Carbohydrate Research
Elsevier Publishing Company, Amsterdam
Printed in Belgium

NUCLEOSIDES

PART LXII. SYNTHETIC STUDIES ON NUCLEOSIDE ANTIBIOTICS. 2. SYNTHESES OF METHYL 4-AMINO-4-DEOXY-D-GLUCOSIDURONIC ACID DERIVATIVES RELATED TO THE CARBOHYDRATE MOIETY OF GOUGEROTIN*

M. P. KOTICK, R. S. KLEIN, K. A. WATANABE, AND J. J. FOX

Division of Biological Chemistry, Sloan-Kettering Institute For Cancer Research, Sloan-Kettering Division of Cornell University Medical College, New York, N. Y. 10021 (U.S.A.)

(Received April 24th, 1969)

ABSTRACT

The first synthesis of 4-amino-4-deoxy-D-hexuronic acid has been achieved. Tritylation of methyl 4-azido-4-deoxy-α-D-glucopyranoside (**1**) gave the 6-trityl ether (**2**) which was converted into its 2,3-dibenzoate (**3**) and deritylated to methyl 4-azido-2,3-di-*O*-benzoyl-α-D-glucopyranoside (**5**). Oxidation of **5** afforded the glucuronic acid derivative **6** which upon esterification to **7**, followed by reduction and benzoylation yielded methyl (methyl 4-benzamido-2,3-di-*O*-benzoyl-4-deoxy-α-D-glucopyranosid)uronate (**8**), the structure and conformation of which were firmly established by n.m.r. analysis. De-benzoylation of methyl (methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-D-glucopyranosid)uronate (**7**) with sodium methylate to **9**, followed by de-esterification and subsequent hydrogenation afforded crystalline methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**), the structure of which was established by esterification and benzoylation to **8**. *N*-Acetylation of **11** yielded methyl 4-acetamido-4-deoxy-α-D-glucopyranosiduronic acid (**14**) which was esterified and peracetylated to the methyl ester **16**. Derivative **16** was also obtained by hydrogenation and peracetylation of **9**. Epimerization at C-5 was not observed in the conversion of 7→11, which suggests that a total synthesis of the gougerotin-derived C-substance from the 4-amino-4-deoxy-hexuronic acid derivatives reported herein is feasible.

INTRODUCTION

The nucleoside antibiotics[1], gougerotin[2] and blasticidin S[3] contain 4-amino-4-deoxy-D-hexopyranuronic acid moieties. The structures of these antibiotics have recently been firmly established[4]. As part of our program[5] designed toward the total syntheses of these antibiotics, we have investigated the chemistry of aminodeoxyhexuronic acid derivatives. Although interesting biological properties have been reported for aminodeoxyhexuronic acid-containing polysaccharides[6-8], literature on the chemistry of the monomers is sparse. To our best knowledge, only

*This investigation was supported in part by funds from the National Cancer Institute, National Institutes of Health, U. S. Public Health Service (Grant No. CA-08748).

