Carbohydrate Research, 135 (1985) 337–341
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

Synthesis of novel bis(D-mannose) compounds

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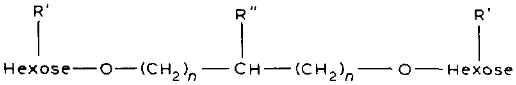
(Received June 4th, 1984; accepted for publication, July 20th, 1984)

Bis(hexoses), which are cross-linked sugars having the general structure **1**, are of interest as potential substrates and inhibitors of cell-membrane transport systems. The bridge length may be varied as well as the position on the sugar to which it is attached. The choice of this position will depend on the specificity of the transport system being studied and, for the mammalian facilitative transport system, specificity investigations have indicated that position 4 in the sugar is most suitable[1–5]. Reactive substituents may be introduced into the bridge in position R″ or into a suitable position on the sugars as indicated by R′.



$$\overset{R'}{\overset{|}{\text{Hexose}}}\text{—O—}(\text{CH}_2)_n\text{—}\overset{R''}{\overset{|}{\text{CH}}}\text{—}(\text{CH}_2)_n\text{—O—}\overset{R'}{\overset{|}{\text{Hexose}}}$$

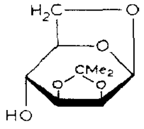
1

We now describe the synthesis of two bis(D-mannose) compounds linked via the 4-positions of the sugar by 2-propylamine and hexane bridges. The amino group was introduced into the bridge for subsequent attachment of such photolabile groups as nitrophenylazides. An advantage of introducing a bulky photoreactive group in the bridge rather than into the sugar moieties is that, in such a position, the bulky group should not interfere with the approach of groups on the transport system to binding positions on the sugar. Compounds of this type are thus called exoaffinity labels[6].

1,3-Bis(4-deoxy-D-mannopyranose-4-yloxy)-2-propylamine was synthesised as follows. 1,6-Anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[7] (**2**) was cross-linked using 1-chloro-2,3-epoxypropane (epichlorohydrin) in the presence of base for 7 days at 70° to give 1,3-bis(1,6-anhydro-4-deoxy-2,3-*O*-isopropylidene-β-D-mannopyranose-4-yloxy)-2-propanol (**3**). Oxidation of **3** with methyl sulphoxide–phosphorus pentaoxide gave 1,3-bis(1,6-anhydro-4-deoxy-2,3-*O*-isopropylidene-β-D-mannopyranose-4-yloxy)-2-propanone (**4**), borohydride reduction of which

0008-6215/85/$ 03.30

regenerated **3**. Reductive amination of **4** with ammonium acetate and sodium cyanoborohydride gave 1,3-bis(1,6-anhydro-4-deoxy-2,3-*O*-isopropylidene-β-D-mannopyranose-4-yloxy)-2-propylamine (**5**). Acid hydrolysis of **5** then gave 1,3-bis(4-deoxy-D-mannopyranose-4-yloxy)-2-propylamine (**6**). The reductive amination was also carried out with cyanoborotritide to yield [2-^{3}H]-1,3-bis(4-deoxy-D-mannopyranose-4-yloxy)-2-propylamine.



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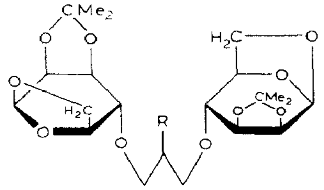
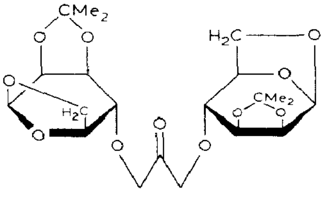


3 R = OH

5 R = NH$_2$



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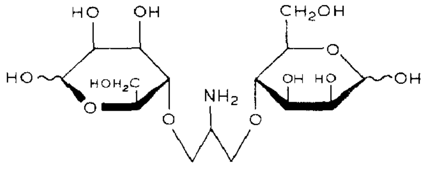

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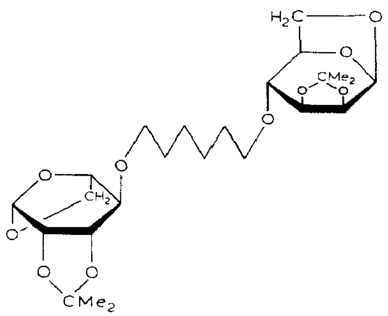

