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SYNTHESIS OF *N*-GLYCOPEPTIDES AND A NEOGLYCOPROTEIN*

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ABSTRACT

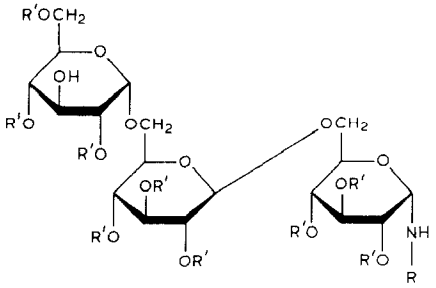
O-(α-D-Glucopyranosyl)-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-1-*N*-(glycyl-glycylglycyl-L-seryl-L-leucyl-L-glutam-5-oyl)-α-D-glucopyranosylamine has been prepared, as a model of a derivative possibly present in the glomerular basement membrane of rats, by condensation of the corresponding L-glutamyl derivative of the trisaccharide with the pentapeptide in the presence of *O,O*-diethyl cyanophosphonate. Protective groups were removed by *O*-deacetylation, deethoxylation, and hydrogenolysis. The glutamyl derivative of the trisaccharide was also converted into the acyl azide which was condensed with bovine serum albumin to form a neoglycoprotein.

INTRODUCTION

Shibata *et al.*[2] isolated and purified from the glomerular basement membrane of rats a new glycopeptide that has activity for the induction of glomerulonephritis in homologous animals[3]. From methylation analysis[4] and ^{13}C-n.m.r. data compared with those of related synthetic glycosylamine derivatives[5], Shibata *et al.*[6] proposed structure **1** for the nephritogenoside. Thus, **1** contains a new type of carbohydrate-peptide linkage having the α-D configuration. The synthesis of model glycoproteins and glycopeptides is important because these compounds may have many biological properties.

In previous papers[7,8], we reported the synthesis of an *N*-glycosyl linkage between the trisaccharide glycosylamine, *O*-α-D-glucopyranosyl-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-*O*-α-D-glucopyranosylamine, and L-glutamic acid. We report herein the synthesis of the glycopeptide, *O*-(α-D-glucopyranosyl)-(1→6)-*O*-β-D-glucopyranosyl-(1→6)-1-*N*-(glycylglycylglycyl-L-seryl-L-leucyl-L-glutam-5-oyl)-α-D-glucopyranosylamine (**2**), and of the neoglycoprotein, α-D-Glc*p*-(1→6)-β-D-

*Part VI. The Nephritogenic Glycopeptide from Rat Glomerular Basement Membrane. For Part V, see ref. 1. A part of this work has been presented at the XIth International Carbohydrate Symposium, Vancouver, Canada, August 1982.



1 R = Peptide, R′ = H

2 R = H-Gly-Gly-Gly-Ser-Leu-GluOH, R′ = H

3 R = CONH-BSA]n, R′ = H

H_2NCH

$(CH_2)_2$

CO

4 R = CO_2Et, R′ = Ac

H_2NCH

$(CH_2)_2$

CO

5 R = CbzGly-Gly-Gly-L-Ser-L-Leu-L-GluOEt, R′ = Ac

6 R = CbzGly-Gly-Gly-L-Ser-L-Leu-L-GluOH, R′ = H

7 R = CO_2Et, R′ = Ac

CbzNCH

$(CH_2)_2$

CO

8 R = $CONHNH_2$, R′ = H

H_2NCH

$(CH_2)_2$

CO

Cbz = $C_6H_5CH_2OCO$

9 R = CON_3, R′ = H

H_2NCH

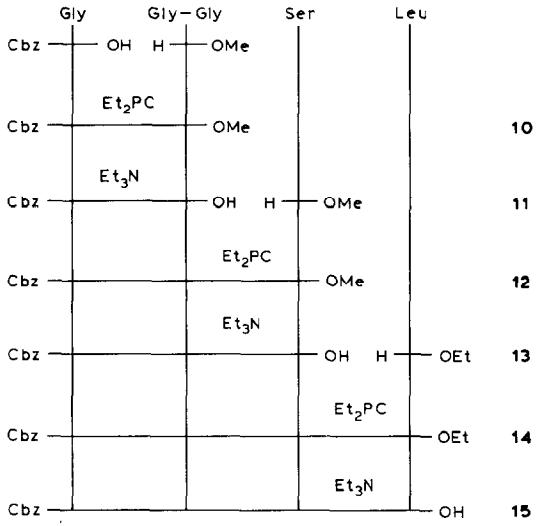
$(CH_2)_2$

CO

Glc*p*-(1→6)-α-D-Glc*p*-1-*N*-(1→5)-L-Glu·BSA (**3**), as models of corresponding derivatives possibly present in the glomerular basement membrane of rats.

