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Synthesis of the unique trisaccharide repeating unit, isolated from lipopolysaccharides *Rhizobium leguminosarum bv. trifolii* 24, and its analogue

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Abstract

The synthesis of trisaccharide: 6-d-α-L-Tal*p*(1→2)-α-L-Rhap(1→5)-DHA, and its analogue: 6-d-α-L-Tal*p*(1→2)-β-L-Rha*f*(1→5)DHA is described. In the first step a disaccharide, composed of 6-d-L-Tal*p* and L-Rha*p* was obtained. This, in turn, was converted to the corresponding 1-trichloroacetimidate and coupled with DHA alcohol to afford the required trisaccharide. Its analogue was achieved by the conversion of the above disaccharide to the glycosyl bromide, involving the rhamnopyranose ring scission, followed by condensation with DHA in Koenigs-Knorr procedure.

Keywords: Trisaccharides; DHA; 6-d-L-Talose; L-Rhamnopyranose

1. Introduction

Gram-negative bacteria belonging to the genus *Rhizobium* have the unique ability to induce N_2 fixing nodules on roots of legumes by a complex multistep interaction between the microsymbiont and its host plant [1]. Experimental evidence suggests that the conversion from the bacteria to nitrogen-fixing bacteroides involves the alteration of their antigenic specifity [2]. In order to get a deeper insight into the precise role of the carbohydrate chain in *R. leguminosarum bv. trifolii* and its exo$^-$ mutant AR20 [3], a detailed structural analysis of their lypopolysaccharides (LPS) was performed [4]. It was found that their *O*-specific longer chain consists of unusual trisaccharide repeating units, composed of solely rare sugars: 3-deoxy-D-*lyxo*-heptulosaric acid (DHA), 6-deoxy-L-Tal, and L-Rha (Fig. 1). Synthetic approaches to this trisaccharide were not described, however, shortly after isolation of DHA [5] we elaborated a method of preparation of this particularly attractive sugar [6] as well as 6-deoxy-L-Tal [7].

2. Results and discussion

A retrosynthetic analysis of the target compound suggested a preparation in the first step of the disaccharide **3a** (Scheme 1). Assuming the structural similarity of 6-deoxy-sugar units, both the required glycosydic acceptor **1c** and glycosydic donor **2d**

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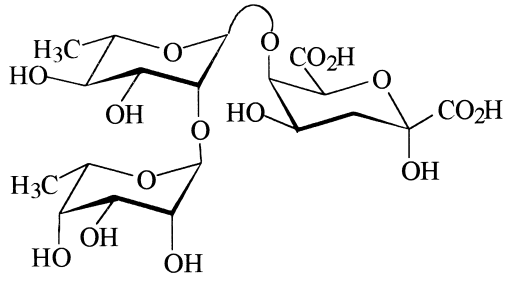
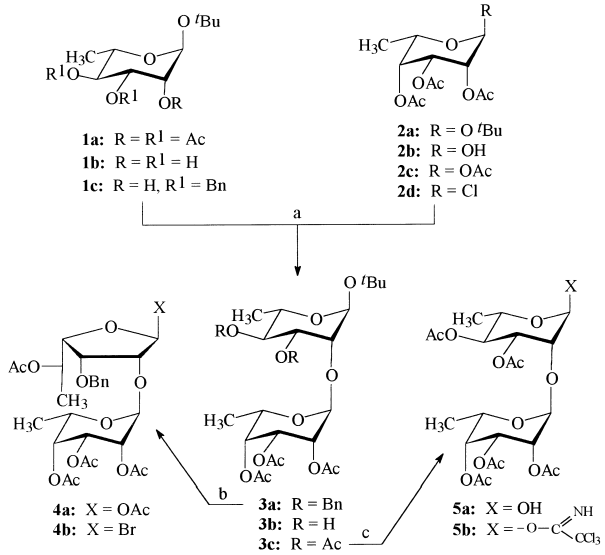


Fig. 1.

could be prepared from the common substrate i.e. *tert*-butyl L-Rha*p* **1b**. Thus, the glycosyl acceptor **1c** was readily obtained by a selective protection of **1b**, analogous to the reported procedure [8]. Preparation of the glycosyl donor **2d** was performed by a conversion of **1b** to **2a** in a known reaction sequence [9]. Subsequent hydrolysis of the *tert*-butyl group with TFA in CH_2Cl_2 under the conditions applied for the hydrolysis of a *tert*-butyl ester [10], then acetylation and reaction with DCMME [11] afforded the required chloride **2d**.

Ideally suited to construct the α-(1→2)-*trans* linkage was a classical procedure involving AgOTf-sym. collidine as promoters [12]. Disaccharide **3a** thus obtained was isolated in 72% yield as a sole product. For the conversion of **3a** to the glycosyl donor, needed for condensation with DHA acceptor, removal of the anomeric *tert*-butyl group was necessary. With this aim, acetolysis using TFA–Ac_2O (1:15, 0 °C→rt) was attempted. However, despite the mild reaction conditions, acetolysis was accompanied by transformation of Rha*p* ring to the furanose one giving rise to 1-*O*-acetyl derivative **4a**. Disaccharide **4a** thus formed was considered as a kinetic product of dealkylation of 1-OH and 4-OH groups in the Rha*p* unit, with spontaneous ring scission, followed by acetylation [13]. To overcome this undesired transformation, the benzyl groups in **3a** were removed and the product, after acetylation and deprotection of the anomeric hydroxyl group with TFA in CH_2Cl_2, was converted to the trichloroacetimidate **5b** [14] (Scheme 1).



