m.p. 135–136°, after recrystallization from 50% (v./v.) aqueous ethanol.

Hydrolysis of the Monohydrate of the Mono-*O*-benzylidenedi-*O*-methyl-*aldehydo*-pentose to Form 3,5-Di-*O*-methyl-L-xylose.—The mono-*O*-benzylidenedi-*O*-methyl-*aldehydo*-pentose monohydrate (80 mg.) was suspended in 2 ml. of aqueous acetic acid (1:1 by volume) contained in a flask fitted with a reflux condenser and the mixture was heated in a boiling water bath for 4 hr. At the end of this time no solid particles could be seen; the solution, however, had a cloudy appearance and had an odor of benzaldehyde. The solution was cooled to room temperature, diluted with 5 ml. of water, and extracted four times with 10-ml. portions of ether to remove the liberated benzaldehyde. The aqueous solution was then evaporated to dryness under reduced pressure at 40–50°. The resulting sirup, when dried under high vacuum over P_2O_5 overnight, weighed 50 mg. It analyzed for a di-*O*-methylpentose and had an optical rotation of $[\alpha]^{20}D$ −11° (*c* 1, $CHCl_3$). The optical rotation is of the same magnitude but opposite in sign to that reported for 3,5-di-*O*-methyl-D-xylose.[3,4]

Anal. Calcd. for $C_7H_{14}O_5$: C, 47.18; H, 7.92; OMe, 34.84. Found: C, 47.21; H, 7.85; OMe, 34.02.

Preparation of the *p*-Bromophenylosazone of the 3,5-Di-*O*-methyl-L-xylose.—The 3,5-di-*O*-methyl-L-xylose (36 mg.) was treated after the manner of Levene and Raymond[4] with 120 mg. of *p*-bromophenylhydrazine in 2 ml. of aqueous acetic acid (1:1 v./v.). The solution was heated in the boiling water bath for 0.5 hr., then cooled. The thick oil that had separated was recovered by decanting off the aqueous phase. The oil was then crystallized from aqueous methanol, m.p. 107°, $[\alpha]^{20}D$ +30° (*c* 1, C_5H_5N–EtOH, 2:3 v./v.) after 12 hr.

Anal. Calcd. for $C_{19}H_{22}Br_2N_4O_3$: N, 10.89; OMe, 12.07. Found: N, 10.70; OMe, 11.81.

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An Improved, Stereoselective Synthesis of 2-Amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (Muramic Acid)[1]

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Reaction of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside with an excess of DL-chloropropionic acid in the presence of sodium hydride gave 76% of crystalline benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (muramic acid derivative) and 1–3% of the 3-*O*-(L-1-carboxyethyl) derivative (isomuramic acid derivative). Removal of the benzylidene group by weak acid hydrolysis gave benzyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside, which forms easily a lactone at C-4. Hydrogenolysis of the benzyl group of the free acid gave 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (*N*-acetylmuramic acid) in 92% yield, from which 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (muramic acid) could be obtained in 70% yield.

2-Amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose or muramic acid (IX) has been found to be a constituent of numerous cell walls of microorganisms.[3] It was first isolated in 1956 by Strange and Dark[4] and it was first synthesized by Strange and Kent.[5] This synthesis and the following ones are based on the formation of an ether link between D-lactic acid and the hydroxyl group at C-3 of 2-amino-2-deoxy-D-glucose (D-glucosamine). Most syntheses start from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside; after formation of the sodium salt at C-3, this compound is condensed with various α-halogenopropionic acid derivatives. The use of racemic α-halogenopropionic acid derivatives leads to the formation of the D- and L-3-ethers (muramic and isomuramic acid derivatives) and necessitates a chromatographic separation which is carried out either at the final stage on the mixture of muramic and isomuramic acid,[5] or on the mixture of the methyl 2-acetamido-2-deoxyglycosides.[6] Both methods give low yields since many reactions are carried out before final purification. By the use of an L-α-chloropropionic acid derivative, formation of the isomuramic acid derivative is avoided, and improved yields are given. The preparation of the required reagent is, however, cumbersome.[7,8] The chromatographic separation of the isomers can also be sidestepped by use of the alloxazine derivative of 2-benzamido-2-deoxy-5,6-*O*-isopropylidene-D-glucofuranose as starting material and separating the D- and L-isomers by fractional crystallization.[9,10]

Since both the starting material and the condensing agent possess an asymmetric structure, conditions in which formation of one of the isomers would be preferred were investigated. Therefore a careful separation of the two resulting isomers was made by chromatography at the stage of the benzylidene methyl ester derivatives. Variations of the nature of the aglycone showed the influence of the group attached at C-1 on the stereoselectivity of the reaction at C-3.[2] Thus, when the benzyl α-D-glycoside was used the reaction was practically stereospecific giving in high yield the known benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-(methyl D-1-ethylcarboxylate)-α-D-glucopyranoside (IV).[11] The fact that practically only one of the two isomers of DL-chloropropionic acid takes part in the reaction presents a stereoselectivity of a degree which

