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Note

Synthesis of a tetrasaccharide analogue related to the repeating unit of the antigen from *Klebsiella* type 2

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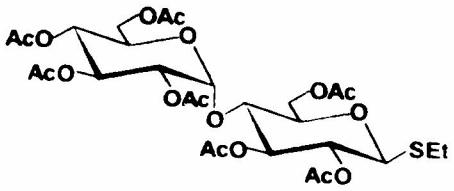
The disaccharide methyl 2,6-di-*O*-benzyl-3-*O*-(methyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-β-D-mannopyranoside **2** has been allowed to react with ethyl 2,3,6,2′,3′,4′,6′-hepta-*O*-acetyl-1-thio-β-D-maltoside **1** with methyl triflate as promoter. Removal of blocking groups from the product affords methyl 3-*O*-(methyl α-D-glucopyranosyluronate)-4-*O*-[4-*O*-(α-D-glucopyranosyl) -β -D- glucopyranosyl]-β-D-mannopyranoside **4** which is the tetrasaccharide analogue related to the repeating unit of the antigen from *Klebsiella* type 2. The structure of **4** has been confirmed by its ^{1}H and ^{13}C NMR spectra.

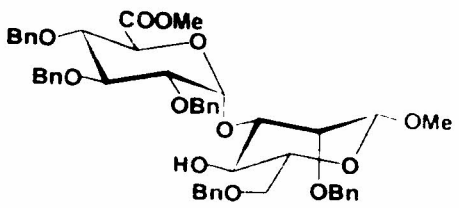
β-Mannopyranosidic linkages are frequently encountered in polysaccharides[1,2] and glycoconjugates[3,4] and they participate in a variety of biological functions. Synthesis of oligosaccharides involving β-D-mannopyranosides is therefore important for understanding the structure-function relationship of the biopolymers containing such residues. Recently, we have synthesised the tetrasaccharide repeating unit of the antigen from *Klebsiella* type 2[5] (K2). In that connection, it was also deemed interesting to synthesise a tetrasaccharide analogue related to the repeating unit of K2, where the two D-glucose moieties are α(1→4) linked instead of α(1→3) as found in the native antigen. Immunochemical studies (to be taken up in a separate project) including both K2 repeating unit and its tetrasaccharide analogue, whose synthesis is reported here, will provide useful information on the relation between structure and immunological specificity of the K2 antigen.

α-D-Glcp-(1→4)-β-D-Glcp-(1→4)-β-D-Manp-OMe
3
↓
1
α-D-Glcp A

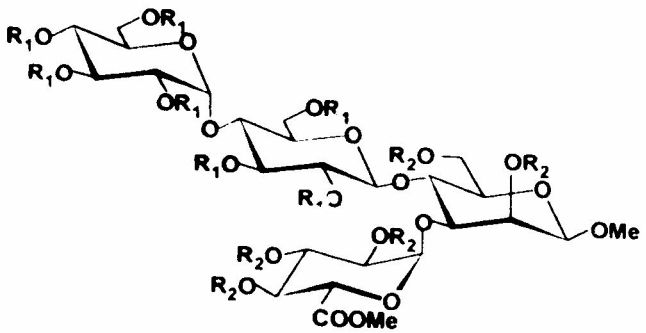
Analogue of K2



1



2



3. R_1 = Ac; R_2 = Bn
4. $R_1 = R_2$ = H

The strategy of block synthesis was utilised in the present report. Maltose octaacetate was treated with ethanethiol and borontrifluoride etherate in dichloromethane to give the thioglycoside **1**[6] in 94% yield. The thioglycoside **1** was allowed to react with the aceeptor methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-β-D-mannopyranoside **2**[5] in the presence of methyl triflate as promoter[7] to give the tetrasaccharide derivative methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glycopyranosyl)-β-D-glucopyranosyl]-β-D-mannopyranoside **3** in 73% yield. Zemplén deacetylation[8] of **3** followed by hydrogenolysis in the presence of 10% Pd/C gave the tetrasaccharide analogue methyl 3-*O*-(methyl α-D-glucopyranosyluronate)-4-*O*- [4-*O*-(α-D-glucopyranosyl)-β-D-glucopyranosyl]-β-D-mannopyranoside **4** in 60% yield. The ^{1}H NMR spectrum of **4** showed peaks for methoxyl (δ 3.54), methyl ester (δ 3.79), β-glucosidic (δ 4.39, *J*=7 Hz), β-mannosidic H-1 (δ 4.50), α-glucosidic H-1 (δ 5.14, *J*= 3.5 Hz) and α-glucouronosidic H-1 (δ 5.20, *J*=2.4 Hz). The ^{13}C NMR spectrum showed signals for 26 carbons which included 4 anomeric carbons at δ 100.07 (C-1‴), 100.26 (C-1′), 101.17 (C-1) and 102.58 (C-1″), one methoxyl and one methyl ester at δ 57.37 and one ester carbonyl at δ 173.0.

