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SYNTHESIS OF VARIOUS GLYCOSIDES OF 2-AMINO-3-*O*-(D-1-CARBOXYETHYL)-2-DEOXY-D-GLUCOPYRANOSE (MURAMIC ACID)*

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ABSTRACT

Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside, and methyl, benzyl, and *p*-nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside were condensed with DL-2-chloropropionic acid to give, in preponderant yields (64, 46, 68, and 25%, respectively), the respective 3-*O*-(D-1-carboxyethyl) derivatives, separated as their methyl esters. Only from the condensation of the methyl α-D-glucoside was the separation of a significant proportion (11% yield) of the 3-*O*-(L-1-carboxyethyl) derivative achieved. Acetolysis of methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[(D-1-methoxycarbonyl)ethyl]-α-D-glucopyranoside gave an oxazoline; this was treated with hydrobromic acid in acetic acid, and then with silver oxide and methanol, to give a methyl β-D-glucoside derivative identical with that obtained from methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside. Benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside was also obtained by condensation with pure L-chloropropionic acid. Benzyl and *p*-nitrophenyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside were resistant to the action of egg-white lysozyme.

INTRODUCTION

The synthesis of 2-amino-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (muramic acid), a constituent of the cell wall of bacteria[2], is 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. Most syntheses[3–8] start from the sodium salt (at O-3) of the methyl[3–6], ethyl[4], or benzyl[7,8] glycoside of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-gluco-

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pyranose. Condensation with a racemic 2-halopropionic acid derivative leads to the formation of both the D- and L-(1-carboxyethyl) derivatives (muramic acid and isomuramic acid, respectively[3,4,8]).

In the present work, we investigated the influence of the chemical constitution of the aglycon group, and of the isomerism at C-1, on the relative proportion of muramic and isomuramic acid derivatives obtained by condensation with racemic 2-chloropropionic acid. The starting materials were methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside and methyl, benzyl, and *p*-nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside. The synthesis starting from the benzyl α-D-glycoside has been reported previously[8].

An additional motive for the preparation of various glycosides of muramic acid was an attempt to find synthetic, easily prepared substrates for egg-white lysozyme. This enzyme, which has been classified[9] as a "muramidase", was found to split the glycosidic linkage of the 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranosyl residues of the peptidoglycan that forms the backbone of the bacterial cell-wall[10].

DISCUSSION

Condensation of the sodium salt of methyl 2-acetamido-4,6-*O*-benzylidene-α-D-glucopyranoside (**1**) with a derivative of a DL-2-halopropionic acid was the main step in the original preparation[3] of muramic acid. This method has the advantage of avoiding the tedious separation of the isomers of the 2-halopropionic acid[6], or their respective preparation from the costly D- or L-alanine[5]. Its disadvantage is the low yield of the final product; this probably results from the separation of muramic acid from isomuramic acid on ion-exchange resins at the last stage of the preparation. A better yield of muramic acid has been obtained by separating the glycosides of muramic and isomuramic acid on a charcoal column[4]. Thus, it was of interest to study the separation one step earlier in the preparation, namely, at the stage of the 4,6-*O*-benzylidene derivatives, to take advantage of the separative effect of adsorption chromatography in an organic medium. In this type of separation, amounts of substance larger than those separated by adsorption on charcoal or on ion-exchange resin can be manipulated.

Since 2-chloropropionic acid has been shown[5] to give higher yields than the other 2-halopropionic acids, condensation of DL-2-chloropropionic acid with the sodium salt of **1** was first studied. The condensation was followed by esterification, with diazomethane, of the resulting mixture of 3-*O*-(1-carboxyethyl) derivatives (**3** and **6**). Separation on a silica gel column showed that the 3-*O*-[D-1-(methoxycarbonyl)-ethyl] ester **4** had been formed in preponderant amount, as compared to the 3-*O*-[L-1-(methoxycarbonyl)ethyl] ester **7**. A similar observation has been reported[8] for the condensation of the racemic 2-chloropropionic acid with benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**2**).

Condensation of DL-2-chloropropionic acid with the sodium salt of methyl (**9**), benzyl (**10**), and *p*-nitrophenyl (**11**) 2-acetamido-4,6-*O*-benzylidene-β-D-glucopyrano-

side gave almost exclusively the corresponding 3-*O*-(D-1-carboxyethyl) derivatives, characterized as the methyl esters **12**, **15**, and **16**, respectively. The yield of the benzyl β-D-glycosides (**14** and **15**) was 68%, whereas that of the methyl β-D-glycosides (**12**) was 40%, and that of the *p*-nitrophenyl β-D-glycoside (**16**) only 25% (30%, if the recovered starting material is considered). However, our inability to isolate significant amounts of the 3-*O*-(L-1-carboxyethyl) derivatives of the β-D anomers does not indicate with certainty that these derivatives are formed in very small proportions. Re-investigation, by thin-layer chromatography, of the product of condensation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside (**2**), which had previously[8] been thought to afford mainly the 3-*O*-(D-1-carboxyethyl) derivative (**5**) and traces of the 3-*O*-(L-1-carboxyethyl) derivative, has shown, indeed, that the latter compound may be formed in a proportion up to 20%. It was also found that the respective yields of both isomers were variable, because of the difficulty in exactly reproducing the conditions of the condensation in a two-phase system[11]. In addition, the 3-*O*-(L-1-carboxyethyl) derivatives of the β-D-glycoside series show very low solubilities in most solvents, and it is probable that these derivatives crystallize in the column during chromatographic separation. Finally, the conditions of the condensation are quite drastic, and this may, in part, explain the low yield resulting from the condensation of the (less stable) *p*-nitrophenyl β-D-glucoside **11**. Despite the great variations in the yield of the products obtained, it is nevertheless possible to conclude that the condensation reaction with DL-2-chloropropionic acid is stereoselective for all of the glucosides investigated, and that the 3-*O*-(D-1-carboxyethyl) derivatives are obtained in preponderant yield.

