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SYNTHESIS AND REACTIONS OF D-GLUCOPYRANOSYL ESTERS OF PHENYLALANINE AND TYROSINE: A STUDY OF THE DIAZOMETHANE-CATALYSED 1→2 ACYL MIGRATION OF THE *N*-ACYLATED α-D ANOMERS*

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ABSTRACT

The fully benzylated α- and β-D-glucopyranosyl esters of *N*-benzyloxycarbonyl- and *N*-*tert*-butoxycarbonyl-L-phenylalanine (**1** and **5**, respectively) have been converted into 1-*O*-(L-phenylalanyl)-α- and -β-D-glucopyranose, which were isolated as trifluoroacetate salts (**2α** and **2β**). On dissolution in water, 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranose (**6α**) gave the anomerised C-2 isomer **8** and hydrolysis products of **6α**. Treatment of **6α** with *N*,*N*-dimethylformamide–ethereal diazomethane gave the 2-*O*-acyl derivative **8α** (>80%). 1-*O*-(L-Tyrosyl)-α- (**12α**) and -β-D-glucopyranose (**12β**), isolated as the trifluoroacetate salts, were prepared from 2,3,4,6-tetra-*O*-benzyl-1-*O*-[*N*-benzyloxycarbonyl-*O*-(*tert*-butyl)-L-tyrosyl]-α- and -β-D-glucopyranose (**10**) and characterised as the *N*-acetyl- (**13**) and tetra-acetate derivatives (**14**). Treatment of **13α** with diazomethane caused extensive hydrolysis, but some rearrangement into the 2-*O*-acyl derivative **15α**, which was characterised as crystalline 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*N*-acetyl-*O*-acetyl-L-tyrosyl)-α-D-glucopyranose (**16α**). The foregoing 1→2 acyl migrations and hydrolyses are competitive reactions, the relative rates of which are strongly affected by reaction temperature and the nature of the acyl group.

INTRODUCTION

D-Glucopyranosyl esters of amino acids, although readily hydrolysed, may also undergo reaction through nucleophilic attack at the 1-ester carbonyl group[1–3]. We have shown[4] that diazomethane catalyses the 1→2 rearrangement of 1-*O*-(*N*-acylaminoacyl)-α-D-glucopyranoses with high retention of anomeric configuration. The diazomethane functions as a base towards HO-2, thus rendering O-2 more prone for nucleophilic attack at the 1-ester carbonyl carbon.

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*Glycosyl Esters of Amino Acids: Part X. For Part IX, see ref. 4.

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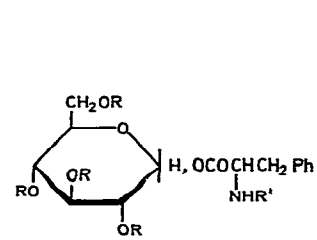
In studying structure–reactivity relations in this series, the 1-esters of phenylalanine and tyrosine have been investigated. The greater resistance toward hydrolysis of the former derivatives allowed an examination of factors that influence the 1→2 migration.

RESULTS AND DISCUSSION

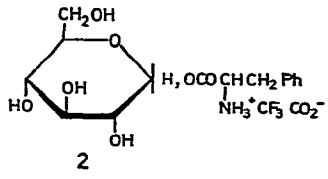
The β-D-glucopyranosyl ester of L-phenylalanine, characterised as the trifluoroacetate salt (**2β**), was obtained by hydrogenolysis of 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyl-L-phenylalanyl)-β-D-glucopyranose[5] (**1β**) in the presence of trifluoroacetic acid or by treating 1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucopyranose (**6β**), obtained from the benzylated 1-ester[5] **5β**, with trifluoroacetic acid at −10°. Likewise, **1α** and **6α** were converted into **2α**, which was obtained crystalline.



1 R = Bzl, R' = Z 5 R = Bzl, R' = Boc
3 R = H , R' = Ac 6 R = H , R' = Boc
4 R = Ac, R' = Ac 7 R = Ac, R' = Boc

Z = $PhCH_2OC(=O)-$

Boc = $Me_3COC(=O)-$



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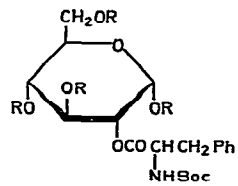


8α R = H
9α R = Ac

The structures **2β** and **2α** were assigned on the basis of microanalytical data, optical rotations, and p.m.r. data (see Experimental). The compounds were characterised as the *N*-acetyl- (**3β** and **3α**) and tetra-acetate derivatives (**4β** and **4α**). Both anomers of **4** were also obtained after condensation of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and *N*-acetyl-L-phenylalanine. The structures of **6α** and **6β** were confirmed by analytical and spectral data together with their conversion into the tetra-acetate derivatives **7α** and **7β**, which were also synthesised by an unambiguous route. Furthermore, treatment of **7α** with trifluoroacetic acid, followed by *N*-acetylation, afforded **4α** (66%).

Compared to the members of the series investigated so far, the D-glucopyranosyl esters **2** and **6** were much more stable toward hydrolysis. Both **2α** and **2β** were stable under dry conditions, but in aqueous solution, after 24 h, the extents of cleavage into D-glucose and phenylalanine were ~20 and 60%, respectively. In aqueous solution,

6β underwent ~50% hydrolysis during 5 days at room temperature. Under similar conditions, ~30% of **6α** survived and, in addition to hydrolysis products (~10%), two major ninhydrin- and silver nitrate-positive components were detected and identified as the α and β anomers of 2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranose (**8**).

