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 glycosyl donor 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 sanitation 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 5 (Figure 1).

In order to synthesize the target pentasaccharide 1, a sequential glycosylation strategy has been adopted. The suitably functionalized monosaccharide derivatives 2, 3, 4, 5, and 6 were prepared following the reaction conditions reported earlier. Thioglycoside derivatives 3, 4, 5, and 6 were used as glycosyl donors for the elongation of the oligosaccharide chain under a generalized stereoselective glyco-
benzylation of the C-4 hydroxyl group using (PMBCl) in the presence of tetrabutylammonium bromide followed by treatment with formation of stannylidene acetal using dibutyltin oxide.

- Benzyl bromide in the presence of sodium hydride; and oxidative removal of the PMB group using DDQ in a biphasic reaction condition to give 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-D-gulose derivative, which on de-O-benzylation using sodium methoxide resulted p-methylyphenyl 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 p-methylyphenyl 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) S_{0,2} 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 hydroxyl group to furnish p-methylyphenyl 2-O-acetyl-3-azido-4-O-benzyl-3-deoxy-1-thio-D-galactopyranoside (5) in 65% overall yield (Scheme 1). All synthetic intermediates were characterized by their NMR and mass spectral analysis.

The rare sugar derivative 5, was prepared from the p-Methylphenol 2-O-benzoyl-1-thio-β-D-fucopyranoside (7), prepared from d-galactose in eight steps was subjected to a number of reactions involving: (a) selective p-methoxybenzylidation at the C-3 hydroxyl group via the formation of stannylidene acetal using dibutyltin oxide followed by treatment with p-methoxybenzyl chloride (PMBCl) in the presence of tetrabutylammonium bromide (TBAB); (b) benzylation of the C-4 hydroxyl group using benzyl bromide in the presence of sodium hydride; and (c) oxidative removal of the PMB group using DDQ in a biphasic reaction condition to give p-methylphenol 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-D-gulose derivative, which on de-O-benzylation using sodium methoxide resulted p-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 p-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) S_{0,2} 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 hydroxyl 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 set of 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 (HClO_{4}-SiO_{2}) as thiophilic activator. Stereoselective glycosylation of compound 2 with 2-azido-2-deoxy-d-galactose thioglycoside derivative 3 in the presence of a combination of NIS and HClO_{4}-SiO_{2} furnished disaccharide de-
derivative 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, $H-1_A$), 5.64 (s, PhCH$_2$), 5.37 (d, $J = 3.5$ Hz, $H-1_B$), 5.42 (d, $J = 3.5$ Hz, $H-1_C$) in $^1$H NMR and $\delta = 101.5$ (PhCH$_3$), 98.6 (C-1$_B$), 98.2 (C-1$_C$) in $^{13}$C NMR spectra]. Although, the C-3 hydroxyl group is quite congested for the glycosylation reaction, the a-glycosidic linkage was formed in compound 12 with satisfactory yield without formation of the other stereoisomer. Stereoselective glycosylation of compound 12 with D-glucose derived thioglycoside donor 4 in the presence of a combination$^{19_a,24}$ 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, $H-1_A$), 5.66 (s, PhCH$_2$), 5.42 (d, $J = 3.5$ Hz, $H-1_B$), 4.62 (br s, $H-1_C$) in $^1$H NMR and $\delta = 101.7$ (PhCH$_3$), 99.4 (C-1$_C$), 98.8 (C-1$_B$), 98.1 (C-1$_A$) in $^{13}$C NMR spectra]. NIS and HClO$_4$-SiO$_2$ mediated$^{19_a,24}$ stereoselective glycosylation of trisaccharide 14 with 3-azido-3-deoxy-D-fucose 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, $H-1_A$), 5.58 (s, PhCH$_2$), 5.44 (d, $J = 3.0$ Hz, $H-1_B$), 4.67 (br s, $H-1_C$), 4.01 (d, $J = 8.0$ Hz, $H-1_D$, H-1$_E$) in $^1$H NMR and $\delta = 101.6$ (PhCH$_3$), 100.2 (C-1$_D$), 99.4 (C-1$_C$), 99.0 (C-1$_B$), 98.2 (C-1$_A$) 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$^{19_a,24}$ 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, $H-1_A$), 4.95 (d, $J = 3.0$ Hz, $H-1_B$), 4.65 (d, $J = 3.0$ Hz, $H-1_C$), 4.00–3.98 (2 d, $J = 8.0$ Hz, $H-1_D$, $H-1_E$) in $^1$H NMR and $\delta = 100.5$ (PhCH$_3$), 100.4 (C-1$_D$), 99.4 (C-1$_C$), 98.9 (C-1$_B$), 98.1 (C-1$_A$) 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-deacetylation using acetic anhydride and pyridine; (iii) transformation of the azido group into an acetamido group by treatment with thioacetic acid in pyridine;$^{37}$ (iv) O-deacetylation using sodium methoxide; and finally (v) removal of benzyl ethers and benzylidene acetal by hydrolysis using hydrogen gas in the presence of Pearlman’s catalyst$^{38}$ 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 $\delta = 8.25$ (br s, $H-1_A$), 4.97 (br s, $H-1_B$), 4.95 (d, $J = 9.5$ Hz, $H-1_C$), 4.52 (d, $J = 9.0$ Hz, $H-1_D$), 4.32 (d, $J = 9.0$ Hz, 1 H, $H-1_E$) in $^1$H NMR and $\delta = 102.8$ (C-1$_D$), 102.6 (C-1$_B$), 101.1 (C-1$_C$), 101.0 (C-1$_A$), 98.9 (C-1$_C$) 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-fucose 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-
ophilic activator for the stereoselective glycosylations of
thioglycosides in generalized reaction conditions. The
yields of the glycosylation steps were very good with excel-
 lent 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% Ce₂(SO₄)₃ 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-fucopyranoside (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 mmol) and the mixture was stirred at room temperature for 3 h,
neutralized with Amberlite IR-120 (H⁺) resin, filtered and concentrated
to give compound 9 (1.0 g, 65%) as a colorless oil.

