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Synthesis of the tetrasaccharide repeating unit of the antigen from *Klebsiella* type 2

Anup Kumar Misra, Nirmolendu Roy *

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

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Abstract

The disaccharide ethyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-1-thio-β-D-glucopyranoside (**6**) and methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyluronate)-β-D-mannopyranoside (**21**) have been synthesized and condensed in the presence of methyl triflate to afford a tetrasaccharide derivative. Removal of protecting groups gave methyl 3-*O*-(methyl α-D-glucopyranosyluronate)-4-*O*-(3-*O*-α-D-glucopyranosyl-β-D-glucopyranosyl)-β-D-mannopyranoside (**23**), the repeating unit of the antigen from *Klebsiella* type 2, in the form of its methyl ester methyl glycoside.

Keywords: Oligosaccharide, synthesis; Tetrasaccharide repeating unit; *Klebsiella* type 2

1. Introduction

Klebsiella aerogenes are non-motile gram-negative enterobacteria which cause a variety of specific infections in man and animal giving rise to occasional cases of acute broncho-pneumoniae [1]. The genus *Klebsiella* was classified by Ørskov [2] into approximately 80 serotypes based on their antigenic capsular polysaccharides. Primary structures of the antigens from almost all these serotypes have been established.

The repeating unit of the capsular polysaccharide from *Klebsiella* type 2 [3] has the structure **I**.

* Corresponding author.

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$$\rightarrow 4)\text{-}\alpha\text{-D-Glc}p\text{-}(1 \rightarrow 3)\text{-}\beta\text{-D-Glc}p\text{-}(1 \rightarrow 4)\text{-}\beta\text{-D-Man}p\text{-}(1 \rightarrow$$
$$\begin{array}{l} 3 \\ \uparrow \\ 1 \\ \alpha\text{-D-Glc}p\text{A} \end{array}$$

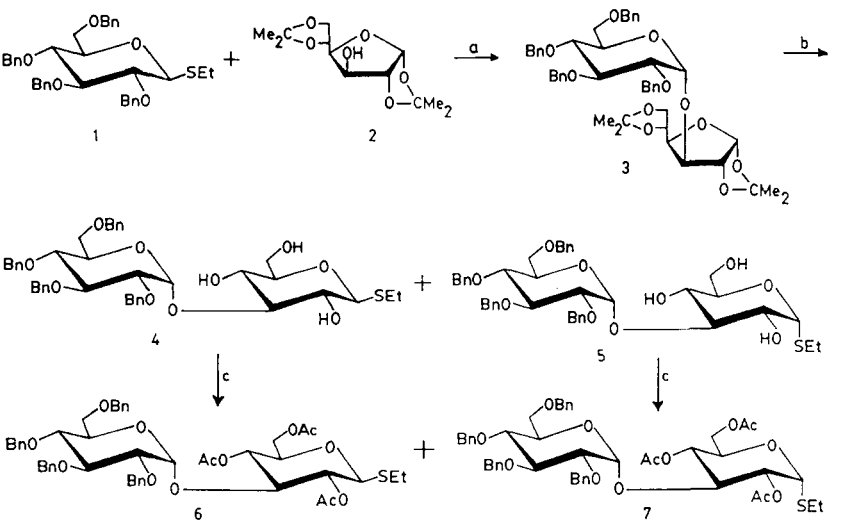
I

As a part of our programme aimed to determine the structure and immunological specificity [4,5] relationship of carbohydrate moieties, it was necessary to synthesize the tetrasaccharide repeating unit of the antigen from *Klebsiella* type 2 together with some related disaccharides. Their synthesis is the subject of this communication. Our strategy was to synthesize two disaccharide blocks and then to condense them to obtain the tetrasaccharide repeating unit I.

