Carbohydrate Research, 29 (1973) 25–39

GLYCOSYL ESTERS OF AMINO ACIDS

PART V*. SYNTHESIS AND PROPERTIES OF 1-*O*-ACYLAMINOACYL-α- AND -β-D-GLUCOPYRANOSES AND 1-*O*-(L-β-ASPARTYL)-β-D-GLUCOPYRANOSE

D. KEGLEVIĆ, Š. VALENTEKOVIĆ, G. ROGLIĆ, D. GOLEŠ, AND F. PLAVŠIĆ**
Tracer Laboratory, Institute "Rudjer Bošković", Zagreb (Yugoslavia)
(Received October 6th, 1972; accepted for publication, November 1st, 1972)

ABSTRACT

Catalytic hydrogenation of the tetrabenzyl ethers of 1-*O*-acetamidoacyl- and 1-*O*-*tert*-butyloxycarbonylaminoacyl-α- and -β-D-glucopyranoses (**1–6**) afforded the corresponding 1-*O*-acylaminoacyl-D-glucopyranoses **8–13** which were fully characterised by physical methods and by conversion into the peracetylated derivatives **14–19**. The α anomers of 1-*O*-*tert*-butyloxycarbonylaminoacyl-D-glucopyranoses underwent 1→2 acyl migration, and, in order to characterize the rearrangement product of 1-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-α-D-glucopyranose (**12α**), 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-α- and -β-D-glucopyranoses (**22** and **23**) were synthesized by definitive methods. Initial studies of the simultaneous deprotection of the amino and hydroxyl functions were performed with D-glucose–amino acid 6-esters; catalytic hydrogenation of methyl 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-benzyloxycarbonylglycyl)-β-D-glucopyranose (**24**) gave methyl 6-*O*-glycyl-β-D-glucopyranose (**25**) as the stable hydrochloride. Hydrogenolysis of the β anomer of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-D-glucopyranose (**7**) afforded 1-*O*-(L-β-aspartyl)-β-D-glucopyranose (**27**). The rates of hydrolysis of the unprotected D-glucose–amino acid 1-ester **27** in water and in 0.1M hydrochloric acid were compared with those of the D-glucose–amino acid 6-ester **25**.

INTRODUCTION

Previous reports[1–3] from this laboratory have shown that fully protected 1-*O*-acylaminoacyl-D-glucopyranoses and -glucuronates can be prepared in high yields by two routes involving direct participation of imidazole in the formation of the 1-ester linkage: (*a*) the "accelerated active-ester" (AAE) method and (*b*) the imidazole-promoted dicyclohexylcarbodiimide (DCC) condensation. It was established[2] that, for fully benzylated D-glucosyl esters having the amino function in the aglycon group protected by the benzyloxycarbonyl or *tert*-butyloxycarbonyl group, the configuration of the amino acid component was retained to a high degree in both methods.

Model compounds in which an *N*-acylated or free amino acid is linked by the

*Part IV: Ref. 2.
**Present address: PLIVA, Pharmaceutical and Chemical Works, Zagreb (Yugoslavia).

glycosidic ester bond to a neutral, unprotected carbohydrate moiety have not yet been described. Yoshimura and Funabashi[4] reported the synthesis of some 2-acetamido-1-*O*-aminoacyl-2-deoxy-D-glucoses by the DCC condensation of 2-acetamido-4,6-*O*-benzylidene-2-deoxy-D-glucose and the appropriate *N*-benzyloxycarbonylamino acid, followed by the removal of the protecting groups by hydrolysis and catalytic hydrogenolysis. However, the authors gave very few data in assigning the structure and configuration to these compounds.

We have examined whether selective removal of blocking groups (either from the aglycon or sugar moiety) in a fully protected glucosyl or glucuronic ester of an amino acid could be achieved without affecting the 1-ester linkage. Recently, we reported[3] that catalytic hydrogenation of fully methylated 1-*O*-benzyloxycarbonylglycyl-β-D-glucofuranuronate in the presence of oxalic acid led to the crystalline mono-oxalate salt of the fully methylated 1-*O*-glycyl ester. We now report on the deprotection of the hydroxyl functions in fully benzylated glucosyl esters of *N*-acylamino acids, and describe the preparation and characterization of 1-*O*-(L-β-aspartyl)-β-D-glucopyranose. In addition, for comparison purposes, some compounds belonging to the known class of D-glucose–amino acid 6-esters[5,6] have been prepared.

RESULTS AND DISCUSSION

Two groups of fully benzylated 1-*O*-acylaminoacyl-D-glucopyranoses were selected as model systems for the deprotection studies by catalytic hydrogenolysis, namely the 1-*O*-acetamidoacyl- and 1-*O*-*tert*-butyloxycarbonylaminoacyl-D-glucopyranoses, **1–3** and **4–6**, respectively.

The fully benzylated 1-*O*-acetylglycyl derivative **1** was obtained as an anomeric mixture by the imidazole-promoted DCC condensation, and the anomers were resolved and characterized. The homologous acetyl-L- and -D-alanine derivatives **2** and **3** were reported earlier[2]. Catalytic hydrogenation of the α and β anomers of **1–3** proceeded smoothly in neutral medium over palladium-on-charcoal, to give the amorphous glucosyl esters **8–10** which were characterized by the data in Table I. The i.r. spectra revealed the presence of hydroxyl absorption at 3350–3450 cm^{-1} and the absence of bands associated with the aromatic ring. The n.m.r. spectra in deuterium oxide showed H-1 signals having $J_{1,2}$ values consistent with the observed optical rotations; one 3-proton singlet at τ 7.98–8.00 assigned to the *N*-acetyl group was present in all cases.

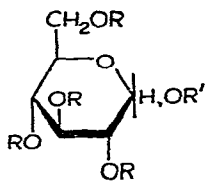
For further characterization, both anomers of **8–10** were treated with acetic anhydride in pyridine; the β anomers gave crystalline products identical with the already known 1-*O*-acetamidoacyl-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoses **14**[7], **15**[8], and **16**[8], respectively. The corresponding α anomers yielded highly dextrorotatory, hygroscopic foams which were identified by comparison of their n m.r. spectra with those of the α anomers of **14**, **15**[2], and **16** obtained directly from 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and the appropriate *N*-acetylamino acid by the imidazole-promoted DCC condensation. In order to reinforce evidence that no 1→2 acyl migra-

