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Synthesis of an apiose-containing disaccharide fragment of rhamnogalacturonan-II and some analogues

Anne-Laure Chauvin, Sergey A. Nepogodiev* and Robert A. Field

Centre for Carbohydrate Chemistry, School of Chemical Sciences and Pharmacy, University of East Anglia, Norwich NR4 7TJ, UK

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Abstract—β-Rhamnosylation of methyl 2-*C*-hydroxymethyl-2,3-*O*-isopropylidene-β-D-erythrofuranoside and methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside was achieved using 4-*O*-acetyl-2,3-*O*-carbonyl-α-L-rhamnopyranosyl bromide and Ag_2O as a promoter. Deprotected disaccharides β-L-Rha*p*-(1 → 3′)-β-D-Api*f*-OMe and β-L-Rha*p*-(1 → 3′)-β-D-Rib*f*-OMe were compared to their α-rhamnosyl isomers which were prepared using conventional Helferich glycosylation.

Keywords: Cell walls; Higher plants; Pectins

1. Introduction

Primary cell walls of all higher plants contain rhamnogalacturonan-II (RG-II), a unique 'mega-oligosaccharide' that belongs to the pectic polysaccharide family.[1] The uniqueness of RG-II within this family is in its very elaborate, but highly conserved glycan sequence. The RG-II structure is composed of four oligosaccharide side chains attached to an oligogalacturonan backbone. Details of the side chain structures have been elucidated on the basis of methylation analysis of RG-II, as well as mass spectrometric analysis of RG-II fragments generated by acid or enzymatic hydrolysis.[2,3] Some insight into the three-dimensional structure of RG-II has been obtained through the use of high-field NMR spectroscopy and molecular modelling.[4] It has also been shown[5,6] that RG-II self-assembles into a dimer that is cross-linked by a 1:2 borate–diol ester. This cross-linking happens on account of the 1,2-*cis*-diol present in apiofuranose residues of RG-II and it is believed that only Api*f* of the so-called side chain A (Fig. 1) is involved in this process.[6] Some experiments indicate[7] that plant growth and health depends on RG-II cross-linking, which defines organisation of wall pectic polysaccharide networks.

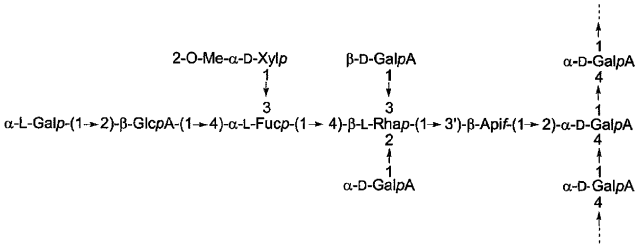

Figure 1. Structure of the side chain A octasaccharide of rhamnogalacturonan-II shown with a trisaccharide region of polygalacturonan backbone.

To obtain a better understanding of this intriguing complexation phenomenon, as well as for comprehensive structural characterisation of RG-II, synthetic fragments of this mega-oligosaccharide would be very useful. In this article we describe the synthesis of β-rhamnosyl-apiose disaccharide **1** (Fig. 2), which is located at a focal point of two different side chains of RG-II, the so-called side chain A (Fig. 1) and side chain B. We have also synthesised a stereoisomer of **1** (the α-rhamnoside **3**), and regioisomers of **1** and **3** (disaccharides **2** and **4**), which incorporate ribofuranose instead

* Corresponding author. Fax: +44-1603-592003; e-mail: s.nepogodiev@uea.ac.uk

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β-L-Rha*p*-(1→3')-β-D-Api*f*-OMe (1)
β-L-Rha*p*-(1→5)-β-D-Rib*f*-OMe (2)
α-L-Rha*p*-(1→3')-β-D-Api*f*-OMe (3)
α-L-Rha*p*-(1→5)-β-D-Rib*f*-OMe (4)

Figure 2. Structures of target disaccharides.

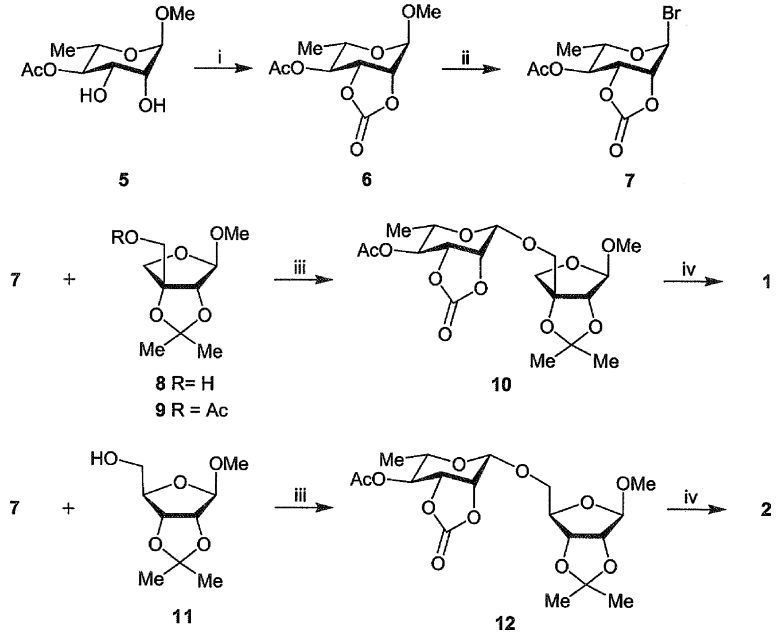
of apiofuranose. D-Ribofuranose is a diastereoisomer of apiose, which differs from the D-erythrofuranose form of apiose only by the position of the exocyclic hydroxymethyl group.

