Synthesis of 2-*O*-, 3-*O*-, and 4-*O*-β-D-Glucopyranosyl-L-rhamnose, and of 2-*O*-β-D-Galactopyranosyl-L-rhamnose[1]

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Received August 16, 1974

RUSSELL R. KING and CLAUDE T. BISHOP. Can. J. Chem. **52**, 3913 (1974).

Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with benzyl-α-L-rhamnopyranoside in the presence of silver carbonate yielded benzyl 2-*O*-, 3-*O*-, and 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranosides in a molar ratio of 1.0:3.6:1.6. The products were separated by preparative t.l.c. Deacetylation followed by hydrogenolysis afforded the unsubstituted disaccharides. Proton magnetic resonance spectra of each derivative and methylation analysis of the disaccharide benzyl glycosides provided confirmation of the structures. The preparation, by a similar method, of benzyl 2-*O*-β-D-galactopyranosyl-α-L-rhamnopyranoside, characterized as its crystalline acetate, is also described.

RUSSELL R. KING et CLAUDE T. BISHOP. Can. J. Chem. **52**, 3913 (1974).

La réaction du bromure de 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyle avec benzyl-α-L-rhamnopyranoside, en présence du carbonate d'argent, conduit aux benzyl 2-*O*-, 3-*O*-, et 4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranosides dans la proportion molaire de 1.0:3.6:1.6. On a separé les trois produits par la chromatographie sur couche mince. La déacétylation suivit par l'hydrogenolyse conduit aux disaccharides non substitués. La spectroscopie r.m.n. de chaque dérivatif et l'analyse par méthylation des glycosides benzyliques des disaccharides ont confirmé les structurs. On décrit aussi la synthèse, par un procédé semblable, de benzyl 2-*O*-β-D-galactopyranosyl-α-L-rhamnopyranoside, caracterisé comme son cristallin dérivatif acetylé.

The successful syntheses of 3-*O*- and 4-*O*-β-D-galactopyranosyl-L-rhamnose and of 3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-rhamnose (1) indicated a potential route to a number of other useful rhamnose-containing disaccharides. That work showed that the variation in reactivity of hydroxyl groups in benzyl-α-L-rhamnopyranoside resulted in preferential formation of the 3-*O*-linked disaccharide and that secondary products could be recovered by chromatography.

The present paper reports an extension of those studies to clarify the generality of the method and to provide β-D-glucopyranosyl-rhamnoses for structural and serological studies on a number of bacterial polysaccharides that are reported (2) to contain these two sugars.

Reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**1**) with benzyl-α-L-rhamnopyranoside (**2**) in the presence of silver carbonate furnished, by t.l.c., two seemingly homogeneous disaccharide fractions. One of these was oxidizable by periodate indicating a 1 → 2 or 1 → 4 linkage. The periodate resistant fraction crystallized readily and t.l.c. of a fully acetylated sample confirmed that the compound was homogeneous. It was tentatively assigned the structure, benzyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**3**).

The fraction that was oxidizable by periodate also gave an isopropylidene derivative when reacted with acetone and concentrated sulfuric acid. This result suggested that the material was predominantly the 1 → 4 linked derivative (**4**). However, because the compound could not be crystallized its homogeneity was checked by t.l.c. of a fully acetylated sample. Another minor component was thereby revealed and the mixture was separated to yield two crystalline hexaacetates tentatively identified as benzyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**6**, major component) and benzyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside (**7**, minor component).

Deacetylation of the tetraacetate **3** and the hexaacetates **6** and **7** yielded benzyl 3-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (**8**), benzyl 4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (**9**), and benzyl 2-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside (**10**). Hydro-

[1]Issued as NRCC No. 14271.