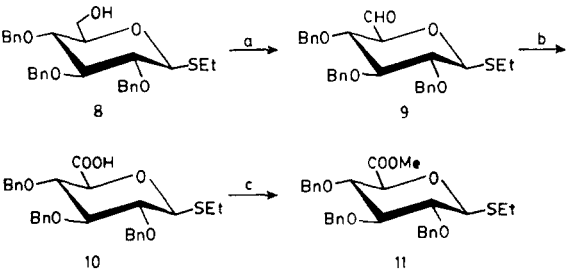
2. Results and discussion

The thioglycoside **1** [6] was allowed to react with **2** [7] using copper(II) bromide and tetrabutylammonium bromide as promoter [8] to give the crystalline disaccharide derivative **3** in 64% yield. Removal of isopropylidene groups from **3** and formation of the corresponding ethyl 1-thioglycopyranoside was achieved in one step by treatment of **3** with boron trifluoride etherate in ethanethiol solution, giving the isomeric β- and α-thioglycoside **4** and **5** in 44% and 21% yield, respectively. Acetylation [9] of the thioglycosides gave the corresponding acetates **6** and **7**, respectively. The donor **6** was crystallised from ethanol but **7** could not be crystallised (Scheme 1).

The D-glucopyranosyluronic acid part was prepared from D-glucose. The primary hydroxyl group of the thioglycoside **8** [10] was oxidised [11] with dimethyl sulfoxide–



Scheme 1. (a) $CuBr_2$, Bu_4NBr, $(CH_2Cl)_2$, DMF; (b) $BF_3 \cdot OEt_2$, EtSH; (c) Ac_2O, pyridine.

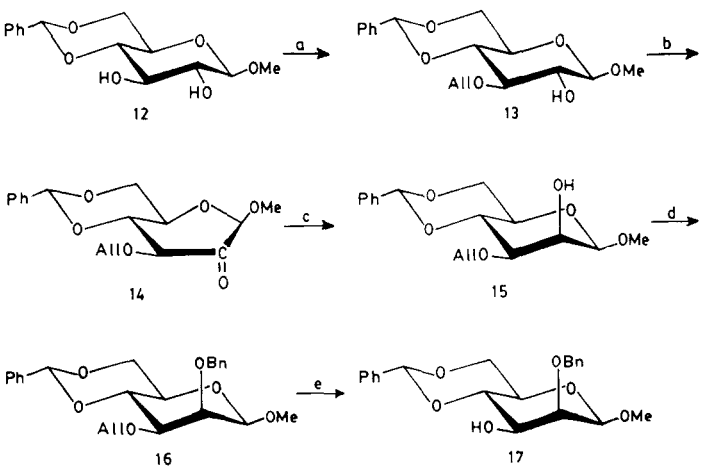


Scheme 2. (a) 2:1 Me_2SO–Ac_2O; (b) $NaClO_2$, NaH_2PO_4, *t*-BuOH, 2-methyl–2-butene; (c) CH_2N_2, Et_2O.

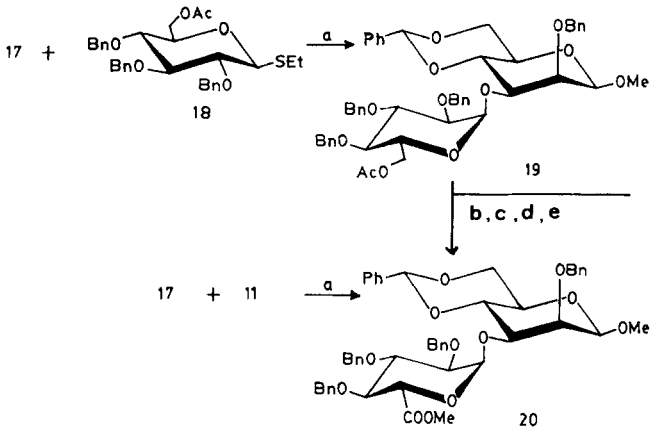
acetic anhydride and the resulting aldehyde **9** further oxidised with sodium chlorite giving the acid **10**. Esterification gave methyl (ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranosid)uronate (**11**) as crystals in 56% yield (Scheme 2).

The β-mannopyranosidic moiety was synthesised from methyl β-D-glucopyranoside. Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside [12] (**12**) was regioselectively allylated [13,14] with allyl bromide and copper(II) chloride in tetrahydrofuran to give the 3-*O*-allyl derivative (**13**) as crystals in 53% yield. Oxidation [15] of **13** with dimethyl sulfoxide–acetic anhydride followed by reduction of the resulting ketone **14** with sodium borohydride gave methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-D-mannopyranoside (**15**) as crystals in 62% yield. The *gluco* isomer was obtained in 15% yield. Benzylation [16] of **15** followed by deallylation [17] of **16** gave the mannose acceptor **17** in crystalline form (Scheme 3).

