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SYNTHESIS AND PROPERTIES OF 2,3,4,6-TETRA-*O*-BENZYL-1-*O*-(*N*-BENZYLOXYCARBONYLDIPEPTIDYL)-α- AND -β-D-GLUCOPYRANOSES AND 1-*O*-DIPEPTIDYL-D-GLUCOPYRANOSES. PIPERAZINEDIONE FORMATION FROM 1-*O*-DIPEPTIDYL-D-GLUCOPYRANOSES BY INTRAMOLECULAR AMINOLYSIS*

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ABSTRACT

2,3,4,6-Tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyldipeptidyl)-D-glucopyranoses (**1–5**) were synthesized from 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose and pentachlorophenyl esters of *N*-benzyloxycarbonyldipeptides in the presence of imidazole; the anomeric mixtures were resolved and the α and β anomers were characterized. Catalytic hydrogenation of the β anomers of **1–3**, having aglycon groups containing aliphatic amino acid residues, afforded the corresponding 1-*O*-dipeptidyl-β-D-glucopyranoses, which were characterized as the mono-oxalates **6–8**; **6** and **7** were converted into the *N*-acetyl derivatives **9** and **10**, which were also prepared by definitive methods. Hydrogenolysis of the β anomers of **4** and **5**, having aglycon groups containing Phe-Gly and Gly-Phe residues, led to intramolecular aminolysis with scission of the glycosidic ester bond to give 3-benzylpiperazine-2,5-dione and D-glucose. Selective *N*-deprotection of **5β** afforded 2,3,4,6-tetra-*O*-benzyl-1-*O*-(glycyl-DL-phenylalanyl)-β-D-glucopyranose (**13β**), and complete deprotection of **5α** gave 1-*O*-(glycyl-DL-phenylalanyl)-α-D-glucopyranose (**14**) as the preponderant products; in both cases, intramolecular cyclisation of the aglycon group was a minor reaction. The results suggest that the balance between the formation of free D-glucosyl ester and the respective piperazinedione derivative depends primarily upon the nature and the sequence of the amino acids involved, and to a lesser extent upon the nature of substituents and the anomeric configuration of the sugar component.

INTRODUCTION

We have shown that protected glucosyl and glucosyluronic esters of amino acids can be synthesized by the imidazole-promoted reactions of sugar derivatives, fully protected other than at C-1, with activated esters of acylamino acids or with acylamino

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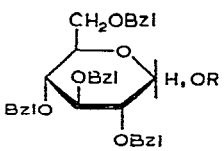
acids in the presence of dicyclohexylcarbodiimide (DCC)[1–3]. Selective removal of the benzyl blocking groups from the D-glucosyl moiety gave the corresponding 1-*O*-acylaminoacyl-D-glucopyranoses without affecting the 1-ester linkage, and simultaneous deprotection of the amino and hydroxyl functions was applied in the preparation of 1-*O*-(L-*β*-aspartyl)-*β*-D-glucopyranose[4].

We now report on the synthesis and properties of some D-glucosyl esters of dipeptides, the first model compounds of glycopeptides containing the glycosidic ester type of linkage.

RESULTS AND DISCUSSION

2,3,4,6-Tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyldipeptidyl)-D-glucopyranoses (**1–5**) were synthesized by the "accelerated active ester" (AAE) method from tetra-*O*-benzyl-*α*-D-glucopyranose and the appropriate benzyloxycarbonyldipeptide pentachlorophenyl ester in the presence of five equivalents of imidazole. The products were obtained in high yields as anomeric mixtures which were resolved by silica-gel chromatography and fully characterized (Table I); the *β*-D anomers were crystalline and the corresponding *α*-D anomers, with the exception of the benzyloxycarbonyl-L-phenylalanylglycine ester **4**, were viscous oils.

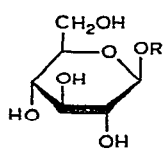
The tendency of the above method to cause racemization was estimated from the optical rotation of benzyloxycarbonyldipeptide methyl esters formed through

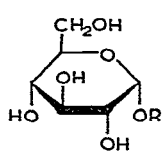


1 R = Z—Gly—Gly—
2 R = Z—Ala—Gly—
3 R = Z—Ala—Ala—
4 R = Z—Phe—Gly—
5 R = Z—Gly—DL-Phe—

11 R = Ac—Gly—Gly—
12 R = Ac—Ala—Gly—
13 R = $(COOH)_2$ x H—Gly—DL-Phe—

Z = $PhCH_2OCO$





6 R = $(COOH)_2$ x H—Gly—Gly—
7 R = $(COOH)_2$ x H—Ala—Gly—
8 R = $(COOH)_2$ x H—Ala—Ala—
9 R = Ac—Gly—Gly—
10 R = Ac—Ala—Gly—

14 R = $(COOH)_2$ x H—Gly—DL-Phe—

transesterification of the protected D-glucosyl esters with methanol in the presence of one equivalent of sodium methoxide[2]. The D-glucosyl ester **3β**, containing two aliphatic amino acid residues, and **4β**, having glycine as the *C*-terminal amino acid in the aglycon portion, yielded optically pure benzyloxycarbonyl-L-alanyl-L-alanine methyl ester and benzyloxycarbonyl-L-phenylalanylglycine methyl ester, respectively.