RESULTS AND DISCUSSION

O-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-ace-



Scheme 1

tyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-1-*N*-[L-glutam-1-oyl-(ethyl ester)-5-oyl]-α-D-glucopyranosylamine (**4**), obtained by the procedure described previously[8], was coupled with the pentapeptide, *N*-(benzyloxycarbonyl)glycylglycylglycyl-L-seryl-L-leucine (**14**), synthesized as shown in Scheme 1, in the presence of *O,O*-diethyl cyanophosphonate[9] (Et_2PC) to form *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-

TABLE I

^{13}C CHEMICAL SHIFTS OF PEPTIDE DERIVATIVES[a] **11–15**

Carbon atom	*Compound*				
	11	**12**	**13**	**14**	**15**
Amino acid residue					
Gly Cα	43.0	44.7	44.8	45.0	45.0
Ser Cα		55.9	57.9	56.3	56.3
Cβ		62.7	63.3	62.1	62.3
Leu $C\delta_2$				21.9	22.0
$C\delta_1$				23.2	23.5
Cγ				25.7	25.7
Cβ				41.3	41.5
Cα				52.2	54.4
Ph	137.7	137.8	137.3	137.8	137.2
	129.2	129.3	129.8	129.3	129.8
	129.0	128.8	129.4	128.8	129.4
	128.8	128.6	128.7	128.6	128.7
$PhCH_2$	67.6	67.7	68.1	67.6	68.4
CO_2CH_3		52.8			
$CO_2CH_2CH_3$				63.0, 14.4	

[a]For a solution in CD_3OD.

TABLE II

^{13}C CHEMICAL SHIFTS OF GLYCOPEPTIDE DERIVATIVES **2**, **5**, AND **6**

Carbon atom	*Compound*		
	2[a]	**5**[b]	**6**[c]
Sugar residues			
C-1	78.3	73.2	78.1
2	70.5	69.1	71.1
3	72.7	69.9	73.1
4	70.5	67.1	71.0
5	73.6	70.7	73.4
6	68.1	67.1	70.1
1′	104.5	99.9	104.2
2′	73.8	71.2	74.6
3′	77.3	72.8	77.5
4′	71.4	68.5	71.1
5′	75.2	72.7	75.9
6′	66.0	67.1	67.5
1″	99.7	96.0	99.5
2″	73.6	69.1	73.4
3″	73.8	70.7	74.8
4″	71.7	64.6	71.3
5″	73.8	70.0	74.8
6″	63.2	62.7	62.2
Amino acid residues			
Gly Cα	45.4	44.4	45.5
Ser Cα	56.9	54.6	56.7
Cβ	62.6	61.9	62.9
Leu $C\delta_2$	21.9	21.6	23.6
$C\delta_1$	23.3	22.8	23.9
Cγ	25.9	24.8	25.9
Cβ	41.5	40.5	42.5
Cα	55.1	52.4	53.1
Glu Cβ	26.2	26.9	26.2
Cγ	30.2	29.6	32.6
Cα	56.5	54.6	55.8

[a]For a solution in CD_3OD. [b]For a solution in $CDCl_3$. [c]For a solution in D_2O.

glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)glycylglycylglycyl-L-seryl-L-leucyl-L-glutam-1-oyl-(ethyl ester)-5-oyl]-α-D-glucopyranosylamine (**5**). The key intermediate[15] was synthesized by stepwise elongation from the amino terminal by use of the Et_2PC method. Coupling of *N*-(benzyloxycarbonyl)glycine with glycylglycine methyl ester gave the tripeptide **10**, which was treated with triethylamine to give the tripeptide[10] **11**. This was coupled with L-serine methyl ester hydrochloride to form a tetrapeptide **12**. The tetrapeptide **13**, obtained by treatment of **12** with triethylamine, was also coupled, in the same manner, with L-leucine ethyl ester to form the pentapeptide **14**. The protected tetra- and penta-peptides were purified by silica gel column

chromatography. Treatment of **14** with triethylamine gave **15**. The ^{13}C shifts of peptide derivatives are listed in Table I. The protected glycopeptide **5** was *O*-deacetylated and deethoxylated with triethylamine in 50% methanolic solution at room temperature to give **6**, and removal of the benzyloxycarbonyl group by catalytic hydrogenation afforded the target compound **2**. The ^{13}C shifts of the glycopeptide derivatives are listed in Table II.