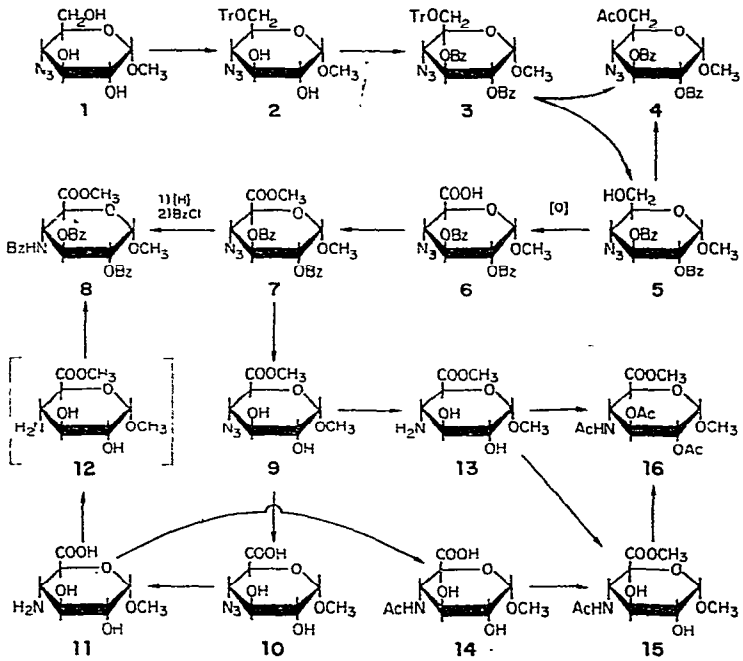
three examples of aminodeoxyhexuronic acids had been reported, namely, the 2-amino-2-deoxyuronic acid derivatives of the *gluco*[6], *galacto*[7], and *manno*[8] configuration. Of these, the former two were prepared[9,10] by catalytic oxidation of their corresponding hexosamine derivatives prior to their discovery in Nature. More recently, a 5-amino-5-deoxy-D-allofuranuronic acid has been discovered as a component of the nucleoside antibiotics, the polyoxins[11a], and 3-amino-3-deoxy-D-allouronic acid[11b] was prepared chemically. No other aminodeoxyhexuronic acid has either been synthesized chemically or (except for the above-mentioned nucleoside antibiotics) been found in Nature. This paper deals with the first chemical synthesis of a 4-amino-4-deoxyhexuronic acid, namely, 4-amino-4-deoxy-D-glucuronic acid from methyl α-D-galactopyranoside.

Conversion of methyl α-D-galactopyranoside[12] to methyl 4-azido-4-deoxy-α-D-glucopyranoside (**1**) was achieved in 4 steps, in improved yields, using a modification[5] of the procedure of Reist *et al.*[13] in which hexamethylphosphoric triamide was employed as solvent for nucleophilic displacement of the 4-mesyloxy function by an azide group. Attempts to oxidize compound **1** with either platinum-on-carbon[14] or platinum black[9,10] were unsuccessful.

Tritylation of **1** gave a yield of about 70% of the crystalline 6-trityl ether **2** which was benzoylated in high yield to **3**. On a preparative scale, **3** was isolated in 70% overall yield from **1** without isolation of the intermediate **2**. Detritylation of **3** in 80% acetic acid afforded **5** and a by-product (6%). This by-product was characterized as the 6-acetate **4** by the identity of its n.m.r. and i.r. spectra with those of the product obtained by direct acetylation of **5**. All attempts to crystallize chromatographically-homogeneous **5** or its 6-acetate **4** were unsuccessful. Oxidation of **5** with potassium permanganate in a mixture of acetic acid and acetone[15], followed by esterification of sirupy **6** with diazomethane gave the methyl ester **7** as a chromatographically-pure syrup in 55–60% overall yield from **5**.

Hydrogenation of ester **7** in the presence of palladium-on-charcoal in methanol afforded two products in approximately equal amounts, as judged by t.l.c. Benzoylation of this mixture gave only a single crystalline product (**8**) and trace amounts of other products which were not further characterized. These observations are best explained by partial $O \rightarrow N$ benzoyl migration during the reduction of **7**. Addition of triethylamine to the reaction mixture prior to the hydrogenation of **7** prevented this transbenzoylation. The n.m.r. spectrum (in chloroform-*d*) of **8** showed two methoxy resonances at δ 3.48 (aglycon) and δ 3.79 (ester). The aromatic proton signals (between δ 7.2 and 8.2) integrated for 15 protons corresponding to three benzoyl groups. On addition of deuterium oxide, the lower field NH doublet (δ 6.78; $J_{4,NH} \cong 9.5$ Hz) disappeared and the higher field H-4 quartet (δ 4.87) collapsed to a triplet ($J_{3,4} \cong 9.5$). Signals for H-2, H-3, and H-5 were observed at δ 5.48 (quartet, $J_{1,2} \cong 3.5$; $J_{2,3} \cong 9.5$), δ 6.03 (triplet, $J_{2,3} \cong J_{3,4} \cong 9.5$), and δ 4.47 (doublet, $J_{4,5} \cong 10.0$), respectively. These data establish the axial orientation for H-2, H-3, H-4, and H-5, and the equatorial orientation for the anomeric proton consistent with the D-*gluco* configuration in the *C1* conformation for **8**.

Methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**) was synthesized from **7** in good overall yield. Treatment of **7** with sodium methoxide in methanol afforded the crystalline, debenzoylated product (**9**) which was de-esterified with aqueous alkali to give **10** in high yield. Catalytic reduction of the azido derivative **10** gave methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**), as the crystalline monohydrate, in 83% overall yield from **9**. The structure of **11** was established by esterification of the latter with diazomethane to give **12** (not isolated), followed by benzoylation to a crystalline product which was identical with **8**.



Catalytic hydrogenation of the azido derivative **9** in presence of palladium-on-charcoal gave the amino sugar **13** which, without isolation, was *N*-acetylated to give the 4-acetamido derivative **15**. Acetylation of **15** gave the peracetylated derivative **16** which was isolated in crystalline form only after passage through a Silica Gel G column. Compound **16** was also obtained by direct acetylation of **13**.

Treatment of the 4-amino-4-deoxyuronic acid derivative **11** with aqueous alkali and acetic anhydride yielded methyl 4-acetamido-4-deoxy-α-D-glucopyranosiduronic acid (**14**) in crystalline form. Esterification of **14** with diazomethane yielded **15**, identical with that obtained by N-acetylation of **13**.

It should be noted, that compounds **9** and **11** were the *sole* products obtained, even though strongly basic conditions were employed during these reactions, that is, no isomerization at C-5 to the β-L-*ido* configuration was observed. Such an epimerization might have been expected on the basis of a reported[16] epimerization of alduronic acids at C-5 which occurred when the acids were heated in aqueous solution. Perry and Hulyalkar[17] later failed to observe this epimerization by g.l.c. A simple

explanation for the apparent absence of C-5 epimerization in our case may be rationalized on the basis that the α-D-*gluco* configuration for the uronic acid derivatives **9**→**11** is thermodynamically more stable than the corresponding β-L-*ido* configuration.

The data herein suggest that it should be possible to synthesize the gougerotin-derived C-substance [1-(4-amino-4-deoxy-β-D-glucopyranosyluronic acid)cytosine] from some of the glucuronic acid derivatives described in this report. Studies directed toward the total synthesis of gougerotin are in progress.

EXPERIMENTAL

Melting points were determined with a Hoover-Thomas capillary apparatus and are corrected. Thin-layer chromatography (t.l.c.) was performed on microscope slides coated with Silica Gel GF 254 (Merck), and preparative layer-chromatography (p.l.c.) on 2 mm layer plates of Silica Gel PF 254 (Merck) in the indicated solvent systems. Compounds were detected with u.v. light and/or by spraying with 20% (v/v) H_2SO_4 in ethanol, followed by heating to 130°. All evaporations were carried out *in vacuo*.

N.m.r. spectra were obtained on a Varian A-60 instrument, using tetramethylsilane as an internal standard: Chemical shifts are reported in p.p.m. (δ), and signals are quoted as s (singlet), d (doublet), t (triplet), q (quartet), or m (complex multiplet). Coupling constants are first order. I.r. spectra were determined on a Perkin-Elmer model 221 spectrophotometer. Elemental analyses were performed by Spang Microanalytical Laboratory, Ann Arbor, Michigan.

Methyl 4-azido-4-deoxy-α-D-glucopyranoside (**1**). — Methyl 2,3,6-tri-*O*-benzoyl-4-*O*-mesyl-α-D-galactopyranoside[13] (104 g, 0.178 mole) was dissolved in hexamethylphosphoric triamide (250 ml) and treated with sodium azide (42 g, 0.645 mole) at 80° with stirring. After 16 h, the reaction was diluted with an ice–water mixture (4 l). The precipitate was filtered off, washed with water, and dissolved in methylene chloride, and the solution was dried with magnesium sulfate. The solvent was evaporated, and traces of methylene chloride were removed by azeotropic distillation with methanol. The colorless residue was dissolved in methanol (700 ml), M methanolic sodium methoxide (20 ml) was added, and the mixture was kept overnight at room temperature.