2. Results and discussion

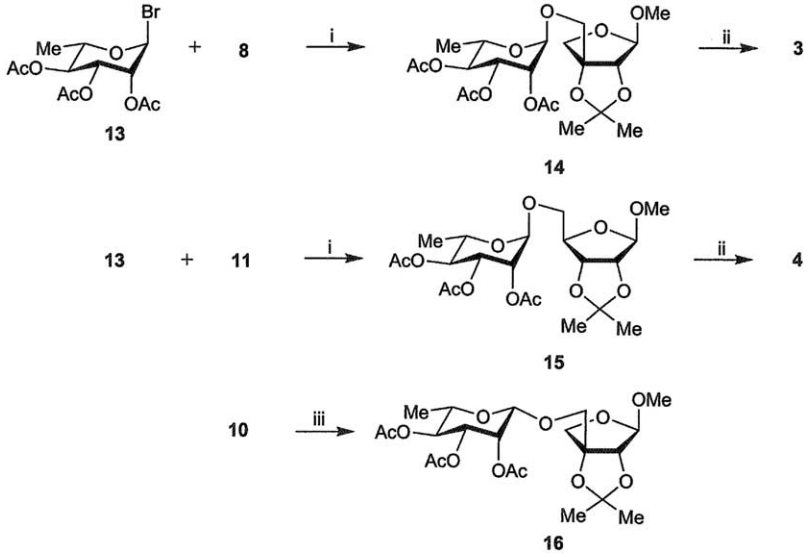
Target disaccharides **1** and **2** incorporate synthetically challenging β-rhamnopyranosidic linkages, the construction of which is similar to the problem of β-mannosylation. The latter has received special attention from synthetic chemists on account of the importance of this type of glycosidic linkage in natural glycoconjugates and several direct and indirect glycosylation methods have been developed.[8–10] Though rhamnose and mannose systems are structurally similar, the 6-deoxy function present in rhamnose imposes certain limitation on the applicability of some methods developed for β-mannosylation. Thus, in an attempt to synthesise disaccharide **1** using Lichtenthaler's 2-oxoglycosyl donor method, the target compound was not formed.[11] Instead, reduction of the intermediate 2′-oxoglycoside led to the undesirable C-2 epimeric L-quinovose-containing disaccharide. Procedures specific for making β-rhamnopyranosides have been reported very recently.[12,13]

For the preparation of **1** and **2** initially we have tried to employ a direct β-rhamnosylation method[12] consisting of coupling sugar triflates with the unprotected 1,2-*O*-stannylene acetal of L-rhamnopyranose. However, reaction of the model 5-triflate of methyl 2,3-*O*-isopropylidene β-D-ribofuranose with this stannylene acetal led to disaccharide in very poor yield. Apparently, the 5-*O*-triflyl derivative of ribose was unstable under reaction conditions used for this coupling. We subsequently adopted a classical glycosylation approach with glycosyl bromides and insoluble silver salt promoters. In the particular case of β-rhamnoside synthesis, two procedures based either on 2,3-*O*-carbonyl[14] or 2,3-*O*-cyclohexylidene[15] derivatives are available. In our syntheses, to provide the possibility for further use of synthetic disaccharides in preparation of larger RG-II fragments, we employed 2,3-carbonate **7** (Scheme 1) and known glycosyl acceptors **8**[16] and **11**,[17] which have isopropylidene protecting groups that allow selective deprotection of individual monosaccharide residues. Cyclic carbonate **6** was prepared in excellent yield from known diol **5** using triphosgene,[18] rather than the previously reported methyl chloroformate procedure,[14] and then converted in two steps into known glycosyl bromide **7**.[14]

Glycosylation of 2,3-*O*-isopropylidene derivatives of apiofuranose **8** and ribofuranose **11** with glycosyl bromide **7** was carried out in the presence of Ag_2O and afforded β-rhamnosides **10** and **12** in 57% and 61% yield,



Scheme 1. Synthesis of β-rhamnopyranosides. Reagents and conditions: (i) $(Cl_3CO)_2CO$, pyridine, CH_2Cl_2, −70 °C, 97%; (ii) 1. 0.5% H_2SO_4 in Ac_2O, 2. HBr, AcOH, 87%; (iii) Ag_2O, mol sieves 4 Å, CH_2Cl_2; 57% (for **10**), 61% (for **12**); (iv) 1. 0.1 M NaOMe in MeOH; 2. Amberlite IRA-120 (H^+), MeOH, 92% (for **1**), 68% (for **2**).



Scheme 2. Synthesis of α-rhamnopyranosides. Reagents and conditions: (i) $Hg(CN)_2$, MeCN, 47% (for **14**), 87% (for **15**); (ii) 1. 0.1 M NaOMe in MeOH; 2. Amberlite IRA-120 (H^+), MeOH, 45% (for **3**), 94% (for **4**); (iii) 1. NaOMe, MeOH, 2. Ac_2O, pyridine, 59%.

respectively. In order to facilitate chromatographic separation of **10** from unreacted alcohol **8**, the mixture of reaction products was treated with Ac_2O–pyridine to convert the latter into methyl 3-*C*-acetoxymethyl-2,3-*O*-isopropylidene-β-D-erythrofuranoside (**9**). 1,2-*trans*-Rhamnosides **14** and **15** were synthesised in 87% and 92% yield, respectively, by $Hg(CN)_2$-promoted reaction of alcohols **8** and **11** with rhamnosyl bromide **13** (Scheme 2).

