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THE SYNTHESIS, AND STUDY OF THE β-ELIMINATION REACTION, OF DI- AND TRI-PEPTIDES HAVING A 3-*O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-L-SERINE RESIDUE*

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ABSTRACT

2-Acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride was condensed with the *N*-(benzyloxycarbonyl) derivatives of, respectively, L-seryl-glycine ethyl, L-seryl-L-alanine methyl, L-seryl-L-phenylalanine methyl, and L-seryl-L-aspartic dibenzyl esters to give (3-*O*-Glc*p*NAc-CbzN-L-Ser)-GlyOEt (**8**), (3-*O*-Glc*p*NAc-CbzN-L-Ser)-L-AlaOMe (**9**), (3-*O*-Glc*p*NAc-CbzN-L-Ser)-L-PheOMe (**10**), and (3-*O*-Glc*p*NAc-CbzN-L-Ser)-L-Asp(diOBzl) (**11**), respectively; *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)-L-serine methyl ester was deblocked by treatment with hydrobromic acid in glacial acetic acid, followed by triethylamine, to give a glycoamino acid that was condensed with the *N*-(benzyloxycarbonyl) derivatives of the *p*-nitrophenyl ester of glycine, L-alanine, and L-proline, respectively, to give CbzNGly-(3-*O*-Glc*p*NAc-L-SerOMe) (**17**), CbzN-L-Ala-(3-*O*-Glc*p*NAc-L-SerOMe), and CbzN-L-Pro-(3-*O*-Glc*p*NAc-L-SerOMe), respectively. Similarly, the glycopeptide resulting from **8** was condensed with the activated esters of glycine, L-alanine, L-phenylalanine, L-proline, and L-serine, respectively, to give CbzNGly-(3-*O*-Glc*p*NAc-L-Ser)-GlyOEt, CbzN-L-Ala-(3-*O*-Glc*p*NAc-L-Ser)-GlyOEt, CbzN-L-Phe-(3-*O*-Glc*p*NAc-L-Ser)-GlyOEt, and CbzN-L-Ser-(3-*O*-Glc*p*NAc-L-Ser)-GlyOEt, respectively; that from **9**, with the *p*-nitrophenyl esters of glycine, L-alanine, L-phenylalanine, L-proline, and L-leucine, respectively, to give CbzNGly-(3-*O*-Glc*p*NAc-L-Ser)-L-AlaOMe, CbzN-L-Ala-(3-*O*-Glc*p*NAc-L-Ser)-L-AlaOMe, CbzN-L-Phe-(3-*O*-Glc*p*NAc-L-Ser)-L-AlaOMe, CbzN-L-Pro-(3-*O*-Glc*p*NAc-L-Ser)-L-AlaOMe, and CbzN-L-Leu-(3-*O*-Glc*p*NAc-L-Ser)-L-AlaOMe, respectively; that from **10**, with the derivatives of glycine, L-alanine, L-phenylalanine, and L-leucine, respectively, to give CbzN-Gly-(3-*O*-Glc*p*NAc-L-Ser)-L-PheOMe, CbzN-L-Ala-(3-*O*-Glc*p*NAc-

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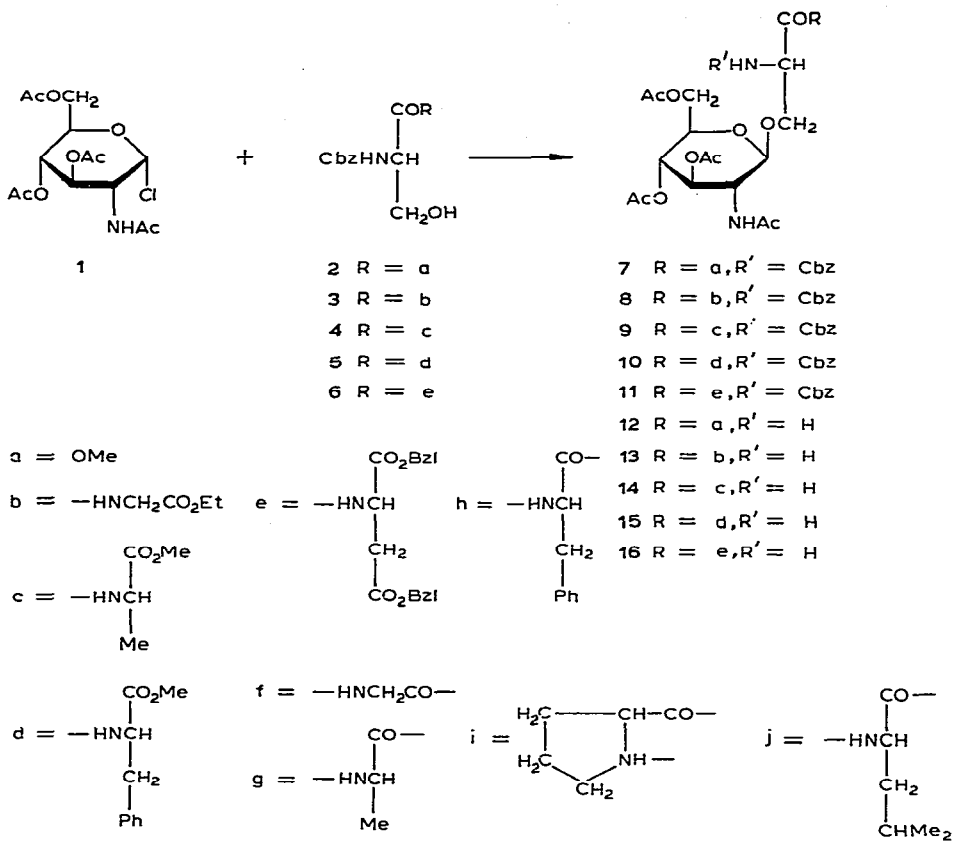
L-Ser)-L-PheOMe, CbzN-L-Phe-(3-*O*-Glc*p*NAc-L-Ser)-L-PheOMe, and CbzN-L-Leu-(3-*O*-Glc*p*NAc-L-Ser)-L-PheOMe, respectively; and that from **11**, with the derivatives of glycine, L-alanine, L-phenylalanine, L-proline, and L-leucine, respectively, to give CbzNGly-(3-*O*-Glc*p*NAc-L-Ser)-L-Asp(diOBzl), CbzN-L-Ala-(3-*O*-Glc*p*NAc-L-Ser)-L-Asp(diOBzl), CbzN-L-Phe-(3-*O*-Glc*p*NAc-L-Ser)-L-Asp(diOBzl), CbzN-L-Pro-(3-*O*-Glc*p*NAc-L-Ser)-L-Asp(diOBzl), and CbzN-L-Leu-(3-*O*-Glc*p*NAc-L-Ser)-L-Asp-(diOBzl), respectively. *O*-(2-Acetamido-3,4,5-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)-L-asparaginylglycyl-L-serine methyl ester (**20**) was synthesized by treating the free amine of **17** with the *p*-nitrophenyl ester of *N*-(benzyloxycarbonyl)-L-asparagine. 2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(glycyl-L-serine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**41**) was synthesized by the condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine with glycyl-L-serine methyl ester. Attempts to transfer the 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-D-glucopyranosyl group from the hydroxyl group of L-serine in **20** to the amido group of L-asparagine, to give **41**, were unsuccessful. The β-elimination of some of the glycodi- and glycotri-peptides was studied.

