Synthesis of a Trisaccharide and a Tetrasaccharide from the Cell-Wall Lipopolysaccharides of *Azospirillum brasilense* S17

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Abstract: A trisaccharide containing a D-mannosamine moiety and a tetrasaccharide containing an L-rhamnan chain that are found in the cell walls of *Azospirillum brasilense* S17 were synthesized concisely and in excellent yields. The key features of the synthetic strategy were stereoselective glycosylations and a minimum number of protecting-group manipulations.

Key words: oligosaccharides, glycosylations, D-mannosamine, *Azospirillum brasilense*, rhizobacteria

Azospirilla are Gram-negative rhizobacteria that promote plant growth through their association with various plants in the rhizosphere.[1] Although azospirilla do not associate with particular plant species, they are widely distributed in soils and establish close relationships with the roots of grasses, cereals, and other nonleguminous plants.[2] They fix atmospheric nitrogen and have a positive effect on plant growth through the action of pectolytic enzymes and by excreting phytohormones, vitamins, and other biologically active substances into the rhizosphere.[2,3] Macromolecules such as exopolysaccharides, capsular polysaccharides, and lipopolysaccharides that are present in the bacterial cell surface are actively involved in the interactions of azospirilla with plants.[4] It is therefore essential to understand the roles of the cell-surface lipopolysaccharides of various strains of azospirilla in the interactions of the bacteria with plant roots.

For a detailed biological study on the role of cell-surface oligosaccharides of such bacteria with plants, it will be necessary to obtain greater quantities of the relevant oligosaccharides than can be obtained by isolation from the natural source. The development of concise strategies for the chemical syntheses of these oligosaccharides in large quantities for use in studies on plant–bacterial interactions is therefore a highly desirable goal. A trisaccharide containing a D-mannosamine moiety and tetrasaccharide containing an L-rhamnan chain from *Azospirillum brasilense* S17, which were recently isolated and characterized by Zdorovenko and co-workers,[5] were synthesized as their methyl glycosides (**1** and **2**, respectively; Figure 1 and Figure 2) in high overall yields by a sequence of reactions

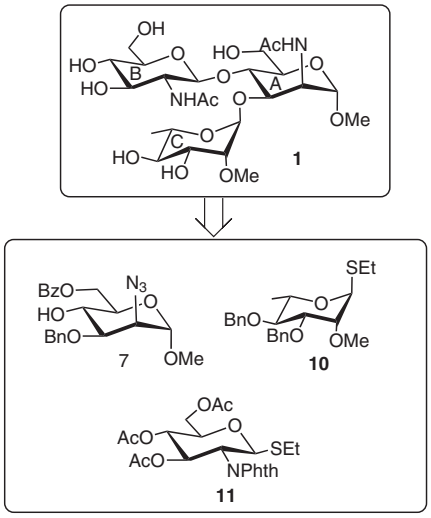

Figure 1 Structures of trisaccharide methyl glycoside **1** and its synthetic precursors

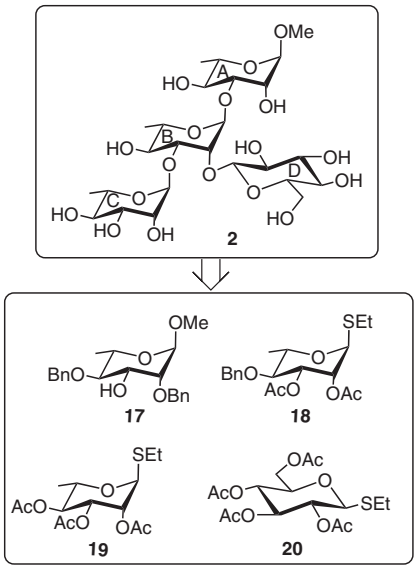

Figure 2 Structure of tetrasaccharide methyl glycoside **2** and its synthetic precursors

starting from monosaccharide intermediates prepared from commercially available reducing sugars.

Methyl 4,6-*O*-benzylidene-α-D-glucopyranoside (**3**)[6] was selectively benzylated to give the 3-*O*-benzyl derivative **4**[7] through formation of the stannylidene acetal. Glycoside

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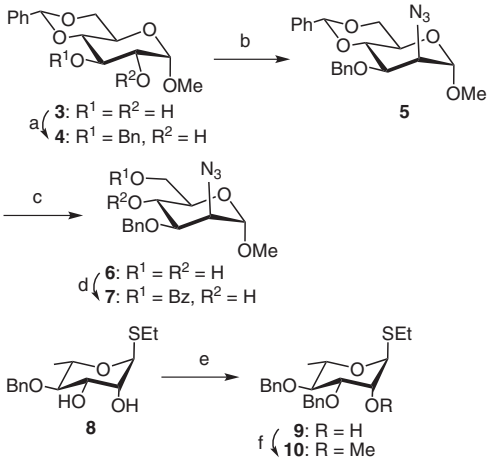
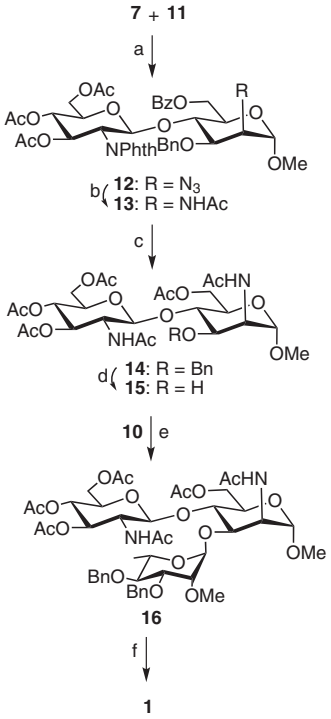
4 was treated with triflic anhydride in the presence of pyridine, and the resulting triflate derivative was treated with sodium azide to give methyl 2-azido-3-*O*-benzyl-2-deoxy-4,6-*O*-benzylidene-α-D-mannopyranoside (**5**) in 82% yield. Acidic hydrolysis of the benzylidene acetal in compound **5** with 80% aqueous acetic acid, followed by selective benzoylation of the primary hydroxy group of the ring-opened product **6** with benzoyl cyanide gave methyl 2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-mannopyranoside (**7**) in 90% yield. In another experiment, ethyl 4-*O*-benzyl-1-thio-α-L-rhamnopyranoside (**8**),[8] derived from L-rhamnose, was selectively benzylated via its stanylidene acetal to give the monodeprotected compound **9**,[9] which was methylated under alkaline condition to give ethyl 3,4-*O*-benzyl-2-*O*-methyl-1-thio-α-L-rhamnopyranoside (**10**) in 90% yield (Scheme 1). Compounds **11**,[10] **17**,[11] **18**,[12] **19**,[13] and **20**[14] were prepared in excellent yields by following the reaction conditions reported in the relevant literature.



Scheme 1 *Reagents and conditions*: (a) (i) see ref 7; (b) triflic anhydride, py, CH_2Cl_2, –10 °C, 1 h then NaN_3, HMPT–DMF (1:1), 70 °C, 24 h, 82%; (c) 80% aq AcOH, 80 °C, 2 h, quant.; (d) BzCN, py, CH_2Cl_2, 2 h, 90%; (e) see ref. 9; (f) MeI, NaH, DMF, 90%.

