THE SYNTHESIS OF 6-*O*-(2-ACETAMIDO-2-DEOXY-β-D-GLUCOPYRANOSYLURONIC ACID)-D-GLUCOSE

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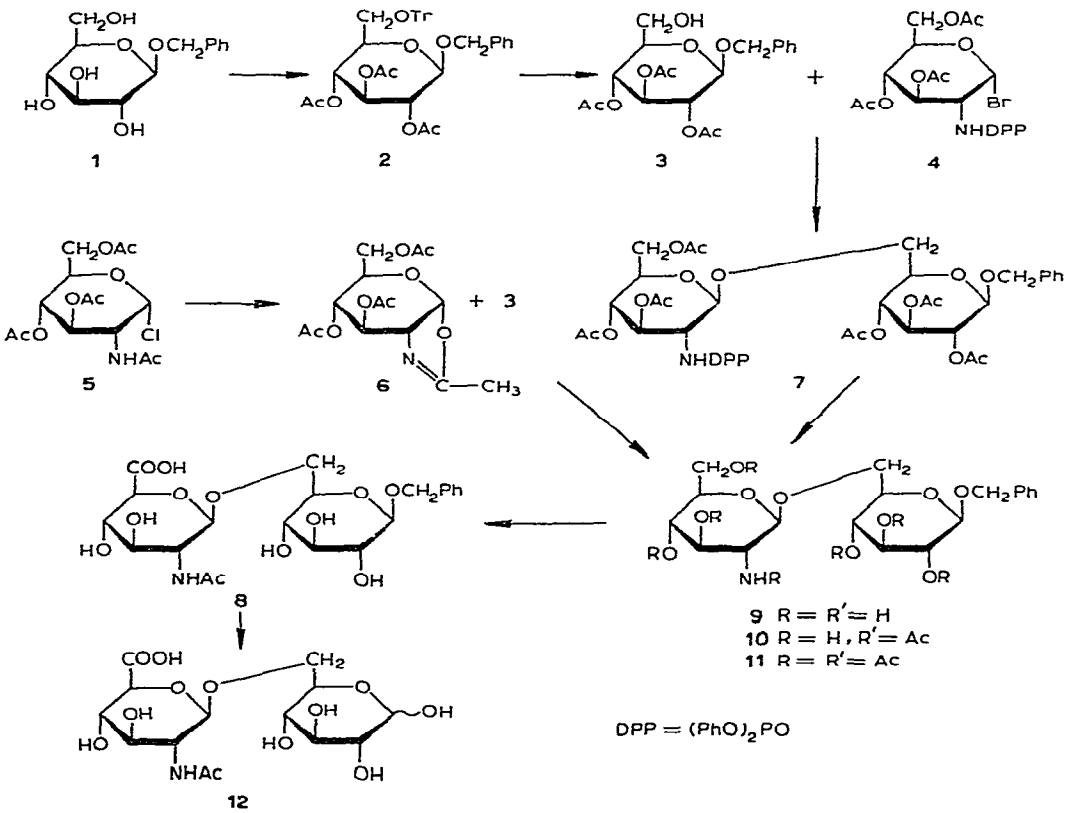
ABSTRACT

Two routes for the synthesis of 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyluronic acid)-D-glucose (**12**) were studied In the first, benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranoside (**10**) was obtained by condensation of benzyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (obtained *via* the 6-*O*-trityl derivative) with 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenylphosphorylamino-α-D-glucopyranosyl bromide, followed by removal of the diphenylphosphoryl and *O*-acetyl groups and



subsequent *N*-acetylation In the second route, 2-methyl-4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyrano)-2-oxazoline was used as 2-acetamido-2-deoxy-D-glucose moiety in the condensation reaction to give benzyl 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (**11**), and subsequent removal of *O*-acetyl groups gave compound **10** Oxidation of the primary hydroxyl group of **10** gave benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyluronic acid)-β-D-glucopyranoside (**8**), which was converted by catalytic hydrogenolysis into the free disaccharide **12**

INTRODUCTION

2-Amino-2-deoxy-D-galacturonic acid has been isolated from the V_1 antigen of *Salmonella typhosa*[1] and 2-amino-2-deoxy-D-glucuronic acid has been reported to occur in *Hemophillus influenzae*, Type d[2] These aminohexuronic acid monomers, 2-amino-2-deoxy-D-glucuronic acid[3] and 2-amino-2-deoxy-D-galacturonic acid[4], as well as 2-amino-2-deoxy-D-mannuronic acid[5] have been synthesized

