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Synthesis of the α-D-GlcpA-(1→3)-α-L-Rhap-(1→2)-L-Rha trisaccharide isolated from the cell wall hydrolyzate of the green alga, *Chlorella vulgaris*

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Dedicated to Professor Peter Köll on the occasion of his 60th birthday

Abstract

The title trisaccharide was synthesized from 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride (**10**), ethyl 2,4-di-*O*-benzyl-1-thio- (**5**) and benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside (**9**). The disaccharide **11** obtained from compounds **5** and **10** was used as the glycosyl donor to glycosylate the rhamnopyranoside derivative **9** having free OH-2 using the NIS–AgOTf-mediated glycosylation methodology. Zemplén deacetylation of the trisaccharide **12** resulted in the 6″-OH derivative (**13**), which was selectively oxidized with CrO_3 to the uronic acid derivative **14**. The benzyl groups were removed by catalytic hydrogenolysis to furnish the target trisaccharide (**1**). © 2001 Elsevier Science Ltd. All rights reserved.

Keywords: *Chlorella vulgaris*; Hydrogenolysis; Oligosaccharide synthesis; α-D-Glucopyranuronosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-L-rhamnopyranose

1. Introduction

Japanese authors reported the isolation of α-D-glucopyranuronosyl-(1→3)-L-rhamnose[1] and α-D-glucopyranuronosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-α-L-rhamnose[2] from the acid hydrolyzate of the cell wall of the green alga *Chlorella vulgaris*, whose structures were determined by chemical and modern spectroscopic methods, involving positive- and negative-ion FABMS, and 1D and 2D NMR techniques. A search among the synthesized oligosaccharides revealed that neither the above disaccharide, nor the trisaccharide have been prepared as yet. Among the glycopyranuronosyl-L-rhamnoses, only the following three disaccharides were synthesized: β-D-glucopyranuronosyl-(1→4)-,[3] β-D-galactopyranuronosyl-(1→4)-,[3] and β-D-glucopyranuronosyl-(1→3)-L-rhamnose.[4] All of these three synthetic routes applied glycopyranuronosyl

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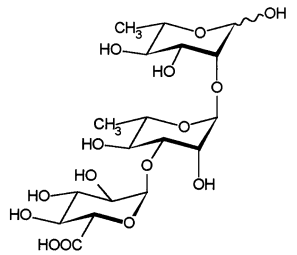
donors and no glycopyranosyl → glycopyranuronosyl transformation was used.

In the present paper, the preparation of a suitably protected α-D-glucopyranosyl-(1 → 3)-α-L-rhamnopyranoside derivative in a glycosyl donator form is reported, which was used to glycosylate benzyl 3,4-di-*O*-benzyl-α-L-rhamnopyranoside. The CH_2OH group of the produced trisaccharide was oxidized to a carboxylic function, and then the molecule was deprotected to the title trisaccharide (**1**).



1

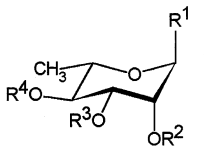
2. Results and discussion

Our synthetic strategy was based on the protection of the hydroxyl functions with benzyl ethers as 'permanent', and acetates as 'temporary' protecting blocking groups. In order to avoid difficulties, oxidation to uronic acid was planned at the trisaccharide level, and thus the target structure **1**[2] was synthesized via a stepwise approach from the monosaccharide building blocks **5**, **9** and **10**.

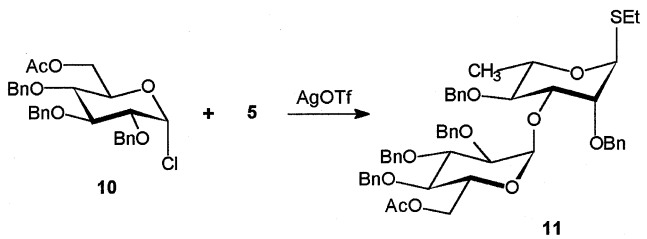
The synthetic schemes reported for the preparation of the rhamnopyranosides **5**[6] and **9**[5] include the common intermediates **2**–**4** to minimize the number of the synthetic steps. Thus, treatment of ethyl 1-thio-α-L-rhamnopyranoside[7] (**2**) with α,α-dimethoxytoluene in the presence of *p*-toluenesulfonic acid (TsOH) in *N*,*N*-dimethylformamide (DMF) gave **3** with an 1.3:1 exo:endo ratio (inseparable mixture) as indicated by ^{1}H NMR analysis. Compound **3** was benzylated using benzyl bromide and NaH in DMF, affording **4** with a BnO-4 group, that could be hydrogenolyzed with the $LiAlH_4$–$AlCl_3$ reagent[8] in dichloromethane–diethyl ether to furnish an easily separable mixture of the 2,4-di-*O*-benzyl (**5**) and 3,4-di-*O*-benzyl (**6**) derivatives.