Dowex 50-W (H^+, previously washed with methanol) was added to neutralize the reaction. The resin was filtered off, and the filtrate was evaporated to dryness. The residue was partitioned between water and ether. The aqueous layer was separated and evaporated to a syrup which was then dissolved in hot acetonitrile (*ca* 100 ml). The crystals were filtered off (29.9 g, m.p. 63–64°), and dried over phosphorus pentoxide, for 24 h at 40°, under vacuum to yield anhydrous material (27.7 g, m.p. 107.5–110°; lit.[13], m.p. 108–109°). From the mother liquor, an additional amount of crystalline **1** (6.8 g) was obtained for an 80% overall yield of **1**.

Methyl 4-azido-4-deoxy-6-O-trityl-α-D-glucopyranoside (**2**). — A solution of **1** (8.8 g) and chlorotriphenylmethane (14.0 g) in dry pyridine (35 ml) was heated on the

steam bath for 1 h, and then stirred overnight at room temperature. The solution was diluted with chloroform and washed twice with water, sodium hydrogen carbonate solution, and water. After drying (magnesium sulfate), the chloroform solution was evaporated to a thick syrup, which was crystallized from benzene to give **2** in three crops (12.7 g, 69%), sintering at 100°, m.p. 102–104°. Recrystallization from benzene–petroleum ether (30–60°) gave an analytical sample, sintering at 100°, m.p. 102–105°, $[\alpha]_D^{26}$ +87° (*c* 0.9, chloroform); λ_{max}^{KBr} 2.7 (OH), 4.7 (N_3), and 9.6 μm (C–O–C); n.m.r. in chloroform-*d*: δ 2.75 (OH, s, 2), δ 3.46 (OMe, s, 3), δ 3.24–3.81 (ring and CH_2 protons, 6), δ 4.91 (H-1, d, 1, $J_{1,2}$ ~3 c.p.s.), and δ 7.27–7.68 (trityl, 15).

Anal. Calc. for $C_{26}H_{27}N_3O_5$: C, 67.66; H, 5.90; N, 9.10. Found: C, 67.59; H, 5.93; N, 9.06.

*Methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-6-*O*-trityl-α-*D*-glucopyranoside* (**3**). *From* **2**. — A solution of **2** (5.00 g) in pyridine (25 ml) was cooled in an ice-bath. Benzoyl chloride (4.5 ml) was added dropwise, and, after stirring for 2 h at room temperature, the pyridine solution was diluted with chloroform and treated as in the preparation of **2**. Concentration of the chloroform solution and removal of the residual pyridine by azeotrope distillation with water gave a syrup which crystallized on addition of ethanol. The crystalline residue was recrystallized from ethanol to give 4.74 g of **3**, m.p. 163–165°. Concentration of the mother liquor yielded an additional 1.15 g of **3** for an overall yield of 81%. A sample for analysis was recrystallized from ethanol, to give cube-like crystals, m.p. 164–165°, $[\alpha]_D^{26}$ +114° (*c* 1.0, chloroform); λ_{max}^{KBr} 4.7 (N_3), and 5.76 μm (ester carbonyl).

Anal. Calc. for $C_{40}H_{35}N_3O_7$: C, 71.74; H, 5.27; N, 6.27. Found: C, 71.67; H, 5.31; N, 6.29.

From **1**. — Compound **1** (14.5 g, 0.066 mole) and chlorotriphenylmethane (23.6 g, 0.088 mole) were dissolved in dry pyridine (100 ml). The mixture was heated on a steam-bath for 3 h, then stirred overnight at room temperature. To the reaction mixture was added benzoyl chloride (25 ml), and, after 2 h, the mixture was poured into a stirred ice–water mixture. The precipitate was dissolved in chloroform, and washed with water, aqueous sodium hydrogen carbonate, and water. Evaporation of the chloroform extract, followed by azeotropic removal of pyridine with water and then with ethanol gave a crystalline residue. Recrystallization of the residue from acetone–ethanol gave **3** (32.2 g, 74%, m.p. 160–162°) of sufficient purity for the next step.

*Methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-*D*-glucopyranoside* (**5**). — A suspension of **3** (3.35 g) in 80% aqueous acetic acid (30 ml) was heated at reflux for 30 min, and the resultant clear solution was kept in an ice-bath for 1 h. After removal of the triphenylcarbinol by filtration, the filtrate was evaporated to a syrup which was chromatographed[18] on Silica Gel G (150 g) with 5:1 benzene–ethyl acetate as the eluent. Eluted first from the column was the 6-*O*-acetyl derivative **4** (0.15 g, 6%) which was obtained as a clear syrup, $[\alpha]_D^{26}$ +158° (*c* 1.0, chloroform); λ_{max}^{film} 4.7 (N_3), 5.75 (ester carbonyl), and 6.9 μm (aromatic); n.m.r. in chloroform-*d*: δ 2.16 (Ac, s, 3) δ 3.42 (OMe, s, 3), δ 3.82–4.00 (H-4, H-5, m, 2), δ 4.46 (H-6, H-6, d, 2), δ 5.19 (H-1, d, 1), δ 5.25 (H-2, q, 1), δ 6.00 (H-3, t, 1), and δ 7.12–8.12 (aromatic, 10).

Continued elution of the column with the same solvent pair yielded the major product **5** (1.9 g, 91%) as a clear syrup which resisted all attempts at crystallization, $[\alpha]_D^{26}$ +182° (*c* 1.7, chloroform), λ_{max}^{film} 2.45 (OH), 4.7 (N_3), and 5.75 μm (ester carbonyl); n.m.r. in chloroform-*d*: δ 2.50 (OH, s, 1 proton exchangeable with D_2O), δ 3.40 (OMe, s, 3), δ 3.80–4.03 (H-4, H-5, m, 2), δ 5.12 (H-2, q, 1), δ 5.20 (H-1, t, 1), δ 5.99 (H-3, t, 1), and δ 7.17–8.10 (aromatic, 10).

Acetylation of **5** with acetic anhydride in pyridine gave the 6-*O*-acetyl derivative **4** which was identical in all respects (t.l.c., i.r., and n.m.r.) with the material previously described.

*Methyl (methyl 4-azido-2,3-di-*O*-benzoyl-4-deoxy-α-*D*-glucopyranosid)uronate* (**7**). — Compound **5** (1.90 g) was dissolved in a mixture of acetic acid–acetone (1:1, 40 ml) and powdered potassium permanganate (1.00 g) was added with stirring over a period of 10 min. After stirring of the reaction mixture for 1 h at room temperature, additional potassium permanganate (0.50 g) was added, followed by another charge (0.50 g) after 1 h. The dark suspension was stirred overnight at room temperature, after which the excess permanganate was decomposed with sulfur dioxide. The resultant gel was evaporated to a volume of *ca* 10 ml to which was added water (150 ml). The aqueous suspension was extracted thrice with methylene chloride (100 ml), and the combined methylene chloride extracts were washed four times with water (100 ml). After drying (magnesium sulfate), the methylene chloride solution was evaporated to a clear syrup which was dissolved in methanol (25 ml), cooled, and treated with an excess of diazomethane. The solution was evaporated to dryness and chromatographed[18] on Silica Gel G (100 g) with benzene–ethyl acetate (15:1) as the eluent.

Fractions eluted just prior to the methyl ester **7** were contaminated by a substance moving slightly faster on t.l.c., which was not apparent on thin-layer plates and tended to elute with the major product from the column, thereby lowering the yield of chromatographically-homogeneous ester **7** to about 55–60%. Fractions contaminated with the faster-moving component were partially separated in another experiment. The minor product did not show an azide band in the i.r. spectrum, and was not investigated further. Homogeneous **7** was obtained as a clear syrup, $[\alpha]_D^{26}$ +152° (*c* 1.0, chloroform); λ_{max}^{film} 4.7 (N_3), 5.75 (ester carbonyl), and 7.9 μm (ester C–O–C).

