roform–methanol (90:10) as developing solvent were (mobilities of authentic standards in parentheses) **6a**, 0.37, (20α = 0.26; 20β = 0.37); **6b**, 0.47 (20α = 0.37; 20β = 0.47); (chloroform–methanol, 85:15) **6c**, 0.32 (20α = 0.24; 20β = 0.32); **6d**, 0.25 (20α = 0.18, 20β = 0.25).

Acknowledgments. This investigation was supported by U.S. Public Health Service Grants CA 14914, AM 09006, and RR 5589. S. w. O. is a trainee of the National Cancer Institute (Grant CA 5215).

Registry No.—**1a**, 64-85-7; **1b**, 152-58-9; **1c**, 50-22-6; **1d**, 50-23-7; **2a**, 853-27-0; **2b**, 20287-95-0; **2c**, 20287-97-2; **2d**, 14760-49-7; **3a**, 59005-48-0; **3b**, 59005-49-1; **3c**, 59005-50-4; **3d**, 59005-51-5; **4a**, 59005-52-6; **4b**, 59005-53-7; **4c**, 59005-54-8; **4d**, 59005-55-9; **5a**, 59005-56-0; **5b**, 59005-57-1; **5c**, 59005-58-2; **5d**, 59005-59-3.

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Synthesis of Substituted Glycopeptides Containing a 2-Acetamido-2-deoxy-β-D-glucopyranosyl Residue and the Amino Acid Sequence 18–22 of Bovine Pancreatic Deoxyribonuclease A[1]

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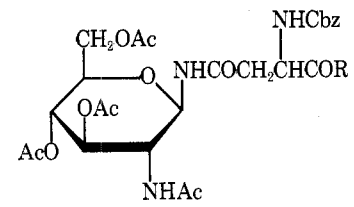
Received December 22, 1975

Condensation of 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl]-2-deoxy-β-D-glucopyranosylamine (1) with protected L-alanine, L-alanyl-L-threonine, L-alanyl-L-threonyl-L-leucine, and L-alanyl-L-threonyl-L-leucyl-L-alanine derivatives gave glycodi-, -tri-, -tetra-, and -pentapeptides corresponding to the sequences 18–19, 18–20, 18–21, and 18–22 of deoxyribonuclease A.

Glycoproteins containing the 2-acetamido-1-*N*-(L-aspart-4-oyl)-β-D-glucosylamine carbohydrate–protein linkage include many of the biologically important proteins, such as hormones, enzymes, plasma proteins, etc.[2] The mechanism of the biosynthesis of the carbohydrate chain and its attachment to the protein backbone are not as yet completely elucidated, because of the difficulty in the separation and identification of the final product, in addition to the instability of the possible carbohydrate intermediates. For this reason, a study of the biosynthesis of glycoproteins based on chemically synthesized peptide acceptors and carbohydrate intermediates[3,4] has been undertaken in this laboratory. Glycoproteins from pancreatic tissues were selected because this tissue has been shown to synthesize rapidly the possible intermediates.

In a preceding paper,[3] we have described the synthesis of glycopeptides derived from beef ribonuclease B and in the present paper we describe the synthesis of glycopeptides derived from beef deoxyribonuclease A. This enzyme exists in bovine pancreatic tissue in three forms, A, B, and C, which differ in the carbohydrate composition of the chain attached to Asn-18 as well as in their amino acid sequences.[5-8] In addition, the amino acid sequence Asn-X-Ser, which is generally assumed to be a prerequisite for the linkage of a carbohydrate chain to an asparagine residue,[2] exists at Asn-103, X being Asp-104, but no carbohydrate chain is linked to Asn-103. Thus, biosynthetic experiments with glycopeptides derived from the Asn-18 and Asn-103 regions could give important information on the role played by the amino acid sequence in the formation and structure of the carbohydrate chain. As model glycopeptides, the synthesis of di-, tri-, tetra-, and pentapeptides related to the sequence 18–22 (Asn-Ala-Thr-Leu-Ala) of deoxyribonuclease A, where a 2-acetamido-2-deoxy-β-D-glycopyranosyl residue is linked to the amide group of the Asn-18 residue, is described.