Assignment of NMR spectra of protected disaccharides **10**, **12**, **14** and **15** was made on the basis of 2D COSY and HSQC experiments. However, stereochemical assignment of rhamnopyranosidic linkages was not obvious from these spectra. Coupling constants ($^1J_{CH}$) for anomeric carbon, which are often employed for determination of anomeric configuration,[19] were also not diagnostic. Thus, β-rhamnosides **10** and **12** have $^1J_{CH}$ values that are rather close to those for α-rhamnosides **14** and **15** and methyl rhamnoside **6** with an established α-anomeric configuration (Table 1). In rhamnopyranosides **10** and **12**, the pyranose ring is flattened by the fused 2,3-*O*-carbonyl group, which results in anomalous values for anomeric $^1J_{CH}$ coupling constants. Crich and Li suggested[20] another way for the assignment of glycosidic linkages in rhamnosides bearing 2,3-*O*-carbonyl group based on the observation of a very small but detectable difference between $^3J_{H\text{-}1,H\text{-}2}$ coupling constants for α-rhamnosides (<1 Hz) and β-rhamnosides (~3 Hz). However, accurate measurement of these coupling constants in ^1H NMR spectra of **10** and **12** was not possible since resonances of H-1 of rhamnopyranosyl residues in these spectra appear as broad singlets whereas signals of H-2 and H-3 chemical shifts close to unresolved multiplets. Therefore, to confirm the β-linkage for the rhamnoside **10**, its rhamnose moiety was deprotected and acetylated to give compound **16**, which allowed direct comparison with assumed α-rhamnoside **14**. Indeed, coupled ^{13}C NMR spectrum of **16** showed a typical β-rhamnoside $^1J_{CH}$ value (156 Hz), which differs noticeably from $^1J_{CH}$ (172 Hz) for the isomeric α-rhamnoside **14**. Since rhamnopyranosidic signals are essentially the same in NMR spectra of **10** and **12**, the latter disaccharide is obviously a β-rhamnoside as well.

Table 1. ^{13}C chemical shifts and coupling constants $^1J_{CH}$ for C-1 of methyl rhamnopyranoside **6** and rhamnopyranosyl residues of disaccharides **10**, **12** and **14**–**16**

	Compound					
	6	**10**	**12**	**14**	**15**	**16**
δ C-1 Rha	96.0	95.2	96.0	97.7	97.9	98.2
$^1J_{CH}$ (Hz)	172	168	164	172	173	159

Deprotection of disaccharide derivatives was carried out in two steps; they were first saponified by NaOMe in methanol and then isopropylidene groups were removed by careful treatment with cation-exchange resin (H^+) in methanol. The use of stronger acidic conditions (e.g. 90% aq CF_3CO_2H) led not only to the desired deacetonation, but also to the partial hydrolysis of the methyl apiofuranoside unit. Finally, purification on silica gel with CH_2Cl_2–MeOH afforded target disaccharides **1**–**4**. ^1H and ^{13}C NMR spectroscopic data of **1**–**4** were in agreement with the expected disaccharide structures. Values of the $J_{1,2}$ coupling constants observed for apiofuranoside residues in compounds **1** and **3** were 3.8 and 3.5 Hz, respectively. These values are unusually large for 1,2-*trans*-furanosides, which typically have $J_{1,2} < 1$ Hz, but they are in a good agreement with $J_{1,2}$

value of 3.7 Hz reported for methyl 3-*C*-hydroxymethyl-β-D-erythrofuranoside.[21]

In summary, synthesis of a disaccharide fragment of RG-II incorporating β-linked rhamnopyranose and apiofuranose has been accomplished for the first time, along with the preparation of its analogues and isomers incorporating β-D-ribofuranose and/or α-L-rhamnopyranose residues. The combination of protecting groups in β-L-Rha*p*-(1→3′)-β-D-Api*f* derivative (**14**) makes this disaccharide a suitable precursor for construction of larger oligosaccharide fragments of both chain A and chain B of RG-II, which is currently underway in our laboratories.

3. Experimental

All solvents were used as supplied, except for CH_2Cl_2, which was freshly distilled from CaH_2, MeOH, which was distilled from $Mg(OMe)_2$ and pyridine, which was distilled from P_2O_5 and kept dry over 4 Å molecular sieves. Cation-exchange resin (Amberlite IRA120, H^+-form, Fluka) was prewashed with water and dry MeOH before use. Thin-layer chromatography (TLC) was performed on aluminium-backed, precoated silica gel plates (Silica Gel 60 F_{254}, Merck). Spots were detected by immersion in a 5% ethanolic solution of H_2SO_4, followed by heating to 200 °C. Column chromatography was performed on silica gel (40–70 μm, BDH–Merck). Evaporation of solvents was performed under reduced pressure at 25–40 °C. Reagents and dry solvents were added via syringes through septa. Melting points were determined using a Gallenkamp melting point apparatus and are uncorrected. Optical rotations were measured at 18 °C using a Perkin–Elmer 141 polarimeter. ^1H and ^{13}C NMR spectra were recorded at 24 °C with a Varian Gemini 2000 spectrometer at 300 and 75 MHz, respectively, or with a Varian Unity Plus spectrometer at 400 and 100.6 MHz, respectively, using Me_4Si (for solutions in $CDCl_3$) or MeOH (δ 49.9, for solutions in D_2O). Resonance allocations were made with the aid of COSY and HSQC experiments when necessary. IR spectra were recorded in Nujol on a Perkin–Elmer 298 spectrometer. Accurate mass electrospray ionisation (ESI) mass spectra were obtained on Finnigan MAT 900 XLT mass spectrometer.

