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Note

Synthesis of *p*-trifluoroacetamidophenyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosiduronic acid, an artificial antigen corresponding to a disaccharide repeating unit of the capsular polysaccharide of *Haemophilus influenzae* type e

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The *Haemophilus influenzae* type e capsular antigen contains[1] the repeating unit →3)-β-D-Glc*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→. This disaccharide unit, joined to a spacer suitable for attachment to free amino groups in a protein[2], was required for immunological studies and its synthesis is now reported. The present synthesis parallels that[3] of the (1→3)-linked analogue which is related to the disaccharide repeating unit[4] of *Haemophilus influenzae* type d.

p-Nitrophenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranoside[3] (**1**) was benzylated to give **2** (68%), which was treated with aqueous acetic acid to remove the 4,6-*O*-benzylidene group, and the product was 6-*O*-*tert*-butyldimethylsilylated to give **3** (96%). Glycosylation of HO-4 in **3** with ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[5] (**4**) in dichloromethane containing dimethyl(methylthio)sulfonium triflate as promoter and 2,6-di-*tert*-butyl-4-methylpyridine as acidity modifier gave the disaccharide **5** (96%), which was processed following the protocol described[3]. Thus, dephthalimidation of **5**, acetylation, and desilylation gave **7** (69%). Oxidation of **7** with pyridinium dichromate–acetic anhydride in the presence of *tert*-butyl alcohol[6] gave the uronide **8** (32%). The azido group in **8** was reduced to an amino group which was acetylated (→**9**), the aromatic nitro group was reduced with aluminium amalgam, and the new aromatic amino group was trifluoroacetylated (→**10**). Debenzylation by catalytic hydrogenolysis (→**11**), hydrolysis of the *tert*-butyl group with formic acid, and finally *O*-deacetylation then gave the target compound *p*-trifluoroacetamidophenyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosiduronic acid (**12**, 36% from **8**).

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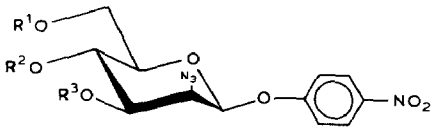
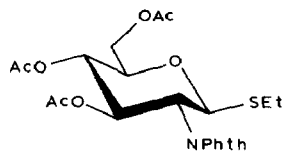


1 R^1, R^2 = PhCH, R^3 = H

2 R^1, R^2 = PhCH, R^3 = Bn

3 R^1 = ^{t}BuMe$_2$Si, R^2 = H, R^3 = Bn



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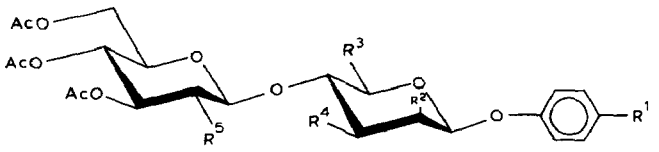


5 R^1 = NO_2, R^2 = N_3, R^3 = $CH_2OSi^tBuMe_2$, R^4 = OBn, R^5 = NPhth

6 R^1 = NO_2, R^2 = N_3, R^3 = $CH_2OSi^tBuMe_2$, R^4 = OBn, R^5 = NHAc

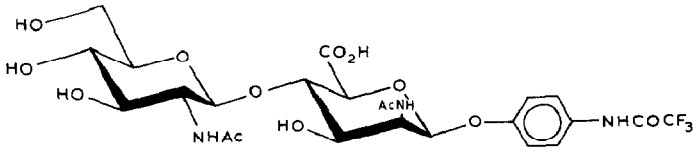
7 R^1 = NO_2, R^2 = N_3, R^3 = CH_2OH, R^4 = OBn, R^5 = NHAc

8 R^1 = NO_2, R^2 = N_3, R^3 = COO^tBu, R^4 = OBn, R^5 = NHAc

9 R^1 = NO_2, R^2 = NHAc, R^3 = COO^tBu, R^4 = OBn, R^5 = NHAc

10 R^1 = $NHCOCF_3$, R^2 = NHAc, R^3 = COO^tBu, R^4 = OBn, R^5 = NHAc

11 R^1 = $NHCOCF_3$, R^2 = NHAc, R^3 = COO^tBu, R^4 = OH, R^5 = NHAc



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EXPERIMENTAL

General methods. — These were as described[3]. N.m.r. spectra were recorded at 25° on solutions in D_2O. The f.a.b.-mass spectrum was obtained with a JEOL SX-102 instrument, with Xe atoms at 6 keV and a matrix of *p*-nitrobenzyl alcohol.

