Carbohydrate Research, 103 (1982) 15–28
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESES OF MODEL OLIGOSACCHARIDES OF BIOLOGICAL SIGNIFICANCE. SYNTHESIS OF METHYL 3,6-DI-*O*-(α-D-MANNOPYRANOSYL)-α-D-MANNOPYRANOSIDE AND THE CORRESPONDING MANNOBIOSIDES

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(Received March 16th, 1981; accepted for publication in revised form, August 5th, 1981)

ABSTRACT

Methyl 2-*O*-allyl-4,6-*O*-benzylidene-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**12**) was prepared in 90% yield by Helferich glycosylation of methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**9**) with tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**11**). Removal of the benzylidene group and second Helferich glycosylation with **11** led to methyl 2-*O*-allyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**14**) which, after deallylation and Zemplén deacetylation, gave the title compound **5**. The disaccharides methyl 3-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**7**) and methyl 6-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside (**6**) have also been synthesized. Complete assignments of the ^{1}H-n.m.r. spectra of the compounds **5**, **6**, and **7** are given.

INTRODUCTION

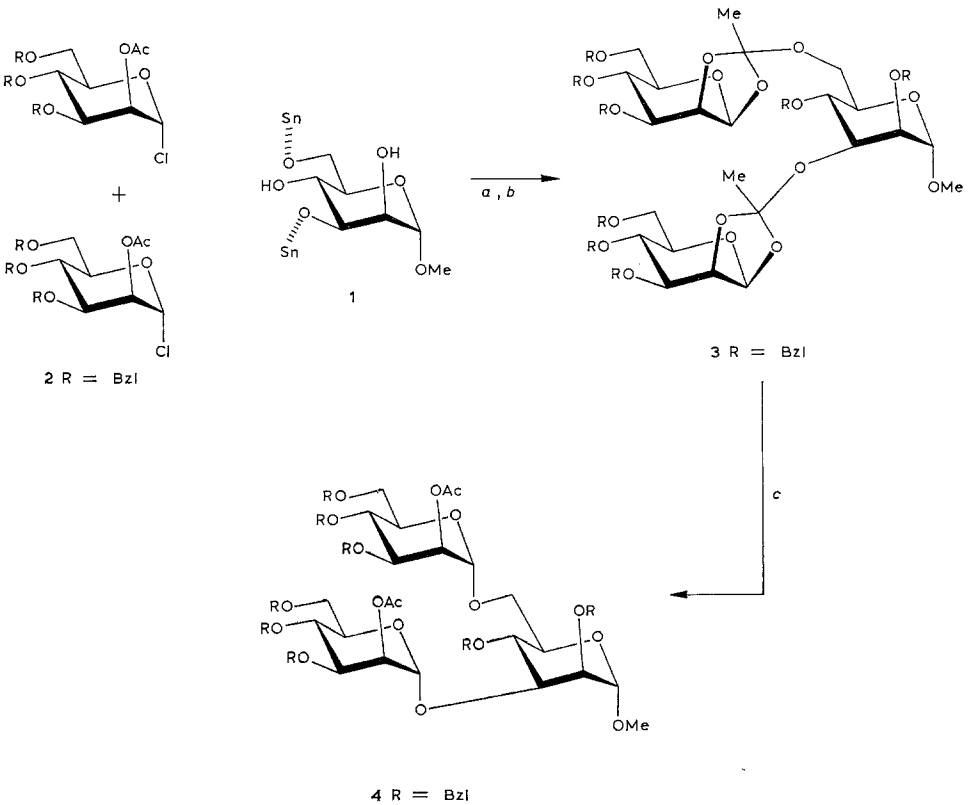
Glycoproteins are ubiquitous components of the cell membrane. Important progress has been made toward unravelling the structures of their carbohydrate components and their role in such crucial biological processes, as differentiation and cell–cell recognition[1]. The specific function of glycopeptides appears to be dictated by their secondary structures, in particular by that of their carbohydrate moieties.

We are developing a general method for the determination of both the primary and secondary structures of glycopeptides by a combination of n.m.r. spectroscopy and chemical synthesis of model compounds. N.m.r. spectroscopy has been used to determine the monosaccharide sequences of glycopeptides and also to detect specific intramolecular interactions present in glycopeptides in aqueous solution[1,2]. Despite difficulties with n.m.r. spectroscopy that has set limitations upon its application to very complex structures, assignments of complex spectra may be achieved by comparing the spectra of a series of synthetic analogues.

The 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranosyl structure is a feature common to all *N*-linked oligosaccharides[1]. Therefore it was an obvious choice for our first synthetic model-compound. Thus far, two syntheses of the 3,6-di-*O*-(α-D-mannopyranosyl)-D-mannopyranosyl structure have been reported. Both suffer from

0008-6215/82/0000–0000/$ 02.75,

an intrinsic limitation in that the substituents at O-3 and O-6 of the central mannose residue must be identical. Ogawa and his co-workers used a very direct approach based on the selective activation toward *O*-alkylation of O-3 and O-6 of stannylated methyl α-D-mannopyranoside[3] (**1**). However, the glycosylation of compound **1** with 2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl chloride (**2**) led to the diortho-acetate **3** rather than to the desired methyl mannotrioside **4**. The latter was obtained in mediocre yield by a mercuric bromide-catalyzed orthoester rearrangement of **3** (Scheme 1). A slightly more circuitous route was described by Arnarp and Lönngren[4]. It starts with stannylated benzyl α-D-mannopyranoside, and *via* a blocking–de-blocking sequence that circumvents the orthoesterglycoside rearrangement it gives the mannotrioside in satisfactory yields.

