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Synthesis of an xylosylated rhamnose pentasaccharide, the repeating unit of the O-chain polysaccharide of the lipopolysaccharide of *Xanthomonas campestris* pv. *begoniae* GSPB 525

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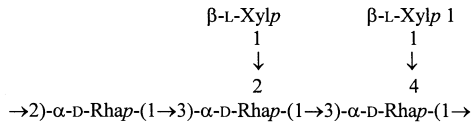
Abstract

A xylosylated rhamnose pentasaccharide, α-L-Rha*p*-(1→3)-[β-L-Xyl*p*-(1→2)-]-α-L-Rha*p*-(1→3)-[β-L-Xyl*p*-(1→4)]-L-Rha*p*, the repeating unit of the O-chain polysaccharide (OPS) of the lipopolysaccharides of *Xanthomonas campestris* pv. *begoniae* GSPB 525 was synthesized by a highly regio- and stereoselective way. Thus coupling of 1,2-*O*-ethylidene-β-L-rhamnopyranose (**1**) with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**2**) to give (1→3)-linked disaccharide (**3**), subsequent benzoylation, deethylidenation, acetylation, 1-*O*-deacetylation, and trichloroacetimidation afforded the disaccharide donor **11**. Condensation of **11** with **1** yielded 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**12**), and selective deacetylation of **12** yielded the trisaccharide diol acceptor **15**. Coupling of **15** with 2,3,4-tri-*O*-benzoyl-α-L-xylopyranosyl trichloroacetimidate (**16**), followed by deprotection, gave the target pentasaccharide **19**.

Keywords: Oligosaccharide; Rhamnose; Xylose

1. Introduction

It was reported recently that the repeating unit of the O-chain polysaccharide (OPS) of the lipopolysaccharides of *Xanthomonas campestris* pv. *begoniae* GSPB 525 is an xylosylated rhamnan pentasaccharide as shown below:[1]



It was known that *Xanthomonas* are phytopathogens and cause leaf spots by colonizing the intercellular leaf space.[2] *X. Campestris* pv. *begoniae* causes a disease characterized by large water-soaked lesions on the leaves. Several data indicate that the lipopolysaccharides (LPSs) contribute to bacterial virulence.[3] For investigation of structure–bioactivity relationships of oligosaccharides, we report herewith a concise and efficient synthesis of the OPS repeating unit.

Although the pentasaccharide-repeating unit is not very complex, its synthesis will need orthogonal masking groups and multiprotection–deprotection steps if the traditional stepwise method is used. A stepwise synthesis of the hexasaccharide with (1→2)- and (1→3)-linked rhamnotetraose as the backbone and two glucosamine units as the side chains has been reported.[4] Our previous work described highly regio- and stereoselective syntheses of oligosaccharides using unprotected sugars via orthoester formation–rearrangement strategy.[5–7] Later on we found that high regio- and stereoselectivity were achieved in a one-pot manner using glycosyl trichloroacetimidates as the donors and partly protected sugars as the acceptors.[8,9] Based on these new findings, we readily accomplished the synthesis of the title pentasaccharide.

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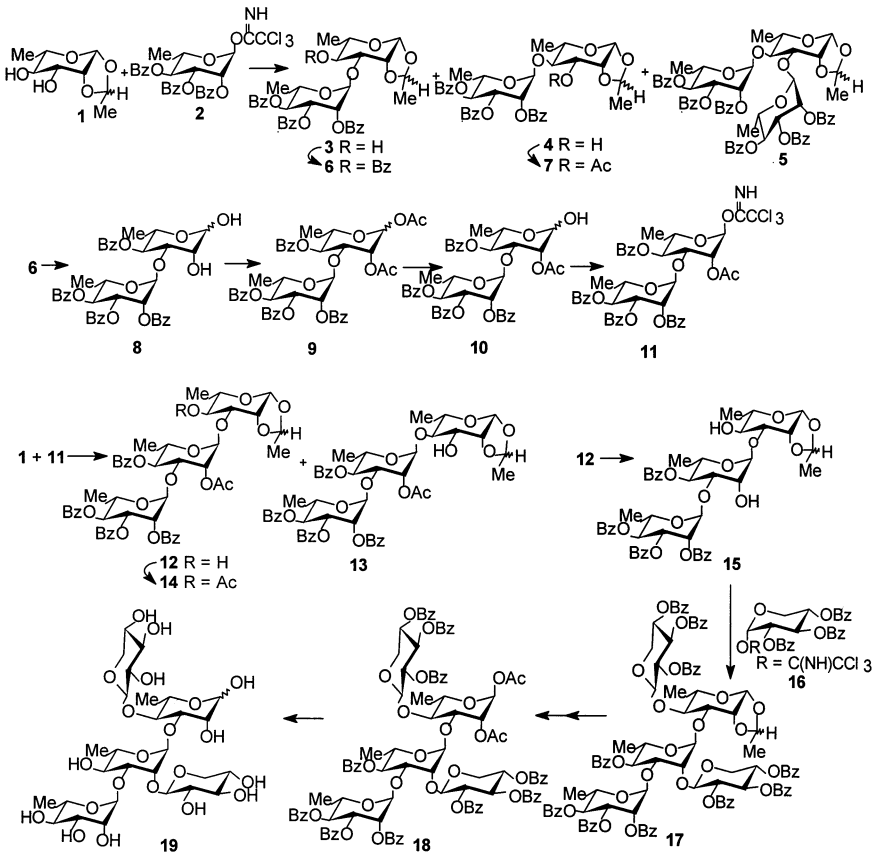
2. Results and discussion

