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SYNTHESIS OF SOME GLYCODIPEPTIDES CONTAINING HYDROXYAMINO ACIDS, AND THEIR STABILITIES TO ACIDS AND BASES*†

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ABSTRACT

The following new compounds were prepared and characterized: *N*-benzyloxycarbonyl-*O*-(tetra-*O*-acetyl-β-D-glucopyranosyl)-*N*-glycyl-L-serine methyl ester (**1**) and L-threonine methyl ester (**2**), *N*-benzyloxycarbonyl-*O*-(β-D-glucopyranosyl)-*N*-glycyl-L-serine amide (**3**), *N*-benzyloxycarbonyl-*O*-(β-D-glucopyranosyl)-*N*-glycyl-L-threonine methyl ester (**4**) and L-threonine amide (**5**), *N*-benzyloxycarbonyl-*O*-(tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)-*N*-glycyl-L-serine methyl ester (**6**), and *N*-benzyloxycarbonyl-*O*-(2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)-*N*-glycyl-L-serine amide (**7**). Although various modifications of the Koenigs–Knorr synthesis were used, the best, over-all yields of the deacetylated dipeptide derivatives were only 5–10%. Although the products are alkali-labile, deacetylation was accomplished with methanolic ammonia. Of the deacetylated products, the threonine derivatives (**4** and **5**) were more rapidly hydrolyzed by acids than phenyl β-D-glucopyranoside, which in turn was more rapidly cleaved than the serine derivatives (**3** and **7**). The stabilities of **3**, **4**, **5**, and **7** to sodium hydroxide and sodium borohydride were similar, and essentially complete β-elimination of the glycosyl residue occurred for the amide derivatives (**3**, **5**, and **7**). For the ester derivative **4**, pH 9 was optimal; above this pH, ester hydrolysis was more rapid than β-elimination, and the resulting carboxyl derivatives did not undergo β-elimination. Under optimal conditions with sodium borohydride, the β-elimination reaction was complete, but the corresponding alanine and α-aminobutyric acid residues were not formed; presumably reductions to the amino alcohols occurred. A mechanism for the β-elimination is proposed.

*Dedicated to Dr. Horace S. Isbell, in honor of his 75th birthday.
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INTRODUCTION

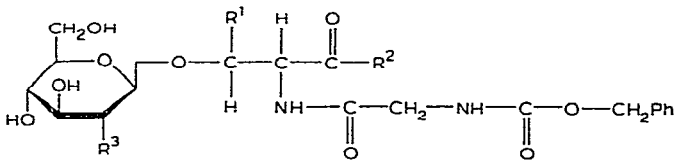
Some glycoproteins of animal origin, as in submaxillary mucins[1], blood-group substances[2], and some mucopolysaccharide–protein complexes[3,4] contain predominantly carbohydrate chains linked through *O*-glycosidic linkages to hydroxyamino acids in the polypeptide core. The existence of this type of linkage was proved by base-catalyzed β-elimination of carbohydrate side-chains from the *O*-substituted serine and threonine residues in the protein cores of bovine submaxillary mucin[5,6] and other products[6], and also of ovine submaxillary mucin[7].

Recently, several studies[8–20] of the base-lability of the *O*-glycosidic linkage in model compounds have been reported. Since the ease of elimination of glycosyl residues was usually studied for substituents on the amino and carboxyl groups of free hydroxyamino acids, a more-detailed study of the properties of this linkage type in glycodipeptides is desirable for a more complete understanding of the properties of the linkage in glycoproteins.

We now report on the synthesis of four glycodipeptide derivatives containing *O*-glycosylated serine or threonine, and on their stability towards acid and base.

RESULTS

Four glycodipeptide derivatives containing hydroxyamino acids were synthesized by the Koenigs–Knorr procedure. The best results were achieved by treatment of *N*-benzyloxycarbonyl-*N*-glycyl-L-serine and -L-threonine methyl esters with tetra-*O*-acetyl-α-D-glucopyranosyl bromide in boiling, dry benzene in the presence of silver carbonate, followed by deacetylation with methanolic ammonia. After purification by column chromatography on silica gel, the desired products (**3**, **4**, **5**, and **7**) were obtained as crystalline solids in 5–10% overall yields.