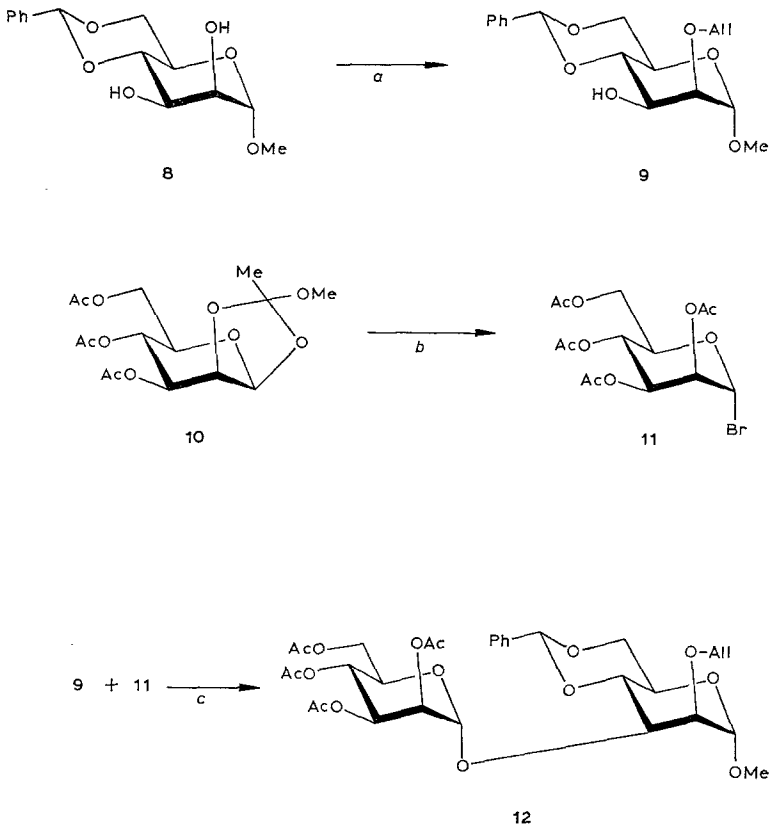


a: NEt_4Br, $C_2H_4Cl_2$; b: BzlBr, NaH, $NCONMe_2$; c: $HgBr_2$, 120°.

Scheme 1

RESULTS AND DISCUSSION

The mannotriosyl structure was built around a central unit, compound **9** (Scheme 2). In this monosaccharide, only O-3 is available for direct glycosylation, but the stage is set for further substitution at O-6.



a: All-Br, NBu_4HSO_4, NaOH 50%, H_2O, $CHCl_3$; b: Me_3SiBr, CH_2Cl_2; c: $HgBr_2$, $Hg(CN)_2$, molecular sieves, CH_3CN.

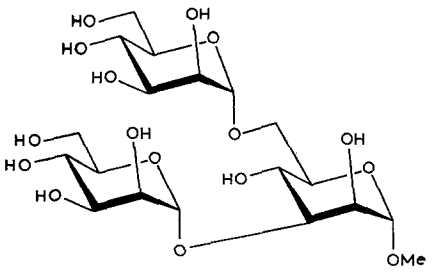
Scheme 2

Compound **9** was synthesized from methyl 4,6-*O*-benzylidene-α-D-manno-pyranoside[5] (**8**) by phase transfer-catalyzed allylation of the axial 2-hydroxyl group (Scheme 2). The regioselectivity of phase transfer-catalyzed ether formation has been observed previously with partially protected mannopyranosides[6]. That only the axial hydroxyl group in **8** is allylated was confirmed as follows. In the ^{1}H-n.m.r. spectrum of **9**, in chloroform-*d*, a doublet at δ 4.77 (J 1.5 Hz) is assigned to the resonance of the anomeric proton (H-1). A doublet of doublets at δ 3.75 (J 3.6, 1.5 Hz) is ascribed to the resonance of H-2, which is coupled to the vicinal protons H-3 and H-1. The proton H-3 is vicinally coupled to H-2 and H-4, and its resonance is a complex multiplet centered at δ 4.06 which simplifies to a doublet of doublets (J 3.6, 9 Hz) upon addition of deuterium oxide. These assignments were confirmed by spin-decoupling of D_2O-exchanged **9**: irradiation of the signal at δ 3.75 (H-2) results in collapse of the doublet at δ 4.77 into a singlet and of the doublet of doublets at δ 4.06 into a doublet (J 9 Hz).

We found that Helferich glycosylation[7] of compound **9** with tetra-*O*-acetyl-α-D-mannopyranosyl bromide **11** was the most efficient route to the disaccharide **12**. The bromide **11** was prepared immediately before use by opening of the orthoacetate **10** with bromotrimethylsilane[8,9]. As the orthoacetate **10** itself was prepared from the bromide **11**, this detour may seem unnecessary. However, **10** is a very stable compound which can be extensively purified. Its bromination with bromotrimethylsilane gives "acetobromomannose" **11** in a high degree of purity and completely anhydrous. The results of glycosylations using **11** prepared in this way were consistently much superior to those using **11** prepared directly as in ref. 15 (compare also ref. 9a). The protected disaccharide **12** was obtained crystalline in 90% yield. The structure assigned to **12** is consistent with the presence, in the ^{1}H-n.m.r. spectrum, of two doublets (J 1.5 Hz) at δ 4.71 and 5.41 assigned to the anomeric protons H-1 and H-1′, respectively. The ^{1}H-n.m.r. spectrum of **12** shows also four singlets (δ 1.99 2.06, 2.09, 2.11) characteristic of acetyl proton resonances. The configuration of the newly formed glycosidic bond, expected to be α from mechanistic considerations, was determined as follows. After complete deprotection of **12** by treatment with diluted acetic acid[10], followed by deallylation with 10% palladium-on-carbon[11] and finally Zemplén deacetylation, we obtained a crystalline compound that had an $[\alpha]_D$ value identical to that of methyl 3-*O*-α-D-mannopyranosyl-α-D-mannopyranoside[12] (**7**). The structure of **7** is further corroborated by study of its ^{1}H-n.m.r. spectrum in D_2O. Doublets (J 1.7 Hz) at δ 4.74 and 5.11 are assigned to the resonances of the anomeric protons H-1 and H-1′, respectively. Doublets of doublets (J 1.7, 3.4 Hz) at δ 4.08 and 4.06 are assigned to the resonances of H-2 and H-2′, respectively.

