Synthesis of Pentasaccharide Repeating Unit Corresponding to the Cell Wall O-Polysaccharide of *Salmonella enterica* O55 Strain Containing a Rare Sugar 3-Acetamido-3-deoxy-D-fucose

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**Abstract** A pentasaccharide repeating unit corresponding to the cell wall O-antigen of *Salmonella enterica* O55 containing a rare sugar, 3-acetamido-3-deoxy-D-fucose has been synthesized as its p-methoxymethyl glycoside using a sequential stereoselective glycosylation strategy. A suitably functionalized 3-azido-3-deoxy-D-fucose thioglycoside derivative was prepared in very good yield and used in the stereoselective glycosylation reaction. Functionalized monosaccharide intermediates were prepared judiciously and stereoselectively assembled to get the desired pentasaccharide derivative in excellent yield.

**Key words** pentasaccharide, glycosylation, 3-acetamido-3-deoxy-D-fucose, *Salmonella enterica*, stereoselective

Food borne gastrointestinal disorders causing hospitalization and deaths are serious concern all over the world and particularly in the developing countries. Lack of adequate sanitization and intake of contaminated food and water are major cause of diarrheal infections. There are several pathogenic bacteria causing diarrheal outbreaks, which include *Escherichia coli* (*E. coli*), *Shigella*, *Vibrio cholerae*, *Proteus*, and *Salmonella* strains. The gastrointestinal disorders caused by the *Salmonella* infection are termed as salmonellosis, which are generally being treated with antimicrobial agents. The causative agent of most of the occurrence of salmonellosis in humans and animals are *Salmonella enterica* (*S. enterica*) strains. Most common symptoms of *Salmonella* infections are diarrhea, fever, vomiting with dehydration etc. Although a variety of therapeutic agents are being used for controlling food borne illness or diarrheal infections, they become ineffective because of the emergence of multidrug-resistant bacterial strains. As a result, there is a strong need to develop alternative approaches for controlling salmonellosis. In general, the polysaccharides present in the cell wall of the virulent bacteria play the pivotal role in their pathogenicity and initial stage of infection to the host. Among several strains of *S. enterica*, responsible for diarrheal infections in humans, *S. enterica* O55 deserves special attention due to its unique cell wall polysaccharide structure containing a rare sugar, 3-amino-3-deoxy-D-fucose moiety. Liu et al. reported the structure of the pentasaccharide repeating unit of the cell wall polysaccharide of *S. enterica*, which is composed of five monosaccharide moieties namely, β-D-glucose, α-D-glucose, N-acetyl-α-D-galactosamine, N-acetyl-β-D-glucosamine, and β-3-acetamido-3-deoxy-D-fucose. In the past, polysaccharide-based glycoconjugates have emerged as effective vaccine candidates against several bacterial infections such as influenza, pneumococcal, and meningitis infections. Despite the possibility of obtaining the polysaccharides from bacterial sources using biofermentation techniques, it suffers from several drawbacks, such as heterogeneity of isolated polysaccharides, handling of live bacterial strains, difficult-to-remove biological impurities etc. In contrast, chemical synthesis of the polysaccharide fragments could provide homogeneous oligosaccharides with confirmed structures. In the recent past, a number of reports appeared from our laboratory towards the synthesis of cell wall oligosaccharides and their glycoconjugates of *Salmonella* strains. In continuation, a concise synthesis of the pentasaccharide repeating unit of the cell wall polysaccharide of *S. enterica* O55 is reported herein. The synthetic strategy involves the synthesis of a rare sugar derivative, i.e. 3-azido-3-deoxy-β-D-fucosyl thioglycoside (Figure 1).