The compounds obtained by condensation of **1** with DL-2-chloropropionic acid were identified by comparison with the compound obtained by condensation of **1** with L-2-chloropropionic acid[5,6] and D-2-chloropropionic acid[5], respectively.

Roth and Pigman[12] have described the preparation of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**9**) by methylation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucose in aqueous solution. Re-investigation of this reaction showed that the product **9** previously described contained as an impurity the α-D anomer; purification could not be achieved by fractional recrystallization, but only by chromatography. The physical constants of the product thus obtained were in good agreement with those of the compound formed by alkaline hydrolysis of methyl 2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[13]. Condensation of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**9**) with DL-2-chloropropionic acid led to the isolation of only one isomer (**12**). This compound gave, after alkaline hydrolysis, the acid (**13**), which had the same properties as those of the compound described by Matsushima and Park[5]. Further proof of the configuration of the lactyl residue of **12** was obtained by acetolysis of methyl 2-acetamido-4, 6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside[6] (**22**), followed by treatment with hydrogen bromide in methanol in the presence of silver oxide. Although the crystalline product resulting from the acetolysis was obtained in 67% yield and had an elementary analysis corresponding to that of a

2-acetamido-tri-*O*-acetyl-2-deoxy-3-*O*-[(methoxycarbonyl)ethyl]hexose, its i.r. spectrum was that of an oxazoline structure, and therefore structure **23** was attributed to this product. Oxazolines have been obtained in the past by treatment of *N*-benzoyl derivatives of acetylated hexosamines with hydrobromic acid in glacial acetic acid[14] and with aluminum chloride[15], and, more recently, by treatment of *N*-acetyl derivatives with acetic anhydride and zinc chloride[16], but oxazolines obtained by treatment of *N*-acetyl derivatives with an acetolysis mixture (acetic anhydride, acetic acid, and sulfuric acid) have not hitherto been reported. Treatment of the oxazoline **23** with hydrobromic acid in glacial acetic acid, and then with methanol in the presence of silver oxide gave the acetylated methyl β-D-glucoside **17**. This compound was identical with the compound obtained from **12** by removal of the benzylidene group followed by acetylation. Definite identification of the oxazoline **23** could not be achieved by treatment with hydrated *p*-toluenesulfonic acid in methanol, as described by Pravdić, Inch, and Fletcher[16], because application of these reagents to methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**22**) resulted in rapid de-*O*-acetylation, to give a compound moving, on thin-layer chromatograms, similarly to methyl 2-acetamido-4-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside[6]. Direct treatment of the α-D-glucoside **22** with hydrobromic acid in glacial acetic acid, followed by condensation with methanol in the presence of silver oxide, gave unchanged starting-material **22**.

For the preparation of benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**10**), the intermediate benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranoside (**21**) was obtained from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-D-glucopyranose *via* the corresponding α-D-glucopyranosyl bromide (which was not isolated). Treatment of this bromide with the Koenigs–Knorr reagent had been reported to give syrupy products, and use of mercuric cyanide as a catalyst was recommended[17]. Modification of the Koenigs–Knorr procedure led to the crystalline glycoside (**21**) previously described[17]. Condensation of the benzyl glycoside (**10**) with DL-2-chloropropionic acid gave, in addition to the expected 3-*O*-(D-1-carboxyethyl) derivative **14**, a compound having a higher m.p. which may be the 3-*O*-(L-1-carboxyethyl) isomer. Identification of the configuration of the lactyl group of **14** was obtained by condensation of **10** with L-2-chloropropionic acid. Removal of the benzylidene group of **14** gave benzyl 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside (**19**), which was characterized by the crystalline 4-(cyclic ester) **24** and tested as a substrate for lysozyme.

p-Nitrophenyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**11**) was prepared from the known *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside[18]. The yield of product from its condensation with DL-2-chloropropionic acid could not be raised above 25%, and the product was quite unstable. Because the final product **20**, obtained by removal of the benzylidene group from **16**, was shown not to be a substrate for lysozyme, further study of the condensation reaction was not pursued.

