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# *p*-NITROPHENYL 2-, AND 3-*O*-α-D-MANNOPYRANOSYL-α-D-MANNOPYRANOSIDE*,†

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ABSTRACT

*p*-Nitrophenyl 3- and 2-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside were each condensed with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide, and the products were deprotected, to yield, respectively, *p*-nitrophenyl 2- and 3-*O*-α-D-mannopyranosyl-α-D-mannopyranoside.

## INTRODUCTION

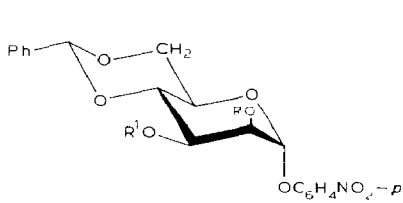
Our laboratory is preparing an extensive number of disaccharide determinants that occur on glycoproteins. These are being synthesized in a form suitable for linkage to a protein carrier, so as to produce immunogens[1,2]. We here report the synthesis of two additional such determinants.

## RESULTS AND DISCUSSION

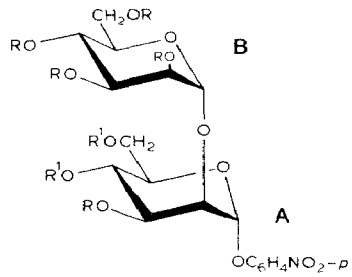
The preparation of *p*-nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**1**) has been reported[3]. The corresponding 2-benzoate (**2**) was prepared in 60% yield by treatment of *p*-nitrophenyl 4,6-*O*-benzylidene-α-D-mannopyranoside with trimethyl orthobenzoate, using *p*-toluenesulfonic acid as the catalyst, followed by treatment of the resulting, cyclic orthoester with aqueous acetic acid[4]. The two monobenzoates **1** and **2** were each condensed with 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide, using the silver triflate–2,4,6-collidine complex[5], and, after a short reaction-time, good yields (>60%) of the desired, protected disaccharides were obtained. Deprotection then yielded the crystalline compounds **4** and **6**.

Awad *et al.*[6] reported that the condensation of tetra-*O*-acetyl-α-D-man-

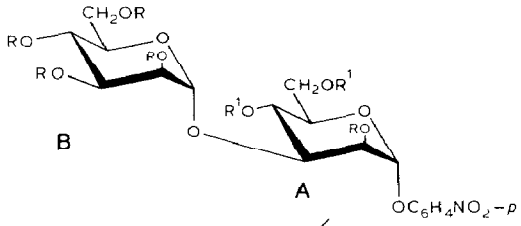
*The Preparation of Immunogens Bearing Immunodeterminants Known to Occur on Glycoproteins, Part III. For Part II, see ref. 1.
†This paper is dedicated to R. Stuart Tipson upon his retirement as a Regional Editor of this Journal.
**Visiting Associate, 1980–1984.



1 R = H, $R^1$ = Bz
2 R = Bz, $R^1$ = H



3 R = Bz, $R^1$ = PhCH
4 R = $R^1$ = H



5 R = Bz, $R^1$ = PhCH
6 R = $R^1$ = H

nopyranosyl bromide (**7**) with 2-(4-nitrophenyl)ethyl 2-*O*-acetyl-4,6-di-*O*-benzyl-α-D-mannopyranoside, using silver triflate, gave decomposition products, and that the corresponding condensation of **7** with methyl 2,4,6-tri-*O*-benzyl-α-D-mannopyranoside afforded methyl 2,4,6-tri-*O*-benzyl-3-*O*-(2,3,4,6-tetra-*O*-acetyl-α-D-mannopyranosyl)-α-D-mannopyranoside in only 36% yield. It appears that the presence of a 2-*O*-acetyl group on the nucleophile, or the use of the fully acetylated mannopyranosyl bromide, or both, is not conducive to a proper condensation reaction. In our case, the use of benzoate blocking groups gave good yields, and little decomposition in the condensation.

Compound **4**, here obtained crystalline and fully characterized by n.m.r. spectroscopy, had been reported as a syrup without any analytical or chemical characterization[7].

