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Note

Synthesis of the trisaccharide repeating unit related to *Klebsiella* 012 serotype

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ABSTRACT

Synthesis of the trisaccharide, allyl α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside related to O-chain glycans isolated from the deaminated LPSs of *Klebsiella pneumoniae* serotype 012, was achieved through condensation of suitably synthesized disaccharide, allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-L-rhamnopyranoside and donor, ethyl 2,3,4-tri-*O*-acetyl-1-thio α-L-rhamnopyranoside starting from L-rhamnose and D-glucosamine hydrochloride. The trisaccharide can be utilized for the synthesis of neoglycoconjugates for use as a synthetic vaccine by coupling it with a suitable protein after deprotection. Various regio- and stereoselective protecting group strategies have been carefully considered, as protecting groups can influence the reactivity of the electrophile and nucleophile in glycosylation reactions on the basis of steric and electronic requirements.

The study in the field of complex oligosaccharides related to bacterial *O*-LPS has proven to be a principal component of many biological and medicinal studies in recent years.[1–8] The synthesis of terminal units of the bacterial *O*-antigens, in combination with the strategies for the covalent attachment of the hapten to the solid support, permits the use of these compounds as substitutes for polysaccharides of bacterial origin in several serological tests and eventually as vaccines.[9–14] Vaccines containing bacterial toxins, for example, lipids, when incorporated as part of the lipopolysaccharide, cause undesired side effects, thus restraining vaccine development. With an objective to gain detailed insight into the structural requirements for studying the pharmacological parameters of the biological repeating units of bacterial *O*-LPS, attention has been focused on the synthesis of hapten moieties, since it has been reported that the small carbohydrate epitopes can provoke the formation of antibodies.[15–17] *Klebsiella pneumoniae* is a Gram-negative, non-motile, encapsulated, lactose-fermenting, facultative anaerobic found in the normal flora of the mouth, skin, and intestines.[18] Members of the *Klebsiella* genus typically express two types of antigens on their cell surface. The first, O antigen, is a lipopolysaccharide of which nine varieties exist. The second is K antigen, a capsular polysaccharide with more than 80 varieties.[19] Both contribute to pathogenicity and form the basis for subtyping.

This paper illustrates the synthesis of the repeating unit of the oligosaccharide from *K. pneumoniae* O12 serotype in the form of allyl glycosides that may be conjugated[20] to BSA. The allyl group provides a two-carbon chain linker arm that is superior to other widely known linker arms.[21] The structure of the repeating unit has the following structural framework:[22]

-α-L-Rhap (1→3)-β-D-GlcNHAc-(1→4)-α-L-Rhap.

For the synthesis of this trisaccharide, allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranoside[23] (**1**) was prepared from L-rhamnose through an unreported method by acetylating it to the tetra-*O*-acetyl derivative, followed by treatment with allyl alcohol and boron trifluoride diethyl etherate,[24] affording **1** as syrup in 70% yield. Compound **1** was deacetylated using the Zemplén method, followed by isopropylidenation with 2,2-dimethoxypropane to yield allyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**2**).[25] Compound **2** was suitably chloroacetylated[26] at C-4 using chloroacetyl chloride in pyridine to afford allyl 4-*O*-chloroacetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (**3**). Removal of the isopropylidene ring of **3** was accomplished by aqueous acetic acid to give allyl 4-*O*-chloroacetyl-α-L-rhamnopyranoside (**4**). Compound **4** was then benzoylated to give allyl 2,3-di-*O*-benzoyl-4-*O*-chloroacetyl-α-L-rhamnopyranoside (**5**), which was then dechloroacetylated using thiourea[26] to give allyl 2,3-di-*O*-benzoyl-α-L-rhamnopyranoside (**6**). Compound **6** served as an acceptor for the synthesis of the disaccharide fragment present at the reducing end of *Klebsiella* serotype 012 strain (Scheme 1).

