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DIAZOMETHANE-CATALYSED REARRANGEMENT OF α-D-GLUCOPYRANOSYL ESTERS OF *N*-ACYLAMINO ACIDS INTO 2-*O*-ACYLAMINOACYL-α-D-GLUCOPYRANOSES*

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ABSTRACT

Both anomers of 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-α-glutamyl]-D-glucopyranose (**2**) were converted into the unprotected 1-esters, characterised as the trifluoroacetate salts **3α** and **3β**. On esterification with diazomethane and acetylation, the *N*-acetylated derivative of **3β** and **2β** gave the peracetylated 1-*O*-[5-methyl *N*-acetyl- and -*tert*-butoxycarbonyl-L-glutam-1-oyl]-β-D-glucopyranoses (**4β** and **6β**), respectively. Similar treatment of **2α** and **3α** led to acyl migration, to yield 1,3,4,6-tetra-*O*-acetyl-2-*O*-[5-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-1-oyl]-α-D-glucopyranose (**7α**, 64%) with traces of **7β**, and a mixture (~2:1:0.2) of the *N*-acetyl analogue of **7α** (**8α**), **4α**, and **8β**, respectively. Treatment of 1-*O*-[5-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-1-oyl]-α-D-glucopyranose (**10**) and the corresponding glutam-5-oyl isomer **12** in *N*,*N*-dimethylformamide with diazomethane for 1 h resulted in 1→2 *O*-acyl transfer to give, upon acetylation, **7α** and the fully acetylated 2-*O*-[1-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose in yields of 70 and 90%, respectively; in the absence of diazomethane, **10** and **12** remained unchanged. Similar experiments with α-D-glucopyranosyl esters of *N*-acetylglycine, *N*-acetylalanine, and *N*-(*tert*-butoxycarbonyl)phenylalanine yielded the 2-*O*-acyl derivatives in high yields and with high retention of anomeric configuration. The structures of the rearrangement products were proved both spectroscopically and chemically. The results imply that diazomethane functions as a base in the migration process.

INTRODUCTION

In studies[1–4] of the preparation of unprotected D-glucopyranosyl esters of amino acids and *N*-acylamino acids, we have observed that the relative stabilities of these compounds are significantly different, depending on the aglycon structure and anomeric configuration of the sugar moiety. During the characterisation of α-D-

*Glycosyl Esters of Amino Acids: Part IX. For Part VIII, see Ref. 4.

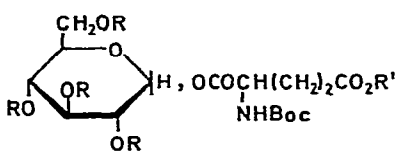
glucopyranosyl esters of *N*-acyl-aspartic and -glutamic acids, it was found that diazomethane promoted 1→2 migration of the amino acid residues.

For example, hydrogenolysis of the fully benzylated α-D-glucopyranosyl ester of *tert*-butoxycarbonyl(Boc)-glutamic acid having the side-chain carboxyl group involved in the glycosyl linkage yielded 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-γ-glutamyl]-α-D-glucopyranose, which, on storage for several days under anhydrous conditions, underwent a slow O-1→O-2 acyl migration accompanied by minor cleavage of the sugar–amino acid bond[4]. However, when a solution of the freshly prepared glycoside in *N*,*N*-dimethylformamide was treated with an ethereal solution of diazomethane at 0°, the corresponding 2-*O*-acyl derivative was formed within 1 h.

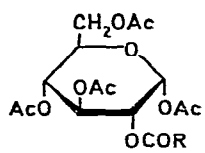
The present study was undertaken to provide a better understanding of factors that influence the acyl migration of the 1-esters involved. For comparison purposes, D-glucopyranosyl esters of Boc-glutamic acid linked through the 1-CO_2H group to the sugar moiety were synthesised, and the migratory tendencies of the two isomeric acyl groups were investigated.

RESULTS AND DISCUSSION

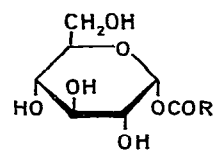
Treatment of 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-α-glutamyl]-β-D-glucopyranose[1] (**2β**) with trifluoroacetic acid at −10° afforded the unprotected D-glucopyranosyl ester **3β** as the unstable trifluoroacetate salt in practically quantitative yield. The identity of **3β** was confirmed by its three-step conversion into the peracetylated methyl ester **4β**, which was also prepared by direct synthesis. The structure of **2β** was proved



1 R = R' = CH_2Ph
2 R = R' = H
5 R = Ac, R' = H
6 R = Ac, R' = Me
9 R = CH_2Ph, R' = Me

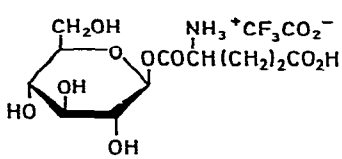


7α R = $CH(CH_2)_2CO_2Me$ (NHBoc)
8α R = $CH(CH_2)_2CO_2Me$ (NHAc)
15α R = CH_2NHAc