TABLE I

1-*O*-(2-ACYLAMINOACYL)-D-GLUCOPYRANOSES

Compound No.	*Acylaminoacyl group*	*Anomer*[a]	$[\alpha]_D^{22}$ *(degrees)*[b]		*N.m.r. data*[c], τ(J)		*Found* (%)			*Formula*	*Calc.* (%)		
			Methanol	*Water*	*H-1 (doublet)*	*N-Ac*[d] *or* Me_3C[e]	*C*	*H*	*N*		*C*	*H*	*N*
8	Ac-Gly-	β	0	−8.9	4.41 (7)	7.99[d]	42.87	6.19	5.11	$C_{10}H_{17}NO_8$	43.01	6.15	5.01
		α	+107.0	+101.0	3.87 (3)	7.98[d]	42.87	6.07	5.08				
9	Ac-L-Ala-	β	−31.2	−46.6	4.39 (7)	7.99[d]	44.82	6.72	4.73				
		α	+83.0	+74.1	3.83 (3)	7.99[d]	45.10	6.56	4.97				
10	Ac-D-Ala-	β	+30.4	+43.5	4.40 (7)	7.99[d]	45.15	6.72	4.88	$C_{11}H_{19}NO_8$	45.05	6.53	4.77
		α	+106.0	+110.0	3.88 (3)	8.00[d]	44.77	6.32	4.88				
11	BOC-Gly-	β	+5.7	−9.0	4.39 (7)	8.59[e]	46.46	7.03	3.99	$C_{13}H_{23}NO_9$	46.28	6.87	4.15
12	BOC-L-Ala-	β	−33.4	−32.7	4.40 (7)	8.58[e]	47.59	7.18	4.15	$C_{14}H_{25}NO_9$	47.86	7.17	3.98
13	BOC-L-Glu(OH)-	β	+12.1	−18.8	4.40 (7)	8.58[e]	46.94	6.97	3.36	$C_{16}H_{27}NO_{11}$	46.94	6.65	3.42

[a]Except for **11**, m.p. 91–93° (ethyl acetate–light petroleum), all compounds were solid foams with no definite melting-points. [b]*c*, 1–2. [c]Measured in D_2O. [d]Three-proton singlets. [e]Nine-proton singlets.

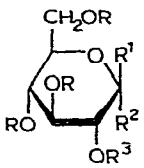


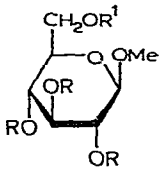
1–7 R = $PhCH_2$;
8–13 R = H;
14–19 R = Ac

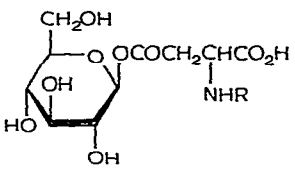
1 R′ = Ac-Gly-
2 R′ = Ac-L-Ala-
3 R′ = Ac-D-Ala-
4 R′ = BOC-Gly-
5 R′ = BOC-L-Ala-OBZL
6 R′ = BOC-L-Glu-(OBZL)-
7 R′ = Z-L-Asp-OBZL
8 R′ = Ac-Gly-
9 R′ = Ac-L-Ala-
10 R′ = Ac-D-Ala-
11 R′ = BOC-Gly-
12 R′ = BOC-L-Ala-OH
13 R′ = BOC-L-Glu-(OH)-
14 R′ = Ac-Gly-
15 R′ = Ac-L-Ala-
16 R′ = Ac-D-Ala-
17 R′ = BOC-Gly-
18 R′ = BOC-L-Ala-OH
19 R′ = BOC-L-Glu-(OH)-

BOC = *tert*-butyloxycarbonyl;
BZL = benzyl;
Z = benzyloxycarbonyl.

tion had taken place during hydrogenolysis of the α anomers of **1–3**, 1,3,4,6-tetra-*O*-acetyl-2-*O*-(acetyl-D-alanyl)-α-D-glucopyranose (**20**) was synthesised and compared with the acetylated hydrogenation product of **3α** (Table II). The positions of the acetyl signals in the n.m.r. spectrum of **20** in deuteriochloroform and methyl sulphoxide-d_6 are clearly different from those for the acetylated product of **3α**; on the other hand, the spectrum of the latter compound is identical with that of authentic **16α**.







20 R = R^2 = Ac, R^1 = H, R^3 = Ac-D-Ala-
21 R = H, R^1 = R^2 = H,OH, R^3 = BOC-L-Ala-
22 R = R^2 = Ac, R^1 = H, R^3 = BOC-L-Ala-
23 R = R^1 = Ac, R^2 = H, R^3 = BOC-L-Ala-
24 R = $PhCH_2$, R^1 = ZGly-
25 R = H, R^1 = HCl x H-Gly-
26 R = Ac, R^1 = AcGly-
27 R = H
28 R = Ac

2,3,4,6-Tetra-*O*-benzyl-1-*O*-[5-benzyl *N*-(*tert*-butyloxycarbonyl)-L-glutam-1-oyl]-D-glucopyranose (**6**) was synthesized by the AAE method from tetra-*O*-benzyl-α-D-glucopyranose and 5-benzyl 1-pentachlorophenyl *tert*-butyloxycarbonyl-L-glutamate; the anomeric products were resolved by chromatography on silica gel and carbon–Celite. Hydrogenolysis of the β anomers of the fully benzylated 1-*O*-*tert*-butyloxycarbonyl derivatives 4^2, 5^2, and **6** yielded chromatographically homogeneous, solid foams having physical data consistent with the β-D-anomeric structures of the hydroxyl-free D-glucosyl esters **11**, **12**, and **13** (Table I). Acetylation of these compounds afforded the crystalline β anomers of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*tert*-butyloxycarbonylaminoacyl)-D-glucopyranoses **17**, **18**, and **19**. For comparison, anomeric mixtures of **17** and **18** were prepared directly by the AAE method from 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and *tert*-butyloxycarbonyl-glycine and -L-alanine penta-

