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Note

Synthesis of 2-*O*- and 3-*O*-α-L-rhamnopyranosyl-L-rhamnose*

DOMINIQUE SCHWARZENBACH† AND ROGER W. JEANLOZ‡

Laboratory for Carbohydrate Research, Departments of Biological Chemistry and Medicine, Harvard Medical School and Massachusetts General Hospital, Boston, Massachusetts 02114 (U.S.A.)

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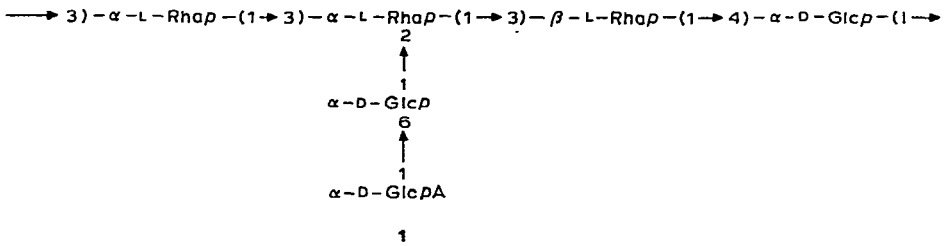
The type II capsular polysaccharide of *Streptococcus pneumoniae*, the structure (**1**) of which was established by Kenne *et al.*[2], induces antibodies of restricted heterogeneity[3]. The immunodominant group is the α-D-glucopyranosyluronic acid residue of the side chain, but the *O*-(α-D-glucopyranosyluronic acid)-(1→6)-α-D-glucopyranosyl side-chain inhibits only 70% of the precipitation of antibodies[4]. Various oligosaccharides derived from the polysaccharide are required in order to ascertain



the contribution of the remaining residues to the antigenic activity. These same oligosaccharides, bound to a polymer column, may be used for further fractionation of the "homogeneous antibodies". Because of the difficulty in isolating, by acid hydrolysis, pure oligosaccharides in sufficient amount for testing, it was of interest to synthesize the various chemical structures where the antigenic activity may reside. In the present communication, we describe the synthesis of 2-*O*- (**10**) and 3-*O*-α-L-rhamnosyl-L-rhamnose (**11**), the latter compound being part of the main chain of the *S. pneumoniae*

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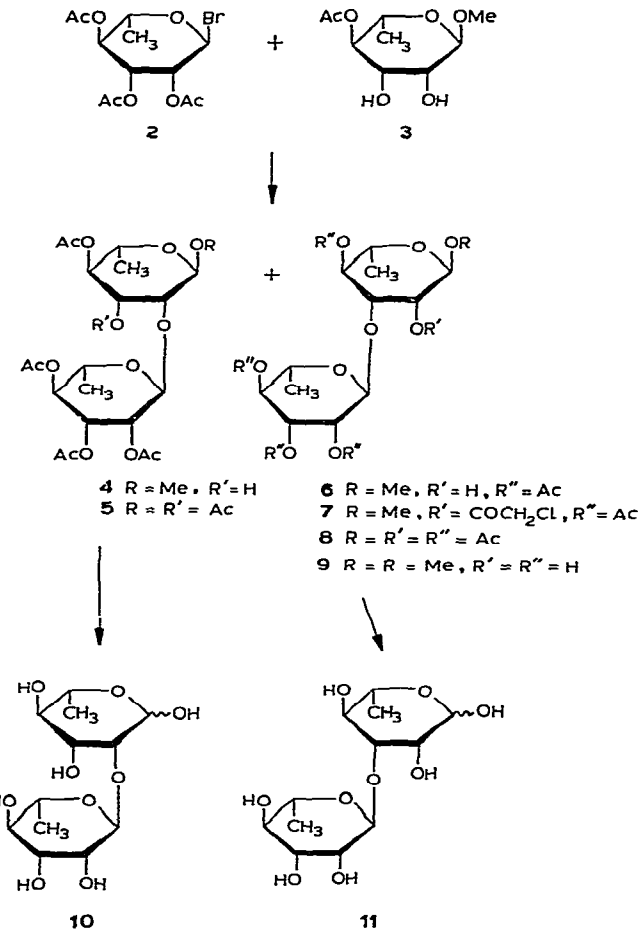
†Fellow of the Swiss National Science Foundation (No. 83.559.0.77).
‡To whom inquiries should be sent.