Both benzyl (**19**) and *p*-nitrophenyl (**20**) 2-acetamido-3-*O*-(D-1-carboxyethyl)-

2-deoxy-β-D-glucopyranoside were investigated as substrates for egg-white lysozyme. These aglycons were selected because (*a*) numerous studies[19] had shown that aryl aglycons are more reactive with hydrolases than are alkyl aglycons, and (*b*) the products of the reaction, benzyl alcohol and *p*-nitrophenol, can be readily detected on chromatograms. In addition, *p*-nitrophenol can be determined colorimetrically and, thus, the kinetics of the enzyme reaction may be studied. The β-D-glycosides were selected because, for chitin, egg-white lysozyme splits a 2-acetamido-2-deoxy-β-D-glucosyl linkage[20] which is assumed to be of the same type as that of the peptidoglycan of bacterial cell-wall[21]. No reaction of compounds **19** and **20** could be detected with egg-white lysozyme after an incubation period of up to 48 h, under the conditions known to be optimal for the action of the enzyme[22]. Thus, it may be concluded that egg-white lysozyme is not a "muramidase" of wide specificity, but rather an "endo-hexosaminidase" acting on substrates having three or more monosaccharide units. A similar conclusion had been reached by Matsushima and associates, who synthesized phenyl[23] and *p*-aminophenyl[24] 2-acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside and observed their resistance to degradation by lysozyme.

EXPERIMENTAL

Melting points were determined on a hot stage equipped with a microscope, and correspond to "corrected melting points". Rotations were determined with a polarimeter equipped with a Rudolph photoelectric polarimeter attachment Model 200, or with the Perkin–Elmer No. 141 polarimeter. The chloroform used was A. R. grade and contained approximately 0.75% of ethanol. Infrared spectra were recorded, for potassium bromide discs, with a Perkin–Elmer spectrophotometer Model 237. The homogeneity of the compound synthesized was determined by chromatography on plates covered with a thin layer of a 3:1 mixture of silica gel G (Merck) and silica gel GF (Merck). Column chromatography was performed on "Silica Gel Davison", from the Davison Co., Baltimore, Maryland 21201 (grade 950, 60–200 mesh), which was used without pretreatment. When deactivation by contact with moist air occurred, reactivation was conducted by heating to 170–200° (manufacturer's instructions). The sequence of eluents was hexane, benzene (or 1,2-dichloroethane), ether, ethyl acetate, acetone, and methanol, individually or in binary mixtures. The ratio of weight of substance to weight of adsorbent was 1:50 to 1:100. The ratio of weight of substance (in g) to volume of fraction of eluent (in ml) was 1:100. The ratio of diameter to length of the column was 1:20. Evaporations were conducted *in vacuo*, with the bath temperature below 45°. Volumes of volatile solvent smaller than 20 ml were evaporated under a stream of dry nitrogen. The microanalyses were performed by Dr. M. Manser, Zurich, Switzerland.

Condensation of DL-*2-chloropropionic acid with methyl 2-acetamido-4,6-O-benzylidene-2-deoxy-α-*D-*glucopyranoside* (**1**). — A solution of dry **1** (5.0 g)[25] in *p*-dioxane (450 ml, freshly distilled in presence of sodium) was kept at 60–70°. Under vigorous stirring and protection from moisture, a suspension of sodium hydride (2.5 g) in dry *p*-dioxane (25 ml) [prepared from a commercially available suspension of the

hydride in oil (Alfa Inorganics, Beverly, Massachusetts)] was added during 10 min. After 15 min of stirring, a solution of freshly distilled DL-2-chloropropionic acid (16.4 g) in dry *p*-dioxane (50 ml) was slowly added, and the mixture was stirred for one h at 60–70°. A suspension of sodium hydride (10 g) in dry *p*-dioxane (50 ml) was then slowly added at 40°, the mixture was treated with water (75–100 ml) to decompose the excess sodium hydride, and the solution was concentrated to a syrup, which was dissolved in water (100 ml). Unreacted starting material and traces of oil (from the sodium hydride suspension) were removed by washing the solution with chloroform. To the aqueous layer was then added 300 ml of chloroform, ice to maintain the temperature at 0°, and 3M hydrochloric acid (about 75 ml) in small portions with vigorous shaking between additions, until pH 3 was reached. The chloroform layer was washed 3 times with ice-cold water, dried (sodium sulfate), and evaporated to dryness. The residue was dissolved in methanol (300 ml), and the solution was treated with a solution of diazomethane (6 g) in ether–ethanol. After 30 min, the solution was evaporated, and the residue was dissolved in 1,2-dichloroethane and chromatographed on silica gel. Elution with 1,2-dichloroethane–ether (1:1) gave two crystalline compounds. Intermediate fractions containing a mixture of these compounds were rechromatographed. After recrystallization from acetone, the material first eluted gave 4.54 g (71%) of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-D-glucopyranoside (**4**); m.p. 211–212°, $[\alpha]_D^{25}$ +115° (*c* 0.58, chloroform), showing no depression of m.p. on admixture with the compound previously described[6], and an identical i.r. spectrum.

The second compound eluted with the same solvent mixture gave, after crystallization from acetone, 0.68 g (11%) of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[L-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside (**7**), as long needles, m.p. 267–270°, $[\alpha]_D^{18}$ +33° (c 0.48, chloroform).

Anal. Calc. for $C_{20}H_{27}NO_8$: C, 58,67; H, 6.65; OCH_3, 15.16. Found: C, 58.65; H, 6.63; OCH_3, 15.36.