[α]D = −17.0 (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), 3.61–3.58 (m, 2 H, H-3, H-4), 2.25 (s, 3 H, CH₃),
1.30 (d, J = 6.5 Hz, 3 H, CH₃). ²⁶

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 (CH₂). ²⁶

HRMS (ESI): m/z [M + H]+ calcd for C₂₅H₂₄O₄S (500.2021): 501.2099; found:
501.2082.

p-Methylphenyl 2-O-Acetyl-3-azido-4-O-benzyl-3-deoxy-1-thio-β-D-
fucopyranoside (5)

A solution of 10 (1.0 g, 2.0 mmol) in dry CH₂Cl₂ (15 mL) was cooled to
−10 °C. To the cooled mixture were added pyridine (0.5 mL) and TF₂O
(850 μL, 5.06 mmol) and it was stirred at −10 °C for 2 h. The solvents
were removed and co-evaporated with toluene (2 × 20 mL) under reduced pressure. To a solution of the

The crude product in dry DMF (10 mL) was added NaNO₂ (2.4 g, 33 mmol) and it was stirred at 60 °C for 12 h. The reaction mixture was diluted with
H₂O (50 mL) and extracted with CH₂Cl₂ (50 mL). The organic layer
was washed with water (50 mL), dried (Na₂SO₄), and concentrated. A solution of the crude product in 0.1 M CH₃ONa in CH₂OH (10 mL) was stirred at room temperature for 3 h, neutralized with Amberlite IR-120 (H⁺) resin, filtered and concentrated to give compound 9 (910 mg, 65%) as a colorless oil.

[α]D = −12.0 (c 1.0, CHCl₃).

1H NMR (500 MHz, CDCl₃): δ = 7.36–7.01 (m, 9 H, Ar-H), 4.71 (d, J =
10.0 Hz, 1 H, H-1), 4.59 (d, J = 12.0 Hz, 1 H, CH₂Ph), 4.48 (d, J = 12.0 Hz,
1 H, CH₂Ph), 4.14–4.13 (m, 1 H, H-5), 3.96–3.95 (m, 1 H, H-3), 3.67
(dd, J = 10.0, 3.5 Hz, 1 H, H-2), 3.30 (d, J = 2.5 Hz, 1 H, H-4), 2.27 (s, 3 H,
CH₃), 1.19 (d, J = 6.5 Hz, 3 H, CH₃). ²⁶

