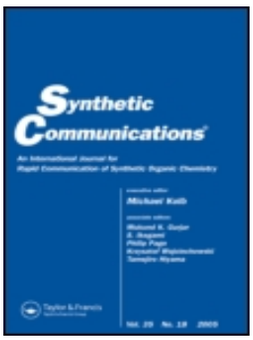

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SYNTHESIS OF A TRISACCHARIDE RELATED TO THE K-ANTIGEN FROM *STREPTOCOCCUS PNEUMONIAE* TYPE 29

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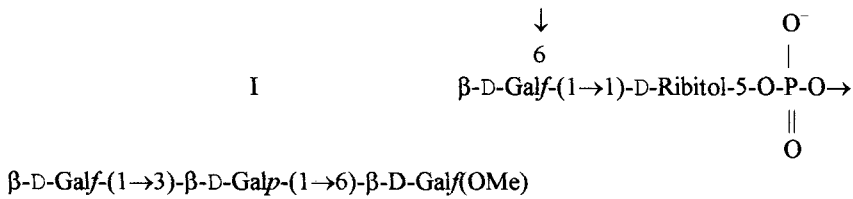
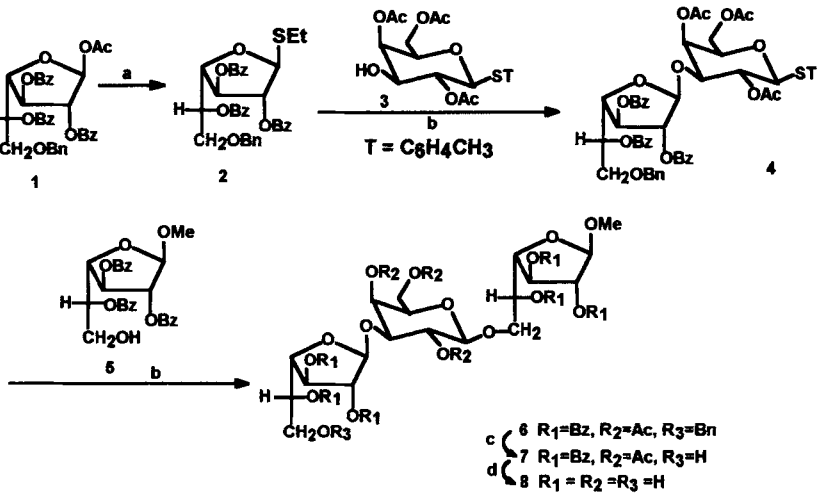
Abstract: Ethyl 2,3,5-tri-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactofuranoside was utilized as glycosyl donor for the synthesis of a disaccharide donor as a *p*-tolyl thioglycoside, which reacted with the suitable furanoside acceptor to afford the trisaccharide derivative related to the antigen of *Streptococcus pneumoniae* type 29 in the form of its methyl glycoside.

The structure of the repeating unit of capsular polysaccharide from *Streptococcus pneumoniae* type 29 has been established[1] to be the pentasaccharide I. In continuation of our programme to determine the relation between the structure and the immunological specificity of bacterial antigens, we report here the synthesis of the trisaccharide II related to this repeating unit. This



I

II



Reagents: a) EtSH, BF_3•OEt_2, CH_2Cl_2, 0 °C, 2 h; b) NIS/TfOH, CH_2Cl_2, 0 ^{0}C, ; c) 10% Pd-C, AcOH, 24h, 22 ^{0}C; d) 0.1 M NaOMe, MeOH, Dowex 50W (H^+).

trisaccharide contains two galactofuranoside moieties. Such furanoside moieties are present in many bacterial antigens and are claimed to be immunodominant in many cases.[2]

Ethyl 2,3,5-tri-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactofuranoside (**2**) was prepared from 1-*O*-acetyl-2,3,5-tri-*O*-benzoyl-6-*O*benzyl-β-D-galactofuranose[3] (**1**) by treatment with ethanethiol-boron trifluoride diethyl etherate-dichloromethane.[4] This novel thioglycoside derivative was allowed to react with *p*-tolyl 2,4,6-tri-*O*-acetyl-1-thio-β-D-galactopyranoside (**3**) in presence of N-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH)[5] to obtain the disaccharide **4** as its *p*-tolyl thioglycoside in 80% yield. Although both **2** and **3** were disarmed[6] because of the presence of 2-*O*-benzoyl and 2-*O*-acetyl groups, the formation of **4** was favoured because of the greater reactivity of ethyl thioglycoside[7]. The same disaccharide was obtained by us earlier[3] by the reaction of 2,3,5-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactofuranosyl trichloroacetimidate and **3** in presence of