TABLE II

N.M.R. PARAMETERS OF THE PERACETYLATED 1- AND 2-*O*-(ACYLAMINOACYL)-D-GLUCOPYRANOSES

Compound			*Solvent*[a]	*Chemical shifts*[b], τ				
	Anomeric form	*Acylaminoacyl, position of linkage and chemical structure*		*H-1 Doublet*[c]	*O-Ac* and *N-Ac*[d]		*Other signals* [f]	[g]
16	α	1-*O*-(Acetyl-D-alanyl)-	A	3.63 (3)	7.92–7.98 [5][e]		8.50	
			B	3.93 (3)	8.12–8.14 [4][e], 8.24		8.79	1.68
Acetylated hydrogenolysis product of 3α			A	3.63 (3)	7.92–7.98 [5][e]		8.49	
			B	3.91 (3)	8.10–8.12 [4][e], 8.22		8.75	1.65
20	α	2-*O*-(Acetyl-D-alanyl)-	A	3.61 (3)	7.80, 7.90, 7.95, 7.97, 8.00		8.65	
			B	3.99 (3)	7.92, 8.11 [3][e], 8.29		8.90	1.65
					O-Ac[d]	*Me_3C*[h]		
18	α[i]	1-*O*-(*tert*-Butyloxycarbonyl-L-alanyl)-	A	3.62 (3)	7.92, 7.97, 7.99, 8.01	8.60		
			B	3.77 (3)	8.02–8.07 [4][e]	8.65	8.78	2.68
Acetylated crude hydrogenolysis product of 5α			A	3.62 (3)	7.92, 7.97, 7.99, 8.01	8.55		
			B	3.79 (3)	8.02–8.07 [4][e]	8.63		2.60
22	α	2-*O*-(*tert*-Butyloxycarbonyl-L-alanyl)-	A	3.63 (3)	7.80, 7.90–7.98 [3][e]	8.57	8.67	
			B	3.95 (3)	7.92, 8.12–8.15 [3][e]	8.77	8.93	2.91
23	β	2-*O*-(*tert*-Butyloxycarbonyl-L-alanyl)-	A	4.29 (7)	7.92, 7.99, 8.03, 8.09	8.63	8.75	
			B	4.05 (7)	7.85–8.03 [4][e]	8.67	8.85	2.78
Acetylation product of **21**			A	3.65 (3), 4.23 (7)	7.87, 7.92, 7.99, 8.03	8.63	8.75	
			B	3.87 (3), 4.05 (7)	7.85–7.99–8.12 [4][e]	8.69	8.88	2.78

[a] A = chloroform-*d*, B = methyl sulphoxide-d_6. [b] Data taken from spectra measured at 60 MHz. [c] Coupling constants (*J*) in Hz. [d] Three-proton singlets unless otherwise indicated. [e] Unresolved, the numbers in parenthesis indicate number of acetyl groups. [f] Doublet, 3 H, *J* 7 Hz, *Me*-CH. [g] Doublet, 1 H, *J* 7 Hz, removed by D_2O exchange, NH. [h] Nine-proton singlets. [i] Not completely resolved from the β anomer.

chlorophenyl ester, respectively; the β anomers of **17** and **18** were indistinguishable from the acetylation products of **11β** and **12β**.

The pure β-anomers of the D-glucosyl esters **8–13** were stable when stored under anhydrous conditions; in aqueous solutions kept at room temperature, the cleavage of the glycosidic ester bond did not exceed 5% after one week (monitoring by t.l.c.). On the other hand, the α anomers of the *N*-acetyl derivatives **8–10** underwent ~20% hydrolysis within the first 24 h. Incubation of the β anomers of **8–13** with β-D-glucosidase resulted in a complete splitting into D-glucose and the parent N-acylamino acid within 1-2 h; the α anomers of **8–10** were not affected by the enzyme.

Debenzylation of the α anomers of the *tert*-butyloxycarbonyl derivatives **4** and **5** gave products which underwent 1→2 acyl migration during the isolation procedure. The sequence of the reactions was studied in detail with **5α**. The n.m.r. spectrum (D_2O) of the crude hydrogenolysis product of **5α** contained a doublet ($J_{1,2}$ 3 Hz) for H-1 at τ 3.90; during several days, the intensity of this signal decreased substantially as a multiplet appeared at τ 4.54–4.78 attributable to HO-1. Acetylation of the crude, hydrogenolysis product afforded the α anomer of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranose (**18**); comparison of the n.m.r. spectrum with that of authentic **18** (highly enriched in the α anomer) confirmed the structure proposed (Table II). Thus, 1-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-α-D-glucopyranose (**12α**) is the initial product of debenzylation of **5α**. The rearrangement of **12α** occurred rapidly in aqueous solutions containing silica gel to give 2-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranose (**21**). Acetylation of **21** yielded 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranose as an anomeric mixture **22** and **23**; the n.m.r. spectrum of the product is given in Table II. For comparison, the authentic anomers were synthesized separately by the imidazole-promoted DCC condensation of 1,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranose with *tert*-butyloxycarbony'-L-alanine (Table II).

The ease with which the α anomers of *tert*-butyloxycarbonyl derivatives **11** and **12** underwent acyl migration contrasts with the stability of the 1-*O*-acetamidoacyl-α-D-glucopyranoses **8–10**. Presuming that the rearrangement of the former compounds proceeds *via* a cyclic ortho-ester intermediate, the above results suggest that the *tert*-butyloxycarbonyl group confers a higher positive charge on the ester carbonyl carbon than does the acetyl group, thereby facilitating nucleophilic attack by the adjacent hydroxyl group. The difference between the acyl and alkoxycarbonyl residues as blocking agents of the amino function was studied by Determann[9,10] who found, on the basis of i.r. and n.m.r. data, that the nitrogen atom in urethane-type protecting groups retains its basic character in contrast to that of normal amides. The fact that acetylation of the rearrangement product **21** gave an anomeric mixture of the corresponding tetra-acetyl derivative (compounds **22** and **23**) suggests that acyl migration is followed by anomerization of the initial form (presumably β) of **21** released.

For studies of simultaneous deprotection of the amino and hydroxyl functions, we initially examined the more-stable class of sugar–amino acid 6-esters. Kochetkov and his collaborators[5] prepared a number of 6-*O*-aminoacyl-D-glucoses as neutral

oxalate salts from D-glucose and the appropriate *N*-benzyloxycarbonylamino acid by a DCC condensation followed by catalytic hydrogenation in the presence of oxalic acid. In order to exclude the effect of the glycosidic hydroxyl group, we used methyl 2,3,4-tri-*O*-benzyl-6-*O*-(*N*-benzyloxycarbonylglycyl)-β-D-glucopyranoside (**24**) as a model for the hydrogenolysis studies. Compound **24** was prepared by the imidazole-promoted DCC and the AAE methods by reacting methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside[11] with *N*-benzyloxycarbonylglycine and its pentachlorophenyl ester, respectively; the high yields of **24** indicate the general applicability of these methods for the synthesis of sugar–amino acid esters.

Hydrogenolysis of **24** in the presence of oxalic acid proceeded very slowly. However, when the reaction was performed in the presence of one equivalent of M hydrochloric acid, methyl 6-*O*-glycyl-β-D-glucopyranoside (**25**) was readily obtained as the crystalline hydrochloride; its structure was confirmed by conversion into methyl 2,3,4-tri-*O*-acetyl-6-*O*-(acetylglycyl)-β-D-glucopyranoside (**26**). In order to investigate the stability of the 6-ester bond, solutions of **25** (10mM) in water (pH 5.25) and 0.1M hydrochloric acid (pH 1.25) were kept at room temperature and 38°, respectively, for 6 days; the amount of glycine released did not exceed 5% in water and 10% in 0.1M hydrochloric acid.

