Carbohydrate Research, 152 (1986) 323–328
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

A synthesis of methyl 3-*O*-α-D-mannopyranosyl-α-D-mannopyranoside and related disaccharides*

MANJIT S. CHOWDHARY, RAKESH K. JAIN, SURJIT S. RANA, AND KHUSHI L. MATTA**
Department of Gynecologic Oncology, Roswell Park Memorial Institute, 666 Elm Street, Buffalo, New York 14263 (U.S.A.)

(Received June 10th, 1985; accepted for publication in revised form, November 12th, 1985)

For the past decade, we have been involved in the chemical synthesis of such oligosaccharides as *O*-α-L-fucosyl glycose derivatives which can be effectively employed for the study of glycosidases and glycosyltransferases[2–4]. Recently, we focused our attention on *O*-α-D-mannopyranosylglycose derivatives for the study of key enzymes involved in lysosomal-enzyme targeting. We have described[5] the synthesis of certain methyl D-mannobiosides and their use for specificity studies of *N*-acetylglucosamine-1-phosphotransferase (EC 2.7.8.17), one of the two enzymes responsible for such targeting. Among the various disaccharides tested[5], methyl 2-*O*-α-D-mannopyranosyl-α-D-mannopyranoside was found to be a better acceptor than methyl α-D-mannopyranoside, whereas methyl 3-*O*-α-D-mannopyranosyl-α-D-mannopyranoside (**8**) showed the least activity toward this enzyme. It was not clear if the disaccharide **8** can act as an inhibitor for *N*-acetylglucosamine-1-phosphotransferase. To elucidate this point, we desired a large-scale preparation of **8**, and the similarly linked disaccharides **10** and **12** having different aglycon moieties. We here describe a practical approach to this synthesis.

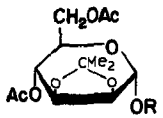
In the past, various types of protected intermediates were used for the synthesis of 3-*O*-D-mannopyranosyl-D-mannopyranosides. For example, Ekborg and Glaudemans[6] employed *p*-nitrophenyl 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside for the synthesis of *p*-nitrophenyl 3-*O*-α-D-mannopyranosyl-α-D-mannopyranoside (**10**), whereas Winnik *et al.*[7] condensed benzyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside with 2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl bromide, to give the desired disaccharide derivative. Similarly, benzyl 2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranoside and methyl 2,4,6-tri-*O*-benzyl-

*Synthetic Studies in Carbohydrates, Part XLIV. For Part XLIII, see ref. 1.
**To whom correspondence should be addressed.

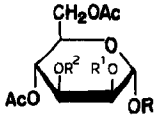
0008-6215/86/$ 03.50

α-D-mannopyranoside have been used for the synthesis of the target compounds[5,8]. Lee and Wood[9] condensed methyl 4,6-*O*-benzylidene-α-D-mannopyranoside directly with tetra-*O*-acetyl-α-D-mannopyranosyl bromide, and obtained the 3-*O*-α-D-mannopyranosyl derivative in 24% yield. Herein, we describe the use of methyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**4**), *p*-nitrophenyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**6**), and allyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside[10] for the synthesis of disaccharides **8** (refs. 5, 9, and 11), **10** (ref. 6), and **12**, respectively.

Conventional acetylation of methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[12] with acetic anhydride and pyridine gave **1**, which, on treatment with trifluoroacetic acid, produced crystalline **3** in 80% yield. The diol **3** was then converted into its 2,3-(ethyl orthoacetate), which was hydrolyzed with 80% acetic acid to give the key intermediate, namely methyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**4**), in 90% yield. The ^{1}H-n.m.r. spectrum of **4** exhibited a low-field shift of the H-2 resonance, compared to **3**, confirming that compound **4** had been acetylated at O-2.



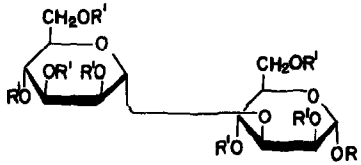
1 R = Me
2 R = $C_6H_4NO_2$-*p*



3 R = Me, R^1 = R^2 = H
4 R = Me, R^1 = Ac, R^2 = H
5 R = $C_6H_4NO_2$-*p*, R^1 = R^2 = H
6 R = $C_6H_4NO_2$-*p*, R^1 = Ac, R^2 = H

Starting from *p*-nitrophenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[13], the synthesis of *p*-nitrophenyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside (**6**) was accomplished by a similar reaction-sequence conducted under identical conditions; its structure was confirmed by ^{1}H-n.m.r. spectroscopy. Allyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside, the key intermediate for the synthesis of disaccharide **12**, was prepared as described[10].

