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SYNTHESIS OF THE IMMUNODOMINANT TRISACCHARIDE RELATED TO THE ANTIGEN FROM *E. COLI* O126

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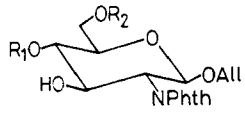
ABSTRACT : The trisaccharide derivative methyl 2-*O*-[4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-gal-actopyranosyl)-2-deoxy-2-phthalimido-β-D-gluco-pyranosyl]-4,6-*O*-benzylidene-β-D-mannopyranoside (**12**) was obtained when 3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-4,6-di-*O*-acetyl-2-deoxy-2-phtha-limido-β-D-glucopyranosyl trichloroacetimidate (**8**) was allowed to react with methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**11**) in presence of trimethylsilyl triflate. Removal of protecting groups then gave the desired trisaccharide.

The enteropathogenic strains of *E. coli* are known to be associated[1] with infantile diarrhoea which is one of the major cause of illness and death among children in tropical countries. Serotypes from diarrhoeal diseases are largely specific to the host-species in which the disease occurs.[2] The precise diagnosis based on O-serotyping is possible only when we could explore the detailed structural features of the O-specific polysaccharide (O-SP) of the lipopolysaccharide isolated from appropriate strains.

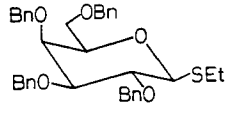
The structure of the O-SP from the enteropathogenic strain of *E. coli* O126 have already been known[3] and the specificities of the polyclonal antiserum against this O-SP O126 were also determined.[4] In the light of increasing drug resistance of pathogenic bacterial infections the selective chemical synthesis of the immunodominant block of the O-antigens has gained considerable interest.

This will help to study the potential of synthetic antigens for precise diagnosis and protection. In this communication we report the synthesis of the immunodominant sugar block of *E. coli* O126 antigen.

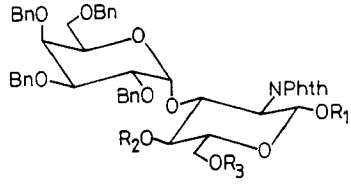
Allyl 2-deoxy-2-phthalimido-β-D-glucopyranoside[5] (**1**) was allowed to react with benzaldehyde dimethylacetal in presence of Toluene *p*-sulfonic acid in acetonitrile[6] to give allyl 4,6-*O*-benzylidene-2-deoxy-2-phthalimido-β-D-glucopyranoside (**2**) as crystals in 70% yield. Ethyl 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-galactopyranoside[7] (**3**), prepared from galactose, was allowed to react with the acceptor **2** in the presence of copper(II) bromide and tetrabutylammonium bromide[8] to afford the disaccharide allyl 4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (**4**) in 74% yield. Debenzylidenation of **4** with 85% acetic acid[9] followed by acetylation of the diol, gave allyl 4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranoside (**6**) in quantitative yield. Compound **6** was deallylated[10] with palladium(II) chloride and sodium acetate, and the resulting hemiacetal(**7**),on treatment with trichloroacetonitrile[11] and potassium carbonate in dichloromethane gave the donor 4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl trichloroacetimidate (**8**) donor in 69% yield.



1. $R_1 = R_2 = H$
2. $R_1, R_2 = PhCH$



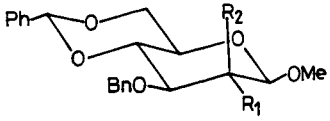
3



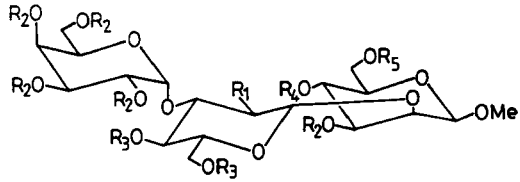
4. $R_1 = Allyl; R_2, R_3 = PhCH$
5. $R_1 = Allyl; R_2 = R_3 = H$
6. $R_1 = Allyl; R_2 = R_3 = Ac$
7. $R_1 = H; R_2 = R_3 = Ac$
8. $R_1 = CNHCCl_3; R_2 = R_3 = Ac$

In a separate experiment, methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-D-glucopyranoside[12] (**9**) was oxidised[13] with dimethyl sulfoxide-acetic anhydride to give the corresponding 2-keto compound **10** which was reduced with $NaBH_4$[13] to give methyl 3-O-benzyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**11**) in 70% yield.

