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Synthesis of a D-rhamnose branched tetrasaccharide, repeating unit of the *O*-chain from *Pseudomonas syringae* pv. *Syringae (cerasi)* 435

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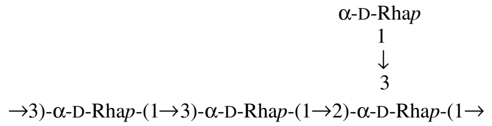
Abstract—The first synthesis of a D-rhamnose branched tetrasaccharide, corresponding to the repeating unit of the *O*-chain from *Pseudomonas syringae* pv. *cerasi* 435, as methyl glycoside is reported. The approach used is based on the synthesis of an opportune building-block, that is the methyl 3-*O*-allyl-4-*O*-benzoyl-α-D-rhamnopyranoside, which was then converted into both a glycosyl acceptor and two different protected glycosyl trichloroacetimidate donors. Successive couplings of these three compounds afforded the target oligosaccharide. The reported synthesis is also useful to perform the oligomerization of the repeating unit.

Keywords: *O*-Chain; *Pseudomonas cerasi*; Repeating unit; D-Rhamnose; Oligosaccharide; Synthesis

1. Introduction

Little is known about the effects of bacterial LPS[1–3] on plant cells. The only extensive study is on the ability of LPS to prevent the hypersensitive response (HR) caused in plants by avirulent bacteria,[4,5] but almost nothing is known about the molecular basis of the LPS-plant recognition that leads to this and, eventually, other unknown interactions.[6] Due to its extension out from the bacterial cell, the *O*-chain should be highly involved in recognition mechanism.[5] In order to investigate its role, the availability of *O*-chain repeating unit oligosaccharides is necessary. In particular any synthesis should be directed towards oligosaccharides, which may be oligomerized, in order to investigate also the influence of the *O*-chain length on the biological activity.

A recent review[7] showed that the *O*-chains from phytopathogenic bacteria are typically made of repeating units with an L- and/or D-rhamnose backbone, bearing, as branches, single monosaccharides, that are usually from a very small group, comprising L- and D-Xyl*p*, L- and D-Rha*p*, D-Glc*p*NAc, D-Fuc*f* and an unusual sugar, 3-acetamido-3,6-dideoxy-D-galactopyranose (D-Fuc*p*3NAc). In particular, the *O*-chain from *Pseudomonas syringae* pv. *cerasi* 435, a general phytopathogenic agent,[8] shows the following D-rhamnose branched tetrasaccharide repeating unit **1**:[9]



$$\begin{array}{rcl} & \alpha\text{-D-Rha}p & \\ & 1 & \\ & \downarrow & \\ & 3 & \\ \rightarrow 3)\text{-}\alpha\text{-D-Rha}p\text{-}(1\rightarrow 3)\text{-} & \alpha\text{-D-Rha}p & \text{-}(1\rightarrow 2)\text{-}\alpha\text{-D-Rha}p\text{-}(1\rightarrow \end{array}$$

1

Whereas various L-rhamnan oligosaccharides related to the *O*-chains of phytopathogenic bacteria have been recently synthesized,[10–17] few synthesis of D-rhamnose oligosaccharides have been hitherto reported.[18–21]

In this paper the first synthesis of the tetrasaccharide unit **1** as methyl glycoside is described. It is noteworthy that the synthetic approach used aims at the synthesis of a tetrasaccharide building-block, whose protecting group pattern could allow its smooth oligomerization to obtain higher oligosaccharides.

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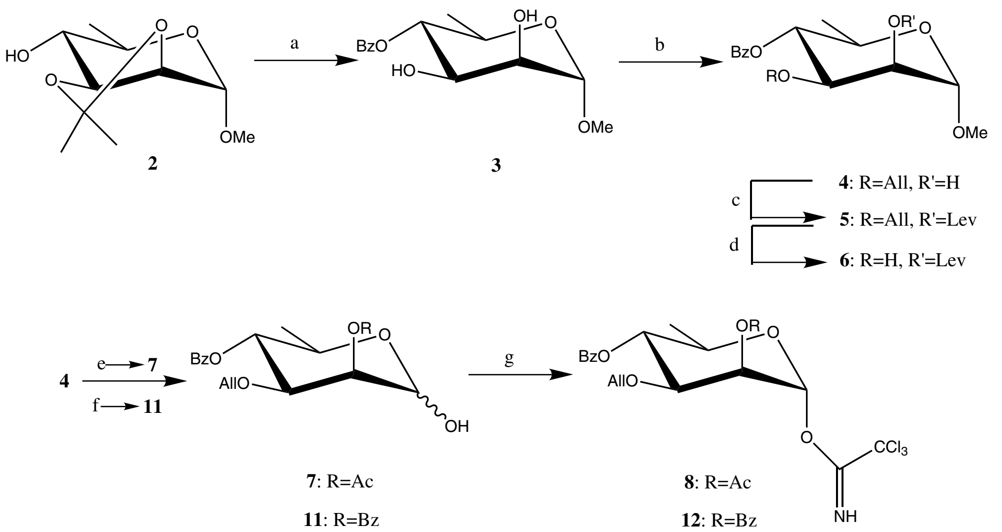
2. Results and discussion

Since **1** consists of a single monosaccharide type, that is D-rhamnose, an efficient synthetic strategy was based on the choice of a convenient D-rhamnose building-block, which would be easily converted into both a glycosyl donor and 2-*O*- and 3-*O*-glycosyl acceptors. The chosen precursor was the alcohol **4**, bearing a methoxy group at the anomeric position, a benzoyl group on *O*-4 and a selectively removable allyl protecting group at *O*-3 position (Scheme 1). Building-block **4** was synthesized from the known methyl 2,3-*O*-isopropylidene-α-D-rhamnopyranoside **2**[22] by benzoylation and ketal cleavage to afford **3** (77%), and subsequent dibutylstannylidene mediated allylation of position *O*-3; the very good yield of **4** (88%) confirmed the excellent regioselectivity of this alkylation method.[23]

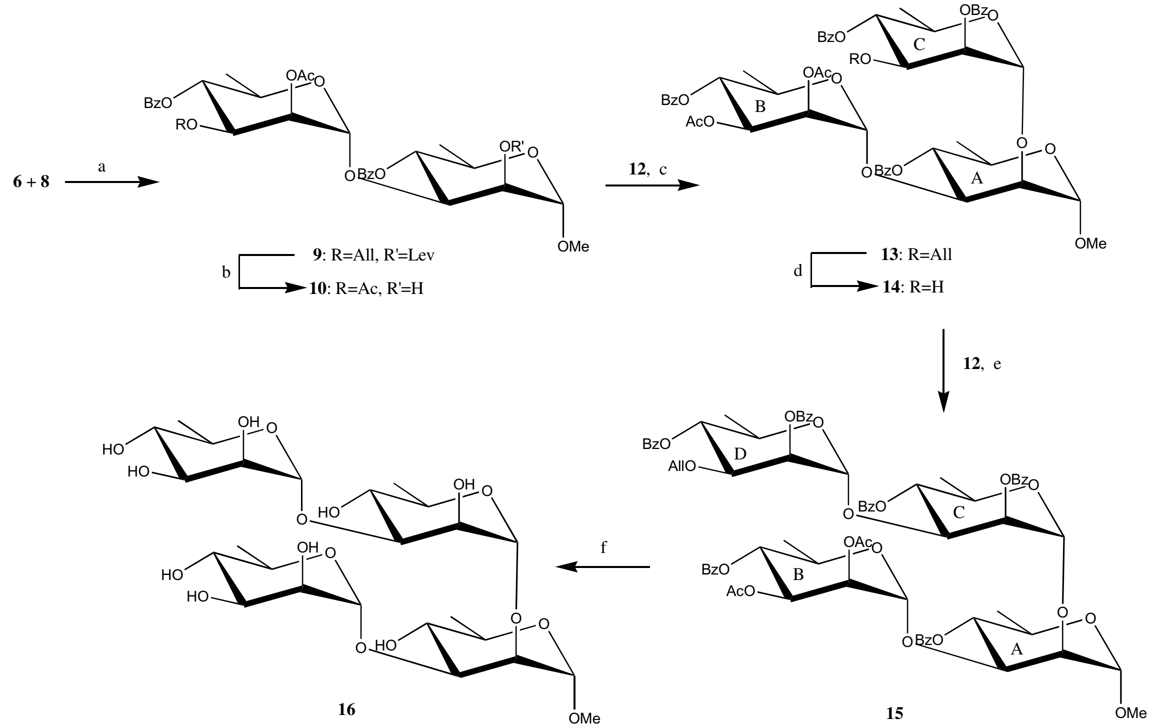
From compound **4** two routes had to be developed, respectively, towards a glycosyl donor and a glycosyl acceptor respectively. In particular **4** could be used directly as 2-*O*-glycosyl acceptor, nevertheless this compound was discarded since an oligomerizable tetrasaccharide building-block could be not easily obtained by such a strategy. Thus, it was decided to convert **4** into a 3-*O*-glycosyl acceptor, bearing a selective removable protecting group on *O*-2 position. The first attempt was to chloroacetylate this position, but subsequent palladium-catalyzed de-*O*-allylation caused almost total migration of the chloroacetyl group from *O*-2 to *O*-3. Better results were achieved by using a levulinoyl (Lev) protecting group, which is well-known to be less prone to acyl migration.[24] Actually, compound **5**, obtained from **4** by treatment with LevOH in the presence of *N*,*N*′-diisopropylcarbodiimide (DIPC) and 4-dimethylaminopyridine (DMAP) (79%), afforded acceptor **6** in good yield (78%) when de-*O*-allylated with $PdCl_2$.