Synthesis of the 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl structure was accomplished by selective Helferich glycosylation at the primary hydroxyl group of the partially protected disaccharide **13**, obtained by acid treatment of the benzylidene derivative **12** (Scheme 3). The reaction of **13** with one equivalent of tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**11**) was complete after one h at room temperature. The ^{1}H-n.m.r. spectrum of the product **14** showed three doublets (J 1.5 Hz) at δ 4.73, 5.23, and 4.93 ascribed to the resonances of the anomeric protons H-1, H-1′, and H-1″, respectively, and eight singlets from δ 1.98–2.15, characteristic of acetyl protons. The site of glycosylation of **13** was expected to be O-6, because of the higher reactivity[13] of the primary hydroxyl group as compared to the secondary hydroxyl group on C-4. Deprotection of **14** by deallylation and Zemplén deacetylation gave a trisaccharide to which we were able to assign unambiguously the 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranosyl structure **5**, as its physical properties (^{1}H-n.m.r. spectrum, $[\alpha]_D$) were identical to those of the product of Ogawa's synthesis[3]*. Methylation analysis[14] further confirmed the substitution pattern of the central

*Our attempts to increase the yield of the orthoester rearrangement (Scheme 1, step *c*) were unsuccessful; in all cases we observed the formation of a side product (5–10%), identified as methyl 6-*O*-(2-*O*-acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl)-2,4-di-*O*-benzyl-α-D-mannopyranoside (**15**), in view of its origin and of the fact that it was converted into **6** by deprotection of the hydroxyl groups.



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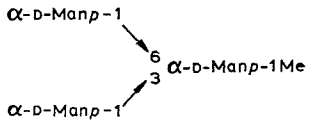

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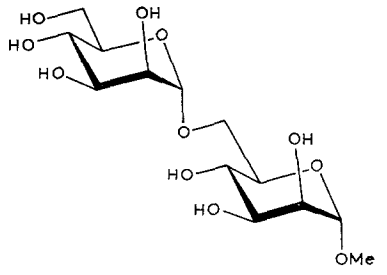

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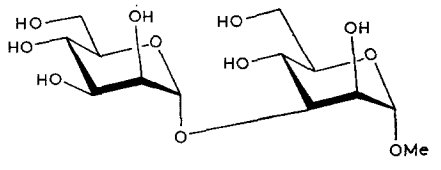

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α-D-Manp-(1→6)-α-D-Manp-1Me

α-D-Manp-(1→3)-α-D-Manp-1Me

TABLE I

^{1}H CHEMICAL SHIFTS[a,b]

Compound	*H-1*	*H-2*	*H-3*	*H-4*	*H-5*	*H-6a*	*H-6b*	*MeO*
α-D-Manp-1-Me	4.762	3.930	3.756	3.636	3.611	3.899	3.754	3.407
α-D-Manp-(1→6)-α-D-Manp-1Me	4.913	3.989	3.847	3.661	3.710	3.894	3.763	
	4.754	3.941	3.744[c]	3.744[c]	3.744[c]	3.772	3.977	3.402
α-D-Manp-(1→3)-α-D-Manp-1Me	4.742	4.078	3.867	3.752	3.657	3.900	3.766	3.412
	5.110	4.064	3.878	3.662	3.754	3.883	3.760	
α-D-Manp-1 ↘								
	4.908	3.944	3.838	3.661	3.689	3.892	3.763	
6	4.729	4.089	3.856	3.898	3.804	3.727	4.019	3.407
3α-D-Manp-1Me	5.097	4.062	3.879	3.665	3.760	3.883	3.761	
↗								
α-D-Manp-1								

[a]Chemical shifts are in p.p.m. relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (using internal acetone at 2.225 p.p.m.) in D_2O at 23 ±2°. [b]±0.002 p.p.m. error except where indicated. [c]Strong coupling between four protons, ±0.01 p.p.m. error.

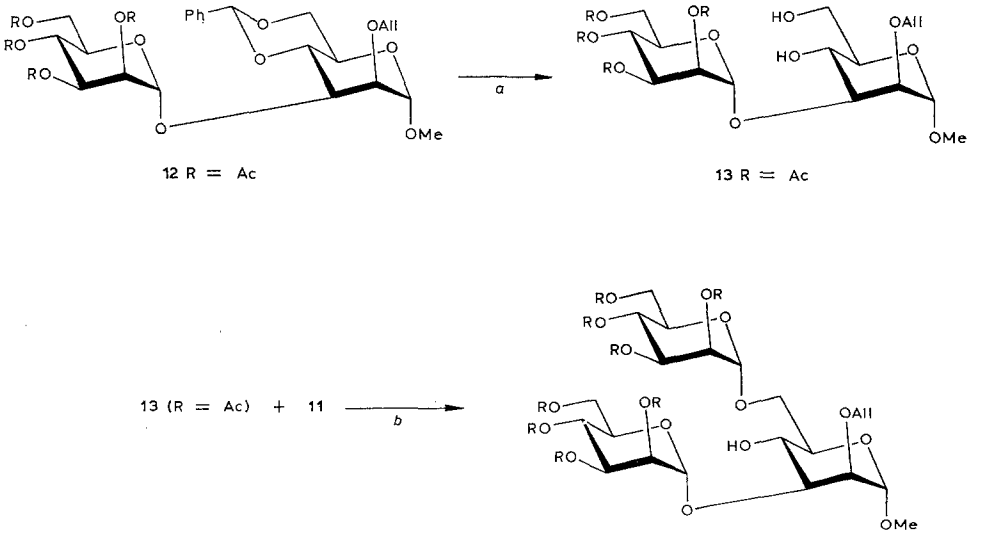
TABLE II

COUPLING CONSTANTS[a,b]