Iodonium ion-promoted stereoselective glycosylation of compound **7** with thioglycoside donor **11** in the presence of *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate[15] gave the disaccharide derivative **12**, which on treatment with triethylsilane[16] and 20% palladium(II) hydroxide/carbon followed by conventional acetylation gave the disaccharide derivative **13** in 91% yield. The presence of signals in the ^{1}H NMR spectrum at δ = 5.72 (d, J = 8.5 Hz, H-1_B) and 4.58 (d, J = 1.6 Hz, H-1_A)] and in the ^{13}C NMR spectrum at δ = 98.9 (C-1_A) and 98.5 (C-1_B) confirmed the formation of compound **12**. Treatment of disaccharide **13** with ethane-1,2-diamine in butan-1-ol followed by conventional acetylation gave disaccharide derivative **14** in 85% yield. Disaccharide **14** was hydrogenolyzed over 20% palladium(II) hydroxide/carbon[17] to give disaccharide acceptor **15** in 92% yield. Stereoselective glycosylation of compound **15** with thioglycoside donor **10** in the presence of *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate gave the trisaccharide derivative **16** in 89% yield. The stereoselective formation of compound **16** was confirmed by its one- and two-dimensional NMR spectra [δ = 5.10 (br s, H-1_C), 4.81 (d, J = 8.3 Hz, H-1_B), 4.55 (br s, H-1_A) in the ^{1}H NMR spectrum, and δ = 100.2 (2 C, C-1_B, C-1_C), 99.5 (C-1_A) in the ^{13}C NMR spectra] and by mass spectral analysis. Hydrogenolysis over Pearlmann's catalyst[17] followed by deacetylation gave the required trisaccharide **1** as its methyl glycoside in 81% yield; this was purified over LH-20 Sephadex by using MeOH–H_2O (4:1) as the eluant (Scheme 2). The presence of signals at δ = 4.94 (br s, 1 H, H-1_C), 4.63 (br s, 1 H, H-1_A), and 4.56 (d, J = 7.5 Hz, 1 H, H-1_B) in ^{1}H NMR spectrum and at δ = 102.4 (C-1_B), 101.4 (C-1_A), and 94.5 (C-1_C) in the ^{13}C NMR spectrum confirmed the formation of compound **1**.



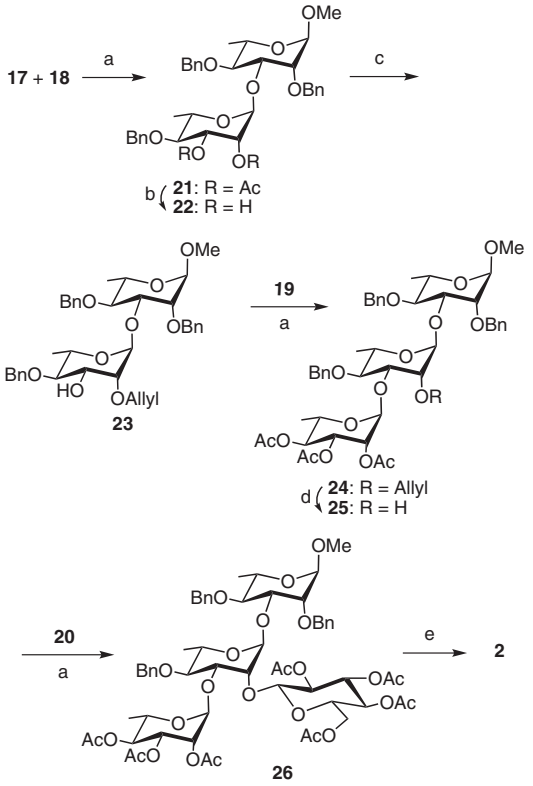
Scheme 2 *Reagents and conditions*: (a) NIS, TMSOTf, CH_2Cl_2, –20 °C, 1.5 h, 90%; (b) Et_3SiH, 20% $Pd(OH)_2$/C, MeOH–MeOH (5:1), r.t., 4 h, 91%; (c) (i) $H_2N(CH_2)_2NH_2$, BuOH, 110 °C, 10 h; (ii) Ac_2O, py, 2 h, r.t., 85%; (d) H_2, 20% $Pd(OH)_2$/C, r.t., 8 h, 92%; (e) NIS, TMSOTf, CH_2Cl_2, –10 °C, 2 h, 89%; (f) H_2, 20% $Pd(OH)_2$/C, r.t., 8 h then MeONa, MeOH, r.t., 12 h, 81%.

Stereoselective glycosylation of monodeprotected compound **17** with the thioglycoside donor **18** in the presence of *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate gave the disaccharide derivative **21** in 90% yield; this was then deacetylated by sodium methoxide to give disaccharide diol derivative **22** quantitatively. The formation of compound **21** was confirmed by the presence of signals at δ = 98.9 (C-1_A) and 98.4 (C-1_B) in the ^{13}C NMR spectrum.

Selective allylation of compound **22** under phase-transfer reaction conditions gave the disaccharide acceptor **23** in

84% yield. Iodonium ion-promoted coupling of disaccharide **23** with thioglycoside **19** in the presence of *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate gave trisaccharide derivative **24** in 89% yield. The formation of trisaccharide **24** was confirmed by the presence of signals in at δ = 99.3 (C-1_B), 99.2 (C-1_C), 98.5 (C-1_A) in the ^{13}C NMR spectrum. Removal of the allyl group from compound **24** by using palladium chloride in methanol gave trisaccharide acceptor **25** in 87% yield. Glycosylation of this product with thioglycoside **20** in the presence of *N*-iodosuccinimide and trimethylsilyl trifluoromethanesulfonate gave the tetrasaccharide derivative **26** in 83% yield. This was deprotected by hydrogenation and saponification to give the tetrasaccharide methyl glycoside **2** in 79% overall yield. Tetrasaccharide **2** was purified by passage through a column of LH-20 Sephadex using methanol–water (4:1) as the eluant. The formation of compound **2** was confirmed by the presence of signals at δ = 5.04 (br s, H-1_A), 5.01 (br s, H-1_C), 4.76 (d, *J* = 7.8 Hz, H-1_D), and 4.57 (br s, H-1_B) in the ^{1}H NMR and at δ = 104.2 (C-1_D), 104.0 (C-1_A), 103.9 (C-1_C), 102.6 (C-1_B)] in the ^{13}C NMR spectrum (Scheme 3).

In summary, efficient synthetic strategies have been developed for the synthesis of the methyl glycoside of an Azospirillum trisaccharide containing a D-mannosamine moiety and an Azospirillum tetrasaccharide containing an L-rhamnan chain. All the intermediate steps are high yielding, and can be used in large-scale preparations.