The thioglycoside **18** [10] was condensed [18] with **17** using methyl triflate as the promoter to afford the crystalline disaccharide derivative **19** in 72% yield. Zemplén deacetylation of **19** gave the corresponding 6′-hydroxy derivative. The primary hydroxyl



Scheme 3. (a) Allyl bromide, $CuCl_2$, NaH, *t*-Bu_4NI; (b) 2:1 Me_2SO–Ac_2O; (c) $NaBH_4$; (d) BnBr, NaH; (e) $PdCl_2$, 20:1 AcOH–H_2O, NaOAc.



Scheme 4. (a) MeOTf, Et_2O, MS 4 Å; (b) NaOMe; (c) 2:1 Me_2SO–Ac_2O; (d) $NaClO_2$, NaH_2PO_4, *t*-BuOH, 2-methyl–2-butene; (e) CH_2N_2.

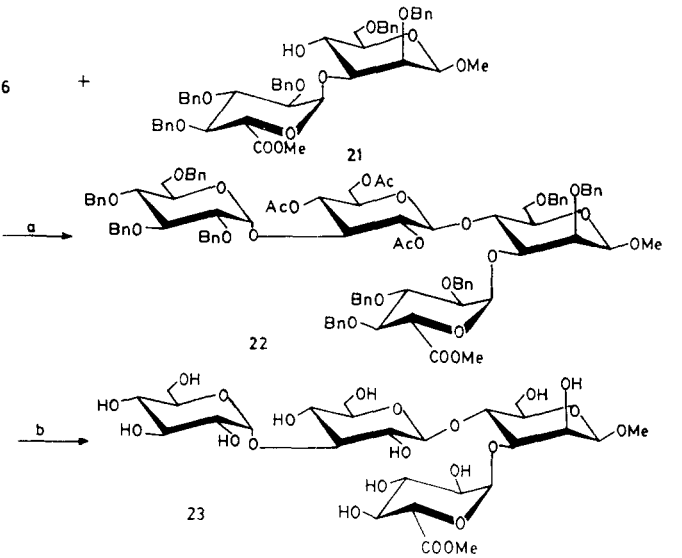
group of **19** was oxidised [11] as described for **8** and the resulting acid esterified with diazomethane to give the disaccharide **20** as crystals in 66% yield. Compound **20** was also prepared with the glucopyranosyluronic acid derivative **11** as donor. In this alternative route, **17** was allowed to react [18] with **11** using methyl triflate as promoter to obtain **20** in 53% yield. Regioselective ring opening [19] of the benzylidene acetal of **20** gave methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl-uronate)-β-D-mannopyranoside (**21**) in 51% yield together with a small amount (~ 5%) of 6-hydroxy derivative and ~ 20% of 4,6-dihydroxy derivative (Scheme 4).

Condensation [18] of **6** with **21** using methyl triflate as promoter gave the tetrasaccharide derivative **22** in 71% yield. Hydrogenolysis of **22** with H_2/Pd-C followed by Zemplén [20] deacetylation of the product afforded methyl 3-*O*-(methyl α-D-glucopyranosyluronate)-4-*O*-(3-*O*-α-D-glucopyranosyl-β-D-glucopyranosyl)-β-D-mannopyranoside (**23**) in 58% yield. The ^1H and ^{13}C NMR spectra of **23** showed the presence of methyl ester and acetal signals together with the presence of one β-glucosidic, one α-glucosidic, one β-mannosidic and one α-glucosiduronic linkage (Scheme 5).

3. Experimental

General.—All reactions were monitored by TLC on Silica Gel G (E. Merck, India). Column chromatography was performed using silica gel (SRL, India), and all concentrations were conducted below 50°C unless stated otherwise. Optical rotations were measured at 23°C with a Perkin–Elmer 241 MC polarimeter. The ^1H and ^{13}C NMR spectra were recorded with a Jeol FX-100 or a Varian Gemini-200 instrument using $CDCl_3$ as solvent and Me_4Si as internal standard unless stated otherwise. Melting points were determined on a paraffin oil bath and reported uncorrected.