3 $R^1 = H, R^2 = NH_2, R^3 = OH$
4 $R^1 = Me, R^2 = OMe, R^3 = OH$
5 $R^1 = Me, R^2 = NH_2, R^3 = OH$
7 $R^1 = H, R^2 = NH_2, R^3 = NHCOCF_3$

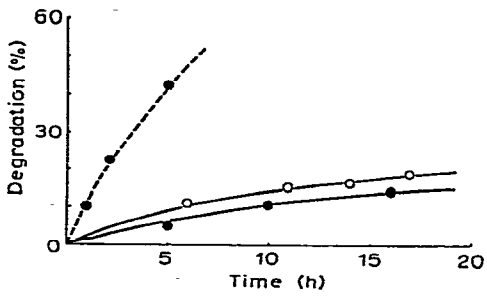
In general, the difficulty in the synthesis of glycodipeptides containing hydroxyamino acids seems to arise from the higher lability to alkaline media of the glycosidic linkages in these peptides, as compared to most glycosides, and/or the steric factors unique to the dipeptide derivatives. Apparently, Jones *et al.*[8] reported the first synthesis of an *O*-glycosylserine by a Koenigs–Knorr coupling of 3,4,6-tri-*O*-acetyl-

2-acetamido-2-deoxy-α-D-glucopyranosyl chloride with *N*-benzyloxycarbonyl-L-serine methyl ester. However, in the present work, this condensation was not successful with *N*-benzyloxycarbonyl-*N*-glycyl-L-serine and -L-threonine methyl esters. Because 3,4,6-tri-*O*-acetyl-2-acetamido-2-deoxy-α-D-glucopyranosyl bromide is very unstable and rapidly undergoes an acetyl migration[21] to form 1,3,4,6-tetra-*O*-acetyl-2-amino-2-deoxy-α-D-glucopyranose hydrobromide, the condensation of 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide with *N*-benzyloxycarbonyl-*N*-glycyl-L-threonine methyl ester was attempted. However, this reaction was not efficient, possibly as a result of steric hindrance caused by the methyl group on the β-carbon of the threonine residue and/or the higher lability of the glycosidic linkage in the glycodipeptide containing threonine. Because of the alkali-lability of the glycosidic linkage in these glycopeptides, successful deacetylations could only be carried out under mild conditions with methanolic ammonia.

Recently, Ozeki and Yosizawa[22] reported that the *O*-(2-acetamido-2-deoxy-D-galactosyl) linkages of the glycopeptides isolated by pronase digestion from bovine submaxillary mucin had the α-anomeric configuration, as indicated by the absorption at 840 cm^{-1} in the infrared spectra and by the high dextrorotation, and as already shown by other enzymic studies[23–25]. However, the four glycodipeptides synthesized in the present work had an i.r. absorption band near 910 cm^{-1} for the threonine derivatives (**4** and **5**), 900 for the D-glucosylserine amide **3**, and 870 and 930 cm^{-1} for the corresponding 2-deoxy-2-trifluoroacetamido derivative (**7**), and showed low dextrorotations. Hence, the configurations of the glycosyl linkages of these glycodipeptides are apparently β-D[26]. The threonine methyl ester derivatives also showed a distinct i.r. absorption near 1725 cm^{-1} attributable to ester or ketone C=O, whereas the threonine amide derivative was devoid of this absorption band. The i.r. absorption at 1725 cm^{-1} is thus a measure of the ester content[37] of the threonine derivative.

The glycosidic linkages of the four glycodipeptides were completely stable at pH 1–7 during 24 h at room temperature. Under more-drastic conditions (0.1M HCl, at 80°), the glycosidic linkages of the serine or threonine derivatives were cleaved with the formation of monosaccharides and hydroxyamino acid derivatives. The course of cleavage was monitored by the quantitative determination of the free D-glucose and 2-amino-2-deoxy-D-glucose by g.l.c. and by use of the amino acid analyzer, respectively. Figs. 1 and 2 represent the rates of cleavage of the glycosidic linkages in the glycosylserine amide and threonine derivatives, respectively, compared with that of phenyl β-D-glucopyranoside.