*Methyl (methyl 4-benzamido-2,3-di-*O*-benzoyl-4-deoxy-α-*D*-glucopyranosid)-uronate* (**8**). — A solution of **7** (0.583 g) in methanol (25 ml) was hydrogenated at atmospheric pressure and room temperature in the presence of 10% palladium-on-carbon (75 mg). T.l.c. (9:1 benzene–ethyl acetate) at 1.5 h indicated the presence of starting material, in addition to two slower-moving components in approximately equal amounts. Hydrogenation was continued for an additional 45 min, at which time no more starting material could be detected by t.l.c. Filtration of the catalyst, followed by evaporation of the filtrate gave a syrup to which pyridine was added, and then benzoyl chloride (1 ml). After being stirred for 2 h at room temperature, the solution was poured into ice–water and extracted with chloroform, and the chloroform solution was washed with water, sodium hydrogen carbonate solution, and

water, and dried (magnesium sulfate). After filtration of the salts, the chloroform solution was evaporated to dryness, and the pyridine was removed by azeotropic distillation with water, and then with ethanol to give 560 mg of a partly crystalline residue. T.l.c. (5:1 benzene–ethyl acetate) indicated the presence of a single major spot, along with traces of faster-migrating impurities. Crystallization of this residue from ethanol gave 363 mg (55%), of crystalline tribenzoate **8**, m.p. 216–217°. A sample for analysis was prepared by recrystallization of **8** from ethanol to afford needles, m.p. 217–218°; $[\alpha]_D^{26}$ +101° (*c* 1.1, chloroform).

Anal. Calc. for $C_{29}H_{27}NO_9$: C, 65.28; H, 5.10; N, 2.63. Found: C, 65.18; H, 5.17; N, 2.54.

When the hydrogenation of **7** was conducted in the presence of triethylamine, the time required for reduction was reduced to ~1.5 h, as indicated by t.l.c. In addition, only a single spot was noted prior to benzoylation. T.l.c. of the benzoylation mixture prior to isolation of crystalline **8** indicated the presence of a single spot, unaccompanied by traces of impurities.

Methyl (methyl 4-azido-4-deoxy-α-D-glucopyranosid)uronate (**9**). — To **7** (6.90 g) in methanol (75 ml) was added a solution of sodium (45 mg) in methanol (20 ml), and the mixture was stirred for 3 h at room temperature. Dowex 50-W resin (H^+, previously washed with methanol) was added, the neutral solution was filtered, and the resin washed with methanol. The combined filtrates were evaporated to a thick syrup which was partitioned between water and ether. The aqueous solution was evaporated to a clear syrup which was dried by several azeotropic distillations with benzene, and finally crystallized from benzene–petroleum ether (30–60°) to give 3.16 g of **9** (85%), sintering at 79°, m.p. 80–81°. A sample for analysis was recrystallized from the same solvent pair to give fine needles, sintering at 80°, m.p. 82–83°, $[\alpha]_D^{26}$ +166° (*c* 1.0, chloroform).

Anal. Calc. for $C_8H_{13}N_3O_6$: C, 38.87; H, 5.30; N, 17.00. Found: C, 39.71; H, 5.14; N, 16.81.

Methyl 4-amino-4-deoxy-α-D-glucopyranosiduronic acid (**11**). — To a solution of **9** (1.00 g) in water (5 ml) was added dropwise M sodium hydroxide (4 ml). Dowex 50-W (H^+) was added and the acidic solution filtered from the resin. The resin was washed with water (3 × 5 ml), the combined filtrates were treated with triethylamine (6 drops), and the mixture was hydrogenated in presence of 10% palladium-on-carbon (95 mg) for 1.5 h. After removal of the catalyst by filtration, the solution was evaporated to a crystalline residue which was recrystallized from water–ethanol to give 0.76 g of **11** (83%), as the monohydrate which did not melt below 250°. Recrystallization from water–ethanol, followed by drying in presence of phosphorus pentoxide for 18 h at 100° gave an analytical sample of the monohydrate, $[\alpha]_D^{26}$ +95° (*c* 1.1, water).