Condensation of compound **4** with 2,3,4,6-tetra-*O*-acetyl-α-D-manno-



7 R = Me, R^1 = Ac
8 R = Me, R^1 = H
9 R = $C_6H_4NO_2$-*p*, R^1 = Ac
10 R = $C_6H_4NO_2$-*p*, R^1 = H
11 R = All, R^1 = Ac
12 R = All, R^1 = H

All = $-CH_2-CH=CH_2$

pyranosyl bromide[14] gave the disaccharide derivative **7** in 45% yield. Under identical conditions of glycosylation, compound **6** and allyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside[10] produced **9** and **11**, respectively. Zemplén *O*-deacetylation of the disaccharide derivatives **7**, **9**, and **11** provided the title disaccharides **8** (refs. 5, 9, and 11), **10** (ref. 6), and **12**, respectively, in almost quantitative yield. The structures of **8**, **10** and **12** were confirmed by ^{1}H- and ^{13}C-n.m.r. spectroscopy.

EXPERIMENTAL

General methods. — These were the same as those described[5].

*Methyl 4,6-di-*O*-acetyl-2,3-*O*-isopropylidene-α-*D*-mannopyranoside* (**1**). — A solution of methyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[12] (4 g) in pyridine (40 mL) and acetic anhydride (10 mL) was stirred for 2 h at 60°, cooled, and evaporated to a thick syrup that crystallized from ether–hexane, to give **1** (5.2 g) in 96% yield; m.p. 52–53°, $[\alpha]_D$ +0.4° (*c* 1.8, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.33 and 1.53 (each s, 2 × 3 H, isopropylidene methyls), 2.07 (s, 6 H, Ac), 3.37 (s, 3 H, OMe), 3.67–4.33 (5 H, H-2,3,5,6,6'), 4.9 (s, 1 H, H-1), and 5.0 (dd, 1 H, $J_{3,4}$ 7, $J_{4,5}$ 10 Hz, H-4).

Anal. Calc. for $C_{14}H_{22}O_8$: C, 52.82; H, 6.97. Found: C, 52.75; H, 6.98.

p-*Nitrophenyl 4,6-di-*O*-acetyl-2,3-*O*-isopropylidene-α-*D*-mannopyranoside* (**2**). — Conventional acetylation of *p*-nitrophenyl 2,3-*O*-isopropylidene-α-D-mannopyranoside[13] (0.5 g) with acetic anhydride in pyridine gave a syrup that crystallized from ether–hexane, to afford **2** in 87% yield (540 mg); m.p. 102–103°, $[\alpha]_D$ +74.6° (*c* 1, chloroform); R_F 0.73 in 19:1 chloroform–methanol; n.m.r. data ($CDCl_3$): δ 1.4 and 1.63 (each s, 2 × 3 H, isopropylidene methyls), 1.9 and 2.1 (each s, 2 × 3 H, 2 Ac), 4.36 (d, 2 H, $J_{5,6}$ 3 Hz, H-6,6'), 5.83 (d, 1 H, $J_{1,2}$ <1 Hz, H-1), and 7.13 and 8.2 (2 m, 2 × 2 H, aromatic).

Anal. Calc. for $C_{19}H_{23}NO_{10}$: C, 53.64; H, 5.45; N, 3.29. Found: C, 53.69; H, 5.55; N, 3.30.

*Methyl 4,6-di-*O*-acetyl-α-*D*-mannopyranoside* (**3**). — A solution of **1** (4 g) in trifluoroacetic acid (8 mL) and water (0.5 mL) was stirred for 1 h at room temperature, and then evaporated to dryness. Residual acid was removed by several coevaporations with toluene, to give a thick syrup that crystallized from ether–hexane to afford **3** in 80% yield (2.8 g); m.p. 76–77°, $[\alpha]_D$ +66.7° (*c* 0.9, chloroform), lit.[15] $[\alpha]_D$ +38° (chloroform); n.m.r. data ($CDCl_3$): δ 2.07 and 2.08 (each s, 2 × 3 H, 2 Ac), 3.37 (s, 3 H, OMe), 4.17 (d, 1 H, $J_{2,3}$ 3.3 Hz, H-2), 4.73 (s, 1 H, H-1), and 5.02 (t, 1 H, $J_{3,4} = J_{4,5}$ = 10 Hz, H-4).