As can be seen from Figs. 1 and 2, the *O*-D-glucosylthreonine derivatives were much more labile in acidic medium, than was phenyl β-D-glucopyranoside (Fig. 2), and the stability of the threonine methyl ester derivatives was similar to that of the threonine amide derivative (Fig. 2). On the other hand, the D-glucosyl and 2-deoxy-2-trifluoroacetamidoglucosyl linkages of the serine amide derivatives were more stable than that of phenyl β-D-glucopyranoside. Thus, the influence of the type of monosaccharide in the glycosyl derivatives was rather small, but the glycosidic linkage of the threonine amide derivatives was much more labile to acid hydrolysis than that of the



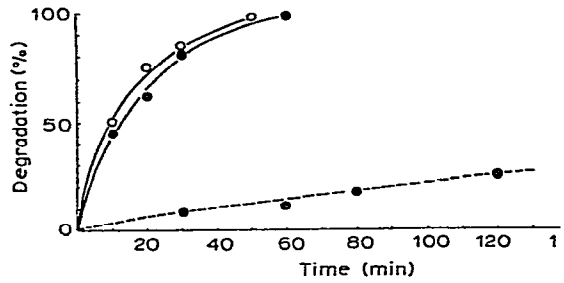


Fig. 1. Rates of hydrolysis of D-glucopyranosyl-serine derivatives by 0.1M HCl at 80°. —●—, amide **3**; —○—, trifluoroacetamido amide **7**; ...●..., phenyl β-D-glucopyranoside.

Fig. 2. Rates of hydrolysis of D-glucopyranosyl-threonine derivatives by 0.1M HCl at 80°: —●—, methyl ester **4**; —○—, amide **5**; ...●..., phenyl β-D-glucopyranoside.

serine amide derivatives. Since the methyl group on the β carbon of the threonine residue is weakly electron releasing (hyperconjugation effect), the oxygen of the glycosidic linkage will be more electronegative by electron localization at the oxygen at the β carbon atom. This effect enhances the attack of the proton on the oxygen at the β carbon atom. Thus, the order of stability of the *O*-glycosidic linkages in the glycodipeptides in acidic medium is: [*N*-benzyloxycarbonyl-*O*-(β-D-glucopyranosyl)-*N*-glycyl-L-threonine methyl ester (**4**)] = [the corresponding amide **5**] < phenyl β-D-glucopyranoside < [*N*-benzyloxycarbonyl-*O*-(2-deoxy-2-trifluoroacetamido-β-D-glucopyranosyl)-*N*-glycyl-L-serine amide (**7**)] < [*N*-benzyloxycarbonyl-*O*-(β-D-glucopyranosyl)-*N*-glycyl-L-serine amide (**3**)].

The glycosyldipeptides containing hydroxyamino acids underwent β-elimination in alkaline media with the formation of monosaccharides and unsaturated amino acid derivatives. The presence of the latter compounds was shown by the u.v.

TABLE I

CHANGES IN ABSORPTION AT 240 nm DURING β-ELIMINATION AT 20°

Compound	*pH*	*Time (min)*	*% Abs.*	*Compound*	*pH*	*Time (min)*	*% Abs.*
4	9	0	40	4	12	0	50
		10	41			60	52
		30	50			120	54
		40	56			240	62
		60	70			300	69
3	11	0	62	7	11	0	52
		30	67			30	59
		60	70			60	65
		120	78			120	70
		180	87			240	82
		240	93			420	87

absorption at 240 nm and by a decrease in the serine and threonine contents. Table I shows changes in u.v. absorbancy for the reaction of **3**, **4**, and **7** in alkaline media at 20°. The D-glucosylthreonine ester (**4**) showed a more rapid increase of absorption at 240 nm at pH 9 than at pH 12 in sodium hydroxide. For the *O*-glycosyl derivatives of the serine amide at pH 11, the D-glucosyl derivative (**3**) and the 2-deoxy-2-trifluoroacetamido-D-glucosyl derivative (**7**) showed about the same rates of increase of absorption.

The quantitative determination of the stability of the glycosidic linkages in alkaline media also was made by measurements of the decrease of the amount of

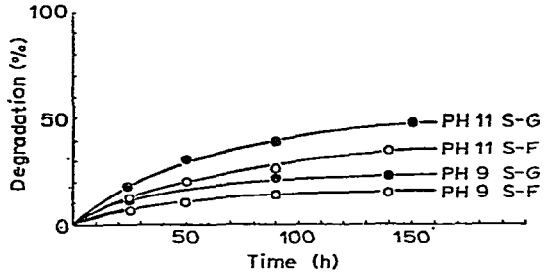


Fig. 3. The rates of cleavage of D-glucopyranosyl-L-serine amide derivatives at different pH values: S-G, amide **3**; S-F, trifluoroacetamido amide **7**.