Treatment of the L isomer **7** with sodium hydroxide in methanol gave, in 83% yield, methyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(L-1-carboxyethyl)-2-deoxy-α-D-glucopyranoside (**6**), m.p. 283–286°, $[\alpha]_D^{18}$ +39° (*c* 0.47, ethanol); lit.[5] m.p. 280–3°, $[\alpha]_D^{25}$ +39.1° (*c* 0.20, ethanol).

*Methyl 2-acetamido-2-deoxy-3-*O*-[-L-1-(methoxycarbonyl)ethyl]-α-D-glucopyranoside* (**8**). — Treatment of compound **7** (150 mg) with 60% acetic acid (5 ml) for 30 min at 100° was followed by evaporation to dryness in the presence of abs. toluene. The residue was dissolved in chloroform, and chromatographed on silica gel. Crystalline fractions eluted with 2:1 ethyl acetate–acetone were recrystallized from acetone to give short needles (74% yield), m.p. 165–168°, $[\alpha]_D^{24}$ +36° (*c* 0.45, chloroform).

Anal. Calc. for $C_{13}H_{23}NO_8$: C, 48.59; H, 7.22; OCH_3, 19.32. Found: C, 48.53; H, 7.17; OCH_3, 19.20.

*Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside* (**9**). — Methyl glycosidation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranose

in water was performed as described by Roth and Pigman[12], and gave a product having m.p. 273–6°; $[\alpha]_D^{24}$ −83° (*c* 0.25, methanol), −68° (*c* 0.60, methyl sulfoxide); lit.[12] m.p. 278–9°, $[\alpha]_D^{20}$ −63.8° (*c* 0.5, methyl sulfoxide). Part of this product (6.0 g) was dissolved in chloroform and chromatographed on silica gel. Elution with pure ethyl acetate gave 850 mg of crude methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-α-D-glucopyranoside. Further elution with ethyl acetate gave 4.3 g of compound **9**, with some intermediate fractions containing a mixture of both anomers. Recrystallization of **9** gave 3.27 g of needles, m.p. 298–300°, with sublimation starting at 285°; $[\alpha]_D^{24}$ −95° (*c* 0.33, methanol), $[\alpha]_D^{24}$ −82° (*c* 0.53, methyl sulfoxide). The product did not show a depression in m.p. on admixture with the product described below.

A solution of methyl 2-acetamido-3-*O*-benzoyl-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside[13] (50 mg) in 0.2M barium methoxide (2 ml) was kept overnight at 0°. After neutralization with sulfuric acid and evaporation of the solution, the residue resulting was extracted with methanol. Crystallization from the same solvent gave 23 mg of long needles, m.p. 306–308°, $[\alpha]_D^{27}$ −98° (*c* 0.27, methanol).

Anal. Calc. for $C_{16}H_{21}NO_6$: C, 59.43; H, 6.55. Found: C, 59.43; H, 6.37.

Acetylation of **9** with acetic anhydride and pyridine gave the 3-*O*-acetyl derivative, m.p. 304–305°, $[\alpha]_D^{30}$ −94° (*c* 0.43, chloroform); lit. m.p. 158°, $[\alpha]_D$ −12.9° (*c* 0.9, chloroform)[26]; m.p. 300–301°, $[\alpha]_D$ −95.1° (*c* 0.48, chloroform)[27].

*Methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside* (**12**). — The condensation of methyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-β-D-glucopyranoside (**9**) (3.0 g) with DL-2-chloropropionic acid was performed as described for the α-D anomer, with the following modifications. Because of the low solubility of the starting material and final products, the proportions of *p*-dioxane and of chloroform, used for the reaction and for the extraction, were increased by a factor of 1.5–2. A small amount of starting material (45 mg) was recovered from the first chloroform extract. The residue obtained after esterification (2.09 g) was chromatographed on silica gel, and crystalline fractions were eluted with 1,2-dichloroethane–ether (1:1). Recrystallization from acetone gave 1.75 g (46%) of long needles, m.p. 282–285°, $[\alpha]_D^{22}$ −22° (*c* 0.42, chloroform). All of the crystalline fractions eluted from the column were homogeneous, as shown by optical rotation and by t.l.c. on silica gel.

Anal. Calc. for $C_{20}H_{27}NO_8$: C, 58.67; H, 6.65; OCH_3, 15.16. Found: C, 58.72; H, 6.83; OCH_3, 15.01.

Treatment of **12** with sodium hydroxide in methanol gave methyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside (**13**) in 80% yield, after two recrystallizations from acetone; small needles, m.p. 277–280°, $[\alpha]_D^{24}$ −8.0° (*c* 0.81, ethanol); lit.[5] m.p. 277–280° (dec.), $[\alpha]_D^{25}$ −79° (*c* 0.17, ethanol).

*Methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside* (**17**) *from* **12**. — Treatment of compound **12** (50 mg) with 60% acetic acid for 30 min at 100°, followed by evaporation, and acetylation with acetic anhydride (2 ml) and pyridine (3 ml) overnight, gave, after crystallization from acetone–ether, needles (32 mg, 65%), m.p. 174–176°, $[\alpha]_D^{23}$ +42° (*c* 0.19, chloroform).