1,2:5,6-Di-O-isopropylidene-3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-α-D-glucofuranose (**3**).—To a flask containing $CuBr_2$ (3.10 g, 13.9 mmol), Bu_4NBr (0.50 g,



Scheme 5. (a) MeOTf, toluene, MS 4 Å; (b) Pd–C/H_2; NaOMe.

1.6 mmol) and molecular sieves 4 Å (15.0 g) was added a solution of 2,3,4,6-tetra-*O*-benzyl-1-thio-β-D-glucopyranoside [6] (**1**; 5.38 g, 9.2 mmol) and 1,2:5,6-di-*O*-isopropylidene-α-D-glucofuranose [7] (**2**; 2.0 g, 7.7 mmol) in 5:1 1,2-dichloroethane–DMF (154 mL) and the mixture was stirred vigorously under argon at 24°C for 72 h. 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 1:5 Et_2O–petroleum ether (60–80°C) giving **3** (3.85 g, 64%); mp 89–90°C (EtOH); $[\alpha]_D$ +45.7° (*c* 2.7, $CHCl_3$); Lit. [21] mp 90–91°C, $[\alpha]_D$ +46° ($CHCl_3$). ^{1}H NMR: δ 1.22 (2s, 6 H, 5,6-Me_2C), 1.42 (2s, 6 H, 1,2-Me_2C), 4.72 (d, $J_{1',2'}$ 2 Hz, H-1'), 5.24 (d, *J* 4 Hz, H-2), 5.88 (d, $J_{1,2}$ 4 Hz, H-1), 7.0–7.5 (m, 20 H, 4 Ph).

Ethyl 2,4,6-tri-O-acetyl-3-O-(2,3,4,6-tetra-O-benzyl-α-D-glucopyranosyl)-1-thio-β- and α-D-glucopyranoside (**6** *and* **7**).—To a mixture of **3** (3.0 g, 3.8 mmol) and EtSH (0.6 mL, 8.1 mmol) in CH_2Cl_2 (100 mL), $BF_3 \cdot Et_2O$ (0.9 mL, 7.3 mmol) was added and the mixture was stirred at 0–5°C for 4 h. The reaction mixture was then washed successively with water, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated. The crude product was chromatographed with 5:1 toluene–Et_2O giving **4** (1.25 g, 43.8%) having $[\alpha]_D$ +29.5° (*c* 0.7, $CHCl_3$) and **5** (587 mg, 20.6%) having $[\alpha]_D$ +122.5° (*c* 2.6, $CHCl_3$). ^{13}C NMR ($CDCl_3$) of **4**: δ 14.9 (SCH_2CH_3), 24.2 (SCH_2CH_3), 62.4 (C-6), 68.3 (C-6'), 69.7, 71.2, 74.0, 74.8, 75.5, 77.7, 79.1, 79.3, 82.0, 85.2, 90.6 (C-1), 99.5 (C-1') and 127.0–138.3 (aromatic carbons). ^{13}C NMR of **5**: δ 14.6 (SCH_2CH_3), 24.3 (SCH_2CH_3), 62.3 (C-6), 68.4 (C-6'), 69.7, 70.4, 71.2, 73.4, 74.0, 74.9, 75.5, 77.8, 79.3, 82.1, 85.1, 87.7 (C-1), 99.3 (C-1') and 127.0–138.3 (aromatic carbons).

In separate experiments, **4** (0.9 g) and **5** (0.5 g) were acetylated conventionally [9] with Ac_2O and pyridine at room temperature to give, in quantitative yield, the acetates **6**

and **7**, respectively, which were purified by column chromatography with 2:1 toluene–Et_2O. Compound **6** was crystallised from EtOH; mp 136°C; $[\alpha]_D$ +16.6° (*c* 1.6, $CHCl_3$). ^1H NMR: δ 1.24 (t, 3 H, SCH_2CH_3), 1.8–2.06 (3s, 9 H, 3 OAc), 2.70 (q, 2 H, SCH_2CH_3), 4.32 (d, $J_{1,2}$ 7.5 Hz, H-1), 4.72 (d, $J_{1',2'}$ 0.5 Hz, H-1'), 7.00–7.52 (m, 20 H, 4 Ph). Anal. Calcd for $C_{48}H_{56}O_{13}S$: C, 66.03; H, 6.46. Found: C, 65.76; H, 6.69.