On the other hand the sequence of two reactions (acetolysis and cleavage of anomeric acetate) converted **4** into the hemiacetal **7** with 59% yield over two steps. It is noteworthy that the condition used for the acetolysis (100:40:1 v/v/v $Ac_2O/AcOH/H_2SO_4$)[25] did not affect the allyl group. Hemiacetal **7** was then activated as trichloroacetimidate glycosyl donor by treatment with Cl_3CCN and DBU, affording **8** in 72% yield. Subsequent coupling of donor **8** with acceptor **6** was smoothly performed at −50 °C using $BF_3{\cdot}OEt_2$ as acid catalyst: disaccharide **9** was obtained with 86% yield (Scheme 2). The α-configuration of the glycosidic bond was ascertained by measuring the heteronuclear $^1J_{C,H}$ coupling constant value ($J_{C-1,H-1} = 173$ Hz) in a coupled HMQC-COSY experiment.[26]

The building of the target tetrasaccharide would have required at this point the conversion of **9** into a glycosyl acceptor by mere selective removal of the Lev group, but the two-steps conversion of the allyl into an acetyl group was at this point included in the synthetic strategy, in order to ensure a different protection on the 3-*O*-positions of residue B and D at tetrasaccharide level and to allow its future oligomerization. Thus, **9** was firstly subjected to de-*O*-allylation and acetylation, then, without any intermediate chromatographic purification, the Lev group cleavage was achieved by treatment with hydrazinium acetate in 4:1 CH_2Cl_2/MeOH, affording **10** in excellent yield (87% over three-steps). It is noteworthy that palladium-catalyzed de-*O*-allylation of **9** produced, as determined by TLC, a mixture of two compounds,



Scheme 1. Reagents and conditions: (a) (i) BzCl, pyridine, 0 °C, 60 min; (ii) 4:1 TFA/H_2O, rt, 20 min; 77%; (b) (i) Bu_2SnO, 10:1 benzene/MeOH 0 °C, 90 min; (ii) Bu_4NBr, AllBr, toluene, 65 °C, 2 h; 88%; (c) LevOH, DIPC, DMAP, CH_2Cl_2, rt, 60 min, 79%; (d) $PdCl_2$, 3:2 $MeOH/CH_2Cl_2$, rt, overnight, 78%; (e) (i) 100:40:1 $Ac_2O/AcOH/H_2SO_4$, rt, 30 min; (ii) hydrazine acetate, DMF, rt, 40 min; 59% ($\alpha/\beta = 3.5/1$); (f) (i) BzCl, pyridine, rt, 30 min; (ii) 100:40:1 $Ac_2O/AcOH/H_2SO_4$, rt, 60 min; (iii) hydrazine acetate, DMF, rt, 45 min; 58% ($\alpha/\beta = 5:1$); (g) Cl_3CCN, DBU, CH_2Cl_2, 0 °C, 60 min; 72% for **8**, 55% for **12**.



Scheme 2. Reagents and conditions: (a) $BF_3\cdot OEt_2$, 4 Å HW-300 MS, CH_2Cl_2, −50 °C, 150 min; 86%; (b) (i) $PdCl_2$, 2:1 $MeOH/CH_2Cl_2$, rt, 4 h; (ii) Ac_2O, pyridine, rt, overnight; (iii) hydrazine acetate, 4:1 $CH_2Cl_2/MeOH$; 87%; (c) TMSOTf, 4 Å HW-300 MS, CH_2Cl_2, −50 °C, 3 h; 71%; (d) $PdCl_2$, 3:2 $MeOH/CH_2Cl_2$, rt, 4 h; 92%; (e) TMSOTf, 4 Å HW-300 MS, CH_2Cl_2, −50 °C, 90 min; 70%; (f) (i) $PdCl_2$, 1:1 $MeOH/CH_2Cl_2$, rt, overnight; (ii) 3.4 M NaOMe, 1:1 $MeOH/CH_2Cl_2$, rt, overnight; 72%.

which were converted into the same product by the following acetylation. A more detailed analysis, performed with NMR spectroscopy, of the de-*O*-allylation products, revealed that treatment of **9** with $PdCl_2$ induced a partial migration of the acetyl group from 2_B-*O*- to 3_B-*O*-position. This disclosed a crucial problem for the completion of the synthesis, that was based on the possibility of smoothly removing a 3-*O*-allyl group, contiguous to a 2-*O*-acetyl group, at trisaccharide level. Thus, it was decided to couple acceptor **10** not with donor **8**, but with the 2-*O*-benzoylated donor **12**, since benzoyl groups are usually less prone to give acyl migration than acetates.[27] In fact, a test $PdCl_2$ deallylation on benzoylated **4** afforded cleavage of allyl group with no acyl migration. Glycosyl donor **12** was therefore synthesized from **4**, by benzoylation, acetolysis, anomeric acetate cleavage (58% yield over three steps) and conversion of the hemiacetal **11** into tricholoracetimidate (55%) (Scheme 1).

The 'disarming' nature of the benzoyl group on *O*-2 of donor **12**, required the use of TMSOTf as catalyst for the coupling reaction with acceptor **10**, that proceeded at −50 °C with 71% yield (Scheme 2). The α-configuration of the new glycosidic bond was ascertained again by a coupled HSQC experiment ($J_{C-1,H-1} = 174$ Hz). Trisaccharide **13** was then smoothly deallylated to alcohol **14** (92%), that was again coupled with donor **12**, affording **15** ($J_{C-1,H-1} = 173$ Hz) with 70% yield. The target methyl tetrasaccharide **16** was then finally obtained by a two-steps deprotection (de-*O*-allylation and Zemplén deacylation) in 72% yield. NMR chemical shifts for **16** and the natural *O*-chain show good accordance (see Section 3).[28] The ^1H NMR spectrum of **16** is shown in Figure 1 (Fig. 1).

In conclusion, a D-rhamnose branched tetrasaccharide corresponding to the *O*-chain repeating unit from *P. syringae* pv. *cerasi* 435, has been synthesized as its methyl glycoside. It is noteworthy that the synthetic approach used aimed also at the synthesis of a tetrasaccharide building-block, whose protecting group pattern could allow its smooth oligomerization to obtain higher oligosaccharides suitable for structure–activity studies. Work is in progress in this direction and results will be published at due time, together with the biological assays.

