SYNTHESIS OF 2-ACETAMIDO-6-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYL)-2-DEOXY-D-GLUCOPYRANOSE

D. BUNDLE AND N. SHAW
Microbiological Chemistry Research Laboratory, Department of Organic Chemistry, The University, Newcastle upon Tyne, NE1 7RU (Great Britain)
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ABSTRACT

A β-(1→6)-linked disaccharide of 2-amino-2-deoxy-D-glucose is known to be the basic structural unit of the lipid-A component in the lipopolysaccharides from *Salmonella typhimurium* and *Escherichia coli*. Synthesis of the di-*N*-acetyl derivative has been achieved by treating 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphoryl-amino-α-D-glucopyranosyl bromide with various 6-unsubstituted derivatives of 2-amino-2-deoxy-D-glucose followed by removal of protecting groups and *N*-acetylation. The maximal yield was obtained with benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside, which was also the most conveniently prepared derivative of 2-amino-2-deoxy-D-glucose suitable for use in the Koenigs–Knorr condensation. Hydrogenolysis of the diphenoxyphosphoryl protecting group was carried out at room temperature and pressure and did not require the high pressures reported previously.

INTRODUCTION

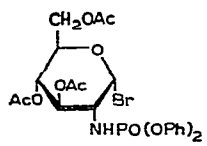
A common feature of the lipopolysaccharides isolated from Gram-negative bacteria is the presence of a lipid component, lipid A, which is only released on acid hydrolysis[1]. Where chemical studies have been carried out, lipid A is composed of 2-amino-2-deoxy-D-glucose, fatty acids, and phosphate, but its precise chemical structure is still unknown. Recent investigations have shown that in *Salmonella typhimurium*[2] and *Escherichia coli* ATCC 12408[3] the basic structure unit of lipid A is 2-amino-6-*O*-(2-amino-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose, and in order to facilitate further studies an authentic sample of the disaccharide was required.

Although both anomers of the (1→6)-linked disaccharide have been isolated[4] from the acid-reversion products of 2-acetamido-2-deoxy-D-glucose, a conventional chemical synthesis was favoured, which, with suitable modifications, would also enable the synthesis of potential analogues of lipid A to be undertaken. A synthesis of the octa-acetate of the disaccharide by a Koenigs–Knorr condensation between 2-acetamido-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl bromide and 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose has been reported[5], but a yield of only 5% was obtained. This low yield was presumably due to the instability of the acetobromo

derivative which readily rearranges to the hydrobromide[6]. A more suitable derivative is 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphorylamino-α-D-glucopyranosyl bromide (**1**) which has been used to prepare simple glycoside[7] and disaccharide[8,9] derivatives of 2-amino-2-deoxy-D-glucose. We have utilised the acetobromo sugar **1** to prepare derivatives of the β-(1→6)-linked disaccharide in yields of 40–60% by condensation with various 6-unsubstituted derivatives of 2-amino-2-deoxy-D-glucose.

RESULTS AND DISCUSSION

Condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphorylamino-α-D-glucopyranosyl bromide (**1**) with 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose (**2**) gave the crystalline disaccharide **3** in 40% yield. Chromatography of the mother liquors yielded a further quantity of material. The diphenoxyphosphoryl protecting group was removed by hydrogenolysis at atmospheric pressure and room temperature, and did not require the high pressures (20 atm.) previously reported[7]. No attempt was made to isolate the hydrogenolysis product which was acetylated directly to yield the known octa-acetate **4**. *O*-Deacetylation with sodium or barium methoxide did not proceed smoothly, presumably due to the formation of artefacts as previously reported during similar experiments on oligosaccharides derived from chitin[10,11]. However, treatment with 1% magnesium methoxide[11] yielded the di-*N*-acetyldisaccharide **5** as a very hygroscopic, white solid.



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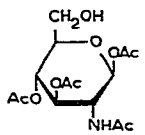

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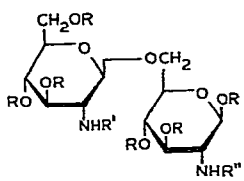


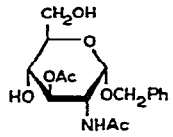
3 R,R" = Ac; R' = PO(OPh)$_2$

4 R,R',R" = Ac

5 R = H; R',R" = Ac

Replacement of **2** in the condensation with the acetabromo sugar **1** by either benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**6**) or benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-D-glucopyranoside (**7**) gave the corresponding disaccharide derivatives **8** and **9** in yields of 60%. Acetylation of **8** gave **9**. The increased yield may be due to the greater solubility of **6** and **7** in benzene, the solvent used for the condensation. The aglycone **7** was prepared by tritylation, acetylation, and detritylation of benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[12], and **6** was prepared from benzyl 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[13] by treatment with 60% acetic acid at 100° for 30 min. The disaccharide **9** was dissolved in a saturated solution of ammonia in benzyl alcohol and allowed to stand at room temperature for several days. The dibenzylphosphorylamino derivative **10**

so produced by transesterification was smoothly hydrogenated, and the resulting product was *N*-acetylated to give **5**. Hydrogenolysis of the dibenzylphosphoryl group was accomplished more readily (in 6 h) than for the diphenoxyphosphoryl group (48 h).



