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Carbohydrate Research 271 (1995) 177–183

CARBOHYDRATE RESEARCH

Synthesis of the tetrasaccharide repeating unit of the antigen from *Klebsiella* type 20

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Received 1 September 1994; accepted 16 January 1995

Abstract

Starting from D-mannose, D-galactose and D-glucuronolactone, two disaccharide blocks, namely methyl 4,6-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside, acting as acceptor, and ethyl 4,6-di-*O*-acetyl-2-*O*-allyl-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-1-thio-β-D-galactopyranoside, acting as donor, were synthesised. The two disaccharides were then allowed to react to give, after deprotection, methyl 2-*O*-β-D-galactopyranosyl-3-*O*-(3-*O*-β-D-glucopyranosyluronic acid-α-D-galactopyranosyl)-α-D-mannopyranoside which is the methyl glycoside of the tetrasaccharide repeating unit of the said antigen.

Keywords: Synthesis; Tetrasaccharide repeating unit; *Klebsiella* type 20

1. Introduction

The structure of the tetrasaccharide repeating unit of the capsular polysaccharide from *Klebsiella* type 20 was established by Choy and Dutton [1] and this structure is identical with the structure of the K-antigen from *E. coli* type 30 [2]. As a part of our programme to determine the relation between the structure and the immunological specificity of the carbohydrate moieties, it was necessary to synthesise the repeating unit of the specific antigen which is now reported.

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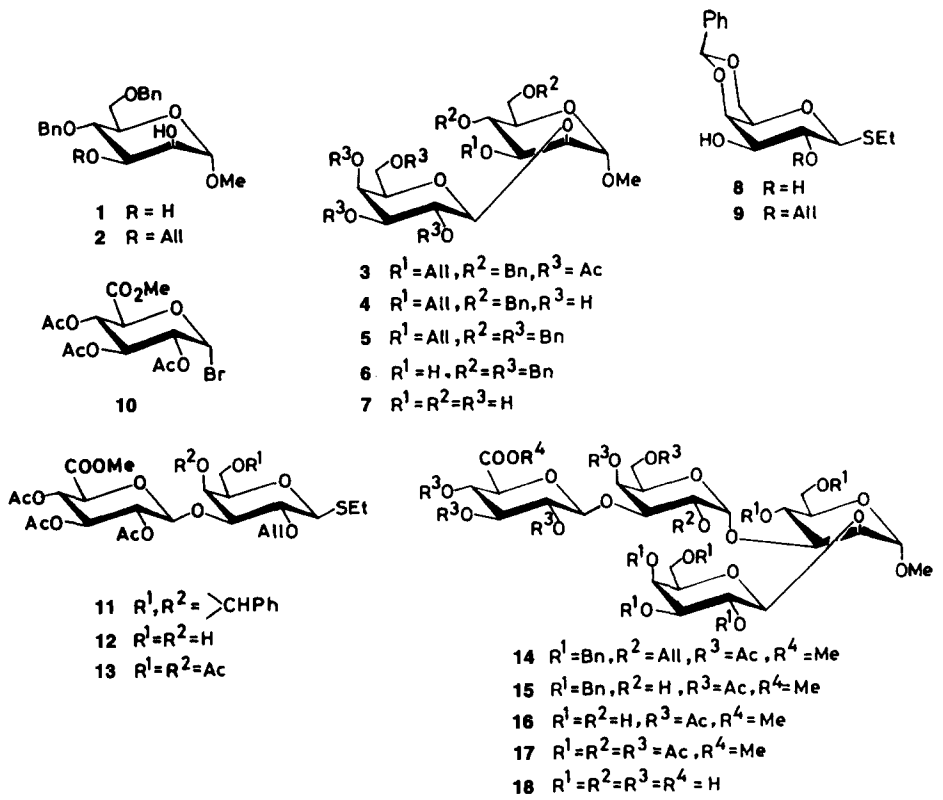
SSDI 0008-6215(95)00064-X