In contrast, the ester-exchange reactions of **5β** and **5α** gave completely racemized benzyloxycarbonylglycylphenylalanine methyl ester. It is known that amino acids having electronegative substituents, such as phenylalanine, are easily racemized, and that benzyloxycarbonyldipeptide active esters having the sequence Gly-Phe are particularly prone to extensive racemization[5,6]. We prepared the latter compound with 95% optical purity by condensing[7] benzyloxycarbonylglycyl-L-phenylalanine with a crystalline DCC–pentachlorophenol complex in ethyl acetate; it was claimed[7] that the same procedure in *N*,*N*-dimethylformamide led to a 50% racemized product. Since the latter solvent was used in the synthesis of **1–5**, we tested its possible effect on racemization of the active ester involved: when solutions of benzyloxycarbonylglycyl-L-phenylalanine pentachlorophenyl ester in *N*,*N*-dimethylformamide were kept at room temperature for 24 h, *i.e.*, under conditions of the coupling reaction, 70–75% of the initial optical activity was lost.

Hence, although the influence of imidazole on the racemization of the aglycon moiety of **5** cannot be completely ruled out, the above results indicate that the optical purity of the D-glucosyl ester formed depends critically on the nature and the sequence of the amino acid residues in the peptide component, as well as on the solvent used in coupling with the sugar component.

Deprotection of the β anomers of D-glucosyl esters **1–5** was performed by catalytic hydrogenation in acetic acid–2-methoxyethanol over palladium-on-charcoal in the presence of the equimolar amounts of oxalic acid dihydrate. The reaction was conducted in two successive steps: *1*, removal of the *N*-benzyloxycarbonyl group in the aglycon portion; and *2*, removal of the benzyl ether groups in the sugar moiety. D-Glucosyl esters **1β**, **2β**, and **3β**, having aglycon groups containing aliphatic amino acid residues, afforded the corresponding unprotected esters **6**, **7**, and **8** in good yields. The compounds were isolated as crystallisable, highly hygroscopic, mono-oxalate salts. The crude hydrogenolysis product of **3β** revealed (t.l.c.) two additional, minor products: one was coincident with D-glucose and the other with 3,6-dimethylpiperazine-2,5-dione. Thus, deprotection of **3β** was accompanied, to a low extent, by intramolecular cyclisation of the aglycon portion with concomitant scission of the glycosidic ester bond.

D-Glucosyl esters **6–8** gave elemental analyses and spectral data fully consistent with the structures proposed. The i.r. spectra contained absorptions characteristic of hydroxyl and ester functions, and the n.m.r. spectra in deuterium oxide and methyl sulphoxide-d_6 showed H-1 signals having positions and $J_{1,2}$ values indicative of the β-D configuration. When stored under anhydrous conditions at room temperature, the crystalline mono-oxalate salts **6–8** decomposed (t.l.c.) within 2–3 months into D-glucose and the parent, linear peptide. In the case of **8**, a spot associated with the

TABLE I

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

Compound	*Aglycon group* ($Z = PhCH_2OCO$)	*Yield*[a] (%)	*Anomeric form*	*M.p.* (*degrees*)	$[\alpha]_D$ (*degrees*, *c*1–2)[b]	*Chemical shifts*[c], τ *H-1* (*doublet*)
1	Z–Gly–Gly	60	*β*	102–103[e]	+6.0	4.32 (7)
			α	oil	+43.0	3.62 (3)
2	Z–Ala–Gly	68	*β*	137–138[f]	−7.4	4.45 (7)
			α	oil	+36.8	3.68 (3)
3	Z–Ala–Ala	77	*β*	118–119[e]	+2.6	4.36 (7)
			α	oil	+37.3	3.65 (3)
4	Z–Phe–Gly	57	*β*	126–128[e]	+5.7	4.32 (7)
		15	*α*	95–96[h]	+33.0	3.62 (3)
5	Z–Gly–DL-Phe	83	*β*	111–113[e]	+12.0	4.37 (7)
			α	oil	+41.0	3.55–3.82 m[i]
11	Ac–Gly–Gly	53	*β*	141–142[j]	+4.2	4.36 (7)
			α	116–118[f]	+55.2	3.85 (2.5)
12	Ac–Ala–Gly	53	*β*	149–151[j]	−19.0	4.37 (7)
			α	57–59[f]	+22.0	3.92 (2.5)