3. Experimental

3.1. General methods

^1H (400; 200 MHz) and ^{13}C NMR (100; 50 MHz) spectra were respectively recorded on a Bruker DRX-400 or on a Varian XL-200 NMR, in $CDCl_3$ (internal standard,

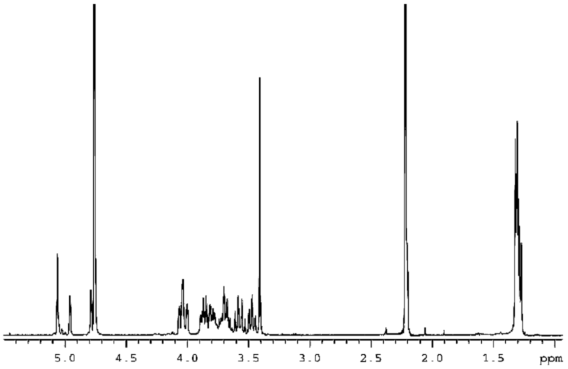


Figure 1. ^{1}H NMR spectrum (400 MHz, $(CH_3)_2CO$ as internal standard) of the target tetrasaccharide **16**.

for ^{1}H: $CHCl_3$ at δ 7.26; for ^{13}C: $CDCl_3$ at δ 77.0) or in D_2O (internal standard, for ^{1}H and ^{13}C: $(CH_3)_2CO$ at δ 2.22 and at δ 31.5, respectively). Assignment of proton and carbon chemical shifts for compounds **12**–**15** were based on COSY, TOCSY, ROESY, HSQC and coupled HSQC-COSY experiments. Positive ESI-MS spectra were recorded on a Finnigan LCQ-DECA ion trap mass spectrometer. Optical rotations were measured on a JASCO P-1010 polarimeter. Elemental analysis were performed on a Carlo Erba 1108 instrument. Analytical thin layer chromatography (TLC) was performed on aluminium plates precoated with Merck Silica Gel 60 F_{254} as the adsorbent. The plates were developed with 5% H_2SO_4 ethanolic solution and then heating to 130 °C. Column chromatography was performed on Kieselgel 60 (63–200 mesh). Gel filtration chromatography was performed on a Sephadex G-10 column (1.0 × 20 cm) with H_2O as eluant. Solvents used were purchased from Fluka and not further purified before use.

3.2. Methyl 4-*O*-benzoyl-α-D-rhamnopyranoside (3)

To a 0 °C cooled solution of **2** (0.869 g, 4.02 mmol) in pyridine (5.8 mL), BzCl (1.3 mL, 10.9 mmol) was added and the mixture was stirred at 0 °C for 1 h, after that CH_2Cl_2 (20 mL) was added. The mixture was washed with 0.5 M HCl. The organic phase was collected, dried and concentrated to afford a brown residue, that was subsequently suspended in 4:1 TFA/H_2O (9 mL) and stirred at rt. After 20 min the mixture was concentrated to give a residue, that, after silica gel chromatography (2:1, petroleum ether/ethyl acetate), afforded **3** (0.878 g, 77%) as a white foam. $[\alpha]_D$ +114.5 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10–7.41 (m, 5H, H-Ar), 5.04 (t, $J_{4,3} = J_{4,5} = 9.8$ Hz, 1H, H-4), 4.77 (br s, 1H, H-1), 4.14–3.91 (m, 3H, H-2, H-3, H-5), 3.42 (s, 3H, OMe), 1.30 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 167.5 (C=O), 133.5 (C_{ipso}), 129.8–128.5 (C–Ar), 100.4 (C-1), 70.8, 70.6, 70.4 (C_2, C_3, C_4), 65.5 (C_5), 55.1 (OMe), 17.7 (C_6). ESI-MS for $C_{14}H_{18}O_6$ (*m*/*z*): M_r (calcd) 282.11, M_r (found) 305.25 $(M+Na)^+$. Anal. Calcd C 59.57; H 6.43. Found: C 59.25; H 6.53.

3.3. Methyl 3-*O*-allyl-4-*O*-benzoyl-α-D-rhamnopyranoside (4)

A mixture of **3** (0.878 g, 3.14 mmol) and Bu_2SnO (0.978 g, 3.91 mmol) was suspended in 10:1 benzene/methanol (23 mL) and then heated and stirred at 60 °C. After 90 min solvent was removed. The residue was mixed under argon atmosphere with Bu_4NBr (0.995 g, 3.21 mmol) and the solid mixture suspended in toluene (12 mL). Allyl bromide (2.91 mL, 34.1 mmol) was added and the mixture was stirred at 65 °C. After 2 h the residue was concentrated. Silica gel chromatography (7:1, petroleum ether/ethyl acetate) of the residue afforded **4** (2.76 mmol, 88%) as a yellowish oil. $[\alpha]_D$ +42.0 (*c* 1.0, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.10–7.40 (m, 5H, H-Ar), 5.72 (m, 1H, $OCH_2CH{=}CH_2$), 5.28 (t, $J_{4,3} = J_{4,5} = 9.8$ Hz, 1H, H-4), 5.16 (br d, $J_{vic} = 17.4$ Hz, 1H, $OCH_2CH{=}CH_2$ *trans*), 5.07 (br d, $J_{vic} = 10.2$ Hz, 1H, $OCH_2CH{=}CH_2$ *cis*), 4.78 (br s, 1H, H-1), 4.16–3.89 (m, 4H, H-2, H-5, $OCH_2CH{=}CH_2$), 3.81 (dd, $J_{3,4} = 9.8$ Hz, $J_{3,2} = 3.4$ Hz, 1H, H-3), 3.41 (s, 3H, OMe), 1.25 (d, $J_{6,5} = 6.4$ Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.7 (C=O), 134.1 ($OCH_2CH{=}CH_2$), 133.1 (C_{ipso}), 129.7–128.4 (C–Ar), 117.7 ($OCH_2CH{=}CH_2$), 100.2 (C-1), 76.6 (C-3), 73.1, 71.0, 68.7, 66.1 (C-2, C-4, C-5, $OCH_2CH{=}CH_2$), 55.0 (OMe), 17.5 (C-6). ESI-MS for $C_{17}H_{22}O_6$ (*m*/*z*): M_r (calcd) 322.14, M_r (found) 345.39 $(M+Na)^+$. Anal. Calcd C 63.34; H 6.88. Found: C 63.45; H 6.99.

3.4. Methyl 3-*O*-allyl-4-*O*-benzoyl-2-*O*-levulinoyl-α-D-rhamnopyranoside (5)

To a solution of **4** (0.499 g, 1.55 mmol) in CH_2Cl_2 (11 mL), levulinic acid (1.0 mL, 8.64 mmol), DMAP (0.120 g, 0.98 mmol) and then DIPC (1.6 mL, 10.2 mmol) were added. The mixture was stirred at rt for 60′, after that it was filtered over a Celite pad, washed with water, dried and concentrated to afford a brown residue. Silica gel chromatography (6:1, petroleum ether/ethyl acetate) of the residue afforded **5** (0.516 g, 79%) as a yellowish oil. $[\alpha]_D$ −3.3 (*c* 0.7, CH_2Cl_2); ^{1}H NMR (200 MHz, $CDCl_3$): δ 8.08–7.40 (m, 5H, H-Ar), 5.65 (m, 1H, $OCH_2CH{=}CH_2$), 5.30 (dd, $J_{2,3} = 3.2$ Hz, $J_{2,1} = 1.6$ Hz, 1H, H-2), 5.22 (t, $J_{4,3} = J_{4,5} = 10.0$ Hz, 1H, H-4), 5.13 (br d, $J_{vic} = 17.4$ Hz, 1H, $OCH_2CH{=}CH_2$ *trans*), 5.02 (br d, $J_{vic} = 10.4$ Hz, 1H, $OCH_2CH{=}CH_2$ *cis*), 4.67 (br s, 1H, H-1), 4.12–3.81 (m, 4H, H-3, H-5, $OCH_2CH{=}CH_2$), 3.39 (s, 3H, OMe), 2.82–2.62 (m, 4H, CH_2CH_2), 2.20 (s, 3H, CH_3CO), 1.25 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 206.3 ($CH_3C{=}O$), 171.9 (C=OLev), 165.6 (C=OBz), 134.3

(OCH$_2$*C*H=CH$_2$), 133.1 (C$_{ipso}$), 130.0–128.4 (C–Ar), 117.2 (OCH$_2$CH=*C*H$_2$), 98.7 (C-1), 74.4, 73.1, 70.5, 69.0, 66.4 (C-2, C-3, C-4, C-5, O*C*H$_2$CH=CH$_2$), 55.1 (OMe), 38.0, 29.8, 28.2 *C*H$_2$*C*H$_2$, *C*H$_3$C=O), 17.6 (C-6). ESI-MS for $C_{22}H_{28}O_8$ (*m*/*z*): M_r (calcd) 420.18, M_r (found) 443.40 $(M+Na)^+$. Anal. Calcd C 62.85; H 6.71. Found: C 62.95; H 6.78.

