Synthesis of Oligosaccharides Corresponding to the Polysaccharides of *Lactobacillus* and *Thermophilus* Strains

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**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:** oligosaccharide, glycoside, glycosylation, *Thermus thermophilus*, *Lactobacillus* spp. G-77

A wide variety of exopolysaccharides (EPS) 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 immunomodulators. Recently it has been observed that exopolysaccharides produced by lactic acid bacteria have suitable rheological properties for the dairy industry. In the wine industry, the polysaccharides produced by lactic acid bacteria can cause the alteration known as oiliness or ropiness by increasing the consistency of the beverage. To date a number of bacteria are known to produce exopolysaccharides in ciders and wines and some of the structures of their exopolysaccharides have been elucidated. The structure of a ropy strain of *Lactobacillus* spp. G-77 has been characterized recently that contains a unique 1,2-*cis*-linked branched trisaccharide (Figure 1).

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

In order to analyze and assess the role of exopolysaccharides produced by *Lactobacillus* spp. G-77 and glycolipids in the adaptation process of *Thermus thermophilus*, reasonable quantities of the corresponding tri- and tetrasaccharides are required. Recently, we engaged in the synthesis of some oligosaccharide fragments corresponding to lactic acid bacteria and some heat-stable bacteria for their evaluation in several biological studies. 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).

![Figure 1](image1.png)

**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

![Figure 2](image2.png)

**Figure 2** Synthesized trisaccharide 1 and tetrasaccharide 2 as their methyl glycosides

In order to synthesize the 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)14 was prepared from D-glucose (3) following two recently developed methodologies from our laboratory, one-pot acetylation-thioglycosidation12 and one-pot deacetylation-benzyla- tion. Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-*α*-D-glucopyranoside (8)14 was prepared from methyl *α*-D-glucopyranoside (6) in two steps that consisted of
benzylideneation\textsuperscript{15} and selective benzylolation through the formation of a stannylidene acetal.\textsuperscript{16} Ethyl 3,4-di-O-acetyl-6-O-tert-butylmethylsilyl-2-deoxy-2-pthalimido-1-thio-β-D-glucopyranoside (11) was prepared from ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-pthalimido-1-thio-β-D-glucopyranoside (9) in three steps involving deacetylation,\textsuperscript{17} selective silyl group protection,\textsuperscript{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 deacylation, benzylidene acetal formation,\textsuperscript{15} selective benzylolation through stannylidene acetal formation,\textsuperscript{16} and 4-methoxybenzylolation. Ethyl 2,3,5,6-tetra-O-benzyl-1-thio-α-D-galactofuranoside (18) was prepared from D-galactose diethyl dithioacetal (16) by a two-step reaction sequence involving mercury-catalyzed thiofuranoside formation\textsuperscript{19} and per-O-benzylolation 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 5 with 8 using N-iodosuccinimide (NIS) and trimethylsilyl trifluoromethanesulfonate (TMSOTf)\textsuperscript{20} furnished disaccharide 19 in 80% yield. Formation of 19 was confirmed by its NMR and mass spectral analysis. Removal of the benzylidene acetal in 19 using perchloric acid supported on silica gel\textsuperscript{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 N-iodosuccinimide/triethylsilyl trifluoromethanesulfonate\textsuperscript{20} furnished trisaccharide derivative 21 in 78% yield. Global debenzylation\textsuperscript{22} of trisaccharide derivative 21 by hydrogenation using hydrogen and palladium hydroxide on carbon (Pearlman’s catalyst) gave the target trisaccharide 1 in 80% yield. The presence of three anomeric protons in the \textsuperscript{1}H NMR [\(\delta = 5.06\) (d, \(J = 3.6\) Hz, 2 H) and 4.94 (d, \(J = 3.6\) Hz, 1 H)] and \textsuperscript{13}C NMR [\(\delta = 96.7\) (2 C) and 96.4] spectra of trisaccharide 1 supported the fact 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 a high yield in each step.