Treatment[4] of a solution of **6α** in *N*,*N*-dimethylformamide with 5 equiv. of ethereal diazomethane at 0° for 1 h afforded **8α**, the p.m.r. spectrum (D_2O) of which contained a doublet for H-1 (τ 4.58, $J_{1,2}$ 4 Hz) 0.90 p.p.m. upfield of that ($J_{1,2}$ 3 Hz) of **6α**, thus indicating HO-1 to be unsubstituted. The tetra-acetate (**9α**) of **8α** was identical with authentic 1,3,4,6-tetra-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranose. Thus, the diazomethane-induced rearrangement **6α**→**8** proceeded with retention of anomeric configuration.

In aqueous solution, **8α** slowly anomerised to give an ~1:1 equilibrium mixture after ~6 days; hydrolysis products were not detectable. This result indicates that the C-2 isomer is the thermodynamically preferred product, as found[6] for the corresponding acetates. Conventional acetylation of **8αβ** gave the tetra-acetate **9** (αβ ratio 3:1). Partial anomerisation of **8α** occurred during purification on silica gel. The influence of diazomethane concentration on the acyl migration **6α**→**8α** is shown by the results in Table I. With 0.5 equiv. of diazomethane at 0° and −10° (Table I, 2 and 3), almost complete rearrangement of **6α** into **8α** occurred within 1 h. In the latter experiment, the difference in rate between isomerisation and hydrolysis was sufficiently high to yield almost pure **8α**. At room temperature for 10 min (Table I, 4), ~40% hydrolysis occurred. With 0.25 equiv. of diazomethane (Table I, 5), ⩽10% hydrolysis products were formed. The above results suggest that 1→2 acyl migration

TABLE I

EFFECTS OF DIAZOMETHANE CONCENTRATION AND TEMPERATURE ON THE REARRANGEMENT OF 1-*O*-[*N*-(*tert*-BUTOXYCARBONYL)-L-PHENYLALANYL]-α-D-GLUCOPYRANOSE (**6α**) INTO THE 2-*O*-ACYL DERIVATIVE **8α**

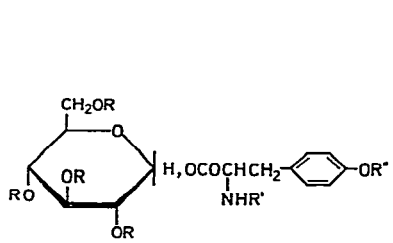
Expt.	*Reaction conditions*[a]				*Found*[b] *(%)*	
	Diazomethane[c] *added*		*Temp. (degree)*	*Time (min)*	**8α**	**6α**
	(ml)	*(molar ratio to 6α)*				
1	0.4	1	0	60	>80	n.d.
2	0.2	0.5	0	60	>80	n.d.
3	0.2	0.5	−10	60	>90	n.d.
4	0.2	0.5	23	10	~60	n.d.
5	0.1	0.25	0	60	~25	~65
6	—	—	0	60	n.d.[d]	100

[a]Each experiment was performed with 0.1 mmol (43 mg) of **6α** dissolved in dry *N*,*N*-dimethylformamide (1 ml). After the required time, the solvent was evaporated (0.1 torr), and the residue was analysed. [b]Estimated on the basis of t.l.c. (solvent *C*) and p.m.r. spectroscopy [D_2O, internal sodium 4,4-dimethyl-4-silapentane-1-sulfonate (DSS)]. [c]0.25M in dry ether (redistilled before use, and concentration determined by titration of an aliquot with benzoic acid). [d]n.d., Not determined.

and hydrolysis are competitive reactions involving the intermediate cyclic 1,2-(ortho ester)[4,7]. The relative rates of the two reactions are markedly and oppositely affected by reaction temperature.

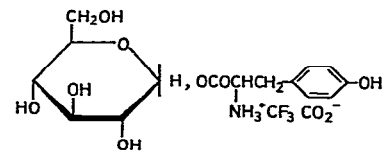
The effect of configuration on acyl migration[8-10] was also shown in parallel experiments with **6β**. Treatment of **6β**, as for **6α**, with 0.5 or 1 equiv. of diazomethane at 0° for 1 h effected neither migration nor hydrolysis. However, with 5 equiv. of diazomethane (0°, 1 h), **6β** gave (t.l.c.) several products indicative of several acyl migrations.

The synthesis of D-glucopyranosyl esters of tyrosine was effected as follows. Treatment of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[*N*-benzyloxycarbonyl-*O*-(*tert*-butyl)-L-tyrosyl]-α- or -β-D-glucopyranose (**10**, see Experimental) with trifluoroacetic acid at −10° liberated the phenolic hydroxyl group, to give **11α** and **11β**. Catalytic hydrogenation of the appropriate anomer of **10** or **11**, in the presence of trifluoroacetic acid, yielded 1-*O*-(L-tyrosyl)-α- and -β-D-glucopyranose isolated as the trifluoroacetate salt (**12**). Stepwise deprotection of **10**, although more tedious, gave a cleaner product **12**.