3.1. Methyl 4-*O*-acetyl-2,3-*O*-carbonyl-α-L-rhamnopyranoside (6)

A solution of methyl 4-*O*-acetyl-α-L-rhamnopyranoside[22] (**5**, 2.30 g, 10.45 mmol) in CH_2Cl_2 (40 mL) containing pyridine (9 mL) was stirred at −70 °C. Triphosgene (1.60 g, 5.25 mmol) in CH_2Cl_2 (25 mL) was added dropwise during 10 min and the mixture was allowed to warm to room temperature. The mixture was treated with aq satd NH_4Cl solution, washed with 1 M aq HCl solution, satd aq $NaHCO_3$, dried and concentrated in vacuo. Crystallisation from toluene–hexane afforded the title compound **6** (2.51 g, 97%). $R_f = 0.49$ [toluene–EtOAc (2:1)]; mp: 132–134 °C (lit.,[14] 137–138 °C); $[\alpha]_D$ −28.1 (*c* 0.52, $CHCl_3$) (lit.,[14] −22.1); ν_{max} (cm^{-1}, Nujol): 1749 (C=O), 1826 cm^{-1} (C=O carbonate); ^1H NMR (400 MHz, $CDCl_3$): δ 1.25 (3H, d, $J_{5,6}$ 6.4 Hz, H-6), 2.13 (3H, s, OCOCH_3), 3.40 (3H, s, OCH_3), 3.80 (1H, m, H-5), 4.65 (1H, d, $J_{2,3}$ 7.0 Hz, H-2), 4.74 (1H, pseudo t, $J_{2,3} \approx J_{3,4} \sim 7.1$ Hz, H-3), 4.90 (1H, dd, $J_{3,4}$ 7.3 Hz, $J_{4,5}$ 9.7 Hz, H-4), 4.97 (1H, s, H-1); δ_C (100.6 MHz, $CDCl_3$): 17.0 (C-6), 21.2 (OCO*C*H_3), 55.2 (O*C*H_3), 63.0 (C-5), 72.7, 76.1 (C-2, C-3), 76.4 (C-4), 96.2 (C-1), 153.15 (C=O carbonate), 169.6 (O*C*OCH$_3$).

3.2. Methyl 3-*C*-(4-*O*-acetyl-2,3-*O*-carbonyl-β-L-rhamnopyranosyloxymethyl)-2,3-*O*-isopropylidene-β-D-erythrofuranoside (10)

A mixture of methyl 3-*C*-hydroxymethyl-2,3-*O*-isopropylidene-β-D-erythrofuranoside[16] (**8**, 480 mg, 2.35 mmol), Ag_2O (1.25 g, 5.4 mmol) and molecular sieves 4 Å (6.0 g) in CH_2Cl_2 (10 mL) was stirred for 1 h in the dark and a solution of rhamnopyranosyl bromide **7** (900 mg, 3.05 mmol) in CH_2Cl_2 (10 mL) was added over 1 h. After stirring the mixture in the dark for 24 h at 20 °C, it was treated with pyridine (1 mL) and filtered through Celite, which was washed with CH_2Cl_2. The filtrate was washed with 10% satd aq $Na_2S_2O_3$, water and the organic layer was dried ($MgSO_4$) and concentrated. The resulting residue was treated with Ac_2O–pyridine (1:5, 6 mL) for 16 h, then with MeOH (1 mL) for 0.5 h and concentrated with added toluene. Column chromatography [toluene–EtOAc (70:30)→(65:35)] afforded disaccharide **10** (555 mg, 57%) and acetylated glycosyl acceptor **9** (95 mg, 16%).

Disaccharide **10**: $R_f = 0.50$ [toluene–EtOAc (1:1)]; $[\alpha]_D$ −15 (*c* 1.00, $CHCl_3$); ^1H NMR (300 MHz, $CDCl_3$): δ 1.28 (3H, d, $J_{5,6}$ 6.6 Hz, H-6 Rha), 1.40, 1.46 (6H, 2s, C(CH_3)$_2$), 2.12 (3H, s, OCOCH_3), 3.30 (3H, s, OCH_3), 3.76–3.81 (2H, m, H-3′a Api and H-5 Rha), 3.87 (1H, d, $J_{4a,4b}$ 10.0 Hz, H-4a Api), 3.96 (1H, d, $J_{4a,4b}$ 10.0 Hz, H-4b Api), 4.08 (1H, d, $J_{3'a,3'b}$ 10.6 Hz, H-3′b Api), 4.28 (1H, s, H-2 Api), 4.80–4.88 (2H, m, H-2 and H-3 Rha), 4.93 (1H, s, H-1 Api), 5.09 (1H, broad s, H-1 Rha), 5.34–5.42 (1H, m, H-4 Rha); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.7 (C-6 Rha), 21.0 (OCO*C*H_3), 27.5, 27.7 (C(*C*H_3)$_2$ Api), 54.6 (O*C*H_3 Api), 70.3 (C-5 Rha), 70.9 (C-3′ Api), 71.5 (C-4 Rha), 72.6, 76.4 (C-2, C-3 Rha), 74.4 (C-4 Api), 86.4 (C-2 Api), 91.2 (C-3 Api), 95.2 (C-1 Rha), 107.9 (C-1 Api), 113.9 (*C*(CH$_3$)$_2$ Api), 153.8 (*C*=O), 169.4 (O*C*OCH$_3$). ES-MS found *m*/*z* 436.1822 [M + NH_4^+]. Calcd for $C_{18}H_{26}O_{11}\cdot NH_4$ 436.1819.