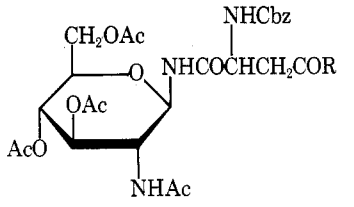
The present synthesis of the protected glycopeptides Asn(GlcNAc)-Ala (**2** and **3**), Asn(GlcNAc)-Ala-Thr (**11** and **12**), Asn(GlcNAc)-Ala-Thr-Leu (**13**), and Asn(GlcNAc)-Ala-Thr-Leu-Ala (**14**) is based on the synthesis of the peptide chain, unmasking of the terminal amino group, and condensation with 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl]-2-deoxy-β-D-glucopyranosylamine (**1**), in a sequence of reactions similar to that described[3] for the synthesis of glycopeptides derived from the region of Asn-34 of beef ribonuclease B. Of the two reagents for peptide synthesis, *N*-ethyl-5-phenylisoxazolium 3'-sulfonate[9] (WRK) and 2-ethoxy-*N*-ethoxycarbonyl-1,2-dihydroquinoline[10] (EEDQ), the latter-named reagent was found to be more efficient for the synthesis of N^4-glycosylasparagine,[11] but less efficient for peptides of high molecular weight.[12] Both reagents were tested for the condensation of the glycopyranosylamine **1** with L-alanine *p*-nitrobenzyl ester to give crystalline 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanine *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**2**) in similar yields. The *p*-nitrobenzyl ester group, protective of the C-terminal group, is stable under acid conditions and, thus, is useful for elongation of the peptide chain from the N-terminal group. In order to elongate the chain at the C-terminal group, the methyl ester derivatives, which can be easily converted into reactive hydrazides,[14] were selected. Condensation of **1** with L-alanine methyl ester in the presence of the WRK re-



1, R = OH

2, R = NHCHCO$_2$CH$_2$C$_6$H$_4$NO$_2$(*p*) (CH$_3$)

3, R = NHCHCO$_2$Me (CH$_3$)



4, R = OH

5, R = NHCHCH$_3$ (CO$_2$CH$_2$C$_6$H$_4$NO$_2$(*p*))

6, R = NHCHCH$_2$Ph (CO$_2$Me)

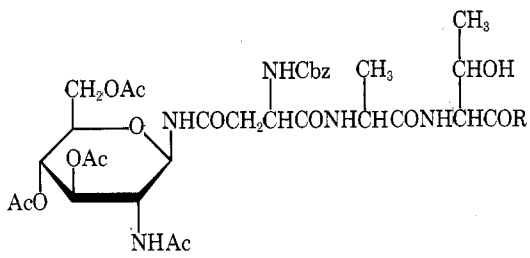
R–Ala–Thr–OR′

7, R = Cbz; R′ = Me

8, R = H; R′ = Me

9, R = Cbz; R′ = CH$_2$C$_6$H$_4$NO$_2$(*p*)

10, R = H; R′ = CH$_2$C$_6$H$_4$NO$_2$(*p*)



11, R = OMe

12, R = OCH$_2$C$_6$H$_4$NO$_2$(*p*) (CH(CH$_3$)$_2$)

13, R = NHCHCO$_2$CH$_2$C$_6$H$_4$NO$_2$(*p*) (CH(CH$_3$)$_2$)

14, R = NHCHCONHCHCO$_2$CH$_2$C$_6$H$_4$NO$_2$(*p*) (CH$_2$, CH$_3$)

agent gave, however, the methyl ester analogue **3** of **2** only in 25% yield.

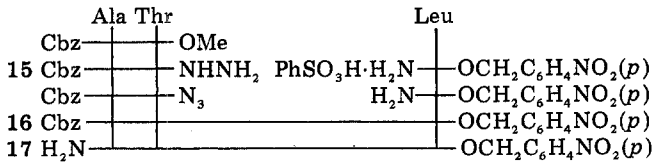
In order to ascertain whether the alkaline condition of the peptide chain elongation might cause a translocation of the glycosylamine group from C-4 to C-1 of the asparagine moiety, the isomeric glycosylamine of **1**, namely 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl]-2-deoxy-β-D-glucoypyranosylamine (**4**), was condensed with L-alanine *p*-nitrobenzyl ester and L-phenylalanine methyl ester in the presence of the WRK or EEDQ reagent to give 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl-(L-alanine nitrobenzyl ester and L-phenylalanine methyl ester)-1-oyl]-2-deoxy-β-D-glucopyranosylamine (**5** and **6**), respectively. Both **5** and **6** showed properties different from those of the analogue **3** and of the previously synthesized 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-phenylalanine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine,[3] respectively, and no transposition products were observed.

Removal of the protective *N*-benzyloxycarbonyl group from the methyl[15] and *p*-nitrobenzyl esters of *N*-(benzyloxycarbonyl)-L-alanyl-L-threonine (**7** and **9**), obtained by condensation of *N*-benzyloxycarbonyl-L-alanine with L-threonine methyl or *p*-nitrobenzyl ester in the presence of dicyclohexacarbodiimide, gave the corresponding dipeptides **8** and **10**. These were coupled with **1** in the presence of the WRK reagent to give the crystalline glycotripeptides 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonine methyl and *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**11** and **12**), in 38 and 30% yields, respectively. These protected glycotripeptides correspond to the amino acid sequence 18–20 of deoxyribonuclease A.