Ring opening of the dioxolane-type benzylidene acetals of pyranosides under hydrogenolytic conditions with various reagents is well-documented.[9] Since the direction of the ring cleavage is determined by the stereochemistry at the acetal carbon atom,[10,11] hydrogenolysis of an exo–endo mixture of benzylidene acetals generally results in a mixture of regioisomeric benzyl ethers. Indeed, hydrogenolysis of **4** (1.2:1 exo–endo) gave the two regioisomeric benzyl ethers **6** and **5** in 30.6 and 53.2% yield, respectively. The outcome of this experiment clearly indicated that isomerization of the benzylidene ring[11] due to the Lewis acid character of the reagent applied.

For the synthesis of **5** and **6** alternative reaction sequences are described via the regioselective benzylation of ethyl 4-*O*-benzyl-1-thio-α-L-rhamnopyranoside[12] using phase-transfer conditions[6] (→**5**), or via a stannylidene acetal[12] (→**6**). However, the convenient separation of the regioisomers **5** and **6** (R_f 0.30 and 0.14 in 8:2 hexane–EtOAc) makes hydrogenolysis of **4** superior to the previous methods.



	R^1	R^2	R^3	R^4
2	SEt	H	H	H
3	SEt	-Ph, H-		H
4	SEt	-Ph, H-		Bn
5	SEt	Bn	H	Bn
6	SEt	H	Bn	Bn
7	SEt	Ac	Bn	Bn
8	OBn	Ac	Bn	Bn
9	OBn	H	Bn	Bn

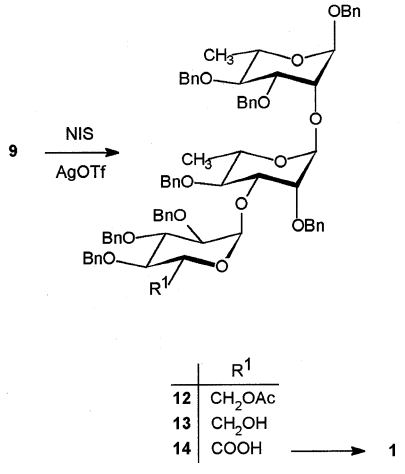


10 **11**

For the synthesis of compound **9**,[5] the HO-2 group of **6** was acetylated to give the glycosyl donor **7**. Glycosylation of benzyl alcohol with **7** in dichloromethane at room temperature in the presence of methyl trifluoromethanesulfonate (MeOTf)[14] and powdered 4 Å molecular sieves afforded the expected benzyl rhamnopyranoside **8**[15] (85.7%), and subsequent Zemplén deacetyla-tion (NaOMe in MeOH) yielded compound **9**.[5]

For the synthesis of **11**, 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl chloride[16] (**10**) was coupled with **5**[6] in dichloromethane–toluene in the presence of silver trifluoromethanesulfonate (AgOTf)[17] as the promoter, furnishing the desired disaccharide with a complete α-stereoselectivity in an isolated yield of 59.4, and 21.8 of the starting **5** was recovered. The configuration of the glycosidic linkage was confirmed by the $^1J_{C\text{-}1,H\text{-}1}$ coupling constant (167.1 Hz).[18] Attempts to obtain higher yield of the disaccharide **11** failed, however the formation of the corresponding β-linked disaccharide was not detected at all. The thioglycosyl donor **11** was activated with *N*-iodosuccinimide (NIS–AgOTf)[19] and coupled to acceptor **9** to produce the trisaccharide **12** exclusively. The large value of the $^1J_{C\text{-}1,H\text{-}1}$ coupling constant (171.1 Hz) assigned for the newly formed glycosidic bond was in full agreement with the expected α-rhamnosidic linkage.

