SYNTHESIS OF MODEL OLIGOSACCHARIDES OF BIOLOGICAL SIGNIFICANCE. 3.

SYNTHESIS OF CARBON-13 LABELLED TRIMANNOSIDES

F. M. Winnik[a,†], J. P. Carver[a], and J. J. Krepinsky[a,b]

a. Departments of Medical Genetics and Medical Biophysics,University of Toronto, Toronto, Ontario, Canada M5S 1A8

b. Ludwig Institute for Cancer Research, Toronto Branch9, Earl Street, Toronto, Ontario, Canada M4Y 1M4

SUMMARY

Two isomeric trimannosides labelled with carbon-13 at one specific anomeric position have been synthesized. Methyl 2-O-allyl-4,6-O-benzylidene-α-D-mannopyranoside was reacted with tetra-O-acetyl-D-mannopyranosyl-1-^{13}C bromide. The disaccharide obtained was glycosylated with tetra-O-acetyl-D-mannopyranosyl bromide after hydrolysis of the benzylidene group. This sequence led to methyl 3-O-α-D-mannopyranosyl-1-^{13}C-6-O-α-D-mannopyranosyl-α-D-mannopyranoside after deblocking of all hydroxyl groups. When the ^{13}C-labelled and unlabelled glycosyl bromides were added to the methyl mannoside in the reversed order, methyl 3-O-α-D-mannopyranosyl-6-O-α-D-mannopyranosyl-1-^{13}C-α-D-mannopyranoside was obtained.
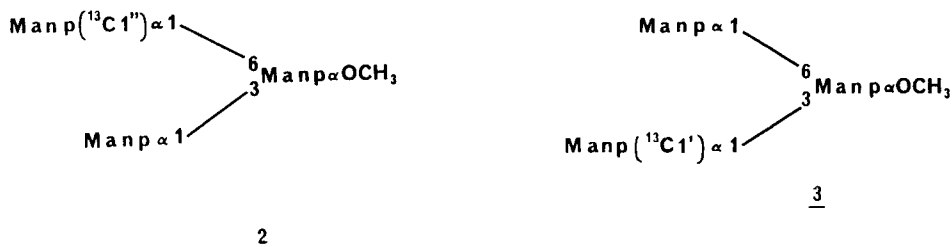
Key words: methyl 3,6-di-O-(α-D-mannopyranosyl)-α-D-mannopyranoside, carbon-13, D-mannose-1-[13]C.

INTRODUCTION

The trisaccharide methyl 3,6-di-O-α-D-mannopyranosyl-α-D-mannopyranoside <u>1</u> is a useful model for the study of the conformation of complex oligosaccharides (1). It also binds with high affinity to the lectin Concanavalin A, as shown by fluorescence displacement[†]studies (2). We report here the synthesis of two trisaccharides labelled at a specific anomeric position with carbon-13. In compound <u>2</u>, the C-6 substituent of the central mannoside is labelled at the anomeric position C-1"[*]. In compound <u>3</u> the ^{13}C label is placed at the anomeric position of the C-3 substituent of the central mannoside. The regiospecific synthesis of the isomers <u>2</u> and <u>3</u> was achieved by a sequence in which the two substituents of the central mannopyranoside are added in two consecutive steps.

$$Manp\left(^{13}C1''\right)\alpha 1$$
$$\overset{6}{\underset{3}{}}Manp\alpha OCH_3$$
$$Manp\alpha 1$$

2

$$Manp\alpha 1$$
$$\overset{6}{\underset{3}{}}Manp\alpha OCH_3$$
$$Manp\left(^{13}C1'\right)\alpha 1$$

3

RESULTS AND DISCUSSION

Scheme 1 outlines the synthesis of the trisaccharide **2** by glycosylation of the partially protected disaccharide **6** (3) with tetra-O-acetyl-α-D-mannopyranosyl-1-^{13}C bromide **7**. The bromide **7** was prepared immediately before use by reaction of trimethylsilyl bromide with the orthoester **5** (4, 5). The latter crystalline compound was synthesized in excellent yield from D-mannose-1-^{13}C (**4**) by standard techniques : peracetylation, bromination of the anomeric position, and treatment with methanol in the presence of 2,6-lutidine. The use of the orthoester might appear circuitous, but it resulted in a significant increase of the glycosylation yields, especially for reactions run on a small scale.