The synthesis of the *p*-nitrobenzyl ester of the tri- and tetrapeptides *N*-(benzyloxycarbonyl)-L-alanyl-L-threonyl-leucine (**16**) and *N*-(benzyloxycarbonyl)-L-alanyl-L-threonyl-L-leucyl-L-alanine (**19**) by coupling *N*-(benzyloxycarbonyl)-L-alanyl-L-threonine hydrazide (**15**) via the azide derivative with L-leucine *p*-nitrobenzyl ester and with *N*-(benzyloxycarbonyl)-L-leucinyl-L-alanine *p*-nitrobenzyl ester (**18**), after removal of the *N*-(benzyloxycarbonyl) group, respectively, is illustrated in Schemes I and II. After removal of the protective *N*-(benzyloxycarbonyl) group, the tripeptide **17** was condensed with **1** in the presence of either the WRK or the EEDQ reagent to give crystalline 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonyl-L-leucine *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**13**), which corresponds to the amino acid sequence 18–21 of deoxyribonuclease A. In both cases, the yields of **13** were low (19 and 13%, respectively).

A similar condensation of the deblocked tetrapeptide **20**, obtained from **19**, with **1** in the presence of the WRK or EEDQ reagent gave the crystalline, protected glycopentapeptide 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonyl-L-leucyl-L-alanine

Scheme I



Scheme II

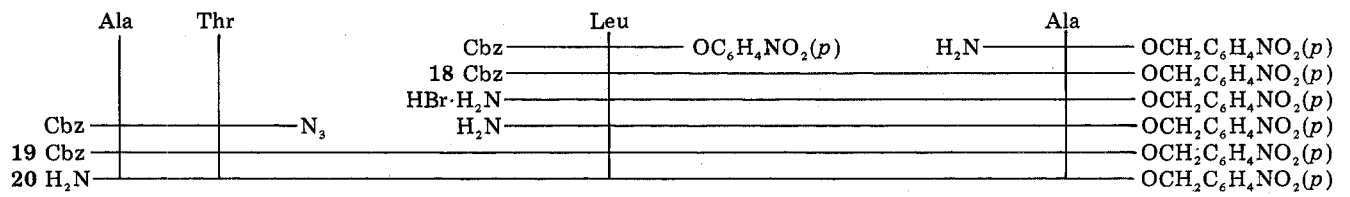


Table I. Amino Acid Composition of the Synthetic Glycopeptides[a]

	Amino acid analysis			
	Theory		Found (GLC)	Found (Beckman)
L-Alanine *p*-nitrobenzyl ester (**2**)	L-Asp	1.00	1.00	
	L-Ala	1.00	0.81	
L-Alanine methyl ester (**3**)	L-Asp	1.00	1.00	
	L-Ala	1.00	1.06	
L-Alanyl-L-threonine methyl ester (**11**)	L-Asp	1.00	1.00	
	L-Ala	1.00	0.86	
	L-Thr	1.00	0.91	
L-Alanyl-L-threonine *p*-nitrobenzyl ester (**12**)	L-Asp	1.00	1.00	1.00
	L-Ala	1.00	0.92	0.93
	L-Thr	1.00	1.04	0.91
L-Alanyl-L-threonyl-L-leucine *p*-nitrobenzyl ester (**13**)	L-Asp	1.00	1.00	1.00
	L-Ala	1.00	0.82	0.98
	L-Leu	1.00	0.90	0.98
	L-Thr	1.00	1.00	0.96
L-Alanyl-L-threonyl-L-leucyl-L-alanine *p*-nitrobenzyl ester (**14**)	L-Asp	1.00	1.00	1.00
	L-Ala	2.00	2.08	1.89
	L-Leu	1.00	0.99	0.93
	L-Thr	1.00	0.84	0.78

[a] 2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(peptide ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine.

p-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**14**), corresponding to the amino acid sequence 18–22 of ribonuclease A. The respective yields were 11 and 3%. The low yield of the condensation in the presence of the EEDQ reagent is in agreement with the low yields obtained previously in the synthesis of high molecular weight polypeptides with this reagent.[12]

Experimental Section

General Methods. Melting points were determined with a Mettler FP-2 apparatus and correspond to "corrected melting points". Rotations were determined for solutions in 1-dm semimicrotubes with a Perkin-Elmer No. 141 polarimeter. The *N,N*-dimethylformamide used was Spectro-reagent grade. Ir spectra were recorded for potassium bromide disks, with a Perkin-Elmer spectrophotometer Model 237. Evaporations were performed in vacuo, the bath temperature being kept below 45 °C. Column chromatography was performed on silica gel Merck (70–325 mesh, E. Merck, Darmstadt, Germany), used without pretreatment; the ratio of the weight of substance to the weight of silica gel was 1:60; the volume of the fractions collected was 2 ml/g of the substance; the ratio was verified by ascending TLC on precoated plates of silica gel (Merck); solvents (v/v) used were A, chloroform–methanol, 19:1; B, chloroform–ethanol, 19:1; C, chloroform–methanol, 14:1; D, chloroform–methanol, 9:1; E, chloroform–ethanol, 14:1; the spots were detected by spraying the plates with 20% sulfuric acid and heating them at 200 °C for a few minutes. The microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