The donor **8** and the acceptor **11** were then allowed to react in the presence of trimethylsilyl triflate[14] to afford the trisaccharide derivative methyl 2-*O*-[4,6-di-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-galactopyranosyl)-2-deoxy-2-phthalimido-β-D-glucopyranosyl]-4,6-*O*-benzylidene-β-D-mannopyranoside (**12**) in 54% yield. Removal of benzyl groups from **12** followed by treatment of the product with hydrazine hydrate gave a free amine which was N-acetylated giving the desired methyl 2-*O*-[3-*O*-(α-D-galactopyranosyl)-2-acetamido-2-deoxy-β-D-glucopyranosyl]-β-D-mannopyranoside (**13**). The trisaccharide **13** was characterised by its ^1H and ^{13}C NMR spectra.



9. R_1=OH ; R_2=H
11. R_1=H ; R_2=OH



12. R_1 =NPhth ; R_2=Bn ; R_3 =Ac ; R_4,R_5 =PhCH
13. R_1 =NHAc ; R_2=R_3 =R_4=R_5 =H

EXPERIMENTAL

General- The general methods are the same as used previously[15].

Preparation of **2**.- A solution of **1** (1.80 g, 5.15 mmol) in dry MeCN (20 mL) was stirred with MS 3Å (2 g) for 1h at r.t. Benzaldehyde dimethylacetal (1.93 mL, 12.6 mmol) and *p*-TsOH (50 mg) were added, and the mixture was stirred for 24h at r.t. It was then neutralised with Et_3N (500 μL), filtered through celite and concentrated. Column chromatography (4:1 toluene-EtOAc) afforded pure **2** (1.58 g, 70%) which crystalised from EtOH; mp 179-180°C; $[\alpha]_D$ -28.6° (*c* 0.9, $CHCl_3$). ^1H NMR:δ 5.34 (d, $J_{1,2}$=8.0 Hz, 1H, H-1), 5.58 (s, 1H, PhC*H*), 5.76 (m, 1H, CH_2=C*H*-CH_2), 7.32-7.92 (m, 9H, Ph & Phth). Anal. Calcd. for $C_{24}H_{23}O_7N$: C, 65.89; H, 5.30. Found: C, 65.67; H, 5.52.

Preparation of **4**.- To a flask containing $CuBr_2$ (1.45 g, 6.50 mmol), Bu_4NBr (217 mg, 0.67 mmol) and MS 4Å (10 g) was added a solution of **3** (3.15 g, 5.39 mmol) and **2** (1.47 g, 3.36 mmol) in 5:1 1,2-dichloroethane-DMF (96 mL) and the mixture was stirred vigorously under argon at 24°C for 72h. The contents were

filtered through celite and diluted with CH_2Cl_2. The organic layer was washed successively with water, aq. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. The residue was chromatographed with 60:1 toluene-Et_2O giving **4** (2.38 g, 74%); $[\alpha]_D$ +26.5° (*c* 1.6, $CHCl_3$). ^1H NMR: δ 5.34 (d, $J_{1,2}$=7.8 Hz, 1H, H-1), 5.36 (s, 1H, PhC*H*), 5.18 (d, $J_{1',2'}$=2.0 Hz, 1H, H-1'), 5.76 (m, 1H, CH_2=C*H*-CH_2), 7.24-7.86 (m, 29H, 5Ph and Phth). Anal. Calcd. for $C_{58}H_{57}O_{12}N$: C, 72.56; H, 5.98. Found: C, 72.32; H, 6.22.

