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Synthesis of oligosaccharides corresponding to the polysaccharides of *Lactobacillus* and *Thermophlilus* strains[1]

Pintu Kumar Mandal, Anup Kumar Misra*

Medicinal and Process Chemistry Division, Central Drug Research Institute, Chattar Manzil
Palace, Lucknow 226001, UP, IndiFax: +91-522-2223938, +91-522-2223405E-mail:
akmisra69@rediffmail.com

Abstract: Synthesis of a branched trisaccharide and a tetrasaccharide repeating units corresponding to the polysaccharides of *Lactobacillus* spp. G-77 and *Thermus thermophilus* Samu-SA1 as their methyl glycosides has been achieved in excellent yield. Most of the glycosyl linkages are 1,2-*cis* in these oligosaccharide fragments.

Key words: Oligosaccharides, glycosides, glycosylation, *Thermus thermophilus*, *Lactobacillus* *s*sp. G-77.

A wide variety of exopolysaccharides (EPS's) produced by Lactic acid bacteria (LAB) present in the body provide an important contribution to human health by acting as prebiotic substrates, nutraceuticals, cholesterol lowering agents or immunomodulants.[2] Recently it has been observed that EPS produced by lactic acid bacteria have suitable rheological properties for the dairy industry.[3] In wine industry, the polysaccharides produced by LAB can cause the alteration known as oiliness or ropiness by increasing the consistency of the beverage. Till to date a number of bacteria known to produce exopolysaccharides in ciders and wines and some of their structures of EPS have been elucidated.[4] The structure of a ropy strain of *Lactobacillus* spp. G-77 has been characterized recently,[5] which contains a unique 1,2-*cis* linked branched trisaccharide (Figure 1).

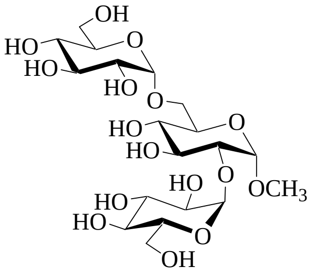
Another heat stable bacteria belong to genus *Thermus*, which are able to grow at extremely elevated temperature. Some of these species has an outer membrane in the cell envelope and can be classified as Gram-negative bacteria.[6] In contrast to the Gram-negative bacteria the outer membrane of *Thermus* does not composed of lipopolysaccharides but some unique glycolipids.[7] It is believed that the glycolipids present in the outer layer of cell envelope have an important role in adaptation to high temperature.[8] Recently, the structure of a tetrasaccharide repeating unit of the glycolipid isolated from *Thermus thermophilus* Samu-SA1, a thermohalophilic bacterium has been demonstrated[9] in which a unique D-galactofuranosyl moiety present at the non reducing end through an α-linkage (Figure 1).

In order to analyze and assess the role of EPS produced by *Lactobacillus* spp. G-77 and glycolipids in the adaptation process of *Thermus thermophilus*, a reasonable quantity of the tri- and tetrasaccharide corresponding to them is required. Recently, we are engaged in synthesizing some oligosaccharide fragments corresponding to lactic acid bacteria and some heat stable bacteria for their evaluation in several biological studies.[10] We herein report a concise synthesis of a unique trisaccharide and a tetrasaccharide as their methyl glycosides corresponding to the *Lactobacillus* spp. G-77 and *Thermus thermophilus* Samu-SA1 respectively (Figure 2).

→6)-α-D-Glc*p*-(1→6)-α-D-Glc*p*-(1→
2

↑
1
α-D-Glc*p*
A
→)-α-D-Gal*f*-(1→2)-α-D-Gal*p*-(1→6)-β-D-Glc*p*NAc
1
↓
2
α-D-Glc*p*-(1→
B

Figure 1: (A) Trisaccharide repeating unit of the exopolysaccharide produced by *Lactobacillus* spp. G-77 and (B) tetrasaccharide repeating unit of the glycolipid of *Thermus thermophilus* Samu-SA1.



1

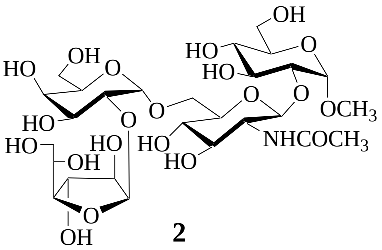


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Figure 2: Synthesized trisaccharide (**1**) and tetrasaccharide (**2**) as their methyl glycosides.

In order to synthesize target tri- and tetrasaccharides (**1** and **2**) a series of differentially protected monosaccharide derivatives were prepared from commercially available reducing sugars (Scheme 1). Phenyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside (**5**)[11] was prepared from D-glucose (**3**) following two recently developed methodologies from our laboratory such as, one-pot acetylation-thioglycosidation[12] and one-pot deacetylation-benzylation.[13] Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (**8**)[14] was prepared from methyl α-D-glucopyranoside (**6**) in two steps consisting of benzylidenation[15] and selective benzylation through the formation of stannylidene acetal.[16] Ethyl 3,4-di-*O*-acetyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (**11**) was prepared from ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (**9**) in three steps involving de-acetylation,[17] selective silyl group protection[18] and acetylation. Phenyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (**15**) was prepared from phenyl 2,3,4,6-tetra-*O*-acetyl-1-thio-β-D-galactopyranoside (**12**) in four steps involving de-acetylation, benzylidene acetal formation,[15] selective benzylation through stannylidene acetal formation[16] and 4-methoxybenzylation. Ethyl 2,3,5,6-tetra-*O*-benzyl-1-thio-α-D-galactofuranoside (**18**) was prepared from D-galactose diethyldithioacetal (**16**) in a two step reaction sequence involving mercury catalyzed thiofuranoside formation [19] and per-*O*-benzylation using benzyl bromide in the presence of sodium hydroxide.

Having prepared a number of suitably functionalized monosaccharide intermediates, synthesis of the trisaccharide **1** consisting of all 1,2-*cis* linked D-glucose moieties was attempted, which is presented in scheme 2. Glycosylation of compound **5** with compound **8** using *N*-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)[20] furnished disaccharide **19** in 80% yield. Formation of compound **19** was confirmed by its NMR and mass spectral analysis. Removal of benzylidene acetal in compound **19** using perchloric acid supported over silica gel ($HClO_4$-SiO_2)[21] under a modified protocol developed in our laboratory afforded disaccharide diol **20** in 90% yield. Selective glycosylation of disaccharide diol **20** with thioglycoside derivative **5** using NIS-TMSOTf [20] furnished trisaccharide derivative **21** in 73% yield. Global debenzylation[22] of trisaccharide derivative **21** under hydrogenation using H_2/20% $Pd(OH)_2$-C gave target trisaccharide **1** in 80% yield. Presence of three anomeric protons in the ^1H NMR [δ 5.06 (d, J = 3.6 Hz, 2 H) and 4.94 (d, J = 3.6 Hz, 1 H)] and ^{13}C NMR (δ 96.7 (2 C) and 96.4) spectra of trisaccharide **1** supported that all monosaccharide derivatives are linked together by 1,2-*cis* linkages (Scheme 2). The key features of this synthetic sequence are the use of similar protecting groups and high yield in each step.

