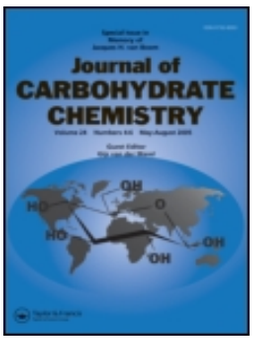


Journal of Carbohydrate Chemistry

ISSN: 0732-8303 (Print) 1532-2327 (Online) Journal homepage: http://www.tandfonline.com/loi/lcar20

Synthesis of Disaccharides Related to the O-Specific Side Chains from E.coli O126 & O128 Lipopolysaccharides

Prabal Sengupta , Sumita Sarbajna & Sumanta Basu

To cite this article: Prabal Sengupta , Sumita Sarbajna & Sumanta Basu (1999) Synthesis of Disaccharides Related to the O-Specific Side Chains from E.coli O126 & O128 Lipopolysaccharides, Journal of Carbohydrate Chemistry, 18:1, 87-97, DOI: 10.1080/07328309908543981

To link to this article: http://dx.doi.org/10.1080/07328309908543981

J. CARBOHYDRATE CHEMISTRY, 18(1), 87-97 (1999)

SYNTHESIS OF DISACCHARIDES RELATED TO THE O-SPECIFIC SIDE CHAINS FROM *E.coli* O126 & O128 LIPOPOLYSACCHARIDES

Prabal Sengupta,* Sumita Sarbajna and Sumanta Basu

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

Received August 18, 1997 - Final Form August 12, 1998

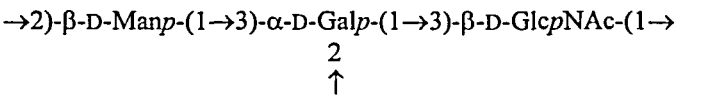
ABSTRACT

Starting from D-galactose and L-fucose, four disaccharides, namely methyl β-L-fucopyranosyl-(1→2)-α-D-galactopyranoside, methyl α-L-fucopyranosyl-(1→2)-α-D-galactopyranoside, methyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranoside and methyl β-L-fucopyranosyl-(1→2)-β-D-galactopyranoside related to the repeating units of *E.coli* O126 and O128 have been synthesized using NIS and TfOH as promoter.

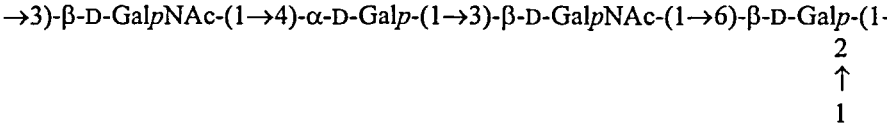
INTRODUCTION

Escherichia coli is a gram-negative, opportunistic pathogen[1] causing intestinal or extra-intestinal diseases both in man and animals.[2] It is reported that natural immunity to gram-negative bacteria is often provided by antibodies that recognize lipopolysaccharide (LPS) antigens[3] found on the outer leaflet of the outer membrane of the bacteria. Moreover, it has been claimed that the antigenicity of the LPS is due to the polysaccharide part. The enteropathogenic (EPEC) strains of *E.coli* O126 and O128 are known to be associated with infantile diarrhea.

The repeating units of the O-antigenic polysaccharides from *E.coli* types O126 and O128 have already been reported.[4,5]



***E. coli* O126**

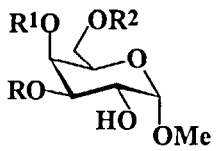
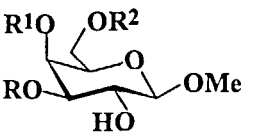
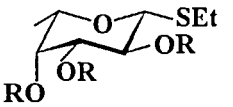


***E. coli* O128**

In order to understand the relationship between structural and immunological specificity of these antigens, it is necessary to study the inhibitory effect of the related sugar groupings in the corresponding polysaccharides. The disaccharide determinant, α-L-Fuc*p*-(1→2)-β-D-Gal*p*, of the O-antigen from *E. coli* O127 is reported[6] to be responsible for expression of the specific blood group H activity. The branched part of the repeating unit of O-antigenic polysaccharides often plays the role of immunodominant sugar blocks.[7] The L-fucose and D-galactose constitute the branched part of the repeating unit of *E. coli* O126 and O128 polysaccharides. It is, therefore, of interest to synthesise the branched disaccharides related to these antigens.

