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Synthesis of an artificial antigen that corresponds to a disaccharide repeating unit of the capsular polysaccharide of *Haemophilus influenzae* type d. A facile synthesis of methyl 2-acetamido-2-deoxy-β-D-mannopyranoside*

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ABSTRACT

The synthesis is described of *p*-nitrophenyl 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosiduronic acid, corresponding to the disaccharide repeating unit of the capsular polysaccharide of *Haemophilus influenzae* type d, which, after conversion of the *p*-nitro- into a *p*-amino-phenyl residue, may be attached to a protein to make an artificial antigen for immunological studies. The synthesis incorporates a facile route to the 2-acetamido-2-deoxy-β-D-mannopyranosyl unit.

INTRODUCTION

The *Haemophilus influenzae* type d and e capsular antigens contain a repeating unit of a 2-acetamido-2-deoxy-β-D-glucopyranosyl residue linked to the 3-position[1] (type d) or 4-position[2] (type e) of a 2-acetamido-2-deoxy-β-D-mannopyranosyluronic acid residue:

type d →4)-β-D-Glc*p*NAc-(1→3)-β-D-Man*p*NAcA-(1→

type e →3)-β-D-Glc*p*NAc-(1→4)-β-D-Man*p*NAcA-(1→

These disaccharide units, joined to a linking arm suitable for attachment to free amino groups in a protein[3], were required for immunological studies which will be described elsewhere. We now describe the synthesis of the type d partial-structure antigen *p*-nitrophenyl 2-acetamido-3-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosiduronic acid (**21**).

One route to a 2-acetamido-2-deoxy-β-D-mannopyranosyluronic acid residue would be the construction of a suitably substituted 2-azido-2-deoxy-β-D-mannopyranoside derivative with HO-6 unsubstituted, which, at a suitable stage, could be oxidised at

* Dedicated to Professor Grant Buchanan on the occasion of his 65th birthday.
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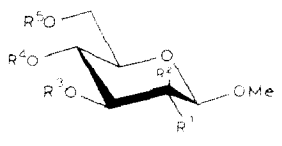
position 6. We now report that readily available methyl 3-*O*-benzyl-4,6-*O*-benzylidene-2-*O*-trifluoromethanesulfonyl-β-D-glucopyranoside undergoes smooth azide displacement to give the corresponding 2-azido-2-deoxy-β-D-*manno* derivative **3**. This displacement is the key step in the subsequent synthesis of the artificial antigen, and has yielded a facile stereospecific route to methyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**5**). Since all intermediates, and also methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-mannopyranoside (**4**), were obtained in high yields and are crystalline, this synthesis compares favourably with those published[3-6]. A synthesis of an α anomer, also *via* azide displacement at C-2, has been described[7].

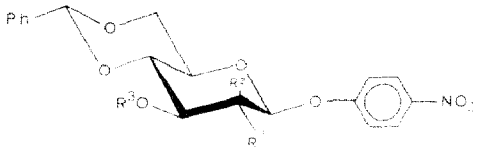
RESULTS AND DISCUSSION

Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**1**) was treated in sequence with sodium hydride, 1 mol of copper(II) chloride, and then benzyl bromide and tetrabutylammonium iodide[8,9] in tetrahydrofuran, to yield the 3-benzyl ether **2** (70%). Compound **2** was converted into the 2-triflate, and the crude product was subjected, in *N,N*-dimethylformamide, to azide displacement to give the 2-azido-mannoside **3** (85%). Hydrogenolysis (Pd/C) of **3**, followed by hydrolysis of the 4,6-benzylidene acetal and acetylation, then afforded the triacetate **4** (83%), from which syrupy methyl 2-acetamido-2-deoxy-β-D-mannopyranoside (**5**) was obtained by *O*-deacetylation.

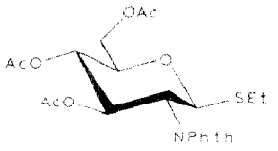
Alternatively, **1** was 3-acetylated *via* chelation with copper(II) chloride as described above[8,9], to give **6**. Conversion of **6** into the 2-triflate, followed by azide displacement at C-2, then gave methyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranoside (**7**, 84% from **6**). All of the above compounds, except **5**, were crystalline.



