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Note

The synthesis and characterisation of 6-*O*-L-*glycero*-α-D-*manno*-heptopyranosyl-D-glucopyranose

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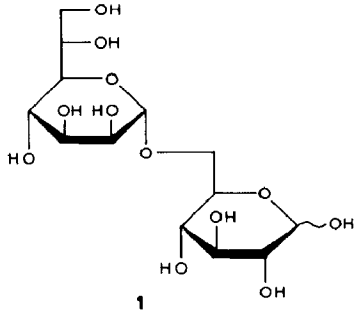
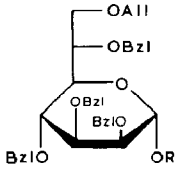
Investigation of the hexose–heptose region of the core oligosaccharide from the lipopolysaccharide (LPS) of *Escherichia coli* K-12 strains W3100 and W3110 indicated L-*glycero*-D-*manno*-heptose to be 6-linked to a D-glucose residue[1]. In order to confirm this unusual substitution and in view of the fact that syntheses of heptose-containing oligosaccharides are still rare[2–6], the synthesis of the title disaccharide **1** was carried out using the imidate method[7].

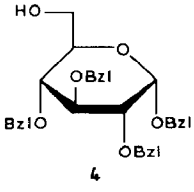
The trichloroacetimidate **3**, prepared from benzyl 7-*O*-allyl-2,3,4,6-tetra-*O*-benzyl-L-*glycero*-α-D-*manno*-heptopyranoside[8] (**2**), when condensed with benzyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside[9] (**4**) in the presence of toluene-*p*-sulfonic acid gave the α- (**5**, 51.3%) and β-linked (**6**, 15.3%) disaccharide derivatives. Deallylation of **5** followed by hydrogenolysis yielded **1**, which was characterised by analytical, physicochemical, and spectral data, and converted into the nona-acetate **7**. Treatment of **1** with methanolic M hydrogen chloride (16 h, room temperature) followed by methylation gave the nona-*O*-methyl derivative **8**.



1



2 R = Bzl
3 R = C(=NH)CCl$_3$



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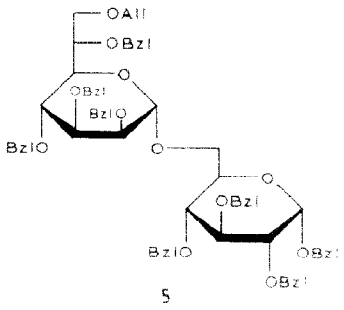

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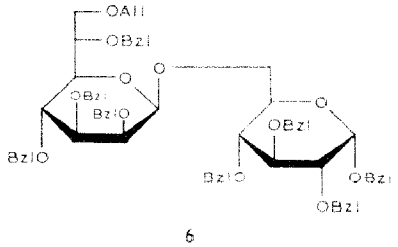


6

For comparison with the disaccharide isolated[1] from LPS of *E. coli* K-12, **1** was reduced ($NaBH_4$) and then acetylated to give 1,2,3,4,5-penta-*O*-acetyl-6-*O*-(2,3,4,6,7-penta-*O*-acetyl-L-*glycero*-D-*manno*-heptopyranosyl)-D-glucitol (**9**). Alternatively, **1** was reduced with NaB^2H_4 and methylated to yield **10**. Acid hydrolysis of **8** followed by reduction (NaB^2H_4) and acetylation gave **11**. The c.i.-mass spectral fragmentation patterns of **7**–**11** are shown in the formulae (Figs. 1–5), and corresponding *T* values are shown in Table I. The ions recorded correspond to characteristic fragments found in other L-*glycero*-D-*manno*-heptose-containing oligosaccharides[10,11]. The mol. wts. of **7**–**11** were determined by c.i.(ammonia)-m.s.

Although the anomers **7** were not separated in the h.p.l.c. system used, the assignment of overlapping signals in the n.m.r. spectrum was achieved using $^1H,^1H$- and $^1H,^{13}C$-COSY methods. The n.m.r. and mass spectral data of synthetic **1** were identical with those of the compound[1] isolated from the core-oligosaccharide of *E. coli* K-12 LPS.

EXPERIMENTAL

General methods. — T.l.c. was performed on Silica Gel G (Merck) and column chromatography on Silica Gel (230–400 mesh, Merck). Optical rotations were measured with a Jasco DIP 360 automatic polarimeter.