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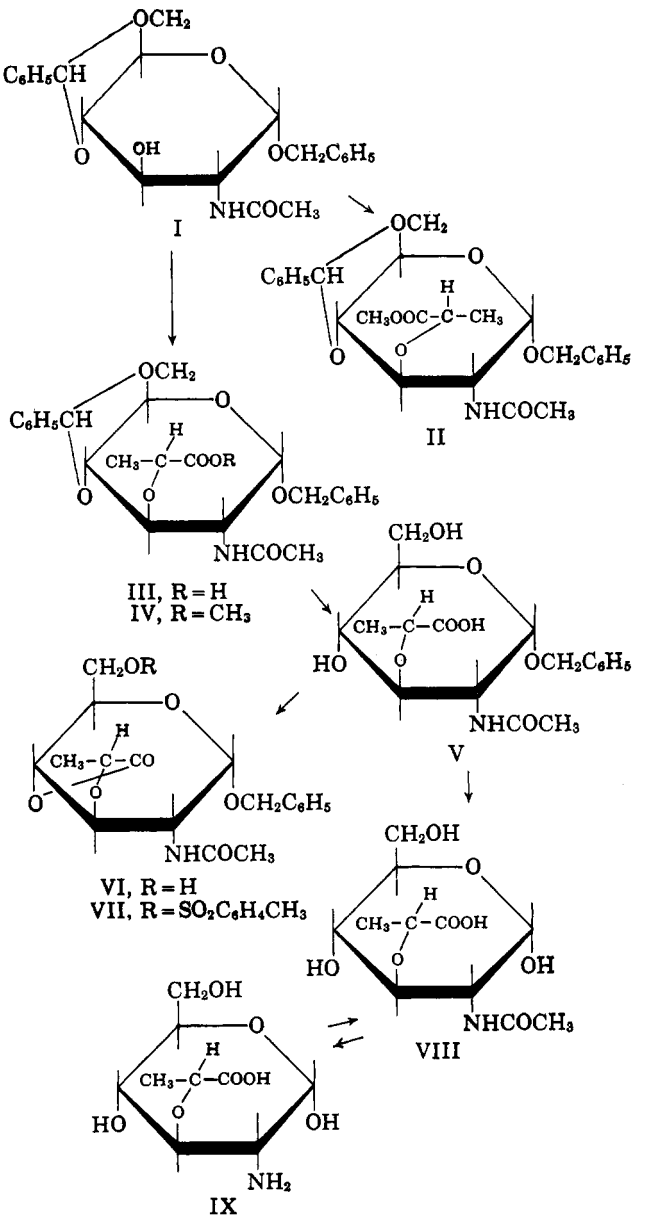

has not yet, to our knowledge, been encountered.[12] It is of interest that the isomer obtained in this reaction is the same as the one found in nature.

Additional advantage of the benzyl α-D-glycoside resides in the possibility to remove the aglycone under very mild conditions by catalytic hydrogenation. Thus, 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (*N*-acetylmuramic acid) (VIII)[11] is directly obtained from IV. The mild conditions used in the removal of the aglycone are of special importance for the preparation of *O*-acetyl derivatives of VIII, which will be described in a forthcoming publication.

Condensation of DL-chloropropionic acid with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (I)[13] in the presence of sodium hydride gave a product partially soluble in methanol. After recrystallization from this solvent benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (III) was obtained in 76% yield. Esterification with diazomethane of the part insoluble in methanol followed by chromatography gave benzyl 2-acetamido 4,6-*O*-benzylidene-2-deoxy-3-*O*-(methyl L-1-ethylcarboxylate)-α-D-glucopyranoside (II) in 1–3% yield.

The benzylidene group of the free acid III can be removed directly by hydrolysis with dilute acetic acid. The resulting benzyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (V), obtained in 65% yield, forms readily a lactone VI with the hydroxyl at C-4. The lactone VI is opened by dilute sodium hydroxide treatment before crystallization of V. Evidence for the formation of the lactone VI with C-4 and not C-6 was obtained by preparation of the 6-tosylate VII of VI. The tosyl group was displaced almost quantitatively by treatment with sodium iodide in acetone.[14]

Catalytic hydrogenation of V[11] gives a 92% yield of crystalline 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (*N*-acetylmuramic acid) (VIII) which is thus obtained in four intermediate steps from 2-acetamido-2-deoxy-D-glucose (*N*-acetylglucosamine) in an over-all yield of 13%. Mild acid hydrolysis of the *N*-acetyl group of VIII gives crystalline 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (muramic acid) (IX) in 70% yield. By *N*-acetylation of VIII using 2 moles of *N*-acetoxyphthalimide 70% of crystalline VIII was obtained. The same reaction carried out with 1 mole of *N*-acetoxyphthalimide as described by Carroll[15] gives a mixture of VIII and IX.

