Carbohydrate Research, 104 (1982) 271–283
Elsevier Scientific Publishing Company, Amsterdam – Printed in The Netherlands

SYNTHESIS OF LINEAR D-MANNOTETRAOSE AND D-MANNOHEXAOSE, PARTIAL STRUCTURES OF THE CELL-SURFACE D-MANNAN OF *Candida albicans* AND *Candida utilis**

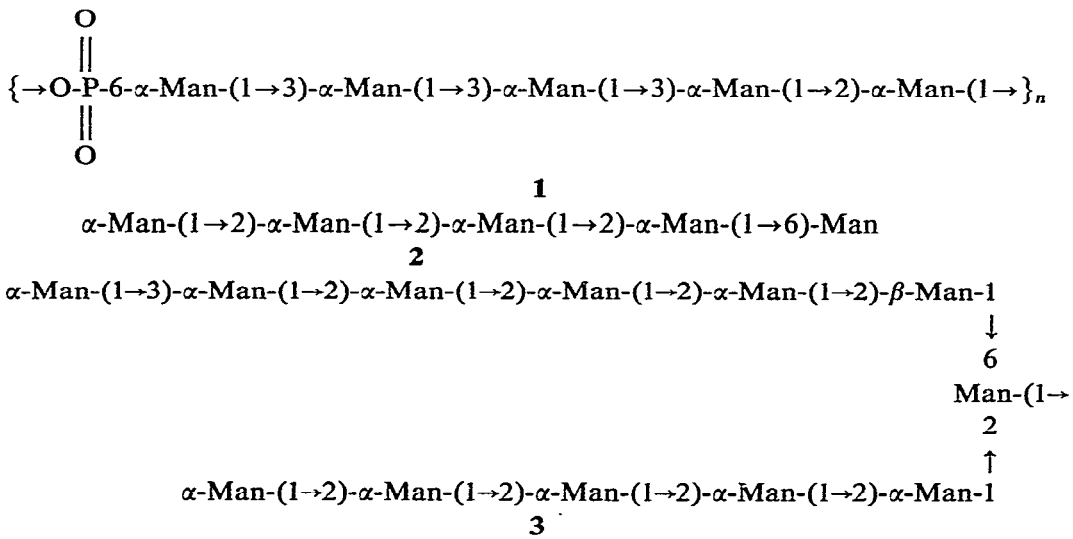
TOMOYA OGAWA** AND HISAO YAMAMOTO
The Institute of Physical and Chemical Research, Wako-shi, Saitama, 351 (Japan)
(Received December 8th, 1981; accepted for publication, December 28th, 1981)

ABSTRACT

Syntheses of linear D-manno-oligosaccharides, *O*-α-Man-(1→2)-*O*-α-Man-(1→2)-*O*-α-Man-(1→2)-Man and *O*-α-Man-(1→2)-*O*-α-Man-(1→2)-*O*-α-Man-(1→2)-*O*-α-Man-(1→2)-*O*-α-Man-(1→2)-Man, which correspond to part of the structure of the cell-wall D-mannan chain of *Candida utilis* and *Candida albicans*, respectively, are described.

INTRODUCTION

Linear D-manno-oligosaccharides containing mainly α-D-(1→2) interglycosidic linkages have been isolated from the acetolysis product of yeast cell-wall D-mannans, and structures **1**, **2**, and **3** have recently been proposed for the D-manno-oligosaccharide chains of *Hansenula wingei*[2], *Candida utilis*[3], and *Candida albicans*[4].



*Part 17 in the series "Synthetic Studies on Cell-surface Glycans". For part 16, see ref. 1.
**To whom enquiries should be addressed.

0008-6215/82/0000–0000/$ 02.75,

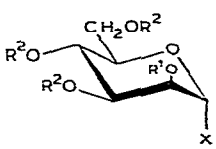
α-Man-{(1→2)-α-Man}$_n$-(1→2)-Man

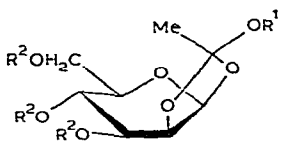
4 $n = 0$
5 $n = 1$
6 $n = 2$
7 $n = 4$

As part of a project on the synthesis of high-D-mannose types of glycans[5], we describe here a regio- and stereo-controlled synthesis of D-mannotetraose (**6**) and D-mannohexaose (**7**), which respectively correspond to part of the structure of glycans **2** and **3**.

RESULTS AND DISCUSSION

A. Stepwise synthesis of D-*mannotetraose.* — In order to synthesize D-mannotetraose (**6**) in a stepwise manner using the mono-D-mannosyl donor **8**, which was readily available[6] from the orthoester **12**, properly protected α-D-mannopyranoside **9** was chosen as the glycosyl acceptor. The acceptor **9** was prepared in the conventional way from tetra-*O*-acetyl-α-D mannopyranosyl bromide (**10**), *via* orthoesters **13** and **14**, in 5 steps in 27% overall yield. The α-D-configuration at C-1 of **9** was confirmed[7] by the ^{13}C-n.m.r. spectrum, which contained a signal for C-1 at 98.4 p.p.m., with $^1J_{CH}$ 168.5 Hz. Glycosylation of **9** with **8** was performed in the presence of $AgOSO_2CF_3$–powdered molecular sieve 4A in dichloroethane, to give an 85% yield of protected D-mannobioside **15**. Deacetylation of **15** afforded the glycosyl acceptor **16**, the structure of which was confirmed by transformation into D-mannobiose **4** (refs. 8 and 9).