SCHEME 1 *
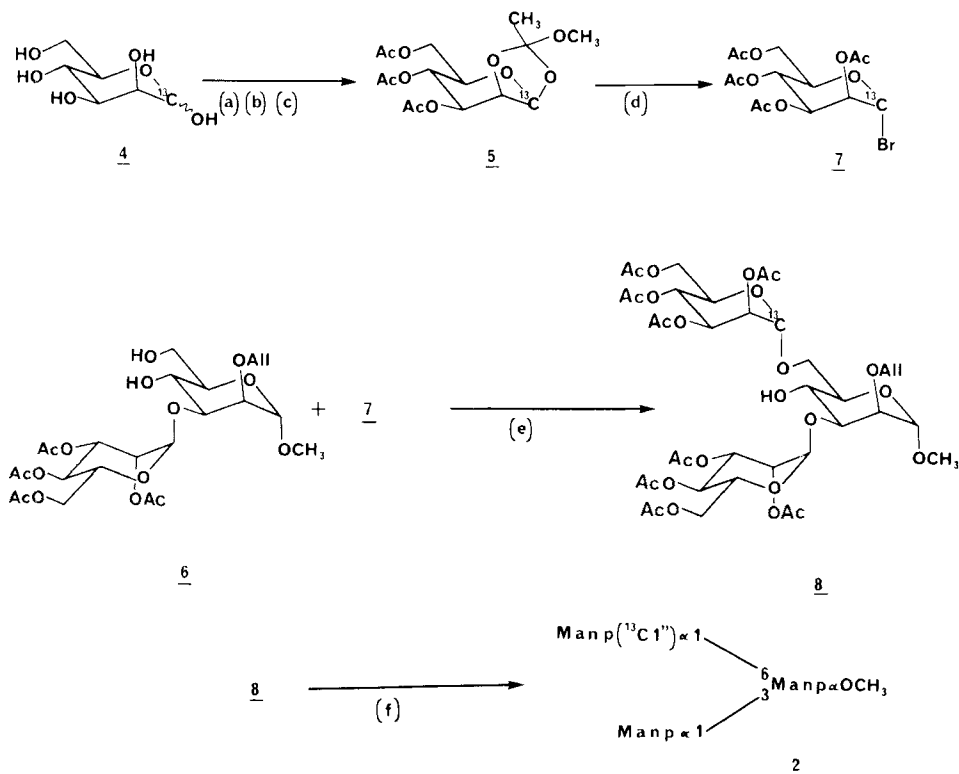
The glycosylation and the subsequent deprotection of the hydroxyl groups have been optimized for the preparation of the unlabelled trisaccharide **1** (3). They were performed without any modification, with the carbon-13 labelled compounds to give the trisaccharide **2**. The ^{1}H NMR spectrum of **2** (experimental section) is consistent with the assigned structure. Of particular interest is the presence in the anomeric region of a doublet of doublets (*J* 1.5 Hz, 176 Hz) at δ 4.92 assigned to the proton H-1", coupled to H-2" and to [13]C-1".

Scheme 2 illustrates the synthesis of the trimannoside **3**. The carbon-13 labelled disaccharide **10** was prepared by glycosylation of methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside with tetra-*O*-acetyl-α-D-mannopyranosyl-1-^{13}C bromide, following the procedure developed for the glycosylation of **9** with methyl 2-*O*-allyl-4,6-*O*-benzylidene-α-D-mannopyranoside (3). Hydrolysis of the benzylidene group in **10**, condensation of the resulting diol with one equivalent of tetra-*O*-acetyl-α-D-mannopyranosyl bromide, and subsequent deblocking of all hydroxy groups gave the trisaccharide **3**. This compound was characterized by its optical rotation and its ^{1}H NMR spectrum (see experimental section). In accord with the structural assignment, the resonance of the anomeric proton H-1' appears as a doublet of doublets (*J* 1.5 Hz, 176 Hz) at δ 5.10.

The binding of the carbon-13 labelled trimannosides **2** and **3** to Concanavalin A was examined by ^{13}C NMR spectroscopy. The results of this study will be reported and discussed in a separate communication (2).

EXPERIMENTAL

The ^{1}H NMR spectra were recorded at 360 MHz at 23 ± 2°C either in $CDCl_3$ containing 1% TMS as an internal standard or in D_2O with acetone (0.1%, 2.225 ppm relative to internal DSS) as the internal standard. Dichloromethane was dried by distillation under dry nitrogen in the presence of P_2O_5. Acetonitrile was dried by a 3h-reflux over CaH_2 and subsequent distillation under nitrogen onto 4 A molecular sieves. Methanol was dried by a 4h-reflux over Mg and a trace of I_2 and subsequent distillation under dry nitrogen onto 3 A molecular sieves. Mercuric bromide was dissolved in hot toluene, dried by azeotropic distillation of some toluene, and crystallized upon cooling. D-Mannose-1-[13]C (90 mol % [13]C) was purchased from Merck, Sharp, and Dohme. Gel filtration chromatography was performed on Bio Gel P-2, 200-400 mesh (Bio-Rad), the column effluent being monitored by a Flow Cell Refractive Index detector.