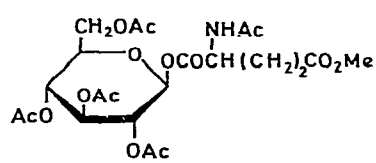


10 R = $CH(CH_2)_2CO_2Me$ (NHBoc)
12 R = $(CH_2)_2CH(NHBoc)CO_2Me$
13 R = CH_2NHAc
14 R = $CH(CH_3)NHAc$

Boc = $Me_3COC(=O)-$



3β



4β

by converting it into the fully protected D-glucopyranosyl ester **6β**, which was also obtained by two additional routes: (*a*) diazomethane treatment of the known[1] tetra-*O*-acetyl derivative **5β**, and (*b*) direct synthesis.

The synthesis in the α-D series started with the fully benzylated D-glucopyranosyl ester[1] **1α**, which, upon catalytic hydrogenation, gave the *N*-Boc-protected ester **2α** as a hygroscopic solid. T.l.c. revealed that **2α** and its slightly slower-moving rearrangement product decomposed at much higher rates than the corresponding 1- and 2-*O*-(Boc-γ-glutamyl) isomers[4], possibly because of the closer proximity of the Boc-amino substituent to the ester functional groups in the former two compounds. However, when **2α** was immediately treated with diazomethane, followed by conventional acetylation, 1,3,4,6-tetra-*O*-acetyl-2-*O*-[5-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-1-oyl]-α-D-glucopyranose (**7α**), contaminated (<10%) with its β anomer, was isolated in 64% yield, thus indicating that the 1→2 acyl rearrangement that had occurred during the foregoing treatment was faster than the hydrolysis of the glycosylic ester bond. The structure of **7α** was established from analytical and p.m.r. data and by comparison with an authentic sample synthesised by the imidazole-promoted condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose and 5-methyl 1-pentachlorophenyl Boc-L-glutamate. The structure of the contaminant **7β** was deduced from the elemental analysis, t.l.c. behaviour, and optical and spectral data of the product. In the p.m.r. spectrum of the latter, the signal assigned to AcO-1 appeared as two singlets of different intensities (ratio *ax*:*eq* ~9:1).

Deprotection of **2α** by trifluoroacetic acid yielded 1-*O*-(L-α-glutamyl)-α-D-glucopyranose (**3α**) as the highly unstable trifluoroacetate salt, which was immediately submitted to *N*-acetylation, esterification with diazomethane, and peracetylation; the first two steps were accompanied by considerable cleavage of the glycosyl bond. The final, stable product (15% yield based on **3α**) was a mixture of 1,3,4,6-tetra-*O*-acetyl-2-*O*-(5-methyl *N*-acetyl-L-glutam-1-oyl)-α-D-glucopyranose (**8α**), 2,3,4,6-tetra-*O*-acetyl-1-*O*-(5-methyl *N*-acetyl-L-glutam-1-oyl)-α-D-glucopyranose (**4α**), and the β anomer of **8** in the ratios ~2:1:0.2. Assignment of the structures **8α** and **4α** to the major components was based on analytical, t.l.c., and spectral data of the product, as well as by comparison with the authentic samples prepared by the direct condensation of the appropriate sugar and amino acid components. The structure **8β** for the minor component was deduced, *inter alia*, from the p.m.r. spectrum of the heterogeneous product, in which the integrated peak intensities of the two AcO-1 singlets (ratio *ax*:*eq* ~6:1), well-separated from the other acetyl signals, accounted for less than 3 protons, whereas the integral of the remaining acetyl signals, which appeared as unresolved singlets at higher field, corresponded (~13 H) to more than three acetoxyl and one *N*-acetyl groups.

The finding that hydrolysis of the α-D-glucopyranosyl ester **2** was practically as fast as the formation of the 2-*O*-acyl derivative, whereas, in the presence of diazomethane, the difference in rate of the two reactions was sufficiently high to give, upon acetylation, the protected 2-*O*-acyl derivative **7α** in 64% yield, suggested that diazomethane was directly involved in the 1→2 acyl-migration process. In order to ascertain

that esterification of the aglycon 5-CO_2H group was not responsible for the enhanced migration mobility, 1-*O*-[5-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-1-oyl]-α-D-glucopyranose (**10**) and the corresponding glutam-5-oyl isomer **12** were synthesised *via* their fully benzylated derivatives **9** and **11**. Treatment of **10** and **12** in *N*,*N*-dimethylformamide with ethereal diazomethane at 0° for 1 h, followed by acetylation of the product, gave **7α** and 1,3,4,6-tetra-*O*-acetyl-2-*O*-[1-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-5-oyl]-α-D-glucopyranose[4] in yields of 70 and 90%, respectively. In parallel experiments performed in the *absence* of diazomethane, **10** and **12** remained unchanged, thus providing evidence for the catalytic role of diazomethane and the inertness of the solvents used.