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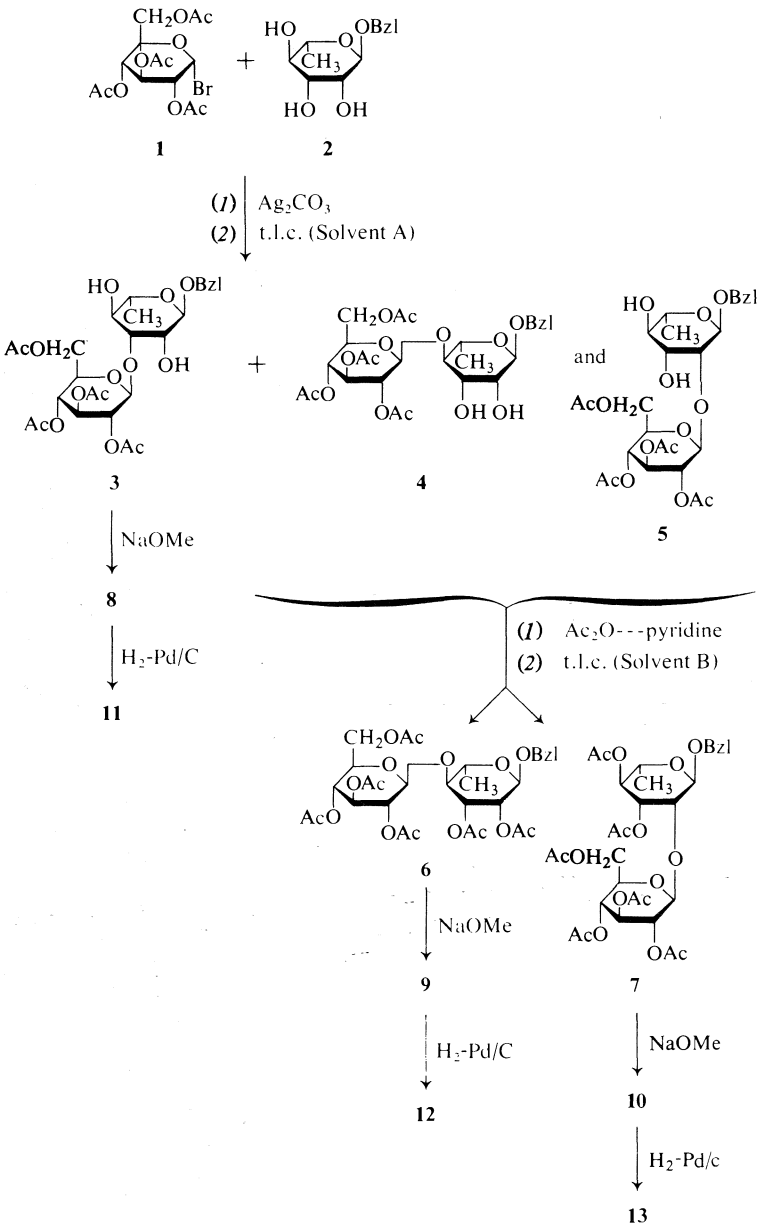

genolysis of these benzyl glycosides furnished the disaccharides 3-*O*-β-D-glucopyranosyl-L-rhamnose (**11**), 4-*O*-β-D-glucopyranosyl-L-rhamnose (**12**), and 2-*O*-β-D-glucopyranosyl-L-rhamnose (**13**). The p.m.r. spectra of the disaccharides **11**, **12**, and **13** indicated $J_{1',2'} =$ 7.5, 8.5, and 7.5 Hz, respectively, consistent with a β-D-glucopyranosyl linkage (3). The structures were confirmed by methylation analysis of the benzyl glycosides **8**, **9**, and **10**, the hydrolysis products after methylation being characterized by mass spectrometry of their alditol acetates (4).