8 R^1 = Ac, R^2 = Bn, X = Cl
9 R^1 = H, R^2 = Bn, X = OBn
10 R^1 = R^2 = Ac, X = Br
11 R^1 = Ac, R^2 = Bn, X = OBn

12 R^1 = Me, R^2 = Bn
13 R^1 = Bn, R^2 = Ac
14 R^1 = R^2 = Bn

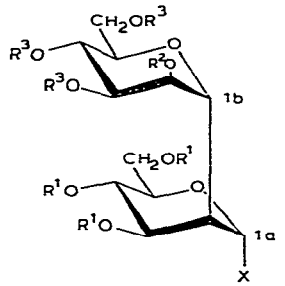
Bn = $PhCH_2$

Glycosylation of the D-mannobioside **16** with the mono-D-mannosyl donor **8** gave **21**, and deacetylation of **21** led to the isolation of the D-mannotrioside **22** in 71% yield. The α-D configuration at the anomeric carbon atom of the newly introduced D-mannosyl group was determined from the ^{13}C-n.m.r. spectrum, which

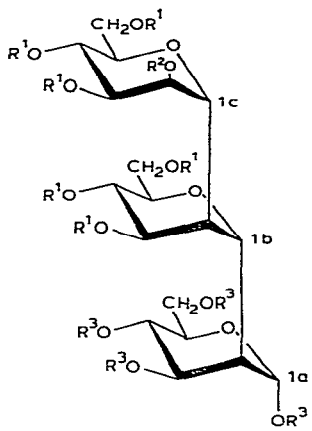
showed two signals for three anomeric carbon atoms, at δ 100.9 with $^1J_{CH}$ 170.9 Hz, and at δ 98.1 with $^1J_{CH}$ 171.9 Hz, in the ratio of 2:1, and was further confirmed by hydrogenolysis of **22** to free D-mannotriose **5** (refs. 8 and 9).

Similar glycosylation of the acceptor **22** with the D-mannosyl donor **8** gave the fully protected D-mannotetraoside **25** in 89% yield. *O*-Deacetylation of **25** to **26**, and hydrogenolysis of **26**, afforded free D-mannotetraose (**6**), which corresponds to part of the structure of *Candida utilis* D-mannan.

B. Synthesis of D-*mannohexaose by use of* D-*mannobiosyl donor* **20**. — An efficient elongation of the mannan chain in the α-(1→2) direction could also be achieved by using the D-mannobiosyl donor **20**. The donor (**20**) was prepared from **9** in 4 steps in 59% overall yield: (*i*) glycosylation with **10** in the presence of[10] $HgBr_2$–powdered molecular sieve 4A, (*ii*) H_2 in the presence of 10% Pd–C, (*iii*) Ac_2O–pyridine, and (*iv*) HBr–AcOH. The reactivity of donor **20** was examined by reaction with the acceptors **9** and **16**. Glycosylation of **9** with **20** in the presence of $AgOSO_2CF_3$–powdered molecular sieve 4A gave **23**, and deprotection of **23** afforded crystalline D-mannotriose (**5**) in 3 steps, in 41% overall yield. Similarly, the protected D-mannobioside **16** was used as the glycosyl acceptor, and converted into free D-mannotetraose (**6**) in 3 steps, in 51% overall yield. It may be noted that, by using the stepwise approach described in (*A*), D-mannotriose (**5**) and D-mannotetraose (**6**) were respectively obtained from **9** and **16**, in 26% (5 steps) and 51% (5 steps) yield.



15 $R^1 = R^3 = Bn, R^2 = Ac, X = OBn$
16 $R^1 = R^3 = Bn, R^2 = H, X = OBn$
4 $R^1 = R^2 = R^3 = H, X = OH$
17 $R^1 = Bn, R^2 = R^3 = Ac, X = OBn$
18 $R^1 = H, R^2 = R^3 = Ac, X = OH$
19 $R^1 = R^2 = R^3 = Ac, X = OAc$
20 $R^1 = R^2 = R^3 = Ac, X = Br$



21 $R^1 = R^3 = Bn, R^2 = Ac$
22 $R^1 = R^3 = Bn, R^2 = H$
23 $R^1 = R^2 = Ac, R^3 = Bn$
24 $R^1 = R^2 = H, R^3 = Bn$
5 $R^1 = R^2 = R^3 = H$

Finally, glycosylation of **26** with **20** was performed in the presence of $AgOSO_2CF_3$–powdered molecular sieve 4A, to afford the fully protected D-mannohexaoside **29** in 73% yield. The structural assignment of **29** was supported by its ^{1}H-n.m.r. spectrum, which contained, in addition to the signals for 13 benzyl groups,

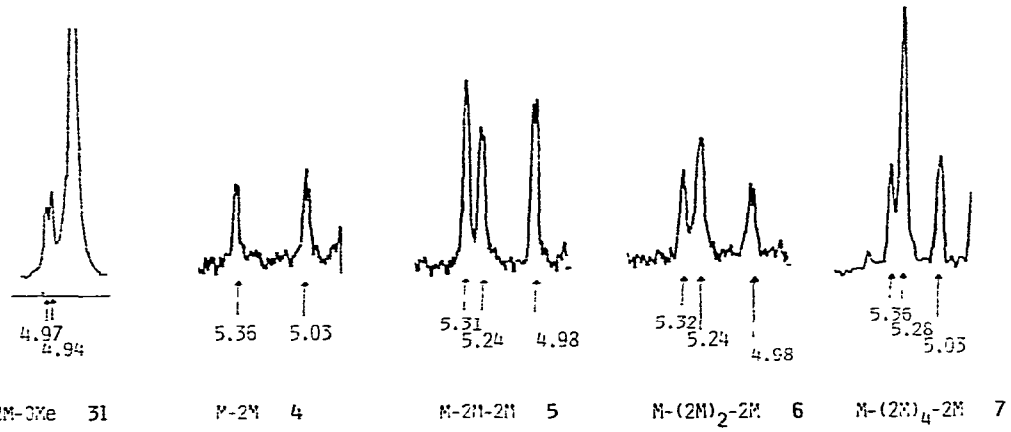


Fig. 1. ^{1}H-N.m.r. spectra of synthetic D-manno-oligosaccharides.