In a separate experiment ethyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**7**) was synthesized[27] from D-glucosamine·HCl that served as donor for the synthesis of the disaccharide fragment present at the reducing end of the trisaccharide. Compound **7** was condensed with

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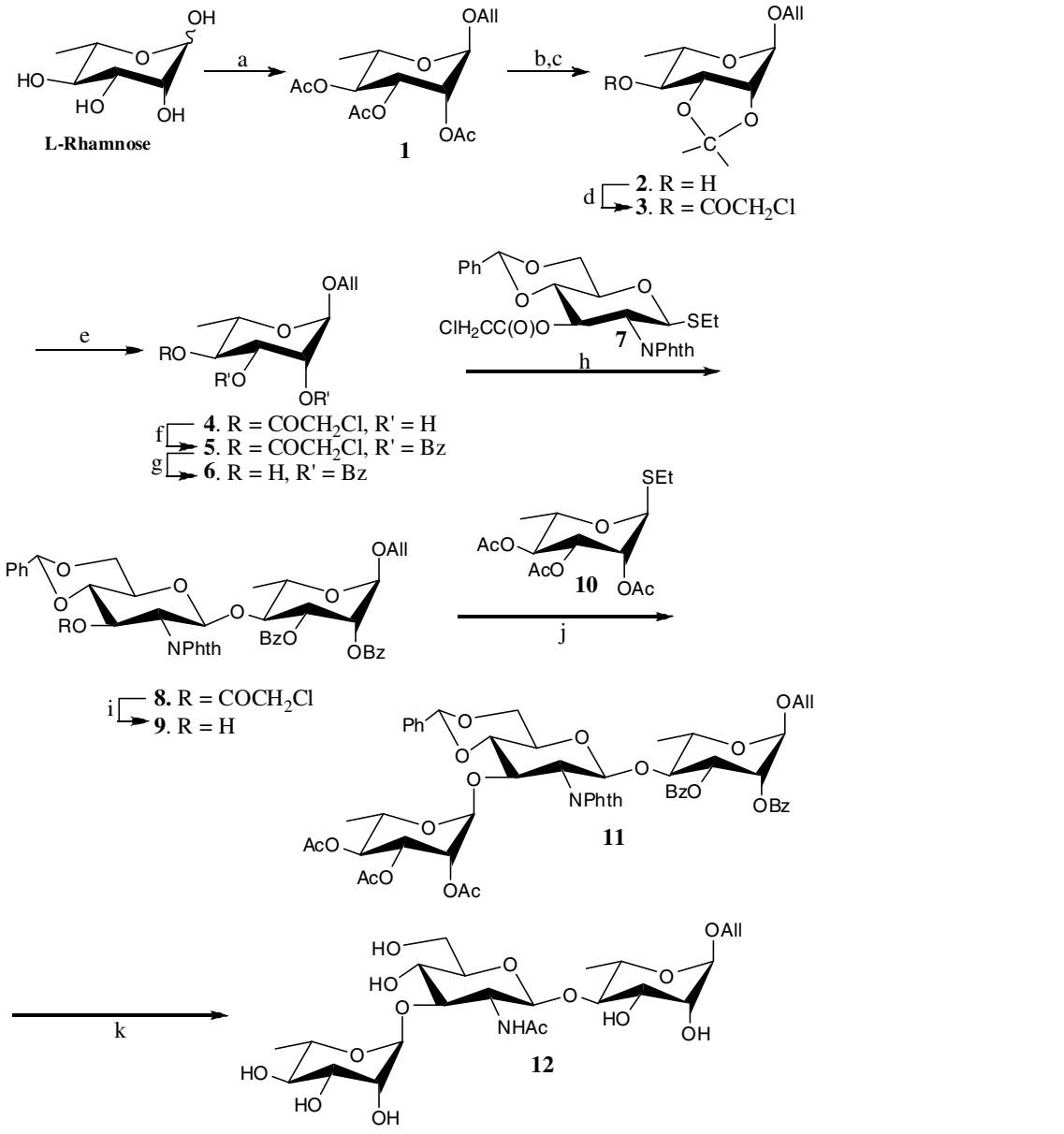