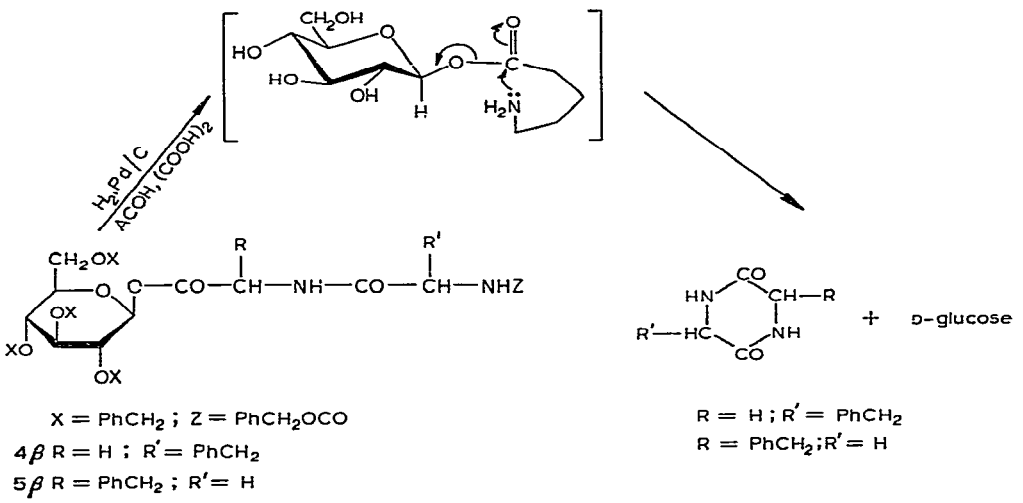
[a]The yields are given for chromatographically homogeneous, anomeric mixtures, except for 4*β* which crystal from the crude product. [b]Determined in chloroform. [c]Data taken from spectra measured at 60 MHz in chl form-*d* for **1–5**; in methyl sulphoxide-d_6 for **11–12**. Coupling constants (*J*) in Hz. [d]Removed by D_2O excha integrated intensity: 1 proton for **1–5**; two protons for **11–12**. Peak multiplicities: d, doublet; t, triplet multiplet. [e]From ethanol. [f]From ethyl acetate–light petroleum. [g]Doublet, 3 H for **2** and **12**, 6 H for **3**, *J* 7 *Me*–CH. [h]From ether–light petroleum. [i]Two-proton signal; after D_2O exchange: 1 H, τ 3.62 d (3), H-1. [j]F methanol. [k]Three-proton singlet, N–*Ac*.

cyclic dipeptide was also detectable. Aqueous and acetic acid solutions of **6–8** showed remarkable differences in stability with respect to the aglycon structure. Whereas, in aqueous solution, **6** decomposed almost completely into D-glucose and glycylglycine within the first 24 h, the cleavage of the C-1 ester bond in **7** and **8** did not exceed 20 and 30%, respectively, under identical conditions.

For further characterization, compounds **6** and **7** were selectively acetylated at the terminal amino group with 2% acetic anhydride in acetone–water to give 1-*O*-(*N*-acetylglycylglycyl)-*β*-D-glucopyranose (**9**) and 1-*O*-(*N*-acetyl-L-alanylglycyl)-*β*-D-glucopyranose (**10**), respectively. The products were obtained pure by chromatography on cellulose, and their structures were assigned by comparison with **9** and **10** prepared by an alternative route, as follows. Imidazole-promoted DCC condensation of tetra-*O*-benzyl-*α*-D-glucopyranose with *N*-acetylglycylglycine and *N*-acetyl-L-alanylglycine, respectively, afforded the corresponding, fully protected D-glucosyl esters **11** and **12**, as anomeric mixtures which were resolved and fully characterized (Table I). Catalytic hydrogenation of the *β* anomers of **11** and **12** yielded chromatographically homogeneous, hygroscopic solids having physical data ($[\alpha]_D$, n.m.r., i.r.) indistinguishable from those of the product obtained by *N*-acetylation of **6** and **7**, respectively.

Catalytic hydrogenolysis of D-glucosyl esters **4*β*** and **5*β***, performed as described for the *β* anomers of **1–3**, led almost exclusively to intramolecular aminolysis of the

		Found (%)			Formula	Calc. (%)		
N–H[d]	Other signals	C	H	N		C	H	N
4.48t		69.78	6.30	3.74	$C_{46}H_{48}N_2O_{10}$	70.03	6.13	3.55
4.50t		69.75	6.04	3.42				
4.80d	8.70[g]	70.38	6.14	3.69	$C_{47}H_{50}N_2O_{10}$	70.30	6.28	3.49
4.75d	8.68[g]	70.57	6.55	3.38				
4.70d	8.68[g]	70.59	6.54	3.41	$C_{48}H_{52}N_2O_{10}$	70.57	6.42	3.43
4.68d	8.68[g]	70.37	6.54	3.52				
4.66d		72.65	6.28	2.94	$C_{53}H_{54}N_2O_{10}$	72.42	6.19	3.19
4.65d		72.18	6.29	3.09				
3.70d		72.61	6.48	3.10				
		72.26	6.34	3.46				
1.70–2.30m	8.18[k]	68.85	6.46	4.17	$C_{40}H_{44}N_2O_9$	68.95	6.36	4.02
1.70–2.30m	8.28[k]	69.24	6.15	4.19				
1.71–2.33m	8.81[g] 8.22[k]	69.14	6.67	3.80	$C_{41}H_{46}N_2O_9$	69.28	6.52	3.94
1.71–2.33m	8.90[g] 8.31[k]	69.26	6.71	3.87				



X = $PhCH_2$; Z = $PhCH_2OCO$

4β R = H ; R' = $PhCH_2$

5β R = $PhCH_2$; R' = H

R = H ; R' = $PhCH_2$

R = $PhCH_2$; R' = H

Scheme 1

ester with cleavage of the C-1 ester bond (Scheme 1). For each reaction, 3-benzylpiperazine-2,5-dione and D-glucose were identified as the major products of the reaction, together with small proportions (t.l.c.) of a ninhydrin-, peptide-, and silver

nitrate-posi ive component; the latter product, presumably the unprotected dipeptide D-glucosyl ester, disappeared successively during purification of the crude product. 3-Benzylpiperazine-2,5-dione, isolated from the hydrogenolysis product of **4β**, showed $[\alpha]_D$ +88.6° (acetic acid), which was similar to that of the L-form of an authentic sample, whereas the product formed from **5β** showed $[\alpha]_D$ +4.4° and was practically indistinguishable from the racemic form.