The amino acid composition of the peptides was determined after hydrolysis by heating the solution for 24 h at 108 °C with hydrochloric acid (ca. 5.8 M, constant boiling point), followed by evaporation in the presence of NaOH pellets. (a) The dry residue was heated with 3 M hydrochloric acid (5 ml) for 1 h at 100 °C, followed by treatment with a 25% solution of trifluoroacetic anhydride in dichloromethane (0.1 ml) for 1 h at 100 °C. GLC analysis of the *N*-trifluoroacetyl butyl esters was performed on a column of Tabsorb (Regis Chemical Co., Chicago, Ill.) programmed for a rise of 4 °C/min from 75 to 225 °C. (b) The amino acid composition of the residue was determined with a Beckman Spinco Model 117 amino acid analyzer.

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanine *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (2). A. To a solution of *N*-ethyl-5-phenylisoxazolium 3′-sulfonate (63 mg) in acetonitrile (10 ml) at 10 °C was added **1** (0.149 g) and *N*-methylmorpholine (25 μl) in acetonitrile (20 ml). The reaction mixture was stirred and the ice bath was removed. After 65 min, all compounds were in solution. L-Alanine *p*-nitrobenzyl ester hydrobromide[17] (80 mg) in acetonitrile (10 ml) and *N*-methylmorpholine (25 μl) were added. The mixture was stirred for 24 h at room temperature, and the solvents were removed in vacuo. The residue was dissolved in chloroform, and the solution was successively washed with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, and evaporated in vacuo. The residue was crystallized from chloroform–methanol (0.125 g, 62.5%): mp 271–273 °C dec; $[\alpha]^{22}D$ +4.7° (*c* 0.94, *N,N*-dimethylformamide); ir 3300 (NH), 1740 (OAc), 1650 (benzyloxycarbonyl group CO), 1580–1680 cm^{-1} (peptide amide I); R_f 0.63 (D), 0.54 (E).

Anal. Calcd for $C_{36}H_{43}N_5O_{16}$: C, 53.93; H, 5.41; N, 8.73; O, 31.93. Found: C, 53.93; H, 5.50; N, 8.78; O, 32.04.

B. A solution of **1** (0.149 g) in tetrahydrofuran–acetonitrile (20 ml) was mixed with L-alanine *p*-nitrobenzyl ester hydrobromide (80 mg) in the same solvent mixture (1 ml) containing triethylamine (35 μl), and treated with *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 0.62 g). The mixture was stirred overnight and evaporated in vacuo. The residue was dissolved in chloroform and processed as described in method A. The residue crystallized as long needles from chloroform–methanol (0.13 g, 65%), mp 274–275 °C; ir spectrum and mobility on TLC were identical with those of material obtained by method A.

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (3). To a solution of *N*-ethyl-5-phenylisoxazolium 3′-sulfonate (0.125 g) in acetonitrile (20 ml) at 0 °C was added **1** (0.298 g) and *N*-methylmorpholine (50 μl) in acetonitrile (40 ml). The suspension was treated as described for **2**, and a solution of L-alanine methyl ester hydrochloride (70 mg)[16] in acetonitrile (12 ml) and *N*-methylmorpholine (50 μl) was added. The mixture was treated as described for **2** and the residue was crystallized from hot methanol (85 mg, 25%): mp 268–269 °C dec (sintered at 255–257 °C); $[\alpha]^{25}D$ +7.9° (*c* 0.94, *N,N*-dimethylformamide); ir 3300 (NH), 1650 (benzyloxycarbonyl group CO), 1550–1740 cm^{-1} (peptide amide I); R_f 0.58 (C).

Anal. Calcd for $C_{30}H_{40}N_4O_{14}$: C, 52.94; H, 5.92; N, 8.23; O, 32.91. Found: C, 52.86; H, 5.96; N, 8.28; O, 32.91.

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-4-oyl-(L-alanine *p*-nitrobenzyl ester)-1-oyl]-2-deoxy-β-D-glucopyranosylamine (5). A. A solution of **4** (0.15 g)[18] in benzene–ethanol (1:1 v/v, 20 ml) was mixed with a solution of L-alanine *p*-nitrobenzyl ester hydrobromide (80 mg) in the same solvent mixture (5 ml) containing triethylamine (35 μl), and EEDQ (65 mg) was added. The mixture was stirred overnight and the solvents were removed in vacuo. The residue was washed successively with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, evaporated in vacuo, and crystallized from

acetonitrile (95 mg, 47.5%): mp 238–239 °C dec; $[\alpha]^{18}D$ −10.4° (c 1.1, N,N-dimethylformamide); ir 3300 (NH), 1740 (OAc), 1650 (benzyloxycarbonyl group CO), 1550–1700 cm^{-1} (peptide amide I); R_f 0.25 (E).

Anal. Calcd for $C_{36}H_{43}N_5O_{16}$: C, 53.93; H, 5.41; N, 8.73; O, 31.93. Found: C, 53.89; H, 5.72; N, 8.84; O, 31.65.

