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## Note

# Synthesis of 4-nitrophenyl 2,3,6-tri-*O*-acetyl-4-deoxy-β-D-*lyxo*-hexopyranoside and a new synthesis of 4-nitrophenyl β-D-mannopyranoside

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The utility of 4-nitrophenyl β-D-mannopyranoside (**1**) as a substrate for use in the assay of β-D-mannosidase (EC 3.2.1.25) is well established[1]. In general, there are difficulties associated with the synthesis of 1,2-*cis*-glycosides because of neighbouring-group participation[2], but these can be overcome[3] by using non-participating groups at O-2. An alternative approach is to start from β-D-glucopyranoside derivatives and invert the configuration at C-2 by oxidation and then reduction[4]. The viability of this approach depends on the accessibility of suitably blocked derivatives. We now report on the synthesis of **1**, using the latter strategy, in which the correctly blocked derivative is obtained directly from the glycoside-forming reaction.

The general strategy for the synthesis of β-D-mannosides involving isomerisation of suitably protected β-D-glucosides employed[4] benzyl ether or benzylidene blocking-groups. Such protecting groups require hydrogenolysis for removal and are not appropriate for the synthesis of 4-nitrophenyl glycosides because of possible reduction of the nitro group. Ester protecting-groups were therefore considered, but a problem then arose due to the increased lability of an ester group in the β-position to a carbonyl group. Thus, oxidation of 1,3,4,6-tetra-*O*-acetyl-α-D-gluco- or -galacto-pyranose gave 5-acetoxy-2-acetoxymethyl-4*H*-pyran-4-one (**2**) by oxidation and elimination of two molecules of acetic acid[5]. Oxidation of methyl 3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (**3**) occurred with β-elimination of acetic acid[6] when dimethyl sulphoxide–acetic anhydride was used as oxidant. Using ruthenium tetraoxide as oxidant, simple oxidation of an ester-protected glycoside was achieved, and then a molecule of carboxylic acid was readily eliminated during column chromatography[7] or thermally[8]. Oxidation with dimethyl sulphoxide–phosphorus(V) oxide has been recommended for use with ester protecting-groups[9].

For the synthesis of **1**, a suitably protected intermediate with HO-2 unsubsti-

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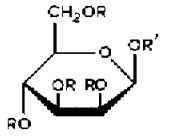
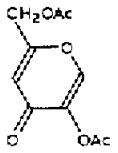
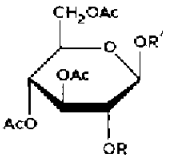
tuted was required. For this purpose, 4-nitrophenyl 3,4,6-tri-*O*-acetyl-β-D-glucopyranoside (**4**) was readily obtained by the reaction of sodium 4-nitrophenoxide with 3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride[10]. Oxidation[11] (40°, 1.5 h) of **4** with dimethyl sulphoxide gave 59% of the diacetate **2**. Thus, under these conditions, both acetic acid and 4-nitrophenol were eliminated from the product, presumably in a process (similar to that proposed earlier[5,6]) involving oxidation, β-elimination of acetic acid, rearrangement, and then further β-elimination of 4-nitrophenol. When the reaction was repeated at a lower temperature, **2** was also obtained, as was **5**, the product of acetylation of the starting material. Such acetylations have been observed previously[11].



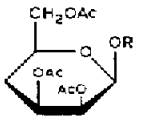
1 R = H, R′ = *p*NO$_2$Ph
9 R = Ac, R′ = *p*NO$_2$Ph



