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GLYCOSIDATION AT C-4 OF 2-ACETAMIDO-2-DEOXY-D-GLUCOPYRANOSE DERIVATIVES BY KOENIGS–KNORR TYPE CONDENSATIONS

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ABSTRACT

The condensation of 2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl bromide and 2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl chloride with benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**1**), under Koenigs–Knorr conditions, gave the fully benzylated derivatives of benzyl 2-acetamido-2-deoxy-4-*O*-α-D-glucopyranosyl-α-D-glucopyranoside, benzyl 2-acetamido-2-deoxy-4-*O*-β-D-glucopyranosyl-α-D-glucopyranoside, and benzyl 2-acetamido-2-deoxy-4-*O*-α-D-mannopyranosyl-α-D-glucopyranoside. Three further compounds, namely, benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-α-D-glucopyranoside, benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-α-D-glucopyranoside, and benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-α-D-glucopyranoside, were formed by reaction of the respective glycosyl halide with benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside present as contaminant in **1**.

INTRODUCTION

In investigating methods for the synthesis of the biologically important disaccharide 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucopyranose, the condensation, under classical Koenigs–Knorr conditions, of mannose or glucose derivatives having a non-participating group at C-2 with a derivative of 2-acetamido-2-deoxy-D-glucopyranose having only HO-4 unsubstituted has been studied. The use of the non-participating group at C-2 has been proposed as a means of increasing the relative yield of products having the substituents at positions 1 and 2 *cis*-related[1]. Thus, the presence of a 2-*O*-benzyl group should increase the yield of α-glucopyranosyl products and give some β-mannopyranosyl products. Although it has been reported that position 4 of 2-acetamido-2-deoxy-D-glucopyranose is relatively unreactive in Koenigs–Knorr condensations[2,3], its reactivity in benzyl 2-acetamido-

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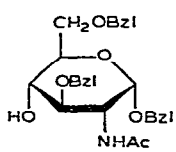
3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**1**) has not been assessed. A convenient route to **1** from readily obtainable benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside[3] has been reported[4], and the use of **1** in condensations with tetra-*O*-benzylhexopyranosyl halides has been studied. Whilst this work was in progress, the synthesis of 2-acetamido-2-deoxy-4-*O*-β-D-mannopyranosyl-D-glucose was reported[5,6].

RESULTS AND DISCUSSION

Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside (**1**) was synthesised by the published route[4], and 2,3,4,6-tetra-*O*-benzyl-D-gluco- and -D-manno-pyranose were best obtained by a route involving conversion[7] of an allyl into an acid-labile prop-1-enyl glycoside. The last two compounds were then treated as described by Austin *et al.*[8] to give 2,3,4,6-tetra-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-glucopyranose and 2,3,4,6-tetra-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-mannopyranose. The *manno* compound has recently been reported by Koto *et al.*[9].

The *p*-nitrobenzoic esters were converted into their glycosyl halides under strictly anhydrous conditions, in order to minimise loss of halides by hydrolysis. The use of dilute solutions of the appropriate hydrogen halide in dichloromethane for these reactions, coupled with low temperatures, ensured that no secondary reactions occurred. When tetra-*O*-benzylhexosyl bromides formed by saturation of solutions of the 1-*p*-nitrobenzoate with hydrogen bromide at room temperature[10] were used in condensations with **1**, large proportions of benzyl 2-acetamido-3,4,6-tri-*O*-benzyl-2-deoxy-β-D-glucopyranoside[11] were formed, probably *via* a reaction of benzyl bromide, which could be detected in the reaction mixture, with HO-4 of **1**. The only published reference to this undoubtedly common problem is that of Lemieux *et al.*[12], whose solution was to use only a small (~200%) molar excess of hydrogen bromide at 0°. Using similar conditions, >80% yields of the desired glycosyl halides were usually obtained in less than 1 h.



