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Concise chemical synthesis of a tetrasaccharide repeating unit of the *O*-antigen of *Hafnia alvei* 10457

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Abstract Concise chemical synthesis of a tetrasaccharide repeating unit of the *O*-antigen of *Hafnia alvei* 10457 is reported. Construction of the tetrasaccharide as its 4-methoxyphenyl glycoside was achieved by condensation of less abundant monosaccharide units such as, D-galactofuranose, *N*-acetyl-D-galactosamine and *N*-acetylneuraminic acid. The synthetic strategy consists of the preparation of suitably protected required monosaccharide intermediates from the commercially available reducing sugars and high yielding glycosylation reactions.

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

Hafnia alvei, a member of the Enterobacteriaceae family is a motile, facultatively anaerobic, Gram-negative bacterium [1, 2]. Although these bacteria have been found in stool samples of healthy human, several reports of nosocomial infections associated with *Hafnia* have appeared [3]. Immunochemical studies on lipopolysaccharides suggested that *Hafnia alvei* has an active role in causing human infections, such as diarrhoea [4], extraintestinal infections [5], urogenital, respiratory tract, skin and soft tissue infections [6–9]. It is well established that the *O*-antigenic lipopolysaccharides of the cell wall components are responsible for the immunospecificity of *Hafnia alvei* [10, 11]. *H. alvei* have been divided into 39 *O*-serotypes depending on the structure of their *O*-antigens [12]. A number of clinical isolates of *H. alvei* possesses homologous pathogenicity like enteropathogenic *E. coli* and have been found to be associated with diarrhoea in human [4, 13]. Recently, Eserstam *et al*. [14] reported the structure of the *O*-antigenic lipopolysaccharide of a diarrheagenic strain of *Hafnia alvei* 10457 that has characteristic similarity with enteropathogenic *E. coli* (Fig. 1). The lipopolysaccharide consists of a tetrasaccharide repeating unit containing unique D-galactofuranose, *N*-acetyl-D-galactosamine and *N*-acetyl-neuraminic acid moieties.

The development of anti-microbial vaccines for the protection from future infections is a thrust area in medicinal chemistry. Because of the antigenicity of the lipopolysaccharides, glycoconjugate vaccines have been considered to be very effective against several bacterial infections. In most of the glycoconjugate vaccines developed so far have used polysaccharides isolated from natural sources. Recently, Verez-Bencomo *et al*. [15] reported a synthetic strategy for the preparation of Hib vaccine against *Haemophilus influenzae* type b. Besides this, a number of reports appeared in the literature aiming towards the development of synthetic carbohydrate vaccine candidates to control cancer, anthrax, malaria, leishmania etc [16–19]. However, the development of glycoconjugate vaccines has its own limitation because of the scarcity of larger quantity of isolated natural polysaccharides. In order to achieve large quantities of oligosaccharides as well as analogues of the natural oligosaccharides for their use in the development of carbohydrate based vaccine leads, efficient chemical synthetic strategies are always welcome. In this endeavor, we

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→6)-β-D-Gal*f*-(1→3)-β-D-Gal*p*NAc-(1→3)-β-D-Gal*p*-(1→
6
↑
2
α-NeuAc

Fig. 1 Structure of the repeating unit of the *O*-antigen of *Hafnia alvei* 10457

would like to report herein a total synthesis of the tetrasaccharide repeating unit of the *O*-antigen of *H. alvei* 10457 as its 4-methoxyphenyl glycoside (Fig. 2) in which suitably protected monosaccharide units have been linked together sequentially in a minimum number of steps.

The synthesis of the target tetrasaccharide 1 as its 4-methoxyphenyl glycoside is presented in Scheme 1. The tetrasaccharide 1 contains three monosaccharide units (*e.g.* D-galactofuranose, D-galactosamine and *N*-acetylneuraminic acid or sialic acid), which are commercially expensive. Therefore, it was planned to synthesize them from relatively cheaper starting materials. The synthesis of the tetrasaccharide 1 as its 4-methoxyphenyl glycoside was achieved by condensation of four suitably protected monosaccharide intermediates (4, 7, 10 and 13), which were prepared from commercially available D-galactose, D-glucosamine hydrochloride and *N*-acetylneuraminic acid following reported methodologies. In order to synthesize the suitably functionalized D-galactosamine moiety we have used D-glucosamine hydrochloride as the starting material instead of using D-galactosamine hydrochloride itself. Ethyl 3,6-di-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (2) [20] was prepared from D-glucosamine hydrochloride following a sequence of reactions involving *N*-phthaloylation, acetylation, thioglycosidation, deacetylation and selective benzoylation. Reaction of compound 2 with trifluoromethanesulfonic anhydride followed by hydrolysis of the triflate group in a S_N2 manner furnished ethyl 3,6-di-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (3) [20] in 80% yield, which was converted into compound 4 after debenzoylation and benzylidenation followed by acetylation. Yields were excellent in every step and quite comparable to the earlier reports. 4-Methoxyphenyl 2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside (5) [21] was prepared from D-galactose by acetylation followed by acid catalyzed glycosylation with 4-methoxyphenol. Following a set of reactions consisting of deacetylation, isopropylidenation, benzylation and removal of the isopropylidene group, compound 5 was transformed to compound 6 [22] in excellent yield. Selective acetylation of compound 6 via an orthoester formation furnished monosaccharide acceptor **7** in 75% yield. Ethyl 2,3,5,6-tetra-*O*-benzoyl-1-thio-α-D-galactofuranoside (10) [23, 24] was prepared from D-galactose following the reported literature. Compound 13 [25] was prepared from commercially available *N*-acetylneuraminic acid following a three-step reaction sequence consisting of methyl ester formation, acetylation and thioglycosidation.

Condensation of compound 4 with compound 7 in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) [26, 27] furnished disaccharide 8 in 82% yield. Presence of signals in ^{1}H [δ 5.61 (d, J=8.4 Hz, H-1′), 5.54 (s, PhC*H*) and 4.78 (d, J=9.3 Hz, H-1)] and ^{13}C NMR [δ 102.4 (C-1), 101.0 (Ph*C*H), 98.0 (C-1′)] confirmed the formation of disaccharide derivative 8. The stereoselective formation of disaccharide 8 was also confirmed from its single crystal X-ray crystallographic study. From the ORTEP diagram of compound 8 it was established that both D-galactose and D-galactosamine moieties were possessing $^4C_1$ beta-oriented chair conformation and the phenyl ring of the benzylidene acetal was equatorially positioned (Fig. 3). From the X-ray study ($R1$=0.0577) it has been found that there is a weak intra-molecular C–H…O interaction between C6′–H6′B…O16. [The distance d between H6′B…O16 is 2.505 Å (distance between C6′…O16 D=3.317 Å) and the angle between C6′–H6′B–O16 is 141.12°. (Fig. 3 and supporting information)]. Due to the presence of a weak hydrogen bonding it is expected that 4-*O*-acetyl group of the D-galactose moiety is hydrolytically more stable. Besides the presence of weak intra-molecular hydrogen bonding between C6′–H6′B…O16, 4-*O*-acetyl group of D-galactose moiety is sterically crowded too, which may exert extra stability to the 4-*O*-acetyl group towards its hydrolysis. With this presumption, selective

