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First total synthesis of a pentasaccharide repeating unit of the *O*-antigen of *Hafnia alvei* PCM 1529

Chinmoy Mukherjee · Anup Kumar Misra

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Abstract A pentasaccharide repeating unit of the *O*-antigen of *Hafnia alvei* has been synthesized in a concise manner. High yielding glycosylation steps and minimum number of protecting group manipulation steps are the key features of this synthesis. A two-step, one-pot phase transfer oxidation protocol has been applied for the preparation of D-galacturonic acid.

Keywords Carbohydrates · Oligosaccharides · Total synthesis · Vaccines · *Hafnia alvei*

Extra intestinal invasive infections frequently occur in patients with chronic debilitating disorders after antibiotic treatment. In several cases of invasive infections with septicemia, endocarditis, meningitis, pneumonia, abscesses and surgical wound infections it has been found that *Hafnia alvei* was highly associated with them. *Hafnia alvei* is a motile, facultatively anaerobic, Gram-negative bacterium that belonging to the Enterobacteriaceae family [1, 2]. This is an opportunistic pathogen, which causes many nosocomial infections including wounds, enteric diarrhea, and urinary and respiratory tract disorders [3–5]. The pathogenicity of *H. alvei* is due to the endotoxic lipopolysaccharide (LPS, O-antigen), which is present in the O-specific polysaccharide chain (O-PS) attached via a core oligosaccharide to lipid A [6, 7]. From the immunochemical studies on LPSs, about 29 O-specific polysaccharides of *H. alvei* have been characterized [8, 9]. Based on the structure of O-antigens, *H. alvei* have been divided into 39 O-serotypes [10]. A new acidic *O*-antigenic pentasaccharide repeating unit of the *O*-antigen of *H. alvei* strain PCM 1529 has been reported by Katzenellenbogen *et al*. [11] (Fig. 1). Based on the genetic data, this particular strain has recently been reclassified and included in *Hafnia* from *Citrobacter* genus [12, 13].

A long-standing goal for medicinal chemists is to develop anti-microbial vaccines to induce immune response for the protection from future infections. Glycoconjugate vaccines are well known for their efficacy against bacterial infections. However, there are no commercially available carbohydrate vaccines in which the carbohydrate antigens are prepared synthetically. In the recent past several reports have appeared in the literature aiming to develop synthetic version of the polysaccharide based carbohydrate vaccines like *Haemophilus influenzae* type b [14] or towards the development of synthetic carbohydrate vaccine candidates against cancer, anthrax, malaria, leishmania etc [15–18]. In order to study the antigen-antibody interactions to induce a specific immune response in the host with the help of a carbohydrate vaccine candidate, *O*-antigenic oligosaccharide is an attractive target. Although, oligosaccharides can be isolated from the natural sources, efficient chemical synthetic strategies offer the advantage of having access to large quantities of oligosaccharides as well as analogues of the natural oligosaccharides. Most often, it is required to conjugate the oligosaccharide with a carrier protein through a spacer to produce an immune response. To serve this purpose, a temporary protecting group is recommended at the reducing terminus of the oligosaccharides, which can be removed to attach the oligosaccharide unit with the carrier protein. However, at the beginning it is very much essential to establish a concise chemical synthetic strategy for the synthesis of a target oligosaccharide, which can be

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C. Mukherjee · A. K. Misra (✉)
Medicinal and Process Chemistry Division,
Central Drug Research Institute,
Chattar Manzil Palace,
Lucknow 226 001, India
e-mail: akmisra69@rediffmail.com

Fig. 1 Structure of the repeating unit of the *O*-antigen of *Hafnia alvei* PCM 1529

→4)-α-D-GalpA-(1→3)-β-D-GlcpNAc-(1→2)-α-L-Rhap-(1→3)-α-L-Rhap-(1→
3
↑
1
α-L-Rhap

extended having a temporary protecting group at the reducing terminus. In this direction we report herein the first total synthesis of the pentasaccharide repeating unit of the *O*-antigen of *H. alvei* PCM 1529 as its methyl glycoside (Fig. 2) in which monosaccharides have been linked together sequentially in minimum number of steps.

The synthesis of the pentasaccharide **1** as its methyl glycoside from the suitably protected monosaccharide derivatives (Fig. 3) prepared from commercially available sugars using reported methodologies is presented in Scheme 1 and 2.

Methyl 2,4-di-*O*-benzyl-α-L-rhamnopyranoside (**2**) [19] prepared from L-rhamnose in five steps, was coupled with ethyl thioglycoside donor **3**, [20] prepared from L-rhamnose in seven steps, in the presence of *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf) [21] to furnish disaccharide derivative **7** in 80% yield. Presence of signals at δ 5.02 (br s, H-1′) and 4.62 (br s, H-1) in ^{1}H NMR and at δ 99.4 and 98.9 in the ^{13}C NMR confirmed the formation of disaccharide derivative **7**. Removal of acetyl groups from the disaccharide **7** under using sodium methoxide gave the disaccharide derivative **8** in quantitative yield. Selective acetylation [22] of compound **8** via the formation of an orthoester using triethylorthoacetate followed by hydrolysis of orthoacetate furnished disaccharide derivative **9** in 86% yield. Glycosylation of compound **9** with ethyl thioglycoside **4**, [23] prepared from L-rhamnose in four steps, in the presence of NIS-TMSOTf [21] furnished trisaccharide derivative **10** in 83% yield, which on deacetylation using sodium methoxide gave trisaccharide acceptor **11** in quantitative yield. Presence of signals at δ 5.19 (br s, H-1′), 5.15 (br s, H-1) and 4.71 (br s, H-1″) in ^{1}H NMR and at δ 100.1, 98.8 and 98.2 in the ^{13}C NMR confirmed the formation of trisaccharide derivative **10**. Exclusive formation of alpha-glycoside **10** was confirmed from the $J_{\text{H-1/C-1}}$ values (J=172, 171 and 168 Hz) in the partially coupled ^{13}C spectrum.