Scheme 1: Reagents and conditions: (a) Ac\textsubscript{2}O, BF\textsubscript{3}·OEt\textsubscript{2}, 5 min, then PhSH, 5 °C, 6 h, 85%; (b) BnBr, NaOH, TBAB, THF, r.t., 8 h, 90%; (c) benzaldehyde dimethyl acetal, p-TsOH, MeCN, r.t., 12 h, 90%; (d) 1. Bu\textsubscript{2}SnO, MeOH, 80 °C, 2 h, 2. BnBr, CsF, DMF, r.t., 12 h, 80%; (e) 1. NaOMe, MeOH, r.t., 20 min; 2. TBDMSCl, pyridine, DMAP, 50 °C, 6 h, 90% (2 steps); (f) Ac\textsubscript{2}O, pyridine, r.t., 6 h, quantitative; (g) 1. NaOMe, MeOH, r.t., 3 h; 2. benzaldehyde dimethyl acetal, p-TsOH, MeCN, r.t., 12 h, 85%; (h) 1. Bu\textsubscript{2}SnO, MeOH, 80 °C, 2 h, 2. BnBr, CsF, DMF, r.t., 12 h, 85%; (i) 4-methoxybenzyl chloride, NaOH, TBAB, THF, r.t., 4 h, 90%; (j) HgCl\textsubscript{2}, HgO, H\textsubscript{2}O, r.t., 2 h, 76%; (k) BnBr, NaOH, TBAB, THF, r.t., 6 h, 85%.
In another set experiments, methyl α-D-galactofuranosyl-
(1→2)-α-D-galactopyranosyl-(1→6)-(2-acetamido-2-de-
oxy-β-D-glucopyranosyl)-(1→2)-α-D-glucopyranoside (2) was synthesized following Scheme 3. Condensation of 8
with 11 in the presence of N-iodosuccinimide/triethylsilyl
trifluoromethanesulfonate furnished disaccharide 22 in
78% yield. Removal of tert-butylmethysilyl group23
using tetrabutylammonium fluoride in tetrahydrofuran–
acetic acid afforded disaccharide acceptor 23 in 75% yield.
Sequential glycosylation of 23 with thiglycoside donor
15 and removal of 4-methoxybenzyl group was achieved
in one pot24 in the presence of N-iodosuccini
imide/triethylsilyl trifluoromethanesulfonate to furnish
trisaccharide acceptor 24 in 74% yield. Final glycosyla
tion of trisaccharide derivative 24 with thiglycoside dona
or 18 in the presence N-iodosuccinimide/triethylsilyl
trifluoromethanesulfonate afforded the tetrasaccharide
derivative 25 in 70% yield. Removal of the N-phthalamido
by treatment with hydrazine hydrate25 and N-acetylation
followed by hydrogenolysis over palladium hydroxide on
carbon22 afforded pure tetrasaccharide 2 as its methyl glycoside in 76% yield. The presence of signals for four anoma
eric 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 anomer carbons [δ = 109.5, 102.7, 99.4 and 98.2] in
the 13C NMR spectra confirmed formation of the teta
saccharide 2. The key features of Scheme 3 include glycosyla
tion and removal of the 4-methoxybenzyl group in one
pot without the addition of any extra reagent and high
yields in the glycosylations.

In summary, the synthesis of a branched trisaccharide
w
responding to the repeating unit of the exopolysaccharide
produced by Lactobacillus spp. G-77 and a tetrasacca
ride corresponding to the cell-wall glycolipid of heat sta
ble 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 the 4-
methoxybenzyl group was achieved in one pot without the
addition of extra reagent, yields were excellent, and a gen
eral glycosylation reaction condition has been applied.

All the reactions were monitored by TLC over silica gel coated TLC
plates; TLC was visualized by warming with 2% Ce(SO 4)2 in 1 M
H 2SO 4 sprayed plates on a hot plate. Silica gel 230–400 mesh was
used for column chromatography. 1H and 13C NMR were recorded on
Bruker Advance DPX 200 and 300 MHz using CDCl 3 and D 2O
as solvents and TMS as internal reference and acetone as external
reference. ESI-MS were recorded on a MICROMASS QUTTRO II
triple quadrupole mass spectrometer. Elementary analysis was car

Scheme 2  Reagents and conditions: (a) NIS, TMSOTf, 4 Å MS, CH 3Cl 2, –45 °C, 30–45 min (80% for 19 and 73% for 21); (b) HClO 4 on
silica gel, MeCN, r.t., 30 min, 90%; (c) H 2, 20% Pd(OH) 2-C, MeOH, r.t., 24 h, 80%.