Acetate **9**: $R_f = 0.72$ [toluene–EtOAc (1:1)]; $[\alpha]_D$ −157 (*c* 1.9, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.40,

1.43 (6H, 2s, C(CH_3)$_2$), 2.08 (3H, s, OCOCH_3), 3.31 (3H, s, OCH_3), 3.83 (1H, d, $J_{4a,4b}$ 10.0 Hz, H-4a), 3.95 (1H, d, $J_{4a,4b}$ 10.0 Hz, H-4b), 4.20 (1H, d, $J_{3'a,3'b}$ 11.6 Hz, H-3′a), 4.32 (1H, s, H-2), 4.39 (1H, d, $J_{3'a,3'b}$ 11.6 Hz, H-3′b), 4.96 (1H, s, H-1); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 20.6 (OCO*C*H$_3$), 27.1, 27.4 (C(*C*H$_3$)$_2$), 54.4 (O*C*H$_3$), 65.7 (C-3′), 74.0 (C-4), 86.4 (C-2), 90.0 (C-3), 107.8 (C-1), 113.8 (*C*(CH$_3$)$_2$), 170.7 (O*C*OCH$_3$).

3.3. Methyl 5-*O*-(4-*O*-acetyl-2,3-*O*-carbonyl-β-L-rhamnopyranosyl)-2,3-*O*-isopropylidene-β-D-ribofuranoside (12)

Methyl 2,3-*O*-isopropylidene-β-D-ribofuranoside[17] (**11**, 390 mg, 1.90 mmol) was stirred for 1 h with Ag_2O (670 mg, 2.9 mmol) and molecular sieves (4 Å, 3.75 g) in CH_2Cl_2 (12.5 mL). A solution of rhamnopyranosyl bromide (**7**, 610 mg, 2.12 mmol) in dry CH_2Cl_2 (12.5 mL) was added dropwise over 2 h in the dark and stirring was continued for an additional 24 h. The mixture was filtered, the filtrate was concentrated and the residue was purified by chromatography to give crystalline disaccharide **12** (510 mg, 61%); $R_f = 0.28$ [toluene–EtOAc (3:1)]; mp: 38–39 °C, $[\alpha]_D$ −13 (*c* 1.3, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.31 (3H, d, $J_{5,6}$ 6.4 Hz, H-6 Rha), 1.32, 1.48 (6H, 2s, C(CH_3)$_2$), 2.11 (3H, s, OCOCH_3), 3.33 (3H, s, OCH_3), 3.60 (1H, dd, $J_{4,5a}$ 7.9 Hz, $J_{5a,5b}$ 10.4 Hz, H-5a Rib), 3.77 (1H, m, H-5 Rha), 3.90 (1H, dd, $J_{4,5b}$ 5.9 Hz, $J_{5a,5b}$ 10.4 Hz, H-5b Rib), 4.41 (1H, dd, $J_{4,5a}$ 7.9 Hz, $J_{4,5b}$ 5.9 Hz, H-4 Rib), 4.59 (1H, d, $J_{2,3}$ 6.0 Hz, H-3 Rib), 4.70 (1H, d, $J_{2,3}$ 6.0 Hz, H-2 Rib), 4.77–4.79 (2H, m, H-2, H-3 Rha), 4.91 (1H, broad s, H-1 Rha), 4.96 (1H, s, H-1 Rib), 5.30–5.34 (1H, m, H-4 Rha); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 19.5 (C-6 Rha), 21.0 (OCO*C*H$_3$), 25.1, 26.6 (C(*C*H$_3$)$_2$), 55.3 (O*C*H$_3$), 70.4 (C-5 Rib), 71.0 (C-5 Rha), 71.3 (C-4 Rha), 72.6, 76.3 (C-2, C-3 Rha), 82.0 (C-3 Rib), 84.6 (C-4 Rib), 85.4 (C-2 Rib), 96.0 (C-1 Rha), 110.0 (C-1 Rib), 112.7 (*C*(CH$_3$)$_2$), 153.7 (*C*=O), 169.3 (O*C*OCH$_3$). ES-MS found *m*/*z* 436.1814 [M + NH$_4^+$]. Calcd for $C_{18}H_{26}O_{11}\cdot NH_4$ 436.1813.

3.4. Methyl 2,3-*O*-isopropylidene-3-*C*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyloxymethyl)-β-D-erythrofuranoside (14)