Glycosylation of the trisaccharide derivative **11** with ethyl thioglycoside **5**, [24] prepared from D-glucosamine hydrochloride in six steps, in the presence of NIS-TMSOTf furnished tetrasaccharide derivative **12** in 78% yield. Presence of signals at δ 5.77 (d, J=8.2 Hz), 5.03 (br s), 4.95 (br s), 4.59 (br s) in the ^{1}H NMR and at δ 101.9 (PhCH), 101.3 (C-1‴), 100.0 (C-1″), 98.7 (C-1), 98.0 (C-1′) in the ^{13}C NMR spectrum confirmed the formation of compound **12**. Removal of *N*-phthalimido group [25] from the tetrasaccharide derivative **12** using hydrazine hydrate followed by *N*-acetylation and *O*-deacetylation afforded tetrasaccharide acceptor **13** in 77% yield. At this point, it was envisioned that incorporation of a suitably protected D-galactosyl moiety to the compound **13** through an alpha-linkage and further oxidation of the product could produce the required D-galacturonic acid containing pentasaccharide derivative. In order to condense compound **13** via an alpha-linkage with ethyl thioglycoside **6**, prepared from D-galactose, several trials have been made using NIS-TfOH or DMTST as thioglycoside activators in a variety of solvents. Unfortunately, a very low yield was obtained in every case. However, use of $CuBr_2$-Bu_4NBr-AgOTf [26] in DMF-$(CH_2Cl)_2$ as solvent furnished pentasaccharide derivative **14** in 65% yield. Presence of signals at δ 5.58 (d, J= 3.2 Hz), 5.14 (br s), 5.11 (br s), 4.82 (d, J=8.2 Hz), 4.68 (br s) in ^{1}H NMR and at δ 102.3 (PhCH), 101.9 (C-1‴), 101.8

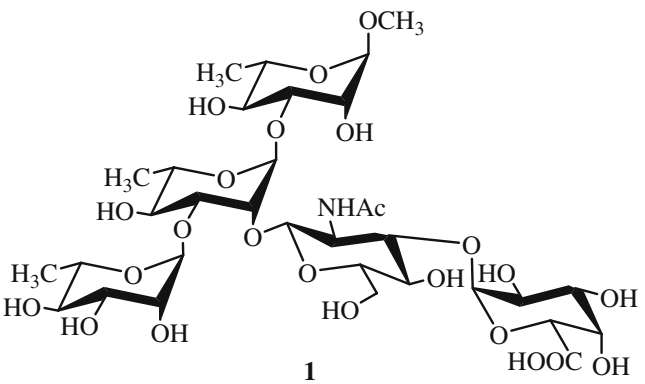


Fig. 2 Structure of the synthesized pentasaccharide as its methyl glycoside (**1**)

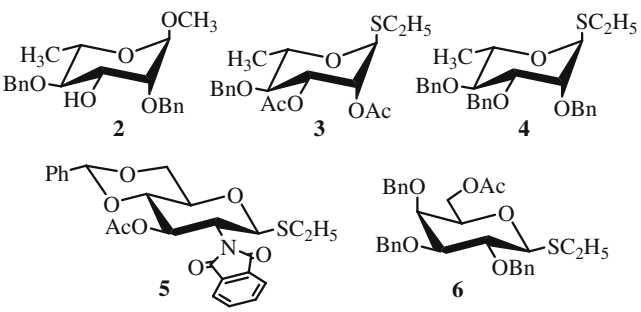
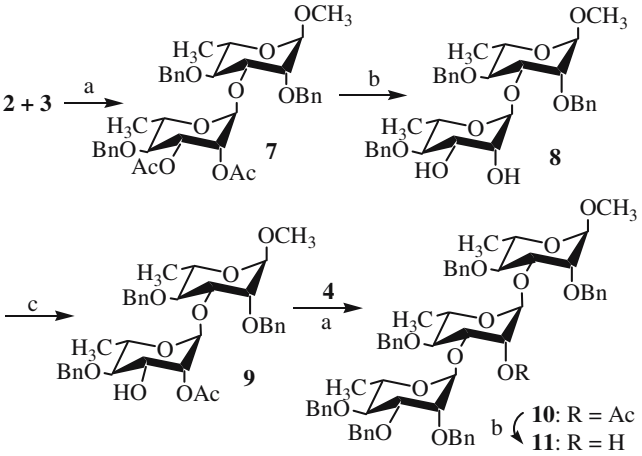


Fig. 3 Suitably functionalized monosaccharide derivatives used for the synthesis of pentasaccharide **1**

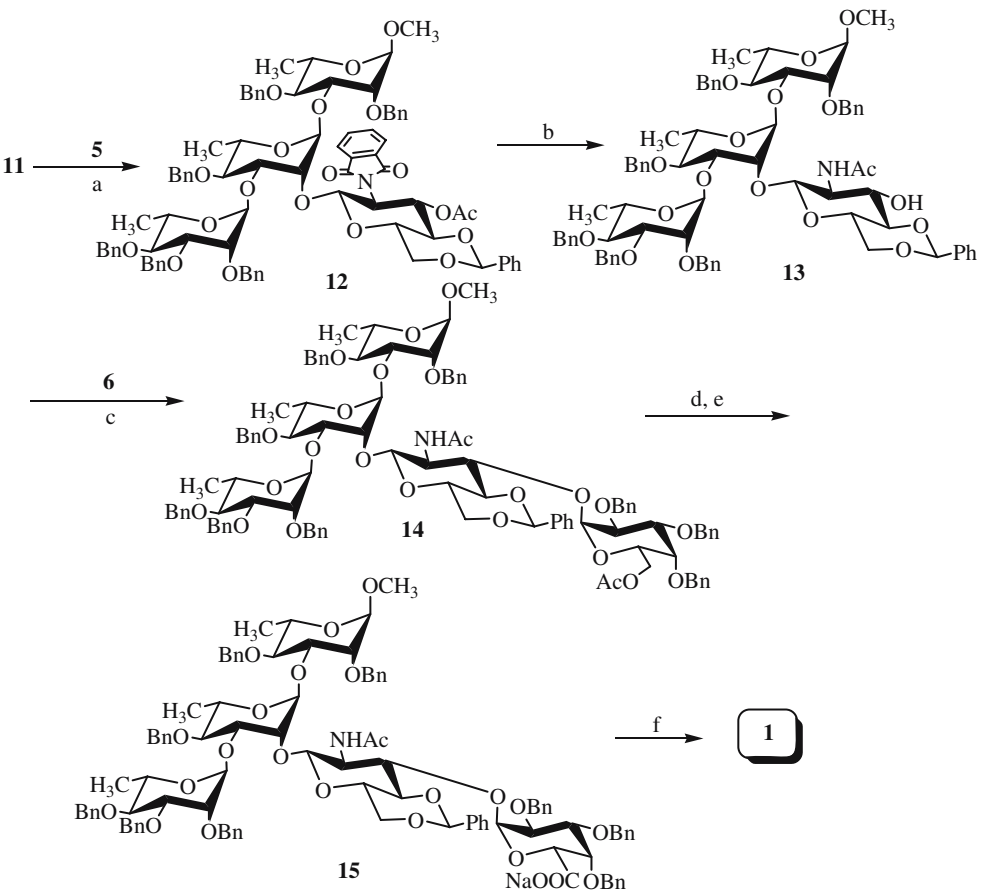


Scheme 1 Reagents: (a) *N*-Iodosuccinimide, TMSOTf, MS 4 Å, CH_2Cl_2, −40°C, 45 min., 80% for **7** and 83% for **10**; (b) CH_3ONa, CH_3OH, r t, 2 h, quantitative; (c) (1) triethyl orthoacetate, DMF, *p*-TsOH, r t, 2 h; (2) 80% aq. AcOH, r t, 1 h, 86%