p-*Nitrophenyl 2-azido-3-O-benzyl-4,6-O-benzylidene-2-deoxy-β-*D-*mannopyranoside* (**2**).— A solution of *p*-nitrophenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranoside[3] (**1**, 430 mg) and benzyl bromide (136 μL) in *N,N*-dimethylformamide (2 mL) was added dropwise to sodium hydride (27 mg) at 0°.The mixture was allowed to attain room temperature and, after 1 h, cooled to 0°, and methanol was added dropwise to decompose the excess of sodium hydride.The solution was added to a column of silica gel and eluted with 3:1 toluene–ethyl acetate to give **2** (357 mg, 68%), m.p. 166° (from ethanol), $[\alpha]_D$ −66° (*c* 0.96, $CHCl_3$). ^{13}C-N.m.r. data ($CDCl_3$): δ 63.2, 67.6, 68.2, 73.2,

76.1, 78.2 (C-2,3,4,5,6 and OCH_2Ph), 97.2, 101.7 (C-1 and PhCH), 116.3–160.7 (aromatic C).

Anal. Calc. for $C_{26}H_{24}N_4O_7$: C, 61.9; H, 4.8; N, 11.1; O, 22.2. Found C, 61.9; H, 4.5; N,11.4.

p-*Nitrophenyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-azido-3-O-benzyl-6-O-tert-butyldimethylsilyl-2-deoxy-β-D-mannopyranoside* (**6**). — A solution of **2** (415 mg) in aq. 70% acetic acid (8 mL) was kept for 1.5 h at 70°, when t.l.c. (9:1 $CHCl_3$–MeOH) indicated that reaction was complete. The mixture was concentrated and toluene was evaporated twice from the residue. *tert*-Butyldimethylsilyl chloride (372 mg) was added at room temperature to a stirred solution of the crude 4,6-diol in pyridine(12 mL). The mixture was stirred overnight and then concentrated, and toluene was evaporated from the residue. Column chromatography (3:1 toluene–ethyl acetate) then gave the 6-*O*-*tert*-butyldimethylsilyl derivative **3**. ^{13}C-N.m.r. data ($CDCl_3$): δ −5.5, −5.4 (CH_3Si), 18.2 (CSi), 25.8 [(CH_3)$_3$CSi], 61.0, 63.7, 68.1, 72.5, 76.5, 80.2 (C-2,3,4,5,6 and OCH_2Ph), 97.1 (C-1), 116.4–161.1 (aromatic C).

Dimethyl(methylthio)sulfonium triflate (1.6 g) was added at room temperature to a stirred mixture of **3** (551 mg), ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[5] (**4**, 747 mg), 2,6-di-*tert*-butyl-4-methylpyridine (1.3 g), and 4A molecular sieves in dichloromethane (40 mL). After 3 h, the reaction was complete (t.l.c.; 3:1 toluene–ethyl acetate), triethylamine (1 mL) was added, and stirring was continued for 30 min. The mixture was subjected to column chromatography (3:1 toluene–ethyl acetate) and gave *p*-nitrophenyl 2-azido-3-*O*-benzyl-6-*O*-*tert*-butyldimethylsilyl-2-deoxy-4-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-β-D-mannopyranoside (**5**; 929 mg, 96%). ^{13}C-N.m.r. data (C_5D_5N): δ −5.2 (CH_3Si), 18.4 (CSi), 20.1, 20.4, 20.5 (CH_3CO), 26.0 [(CH_3)$_3$CSi], 56.2, 61.8, 62.3, 62.6, 70.1, 71.5, 72.6, 72.8, 73.6, 76.9, 78.7 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, and OCH_2Ph), 97.3, 98.2 (C-1,1′), 117.0–161.9 (aromatic C), 168.2, 170.2, 170.3 (carbonyl C).

Hydrazine hydrate (853 μL) was added to a solution of **5** (819 mg) in ethanol (80 mL). The mixture was boiled under reflux overnight, then concentrated, and toluene was evaporated twice from the residue. Pyridine (4 mL) and acetic anhydride (4 mL) were added. When t.l.c. (15:1 $CHCl_3$–MeOH) showed acetylation to be complete, the mixture was concentrated and toluene was evaporated twice from the residue. Column chromatography (15:1 $CHCl_3$–MeOH) then gave **6** (530 mg, 70%), $[\alpha]_D$ −56° (*c* 1.1, $CHCl_3$) ^{13}C-N.m.r. data ($CDCl_3$): δ −5.3 (CH_3Si), 18.1 (CSi), 20.7, 21.5 (CH_3CO), 23.3 (CH_3CON), 25.8 [(CH_3)$_3$CSi], 54.7, 60.9, 61.9 (2 C), 68.1, 71.8, 72.7, 72.9, 74.1, 76.6, 78.7 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, and OCH_2Ph), 96.8, 101.2 (C-1,1′), 116.4–161.3 (aromatic C), 169.3, 170.2, 170.7, 171.1 (carbonyl C).