B. To a clarified mixture of N-ethyl-5-phenylisoxazolium 3′-sulfonate (63 mg), 4 (0.15 g), and N-methylmorpholine (25 μl) in acetonitrile (20 ml) obtained under conditions described for **2** was added a solution of L-alanine p-nitrobenzyl ester hydrobromide (80 mg) containing N-methylmorpholine (25 μl). The mixture was stirred overnight and the solvents were removed in vacuo. The residue was dissolved in chloroform, and the solution was washed, dried, and evaporated as described for **5**. The residue was crystallized from acetonitrile (26 mg, 13%): mp 238.5–239 °C dec; ir spectrum and mobility on TLC were identical with those of the compound obtained by method A.

2-Acetamido-3,4,6-tri-O-acetyl-1-N-[N-(benzyloxycarbonyl)-L-aspart-4-oyl-(L-phenylalanine methyl ester)-1-oyl]-2-deoxy-β-D-glucopyranosylamine (6). A solution of **4** (0.15 g) in benzene–ethanol (1:1 v/v, 20 ml) was mixed with a solution of L-phenylalanine methyl ester hydrochloride[19] (55 mg) in the same solvent mixture (2 ml) containing triethylamine (35 μl), and EEDQ (65 mg) was added. The mixture was stirred overnight and the solvents were evaporated. The residue was filtered off, washed successively with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, and crystallized from hot acetonitrile as needles (0.135 g, 71%): mp 263–264 °C dec (sintered at 260 °C); $[\alpha]^{21}D$ +1.0° (c 0.8, N,N-dimethylformamide); ir 3300 (NH), 1740 (OAc), 1675 (benzyloxycarbonyl group CO), 1555–1695 cm^{-1} (peptide amide I); R_f 0.34 (E).

Anal. Calcd for $C_{36}H_{44}N_4O_{14}$: C, 57.14; H, 5.86; N, 7.40; O, 29.60. Found: C, 57.00; H, 5.83; N, 7.38; O, 29.48.

N-(Benzyloxycarbonyl)-L-alanyl-L-threonine p-Nitrobenzyl Ester (9). A solution of L-threonine p-nitrobenzyl ester was prepared by treatment of a solution of N-(benzyloxycarbonyl)-L-threonine p-nitrobenzyl ester[20] (1.94 g) in glacial acetic acid (5 ml) with 30% hydrogen bromide in acetic acid (5 ml), keeping the mixture at room temperature for 1 h and precipitating the resulting hydrobromide with anhydrous ether; the precipitate was rapidly filtered off and treated with triethylamine (0.7 ml) in N,N-dimethylformamide (5 ml). This solution was added to a cooled solution (0 °C) of N-(benzyloxycarbonyl)-L-alanine (1.12 g) and N,N-dicyclohexylcarbodiimide (1.03 g) in dichloromethane–N,N-dimethylformamide (2:1 v/v, 30 ml). This mixture was stirred for 3 h at 0 °C and then at room temperature overnight. The solvents were evaporated and the residue was dissolved in ethyl acetate. The N,N-dicyclohexylurea was filtered off and the filtrate was successively washed with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, and dried with sodium sulfate. After evaporation, the residual syrup crystallized on trituration with hexane. The crystals were recrystallized from ethyl acetate as long needles (1.3 g, 57%): mp 133–134 °C; $[\alpha]^{25}D$ −55.5° (c 0.76, N,N-dimethylformamide); ir 3325–3375 (NH), 1660 (benzyloxycarbonyl group CO), 1525–1746 cm^{-1} (peptide amide I); R_f 0.21 (A).

Anal. Calcd for $C_{22}H_{25}N_3O_8$: C, 57.51; H, 5.48; N, 9.15; O, 27.86. Found: C, 57.60; H, 5.68; N, 9.14; O, 27.66.

2-Acetamido-3,4,6-tri-O-acetyl-1-N-[N-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (11). To a solution of N-ethyl-5-phenylisoxazolium 3′-sulfonate (63 mg), 1 (0.15 g), and N-methylmorpholine (25 μl) in acetonitrile (20 ml), prepared under the conditions described for **2**, was added L-alanyl-L-threonine methyl ester hydrobromide in acetonitrile (10 ml) containing N-methylmorpholine (25 μl). L-Alanyl-L-threonine methyl ester hydrobromide was prepared from N-(benzyloxycarbonyl)-L-alanyl-L-threonine methyl ester[15] (85 mg) by treatment in acetic acid (1 ml) with 30% hydrogen bromide in acetic acid (1 ml) at room temperature for 1 h, subsequent precipitation, and rapid filtration. The mixture was stirred overnight and the solvent evaporated in vacuo. The residue was dissolved in dichloromethane and the solution washed, dried with sodium sulfate, and evaporated as described for **2**. The residue was crystallized from ethyl alcohol (75 mg, 38%): mp 252–254 °C dec (sintered at 247 °C); $[\alpha]^{25}D$ +9.4 °C (c 0.54, N,N-dimethylformamide); ir 3300 (NH), 1650 (benzyloxycarbonyl group CO), 1540–1690 cm^{-1} (peptide amide I); R_f 0.47 (C).

