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SYNTHESIS OF THE TETRASACCHARIDE REPEATING-UNIT OF THE POLYSACCHARIDE FROM *Klebsiella* TYPE 23

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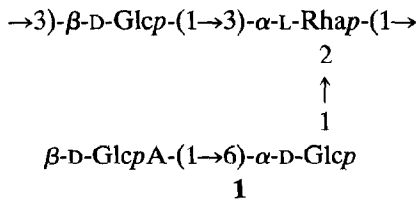
ABSTRACT

Methyl 2-*O*-allyl-4-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnospyranoside was obtained by condensing methyl 2-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranoside with tetra-*O*-acetyl-α-D-glucopyranosyl bromide. Benzylation, removal of the allyl group, and condensation of the product with ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside gave methyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-4-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside. *O*-Deacetylation, condensation of the product with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)-uronate, and removal of the protecting groups gave methyl 3-*O*-β-D-glucopyranosyl-2-*O*-[6-*O*-(β-D-glucopyranosyluronic acid)-α-D-glucopyranosyl]-α-L-rhamnopyranoside (**13**).

INTRODUCTION

Bacterial polysaccharides exhibit a broad range of biological activities and specificities, and a part of our programme involves the synthesis of immunodominant fragments related to various bacterial antigens.



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The repeating unit **1** of the capsular polysaccharide from *Klebsiella* Type 23, established by Dutton *et al.*[1], contains three 1,2-*trans*-linked and one 1,2-*cis*-linked glycosidic bonds. We now report the synthesis of the methyl glycoside of the tetrasaccharide repeating-unit of this antigen.

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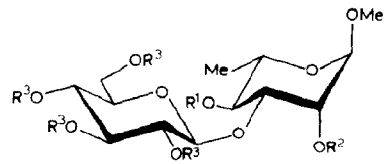
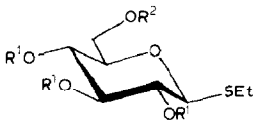
RESULTS AND DISCUSSION

Methyl 2-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranoside[2] reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide[3] in the presence of mercury(II) cyanide and mercury(II) bromide in acetonitrile[4] to give 73% of syrupy methyl 2-*O*-allyl-4-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**2**). Treatment[5] of **2** with benzyl chloride and KOH in 1,4-dioxane gave **3**, which, on deallylation with selenium(IV) oxide and acetic acid in 1,4-dioxane[6], yielded crystalline methyl 4-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**4**).

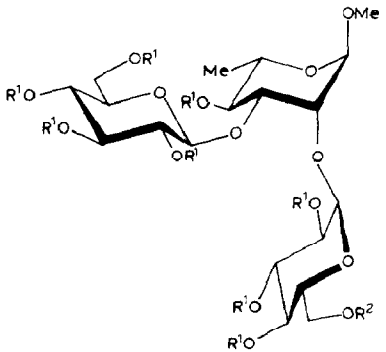
Condensation of **4** with ethyl 6-*O*-acetyl-2,3,4-tri-*O*-benzyl-1-thio-β-D-glucopyranoside (**8**), in the presence of copper(II) bromide, tetraethylammonium bromide, and silver triflate[7] in dichloromethane–*N*,*N*-dimethylformamide, gave methyl 2-*O*-(6-*O*-acetyl-2,3,4-tri-*O*-benzyl-α-D-glucopyranosyl)-4-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**9**). Deacetylation of **9** gave **10**, which contained a single primary hydroxyl group. Reaction of **10** with methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate[8], in the presence of silver triflate[9] in dichloromethane, gave crystalline methyl 4-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-2-*O*-[2,3,4-tri-*O*-benzyl-6-*O*-



2 R^1 = Bn, R^2 = allyl, R^3 = Ac
3 R^1 = R^3 = Bn, R^2 = allyl
4 R^1 = R^3 = Bn, R^2 = H



6 R^1 = H, R^2 = Tr
7 R^1 = Bn, R^2 = H
8 R^1 = Bn, R^2 = Ac



9 R^1 = Bn, R^2 = Ac
10 R^1 = Bn, R^2 = H
11 R^1 = R^2 = H
12 R^1 = Bn, R^2 = Methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate
13 R^1 = H, R^2 = β-D-Glucopyranosyluronic acid

(methyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranosyl]-α-L-rhamnopyranoside (**12**).

