Carbohydrate Research, 242 (1993) 161–166
Elsevier Science Publishers B.V., Amsterdam

Chemical synthesis of a comb-shaped, branched stereoregular polysaccharide, 4-*O*-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranan

Kazukiyo Kobayashi *, Koji Nomura and Masahiko Okada
Faculty of Agriculture, Nagoya University, Chikusa, Nagoya 464-01 (Japan)

(Received July 12th, 1992; accepted October 20th, 1992)

ABSTRACT

4-*O*-α-D-Mannopyranosyl-(1 → 6)-α-D-mannopyranan (**7**) was prepared via ring-opening polymerization of 1,6-anhydro-2,3-di-*O*-benzyl-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-β-D-mannopyranose (**5**) using phosphorus pentafluoride as initiator in dichloromethane at −60°C, followed by debenzylation. Compound **5** was obtained via glycosidation of 1,6-anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (**1**) with 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-mannopyranose (**2**) using *p*-toluenesulfonic acid as catalyst and subsequent transformation of the protecting groups of the resulting 1,6-anhydro-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-2,3-*O*-isopropylidene-β-D-mannopyranose (**3**).

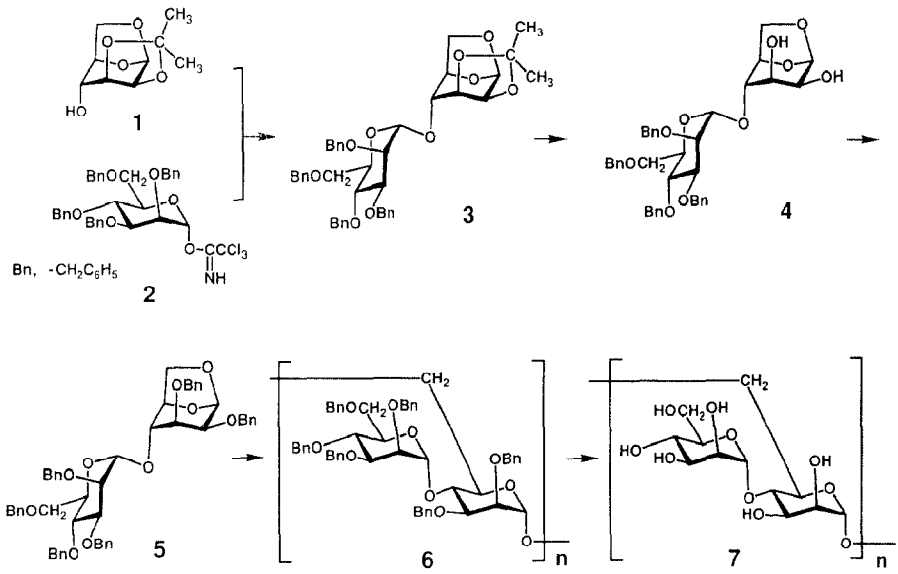
INTRODUCTION

This paper reports the synthesis of 4-*O*-(α-D-mannopyranosyl)-(1 → 6)-α-D-mannopyranan (**7**) via ring-opening polymerization of 1,6-anhydro disaccharide derivative **5** according to the route shown in Scheme 1. The branched polysaccharide has a pendant sugar moiety regularly substituted on each sugar unit of the linear backbone chain[1–3]. Such a comb-shaped, branched polysaccharide consists of densely packed α-D-mannopyranose units alone. D-Mannose occurring on cell surfaces is one of the key recognition markers in some biological interactions[4–6]. We expect that well-defined comb-shaped branched polysaccharides could serve as model compounds for physicochemical and biological studies and cell-specific biomedial materials using α-D-mannose as a recognition signal.

RESULTS AND DISCUSSION

Glycosidation of the 1,6-anhydromannose derivative[7] **1** with the tetra-*O*-benzyl-D-mannose imidate[8] **2** was attempted with *p*-toluenesulfonic acid and boron

* Corresponding author.



Scheme 1. Synthesis of 4-*O*-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranan (**7**).

trifluoride diethyl etherate as catalysts[9]. Use of boron trifluoride diethyl etherate, even at −30°C, gave a mixture of α- and β-anomeric products and also other unassignable by-products. In contrast, a high α-anomeric glycosidation selectivity was attained with *p*-toluenesulfonic acid as catalyst at room temperature. Only one disaccharide derivative was produced and was isolated by silica gel chromatography in 70% yield. Removal of the isopropylidene protecting groups, followed by benzylation, gave the perbenzylated anhydromannobiose monomer **5**.