In another set experiments, methyl α-D-galactofuranosyl-(1→2)-α-D-galactopyranosyl-(1→6)-2-acetamido-2-deoxy-β-D-glucopyranosyl-(1→2)-α-D-glucopyranoside (**2**) was synthesized following Scheme 3. Condensation of compound **8** with compound **11** in the presence of NIS-TMSOTf furnished disaccharide **22** in 78% yield. Removal of *tert*-butyldimethylsilyl group[23] using tetrabutylammonium fluoride in THF-acetic acid afforded disaccharide acceptor **23** in 75% yield. Sequential glycosylation of compound **23** with thioglycoside donor **15** and removal of 4-methoxybenzyl group was achieved in one-pot[24] in the presence of NIS-TMSOTf to furnish trisaccharide acceptor **24** in 74% yield. Final glycosylation of trisaccharide derivative **24** with thioglycoside donor **18** in the presence NIS-TMSOTf afforded the tetrasaccharide derivative **25** in 70% yield. Removal of *N*-phthalimido by the treatment of hydrazine hydrate [25] and *N*-acetylation followed by hydrogenolysis over Pearlman's catalyst[22] afforded pure tetrasaccharide **2** as its methyl glycoside in 76% yield. Presence of signals for four anomeric protons [δ 5.81 (br s, 1 H), 5.29 (br s, 1 H), 5.10 (br s, 1 H), 4.82 (d, J = 8.4 Hz, 1 H)] in the ^1H NMR and four anomeric carbons [δ 109.3, 100.1, 99.1 and 98.4] in the ^{13}C NMR spectra confirmed the formation of the tetrasaccharide **2**. The key features of the scheme 3 include glycosylation and removal of 4-methoxybenzyl group in one-pot without addition of any extra reagent and high yields in glycosylations.

In summary, the synthesis of a branched trisaccharide corresponding to the repeating unit of the exopolysaccharide produced by *Lactobacillus* spp. G-77 and a tetrasaccharide corresponding to the cell-wall glycolipid of heat stable *Thermus thermophilus* Samu-SA1 as their methyl glycosides has been achieved in high yield. Most of the monosaccharide moieties are linked together through 1,2-*cis* glycosyl linkages, glycosylation and removal of 4-methoxybenzyl group was achieved in one pot without addition of extra reagent, yields were excellent, and a general glycosylation reaction condition has been applied.

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 was recorded on Brucker Advance DPX 200 and 300 MHz using $CDCl_3$ and D_2O as solvents and TMS as internal reference and acetone as external reference. 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 3,4-di-*O*-acetyl-6-*O*-*tert*butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-1-thio-β-D-glucopyranoside (11): A solution of ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**9**; 4.0 g, 8.34 mmol) in 0.05 M sodium methoxide in CH_3OH (50 mL) was allowed to stir at room temperature for 20 min and neutralized with Amberlite IR-120 (H^+) resin. The reaction mixture was filtered and evaporated to dryness. To a solution of the crude mass in pyridine (30 mL) were added *tert*-butyldimethylsilylchloride (1.4 g, 9.4 mmol) and DMAP (100 mg) and the reaction mixture was allowed to stir at 50 °C for 6 h. After complete consumption of the starting material, Ac_2O (20 mL) was added to the reaction mixture and allowed to stir at room temperature for 6 h. The solvents were removed under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (5:1) as eluant to furnish pure compound **11** (4.14 g, 90%).

Syrup.

$[\alpha]_D^{25}$ + 41.5 (*c* 1.5, $CHCl_3$).

IR (neat): 2367, 1594, 1383, 1351, 769 cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ 7.78–7.65 (m, 4 H, ArH), 5.76 (t, *J* = 10.5 and 10.5 Hz, 1 H, H-3), 5.35 (d, *J* = 10.5 Hz, 1 H, H-1), 5.06 (t, *J* = 10.0 and 10.0 Hz, 1 H, H-4), 4.26 (t, *J* = 10.5 and 10.5 Hz, 1 H, H-2), 3.72–3.64 (m, 3 H, H-5 and H-6_{ab}), 2.75–2.45 (m, 2 H, $SC\underline{H}_2CH_3$), 1.95, 1.79 (2 s, 6 H, 2 $COC\underline{H}_3$), 1.15 (t, *J* = 7.4 and 7.4 Hz, 3 H, $SCH_2C\underline{H}_3$), 0.84 (s, 9 H, $C(C\underline{H}_3)_3$), 0.02, 0.01 (2 s, 6 H, -$Si(C\underline{H}_3)_2$-).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.3, 169.4 (2 $\underline{C}OCH_3$), 167.9, 167.4 (2 $\underline{C}O$, Phth), 134.5-124.0 (Ar-C), 80.8 (C-1), 79.3, 72.2, 69.8, 63.0 (C-6), 54.1 ($O\underline{C}H_3$), 26.3 (3 C, $C(\underline{C}H_3)_3$), 23.9 ($S\underline{C}H_2CH_3$), 21.0, 20.8 ($2CO\underline{C}H_3$), 18.7 ($\underline{C}(CH_3)_3$), 15.3 ($SCH_2\underline{C}H_3$), –4.9 (2 C, -$Si(\underline{C}H_3)_2$-).

ESI-MS: *m/z* = 574.2 $[M+Na]^+$.

Anal. Calcd. for $C_{26}H_{37}NO_8SSi$ (551.2): C, 56.60; H, 6.76%; found: C, 56.42; H, 6.95%.

Phenyl 2-*O*-(4-methoxybenzyl)-3-*O*-benzyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (15): To a solution of compound **14** (3.0 g, 6.6 mmol) in dry THF (25 mL) were added powdered NaOH (800 mg, 20 mmol), 4-methoxybenzyl chloride (1.4 mL, 9.9 mmol) and tetrabutylammonium bromide (100 mg) and the reaction mixture was allowed to stir at room temperature for 4 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (3 x 50 mL). The organic layer was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give the crude product, which was purified over SiO_2 using hexane-EtOAc (7:1) as eluant to afford pure compound **15** (3.42 g, 90%).

Syrup.

$[\alpha]_D^{25}$ – 28.4 (*c* 1.5, $CHCl_3$).

IR (neat): 2922, 1592, 1453, 1351, 1252, 1165, 1054, 734, 695 cm^{-1}.

^1H NMR ($CDCl_3$, 300 MHz): δ 7.73-6.86 (m, 19 H, Ar H), 5.49 (s, 1 H, $PhC\underline{H}$), 4.74 (Abq, *J* = 12.3 Hz, 2 H, 4 $MeOPhC\underline{H}_2$), 4.65 (br s, 2 H, $PhC\underline{H}_2$), 4.59 (d, *J* = 9.5 Hz, 1 H, H-1), 4.38 (dd, *J* = 12.5 and 1.4 Hz, 1 H, H-6_a), 4.14 (d, *J* = 3.1 Hz, 1 H, H-4), 3.99 (dd, *J* = 12.2 and 1.4 Hz, 1 H, H-6_b), 3.90 (t, *J* = 9.4, 9.4 Hz, 1 H, H-2), 3.83 (s, 3 H, $OC\underline{H}_3$), 3.60 (dd, *J* = 9.2 and 3.4 Hz, 1 H, H-3), 3.41 (m, 1 H, H-5). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 159.2, 138.2-126.6 (Ar-C), 113.7, 101.2 ($Ph\underline{C}H$), 86.6 (C-1), 81.4, 75.2, 75.1 ($Ph\underline{C}H_2$), 73.8, 71.8 ($Ph\underline{C}H_2$), 69.8, 69.4 (C-6), 55.2 ($O\underline{C}H_3$).