(C-1‴), 101.4 (C-1″), 101.0 (C-1) and 99.9 (C-1′) in the ^{13}C NMR confirmed the formation of the compound **14**. Although a number of useful methodologies such as Swern oxidation [27] followed by sodium chlorite oxidation have been applied for the transformation of D-galactosyl moiety into the D-galacturonic acid, none of them furnished satisfactory yield. Finally, application of a two-step one-pot phase transfer reaction condition using TEMPO [28–30] provided satisfactory yield of the oxidized product. Thus removal of the acetyl group from the pentasaccharide derivative **14** using sodium methoxide followed by TEMPO mediated oxidation of the primary hydroxyl group furnished pentasaccharide derivative **15** in 75% yield. Global deprotection of the pentasaccharide acid derivative **15** under hydrogenolysis [31] using 20% Pd $(OH)_2$-C afforded pure pentasaccharide **1** as methyl glycoside in 74% yield. Presence of signals at δ 5.28 (br s, 1 H), 5.21 (br s, 1 H), 5.04 (br s, 1 H), 4.65 (br s, 1 H) and 4.56 (d, J=7.6 Hz, 1 H) in the ^1H NMR and at δ 103.5 (C-1‴), 102.9 (C-1), 101.8 (C-1″), 101.4 (C-1‴), 101.2 (C-1′) in the ^{13}C NMR confirmed the presence of required glycosyl linkages in the target pentasaccharide **1**.

Conclusion

In summary, the synthesis of a alpha-D-galacturonic acid containing pentasaccharide repeating unit of the *O*-antigen of *Hafnia alvei* as its methyl glycoside has been achieved in a concise manner. All glycosylation steps were high yielding and minimum number of protecting group manipulation steps were involved in the synthesis. Application of



Scheme 2 Reagents: (a) *N*-Iodosuccinimide, TMSOTf, MS 4 Å, CH_2Cl_2, −40°C, 45 min, 78%; (b) (1) hydrazine hydrate, C_2H_5OH, 80°C, 5 h, (2) acetic anhydride, pyridine, r t, 1 h, (3) CH_3ONa, CH_3OH, r t, 2 h, 77% in three steps; (c) $CuBr_2$, tetrabutylammonium bromide (TBAB), silver trifluoromethanesulfonate, MS 4 Å, 1,2-dichloroethane-DMF (5:1, *v*/*v*), 72 h, r t, 65%; (d) CH_3ONa, CH_3OH, r t, 3 h, quantitative; (e) TEMPO, NaBr, NaOCl, TBAB, $NaClO_2$, 2-methyl-but-2-ene, NaH_2PO_4, $NaHCO_3$, CH_2Cl_2, 0°C-r t, 5 h, 75%; (f) H_2, 20% $Pd(OH)_2$-C, CH_3OH, r t, 24 h, 74%

a two-step, one-pot phase transfer oxidation protocol is an added advantage of this synthesis.

Experimental section

General methods All the reactions were monitored by thin layer chromatography over silica gel coated TLC plates. The spots on TLC were visualized by warming ceric sulphate (2% $Ce(SO_4)_2$ in 2N H_2SO_4) sprayed plates in hot plate. Silica gel 230–400 mesh was used for column chromatography. ^1H and ^{13}C NMR, 2DCOSY, HMQC spectra were recorded on Brucker Advance DPX 200 and 300 MHz using $CDCl_3$ and D_2O as solvents and TMS as internal reference unless stated otherwise. Chemical shift value is expressed in δ ppm. ESI-MS were recorded on a MICROMASS QUTTRO 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 are used in many reactions.

Ethyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio-β-D-galactopyranoside (6) To an ice-cooled suspension of D-galactose (3.6 g, 20.0 mmol) in Ac_2O (9.6 mL, 102.0 mmol) was added $BF_3{\cdot}OEt_2$ (3.8 mL, 30.0 mmol) and the mixture was allowed to stir for 5.0 min. After completion (TLC, hexane–EtOAc 1:1), ethanethiol (2.3 mL; 30.7 mmol) was added and the reaction mixture was allowed to stir at 5°C for another 5 h. The reaction was quenched by addition of aq $NaHCO_3$ and extracted with CH_2Cl_2 (100 mL). The organic layer was washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. Column chromatography of the crude product over SiO_2 using hexane–EtOAc (3:1) as the eluant furnished pure ethyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside, which was crystallized from Et_2O–hexane (7.0 g, 90%); A solution of the thioglycoside in 0.1 M CH_3ONa in CH_3OH (100 mL) was allowed to stir at room temperature for 5 h and neutralized with Amberlite IR-120 (H^+) resin. The reaction mixture was filtered and concentrated under reduced pressure. To a solution of ethyl 1-thio-β-D-galactopyranoside thus obtained in pyridine (50 mL) was added triphenylmethyl chloride (10 g, 35.8 mmol) and the reaction mixture was allowed to stir at 70°C for 8 h. The solvents were removed under reduced pressure to give a crude product. To a solution of the crude product in DMF (50 mL) were added benzyl bromide (8.0 mL; 67 mmol), powdered NaOH (5 g, 125 mmol) and tetrabutylammonium bromide (100 mg) and the reaction mixture was allowed to stir at room temperature for 8 h. The solvents were removed under reduced pressure to give the crude product. To a solution of the crude product in CH_2Cl_2 (100 mL) was added trifluoroacetic acid (2 mL) and H_2O (2 mL) and the reaction mixture was allowed to stir at 15°C for 1 h. The solvents were removed under reduced pressure and the crude product was acetylated by stirring the solution of the crude product in Ac_2O-pyridine (20 mL; 1:1, *v*/*v*) at room temperature. The solvents were removed under reduced pressure and the crude product was purified over SiO_2 using hexane–EtOAc (7:1) as eluant to give pure compound **6** (6.8 g, 70%) as white solid; m.p. 78°C; $[\alpha]_D^{25}$ − 16.5 (*c* 1.5, $CHCl_3$); IR (KBr): 2365, 1740, 1597, 1359, 1226, 1049, 735, 695 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.42–7.28 (m, 15 H, aromatic protons), 5.02 (d, *J*=11.7 Hz, 1 H, PhC*H*$_2$), 4.94 (d, *J*=11.7 Hz, 1 H, PhC*H*$_2$), 4.85 (d, *J*=11.7 Hz, 1 H, PhC*H*$_2$), 4.79 (d, *J*=11.8 Hz, 1 H, PhC*H*$_2$), 4.76 (d, *J*=11.8 Hz, 1 H, PhC*H*$_2$), 4.70 (d, *J*=11.7 Hz, 1 H, PhC*H*$_2$), 4.44 (d, *J*=9.6 Hz, 1 H, H-1), 4.24 (dd, *J*=11.2 and 6.7 Hz, 1 H-6$_a$), 4.08 (dd, *J*=11.2 and 6.7 Hz, 1 H, H-6$_b$), 3.89 (t, *J*=9.4 and 9.4 Hz, 1 H, H-2), 3.83 (br s, 1 H, H-4), 3.58 (dd, *J*=9.4 and 2.3 Hz, 1 H, H-3), 3.56-3.55 (m, 1 H, H-5), 2.81-2.71 (m, 2 H, SC*H*$_2$CH$_3$), 2.0 (s, 3 H, COC*H*$_3$), 1.34 (t, *J*=7.4 Hz, 3 H, SCH$_2$C*H*$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.0 (*C*OCH$_3$), 138.1-127.4 (aromatic carbons), 85.2 (C-1), 84.0, 78.2, 75.8, 75.6 (Ph*C*H$_2$), 74.1 (Ph*C*H$_2$), 73.2 (Ph*C*H$_2$), 73.0, 63.2 (C-6), 24.6 (S*C*H$_2$CH$_3$), 20.6 (CO*C*H$_3$), 15.0 (SCH$_2$*C*H$_3$); ESI-MS: *m*/*z*=559.3 $[M+ Na]^+$; Anal. Calcd. for $C_{31}H_{36}O_6S$ (536.2): C, 69.38; H, 6.76; found: C, 69.17; H, 6.92.