It was shown previously that, in contrast to their Boc-analogues, catalytic hydrogenation of the fully benzylated α-D-glucopyranosyl esters of *N*-acetyl-glycine and -alanine proceeds without any notable rearrangement, to give the relatively stable 1-esters **13** and **14** for which hydrolysis appeared to be the only process occurring during prolonged storage[1]. The reluctance of the acetylaminoacyl residue to undergo migration may be explained by a lower electrophilicity of its ester carbonyl group, as compared to that in the analogous Boc-protected aminoacyl residue. However, treatment of **13** and **14** with diazomethane, under the conditions described above, followed by acetylation, yielded 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*N*-acetylglycyl)-α-D-glucopyranose (**15**) and the corresponding alanyl derivative[1], respectively, in >70% yields. The peracetylated products were identical with the authentic samples prepared by the condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose with the appropriate *N*-acetylamino acid. In addition, 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranose[5], the acyl group of which revealed a remarkable stability toward migration, rearranged into the 2-*O*-acyl derivative within 1 h of the addition of diazomethane.

Compared with the parent α-D-glucopyranosyl esters, the 2-*O*-acyl derivatives showed slightly lower t.l.c. mobilities and less-positive optical rotations. The relative

TABLE I

CHEMICAL SHIFT (τ VALUES) AND COUPLING CONSTANT (Hz) DATA FOR THE ANOMERIC PROTONS IN ^{1}H-N.M.R. SPECTRA OF 1- AND 2-*O*-ACYLAMINOACYL-α-D-GLUCOPYRANOSES[a]

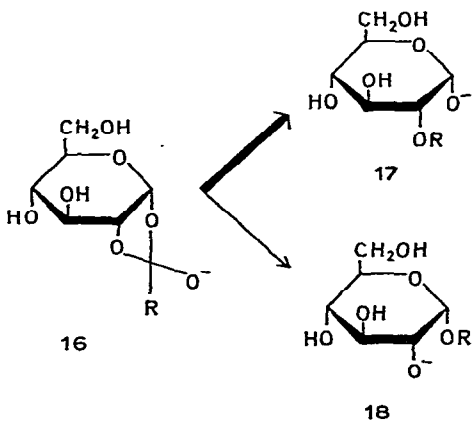
Substituent at O-1 or O-2	*O-1*		*O-2*	
	H-1	$J_{1,2}$	*H-1*	$J_{1,2}$
OMe | Boc-Glu-	3.78	3	4.57	4
OMe | Boc-Glu-OMe	3.78	3	4.54	4
Ac-Ala-	3.88	3	4.68	4
Ac-Gly-	3.58	3	4.72	4
Boc-Phe-	3.82	3	4.58	4

[a]Measured in deuterium oxide with tetramethylsilane as the external standard.

amounts of the 1- and 2-esters were indicated by the p.m.r. data for the diazomethane-treated material (Table I); the chemical shifts and coupling constants of the anomeric protons allow an α-D-glucopyranosyl ester and its HO-1 unsubstituted isomer to be distinguished.

Transesterification of some simple esters in the presence of alcohol and diazomethane has been ascribed[6,7] to an enhanced nucleophilicity of the alcoholic component, achieved through the formation of a diazomethane–alcohol complex[8]. The finding[9] that dimethyl oxalate underwent such transesterification, whereas the methyl esters of homologous dicarboxylic acids were resistant to these conditions, was rationalised in terms of relative electrophilicity of the carbonyl carboxylate groups involved. Transesterification with methanol in the presence of diazomethane was claimed[10] to be a particularly suitable reaction in solid-phase synthesis, for liberating ester-bound peptides from the polymer support as their methyl esters.

The results of the present study may be rationalised in terms of a base-catalysed ester interchange, in which diazomethane functions as a base toward the 2-hydroxyl group, thus rendering O-2 more nucleophilic. The fact that, in all cases investigated, the catalysed 1→2 acyl-transfer proceeded with high retention of anomeric configuration implies an intermediate, such as **16**, that may control the stereochemistry at C-1. Schroeder *et al.*[11], by studying the acid-catalysed hydrolysis of 1,2-*O*-(1-alkoxyethylidene)-3,4,6-tri-*O*-methyl-α-D-glucopyranoses, came to the conclusion that, due to the greater basicity of O-2 relative to that of O-1, the conjugate base of the type **16** should yield more of the anion of type **17** than that of type **18** and, consequently, more of the 2- than the 1-*O*-acyl isomer.