2. Results and discussion

Methyl 4,6-di-*O*-benzyl-α-D-mannopyranoside [3] (**1**) was selectively allylated at C-3 via a 2,3-stannylidene complex [4] to give the 3-*O*-allyl derivative (**2**). Compound **2** was allowed to condense with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide [5] in the presence of mercury (II) cyanide [6] in acetonitrile to give methyl 2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-3-*O*-allyl-4,6-di-*O*-benzyl-α-D-mannopyranoside (**3**). This compound could not be purified by column chromatography. The mixture was therefore deacetylated [7] and the product was then purified by chromatography to give methyl 3-*O*-allyl-4,6-di-*O*-benzyl-2-*O*-β-D-galactopyranosyl-α-D-mannopyranoside (**4**) in good yield. A portion of **4** was reacetylated to obtain pure **3** which was characterised by its specific rotation, ^{1}H NMR spectrum and elemental analysis. The remaining portion of **4** was benzylated using benzyl bromide, and sodium hydride in *N*,*N*-dimethylformamide [8] to give the hexa-*O*-benzyl derivative **5** which, on deallylation [9] with palladium (II) chloride in methanol, gave the disaccharide acceptor, methyl 4,6-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (**6**). A portion of **6** was hydrogenolysed to afford methyl 2-*O*-β-D-galactopyranosyl-α-D-mannopyranoside (**7**). The ^{13}C NMR spectrum of the compound exhibited signals for 13 carbons. The signals at δ 99.31 (C-1) and 102.97 (C-1′) proved the presence of α-mannosidic and β-galactosidic anomeric carbon atoms.

Ethyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside [10] (**8**) was allylated at C-2 using phase transfer catalysis [11] to give ethyl 2-*O*-allyl-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**9**). Compound **9** was allowed to react with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate [12] (**10**) in nitromethane at −30°C using silver triflate [13] as promoter to give ethyl 2-*O*-allyl-4,6-*O*-benzylidene-3-*O*-(methyl 2,3,4-*O*-acetyl-β-D-glucopyranosyluronate)-1-thio-β-D-galactopyranoside (**11**) in 50% yield. This compound exhibited ^{1}H NMR signals for SCH_2CH_3, COOMe, PhCH and CH_2–CH=CH_2 groups together with a doublet at δ 4.59 (J = 9.0 Hz) for a single proton (H-1′) confirming its β-glucuronosidic linkage. Benzylidene group of **11** was then removed with 85% acetic acid at 70°C and the product (**12**) was acetylated to obtain the acetate **13** as the donor.

Compound **6** was allowed to react with **13** in the presence of methyl triflate [14] as promoter to give methyl 3-*O*-[4,6-di-*O*-acetyl-2-*O*-allyl-3-*O*-(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-galactopyranosyl]-4,6-di-*O*-benzyl-2-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (**14**) in 66% yield. ^{1}H NMR spectrum of the compound showed the presence of glycosidic and ester methyl groups and allylic protons. The tetrasaccharide derivative (**14**) was deallylated using palladium (II) chloride in methanol [9] to afford the corresponding hydroxy compound **15**. Compound **15** was hydrogenolysed in acetic acid using 10% Pd–C to afford **16** which, on acetylation, gave the peracetate **17** as fine crystals which was characterised by proton NMR and analysis. Deacetylation [7] and subsequent saponification of **17** gave methyl 2-*O*-β-D-galactopyranosyl-3-*O*-(3-*O*-β-D-glucopyranosyluronic acid-α-D-galactopyranosyl)-α-D-mannopyranoside (**18**). The signals in the ^{1}H NMR spectrum at δ 3.4, 4.42 (d, J = 7.2 Hz), 4.77 (d, J = 7.8 Hz), 4.84 (s) and 5.32 (br s) indicated the presence of methoxyl group, H-1′, H-1‴, H-1 and H-1″, respectively. The ^{13}C NMR spectrum of **18**



Scheme 1

exhibited the presence of 25 carbon atoms. The four anomeric carbon signals appearing at δ 99.63 (C-1), 101.00 (C-1″), 103.01 (C-1′), 104.90 (C-1‴) and the acidic carbon signal appearing at δ 173.52 (COOH) confirmed the structure.