INTRODUCTION

The 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine and the *O*-(2-acetamido-2-deoxy-α-D-galactopyranosyl)-L-serine or -threonine linkages are the most widely distributed carbohydrate–protein linkages in animal glycoproteins[2,3]. It is of interest that neither the 2-acetamido-2-deoxy-D-galactosyl group linked with an L-asparagine residue, nor the 2-acetamido-2-deoxy-D-glucosyl group linked with an L-serine (or L-threonine) residue, has been found. On the other hand, L-asparagine residues involved in the carbohydrate–protein linkage have always been found located one amino acid away (in the direction of the N-terminal amino acid) from an L-serine (or L-threonine) residue[2]. This suggests the possibility that L-serine (or L-threonine) may serve to form an intermediate in the biosynthesis of the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine linkage. In order to test the possible transfer, by chemical or biochemical agents, of a 2-acetamido-2-deoxy-D-glucopyranosyl group from an L-serine to an L-asparagine residue, synthetic glycotripeptides involving L-asparagine as the N-terminal amino acid and *O*-(2-acetamido-2-deoxy-D-glucopyranosyl)-L-serine as the C-terminal were prepared. These glycopeptides also provide a direct way to study the influence of vicinal amino acids on the base-catalyzed β-elimination of the carbohydrate residue present in naturally occurring *O*-glycoproteins[3]. The extension of the peptide chain on both sides of an *O*-(2-acetamido-2-deoxy-β-D-glucopyranosyl)-L-serine residue is described.

RESULTS AND DISCUSSION

The synthesis of *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl)-*N*-



(benzyloxycarbonyl)-L-serine methyl ester (**7**) by condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-α-D-glucopyranosyl chloride[4] (**1**) with *N*-(benzyloxycarbonyl)-L-serine methyl ester[5] (**2**) in the presence of mercuric cyanide has been reported[6]. Under similar conditions, **1** was condensed with *N*-(benzyloxycarbonyl)-L-serylglycine (ethyl ester)[7] (**3**), *N*-benzyloxycarbonyl)-L-seryl-L-alanine (methyl ester)[7] (**4**), *N*-(benzyloxycarbonyl)-L-seryl-L-phenylalanine (methyl ester)[8] (**5**), and dibenzyl *N*-(benzyloxycarbonyl)-L-seryl-L-aspartate[9] (**6**), to give *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)-L-serylglycine (ethyl ester) (**8**), *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)-L-seryl-L-alanine (methyl ester) (**9**), *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)-L-seryl-L-phenylalanine (methyl ester) (**10**), and *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)-L-seryl-L-aspartic dibenzyl ester (**11**), respectively. The yield of glycodipeptides was in the range 50–60%.

Attempts to synthesize *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-*N*-(benzyloxycarbonyl)glycyl-L-serine (methyl ester) (**17**), a possible intermediate for the preparation of *N*-(benzyloxycarbonyl)-L-asparaginylglycyl-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl)-L-serine (methyl ester)(**20**),

TABLE I

PROPERTIES AND ANALYTICAL DATA FOR *O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-*N*-(BENZYLOXYCARBONYLAMINO ACID)-L-SERINE METHYL ESTERS

Compound	*M.p. (degrees)*	$[\alpha]_D^{21}$ *(degrees)*[a]	R_F[b]	*Yield (%)*	*Formula*	*Anal.*		*Amino acids (Found)*	ν_{max}^{KBr} *(cm^{-1})*
						Calc.	*Found*		
17	168–169	+31.5 (1.7)	0.64 (C)	50	$C_{28}H_{37}N_3O_{14}$	C, 52.57 H, 5.85 N, 6.57 O, 35.01	52.50 5.79 6.49 34.95	glycine 0.99, and L-serine 1.00	3300 (NH), 1730 (OAc), 1650 (Cbz group CO), and 1680–1525 (peptide Amide I)
18	223–225	−21.6 (1.1)	0.37 (A)	34	$C_{29}H_{39}N_3O_{14} \cdot HCONMe_2$	C, 52.89 H, 6.38 N, 7.71 O, 33.02	53.05 6.14 7.64 32.93	[c]	3300 (NH), 1730 (OAc), 1660 (Cbz group CO), and 1680–1530 (peptide Amide I)
19	191–191.5	+8.3 (1.8)	0.64 (B)	60	$C_{31}H_{41}N_3O_{14}$	C, 54.78 H, 6.08 N, 6.18 O, 32.95	54.75 6.05 6.10 32.78	L-proline 0.90, and L-serine 1.00	3300 (NH), 1735 (OAc), 1660 (Cbz group CO), and 1710–1530 (peptide Amide I)

[a]In chloroform, concentration in parentheses. [b]Solvent system in parentheses. [c]Satisfactory analyses could not be obtained.

