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SYNTHESIS, PROPERTIES, AND REACTIONS OF α- AND β-D-GLUCOPYRANOSYL ESTERS OF SOME TRIPEPTIDES*

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ABSTRACT

The 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyltripeptidyl)-D-glucopyranoses **1**, **8**, and **13** were synthesised from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose and the active esters of the appropriate *N*-protected tripeptides (Gly-Gly-Gly-, L-Phe-Gly-Gly-, and Gly-Gly-L-Phe-) in the presence of imidazole; the anomeric mixtures were resolved and the α and β anomers characterised. The β anomer of **13**, containing the L and D enantiomers (ratio ~3:1) of Gly-Gly-Phe- as the aglycon, could be resolved by column chromatography into the pure isomeric forms. Catalytic hydrogenolysis of the β anomers, in the presence and absence of a strong acid, yielded the free 1-esters **2β**, **9β**, and **14β**, which were characterised as the monooxalate or trifluoroacetate salts and as free bases. Similarly, the α anomers afforded **2α**, **9α**, and **14α**, whereas omission of the strong acid led to accompanying 1→2 acyl migration, to give the 2-*O*-acyl derivatives. All of the compounds prepared were converted into the *N*-acetyl and/or peracetylated derivatives. The 1-esters **2β** and **9β**, both in the charged and uncharged form, and the trifluoroacetate salt of **14β**, are susceptible to cleavage by β-D-glucosidase; the enzyme had no effect on the uncharged form of **14β**. This difference between **14β** and its salt is discussed in conformational terms.

INTRODUCTION

In a previous paper[1], we reported on the synthesis of some 1-*O*-dipeptidyl-D-glucopyranoses and their tendency to undergo intramolecular aminolysis with scission of the glycosidic ester bond. The balance between the formation of free D-glucosyl ester and the respective piperazinedione derivative depends on the nature and sequence of the amino acids, as well as on the nature of substituents and the anomeric configuration of the sugar component.

In continuation of our studies, we turned to D-glucopyranosyl esters of tripeptides, which, as expected, are resistant to intramolecular cyclisation of the aglycon

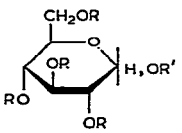
*Glycosyl Esters of Peptides, Part II. For Part I, see ref. 1.

group, because of an unfavourable spatial disposition of the amino and the 1-ester carbonyl groups. We now report on the synthesis and reactions of D-glucopyranosyl esters having glycylglycylglycine, L-phenylalanylglycylglycine, and glycylglycyl-L- and -D-phenylalanine, respectively, as the aglycon.

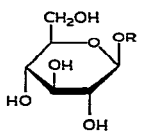
RESULTS AND DISCUSSION

The reaction of 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose with the pentachlorophenyl esters of *N*-benzyloxycarbonyl-glycylglycylglycine, -L-phenylalanylglycylglycine, and -glycylglycyl-L-phenylalanine, in the presence of imidazole, gave the fully protected 1-esters **1**, **8**, and **13**, respectively. The resulting, anomeric mixtures were resolved and the α and β anomers characterised (Table I). T.l.c. (solvent *A*) revealed **13β** to be a mixture of two components of similar mobility that could be separated from one another by repeated column chromatography. They were shown (see Table I and the section dealing with carboxypeptidase A) to be the pair of diastereoisomers containing the enantiomeric Gly-Gly-L-Phe and Gly-Gly-D-Phe aglycon groups. Since the active peptide ester used[2] in the synthesis was >90% optically pure, racemisation of the L-phenylalanyl residue must have occurred during the coupling of the peptide portion with the sugar component. It is known[2–4] that active esters of *N*-acylglycyl-L-phenylalanine are readily racemised, and that storage of solutions of *N*-benzyloxycarbonylglycyl-L-phenylalanine pentachlorophenyl ester in *N*,*N*-dimethylformamide causes ~70% loss of optical activity during 24 h.

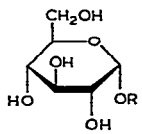
Complete deprotection of the α and β anomers of **1**, **8**, and **13** was performed



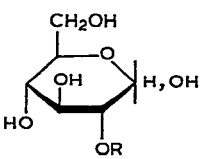
1 R = CH_2Ph, R′ = Z-Gly-Gly-Gly-
4 R = Ac, R′ = Ac-Gly-Gly-Gly-
8 R = CH_2Ph, R′ = Z-Phe-Gly-Gly-
10 R = Ac, R′ = Ac-Phe-Gly-Gly-
13 R = CH_2Ph, R′ = Z-Gly-Gly-Phe-
16 R = Ac, R′ = Ac-Gly-Gly-Phe-



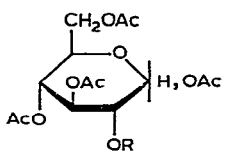
2β R = H-Gly-Gly-Gly-
3β R = Ac-Gly-Gly-Gly-
9β R = H-Phe-Gly-Gly-
14β R = H-Gly-Gly-Phe-
15β R = Ac-Gly-Gly-Phe-



2α R = $(CO_2H)_2$ • H-Gly-Gly-Gly-
3α R = Ac-Gly-Gly-Gly-
9α R = CF_3CO_2H • H-Phe-Gly-Gly-
14α R = CF_3CO_2H • H-Gly-Gly-Phe-
15α R = Ac-Gly-Gly-Phe-