We report here the chemical synthesis of four disaccharides related to the branched part of the *E.coli* O126 and O128 polysaccharides. These disaccharides were difficult to isolate from the native polysaccharides by partial hydrolysis because of the acid lability of 6-deoxyhexose sugar. Synthesis will help us to undertake the comparative immuno-chemical study of the two different polysaccharides which will aid our understanding of the nature of the biological repeating units and the chemical basis of their serological specificities.

The synthesis of the target compounds was reported earlier[8] using the Koenigs-Knorr glycosylation method. We have developed an alternate synthesis of these disaccharides, based on stable thioglycoside donors. Key glycosylation steps were carried out with high stereoselectivies, while reaction times were short. The deblocked

		
(1) R,R^1 = CMe$_2$, R^2 = H (2) R,R^1 = CMe$_2$, R^2 = SiPh$_2$CMe$_3$	(3) R = Bn, R^1 = R^2 = H (4) R = Bn, R^1 ,R^2 = CHPh	(5) R = Ac (6) R = H (7) R = Bn

Scheme 1

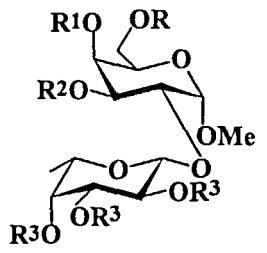
disaccharides were characterized by considering the glycosidation effects for the anomeric residues as well as by comparison of the ^{1}H and ^{13}C NMR spectra.[9,10]

RESULTS AND DISCUSSION

Methyl α-D-galactopyranoside[11] was allowed to react with 2,2-dimethoxypropane to afford methyl 3,4-*O*-isopropylidene-α-D-galactopyranoside[12] **1** which was selectively silylated[13] at C-6 to give methyl 3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside **2**. In another experiment, methyl β-D-galactopyranoside[14] was regioselectively benzylated[15] at C-3 via stannylidene complex to give methyl 3-*O*-benzyl-β-D-galactopyranoside[16] **3** which was benzylidenated[17] to give the corresponding 4,6-*O*-benzylidene derivative **4**.[18] In a separate experiment, ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-L-fucopyranoside **7** was prepared according to Lönn[19] (Scheme 1).

The donor **5** and acceptor **2** were then allowed to condense in presence of *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[20] as promoter to afford methyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranosyl-(1→2)-3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside **8**. Removal of the *tert*-BuPh$_2$Si group with Bu$_4$NF-THF[21] followed by deisopropylidenation with 85% AcOH[22] gave **10** which was deacetylated[23] to afford methyl β-L-fucopyranosyl-(1→2)-α-D-galactopyranoside **11**.[8]

The acceptor **2** was also condensed with **7** in presence of NIS-TfOH to give methyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3,4-*O*-isopropylidene-6-*O*-*tert*-

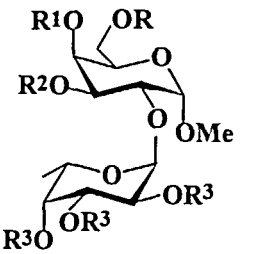


(8) R = $SiPh_2CMe_3$, R^1,R^2 = CMe_2, R^3 = Ac

(9) R = H, R^1,R^2 = CMe_2, R^3 = Ac

(10) R = R^1 = R^2 = H, R^3 = Ac

(11) R = R^1 = R^2 = R^3 = H



(12) R = $SiPh_2CMe_3$, R^1,R^2 = CMe_2, R^3 = Bn

(13) R = H, R^1,R^2 = CMe_2, R^3 = Bn

(14) R = R^1 = R^2 = H, R^3 = Bn

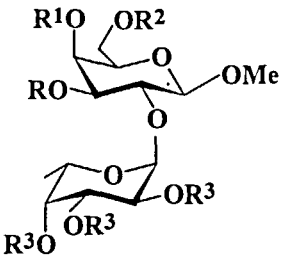
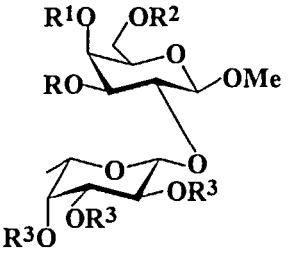
(15) R = R^1 = R^2 = R^3 = H

Scheme 2

butyl-diphenylsilyl-α-D-galactopyranoside **12**. The *tert*-$BuPh_2Si$ and isopropylidene groups were removed as previously mentioned to give **14** which was debenzylated with 10% Pd-C to afford the deblocked disaccharide methyl α-L-fucopyranosyl-(1→2)-α-D-galactopyrano-side **15**[8] (Scheme 2).