Anal. Calcd for $C_{34}H_{47}N_5O_{16}$: C, 52.23; H, 6.06; N, 8.96; O, 32.75. Found: C, 51.99; H, 6.00; N, 8.67; O, 32.48.

2-Acetamido-3,4,6-tri-O-acetyl-1-N-[N-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonine p-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (12). L-Alanyl-L-threonine p-nitrobenzyl ester hydrobromide was prepared by treatment of **9** (0.115 g) in glacial acetic acid (2 ml) with 30% hydrogen bromide in acetic acid (2 ml) at room temperature for 1 h, subsequent precipitation with anhydrous ether, and filtration. A solution in acetonitrile (10 ml) containing N-methylmorpholine (25 μl) was added to a solution of N-ethyl-5-phenylisoxazolium 3′-sulfonate (63 mg), **1** (0.15 g), and N-methylmorpholine (25 μl) in acetonitrile (10 ml) prepared as described for **2**. The reaction mixture was stirred for 24 h and the solvents were removed in vacuo. The residue was dissolved in chloroform, and the solution was washed, dried, and evaporated as described for **2**. The residue crystallized from methanol (75 mg, 30%): mp 244.5–245.5 °C dec (sintered 241.5 °C); $[\alpha]^{21}D$ −18.0° (c 0.77, N,N-dimethylformamide); ir 3300 (NH), 1740 (OAc), 1640 (benzyloxycarbonyl group CO), 1525–1700 cm^{-1} (peptide amide I); R_f 0.77 (D).

Anal. Calcd for $C_{40}H_{50}N_6O_{18}$: C, 53.21; H, 5.58; N, 9.31; O, 31.90. Found: C, 53.17; H, 5.66; N, 9.38; O, 31.78.

N-(Benzyloxycarbonyl)-L-alanyl-L-threonine Hydrazide (15). Hydrazine[22] (95%, 0.6 ml) was added to a solution of methyl N-(benzyloxycarbonyl)-L-alanyl-L-threonate[15] (2.0 g) in methanol (10 ml) and the mixture was kept at room temperature overnight. The hydrazide was obtained after evaporation of the solvent and recrystallized from methanol (1.7 g, 85%): mp 208–210 °C; $[\alpha]^{25}D$ −37.4° (c 0.53, 0.2 M hydrochloric acid); ir 3250–3275 (NH), 1630 (benzyloxycarbonyl group CO), 1525–1675 cm^{-1} (peptide amide I).

Anal. Calcd for $C_{15}H_{22}N_4O_5$: C, 53.26; H, 6.56; N, 16.54; O, 23.64. Found: C, 53.08; H, 6.47; N, 16.41; O, 23.69.

N-(Benzyloxycarbonyl)-L-alanyl-L-threonyl-L-leucine p-Nitrobenzyl Ester (16). N-(Benzyloxycarbonyl)-L-alanyl-L-threonine hydrazide (**15**, 0.85 g) was dissolved in a mixture of concentrated hydrochloric acid (0.25 ml), glacial acetic acid (0.75 ml), and water (6.5 ml), and the solution was cooled to 0 °C. Sodium nitrite (0.175 g) was added with stirring and the syrupy azide formed was extracted with ethyl acetate precooled to 0 °C. The extract was washed with cold water and dried with sodium sulfate. It was added to a precooled solution of L-leucine p-nitrobenzyl ester prepared from a solution of its benzenesulfonic salt[21] (1.06 g) in N,N-dimethylformamide (5 ml) and triethylamine (0.35 ml). After 24 h at 6 °C and several hours at room temperature, the solvents were evaporated. The residue was dissolved in ethyl acetate and washed successively with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, and dried with sodium sulfate. The solution was evaporated and the residue dissolved in a small amount of ethyl acetate. The insoluble material was filtered off, and the filtrate crystallized on cooling (0.975 g, 69%): mp 79–80 °C; $[\alpha]^{22}D$ −12.3° (c 0.61, N,N-dimethylformamide); ir 3275 (NH), 1630 (benzyloxycarbonyl group CO), 1550–1750 cm^{-1} (peptide amide I); R_f 0.36 (B).

Anal. Calcd for $C_{28}H_{36}N_4O_9$: C, 58.73; H, 6.34; N, 9.78; O, 25.15. Found: C, 58.63; H, 6.36; N, 9.84; O, 25.22.