1 $R^1 = OH, R^2 = R^3 = H, R^4, R^5 = PhCH$
2 $R^1 = OH, R^2 = H, R^3 = Bn, R^4, R^5 = PhCH$
3 $R^1 = H, R^2 = N_3, R^3 = Bn, R^4, R^5 = PhCH$
4 $R^1 = H, R^2 = NHAc, R^3 = R^4 = R^5 = Ac$
5 $R^1 = R^3 = R^4 = R^5 = H, R^2 = NHAc$
6 $R^1 = OH, R^2 = H, R^3 = Ac, R^4, R^5 = PhCH$
7 $R^1 = H, R^2 = N_3, R^3 = Ac, R^4, R^5 = PhCH$



8 $R^1 = OH, R^2 = R^3 = H$
9 $R^1 = OH, R^2 = H, R^3 = Bz$
10 $R^1 = H, R^2 = N_3, R^3 = Bz$
11 $R^1 = R^2 = H, R^3 = N_3$



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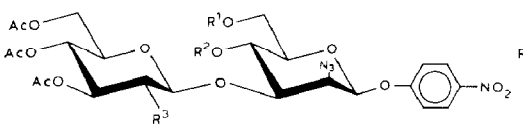
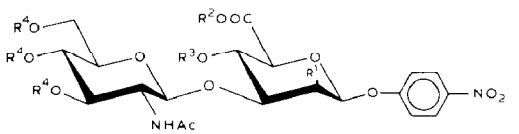
p-Nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside (**8**) was 3-benzoylated to yield crystalline **9** (77%), for which homonuclear decoupling experiments showed the position of the benzoyl group. Conversion of **9** into the 2-triflate was followed, without isolation of the intermediate, by azide displacement at C-2 to give the 2-azido-2-deoxy-mannoside derivative **10**. Debenzoylation of **10** exposed HO-3 for glycosylation in the product **11**. The purification of **9–11** was complicated by solubility problems. The conversion of **8** into **11** could be performed, without purification of the intermediates, in an overall yield of 52% [method (*b*) in the Experimental]. Compound **11** was then condensed, in a dimethyl(methylthio)sulfonium triflate-promoted reaction[10], with ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside (**12**) in dichloromethane, to yield the disaccharide derivative **13** (82%). The phthalimido group in **13** was converted into an acetamido group by treatment with hydrazine hydrate, followed by acetylation to give **14** (58%). Treatment of **14** with aqueous acetic acid removed the 4,6-*O*-benzylidene group and yielded **15** that was 6-*tert*-butyldimethylsilylated (→**16**) and then 4-benzoylated to give **17** (73% from **14**). Exposure of HO-6 by desilylation of **17** with aqueous acetic acid gave **18**, which was oxidised at position 6 with pyridinium dichromate–acetic anhydride in dichloromethane, in the presence of *tert*-butyl alcohol[11,12], to give the 6-*tert*-butyl ester **19** (57% from **17**). The following deprotection steps were then performed on **19**. Reduction of the azide group in the presence of the nitro group, with triphenylphosphine[13], followed by aqueous hydrolysis of the intermediate phosphine imine and *N*-acetylation gave **20**. Hydrolysis of the *tert*-butyl group with formic acid followed by *O*-deacylation with sodium methoxide in methanol gave the disaccharide derivative **21** (84% from **19**). Hydrogenation of the aromatic nitro group in **21** to an amino group gives an artificial antigen suitable for joining to a protein *via* a thiocarbamoyl linkage[3].

EXPERIMENTAL

General methods. — Melting points are corrected. Concentrations were performed under reduced pressure at <40° (bath), except for *N,N*-dimethylformamide for which 60° was used. Optical rotations were recorded at room temperature with a Perkin–Elmer 241 polarimeter. N.m.r. spectra were recorded with a JEOL FX-100 or GX-270 instrument. The 400-MHz ^{1}H-n.m.r. spectra were recorded with a JEOL





13 R^1, R^2 = PhCH, R^3 = NPhth
14 R^1, R^2 = PhCH, R^3 = NHAc
15 R^1 = R^2 = H, R^3 = NHAc
16 R^1 = Bu^tMe_2Si, R^2 = H, R^3 = NHAc
17 R^1 = Bu^tMe_2Si, R^2 = Bz, R^3 = NHAc
18 R^1 = H, R^2 = Bz, R^3 = NHAc

19 R^1 = N_3, R^2 = CMe_3, R^3 = Bz, R^4 = Ac
20 R^1 = NHAc, R^2 = CMe_3, R^3 = Bz, R^4 = Ac
21 R^1 = NHAc, R^2 = R^3 = R^4 = H