ESI-MS: *m/z* = 588.2 $[M+NH_4]^+$.

Anal. Calcd. for $C_{34}H_{34}O_6S$ (570.2): C, 71.56; H, 6.0%; found: C, 71.34; H, 6.24%.

Ethyl 2,3,5,6-tetra-*O*-benzyl-1-thio-α-D-galactofurano-side (18): To a solution of diethyl D-galactose dithioacetal (**16**; 5.0 g, 17.5 mmol) in water (100 mL) was added a solution of mercuric chloride (9.9 g, 20.9 mmol) in water (70 mL). A white precipitate was formed instantaneously. To the resulting reaction mixture 1 N NaOH was added till the pH of the solution reached ~6. The reaction mixture was allowed to stir at room temperature for 2 h and was then filtered. The filtrate was made alkaline by addition of ammonia solution and the residue was concentrated to a dry mass and the reaction mixture was extracted with CH_2Cl_2 (250 mL). The organic layer was washed with water, dried (Na_2SO_4) and concentrated under reduced pressure to give crude ethyl 1-thio-α-D-galactofuranoside (**17**). To a solution of crude product **17** in THF (25 mL) were added benzyl bromide (12.5 mL, 105 mmol), powdered NaOH (5.6 g, 140 mmol) and tetrabutylammonium bromide (100 mg) and the reaction mixture was allowed to stir at room temperature for 6 h. The excess reagents were quenched by addition of methanol (2 mL) and the resulting solution was concentrated under reduced pressure. The crude mass was dissolved in dichloromethane (100 mL) and the organic layer was washed with water, dried (Na_2SO_4), and concentrated under reduced pressure. Purification of the crude reaction product over SiO_2 using hexane–EtOAc (12:1) as the eluant furnished pure compound **18** (8.7 g, 85%).

Syrup.

$[\alpha]_D^{25}$ + 37.2 (*c* 1.5, $CHCl_3$).

IR (neat): 2922, 2867, 1743, 1707, 1495, 1454, 1362, 1208, 1099, 739, 698 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.40–7.20 (m, 20 H, Ar-H), 5.38 (d, *J* = 4.7 Hz, 1 H, H-1), 4.82–4.71 (AB_q, *J* = 11.7 Hz, 2 H, $PhC\underline{H}_2$), 4.62 (d, *J* = 11.7 Hz, 1 H, $PhC\underline{H}_2$), 4.54–4.46 (m, 4 H, 2 $PhC\underline{H}_2$), 4.42 (d, *J* = 11.7 Hz, 1 H, $PhC\underline{H}_2$), 4.19–4.13 (m, 2 H, H-3 and H-4), 4.06 (dd, *J* = 6.6 and 4.7 Hz, 1 H, H-2), 3.93–3.88 (m, 1 H, H-5), 3.65 (dd, *J* = 10.5 and 4.1 Hz, 1 H, H-6_a), 3.57 (dd, *J* = 10.5, 5.9 Hz, 1 H, H-6_b), 2.74 (dd, *J* = 14.7 and 7.3 Hz, 2 H, $SC\underline{H}_2CH_3$), 1.33 (t, *J* = 7.4 Hz, 3 H, $SCH_2C\underline{H}_3$).

^{13}CNMR ($CDCl_3$, 75MHz): δ 139.3–127.3 (Ar-C), 87.0 (C-1), 84.7, 84.0, 82.4, 78.8, 76.1, 73.6, 72.6, 72.1 (4 $Ph\underline{C}H_2$), 71.1 (C-6), 25.3 ($S\underline{C}H_2CH_3$), 15.6 ($SCH_2\underline{C}H_3$).

ESI-MS: *m/z* = 602.2 $[M+NH_4]^+$.

Anal. Calcd. for $C_{36}H_{40}O_5S$ (584.2): C, 73.94; H, 6.89%; found: C, 73.72; H, 7.15%.

Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyrano-side (19): To a solution of compound **5** (5.1 g, 8.0 mmol) and compound **8** (2.5 g, 6.7 mmol) in CH_2Cl_2 (20 mL) was added 4Å MS (2 g) and the reaction mixture was stirred under argon for 30 min at room temperature. *N*-Iodosuccinimide (2.2 g, 9.6 mmol) was added to the reaction mixture and it was cooled to –45 °C. To the cooled reaction mixture trimethylsilyl trifluoromethanesulfonate (50 µL, 0.27 mmol) was injected and stirring continued at –45 °C for 45 min. The reaction mixture was filtered through a celite bed. The filtrate was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$, water, dried (Na_2SO_4) and concentrated to a syrup. Column chromatography of the crude product over SiO_2 using hexane-EtOAc (5:1) furnished pure compound **19** (4.8 g, 80%).

Syrup.

$[\alpha]_D^{25}$ + 37.6 (*c* 1.5, $CHCl_3$).

IR (neat): 1600, 1595, 1382, 1352, 1059, 696 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.55–7.03 (m, 30 H, Ar-H), 5.57 (s, 1 H, $PhC\underline{H}$), 5.05 (d, *J* = 11.0 Hz, 1 H, $PhC\underline{H}_2$), 4.97 (d, *J* = 3.6 Hz, 1 H, H-1), 4.91–4.85 (m, 2 H, $PhC\underline{H}_2$), 4.83–4.81 (m, 2 H, $PhC\underline{H}_2$), 4.80 (d, *J* = 1.6 Hz, 1 H, H-1′), 4.76–4.70 (m, 2 H, $PhC\underline{H}_2$), 4.59–4.48 (m, 2 H, $PhC\underline{H}_2$), 4.34 (d, *J* = 12.0 Hz, 1 H, $PhC\underline{H}_2$), 4.30 (t, *J* = 8.4 Hz, 1 H, H-3), 4.16 (dt, *J* = 9.3, 1.4 Hz, 2 H, H-3′ and H-4′), 3.92–3.85 (ddd, *J* = 9.5, 3.7 Hz, 2 H, H-2 and H-2′), 3.81 (d, *J* = 10.1 Hz, 1 H, H-6_a), 3.74 (d, *J* = 10.4 Hz, 1 H, H-$6'_a$), 3.65–3.58 (m, 2 H, H-6_b and H-$6'_b$), 3.53–3.49 (m, 1 H, H-4), 3.48 (s, 3 H, $OC\underline{H}_3$), 3.45–3.39 (m, 2 H, H-5 and H-5′).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 139.1–126.2 (Ar-C), 101.5 ($Ph\underline{C}H$), 97.5 (C-1), 94.5 (C-1′), 82.7, 82.2, 79.4, 77.7, 76.9, 75.9 ($Ph\underline{C}H_2$), 75.7 ($Ph\underline{C}H_2$), 75.1 ($Ph\underline{C}H_2$), 74.6, 73.3 ($Ph\underline{C}H_2$), 73.0 ($Ph\underline{C}H_2$), 70.2, 69.2 (C-6′), 68.4 (C-6), 62.5, 55.2 ($O\underline{C}H_3$).