Scheme 1. Reagents and conditions: (a) Ac_2O/Pyr/EtSH/$BF_3{\cdot}OEt_2$/0 °C, rt/4 h; (b) NaOMe/MeOH (1 M)/4 h; (c) 2,2 DMP/acetone/*p*-TSA, rt/6 h; (d) $ClCH_2COCl$/Pyr/CH_2Cl_2/0 °C, rt/16 h; (e) 80% aqueous HOAc/80 °C/7 h; (f) BzCl/Pyr/0 °C, rt/2 h; (g) thiourea/3:2 MeOH–H_2Cl_2, rt/3 h; (h)NIS/TMSOTf /CH_2Cl_2/MS-4 Å/0 °CC/20 min; (i) thiourea/3:2 $MeOHCH_2Cl_2$/rt/3 h; (j) NIS/TMSOTf/CH_2Cl_2/MS-4 Å/0 °C/20 min; (k) ethylinediamine, *n*-butanol, 90 °C/20 h, Ac_2O-Pyr, rt/14 h, 80% aqueous HOAc, 80 °C/4 h, MeONa/MeOH, rt/6 h.

acceptor **6** in the presence of *N*-iodosuccinimide and trimethylsilyl triflate as promoter[28] to afford the disaccharide, allyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-L-rhamnopyranoside (**8**), as a foam in 69% yield. The ^{1}H NMR spectrum showed a doublet of 9.3 Hz at δ 5.55 for H-1′ of β-GlcNPhth and a doublet of 1.2 Hz at δ 4.86 for H-1 of α-Rha_1. Selective removal of the chloroacetyl group of the disaccharide **8** using thiourea[26] yields the acceptor, allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-L-rhamnopyranoside (**9**), which was condensed with ethyl 2,3,4-tri-*O*-acetyl-1-thio-α-L-rhamnopyranoside[29] (**10**) in the presence of *N*-iodosuccinimide and trimethylsilyl triflate as promoter[28] to yield the trisaccharide, allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-L-rhamnopyranoside (**11**) in 40% yield. The ^{1}H NMR spectrum of **11** showed a doublet of 9.0 Hz for one proton at δ 5.35 for the H-1′ of β-GlcNPhth along with a doublet of 1.2 Hz at 4.85 for H-1 of α-Rha_1 and a singlet at 4.75 for H-1″ of α-Rha_2. The ^{13}C NMR spectrum showed peaks at 98.6 (C-1′), 97.1 (C-1″) and 96.3 (C-1) for the three anomeric carbons. The structure of the trisaccharide (**11**) is also supported by its 2D HSQC spectrum and ESIMS, which showed *m*/*z* 1086 $(M+Na)^+$. Removal of the phthalimido group using ethylenediamine and *n*-butanol,[30] followed by debenzylidenation and deacylation, afforded the target trisaccharide (**12**) in 58% yield (Scheme 1). The structure of **12** was confirmed by NMR spectroscopy and ESIMS. The ^{1}H NMR spectrum of **12** showed a doublet of 7.8 Hz for one proton at δ 4.99 for the H-1′ of β-GlcNAc along with two singlets at δ 4.75 and 4.59 of one proton each for the H-1 and H-1″ of α-Rha_1 and α-Rha_2,

respectively. The structure of the trisaccharide (**12**) is also supported by its by ESIMS at *m/z* 554 $(M + H)^+$.

1. Experimental

1.1. General experimental methods

All reactions were monitored by TLC on Silica Gel G (E. Merck). Column chromatography was performed using silica gel (SRL, 60–120 mesh). Solvents were dried and distilled before use, and evaporations were conducted at 40 °C unless otherwise stated. Optical rotations were measured at 25 °C on AA-5 series polarimeter. ^{1}H NMR spectra were recorded on a Bruker DPX 300 spectrometer using $CDCl_3$ as solvent (TMS as internal standard) unless otherwise stated. Melting points were determined on Büchi 540 melting point apparatus. The mass spectra were recorded on a Jeol SX 102 mass spectrometer for FABMS and Micromass Quattro II ESIMS. The disaccharides **8** and **9** and the trisaccharide **11** were confirmed by a 2D HSQC experiment as well.

