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Note

p-(Trifluoroacetamido)phenyl 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-β-D-glucopyranoside*

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We have previously reported on the synthesis of three disaccharide glycosides having immunodeterminants that occur on glycoproteins[1]. Efforts in this laboratory to prepare all such possible combinations continue. These oligosaccharidic determinants are being prepared in a form suitable for linkage to a protein carrier; here, we report the synthesis of an additional determinant, namely, β-D-Man*p*-(1→4)-β-D-Glc*p*NAc-(1→ $OC_6H_4NHCOCF_3$-*p* (**1**). A route involving an oxidation–reduction sequence at O-2 of D-glucose in the readily accessible, corresponding *p*-(trifluoroacetamido)phenyl glycoside of β-D-glucosyl-(1→4)-D-Glc*p*NAc was chosen. This approach had previously been successfully applied to the preparation of β-D-mannosides and α-D-glucosides[2–4]. Other routes employing D-mannopyranosyl halides having a nonparticipating group on O-2 have also been used, but, when the reactivity of the "aglycon" is low, they tend to give anomeric mixtures containing a large proportion of the α-anomer[5].

The preparation of **1** was achieved by glycosylation of *p*-nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside[1] (**2**) with 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-α-D-glucopyranosyl bromide[2] (**7**), followed by transformation of the D-glucosyl moiety of the resulting *p*-nitrophenyl 2-acetamido-4-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**8**) into the desired D-mannosyl epimer.

Preparation of **7** was achieved as follows. Tetra-*O*-benzoyl-α-D-glucopyranosyl bromide (**3**) was converted[6] into tri-*O*-benzoyl-1,2-*O*-(1-methoxybenzylidene)-α-D-glucopyranose (**4**) in quantitative yield, compound **4** was *O*-debenzoylated, and the resulting product **5** was benzylated[7], to yield 3,4,6-tri-*O*-benzyl-

*The Preparation of Immunogens Bearing Immunodeterminants Known to Occur on Glycoproteins, Part II. For Part I, see ref. 1.
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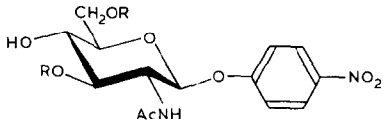
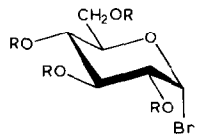
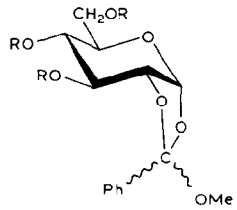


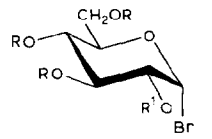
2 R = En
Bn = $PhCH_2$



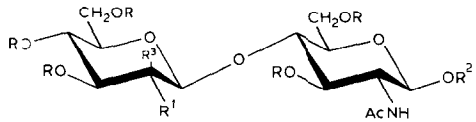
3



4 R = Bz
5 R = H
6 R = Bn



7 R = Bn, R' = Bz



8 R = Bn, R^1 = OBz, R^2 = $C_6H_4NO_2$-*p*, R^3 = H
9 R = Bn, R^1 = OH, R^2 = $C_6H_4NO_2$-*p*, R^3 = H
10 R = Bn, R^1 = H, R^2 = $C_6H_4NO_2$-*p*, R^3 = OH
11 R = Bn, R^1 = H, R^2 = $C_6H_4NHCOCF_3$-*p*, R^3 = OH
1 R = R^1 = H, R^2 = $C_6H_4NHCOCF_3$-*p*, R^3 = OH

1,2-*O*-(1-methoxybenzylidene)-α-D-glucopyranose (**6**). Compound **6** was then converted into **7** by treatment with bromotrimethylsilane[8].