Removal of the primary acetyl function (**12** → **13**), followed by Jones oxidation (CrO_3–H_2SO_4)[20] in acetone, led to the uronic acid **14**. Deprotection of the trisaccharide **14** upon reductive cleavage of the benzyl groups ($Pd(OH)_2$–C/H_2) in *tert*-butanol and water containing acetic acid (pH 4–5) gave, after purification on Toyopearl HW-40F, the target trisaccharide **1** in a yield of 44.3%. The structure (86% in an α-L-rhamnopyranosidic form) of **1** was confirmed by ^1H and ^{13}C NMR spectroscopic methods. The NMR data were in full accordance with the data published recently.[1]



	R^1
12	CH_2OAc
13	CH_2OH
14	COOH

→ **1**

3. Experimental

General.—All moisture-sensitive reactions were performed under an Ar atmosphere using oven-dried glassware. Solvents were dried over standard drying agents. Organic solutions were concentrated under reduced pressure at 40 °C (bath). Column chromatography was performed on Kieselgel 60 (0.063–0.2 mm, Merck). TLC was carried out on Kieselgel 60 F_{254} (Merck) plates. Compounds were visualized by spraying with 50% aq H_2SO_4 followed by heating. Melting points (uncorrected) were determined on a Kofler hot-stage apparatus. Optical rotations were measured with a Perkin–Elmer 241 polarimeter at rt, using a 10-cm (1-mL) cell. NMR spectra were recorded with a Bruker WP-200 SY (^1H, 200.13 MHz; ^{13}C, 50.31 MHz) or a Bruker Avance DRX 500 (^1H, 500.13 MHz; ^{13}C, 125.76 MHz) spectrometer for solutions in $CDCl_3$ (internal Me_4Si) or in D_2O (internal DSS). Proton chemical shifts (δ) are given in ppm relative to the signal for internal Me_4Si ($CDCl_3$) or DSS (D_2O). Carbon chemical shifts were referenced to the solvent signal.

Ethyl 2,3-O-benzylidene-1-thio-α-L-rhamnopyranoside (**3**).—To a solution of **2** (5.0 g, 24.0 mmol) in α,α-dimethoxytoluene (25 mL) were added *p*-toluenesulfonic acid (456 mg, 2.40 mmol) and DMF (5 mL). The mixture was stirred overnight at rt, then diluted with toluene (200 mL), washed with satd $NaHCO_3$ (2 × 30 mL) and water (3 × 30 mL), dried, filtered, and concentrated. Column chromatography (3:1 hexane–EtOAc) of the residue yielded **3** (1.3:1 exo:endo, 4.7 g, 66.1%); $[\alpha]_D$ −156.0° (*c* 0.71, $CHCl_3$); ^1H NMR (200 MHz, $CDCl_3$): δ 7.55–7.32 (m, 5 H, Ph), 6.15 (s, 0.57 H, PhCH_{exo}), 5.91 (s, 0.43 H, PhCH_{endo}), 5.66 (s, 0.43 H, H_{endo}-1), 5.56 (s, 0.57 H, H_{exo}-1), 1.38–1.21 (m, 6 H, CCH_3 and SCH_2CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 138.5 and 137.0 (2 $C_{quat.}$), 129.5-126.0 (Ph), 104.0 and 102.9 (2 PhCH), 79.6 and 79.4 (2 C-1), 78.0 and 75.6 (C-2,3), 72.5 (C-4), 66.0 and 65.7 (2 C-5), 24.5 (SCH_2CH_3), 17.2 (C-6), 14.6 (SCH_2CH_3). Anal. Calcd for $C_{15}H_{20}O_4S$: C, 60.79; H, 6.80. Found: C, 61.03; H, 6.82. The exo-isomer **3** was crystallized from dry

EtOH (10 mL); mp 114–116 °C; $[\alpha]_D$ −142.9° (*c* 0.36, $CHCl_3$).