Glycosidation proceeded with retention of the α-anomeric configuration, shown by the anomeric ^1H and ^{13}C NMR signals of compounds **3**, **4**, and **5**. Each ^1H NMR spectrum showed a singlet assignable to the β-anomeric proton of the anhydromannose unit (H-1β) and a doublet assignable to the α-anomeric proton of a nonreducing mannose unit (H-1′α), respectively at 5.30 and 5.01 ppm for **3**, 5.31 and 4.98 ppm for **4**, and 5.42 and 4.74 ppm for **5**. The anomeric C-1 signals of **3** were almost overlapped at 98.9 and 98.7 ppm. The resonances at 101.5 and 98.7 ppm of **4** and at 99.8 and 97.9 ppm of **5** were respectively assignable to a β-anomeric carbon of an anhydromannose unit (C-1β) and an α-anomeric carbon of a nonreducing mannose unit (C-1′α). The coupling constant $J_{C\text{-}1,H\text{-}1}$ of 166.0 Hz at 97.9 ppm of **5** was attributable to coupling between the C-1′α anomeric carbon and the equatorial H-1 proton of the $^4C_1$ form of the nonreducing mannopyranose[10]. The coupling constant $J_{C\text{-}1,H\text{-}1}$ of 175.8 Hz at 99.8 ppm of **5** was due to coupling between the C-1β anomeric carbon and the equatorial H-1 proton of the $^1C_4$ form of the anhydropyranose ring [11].

Ring-opening polymerization of **5** was carried out using 20 mol% PF_5 initiator in dichloromethane for 26 h at −60°C. The yield was 70%, and the $\overline{M}_n$ value was

2.3×10^4 ($\overline{DP_n} = 26$) and $\overline{M_w}/\overline{M_n} = 2.2$. A high concentration of initiator was required, probably because PF_5 was consumed through coordination to the numerous benzyl ether oxygen atoms of the monomer and polymer[1–3].

Inspection of CPK molecular models suggests that the polymer backbone is very crowded by a large volume of pendant tetrabenzylated sugar units. As a result, the polymer sequence is thermodynamically less stable than that of the polymerizate of the corresponding anhydro-monosaccharide derivatives. Highly reactive disaccharide anhydrides are thus required if high molecular-weight polysaccharides are to be obtained. It has been reported that 1,6-anhydro-β-D-mannopyranose derivatives show the highest polymerization reactivity among the 1,6-anhydro-D-glycopyranoses[12,13], and that 4-*O*-substituted anhydro-glycopyranoses are more reactive than 3-*O*- and 2-*O*-substituted ones[3]. The monomer **5**, effectively meets these criteria and gives a stereoregular polysaccharide.

Debenzylation of polymer **6** with sodium in liquid ammonia gave the polysaccharide **7**, which has a regio- and stereo-specifically substituted mannose branch on each repeating unit. The average degree of polymerization, referred to the disaccharide unit, was 28. One molecular chain had 56 units of the α-mannose residues.

It was confirmed from the ^1H and ^{13}C NMR spectra of polymers **6** and **7** that both the pendant and backbone mannose units have the α-anomeric configurations. Polymer **6** showed two singlet proton signals at 5.40 ppm attributable to the pendent α-mannose moiety (H-1′α) and at 5.06 ppm due to the backbone α-mannose moiety (H-1α). ^{13}C NMR resonances at 100.7 and 100.1 ppm were due respectively to the pendant α-mannose (C-1′α) and the backbone α-mannose (C-1α) moiety. Polysaccharide **7** also showed similar NMR resonances to **6**, except that signals of benzyl ethers were absent. The singlet peaks at 5.31 and 4.96 ppm were respectively assignable to H-1′α [α-(1 → 4)-linked mannoside] and H-1α [α-(1 → 6)-linked mannoside][14]. The ^{13}C NMR spectrum of **7** is shown in Fig. 1. The coupling constants $J_{C\text{-}1,H\text{-}1}$ of 171.4 Hz at 100.9 ppm for the C-1′α carbon and $J_{C\text{-}1,H\text{-}1}$ of 170.4 Hz at 100.1 ppm for the C-1α carbon were diagnostic of the α configuration. Complete assignment of the ^1H and ^{13}C NMR spectra of **6** and **7** has not yet been made, owing to excessive overlapping of similar resonances of the pendant and backbone moieties.

