Carbohydrate Research, 47 (1976) 49–61

SYNTHESIS AND CHEMICAL BEHAVIOUR OF D-GLUCOSYL ESTERS OF GLUTAMIC ACID HAVING THE SIDE-CHAIN CARBOXYL GROUP INVOLVED IN THE GLYCOSIDIC LINKAGE*

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(Received July 24th, 1975; accepted for publication, August 18th, 1975)

ABSTRACT

Simultaneous and stepwise deprotection of the fully benzylated D-glucosyl esters of 1-benzyl *N*-benzyloxycarbonyl- and *N*-*tert*-butyloxycarbonyl-L-glutamic acid (**1** and **5**, respectively) have been examined. Catalytic hydrogenation of **1** led to intramolecular aminolysis to give pyroglutamic acid and D-glucose, but similar treatment in the presence of trifluoroacetic acid afforded both anomers of 1-*O*-(L-γ-glutamyl)-D-glucopyranose, which were characterized as trifluoroacetates (**2α** and **2β**) and converted into 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(acetyl)-L-glutam-5-oyl]-D-glucopyranose (**4**) which was also prepared by a definitive method. Hydrogenolysis of **5** gave both anomers of 1-*O*-[*N*-(*tert*-butyloxycarbonyl)-L-γ-glutamyl]-D-glucopyranose (**6**), which, upon treatment with trifluoroacetic acid at −10°, afforded **2α** and **2β**, respectively. The structure of **6β** was established by its conversion into 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-β-D-glucopyranose (**7β**), whereas similar treatment of **6α** gave a mixture of 1,3,4,6-tetra-*O*-acetyl-2-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose (**9**) and **7α**. A 1→2 acyl migration occurred during esterification of the aglycon carboxyl group of **6α** with diazomethane to give 2-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose (**8**).

INTRODUCTION

In the preceding paper[1], D-glucosyl esters of aspartic acid having the 1- or 4-carboxyl group linked to the sugar moiety were synthesized as simple model systems to represent potential intermediates in glycosidase–substrate interactions. The chemical behaviour of these compounds depends strongly upon the mutual positions of their functional groups which account for substantial differences in reaction products.

In a parallel study, we have investigated the relative ease of formation of D-glucosyl esters of glutamic acid having the carboxyl side-chain group involved in

*Glycosyl Esters of Amino Acids: Part VIII. For Part VII, see Ref. 1.
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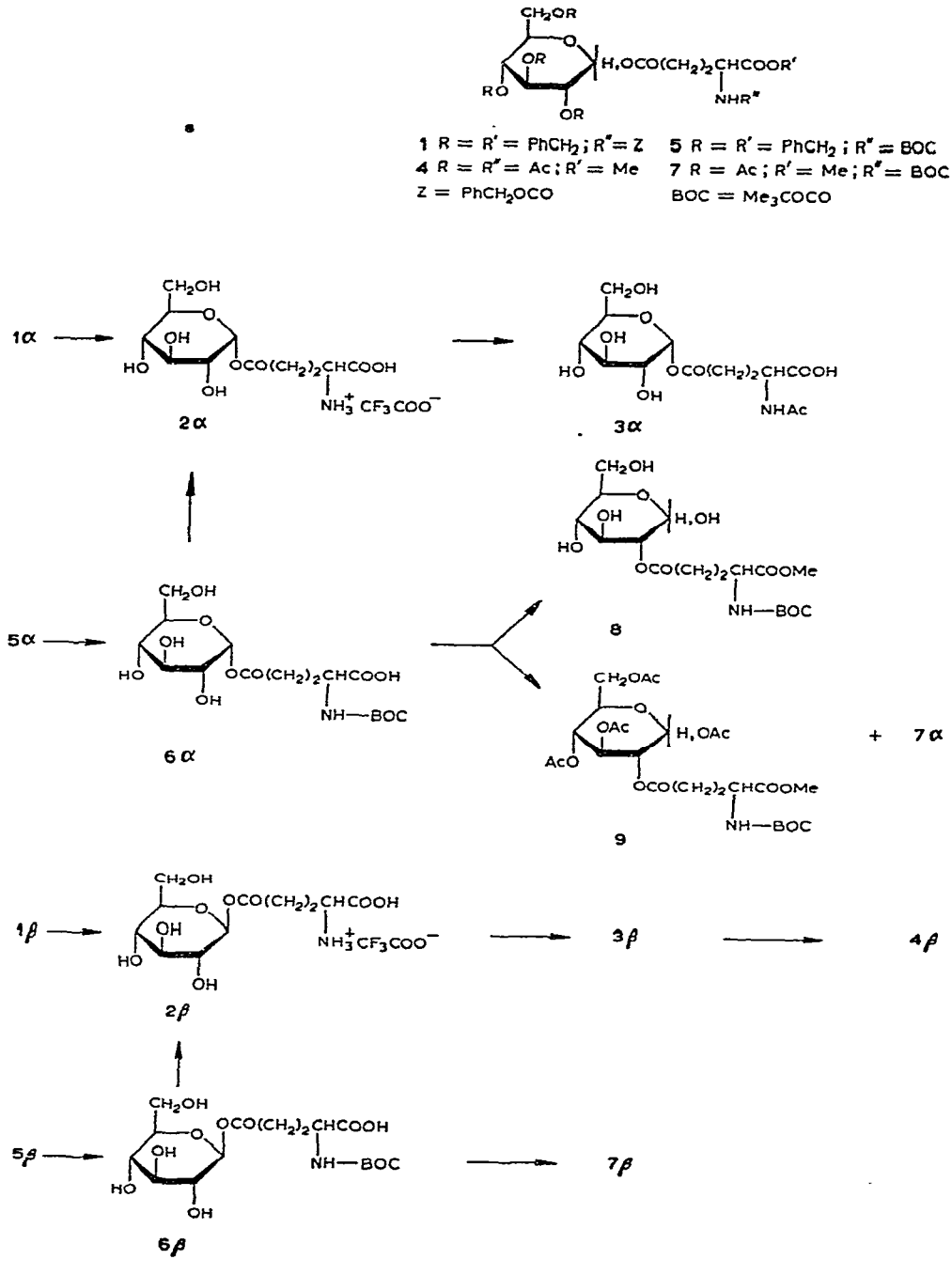
the glycosidic linkage. We now report two convenient routes for the synthesis of these compounds and derivatives thereof, and comment on their reactivity.

