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Synthesis of a mannotetraose derivative related to the antigen from *Escherichia coli* O9a:K26:H⁻

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The disaccharide blocks ethyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-2-*O*-acetyl-4, 6-*O*-benzylidene-1-thio-α-D-mannopyranoside **7** and methyl *O*-(3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranoside **11** have been prepared from D-mannose and allowed to react in the presence of methyl triflate as promoter to give the tetrasaccharide derivative methyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1→3)-*O*-(2-*O*-acetyl-4, 6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-*O*-(3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranosyl)-(1→2)-3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranoside **12**.

The O-antigens associated with strains of *Escherichia coli* that form thick capsules are commonly O8 and O9[1]. The O-antigenic lipopolysaccharide, found in the outer membrane of these *E. coli* strains are homopolysaccharides consisting only of mannose. The O-antigen carried by the serotype O9a:K26:H⁻ (O9a PS) (Figure 1) is almost identical to, yet slightly different serologically from the O9:K26:H⁻ (O9 PS) antigen[2].

Ogawa and Yamamoto[3] have synthesised the O9 PS repeating unit (Figure 2) by sequential addition of mannose unit using a mannosyl chloride as donor. Our interest is to synthesise the mannotetraose repeating unit of O9a PS utilising more stable D-mannopyranosyl donors in the form of thioglycoside or trichloroacetimidates using a more convenient block synthesis strategy.

α-D-Manp(1→3)-α-D-Manp(1→ 2)-α-D-Manp(1→2)-α-D-Manp(1→

Figure 1

α-D-Manp(1→3)-α-D-Manp(1→ 2)-α-D-Manp(1→2)-α-D-Manp(1→2)-α-D-Manp(1→

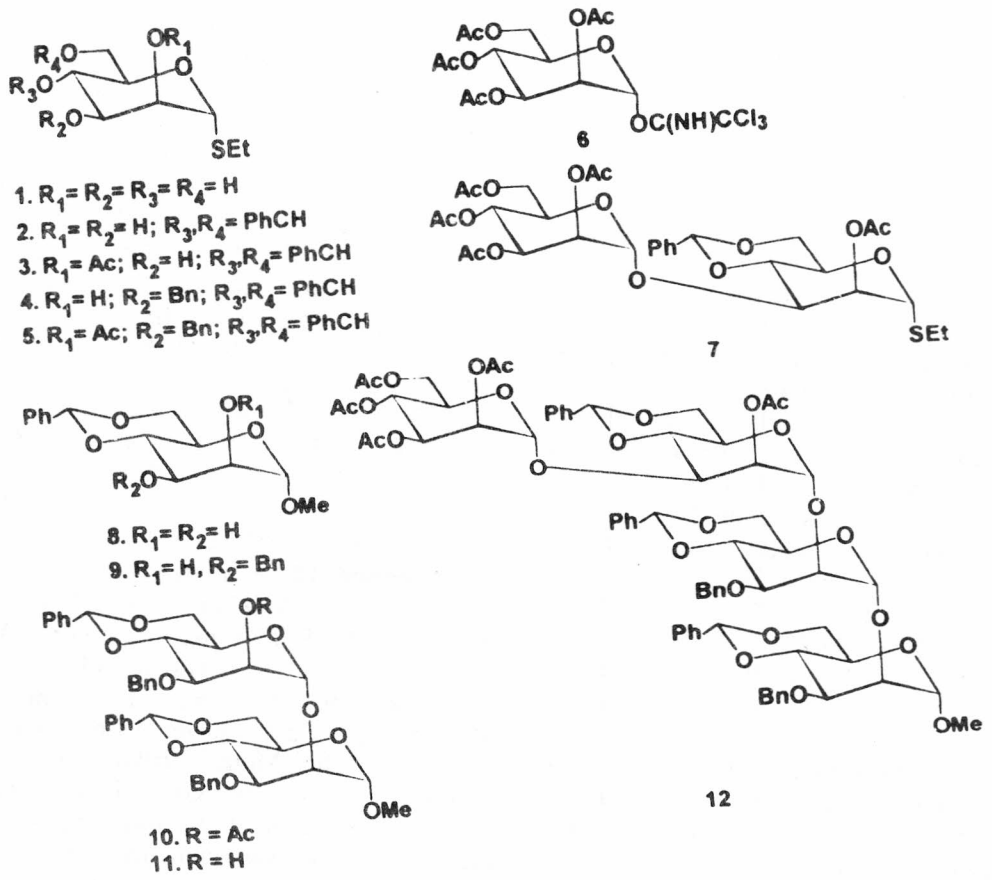
Figure 2

Ethyl 1-thio-α-D-mannopyranoside[4] **1** was converted to ethyl 4, 6-*O*-benzylidene-1-thio-α-D-mannopyranoside **2** with benzaldehyde dimethylacetal (1.2 eqv.) and *p*-toluenesulphonic acid[5] using acetonitrile as solvent in 69% yield. Compound **2** was allowed to react with trimethyl orthoacetate[6] in the presence of *p*-toluenesulphonic acid followed by treatment with 80% acetic acid to give ethyl 2-*O*-acetyl-4, 6-*O*-benzylidene-1-thio-α-D-mannopyranoside **3** in 83% yield.