Methyl (2,3-di-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (7) To a solution of compound **2** (3.6 g, 10.0 mmol) and compound **3** (4.6 g, 12.0 mmol) in anhydrous CH_2Cl_2 (50 mL) was added freshly activated powdered MS 4 Å (4 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. After cooling the reaction mixture to −40°C, *N*-iodosuccinimide (NIS; 3.3 g, 14.6 mmol) was added to it followed by trimethylsilyl trifluoromethanesulfonate (TMSOTf; 50 μl, 0.27 mmol) and the reaction mixture was allowed to stir at −40°C for 45 min. The reaction mixture was quenched by the addition of triethylamine (0.5 mL), filtered through a Celite® bed and washed with CH_2Cl_2 (3×20 mL). The organic layer was washed successively with 10% aq. $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to afford pure compound **7** (5.42 g, 80%) as yellow oil; $[\alpha]_D^{25}$ − 47.7 (*c* 1.0, $CHCl_3$); IR (neat): 2927, 1750, 1599, 1455, 1367, 1242, 1133, 1066, 745 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.44–7.19 (m, 15 H, aromatic protons), 5.43–5.04 (dd, *J*=9.3, 1.8 Hz, 1 H, H-3′), 5.37-5.33 (m, 1 H, H-2′), 5.02 (br s, 1 H, H-1′), 4.85-4.81 (d, *J*=

11.1 Hz, 1 H, PhCH_2), 4.77-4.75 (m, 2 H, PhCH_2), 4.72-4.68 (d, J=11.1 Hz, 1 H, PhCH_2), 4.64-4.60 (m, 3 H, PhCH_2 and H-1), 4.04-4.03 (m, 1 H, H-2), 3.90-3.81 (m, 1 H, H-3), 3.62-3.58 (m, 3 H, H-4, H-5 and H-5′), 3.47-3.41 (t, J=9.9, 9.9 Hz, 1 H, H-4′), 3.34 (s, 3 H, OCH_3), 2.05, 1.98 (2 s, 6 H, 2 COCH_3), 1.35-1.32 (d, J=6.3 Hz, 3 H, CH_3), 1.29–1.27 (d, J=6.0 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 169.6 (2 C, 2 COCH$_3$), 138.7–127.8 (aromatic carbons), 99.4 (C-1), 98.9 (C-1′), 81.3, 79.2, 78.1, 78.0, 75.6 (PhCH$_2$), 75.1 (PhCH$_2$), 73.1 (PhCH$_2$), 72.0, 70.8, 68.5 (2 C), 54.9 (OCH$_3$), 21.2, 21.1 (2 COCH$_3$), 18.4 (2 C, 2 CH$_3$); ESI-MS: m/z=701.4 [M+Na]$^+$; Anal. Calcd. for $C_{38}H_{46}O_{11}$ (678.3): C, 67.24; H, 6.83; found: C, 67.0; H, 7.05.

Methyl (4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (8) To a solution of **7** (5.1 g, 7.5 mmol) in MeOH (30 mL) was added solid sodium methoxide until the pH of the solution reached ~10. The reaction mixture was allowed to stir at room temperature for 2 h and neutralized with Dowex-50W X8 (H^+). The reaction mixture was filtered and evaporated to dryness to give pure compound **8** (4.4 g, quantitative) as syrup. $[\alpha]_D^{25}$ − 45.6 (c 1.0, $CHCl_3$); IR (neat): 2927, 2365, 1597, 1456, 1383, 1353, 1102, 1065, 745, 669 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.41–7.22 (m, 15 H, aromatic protons), 5.07 (br s, 1 H, H-1′), 4.83–4.70 (m, 4 H, PhCH_2), 4.68–4.60 (m, 2 H, H-1 and PhCH_2), 4.64 (br s, 1 H, PhCH_2), 4.06–4.02 (dd, J=9.0, 3.0 Hz, 1 H, H-3), 3.96–3.92 (dd, J=9.0, 3.0 Hz, 1 H, H-3′), 3.90–3.87 (m, 1 H, H-2′), 3.85–3.80 (dd, J=9.3, 6.3 Hz, 1 H, H-4), 3.72–3.70 (m, 1 H, H-2), 3.68–3.56 (m, 2 H, H-5 and H-5′), 3.36–3.34 (m, 1 H, H-4′), 3.33 (s, 3 H, OCH_3), 1.34–1.32 (d, J=6.0 Hz, 3 H, CH_3), 1.30–1.28 (d, J=6.3 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 139.1–128.1 (aromatic carbons), 101.8 (C-1), 98.9 (C-1′), 82.0, 81.4, 78.4, 78.2, 75.0 (PhCH$_2$), 74.8 (PhCH$_2$), 73.5 (PhCH$_2$), 72.0, 71.8, 68.4, 68.2, 55.0 (OCH$_3$), 18.5, 18.4 (2 CH$_3$); ESI-MS: m/z=617.4 [M+Na]$^+$; Anal. Calcd. for $C_{34}H_{42}O_9$ (594.3): C, 68.67; H, 7.12; found: C, 68.45; H, 7.38.