RESULTS AND DISCUSSION

In our first approach to the unprotected D-glucosyl ester of glutamic acid having the 5-carboxyl group involved in the glycosidic linkage, 2,3,4,6-tetra-*O*-benzyl-1-*O*-[1-benzyl *N*-(benzyloxycarbonyl)-L-glutam-5-oyl]-D-glucopyranose (**1**) was chosen as the starting material, with the objective of removing the protecting groups simultaneously in a single-step reaction. Compound **1** was obtained by the imidazole-promoted condensation[2] of tetra-*O*-benzyl-α-D-glucopyranose and 1-benzyl 5-pentachlorophenyl *N*-benzyloxycarbonyl-L-glutamate as an anomeric mixture (yield 60%) in which the β anomer was strongly preponderant; the anomers were separated and characterized.

Catalytic hydrogenation of **1α** and **1β** in acetic acid—2-methoxyethanol over palladium-on-charcoal, *i.e.*, under conditions which were successfully employed[1] in the preparation of analogously linked α -and β-D-glucosyl esters of aspartic acid, led almost exclusively to intramolecular aminolysis with cleavage of the C-1 ester bond: in both cases, D-glucose and pyroglutamic acid were the only isolable products. Monitoring of the hydrogenation mixtures by t.l.c. revealed that the unprotected D-glucosyl ester formed from **1α** decomposed at a lower rate than that arising from **1β**. The lower tendency of the glutamyl aglycon of the α anomer to cyclise, as compared with its β counterpart, may be explained by a lower extent of protonation of the glucosidic oxygen atom on the axial than on the equatorial position in the ester bond-breaking step. When the hydrogenolysis mixture from **1α** was immediately treated with acetic anhydride in acetone–water, a hygroscopic solid could be isolated (after purification on a cellulose column) in low yield (12%); its analytical data, optical rotation ($[\alpha]_D$ +49.2°), and i.r. and n.m.r. spectra (see Experimental section) suggested the structure 1-*O*-[*N*-(acetyl)-L-γ-glutamyl]-α-D-glucopyranose (**3α**).

In order to prevent nucleophilic attack of the glutamate amino group on the glycosidic ester carbonyl, hydrogenation of **1** was performed in the presence of an excess of trifluoroacetic acid[1,3]. Under these conditions, both anomers of **1** afforded the corresponding 1-*O*-(L-γ-glutamyl)-D-glucopyranose trifluoroacetate salt, **2α** and **2β**, as very hygroscopic solids in high yields (86 and 74%, respectively). Their structures were assigned on the basis of analytical and spectral data and chemical transformations. The i.r. spectra revealed the ionic carboxyl absorption (~1650 cm^{-1}) and two characteristic bands in the region of 1690–1525 cm^{-1} associated with the NH_3^+ deformations, and the n.m.r. spectra in deuterium oxide showed H-1 signals with chemical shifts and $J_{1,2}$ values indicative of the α- and β-D configuration, respectively. When kept under anhydrous conditions at room temperature, both anomers of **2** were stable for more than one month, whereas their aqueous solutions (pH ~2.3) underwent a ~30% cleavage (t.l.c.) within the first 24–48 h, the β anomer being the more labile. In both cases, D-glucose, and glutamic and pyroglutamic acid


1 R = R' = PhCH2 ; R" = Z
4 R = R" = Ac ; R' = Me
Z = PhCH2OCO
5 R = R' = PhCH2 ; R" = BOC
7 R = Ac ; R' = Me ; R" = BOC
BOC = Me3COCO
1α
2α
3α
5α
6α
8
9
+ 7α
1β
2β
3β
4β
5β
6β
7β

were detected (t.l.c.) as the decomposition products, thus indicating that hydrolysis and intramolecular aminolysis are operative under these conditions.

Selective *N*-acetylation of **2α** gave a product whose optical rotation and spectral data were indistinguishable from those of **3α** derived from the hydrogenolysis mixture to which trifluoroacetic acid had not been added. The β anomer of **2**, available in greater amount, was submitted to *N*-acetylation, esterification of the aglycon carboxyl group with diazomethane, and *O*-acetylation of the sugar moiety, without isolation of the intermediates. The final product, obtained in crystalline form, had physical properties identical to those of an authentic sample of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(acetyl)-L-glutam-5-oyl]-β-D-glucopyranose (**4β**), which was synthesized by the imidazole-promoted DCC condensation[2] of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and 1-methyl *N*-acetyl-L-glutamic acid followed by fractionation of the resulting mixture of anomers.

In the n.m.r. spectrum of **4β**, the signal for the ester methyl group of glutamate was a 3-proton singlet at τ 6.28 (chloroform-*d*) or 6.39 (methyl sulphoxide-d_6). A comparison with the n.m.r. spectra of 1-methyl *N*-acetyl-L-glutamic acid and 1,5-dimethyl *N*-acetyl-L-glutamate, prepared by definitive methods, established that the position of the singlet coincided with that of the methyl ester group at position 1. Hence, the sample of **4β** derived from **2β** was free of the 1-yl isomeric form of the aglycon.