TABLE I

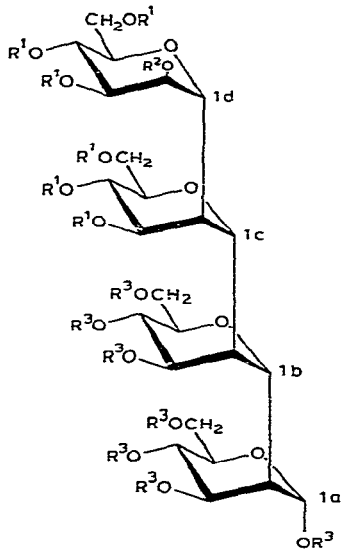
CHEMICAL SHIFTS[a] OF ANOMERIC PROTONS OF D-MANNO-OLIGOSACCHARIDES IN D_2O AT 25°

Structure	*Proton*					
	H-1a	*H-1b*	*H-1c*	*H-1d*	*H-1e*	*H-1f*
M_2 (4)	5.36	5.03				
M_3 (5)	5.31	5.24	4.98			
M_4 (6)	5.32	5.24	5.24	4.99		
M_6 (7)	5.36	5.28	5.28	5.28	5.28	5.03

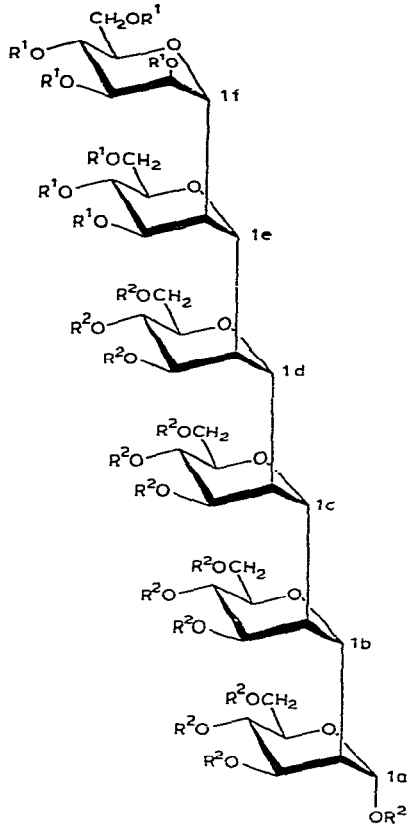
[a]The values of δ_H are expressed in p.p.m. downward from the internal standard, sodium 2,2,3,3-tetradeuterio-4,4-dimethyl-4-silapentanoate. Samples were prepared by "exchanging" them with D_2O by several times dissolving them in 99.8% D_2O and evaporating *in vacuo*.

five singlets (for seven acetyl groups), at δ 2.08, 2.05, 2.02, 2.00, and 1.96, in the ratios of 1:1:1:3:1. *O*-Deacetylation of **29** to **30**, and hydrogenolysis of **30** in the presence of 10% Pd–C in AcOH, afforded D-mannohexaose (**7**) as crystals in 65% yield.

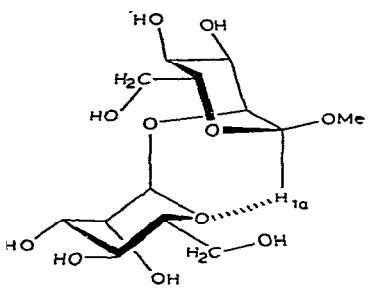
C. ^{1}H- and ^{13}C-N.m.r. spectroscopy of synthetic D-*manno-oligosaccharides.* — ^{1}H-N.m.r. data for synthetic D-manno-oligosaccharides are shown in Fig. 1 and Table I. These data are in good agreement with those previously reported for D-mannobiose **4** (ref. 9), D-mannotriose **5** (ref. 9), and D-mannotetraose **6** (refs. 11 and 12) of natural origin. Deshielding of H-1b in the spectrum of **5** by 0.21 p.p.m., as compared with H-1b in **4**, may be explained by the favored conformation **32**, stabilized by the exo-anomeric effect[13], as in the case[14] of D-mannobioside **31**, wherein the ring-oxygen atom of the D-mannosyl group is situated close to H-1a. As the same amount of deshielding of the signals was observed for the all of the anomeric protons of the internal, D-mannosyl residues in D-mannohexaose (**7**), a regular, helical conformation,



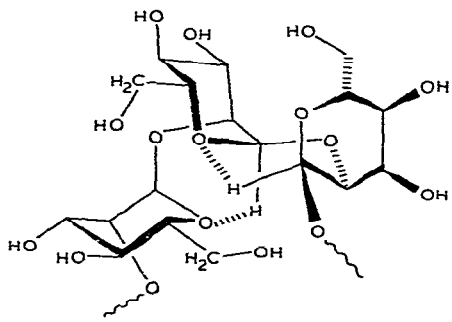
25 $R^1 = R^3 = Bn, R^2 = Ac$
26 $R^1 = R^3 = Bn, R^2 = H$
27 $R^1 = R^2 = Ac, R^3 = Bn$
28 $R^1 = R^2 = H, R^3 = Bn$
6 $R^1 = R^2 = R^3 = H$