Anal. Calc. for $C_{11}H_{18}O_8$: C, 47.48; H, 6.52. Found: C, 47.23; H, 6.57.

*Methyl 2,4,6-tri-*O*-acetyl-α-*D*-mannopyranoside* (**4**). — To a solution of **3** (5.4 g) in dry benzene (50 mL) was added triethyl orthoacetate (25 mL) and *p*-toluenesulfonic acid monohydrate (0.27 g). After stirring for 1 h at room temperature, triethylamine (15 mL) was added, and, after 5 min, the solution was poured into iced water, and the mixture extracted thrice with chloroform. The extracts were

combined, washed with water, dried (sodium sulfate), and evaporated, to give the 2,3-orthoester in quantitative yield. It was dissolved in 80% acetic acid (100 mL), and the solution stirred for 15 min at room temperature and evaporated; residual acetic acid was removed by coevaporation with toluene, to give a syrup which was purified by chromatography on a column of silica gel (chloroform) to give **4** (5.6 g, 90.1%); $[\alpha]_D$ +21.3° (*c* 1.1, chloroform); n.m.r. data ($CDCl_3$): δ 2.0–2.1 (3 s, 12 H, Ac), 3.3 (s, 3 H, OMe), 3.7–4.4 (m, 4 H, H-3,5,6,6'), 4.7 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), 5.0 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-2), and 5.0 (dd, 1 H, $J_{1,2}$ 2, $J_{2,3}$ 3.5 Hz, H-2).

Anal. Calc. for $C_{13}H_{19}O_9$: C, 48.90; H, 6.00. Found: C, 49.03; H, 6.17.

p-*Nitrophenyl 4,6-di-*O*-acetyl-*α*-*D*-mannopyranoside* (**5**). — The isopropylidene group of compound **2** (0.7 g) was removed as described for the preparation of **3**, to give a thick syrup that crystallized from ethyl acetate–hexane to afford **5** (0.56 g) in 88% yield; m.p. 117–119°, $[\alpha]_D$ +126.2° (*c* 0.9, chloroform); R_F 0.4 in 9:1 chloroform–methanol; n.m.r. data ($CDCl_3$): δ 1.98 and 2.13 (each s, 2 × 3 H, Ac), 5.13 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 5.68 (d, 1 H, $J_{1,2} \sim 1$ Hz, H-1), 7.17 and 8.2 (2 m, 2 × 2 H, aromatic).

Anal. Calc. for $C_{16}H_{19}NO_{10}$: C, 49.87; H, 4.97; N, 3.64. Found: C, 49.82; H, 5.08; N, 3.47.

p-*Nitrophenyl 2,4,6-tri-*O*-acetyl-*α*-*D*-mannopyranoside* (**6**). — Compound **6** was prepared from **5** (1.5 g) as described for **4**. After purification by column chromatography, a syrup was obtained in a yield of 85% (1.41 g); $[\alpha]_D$ +81.3° (*c* 1.2, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.0–2.3 (3 s, 12 H, Ac), 4.4 (m, 4 H, H-3,5,6,6'), 5.2 (t, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4), 5.3 (dd, 1 H, $J_{1,2}$ 2, $J_{2,3}$ 4 Hz, H-2), 5.7 (d, 1 H, $J_{1,2}$ 2 Hz, H-1), and 7.2 and 8.15 (2 m, 2 × 2 H, aromatic).

Anal. Calc. for $C_{18}H_{21}NO_{11}$: C, 50.58; H, 4.95; N, 3.28. Found: C, 50.30; H, 5.20; N, 3.10.