3. Experimental

General. — All reactions were monitored by TLC on Silica Gel G (E. Merck). Column chromatography was performed using 100–200 mesh silica gel (SRL, India). The weight of silica gel taken for individual separations was approximately 10 to 25 times that of the weight of crude reaction mixture, depending on the extent of separation. All solvents were dried and/or distilled before use, and all evaporations were conducted below 40°C unless otherwise stated. Optical rotations were measured with a Perkin–Elmer 241 MC polarimeter. ^{1}H and ^{13}C NMR spectra were recorded (internal standard tetramethylsilane) with a Jeol FX-100 and Bruker 300 MHz spectrometer, using $CDCl_3$ as solvent unless stated otherwise. The organic extracts were dried over anhydrous Na_2SO_4.

Methyl 3-O-*allyl-4,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**2**). — Methyl 4,6-di-*O*-benzyl-α-D-mannopyranoside [3] (**1**, 10.7 g, 28.6 mmol) was refluxed in benzene (170 mL) with dibutyltin oxide (8.5 g, 34.3 mmol) with azeotropic removal of water for 12 h. Allyl bromide (30 mL, 42.9 mmol) and Bu_4NBr (11.1 g, 34.3 mmol) were then added and the mixture was stirred at 63°C for 6 h. The solvent was removed by evaporation. The unwanted solids obtained on addition of cold MeOH were filtered off and the filtrate was evaporated to dryness. This residue was chromatographed using 5:3 toluene–ether as eluent to give pure **2** (9.36 g, 79%); $[\alpha]_D^{30}$ + 79.8° (*c* 2.0, $CHCl_3$). ^1H NMR: δ 3.36 (s, 3 H, OMe), 4.12–4.24 (m, 2 H, CH_2–CH=CH_2), 4.82 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 5.14–5.42 (m, 2 H, CH_2–CH=CH_2), 5.78–6.18 (m, 1 H, CH_2–C*H*=CH_2), 7.24–7.42 (m, 10 H, 2 Ph). Anal. Calcd for $C_{24}H_{30}O_6$: C, 69.54; H, 7.29. Found: C, 69.32; H, 7.39.

Methyl 2-O-*(2,3,4,6-tetra*-O-*acetyl-β*-D-*galactopyranosyl)-3*-O-*allyl-4,6-di*-O-*benzyl-α*-D-*mannopyranoside* (**3**). — Compound **2** (7.4 g, 17.85 mmol) and mercury(II) cyanide (9.1 g, 35.46 mmol) were dissolved in MeCN (90 mL) and 3 Å molecular sieves (10 g) were added to it. The mixture was stirred for 1 h under Ar. 2,3,4,6-Tetra-*O*-acetyl-α-D-galactopyranosyl bromide [5] (13.2 g, 32.1 mmol) in MeCN (10 mL) was then injected into the reaction flask. After 24 h, the reaction mixture was diluted with CH_2Cl_2 and filtered through Celite. The filtrate was washed with 5% KI solution, saturated aqueous $NaHCO_3$ solution and water in succession. The organic layer was dried and evaporated to dryness. Column chromatography with 3:2 ether–petroleum ether (bp 60–80°C) gave **3** together with some impurities which could not be separated at this stage. This mixture was then treated with 0.05 M NaOMe (80 mL) for 3 h and decationised with Dowex 50W-X8 (H^+) resin. Column chromatography with 5% MeOH in EtOAc gave pure **4** (7.05 g, 68.4% from **2**). A small amount of **4** was reacetylated as usual with pyridine–Ac_2O giving **3**; $[\alpha]_D^{24}$ + 2.7° (*c* 1.0, $CHCl_3$). ^1H NMR: δ 2.00, 2.08, 2.12 and 2.20 (4s, 12 H, 4 Ac), 3.4 (s, 3 H, OMe), 4.4 (d, 1 H, $J_{1',2'}$ = 7.0 Hz, H-1′), 4.68 (d, 1 H, $J_{1,2}$ = 2.0 Hz, H-1), 5.80–6.20 (m, 1 H, CH_2–C*H*=CH_2), 7.24–7.40 (m, 10 H, 2 Ph). Anal. Calcd for $C_{38}H_{48}O_{15}$: C, 61.28; H, 6.5. Found: C, 61.13; H, 6.7.