Experimental

Melting points were taken on a hot stage, equipped with a microscope, and correspond to "corrected melting point." Rotations were determined in semimicro- or micro- (for amounts smaller than 3 mg.) tubes with lengths of 100 or 200 mm., using a Rudolph photoelectric polarimeter attachment, Model 200; the chloroform used was A.R. grade and contained approximately 0.75% of ethanol. Chromatography was performed as follows: "Silica Gel Davison," from the Davison Co., Baltimore 3, Md. (grade 950, 60–200 mesh), was used without pretreatment. When deactivation by contact with moist air occurred, reactivation was obtained by heating to 170–200° (manufacturer's instructions). The sequence of eluents was hexane, benzene or ethylene dichloride, ether, ethyl acetate, acetone, and methanol individually or in binary mixtures. Mixtures were chromatographed using 50–100 times their weights of adsorbent. The amount of eluent in each fraction (ml.) was about 100 times the weight (g.) of the applied mixture. The ratio of diameter to length of the column was 1 to 20. Evaporations were carried out *in vacuo*, with an outside bath temperature kept below 45°. Amounts of volatile solvent smaller than 20 ml. were evaporated under a stream of dry nitrogen. The microanalyses were done by Dr. M. Manser, Zurich, Switzerland.

Condensation of Benzyl 2-Acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (I) with DL-α-Chloropropionic Acid.—To a solution of 3.2 g. of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (I, m.p. 257°, $[\alpha]^{26}D$ +121°, *c* 0.54 in pyridine)[13] in 200 ml. of dioxane was added 0.9 g. of sodium hydride, and the mixture was stirred at 95° for 1 hr. An addition of 4.5 g. of DL-α-chloropropionic acid was made when the temperature had cooled to 65°. After 1 hr., 3.7 g. of sodium hydride was added. The mechanical stirring was continued at 65° overnight. To the reaction mixture, 100 ml. of water was added carefully, and the dioxane was evaporated. The yellowish aqueous solution was extracted once with chloroform. It was then acidified with 6 *N* hydrochloric acid under ice cooling, and the resulting precipitate was immediately extracted three times with 100 ml. of chloroform. The combined chloroform extracts were washed with water, dried over sodium sulfate, and evaporated to give 3.3 g. of a crystalline residue. It was

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dissolved in 80 ml. of hot methanol and the insoluble material (80 mg.) was filtered off. The filtrate was evaporated, and the residue was recrystallized from methanol to give 2.9 g. (76%) of **benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (III)**, m.p. 243–244°, $[\alpha]^{22}D$ +98° (*c* 0.63, methanol).[16]

A solution of 2.0 g. of this compound in the minimum of methanol was esterified by the addition of a slight excess of diazomethane in ether. Evaporation of the solution and recrystallization of the residue from methanol gave 1.7 g. of **benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[D-1-(methyl carboxylate)ethyl]-α-D-glucopyranoside (IV)**, m.p. 213–214°, $[\alpha]^{25}D$ +100° (*c* 1.09, chloroform).[17] This compound showed no depression of the melting point in admixture with an authentic sample.

The material insoluble in methanol was esterified with diazomethane in ether. After evaporation, the residue was dissolved in ethylene dichloride and chromatographed on silica gel. Elution with a mixture of ethylene dichloride and ether 4:1 gave 17 mg. of IV and 38 mg. (1%) of **benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[L-1-(methyl carboxylate)ethyl]-α-D-glucopyranoside (II)**, m.p. 253–254°, $[\alpha]^{20}D$ +54° (*c* 0.60, chloroform).

Anal. Calcd. for $C_{26}H_{31}NO_8$: C, 64.32; H, 6.44. Found: C, 64.38; H, 6.64.

Benzyl 2-Acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (V).—A solution of 0.20 g. of III in 5 ml. of 60% acetic acid was heated on a boiling water bath for 1 hr. After cooling to room temperature, the solution was evaporated to a sirup and the last traces of acetic acid and benzaldehyde were removed by codistillation with water. The residue was dissolved in 4.5 ml. of methanol and 0.5 ml. of 2 *N* sodium hydroxide was added. The mixture was kept at room temperature overnight. The solution was then neutralized with acetic acid and evaporated. The residue was dissolved in water and a small amount of Dowex-50 (H^+ form) was added to the solution. After filtration, the solution was treated with a small amount of charcoal, then evaporated to a sirup. Crystallization from a mixture of methanol and ethyl acetate gave 80 mg. of needles, m.p. 162–163°, $[\alpha]^{25}D$ +168° (*c* 0.62, methanol).[18] Treatment of the mother liquors using the same procedure gave an additional 25 mg. of needles, m.p. 162–163°, resulting in a total yield of 65%. This compound showed no depression of the melting point in admixture with an authentic sample.