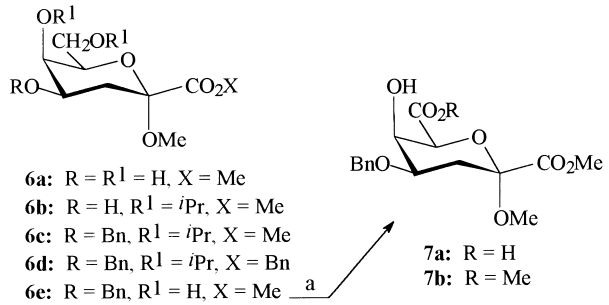
Scheme 1. (a) AgOTf, sym. collidine, (b) TFA, Ac_2O; (c) TFA, CH_2Cl_2.

The required glycosyl partner **7b** (Scheme 2) was prepared from the heptulosonic acid **6a** [6], the 5,7-OH groups of which were selectively blocked [15] as *O*-isopropylidene derivative **6b**. This, in turn, was benzylated at the 4-*O*-position with BnBr/Ag_2O (Scheme 2). After removal of *O*-isopropylidene protection, the primary 7-OH group in **6e** was oxidized using NaOCl/TEMPO [16] to afford **7a**. Esterification of 7-CO_2H group with methanol, promoted by Me_3SiCl [6] furnished the acceptor **7b**.

Coupling of the trichloroacetimidate **5b** with the acceptor **7b** promoted by $BF_3{\cdot}OEt_2$ afforded solely the α-coupled trisaccharide **8a**. This, in turn, was subjected to hydrogenolysis, followed by acetylation, leading to the fully *O*-acetylated derivative **8c**. Thus, the first synthesis of the *Rhizobium* trisaccharide in a protected form was achieved.

Having in hand the disaccharide **4a** it was of interest to accomplish the synthesis of an analogous trisaccharide, containing the Rha unit in the furanose form. Additionally, we were stimulated by the absence of information in literature on the use of a Rha*f* donor for condensation with sugar acceptors. It is worth noting that hexofuranoses have been identified as components of the bacterial LPSs [17].

For the formation of an interglycosidic linkage leading to the "new" trisaccharide the Koenigs–Knorr procedure was employed. Thus,



Scheme 2. (a) NaOCl, TEMPO; TEMPO = 2,2,6,6,-tetramethylpiperidine 1-oxyl, radical.

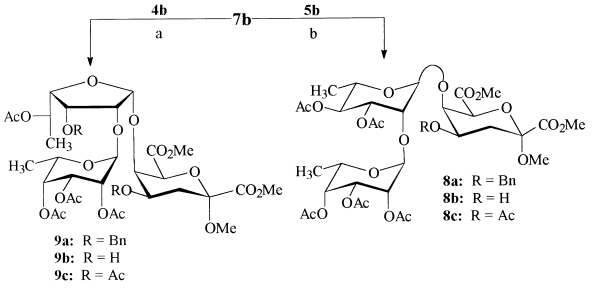
disaccharide bromide **4b** obtained in the reaction of **4a** with $TiBr_4$ [15] was condensed with the DHA alcohol **6b** in the presence of $Hg(CN)_2$. The reaction proceeded smoothly, furnishing the sole trisaccharide **9a** (Scheme 3) with the new glycosidic linkage assigned to the β-configuration. It is known that the NMR data i.e. chemical shifts and coupling constants for establishing α or β-configuration of the *manno* type anomers, especially those of furanosides, are uncertain due to their great conformational diversity [18a]. Fortunately, the per-*O*-acetylated derivative of this trisaccharide **9c** was crystalline, so allowing to confirmation of the structure by the X-ray analysis [13].

A creation of the β-glycosidic linkage between the bromide **4b** and alcohol **7b** deserves some notes. According to the knowledge on a particularly disfavoring creation of the *cis*-glycosidic linkage in furanosides: *manno*, *rhamno*, *lyxo* [18] we could expect a selective formation of the *trans* α-glycoside. The creation of the opposite β-*cis* linkage implicit so far.

3. Experimental

General methods.—Optical rotations were measured with JASCO DIP Digital Polarimeter at room temperature. ^{1}H NMR spectra were recorded on Bruker AM-500 (500 MHz) with Me_4Si as internal standard. Mass spectra were taken on a AMD-604 mass spectrometer. Reactions were monitored by TLC on silica (Merck alu-plates (0.2 mm)). Reaction products were purified by flash chromatography, using Merck's Kieselgel 60 (240–400 mesh or 70–230 mesh).

t-*Butyl 2,3,4-tri*-O-*acetyl*-α-L-*rhamnopyranoside* (**1a**).—To a mixture of *t*-butyl alcohol (7.2 mL, 79 mmol), $Hg(CN)_2$ (15 g, 59.5 mmol) and molecular sieves 3Å (10 g) in CH_2Cl_2 (100 mL), was added a solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide (17.2 g, 45 mmol) in CH_2Cl_2 (50 mL) at −5 °C under argon. The mixture was stirred for 1 h at room temperature, then filtered and purified by chromatography (hexane–AcOEt, 17:3), yield: 12.5 g (76%): mp 71–73 °C; $[\alpha]_D$ −54.6° (*c* 2.32, $CHCl_3$); ^{1}H NMR (C_6D_6): δ 5.81 (dd, 1H, H-3), 5.55 (5, 1H, H-4), 5.47 (dd, 1H, H-2), 5.20 (d, 1H, H-1), 4.20 (pq, 1H, H-5), 1.72, 1.69, 1.68, (3s, 3×3H, Ac), 1.24 (d, 3H, CH_3), 1.04 (s, 9H, *t*-Bu); $J_{1,2}$ 1.9, $J_{2,3}$ 3.3, $J_{3,4}$ 10.0, $J_{4,5}$ 9.9, $J_{5,6}$ 9.8, $J_{5,3}$ 1.8 Hz; Anal. Calcd for $C_{16}H_{26}O_8$ (346.37): C 55.48%, H 7.57%, Found C 55.13%, H 7.46%.



Scheme 3. (a) $Hg(CN)_2$, CH_2Cl_2, rt; (b) $BF_3{\cdot}OEt_2$, CH_2Cl_2, −40 °C.