10 R = Bzl, R′ = Z, R″ = t-Bu
11 R = Bzl, R′ = Z, R″ = H
13 R = R″ = H, R′ = Ac
14 R = R′ = R″ = Ac

Z = $PhCH_2OC(=O)-$



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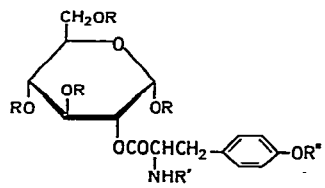

15 R = R″ = H, R′ = Ac
16 R = R′ = R″ = Ac

Both **12α** and **12β** were very hygroscopic solids that were more readily hydrolysed than the phenylalanine analogues **2α** and **2β**. *N*-Acetylation and conventional acetylation of **12α** and **12β** gave the respective anomers of 1-*O*-(*N*-acetyl-L-tyrosyl)-D-glucopyranose (**13**) and 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetyl-*O*-acetyl-L-tyrosyl)-D-glucopyranose (**14**). The latter products were identical with the authentic compounds obtained after condensation of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and *N*-acetyl-*O*-acetyl-L-tyrosine.

The effect of diazomethane on **13α** shows that the nature of the acyl groups in the *N*-acylaminoacyl residue greatly influences the relative distribution of products. Thus, treatment (0°, 1 h) of a solution of **13α** in *N*,*N*-dimethylformamide with 0.5 equiv. of diazomethane caused extensive hydrolysis, but some 1→2 rearrangement, to give **15α** (~60%). The finding that only 10% hydrolysis of **13α** in *N*,*N*-dimethyl-

formamide at 0° occurred during 24 h accords with the intermediacy of the ortho acid. Attempts to obtain pure **15α** failed, but acetylation of the crude product gave the crystalline *N*-acetyl penta-acetate, which was identical with 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*N*-acetyl-*O*-acetyl-L-tyrosyl)-α-D-glucopyranose (**16α**) prepared by direct synthesis.

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) or cellulose (Standard grade, Whatman), and t.l.c. on Kieselgel G (Merck), if not stated otherwise. The solvents employed were: *A*, benzene–ethyl acetate (proportions are given in the text); *B*, chloroform; *C*, 5:3:1 2-propanol–light petroleum–water. Detection on t.l.c. plates was effected by charring with sulphuric acid, with ninhydrin reagent, with alkaline silver nitrate, or with tetrazotized *o*-dianisidine (Fast Blue B salt, Sigma, 0.5% in water) for phenolic hydroxyl. 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.

N-Benzyloxycarbonyl-*O*-(*tert*-butyl)-L-tyrosine *p*-nitrophenyl ester (70%) was prepared by the DCC condensation of the corresponding acid[11] and *p*-nitrophenol in ethyl acetate; after recrystallisation (twice) from ethanol, the product had m.p. 86–87°, $[\alpha]_D$ −7° (*c* 2, *N*,*N*-dimethylformamide).

Anal. Calc. for $C_{27}H_{28}N_2O_7$: C, 65.83; H, 5.73; N, 5.69. Found: C, 65.84; H, 5.58; N, 5.85.

The α anomer of 2,3,4,6-tetra-*O*-benzyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranose[5] (**5**) was obtained anomerically pure after repeated chromatography on silica gel with solvents *A* (10:1) (2×) and *B* (2×); syrup, $[\alpha]_D$ +60°; ν_{max}^{KBr} 3500 (NH), 1765 (C=O), 1730 and 1510 (Amide I and II), 1390 and 1375 cm^{-1} (Me_3C). P.m.r. data: τ 2.71–2.28 (m, 5 Ph), 3.57 (d, $J_{1,2}$ 3 Hz, H-1), 6.91 (d, *J* 6 Hz, CH-CH_2-Ph), and 8.62 (s, Me_3C).

Anal. Calc. for $C_{48}H_{53}NO_9$: C, 73.16; H, 6.78; N, 1.78. Found: C, 73.10; H, 7.02; N, 1.94.

1-O-(L-Phenylalanyl)-β-D-glucopyranose trifluoroacetate salt (**2β**). — (*a*) *From* **1β**. A solution of **1β**[5] (263 mg) in 2-methoxyethanol (18 ml) was hydrogenated in the presence of 10% palladium-on-charcoal (210 mg) and trifluoroacetic acid (98%, 0.6 ml) at atmospheric pressure until termination of hydrogen uptake (~24 h; monitoring by t.l.c., cellulose, solvent *C*). After removal of the catalyst and concentration of the solvent (0.1 torr), the residue was triturated with dry ether (3×) and then dissolved in water (1 ml), and the solution lyophilised to give **2β** as a hygroscopic solid (122 mg, 86.5%), $[\alpha]_D$ −16° (water); ν_{max}^{KBr} 3480 vs broad (OH), 1770 (C=O),

1680 and 1535 (Amino acid I and II), and 725 cm^{-1} (CF_3). P.m.r. data (D_2O): τ 2.55 (s, Ph) and 4.24 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{17}H_{22}F_3NO_9$: C, 46.26; H, 5.02; N, 3.17. Found: C, 46.23; H, 5.05; N, 3.04.

N-Acetylation of **2β** (133 mg) was performed with 10% (v/v) acetic anhydride in 1:1 acetone–water (20 ml) at 4° overnight. After removal of the solvent (0.1 torr), the residue was passed through a cellulose column with solvent *C*; the fractions containing chromatographically homogeneous material were combined and concentrated, to give 1-*O*-(*N*-acetyl-L-phenylalanyl)-β-D-glucopyranose (**3β**; 71 mg, 64%) as a hygroscopic solid, $[\alpha]_D$ +4.2° (*c* 2, water); ν_{max}^{KBr} 3500 (OH), 1770 (C=O), 1670 and 1560 (Amide I and II). P.m.r. data (D_2O): τ 2.60 (s, Ph), 4.32 (d, $J_{1,2}$ 7 Hz, H-1), and 8.01 (NAc).