In order to synthesize the target pentasaccharide, a sequential glycosylation strategy was adopted. The suitably functionalized monosaccharide derivatives were prepared following the reaction conditions reported earlier. Thioglycoside derivatives were used as glycosyl donors for the elongation of the oligosaccharide chain under a generalized stereoselective glyco-
4-O-benzyl-2-O-benzoyl-1-thio-β-D-fucopyranoside (8) in 72% overall yield. Compound 8 was treated with triflic anhydride in the presence of pyridine to give the triflyl derivative, which was immediately treated with sodium nitrite to furnish corresponding β-gulose derivative, which on de-O-benzoylation using sodium methoxide resulted in methylphenyl 4-O-benzyl-1-thio-β-D-gulopyranoside (9) in overall 58% yield. Selective protection of the 2-hydroxy group in compound 9 with the 2-naphthylmethyl (NAP) group via the formation of stannylidene acetal by the treatment with dibutyltin oxide followed by treatment of the stannylidene acetal with 2-naphthylmethyl bromide (NAP-Br) in the presence of cesium fluoride furnished methylphenyl 4-O-benzyl-2-O-(2-naphthylmethyl)-1-thio-β-D-gulopyranoside (10) in 80% yield. Compound 10 was subjected to a sequence of functional group transformations which include: (i) treatment with triflic anhydride in the presence of pyridine to give the 3-O-triflyl derivative; (ii) Sβ2 substitution of the 3-O-triflyl group with an azido group by treatment with sodium azide; (iii) oxidative removal of the NAP group using DDQ in a biphasic reaction condition and finally (iv) acetylation of the free hydroxy group to furnish p-methylphenyl 2-O-acetyl-3-azido-4-O-benzyl-3-deoxy-1-thio-β-D-fucopyranoside (5) in 65% overall yield (Scheme 1). All synthetic intermediates were characterized by their NMR and mass spectral analysis.

Having a suitably functionalized thioglycoside donors and acceptors in hand, attempts were made to couple monosaccharide derivatives by stereoselective glycosylations in the presence of a combination of N-iodosuccinimide (NIS) and perchloric acid supported over silica (HClO4-SiO2) as thiophilic activator. Stereoselective glycosylation of compound 2 with 2-azido-2-deoxy-β-D-galactose thioglycoside derivative 3 in the presence of a combination of NIS and HClO4-SiO2 furnished disaccharide de-

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**Figure 1** Structure of the synthesized pentasaccharide corresponding to the repeating unit of the cell wall polysaccharide of *Salmonella enterica* O55 strain

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Reagents and conditions: (a) (i) Bu2SnO, CH3OH, 80 °C, 3 h; (ii) PMBCl, TBAB, DMF, 65 °C, 6 h; (b) benzyl bromide, NaH, DMF, r.t., 2 h; (c) DDQ, CH2Cl2/H2O (10:1), r.t., 3 h, 72% (3 steps); (d) Tf2O, pyridine, CH2Cl2, –10 °C, 2 h; (e) NaNO2, DMF, 60 °C, 12 h; (f) 0.1 M CH3ONa, CH2OH, r.t., 3 h, 65% (3 steps); (g) (i) Bu4NNO2, CH3OH, 80 °C, 3 h; (ii) 2-(bromomethyl)naphthalene (NAPBr), CsF, DMF, 65 °C, 6 h, 80%; (h) TfO, pyridine, CH2Cl2, –10 °C, 2 h; (i) NaNO2, DMF, 60 °C, 12 h; (j) DDQ, CH2Cl2/H2O (10:1), r.t., 3 h; (k) Ac2O, pyridine, r.t., 3 h, 65% (4 steps).