trimethylsilyl trifluoromethanesulfonate. The disaccharide donor **4** was then allowed to react with methyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranoside (**5**), obtained by removal of benzyl group from methyl 2,3,5-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactofuranoside,[3] in presence of NIS-TfOH to afford the trisaccharide derivative **6** in 75% yield. ^{1}H NMR spectrum of **6** showed the signals at δ 5.25 and 5.27 for two β-galactofuranosidic anomeric protons and at δ 4.87 for one β-galactopyranosidic anomeric proton in addition to other characteristic peaks. The ^{13}C NMR spectrum of **6** gave signals at δ 107.5 (C-1"), 106.9 (C-1) and 102.1 (C-1') together with other peaks which supported the assignments of the anomeric linkages. Hydrogenolysis of **6** with 10% Pd-C followed by removal of the acyl groups from the product **7** with sodium methoxide gave the desired trisaccharide in the form of its methyl glycoside **8** in 68% yield. The ^{1}H NMR spectrum of **8** showed signals for two β-galactofuranosidic anomeric protons (δ 5.25 and 5.27) and one β-galactopyranosidic anomeric protons (δ 4.78). ^{13}C NMR spectrum of **8** having anomeric carbon signals at δ 109.7, 109.5 and 102.8 confirmed the configuration of the anomeric positions[3,8] as assigned above.

Experimental

General methods. — All reactions were monitored by TLC on Silica Gel G (E. Merck). Column chromatography was performed on 100-200 mesh silica gel (SRL, India). All solvents were distilled and or dried before use and all evaporations were conducted below 40 °C under reduced pressure unless stated otherwise. Optical rotations were measured with a Perkin-Elmer model 241 MC polarimeter. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol FX-100 or Bruker 300 MHz Spectrometer using $CDCl_3$ as solvent (internal standard TMS) unless otherwise stated.

Ethyl 2,3,5-tri-*O*-benzoyl-6-*O*-benzyl-1-thio-β-D-galactofuranoside (2). To a solution of **1** (375 mg, 0.6 mmol) and EtSH (90 μL, 1.2 mmol) in CH_2Cl_2 (8 mL), cooled to 0 °C, BF_3.OEt2 (0.23 mL, 1.8 mmol) was added and the mixture was

stirred for 2 h. After dilution with CH_2Cl_2. the mixture was washed with water, aq $NaHCO_3$ and water in succession. The organic layer was dried, filtered and concentrated to a syrup. Column chromatography with 8:1 toluene-ethylacetate gave 2 (300 mg, 79.8%) ; $[\alpha]_D^{25}$ -58.5° (c 0.7, $CHCl_3$); ^{1}H NMR : δ 1.30 (t, 3 H, SCH_2CH_3), 2.72 (q, 2 H, SCH_2CH_3), 4.60 (d, 1 H, $J_{1,2}$ =0.9 Hz, H-1), 7.40-8.10 (m, 20 H, aromatic protons). Anal. Calcd for $C_{36}H_{34}O_8S$: C, 68.99; H, 5.47. Found: C, 68.75; H, 5.65.