1.2. Allyl 2,3,4 tri-*O*-acetyl-α-L-rhamnopyranoside (1)

Rhamnose (5.0 g, 30.48 mmol) was conventionally acetylated using Ac_2O (25 mL) and pyridine (25 mL) to give the acetylated product. The crude product (8.14 g, 24.68 mmol) was dissolved in dry CH_2Cl_2 (95 mL) and allyl alcohol (3.90 mL, 49.4 mmol), and $BF_3{\cdot}OEt_2$ (7.8 mL, 61.7 mmol) was added dropwise at 0 °C. The solution was then allowed to stir for 4 h at 0 °C and washed successively with satd aq $NaHCO_3$, brine, dried, and concentrated. Chromatography (8:2 *n*-hexane–EtOAc,) of the residue afforded **1**[23] (5.7 g, 70%) as syrup: $[\alpha]_D^{25}$ −53.7 (*c* 1.3, $CHCl_3$). ^{1}H NMR: δ 5.94–5.83 (m, 1H, –CH_2CHCH_2), 5.35–5.20 (m, 4H, H-2, H-3, –CH_2CHCH_2), 5.07 (t, 1H, $J_{3,4,5}$ 9.9 Hz, H-4), 4.78 (d, 1H, $J_{1,2}$ 1.5 Hz, H-1), 4.22–4.15 (m, 1H, $CHCHCH_2$), 4.04–3.97 (m, 1H, –$CHCHCH_2$), 3.95–3.87 (m, 1H, H-5), 2.14, 2.04, 1.98 (s, 3H each, 3×–$OCOCH_3$), 1.26 (d, 3H, *J* 6.3 Hz, 6-CH_3). Anal. Calcd for $C_{15}H_{22}O_8$: C, 54.54; H, 6.71. Found: C, 54.41; H, 6.80.

1.3. Allyl 4-*O*-chloroacetyl-2,3-*O*-isopropylidene-α-L-rhamnopyranoside (3)

Chloroacetyl chloride (0.7 mL, 9.0 mmol) was added dropwise at 0 °C to the solution of compound **2**[25] (1.7 g, 7.0 mmol) and pyridine (0.7 mL, 9.0 mmol) in CH_2Cl_2 (35 mL). The mixture was stirred for 16 h at rt and poured into water. The organic layer was separated, washed successively with aq HCl and aq $NaHCO_3$, and brine, dried, and concentrated. Chromatography (9:1 *n*-hexane–EtOAC) of the residue afforded **3** as syrup (1.8 g, 80%): $[\alpha]_D^{25}$ −25.8 (*c* 1.7, $CHCl_3$). ^{1}H NMR: δ 5.98–5.84 (m, 1H, –CH_2CHCH_2), 5.34–5.21 (m, 2H, –CH_2CHCH_2), 5.06 (s, 1H, H-1), 4.91 (dd, 1H, *J* 10.2 Hz, H-4), 4.28–4.15 (m, 3H, –$CHCHCH_2$, H-2, H-3), 4.09 (s, 2H, –$COCH_2Cl$), 4.04–3.98 (m, 1H, –$CHCHCH_2$), 3.89–3.75 (m, 1H, H-5), 1.56 (s, 3H, –CCH_3CH_3), 1.34 (s, 3H, –C CH_3CH_3), 1.18 (d, 3H, *J* 6.3 Hz, 6–CH_3). Anal. Calcd for $C_{14}H_{21}ClO_6$: C, 52.42; H, 6.60. Found: C, 52.35; H, 6.67.