Compound	$J_{1,2}$	$J_{2,3}$	$J_{3,4}$	$J_{4,5}$[c]	$J_{5,6a}$	$J_{5,6b}$	$J_{6a,6b}$
α-D-Man*p*-1-Me	1.7	3.5	9.55	9.8	2.0	6.0	−12.2
α-D-Man*p*-(1→6)-α-D-Man*p*-1Me	1.7	3.4	9.5	9.8	2.3	6.0	−12.3
	1.7	3.4[d]	9.5[d]	9.8[d]	1.8[d]	5.0[d]	−11.0[d]
α-D-Man*p*-(1→3)-α-D-Man*p*-1Me	1.7	3.4	9.5	9.8	2.2	6.0	−12.2
	1.7	3.4	9.5	9.8	2.2	6.0	−12.2
α-D-Man*p*-1 ↘	1.7	3.4	9.5	9.8	2.2	6.0	−12.2
6 3 α-D-Man*p*-1Me	1.7	3.4	9.5	9.8	1.8	5.0	−11.5
α-D-Man*p*-1 ↗	1.7	3.4	9.5	9.8	2.2	6.0	−12.2

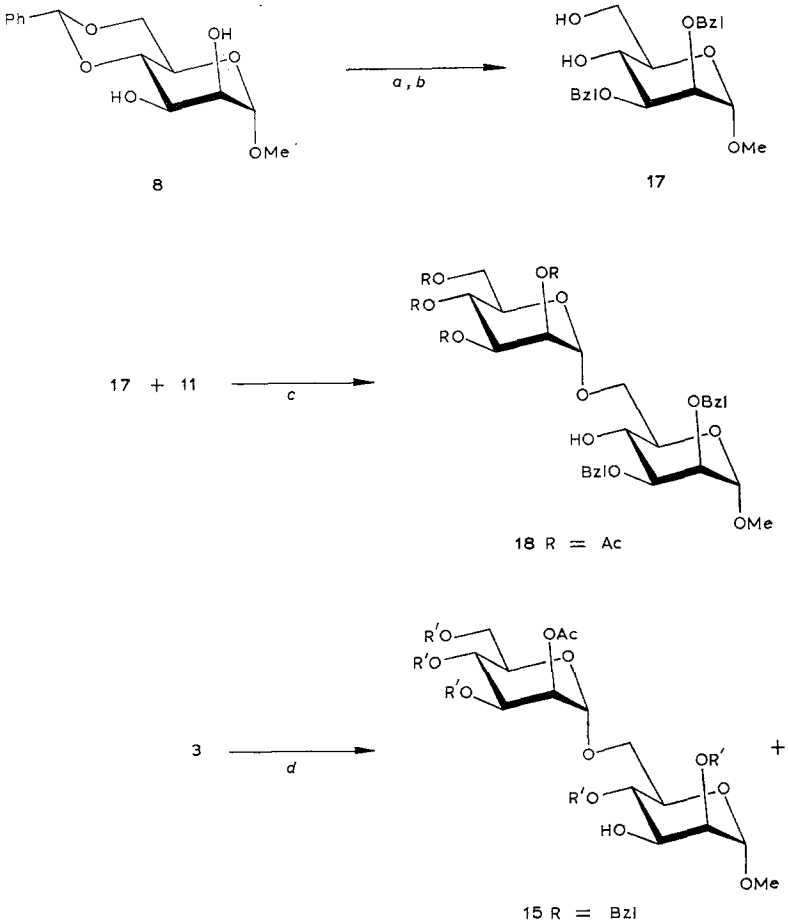
[a]in Hz, at 23 ±2°. [b]±0.1 Hz error except when indicated. [c]±0.3 Hz error due to overlapping doublets. [d]±0.5 Hz error due to strong coupling and virtual coupling.

methyl α-D-mannopyranoside in **5**. The only two products of the analysis were identified as 1,5-di-*O*-acetyl-2,3,4,6-tetra-*O*-methylhexitol and 1,3,5,6-tetra-*O*-acetyl-2,4-di-*O*-methylhexitol by gas chromatography–mass spectrometry analysis. The ^{1}H-n.m.r. spectrum of **5**, consistent with the structural assignment, is reported in Tables I and II.



Scheme 3

In order to make unambiguous assignments of all signals in the ^{1}H-n.m.r. spectrum of **5**, we needed to know the proton assignments in the spectrum of the disaccharide **6**. To our knowledge the synthesis of this mannobioside having an α-D-(1→6) linkage has not yet been reported. (A different synthesis of a protected form of methyl 6-*O*-α-D-mannopyranosyl-α-D-mannopyranoside was reported recently after completion of this work[16]). We devised a simple, three-step synthesis of the 6-*O*-α-D-mannopyranosyl-α-D-mannopyranosyl structure, starting from the *O*-benzylidenemannopyranoside **8** (Scheme 4). This synthesis once again takes advantage of the higher reactivity of the primary 6-hydroxyl group compared to the secondary 4-hydroxyl group in methyl α-D-mannopyranoside bearing protected hydroxyl groups at C-2 and C-3. The methods previously reported for the preparation of the products of both reaction (*a*)[17-19] and reaction (*b*)[17] (Scheme 4) were modified in order to



8 17

18 R = Ac

15 R = Bzl

a: Bzl Br, NaH, $HCONMe_2$; *b*: AcOH 60%, 80°; *c*: $HgBr_2$, $Hg(CN)_2$, molecular sieves, CH_3CN ; *d*: $HgBr_2$, 120°.