GX-400 instrument. Spectra were recorded at 25° on solutions in $CDCl_3$ (internal Me_4Si) and at 70° on solutions in C_5D_5N (internal Me_4Si) and D_2O (internal acetone δ_C 31.0, δ_H 2.221). All ^1H assignments are based on 2D experiments, as well as the ^{13}C assignments of **21**. The f.a.b. mass spectrum was obtained with a JEOL SX-102 instrument, with Xe atoms at 6 keV and a matrix of triethanolamine. T.l.c. was performed on Silica Gel F_{254} (Merck), with detection by u.v. light and by charring with sulfuric acid. Column chromatography was performed using silica gel (0.040–0.063 mm, Merck) in the flash mode unless otherwise stated. I.r. spectra were recorded with a Perkin–Elmer grating i.r. spectrophotometer. Tetrahydrofuran was distilled from lithium aluminium hydride. Pyridine, *N,N*-dimethylformamide, and dichloromethane were distilled from phosphorus pentaoxide. All dry solvents were stored over molecular sieves (4A). Cupric chloride was dried at 100° in a vacuum immediately before use.

*Methyl 3-*O*-benzyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside*[14] (**2**). — Methyl 4,6-*O*-benzylidene-β-D-glucopyranoside[15] (**1**; 200 mg, 0.71 mmol) was stirred with sodium hydride (34 mg, 1.4 mmol) in dry tetrahydrofuran (10 mL). After the evolution of hydrogen ceased (1–2 h), cupric chloride (95 mg, 0.71 mmol) was added. After ~ 10 min, a green solution of the copper chelate resulted. Benzyl bromide (0.42 mL, 0.71 mmol) and tetrabutylammonium iodide (50 mg) were added, and the mixture was boiled under reflux for 24 h (t.l.c.; toluene–ethyl acetate, 1:1), then cooled, treated with dilute ammonium hydroxide, and concentrated to dryness. A solution of the residue in ethyl acetate was washed with dilute ammonium hydroxide until the aqueous layer was colourless, then with water, dried (Na_2SO_4), and concentrated to a syrup. Column chromatography (toluene–ethyl acetate, 3:1) of the product gave **2** (185 mg, 70%), m.p. 182–183°, $[\alpha]_D$ −48° (*c* 1.0, chloroform); lit.[14] m.p. 184–185°, $[\alpha]_D$ −48° (chloroform).

*Methyl 2-azido-3-*O*-benzyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyranoside*[16] (**3**). — Trifluoromethanesulfonic anhydride (0.75 mL, 4.6 mmol) was added at 0° to a cooled solution of **2** (700 mg, 1.9 mmol) in dichloromethane–pyridine (2:1, 18 mL), and the mixture was stirred at 0° for 30 min (t.l.c.; toluene–ethyl acetate, 6:1). The solution was diluted with dichloromethane, washed with saturated aqueous sodium hydrogencarbonate, and concentrated to dryness. Sodium azide (600 mg, 9.4 mmol) was added to the stirred solution of the crude product in *N,N*-dimethylformamide (20 mL), and the mixture was kept at 70° for 2.5 h (t.l.c.; toluene–ethyl acetate, 6:1), filtered through Celite, and concentrated. Column chromatography (toluene–ethyl acetate, 6:1) of the residue gave **3** (635 mg, 85%), m.p. 70–74° (from ethanol), $[\alpha]_D$ −73° (*c* 1.0, chloroform); lit.[16] $[\alpha]_D$ −100° (chloroform); $\nu_{max}^{CHCl_3}$ 2110 cm^{-1} (CN_3). N.m.r. data ($CDCl_3$): ^{13}C, δ 57.2, 63.4, 67.3, 68.4, 72.9, 76.7, 78.6 (C-2,3,4,5,6 PhCH$_2$O, and OCH$_3$), 101.1, 101.5 (C-1 and PhCH), 126.0–137.4 (aromatic C); ^1H (400 MHz), δ 4.46 (d, 1 H, $J_{1,2}$ 1.5 Hz, H-1).

Anal. Calc. for $C_{21}H_{23}N_3O_5$: C, 63.5; H, 5.8; N, 10.6. Found: C, 63.5; H, 6.0; N, 10.5.

*Methyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-mannopyranoside* (**4**). — A solution of **3** (200 mg, 0.53 mmol) in aqueous 95% ethanol (12 mL) was hydrogenolysed over Pd/C (10%, 280 mg) at 400 kPa for 2 days (t.l.c.; chloroform–methanol, 4:1), then

filtered through Celite, and concentrated to a syrup. The crude syrupy product was added to aqueous 70% acetic acid (5 mL), the mixture was kept at 70° for 2 h and then concentrated, and toluene was evaporated twice from the residue which was acetylated in acetic anhydride–pyridine (1:2, 6 mL) (t.l.c.; chloroform–methanol, 9:1). The mixture was concentrated, and toluene (5 mL) was evaporated twice from the residue, which was crystallised from toluene to give **4** (159 mg, 83%), m.p. 154–156°, $[\alpha]_D$ −36° (*c* 0.5, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 20.6 (CH_3CO), 23.2 (CH_3CON), 50.1, 56.9, 62.5, 66.1, 71.6, 72.5 (C-2,3,4,5,6 and OCH_3), 100.1 (C-1), 170.7 (carbonyl C).