1.4. Allyl 4-*O*-chloroacetyl-α-L-rhamnopyranoside (4)

Aq HOAc (80%, 20 mL) was added to a solution of **3** (1.5 g, 4.7 mmol) in CH_2Cl_2 (5 mL). The mixture was stirred for 7 h at 80 °C, cooled to rt and concentrated. Chromatography (4:1 *n*-hexane–EtOAc,) of the residue afforded **4** as syrup (1.1 g, 85%): $[\alpha]_D^{25}$ −62.6 (c 1.1, $CHCl_3$). ^{1}H NMR: δ 5.96–5.83 (m, 1H, –CH_2CHCH_2), 5.39–5.19 (m, 2H, –CH_2CHCH_2), 4.92 (t, 1H, $J_{3,4,5}$ 9.6 Hz, H-4), 4.85 (s, 1H, H-1), 4.19–4.15 (m, 1H, –$CHCHCH_2$), 4.14, 4.13 (2s, 2H, –$COCH_2Cl$), 4.03–3.91 (m, 3H, –$CHCHCH_2$, H-2, H-3), 3.86–3.78 (m, 1H, H-5), 1.21 (d, 3H, *J* 6.3 Hz, 6–CH_3). Anal. Calcd for $C_{11}H_{17}ClO_6$: C, 47.07; H, 6.10. Found C, 47.02; H, 6.13.

1.5. Allyl 2,3-di-*O*-benzoyl-4-*O*-chloroacetyl-α-L-rhamnopyranoside (5)

A mixture of BzCl (2.2 mL, 18.8 mmol) and pyridine (1.5 mL, 18.4 mmol) was added dropwise at 0 °C into a solution of **4** (1.0 g, 3.6 mmol) in CH_3CN (15 mL). The mixture was stirred for 2 h at rt, poured into water and extracted with CH_2Cl_2. The combined extract was washed successively with dil HCl and aq $NaHCO_3$, and brine, dried, and concentrated. Chromatography (9:1 *n*-hexane–EtOAc) of the residue afforded **5** (1.5 g, 85%), as crystals: mp 80.5–82.3 °C, $[\alpha]_D^{25}$ +55.2 (*c* 0.4, $CHCl_3$). ^{1}H NMR: δ 8.13–7.31 (m, 10H, aromatic protons) 6.01–5.89 (m, 1H, –CH_2CHCH_2), 5.70 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.9 Hz, H-3), 5.63 (d, 1H, $J_{1,2}$ 1.5 Hz, H-2), 5.49 (t, 1H, $J_{3,4,5}$ 10.2 Hz, H-4), 5.42–5.24 (m, 2H, –CH_2CHCH_2), 5.01 (s, 1H, H-1), 4.29–4.23 (m, 1H, –$CHCHCH_2$), 4.16–4.01 (m, 2H, –$CHCHCH_2$, H-5), 3.97, 3.95 (2s, 2H, –$COCH_2Cl$), 1.34 (d, 3H, *J* 6.3 Hz, 6-CH_3). Anal. Calcd for $C_{25}H_{25}ClO_8$: C, 61.42; H, 5.15. Found: C, 61.39; H, 5.17.

1.6. Allyl 2,3-di-*O*-benzoyl-α-L-rhamnopyranoside (6)

A solution of compound **6** (1.2 g, 2.5 mmol) and thiourea (0.93 g, 12.3 mmol) in a mixture of MeOH (30 mL) and CH_2Cl_2 (20 mL) was stirred at rt for 3 h and concentrated. The residue was dissolved in CH_2Cl_2, washed with water, dried, and concentrated. Chromatography (9:1 *n*-hexane–EtOAc) of the residue afforded **6** as syrup (0.8 g, 80%), $[\alpha]_D^{25}$ +35 (*c* 1.3, $CHCl_3$). ^{1}H NMR: δ 8.13–7.32 (m, 10H, aromatic protons) 5.97–5.88 (m, 1H, –CH_2CHCH_2), 5.60 (d 1H, $J_{2,3}$ 1.5 Hz, H-2), 5.54 (dd, 1H, $J_{2,3}$ 3.3 Hz, $J_{3,4}$ 9.6 Hz, H-3), 5.38–5.22 (m, 2H, –CH_2CHCH_2), 4.99 (s, 1H, H-1), 4.29–4.23 (m, 1H, –$CHCHCH_2$), 4.17–4.04 (m, 1H, –$CHCHCH_2$,), 3.98–3.94 (m, 2H, H-4, H-5), 1.34 (d, 3H, *J* 6.0 Hz, 6-CH_3). Anal. Calcd for $C_{23}H_{24}O_7$: C, 66.98; H, 5.87. Found: C, 66.91; H, 5.93.