The tendency of peptide esters to cyclise by intramolecular aminolysis[8] depends on many factors, among which are the nature, configuration, and sequence of the amino acids involved in the peptide chain. For piperazinedione-ring formation, the linear dipeptide must have the peptide bond in the *cis*-form, so that interaction of the terminal amino group and the ester carbonyl is possible. In order to examine the effect of the hydroxyl groups of the sugar moiety on cyclisation, **5β** was subjected to selective deprotection of the amino-terminal group.

Catalytic hydrogenolysis of **5β**, performed under conditions which removed the *N*-benzyloxycarbonyl group but not the *O*-benzyl groups, gave a mixture containing a major ninhydrin-, peptide-, and silver nitrate-positive component, together with two minor components coincident (t.l.c.) with 3-benzylpiperazine-2,5-dione and tetra-*O*-benzyl-D-glucopyranose. The major product was isolated crystalline (34.7%), and its elemental analysis, and n.m.r. and i.r. spectra were consistent with the structure 2,3,4,6-tetra-*O*-benzyl-1-*O*-(glycyl-DL-phenylalanyl)-β-D-glucopyranose mono-oxalate (**13β**). In the dry state, the compound was stable, whereas in methanolic solution at room temperature, cyclisation of the aglycon group proceeded (t.l.c.) very slowly; it is noteworthy that the corresponding linear peptide could not be detected in the solution. Thus, the sluggish cyclisation of **13β** and the fact that the cyclic dipeptide was produced in only small proportion during *N*-deprotection of **5β**, whereas it was the principal product formed after the removal of *O*-benzyl groups, suggest that intramolecular aminolysis was facilitated by the presence of hydroxyl group(s) on the sugar moiety.

Unlike the β anomer, complete deprotection of the α anomer of **5** afforded a ninhydrin-positive component as the major product, together with small proportions of 3-benzylpiperazine-2,5-dione and D-glucose. The main component, isolated crystalline (46.7%), was identified by elemental analysis and spectral data as 1-*O*-(glycyl-DL-phenylalanyl)-α-D-glucopyranose mono-oxalate (**14**); the n.m.r. spectrum in deuterium oxide contained the doublet for H-1 at τ 3.77, having a $J_{1,2}$ value (3 Hz) consistent with the observed optical rotation. Thus, the axial aglycon group in **14**, having a *cis*-relation to HO-2, appears to be less readily involved in cyclisation than the equatorial aglycon group, *trans*-related to HO-2.

Bimolecular aminolysis reactions have been intensively studied in hydroxylic media, and explained by assuming the existence of a tetrahedral intermediate adduct, or in terms of a synchronous mechanism[9,10]. From a study on the kinetics and mechanism of intramolecular aminolysis of methyl and ethyl esters of dipeptides in slightly alkaline, aqueous solution[11], the reaction was found to be a self-catalysed process and other amines also served as catalysts. It is not yet possible to identify

and compare the behaviour of the unprotected glucosyl esters of dipeptides with various mechanisms postulated for intermolecular and intramolecular[12] ester aminolysis by more conventional reagents and solvents; nevertheless, some speculations on the data available are still possible.

The results of the catalytic hydrogenolysis of protected esters, performed under conditions which, in general, are not favourable for an ester aminolysis-type reaction, suggest that the product balance between the free D-glucosyl ester and the respective cyclic dipeptide depends primarily on the nature and the sequence of the amino acids involved. Different tendencies to cyclisation exhibited by the aglycon portions of compounds **1–5** may be attributed mainly to relative spatial dispositions governed by geometrical, sterical, and electronic factors in the peptide chain. If interaction of the aglycon, terminal amino-group and the ester carbonyl carbon is possible, then formation of the piperazinedione ring may proceed *via* a carbonyl addition intermediate, from which there is proton-assisted departure of the leaving group. It is believed that the fission occurs at the aglycon-oxygen bond, *i.e.*, analogously to the mode of cleavage in acid-catalyzed hydrolysis of ether glycosides having aglycons that can afford a stable carbonium ion[13,14]. This suggestion is supported by the observation (t.l.c., solvent *E*) that the intramolecular aglycon cyclisation of 1-*O*-(glycyl-DL-phenylalanyl)-α-D-glucopyranose (**14**) in methanol at room temperature was accompanied by the appearance of a spot coincident with D-glucose; methyl D-glucoside, the product diagnostic of glycosyl-oxygen fission[13], was not detectable.