ESI-MS: *m/z* = 917.6 $[M+Na]^+$.

Anal. Calcd. for $C_{55}H_{58}O_{11}$ (894.4): C, 73.81; H, 6.53%; found: C, 73.60; H, 6.90%.

Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→2)-3-*O*-benzyl-α-D-glucopyranoside (20): To a solution of compound **19** (4 g, 4.5 mmol) in CH_3CN (50 mL) was added $HClO_4$-SiO_2 (1 g) and reaction mixture was stirred at room temperature for 30 min. The reaction mixture was filtered through a celite bed and evaporated to dryness. Column chromatography of the crude product over silica gel using hexane-EtOAc (2:1) as eluant afforded pure compound **20** (3.24 g, 90%).

Syrup.

$[\alpha]_D^{25}$ + 33.7 (*c* 1.5, $CHCl_3$).

IR (neat): 2926, 2366, 1596, 1459, 1352, 1070, 743 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.41–7.11 (m, 25 H, Ar-H), 5.04–4.96 (m, 2 H, PhC$\underline{H}_2$), 4.92 (d, *J* = 3.0 Hz, 1 H, H-1), 4.87–4.82 (m, 2 H, PhC$\underline{H}_2$), 4.77–4.68 (m, 4 H, H-1′ and PhC$\underline{H}_2$), 4.59 (d, *J* = 12.0 Hz, 1 H, PhC$\underline{H}_2$), 4.51 (d, *J* = 11.6 Hz, 1 H, PHC$\underline{H}_2$), 4.40 (d, *J* = 12.0 Hz, 1 H, PhC$\underline{H}_2$), 4.10–3.97 (m, 2 H, H-3′ and H-4′), 3.92 (t, *J* = 9.0, 9.0 Hz, 1 H, H-4), 3.85–3.75 (m, 2 H, H-2 and H-2′), 3.71–3.52 (m, 5 H, H-3, H-6$_{ab}$ and H-6′$_{ab}$), 3.47 (s, 3 H, OC$\underline{H}_3$), 3.45–3.41 (m, 2 H, H-5 and H-5′).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 138.9–127.6 (Ar-C), 96.8 (C-1), 94.0 (C-1′), 81.9, 80.4, 79.2, 77.6, 75.9 (Ph$\underline{C}H_2$), 75.5 (Ph$\underline{C}H_2$), 74.9 (Ph$\underline{C}H_2$), 74.5, 73.3 (Ph$\underline{C}H_2$), 72.7 (Ph$\underline{C}H_2$), 70.9, 70.7, 70.3, 68.3 (C-6′), 62.4 (C-6), 54.9 (O$\underline{C}H_3$).

ESI-MS: *m/z*: 829.6 $[M+Na]^+$.

Anal. Calcd. for $C_{48}H_{54}O_{11}$ (806.4): C, 71.44; H, 6.75%; found: C, 71.20; H, 7.0%.

Methyl (2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-(1→6)-[2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl-(1→2)]-3-*O*-benzyl-α-D-glucopyranoside (21): To a solution of compound **5** (2.2 g, 3.4 mmol) and compound **20** (2.5 g, 3.1 mmol) in CH_2Cl_2 (25 mL) was added 4Å MS (3 g) and the reaction mixture was stirred under argon for 30 min at room temperature. *N*-Iodosuccinimide (925 mg, 4.1 mmol) was added to the reaction mixture and it was cooled to –45 °C. To the cooled reaction mixture trimethylsilyl trifluoromethanesulfonate (20 µL, 0.11 mmol) was injected and stirring continued at –45 °C for 30 min. The reaction mixture was filtered through a celite bed. The filtrate was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$, water, dried (Na_2SO_4) and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 using hexane-EtOAc (5:1) furnished pure compound **21** (3 g, 73%).

Syrup.

$[\alpha]_D^{25}$ + 34.2 (*c* 1.5, $CHCl_3$).

IR (neat): 1595, 1351, 1066, 697 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.42–7.14 (m, 45 H, Ar-H), 5.06–5.0 (m, 3 H, PhC$\underline{H}_2$), 4.98–4.95 (m, 2 H, PhC$\underline{H}_2$), 4.93–4.83 (m, 5 H, PhC$\underline{H}_2$), 4.82–4.74 (m, 4 H, H-1 and PhC$\underline{H}_2$), 4.70 (d, *J* = 2.0 Hz, 1 H, H-1″), 4.66 (d, *J* = 3.0 Hz, 1 H, H-1′), 4.60–4.50 (m, 5 H, PhC$\underline{H}_2$), 4.40–4.35 (t, *J* = 10.3 Hz, 1 H, H-4), 4.14–3.98 (m, 2 H, H-3′ and H-4′), 3.97– 3.91 (m, 2 H, H-2 and H-2′), 3.88–3.81 (m, 4 H, H-3, H-3″, H-4″ and H-2″), 3.76–3.66 (m, 5 H, H-6″$_a$, H-6′$_{ab,}$ H-6$_{ab}$), 3.64–3.53 (m, 4 H, H-5, H-5′, H-5″ and H-6″$_b$), 3.50 (s, 3 H, OC$\underline{H}_3$). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 138.1–127.9 (Ar-C), 97.6 (C-1″), 96.4 (C-1), 94.0 (C-1′), 82.0, 81.9, 80.4, 80.2, 80.0, 79.3, 77.7, 77.6, 76.0, 75.9, 75.5, 75.0, 74.9, 74.8, 74.4, 73.5, 73.4, 73.3 (9 Ph$\underline{C}H_2$), 70.5, 70.2, 69.6, 68.8 (C-6″), 68.6 (C-6), 68.2 (C-6′), 54.9 (O$\underline{C}H_3$).

ESI-MS: *m/z* = 1351.6 $[M+Na]^+$.

Anal. Calcd. for $C_{82}H_{88}O_{16}$ (1328.6): C, 74.08; H, 6.67%; found: C, 73.84; H, 6.95%.

Methyl (3,4-di-*O*-acetyl-6-*O*-*tert*butyldimethylsilyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (22): To a solution of compound **8** (2.5 g, 6.7 mmol) and compound **11** (4.8 g, 8.7 mmol) in CH_2Cl_2 (25 mL) was added 4Å MS (3 g) and the reaction mixture was stirred under argon for 30 min at room temperature. *N*-Iodosuccinimide (2.3 g, 10.2 mmol) was added to the reaction mixture and it was cooled to –45 °C. To the cooled reaction mixture trimethylsilyl trifluoromethanesulfonate (40 µL, 0.22 mmol) was injected and stirring continued at –45 °C for 30 min. The reaction mixture was filtered through a celite bed. The filtrate was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$, water, dried (Na_2SO_4) and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 using hexane-EtOAc (5:1) furnished pure compound **22** (4.5 g, 78%).

Syrup.

$[\alpha]_D^{25}$ + 24.2 (*c* 1.5, $CHCl_3$).