Type II polysaccharide. After this work had been undertaken, Schalch *et al.*[5] described in a preliminary communication the synthesis of **11**; Laffite *et al.*[6] the synthesis of **10**, obtained in admixture with the (1→3) and (1→4) isomers; and Liptak *et al.*[7] in a preliminary communication the synthesis of the methyl α-D-glycosides of **10** and **11**.



Condensation of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[8] with methyl 4-*O*-acetyl-α-L-rhamnopyranoside[9] (**3**) in dry acetonitrile at room temperature in the presence of mercuric cyanide as catalyst gave, in 60% yield, a 57:43 mixture of methyl 4-*O*-acetyl-2-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**4**) and methyl 4-*O*-acetyl-3-*O*-(2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**6**), which could be separated by chromatography. The linkages between the two carbohydrate moieties of **4** and **6** were ascertained by study of the n.m.r. data of the 2-*O*-chloroacetyl derivative **7**, obtained by treatment of **6** with chloroacetyl chloride in acetonitrile. Acetolysis of **4** and **6** gave the fully acetylated disaccharides **5** and **8**, respectively. The n.m.r. spectra of all compounds showed

TABLE I

N.M.R. DATA (270 MHz) FOR 4–8[a]

Compounds	*Chemical shifts (δ)*					
	H-2	*H-3*	*H-4*	*H-2'*	*H-3'*	*H-4'*
4	~3.9	~3.9	4.82	5.37	5.31	5.07
5	4.01	5.20	5.08	5.26	5.33	5.11
6	~4.0	3.95	5.09	5.09	5.26	5.08
7	5.24	4.40	5.07	5.03	5.15	5.04
8	5.17	4.09	5.17	5.02	5.14	5.04

[a]For solutions in chloroform-*d*.

clearly the α-L configuration of C-1 ($J_{1',2'}$ <2 Hz), H-1' resonating at lower field[10,11]. In addition, the mode of synthesis leads preferentially to the 1,2-*trans* configuration[12]. Deshielding of H-2 and H-3 in **4**, **6**, and **7** established unambiguously the linkage between the two carbohydrate moieties of **4** and **6**. The difference in the chemical shifts of H-2 between **6** and **7** is 1.24 p.p.m., whereas that for H-3 is only 0.45 p.p.m. Study of the chemical shifts of H-2 and H-3 of the acetylated compounds **5** and **8** confirms these attributions, H-2 and H-3 linked to the carbon atom bearing the acetoxyl group being deshielded: δ 5.17 and 4.09 for H-2 and H-3, respectively, of **5**; and δ 4.01 and 5.20 for H-2 and H-3, respectively, of **8**. In contrast, these values for the corresponding protons of the nonreducing moiety are very constant (see Table I). Compounds **10** and **11** were obtained by *O*-deacetylation of **5** and **8**, respectively, under conventional conditions, and disaccharide **9** by *O*-deacetylation of **6**. Their n.m.r. spectra accord with the expected structures, and periodate oxidation of **9** showed the resistance to degradation of one residue of L-rhamnose.

EXPERIMENTAL

General methods. — Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points". Optical rotations were determined in 1-dm semimicro tubes with a Perkin–Elmer No. 141 polarimeter. Evaporations were conducted *in vacuo*, the bath temperature being kept below 40°. Ascending t.l.c. was performed on precoated plates of silica gel (Merck 60 F-254) with solvents (v/v) (*A*) 1:1 ethyl acetate–hexane and (*B*) 2:1 diisopropyl ether–methanol and detection with the phosphomolybdic reagent; the R_F values were measured on 2.5 × 7.5-cm plates, thickness 0.25 mm. Column chromatography was performed on silica gel (Merck 60 F 254, 70–230 mesh). N.m.r. spectra were recorded with a Bruker HX-270 instrument unless otherwise indicated, with tetramethylsilane or sodium 4,4-dimethyl-4-silapentane-1-sulfonate as internal standard; and i.r. spectra, for potassium bromide discs, with a Perkin–Elmer spectrophotometer Model

237. Microanalyses were performed by Dr. W. Manser, Zürich, Switzerland; and Galbraith Laboratories, Inc., Knoxville, TN.