5 R = H-Gly-Gly-Gly-
6 R = Ac-Gly-Gly-Gly-
11 R = H-Phe-Gly-Gly-
17 R = H-Gly-Gly-Phe-



7 R = Ac-Gly-Gly-Gly-
12 R = Ac-Phe-Gly-Gly-
18 R = Ac-Gly-Gly-Phe-

TABLE I

2,3,4,6-TETRA-*O*-BENZYL-1-*O*-(*N*-BENZYLOXYCARBONYLTRIPEPTIDYL)-D-GLUCOPYRANOSES

Compound	*Aglycon group (Z = $PhCH_2OCO$)*	*Yield*[a] *(%)*	*Anomeric form*	*M.p. (degrees)*	$[\alpha]_D$[b] *(degrees)*	*P.m.r. data*[c] *[τ ($J_{1,2}$)] for H-1*	*Found (%)*		
							C	*H*	*N*
1[d]	Z-Gly-Gly-Gly	72	β	116–118[e]	+5.0	4.38 (7)[f]	67.89	6.34	5.04
			α	syrup	+47.6	3.67 (3)	68.43	6.19	5.14
8[g]	Z-Phe-Gly-Gly	74.5	β	169–171[h] (sintering at 133)	+7.1	4.35 (7)[f]	70.52	6.08	4.29
			α	syrup	+53.0	3.60 (3)	70.54	6.23	4.36
13[g]	Z-Gly-Gly-L,D-Phe	74.4	β[i]	134–137[h] (sintering at 96)	+11.0	4.32 (6.5)[f]	70.62	6.20	4.39
			α[i]	syrup	+48.2	3.65 (3)	70.44	6.10	4.49
13a[g]	Z-Gly-Gly-Phe[j]		β	137–139[h]	+12.0	4.31 (6.5)[f]	70.32	6.15	4.46
13b[g]	Z-Gly-Gly-D-Phe[j]		β	137–139[h] (sintering at 96)	+11.5	4.35 (6.5)[f]	70.58	6.15	4.72

[a]The yields are given for chromatographically homogeneous, anomeric mixtures. [b]In chloroform (*c* 1–2). [c]Measured in chloroform-*d*; coupling constants in Hz. [d]Calc. for $C_{48}H_{51}N_3O_{11}$: C, 68.15; H, 6.08; N, 4.96. [e]From ethyl acetate. [f]After deuterium exchange of NH. [g]Calc. for $C_{55}H_{57}N_3O_{11}$: C, 70.57; H, 6.14; N, 4.49. [h]From 96% ethanol. [i]Ratio of L-:D-Phe ~3:1. [j]Isolated by fractionation of **13**β on silica gel.

by catalytic hydrogenolysis in the presence, and in the absence, of a strong acid. Thus, hydrogenolysis of **1β** in the presence of an equimolar amount of oxalic acid dihydrate afforded 1-*O*-(glycylglycylglycyl)-β-D-glucopyranose (**2β**) mono-oxalate, the structure of which was confirmed by analytical and spectral data and by its conversion into the *N*-acetyl derivative **3β**. Gel filtration of **2β** mono-oxalate on a column of Sephadex G-10 resulted in separation of oxalic acid to give **2β** as the free base; the latter compound was also obtained directly from **1β** by hydrogenolysis in acetic acid–2-methoxyethanol. Compared to the β-D-glucopyranosyl ester of glycylglycine, which could be characterised only in the form of its mono-oxalate salt, both **2β** and its salt were much more stable in water and in 0.1M HCl; after 4 days, the extents of their decomposition into glucose and tripeptide were ~30 and 50%, respectively. By contrast, on dissolution in methanol, **2β** underwent rapid, intermolecular transesterification, to give, within 12 h at room temperature, ~70% of glucose and the corresponding tripeptide methyl ester; under these conditions, the scarcely soluble **2β** mono-oxalate remained unchanged.

Conventional acetylation of **3β** yielded a sole product, whose chromatographic and spectral data were consistent with the structure 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetylglycylglycylglycyl)-β-D-glucopyranose (**4β**). However, attempts to purify the crude product on silica gel with ethyl acetate–methanol (5:1; solvent *C*) were unsuccessful, because of the transfer of the aglycon group to the alcoholic component of the eluent; 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and *N*-acetylglycylglycylglycine methyl ester were identified as the principal components in the eluate. A closer examination revealed that intermolecular transesterification of **4β** in solvent *C* proceeded at a remarkably higher rate in the presence of silica gel. It should also be noted that all attempts to prepare 1-*O*-(*N*-acetyltripeptidyl)-D-glucopyranose tetra-acetates (**4**, **10**, and **16**, respectively) by direct condensation of 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose and the appropriate *N*-acetyltripeptide failed, because of decomposition of the crude products on silica gel during chromatography.

Catalytic hydrogenolysis of **1α**, performed in the presence of oxalic acid, gave the α-D-glucopyranosyl ester mono-oxalate **2α**, which was converted into the *N*-acetyl and tetra-acetate derivatives (**3α** and **4α**, respectively); the isolation of the latter could be achieved without recourse to chromatography on silica gel. When deprotection of **1α** was performed in the absence of a strong acid, accompanying 1→2 acyl migration occurred, to give 2-*O*-(glycylglycylglycyl)-D-glucopyranose (**5**, 71% yield). The p.m.r. spectrum (D_2O) of **5** contained a multiplet for H-1 (τ 4.62–4.80) that was 0.85 p.p.m. upfield from the H-1 doublet ($J_{1,2}$ 2.5 Hz) of **2α**, thus indicating that HO-1 was unsubstituted. Compound **5** was converted into the *N*-acetyl derivative **6**, which was treated with acetic anhydride–pyridine to give the peracetylated 2-*O*-acyl derivative **7**; the p.m.r. spectrum of **7** was indicative of an ~2:1 mixture of the α and β anomers.