Scheme 3 *Reagents and conditions*: (a) NIS, TMSOTf, CH_2Cl_2, –30 °C, 1.5 h, 90% for **21**, 89% for **24**, and 83% for **26**; (b) MeONa, MeOH, r.t., 5 h, quant; (c) allyl bromide, 5% aq NaOH, CH_2Cl_2, Bu_4NHSO_4, r.t., 3 h, 84%; (d) $PdCl_2$, MeOH, r.t., 2 h, 87%; (e) H_2, 20% $Pd(OH)_2$/C, r.t., 12 h then MeONa, MeOH, r.t., 8 h, 79%.

All the reactions were monitored by TLC on silica gel-coated plates; the spots were visualized by spraying the plates with 2% $Ce(SO_4)_2$ in 4 M H_2SO_4 and then warming the plates on a hotplate. Silica gel (230–400 mesh) was used for column chromatography. ^{1}H and ^{13}C NMR, 2D COSY, and HSQC spectra were recorded on a Bruker Avance DPX at 300 MHz using $CDCl_3$ and D_2O as solvents and TMS as the internal reference, unless otherwise stated. Chemical shifts are expressed in δ (ppm). ESI-MS were recorded on a Micromass Quattro II triple quadrupole mass spectrometer. Elementary analysis was carried out on Carlo ERBA-1108 analyzer. Optical rotations were measured at 25 °C on a Rudolf Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity were used in most reactions.

Methyl 2-Azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-α-D-mannopyranoside (5)

Pyridine (5 mL) was added to a soln of compound **4** (1.7 g, 4.57 mmol) in anhyd CH_2Cl_2 (10 mL) and the mixture was cooled to –10 °C. Triflic anhydride (1.6 mL, 9.51 mmol) was added and the cold mixture was stirred at –10 °C for 1 h. The solvents were removed under reduced pressure and the crude product was used directly in the next step.

NaN_3 (1.5 g, 23 mmol) was added to a soln of the crude product in HMPT (5 mL), and the mixture was stirred at 70 °C for 24 h. The mixture was then poured into cold H_2O and extracted with EtOAc (100 mL). The organic layer was washed with sat. aq $NaHCO_3$, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography [hexane–EtOAc (3:1)] to give pure **5** as a yellow oil; yield: 1.5 g (82%); $[\alpha]_D$ +93 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.47–7.17 (m, 10 H, Ar-H), 5.59 (s, 1 H, PhC*H*) 4.89–4.85 (d, *J* = 12.2 Hz, 1 H, PhCH_2), 4.73–4.69 (d, *J* = 12.2 Hz, 1 H, PhCH_2), 4.62 (s, 1 H, H-1), 4.25–4.20 (dd, *J* = 9.4, 3.9 Hz, 1 H, H-6_a), 4.12–4.03 (m, 2 H, H-3, H-5), 3.93 (br s, 1 H, H-2), 3.80 (d, *J* = 9.6 Hz, 1 H, H-4), 3.85–3.73 (m, 1 H, H-6_b), 3.35 (s, 3 H, OCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.1–126.1 (Ar-C), 101.7 (Ph*C*H), 100.2 (C-1), 79.2 (C-3), 75.6 (C-5), 73.4 (Ph*C*H_2), 68.7 (C-6), 63.8 (C-4), 62.8 (C-2), 55.0 (O*C*H_3).

MS (EI, 70 eV): 420.1 $[M + Na]^+$.

Anal. Calcd for $C_{21}H_{23}N_3O_5$: C, 63.46; H, 5.83. Found: C, 63.30; H, 6.0.

Methyl 2-Azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-mannopyranoside (7)

10% $HClO_4$/silica gel (200 mg) was added to a soln of compound **5** (1.5 g, 3.77 mmol) in MeCN (20 mL), and the reaction was stirred at r.t. for 30 min then filtered through a Celite bed and evaporated to dryness. The crude mass was dissolved in anhyd CH_2Cl_2 (10 mL) then pyridine (5 mL) and BzCN (500 mg, 3.81 mmol) were added sequentially. The mixture was stirred at r.t. for 2 h and concentrated under reduced pressure to give a crude product that was purified by chromatography [silica gel, hexane–EtOAc (5:1)] to give pure compound **7** as a yellow oil; yield: 1.4 g (90%); $[\alpha]_D$ +62 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.07–7.29 (m, 10 H, Ar-H), 4.76 (d, *J* = 11.5 Hz, 1 H, PhCH_2), 4.70 (br s, 1 H, H-1), 4.64 (d, *J* = 11.5 Hz, 1 H, PhCH_2), 4.61–4.52 (m, 2 H, H-6_{ab}), 3.95–3.79 (m, 4 H, H-2, H-4, H-5, and H-3), 3.37 (s, 3 H, OCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 166.6 (Ph*C*O), 137.5–128.0 (Ar-C), 99.4 (C-1), 79.2 (C-3), 72.6 (Ph*C*H_2), 70.7 (C-5), 66.7 (C-4), 63.8 (C-6), 60.5 (C-2), 55.2 (O*C*H_3).

MS (EI, 70 eV): 436.1 $[M + Na]^+$.

Anal. Calcd for $C_{21}H_{23}N_3O_6$: C, 61.01; H, 5.61. Found: C, 60.85; H, 5.80.

Ethyl 3,4-Di-*O*-benzyl-2-*O*-methyl-1-thio-α-L-rhamnopyranoside (10)

Crushed NaOH (500 mg, 12.5 mmol) and MeI (1.0 mL, 16 mmol) were added to a soln of thioglycoside **9** (1.5 g, 3.86 mmol) in anhyd THF (20 mL), and the mixture was stirred at 0–5 °C for 4 h. The mixture was then diluted with H_2O and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated to dryness. The crude product was purified by chromatography [silica gel, hexane–EtOAc (10:1)] to give pure compound **10** as a yellow oil; yield: 1.4 g (90%); $[\alpha]_D$ –134 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.24 (m, 10 H, Ar-H), 5.27 (br s, 1 H, H-1), 4.93–4.89 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.71–4.66 (m, 2 H, PhC*H*$_2$), 4.62–4.59 (d, *J* = 11.0 Hz, 1 H, PhC*H*$_2$), 4.03–3.91 (m, 1 H, H-5), 3.77–3.73 (dd, *J* = 9.4, 3.1 Hz, 1 H, H-3), 3.53–3.50 (m, 2 H, H-2, H-4), 3.47 (s, 3 H, OC*H*$_3$), 2.66–2.52 (m, 2 H, SC*H*$_2$CH$_3$), 1.30–1.25 (m, 6 H, SCH$_2$C*H*$_3$, C*H*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.7–127.5 (Ar-C), 81.2 (C-1) 80.6 (C-4), 80.2 (Ph*C*H$_2$), 79.8 (C-2), 75.3 (Ph*C*H$_2$), 72.3 (C-3), 68.3 (C-5), 58.5 (O*C*H$_3$), 25.3 (S*C*H$_2$CH$_3$), 17.9 (C*C*H$_3$), 15.0 (SCH$_2$*C*H$_3$).

MS (EI, 70 eV): 425.2 $[M + Na]^+$.

Anal. Calcd for $C_{23}H_{30}O_4S$: C, 68.62; H, 7.51. Found: C, 68.45; H, 7.72.