Simultaneous deprotection in the glucose–amino acid 1-ester class was studied with the β anomer of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[1-benzyl *N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-D-glucopyranose (**7**). This compound was chosen as model substance because of its aglycon structure; it is to be expected that the presence of a free carboxylic group, as well as the remoteness of the amino group from the ester bond, will add to the stability of the glucosidic ester linkage. Crystalline **7β** was obtained by the AAE method from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose and 1-benzyl 4-pentachlorophenyl *N*-benzyloxycarbonyl-L-aspartate, followed by separation of the anomeric products. Hydrogenolysis of **7β** was carried out over palladium-on-charcoal in acetic acid–2-methoxyethanol to give crystalline 1-*O*-(L-β-aspartyl)-β-D-glucopyranose (**27**). The i.r. spectrum of **27** showed hydroxyl absorption at 3500 cm^{-1}, as well as absorptions at 1640 and 1500 cm^{-1} characteristic of α amino acids possessing dipolar, ionic structures; the n.m.r. spectrum revealed a one-proton doublet at τ 4.40 (J 7 Hz) indicative of the β-D-configuration. Attempts to acetylate **27** in the standard manner with pyridine–acetic anhydride gave a complex mixture of products (t.l.c.). However, treatment of an aqueous solution of **27** with 2% acetic anhydride in acetone[12] resulted in selective acetylation of the amino group to give 1-*O*-(*N*-acetyl-L-β-aspartyl)-β-D-glucopyranose (**28**).

Incubation of **27** with β-D-glucosidase resulted in quantitative liberation of aspartic acid, whereas in the absence of enzyme ~10% of the compound was hydrolysed. At room temperature, an aqueous solution of **27** is remarkably stable, but the compound is more labile at lower pH values (Fig. 1). This finding agrees with that of Yoshimura *et al.*[13] for the relative stability of 2-acetamido-6-*O*-(L-α-aspartyl)-2-deoxy-D-glucose; these authors ascribed the increase in hydrolytic rate in acidic media to the fact that, in lower pH regions, the proton-attractive effect of the carboxylate

anion diminishes the electrostatic repulsion of the protonated amino group. With an increase in temperature to 38°, the cleavage of the glucosidic ester bond in **27** is rapidly accelerated in both media; the effect of the temperature on the hydrolytic rate of **27** is even more apparent when compared with the glycyl 6-ester **25** which is hydrolysed at a nearly identical rate at both temperatures.

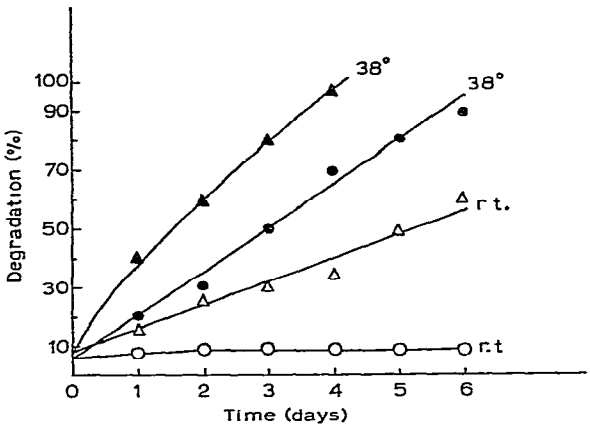


Fig. 1. The rate of cleavage of 10mM solutions of 1-*O*-(L-*β*-aspartyl)-*β*-D-glucopyranose (**27**) in water (pH 3.12) and 0.1M hydrochloric acid (pH 1.20) as a function of temperature; ○ and ● = water; △ and ▲ = 0.1M hydrochloric acid; r.t. = room temperature.

EXPERIMENTAL

General. — Melting points are uncorrected. Evaporations were performed in a rotary evaporator *in vacuo* at bath temperature below 40°, if not stated otherwise. Column chromatography was performed on silica gel (Merck, 0 05–0 2 mm); carbon-Celite (Charcoal activated, BDH; Kieselguhr, BDH), prepared as a 2:1 (w/w) mixture; or cellulose powder (Whatman, standard grade), packed as a slurry by using a plunger. Solvent systems: *A* benzene–ethyl acetate (proportions are given in the text); *B* 15:4:1 chloroform–methanol–acetic acid; *C* 5:4:1 methanol–ethyl acetate–water; *D* 5:1:1 ether–acetone–light petroleum; *E* 5:3:1 propan-2-ol–light petroleum–water; *F* 5:2:1 chloroform–acetone–methanol. T.l.c. was performed on Kieselgel G (Merck), followed by detection with 10% sulphuric acid and heating, with ninhydrin reagent, or with chlorine–starch–iodide reagent for acylated amino acids. Optical rotations were determined for 1% solutions in chloroform unless otherwise stated. I.r. spectra were determinated on a Perkin–Elmer Model 137 spectrometer. N.m.r. spectra were recorded on solutions in chloroform-*d*, unless otherwise stated, with tetramethylsilane as internal standard, using a Varian A-60A spectrometer.

1-Benzyl 4-pentachlorophenyl benzyloxycarbonyl-L-aspartate was prepared by the method of Fujino and Hatanake[14]. 5-Benzyl 1-pentachlorophenyl *tert*-butyloxy-carbonyl-L-glutamate was synthesized (yield 72%) by the DCC method from equi-

molar amounts of 5-benzyl *tert*-butyloxycarbonyl-L-glutamic acid[15] and pentachlorophenol in dichloromethane; after two recrystallisations from ethanol, the product had m.p. 133–135°, $[\alpha]_D - 14.9°$.

Anal. Calc. for $C_{23}H_{22}Cl_5NO_6$: C, 47.16; H, 3.79; N, 2.39. Found: C, 46.90; H, 3.60; N, 2.23.

Incubations with β-D-glucosidase (from sweet-almond meal, BDH) were performed in citric acid–sodium citrate buffer (50mM, pH 4.5) at 38° for 1 and 2 h. The amount of *N*-acylamino or amino acid liberated was estimated by co-chromatography with authentic samples.

Determination of ester bond-stability in compounds **25** and **27** was performed with 10mM solutions in a thermostat at 38° or at room temperature; aliquots were taken at intervals, and the amino acid liberated was determined as described above.