1D ^1H-N.m.r. spectra were recorded with Bruker AM 360 L and AM 500 instruments. The homonuclear $^1H,^1H$ chemical-shift-correlated 2D-spectrum [COSY, 90° pulse sequence, spectral width F2 = F1 = 2520 Hz, 2048 × 1024 blocks of data,

TABLE I

Relative retention times (*T*, relative to that of α-D-glucose penta-acetate) of **7**–**11**

Compound	T
7	4.47/4.52[a]
8	2.66/2.81[a]
9	4.55
10	3.18
11	3.35

[a] Values for the anomers.

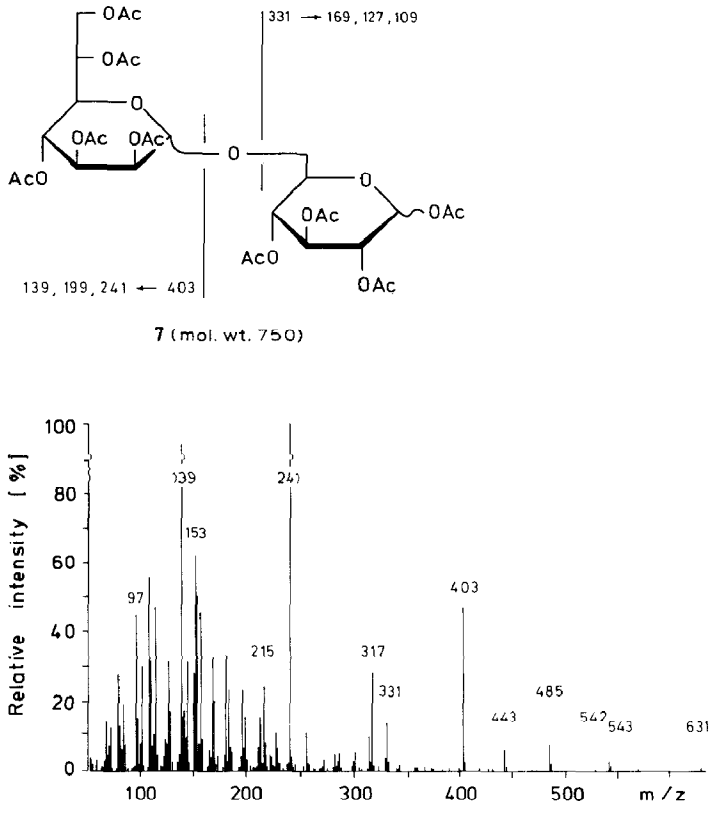


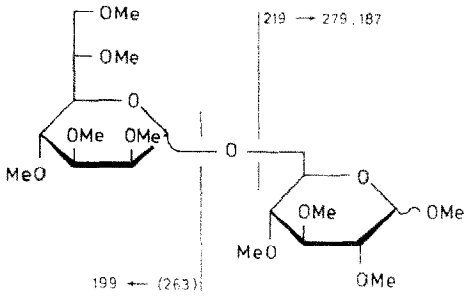
Fig. 1. E.i.-mass spectrum and fragmentation pattern of 7.

processed used sinusoidal multiplication in each dimension followed by symmetrisation of the final data matrix (512 increments in t_1, 48 scans, 1-s relaxation delay)] was recorded in benzene-d_6 at 23° with a Bruker AM 360 L instrument. ^{13}C-N.m.r. spectra were recorded for solutions in $CDCl_3$ and benzene-d_6 at 23°, using Bruker AM 360 L (90 MHz) and AM 500 (125 MHz) spectrometers.

G.l.c. was performed with a Varian 3700 gas chromatograph equipped with a flame-ionisation detector and a fused-silica capillary column (25 m × 0.32 mm i.d.) with chemically bonded SE-54 (0.2 μm, Weeke, Mühlheim) for partially methylated or acetylated alditols [H_2 as carrier gas (0.15 MPa)]. Temperature programme: 150° for 5 min, then 5°/min → 300°. G.l.c.–m.s. was carried out with a Hewlett–Packard 5985 instrument equipped with an SE-54 column and an HP-1000 data system. E.i.-mass spectra were recorded at 70 eV and c.i.-mass spectra were obtained with ammonia as reactant gas. The ion-source temperature was 200°.

Acetylation was effected with acetic anhydride–pyridine overnight with catalytic amounts of 4-dimethylaminopyridine.