Ethyl 4-O-benzyl-2,3-O-benzylidene-1-thio-α-L-rhamnopyranoside (**4**).—To a stirred solution of **3** (1.2:1 exo:endo, 4.39 g, 14.81 mmol) in dry DMF (20 mL) was added NaH (0.71 g, 29.62 mmol) at 0 °C. After 30 min, BnBr (2.60 mL, 22.22 mmol) was added, and the mixture was stirred overnight at rt. The excess of the reagent was decomposed by subsequent addition of MeOH and water. The mixture was then diluted with toluene (600 mL), and washed with water (4 × 100 mL). The organic layer was dried, and concentrated. The residue was purified by column chromatography (19:1 hexane–EtOAc) to obtain **4** (5.1 g, 89.1%). Crystallization from abs EtOH (10 mL) resulted in 3.0 g of **4** (5:1 exo:endo); mp 47–48 °C; ^{1}H NMR (200 MHz, $CDCl_3$): δ 7.52–7.21 (m, 10 H, 2 Ph), 6.07 (s, 0.83 H, PhCH_{exo}), 5.91 (s, 0.17 H, PhCH_{endo}), 5.65 (s, 0.17 H, H_{endo}-1), 5.55 (s, 0.83 H, H_{exo}-1), 4.96 and 4.72 (2 d, 1.66 H, PhCH_{2exo}), 4.83 and 4.53 (2 d, 0.34 H, PhCH_{2endo}), 2.77–2.41 (m, 2 H, SCH_2CH$_3$), 1.37–1.21 (m, 6 H, CCH$_3$ and SCH$_2$CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 138.6 and 138.0 (2 $C_{quat.}$), 129.2–126.2 (2 Ph), 103.8 and 102.9 (2 Ph*C*H), 79.8 (C-1), 79.4, 78.0 and 76.6 (C-2,3,4), 73.0 (PhCH_2), 65.0 (C-5), 24.4 (SCH_2CH$_3$), 17.8 (C-6), 14.6 (SCH$_2$$CH_3$). Anal. Calcd for $C_{22}H_{26}O_4S$: C, 68.37; H, 6.78. Found: C, 68.59; H, 6.76.

Ethyl 2,4-di-O-benzyl- (**5**) *and ethyl 3,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside* (**6**).—A mixture of $LiAlH_4$ (857 mg, 22.59 mmol) and $AlCl_3$ (2.54 g, 19.06 mmol) in Et_2O (20 mL) was heated to 35 °C. Then, a solution of **4** (2.73 g, 7.06 mmol) in dry 1:1 CH_2Cl_2–Et_2O (70 mL) was added dropwise, and the mixture was stirred for 15 min at reflux temperature. The excess of $LiAlH_4$ was decomposed with EtOAc (10 mL), and $Al(OH)_3$ was precipitated by the addition of water (15 mL). After dilution with EtOAc, the organic layer was decanted, washed with water (3 × 25 mL), dried, and concentrated. Column chromatography (4:1 hexane–EtOAc) of the residue gave **5** (1.46 g, 53.2%) and **6** (0.84 g, 30.6%).

Compound **5** had $[\alpha]_D$ −92.1° (*c* 0.60, $CHCl_3$), lit. $[\alpha]_D$ −91.3° (*c* 0.64, $CHCl_3$);[6] ^{1}H NMR shifts were in agreement with those previously reported;[6] ^{13}C NMR (50 MHz, $CDCl_3$): δ 138.5 and 137.5 (2 $C_{quat.}$), 128.5–127.6 (2 Ph), 82.5 (C-1), 81.0 and 80.2 (C-2,4), 74.9 and 72.3 (2 PhCH_2), 72.1 (C-3), 67.6 (C-5), 25.1 (SCH_2CH$_3$), 17.9 (C-6), 14.9 (SC$_2$$CH_3$).

Compound **6** had $[\alpha]_D$ −140.7° (*c* 0.60, $CHCl_3$), lit. $[\alpha]_D$ −152.4° (*c* 1.00, $CHCl_3$);[13] ^{1}H and ^{13}C NMR shifts were in full accordance with the data described previously.[13]

Ethyl 2-O-acetyl-3,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**7**).—Compound **6** (980 mg, 2.52 mmol) was treated with 1:1 pyridine–Ac_2O (10 mL) for 5 h. The mixture was concentrated and co-evaporated with toluene (3 × 10 mL). Column chromatography (15:1 hexane–EtOAc) of the residue furnished **7** (1.02 g, 94.0%); $[\alpha]_D$ −77.6° (*c* 0.84, $CHCl_3$), lit. (D enantiomer) $[\alpha]_D$ +61° (*c* 2.1, $CHCl_3$);[21] ^{1}H NMR shifts were in full accordance with the data reported previously;[21] ^{13}C NMR (50 MHz $CDCl_3$): δ 170.3 (C=O), 138.4 and 137.7 (2 $C_{quat.}$), 128.4–127.7 (2 Ph), 82.3 (C-1), 80.3 and 78.3 (C-3,4), 75.4 and 71.8 (2 PhCH_2), 70.8 (C-2), 68.3 (C-5), 25.5 (SCH$_2$$CH_3$).