Methyl 4-O-acetyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**6**). — Methyl 4-*O*-acetyl-α-L-rhamnopyranoside[9] (**3**; 2.2 g, 10 mmol) was added to a solution of 2,3,4-tri-*O*-acetyl-α-L-rhamnopyranosyl bromide[8] (**1**; 3.53 g, 10 mmol) in dry acetonitrile (20 mL) containing mercuric cyanide (2.0 g). After 1 h, the acetonitrile was evaporated, and the resulting syrup dissolved in chloroform (100 mL). The solution was washed with 30% potassium iodide solution (50 mL), water (2 × 50 mL), saturated sodium hydrogencarbonate solution (50 mL), and water (50 mL), and evaporated to give a syrup (5.2 g), which was chromatographed on silica gel (500 g, *A*) to give **6** (1.1 g, 23%) and **4** (1.5 g, 30%). Compound **6** was crystallized from ether–ethyl acetate; m.p. 166.0–166.5°, $[\alpha]_D^{20}$ −61° (*c* 1.2, chloroform); t.l.c. (*A*): R_F 0.4; ν_{max}^{KBr} 3430 (OH), 1745 (CO), 1230, and 1060 cm^{-1}; ^{1}H-n.m.r. (270 MHz, $CDCl_3$): δ 5.26 (dd, 1 H, $J_{2',3'}$ 3.4, $J_{3',4'}$ 10 Hz, H-3'), 5.09 (m, 2 H, $J_{1',2'}$ 1.5, $J_{3,4}$ 9.5, $J_{4,5}$ 9.5 Hz, H-2' and -4), 5.08 (dd, 1 H, $J_{4',5'}$ 10.3 Hz, H-4'), 4.91 (d, 1 H, H-1'), 4.70 (d, 1 H, $J_{1,2}$ <1 Hz, H-1), 4.01 (dd, 1 H, $J_{5,6}$ 6 Hz, H-5), 4.0–3.95 (m, 1 H, H-2), 3.95 (dd, 1 H, $J_{2,3}$ 3 Hz, H-3), 3.74 (dd, 1 H, $J_{5',6'}$ 6 Hz, H-5'), 3.36 (s, 3 H, OMe), 2.15, 2.17, 2.05, and 1.99 (4s, 4 × 3 H, OAc), 1.21 and 1.20 (2d, 2 × 3 H, H_3-6 and -6').

Anal. Calc. for $C_{21}H_{32}O_{13}$: C, 51.22; H, 6.55; O, 42.23. Found: C, 51.02; H, 6.60; O, 42.35.

Methyl 4-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**4**). — Compound **4** was obtained amorphous (m.p. 66.5–68.5°), $[\alpha]_D^{20}$ −56° (*c* 1.0, chloroform); t.l.c. (*A*): R_F 0.3; ν_{max}^{KBr} 3475 (OH), 1740 (CO), 1230, and 1045 cm^{-1}; ^{1}H-n.m.r. (270 MHz, $CDCl_3$): δ 5.37 (dd, 1 H, $J_{1',2'}$ 1.9, $J_{2',3'}$ 3.7 Hz, H-2'), 5.31 (dd, 1 H, $J_{3',4'}$ 9.9 Hz, H-3'), 5.07 (dd, 1 H, $J_{4',5'}$ 9.9 Hz, H-4'), 4.96 (d, 1 H, H-1'), 4.82 (dd, 1 H, $J_{3,4}$ 9.1, $J_{4,5}$ 9.0 Hz, H-4), 4.75 (d, 1 H, $J_{1,2}$ <1 Hz, H-1), 4.04 (dd, 1 H, $J_{5',6'}$ 6.0 Hz, H-5'), 3.94–3.87 (m, 2 H, H-3 and -2), 3.75 (dd, 1 H, $J_{5,6}$ 6.0 Hz, H-5), 3.35 (s, 3 H, OMe), 2.13, 2.1, 2.05, and 1.98 (4s, 4 × 3 H, OAc), 1.23 and 1.21 (2d, 2 × 3 H, H_3-6 and -6').