Compound **3** was then allowed to react with 2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate[7] **6** in the presence of trimethylsilyl triflate[8] to give ethyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-2-*O*-acetyl-4, 6-*O*-benzylidene-1-thio-α-D-mannopyranoside **7** in 67% yield.

In another experiment, compound **2** was selectively benzylated at 3-position with dibutyltin oxide[9] and benzyl bromide to give ethyl 3-*O*-benzyl-4, 6-*O*-benzylidene-1-thio-α-D-mannopyranoside **4** in 87% yield. Conventional acetylation[10] of **4** using pyridine and acetic anhydride gave ethyl 2-*O*-acetyl-3-*O*-benzyl-4, 6-*O*-benzylidene-1-thio-α-D-mannopyranoside **5** in quantitative yield.

In a separate experiment, methyl 4, 6-*O*-benzylidene-α-D-mannopyranoside[11] **8** was selectively benzylated[9] with dibutyltin oxide and benzyl bromide to give methyl 3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranoside **9** in 83.5% yield. The donor **5** and the acceptor **9** were then allowed to condense[12] in the presence of methyl triflate as promoter to give methyl *O*-(2-*O*-acetyl-3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mennopyranosyl)-(1 → 2)-3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranoside **10** in 73% yield. Zemplén deacetylation[13] of **10** afforded the disaccharide acceptor methyl *O*-(3-*O*-benzyl-4, 6-*O*-benzylidene) α-D-mannopyranosyl-(-(1 → 2)-3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranoside **11** in quantitative yield.



The disaccharide donor **7** and the disaccharide acceptor **11** were then allowed to react in the presence of methyl triflate[12] as promoter to afford the mannotetraose derivative methyl *O*-(2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl)-(1 → 3)-*O*-(2-*O*-acetyl-4, 6-*O*-benzylidene-α-D-mannopyranosyl)-(1 → 2)-*O*-(3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranosyl)-(1 → 2)-3-*O*-benzyl-4, 6-*O*-benzylidene-α-D-mannopyranoside **12** in 68% yield, which was confirmed by its ^{1}H NMR spectrum [δ 1.91-2.11 (5s, 5 $COCH_3$); δ5.57, 5.61 and 5.66 (3s, 3 Ph-C*H*); δ3.33 (s, OCH_3); δ4.93 (bs, H-1); δ5.10 (d, H-1′); δ5.18 (d, H-1″); δ 5.25 (bs, H-1‴)].

Experimental Section

General. Optical rotations were measured at 25°C on a Perkin-Elmer 241 MC polarimeter. ^{1}H NMR spectra were recorded (internal standard tetramethyl silane) on a Jeol FX-100 or a Bruker 200 MHz spectrometer, using $CDCl_3$ as the solvent. All column chromatography was performed using silica gel (SRL, 100-200 mesh) solvents were distilled before use and all evaporations were conducted at 40°C under reduced pressure unless stated otherwise.

Compound 2. A solution of ethyl 1-thio-α-D-mannopyranoside **1** (2g, 8.93 mmoles) in dry acetonitrile (20 mL) was stirred with benzaldehyde dimethylacetal (1.47 mL, 9.82 mmoles) in the presence of *p*-TsOH (150 mg) and MS-3Å (2.5g) at room temperature for 18 hr. The reaction mixture was neutralised with Et_3N (~0.5 mL) and filtered through a Celite bed and concentrated to a white solid, which crystallised from $CHCl_3$-pet. ether giving pure **2** (1.92 g, 69%), m.p. 173-74°, $[\alpha]_D^{24}$ + 172° (*c* 2.3, $CHCl_3$) [Lit.[14] m.p. 174-75°; $[\alpha]_D$ + 167.5° (*c* 1.22, $CHCl_3$)]; ^{1}H NMR ($CDCl_3$):δ1.38 (t, 3H, SCH_2CH_3), 2.56 (q, 2H, SCH_2CH_3), 3.24 (bs, 2 O*H*), 5.36 (bs, 1H, H-1), 5.56 (s, 1H, PhC*H*), 7.32-7.62 (m, 5H, Ph).