By using the reactions described above, 1-*O*-(L-phenylalanylglycylglycyl)-β-D-glucopyranose (**9β**) was prepared from **8β** and characterised as the mono-oxalate and the free base; acetylation of each form yielded 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-

acetyl-L-phenylalanylglycylglycyl)-β-D-glucopyranose (**10β**). Catalytic hydrogenolysis of **8α** in the presence of trifluoroacetic acid gave the 1-ester trifluoroacetate salt **9α**, whereas the same treatment in the absence of a strong acid gave 2-*O*-(L-phenylalanylglycylglycyl)-D-glucopyranose (**11**). Both **9α** and **11** were characterised as the *N*-acetyl 1- and 2-*O*-acyl tetra-acetates (**10α** and **12**, respectively). In the p.m.r. spectrum ($CDCl_3$) of **10α**, the signal assigned to AcO-2 was shifted to higher field (τ 8.15) relative to the corresponding signal for **10β**. Inspection of Dreiding models revealed that the phenyl group of the *N*-terminal L-phenylalanyl residue in **10α** can easily approach the protons of the AcO-2 group of the sugar moiety, whereas such shielding is not possible for **10β**.

Definitive evidence that the two components (**a** and **b**) of the fully protected β-D-glucopyranosyl ester **13** contained peptidic aglycons of opposite enantiomeric configuration was provided by subjecting each of them to catalytic hydrogenolysis, followed by incubation of the residue with carboxypeptidase A, which attacks the end of a peptide chain producing the C-terminal amino acid and a smaller peptide. Thus, monitoring of the reaction mixture by t.l.c. (solvent *D*) revealed that >80% of the aglycon of the free C-1 ester derived from **13βa** was split into phenylalanine and glycylglycine (ratio 1:1) within 12 h, whereas that for **13βb** remained unchanged, even after 24 h; in both cases (buffer pH 7.5), hydrolysis of the glycosidic ester bond was complete within 6 h. Catalytic hydrogenolysis of **13α** (which moved as a single spot in all of the chromatography systems tried), followed by incubation of the residue with carboxypeptidase A, yielded phenylalanine, glycylglycine, and glycylglycylphenylalanine in ~3:3:1 ratios, thus indicating that the aglycon contained the L- and D-enantiomeric tripeptide in an ~3:1 ratio.

Catalytic hydrogenolysis of **13βa** was performed on a larger scale in the presence and absence of trifluoroacetic acid. The structure of 1-*O*-(glycylglycyl-L-phenylalanyl)-β-D-glucopyranose (**14β**) trifluoroacetate was easily determined, *inter alia*, from its p.m.r. spectrum (D_2O; H-1: τ 4.37, $J_{1,2}$ 7 Hz) and that of the *N*-acetylated tetra-acetate **16β** ($CDCl_3$; H-1: τ 4.30, $J_{1,2}$ 7 Hz); in the spectrum of **14β**, with the tripeptide chain uncharged, the signal due to H-1 could not be assigned, as it occurred further upfield in the region (τ 5–6) obscured by the large HDO peak. Treatment of the latter compound with acetic anhydride in aqueous acetone gave the corresponding *N*-acetyl derivative **15β**, in whose p.m.r. spectrum (D_2O) the anomeric proton resonated at τ 4.46 ($J_{1,2}$ 7 Hz); furthermore, conventional acetylation of **15β** yielded **16β** as the sole product.

It is well-established that protons situated above or below an aromatic ring are subject to shielding effects in n.m.r. spectroscopy[5]. The difference in the spectra for the uncharged and charged molecule of **14β** may be rationalised in these terms. Thus, if the terminal amino group in **14β** is not protonated, the preferred conformation of the tripeptidyl chain could be that in which the aromatic ring of the phenylalanyl residue is turned perpendicularly toward the anomeric proton of the sugar moiety. In such a case, restriction of flexibility in the peptide chain might also cause

distortion of the pyranoid ring, but elucidation of this problem requires further investigation.

The 1-*O*-tripeptidyl-β-D-glucopyranoses **2β** and **9β**, whether in the charged or uncharged forms, were good substrates for almond β-D-glucosidase. In contrast, whereas the trifluoroacetate of **14β** was completely cleaved by β-D-glucosidase into D-glucose and the tripeptide after ~6 h (as was also the case with **2β** and **9β** monooxalates), the free base was unaffected, even after 24 h of incubation. This resistance may reflect a strong interaction between the aromatic ring on the non-flexible peptide chain and H-1 of the sugar moiety, which makes the glycosyl ester bond not easily accessible to the active site of the enzyme.

Catalytic hydrogenolysis of **13α** in the presence of trifluoroacetic acid yielded chromatographically homogeneous 1-*O*-(glycylglycylphenylalanyl)-α-D-glucopyranose (**14α**) trifluoroacetate, which was characterised as the *N*-acetyl and tetraacetate derivatives (**15α** and **16α**, respectively). However, the p.m.r. spectra of **14α** and **15α** did not allow assignment of the H-1 signals, as they were obscured by the signals for the other ring protons. By contrast, the spectrum of **16α** was amenable to simple analysis and fully supported the 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetylglycylglycylphenylalanyl)-α-D-glucopyranose structure. Additional experiments with **14α** will be necessary before a discussion on the effects observed can be presented. Deprotection of **13α**, in the absence of a strong acid, yielded the corresponding 2-*O*-acyl derivative **17**, which was further characterised as the *N*-acetyl-1,3,4,6-tetra-*O*-acetyl derivative **18**; the p.m.r. spectrum of **18** indicated an ~1:1 anomeric mixture.

The conformational changes that occur when a biologically active peptide binds to its receptor have been studied[6-9] extensively. The possible existence of a preferred conformation of the glycosidically linked tripeptidyl chain of the uncharged **14β** suggests that glycosyl esters of peptides may be of interest in such studies.