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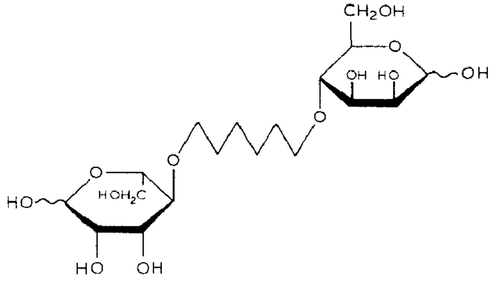

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1,6-Anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (**2**) was also cross-linked with dibromohexane. This reaction was slow but gave a good yield of 1,6-bis(1,6-anhydro-4-deoxy-2,3-*O*-isopropylidene-β-D-mannopyranose-4-yloxy)-hexane (**7**), acid hydrolysis of which yielded 1,6-bis(4-deoxy-D-mannopyranose-4-yloxy)hexane (**8**).

EXPERIMENTAL

Melting points were determined with a Gallenkamp melting-point apparatus and are uncorrected. Reactions were monitored by t.l.c. on Keiselgel G60 (Merck), using the solvents indicated. ^{1}H-N.m.r. spectra were recorded with a Bruker WM 250 (250 MHz) or a JEOL P.S. 100 (100 MHz) spectrometer. Optical rotations were measured with a Perkin–Elmer 141 polarimeter. Mass spectra (e.i. at 70 eV, c.i. using 2-propanol, or f.a.b. using glycerol) were obtained with a VG 7070E spectrometer and a VG 2025 data processor. Microanalyses were carried out by Butterworths Laboratories.

*1,3-Bis(1,6-anhydro-4-deoxy-2,3-*O*-isopropylidene-β-*D*-mannopyranose-4-yloxy)-2-propanol* (**3**). — To a solution of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose[7] (**2**; 5 g, 24.8 mmol) in 1,4-dioxane (25 mL) was added 1-chloro-2,3-epoxypropane (1.14 g, 12.4 mmol) and finely ground sodium hydroxide (5 g, 125 mmol). The mixture was stirred at 70° for 7 days and then filtered, and the insoluble material was washed with chloroform (3 × 150 mL). The combined filtrate and washings were washed with aqueous 5% sodium chloride (50 mL) and water (3 × 50 mL), dried (Na_2SO_4), and concentrated to dryness *in vacuo*. Crystallisation of the residue from chloroform–ether and recrystallisation from 2-propanol yielded **3** (1.95 g, 34%), m.p. 134–136°, $[\alpha]^{25}_{589}$ −81° (*c* 0.45, methanol). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.36 (d, 2 H, $J_{1,2}$ 3.0 Hz, H-1), 4.64 (dd, 2 H, $J_{5,6}$ 1.5, $J_{5,6'}$ 6.2 Hz, H-5), 4.23 (d, 2 H, H-3), 4.06 (dd, 2 H, $J_{2,3}$ 6.8 Hz, H-2), 4.00 (t, 1 H, $CH_2C\mathit{H}CH_2$), 3.96 (dd, 2 H, $J_{6,6'}$ 7.5 Hz, H-6), 3.77 (dd, 2 H, H-6′), 3.71 (m, 4 H, $C\mathit{H}_2CHC\mathit{H}_2$), 3.65 (s, 2 H, H-4), 2.64 (d, 1 H, exchangeable with D_2O, $J_{2,OH}$ 5.25 Hz, OH), 1.54 and 1.34 (2 s, each 6 H, 2 CMe_2). Mass spectra: e.i., *m/z* 445 (M^+ − Me); c.i., *m/z* 461 (M^+ + 1).

Anal. Calc. for $C_{21}H_{32}O_{11}$: C, 54.77; H, 7.01. Found: C, 54.66; H, 7.16.