Scheme 3  Reagents and conditions: (a) NIS, TMSOTf, 4 Å MS, CH 3Cl 2, –45 °C, 30 min (78% for 22 and 70% for 25); (b) TBAF, THF,
AcOH, r.t., 6 h, 75%; (c) NIS, TMSOTf, 4 Å MS, CH 3Cl 2, –45 °C, 30 min then 0 °C, 30 min, 74%; (d) NH 2NH 2·H 2O, EtOH, 80 °C, 5 h; (ii)
Ac 2O, pyridine, r.t., 1 h; (iii) NaOMe, MeOH, r.t., 5 h, 80%; (e) H 2, 20% Pd(OH) 2-C, MeOH, r.t., 24 h, 76%.

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ried out on Carlo ERBA-1108 analyzer. Optical rotations were measured at 25 °C on a Rudolf Autopol III polarimeter. Commercially available grades of organic solvents of adequate purity were used in many reactions.

**Ethyl 3,4-Di-O-acetyl-6-O-tert-butylidimethylsilyl-2-deoxy-2-thiaphthimido-1-thio-β-D-glucopyranoside (11)**

A solution of ethyl 3,4,6-tri-O-acetyl-2-deoxy-2-thiaphthimido-1-thio-β-D-glucopyranoside (9, 4.0 g, 8.34 mmol) in 0.05 M NaOMe in MeOH (50 mL) was allowed to stir at r.t. for 20 min and neutralized with Amberlite IR-120 (H⁺) resin. The mixture was filtered and evaporated to dryness. To a solution of the crude mass in pyridine (30 mL) were added TBDMSCl (1.4 g, 9.4 mmol) and DMAP (100 mg) and the mixture was allowed to stir at 50 °C for 6 h. After complete consumption of the starting material, Ac₂O (20 mL) was added to the mixture and it was allowed to stir at r.t. for 6 h. The solvents were removed under reduced pressure to give the crude product, which was purified by chromatography (silica gel, hexane–EtOAc, 5:1) to furnish pure 11 (4.14 g, 90%) as a syrup.

\[\text{[c]} +24.1 \text{ (c 1.5, CHCl}_3\text{).} \]

IR (neat): 3,720, 1,760, 1,370, 1,340, 1,290, 1,150, 1,090, 950, 840, 750, 690 cm⁻¹.

**Phenyl 3-O-Benzyl-4,6-O-benzylidene-2-O-(4-methoxybenzyl)-1-thio-β-D-galactopyranoside (15)**

To a solution of 14 (3.0 g, 6.6 mmol) in anhyd THF (25 mL) were added powdered NaOH (800 mg, 20 mmol), 4-methoxybenzyl chloride (1.4 mL, 9.9 mmol), and TBAB (100 mg) and the mixture was allowed to stir at r.t. for 4 h. The mixture was diluted with H₂O and extracted with CH₂Cl₂ (3 × 50 mL). The organic layer was washed with H₂O, dried (Na₂SO₄), and concentrated under reduced pressure to give the crude product, which was purified by chromatography (silica gel, hexane–EtOAc, 7:1) to afford pure 15 (3.42 g, 90%) as a syrup.

\[\text{[c]} +28.4 \text{ (c 1.5, CHCl}_3\text{).} \]

IR (neat): 2922, 1592, 1453, 1351, 1252, 1165, 1054, 734, 695 cm⁻¹.