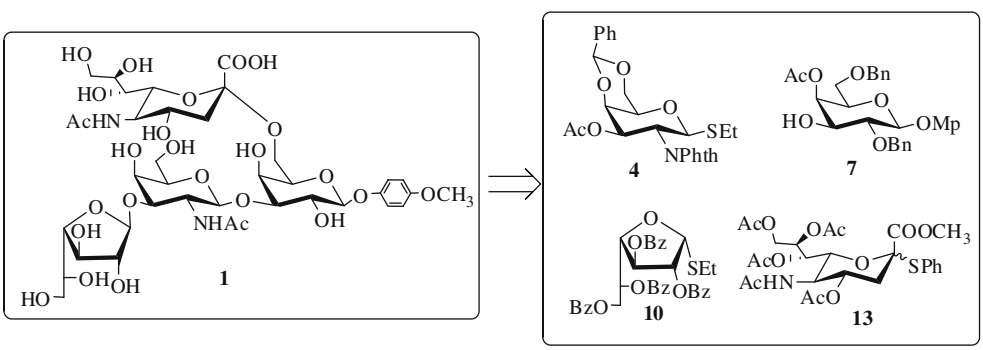
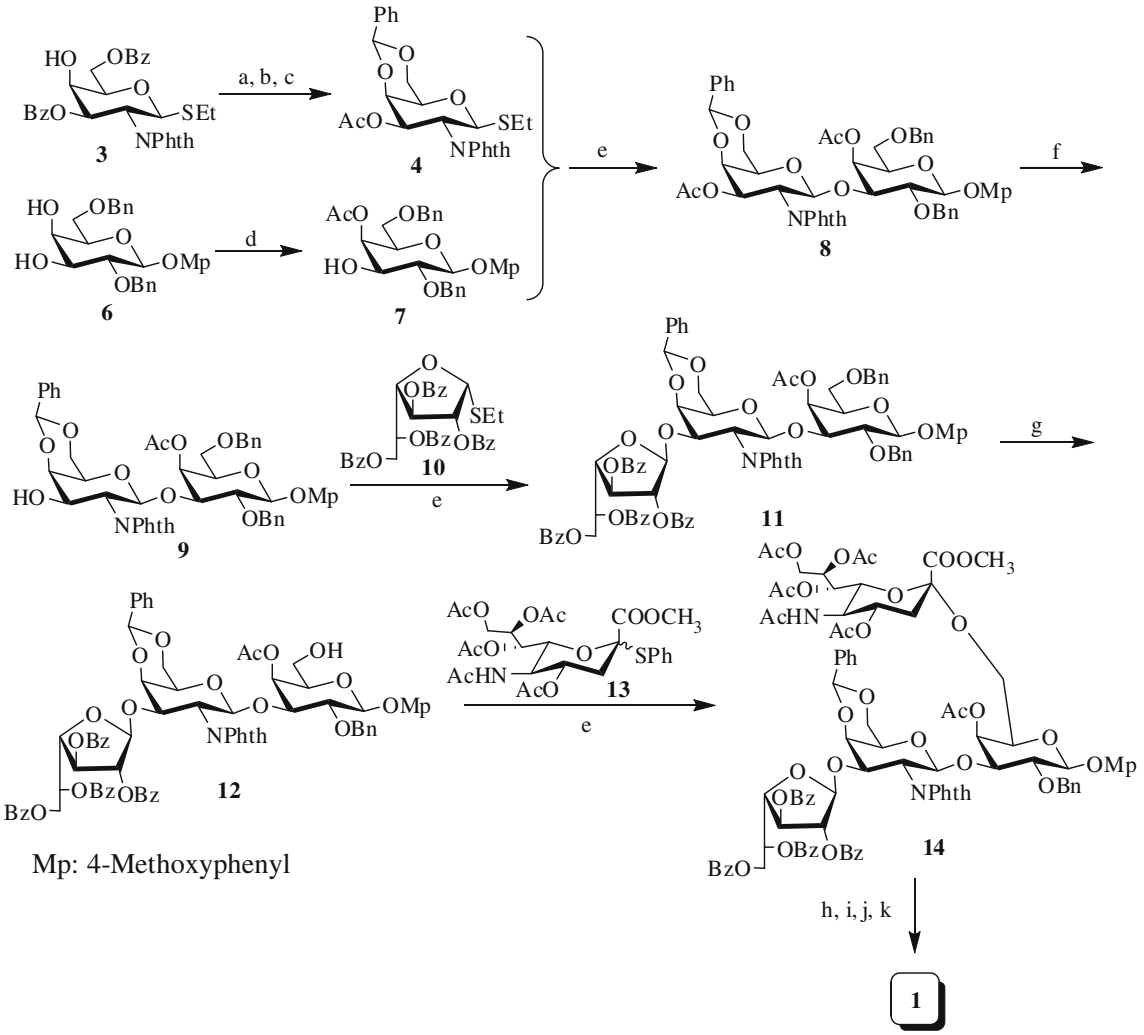


Fig. 2 Structure of synthesized tetrasaccharide as 4-methoxyphenyl glycoside (*1*) and its synthetic intermediates



Scheme 1 Reagents: **a** 0.01 M NaOMe, MeOH, r t, 30 min; **b** PhCH$(OMe)_2$, *p*-TsOH, CH_3CN, r t, 10 h; **c** Ac_2O, pyridine, r t, 76% in three steps; **d** triethylorthoacetate, *p*-TsOH, DMF, r t, 2 h, then 80% aq. AcOH, r t, 1 h, 75%; **e** *N*-iodosuccinimide, TfOH, CH_2Cl_2, −30°C (82% for 8; 85% for 11; 52% for 14); **f** 0.01 M NaOMe, MeOH, r t, 20 min, 92%; **g** H_2, 20% $Pd(OH)_2$–C, MeOH–toluene (1:1), 2 h, r t, 72%; **h** H_2, $Pd(OH)_2$–C, MeOH, r t, 24 h; **i** $NH_2NH_2H_2O$, EtOH, 80° C, 6 h; **j** Ac_2O, pyridine, r t, 2 h; **k** 0.1 M NaOMe, MeOH, r t, 8 h, then few drops of water, r t, 12 h, 68% overall yield