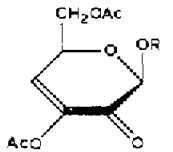
**2**



3 R = H, R′ = Me
4 R = H, R′ = *p*NO$_2$Ph
5 R = Ac, R′ = *p*NO$_2$Ph



6 R = *p*NO$_2$Ph



7 R = *p*NO$_2$Ph
8 R = Me

The oxidation was next attempted using dimethyl sulphoxide and phosphorus(V) oxide, which has been recommended[9] for use in the presence of ester substituents. Treatment[12] of **4** with 4 mol of dimethyl sulphoxide and 1.5 mol of phosphorus(V) oxide in *N*,*N*-dimethylformamide at 65–70° for 2 h gave a syrupy mixture of products (t.l.c.). Column chromatography of this mixture was not successful, and its composition changed during chromatography as previously observed with similar compounds[7]. Consequently, reduction of the carbonyl group was carried out on the crude reaction-product. Catalytic hydrogenation[9] was precluded, because of the nitro group, but the use of sodium borohydride[4] gave a mixture of products that could not be completely purified by column chromatography; after acetylation, 24% of crystalline 4-nitrophenyl 2,3,6-tri-*O*-acetyl-4-deoxy-β-D-*lyxo*-hexopyranoside (**6**) could be isolated.

The structure of **6** was established as follows. The elemental analysis was consistent with a 4-nitrophenyl tri-*O*-acetyldeoxyhexoside. The signal at 96.5 p.p.m. in

the $^{13}$C-n.m.r. spectrum was assigned to C-1, and the observation that irradiation at $\delta$ 5.25 caused this signal to collapse to a singlet showed that the doublet at $\delta$ 5.25 was due to H-1. Irradiation at $\delta$ 5.25 also caused the $^1$H signal at $\delta$ 5.59 to simplify to a doublet, showing that the latter signal was due to H-2. Irradiation at $\delta$ 5.59 collapsed the signal at $\delta$ 5.25 to a singlet, and also decoupled the signal at $\delta$ 5.12 which was therefore assigned to H-3. Irradiation at $\delta$ 5.12 decoupled the signals at $\delta$ 5.59 and 1.98–1.78, showing that the deoxy group, which is the only group that could give signals at such high field, was at position 4. Irradiation at $\delta$ 3.97 also collapsed the signal for the deoxy group, showing that the $\delta$ 3.97 signal was due to H-5. The magnitude of the various $J$ values together with the optical rotation were used to assign configuration.

The relatively high, negative rotation suggested that the original $\beta$-D configuration was retained, *i.e.*, the configuration was unchanged at C-1 and C-5. As a consequence, both H-1 and H-5 are expected to adopt axial positions. The small value (1 Hz) of $J_{1,2}$ showed that H-2 was equatorial[13] and that **6** was a 1,2-*cis*-glycoside. The signal for H-4 was deceptively simple, because of accidental chemical-shift equivalence of H-4,4′, and hence the $J_{3,4}$ and $J_{5,4}$ values were averages of the separate $J_{n,4a}$ and $J_{n,4e}$ values. The observed, relatively high, averaged values of 7.5 Hz indicate averaging of a large and a small value, which could only arise if both H-3 and H-5 were axial. Thus, the structure of **6** was established. Presumably, **6** arose from **4** by oxidation, followed by $\beta$-elimination to give **7**, and then stereoselective reduction from the less-hindered side to give the product having all non-hydrogen substituents on the same side of the ring. The conversion of the corresponding methyl glycoside into **8** by oxidation with $\beta$-elimination has been reported[6].

In an attempt to avoid the $\beta$-elimination, the oxidation was repeated using a stoichiometric amount of phosphorus(V) oxide and a lower temperature. The crude reaction-product was reduced with sodium borohydride in methanol, but again the product could not be completely purified by chromatography. During chromatography, the composition of the mixture changed (as indicated by t.l.c.), presumably because of acetyl migration, which is a facile process under a variety of conditions[14]. After acetylation of the product mixture, 21% of crystalline 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-$\beta$-D-mannopyranoside (**9**) was obtained, and chromatography of the mother liquor gave more **9** (5%) and the corresponding $\beta$-D-glucoside **5** (3%). Zemplén deacetylation of **9** then gave **1**.