Condensation of **4** with **7** in presence of the same promoter gave the blocked disaccharide methyl 2,3,4-tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside **16**. This compd was hydrogenolyzed as mentioned previously affording methyl α-L-fucopyranosyl-(1→2)-β-D-galactopyranoside **17**.[8]

Lastly, this acceptor **4** was allowed to condense with the per-*O*-acetylated donor **5** using the same promoter and reaction conditions to yield methyl 2,3,4-tri-*O*-acetyl-β-L-fucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside **18**. The compd **18** was subjected to Zemplén deacetylation followed by hydrogenolysis to give the deblocked disaccharide methyl β-L-fucopyranosyl-(1→2)-β-D-galactopyranoside **20**[8] (Scheme 3).

	
(16) R = R^3 = Bn, R^1 = R^2 = CHPh. (17) R = R^1 = R^2 = R^3 = H.	(18) R = Bn, R^1 = R^2 = CHPh, R^3 = Ac. (19) R = Bn, R^1 = R^2 = CHPh, R^3 = H. (20) R = R^1 = R^2 = R^3 = H.

Scheme 3

EXPERIMENTAL

General. All reactions were monitored by TLC on Silica Gel (E. Merck). Column chromatography was performed using 100-200 silica gel (SRL, India). All solvents were dried and/or distilled before use, and all reactions were conducted below 50 °C unless stated otherwise. Optical rotations were measured at 24 °C with a Perkin Elmer 241MC polarimeter. ^{1}H NMR and ^{13}C NMR spectra were recorded with a Jeol FX-100 and DPX-300 spectrometer using $CDCl_3$ as the solvent unless stated otherwise. The organic extracts were dried over anhydrous Na_2SO_4.

Methyl 3,4-*O*-Isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (2). To the stirred mixture of compd **1**[12] (1.13 g, 4.82 mmol), imidazole (0.65 g, 2 equiv) in dry DMF (10 mL) at 0 °C under nitrogen, was added *tert*-butyldiphenylchlorosilane (1.5 mL, 1.1 equiv) slowly, after which the mixture was allowed to warm up to rt during 3 h. After 22 h DMF was evaporated and the crude mixture was shaken with water (10 mL). CH_2Cl_2 (20 mL) was added and the layers were separated after shaking. The combined organic extracts were washed with saturated aq $(NH_4)_2CO_3$ (4x25 mL) and concentrated. Column chromatography using 6:1 toluene-EtOAc gave **2** as syrup (1.68 g, 73.6%); $[\alpha]_D$ + 57.9° (*c* 2.04, $CHCl_3$); ^{1}H NMR δ 1.16 (s, 9H, CMe_3), 1.43 and 1.56 (2s,

6H, CMe_2), 3.5 (s, 3H, OCH_3), 3.9-4.32 (m, 5H, H-2, H-3, H-4, H-5, H-6), 4.80 (d, 1H, H-1, J = 4.2 Hz), 7.33-7.9 (m, 10H, aromatic protons).

Anal. Calcd for $C_{26}H_{36}O_6Si$: C, 66.07; H, 7.67. Found: C, 65.92; H, 7.88.