29 $R^1 = Ac, R^2 = Bn$
30 $R^1 = H, R^2 = Bn$
7 $R^1 = R^2 = H$



31



32

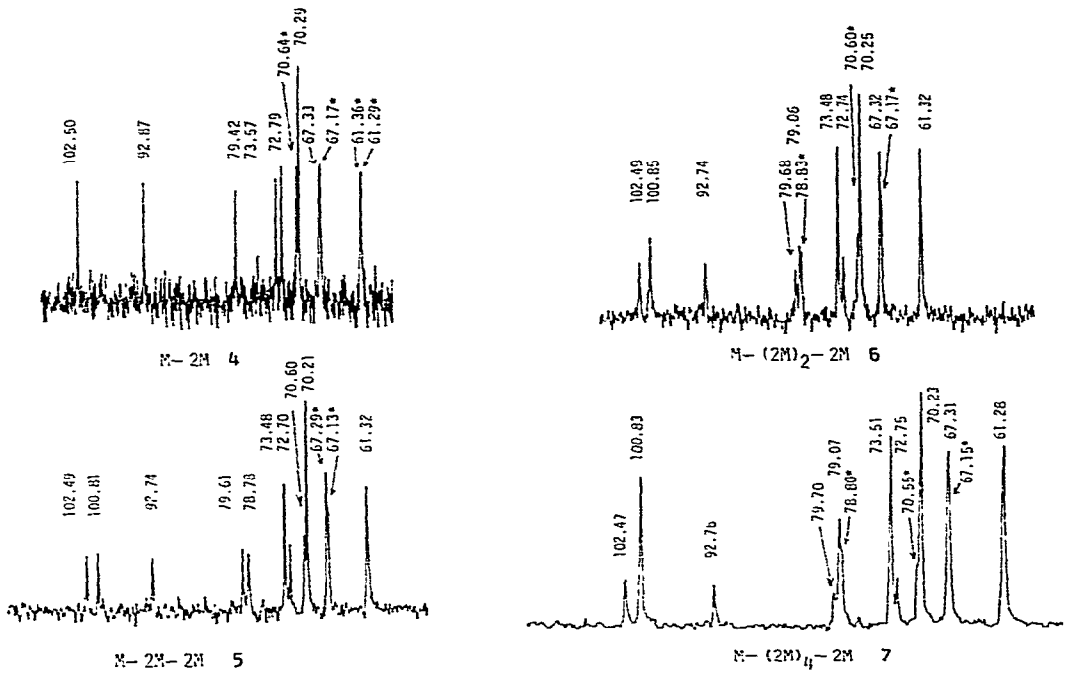


Fig. 2. ^{13}C-N.m.r. spectra of synthetic D-manno-oligosaccharides. (The signals at the chemical shifts marked with * could be detected by enlargement of the spectrum.)

as depicted in **32**, also seems to be favored in the case of longer (1→2)-α-D-mannan chains.

The ^{13}C-n.m.r. data for some synthetic D-manno-oligosaccharides are shown in Fig. 2 and Table II. The $^{1}J_{CH}$ values observed for the signals of all of the anomeric carbon atoms were in the range of 168.9–173.8 Hz, thus proving the α configuration at all inter-D-mannosidic linkages in the synthetic products. Each signal was assignable, based on the data for α-D-mannopyranose[15] and on our previous observations[14]. The signals for the anomeric carbon atoms of the D-mannose residues (at the reducing end) were the most shielded, and appeared at 92.74–92.87 p.p.m. Those of the (internal) D-mannosyl residues were more shielded, compared with those of the D-mannosyl groups (at the nonreducing end). The former appeared at 100.81–100.85 p.p.m., the latter at 102.47–102.50 p.p.m. Therefore, by measuring the peak area of these signals, the chain length of a (1→2)-α-D-mannan may be estimated.

The signals for C-2 may be classified into four groups: *1*, the most deshielded, for those of the D-mannose residues (at the reducing end) appeared at 79.42–79.70 p.p.m.; *2*, for those of the internal D-mannosyl residues, at 79.06–79.07 p.p.m.; *3*, for those of the D-mannosyl residues located next to the nonreducing end, at 78.78–78.83 p.p.m.; and *4*, for those of the D-mannosyl groups (at the nonreducing end), at 70.55–70.64 p.p.m.

TABLE II

^{13}C-CHEMICAL SHIFTS OF D-MANNO-OLIGOSACCHARIDES[a]

Structure	*Chemical shift for each carbon atom (p.p.m.) Reducing end*					
	1	*2*	*3*	*4*	*5*	*6*
α-D-Mannopyranose	95.20	71.85	71.35	68.05	73.35	62.15
M→2M (4)	92.87 (171.9)[b]	79.42	70.29	67.33	73.57	61.36
M→2M→2M (5)	92.74 (171.9)	79.61	70.21	67.29	73.48	61.32
M→(2M)$_2$→2M (6)	92.74 (170.9)	79.68	70.25	67.32	73.48	61.32
M→(2M)$_4$→2M (7)	92.76 (171.4)	79.70	70.23	67.31	73.51	61.28

Structure	*Chemical shift for each carbon atom (p.p.m.) Internal*					
	1	*2*	*3*	*4*	*5*	*6*
M→(2M)$_2$→2M (6)	100.85 (170.9)	79.06	70.25	67.32	73.48	61.32
M→(2M)$_4$→2M (7)	100.83 (171.4)	79.07	70.23	67.31	73.51	61.28

Structure	*Chemical shift for each carbon atom (p.p.m.) Next to nonreducing end*					
	1	*2*	*3*	*4*	*5*	*6*
M→2M→2M (5)	100.81 (173.8)	78.78	70.21	67.29	73.48	61.32
M→(2M)$_2$→2M (6)	100.85 (170.9)	78.83	70.25	67.32	73.48	61.32
M→(2M)$_4$→2M (7)	100.83 (171.4)	78.80	70.23	67.31	73.51	61.28

Structure	*Chemical shift for each carbon atom (p.p.m.) Nonreducing end*					
	1	*2*	*3*	*4*	*5*	*6*
M→2M (4)	102.50 (172.9)	70.64	70.29	67.17	72.79	61.29
M→2M→2M (5)	102.49 (171.9)	70.60	70.21	67.13	72.70	61.32
M→(2M)$_2$→2M (6)	102.49 (168.9)	70.60	70.25	67.17	72.74	61.32
M→(2M)$_4$→2M (7)	102.47 (171.4)	70.55	70.23	67.15	72.75	61.28

[a]The values of δ_C are expressed in p.p.m. downward from tetramethylsilane, referenced indirectly with an internal standard of 1,4-dioxane (δ 66.90). [b]Values in parentheses correspond to $^1J_{CH}$ (in Hz).

These signal assignments for C-1 and C-2 of **4**, **5**, and **6** were found to be in rough agreement with the data for natural samples discussed by Gorin[16].

In conclusion, D-mannotetraose (**6**) and D-mannohexose (**7**) corresponding to the partial structures of yeast cell-wall D-mannan were synthesized unambiguously, and their ^{1}H- and ^{13}C-n.m.r. data were found to be in agreement with those of natural samples.