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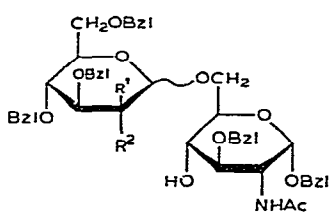
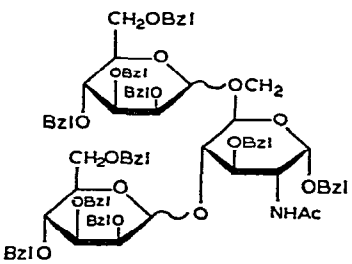


2 R^1 = H, R^2 = OBzl
3 R^1 = OBzl, R^2 = H

The glycosyl halides were used immediately in condensations under strictly anhydrous conditions with limiting amounts of **1**. The retrieval of up to 50% of **1** after these reactions reflected the low reactivity of HO-4 in **1**, which was further

confirmed by the formation of benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-α-D-glucopyranoside (**2**) and benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-α-D-glucopyranose (**3**). These products had i.r. absorption for hydroxyl, and each gave a new product on acetylation[13]. That **2** and **3** contained (1→6)-links was established by subjecting each to the reaction sequence methylation, acetolysis, hydrolysis, hydrogenolysis, reduction, and acetylation. The resulting methylated alditol acetates of 2-deoxy-2-(*N*-methylacetamido)-D-glucopyranose were analysed by g.l.c., and in each case gave a large peak with retention time (116 min) identical to that of the product obtained by applying the same reaction sequence to benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-D-glucopyranoside. Thus, it appeared that **1** was contaminated with benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-α-D-glucopyranoside, which was readily glycosylated to give **2** and **3**. In the condensation of **1** with a larger excess of 2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl chloride, further glycosidation occurred at HO-4 to give the trisaccharide derivative benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-α-D-glucopyranoside (**4**). That HO-4 did undergo glycosidation was further demonstrated by its reaction with 2,3,4,6-tetra-*O*-benzyl-D-glucosyl bromide, which yielded the fully benzylated disaccharides **5** and **6** in a combined yield of 12.5%, and reaction with 2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl chloride to yield 7% of **7**. Compound **6** was also obtained by an alternative procedure involving treatment of **1** with sodium methylsulphinylmethanide in methyl sulphoxide. The resulting alkoxide should attack the subsequently added α-glycosyl halide in an S_N2-type reaction to give a product having the β configuration. On this basis, **6** was provisionally assigned the β configuration. The ratio of **5** to **6** was ~1:2, suggesting steric hindrance by the 2-*O*-benzyl group in the formation of products having the α configuration. Analogous steric hindrance would be expected to lead to the α-D-*manno* anomer, and thus **7** was provisionally assigned the α configuration.



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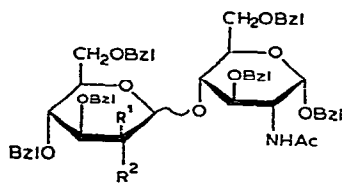

5(α) R^1 = H, R^2 = OBzl
6(β) R^1 = H, R^2 = OBzl
7(α) R^1 = OBzl, R^2 = H

The foregoing results show that HO-4 of a suitably protected 2-acetamido-2-deoxy-D-glucopyranose derivative can be glycosidated under Koenigs–Knorr conditions. However, the much greater reactivity of HO-6 necessitates the most

stringent purification of starting material, in order to avoid contamination of the product with (1→6)-linked disaccharides and (1→4),(1→6)-linked trisaccharides. With recent improvements in the preparation of 2,3,4,6-tetra-*O*-benzylhexopyranoses[7,9], the present route is attractive for the synthesis of (1→4)-linked disaccharides containing 2-acetamido-2-deoxy-D-glucopyranose as the reducing sugar, compounds that have previously been obtained *via* more complicated routes (*cf.* Ref. 14).

EXPERIMENTAL

General methods. — All solvents were distilled and dried before use. Melting points were determined with a Reichart hot-stage microscope apparatus or an Electrothermal melting-point apparatus, and are uncorrected. Optical rotations were determined with a Carl Zeiss polarimeter. I.r. spectra were recorded on a Perkin–Elmer 257 grating spectrometer. N.m.r. spectra (60 MHz) were recorded for solutions in $CDCl_3$ (internal Me_4Si) with a Varian EM 360 spectrometer. Elemental analyses were carried out by B.M.A.C. Teddington. T.l.c. was performed on silica gel GF_{254} (Merck), with detection by charring with sulphuric acid. P.l.c. was performed on 2-mm layers of silica gel GF_{254} (Merck) preheated at 90° for 4 h. Hydrogenation employing a modified Brown hydrogenator system (Delmar Scientific Laboratories, Illinois), with an external hydrogen-generating system, was carried out for 24 h at atmospheric pressure on solutions in glacial acetic acid in the presence of 10% palladium-on-charcoal catalyst.