As outlined in Scheme 1, coupling of the 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**2**) with the 1,2-*O*-ethylidene-β-L-rhamnopyranose (**1**) in the presence of a catalytic amount of TMSOTf selectively gave (1→3)-linked disaccharide **3** (74.2%) as the main product, together with a small amount of (1→4)-linked disaccharide **4** (6.9%) and (1→3) (1→4)-linked trisaccharide **5** (1.9%). Their structures were confirmed by benzoylation or acetylation to give 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzoyl-1,2-*O*-ethylidene-β-L-rhamnopyranose (**6**), showing characteristic signals at δ 5.48 ppm (dd, $J_{3,4} = J_{4,5}$ 9.5 Hz) for H-4, or 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-3-*O*-acetyl-1,2-*O*-ethylidene-β-L-rhamnopyranose (**7**), showing characteristic signals at δ 5.18 ppm (dd, $J_{2,3}$ 4.0, $J_{3,4}$ 9.7 Hz) for H-3 in its ^{1}H NMR spectrum. It was determined that it was necessary to maintain the temperature during addition of TMSOTf below −20 °C to ensure the formation of the orthoester intermediate. Otherwise, for example at room temperature, the regioselectivity was poor. Compound **6** was deethylidenated with 90% trifluroacetic acid (TFA), and the product was acetylated with acetic anhydride in pyridine, selectively 1-*O*-deacetylated with ammonia–methanol, and converted to the trichloroacetimidate with trichloroactonitrile in the presence of DBU or potassium carbonate, to furnish the disaccharide donor 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**11**). These four steps were performed continuously without separation, giving 86.9% overall yield. Condensation of the disaccharide donor **11** with acceptor **1** using TMSOTf as the catalyst selectively gave 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**12**) (76.7%) as the major product, together with 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**13**) (3.2%) as the minor one. No pentasaccharide was detected perhaps due to steric hindrance. Because of the presence of the ethylidene group, an attempt for selective deacetylation of **12** with 3% CH_3COCl–MeOH[10] was not successful since a very complex product was obtained. Later, we found that the acetyl group can be selectively removed with 0.5 N ammonia–methanol without affecting either the ethylidene or the benzoyl group, giving **15** in 83% yield. Coupling of the trisaccharide acceptor **15** with the xylose donor **16** gave the pentasaccharide **17**, and sub-



Scheme 1.

sequent deethyledenation and acetylation furnished pentasaccharide **18** as its α isomer, only. Finally deacylation of **18** in ammonia–methanol gave the target pentasaccharide **19**. Bioassay of the resultant pentasaccharide is in progress.

In summary, a branched xylosylated rhamnan pentasaccharide was synthesized in a highly regioselective way with a simple procedure. Large-scale preparations can be performed with this method.

3. Experimental

General methods.—Melting points were determined with a Mel-Temp apparatus. Optical rotations were determined with a Perkin–Elmer model 241-MC automatic polarimeter at 20 °C for solutions in a 1-dm, jacketed cell. ^{1}H and ^{13}C NMR spectra were recorded with Varian XL-400 and Varian XL-200 spectrometers, for solutions in $CDCl_3$ with tetramethylsilane (Me_4Si) as the internal standard. Chemical shifts are expressed in ppm downfield from the internal Me_4Si absorption. Mass spectra were recorded with a VG PLATFORM mass spectrometer using the ESI mode. Thin-layer chromatography (TLC) was performed on silica gel HF with detection by charring with 30% (v/v) H_2SO_4 in MeOH or by UV detection. Column chromatography was conducted by elution of a column (8 × 100 mm, 16 × 240 mm, 18 × 300 mm, and 35 × 400 mm) of silica gel (100–200 mesh) and EtOAc–petroleum ether (bp 60–90 °C) as the eluent. Analytical LC was performed with a Gilson HPLC consisting of a pump (model 306), stainless steel column packed with silica gel (Spherisorb SiO_2, 10 × 300 mm or 4.6 × 250 mm), differential refractometer (132-RI detector), UV–Vis detector (model 118). EtOAc–petroleum ether (bp 60–90 °C) was used as the eluent at a flow rate of 1–4 mL/min. Solutions were concentrated at a temperature < 60 °C under diminished pressure.