1.7. Allyl 4,6-*O*-benzylidene-3-*O*-chloroacetyl-2-deoxy-2-phthalimido-β-D-gluco-pyranosyl (1→4)-2,3-di-*O*-benzoyl-α-L-rhamnopyranoside (8)

A mixture of **6** (400 mg, 0.97 mmol), **7**[29] (553 mg, 1.1 mmol) and 4 Å molecular sieves (1.0 g) in CH_2Cl_2 (13 mL) was cooled under nitrogen to 0 °C and stirred for 10 min. NIS (313 mg, 1.4 mmol) and TMSOTf (97 μL, 0.53 mmol) were successively added, the mixture was stirred for 20 min, neutralized by the addition of Et_3N and filtered through a layer of Celite. The filtrate was washed successively with aq $Na_2S_2O_3$, followed by water, dried and concentrated. Chromatography (17:3 *n*-hexane–EtOAc) of the residue afforded **8** as a foam (613 mg, 69%): $[\alpha]_D^{25}$ +31 (*c* 1.0, $CHCl_3$). ^{1}H NMR: δ 7.94–7.33 (m, 19H, aromatic protons), 5.89 (dd, 1H, *J* 10.8 Hz, H-3), 5.85–5.81 (m, 1H, –CH_2CHCH_2), 5.55 (d, 1H, $J_{1,2}$ 9.3 Hz, H-1′), 5.52 (s, 1H, –CHC_6H_5), 5.41 (d, 1H, $J_{1,2}$ 1.2 Hz, H-2), 5.36 (dd, 1H, $J_{2,3}$ 3.6 Hz, $J_{3,4}$ 9.3 Hz, H-3), 5.31–5.18 (m, 2H, –CH_2CHCH_2), 4.86 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1), 4.59–4.48 (m, 1H, –$CHCHCHH$), 4.31–4.15 (m, 2H, H-2′ –$CHCHCHH$), 4.03–3.89 (m, 5H, H-4, H-5, H-4′, H-5′, H-6a′), 3.81 (s, 2H, –$COCH_2Cl$), 3.80–3.76 (m, 1H, H-6b′), 1.39 (d, 3H, *J* 6.0 Hz, 6-CH_3). ^{13}C NMR: δ 167.3 (–N[*C*O]$_2$Ph), 167.2 (–N[*C*O]$_2$Ph), 166.7 (–*C*OCH_2Cl), 165.3 (–*C*OPh), 164.9 (–*C*OPh), 136.7 (–OCH_2CHCH_2), 133.9–123.4 (aromatic carbons), 118.0 (–OCH_2CHCH_2), 101.7 (–CHPh), 98.3 (*C*-1′), 96.3 (*C*-1), 78.9, 71.9, 71.1, 70.3 (2C), 68.5,68.3 (2C), 66.9, 65.5, 55.1 (C-2′), 40.2 (–$COCH_2Cl$), 18.0 (C-6, Rha_1). FABMS $[M]^+$ *m/z* 867. Anal. Calcd for $C_{46}H_{42}ClNO_{14}$: C, 63.63; H, 4.88. Found: C, 63.56; H, 4.93.