R = Boc- or Acetyl-aminoacyl

It is likely that the diazomethane-catalysed 1→2 intramolecular transesterification would also affect other glycosylic ester conjugates possessing a 1,2-*cis* arrangement, and possible isomerisations of this kind should be taken into account when using the reagent for preparation of methyl ester derivatives.

EXPERIMENTAL

General. — Melting points are uncorrected. Concentrations were carried out at reduced pressure on a rotary evaporator at <35°, if not stated otherwise, and solutions were dried with sodium sulphate. Column chromatography was performed on silica gel (Merck, 0.05–0.2 mm), and t.l.c. on Kieselgel G (Merck) or cellulose (Microcrystalline, Merck), with *A*, benzene–ethyl acetate (proportions are given in the text); *B*, ethyl acetate; *C*, 3:1 acetonitrile–water; and *D*, 60:15:25 1-butanol–acetic acid–water. Detection on t.l.c. plates was effected by charring with sulphuric acid, with ninhydrin reagent, or with alkaline silver nitrate. Optical rotations were determined for 1% solutions in chloroform, unless otherwise stated. I.r. spectra were recorded with a Perkin–Elmer Model 297 spectrometer, and p.m.r. spectra with a Varian A-60A spectrometer for solutions in chloroform-*d* with tetramethylsilane as the internal standard, if not stated otherwise.

Glutamic acid derivatives. — 5-Methyl 1-pentachlorophenyl *N-tert*-butoxycarbonyl-L-glutamate (80%) was prepared by the dicyclohexylcarbodiimide-mediated condensation of 5-methyl *N-tert*-butoxycarbonyl-L-glutamic acid[12] and pentachlorophenol in dichloromethane: after recrystallisation from chloroform–light petroleum, the product had m.p. 134–135°, $[\alpha]_D$ −25°. P.m.r. data: τ 6.32 (s, 5-CO_2Me) and 8.57 (s, Me_3C).

Anal. Calc. for $C_{17}H_{18}Cl_5NO_6$: C, 40.07; H, 3.56; N, 2.75. Found: C, 40.31; H, 3.77; N, 2.94.

5-Methyl *N*-acetyl-L-glutamic acid dicyclohexylamine salt (90%) was prepared by treating 5-methyl L-glutamic acid and dicyclohexylamine with acetic anhydride–chloroform, as described[13] for the 1-methyl isomer; m.p. 176–180°, $[\alpha]_D$ +24°. P.m.r. data: τ 3.28 (d, *J* 8 Hz, NH), 6.32 (s, 5-CO_2Me), and 7.98 (s, N*Ac*).

Anal. Calc. for $C_{20}H_{36}N_2O_5$: C, 62.46; H, 9.44; N, 7.29. Found: C, 62.70; H, 9.55; N, 7.42.

1-O-(L-α-Glutamyl)-β-D-glucopyranose trifluoroacetate salt (**3β**). — 1-*O*-[*N*-(*tert*-Butoxycarbonyl)-L-α-glutamyl]-β-D-glucopyranose[3] (**2β**, 70 mg), $[\alpha]_D$ −12° (*c* 2, methanol) [previously reported[3] erroneously as +12° (methanol)], was treated with trifluoroacetic acid (98%, 2 ml) at −10° for ~1 h (monitoring by t.l.c., cellulose, solvent *C*). The solution was then concentrated, and traces of trifluoroacetic acid were removed by co-distillation with dry ether. The residue was dissolved in iced water (2 ml) and immediately lyophilised to give **3β** (72 mg, 96%) as a hygroscopic, fluffy mass, R_F 0.30 (solvent *C*), $[\alpha]_D$ +10.8° (*c* 4, methanol); ν_{max}^{KBr} 3500 (vs, broad; OH), 1760 (C=O), 1640 and 1525 (amino acid I and II), and 735 cm^{-1} (CF_3). P.m.r. data (D_2O): τ 4.28 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{13}H_{20}F_3NO_{11}$: C, 36.64; H, 4.98; N, 3.31. Found: C, 36.44; H, 4.83; N, 3.10.

Subsequent *N*-acetylation, esterification, and peracetylation of **3β** was performed as follows. To a sample (142 mg) of **3β** was added 10% acetic anhydride in 1:1 acetone–water (10 ml) at 0°, and the solution was kept overnight in a refrigerator

and then concentrated (0.1 Torr); traces of anhydride were removed by co-distillation with water. To a solution of the residue in anhydrous *N,N*-dimethylformamide (1 ml) was added an ethereal solution of diazomethane (2 ml), and the mixture was kept at 0° for 1 h. After evaporation (0.1 Torr) of the solvents, the residual oil was treated at 0° with 4:1 (v/v) pyridine–acetic anhydride (4 ml) for 4 h. Chloroform was added, the solution was washed twice with iced water, and the organic layer was dried and evaporated to a syrup that was passed through silica gel with solvent *B*. The fractions containing chromatographically homogeneous material were combined, and evaporated to a residue (44 mg, 25% based on **3β**) that crystallised from chloroform–light petroleum, to give a product, m.p. 86–88°, which was identical with authentic 2,3,4,6-tetra-*O*-acetyl-1-*O*-(5-methyl *N*-acetyl-L-glutam-1-oyl)-β-D-glucopyranose (**4β**), by mixture m.p., $[\alpha]_D$, and comparative i.r. and p.m.r. spectra.