Anal. Calc. for $C_{17}H_{23}NO_8$: C, 55.27; H, 6.28; N, 3.79. Found: C, 55.20; H, 6.45; N, 3.94.

A sample (40 mg) of **3β** was conventionally acetylated in acetic anhydride–pyridine (1:4, 5 ml) at 4° overnight. After work-up and evaporation of the solvent, the residue was crystallised from ether–light petroleum (1:1), to give a product (46 mg, 79%), m.p. 138–140°, $[\alpha]_D$ +10°, which was indistinguishable (mixture m.p., i.r. and p.m.r. spectra) from an authentic sample of 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanyl)-β-D-glucopyranose (**4β**) described below.

(*b*) *From* **6β**. Trifluoroacetic acid (98%, 3 ml) was added to **6β** (220 mg) at −10°, and the solution was kept at this temperature for ~30 min (t.l.c., cellulose, solvent *C*), whereupon it was concentrated, and the residue was triturated with dry ether and dissolved in water (~1 ml). Lyophilisation afforded **2β** (179 mg, 79%), $[\alpha]_D$ −15° (*c* 2, water), indistinguishable (t.l.c., i.r. and p.m.r. spectra) from that prepared from **1β** (Found: C, 46.23; H, 5.05; N, 3.04).

1-O-(L-*Phenylalanyl*)-α-D-*glucopyranose trifluoroacetate* salt (**2α**). — Catalytic hydrogenation of **1α**[5] (350 mg) was performed as described for **1β**, to give, after lyophilisation, the title compound (137 mg, 72.8%) as a solid that crystallised from 2-propanol upon addition of dry ether; m.p. 111–112° (dec.), $[\alpha]_D$ +64° (water). P.m.r. data (D_2O): τ 2.55 (s, Ph) and 3.68 (d, $J_{1,2}$ 3 Hz, H-1).

Anal. Calc. for $C_{17}H_{22}F_3NO_9$: C, 46.40; H, 5.25; N, 3.40. Found: C, 46.50; H, 5.24; N, 3.37.

N-Acetylation of **2α** (70 mg), performed as described for **3α**, gave, after cellulose column chromatography, 1-*O*-(*N*-acetyl-L-phenylalanyl)-α-D-glucopyranose (**3α**; 42 mg, 71%) as a very hygroscopic solid, $[\alpha]_D$ +54° (water). P.m.r. data (D_2O): τ 2.58 (s, Ph), 3.79 (d, $J_{1,2}$ 3 Hz, H-1), and 7.99 (NAc).

Anal. Calc. for $C_{17}H_{23}NO_8$: C, 55.27; H, 6.28; N, 3.79. Found: C, 55.09; H, 6.46; N, 3.78.

Conventional acetylation of **3α** (85 mg), followed by chromatography (solvent *A*, 1:2) of the product on silica gel, gave a chromatographically homogeneous foam (63 mg, 51%), $[\alpha]_D$ +70°, whose i.r. and p.m.r. spectra were superposable on those

of authentic 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanyl)-α-D-glucopyranose (**4α**).

(*b*) *From* **6α**. Treatment of **6α** (110 mg) with trifluoroacetic acid (1.5 ml), as described for the β anomer, gave crystalline **2α** (90 mg, 80%), indistinguishable (t.l.c., i.r. and p.m.r. spectra) from the sample prepared from **1α**.

2,3,4,6-Tetra-O-*acetyl-1*-O-(N-*acetyl*-L-*phenylalanyl*)-D-*glucopyranose* (**4**). — Condensation of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (1.39 g) and *N*-acetyl-L-phenylalanine (829 mg) was performed in the presence of dicyclohexylcarbodiimide (DCC) (815 mg) and imidazole (545 mg) in dichloromethane (25 ml) with stirring at 0° and then at room temperature (24 h). After removal of *N*,*N'*-dicyclohexylurea and work-up of the filtrate, the residue was crystallised from ethanol–water (1:1) to give **4β** (590 mg), m.p. 139–140°, $[\alpha]_D$ +8.5°, lit.[12] m.p. 138–140°, $[\alpha]_D$ +11°. P.m.r. data: τ 2.79 (s, Ph), 4.15 (d, *J* 8 Hz, disappeared on deuteration, NH), 4.26 (d, $J_{1,2}$ 8 Hz, H-1), 6.84 (d, *J* 6 Hz, >CH-CH_2-Ph), 7.94, 8.00, 8.02, and 8.05 (4 OAc + NAc) (Found: C, 56.00; H, 5.62; N, 2.67).

The residue left upon concentration of the mother liquor was passed through a column of silica gel with solvent *A* (1:2), to give an additional amount (350 mg) of **4β** (total yield: 44%), and the α anomer of **4** (185 mg, 8.6%) as a solid foam, $[\alpha]_D$ +78°. P.m.r. data: τ 2.76 (s, Ph), 3.69 (d, $J_{1,2}$ 3 Hz, H-1), 4.07 (d, *J* 8 Hz, NH), 7.94, 7.98, 8.01, and 8.06 (4 OAc + NAc).

Anal. Calc. for $C_{25}H_{31}NO_{12}$: C, 55.86; H, 5.81; N, 2.61. Found: C, 56.00; H, 5.62; N, 2.67.