In general, the rates of aminolysis reactions increase with the decreasing basicity of the leaving group[10]. Although the D-glucosyl moiety represents a rather special and complex type of leaving group, one can assume that in **13β**, having all the hydroxyl hydrogen atoms replaced by benzyl groups, the lower tendency to cyclise is due, at least partly, to the lower electron-withdrawing ability of the leaving group as compared to that in the unprotected ester. The different behaviour of the α and β anomers of **5** during catalytic hydrogenolysis may be explained by supposing that the negative charge on the oxygen atom of the equatorial alcohol is greater than that on the axial O-1, thereby accounting for the more extensive protonation at the former site in the bond-breaking step. The lower reactivity in acid-catalyzed hydrolysis of α-D-glucosides, as compared to that of the corresponding β anomers, has been explained[15] by a lower extent of protonation of the glucosidic oxygen atom of the former and attributed to the reverse anomeric effect. However, further experiments with glucosyl esters of dipeptides will be necessary before a detailed discussion of the reactions observed can be presented.

EXPERIMENTAL

General. — Melting points are uncorrected. Evaporations were performed in a rotary evaporator *in vacuo* at bath temperatures below 40°, if not stated otherwise. Column chromatography was performed on silica gel (Merck, 0.05–0.2 mm); cellulose powder (Whatman standard grade), packed as a slurry by using a plunger; or carbon–

Celite (Charcoal activated, B.D.H.; Kieselguhr, B.D.H.), prepared as a 2:1 (w/w) mixture. Solvent systems: *A* benzene–ethyl acetate (proportions are given in the text); *B* 4:1 benzene–ether; *C* 60:15:25 1-butanol–acetic acid–water; *D* 5:4:1 methanol–ethyl acetate–water; *E* 16:9:2 chloroform–methanol–water; *F* 3:1 acetonitrile–water. T.l.c. was performed with Kieselgel G (Merck) or cellulose (microcrystalline, Merck), followed by detection with 10% sulphuric acid and heating, with ninhydrin reagent, or with chlorine–starch–iodide reagent for peptides. Optical rotations were determined for 1% solutions in chloroform, unless otherwise stated. I.r. spectra were determined 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.

N-Benzyloxycarbonylglycylglycine and *N*-benzyloxycarbonyl-L-alanyl-L-alanine pentachlorophenyl esters were prepared by the method of Kapoor and Gerenscer[16]. *N*-Benzyloxycarbonylglycyl-L-phenylalanine pentachlorophenyl ester was synthesized by using the crystalline DCC–pentachlorophenol (1:3 equivalent) complex, as described by Kovacs *et al.*[7]; by this way, a 95% retention of optical activity was achieved. *N*-Benzyloxycarbonyl-L-alanylglycine pentachlorophenyl ester (66%) was obtained by the DCC method from equimolar amounts of *N*-benzyloxycarbonyl-L-alanylglycine[17] and pentachlorophenol in *N,N*-dimethylformamide–dichloromethane; after two recrystallisations from hot ethyl acetate–*N,N*-dimethylformamide (10:0.3), the product had m.p. 196–199°, $[\alpha]_D$ −11.8° (*N,N*-dimethylformamide).

Anal. Calc. for $C_{19}H_{15}Cl_5N_2O_5$: C, 43.17; H, 2.86; Cl, 33.54. Found: C, 43.33; H, 2.67; Cl, 33.71.

N-Benzyloxycarbonyl-L-phenylalanylglycine pentachlorophenyl ester (60%) prepared by the DCC condensation of *N*-benzyloxycarbonyl-L-phenylalanylglycine[18] and pentachlorophenol in *N,N*-dimethylformamide had m.p. 208–210° (from acetone plus some drops of *N,N*-dimethylformamide) $[\alpha]_D$ −8.0° (*N,N*-dimethylformamide).

Anal. Calc. for $C_{25}H_{19}Cl_5N_2O_5$: C 49.65; H 3.17; N, 4.63. Found: C, 49.44; H, 3.20; N, 4.65.

*2,3,4,6-Tetra-*O*-benzyl-1-*O*-(*N*-benzyloxycarbonyldipeptidyl)-*D*-glucopyranoses* (**1–5**). — 2,3,4,6-Tetra-*O*-benzyl-α-D-glucopyranose (3 mmoles) and imidazole (15 mmoles) were dissolved in dichloromethane (20 ml, for preparation of compounds **1–2**) or *N,N*-dimethylformamide (20 ml, for preparation of **3–5**) and the appropriate *N*-benzyloxycarbonyldipeptide pentachlorophenyl ester (3 mmoles) in the solvent (20 ml) indicated above was added at room temperature with shaking; in the preparation of **2**, the powdered peptide component and the solvent were added alternatively with mechanical stirring. After 2 h, more peptide ester (20–30% excess) was added, and the reaction mixture was kept at room temperature for a total of 24 h. In reactions using dichloromethane as the solvent, the precipitated pentachlorophenol was filtered off and washed with the same solvent, and the combined filtrate and washings were treated successively with water, 10% citric acid, water, aqueous sodium hydrogen carbonate, and water, and dried (sodium sulphate). In the reactions with *N,N*-

dimethylformamide, the solvent was removed *in vacuo* (0.1 torr), the residue was dissolved in ethyl acetate, and the solution was further treated as described above. After evaporation of the solvent, the crude products were chromatographed on silica gel; the eluting solvents for **1**, **2**, **3**, **4**, and **5** were *A* (3:1), *A* (7:3), *B*, *A* (7:3), and *B*, respectively. Combination and concentration of the appropriate fractions gave the products as chromatographically homogeneous, anomeric mixtures from which the β-D anomers were obtained by crystallisation; the β anomer of **4** could also be obtained by direct crystallisation of the crude product.