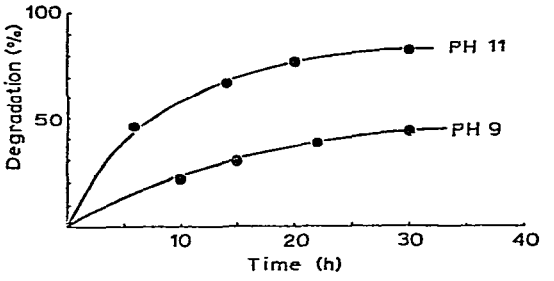


Fig. 4. The rates of cleavage of the L-threonine amide **5** at different pH values.

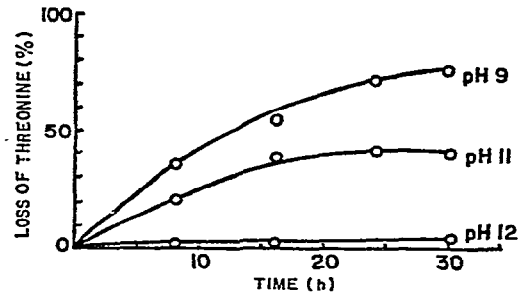


Fig. 5. The rates of cleavage of the L-threonine methyl ester **4** at different pH values.

hydroxyamino acids or by the release of monosaccharides. In Figs 3–5 are shown the rates of cleavage of the glycosidic linkage at different pH values at 45°. As may be seen from Fig. 3, the extents of cleavage of the two serine amide derivatives at pH 9 and 11 were approximately 15, 25, 35, and 45%, respectively, after 150 h, and the glycosidic linkage of the D-glucosyl derivative was only slightly more labile than that of the 2-amino-2-deoxy-D-glucosyl derivative. The rates of cleavage of the threonine amide derivative at pH 9 and 11 were approximately 40 and 80%, respectively, after 30 h (Fig. 4).

The nature of the monosaccharide unit had little influence on the stability of the glycosidic linkage in alkaline media, since the changes in u.v. absorbancy of the D-glucosyl derivative (Table I) and the loss of oligosaccharide (Fig. 3) were comparable with those of the 2-amino-2-deoxy-D-glucosyl derivative under the same conditions.

The rates of cleavage of the glycosidic linkage in the threonine methyl ester derivative (**4**) at different pH values and 45° are shown in Fig. 5. The cleavage of the glycosidic linkage at pH 9 and 45° proceeded more rapidly than that at pH 11, and at pH 11 ceased at 40% after 20 h. At pH 12, very little cleavage took place. This difference was also indicated by the change of u.v. absorbancy at 240 nm for the threonine methyl ester derivative, which was faster at pH 9 than at pH 12 and 20°, as shown in Table I. The resistance to cleavage of the glycosidic linkage at higher pH values is attributed to the formation of a free carboxyl group of the threonine residue, resulting from faster hydrolysis of the ester. This interpretation was validated by measurements of ester content by the reaction with alkaline hydroxylamine. Hence, the rates of elimination of the glycosidic group at different pH values at 45° are in the following apparent order: $12 < 11 < 9$. However, the results show that β-elimination is more rapid at low alkalinities, but ester hydrolysis increases relatively as the pH increases. They also show that the presence of a free carboxyl group in the hydroxyamino acid decreases or prevents β-elimination.

Four glycopeptides (**3**, **4**, **5**, and **7**) were incubated with 0.3M sodium borohydride in 0.1M sodium hydroxide at different temperatures. The results are represented in Figs. 6–9. The rates of cleavage of the three amide derivatives were maximal after 90 min at 45°, 60–70% at 20°, and ~30% at 5° (Figs. 6–8). However, although the treatment of these derivatives with alkaline sodium borohydride caused, progressively, a great loss of serine or threonine with elimination of the glycosidic residues, the corresponding amounts of alanine and α-aminobutyric acid were not obtained from the serine and threonine residues. As this result might arise from an incomplete reduction of the dehydroserine and dehydrothreonine residues, further hydrogenation to recover the saturated amino acids was done with alkaline sodium borohydride, using palladium chloride by the procedure of Tanaka and Pigman[5]. Again, no alanine and α-aminobutyric acid were detected after amino acid analysis. This result suggests that, during the treatment of the glycodipeptides with alkaline sodium borohydride, the dehydrohydroxyamino acid amide and ester residues were reduced to α-amino alcohols[27]. Although not ordinarily reduced under such con-

ditions, the presence of the double bond and the absence of substituents on the amide group may make the dehydroserine and dehydrothreonine residues more reactive than usual. This subject is under further investigation.