TABLE II

PROPERTIES AND ANALYTICAL DATA FOR *O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-*N*-(BENZYLOXYCARBONYLAMINO ACID)-L-SERYLGLYCINE ETHYL ESTERS

Compound	*M.p. (degrees)*	$[\alpha]_D^{21}$ *(degrees)*[a]	R_F[b]	*Yield (%)*	*Formula*	*Anal.*		*Amino acids (Found)*	ν_{max}^{KBr} *(cm^{-1})*
						Calc.	*Found*		
21	194–195	−9.1 (0.55)	0.64 (C)	42	$C_{31}H_{42}N_4O_{15}$	C, 52.39 H, 5.96 N, 7.88 O, 33.77	52.31 5.94 7.93 33.66	glycine 1.79, and L-serine 1.00	3300 (NH), 1750 (OAc), 1660 (Cbz group CO), and 1640–1530 (peptide Amide I)
22	200–201 (dec.)	−13.3 (0.87)	0.28 (C)	52	$C_{32}H_{44}N_4O_{15}$	C, 53.03 H, 6.12 N, 7.73 O, 33.11	53.13 6.14 7.69 33.14	L-alanine 0.92, glycine 0.92, and L-serine 1.00	3300 (NH), 1760 (OAc), 1680 (Cbz group CO), and 1640–1550 (peptide Amide I)
23	225-226 (dec.)	−6.2 (0.45)	0.32 (C)	75	$C_{38}H_{48}N_4O_{15}$	C, 56.99 H, 6.04 N, 7.00 O, 29.97	56.89 6.03 6.94 30.00	glycine 1.12, L-phenylalanine 1.18, and L-serine 1.00	3300 (NH), 1760 (OAc), 1660 (Cbz group CO), and 1640–1530 (peptide Amide I)
24	193–195 (shrinking at 180)	−8.7 (0.60)	0.30 (C)	30	$C_{34}H_{46}N_4O_{15}$	C, 54.39 H, 6.18 N, 7.46 O, 31.97	54.44 6.13 7.39 31.91	glycine 0.94, L-proline 0.98, and L-serine 1.00	3280 (NH), 1730 (OAc), 1680 (Cbz group CO), and 1640–1550 (peptide Amide I)
25	191–193 (dec.)	−15.4 (0.86)	0.10 (C)	23	$C_{32}H_{44}N_4O_{16}$	C, 51.89 H, 5.99 N, 7.56 O, 34.56	51.85 5.87 7.45 34.53	glycine 0.96, and L-serine 2.00	3300 (NH), 1730 (OAc), 1680 (Cbz group CO), and 1640–1540 (peptide Amide I)

[a]In chloroform, concentration in parentheses. [b]Solvent system in parentheses.

TABLE III

PROPERTIES AND ANALYTICAL DATA FOR *O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-*N*-(BENZYLOXYCARBONYLAMINO ACID)-L-SERYL-L-ALANINE METHYL ESTERS

Compound	*M.p. (degrees)*	$[\alpha]_D^{21}$ *(degrees)*[a]	R_F[b]	*Yield (%)*	*Formula*	*Anal.*		*Amino acids (Found)*	ν_{max}^{KBr} *(cm^{-1})*
						Calc.	*Found*		
26	193–194.5 (dec.)	−12.3 (1.2)	0.40 (C)	42	$C_{31}H_{42}N_4O_{15}$	C, 52.39 H, 5.96 N, 7.88 O, 33.77	52.33 5.88 7.96 33.86	L-alanine 1.06, glycine 0.99, and L-serine 1.00	3320 (NH), 1750 (OAc), 1680 (Cbz group CO), and 1630–1540 (peptide Amide I)
27	206–206.5 (swells at 96)	−17.8 (0.9)	0.20 (C)	65	$C_{32}H_{44}N_4O_{15}$	C, 53.03 H, 6.12 N, 7.73 O, 33.11	52.98 6.11 7.78 33.13	L-alanine 1.91, and L-serine 1.00	3300 (NH), 1760 (OAc), 1680 (Cbz group CO), and 1640–1520 (peptide Amide I)
28	220–221	−11.0 (0.5)	0.70 (D)	35	$C_{38}H_{48}N_4O_{15}$	C, 56.99 H, 6.04 N, 7.00 O, 29.97	57.13 6.07 6.82 29.83	L-alanine 1.06, glycine 0.99, and L-serine 1.00	3300 (NH), 1735 (OAc), 1680 (Cbz group CO), and 1640–1550 (peptide Amide I)
29	171–172 (shrinking at 166)	−25.8 (1.1)	0.27	36	$C_{34}H_{46}N_4O_{15}$	C, 54.39 H, 6.18 N, 7.46 O, 31.97	54.33 6.15 7.52 31.80	L-alanine 1.13, L-proline 0.96, and L-serine 1.00	3300 (NH), 1760 (OAc), 1660 (Cbz group CO), and 1650–1550 (peptide Amide I)
30	212–213 (dec.)	−18.5 (0.9)	0.66 (D)	42	$C_{35}H_{50}N_4O_{15}$	C, 54.82 H, 6.57 N, 7.31 O, 31.30	54.78 6.57 7.26 31.52	L-alanine 0.92, L-leucine 1.05, and L-serine 1.00	3300 (NH), 1740 (OAc), 1655 (Cbz group CO), and 1645–1540 (peptide Amide I)

[a]In chloroform, concentration in parentheses. [b]Solvent system in parentheses.