All of the intermediates containing the ethylidene group were composed of R and S isomers. These two isomers had no apparent difference in reactivity, and thus no separation was conducted in the synthesis. However, for the convenience of identification by NMR spectrometry, the predominant R isomer was isolated in pure form in most of the cases.

*Preparation of 2,3,4-tri-*O*-benzoyl-α-*L*-rhamnopyranosyl-(1→3)-1,2-*O*-ethylidene-β-*L*-rhamnopyranose* (**3**). —1,2-*O*-Ethylidene-β-L-rhamnopyranose (**1**, 1.90 g, 10.0 mmol) and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**2**, 6.2 g, 10.0 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (40 mL). TMSOTf (90 μL, 0.5 mmol) was added dropwise at −25 °C with N_2 protection. The reaction mixture was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. Then the mixture was neutralized with triethylamine, and concentrated to dryness. Purification of the residue by column chromatography (1:1 petroleum ether–EtOAc) gave **3** (4.81 g, 74.2%), and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**4**, 0.45 g, 6.9%), and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-[2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)]-1,2-*O*-ethylidene-β-L-rhamnopyranose (**5**, 0.10 g, 1.9%) as foamy solids. For **3** (R isomer): $[\alpha]_D$ +132.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.08–7.27 (m, 15 H, 3 Ph), 5.89 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9.9 Hz, H-3), 5.77 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.1 Hz, H-2), 5.69 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 5.35–5.23 (m, 2 H, H-1, CH_3CHO_2), 5.26 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.53 (m, 1 H, H-5), 4.28 (dd, 1 H, $J_{1,2}$ 1.7, $J_{2,3}$ 3.2 Hz, H-2), 3.81 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.7 Hz, H-3), 3.74 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 3.40 (m, 1 H, H-5), 1.56 (d, 3 H, *J* 4.8 Hz, CH_3CHO_2), 1.37 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.32 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.9, 165.8, 165.7 (3 C, 3 *C*OPh), 104.6 (1 C, CH_3CHO_2), 100.0, 96.5 (2 C, 2 C-1), 81.8, 79.8, 71.7, 71.0, 71.0, 70.9, 70.3, 67.4, 21.9, 17.9, 17.6. Anal. Calcd for $C_{35}H_{36}O_{12}$: C, 64.81; H, 5.59. Found: C, 64.93; H, 5.40. For **4** (R isomer): $[\alpha]_D$ +105.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.10–7.25 (m, 15 H, 3 Ph), 5.78 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 5.75 (dd, 1 H, $J_{1,2}$ 1.6, $J_{2,3}$ 3.2 Hz, H-2), 5.69 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.60 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.30 (q, *J* 4.8 Hz, 1 H, CH_3CHO_2), 5.25 (d, 1 H, $J_{1,2}$ 2.3 Hz, H-1), 4.28 (m, 1 H, H-5), 4.26 (dd, 1 H, $J_{1,2}$ 2.3, $J_{2,3}$ 4.4 Hz, H-2), 4.06 (dd, 1 H, $J_{2,3}$ 4.4, $J_{3,4}$ 8.9 Hz, H-3), 3.71 (dd, 1 H, $J_{3,4} = J_{4,5}$ 8.9 Hz, H-4), 3.49 (m, 1 H, H-5), 1.51 (d, 3 H, *J* 4.9 Hz, CH_3CHO_2), 1.42 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.37 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 165.8, 165.8, 165.7 (3 C, 3 *C*OPh), 104.1 (1 C, CH_3CHO_2), 98.8, 96.5 (2 C, 2 C-1), 80.1, 80.0, 72.5, 71.7, 71.1, 70.1, 69.7, 67.7, 21.6, 18.5, 17.7. Anal. Calcd for $C_{35}H_{36}O_{12}$: C, 64.81; H, 5.59. Found: C, 64.89; H, 5.67. For **5** (R isomer): $[\alpha]_D$ +139.5° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.99–7.17 (m, 30 H, 6 Ph), 6.04–5.99 (m, 2 H, H-2, H-3), 5.90 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.0 Hz, H-3), 5.81 (dd, 1 H, $J_{1,2}$ 1.8, $J_{2,3}$ 2.7 Hz, H-2), 5.76 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.0 Hz, H-4), 5.65 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.61 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.35 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 5.30–5.26 (m, 2 H), 4.59 (m, 1 H, H-5), 4.41 (dd, 1 H, $J_{1,2}$ 2.5, $J_{2,3}$ 3.5 Hz, H-2), 4.30 (m, 1 H, H-5), 4.07 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.1 Hz, H-3), 3.99 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4), 3.60 (m, 1 H, H-5), 1.57 (d, 3 H, *J* 4.9 Hz, CH_3CHO_2), 1.49 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.38 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 1.26 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 165.9, 165.6, 165.5, 165.4, 165.0 (6 C, 6 *C*OPh), 104.3 (1 C, CH_3CHO_2), 100.1, 99.5, 96.5 (3 C, 3 C-1), 82.5, 79.8, 72.3, 72.0, 71.7, 71.7, 70.5, 69.6, 69.4, 67.8, 67.6, 21.7, 19.0, 17.7,