Anal. Calc. for $C_{15}H_{23}NO_9$: C, 49.9; H, 6.4; N, 3.9. Found: C, 49.9; H, 6.5; N, 3.7.

*Methyl 2-acetamido-2-deoxy-β-*D*-mannopyranoside*[17] (**5**). — A catalytic amount of sodium methoxide was added to a solution of **4** (182 mg, 0.50 mmol) in methanol (3 mL), and the solution was stirred at room temperature until the deacetylation was complete (t.l.c.; chloroform–methanol, 4:1). The reaction mixture was neutralised with Dowex 50 (H^+) resin, filtered, and concentrated to yield **5** (115 mg, 97%), $[\alpha]_D$ −62° (*c* 1.0, water); lit.[17] $[\alpha]_D$ −68° (water). ^{13}C-N.m.r. data (D_2O from external Me_4Si): δ 23.8 (CH_3CON), 54.6, 58.5, 62.4, 68.8, 73.9, 78.2 (C-2,3,4,5,6 and OCH_3), 102.2 (C-1).

*Methyl 3-*O*-acetyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside*[18] (**6**). — Compound **1** (300 mg, 1.06 mmol) was stirred with sodium hydride (51 mg, 2.1 mmol) in dry tetrahydrofuran (15 mL). After the evolution of hydrogen ceased (1–2 h), cupric chloride (143 mg, 1.1 mmol) was added. After ~10 min, a green solution of the copper chelate resulted. The solution was cooled to 0°, acetic anhydride (0.15 mL, 1.59 mmol) was added, and the temperature was kept at 0° for 30 min and then for 1.5 h at room temperature (t.l.c.; toluene–ethyl acetate, 1:1). The mixture was concentrated, and a solution of the residue in dichloromethane was washed successively with water, aqueous sodium hydrogencarbonate, and saturated aqueous sodium chloride, dried (Na_2SO_4), and concentrated. Column chromatography (toluene–ethyl acetate, 5:1) of the solid residue gave **6** (274 mg, 80%), m.p. 161–163°, $[\alpha]_D$ −52° (*c* 1.0, chloroform); lit.[18] m.p. 162–163°, $[\alpha]_D$ −55.2° (chloroform).

*Methyl 3-*O*-acetyl-2-azido-4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyranoside* (**7**). — Compound **6** (610 mg, 1.9 mmol) was processed, as described above for **2**, to give, after crystallisation from ethanol (no chromatography), **7** (551 mg, 84%), m.p. 131–133°, $[\alpha]_D$ −122° (*c* 0.31, chloroform); $\nu_{max}^{CHCl_3}$ 2105 cm^{-1} (CN_3). N.m.r. data ($CDCl_3$). ^{13}C, δ 20.8 (CH_3CO), 57.5, 62.5, 67.5, 68.5, 71.3, 75.6 (C-2,3,4,5,6 and OCH_3), 101.2, 102.0 (C-1 and PhCH), 126.2, 128.3, 129.2 (aromatic C); ^{1}H (400 MHz): δ 4.63 (d, 1 H, $J_{1,2}$ 1.2 Hz, H-1).

Anal. Calc. for $C_{16}H_{19}N_3O_6$: C, 55.0; H, 5.4; N, 12.0. Found: C, 55.1; H, 5.4; N, 12.1.

p-*Nitrophenyl 3-*O*-benzoyl-4,6-*O*-benzylidene-β-*D*-glucopyranoside* (**9**). — Benzoyl chloride (170 μL, 1.5 mmol) was added at 0° to a stirred solution of *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside[19] (**8**; 520 mg, 1.3 mmol) in dichloromethane–pyridine (25:1, 50 mL). The mixture was allowed to attain room temperature and, after 4 h (t.l.c.; toluene–ethyl acetate, 3:1), benzoyl chloride (85 μL, 0.73 mmol) was added at 0°. After another 3 h, methanol (2 mL) was added, the mixture was concentrated, and

toluene was evaporated twice from the residue, a solution of which in acetone was concentrated with silica gel. The residue was dried in a vacuum and put on top of a column of silica gel packed in toluene. Elution with toluene–ethyl acetate (1:1) gave **9** (510 mg, 77%), m.p. >250° (from ethanol), $[\alpha]_D$ −1.6° (*c* 1.1 pyridine). ^{13}C-N.m.r. data (C_5D_5N): δ 67.4, 69.0, 73.3, 75.7, 79.3 (C-2,3,4,5,6), 102.0, 102.1 (C-1 and PhCH), 117.3–162.7 (aromatic C), 166.3 (carbonyl C).