EXPERIMENTAL

General. — Melting points are uncorrected. Concentrations were performed at diminished 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*, chloroform–ethyl acetate (proportions are given in the text); *B*, 60:15:25 1-butanol–acetic acid–water; *C*, 5:1 ethyl acetate–methanol; and *D*, 2:2:1 acetonitrile–2-propanol–water. Detection in t.l.c. was effected by charring with sulphuric acid, or by using ninhydrin, alkaline silver nitrate, or the chlorine–starch–iodine reagent for peptides. 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.

N-Benzyloxycarbonylglycylglycylglycine pentachlorophenyl ester (70%) was

prepared by the carbodi-imide method from equimolar amounts of *N*-benzyloxycarbonylglycylglycylglycine[10] and pentachlorophenol in *N,N*-dimethylformamide; m.p. 193–195° (from ethanol–water); lit.[11] m.p. 189–190°. *N*-Benzyloxycarbonyl-L-phenylalanylglycylglycine pentachlorophenyl ester (74%) was obtained from $PhCH_2OCO$-Phe-Gly-Gly-OH[12] by using the crystalline dicyclohexylcarbodi-imide–pentachlorophenol (1:3 equiv.) complex, as described by Kovacs *et al.*[2]; after dissolution in *N,N*-dimethylformamide and precipitation with ether, the product had m.p. 200–202°, $[\alpha]_D$ −7.9° (*N,N*-dimethylformamide).

Anal. Calc. for $C_{27}H_{22}Cl_5N_3O_6$: C, 49.00; H, 3.35; N, 6.35. Found: C, 48.81; H, 3.49; N, 6.44.

N-Benzyloxycarbonylglycylglycyl-L-phenylalanine pentachlorophenyl ester[2] was prepared as just described; hydrolysis (6M HCl) of a sample showed that >90% of the optical activity of phenylalanine had been retained.

N-Acetylglycylglycylglycine methyl ester (70%) was prepared from the corresponding *N*-acetyltripeptide by the action of diazomethane; m.p. 231–232°.

Anal. Calc. for $C_9H_{15}N_3O_5$: C, 44.08; H, 6.17; N, 17.13; Found: C, 43.85; H, 6.16; N, 6.94.

2,3,4,6-Tetra-O-benzyl-1-O-(N-benzyloxycarbonyltripeptidyl)-D-glucopyranoses (**1**, **8**, *and* **13**). — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (4 mmol), imidazole (20 mmol), and the appropriate *N*-benzyloxycarbonyl-tripeptide pentachlorophenyl ester (4 mmol) were dissolved in anhydrous *N,N*-dimethylformamide (20–40 ml) at room temperature with shaking, and the reaction mixture was kept [monitoring by t.l.c. in solvent *A* (3:7) for **1**, and *A* (5:2) for **8** and **13**] at room temperature for a total of 4 days, 24 h, and 48 h, for **1**, **8**, and **13**, respectively; in the preparation of **1**, more peptide component (20% excess) was added after 2 h. The solvent was removed *in vacuo* (0.1 torr), the residue was dissolved in chloroform, and, after ~3 h, the precipitate was filtered off. The filtrate was washed successively with water, 10% citric acid in water, water, aqueous sodium hydrogencarbonate, and water, dried, and concentrated. The residue was eluted from silica gel [solvent *A* (3:7) for **1**; *A* (5:2) for **8** and **13**], the appropriate fractions were combined and concentrated, and the β-D anomers were obtained by crystallisation of the residues.

The material left after concentration of the mother liquor was re-chromatographed with the same solvent system, and fractions containing the slightly faster-moving α-D anomer were combined and concentrated. Yields and the physical and analytical data of the α and β anomers are given in Table I.

Chromatography of the diastereoisomers of **13β** (1.1 g) was performed on a column (1.5 × 70 cm) of silica gel with solvent *A* (2:5, fractions: 3 ml/10 min). Fractions 36–38 contained the D-Phe isomer (**b**, R_F 0.55, minor component, 60 mg), and fractions 55–57 the L-Phe isomer (**a**, R_F 0.45, major component, 282 mg). By repeating the above procedure with mixtures of different L-:D-Phe ratio (obtained by combination and concentration of the appropriate middle fractions), ~65% of **13β** could be separated into the pure isomeric forms (**a**:**b** ratio ~3:1).

1-O-(Glycylglycylglycyl)-β-D-glucopyranose (**2β**). — (*a*) *In the presence of*

oxalic acid. A solution of 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonylglycyl-glycylglycyl)-*β*-D-glucopyranose (**1*β***, 749 mg) in acetic acid–2-methoxyethanol (2:1, 30 ml) was shaken with hydrogen in the presence of 10% palladium-on-charcoal (500 mg) and the equivalent amount (111 mg) of oxalic acid dihydrate for ~24 h (monitoring by t.l.c., solvent *B*). The catalyst was removed by centrifugation, and dry ether was added to the supernatant solution at 0°; **2*β*** mono-oxalate (319 mg, 82%) precipitated as a chromatographically homogeneous, hygroscopic solid, m.p. 119–120°, $[\alpha]_D$ −9.1° (water); ν_{max}^{KBr} 3310 (vs, broad, OH), 1770 (C=O), and 720 cm^{-1} (CO_2^- of oxalic acid). P.m.r. data (D_2O): τ 4.43 (d, $J_{1,2}$ 7 Hz, H-1), 5.88, 5.98, and 6.13 (3 s, 3 CH_2 of glycine).

Anal. Calc. for $C_{12}H_{21}N_3O_9 \cdot C_2H_2O_4$: C, 38.10; H, 5.25; N, 9.52. Found: C, 37.84; H, 5.55; N, 9.34.