Methyl 2,3,4-Tri-*O*-acetyl-β-L-fucopyranosyl-(1→2)-3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (8). To a soln of **2** (204 mg, 0.43 mmol), **5**[19] (240 mg, 0.717 mmol), and molecular sieves 4Å (1 gm) in CH_2Cl_2 (10 mL) were added NIS (209 mg, 0.93 mmol) and TfOH[20] (5.9 μL, 0.06 mmol). The mixture was then stirred for 1 h at 0 °C, filtered, and the filtrate diluted with aq 5% $Na_2S_2O_3$ soln, (M) $NaHCO_3$, water and then dried (Na_2SO_4). Column chromatographic purification (19:1 toluene-EtOAc) gave **8** as syrup (205 mg, 63%); $[\alpha]_D$ +37.8° (*c* 1.1, $CHCl_3$); ^1H NMR δ 1.05 (s, 9H, CMe_3), 1.17 (d, 3H, CH_3, J = 6.2 Hz), 1.4 and 1.52 (2s, 6H, CMe_2), 1.99, 2.04 and 2.15 (3s, 9H, 3 OAc), 3.37 (s, 3H, OCH_3), 4.61 (d, 1H, H-1', J = 7.8 Hz), 4.78 (d, 1H, H-1, J = 3.7 Hz), 5.03 (dd, 1H, $J_{2,3}$ = 9.8 Hz, $J_{3,4}$ = 3.9 Hz, H-3'), 5.18 (dd, 1H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 8 Hz, H-2'), 5.23 (q, 2H, H-6), 7.29-7.76 (m, 10H, aromatic protons); ^{13}C NMR δ 15.8 (CH_3), 19.12, 19.69 [$C(CH_3)_2$], 20.63 [$C(CH_3)_3$], 25.99, 26.62 and 27.73 ($COCH_3$), 26.67 (CMe_3), 55.27 (OCH_3), 63.2 (C-6), 68.54, 69.02, 69.33, 70.25, 71.26, 73.99, 75.75, 76.06, 97.43 (C-1), 99.91 (C-1'), 109.11 (CMe_2), 127.52-135.53 (aromatic carbons), 170.14, 170.2 and 171.28 ($COCH_3$).

Anal. Calcd for $C_{38}H_{52}O_{13}Si$: C, 61.27; H, 7.03. Found: C, 61.16; H, 7.21.

Methyl 2,3,4-Tri-*O*-acetyl-β-L-fucopyranosyl-(1→2)-3,4-*O*-isopropylidene-α-D-galactopyranoside (9). To a stirred soln of **8** (200 mg, 0.27 mmol) and acetic acid (46 μL, 0.81 mmol) in THF (10 mL) was added 0.23 M tetrabutylammonium fluoride[21] in THF (9.2 mL, 4 equiv) at 0 °C. Stirring was continued at 0 °C to rt overnight. The mixture was then concentrated and the residue was extracted with water and aq $NaHCO_3$, dried (Na_2SO_4) and concentrated. Column chromatography of the residue on silica gel with 4:1 toluene-EtOAc gave **9** as syrup (75 mg, 55%); $[\alpha]_D$ +59.2° (*c* 1.2, $CHCl_3$); ^1H NMR δ 1.19 (d, 3H, CH_3, J = 6.2 Hz), 1.33 and 1.50 (2s, 6H, CMe_2), 1.99, 2.05 and 2.16 (3s, 9H, 3 OAc), 3.41 (s, 3H, OCH_3), 3.74 (m, 2H, H-6), 4.14 (q, 1H, H-5'), 4.25 (dd, 1H, H-4'), 4.60 (d, 1H, H-1', J = 8.3 Hz), 4.79 (d, 1H, H-1, J = 3.7 Hz), 5.02 (dd, 1H, $J_{2,3}$ = 9.5 Hz, $J_{3,4}$ = 3.4 Hz, H-3'), 5.20 (dd, 1H, $J_{1,2}$ = 8.3 Hz, $J_{2,3}$ = 7.4 Hz, H-2').

Anal. Calcd for $C_{22}H_{34}O_{13}$: C, 47.86; H, 6.76. Found: C, 47.82; H, 6.91.