Anal. Calc. for $C_{26}H_{23}NO_9$: C, 63.3; H, 4.7; N, 2.8; O, 29.2. Found: C, 63.3; H, 4.7; N, 2.8.

p-*Nitrophenyl 2-azido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyranoside* (**10**). — Triflic anhydride (0.26 mL, 1.6 mmol) was added at 0° to a stirred solution of **9** (320 mg, 0.65 mmol) in dichloromethane–pyridine (2:1, 12 mL). The mixture was kept at 0° for 30 min, then allowed to attain room temperature. After another 30 min, the reaction was complete (t.l.c.; toluene–ethyl acetate, 3:1) and water (10 mL) was added. The organic phase was separated and concentrated, and toluene was evaporated twice from the residue. Sodium azide (211 mg, 3.2 mmol) was added at room temperature to a stirred solution of the crude triflate in *N*,*N*-dimethylformamide (12 mL), and the mixture was stirred overnight, then filtered through Celite, and concentrated. Column chromatography (toluene–ethyl acetate, 6:1) of the residue yielded **10** (306 mg, 91%), $[\alpha]_D$ −50° (*c* 1.2, chloroform); $\nu_{max}^{CHCl_3}$ 2105 cm^{-1} ($C{\equiv}N_3$). N.m.r. data: ^{13}C (C_5D_5N), δ 63.2, 68.3, 68.6, 71.8, 76.2, (C-2,3,4,5,6), 98.0, 102.4 (C-1 and PhCH), 117.0–161.3 (aromatic C), 165.9 (carbonyl C); ^{1}H (400 MHz, $CDCl_3$), δ 6.18 (d, $J_{1,2}$ <0.5 Hz, H-1).

Anal. Calc. for $C_{26}H_{22}N_4O_8$: C, 60.2; H, 4.3; N, 10.8; O, 24.7. Found: C, 60.1; H, 4.1; N, 10.2.

p-*Nitrophenyl 2-azido-4,6-*O*-benzylidene-2-deoxy-β-*D*-mannopyranoside* (**11**). — (*a*) A catalytic amount of methanolic sodium methoxide was added at room temperature to a stirred solution of **10** (190 mg, 0.37 mmol) in methanol (4 mL). After 3 h, the reaction was complete (t.l.c.; toluene–ethyl acetate, 3:1), and the mixture was neutralised with glacial acetic acid and concentrated. The residue was dissolved in the minimum amount of dichloromethane and subjected to column chromatography (toluene–ethyl acetate, 3:1), to give **11** (149 mg, 98%), m.p. 205° [from toluene–light petroleum (b.p. 40–60°)], $[\alpha]_D$ −126° (*c* 1.0, chloroform). ^{13}C-N.m.r. data ($CDCl_3$): δ 64.2, 67.6, 68.2, 70.1, 78.1 (C-2,3,4,5,6), 97.5, 102.4 (C-1 and PhCH), 116.4–160.6 (aromatic C).

Anal. Calc. for $C_{19}H_{18}N_4O_7$: C, 55.1; H, 4.4; N, 13.5; O, 27.0. Found: C, 55.1; H, 4.4; N, 13.4.

(*b*) Benzoyl chloride (629 μL, 5.4 mmol) was added at 0° to a stirred solution of *p*-nitrophenyl 4,6-*O*-benzylidene-β-D-glucopyranoside[19] (**8**; 1.92 g, 4.9 mmol) in dichloromethane (80 mL) and pyridine (4.8 mL). The mixture was allowed to attain room temperature and, when the reaction had stopped (t.l.c.; toluene–ethyl acetate, 3:1), more benzoyl chloride (629 μL, 5.4 mmol) was added at 0°. When t.l.c. showed that no **8** was left, methanol (2 mL) was added, the mixture was concentrated, and toluene was evaporated twice from the crude residue which was dried in vacuum. Triflic anhydride

(1.94 mL, 12 mmol) was added dropwise at 0° to a stirred solution of the crude residue in dichloromethane (30 mL) and pyridine (15 mL). The mixture was kept at 0° for 30 min and then allowed to attain room temperature. After another 30 min, the reaction was complete (t.l.c.; toluene–ethyl acetate, 3:1) and water (40 mL) was added. The organic phase was separated and concentrated, and toluene was evaporated twice from the residue. Sodium azide (1.6 g, 25 mmol) was added at room temperature to a stirred solution of the crude triflate in *N,N*-dimethylformamide (45 mL). After 12 h, the mixture was filtered through Celite and concentrated, and toluene was evaporated twice from the residue.