*1,3-Bis(1,6-anhydro-4-deoxy-2,3-*O*-isopropylidene-β-*D*-mannopyranose-4-yloxy)-2-propanone* (**4**). — To a solution of **3** (1 g, 2.2 mmol) in *N,N*-dimethylformamide (20 mL) and methyl sulphoxide (550 μL) was added phosphorus pentaoxide (0.8 g, 5.6 mmol) with stirring. The mixture was then heated at 60° for 3.5 h, cooled, and partitioned between chloroform (150 mL) and ice–water (150 mL). The water layer was extracted with chloroform (4 × 150 mL), and the combined extracts were dried (Na_2SO_4) and concentrated to dryness to yield a crystalline mixture of **3** and **4** in the ratio 1:2. Crystallisation from chloroform–ethanol and recrystallisation from ethanol yielded **4** (0.5 g, 50%), m.p. 144–145°, $[\alpha]^{25}_{589}$ +31° (*c* 0.26, methanol). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.37 (d, 2 H, $J_{1,2}$ 3.0 Hz, H-1), 4.73 (dd, 2 H, $J_{5,6}$ 1.5, $J_{5,6'}$ 6.2 Hz, H-5), 4.45 (s, 4 H, $C\mathit{H}_2CC\mathit{H}_2$), 4.23 (d, 2 H, H-3), 4.11 (dd, 2 H, $J_{2,3}$ 6.8 Hz, H-2), 3.94 (dd, 2 H, $J_{6,6'}$ 7.5 Hz, H-6), 3.78 (dd, 2 H, H-6′), 3.66 (s, 2 H, H-4), 1.54 and 1.35 (2 s, each 6 H, 2 CMe_2). Mass spectra: e.i. and c.i., *m/z* 443 (M^+ − Me).

Anal. Calc. for $C_{21}H_{30}O_{11}$: C, 55.02; H, 6.60. Found: C, 55.32; H, 6.72.

*1,3-Bis(1,6-anhydro-4-deoxy-2,3-*O*-isopropylidene-β-*D*-mannopyranose-4-*

yloxy)-2-propylamine (**5**). — To a solution of **4** (1.5 g, 3.27 mmol) and ammonium acetate (2.5 g, 32.5 mmol) in methanol (20 mL) and tetrahydrofuran (20 mL) was added, dropwise with stirring, a solution of sodium cyanoborohydride (110 mg, 1.75 mmol) in methanol (2 mL). The mixture was stirred for a further 48 h at 25° and then added to a mixture of chloroform (300 mL) and ice–water (300 mL). The water layer was further extracted with chloroform (4 × 300 mL), and the combined organic phases were dried (Na_2SO_4) and concentrated *in vacuo*. The syrupy product was eluted from a column of Kieselgel G60 (Merck, 300 g) with ethyl acetate–light petroleum (4:3, 500 mL). Elution with chloroform–ethanol (10:1, 500 mL) then gave **5** (1.05 g, 70%), m.p. 133–134° (from ethyl acetate), $[\alpha]_{589}^{25}$ −61° (*c* 0.29, methanol). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.35 (d, 2 H, $J_{1,2}$ 3.0 Hz, H-1), 4.63 (dd, 2 H, $J_{5,6}$ 1.5, $J_{5,6'}$ 6.2 Hz, H-5), 4.23 (d, 2 H, H-3), 4.05 (dd, 2 H, $J_{2,3}$ 6.8 Hz, H-2), 3.96 (dd, 2 H, $J_{6,6'}$ 7.5 Hz, H-6), 3.76 (dd, 2 H, H-6′), 3.62 (s, 2 H, H-4), 3.72–3.48 (m, 4 H, $\mathrm{C}H_2\mathrm{CHC}H_2$), 3.21 (t, 1 H, $J_{2,3}$ 5.5 Hz, $\mathrm{CH_2C}H\mathrm{CH_2}$), 1.70 (s, 2 H, exchangeable with D_2O, NH_2), 1.53 and 1.35 (2 s, each 6 H, 2 CMe_2). Mass spectra: e.i., *m/z* 444 (M^+ − Me); c.i., *m/z* 460 (M^+ + 1).

Anal. Calc. for $C_{21}H_{33}NO_{10}$: C, 54.89; H, 7.24; N, 3.04. Found: C, 54.82; H, 7.30; N, 2.87.

*1,3-Bis(4-deoxy-*D*-mannopyranose-4-yloxy)-2-propylamine* (**6**). — A suspension of **5** (1.0 g, 2.2 mmol) in M HCl (100 mL) was heated at ~100° for 3.5 h. The resulting solution was neutralised with Amberlite IRA-93 (HO^-) resin (70 g), filtered, and concentrated to dryness *in vacuo*. The product was purified by p.c. (Whatman 3MM paper, 40:11:19 1-butanol–ethanol–water), acidified with M HCl, and crystallised from ethanol to yield **6** as the hydrochloride (0.89 g, 91%), m.p. 77–78° (dec.), $[\alpha]_{589}^{25}$ +13° (*c* 0.22, methanol). Mass spectrum: +ve ion f.a.b., *m/z* 416 (M^+ + 1).