To a cooled solution of the above salt (126 mg) in water (30 ml) was added 20% acetic anhydride in acetone (30 ml), and the solution was kept at 0° for ~20 h (monitoring by t.l.c., solvent *B*). After removal of traces of anhydride by co-distillation with water (0.1 torr), the residue was dissolved in hot methanol; subsequent addition of ether to the cooled solution gave 1-*O*-(*N*-acetylglycylglycylglycyl)-*β*-D-glucopyranose (**3*β***; 91 mg, 81%), m.p. 150–152°, $[\alpha]_D$ −8° (water); ν_{max}^{KBr} 3400 (vs, broad; OH, NH), 1650 and 1560 cm^{-1} (Amide I and II). P.m.r. data (D_2O): τ 4.27 (d, $J_{1,2}$ 7 Hz, H-1), 5.78, 5.92, 5.96 (3 CH_2 of glycine), and 7.87 (NAc).

Anal. Calc. for $C_{14}H_{23}N_3O_{10}$: C, 42.75; H, 5.89; N, 10.68. Found: C, 42.71; H, 6.07; N, 10.45.

(*b*) Catalytic hydrogenolysis of **1*β*** (688 mg), as described in (*a*) but without addition of oxalic acid, afforded the free base **2*β*** (280 mg) as a hygroscopic solid, $[\alpha]_D$ −7.8° (*c* 2, water); no i.r. absorption at 720 cm^{-1}. P.m.r. data (D_2O): τ 4.48 (d, $J_{1,2}$ 7 Hz, H-1), 5.94, 6.04, and 6.21 (3 CH_2 of glycine).

Anal. Calc. for $C_{12}H_{21}N_3O_9$: C, 41.02; H, 6.03; N, 11.96. Found: C, 41.26; H, 6.20; N, 12.05.

N-Acetylation of **2*β*** (35 mg), as described in (*a*), yielded chromatographically homogeneous product (35 mg, 90%) that was indistinguishable (spectral and analytical data) from **3*β*** obtained from **2*β*** mono-oxalate.

In a conventional acetylation, **3*β*** (50 mg) was treated with pyridine–acetic anhydride (5:1, 12 ml) at 0° for 24 h, the solution was then concentrated (0.1 torr), and final traces of anhydride were removed by distillation of water (3 × 1 ml) from the residue. The resulting solid showed (t.l.c.) one major (R_F 0.3, solvent *C*; 0.2, solvent *B*) and several very weak spots; it was practically insoluble in chloroform, ethyl acetate, and benzene, and fairly soluble in methanol. The p.m.r. spectrum [(CD_3OD): τ 4.10 (d, $J_{1,2}$ 7 Hz, H-1), 5.98, 6.08, 6.15 (3 CH_2 of glycine), 7.97, 7.99, and 8.02 (15 H, 4 OAc + NAc)] was consistent with the structure 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetylglycylglycylglycyl)-*β*-D-glucopyranose (**4*β***). Elution of this material from silica gel with solvent *C* led to extensive decomposition, to give a very small amount of **4*β*** heavily contaminated with 2,3,4,6-tetra-*O*-acetyl-D-glucopyranose (t.l.c.; solvent *C*, R_F 0.8). Further elution of the column with methanol deposited a

solid identified by comparison (mixture m.p., t.l.c., and p.m.r. data) with an authentic sample as *N*-acetylglycylglycylglycine methyl ester.

(*c*) A solution of **2β** mono-oxalate (30 mg) in water (1 ml) was passed through a column (25 × 1 cm) of Sephadex G-10 with water. The fractions (1.5 ml) were assayed for absorption at 280 nm; **2β** (21 mg, 90%) emerged as a sharp peak in 3 fractions, well ahead of oxalic acid. Thus obtained, the product was indistinguishable (i.r. and analytical data) from **2β** obtained in (*b*).

1-O-(*Glycylglycylglycyl*)-α-D-*glucopyranose mono-oxalate* (**2α**). — Catalytic hydrogenolysis of **1α** (100 mg) in the presence of oxalic acid dihydrate (15 mg), as described for **2β**, afforded **2α** (48 mg, 92%) as a hygroscopic solid, $[\alpha]_D$ +66° (water); ν_{max}^{KBr} 720 cm^{-1} (CO_2^-). P.m.r. data (D_2O): τ 3.86 (d, $J_{1,2}$ 2.5 Hz, H-1), 5.90, 6.00, and 6.18 (3 CH_2 of glycine).

Anal. Calc. for $C_{12}H_{21}N_3O_9 \cdot C_2H_2O_4$: C, 38.10; H, 5.25; N, 9.52. Found: C, 38.38; H, 5.31; N, 9.27.

N-Acetylation of **2α** (52 mg), as described for **3β**, afforded 1-*O*-(*N*-acetylglycylglycylglycyl)-α-D-glucopyranose (**3α**; 41 mg, 88%) as a hygroscopic solid, $[\alpha]_D$ +80° (water). P.m.r. data (D_2O): τ 3.90 (d, $J_{1,2}$ 2.5 Hz, H-1), 5.98, 6.16, 6.18 (3 CH_2 of glycine), and 8.01 (NAc).

Anal. Calc. for $C_{14}H_{23}N_3O_{10} \cdot H_2O$: C, 40.87; H, 6.12; N, 10.21. Found: C, 41.23; H, 6.19; N, 10.27.

Conventional acetylation of **3α** (35 mg), followed by processing as described for **4β** and precipitation of the product from solution in ethyl acetate with light petroleum at 0°, gave chromatographically homogeneous 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetylglycylglycylglycyl)-α-D-glucopyranose (**4α**; 49 mg, 98%) as a solid foam, $[\alpha]_D$ +83.7° (*c* 2). P.m.r. data (after D_2O exchange): τ 3.72 (d, $J_{1,2}$ 3 Hz, H-1), 7.92, and 7.97 (4 OAc + NAc).