A catalytic amount of methanolic sodium methoxide was added to the solution of the crude azide in methanol (50 mL) at room temperature. After 3 h, the reaction was complete (t.l.c.; toluene–ethyl acetate, 3:1), the mixture was neutralised with acetic acid and concentrated, and a solution of the residue in the minimum amount of dichloromethane was subjected to column chromatography (toluene–ethyl acetate, 3:1) to give **11** (1.06 g, 52%). Elution with toluene–ethyl acetate (1:1) gave the diol (0.55 g, 29%).

p-*Nitrophenyl 3*-O-*(2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl)-2-azido-4,6*-O-*benzylidene-2-deoxy-β*-D-*mannopyranoside* (**14**). — Dimethyl(methylthio) sulfonium triflate (343 mg, 1.3 mmol) was added at room temperature to a stirred mixture of **11** (92 mg, 0.22 mmol), ethyl 3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-1-thio-β-D-glucopyranoside[20] (**12**; 160 mg, 0.33 mmol), 2,6-di-*tert*-butyl-4-methylpyridine (274 mg, 1.3 mmol), 4Å molecular sieves, and dichloromethane (10 mL). After 2 h, the reaction was complete (t.l.c.; toluene–ethyl acetate, 3:1), triethylamine (0.5 mL) was added, and stirring was continued for 30 min. The mixture was subjected to column chromatography (toluene–ethyl acetate, 3:1), the fraction that contained *p*-nitrophenyl 3-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl)-2-azido-4,6-*O*-benzylidene-2-deoxy-β-D-mannopyranoside (**13**) and the elimination product formed from the thio sugar was collected. Column chromatography (toluene–ethyl acetate, 1:1; not flash mode) gave **13** (152 mg, 82%). ^{13}C-N.m.r. data ($CDCl_3$): δ 20.4, 20.6, 20.7 (CH_3CO), 54.2, 61.8, 62.3, 67.9, 68.1, 68.7, 70.9, 72.2, 75.6, 75.7 (C-2,3,4,5,6 and C-2′,3′,4′,5′,6′), 96.6, 97.4, 101.7 (C-1,1′ and Ph*C*H), 116.3–160.5 (aromatic C), 169.4, 170.2, 170.7 (carbonyl C).

Hydrazine hydrate (1.9 mL, 39 mmol) was added to a solution of **13** (546 mg, 0.66 mmol) in ethanol (20 mL). The mixture was boiled under reflux overnight, then concentrated, and toluene was evaporated twice from the residue. Pyridine (4 mL, 50 mmol) and acetic anhydride (4 mL, 42 mmol) were added. When t.l.c. (chloroform–methanol, 9:1) showed acetylation to be complete, the mixture was concentrated and toluene was evaporated twice from the residue. Column chromatography (toluene–ethyl acetate, 1:1) then gave **14** (281 mg, 58%), $[\alpha]_D$ −65° (*c* 1.0, chloroform). ^{13}C-N.m.r. data (C_5D_5N): δ 20.4, 20.5 (CH_3CO), 23.2 (CH_3CON), 55.4, 62.9, 64.2, 68.4, 68.7, 70.1, 72.9, 73.3, 75.5, 77.0 (C-2,3,4,5,6 and C-2′,3′,4′,5′,6′), 98.1, 99.7, 102.3 (C-1,1′ and Ph*C*H), 117.0–161.5 (aromatic C), 169.7, 170.3, 171.0 (carbonyl C).

Anal. Calc. for $C_{33}H_{37}N_5O_{15}$: C, 53.3; H, 5.0; N, 9.4; O, 32.3. Found: C, 53.9; H, 5.1; N, 9.2.

p-*Nitrophenyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-azido-2-deoxy-β-D-mannopyranoside* (**15**). — A solution of **14** (227 mg, 0.31 mmol) in aqueous 70% acetic acid was kept at 70° for 1 h, when t.l.c. (chloroform–methanol, 9:1) indicated complete reaction. The mixture was concentrated and subjected to column chromatography (chloroform–methanol, 15:1) to yield **15** (149 mg, 75%), m.p. >250° (from ethanol), $[\alpha]_D$ −83° (*c* 0.57, pyridine). ^{13}C-N.m.r. data (C_5D_5N): δ 20.5 (CH_3CO), 23.3 (CH_3CON), 56.3, 62.2, 62.9, 63.1, 66.0, 70.3, 72.7, 73.0, 79.6, 80.9 (C-2,3,4,5,6 and C-2′,3′,4′,5′,6′), 97.9, 99.5 (C-1 and C-1′), 116.9–161.9 (aromatic C), 169.8, 170.4, 171.2 (carbonyl C).