Further transformation of **1a** to **1c** was performed according to the procedure described for an analogous methyl glycoside [8].

t-*Butyl* α-L-*rhamnopyranoside* (**1b**).—Characteristic data: mp 45–46 °C; $[\alpha]_D$ −79.8° (*c* 1.2, $CHCl_3$); Anal. Calcd for $C_{10}H_{20}O_5$ (220.26): C 54.53%, H 9.15%, Found C 54.36%, H 9.16%.

t-*Butyl 3,4-di*-O-*benzyl-6-deoxy*-α-L-*rhamnopyranoside* (**1c**).—Characteristic data: colourless syrup; $[\alpha]_D$ −46.5° (*c* 1.3, $CHCl_3$); ^{1}H NMR (200 MHz, C_6D_6): δ 7.05–7.35 (m, 10H, 2×Ph), 5.27 (s, 1H, H-1), 4.85 (d, 1H, Bn), 4.52 (d, 1H, Bn), 4.36 (s, 2H, Bn), 4.12 (pq, 1H, H-5), 3.88 (m, 2H, H-2, H-4), 3.58 (t, 1H, H-3), 2.4 (bs, 1H, OH), 1.8 (d, 3H, CH_3), 1.11 (s, 9H, *t*-Bu); $J_{1,2}$ ∼0, $J_{2,3}$ ∼1.8, $J_{3,4}$ 9.1, $J_{4,5}$ 9.6, $J_{5,6}$ 6.2 Hz; ^{1}H NMR ($CDCl_3$): δ 7.28–7.38 (m, 10H, Ph), 5.07 (d, 1H, H-1), 4.88 (d, 1H, Bn), 4.66–4.73 (m, 2H, Bn), 4.63 (d, 1H, Bn), 3.93 (pq, 1H, H-5), 3.85–3.90 (m, 2H, H-2, H-4), 3.42 (t, 1H, H-3), 2.4 (d, 1H, OH), 1.27 (d, 3H, CH_3), 1.22 (s, 9H, *t*-Bu); $J_{1,2}$ 0.7, $J_{3,4}$ 9.5, $J_{4,5}$ 9.6, $J_{5,6}$ 6.2, $J_{2,OH}$ 2.1 Hz; Anal. Calcd for $C_{24}H_{32}O_5$ (400.5): C 71.97%, H 8.05%, Found C 71.63%, H 8.12%.

t-*Butyl 2,3,4-tri*-O-*acetyl-6-deoxy*-α-L-*talopyranoside* (**2a**).—Prepared from **1b** analogously to the described procedure for methyl glycoside [9]. Characteristic data: mp 122 °C; $[\alpha]_D$ −72.3° (*c* 1.0, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.31 (t, 1H, H-3), 5.15 (m, 1H, H-2), 5.12 (d, 1H, H-1), 4.91 (ddd, 1H, H-4), 4.31 (pq, 1H, H-5), 2.15, 2.14, 1.99 (3s, 3×3H, Ac), 1.25 (s, 9H, *t*-Bu), 1.17 (d, 3H, CH_3); $J_{1,2}$ 1.6, $J_{2,3}=J_{3,4}$ 3.7, $J_{4,5}$ 1.7, $J_{4,2}$ 1.0, $J_{5,6}$ 6.6 Hz; Anal. Calcd for $C_{16}H_{26}O_8$ (346.37): C 55.48%, H 7.57%, Found C 55.25%, H 7.46%.

1,2,3,4,-Tetra-O-*acetyl-6-deoxy*-α-L-*talopyranose* (**2c**).—A solution of **3a** (0.52 g, 1.5 mmol) in anhydrous CH_2Cl_2 (30 mL) was stirred with TFA (15 mL) at room temperature until TLC (hexane–Et_2O, 3:7) showed disappearance of the substrate.

Evaporation left a syrup which was treated with Ac_2O-Py. After usual work-up the product was purified by chromatography to yield 0.31 g (62%) of **2c**: mp 97 °C; $[\alpha]_D$ −72.3° (*c* 0.26, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 6.13 (d, 1H, H-1), 5.32 (t, 1H, H-3), 5.20 (m, 1H, H-2), 5.09 (ddd, 1H, H-4), 4.22 (pq, 1H, H-5), 2.7, 2.16, 2.14, 2.0 (4s, 4×3H, Ac), 1.22 (d, 3H, CH_3); $J_{1,2}$ 1.5, $J_{2,3}$ 3.7, $J_{3,4}$ 3.7, $J_{4,5}$ 1.0, $J_{5,6}$ 6.5 Hz; Anal. Calcd for $C_{14}H_{20}O_9$ (332.30): C 50.60%, H 6.07%, Found C 50.48%, H 6.14%.

2,3,4,-Tri-O-*acetyl-6-deoxy*-α-L-*talopyranosyl chloride* (**2d**).—To a solution of **2c** (0.664 g, 2 mmol) in anhydrous CH_2Cl_2 (5 mL) were added DCMME (Cl_2CHOCH_3) (0.89 mL, 10 mmol) and freshly fused $ZnCl_2$ (13 mg) under argon. The reaction mixture was stirred at room temperature until all starting material was consumed (TLC, hexane–Me_2CO, 17:3) and the single faster moving product was formed. The mixture was diluted with dry toluene, filtered through Celite and concentrated. The residue was eluted from a short column of silica gel to give 0.49 g (79.5%) of **2d** as a syrup.