Methyl (2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (9) To the solutions of compound **8** (4.1 g, 6.9 mmol) in dry DMF (20 mL) were added triethyl orthoacetate (6.3 mL, 34.5 mmol) and p-TsOH (200 mg) and the reaction mixture was allowed to stir at room temperature for 2 h. After complete consumption of the starting material, the reaction mixture was neutralized with triethylamine (0.5 mL) and solvents removed under reduced pressure. A solution of the crude mass in 80% aq. AcOH (30 mL) was allowed to stir at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure and co-evaporated with toluene (3x 20 mL) to give the crude product, which was purified over SiO_2 using hexane–EtOAc (5:1) as eluant to give pure compound **9** (3.8 g, 86%) as syrup. $[\alpha]_D^{25}$ − 56.4 (c 1.5, $CHCl_3$); IR (neat): 2362, 1741, 1596, 1460, 1352, 1064, 755 cm^{-1}; ^{1}H NMR ($CDCl_3$, 200 MHz): δ 7.35–7.22 (m, 15 H, aromatic protons), 5.18 (br s, 1 H, H-2′), 4.98 (br s, 1 H, H-1′), 4.86–4.74 (m, 2 H, PhCH_2), 4.70–4.52 (m, 5 H, H-1 and PhCH_2), 4.09–3.96 (m, 2 H, H-3 and H-3′), 3.79–3.71 (m, 1 H, H-4), 3.65–3.50 (m, 3 H, H-2, H-5 and H-5′), 3.31–3.22 (m, 1 H, H-4′), 3.27 (s, 3 H, OCH_3), 2.04 (s, 3 H, COCH_3), 1.32–1.23 (m, 6 H, 2 CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.8 (COCH$_3$), 138.1–127.0 (aromatic carbons), 100.0 (C-1), 98.3 (C-1′), 80.9, 80.6, 77.5, 77.4, 75.0 (PhCH$_2$), 74.8 (PhCH$_2$), 72.6 (PhCH$_2$), 72.4, 70.3 (C-2′), 67.9, 67.8, 54.1 (OCH$_3$), 20.4 (COCH$_3$), 17.6 (2 C, 2 CH$_3$); ESI-MS: m/z=659.4 [M+Na]$^+$; Anal. Calcd. for $C_{36}H_{44}O_{10}$ (636.3): C, 67.91; H, 6.97; found: C, 67.70; H, 7.20.

Methyl (2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(2-O-acetyl-4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (10) To a solution of compound **9** (3.5 g, 5.5 mmol) and compound **4** (3.2 g, 6.7 mmol) in anhydrous CH_2Cl_2 (30 mL) was added freshly activated powdered MS 4 Å (4 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. After cooling the reaction mixture to −40 °C, NIS (1.8 g, 8.0 mmol) was added to it followed by TMSOTf (25 μl, 0.13 mmol) and the reaction mixture was allowed to stir at −40°C for 45 min. The reaction mixture was quenched by the addition of triethylamine (0.2 mL), filtered through a Celite® bed and washed with CH_2Cl_2 (3× 25 mL). The organic layer was washed successively with aq. $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (3:1) as eluant to afford pure compound **10** (4.8 g, 83%) as syrup; $[\alpha]_D^{25}$ − 37.5 (c 1.0, $CHCl_3$); IR (neat): 2924, 1744, 1596, 1455, 1352, 1237, 1068, 746, 699 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.54–7.31 (m, 30 H, aromatic protons), 5.44 (br s, 1 H, H-2′), 5.19 (br s, 1 H, H-1′), 5.15 (br s, 1 H, H-1), 5.09–4.92 (m, 2 H, PhCH_2), 4.89–4.72 (m, 4 H, PhCH_2), 4.71 (br s, 1 H, H-1″), 4.71–4.63 (m, 5 H, PhCH_2), 4.62–4.58 (m, 2 H, PhCH_2), 4.30–4.27 (m, 1 H, H-3), 4.18–4.15 (m, 1 H, H-3″), 3.97–3.91 (m, 1 H, H-4), 3.90–3.87 (m, 1 H, H-3′), 3.80–3.75 (m, 3 H, H-2, H-2″ and H-4″), 3.74–3.67 (m, 3 H, H-5, H-5′ and H-5″), 3.54–3.48 (m, 1 H, H-4′), 3.42 (s, 3 H, OCH_3), 2.18 (s, 3 H, COCH_3), 1.39–1.34 (3 d, J=6.0 Hz, 9 H, 3 CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.3 (COCH$_3$), 138.7–126.8 (aromatic carbons), 100.1 (C-1″), 98.8 (C-1), 98.2 (C-1′), 80.6, 80.4, 80.2, 79.6, 78.1 (2 C), 77.1, 75.7, 75.2 (PhCH$_2$), 74.8 (PhCH$_2$), 74.5 (PhCH$_2$), 72.5 (PhCH$_2$), 72.4 (2 C, PhCH$_2$ and C-2″), 71.9 (PhCH$_2$), 68.9, 68.1, 67.9, 54.4

(OCH_3), 20.7 ($COCH_3$), 18.0 (2 C, 2 CH_3), 17.9 (CH_3); ESI-MS: m/z=1075.6 $[M+Na]^+$; Anal. Calcd. for $C_{63}H_{72}O_{14}$ (1052.5): C, 71.84; H, 6.89; found: C, 71.62; H, 7.02.

Methyl (2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-(4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (11) To a solution of compound **10** (4.6 g, 4.4 mmol) in MeOH (25 mL) was added solid sodium methoxide until the pH of the solution reached to ~10. The reaction mixture was allowed to stir at room temperature for 2 h, neutralized with Dowex-50W X-8 (H^+), filtered and evaporated to dryness to give pure compound **11** (4.4 g, quantitative) as syrup. $[\alpha]_D^{25}$ − 30.3 (*c* 1.0, $CHCl_3$); IR (neat): 2926, 1597, 1458, 1352, 1076, 1064, 742, 699 cm^{-1}; ^1H NMR ($CDCl_3$, 200 MHz): δ 7.38–7.16 (m, 30 H, aromatic protons), 5.03 (br s, 1 H, H-1′), 5.00 (br s, 1 H, H-1), 4.92–4.86 (d, J=11.0 Hz, 1 H, PhCH_2), 4.76–4.52 (m, 10 H, PhCH_2 and H–1″), 4.44–4.42 (m, 2 H, PhCH_2), 3.98–3.90 (m, 3 H, H-3, H-3′ and H-3″), 3.80–3.50 (m, 8 H, H-2, H-2′, H-2″, H-4, H-4″, H-5, H-5′ and H-5″), 3.37–3.32 (m, 1 H, H-4′), 3.30 (s, 3 H, OCH_3), 1.29–1.19 (3 d, J=6.3, 6.3 and 6.0 Hz, 9 H, 3 CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 138.6–138.1 (aromatic carbons), 128.4–126.9 (aromatic carbons), 101.2 (C-1″), 99.8 (C-1), 98.4 (C-1′), 80.9, 80.3, 80.2, 79.6, 79.3, 78.2, 78.1, 75.7, 75.3 (PhCH$_2$), 75.2 (PhCH$_2$), 74.6 (PhCH$_2$), 72.6 (PhCH$_2$), 72.5, 72.1 (PhCH$_2$), 71.2 (PhCH$_2$), 68.9, 68.0 (2 C), 54.5 (OCH$_3$), 18.1 (2 C, 2 CH$_3$), 18.0 (CH$_3$); ESI-MS: m/z= 1033.6 $[M+Na]^+$; Anal. Calcd. for $C_{61}H_{70}O_{13}$ (1010.5): C, 72.45; H, 6.98; found: C, 72.21; H, 7.22.

