

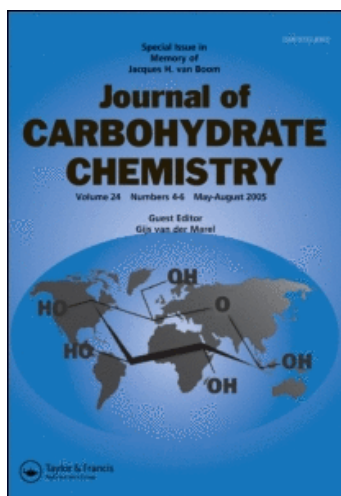
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SYNTHESIS OF A DI- AND A TRISACCHARIDE RELATED TO THE REPEATING UNIT OF *E.coli* O128 LIPOPOLYSACCHARIDE

Prabal Sengupta, Sumanta Basu and Bishnu P. Chatterjee*

Department of Biological Chemistry
Indian Association for the Cultivation of Science
Jadavpur, Calcutta 700 032, India.

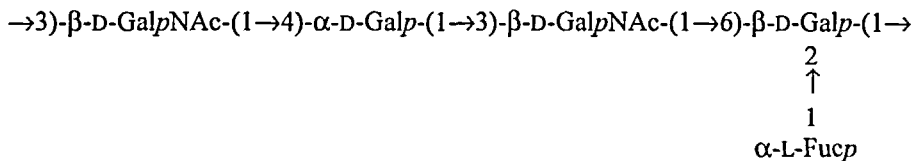
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ABSTRACT

Starting from L-fucose, D-galactose and 2-amino-2-deoxy-D-galactose, methyl 2-acetamido-2-deoxy- β -D-galactopyranosyl-(1 \rightarrow 4)- α -D-galactopyranoside and methyl 2-acetamido-2-deoxy- β -D-galactopyranosyl-(1 \rightarrow 6)-[α -L-fucopyranosyl-(1 \rightarrow 2)]- β -D-galactopyranoside, which are related to the repeating unit of *E.coli* O128, have been synthesized using NIS and TfOH as promoter.

INTRODUCTION

The structure of the repeating unit of the O-antigenic polysaccharide from *E.coli* O128 has been reported¹ as:



In continuation of our previous efforts to understand the relationship between structure and immunological specificity of the antigen, it is necessary to study the inhibitory effect of the related sugar groupings in the corresponding polysaccharide. As a

part of our program to undertake the immunochemical study of the polysaccharide, which will help us to understand the nature of the biological repeating units, and also the chemical basis of their serological specificities, we recently reported² the synthesis of some branched disaccharides related to this antigen. The purpose of this program was to provide a variety of oligosaccharides and their corresponding synthetic glycoconjugates which could be used in inhibition studies to determine the immunodominant sugar of the repeating unit of *E.coli* O128 lipopolysaccharide by enzyme-linked immunosorbent assay (ELISA). As a further extension of our synthetic program, we now report a route to the synthesis of a di- and a trisaccharide related to the repeating unit of this O-antigenic polysaccharide. The synthesis of the methyl glycosides of these oligosaccharides, β -D-GalpNAc-(1 \rightarrow 4)- α -D-Galp and β -D-GalpNAc-(1 \rightarrow 6)-[α -L-Fucp-(1 \rightarrow 2)]- β -D-Galp have not yet been previously reported, as far as we are aware.

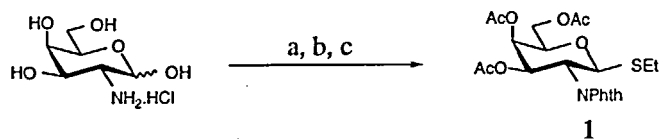
The immunological work was described separately (Communicated in *FEMS Immunol. Med. Microbiol.*). Briefly, the antiserum against *E.coli* O128 was raised in rabbits and ELISA was performed with the purified LPS as well as the inhibitors (oligosaccharides) prepared synthetically with the antibody raised to get an antigen-antibody reaction in microtiter plates which can be monitored by HRP-conjugated goat anti-rabbit IgG. The inhibitory effect of the sugars was calculated by comparing the absorbance values of the LPS and the 50% inhibition value (IC₅₀) of each sugar, which was obtained from the semilogarithmic curves.