*Methyl (ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-*D*-glucopyranosid)uronate* (**11**).—To a solution of ethyl 2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside [10] (**8**; 1.18 g, 2.4 mmol) in Me_2SO (11 mL) was added 1:1 Ac_2O–Me_2SO (22 mL) and the mixture was stirred at room temperature for 16 h. The reagents were removed by evaporation under reduced pressure and the solid mass of crude aldehyde **9** was dissolved in a mixture of *tert*-butanol (90 mL) and 2-methyl-2-butene (47 mL). A solution of $NaClO_2$ (6.1 g) and NaH_2PO_4 (6.1 g) in water (61 mL) was then added and the mixture was stirred for 16 h. The reaction mixture was concentrated under reduced pressure, diluted with EtOAc (100 mL), washed with water (2 × 50 mL), dried (Na_2SO_4) and concentrated to give the acid **10**. A solution of this material in Et_2O (30 mL) was treated with excess of ethereal CH_2N_2, then concentrated. Column chromatography of the residue with 6:1 toluene–EtOAc gave pure **11** (0.69 g, 56%), which crystallised from EtOH; mp 124–125°C, $[\alpha]_D$ −1.8° (*c* 2.4, $CHCl_3$). ^1H NMR: δ 1.30 (t, 3 H, SCH_2CH_3), 2.86 (q, 2 H, SCH_2CH_3), 3.70 (s, 3 H, COOMe), 4.66 (d, $J_{1,2}$ 7 Hz, H-1), 7.32 (m, 15 H, 3 Ph). ^{13}C NMR: δ 7 (CH_3CH_2S), 42.2 (CH_3CH_2S), 52.4 ($COOCH_3$), 74.1, 74.5, 75.0, 75.7, 77.2, 78.2, 83.7, 92.0 (C-1), 127.6–137.6 (aromatic carbons), 168.7 ($COOCH_3$). Anal. Calcd for $C_{30}H_{34}O_6S$: C, 68.94; H, 6.56. Found: C, 68.75; H, 6.79.

*Methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside* (**13**).—To a solution of methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**12**; 1.70 g, 6.0 mmol) in dry THF (80 mL) was added NaH (50% oil coated, 580 mg, 12 mmol) with stirring. When the evolution of H_2 ceased, $CuCl_2$ (810 mg, 6.0 mmol) was added, while stirring was continued for 15 min. Allyl bromide (0.5 mL, 6.0 mmol) and Bu_4NI (500 mg) were then added and the mixture was boiled under reflux for 24 h. The reaction mixture was cooled, treated with dilute NH_4OH and concentrated to dryness. The residue was dissolved in EtOAc and washed successively with dilute NH_4OH and water, dried (Na_2SO_4) and evaporated to dryness. Column chromatography of the residue with 8:1 toluene–EtOAc gave pure **13** (1.02 g, 53%); mp 147°C; $[\alpha]_D$ −41.46° (*c* 1.28, $CHCl_3$). ^1H NMR: δ 3.36 (s, 3 H, OMe), 5.65 (s, Ph–C*H*), 5.50 (s, 2 H, CH_2=CH–CH_2), 5.26 (d, 2 H, CH_2=CH–CH_2), 6.1 (m, CH_2=C*H*), 6.9 (m, 5 H, Ph), 4.4 (d, $J_{1,2}$ 8 Hz, H-1). Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.33; H, 6.88. Found: C, 63.12, H, 7.09.