Benzyl 2-O-acetyl-3,4-di-O-benzyl-α-L-rhamnopyranoside (**8**).—To a solution of **7** (770 mg, 1.79 mmol) in dry CH_2Cl_2 (15 mL) were added 4 Å powdered molecular sieves (4 g) and benzyl alcohol (278 μL, 2.68 mmol), and the mixture was stirred under Ar overnight. After the addition of MeOTf (810 μL, 7.15 mmol) at 0 °C, the mixture was stirred for 24 h at rt. Triethylamine (800 μL) was added, and the mixture was filtered through Celite, then the cake was washed with CH_2Cl_2 (3 × 10 mL). The combined filtrates were washed with water (3 × 20 mL), dried and concentrated. The product was purified by column chromatography (3:1 hexane–EtOAc) to obtain pure **8** (730 mg, 85.7%); $[\alpha]_D$ −19.6° (*c* 0.55, $CHCl_3$), lit. $[\alpha]_D$ −25.0° (*c* 2.0, $CHCl_3$);[15] ^{1}H NMR shifts were in full accordance with the data reported previously;[15] ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.3 (C=O), 138.4, 138.0 and 136.9 (3 $C_{quat.}$), 128.4–127.7 (3 Ph), 97.0 (C-1), 80.0 and 78.1 (C-3,4), 75.4, 71.8 and 69.2 (3 PhCH_2), 69.0 (C-2), 67.9 (C-5), 21.1 (*C*H$_3$CO), 17.9 (C-6).

Benzyl 3,4-di-O-benzyl-α-L-rhamnopyranoside (**9**).—To a solution of **8** (730 mg, 1.53 mmol) in dry MeOH (20 mL) was added NaOMe (pH 9–10). The mixture was kept at rt overnight, and neutralized with Amberlite IR-120 (H^+) resin. Then, the resin was filtered off, and the filtrate was concentrated and co-evaporated with dry CH_2Cl_2 (2 × 10 mL). The residue was purified by column chromatography (7:3 hexane–EtOAc) to give pure **9** (630 mg, 94.8%); $[\alpha]_D$ −52.4° (*c* 0.63, $CHCl_3$), lit. $[\alpha]_D$ −58° (*c* 0.6, $CHCl_3$);[5] ^1H and ^{13}C NMR shifts were in agreement with those previously published.[22]

Ethyl (6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-2,4-di-O-benzyl-1-thio-α-L-rhamnopyranoside (**11**).—To a solution of **10**[15] (1.2 g, 2.35 mmol) and **5** (540 mg, 1.39 mmol) in dry CH_2Cl_2 (20 mL) was added 4 Å powdered molecular sieves (2 g), and the mixture was stirred under Ar for 30 min. The mixture was cooled to −40 °C, then a solution of AgOTf (890 mg, 3.48 mmol) in dry toluene (20 mL) was added dropwise and the mixture was stirred for 30 min at −40 °C. After the addition of pyridine (1 mL) and CH_2Cl_2 (150 mL), the mixture was filtered through Celite. The filtrate was washed with 10% aq $Na_2S_2O_3$ (2 × 30 mL), satd $NaHCO_3$ (20 mL) and water (20 mL), dried, and evaporated. The product was purified by column chromatography (3:1 hexane–EtOAc) giving pure **11** (713 mg, 59.4%), and 118 mg (21.8%) of **5** was also collected; $[\alpha]_D$ +5.6° (*c* 1.60, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.38–7.09 (m, 25 H, 5 Ph), 5.35 (d, 1 H, $J_{1,2}$ 1.8 Hz, H-1), 5.22 (d, 1 H, $J_{1',2'}$ 3.5 Hz, H-1′), 5.05–4.60 (m, 10 H, 5 PhCH_2), 4.26–4.11 (m, 4 H, H-5,5′,6′a,6′b), 4.21 (t, 1 H, $J_{3',4'}$ 9.4 Hz, H-3′), 4.13 (dd, 1 H, $J_{3,4}$ 9.2 Hz, H-3), 4.09 (dd, 1 H, $J_{2,3}$ 2.8 Hz, H-2), 3.78 (t, 1 H, $J_{4,5}$ 9.2 Hz, H-4), 3.70 (dd, 1 H, $J_{2',3'}$ 9.4 Hz, H-2′), 3.59 (t, 1 H, $J_{4',5'}$ 9.2, H-4′), 2.63 (m, 2 H, SCH_2CH$_3$), 1.99 (s, 3 H, Ac), 1.35 (d, 3 H, $J_{5,6}$ 5.0 Hz, CCH_3), 1.20 (t, 3 H, SCH_2CH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.6 (C=O), 138.3–137.8 (5 $C_{quat.}$), 128.3–127.6 (5 Ph), 93.9 ($J_{C1',H1'}$ 167.1 Hz, C-1′), 82.0 (C-1), 81.8 (C-3′), 79.9 (C-4), 79.3 (C-2′), 77.5 (C-4′), 76.7 (C-2), 75.9 (C-3), 68.9 and 68.5 (C-5,5′), 62.9 (C-6′), 25.3 (SCH_2CH$_3$), 20.8 (CH_3CO), 17.8 (C-6), 14.9 (SCH_2CH_3). Anal. Calcd for $C_{51}H_{58}O_{10}S$: C, 70.97; H, 6.77. Found: C, 71.23; H, 6.79.