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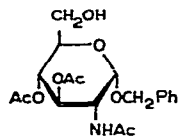

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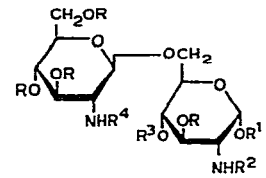

8 R, R^2 = Ac; R^1 = CH_2Ph; R^3 = H; R^4 = $PO(OPh)_2$

9 R, R^2, R^3 = Ac; R^1 = CH_2Ph; R^4 = $PO(OPh)_2$

10 R, R^3 = H; R^1 = CH_2Ph; R^2 = Ac; R^4 = $PO(OCH_2Ph)_2$

The physical properties of the disaccharide **5** and the corresponding derivative prepared by reduction with sodium borohydride were in agreement with those previously reported[4]. The disaccharide was completely hydrolysed to 2-acetamido-2-deoxy-D-glucose by incubation with 2-acetamido-2-deoxy-β-D-glucosidase. These syntheses provide further examples of the efficiency of the acetobromo sugar derivative **1** in effecting high-yield, Koenigs–Knorr condensations.

EXPERIMENTAL

Melting points were measured on a Kofler block and are uncorrected. Optical rotations were determined on an ETL-NPT automatic polarimeter. Column chromatography was carried out on silicic acid (Merck, 0.05–0.2 mm, 70–325 mesh) and thin-layer chromatography with silica gel G (Merck). The solvents used were: *A*, benzene–methanol 10:1 and B, chloroform–acetone 3:1. Components were detected by charring with 50% sulphuric acid. N.m.r. spectra were measured on a Bruker 90-MHz spectrometer using 10–15% solutions in chloroform-*d* with tetramethylsilane as internal standard.

*2-Acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphorylamino-β-*D*-glucopyranosyl)-β-*D*-glucopyranose* (**3**). — A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenoxyphosphorylamino-α-D-glucopyranosyl bromide[7] (**1**, 6.0 g), 2-acetamido-1,3,4-tri-*O*-acetyl-2-deoxy-β-D-glucopyranose[14] (**2**, 3.47 g), and finely divided mercuric cyanide (4.5 g) in dry benzene (55 ml) was boiled under reflux for 3 h with vigorous stirring under anhydrous conditions. Whilst still hot, the solution was filtered, and the residue was washed with benzene (35 ml). Chloroform (250 ml) was added to the filtrate, and the solution was washed twice with ice-cold, aqueous sodium chloride and three times with water, and finally dried over sodium sulphate. Evaporation of the solvents yielded a cream-coloured solid (8.3 g) which was recrystallised from ethanol to yield **3** as colourless needles (3.5 g, 41%), m.p. 206–207°, $[\alpha]_D$ −5° (*c* 2, chloroform), R_F 0.25 (t.l.c., solvent *B*).

Anal. Calc. for $C_{38}H_{47}N_2O_{19}P$: C, 52.66; H, 5.46; N, 3.23; P, 3.57. Found: C, 52.54; H, 5.44; N, 3.23; P, 3.58.

N.m.r. data: τ 3.75 (doublet, J 9.2 Hz, –CO.NH–), 4.59 (doublet, J 8.8 Hz, phosphoramidate H), 5.38 (doublet, J 8.36 Hz, anomeric H), 7.87–8.13 (21 protons, Ac and NHAc).

*2-Acetamido-6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranosyl)-1,3,4-tri-*O*-acetyl-2-deoxy-β-*D*-glucopyranose* (**4**). — The disaccharide **3** (2.5 g) in acetic acid (80 ml) was hydrogenated over Adams' catalyst (0.2 g) at atmospheric pressure and room temperature until uptake of hydrogen ceased (*ca.* 48 h). The catalyst was removed by filtration, water (10 ml) was added, and the solution was evaporated to dryness. The residue was dissolved in water (20 ml), and the solution was basified with aqueous potassium hydrogen carbonate. The free base was extracted into chloroform (3 × 200 ml), and the extract was dried over sodium sulphate. Evaporation of the solvent yielded a syrup (1.5 g) which was treated with acetic anhydride–pyridine (1:1, 20 ml) for 24 h. The mixture was poured into ice–water and extracted with chloroform (5 × 150 ml). The extracts were washed with aqueous potassium hydrogen carbonate and ice–water and then dried over sodium sulphate. Evaporation of the solvent and recrystallisation of the residue from ethanol yielded the octa-acetate **4** (1.5 g, 77%), m.p. 242–243°, $[\alpha]_D$ −1.5° (*c* 2, chloroform).