EXPERIMENTAL

General methods.—200-MHz ^1H and 50-MHz ^{13}C NMR spectra were obtained with a Japan Electron Optics Laboratory JNM-FX-200 Fourier transform NMR spectrometer using solutions in $CDCl_3$ with Me_4Si as the internal reference. The ^1H NMR spectrum of the deprotected polysaccharide **7** was obtained with a Bruker 600-MHz NMR spectrometer using a solution in D_2O with CH_3OH as the reference (3.40 ppm). Optical rotations were determined with a Jasco DIP-181 digital polarimeter using a jacketed 1-dm cell. Gel-permeation chromatography of

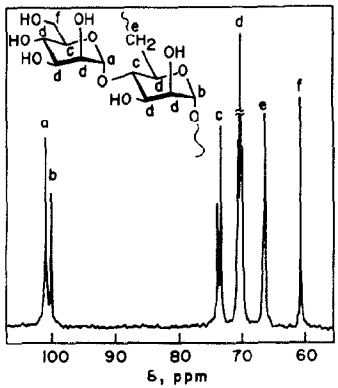


Fig. 1. ^{13}C NMR spectrum of 4-*O*-α-D-mannopyranosyl-(1 → 6)-α-D-mannopyranan (**7**). (5% in D_2O; at 70°C; reference, MeOH 50 MHz).

6 was carried out on a Toso HLC-8020 high-speed chromatograph using TSK-gel $GMH_{XL} \times 2$ and $G2000H_{XL}$, $G3000H_{XL}$, $G4000H_{XL}$, and $G5000H_{XL}$ columns (solvent, $CHCl_3$; polystyrene standards). Gel-permeation chromatography of **7** was performed with a Jasco Trirotor high-speed chromatograph using Shodex B805 → 804 columns (solvent, water; pullulan standards). Microanalysis was made with a Perkin–Elmer 240C elemental analyzer.

Materials.—1,6-Anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (**1**) was prepared from D-mannose by the method of Fraser-Reid[7]; mp 154–165°C (lit., 159–169°C); $[\alpha]_D^{25}$, −53.1° (*c* 1.0, $CHCl_3$). 2,3,4,6-Tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-mannopyranose[8] (**2**) was prepared from mannose via methyl α-D-mannopyranoside, methyl 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranoside, and then 2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranose[15]. ^{13}C NMR: 95.9 ppm (C-1); ^1H NMR: 6.37 ppm (H-1).

1,6-Anhydro-4-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-2,3-O-isopropylidene-β-D-mannopyranose (**3**).—1,6-Anhydro-2,3-*O*-isopropylidene-β-D-mannopyranose (**1**; 0.40 g, 2.0 mmol) and 2,3,4,6-tetra-*O*-benzyl-1-*O*-trichloroacetimidoyl-α-D-mannopyranose (**2**; 1.64 g, 2.4 mmol) were dissolved in dry CH_2Cl_2 (20 mL). *p*-Toluenesulfonic acid (0.40 mmol) and 4A molecular sieves (800 mg) were added and the mixture was stirred for 5.5 h at a room temperature. The reaction was monitored by TLC [R_f 0.58, 2:1 hexane–EtOAc] and terminated with Et_3N (0.1 mL). The solid pellet was removed by filtration and washed with water (30 mL). The organic layer was dried ($MgSO_4$) and concentrated in a rotary evaporator. The resulting syrup was chromatographed through silica gel [2:1 hexane–EtOAc]; yield 1.0 g (70%); ^1H NMR (3% in $CDCl_3$, 200 MHz): δ 7.34–7.15 (m, 20 H, C_6H_5), 5.30 (s, 1 H, H-1), 5.01 (d, 1 H, *J* 1.5 Hz H-1′), 4.89–4.47 (m), 3.98–3.54 (m), 1.51 and 1.31 (2s, 6 H, CH_3); ^{13}C NMR (3% in $CDCl_3$, 50 MHz): δ 138.1 (*ipso*-phenyl), 128.1–127.4 (phenyl), 109.7 (CMe_2), 98.9 and 98.7 (C-1 and C-1′), 79.8 (C-3′), 75.6–72.1, 69.4 (C-6′), 64.4 (C-6), and 25.9 (CH_3). *Anal.* Calcd for $C_{43}O_{10}H_{48}$: C, 71.25; H, 6.68. Found: C, 71.28; H, 6.84.