1-O-[N-(tert-*Butoxycarbonyl*)-L-*phenylalanyl*]-β-D-glucopyranose (**6β**). — A solution of **5β**[5] (394 mg) in 2-methoxyethanol (13 ml) was hydrogenated in the presence of 10% palladium-on-charcoal (200 mg) and a few drops of acetic acid (monitoring by t.l.c., solvent *C*), filtered, and evaporated (0.1 torr). A solution of the residue in water was lyophilised; to yield amorphous **6β** (165 mg, 77.5%), $[\alpha]_D$ −11.5° (*c* 2, water). P.m.r. data (D_2O): τ 2.68 (s, Ph), 4.34 (d, $J_{1,2}$ 7 Hz, H-1), and 8.67 (s, Me_3C).

Conventional acetylation of a sample of **6β** gave, after chromatography on silica gel (solvent *A*, 1:2), a homogeneous product (86% yield, m.p. 78–80°), which was indistinguishable (mixture m.p., p.m.r. spectrum) from authentic 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-β-D-glucopyranose (**7β**) described below.

1-O-[N-(tert-*Butoxycarbonyl*)-L-*phenylalanyl*]-α-D-*glucopyranose* (**6α**). — Catalytic hydrogenation of **5α** (195 mg), performed as described for **6β**, gave, after lyophilisation, **6α** (75 mg, 71%) as a hygroscopic solid, $[\alpha]_D$ +52° (water), $[\alpha]_D$ +58° (methanol). P.m.r. data (D_2O): τ 2.65 (s, Ph), 3.79 (d, $J_{1,2}$ 3 Hz, H-1), and 8.61 (Me_3C).

Anal. Calc. for $C_{20}H_{29}NO_9$: C, 56.19; H, 6.84; N, 3.28. Found: C, 56.11; H, 7.06; N, 3.46.

Conventional acetylation of a sample (120 mg) of **6α**, followed by chromatography on silica gel, gave a chromatographically homogeneous, amorphous product

(133 mg, 80%), $[\alpha]_D$ +78°, which was indistinguishable from an authentic sample of 2,3,4,6-tetra-*O*-acetyl-1-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranose (**7α**) (Found: C, 56.66; H, 6.43; N, 2.40).

Treatment of the above product (65 mg) with trifluoroacetic acid (1 ml) at −10° for 20 min, followed by *N*-acetylation (as described for **3β**) of the residue, gave, after chromatography on silica gel (solvent *A*, 1:2), an amorphous product (39 mg, 66%), $[\alpha]_D$ +78°, whose i.r. and p.m.r. spectra were superposable on those of authentic 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanyl)-α-D-glucopyranose(**4α**) (Found: C, 55.87; H, 5.55; N, 2.39).

*2,3,4,6-Tetra-*O*-acetyl-1-*O*-[*N*-(tert-butoxycarbonyl)-L-phenylalanyl]-D-glucopyranose* (**7**). — 2,3,4,6-Tetra-*O*-acetyl-D-glucopyranose (246 mg) and *N*-*tert*-butoxycarbonyl-L-phenylalanine pentachlorophenyl ester[13] (385 mg) were condensed in the presence of imidazole (255 mg) in dichloromethane (12 ml) at room temperature for 24 h. After removal of pentachlorophenol and work-up of the filtrate, the residue was eluted from silica gel with solvent *A* (2:1), to give **7β** (75 mg, 18%) as the faster-moving fraction, m.p. 79–80° (from di-isopropyl ether), $[\alpha]_D$ +10°. P.m.r. data: τ 2.76 (s, Ph), 4.21 (d, $J_{1,2}$ 7 Hz, H-1), 7.92, 7.99, 8.00 (4 OAc), and 8.54 (s, Me_3C).

Anal. Calc. for $C_{28}H_{37}NO_{13}$: C, 56.47; H, 6.26; N, 2.35. Found: C, 56.72; H, 6.25; N, 2.51.

The slower-moving fraction was passed through a second column of silica gel, to give **7αβ** (237 mg, 53%) and amorphous **7α** (23 mg), $[\alpha]_D$ +76°. P.m.r. data: τ 2.76 (s, Ph), 3.78 (d, $J_{1,2}$ 3 Hz, H-1), 7.92, 7.99, 8.00 (4 OAc), and 8.54 (s, Me_3C) (Found: C, 56.39; H, 6.35; N, 2.58).

2-O-[N-(tert-Butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranose (**8α**). — To a solution of **6α** (70 mg; t.l.c. in solvent *C*: R_F 0.80) in dry *N*,*N*-dimethylformamide (2 ml) was added diazomethane in dry ether (re-distilled before use, 0.43 mmol/ml, 1 ml), and the solution was kept at 0° for 1 h, whereupon it was concentrated (0.1 torr) and lyophilised, to give a solid product (t.l.c. in solvent *C*: R_F 0.82) contaminated with D-glucose and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine; $[\alpha]_D$ +46° (methanol). P.m.r. data (D_2O): τ 2.68 (s, Ph), 4.74 (d, $J_{1,2}$ 4 Hz, H-1), and 8.62 (s, Me_3C). Elution of the crude product from silica gel with solvent *C* afforded the analytically pure sample (yield 77%, calc. on **6α**), which was estimated (t.l.c.) to be a 3:1 mixture of **8α** and **8β** (R_F 0.82 and 0.85, respectively).

Anal. Calc. for $C_{20}H_{29}NO_9$: C, 56.19; H, 6.84; N, 3.27. Found: C, 56.44; H, 7.10; N, 3.49.