**Ethyl 2,3,5,6-Tetra-O-benzyl-1-thio-β-D-galactofuranoside (18)**

To a solution of 3,4,6-tri-O-acetyl-2-deoxy-2-thiaphthimido-1-thio-β-D-galactofuranoside (17) in THF (25 mL) were added BnBr (12.5 mL, 105 mmol), powdered NaOH (5.6 g, 140 mmol), and TBAB (100 mg) and the mixture was allowed to stir at r.t. for 6 h. The excess reagents were quenched by addition of MeOH (2 mL) and the resulting solution was concentrated under reduced pressure. The crude mass was dissolved in CH₂Cl₂ (100 mL) and the organic layer was washed with H₂O, dried (Na₂SO₄), and concentrated under reduced pressure. Purification of the crude product by chromatography (silica gel, hexane–EtOAc, 12:1) furnished pure 18 (8.7 g, 85%) as a syrup.

\[\text{[c]} +37.2 \text{ (c 1.5, CHCl}_3\text{).} \]

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

**Methyl (2,3,4,6-Tetra-O-benzyl-β-D-glucopyranosyl)-(1→2)-3-O-Benzyl-4,6-O-benzylidene-α-D-glucopyranosyl (19)**

To a solution of 5 (5.1 g, 8.0 mmol) and 8 (2.5 g, 6.7 mmol) in CH₂Cl₂ (20 mL) was added 4 Å MS (2 g) and the mixture was stirred under argon at r.t. for 30 min. NIS (2.2 g, 9.6 mmol) was added to the mixture and it was cooled to –45 °C. To the cooled mixture TMSOTf (20 mL) was added 4 Å MS (2 g) and the mixture was stirred under argon at r.t. for 4 h. The mixture was diluted with H₂O and extracted with CH₂Cl₂ (3 × 50 mL). The organic layer was washed with H₂O, dried (Na₂SO₄), and concentrated under reduced pressure to give the crude product, which was purified by chromatography (silica gel, hexane–EtOAc, 7:1) to afford pure 19 (4.8 g, 80%) as a syrup.

\[\text{[c]} +37.6 \text{ (c 1.5, CHCl}_3\text{).} \]

IR (neat): 1600, 1595, 1382, 1352, 1059, 696 cm⁻¹.

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Methyl (3,4-Di-acetyl-6-O-tert-butylidimethylsilyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-benzylidine-α-D-glucopyranoside (22)

To a soln of 8 (2.5 g, 6.7 mmol) and 11 (4.8 g, 8.7 mmol) in CH2Cl2 (25 mL) was added 4 Å MS (3 g) and the mixture was stirred under argon at r.t. for 30 min. NIS (2.5 g, 10.2 mmol) was added to the mixture and it was cooled to −45 °C. To the cooled mixture TMSOTf (40 μL, 0.22 mmol) was added by injection and stirring continued at −45 °C for 30 min. The mixture was filtered through a Celite bed. The filtrate was washed with 5% aq Na2S2O3, sat. NaHCO3, and H2O, dried (Na2SO4), and concentrated to dryness to give the crude product. Column chromatography of the crude product (silica gel, hexane–EtOAc, 5:1) furnished pure 22 (4.5 g, 78%) as a syrup.

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

IR (neat): 3292, 1753, 1719, 1597, 1461, 1384, 1355, 1237, 1052, 761 cm⁻¹.

1H NMR (300 MHz, CDCl3): δ = 7.76–6.89 (m, 14 H, Ar-H), 5.77 (dd, J = 9.0, 9.0 Hz, 1 H, H3), 5.62 (dd, J = 8.4 Hz, 1 H, H1'), 5.41 (s, 1 H, PhCH), 5.13 (t, J = 9.3 Hz, 1 H, H4'), 4.95 (dd, J = 3.6 Hz, 1 H, H1), 4.45 (dd, J = 8.4 Hz, 1 H, H1', 4.36 (dd, J = 12.0 Hz, 1 H, PhCH3), 4.22 (dd, J = 9.6, 4.2 Hz, 1 H, H1), 4.18 (dd, J = 15.0 Hz, 1 H, PhCH3), 3.82 (t, J = 9.3 Hz, 1 H, H4), 3.80–3.72 (m, 2 H, H2, H2'), 3.68–3.62 (m, 2 H, H2, H2'), 3.50 (t, J = 9.3 Hz, 1 H, H5'), 3.44 (s, 3 H, OCH3), 2.04 (s, 2 H, H6, H6'), 2.04, 1.97 (2 s, 6 H, (CH3)3), 1.01, 0.99 (2 s, 6 H, Si(CH3)3).