An alternative approach to the α and β anomers of **2** involved stepwise deprotection of the fully blocked precursor. Accordingly, 2,3,4,6-tetra-*O*-benzyl-1-*O*-[1-benzyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-D-glucopyranose (**5**) was synthesised. In **5**, the aglycon amino function is protected by a group which is stable towards catalytic hydrogenation but is readily removed by treatment with trifluoroacetic acid. Compound **5** was obtained analogously by using 1-benzyl 5-pentachlorophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate as the amino acid component; the anomeric mixture (yield 66%) contained, in contrast to **1**, a very high proportion of the α anomer. Since **1** and **5** each possesses a urethane-type amino protecting-group, the difference in anomeric ratio between the two compounds seems to be due more to steric than to electronic effects; indeed, the Dreiding model of **5β** indicates a considerable restriction in movement of the bulky *tert*-butyloxycarbonyl (BOC) group.

Catalytic hydrogenation of **5β**, performed in 2-methoxyethanol in the presence of catalytic amounts of acetic acid, completely removed the ether and ester benzyl groups to give 1-*O*-[*N*-(*tert*-butyloxycarbonyl)-L-γ-glutamyl]-β-D-glucopyranose (**6β**) as a hygroscopic solid in almost quantitative yield. The structure assignment was deduced from analytical and spectral data; the n.m.r. spectrum in deuterium oxide contained a doublet for H-1 at τ 4.31 ($J_{1,2}$ 7 Hz) and a singlet for Me_3C at τ 8.58. Hydrolysis of **6β** (2M HCl, 100°, 2 h) afforded 80% optically pure L-glutamic acid. Unambigous structural proof was provided by converting **6β** into 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-β-D-glucopyranose (**7β**) by esterification and *O*-acetylation. Compound **7β** was identical with an authentic sample synthesized by the imidazole-promoted condensation of 2,3,4,6-

tetra-*O*-acetyl-D-glucopyranose and 1-methyl 5-pentachlorophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate, followed by anomeric fractionation of the product.

The BOC-derivative **6β** was more labile than the corresponding unprotected D-glucosyl ester **2β**, the former decomposing within a few days even when stored under strictly anhydrous conditions. However, when a freshly prepared sample of **6β** was treated[3] with trifluoroacetic acid at −10°, a rapid (~30 min) and clean cleavage of the *tert*-butyloxycarbonyl (BOC) group took place (t.l.c.), and 1-*O*-(L-γ-glutamyl)-β-D-glucopyranose trifluoroacetate salt (**2β**) was isolated in 92% yield. The identity of **2β** was established by comparing its physical data (t.l.c., $[\alpha]_D$, i.r. and n.m.r. spectra) with those of the product formed from the hydrogenation of **1β** in the presence of trifluoroacetic acid, as well as by converting it into the peracetylated methyl ester **4β**.

Similar debenzylation of **5α** afforded the BOC-protected D-glucosyl ester **6α**, the analytical and spectral data of which were fully consistent with the structure assigned; the signal for the anomeric proton in the n.m.r. spectrum was a narrow doublet ($J_{1,2}$ 3 Hz) at τ 3.82, thus confirming the α-D configuration. Treatment of **6α** with trifluoroacetic acid cleaved the amino protecting-group to give the unsubstituted D-glucosyl ester trifluoroacetate salt **2α**, indistinguishable from the product formed from **1α** in the one-step deprotection operation.

T.l.c. indicated that **6α** decomposed at a considerably lower rate than **6β**; monitoring of a dry sample of **6α** during 14 days revealed a gradual conversion of the original spot into a slightly faster-moving, silver nitrate- and ninhydrin- (after heating) positive component, together with the appearance of several minor spots, two of which coincided with D-glucose and BOC-glutamic acid. The above data suggest a slow, intramolecular O→O acyl migration reaction accompanied by the scission of the sugar–amino acid linkage.

Treatment of a freshly prepared sample of **6α** with diazomethane, followed by conventional acetylation with acetic anhydride–pyridine, gave a product which was shown to be a mixture of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose (**7α**) and 1,3,4,6-tetra-*O*-acetyl-2-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose (**9**). Thus, the product moved as a single component in several t.l.c. solvent systems, but its n.m.r. spectrum in chloroform-*d*, although closely similar to that of an authentic sample of **7α**, showed some differences in the acetoxyl region. The presence of a singlet at τ 7.83, assinged to the axial AcO-1 group (ratio of intensities of H-1 and *ax* AcO-1 was ~1:2.1), suggested that the product was a mixture of peracetylated 1- and 2-*O*-substituted derivatives, with the latter being preponderant. A second elution from silica gel led to partial fractionation of the mixture, affording, in the slower fractions, material highly enriched (>85%, estimated on the basis of n.m.r. data) in the 2-*O*-substituted component. Characterization required the synthesis of an authentic sample of **9**, and this was achieved by the imidazole-promoted condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose and 1-methyl 5-pentachlorophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate; direct comparison of physical data allowed assignments of structures **7α** and **9** to the product above.

The fact that **7α** and **9** were formed from a sample of **6α** that was completely free (t.l.c.) of its faster-moving rearrangement product suggested that the 1→2 migration of the glutamyl residue took place during or after esterification of the aglycon carboxyl group with diazomethane. Chromatography of the esterification product on a cellulose column gave a chromatographically homogeneous glass (63.5% yield) whose analytical and spectral data were consistent with the structure 2-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose (**8**). Comparison of the n.m.r. spectra of **8** with those of **6α** indicated that the H-1 resonance for **8** had undergone an upfield shift (0.76 and 0.92 p.p.m. in deuterium oxide and methyl sulphoxide-d_6, respectively), in accordance with the structure assigned. In addition, the spectrum of **8** in methyl sulphoxide-d_6 revealed, after removal of OH protons, only one signal at low field (τ 4.92, $J_{1,2}$ 4 Hz), assigned to H-1α.