6-*O*-L-*glycero*-D-*manno*-heptopyranosyl-D-glucose (**1**) was treated with methanolic M hydrogen chloride (16 h, room temperature), followed by methylation[12,13] to



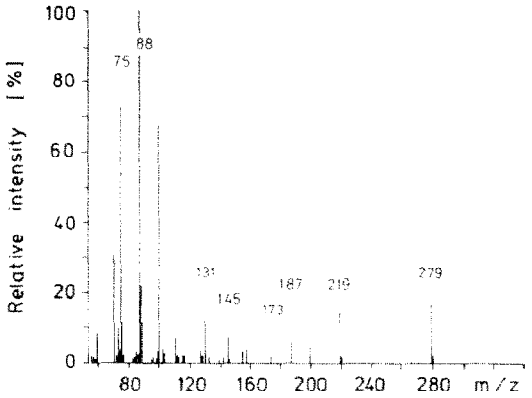
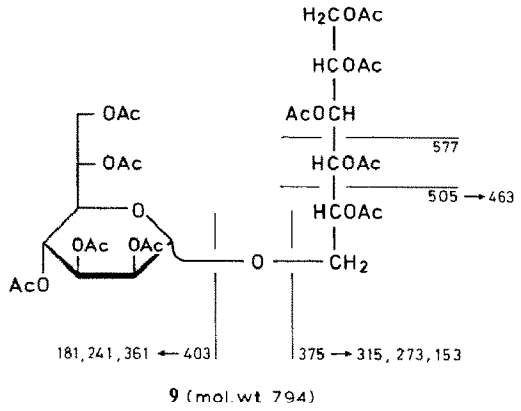


Fig. 2. E.i.-mass spectrum and fragmentation pattern of **8**.

yield **8**, which then was hydrolysed (120°, 1 h) in M trifluoroacetic acid, reduced (NaB^2H_4), and acetylated to give **11**. Reduction ($NaBH_4$ or NaB^2H_4) of **1** and acetylation provided **9** and **10**, respectively.

*Benzyl 6-O-(7-O-allyl-2,3,4,6-tetra-O-benzyl-*L*-glycero-α-* (**5**) *and -β-*D*-manno-heptopyranosyl)-2,3,4-tri-O-benzyl-α-*D*-glucopyranoside* (**6**). — To a solution of 7-*O*-allyl-2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-L-*glycero*-α-D-*manno*-heptopyranose[2] (**3**; 870 mg, 1.15 mmol) and benzyl 2,3,4-tri-*O*-benzyl-α-D-glucopyranoside[9] (**4**; 630 mg, 1.16 mmol) in dichloromethane (8 mL) was added molecular sieve (4 Å, 1.2 g), and the suspension was stirred at room temperature under argon. After 45 min, a solution of anhydrous toluene-*p*-sulfonic acid (60 mg) in dichloromethane (0.5 mL) was added and stirring at room temperature was continued for 24 h. The mixture was poured into saturated aqueous sodium hydrogencarbonate (50 mL) and filtered through a Celite pad, and the organic layer was washed with water, dried, and concentrated. Column chromatography (benzene–ethyl acetate, 9:1) of the residue gave, first, **5** (670 mg, 51.3%), isolated as a thick syrup, $[\alpha]_D^{20}$ +55° (*c* 1.2, chloroform). N.m.r. data ($CDCl_3$): ^1H, *inter alia*, δ 5.00 (d, 1 H, $J_{1',2'}$ 1.7 Hz, H-1'), 4.76 (d, 1 H, $J_{1,2}$ 3.7 Hz, H-1), 4.32–5.20 (Abq, 16 H, 8 PhCH_2), 4.19 (t, 1 H, $J_{2,3} = J_{3,4}$ = 9.5 Hz, H-3), 4.02 (t, 1 H, $J_{4,5}$



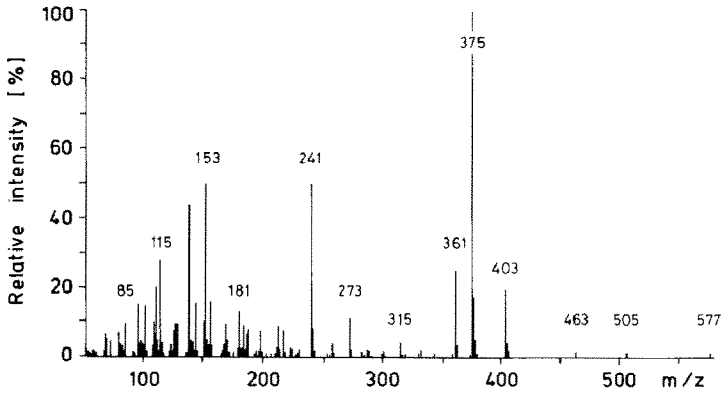


Fig. 3. E.i.-mass spectrum and fragmentation pattern of **9**.