Anal. Calc. for $C_{17}H_{17}NO_{10}$: C, 50.37; H, 6.71. Found: C, 50.29; H, 6.56.

2-Phenyl-4,5-[3,4,6-tri-O-acetyl-D-glucopyrano]-2-oxazoline (**23**). — A solution of methyl 2-acetamido-4,6-di-*O*-acetyl-2-deoxy-3-*O*-[D-1-(methoxylcarbonyl)ethyl]-α-D-glucopyranoside (**22**, 1.0 g)[22] in a mixture of acetic anhydride (15 ml), acetic acid (10 ml), and conc. sulfuric acid (0.175 ml) was kept for 48 h at room temperature, and extracted with ice-cold chloroform (25 ml). The organic layer was washed 3 times with ice-cold, saturated sodium hydrogen carbonate solution and 3 times with ice-cold water, dried (sodium sulfate), and evaporated. After a few days, the residue crystallized, and recrystallization from ether gave needles (302 mg, 67%), m.p. 96–97° $[\alpha]_D^{24}$ +122° (*c* 0.45, chloroform); ν_{max}^{KBr} 1670 (C=N), 1725, and 1740 (OAc) cm^{-1}; no absorption band in the regions 1460–1670 and 3000–3600 (amide) cm^{-1}.

Anal. Calc. for $C_{18}H_{27}NO_{11}$: C, 49.88; H, 6.28; N, 3.23. Found: C, 50.00; H, 6.40; N, 3.30.

Methyl 2-acetamido-4,6-di-O-acetyl-2-deoxy-3-O-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside (**17**) *from* **23**. — A solution of **23** (150 mg) in glacial acetic acid presaturated at 0° with hydrobromic acid (2 ml) was kept in the dark for 6 h at room temperature, and then diluted with chloroform (10 ml). The solution was rapidly washed twice with ice-cold water, twice with ice-cold, saturated sodium hydrogen carbonate solution, and twice with ice-cold water, and dried (sodium sulfate). To the solution was added anhydrous sodium sulfate (0.8 g), freshly prepared silver oxide (0.75 mg), methanol (1.5 ml), and a crystal of iodine, according to Inouye *et al.*[28], and the suspension was shaken for 2 days in the dark, and filtered. The filtrate was washed twice with water, dried (sodium sulfate), and evaporated, giving a residue which was crystallized from acetone–ether to give 51 mg (38%) of sharp needles, m.p. 174–176°, $[\alpha]_D^{25}$ +42° (*c* 0.19, chloroform), The i.r. spectrum was similar to that of the product previously described, and on admixture, the m.p. was not depressed.

Anal. Calc. for $C_{17}H_{27}NO_{10}$: C, 50.37; H, 6.71; OCH_3, 15.31. Found: C, 50.29; H, 6.85; OCH_3, 15.21.

Benzyl 2-acetamido-3,4,6-tri-O-acetyl-2-deoxy-β-D-glucopyranoside. — 2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl bromide was prepared from 2-acetamido-1,3,4,6-tetra-*O*-acetyl-2-deoxy-D-glucopyranose (5.0 g) according to Inouye *et al.*[28]. The resulting chloroform solution (200 ml) was dried with sodium sulfate and filtered, and used immediately. Anhydrous sodium sulfate (15 g), benzyl alcohol (5.0 g), silver oxide (10 g), and iodine (0.2 g) were added, and the mixture was shaken for 60 h at room temperature in the dark, and then filtered. The residue was washed with alcohol-free chloroform, and the filtrate and washings were combined, washed with water, dried (sodium sulfate), and evaporated. The residue was triturated with ether, to give a gel which was filtered off, and washed exhaustively with ether. Recrystallization from boiling water gave 2.25 g (42%) of needles, m.p. 167–8°, $[\alpha]_D^{20}$ −44° (methanol); lit.[17] m.p. 165–7°, $[\alpha]_D$ −43.3° (methanol).

Anal. Calc. for $C_{21}H_{27}NO_9$: C, 57.66; H, 6.22. Found: C, 57.69; H, 6.26.

Benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**10**). — Treatment of benzyl 2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranoside with barium

methoxide in methanol at 0°, followed by removal of the barium ions with Dowex-50, gave, in 80% yield, benzyl 2-acetamido-2-deoxy-β-D-glucopyranoside having physical constants similar to those of the product described by Kuhn and Kirschenlohr[17]. This product (2.0 g) was shaken for 24 h at room temperature with benzaldehyde (20 ml) and freshly fused zinc chloride (200 mg). Hexane (30 ml) and water (30 ml) were added, the mixture was shaken for 1 h, and the supernatant liquor was discarded. The addition and removal of hexane and water was repeated twice, and the resulting precipitate was filtered off, and washed exhaustively with water and then with hexane. It was recrystallized from methanol and from methanol–water to give needles (1.60 g, 62%), m.p. 266–8°, $[\alpha]_D^{20}$ −64° (*c* 0.55, methanol). This product was identical with the product described previously[29].

Anal. Calc. for $C_{22}H_{25}NO_6 \cdot H_2O$: C, 63.30; H, 6.52. Found: 63.87; H, 6.50.