t-*Butyl 2*-O-*(2,3,4-tri*-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-3,4-di*-O-*benzyl*-α-L-*rhamnopyranoside* (**3a**).—To a stirred suspension of AgOTf (0.46 g, 1.8 mmol) in dry CH_2Cl_2 (3 mL) was added a mixture of glycosyl donor **2d** (0.49 g, 1.55 mmol), glycosyl acceptor **1c** (0.44 g, 1.25 mmol), and sym.-collidine (1.65 mL 1 M solution in CH_2Cl_2, 1.25 mmol) at −40 °C under argon. After stirring for 0.5 h at this temperature (TLC, hexane–Me_2CO, 4:1; hexane–Et_2O-MeOH, 1:1:0.05) the reaction mixture was diluted with CH_2Cl_2 and filtered through a Celite. The filtrate was washed with aqueous solution of saturated $NaHCO_3$, $Na_2S_2O_3$ and H_2O. The organic phase was dried with $MgSO_4$, evaporated and the residue was purified by chromatography to give amorphous **3a** (0.57 g, 73%): $[\alpha]_D$ −61.4° (*c* 0.8, $CHCl_3$); ^1H NMR (C_6D_6): δ 7.5–7.35 (m, 10H, 2Ph), 5.67 (m, 1H, H-4′), 5.61 (t, 1H, H-3′), 5.29 (m, 1H, H-2′), 5.28 (d, 1H, H-1′), 5.23 (d, 1H, H-1), 4.93, 4.59, 4.51, 4.42 (4d, 4×1H, 2Bn), 4.23 (pq, 1H, H-5′), 4.11 (pq, 1H, H-5), 4.08 (dd, 1H, H-3), 3.95 (t, 1H, H-2), 3.77 (t, 1H, H-4), 1.83, 1.73, 1.72 (3s, 3×3H, Ac), 1.39 (d, 3H, CH_3), 1.18 (s, 9H, *t*-Bu), 1.06 (d, 3H, CH_3'); $J_{1,2}$ 2.0, $J_{1',2'}$ 1.4, $J_{2,3}$ 3.0, $J_{2',3'}=J_{3',4'}$ 3.6, $J_{3,4}$ 9.3, $J_{4,5}$ 9.3, $J_{4',5'}$ 1.1, $J_{5,6}$ 6.3, $J_{5',6'}$ 6.5 Hz; ^1H NMR ($CDCl_3$): δ 7.24–7.34 (m, 10H, 2Ph), 5.32 (t, 1H, H-3′), 5.29 (dt, 1H, H-4′), 5.19 (m, 1H, H-2′), 5.1 (bs, 2H, H-1, H-1′), 4.88 (d, 1H, Bn), 4.61–4.72 (m, 3H, Bn), 4.31 (pq, 1H, H-5), 3.90 (dd, 1H, H-3), 3.85 (pq, 1H, H-5), 3.75 (t, 1H, H-2), 3.46 (t, 1H, H-4), 2.15, 2.10, 1.99 (3s, 3×3H, Ac), 1.27 (d, 3H, CH_3), 1.2 (d, 3H, CH_3'), 1.19 (s, 9H, *t*-Bu); $J_{2,3}$ 3.0, $J_{2',3'}=J_{3',4'}$ 3.8, $J_{3,4}$ 9.3, $J_{4,5}$ 9.5, $J_{4',5'}$ 1.1, $J_{5,6}$ 6.3, $J_{5',6'}$ 6.6 Hz; HRMS (LSIMS): Calcd for $C_{36}H_{48}O_{12}$ $[M+Na]^+$ *m/z* 695.3044, Found *m/z* 695.3051.

t-*Butyl 2*-O-*(2,3,4-tri*-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-3,4-di*-O-*acetyl*-α-L-*rhamnopyranoside* (**3c**).—A solution of **3a** (0.67 g, 1 mmol) in EtOH (15 mL) was stirred with Pd/C (0.2 g) overnight. After filtration, and evaporation of the solvent the residue was acetylated with Ac_2O/Py, to afford **3c** (0.48 g, 83%): $[\alpha]_D$ −72.9° (*c* 0.9, $CHCl_3$); ^1H NMR (C_6D_6): δ 5.65 (dd, 1H, H-3), 5.53 (t, 1H, H-4), 5.48 (t, 1H, H-3′), 5.40 (dt, 1H, H-4′), 5.30 (m, 1H, H-2′), 5.21 (d, 1H, H), 5.07 (d, 1H, H-1′), 4.20 (pq, 1H, H-5′), 4.17 (pq, 1H, H-5), 4.8 (q, 1H, H-2), 1.99, 1.83, 1.69, 1.68, 1.66 (5s, 5×3H, Ac), 1.25 (d, 3H, CH_3), 1.11 (s, 9H, *t*-Bu), 1.0 (d, 3H, CH_3'); $J_{1,2}$ 1.9, $J_{1',2'}$ 1.3, $J_{2,3}$ 3.4, $J_{2',3'}=J_{3',4'}$ 3.7, $J_{3,4}$ 9.9, $J_{4',5'}$ 1.1, $J_{5,6}$ 6.3, $J_{5',6'}$ 6.5 Hz; HRMS (LSIMS): Calcd for $C_{26}H_{40}O_{14}$ $[M+Na]^+$ *m/z* 599.2315, Found *m/z* 599.2316.

2-O-*(2,3,4-Tri*-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-3,4-di*-O-*acetyl*-α-L-*rhamnopyranose* (**5a**).—A solution of **3c** (0.58 g, 1 mmol) in CH_2Cl_2 (3 mL) was stirred with TFA (1.5 mL) for 4 h at room temperature. Evaporation followed by filtration through silica gel (hexane–Me_2CO, 7:3) afforded **5a** (0.42 g, 82%): mp 73 °C; $[\alpha]_D$ −34.8° (*c* 0.52, $CHCl_3$).