17.6. Anal. Calcd for $C_{62}H_{58}O_{19}$: C, 67.26; H, 5.28. Found: C, 67.33; H, 5.25.

2,3,4-Tri-O-*benzoyl*-α-L-*rhamnopyranosyl*-(1→3)-4-O-*benzoyl*-1,2-O-*ethylidene*-β-L-*rhamnopyranose* (**6**).—To a solution of 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**3**, 4.8 g, 7.4 mmol) in pyridine (20 mL) was added benzoyl chloride (1.5 mL, 13 mmol) at 0 °C. The reaction mixture was slowly raised to rt and stirred for 12 h, at the end of which time TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. Water (100 mL) was added to the reaction mixture, and stirring was continued for 30 min. The aqueous solution was extracted with CH_2Cl_2 (3 × 100 mL), the extract was washed with HCl (1 N) and satd aq $NaHCO_3$, and dried (Na_2SO_4). The solution was concentrated, and purification of the residue by flash-column chromatography on a silica gel column (3:1 petroleum ether–EtOAc) gave **6** (5.16 g, 92.6%) as a syrup. For R isomer: $[\alpha]_D$ +162.0° (*c* 1.0, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09–7.20 (m, 20 H, 4 Ph), 5.83 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 10.1 Hz, H-3), 5.60 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 5.48 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 5.40–5.38 (m, 2 H, H-2, CH_3CHO_2), 5.30 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 5.22 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.45 (m, 1 H, H-5), 4.34 (dd, 1 H, $J_{1,2}$ 2.1, $J_{2,3}$ 3.9 Hz, H-2), 5.18 (dd, 1 H, $J_{3,4}$ 3.9, $J_{4,5}$ 9.5 Hz, H-3), 3.64 (m, 1 H, H-5), 1.62 (d, 3 H, *J* 4.8 Hz, CH_3CHO_2), 1.33 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.29 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 166.0, 165.8, 165.1, 165.0 (4 C, 4 *C*OPh), 105.0 (1 C, CH_3CHO_2), 99.6, 96.6 (2 C, 2 C-1), 79.6, 78.5, 72.4, 71.9, 71.0, 70.0, 69.5, 67.5, 21.9, 17.9, 17.6. Anal. Calcd for $C_{42}H_{40}O_{13}$: C, 67.01; H, 5.36. Found: C, 66.94; H, 5.28.

2,3,4-Tri-O-*benzoyl*-α-L-*rhamnopyranosyl*-(1→4)-3-O-*acetyl*-1,2-O-*ethylidene*-β-L-*rhamnopyranose* (**7**).—To a solution of 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**4**, 400 mg, 0.60 mmol) in pyridine (5 mL) was added acetyl anhydride (1.0 mL, 1 mmol). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated, and purification of the residue by flash-column chromatography on a silica gel column (3:1 petroleum ether–EtOAc) gave **7** (0.38 g, 92.0%) as a syrup. For R isomer: $[\alpha]_D$ +131.0° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.09–7.25 (m, 15 H, 3 Ph), 5.74 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 10.1 Hz, H-3), 5.69 (dd, 1 H, $J_{3,4} = J_{4,5}$ 10.1 Hz, H-4), 5.58 (dd, 1 H, $J_{1,2}$ 1.9, $J_{2,3}$ 3.1 Hz, H-2), 5.29–5.28 (m, 2 H, H-1, H-1), 5.25 (q, 1 H, *J* 4.8 Hz, CH_3CHO_2), 5.18 (dd, 1 H, $J_{2,3}$ 4.0, $J_{3,4}$ 9.7 Hz, H-3), 4.65 (dd, 1 H, $J_{1,2}$ 2.4, $J_{2,3}$ 4.0 Hz, H-2), 4.27 (m, 1 H, H-5), 3.92 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 3.62 (m, 1 H, H-5), 2.26 (s, 3 H, $COCH_3$), 1.51 (d, 3 H, *J* 4.9 Hz, CH_3CHO_2), 1.46 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6), 1.36 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.9 (1 C, $COCH_3$), 165.8, 165.6, 165.5 (3 C, 3 *C*OPh), 104.3(1 C, CH_3CHO_2), 99.7, 96.4 (2 C, 2 C-1), 78.6, 77.6, 73.4, 71.6, 71.1, 70.2, 69.7, 67.8, 21.6, 21.0, 18.6, 17.6. Anal. Calcd for $C_{37}H_{38}O_{13}$: C, 64.34; H, 5.55. Found: C, 64.40; H, 5.41.