Presumably, **8** must have been formed *via* a cyclic 1,2-orthoester. An analogous, although much faster 1→2 acyl migration has been observed[4] with the α anomer of 1-*O*-[*N*-(*tert*-butyloxycarbonyl)-L-alanyl]-D-glucopyranose and ascribed to an enhancement of the positive charge on the ester carbonyl carbon caused by the presence of the adjacent BOC group. The fact that the above migration of the BOC-glutamyl residue proceeded at a considerably lower rate might be rationalized by the remoteness of the BOC-protected amino function from the glycosidic ester linkage.

EXPERIMENTAL

For general procedures, see Part VII[1]. Column chromatography was performed on silica gel Merck (0.05–0.2 mm) or cellulose powder (Whatman, standard grade), and t.l.c. was performed on Kieselgel G (Merck) if not stated otherwise. The solvent systems used were: *A* benzene–ethyl acetate (proportions are given in the text); *B* acetonitrile–water (proportions are given in the text); *C* 5:3:1 propan-2-ol–light petroleum–water. Unless otherwise stated, optical rotations were measured for 1% solutions in chloroform. I. r. spectra were recorded with a Perkin–Elmer Model 137 spectrometer, and n.m.r. spectra with a Varian A-60A spectrometer (chloroform-*d*, unless otherwise stated.

Glutamic acid derivatives. — 1-Benzyl 5-pentachlorophenyl *N*-benzyloxycarbonyl-L-glutamate (52%) was prepared by the DCC method from 1-benzyl *N*-benzyloxycarbonyl-L-glutamic acid[5] and pentachlorophenol in dichloromethane; after recrystallisation from ethyl acetate, the product had m.p. 137–139°, $[\alpha]_D$ −16.0°.

Anal. Calc. for $C_{26}H_{20}Cl_5NO_6$: C, 50.39; H, 3.25; N, 2.26. Found: C, 50.42; H, 3.41; N, 2.01.

1-Benzyl 5-pentachlorophenyl *N*-*tert*-butyloxycarbonyl-L-glutamate (80%), prepared by the DCC condensation of 1-benzyl *N*-*tert*-butyloxycarbonyl-L-glutamic acid[6] and pentachlorophenol, had m.p. 114–116° (from chloroform–light petroleum), $[\alpha]_D$ −16.5°.

Anal. Calc. for $C_{23}H_{22}Cl_5NO_6$: C, 47.15; H, 3.78; N, 2.40. Found: C, 47.42; H, 3.82; N, 2.31.

1-Methyl 5-benzyl *N-tert*-butyloxycarbonyl-L-glutamate was prepared by treating 5-benzyl *N-tert*-butyloxycarbonyl-L-glutamic acid (liberated from the dicyclohexylamine salt[7]) with diazomethane in ether at 0°. The reaction product was purified on a silica gel column with solvent *A* (10:1) to give an oil (63%), $[\alpha]_D$ +15.5°.

Anal. Calc. for $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.34; H, 6.95; N, 4.15.

Catalytic hydrogenation of the foregoing compound gave 1-methyl *N-tert*-butyloxycarbonyl-L-glutamic acid (characterized as the dicyclohexylamine salt[8]) which was treated with pentachlorophenol in the presence of DCC. The resulting 1-methyl 5-pentachlorophenyl *N-tert*-butyloxycarbonyl-L-glutamate (66%), when crystallised from chloroform–light petroleum, had m.p. 119–121°, $[\alpha]_D$ +17.0°. N.m.r. data: τ 6.22 (s, 3H, 1-COO*Me*), 8.55 (s, 9H, Me_3C).

Anal. Calc. for $C_{17}H_{18}Cl_5NO_6$: C, 40.07; H, 3.56; N, 2.75. Found: C, 40.26; H, 3.53; N, 2.90.

1,5-Dimethyl *N-tert*-butyloxycarbonyl-L-glutamate was prepared by treating the *N*-protected amino acid with diazomethane in ether at 0°. The product was eluted from a silica gel column with solvent *A* (1:1) to give an oil (87%), $[\alpha]_D$ +12.2°. N.m.r. data: τ 4.81 (d, *J* 8 Hz, NH), 6.27 (s, 1-COO*Me*), 6.33 (s, 5-COO*Me*), 8.58 (s, Me_3C); (in methyl sulphoxide-d_6): τ 2.87 (d, *J* 9 Hz, NH), 6.38 (s, 1-COO*Me*), 6.43 (s, 5-COO*Me*), 8.65 (s, Me_3C).

Anal. Calc. for $C_{12}H_{21}NO_6$: C, 52.35; H, 7.69; N, 5.09. Found: C, 52.15; H, 7.42; N, 5.25.

1-Methyl *N*-acetyl-L-glutamic acid, prepared by the method of Hawkins *et al.*[9], was treated with diazomethane in ether to afford 1,5-dimethyl *N*-acetyl-L-glutamate as an oil, $[\alpha]_D$ +12.0°. N.m.r. data: τ 6.28 (s, 1-COO*Me*), 6.34 (s, 5-COO*Me*), 8.04 (s, N*Ac*).

2,3,4,6-Tetra-O-*benzyl-1*-O-[*1-benzyl* N-(*benzyloxycarbonyl*)-L-*glutam-5-oyl*]-D-*glucopyranose* (**1**). — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (2.7 g), 1-benzyl 5-pentachlorophenyl *N*-benzyloxycarbonyl-L-glutamate (3.1 g), and imidazole (1.85 g) were dissolved in dichloromethane (30 ml) at room temperature; after 1 h, an additional amount (310 mg) of the amino acid component was added, and the mixture was kept at room temperature for 24 h. Pentachlorophenol was filtered off, and the filtrate was washed with water, 10% aqueous citric acid, water, aqueous sodium hydrogen carbonate, and water, dried (Na_2SO_4), and concentrated. The residue was eluted from a silica gel column with solvent *A* (10:1) to give chromatographically homogeneous **1** (2.41 g, 60%) as an anomeric mixture. Crystallisation from ethanol afforded the β anomer of **1** (1.3 g), m.p. 106–107°, $[\alpha]_D$ +7.0°. N.m.r. data: τ 2.68–2.75 (m, 30H, 6Ph), 4.44 (d, $J_{1,2}$ 7 Hz, H-1). Treatment of a sample with methanolic sodium methoxide[2] gave 85% optically pure 1,5-dimethyl *N*-benzyloxycarbonyl-L-glutamate.