Condensation of bromide **7** with **2** yielded the fully protected disaccharide **8** in 57% yield, and *O*-debenzoylation of **8** afforded **9** in almost quantitative yield. The 2-hydroxyl group on the D-glucosyl group of **9** was oxidized to the ketone by the use of acetic anhydride in dimethyl sulfoxide[9], and the ketone, not isolated, was reduced directly to the D-mannosyl epimer **10** by the action of lithium tris(1-methylpropyl)borohydride[4,10]. Reduction of the glycosidic *p*-nitrophenyl to a *p*-aminophenyl group, followed by trifluoroacetylation, then yielded **11**, which was deprotected to afford **1**.

EXPERIMENTAL

General methods. — These were as previously described[1].

3,4,6-Tri-O-benzyl-1,2-O-(1-methoxybenzylidene)-α-D-glucopyranose (**6**). —

A solution of **3** (28.25 g, 42.8 mmol) in nitromethane (60 mL) containing methanol (5 mL) and 2,4,6-collidine (6 mL) was kept for 3 days at 37°. Examination by t.l.c. (8:1 toluene–EtOAc) then showed essentially a single spot having R_F 0.53 (the starting material had R_F 0.61). Aqueous 2M silver nitrate solution (20 mL) was added, followed by water (35 mL) and acetone (60 mL), the mixture was filtered through Celite, and the filtrate was diluted with a mixture of chloroform (100 mL) and hexane (250 mL), and washed with water (2 × 100 mL), dried (Na_2SO_4), and concentrated; crude 3,4,6-tri-*O*-benzoyl-1,2-*O*-(1-methoxybenzylidene)-α-D-glucopyranose (**4**) (27 g, 100%, crude yield) was obtained as a thick, glassy syrup that was not characterized or purified, but used directly in the next step.

Compound **4** (27 g) was suspended in methanol (200 mL), and a 0.2M solution of sodium methoxide in methanol (10 mL) was added. The mixture was stirred overnight at room temperature, the substrate gradually dissolving as the reaction proceeded. The base was neutralized by addition of solid CO_2, the suspension filtered, and the filtrate concentrated. The residue was mixed with ethyl acetate (100 mL), the suspension filtered to remove sodium carbonate, and the filtrate concentrated. The oily residue was dissolved in methanol (300 mL), and the solution washed with hexane (4 × 100 mL) to remove methyl benzoate, and evaporated to dryness; yield of **5**, 12.9 g. This was dissolved in *N,N*-dimethylformamide (DMF; 120 mL), and sodium hydride (3.6 g, oil-free) was added in small portions to the stirred solution. After gas evolution had ceased, benzyl bromide (16 mL) was added dropwise during ~45 min to the vigorously stirred solution, and stirring was continued overnight, while the temperature was allowed to rise slowly to the ambient temperature. Examination by t.l.c. (8:1 toluene–ethyl acetate) showed only one major product, R_F 0.57. Work-up was effected by dropwise addition of methanol (10 mL) and, after gas evolution had ceased, the mixture was partitioned between dichloromethane and water. The organic layer was dried (Na_2SO_4) and concentrated to an oily residue, first with a water aspirator and finally with an oil pump. Dibenzyl ether was removed by passing this product through a column of Sephadex LH-20 with methanol containing 0.2% of triethylamine; the yield of crude **6** was 16.2 g. Final purification was effected by chromatography on silica gel with 8:1 toluene–ethyl acetate + 0.5% (v/v) of pyridine; yield, 13.9 g (57%) of chromatographically homogeneous **6**; $[\alpha]_{578}$ +67.5° (*c* 2.33, $CHCl_3$); ^{1}H-n.m.r. (220 MHz): δ 7.70–7.00 (m, 20 H, aromatic H), 5.93 (d, 1 H, $J_{1,2}$ 5.5 Hz, H-1), and 3.14 (s, 3 H, OCH_3).

Anal. Calc. for $C_{35}H_{36}O_2$: C, 73.9; H, 6.38. Found: C, 74.1; H, 6.53.

The isomeric methyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranoside[2] is crystalline {m.p. 93–96° and $[\alpha]^{22}_{578}$ +41° (*c* 1.0, $CHCl_3$)}, and its ^{1}H-n.m.r. spectrum shows a resonance at δ 3.45 for its methoxyl group.