Preparation of **6**.- Compound **4** (2.20 g, 2.29 mmol) in 85% AcOH (40 mL) was stirred at 90°C for 2h. The solvent was removed under vacuum and the residue was co-evaporated with toluene to give **5** in quantitative yield. Acetylation of **5** with Ac_2O and pyridine gave **6** in quantitative yield; $[\alpha]_D$ +33.8° (*c* 0.4, $CHCl_3$). ^1H NMR: δ 1.78, 2.12 (2s, 6H, 2C*H*$_3$CO), 5.13 (d, $J_{1,2}$=8.0 Hz, 1H, H-1), 5.04 (d, $J_{1',2'}$=1.5 Hz, 1H, H-1'), 5.74 (m, 1H, CH_2=C*H*-CH_2), 7.18-7.68 (m, 24H, 4Ph and Phth). Anal. Calcd. for $C_{55}H_{57}O_{14}N$: C, 69.09; H, 6.01. Found: C, 68.85; H, 6.27.

Preparation of **8**.- A mixture of **6** (1.30 g, 1.36 mmol), $PdCl_2$ (194 mg, 1.09 mmol), NaOAc.3H_2O (770 mg, 5.58 mmol) in 20:1 AcOH-H_2O (32 mL; v/v) were stirred at 20°C for 18h. The reaction mixture was filtered through celite and concentrated to dryness. The product was dissolved in CH_2Cl_2 and washed in succession with water, aq. $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated to a syrup. Column chromatography with 10:1 toluene-EtOAc gave compound **7** (0.97 g, 78%) as a colourless syrup. To a solution of **7** (522.5 mg, 0.57 mmol) in CH_2Cl_2 (6 mL) was added CCl_3CN(1.14 mL, 11.4 mmol) and K_2CO_3 (2.85 g) and stirred at 20°C for 10h. The reaction mixture was then filtered through celite and concentrated to a syrupy mass. Column chromatography with 12:1 toluene-EtOAc gave **8** (0.40 g, 69%) as a colourless syrup; $[\alpha]_D$ +39.5° (*c* 1.6, $CHCl_3$). ^1H NMR: δ 1.79, 2.15 (2s, 6H, 2C*H*$_3$CO), 5.22 (d, $J_{1,2}$=7.5 Hz, 1H, H-1), 5.03 (bs, 1H, H-1'), 7.18-7.57 (m, 24H, 4Ph and Phth), 8.57 (s, 1H, OCN*H*CCl_3). Anal. Calcd. for $C_{54}H_{53}O_{14}N_2Cl_3$: C, 61.16; H, 5.04. Found: C, 61.01; H, 5.27.

Preparation of **11**.- To a solution of **9** (2.38 g, 6.60 mmol) in Me_2SO (40 mL) was added 1:2 Ac_2O-Me_2SO (80 mL, v/v) and the mixture was stirred for 16h at r.t. The solvents were removed by evaporation under reduced pressure when the

keto compound **10** (2.10 g) was obtained as a solid mass. To a solution of this product in 1:1 CH_2Cl_2-MeOH (120 mL), $NaBH_4$ (10 g) was added and the mixture was stirred at 5-10°C for 5h. The reaction mixture was concentrated in vacuo and diluted with CH_2Cl_2 (150 mL). The organic layer was washed in succession with 5% citric acid solution, aq. $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated to a solid mass. Column chromatography using toluene-Et_2O gave pure **11** (1.66 g, 70%) together with some gluco-isomer. The compound **11** was crystallised from EtOH, mp 113-114°C; $[\alpha]_D$ -26.12° (*c* 1.0, $CHCl_3$). ^1H NMR: δ 3.56 (s, 3H, OCH_3), 4.42 (d, $J_{1,2}$=2.0 Hz, 1H, H-1), 4.83 (dd, 2H, $PhCH_2$), 5.63 (s, 1H, PhC*H*), 7.4 (m, 10H, 2Ph). Anal. Calcd. for $C_{20}H_{24}O_6$: C, 66.65; H, 6.71. Found: C, 66.52; H, 6.85.