removal of acetyl group from compound 8 using a very dilute solution of sodium methoxide in a short interval of time gave disaccharide acceptor 9 having the 4-*O*-acetyl group in the D-galactose moiety intact, which on glycosylation with ethyl thioglycoside donor 10 in the presence of NIS-TfOH [26, 27] afforded trisaccharide derivative 11 in 85% yield. The presence of β-linked D-galactofuranosyl residue in compound 11 was confirmed from its ^{13}C NMR spectrum (C-1″ at δ 107.6), which is quite diagnostic and comparable with the earlier report [28]. Appearance of signals in ^1H NMR [δ 5.50 (s, PhCH), 5.47 (d, J=8.3 Hz, H-1′), 5.30 (brs, H-1″), 4.79 (d, J=7.1 Hz, H-1)] and ^{13}C NMR [δ 107.6 (C-1″), 102.5 (C-1), 100.8 (PhCH), 98.4 (C-1′)] confirmed the formation of trisaccharide derivative 11. In order to incorporate sialic acid derivative selectively to the 6-hydroxy group of the D-galactose moiety, it was essential to remove the primary benzyl group from compound 11 having benzylidene acetal unaffected. For the selective removal of primary benzyl group from the trisaccharide derivative 11 in the presence of benzylidene acetal, we applied our earlier reported methodology for the time dependent hydrogenolysis reaction over Pearlmans catalyst [29]. Thus, selective hydrogenation of compound 11 over 20% $Pd(OH)_2$–C in a methanol–toluene mixture furnished trisaccharide acceptor 12 in 72% yield. Presence of a signal for the benzylidene acetal in the NMR spectra [δ 5.49 (s, PhCH) in ^1H NMR and δ 100.6 in ^{13}C NMR] supported the selective removal of the primary benzyl group leaving the secondary benzyl group and benzylidene acetal intact. Glycosylation of compound 12 with phenyl thioglycoside donor 13 in the presence of NIS-TfOH [26, 27] furnished tetrasaccharide derivative 14 in 52% yield

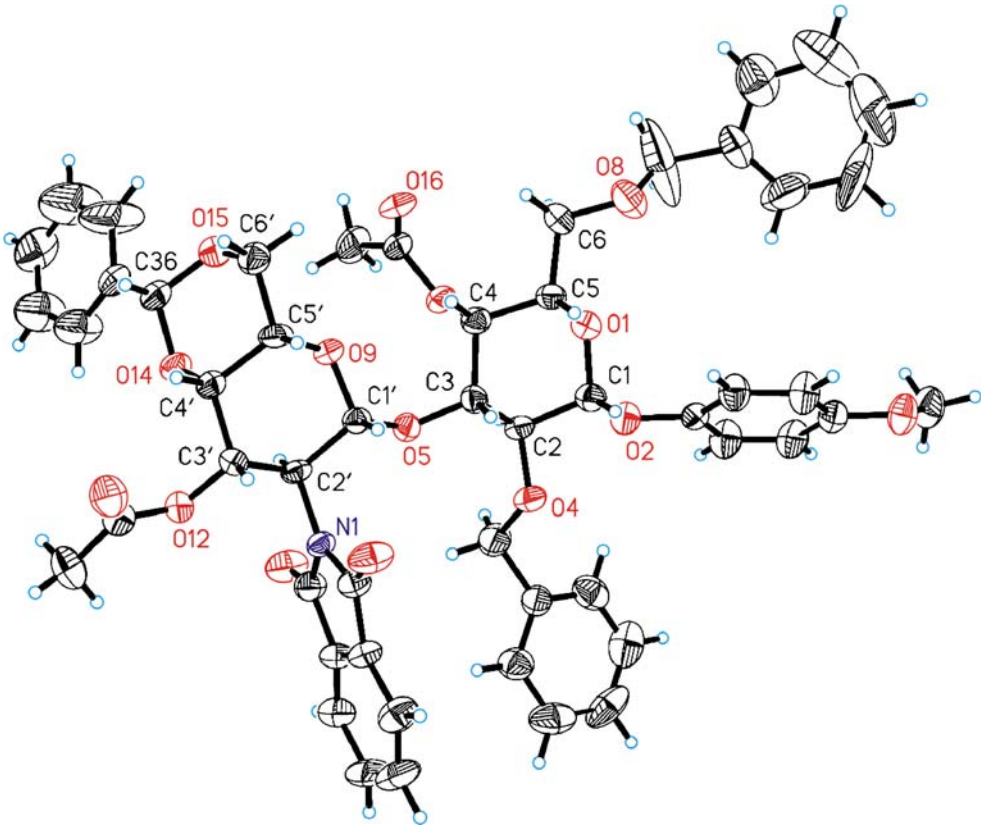


Fig. 3 ORTEP diagram of compound 8. Some of the atoms are not being numbered for clarity

together with some beta-isomer (~10%). The formation of compound 14 having α-linked sialic acid moiety as major product was confirmed from its NMR spectral analysis. In the ^{1}H NMR spectrum of compound 14, H-3$_e$ of sialic acid moiety appeared at δ 2.55 (dd, J=11.8 and 3.9 Hz) and H-3$_a$ at δ 1.95 (t, J=11.9 Hz) indicating the formation of α-linkage of sialic acid. Complete deprotection of compound 14 following a series of reactions consisting of hydrogenolysis [30], hydrazinolysis [31], acetylation and saponification furnished target tetrasaccharide 1 as its 4-methoxyphenyl glycoside in 68% over all yield, which was confirmed from its NMR and mass spectral studies. Presence of α-linked sialic acid moiety in the deprotected tetrasaccharide 1 was further confirmed from its ^{1}H NMR spectra [H-3$_e$‴ appeared at δ 2.76 (dd, J=12.4 and 3.4 Hz) and H-3$_a$‴ appeared at δ 1.68 (t, J=12.2 Hz)] and compared with the data reported earlier [32].

Conclusion

In summary, the synthesis of a tetrasaccharide repeating unit of the *O*-antigen of *Hafnia alvei* 10457 as its 4-methoxyphenyl glycoside containing D-galactofuranose, *N*-acetyl-D-galactosamine and sialic acid has been achieved in a concise manner. All glycosylation steps, carried out in gram scale, were high yielding and minimum number of protecting group manipulation steps were involved in the synthesis. It is noteworthy that two elegant methodologies for the selective removal of one acetyl group in the presence of other using saponification condition and removal of the primary benzyl group in the presence of a secondary benzyl group and a benzylidene acetal under hydrogenation conditions have been optimized and successfully applied for the synthesis of target tetrasaccharide 1. 4-Methoxybenzyl group at the reducing terminus serves as a temporary protecting group, which can be removed for the conjugation of the tetrasaccharide with a protein as and when needed.