2-O-Benzoyl-3,4,6-tri-O-benzyl-α-D-glucopyranosyl bromide (**7**). — A solution of compound **6** (3.4 g) in toluene (10 mL, dried by azeotropic distillation) was treated under dry argon with bromotrimethylsilane (3.5 mL) for 1.5 h at room temperature, and then concentrated to a syrup that showed in t.l.c. (16:1 toluene–

EtOAc), *inter alia*, a major spot at R_F 0.56; no starting material could be detected, but owing to the high reactivity of the bromide, a hydrolysis product (very low R_F) was also observed. The ^{1}H-n.m.r. spectrum (220 MHz) showed, *inter alia*, a one-proton doublet at δ 6.73 ($J_{1,2}$ 4 Hz, H-1) and a one-proton double-doublet at δ 5.07 ($J_{1,2}$ 4, $J_{2,3}$ 9.5 Hz, H-2) which indicated that **7** had the designated structure. The crude **7** was not further characterized, but was used directly in the next step. This bromide had previously been prepared by an alternative route[2].

p-*Nitrophenyl 2-acetamido-4-*O*-(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-3,6-di-*O*-benzyl-2-deoxy-β-*D*-glucopyranoside* (**8**). — Bromide **7** (from 3.4 g, 6 mmol, of compound **6**) and *p*-nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (**2**; 1.56 g, 3 mmol) were dissolved in 1:1 anhydrous toluene–nitromethane (45 mL) at −25°. A solution of silver triflate (1.65 g, 6 mL) and 2,4,6-collidine (400 μL) in the same solvent (10 mL) was added dropwise, with stirring[11], during 15 min under argon (to ensure anhydrous conditions), and stirred overnight, the mixture slowly attaining room temperature; t.l.c. then showed the presence of one major product (R_F 0.50 in 2:1 toluene–ethyl acetate). The mixture was made neutral with pyridine, filtered through Celite, and the filtrate diluted with toluene (50 mL), successively washed with 0.5M sodium thiosulfate (2 × 25 mL) and water (2 × 25 mL), dried (Na_2SO_4), and concentrated to a residue that was separated by chromatography on a column of silica gel (2:1 toluene–EtOAc), to give 1.79 g (57%) of **8**, which crystallized on standing. Recrystallization from 95% ethanol afforded pure **8**, m.p. 149–151°, $[\alpha]_{578}^{23}$ −19.8° (*c* 2.18, $CHCl_3$); ^{1}H-n.m.r. (220 MHz; $CDCl_3$ + 10% of CD_3OD): δ 7.91 and 6.65 (both d, 2 H each, $J_{H,H}$ 10 Hz, AB spectrum *p*-nitrophenyl, H), 7.61–6.93 (m, 30 H, other aromatic H), 5.27 (dd, 1 H, $J_{2,3}$ 9, $J_{1,2}$ 8 Hz, Glc H-2), 5.05 (d, 1 H, $J_{1,2}$ 5 Hz, GlcNAc H-1), and 2.05 (s, 3 H, NCOCH_3); the ^{13}C-n.m.r. spectrum (25.05 MHz) showed, *inter alia*, δ 99.13 (C-1, Glc), 97.33 (C-1, GlcNAc), 82.07 (C-4, GlcNAc), 77.69 (C-5, Glc), 76.08 (C-5, GlcNAc), 69.35 and 68.28 (C-6, Glc and C-6, GlcNAc), 48.54 (C-2, GlcNAc), and 23.05 (NCOCH_3).