3.5. Methyl 4-*O*-benzoyl-2-*O*-levulinoyl-α-D-rhamnopyranoside (6)

Compound **5** (0.487 g, 1.16 mmol) was dissolved in 3:2 MeOH/CH$_2$Cl$_2$ (20 mL) and PdCl$_2$ (82 mg, 0.46 mmol) was added. The mixture was stirred at rt overnight, after that it was filtered over a Celite pad, washed with 5 N NaCl, dried and concentrated. Silica gel chromatography (4:1, petroleum ether/ethyl acetate) afforded **6** (0.346 g, 78%) as a white foam. $[\alpha]_D$ +30.8 (*c* 0.9, CH$_2$Cl$_2$); ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.12–7.41 (m, 5H, H-Ar), 5.19 (dd, $J_{2,3} = 3.2$ Hz, $J_{2,1} = 1.6$ Hz, 1H, H-2), 5.09 (t, $J_{4,3} = J_{4,5} = 10.0$ Hz, 1H, H-4), 4.69 (br s, 1H, H-1), 4.23–3.88 (m, 2H, H-3, H-5), 3.40 (s, 3H, OMe), 2.86–2.65 (m, 4H, C*H$_2$*C*H$_2$*), 2.22 (s, 3H, C*H$_3$*CO), 1.27 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6). ^{13}C NMR (50 MHz, CDCl$_3$): δ 207.0 (CH$_3$*C*=O), 172.2 (C=OLev), 166.7 (C=OBz), 133.3 (C$_{ipso}$), 129.8–128.4 (C–Ar), 98.4 (C-1), 75.3, 72.7, 68.7, 66.1 (C-2, C-3, C-4, C-5), 55.2 (OMe), 38.3, 29.8, 28.3 (*C*H$_2$*C*H$_2$, *C*H$_3$C=O), 17.6 (C-6). ESI-MS for $C_{19}H_{24}O_8$ (*m*/*z*): M_r (calcd) 380.15, M_r (found) 403.37 $(M+Na)^+$. Anal. Calcd C 59.99; H 6.36. Found: C 60.18; H 6.29.

3.6. 2-*O*-Acetyl-3-*O*-allyl-4-*O*-benzoyl-D-rhamnopyranose (7)

Compound **4** (1.337 g, 4.15 mmol) was dissolved in Ac$_2$O (10 mL). To this solution 25:20:0.5 v/v/v Ac$_2$O/AcOH/H$_2$SO$_4$ (18 mL) was added. The solution was stirred for 30 min at rt, then water (5.0 mL) was dropwise added and stirring was continued for additional 10 min, after that the solution was diluted with CH$_2$Cl$_2$ (400 mL). After successive washings with water, 1 M NaHCO$_3$ and then with water again, the organic layer was collected, dried and concentrated. The residue was dissolved in DMF (15 mL) and then hydrazine acetate (0.295 g, 3.11 mmol) was added. After 40 min stirring at rt, the solution was diluted with CH$_2$Cl$_2$ (400 mL) and washed with 5 N NaCl, dried and concentrated to give a residue, that after silica gel chromatography (5:1 petroleum ether/ethyl acetate), afforded **7** (0.858 g, 59%; $\alpha/\beta = 3.5/1$) as a yellowish oil. ^{1}H NMR (200 MHz, CDCl$_3$; α-anomer): δ 8.09–7.43 (m, 5H, H-Ar), 5.68 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.36 (dd, $J_{2,3} = 3.3$ Hz, $J_{2,1} = 1.8$ Hz, 1H, H-2), 5.28 (t, $J_{4,3} = J_{4,5} = 9.9$ Hz, 1H, H-4), 5.22 (br s, 1H, H-1), 5.16 (dd, $J_{vic} = 17.4$ Hz, $J_{gem} = 1.5$ Hz, 1H, OCH$_2$CH=C*H$_2$* *trans*), 5.05 (dd, $J_{vic} = 10.2$ Hz, $J_{gem} = 1.5$ Hz, 1H, OCH$_2$CH=C*H$_2$* *cis*), 4.21 (dq, $J_{5,4} = 9.9$ Hz, $J_{5,6} = 6.2$ Hz, 1H, H-5), 4.17-3.94 (m, 3H, H-3, OC*H$_2$*CH=CH$_2$), 2.24 (s, 3H, C*H$_3$*CO), 1.25 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6). ^{13}C NMR (50 MHz, CDCl$_3$; α-anomer): δ 170.3 (C=OAc), 166.5 (C=OBz), 134.1 (OCH$_2$*C*H=CH$_2$), 133.2 (C$_{ipso}$), 129.8–128.5 (C–Ar), 117.3 (OCH$_2$CH=*C*H$_2$), 93.0 (C-1), 74.4, 73.2, 70.4, 70.2, 66.9 (C-2, C-3, C-4, C-5, O*C*H$_2$CH=CH$_2$), 20.4 (*C*H$_3$C=OAc), 17.7 (C-6). ESI-MS for $C_{18}H_{22}O_7$ (*m*/*z*): M_r (calcd) 350.14, M_r (found) 373.27 $(M+Na)^+$. Anal. Calcd C 61.71; H 6.33. Found: C 61.88; H 6.20.

3.7. 2-*O*-Acetyl-3-*O*-allyl-4-*O*-benzoyl-α-D-rhamnopyranosyl trichloroacetimidate (8)

To a 0 °C cooled solution of **7** (0.429 g, 1.23 mmol) in CH$_2$Cl$_2$ (10 mL), Cl$_3$CCN (0.610 mL, 6.08 mmol) and DBU (0.105 mL, 0.703 mmol) were added under argon atmosphere. After 60 min stirring at 0 °C, the solution was concentrated at 20 °C. Silica gel chromatography (14:1 petroleum ether/ethyl acetate) of the residue afforded **8** (0.435 g, 72%) as a yellowish oil. $[\alpha]_D$ +14.4 (*c* 1.0, CH$_2$Cl$_2$); ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.73 (s, 1H, NH), 8.10–7.44 (m, 5H, H-Ar), 6.25 (d, $J_{1,2} = 2.0$ Hz, H-1), 5.68 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.51 (dd, $J_{2,3} = 3.2$ Hz, $J_{2,1} = 2.0$ Hz, 1H, H-2), 5.37 (t, $J_{4,3} = J_{4,5} = 10.0$ Hz, 1H, H-4), 5.16 (dd, $J_{vic} = 17.4$ Hz, $J_{gem} = 1.6$ Hz, 1H, OCH$_2$CH=C*H$_2$* *trans*), 5.08 (dd, $J_{vic} = 10.2$ Hz, $J_{gem} = 1.6$ Hz, 1H, OCH$_2$CH=C*H$_2$* *cis*), 4.23–3.90 (m, 4H, H-3, H-5, OC*H$_2$*CH=CH$_2$), 2.21 (s, 3H, C*H$_3$*C=OAc), 1.30 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6). ^{13}C NMR (50 MHz, CDCl$_3$): δ 170.1 (C=OAc), 165.6 (C=OBz), 159.9 (C=NH), 134.0 (OCH$_2$*C*H=CH$_2$), 133.3 (C$_{ipso}$), 129.8–128.5 (C–Ar), 118.0 (OCH$_2$CH=*C*H$_2$), 95.1 (C-1), 77.2 (C-3), 72.2, 70.9, 69.7, 67.4 (C-2, C-4, C-5, O*C*H$_2$CH=CH$_2$), 20.9 (*C*H$_3$C=O), 17.6 (C-6). ESI-MS for $C_{20}H_{22}Cl_3NO_7$ (*m*/*z*): M_r (calcd) 493.05, M_r (found) 516.41 $(M+Na)^+$. Anal. Calcd C 48.55; H 4.48; N 2.83. Found: C 49.00; H 4.44; N.2.78.