Methyl β-L-Fucopyranosyl-(1→2)-α-D-galactopyranoside (11). Compd **9** (75 mg, 0.15 mmol) was stirred with 85% aq AcOH[22] (10 mL) at 90 °C for 2 h. AcOH was then removed by coevaporation with water and toluene to give compd **10**. This material was deacetylated[23] and purified by column chromatography (toluene-EtOAc 19:1) to give **11** as syrup (45 mg, 89%); $[\alpha]_D$ + 63.1° (*c* 0.9, H_2O);[24] lit.[8] $[\alpha]_D$ + 109° (*c* 0.89, H_2O); ^1H NMR (D_2O) δ 1.16 (d, 3H, CH_3, J = 6.2 Hz), 3.43 (s, 3H, OCH_3), 3.56 (dd, 1H, $J_{1,2}$ = 8.9 Hz, $J_{2,3}$ = 8 Hz, H-2'), 3.59 (dd, 1H, $J_{2,3}$ = 9.9 Hz, $J_{3,4}$ = 3.5 Hz, H-3'), 3.65-3.74 (m, 4H, H-4', H-6, H-3), 3.80-3.83 (m, 2H, H-2, H-5'), 3.89-3.94 (m, 2H, H-4, H-5), 4.38 (d, 1H, H-1', J = 6.2 Hz), 4.90 (d, 1H, H-1, J = 3.5 Hz); ^{13}C NMR (D_2O) δ 15.2 (CH_3), 54.62 (OCH_3), 61.05 (C-6), 67.94, 68.79, 70.25, 70.50, 70.96, 71.10, 72.68, 75.96, 97.30 (C-1), 101.83 (C-1').

Anal. Calcd for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.10. Found: C, 45.71; H, 7.18.

Methyl 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3,4-*O*-isopropylidene-6-*O*-*tert*-butyldiphenylsilyl-α-D-galactopyranoside (12). To a soln of **2** (254 mg, 0.53 mmol), **7**[19] (301 mg, 0.62 mmol), molecular sieves 4Å (1 gm) in CH_2Cl_2 (10 mL) were added NIS (181 mg, 0.81 mmol) and TfOH[20] (7.1 μL, 0.08 mmol) and the mixture was stirred for 2 h at 0 °C. The work-up was the same as described for the preparation of **8**. Column chromatography using 19:1 toluene-EtOAc gave pure **12** as syrup (216 mg, 45%); $[\alpha]_D$ -4.3° (*c* 0.5, $CHCl_3$); ^1H NMR δ 1.08 (s, 9H, CMe_3), 1.13 (d, 3H, CH_3, J = 5.6 Hz), 1.38 and 1.47 (2s, 6H, CMe_2), 3.31 (s, 3H, OCH_3), 3.83-4.05 (m, 5H, H-2', H-3', H-4', H-5', H-5), 4.48 (m, 2H, H-3, H-4), 4.68-5.06 (m, 6H, $3PhCH_2$), 4.92 (d, 1H, H-1, J = 3.1 Hz), 5.04 (d, 1H, H-1', J = 4.2 Hz), 7.30-7.71 (m, 25H, aromatic protons); ^{13}C NMR δ 16.78 (CH_3), 19.12 and 19.67 [$C(CH_3)_2$], 26.69 [$C(CH_3)_3$], 55.12 (OCH_3), 62.94 (C-6), 68.44, 70.47, 70.96, 72.22, 73.20, 74.57, 74.65, 75.20, 82.62, 83.02, 83.79, 97.67 (C-1), 98.71 (C-1'), 108.97 (CMe_2), 127.20-139.16 (aromatic carbons).

Anal. Calcd for $C_{53}H_{64}O_{10}Si$: C, 71.59; H, 7.25. Found: C, 71.43; H, 7.39.

Methyl 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3,4-*O*-isopropylidene-α-D-galactopyranoside (13). To a stirred mixture of **12** (200 mg, 0.224 mmol) and AcOH (38 μL, 0.672 mmol) in THF (7 mL) was added 0.23 molar Bu_4NF in THF[21] (6.3 mL, 4 equiv) at 0 °C. The work-up was the same as described for the preparation of **9**. Column chromatography of the product using 4:1 toluene-EtOAc gave pure **13** as syrup (90 mg,

62%); $[\alpha]_D$ +37.8° (*c* 1.3, $CHCl_3$); ^1H NMR δ 1.16 (d, 3H, C*H*$_3$, J = 5.9 Hz), 1.33 and 1.50 (2s, 6H, CMe_2), 3.33 (s, 3H, OC*H*$_3$), 3.53 (m, 2H, H-6), 3.83-4.07 (m, 5H, H-2', H-3', H-4', H-5', H-5), 4.26 (dd, 1H, $J_{1,2}$ = 2.4 Hz, $J_{2,3}$ = 5.7 Hz, H-2), 4.41-4.46 (m, 2H, H-3, H-4), 4.64-5.06 (m, 6H, 3PhC*H*$_2$), 4.88 (d, 1H, H-1, J = 3.2 Hz), 5.03 (d, 1H, H-1', J = 3.9 Hz), 7.24-7.42 (m, 15H, aromatic protons).