TABLE IV

PROPERTIES AND ANALYTICAL DATA FOR *O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-*N*-(BENZYLOXYCARBONYLAMINO ACID)-L-SERYL-L-PHENYLALANINE METHYL ESTERS

Compound	*M.p. (degrees)*	$[\alpha]_D^{21}$ *(degrees)*[a]	R_F[b]	*Yield (%)*	*Formula*	*Anal.*		*Amino acids (Found)*	ν_{max}^{KBr} *(cm^{-1})*
						Calc.	*Found*		
31	183–185 (dec.)	+4.5 (0.9)	0.34 (D)	44	$C_{37}H_{46}N_4O_{15}$	C, 56.48 H, 5.89 N, 7.12 O, 30.50	56.10 5.87 7.06 30.30	glycine 1.15, L-phenylalanine 1.23, and L-serine 1.00	3300 (NH), 1735 (OAc), 1660 (Cbz group CO), and 1685–1525 (peptide Amide I)
32	210–213 (shrinking at 146)	+1.3 (0.6)	0.40 (D)	45	$C_{38}H_{48}N_4O_{15}$	C, 56.99 H, 6.04 N, 7.00 O, 29.97	56.98 6.06 7.03 29.94	L-alanine 1.21, L-phenylalanine 1.23, and L-serine 1.00	3300 (NH), 1735 (OAc), 1660 (Cbz group CO), and 1680–1530 (peptide Amide I)
33	210–211 (dec.; shrinking at 208)	+9.6 (2.0)	0.35 (C)	32	$C_{44}H_{52}N_4O_{15}$	C, 60.27 H, 5.98 N, 6.39 O, 27.37	60.19 5.90 6.35 27.28	L-phenylalanine 2.47, and L-serine 1.00	3300 (NH), 1730 (OAc), 1640 (Cbz group CO), and 1685–1530 (peptide Amide I)
34	212–213 (dec.)	+1.3 (2.8)	0.3 (C)	23	$C_{41}H_{54}N_4O_{15}$	C, 58.42 H, 6.47 N, 6.65 O, 28.47	58.33 6.44 6.55 28.60	L-leucine 1.01, L-phenylalanine 1.03, and L-serine 1.00	3300 (NH), 1730 (OAc), 1660 (Cbz group CO), and 1690–1530 (peptide Amide I)

[a]In chloroform, concentration in parentheses. [b]Solvent system in parentheses.

TABLE V

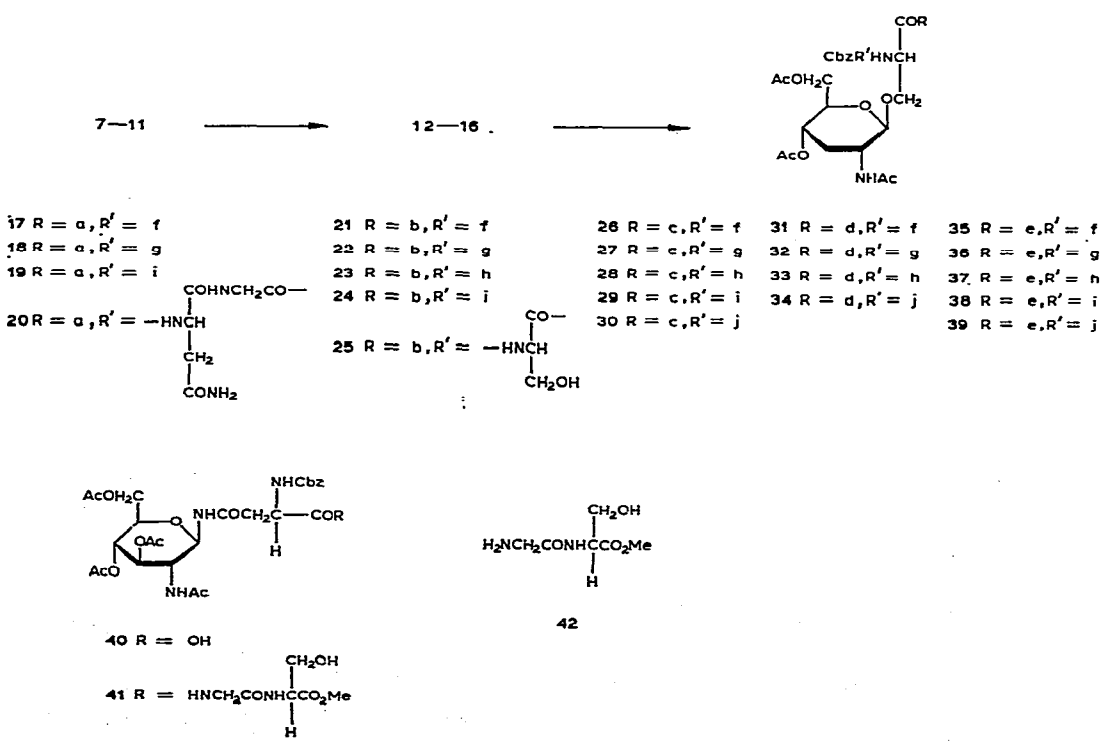
PROPERTIES AND ANALYTICAL DATA FOR *O*-(2-ACETAMIDO-3,4,6-TRI-*O*-ACETYL-2-DEOXY-β-D-GLUCOPYRANOSYL)-*N*-(BENZYLOXYCARBONYLAMINO ACID)-L-SERYL-L-ASPARTIC DIBENZYL ESTERS