Compound 3. Compound **2** (1.72 g, 5.51 mmoles) was added to a mixture of dry benzene (20 mL), triethyl orthoacetate (3 mL, 16.44 mmoles) and *p*-TsOH (50 mg), and the mixture stirred at room temperature for 2 hr. The reaction was quenched with Et_3N (1 mL) and the reaction mixture evaporated to dryness. The crude was dissolved in 80% aq. AcOH (50 mL) and kept at room temperature for 1 hr. The reaction mixture was evaporated to a syrup and column chromatographed using toluene-EtOAc (5:1)

as eluent to give **3** (1.62 g, 83%) which crystallised from ethanol, m.p. 110-11°, $[\alpha]_D^{24}$ + 116.7° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.31 (t, 3H, SCH_2CH_3), 2.18 (s, 3H, $COCH_3$), 2.66 (q, 2H, SCH_2CH_3), 5.31 (bs, 1H, H-1), 5.64 (s, 1H, PhC*H*), 7.33-7.52 (m, 5H, Ph).

Compound 4. A solution of **2** (2g, 6.41 mmoles) and dibutyltin oxide (1. 6 g, 6.42 mmoles) in dry methanol (70 mL) was refluxed for 1 hr and the reaction mixture concentrated under reduced pressure. The product was dissolved in *N*, *N*-dimethylformamide (50 mL), benzyl bromide (0.95 ml, 8.0 mmoles) added to it and the mixture stirred for 24 hr at 80°. The reaction mixture was diluted with CH_2Cl_2, washed with water, dried and concentrated. The crude residue was chromatographed using toluene-EtOAc (20:1) to give pure **4** (2.25g, 87%), $[\alpha]_D^{25}$ + 162°) *c* 0.7, $CHCl_3$) [Lit.[15] $[\alpha]_D$ + 195.1° ($CHCl_3$)]; ^1H NMR ($CDCl_3$) : δ 1.28 (t, 3H, SCH_2CH_3), 2.60 (q, 2H, SCH_2CH_3), 4.74 (dd, 2H, PhCH_2), 5.33 (bs, 1H, H-1), 5.37 (s, 1H, PhC*H*), 7.30-7.52 (m, 10H, 2Ph).

Compound 5. Compound **4** (3.0 g, 7.46 mmoles) was treated with acetic anhydride (10 mL) and pyridine (15 mL) at room temperature for 3 hr to give **5** (3.3 g; 99%), $[\alpha]_D^{25}$ + 79.5° (*c* 0.7, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 1.29 (t, 3H, SCH_2CH_3), 2.16 (s, 3H, $COCH_3$), 2.62 (q, 2H, SCH_2CH_3), 5.18 (bs, 1H, H-1), 5.56 (s, 1H, PhC*H*), 7.30-7.48 (m, 10H, 2Ph).

Compound 7. A mixture of 2, 3, 4, 6-tetra-*O*-acetyl-α-D-mannopyranosyl trichloroacetimidate[7] **6** (585.5 mg, 1.20 mmoles) compound **3** (388 mg, 1.09 mmol) and MS-4Å (1 g) in dichloromethane (6 mL) was cooled to −40°C and trimethylsilyl triflate (497.1 μl, 2.41 mmol) was added to it. After stirring for 4 hr at −40°C, pyridine (1 mL) was added and the mixture filtered through a Celite bed, concentrated and co-concentrated with toluene (3 × 15 mL), ethanol (2 × 10 mL) and dichloromethane (2 × 15 mL). A solution of the residue in dichloromethane (30 mL) was washed successively with water, aq. $NaHCO_3$ and water, dried and concentrated. The residue was chromatographed using toluene-ether (6:1) to give pure **7** (506.1 mg, 67%) which was crystallised from ethanol, m.p. 78-79°, $[\alpha]_D^{24}$ + 74° (*c* 1.8, $CHCl_3$); ^1H NMR ($CDCl_3$) : δ 1.30 (t, 3H, SCH_2CH_3), 1.95-2.26 (5s, 15H, $5COCH_3$), 2.64 (q, 2H, SCH_2CH_3), 5.18 (d, $J_{1,2}$ = 2 Hz, 1H, H-1), 5.26 (d, $J_{1',2'}$ = 1Hz, 1H, H-1'), 5.60(s, 1H, PhC*H*), 7.29-7.44 (m, 5H, Ph). Anal: Calcd for $C_{31}H_{33}O_{15}S$: C, 54.94; H, 4.90. Found: C, 54.78; H, 5.08%.