A mixture of acceptor **8**[16] (230 mg, 1.13 mmol), $Hg(CN)_2$ (330 mg, 1.3 mmol) and mol seives 4 Å in dry MeCN (8 mL) was stirred for 1 h and a solution of rhamnopyranosyl bromide **13** (800 mg, 2.26 mmol) in MeCN (8 mL) was added. The reaction mixture was stirred for 17 h at 22 °C, diluted with CH_2Cl_2, washed with 10% aq NaBr solution, aq $NaHCO_3$ solution, dried over $MgSO_4$ and concentrated. The title compound (**14**) was purified by chromatography [hexane–EtOAc (4:1)] to give **14** (250 mg, 47%); $[\alpha]_D$ −86 (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (3H, d, $J_{5,6}$ 6.3 Hz, H-6 Rha), 1.41, 1.48 (6H, 2s, C(CH_3)$_2$), 1.98, 2.06, 2.15 (9H, 3s, OCOCH_3), 3.33 (3H, s, OCH_3), 3.69 (1H, d, $J_{3'a,3'b}$ 10.4 Hz, H-3′a, Api), 3.83 (1H, d, $J_{4a,4b}$ 10.2 Hz, H-4a Api), 3.88 (1H, d, $J_{3'a,3'b}$ 10.4 Hz, H-3′b, Api), 3.92–4.02 (2H, m, H-4b Api, H-5 Rha), 4.34 (1H, s, H-2 Api), 4.80 (1H, s, H-1 Rha), 4.96 (1H, s, H-1 Api), 5.05–5.14 (1H, m, H-4 Rha), 5.26–5.34 (2H, m, H-2 and H-3 Rha); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 17.1 (C-6 Rha), 20.4, 20.6, 20.6 (OCO*C*H$_3$), 27.05, 27.45 (C(*C*H$_3$)$_2$), 54.3 (O*C*H$_3$), 66.5 (C-5 Rha), 68.9, 69.3, 69.4 (C-3′ Api, C-2 and C-3 Rha), 70.8 (C-4 Rha), 74.2 (C-4 Api), 86.1 (C-2 Api), 90.6 (C-3 Api), 97.7 (C-1 Rha), 107.7 (C-1 Api), 113.5 (C(CH$_3$)$_2$ Api), 170.0 (O*C* OCH$_3$); ES-MS found *m*/*z* 494.2237 [M + NH$_4^+$]. Calcd for $C_{21}H_{32}O_{12}\cdot NH_4$ 494.2238.

3.5. Methyl 2,3-*O*-isopropylidene-5-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-β-D-ribofuranoside (15)

To a mixture of compound **11**[17] (710 g, 3.47 mmol) and $Hg(CN)_2$ (1.32 g, 3.47 mmol) in dry MeCN (20 mL) was added rhamnopyranosyl bromide **13** (1.85 g, 5.24 mmol) and the mixture was stirred for 17 h at 20 °C. The mixture was diluted with CH_2Cl_2, and washed successively with 1 M aq KBr solution and aq $NaHCO_3$ solution, dried and concentrated. Column chromatography [hexane–EtOAc (3:1)] gave **15** (1.44 g, 87%); $R_f = 0.29$ [hexane–EtOAc (3:1)]; $[\alpha]_D$ −78 (*c* 1.2, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.22 (3H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 1.33, 1.49 (6H, 2s, C(CH_3)$_2$), 1.99, 2.05, 2.15 (9H, 3s, OCOCH_3), 3.34 (3H, s, OCH_3), 3.54 (1H, dd, $J_{4,5a}$ 6.2 Hz, $J_{5a,5b}$ 10.5 Hz, H-5a Rib), 3.68 (1H, dd, $J_{4,5b}$ 6.5 Hz, $J_{5a,5b}$ 10.5 Hz, H-5b Rib), 3.90–3.94 (1H, m, H-5 Rha), 4.37 (1H, pseudo t, $J_{4,5a} \approx J_{4,5b}$ 6.5 Hz, H-4 Rib), 4.60 (1H, d, $J_{2,3}$ 6.0 Hz, H-3 or H-2 Rib), 4.65 (1H, d, $J_{2,3}$ 6.0 Hz, H-2 or H-3 Rib), 4.77 (1H, d, $J_{1,2}$ 1.7 Hz, H-1 Rha), 4.97 (1H, s, H-1 Rib), 5.07 (1H, pseudo t, $J_{3,4} \approx J_{4,5}$ 10.0 Hz, H-4 Rha), 5.26 (1H, dd, $J_{1,2}$ 1.7 Hz, $J_{2,3}$ 3.6 Hz, H-2 Rha), 5.34 (1H, dd, $J_{2,3}$ 3.6 Hz, $J_{3,4}$ 10.0 Hz, H-3 Rha); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 17.6 (C-6 Rha), 20.9, 21.0, 21.1 (OCO*C*H$_3$), 25.2, 26.7 (C(*C*H$_3$)$_2$), 55.3 (O*C*H$_3$), 66.7 (C-5 Rha), 69.2 (C-3 Rha), 69.6 (C-5 Rib), 70.0 (C-2 Rha), 71.2 (C-4 Rha), 82.0, 85.5 (C-2, C-3 Rib), 85.1 (C-4 Rib), 97.9 (C-1 Rha), 110.0 (C-1 Rib), 112.7 (*C*(CH$_3$)$_2$), 170.1, 170.2, 170.3 (O*C*OCH$_3$); ES-MS found *m*/*z* 494.2242 [M + NH$_4^+$]. Calcd for $C_{21}H_{32}O_{12}\cdot NH_4$ 494.2238.