The mother liquors were evaporated to dryness, and the oily residues were re-chromatographed on silica gel with the solvent systems indicated above. The fractions containing the slightly faster moving α-D anomer were combined and evaporated to dryness. Physical constants, yields, and analytical data of the α and β anomers of compounds **1**–**5** are given in Table I.

Treatment of the protected D-*glucosyl esters with methanolic sodium methoxide.* — To a suspension of each D-glucosyl ester (0.2 mmole) in methanol (2 ml), 0.1M methanolic sodium methoxide (2 ml) was added, and the mixture was shaken for 1 h at room temperature; the reaction was monitored by t.l.c. in solvent *A* (1:1). Precipitated tetra-*O*-benzyl-α-D-glucopyranose (80–95%) was filtered off and washed with cold methanol, and the combined filtrate and washings were passed through a column of Amberlite IR-120(H^+) resin (5 ml) prewashed with methanol. The effluent was evaporated to dryness, and the residue was eluted through a column of silica gel with solvent *A* (1:1) to afford the corresponding benzyloxycarbonyldipeptide methyl ester (80–90%) which, after one recrystallisation from ethyl acetate–light petroleum, was checked for optical purity.

Starting compound	*Benzyloxycarbonyldipeptide methyl ester isolated*		
	Dipeptide	*M.p. (degrees)*	$[\alpha]_D$ *(degrees, c 1–2)*
3β	Ala–Ala	103–108	−17.3 ($CHCl_3$)[a]
4β	Phe–Gly	116–117	−24.8 ($HCONMe_2$)[b]
5β	Gly–Phe	71–73	0 (EtOH and $CHCl_3$)[c]
5α	Gly–Phe	71–73	0 (EtOH and $CHCl_3$)[c]

[a]Z–Ala–Ala–OMe; lit.[19] m.p. 105–107°, $[\alpha]_D$ −16° (*c* 1, chloroform). [b]Z–Phe–Gly–OMe; lit.[20] m.p. 121–123°, $[\alpha]_D$ −23° (*c* 1.8, *N,N*-dimethylformamide). [c]Z–Gly–Phe–OMe; lit.[21] m.p. 55–58°, $[\alpha]_D$ +13.7° (*c* 2, ethanol), unpublished results, $[\alpha]_D$ +50.0° (*c* 1, chloroform); Z–Gly–DL-Phe–OMe; lit.[22] m.p. 83–84°.

*1-O-Glycylglycyl-β-*D-*glucopyranose mono-oxalate* (**6**). — To a solution of the β anomer of **1** (394 mg, 0.5 mmole) in acetic acid–2-methoxyethanol (2:1, 15 ml) were added oxalic acid dihydrate (63 mg, 0.5 mmole) and 10% palladium-on-charcoal (100 mg); hydrogen was passed through the stirred suspension until evolution of carbon dioxide [$Ba(OH)_2$ solution] ceased (~1 h). More catalyst (250 mg) was added, and the mixture was shaken with hydrogen at atmospheric pressure and room

temperature until termination of hydrogen uptake. The catalyst was centrifuged off, the supernatant was evaporated to dryness (0.1 torr, bath 30°), and the residual syrup was triturated with dry ether to give **6** as a chromatographically homogeneous (t.l.c., silica gel, solvent *C*) solid (189 mg, 98%). The product was dissolved in warm acetic acid (45°), and to the cooled solution dry ether was added; on storage at 0°, analytically pure **6** was deposited as a hygroscopic powder with no definite melting-point (dec., ~90°); $[\alpha]_D$ 0° (water), 0° (acetic acid); ν_{max}^{KBr} 3450 broad, vs (OH), 1770 s (C=O), 1080 cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.42 (d, $J_{1,2}$ 7 Hz, H-1); $(CD_3)_2SO$: τ 4.62 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{12}H_{20}N_2O_{12}$: C, 37.50; H, 5.25; N, 7.29. Found: C, 37.78; H, 5.40; N, 7.34.

N-Acetylation of compound **6** (150 mg) in water (25 ml) was performed with a 2% solution of acetic anhydride in acetone (25 ml) at room temperature; after 1 h (t.l.c., silica gel, solvent *C*), the reaction was complete. The solvent was evaporated (0.1 torr, bath 30°), traces of the anhydride were removed by repeated addition and distillation of ethanol, and the residue was eluted from a column of cellulose (25 g, 63 × 1.2 cm) with solvent *D*; fractions (1 ml/0.5 h) were examined by t.l.c. (silica gel, solvent *C*), and those containing the chromatographically homogeneous *N*-acetyl derivative **9** were combined and evaporated to dryness. The residue (73 mg, 55.6%) was crystallised from ethanol–ether to give a hygroscopic powder (51 mg) which was identical with the authentic sample of 1-*O*-(*N*-acetylglycylglycyl)-β-D-glucopyranose (**9**), as judged by elemental analysis, optical rotation, and i.r. and n.m.r. spectra.