Anal. Calc. for $C_{26}H_{33}N_5O_{15}$: C, 47.6; H, 5.1; N, 10.7; O, 36.6. Found: C, 47.4; H, 5.2; N, 10.7.

*p-Nitrophenyl 3-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2-azido-4-O-benzoyl-6-O-*tert*-butyldimethylsilyl-2-deoxy-β-D-mannopyranoside* (**17**). — *tert*-Butyldimethylsilyl chloride (51 mg, 0.34 mmol) was added at room temperature to a stirred solution of **15** (108 mg, 0.16 mmol) in pyridine (5 mL). The mixture was stirred overnight, then co-concentrated with toluene twice. Column chromatography (chloroform–methanol, 15:1; not flash mode) of the residue gave the 6-*O*-silylated product **16**. ^{13}C-N.m.r. data ($CDCl_3$): δ −5.4, −5.3 (CH_3Si), 18.3 (CSi), 20.6 (CH_3CO), 23.5 (CH_3CON), 25.8 [$(CH_3)_3CSi$], 56.2, 62.0, 62.9, 63.1, 65.9, 68.9, 71.3, 72.3, 78.0, 83.1 (C-2,3,4,5,6 and C-2′,3′,4′,5′,6′), 96.9, 99.8 (C-1 and C-1′), 116.6–161.3 (aromatic C), 169.5, 170.4, 170.6, 171.3 (carbonyl C).

Benzoyl chloride (39 μL, 0.34 mmol) was added at 0° to a solution of the silylated compound in pyridine (5 mL). After 2 h, the mixture was concentrated and toluene was evaporated twice from the residue, a solution of which in the minimum amount of dichloromethane was subjected to column chromatography (toluene–ethyl acetate, 1:1) to yield **17** (140 mg, 97%), $[\alpha]_D$ −57° (*c* 1.0, chloroform). ^{13}C-N.m.r. data (C_5D_5N): δ −5.3 (2 CH_3Si), 18.4 (CSi), 20.4, 20.5 (CH_3CO), 23.4 (CH_3CON), 26.0 [$(CH_3)_3CSi$], 56.8, 62.9, 63.0, 63.2, 67.9, 70.2, 72.5, 72.6, 76.6, 77.3 (C-2,3,4,5,6 and C-2′,3′,4′,5′,6′), 97.9, 99.0 (C-1 and C-1′), 117.2–161.7 (aromatic C), 165.7, 169.7, 170.2, 170.3, 171.4 (carbonyl C).

Anal. Calc. for $C_{39}H_{51}N_5O_{16}Si$: C, 53.6; H, 5.9; N, 8.0; O, 29.3; Si, 3.2. Found: C, 53.9; H, 5.8; N, 7.6.

p-*Nitrophenyl 2-acetamido-3-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-β-D-mannopyranosiduronic acid* (**21**). — A solution of **17** (147 mg, 0.17 mmol) in aqueous 70% acetic acid (5 mL) was stirred at room temperature. After 3 h, the reaction was complete (t.l.c.; chloroform–methanol, 15:1). The mixture was concentrated, and toluene was evaporated twice from the residue which was then washed with ether (4 mL) to give the crude 6-hydroxy compound **18** (129 mg). ^{13}C-N.m.r. data (C_5D_5N): δ 20.4, 20.6 (CH_3CO), 23.4 (CH_3CON), 56.9, 61.9, 2 × 63.0, 68.1, 70.2, 72.5, 72.6, 77.3, 77.5 (C-2,3,4,5,6 and C-2′,3′,4′,5′,6′), 97.9, 99.0 (C-1 and C-1′), 117.0–161.6 (aromatic C), 165.9, 169.7, 170.2, 171.4 (carbonyl C).