Methyl 3-O-*allyl-4,6-di*-O-*benzyl-2*-O-*(2,3,4,6-tetra*-O-*benzyl-β*-D-*galactopyranosyl)-α*-D-*mannopyranoside* (**5**). — Compound **4** (7 g, 12.1 mmol) in DMF (50 mL) was cooled and NaH (3.67 g, 50% oil coated) and benzyl bromide (7 mL, 58.0 mmol) were added. The mixture was stirred for 6 h at room temperature. Excess NaH was decomposed by adding MeOH (3 mL). Water (400 mL) was then added and the mixture was extracted with CH_2Cl_2 (3 × 150 mL). The dichloromethane extract was washed with water, dried and evaporated to dryness. Column chromatography with 6:1 toluene–ether gave pure **5** as a syrup (10.7 g, 94%); $[\alpha]_D^{24}$ + 2.9° (*c* 1.5, $CHCl_3$). ^1H NMR: δ 3.34 (s, 3 H, OMe), 4.68 (d, 1 H, $J_{1',2'}$ = 8.0 Hz, H-1′), 4.9 (br s, 1 H, H-1), 5.76–6.20 (m, 1 H, CH_2–C*H*=CH_2), 7.24–7.40 (m, 30 H, 6 Ph). Anal. Calcd for $C_{58}H_{64}O_{11}$: C, 74.34; H, 6.88. Found: C, 74.30; H, 6.90.

Methyl 4,6-di-O-*benzyl-2*-O-*(2,3,4,6-tetra*-O-*benzyl-β*-D-*galactopyranosyl)-α*-D-*mannopyranoside* (**6**). — To a solution of **5** (10 g, 10.67 mmol) in MeOH (130 mL), $PdCl_2$ (935 mg, 5.3 mmol) was added and the solution was stirred at room temperature for 2.5 h. After evaporation of MeOH, the crude product was immediately charged on a

column and eluted with 7:1 toluene–ether to give pure syrupy **6** (9.19 g, 96%); $[\alpha]_D^{24}$ + 20.41° (*c* 1.0, $CHCl_3$). ^1H NMR: δ 3.31 (s, 3 H, OMe), 4.63 (d, 1 H, $J_{1',2'}$ = 6.0 Hz, H-1'), 4.93 (br s, 1 H, H-1), 7.22–7.42 (m, 30 H, 6 Ph). Anal. Calcd for $C_{55}H_{60}O_{11}$: C, 73.64; H, 6.74. Found: C, 73.50; H, 6.90.

Methyl 2-O-β-D-galactopyranosyl-α-D-mannopyranoside (**7**). — Compound **6** (300 mg, 0.33 mmol) was hydrogenolysed using 10% Pd–C in AcOH (10 mL) at room temperature for 76 h. The reaction mixture was filtered through Celite and evaporated to dryness. The traces of acid were removed by co-evaporation with toluene. Column chromatography with 10:5:1 $CHCl_3$–MeOH–H_2O gave pure **7** (98 mg, 82%); $[\alpha]_D^{28}$ + 22.95° (*c* 0.6, H_2O). ^1H NMR (D_2O, internal standard 1,4-dioxane): δ 3.44 (s, 3 H, OMe), 4.49 (d, 1 H, $J_{1',2'}$ = 7.0 Hz, H-1'), 4.9 (br s, 1 H, H-1). ^{13}C NMR (D_2O, internal standard 1,4-dioxane): δ 55.64 (OCH_3), 61.32 (C-6), 61.96 (C-6'), 67.75, 69.45, 70.62, 71.38, 73.31 (2C), 76.06, 77.99, 99.31 (C-1), 102.97 (C-1').