As mentioned previously,² the de-blocked di- and trisaccharides were characterized by considering the glycosidation effects for the anomeric residues as well as by comparison of their ¹H and ¹³C NMR spectra.^{3,4}

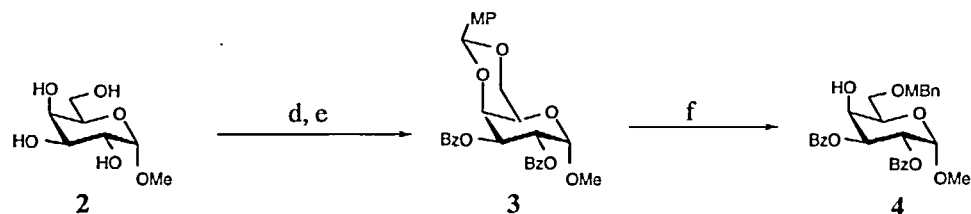
RESULTS AND DISCUSSION

Ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio- β -D-galactopyranoside 1 was prepared as described before.⁵ In a set of experiments, methyl α -D-galactopyranoside 2 was first treated with 4-methoxybenzaldehyde dimethyl acetal to give the corresponding 4,6-*O*-(4-methoxybenzylidene) derivative which was then benzoylated to afford methyl 2,3-di-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)- α -D-galactopyranoside 3. Regioselective opening of the 4-methoxybenzylidene ring with NaCNBH₃ and

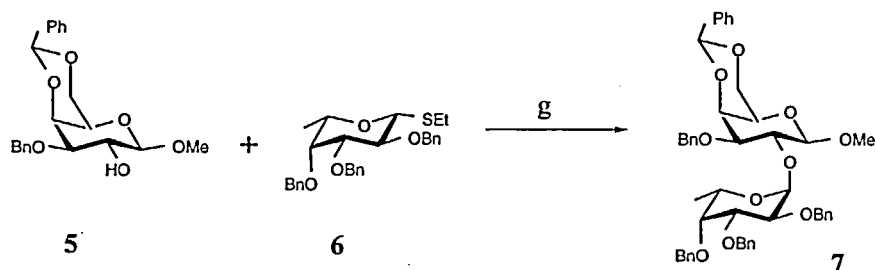
trifluoroacetic acid gave methyl 2,3-di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)- α -D-galactopyranoside **4**.⁶ In a separate experiment, disaccharide methyl 2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl-(1 \rightarrow 2)-3-*O*-benzyl-4,6-*O*-benzylidene- β -D-galactopyranoside **7**,² prepared by condensation of methyl 3-*O*-benzyl-4,6-*O*-benzylidene- β -D-galactopyranoside **5**⁷ and ethyl 2,3,4-tri-*O*-benzyl-1-thio- β -L-fucopyranoside **6**⁸ with CuBr_2 and Bu_4NBr ,⁹ was treated with LiAlH_4 and AlCl_3 ¹⁰ to give methyl 2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl-(1 \rightarrow 2)-3,4-di-*O*-benzyl- β -D-galactopyranoside **8** (Scheme 1).



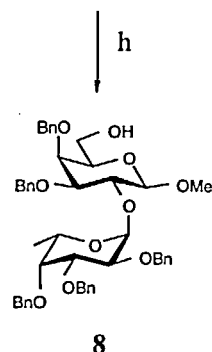
a) Phthalic Anhydride, NaOH ; b) Acetic Anhydride / Pyridine ; c) Ethanethiol



d) *p*-Methoxybenzaldehyde dimethyl acetal / *p*-Toluenesulfonic Acid ;
e) Benzoyl chloride / Pyridine ; f) Sodium cyanoborohydride / Trifluoroacetic acid / DMF