Compound	*M.p. (degrees)*	$[\alpha]_D^{21}$ *(degrees)*[a]	R_F[b]	*Yield (%)*	*Formula*	*Anal.* *Calc.*	*Found*	*Amino acids (Found)*	ν_{max}^{KBr} *(cm^{-1})*
35	172–173 (dec.; shrinking at 170)	+5.0 (1.1)	0.47 (D)	23	$C_{45}H_{52}N_4O_{17}$	C, 58.69 H, 5.69 N, 6.08 O, 29.53	58.57 5.78 6.00 29.60	L-aspartic acid 1.00, glycine 0.84, and L-serine 1.00	3300 (NH), 1740 (OAc), 1655 (Cbz group CO), and 1700–1550 (peptide Amide I)
36	193–194 (shrinking at 159)	+1.2 (0.90)	0.34 (D)	23	$C_{46}H_{54}N_4O_{17}$	C, 59.09 H, 5.82 N, 5.99 O, 29.09	59.00 5.82 5.97 28.87	L-alanine 1.06, L-aspartic acid 1.02, and L-serine 1.00	3300 (NH), 1735 (OAc), 1635 (Cbz group CO), and 1680–1540 (peptide Amide I)
37	182–184 (dec.; shrinking at 179)	+5.3 (1.5)	0.36 (C)	27	$C_{52}H_{58}N_4O_{17} \cdot H_2O$	C, 60.69 H, 5.88 N, 5.44	60.75 5.81 5.54	L-aspartic acid 1.00, L-phenylalanine 1.05, and L-serine 1.00	3300 (NH), 1730 (OAc), 1640 (Cbz group CO), and 1680–1530 (peptide Amide I)
38	169–171 (dec.; browning at 166)	−8.5 (1.0)	0.51 (D)	27	$C_{48}H_{56}N_4O_{17}$	C, 59.99 H, 5.87 N, 5.83 O, 28.30	59.86 5.89 5.86 28.38	L-aspartic acid 1.00, L-proline 1.02, and L-serine 1.00	3290 (NH), 1735 (OAc), 1640 (Cbz group CO), and 1660–1545 (peptide Amide I)
39	182–187 (dec.)	−14.6 (0.8)	0.38 (C)	28	$C_{49}H_{60}N_4O_{17} \cdot 2H_2O$	C, 57.75 H, 6.33 N, 5.50	57.92 6.11 5.59	L-aspartic acid 1.07, L-leucine 0.77, and L-serine 1.00	3300 (NH), 1735 (OAc), 1660 (Cbz group CO, and Amide I)

[a]In chloroform, concentration in parentheses. [b]Solvent system in parentheses.

by direct condensation of **1** with *N*-benzyloxycarbonyl)glycyl-L-serine (methyl ester)[10], was not successful. In another approach, the *N*-(benzyloxycarbonyl) group of **7** was removed by treatment with hydrogen bromide in acetic acid, followed by treatment with triethylamine under the conditions described earlier[11], to give the free amine **12**. The *p*-nitrophenyl esters of glycine, L-alanine, and L-proline were each coupled with **12** to give the derivatives **17**, **18**, and **19**, respectively (see Table I). The *N*-(benzyloxycarbonyl) group of **17** was removed by treatment with hydrogen bromide in acetic acid, followed by treatment with triethylamine, to give the free amine of **17**, which was condensed with the *p*-nitrophenyl ester of *N*-(benzyloxycarbonyl)-L-asparagine[12] to give **20**.

Similarly, the amines **13**, **14**, **15**, and **16**, respectively obtained by removal of the *N*-(benzyloxycarbonyl) group of **8**, **9**, **10**, and **11** with hydrogen bromide in acetic acid, were each treated (in part) with the *p*-nitrophenyl esters of the *N*-(benzyloxycarbonyl) derivatives of glycine[13], L-alanine[14], L-phenylalanine[14], L-proline[12], and L-leucine[12], to give the derivatives **21** to **39** (see Tables II, III, IV, and V). The condensation of *O*-(2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-*β*-D-glucopyranosyl)-L-serylglycine (ethyl ester) (**13**) with the 2,3,4,5,6-pentachlorophenyl ester of *N*-(benzyloxycarbonyl)-L-serine[15] gave *N*-(benzyloxycarbonyl-L-seryl)-3-*O*-(2-acetamido-3,4,6-tri-*O*-acetyl-*β*-D-glucopyranosyl)-L-serylglycine ethyl ester (**25**) in poor yield; the



p-nitrobenzyl ester of *N*-(benzyloxycarbonyl)-L-serine[16] could not be employed as an alternative condensation reagent as its purity had not been ascertained[17].

In order to study the possibility of the transfer in **20** of the 2-acetamido-2-deoxy-β-D-glucopyranosyl group from the hydroxyl group of the L-serine to the amide group of an L-asparagine residue, 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine[18] (**40**) was coupled with glycyl-L-serine (methyl ester) (**42**) in the presence of *N*-ethyl-5-phenylisoxazolium 3'-sulfonate (the WRK reagent)[19] to give 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-benzyloxycarbonyl)-L-aspart-1-oyl-(glycyl-L-serine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**41**).

Attempts to transfer the 2-acetamido-3,4,6-tri-*O*-acetyl-β-D-glucopyranosyl group from **20** to give **41**, under conditions described earlier[20] for O→N migration of the glycosyl group in pyrimidine nucleosides (*i.e.*, in the presence of stannic chloride) were unsuccessful; **20** was decomposed to such an extent that, on examination by t.l.c., no spot migrating at the same rate as that of **41** was detected.