In 1962, Perkins[6 7] found a heteropolymer which consisted of an equimolar proportion of D-glucose and 2-acetamido-2-deoxymannuronic acid residues in the cell wall of *Micrococcus lysodeikticus* Periodate oxidation of the polymer and isolation of a disaccharide from it suggested[8] the structure to be composed of alternative residues of these two sugars, in which D-glucose was substituted at C-6 Jeanloz[9] also showed the linkage at C-6 of glucose by permethylation of the polymer fraction Recently, it was shown that the 2-acetamido-2-deoxymannuronic acid residues were linked through C-4 and C-1 and had the D-configuration[10 11] In this work, 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyluronic acid)-D-glucose was synthesized as an analog of the disaccharide portion of the polymer To our knowledge, this is the first disaccharide synthesized that contains an aminodeoxyhexuronic acid

RESULTS AND DISCUSSION

Benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranoside (**10**) was synthesized *via* two different routes In the first route, 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenylphosphorylamino-α-D-glucopyranosyl bromide[12] (**4**) was condensed with benzyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (**3**) in dry benzene in the presence of mercuric cyanide to give in 60% yield benzyl 2,3,4-tri-*O*-acetyl-6-*O*-(3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenylphosphorylamino-β-D-glucopyranosyl)-β-D-glucopyranoside (**7**) Compound **3** had been obtained from benzyl β-D-glucopyranoside (**1**) by tritylation and acetylation to give benzyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-β-D-glucopyranoside (**2**) followed by detritylation with 60% acetic acid Removal of the acetyl and diphenylphosphoryl groups of **7** was performed with 0 5M sodium hydroxide (catalytic hydrogenolysis of the diphenylphosphoryl group may also remove the benzyl group) The intermediate **9** was not isolated and *N*-acetylation with acetic anhydride at pH 7–8 gave crystalline **10**

Zurabyan *et al*[13] have shown 2-methylglyco[l',2' 4,5]-2-oxazolines to be effective glycosylating agents Benzyl 6-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*2,3,4*-tri-*O*-acetyl-β-D-glucopyranoside (**11**) was obtained in 46% yield by heating the 4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyrano)-2-methyl-2-oxazoline[14] (**6**) with **3** in dry benzene–nitromethane in the presence of *p*-toluenesulfonic acid at pH 3–4 Deacetylation with 0 4M sodium methoxide gave **10** in 86% yield

Periodate oxidation studies of **10** showed a (1→6) glycoside bond and a ratio of D-glucose to 2-amino-2-deoxy-D-glucose of 1.1 These results are consistent with the proposed structure Compound **10** was oxidized with oxygen in the presence of Adams' platinum catalyst to give benzyl 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyluronic acid)-β-D-glucopyranoside (**8**) in 55% yield This compound showed a single acidic spot on paper chromatogram After hydrolysis, D-glucose and 2-amino-2-deoxy D-glucuronic acid were detected, the latter giving with ninhydrin the characteristic brownish color which turned purple with time The i r spectra of **8** showed the absorption of the carboxyl group of 1750 cm^{-1} Hydrogenolysis of **8**, in the presence of palladium-on-charcoal, gave 6-*O*-(2-acetamido-2-deoxy-β-D-glucopyranosyluronic acid)-D-glucose (**12**) in 80% yield On paper chromatograms, **12** migrated as a single spot reacting with Bromophenol Blue and with the silver nitrate–sodium hydroxide reagents to show the presence of a reducing group The i r spectra of **12** showed the absorption of the carboxyl group The β-D-configuration of the (1→6) glycosidic bond in the disaccharides was ascertained by the enzymic hydrolysis of **10** with a *N*-acetyl-β-D-glucosaminidase and from the n m r. spectra of **11** (H-1 doublet at δ 5 10 p p m , $J_{1,2}$ 7–8 Hz, anomeric 2 H, 2-acetamido-2-deoxy-β-D-glucoside and β-D-glucoside)

EXPERIMENTAL

General methods — All melting points are uncorrected Optical rotations were determined in a 0 5-dm tube with a Hitachi Model No 042-4 polarimeter I r spectra were recorded for potassium bromide discs, with a Nippon Bunko model IR-S spectrophotometer N m r spectra were obtained with a Varian T-60 spectrometer on *ca* 20% solution in chloroform-*d* with tetramethylsilane as internal standard Paper chromatography was performed by the ascending method on Toyo filter paper No 51 T l c was performed on silica gel G (Nakarai Chemicals, Ltd) The solvent systems for chromatography were (A) 7 7 6 butyl alcohol–pyridine–water, (B) 3 1 1 butyl alcohol–methanol–water, (C) 3 1 1 butyl alcohol–formic acid–water, and (D) ether. The paper chromatograms were detected with a solution of 1% ninhydrin in butyl alcohol saturated with water[15] for free amino groups, with chlorine–iodine–starch[16] for *N*-acetylated amino groups, with 0 05% Bromophenol Blue in water for free carboxylic groups, with silver nitrate in acetone–sodium hydroxide[17] for reducing groups, and with the Elson–Morgan reagent[18] for aminoaldehyde groups T l c plates were sprayed with 1·1 sulfuric acid–ethanol and heated to detect carbo-

hydrates D-Glucose was determined by the orcinol method[19], 2-amino-2-deoxy-D-glucose by the Elson–Morgan method[20], and phosphoric acid by the Fiske–Subbarow method[21] with ashing of the sample according to the Chen–Toribara–Warner method[22] Elementary analyses were performed at the Institute for Protein Research, Osaka University