Anal. Calc. for $C_{54}H_{55}NO_{11}$: C, 72.54; H, 6.21; N, 1.57. Found: C, 72.47; H, 6.16; N, 1.69.

The mother liquor was evaporated to dryness, and the residue was submitted to

chromatography on silica gel in solvent *A* to give, in the first fractions, the pure α anomer of **1** as a colourless syrup, $[\alpha]_D$ +38.0°. N.m.r. data: τ 2.70–2.78 (m, 30H, 6Ph), 3.63 (d, $J_{1,2}$ 3 Hz, H-1) (Found: C, 72.44; H, 6.09; N, 1.31).

Catalytic hydrogenation of **1**. — (*a*) *Without trifluoroacetic acid.* When **1α** or **1β** was hydrogenated in acetic acid—2-methoxyethanol (2:1) in the presence of 10% palladium-on-charcoal, t.l.c. (cellulose, solvent *B*, 3:1) revealed two major (coincident with pyroglutamic acid and D-glucose, respectively) and one minor (ninhydrin- and silver nitrate-positive) spots; the last product decomposed gradually during attempted purification of the mixture.

The crude hydrogenolysis product of **1α** was immediately treated with a 5% solution of acetic anhydride in acetone–water (1:1) at 0° for 16 h; after removal of the solvent (0.1 torr), the residue was eluted from a cellulose column with solvent *B* (3:1) to give a chromatographically homogeneous, hygroscopic foam which, on crystallisation from methanol–ether, deposited 1-*O*-[*N*-(acetyl)-L-γ-glutamyl]-α-D-glucopyranose (**3α**) as highly deliquescent crystals (50 mg, 12% calc. on **1α**), $[\alpha]_D$ +49.2° (*c* 0.52, water); ν_{max}^{KBr} 3420 broad vs (OH and NH), 1720 s (C=O), 1630 and 1560 (amide I and II). N.m.r. data (D_2O): τ 3.83 (d, $J_{1,2}$ 3 Hz, H-1), 7.95 (s, N*Ac*).

Anal. Calc. for $C_{13}H_{21}NO_{10}$: C, 44.44; H, 6.03; N, 3.99. Found: C, 44.64; H, 6.31; N, 3.84.

(*b*) *In the presence of trifluoroacetic acid: deprotection of* **1α**. To a suspension of **1α** (270 mg) in 2-methoxyethanol (10 ml), trifluoroacetic acid (2 ml) and 10% palladium-on-charcoal (50 mg) were added; hydrogen was passed through the stirred suspension until evolution of carbon dioxide [$Ba(OH)_2$] solution ceased. Additional catalyst (100 mg) was then added, and the mixture was shaken with hydrogen at atmospheric pressure and room temperature until termination of hydrogen uptake (~20 h). The catalyst was centrifuged off, the supernatant was evaporated (0.1 torr), traces of trifluoroacetic acid were removed by co-distillation with ether, and a solution of the residue in water (2 ml) was freeze-dried to give 1-*O*-(L-γ-glutamyl)-α-D-glucopyranose trifllluoroacetate salt (**2α**) as a chromatographically homogeneous, highly hygroscopic solid (110 mg, 86%); $[\alpha]_D$ +55.5° (methanol), +53.2° (*c* 2, water); ν_{max}^{KBr} 3500 (broad (OH), 1760 s (C=O), 1695 and 1530 (NH_3^+ deformations), 1660 sh (ionized trifluoroacetic carboxyl), 1070 vs cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 3.82 (d, $J_{1,2}$ 3 Hz, H-1). Hydrolysis of a sample of **2α** (2M HCl, 100°, 2 h)[1] afforded 71% optically pure L-glutamic acid.

Anal. Calc. for $C_{13}H_{20}F_3NO_{11}$: C, 36.87; H, 4.77; N, 3.31. Found: C, 36.74; H, 4.89; N, 3.11.

To a sample (30 mg) of the above compound in water (5 ml), 20% acetic anhydride in acetone (5 ml) was added, and the solution was kept at 0° for 16 h. After removal of the solvent (0.1 torr), the residue was dissolved in methanol, and ether was added at 0°; the precipitate was centrifuged off and dissolved in water (2 ml), and the solution was freeze-dried to give a fluffy solid (15 mg, 60%), $[\alpha]_D$ +47.0° (*c* 2, water), for which the i.r. and n.m.r. spectra were indistinguishable from those of 1-*O*-[*N*-(acetyl)-L-γ-glutamyl]-α-D-glucopyranose (**3α**) described above.

(*c*) *Deprotection of* **1β**. Hydrogenation of the β anomer of **1** (268 mg) in the presence of trifluoroacetic acid, as in (*b*), afforded 1-*O*-(L-γ-glutamyl)-β-D-glucopyranose trifluoroacetate salt (**2β**) (97 mg, 74%) as a hygroscopic solid. For analysis, the sample was dissolved in methanol and precipitated with ether; $[\alpha]_D$ +14.0° (methanol), +12.8° (water); ν_{max}^{KBr} 3500 broad (OH), 1760 s (C=O), 1690 and 1525 (NH_3^+ deformations), 1650 sh (ionized trifluoroacetic carboxyl), 1080 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.44 (d, $J_{1,2}$ 7 Hz, H-1) (Found: C, 37.04; H, 4.96; N, 3.54).