1-O-(Acetylglycyl)-2,3,4,6-tetra-O-benzyl-D-glucopyranose (**1**).—To 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (1.640 g) in dichloromethane (5 ml), solutions of *N*-acetylglycine (351 mg) in *N,N*-dimethylformamide (8 ml), imidazole (408 mg) in dichloromethane (2 ml), and DCC (618 mg) in dichloromethane (5 ml) were added at 0°; after 2 h, more *N*-acetylglycine (35 mg) was added, and the mixture was kept overnight at room temperature. *N,N'*-Dicyclohexylurea was filtered off, the filtrate was evaporated (traces of *N,N*-dimethylformamide were removed at 0.1 Torr), and the residue was extracted with chloroform; the extracts were washed with water, 1.5% sulphuric acid, water, aqueous sodium hydrogen carbonate, and water. After drying (sodium sulphate) and evaporation, the residue was eluted from a column of silica gel with solvent *A* (2:1); crystallisation of the chromatographically homogeneous, anomeric mixture (1.257 g, 65.5%) from dry ether–acetone (2:1) afforded the β anomer of **1** (851 mg), m.p. 136–138°, $[\alpha]_D$ 0°, $[\alpha]_D + 5.8°$ (ethyl acetate). N.m.r. data: τ 4.12 (broad, 1 proton, removed by D_2O exchange, NH), 4.38 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1), 8.02 (3-proton singlet, NAc).

Anal. Calc. for $C_{38}H_{41}NO_8$: C, 71.34; H, 6.46; N, 2.18. Found: C, 71.52; H, 6.33; N, 2.25.

The mother liquor was evaporated to dryness, and the residue was submitted to chromatography on carbon–Celite and silica gel (solvent *A*, 2:1) to give the α anomer of **1** as a colourless syrup, $[\alpha]_D + 56.0°$, $[\alpha]_D + 66.5°$ (ethyl acetate). N.m.r. data: τ 3.65 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), 3.85 (broad, 1 proton, removed by D_2O exchange, NH), 8.02 (3-proton singlet, NAc).

Anal. Found: C, 71.61; H, 6.25; N, 2.31.

2,3,4,6-Tetra-O-benzyl-1-O-[5-benzyl N-(tert-*butyloxycarbonyl)-L-glutam-1-oyl]-D-glucopyranose* (**6**). — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (540 mg), 5-benzyl 1-pentachlorophenyl *tert*-butyloxycarbonyl-L-glutamate (585 mg) and imidazole (340 mg) were dissolved in dichloromethane (20 ml) at room temperature with shaking. After 2 h, an additional amount of the amino acid component (59 mg) was added to the mixture. After a further 3 h, the pentachlorophenol was filtered off, and the filtrate was treated as described for **1** except that 10% citric acid was used in place of sulphuric acid. The residue was eluted from a silica gel column with solvent *A* (10:1); crystallisa-

tion of the chromatographically homogenous, anomeric mixture (742 mg, 86.5%) from ether–light petroleum afforded the β anomer of **6** (290 mg), m.p. 75–76°, $[\alpha]_D$+3.8°. N.m.r. data: τ 4.33 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1), 8.59 (9-proton singlet, Me_3C).

Anal. Calc. for $C_{51}H_{57}NO_{11}$: C, 71.22; H, 6.68; N, 1.63. Found: C, 71.29; H, 6.61; N, 1.55.

The mother liquor was evaporated and the residue was chromatographed over carbon–Celite and silica gel in solvent *A* (10:1) to give the α anomer of **6** as a syrup, $[\alpha]_D$+53.8°. N.m.r. data: τ 3.58 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), 8.59 (9-proton singlet, Me_3C).

Anal. Found: C, 71.26; H, 6.88; N, 1.75.

*2,3,4,6-Tetra-*O*-benzyl-1-*O*-[1-benzyl* N*-(benzyloxycarbonyl)-*L*-aspart-4-oyl]-*D*-glucopyranose* (**7**). — The condensation of 2,3,4,6-tetra-O-benzyl-α-D-glucopyranose (810 mg) and 1-benzyl 4-pentachlorophenyl benzyloxycarbonyl-L-aspartate (909+91 mg) was performed in the presence of imidazole (510 mg) as described for **6**. After working up, the residue was chromatographed over silica gel in solvent *A* (10:1) to give chromatographically homogeneous **7** (958 mg, 72%). Recrystallisation from ethanol afforded the β anomer of **7** (427 mg), m.p. 114–115°, $[\alpha]_D$+7.5°. N.m.r. data: τ 4.18 (1-proton doublet, *J* 8.5 Hz, removed by D_2O exchange, NH), 4.40 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{53}H_{53}NO_{11}$: C, 72.34; H, 6.07; N, 1.59. Found: C, 72.23; H, 6.15; N, 1.54.

The mother liquor was evaporated, and the residual oil was rechromatographed three times over silica gel in solvent *A* (10:1) to give the α anomer of **7** as a syrup, $[\alpha]_D$+41.0°. N.m.r. data: τ 3.66 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), 4.10 (1-proton doublet, *J* 8.5 Hz, NH).

Anal. Found: C, 72.57; H, 6.09; N, 1.80.

*Catalytic debenzylation of 1-*O*-(acylaminoacyl)-2,3,4,6-tetra-*O*-benzyl-*D*-glucopyranoses* **1–6**. — (*a*) Hydrogenation of the α and β anomers of 1-*O*-(2-acetamidoacyl) derivatives **1–3** (1 mmole) was performed in 2-methoxyethanol (15 ml) over 10% palladium-on-charcoal (200 mg) at room temperature and pressure; the reaction was monitored by t.l.c. in solvent *B*. After completion of the reaction (18–24 h), the catalyst was removed by centrifugation and washed with 2-methoxyethanol, and the combined supernatant and washings were evaporated to dryness (0.1 Torr, bath 30°). The residue was eluted from a column (49 × 1.5 cm) of cellulose with solvent *C*: fractions (1 ml/0.5 h) were examined by t.l.c. in solvent *B* and those containing the chromatographically homogenous material were combined and evaporated to dryness. The α and β anomers of 1-*O*-(2-acetamidoacyl)-D-glucopyranoses **8–10** were obtained as hygroscopic foams (yields 75–85%); physical constants and analytical data are given in Table I.

Acetylation of the above compounds was performed with pyridine–acetic anhydride (5:1) at 0° for 5–10 h; the progress of the reaction was monitored by t.l.c. in solvent *D*. The mixtures were poured on to ice and extracted with chloroform, and

after working up the residues were eluted from silica gel with solvent *D* to give products (60–75% yields) identical with authentic samples of 1-*O*-(2-acetamidoacyl)-2,3,4,6-tetra-*O*-acetyl-α- and -β-D-glucopyranoses **14–16**, as judged by spectroscopic data, elemental analyses, and optical rotations.