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Rivervative 11, which on subsequent de-O-acetylation using sodium methoxide gave disaccharide acceptor 12 in 69% over all yield. NMR spectroscopic analysis of compound 12 confirmed its stereoselective formation [signals at $\delta = 5.74$ ($d, J = 8.5$ Hz, H-1A), 5.64 ($s$, PhCH) in $^1$H NMR and $\delta = 101.5$ (PhCH), 98.6 (C-1B), 98.2 (C-1C) in $^{13}$C NMR spectra]. Although, the C-3 hydroxyl group is quite congested for the glycosylation reaction, the $\alpha$-glycosidic linkage was formed in compound 12 with satisfactory yield without formation of the other stereoisomer. Stereoselective glycosylation of compound 12 with d-glucose thioglycoside donor 4 in the presence of a combination$^{19a,24}$ of NIS and HClO$_4$·SiO$_2$ produced trisaccharide derivative 13, which was immediately de-O-acetylated using sodium methoxide to furnish trisaccharide acceptor 14 in 73% yield. The formation of new glycosyl linkages in compound 14 was confirmed from its NMR spectroscopic analysis [signals at $\delta = 5.75$ ($d, J = 8.5$ Hz, H-1A), 5.66 ($s$, PhCH), 5.42 ($d, J = 3.5$ Hz, H-1B), 4.62 ($br s$, H-1C) in $^1$H NMR and $\delta = 100.4$ (C-1D), 99.4 (C-1C), 98.8 (C-1B), 98.1 (C-1A) in $^{13}$C NMR spectra]. NIS and HClO$_4$·SiO$_2$ mediated$^{19a,24}$ stereoselective glycosylation of trisaccharide 14 with 3-azido-3-deoxy-d-fucosyl thioglycoside derivative 5 furnished tetrasaccharide derivative 15 in 63% yield. NMR spectroscopic analysis of compound 15 confirmed its stereoselective formation [signals at $\delta = 5.67$ ($d, J = 8.0$ Hz, H-1A), 5.58 ($s$, PhCH), 5.44 ($d, J = 3.0$ Hz, H-1B), 4.67 ($br s$, H-1C), 4.01 ($d, J = 8.0$ Hz, H-1D) in $^1$H NMR and $\delta = 101.6$ (PhCH), 100.2 (C-1D), 99.4 (C-1C), 99.0 (C-1B), 98.2 (C-1A) in $^{13}$C NMR spectra]. De-O-acetylation of compound 15 by treatment with sodium methoxide furnished tetrasaccharide acceptor 16 in 84% yield, which was characterized by its NMR spectral analysis. Compound 16 was allowed to couple stereoselectively with d-glucose thioglycoside derivative 6 in the presence of a combination$^{19a,24}$ of NIS and HClO$_4$·SiO$_2$ to furnish pentasaccharide derivative 17 in 64% yield. The formation of new glycosyl linkages in compound 17 was confirmed from its NMR spectroscopic analysis [signals at $\delta = 5.52$ ($d, J = 8.0$ Hz, H-1A), 4.95 ($d, J = 3.0$ Hz, H-1B), 4.65 ($d, J = 9.0$ Hz, H-1C), 4.00–3.98 (2 $d, J = 8.0$ Hz, H-1D, H-1E) in $^1$H NMR and $\delta = 100.5$ (C-1D), 100.4 (C-1B), 99.4 (C-1C), 98.9 (C-1A), 98.1 (C-1E) in $^{13}$C NMR spectra]. Compound 17 was subjected to a series of functional group transformations, which include (i) treatment with hydrazine hydrate monohydrate to remove phthaloyl group;$^{36}$ (ii) N- and O-acetylation using acetic anhydride and pyridine; (iii) transformation of the azido group into an acetamido group by treatment with thioacetic acid in pyridine;$^{17}$ (iv) de-O-acetylation using sodium methoxide; and finally (v) removal of benzyl ethers and benzylidene acetal by hydrogenolysis using hydrogen gas in the presence of Pearlman's catalyst$^{20}$ to give target pentasaccharide 1 as its p-methoxyphenyl glycoside in 52% over all yield. NMR spectroscopic analysis of compound 1 unambiguously confirmed its formation [signals at $\delta = 5.25$ ($br s$, H-1), 4.97 ($br s$, H-1), 4.95 ($d, J = 9.5$ Hz, H-1), 4.52 ($d, J = 9.0$ Hz, H-1), 4.32 ($d, J = 9.0$ Hz, 1 H, H-1C) in $^1$H NMR and $\delta = 102.8$ (C-1D), 102.6 (C-1B), 101.1 (C-1A), 101.0 (C-1B), 98.9 (C-1C) in $^{13}$C NMR spectra] (Scheme 2).

In summary, a pentasaccharide repeating unit of the O-specific polysaccharide of Salmonella enterica O55 containing 3-acetamido-3-deoxy-d-fucose moiety has been synthesized in very good yield using a sequential glycosylation strategy. To the best of our knowledge, a suitably functionalized 3-azido-3-deoxy-d-fucosyl thioglycoside derivative was prepared in excellent yield and used in the stereoselective glycosylation reaction for the first time. A-
bination of NIS and HClO₄-SiO₂ has been used as the thio-
philic activator for the stereoselective glycosylations of
thioglycosides in generalized reaction conditions. The
yields of the glycosylation steps were very good with ex-
cellent stereo outcome.