2-O-*(2,3,4-Tri*-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-3,4-di*-O-*acetyl*-α-L-*rhamnopyranosyl trichloroacetimidate* (**5b**).—To a solution of **5a** (0.26 g, 0.5 mmol) and Cl_3CCN (0.5 mL, 4.45 mmol) in dry CH_2Cl_2 (15 mL) was added DBU (0.47 mL, 0.51 mmol) at −20 °C under argon. After stirring for 10 min, the mixture was directly poured on silica gel, and eluted with hexane–Et_2O (1:1) to give **5b** (0.29 g, 82%) as a colourless syrup: $[\alpha]_D$ −51.7° (*c* 1.0, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 8.68 (s, 1H, NH), 6.25 (d, 1H, H-1), 5.29 (t, 1H, H-3′), 5.26 (dd, 1H, H-3), 5.22–5.24 (m, 1H, H-4′), 5.13–5.17 (m, 2H, H-2′, H-4), 4.98 (d, 1H, H-1′), 4.29 (pq, 1H, H-5′), 4.27 (q, 1H, H-2), 4.06 (pq, 1H, H-5), 2.16, 2.14, 2.09, 2.06, 2.0 (5s, 5×3H, Ac), 1.27 (d, 3H, CH_3), 1.23 (d, 3H, CH_3'); $J_{1,2}$ 2.0, $J_{1',2'}$ 1.0, $J_{2,3}$ 3.3, $J_{3,4}$ 9.7, $J_{4,5}$ 9.8, $J_{4',5'}$ 1.0, $J_{5,6}$ 6.3, $J_{5',6'}$ 6.6 Hz.

Methyl (methyl 3-deoxy-5,7-O-*isopropylidene*-α-D-lyxo-*hept-2-ulopyranosid)onate* (**6b**).—A mixture

of **6a** [6] (0.354 g, 1.5 mmol), dimethoxypropane (2 mL), DMF (3 mL), and CSA (15 mg) was stirred for 3 h at room temperature, then the mixture was neutralized with TEA (0.2 mL) and evaporated with toluene. Purification by chromatography (hexane–Me_2CO, 3:2) gave **6b** (0.38 g, 92%) as a crystalline product: mp 53 °C; $[\alpha]_D$ +61.3° (*c* 0.63, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 4.48 (ddd, 1H, H-4), 4.18 (dd, 1H, H-5), 3.79–3.82 (m, 1H, H-6), 3.78 (s, 3H, CO_2CH_3), 3.70–3.71 (m, 2H, H-7, H-7a), 3.70 (bs, 1H, OH), 3.26 (s, 3H, OCH_3), 2.63 (dd, 1H, H-3_{eq}), 1.90 (dd, 1H, H-3_{ax}), 1.43, 1.36 (2s, 2×3H, *i*-Pr),; $J_{3ax,3eq}$ 15.1, $J_{3ax,4}$ 3.5, $J_{3eq,4}$ 4.7, $J_{4,5}$ 7.2, $J_{5,6}$ 1.9, $J_{6,7}$ 1.9 Hz; HRMS (LSIMS): Calcd for $C_{12}H_{20}O_7$ $[M+Na]^+$ *m/z* 299.1106, Found *m/z* 299.1104.

*Methyl (methyl 4-*O*-benzyl-3-deoxy-5,7-*O*-isopropylidene-*α*-*D*-lyxo-hept-2-ulopyranosid)onate* (**6c**)*, and benzyl (methyl 4-*O*-benzyl-3-deoxy-5,7-*O*-isopropylidene-*α*-*D*-lyxo-hept-2-ulopyranosid)-onate* (**6d**).—A suspension of **6b** (0.184 g, 0.66 mmol), BnBr (0.3 mL, 2.53 mmol) and a freshly prepared Ag_2O (0.58 g, 2.5 mmol) in a mixture of MeCN–DMF (2:1, 6 mL) was stirred overnight at room temperature. The suspension was diluted with Et_2O, filtered, poured into aq. $NaHCO_3$, and extracted with Et_2O. The organic layer was washed with aq. $Na_2S_2O_3$, dried, and evaporated. The residue was applied to a silica gel column. Elution with hexane–Et_2O (1:2) gave **6c** (0.17 g, 71%): mp 108 °C; $[\alpha]_D$ +63.8° (*c* 0.55, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.26–7.36 (m, 5H, Ph), 4.63 (d, 1H, Bn), 4.57 (d, 1H, Bn), 4.10–4.12 (m, 1H, H-5), 4.05 (d, 1H, H-7a), 3.96 (d, 1H, H-7), 3.88 (ddd, 1H, H-4), 3.81 (s, 3H, CO_2CH_3), 3.34–3.36 (m, 1H, H-6), 3.21 (s, 3H, OCH_3), 2.1–2.22 (m, 2H, H-3_{ax}, H-3_{eq}), 1.49, 1.46, (2s, 2(3H, *i*-Pr); $J_{3ax,3eq}$ 12.5, $J_{3ax,4}$ 11.5, $J_{3eq,4}$ 5.2, $J_{4,5}$ 3.2, $J_{5,6}$ 2.4, $J_{6,7}$ 1.7, $J_{6,7a}$ 2.1, $J_{7,7a}$ 12.8 Hz; HRMS (LSIMS): Calcd for $C_{19}H_{26}O_7$ $[M+Na]^+$ *m/z* 389.1576, Found *m/z* 389.1577. As a side product was isolated the product of transesterification of carbomethoxy group leading to the carbobenzyloxy derivative **6d** in 4.8% yield (14 mg): mp 73–74 °C; $[\alpha]_D$ +43.0° (*c* 1.59, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.27–7.40 (m,5H, Ph), 5.20 (d, 1H, OCH_2Ph), 4.59 (d, 1H, OCH_2), 4.56 (d, 1H, OCH_2Ph), 4.30 (d, 1H, OCH_2Ph), 4.10 (m, 1H, H-5), 4.04 (dd, 1H, H-7a), 3.97 (dd, 1H, H-7), 3.87 (ddd, 1H, H-4), 3.35 (m, 1H, H-6), 3.19 (s, 3H, OCH_3), 2.12–2.24 (m, 2H, H-3_{ax}, H-3_{eq}), 1.47, 1.45 (2s, 2×3H, *i*-Pr); $J_{3ax,3eq}$ 12.4, $J_{3ax,4}$ 11.8, $J_{3eq,4}$ 4.9, $J_{4,5}$ 3.2, $J_{6,7}$ 1.8, $J_{6,7a}$ 2.2, $J_{7,7a}$ 12.8 Hz; HRMS (LSIMS): Calcd for $C_{25}H_{30}O_7$ $[M+Na]^+$ *m/z* 465.1889, Found *m/z* 465.1889.