Methyl (2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranosyl)-(1→2)]-(4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (12) To a solution of compound **11** (3.5 g, 3.5 mmol) and compound **5** (2.0 g, 4.1 mmol) in anhydrous CH_2Cl_2 (30 mL) was added activated powdered MS 4 Å (4 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. After cooling the reaction mixture to −40 °C, NIS (1.1 g, 4.9 mmol) was added to it followed by TMSOTf (25 μl, 0.13 mmol) and the reaction mixture was allowed to stir at −40°C for 45 min. The reaction mixture was quenched by the addition of triethylamine (0.2 mL), filtered through a Celite® bed and washed with CH_2Cl_2 (3×25 mL). The organic layer was washed successively with aq. $Na_2S_2O_3$ and water, dried (Na_2SO_4) and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (4:1) as eluant to afford pure compound **12** (3.9 g, 78%) as white solid; m.p. 85°C; $[\alpha]_D^{25}$ − 9.0 (*c* 1.0, $CHCl_3$); IR (KBr): 2926, 2367, 1720, 1598, 1459, 1383, 1354, 1225, 1102., 737 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.46–6.93 (m, 39 H, aromatic protons), 5.94–5.87 (t, J=9.3, 9.3 Hz, 1 H, H-3‴), 5.77 (d, J=8.2 Hz, 1 H, H-1‴), 5.40 (s, 1 H, PhCH), 5.03 (br s, 1 H, H-1′), 4.95 (br s, 1 H, H-1), 4.93–4.89 (m, 3 H, PhCH_2), 4.78–4.74 (d, J=11.0 Hz, 1 H, PhCH_2), 4.69–4.56 (m, 4 H, PhCH_2 and H-1″), 4.52–4.48 (d, J=11.0 Hz, 1 H, PhCH_2), 4.27–4.18 (m, 4 H, PhCH_2), 4.13 (br s, 1 H, H-2′), 4.00–3.91 (m, 3 H, H-2‴, H-3″ and H-4‴), 3.77–3.73 (m, 1 H, H-3′), 3.67–3.50 (m, 10 H, H-2, H-3, H-4, H-2″, H-5, H-5′, H-5″, H-5‴, H-6_{ab}‴), 3.43–3.35 (m, 2 H, H-4′ and H-4″), 3.28 (s, 3 H, OCH_3), 1.92 (s, 3 H, COCH_3), 1.25–1.22 (m, 6 H, 2 CH_3), 1.18–1.16 (d, J=6.1 Hz, 3 H, CH_3); ^{13}C NMR ($CDCl_3$, 50 MHz): δ 170.0 (*C*OCH$_3$), 167.4 (2 C, 2 *C*O, Phth), 139.6–123.9 (aromatic carbons), 101.9 (Ph*C*H), 101.3 (C-1‴), 100.0 (C-1″), 98.7 (C-1), 98.0 (C-1′), 82.3, 81.0, 80.8, 80.3, 79.8, 78.9, 78.6, 76.2, 76.0, 75.6 (2 C, Ph*C*H$_2$), 75.5 (Ph*C*H$_2$), 74.0, 72.9 (Ph*C*H$_2$), 72.6 (Ph*C*H$_2$), 72.5 (Ph*C*H$_2$), 69.8, 69.3 (2 C), 68.6 (C-6″), 68.4, 66.3, 56.2 (O*C*H$_3$), 54.9 (C-2‴), 21.0 (CO*C*H$_3$), 18.5, 18.4, 18.2 (3 *C*H$_3$); ESI-MS: m/z=1454.4 $[M+Na]^+$; Anal. Calcd. For $C_{84}H_{89}NO_{20}$ (1431.6): C, 70.42; H, 6.26; found: C, 70.18; H, 6.47.

Methyl (2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)]-(4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (13) To the solution of compound **12** (3.0 g, 2.1 mmol) in ethanol (50 mL) was added hydrazine hydrate (10 mL) and the reaction mixture was allowed to stir at 80°C for 5 h. The solvents were evaporated off under reduced pressure and co-evaporated with toluene (3×20 mL). A solution of the crude product in Ac_2O and pyridine (20 mL, 1:1, v/v) was stirred at room temperature for 1 h. The solvents were removed under reduced pressure and co-evaporated with toluene (3× 20 mL). To a solution of the crude acetylated product in MeOH (25 mL) was added solid sodium methoxide until the pH of the solution reached ~10. The reaction mixture was allowed to stir at room temperature for 2 h, neutralized with Dowex 50 W-X8 (H^+) and filtered. The reaction mixture was concentrated under reduced pressure to give crude product, which was purified over SiO_2 using hexane–EtOAc (1:1) as eluant to give pure compound **13** (2.1 g, 77%) as white solid; m.p. 79°C; $[\alpha]_D^{25}$ − 38.7 (*c* 1.0, $CHCl_3$); IR (KBr): 2928, 2370, 1657, 1597, 1456, 1356, 1208, 1089, 740, 697 cm^{-1}; ^1H NMR ($CDCl_3$, 300 MHz): δ 7.47–7.16 (m, 35 H, aromatic protons), 5.44 (s, 1 H, PhCH), 5.25–5.24 (d, J=1.0 Hz, 1 H, H-1′), 5.16 (s, 1 H, H-1), 4.96–4.92 (d, J=11.0 Hz, 1 H, PhCH_2), 4.83–4.80 (d, J=11.1 Hz, 1 H, PhCH_2), 4.79–4.75 (d, J=12.3 Hz, 1 H, PhCH_2), 4.73 (d, J=7.8 Hz, 1 H, H-1‴), 4.72–4.68 (m, 3 H, PhCH_2), 4.64–4.55 (m, 5 H, PhCH_2 and H-1″), 4.49–4.45 (d, J=12.3 Hz, 1 H, PhCH_2), 4.41–4.37 (d, J= 12.3 Hz, 1 H, PhCH_2), 4.33–4.29 (m, 1 H, H-2′), 4.05–