*Methyl 3-*O*-allyl-4,6-*O*-benzylidene-β-*D*-mannopyranoside* (**15**).—To a solution of **13** (1.68 g, 5.2 mmol) in Me_2SO (9 mL) was added 1:2 Ac_2O–Me_2SO (18 mL) and the mixture was stirred for 16 h at room temperature. The solvents were removed by evaporation under reduced pressure yielding the keto compound **14** (1.64 g) as a solid mass. To a solution of this product in 1:1 CH_2Cl_2–MeOH (122 mL), $NaBH_4$ (9.5 g) was added and the mixture was stirred at 5–10°C for 5 h. The reaction mixture was concentrated in vacuo and diluted with CH_2Cl_2 (150 mL). The organic layer was washed successively with 5% citric acid solution, aq $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated to a glassy mass. Column chromatography using 8:1 toluene–Et_2O gave pure **15** (1.03 g, 62%) and its *gluco* isomer (15%). Compound **15**

had mp 146°C and $[\alpha]_D$ −62.1° (*c* 1.1, $CHCl_3$). ^1H NMR: δ 3.60 (s, 3 H, OCH_3), 4.48 (d, $J_{1,2}$ 1 Hz, H-1), 5.6 (s, Ph–C*H*), 5.96 (m, CH_2=C*H*), 7.44 (m, 5 H, Ph). Anal. Calcd for $C_{17}H_{22}O_6$: C, 63.34; H, 6.88. Found: C, 63.12; H, 6.95.

Methyl 3-O-allyl-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (**16**).—Compound **15** (1.63 g, 5.05 mmol) was benzylated conventionally [16]. The product crystallised from EtOH to give **16** (2.04 g, 98%); mp 63–64°C; $[\alpha]_D$ −85.5° (*c* 1.9, $CHCl_3$). ^1H NMR: δ 3.56 (s, 3 H, OMe), 4.42 (s, $J_{1,2}$ 1 Hz, H-1), 4.92 (dd, 2 H, Ph–CH_2), 5.62 (s, Ph–C*H*), 5.94 (m, 1 H, CH_2=C*H*), 7.48 (m, 10 H, 2 Ph). Anal. Calcd for $C_{24}H_{28}O_6$: C, 69.88; H, 6.84. Found: C, 69.68; H, 7.02.

Methyl 2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (**17**).—A mixture of **16** (560 mg, 1.36 mmol), $PdCl_2$ (172 mg, 0.97 mmol), and NaOAc (725 mg, 5.56 mmol) in 20:1 AcOH–H_2O (13.5 mL) was stirred at 0–5°C for 18 h. The reaction mixture was filtered through Celite and concentrated to dryness. The product was dissolved in CH_2Cl_2 and washed successively with water, aq $NaHCO_3$ and water, dried (Na_2SO_4), and concentrated to a syrup. Column chromatography with 10:1 toluene–Et_2O gave pure **17** (393 mg,78%); mp 150–151°C (EtOH); $[\alpha]_D$ −131.6° (*c* 0.9, $CHCl_3$). ^1H NMR: δ 3.58 (s, 3 H, OMe), 4.52 (s, $J_{1,2}$ 1 Hz, H-1), 4.90 (dd, 2 H, Ph–CH_2), 5.56 (s, 1 H, PH–C*H*), 7.38 (m, 10 H, 2 Ph). Anal. Calcd for $C_{21}H_{24}O_6$: C, 57.73; H, 6.49. Found: C, 67.60; H, 6.57.

Methyl 3-O-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-2-O-benzyl-4,6-O-benzylidene-β-D-mannopyranoside (**19**).—To a solution of **17** (302 mg, 0.81 mmol) and ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**18**; 783 mg, 1.46 mmol) in Et_2O (23 mL), MS 4 Å (2.5 g) was added and the mixture was stirred under argon for 1 h at room temparature. Methyl triflate (0.82 mL, 7.3 mmol) was then added and stirring was continued at 25°C for 24 h. The reaction was then quenched with Et_3N, the mixture was stirred for 1 h and filtered through Celite. The filtrate was concentrated and the syrupy product was chromatographed with 15:1 toluene–Et_2O to afford pure **19** (0.5 g, 72%); mp 116–117°C (EtOH); $[\alpha]_D$ +20.3° (*c* 0.9, $CHCl_3$). ^1H NMR: δ 1.76 (s, 3 H, OAc), 3.37 (s, 3 H, OMe), 4.50 (d, $J_{1,2}$ 2 Hz, H-1), 5.27 (s, 1 H, PhC*H*), 5.36 (d, $J_{1',2'}$ 7 Hz, H-1'), 7.15 (m, 25 H, 4 Ph). Anal. Calcd for $C_{50}H_{54}O_{12}$: C, 70.90; H, 6.43. Found: C, 70.72; H, 6.57.