Anal. Calc. for $C_{21}H_{32}O_{13}$: C, 51.22; H, 6.55; O, 42.23. Found: C, 51.13; H, 6.59; O, 42.30.

1,3,4-Tri-O-acetyl-2-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranose (**5**). — A solution of **4** (350 mg, 0.71 mmol) in acetic anhydride (2.5 mL) was stirred for 12 h with 2% (v/v) conc. sulfuric acid in acetic anhydride (4 mL). The mixture was then diluted with chloroform (50 mL), and washed successively with water, saturated sodium hydrogencarbonate solution, and water, and evaporated. The resulting syrup was chromatographed on silica gel (*A*) to give 210 mg (53%) of **5** as an amorphous powder (m.p. 57–60°), $[\alpha]_D^{20}$ −47° (*c* 1.0, chloroform); t.l.c. (*A*): R_F 0.43; ν_{max}^{KBr} 1750 (CO), 1377, 1225, and 1050 cm^{-1}; ^{1}H-n.m.r. (270 MHz, $CDCl_3$): δ 6.05 (d, 1 H, $J_{1,2}$ 2.0 Hz, H-1), 5.33 (dd, 1 H, $J_{2',3'}$ 3.9, $J_{3',4'}$ 10.2 Hz, H-3'), 5.26 (dd, 1 H, $J_{1',2'}$ 1.5 Hz, H-2'), 5.20 (dd, 1 H, $H_{2,3}$ 3.5, $J_{3,4}$ 10.6 Hz, H-3), 5.11 (dd, 1 H, $J_{4',5'}$ 9 Hz, H-4'), 5.08 (dd, 1 H, $J_{4,5}$ 2.5 Hz, H-4), 4.84 (d, 1 H, H-1'),

4.01 (m, 1 H, H-2), 3.96 (dd, 1 H, $J_{5,6}$ 10 Hz, H-5), 3.90 (dd, 1 H, $J_{5',6'}$ 5.5 Hz, H-5'), 2.15, 2.09, 2.05, 2.0 (4s, 4 × 3 H, OAc), 1.26 and 1.22 (2d, 2 × 3 H, H_3-6 and -6'); lit.[7] m.p. 118–120°, $[\alpha]_D$ −48° (chloroform).

Anal. Calc. for $C_{24}H_{34}O_{15}$: C, 51.25; H, 6.09; O, 42.66. Found: C, 51.08; H, 6.05; O, 42.57.

Methyl 4-O-acetyl-2-O-chloroacetyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranoside (**7**). — A solution of **6** (122 mg, 0.25 mmol) in dry acetonitrile (3 mL) was cooled to 0°, and pyridine (25 μL) and a solution of chloroacetyl chloride (25 μL) in acetonitrile (1 mL) were successively added. The solvent was evaporated after 12 h, and the syrupy residue was dried by addition and evaporation of toluene (10 mL), and dissolved in chloroform (10 mL). The solution was washed with water (5 mL), saturated sodium hydrogencarbonate solution (5 mL), and water (5 mL), and evaporated. The residue was chromatographed on silica gel (*A*) to give **7** (110 mg, 77%) as an amorphous compound (m.p. 57–59°), $[\alpha]_D^{20}$ −38° (*c* 1.2, chloroform); t.l.c. (*A*): R_F 0.6; ν_{max}^{KBr} 1745 (CO), 1375, 1220, 1135, 1085, and 1035 cm^{-1}; ^{1}H-n.m.r. (270 MHz, $CDCl_3$): δ 5.24 (dd, 1 H, $J_{1,2}$ 1.2, $J_{2,3}$ 3.5 Hz, H-2), 5.15 (dd, 1 H, $J_{2',3'}$ 3.2, $J_{3',4'}$ 10.5 Hz, H-3'), 5.07 (dd, 1 H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4), 5.04 (dd, 1 H, $J_{4',5'}$ 9.5 Hz, H-4'), 5.03 (dd, 1 H, $J_{1',2'}$ 1.6 Hz, H-2'), 4.89 (d, 1 H, H-1'), 4.65 (d, 1 H, H-1), 4.40 (dd, 1 H, H-3), 4.26 and 4.19 (2d, 2 × 1 H, $J_{2a,2b}$ 15 Hz, $-CH_2-$), 3.86 (dd, 1 H, $J_{5',6'}$ 6.5 Hz, H-5'), 3.77 (dd, 1 H, $J_{5,6}$ 6.5 Hz, H-5), 3.38 (s, 3 H, OMe), 2.13, 2.05, and 2.0 (3s, 1 × 3 H and 2 × 3 H, OAc), 1.21 and 1.19 (2d, 2 × 3 H, H_3-6 and -6').