Scheme 4

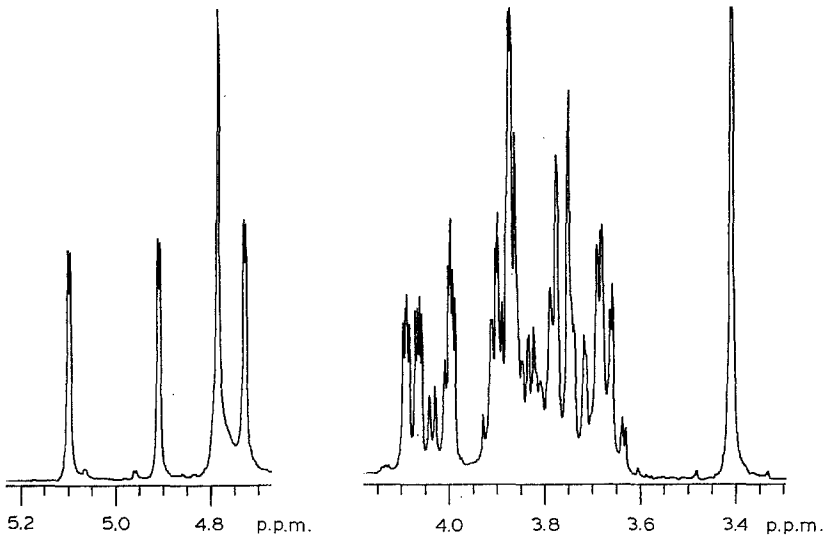


Fig. 1. ^{1}H-N.m.r. spectrum at 360 MHz of methyl 3,6-di-*O*-(α-D-mannopyranosyl)-α-D-mannopyranoside in D_2O.

improve the yields of the desired compounds. Thus, methyl 2,3-di-*O*-benzyl-α-D-mannopyranoside (**17**) was prepared by benzylation of the free hydroxyl groups in **8** using sodium hydride and benzyl bromide (reaction *a*) and subsequent removal of the benzylidene group by mild acid treatment (reaction *b*). Helferich glycosylation of **17** with one equivalent of 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide (**11**) gave the protected disaccharide **18**. The ^{1}H-n.m.r. spectrum of **18**, in chloroform-*d*, has two doublets (J = 1.5 Hz) at δ 4.76 and 4.89 characteristic of anomeric proton resonances and four singlets in the acetyl-proton resonance region (δ 1.95–2.11). Exchange by D_2O resulted in the disappearance of a broad one-proton singlet at δ 2.50 (exchangeable hydroxyl proton) and in a sharpening of the signal at δ 3.94 (H-4). This confirms the substitution pattern of the methyl α-D-mannopyranoside in **18**. Methyl 6-*O*-α-D-mannopyranosyl-α-D-mannopyranoside (**6**) was obtained from **18** by hydrogenolysis over palladium-on-carbon of the benzyl ether groups, followed by Zemplén deacetylation.

^{1}H-n.m.r. spectroscopy of the mannosides. — Detailed analysis of the ^{1}H-n.m.r. spectra and the methods used for assignments of the signals will be published in a separate communication. As the assignments constitute a part of the general characterization of the compounds, we have summarized in Tables I and II the values of the chemical shifts and coupling constants. As an example, the spectrum of the mannotrioside **5** is shown in Fig. 1.

EXPERIMENTAL

General methods. — Melting points were determined with a Fisher–Johns

melting-point apparatus and are uncorrected. Optical rotations were measured with a Jasco (Model ORD/UV-5) polarimeter at 26 ±1° Infrared spectra were recorded with a Unicam SP 1025 spectrophotometer. Solutions were dried with sodium sulfate and were concentrated *in vacuo* with the bath temperature kept below 35°. All solvents were distilled before use. Dichloromethane was dried by distillation under dry nitrogen in the presence of phosphorus pentaoxide and kept over 4A molecular sieves. Acetonitrile was dried by 3-h reflux over calcium hydride and subsequent distillation under dry nitrogen onto 4A molecular sieves, the first 15% of the distillate being discarded. Methanol was dried by 4-h reflux over magnesium and a trace of iodine and subsequent distillation under dry nitrogen onto 3A molecular sieves. Mercuric bromide was dissolved in hot toluene, dried by azeotropic distillation, and crystallized upon cooling from toluene. Aqueous solutions were desalted with mixed-bed resin (AG 501-X8, 20-50 mesh, Bio Rad). Microanalyses were performed by the Microanalytical Laboratory, Ltd., Markham, Ontario.

Chromatographic methods. — T.l.c. was performed on precoated plates of silica gel 60F, 0.20 mm thick (Merck). All proportions of solvents are v/v. For detection, the plates were sprayed with 50% sulfuric acid and heated for 3–5 min at ~130°. Gel-filtration chromatography was performed on Bio Gel P-2, 200–400 mesh (Bio Rad), the column effluent being monitored with a flow-cell refractive-index detector (Pharmacia). G.l.c.–m.s. spectra were recorded on a Hewlett–Packard 5985 B g.l.c.–m.s. system in the Banting and Best Department of Medical Research, University of Toronto using a 50-m fused-silica column (SP-2100), and 70 eV.

^{1}H-N.m.r. spectra. — ^{1}H-N.m.r. spectra were recorded at 360 MHz with a Nicolet spectrometer located at the Toronto Biomedical NMR Centre, University of Toronto. They were obtained at 23 ±2° either in chloroform-*d* containing 1% of tetramethylsilane as the internal standard or in deuterium oxide (99.996%, Merck, Sharp, and Dohme) with acetone (0.1%, 2.225 p.p.m. relative to internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate) as the internal standard. For a 10mM solution of sugar in D_2O, 128 transients were accumulated and a pulse angle of 90° was used in all cases.

Methyl 2-O-allyl-4,6-O-benzylidene-α-D-mannopyranoside (**9**). — A solution of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside[5] (**8**) (3.0 g, 1.06 mmol), allyl bromide (3.25 g, 18 mmol, freshly distilled), and tetra-butylammonium hydrogen sulfate (0.72 g, 2 mmol) in dichloromethane (200 mL) was stirred vigorously with 50% aqueous sodium hydroxide (15 mL) and boiled for 20 h under reflux. The aqueous phase was removed from the cold mixture and the organic phase washed with water (20 mL, 3 times), aqueous saturated sodium hydrogencarbonate solution (20 mL, 3 times), brine (20 mL, once), dried, and evaporated. Crystallization of the residue from 19:1 hexane–ethyl acetate gave **9** (2.6 g, 76%); m.p. 100–101°, $[\alpha]_D$ +17.2° (*c* 0.31, chloroform); t.l.c. (3:2 carbon tetrachloride–acetone) R_F 0.75; $\nu_{max}^{CHCl_3}$ 3570 (OH, w), 2940 (m), 2880, 2850, 1700, 1465, 1385, 1285, 1105, and 1075 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 2.30 (d, 1 H, *J* 9 Hz, exchangeable, OH-3), 3.39 (s, 3 H, OCH_3), 3.75 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.6 Hz, H-2), 3.76–3.90 (m, 3 H), 4.03–4.10

(m, 1 H, H-3), 4.13–4.30 (m, 3 H), 4.77 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.23–5.38 (m, 2 H, ABX system, CH_2=CH), 5.58 (s, 1 H, Ph-C*H*), 5.88–6.00 (m, 1 H, CH_2=C*H*), and 7.30–7.52 (m, 5 H, aromatic H).