Methyl 2-O-benzyl-4,6-O-benzylidene-3-O-(methyl 2,3,4-tri-O-benzyl-α-D-glucopyranosyluronate)-β-D-mannopyranoside (**20**).—The disaccharide derivative **19** (0.5 g, 0.59 mmol) was deacetylated according to Zemplén [20] to give methyl 2-*O*-benzyl-4,6-*O*-benzylidene-3-*O*-(2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-β-D-mannopyranoside (0.45 g, 94.7%), which was oxidised to the carboxylic acid and subsequently transformed into its methyl ester as described for the preparation of **11** to afford **20**. The crude product was purified by column chromatography with 10:1 toluene–Et_2O to give pure **20** (324 mg, 66%), which crystallised from EtOH; mp 117–118°C; $[\alpha]_D$ +6.4° (*c* 0.8, $CHCl_3$). ^1H NMR: δ 3.58 (s, 3 H, OMe), 3.68 (s, 3 H, COOMe), 4.72 (d, $J_{1,2}$ 1.5 Hz, H-1), 5.4 (s, PhC*H*), 5.58 (d, $J_{1',2'}$ 3 Hz, H-1'), 7.24 (m, 25 H, 5 Ph). ^{13}C NMR: δ 52.40 (COO*C*H_3), 57.3 (O*C*H_3), 68.6 (C-6), 70.8, 74.9, 75.1, 75.57, 75.63, 76.4, 77.6, 78.2, 78.3, 79.0, 79.2, 80.5, 97.2 (C-1'), 102.2 (C-1), 102.96 (Ph–*C*H), 126.3–138.4 (aromatic carbons), 170.0 (*C*OOCH$_3$). Anal. Calcd for $C_{49}H_{52}O_{12}$: C, 70.65; H, 6.29. Found: C, 70.47; H, 6.50.

In a separate series of experiments, **20** was prepared following a different route. To a solution of **17** (130.1 mg, 0.35 mmol) in Et_2O were added **11** (365.4 mg, 0.69 mmol) and MS 4 Å (1.5 g) and the mixture was stirred under argon for 1 h at room temperature. Methyl triflate (0.39 mL, 3.4 mmol) was then added and stirring was continued at 22°C for 16 h. The reaction was then quenched with Et_3N, and after 1 h the mixture was filtered through Celite and concentrated to a syrup. Chromatography of the crude product with 10:1 toluene–Et_2O gave pure **20** (154.3 mg, 53%) which was crystallised from EtOH; mp 117–118°C; $[\alpha]_D$ +6.4° (*c* 0.8, $CHCl_3$). The ^1H NMR and ^{13}C NMR of this product was identical to the one previously recorded. Anal. Calcd for $C_{49}H_{52}O_{12}$: C, 70.65; H, 6.29. Found: C, 70.43; H, 6.52.

*Methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyluronate)-β-*D*-mannopyranoside* (**21**).—To a solution of **20** (231 mg, 0.27 mmol) in dry THF (5 mL) were added MS 3 Å (2 g) and $NaCNBH_3$ (157 mg, 2.49 mmol) at 0°C. A saturated solution of HCl in Et_2O (2 mL) was added dropwise with vigorous stirring till pH came down to about 2–3. The suspension was stirred at 0°C for a further 45 min, filtered through Celite and washed with CH_2Cl_2. The combined filtrate and washing was washed with aq $NaHCO_3$ and water, dried (Na_2SO_4) and concentrated. Column chromatography of the syrupy product with 5:1 toluene–Et_2O gave pure **21** (118 mg, 51%); $[\alpha]_D$ −14.7° (*c* 0.3, $CHCl_3$). ^1H NMR: δ 3.55 (s, 3 H, OMe), 3.64 (s, 3 H, COOMe), 4.40 (d, $J_{1,2}$ 1.5 Hz, H-1), 5.1 (d, $J_{1',2'}$ 3.5 Hz, H-1'), 7.31 (m, 25 H, 5 Ph). Anal. Calcd for $C_{49}H_{54}O_{12}$: C, 70.48; H, 6.52. Found: C, 70.20; H, 6.76. About 5% of the 6-hydroxy compound and 20% of the 4,6-di-hydroxy compound were also isolated.