Ruthenium(IV) oxide has been recommended for oxidation of partially protected glycosides[15], particularly because of the mild conditions used and good yields obtained, but a tendency to give $\beta$-elimination with esters was noted[7]. A variation of the method using a catalytic quantity of ruthenium(II) oxide and sparingly soluble potassium periodate has been recommended[16], to avoid over-oxidation. Attempts to oxidise **4** under the above conditions gave (t.l.c.) complex mixtures of products which were not further examined. Attempted oxidation with pyridinium chlorochromate[17] also gave a complex mixture of products (t.l.c.).

The strategy described herein for the synthesis of 4-nitrophenyl β-D-mannopyranoside is attractive, because of the significantly lower cost of glucose compared to mannose. The general strategy has proved successful[4], but, in this particular case, problems associated with β-elimination of acetic acid and 4-nitrophenol (brought about by the enhanced leaving-tendency of anions of stronger acids) make the approach less attractive than an alternative[21] starting from mannose.

## EXPERIMENTAL

*General methods.* — Melting points are uncorrected. Solvents were dried as follows. Acetone was stored over potassium carbonate and distilled from potassium permanganate; ether was stored over sodium wire; methanol was distilled from magnesium methoxide; pyridine was distilled from phosphorus(V) oxide and stored over potassium hydroxide; *N*,*N*-dimethylformamide was azeotropically distilled with benzene, shaken with barium oxide, distilled under nitrogen at reduced pressure, and stored over 4Å molecular sieve; methyl sulphoxide was stored overnight over barium oxide, and then distilled from calcium hydride under reduced pressure and stored over 4Å molecular sieves. Organic solutions were dried with $MgSO_4$.

T.l.c. was performed on silica gel (Merck, 5735) with detection by iodine vapour, vanillin–sulphuric acid, or u.v. light. Column chromatography was carried out on silica gel (Merck, 7734).

$^1$H-N.m.r. spectra (internal $Me_4Si$) were recorded with Perkin–Elmer R-14 or Varian X-L100 spectrometers. All coupling constants refer to measured splittings. $^{13}$C-N.m.r. spectra (internal $Me_4Si$) were recorded with a Jeol JNM FX60 FT spectrometer. Optical rotations were determined with a Perkin–Elmer 141 polarimeter (1-dm tube).

*4-Nitrophenyl 3,4,6-tri-O-acetyl-β-D-glucopyranoside* (**4**). — (*a*) A solution of 3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride[10] (**10**, 2 g) in acetone (25 mL) was added to a solution of 4-nitrophenol (1.3 g) and sodium hydroxide (0.5 g) in water (15 mL). After 5 h at ambient temperature, the solution was concentrated at 30° (bath). T.l.c. (toluene–ether, 1:2) of the syrupy residue showed two components [$R_F$ 0.34 (major) and 0.26]. Column chromatography, using a toluene–ether gradient, gave the major product as a pale-yellow syrup (1.15 g, 44%) that crystallised from chloroform–carbon tetrachloride, to give **4** (0.82 g, 31%), m.p. 105–107°, $[\alpha]_D^{20}$ −42° (*c* 1, chloroform). $^1$H-N.m.r. data ($CDCl_3$): δ 8.26–7.04 (m, 4 H, aromatic), 5.33–4.97 (m, 3 H, H-1,3,4), 4.32–3.80 (m, 4 H, H-2,5,6,6'), 3.12 (d, 1 H, $J_{HO,2}$ 4.5 Hz, OH), 2.11 and 2.09 (2 s, 9 H, 3 OAc).

*Anal.* Calc. for $C_{18}H_{21}NO_{11}$: C, 50.6; H, 5.0; N, 3.3. Found: C, 50.3; H, 5.2; N, 3.3.

(*b*) A mixture of dry sodium 4-nitrophenoxide (3.0 g), dry active silver(I) carbonate[18] (2.75 g), anhydrous calcium sulphate (5 g), and dry *N*,*N*-dimethylformamide (40 mL) was stirred at 40°, **10** (3.0 g) was then added, and stirring was con-

tinued overnight. T.l.c. (toluene–ether, 1:2) then showed that **10** ($R_F$ 0.42) had almost completely reacted. The mixture was filtered, diluted with chloroform (80 mL), washed with aqueous sodium hydrogencarbonate (3 × 25 mL) and water (2 × 25 mL), dried, and concentrated. The syrupy residue (3.1 g) was purified by column chromatography and then crystallised as in (*a*), to give **4** (2.7 g, 51%).