Methyl 4-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-2-azido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-mannopyranoside (12)

A soln of azide **7** (1.0 g, 2.42 mmol) and thioglycoside **11** (1.4 g, 2.92 mmol) in anhyd CH_2Cl_2 (20 mL) was treated with 4-Å MS (2 g), and the mixture was stirred under argon for 1 h. The mixture was then cooled to –20 °C, and NIS (850 mg, 3.77 mmol) and TMSOTf (15 μL) were added sequentially. The mixture was stirred at –20 °C for 1.5 h then diluted with CH_2Cl_2 (100 mL) and filtered through a Celite bed. The organic layer was washed successively with sat. aq $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography [silica gel, hexane–EtOAc (3:1)] to give pure disaccharide derivative **12** as a yellow oil; yield: 1.8 g (90%); $[\alpha]_D$ +152 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.80–7.28 (m, 14 H, Ar-H), 5.72 (d, *J* = 8.5 Hz, 1 H, H-1$_B$), 5.66 (d, *J* = 9.5 Hz, 1 H, H-3$_B$), 5.11 (t, *J* = 9.5 Hz, 1 H, H-4$_B$), 4.84 (d, *J* = 12.3 Hz, 1 H, PhC*H*$_2$), 4.79 (d, *J* = 12.3 Hz, 1 H, PhC*H*$_2$), 4.58 (d, *J* = 1.6 Hz, 1 H, H-1$_A$), 4.77–4.43 (dd, *J* = 12.3, 1.6 Hz, 1 H, H-6$_{aA}$), 4.31–4.24 (dd, *J* = 8.5 Hz each, 1 H, H-2$_B$), 4.14 (d, *J* = 9.1 Hz, 1 H, H-4$_A$), 4.10–4.01 (m, 2 H, H-3$_A$, H-6$_{aB}$), 3.94–3.83 (m, 2 H, H-6$_{bA}$, H-6$_{bB}$), 3.79–3.74 (m, 2 H, H-2$_A$, H-5$_A$), 3.57–3.54 (m, 1 H, H-5$_B$), 3.28 (s, 3 H, OC*H*$_3$), 2.04, 1.98, 1.81 (3 s, 9 H, 3 COC*H*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.3, 169.8, 169.1 (3 *C*OCH$_3$), 167.6, 166.7 (2 *C*O; Phth), 165.3 (Ph*C*O), 137.9–123.6 (Ar-C), 98.9 (C-1$_A$), 98.5 (C-1$_B$), 78.1 (C-3$_A$), 74.7 (C-4$_A$), 71.9 (Ph*C*H$_2$), 71.7 (C-5$_B$), 70.7 (C-3$_B$), 68.9 (C-5$_A$), 68.4 (C-4$_B$), 62.5 (C-6$_A$), 61.3 (C-6$_B$), 60.6 (C-2$_A$), 55.2 (C-2$_B$), 55.0 (O*C*H$_3$), 20.6, 20.5, 20.3 (3 C, CO*C*H$_3$).

MS (EI, 70 eV): 853.2 $[M + Na]^+$.

Anal. Calcd for $C_{41}H_{42}N_4O_{15}$: C, 59.27; H, 5.10. Found: C, 59.10; H, 5.26.

Methyl 4-(3,4,6-Tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-2-acetamido-6-*O*-benzoyl-3-*O*-benzyl-2-deoxy-α-D-mannopyranoside (13)

20% $Pd(OH)_2$/C (100 mg) and TES (3.2 mL, 50 mmol) were added to a soln of azide **12** (1.3 g, 1.56 mmol) in MeOH–$CHCl_3$ (5:1) under argon, and the mixture was stirred at r.t. for 6 h, filtered through a Celite bed, and evaporated to dryness. A soln of the crude product in Ac_2O–pyridine (5 mL, 1:1) was kept at r.t. for 2 h. The solvents were removed under reduced pressure and the crude product was purified by chromatography [silica gel, hexane–EtOAc (3:1)] to give pure compound **13** as a yellow oil; yield: 1.2 g (91%); $[\alpha]_D$ +108 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.87–7.27 (m, 14 H, Ar-H), 5.74 (d, *J* = 8.5 Hz, 1 H, H-1$_B$), 5.75–5.72 (m, 1 H, N*H*COCH$_3$), 5.67–5.60 (dd, *J* = 9.3, 9.3 Hz, 1 H, H-3$_B$), 5.06 (t, *J* = 9.5 Hz, 1 H, H-4$_B$), 4.66–4.63 (m, 2 H, H-1$_A$, PhC*H*$_2$), 4.52–4.44 (m, 3 H, H-2$_A$, H-6$_{aA}$, PhC*H*$_2$), 4.32–4.26 (dd, *J* = 8.5, 8.5 Hz, 1 H, H-2$_B$), 4.10–4.03 (m, 2 H, H-3$_A$, H-6$_{bA}$), 3.96–3.91 (m, 3 H, H-4$_A$, H-6$_{abB}$), 3.85–3.82 (m, 1 H, H-5$_A$), 3.52–3.46 (m, 1 H, H-5$_B$), 3.29 (s, 3 H, OC*H*$_3$), 2.0, 1.97, 1.92, 1.79 (4 s, 12 H, 4 COC*H*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.4, 169.9, 169.8, 169.0 (4 *C*OCH$_3$), 167.7, 166.8 (2 *C*O; Phth), 165.2 (Ph*C*O), 137.8–123.5 (Ar-C), 99.6 (C-1$_A$), 97.5 (C-1$_B$), 75.1 (C-3$_A$), 73.7 (C-4$_A$), 71.8 (C-5$_B$), 70.8 (C-3$_B$), 70.4 (Ph*C*H$_2$), 68.4 (C-4$_B$), 68.2 (C-5$_A$), 55.1 (O*C*H$_3$), 55.0 (C-2$_B$), 49.1 (C-2$_A$), 23.1 (NHCO*C*H$_3$), 20.6, 20.5, 20.3 (3 CO*C*H$_3$).

MS (EI, 70 eV): 869.3 $[M + Na]^+$.

Anal. Calcd for $C_{43}H_{46}N_2O_{16}$: C, 60.99; H, 5.48. Found: C, 60.82; H, 5.64.