1-O-(L-*Alanylglycyl*)-β-D-*glucopyranose mono-oxalate* (**7**). — Hydrogenation of the β anomer of **2** (300 mg) was performed as described for **6**. After removal of the solvent, **7** was obtained as a highly hygroscopic solid which, after recrystallisation from acetic acid–ether, gave 89 mg (60.6%) of an analytical sample, $[\alpha]_D$ −5.0° (*c* 1.2, water); ν_{max}^{KBr} 3450 broad, vs (OH), 1760 s (C=O), 1070 s cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.42 (d, $J_{1,2}$ 7 Hz, H-1), 8.45 (d, *J* 7 Hz, *Me*–CH).

Anal. Calc. for $C_{13}H_{22}N_2O_{12}$: C, 39.20; H, 5.57; N, 7.03. Found: C, 39.49; H, 5.74; N, 6.89.

N-Acetylation of an aqueous solution of **7** (100 mg) was performed as described for **9**; the resulting syrup was chromatographed on a cellulose column with solvent *D* to give the acetylated product (54 mg, 61.7%) as a homogeneous, hygroscopic solid. Recrystallisation from dry ethanol–ether afforded an analytical sample identical with the authentic 1-*O*-(*N*-acetyl-L-alanylglycyl)-β-D-glucopyranose (**10**) described below.

1-O-(L-*Alanyl*-L-*alanyl*)-β-D-*glucopyranose mono-oxalate* (**8**). — Complete deprotection of **3β** (169 mg), performed as described for **1β**, yielded an oily product; on t.l.c. (silica gel, solvent *C*), one major (R_F ~0.3) and two minor (R_F ~0.5 and 0.75, coincident with D-glucose and 3,6-dimethylpiperazine-2,5-dione, respectively) components were detectable. Crystallisation from 2-methoxyethanol–ether at 0° gave **8** (47 mg, 55%) as a white, hygroscopic solid. A second crystallisation afforded the analytical sample, m.p. 113–118° (dec.), sintering at 107°, $[\alpha]_D$ +26.6° (water); ν_{max}^{KBr} 3420 broad, vs (OH), 1770 and 1690 s (C=O), 1630 and 1570 s (amide I and II),

1080 vs cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.45 (d, $J_{1,2}$ 7 Hz, H-1), 8.50 and 8.56 (2 d, J 7 Hz, 2 × *Me*CH).

Anal. Calc. for $C_{14}H_{24}N_2O_{12}$: C, 40.78; H, 5.87; N, 6.79. Found: C, 41.01; H, 6.02; N, 6.65.

Catalytic hydrogenation of the β anomers of **4** *and* **5**. — When compounds **4β** and **5β** were hydrogenated as described for **1β**, t.l.c. (silica gel, solvent *C*) revealed two major (coincident with 3-benzylpiperazine-2,5-dione and D-glucose, respectively) and one minor (ninhydrin-, peptide-, and silver nitrate-positive) spots. Elution of the crude products from silica gel with solvent *E* afforded the major, peptide-positive component as a homogeneous solid; after crystallisation from water, the compound gave elemental analysis consistent with the 3-benzylpiperazine-2,5-dione structure. The n.m.r. spectra in methyl sulphoxide-d_6 and trifluoroacetic acid-*d* of the product obtained from **4β** were indistinguishable from those of the product obtained from **5β** and from those[23] of an authentic sample. The product (60%) from **4β** showed m.p. 250–252°, $[\alpha]_D$ +88.6° (acetic acid); lit.[24] for the L-form: m.p. 268°, $[\alpha]_D$ +97.8° (*c* 1, acetic acid). The product (71%) from **5β** had m.p. 260–261°, $[\alpha]_D$ +4.4° (acetic acid); lit.[25] for the DL-form: m.p. 282–283°.

*2,3,4,6-Tetra-O-benzyl-1-O-(glycyl-*DL*-phenylalanyl)-β-*D*-glucopyranose mono-oxalate* (**13β**). — Through a suspension of **5β** (200 mg), oxalic acid dihydrate (29 mg), and 10% palladium-on-charcoal (50 mg) in 2-methoxyethanol–acetic acid (1:2, 15 ml), hydrogen was passed until evolution of carbon dioxide ceased. The catalyst was immediately centrifuged off, the supernatant was evaporated to dryness, and the remaining syrup was triturated with dry ether. The resulting white solid was crystallised from methanol to give chromatographically homogeneous (t.l.c., cellulose, solvent *F*) **13β** (67 mg, 34.7%); a second crystallisation afforded the analytical sample, m.p. 130–132°, $[\alpha]_D$ +7.5° (*N*,*N*-dimethylformamide); ν_{max}^{KBr} 3500 vs (NH), 3070 s (NH_2), 1760 vs (C=O), 1670 vs and 1540 w (amide I and II), 1080 vs (C–O–C), 750, 733, and 695 vs cm^{-1} (aromatic CH). N.m.r. data (methyl sulphoxide-d_6): τ 0.95–1.47 broad (1 H, NH), 2.58–2.90 (m, 25 H, 5 × Ph), 4.30 (d, $J_{1,2}$ 7 Hz, H-1).