3.6. Methyl-3-*C*-(2,3,4-tri-*O*-acetyl-β-L-rhamnopyranosyloxymethyl)-2,3-*O*-isopropylidene-β-D-erythrofuranoside (16)

Disaccharide **10** (165 mg, 0.39 mmol) was treated with 0.1 M NaOMe in MeOH (3.0 mL) for 4 h at 20 °C, the solution was neutralised with Amberlite IRA-120 (H^+),

filtered and the filtrate was concentrated. The residue was acetylated with Ac_2O–pyridine (1:4, 5 mL) for 16 h, treated with MeOH (1 mL) for 0.5 h and concentrated with toluene. Column chromatography (hexane–EtOAc, 7:3) gave **16** (110 mg, 59%); $R_f = 0.63$ [hexane–EtOAc (3:2)]; $[\alpha]_D$ −12 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 1.23 (3H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 1.25, 1.31, (6H, 2s, $C(CH_3)_2$), 1.94, 2.01, 2.11 (9H, 3s, $OCOCH_3$), 3.25 (3H, s, OCH_3), 3.41–3.49 (1H, m, H-5 Rha), 3.68 (1H, d, $J_{3'a,3'b}$ 10.6 Hz, H-3′a Api), 3.79 (1H, d, $J_{4a,4b}$ 9.9 Hz, H-4a Api), 3.85 (1H, d, $J_{4a,4b}$ 9.9 Hz, H-4b Api), 4.01 (1H, d, $J_{3'a,3'b}$ 10.6 Hz, H-3′b Api), 4.19 (1H, broad s, H-2 Api), 4.71 (1H, s, H-1 Rha), 4.91–5.03 (2H, m, H-3 Rha, H-4 Rha), 5.42 (1H, broad s, H-2 Rha); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 17.2 (C-6 Rha), 20.5, 20.6 (2C), ($OCOCH_3$), 27.2, 27.45 ($C(CH_3)_2$), 54.3 (OCH_3), 68.8 (C-2 Rha), 70.6 (2C), 71.0, 71.3 (C-3 Rha, C-4 Rha, C-5 Rha, C-3′Api), 74.1 (C-4 Api), 86.3 (C-2 Api), 91.1 (C-3 Api), 98.2 (C-1 Rha), 107.9 (C-1 Api), 113.6 ($C(CH_3)_2$), 169.9, 170.35 ($OCOCH_3$); ES-MS found *m/z* 494.2238 $[M + NH_4^+]$. Calcd for $C_{21}H_{32}O_{12}\cdot NH_4$ 494.2238.

3.7. General method for deprotection of disaccharides 10, 12, 14 and 15

The protected disaccharide (0.15 mmol) was treated with 0.1 M NaOMe in MeOH (3 mL) at 20 °C until complete conversion (~1 h) of starting material into a single product having $R_f = 0.4$–0.7 (CH_2Cl_2–MeOH, 9:1). The solution was treated with Amberlite IRA-120 (H^+) until the pH was neutral, the resin was filtered off, washed with MeOH and the methanolic solution was concentrated to dryness. The residue was redissolved in MeOH (10 mL) and the solution was stirred with Amberlite IRA-120 (H^+, 2 g) at 20 °C. The progress of the reaction was monitored by TLC (CH_2Cl_2–MeOH, 4:1), which showed complete disappearance of starting material in 2–7 days. The mixture was filtered, the resin was washed with MeOH, combined filtrates were concentrated and the product was purified by column chromatography (CH_2Cl_2–MeOH, 95:5).

3.7.1. Methyl 3-*C*-(β-L-rhamnopyranosyloxymethyl)-β-D-erythrofuranoside (1). Disaccharide **1**, yield 92%, $R_f = 0.22$ [CH_2Cl_2–MeOH (9:1)]; $[\alpha]_D$ +5 (*c* 0.8, MeOH); ^1H NMR (400 MHz, $CDCl_3$): δ 1.12 (3H, d, $J_{5,6}$ 5.9 Hz, H-6 Rha), 3.17–3.22 (2H, m, H-4 Rha and H-5 Rha), 3.26 (3H, s, OCH_3), 3.39–3.42 (1H, m, H-3 Rha), 3.53 (1H, d, $J_{4a,4b}$ 10.8 Hz, H-4a Api), 3.74 (1H, d, $J_{3'a,3'b}$ 10.6 Hz, H-3′a Api), 3.75 (1H, d, $J_{4a,4b}$ 10.8 Hz, H-4b Api), 3.80 (1H, d, $J_{1,2}$ 3.8 Hz, H-2 Api), 3.85 (1H, dd, $J_{1,2} < 1$ Hz, $J_{2,3}$ 2.4 Hz, H-2 Rha), 3.90 (1H, d, $J_{3'a,3'b}$ 10.8 Hz, H-3′b Api), 4.46 (1H, d, $J_{1,2} < 1$ Hz, H-1 Rha), 4.78 (1H, d, $J_{1,2}$ 3.8 Hz, H-1 Api); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.7 (C-6 Rha), 56.1 (OCH_3), 70.5 (C-2 Rha), 71.3 (C-4 Api), 72.1, 72.3 (C-4 and C-5 Rha), 72.8 (C-3 Rha), 73.7 (C-3′ Api), 76.8 (C-2 Api), 78.5 (C-3 Api), 100.4 (C-1 Rha), 109.3 (C-1 Api). ES-MS found *m/z* 328.1604 $[M + NH_4^+]$. Calcd for $C_{12}H_{22}O_9\cdot NH_4$ 328.1602.