Methyl 4-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-acetamido-6-*O*-acetyl-3-*O*-benzyl-2-deoxy-α-D-mannopyranoside (14)

$H_2N(CH_2)_2NH_2$ (0.8 mL, 12 mmol) was added to a soln of acetamide **13** (1.1 g, 1.3 mmol) in BuOH (20 mL), and the mixture was stirred at 110 °C for 6 h. The solvents were removed under reduced pressure and a soln of the crude product in Ac_2O–py (10 mL, 1:1) was kept at r.t. for 2 h. The solvents were removed under reduced pressure, and the crude product was purified by chromatography [silica gel, hexane–EtOAc (3:1)] to give pure compound **14** as a yellow oil; yield: 770 mg (85%); $[\alpha]_D$ +28 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.38–7.28 (m, 5 H, Ar-H), 5.92 (t, *J* = 9.2 Hz, 2 H, 2 N*H*COCH$_3$), 5.24 (t, *J* = 10.2 Hz, 1 H, H-3$_B$), 5.05–4.89 (m, 2 H, H-1$_B$, H-4$_B$), 4.74–4.71 (m, 2 H, H-1$_A$, PhC*H*$_2$), 4.59–4.55 (m, 1 H, H-2$_A$), 4.49 (d, *J* = 11.4 Hz, 1 H, PhC*H*$_2$), 4.41–4.38 (dd, *J* = 11.8, 1.6 Hz, 1 H, H-6$_{bA}$), 4.31–4.25 (dd, *J* = 11.8, 5.6 Hz, 1 H, H-6$_{bA}$), 4.07–4.02 (m, 2 H, H-3$_A$, H-6$_{aB}$), 3.92–3.83 (m, 2 H, H-3$_A$, H-6$_{bB}$), 3.77–3.69 (m, 2 H, H-2$_B$, H-5$_A$), 3.47–3.44 (m, 1 H, H-5$_B$), 3.35 (s, 3 H, OC*H*$_3$), 2.13, 2.02, 1.99, 1.87 (4 s, 18 H, 6 COC*H*$_3$).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 170.6, 170.5, 170.3, 169.3, 169.1 (6 *C*OCH$_3$), 137.8–127.3 (Ar-C), 99.9 (C-1$_B$), 99.7 (C-1$_A$), 75.3 (C-3$_A$), 74.4 (C-5$_A$), 72.2 (C-3$_B$), 71.7 (C-5$_B$), 70.8 (Ph*C*H$_2$), 68.5 (C-4$_A$), 68.2 (C-4$_B$), 63.3 (C-6$_A$), 61.7 (C-6$_B$), 55.3 (C-2$_B$), 55.1 (O*C*H$_3$), 49.3 (C-2$_A$), 23.3, 23.2 (2 NHCO*C*H$_3$), 20.9, 20.6, 20.5 (2 C) (4 CO*C*H$_3$).

MS (EI, 70 eV): 719.3 $[M + Na]^+$.

Anal. Calcd for $C_{32}H_{44}N_2O_{15}$: C, 55.17; H, 6.37. Found: C, 55.0; H, 6.55.

Methyl 4-(2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-acetamido-6-*O*-acetyl-2-deoxy-α-D-mannopyranoside (15)

20% $Pd(OH)_2$-C (100 mg) was added to a soln of disaccharide **14** (750 mg, 1.07 mmol) in MeOH (10 mL), and the mixture was stirred under a positive pressure of H_2 at r.t. for 10 h. The mixture was then filtered through a Celite bed and the solvents were evaporated to dryness. The crude product was purified by chromatography [silica gel, hexane–EtOAc (2:1)] to give pure compound **15** as a yellow oil; yield: 600 mg (92%); $[\alpha]_D$ +7.7 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 6.32 (d, *J* = 8.8 Hz, 1 H, N*H*CH_3), 5.76 (d, *J* = 6.51 Hz, 1 H, N*H*$COCH_3$), 5.31–5.24 (m, 1 H, H-3_B), 5.00 (t, *J* = 9.6 Hz, 1 H, H-4_B), 4.83 (br s, 1 H, H-1_A), 4.70 (d, *J* = 8.4 Hz, 1 H, H-1_B), 4.44–4.41 (m, 1 H, H-6_{aB}), 4.33–4.31 (m, 1 H, H-2_A), 4.27–4.19 (m, 2 H, H-6_{aA}, H-6_{bB}), 4.15–4.07 (m, 2 H, H-3_A, H-6_{bA}), 3.97–3.93 (m, 1 H, H-2_B), 3.84–3.77 (m, 2 H, H-4_A, H-5_B), 3.35 (s, 3 H, OCH_3), 3.38–3.32 (m, 1 H, H-5_A), 2.16, 2.08, 2.03, 2.02, 1.97 (5 s, 18 H, 6 COCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 171.4, 170.8, 170.7, 169.3, 169.1, 169.0 (6 *C*OCH_3), 101.4 (C-1_B), 99.4 (C-1_A), 80.2 (C-5_A), 72.1 (C-3_B), 71.9 (C-4_A), 68.6 (C-4_B), 67.5 (C-5_B), 67.3 (C-3_A), 62.7 (C-6_A), 62.1 (C-6_B), 55.1 (O*C*H_3), 54.6 (C-2_B), 52.3 (C-2_A), 23.3, 23.1 (2 NHCO*C*H_3), 20.9, 20.6, 20.5, 20.4 (4 CO*C*H_3).

MS (EI, 70 eV): 629.2 $[M + Na]^+$.

Anal. Calcd for $C_{25}H_{38}N_2O_{15}$: C, 49.50; H, 6.31. Found: C, 49.34; H, 6.50.

Methyl (2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-(1→4)-[(3,4-di-*O*-benzyl-2-*O*-methyl-α-L-rhamnopyranosyl)-(1→3)]-2-acetamido-6-*O*-acetyl-2-deoxy-α-D-mannopyranoside (16)

A soln of acetamide **15** (550 mg, 0.9 mmol) and thioglycoside **10** (540 mg, 1.34 mmol) in anhyd CH_2Cl_2 (10 mL) was treated with 4-Å MS (1 g), and the mixture was stirred at r.t. under argon for 1 h. The mixture was then cooled to –10 °C then NIS (360 mg, 1.6 mmol) and TMSOTf (5 μL) were added successively. The mixture was stirred at –10 °C for 1.5 h, diluted with CH_2Cl_2 (100 mL), and filtered through a Celite bed. The organic layer was washed successively with sat. aq $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography [silica gel, hexane–EtOAc (3:1)] to give pure compound **16** as a yellow oil; yield: 760 mg (89%); $[\alpha]_D$ –1.4 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.26 (m, 10 H, Ar-H), 6.32 (d, *J* = 8.8 Hz, 1 H, N*H*$COCH_3$), 5.83 (d, *J* = 6.8 Hz, 1 H, N*H*$COCH_3$), 5.19 (t, *J* = 9.6 Hz, 1 H, H-3_B), 5.10 (br s, 1 H, H-1_C), 4.96–4.89 (m, 2 H, H-4_B, PhCH_2), 4.81 (d, *J* = 8.3 Hz, 1 H, H-1_B), 4.71–4.62 (m, 3 H, PhCH_2), 4.55 (br s, 1 H, H-1_A), 4.53–4.48 (m, 1 H, H-6_{aB}), 4.33 (br s, 2 H, H-2_A, H-3_A), 4.17–4.15 (m, 1 H, H-2_B), 4.12–4.08 (m, 1 H, H-6_{bB}), 3.96–3.88 (m, 1 H, H-5_C), 3.85–3.76 (m, 4 H, H-3_C, H-4_A, H-6_{abA}), 3.64–3.59 (m, 2 H, H-4_C, H-5_B), 3.54–3.84 (m, 1 H, H-5_A), 3.50 (s, 3 H, OCH_3), 3.41 (br s, 1 H, H-2_C), 3.35 (s, 3 H, OCH_3), 2.16, 2.02, 2.0, 1.95, 1.79 (5 s, 18 H, 6 COCH_3), 1.30 (d, *J* = 6.4 Hz, 3 H, CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.7, 170.4 (2 C), 169.9, 169.8, 169.0 (6 *C*OCH_3), 138.6–127.6 (Ar-C), 100.2 (2 C, C-1_B, C-1_C), 99.5 (C-1_A), 80.6 (C-4_C, C-5_A), 79.2 (C-3_B), 77.6 (C-2_C), 75.6 (Ph*C*H_2), 72.4 (C-5_B), 71.9 (2 C, C-3_A, C-4_A), 71.5 (PhCH_2), 69.3 (C-3_C), 69.1 (C-4_B), 67.7 (C-5_C), 63.2 (C-6_A), 62.3 (C-6_B), 58.9 (O*C*H_3), 55.1 (OCH_3), 54.5 (C-2_B), 49.0 (C-2_A), 23.2, 23.1 (2 NHCO*C*H_3), 20.8, 20.7 (2 C), 20.6 (4 CO*C*H_3), 17.9 (C*C*H_3).