Anal. Calc. for $C_{15}H_{30}ClNO_{12}$: C, 39.87; H, 6.69; N, 3.10. Found: C, 39.75; H, 6.64; N, 2.96.

*1,6-Bis(1,6-anhydro-4-deoxy-2,3-O-isopropylidene-β-*D*-mannopyranose-4-yloxy)hexane* (**7**). — To a solution of **2** (1.0 g, 4.95 mmol) in 1,4-dioxane (5 mL) was added dibromohexane (0.60 g, 2.48 mmol) and powdered sodium hydroxide (1.0 g, 25 mmol). The mixture was stirred at 70° for 7 days and then filtered, the insoluble material was washed with chloroform (3 × 50 mL), and the combined filtrate and washings were washed with aqueous 5% sodium chloride (25 mL) and water (2 x 25 mL), dried (Na_2SO_4), and concentrated to dryness *in vacuo*. Crystallisation of the residue from chloroform–ether yielded **7** (0.517 g, 43%), m.p. 135–138°, $[\alpha]_{589}^{25}$ +22.5° (*c* 0.2, methanol). ^{1}H-N.m.r. data ($CDCl_3$): δ 5.33 (d, 2 H, $J_{1,2}$ 3.0 Hz, H-1), 4.60 (dd, 2 H, $J_{5,6}$ 1.5, $J_{5,6'}$ 6.2 Hz, H-5), 4.21 (d, 2 H, H-3), 4.06 (dd, 2 H, $J_{2,3}$ 6.8 Hz, H-2), 3.96 (dd, 2 H, $J_{6,6'}$ 7.5 Hz, H-6), 3.76 (dd, 2 H, H-6′), 3.60 (s, 2 H, H-4), 3.72–3.49 (m, 4 H, $\mathrm{C}H_2(\mathrm{CH_2})_4\mathrm{C}H_2$), 1.56 and 1.35 (2 s, each 6 H, 2 CMe_2), and 1.80–1.08 (m, 8 H, $\mathrm{CH_2(C}H_2)_4\mathrm{CH_2}$). Mass spectra: e.i., *m/z* 471 (M^+ − Me); c.i., *m/z* 487 (M^+ +1).

Anal. Calc. for $C_{24}H_{38}O_{10}$: C, 59.24; H, 7.87. Found: C, 59.28; H, 8.06.

*1,6-Bis(4-deoxy-*D*-mannopyranose-4-yloxy)hexane* (**8**). — A suspension of **7** (0.5 g, 1.13 mmol) in M HCl (50 mL) was heated at ~100° for 3.5 h. The resulting solution was neutralised with Amberlite IRA-93 (HO^-) resin (35 g), filtered, and concentrated to dryness *in vacuo*. The product was purified by p.c. (Whatman 3MM paper, 40:11:19 1-butanol–ethanol–water), and crystallised from ethanol to yield **8** (0.42 g, 92%), m.p. 81° (dec.), $[\alpha]_{589}^{25}$ +35° (*c* 0.23, methanol). Mass spectrum: −ve ion f.a.b., *m/z* 441 ($M^+ - 1$). The difference in weight estimated for a sample dried at room temperature and dried *in vacuo* at 60° gave ~0.93 mol of H_2O.

Anal. Calc. for $C_{18}H_{34}O_{12} \cdot H_2O$: C, 46.95; H, 7.88. Found: C, 46.94; H, 8.03.

ACKNOWLEDGMENTS

We thank the M.R.C. and the S.E.R.C. for financial support.

REFERENCES

1 J. E. G. BARNETT, G. D. HOLMAN, AND K. A. MUNDAY, *Biochem. J.*, 135 (1973) 537–541.

2 J. E. G. BARNETT, G. D. HOLMAN, R. A. CHALKLEY, AND K. A. MUNDAY, *Biochem. J.*, 145 (1975) 417–429.

3 W. D. REES AND G. D. HOLMAN, *Biochim. Biophys. Acta*, 646 (1981) 251–260.

4 G. D. HOLMAN, E. J. PIERCE, AND W. D. REES, *Biochim. Biophys. Acta*, 646 (1981) 382–388.

5 G. D. HOLMAN AND W. D. REES, *Biochim. Biophys. Acta*, 685 (1982) 78–86.

6 M. CORY, J. M. ANDREWS, AND D. H. BING, *Methods Enzymol.*, 46 (1977) 115–130.

7 K. HEYNS, P. KOLL, AND H. PAULSEN, *Chem. Ber.*, 104 (1971) 830–836.