9.3 Hz, H-4); ^{13}C, *inter alia*, δ 98.25 (C-1'), 94.65 (C-1), 82.14 (C-3), 80.11 (C-3'), 80.05 (C-2), 77.68 (C-4), 75.19 (C-2'), 74.33 (C-4'), 74.27 (C-6'), 71.87 (C-5'), 70.31 (C-5), 68.74 (C-7'), 65.62 (C-6).

Anal. Calc. for $C_{72}H_{76}O_{12}\cdot 3H_2O$: C, 72.83; H, 6.96. Found: C, 73.12; H, 6.48.

Eluted second was **6** (200 mg, 15.3%), isolated as a thick syrup, $[\alpha]_D^{24}$ +17° (*c* 1.0, chloroform). N.m.r. data ($CDCl_3$): ^1H, *inter alia*, δ 4.80 (d, 1 H, $J_{1,2}$ 3.6 Hz, H-1), 4.08 (t, 1 H, $J_{3,2} = J_{3,4} = 9.3$ Hz, H-3); ^{13}C, *inter alia*, δ 102.75 (C-1'), 94.78 (C-1), 83.08 (C-3'), 82.76 (C-2'), 82.12 (C-3), 80.06 (C-2), 78.08 (C-4), 75.48, 75.18, 74.14 (C-4',5',6'), 70.26 (C-5), 68.82 (C-7'), 68.63 (C-6).

Anal. Calc. for $C_{72}H_{76}O_{12}\cdot 2H_2O$: C, 73.95; H, 6.89. Found: C, 74.05; H, 6.66.

6-O-L-glycero-α-D-manno-*heptopyranosyl*-α-D-*glucopyranose* (**1**). — To a solution of **5** (640 mg) in methanol (15 mL), oxolane (1 mL), and water (0.5 mL) were added a few crystals of toluene-*p*-sulfonic acid, and the solution was stirred at 40–50°. After 24 h, the mixture was filtered and concentrated to dryness. Column chromatography (light petroleum–ether–methanol, 70:30:1.5) yielded the *O*-deallylated derivative (470 mg, 76%), $[\alpha]_D^{19}$ +63° (*c* 1.0, chloroform).

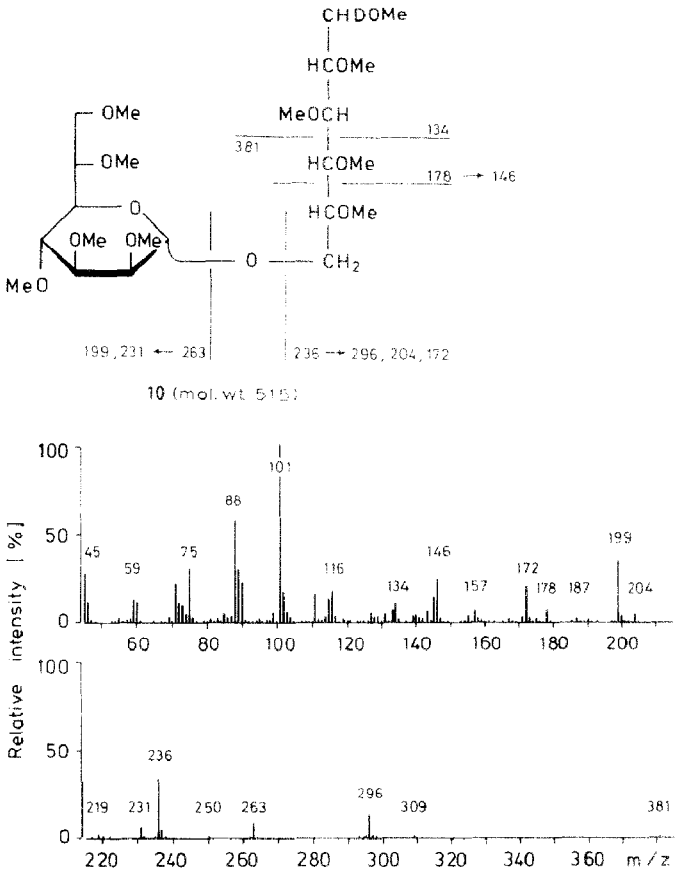


Fig. 4. E.i.-mass spectrum and fragmentation pattern of **10**.