IR (neat): 2932, 1753, 1719, 1597, 1461, 1384, 1355, 1237, 1052, 761 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.76–6.89 (m, 14 H, ArH), 5.77 (dd, *J* = 9.0 and 9.0 Hz, 1 H, H-3′), 5.62 (d, *J* = 8.4 Hz, 1 H, H-1′), 5.41 (s,1 H, PhC<u>H</u>), 5.13 (t, *J* = 9.3 and 9.3 Hz, 1 H, H-4′), 4.95 (d, *J* = 3.6 Hz, 1 H, H-1), 4.45 (dd, *J* = 8.4 and 8.4 Hz, 1 H, H-2′), 4.36 (d, *J* = 12.0 Hz, 1 H, PhC$\underline{H}_2$), 4.22 (dd, *J* = 9.6 and 4.2 Hz, 1 H, H-3), 4.18 (d, *J* = 12.0 Hz, 1 H, PhC$\underline{H}_2$), 3.82 (t, *J* = 9.3 and 9.3 Hz, 1 H, H-4), 3.80–3.72 (m, 4 H, H-5, H-6_{ab}, H-6′$_a$), 3.68–3.62 (m, 2 H, H-2, H-6′$_b$), 3.50 (t, *J* = 9.3 and 9.3 Hz, 1 H, H-5′), 3.44 (s, 3 H, OC$\underline{H}_3$), 2.04,1.87 (2 s, 6 H, 2 COC$\underline{H}_3$), 0.94 (s, 9 H, -C(C$\underline{H}_3$)$_3$), 0.10, 0.09 (2 s, 6 H, -Si(C$\underline{H}_3$)$_2$-).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.4, 169.5 (2 $\underline{C}OCH_3$), 167.7, 167.6 (2 CO, Phth), 138.8–123.8 (Ar-C), 101.7 (Ph$\underline{C}$H), 100.5 (C-1), 100.1 (C-1′), 82.5, 81.8, 76.1, 75.3, 74.2 (Ph$\underline{C}H_2$), 71.5, 69.7, 69.4 (C-6′), 62.8 (C-6), 62.6, 55.9 (C-2′), 55.2 (O$\underline{C}H_3$), 26.3 (3 C, C($\underline{C}H_3$)$_3$), 21.0, 20.8 (2 CO$\underline{C}H_3$), 18.7 ($\underline{C}(CH_3)_3$), –4.9 (2 C, -Si($\underline{C}H_3$)$_2$-).

ESI-MS: *m/z* = 884.3 $[M+Na]^+$.

Anal. Calcd. for $C_{45}H_{55}NO_{14}Si$ (861.3): C, 62.70; H, 6.43%; found: C, 62.51; H, 6.65%.

Methyl (3,4-di-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (23): To a solution of compound **22** (3.0 g, 3.5 mmol) in acetic acid (25 mL) was added 1 M solution of tetrbutylammonium fluoride in THF (25 mL) and the reaction mixture was allowed to stir at room temperature for 6 h. The reaction mixture was diluted with water and extracted with CH_2Cl_2 (3 x 50 mL). The organic layer was washed with satd. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated to dryness to give the crude product. Column chromatography of the crude product over SiO_2 using hexane-EtOAc (3:1) furnished pure compound **23** (2.0 g, 75%).

Syrup.

$[\alpha]_D^{25}$ – 9 (*c* 1.5, $CHCl_3$).

IR (neat): 2926, 2858, 1719, 1717, 1458, 1387, 1227, 1088, 1046, 994, 766 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.71–6.88 (m, 14 H, Ar-H), 5.65 (d, *J* = 8.4 Hz, 1 H, H-1′), 5.62 (t, *J* = 8.4 and 8.4 Hz, 1 H, H-3′), 5.44 (s, 1 H, PhC<u>H</u>), 4.96 (d, *J* = 3.6 Hz, 1 H, H-1), 4.53-4.43 (m, 2 H, H-2′ and H-4′), 4.34 (d, *J* = 12.3 Hz, 1 H, PhC$\underline{H}_2$), 4.23 (dd, *J* = 9.9 and 4.5 Hz, 1 H, H-3), 4.21 (d, *J* = 12.3 Hz, 1 H, PhC$\underline{H}_2$), 4.10 (t, *J* = 7.2 and 7.2 Hz, 1 H, H-4), 3.89–3.81 (m, 1 H, H-5), 3.79–3.65 (m, 5 H, H-2, H-6_{ab} and H-6′$_{ab}$), 3.54–3.47 (m, 1 H, H-5′), 3.43 (s, 3 H, OC$\underline{H}_3$), 2.14, 1.94 (2 s, 6 H, 2 COC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$,75 MHz): δ 171.7, 171.4 (2 $\underline{C}OCH_3$), 167.9, 167.4 (2 CO, Phth), 138.7–123.8 (Ar-C), 101.7 (Ph$\underline{C}$H), 100.4 (C-1), 100.3 (C-1′), 82.7, 82.2, 76.5, 74.5, 74.3 (Ph$\underline{C}H_2$), 73.9, 69.9, 69.4 (C-6′), 63.6 (C-6), 62.6, 55.7 (C-2′), 54.9, 21.3, 21.0 (2 CO$\underline{C}H_3$).

ESI-MS: *m/z* = 770.3 $[M+Na]^+$.

Anal. Calcd. for $C_{39}H_{41}NO_{14}$ (747.2): C, 62.64; H, 5.53%; found: C, 62.41; H, 6.85%.

Methyl (3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galacto-pyranosyl)-(1→6)-(3,4-di-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (24): To a solution of compound **23** (2.0 g, 2.7 mmol) and compound **15** (2.3 g, 4.0 mmol) in CH_2Cl_2 (20 mL) was added 4Å MS (3 g) and the reaction mixture was stirred under argon for 30 min at room temperature. *N*-Iodosuccinimide (1.1 g, 4.8 mmol) was added to the reaction mixture and it was cooled to –45 °C. To the cooled reaction mixture trimethylsilyl trifluoromethane sulfonate (40 µL, 0.22 mmol) was injected and stirring continued at –45 °C for 30 min. After consumption of compound 23 (monitored by TLC) the reaction mixture was allowed to stir at 0 °C for 20 min. The reaction mixture was filtered through a Celite bed and washed with CH_2Cl_2 (50 mL). The organic layer was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$, water, dried (Na_2SO_4) and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 using hexane-EtOAc (5:1) furnished pure compound **24** (2.2 g, 74%).

Syrup.

$[\alpha]_D^{25}$ + 66.5 (*c* 1.5, $CHCl_3$).