Anal. Calc. for $C_{39}H_{53}N_5O_{15}Si$: C, 54.5; H, 6.2; N, 8.1; O, 27.9; Si, 3.3. Found: C, 54.6; H, 6.2; N, 8.1.

p-*Nitrophenyl 4-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-azido-3-O-benzyl-2-deoxy-β-D-mannopyranoside* (**7**). — Compound **6** (277 mg) was added to M triethylammonium fluoride in tetrahydrofuran (10 mL), and the solution was stirred overnight and then concentrated. A solution of the residue in

dichloromethane was washed with water and concentrated. Column chromatography (15:1 $CHCl_3$–MeOH) of the residue gave **7** (239 mg, 99%), m.p. 233–235° (from EtOH), $[\alpha]_D$ −46° (*c* 0.54, $CHCl_3$). N.m.r. data (C_5D_5N): ^{13}C, δ 20.5 (CH_3CO), 23.2 (CH_3CON), 55.8, 61.2, 62.3, 62.7, 69.9, 72.3, 72.7, 74.1, 75.1, 77.8, 80.2 (C-2,3,4,5,6, C-2′,3′,4′,5′,6′, and OCH_2Ph), 97.3, 102.3 (C-1,1′), 116.9–161.8 (aromatic C), 169.7, 170.4, 170.6 (carbonyl C). ^1H, δ 5.64 (d, $J_{1,2}$ 1.3 Hz, H-1).

Anal. Calc. for $C_{33}H_{39}N_5O_{15}$: C, 53.2; H, 5.3; N, 9.4; O, 32.2. Found: C, 53.2; H, 5.2; N, 9.3.

p-*Trifluoroacetamidophenyl 2-acetamido-4-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-β-*D*-mannopyranosiduronic acid* (**12**). — Acetic anhydride (150 μL), was added to a stirred suspension of **7** (79 mg), pyridinium dichromate (125 mg), and *tert*-butyl alcohol (235 mg) in dichloromethane (16 mL). The mixture was stirred at room temperature for 5 h in the dark, more pyridinium dichromate (60 mg) was added, and the mixture was left overnight. Methanol (0.5 mL) was added and stirring was continued for 30 min. The mixture was washed through silica gel with ethyl acetate and concentrated. Toluene was evaporated twice from the residue. Column chromatography (20:10:1 toluene-ethyl acetate–methanol) then gave crude *tert*-butyl [*p*-nitrophenyl 4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-azido-3-*O*-benzyl-2-deoxy-β-D-mannopyranosid]uronate (**8**; 27 mg, 32%). ^{13}C-N.m.r. data (C_5D_5N): δ 20.4, 20.5 (CH_3CO), 23.2 (CH_3CON), 27.7 [$(CH_3)_3CO$], 55.5, 57.6, 62.8, 70.0, 72.7, 72.9, 73.6, 74.3, 75.0, 77.0, (C-2,3,4,5, C-2′,3′,4′,5′,6′, and OCH_2Ph), 82.6 [$(CH_3)_3CO$], 97.4, 101.0 (C-1,1′), 117.4–162.9 (aromatic C), 167.7, 169.7, 170.3, 170.5 (carbonyl C).

Triphenylphosphine (23 mg) was added at room temperature to a stirred solution of **8** (49 mg) in dichloromethane (4 mL) and stirring was continued at room temperature overnight. Water (4 mL) was added and stirring was continued under reflux until all of the phosphine imine was hydrolysed (12 h, t.l.c.; 15:1 $CHCl_3$–MeOH). The organic layer was concentrated and toluene was evaporated twice from the residue. Acetyl chloride (4.7 μL) was added at 0° to a stirred solution of the crude amine in 1:1 dichloromethane–pyridine (4 mL). After 1 h, the mixture was concentrated and toluene was evaporated twice from the residue. Column chromatography (15:1 $CHCl_3$–MeOH) then yielded *tert*-butyl [*p*-nitrophenyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-benzyl-2-deoxy-β-D-mannopyranosid]uronate (**9**; 39 mg, 80%). ^{13}C-N.m.r. data (C_5D_5N): δ 20.4, 20.5 (CH_3CO), 22.8, 23.2 (CH_3CON), 27.7 [$(CH_3)_3CO$], 46.3, 55.8, 62.8, 70.1, 72.2, 72.4, 72.9, 73.2, 73.7, 75.3 (C-2,3,4,5, C-2′,3′,4′,5′,6′, and OCH_2Ph), 82.1 [$(CH_3)_3CO$], 96.5, 100.1 (C-1,1′), 117.3–164.0 (aromatic C), 168.1, 169.7, 170.1, 170.4, 170.8 (carbonyl C).