Anal. Calc. for $C_7H_{13}NO_6 \cdot H_2O$: C, 37.35; H, 6.72; N, 6.22. Found: C, 37.46; H, 6.73; N, 6.28.

A solution of **11** (200 mg) in methanol (25 ml) was treated with an excess of

diazomethane, and subsequently benzoylated in pyridine in the usual manner to give crystalline **8**, identical in all respects to the material obtained previously.

Methyl 4-acetamido-4-deoxy-α-D-glucopyranosiduronic acid (**14**). — To a solution of **11** (414 mg) in water (5 ml), cooled in an ice-bath, was added M sodium hydroxide (3 ml) followed by acetic anhydride (0.3 ml). Rapid stirring and cooling were continued for 15 min, Dowex 50-W (H^+) was added, and the stirring continued for 5 min. After filtration, the solution was evaporated to give a crystalline residue which was recrystallized from ethanol to give 382 mg of **14** (71%) as the monoethanolate as determined by the n.m.r. spectrum in D_2O, sintering at ~125°, m.p. 133–137° (with effervescence), $[\alpha]_D^{26}$ +117° (*c* 0.5, water). A sample for analysis was dried *in vacuo* for 24 h at 100°.

Anal. Calc. for $C_9H_{15}NO_7$: C, 43.35; H, 6.07; N, 5.62. Found: C, 43.55; H, 6.40; N, 5.26.

Methyl (methyl 4-acetamido-4-deoxy-α-D-glucopyranosid)uronic acid (**15**). — A solution of **9** (500 mg) in methanol (25 ml) containing triethylamine (5 drops) was hydrogenated in the presence of 10% palladium-on-carbon (60 mg) for 1 h. After removal of the catalyst by filtration, acetic anhydride (0.5 ml) was added to the filtrate, and the clear solution was stirred for 1 h at room temperature. Evaporation gave **15** which was purified by p.l.c. with 6:1 chloroform–methanol as the developing phase to yield a foam, $[\alpha]_D^{26}$ +92° (*c* 0.79, chloroform), which crystallized on prolonged standing at room temperature. Recrystallization from ethanol–petroleum ether gave fine needles, m.p. 159–160°.

Anal. Calc. for $C_{10}H_{17}NO_7$: C, 45.64; H, 6.45; N, 5.27. Found: C, 45.70; H, 6.47; N, 5.24.

Methyl (methyl 4-acetamido-2,3-di-O-acetyl-4-deoxy-α-D-glucopyranosid)uronate (**16**). — A solution of **14** (200 mg) dissolved in methanol (15 ml) was treated with an excess of diazomethane at 0°. After evaporation of the solution to dryness, the residual syrup was dissolved in pyridine, and acetic anhydride (1 ml) was added. The solution was stirred overnight at room temperature, and then it was poured into water and extracted with chloroform. After the chloroform extract had been washed in the usual manner and evaporated, compound **14** (193 mg) was obtained as a foam in 82% yield. A sample for analysis was purified by p.l.c. with 4:1 ethyl acetate–acetone as the developing solvent; $[\alpha]_D^{26}$ +107° (*c* 1.3, chloroform); n.m.r. in pyridine-d_5: δ 1.94, 2.02, 2.05 (Ac), δ 3.26 (aglycon OMe, s, 3), δ 3.82 (ester OMe, s, 3), δ 4.40–5.50 (H-4, H-5, m, 2), δ 5.20 (H-1, d, 1), δ 5.33 (H-2, q, 1, $J_{1,2}$ ~3.5, $J_{2,3}$ ~9.5 c.p.s.), δ 5.98 (H-3, t, 1, $J_{3,4}$ ~10 c.p.s.), and δ 9.15 (NH, d, 1, $J_{4,NH}$ ~9.5 c.p.s.).