IR (neat): 2929, 1600, 1593, 1356, 1352, 1055, 665 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.67–6.86 (m, 24 H, Ar-H), 5.72 (t, *J* = 8.8 and 8.8 Hz, 1 H, H-3′), 5.66 (d, *J* = 8.4 Hz, 1 H, H-1′), 5.40, 5.38 (2 s, 2 H, 2 PhC$\underline{H}$), 5.22 (br s, 1 H, H-1″), 4.89 (br s, 1 H, H-1), 4.64–4.58 (m, 3 H, PhC$\underline{H}_2$ and H-3″), 4.38 (t, *J* = 9.1 and 9.1 Hz, 1 H, H-2′), 4.29 (d, *J* = 12.5 Hz, 1 H, PhC$\underline{H}_2$), 4.22–4.02 (m, 6 H, H-2″, H-4, H-4′, H-4″, H-6$_a$, PhC$\underline{H}_2$), 3.98 (d, *J* = 12.7 Hz, 1 H, H-6$_b$), 3.90–3.55 (m, 8 H, H-2, H-3, H-5, H-5″, H-6′$_{ab}$, H-6″$_{ab}$), 3.49–3.43 (m, 1 H, H-5′), 2.13, 1.86 (2 s, 6 H, 2 COC$\underline{H}_3$).

^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.4, 170.3 (2 $\underline{C}OCH_3$), 167.9, 167.2 (2 $\underline{C}$O, Phth), 138.3–123.4 (Ar-C), 102.2 (Ph$\underline{C}$H), 101.2 (Ph$\underline{C}$H), 100.6 (C-1′), 100.0 (C-1″), 99.5 C-1), 82.1, 81.9, 77.4, 76.2, 76.0, 73.9 (Ph$\underline{C}H_2$), 73.1, 72.9 (2 C), 71.2 (Ph$\underline{C}H_2$), 69.3 (C-6″), 68.9 (C-6′), 67.3, 63.8, 63.1 (C-6), 62.2, 55.3 (O$\underline{C}H_3$), 54.9(C-2′), 20.8, 20.6 (2 CO$\underline{C}H_3$). ESI-MS: *m/z* = 1110.3 [M+Na]$^+$.

Anal. Calcd. for $C_{59}H_{61}NO_{19}$ (1087.4): C, 65.12; H, 5.65%; found: C, 64.92; H, 5.90%.

Methyl (2,3,5,6-tetra-*O*-benzyl-α-D-galactofuranosyl-(1→2)-(3-*O*-benzyl-4,6-*O*-benzylidene-α-D-galacto-pyranosyl)-(1→6)-(3,4-di-*O*-acetyl-2-deoxy-2-*N*-phthalimido-β-D-glucopyranosyl)-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranoside (25): To a solution of compound **24** (2.0 g, 1.8 mmol) and compound **18** (1.6 g, 2.8 mmol) in CH_2Cl_2 (20 mL) was added 4Å MS (3 g) and the reaction mixture was stirred under argon for 30 min at room temperature. *N*-Iodosuccinimide (745 mg, 3.3 mmol) was added to the reaction mixture and it was cooled to –45 °C. To the cooled reaction mixture trimethylsilyl trifluoromethanesulfonate (20 µL, 0.11 mmol) was injected and stirring continued at –45 °C for 30 min. The reaction mixture was filtered through a celite bed. The filtrate was washed with 5% aq. $Na_2S_2O_3$, satd. $NaHCO_3$, water, dried (Na_2SO_4) and concentrated to a syrupy product. Column chromatography of the crude product over SiO_2 using hexane-EtOAc (5:1) furnished pure compound **25** (2.0 g, 70%).

Syrup.

$[\alpha]_D^{25}$ + 30.6 (*c* 1.5, $CHCl_3$).

IR (neat): 2926, 2861, 2370, 1743, 1718, 1458, 1384, 1224, 1094, 745 cm^{-1}.

^{1}H NMR ($CDCl_3$, 300 MHz): δ 7.70–6.82 (m, 44 H, Ar-H), 5.80 (t, *J* = 10.2 and 10.2 Hz, 1 H, H-3′), 5.52 (d, *J* = 8.5 Hz, 1 H, H-1′), 5.40, 5.37 (2 s, 2 H, 2 PhC$\underline{H}$), 5.32 (br s, 1 H, H-1‴), 5.20 (br s, 1 H, H-1″), 4.90 (d, *J* = 12.2 Hz, PhC$\underline{H}_2$), 4.85 (br s, 1 H, H-1), 4.78 (d, *J* = 12.2 Hz, 1 H, PhC$\underline{H}_2$), 4.68–4.61 (m, 3 H, PhC$\underline{H}_2$), 4.56–4.47 (m, 2 H, PhC$\underline{H}_2$), 4.45–4.39 (m, 3 H, H-2′ and PhC$\underline{H}_2$), 4.30 (d, *J* = 12.0 Hz, 1 H, PhC$\underline{H}_2$), 4.28–4.18 (m, 6 H, H-2″, H-3‴, H-4, H-4′, H-6‴$_a$, PhC$\underline{H}_2$), 4.15–4.08 (m, 4 H, H-4″, H-4‴, H-6‴$_b$, PhC$\underline{H}_2$), 4.05–3.90 (m, 4 H, H-2‴, H-3″ and H-6″$_{ab}$), 3.88–3.85 (m, 1 H, H-5‴), 3.80–3.72 (m, 3 H, H-3, H-5 and H-5′), 3.70–3.60 (m, 4 H, H-6$_{ab}$ and H-6′$_{ab}$), 3.57–3.52 (m, 1 H, H-2), 3.44–3.39 (m, 1 H, H-5″), 3.27 (s, 3 H, OC$\underline{H}_3$), 2.02, 1.74 (2 s, 6 H, 2 COC$\underline{H}_3$).

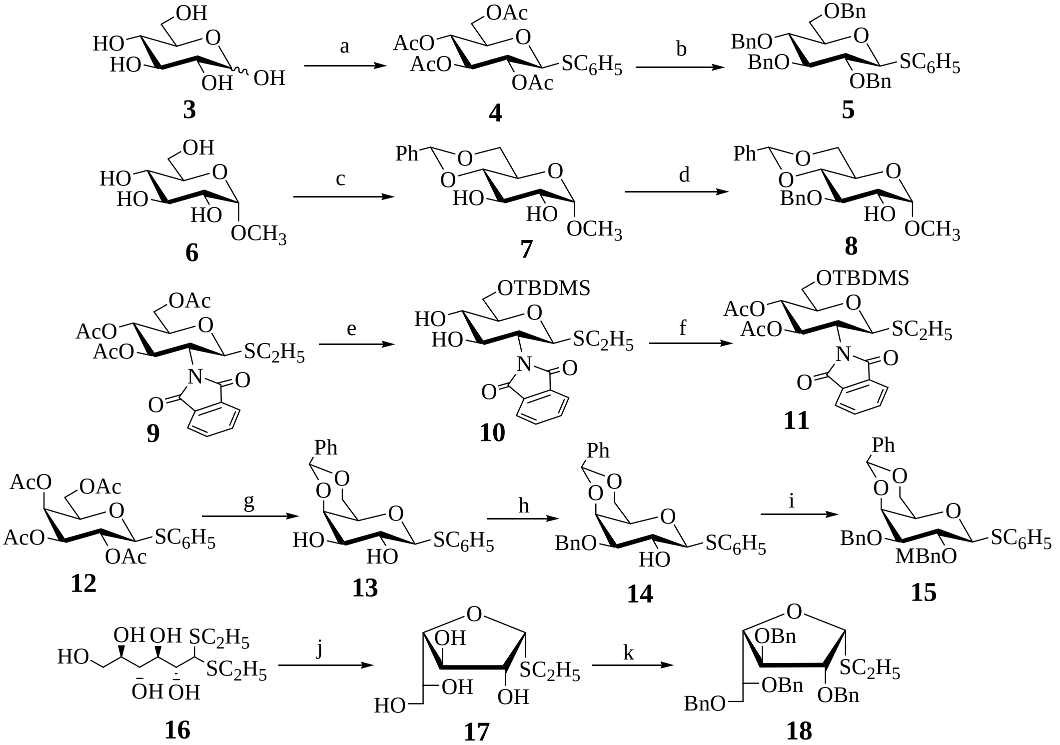
^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.0 (2 C, 2 $\underline{C}OCH_3$), 167.9, 167.3 (2 C, 2 $\underline{C}$O, Phth), 138.4-123.2 (Ar-C), 108.3 (C-1‴), 101.3 (Ph$\underline{C}$H), 100.8 (Ph$\underline{C}$H), 100.0 (C-1′), 99.8 (C-1), 99.4 (C-1″), 87.9, 82.4, 82.2, 82.0, 81.3, 76.7, 76.0 (2 C), 75.1, 74.7, 73.8, 73.7 (Ph$\underline{C}H_2$), 73.4, 73.3 (Ph$\underline{C}H_2$), 73.1