*Methyl (methyl 4-*O*-benzyl-3-deoxy-*α*-*D*-lyxo-hept-2-ulopyranosid)onate* (**6e**).—A solution of **6c** (0.183 g, 0.5 mmol) and CSA (5 mg) in a mixture of CH_2Cl_2–MeOH (6 mL, 2:1) was stirred for 1 h at room temperature (TLC, hexane–Me_2CO, 3:2) $NaHCO_3$ was added and stirring was continued for 15 min. Filtration through a Celite followed by evaporation afforded crystalline **6e** (0.152 g, 93%): mp 47 °C; $[\alpha]_D$ +49.9° (*c* 0.61, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.30–7.37 (m, 5H, Ph), 4.57–4.62 (m, 2H, Bn), 4.01–4.05 (m, 1H, H-5), 3.89 (ddd, 1H, H-4), 3.84–3.87 (m, 1H, H-6), 3.81 (s, 3H, CO_2CH_3), 3.67 (ddd, 2H, H-7, H-7a), 3.23 (s, 3H, OCH_3), 2.8–2.86 (bs, 1H, 7-OH), 2.69 (bs, 1H, 5-OH), 2.23 (ddd, 1H, H-3_{eq}), 2.03 (dd, 1H, H-3_{ax}); $J_{3ax,3eq}$ 12.8, $J_{3ax,4}$ 11.6, $J_{3eq,4}$ 5.0, $J_{3eq,5}$ 0.9, $J_{7,6}$ 1.3, $J_{7a,6}$ 1.2, $J_{7a,7b}$ 4.3, $J_{4,5}$ 3.0 Hz; HRMS (LSIMS): Calcd for $C_{16}H_{22}O_7$ $[M+Na]^+$ *m/z* 349.1263, Found *m/z* 349.1264.

*1,7-Dimethyl (methyl 4-*O*-benzyl-3-deoxy-*α*-*D*-lyxo-hept-2-ulopyranosid)arate* (**7b**).—To a solution of **6e** (0.2 g, 0.6 mmol) in CH_2Cl_2 (2 mL) saturated aq. $NaHCO_3$ (5 mL), KBr (8 mg), and TEMPO (8 mg) were added. The mixture was cooled in the ice-bath, then NaOCl (4.1 mL) was added dropwise with stirring. After 1 h the solvents were evaporated and the residue was filtered through a silica gel column (CH_2Cl_2–MeOH, 10:1). Evaporation left a syrup which was redissolved in dry MeOH, treated with 1 M solution of Me_3SiCl in CH_2Cl_2 and stirred overnight. The reaction mixture was concentrated, and purified by chromarography (CH_2Cl_2–Me_2CO, 95:5) to yield **7b** (0.18 g, 82%): $[\alpha]_D$ +56.6° (*c* 0.41, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 7.1–7.4 (m, 5H, Ph), 4.61 (s, 2H, Bn), 4.36 (d, 1H, H-5), 4.27 (d, 1H, H-6), 3.97 (ddd, 1H, H-4), 3.86, 3.84 (2s, 2×3H, CO_2CH_3), 3.26 (s, 3H, OCH_3), 2.25 (ddd, 1H, H-3_{eq}), 2.09 (dd, 1H, H-3_{ax}), 1.92 (bs, 1H, OH); $J_{3ax,3eq}$ 12.9, $J_{3ax,4}$ 11.6, $J_{3eq,4}$ 5.1, $J_{3eq,5}$ 0.9, $J_{4,5}$ 3.0, $J_{5,6}$ 1.5 Hz; HRMS (LSIMS): Calcd for $C_{17}H_{22}O_8$ $[M+Na]^+$ *m/z* 377.1212, Found *m/z* 377.1220.

*1,7-dimethyl (2,3,4-tri-*O*-acetyl-6-deoxy-*α*-*L*-talopyranosyl)-(1→2)-(3,4-di-*O*-acetyl-*α*-*L*-rhamnopyranosyl)-(1→5)-(methyl 4-*O*-benzyl-3-deoxy-*α*-*D*-lyxo-hept-2-ulopyranosid)arate* (**8a**).—A mixture of alcohol **7b** (65 mg, 0.177 mmol), trichloroacetimidate **5b** (118 mg, 0.177 mmol) and molecular sieves 3 Å (100 mg) in CH_2Cl_2 (2 mL),