All reactions were monitored by TLC over silica gel coated TLC plates. The
spots on TLC were visualized by warming ceric sulfate (2% Ce(SO₄)₂ in 2 N H₂SO₄) sprayed plates on a hot plate. Silica gel 230–
400 mesh was used for column chromatography. NMR spectra were
recorded on Bruker Avance 500 MHz using CDCl₃ as solvent and TMS as internal reference unless stated otherwise. MS were recorded on a
Bruker mass spectrometer. Optical rotations were recorded in a Jasco
P-2000 spectrometer at 25 °C. Commercially available grades of or-
ganic solvents of adequate purity are used in all reactions. HClO₄-SiO₂
was prepared following the reported method.25

p-Methylphenyl 4-O-Benzyl-2-O-benzoyl-1-thio-β-D-fucopyrano-
side (8)
To a solution of 7 (3 g, 8.02 mmol) in CH₂OH (45 mL) was added Bu₂SnO (2.4 g, 9.62 mmol) and the mixture was stirred at 80 °C for 3 h.
the solvents were evaporated and co-evaporated with toluene (3 × 30 mL) under reduced pressure. To a solution of the crude product in
dry DMF (20 mL) were added PMBCl (1.2 mL, 8.82 mmol) and TBAB (2.25 g) and the mixture was stirred at 65 °C for 6 h. The mixture was
diluted with H₂O (100 mL) and extracted with EtOAc (100 mL). The organic layer was successively washed with 2 M HCl (50 mL) and H₂O
(50 mL), dried (Na₂SO₄), and concentrated. To a solution of the crude product in DMF (20 mL) was added NaH (60% oil coated; 300 mg) and the
mixture was stirred at 0 °C. To the stirred solution was added benz-
yl bromide (1 mL, 9.25 mmol) and the mixture was stirred at r.t. for 2 h. The mixture was quenched with aq NH₄Cl, diluted with H₂O (50 mL)
and it was stirred at 60 °C for 12 h. The reaction mixture was diluted with H₂O (50 mL) and extracted with EtOAc (100 mL), dried (Na₂SO₄), and concentrated.
The obtained crude product was purified by column chromatography (silica gel, hexane/EtOAc 3:1) to give pure 8 (2.68 g, 72%) as a colorless oil.

[a]₀ = −7.0 (c 1.0, CHCl₃).

1H NMR (500 MHz, CDCl₃): δ = 7.99–6.96 (m, 14 H, Ar-H), 5.10 (s, J = 10.0 Hz, 1 H, H-2), 4.72 (br s, 2 H, CH₂Ph), 4.62 (d, J = 10.0 Hz, 1 H, H-1), 3.72 (m, 1 H, H-5), 3.61–3.38 (m, 2 H, H-3, H-4), 2.25 (s, 3 H, CH₃), 1.30 (d, J = 6.5 Hz, 3 H, CH₃).

13C NMR (125 MHz, CDCl₃): δ = 166.6 (COPh), 138.1–127.6 (Ar-C), 85.9 (C-1), 80.1 (C-3), 75.9 (CH₂Ph), 74.9 (C-5, C-4), 72.3 (C-2), 21.2 (CH₃), 17.2 (CCH₃).


p-Methylphenyl 4-O-Benzyl-1-thio-β-D-gulopyranoside (9)
A solution of compound 8 (1.8 g, 3.87 mmol) in dry CH₂Cl₂ (25 mL) was
cooled to 10 °C. To the cooled reaction mixture were added pyridine (1 mL) and TfO (715 μL, 4.26 mmol) and it was stirred at same
temperature for 2 h. The solvents were removed and co-evaporated with toluene (2 × 20 mL) under reduced pressure. To a solution of the

[a]₀ = +7.0 (c 1.0, CHCl₃).

1H NMR (500 MHz, CDCl₃): δ = 7.50–6.79 (m, 16 H, Ar-H), 4.95 (d, J = 10.0 Hz, 1 H, H-1), 4.89 (d, J = 10.0 Hz, 1 H, CH₂Ph), 4.68 (d, J = 11.5 Hz, 1 H, CH₂Ph), 4.44–4.43 (m, 2 H, CH₂Ph), 4.01–4.00 (m, 2 H, H-3, H-5), 3.76 (dd, J = 10.0, 3.0 Hz, 1 H, H-2), 3.35 (d, J = 2.5 Hz, 1 H, H-4), 2.37 (s, 3 H, CH₃), 1.27 (d, J = 6.5 Hz, 3 H, CH₃).