13C NMR (125 MHz, CDCl₃): δ = 138.0–126.9 (Ar-C), 86.0 (C-1), 78.0
(C-3), 72.8 (CH₂Ph), 71.7 (C-4), 67.5 (C-5), 66.9 (C-2), 21.2 (CH₃), 16.4
(CH₂). ²⁶

HRMS (ESI): m/z [M + H]+ calcd for C₂₇H₂₈O₅S (464.1657): 465.1735; found:
465.1671.

p-Methylphenyl 4-O-Benzyl-1-thio-β-D-galactopyranoside (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 pyri-
dine (1 mL) and TF₂O (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

The obtained crude was purified by column chromatography (silica gel, hexane/EtOAc 3:1) to give pure 8 (2.68 g, 72%) as a colorless oil.

[α]D = −7.9 (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), 3.61–3.58 (m, 2 H, H-3, H-4), 2.25 (s, 3 H, CH₃),
1.30 (d, J = 6.5 Hz, 3 H, CH₃). ²⁶

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 (CH₂). ²⁶

HRMS (ESI): m/z [M + H]+ calcd for C₂₅H₂₄O₄S (464.1657): 465.1735; found:
465.1671.
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 x 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.

\[ \text{[M + H]}^{+} \text{calcd for C}_{22}\text{H}_{25}\text{N}_{3}\text{O}_{4}\text{S (427.1566): 428.1656;} \]

p-Methoxyphenyl (2-Azido-3,6-di-O-benzyl-2-deoxy-o-D-glucopyranosyl)-(1-4)-6-O-benzylidene-2-deoxy-2-N-phthalimido-ß-o-glucopyranoside (12)

To a solution of 12 (1.1 g, 1.26 mmol) and 4 (900 mg, 1.51 mmol) in anhyd CH2Cl2 (10 mL) was added MS 4Å (1.0 g) and the mixture was cooled to −10 °C under argon. To the cooled mixture was 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. 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 3:1) to give pure 14 (1.2 g, 73%) as a colorless oil.

\[ \text{[M + H]}^{+} \text{calcd for C}_{75}\text{H}_{74}\text{N}_{4}\text{O}_{17 (1302.5049): 1303.5118;} \]

p-Methoxyphenyl (2-O-Acetyl-3-azido-4-O-benzyl-3-deoxy-o-D-glucopyranosyl)-(1-6)-(2,3,4-tri-O-benzyl-ß-o-glucopyranosyl)-(1-4)-(2-azido-3,6-di-O-benzyl-2-deoxy-o-D-galactopyranosyl)-(1-3)-4,6-benzylidene-2-deoxy-2-N-phthalimido-ß-o-glucopyranoside (15)

To a solution of 15 (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 was added NIS (225 mg, 1.00 mmol) and HClO4-SiO2 (25 mg) and it was stirred at −70 °C for 3 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 (25 mL), dried (Na2SO4), and concentrated. The crude product was purified by column chromatography (silica gel, hexane/EtOAc 3:1) to give pure 16 (520 mg, 63%) as a colorless oil.

\[ \text{[M + H]}^{+} \text{calcd for C}_{73}\text{H}_{82}\text{N}_{6}\text{O}_{16 (1512.8258): 1514.8302;} \]
H, H-2c, H-3a, H-4d), 3.49–3.32 (m, 5 H, H-4a–4c), 3.30–3.25 (m, 2 H, H-2h, H-3g), 2.30–3.11 (m, 2 H, H-1h, H-2c), 2.52–
2.48 (m, 1 H, H-6a), 1.74 (s, 3 H, COCH3), 1.17 (s, 3 H, CH3).

11C NMR (125 MHz, CDCl3): δ = 168.9 (COCH3), 155.6–114.5 (Ar-C), 101.6 (PhCH3), 100.2 (C-1c), 99.4 (C-1a), 99.0 (C-1e), 98.2 (C-1f), 82.4 (C-4d), 61.8–61.1 (m, 2 H, H-2h, H-3g), 49.7 (C-3C), 48.9 (C-3B), 48.1 (C-3A), 48.0 (C-3E), 47.3 (C-4D), 47.0 (C-4E), 47.0 (C-4A), 79.8 (C-6A), 79.1 (C-6B, C-6C), 78.3 (C-6D), 72.8 (C-5C, C-5D), 72.0 (C-5B), 71.9 (C-5E), 71.6 (C-5A), 70.4 (C-5H), 55.3 (C-2A), 55.2 (C-2c), 20.8 (COCH3), 74.2 (CH2).