($\mathrm{Ph\underline{C}H_2}$), 71.7 ($\mathrm{Ph\underline{C}H_2}$), 71.5, 71.4 (2 C, $\mathrm{Ph\underline{C}H_2}$), 70.4 (C-6‴), 69.2 (C-6′), 69.0 (2 C, C-6 and C-6″), 63.7, 62.1, 55.2 ($\mathrm{O\underline{C}H_3}$), 55.0 (C-2″), 20.8, 20.7 (2 $\mathrm{CO\underline{C}H_3}$).
ESI-MS: *m/z* = 1628.4 $[\mathrm{M+NH_4}]^+$.
Anal. Calcd. for $C_{93}H_{95}NO_{24}$ (1609.6): C, 69.35; H, 5.94%; found: C, 69.12; H, 6.20%.

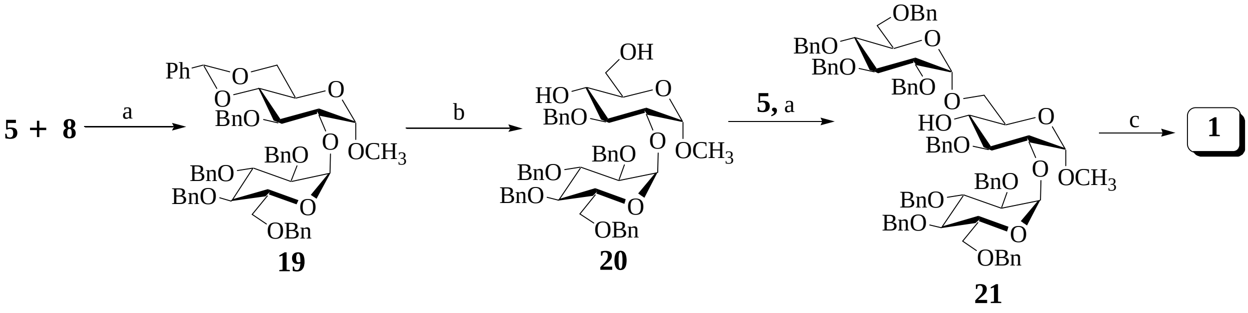
Methyl (α-D-glucopyranosyl)-(1→6)-[α-D-glucopyrano-syl-(1→2)]-α-D-glucopyranoside (1): To a solution of compound **21** (1.4 g, 1 mmol) in methanol (30 mL), was added 20% $Pd(OH)_2$-C (500 mg) and reaction mixture was stirred under hydrogen at room temperature for 24 h. The mixture was filtered through celite bed and concentrated to give a solid mass, which was purified through Sephadex LH-20 using 80% aqueous EtOH as eluant to give pure trisaccharide **1** (435 mg, 80%).
Amorphous powder.
$[\alpha]_D^{25}$ + 16 (*c* 1.0, H_2O).
IR (KBr): 1595, 1460, 1351, 1066, 697 cm^{-1}.
^{1}H NMR (D_2O, 300 MHz): δ 5.06 (d, *J* = 3.6 Hz, 2 H, H-1 and H-1′), 4.94 (d, *J* = 3.6 Hz, 1 H, H-1″), 4.0 (dd, *J* = 10.0, 3.6 Hz, 1 H, H-2), 3.93–3.85 (m, 2 H, H-6$_a$″ and H-3″), 3.81–3.64 (m, 7 H, H-3, H-4, H-3′, H-4′, H-4″, H-5″, H-6″$_b$), 3.59–3.51 (m, 4 H, H-6$_{ab}$ and H-6′$_{ab}$), 3.49–3.44 (m, 2 H, H-2′ and H-2″), 3.43 (s, 3 H, $\mathrm{OC\underline{H}_3}$), 3.40-3.25 (m, 2 H, H-5 and H-5′).
^{13}C NMR (D_2O, 75 MHz): δ 96.7 (2 C, C-1 and C-1″), 96.4 (C-1′), 75.9, 75.5, 73.1, 72.7, 71.8 (2 C), 71.5, 71.3 (2 C), 69.9, 69.4 (2 C), 60.8 (C-6′), 60.5 (C-6), 60.4 (C-6″), 55.1 ($\mathrm{O\underline{C}H_3}$).
ESI-MS: *m/z* = 541.2 $[\mathrm{M+Na}]^+$.
Anal. Calcd. for $C_{19}H_{34}O_{16}$ (518.2): C, 44.02; H, 6.61%; found: C, 43.78; H, 6.95%.