A solution of **9** (54 mg) in aq. 80% tetrahydrofuran (2 mL) was treated with aluminium amalgam for 1 h at room temperature. The mixture was filtered through Celite and the solvent was evaporated. To a solution of the residue in 1:1 dichloromethane–pyridine (2 mL) at 0° was added trifluoroacetic anhydride (16 μL). After 1 h, the mixture was concentrated. Column chromatography (15:1 $CHCl_3$–MeOH) of the residue gave *tert*-butyl [*p*-trifluoroacetamidophenyl 2-acetamido-4-*O*-(2-acetamido-

3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-3-*O*-benzyl-2-deoxy-β-D-mannopyranosid]uronate (**10**; 39 mg, 67%). N.m.r. data (C_5D_5N): ^{13}C, δ 20.4, 20.6 (CH_3CO), 22.9, 23.2 (CH_3CON), 27.8 [$(CH_3)_3CO$], 46.9, 55.9, 62.8, 70.1, 72.2, 72.7, 73.3, 73.8, 75.9, 75.3 (C-2,3,4,5, C-2',3',4',5',6', and OCH_2Ph), 82.0 [$(CH_3)_3CO$], 97.3, 100.1 (C-1,1'), 117.7–157.0 (aromatic C), 168.4, 169.7, 170.0, 170.4, 170.8 (carbonyl C).^1H, δ 5.72 (d, $J_{1,2}$ 3.7 Hz, H-1).

10% Pd/C (10 mg) was added to a solution of **10** (43mg) in 4:1 ethyl acetate–water (5 mL), and the mixture was hydrogenolysed in a Parr apparatus (400 kPa) overnight, then filtered, and concentrated.Column chromatography (15:1 $CHCl_3$–MeOH) of the residue gave *tert*-butyl [*p*-trifluoroacetamidophenyl 2-acetamido-4-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosid]uronate (**11**; 35 mg, 91%). ^{13}C-N.m.r. data (C_5D_5N): δ 20.4, 20.5 (CH_3CO), 23.0, 23.2 (CH_3CON), 27.9 [$(CH_3)_3CO$], 49.7, 55.8, 62.8, 69.5, 70.2, 72.5, 73.5, 74.2, 77.8 (C-2,3,4,5 and C-2',3',4',5',6'), 82.0 [$(CH_3)_3CO$], 98.6, 100.3 (C-1,1'), 118.0–156.5 (aromatic C), 168.5, 169.7, 170.4, 170.6 (carbonyl C).

A solution of **11** (35 mg) in formic acid (2 mL) was stirred for 6 h at room temperature, then concentrated, and toluene was evaporated twice from the residue. Sodium methoxide in methanol was added at room temperature to a stirred solution of the crude acid in methanol (3 mL) and stirring was continued overnight (t.l.c.; 5:3:3:2 ethyl acetate–acetic acid–methanol–water). The solution was neutralised with Dowex 50 (H^+) resin, filtered, and concentrated. Filtration of the residue through a Sep pak C18 cartridge followed by gel filtration on Bio-Gel P-2 (200–400 mesh) gave **12** (20 mg, 74%), $[\alpha]_D$ −22° (*c* 0.28, H_2O).F.a.b.-mass spectrum: *m/z* 664.2 $(M + K)^+$. N.m.r. data D_2O, pD 4): ^{13}C, δ 22.8, 23.2 (CH_3CON), 53.1, 56.2, 61.3, 70.5, 70.6, 74.5, 76.8, 78.1, 78.3 (C-2,3,4,5 and C-2',3',4',5',6'), 97.9, 101.6 (C-1,1'), 117.6–155.2 (aromatic C), 175.1, 175.7, 176.3 (carbonyl C); ^1H, δ 2.07, 2.13 (CH_3CON), 3.45 (H-4',5'), 3.53 (H-3'), 3.70 (H-2'), 3.75 (H-6'a), 3.86 (H-5), 3.94 (H-4,6'b), 3.98 (H-3), 4.53 (d, $J_{1',2'}$ 8.4 Hz, H-1'), 4.72 (H-2), 5.46 (d, $J_{1,2}$ 1.5 Hz, H-1), 7.11, 7.47 (aromatic H).

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