Anal. Calcd for $C_{37}H_{46}O_{10}$: C, 68.28; H, 7.12. Found: C, 68.14; H, 7.25.

Methyl α-L-Fucopyranosyl-(1→2)-α-D-galactopyranoside (15). Compd **13** (90 mg, 0.13 mmol) was stirred with 85% aq. AcOH[22] (10 mL) at 90 °C for 2 h and the work-up was the same as that described for preparing **10** to give compd **14**. A soln of **14** in AcOH (15 mL) was hydrogenolyzed for 48 h in presence of 10% Pd-C (40 mg) at 24 °C. The reaction mixture was filtered through a celite bed, purified by column chromatography and concd to give **15** as syrup (35 mg, 74%); $[\alpha]_D$ +19° (*c* 0.91, H_2O);[24] lit.[8] $[\alpha]_D$ +1.3° (*c* 1.12, H_2O); ^1H NMR (D_2O) δ 1.14 (d, 3H, C*H*$_3$, J = 5.9 Hz), 3.34 (s, 3H, OC*H*$_3$), 3.49 (m, 2H, H-6), 3.70-3.91 (m, 6H, H-2, H-3, H-4, H-2', H-3', H-4'), 4.0-4.17 (m, 2H, H-5, H-5'), 4.89 (d, 1H, H-1, J = 3.8 Hz), 5.03 (d, 1H, H-1', J = 4.2 Hz); ^{13}C NMR (D_2O) δ 15.79 (*C*H$_3$), 55.06 (O*C*H$_3$), 61.68 (C-6), 66.18, 68.03, 69.11, 70.24, 70.73, 71.29, 71.51, 71.74, 97.27 (C-1), 99.25 (C-1').

Anal. Calcd for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.10. Found: C, 45.74; H, 7.17.

Methyl 2,3,4-Tri-*O*-benzyl-α-L-fucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (16). To a soln of **4**[18] (210 mg, 0.44 mmol), **7**[19] (315 mg, 0.66 mmol), molecular sieves 4Å (1 gm) in CH_2Cl_2 (10 mL) were added NIS (193 mg, 0.86 mmol) and TfOH[20] (7.5 μL, 0.08 mmol) and the mixture was stirred for 2 h. The workup is exactly the same as described before to give **16** as syrup (230 mg, 65%), $[\alpha]_D$ -69.3° (*c* 1.1, $CHCl_3$); ^1H NMR δ 1.1 (d, 3H, C*H*$_3$, J = 6.1 Hz), 3.39 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 7.9 Hz, H-2), 3.47 (s, 3H, OC*H*$_3$), 3.59-3.63 (m, 2H, H-3, H-5), 3.75-3.85 (m, 2H, H-2', H-4'), 4.00 (dd, 1H, $J_{2,3}$ = 9.6 Hz, $J_{3,4}$ = 2.7 Hz, H-3'), 4.24 (d, 1H, H-1, J = 7.8 Hz), 4.48-4.90 (m, 8H, 4PhC*H*$_2$), 5.02 (d, 1H, H-1', J = 3.1 Hz), 5.50 (s, 1H, PhC*H*), 7.23-7.52 (m, 25H, aromatic protons); ^{13}C NMR δ 16.69 (*C*H$_3$), 56.74 (O*C*H$_3$), 66.33 (C-6), 69.05, 70.01, 71.60, 72.97, 74.35, 74.82, 75.62, 78.46, 80.12, 80.53, 82.63, 101.19 (C-1'), 102.62 (C-1), 104.32 (Ph*C*H), 126.44-138.90 (aromatic carbons).

Anal. Calcd for $C_{48}H_{52}O_{10}$: C, 73.07; H, 6.64. Found: C, 72.91; H, 6.78.