Ethyl 2-O-allyl-4,6-O-benzylidene-1-thio-β-D-galactopyranoside (**9**). — To a solution of **8** (10 g, 32.0 mmol) in CH_2Cl_2 (175 mL), 10% NaOH solution (70 mL), tetrabutylammonium bromide (12 g, 36.8 mmol) and allyl bromide (3.1 mL, 36.6 mmol) were added and the mixture was stirred vigorously at room temperature for 48 h. The mixture was washed thrice with water and the organic layer was dried and concentrated to a syrup. Column chromatography of the residue using 7:1 toluene–ether gave pure **9** (4.51 g, 40%) together with some 3-*O*-allyl and 2,3-di-*O*-allyl compounds. Compound **9** was crystallised from CH_2Cl_2–petroleum ether (bp 60–80°C); mp 92.5–93.5°C; $[\alpha]_D^{25}$ + 47.98° (*c* 1.0, $CHCl_3$). ^1H NMR: δ 1.32 (t, 3 H, *J* = 8.0 Hz, SCH_2CH_3), 2.78 (q, 2 H, *J* = 7.5 Hz, SCH_2CH_3), 4.39 (d, 1 H, $J_{1,2}$ = 10.0 Hz, H-1), 5.54 (s, 1 H, PhC*H*), 5.83–6.24 (m, 1 H, CH_2–C*H*=CH_2), 7.34–7.52 (m, 5 H, Ph). Anal. Calcd for $C_{18}H_{24}O_5S$: C, 61.34; H, 6.86. Found: C, 61.21; H, 6.94.

Ethyl-2-O-allyl-4,6-O-benzylidene-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-1-thio-β-D-galactopyranoside (**11**). — A solution of silver triflate (1.95 g, 7.54 mmol) in toluene–benzene (5:1, 6 mL) was added to a stirred mixture of **9** (970 mg, 2.75 mmol), methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide) uronate (**10**, 949 mg, 2.39 mmol) and molecular sieves (4 Å, 2 g) in dry nitromethane (10 mL) at −30°C under nitrogen. Stirring was continued for 30 min at that temperature. Pyridine (3.9 mL) and aqueous sodium thiosulphate (10%, 40 mL) were then added; the reaction mixture was allowed to attain room temperature and filtered through Celite. The filtrate was washed with water, dried and concentrated. The residue was purified by column chromatography using 3:1 toluene–EtOAc to give pure **11** (799 mg, 50%); $[\alpha]_D^{28}$ + 0.93° (*c* 1.5, $CHCl_3$). ^1H NMR: δ 1.31 (t, 3 H, *J* = 8.0 Hz, SCH_2CH_3), 2.01, 2.02 and 2.06 (3s, 9 H, 3 Ac), 2.8 (q, 2 H, *J* = 7.5 Hz, SCH_2CH_3), 3.74 (s, 3 H, COOMe), 4.39 (d, 1 H, $J_{1,2}$ = 10.0 Hz, H-1), 4.59 (d, 1 H, $J_{1',2'}$ = 9.0 Hz, H-1'), 5.54 (s, 1 H, PhC*H*), 5.82–6.20 (m, 1 H, CH_2–C*H*=CH_2), 7.31–7.56 (m, 5 H, Ph). Anal. Calcd for $C_{31}H_{40}O_{14}S$: C, 55.68; H, 6.03. Found: C, 55.70; H, 6.00.