*Methyl 2,4,6-tri-*O*-acetyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-*α*-*D*-mannopyranosyl)-*α*-*D*-mannopyranoside* (**7**). — To a solution of compound **4** (6 g, 18.75 mmol) in freshly distilled, dry acetonitrile (30 mL) were added 4A molecular sieves (6 g), mercuric bromide (13.5 g), and mercuric cyanide (9.5 g), and the mixture was stirred at room temperature with exclusion of moisture while a solution of 2,3,4,6-tetra-*O*-acetyl-*α*-D-mannopyranosyl bromide[14] (11.7 g, 28.5 mmol) in freshly distilled acetonitrile (27 mL) was added. After 4 h, more of the bromide (3.9 g, 9.5 mmol) in acetonitrile (9 mL) was added, and the mixture was kept overnight, with stirring, filtered through Celite and the filtrate evaporated, to give a yellowish-brown syrup which was extracted twice with chloroform (200 mL). The extracts were combined, washed successively with saturated aqueous solutions of sodium chloride, $NaHCO_3$, and water, dried (magnesium sulfate), and evaporated to a light-yellow syrup. Purification by chromatography on a column of silica gel (chloroform) afforded **7** (5.5 g) in 45% yield; $[\alpha]_D$ +34.9° (*c* 1.1, chloroform), lit.[9] $[\alpha]_D$ +39.0° (*c* 0.9, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.9–2.2 (cluster of singlets, 21 H, Ac), 3.35 (s, 3 H, OMe), and 4.72 (d, 1 H, $J_{1,2}$ 1 Hz, H-1).

*Methyl 3-O-α-*D*-mannopyranosyl-α-*D*-mannopyranoside* (**8**). — A solution of

7 (1 g) in dry methanol (10 mL) was treated with a catalytic amount of M methanolic sodium methoxide for 1 h, the base neutralized with Amberlite IR-120 (H^+) ion-exchange resin, the suspension filtered, and the filtrate evaporated, to give **8** in 95% yield (0.52 g); $[\alpha]_D$ +101° (*c* 0.95, water), lit.[9] $[\alpha]_D$ +91° (*c* 0.8, water), lit.[5] $[\alpha]_D$ +103.3° (*c* 0.62, water); its ^{1}H- and ^{13}C-n.m.r. data were comparable to those reported in the literature[5,16].

p-*Nitrophenyl 2,4,6-tri*-O-*acetyl-3*-O-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (**9**). — Compound **9** was prepared from **6** (1 g, 2.34 mmol) as described for **7**. The syrup was purified by chromatography on a column of silica gel (chloroform) to afford **9** in 76% yield (1.35 g); $[\alpha]_D$ +72.7° (*c* 1.2, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 2.0–2.3 (cluster of singlets, 21 H, Ac), 5.7 (c, 1 H, $J_{1,2}$ 1.5 Hz, H-1), and 7.2 and 8.3 (2 m, 2 × 2 H, aromatic).

Anal. Calc. for $C_{32}H_{39}NO_{20}$: C, 50.73; H, 5.19; N, 1.85. Found: C, 50.53; H, 5.31; N, 1.72.

p-*Nitrophenyl 3*-O-α-D-*mannopyranosyl*-α-D-*mannopyranoside* (**10**). — *O*-Deacetylation of **9** (1 g) as described for **8** gave amorphous **10** (0.5 g, 82%); $[\alpha]_D$ +157.3° (*c* 0.9, water), lit.[6] $[\alpha]_{578}^{23}$ +185° (*c* 1.18, H_2O); the ^{1}H-n.m.r. data were comparable to those reported in the literature[6]; ^{13}C-n.m.r. data (D_2O): δ 103.4 (C-1′), 98.7 (C-1), 78.8 (C-3), 75.0 (C-5), 74.5 (C-5′), 71.5 (C-3′), 71.1 (C-2′), 70.4 (C-2), 67.9 (C-4′), 66.8 (C-4), 62.1 (C-6′), and 61.6 (C-6).

Allyl 2,4,6-tri-O-*acetyl-3*-O-*(2,3,4,6-tetra*-O-*acetyl*-α-D-*mannopyranosyl)*-α-D-*mannopyranoside* (**11**). — Glycosylation of allyl 2,4,6-tri-*O*-acetyl-α-D-mannopyranoside[10] (5.5 g) as described for **7** gave a syrup that was purified by chromatography on a column of silica gel with 3:1 (v/v) toluene–ethyl acetate, to afford **11** in 60% yield (6.5 g); $[\alpha]_D$ +34° (*c* 0.5, chloroform); ^{1}H-n.m.r. data ($CDCl_3$): δ 1.9–2.03 (cluster of singlets, 21 H, Ac).