SCHEME 2[*]
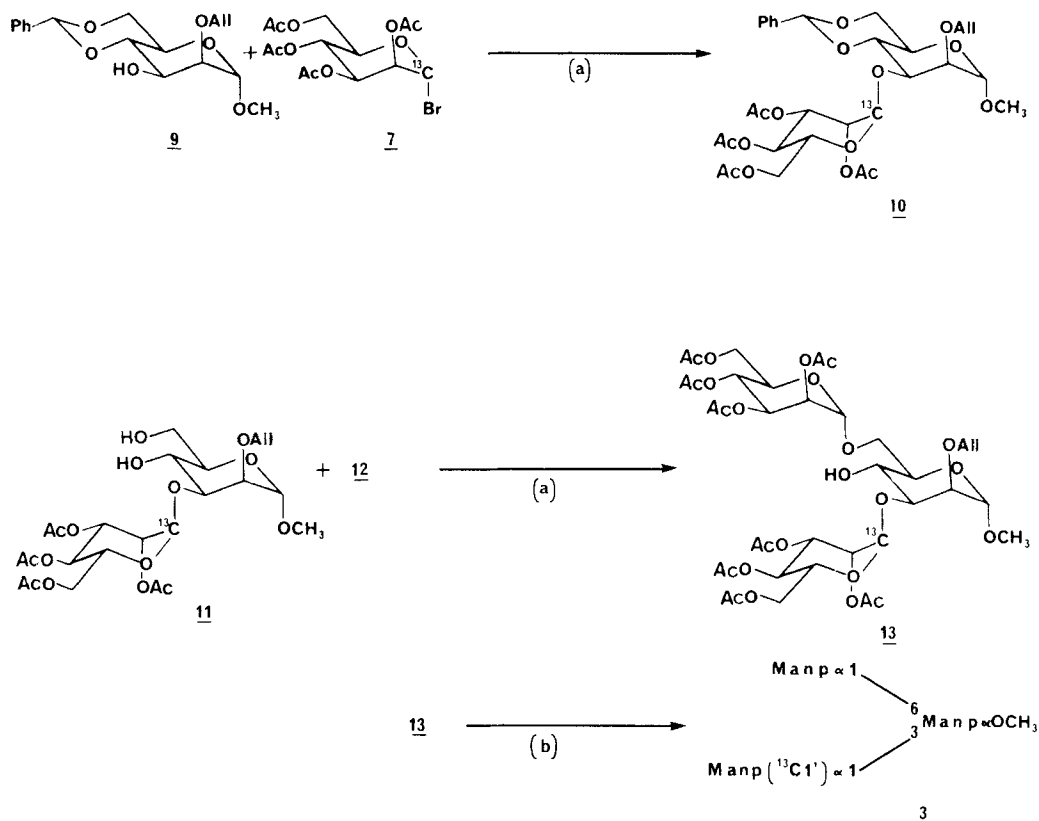
[•] Reagents : (a) HgBr$_2$, Hg(CN)$_2$, molecular sieves, CH$_3$CN; (b) 10% Pd/C, C$_2$H$_5$OH, CH$_3$CO$_2$H, H$_2$O, 70°C, then CH$_3$ONa in CH$_3$OH

General Deprotection Method

The allyl and acetyl substituted carbohydrates were deprotected as follows. The oligosaccharide (1 equivalent) was added to a suspension of Pd on carbon (10%, 0.1 equivalent) in ethanol/water/glacial acetic acid 2/1/1. The suspension was heated at 75°C for 17 h under nitrogen. The cold reaction mixture was filtered through celite. The filtrate was neutralized with NaHCO$_3$ and evaporated. The residue was taken up in CH$_2$Cl$_2$, washed with water, saturated NaHCO$_3$ and brine. It was dried over Na$_2$SO$_4$, filtered and evaporated to give an amorphous solid. This was dissolved in dry CH$_3$OH and treated with CH$_3$ONa (5 equivalents, 2 M in CH$_3$OH). After 20 min the solution was evaporated to dryness. The residue was dissolved in water and desalted with mixed ·bed resin (AG 501·X8, 20-50 mesh, Bio Rad). Filtration and lyophilization gave the crude oligosaccharide.