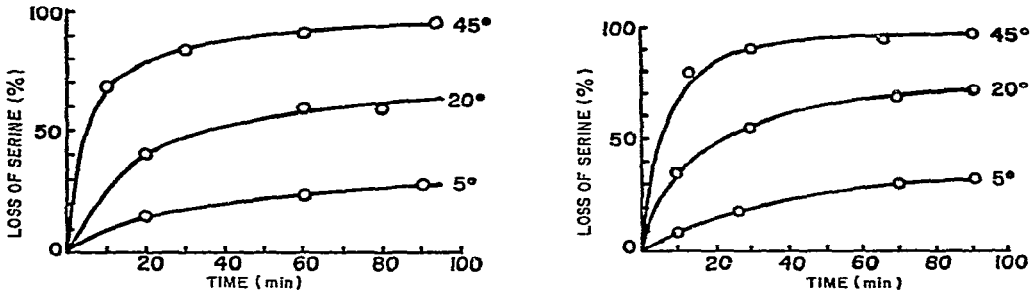


Fig. 6. Rate of β-elimination for the L-serine amide 3 by 0.3M $NaBH_4$ in 0.1M NaOH at different temperatures.

Fig. 7. Rate of β-elimination for the trifluoroacetamido L-serine amide 7 by 0.3M $NaBH_4$ in 0.1M NaOH at different temperatures.

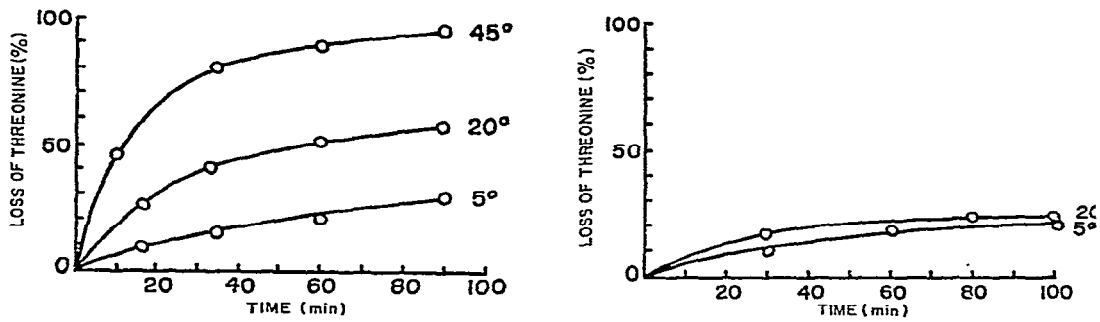


Fig. 8. Rate of β-elimination of the L-threonine amide 5 by 0.3M $NaBH_4$ in 0.1M NaOH at different temperatures.

Fig. 9. Rate of β-elimination of the L-threonine methyl ester 4 by 0.3M $NaBH_4$ in 0.1M NaOH at different temperatures.

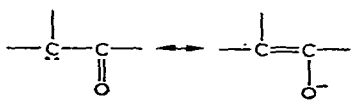
The rates of β-elimination of the three amide derivatives by alkaline sodium borohydride increased with an increase of the reaction temperature and also were approximately equal to each other at the same temperature, although the stabilities of *O*-D-glucosylserine and *O*-D-glucosylthreonine amide derivatives were considerably greater for the latter (Figs. 3 and 4) under similar conditions in alkaline media in the absence of borohydride. Consequently, when β-elimination and reduction in alkaline sodium borohydride took place simultaneously, the rates of cleavage of the seryl and threonyl linkages were approximately equal to each other, regardless of difference in degree of sensitivities of the glycosidic linkages to alkali.

The rates of cleavage of the glycosidic linkage in the threonine methyl ester derivative (**4**) by alkaline sodium borohydride were approximately constant over the temperature range 5–20°, and reached a plateau with only 20% β-elimination after 100 min (Fig. 9). However, no cleavage of the glycosidic linkage occurred at 45° under the same conditions. Thus, it may be deduced that, with an increase of the reaction temperature from 5° to 45°, the competing hydrolysis of the ester linkage progressively predominated, and at higher temperature, the ester linkage was hydrolyzed more rapidly than the β-elimination of the glycosidic linkage.