Conventional acetylation of the crude product (30 mg), followed by chromatography on silica gel (solvent *A*, 2:1), gave a homogeneous product (33 mg, 80%), $[\alpha]_D$ +68°, which crystallised from di-isopropyl ether and was indistinguishable (mixture m.p., p.m.r. spectrum) from 1,3,4,6-tetra-*O*-acetyl-2-*O*-[*N*-(*tert*-butoxycarbonyl)-L-phenylalanyl]-α-D-glucopyranose (**9α**) described below.

The same treatment performed with the analytical sample of **8** (10 mg) afforded

a solid (10 mg, 80%) that gave correct analyses for **9**; p.m.r. data for OAc-1: τ 7.82 and 7.88, intensity ratio ~3:1.

1,3,4,6-Tetra-O-*acetyl-2*-O-[N-(tert-*butoxycarbonyl*)-L-*phenylalanyl*]-α-D-*glucopyranose* (**9α**). — Condensation of 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (246 mg) and *N*-(*tert*-butoxycarbonyl)-L-phenylalanine pentachlorophenyl ester (385 mg), in the presence of imidazole (255 mg), was performed as described for **7**, to give, after chromatography on silica gel (solvent *A*, 2:1), **9α** (250 mg, 58%), m.p. 107–109° (from di-isopropyl ether), $[\alpha]_D$ +69°; ν_{max}^{KBr} 3400 (NH), 1755 (C=O), 1690 and 1515 (Amide I and II), 1365 and 1385 cm^{-1} (Me_3C). P.m.r. data: τ 2.76 (s, Ph), 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 7.82 (s, *ax* OAc-1), 7.92, 7.99, 8.00 (3 OAc), and 8.54 (s, Me_3C).

Anal. Calc. for $C_{28}H_{37}NO_{13}$: C, 56.47; H, 6.26; N, 2.35. Found: C, 56.31; H, 6.40; N, 2.40.

2,3,4,6-Tetra-O-*benzyl-1*-O-[N-*benzyloxycarbonyl*-O-(tert-*butyl*)-L-*tyrosyl*]-D-*glucopyranose* (**10**). — The condensation of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (2.94 g) and *N*-benzyloxycarbonyl-*O*-(*tert*-butyl)-L-tyrosine *p*-nitrophenyl ester (2.95 g), in the presence of imidazole (1.87 g), was performed as described for **7**. The reaction mixture was poured onto ice, the organic layer was worked up, and the product was dissolved in hot ethanol (15 ml); on cooling, crystals of unreacted sugar component (160 mg) were deposited. The material in the mother liquor was passed through a column of silica gel with solvent *A* (10:1), to give pure **10** (3.03 g, 62.5%) as an anomeric mixture. Material left in slower-moving fractions was crystallised twice from ethanol, to give **10β** (214 mg), m.p. 77–78°, $[\alpha]_D$ +4° (*c* 3). P.m.r. data: τ 2.70–2.77 (m, 5 Ph), 4.35 (d, $J_{1,2}$ 7 Hz, H-1), 6.95 (d, *J* 6 Hz, >CH-CH_2-C_6H_4-), and 8.72 (s, Me_3C).

Anal. Calc. for $C_{55}H_{59}NO_{10}$: C, 73.89; H, 6.65; N, 1.57. Found: C, 73.85; H, 6.88; N, 1.38.

Material left in faster-moving fractions was eluted twice from columns of silica gel with solvent *A* and then twice with solvent *B*, to give **10α** (300 mg) as a colourless syrup, $[\alpha]_D$ +63°. P.m.r. data: τ 2.76–2.84 (m, Ph), 3.62 (d, $J_{1,2}$ 3 Hz, H-1), 6.97 (d, *J* 6 Hz, >CH-CH_2-C_6H_4-), and 8.74 (s, Me_3C) (Found: C, 74.00; H, 6.62; N, 1.49).

2,3,4,6-Tetra-O-*benzyl-1*-O-(N-*benzyloxycarbonyl*-L-*tyrosyl*)-β-D-*glucopyranose* (**11β**). — (*a*) Trifluoroacetic acid (98%, 4 ml) was added to **10β** (448 mg) at −10°, and the solution was kept at this temperature for ~1 h (monitoring by t.l.c.; solvent *A*, 5:1). After removal of trifluoroacetic acid by co-distillation with dry ether, the residue was crystallised from ethanol, to give **11β** (306 mg, 73%), m.p. 125–126°, $[\alpha]_D$ +11°; ν_{max}^{KBr} 3420 sh and 3350 (NH, OH), 1760 (C=O), 1700 and 1520 cm^{-1} (Amide I and II). P.m.r. data: τ 2.72–2.79 (m, Ph), 4.36 (d, $J_{1,2}$ 7 Hz, H-1), and 7.01 (d, 6 Hz, >CH-CH_2-C_6H_4-).

Anal. Calc. for $C_{51}H_{51}NO_{10}$: C, 73.10; H, 6.14; N, 1.67. Found: C, 73.22; H, 6.35; N, 1.58.

(*b*) The above procedure was applied to **10α** (226 mg), to give, after chromatography on silica gel (solvent *A*, 10:1), **11α** (112 mg, 53%) as a solid foam, $[\alpha]_D$

+57°. P.m.r. data: τ 3.62 (d, $J_{1,2}$ 3 Hz, H-1) (Found: C, 73.15; H, 6.19; N, 1.60).