Condensation of benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**10**) *with* DL-*2-chloropropionic acid.* — Condensation of compound **10** (1.5 g), prepared as already described or as reported by Gross and Jeanloz[29], with DL-2-chloropropionic acid was performed as described for the preparation of **12**. Extraction of the alkaline phase with chloroform resulted in the recovery of some starting material (55 mg). After acidification of the aqueous solution and extraction with chloroform, the aqueous phase contained, in suspension, an insoluble material which was filtered off, washed with water, and dried (0.60 g). It was recrystallized twice from abs. ethanol to give benzyl 2-acetamido-4,6-*O*-benzylidene-3-*O*-(D-1-carboxyethyl)-2-deoxy-β-D-glucopyranoside (**14**), needles, m.p. 264–5°; $[\alpha]_D^{20}$ −52° (*c* 0.41, ethanol).

Anal. Calc. for $C_{22}H_{22}NO$: C, 63.68; H, 6.20. Found: C, 63.59; H, 6.21.

The chloroform extract of the acid phase was evaporated, and the residue was dissolved in methanol. The solution was treated with diazomethane, and evaporated, and the residue (0.80 g) was dissolved in 1,2-dichloroethane and chromatographed on silica gel. 1,2-Dichloroethane–ether (4:1) eluted crystalline fractions (0.62 g), which were recrystallized from acetone–methanol to give benzyl 2-acetamido-4,6-*O*-benzylidene-2-deoxy-3-*O*-[D-1-(methoxycarbonyl)ethyl]-β-D-glucopyranoside (**15**), long needles, m.p. 250–1° (sublimation at 205°); $[\alpha]_D^{18}$ −51° (*c* 0.37, chloroform). The same compound was obtained by esterification of **14** (described above) with diazomethane.

Anal. Calc. for $C_{26}H_{31}NO_8$: C, 64.32; H, 6.44; N, 2.88; OCH_3, 6.39. Found: C, 64.38; H, 6.64; N, 3.08; OCH_3, 6.89.

The crystalline fractions next obtained by elution with 1,2-dichloroethane–ether (4:1) were recrystallized from ethanol–acetone to give 12 mg of small needles, m.p. 281–2° (sublimation at 230°), not further investigated. Pure ether and 9:1 ether–ethyl acetate eluted the crystalline starting material (50 mg). The yield of D-1-carboxyethyl derivatives (**14** and **15**) was 68%, and that of recovered starting material was 7%.

Condensation of benzyl 2-acetamido-4,6-O-benzylidene-2-deoxy-β-D-glucopyranoside (**10**) *with* L-*2-chloropropionic acid.* — The procedure used for this condensation was a modification of that just described for condensation with DL-chloropropionic acid. To a solution of compound **10** (1.6 g) in dry *p*-dioxane (100 ml), at 95°, was added in small portions a suspension (50%) of sodium hydride (0.9 g) in mineral oil.

The mixture was stirred for 7 h at 95°, and was then cooled to 65°. L-2-Chloropropionic acid (prepared by a modification[30] of the method of Fischer and Raske[31] for D-2-bromopropionic acid) (1.75 ml) in dry *p*-dioxane (10 ml) was added dropwise to the mixture; after 1 h, a 50% suspension of sodium hydride (3.7 g) was added in small portions, and the mixture was stirred overnight at 65°. The mixture was cooled to 0°, and treated with water (50 ml), and the upper, organic layer was decanted, filtered, and partially concentrated (about 100 ml was evaporated). Water (25 ml) was added, and the solution was washed with chloroform, cooled to 0°, and acidified with 6M hydrochloric acid (about 1 ml) to pH 3. The resulting precipitate was immediately filtered off, carefully washed with cold water, and dried in a desiccator. Recrystallization from methanol gave 1.1 g (58%), m.p. 264–5°, $[\alpha]_D^{20}$ −76° (*c* 0.40, pyridine), which was identical with compound **14** already described.

*Benzyl 2-acetamido-2-deoxy-3-*O-[D-*1-(methoxycarbonyl)ethyl]-β-*D *glucopyranoside* (**18**). — Treatment of compound **15** with 60% acetic acid for 30 min at 100°, followed by evaporation, gave a crystalline residue which was recrystallized from ethyl acetate to give small needles (80% yield), m.p. 169–170°, $[\alpha]_D^{25}$ −53° (*c* 0.57, chloroform).

Anal. Calc. for $C_{19}H_{27}NO_8$: C, 57.42; H, 6.85; N, 3.52. Found: C, 57.06; H, 6.24; N, 3.23.

*Benzyl 2-acetamido-3-*O-(D-1-*carboxyethyl)-2-deoxy-β-*D-*glucopyranoside* (**19**). — A solution of compound **18** (125 mg) in 0.2M sodium hydroxide (10 ml) was kept overnight at room temperature, and was then passed through a column of Dowex-50 (H^+) ion-exchange resin. Evaporation of the eluate, followed by crystallization of the residue from ethanol–water, gave long needles (86 mg, 79%), m.p. 189–191° and 225–227°, $[\alpha]_D^{20}$ −36 (*c* 2.2, methanol).

Anal. Calc. for $C_{18}H_{25}NO_8$: C, 56.39; H, 6.57; N, 3.65. Found: C, 55.99; H, 6.56; N, 3.49.