(*c*) The reaction was repeated essentially as in (*b*), but using 2-methoxyethyl ether (50 mL) as solvent, to give 40% of **4**.

(*d*) A mixture of dry sodium 4-nitrophenoxide (2.6 g), dry active silver(I) carbonate (2.2 g), anhydrous calcium sulphate (1.0 g), and dry acetone (60 mL) was stirred and then a solution of **10** (2.6 g) in acetone (20 mL) was added. Stirring was continued at ambient temperature for 3 h, and t.l.c. (toluene–ether, 1:2) then revealed that **10** had reacted. The mixture was filtered and concentrated to a syrup. The insoluble material was washed with chloroform (3 × 20 mL), the washings were combined with the syrup, the resulting mixture was filtered, the insoluble material was washed with chloroform, and the combined filtrate and washings were concentrated. The residue was treated with carbon tetrachloride, to give a crystalline product (2.4 g) that was recrystallised from chloroform–carbon tetrachloride to give **4** (2.1 g, 62%).

(*e*) The reaction was repeated using sodium 4-nitrophenoxide (9.5 g), anhydrous calcium sulphate (4.0 g), and **10** (9.5 g) in dry acetone (190 mL) for 1.5 h at amient temperature. The mixture was processed as in (*d*), to give **4** (8.7 g, 70%).

*Oxidation of* **4**. — (*a*) To a mixture of dry dimethyl sulphoxide (10 mL) and acetic anhydride (7 mL) was added **4** (0.5 g). The solution was kept at 40° for 1.5 h, and then poured into chloroform (30 mL) and water (30 mL). The aqueous layer was washed with chloroform (2 × 10 mL), and then the chloroform solutions were combined, washed with aqueous sodium hydrogencarbonate (10 mL) and water (20 mL), and dried. T.l.c. (toluene–ether, 1:2) revealed a major component ($R_F$ 0.11) and several minor components ($R_F$ 0.63, 0.45, 0.28, 0.20). The chloroform solution was concentrated, and light petroleum (b.p. 60–80°) was added to the solution, to give a crystalline product (0.17 g, 64%), $R_F$ 0.11, which was recrystallised from chloroform–light petroleum (b.p. 40–60°) to yield 5-acetoxy-2-acetoxymethyl-4*H*-pyran-4-one (**2**; 0.16 g, 60%), m.p. 97–99°; lit.[5] m.p. 101–102°. $^1$H-N.m.r. data ($CDCl_3$): $\delta$ 7.91 (s, 1 H, H-6), 6.48 (s, 1 H, H-3), 4.90 (s, 2 H, $CH_2$-O), 2.30 (s, 3 H, enol Ac), and 2.14 (s, 3 H, OAc).

(*b*) The reaction was repeated as in (*a*), but at ambient temperature for 3 h, to give **2** (45 mg, 17%). The mother liquor was purified by column chromatography (toluene–ether, 1:1), to give 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**5**; 140 mg, 26%), m.p. 175–178° [from chloroform–light petroleum (b.p. 40–60°)], $[\alpha]_D^{22}$ −40° (*c* 1, chloroform); lit.[19] m.p. 174–175°, $[\alpha]_D$ −40° (chloroform).