Anal. Calc. for $C_{23}H_{33}ClO_{14}$: C, 48.55; H, 5.85; Cl, 6.23; O, 39.37. Found: C, 48.67; H, 6.02; Cl, 6.31; O, 39.60.

1,2,4-Tri-O-acetyl-3-O-(2,3,4-tri-O-acetyl-α-L-rhamnopyranosyl)-α-L-rhamnopyranose (**8**). — Compound **6** (500 mg, 0.89 mmol) was acetolyzed with acetic anhydride–sulfuric acid (2.5 mL) for 8 h, and the resulting product processed as described for **5** to give 320 mg (56%) of **8** as an amorphous powder (m.p. 78–80.5°), $[\alpha]_D^{20}$ −37° (*c* 1.0, chloroform); t.l.c. (*A*): R_F 0.56; ν_{max}^{KBr} 1750 (CO), 1380, 1230, and 1050 cm^{-1}; ^{1}H-n.m.r. (270 MHz, $CDCl_3$): δ 5.98 (d, 1 H, $J_{1,2}$ 1.9 Hz, H-1), 5.22–5.14 (m, 2 H, $J_{2,3}$ 3.6, $J_{3,4}$ 9.9, $J_{4,5}$ 6.5 Hz, H-2 and -4), 5.14 (dd, 1 H, $J_{2',3'}$ 4.1, $J_{3',4'}$ 7.5 Hz, H-3'), 5.04 (dd, 1 H, $J_{4',5'}$ 6 Hz, H-4'), 5.02 (dd, 1 H, $J_{1',2'}$ 1.7 Hz, H-2'), 4.91 (d, 1 H, H-1'), 4.09 (dd, 1 H, H-3), 3.94–3.78 (m, 2 H, $J_{5,6}$ 9, $J_{5',6'}$ 9 Hz, H-5 and -5'), 2.2, 2.12, 2.02, and 1.96 (4s, 4 × 3 H, OAc), 1.22 and 1.18 (2d, 2 × 3 H, H-6 and -6'); [lit.[6] amorphous, $[\alpha]_D^{25}$ −32° (*c* 4.5, chloroform); t.l.c. (1 : 1, v/v, benzene–ether): R_F 0.75; ^{1}H-n.m.r. (100 MHz, $CDCl_3$): δ 6.02 (d, $J_{1,2}$ 1.8 Hz, H-1), 5.25 ($J_{2,3}$ 3.5 Hz, H-2 and -2'), 5.14 ($J_{4',5'}$ 10 Hz, H-4 and -4'), 5.05 (H-3), 4.96 (d, $J_{1',2'}$ 1.0 Hz, H-1'), 3.92 (m, $J_{5,6}$ 6.0 Hz, H-5), 3.78 (m, $J_{5',6'}$ 6.0 Hz, H-5'), 1.20 (d, H-6), and 1.18 (d, H-6'); lit.[7] m.p. 75–76°, $[\alpha]_D$ −38° (chloroform)].

Anal. Calc. for $C_{24}H_{34}O_{15}$: C, 51.25; H, 6.09. Found: C, 51.04; H, 6.35.