The same compound was obtained in 87% yield by treatment of **14** with 60% acetic acid.

Sublimation at 220–220° under high vacuum gave benzyl 2-acetamido-3-*O*-[(*R*)-1-carboxyethyl]-2-deoxy-β-D-glucopyranoside 4-(inner ester) (**24**), m.p. 223–5°, $[\alpha]_D^{20}$ −4° (*c* 0.10, pyridine).

Anal. Calc. for $C_{18}H_{23}NO_7$: C, 59.17; H, 6.34; N, 3.83. Found: C, 59.10; H, 6.40; N, 3.84.

p-*Nitrophenyl 2-acetamido-4,6-*O-*benzylidene-2-deoxy-β-*D-*glucopyranoside* (**11**). — A mixture of *p*-nitrophenyl 2-acetamido-2-deoxy-β-D-glucopyranoside[18] (450 mg), freshly fused zinc chloride (350 mg), and freshly distilled benzaldehyde (5 ml) was shaken for 24 h at room temperature, hexane (4 ml) and water (4 ml) were added, the mixture was shaken for 1 h, and the liquid phase was decanted. Hexane and water were added to the solid, and the procedure was repeated three times. Finally, the solid was filtered off, washed thoroughly with water and hexane, dried in a desiccator, and recrystallized from methanol–water to give white needles (460 mg, 81%), m.p. 245–7° (dec.), $[\alpha]_D^{20}$ −52° (*c* 0.57, chloroform).

Anal. Calc. for $C_{21}H_{22}N_2O_8$: C, 58.60; H, 5.15; N, 6.51. Found: C, 58.48; H, 5.02; N, 6.42.

p-*Nitrophenyl 2-acetamido-4,6-O-benzylidene-2-deoxy-3-O-*[D-*1-(methoxycarbonyl)ethyl]-β-*D-*glucopyranoside* (**16**). — To a stirred solution of compound **11** (190 mg) in dry *p*-dioxane (100 ml) at 60–70° was added sodium hydride (150 mg, as a 54% suspension in mineral oil). After the mixture had been stirred for 15 min, DL-2-chloropropionic acid (0.75 ml) and *p*-dioxane (5 ml) were added, and the suspension was stirred for 2 h at 60–70°. Sodium hydride (2 g) in mineral oil and *p*-dioxane were added portionwise, and the mixture became very thick. It was shaken vigorously by hand for 10 min, and then mechanically overnight at 45–50°, and processed by to the method used for the preparation of **12**. Starting material (38 mg, 20%) was recovered from the chloroform extract of the alkaline phase and from the fractions eluted with ether–ethyl acetate (4:1 and 1:1) from the silica gel column. The fractions eluted with (9:1) 1-2-dichloroethane–ether gave, after recrystallization from acetone–ether cream-colored needles (56 mg, 25%), m.p. 254–5°, $[\alpha]_D^{20}$ −16° (*c* 0.21, chloroform).

Anal. Calc. for $C_{25}H_{28}N_2O_{10}\cdot 0.5\ H_2O$: C, 57.14; H, 5.53; N, 5.33. Found: C, 57.16; H, 5.62; N, 5.76.

p-*Nitrophenyl 2-acetamido-3-O-*(D-*1-carboxyethyl)-β-*D-*glucopyranoside* (**20**). — Treatment of compound **16** (40 mg) with 60% acetic acid at 100° and removal of the acetic acid and benzaldehyde, as described for the preparation of **17**, was followed by treatment with 0.5M sodium hydroxide, as described for the preparation of **19**. The residue was purified by dissolution in benzene and chromatography on silica gel. Ether–ethyl acetate (1:1) eluted fractions which were crystallized, and which gave, after recrystallization from ethanol–ether, 16 mg (50%) of small clusters of needles, m.p. 167–170° (turning yellow at 155°), $[\alpha]_D^{20}$ −27° (*c* 0.26, ethanol).

Anal. Calc. for $C_{17}H_{22}N_2O_{10}$: C, 49.28; H, 5.35. Found: C, 49.01; H, 5.99.

Action of egg-white lysozyme on compounds **19** *and* **20**. — To a 4% solution (1 ml) of **19** or **20** in water was added 0.1M ammonium acetate buffer (0.025 ml; adjusted to pH 6.30 with acetic acid) and a 0.0025% solution (0.05 ml) of thrice-recrystallized, egg-white lysozyme (General Biochemicals, Chagrin Falls, Ohio). Control solutions without lysozyme were prepared, and the mixtures were incubated at 37°. Aliquots were examined by descending chromatography on Whatman No. 1 paper with butanol–acetic acid–water (4:1:5, upper phase). 2-Acetamido-3-*O*-(D-1-carboxyethyl)-2-deoxy-D-glucose (*N*-acetylmuramic acid), benzyl alcohol, and *p*-nitrophenol, were chromatographed as standards. The spots were revealed by the silver nitrate method and by dipping of the dried chromatograms in a solution of 0.5M sodium hydroxide in 6:4 ethanol–propanol, heating for 5 to 10 min at 120°, and then examining under u.v. light[32].

No product of hydrolysis could be observed with either **19** or **20** for incubation times of up to 48 h.