13C NMR (125 MHz, CDCl₃): δ = 132.2–125.9 (Ar-C), 83.9 (C-1), 78.0 (C-3), 73.7 (C-4), 73.1 (CH₂Ph), 72.9 (CH₂Ph), 71.1 (C-5), 67.1 (C-2), 21.2 (CH₃), 16.3 (CCH₃).


p-Methylphenyl 4-O-Benzyl-2-O-naphthylmethyl-1-thio-β-D-
gulopyranoside (10)
To a solution of 9 (900 mg, 2.50 mmol) in CH₂OH (30 mL) was added Bu₂SnO (750 mg, 3.00 mmol) and the mixture was stirred at 80 °C for 3 h. The solvents were evaporated and co-evaporated with toluene (3 × 20 mL) under reduced pressure. To a solution of the crude product in dry DMF (10 mL) were added 2-[bromomethyl]napththalene (610 mg, 2.75 mmol) and CS₂ (380 mg, 2.5 mmol) and the mixture was stirred at 65 °C for 6 h. The mixture was diluted with H₂O (50 mL) and ex-
ttracted with EtOAc (50 mL). The organic layer was successively washed with 2 M HCl (50 mL) and H₂O (50 mL), dried (Na₂SO₄), and concentrated. The crude product was purified by column chromatography (silica gel, hexane/EtOAc 2:1) to give pure 10 (1.0 g, 80%) as a colorless oil.

[a]₀ = −17.0 (c 1.0, CHCl₃).

1H NMR (500 MHz, CDCl₃): δ = 7.50–7.07 (m, 16 H, Ar-H), 4.95 (d, J = 10.0 Hz, 1 H, H-1), 4.89 (d, J = 10.0 Hz, 1 H, CH₂Ph), 4.68 (d, J = 11.5 Hz, 1 H, CH₂Ph), 4.44–4.43 (m, 2 H, CH₂Ph), 4.01–4.00 (m, 2 H, H-3, H-5), 3.76 (dd, J = 10.0, 3.0 Hz, 1 H, H-2), 3.35 (d, J = 2.5 Hz, 1 H, H-4), 2.37 (s, 3 H, CH₃), 1.27 (d, J = 6.5 Hz, 3 H, CH₃).

13C NMR (125 MHz, CDCl₃): δ = 132.2–125.9 (Ar-C), 83.9 (C-1), 78.0 (C-3), 73.7 (C-4), 73.1 (CH₂Ph), 72.9 (CH₂Ph), 71.1 (C-5), 67.1 (C-2), 21.2 (CH₃), 16.3 (CCH₃).

mixture was diluted with H2O (50 mL) and extracted with CH2Cl2 (50 mL). The organic layer was washed with H2O (50 mL), dried (Na2SO4), and concentrated. To a solution of the crude product in pyridine (5 mL) was added Ac2O (2 mL) and the mixture was stirred at r.t. for 3 h. The mixture was concentrated under reduced pressure and co-evaporated with toluene (3 × 20 mL). The crude product was purified by column chromatography (silica gel, hexane/EtOAc 3:1) to give pure 5 (555 mg, 65%) as a colorless oil.

[a]D −10.0 (c 1.0, CHCl3).

1H NMR (500 MHz, CDCl3): δ = 7.32–6.98 (m, 9 H, Ar-H), 5.23 (t, J = 5.0 Hz, 1 H, H-1A), 8.45 (d, J = 11.5 Hz, 1 H, CH2Ph), 4.53 (d, J = 11.5 Hz, 1 H, CH2Ph), 4.47 (d, J = 10.0 Hz, 1 H, H-1), 3.54–3.47 (m, 2 H, H-4, H-5), 3.41 (dd, J = 10.5, 3.0 Hz, 1 H, H-3), 2.26 (s, 3 H, CH3), 2.08 (s, 3 H, COCH3), 1.17 (d, J = 6.5 Hz, 3 H, CH2). 13C NMR (125 MHz, CDCl3): δ = 170.1 (COCH3), 132.9–128.0 (Ar-C), 86.8 (C-1), 77.9 (C-3), 75.4 (CH2Ph), 75.3 (C-4), 68.7 (C-5), 65.2 (C-2), 21.2 (CH3), 20.9 (COCH3), 17.0 (CH3). HRMS (ESI): m/z [M + H]+ calcd for C48H46N4O12 (870.3112): 871.3190; found: 871.3190.