Acetic anhydride (239 μL, 2.5 mmol) was added to a stirred suspension of crude **18**, pyridinium dichromate (199 mg, 0.68 mmol), and *tert*-butyl alcohol (374 mg, 5.1

TABLE I

^{1}H- and ^{13}C-n.m.r. data for **21** at pD 8 (δ in p.p.m., J in Hz)

H-1′	$J_{1',2'}$	*C-1′*	*H-2′*	*C-2′*	*H-3′*	*C-3′*	*H-4′*	*C-4′*[a]	*H-5′*	*C-5′*[a]	*H-6′*		*C-6′*
4.67	8.2	98.7	3.76	56.4	3.58	74.9	3.48	71.0	3.48	76.8	3.94	3.76	61.7

H-1	$J_{1,2}$	*C-1*	*H-2*	*C-2*	*H-3*	*C-3*	*H-4*	*C-4*	*H-5*	*C-5*	–	*C-6*
5.53	1.5	97.5	4.84	50.2	4.19	77.2	3.82	68.1	3.92	78.1		175.6[c]

NAc			*Aromatic*[b] –O– 					
C=O	*–CH$_3$*	*–CH$_3$*	*C-1*	*H-2*	*C-2*	*H-3*	*C-3*	*C-4*
175.2[c] 175.6	2.05 2.13	23.0 23.3	162.1	7.18	117.6	8.24	126.9	143.6

[a] Tentative assignments[21]. [b] Tentative assignments[22]. [c] May be interchanged.

mmol) in dichloromethane (13 mL). The mixture was stirred at room temperature for 5 h in the dark, more pyridinium dichromate (100 mg, 0.34 mmol) was added, and the mixture was left overnight. Methanol (1 mL) was added and stirring was continued for 30 min. The mixture was washed through silica gel with ethyl acetate, then with ethyl acetate–acetic acid–methanol–water (12:3:3:1), and the combined filtrates were concentrated. Toluene was evaporated twice from the residue, a solution of which in the minimum amount of chloroform was subjected to column chromatography (chloroform–methano, 30:1), to give crude *tert*-butyl [*p*-nitrophenyl 3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2-azido-4-*O*-benzoyl-2-deoxy-β-D-mannopyranosid]uronate (**19**; 80 mg, 57%). ^{13}C-N.m.r. data (C_5D_5N): δ 20.4, 20.5, 20.5 (CH_3CO), 23.4 (CH_3CON), 27.8 [$(CH_3)_3CO$], 56.6, 62.8, 62.9, 68.5, 70.1, 2 × 72.7, 74.8, 77.0 (C-2,3,4,5 and C-2′,3′,4′,5′,6′), 83.0 [$(CH_3)_3CO$], 97.8, 99.4 (C-1 and C-1′), 117.2–161.4 (aromatic C), 165.5, 166.2, 169.7, 170.2, 170.3, 171.4 (carbonyl C).

Triphenylphosphine (22 mg, 0.083 mmol) was added at room temperature to a stirred solution of **19** (47 mg, 0.057 mmol) in dichloromethane (4 mL), and the mixture was heated to 35° and stirred for 5 h. Water (4 mL) was added and stirring was continued overnight until all of the phosphine imine was hydrolysed (t.l.c.; chloroform–methanol, 9:1). The organic layer was separated, then concentrated, and toluene was evaporated twice from the residue. Acetyl chloride (7 μL, 0.090 mmol) was added at 0° to a stirred solution of the crude amine in dichloromethane–pyridine (1:1, 4 mL). After 1 h, the mixture was concentrated and toluene was evaporated twice from the residue. Column chromatography (chloroform–methanol, 15:1) then yielded *tert*-butyl [*p*-nitrophenyl 2-acetamido-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-4-*O*-

benzoyl-2-deoxy-β-D-mannopyranosid]uronate (**20**; 46 mg, 96%). ^{13}C-N.m.r. data (C_5D_5N): δ 20.4, 20.5 (CH_3CO), 23.1, 23.5 (CH_3CON), 27.8 [$(CH_3)_2CO$], 49.5, 56.1, 62.9, 69.5, 70.1, 72.6, 73.9, 74.1, 76.7 (C-2,3,4,5 and C-2′,3′,4′,5′,6′), 82.5 [$(CH_3)_2CO$], 97.3, 100.7 (C-1 and C-1′), 117.3–162.6 (aromatic C), 166.3, 169.7, 170.3, 170.9, 171.5 (carbonyl C).

A solution of **20** (105 mg) in formic acid (8 mL) was stirred at room temperature for 6 h, 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 (5 mL) and stirring was continued overnight, to yield **21** (t.l.c.: ethyl acetate–acetic acid–methanol–water 4:3:3:2), whereafter the solution was neutralised with Dowex 50 (H^+) resin, filtered, and concentrated. Chromatography of the residue on DEAE-Sepharose CL-68 followed by gel filtration on Bio-Gel P-2 (200–400 mesh) gave **21** (60 mg, 87%), $[\alpha]_D$ −81° (*c* 0.37, water). F.a.b.-mass spectrum: pseudomolecular ion *m/z* 558.4 ($M - H$)$^-$. The ^{1}H- and ^{13}C-n.m.r. data for **21** are shown in Table I.

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