Conversion of **2β** *into* **6β**. — (*a*) A sample (41 mg) of **2β** in *N,N*-dimethylformamide (0.5 ml) was treated with an ethereal solution of diazomethane (2 ml) and then with pyridine–acetic anhydride as described above. Elution of the residual oil from silica gel with solvent *A* (1:1) afforded a chromatographically homogeneous foam (25 mg, 42% based on **2β**), $[\alpha]_D$ +11°, whose i.r. and p.m.r. spectra were indistinguishable from those of authentic 2,3,4,6-tetra-*O*-acetyl-1-*O*-[5-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-1-oyl]-β-D-glucopyranose (**6β**).

(*b*) When a sample (50 mg) of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-glutam-1-oyl]-β-D-glucopyranose[3] (**5β**) was subjected to esterification as described above, the product (28 mg, 55%) obtained by silica gel chromatography was indistinguishable (t.l.c., $[\alpha]_D$, p.m.r. spectrum) from that of authentic **6β**.

*2,3,4,6-Tetra-*O*-acetyl-1-*O*-(5-methyl* N*-acetyl-*L*-glutam-1-oyl)-*D*-glucopyranose* (**4**). — To a solution of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (700 mg), 5-methyl *N*-acetyl-L-glutamic acid (400 mg, liberated from the dicyclohexylamine salt), and imidazole (280 mg) in dichloromethane (8 ml) was added a solution of dicyclohexylcarbodiimide (DCC) (400 mg) in dichloromethane (2 ml) at 0°. The mixture was stirred at 0° for 1 h and then at room temperature for 24 h. *N,N'*-Dicyclohexylurea was filtered off, and the filtrate was washed with water, 10% citric acid, water, saturated, aqueous hydrogen carbonate, and water, dried, and concentrated. The residue was passed through a column of silica gel with solvent *A* (1:2), and the fractions containing the faster-moving β anomer of **4** were combined and concentrated. Crystallisation of the residue from chloroform–light petroleum afforded pure **4β** (130 mg, 12%), m.p. 87–89°, $[\alpha]_D$ +6.5° (*c* 2); ν_{max}^{KBr} 3500 (NH), 1790 (C=O), 1690 and 1550 (Amide I and II), and 1380 cm^{-1} (OAc). P.m.r. data: τ 4.20 (d, $J_{1,2}$ 7 Hz, H-1), 6.33 (s, CO_2Me), 7.93, 7.99, and 8.01 (4 O*Ac* + N*Ac*).

Anal. Calc. for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.63. Found: C, 49.64; H, 5.65; N, 2.74.

Concentration of the slower-moving fractions, followed by chromatography on silica gel, afforded **4α** (190 mg, 17%) as an oil, $[\alpha]_D$ +48.3°. P.m.r. data: τ 3.71 (d, $J_{1,2}$ 3 Hz, H-1), 6.32 (s, CO_2Me), 7.94, 7.99, and 8.01 (4 O*Ac* + N*Ac*) (Found: C, 49.39; H, 5.83; N, 2.88).

2,3,4,6-Tetra-O-acetyl-1-O-[5-methyl N-(tert-*butoxycarbonyl*)-L-*glutam-1-oyl*]-D-*glucopyranose* (**6**). — Condensation of 2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose (260 mg) and 5-methyl 1-pentachlorophenyl *N*-(*tert*-butoxycarbonyl)-L-glutamate (180 mg) was performed in dichloromethane (10 ml) in the presence of imidazole (260 mg) at room temperature for 24 h. The precipitated pentachlorophenol was filtered off, the filtrate was worked-up as described above, and the residue was passed through a column of silica gel with solvent *A* (1 : 1) to give chromatographically pure **6** (200 mg, 45.5%) as an anomeric mixture. The faster-moving fractions were combined and concentrated, and the residue was passed through a column of silica gel to give **6β** (60 mg) as a solid foam, $[\alpha]_D$ +9.6° (*c* 3); ν_{max}^{KBr} 3475 (NH), 1780 (C=O), 1730 sh and 1520 (Amide I and II), and 1370 cm^{-1} (OAc). P.m.r. data: τ 4.27 (d, $J_{1,2}$ 7 Hz, H-1), 6.32 (s, 5-CO_2Me), 7.94, 7.97, 8.01 (4 O*Ac*), and 8.57 (s, Me_3C).