MS (EI, 70 eV): 969.3 $[M + Na]^+$.

Anal. Calcd for $C_{46}H_{62}N_2O_{19}$: C, 58.34; H, 6.60. Found: C, 58.17; H, 6.72.

Methyl (2-Acetamido-2-deoxy-β-D-glucopyranosyl)-(1→4)-[(2-*O*-methyl-α-L-rhamnopyranosyl)-(1→3)]-2-acetamido-2-deoxy-α-D-mannopyranoside (1)

20% $Pd(OH)_2$/C (150 mg) was added to a soln of the protected compound **16** (700 mg, 0.74 mmol) in MeOH (10 mL), and the mixture was stirred under a positive pressure of H_2 at r.t. for 10 h. The mixture was then filtered through a Celite bed and the solvents were evaporated to dryness. A soln of the crude product in 0.1 M methanolic NaOMe (5 mL) was stirred at r.t. for 6 h and then neutralized with Dowex 50W X-8 (H^+). The mixture was filtered and evaporated to dryness to give crude target compound **1**, which was purified as an amorphous powder by chromatography [LH-20 Sephadex, MeOH–H_2O (4:1)]; yield: 360 mg (81%); $[\alpha]_D$ +20 (*c* 1.0, H_2O).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 4.94 (br s, 1 H, H-1_C), 4.63 (br s, 1 H, H-1_A), 4.56 (d, *J* = 7.5 Hz, 1 H, H-1_B), 4.50–4.49 (m, 1 H, H-2_A), 4.31–4.21 (m, 1 H, H-5_C), 4.04–4.00 (m, 1 H, H-3_A), 3.97–3.84 (m, 5 H, H-4_A, H-4_B, H-6_{abB}, H-6_{aA}), 3.70–3.65 (m, 2 H, H-2_B, H-5_B), 3.62–3.59 (m, 3 H, H-3_C, H-5_B, H-6_{bA}), 3.46 (s, 3 H, OCH_3), 3.37 (s, 3 H, OCH_3), 3.34–3.22 (m, 3 H, H-2_C, H-4_C, H-5_A), 2.05, 2.02 (2 s, 6 H, 2 COCH_3), 1.31 (d, *J* = 6.3 Hz, 3 H, CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 173.7, 173.4 (2 *C*OCH_3), 102.4 (C-1_B), 101.4 (C-1_A), 94.5 (C-1_C), 82.0 (C-2_C), 77.6 (C-5_A), 75.3 (C-5_B), 74.5 (C-4_C), 73.1 (C-3_C), 72.6 (C-3_B), 72.4 (C-3_A) 71.6 (2 C, C-4_A, C-4_B), 69.5 (C-5_C), 62.3 (C-6_B), 61.4 (C-6_A), 59.1 (O*C*H_3), 58.0 (C-2_B), 55.4 (O*C*H_3), 49.9 (C-2_A), 22.9, 22.6 (2 *C*OCH_3), 17.8 (C*C*H_3).

MS (EI, 70 eV): 621.7 $[M + Na]^+$.

Anal. Calcd for $C_{24}H_{42}N_2O_{15}$: C, 48.16; H, 7.07. Found: C, 47.97; H, 7.24.

Methyl 3-(2,3-Di-*O*-acetyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (21)

A soln of monodeprotected compound **17** (1.4 g, 3.9 mmol) and thioglycoside **18** (1.8 g, 4.7 mmol) in anhyd CH_2Cl_2 (20 mL) was treated with 4-Å MS (2 g), and the mixture was stirred at r.t. under argon for 1 h. The mixture was then cooled to –10 °C, and NIS (1.3 g, 5.77 mmol) and TMSOTf (20 μL) were added sequentially. The mixture was stirred at –10 °C for 1.5 h then diluted with CH_2Cl_2 (100 mL) and filtered through a Celite bed. The organic layer was washed successively with sat. aq $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography [silica gel, hexane–EtOAc (4:1)] to give pure compound **21** as a yellow oil; yield: 2.4 g (90%); $[\alpha]_D$ –47 (*c* 1.1, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.41–7.18 (m, 15 H, Ar-H), 5.43–5.37 (m, 2 H, H-2_B, H-3_B), 5.02 (s, 1 H, H-1_B), 4.81–4.57 (m, 7 H, H-1_A, 3 PhCH_2), 4.04–4.01 (m, 1 H, H-5_A), 3.89–3.80 (m, 1 H, H-5_B), 3.67–3.62 (m, 3 H, H-2_A, H-3_A, H-4_B), 3.47–3.41 (t, *J* = 9.4 Hz, 1 H, H-4_A), 3.29 (s, 3 H, OCH_3), 2.01, 1.98 (2 s, 6 H, 2 COCH_3), 1.30, 1.27 (2 d, *J* = 6.3 Hz each, 6 H, 2 CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.4, 169.3 (2 *C*OCH_3), 138.5–127.3 (Ar-C), 98.9 (C-1_A), 98.4 (C-1_B), 80.7 (C-4_B), 78.7 (C-4_A), 77.7 (C-2_B), 77.4 (C-3_A), 75.1 (C-2_A), 74.6 (Ph*C*H_2), 72.6 (Ph*C*H_2), 71.5 (Ph*C*H_2), 70.2 (C-3_B), 68.0 (C-5_A), 67.9 (C-5_B), 54.5 (O*C*H_3), 20.7, 20.6 (2 CO*C*H_3), 18.0, 17.9 (2 C*C*H_3).

MS (EI, 70 eV): 701.3 $[M + Na]^+$.

Anal. Calcd for $C_{38}H_{46}O_{11}$: C, 67.24; H, 6.83. Found: C, 67.07; H, 7.0.