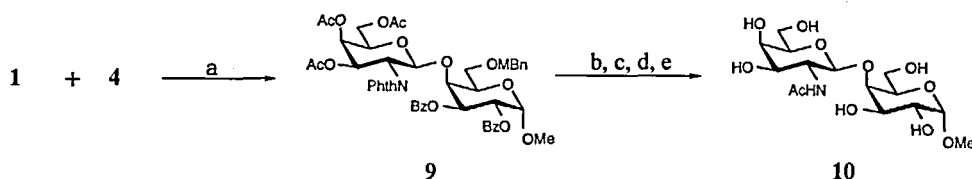


g) CuBr_2 / Bu_4NBr ; h) LiAlH_4 / AlCl_3



Scheme 1

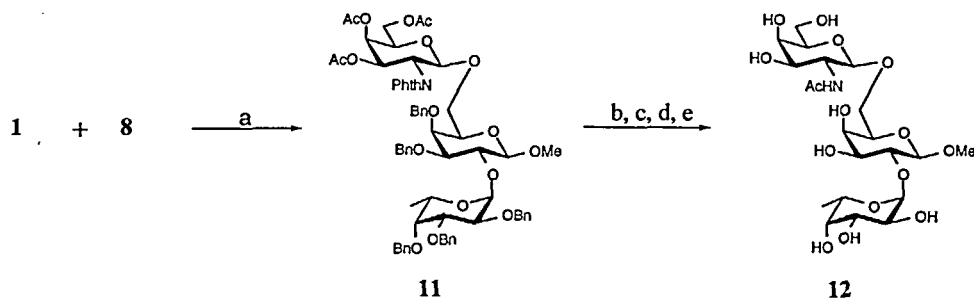
The donor **1** and acceptor **4** were then allowed to condense in the presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)¹¹ as promoter to yield methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-galactopyranosyl-(1 \rightarrow 4)-2,3-di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)- α -D-galactopyranoside **9** in 67% yield. Dephthaloylation of **9** with hydrazine hydrate,¹² followed by *N*-acetylation and Zemplén deacetylation¹³ and finally oxidative removal of the 4-methoxybenzyl group with ceric ammonium nitrate (CAN) gave methyl 2-acetamido-2-deoxy- β -D-galactopyranosyl-(1 \rightarrow 4)- α -D-galactopyranoside **10** in 85% yield (Scheme 2).



a) NIS-TfOH; b) $N_2H_4 \cdot H_2O$, EtOH; c) Ac_2O / Py; d) NaOMe / MeOH; e) CAN

Scheme 2

The donor **1** was also condensed with acceptor **8** using the same promoter to yield the trisaccharide derivative methyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-galactopyranosyl-(1 \rightarrow 6)-[2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl-(1 \rightarrow 2)]-3,4-di-*O*-benzyl- β -D-galactopyranoside **11** in 61% yield. Removal of the phthalimido group and subsequent *N*-acetylation of the compound, followed by Zemplén deacetylation and hydrogenolysis with 10% Pd-C afforded the de-blocked trisaccharide methyl 2-acetamido-2-deoxy- β -D-



a) NIS-TfOH; b) $N_2H_4 \cdot H_2O$, EtOH; c) Ac_2O / Py; d) NaOMe / MeOH; e) H_2 / Pd-C

Scheme 3

galactopyranosyl-(1 \rightarrow 6)-[α -L-fucopyranosyl-(1 \rightarrow 2)]- β -D-galactopyranoside **12** in 74% yield (Scheme 3).

EXPERIMENTAL

General. All reactions were monitored by TLC on Silica Gel G (E. Merck, India). Column chromatography was performed using 100 - 200 mesh silica gel (SRL, India). All solvents were dried and/or distilled before use and all evaporations were conducted below 50 °C under reduced pressure, unless stated otherwise. Optical rotations were measured at 24 °C with a Perkin-Elmer 241 MC polarimeter. The ¹H and ¹³C NMR spectra were recorded with a Bruker DPX-300 spectrometer using CDCl₃ as the solvent (internal standard TMS) unless otherwise stated. The organic extracts were dried over anhydrous Na₂SO₄.

Ethyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-galactopyranoside (1). 1,3,4,6-Tetra-*O*-acetyl-2-deoxy-2-phthalimido-β-D-galactopyranose¹⁴ (2.00 g, 4.18 mmol) was treated as mentioned⁵ to give 1 (1.6 g, 82%); [α]_D +20.4° (*c* 0.1, CHCl₃); ¹H NMR δ 1.15 (t, 3H, SCH₂CH₃), 1.78, 1.91, 2.10 (3s, 3H each, 3xAc), 2.60-2.65 (m, 2H, SCH₂CH₃), 4.02-4.18 (m, 2H, H-5, H-6), 4.54 (t, 1H, *J* = 10.7 Hz, H-2), 5.40 (d, 1H, *J* = 10.5 Hz, H-1), 5.45 (bd, 1H, *J* = 3 Hz, H-4), 5.78 (dd, 1H, *J* = 3.3 Hz, 10.8 Hz, H-3), 7.66-7.79 (m, 4H, NPhth).

Anal. Calcd for C₂₂H₂₅O₉NS: C, 55.10; H, 5.25. Found: C, 54.89, H, 5.67.

Methyl 2,3-Di-*O*-benzoyl-6-*O*-(4-methoxybenzyl)-α-D-galactopyranoside (4). To a stirred mixture containing methyl 2,3-di-*O*-benzoyl-4,6-*O*-(4-methoxybenzylidene)-α-D-galactopyranoside (1.17 g, 2.24 mmol) and NaCNBH₃ (0.7 g, 11.2 mmol) was added dropwise a chilled soln (0 °C) of TFA (1.72 mL, 22.4 mmol) in DMF (14 mL). After 7 h, the mixture was filtered through celite and poured into ice-cold saturated aq NaHCO₃. The aq phase was repeatedly extracted with CH₂Cl₂. The combined extracts were washed with saturated aq NaHCO₃, dried, filtered and concentrated to give 4 as syrup (0.88 g, 75%); [α]_D +29.7° (*c* 0.8, CHCl₃); ¹H NMR δ 3.39 (s, 3H, OCH₃), 3.76 (s, 3H, CH₂C₆H₄OCH₃), 5.67 (d, 1H, *J* = 3.3 Hz, H-1), 7.23-7.98 (m, 14H, aromatic protons).

Anal. Calcd for C₂₉H₃₂O₉: C, 66.40; H, 6.14. Found: C, 65.76; H, 6.82.

Methyl 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (7). To a stirred mixture of CuBr₂ (0.76 g, 3.43 mmol), Bu₄NBr⁹ (0.11 g, 0.34 mmol) and molecular sieves 4Å was added a soln of 6⁸ (1.1 g, 2.29 mmol) and 5⁷ (0.7 g, 1.87 mmol) in 1,2-dichloroethane-DMF (5:1) at 20 °C. After stirring for 48 h at 20 °C, the mixture was diluted with 1,2-dichloroethane and filtered through celite. The organic layer was washed successively with water, aq NaHCO₃ and water,