Methyl α-L-Fucopyranosyl-(1→2)-β-D-galactopyranoside (17). A soln of **16** (100 mg, 0.126 mmol) in AcOH (10 mL) was hydrogenolyzed as described previously and purified by column chromatography to give **17** as syrup (38 mg, 88%); $[\alpha]_D$ - 17.3° (*c* 0.62, H_2O);[24] lit.[8] $[\alpha]_D$ -70.1° (*c* 0.88, H_2O); ^1H NMR (D_2O) δ 1.15 (d, 3H, C*H*$_3$, J = 5.3 Hz), 3.36 (dd, 1H, $J_{1,2}$ = 8.1 Hz, $J_{2,3}$ = 8.4 Hz, H-2), 3.45 (s, 3H, OC*H*$_3$), 3.53-3.61 (m, 2H, H-3, H-5), 3.74-3.82 (m, 2H, H-2', H-4'), 3.97 (m, 2H, H-3, H-3'), 4.45 (d, 1H, H-1, J = 7.8 Hz), 5.04 (d, 1H, H-1', J = 2.7 Hz); ^{13}C NMR (D_2O) 15.30 (*C*H$_3$), 57.04 (OCH$_3$), 60.83 (C-6), 68.16, 70.52, 70.62, 70.71, 72.60, 74.84, 75.00, 77.81, 102.25 (C-1'), 103.71 (C-1).

Anal. Calcd for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.10. Found: C, 45.76; H, 7.27.

Methyl 2,3,4-Tri-*O*-acetyl-β-L-fucopyranosyl-(1→2)-3-*O*-benzyl-4,6-*O*-benzylidene-β-D-galactopyranoside (18). To a soln of **4**[18] (201.9 mg, 0.54 mmol), **5**[19] (257 mg, 0.77 mmol), and molecular sieves 4Å (1 gm) in CH_2Cl_2 (10 mL) were added NIS (224 mg, 0.99 mmol) and TfOH[20] (8.8 μL, 0.09 mmol). The mixture was stirred under nitrogen for 30 min and the workup was exactly the same as described for **18** as syrup (270 mg, 77.2%); $[\alpha]_D$ +2.8° (*c* 1.26, $CHCl_3$); ^1H NMR δ 1.16 (d, 3H, C*H*$_3$, J = 6.3 Hz), 1.97, 2.06 and 2.14 (3s, 9H, 3 OAc), 3.5 (s, 3H, OC*H*$_3$), 3.56 (dd, 1H, $J_{1,2}$ = 7.5 Hz, $J_{2,3}$ = 10 Hz, H-2), 4.22 (d, 1H, H-1, J = 7.5 Hz), 4.86 (d, 1H, H-1', J = 7.8 Hz), 4.98 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3.3 Hz, H-3'), 5.17 (dd, 1H, $J_{1,2}$ = 8 Hz, $J_{2,3}$ = 7.9 Hz, H-2'), 5.44 (s, 1H, PhC*H*), 7.32-7.54 (m, 10 H, aromatic protons); ^{13}C NMR δ 16.01 (*C*H$_3$), 20.61, 20.65, 20.90 (3 CO*C*H$_3$), 57.33 (O*C*H$_3$), 66.26 (C-6), 69.05, 69.74, 70.31, 71.40, 71.82, 73.69, 76.58, 79.12, 101.20 (C-1'), 101.42 (C-1), 103.44 (Ph*C*H), 126.34-138.47 (aromatic carbons), 169.68, 170.19, 170.64 (3 *C*OCH$_3$).

Anal. Calcd for $C_{33}H_{40}O_{13}$: C, 61.48; H, 6.25. Found: C, 61.30; H, 6.43.

Methyl β-L-Fucopyranosyl-(1→2)-β-D-galactopyranoside (20). Compd **18** (76.4 mg, 0.118 mmol) was stirred with 0.05 M NaOMe (10 mL) for 3 h. The workup was the same as that described for the preparation of **10** to give **19**. A soln of **19** in AcOH (5 mL) was hydrogenolyzed for 72 h as described previously and purified by column chromatography to give **20** as syrup (31.8 mg, 79%); $[\alpha]_D$ -13.2° (*c* 2.5, H_2O);[24] lit.[8] $[\alpha]_D$ +11.5° (*c* 1.04, H_2O); ^1H NMR (D_2O) δ 1.09 (d, 3H, C*H*$_3$, J = 6 Hz), 3.35 (m, 2H, H-2, H-2'), 3.41 (s, 3H, OC*H*$_3$), 3.75 (dd, 1H, $J_{2,3}$ = 10.5 Hz, $J_{3,4}$ = 3 Hz, H-3), 4.16 (d, 1H, H-