Benzyl 2-Acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-6-*O*-*p*-tolylsulfonyl-α-D-glucopyranoside δ-Lactone (VII).—A solution of 0.38 g. of III in 5 ml. of 60% acetic acid was heated on a boiling water bath for 1 hr. and treated as just described. The residue, dried by codistillation with toluene, was dissolved in 5 ml. of pyridine. To this solution was added at 0° 0.33 g. (2.2 moles) of *p*-toluenesulfonyl chloride, and the mixture was kept at room temperature overnight. The mixture was then poured into ice–water and extracted with chloroform. The chloroform layer was washed three times with water, dried over sodium sulfate, and evaporated. The resulting crystalline mass was recrystallized from methanol to give 0.22 g. of needles (53%), m.p. 183–185°. A second recrystallization from the same solvent raised the melting point to 186–187°, $[\alpha]^{19}D$ +130° (*c* 0.74, chloroform).

Anal. Calcd. for $C_{25}H_{29}NO_9S$: C, 57.79; H, 5.63; N, 2.70; S, 6.17. Found: C, 57.09; H, 5.48; N, 2.82; S, 6.44.

To a solution of 6.32 mg. of this compound in 0.5 ml. of dry acetone was added 6.3 mg. of sodium iodide. After this mixture was heated for 2 hr. at 100°, then cooled, 2.02 mg. of plate-shaped crystals of sodium *p*-toluenesulfonate were filtered, corresponding to the elimination of 0.86 tosyl group/sugar molecule.

2-Acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (*N*-Acetylmuramic Acid) (VIII). From V.—Hydrogenolysis of V as previously described[11] gave VIII, m.p. 119–120°, in 92% yield; $[\alpha]^{20}D$ +60° (after 10 min.), $[\alpha]^{21}D$ +40° at equilibrium (after 24 hr.) (*c* 1.18, water).[19] This compound showed no depression of the melting point in admixture with an authentic sample.

From IX.—To a solution of 358 mg. (1 equiv.) of IX in methanol were added at 0° 144 mg. of triethylamine (1 equiv.) and 860 mg. of *N*-acetoxyphthalimide (2 equiv.). The mixture was kept at room temperature for 20 hr. After evaporation, the residue was extracted with 10 ml. of water and the insoluble material was filtered off. The solution was treated with Amberlite IR 120 (H^+ form) until the pH reached 3.5. It was then extracted with portions of ethyl acetate until no ethyl acetate extracts gave color when shaken with sodium bicarbonate. Evaporation of the aqueous layer gave a sirup which crystallized spontaneously on standing. Recrystallization from a mixture of ethyl acetate and methanol gave 300 mg. (70%) of prisms, m.p. 119–121°, showing a mutarotation from $[\alpha]^{20}D$ +56° (after 10 min.) to $[\alpha]^{20}D$ +40° at equilibrium (after 24 hr.) (*c* 0.68, water).[19] This compound showed no depression of melting point in admixture with a sample obtained from V.

2-Amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-α-D-glucose (IX).—A solution of 35 mg. of VIII in 1 ml. of 2.5 *N* hydrochloric acid was heated at 100° for 6 hr., then evaporated to dryness. This residue was dissolved in a small amount of water and treated with Dowex-1 (OH^- form) until pH 5.5 was reached. The solution was filtered and evaporated, and the residue was crystallized from 90% ethanol to give 21 mg. (70%), m.p. 160–162° dec. The product showed a mutarotation from $[\alpha]^{22}D$ +146° (after 6 min.) to $[\alpha]^{23}D$ +116° at equilibrium (after 31 hr.) (*c* 0.57, water).[20]

Acknowledgment.—The authors wish to thank Chas. Pfizer and Company for a kind gift of 2-acetamido-2-deoxy-D-glucose.

(16) Flowers and Jeanloz[11] reported m.p. 237–239°, $[\alpha]^{18}D$ +115° (*c* 1.28 methanol).

(17) Flowers and Jeanloz[11] reported m.p. 212–213°, $[\alpha]^{21}D$ +94° (*c* 0.70, chloroform).

(18) Flowers and Jeanloz[11] reported m.p. 160–161°, $[\alpha]^{22}D$ +168° (*c* 1.25, chloroform).

(19) Flowers and Jeanloz[11] reported m.p. 122–124°, $[\alpha]^{23}D$ +39° at equilibrium (after 6 hr.) (*c* 1.58, water).

(20) Gigg and Carroll[9] reported m.p. 150–° dec., $[\alpha]^{22}D$ 151+112° (after 23 hr., *c* 2, water).