13C NMR (75 MHz, CDCl3): δ = 170.4, 169.5 (2 COCH3), 167.7, 167.6 (CO, Phth), 138.8–123.8 (Ar-C), 101.7 (PhCH), 100.1 (C1), 82.5, 81.8, 76.1, 75.3, 74.2 (PhCH3), 71.5, 69.7, 69.4 (C6), 62.8 (C6), 62.6, 55.5 (C2), 55.2 (OCH3), 26.3 (C, C(CH3)2), 21.0, 20.8 (2 COCH3), 18.7 (C(CH3)), −4.9 [2 s, Si(CH3)].

ESI-MS: \( m/z = 884.3 [M + Na]^+ \).


Methyl (3,4-Di-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-(1→3)-O-benzylidine-α-D-glucopyranoside (23)

To a soln of 22 (3 g, 3.5 mmol) in AcOH (25 mL) was added 1 M TBAF in THF (25 mL); the mixture was allowed to stir at r.t. for 6 h. The mixture was diluted with H2O and extracted with CH2Cl2 (3 × 50 mL). The organic layer was washed with sat. NaHCO3, and H2O, dried (Na2SO4), and concentrated to dryness to give the crude product. Column chromatography of the crude product (silica gel, hexane–EtOAc, 3:1) furnished pure 23 (2.0 g, 75%) as a syrup.

\[ [\alpha]_D^{25} +9.5 (c 1.5, CHCl_3). \]

IR (neat): 2926, 2858, 2719, 1717, 1458, 1387, 1227, 1088, 1046, 994, 776 cm⁻¹.

1H NMR (300 MHz, CDCl3): δ = 7.71–6.88 (m, 14 H, Ar-H), 5.65 (d, J = 8.4 Hz, 1 H, H1'), 5.12 (s, J = 8.4, 8.4 Hz, 1 H, H3'), 5.44 (s, 1 H, PhCH), 4.96 (d, J = 3.6 Hz, 1 H, H1'), 4.53–4.43 (m, 2 H, H2, H2'), 4.34 (dd, J = 12.3 Hz, 1 H, PhCH3), 4.23 (dd, J = 9.9, 4.5 Hz, 1 H, H1), 4.21 (d, J = 12.3 Hz, 1 H, H1'), 4.10 (t, J = 7.2, 7.2 Hz, 1 H, H4), 3.89–3.81 (m, 1 H, H5), 3.79–3.65 (m, 5 H, H2, H2', H6, H6'), 3.54–3.47 (m, 1 H, H5'), 3.43 (s, 3 H, OCH3), 2.14, 1.94 (2 s, 6 H, 2 COCH3).

13C NMR (75 MHz, CDCl3): δ = 171.7, 171.4 (2 COCH3), 167.9, 167.4 (CO, Phth), 138.7–123.8 (Ar-C), 101.7 (PhCH), 100.4 (C1), 100.3 (C2), 82.7, 82.2, 76.5, 74.5, 74.3 (PhCH3), 73.9, 69.9, 69.4 (C6'), 63.6 (C6), 62.6, 55.5 (C2'), 54.9, 21.3, 21.0 (2 COCH3).

ESI-MS: \( m/z = 770.3 [M + Na]^+ \).

Methyl (3-O-Benzyl-4,6-O-benzylidene-a-D-galactopyranosyl)-(1→6)-(3,4-di-O-acetyl-2-deoxy-2-phenylamido-b-D-glucopyranosyl)-(1→2)-3-O-benzyl-4,6-O-benzylidene-a-D-galactopyranosyl-(1→2)-3-O-benzyl-4,6-O-benzylidene-a-D-galactopyranoside (24)

To a soln of 23 (2.0 g, 2.7 mmol) and 15 (2.3 g, 4.0 mmol) in CHCl₃ (20 mL) was added 4 Å MS (3 g) and the mixture was stirred under argon at t.r.t. for 30 min. NIS (1.1 g, 4.8 mmol) was added to the mixture and it was cooled to –45 °C. To the cooled mixture TMSOTf (40 µL, 0.22 mmol) was added by injection and stirring was continued at –45 °C for 30 min. After consumption of 23 (monitored by TLC) the mixture was allowed to stir at 0 °C for 20 min. The mixture was filtered through a Celite bed and washed with CH₂Cl₂ (50 mL). The organic layer was washed with 5% aq Na₂SO₄ sat. NaHCO₃, and H₂O, dried (Na₂SO₄), and concentrated to a syrupy product. Column chromatography of the crude product (silica gel, hexane–EtOAc, 5:1) furnished pure 24 (2.2 g, 74%) as a syrup.

\[ \delta \text{H}_1^{13} +66.5 (c. 1.5, CHCl₃). \]

IR (neat): 2929, 1600, 1593, 1352, 1055, 665 cm⁻¹.