Discussion of mechanisms of β-elimination. In previous studies of the stabilities of *O*-glycosides a number of substituted *O*-glycosyl-serine and -threonine derivatives have been prepared, including a few dipeptides. The mechanism and conditions for β-elimination of the glycosyl group have been reviewed by Pigman and Moschera[28].

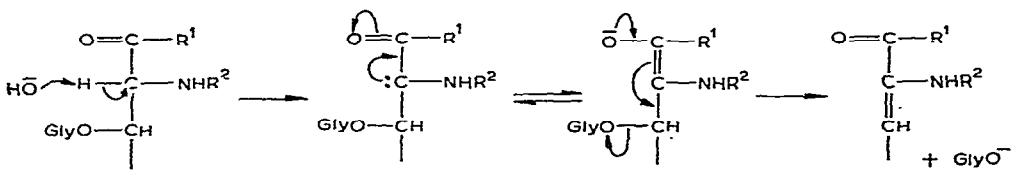
The *O*-(2-acetamido-2-deoxy-β-D-glycosyl) derivatives of serine and threonine were stable to 0.1M sodium hydroxide, and 0.3M sodium borohydride at 20° for 24 h, but the corresponding amides were partially cleaved[14]. Derevitskaya *et al.*[15] found that *O*-β-D-glucosyl derivatives of serine and seryl methylamide were stable at pH 11 (37°, 24 h), as was the serylglycine derivative. The corresponding benzyloxycarbonyl derivatives were cleaved when the carboxyl group was substituted as the methylamide, but the methyl esters and the free-carboxylate ions were stable. The work in the present paper shows that the stability of the esters arises from a competing, more-rapid hydrolysis of the ester group, which is markedly affected by the pH. Usually, β-elimination does not occur when either the amino or the carboxyl groups are unsubstituted.

The earlier results[8–20] and our new results can be explained on the basis of the formation as postulated by Isbell[29], of an enolic anion:



The presence of such enolic ions in alkaline solutions of sugars was shown later by Isbell[30].

The mechanism of the alkaline β-elimination reaction of substituted serine and threonine glycosides based on this concept is as follows:



Gly = glycosyl

Differences in alkali-lability are related to the stability of the enolic intermediates. When a free carboxyl group is present, the carboxyl ion causes resonance stabilization. Esters and amides do not show resonance, and the formation of the intermediate enolic ion will result in alkali-lability.

When a free amino group is present, the unpaired electrons of the nitrogen atom may influence the electronegativity of the carbonyl carbon atom and thereby decrease its tendency to withdraw electrons from the adjacent α-carbon atom.

EXPERIMENTAL

General. — Silica gel C-300 (200–300 mesh, Wako Pure Chemical Industries Ltd., Tokyo) was employed, without pretreatment, for column chromatography. Tetra-*O*-acetyl-α-D-glucopyranosyl bromide (m.p. 90–91°) was prepared by a standard method[31], and active silver carbonate by the method of Wolfrom *et al.*[32].

All m.p.s. are uncorrected. Optical rotations were measured with a Hitachi polarimeter model PO-B. Glucose was determined by the phenol–sulfuric acid method[33], and 2-amino-2-deoxyglucose by the Elson–Morgan reaction as modified by Boas[35], after hydrolysis in sealed tubes for 1 h with 6M HCl at 100°, or assayed with an amino acid analyzer. Ester content was determined by the alkaline hydroxylamine method of Hestrin[34]. Benzyl groups were determined for methanolic solutions by the absorbance at 258 nm, using a Hitachi spectrophotometer model ESP-3; *N*-benzyloxycarbonyl-*N*-glycyl-L-serine methyl ester was used as the reference compound.

T.l.c. was performed on Silica gel G, B-10 (Wako Pure Chemical Industries Ltd., Tokyo, activated at 110° for 1 h) with ethyl acetate–chloroform mixtures *A* (1:1), *B* (2:1), and *C* (3:1), ethyl acetate–methanol mixtures *D* (1:1), *E* (3:1), *F* (4:1), and *G* (5:1). Detection was effected by spraying with 30% sulfuric acid and charring.

For the quantitative analysis of amino acids, samples were hydrolyzed in sealed tubes with 6M HCl at 103° for 22 h, and the hydrolyzates were analysed by using a Hitachi amino acid analyzer model KLA-3B. U.v. spectra were obtained with a Hitachi spectrophotometer model FPW-4, and i.r. spectra with a Japan Spectroscopic Co., model DS-301 spectrophotometer on KBr pellets.