Methyl 3-(2-*O*-Allyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (23)

A soln of compound **21** (2.3 g, 3.38 mmol) in 0.1 M methanolic NaOMe (50 mL) was stirred at r.t. for 3 h and then neutralized with Dowex 50W-X8 (H^+) resin. The mixture was filtered and evaporated to dryness. To a soln of the crude product **22** in CH_2Cl_2 (25 mL) were added 5% aq NaOH (15 mL), allyl bromide (440 μL, 5.08 mmol), and Bu_4NHSO_4 (100 mg), and the biphasic reaction mixture was stirred briskly at r.t. for 3 h. The mixture was diluted with CH_2Cl_2 (100 mL), and the organic layer was washed with H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography [silica gel, hexane–EtOAc (4:1)] to give pure compound **23** as a yellow oil; yield: 1.8 g (84%); $[\alpha]_D$ –58 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.39–7.22 (m, 15 H, Ar-H), 5.78–5.65 (m, 1 H, CH_2=C*H*), 5.09 (br s, 1 H, H-1_B), 5.08–5.02 (m, 2 H, CH=CH_2), 4.93–4.89 (d, *J* = 11.3 Hz, 1 H, PhCH_2), 4.81–4.78 (d, *J* = 11.7, Hz, 1 H, PhCH_2), 4.73–4.63 (m, 7 H, H-1_A, 3 PhCH_2), 4.09–4.05 (dd, *J* = 12.3, 3.0 Hz, 1 H, H-3_A), 4.01–3.93 (m, 1 H, H-3_B), 3.82–3.57 (m, 7 H, H-2_A, H-2_B, H-4_A, H-5_A, H-5_B, OCH_2–CH=CH_2), 3.31 (s, 3 H, OCH_3), 3.30–3.24 (m, 1 H, H-4_B), 1.32, 1.26 (2 d, *J* = 6.4 Hz each, 6 H, 2 CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 138.8–126.8 (Ar-C), 117.3 (–*C*H=CH_2), 98.9 (C-1_A), 98.7 (C-1_B), 82.2 (C-4_B), 80.9 (C-4_A), 78.8 (C-2_B), 77.8 (C-3_A), 76.6 (C-2_A), 74.8 (Ph*C*H$_2$), 74.7 (Ph*C*H$_2$), 72.7 (Ph*C*H$_2$), 71.6 (O*C*H$_2$–CH=CH_2), 71.5 (C-3_B), 68.1 (C-5_A), 67.7 (C-5_B), 54.6 (O*C*H$_3$), 18.0, 17.9 (2 C*C*H$_3$).

MS (EI, 70 eV): 657.3 $[M + Na]^+$.

Anal. Calcd for $C_{37}H_{46}O_9$: C, 70.01; H, 7.30. Found: C, 69.84; H, 7.48.

Methyl (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(2-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (24)

A soln of allyl derivative **23** (1.5 g, 2.36 mmol) and thioglycoside **19** (1.0 g, 3.0 mmol) in anhyd CH_2Cl_2 (20 mL) was added 4-Å MS (2 g), and the soln was stirred under argon for 1 h. The reaction mixture was cooled to –10 °C, and NIS (560 mg, 2.48 mmol) and TMSOTf (10 μL) were added sequentially. The mixture was stirred at –10 °C for 1.5 h then diluted with CH_2Cl_2 (100 mL) and filtered through a Celite bed. The organic layer was washed successively with sat. aq $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography [silica gel, hexane–EtOAc (4:1)] to give pure compound **24** as a yellow oil; yield: 1.9 g (89%); $[\alpha]_D$ –35 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.36–7.19 (m, 15 H, Ar-H), 5.77–5.72 (m, 1 H, CH_2=C*H*–), 5.34–5.27 (m, 2 H, H-2_C, H-4_C), 5.15–4.96 (m, 5 H, H-1_B, H-1_C, H-3_C, CH_2=CH–), 4.81–4.58 (m, 7 H, H-1_A, 3 PhCH_2), 4.04–3.99 (m, 2 H, H-3_B, H-5_C), 3.97–3.94 (m, 1 H, H-4_A), 3.90–3.84 (m, 1 H, H-3_A), 3.74–3.62 (m, 5 H, H-2_A, H-2_B, H-5_B, OCH_2–CH=CH_2), 3.59–3.53 (m, 2 H, H-4_B, H-5_A), 3.31 (s, 3 H, OCH_3), 2.07, 2.02, 1.97 (3 s, 9 H, 3 COCH_3), 1.27, 1.24, 1.11 (3 d, *J* = 6.4 Hz each, 9 H, 3 CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.6, 169.5, 169.4 (3 *C*OCH$_3$), 138.5–126.8 (Ar-C), 99.3 (C-1_B), 99.2 (C-1_C), 98.5 (C-1_A), 80.8 (C-5_A), 80.5 (C-4_B), 78.8 (C-3_A), 78.3 (C-3_B), 78.1 (C-5_C), 78.0 (C-5_B), 75.2 (Ph*C*H$_2$), 74.7 (Ph*C*H$_2$), 72.7 (Ph*C*H$_2$), 71.5 (O*C*H$_2$-CH = CH_2), 71.1 (C-1_C), 69.9 (C-4_C), 69.1 (C-2_C), 68.7 (C-2_B), 68.1 (C-2_A), 66.8 (C-4_A), 54.7 (O*C*H$_3$), 20.8, 20.7, 20.6 (3 CO*C*H$_3$), 18.1, 18.0, 17.5 (3 C*C*H$_3$).

MS (EI, 70 eV): 929.4 $[M + Na]^+$.

Anal. Calcd for $C_{49}H_{62}O_{16}$: C, 64.89; H, 6.89. Found: C, 64.72; H, 7.08.

Methyl (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (25)

$PdCl_2$ (180 mg, 1.0 mmol) was added to a soln of allyl derivative **24** (1.8 g, 1.98 mmol) in anhyd MeOH (25 mL), and the mixture was stirred at r.t. for 3 h. The mixture was filtered through a Celite bed and evaporated to dryness. The crude product was purified by chromatography [silica gel, hexane–EtOAc (4:1)] to give pure compound **25** as a yellow oil; yield: 1.5 g (87%); $[\alpha]_D$ –49 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.35–7.19 (m, 15 H, Ar-H), 5.31–5.28 (m, 2 H, H-2_C, H-4_C), 5.04 (br s, 1 H, H-1_B), 5.01–4.98 (m, 2 H, H-1_C, H-3_C), 4.80–4.59 (m, 7 H, H-1_A, 3 PhCH_2), 4.03–3.95 (m, 4 H, H-2_B, H-3_A, H-5_A, H-5_C), 3.79–3.76 (m, 1 H, H-5_B), 3.69–3.66 (m, 1 H, H-2_A), 3.63–3.20 (m, 2 H, H-3_B, H-4_A), 3.46 (t, *J* = 9.3 Hz, 1 H, H-4_B), 3.31 (s, 3 H, OCH_3), 2.08, 2.06, 1.97 (3 s, 9 H, 3 COCH_3), 1.29, 1.24, 1.10 (3 d, *J* = 6.6 Hz each, 9 H, 3 CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 169.7, 169.6, 169.5 (3 *C*OCH$_3$), 138.2–127.6 (Ar-C), 101.3 (C-1_B), 99.3 (C-1_C), 98.4 (C-1_A), 80.9 (C-4_A), 80.1 (C-4_B), 80.0 (C-5_A), 78.3 (C-5_C), 77.9 (C-2_A), 75.3 (2 C, Ph*C*H$_2$), 72.6 (Ph*C*H$_2$), 71.3 (C-3_A), 70.9 (C-3_C), 69.8 (C-2_C), 69.2 (C-4_C), 68.2 (C-3_B), 68.0 (C-5_B), 67.1 (C-2_B), 54.7 (O*C*H$_3$), 20.8, 20.7 (2 C) (3 CO*C*H$_3$), 18.1, 18.0, 17.4 (3 C*C*H$_3$).