Experimental section

General methods All the reactions were monitored by thin layer chromatography over silica gel GF$_{254}$ coated TLC plates. The spots on TLC were visualized by UV lamp and warming ceric sulphate (2% $Ce(SO_4)_2$ in 1M H_2SO_4) sprayed plates on a hot plate. Silica gel 230–400 mesh was used for flash column chromatography. ^{1}H and ^{13}C NMR, 2DCOSY, HMQC spectra were recorded on Bruker Avance DPX 200, 300 and 600 MHz using $CDCl_3$ and D_2O as solvents and TMS as internal reference unless stated otherwise. Chemical shift values were expressed in δ ppm.

ESI-MS spectra 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 3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-1-thio-β-D-galactopyranoside (4) A solution of ethyl 3,6-di-*O*-benzoyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (3) (5.6 g, 10 mmol) in 0.01 M sodium methoxide in MeOH (60 ml) was allowed to stir at room temperature for 30 min and neutralized with Dowex-50W X8 (H^+) resin. The reaction mixture was filtered and evaporated to dryness. The dried mass was dissolved in anhydrous CH_3CN (20 ml) and benzaldehyde dimethylacetal (1.8 ml, 12 mmol) was added to it followed by *p*-toluenesulfonic acid (200 mg). After stirring at room temperature for 10 h, the reaction mixture was quenched with Et_3N (0.5 ml) and solvents were removed under reduced pressure. To a solution of the crude reaction mixture in pyridine (20 ml) was added acetic anhydride (15 ml) and the reaction mixture was allowed to stir at room temperature for 4 h. The solvents were removed under reduced pressure and the crude mass was purified over SiO_2 using hexane-EtOAc (3:1) as eluant to furnish pure compound 4 (3.7 g, 76%) as a syrup; $[\alpha]_D^{25}$−19 (*c* 2.0, $CDCl_3$); IR (neat): 2,375.5, 2,136.6, 1,761.1, 1,652.0, 1,386.4, 1,235.2, 1,085.9, 1,042.1, 755.9, 720.8, 624.4, 531.1 cm^{-1}; ^1H NMR (200 MHz, $CDCl_3$): δ 7.88–7.82 (m, 2 H, Ar–H), 7.76–7.71 (m, 2 H, Ar–H), 7.57–7.52 (m, 2 H, Ar–H), 7.41–7.38 (m, 3 H, Ar–H), 5.79 (dd, *J*=10.9 and 3.5 Hz, 1 H, H-3), 5.56 (s, 1 H, PhC*H*), 5.43 (d, *J*=10.2 Hz, 1 H, H-1), 4.90 (t, *J*=10.7 Hz, 1 H, H-2), 4.53 (d, *J*=3.4 Hz, 1 H, H-4), 4.38 (dd, *J*=12.4 and 1.2 Hz, 1 H, H-6_a), 4.10–4.03 (dd, *J*=12.5 and 1.2 Hz, 1 H, H-6_b), 3.72 (brs, 1 H, H-5), 2.92–2.61 (m, 2 H, SCH_2CH$_3$), 1.92 (s, 3 H, COCH_3), 1.29–1.20 (m, 3 H, SCH$_2$CH_3); ^{13}C NMR (50 MHz, $CDCl_3$): δ 170.4 (*C*OCH$_3$), 168.4 (*C*OPhth), 167.6 (*C*OPhth), 138.1, 134.5 (2 C), 132.1, 131.8, 129.5, 128.6 (2 C), 126.8 (2 C), 124.1, 123.8, 101.5 (Ph*C*H), 80.7 (C-1), 73.5 (C-5), 70.3 (C-3 and C-4), 69.7 (C-6), 49.9 (C-2), 23.3 (S*C*H$_2$), 21.1 (CO*C*H$_3$), 15.2 (SCH$_2$*C*H$_3$); ESI-MS: *m/z*=506.2 $[M+Na]^+$; Anal. Calcd. For $C_{25}H_{25}NO_7S$ (483.14): C, 62.10; H, 5.21; found: C, 61.87; H, 5.48.

4-Methoxyphenyl 4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (7) To a solution of compound 6 (5 g, 10.7 mmol) in DMF (10 ml) were added triethyl orthoacetate (10 ml, 54.5 mmol) and *p*-toluenesulfonic acid (200 mg) and the reaction mixture was allowed to stir at room temperature for 2 h. After completion (TLC; hexane-EtOAc 2:1), the reaction mixture was neutralized with triethylamine (1 ml) and evaporated to dryness. A solution of the crude mass in 80% aq. acetic acid (80 ml) was allowed to stir at room temperature for 1 h. The reaction mixture was concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to furnish pure compound 7 (4.1 g, 75%) as syrup; $[\alpha]_D^{25}$+36 (*c* 2.0, $CDCl_3$); IR (neat): 3,452.3, 2,923.4, 2,870.0, 1,742.4, 1,507.5, 1,456.6, 1,374.5, 1,222.7, 1,102.1, 1,068.0, 944.5, 829.4, 746.5, 700.3 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.36–7.27 (m, 10 H, Ar–H), 7.03 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.79 (d, *J*=9.0 Hz, 2 H, Ar–H), 5.40 (d, *J*=2.9 Hz, 1 H, H-4), 5.07 (d, *J*=11.2 Hz, 1 H, PhCH_{2a}), 4.87 (d, *J*=7.5 Hz, 1 H, H-1), 4.80 (d, *J*=11.2 Hz, 1 H, PhCH_{2b}), 4.56 (d, *J*=11.7 Hz, 1 H, PhCH_{2a}), 4.47 (d, *J*=11.7 Hz, 1 H, PhCH_{2b}), 3.86–3.70 (m, 6 H, H-2, H-6_{ab} and OCH_3), 3.59–3.57 (m, 2 H, H-3 and H-5), 2.11 (s, 3 H, OCOCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.7 (*C*OCH$_3$), 155.3, 151.3, 138.2, 137.8, 128.5 (2 C), 128.4 (2 C), 128.1 (2 C), 127.9 (2 C), 127.8 (2 C), 118.3 (2 C), 114.6 (2 C), 102.8 (C-1), 79.1, 75.0 (Ph*C*H$_2$), 73.6 (Ph*C*H$_2$), 72.9, 71.9, 69.5, 68.4 (C-6), 55. 6 (O*C*H$_3$), 20.8 (CO*C*H$_3$); ESI-MS: *m/z*=531.4 $[M+Na]^+$; Anal. Calcd. For $C_{29}H_{32}O_8$ (508.21): C, 68.49; H, 6.34; found: C, 68.23; H, 6.60.