4.01 (m, 2 H, H-2‴ and H-3″), 3.92–3.78 (m, 3 H, H-3, H-3‴ and H-4‴), 3.76–3.70 (m, 3 H, H-2, H-2″ and H-5), 3.68–3.57 (m, 5 H, H-3, H-4, H-5′ and H-6_{ab}‴), 3.49–3.41 (m, 3 H, H-4″, H-5″ and H-5‴), 3.33 (s, 3 H, OCH_3), 3.28–3.20 (m, 1 H, H-4′), 1.83 (s, 3 H, NHAc), 1.31–1.25 (3 d, J=6.0, 6.0 and 6.0 Hz, 9 H, 3 CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 171.3 (NH*C*OCH$_3$), 138.3–126.3 (aromatic carbons), 101.9 (Ph*C*H), 101.6 (C-1‴), 101.1 (C-1″), 99.9 (C-1), 98.1 (C-1′), 81.5, 81.1, 80.4, 80.2, 79.5, 79.1, 78.3, 78.2, 77.2, 75.3 (2 C, 2 Ph*C*H$_2$), 75.1, 74.5 (Ph*C*H$_2$), 72.5 (Ph*C*H$_2$), 72.4 (Ph*C*H$_2$), 71.8 (Ph*C*H$_2$), 71.5, 69.1, 68.8, 68.2 (C-6‴), 67.9, 66.4, 58.5 (O*C*H$_3$), 54.6 (C-2‴), 23.4 (NHCO*C*H$_3$), 18.2, 18.0, 17.9 (3 *C*H$_3$); ESI-MS: m/z=1324.6 $[M+Na]^+$; Anal. Calcd. for $C_{76}H_{87}NO_{18}$ (1301.6): C, 70.08; H, 6.73; found: C, 69.84; H, 6.97.

Methyl (2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-galactopyranosyl)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)]-(4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (14) To a solution of compound **13** (1.9 g, 1.4 mmol) and compound **6** (940 mg, 1.7 mmol) in 1,2-dichloroethane and *N*,*N*-dimethylformamide (12 mL; 5:1; v/v) was added activated powdered MS 4 Å (4 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. To the reaction mixture were added anhydrous $CuBr_2$ (450 mg, 2.0 mmol), TBAB (130 mg, 0.4 mmol) and AgOTf (465 mg, 1.8 mmol) and allowed to stir under darkness at room temperature for 72 h. The reaction mixture was filtered through a Celite® bed and washed with CH_2Cl_2 (4× 20 mL). The organic layer was washed with satd. aq. $NaHCO_3$ and water, dried (Na_2SO_4) and evaporated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (1:1) as eluant to give pure compound **14** (1.6 g, 65%) as white solid; m.p. 75°C; $[\alpha]_D^{25}$ + 3.6 (c 1.0, $CHCl_3$); IR (KBr): 2371, 1741, 1597, 1455, 1355, 1093, 738, 697 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.39–7.10 (m, 50 H, aromatic protons), 5.58 (d, J=3.2 Hz, 1 H, H-1‴), 5.16 (s, 1 H, PhC*H*), 5.14 (br s, 1 H, H-1′), 5.11 (br s, 1 H, H-1), 4.96–4.85 (m, 3 H, PhCH_2), 4.82 (d, J=8.2 Hz, 1 H, H-1‴), 4.76–4.72 (m, 3 H, PhCH_2), 4.70–4.66 (m, 3 H, PhCH_2 and H-1″), 4.65–4.60 (m, 4 H, PhCH_2), 4.58–4.42 (m, 5 H, PhCH_2), 4.40–4.36 (m, 2 H, PhCH_2 and H-4‴), 4.30–4.18 (m, 2 H, H-2′ and H-3″), 4.10–3.90 (m, 7 H, H-2, H-2″, H-2‴, H-3′, H-3‴, H-3‴′ and H-4‴), 3.88–3.84 (m, 1 H, H-2‴′), 3.82–3.75 (m, 4 H, H-3, H-5′, H-6_{ab}‴′), 3.74–3.65 (m, 3 H, H-4 and H-6_{ab}‴), 3.64–3.50 (m, 4 H, H-4″, H-5, H-5″ and H-5‴), 3.45–3.30 (m, 2 H, H-4′ and H-5‴′), 3.29 (s, 3 H, OCH_3), 1.86, 1.83 (2 s, 6 H, 2 COCH_3), 1.28–1.20 (m, 9 H, 3 CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz): δ 169.8 (2 C, *C*OCH$_3$ and NH*C*OCH$_3$), 138.8–126.3 (aromatic carbons), 102.3 (Ph*C*H), 101.9 (C-1‴), 101.8 (C-1‴′), 101.4 (C-1″), 101.0 (C-1), 99.9 (C-1′), 82.1, 80.0, 79.7, 78.6, 78.3, 77.1, 77.0, 76.0, 75.1, 74.3, 74.1 (2 C, 2 Ph*C*H$_2$), 73.6, 73.5 (Ph*C*H$_2$), 72.6 (2 C; 2 Ph*C*H$_2$), 71.9, 71.6 (Ph*C*H$_2$), 71.5 (Ph*C*H$_2$), 71.2, 71.1 (Ph*C*H$_2$), 70.5 (Ph*C*H$_2$), 70.2, 69.7, 69.6 (C-6‴′), 67.2 (2 C), 66.5 (C-6‴), 65.4, 61.4, 54.6 (O*C*H$_3$), 52.3 (C-2‴), 23.2 (2 C, CO*C*H$_3$ and NHCO*C*H$_3$), 18.0, 17.9 (2 C) (3 *C*H$_3$); ESI-MS: m/z= 1799.7 $[M+Na]^+$; Anal. Calcd. for $C_{105}H_{117}NO_{24}$ (1775.8): C, 70.97; H, 6.64; found: C, 70.75; H, 6.88.