Anal. Calc. for $C_{29}H_{40}O_{18}$: C, 51.48; H, 5.96. Found: C, 51.25; H, 6.03.

Allyl 3-O-α-D-*mannopyranosyl*-α-D-*mannopyranoside* (**12**). — *O*-Deacetylation of compound **11** (4 g) as described for **8** gave a solid mass which crystallized from methanol–ether to give **12** in 91% yield (2.05 g); m.p. 187–188°, $[\alpha]_D$ +94° (*c* 0.5, water); ^{13}C-n.m.r. data (Me_2SO-d_6): δ 134.39 (CH_2-*C*H=CH_2), 116.36 (CH_2-CH = *C*H_2), 102.11 (C-1′), 98.95 (C-1), 78.71 (C-3), 74.05 (C-5), 73.41 (C-5′), 70.58 (C-3′), 70.12 (C-2′), 69.29 (C-2), 66.95 (C-4′), 66.51 (*C*H_2-CH=CH_2), 65.65 (C-4), and 60.96 (C-6, C-6′).

Anal. Calc. for $C_{15}H_{26}O_{11}$: C, 47.12; H, 6.80. Found: C, 46.88; H, 7.07.

ACKNOWLEDGMENTS

We are grateful to Mrs. Onda D. Simmons for recording the n.m.r. spectra, and to Mrs. Martie Vallina for kindly typing the manuscript. The n.m.r. studies were supported by N.I.H Grant CA-08793. This investigation was supported by PHS Grant No. GM-31425 awarded by the National Institute of General Medical Sciences, DHHS.

REFERENCES

1 K. KOHATA, S. A. ABBAS, AND K. L. MATTA, *Carbohydr. Res.*, 132 (1984) 345–350.
2 R. A. DICIOCCIO, J. J. BARLOW, AND K. L. MATTA, *J. Biol. Chem.*, 257 (1982) 714–718.
3 R. A. DICIOCCIO, J. J. BARLOW, AND K. L. MATTA, *Clin. Chim. Acta*, 108 (1980) 41–48.
4 D. E. SYKES, S. S. RANA, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 112 (1983) 221–232.
5 R. MADIYALAKAN, M. S. CHOWDHARY, S. S. RANA, AND K. L. MATTA, *Carbohydr. Res.*, 152 (1986) 183–194.
6 G. EKBORG AND C. P. J. GLAUDEMANS, *Carbohydr. Res.*, 134 (1984) 83–87.
7 F. M. WINNIK, J. P. CARVER, AND J. J. KREPINSKY, *J. Org. Chem.*, 47 (1982) 2701–2707.
8 L. F. AWAD, E. S. H. EL ASHRY, AND C. SCHUERCH, *Carbohydr. Res.*, 122 (1983) 69–79.
9 E. E. LEE AND J. O. WOOD, *Carbohydr. Res.*, 75 (1979) 322-324.
10 A. YA. CHERNYAK, A. B. LEVINSKY, B. A. DMITRIEV, AND N. K. KOCHETKOV, *Carbohydr. Res.*, 128 (1984) 269–282.
11 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 93 (1981) 53–66.
12 M. E. EVANS AND F. W. PARRISH, *Carbohydr. Res.*, 54 (1977) 105–114.
13 S. S. RANA, J. J. BARLOW, AND K. L. MATTA, *Carbohydr. Res.*, 96 (1981) 79–85.
14 P. A. LEVENE AND R. S. TIPSON, *J. Biol. Chem.*, 90 (1931) 89–98; E. A. TALLEY, D. D. REYNOLDS, AND W. L. EVANS, *J. Am. Chem. Soc.*, 65 (1943) 575–582.
15 H. B. BOREN, P. J. GAREGG, L. KENNE, Å. PILOTTI, S. SVENSSON, AND C.-G. SWAHN, *Acta. Chem. Scand.*, 27 (1973) 2740–2748.
16 T. OGAWA AND K. SASAJIMA, *Carbohydr. Res.*, 97 (1981) 205–227.