N-(Benzyloxycarbonyl)-L-leucyl-L-alanine p-Nitrobenzyl Ester (18). A solution of p-nitrophenyl N-(benzyloxycarbonyl)-L-leucinate[23] (1.93 g) in chloroform (10 ml) was added to a N,N-dimethylformamide solution (5 ml) containing L-alanine p-nitrobenzyl ester hydrobromide[17] (1.52 g) and triethylamine (0.7 ml). The mixture was stirred for 24 h, the solvents were removed, and the residue was dissolved in ethyl acetate. The organic layer was washed successively with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, and dried with sodium sulfate. The solvent was evaporated and the syrup crystallized from ethyl alcohol on cooling (1.7 g, 72%): mp 108–109 °C; $[\alpha]^{25}D$ −17.3° (c 0.87, N,N-dimethylformamide); ir 3300 (NH), 1630 (benzyloxycarbonyl group CO), 1560–1725 cm^{-1} (peptide amide I); R_f 0.7 (B).

Anal. Calcd for $C_{24}H_{29}N_3O_7$: C, 61.14; H, 6.20; N, 8.91; O, 23.75. Found: C, 61.14; H, 6.24; N, 8.88; O, 23.89.

N-(Benzyloxycarbonyl)-L-alanyl-L-threonyl-L-leucyl-L-alanine p-Nitrobenzyl Ester (19). N-(Benzyloxycarbonyl)-L-leucyl-L-alanine p-nitrobenzyl ester (**18**, 0.24 g) was converted into its hydrobromide derivative by dissolving it in glacial acetic acid (1.5 ml) and treating the solution with 30% hydrogen bromide in acetic acid (1.5 ml) for 1 h at room temperature. The hydrobromide was precipitated by anhydrous ether and rapidly filtered off, dissolved in N,N-dimethylformamide (1 ml), and treated with triethylamine (70 μl). The resulting product was condensed with the azide prepared from N-(benzyloxycarbonyl)-L-alanyl-L-threonine hydrazide (**15**, 0.17 g) in the same manner as described for **16**. The mixture was stirred for 20 h, and the solvents were removed in vacuo. The residue was dissolved in ethyl acetate, and the solution was washed successively with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, and concentrated in vacuo. The residue was crystallized from absolute ethanol as granules

(0.19 g, 60%); mp 187–189 °C dec; $[\alpha]^{22}D$ −11.7° (c 0.71, N,N-dimethylformamide); ir 3250 (NH), 1660 (benzyloxycarbonyl group CO), 1630–1730 cm^{-1} (peptide amide I); R_f 0.3 (B).

Anal. Calcd for $C_{31}H_{41}N_5O_{10}$: C, 57.85; H, 6.42; N, 10.88; O, 24.85. Found: C, 57.83; H, 6.47; N, 10.86; O, 24.80.

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonyl-L-leucine *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (13). A. A solution of N-ethyl-5-phenylisoxazolium 3′-sulfonate (63 mg) in acetonitrile (10 ml) cooled to 0 °C was added to 1 (0.149 g) and N-methylmorpholine (25 μl) in acetonitrile (20 ml). The reaction mixture was stirred for 65 min at room temperature until dissolution. A solution of L-alanyl-L-threonyl-L-leucine p-nitrobenzyl ester hydrobromide was prepared from **16** (0.143 g) in glacial acetic acid (2 ml) by treatment with 30% hydrogen bromide in acetic acid (2 ml) at room temperature for 1 h, removal of the acids by evaporation in vacuo, washing the residue with anhydrous ether, and drying in a vacuum desiccator. This residue was dissolved in acetonitrile (10 ml) containing N-methylmorpholine (25 μl), and the solution was added to the previously described solution. The mixture was stirred for 24 h and the acetonitrile was evaporated in vacuo. The residue was dissolved in chloroform, and the solution was successively washed with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, and evaporated in vacuo. The crude material (0.15 g) showed on TLC two major spots [R_f 0.55 and 0.43 (C)] and a few minor spots moving faster. It was purified by column chromatography on silica gel. Chloroform eluted fast-moving products, and successive elution with a linear gradient (1:100 to 3:100, v/v) of methanol–chloroform gave in the earlier fractions 0.21 mg of a compound having R_f 0.53 (C) and then pure **13** [R_f 0.43 (C), 0.56 g]. This was crystallized from acetonitrile–methanol (48 mg, 19%): mp 263–264 °C dec; $[\alpha]^{21}D$ −2.0° (c 0.68, N,N-dimethylformamide); ir 3285 (NH), 1740 (OAc), 1630 (benzyloxycarbonyl group CO), 1530–1650 cm^{-1} (peptide amide I).

Anal. Calcd for $C_{46}H_{61}N_7O_{19}$: C, 54.38; H, 6.05; N, 9.65; O, 29.92. Found: C, 54.29; H, 6.08; N, 9.63; O, 30.02.

B. A solution of 1 (0.149 g) in tetrahydrofuran (15 ml) was treated with a solution of L-alanyl-L-threonyl-L-leucine p-nitrobenzyl ester hydrobromide [prepared from **16** (0.143 g) under the same conditions as described in method A] in acetonitrile (5 ml) containing triethylamine (35 μl) and EEDQ reagent (62 mg). The mixture was stirred overnight at room temperature. The solvents were removed in vacuo and the residue was dissolved in chloroform. The solution was washed successively with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, and evaporated. The residue was crystallized from acetonitrile–methanol (35 mg, 13%), mp 261–263 °C. The ir spectrum and the mobility on TLC were identical with those of the material obtained by method A.