p-Tolyl 2,3,5-tri-*O*-benzoyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-O-acetyl-1-thio-β-D-galactopyranoside (4).). A mixture of the donor **2** (125 mg, 0.20 mmol), the acceptor **3** (83mg, 0.20 mmol) and 4Å molecular sieves (200 mg) in CH_2Cl_2 (3 mL) were stirred for 3 h at 25 °C. The mixture was then cooled to 0 °C, NIS (0.24 mmol) and TfOH (0.08 mmol) were added and stirring was continued for 20 min. The reaction mixture was then diluted with CH_2Cl_2, filtered, and washed successively with $Na_2S_2O_3$, $NaHCO_3$ and water. The organic layer was dried and then concentrated. Column chromatography with 8:1 toluene-ethylacetate gave pure **4** (160 mg, 82%) ; $[\alpha]_D^{25}$ + 8.3° (c 1.1, $CHCl_3$). ^{1}H-NMR : δ 2.05, 2.08 and 2.21 (3 s, 9 H, 3 OAc), 2.34 (s, 3 H, $SC_6H_4CH_3$), 4.55 (d, 1 H, $J_{1,2}$ 12 Hz, H-1), 4.63 (dd, 2 H, $CH_2C_6H_5$), 4.89 (dd, 1 H, $J_{3,4}$ = 3.6 Hz, $J_{4,5}$ 5.1 Hz, H-4), 5.26 (d, 1 H, $J_{1',2'}$ = 0.9 Hz, H-1'), 5.31 (t, 1 H, J =12 Hz, H-2), 5.36 (d, 1 H, J = 0.9 Hz, H-2'), 5.46 (d, 1 H, $J_{3,4}$ = 3.6 Hz, H-3), 7.10-8.13 (m, 24 H, aromatic protons). ^{13}C NMR: δ 20.4, 20.5 and 20.8 (3 $COCH_3$), 21.0 ($SC_6H_4CH_3$), 62.3 (C-6), 68.3 (C-6'), 68.8, 69.2, 71.2, 73.0, 74.8, 76.4, 77.5, 82.4 (C-2'), 82.8 (C-4'), 87.0 (C-1), 107.4 (C-1'), 126.6-137.9 (aromatic carbons), 170.2-165.3 (3 $COCH_3$, 3 COPh). Anal. Calcd for $C_{53}H_{52}O_{16}S$: C, 65.15; H, 5.36. Found: C, 65.02; H, 5.50.

Methyl 2,3,5-tri-*O*-benzoyl-6-*O*-benzyl-β-D-galactofuranosyl-(1→3)-2,4,6-tri-*O*-acetyl-β-D-galactopyranosyl-(1→6)-2,3,5-tri-*O*-benzoyl-β-D-galacto-furanoside (6). A mixture of **4** (98mg, 0.10 mmol), and **5** (61 mg, 0.12 mmol), were allowed to react in presence of NIS-TfOH as described for the preparation of

4. Column chromatography of the crude product with 8:1 toluene-ethylacetate gave pure **6** (102 mg, 75%); $[\alpha]_D = +26.6^0$ (*c* 3.4, $CHCl_3$). ^{1}H-NMR : δ 1.86, 1.97 and 2.07 (3s, 9H, 3OAc), 3.43 (s, 3H, OCH_3), 5.55 (bs, 1H, H-1"), 5.28 (d, 1H, $J_{1',2'} = 6.3$ Hz, H-1'), 5.12 (bs, 1H, H-1), 8.22-7.12 (m, 35H, aromatic protons). ^{13}C NMR : δ 20.5, 20.6 and 20.7 (3 $COCH_3$), 54.8 (OCH_3), 61.8, 64.8, 69.0, 70.5, 71.2, 71.3, 76.5, 76.6, 76.8, 77.4, 77.7, 78.2, 80.9 (C-2'), 81.7 (C-4), 82.5 (C-4), 82.7 (C-4"), 102.1.0 (C-1'), 106.9 (C-1), 107.5 (C-1"), 127.4-133.3 (aromatic carbons), 165.5-169.9 ($3COCH_3$, $6COPh$). Anal. Calcd for $C_{74}H_{70}O_{25}$: C, 65.39; H, 5.19. Found: C, 64.83; H, 5.14.

Methyl β-D-galactofuranosyl-(1→3)-β-D-galactopyranosyl-(1→6)-β-D-galactofuranoside (8). A solution of **6** (0.1 mmol) in acetic acid (3 mL) was hydrogenolysed over 10% Pd-C (100 mg) for 24 h. The product **7** was isolated and treated with 0.1 M NaOMe in MeOH by the conventional method. Column chromatography with 2:1 EtOAc-MeOH afforded **8** (0.07mmol); $[\alpha]_D = +37.3^0$ (*c* 0.25, MeOH). ^{1}H NMR (D_2O) : δ 3.27 (s, 3H, OCH_3), 5.31 (bs, 1H, H-1"), 5.18 (bs, 1H, H-1), 4.66 (d, 1H, J=6.0 Hz, H-1'). ^{13}C NMR (D_2O) : δ 62.0, 63.4, 63.5, 69.5, 71.0, 72.0, 72.1, 73.5, 75.4, 75.7, 77.1, 82.3 (C-2), 82.5 (C-2"), 84.4 (C-4), 84.6 (C-4"), 109.0 (C-1), 109.7 (C-1"). Anal. Calcd for $C_{19}H_{34}O_{16}$: C, 44.02; H, 6.61. Found : C, 44.21, H, 6.75.

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