β-Elimination reaction. — When the substituted glycodipeptides Gly-(GlcNAc)-Ser (**17**) and Pro-(GlcNAc)Ser (**19**), and glycotripeptides Ala-(GlcNAc)Ser-Gly (**22**), Phe-(GlcNAc)Ser-Gly (**23**), Gly-(GlcNAc)Ser-Ala (**26**), Ala-Glc(NAc)Ser-Ala (**27**), Pro-(GlcNAc)Ser-Ala (**29**), Gly-(GlcNAc)Ser-Phe (**31**), Ala-(GlcNAc)Ser-Phe (**32**), Gly-(GlcNAc)Ser-Asp (**35**), Ala-(GlcNAc)Ser-Asp (**36**), and Pro-(GlcNAc)-Ser-Asp (**38**) were treated with alkaline borohydride under the conditions described earlier[6], a very large decrease in the content of L-serine and some increase in that of DL-alanine was observed (see Table VI). As reported by others[21], the percentage of

TABLE VI

TREATMENT OF COMPOUNDS **17**, **19**, **22**, **23**, **26**, **27**, **29**, **31**, **32**, **35**, **36**, AND **38** UNDER THE CONDITIONS OF THE β-ELIMINATION[a]

Derivatives of GlcNAc-Ser		*Compounds obtained after hydrolysis[b] (%)*							
		Ser	*Ala[c]*	*Gly*	*Pro*	*Asp*	*Phe*	*GlcNAc*	*GlcNAcol[d]*
Gly-Ser	(**17**)	41	8	41				48	13
Pro-Ser	(**19**)	76	20		60			51	12
Ala-Ser-Gly	(**22**)	17	49	98				22	83
Phe-Ser-Gly	(**23**)	95	10	100			81	86	8
Gly-Ser-Ala	(**26**)	15	138	21				21	35
Ala-Ser-Ala	(**27**)	4	121					6	60
Pro-Ser-Ala	(**29**)	25	128					36	62
Gly-Ser-Phe	(**31**)	11	19	17			83	21	57
Ala-Ser-Phe	(**32**)	42	57				91	48	47
Gly-Ser-Asp	(**35**)	31	9	12		54		39	45
Ala-Ser-Asp	(**36**)	8	20			39		10	32
Pro-Ser-Asp	(**38**)	7	58		61	38		24	86

[a]2M Sodium borohydride and 50mM sodium hydroxide for 20 h at 40°. [b]With ~5.8M hydrochloric acid for 24 h at 110°. [c]DL-Alanine. [d]2-Acetamido-2-deoxy-D-glucitol.

amino acids recovered from the alkali–borohydride-treated glycopeptides was lower than that of the initial material. The degree of decomposition of the terminal amino acid linked to the amino group of L-serine varied widely, from a low value of ~20% for L-phenylalanine to almost 100% for L-alanine and glycine, whereas the terminal amino acid linked to the carboxyl group of L-serine was more resistant (recovery of 80–100%), except for L-aspartic acid, where the recovery was <55%. Thus, the release of carbohydrate chains from *O*-glycopeptides seems to depend greatly on the nature of the amino acids linked both to amino and carboxylic groups of the glycosylated L-serine residue. It is not possible to deduce general conclusions from the limited number of glycopeptides described here, but it is quite clear that, in contrast to the results obtained with *O*-glycoproteins, the β-elimination procedure is of very limited value for the study of glycopeptides of low molecular weight.

The total recovery of 2-acetamido-2-deoxy-D-glucitol derived from the 2-acetamido-2-deoxy-D-glucose linked to the L-serine residue, and of untransformed 2-acetamido-2-deoxy-D-glucose, was, in general, in the range of yields previously reported[22], but some very low values, and some values in excess of 100% show the difficulty in obtaining meaningful results. The low values may be explained by condensation between the products of alkaline degradation of the amino acids with 2-acetamido-2-deoxy-D-glucose or -D-glucitol, or both.

EXPERIMENTAL

General methods. — Evaporations were performed *in vacuo*. Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points". Rotations were determined, for solutions in 1-dm, semimicro tubes, with a Perkin–Elmer Model 141 polarimeter. I.r. spectra were recorded, for potassium bromide discs, with a Perkin–Elmer spectrophotometer Model 237. The homogeneity of compounds was verified by ascending t.l.c. on precoated plates of Silica gel (Merck) with solvents (v/v): (*A*) 19:1 chloroform–methanol, (*B*) 14:1 chloroform–methanol, (*C*) 19:1 chloroform–ethanol, and (*D*) 14:1 chloroform–ethanol. The spots were detected by spraying the plates with 20% sulfuric acid and heating for a few min at 200°. The amino acid composition of hydrolyzates of glycopeptides was determined by g.l.c. of the *N*-(trifluoroacetyl)ated butyl esters with a Perkin–Elmer Model 900 gas chromatograph having a column of Tabsorb (Regis Chemical Co., Chicago, IL 60610), programmed for a rise of 4°/min from 100 to 225°. The glycopeptides were hydrolyzed by heating with ~5.8M hydrochloric acid for 24 h at 110°, followed by evaporation of the solution under a stream of nitrogen. The residue was heated with 3M hydrochloric acid in 1-butanol (0.5 mL) for 1 h at 100°, followed by treatment with a 25% solution of trifluoroacetic anhydride in dichloromethane (0.1 mL) for 1 h at 100°. The results are reported in molecular proportions relative to the L-serine residue of the compounds. The microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

O-(*2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-N-(*benzyloxy-*

carbonyl)-L-*serylglycine ethyl ester* (**8**). — To a solution of *N*-(benzyloxycarbonyl)-L-serylglycine ethyl ester[7] (**3**, 2.0 g) in benzene (100 mL), predried by addition and distillation of benzene (25 mL), were added mercuric cyanide (1.6 g) and benzene (25 mL). Benzene (25 mL) was distilled off, 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[4] (**1**, 2.2 g) was added, and the mixture was stirred and boiled overnight under reflux. The dark-brown solution was evaporated, and the residue extracted with chloroform (200 mL). The extract was successively washed several times with 30% aqueous potassium iodide and water, dried (sodium sulfate), and evaporated to a syrup that crystallized from hot ethanol, to give **8** (2.6 g, 57%), m.p. 166–166.5°, $[\alpha]_D^{20}$ −6.0° (*c* 0.5, chloroform); t.l.c. (*C*): R_F 0.37; ν_{max}^{KBr} 3350–3290 (NH), 1725 (OAc), 1660 (Cbz group CO), and 1600–1550 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{29}H_{39}N_3O_{14}$: C, 53.29; H, 6.01; N, 6.43; O, 34.27; Gly, 1.00; Ser, 1.00. Found: C, 53.28; H, 6.02; N, 6.51; O, 34.08; Gly, 1.28; Ser, 1.00.