Benzyl 2,3,4-tri-O-acetyl-β-D-glucopyranoside (**3**) — A mixture of benzyl β-D-glucopyranoside[23] (**1**, 3 g) and chlorotriphenylmethane (5 g) in dry pyridine (30 ml) was stirred for 2 h at 100°, followed by the addition of acetic anhydride (18 ml) The reaction mixture was kept overnight at room temperature, and then poured into ice–water, and stirred for 4 h The crude benzyl 2,3,4-tri-*O*-acetyl-6-*O*-trityl-β-D-glucopyranoside (**2**) was filtered off and detritylated without further purification by treatment with 60% acetic acid (39 ml) for 2–3 h at 100°. The reaction mixture was cooled and the triphenylmethanol filtered off After the filtrate had been concentrated, it was codistilled with toluene, and the residue was crystallized from 99% ethanol to give 2 4 g of **3** (54% yield from compound **1**), m p 128–130° Recrystallization from acetone–ether raised the m p to 132–134°, $[\alpha]_D^{20}$ −39° (*c* 2, chloroform), t l c. in ether R_F 0 74 (sulfuric acid)

Anal Calc for $C_{19}H_{24}O_9$, C, 57 57, H, 6 05 Found C, 57 48, H, 6 12

Benzyl 6-O-(3,4,6-tri-O-acetyl-2-deoxy-2-diphenylphosphorylamino-β-D-glucopyranosyl)-2,3,4-tri-O-acetyl-β-D-glucopyranoside (**7**) — A mixture of 3,4,6-tri-*O*-acetyl-2-deoxy-2-diphenylphosphorylamino-α-D-glucopyranosyl bromide[12] (**4**, 3 g), benzyl 2,3,4-tri-*O*-acetyl-β-D-glucopyranoside (**3**, 2 g), and mercuric cyanide (2 g) in dry benzene (30 ml) was stirred for 2 h at 90° and then filtered. The filtrate was diluted with chloroform (50 ml), washed successively twice with 10% sodium chloride and thrice with water, dried (sodium sulfate), and evaporated The residue was triturated with dry ether and crystallized from 2-propanol to give 2 8 g (61%) of needles, m p 200–201°, raised by recrystallization to 202–203°, $[\alpha]_D^{20}$ −20° (*c* 2, chloroform)

Anal Calc For $C_{43}H_{50}NO_{19}P$ C, 56 38, H, 5 51, N, 1 53, P, 3 38 Found C, 56 45, H, 5 70, N, 1 34, P, 3 51

Benzyl 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranoside (**10**) — *From compound* **7** A mixture of compound **7** (2.5 g) in acetone (125 ml) and M sodium hydroxide (125 ml) was kept for 4 h at room temperature and overnight in the cold After the acetone had been evaporated, the solution was treated with acetic acid until pH 8 was reached and *N*-acetylation was performed with acetic anhydride in keeping the pH at 7–8 by addition of sodium carbonate The reaction mixture was then passed successively through a column of Dowex 50 (X–8, H^+) and of Dowex 2 (CO_3^{2-}), and the deionized solution was evaporated Crystallization of the residue from methanol gave 0 5 g of **10** (38%), m p 237–238°, raised by recrystallization to 240–241°, $[\alpha]_D^{20}$ −50° (*c* 1 6, methanol), paper chromatography in solvent C. R_F 0 74 (chlorine–iodine–starch method)

Anal Calc for $C_{21}H_{31}NO_{11}\ H_2O$. C, 51 30, H, 6 78, N, 2 85 Found C, 51 82, H, 6.96, N, 2 91.

A solution of **10** (4 37 mg, 9 6 μmoles) in water (8 ml) was mixed with a per-

iodate solution prepared by dissolving 13 8 mg (60 μmoles) of potassium metaperiodate in water (4 ml) The reaction mixture was kept at 30° in the dark and the consumption of periodate was followed by measurement of the absorption at 290 nm[24] against a blank of water with a Hitachi Model Perkin–Elmer 139 spectrophotometer An absorbance of A_{290} 0 248 corresponded to 1 μmole of periodate per ml of solution, and formic acid was determined by titration of the reaction mixture with 0 1M sodium hydroxide A consumption of 3 07 moles of periodate with formation of 1 03 moles of formic acid per mole of disaccharide was determined