Methanolyses. — Samples were methanolysed, and the products were re-*N*-acetylated by the method of Bhatti *et al.*[15] and then analysed by t.l.c. (dichloromethane–ethyl acetate, 3:1) for the presence of methyl 2,3,4,6-tetra-*O*-benzyl-D-mannopyranoside or -D-glucopyranoside.

Methylation analysis. — Samples (1–2 mg), dried overnight over phosphorus pentaoxide under vacuum, were dissolved in anhydrous methyl sulphoxide (1 ml). Sodium methylsulphinylmethanide (1 ml of a 2-mequiv./ml solution) was added, and the solution was stirred rapidly under a nitrogen atmosphere for 1 h and then cooled. Methyl iodide (1 ml) was added under a stream of nitrogen, and the mixture was stirred at room temperature for a further 2 h. Water (5 ml) was added and the mixture was extracted with dichloromethane (2 × 5 ml). The combined extracts were washed with water (6 × 5 ml), and concentrated to dryness under reduced pressure. The residue was subjected to acetolysis, hydrolysis, reduction, and acetylation, as described by Stellner *et al.*[13]. G.l.c. of the resulting, partially methylated alditol acetates was performed on an OV-225 column (3% on Gas Chrom Q) with a temperature programme (170°, 40 min, 2°/min to 220°), using a Pye 104 chromatograph with flame-ionisation detection.

*Condensation of 2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranosyl bromide with benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-α-*D*-glucopyranoside* (**1**). — A solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-glucopyranose[7,8] (1.0 g, 1.5 mmol) in anhydrous

dichloromethane (10 ml) at −20° was added under anhydrous conditions and in a stream of nitrogen to a 2–3% solution of hydrogen bromide in dichloromethane (20 ml) also at −20°. The mixture, under nitrogen, was allowed to warm up to 4° during 1 h and then filtered from *p*-nitrobenzoic acid which was washed with dichloromethane (2 × 5 ml) under anhydrous conditions. The combined filtrate and washings were concentrated to dryness under reduced pressure at 35°, and toluene (2 × 20 ml) was distilled from the residue. T.l.c. (dichloromethane–ethyl acetate, 25:1) gave one main spot, R_F 0.91 (*cf.* R_F 0.81 for the starting 1-*p*-nitrobenzoate).

A solution of the glycosyl bromide in anhydrous nitromethane (50 ml) containing **1** (0.5 g, 0.8 mmol) and mercuric cyanide (0.3 g, 1.3 mmol) was stirred under an atmosphere of dry nitrogen for 24 h at room temperature and then boiled under reflux for a further 16 h. The cooled mixture was concentrated to dryness under reduced pressure at 35°, and a solution of the residue in dichloromethane (50 ml) was washed with aqueous 10% potassium iodide (20 ml) and water (3 × 50 ml), dried ($MgSO_4$), and concentrated to dryness under reduced pressure. T.l.c. (dichloromethane–ethyl acetate, 3:1) of the resulting amber oil revealed **1** (R_F 0.15), unreacted *p*-nitrobenzoate (R_F 0.95), 2,3,4,6-tetra-*O*-benzyl-D-glucopyranose (R_F 0.85), and suspected disaccharide products **5**, **6**, and **2** (R_F 0.81, 0.5, and 0.45). The products **5**, **6**, and **2** were purified by p.l.c. (dichloromethane–ethyl acetate, 85:15). Each gave methyl 2,3,4,6-tetra-*O*-benzyl-D-glucopyranoside after methanolysis, and n.m.r. and i.r. data consistent with the presence of benzyl and acetamido groups: ν_{max}^{film} 3,300 (N–H), 1650, 1510–1540 (acetamido C=O), and 700–740 cm^{-1} (aromatic C–H).

Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranosyl)-α-D-glucopyranoside (**5**; 45 mg, 4.5%), $[\alpha]_D^{28}$ +73° (*c* 1.4, dichloromethane). No reaction under *O*-acetylating conditions.

Anal. Calc. for $C_{63}H_{67}NO_{11}$: C, 74.61; H, 6.66; N, 1.38. Found: C, 74.43; H, 6.67; N, 1.22.

Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl)-α-D-glucopyranoside (**6**; 77 mg, 8%), m.p. 133–136°, $[\alpha]_D^{28}$ +55° (*c* 1.4, dichloromethane). No reaction under *O*-acetylating conditions.

Anal. Calc. for $C_{63}H_{67}NO_{11}$: C, 74.61; H, 6.66; N, 1.38. Found: C, 74.48; H, 6.48; N, 1.56.

Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-glucopyranosyl)-α-D-glucopyranoside (**2**; 23.5 mg, 2.5%), m.p. 192–195°, $[\alpha]_D^{28}$ +47.5° (*c* 1.2, dichloromethane); ν_{max}^{film} 3,500 and 3,580 cm^{-1} (OH). On acetylation, the R_F (dichloromethane–ethyl acetate, 3:1) changed from 0.45 to 0.73.

Anal. Calc. for $C_{56}H_{62}NO_{11}$: C, 72.78; H, 6.65; N, 1.52. Found: C, 72.66; H, 6.91; N, 1.88.

*Condensation of 2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl chloride with* **1**. — A solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-*p*-nitrobenzoyl-D-mannopyranose (4.6 g, 6.6 mmol) in anhydrous dichloromethane (10 ml) at −20° was treated with a 2.5% solution of hydrogen chloride in dichloromethane (25 ml) also at −20°, by essentially the same procedure described above, to give 2,3,4,6-tetra-*O*-benzyl-D-manno-

pyranosyl chloride which showed two main spots, R_F 0.87 and 0.81 (t.l.c., dichloromethane–ethyl acetate, 25:1) (*cf.* R_F 0.68 and 0.64 for the starting 1-*p*-nitrobenzoate).

A solution of the glycosyl chloride in anhydrous nitromethane (50 ml) was added slowly, under a stream of dry nitrogen, to a solution of **1** (0.6 g, 1.2 mmol) and mercuric cyanide (0.7 g, 3.0 mmol) in nitromethane (50 ml) at 40°. The mixture was stirred under an atmosphere of dry nitrogen for 24 h at room temperature. A further quantity of freshly prepared glycosyl chloride (2.8 g, 4.9 mmol) was then added and the solution was boiled under reflux for 48 h. The cooled mixture was then concentrated to dryness under reduced pressure at 35°. A solution of the residue in dichloromethane (250 ml) was washed with water (3 × 200 ml), dried ($MgSO_4$), and concentrated to dryness under reduced pressure. T.l.c. (dichloromethane–ethyl acetate, 3:1) of the resulting yellow oil (7.7 g) revealed **1** (R_F 0.15), unreacted *p*-nitrobenzoate (R_F 0.9), 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose (R_F 0.72), and suspected disaccharide products **7**, **3**, and **4** (R_F 0.58, 0.48, and 0.33). These products were purified by p.l.c. (dichloromethane–ethyl acetate, 85:15). Each gave methyl 2,3,4,6-tetra-*O*-benzyl-D-mannopyranoside on methanolysis, and n.m.r. and i.r. data consistent with the presence of benzyl and acetamido groups: ν_{max}^{film} 3290 (N–H), 1650, 1510–1540 (acetamido C=O), 690 and 740 cm^{-1} (aromatic C–H).

Benzyl 2-acetamido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(2,3,4,6-tetra-*O*-benzyl-α-D-mannopyranosyl)-α-D-glucopyranoside (**7**) was a yellow oil (79 mg, 7%), $[\alpha]_D^{23}$ +50° (*c* 7.8, dichloromethane). No reaction under *O*-acetylating conditions.

Anal. Calc. for $C_{63}H_{67}NO_{11}$: C, 74.61; H, 6.66; N, 1.38. Found: C, 74.92; H, 6.87; N, 1.26.

Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-6-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-α-D-glucopyranoside (**3**) was a yellow oil (78 mg, 7%), $[\alpha]_D^{23}$ +59° (*c* 4.9, dichloromethane), ν_{max}^{film} 3420 cm^{-1} (OH). Under *O*-acetylating conditions[13], the R_F (t.l.c.; dichloromethane–ethyl acetate, 3:1) changed from 0.48 to 0.69.

Anal. Calc. for $C_{56}H_{62}NO_{11}$: C, 72.78; H, 6.65; N, 1.52. Found: C, 72.66; H, 6.91; N, 1.88.

Benzyl 2-acetamido-3-*O*-benzyl-2-deoxy-4,6-di-*O*-(2,3,4,6-tetra-*O*-benzyl-D-mannopyranosyl)-α-D-glucopyranoside (**4**) was a yellow oil (73 mg, 4%), $[\alpha]_D^{23}$ +44° (*c* 7.3, dichloromethane). No reaction under *O*-acetylating conditions.

Anal. Calc. for $C_{90}H_{95}NO_{16}$: C, 74.72; H, 6.62; N, 0.97. Found: C, 74.87; H, 7.04; N, 1.26.

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