2}$ 7.9 Hz, H-3 GalA), 4.16, 4.13 (2d, 2H, $J_{4a,4b}$ 10.4 Hz, H-4a, H-4b Api), 4.12, 3.91 (2d, 2H, $J_{3a',3b'}$ 10.5 Hz, H-3′a, H-3′b Api), 3.81 (s, 3H, CO_2CH_3), 3.74 (dd, 1H, H-2 GalA), 3.31 (s, 3H, OCH_3), 2.06, 2.03 (2s, 6H, OCOCH_3), 1.47, 1.30 (2s, 6H, C(CH_3)$_2$); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C, 170.2, 169.2, 168.6 (*C*=O), 138.1, 128.6, 127.8, 127.7 (aromatic), 110.0 (*C*(CH$_3$)$_2$), 106.5 (C-1 Api), 99.8 (C-1 GalA), 85.7 (C-3 Api), 76.8 (C-2 Api), 75.3 (C-2 GalA), 75.0 (C-3 GalA), 73.0 (C-4 GalA), 73.6 (C-4 Api, OCH$_2$Ph), 69.9 (C-3′Api), 67.4 (C-5 GalA), 56.3 (OCH$_3$), 52.7 (CO_2CH_3), 28.2, 26.5 (C(*C*H$_3$)$_2$), 21.5, 20.8 (OCO*C*H$_3$). Anal. Calcd for $C_{27}H_{36}O_{13}$: C, 57.04; H, 6.38. Found: C, 56.97; H, 6.53.

3.7. Methyl (2,3-di-*O*-acetyl-3-*C*-(hydroxymethyl)-β-D-erythrofuranosyl)-(1→2) (methyl 3,4-*O*-isopropylidene-α-D-galactopyranosid)uronate (13)

Compound **12** (0.111 g 0.195 mmol) was dissolved in 23 mL of ethyl acetate–methanol–acetic acid (5:5:1). Pal-

ladium(II) acetate (0.142 g, 0.633 mmol) was added and the solution was shaken under H_2 (44 p.s.i) for 45 min. The black precipitate that formed was removed by filtration through a bed of Celite, and the filtrate was concentrated to dryness. The crude syrup was purified by chromatography using hexane–ethyl acetate (2:1) as eluant. The title compound **13** was obtained as a syrup (0.0842 g, 90.2%); $[\alpha]_D^{25}$ +8.50 (*c* 0.60, $CHCl_3$); ^{1}H NMR (300.13 MHz, $CDCl_3$): δ_H, 5.33 (br s, 1H, H-1 Api), 5.04 (br s, 1H, H-2 Api), 4.91 (d, 1H, $J_{1,2}$ 3.5 Hz, H-1 GalA), 4.58 (d, 1H, $J_{5,4}$ 2.8 Hz, H-5 GalA), 4.47 (dd, 1H, $J_{4,3}$ 5.5 Hz, H-4 GalA), 4.43, 4.27 (2d, 2H, $J_{4a,4b}$ 11.9 Hz, H-4a, H-4b Api), 4.28 (dd, 1H, $J_{3,2}$ 7.8 Hz, H-3 GalA), 3.92, 3.87 (2d, 2H, $J_{3a',3b'}$ 9.9, H-3′a, H-3′b Api), 3.83 (s, 3H, CO_2CH_3), 3.78 (dd, 1H, H-2 GalA), 3.41 (s, 3H, OCH_3), 2.14, 2.11 (2s, 6H, $OCOCH_3$), 1.50, 1.33 (2s, 6H, $C(CH_3)_2$); ^{13}C NMR (75.03 MHz, $CDCl_3$): δ_C, 171.4, 170.4, 168.6 (*C*=O), 110.0 ($C(CH_3)_2$), 107.4 (C-1 Api), 99.9 (C-1 GalA), 79.1 (C-3 Api), 78.7 (C-2 Api), 75.6 (C-2 GalA), 75.2 (C-3 GalA), 74.5 (C-3′ Api), 73.9 (C-4 GalA), 67.7 (C-4 Api), 67.3 (C-5 GalA), 56.4 (OCH_3), 52.7 (CO_2CH_3), 28.3, 26.6 ($C(CH_3)_2$), 20.95, 20.90 ($OCOCH_3$). Anal. Calcd for $C_{20}H_{30}O_{13}$: C, 50.21; H, 6.32. Found: C, 50.14; H, 6.33.

3.8. Methyl ((3-*C*-hydroxymethyl)-β-D-erythrofuranosyl)-(1→2)-α-D-galactopyranosiduronic acid (1)

Disaccharide **13** (0.446 g, 0.933 mmol) was dissolved in CH_3OH (25 mL). The solution was made basic (pH 9.0) by the dropwise addition of $NaOCH_3$ (1.0 M). After 15 min, the reaction was complete and the solution was neutralized by the addition of Amberlyst 15 H^+ resin beads. The resin was removed by filtration and the filtrate concentrated to dryness. The residue was taken up in H_2O (45 mL) and made acidic (pH 2.5) by the addition of Amberlyst 15 H^+ resin beads. After 18 h at room temperature, the resin was removed by filtration. The filtrate was made basic (pH 10–11) by the dropwise addition of NaOH (1.0 M). The pH was maintained at 10–11 by adding addition amounts of NaOH as needed. After 2 h, the solution was neutralized by the addition of HCl (1.0 M). The solution was concentrated to dryness. The title compound **1** was obtained following chromatography of the residue on a column of BioGel P2 using H_2O as eluant (0.268 g, 84.5%); $[\alpha]_D^{25}$ +3.53 (*c* 0.85, H_2O); ^{1}H NMR (300.13 MHz, D_2O): δ_H, 5.10 (d, 1H, $J_{1,2}$ 3.0 Hz, H-1 Api), 4.88 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1 GalA), 4.19 (dd, 1H, $J_{4,3}$ 3.5 Hz, $J_{4,5}$ 1.3 Hz, H-4 GalA), 3.95 (d, 1H, H-2 Api), 3.94, 3.81 (2d, 2H, $J_{4a,4b}$ 10.2 Hz, H-4a, H-4b Api), 3.85 (dd, 1H, H-3 GalA), 3.72 (dd, 1H, $J_{2,3}$ 10.2 Hz, H-2 GalA), 3.59 (s, 2H, H-3a, H-3b Api), 3.30 (s, 3H, OCH_3); ^{13}C NMR (75.03 MHz, D_2O): δ_C, 177.1 (CO_2H), 111.9 (C-1 Api), 100.5 (C-1 GalA), 81.8 (C-3 Api), 78.7 (C-2 Api), 77.8 (C-2 GalA), 75.2 (C-4 Api), 72.7 (C-5 GalA), 72.2 (C-4 GalA), 70.1 (C-3 GalA), 65.2 (C-3′ Api), 56.6 (OCH_3). HRMS (negative mode) found *m/z* 339.0922 $[MH]^-$. Calcd for $C_{12}H_{19}O_{11}$ 339.0922.

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