1.8. Allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-L-rhamnopyranoside (9)

A solution of **8** (400 mg, 0.46 mmol) and thiourea (175 mg, 2.3 mmol) in a mixture of MeOH (9.0 mL) and CH_2Cl_2 (6.0 mL) was stirred at rt for 3 h and concentrated. The residue was dissolved in CH_2Cl_2, washed successively with water, dried and concentrated. Chromatography (8:2 *n*-hexane–EtOAc) of the residue afforded **9** (274 mg, 75%) as a foam: $[\alpha]_D^{25}$ +15.9 (*c* 1.1, $CHCl_3$). ^1H NMR: δ 7.94–7.33 (m, 19H, aromatic protons), 5.99–5.81 (m, 1H, $-CH_2CHCH_2$), 5.54 (s, 1H, $-CHC_6H_5$), 5.37 (d, 1H, $J_{1,2}$ 9.0 Hz, H-1′), 5.40–5.35 (m, 2H, H-2, H-3), 5.31–5.18 (m, 2H, $-CH_2CHCH_2$), 4.86 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1), 4.60 (t, 1H, $J_{2,3,4}$ 9.0 Hz, H-3′), 4.45–4.41 (m, 1H, $CHHCHCH_2$), 4.18–4.10 (m, 2H, H-2′, $-CHHCHCH_2$), 4.01–3.80 (m, 4H, H-4, H-5, H-4′, H-5′), 3.73–3.56 (m, 2-H, H-6a′, 6b′), 1.39 (d, 3H, *J* 6.0 Hz, 6-CH_3). ^{13}C NMR: δ 167.7 (2 × $-N[CO]_2Ph$), 165.3 (–COPh), 164.9 (–COPh), 136.9 ($-OCH_2CHCH_2$), 134.3–123.6 (aromatic carbons), 118.0 ($-OCH_2CHCH_2$), 101.9 (–CHPh), 98.6 (C-1′), 96.3 (C-1), 82.0, 72.0, 70.4, 68.6, 68.3, 68.1, 67.9, 67.0, 65.6, 60.3, 56.6 (C 2′), 18.0 (C-6, Rha_1). ESIMS $(M + H)^+$ at *m/z* 792 and $(M+Na)^+$ at *m/z* 814. Anal. Calcd for $C_{44}H_{41}NO_{13}$: C, 66.74; H, 5.22. Found: C, 66.68; H, 5.26.

1.9. Allyl 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl-(1→3)-4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→4)-2,3-di-*O*-benzoyl-α-L-rhamno-pyranoside (11)