N-Acetylation of **2β** (350 mg) was performed as described in (*b*); the crude product was eluted from a cellulose column with solvent *B* (2:1) to give 1-*O*-[*N*-(acetyl)-L-γ-glutamyl]-β-D-glucopyranose (**3β**; 150 mg, 51.5%) as a highly hygroscopic solid, $[\alpha]_D$ +8.8° (*c* 2.3, water); ν_{max}^{KBr} 3500 (OH), 1755 (C=O), 1655 and 1560 (amide I and II), 1080 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.41 (d, $J_{1,2}$ 7 Hz, H-1), 7.96 (s, N*Ac*). Due to the high hygroscopicity of **3β**, correct results could not be obtained for elemental analysis.

To a sample (100 mg) of **3β** in *N*,*N*-dimethylformamide (1.5 ml), an ethereal solution of diazomethane (~20 ml) was added at 0°; after ~1 h, the solvent was removed (0.1 torr), and the residue was conventionally acetylated with pyridine–acetic anhydride (4:1, 13 ml) at 0° for 72 h. The crude product was eluted from a silica gel column with solvent *A* (1:3) to give a homogeneous syrup (30 mg, 20%), $[\alpha]_D$ +18.0°, which crystallised from chloroform–light petroleum and was identical with an authentic sample of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(acetyl)-L-glutam-5-oyl]-β-D-glucopyranose (**4β**) by mixture m.p. and comparative i.r. and n.m.r. spectra.

2,3,4,6-Tetra-O-*acetyl-1*-O-[*1-methyl* N-(*acetyl*)-L-*glutam-5-oyl*]-D-*glucopyranose* (**4**). — To 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (696 mg) and 1-methyl *N*-acetyl-L-glutamic acid (452 mg) in dichloromethane (15 ml), DCC (412 mg) and imidazole (272 mg) were added at 0°. After shaking the mixture at room temperature for 20 h (monitoring by t.l.c. in solvent *A*, 1:2), *N*,*N'*-dicyclohexylurea was filtered off, and the mixture was worked-up as described for **1**. Elution of the product from silica gel with solvent *A* (1:2) resulted in partial separation of the anomers (total yield: 267 mg, 25%). On recrystallisation from chloroform–light petroleum, the faster-moving fractions yielded the pure β anomer of **4**, m.p. 129–130°, $[\alpha]_D$ +16.6°. N.m.r. data: τ 3.70 (d, *J* 8 Hz, NH), 4.28 (d, $J_{1,2}$ 7 Hz, H-1), 6.28 (s, 3H, 1-COO*Me*), 7.94, 7.98, 8.01 (unresolved, 15H, 4*Ac*O+N*Ac*); (methyl sulphoxide-d_6): τ 1.82 (d, *J* 8 Hz, NH), 4.04 (d, $J_{1,2}$ 8 Hz, H-1), 6.39 (s, 1-COO*Me*), 8.03–8.08 (unresolved, 12H, 4*Ac*O), 8.18 (s, N*Ac*).

Anal. Calc. for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.63. Found: C, 49.46; H, 5.98; N, 2.86.

The mother liquor and the slower-moving fractions were evaporated to dryness, and the residue was rechromatographed in solvent *A* (1:2); on concentration, the slower-moving fractions gave the pure α anomer of **4** which, when crystallised from chloroform–light petroleum, had m.p. 94–97°, $[\alpha]_D$ +83.0°. N.m.r. data: τ 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 6.26 (s, 1-COO*Me*), 7.96–8.01 (unresolved, 15H, 4*Ac*O+N*Ac*);

ν_{max}^{KBr} 3500 (NH), 1800 (C=O), 1690 and 1520 (amide I and II) (Found: C, 49.62; H, 6.04; N, 2.87).

2,3,4,6-Tetra-O-*benzyl-1*-O-[*1-benzyl* N-(tert-*butyloxycarbonyl*)-L-*glutam-5-oyl*]-D-*glucopyranose* (**5**). — Using 1-benzyl 5-pentachlorophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate (2.81 g) as the amino acid component, the reaction was performed as described for **1**. After working-up, the crude product was eluted from silica gel with solvent *A* (10:1) to give **5** (2.28 g, 66.3%) as an anomeric mixture in which the slightly faster-moving α anomer strongly preponderated. The residue from the slower-moving fractions was rechromatographed (twice) as just described, and the material highly enriched in the less-mobile component was dissolved in hot ethanol to which some drops of water were added; on keeping the solution at room temperature for several days, white crystals of the β anomer of **5** were deposited, m.p. 90–92°, $[\alpha]_D$ +4.0° (*c* 2); ν_{max}^{KBr} 3420 (NH), 1760 (C=O), 1680 and 1530 (amide I and II), 1360 and 1390 cm^{-1} (Me_3C). N.m.r. data: τ 2.78–2.87 (m, 5Ph), 4.38 (d, $J_{1,2}$ 7 Hz, H-1), 8.59 (s, 9H, Me_3C).

Anal. Calc. for $C_{51}H_{57}NO_{11}$: C, 71.22; H, 6.68; N, 1.64. Found: C, 71.26; H, 6.59; N, 1.63.

The faster-moving fractions furnished the α anomer of **5** as a colourless oil (1.6 g, 46.5%), $[\alpha]_D$ +31.8°. N.m.r. data: τ 2.60–2.80 (m, 5 Ph), 3.66 (d, $J_{1,2}$ 3 Hz, H-1), 8.59 (s, Me_3C) (Found: C, 71.31; H, 6.60; N, 1.55).