Glucose was determined by g.l.c., using a Shimadzu gas chromatograph GC-4APF equipped with a flame-ionization detector, a glass column (4 mm × 1.5 m) packed with 5% SE-30 (neopentylglycol succinate) on Chromosorb W (60–80 mesh) with nitrogen (66 ml/min) as the carrier gas, and a column temperature of 180°. Phenyl α-D-galactopyranoside (m.p. 138–140°) was used as an internal standard, together with a standard silylation procedure[36].

Nessler-positive materials of low molecular weight, present in the reaction mixtures after deacetylation with methanolic ammonia, and silica gel were removed by molecular-sieve chromatography on a column (1.2 × 110 cm) of Sephadex LH-20 (Pharmacia, Uppsala, Sweden) with methanol as eluant.

Determination of the stability of the glycosidic linkages. — The reactions were

performed in 0.1M HCl, 0.2M phosphate–citrate buffer (pH 9), and 0.2M phosphate–sodium hydroxide buffer (pH 11 and 12). Solutions (3.3mM) of substituted dipeptides were incubated in small, sealed tubes, and the reactions were monitored by quantitative determination of the glucose or 2-amino-2-deoxyglucose liberated and the decrease of the serine and threonine.

Treatments with alkaline borohydride. — Solutions (3.3mM) of glycodipeptides were each treated[37] with 0.3M sodium borohydride in 0.1M NaOH at different temperatures. Aliquots were acidified to pH 5.3 with cold, M acetic acid to remove the excess sodium borohydride, adjusted to pH 6.8 with 0.2M NaOH, and then concentrated to dryness. Methanol was distilled several times from the residue in order to remove borate.

N-*Benzyloxycarbonyl*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-N-*glycyl*-L-*serine methyl ester* (**1**). — Using the Koenigs–Knorr reaction[26,38], *N*-benzyloxycarbonyl-*N*-glycyl-L-serine methyl ester (1 g) and anhydrous silver carbonate (2.2 g) were added to dry benzene (24 ml), and the mixture was boiled under reflux in the dark with stirring. Benzene (10 ml) was distilled from the mixture, and a solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (3.3 g) in dry benzene (20 ml) was added dropwise during 10 min to the stirred, boiling mixture, which was protected from moisture. Additional benzene (20 ml) was then added during 20 min under the same conditions. The reaction was monitored by t.l.c. (solvent *B*) until only traces of the initial dipeptide derivative could be detected. The cooled mixture was diluted with benzene (50 ml), filtered, washed successively with M sodium chloride (80 ml) and water (100 ml), dried ($MgSO_4$), and concentrated.

The resulting, syrupy product was eluted with solvent *B* from a column (1.8 × 90 cm) of a slurry of Silica gel C-300 in solvent *B*. Fractions (10 ml) were examined by t.l.c. The product was detected in fractions 25–35, but was contaminated. The fractions were combined and concentrated, and the residue was subjected to column chromatography. This procedure was repeated until **1** was obtained as an amorphous compound (384 mg, 17%), which appeared as a single spot on t.l.c. Analysis of **1** by the methods described above showed that it contained glucose, glycine, serine, and benzyl residues in molar ratios of 1:1:1:1, and the compound had m.p. 55–57°, $[\alpha]_D^{20}$ +3.5° (*c* 2, chloroform).

Anal. Calc. for $C_{28}H_{36}N_2O_{15}$: C, 52.50; H, 5.63; N, 4.37. Found: C, 52.06; H, 5.66; N, 4.19.

N-*Benzyloxycarbonyl*-O-(*2,3,4,6-tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-N-*glycyl*-L-*threonine methyl ester* (**2**). — Using the method given for **1**, *N*-benzyloxycarbonyl-*N*-glycyl-L-threonine methyl ester (0.5 g) and 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (1.6 g) were reacted in dry benzene in the presence of silver carbonate (1.1 g). Elution of the product from Silica gel C-300 with solvent *A* gave amorphous **2** (122 mg, 11%), m.p. 63–65°, $[\alpha]_D^{20}$ +1.5° (*c* 5, chloroform).

Anal. Calc. for $C_{29}H_{38}N_2O_{15}$: C, 53.21; H, 5.81; N, 4.28. Found: C, 53.21; H, 5.12; N, 4.23.