Anal. Calc. for $C_{22}H_{31}N_3O_{14}$: C, 47.06; H, 5.56; N, 7.48. Found: C, 46.99; H, 5.56; N, 7.57.

2-O-(*Glycylglycylglycyl*)-D-*glucopyranose* (**5**). — Catalytic hydrogenolysis of **1α** (150 mg) in the absence of a strong acid, as described for **2β**, followed by lyophilisation of a solution of the product in water (1 ml), gave amorphous **5** (44 mg, 71%), $[\alpha]_D$ +26° (*c* 2, water). P.m.r. data (D_2O): τ 4.62–4.80 (m, H-1).

Anal. Calc. for $C_{12}H_{21}N_3O_9$: C, 41.02; H, 6.03; N, 11.96. Found: C, 40.87; H, 6.30; N, 11.84.

N-Acetylation of **5** (40 mg), as described for **3β**, gave 2-*O*-(*N*-acetylglycylglycylglycyl)-D-glucopyranose (**6**; 29 mg, 65%) as a white, hygroscopic solid, $[\alpha]_D$ +46° (water); ν_{max}^{KBr} 3430 (vs, broad; OH, NH), 1770 (C=O), 1670 and 1570 cm^{-1} (Amide I and II). P.m.r. data (D_2O): τ 4.62–4.80 (m, H-1), 5.91, 6.05, 6.10 (3 CH_2 of glycine), and 8.00 (NAc).

Anal. Calc. for $C_{14}H_{23}N_3O_{10}$: C, 42.75; H, 5.89; N, 10.68. Found: C, 43.02; H, 6.16; N, 10.86.

Conventional acetylation of **6** (27 mg), as described for **4β**, followed by precipitation of the product from solution in ethyl acetate with light petroleum, gave 1,3,4,6-

tetra-*O*-acetyl-2-*O*-(*N*-acetylglycylglycylglycyl)-D-glucopyranose (**7**; 20 mg, 52%) as a solid foam, $[\alpha]_D$ +45°. P.m.r. data (after D_2O exchange): τ 3.72 (d, ~0.6 H, $J_{1,2}$ 3 Hz) +4.29 (d, ~0.3 H, $J_{1,2}$ 7 Hz), H-1; 5.88, 6.10 (2 + 4 H, 3 CH_2 of glycine); 7.82 (~0.7 × 3 H, AcO-1*ax*) +7.92 and 7.97 (~13 H), 4 OAc + NAc.

Anal. Calc. for $C_{22}H_{31}N_3O_{14}$: C, 47.06; H, 5.56; N, 7.48. Found: C, 46.88; H, 5.69; N, 7.33.

1-O-(L-*Phenylalanylglycylglycyl*)-β-D-*glucopyranose* (**9β**). — (*a*) *In the presence of oxalic acid.* Deprotection of **8β** (300 mg) was performed as described for **2β** mono-oxalate, to give, after concentration of the solvent (~ half of the volume) and precipitation with anhydrous ether, the hygroscopic **9β** mono-oxalate (156 mg, 92%), m.p. 130° (dec.), $[\alpha]_D$ +30.5° (water); ν_{max}^{KBr} 722 cm^{-1} (CO_2^-). P.m.r. data (D_2O): τ 2.70 (Ph) and 4.37 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{19}H_{27}N_3O_9 \cdot C_2H_2O_4$: C, 47.46; H, 5.50; N, 7.90. Found: C, 47.24; H, 5.79; N, 7.66.

Conventional acetylation of the foregoing compound (43 mg) gave, after addition of light petroleum to a solution of the residue in ethyl acetate, 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanylglycylglycyl)-β-D-glucopyranose (**10β**; 36.5 mg, 71%), m.p. 192–193°, $[\alpha]_D$ +7°. P.m.r. data (after D_2O exchange): τ 2.80 (Ph), 4.35 (d, $J_{1,2}$ 7 Hz, H-1), and 7.95–8.06 (4 OAc + NAc).

Anal. Calc. for $C_{29}H_{37}N_3O_{14}$: C, 53.45; H, 5.72; N, 6.45. Found: C, 53.54; H, 5.91; N, 6.26.

(*b*) Catalytic hydrogenolysis of **8β** (300 mg) in the absence of a strong acid gave the hygroscopic, free base **9β** (126 mg, 90%), m.p. 90° (dec.), $[\alpha]_D$ +21.5° (water). P.m.r. data (D_2O): τ 2.66 (Ph) and 4.37 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{19}H_{27}N_3O_9$: C, 51.69; H, 6.17; N, 9.52. Found: C, 51.50; H, 5.97; N, 9.54.

Conventional acetylation of **9β** (43 mg) gave a product (49 mg, 76.5%) whose m.p., mixture m.p., and spectral data were indistinguishable from those of **10β** prepared from **9β** mono-oxalate.

1-O-(L-*Phenylalanylglycylglycyl*)-α-D-*glucopyranose trifluoroacetate* (**9α**). — A solution of **8α** (50 mg) in 2-methoxyethanol (15 ml) was shaken with hydrogen in the presence of 10% palladium-on-charcoal (30 mg) and trifluoroacetic acid (98%, 0.5 ml) for ~20 h. After removal of the catalyst and solvent (0.1 torr), traces of trifluoroacetic acid were removed by co-distillation with ether, and to a solution of the residue in methanol was added anhydrous ether at 0°. The title compound (26 mg, 88%) precipitated as a hygroscopic solid, $[\alpha]_D$ +90° (water); ν_{max}^{KBr} 3420 (vs, broad; OH, NH), 1770 (C=O), 1555 (Amide II), and 725 cm^{-1} ($CF_3CO_2^-$). P.m.r. data (D_2O): τ 2.59 (Ph), 3.74 (d, $J_{1,2}$ 3 Hz, H-1), and 6.77 (d, J 6 Hz, CH_2 of phenylalanine).