Methyl (2,3,4-tri-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-[(2,3,4-tri-O-benzyl-α-D-galactopyranosyl uronic acid)-(1→3)-(2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranosyl)-(1→2)]-(4-O-benzyl-α-L-rhamnopyranosyl)-(1→3)-2,4-di-O-benzyl-α-L-rhamnopyranoside (15) To a solution of compound **14** (1.5 g, 0.84 mmol) in MeOH (25 mL) was added solid sodium methoxide until the pH of the solution reached to ~10. The reaction mixture was allowed to stir at room temperature for 3 h, neutralized with Dowex 50W-X8 (H^+), filtered and concentrated under reduced pressure. To a solution of the product (1.45 g) in CH_2Cl_2 (25 mL) and H_2O (4.5 mL) were added aq. solution of NaBr (585 μL; 1 M), aq. solution of TBAB (1.2 mL; 1 M), TEMPO (62 mg, 0.4 mmol), satd. aq. solution of $NaHCO_3$ (3 mL) and 4% aq. NaOCl (4.5 mL) in succession and the reaction mixture was allowed to stir at 0–5°C for 2 h. The reaction mixture was neutralized with the addition of 1 N aq. HCl solution. To the reaction mixture were added *tert*-butanol (18 mL), 2-methyl-but-2-ene (38 mL; 2 M solution in THF), aq. solution of $NaClO_2$ (800 mg in 3 mL) and aq. solution of NaH_2PO_4 (800 mg in 3 mL) and the reaction mixture was allowed to stir at room temperature for 3 h. The reaction mixture was diluted with satd. aq. NaH_2PO_4 and extracted with CH_2Cl_2 (3×50 mL). The combined organic layer was washed with water, dried (Na_2SO_4) and concentrated to dryness. The crude product was purified over SiO_2 using hexane–EtOAc (1:2) to give pure compound **15** (1.1 g, 75%) as yellowish solid; m.p. 84°C; $[\alpha]_D^{25}$ + 15.3 (c 1.0, $CHCl_3$); IR (KBr): 2924, 1727, 1600, 1455, 1357, 1280, 1094, 738, 697 cm^{-1}; ^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.41–7.05 (m, 50 H, aromatic protons), 5.28 (s, 1 H, PhC*H*), 4.78 (br s, 1 H, H-1‴′), 4.75 (d, J=7.8 Hz, 1 H, H-1‴), 4.66 (br s, 1 H, H-1), 4.65 (br s, 1 H, H-1′), 4.63–4.33 (m, 19 H, H-1″ and 9 PhCH_2), 4.30–4.15 (m, 4 H, H-2′, H-2‴, H-3′ and H-3′), 4.10–3.86 (m, 5 H, H-2, H-2″, H-3‴, H-3‴′ and H-4‴), 3.70–3.68 (m, 1 H, H-2‴′), 3.67–3.50 (m, 9 H, H-3, H-4, H-4″, H-5, H-5′, H-5″, H-5‴ and H-$6_{a,b}$‴), 3.40–3.35 (m, 2 H, H-4′ and H- 4‴′), 3.29 (s, 3 H, OCH_3), 3.28–3.27 (m, 1 H, H-5‴′), 1.88 (s, 3 H, NHCOCH_3), 1.27–1.19 (m, 9 H, 3 CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 174.0 (2 C, *C*OOH and NH*C*OCH$_3$), 139.7–126.3 (aromatic carbons), 102.4 (Ph*C*H),

101.1 (C-1‴), 100.2 (C-1‴), 98.4 (2 C, C-1 and C-1″), 95.9 (C-1′), 83.2, 80.8, 80.5, 80.1, 78.7, 77.9 (2 C), 77.2, 76.0, 75.7, 75.3 (3 C), 74.4 (Ph*C*H$_2$), 72.5 (3 C, 3 Ph*C*H$_2$), 72.4 (2 C, 2 Ph*C*H$_2$), 72.3 (2 C, 2 Ph*C*H$_2$), 71.6 (C-6‴ and Ph*C*H$_2$), 70.7, 69.9, 67.9 (2 C), 65.1, 60.3, 54.5 (2 C, C-2‴ and O*C*H$_3$), 20.7 (NHCO*C*H$_3$), 17.9, 17.8, 17.6 (3 *C*H$_3$); ESI-MS: m/z=1771.6 $[M+Na]^+$; Anal. Calcd. for $C_{103}H_{113}NO_{24}$ (1747.7): C, 70.73; H, 6.51; found: C, 70.54; H, 6.75.

Methyl (α-L-rhamnopyranosyl)-(1→3)-[(α-D-galactopyranosyl uronic acid)-(1→3)-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-(1→2)]-(α-L-rhamnopyranosyl)-(1→3)-α-L-rhamnopyranoside (1) To the solution of compound **15** (1.0 g, 0.57 mmol) in methanol (25 mL) was added 20% Pd $(OH)_2$/C (500 mg) and the reaction mixture was allowed to stir at room temperature under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a Celite® bed and evaporated to dryness to give pentasaccharide **1** (360 mg, 74%) as a white powder, which was further purified by passing through a column of Sephadex-LH-20 using CH_3OH–H_2O (4:1) as eluant. $[\alpha]_D^{25}$ − 7 (*c* 1.0, H_2O); IR (KBr): 3427, 2927, 1597, 1353, 1129, 1073, 635 cm^{-1}; ^{1}H NMR (D_2O, 300 MHz): δ 5.28 (br s, 1 H, H-1‴), 5.21 (br s, 1 H, H-1″), 5.04 (br s, 1 H, H-1), 4.65 (br s, 1 H, H-1′), 4.56 (d, J=7.6 Hz, 1 H, H-1‴), 4.25 (br s, 1 H, H-3‴), 4.17 (br s, 1 H, H-3″), 4.12 (br s, 1 H, H-2″), 4.02 (br s, 1 H, H-2), 3.99 (br s, 1 H, H-2′), 3.94–3.89 (dd, J=9.8, 2.6 Hz, 1 H, H-4‴), 3.88–3.60 (m, 12 H, H-2‴, H-2‴, H-3, H-3′, H-3‴, H-4, H-4‴, H-5, H-5′, H-5″, H-6_{ab}‴), 3.56–3.40 (m, 4 H, H-4′, H-4″, H-5‴ and H-5‴), 3.37 (s, 3 H, O*C*H$_3$), 1.98 (s, 3 H, NHCOC*H*$_3$), 1.30–1.26 (m, 9 H, 3 C*H*$_3$); ^{13}C NMR (D_2O, 75 MHz): δ 174.8 (*C*OOH), 171.2 (NH*C*OCH$_3$), 103.5 (C-1‴), 102.9 (C-1), 101.8 (C-1″), 101.4 (C-1‴), 101.2 (C-1′), 81.7 (C-3), 78.9 (C-2″), 78.3 (C-4‴), 77.1 (C-3′), 75.7 (C-4′), 72.9 (C-5‴), 72.6 (2 C, C-3″ and C-4″), 72.2 (C-5‴), 71.4 (C-3‴), 71.0 (C-2), 70.8 (C-2′), 70.7 (C-4), 70.4 (C-4‴), 70.1 (2 C, C-5 and C-5′), 69.8 (C-5″), 69.2 (C-3‴), 68.8 (C-2‴), 60.8 (C-6‴), 55.4 (O*C*H$_3$), 54.9 (C-2‴), 22.9 (NHCO*C*H$_3$), 17.2 (2 C, 2 *C*H$_3$), 17.1 (*C*H$_3$); ESI-MS: m/z=872.4 $[M+Na]^+$; Anal. Calcd. For $C_{33}H_{55}NO_{24}$ (849.3): C, 46.64; H, 6.52; found: C, 46.35; H, 6.80.

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