2,3,4-Tri-O-*benzoyl*-α-L-*rhamnopyranosyl*-(1→3)-2-O-*acetyl*-4-O-*benzoyl*-α-L-*rhamnopyranosyl trichloroacetimidate* (**11**).—Compound **6** (5.03 g, 6.69 mmol) was dissolved in 90% TFA (70 mL) and stirred for 2 h, at the end of which time the reaction mixture was poured directly to toluene (250 mL) and concentrated. Drying the residue under high vacuum gave **8** as a white foamy solid in a quantitative yield. To the solution of compound **8** in pyridine (100 mL) was added Ac_2O (20.0 mL). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (4:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated to dryness, and the residue was dissolved in CH_2Cl_2 (300 mL), washed with water and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated to give **9**. Compound **9** was dissolved in a 1 M solution of ammonia–MeOH (200 mL) and stirred for 6 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The solution was concentrated directly to give **10** as a syrup. A mixture of **10**, trichloroacetonitrile (4.2 mL, 20 mmol), and 1,8-diazabicyclo[5.4.0]undecene (DBU) (0.50 mL, 4.04 mmol) in dry CH_2Cl_2 (50 mL) was stirred under nitrogen for 3 h, and then concentrated. The residue was purified by flash chromatography (4:1 petroleum ether–EtOAc) to give **11** (5.3 g, 86.9% for four steps) as a yellow syrup: $[\alpha]_D$ +143.3° (*c* 1.1, $CHCl_3$); ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.78 (s, 1 H, N*H*), 8.09–7.25 (m, 20 H, 4 Ph), 6.32 (d, 1 H, $J_{1,2}$ 0.8 Hz, H-1), 5.66 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.7 Hz, H-3), 5.61–5.54 (m, 3 H), 5.36 (dd, 1 H, $J_{1,2}$ 0.8, $J_{2,3}$ 3.2 Hz, H-2), 5.19 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 4.52 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 4.27 (m, 1 H, H-5), 4.19 (m, 1 H, H-5), 2.38 (s, 3 H, $COCH_3$), 1.35 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.26 (d, 3 H, $J_{5,6}$ 6.1 Hz, H-6). Anal. Calcd for $C_{44}H_{40}Cl_3NO_{14}$: C, 57.86; H, 4.41. Found: C, 57.88; H, 4.50.

2,3,4-Tri-O-*benzoyl*-α-L-*rhamnopyranosyl*-(1→3)-2-O-*acetyl*-4-O-*benzoyl*-α-L-*rhamnopyranosyl*-(1→3)-1,2-O-*ethylidene*-β-L-*rhamnopyranose* (**12**).—2,3,4-Tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnopyranosyl trichloroacetimidate (**11**, 4.8 g, 5.26 mmol) and 1,2-*O*-ethylidene-β-L-rhamnopyranose (**1**, 1.00 g, 5.26 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (40 mL). TMSOTf (90 μL, 0.5 mmol) was added dropwise at −25 °C with N_2 protection. The reaction mixture was stirred for 3 h, during which time the temperature was gradually raised to ambient temperature. Then the mixture was neutralized with

triethylamine, and concentrated to dryness. Purification of the residue by column chromatography (1:1 petroleum ether–EtOAc) gave **12** (3.79 g, 76.7%) and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-2-*O*-acetyl-4-*O*-benzoyl-α-L-rhamnopyranosyl-(1→4)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**13**, 0.158 g, 3.2%).

For **12** (R isomer): $[\alpha]_D$ +117.3° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.09–7.21 (m, 20 H, 4 Ph), 5.60 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 10.0 Hz, H-3), 5.58 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.47–5.45 (m, 2 H, H-2, H-4), 5.31–5.29 (m, 2 H, H-2, CH_3CHO_2), 5.13 (d, 1 H, $J_{1,2}$ 1.4 Hz, H-1), 5.12 (d, 1 H, $J_{1,2}$ 2.1 Hz, H-1), 5.09 (d, $J_{1,2}$ 1.2 Hz, 1 H, H-1), 4.81 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.6 Hz, H-3), 4.29–4.20 (m, 3 H, H-2, H-5, H-5), 3.77 (dd, 1 H, $J_{2,3}$ 4.0, $J_{3,4}$ 9.2 Hz, H-3), 3.68 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.2 Hz, H-4), 3.37 (m, 1 H, H-5), 2.32 (s, 3 H, $COCH_3$), 1.54 (d, 3 H, *J* 4.8 Hz, CH_3CHO_2), 1.37 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6), 1.32 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.8 (1 C, $COCH_3$), 165.8, 165.7, 165.1, 164.9 (4 C, 4 COPh), 104.6 (1 C, CH_3CHO_2), 100.0, 99.0, 96.4 (3 C, 3 C-1), 81.1, 79.8, 74.8, 73.1, 71.7, 71.4, 71.3, 70.9, 70.7, 69.2, 67.7, 67.5, 21.8, 17.7, 17.6, 17.6. Anal. Calcd for $C_{50}H_{52}O_{18}$: C, 63.82; H, 5.57. Found: C, 63.71; H, 5.50. For **13** (R isomer): $[\alpha]_D$ +99.2° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.06–7.20 (m, 20 H, 4 Ph), 5.65 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 5.55 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.47 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.9 Hz, H-4), 5.43–5.42 (m, 2 H, H-1, CH_3CHO_2), 5.35 (d, $J_{1,2}$ 1.9 Hz, H-1), 5.32 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.2 Hz, H-2), 5.20–5.17 (m, 2 H, H-1, H-2), 4.37–4.21 (m, 3 H), 4.04–3.99 (m, 2 H), 3.48 (m, 1 H, H-5), 2.33 (s, 3H, $COCH_3$), 1.48 (d, 3 H, *J* 4.9 Hz, CH_3CHO_2), 1.34–1.25 (m, 9 H); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.6 (1 C, $COCH_3$), 165.9, 165.7, 165.1, 164.9 (4 C, 4 COPh), 102.5 (1 C, CH_3CHO_2), 99.0, 96.5, 91.8 (3 C, 3 C-1), 79.1, 78.3, 74.1, 73.5, 71.6, 71.5, 70.7, 69.3, 67.6, 67.7, 67.5, 64.2, 21.2, 18.2, 17.7, 17.6. Anal. Calcd for $C_{50}H_{52}O_{18}$: C, 63.82; H, 5.57. Found: C, 63.88; H, 5.61.