Removal of protecting groups from **4**, **10**, and **12** gave methyl glucosides of the di- (**5**), tri- (**11**), and tetra-saccharide (**13**), respectively. The glycoside **13** corresponds to **1**, and the compounds **5**, **11**, and **13** were characterised by their ^{1}H-n.m.r. spectra, acid hydrolysis, and methylation analysis in the usual way[10].

EXPERIMENTAL

General. — Reactions were monitored by t.l.c. on Silica Gel G (Merck). Column chromatography was performed on Silica Gel 60 (Merck, 70–230 mesh ASTM). G.l.c. of alditol acetates[10] was performed at 180° for neutral sugars and 170° for methylated sugars with a Hewlett–Packard 5730A gas chromatograph equipped with a flame-ionisation detector and a glass column (1.83 m × 6 mm) containing 3% of ECNSS-M on Gas Chrom Q (100–120 mesh).

^{1}H-N.m.r. spectra were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Jeol FX-100 spectrometer, and optical rotations with a Perkin–Elmer 241MC polarimeter. Melting points were determined on a Fisher–Johns apparatus and are uncorrected. The glycoside syntheses were performed under dry nitrogen. All solvents were distilled before use and all evaporations were conducted at 50° under diminished pressure unless otherwise stated.

Methyl 2-O-allyl-4-O-benzyl-3-O-(2,3,4,6-tetra-O-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**2**). — A solution of methyl 2-*O*-allyl-4-*O*-benzyl-α-L-rhamnopyranoside[2] (10 g, 30 mmol), prepared from methyl 4-*O*-benzyl-α-L-rhamnopyranoside[11], in dry acetonitrile (150 mL) was stirred with tetra-*O*-acetyl-α-D-glucopyranosyl bromide[3] (14.8 g, 36 mmol), mercury(II) cyanide (10.1 g, 40 mmol), mercury(II) bromide (14.4 g, 40 mmol), and molecular sieves (3 Å, 6 g) for 20 h at room temperature. The mixture was filtered through Celite, concentrated to a small volume, diluted with chloroform (300 mL), washed with aq. 10% potassium iodide, saturated aq. sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Column chromatography (5:1 benzene–ether) of the residue gave **2** (14.2 g, 73%), $[\alpha]_D^{24}$ −39° (*c* 2.2, chloroform). ^{1}H-N.m.r. data: δ 1.30 (d, 3 H, *J* 6 Hz, H-6,6,6), 2.00–2.18 (4 s, 12 H, 4 OAc), 3.42 (s, 3 H, OMe), 4.54 (d, 1 H, *J* 8 Hz, H-1), 4.98 (d, 1 H, *J* 1.5 Hz, H-1), 4.68 (s, 2 H, PhC*H*$_2$), 5.72–6.06 (m, 1 H, CH_2=C*H*), 7.34–7.40 (m, 5 H, Ph).

Anal. Calc. for $C_{31}H_{42}O_{14}$: C, 58.29; H, 6.63. Found: C, 58.01; H, 6.78.

Methyl 2-O-allyl-4-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**3**). — A solution of **2** (10 g) in 1,4-dioxane was stirred at 80° with powdered potassium hydroxide (20 g), then benzyl chloride (18 mL) was added dropwise during 1 h. Heating and stirring were continued for 4 h. Water (100 mL) was added, the mixture was extracted with chloroform (4 × 100 mL), and the combined extracts were washed with water (5 × 200 mL) and concentrated. Benzyl alcohol was removed from the residue by azeotropic distillation with water. Column

chromatography (8:1 benzene–ether) then gave **3** (12 g, 91%), $[\alpha]_D^{24}$ −8° (*c* 3.1, chloroform). ^{1}H-N.m.r. data: δ 1.33 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.33 (s, 3 H, OMe), 4.42–4.74 (m, 10 H, 5 PhCH_2), 4.48 (d, 1 H, *J* 7.6 Hz, H-1′), 4.94 (d, 1 H, *J* 1 Hz, H-1), 5.64–6.02 (m, CH_2=C*H*), 7.24–7.38 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{51}H_{58}O_{10}$: C, 73.69; H, 7.03. Found: C, 73.43; H, 7.21.

*Methyl 4-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-*D*-glucopyranosyl)-α-*L*-rhamnopyranoside* (**4**). — To a solution of **3** (8 g, 9.6 mmol) in 1,4-dioxane (40 mL) were added acetic acid (0.6 mL, 10 mmol) and selenium(IV) oxide[6] (1.11 g, 10 mmol). The mixture was stirred under reflux for 40 min, then filtered, and concentrated. Column chromatography (5:1 benzene–ether) of the residue gave **4** (5.8 g, 76%), which crystallised from ethanol–ethyl acetate to give material (4.9 g, 64%) having m.p. 112–114°, $[\alpha]_D^{24}$ −15° (*c* 2.4, chloroform). ^{1}H-N.m.r. data: δ 1.24 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.30 (s, 3 H, OMe), 4.40–4.70 (m, 10 H, 5 PhCH_2), 4.46 (d, 1 H, *J* 7.6 Hz, H-1′), 4.96 (d, 1 H, H-1), 7.32–7.51 (m, 25 H, 5 Ph).