EXPERIMENTAL

General. — Melting points were determined with a Yanagimoto micro melting-point apparatus and are uncorrected. Optical rotations were determined with a Perkin–Elmer Model 141 polarimeter, for solutions in $CHCl_3$ at 25°, unless noted otherwise. Column chromatography was performed on columns of Silica Gel Merck (70–230 mesh; E. Merck, Darmstadt, Germany). Thin-layer chromatography (t.l.c.) was performed on plates (layer thickness, 0.25 mm) precoated with Silica Gel 60 F_{254} (E. Merck, Darmstadt, Germany). I.r. spectra were recorded with an EPI-G2 Hitachi spectrophotometer, using KBr pellets for the crystalline samples, and neat films for the liquid samples. ^{1}H-N.m.r. spectra were recorded with a Varian HA-100 n.m.r. spectrometer, using tetramethylsilane as the internal standard. ^{13}C-N.m.r. spectra were recorded with a JNM-FX 100FT n.m.r. spectrometer operated at 25.05 MHz. The values of δ_C and δ_H are expressed in p.p.m. downwards from the internal standard, for solutions in $CDCl_3$, unless noted otherwise.

*3,4,6-Tri-*O*-acetyl-1,2-*O*-(1-benzyloxyethylidene)-β-*D*-mannopyranose* (**13**). — A mixture of **10** (prepared[17] from 100 g of D-mannose), benzyl alcohol (52.8 mL), and Et_3N (108 mL) in $Cl(CH_2)_2Cl$ (500 mL) was boiled for 16 h under reflux, cooled, washed with aq. $NaHCO_3$, dried ($MgSO_4$), treated with charcoal, and evaporated *in vacuo*. The residue crystallized from EtOAc–iPr_2O, to give **13** (144.5 g, 59% from D-mannose), m.p. 140–141°, $[\alpha]_D$ +95.2° (*c* 7.2).

Anal. Calc. for $C_{21}H_{26}O_{10}$: C, 57.53; H, 5.98. Found: C, 57.40; H, 5.80.

*3,4,6-Tri-*O*-benzyl-1,2-*O*-(1-benzyloxyethylidene)-β-*D*-mannopyranose* (**14**). — A solution of **13** (50 g) in MeOH (200 mL) was deacetylated with a catalytic amount of NaOMe. The usual processing, and subsequent benzylation with benzyl bromide and NaH, afforded crystalline **14** (57.7 g, 91%), m.p. 82–83° (iPr_2O–hexane), $[\alpha]_D$ +25.8° (*c* 2.8); R_F 0.57 in 10:1 CCl_4–Me_2CO.

Anal. Calc. for $C_{36}H_{38}O_7$: C, 74.20; H, 6.57. Found: C, 74.21; H, 6.37.

*Benzyl 3,4,6-tri-*O*-benzyl-α-*D*-mannopyranoside* (**9**). — A mixture of **14** (108 g) and $HgBr_2$ (5 g) was stirred under argon for 9 h at 140°. T.l.c. examination then showed the disappearance of starting **14** (at R_F 0.57) and the appearance of a new product (at R_F 0.51 in 10:1 CCl_4–Me_2CO). After being cooled, the mixture was dissolved in EtOAc, and the solution washed with water, dried ($MgSO_4$), and evaporated *in vacuo*, to give syrupy **11** (92 g). A solution of **11** (82 g) in oxolane (THF; 100 mL) and 25mM NaOMe in MeOH (200 mL) was stirred for 4 h at 15–20°, made neutral with Amberlist A-15, and evaporated; chromatography of the residue on

SiO_2 (1 kg) in 5:1 toluene–EtOAc afforded **9** (55 g; 50% from **14**), $[\alpha]_D$ −37.8° (*c* 8.6); δ_H: 7.4–7.1 (m, 20 H, 4 benzyl), and 5.61 (d, 1 H, *J* 2.5 Hz, H-1); δ_C: 98.4 ($^1J_{CH}$ 168.5 Hz, C-1), 75.1 (O-CH_2Ph-4), 74.3 (C-4), 73.4 (O-CH_2Ph-6), 71.9 (O-CH_2Ph-3), 71.2 (C-5), 69.0 (C-6), 68.8 (O-CH_2Ph-1), and 68.4 (C-2).

Anal. Calc. for $C_{34}H_{36}O_6$: C, 74.20; H, 6.57. Found: C, 74.24; H, 6.52.

Benzyl 2-O-(2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-3,4,6-tri-O-benzyl-α-D-mannopyranoside (**15**). — To a stirred mixture of **9** (4.3 g, 8 mmol), powdered molecular sieves 4A (20 g), and $AgOSO_2CF_3$ (3.9 g, 15 mmol) in $Cl(CH_2)_2Cl$ (50 mL) was added dropwise a solution of **8** [prepared[6] from **12** (5.06 g, 10 mmol)] in $Cl(CH_2)_2Cl$ (20 mL) at −30°. The mixture was stirred for 1 h at 15–20°; t.l.c. then showed the formation of a major product, at R_F 0.85, and a minor product, at R_F 0.81 (in 5:1 toluene–EtOAc). The mixture was diluted with $Cl(CH_2)_2Cl$ (200 mL), filtered through Celite, washed with aq. $NaHCO_3$, dried ($MgSO_4$), and evaporated *in vacuo*. The residue was chromatographed on SiO_2 (75 g) with 10:1 toluene–EtOAc, to give **15** (6.9 g, 85%), $[\alpha]_D$ +35.9° (*c* 5.1); R_F 0.85 in 5:1 toluene–EtOAc; δ_H: 7.4–7.1 (m, 35 H, 7 benzyl), 5.50 (bs, 1 H, H-1a or H-1b), and 2.07 (s, 3 H, Ac).

Anal. Calc. for $C_{63}H_{66}O_{12}$: C, 74.54; H, 6.55. Found: C, 74.13; H, 6.54.

Benzyl 3,4,6-tri-O-benzyl-2-O-(3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-α-D-mannopyranoside (**16**). — A solution of **15** (6.9 g) in THF (50 mL) and 75mM NaOMe in MeOH (100 mL) was stirred for 2 h at 20°, and evaporated *in vacuo*. The residue was chromatographed on SiO_2 (50 g) with 5:1 toluene–EtOAc, to give **16** (5.0 g, 76%), $[\alpha]_D$ +35.9° (*c* 8.1); R_F 0.35 in 5:1 toluene–EtOAC; δ_H: 7.4–7.1 (m, 35 H, 7 benzyl), 5.12 (d, 1 H, *J* 2 Hz, H-1a or H-1b), and 4.99 (d, 1 H, *J* 2 Hz, H-1b or H-1a).