2,3,4-Tri-O-*benzoyl-α-L-rhamnopyranosyl-(1→3)-2*-O-*acetyl-4*-O-*benzoyl-α-L-rhamnopyranosyl-(1→3)-4*-O-*acetyl-1,2*-O-*ethylidene-β-L-rhamnopyranose* (**14**).—To a solution of **12** (100 mg, 0.11 mmol) in pyridine (5 mL) was added Ac_2O (1.0 mL, 1 mmol). The reaction mixture was stirred at rt for 12 h, at the end of which time TLC (3:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated, purification of the residue by flash-column chromatography on a silica gel column (3:1 petroleum ether–EtOAc) gave **14** (92 mg, 85.0%) as a syrup. For R isomer: $[\alpha]_D$ +111.8° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.08–7.20 (m, 20 H, 4 Ph), 5.66 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.7 Hz, H-3), 5.57 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.45 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.7 Hz, H-4), 5.33–5.30 (m, 2 H, H-2, CH_3CHO_2), 5.22–5.16 (m, 3 H, H-2, H-1, H-1), 5.13 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.0 Hz, H-4), 4.95 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1), 4.43 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.0 Hz, H-3), 4.24 (m, 1 H, H-5), 4.20 (dd, 1 H, $J_{1,2}$ 2.2, $J_{2,3}$ 3.4 Hz, H-2), 4.13 (m, 1 H, H-5), 3.86 (dd, 1 H, $J_{1,2}$ 3.2, $J_{3,4}$ 9.8 Hz, H-3), 3.47 (m, 1 H, H-5), 2.31 (s, 3 H, $COCH_3$), 2.08 (s, 3 H, $COCH_3$), 1.56 (d, 3 H, *J* 4.7 Hz, CH_3CHO_2), 1.37 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.27 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 1.23 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 170.7, 170.0 (2 C, 2 $COCH_3$), 165.8, 165.8, 165.1, 164.9 (4 C, 4 COPh), 104.9(1 C, CH_3CHO_2), 100.1, 99.2, 96.4 (3 C, 3 C-1), 79.7, 79.0, 74.4, 73.1, 71.7, 71.7, 71.6, 70.6, 69.5, 69.3, 67.6, 67.5, 21.8, 21.2, 17.6, 17.6, 17.4. Anal. Calcd for $C_{52}H_{54}O_{19}$: C, 63.54; H, 5.54. Found: C, 63.28; H, 5.66.