(*b*) Debenzylation of the *tert*-butyloxycarbonylaminoacyl derivatives **4–6** was performed as described above, except that a catalytic amount of acetic acid (0.3 ml/mmole of ester) was added in order to accelerate the uptake of hydrogen. The crude hydrogenolysis products, obtained from the β anomers of **4–6** were purified by chromatography on cellulose (solvent *E*) to give 1-*O*-(2-*tert*-butyloxycarbonylaminoacyl)-β-D-glucopyranoses **11–13** in 70–80% yields; physical constants and analytical data are given in Table I.

Acetylation of **11β–13β** was performed as described above; the products were eluted from silica gel with solvent *A* (1:1) to give the crystalline β anomers of tetraacetates **17–19**. Compounds **17β** and **18β** (yields 69 and 64%, respectively) were identical with the authentic samples described below. The β anomer of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butyloxycarbonyl)-L-glutam-1-yl]-D-glucopyranose (**19**) was obtained in 61% yield; m.p. 143–145° (from ether–light petroleum), $[\alpha]_D$ 0°, $[\alpha]_D$ −18.8° (methanol). N.m.r. data: τ 4.28 (1-proton doublet, $J_{1,2}$ 8 Hz, H-1), 7.98–8.05 (two singlets, 12 protons, OAc), 8.62 (9-proton singlet, Me_3C); in methyl sulphoxide-d_6: τ 2.75 (1-proton doublet, *J* 8 Hz, NH), 3.99 (1-proton doublet, $J_{1,2}$ 8 Hz, H-1), 8.05–8.09 (two singlets, 12 protons OAc), 8.68 (9-proton singlet, Me_3C).

Anal. Calc. for $C_{24}H_{35}NO_{15}$: C, 49.91; H, 6.11; N, 2.42. Found: C, 49.88; H, 6.05; N, 2.49.

Hydrogenolysis products of the α anomers of **4** and **5** revealed (t.l.c., solvent *B*) a major spot with mobility identical to that of **11β** and **12β**, respectively, and a minor, slightly faster-moving spot; the intensity of the latter increased during the isolation procedure. A sample (150 mg) of the crude hydrogenolysis product of **5α** was acetylated with pyridine–acetic anhydride in the usual manner. Elution of the residue from silica gel with solvent *A* (1:1) afforded **18α** (65 mg, 30%) as a chromatographically homogeneous foam, $[\alpha]_D$ +70°, identical with the authentic compound described below.

A sample (149 mg) of the crude hydrogenolysis product of **5α** was dissolved in water (5 ml) and kept with silica gel (500 mg) at room temperature for 20 h; by this time, the initial product had rearranged almost completely into the faster-moving component. The silica gel was filtered off, the filtrate evaporated to dryness, and the residue eluted from silica gel with solvent *F* to give chromatographically homogeneous 2-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-D-glucopyranose (**21**; 120 mg, 81%) as an amorphous solid which was recrystallised from dry ethanol–dry ether; m.p. 40–45°, $[\alpha]_D$ +35.0° (ethanol). N.m.r. data (D_2O): τ 4.52–4.78 (1-proton multiplet, H-1), 8.56–8.68 (singlet, 12 protons, Me_3C and Me-CH).

Anal. Calc. for $C_{14}H_{25}NO_9$: C, 47.86; H, 7.17; N, 3.99. Found: C, 47.72; H, 7.17; N, 3.89.

Acetylation of **21** (60 mg) gave a mixture of two components which were partly resolved by elution from silica gel with solvent *A* (1:1). Fractions containing only the

slower-moving component were combined and evaporated, and the residue (27 mg) was recrystallised from ether–light petroleum to give 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*tert*-butyloxycarbonyl-L-alanyl)-α-D-glucopyranose (**22**), m.p. 88–90° alone and in admixture with the authentic compound described below.

2,3,4,6-Tetra-O-*acetyl-1*-O-*acetylglycyl*-D-*glucopyranose* (**14**). — This compound was prepared[7] by the DCC method in the presence of triethylamine; the mother liquor left after crystallisation of the β anomer[7] was evaporated to dryness, and the residue was submitted to chromatography on carbon–Celite and silica gel in solvent *D* to give the α anomer of **14** as a hygroscopic foam, $[\alpha]_D$ +110°. N.m.r. data: τ 3.62 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), 3.92 (broad, 1 proton, removed by D_2O exchange, NH), 7.92, 7.96, and 7.99 (singlets, 15 protons, 3 × OAc and NAc).

Anal. Calc. for $C_{18}H_{25}NO_{12}$: C, 48.33; H, 5.63; N, 3.13. Found: C, 48.55; H, 5.65; N, 3.23.

2,3,4,6-Tetra-O-*acetyl-1*-O-(*acetyl*-D-*alanyl*)-D-*glucopyranose* (**16**). — This compound was prepared by the DCC method in the presence of two equivalents of imidazole, as described for the L-alanine homologue[2]. Crystallisation of the chromatographically homogeneous product (solvent *D*, yield 54%) from ether–acetone–light petroleum afforded the β anomer of **16**, m.p. 149–150°, $[\alpha]_D$ +15.9°; lit.[8] m.p. 150–151°, $[\alpha]_D$ +15.0° (chloroform), for the product obtained by the silver salt method.

The mother liquor was evaporated to dryness, and the residue was eluted from carbon–Celite with solvent *D* to give the α anomer of **16** as a hygroscopic foam, $[\alpha]_D$ +83.5°, $[\alpha]_D$ +90.0° (methanol). N.m.r. data are given in Table II.

Anal. Calc. for $C_{19}H_{27}NO_{12}$: C, 49.46; H, 5.90; N, 3.04. Found: C, 49.22; H, 5.95; N, 3.32.

2,3,4,6-Tetra-O-*acetyl-1*-O-(tert-*butyloxycarbonylglycyl*)-D-*glucopyranose* (**17**). — The condensation was performed with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (348 mg) and *tert*-butyloxycarbonylglycine pentachlorophenyl ester (423 + 42 mg) in the presence of imidazole (340 mg), as described for compound **6**. After working up, the residue was eluted from silica gel with solvent *A* (1:1) to give chromatographically homogeneous **17** (421 mg, 83.5%). Crystallisation from isopropyl ether, to which a few drops of ethanol were added, afforded the β anomer of **17** (137 mg), m.p. 146–147°, $[\alpha]_D$ +3.8°. N.m.r. data: τ 4.18 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1), 7.91, 7.96, and 8.00 (singlets, 12 protons OAc), 8.55 (9-proton singlet, Me_3C).