Anal. Calc. for $C_{14}H_{21}NO_9$: C, 48.41; H, 6.10; N, 4.03. Found: C, 48.29; H, 6.06; N, 3.84.

The n.m.r. spectrum (in pyridine-d_5) and t.l.c. of this compound were identical to those of the compound obtained by reduction of **9** in methanol containing triethylamine, followed by acetylation in pyridine. After the reaction mixture had been processed in the usual manner, the product was chromatographed on silica gel G

with 4:1 ethyl acetate–acetone as the eluting solvent. In this case, complete removal of the solvents gave **16** in a crystalline form, but it could not be recrystallized.

REFERENCES

1 J. J. Fox, K. A. Watanabe, and A. Bloch, *Progr. Nucleic Acid Res. Mol. Biol.*, 5 (1966) 251.
2 T. Kanzaki, E. Higashide, H. Yamamoto, M. Shibata, K. Nakazawa, H. Iwasaki, T. Takewaka, and A. Miyake, *J. Antibiot.* (Tokyo), *Ser. A*, 15 (1962) 93.
3 S. Takeuchi, K. Hirayama, K. Ueda, H. Sakai, and H. Yonehara, *J. Antibiot.* (Tokyo), *Ser. A*, 11 (1958) 1.
4 J. J. Fox, Y. Kuwada, K. A. Watanabe, T. Ueda, and E. B. Whipple, *Antimicrob. Agents Chemother.*, (1964) 518; J. J. Fox, Y. Kuwada, and K. A. Watanabe, *Tetrahedron Lett.*, (1968) 6029; N. Otake, S. Takeuchi, T. Endo, and H. Yonehara, *Tetrahedron Lett.*, (1965) 1404 and 1411; J. J. Fox and K. A. Watanabe, *Tetrahedron Lett.*, (1966) 897; H. Yonehara and N. Otake, *Tetrahedron Lett.*, (1966) 3785.
5 K. A. Watanabe, M. P. Kotick and J. J. Fox, *Chem. Pharm. Bull.* (Tokyo), 17 (1969) 416.
6 A. R. Williamson and S. Zammenhof, *J. Biol. Chem.*, 238 (1963) 2255; S. Hanessian and T. H. Haskell, *J. Biol. Chem.*, 239 (1964) 2758, and references therein.
7 K. Heyns, G. Kiessling, W. Lindenberg, H. Paulsen, and M. E. Webster, *Chem. Ber.*, 92 (1959) 2435.
8 H. R. Perkins, *Biochem. J.*, 86 (1963) 475.
9 K. Heyns and H. Paulsen, *Chem. Ber.*, 88 (1955) 188.
10 K. Heyns and M. Beck, *Chem. Ber.*, 90 (1957) 2443.
11 (*a*) K. Isono and S. Suzuki, *Tetrahedron Lett.*, (1968) 203; (*b*) A. Tsuji, T. Kinoshita, and M. Maeda, *Chem. Pharm. Bull.* (Tokyo), 16 (1968) 539.
12 J. L. Frahn and J. A. Mills, *Aust. J. Chem.*, 18 (1965) 1303.
13 E. J. Reist, R. R. Spencer, D. F. Calkins, B. R. Baker, and L. Goodman, *J. Org. Chem.*, 30 (1965) 2321.
14 C. L. Mehltretter, B. H. Alexander, R. L. Mellies, and C. E. Rist, *J. Amer. Chem. Soc.*, 73 (1951) 2424.
15 M. Stacey, *J. Chem. Soc.*, (1939) 1529.
16 F. G. Fischer and H. Schmidt, *Chem. Ber.*, 92 (1959) 2184.
17 M. B. Perry and R. K. Hulyalkar, *Can. J. Biochem.*, 43 (1965) 573.
18 B. J. Hunt and W. Rigby, *Chem. Ind.* (London), (1967) 1868.