Both the optical rotation and the p.m.r. spectrum of compound **12** were similar to those given by the disaccharide derived from condensation of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide with methyl 2,3-*O*-isopropylidene-α-L-rhamnopyranoside in the presence of mercuric cyanide in acetonitrile (5). A disaccharide identified as 3-*O*-β-D-glucopyranosyl-L-rhamnose by methylation analysis and

p.m.r. spectroscopy has been isolated recently by partial hydrolysis of *Klebsiella* K72 capsular polysaccharide (6). The p.m.r. spectrum reported for that compound is in agreement with that found for the synthetic product, **11**.

Discovery of the $1 \rightarrow 2$ linked disaccharide in the glucosyl-L-rhamnose series prompted a reexamination of the products from reaction of 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide with benzyl α-L-rhamnopyranoside (1). Following the same separation scheme, t.l.c. of the acetylated, periodate-sensitive fraction from this latter condensation led to isolation of benzyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-L-rhamnopyranoside (**14**). The structure was confirmed by methylation analysis of the benzyl glycoside and p.m.r. spectroscopy of the free disaccharide as outlined above.

It may be noted that yields of disaccharides from benzyl α-L-rhamnopyranoside were in the same order, *i.e.* $1 \rightarrow 3 > 1 \rightarrow 4 > 1 \rightarrow 2$, with either the D-glucosyl or D-galactosyl halide tetraacetate. There is little doubt that this order of reactivity is determined almost exclusively by the relative reactivities of the free hydroxyls in the aglycone and that similar results would be obtained with other glycosyl halide tetraacetates. The method therefore provides a facile route to other disaccharides in which L-rhamnose is the aglycone. Furthermore, this condensation of unprotected glycosides with glycosyl halides and subsequent separation of products offers an attractive route to disaccharides where a specifically blocked aglycone is either inaccessible or difficult to prepare. Clearly, the greater reactivity of primary hydroxyl groups would lead to a predominance of $1 \rightarrow 6$ linked disaccharides from hexopyranosides. However, specific blocking at that position is easily achieved, *e.g.* *O*-trityl group, thus opening the route for reaction at the other hydroxyls.

Experimental

Melting points are uncorrected and were determined on a Kofler hot stage microscope. Optical rotations were measured with a Perkin-Elmer 141 polarimeter and are equilibrium values unless stated otherwise. Proton magnetic resonance spectra were recorded on a Varian Anaspect EM360 NMR spectrometer with tetramethylsilane as an internal standard except as noted. A Hewlett-Packard Model 402 gas chromatograph with a flame ionization detector was used for g.l.c.; the column was a glass U-tube (150 × 0.3 cm) filled with 3% SE30 on Chromosorb W. Thin-layer chromatograms were run on glass plates coated with Silica Gel F-254 unless noted otherwise. Solvent mixtures used were (v/v): (A) ethyl acetate – petroleum ether (40–60), 3:2, (B) ethyl acetate – petroleum ether 4:5, (C) ethyl acetate – petroleum ether, 3:7, (D) *n*-butanol–pyridine–water, 6:4:3. Separated components were detected by u.v. irradiation (for all benzyl glycosides) or by charring after a spray of 5% sulfuric acid in ethanol. Reducing sugars were detected by *p*-anisidine. Mass spectra were recorded on a Hitachi RMU-6D mass spectrometer.

Reaction of 2,3,4,6-Tetra-O-acetyl-α-D-glucopyranosyl Bromide with Benzyl α-L-Rhamnopyranoside

Benzyl α-L-rhamnopyranoside, **2** (1) (2.8 g) was dissolved in chloroform (70 ml) and the solution was stirred with silver carbonate (6 g) and Drierite (10 g) for 18 h. 2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl bromide, **1** (7) (6.5 g) was added to the stirred mixture over a period of 1 h and stirring was continued for 18 h. The inorganic salts were removed by filtration, washed with chloroform (2 × 50 ml), and the combined filtrate and washings were evaporated to dryness under diminished pressure. The sirupy residue was fractionated by t.l.c. (solvent A) to yield two fractions.