Preparation of **12**.-A mixture of **8** (266.8 mg, 0.26 mmol), **11** (188 mg, 0.52 mmol) and MS 4A (1.0 g) in CH_2Cl_2 (5 mL) was stirred and cooled to -40°C. After 1h, a solution of trimethylsilyl triflate (107 µL, 0.5 mmol) in CH_2Cl_2 (2 mL) was added and the mixture was stirred for 3.5h. Pyridine (1 mL) was then added and the mixture was filtered through celite, concentrated and co-evaporated with toluene (3x5 mL) and EtOH (2x5 mL). A solution of this residue in CH_2Cl_2 (25 mL) was washed with water, aq. $NaHCO_3$ and water in succession, dried (Na_2SO_4) and concentrated. Column chromatography of the residue with 8:1 toluene-Et_2O gave pure **12** (356.7 mg, 54%) as a colourless syrup; $[\alpha]_D$ -20.7° (*c* 0.6, $CHCl_3$). ^1H NMR: δ 1.79, 2.07 (2s, 6H, $2CH_3CO$), 3.37 (s, 3H, OCH_3), 4.34 (d, $J_{1,2}$=3.0 Hz, 1H, H-1), 5.02 (bs, 1H, H-1"), 5.36 (d, $J_{1',2'}$=8.4 Hz, 1H, H-1'), 5.65 (s, 1H, PhC*H*), 7.17-7.63 (m, 34H, 6Ph and Phth). ^{13}C NMR: δ 20.67 (2C, $2CH_3CO$), 56.78 (OCH_3), 98.78 (C-1"), 99.76 (C-1'), 101.38 (PhCH), 101.89 (C-1), 122.6-138.99 (aromatic carbons), 167.68-170.71 (4C, $2COCH_3$ and 2*C*O of Phth).Anal. Calcd. for $C_{73}H_{75}O_{19}N$: C, 69.01; H, 5.95. Found: C, 68.79; H, 6.18.

Preparation of **13**.-A solution of **12** (170 mg, 0.13 mmol) in acetic acid (10 mL) was hydrogenolised using 10% Pd-C (100 mg) for 48h at room temparature. The reaction mixture was filtered through a celite bed and dried. To a solution of the dry mass in ethanol (10 mL) hydrazine monohydrate (4 mL) was added and stirred at 70°C for 2h. The reaction mixture was concentrated and co-evaporated with toluene (2x10 mL) and ethanol (2x10 mL). The residue was treated with pyridine (1.5 mL) and Ac_2O (2.0 mL) for 3h at room temparature. The reagents

were then removed in the usual way to afford a syrupy mass which was then treated with 0.05M NaOMe (3 mL) for 3h. The reaction mixture decationized with Dowex 50W (H+) resin, filtered and dried to yield a glassy solid (49.2 mg,67%); $[\alpha]_D$ +14.2° (*c* 1.09, H_2O). ^1H NMR: δ 1.87(s, 3H, NHCOC*H*$_3$), 3.34 (s, 3H, OC*H*$_3$), 4.37(bs, 1H, H-1), 4.60 (bs, 1H, H-1''), 5.23(bs, 1H, H-1'). ^{13}C NMR: δ 22.16 (CH_3CONH), 53.88, 55.34, 56.54 (OCH_3), 59.95, 60.55, 66.90, 67.93, 68.47, 68.73, 69.94, 70.25, 71.67, 74.84, 75.88, 76.70, 78.72, 98.56 (C-1''), 100.67 (C-1'), 100.95 (C-1) and 174.30 (NCH_3CO). Anal.Calcd. for $C_{21}H_{27}O_{16}N$:C, 45.90; H, 4.95. Found: C, 45.78; H, 5.08.

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