p-Methoxophenyl (2-Azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyra- nosyl)-(1 → 3)-4,6-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (12)

To a solution of 2 (1.5 g, 2.98 mmol) and 3 (1.85 g, 3.57 mmol) in anhyd CH2Cl2 (10 mL) was added MS 4Å (1.0 g) and the mixture was cooled to −10 °C under argon. The mixture was filtered through a Celite bed and washed with CH2Cl2 (50 mL). The combined organic layers were successively washed with 5% Na2SO4 (50 mL), sat. NaHCO3 (50 mL), and H2O (50 mL), dried (Na2SO4), and concentrated. A solution of the trisaccharide derivative in 0.01 M CH3ONa in CH3OH (20 mL) was stirred at r.t. Neutralized with Amberlite IR-120 (H+), filtered, and concentrated. The crude product was purified by column chromatography (silica gel, hexane/EtOAc 3:1) to give pure 12 (1.4 g, 73%) as a colorless oil.

[a]D −21.0 (c 1.0, CHCl3).

1H NMR (500 MHz, CDCl3): δ = 7.74–6.69 (m, 38 H, Ar-H), 5.75 (d, J = 8.5 Hz, 1 H, H-1A), 5.66 (s, 1 H, PhCH), 5.42 (d, J = 3.5 Hz, 1 H, H-1B), 4.85–4.81 (m, 3 H, H-3A, 2 CHPh), 4.80 (d, J = 11.5 Hz, 1 H, CHPh), 4.77 (d, J = 12.0 Hz, 1 H, CHPh), 4.71–4.63 (m, 2 H, 2 CHPh), 4.62 (br s, 1 H, H-1C), 4.59–4.54 (m, 2 H, H-2B, CHPh), 4.45–4.40 (m, 2 H, H-6B, CHPh), 4.00 (t, J = 9.0 Hz, 1 H, H-1), 3.91–3.83 (m, 3 H, H-3A, H-3B, H-6B), 3.83 (s, 1 H, H-4A), 3.78–3.76 (m, 2 H, H-5A-H-5B), 3.70 (s, 3 H, OCH3), 3.67 (d, J = 12.5 Hz, 1 H, CHPh), 3.62–3.60 (m, 2 H, H-2B, H-6A), 3.55 (d, J = 12.0 Hz, 1 H, CHPh), 3.47–3.43 (m, 2 H, H-2B, H-6A), 3.29–3.27 (m, 2 H, H-2B, H-6A), 3.20 (d, J = 10.0 Hz, 1 H, H-6A), 2.59–2.56 (m, 1 H, H-6A).

13C NMR (125 MHz, CDCl3): δ = 155.7–114.5 (Ar-C), 101.7 (PhCH), 99.4 (C-1C), 98.8 (C-1A), 98.1 (C-1B), 82.4 (C-4A), 81.7 (C-3), 80.0 (C-2), 77.4 (C-4C), 75.3 (CH2Ph), 75.1 (C-4B), 74.9 (CH2Ph), 74.3 (C-3), 74.2 (C-4B), 73.7 (CH2Ph), 72.4 (CH2Ph), 72.1 (CH1Ph), 71.3 (C-3), 69.9 (C-5B), 68.6 (C-6B), 66.9 (C-6B), 66.1 (C-6B), 60.9 (C-6B), 59.1 (C-6B), 55.4 (OCH3), 55.2 (C-2).


p-Methoxophenyl (2-O-Acetyl-3-azido-4-O-benzyl-3-deoxy-β-D-fucopyranosyl)-(1 → 6) → (2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-(1 → 4) → (2-azido-3,6-di-O-benzyl-2-deoxy-α-D-glucopyranosyl)-(1 → 3)-4,6-benzylidene-2-deoxy-2-N-phthalimido-β-D-glucopyranoside (15)

To a solution of 14 (800 mg, 0.61 mmol) and 5 (395 mg, 0.91 mmol) in anhyd CH2Cl2 (10 mL) was added MS 4Å (0.5 g) and the mixture was cooled to −70 °C under argon. To the cooled mixture were added NIS (375 mg, 1.66 mmol) and HClO4–SiO2 (30 mg) and it was stirred at −10 °C for 2 h. The mixture was filtered through a Celite bed and washed with CH2Cl2 (50 mL). The combined organic layers were successively washed with 5% Na2SO4 (50 mL), sat. NaHCO3 (50 mL), and H2O (50 mL), dried (Na2SO4), passed through a short pad of silica gel, and concentrated. A solution of the trisaccharide derivative in 0.01 M CH3ONa in CH3OH (20 mL) was stirred at r.t. Neutralized with Amberlite IR-120 (H+) resin, filtered, and concentrated. The crude product was purified by column chromatography (silica gel, hexane/EtOAc 3:1) to give pure 15 (620 mg, 63%) as a colorless oil.