N-*Benzyloxycarbonyl*-O-(β-D-*glucopyranosyl*)-N-*glycyl*-L-*serine amide* (**3**). — A

solution of **1** (0.5 g) in anhydrous methanol (30 ml) at 0° was mixed with methanol (30 ml) saturated with ammonia at 0°. The mixture was stored at 0° under protection from moisture, until t.l.c. (solvent *D*) indicated the disappearance of **1** (~20 h). The solution was evaporated at <10°, the syrupy residue was washed three times with anhydrous methanol and then eluted with solvent *D* from a column (1.2 × 90 cm) of a slurry of Silica gel C-300 in solvent *D*. The fractionation was monitored by t.l.c. (solvent *D*). The fractions containing **3** were combined and concentrated, and the residue was again subjected to column chromatography under the same conditions. To remove ammonia compounds and silica gel, crude **3** was eluted from Sephadex LH-20 with methanol. Two crystallizations from methanol–ether then gave **3** (165 mg, 60%), as colourless plates, m.p. 80–82°, $[\alpha]_D^{18}$ +1.5° (*c* 2, methanol).

Anal. Calc. for $C_{19}H_{27}N_3O_{10}\cdot H_2O$: C, 48.00; H, 6.10; N, 8.84. Found: C, 48.23; H, 6.33; N, 8.70.

N-*Benzyloxycarbonyl*-O-(*β*-D-*glucopyranosyl*)-N-*glycyl*-L-*threonine methyl ester* (**4**). — Deacetylation of **2** (0.3 g) as for **1**, with similar purification of the product but using solvent *G* for chromatography, gave **4** as colorless needles (106 mg, 48%), m.p. 128–129° (from methanol), $[\alpha]_D^{18}$ +3.8° (*c* 2, methanol).

Anal. Calc. foi $C_{21}H_{30}N_2O_{11}$: C, 51.84; H, 6.21; N, 5.76. Found: C, 51.92; H, 6.24; N, 5.09.

N-*Benzyloxycarbonyl*-O-(*β*-D-*glucopyranosyl*)-N-*glycyl*-L-*threonine amide* (**5**). — Treatment of **2** (0.3 g) with saturated, anhydrous, methanolic ammonia at 0°, with purification of the product as for **3** but using solvent *F* for column chromatography, gave **5** (97 mg, 45%) as colourless needles, m.p. 116–118° (from methanol–ether), $[\alpha]_D^{20}$ +4° (*c* 2, methanol).

Anal. Calc. for $C_{20}H_{29}N_3O_{10}\cdot 0.5H_2O$: C, 50.00; H, 6.25; N, 8.75. Found: C, 49.70; H, 6.36; N, 8.80.

N-*Benzyloxycarbonyl*-O-(*3,4,6-tri*-O-*acetyl*-2-*deoxy*-2-*trifluoroacetamido*-*β*-D-*glucopyranosyl*)-N-*glycyl*-L-*serine methyl ester* (**6**). — *N*-Benzyloxycarbonyl-*N*-glycyl-L-serine methyl ester (0.5 g) and 3,4,6-tri-*O*-acetyl-2-deoxy-2-trifluoroacetamido-α-D-glucopyranosyl bromide[39] (1.8 g) were condensed by a procedure similar to that used for **1**, but with dry toluene as the solvent. The crude product was eluted from Silica gel C-300 with solvent *C* and crystallized from methanol to give **6** (190 mg, 16%) as colorless, long needles, m.p. 176–177°, $[\alpha]_D^{20}$ +2° (*c* 2, chloroform).

Anal. Calc. for $C_{28}H_{34}F_3N_3O_{14}$: C, 48.48; H, 4.91; N, 6.06. Found: C, 48.49; H, 4.56; N, 6.18.

N-*Benzyloxycarbonyl*-O-(*2-deoxy*-2-*trifluoroacetamido*-*β*-D-*glucopyranosyl*)-N-*glycyl*-L-*serine amide* (**7**). — Deacetylation of **6** (0.5 g) with 50% saturated ammonia at 0°, as described for **3** but using solvent *E* for t.l.c. and column chromatography, followed by three recrystallisations of the product from methanol–ether gave **7** (248 mg, 64%) as colourless plates, m.p. 201–203°, $[\alpha]_D^{18}$ +0.8° (*c* 2, methanol).

Anal. Calc. for $C_{21}H_{27}F_3N_4O_{10}\cdot 0.5H_2O$: C, 44.92; H, 5.03; N, 9.98. Found: C, 44.70; H, 5.38; N, 9.92.

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