Experimental Section

General. All reactions were monitored by TLC on silica gel G (Merck). Column chromatography was performed using silica gel (SRL, 100-200 mesh). All solvents were distilled before use and all evaporations were conducted at 40° unless stated otherwise. Optical rotations were measured on a Perkin-Elmer model 241 MC polarimeter at 25°. ^{1}H and ^{13}C NMR spectra were recorded on a Jeol FX-100 and Bruker 200 spectrometer respectively in $CDCl_3$ (internal standard TMS or dioxane).

Methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-4-*O*-[2,3,6-tri-*O*-acetyl-4-*O*- (2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl) -β-D-glucopyranosyl] -β-D-mannopyranoside 3. A solution of **1** (79.6 mg, 0.12 mmole), **2** (48.8 mg, 0.06 mmole) and MS 4Å (500 mg) in dry toluene (4 mL) was stirred at room temperature for 1 hr. Methyl triflate (66 μL, 0.58 mmole) was then injected into the mixture and stirring continued for 20 hr at 25°C. The reaction was quenched with triethylamine (0.5 mL) and the reaction mixture filtered through a Celite bed. The filtrate was concentrated to a syrup. Column chromatography of the syrup using toluene-ether (2:1) gave pure **3** (62 mg, 73%), $[\alpha]_D$+38.5° (*c* 1.15, $CHCl_3$); ^{1}H NMR ($CDCl_3$): δ 2.0-2.12 (7s, 21H, 7 $COCH_3$), 3.48 (s, $J_{1'',2''}$7.5 Hz, 1H, H-1‴, 5.06 (d, $J_{1',2'}$ =2 Hz, 1H, H-1′), 7.34 (m, 25H, 5Ph). Anal: Calcd for $C_{47}H_{88}O_{29}$: C, 50.52; H, 7.94. Found: C, 50.46: H, 8.16%.

Methyl 3-*O*-(methyl α-D-glucopyranosyluronate) -4-*O*-[4-*O*-(α-D-glucopyranosyl) -β-D-glucopyranosyl] -β-D-mannopyranoside 4. A solution of **3** (60 mg, 0.05 mmole) in acetic acid (10 mL) was stirred with Pd-C (150 mg) under hydrogen for 24 hr at room temperature. The reaction mixture was filtered through a Celite bed and evaporated to dryness. Traces of acetic acid and water were removed by repeated coevaporation with toluene. The dried mass was dissolved in 0.05 *M* sodium methoxide (5 mL) and stirred at room temperature for 4 hr. The reaction mixture was neutralised with Dowex 50W-X8 (H^+) resin, filtered and evaporated to a glassy material (23 mg, 60%), $[\alpha]_D$+70° (*c*, 2.4, H_2O); ^{1}H NMR (D_2O): δ 3.54 (s, 3H, OCH_3), 3.79 (s, 3H, $COOCH_3$), 4.39 (d, $J_{1''',2'''}$=7 Hz, 1H, H-1‴), 4.50 (bs, 1H, H-1), 5.14 (d, $J_{1'',2''}$=3.4 Hz, 1H, H-1‴), 5.20 (d, $J_{1',2'}$=3.5 Hz, 1H, H-1′); ^{13}C NMR (D_2O, 1,4-dioxane as internal standard): δ 57.37 (OCH_3 and $COOCH_3$), 60.96, 61.38, 61.58 (C-6, C-6‴ and C-6‴), 69.73, 69.80, 71.72, 72.09, 72.20, 73.16 (2 carbons), 73.26, 73.32, 73.74, 73.69, 76.29, 77.76, 78.37, 79.30, 81.42, 100.07 (C-1‴), 100.26 (C-1′), 101.17 (C-1), 102.58 (C-1‴) and 173.0 ($COOCH_3$). Anal: Calcd for $C_{26}H_{44}O_{22}$: C, 44.07; H, 6.26. Found: C, 43.85; H, 6.40%.

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