3.8. Methyl (2-*O*-acetyl-3-*O*-allyl-4-*O*-benzoyl-α-D-rhamnopyranosyl)-(1 → 3)-4-*O*-benzoyl-2-*O*-levulinoyl-α-D-rhamnopyranoside (9)

A suspension of acceptor **6** (0.243 g, 0.64 mmol), imidate **8** (0.413 g, 0.84 mmol) and freshly powdered 4 Å HW-300 molecular sieves in CH$_2$Cl$_2$ (15 mL) was stirred at −50 °C under argon atmosphere. BF$_3$·OEt$_2$ (32 μL, 0.25 mmol) was added and the mixture was kept at −50 °C for 150 min, after that it was filtered on a Celite pad and washed with 1 M NaHCO$_3$ and water. The organic layer was collected, dried and concentrated. Silica gel chromatography (3:1, petroleum ether/ethyl acetate) of the residue afforded **9** (0.389 g, 86%) as a white foam. $[\alpha]_D$ −21.4 (*c* 1.0, CH$_2$Cl$_2$); ^{1}H NMR

(200 MHz, CDCl$_3$): δ 8.10–7.41 (m, 10H, H-Ar), 5.52 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.34 (t, $J_{4,3} = J_{4,5} = 9.8$ Hz, 1H, H-4$_A$), 5.27 (dd, $J_{2,3} = 3.2$ Hz, $J_{2,1} = 1.6$ Hz, 1H, H-2$_A$), 5.13 (t, $J_{4,3} = J_{4,5} = 10.0$ Hz, 1H, H-4$_B$), 5.02–4.85 (m, 3H, H-2$_B$, OCH$_2$CH=C*H*$_2$), 4.69 (br s, 1H, H-1$_A$), 4.67 (br s, 1H, H-1$_B$), 4.26 (dd, $J_{3,4} = 10.2$ Hz, $J_{3,2} = 3.2$ Hz, 1H, H-3$_A$), 3.96 (m, 2H, H-5$_A$, H-5$_B$), 3.84–3.63 (3H, H-3$_B$, OC*H*$_2$CH=CH$_2$), 3.41 (OMe), 2.77 (m, 4H, C*H*$_2$*H*$_2$), 2.21 (s, 3H, *C*H$_3$C=OLev), 1.92 (s, 3H, C*H*$_3$ C=OAc), 1.29 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6$_A$), 1.19 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6$_B$). ^{13}C NMR (50 MHz, CDCl$_3$): δ 206.6 (CH$_3$*C*=O), 171.8 (C=OLev), 169.6 (C=OAc), 165.7 (C=OBz), 134.1 (OCH$_2$*C*H=CH$_2$), 133.4, 133.0 (2C$_{ipso}$), 129.8–128.3 (C-Ar), 117.0 (OCH$_2$CH=*C*H$_2$), 99.8 (C-1$_B$, $^1J_{C,H} = 173$ Hz), 98.3 (C-1$_A$), 75.3, 74.0, 73.2, 72.9, 71.6, 70.5, 68.8, 67.5, 66.5 (C-2$_A$, C-2$_B$, C-3$_A$, C-3$_B$, C-4$_A$, C-4$_B$, C-5$_A$, C-5$_B$, O*C*H$_2$CH=CH$_2$), 55.2 (OMe), 37.8, 29.8, 28.1 (*C*H$_2$*C*H$_2$, *C*H$_3$C=OLev), 20.7 (*C*H$_3$C=OAc), 17.5, 17.4 (C-6$_A$, C-6$_B$). ESI-MS for $C_{37}H_{44}O_{14}$ (*m*/*z*): M_r (calcd) 712.27, M_r (found) 735.49 (M + Na)$^+$. Anal. Calcd C 62.35; H 6.22. Found: C 62.50; H 6.34.

3.9. Methyl (2,3-di-*O*-acetyl-4-*O*-benzoyl-α-D-rhamnopyranosyl)-(1 → 3)-4-*O*-benzoyl-α-D-rhamnopyranoside (10)

To a solution of **9** (0.350 g, 0.49 mmol) in 2:1 MeOH/CH$_2$Cl$_2$ (8.6 mL), PdCl$_2$ (18 mg, 0.10 mmol) was added and the mixture was stirred vigorously at rt for 4 h. The mixture was then filtered over a Celite pad, diluted with CH$_2$Cl$_2$ (100 mL) and washed with 5 N NaCl. The organic layer was collected, dried and concentrated to afford a brownish residue, that was dissolved in pyridine (4.0 mL) and then Ac$_2$O (4.0 mL) was added to the mixture. The solution was stirred at rt overnight, then it was concentrated, diluted with CH$_2$Cl$_2$ (100 mL) and washed with 1 M HCl (100 mL) and 1 M NaHCO$_3$ (100 mL). The organic layer was collected, dried and concentrated. The residue was dissolved in 4:1 CH$_2$Cl$_2$/MeOH (8.0 mL) and then hydrazine acetate (65 mg, 0.68 mmol) was added. The solution was stirred 4 h at rt, then it was concentrated to give a residue, that, after silica gel chromatography (4:1, petroleum ether/ethyl acetate) afforded **10** (0.278 g, 87%) as a white foam. $[\alpha]_D$+0.9 (*c* 1.0, CH$_2$Cl$_2$); ^{1}H NMR (200 MHz, CDCl$_3$): δ 8.09–7.40 (m, 10H, H-Ar), 5.47–5.35 (m, 2H, H-4$_A$, H-3$_B$), 5.23 (t, $J_{4,3} = J_{4,5} = 9.6$ Hz, 1H, H-4$_B$), 5.02 (dd, $J_{2,3} = 3.6$ Hz, $J_{2,1} = 1.6$ Hz, 1H, H-2$_B$), 4.94 (d, $J_{1,2} = 1.6$ Hz, 1H, H-1$_B$), 4.76 (br s, 1H, H-1$_A$), 4.23–4.05 (m, 3H, H-2$_A$, H-3$_A$, H-5$_B$), 3.93 (dq, $J_{5,4} = 9.8$ Hz, $J_{5,6} = 6.2$ Hz, 1H, H-5$_A$), 3.43 (s, 3H, OMe), 1.90 (s, 3H, C*H*$_3$C=O), 1.79 (s, 3H, C*H*$_3$C=O), 1.26 (m, 6H, H-6$_A$, H-6$_B$). ^{13}C NMR (50 MHz, CDCl$_3$): δ 169.5, 169.2 (2C=OAc), 165.6, 165.4 (2C=OBz), 133.4, 133.2 (2C$_{ipso}$), 129.8–128.3 (C–Ar), 100.3, 99.1 (C-1$_A$, C-1$_B$), 77.2 (C-3$_A$), 73.0, 71.4, 70.8, 69.7, 68.4, 67.4, 66.3 (C-2$_A$, C-2$_B$, C-3$_B$, C-4$_A$, C-4$_B$, C-5$_A$, C-5$_B$), 55.1 (OMe), 20.5, 20.4 (2*C*H$_3$C=OAc), 17.5, 17.4 (C-6$_A$, C-6$_B$). ESI-MS for $C_{31}H_{36}O_{13}$ (*m*/*z*): M_r (calcd) 616.22, M_r (found) 639.31 (M + Na)$^+$. Anal. Calcd C 60.38; H 5.88. Found: C 60.55; H 6.00.