2,3,4-Tri-O-*benzoyl-α-L-rhamnopyranosyl-(1→3)-4*-O-*benzoyl-α-L-rhamnopyranosyl-(1→3)-1,2*-O-*ethylidene-β-L-rhamnopyranose* (**15**).—Ammonia was bubbled in to a solution of 2:1 THF–MeOH (120 mL) until concentration of the solution reached 1.5 M. Compound **12** (3.20 g, 0.34 mmol) was dissolved in THF (40 mL), the solution was mixed with the ammonia solution (20 mL), and the mixture was stirred for 24 h. TLC (3:1 petroleum ether–EtOAc) indicated that more than half of the starting material had reacted. The solution was concentrated, and purification of the residue by column chromatography on a silica gel column (1:1 petroleum ether–EtOAc) gave compound **15** (1.7 g, 80.2%, corrected yield, 0.98 g **12** was recovered) as a syrup: $[\alpha]_D$ +85.2° (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.06–7.20 (m, 20 H, 4 Ph), 5.76 (dd, 1 H, $J_{2,3}$ 3.0, $J_{3,4}$ 9.7 Hz, H-3), 5.61 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.8 Hz, H-4), 5.47–5.42 (m, 3 H, H-1, H-2, H-4), 5.30 (q, 1 H, *J* 4.8 Hz, CH_3CHO_2), 5.26 (d, 1 H, $J_{1,2}$ 1.1 Hz, H-1), 5.23 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 4.36–4.25 (m, 3 H), 4.16–4.06 (m, 2 H), 3.91 (dd, 1 H, $J_{1,2}$ 1.1, $J_{2,3}$ 3.1 Hz, H-2), 3.65 (dd, 1 H, $J_{3,4} = J_{4,5}$ 9.1 Hz, H-4), 3.41 (m, 1 H, H-5), 1.51 (d, 3 H, *J* 4.8 Hz, CH_3CHO_2), 1.39 (d, 3 H, $J_{5,6}$ 6.3 Hz, H-6), 1.35 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.32 (d, 3 H, $J_{5,6}$ 6.0 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$); δ 165.8, 165.7, 165.3, 165.0 (4 C, 4 COPh), 104.3 (1 C, CH_3CHO_2), 100.8, 98.7, 96.5 (3 C, 3 C-1), 80.1, 79.8, 73.1, 73.0, 71.7, 71.0, 70.8, 69.9, 69.5, 67.8, 67.7, 21.7, 18.4, 17.7, 17.6. Anal. Calcd for $C_{48}H_{50}O_{17}$: C, 64.13; H, 5.61. Found: C, 64.24; H, 5.68.

2,3,4-Tri-O-*benzoyl-α-L-rhamnopyranosyl-(1→3)-[2,3,4-tri*-O-*benzoyl-β-L-xylopyranosyl-(1→2)]-4*-O-*benzoyl-α-L-rhamnopyranosyl-(1→3)-[2,3,4-tri*-O-*benzoyl-β-L-xylopyranosyl-(1→4)]-1,2*-O-*ethylidene-β-L-rhamnopyranose* (**17**).—2,3,4-Tri-*O*-benzoyl-α-L-xylopyranosyl trichloroacetimidate (**16**, 1.94 g, 3.20 mmol) and 2,3,4-tri-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-4-*O*-benzoyl-α-L-rhamnopyranosyl-(1→3)-1,2-*O*-ethylidene-β-L-rhamnopyranose (**15**, 1.20 g, 1.34 mmol) were dried together under high vacuum for 2 h, then dissolved in anhyd CH_2Cl_2 (50 mL). TMSOTf (58 μL, 0.32 mmol) was added dropwise at −20 °C with N_2 protection. The reaction mixture was stirred for 2 h, during

which time the temperature was gradually raised to ambient temperature. Then the mixture was neutralized with triethylamine, and concentrated to dryness. Purification of the residue by column chromatography (3:1 petroleum ether–EtOAc) gave **17** (1.93 g, 80.7%) as a syrup: $[\alpha]_D$ +98.3° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 8.10–6.78 (m, 50 H, 10 Ph), 6.08–5.82 (m, 2 H), 5.75–5.61 (m, 3 H), 5.53–5.29 (m, 6 H), 5.22–4.94 (m, 4 H), 4.68–4.46 (m, 3 H), 4.42–4.23 (m, 3 H), 4.11–4.02 (m, 2 H), 3.91–3.83 (m, 1 H), 3.68–3.31 (m, 3 H), 1.52–0.94 (m, 12 H). Anal. Calcd for $C_{100}H_{90}O_{31}$ C, 67.18; H, 5.07. Found: C, 67.02; H, 5.13.