The fraction (734 mg) of higher mobility crystallized from ether. The compound did not react with an excess of aqueous sodium metaperiodate at 24° and it was therefore designated as benzyl 3-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside, **3**; m.p. 138–139°; $[\alpha]_D^{24}$ −37.5° (*c*, 1.0 in chloroform); p.m.r. ($CDCl_3$) τ 2.62 (5H singlet, aromatic protons), 7.96 (12H singlet, 4 × 0Ac), 8.65 (3H doublet, $J = 6.0$ Hz, CH_3).

Anal. Calcd. for $C_{27}H_{36}O_{14}$: C, 55.47; H, 6.17. Found: C, 55.41; H, 5.91.

As a further test of homogeneity this fraction was fully acetylated (acetic anhydride and pyridine) and the hexaacetate was examined by t.l.c. (solvent B); only a single component was found.

The fraction (520 mg) of lower mobility was sensitive to oxidation by aqueous sodium metaperiodate (reaction monitored t.l.c.). Reaction of a small sample of this fraction with acetone containing a trace of concentrated sulfuric acid gave rise to a new, faster running compound on thin-layer chromatograms (solvent B) indicating that an *O*-isopropylidene derivative had been formed. Acetylation of this fraction (500 mg) with acetic anhydride (10 ml) and pyridine (15 ml) yielded a mixture of hexaacetates that was separable into two compounds by t.l.c. in solvent B.

The hexaacetate of higher mobility in solvent B crystallized from ether and was shown subsequently to be benzyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside, **7** (149 mg): m.p. 147–148°; $[\alpha]_D^{24}$ −46.6° (*c*, 1.0 in chloroform).

Anal. Calcd. for $C_{31}H_{40}O_{16}$: C, 55.69; H, 5.99. Found: C, 55.83; H, 6.02.

The hexaacetate of lower mobility in solvent B crystallized from absolute ethanol and was shown subsequently to be benzyl 2,3-di-*O*-acetyl-4-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranosyl)-α-L-rhamnopyranoside, **6** (232 mg): m.p. 104–105°; $[\alpha]_D^{24}$ −76.8° (*c*, 1.0 in chloroform).

Anal. Calcd. for $C_{31}H_{40}O_{16}$: C, 55.69; H, 5.99. Found: C, 55.75; H, 6.08.

*Benzyl 2-O-, 3-O-, and 4-O-β-D-Glucopyranosyl-α-L-rhamnopyranoside (**10**, **8**, and **9**)*

The tetraacetate **3** and the hexaacetates **6** and **7** (690, 210, and 140 mg, respectively) were each deacetylated in methanol (10 ml) by sodium methoxide (0.1 *N*, 25, 10, and 10 ml, respectively). The solutions were deionized with Amberlite IR-120 and evaporated to dryness to yield the following benzyl glycosides.

(*a*) Benzyl 3-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside, **8** (506 mg) was obtained as a sirup; $[\alpha]_D^{24}$ −68.4° (*c*, 1.0 in ethanol); p.m.r. (D_2O, external TMS) τ, 2.67 (5H singlet, aromatic protons), 5.15 (1H doublet, $J_{1,2}$ = 1.6 Hz, H-1), 5.41 (1H doublet, $J_{1',2'}$ = 7.4 Hz, H′-1), 5.44 (2H doublet, J = 3.0 Hz, benzylic protons), 8.70 (3H doublet, J = 6.0 Hz, CH_3).

Anal. Calcd. for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 54.58; H, 6.54.

(*b*) Benzyl 4-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside, **9** (129 mg) crystallized from acetone, m.p. 96–98°; $[\alpha]_D^{24}$ −75.6° (*c*, 1.0 in ethanol); p.m.r. (D_2O, external TMS) τ, 2.68 (5H singlet, aromatic protons), 5.25 (1H doublet, $J_{1,2}$ = 1.5 Hz, H-1), 5.30 (1H doublet, $J_{1',2'}$ = 7.5 Hz, H′-1), 5.51 (2H doublet, J = 2.5 Hz, benzylic protons), 8.70 (3H doublet, J = 6.0 Hz, CH_3).