Anal. Calc. for $C_{62}H_{62}N_2O_{14}$: C, 70.30; H, 5.90; N, 2.65. Found: C, 70.04; H, 5.90; N, 2.79.

p-*Nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-glucopyranosyl)-β-*D*-glucopyranoside* (**9**). — A solution of **8** (1.51 g, 1.4 mmol) in 1:1 methanol–dichloromethane (50 mL) containing barium oxide (2 g) was boiled under relux for 2 h; t.l.c. (2:1 toluene-ethyl acetate) then showed only one major component, having a lower mobility than the starting material. The mixture was cooled, filtered through Celite, and the filtrate concentrated to a residue, chromatography of which on a column of silica gel (1:2 toluene–ethyl acetate) afforded 1.30 g (92%) of chromatographically homogeneous **9**, which crystallized on standing. Recrystallized from 95% ethanol, **9** had m.p. 176–177°, $[\alpha]_{578}^{24}$ −9.8° (*c* 0.8, $CHCl_3$); ^{1}H-n.m.r. (220 MHz; $CDCl_3$ + 20% of CD_3OD): δ 8.11 and 7.05 (both d, 2 H each, $J_{H,H}$ 9.5 Hz, *p*-$NO_2C_6H_4O$), 7.43–7.11 (m, 25 H, other aromatic H), 5.45 (d, 1 H, $J_{1,2}$ 6 Hz, GlcNAc H-1), and 1.84 (s, 3 H, NCOCH_3); ^{13}C-n.m.r.

(25.05 MHz; $CDCl_3$ + 20% of CD_3OD) showed, *inter alia*, δ 102.93 (C-1, Glc), 97.43 (C-1, GlcNAc), 84.80 (C-4, GlcNAc), 77.74 and 77.19 (C-3 and C-5, GlcNAc), 69.11 and 68.87 (C-6, Glc and C-6, GlcNAc), 53.32 (C-2, GlcNAc), and 23.00 ($NCOCH_3$).

p-*Nitrophenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-mannopyranosyl)-β-*D*-glucopyranoside* (**10**). — A solution of the alcohol **9** (0.27 g) in a mixture of dimethyl sulfoxide (40 mL) and acetic anhydride (2 mL) was kept, under argon, for two days at room temperature. The mixture was then lyophilized, and the oxidation product (0.27 g) was dissolved in anhydrous oxolane (THF, 10 mL), and the solution added (under argon) to a stirred, M solution of lithium tris(1-methylpropyl)borohydride in THF (10 mL; L-Selectride, Aldrich) at −75°. After 3 h at −75°, methanol (1 mL) was added to decompose the excess of hydride, and the alkylboranes were oxidized by addition of 30% aqueous hydrogen peroxide. The salts were removed by decantation, and the supernatant liquor was concentrated to dryness. T.l.c. of the residue showed only one major product (R_F 0.27, 1:1 toluene–EtOAc), moving more slowly than the starting *gluco* compound **9** (R_F 0.51, same solvent). Chromatography on a column of silica gel yielded 0.20 g (75%) of compound **10**. Crystallization from 2-propanol afforded material having m.p. 201–203°, $[\alpha]_{578}^{24}$ −57.8° (*c* 0.85, $CHCl_3$); ^{1}H-n.m.r. (220 MHz, $CDCl_3$ + 10% of CD_3OD) showed, *inter alia*, δ 8.16 and 7.05 (both d, 2 H each, $J_{H,H}$ 10 Hz, AB spectrum, *p*-$NO_2C_6H_4O$ group), 7.43–7.11 (m, 25 H, other aromatic H), 5.48 (d, 1 H, $J_{1,2}$ 4.5 Hz, H-1 GlcNAc), 4.95 (s, 1 H, $J_{1,2} \leq 1$ Hz, H-1 Man), and 1.93 (s, 3 H, $NCOCH_3$); ^{13}C-n.m.r. (25.05 MHz; $CDCl_3$ + 10% of CD_3OD) showed, *inter alia*, δ 100.25 (C-1, Man), 97.33 (C-1, GlcNAc), 81.59 (C-4, GlcNAc), 72.27 and 75.93 (C-3 and C-5, both GlcNAc), 69.06 and 68.33 (C-6, Man and C-6, GlcNAc), 50.39 (C-2, GlcNAc), and 22.91 (*N*-acetyl CH_3).