1-O-(L-*Tyrosyl*)-β-D-*glucopyranose trifluoroacetate salt* (**12β**). — (*a*) *From* **11β**. Catalytic hydrogenation of **11β** (167 mg) in 2-methoxyethanol (10 ml) was performed in the presence of trifluoroacetic acid (0.35 ml), as described for **2β**, to give, after lyophilisation, **12β** (81 mg, 88%), as a very hygroscopic solid, $[\alpha]_D$ −13° (water); ν_{max}^{KBr} 3500 broad vs (OH), 1765 (C=O), 840 (-C_6H_4-), and 723 cm^{-1} (CF_3). P.m.r. data (D_2O): τ 2.60 and 3.09 (2 d, each 2 H, $J_{H,H}$ 8.5 Hz, -C_6H_4-), and 4.23 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{17}H_{22}F_3NO_{10} \cdot H_2O$: C, 42.94; H, 5.09; N, 2.95. Found: C, 43.05; H, 5.30; N, 2.83.

(*b*) *From* **10β**. A solution of **10β** (1.35 g) in 2-methoxyethanol (20 ml) was hydrogenated in the presence of trifluoroacetic acid (3 ml), as described above, to give a hygroscopic solid (670 mg, 98%), $[\alpha]_D$ −11° (water), which contained (t.l.c., solvent *C*) mainly **12β** (R_F 0.45), and traces of D-glucose and tyrosine.

N-Acetylation of the above compound (600 mg), performed as described for **3β**, gave, after chromatography of the reaction mixture on a cellulose column with solvent *C*, 1-*O*-(*N*-acetyl-L-tyrosyl)-β-D-glucopyranose (**13β**; 250 mg, 50%) as a hygroscopic solid, $[\alpha]_D$ +2° (*c* 4, water). P.m.r. data (D_2O): τ 2.66 and 3.15 (2 d, each 2 H, $J_{H,H}$ 8.5 Hz, -C_6H_4-), 4.36 (d, $J_{1,2}$ 7 Hz, H-1), and 8.01 (NAc).

Anal. Calc. for $C_{17}H_{23}NO_9$: C, 52.98; H, 6.01; N, 3.63. Found: C, 52.88; H, 6.06; N, 3.50.

Conventional acetylation of **13β** (72 mg), followed by elution of the product from silica gel (solvent *A*, 1:2), afforded a chromatographically homogeneous syrup (67 mg, 60%) that crystallised on trituration with dry ether, to give a compound, m.p. 122–123°, which was identical (mixture m.p., $[\alpha]_D$, p.m.r. spectrum) with authentic 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetyl-*O*-acetyl-L-tyrosyl)-β-D-glucopyranose (**14β**) described below.

1-O-(L-*Tyrosyl*)-α-D-*glucopyranose trifluoroacetate salt* (**12α**). — The compound was prepared from **11α** (1.57 g) and **10α** (900 mg), respectively, as described for **12β**; t.l.c. (solvent *C*) of the crude product ($[\alpha]_D$ +60°, water) showed one main spot (R_F 0.45, cellulose: 0.07) and traces of D-glucose and tyrosine. Attempts to purify the product by chromatographic methods failed, due to decomposition.

N-Acetylation of a freshly prepared sample (685 mg), performed as for **3β**, gave, after elution of the crude product from a cellulose column (solvent *C*), 1-*O*-(*N*-acetyl-L-tyrosyl)-α-D-glucopyranose (**13α**; 290 mg, 50%) as a chromatographically homogeneous syrup, $[\alpha]_D$ +74° (water); ν_{max}^{KBr} 3420 vs broad (OH), 1760 (C=O), 1650 and 1540 (Amide I and II). P.m.r. data (D_2O): τ 2.72 and 3.16 (2 d, each 2 H, $J_{H,H}$ 8.5 Hz, -C_6H_4-), 3.84 (d, $J_{1,2}$ 3 Hz, H-1), and 8.04 (NAc).

Anal. Calc. for $C_{17}H_{23}NO_9 \cdot H_2O$: C, 50.62; H, 6.25; N, 3.47. Found: C, 50.58; H, 6.38; N, 3.28.

Peracetylation of **13α** (72 mg) with pyridine–acetic anhydride (5:1, 5 ml), followed by chromatography on silica gel (solvent *A*, 1:2), afforded a chromatographically homogeneous, amorphous product (110 mg, 61.5%), $[\alpha]_D$ +87°, whose

t.l.c. (solvent *A*, 1:4) behaviour and i.r. and p.m.r. spectra were indistinguishable from those of authentic **14α**.

*2,3,4,6-Tetra-*O*-acetyl-1-*O*-(*N*-acetyl-*O*-acetyl-*L*-tyrosyl)-*D*-glucopyranose* (**14**). — This compound was prepared by condensing 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (700 mg) and *N*-acetyl-*O*-acetyl-L-tyrosine[14] in the presence of DCC (410 mg) and imidazole (280 mg), as described for **4**. The crude product was eluted from silica gel with solvent *A* (1:4); combination and rechromatography of the appropriate fractions resulted in separation of the anomers (total yield: 180 mg, 30%). The residue left in the slower-moving fractions afforded, upon trituration with dry ether, **14β** (70 mg), m.p. 122–124°, $[\alpha]_D$ +13° (*c* 2). P.m.r. data: τ 2.92–3.10 (m, 4 H, $-C_6H_4-$), 4.18 (d, *J* 8 Hz, disappeared on deuteration, NH), 4.35 (d, $J_{1,2}$ 7 Hz, H-1), 6.68 (d, *J* 6 Hz, $>CH-CH_2-C_6H_4-$), 7.78 (s, $-C_6H_4-OAc$), 7.93, 8.00, 8.01, and 8.04 (4 OAc, NAc).