4-Methoxyphenyl (3-O-acetyl-4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranosyl)-(1→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (8) To a solution of compound 7 (4.0 g, 7.86 mmol) and ethyl thioglycoside donor 4 (4.5 g, 9.3 mmol) in anhydrous CH_2Cl_2 (50 ml) was added freshly activated powdered MS 4 Å (5 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. *N*-Iodosuccinimide (NIS; 2.5 g, 11.1 mmol) was added to the reaction mixture and the reaction mixture was cooled to −30°C. To the cooled reaction mixture was added trifluoromethanesulfonic acid (TfOH; 50 μl, 0.57 mmol) and the reaction mixture was allowed to stir at −30°C for 1 h. The reaction mixture was quenched by the addition of triethylamine (0.5 ml), filtered through a Celite® bed and washed with CH_2Cl_2 (3×50 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 (2:1) as eluant to afford pure compound 8 (6.0 g, 82%) as colourless solid; m.p. 180°C (EtOAc–hexane, 3:1 *v/v*);$[\alpha]_D^{25}$+28.8 (*c* 1.6, $CDCl_3$); IR (KBr): 2,928.3, 2,372.8, 2,127.4, 1,630.0, 1,461.9, 1,390.4, 1,080.9, 795.9, 703.0 cm^{-1}; ^1H NMR (300 MHz, $CDCl_3$): δ 7.72–7.69 (m, 1 H, Ar–H), 7.61–7.53 (m, 5 H, Ar–H), 7.45–7.40 (m, 3 H, Ar–H), 7.33–7.28 (m, 5 H, Ar–H), 7.17–7.14 (m, 3 H, Ar–H), 7.05–7.02 (m, 2 H, Ar–H), 6.89 (d, *J*=9.1 Hz, 2 H, Ar–H), 6.70 (d, *J*=9.1 Hz, 2 H, Ar–H), 5.67 (dd, *J*=11.4 and 3.6 Hz, 1 H, H-3′), 5.61 (d, *J*=8.4 Hz,

1 H, H-1′), 5.54 (s, 1 H, PhC*H*), 5.43 (d, *J*=3.0 Hz, 1 H, H-4), 4.82 (d, *J*=8.4 Hz, 1 H, H-1), 4.78 (t, *J*=9.3 Hz, 1 H, H-2′), 4.72 (d, *J*=11.5 Hz, 1 H, Ph CH_{2a}), 4.52–4.44 (m, 4 H, PhCH_{2ab}, PhCH_{2b}, H-4′), 4.38 (d, *J*=12.2 Hz, 1 H, H-6_a), 4.01 (d, *J*=12.3 Hz, 1 H, H-6_b), 3.86–3.79 (m, 3 H, H-5, H-$6'_{ab}$), 3.73 (s, 3 H, OCH_3), 3.69 (dd, *J*=10.4 and 3.9 Hz, 1 H, H-3), 3.57–3.54 (m, 2 H, H-2, H-5′), 2.11, 1.91 (2 s, 6 H, 2 COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9 (*C*OCH_3), 170.4 (*C*OCH_3), 168.6 (*C*OPhth), 167.3 (*C*OPhth), 155.1, 151.3, 138.3 (2 C), 138.0 (2 C), 137.8, 134.0, 133.9, 128.9, 128.3 (2 C), 128.1 (2 C), 127.9 (2 C), 127.6 (2 C), 127.5 (2 C), 127.0 (2 C), 126.5 (2 C), 123.3, 123.2, 118.1 (2 C), 114.3 (2 C), 102.4 (C-1), 101.0 (Ph*C*H), 98.0 (C-1′), 79.3 (C-3), 78.1 (C-2), 74.7 (Ph*C*H_2), 73.6 (Ph*C*H_2), 73.4 (C-3′), 72.7 (C-5), 69.5 (C-6), 69.2 (C-4), 69.1 (C-5′), 68.7 (C-6′), 66.5 (C-4), 55.5 (O*C*H_3), 51.3 (C-2′), 20.9, 20.7 (2 CO*C*H_3); ESI-MS: *m*/*z*=952.4 $[M+Na]^+$; Anal. Calcd. For $C_{52}H_{51}NO_{15}$ (929.33): C, 67.16; H, 5.53; found: C, 66.92; H, 5.78.

Crystal data for compound 8 $C_{52}H_{51}NO_{15}$, *M*=929.96, monoclinic, $P2_1$, *a*=10.213(2), *b*=9.590(2), *c*=24.734(4)Å, β=92.04 (1)°, *V*=2,421.0(8)Å^3, *T*=293(2)K, *Z*=2, D_c= 1.276 gcm^{-3}, μ=0.094 mm^{-1}, $F_{(000)}$=980, λ (Mo K_α)= 0.71073 Å, colorless transparent block, crystal size 0.350× 0.250×0.150 mm, 6,011 reflections measured (R_{int}= 0.0382), 5,101 unique, *R*1=0.0577 for 2,450 *Fo*>4σ (*Fo*) and 0.1522 for all 5,101 data, *S*=0.984 for all data and 604 parameters. Unit cell determinations and intensity data collection (2θ=49.15°) was performed on a Bruker P4 diffractometer at 293(2)K. Structure solutions by direct methods and refinements by full-matrix-least-squares methods on F^2. Programs: *XSCANS* [(Siemens Analytical X-ray Instruments Inc.: Madison, Wisconsin, USA 1996) were used for data collection and data processing], *SHELXTL-NT* [(Bruker AXS Inc.: Madison, Wisconsin, USA 1997) was used for structure determination, refinements and molecular graphics]. Further details of the crystal structure investigation can be obtained from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB21EZ, UK (CCDC deposit No. 661562).