*Methyl 2,6-di-*O*-benzyl-3-*O*-(methyl 2,3,4-tri-*O*-benzyl-α-*D*-glucopyranosyluronate)-4-*O*-[2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-α-*D*-glucopyranosyl)-β-*D*-glucopyranosyl]-β-*D*-mannopyranoside* (**22**).—A mixture of **6** (314 mg, 0.36 mmol), **21** (167 mg, 0.2 mmol) and MS-4 Å (1 g) in toluene (10.5 mL) was stirred under argon for 1 h at 25°C. Methyl triflate (0.2 mL, 1.8 mmol) was then added and stirring was continued for 20 h. The reaction was then quenched with Et_3N, stirred for 1 h, filtered through Celite and washed with toluene. The filtrate and washings were concentrated to a syrup. Column chromatography of the product with 5:1 toluene–Et_2O gave pure **22** (234 mg, 71%); $[\alpha]_D$ +6° (*c* 3.4, $CHCl_3$). ^1H NMR: δ 1.76–1.92 (3s, 9 H, 3OAc), 3.46 (s, 3 H, OMe), 3.54 (s, 3 H, COOMe), 4.44 (bs, H-1), 4.74 (d, $J_{1''',2'''}$ 2 Hz, H-1'''), 4.50 (d, $J_{1'',2''}$ 7 Hz, H-1''), 5.08 (d, $J_{1',2'}$ 2.5 Hz, H-1'), 7.28 (m, 40 H, 8 Ph). Anal. Calcd for $C_{95}H_{104}O_{25}$: C, 69.33; H, 6.37. Found: C, 69.07; H, 6.59.

*Methyl 3-*O*-(methyl α-*D*-glucopyranosyluronate)-4-*O*-(3-*O*-α-*D*-glucopyranosyl-β-*D*-glucopyranosyl)-β-*D*-mannopyranoside* (**23**).—Compound **22** (218.3 mg, 0.13 mmol) and 10% Pd–C (300 mg) in acetic acid (15 mL) were stirred under hydrogen for 18 h at 25°C. The mixture was filtered through Celite and concentrated. The glassy product was treated with methanolic 0.05 M NaOMe (15 mL) for 5 h at room temperature. The solution was demineralised with Dowex-50W X8 (H^+) resin, filtered and concentrated to dryness. The product was chromatographed with 10:5:1 $CHCl_3$–MeOH–H_2O and then filtered through a 0.45 μm Millipore membrane to afford pure **23** (55 mg, 58%); $[\alpha]_D$ +50.4° (*c* 0.6, H_2O). ^1H NMR: δ 3.53 (s, 3 H, OMe), 3.78 (s, 3 H, COOMe), 4.54 (d, $J_{1'',2''}$ 7.5 Hz, H-1''), 4.60 (bs, H-1), 5.15 (d, $J_{1''',2'''}$ 3.8 Hz, H-1'''), 5.30 (d, $J_{1',2'}$ 3.9 Hz, H-1'). ^{13}C NMR: δ 57.72 (OCH_3 and $COOCH_3$), 60.84, 61.04, 61.84 (C-6,

C-6″, C-6‴), 70.14, 71.22 (2-carbons), 72.6, 72.85 (2-carbons), 73.14, 73.23 (2-carbons), 73.75, 74.38, 76.16, 77.15, 81.82, 83.12, 100.14 (C-1‴), 101.65 (C-1′), 102.32 (C-1), 103.28 (C-1″), 175 ($COOCH_3$). Anal. Calcd for $C_{26}H_{44}O_{22}$: C, 44.07; H, 6.26. Found: C, 43.89; H, 6.45.

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