O-(*2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-N-(*benzyloxycarbonyl*)-L-*seryl*-L-*alanine methyl ester* (**9**). — A solution of *N*-(benzyloxycarbonyl)-L-seryl-L-alanine methyl ester[7] (**4**, 3.25 g) in benzene (100 mL) was treated with mercuric cyanide (2.5 g) and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[4] (**1**, 3.65 g) in benzene (25 mL), as described for the preparation of **8**. Crystallization from ethanol–ether gave 3.5 g (53%) of **9**, m.p. 194.5–195.5° (dec.), $[\alpha]_D^{20}$ −6.3° (*c* 0.9, chloroform); t.l.c. (*C*): R_F 0.37; ν_{max}^{KBr} 3290 (NH), 1730 (OAc), 1690 (Cbz group CO), and 1650–1550 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{29}H_{39}N_3O_{14}$: C, 53.29; H, 6.01; N, 6.43; O, 34.27; Ala, 1.00; Ser, 1.00. Found: C, 53.29; H, 5.96; N, 6.26; O, 33.91; Ala, 1.01; Ser, 1.00.

O-(*2-Acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-N-(*benzyloxycarbonyl*)-L-*seryl*-L-*phenylalanine methyl ester* (**10**). — A solution of *N*-(benzyloxycarbonyl)-L-seryl-L-phenylalanine methyl ester[8] (**5**, 4.0 g) in benzene (100 mL) was treated with mercuric cyanide (2.5 g) and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[4] (**1**, 3.65 g) in benzene (25 mL), as described for the preparation of **8**. Crystallization from ethanol–ether gave 3.9 g (54%) of **10**, m.p. 179.5–180.5° (dec.), $[\alpha]_D^{20}$ +15.4° (*c* 1.6, chloroform); t.l.c. (*C*): R_F 0.34; ν_{max}^{KBr} 3300 (NH), 1725 (OAc), 1680 (CBz CO), and 1670–1530 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{35}H_{43}N_3O_{14}$: C, 57.61; H, 5.94; N, 5.76; O, 30.69; Phe, 1.00; Ser, 1.00. Found: C, 57.47; H, 5.93; N, 5.75; O, 30.56; Phe, 1.11; Ser, 1.00.

Dibenzyl O-(*2-acetamido-3,4,6-tri*-O-*acetyl-2-deoxy-β*-D-*glucopyranosyl*)-N-(*benzyloxycarbonyl*)-L-*seryl*-L-*aspartate* (**11**). — A solution of dibenzyl *N*-(benzyloxycarbonyl)-L-seryl-L-aspartate[9] (**6**, 2.67 g) in benzene (100 mL) was treated with mercuric cyanide (1.25 g) and 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-α-D-glucopyranosyl chloride[4] (**1**, 1.83 g) in benzene (25 mL), as described for the preparation of **8**. Crystallization from ethanol–ether gave 2.8 g (65%) of **11**, m.p. 173–174° (shrinking at 170°), $[\alpha]_D^{20}$ +17.7° (*c* 1.1, chloroform); t.l.c. (*C*): R_F 0.40; ν_{max}^{KBr} 3300 (NH), 1725 (OAc), 1630 (Cbz CO), and 1680–1530 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{43}H_{49}N_3O_{16}$: C, 59.79; H, 5.72; N, 4.86; O, 29.63; Asp, 1.00; Ser, 1.00. Found: C, 59.81; H, 5.70; N, 4.72; O, 29.44; Asp, 0.93; Ser, 1.00.

General procedure for the condensation of the p-*nitrophenyl esters of amino acids, or the 2,3,4,5,6-pentachlorophenyl ester of* L-*serine, with* O-(*2-acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-β-*D-*glucopyranosyl*)-L-*serine methyl ester* (**12**), -L-*serylglycine ethyl ester* (**13**), -L-*seryl*-L-*alanine methyl ester* (**14**), -L-*seryl*-L-*phenylalanine methyl ester* (**15**), *and* -L-*seryl*-L-*aspartic dibenzyl ester* (**16**). — To a solution of **7** (ref. 6), **8**, **9**, **10**, or **11** (0.25 mmol) in glacial acetic acid (1 mL) was added 30% hydrogen bromide in acetic acid (1 mL). The mixture was kept for 1 h at room temperature, and the resulting hydrobromide was precipitated by addition of anhydrous ether and rapidly filtered off. Treatment of the hydrobromide with triethylamine (35 μL) in *N*,*N*-dimethylformamide (2 mL) gave the free amine **12**, **13**, **14**, **15**, or **16**, respectively. The *p*-nitrophenyl esters of *N*-(benzyloxycarbonyl)-glycine[13], -L-alanine[14], -L-phenylalanine[14], -L-leucine[12], or L-proline[12], or the 2,3,4,5,6-pentachlorophenyl ester of *N*-(benzyloxycarbonyl)-L-serine[15], were each added to **12**, **13**, **14**, **15**, or **16**, respectively, followed by the addition of chloroform (10 mL). The mixture was stirred overnight, the solvents were removed *in vacuo*, the residue was dissolved in chloroform, and the solution was successively washed with M hydrochloric acid and water, dried (sodium sulfate), and evaporated; the syrup was crystallized from ethanol, methanol, ethanol–ether, or methanol–ether. The yields and characteristics of the resulting compounds are reported in Tables I–V.