2-O-α-L-Rhamnopyranosyl-L-rhamnopyranose (**10**). — Compound **5** (210 mg, 0.37 mmol) was treated with 0.1M sodium methoxide (20 mL) for 2 h at room temperature. The solution was passed through a column of Dowex-50 (H^+) and evaporated

to give amorphous **10** (78%) (m.p. 80–85°), $[\alpha]_D^{20}$ −21° (*c* 1.0, water); t.l.c. (*B*): R_F 0.24; ν_{max}^{KBr} 3410 (OH), 2945, and 1065 cm^{-1}; ^{1}H-n.m.r. (270 MHz, D_2O): δ 5.22 (s, 1 H, H-1′), 4.96 (s, 0.75 H, H-1, α anomer), 4.88 (s, 0.25 H, H-1, β anomer), 4.16–3.65 (m, 6 H, $J_{4,5} = J_{4',5'} = 7$ Hz, H-2,-2′,-3, -3′, -4, and -4′), 3.51–3.38 (m, 2 H, $J_{5,6} = J_{5',6'} = 7$ Hz, H-5 and -5′), 1.28 and 1.27 (2d, 2 H, H-6 and -6′); [lit.[5] amorphous, $[\alpha]_D^{25}$ −28.27°; ^{1}H-n.m.r. (D_2O, 270 MHz): δ 5.24 (s, 0.9 H, H-1), 5.04 (d, 0.1 H, *J* 2 Hz), 4.98 (d, 0.9 H, $J_{1',2'}$ 1.7 Hz, H-1′), and 4.90 (s, 0.1 H)].

Anal. Calc. for $C_{12}H_{22}O_9$: C, 46.45; H, 7.15; O, 46.40. Found: C, 46.15; H, 7.29; O, 46.14.

Methyl 3-O-α-L-rhamnopyranosyl-α-L-rhamnopyranoside (**9**). — Compound **6** (80 mg, 0.16 mmol) was deacetylated with sodium methoxide (0.1M, 5 mL) for 2 h at room temperature. Neutralization with Dowex-50 (H^+), chromatography (silica gel, *B*), and crystallization from diisopropyl ether–methanol gave **9** (41 mg, 79%), m.p. 181–183.5°, $[\alpha]_D^{20}$ −59° (*c* 1.0, chloroform); t.l.c. (*B*): R_F 0.37; ν_{max}^{KBr} 3450 (OH), 2940, 1135, 1070, and 985 cm^{-1}; ^{1}H-n.m.r. (60 MHz, D_2O): δ 5.05 (d, 1 H, $J_{1',2'}$ 2 Hz, H-1′), 3.42 (s, 3 H, OMe), 1.28 (d, 2 × 3 H, $J_{5,6}$ ~6 Hz, $J_{5',6'}$ ~6 Hz, H-6 and -6′). Oxidation with an excess of sodium metaperiodate, followed by hydrolysis, gave L-rhamnose (t.l.c.). Despite extensive drying, the compound retained traces of diisopropyl ether.

Anal. Calc. for $C_{13}H_{24}O_9 \cdot 0.1C_6H_{14}O$: C, 48.82; H, 7.65; O, 43.52. Found: C, 49.02; H, 7.40; O, 43.59.

3-O-α-L-Rhamnopyranosyl-L-rhamnopyranose (**11**). — Compound **8** (220 mg, 0.39 mmol) was deacetylated, as described for the preparation of **9**, to give a residue that crystallized from methanol–chloroform (105 mg, 86%), m.p. 141–142°, $[\alpha]_D^{20}$ −41° (*c* 0.9, water); t.l.c. (*B*): R_F 0.23; ν_{max}^{KBr} 3380 (OH), 2945, 1378, and 1060 cm^{-1}; ^{1}H-n.m.r. (270 MHz, D_2O): δ 5.07 (s, 1 H, H-1′), 5.04 (s, 0.7 H, H-1, α anomer), 4.87 (s, 0.3 H, H-1, β anomer), 4.10–3.74 (m, 6 H, H-2,-2′,-3,-3′,-4, and -4′), 3.56–3.38 (m, 2 H, $J_{5,6} = J_{5',6'} = 6$ Hz, H-5 and -5′), 1.28 and 1.26 (2d, 2 H, H-6 and -6′); lit.[6] amorphous, $[\alpha]_D^{25}$ −21° (*c* 3.2, water).

Anal. Calc. for $C_{12}H_{22}O_9$: C, 46.45; H, 7.15; O, 46.40. Found: C, 46.40; H, 7.23; O, 46.08.

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