## EXPERIMENTAL

*p-Nitrophenyl 2-O-benzoyl-4,6-O-benzylidene-α-D-mannopyranoside* (**2**). — To a solution of *p*-nitrophenyl 4,6-*O*-benzylidene-α-D-mannopyranoside (0.5 g, 1.25 mmol) in *N,N*-dimethylformamide (DMF; 5 mL) were added trimethyl orthobenzoate (5 mL) and *p*-toluenesulfonic acid (50 mg). After standing for two days at room temperature, the acid was neutralized with triethylamine (0.5 mL), and the DMF was removed by co-evaporation with toluene. The oily residue was treated with 80% aqueous acetic acid (50 mL) for 15 min at room temperature, the

solution evaporated *in vacuo,* and residual acetic acid removed by codistillation with toluene. Methyl benzoate was removed from the residue by trituration with hexane. Examination by t.l.c. with 4:1 toluene–EtOAc showed a major product at $R_F$ 0.48 (the 3-benzoate has $R_F$ 0.36), some minor, slower-moving components, and only a trace of material having the same mobility as the 3-benzoate. After chromatography of the crude product on a column of silica gel with 4:1 toluene–EtOAc, compound **2** (0.37 g, 60%) was obtained as a chromatographically homogeneous, amorphous solid; $[\alpha]_{578}$ +107° (*c* 1.1, $CHCl_3$); $^1$H-n.m.r. (220 MHz, $CDCl_3$): δ 8.25–7.00 (m, 14 H, aromatic H), 5.72 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1), 5.64 (s, 1 H, benzal H), 5.36 (dd, 1 H, $J_{1,2}$ 1.5, $J_{2,3}$ 4, $J_{3,4}$ 9 Hz, H-2), 4.47 (dd, 1 H, $J_{2,3}$ 4, $J_{3,4}$ 9 Hz, H-3), 4.20 (dd, 1 H, $J_{5,6}$ 3, $J_{6,6'}$ 8.5 Hz, H-6), 4.14 (dd, 1 H, $J_{3,4} = J_{4,5}$ = 9 Hz, H-4), and 3.98–3.75 (m, 2 H, H-5,6'); $^{13}$C-n.m.r. (25.05 MHz, $CDCl_3$), *inter alia*: δ 102.40 (benzal C), 96.55 (C-1), 79.00 (C-4), 72.33 (C-2), 68.48 (C-6), 66.72 (C-3), and 64.92 (C-5).

p-*Nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-2-*O*-(2,3,4,6-tetra-*O*-benzoyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**3**). — The aglycon **1** (0.98 g, 2 mmol) and 2,3,4,6-tetra-*O*-benzoyl-α-D-mannopyranosyl bromide (1.58 g, 2.4 mmol) were dissolved in 1:1 anhydrous toluene–nitromethane (10 mL), and cooled to −20° under argon. A solution of silver triflate (0.57 g, 2.2 mmol) and 2,4,6-collidine (225 μL) in 1:1 anhydrous toluene–nitromethane (5 mL) was added dropwise, with stirring, during 5 min. After 10 min of additional stirring at −20°, examination by t.l.c. with 16:1 toluene–EtOAc showed a new product, at $R_F$ 0.38, and only minor amounts of the starting materials.

The mixture was made neutral by addition of pyridine (1 mL), filtered through Celite, and the filtrate diluted with toluene (100 mL), successively washed with water (50 mL), 0.5M $Na_2S_2O_3$ (2 × 25 mL), and water (50 mL), dried ($Na_2SO_4$), and evaporated to dryness. Chromatography on a column of silica gel with 16:1 toluene–EtOAc yielded chromatographically homogeneous **3** (1.40 g, 66%); amorphous; $[\alpha]^{23}_{578}$ −21° (*c* 0.91, $CHCl_3$); $^1$H-n.m.r. (220 MHz, $CDCl_3$), *inter alia*: δ 8.23–7.00 (m, 34 H, aromatic H), 6.14–6.00 (m, 3 H, H-2,3,4, all Man B), 5.95 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, H-3 Man A), 5.89 (s, 1 H, $J_{1,2} \leq 1$ Hz, H-1 Man A), 5.75 (s, 1 H, benzal H), 5.34 (s, 1 H, $J_{1,2} \leq 1$ Hz, H-1 Man B), and 4.75 (dd, 1 H, $J_{5,6}$ 2.5, $J_{6,6'}$ 11.5 Hz, H-6 Man B); $^{13}$C-n.m.r. (25.05 MHz, $CDCl_3$) (60–102-p.p.m. region only): δ 102.01 (benzal C), 99.86 (C-1 Man A), 97.13 (C-1 Man B), 76.42 and 75.93 (C-4 Man A and C-2 Man A), 69.84 (C-3 Man A, C-2,3,5 Man B), 68.33 (C-6 Man A), 67.26 (C-4 Man B), 65.36 (C-5 Man A), and 63.40 (C-6 Man B).