1-O-[N-(tert-*Butyloxycarbonyl*)-L-*γ-glutamyl*]-*β*-D-*glucopyranose* (**6β**). — Catalytic hydrogenation of **5β** (170 mg) over 10% palladium-on-charcoal (100 mg) was performed in 2-methoxyethanol (10 ml) to which acetic acid (~0.5 ml) was added. Removal of the catalyst and solvent (0.1 torr) left a solid, which was dissolved in water (3 ml) and freeze-dried to give 68 mg (97%) of **6β** as a fluffy mass, $[\alpha]_D$ +3.6° (*c* 3.3, methanol); ν_{max}^{KBr} 3500 vs, broad (OH), 1800 and 1730 (C=O), 1530 (amide I), 1400 and 1380 (Me_3C), 1080 cm^{-1} (C–O–C). N.m.r. data: τ 4.31 (d, $J_{1,2}$ 7 Hz, H-1), 8.58 (s, Me_3C).

Anal. Calc. for $C_{16}H_{27}NO_{11}$: C, 46.94; H, 6.65; N, 3.42. Found: C, 47.08; H, 6.87; N, 3.51.

Esterification of **6β** (66 mg), followed by *O*-acetylation, was effected as described for the conversion of **3β** into **4β**, but without using *N,N*-dimethylformamide as the solvent in the esterification step. The crude product was eluted from a silica gel column with solvent *A* (3:1) to give a chromatographically homogeneous solid (60 mg, 61.4%), $[\alpha]_D$ +15.7° (*c* 2), which gave a satisfactory elemental analysis for 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-*β*-D-glucopyranose (**7β**), and was identical with an authentic sample of **7β** described below, as judged by mixture m.p., and i.r. and n.m.r. spectra.

Treatment of **6β** *with trifluoroacetic acid to give* **2β**. — A solution of the β anomer of **6** (110 mg) in 98% trifluoroacetic acid (3 ml) at −10° was kept at this temperature for ~30 min (monitoring by t.l.c., cellulose; solvent *B*, 2:1). After removal of trifluoroacetic acid by co-distillation with dry ether, the residue was dissolved in water (3 ml) and freeze-dried to give a fluffy, hygroscopic solid (105 mg,

92%); $[\alpha]_D$ +14.0° (*c* 2, water), +15.0° (*c* 5, methanol); whose t.l.c. behaviour, and i.r. and n.m.r. spectra were indistinguishable from those of 1-*O*-(L-γ-glutamyl)-β-D-glucopyranose trifluoroacetate salt (**2β**) prepared by catalytic hydrogenation of **1β**.

In a second preparation, the product (160 mg) was subjected in turn to *N*-acetylation, esterification with diazomethane, and *O*-acetylation with pyridine–acetic anhydride to give crystals (30 mg, 15%), m.p. 128–130°, $[\alpha]_D$ +17.0° (*c* 1.5), identical with **4β**, as judged by elemental analysis, mixture m.p., and comparative n.m.r. spectrum.

1-O-[N-(tert-*Butyloxycarbonyl*)-L-γ-*glutamyl*]-α-D-glucopyranose (**6α**). — Catalytic hydrogenation of **5α** (380 mg) was performed as described for **5β**; after freeze-drying, **6α** was obtained as a hygroscopic, fluffy mass (175 mg, 97%), t.l.c. (cellulose, solvent *B*, 2:1) of which revealed the presence of a single spot; $[\alpha]_D$ +27.0° (methanol); ν_{max}^{film} 3470 (broad OH, NH), 1735 and 1695 (C=O), 1630 sh and 1525 (amide I and II), 1395 and 1375 (Me_3C), 1080 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 3.80 (d, $J_{1,2}$ 3 Hz, H-1), 8.52 (s, Me_3C); *in methyl sulphoxide-d*$_6$: τ 2.98 (d, *J* 8 Hz, disappeared on deuteration, NH), 4.00 (d, $J_{1,2}$ 2.5 Hz, H-1), 8.62 (s, Me_3C). Hydrolysis of a sample afforded 80% optically pure L-glutamic acid.

Anal. Calc. for $C_{16}H_{27}NO_{11}$: C, 46.94; H, 6.65; N, 3.42. Found: C, 46.79; H, 6.82; N, 3.21.

A freshly prepared sample (130 mg) of **6α** was treated with trifluoroacetic acid at −10° as described for the β anomer. After freeze-drying, the hygroscopic solid (130 mg, 97%), $[\alpha]_D$ +54.0°, showed i.r. and n.m.r. spectra indistinguishable from those of **2α** prepared by hydrogenation of **1α** in the presence of trifluoroacetic acid.

A sample (165 mg) of **6α** was immediately treated with diazomethane in ether and then with pyridine–acetic anhydride, as described for the conversion of **6β** into **7β**. Elution of the product from a silica gel column (solvent *A*, 3:1) afforded a mixture of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose (**7α**) and 1,3,4,6-tetra-*O*-acetyl-2-*O*-[1-methyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose (**9**) (total yield: 120 mg, 50%) which on t.l.c. (solvent *A*, 3:1) appeared as a single spot. N.m.r. data: τ 3.66 (d, $J_{1,2}$ 3 Hz, H-1), 6.27 (s, 1-COO*Me*), 7.83 (s ~0.7×3H, *ax* O*Ac*-1), 7.94 and 7.99 (unresolved, ~0.8×12H, 4*Ac*O), 8.58 (s, Me_3C).

Anal. Calc. for $C_{25}H_{37}NO_{15}$: C, 50.76; H, 6.30; N, 2.37. Found: C, 50.79; H, 6.18; N, 2.09.

Elution of the above material from a second silica gel column with solvent *A* (1:1) gave, in the slower fractions, almost pure **9** (>85%), as judged by a comparison of its n.m.r. spectrum with that of authentic **9** described below.