MS (EI, 70 eV): 889.4 $[M + Na]^+$.

Anal. Calcd for $C_{46}H_{58}O_{16}$: C, 63.73; H, 6.74. Found: C, 63.55; H, 6.90.

Methyl (2,3,4-Tri-*O*-acetyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-(1→2)]-(4-*O*-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-*O*-benzyl-α-L-rhamnopyranoside (26)

A soln of monodeprotected derivative **25** (1.4 g, 1.61 mmol) and thioglycoside **20** (950 mg, 2.42 mmol) in anhyd CH_2Cl_2 (20 mL) was treated with 4-Å MS (2 g), and the soln was stirred under argon for 1 h. The mixture was cooled to –10 °C then NIS (650 mg, 2.88 mmol) and TMSOTf (10 μL) were added successively. The mixture was stirred at –10 °C for 1.5 h, diluted with CH_2Cl_2 (100 mL), and filtered through a Celite bed. The organic layer was washed successively with sat. aq $NaHCO_3$ and H_2O, dried (Na_2SO_4), and concentrated under reduced pressure. The crude product was purified by chromatography [silica gel, hexane–EtOAc (4:1)] to give pure compound **26** as a yellow oil; yield: 1.6 g (83%); $[\alpha]_D$ –12 (*c* 1.2, $CHCl_3$).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 7.40–7.22 (m, 15 H, Ar-H), 5.63 (d, *J* = 5.1 Hz, 1 H, H-2_D), 5.31–5.28 (m, 1 H, H-2_C), 5.21–5.17 (m, 2 H, H-3_C, H-3_D), 5.15 (br s, 1 H, H-2_C), 5.01–4.94 (m, 2 H, H-1_B, H-4_C), 4.88–4.60 (m, 8 H, H-1_A, H-1_D, 3 PhCH_2), 4.44–4.42 (m, 1 H, H-4_D), 4.22–4.17 (m, 3 H, H-3_B, H-6_{abD}), 4.04–4.01 (m, 1 H, H-3_A), 3.94–3.75 (m, 5 H, H-2_A, H-2_B, H-4_B, H-5_B, H-5_C), 3.69–3.52 (m, 3 H, H-4_A, H-5_A, H-5_D), 3.34 (s, 3 H, OCH_3), 2.14, 2.12, 2.10, 2.08, 2.04, 1.96 (6 s, 21 H, 7 COCH_3), 18.2, 18.0, 17.5 (3 d, *J* = 6.4 Hz each, 9 H, 3 CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 170.3, 169.7, 169.5, 169.4, 169.2, 168.8, 168.7 (7 *C*OCH$_3$), 138.2–127.4 (Ar-C), 98.6 (C-1_B), 98.0 (C-1_C), 97.2 (C-1_D), 96.9 (C-1_A), 81.1 (C-5_A), 80.0 (C-4_B), 78.3 (C-4_A), 74.7 (C-5_D), 73.3 (2 C, C-5_B, C-5_C), 72.9 (C-3_A), 72.4, 72.3, 70.8 (3 Ph*C*H$_2$), 69.9 (C-2_C), 69.7 (C-4_D), 69.5 (C-2_A), 68.9 (C-3_C), 68.2 (C-3_D), 68.1 (C-4_C), 68.0 (C-3_B), 67.1 (C-2_B), 67.0 (C-2_D), 63.2 (C-6_D), 54.6 (O*C*H$_3$), 20.8 (2 C), 20.7 (2 C), 20.5 (2 C), 20.4 (7 COCH_3), 18.3, 18.1, 17.6 (3 C*C*H$_3$).

MS (EI, 70 eV): 1219.4 $[M + Na]^+$.

Anal. Calcd for $C_{60}H_{76}O_{25}$: C, 60.19; H, 6.40. Found: C, 60.0; H, 6.54.

Methyl (α-L-Rhamnopyranosyl)-(1→3)-[(β-D-glucopyranosyl)-(1→2)]-(α-L-rhamnopyranosyl)-(1→3)-α-L-rhamnopyranoside (2)

20% $Pd(OH)_2$/C (250 mg) was added to a soln of protected derivative **26** (1.5 g, 1.25 mmol) in MeOH (20 mL), and the mixture was stirred under a positive pressure of H_2 at r.t. for 10 h. The mixture was filtered through a Celite bed, and the solvents were evaporated to dryness. A soln of the crude product in 0.1 M methanolic NaOMe (20 mL) was stirred at r.t. for 6 h and neutralized with Dowex 50W X-8 (H^+). The mixture was filtered and evaporated to dryness to give the target compound **2**, which was purified as an amorphous powder by chromatography [LH-20 Sephadex, MeOH-H_2O (4:1)]; yield: 625 mg (79%); $[\alpha]_D$ –20 (*c* 1.0, H_2O).

^{1}H NMR (300 MHz, $CDCl_3$): δ = 5.04 (br s, 1 H, H-1_A), 5.01 (br s, 1 H, H-1_C), 4.76 (d, *J* = 7.8 Hz, 1 H, H-1_D), 4.57 (br s, 1 H, H-1_B), 4.08–4.05 (m, 1 H, H-2_C), 4.0 (br s, 1 H, H-2_A), 3.89–3.65 (m, 9 H, H-2_B, H-3_A, H-3_B, H-3_C, H-3_D, H-4_B, H-5_A, H-5_C, H-6_{aD}), 3.63–3.49 (4 H, H-4_A, H-4_C, H-5_B, H-6_{bD}), 3.44–3.28 (6 H, H-2_D, H-4_D, H-5_D, OCH_3), 18.1, 18.0, 17.7 (m, 9 H, 3 CCH_3).

^{13}C NMR (75 MHz, $CDCl_3$): δ = 104.2 (C-1_D), 104.0 (C-1_A), 103.9 (C-1_C), 102.6 (C-1_B), 79.9 (C-5_A), 79.4 (C-5_C), 78.5 (C-5_D), 76.8 (C-2_D), 76.5 (C-3_D), 74.1 (C-4_B), 73.2 (C-2_B), 73.0 (C-3_B), 72.2 (C-2_C), 72.1 (C-3_C), 72.4 (C-4_D), 71.9 (C-2_A), 71.8 (C-3_A), 70.3 (C-4_A), 70.2 (C-4_C), 70.1 (C-5_B), 62.6 (C-6_D), 55.4 (OCH_3), 18.3, 18.2, 18.0 (3 CCH_3).

MS (EI, 70 eV): 655.2 $[M + Na]^+$.

Anal. Calcd for $C_{25}H_{44}O_{18}$: C, 47.47; H, 7.01. Found: C, 47.30; H, 7.20.

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