Compound 9. A solution of **8** (1.5 g, 5.32 mmoles) and dibutyltin oxide (1.4 g, 5.62 mmoles) in methanol (90 mL) was refluxed for 2 hr. The reaction mixture was concentrated and dried in vacuum. To a solution of the dried mass in *N*, *N*-dimethylformamide (50 mL) benzyl bromide (0.75 mL, 6.38 mmoles) was added and the mixture stirred at 80°C for 24 hr. The reaction mixture was diluted with dichloromethane and the organic layer washed with water, dried and concentrated to a yellow syrup. Column chromatography of the crude product using toluene-ether (8:1) gave pure **9** (1.65 g, 83.5%), $[\alpha]_D^{25}$ + 38.5° (*c* 0.9, $CHCl_3$); ^1H NMR ($CDCl_3$) : δ 3.30 (s, 3H, OCH_3), 4.74 (bs, 1H, H-1), 5.46 (s, 1H, PhC*H*), 7.23-7.48 (m, 10H, 2Ph). Anal: Calcd for $C_{21}H_{24}O_6$: C, 67.72; H, 6.49. Found: C, 67.61; H, 6.59%.

Compound 10. A mixture of ethyl 2-*O*-acetyl-3-*O*-benzyl-4, 6-*O*-benzylidene-1-thio-α-D-mannopyranoside **5** (573 mg, 1.29 mmoles), methyl 2-*O*-benzyl-4- 6-*O*-benzylidene-α-D-mannopyranoside **9** (400 mg, 1.07 mmoles) and MS-4Å (3 g) in dry toluene (18 mL) was stirred at 25°C for 1hr. Methyl triflate (0.72 mL, 6.45 mmoles) was then injected to the reaction mixture and stirring continued at toom temperature for 18 hr. The reaction was quenched with Et_3N (1 mL) and the reaction mixture filtered through a Celite bed and concentrated to a syrup. The crude syrup was chromatographed using toluene-ether (10:1) to give pure **10** (589 mg, 73%), $[\alpha]_D^{25}$ + 5.37° (*c* 2.1, $CHCl_3$); ^1H NMR ($CDCl_3$); δ 2.15 (s, 3H, $COCH_3$), 3.36 (s, 3H, OCH_3), 4.67-4.75 (2dd, 2 × PhCH_2), 4.85 (bs, 1H, H-1), 5.13 (d, $J_{1',2'}$ = 2 Hz, 1H, H-1'), 5.65 (s, 2H, 2 × PhC*H*), 7.25-7.53 (m, 20H, 4Ph). Anal: Calcd for $C_{43}H_{46}O_{12}$: C, 68.42; H, 6.14. Found: C, 68.29; H, 6.31%.

Compound 11. A solution of **10** (589 mg, 0.78 mmoles) in 0.05*M* sodium methoxide (5 mL) was stirred at room temperature for 3 hr. The reaction mixture was decationised by Dowex 50W-X8 (H^+) resin, filtered through a cotton plug and evaporated to dryness to give **11** almost in quantitative yield, $[\alpha]_D^{24}$; + 19.35° (*c* 1.2, $CHCl_3$); ^1H NMR ($CDCl_3$): δ 3.36 (s, 3H, OCH_3), 4.88 (bs, 1H, H-1), 5.17 (d, $J_{1',2'}$ = 2Hz, 1H, H-1'), 5.60, 5.62 (2s, 2H, 2 × PhC*H*), 7.29-7.52 (m, 20H, 4 × Ph).

Compound 12. A mixture of **7** (135.4 mg, 0.19 mmoles), **11** (107 mg, 0.15 mmoles) and MS-4Å (500 mg) in dry toluene (3 mL) was stirred at 25°C for 1hr. Methyl triflate (0.11 mL, 0.97 mmole) was then injected to the reaction mixture and stirring continued at room temperature for 18 hr. The reaction was quenched with Et_3N (0.5

mL), and the reaction mixture filtered through a Celite bed and concentrated to a syrup. Column chromatography of the syrupy product using toluene-ether (8:1) gave pure **12** (137 mg, 68%), $[\alpha]_D^{25}$ + 32.5° (*c* 1.02, $CHCl_3$); ^1H NMR ($CDCl_3$) : δ 1.91-2.11 (5s, 15H, $5COCH_3$), 3.33 (s, 3H, OCH_3), 4.93 (bs, 1H, H-1), 5.10 (d, $J_{1',2'}$ = 2Hz, 1H, H-1′), 5.18 (d, $J_{1'',2''}$ = 2Hz, 1H, H-1″), 5.25 (bs, 1H, H-1‴), 5.57, 5.61 and 5.66 (3s, 3H, 3 × PhC*H*), 7.22-7.51 (m, 25H, 5Ph). Anal: Calcd for $C_{70}H_{78}O_{26}$: C, 62.96; H, 5.88. Found: C, 62.71; H, 6.09%.

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