Ethyl 4,6-di-O-acetyl-2-O-allyl-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-1-thio-β-D-galactopyranoside (**13**). — Compound **11** (750 mg, 1.12 mmol) was stirred in 85% AcOH (5 mL) at 70°C for 3 h. The acid was removed by evaporation to give **12**. Compound **12** was acetylated with Ac_2O in pyridine in the presence of 4-dimethylaminopyridine (10 mg). The mixture was evaporated to dryness and traces of

Ac_2O and pyridine were removed by co-evaporation with toluene. The residue was chromatographed using 1:1 toluene–EtOAc to give pure **13** (597 mg, 80%). The product was crystallised from EtOH; mp 103–104°C; $[\alpha]_D^{20}$ −5.09° (*c* 0.6, $CHCl_3$). ^1H NMR: δ 1.34 (t, 3 H, $J = 7.5$ Hz, SCH_2CH_3), 2.01–2.08 (3s, 15 H, 5 Ac), 2.73 (q, 2 H, $J = 7.0$ Hz, SCH_2CH_3), 3.77 (s, 3 H, COOMe), 4.09 (d, 1 H, $J_{1,2} = 8.0$ Hz, H-1), 4.39 (d, 1 H, $J_{1',2'} = 9.0$ Hz, H-1'), 5.79–6.17 (m, 1 H, $CH_2-CH=CH_2$). Anal. Calcd for $C_{28}H_{40}O_{16}S$: C, 50.60; H, 6.07. Found: C, 50.88; H, 6.10.

Methyl 3-O-[4,6-di-O-acetyl-2-O-allyl-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-α-D-galactopyranosyl]-4,6-di-O-benzyl-2-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (**14**). — Compound **13** (550 mg, 0.83 mmol) and **6** (496 mg, 0.55 mmol) in ether (20 mL) were stirred at room temperature under N_2 with 4 Å molecular sieves (2 g) for 45 min. Methyl triflate (0.45 mL, 4.15 mmol) was then injected into the mixture and stirring was continued at 22°C for 98 h. The reaction was then quenched with triethylamine, filtered through Celite and concentrated. The crude mixture was chromatographed with 9:1 toluene–ether to give syrupy **14** (547 mg, 66%); $[\alpha]_D^{24}$ + 42.5° (*c* 1.4, $CHCl_3$). ^1H NMR: δ 1.96–2.06 (4s, 15 H, 5 Ac), 3.31 (s, 3 H, OMe), 3.6 (s, 3 H, COOMe), 4.66 (d, 1 H, $J_{1',2'} = 6.5$ Hz, H-1'), 4.86 (d, 1 H, $J_{1''',2'''} = 7.0$ Hz, H-1'''), 5.00 (br s, 1 H, H-1), 5.19 (d, 1 H, $J_{1'',2''} = 3.0$ Hz, H-1''), 5.80–6.10 (m, 1 H, $CH_2-CH=CH_2$), 7.22–7.44 (m, 30 H, 6 Ph). Anal. Calcd for $C_{81}H_{94}O_{27}$: C, 64.88; H, 6.32. Found: C, 64.73; H, 6.41.

Methyl 3-O-[4,6-di-O-acetyl-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-α-D-galactopyranosyl]-4,6-di-O-benzyl-2-O-(2,3,4,6-tetra-O-benzyl-β-D-galactopyranosyl)-α-D-mannopyranoside (**15**). — Compound **14** (245 mg, 0.16 mmol) was dissolved in MeOH (5 mL) and $PdCl_2$ (14.5 mg) was added and stirred at room temperature for 2.5 h. The mixture was evaporated to dryness and column chromatographed using 5:1 toluene–ether to give **15** (216 mg, 91%); $[\alpha]_D^{24}$ + 40.2° (*c* 1.4, $CHCl_3$). ^1H NMR: δ 1.96, 2.00 and 2.07 (3s, 15 H, 5 Ac), 3.3 (s, 3 H, OMe), 3.61 (s, 3 H, COOMe), 4.5 (d, 1 H, $J_{1',2'} = 6.0$ Hz, H-1'), 4.84 (d, 1 H, $J_{1''',2'''} = 7.5$ Hz, H-1'''), 5.06 (br s, 1 H, H-1), 5.22 (d, 1 H, $J_{1'',2''} = 2.5$ Hz, H-1''), 7.20–7.40 (m, 30 H, 6 Ph). Anal. Calcd for $C_{78}H_{90}O_{27}$: C, 64.19; H, 6.21. Found: C, 64.02; H, 6.37.