Goebel and Avery[11] first prepared carbohydrate–protein conjugates by way of a diazonium reaction. Lemieux *et al.*[12] prepared the semisynthetic antigens related to human blood-group Lewis[a] from esters by linkage to the free amino groups of bovine serum albumin(BSA). In the present work, the procedure of Lemieux *et al.*[13] was used. Compound[8] **7** was converted into the hydrazide **8**, further transformed into the acyl azide **9**, and attachment of **9** to BSA gave the neoglycoprotein **3**. Reaction of trisaccharide to BSA in the ratio of 60:1 gave a product having 30 trisaccharides/mol of BSA. The level of incorporation was determined on the basis of carbohydrate content as described by Lemieux *et al.*[12].

EXPERIMENTAL

General methods. — ^1H-N.m.r. spectra were recorded with a JNM MH-100 spectrometer, and ^{13}C-n.m.r. spectra with a FX-100 instrument, tetramethylsilane being the internal standard in both cases. Optical rotations were measured with a JASCO DIP-4 digital polarimeter. Thin-layer chromatography was conducted on precoated silica gel plates (Merck GF-254), and compounds were detected by quenching of u.v. fluorescence and by spraying with 10% H_2SO_4 or a 5% methanolic ninhydrin solution; R_F values for t.l.c. refer to the following solvent systems: R_F^1 5:4:1 chloroform–methanol–water, R_F^2 16:2:1 chloroform–methanol–acetic acid. Column chromatography was carried out on silica gel (Merck Kieselgel 60).

Materials. — *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-1-*N*-[1-ethyl *N*-(benzyloxycarbonyl)-L-glutam-5-oyl)-α-D-glucopyranosylamine (**7**) and *O*-(2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-1-*N*-(1-ethyl-L-glutam-5-oyl)-α-D-glucopyranosylamine (**4**) were obtained by the procedure described in a previous paper[8]. Crystalline BSA was obtained from Sigma Chemical Co.

N-*Benzyloxycarbonylglycylglycylglycine methyl ester* (**10**). — A mixture of *N*-benzyloxycarbonylglycine (12 g) in 1:1 (v/v) *N,N*-dimethylformamide–oxolane (132 mL) was treated with triethylamine (6 mL). Glycylglycine methyl ester (10 g) [obtained from glycylglycine (10 g) and thionyl chloride in methanol under conditions similar to those described by Brenner *et al.*[14]] was added, followed by *O,O*-diethyl cyanophosphonate (3 mL) (Et_2PC method). The solution was stirred for 16 h at 0°, and then extracted with ethyl acetate and evaporated. Crystallization of the residue from methanol–ether gave **10** (14.4 g, 78.7%); t.l.c. R_F^1 0.38; ^1H-n.m.r.

(CD_3OD): δ 7.32 (br.s, 5 H, arom.), 5.09 (s, 2 H, PhCH_2), and 3.69 (s, 3 H, OMe).

N-*Benzyloxycarbonylglycylglycylglycine* (**11**). — To a solution of **10** (4.8 g) in 50% methanol (20 mL) was added triethylamine (2 mL). The mixture was kept overnight at room temperature, and then evaporated *in vacuo* to give a syrup (4.5 g, 97.8%); m.p. 184–185° (lit.[10] 184–186°).

N-*(Benzyloxycarbonyl)glycylglycylglycyl*-L-*serine methyl ester* (**12**). — Compound **11** (5.4 g) was coupled with L-serine methyl ester hydrochloride (3.2 g) by the ET_2PC method in 1:10 (v/v) *N,N*-dimethylformamide–dichloromethane (220 mL). After the reaction, the solvent was evaporated. The residue was applied to a silica gel column (5 × 95 cm) which was eluted with 5:1 (v/v) chloroform–methanol. The fractions containing material having R_F^2 0.62 were pooled and evaporated (6.1 g, 86.0%), $[\alpha]_D^{23}$ −4.2° (*c* 1.4, methanol); ^{1}H-n.m.r. (D_2O): δ 7.32 (br.s, 5 H, arom.), 5.09 (s, 2 H, PhCH_2), and 3.69 (s, 3 H, OMe).