4-Methoxyphenyl (4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranosyl)-(1→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (9) A solution of compound 8 (5.8 g, 6.24 mmol) in 0.01 M sodium methoxide in methanol (120 ml) was allowed to stir at room temperature for 20 min. The reaction mixture was neutralized with Dowex 50W-X8 (H^+) resin, filtered and concentrated under reduced pressure to afford the product 9 (5.1 g, 92%) as syrup; $[\alpha]_D^{25}$−19 (*c* 1.3, $CDCl_3$); IR (neat): 3,366.7, 2,925.4, 2,855.7, 2,374.6, 2,134.6, 1,712.1, 1,655.2, 1,508.8, 1,462.4, 1,382.5, 1,239.4, 1,079.4, 719.7 cm^{-1}. ^{1}H-NMR (300 MHz, $CDCl_3$): δ 7.6 (m, 1 H, Ar–H), 7.56–7.47 (m, 5 H, Ar–H), 7.44–7.37 (m, 3 H, Ar–H), 7.38–7.22 (m, 5 H, Ar–H), 7.10–7.06 (m, 3 H, Ar–H), 6.98–6.95 (m, 2 H, Ar–H), 6.84 (d, *J*=9.1 Hz, 2 H, Ar–H), 6.64 (d, *J*=9.1 Hz, 2 H, Ar–H), 5.53 (s, 1 H, PhC*H*), 5.47 (d, *J*=7.7 Hz, 1 H, H-1′), 5.38 (d, *J*=2.6 Hz, 1 H, H-4), 4.73 (d, *J*=7.0 Hz, 1 H, H-1), 4.67 (d, *J*=11.5 Hz, 1 H, PhCH_{2a}), 4.48–4.42 (m, 3 H, PhCH_{2ab}, PhCH_{2b}), 4.40–4.34 (m, 3 H, H-2′, H-3′, H-6_a), 4.18 (d, *J*=2.3 Hz, 1 H, H-4′), 3.96 (d, *J*=11.2 Hz, 1 H, H-6_b), 3.79–3.74 (m, 3 H, H-5, H-$6'_{ab}$), 3.70 (brs, 3 H, O*C*H_3), 3.64 (dd, *J*=10.3 and 4.1 Hz, 1 H, H-3), 3.51–3.43 (m, 2 H, H-2, H-5′), 2.11 (s, 3 H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9 (*C*OCH_3), 168.2 (2 *C*OPhth), 155.1, 151.7, 138.7 (2 C), 138.3, 137.8 (2 C), 133.9, 129.5, 128.5 (5 C), 128.1 (3 C), 127.9 (3 C), 127.2 (2 C), 126.8 (2 C), 123.6, 123.2, 118.5 (2 C), 114.6 (2 C), 102.8 (C-1), 101.8 (Ph*C*H), 98.3 (C-1′), 78.9 (C-3), 78.3 (C-2), 75.3 (C-3′), 75.0 (C-4′), 73.8 (Ph*C*H_2), 73.7 (C-5), 69.7 (Ph*C*H_2), 69.5 (C-6), 69.4 (C-4), 69.0 (C-5′), 68.1 (C-6′), 55.3 (O*C*H_3), 54.9 (C-2′), 21.2 (CO*C*H_3); ESI-MS: *m*/*z*=910.4 $[M+Na]^+$; Anal. Calcd. For $C_{50}H_{49}NO_{14}$ (887.32): C, 67.63; H, 5.56; found: C, 67.40; H, 5.84.

4-Methoxyphenyl (2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl)-(1→3)-(4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranosyl)-(1→3)-4-O-acetyl-2,6-di-O-benzyl-β-D-galactopyranoside (11) To a solution of compound 9 (4.6 g, 5.18 mmol) and ethyl thioglycoside donor 10 (4.0 g, 6.24 mmol) in anhydrous CH_2Cl_2 (50 ml) was added freshly activated powdered MS 4 Å (5 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. *N*-Iodosuccinimide (1.7 g, 7.55 mmol) was added to the reaction mixture and the reaction mixture was cooled to −30°C. To the cooled reaction mixture was added TfOH (50 μl, 0.57 mmol) and the reaction mixture was allowed to stir at −30°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 (2:1) as eluant to afford pure compound 11 (6.5 g, 85%) as syrup; $[\alpha]_D^{25}$+23.5 (*c* 2.0, $CDCl_3$); IR (neat): 2,923.7, 1,776.4, 1,719.1, 1,655.2, 1,603.1, 1,507.8, 1,454.3, 1,391.5, 1,265.1, 1,109.1, 1,027.5, 751.5, 713.1 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.01–7.92 (m, 6 H, Ar–H), 7.64–7.04 (m, 33 H, Ar–H), 6.90 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.68 (d, *J*=9.0 Hz, 2 H, Ar–H), 6.01–5.96 (m, 1 H, H-5″), 5.59 (d, *J*=5.0 Hz, 1 H, H-3″), 5.50 (s, 1 H, PhC*H*), 5.47 (d, *J*=8.3 Hz, 1 H, H-1′), 5.43 (d, *J*=2.9 Hz, 1 H, H-4), 5.30 (brs, 1 H, H-1″), 5.13 (brs, 1 H, H-2″), 4.84 (dd, *J*=11.1 and 8.3 Hz, 1 H, H-2′), 4.79 (d, *J*=7.1 Hz, 1 H, H-1), 4.76–4.61 (m, 5 H,

PhCH_{2a}, H-3′, H-4″, H-6″$_{ab}$), 4.51–4.47 (m, 3 H, PhCH_{2b}, PhCH_{2ab}), 4.42 (d, J=3.0 Hz, 1 H, H-4′), 4.30 (d, J=12.1 Hz, 1 H, H-6$_a$), 3.87–3.79 (m, 4 H, H-2, H-3, H-5, H-6$_b$), 3.72–3.68 (m, 4 H, H-6$_a$′ and OCH_3), 3.53 (dd, J=10.2 and 3.7 Hz, 1 H, H-6$_b$′), 3.42 (brs, 1 H, H-5′), 2.10 (s, 3 H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 170.9 (*C*OCH$_3$), 169.6 (*C*OPhth), 167.7 (*C*OPhth), 166.1 (*C*OPh), 165.6 (2 *C*OPh), 169.9 (*C*OPh), 155.2, 151.4, 138.4 (2 C), 138.1 (2 C), 137.8, 133.9, 133.5, 133.4, 133.3 (2 C), 133.2, 131.8 (2 C), 131.6 (2 C), 130.1 (2 C), 129.9 (2 C), 129.7 (2 C), 129.6 (2 C), 128.7, 128.5 (2 C), 128.5 (2 C), 128.4 (2 C), 128.3 (2 C), 128.2 (2 C), 128.1 (2 C), 128.0 (2 C), 127.7 (2 C), 127.6, 127.3 (2 C), 127.1, 126.2 (2 C), 123.6, 122.9, 118.2 (2 C), 114.4 (2 C), 107.6 (C-1″), 102.5 (C-1), 100.8 (PhCH), 98.4 (C-1′), 82.6 (C-2′), 81.6 (C-4′), 79.3 (C-3), 78.3 (C-2), 76.5 (C-3″), 75.3 (C-4′), 75.2 (C-3′), 74.8 (PhCH_2), 73.7 (PhCH_2), 73.6 (C-5), 70.22 (C-5″), 69.6 (C-6′), 69.3 (C-4), 68.8 (C-6), 66.7 (C-5′), 63.4 (C-6″), 55.6 (OCH_3), 52.2 (C-2′), 20.9 (COCH_3); ESI-MS: m/z=1,488.9 $[M+Na]^+$; Anal. Calcd. For $C_{84}H_{75}NO_{23}$ (1,465.47): C, 68.80; H, 5.15; found: C, 68.54; H, 5.40.