3.7.2. Methyl 5-*O*-(β-L-rhamnopyranosyl)-β-D-ribofuranoside (2). Disaccharide **2**, yield 68%; $R_f = 0.11$ [CH_2Cl_2–MeOH (9:1)]; $[\alpha]_D$ +45 (*c* 2.89, MeOH); ^1H NMR (400 MHz, $CDCl_3$): δ 1.15 (3H, d, $J_{5,6}$ 4.6 Hz, H-6 Rha), 3.19–3.26 (5H, m, H-4 and H-5 Rha, OCH_3), 3.38–3.43 (1H, m, H-3 Rha), 3.67 (1H, dd, $J_{4,5a}$ 3.2 Hz, $J_{5a,5b}$ 11.7 Hz, H-5a Rib), 3.75 (1H, dd, $J_{4,5b}$ 5.3 Hz, $J_{5a,5b}$ 11.7 Hz, H-5b Rib), 3.83 (1H, d, $J_{2,3}$ 3.2 Hz, H-2 Rha), 3.88 (1H, d, $J_{2,3}$ 4.8 Hz, H-2 Rib), 3.92–3.96 (1H, m, H-4 Rib), 4.08 (1H, dd, $J_{2,3}$ 4.8 Hz, $J_{3,4}$ 6.6 Hz, H-3 Rib), 4.50 (1H, s, H-1 Rha), 4.73 (1H, s, H-1 Rib); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.8 (C-6 Rha), 55.5 (OCH_3), 69.9 (C-5 Rib), 70.6 (C-2 Rha), 70.7 (C-3 Rib), 72.1, 72.3 (C-4 and C-5 Rha), 72.7 (C-3 Rha), 74.2 (C-2 Rib), 81.2 (C-4 Rib), 100.2 (C-1 Rha), 108.7 (C-1 Rib). ES-MS found *m/z* 328.1601 $[M + NH_4^+]$. Calcd for $C_{12}H_{22}O_9\cdot NH_4$ 328.1602.

3.7.3. Methyl 3-*C*-(α-L-rhamnopyranosyloxymethyl)-β-D-erythrofuranoside (3). Disaccharide **3**, yield 45%; $R_f = 0.16$ [CH_2Cl_2–MeOH (9:1)]; $[\alpha]_D$ −35 (*c* 1.1, MeOH); ^1H NMR (400 MHz, $CDCl_3$): 1.11 (3H, d, $J_{5,6}$ 6.2 Hz, H-6 Rha), 3.23–3.33 (4H, m, H-4 Rha, OCH_3), 3.34 (1H, d, $J_{4a,4b}$ 10.3 Hz, H-4a Api), 3.50 (1H, m, H-5 Rha), 3.59–3.63 (2H, m, H-3 Rha and H-4b Api), 3.73 (1H, d, $J_{3'a,3'b}$ 10.3 Hz, H-3′a Api), 3.80–3.81 (1H, m, H-2 Rha), 3.82 (1H, d, $J_{1,2}$ 3.5 Hz, H-2 Api), 3.88 (1H, d, $J_{3'a,3'b}$ 10.3 Hz, H-3′b Api), 4.61 (1H, d, $J_{1,2}$ 1.7, H-1 Rha), 4.50 (1H, d, $J_{1,2}$ 3.5 Hz, H-1 Api); δ_C (100.6 MHz, D_2O): 16.7 (C-6 Rha), 55.5 (OCH_3), 68.9 (C-5 Rha), 69.1 (C-4 Api), 70.0 (C-2 Rha), 70.3 (C-3 Rha), 72.1 (C-4 Rha), 73.5 (C-3′Api), 76.6 (C-2 Api), 78.2 (C-3 Api), 100.2 (C-1 Rha), 109.4 (C-1 Api). ES-MS found *m/z* 328.1601 $[M + NH_4^+]$. Calcd for $C_{12}H_{22}O_9\cdot NH_4$ 328.1602.

3.7.4. Methyl 5-*O*-(α-L-rhamnopyranosyl)-β-D-ribofuranoside (4). Disaccharide **4**, yield 94%, $R_f = 0.10$ [CH_2Cl_2–MeOH (9:1)]; $[\alpha]_D$ −291 (*c* 0.18, MeOH); ^1H NMR (400 MHz, $CDCl_3$): δ 1.11 (3H, d, $J_{5,6}$ 6.9 Hz, H-6 Rha), 3.21 (3H, s, OCH_3), 3.25 (1H, pseudo t, $J_{3,4} \approx J_{4,5}$ 9.7 Hz, H-4 Rha), 3.36 (1H, d, $J_{5a,5b}$ 10.0 Hz, H-5a Rib), 3.52–3.56 (1H, m, H-5 Rha), 3.58 (1H, dd, $J_{2,3}$ 3.5 Hz, $J_{3,4}$ 9.7 Hz, H-3 Rha), 3.72 (1H, dd, $J_{5a,5b}$ 10.0 Hz, H-5b Rib), 3.79 (1H, dd, $J_{1,2}$ 1.6 Hz, $J_{2,3}$ 3.5 Hz, H-2 Rha), 3.85 (1H, d, $J_{2,3}$ 4.5 Hz, H-2 Rib), 3.93 (1H, m, H-4 Rib), 4.03 (1H, pseudo t, $J_{2,3} \approx J_{3,4}$ 4.5 Hz, H-3 Rib), 4.65 (1H, broad s, H-1 Rha), 4.72 (1H, s, H-1 Rib); ^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.7 (C-6 Rha), 55.3 (OCH_3),

68.4 (C-5 Rib), 68.7 (C-5 Rha), 70.1 (C-2 Rha), 70.3 (C-3 Rha), 70.9 (C-3 Rib), 72.1 (C-4 Rha), 74.2 (C-2 Rib), 81.2 (C-4 Rib), 100.3 (C-1 Rha), 108.1 (C-1 Rib). ES-MS found m/z 328.1601 $[M + NH_4^+]$. Calcd for $C_{12}H_{22}O_9 \cdot NH_4$ 328.1602.

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