dried (Na_2SO_4) and concentrated. The residue was chromatographed with 9:1 toluene-EtOAc giving **7** as syrup (1.08 g, 72%); $[\alpha]_D -68.7^\circ$ (c 0.9, CHCl_3); $^1\text{H NMR } \delta$ 1.21 (d, 3H, $J = 6.6$ Hz, CH_3), 3.48 (s, 3H, OCH_3), 3.76-3.81 (m, 2H, H-3, H-5), 3.96 (dd, 1H, $J_{1,2} = 8$ Hz, $J_{2,3} = 7.8$ Hz, H-2), 4.00-4.05 (m, 2H, H-2', H-4'), 4.12 (dd, 1H, $J_{2,3} = 9.6$ Hz, $J_{3,4} = 2.7$ Hz, H-3'), 4.15 (dd, 1H, $J = 11$ Hz & 1.8 Hz, H-6), 4.39 (d, 1H, $J = 7.8$ Hz, H-1), 4.53 (dd, 1H, $J = 10.7$ Hz & 2 Hz, H-6'), 4.62-4.95 (m, 8H, 4PhCH₂), 5.40 (s, 1H, PhCH), 5.65 (d, 1H, $J = 3.6$ Hz, H-1'), 7.17-7.39 (m, 25H, aromatic protons); $^{13}\text{C NMR } \delta$ 17.1 (CH_3), 56.75 (OCH_3), 66.79 (C-6), 69.75, 71.11, 72.93, 73.19, 73.48, 75.19, 76.48, 77.84, 78.41, 80.03, 81.69, 97.99 (C-1'), 101.39 (C-1), 103.14 (PhCH), 126.88-139.60 (aromatic carbons).

Anal. Calcd for $\text{C}_{48}\text{H}_{52}\text{O}_{10}$: C, 73.07; H, 6.64. Found: C, 72.76; H, 6.86.

Methyl 2,3,4-Tri-O-benzyl- α -L-fucopyranosyl-(1 \rightarrow 2)-3,4-di-O-benzyl- β -D-galactopyranoside (8). Compound **7** (0.5 g, 0.63 mmol) was dissolved in 1:1 ether- CH_2Cl_2 (20 mL) and to it LiAlH_4 (0.2 g) was added in 3-4 portions at 0 °C. The mixture was heated to reflux temp following addition of AlCl_3 (0.5 g) in ether (10 mL) to the hot soln for 1 h and refluxing was continued for another 45 min. The mixture was cooled and excess LiAlH_4 was decomposed with EtOAc (5 mL). The $\text{Al}(\text{OH})_3$ was precipitated by the addition of water (15 mL) and filtered off. The filtrate was diluted with ether (50 mL) and the organic phase was separated, washed with water (3x20 mL) and dried. The solution was concentrated to dryness to give pure syrup **8** (0.35 g, 70%); $[\alpha]_D -41.5^\circ$ (c 0.65, CHCl_3); $^1\text{H NMR } \delta$ 1.04 (d, 1H, $J = 6.3$ Hz, CH_3), 3.40 (s, 3H, OCH_3), 3.62-3.69 (m, 2H, H-3, H-5), 3.89 (dd, 1H, $J_{1,2} = 8.4$ Hz, $J_{2,3} = 6$ Hz, H-2), 3.94-3.97 (m, 2H, H-2', H-4'), 4.04 (dd, 1H, $J_{2,3} = 7.8$ Hz, $J_{3,4} = 3.9$ Hz, H-3'), 4.32 (d, 1H, $J_{1,2} = 7.8$ Hz, H-1), 5.48 (d, 1H, $J_{1,2'} = 3.6$ Hz, H-1'), 7.08-7.29 (m, 30H, aromatic protons); $^{13}\text{C NMR } \delta$ 16.90 (CH_3), 56.98 (OCH_3), 66.83 (C-6), 71.88, 73.27, 73.37, 73.52, 74.35, 74.84, 75.08, 76.18, 78.23, 80.16, 84.49, 98.01 (C-1'), 103.52 (C-1), 126.86-138.52 (aromatic carbons).

Anal. Calcd for $\text{C}_{48}\text{H}_{54}\text{O}_{10}$: C, 72.89; H, 6.88. Found: C, 72.42; H, 7.02.