was stirred for 1 h under argon. The mixture was cooled to −40 °C and a solution of $BF_3{\cdot}OEt_2$ (1.77 mL, 1 M solution in CH_2Cl_2) was added. After stirring for 15 min at ambient temperature (TLC, CH_2Cl_2–MeOH, 95:5) the mixture was neutralized with pyridine, filtered, and concentrated. Purification by chromatography afforded trisaccharide **8a** (118 mg, 78%) as a sole product: amorphous powder; $[\alpha]_D$ −21.5° (*c* 0.55, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 5.14–5.19 (m, 2H, H-2″, H-3″), 4.8 (dt, 1H, H-4″), 4.7–5.2 (m, 2H, H-3′, H-4′), 4.83 (d, 1H, H-1″), 6.63, 4.55 (2d, 2×1H, Bn), 4.49 (bs, 1H, H-5), 4.27 (d, 1H, H-6), 4.11 (dd, 1H, H-2′), 3.97 (ddd, 1H, H-4), 3.92 (pq, 1H, H-5″), 3.86, 3.85 (2s, 2×3H, CO_2CH_3), 3.82 (pq, 1H, H-5′), 3.24 (s, 3H, OCH_3), 2.27 (dd, 1H, H-3_{eq}), 1.98–2.18 (m, 1H, H-3_{ax}), 2.11, 2.10, 2.03, 2.02, 2.0 (5s, 5×3H, Ac), 1.22 (d, 3H, CH_3′), 0.90 (d, 3H, CH_3″); $J_{1',2'}$ 2.1, $J_{1'',2''}$ 1.0, $J_{2',3'}$ 3.2, $J_{2'',3''}$ 3.3, $J_{3ax,3eq}$ 12.7, $J_{4,3ax}$ 11.8, $J_{4,3eq}$ 4.7, $J_{4,5}$ 2.5, $J_{3',4'}$ 9.9, $J_{3'',4''}$ 3.9, $J_{4',5'}$ 9.9, $J_{4'',5''}$ 1.2, $J_{5,6}$ 1.4, $J_{5',6'}$ 6.3, $J_{5'',6''}$ 6.6 Hz; HRMS (LSIMS): Calcd for $C_{39}H_{52}O_{21}$ $[M+Na]^+$ *m*/*z* 879.2899, Found *m*/*z* 879.2902.

1,7-dimethyl (2,3,4-tri-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-(1→2)-(3,4-di*-O-*acetyl*-α-L-*rhamnopyranosyl)-(1→5)-(methyl 4*-O-*acetyl-3-deoxy*-α-D-lyxo-*hept-2-ulopyranosid)arate* (**8c**).—A mixture of **8a** (43 mg, 0.5 mmol), in EtOH and the catalyst Pd/C (20 mg) was stirred overnight under H_2. Filtration through a Celite, then evaporation gave **8b** (TLC, hexane–Me_2CO, 3:2) which was subjected to acetylation (Ac_2O-Py, 1:1, 1 mL). Usual work-up afforded **9c** (31 mg, 78%): mp 93–94 °C; $[\alpha]_D$ −2.4° (*c* 0.72, $CHCl_3$); ^{1}H NMR (C_6D_6): δ 5.54 (dd, 1H, H-3′), 5.46–5.49 (m, 2H, H-5, H-4′), 5.38 (dt, 1H, H-4″), 5.32 (ddd, 1H, H-4), 5.26 (t, 1H, H-2″), 5.22 (d, 1H, H-1′), 5.05 (d, 1H, H-1″), 4.56 (t, 1H, H-3″), 4.31 (t, 1H, H-2′), 2.20–4.26 (m, 2H, H-5′, H-5″), 4.14 (d, 1H, H-6), 3.70, 3.30 (2s, 2×3H, CO_2CH_3), 3.11 (s, 3H, OCH_3), 2.46 (t, 1H, H-3_{ax}), 2.35 (dddd, 1H, H-3_{eq}), 1.96, 1.81, 1.78, 1.69, 1.66, 1.61 (6s, 6×3H, Ac), 1.32 (d, 3H, CH_3′), 1.05 (d, 3H, CH_3″); $J_{1',2'}$ 2.2, $J_{1'',2''}$ 1.4, $J_{2',3'}$ 3.1, $J_{2'',3''}$ 2.5, $J_{3ax,3eq}$ 12.7, $J_{3ax,4}$ 12.3, $J_{3eq,4}$ 5.0, $J_{3eq,5}$ 0.7, $J_{3',4'}$ 9.7, $J_{3'',4''}$ 3.8, $J_{4,5}$ 2.6, $J_{4',5'}$ 9.5, $J_{4'',5''}$ 0.5, $J_{5,6}$ 1.4, $J_{5',6'}$ 1.3, $J_{5'',6''}$ 1.6 Hz; HRMS (LSIMS): Calcd for $C_{34}H_{48}O_{22}$ $[M+Na]^+$ *m*/*z* 831.2535, Found *m*/*z* 831.2535.

2-O-*(2,3,4-Tri*-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-1,5-di*-O-*acetyl-3*-O-*benzyl-6-deoxy*-α-L-*rhamnofuranose* (**4a**).—A solution of **3a** (0.124 g, 0.2 mmol) in Ac_2O (4 mL) was treated with TfOH (0.1 mL), and the reaction mixture was stirred overnight at room temperature. After addition of dry $NaHCO_3$ and evaporation with toluene the product was purified by chromatography (hexane–Et_2O, 1:1) to yield **4a** (86 mg, 71%): syrup; $[\alpha]_D$ −42.8° (*c* 0.9, $CHCl_3$); ^{1}H NMR (C_6D_6): δ 7.1–7.6 (m, 5H, Ph), 6.50 (d, 1H, H-1), 5.56 (t, 1H, H-5), 5.44 (t, 1H, H-3′), 5.41 (dt, 1H, H-2′), 4.47 (d, 1H, Bn), 4.41 (d, 1H, Bn), 4.17 (dd, 1H, H-4), 3.99 (dd, 1H, H-2), 3.98 (dq, 1H, H-5′), 3.82 (t, 1H, H-3), 1.80, 1.75, 1.72, 1.63, 1.62 (5s, 5×3H, Ac), 1.50 (d, 3H, CH_3′), 1.04 (d, 3H, CH_3); $J_{1,2}$ 3.4, $J_{1',2'}$ 1.0, $J_{2,3}$ 4.6, $J_{2',3'}$ 3.7, $J_{3,4}$ 4.6, $J_{3',4'}$ 3.6, $J_{4,5}$ 6.4, $J_{4',5'}$ 1.4, $J_{5,6}$ 6.4, $J_{5',6'}$ 6.6 Hz; Anal Calcd for $C_{29}H_{38}O_{14}$ (610.61)): C 56.99%, H 6.27%, Found C 57.12%, H 6.11%.