Anal. Calc. for $C_{19}H_{27}N_3O_9 \cdot CF_3CO_2H$: C, 45.41; H, 5.08; N, 7.56. Found: C, 45.15; H, 5.29; N, 7.44.

Conventional acetylation of **9α** (17 mg) gave, after addition of anhydrous ether to a solution of the residue in chloroform, 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetyl-L-phenylalanylglycylglycyl)-α-D-glucopyranose (**10α**; 19 mg, 82%), m.p. 88–90°

(sintering at 65°), $[\alpha]_D$ +55°. P.m.r. data (after D_2O exchange): τ 2.63 (Ph), 3.72 (d, $J_{1,2}$ 3 Hz, H-1), 7.92, 7.97, 8.09, and 8.15 (4 OAc + NAc).

Anal. Calc. for $C_{29}H_{37}N_3O_{14}$: C, 53.45; H, 5.72; N, 6.45. Found: C, 53.70; H, 5.93; N, 6.71.

2-O-(L-*Phenylalanylglycylglycyl*)-D-*glucopyranose* (**11**). — Deprotection of **8α** (50 mg) was performed as described for **2β** under (*b*); after removal of the catalyst and solvent, the residue was dissolved in methanol, and addition of anhydrous ether then gave **11** as a hygroscopic solid (19 mg, 80%), $[\alpha]_D$ +45.6° (water). P.m.r. data (D_2O): τ 2.72 (Ph) and 4.68–4.88 (m, H-1). Satisfactory results could not be obtained in the elemental analysis of **11**, because of its extreme hygroscopicity.

Conventional acetylation of a freshly prepared sample (26 mg) of **11** afforded 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*N*-acetyl-L-phenylalanylglycylglycyl)-D-glucopyranose (**12**; 17 mg, 46%), m.p. 116–120° (from ethyl acetate–light petroleum), $[\alpha]_D$ +24.2°. P.m.r. data (after D_2O exchange): τ 2.72 (Ph); 3.70 (d, ~0.5 H, $J_{1,2}$ 3 Hz) + 4.40 (d, ~0.5 H, $J_{1,2}$ 7 Hz), H-1; 7.81 (~0.5 × 3 H, AcO-1*ax*) + 7.90–8.05 (~13.5 H), 4 OAc + NAc.

Anal. Calc. for $C_{29}H_{37}N_3O_{14}$: C, 53.45; H, 5.72; N, 6.45. Found: C, 53.21; H, 6.11; N, 6.29.

1-O-(*Glycylglycyl*-L-*phenylalanyl*)-β-D-*glucopyranose* (**14β**). — (*a*) *In the presence of trifluoroacetic acid.* Catalytic hydrogenolysis of 2,3,4,6-tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonylglycylglycyl-L-phenylalanyl)-β-D-glucopyranose (**13βa**, 330 mg) was performed in the presence of trifluoroacetic acid (3 ml), as described for **9α**; addition of anhydrous ether to a solution of the product in methanol gave the **14β** trifluoroacetate salt (177 mg, 90%) as a very hygroscopic solid, $[\alpha]_D$ ~0° (water); ν_{max}^{KBr} 3400 (vs, broad; OH, NH), 1755 (C=O), 1680 and 1545 (Amide I and II), and 720 cm^{-1} ($CF_3CO_2^-$). P.m.r. data (D_2O): τ 2.65 (Ph) and 4.37 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{19}H_{27}N_3O_9 \cdot CF_3CO_2H$: C, 45.41; H, 5.08; N, 7.56. Found: C, 45.37; H, 5.33; N, 7.77.

Conventional acetylation of the foregoing salt (21 mg) was performed as described for **4β**; addition of light petroleum to a solution of the product in chloroform gave 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetylglycylglycyl-L-phenylalanyl)-β-D-glucopyranose (**16β**) as a hygroscopic solid (23 mg, 80%), $[\alpha]_D$ +5°. P.m.r. data (after D_2O exchange): τ 2.75 (Ph), 4.30 (d, $J_{1,2}$ 7 Hz, H-1), 7.93, and 8.00 (4 OAc + NAc).

Anal. Calc. for $C_{29}H_{37}N_3O_{14}$: C, 53.45; H, 5.72; N, 6.45. Found: C, 53.18; H, 5.80; N, 6.29.

(*b*) Deprotection of **13βa** (178 mg), performed as described for **2β** under (*b*), yielded, upon addition of anhydrous ether to the concentrated (~half of the volume) supernatant solution, the hygroscopic, free base **14β** (76 mg, 90%), m.p. 124° (dec., shrinking at 60°), $[\alpha]_D$ −7.5° (water); no i.r. absorption at 720 cm^{-1}.

Anal. Calc. for $C_{19}H_{27}N_3O_9$: C, 51.69; H, 6.17; N, 9.52. Found: C, 51.45; H, 6.40; N, 9.45.

N-Acetylation of the foregoing compound (50 mg) was performed as for **3β**;

addition of anhydrous ether to a solution of the product in 2-methoxyethanol precipitated 1-*O*-(*N*-acetylglycylglycyl-L-phenylalanyl)-β-D-glucopyranose (**15β**) as a hygroscopic solid (35 mg, 64%), $[\alpha]_D$ −5.8° (water). P.m.r. data (D_2O): τ 2.70 (Ph), 4.46 (d, $J_{1,2}$ 7 Hz, H-1), and 7.99 (NAc).

Anal. Calc. for $C_{21}H_{29}N_3O_{10} \cdot H_2O$: C, 50.29; H, 6.23; N, 8.37. Found: C, 50.34; H, 6.55; N, 8.47.