Benzyl 6-O-(2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranosyl)-2,3,4-tri-O-acetyl-β-D-glucopyranoside (**11**) — A mixture of 4,5-(3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyrano)-2-methyl-2-oxazoline[14] (**6**, reprecipitated from a dry ether solution by addition of petroleum ether twice to remove collidine but not further purified, 7 0 g), **3** (5,0 g) and *p*-toluenesulfonic acid (*ca* 300 mg to give a pH 3–4) in 1.1 dry benzene–nitromethane (85 ml) was heated for 3 h at 100–105° The reaction was followed by t l c (solvent D) The cooled reaction mixture was washed with water, evaporated, and the residue was triturated with ether The resulting precipitate was recrystallized from ethanol to give 4 2 g, (46%), showing a ratio of D-glucose to 2-amino-2-deoxy-D-glucose of 1 1 after hydrolysis; m p 210–211°, $[\alpha]_D^{20}$ −26° (*c* 1, chloroform), t l c in ether R_F 0 14 (sulfuric acid), n m r data δ 5 10 (doublet, $J_{1,2}$ 7–8 Hz, anomeric 2 H)

Anal Calc for $C_{33}H_{40}NO_{17}$ C, 54 61; H, 5 97, N, 1 93 Found C, 54 24, H, 6 00, N, 1.97

Benzyl 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-β-D-glucopyranoside (**10**) — *From compound* **11** A solution of compound **11** (2 1 g) in abs methanol (25 ml) was treated with 0 4M sodium methoxide in methanol (0 3 ml) for 10 min at room temperature, and the mixture was kept overnight in the cold The crystals were filtered off and recrystallized from methanol to give 1 2 g (85%), m p 237–238°, $[\alpha]_D^{20}$ −42° (*c* 2, methanol), showing no m p depression with **10** just described and an identical behavior on paper chromatograms The crystals lost 0 5 mole of water after being kept for 39 h at 100° *in vacuo*

Anal Calc for $C_{21}H_{31}NO_{11}$ 0 5 H_2O C, 52 57, H, 6 69, N, 2 90 Found. C, 52 36, H, 6 88, N, 2 82

Incubation of **10** with a 2-acetamido-2-deoxy-β-D-glucoside 2-acetamido-2-deoxy-β-D-glucohydrolase (E C 3 2 1 30) (*Aspergillus oryzae*)[25,26] at 37° for 4 h, resulted in 90% hydrolysis (by the Park–Johnson method[27])

Benzyl 6-O-(2-acetamido-2-deoxy-β-D-glucopyranosyluronic acid)-β-D-glucopyranoside (**8**) — In a three-necked flask, equipped with a stirrer and an inlet capillary, oxygen was introduced into a mixture of compound **10** (2 g, prepared from compound **11**) and freshly reduced Adams' platinum catalyst (1 g) in water (20 ml), under vigorous stirring, at 65–70°, the pH was kept at 6–8 by the occasional addition of a 0 5M sodium hydrogen carbonate solution The reaction was continued until the starting material had disappeared by examination on t l c After the mixture had been cooled, the catalyst was filtered off, the solution was deionized with Amberlite IR 120 (H^+)

and evaporated to give a syrup which was crystallized from water (1 1 g, 55%), m p 147–148°, $[\alpha]_D^{20}$ −60° (*c* 1, water); paper chromatography in solvent A R_F 0 64 (sulfuric acid) The crystals lost 1 8 moles of water after 39 h at 100° *in vacuo*

Anal Calc for $C_{21}H_{29}NO_{12}$ 0 5 H_2O C, 50 80; H, 6 09; N, 2 82 Found C, 50 80; H, 6 27; N, 2 68

6-O-(2-Acetamido-2-deoxy-β-D-glucopyranosyluronic acid)-D-glucose (**12**) — A solution of **8** (1 0 g) in water (100 ml) was hydrogenolyzed with hydrogen under normal pressure in the presence of 10% palladium-on-charcoal (1 3 g) for 2 h The catalyst was filtered off and the solution evaporated The residual syrup was dissolved in the minimum amount of water and ethanol was added The precipitate of impurities was filtered off, and the solution was evaporated to give a hygroscopic white powder (0 6 g, 80%) which showed only one spot on paper chromatography and t l c For elementary analysis, the precipitation from water with ethanol was repeated three times, m p 132–134°; $[\alpha]_D^{20}$ −4→−14° (at equilibrium, *c* 0 5, water), paper chromatography in solvent C R_F 0 21 (Bromophenol Blue and silver nitrate–sodium hydroxide); t l c in the same solvent R_F 0 27 (sulfuric acid) The crystals contained a small amount of ash (0 3%), and they lost 0 9 mole of water after 36 h at 60° *in vacuo*

Anal Calc for $C_{14}H_{23}NO_{12}$ C, 42 32, H, 5 83, N, 3 53 Found C, 42 10, H, 6 15, N, 3 17

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