Anal. Calcd. for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 54.68; H, 6.62.

(*c*) Benzyl 2-*O*-β-D-glucopyranosyl-α-L-rhamnopyranoside, **10** (81 mg) was obtained as a sirup; $[\alpha]_D^{24}$ −48.8° (*c*, 1.0 in ethanol); p.m.r. (D_2O, external TMS) τ, 2.64 (5H singlet, aromatic protons), 4.88 (1H doublet, $J_{1,2}$ = 0.8 Hz, H-1), 5.41 (2H doublet, J = 2.5 Hz, benzylic protons), 5.57 (1H doublet, $J_{1',2'}$ = 7.2 Hz, H′-1), 8.74 (3H doublet, J = 6.0 Hz, CH_3).

Anal. Calcd. for $C_{19}H_{28}O_{10}$: C, 54.80; H, 6.78. Found: C, 54.52; H, 6.55.

Methylation Analysis

A portion (10 mg) of each benzyl glycoside (**8**, **9**, and **10**) was methylated (8) with methyl iodide (0.1 ml) and sodium hydride (20 mg) in *N*,*N*-dimethylformamide (5 ml). Each product was purified by t.l.c. (solvent A), hydrolyzed (0.54 *M* sulfuric acid, 1.0 ml, 100°, 6 h), reduced by sodium borohydride, and acetylated (acetic anhydride – pyridine) to furnish a 1:1 mixture (determined by g.l.c.) of two partially methylated alditol acetates. Each mixture was separated by t.l.c. (solvent C) and the products were identified by mass spectrometry (4) as follows: 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-glucitol (*m/e* 117, 161, 205) from each benzyl glycoside **8**, **9**, and **10**; 1,3,5-tri-*O*-acetyl-2,4-di-*O*-methyl-L-rhamnitol (*m/e* 117, 131, 233) from compound **8**; 1,4,5-tri-*O*-acetyl-2,3-di-*O*-methyl-L-rhamnitol (*m/e* 117, 203) from compound **9**; 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol (*m/e* 131, 189) from compound **10**. The identification of these partially methylated L-rhamnitol acetates proved that the benzyl glycosides **8**, **9**, and **10** were respectively the 1 → 3, 1 → 4, and 1 → 2 linked disaccharide derivatives.

*2-O-, 3-O-, and 4-O-β-D-Glucopyranosyl-L-rhamnose (**13**, **11**, and **12**)*

Each benzyl glycoside **8**, **9**, and **10** (400, 112, and 70 mg, respectively) in 95% ethanol (25 ml) was shaken overnight with palladium black (50 mg) under hydrogen at atmospheric pressure. The catalyst was removed by filtration and evaporation of the filtrates yielded the following disaccharides as sirups.

(*a*) 2-*O*-β-D-Glucopyranosyl-L-rhamnose (**13**, 44 mg) showed only one spot on t.l.c. (cellulose, solvent D) with $R_{glucose}$ = 1.38; its trimethylsilyl derivative gave a single peak on g.l.c. with $T_{sucrose}$ = 0.87; $[\alpha]_D^{24}$ +9.5° (*c*, 2.0 in water); p.m.r. (D_2O, external TMS) τ, 4.64 (⅔H doublet, $J_{1,2}$ = 1.5 Hz, H-1 of α-L-form), 5.19 (⅓H doublet, $J_{1,2}$ = 1.0 Hz, H-1 of β-L-form), 5.43 (1H doublet, $J_{1',2'}$ = 8.5 Hz, H′-1), 8.75 (3H doublet, J = 6.0 Hz, CH_3).