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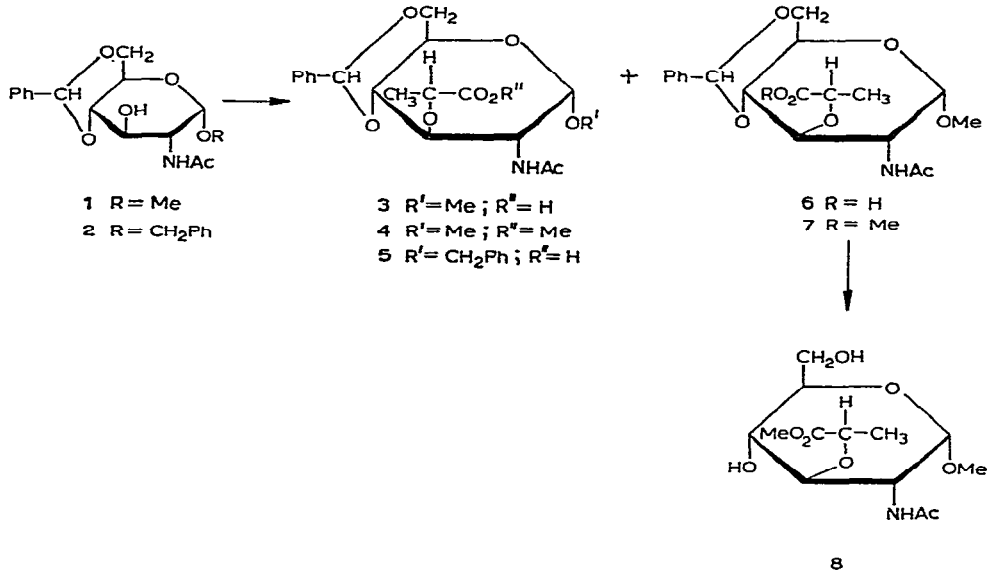
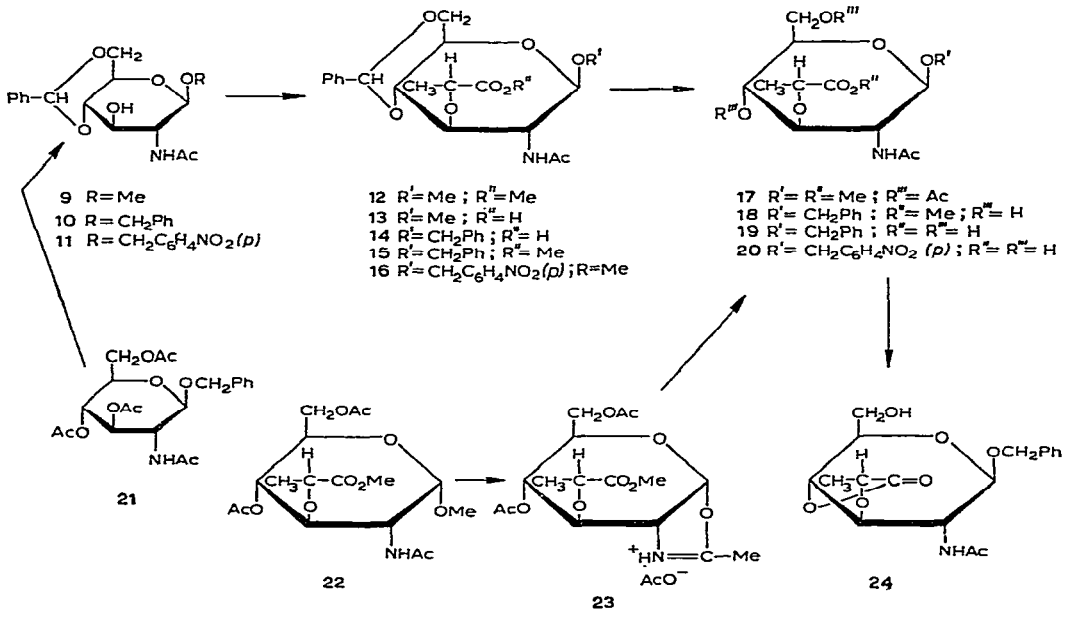


1 R = Me
2 R = CH_2Ph

3 R^{I} = Me ; R^{II} = H
4 R^{I} = Me ; R^{II} = Me
5 R^{I} = CH_2Ph ; R^{II} = H

6 R = H
7 R = Me

8



9 R = Me
10 R = CH_2Ph
11 R = $CH_2C_6H_4NO_2$ *(p)*

12 R^{I} = Me ; R^{II} = Me
13 R^{I} = Me ; R^{II} = H
14 R^{I} = CH_2Ph ; R^{II} = H
15 R^{I} = CH_2Ph ; R^{II} = Me
16 R^{I} = $CH_2C_6H_4NO_2$ *(p)* ; R = Me

17 R^{I} = R^{II} = Me ; R^{III} = Ac
18 R^{I} = CH_2Ph ; R^{II} = Me ; R^{III} = H
19 R^{I} = CH_2Ph ; R^{II} = R^{III} = H
20 R^{I} = $CH_2C_6H_4NO_2$ *(p)* ; R^{II} = R^{III} = H

21

22

23

24

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