2-O-α-D-Mannopyranosyl-α-D-mannopyranose (**4**). — A mixture of **16** (3.4 g) and 10% Pd–C (1.7 g) in AcOH (40 mL) was stirred for 24 h at 50° under H_2. Filtration through Celite, and evaporation *in vacuo*, afforded **4** (1.0 g, 82%) as an amorphous powder, m.p. 186–189° (dec.), $[\alpha]_D$ +60.0° (*c* 0.1, H_2O); R_F 0.44 in 2:1:1 BuOH–AcOH–H_2O; δ_H (D_2O): 5.36 (d, 1 H, *J* 2 Hz, H-1a), and 5.03 (d, 1 H, *J* 2 Hz, H-1b).

Anal. Calc. for $C_{12}H_{22}O_{11} \cdot 0.5\ H_2O$: C, 41.03; H, 6.60. Found: C, 40.79; H, 6.44.

1,3,4,6-Tetra-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-D-mannopyranose (**19**). — (*A*) Compound **4** (1.0 g) was acetylated with Ac_2O (10 mL) and pyridine (5 mL). The usual processing, and chromatography on 5:1 SiO_2–Celite (80 g) with 3:2 toluene–EtOAc, afforded crystalline **19** (1.55 g, 65% from **16**), m.p. 54–57° (EtOAc–cyclohexane), $[\alpha]_D$ +36.9° (*c* 4.8); δ_H: 6.23 (d, 1 H, *J* 2 Hz, H-1a), 4.94 (d, 1 H, *J* 2 Hz, H-1b), 2.13 (s, 6 H, 2 Ac), 2.12 (s, 3 H, Ac), 2.09 (s, 3 H, Ac), 2.07 (s, 3 H, Ac), 2.03 (s, 6 H, 2 Ac), and 2.00 (s, 3 H, Ac).

Anal. Calc. for $C_{28}H_{38}O_{19}$: C, 49.56; H, 5.60. Found: C, 49.34; H, 5.62.

(*B*) To a stirred mixture of **9** (540 mg, 1 mmol), powdered molecular sieves 4A (5 g), and $HgBr_2$ (720 mg) in $Cl(CH_2)_2Cl$ (20 mL) was added a solution of **10** (820 mg, 2 mmol) in $Cl(CH_2)_2Cl$ (5 mL) at 0°, and the mixture was stirred for 16 h

at 15–20°; t.l.c. (2:1 toluene–EtOAc) then showed the disappearance of **9** and **10**, and the formation of **17** (R_F 0.56). Processing, and chromatography on 5:1 SiO_2–Hiflo Super-Cel (50 g) with 2:1 toluene–EtOAc, gave syrupy **17** (740 mg, 89%), $[\alpha]_D$ +35.9° (*c* 0.5); δ_H: 2.10 (s, 3 H, Ac), 2.06 (s, 3 H, Ac), and 2.00 (s, 6 H, 2 Ac). A mixture of **17** (740 mg) and 10% Pd–C (500 mg) in AcOH (20 mL) was stirred for 16 h at 50° under H_2. Filtration, and evaporation of the filtrate, gave crude **18**, which was acetylated with Ac_2O (15 mL) and pyridine (30 mL). The solution was evaporated, and the residue was chromatographed on SiO_2 (10 g) with 1:1 toluene–EtOAc, to give crystalline **19** (400 mg, 59.0% from **9**).

3,4,6-Tri-O-acetyl-2-O-(2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl)-α-D-mannopyranosyl bromide (**20**). — A solution of **19** (250 mg, 0.41 mmol) in 30% HBr–AcOH (15 mL) was stirred for 2 h at 20°. T.l.c. examination (1:1 toluene–EtOAc) then showed the formation of a single product at R_F 0.44. Evaporation of the solvent *in vacuo*, and co-evaporation of traces thereof with toluene (3 × 20 mL), gave **20**, which was pure enough for the next step; δ_H: 6.67 (d, 1 H, *J* 2 Hz, H-1a).

Benzyl O-(*3,4,6-tri-O-benzyl-α-D-mannopyranosyl*)-(*1→2*)-O-(*3,4,6-tri-O-benzyl-α-D-mannopyranosyl*)-(*1→2*)-*3,4,6-tri-O-benzyl-α-D-mannopyranoside* (**22**). — To a stirred mixture of **16** (1.2 g, 1.2 mmol), powdered molecular sieves 4A (5 g), and $AgOSO_2CF_3$ (0.9 g, 3.4 mmol) in $Cl(CH_2)_2Cl$ (20 mL) was added dropwise a solution of **8** [prepared from 875 mg (2.4 mmol) of **12**] in $Cl(CH_2)_2Cl$ (5 mL) at −15°. The mixture was stirred for 16 h at 15–20°, when t.l.c. examination in 5:1 toluene–EtOAc showed the disappearance of **16** (R_F 0.35) and the formation of **21** (R_F 0.65). Processing afforded crude **21**, which was deacetylated in 0.1M NaOMe–MeOH (50 mL) and THF (50 mL). The crude product was chromatographed on 5:1 SiO_2–Hiflo Super-Cel (120 g) with 5:1 toluene–EtOAc, to give **22** (1.2 g, 71%), $[\alpha]_D$ +34.2° (*c* 10.6); R_F 0.41 in 5:1 toluene–EtOAc; δ_C: 100.9 ($^1J_{CH}$ 170.9 Hz, 2 anomeric carbons) and 98.1 ($^1J_{CH}$ 171.9 Hz, one anomeric carbon).

Anal. Calc. for $C_{86}H_{89}O_{15}$: C, 75.27; H, 6.49. Found: C, 75.34; H, 6.60.