p-*Nitrophenyl 2-*O*-α-*D*-mannopyranosyl-α-*D*-mannopyranoside* (**4**). — A solution of compound **3** (1.28 g, 1.2 mmol) in 90% aq. acetic acid (100 mL) was heated for 25 min on a steam bath, cooled, evaporated to dryness, and residual acetic acid removed by codistillation with toluene. Examination by t.l.c. with 16:1 toluene–EtOAc showed no starting material remaining. This product was dissolved in methanol (50 mL) and the solution was made alkaline by addition of 0.2M sodium methoxide in methanol (3 mL). After 3 h at room temperature, sodium ions were

removed by treatment with Amberlite IR-120 ($H^+$) resin, and the solution was evaporated. Methyl benzoate was removed by partitioning the residue between water (50 mL) and hexane (2 × 50 mL). The aqueous layer was evaporated to dryness, to give crude compound **4** (0.56 g, quant.), homogeneous by t.l.c. with 6:2:1 EtOAc–2-propanol–$H_2O$, $R_F$ 0.36. The product was purified by chromatography on a column of silica gel (same solvent system), to give chromatographically pure **4** (0.51 g, 91%), which crystallized on standing. An analytical sample had m.p. 203–208°, $[\alpha]_D^{23}$ +99.1° (*c* 1.62, $H_2O$); lit.[7] $[\alpha]_D^{20}$ +85.1° (*c* 1.2, $H_2O$); $^1$H-n.m.r. (220 MHz, $CD_3OD$), *inter alia*: δ 8.18 and 7.27 (both d, 2 H each, $J_{H,H}$ 10 Hz, AB spectrum, *p*-$NO_2C_6H_4O$ group), 6.11 (s, 1 H, $J_{1,2} \leq 1$ Hz, H-1 Man A), and 5.02 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 Man B); $^{13}$C-n.m.r. (25.05 MHz, $CD_3OD$); carbohydrate carbon atoms only: δ 104.44 (C-1 Man B), 98.16 (C-1 Man A), 80.42 (C-2 Man A), 75.84 and 75.30 (C-5 × 2, Man A and B), 72.38 (C-3 Man B), 71.69 and 71.55 (C-2 Man B C-3 Man A), 68.96 (C-4 B), 68.33 (C-4 A), and 63.31 and 62.48 (2 × C-6, Man A and B).

*Anal.* Calc. for $C_{18}H_{25}NO_{13}$: C, 46.65; H, 5.44; N, 3.02. Found: C, 46.47; H, 5.54; N, 2.88.