Benzyl (6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1→3)-(2,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (**12**).—A mixture of **11** (672 mg, 0.78 mmol), **9** (282 mg, 0.65 mmol) and 4 Å molecular sieves (beads) was stirred for 30 min in dry CH_2Cl_2 (15 mL) under Ar. The mixture was cooled to −20 °C and subsequently a solution of NIS (219 mg, 0.97 mmol) in 3:2 dry MeCN–dry CH_2Cl_2 (5 mL) and AgOTf (25 mg, 97.30 μmol) in dry toluene (1 mL) were added and the reaction mixture was stirred for 10 min. The reaction was quenched by Et_3N (50 μL), diluted with CH_2Cl_2 (40 mL), and filtered. The filtrate was washed with 10% aq $Na_2S_2O_3$ (2 × 20 mL), satd $NaHCO_3$ (20 mL) and water (20 mL), dried and concentrated. The residue was purified by column chromatography (3:1 hexane–EtOAc) to give pure **12** (525 mg, 65.5%); $[\alpha]_D$ +17.9° (*c* 0.43, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$): δ 7.35–7.10 (m, 40 H, 8 Ph), 5.22 (d, 1 H, $J_{1'',2''}$ 3.5 Hz, H-1″), 5.20 (d, 1 H, $J_{1',2'}$ 2.2 Hz, H-1′), 5.04–4.50 (m, 16 H, 8 PhCH_2), 4.78 (d, 1 H, $J_{1,2}$ 2.5 Hz, H-1), 4.30–4.15 (m, 3 H, H-5″,6″a,6″b), 4.20 (t, 1 H, $J_{2'',3''}$ 9.4 Hz, H-3″), 4.18 (dd, 1 H, $J_{3',4'}$ 9.0 Hz, H-3′), 4.15 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.05 (t, 1 H, $J_{2',3'}$ 2.6 Hz, H-2′), 3.97 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 3.79 (m, 2 H, H-5,5′), 3.70 (t, 1 H, $J_{4',5'}$ 9.0 Hz, H-4′), 3.66 (dd, 1 H, $J_{2'',3''}$ 9.7 Hz, H-2″), 3.61 (t, 1 H, $J_{4'',5''}$ 9.4 Hz, H-4″), 3.51 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 1.94 (s, 3 H, Ac), 1.30 (d, 3 H, $J_{5,6}$ 6.2 Hz, CCH_3), 1.24 (d, 3 H, $J_{5',6'}$ 6.5 Hz, CCH_3); ^{13}C NMR (125 MHz, $CDCl_3$): δ 170.5 (C=O), 138.5–137.3 (8 $C_{quat.}$), 128.3–127.3 (8 Ph), 99.5 ($J_{C\text{-}1',H\text{-}1'}$ 171.1 Hz, C-1′), 98.0 (C-1), 94.4 (C-1″), 82.0 (C-3″), 80.7 (C-4), 80.0 (C-4′), 79.8 (C-3), 79.2 (C-2″), 77.4 (C-4″), 75.4 (C-3′), 75.3 (C-2′), 74.3 (C-2), 68.9 (C-5″), 68.5 and 68.1 (C-5,5′), 62.8 (C-6″), 20.8 (CH_3CO), 17.9 (C-6,6′). Anal. Calcd for $C_{76}H_{82}O_{15}$: C, 73.89; H, 6.69. Found: C, 74.13; H, 6.67.