A mixture of **9** (47 mg, 0.14 mmol), **10**[29] (100 mg, 0.13 mmol) and 4 Å molecular sieves (150 mg) in CH_2Cl_2 (4 mL) was cooled under nitrogen to 0 °C and stirred for 10 min. NIS (41 mg, 0.18 mmol) and TMSOTf (13 μL, 0.07 mmol) were successively added. The mixture was stirred for 20 min, neutralized by the addition of Et_3N and filtered through a layer of Celite. The filtrate was washed successively with aq $Na_2S_2O_3$, and brine, dried and concentrated. Chromatography (3:1 *n*-hexane–EtOAc) of the residue afforded **11** (55 mg, 40%) as a syrup: $[\alpha]_D^{25}$ +2.6 (*c* 1.1 $CHCl_3$). ^1H NMR: δ 7.94–7.31 (m, 19H, aromatic protons), 5.94–5.83 (m, 1H, $-CH_2CHCH_2$), 5.59 (s, 1H, $-CHC_6H_5$), 5.39 (d, 1H, $J_{1,2}$ 1.2 Hz, H-2), 5.35 (d, 1H, $J_{1,2}$ 9.0 Hz, H-1′GlcNPhth), 5.31–5.29 (m, 2H, H-2″, H-3), 5.25–5.14 (m, 3H, $-CH_2CHCH_2$, H-3″), 4.85 (d, 1H, $J_{1,2}$ 1.2 Hz, H-1, Rha_1), 4.75 (s, 1H, 1-H″ Rha_2), 4.62 (m, 2H, H-3′, H-4″), 4.45–4.41 (m, 1H, $-CHHCHCH_2$), 4.22–4.16 (m, 2H, H-2′, $-CHHCHCH_2$), 3.99–3.82 (m, 6H, H-4, H-4′, H-5, H-5′, H-5″, H-6a′), 3.70–3.67 (m, 1H, H-6b′), 1.95, 1.91, 1.87 (s, 3H each, 3 × $-COCH_3$), 1.38 (d, 3H, *J* 6.0 Hz, 6-CH_3), 0.50 (d, 3H, *J* 6.3 Hz, 6-CH_3″). ^{13}C NMR: δ 169.8 (2 × $-COCH_3$), 169.7 (1 × $-COCH_3$), 169.2 ($-N[CO]_2Ph$), 169.1 ($-N[CO]_2Ph$), 165.3 (–COPh), 164.9 (–COPh), 136.9 ($-OCH_2CHCH_2$), 134.2–123.7 (aromatic carbons), 118.1 ($-OCH_2CHCH_2$), 102.1 (–CHPh), 98.6(C-1′), 97.1 (C-1″), 96.3 (C-1), 80.2, 76.0, 73.9, 72.0, 71.1 (2C), 70.3, 70.1, 68.7, 68.3 (2C), 66.9, 66.5, 66.3, 65.9, 56.4 (C-2′), 20.6 ($-COCH_3$), 20.3 ($-COCH_3$), 20.2 ($-COCH_3$), (18.0 C-6 Rha_1), 16.3 (C-6 Rha_2). ESIMS $(M+Na)^+$, *m/z* 1086. Anal. Calcd for $C_{56}H_{57}NO_{20}$: C, 63.21; H, 5.40. Found: C, 63.17; H, 5.47.

1.10. Allyl α-L-rhamnopyranosyl-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→4)-α-L-rhamnopyranoside (12)

Compound **11** (30 mg, 0.028 mmol) in *n*-BuOH (5.0 mL) was added to ethylenediamine (1.0 mL) under N_2. The solution was stirred for 20 h at 90 °C, then evaporated to dryness. Toluene (2×) and EtOH were added to and evaporated from the residue to give a syrup. To this Ac_2O (0.6 mL) and pyridine (0.7 mL) were added. After stirring for 14 h at rt, the solution was concentrated to dryness. The residue was purified by column chromatography (5:1 $C_6H_5CH_3$–Et_2O). The product was then heated at 80 °C with 80% aq AcOH (1.5 mL) for 4 h, followed by treatment with a catalytic amount of MeONa in MeOH (2 mL) with stirring for 6 h at rt. The reaction mixture was neutralized with Amberlite IR 120 (H^+) resin and filtered through a Celite bed, concentrated, dissolved in water (1.0 mL) passed through a 0.45 Millipore membrane and dried to afford **12** (9.0 mg, 58%) as colorless syrup: $[\alpha]_D^{25}$ −67.5 (*c* 0.4, H_2O). ^1H NMR (D_2O): δ 5.87–5.83 (m, 1H, $-CH_2CHCH_2$), 5.13–5.09 (m, 2H, $-CH_2CHCH_2$) 4.99 (d, 1H, $J_{1,2}$ 7.8 Hz, H-1′), 4.75 (s, 1H, H-1, Rha_1), 4.59 (s, 1H, H-1″, Rha_2), 4.49–3.69 (m, 16H, $-CHHCHCH_2$, ring protons), 1.95 (s, 3H, 1 × $-NHCOCH_3$), 1.36 (d, 3H, *J* 6.0 Hz, 6-CH_3), 0.90 (d, 3H, *J* 6.3 Hz, 6-CH_3″). ESIMS $(M+H)^+$ at *m/z* 554. Anal. Calcd for $C_{23}H_{39}NO_{14}$: C, 49.90; H, 7.10. Found: C, 49.82; H, 7.15.

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