3.10. 3-*O*-Allyl-2,4-di-*O*-benzoyl-D-rhamnopyranose (11)

Compound **4** (0.982 g, 3.05 mmol) was dissolved in pyridine (5.0 mL) and then BzCl (0.710 mL, 6.14 mmol) was added. The mixture was stirred for 30 min, water (20 mL) was then added. After 10′ additional stirring it was diluted with CH$_2$Cl$_2$ (100 mL) and washed with 0.5 M HCl. The organic layer was collected, dried and concentrated to give a residue that was suspended in Ac$_2$O (7.5 mL). 25:20:0.5 v/v/v Ac$_2$O/AcOH/H$_2$SO$_4$ (12.5 mL) was added and the solution was stirred for 60′ at rt. Water (5.0 mL) was then dropwise added and after 5′ additional stirring the mixture was diluted with CH$_2$Cl$_2$ (300 mL) and washed with 5 M NaCl, 1M NaHCO$_3$ and water. The organic layer was collected, dried and concentrated to give a yellowish oil, that was dissolved in DMF (15 mL). Hydrazine acetate (0.331 g, 3.46 mmol) was added and the mixture was stirred for 45′ at rt, after that it was diluted with CH$_2$Cl$_2$ (400 mL) and washed with 5 M NaCl, dried and concentrated. Silica gel chromatography (5:1, petroleum ether/ethyl acetate) of the residue afforded **11** (0.733 g, 58%; $\alpha/\beta = 5:1$) as a colourless oil. ^{1}H NMR (200 MHz, CDCl$_3$; α-anomer): δ 8.14–7.42 (m, 10H, H-Ar), 5.66 (m, 1H, OCH$_2$C*H*=CH$_2$), 5.58 (dd, $J_{2,3} = 3.3$ Hz, $J_{2,1} = 1.8$ Hz, 1H, H-2), 5.43 (t, $J_{4,3} = J_{4,5} = 9.8$ Hz, 1H, H-4), 5.38 (br s, 1H, H-1), 5.15 (dd, $J_{vic} = 17.2$ Hz, $J_{gem} = 1.6$ Hz, 1H, OCH$_2$CH=C*H*$_2$ *trans*), 5.03 (dd, $J_{vic} = 10.4$ Hz, $J_{gem} = 1.6$ Hz, 1H, OCH$_2$CH=C*H*$_2$ *cis*), 4.27 (dq, $J_{5,4} = 9.8$ Hz, $J_{5,6} = 6.2$ Hz, 1H, H-5), 4.17–3.91 (m, 3H, H-3, OC*H*$_2$CH=CH$_2$), 1.30 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6). ^{13}C NMR (50 MHz, CDCl$_3$; α-anomer): δ 165.9, 165.7 (2C=O), 134.3 (OCH$_2$*C*H=CH$_2$), 133.3, 133.1 (2C$_{ipso}$), 130.0–128.4 (C–Ar), 117.4 (OCH$_2$CH=*C*H$_2$), 92.5 (C-1), 73.9, 73.3, 70.7, 70.0, 66.8 (C-2, C-3, C-4, C-5, O*C*H$_2$CH=CH$_2$), 17.8 (C-6). ESI-MS for $C_{23}H_{24}O_7$ (*m*/*z*): M_r (calcd) 412.15, M_r (found) 435.38 (M + Na)$^+$. Anal. Calcd C 66.98; H 5.87. Found: C 66.74; H 5.99.

3.11. 3-*O*-Allyl-2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl trichloroacetimidate (12)

To a 0 °C cooled solution of **11** (0.728 g, 1.77 mmol) in CH$_2$Cl$_2$ (13 mL), Cl$_3$CCN (0.890 mL, 8.88 mmol) and DBU (0.152 mL, 1.02 mmol) were added under argon atmosphere. After 60 min stirring at rt, the solution was concentrated at 20 °C. Silica gel chromatography (12:1, petroleum ether/ethyl acetate) of the residue afforded **12**

(0.539 g, 55%) as a white foam. $[\alpha]_D$ −50.3 (*c* 1.0, CH_2Cl_2); ^1H NMR (200 MHz, $CDCl_3$): δ 8.77 (s, 1H, NH), 8.12–7.39 (m, 10H, H-Ar), 6.38 (d, $J_{1,2}$ = 2.0 Hz, H-1), 5.72 (dd, $J_{2,3}$ = 3.4 Hz, $J_{2,1}$ = 2.0 Hz, 1H, H-2), 5.67 (m, 1H, $OCH_2C\mathit{H}{=}CH_2$), 5.49 (t, $J_{4,3} = J_{4,5}$ =10.0 Hz, 1H, H-4), 5.13 (dd, J_{vic} = 17.4 Hz, J_{gem} = 1.6 Hz, 1H, $OCH_2CH{=}C\mathit{H}_2$ *trans*), 5.04 (dd, J_{vic} = 10.2 Hz, J_{gem} = 1.6 Hz, 1H, $OCH_2CH{=}C\mathit{H}_2$ *cis*), 4.27-3.92 (m, 4H, H-3, H-5, $OC\mathit{H}_2CH{=}CH_2$), 1.33 (d, $J_{6,5}$ = 6.0 Hz, 3H, H-6). ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.6, 165.4 (2C=OBz), 159.8 (C=NH), 134.0 ($OCH_2\mathit{C}H{=}CH_2$), 133.4, 133.2 ($2C_{ipso}$), 129.9–128.4 (C—Ar), 117.9 ($OCH_2CH{=}\mathit{C}H_2$), 95.1 (C-1), 73.9, 72.4, 70.9, 69.7, 68.0 (C-2, C-3, C-4, C-5, $O\mathit{C}H_2CH{=}CH_2$), 17.7 (C-6). ESI-MS for $C_{25}H_{24}Cl_3NO_7$ (*m/z*): M_r (calcd) 555.06, M_r (found) 578.17 $(M + Na)^+$. Anal. Calcd C 53.93; H 4.34; N 2.52. Found: C 54.10; H 4.24; N 2.73.

3.12. Methyl (3-*O*-allyl-2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl)-(1 → 2)-[2,3-di-*O*-acetyl-4-*O*-benzoyl-α-D-rhamnopyranosyl-(1 → 3)]-4-*O*-benzoyl-α-D-rhamnopyranoside (13)

A suspension of acceptor **10** (0.271 g, 0.44 mmol), imidate **12** (0.342 g, 0.62 mmol) and freshly powdered 4 Å HW-300 molecular sieves in CH_2Cl_2 (16 mL) was stirred at −50 °C under argon atmosphere. TMSOTf (1.1 μL, 6.1 μmol) was added and the mixture was kept at −50 °C. After 60 min other TMSOTf (9.0 μL, 50 μmol) was added and stirring was continued for additional 2 h, after that the reaction was quenched by adding a drop of Et_3N. The mixture was then filtered on a Celite pad and concentrated. Silica gel chromatography (6:1, petroleum ether/ethyl acetate) of the residue afforded **13** (0.315 g, 71%) as a white foam. $[\alpha]_D$ −59.0 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.15–7.29 (m, 20H, H-Ar), 5.76 (m, 1H, $OCH_2C\mathit{H}{=}CH_2$), 5.63 (dd, $J_{2,3}$ = 3.0 Hz, $J_{2,1}$ = 1.6 Hz, 1H, H-2_B), 5.50 (m, 2H, H-4_A, H-3_C), 5.42 (t, $J_{4,3} = J_{4,5}$ = 9.8 Hz, 1H, H-4_B), 5.30 (dd, J_{vic} = 17.2 Hz, J_{gem} = 1.4 Hz, 1H, $OCH_2CH{=}C\mathit{H}_2$ *trans*), 5.26 (t, $J_{4,3} = J_{4,5}$ = 9.9 Hz, 1H, H-4_C), 5.22 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1_B), 5.15 (dd, J_{vic} = 10.2 Hz, J_{gem} = 1.4 Hz, 1H, $OCH_2CH{=}C\mathit{H}_2$ *cis*), 5.05 (dd, $J_{2,3}$ = 3.2 Hz, $J_{2,1}$ = 1.6 Hz, 1H, H-2_C), 4.97 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1_C), 4.88 (d, $J_{1,2}$ = 1.5 Hz, 1H, H-1_A), 4.28–4.11 (m, 6H, H-3_A, H-3_C, H-5_A, H-5_C, $OC\mathit{H}_2CH{=}CH_2$), 4.06 (dd, $J_{2,3}$ = 3.0 Hz, $J_{2,1}$ = 1.6 Hz, 1H, H-2_A), 3.93 (dq, $J_{5,4}$ = 9.8 Hz, $J_{5,6}$ = 6.2 Hz, 1H, H-5_B), 3.42 (s, 3H, OMe), 1.94 (s, 3H, $C\mathit{H}_3C{=}O$), 1.71 (s, 3H, $C\mathit{H}_3C{=}O$), 1.34 (d, $J_{6,5}$ = 6.2 Hz, 3H, H-6_B), 1.31 (d, $J_{6,5}$ = 6.2 Hz, 3H, H-6_C), 1.27 (d, $J_{6,5}$ = 6.2 Hz, 3H, H-6_A). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.3, 169.0 (2C=OAc), 165.9-165.4 (4C=OBz), 134.1 ($OCH_2\mathit{C}H{=}CH_2$), 133.1–133.0 ($4C_{ipso}$), 129.9–128.4 (C—Ar), 118.2 ($OCH_2CH{=}\mathit{C}H_2$), 100.0, 99.9, 99.7 (C-1_A, C-1_B, C-1_C, $^1J_{C,H}$ = 173 Hz, $^1J_{C,H}$ = 173 Hz, $^1J_{C,H}$ = 174 Hz), 79.6 (C-2_A), 77.2 (C-3_A), 73.9, 73.3, 73.2, 71.5, 71.0, 70.1, 70.0, 68.5, 67.7, 67.6, 66.7 (C-2_B, C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C, $O\mathit{C}H_2CH{=}CH_2$), 55.2 (OMe), 20.7, 20.4 ($2\mathit{C}H_3C{=}O$), 17.8–17.7 (C-6_A, C-6_B, C-6_C). ESI-MS for $C_{54}H_{58}O_{19}$ (*m/z*): M_r (calcd) 1010.36, M_r (found) 1033.70 $(M + Na)^+$. Anal. Calcd C 64.15; H 5.78. Found: C 64.00; H 5.72.