Anal. Calc. for $C_{17}H_{22}O_6$ (322.34): C, 63.34; H, 6.88. Found: C, 63.04; H, 6.81.

2,3,4,6-Tetra-O-*acetyl*-α-D-*mannopyranosyl bromide* (**11**). — Bromotrimethylsilane (0.91 mg, 5.95 mmol) was added dropwise, under nitrogen to a solution of 3,4,6-tri-*O*-acetyl-β-D-mannopyranose 1,2-(methyl orthoacetate)[15] (**10**) (1.35 g, 3.7 mmol) in dry dichloromethane (20 mL). The solution was boiled at reflux under nitrogen for 2 h, cooled to room temperature, evaporated, and the residue dried *in vacuo* for 1 h. The resulting oil was used without further purification.

Methyl 2-O-*allyl-4,6*-O-*benzylidene-3*-O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-α-D-*mannopyranoside* (**12**). — To a solution of **9** (1 g, 3.10 mmol) in dry acetonitrile (20 mL), containing 4A molecular sieves were added sequentially, mercuric bromide (1.34 g, 3.72 mmol), mercuric cyanide (0.94 g, 3.72 mmol), and a solution of **11** (from 1.35 g of **10**) in dry acetonitrile (5 mL). The mixture was stirred at room temperature in a closed system for 1 h or until complete disappearance of starting material (as indicated by t.l.c., 2:3 ethyl acetate–toluene). Evaporation of the solvent gave an oily residue which was extracted 3 times with chloroform. The organic extracts were washed with saturated potassium chloride solution (three times), saturated sodium hydrogencarbonate (three times), water, and brine. The dried organic layer was evaporated and the condensation product **12** (1.82 g, 90%) crystallized from the residue (1:9 ether–hexane); m.p. 160–162°, $[\alpha]_D$ +52.4° (*c* 0.25, chloroform); t.l.c. (2:3 ethyl acetate–toluene) R_F 0.50; $\nu_{max}^{CHCl_3}$ 2940, 2880, 2840, 1750 (s), 1375, 1270 (m), 1105 (m), and 1060 cm^{-1}; ^{1}H-n.m.r. ($CDCl_3$): δ 1.99 (s, 3 H, OCOC*H*$_3$), 2.06 (s, 3 H, OCOC*H*$_3$), 2.09 (s, 3 H, OCOC*H*$_3$), 2.11 (s, 3 H, OCOC*H*$_3$), 3.37 (s, 3 H, OC*H*$_3$), 3.72 (dd, 1 H, $J_{1,2}$ 1.5 Hz, $J_{2,3}$ 3.6 Hz, H-2), 3.73–3.79 (m, 1 H), 3.86 (AM_2t, $J_{3,4}$ = $J_{4,5}$ 13 Hz, 1 H, H-4), 4.02–4.09 (m, 1 H), 4.12–4.30 (m, 7 H), 4.71 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.20–5.42 (m, 5 H), 5.58 (s, 1 H, PhC*H*), 5.95–6.06 (m, 1 H, CH_2=C*H*), and 7.30–7.52 (m, 5 H, aromatic-H).

Anal. Calc. for $C_{31}H_{40}O_{15}$ (652.63): C, 57.05; H, 6.18. Found: C, 57.25; H, 6.09.

Methyl 2-O-*allyl-3*-O-(*2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl*)-α-D-*mannopyranoside* (**13**). — A solution of **12** (500 mg, 0.76 mmol) in 60% aqueous acetic acid (15 mL) was heated for 30 min at 80°. The cooled mixture was evaporated in high vacuo, the residue taken up with dichloromethane, and the solution was washed with water, saturated sodium hydrogencarbonate, and brine. It was dried and evaporated to give **13** as an amorphous solid (340 mg, 78%); $[\alpha]_D$ +55.7° (*c* 0.38, chloroform); t.l.c. (1:1 ethyl acetate–toluene) R_F 0.25; ^{1}H-n.m.r. ($CDCl_3$): δ 1.99 (s, 3 H, OCOC*H*$_3$), 2.09 (s, 3 H, OCHOC*H*$_3$), 2.11 (s, 3 H, OCOC*H*$_3$), 2.17 (s, 3 H, OCOC*H*$_3$), 2.45 (br s, 2 H, exchangeable), 3.36 (s, 3 H, OC*H*$_3$), 3.54–3.60 (m, 1 H), 3.70 (dd, 1 H, $J_{1,2}$ 3.7 Hz), 3.85–3.98 (m, 3 H), 4.05–4.26 (m, 6 H), 4.72 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.20–5.40 (m, 8 H), and 5.86–6.00 (m, 1 H, CH_2=C*H*).

Anal. Calc. for $C_{24}H_{36}O_{15}$ (564.53): C, 51.06; H, 6.43. Found: C, 51.29; H, 6.18.