Anal. Calc. for $C_{18}H_{24}N_4O_8$: C, 50.94; H, 5.70; N, 13.20. Found: C, 50.98; H, 5.84; N, 13.40.

N-*(Benzyloxycarbonyl)glycylglycylglycyl*-L-*serine* (**13**). — To a solution of **12** (6.1 g) in 50% methanol (40 mL) was added triethylamine (4.5 mL). The mixture was stirred overnight at room temperature, and then evaporated *in vacuo* to give a syrup (5.5 g, 93.2%), $[\alpha]_D^{23}$ +11.3° (*c* 1.5, methanol); t.l.c. R_F^2 0.22; ^{1}H-n.m.r. (D_2O): δ 7.35 (br.s, 5 H, arom.) and 5.06 (s, 2 H, PhCH_2).

N-*(Benzyloxycarbonyl)glycylglycylglycyl*-L-*seryl*-L-*leucine ethyl ester* (**14**). — Compound **13** (7.4 g) was coupled with L-leucine ethyl ester hydrochloride (4.1 g) by the Et_2PC method as described earlier. After the reaction, the solvent was evaporated to give a syrup which was chromatographed on silica-gel with 5:1 (v/v) chloroform–methanol as an eluent. The eluate containing material having R_F^2 0.75 was evaporated to yield **14** (5.6 g, 56.3%), $[\alpha]_D^{23}$ −30.0° (*c* 2.3, methanol); ^{1}H-n.m.r. (D_2O): δ 7.28 (br.s, 5 H, arom.), 5.06 (s, 2 H, PhCH_2), 4.10 (q, 2 H, –CH_2CH$_3$), 1.19 (t, 3 H, –CH$_2$CH_3), and 0.90 [d, 6 H, *J* 4 Hz, –CH(CH_3)$_2$].

Anal. Calc. for $C_{25}H_{37}N_5O_9$: C, 54.44; H, 6.76; N, 12.70. Found: C, 54.66; H, 6.83; N, 12.83.

N-*(Benzyloxycarbonyl)glycylglycylglycyl*-L-*seryl*-L-*leucine (CbzGly-Gly-Gly*-L-*Ser*-L-*LeuOH)* (**15**). — To a solution of **14** (2.8 g) in 50% methanol (22 mL) was added triethylamine (2.5 mL). The mixture was stirred overnight at room temperature, and then evaporated *in vacuo* to give a syrup (2.1 g, 79.2%); t.l.c. R_F^2 0.25; ^{1}H-n.m.r. (D_2O): δ 7.34 (br.s, 5 H, arom.), 5.11 (s, 2 H, PhCH_2), and 0.80 [d, 6 H, *J* 4 Hz, –CH(CH_3)$_2$].

Anal. Calc. for $C_{23}H_{33}N_5O_9$: C, 52.77; H, 6.35; N, 13.38. Found: C, 52.98; H, 6.59; N, 13.49.

O-*(2,3,4,6-Tetra*-O-*acetyl*-α-D-*glucopyranosyl)-(1→6)*-O-*(2,3,4-tri*-O-*acetyl*-β-D-*glucopyranosyl)-(1→6)-2,3,4-tri*-O-*acetyl-1*-N-[N-*(benzyloxycarbonyl)glycylglycylglycyl*-L-*seryl*-L-*leucyl*-L-*glutam-1-oyl-(ethyl ester)-5-oyl*]-α-D-*glucopyranosylamine* (**5**). — To a solution of **4** (222.6 mg) in 1:1 dichloromethane–*N,N*-dimethylformamide (2 mL) were added pentapeptide **15** (250 mg), *O,O*-diethyl-

cyanophosphonate (160 mg), and triethylamine (80 mg). The mixture was stirred for 40 h at 0°, diluted with ethyl acetate (100 mL), and washed with 5% HCl. Drying, followed by evaporation gave a syrup which was chromatographed on silica gel with 1:1 (v/v) benzene–acetone. The eluate was evaporated to dryness to give **5** (181.5 mg, 55.3%), $[\alpha]_D^{16}$ +28.1° (*c* 6.05, chloroform); t.l.c. R_F^1 0.72; ^{1}H-n.m.r. ($CDCl_3$): δ 7.36 (br. s, 5 H, arom.), 5.14 (s, 2 H, PhCH_2), 2.10–2.04 (30 H, 10 OAc), 1.32 (t, 3 H, *J* 4 Hz, –OCH_2CH$_3$), and 0.92 [d, 6 H, *J* 4 Hz, –CH(CH_3)$_2$].