[a]D −19.0 (c 1.0, CHCl3).

1H NMR (500 MHz, CDCl3): δ = 7.97–6.65 (m, 43 H, Ar-H), 5.67 (d, J = 8.0 Hz, 1 H, H-1A), 5.58 (s, 1 H, PhCH), 5.44 (d, J = 3.0 Hz, 1 H, H-1B), 5.16 (t, J = 8.0 Hz, 1 H, H-2A), 4.84–4.68 (m, 6 H, 6 PhCH), 4.67 (br s, 1 H, H-1C), 4.66–4.33 (m, 7 H, 2 CHPh, 6 CHPh), 4.01 (d, J = 8.0 Hz, 1 H, H-1D), 3.96–3.90 (m, 2 H, H-2B, H-6A), 3.88–3.76 (m, 3 H, H-3A, H-3B, H-4A), 3.72–3.66 (m, 2 H, H-2B, H-6A), 3.64 (s, 3 H, OCH3), 3.62–3.50 (m, 3 H, H-5A).
H, H-2a, H-3a, H-4a); 3.49–3.32 (m, 2 H, H-2b, H-3b), 3.20–3.11 (m, 2 H, H-2c, H-3c), 2.52–2.48 (m, 1 H, H-6b), 1.74 (s, 3 H, CH3C(OH)), 1.17 (s, 3 H, CH3).

11C NMR (125 MHz, CDCl3): δ = 168.9 (COCH3), 155.6–114.5 (Ar-C), 101.6 (PhCH), 100.2 (C-1a), 99.4 (C-1b), 99.0 (C-1c), 98.2 (C-1d), 82.4 (C-3a), 81.8 (C-3b), 80.1 (C-3c), 79.6 (C-2), 77.5 (CH3Ph), 71.5 (C-3d), 74.9 (CHPh), 74.6 (C-3e), 75.4 (CHPh), 74.4 (C-4), 74.0 (C-4a), 73.9 (CH3Ph), 73.8 (CHPh), 72.2 (C-2a), 72.1 (C-2b), 72.0 (2C, 2 CH2Ph), 69.8 (C-5a), 69.7 (C-5c), 69.6 (C-5b), 68.6 (C-6a), 67.0 (C-6c), 66.8 (C-6b), 66.1 (C-5d), 58.9 (C-2a), 55.5 (OCH3), 55.2 (C-2b), 20.8 (COCH3), 17.4 (CH3C(OH)).

HRMS (ESI): m/z [M + H]+ calcd for C90H91N7O21 (1605.6268); found: 1605.6264; calculated mass: 1605.6254.

p-Methoxyphenyl (3-Azido-4-O-benzyl-3-deoxy-β-D-fucopyranosyl)-(1→6)-(2,3,4-tri-O-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→4)-(2-azido-3,6-di-O-benzyl-2-deoxy-α-D-galactopyranosyl)-(1→3)-4,6-benzylidene-2-N-phenylalmidino-β-D-glucopyranoside (16)

A solution of 15 [600 mg, 0.37 mmol] in 0.01 M CH3ONa in CH3OH (15 ml) was stirred at r.t. for 1 h, neutralized with Amberlite IR-120 (H+) resin, filtered, and concentrated. The crude product was purified by column chromatography (silica gel, hexane/ EtOAc: 2:1) to give pure 16 (486 g, 84%) as a colorless oil.

[a]δ = -160.0 (c 1.0, CHCl3).