1, J = 7.9 Hz), 4.86 (d, 1H, H-1', J = 7.8 Hz); ^{13}C NMR (D_2O) δ 15.26 (CH_3), 57.0 (OCH_3), 60.79 (C-6), 68.11, 68.50, 70.71, 71.26, 71.34, 72.74, 74.98, 77.72, 102.20 (C-1'), 103.48 (C-1).

Anal. Calcd for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.10. Found: C, 45.69; H, 7.25.

ACKNOWLEDGEMENTS

The authors express their sincere thanks to Professors N. Roy and B. P. Chatterjee and Drs. A. K. Guha and A. K. Misra of this Department for their constant help and encouragement during the work. This work is supported by Dept. of Science and Technology, Govt. of India (Grant No. SP/SO/D39/94).

REFERENCES AND NOTES

1. F. Ørskov and I. Ørskov, *Can. J. Microbiol.*, **38**, 699 (1992).
2. J. Hacker, *Can. J. Microbiol.*, **38**, 720 (1992).
3. S. R. Munford, *Acta Pathol. Microbiol. Immunol. Scand.*, **99**, 487 (1991).
4. T. Bhattacharyya and S. Basu, *Carbohydr. Res.*, **254**, 221 (1994).
5. P. Sengupta, T. Bhattacharyya, A. S. Shashkov, H. Kochanowski and S. Basu, *Carbohydr. Res.*, **277**, 283 (1995).
6. G. Widmalm and K. Leonteir, *Carbohydr. Res.*, **185**, 211 (1985).
7. W. C. Raschke and C. E. Ballow, *Biochemistry*, **11**, 3807 (1972).
8. H. M. Flowers, *Carbohydr. Res.*, **74**, 177 (1979).
9. N. K. Kochetkov, A. S. Shashkov, G. M. Lipkind and Y. A. Knirel, *Sov. Sci. Rev., Sec B., Chem,* **13**, 1 (1989).
10. P. K. Agrawal, *Phytochemistry*, **31**, 3307 (1992).
11. J. K. Dale and C. S. Hudson, *J. Am. Chem. Soc.*, **52**, 2534 (1930).
12. R. G. Ault, W. N. Haworth and E. L. Hirst, *J. Chem. Soc.*,1012 (1935).
13. W. F. Collin, G. W. J. Fleet, M. Haraldsson, I. C. deBello and B. Winchester, *Carbohydr. Res.*, **202**, 105 (1990).
14. M. T. Campos-Valdes and J. R. Marino-Albernas, *J. Carbohydr. Chem.*, **6**, 509 (1987).
15. M. A. Nashed, M. S. Choudhury and L. Anderson, *Carbohydr. Res.*, **102**, 99 (1982).
16. P. Kovác, C. P. J. Glaudemans and R. B. Taylor, *Carbohydr. Res.*, **142**, 158 (1985).
17. G. Magnusson, S. Ahlfors, J. Dahmen, K. Janson, U. Nilsson, G. Noori, K. Stenvall and A. Tjornebo, *J. Org. Chem.*, **55**, 3932 (1990).

18. J. Kihlberg, T. Frejd, K. Janson and G. Magnusson, *Carbohydr. Res.*, **152**, 113 (1986).
19. H. Lönn, *Carbohydr. Res.*, **139**, 105 (1985).
20. G. H. Veeneman, S. H. vanLeeuwen and J. H. vanBoom, *Tetrahedron Lett.*, 1331 (1990).
21. W. Kinzy and R. R. Schmidt, *Liebigs Ann. Chem.*, 1537 (1985).
22. P. A. Gent and R. Gigg, *J. Chem. Soc., Perkin Trans. 1*, 1446 (1974).
23. G. Zemplén, *Ber. Dtsch. Keram. Ges.*, **59**, 1254 (1926).
24. The optical rotational values are not in agreement with those previously reported.