3.13. Methyl (2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl)-(1 → 2)-[2,3-di-*O*-acetyl-4-*O*-benzoyl- α-D-rhamnopyranosyl-(1 → 3)]-4-*O*-benzoyl-α-D-rhamnopyranoside (14)

A solution of **13** (0.297 g, 0.29 mmol) in 3:2 MeOH/CH_2Cl_2 (10 mL) was treated with $PdCl_2$ (16 mg, 90 μmol). After 7 h stirring at rt, the mixture was filtered on a Celite pad, diluted with CH_2Cl_2 (100 mL) and extracted with 5 M NaCl. The organic layer was collected, dried and concentrated. The residue was subjected to silica gel chromatography (5:1, petroleum ether/ethyl acetate) to afford **14** (0.260 g, 92%) as a white foam. $[\alpha]_D$ −48.0 (*c* 1.0, CH_2Cl_2); ^1H NMR (400 MHz, $CDCl_3$): δ 8.16–7.37 (H-Ar), 5.54 (m, 2H, H-2_B, H-3_C), 5.42 (m, 2H, H-4_A, H-4_B), 5.27 (t, $J_{4,3} = J_{4,5}$ = 9.8 Hz, 1H, H-4_C), 5.21 (d, $J_{1,2}$ = 1.9 Hz, 1H, H-1_B), 5.08 (dd, $J_{2,3}$ = 3.2 Hz, $J_{2,1}$ = 1.8 Hz, 1H, H-2_C), 5.00 (d, $J_{1,2}$ = 1.8 Hz, 1H, H-1_C), 4.86 (d, $J_{1,2}$ = 1.6 Hz, 1H, H-1_A), 4.58 (dd, $J_{3,4}$ = 9.8 Hz, $J_{3,2}$ = 3.4 Hz, 1H, H-3_B), 4.33–4.25 (m, 3H, H-3_A, H-5_B, H-5_C), 4.06 (dd, $J_{2,3}$ = 3.2 Hz, $J_{2,1}$ = 1.6 Hz, 1H, H-2_A), 3.92 (dq, $J_{5,4}$ = 9.6 Hz, $J_{5,6}$ = 6.0 Hz, 1H, H-5_A), 3.43 (s, 3H, OMe), 1.89 (s, 3H, $C\mathit{H}_3C{=}O$), 1.70 (s, 3H, $C\mathit{H}_3C{=}O$), 1.35–1.24 (m, 9H, H-6_A, H-6_B, H-6_C). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.5, 169.0 (2C=OAc),166.4, 165.6, 165.5, 165.2 (4C=OBz), 133.1–133.0 ($4C_{ipso}$), 129.7–128.2 (C—Ar), 99.4–99.2 (C-1_A, C-1_B, C-1_C), 79.2 (C-2_A), 75.6 (C-3_A), 74.4, 73.6, 73.2, 71.2, 69.7, 68.5, 67.9, 67.6, 67.5, 66.4 (C-2_B, C-2_C, C-3_A, C-3_B, C-3_C, C-4_A, C-4_B, C-4_C, C-5_A, C-5_B, C-5_C), 54.9 (OMe), 20.4, 20.3 (2 $\mathit{C}H_3C{=}O$), 17.6–17.5 (C-6_A, C-6_B, C-6_C). ESI-MS for $C_{51}H_{54}O_{19}$ (*m/z*): M_r (calcd) 970.33, M_r (found) 971.02 $(M + H)^+$. Anal. Calcd C 63.09; H 5.61. Found: C 63.09; H 5.58.

3.14. Methyl (3-*O*-allyl-2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl)-(1 → 3)-(2,4-di-*O*-benzoyl-α-D-rhamnopyranosyl)-(1 → 2)-[2,3-di-*O*-acetyl-4-*O*-benzoyl-α-D-rhamnopyranosyl-(1 → 3)]-4-*O*-benzoyl-α-D-rhamnopyranoside (15)

A suspension of acceptor **14** (0.212 g, 0.22 mmol), imidate **12** (0.165 g, 0.30 mmol) and freshly powdered 4 Å HW-300 molecular sieves in CH_2Cl_2 (10 mL) was stirred at −50 °C under argon atmosphere. TMSOTf (0.54 μL, 3.0 μmol) was added and the mixture was kept at −50 °C. After 90′ the reaction was quenched by adding a drop of Et_3N. The mixture was then filtered on a Celite pad and concentrated. Silica gel chromatography (7:1, petroleum ether/ethyl acetate) of the residue afforded **15** (0.210 g, 70%) as a white foam. $[\alpha]_D$ −82.0 (*c* 1.0, CH_2Cl_2);. ^{1}H NMR (400 MHz, $CDCl_3$): δ 8.06–7.34 (H-Ar), 5.67 (dd, $J_{2,3} = 3.2$ Hz, $J_{2,1} = 1.6$ Hz, 1H, H-2$_{\mathrm{C}}$), 5.64 (t, $J_{2,3} = 9.8$ Hz, 1H, H-4$_{\mathrm{C}}$), 5.44 (m, 2H, H-3$_{\mathrm{B}}$, H-4$_{\mathrm{A}}$), 5.33 (d, $J_{2,1} = 1.6$ Hz, 1H, H-1$_{\mathrm{C}}$), 5.30–5.23 (m, 5H, H-1$_{\mathrm{C}}$, H-1$_{\mathrm{D}}$, H-2$_{\mathrm{D}}$, H-4$_{\mathrm{B}}$, H-4$_{\mathrm{D}}$), 5.08 (dd, $J_{2,3} = 3.2$ Hz, $J_{2,1} = 1.6$ Hz, 1H, H-2$_{\mathrm{B}}$), 4.96 (d, $J_{2,1} = 1.6$ Hz, 1H, H-1$_{\mathrm{B}}$), 4.87 (m, 2H, H-1$_{\mathrm{A}}$, $OCH_2CH{=}CH_2$ *trans*), 4.77 (dd, $J_{vic} = 10.2$ Hz, $J_{gem} = 1.4$ Hz, 1H, $OCH_2CH{=}CH_2$ *cis*), 4.59 (dd, $J_{3,4} = 9.8$ Hz, $J_{3,2} = 3.2$ Hz, 1H, H-3$_{\mathrm{C}}$), 4.29 (dd, $J_{3,4} = 9.8$ Hz, $J_{3,2} = 3.3$ Hz, 1H, H-3$_{\mathrm{A}}$), 4.23 (m, 2H, H-5$_{\mathrm{B}}$, H-5$_{\mathrm{C}}$), 4.08 (m, 2H, H-2$_{\mathrm{A}}$, H-5$_{\mathrm{D}}$), 3.93 (m, 1H, H-5$_{\mathrm{A}}$), 3.83 (dd, $J_{3,4} = 9.8$ Hz, $J_{3,2} = 3.4$ Hz, 1H, H-3$_{\mathrm{D}}$), 3.75 (m, 1H, $OCH_2CH{=}CH_2$), 3.62 (m, 1H, $OCH_2CH{=}CH_2$), 3.43 (s, 3H, OMe), 1.34 (m, 6H, H-6$_{\mathrm{A}}$, H-6$_{\mathrm{C}}$) 1.26 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6$_{\mathrm{B}}$), 1.15 (d, $J_{6,5} = 6.2$ Hz, 3H, H-6$_{\mathrm{D}}$). ^{13}C NMR (100 MHz, $CDCl_3$): δ 169.2, 168.6 (2C=OAc), 166.0, 165.8, 165.3, 165.2, 165.0, 164.9 (6 C=OBz), 134.0 ($OCH_2CH{=}CH_2$), 133.4–132.8 (6C$_{ipso}$), 129.8–128.1 (C–Ar), 117.2 ($OCH_2CH{=}CH_2$), 100.0, 99.7, 99.6, 99.2 (C-1$_{\mathrm{A}}$, C-1$_{\mathrm{B}}$, C-1$_{\mathrm{C}}$, C-1$_{\mathrm{D}}$, $^1J_{C,H} = 173$ Hz, $^1J_{C,H} = 173$ Hz, $^1J_{C,H} = 173$ Hz, $^1J_{C,H} = 174$ Hz), 79.0 (C-2$_{\mathrm{A}}$), 76.4, 74.9, 73.8, 73.3, 72.9, 72.4, 71.5, 70.2, 70.0, 69.2, 68.4, 67.7, 67.6, 67.3, 66.9 (C-2$_{\mathrm{B}}$, C-2$_{\mathrm{C}}$, C-2$_{\mathrm{D}}$, C-3$_{\mathrm{A}}$, C-3$_{\mathrm{B}}$, C-3$_{\mathrm{C}}$, C-3$_{\mathrm{D}}$, C-4$_{\mathrm{A}}$, C-4$_{\mathrm{B}}$, C-4$_{\mathrm{C}}$, C-4$_{\mathrm{D}}$, C-5$_{\mathrm{A}}$, C-5$_{\mathrm{B}}$, C-5$_{\mathrm{C}}$, C-5$_{\mathrm{D}}$, $OC_2CH{=}CH_2$), 55.2 (OMe), 20.6, 20.2 ($2CH_3C{=}O$), 17.8–17.5 (C-6$_{\mathrm{A}}$, C-6$_{\mathrm{B}}$, C-6$_{\mathrm{C}}$, C-6$_{\mathrm{C}}$). ESI-MS for $C_{74}H_{76}O_{25}$ (*m*/*z*): M_r (calcd) 1364.47, M_r (found) 1387.43 $(M + Na)^+$. Anal. Calcd C 65.09; H 5.61. Found: C 66.01; H 5.48.