(*c*) A mixture of **4** (1.0 g), dimethyl sulphoxide (0.7 mL), phosphorus(V) oxide (1.0 g), and dry *N*,*N*-dimethylformamide (25 mL) was stirred for 2 h at 65–

70° and then processed, essentially as in (*a*), to give a syrup (0.67 g) which contained (t.l.c.; toluene–ether, 1:2) a major ($R_t$ 0.16) and three minor components ($R_F$ 0.63, 0.55, and 0.23). Attempted purification of the syrup by chromatography resulted in decomposition on the column, to give **2** ($R_t$ 0.11). To a solution of the crude syrup (0.64 g) in methanol (20 mL) was added sodium borohydride (0.1 g), and the mixture was stirred at room temperature for 30 min and then concentrated to dryness. The residue was partitioned between chloroform (50 mL) and water (20 mL). The chloroform solution was washed with water (3 × 10 mL), dried, and concentrated. Attempted purification of the resulting syrup by column chromatography was unsuccessful, but the partially purified material (0.38 g) was treated with acetic anhydride (2.5 mL) in pyridine (2.5 mL) at ambient temperature for 16 h. Conventional work-up and two recrystallisations of the product from chloroform–carbon tetrachloride gave 4-nitrophenyl 2,3,6-tri-*O*-acetyl-4-deoxy-β-D-*lyxo*-hexopyranoside (**6**; 0.22 g, 24%), m.p. 136–138°, $[\alpha]_D^{22}$ −83° (*c* 1, chloroform). N.m.r. data ($CDCl_3$): $^1H$, δ 8.21–7.00 (m, 4 H, aromatic), 5.59 (q, 1 H, $J_{2,1}$ 1, $J_{2,3}$ 3 Hz, H-2), 5.25 (d, 1 H, $J_{1,2}$ 1 Hz, H-1), 5.12 (m, 1 H, $J_{3,2}$ 3, $J_{3,4}$ 7.5 Hz, H-3), 4.28–4.20 (m, 2 H, H-6,6′), 4.12–3.80 (m, 1 H, H-5), 2.22, 2.08, 2.04 (3 s, each 3 H, 3 OAc), and 1.98–1.78 (m, 2 H, H-4,4′); $^{13}C$, δ 170.6, 170.2, 169.9 (C=O), 161.3 (C-1′), 143.0 (C-4′), 125.7 (C-2′,6′), 116.5 (C-3′,5′), 96.5 (C-1), 70.7 (C-5), 68.2 (C-3), 67.1 (C-2), 65.4 (C-6), 27.4 (C-4), and 20.8 ($CH_3CO$).

*Anal.* Calc. for $C_{18}H_{21}NO_{10}$: C, 52.6; H, 5.2; N, 3.4. Found: C, 52.5; H, 5.2; N, 3.3.

(*d*) The oxidation was repeated as in (*c*), but using phosphorus(V) oxide (0.66 g) at 45–50° for 2 h. T.l.c. (toluene–ether, 1:2) revealed two major products ($R_t$ 0.49 and 0.14). The crude product was reduced as in (*c*), to give a syrup (0.47 g) containing seven components (t.l.c.). Attempted chromatographic purification of part of the product was unsuccessful. The remainder (0.39 g) of the crude product was acetylated as in (*c*). Two crystallisations of the syrupy product (0.3 g) from chloroform–light petroleum (b.p. 40–60°) gave 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-mannopyranoside (**9**; 0.18 g, 21%), m.p. 184–186°, $[\alpha]_D^{22}$ −91° (*c* 1, chloroform); lit.[20] m.p. 142–143°, $[\alpha]_D^{18}$ −100° (chloroform).

Chromatography of the mother liquor on silica gel with toluene–ether (1:2) gave more **9** (48 mg, 6%), together with 4-nitrophenyl 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**5**; 30 mg, 3%).

*4-Nitrophenyl β-D-mannopyranoside* (**1**). — To a solution of **9** (0.2 g) in dry methanol (2 mL) was added methanolic sodium methoxide (0.5 mL) [prepared from sodium (25 mg) and dry methanol (5 mL)]. The solution was stored overnight at 4°. The product crystallised (94 mg, 73%), and had m.p. 205–207°, $[\alpha]_D$ −105° (*c* 1, water); lit.[21] m.p. 205–207°, $[\alpha]_D^{20}$ −108° (water).

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