Anal. Calc. for $C_{48}H_{54}O_{10}$: C, 72.89; H, 6.88. Found: C, 72.62; H, 6.94.

*Methyl 3-O-β-*D*-glucopyranosyl-α-*L*-rhamnopyranoside* (**5**). — A solution of **4** (500 mg) in methanol (10 mL) was stirred in the presence of 10% Pd/C (100 mg) under hydrogen at room temperature for 16 h, then filtered, and concentrated to dryness, to give **5** (198 mg, 92%), $[\alpha]_D^{24}$ −27° (*c* 1, water). ^{1}H-N.m.r. data (D_2O): δ 4.78 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.42 (d, 1 H, $J_{1',2'}$ 8 Hz, H-1′), 3.40 (s, 3 H, OMe), 1.30 (d, 3 H, $J_{5',6'}$ 5 Hz, H-6,6,6).

Anal. Calc. for $C_{13}H_{24}O_{10}$: C, 45.88; H, 7.11. Found: C, 45.90; H, 7.00.

*Ethyl 1-thio-6-O-trityl-β-*D*-glucopyranoside* (**6**). — To a solution of ethyl 1-thio-β-D-glucopyranoside[12] (5 g) in pyridine (70 mL) was added trityl chloride (8 g), and the mixture was kept in the dark for 3 days at room temperature with occasional stirring. Methanol (5 mL) was added, and, after 1 h, the mixture was filtered and concentrated. Traces of pyridine were removed from the residue by repeated evaporation of toluene therefrom. Column chromatography (1:1 benzene–ethyl acetate) of the residue gave **6** (9.4 g, 92%), isolated as a glass, $[\alpha]_D^{24}$ −32° (*c* 1.2, methanol).

Anal. Calc. for $C_{27}H_{30}O_5S$: C, 69.50; H, 6.48. Found: C, 70.12; H, 6.78.

*Ethyl 2,3,4-tri-O-benzyl-1-thio-β-*D*-glucopyranoside* (**7**). — To a cold solution of **6** (9 g) in dry *N*,*N*-dimethylformamide (50 mL) was added sodium hydride (3 g) followed by benzyl bromide (9 mL) dropwise, and the mixture was stirred for 4 h at room temperature. After the usual work-up, the crude product was stirred with aq. 80% acetic acid (50 mL) for 2 h at 85°. The solvents were evaporated, and traces of acetic acid were removed by repeated evaporation of water from the residue. Column chromatography (benzene) then gave **7** (8.4 g, 88%). Crystallisation from ethanol gave material with m.p. 79°, $[\alpha]_D^{24}$ −1° (*c* 2, chloroform). ^{1}H-N.m.r. data: δ 1.32 (t, 3 H, SCH_2CH_3), 2.76 (q, 2 H, SCH_2CH_3), 4.36 (d, 1 H, *J* 8 Hz, H-1), 4.56–4.88 (m, 6 H, 3 PhCH_2), 7.32–7.40 (m, 15 H, 5 Ph).

Anal. Calc. for $C_{29}H_{34}O_5S$: C, 70.42; H, 6.93. Found: C, 69.97; H, 7.12.

*Ethyl 6-O-acetyl-2,3,4-tri-O-benzyl-1-thio-β-*D*-glucopyranoside* (**8**). — Com-

pound **7** (7 g) was treated conventionally with acetic anhydride and pyridine to give **8** (7.3 g, 97%). Crystallisation from ethanol gave material having m.p. 94°, $[\alpha]_D^{24}$ +2° (*c* 2.5, chloroform). ^{1}H-N.m.r. data: δ 1.34 (t, 3 H, SCH_2CH_3), 2.10 (s, 3 H, OAc), 2.78 (q, 2 H, SCH_2CH_3), 4.32 (d, 1 H, *J* 8 Hz, H-1), 4.52–4.82 (m, 6 H, 3 $PhCH_2$), 7.30–7.42 (m, 15 H, 3 Ph).

Anal. Calc. for $C_{31}H_{36}O_6S$: C, 69.37; H, 6.76. Found: C, 69.52; H, 6.92.