Anal. Calc. for $C_{47}H_{50}N_2O_{12}$: C, 67.60; H, 6.04; N, 3.36. Found: C, 67.76; H, 6.28; N, 3.16.

A sample of pure **13β** in methanol decomposed into the piperazinedione derivative and tetra-*O*-benzyl-D-glucopyranose at room temperature at a low rate (monitoring by chromatographic and spectroscopic methods); after 5 days, the spot associated with **13β** disappeared completely.

*1-O-(Glycyl-*DL*-phenylalanyl)-α-*D*-glucopyranose mono-oxalate* (**14**). — The α anomer of **5** (230 mg) was hydrogenated as described for **1β**; after removal of the solvent and trituration of the residue with dry ether, a solid was obtained which was crystallised from methanol–ether to give **14** (58 mg, 46.7%), m.p. 110–113°, $[\alpha]_D$ +44.2° (water); ν_{max}^{KBr} 3430 broad vs (OH), 1760 s (C=O), 1080 s cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 2.58 (s, 5 H, Ph), 3.77 (d, $J_{1,2}$ 3 Hz, H-1).

Anal. Calc. for $C_{19}H_{26}N_2O_{12}$: C, 48.10; H, 5.52; N, 5.91. Found: C, 48.37; H, 5.36; N, 6.04.

T.l.c. (silica gel, solvent *C*) of the mother liquor revealed, besides **14**, two spots coincident with D-glucose and 3-benzylpiperazine-2,5-dione, respectively.

1-O-(N-*Acetylglycylglycyl*)- *and 1*-O-(N-*acetyl*-L-*alanylglycyl*)-*2,3,4,6-tetra*-O-*benzyl*-D-*glucopyranose* (**11** *and* **12**). — To a solution of *N*-acetylglycylglycine (522 mg, 3 mmoles) or *N*-acetyl-L-alanylglycine (564 mg, 3 mmoles) in *N,N*-dimethylformamide (20 ml) were added 2,3,4,6-tetra-*O*-benzyl-α-D-glucopyranose (3 mmoles), imidazole (6 mmoles), and DCC (3 mmoles) in dichloromethane (15 ml) at 0°. The mixture was mechanically stirred at room temperature for 20 h, *N,N'*-dicyclohexylurea was then filtered off, the mother liquor was evaporated to dryness (0.1 torr), and the residue was eluted from a column of silica gel with solvent *A* (1:5). Combination and concentration of the appropriate fractions gave the chromatographically homogeneous, anomeric mixture from which the β-D anomer was obtained by crystallisation.

The α anomers of **11** and **12** were isolated from the residues left after evaporation of the mother liquors; **11α** was obtained after fractionation of the residue on a carbon–Celite column (40 × 1 cm) with chloroform followed by crystallisation, and **12α** by direct crystallisation of the residue. Physical constants, yields, and analytical data of the α and β anomers of **11** and **12** are given in Table I.

1-O-(N-*Acetylglycylglycyl*)-β-D-*glucopyranose* (**9**). — To a solution of the β anomer of **11** (95 mg) in acetic acid–2-methoxyethanol (3:1, 15 ml), 10% palladium-on-charcoal was added, and the mixture was shaken at room temperature and pressure until the uptake of hydrogen was complete. The catalyst was centrifuged off, the solvent was removed (0.1 torr, 30° bath), and the residual oil, after thorough drying, was crystallised from dry ethanol–ether to give **9** as a hygroscopic solid (24 mg, 52%), $[\alpha]_D$ +4.9° (water); ν_{max}^{KBr} 3420 broad vs (OH and NH), 1790 vs (C=O), 1650 vs and 1550 s (amide I and II), 1070 s cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.41 (d, $J_{1,2}$ 7 Hz, H-1), 7.99 (3-proton s, N–Ac).

Anal. Calc. for $C_{12}H_{20}N_2O_9$: C, 42.85; H, 6.00; N, 8.33. Found: C, 42.83; H, 5.95; N, 8.39.

1-O-(N-*Acetyl*-L-*alanylglycyl*)-β-D-*glucopyranose* (**10**). — The β anomer of **12** (100 mg) was hydrogenated as described for **9** to give a hygroscopic solid which was crystallised from ethanol–ether to yield **10** (35 mg, 71.5%), $[\alpha]_D$ −47.5° (water); ν_{max}^{KBr} 3350 vs (OH and NH), 1760 s (C=O), 1650 s and 1550 s (amide I and II), 1070 s cm^{-1} (C–O–C). N.m.r. data (D_2O): τ 4.42 (d, $J_{1,2}$ 7 Hz, H-1), 8.01 (3-proton s, N–Ac), 8.64 (3-proton d, *J* 7 Hz, *Me*–CH).

Anal. Calc. for $C_{13}H_{22}N_2O_9$: C, 44.57; H, 6.33; N, 8.00. Found: C, 44.70; H, 6.66; N, 7.86.

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