4-Methoxyphenyl (2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl)-(1→3)-(4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranosyl)-(1→3)-4-O-acetyl-2-O-benzyl-β-D-galactopyranoside (12) To the solution of the compound 11 (6.2 g, 4.23 mmol) in toluene-methanol (3:2; *v*/*v*, 100 ml) was added 20% $Pd(OH)_2$–C (500 mg) and the reaction medium was stirred under a positive pressure of hydrogen gas at room temperature for 2 h. The reaction mixture was filtered through a Celite® bed and concentrated under reduced pressure. The crude product was purified over SiO_2 using hexane–EtOAc (2:1) as eluant to afford pure compound 12 (4.2 g, 72%) as syrup; $[\alpha]_D^{25}$+16.5 (*c* 1.3, $CDCl_3$); IR (neat): 3,449.5, 2,925.0, 2,858.7, 1,719.9, 1,654.5, 1,508.2, 1,456.6, 1,388.3, 1,263.1, 1,107.8, 712.6 cm^{-1}; ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.0–7.9 (m, 6 H, Ar–H), 7.56–6.95 (m, 28 H, Ar–H), 6.81 (d, J=9.2 Hz, 2 H, Ar–H), 6.71 (d, J=9.2 Hz, 2 H, Ar–H), 5.99–5.94 (m, 1 H, H-5″), 5.58 (dd, J=5.5 and 1.4 Hz, 1 H, H-3″), 5.49 (s, 1 H, PhC*H*), 5.45 (d, J=8.4 Hz, 1 H, H-1′), 5.35 (brs, 1 H, H-4), 5.27 (brs, 1 H, H-1″), 5.11 (d, J=1.6 Hz, 1 H, H-2″), 4.85 (dd, J=11.1 and 8.4 Hz, 1 H, H-2′), 4.78 (d, J=7.5 Hz, 1 H, H-1), 4.71–4.61 (m, 5 H, PhCH_{2a}, H-3′, H-4″ and H6′′$_{ab}$), 4.48 (d, J=11.5 Hz, 1 H, PhCH_{2b}), 4.42 (d, J=3.2 Hz, 1 H, H-4′), 4.27 (d, J=11.7 Hz, 1 H, H-6$_a$), 3.84–3.79 (m, 3 H, H-2, H-3 and H-6$_b$), 3.69 (s, 3 H, OCH_3), 3.65–3.61 (m, 2 H, H-5 and H-6′$_a$), 3.47–3.45 (m, 2 H, H-5′, H-6′$_b$), 2.17 (s, 3 H, COCH_3); ^{13}C NMR (75 MHz, $CDCl_3$): δ 173.8 (*C*OCH$_3$), 169.6 (*C*OPhth), 167.8 (*C*OPhth), 166.1 (*C*OPh), 165.6 (2 *C*OPh), 164.9 (*C*OPh), 155.3, 151.2, 138.3 (2 C), 137.8 (2 C), 133.9, 133.5 (2 C), 133.4 (2 C), 133.3 (2 C), 131.7 (2 C), 131.5 (2 C), 130.0 (2 C), 129.9 (3 C), 129.7 (3 C), 129.6 (2 C), 128.7, 128.5 (2 C), 128.5 (2 C), 128.4 (2 C), 128.2 (2 C), 128.0 (2 C), 126.9 (2 C), 126.1 (2 C), 123.6, 123.0, 118.3 (2 C), 114.5 (2 C), 107.6 (C-1″), 102.5 (C-1), 100.6 (PhCH), 98.9 (C-1′), 82.6 (C-2′′), 81.6 (C-4″), 80.4 (C-3), 77.9 (C-2), 76.6 (C-3″), 75.2 (C-3′), 75.1 (C-4′), 74.9 (PhCH_2), 73.1 (C-5), 70.2 (C-5″), 69.5 (C-4), 68.7 (C-6), 66.7 (C-5′), 63.4 (C-6″), 59.9 (C-6′), 55.6 (OCH_3), 52.0 (C-2′), 21.1 (COCH_3); ESI-MS: m/z=1,398.4 $[M+Na]^+$; Anal. Calcd. For $C_{77}H_{69}NO_{23}$ (1,375.43): C, 67.19; H, 5.05; found: C, 66.94; H, 5.27.

4-Methoxyphenyl (2,3,5,6-tetra-O-benzoyl-β-D-galactofuranosyl)-(1→3)-(4,6-O-benzylidene-2-deoxy-2-N-phthalimido-β-D-galactopyranosyl)-(1→3)-[methyl 5-acetamido-4,7,8,9-tetra-O-acetyl-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)]-4-O-acetyl-2-O-benzyl-β-D-galactopyranoside (14) To a solution of compound 12 (4.0 g, 2.9 mmol) and ethyl thioglycoside donor 13 (8.4 g, 5.82 mmol) in anhydrous CH_3CN–CH_2Cl_2 (5:1; *v*/*v*; 50 ml) was added freshly activated powdered MS 4 Å (5 g) and the reaction mixture was allowed to stir under argon at room temperature for 1 h. *N*-Iodosuccinimide (1.6 g, 7.11 mmol) was added to the reaction mixture and the reaction mixture was cooled to −20°C. To the cooled reaction mixture was added TfOH (40 μl, 0.45 mmol) and the reaction mixture was allowed to stir at 0°C for 12 h. 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× 50 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 (1:2) as eluant to afford pure compound 14 (2.8 g, 52%) as syrup; $[\alpha]_D^{25}$+39.6 (*c* 1.3, $CDCl_3$); IR (neat): cm^{-1}. ^{1}H NMR (600 MHz, $CDCl_3$): δ 7.99 (d, 2 H, J=7.2 Hz, Ar–H), 7.93–7.91 (m, 4 H, Ar–H), 7.67 (d, J=7.2 Hz, 1 H, Ar–H), 7.63 (d, J=6.6 Hz, 1 H, Ar–H), 7.59–7.42 (m, 10 H, Ar–H), 7.37 (t, J=7.8 Hz, 2 H, Ar–H), 7.32–7.26 (m, 4 H, Ar–H), 7.20–7.14 (m, 8 H, Ar–H), 7.05 (t, J=7.8 Hz, 2 H, Ar–H), 6.94 (d, J=9.0 Hz, 2 H, Ar–H), 6.75 (d, J=9.0 Hz, 2 H, Ar–H), 5.95 (m, 1 H, H-5″), 5.56 (d, J=4.8 Hz, 1 H, H-3″), 5.49 (d, J=8.4 Hz, 1 H, H-1′), 5.47 (s, 1 H, PhC*H*), 5.41 (d, J=3.6 Hz, 1 H, H-4), 5.33–5.32 (m, 1 H, H-7‴), 5.29–5.28 (m, 2 H, H-1″ and H-8‴), 5.23 (d, J=9.6 Hz, 1 H, N*H*COCH$_3$), 5.12 (brs, 1 H, H-2″), 4.93–4.87 (m, 1 H, H-4‴), 4.80 (dd, J=10.8 and 7.8 Hz, 1 H, H-2′), 4.78 (d, J=10.8 Hz, 1 H, PhCH_{2a}), 4.75 (d, J=7.8 Hz, 1 H, H-1), 4.70 (dd, J=10.8 and 3.6 Hz, 1 H, H-3′), 4.67–4.61 (m, 3 H, H-4″and H-6$_{ab}$″), 4.50 (d, J=11.4 Hz, 1 H, PhCH_{2b}), 4.39 (d, J=3.6 Hz, 1 H, H-4′), 4.28 (dd, J=13.2 and 3.0 Hz, 1 H, H-9$_a$‴), 4.25 (d, J=12.0 Hz, 1 H, H-6$_a$), 4.09–4.06 (m, 2 H, H-6$_a$′ and H-9$_b$‴), 4.01 (m, 1 H, H-5‴), 3.92 (dd, J=9.6 and 3.6 Hz, 1 H, H-6‴), 3.82–3.72 (m, 10 H, H-2, H-3, H-6$_b$, H-6$_b$′,