Methyl 2-O-(6-O-acetyl-2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-4-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**9**). — A mixture of copper(II) bromide (2.46 g, 11 mmol), tetraethylammonium bromide (0.265 g, 1.25 mmol), silver triflate (3.28 g, 11 mmol), molecular sieves 4Å (2 g), and 6:1 CH_2Cl_2–*N,N*-dimethylformamide (25 mL) was stirred in the dark at room temperature for 1 h. A solution of **4** (4 g, 5.06 mmol) and **8** (4 g, 7.45 mmol) in 6:1 CH_2Cl_2–*N,N*-dimethylformamide (25 mL) was added, and the mixture was stirred in the dark for 16 h at room temperature, then filtered through Celite, diluted with dichloromethane (200 mL), washed with aq. 10% sodium thiosulphate, saturated aq. sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated. Column chromatography (10:1 benzene–ether) of the residue gave **9** (5.3 g, 83%). Crystallisation from ethanol–ethyl acetate (2:1) gave material (4.4 g, 69%) with m.p. 156–157°, $[\alpha]_D^{24}$ +25° (*c* 1.2, chloroform). ^{1}H-N.m.r. data: δ 1.30 (d, 3 H, *J* 6 Hz, H-6,6,6), 2.01 (s, 3 H, OAc), 3.32 (s, 3 H, OMe), 4.42–4.81 (m, 16 H, 8 $PhCH_2$), 4.44 (d, 1 H, *J* 7.6 Hz, H-1″). 5.01 (d, 1 H, *J* 1 Hz, H-1), 5.42 (d, 1 H, *J* 3.7 Hz, H-1′), 7.30–7.64 (m, 40 H, 8 Ph).

Anal. Calc. for $C_{77}H_{84}O_{16}$: C, 73.08; H, 6.69. Found: C, 72.86; H, 7.01.

Methyl 4-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-2-O-(2,3,4-tri-O-benzyl-α-D-glucopyranosyl)-α-L-rhamnopyranoside (**10**). — To a solution of **9** (3 g) in dichloromethane (3 mL) was added methanolic 0.2M sodium methoxide (6 mL). The mixture was stirred for 4 h at room temperature, then neutralised with Dowex 50 (H^+) resin, filtered, and concentrated to give **10** (2.76 g, 96%), $[\alpha]_D^{24}$ +41° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 1.31 (d, 3 H, *J* 6 Hz, H-6,6,6), 3.34 (s, 3 H, OMe), 4.40–4.78 (m, 16 H, 8 $PhCH_2$), 4.42 (d, 1 H, *J* 7.6 Hz, H-1″), 5.02 (d, 1 H, *J* 1 Hz, H-1), 5.38 (d, 1 H, *J* 3.6 Hz, H-1′), 7.32–7.68 (m, 40 H, 8 Ph).

Anal. Calc. for $C_{75}H_{82}O_{15}$: C, 73.63; H, 6.76. Found: C, 73.28; H, 6.92.

Methyl 2-O-α-D-glucopyranosyl-3-O-β-D-glucopyranosyl-α-L-rhamnopyranoside (**11**). — A solution of **9** (500 mg) in methanol–ethyl acetate (10 mL) was debenzylated as described for **5**, to give **11** (185 mg, 95%), $[\alpha]_D^{24}$ +34° (*c* 1.1, water). ^{1}H-N.m.r. data (D_2O): δ 4.76 (d, 1 H, $J_{1,2}$ 1.6 Hz, H-1), 4.45 (d, 1 H, $J_{1'',2''}$ 8 Hz, H-1″), 5.25 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1′), 3.40 (s, 3 H, OMe), 1.30 (d, 3 H, $J_{5,6}$ 5 Hz, H-6,6,6).

Anal. Calc. for $C_{19}H_{34}O_{15}$; C, 45.42; H, 6.82. Found: C, 45.31; H, 6.90.