Anal. Calc. for $C_{21}H_{31}NO_{13}$: C, 49.90; H, 6.18; N, 2.77. Found: C, 49.72; H, 6.33; N, 2.79.

Evaporation of the mother liquor left a residue which was chromatographed over silica gel in solvent *A* (1:1) to give the α anomer of **17** as a semi-solid foam, $[\alpha]_D$ +90.0°. N.m.r. data: τ 3.60 (1-proton doublet, $J_{1,2}$ 3 Hz, H-1), 7.91 and 7.98 (singlets, 12 protons OAc), 8.54 (9-proton singlet, Me_3C).

Found: C, 50.09; H, 6.10; N, 3.10.

2,3,4,6-Tetra-O-*acetyl-1*-O-(tert-*butyloxycarbonyl*-L-*alanyl*)-D-*glucopyranose* (**18**). — The reaction was performed with *tert*-butyloxycarbonyl-L-alanine pentachlorophenyl ester as the amino acid component, as described for **17**, to give the anomeric

mixture of **18** in 64% yield. The pure β anomer of **18** was obtained after repeated column chromatography on carbon–Celite with solvent *A* (1:1); m.p. 110–111° (from ether–light petroleum), $[\alpha]_D - 2.1°$. N.m.r. data: τ 4.24 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1), 7.97–8.01 (two singlets, 12 protons, OAc), 8.48 (singlet, 12 protons, Me_3C and Me-CH); in methyl sulphoxide-d_6: τ 2.71 (1-proton doublet, *J* 7 Hz, NH), 3.99 (doublet, $J_{1,2}$ 7 Hz, H-1), 8.01–8.07 (singlet, 12 protons, OAc), 8.63 (9-proton singlet, Me_3C), 8.78 (3-proton doublet, *J* 7 Hz, Me-CH).

Anal. Calc. for $C_{22}H_{33}NO_{13}$: C, 50.86; H, 6.40; N, 2.70. Found: C, 51.06; H, 6.63; N, 2.73.

Attempts to obtain the pure α anomer of **18** failed; n m.r. data of a fraction, $[\alpha]_D + 40°$, highly enriched in **18α**, are given in Table II.

1,3,4,6-Tetra-O-*acetyl-2*-O-(*acetyl*-D-*alanyl*)-α-D-*glucopyranose* (**20**). — The reaction was performed with 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose, *N*-acetyl-D-alanine, DCC (2 mmoles each), and imidazole (4 mmoles), as described for **1**; after working up, the product was eluted from silica gel with solvent *D* to give the title compound (507 mg, 50%) as a hygroscopic foam, $[\alpha]_D + 88.5°$, $[\alpha]_D + 103°$ (methanol). N.m.r. data are given in Table II.

Anal. Calc for $C_{19}H_{27}NO_{12}$: C, 49.46; H, 5.90; N, 3.04. Found: C, 49.22; H, 6.07; N, 3.03.

1,3,4,6-Tetra-O-*acetyl-2*-O-(tert-*butyloxycarbonyl*-L-*alanyl*)-α-D-*glucopyranose* (**22**). — This compound was prepared as described for **20** by using *tert*-butyloxycarbonyl-L-alanine as the amino acid component and dichloromethane as the exclusive solvent. After working up, the residue was eluted from silica gel with solvent *A* (1:1), and the chromatographically homogeneous product (54% yield) was recrystallised from ether–light petroleum to give **22** as white needles, m.p. 88–89°, $[\alpha]_D + 87.5°$, ν_{max}^{KBr} 3510s (NH), 1780vs (C=O), 1740 and 1710sh (C=O), 1530s (amide II), 1030s (C–O–C). N.m.r. data are given in Table II.

Anal. Calc. for $C_{22}H_{33}NO_{13}$: C, 50.86; H, 6.40; N, 2.70. Found: C, 50.97; H, 6.57; N, 2.99.

1,3,4,6-Tetra-O-*acetyl-2*-O-(tert-*butyloxycarbonyl*-L-*alanyl*)-β-D-*glucopyranose* (**23**). — This compound was prepared, as described for **22**, by using 1,3,4,6-tetra-*O*-acetyl-β-D-glucopyranose as the sugar component. Elution of the product from silica gel with solvent *A* (1:1) afforded chromatographically pure **23** in 45% yield; after two recrystallisations from isopropyl ether, the compound had m.p. 101–103°, $[\alpha]_D - 2.8°$, ν_{max}^{KBr} 3450s (NH), 1755vs (C=O), 1720vs (C=O), 1520s (amide II), 1030sh (C–O–C). N.m.r. data are given in Table II.

Anal. Calc. for $C_{22}H_{33}NO_{13}$: C, 50.86; H, 6.40; N, 2.70. Found: C, 50.63; H, 6.31; N, 2.99.

Methyl 2,3,4-tri-O-*benzyl-6*-O-(*benzyloxycarbonylglycyl*)-β-D-*glucopyranoside* (**24**). — The compound was prepared by the AAE method from methyl 2,3,4-tri-*O*-benzyl-β-D-glucopyranoside[11], *N*-benzyloxycarbonylglycine pentachlorophenyl ester, and imidazole, as described for **6**: after working up, the residue was eluted from silica gel with solvent *A* (3:1) to give a 67% yield of chromatographically homogeneous **24**

which was recrystallised from ethanol; m.p. 95–97°, $[\alpha]_D$+23.0°; ν_{max}^{KBr} 3370m (NH), 1780s (C=O), 1710vs and 1570s (amide I and II), 1075vs (C–O–C), 748s and 700vs cm^{-1} (phenyl). N.m.r. data: τ 2.67–2.72 (multiplet, 20 protons, 4 × Ph), 4.87 (2-proton singlet, OCO–CH_2–Ph), 6.08 (2-proton doublet, *J* 6 Hz, CH_2N), and 6.48 (3-proton singlet, OMe).

Anal. Calc. for $C_{38}H_{40}NO_9$: C, 69.71; H, 6.16; N, 2.14. Found: C, 69.44; H, 6.44; N, 1.86.

Compound **24** was also prepared in 65% yield by the imidazole-promoted DCC condensation, using *N*-benzyloxycarbonylglycine as the amino acid component.

Methyl 6-O-glycyl-β-D-glucopyranoside hydrochloride (**25**). — To a solution of **24** (1 mmole) in 2-methoxyethanol (12 ml), M hydrochloric acid (1 ml) and 10% palladium-on-charcoal (200 mg) were added, and hydrogenation was performed as described for the debenzylation of **1–6**; the progress of the reaction was monitored by t.l.c. in solvent *E*. The filtered solution was evaporated, and crystallisation of the residue from methanol–dry ether afforded **25** (840 mg, 83%), m.p. 160–163° (dec.), $[\alpha]_D$−20.0° (methanol), $[\alpha]_D$−24.0° (water); ν_{max}^{KBr} 3470 broad (OH), 1760vs (C=O), 1050vs cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 5.96 (2-proton singlet, CH_2N), 6.43 (3-proton singlet, OMe).