1,6-Anhydro-4-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-β-D-mannopyranose (**4**).—Compound **3** (1.73 g, 2.4 mmol) was stirred in 4:1 CF_3CO_2H–water (20 mL) for 25 min at room temperature[16]. Methanol (20 mL) was added and the solution was evaporated to give crystalline **4**; yield 1.58 g (97%). It was recrystallized from EtOH; mp 150–151°C, $[\alpha]_D^{25}$ +3.8° (*c* 1.0, $CHCl_3$); ^1H NMR (4% in $CDCl_3$, 200 MHz): δ 7.33–7.15 (m, 20 H, phenyl), 5.31 (s, 1 H, H-1), 4.98 (d, 1 H, *J* 1.7 Hz, H-1′), 4.88–4.46 (m), and 4.06–3.52 (m); ^{13}C NMR (4% in $CDCl_3$, 50 MHz): δ 138.3–138.0 (*ipso*-phenyl), 128.3–127.6 (phenyl), 101.5 (C-1), 98.7 (C-1′), 79.8 (C-3′), 75.2–69.1, 66.6 (C-2) and 64.9 (C-6). *Anal.* Calcd for $C_{40}H_{44}O_{10}$: C, 70.16; H, 6.48. Found: C, 70.08; H, 6.63.

1,6-Anhydro-2,3-di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-β-D-mannopyranose (**5**).—Compound **4** (1.37 g, 2.0 mmol) was dissolved in dry tetrahydrofuran (30 mL). Sodium hydride (0.32 g, 8.0 mmol) and Bu_4NI (30 mg, 0.080 mmol) were added and then benzyl bromide (1.0 mL, 8.28 mmol) was added through a dropping funnel. The mixture was stirred for 2 h at 50°C with monitoring by TLC [R_f 0.69, 1:1 hexane–EtOAc]. Water (30 mL) was added, the organic layer was extracted with $CHCl_3$, the extract was dried ($MgSO_4$), concentrated, and then chromatographed through silica gel [2:1 hexane–EtoAc]; yield 1.57 g (91%); $[\alpha]_D^{25}$ +3.4° (*c* 1.0, $CHCl_3$); ^1H NMR (4% in $CDCl_3$, 200 MHz): δ 7.34–7.15 (m, 30 H, phenyl), 5.42 (s, 1 H, H-1); 4.74 (d, 1 H, *J* 1.7 Hz, H-1′), 4.87–4.42 (m), and 4.18–3.34 (m); ^{13}C NMR (5% in $CDCl_3$, 50 MHz): δ 138.2–137.5 (*ipso*-phenyl), 128.1–127.4 (phenyl), 99.8 (C-1), 97.9 (C-1′), 79.8 (C-3′), 75.1–71.1, 69.4 (C-6′), and 65.0 (C-6). *Anal.* Calcd for $C_{54}H_{56}O_{10}$: C, 74.98; H, 6.52. Found: C, 74.94; H, 6.73.

2,3-Di-O-benzyl-4-O-(2,3,4,6-tetra-O-benzyl-α-D-mannopyranosyl)-(1 → 6)-α-D-mannopyranan (**6**).—Polymerization of viscous liquid monomer **5** was carried out by a high-vacuum technique[17] using a vessel previously reported[18]. Monomer **5** (0.43 g, 0.50 mmol) was charged into the round-bottomed flask and dried with CaH_2 in CH_2Cl_2. The solution was transferred through a glass filter into the polymerization tube and the monomer concentration was adjusted to 0.5 mol/L. Initiator PF_5 was generated by pyrolysis of a precursor, *p*-chlorobenzenediazonium hexafluorophosphate (29 mg, 0.10 mmol), and introduced into the monomer solution, which was cooled in a liquid-nitrogen bath.

The polymerization tube was refrigerated for 26 h at −60°C. The polymerization was terminated with an excess amount of cold MeOH. The product was purified by reprecipitation of its $CHCl_3$ solution into MeOH four times, and then freeze-dried from a benzene solution; $[\alpha]_D^{25}$ +16.5° (*c* 1.0, $CHCl_3$); ^1H NMR (9% in $CDCl_3$, 50°C, 200 MHz): δ 7.19–6.80 (m, 30 H, phenyl), 5.40 (s, 1 H, H-1′), 5.06 (m, 1 H, H-1), and 4.73–3.55 (m); ^{13}C NMR (5% in $CDCl_3$, 70°C, 50 MHz): δ 138.6–137.9 (*ipso*-phenyl), 128.1–126.8 (phenyl), 100.7 (C-1′), 100.1 (C-1), 80.4 and 79.4 (C-3 and C-3′), and 74.8–68.0. *Anal.* Calcd for $(C_{54}H_{56}O_{10})_n$: C, 74.98; H, 6.52. Found: C, 74.98; H, 6.62.