Methyl 3,4,6-Tri-O-acetyl-2-deoxy-2-phthalimido- β -D-galactopyranosyl-(1 \rightarrow 4)-2,3-di-O-benzoyl-6-O-(4-methoxybenzyl)- α -D-galactopyranoside (9). To a solution of **1** (201 mg, 0.419 mmol), **4** (132 mg, 0.251 mmol) and molecular sieves 4Å (1 g) in CH_2Cl_2 (15 mL) were added NIS (122 mg, 0.544 mmol) and TfOH^{11} (11 μL , 0.125 mmol). The mixture was then stirred for 3 h at 0 °C, filtered and the filtrate diluted with

aq 5% Na₂S₂O₃ soln, NaHCO₃, water and then dried (Na₂SO₄). Column chromatographic purification (19:1 toluene-EtOAc) gave **9** as syrup (160 mg, 67%); [α]_D +23.7° (*c* 0.9, CHCl₃); ¹H NMR δ 1.83, 2.03 and 2.18 (3s, 9H, 3 OAc), 3.38 (s, 3H, OCH₃), 3.83 (s, 3H, CH₂C₆H₄OCH₃), 3.95-4.01 (m, 1H, H-5), 4.09-4.19 (m, 2H, H-5', H-6'), 4.52-4.64 (m, 4H, H-2, H-3, H-4, H-6), 4.96 (dd, 1H, $J_{1,2'} = 7.6$ Hz, $J_{2,3'} = 9.2$ Hz, H-2'), 5.45 (d, 1H, $J_{1,2'} = 8.4$ Hz, H-1'), 5.48 (dd, 1H, $J_{3,4'} = 2.4$ Hz, $J_{4,5'} = 1.6$ Hz, H-4'), 5.51 (d, 1H, $J_{1,2} = 2.4$ Hz, H-1), 5.80 (dd, 1H, $J_{2,3'} = 8.1$ Hz, $J_{3,4'} = 3.3$ Hz, H-3'), 7.20-7.77 (m, 20H, aromatic protons); ¹³C NMR δ 20.17, 20.33 and 20.39 (3xCOCH₃), 51.32 (C-2'), 54.95 (OCH₃), 55.04 (CH₂C₆H₄OCH₃), 66.18, 67.15, 68.13, 68.98, 69.07, 70.33, 71.58, 72.87, 73.09, 76.31, 76.74, 77.16, 97.09 (C-1), 102.69 (C-1'), 113.51 (CH₂C₆H₄OCH₃), 128.07-134.09 (aromatic carbons), 164.88-166.27 (3xCOCH₃), 169.37 and 169.97 (2xCOPh).

Anal. Calcd for C₄₉H₅₁O₈N: C, 62.48; H, 5.45. Found: C, 62.16; H, 5.69.

Methyl 2-Acetamido-2-deoxy- β -D-galactopyranosyl-(1 \rightarrow 2)- α -D-galactopyranoside (10). To a soln of **9** (100 mg, 0.106 mmol) in 95% aq ethanol (10 mL) was added hydrazine hydrate (1 mL). The mixture was heated at 85 °C for 2 h and then concentrated. The residue was treated with pyridine (5 mL) and Ac₂O (5 mL) at rt for 3 h and concentrated. The residue was purified by column chromatography using 3:1 toluene-EtOAc. This material was deacetylated¹³ and purified by column chromatography (4:1 toluene-EtOAc) and then to the product in acetonitrile-water (9:1) was added ceric ammonium nitrate (50 mg) and the mixture was stirred for 2 h at rt. Usual workup and column chromatographic separation with 4:1 toluene-EtOAc gave **10** as syrup (34 mg, 85%); [α]_D +9.4° (*c* 0.6, H₂O); ¹H NMR (D₂O) δ 3.45 (s, 3H, OCH₃), 3.97-4.02 (m, 1H, H-5), 4.07-4.15 (m, 2H, H-5', H-6'), 4.51 (dd, 1H, $J_{1,2} = 3.6$ Hz, $J_{2,3} = 8.3$ Hz, H-2), 4.54-4.62 (m, 2H, H-3, H-4), 4.97 (dd, 1H, $J_{1,2'} = 6.9$ Hz, $J_{2,3'} = 8.1$ Hz, H-2'), 5.26 (d, 1H, $J_{1,2'} = 8.2$ Hz, H-1'), 5.39 (dd, 1H, $J_{3,4'} = 2.6$ Hz, $J_{4,5'} = 2.1$ Hz, H-4'), 5.48 (d, 1H, $J_{1,2} = 2.4$ Hz, H-1), 5.69 (dd, 1H, $J_{2,3'} = 7.8$ Hz, $J_{3,4'} = 3.3$ Hz, H-3'); ¹³C NMR (D₂O) δ 23.16 (NCOCH₃), 51.49 (C-2'), 56.27 (OCH₃), 67.74, 67.95, 69.46, 70.29, 71.71, 72.61, 74.21, 76.32, 76.85, 98.27 (C-1), 102.19 (C-1'), 175.44 (NCOCH₃).