Benzyl (2,3,4-tri-O-benzyl-α-D-glucopyranuronosyl)-(1→3)-(2,4-di-O-benzyl-α-L-rhamnopyranosyl)-(1→2)-3,4-di-O-benzyl-α-L-rhamnopyranoside (**14**).—To a solution of

12 (519 mg, 0.42 mmol) in dry MeOH (10 mL) and dry CH_2Cl_2 (2 mL) was added a catalytic amount of NaOMe. The mixture was stirred for 4 h, then neutralized with Amberlite IR-120 (H^+) resin. The resin was filtered off, and the filtrate was concentrated to give **13** (488 mg, 97.4%), which was dissolved in acetone (10 mL), then a solution of CrO_3 (410 mg, 4.10 mmol) in aq 3 M H_2SO_4 (2 mL) was added dropwise. The mixture was stirred for 30 min at rt, diluted with CH_2Cl_2 (100 mL), washed with cold water (3×20 mL), dried, and concentrated. The residue was purified by column chromatography (7:3 hexane–acetone) to yield **14** (288 mg, 58.2%); $[\alpha]_D$ +2.3° (*c* 0.92, $CHCl_3$); ^{1}H NMR (500 MHz, $CDCl_3$): δ 7.36–7.08 (m, 40 H, 8 Ph), 5.25 (d, 1 H, $J_{1'',2''}$ 3.1 Hz, H-1″), 5.16 (d, 1 H, $J_{1',2'}$ 2.4 Hz, H-1′), 4.99–4.45 (m, 16 H, 8 $PhCH_2$), 4.81 (d, 1 H, $J_{1,2}$ 1.7 Hz, H-1), 4.19 (dd, 1 H, $J_{3',4'}$ 8.3 Hz, H-3′), 4.14 (t, 1 H, $J_{3'',4''}$ 9.4 Hz, H-3″), 4.11 (dd, 1 H, $J_{2,3}$ 2.9 Hz, H-2), 4.02 (t, 1 H, $J_{2',3'}$ 2.7 Hz, H-2′), 3.94 (dd, 1 H, $J_{3,4}$ 9.4 Hz, H-3), 3.77 (t, 1 H, $J_{4'',5''}$ 9.4 Hz, H-4″), 3.79–3.73 (m, 2 H, H-5,5′), 3.75 (d, 1 H, H-5″), 3.66 (t, 1 H, $J_{4',5'}$ 8.3 Hz, H-4′), 3.64 (dd, 1 H, $J_{2'',3''}$ 9.4 Hz, H-2″), 3.49 (t, 1 H, $J_{4,5}$ 9.4 Hz, H-4), 1.28 (d, 3 H, $J_{5,6}$ 6.1 Hz, CCH_3), 1.19 (d, 3 H, $J_{5',6'}$ 6.6 Hz, CCH_3'); ^{13}C NMR (125 MHz, $CDCl_3$): δ 171.9 (C=O), 138.5–137.3 (8 $C_{quat.}$), 128.4–127.4 (8 Ph), 99.7 (C-1′), 98.1 (C-1), 95.2 (C-1″), 81.4 (C-3″), 80.7 (C-4), 80.6 (C-4′), 80.0 (C-3), 79.5 (C-4″), 78.6 (C-2″), 76.3 (C-3′), 75.8 (C-2′), 75.6-72.4 (8 $PhCH_2$), 74.5 (C-2), 69.8 (C-5″), 68.4 and 68.2 (C-5,5′), 18.0 (C-6,6′). Anal. Calcd for $C_{74}H_{78}O_{15}$: C, 73.61; H, 6.51. Found: C, 73.88; H, 6.53.