To a solution of this product in ethanol (10 mL) was added 10% Pd/C (600 mg), and the suspension was hydrogenated under atmospheric pressure. After 18 h, the mixture was filtered and concentrated to dryness, to yield **1** (147 mg, 92%), m.p. 100–105°, $[\alpha]_D^{20}$ + 70° (*c* 0.9, methanol), after 1 h. ^{13}C-N.m.r. data (D_2O): δ 101.62 (C-1′β), 101.56 (C-1′α), 98.13 (C-1β), 94.23 (C-1α), 78.00 (C-5β), 76.10 (C-3β), 76.07 (C-2β), 75.06 (C-5α), 73.46 (C-3α), 73.39 (C-5′), 72.89 (C-2′α), 72.87 (C-2′β), 71.92 (C-3′), 71.68 (C-2α), 71.51 (C-4α), 71.45 (C-4β), 70.93 (C-6′α), 70.89 (C-6′β), 68.08 (C-4′), 67.36 (C-6β), 67.23 (C-6α), 65.18 (C-7′α), 65.14 (C-7′β).

Anal. Calc. for $C_{13}H_{24}O_{12}$: C, 41.94; H, 6.50. Found: C, 42.14; H, 7.12.

N.m.r. data (C_6D_6) for the nona-acetate (**7**) of **1**: ^{1}H, *inter alia*, δ 6.64 (d, 1 H, $J_{1,2}$ 3.9 Hz, H-1α), 5.86 (dd, 1 H, $J_{3,4}$ 10.5 Hz*, H-3α), 5.79 (ddd, 2 H, $J_{4,5}$ 10.3 Hz, H-4′α,β), 5.73 (d, 1 H, $J_{1,2}$ 7.4 Hz, H-1β), 5.72 (dd, 1 H, $J_{6,7}$ 4.4 Hz, H-6′α), 5.70 (dd, 1 H, $J_{3,4}$ 9.6 Hz, H-3′β), 5.64 (dd, 2 H, $J_{6,7}$ 4.7 Hz, H-6′β, $J_{3,4}$ 9.7 Hz, H-3′α), 5.62 (dd, 1 H, $J_{2,3}$ 3.1 Hz, H-2′β), 5.59 (dd, 1 H, $J_{2,3}$ 3.2 Hz, H-2′α), 5.39 (ddd, 2 H, $J_{2,3}$ 10.4 Hz*, H-2β, $J_{3,4}$ 10.5 Hz*, H-3β), 5.31 (dd, 1 H, $J_{2,3}$ 10.4 Hz*, H-2α), 5.24 (dd, 1 H, $J_{4,5}$ 10.4 Hz, H-4α), 5.14

* Non-resolved high-order multiplet.

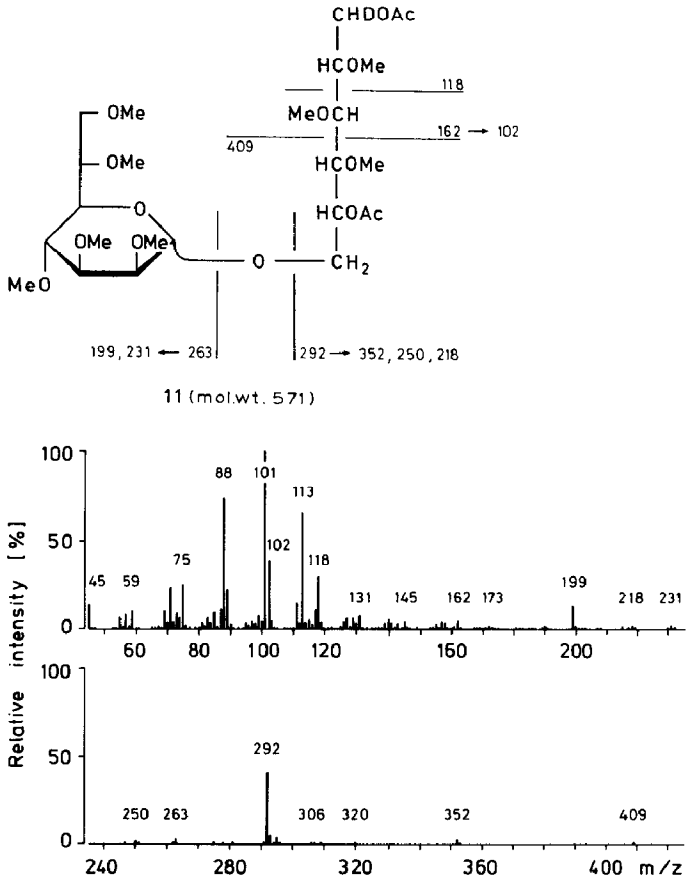


Fig. 5. E.i.-mass spectrum and fragmentation pattern of **11**.