Anal. Calc. for $C_{55}H_{58}N_2O_{13}$: C, 69.16; H, 6.12; N, 2.93. Found C, 69.42; H, 6.21; N, 2.85.

p-*(Trifluoroacetamido)phenyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-benzyl-β-*D*-mannopyranosyl)-β-*D*-glucopyranoside* (**11**). — A solution of **10** (0.20 g, 0.21 mmol) in ethyl acetate (40 mL), containing Adams's catalyst (100 mg), was stirred under hydrogen at room temperature and atmospheric pressure. When hydrogen uptake had ceased, pyridine (3 mL) and trifluoroacetic anhydride (TFAA, 1.5 mL) were added, the tightly stoppered bottle was kept for 20 min at 60°, and cooled, methanol (2 mL) was added, to decompose the excess of TFAA and to hydrolyze any *O*-trifluoroacetyl groups formed, the catalyst was removed by filtration, and the filtrate was concentrated to dryness. The residue was dissolved in 3:2 toluene–ethyl acetate (100 mL), and the solution washed with water (3 × 50 mL), dried (Na_2SO_4), and concentrated to dryness. Chromatography on a column of silica gel (19:1 chloroform–methanol) yielded pure, crystalline compound **11**; 0.21 g (98%). After recrystallization from 95% ethanol, **11** had m.p. 240–242°, $[\alpha]_{578}^{23}$ −12.2° (*c* 1.38, $CHCl_3$ + 25% of MeOH); ^{1}H-n.m.r. (220 MHz; $CDCl_3$ + 25% of CD_3OD) showed, *inter alia*, δ 7.55 and 7.00 (both d, 2 H each, $J_{H,H}$ 7.5 Hz, AB

spectrum, *p*-$CF_3CONHC_6H_4O$ group), 7.41–7.16 (m, 25 H, other aromatic H), 5.20 (d, 1 H, $J_{1,2}$ 6.5 Hz, GlcNAc H-1), and 1.89 (s, 3 H, $NCOCH_3$).

p-*(Trifluoroacetamido)phenyl 2-acetamido-2-deoxy-4*-O-β-D-*mannopyranosyl*-β-D-*glucopyranoside* (**1**). — Compound **11** (0.15 g) was dissolved in glacial acetic acid (70 mL) and hydrogenolyzed in the presence of 10% palladium-on-charcoal (0.35 g). After 16 h, when hydrogen uptake had ceased, examination by t.l.c. (6:2:1 ethyl acetate–2-propanol–water) showed one major spot, at R_F ~0.6 The catalyst was removed by filtration through Celite, and the filtrate concentrated to a residue which was purified by chromatography on a column of silica gel (same solvent), to give compound **1**, 70 mg (70%), which crystallized on standing; m.p. 240–243° (dec.), $[\alpha]_{578}^{23}$ −23° (*c* 1.01, 40% aq. ethanol); ^{1}H-n.m.r. (220 MHz; Me_2SO-d_6 + 2% of D_2O): δ 7.56 and 7.00 (both d, 2 H each, $J_{H,H}$ 9 Hz, AB spectrum, aromatic H), 5.03 (d, 1 H, $J_{1,2}$ 8 Hz, GlcNAc H-1), 4.55 (s, 1 H, $J_{1,2} \leq 1$ Hz, Man H-1), and 1.84 (s, 3 H, *N*-acetyl CH_3); ^{13}C-n.m.r. (25.05 MHz; Me_2SO-d_6 +2% of D_2O); β-D-mannosyl group: δ 100.64 (C-1), 77.35 (C-5, alt. C-5 GlcNAc), 73.30 (C-3, alt. C-3 GlcNAc), 70.33 (C-2), 66.77 (C-4), and 61.07 (C-6, alt. C-6 GlcNAc); 2-acetamido-2-deoxy-β-D-glucoside residue: δ 98.99 (C-1, alt. C-1 Man), 79.59 (C-4), 75.01 (C-5, alt. C-5 Man), 71.79 (C-3, alt. C-3 Man), 59.75 (C-6, alt. C-6 Man), 54.73 (C-2), and 22.86 (*N*-acetyl CH_3).

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