Methyl 4-O-benzyl-3-O-(2,3,4,6-tetra-O-benzyl-β-D-glucopyranosyl)-2-O-[2,3,4-tri-O-benzyl-6-O-(methyl 2,3,4-tri-O-acetyl-β-D-glucopyranosyluronate)-α-D-glucopyranosyl]-α-L-rhamnopyranoside (**12**). — A mixture of **10** (1.5 g, 1.23

mmol), dichloromethane (20 mL), molecular sieves 4 Å (2 g), and tetramethylurea (0.2 mL) was stirred at room temperature. Methyl (2,3,4-tri-*O*-acetyl-α-D-glucopyranosyl bromide)uronate (0.64 g, 1.6 mmol) was added, the mixture was cooled to −30°, silver triflate (0.5 g, 1.68 mmol) was added, and stirring was continued for 10 h at −30° in the dark. The mixture was then filtered through Celite, washed with water, saturated aq. sodium hydrogencarbonate, and water, dried (Na_2SO_4), and concentrated. Column chromatography (10:1 benzene–ether) of the residue and crystallisation from ethanol gave **12** (1.28 g, 64%), m.p. 178–179°, $[\alpha]_D^{24}$ +17° (*c* 1.1, chloroform). ^{1}H-N.m.r. data: δ 1.28 (d, 3 H, *J* 6 Hz, H-6,6,6), 1.84–1.98 (3 s, 9 H, 3 OAc), 3.31 (s, 3 H, OMe), 3.64 (s, 3 H, COOMe), 4.42–4.78 (m, 16 H, 8 PhCH_2), 4.46 (d, 1 H, *J* 7.6 Hz, H-1″), 4.80 (d, 1 H, *J* 8 Hz, H-1‴), 5.02 (d, 1 H, *J* 1.5 Hz, H-1), 5.38 (d, 1 H, *J* 3.6 Hz, H-1′), 7.32–7.68 (m, 40 H, 8 Ph).

Anal. Calc. for $C_{88}H_{98}O_{24}$: C, 70.56; H, 6.02. Found: C, 70.82; H, 6.29.

Methyl 3-O-β-D-glucopyranosyl-2-O-[6-O-(β-D-glucopyranosyluronic acid)-α-D-glucopyranosyl]-α-L-rhamnopyranoside (**13**). — A solution of **12** (700 mg) in methanol (10 mL) was stirred in the presence of 10% Pd/C (200 mg) under hydrogen for 24 h, then filtered through Celite, and concentrated. The syrupy residue was stirred with methanolic 0.1M sodium methoxide (10 mL) for 4 h at room temperature. A few drops of water were added, the solution was stored for 1 h at room temperature, neutralised with Dowex 50 (H^+) resin, and concentrated to give **12** (255 mg, 89%), $[\alpha]_D^{24}$ +25° (*c* 1.1, water). ^{1}H-N.m.r. data (D_2O): δ 4.78 (d, $J_{1,2}$ 1.6 Hz, H-1), 4.48 (d, 1 H, $J_{1'',2''}$ 8 Hz, H-1″), 5.25 (d, 1 H, $J_{1',2'}$ 1.4 Hz, H-1′), 4.70 (d, 1 H, $J_{1''',2'''}$ 8 Hz, H-1‴), 3.40 (s, 3 H, OMe), 1.30 (d, 3 H, $J_{5,6}$ 5 Hz, H-6,6,6).

Anal. Calc. for $C_{25}H_{42}O_{21}$: C, 44.25; H, 6.24. Found: C, 44.12; H, 6.45.

REFERENCES

1 G. G. S. Dutton, K. L. Mackie, A. V. Savage, and M. D. Stephenson, *Carbohydr. Res.*, 66 (1978) 125–131.
2 V. Poszgay, *Carbohydr. Res.*, 69 (1979) 284–286.
3 P. G. Scheurer and F. Smith, *J. Am. Chem. Soc.*, 76 (1954) 3224.
4 H. Paulsen, *Angew. Chem. Int. Ed. Engl.*, 21 (1982) 155–224.
5 C. P. J. Glaudemans and H. G. Fletcher, Jr., *J. Am. Chem. Soc.*, 87 (1965) 4636–4641.
6 S. Ogawa, S. Yokoi, N. Kimura, Y. Shibata, and N. Chida, *Carbohydr. Res.*, 181 (1988) 57–66.
7 S. Sato, M. Mori, Y. Ito, and T. Ogawa, *Carbohydr. Res.*, 155 (1986) c6–c10.
8 G. N. Bollenback, J. W. Long, D. G. Benjamin, and J. A. Lindquist, *J. Am. Chem. Soc.*, 77 (1955) 3310–3315.
9 S. S. Rana, J. J. Barlow, and K. L. Matta, *Carbohydr. Res.*, 84 (1980) 353–357.
10 A. K. Ray, A. Roy, and N. Roy, *Carbohydr. Res.*, 165 (1987) 77–86.
11 A. Haines, *Carbohydr. Res.*, 10 (1969) 466–467.
12 R. U. Lemieux, *Can. J. Chem.*, 29 (1951) 1079–1091.