(dd, 1 H, $J_{4,5}$ 9.2 Hz, H-4β), 4.63 (dd, d, 2 H, $J_{7'a,6'}$ 12.1 Hz, H-7'aα, $J_{1',2'}$ 1.7 Hz, H-1'β), 4.52 (d, 1 H, $J_{1',2'}$ 1.5 Hz, H-1'α), 4.50 (dd, 1 H, $J_{7'a,6'}$ 11.9 Hz, H-7'aβ), 4.43 (dd, 1 H, $J_{5',6'}$ 2.4 Hz, H-5'α), 4.40 (d, 2 H, $J_{7'b,6'}$ 8.2 Hz, H-7'bα,β), 4.29 (dd, 1 H, $J_{5',6'}$ 2.2 Hz, H-5'β), 4.15 (ddd, 1 H, $J_{5,6}$ 5.7 Hz, H-5α), 3.59 (dd, 1 H, $J_{6a,5}$ 11.2 Hz, H-6aα), 3.50 (dd, 1 H, $J_{6a,5}$ 10.8 Hz, H-6aβ), 3.38 (dd, 1 H, $J_{6b,5}$ 3.5 Hz, H-6bα), 3.26 (dd, 1 H, $J_{6b,5}$ 3.4 Hz, H-6bβ), 3.22 (ddd, 1 H, $J_{5,6}$ 6.2 Hz, H-5β); ^{13}C: *inter alia*, δ 98.27 (C-1'β), 98.06 (C-1'α), 92.19 (C-1β), 89.48 (C-1α), 73.41 (C-3β), 73.15 (C-5β), 71.09 (C-2β), 70.81 (C-5α), 70.47 (C-3α), 70.04 (C-2'α), 69.93 (C-4,3',5'α), 69.81 (C-2α), 69.70 (C-5'β), 69.65 (C-4,3'β), 69.27 (C-2'β), 67.86 (C-6'α), 67.55 (C-6α), 67.53 (C-6'β), 67.04 (C-6β), 65.05 (C-4'β), 65.02 (C-4'α), 63.21 (C-7'α), 63.10 (C-7'β).

N.m.r. data (C_6D_6) of the alditol deca-acetate derivative (**9**) of **1**: ^{1}H, *inter alia*, δ 5.83 (dd, 1 H, $J_{4,5}$ 6.4 Hz, H-4), 5.79 (dd, 2 H, $J_{3,4}$ 10.3 Hz, H-3, $J_{4',5'}$ 10.0 Hz, H-4'), 5.73 (ddd, 1 H, $J_{2,3}$ 5.7 Hz, H-2), 5.70 (dd, 1 H, $J_{3',4'}$ 10.3 Hz, H-3'), 5.69 (ddd, 1 H, $J_{6',7'}$ 5.0 Hz, H-6'), 5.65 (dd, 1 H, $J_{2',3'}$ 3.5 Hz, H-2'), 5.38 (ddd, 1 H, $J_{5,6}$ 5.4 Hz, H-5), 4.79 (dd, 1 H, $J_{1',2'}$

1.7 Hz, H-1'), 4.61 (dd, 1 H, $J_{1a,2}$ 4.2 Hz, H-1a), 4.56 (dd, 1 H, $J_{7'a,6'}$ 12.1 Hz, H-7'a), 4.40 (dd, 1 H, $J_{7'b,6'}$ 8.0 Hz, H-7'b), 4.29 (dd, 1 H, $J_{5',6'}$ 2.2 Hz, H-5'), 4.10 (dd, 1 H, $J_{1b,2}$ 6.3 Hz, H-1b), 3.69 (dd, 1 H, $J_{6a,7}$ 10.9 Hz, H-6a), 3.59 (dd, 1 H, $J_{6b,7}$ 5.4 Hz, H-6b); ^{13}C, *inter alia*, δ 98.34 (C-1'), 69.93 (C-5'), 69.87 (C-4,6'), 69.74 (C-2'), 69.68 (C-2), 69.14 (C-3), 68.62 (C-5), 67.42 (C-3'), 65.81 (C-6), 64.93 (C-4'), 62.73 (C-7'), 62.08 (C-1).

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