2-O-*(2,3,4-Tri*-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-5*-O-*acetyl-3*-O-*benzyl*-α-L-*rhamnofuranosyl bromide* (**4b**).—To a solution of **4a** (0.3 g, 0.5 mmol) in a mixture of dry CH_2Cl_2–AcOH (5:0.5, 5.5 mL) was added $TiBr_4$ (0.3 g) under argon. The mixture was stirred for 1 h at room temperature, then dry AcONa was added, and stirring was continued for few minutes. Filtration, followed by evaporation with toluene left a syrup, immediately used for the condensation reaction.

1,7-dimethyl (2,3,4-tri-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-(1→2)-(5*-O-*acetyl-2*-O-*benzyl*-β-L-*rhamnofuranosyl)-(1→5)-(methyl 4*-O-*benzyl-3-deoxy*-α-D-lyxo-*hept-2-ulopyranosid)arate* (**9a**).—A mixture of alcohol **7b** (65 mg, 0.177 mmol), $Hg(CN)_2$ (45 mg, 0.177 mmol) and molecular sieves 3A (0.2 g) was stirred for 1 h under argon. Bromide **4b** (from above experiment) in CH_2Cl_2 was added dropwise and stirring was continued for 3 h. The mixture was diluted with CH_2Cl_2 filtered through a Celite, and the filtrate was washed with aq. KI, then H_2O. Purification by chromatography (hexane–Et_2O-MeOH, 1:1:0.01) afforded **9a** (0.112 g, 70%) as a syrup: $[\alpha]_D$ +26.2° (*c* 0.4, $CHCl_3$); ^{1}H NMR (C_6D_6): δ 7.05–7.25 (m, 5H, Ph), 5.74 (m, 1H, H-4″), 5.64–5.71 (m, 2H, H-5′, H-3″), 5.44 (dt, 1H, H-2″), 5.39 (d, 1H, H-1′), 4.83 (pq, 1H, H-5″), 4.58-4.64 (m, 3H, unresolved), 4.44–4.51 (m, 2H, Bn), 3.96 (d, 1H, H-6), 3.94 (t, 1H, H-3″), 3.87 (ddd, 1H, H-4), 3.84 (dd, 1H, H-3′), 3.62 (t, 1H, H-2′), 3.75, 3.43 (2s, 2×3H, CO_2CH_3), 3.02 (s, 3H, OCH_3), 2.64 (t, 1H, H-3_{ax}), 2.56 (dd, 1H, H-3_{eq}), 1.89, 1.76, 1.69, 1.62 (4s, 4×3H, Ac), 1.54 (d, 3H, CH_3″), 1.34 (d, 3H, CH_3′); $J_{1',2'}$ 4.4, $J_{2'',3''}$ 3.6, $J_{3ax,3eq}$ 12.7, $J_{3ax,4}$ 11.8, $J_{3eq,4}$ 4.6, $J_{4',5'}$ 8.0, $J_{3',4'}$ 5.6, $J_{4',5'}$ 8.1, $J_{5',6'}$ 6.3, $J_{5'',6''}$ 6.6 Hz; HRMS

(LSIMS): Calcd for $C_{44}H_{56}O_{20}$ $[M+Na]^+$ m/z 927.3362, Found m/z 927.3260.

1,7-dimethyl (2,3,4-tri-O-*acetyl-6-deoxy*-α-L-*talopyranosyl)-(1→2)-(2,5-di*-O-*acetyl-2*-O-*benzyl*-β-L-*rhamnofuranosyl)-(1→5)-(methyl 4*-O-*acetyl-3-deoxy*-α-D-lyxo-*hept-2-ulopyranosid)arate*(**9c**).—To a solution of **9a** (94 mg, 0.1 mmol) in EtOH Pd/C was added and the reaction mixture was stirred overnight under hydrogen. After filtration and evaporation of the solvent the residue was acetylated with Ac_2O–Py (1:1, 3 mL). Usuall work-up, then chromatography (hexane–Et_2O, 1:1) gave **9c** (67 mg, 83%) as crystals: mp 213 °C; $[\alpha]_D$ +48.7° (*c* 0.97, $CHCl_3$); ^{1}H NMR (C_6D_6): δ 5.79–5.81 (m, 1H, H-4″), 5.63 (t, 1H, H-3″), 5.42 (dt, 1H, H-2″), 5.32–5.36 (m, 2H, H-4, H-5′), 5.30 (dd, 1H, H-4′), 5.14 (d, 1H, H-1′), 5.09 (d, 1H, H-1″), 4.79 (d, 1H, H-5), 4.72 (pq, 1H, H-5″), 4.03 (bs, 1H, H-6), 3.79 (t, 1H, H-3′), 3.6 (dd, 1H, H-2′), 3.57, 3.35 (2s, 2×3H, CO_2CH_3), 2.98 (s, 3H, OCH_3), 2.57 (t, 1H, H-3_{ax}), 2.40 (dd, 1H, H-3_{eq}), 2.11, 2.08, 1.86, 1.75, 1.72, 1.60 (6s, 6×3H, Ac), 1.53 (d, 3H, CH_3′), 1.33 (d, 3H, CH_3″); $J_{1',2'}$ 4.9, $J_{1'',2''}$ 1.2, $J_{2',3'}$ 9.4, $J_{2'',3''}$ 3.6, $J_{3ax,3eq}$ 12.6, $J_{3ax,4}$ 12.4, $J_{3eq,4}$ 4.5, $J_{4',5'}$ 10.3, $J_{4'',5''}$ 1.8, $J_{5,6}$ 2.3, $J_{5',6'}$ 6.2, $J_{5'',6''}$ 6.4 Hz; HRMS (LSIMS): Calcd for $C_{34}H_{48}O_{22}$ $[M+Na]^+$ m/z 831.2535, Found m/z 831.2533.

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