Anal. Calc. for $C_{27}H_{33}NO_{14}$: C, 54.45; H, 5.59; N, 2.35. Found: C, 54.67; H, 5.77; N, 2.58.

The α anomer of **14** was obtained from the faster-running fractions as a solid foam (50 mg), $[\alpha]_D$ +85°. P.m.r. data: τ 2.85–3.08 (m, 4 H, $-C_6H_4-$), 3.76 (d, $J_{1,2}$ 3 Hz, H-1), 3.92 (d, *J* 8 Hz, NH), 6.68 (d, *J* 6 Hz, $>CH-CH_2-C_6H_4-$), 7.78 (s, $-C_6H_4-OAc$), 7.94, 8.00, 8.01, and 8.06 (4 OAc, NAc) (Found: C, 54.20; H, 5.62; N, 2.66).

Diazomethane-catalysed conversion of **13α** *into* **15α**. — A solution of **13α** (250 mg) in *N*,*N*-dimethylformamide (5 ml) was treated (1 h, 0°) with 0.5 equiv. of diazomethane in dry ether, as described for **8α**, to give, upon lyophilisation, 2-*O*-(*N*-acetyl-L-tyrosyl)-α-D-glucopyranose (**15α**) as a solid contaminated (t.l.c., solvent *C*) with D-glucose and *N*-acetyltyrosine. Purification of the product on either silica gel or cellulose caused additional decomposition. Conventional acetylation of a sample (200 mg), followed by chromatography on silica gel (solvent *A*, 1:2), afforded a chromatographically homogeneous syrup (180 mg, 57.5%). Crystallisation from di-isopropyl ether gave a compound that was indistinguishable (mixture m.p., $[\alpha]_D$, i.r. and p.m.r. spectra) from an authentic sample of 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*N*-acetyl-*O*-acetyl-L-tyrosyl)-α-D-glucopyranose (**16α**) (Found: C, 54.40; H, 5.66; N, 2.32).

*1,3,4,6-Tetra-*O*-acetyl-2-*O*-(*N*-acetyl-*O*-acetyl-*L*-tyrosyl)-α-*D*-glucopyranose* (**16α**). — The reaction was performed with 1,3,4,6-tetra-*O*-acetyl-α-D-glucopyranose (350 mg) and *N*-acetyl-*O*-acetyl-L-tyrosine (290 mg) in the presence of DCC (210 mg) and imidazole (140 mg), as described for **4**. After work-up, the residue was passed through a column of silica gel (solvent *A*, 1:4), to give **16α** (150 mg, 25%), m.p. 142–144° (from di-isopropyl ether), $[\alpha]_D$ +58°; ν_{max}^{KBr} 3395 (NH), 1760 (C=O), 1665 and 1520 (Amide I and II), and 840 cm^{-1} (*p*-C_6H_4). P.m.r. data: τ 2.80–3.18 (m, 4 H, $-C_6H_4-$), 3.68 (d, $J_{1,2}$ 3 Hz, H-1), 3.78 (d, *J* 8 Hz, disappeared on deuteration, NH), 6.68 (d, *J* 6 Hz, $>CH-CH_2-C_6H_4-$), 7.74 (s, $-C_6H_4-OAc$). 7.83 (*ax*-OAc), 7.92, 7.98, 8.01, and 8.05 (3 OAc, NAc).

Anal. Calc. for $C_{27}H_{33}NO_{14}$: C, 54.45; H, 5.59; N, 2.35. Found: C, 54.18; H, 5.40; N, 2.45.

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REFERENCES

1 D. KEGLEVIĆ, DJ. LJEVAKOVIĆ, AND Š. VALENTEKOVIĆ, *Croat. Chem. Acta*, 46 (1974) 115–127.
2 Š. VALENTEKOVIĆ AND D. KEGLEVIĆ, *Carbohydr. Res.*, 47 (1976) 35–48.
3 D. KEGLEVIĆ, J. HORVAT, AND F. PLAVŠIĆ, *Carbohydr. Res.*, 47 (1976) 49–61.
4 J. HORVAT AND D. KEGLEVIĆ, *Carbohydr. Res.*, 75 (1979) 117–127.
5 D. KEGLEVIĆ, A. KORNHAUSER, AND Š. VALENTEKOVIĆ, *Carbohydr. Res.*, 22 (1972) 245–256.
6 W. A. BONNER, *J. Org. Chem.*, 24 (1959) 1388–1390.
7 L. R. SCHROEDER, D. P. HULTMAN, AND D. C. JOHNSON, *J. Chem. Soc., Perkin Trans. 2*, (1972) 1063–1071.
8 S. TEJIMA AND H. G. FLETCHER, JR., *J. Org. Chem.*, 28 (1963) 2999–3004.
9 C. PEDERSEN AND H. G. FLETCHER, JR., *J. Am. Chem. Soc.*, 82 (1960) 3215–3217.
10 A. H. HAINES, *Adv. Carbohydr. Chem. Biochem.*, 33 (1976) 11–109.
11 E. SCHRÖDER, *Justus Liebigs Ann. Chem.*, 670 (1963) 127–136.
12 A. KORNHAUSER AND D. KEGLEVIĆ, *Carbohydr. Res.*, 13 (1970) 433–439.
13 B. J. JOHNSON AND E. G. TRASK, *J. Org. Chem.*, 33 (1968) 4521–4524.
14 M. BERGMANN AND L. ZERVAS, *Biochem. Z.*, 203 (1928) 280–292.