Anal. Calc. for $C_{25}H_{37}NO_{15}$: C, 50.76; H, 6.30; N, 2.37. Found: C, 50.97; H, 6.49; N, 2.46.

Rechromatography of the material recovered from the slower-moving fractions afforded **6α** (100 mg) as a glass, $[\alpha]_D$ +76°. P.m.r. data: τ 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 6.32 (s, 5-CO_2Me), 7.94, 7.98, 8.01 (4 O*Ac*), and 8.58 (Me_3C) (Found: C, 50.69; H, 6.52; N, 2.55).

1-O-[N-(tert-*Butoxycarbonyl*)-L-α-*glutamyl*]-α-D-*glucopyranose* (**2α**). — To a solution of **1α**[3] (100 mg) in 2-methoxyethanol (10 ml) were added 10% palladium-on-charcoal (80 mg) and a few drops of acetic acid, and the mixture was shaken in an atmosphere of hydrogen until termination of hydrogen uptake (~20 h; monitoring by t.l.c., solvent *C*). After removal of the catalyst, the solvent was evaporated, and a solution of the residue in water (1 ml) was lyophilised to give **2α** (43 mg, 94%) as a highly hygroscopic solid, $[\alpha]_D$ +25.6° (*c* 2, methanol); ν_{max}^{KBr} 3500 (NH), 1770 and 1720 (C=O), 1650 sh and 1530 (Amide I and II), 1400 and 1370 cm^{-1} (Me_3C). P.m.r. data (D_2O): τ 3.79 (d, $J_{1,2}$ 2.5 Hz, H-1) and 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, 47.12; H, 6.88; N, 3.25.

A sample (73 mg) of **2α** was immediately esterified with diazomethane and then peracetylated, as described for the conversion of **3β** into **6β**. The crude product was applied to a column of silica gel and eluted with solvent *A* (1 : 1) to give 1,3,4,6-tetra-*O*-acetyl-2-*O*-[5-methyl *N*-(*tert*-butoxycarbonyl)-L-glutam-1-oyl]-α-D-glucopyranose (**7α**) as an amorphous solid (68 mg, 64.5%), $[\alpha]_D$ +52°, that contained traces of **7β**, as indicated by t.l.c. [solvent *A* (1 : 1), R_F 0.15 (major), 0.18 (trace)], p.m.r. data [τ 7.80 + 7.86 (s, ~0.9 × 3 H, *ax AcO*-1 + s, ~0.1 × 3 H, *eq AcO*-1)], and elemental analysis (Found: C, 50.97; H, 6.25; N, 2.39).

1-O-(L-α-*Glutamyl*)-α-D-*glucopyranose trifluoroacetate salt* (**3α**). — Treatment of **2α** (50 mg) with trifluoroacetic acid (2 ml), as described for **3β**, gave the title compound (50 mg, 95%) as a hygroscopic solid, $[\alpha]_D$ +68° (*c* 2.5, methanol); ν_{max}^{KBr} 3500 (vs, broad; OH), 1760 (C=O), 1645 and 1530 (amino acid I and II), and 735 cm^{-1} (CF_3). P.m.r. data (D_2O): τ 3.64 (d, $J_{1,2}$ 2.5 Hz, H-1).

Anal. Calc. for $C_{13}H_{20}F_3NO_{11}$: C, 36.64; H, 4.98; N, 3.31. Found: C, 36.44; H, 4.83; N, 3.10.

Subsequent *N*-acetylation, esterification with diazomethane, and peracetylation of a sample (100 mg) of **3α** was performed as described for the conversion of **3β** into **4β**. The final product was an amorphous solid (18 mg, 15% based on **2α**), identified as a mixture of 1,3,4,6-tetra-*O*-acetyl-2-*O*-(5-methyl *N*-acetyl-L-glutam-1-oyl)-α-D-glucopyranose (**8α**) and 2,3,4,6-tetra-*O*-acetyl-1-*O*-(5-methyl *N*-acetyl-L-glutam-1-oyl)-α-D-glucopyranose (**4α**) (t.l.c. in solvent *B*: R_F 0.58 and 0.62, ratio ~2:1) containing traces of **8β** (not separated by t.l.c. from **4α**), as indicated by p.m.r. data [τ 7.81 + 7.86 (s, 0.6 × 3 H, *ax AcO*-1 + s, 0.1 × 3 H, *eq AcO*-1), 7.92, 7.99, and 8.02 (1.1 × 12 H, O*Ac* + N*Ac*)] and elemental analysis (Found: C, 49.72; H, 5.99; N, 2.86).