2-O-[*1-Methyl* N-(tert-*butyloxycarbonyl*)-L-*glutam-5-oyl*]-α-D-*glucopyranose* (**8**). — A freshly prepared sample (130 mg) of **6α** was shaken with an ethereal solution of diazomethane (~20 ml) at −10° for 1 h. After removal of the solvent, the residue was eluted from a cellulose column with solvent *C* to give **8** (85 mg, 63.5%) as a chromatographically homogeneous glass, $[\alpha]_D$ +17.0° (*c* 1.5, methanol); ν_{max}^{KBr} 3450 vs

broad (OH, NH), 2980 (CH_3 deformation), 1740 vs and 1695 vs (C=O), 1640 sh and 1530 (amide I and II), 1400 and 1375 cm^{-1} (Me_3C). N.m.r. data (D_2O): τ 4.58 (d, $J_{1,2}$ 4 Hz, H-1), 6.21 (s, 1-COO*Me*), 8.57 (s, Me_3C); (in methyl sulphoxide-d_6, after exchange with D_2O): τ 4.92 (d, $J_{1,2}$ 4 Hz, H-1), 6.36 (s, 1-COO*Me*), 8.65 (s, Me_3C).

Anal. Calc. for $C_{17}H_{29}NO_{11}$: C, 48.22; H, 6.90; N, 3.31. Found: C, 48.10; H, 7.12; N, 3.45.

2,3,4,6-Tetra-O-*acetyl-1*-O-[*1-methyl* N-(tert-*butyloxycarbonyl*)-L-*glutam-5-oyl*]-D-*glucopyranose* (7). — 2,3,4,6-Tetra-*O*-acetyl-D-glucopyranose (350 mg) and 1-methyl 5-pentachlorophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate (500 mg + 50 mg) were treated in the presence of imidazole (340 mg), as described for **1**. After working-up, the crude product was eluted from silica gel with solvent *A* (1:1) to give chromatographically homogeneous **7** (410 mg, 68.8%) as an anomeric mixture. After a second column chromatography, the faster moving β anomer of **7** was obtained as a solid foam, m.p. 44–47°, $[\alpha]_D$ +17.0°; ν_{max}^{KBr} 3500 (NH), 1780 (C=O), 1530 cm^{-1} (amide II). N.m.r. data: τ 4.24 (d, $J_{1,2}$ 7 Hz, H-1), 6.27 (s, 1-COO*Me*), 7.93, 7.99, 8.01 (unresolved, 12H, 4*Ac*O), 8.58 (s, Me_3C).

Anal. Calc. for $C_{25}H_{37}NO_{15}$: C, 50.76; H, 6.30; N, 2.37. Found: C, 50.65; H, 6.52; N, 2.36.

The less-mobile α anomer, when crystallised from chloroform–light petroleum, had m.p. 116–119°, $[\alpha]_D$ +53.2°. N.m.r. data: τ 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 6.25 (s, 1-COO*Me*), 7.93, 8.00 (unresolved, 12H, 4*Ac*O), 8.59 (s, Me_3C) (Found: C, 50.99; H, 6.19; N, 2.48).

1,3,4,6-Tetra-O-*acetyl-2*-O-[*1-methyl N*-(tert-*butyloxycarbonyl*)-L-*glutam-5-oyl*]--α-D-*glucopyranose* (**9**). — By using 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (250 mg), 1-methyl 5-pentachlorophenyl *N*-(*tert*-butyloxycarbonyl)-L-glutamate (400+40 mg), and imidazole (270 mg), the reaction was performed as described for **1**. After working-up, the product was eluted from silica gel with solvent *A* (1:1) to give chromatographically homogeneous **9** (190 mg, 40%) which, when crystallised from chloroform–light petroleum, had m.p. 121–123°, $[\alpha]_D$ +59.1°; ν_{max}^{KBr} 3500 (NH), 1760 vs (C=O), 1530 (amide II). N.m.r. data: τ 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 6.24 (s, 1-COO*Me*), 7.83 (s, *ax Ac*O-1), 7.94–8.00 (unresolved, 9H, 3*Ac*O), 8.59 (s, Me_3C).

Anal. Calc. for $C_{25}H_{37}NO_{15}$: C, 50.76; H, 6.30; N, 2.37. Found: C, 50.74; H, 6.43; N, 2.40.

ACKNOWLEDGMENTS

We thank Dr. O. Hadžija and Mrs. Lj. Sesartić for the microanalyses, Mrs. L. Tomić for recording the n.m.r. spectra, and Mrs. Dj. Orlić and Mrs. A. Matijevac for valuable technical assistance.

REFERENCES

1 Š. VALENTEKOVIĆ AND D. KEGLEVIĆ, *Carbohyd. Res.*, 47 (1976) 35–48.
2 D. KEGLEVIĆ, A. KORNHAUSER, AND Š. VALENTEKOVIĆ, *Carbohyd. Res.*, 22 (1972) 245–256.
3 D. KEGLEVIĆ, D. LJEVAKOVIĆ, AND Š. VALENTEKOVIĆ, *Croat. Chem. Acta*, 46 (1974) 115–127.
4 D. KEGLEVIĆ, Š. VALENTEKOVIĆ, G. ROGLIĆ, D. GOLEŠ, AND F. PLAVŠIĆ, *Carbohyd. Res.*, 29 (1973) 25–39.
5 E. KLIEGER AND H. GIBIAN, *Ann.*, 655 (1962) 195–210.
6 J. TOMASZ, *Acta Chim. (Budapest)*, 70 (1971) 255–261.
7 K. P. POLZHOFER, *Tetrahedron Lett.*, (1969) 2305–2307.
8 E. SCHRÖDER AND E. KLIEGER, *Ann.*, 673 (1964) 196–207.
9 M. J. HAWKINS, J. R. KNOWLES, L. WILSON, AND D. WITCHER, *Biochem. J.*, 104 (1967) 762–766.