O-(*2-Acetamido-3,4,6-tri-*O-*acetyl-2-deoxy-β-*D-*glucopyranosyl*)-N-(*benzyloxycarbonyl*-L-*asparaginylglycyl*)-L-*serine methyl ester* (**20**). — To a solution of **17** (0.64 g) in glacial acetic acid (4 mL) was added 30% hydrogen bromide in acetic acid (4 mL). The mixture was kept for 1 h at room temperature, and the resulting hydrobromide was then precipitated by addition of anhydrous ether, and rapidly filtered off. Treatment of the hydrobromide with triethylamine (0.14 mL) in *N*,*N*-dimethylformamide (10 mL) gave the free amine of **17**. The *p*-nitrophenyl ester of *N*-(benzyloxycarbonyl)-L-asparagine[12] (0.38 g) in chloroform (10 mL) was added, and the mixture was stirred overnight. The solvents were removed *in vacuo*, the residue was dissolved in ethyl acetate, and the solution was successively washed with M hydrochloric acid and water, dried (sodium sulfate), and evaporated; the syrup crystallized from ethanol, to give **20** (0.1 g, 28%), m.p. 188–190° (dec.), $[\alpha]_D^{20}$ −11.8° (*c* 0.24, *N*,*N*-dimethylformamide); t.l.c. (*B*): R_F 0.38; ν_{max}^{KBr} 3300 (NH), 1730 (OAc), 1650 (Cbz group CO), and 1680–1550 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{32}H_{43}N_5O_{16}$: C, 51.00; H, 5.76; N, 9.29; Asp, 1.00; Gly, 1.00; Ser, 1.00. Found: C, 50.96; H, 5.79; N, 9.21; Asp, 0.88; Gly, 0.95; Ser, 1.00.

*2-Acetamido-3,4,6-tri-*O-*acetyl-1-*N-[N-(*benzyloxycarbonyl*)-L-*aspart-1-oyl*-(*glycyl*-L-*serine methyl ester*)-*4-oyl*]-*2-deoxy-β-*D-*glucopyranosylamine* (**41**). — To a solution of *N*-ethyl-5-phenylisoxazolium 3′-sulfonate (0.125 g) in acetonitrile (20 mL) at 0° were added **40** (0.298 g; ref. 18) and *N*-methylmorpholine (50 μL) in acetonitrile (40 mL). The mixture was stirred at 0°, and then at room temperature for 65 min, by when, all compounds had dissolved. Separately, a solution of glycyl-L-serine methyl ester (**42**) was prepared by treatment of a solution of *N*-(benzyloxycarbonyl)glycyl-L-serine methyl ester[10] (155 mg) in glacial acetic acid (2 mL) with 30% hydrogen

bromide in acetic acid (2 mL) for 1 h at room temperature, followed by evaporation of the acids *in vacuo*, and addition of *N*-methylmorpholine (50 μL). This solution was added to the solution of **40**, and stirring was continued for 24 h. The acetonitrile was evaporated, chloroform (100 mL) was added, and the solution was successively washed with 1% sodium hydrogencarbonate solution, water, M hydrochloric acid, and water, dried (sodium sulfate), and evaporated; crystallization of the residue from methanol gave 120 mg (32%) of **41**, m.p. 225–228° (dec.) (shrinking at 219°), $[\alpha]_D^{23}$ +57.7° (*c* 1.1, chloroform); t.l.c. (*B*): R_F 0.65; ν_{max}^{KBr} 3300–3100 (NH), 1740 (OAc), 1650 (Cbz group CO), and 1680–1550 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{32}H_{43}N_5O_{16}$: C, 51.00; H, 5.76; N, 9.29; O, 33.97; Asp, 1.00; Glyc, 1.00; Ser, 1.00. Found: C, 50.96; H, 5.69; N, 9.00; O, 33.94; Asp, 1.17; Gly, 1.00; Ser, 1.00.

Attempt to obtain **41** *from* **20**. — To a solution of **20** (10 mg) in dichloromethane (1 mL) was added stannic chloride (0.5 mL) in benzene (2 mL), and the mixture was kept overnight at room temperature, with stirring. The mixture was diluted with water, and extracted with chloroform (20 mL). The dark-brown extract was washed with water and dried (sodium sulfate). On examination by t.l.c., no spot migrating at the same rate as **41** [R_F 0.58 (*D*)] or **20** [R_F 0.32 (*D*)] was detected, the decomposed material giving a streak.

Treatment of glycopeptides with alkaline sodium borohydride. — The glycopeptides **17**, **19**, **22**, **23**, **26**, **27**, **29**, **31**, **32**, **35**, **36**, and **38** (400 μg) were each treated with 2M sodium borohydride in 0.5M sodium hydroxide solution (200 μL) for 20 h at 40°. The reaction was stopped by addition of acetic acid (2–3 drops). Methanol (0.5 mL) was added, and the solution was evaporated (hot-water bath) to dryness under a stream of nitrogen. Methanol was several times added to, and evaporated from, the residue, in order to remove the boric acid. The residue was hydrolyzed by heating it with ~5.8M hydrochloric acid for 24 h at 110°. The solution was evaporated, and the residue, dried under high vacuum in the presence of sodium hydroxide pellets, was treated with 3M hydrogen chloride in 1-butanol (0.5 mL), and then with 1:3 (v/v) trifluoroacetic anhydride–dichloromethane in the same way as given in the General methods section. L-Proline or L-glutamic acid was now added to the hydrolyzate as the standard, and g.l.c. of the amino acids was performed on a column (1.5 m) of Tabsorb (Regis Chemical Co., Chicago, IL 60610), programmed for a rise of 4°/min from 100 to 225°. G.l.c. of 2-acetamido-2-deoxy-D-glucose and 2-acetamido-2-deoxy-D-glucitol was performed on a mixed-phase column (1.5 m) of 2% of OV-17 and 1% of OV-210 (Supelco Inc., Bellefonte, PA 16823), programmed for a rise of 6.5°/min from 75 to 225°. The results are reported in Table VI.

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