Methyl (α-D-galactofuranosyl-(1→2)-(α-D-galacto-pyranosyl)-(1→6)-(2-acetamido-2-deoxy-β-D-gluco-pyranosyl)-(1→2)-α-D-glucopyranoside (2): To a solution of tetrasaccharide derivative **25** (1.6 g, 1.0 mmol) in EtOH (30 mL) was added hydrazine hydrate (4 mL) and the reaction mixture was allowed to stir at 80 °C for 5 h. The solvents were removed under reduced pressure and to the crude mass were added pyridine (5 mL) and acetic anhydride (5 mL) and the reaction mixture was kept at room temperature for 1 h. The solvents were evaporated and co-evaporated with toluene under reduced pressure. To a solution of the crude mass in CH_3OH (25 mL) solid CH_3ONa was added till pH ~10 and the reaction mixture was allowed to stir at room temperature for 5 h. The reaction mixture was neutralized with Amberlite IR-120 (H^+) resin. The reaction mixture was filtered and evaporated to dryness. To a solution of the crude product in CH_3OH (20 mL) was added 20% $Pd(OH)_2$-C (500 mg) and the reaction mixture was stirred at room temperature under a positive pressure of hydrogen for 24 h. The reaction mixture was filtered through a Celite bed and concentrated to a crude mass. Successive purification of the crude mass over SiO_2 using $CHCl_3$-CH_3OH-H_2O (10:5:1) as eluant and through a Sephadex LH-20 using CH_3OH-H_2O (4:1) as eluant furnished pure tetrasaccharide **2** (520 mg, 76%).
Amorphous powder.
$[\alpha]_D^{25}$ + 43 (*c* 1.0, H_2O).
IR (KBr): 3444, 2817, 2367, 1592, 1352, 762 cm^{-1}.
^{1}H NMR (D_2O, 300 MHz): 5.81 (br s, 1 H, H-1″), 5.29 (br s, 1 H, H-1‴), 5.10 (br s, 1 H, H-1), 4.82 (d, *J* = 8.4 Hz, 1 H, H-1′), 4.24 (t, *J* = 9.0 and 9.0 Hz, 1 H, H-4′), 4.18-4.15 (m, 2 H, H-2‴ and H-4″), 4.10–4.0 (m, 4 H, H-3″, H-4 and H-6′), 3.95–3.70 (m, 12 H, H-3, H-3′, H-3‴, H-2″, H-4‴, H-5″, H-6$_{ab}$, H-6″$_{ab}$ and H-6‴$_{ab}$), 3.68–3.65 (m, 1 H, H-2), 3.63–3.55 (m, 2 H, H-5, H-5′ and H-5‴), 3.45 (s, 3 H, $\mathrm{OC\underline{H}_3}$), 3.44-3.42 (m, 1 H, H-2), 2.08 (s, 3 H, $\mathrm{NHCOC\underline{H}_3}$).
^{13}C NMR (D_2O, 75 MHz): δ 170.3 ($\mathrm{\underline{C}OCH_3}$), 109.5 (C-1‴), 102.7 (C-1′), 99.4 (C-1), 98.2 (C-1″), 83.7 (C-3‴), 81.5 (C-2), 81.2 (C-4″), 77.2 (C-2‴), 75.4 (C-4′), 75.3 (C-4‴), 74.8 (C-3), 73.8 (C-2″), 72.0 (2 C, C-3′ and C-5‴), 71.9 (C-5), 71.4 (C-5″), 70.1 (C-4), 69.8 (C-3″), 69.0 (C-5′), 63.0 (C-6″), 61.6 (C-6), 61.3 (C-6‴), 61.1 (C-6′), 57.0 (C-2′), 55.3 ($\mathrm{O\underline{C}H_3}$), 20.8 ($\mathrm{CO\underline{C}H_3}$).
ESI-MS: *m/z* = 744.3 $[\mathrm{M+Na}]^+$.

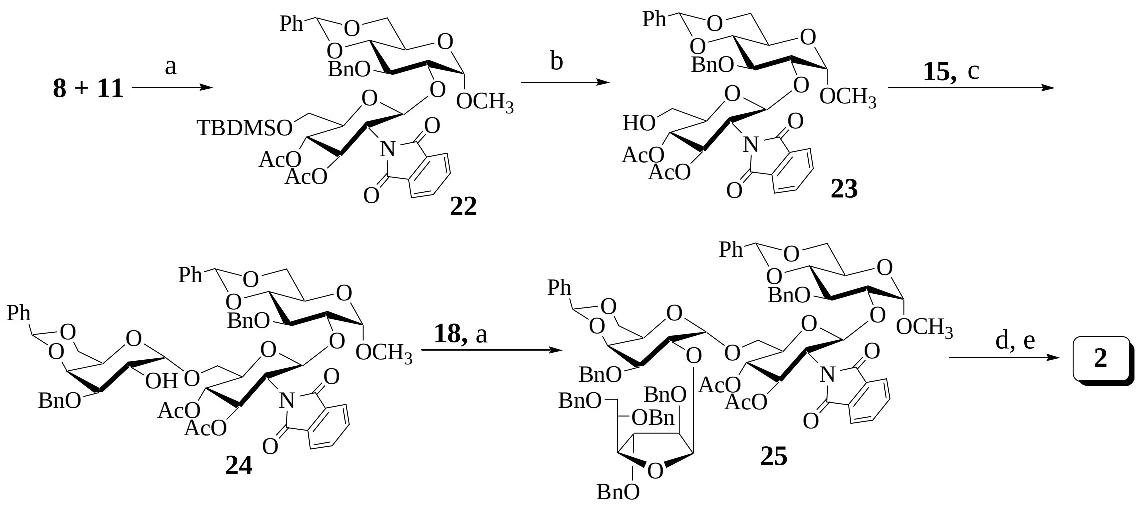
Anal. Calcd. for $C_{27}H_{47}NO_{21}$ (721.2): C, 44.94; H, 6.56; found: C, 44.70; H, 6.85.



Scheme 1: Reagents: (a) Acetic anhydride, $BF_3{\cdot}OEt_2$, 5 min, then C_6H_5SH, 5 °C, 6 h, 85%; (b) benzyl bromide, NaOH, TBAI, THF, r t, 8 h, 90%; (c) benzaldehyde dimethylacetal, *p*-TsOH, CH_3CN, r t, 12 h, 90%; (d) (i) Bu_2SnO, CH_3OH, 80 °C, 2 h, (ii) benzyl bromide, CsF, DMF, r t, 12 h, 80%; (e) (i) CH_3ONa, CH_3OH, r t, 20 min; (ii) *tert*-butyldimethylsilyl chloride, pyridine, DMAP, 50 °C, 6 h, 90% in two steps; (f) acetic anhydride, pyridine, r t, 5 h, quantitative; (g) (i) CH_3ONa, CH_3OH, r t, 3 h; (ii) benzaldehyde dimethylacetal, *p*-TsOH, CH_3CN, r t, 12 h, 85%; (h) (i) Bu_2SnO, CH_3OH, 80 °C, 2 h, (ii) benzyl bromide, CsF, DMF, r t, 12 h, 85%; (i) 4-methoxybenzyl chloride, NaOH, TBAI, 0 °C-r t, 4 h, 90%; (j) $HgCl_2$, HgO, H_2O, r t, 2 h, 76%; (k) benzyl bromide, NaOH, TBAI, THF, r t, 8 h, 85%.



Scheme 2: Reagents: (a) *N*-iodosuccinimide, TMSOTf, MS 4Å, CH_2Cl_2, −20 °C, 45 min. (80% for compound 19 and 73% for compound 21); (b) $HClO_4$-SiO_2, CH_3CN, r t, 20 min, 90%; (c) H_2, 20% $Pd(OH)_2$-C, CH_3OH, r t, 24 h, 80%.



Scheme 3: Reagents: (a) *N*-iodosuccinimide, TMSOTf, MS 4Å, CH_2Cl_2, –30 °C, 45 min. (78% for compound **22** and 70% for compound **25**); (b) tetrabutylammonium fluoride, THF, AcOH, r t, 5 h, 75%; (c) *N*-iodosuccinimide, TMSOTf, MS 4Å, CH_2Cl_2, –40 °C, 45 min. then 0 °C, 30 min., 74%; (d) $NH_2NH_2 \cdot H_2O$, C_2H_5OH, 80 °C, 5 h; (ii) acetic anhydride, pyridine, r t, 2 h; (iii) CH_3ONa, CH_3OH, r t, 5 h, 80%; (e) H_2, 20% $Pd(OH)_2$-C, CH_3OH, r t, 24 h, 76%.

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