Conventional acetylation of **15β** (21 mg) yielded a chromatographically homogeneous product (22 mg, 78%), indistinguishable (t.l.c., analytical, and spectral data) from **16β** described above.

1-O-(Glycylglycylphenylalanyl)-α-D-glucopyranose trifluoroacetate (**14α**). — Deprotection of **13α** (110 mg), as described for **9α**, gave hygroscopic **14α** (39 mg, 61%), m.p. 95–100° (dec.), $[\alpha]_D$ +43.5° (*c* 1.6, water); ν_{max}^{KBr} 3320 (vs, broad; OH, NH), 1745 (C=O), 1670 and 1540 (Amide I and II), and 720 cm^{-1} ($CF_3CO_2^-$).

Anal. Calc. for $C_{19}H_{27}N_3O_9 \cdot CF_3CO_2H$: C, 45.41; H, 5.08; N, 7.56. Found: C, 45.64; H, 5.40; N, 7.84.

N-Acetylation of the above salt (100 mg) afforded, after addition of anhydrous ether to a solution of the residue in methanol, amorphous 1-*O*-(*N*-acetylglycylglycyl-phenylalanyl)-α-D-glucopyranose (**15α**; 80 mg, 91%), $[\alpha]_D$ +38° (water). P.m.r. data (D_2O): τ 2.66 (Ph), 3.83 (d?, $J_{1,2}$ 1.5 Hz, H-1), and 7.92 (NAc).

Anal. Calc. for $C_{21}H_{29}N_3O_{10} \cdot H_2O$: C, 50.29; H, 6.23; N, 8.37. Found: C, 50.53; H, 6.29; N, 8.20.

Conventional acetylation of **15α** (44 mg) afforded, on addition of anhydrous ether to a solution of the residue in chloroform, amorphous 2,3,4,6-tetra-*O*-acetyl-1-*O*-(*N*-acetylglycylglycylphenylalanyl)-α-D-glucopyranose (**16α**; 49 mg, 83%), $[\alpha]_D$ +59°. P.m.r. data (after D_2O exchange): τ 2.75 (Ph), 3.70 (d, $J_{1,2}$ 3 Hz, H-1), 6.82 (d, *J* 6 Hz, PhCH_2 of phenylalanine), 7.91, 7.97, 7.99, and 8.02 (4 OAc + NAc).

Anal. Calc. for $C_{29}H_{37}N_3O_{14}$: C, 53.45; H, 5.72; N, 6.44. Found: C, 53.17; H, 5.85; N, 6.38.

2-O-(Glycylglycylphenylalanyl)-D-glucopyranose (**17**). — Hydrogenolysis of **13α** (100 mg), performed in the absence of a strong acid, gave hygroscopic **17** (38 mg, 81%), m.p. 136–140° (dec.), $[\alpha]_D$ +35.4° (*c* 1.5, water). P.m.r. data (D_2O): τ 2.66 (Ph) and 4.60–4.82 (m, H-1).

Anal. Calc. for $C_{19}H_{27}N_3O_9$: C, 51.69; H, 6.17; N, 9.52. Found: C, 51.46; H, 6.11; N, 9.67.

Conventional acetylation of **17** (30 mg), followed by addition of anhydrous ether to a solution of the product in chloroform, yielded 1,3,4,6-tetra-*O*-acetyl-2-*O*-(*N*-acetylglycylglycylphenylalanyl)-D-glucopyranose (**18**; 20 mg, 50%) as a cream, hygroscopic mass, $[\alpha]_D$ +33°. P.m.r. data (after D_2O exchange): τ 2.76 (Ph); 3.70 (d, $J_{1,2}$ 3 Hz) + 4.24 (d, $J_{1,2}$ 7 Hz), H-1; 7.81 (~0.5 × 3 H, AcO-1*ax*) + 7.92 and 7.99 (~13.5 H), 4 OAc + NAc.

Anal. Calc. for $C_{29}H_{37}N_3O_{14}$: C, 53.45; H, 5.72; N, 6.44. Found: C, 53.52; H, 6.00; N, 6.45.

Enzymic hydrolyses. — (*a*) *With carboxypeptidase A.* Samples (15 mg each)

of the L- and D-phenylalanyl-containing components (**a** and **b**) of **13β**, as well as **13α** and **β**, were catalytically hydrogenolysed in acetic acid–2-methoxyethanol (2:1). The residues [t.l.c. of deprotected products (solvent *D*): R_F 0.25 (from **a**) and 0.20 (from **b**)], obtained after removal of the catalyst and solvent, were made 0.01M in Tris–HCl buffer (0.1M, pH 7.5), and the solutions were incubated in parallel with carboxypeptidase A (EGA, from bovine pancreas, crystalline suspension in water) at 25° together with glycylglycyl-L-phenylalanine as the standard substrate. After 3, 6, and 24 h, aliquots were subjected to t.l.c. with the standard tripeptide, glycylglycine, and phenylalanine. After development (solvent *D*), the amino acid and peptides were located by staining with ninhydrin.

(*b*) *With β-D-glucosidase.* A 5mM solution (0.5 ml) of each 1-*O*-tripeptidyl-β-D-glucopyranose compound (in the form of the salt and as the free base) in 0.05M citrate buffer (pH 5.25) was incubated with a solution (2 mg/ml, 0.2 ml) of β-D-glucosidase (Sigma, from sweet almonds) at 37°, together with 4-methylumbelliferyl β-D-glucopyranoside as the standard substrate. Blank solutions contained the same concentration of the substrate, but no enzyme. After 3, 6, 12, and 24 h, aliquots were subjected to t.l.c. (solvent *D*).

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