p-*Nitrophenyl 2-O-benzoyl-4,6-O-benzylidene-3-O-(2,3,4,6-tetra-O-benzoyl-α-*D*-mannopyranosyl)-α-*D*-mannopyranoside* (**5**). — Compound **2** (0.98 g, 2 mmol) and tetra-*O*-benzoyl-D-mannosyl bromide (1.58 g, 2.4 mmol) were dissolved in 1:1 anhydrous toluene–nitromethanol (10 mL), the solution was cooled to −25° under argon, and a solution of silver triflate (0.52 g, 2.2 mmol) and 2,4,6-collidine (225 μL, 1.7 mmol) in 1:1 anhydrous toluene–nitromethane (5 mL) was added dropwise, with stirring, during 5 min. After additional stirring for 15 min at −25°, examination by t.l.c. with 16:1 toluene–EtOAc showed a new, major product at $R_F$ 0.46, and only small amounts of the starting materials. Work-up was effected as described for compound **2**. Chromatography of the product on a column of silica gel with 16:1 toluene–EtOAc afforded chromatographically homogeneous **5** (1.30 g, 61%); amorphous; $[\alpha]_{578}^{23}$ +9.5° (*c* 1.4, $CHCl_3$); $^1$H-n.m.r. (220 MHz, $CDCl_3$), *inter alia*: δ 8.41–7.00 (m, 34 H, aromatic H), 6.11 (dd, 1 H, $J_{3,4} = J_{4,5} = 10$ Hz, H-4 Man B), 5.91–5.82 (m, 3 H, H-2 Man A and B, H-1 Man A), 5.79 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 10 Hz, H-3 Man B), 5.25 (1 H, benzal H), 5.57 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1 Man B), 4.82–4.68 (m, 2 H, H-6 Man A and Man B), 4.64 (ddd, 1 H, $J_{5,6}$ 2.5, $J_{5,6'}$ 4.5, $J_{4,5}$ 10 Hz, H-5 Man B), 4.50 (dd, 1 H, $J_{5,6'}$ 4.5, $J_{6,6'}$ 12 Hz, H-6′ Man B), 4.41 (dd, 1 H, $J_{3,4} = J_{4,5} = 9$ Hz, H-4 Man A), 4.25 (dd, 1 H, $J_{2,3}$ 3, $J_{3,4}$ 9 Hz, H-3 Man A), and 4.02–3.86 (m, 2 H, H-5,6′, both Man B); $^{13}$C-n.m.r. (25.05 MHz, $CDCl_3$), *inter alia*: δ 101.52 (benzal C), 99.13 (C-1 Man B), 96.50 (C-1 Man A), 78.32 (C-4 Man A), 72.72 and 71.55 (C-2,3, both Man A), 69.94, 69.79 and 69.69 (C-2,3,5, all Man B), 68.28 (C-6 Man A), 66.67 (C-4 Man B), 64.92 (C-5 Man A), and 62.82 (C-6 Man B).

p-*Nitrophenyl 3-O-α-*D*-mannopyranosyl-α-*D*-mannopyranoside* (**6**). — A solution of compound **5** (1.10 g, 1.04 mmol) in 90% aqueous acetic acid (100 mL) was heated on a steam bath for 30 min, cooled, and evaporated to dryness, residual

acetic acid being removed by coevaporation with toluene; examination of the residue by t.l.c., with 16:1 toluene–EtOAc showed no starting material remaining. This product was dissolved in methanol (50 mL), and 0.2M sodium methoxide in methanol (3 mL) was added. After 3 h at room temperature, the base was neutralized with Amberlite IR-120 ($H^+$) resin, and the solution evaporated to dryness. A solution of the residue in water (50 mL) was washed with hexane (2 × 25 mL), to remove methyl benzoate, and evaporated to dryness, to give crude **6** (0.52 g). After chromatography on a column of silica gel with 6:2:1 EtOAc–2-propanol–$H_2O$, pure **6** ($R_F$ 0.33) was obtained as a syrup that crystallized on standing (0.42 g, 81%). After recrystallization from 95% ethanol, **6** had m.p. 155–160°, $[\alpha]_{578}^{23}$ +185° (*c* 1.18, $H_2O$); $^1$H-n.m.r. (220 MHz, $CD_3OD$), *inter alia*: δ 8.18 and 7.27 (both d, 2 H each, $J_{H,H}$ 10 Hz, *p*-$NO_2C_6H_4$), 5.64 (d, 1 H, $J_{1,2}$ 2 Hz, H-1 Man A), 5.18 (d, 1 H, $J_{1,2}$ 1.3 Hz, H-1 Man B), and 4.30 (dd, 1 H, $J_{1,2}$ 2, $J_{2,3}$ 3 Hz, H-2 Man A); $^{13}$C-n.m.r. (25.05 MHz, $CD_3OD$), *inter alia*: δ 103.86 (C-1 Man B), 99.96 (C-1 Man A), 79.73 (C-3 Man A), 76.08 (C-5 Man A), 75.01 (C-5 Man B), 72.42 (C-3 Man B*), 71.99 (C-2 Man B*), 70.86 (C-2 Man A), 68.77 (C-4 Man B), 67.06 (C-4 Man A), and 62.92 and 62.38 (2 × C-6, Man A and B).

*Anal.* Calc. for $C_{18}H_{25}NO_{13} \cdot 2\ H_2O$: C, 43.29; H, 5.85; N, 2.81. Found: C, 43.14; H, 5.31; N, 2.75.

Water of crystallization could be removed only by heating *in vacuo* for 3 h at 160°.

*Anal.* Calc. for $C_{18}H_{25}NO_{13}$: C, 46.65; H, 5.44; N, 3.02. Found: C, 46.55; H, 5.36; N, 2.84.

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*Interchangeable assignments.