Methyl 4,6-di-O-acetyl-3-O-[2,4,6-tri-O-acetyl-3-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-α-D-galactopyranosyl]-2-O-(2,3,4,6-tetra-O-acetyl-β-D-galactopyranosyl)-α-D-mannopyranoside (**17**). — Compound **15** (200 mg, 0.14 mmol) was dissolved in AcOH (6 mL) and hydrogenolysed in 10% Pd–C (100 mg) for 3 days at room temperature. The reaction mixture was filtered through Celite and concentrated to afford **16**. Crude **16** was acetylated using Ac_2O and pyridine in the usual manner. Chromatographic separation gave pure **17** (151 mg, 91%); mp 205°C (EtOH); $[\alpha]_D$ + 54.11° (*c* 0.6, $CHCl_3$). ^1H NMR: δ 2.00–2.25 (6s, 36 H, 12 Ac), 3.38 (s, 3 H, OMe), 3.7 (s, 3 H, COOMe), 4.46 (d, 1 H, $J_{1',2'} = 6.5$ Hz, H-1'), 4.84 (d, 1 H, $J_{1''',2'''} = 7.0$ Hz, H-1'''), 5.08 (br s, 1 H, H-1), 5.24 (d, 1 H, $J_{1'',2''} = 3.0$ Hz, H-1''). Anal. Calcd for $C_{50}H_{68}O_{34}$: C, 49.51; H, 5.65. Found: C, 49.49; H, 5.67.

Methyl 2-O-β-D-galactopyranosyl-3-O-(3-O-β-D-glucopyranosyluronic acid-α-D-galactopyranosyl)-α-D-mannopyranoside (**18**). — Compound **17** (135 mg, 0.11 mmol) was stirred with 0.05 M NaOMe (2 mL) for 4 h at room temperature. Two drops of water were then added and the solution was stirred for 2 h. The solution was

decationised with Dowex 50W-X8 (H^+) resin, filtered and concentrated. The residue was dissolved in water and membrane filtered. The filtrate was evaporated to dryness to give pure **18** (71 mg, 92%); $[\alpha]_D^{25}$ + 50.0° (*c* 0.85, H_2O). ^1H NMR (D_2O): δ 3.4 (s, 3 H, OMe), 4.42 (d, 1 H, $J_{1',2'}$ = 7.2 Hz, H-1′), 4.77 (d, 1 H, $J_{1''',2'''}$ = 7.8 Hz, H-1‴), 4.84 (s, 1 H, H-1), 5.32 (br s, 1 H, H-1″). ^{13}C NMR (D_2O): δ 55.73 (OCH_3), 60.99 (C-6″), 62.08 (C-6), 62.24 (C-6′), 66.19, 67.35, 68.47, 69.41, 70.33, 71.38, 71.98, 72.22, 73.37, 73.49, 73.80, 75.35, 75.88, 76.04, 77.55, 80.56, 99.63 (C-1), 101.00 (C-1″), 103.01 (C-1′), 104.90 (C-1‴), 173.52 (COOH).

Acknowledgements

The authors express their sincere thanks to Dr. Asim K. Ray, University of Alberta, Edmonton for recording some ^1H and ^{13}C NMR spectra of deprotected materials. Financial assistance to S.K.D. by the C.S.I.R., India is gratefully acknowledged.

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