Anal. Calc. for $C_{28}H_{40}N_2O_{17}$: C, 49.70; H, 5.92; N, 4.14. Found: C, 49.78; H, 6.02; N, 4.24.

*2-Acetamido-6-*O*-(2-acetamido-2-deoxy-β-*D*-glucopyranosyl)-2-deoxy-*D*-glucopyranose* (**5**). — The octa-acetate **4** (520 mg) was suspended in a solution of magnesium methoxide in methanol (1% w/v, 30 ml) at 0°. After 10 min, a clear solution was obtained and after standing for 1 h at 0°, this was brought to pH 7 by the addition of solid CO_2. Water (10 ml) was added, and the solution was passed through a column of Dowex-50 (H^+) resin. The eluate was evaporated to dryness to yield a white solid (320 mg) which was recrystallised from ethanol to give **5** as a very hygroscopic, white solid (150 mg, 46%), m.p. 199–201°, $[\alpha]_D$ +10° (*c* 1, water) after 30 min; lit.[4] m.p. 200°.

The disaccharide **5** (60 mg) was dissolved in water (5 ml), sodium borohydride (70 mg) was added, and the solution was left at room temperature for 3 h. Excess sodium borohydride was destroyed by the addition of Dowex-50 (H^+) resin, after filtration the solution was evaporated to dryness, and the borate was removed from the residue by repeated evaporation with methanol. The residue was recrystallised from methanol to yield 2-acetamido-6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucitol, m.p. 200–202°, $[\alpha]_D$ −20° (*c* 0.5, water); lit.[4] m.p. 201–202°.

Incubation of **5** with 2-acetamido-2-deoxy-α-D-glucosidase (pig epididymis) at 37° overnight and examination of the products by paper chromatography showed complete hydrolysis to 2-acetamido-2-deoxy-D-glucose.

*Benzyl 2-acetamido-3-*O*-acetyl-2-deoxy-α-*D*-glucopyranoside* (**6**). — Benzyl 2-acetamido-3-*O*-acetyl-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside[13] (7.2 g) was heated in 60% acetic acid for 30 min at 100°. The acetic acid was evaporated and final traces were removed by repeated evaporation with toluene. The residual syrup

was crystallised from methanol–acetone as colourless needles (4.0 g, 70%), m.p. 122–123°, $[\alpha]_D$ +120° (*c* 1, chloroform)

Anal. Calc. for $C_{17}H_{24}NO_7$: C, 57.62; H, 6.83; N, 3.95. Found: C, 57.57; H, 6.62; N, 4.06.

*Benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-trityl-α-*D*-glucopyranoside.* — Benzyl 2-acetamido-2-deoxy-α-D-glucopyranoside[12] (5.7 g) and chlorotriphenylmethane (5.2 g) were dissolved in dry pyridine (40 ml) and left at room temperature for 2 days with intermittent shaking. Acetic anhydride (13.6 ml) was then added, and the solution was left for a further 3 days. The solution was poured into ice–water (1300 ml) with stirring, and the resulting precipitate was filtered off. The residue (12 g) was purified by chromatography on silicic acid to remove traces of the β-anomer present in the starting material[12]. The α-anomer was eluted with benzene–ether–methanol (50:50:1) and crystallised from ethyl acetate–light petroleum as colourless needles (8.1 g, 68%), m.p. 149–150°, $[\alpha]_D$ +99° (*c* 1, chloroform).

Anal. Calc. for $C_{38}H_{39}NO_8$: C, 71.55; H, 6.17; N, 2.20. Found: C, 71.44; H, 6.28; N, 2.38.

N.m.r. data: τ 2.55–3 (multiplet, aromatic H), 4.26 (doublet, *J* 9.5 Hz, –CO.NH–), 4.93 (doublet, *J* 3.7 Hz, anomeric H). 4.75–6.30 (multiplet, ring H's), 6.84 (doublet, *J* 4 Hz, $-O.CH_2Ph$), 8.01, 8.09, and 8.28 (singlets, Ac and NHAc).

*Benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-α-*D*-glucopyranoside* (**7**). — A solution of the above trityl ether (2.8 g) in acetic acid (15 ml) was cooled in ice–water, and a solution of hydrogen bromide in acetic acid (45% w/v, 1.5 ml) was added. After shaking for 60 sec, the mixture was poured into ice–water. The insoluble residue was removed by filtration, and the solution was extracted four times with chloroform. The extracts were evaporated to dryness and traces of acetic acid removed by evaporation of toluene from the residue. The residual syrup (1.6 g) was crystallised from ethanol–ether to give **7** (1.0 g, 58%), m.p. 167–169°, $[\alpha]_D$ +124° (*c* 1, chloroform).

Anal. Calc. for $C_{19}H_{25}NO_8$: C, 57.71; H, 6.37; N, 3.54. Found: C, 57.92; H, 6.38; N, 3.72.

N.m.r. data: τ 2.60–2.65 (multiplet, aromatic H), 4.17 (doublet, *J* 9 Hz, –CO.NH–), 4.55–6.45 (multiplet, ring H), 5.62 (doublet, *J* 3.6 Hz, anomeric H), 7.93, 7.98, and 8.10 (singlets, OAc and NHAc).

*Benzyl 2-acetamido-3,4-di-*O*-acetyl-2-deoxy-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-di-phenoxyphosphorylamino-β-*D*-glucopyranosyl)-α-*D*-glucopyranoside* (**9**). — The glycoside **6** (2 g) and mercuric cyanide (3.8 g) were stirred in dry benzene (30 ml) at room temperature for 1 h. The acetobromo sugar derivative **1** (3 g) dissolved in benzene (40 ml) was added, and the mixture was refluxed with vigorous stirring for 3 h. The hot solution was filtered, and the residue was washed with benzene (20 ml). Chloroform (80 ml) was added to the filtrate which was then washed twice with saturated, aqueous sodium chloride at 0° and thrice with water, and then dried. Evaporation of the solvent gave **8** as a syrup (4.5 g) which was directly acetylated with acetic anhydride in pyridine, as described for **4**, to give **9** (4 g). The crude product was eluted from a column of silicic acid with benzene–methanol (50:1) to give a product

(3.5 g, 65%) that was crystallised from ethanol to yield **9**, m.p. 179–181°, $[\alpha]_D$ +68.5° (*c* 1, chloroform). T.l.c. in solvent *A*, R_F 0.36; solvent *B*, 0.52.

Anal. Calc. for $C_{43}H_{51}N_2O_{18}P$: C, 56.45; H, 5.63; N, 3.06. Found: C, 56.15; H, 5.51; N, 3.19.

The preparation was repeated using the glycoside **7** (1.9 g), but omitting the acetylation. After column chromatography, the disaccharide **9** (2.8 g) was crystallised from ethanol. The material was identical to that prepared above from **6**, as judged by i.r. spectra, m.p., and optical rotation.

Benzyl 2-acetamido-2-deoxy-6-O-(2-deoxy-2-dibenzyloxyphosphorylamino-β-D-glucopyranosyl)-α-D-glucopyranoside (**10**). — The crude disaccharide **9** (2 g) was dissolved in freshly distilled benzyl alcohol (150 ml) saturated at 0° with ammonia and left at room temperature for 5 days. The ammonia and benzyl alcohol were removed at 70°/0.1 mmHg, and the residual syrup was chromatographed on a column of silicic acid. Elution with benzene–methanol (4:1) gave **10** (0.65 g, 41%) which, after crystallisation from ethanol–light petroleum, had m.p. 230–231°, $[\alpha]_D$ −15° (*c* 1, methanol).

Anal. Calc. for $C_{35}H_{45}N_2O_{13}P$: C, 57.37; H, 6.19; N, 3.82. Found: C, 57.40; H, 6.49; N, 3.97.

2-Acetamido-6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-2-deoxy-D-glucopyranose (**5**). — The disaccharide **10** (200 mg) was dissolved in aqueous acetic acid (60%, 5 ml) and hydrogenated for 6 h at room temperature and pressure over palladium black (50 mg). The catalyst was removed and the filtrate evaporated. A solution of the residue in water was extracted with ethyl acetate and then evaporated to dryness. The syrup was dissolved in water (50 ml) containing 1 ml of saturated, aqueous sodium hydrogen carbonate and 1 ml of 5% aqueous acetic anhydride. The mixture was left at room temperature for 20 min and then heated for 5 min at 100°. The solution was passed through a column of Dowex-50 (H^+) resin, the column was washed well with water, the eluate was evaporated to dryness, and the resulting syrup (110 mg) was crystallised from ethanol to yield **5** as very hygroscopic, white crystals (60 mg, 52%). The product was identical to that prepared above by condensing **1** with **2**, as judged by i.r. spectra, optical rotation, m.p., and analysis.

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