*Methyl 3-*O*-(α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**7**). — Compound **13** (250 mg, 0.44 mmol) was added to a suspension of palladium-on-charcoal (10%, 50 mg) in ethanol (8 mL), water (4 mL), and glacial acetic acid (4 mL), containing sodium acetate (10 mg). The mixture was heated for 17 h at 75° under nitrogen. The cooled mixture was filtered through a bed of Celite. The filtrate was made neutral with solid sodium hydrogencarbonate and evaporated. The residue was taken up with dichloromethane. The solution was washed with water, saturated aqueous sodium hydrogencarbonate, and brine, dried, and evaporated to give an amorphous solid that was dissolved in dry methanol (10 mL) and treated with 2M methanolic sodium methoxide (0.5 mL). After 20 min, the solution was evaporated to dryness, dissolved in water, and desalted with mixed-bed resin. Filtration and evaporation of the filtrate gave the (1→3)-mannobioside **7** (90 mg, 57%). Compound **7** was purified further on a column of Bio Gel P-2 using degassed distilled water as eluent; $[\alpha]_D$ +93.5° (*c* 0.25, water), lit.[12] $[\alpha]_D$ +91° (*c* 0.8, water); ^{1}H-n.m.r. (D_2O), see Tables I and II.

*Methyl 2-*O*-allyl-3,6-di-*O*-(2,3,4,6-tetra-*O*-acetyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**14**). — The bromide **11** (3.19 mmol) was prepared from 3,4,6-tri-*O*-acetyl-β-D-mannopyranose 1,2-(methyl orthoacetate) (1.22 g, 3.19 mmol) and bromotrimethylsilane (1 mL, 6.54 mmol), as described. To a solution of **13** (1.8 g, 3.19 mmol) in dry acetonitrile (30 mL) containing 4A molecular sieves, were added sequentially, mercuric bromide (1.38 g, 3.83 mmol), mercuric cyanide (0.97 g, 3.83 mmol), and a solution of **11** in dry acetonitrile (10 mL). After 1 h, t.l.c. (1:1 toluene–ethyl acetate) indicated complete disappearance of the starting material. The solvent was evaporated and the residue extracted three times with chloroform. The organic extracts were washed with aqueous saturated potassium chloride (three times), saturated aqueous sodium hydrogencarbonate (three times, water (twice), and brine. The organic layer was dried and evaporated to give **14** as a colorless oil (1.90 g, 67%), which was further purified on a column of silica gel (eluent: 1:1 toluene–acetate); $[\alpha]_D$ +53.4° (*c* 0.24, chloroform); t.l.c. (1:1 ethyl acetate–toluene) R_F 0.21; $\nu_{max}^{CHCl_3}$ 3560 (OH, w), 2960 (w), 2850 (w), 1750 (s), 1372 (s), 1140 (s), 1060 (br s), and 985 cm^{-1} (m); ^{1}H-n.m.r. ($CDCl_3$): δ 1.98 (s, 3 H, OCOCH_3), 1.99 (s, 3 H, OCOCH_3), 2.05 (s, 3 H, OCOCH_3), 2.06 (s, 3 H, OCOCH_3), 2.10 (s, 3 H, OCOCH_3), 2.11 (s, 3 H, OCOCH_3), 2.15 (s, 6 H, two OCOCH_3), 3.0 (d, 1 H, *J* 3.5 Hz, exchangeable, O-H), 3.37 (s, 3 H, OCH_3), 3.63–3.72 (m, 2 H), 3.80–4.30 (m, 12 H), 4.73 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.93 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1″), 5.18–5.42 (m, 8 H), and 5.86–6.00 (m, 1 H, CH_2=C*H*).

Anal. Calc. for $C_{38}H_{54}O_{24}$ (894.81): C, 51.00; H, 6.08. Found: C, 50.69; H, 6.01.

*Methyl 3,6-di-*O*-(α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**5**). — Compound **14** (1.0 g, 1.12 mmol) was added to a suspension of palladium-on-carbon (10%, 250 mg) in ethanol (40 mL), water (20 mL), and acetic acid (20 mL) containing

sodium acetate (100 mg). The mixture was heated for 17 h at 75° under nitrogen. The cold mixture was filtered through a bed of Celite. The filtrate was made neutral with solid sodium hydrogencarbonate and the solvent evaporated. The residue was taken up in dichloromethane, and washed with water, saturated aqueous sodium hydrogencarbonate, and brine. It was dried and evaporated to give an amorphous solid that was dissolved in dry methanol (25 mL) and treated with 2M methanolic sodium methoxide (2.0 mL). After 30 min, the solution was evaporated, dissolved in water, and desalted with mixed-bed resin. Filtration and lyophilization of the filtrate gave an amorphous solid (364 mg, 60%). It was purified on a column of Bio Gel P-2 eluted with degassed distilled water; $[\alpha]_D$ +111.0° (*c* 0.31, methanol), lit.[3] $[\alpha]_D$ +96.7° (*c* 0.45, methanol); ^{1}H-n.m.r. (D_2O); see Tables I and II.

*Methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-*D*-mannopyranoside* (**16**). — A 50% suspension of sodium hydride in oil (763 mg, 15.9 mmol) was washed three times with dry benzene under nitrogen, and sodium hydride was resuspended in dry *N,N*-dimethylformamide (5 mL). A solution of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**8**, 1.5 g, 5.3 mmol) in dry *N,N*-dimethylformamide (5 mL) was added dropwise to the stirred suspension kept at 15° with a cold-water bath. After completion of the addition, the grey suspension was stirred for 3 h at room temperature. It was then cooled to 0° and a solution of benzyl bromide (2.17 g, 12.7 mmol, freshly distilled) in *N,N*-dimethylformamide (3 mL) was added dropwise to it. The mixture was stirred for 1 h at 0° and overnight at room temperature. The excess of hydride was decomposed by dropwise addition of distilled water, and the excess of benzyl bromide was hydrolyzed with concentrated ammonium hydroxide (2 mL). After 1 h, the solution was poured over chloroform (50 mL), washed twice with water, three times with phosphate buffer (Na_2HPO_4 0.1M, NaH_2PO_4 0.1M), twice with aqueous saturated sodium hydrogencarbonate, and once with brine. It was dried and evaporated to give **16** as a colorless oil (1.9 g, 77%); t.l.c. (3:7 ethyl acetate–toluene) R_F 0.68; ^{1}H-n.m.r. ($CDCl_3$): δ 3.32 (s, 3 H, OCH_3), 3.74–3.81 (m, 1 H, H-5), 3.83 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.2 Hz, H-2), 3.88 (t, 1 H, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 3.94 (dd, 1 H, $J_{2,3}$ 3.2, $J_{3,4}$ 9.5 Hz, H-3), 4.20–4.30 (m, 2 H, H-6a, H-6b), 4.69 (d, $J_{1,2}$ 1.5 Hz, H-1), 4.65, 4.82 (ABq, 2 H, *J* 11 Hz, $PhCH_2O$), 4.73, 4.83 (ABq, 2 H, *J* 11 Hz, $PhCH_2O$), 5.65 (s, 1 H, PhC*H*), and 7.30–7.55 (m, 15 H, aromatic).