HRMS (ESI): m/z [M + H+] c Wein CH3N=O2 (1941.7477); 1942.7555; found: 1942.7540.

p-Methoxystyryl (2,3,4-Tri-O-acetyl-6-benzyl-β-D-fucopyranosyl)-(1→6)-(2,3,4-Tri-O-acetyl-6-benzyl-β-D-galactopyranosyl)-(1→3)-4-Henzydine-2-N-phthalimido-β-D-glucopyranoside (17)

To a solution of 16 (200 mg, 0.13 mmol) and 6 (130 mg, 0.26 mmol) in anhyd CH2Cl2 (5 mL) was added MeSi (0.3 g) and the mixture was cooled to −10 °C under argon. To the cooled mixture were added NIS (65 mg, 0.26 mmol) and HClO4 (5 mg) and it was stirred at −20 °C for 3 h. The mixture was filtered through a Celite bed and washed with CH2Cl2 (20 mL). The combined organic layers were successively washed with 5% Na2SO4 (10 mL), sat. NaHCO3 (10 mL), and CH3OH (10 mL), dried (Na2SO4), and concentrated. The crude product was purified by column chromatography (silica gel, hexane/EtOAc 2:1) to give pure 17 (160 mg, 64%) as a colorless oil.

[a]D = −0.6 (0.5 H2O).

H NMR (500 MHz, CDCl3): δ = 7.25, 100.5 (H, CH2O), 5.25 (d, J = 9.5 Hz, 1 H, H-1a), 4.52 (d, J = 9.0 Hz, 1 H, H-1c), 3.42 (d, J = 9.0 Hz, 1 H, H-1c), 3.62–3.56 (m, 3 H, H-2h, H-3g), 3.35–3.25 (m, 3 H, H-2h, H-3g), 3.54–3.45 (m, 3 H, H-2h, H-3g), 3.40–3.30 (m, 3 H, H-2h, H-3g), 2.92–2.80 (m, 2 H, H-1c), 2.75–2.65 (m, 2 H, H-1c), 2.65–2.55 (m, 2 H, H-1c), 2.50–2.40 (m, 2 H, H-1c), 1.90–1.80 (m, 2 H, H-1c), 1.70–1.60 (m, 2 H, H-1c), 1.40–1.30 (m, 2 H, H-1c), 1.06 (d, J = 6.5 Hz, 3 H, CH3).

13C NMR (125 MHz, CDCl3): δ = 191.3 (COCH3), 167.9 (COCH3), 154.7–114.5 (Ar-C), 106.8 (PhCH3), 100.2 (C-1c), 99.4 (C-1a), 99.0 (C-1e), 98.2 (C-1f), 82.4 (C-4d), 61.8–61.1 (m, 2 H, H-2h, H-3g), 49.6 (C-3C), 48.9 (C-3B), 48.1 (C-3A), 48.0 (C-3E), 47.3 (C-4D), 47.0 (C-4E), 79.8 (C-6A), 79.1 (C-6B, C-6C), 78.3 (C-6D), 72.8 (C-5C, C-5D), 72.0 (C-5B), 71.9 (C-5E), 71.6 (C-5A), 70.4 (C-5H), 55.3 (C-2a), 55.2 (C-2c), 20.8 (COCH3), 74.2 (CH2).
5.2), 74.6 (C-5), 74.0 (C-2), 73.5 (C-2), 73.0 (2 C, C-5, C-5), 72.6 (2 C, C-3, C-5), 72.0 (3 C, C-3, C-4, C-4), 71.6 (C-4), 71.1 (C-4), 69.6 (C-3), 68.1 (C-6), 67.5 (C-3), 62.8 (C-6), 60.9 (C-6), 59.9 (C-6), 56.1 (OCH3), 55.5 (C-2), 50.8 (C-2), 22.5 (NHCOCH3), 20.4 (NHCOCH3), 16.8 (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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