OCH_3 and COOCH_3), 3.48 (m, 1 H, H-5), 3.33 (m, 1 H, H-5′), 2.55 (dd, J=11.8 and 3.9 Hz, 1 H, H-3$_e$‴), 2.10, 2.05, 2.04, 2.01, 1.98 (5 s, 15 H, 5 COCH_3), 1.95–1.91 (t, J= 11.9 Hz, 1 H, H-3$_a$‴), 1.86 (s, 3 H, NHCOCH_3); ^{13}C NMR (150 MHz, $CDCl_3$): δ 170.9, 170.6 (2 C), 170.2 (2 C), 169.7 (2 C), 167.9, 167.5, 166.1, 165.6, 164.9 (2 C), 155.2, 151.5, 138.5–114.3 (Ar–C), 107.5 (C-1″), 102.6 (C-1), 100.7 (PhCH), 98.5 (C-2‴), 97.8 (C-1′), 82.5 (C-2″), 81.6 (C-4″), 78.6 (C-3), 77.9 (C-5), 76.5 (C-3″), 75.4 (C-3′), 75.1 (C-4′), 74.7 (PhCH_2), 72.6 (C-2), 72.5 (C-5‴), 70.1 (C-5″), 68.9 (C-7‴), 68.8 (C-4), 68.6 (C-4‴), 68.5 (C-6), 67.2 (C-8‴), 66.6 (C-5′), 63.6 (C-6″), 63.4 (C-6′), 62.3 (C-9‴), 55.6 (OCH_3), 52.9 (OCH_3), 52.3 (C-2′), 49.5 (C-6‴), 37.5 (C-3‴), 23.2 (NHCOCH_3), 20.9–20.7 (5 COCH_3); ESI-MS: m/z=1,871.4 $[M+Na]^+$; Anal. Calcd. For $C_{97}H_{96}N_2O_{35}$ (1,848.58): C, 62.98; H, 5.23; found: C, 62.72; H, 5.64.

4-Methoxyphenyl (β-D-galactofuranosyl)-(1→3)-(2-acetamido-2-deoxy-β-D-galactopyranosyl)-(1→3)-[sodium 5-acetamido-3,5-dideoxy-D-glycero-α-D-galacto-2-nonulopyranosylonate)-(2→6)]-4-O-acetyl-2-O-benzyl-β-D-galactopyranoside (1) To a solution of tetrasaccharide derivative 14 (2 g, 1.08 mmol) in methanol (30 ml) was added 20% $Pd(OH)_2$–C (500 mg) and the reaction mixture was allowed to stir at room temperature for 24 h under a positive pressure of hydrogen. The reaction mixture was filtered through a Celite® bed and concentrated under reduced pressure. To a solution of the dry mass in ethanol (50 ml) was added hydrazine monohydrate (1 ml) and the reaction mixture was allowed to stir at 80°C for 6 h. The solvents were removed under reduced pressure and the crude product was acetylated using a mixture of acetic anhydride–pyridine (2:1; v/v; 20 ml) at room temperature. The solvents were removed under reduced pressure and the crude mass was dissolved in 0.1 M sodium methoxide (50 ml) and the reaction mixture was allowed to stir at room temperature for 8 h and then a few drops of distilled water was added to the reaction mixture and allowed to stir for overnight. The reaction mixture was neutralized with Dowex 50W X8 (H^+) resin, filtered and evaporated to dryness and again passed through a short pad of Dowex 50W X8 (Na^+) resin. The crude product was purified by passing through a column of Sephadex-LH-20 using CH_3OH–H_2O (4:1) as eluant to give tetrasaccharide 1 as its sodium salt (710 mg, 68%) as a white powder. $[\alpha]_D^{25}$+18 (c 1.3, H_2O); IR (KBr): 2,356, 1,692, 1,218, 790 cm^{-1}; ^{1}H NMR (300 MHz, D_2O): δ 7.14 (d, J=9.0 Hz, 2 H, Ar–H), 7.00 (d, J=9.0 Hz, 2 H, Ar–H), 5.18 (brs, 1 H, H-1″), 4.97 (d, J=6.8 Hz, 1 H, H-1′), 4.89 (d, J=8.4 Hz, 1 H, H-1), 4.31–4.26 (3 H, m, H-2″, H-4′ and H-8‴), 4.12–4.07 (m, 3 H, H-3″, H-4 and H-7‴), 3.99–3.90 (m, 5 H, H-2, H-2′, H-3, H-4″and H-6‴), 3.87–3.56 (m, 17 H, H-3′, H-4‴, H-5, H-5′, H-5″, H-5‴, H-6$_{ab}$, H-6′$_{ab}$, H-6″$_{ab}$, H-9‴$_{ab}$ and OCH_3), 2.76 (dd, J=12.4 and 3.4 Hz, 1 H, H-3$_e$‴), 2.10, 2.04 (2 s, 6 H, 2 NHOCCH_3), 1.68 (t, J=12.2 Hz, 1 H, H-3$_a$‴); ^{13}CNMR (75 MHz, D_2O): δ 174.4 (COONa), 172.5 (2 COCH_3), 153.9, 150.4, 117.5 (2 C), 114.4 (2 C), 108.7 (C-1″), 102.1 (C-1), 101.0 (C-1′), 99.6 (C-2‴), 82.2, 81.1, 81.0, 77.7, 76.4, 75.6, 74.7, 74.2 (C-6‴), 72.7, 72.1, 71.0, 70.1, 69.8, 69.2 (C-4‴), 67.8, 67.6, 67.5, 66.5, 62.3 (C-6″), 62.1 (C-6 and C-9‴), 60.4 (C-6′), 55.2 (OCH_3), 51.4 (C-2′), 51.3 (C-5‴), 39.5 (C-3‴), 21.5 (2 COCH_3); ESI-MS: m/z= 965.4 $[M+1]^+$; Anal. Calcd. For $C_{38}H_{57}N_2O_{25}Na$ (964.31): C, 47.30; H, 5.95; found: C, 47.0; H, 6.22.

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