Anal. Calcd for C₁₅H₂₇O₁₀: C, 47.24; H, 7.13. Found: C, 47.07; H, 7.32.

Methyl 3,4,6-Tri-*O*-acetyl-2-deoxy-2-phthalimido- β -D-galactopyranosyl-(1 \rightarrow 6)-[2,3,4-tri-*O*-benzyl- α -L-fucopyranosyl-(1 \rightarrow 2)]-3,4-di-*O*-benzyl- β -D-galactopyranoside (11). To a soln of **1** (109 mg, 0.23 mmol), **8** (143 mg, 0.19 mmol), molecular

sieves 4Å (0.5 g) in CH₂Cl₂ (15 mL) were added NIS (66 mg, 0.3 mmol) and TfOH¹¹ (61 μL, 0.07 mmol) and the mixture was stirred for 4 h at 0 °C. The workup was the same as described for the preparation of 9. Column chromatography using 9:1 toluene-EtOAc gave pure 11 as syrup (133 mg, 61%); [α]_D -47.8° (c 0.46, CHCl₃); ¹H NMR δ 1.13 (d, 1H, *J* = 6.1 Hz, CH₃), 1.77, 1.98 and 2.12 (3s, 9H, 3 OCOCH₃), 3.41 (s, 3H, OCH₃), 3.60-3.68 (m, 2H, H-3, H-5), 3.87-3.95 (m, 3H, H-2, H-2', H-4'), 4.01-4.14 (m, 3H, H-5'', H-6'', H-3'), 4.35 (d, 1H, *J*_{1,2} = 8.1 Hz, H-1), 4.59 (t, 1H, *J* = 10.7 Hz, H-2''), 5.38 (d, 1H, *J*_{1'',2''} = 10.1 Hz, H-1''), 5.43 (d, 1H, *J*_{1',2'} = 4.2 Hz, H-1'), 5.49 (d, 1H, *J* = 3.6 Hz, H-4''), 5.83 (m, 1H, H-3''), 7.16-7.33 (m, 30H, aromatic protons), 7.69-7.83 (m, 4H, NPhth); ¹³C NMR δ 15.72 (CH₃), 20.99, 21.07 and 21.12 (3 COCH₃), 51.63 (C-2''), 55.78 (OCH₃), 61.25, 61.51 (C-6', C-6''), 66.27, 67.36, 67.84, 68.16, 69.15, 70.29, 72.65, 73.47, 74.93, 76.98, 77.42, 77.84, 79.09, 82.11, 84.78, 84.96, 98.36 (C-1'), 102.94 (C-1''), 103.25 (C-1'''), 125.47-136.62 (aromatic carbons), 165.27-168.16 (5 COCH₃).

Anal. Calcd for C₆₈H₇₃O₁₉N: C, 67.59; H, 6.08. Found: C, 66.82; H, 6.73.