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonyl-L-leucyl-L-alanine *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (14). A. To a solution of N-ethyl-5-phenylisoxazolium 3′-sulfonate (63 mg) in acetonitrile (10 ml) at 0 °C was added 1 (0.149 g) and N-methylmorpholine (25 μl) in acetonitrile (20 ml). The reaction mixture was stirred for 65 min at room temperature until dissolution. A solution of L-alanyl-L-threonyl-L-leucyl-L-alanine p-nitrobenzyl ester hydrobromide [prepared from **19** (0.161 g), glacial acetic acid (2 ml), and 30% hydrogen bromide in glacial acetic acid (2 ml) under the same conditions as described for **16**] in acetonitrile (10 ml) containing N-methylmorpholine (25 μl) was added to the previously described solution. The mixture was stirred for 24 h and then the acetonitrile was removed in vacuo. The crude material was dissolved in chloroform, and the solution was successively washed with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, and evaporated to give a crude material (33 mg, 12%) that was crystallized from methanol (19 mg): mp 249–251 °C dec (and shrinking at 247 °C); $[\alpha]^{21}D$ +6.4° (c 0.72, N,N-dimethylformamide); ir 3300 (NH), 1740 (OAc), 1660 (benzyloxycarbonyl group CO), 1530–1700 cm^{-1} (peptide amide I); R_f 0.34 (E) and 0.69 (D).

Anal. Calcd for $C_{49}H_{66}N_8O_{20}$: C, 54.14; H, 6.11; N, 10.30; O, 29.43. Found: C, 54.17; H, 6.18; N, 9.62; O, 29.53.

B. A solution of 1 (0.149 g) in tetrahydrofuran (15 ml) was treated with a solution of L-alanyl-L-threonyl-L-leucyl-L-alanine p-nitrobenzyl ester hydrobromide [prepared from **19** (0.161 g) by following the same conditions as described in method A] in acetonitrile (5 ml) containing triethylamine (35 μl) and EEDQ reagent (62 mg). The mixture was stirred overnight at room temperature. The solvents were removed in vacuo and the residue was dissolved in chloroform. The solution was washed successively with 1 M hydrochloric acid, water, 1% sodium hydrogen carbonate, and water, dried with sodium sulfate, and evaporated. The residue crystallized from ethanol–ether (8 mg, 3%), mp 250–252 °C dec, ir identical with that of the product prepared by method A.

Registry No.—**1**, 38877-33-7; **2**, 58944-53-9; **3**, 58944-54-0; **4**, 38877-35-9; **5**, 58944-55-1; **6**, 58944-56-2; **7**, 58944-57-3; **11**, 58944-58-4; **12**, 58944-59-5; **13**, 58944-60-8; **14**, 58944-61-9; **15**, 41961-29-9; **16**, 58944-62-0; **18**, 58944-63-1; **19**, 58944-64-2; L-alanine p-nitrobenzyl ester HBr, 10144-66-8; L-alanine methyl ester HCl, 2491-20-5; L-phenylalanine methyl ester HCl, 7524-50-7; L-threonine p-nitrobenzyl ester, 58944-65-3; N-(benzyloxycarbonyl)-L-alanine, 1142-20-7; L-alanyl-L-threonine methyl ester HBr, 58944-66-4; L-alanyl-L-threonine p-nitrobenzyl ester HBr, 58944-67-5; hydrazine, 302-01-2; methyl N-(benzyloxycarbonyl)-L-alanyl-L-threonate, 19898-16-9; L-leucine p-nitrobenzyl ester, 21691-57-6; p-nitrophenyl N-(benzyloxycarbonyl)-L-leucinate, 1738-87-0; N-(benzloxycarbonyl)-L-alanyl-L-threonine azide, 58944-68-6; L-alanyl-L-threonyl-L-leucine p-nitrobenzyl ester HBr, 58944-69-7; L-alanyl-L-threonyl-L-leucyl-L-alanine p-nitrobenzyl ester HBr, 58944-70-0; L-alanyl-L-threonyl-L-leucyl-L-alanine HBr, 58944-71-1.

References and Notes

(1) Amino Sugars. 101. This is Publication No. 696 of the Robert W. Lovett Memorial Group for the Study of Diseases Causing Deformities, Harvard Medical School at the Massachusetts General Hospital, Boston, Mass. 02114. The work was supported by a research grant from the National Institute of Arthritis and Metabolic Diseases (AM-03864), National Institutes of Health, U.S. Public Health Service. A preliminary communication has been presented; see H. G. Garg and R. W. Jeanloz, Abstracts, 167th National Meeting of the American Chemical Society, Los Angeles, Calif., April 1974, No. CARB-011.

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