*Methyl 2,3-di-*O*-benzyl-α-*D*-mannopyranoside* (**17**). — A solution of **16** (1.9 g, 2.2 mmol) in 60% aqueous acetic acid (2.5 mL) was heated at 80° under nitrogen until dissolution was complete (1 h). The cooled mixture was evaporated and the residue dissolved in chloroform. The solution was washed with water, aqueous sodium hydrogencarbonate, and brine, dried, and evaporated to give a colorless oil (1.08 g, 70%); t.l.c. (1:1 ethyl acetate–toluene) R_F 0.1; ^{1}H-n.m.r. ($CDCl_3$): δ 2.5–2.7 (br m, 2 H, exchangeable), 3.32 (s, 3 H, OCH_3), 3.55–3.62 (m, 1 H, H-5), 3.70 (dd, 1 H, $J_{2,3}$ 3.1, $J_{3,4}$ 9 Hz, H-3), 3.79 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.1 Hz, H-2), 3.81–3.90 (m, 2 H, H-6a, H-6b), 4.03 (t, 1 H, $J_{3,4} = J_{4,5}$ 9 Hz, H-4), 4.47, 4.59 (ABq, 2 H, *J* 11 Hz, $PhCH_2O$), 4.67 (br s, 2 H, $PhCH_2O$), 4.74 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), and 7.25–7.42 (m, 10 H, aromatic).

Methyl 2,3-di-O-benzyl-6-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**18**). — To a solution of **17** (500 mg, 1.34 mmol) in dry acetonitrile (10 mL) containing 4A molecular sieves were added sequentially, mercuric bromide (648 mg, 1.8 mmol), mercuric cyanide (458 mg, 1.8 mmol), and a solution of **11** (from 597 mg of **10**) in dry acetonitrile (5 mL). The mixture was stirred at room temperature in a closed system for 30 min or until complete disappearance of starting material (as indicated by t.l.c., eluent: 1:1 ethyl acetate–toluene). Evaporation of the solvent gave an oil that was extracted three times with chloroform. The organic extracts were washed with saturated potassium chloride solution (three times), saturated sodium hydrogencarbonate (three times), water, and brine. The dried organic layer was evaporated to an oil that was purified by chromatography on a column of silica gel eluted with 3:7 ethyl acetate–toluene. The mannobioside **18** eluted first (400 mg, 42%); t.l.c. (1:1 toluene–ethyl acetate) R_F 0.3; ^{1}H-n.m.r. ($CDCl_3$): δ 1.95 (s, 3 H, CH_3CO), 2.02 (s, 3 H, CH_3CO), 2.04 (s, 3 H, CH_3CO), 2.11 (s, 3 H, CH_3CO), 2.50 (br s, 1 H, exchangeable), 3.35 (s, 3 H, OCH_3), 3.66 (dd, 1 H, $J_{2,3}$ 3.5, $J_{3,4}$ 9.5 Hz, H-3), 3.68–3.75 (m, 1 H, H-5), 3.78 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 3.5 Hz, H-2), 3.81 (dd, 1 H, $J_{6a,6b}$ 9, $J_{5,6a}$ 1.8 Hz, H-6a), 3.91 (dd, 1 H, $J_{6a,6b}$ 9, $J_{5,6b}$ 10 Hz, H-6b), 3.94 (br t, $J_{3,4} = J_{4,5}$ 9.5 Hz, H-4), 4.15–4.25 (m, 3 H), 4.40–4.54 (ABq, 2 H, J 11 Hz, $PhCH_2O$), 4.62–4.68 (ABq, 2 H, J 11 Hz, $PhCH_2O$), 4.76 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 4.89 (d, 1 H, $J_{1'',2''}$ 1.5 Hz, H-1″), 5.27 (t, 1 H, $J_{3'',4''} = J_{4'',5''}$ 9.5 Hz, H-4″), 5.31 (dd, 1 H, $J_{1'',2''}$ 1.5, $J_{2'',3''}$ 3.1 Hz, H-2″), 5.37 (dd, 1 H, $J_{2'',3''}$ 3.1, $J_{3'',4''}$ 9.5 Hz, H-3″), and 7.2–7.4 (m, 10 H, aromatic).

Methyl 6-O-(α-D-mannopyranosyl)-α-D-mannopyranoside (**6**). — The mannobioside **18** (300 mg, 0.43 mmol) in ethanol (15 mL) was treated with hydrogen at atmospheric pressure over palladium-on-carbon (10%, 300 mg) for 17 h. The residue obtained after filtration and evaporation of the solvent, was dissolved in dry methanol and treated with M methanolic sodium methoxide (1 mL) for 1 h. The solution was evaporated, dissolved in water and desalted with mixed-bed resin. Filtration and lyophilization of the filtrate gave **6** as an amorphous solid (61 mg, 73%); $[\alpha]_D$ +94.5° (*c* 0.25, water); ^{1}H-n.m.r. (D_2O), see Tables I and II.

ACKNOWLEDGMENTS

This work was supported by grants from the Medical Research Council of Canada (MT 3732 to J.P.C.) and the National Cancer Institute of Canada (to J.P.C.) as well as by contract funds from the National Cancer Institute (U.S.A.) (to L. Siminovitch). We thank Lorna B. Grey for her expert technical assistance, José Baptista for measuring the optical rotations, and Alan Lee for recording some of the n.m.r. spectra.

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