Anal. Calc. for $C_{68}H_{95}N_7O_{36}$: C, 51.48; H, 6.04; N, 6.18. Found: C, 51.69; H, 6.11; N, 6.29.

*O-α-*D*-Glucopyranosyl-(1→6)-*O*-β-*D*-glucopyranosyl-(1→6)-1-*N*-[N-(benzyloxycarbonyl)glycylglycylglycyl-*L*-seryl-*L*-leucyl-*L*-glutam-5-oyl]-α-*D*-glucopyranosylamine* (**6**). — To a solution of **15** (84.5 mg) in 50% methanolic solution (2 mL) was added triethylamine (0.5 mL). The mixture was kept overnight at room temperature, and then evaporated *in vacuo* to give a syrup (60.5 mg, 74.9%), $[\alpha]_D^{20}$ +18.7° (*c* 1.34, methanol); t.l.c. R_F^1 0.18; ^{1}H-n.m.r. (D_2O): δ 7.42 (br. s, 5 H, arom.), 5.14 (s, 2 H, PhCH_2), and 0.88 [br.s, 6 H, –CH(CH_3)$_2$].

Anal. Calc. for $C_{46}H_{71}N_7O_{26}$: C, 48.55; H, 6.29; N, 8.62. Found: C, 49.04; H, 6.32; N, 8.73.

*O-α-*D*-Glucopyranosyl-(1→6)-*O*-β-*D*-glucopyranosyl-(1→6)-1-*N*-(glycylglycylglycyl-*L*-seryl-*L*-leucyl-*L*-glutam-5-oyl)-α-*D*-glucopyranosylamine* (**2**). — A solution of **6** (26.7 mg) in methanol (1 mL) was hydrogenolyzed in the presence of Pd–C (10 mg) for 6 h at room temperature. The catalyst was filtered off, and the filtrate was evaporated to dryness. The residue was dissolved in a small amount of water, frozen, and dried to give a powder (20 mg, 82.0%), $[\alpha]_D^{19}$ +58.4° (*c* 0.16, water).

Anal. Calc. for $C_{38}H_{65}N_7O_{24}$: C, 45.46; H, 6.53; N, 9.77. Found: C, 45.38; H, 6.66; N, 9.83.

Preparation of O-*α*-D*-glucopyranosyl-(1→6)-*O*-β-*D*-glucopyranosyl-(1→6)-1-*N*-[*L*-glutam-1-oyl-(BSA/n)-5-oyl]-α-*D*-glucopyranosylamine* (**3**). — *O*-(2,3,4,6-Tetra-*O*-acetyl-α-D-glucopyranosyl)-(1→6)-*O*-(2,3,4-tri-*O*-acetyl-β-D-glucopyranosyl)-(1→6)-2,3,4-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-glutam-1-oyl-(ethyl ester)-5-oyl]-α-D-glucopyranosylamine[8] (**7**) (50 mg) was converted into the hydrazide **8** with 80% hydrazine hydrate (1 mL) at room temperature for 24 h. To the solution was added ethanol (5 mL) and the mixture evaporated to dryness. Toluene was added and evaporated. The dry, hydrazine-free compound **8** (39 mg, 36 mmol) was dissolved in dry *N,N*-dimethylformamide (0.8 mL) and 4M HCl in anhydrous 1,4-dioxane (0.12 mL) was added. The solution was cooled to −25° and *tert*-butyl nitrite (10 mg) in *N,N*-dimethylformamide (0.1 mL) added. After 30 min, sulfamic acid (16 mg) in *N,N*-dimethylformamide (0.1 mL) was added and the stirring continued for 15 min. The acyl azide **9** was not isolated and the cold solution used directly for the preparation of the neoglycoprotein. Bovine serum albumin (42 mg, 0.6 mol) was dissolved in an aqueous solution (containing) 0.08M $Na_2B_4O_7$ and 0.35M $KHCO_3$, and the solution cooled to 0°. The cold solution of the acyl azide

was then added dropwise with stirring. The pH of the solution was maintained at 9.0 during the course of the addition. The solution was dialyzed and lyophilized to give **3** (51.4 mg) as a white powder, $[\alpha]_D^{19}$ −45.4° (*c* 0.49, water). The carbohydrate–BSA compound **3** was analyzed for glucose by the phenol–sulfuric acid method. The number of mol of bound carbohydrate was calculated on the basis of a mol. wt. of BSA of 65 000.

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