Methyl 2-Acetamido-2-deoxy-β-D-galactopyranosyl-(1→6)-[α-L-fucopyranosyl-(1→2)]-β-D-galactopyranoside (12). To a soln of 11 (120 mg, 0.993 mmol) in 95% aq ethanol (10 mL) was added hydrazine hydrate (1 mL). The mixture was heated at 85 °C for 2 h and then concentrated. The residue was treated with pyridine (5 mL) and acetic anhydride (5 mL) at rt for 3 h and concentrated. The residue was purified by column chromatography using 3:1 toluene-EtOAc. The material after deacetylation¹³ was purified by column chromatography (4:1 toluene-EtOAc). A soln of this product in AcOH (10 mL) was hydrogenolyzed for 48 h in presence of 10% Pd-C (30 mg) at 24 °C. The reaction mixture was filtered through a celite bed, purified by column chromatography and concentrated to give 12 as syrup (39 mg, 74%); [α]_D -12.7° (c 0.8, H₂O); ¹H NMR (D₂O) δ 1.11 (d, 1H, *J* = 6.5 Hz, CH₃), 2.01 (s, 3H, NCOCH₃), 3.46 (s, 3H, OCH₃), 3.64-3.71 (m, 2H, H-3, H-5), 3.93-3.96 (m, 3H, H-2, H-2', H-4'), 4.02-4.11 (m, 3H, H-3', H-5'', H-6''), 4.42 (d, 1H, *J*_{1,2} = 7.3 Hz, H-1), 4.51 (t, 1H, *J*_{2,3'} = 9.6 Hz, H-2''), 5.47 (d, 1H, *J*_{1'',2''} = 9.2 Hz, H-1''), 5.48 (d, 1H, *J* = 3.9 Hz, H-4''), 5.89 (m, 1H, H-3''), 7.12-7.29 (m, 30H, aromatic protons); ¹³C NMR (D₂O) δ 16.23 (CH₃), 23.31 (NCOCH₃), 52.05 (C-2'), 56.23 (OCH₃), 61.64, 61.70 (2 C-6), 66.35, 67.51, 67.92, 68.34, 69.29, 70.51, 72.83, 73.64, 75.26, 77.31, 77.65, 77.97, 79.32, 82.63, 84.91, 85.34, 99.03 (C-1'), 102.56 (C-1''), 103.45 (C-1'''), 123.45-140.46 (aromatic carbons), 176.32 (NCOCH₃).

Anal. Calcd for C₂₁H₃₇O₁₄N: C, 47.81; H, 7.06. Found: C, 47.25; H, 7.81.

REFERENCES

1. P. Sengupta, T. Bhattacharyya, A. S. Shashkov, H. Kochanowski and S. Basu, *Carbohydr. Res.*, **277**, 283 (1995).
2. P. Sengupta, S. Sarbajna and S. Basu, *J. Carbohydr. Chem.*, **18**, 87 (1999).
3. N. K. Kochetkov, A. S. Shashkov, G. M. Lipkind and Y. A. Knirel, *Sov. Sci. Rev., Sec. B., Chem.*, **13**, 1 (1989).
4. P. K. Agrawal, *Phytochemistry*, **31**, 3307 (1992).
5. U. Ellervik and G. Magnusson, *Carbohydr. Res.*, **280**, 251 (1996).
6. R. Johansson and B. Samuelsson, *J. Chem. Soc., Perkin Trans.1*, 2371 (1984).
7. J. Kihlberg, T. Frejd, K. Jansson and G. Magnusson, *Carbohydr. Res.*, **152**, 113 (1986).
8. H. Lönn, *Carbohydr. Res.*, **139**, 105 (1985).
9. S. Sato, M. Mori, Y. Ito and T. Ogawa, *Carbohydr. Res.*, **155**, C6 (1986).
10. A. Liptak, I. Jodal and P. Nanasi, *Carbohydr. Res.*, **44**, 1 (1975).
11. G. H. Veeneman, S. H. van Leeuwen and J. H. van Boom, *Tetrahedron Lett.*, **31**, 1331 (1990).
12. T. Ehara, A. Kameyama, Y. Yamada, H. Ishida, M. Kiso and A. Hasegawa, *Carbohydr. Res.*, **181**, 237 (1996).
13. G. Zemplén, *Ber. Dtsch. Keram. Ges.*, **59**, 1254 (1926).
14. U. Nilsson, A. K. Ray and G. Magnusson, *Carbohydr. Res.*, **208**, 260 (1990).