1,3,4,6-Tetra-O-*acetyl-2*-O-[*5-methyl* N-(tert-*butoxycarbonyl*)-L-*glutam-1-oyl*]-α-D-*glucopyranose* (**7α**). — Condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (350 mg) and 5-methyl 1-pentachlorophenyl *N*-(*tert*-butoxycarbonyl)-L-glutamate (560 mg) in dichloromethane (10 ml) in the presence of imidazole (340 mg) was performed as described for **6** to give, after work-up, a residue that was passed through a column of silica gel with solvent *A* (1:1). The title compound (280 mg, 47.5%) was obtained as a chromatographically homogeneous glass, $[\alpha]_D$ +64.2° (*c* 4); ν_{max}^{KBr} 3450 (NH), 1770 (C=O), 1730 sh and 1520 (Amide I and II), and 1370 cm^{-1} (OAc). P.m.r. data: τ 3.65 (d, $J_{1,2}$ 4 Hz, H-1), 6.34 (s, CO_2Me), 7.82 (s, *ax AcO*-1), 7.95, 8.00 (3 × O*Ac*), and 8.60 (s, Me_3C).

Anal. Calc. for $C_{25}H_{37}NO_{15}$: C, 50.76; H, 6.30; N, 2.37. Found: C, 50.53; H, 6.44; N, 2.19.

1,3,4,6-Tetra-O-*acetyl-2*-O-(*5-methyl* N-*acetyl*-L-*glutam-1-oyl*)-α-D-*glucopyranose* (**8α**). — The compound was prepared from 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (704 mg) and 5-methyl *N*-acetyl-L-glutamic acid (416 mg) by the DCC (400 mg) condensation in the presence of imidazole (272 mg), as described for **4**. Silica gel chromatography of the crude product (solvent *B*) afforded chromatographically homogeneous **8α** (340 mg, 33%) as a solid foam, $[\alpha]_D$ +59.6°; ν_{max}^{KBr} 3500 (NH), 1760 (C=O), 1670 and 1540 cm^{-1} (Amide I and II). P.m.r. data: τ 3.62 (d, $J_{1,2}$ 3 Hz, H-1), 6.32 (s, CO_2Me), 7.81 (s, *ax AcO*-1), 7.92, 7.99, and 8.02 (3 × O*Ac* + N*Ac*).

Anal. Calc. for $C_{22}H_{31}NO_{14}$: C, 49.53; H, 5.86; N, 2.63. Found: C, 49.72; H, 5.99; N, 2.86.

2,3,4,6-Tetra-O-*benzyl-1*-O-[*5-methyl* N-(tert-*butoxycarbonyl*)-L-*glutam-1-oyl*]-α-D-*glucopyranose* (**9**). — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (540 mg) and 5-methyl 1-pentachlorophenyl *N*-(*tert*-butoxycarbonyl)-L-glutamate (560 + 56 mg) were treated in the presence of imidazole (340 mg), as described for **4**. After work-up, the crude product was eluted from silica gel with solvent *A* (10:1) to give the anomeric mixture of **9** (460 mg, 60%). The residue from the faster-moving fractions, which contained preponderantly the α anomer, was rechromatographed, as just described,

to give the title compound (130 mg) as an oil, $[\alpha]_D$ +45°. P.m.r. data: τ 2.61–2.79 (m, 4 Ph), 3.57 (d, $J_{1,2}$ 3 Hz, H-1), 6.43 (s, CO_2Me), and 8.62 (Me_3C).

Anal. Calc. for $C_{45}H_{53}NO_{11}$: C, 68.94; H, 6.82; N, 1.79. Found: C, 69.17; H, 7.01; N, 1.84.

1-O-[*5-Methyl* N-(tert-*butoxycarbonyl*)-L-*glutam-1-oyl*]-α-D-*glucopyranose* (**10**). — Catalytic hydrogenation of **9** (100 mg), performed as described for **2α**, gave the title compound (40 mg, 93%) as a viscous oil, $[\alpha]_D$ +26° (methanol). P.m.r. data (D_2O): τ 3.78 (d, $J_{1,2}$ 3 Hz, H-1), 6.24 (s, CO_2Me), and 8.56 (s, Me_3C).

Anal. Calc. for $C_{17}H_{29}NO_{11}$: C, 48.22; H, 6.90; N, 3.31. Found: C, 48.03; H, 6.93; N, 3.32.

2,3,4,6-Tetra-O-*benzyl-1*-O-[*1-methyl* N-(tert-*butoxycarbonyl*)-L-*glutam-5-oyl*]-α-D-*glucopyranose* (**11**). — By using 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (540 mg), 1-methyl 5-pentachlorophenyl *N*-(*tert*-butoxycarbonyl)-L-glutamate[4] (560 + 56 mg), and imidazole (340 mg), the reaction was performed as described for **4**. After chromatography (solvent *A*, 10:1), the anomeric mixture (550 mg, 70%) of **11** was passed through a second column of silica gel to give, in the faster-moving fractions, pure **11α** (300 mg, 38%) as an oil, $[\alpha]_D$ +35°; ν_{max}^{film} 3450 (NH), 1750 (C=O), 1710 and 1510 (Amide I and II), and 1370 cm^{-1} (Me_3C). P.m.r. data: τ 2.68–2.88 (m, 4 Ph), 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 6.33 (s, CO_2Me), and 8.60 (Me_3C).