1H NMR (300 MHz, CDCl₃): δ = 7.67–6.86 (m, 24 H, Ar-H), 5.72 (t, J = 8.8, 8.8 Hz, 1 H, H3'), 5.66 (d, J = 8.4 Hz, 1 H, H1'), 3.50, 5.38 (2 s, 2 H, PhCH₂), 5.22 (br s, 1 H, H1'), 4.89 (br s, 1 H, H1), 4.64–4.58 (m, 3 H, PhCH₂, H3'), 3.48 (d, J = 9.1 Hz, 1 H, H2'), 4.29 (d, J = 12.5 Hz, 1 H, PhCH₂), 4.22–4.02 (m, 6 H, H2'), H4', H5', H6', PhCH₂), 3.98 (d, J = 12.7 Hz, 1 H, H6), 3.60–3.55 (m, 8 H, H2, H3, H5, H5', H6',H6″,H6‴,H6‴ᗜ), 3.40–3.43 (m, 1 H, H5'), 3.34 (s, 3 H, OCH₃), 2.13, 1.86 (s, 6 H, 2 COCH₃).

13C NMR (75 MHz, CDCl₃): δ = 170.4, 170.3 (2 C, CO₂CH₃), 167.9, 167.2 (2 C, CO₂Ph), 138.4–123.2 (Ar-C), 108.3 (C1'), 101.3 (PhCH₂), 100.8 (PhCH₂), 100.0 (C1'), 99.8 (C1), 99.4 (C1'), 87.9, 82.4, 82.2, 80.0, 81.3, 76.7, 76.0 (2 C), 75.1, 74.7, 73.8, 73.7 (PhCH₂), 73.4, 73.3 (PhCH₂), 73.1 (PhCH₂), 71.5, 71.4 (2 C, PhCH₂), 70.4 (C6″), 69.2 (C6), 69.0 (2 C, C6″), 63.7, 62.1, 55.2 (OCH₃), 55.0 (C2″), 20.8, 20.7 (2 COCH₃).

ESI-MS: m/z = 1628.4 [M + NH₄]⁺.


Methyl o-D-Galactopyranosyl-(1→6)-a-D-glucopyranosyl-(1→2)-o-D-glucopyranoside (1)

To a soln of 21 (1.4 g, 1.1 mmol) in MeOH (30 mL), was added 20% Pd(OH)₂–C (500 mg) and the mixture was stirred under H₂ at t.r.t. for 24 h. The mixture was filtered through Celite bed and concentrated to give a solid mass that was purified through Sephadex LH-20 (80% aq EtOH) to give pure trisaccharide 1 (435 mg, 80%) as an amorphous powder.

IR (KBr): 1595, 1460, 1066, 697 cm⁻¹.

1H NMR (300 MHz, D₂O): δ = 5.06 (d, J = 3.6 Hz, 2 H, H1, H1'), 4.94 (d, J = 3.6 Hz, 1 H, H1), 4.0 (dd, J = 10.0, 3.6 Hz, 1 H, H2), 3.93–3.85 (m, 2 H, H6‴,H6‴ gốc), 3.81–3.64 (m, 7 H, H3, H4, H4', H5, H5', H6', H6‴ gốc), 3.43–3.51 (m, 4 H, H6, H6‴ gốc, 3.49–3.44 (m, 2 H, H5, H5').

13C NMR (75 MHz, D₂O): δ = 96.7 (2, C1, C1'), 96.4 (C1), 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 (C6'), 60.5 (C6), 60.4 (C6‴ gốc), 55.1 (OCH₃).