4-O-α-D-Mannopyranosyl-(1 → 6)-α-D-mannopyranan (**7**).—Polymer **6** (0.24 g) was dissolved in a mixture of toluene (15 mL) and 1,2-dimethoxyethane (5 mL) and

the solution was added dropwise to liquid NH_3 (50 mL) in a three-necked flask equipped with a cold-finger trap. Small pieces of Na metal (0.31 g) were added until the blue color of the solution persisted, and reaction was continued[16] for 2.5 h at −33°C. The reaction was terminated with aq NH_4Cl. Ammonia was evaporated off and organic materials were extracted with CH_2Cl_2. The water layer was dialyzed in a cellulose tube, concentrated, and freeze-dried to give the product in 80% yield; $[\alpha]_D^{25}$ +119.4° (*c* 0.7, H_2O); ^1H NMR (3% in D_2O, 50°C, 600 MHz): δ 5.31 (s, 1H, H-1′), 4.96 (s, 1 H, H-1), 4.29 (OH), and 4.10–3.73 (m); ^{13}C NMR (5% in D_2O, 70°C, 50 MHz): δ 100.8 (C-1′), 100.1 (C-1), 74.1 and 73.6 (C-5′ and C-4), 70.8–70.2 (C-2, C-3, C-5, C-2′, and C-3′), 66.8 and 66.6 (C-4′ and C-6), and 61.1 (C-6′). *Anal.* Calcd for $(C_{12}H_{20}O_{10})_n$: C, 44.44; H, 6.22. Found: C, 44.43; H, 6.30.

ACKNOWLEDGMENTS

The authors are grateful to Professor Emeritus Hiroshi Sumitomo for his valuable advice, to Professor Isao Ikeda of Osaka University for recording the 600 MHz ^1H NMR spectra, and to Mr. Shigeyuki Kitamura for carrying out the elemental analysis. Financial support by a Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture, Japan is gratefully acknowledged.

REFERENCES

1 B. Veruovic and C. Schuerch, *Carbohydr. Res.*, 14 (1970) 199–206.
2 U. Masura and C. Schuerch, *Carbohydr. Res.*, 15 (1971) 65–72.
3 H. Ichikawa, K. Kobayashi, and H. Sumitomo, *Macromolecules*, 23 (1990) 1884–1886.
4 C.E. Ballou, *Adv. Enzymol.*, 40 (1974) 239–270.
5 N. Sharon, N. Firon, and I. Ofek, *Pure Appl. Chem.*, 55 (1983) 671–675.
6 Y.C. Lee, *Anal. Biochem.*, 189 (1990) 151–162.
7 M. Georges and B. Fraser-Reid, *Carbohydr. Res.*, 127 (1984) 162–164.
8 P. Fügedi, A. Lipták, P. Nánási, and A. Neszmelyi, *Carbohydr. Res.*, 107 (1982) c5–c8.
9 R.R. Schmidt, *Angew. Chem. Int. Ed. Engl.*, 25 (1986) 212–235.
10 K. Bock and C. Pederson, *J. Chem. Soc., Perkin Trans. 2*, (1974) 293–297.
11 H. Paulsen, V. Sinnwell, and W. Greve, *Carbohydr. Res.*, 49 (1976) 27–35.
12 K. Kobayashi and C. Schuerch, *J. Polym. Sci., Polym. Chem. Ed.*, 15 (1977) 913–926.
13 C. Schuerch, *Adv. Carbohydr. Chem. Biochem.*, 39 (1981) 157–212.
14 T. Ogawa and K. Sasajima, *Carbohydr. Res.*, 97 (1981) 205–227.
15 S. Koto, N. Morishima, Y. Miyata, and S. Zen., *Bull. Chem. Soc. Jpn.*, 49 (1976) 2639–2640.
16 J.E. Christensen and L. Goodman, *Carbohydr. Res.*, 7 (1968) 510–512.
17 C. Schuerch and T. Uryu, *Macromol. Synth.*, 4 (1972) 151–155.
18 K. Kobayashi and H. Sumitomo, *Nippon Kagaku Kaishi*, (1982) 1633–1637.