1H NMR (500 MHz, CDCl3): δ = 7.66–6.61 (m, 43 H, Ar-H), 5.67 (d, J = 8.5 Hz, 2H, H-1a, H-1b), 5.58 (s, 1 H, PhCH), 5.36 (d, J = 3.0 Hz, 1 H, H-1c), 4.89 (t, J = 9.0 Hz, 1 H, H-1d), 4.75–4.65 (m, 4 H, 4 CH2Ph), 4.61–4.56 (m, 4 H, H-3a, H-3b, H-3c, H-3d), 4.40–4.34 (m, 3 H, H-3e, H-3f, H-3g), 3.98–3.92 (m, 3 H, H-3h, H-3i, H-3j), 3.80–3.77 (m, 3 H, H-3k, H-3l), 3.70–3.67 (m, 2 H, H-2a, H-2b), 3.63 (s, 3 H, OCH3), 3.57–3.52 (m, 3 H, H-4a, H-4b, H-4c), 3.45–3.40 (m, 3 H, H-4d, H-4e, H-4f, H-4g), 3.32–3.10 (m, 2 H, H-5a, H-5b), 2.49–2.45 (m, 1 H, H-6a), 1.21 (d, J = 6.5 Hz, 3 H, CH3C(OH)).

HRMS (ESI): m/z [M + H]+ calcd for C76H70N7O20 (1536.6162); found: 1536.6240; calculated mass: 1536.6218.

p-Methoxyphenyl (β-D-Glucopyranosyl)-(1→2)-(3-acetamido-3-deoxy-β-D-fucopyranosyl)-(1→6)-(α-D-glucopyranosyl)-(1→4)-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-(1→3)-2-acetamido-2-deoxy-β-D-glucopyranoside (1)

To a solution of 17 (100 mg, 0.05 mmol) in EtOH (10 ml) was added NaNH2/MeOH (0.7 ml) and the mixture was stirred at 70 °C for 2 h. The solvents were removed under reduced pressure and a solution of the crude product in Ac2O (2 ml) and pyridine (2 ml) was kept at r.t. for 4 h. To a solution of the acetylated product in pyridine (2 ml) was added CH2COSH (1.0 ml) and the mixture was stirred at r.t. for 12 h. The solvents were removed and co-evaporated with toluene (3 × 20 ml) under reduced pressure and the crude product was passed through a short pad of silica gel. A solution of the N-acetylated product in 0.1 M CH3ONa in CH3OH (10 ml) was stirred at r.t. for 6 h, neutralized with Amberlite IR-120 (H+) resin, filtered, and concentrated. To the solution of the de-N-acetylated product in CH3OH (5 ml) was added 20% Pd(OH)2/C (25 mg) and the mixture was stirred at r.t. under a positive pressure of H2 for 24 h. The mixture was filtered through a Celite bed, washed with CH3OH/H2O (20 ml, 2:1), and concentrated under reduced pressure. The deprotected product was passed through a Sephadex LH-20 column (CH3OH/H2O:3:1) to give pure 18 (27 mg, 52%) as a white powder.

[a]δ = -160.0 (c 0.5, H2O).

1H NMR (500 MHz, D2O): δ = 7.20–6.91 (m, 4 H, Ar-H), 5.25 (brs, 1 H, H-1), 4.97 (brs, 1 H, H-1a), 4.95 (d, J = 9.5 Hz, 1 H, H-1b), 4.52 (d, J = 9.0 Hz, 1 H, H-1c), 4.32 (d, J = 9.0 Hz, 1 H, H-1d), 4.13–3.96 (m, 3 H, H-2a, H-2b, H-3a, 3.85–3.79 (m, 4 H, H-2c, H-2d, H-2e, H-2f, H-2g), 3.71–3.55 (m, 5 H, H-3a, H-3b, H-3c, H-3d, H-3e, H-3f, H-3g), 3.53–3.50 (m, 2 H, H-5a, H-5b), 3.45–3.40 (m, 3 H, H-2c, H-3a, H-3b), 3.35–3.21 (m, 3 H, H-4a, H-5c, H-5d), 2.05 (s, 3 H, CH3CO2H), 2.0 (s, 3 H, CO2H), 1.97 (s, 3 H, CO2H), 1.07 (d, J = 6.5 Hz, 3 H, CH3).
Conflict of Interest

The authors declare no conflict of interest.

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Supporting Information

Supporting information for this article is available online at https://doi.org/10.1055/s-0037-1610777. Copies of 1D and 2D NMR spectra of compounds 1 and 8–17 are provided.

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