ESI-MS: m/z = 541.2 [M + Na]⁺.

Anal. Caled for C₇₃H₇₄N₀₁₄: C, 44.02; H, 6.61. Found: C, 43.78; H, 6.95.

Methyl o-D-Galactofuranosyl-(1→2)-o-D-galactopyranosyl-(1→6)-(2-acetamido-2-deoxy-b-D-glucopyranosyl)-(1→2)-o-D-glucopyranoside (2)

To a soln of tetrasaccharide derivative 25 (1.6 g, 1.1 mmol) in EtOH (30 mL) was added hydrate hydrate (4 mL) and the 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 Ac₂O (5 mL) and the mixture was kept at r.t. for 1 h. The solvents were evaporated and co-evaporated with tolue under reduced pressure. To a soln of the crude mass in MeOH (25 mL) solid NaOMe was added till pH ~10 and the mixture was allowed to stir at t.r.t for 5 h. The mixture was neutralized with Amberlite IR-120 (H⁺) resin. The mixture was filtered and evaporated to dryness. To a soln of the crude product in MeOH (20 mL) was added 20% Pd(OH)₂–C (500 mg) and the mixture was stirred at t.r.t. under a positive pressure of H₂ for 24 h. The mixture was filtered through a Celite bed and concentrated to a crude mass. Successive purification of the crude mass by chromatography (silica gel, CHCl₃–MeOH–H₂O: 10:5:1) and through a Sephadex LH-20 (MeOH–H₂O: 4:1) furnished pure tetrasaccharide 2 (520 mg, 76%) as an amorphous powder.

\[ \delta \text{H}_1^{13} +43 (c. 1.0, H₂O). \]

IR (KBr): 3444, 2817, 2367, 1592, 1352, 762 cm⁻¹.

1H NMR (300 MHz, D₂O): δ = 5.81 (br s, 1 H, H1), 5.29 (br s, 1 H, H1), 5.10 (br s, 1 H, H1), 4.82 (d, J = 8.4 Hz, 1 H, H1), 4.24 (t, J = 9.0, 9.0 Hz, 1 H, H4', 4.18–4.15 (m, 2 H, H2″, H4″), 4.10–4.0 (m, 4 H, H3, H4, H6), 3.95–3.70 (m, 12 H, H3, H3″, H3‴, H2″, H4″, H5, H6″, H6‴ gốc), 3.68–3.65 (m, 1 H, H2),
3.63–3.55 (m, 2 H, H5, H5’), 3.45 (s, 3 H, OCH3), 3.44–3.42 (m, 1 H, H2), 2.08 (s, 3 H, NHCOCH3).

13C NMR (75 MHz, D2O): δ = 170.3 (COCH3), 109.5 (C1”), 102.7 (C1’), 99.4 (C1), 98.2 (C1”), 83.7 (C3”), 81.5 (C2), 81.2 (C4”), 77.2 (C2”), 75.4 (C4”), 75.3 (C4”), 74.8 (C3), 73.8 (C2”), 72.0 (2 C, C3’, C5”), 71.9 (C5), 71.4 (C5”), 70.1 (C4), 69.8 (C3”), 69.0 (C5”), 63.0 (C6”), 61.6 (C6), 61.3 (C6’”), 61.1 (C6”), 57.0 (C2”), 55.3 (OCH3), 20.8 (COCH3).

ESI-MS: m/z = 744.3 [M + Na]+.

Anal. Calcd for C27H47NO21 (721.2): C, 44.94; H, 6.56. Found: C, 55.3 (C1), 99.4 (C1), 98.2 (C1”), 83.7 (C3”), 81.5 (C2), 81.2 (C4”), 77.2 (C2”), 75.4 (C4”), 75.3 (C4”), 74.8 (C3), 73.8 (C2”), 72.0 (2 C, C3’, C5”), 71.9 (C5), 71.4 (C5”), 70.1 (C4), 69.8 (C3”), 69.0 (C5”), 63.0 (C6”), 61.6 (C6), 61.3 (C6’”), 61.1 (C6”), 57.0 (C2”), 55.3 (OCH3), 20.8 (COCH3).

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References

(1) C.D.R.I. communication no. 7139.

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