3.15. Methyl α-D-rhamnopyranosyl-(1 → 3)-α-D-rhamnopyranosyl)-(1 → 2)-[α-D-rhamnopyranosyl-(1 → 3)]-α-D-rhamnopyranoside (16)

A solution of **15** (30 mg, 22 μmol) in 1:1 $MeOH/CH_2Cl_2$ (1.5 mL) was treated with $PdCl_2$ (4.0 mg, 22 μmol). After stirring overnight at rt, the mixture was filtered on a Celite pad, diluted with CH_2Cl_2 (25 mL) and extracted with 5 M NaCl (25 mL). The organic layer was collected, dried and concentrated. The residue was dissolved in 1:1 $MeOH/CH_2Cl_2$ (1.0 mL) and treated with a 3.4 M methanolic solution of NaOMe (0.1 mL). The solution was stirred overnight at rt, then Amberlist-15 (H^+) was added to adjust the pH value to 6. The mixture was filtered and concentrated to give a residue, that was purified by gel filtration on a G-10 (Sephadex) column using water as eluant, to obtain **16** (9.8 mg, 72%) as a white foamy solid. $[\alpha]_D$ +38.3 (*c* 0.5, CH_2Cl_2); ^{1}H NMR (400 MHz, D_2O): δ 5.06 (br s, 2H, H-1$_{\mathrm{B}}$, H-1$_{\mathrm{D}}$), 4.96 (d, 1H, $J_{1,2} = 1.7$ Hz, 1H, H-1$_{\mathrm{C}}$), 4.78 (d, 1H, $J_{1,2} = 1.6$ Hz, 1H, H-1$_{\mathrm{A}}$), 4.06 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{2,3} = 1.6$ Hz 1H, H-2$_{\mathrm{B}}$), 4.04 (m, 2H, H-2$_{\mathrm{C}}$, H-2$_{\mathrm{D}}$), 4.00 (dd, 1H, $J_{2,3} = 3.2$ Hz, $J_{2,3} = 1.6$ Hz 1H, H-2$_{\mathrm{A}}$), 3.89–3.85 (m, 2H, H-3$_{\mathrm{A}}$, H-3$_{\mathrm{C}}$), 3.82–3.76 (m, 2H, H-3$_{\mathrm{B}}$, H-5$_{\mathrm{C}}$), 3.74–3.65 (m, 4H, H-3$_{\mathrm{D}}$, H-5$_{\mathrm{A}}$, H-5$_{\mathrm{B}}$, H-5$_{\mathrm{D}}$), 3.59 (t, 1H, $J_{4,3} = J_{4,5} = 9.6$ Hz, 1H, H-4$_{\mathrm{A}}$), 3.55 (t, 1H, $J_{4,3} = J_{4,5} = 9.6$ Hz, 1H, H-4$_{\mathrm{C}}$), 3.47 (m, 2H, H-4$_{\mathrm{B}}$, H-4$_{\mathrm{D}}$), 3.41 (s, 3H, OMe), 1.30 (m, 12H, H-6$_{\mathrm{A}}$, H-6$_{\mathrm{B}}$, H-6$_{\mathrm{C}}$, H-6$_{\mathrm{D}}$). ^{13}C NMR (100 MHz, D_2O):[28] δ 103.4 (C-1$_{\mathrm{B}}$, C-1$_{\mathrm{D}}$), 103.0 (C-1$_{\mathrm{C}}$), 100.6 (C-1$_{\mathrm{A}}$), 79.5 (C-2$_{\mathrm{A}}$), 78.5 (C-3$_{\mathrm{A}}$, C-3$_{\mathrm{C}}$), 73.3 (C-4$_{\mathrm{A}}$, C-4$_{\mathrm{B}}$), 73.2 (C-4$_{\mathrm{D}}$), 73.0 (C-4$_{\mathrm{C}}$), 71.5 (C-2$_{\mathrm{B}}$), 71.3 (C-2$_{\mathrm{C}}$), 71.2 (C-2$_{\mathrm{D}}$), 71.1 (C-3$_{\mathrm{B}}$, C-3$_{\mathrm{D}}$), 70.4 (C-5$_{\mathrm{A}}$, C-5$_{\mathrm{B}}$, C-5$_{\mathrm{C}}$, C-5$_{\mathrm{D}}$), 56.0 (OMe), 18.4–18.3 (C-6$_{\mathrm{A}}$, C-6$_{\mathrm{B}}$, C-6$_{\mathrm{C}}$, C-6$_{\mathrm{D}}$). ESI-MS for $C_{25}H_{44}O_{17}$ (*m*/*z*): M_r (calcd) 616.26, M_r (found) 639.58 $(M + Na)^+$. Anal. Calcd C 48.70; H 7.19. Found: C 48.81; H 7.17.

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28. ^{13}C NMR chemical shifts for the synthetic tetrasaccharide **16** and the natural *O*-chain[9] show good accordance (±0.6 ppm), except for few values: the C-3$_{\mathrm{D}}$ signal is shifted highfield (8.0 ppm) in comparison with the value of the natural *O*-chain, due to the absence of glycosylation shift at that position in the synthetic oligosaccharide; on the contrary, the methoxy group on C-1$_{\mathrm{A}}$ explains the highfield shift of the C-1$_{\mathrm{A}}$ signal (+1.3 ppm) and the downfield shift of the C-2$_{\mathrm{A}}$ signal (−1.0 ppm).