O-α-D-*Mannopyranosyl*-(*1→2*)-O-α-D-*mannopyranosyl*-(*1→2*)-α-D-*mannopyranose* (**5**). — (*A*) A mixture of **22** (150 mg) and 10% Pd–C (100 mg) in AcOH (5 mL) was stirred for 16 h at 50° under H_2. The mixture was filtered through Celite, and the filtrate was evaporated *in vacuo*. The residue was purified on Sephadex G-25 (1 g) with H_2O, to give crystalline **5** (30 mg, 56%), m.p. 183–185°, $[\alpha]_D$ +55.3° (*c* 0.11, H_2O); R_F 0.35 in 2:1:1 BuOH–AcOH–H_2O; δ_H (D_2O): 5.31 (bs, 1 H, whh 3.0 Hz, H-1a), 5.24 (d, 1 H, *J* 2 Hz, H-1b), and 4.98 (d, 1 H, *J* 2 Hz, H-1c).

Anal. Calc. for $C_{18}H_{32}O_{16}$: C, 42.86; H, 6.39. Found: C, 42.67; H, 6.52.

(*B*) To a stirred mixture of powdered molecular sieves 4A (1 g), $AgOSO_2CF_3$ (100 mg), and **9** (100 mg, 0.19 mmol) in $Cl(CH_2)_2Cl$ (5 mL) was added dropwise a solution of **20** [prepared from **19** (126 mg, 0.19 mmol)] in $Cl(CH_2)_2Cl$ (2 mL) at −15°. The mixture was stirred for 16 h at 15–20°; t.l.c. in 1:1 toluene–EtOAc then showed the disappearance of both **9** (R_F 0.83) and **20** (R_F 0.40) and the formation of the major product (R_F 0.67). Processing, and chromatography on 5:1 SiO_2–Hyflo Super-Cel (5 g) with 2:1 toluene–EtOAc, afforded the major product

23 (120 mg, 53%). Compound **23** (120 mg) was stirred in 0.01M NaOMe–MeOH (10 mL) for 6 h at 20°; processing, and chromatography on 5:1 SiO_2–Hyflo Super-Cel (5 g) in 1:3 toluene–EtOAc, afforded **24** (77 mg, 87%); R_F 0.1 in 1:1 toluene–EtOAc.

A mixture of **24** (77 mg) and 10% Pd–C (50 mg) in AcOH (5 mL) was stirred for 16 h at 50–60°. Processing as in (*A*) afforded crystalline **5** (38.5 mg, 89%).

Benzyl O-(*2-O-acetyl-3,4,6-tri-O-benzyl-α-D-mannopyranosyl*)-[(*1→2*)-O-(*3,4,6-tri-O-benzyl-α-D-mannopyranosyl*)]$_2$-(*1→2*)-*3,4,6-tri-O-benzyl-α-D-mannopyranoside* (**25**). — To a stirred mixture of **22** (890 mg, 663 μmol), powdered molecular sieves 4A (5 g), and $AgOSO_2CF_3$ (500 mg, 1.92 mmol) in $Cl(CH_2)_2Cl$ (30 mL) was added dropwise a solution of **8** [prepared from **12** (500 mg, 1 mmol)] in $Cl(CH_2)_2Cl$ (5 mL) at −20°. The mixture was stirred for 16 h at 15–20°; processing, and chromatography on 5:1 SiO_2–Hyflo Super-Cel (120 g) with 10:1 toluene–EtOAc, afforded oily **25** (1.08 g, 89%), $[\alpha]_D$ +23.0° (*c* 5.0); R_F 0.74 in 5:1 toluene–EtOAc; δ_C: 101.2 ($^1J_{CH}$ 168.5 Hz), 100.7 ($^1J_{CH}$ 173.3 Hz), 99.4 ($^1J_{CH}$ 173.3 Hz), and 98.1 ($^1J_{CH}$ 172.1 Hz) for 4 anomeric carbon atoms.

Anal. Calc. for $C_{117}H_{122}O_{22}$: C, 74.74; H, 6.54. Found: C, 74.87; H, 6.58.

O-*α-D-Mannopyranosyl*-(*1→2*)-O-*α-D-mannopyranosyl*-(*1→2*)-O-*α-D-mannopyranosyl*-(*1→2*)-*α-D-mannopyranose* (**6**). — (*A*) A solution of **25** (1.0 g) in THF (50 mL) was treated with 0.05M NaOMe–MeOH (50 mL) for 16 h at 20°. Processing, and chromatography on 5:1 SiO_2–Hiflo Super-Cel (120 g) in 5:1 toluene–EtOAc, afforded **26** (843 mg, 79.8%), $[\alpha]_D$ +31.3° (*c* 4.9).

A mixture of **26** (140 mg) and 10% Pd–C (100 mg) in AcOH (5 mL) was stirred for 16 h at 50° under H_2. Processing, and chromatography on Sephadex G-25 (1 g) in H_2O, afforded crystalline **6**, m.p. 180.5–182° (H_2O–EtOH), $[\alpha]_D$ +45.9° (*c* 0.46, H_2O); R_F 0.28 in 2:1:1 BuOH–AcOH–H_2O; δ_H: 5.32 (d, 1 H, *J* 2 Hz, H-1a), 5.24 (bs, 2 H, whh 4 Hz, H-1b and H-1c), and 4.99 (d, 1 H, *J* 2 Hz, H-1e).

Anal. Calc. for $C_{24}H_{42}O_{21} \cdot 2\,H_2O$: C, 41.03; H, 6.59. Found: C, 40.99; H, 6.31.

(*B*) To a stirred mixture of powdered molecular sieves 4A (1 g), $AgOSO_2CF_3$ (100 mg), and **16** (90 mg, 93 μmol) was added dropwise a solution of **20** [prepared from **19** (70 mg, 0.11 mmol)] at −15°, and the mixture was stirred for 16 h at 20°. T.l.c. examination (3:1 toluene–EtOAc) then showed the disappearance of **16** (R_F 0.88) and the formation of the major product (R_F 0.67). Processing, and chromatography on SiO_2 (5 g) with 10:1 toluene–EtOAc, afforded **27** (120 mg, 61%). Compound **27** (120 mg) in 0.01M NaOMe–MeOH (10 mL) was stirred for 2 h at 20°. Processing, and chromatography on SiO_2 (5 g) with 1:2 toluene–EtOAc, afforded syrupy **28** (72 mg, 84%); R_F 0.18 in 1:1 toluene–EtOAc.