Work-up Conditions of the Glycosylation Reactions

In each reaction complete disappearance of the starting material was checked by TLC (eluent: toluene/ethyl acetate 1/1 v/v). The solvent (acetonitrile) was evaporated and the residue was extracted three times with $CHCl_3$. The organic extracts were washed with saturated KCl solution, saturated $NaHCO_3$ solution, water, and brine. The organic layer was dried with anhydrous Na_2SO_4, filtered, and stripped of the solvent.

3,4,6-Tri-O-acetyl-β-D-mannopyranose-1,2-methylorthoacetate-1-^{13}C. 5. - The orthoester **5** was prepared in three steps from D-mannose-1-^{13}C (400 mg) following the procedure described by Mazurek and Perlin (6); yield: 51 % (from D-mannose-1-^{13}C); ^{1}H NMR (CDCl$_3$) δ 1.76 (s, 3H, CH$_3$), 2.06 (s, 3H, OCOCH$_3$), 2.08 (s, 3H, OCOCH$_3$), 2.12 (s, 3H, OCOCH$_3$), 3.28 (s, 3H, OCH$_3$), 3.72- 3.65 (m, 1H, H-5), 4.16 (dd, 1H, 1.3 Hz, 12 Hz, H-6'), 4.25 (dd, 1H, 5 Hz, 12 Hz, H-6), 4.62 (dd, 1H, 1.5 Hz, 4 Hz, H-2), 5.15 (dd, 1H, 4 Hz, 9 Hz, H-2), 5.32 (t, 1H, 9 Hz, H-4), 5.50 (dd, 1H, 1.5 Hz, 176 Hz, H-1).

2,3,4,6-Tetra-O-acetyl-α-D-mannopyranosyl-1-^{13}C Bromide. 7.- Trimethylsilyl bromide (90 mg, 0.58 mmol) was added dropwise, under nitrogen to a solution of **5** (100 mg, 0.27 mmol) in dry CH_2Cl_2 (10 ml). The solution was refluxed under nitrogen for 2 h, cooled to room temperature, evaporated, and dried in high vacuo for 1 h. The resulting oil (310 mg, yield: 98%) was used without further purification.

Methyl 2-O-Allyl-3-O-(tetra-O-acetyl-α-D-mannopyranosyl)-6-O-(tetra-O-acetyl-α-D-mannopyranosyl-1 "-^{13}C)-α-D-mannopyranoside. 8.- The reaction was performed under nitrogen. To a solution of **6** (156 mg, 0.27 mmol) in dry acetonitrile (10 ml) containing molecular sieves (4 A), were added sequentially HgBr$_2$ (117 mg, 0.23 mmol), Hg(CN)$_2$ (81 mg, 0.32 mmol), and a solution of **7** (0.27 mmol) in dry acetonitrile (2 ml). After 1 h, all starting material had disappeared, as indicated by TLC . Usual work-up gave **8** as a colorless oil (165 mg); ^{1}H NMR (CDCl$_3$): δ 1.98 (s, 3H, OCOCH$_3$), 1.99 (s, 3H, OCOCH$_3$), 2.05 (s, 3H, OCOCH$_3$), 2.06 (s, 3H, OCOCH$_3$), 2.10 (s, 3H, OCOCH$_3$), 2.11 (s, 3H, OCOCH$_3$), 2.15 (s, 6H, two OCOCH$_3$), 3.0 (d, 1H, 1.5 Hz, 176 Hz, H-1"), 5.18-5.42 (m, 8H), 5.86-6.00 (m, 1H, CH$_2$=CH-).

Methyl 3-O-α-D-Mannopyranosyl-6-O-α-D-mannopyranosyl-1-^{13}C-α-D-manno-pyranoside. 2.- Deprotection of **8** as described in the general procedure gave an amorphous solid which was purified on a Bio Gel P-2 column eluted with degassed, distilled water. ^{1}H NMR (D$_2$O) δ 3.41 (s, 3H, OCH$_3$), 3.65-4.04 (m, 16H), 4.06 (dd, 1H, 1.7 Hz, 3.4 Hz, H-2), 4.09 (dd, 1H 1.7

Hz. 3.4 Hz, H-2"), 4.73 (d, 1H, H-1), 4.91 (dd, 1H, 1.7 Hz, 176 Hz, H-1"), 5.10 (d, 1H, 1.7 Hz, H-1').