(*b*) 3-*O*-β-D-Glucopyranosyl-L-rhamnose (**11**, 272 mg) also showed one spot on t.l.c. (cellulose, solvent D), $R_{glucose}$ = 1.42 and one peak on g.l.c. of its trimethylsilyl derivative, $T_{sucrose}$ = 0.94; $[\alpha]_D^{24}$ −11.4° (*c*, 2.0 in water); p.m.r. (D_2O, external TMS) τ, 4.96 (⅔H doublet, $J_{1,2}$ = 1.8 Hz, H-1 of α-L-form), 5.19 (⅓H doublet, $J_{1,2}$ = 1.0 Hz, H-1 of β-L-form), 5.40 (1H doublet, $J_{1',2'}$ = 7.5 Hz, H′-1), 8.77 (3H doublet, J = 6.0 Hz, CH_3).

(*c*) 4-*O*-β-D-Glucopyranosyl-L-rhamnose (**12**, 68 mg) showed one spot on t.l.c. (cellulose, solvent D), $R_{glucose}$ = 1.24, and one peak on g.l.c. of its trimethylsilyl derivative, $T_{sucrose}$ = 0.92; $[\alpha]_D^{24}$ −24.5° (*c*, 1.0 in H_2O) (lit. (5) $[\alpha]_D$ −24.2° (in water)); p.m.r. (D_2O, external TMS) τ, 4.87 (⅔H doublet, $J_{1,2}$ = 1.5 Hz, H-1 of α-L-form), 5.12 (⅓H doublet, $J_{1,2}$ = 1.0 Hz, H-1 of β-L-form), 5.27 (1H doublet, $J_{1',2'}$ = 7.5 Hz, H′-1), 8.77 (3H doublet, J = 6.0 Hz, CH_3).

Isolation of 2-O-β-D-Galactopyranosyl-L-rhamnose

The mixture obtained from reaction of benzyl α-L-rhamnopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-galactopyranosyl bromide (1) was separated into periodate-resistant and periodate-sensitive fractions by t.l.c. in solvent A. The periodate-sensitive fraction (490 mg) was acetylated with acetic anhydride (14 ml) and pyridine (16 ml). The mixture of hexaacetates was then resolved by t.l.c. in solvent B and the faster running component proved to be benzyl 3,4-di-*O*-acetyl-2-*O*-(2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranosyl)-α-L-rhamnopyranoside (**14**, 149 mg). The compound crystallized from ether and had m.p. 154–155°; $[\alpha]_D^{24}$ −54.2° (*c*, 1.0 in chloroform).

Anal. Calcd. for $C_{31}H_{40}O_{16}$: C, 55.69; H, 5.99. Found: C, 55.66; H, 5.81.

The compound was deacetylated, methylated, hydrolyzed, reduced, and acetylated as described above to yield 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methyl-D-galactitol (*m/e* 45, 117, 161, 205) and 1,2,5-tri-*O*-acetyl-3,4-di-*O*-methyl-L-rhamnitol (*m/e* 131, 189) analyzed by t.l.c. and mass spectrometry as before. These results prove the 1 → 2 disaccharide linkage.

Deacetylation and hydrogenolysis of compound **14** (135 mg) as described above yielded 2-*O*-β-D-galactopyranosyl-L-rhamnose (**15**, 58 mg) as a sirup. It showed only one spot on t.l.c. (cellulose, solvent D); $[\alpha]_D^{24}$ −7.5° (*c*, 2.0 in water); p.m.r. (D_2O, external TMS) τ, 4.91 (⅔H doublet, $J_{1,2}$ = 1.5 Hz, H-1 of α-L-form), 5.16 (⅓H doublet, $J_{1,2}$ = 1.0 Hz, H-1 of β-L-form), 5.34 (1H doublet, $J_{1',2'}$ = 8.0 Hz, H′-1), 8.70 (3H doublet, J = 6.0 Hz, CH_3).

We thank F. P. Cooper for the p.m.r. spectra and H. Seguin for the microanalyses and mass spectra.

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