2,3,4-Tri-O-*benzoyl*-α-L-*rhamnopyranosyl*-(1→3)-[*2,3,4-tri*-O-*benzoyl*-β-L-*xylopyranosyl*-(1→2)]-*4*-O-*benzoyl*-α-L-*rhamnopyranosyl*-(1→3)-*1,2-di*-O-*acetyl*-[*2,3,4-tri*-O-*benzoyl*-β-L-*xylopyranosyl*-(1→4)]-α-L-*rhamnopyranose* (**18**).—Compound **17** (1.00 g, 0.56 mmol) was dissolved in 90% TFA (20 mL) and stirred for 2 h, at the end of which time the reaction mixture was poured directly to toluene (100 mL), and then the mixture was concentrated. Drying the residue under high vacuum gave a white foamy solid, which was dissolved in pyridine (10 mL), and then Ac_2O (2.0 mL) was added. The reaction mixture was stirred for 12 h at rt, at the end of which time TLC (2:1 petroleum ether–EtOAc) indicated that the reaction was complete. The reaction mixture was concentrated to dryness, and the residue was dissolved in CH_2Cl_2 (300 mL), washed with water and satd aq $NaHCO_3$, dried (Na_2SO_4), and concentrated. The residue was purified by flash chromatography (2:1 petroleum ether–EtOAc) to give **18** (0.97 g, 94.2%) as a syrup: $[\alpha]_D$ +93.5.0°; ^1H NMR (400 MHz, $CDCl_3$): δ 8.13–6.78 (m, 50 H, 10 Ph), 6.08 (dd, 1 H, *J* 9.5 Hz), 5.95 (dd, 1 H, $J_{2,3}$ 3.3, $J_{3,4}$ 9.8 Hz, H-3), 5.87 (dd, 1 H, *J* 10.0 Hz), 5.73–5.57 (m, 7 H), 5.39 (dd, 1 H, $J_{1,2}$ 1.2, $J_{2,3}$ 3.1 Hz, H-2), 5.34 (d, 1 H, $J_{1,2}$ 0.9 Hz, H-1), 5.27 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1), 5.20 (dd, 1 H, $J_{1,2}$ 1.0, $J_{2,3}$ 3.3 Hz, H-2), 4.90 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.6 Hz, H-3), 4.84 (d, 1 H, $J_{1,2}$ 7.3 Hz, H-1), 4.74 (dd, 1 H, $J_{1,2}$ 0.9, $J_{2,3}$ 3.1 Hz, H-2), 4.68 (m, 1 H, H-5), 4.41–4.35 (m, 3 H), 4.27 (dd, 1 H, $J_{2,3}$ 3.4, $J_{3,4}$ 9.9 Hz, H-3), 3.94 (m, 1 H, H-5), 3.80 (m, 1 H, H-5), 3.60–3.54 (m, 2 H), 2.17 (s, 3 H, $COCH_3$), 1.53 (d, 3 H, $J_{5,6}$ 6.2 Hz, H-6), 1.27 (s, 3 H, $COCH_3$), 1.22 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6), 1.07 (d, 3 H, $J_{5,6}$ 6.4 Hz, H-6); ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.8, 168.6 (2 C, 2 $COCH_3$), 166.4, 165.8, 165.6, 165.5, 165.4, 165.1, 165.1, 164.7, 164.6, 164.5 (10 C, 10 *C*OPh), 99.8, 99.4, 98.1, 97.7, 90.6 (5 C, 5 C-1), 79.3, 76.9, 75.1, 74.2, 73.4, 72.3, 71.4, 70.4, 70.3, 69.8, 69.4, 68.1, 66.7, 63.0, 61.2, 21.1, 19.6, 18.1, 18.0, 17.4. Anal. Calcd for $C_{102}H_{92}O_{33}$: C, 66.37; H, 5.02. Found: C, 66.30; H, 5.21.

α-L-*Rhamnopyranosyl*-(1→3)-[β-L-*xylopyranosyl*-(1→2)]α-L-*rhamnopyranosyl*-(1→3)-[β-L-*xylopyranosyl*-(1→4)]-α-L-*rhamnopyranose* (**19**).—Pentasaccharide **18** (800 mg, 0.43 mmol) was dissolved in a satd ammonia solution in MeOH (10 mL). After 96 h at rt, the reaction mixture was concentrated, and the residue was purified by chromatography on Sephadex LH-20 (MeOH) to afford **19** as a foamy solid (256 mg, 82.5%): $[\alpha]_D$ −21.7° (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$): δ 5.34 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 5.25 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.04 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.87 (d, 1 H, $J_{1,2}$ 6.1 Hz, H-1), 4.75 (d, 1 H, $J_{1,2}$ $J_{3,4} = J_{4,5}$ 6.2 Hz, H-1); ^{13}C NMR (100 MHz, $CDCl_3$): δ 103.9, 102.5, 101.4, 99.9, 93.7 (5 C, C-1); MS (*m*/*z*) Calcd for $C_{28}H_{48}O_{21}$: 720.66 $[M]^+$. Found: 743.69 $[M + Na]^+$.

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References

1. Senchenkova, S. N.; Shashkov, A. S.; Laux, P.; Knirel, Y. A.; Rudolph, K. *Carbohydr. Res.* **1999**, *319*, 148–153.
2. Rudolph, K. In *Xanthomonas*; Swings, J. G.; Civerolo, E. L., Eds.; Chapman and Hall: London, 1993; pp. 193–264.
3. Newman, M. A.; Daniels, M. J.; Dow, J. M. *Mol. Plant Microbe Interact.* **1995**, *8*, 778–780.
4. Auzanneau, F.-I.; Forooghian, F.; Pinto, B. M. *Carbohydr. Res.* **1996**, *291*, 21–41.
5. Wang, W.; Kong, F. *Angew. Chem., Int. Ed. Engl.* **1999**, *38*, 1247–1250.
6. Wang, W.; Kong, F. *J. Org. Chem.* **1998**, *63*, 5744–5755.
7. Du, Y.; Kong, F. *J. Carbohydr. Chem.* **1999**, *18*, 655–664.
8. Zhu, Y.; Kong, F. *Synlett* **2000**, 663–669.
9. Zhu, Y.; Kong, F. *Carbohydr. Res.* **2001**, *332*, 1–21.
10. Byramova, N. E.; Ovchinnikov, M. V.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1983**, *124*, C8–C11.