Methyl 2-O-Allyl-4,6-O-benzylidene-3-O-(tetra-O-acetyl-α-D-mannopyranosyl-1'-^{13}C)-α-D-mannopyranoside. 10. - The reaction was done under nitrogen. To a solution of methyl 2-O-allyl-4,6-O-benzylidene-α-D-mannopyranoside **9** (3) (80 mg, 0.25 mmol) in dry acetonitrile (10 ml) containing 4 A molecular sieves were added sequentially, $HgBr_2$ (108 mg, 0.3 mmol), $Hg(CN)_2$ (76 mg, 0.3 mmol), and a solution of **7** (from 100 mg of **5**) in dry acetonitrile (5 ml). After 1 h 10 min the reaction was completed. Usual work-up gave **10**, which was recrystallized from ethyl acetate/hexane 1/5 v/v (150 mg, 92 %); m.p. 160-161°C (m.p. of unlabelled compound: 160-162°C (3)), ^{1}H NMR ($CDCl_3$) δ 1.99(s, 3H, $OCOCH_3$), 2.06 (s, 3H, $OCOCH_3$), 2.09 (s, 3H, $OCOCH_3$), 2.11 (s, 3H, $OCOCH_3$), 3.37 (s, 3H, OCH_3), 3.72 (dd, 1H, 1.5 Hz, 3.6 Hz, H-2), 3.73-3.79 (m, 1H, H-5), 3.86 (AM_2 t, 1H, 13 Hz, H-4), 4.02 -4.30 (m, 8H), 4.71 (d, 1H, 1.5 Hz, H-1), 5.20-5.42 (m, 5H), 5.58 (s, 1H, PhCH), 5.95-6.06 (m, 1H, CH_2=CH), 7.30-7.52 (m, 5H, aromatic).

Methyl 2-O-Allyl-3-O-(tetra-O-acetyl-α-D-mannopyranosyl-1'-^{13}C-α-D-mannopyranoside. 11 . - A solution of **10** (100 mg, 0.15 mmol) in 60% aqueous acetic acid (15 ml) was heated at 80°C for 30 min. The cooled mixture was evaporated and dried under high vacuo overnight. The residue was dissolved in chloroform. The solution was washed with water, saturated $NaHCO_3$, and brine. It was dried over Na_2SO_4 and evaporated to give an amorphous solid (75 mg), which was used in the next reaction without further purification.

Methyl 3-O-α-D-Mannopyranosyl-1'-^{13}C-6-O-α-D-mannopyranosyl-α-D-mannopyranoside. 3 . Tetra-O-acetyl-D-mannopyranosyl bromide **12** was prepared from 3,4,6-tri-O-acetyl-β-D-mannopyranose-1,2-(methyl orthoacetate) (50 mg, 0.14 mmol) and bromotrimethylsilane (0.1 ml, 0.6 mmol) as described (3). To a solution of **11** (75 mg, 0.13 mmol) in dry acetonitrile (10 ml) containing 4 A molecular sieves were added sequentially, $HgBr_2$ (65 mg, 0.18 mmol), $Hg(CN)_2$ (45 mg, 0.18 mmol), and a solution of **12** (~0.14 mmol) in acetonitrile (3 ml). The reaction was completed after 1 h. Usual work-up gave the trisaccharide **13**. Deprotection of the hydroxyl groups under standard conditions gave **3**, which was purified by chromatography on a Bio Gel P-2 column eluted with distilled, degassed water. ^{1}H NMR (D_2O) δ 3.41 (s, 3H, OCH_3), 3.65-4.0 (m, 16H), 4.06 (dd, 1H, 1.7 Hz, 3.4 Hz, H-2), 4.09 (m, 1H, H-2'), 4.73 (d, 1H, 1.7 Hz, H-1), 4.91 (d, 1H, 1.7 Hz, H-1"), 5.10 (dd, 1H, 1.7 Hz, 176 Hz, H-1').

ACKNOWLEDGEMENTS

REFERENCES

1. Brisson J. R. and Carver J. P.- Biochemistry, in press

2. MacKenzie A. E., Winnik F. M., Krepinsky J. J., and Carver J. P. - in preparation

3. Winnik F. M., Brisson J. R., Carver J. P., and Krepinsky J. J. - Carbohydr. Res., **103**: 15 (1982)

4. Newman M. S. and Olson D. R. - J. Org. Chem., **38**: 4203 (1973)

5. Paulsen H. and Lockhoff O. - Tetrahedron Lett., 4027 (1978); Gillard J. W. and Israel M. - *ibid.* 513 (1981)

6. Mazurek M. and Perlin A. S. - Can. J. Chem., **43**: 1918 (1965)