Anal. Calc. for $C_{45}H_{53}NO_{11}$: C, 68.94; H, 6.82; N, 1.79. Found: C, 68.91; H, 6.64; N, 1.78.

1-O-[*1-Methyl* N-(tert-*butoxycarbonyl*)-L-*glutam-5-oyl*]-α-D-*glucopyranose* (**12**). — Catalytic hydrogenation of **11α** (140 mg), as described for **2α**, gave chromatographically homogeneous (solvent *C*) **12** (60 mg, 95%) as a viscous oil, $[\alpha]_D$ +22° (methanol). P.m.r. data (D_2O): τ 3.82 (d, $J_{1,2}$ 3 Hz, H-1), 6.20 (s, CO_2Me), and 8.54 (s, Me_3C).

Conditions for diazomethane-catalysed 1→2 acyl transfer. — The amount of CH_2N_2 in ethereal solutions (redistilled before use) was determined by titration of an aliquot with benzoic acid, and the concentration of the reagent was adjusted to ~0.5 mmol/ml. The respective 1-*O*-acylaminoacyl-α-D-glucopyranose (0.1 mmol, 40–50 mg) was dissolved in *N,N*-dimethylformamide (1 ml), ethereal diazomethane (1–2 ml) was added, and the solution was kept at 0° for 1 h. The residue, upon concentration (0.1 Torr), was analysed for the content of 1- and 2-*O*-acyl isomers by p.m.r. spectroscopy (deuterium oxide) and t.l.c. (solvents *C* and *D*), and the components of the mixture were characterised by conversion into their peracetylated derivatives.

1,3,4,6-Tetra-O-*acetyl-2*-O-(N-*acetylglycyl*)-α-D-*glucopyranose* (**15**). — To a solution of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (680 mg), *N*-acetylglycine (234 mg), and imidazole (280 mg) in dichloromethane–*N,N*-dimethylformamide (1:1, 10 ml) was added DCC (410 mg), and the reaction was further processed as described for **4**. The crude product was passed through a column of silica gel with solvent *B* to give chromatographically homogeneous **15** (300 mg, 33%), which crystallised from isopropyl ether; m.p. 122–124°, $[\alpha]_D$ +89°. P.m.r. data: τ 3.64 (d, $J_{1,2}$ 3 Hz,

H-1), 6.06 (d, *J* 6.5 Hz, CH_2 of glycine), 7.81 (s, *ax* *Ac*O-1), 7.92, 7.97, and 7.99 (4 × O*Ac* + N*Ac*).

Anal. Calc. for $C_{18}H_{25}NO_{12}$: C, 48.33; H, 5.63; N, 3.13. Found: C, 48.11; H, 5.74; N, 3.17.

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REFERENCES

1 D. Keglević, Š. Valenteković, G. Roglić, D. Goleš, and F. Plavšić, *Carbohydr. Res.*, 29 (1973) 25–39.
2 D. Keglević, Dj. Ljevaković, and Š. Valenteković, *Croat. Chem. Acta*, 46 (1974) 115–127.
3 Š. Valenteković and D. Keglević, *Carbohydr. Res.*, 47 (1976) 35–48.
4 D. Keglević, J. Horvat, and F. Plavšić, *Carbohydr. Res.*, 47 (1976) 49–61.
5 D. Keglević, M. Pongračić, and J. Horvat, unpublished data.
6 H. Bredereck, R. Sieber, L. Kamphenkel, and R. Bamberger, *Chem. Ber.*, 89 (1956) 1169–1176.
7 T. Wieland and R. K. Rothhaupt, *Chem. Ber.*, 89 (1956) 1176–1182.
8 B. Eistert, F. Arndt, L. Loewe, and E. Ayça, *Chem. Ber.*, 84 (1951) 156–169.
9 J. C. Young, *Can. J. Chem.*, 53 (1975) 2530–2533.
10 J. Rebek and D. Feitler, *J. Am. Chem. Soc.*, 96 (1974) 1606–1607.
11 L. R. Schroeder, D. P. Hultman, and D. C. Johnson, *J. Chem. Soc., Perkin Trans. 2*, (1972) 1063–1071.
12 J. C. Anderson, M. A. Barton, P. M. Hardy, G. W. Kenner, J. W. Preston, and R. C. Sheppard, *J. Chem. Soc., C*, (1967) 108–115.
13 M. J. Hawkins, J. R. Knowles, L. Wilson, and D. Witcher, *Biochem. J.*, 104 (1967) 762–766.