Anal. Calc. for $C_9H_{18}ClNO_7$: C, 37.57; H, 6 31; N, 4.87. Found: C, 37.53; H, 6.30; N, 4.68.

Acetylation of **25** (150 mg) with pyridine–acetic anhydride was performed as described for **8–10**; after working up, the residue was eluted from silica gel with solvent *A* (1:10) to give the tetra-acetyl derivative **26** (117 mg, 80.5%) as a solid foam, $[\alpha]_D$−11.3°; ν_{max}^{film} 3480m (NH), 1770vs (C=O), 1670s and 1560m (amide I and II), 1040vs (C–O–C). N.m.r. data: τ 3.67 (broad, 1 proton, NH), 5.92 (2-proton doublet, *J* 6 Hz, CH_2N), 6.50 (3-proton singlet, OMe), 7.98–8.01 (two singlets, 12 protons, 3 × OAc and NAc).

Anal. Calc. for $C_{17}H_{25}NO_{11}$: C, 48.69; H, 6.01; N, 3.34. Found: C, 48.93; H, 6.31; N, 3.26.

1-O-(L-β-Aspartyl)-β-D-glucopyranose (**27**). — A solution of the β anomer of **7** (500 mg) in acetic acid–2-methoxyethanol (2:1, 22 ml) was hydrogenated in the presence of 10% palladium-on-charcoal (500 mg) for 5 h at atmospheric pressure and room temperature; the reaction was monitored by t.l.c. in 1-butanol–acetic acid–water (12:3:5). The catalyst was centrifuged off, and the supernatant was evaporated to dryness (0.1 Torr, bath 30°). The remaining solid was shaken with dry ether (3 × 5 ml) and then dissolved in hot acetic acid (65–70°); on cooling and addition of some drops of dry ether, **27** was deposited as white, hygroscopic crystals (101 mg, 60%) with no definite melting point, $[\alpha]_D$−3.8° (water), $[\alpha]_D$−2.7° (methanol); ν_{max}^{KBr} 3500 broad (OH), 1760vs (C=O), 1640vs and 1500s (amino acid I and II), 1070vs cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.40 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{10}H_{17}NO_9$: C, 40.68; H, 5.80; N, 4.75. Found: C, 40.52; H, 6.06; N, 4.55.

1-O-(N-Acetyl-L-β-aspartyl)-β-D-glucopyranose (**28**). — To a solution of com-

pound **27** (295 mg) in water (100 ml), 2% acetic anhydride in acetone (100 ml) was added and the solution was left for 24 h at room temperature with occasional shaking. After removal of the solvent (rotary evaporator and then 0.1 Torr, bath 30°), the residue was evaporated several times with a small amount of water until free of acetic anhydride odour, and dried. The residue thus obtained was a glassy solid which turned to a powder on trituration with dry ether; it was dissolved in methanol, and some drops of ether were added whereupon the unreacted **27** precipitated. The precipitate was centrifuged off, more ether was added to the supernatant, and the turbid solution was kept overnight at 0°; white, hygroscopic crystals (202 mg, 60%) of **28** were deposited. The substance, which was chromatographically homogeneous in solvent *C*, showed no definite melting point; $[\alpha]_D + 5.0°$ (water), $[\alpha]_D + 16.0°$ (methanol); ν_{max}^{KBr} 3450 broad (OH and NH), 1740s (C=O), 1640s and 1550s (amide I and II), 1070vs cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.42 (1-proton doublet, $J_{1,2}$ 7 Hz, H-1), 8.08 (3-proton singlet, NAc).

Anal. Calc. for $C_{12}H_{19}NO_{10}$: C, 42.73; H, 5.67; N, 4.15; Ac, 12.76. Found: C, 42.92; H, 5.93; N, 4.18; Ac, 12.96.

ACKNOWLEDGMENTS

We are indebted to Dr. O. Hadžija for microanalyses. The valuable technical assistance of Mrs. Dj. Orlić is appreciated.

REFERENCES

1 D. KEGLEVIĆ, A. KORNHAUSER, G. ROGLIĆ, AND T. KOVAČ, *Tetrahedron Lett.*, (1970) 2983.
2 D. KEGLEVIĆ, A. KORNHAUSER, AND Š. VALENTEKOVIĆ, *Carbohyd. Res.*, 22 (1972) 245.
3 G. ROGLIĆ AND D. KEGLEVIĆ, *Croat. Chem. Acta*, 44 (1972) 229.
4 J. YOSHIMURA AND M. FUNABASHI, *Bull. Chem. Soc. Japan*, 39 (1966) 2009.
5 N K. KOCHETKOV, V. A. DEREVITSKAYA, L. M. LIKHOSHERSTOV, N. V. MOLODTSOV, AND S. G. KARA-MURZA, *Tetrahedron*, 18 (1962) 273, and references cited therein.
6 N. K. KOCHETKOV, V. A. DEREVITSKAYA, AND L. M. LIKHOSHERSTOV, *Izv. Akad Nauk SSSR, Ser. Khim.*, (1963) 688.
7 A. KORHNAUSER AND D. KEGLEVIĆ, *Carbohyd. Res*, 11 (1969) 407.
8 A. KORNHAUSER AND D. KEGLEVIĆ, *Carbohyd. Res.*, 13 (1970) 433.
9 H. DETERMANN, J. HEUER, P. PFAENDER, AND M. L. REINARTZ, *Ann*, 694 (1966) 190
10 H. DETERMANN, *Peptides, Proc. 8th Europ. Peptide Symp.*, Noordwijk, The Netherlands, 1966, North-Holland Publ. Co., Amsterdam, 1967, pp. 73–79.
11 N. PRAVDIĆ AND D. KEGLEVIĆ, *Tetrahedron*, 21 (1965) 1897.
12 G. A. LEVVY, A. J. HAY, J. CONCHIE, AND I. STRACHAN, *Biochim. Biophys. Acta*, 222 (1970) 333.
13 J. YOSHIMURA, M. FUNABASHI, S. ISHIGE, AND T. SATO, *Carbohyd. Res.*, 3 (1966) 214.
14 M. FUJINO AND C. HATANAKE, *Chem. Pharm. Bull. (Tokyo)* 16 (1968) 929.
15 C. H. LI, B. GORUP, D. CHUNG, AND J. RAMACHANDRAN, *J. Org. Chem.*, 28 (1963) 178.