α-D-Glucopyranuronosyl-(1→3)-α-L-rhamnopyranosyl-(1→2)-L-rhamnopyranose (**1**).—To a solution of **14** (160 mg, 0.13 mmol) in *tert*-butanol (25 mL) and water (5 mL) was added AcOH dropwise until pH 5–6, and then hydrogenolyzed for 24 h in the presence of $Pd(OH)_2$ on carbon (100 mg). The mixture was filtered through Celite, and concentrated. The crude product was purified by gel filtration on Toyopearl HW-40 F to give **1** (28 mg, 44.3%); mp 165–174, lit. 166–176 °C;[2] $[\alpha]_D$ +12.1° (*c* 0.32, water, equil), lit. $[\alpha]_D$ +46° (*c* 0.3, water).[2] The ^{1}H and ^{13}C NMR data found for **1** are consistent with those reported.[2] Anal. Calcd for $C_{18}H_{30}O_{15}$: C, 44.45; H, 6.22. Found: C, 44.31; H, 6.23.

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References

1. Ogawa, K.; Yamaura, M.; Ikeda, Y.; Kondo, S. *Biosci. Biotechnol. Biochem.* **1998**, *62*, 2030–2031.
2. Ogawa, K.; Ikeda, Y.; Kondo, S. *Carbohydr. Res.* **1999**, *321*, 128–131.
3. Betaneli, V. I.; Litvak, M. M.; Backinowsky, L. V.; Kochetkov, N. K. *Carbohydr. Res.* **1981**, *94*, C1–C4.
4. Kaji, E.; Harita, N. *Tetrahedron Lett.* **2000**, *41*, 53–56.
5. (a) Lipták, A.; Fügedi, P.; Nánási, P. *Carbohydr. Res.* **1976**, *51*, C19–C21;
(b) Lipták, A.; Fügedi, P.; Nánási, P. *Carbohydr. Res.* **1978**, *65*, 209–217.
6. Bajza, I.; Kerékgyártó, J.; Hajkó, J.; Szilágyi, L.; Lipták, A. *Carbohydr. Res.* **1994**, *253*, 111–120.
7. (a) van Steijn, A. M. P.; Jetten, M.; Kamerling, J. P.; Vliegenthart, J. F. G. *Recl. Trav. Chim. Pays-Bas* **1989**, *108*, 374–383;
(b) Veeneman, G. H.; Gomes, L. J. F.; van Boom, J. H. *Tetrahedron* **1989**, *45*, 7433–7448.
8. Ashby, E. C.; Prather, J. *J. Am. Chem. Soc.* **1966**, *88*, 729–733.
9. Garegg, P. J. In *Preparative Carbohydrate Chemistry*; Hanessian, S., Ed. Regioselective Cleavage of *O*-Benzylidene Acetals to Benzyl Ethers; Marcel Dekker: New York, 1997; pp. 53–67 and references therein.
10. Lipták, A. *Tetrahedron Lett.* **1976**, 3551–3554.
11. Harangi, J.; Lipták, A.; Oláh, V. A.; Nánási, P. *Carbohydr. Res.* **1981**, *98*, 165–171.
12. Veeneman, G. H.; van Leeuwen, S. H.; Zuurmond, H. M.; van Boom, J. H. *J. Carbohydr. Chem.* **1990**, *9*, 783–796.
13. Lau, R.; Schüle, G.; Schwaneberg, U.; Ziegler, T. *Liebigs Ann.* **1995**, 1745–1754.
14. Lönn, H. *Carbohydr. Res.* **1985**, *139*, 105–113.
15. Ding, X.; Yang, G.; Kong, F. *Carbohydr. Res.* **1998**, *310*, 135–140.
16. Kováč, P.; Sklenář, V.; Glaudemans, C. P. J. *Carbohydr. Res.* **1988**, *175*, 201–213.
17. Hanessian, S.; Banoub, J. *Carbohydr. Res.* **1977**, *53*, C13–C16.
18. Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293–297.

19. Kanie, O.; Ito, Y.; Ogawa, T. *J. Am. Chem. Soc.* **1994**, *116*, 12073–12074.
20. Zissis, E.; Fletcher, H. G. *Carbohydr. Res.* **1970**, *12*, 361–368.
21. Kihlberg, J.; Eichler, E.; Bundle, D. R. *Carbohydr. Res.* **1991**, *211*, 59–75.
22. Kaji, E.; Lichtenthaler, F. W.; Osa, Y.; Takahashi, K.; Zen, S. *Bull. Chem. Soc. Jpn.* **1995**, *68*, 2401–2408.