Hydrogenolysis of **28** (72 mg) in AcOH (3 mL) in the presence of 10% Pd–C (30 mg) for 16 h at 60° under H_2, and processing as in (*A*), afforded **6** (36 mg; quantitative).

Benzyl O-(*2,3,4,6-tetra-O-acetyl-α-D-mannopyranosyl*)-(*1→2*)-O-(*3,4,6-tri-O-acetyl-α-D-mannopyranosyl*)-[(*1→2*)-O-*3,4,6-tri-O-benzyl-α-D-mannopyranosyl*]$_3$-(*1→2*)-*3,4,6-tri-O-benzyl-α-D-mannopyranoside* (**29**). — To a stirred mixture of powdered molecular sieves 4A (5 g), $AgOSO_2CF_3$ (300 mg, 1.15 mmol), and **26** (280 mg, 0.15

mmol) in $Cl(CH_2)_2Cl$ (20 mL) was added dropwise a solution of **20** [prepared from **19** (250 mg, 0.41 mmol)] in $Cl(CH_2)Cl$ (5 mL) at 0°. The mixture was stirred for 16 h at 20°; t.l.c. examination (2:1 toluene–EtOAc) then showed the disappearance of **26** (R_F 0.85) and the formation of the major product (R_F 0.60). Processing, and chromatography on 5:1 SiO_2–Hyflo Super-Cel (20 g) with 2:1 toluene–EtOAc, afforded oily **29** (270 mg, 73%), $[\alpha]_D$ +21.1° (*c* 1.9); δ_H: 7.11 (s, 65 H, 13 Ph), 2.08 (s, 3 H, Ac), 2.05 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 2.00 (s, 9 H, 3 Ac), and 1.96 (s, 3 H, Ac).

Anal. Calc. for $C_{141}H_{148}O_{38}$: C, 69.0; H, 6.09. Found: C, 69.1; H, 6.31.

O-α-D-*Mannopyranosyl*-[(*1→2*)-O-α-D-*mannopyranosyl*]$_4$-(*1→2*)-α-D-*mannopyranose* (**7**). — A solution of **29** (200 mg) in 0.01M NaOMe–MeOH (30 mL) was stirred for 1 h at 20°. Processing, and chromatography on SiO_2 (5 g) in EtOAc, afforded **30** (120 mg, 68%); R_F 0.48 in EtOAc.

A mixture of **30** (120 mg) and 10% Pd–C (60 mg) in AcOH (5 mL) was stirred for 16 h at 50° under H_2. Processing, and chromatography on Sephadex G-25 (2 g) with H_2O, afforded crystalline **7** (51 mg, 95%), m.p. 177–182° (dec.), $[\alpha]_D$ +32.2° (*c* 0.34, H_2O); R_F 0.18 in 2:1:1 BuOH–AcOH–H_2O; δ_H (D_2O): 5.36 (bs, 1 H, whh 4 Hz, H-1a), 5.28 (s, 4 H, whh 6 Hz, H-1b,1c,1d,1e), and 5.03 (bs, 1 H, whh 4 Hz, H-1f).

Anal. Calc. for $C_{36}H_{64}O_{32} \cdot H_2O$: C, 42.11; H, 6.24. Found: C, 42.23; H, 6.24.

ACKNOWLEDGMENTS

We thank Dr. J. Uzawa and Mrs. T. Chijimatsu for recording and measuring the n.m.r. spectra, and Dr. H. Homma and his staff for the elemental analyses. We also thank Emeritus Scientist Prof. W. Matsui for his encouragement, and Miss A. Sone for technical assistance.

REFERENCES

1 T. OGAWA AND K. BEPPU, *Carbohydr. Res.*, 101 (1982) 271–277.
2 C. HASHIMOTO, R. E. COHEN, AND C. E. BALLOU, *Biochemistry*, 19 (1980) 5932–5938.
3 K. OGAWA, K. MATSUDA, K. TAMARI, AND S. KIYO-OKA, *Agric. Biol. Chem.*, 42 (1978) 1101–1109.
4 R. J. YU, C. T. BISHOP, F. P. COOPER, H. F. HASENCLEVER, AND F. BLANK, *Can. J. Chem.*, 45 (1967) 2205–2211; Y. FUKAZAWA, A. NISHIKAWA, M. SUZUKI, AND T. SHINODA, *Med. Mycol. Zentralbl. Bakt., Suppl.*, 8 (1980) 127–136.
5 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 93 (1981) 67–81, 231–241.
6 T. OGAWA, K. KATANO, AND M. MATSUI, *Carbohydr. Res.*, 64 (1978) c3–c9; T. OGAWA, K. KATANO, K. SASAJIMA, AND M. MATSUI, *Tetrahedron*, 37 (1981) 2779–2786.
7 K. BOCK, I. LUNDT, AND C. PEDERSEN, *Tetrahedron Lett.*, (1973) 1037–1040; K. BOCK AND C. PEDERSEN, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297; *Acta Chem. Scand., Ser. B.*, 29 (1975) 258–264.
8 P. A. J. GORIN AND A. S. PERLIN, *Can. J. Chem.*, 35 (1957) 262–267.
9 Y.-C. LEE AND C. E. BALLOU, *Biochemistry*, 4 (1965) 257–264.
10 J.-C. JACQUINET, J.-R. POUGNY, D. DUCHET, AND P. SINAŸ, *Abstr. Joint Conf. Chem. Inst. Can. Am. Chem. Soc., 2nd*, (1977) CARB 44.

11 P. A. J. GORIN, M. MAZUREK, AND J. F. T. SPENCER, *Can. J. Chem.*, 46 (1968) 2305–2310.
12 R. E. COHEN AND C. E. BALLOU, *Biochemistry*, 19 (1980) 4345–4358.
13 R. U. LEMIEUX AND S. KOTO, *Tetrahedron*, 30 (1974) 1933–1944.
14 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.
15 W. VOELTER, V. BILÍK, AND E. BREITMAIER, *Collect. Czech. Chem. Commun.*, 38 (1973) 2054–2071.
16 P. A. J. GORIN, *Can. J. Chem.*, 51 (1973) 2375–2383.
17 R. U. LEMIEUX, *Methods Carbohydr. Chem.*, 2 (1963) 221–228.