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SYNTHESIS OF GLYCOPEPTIDES CONTAINING THE AMINO ACID SEQUENCE 17–23 OF BOVINE PANCREATIC DEOXYRIBONUCLEASE*

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ABSTRACT

2-Acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl-L-seryl)-L-aspart-1-oyl-(*p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine, 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-(benzyloxycarbonyl-L-seryl)-L-aspart-1-oyl-(L-alanine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine, and 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[*N*-benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonyl-L-leucyl-L-alanyl-L-serine *p*-nitrobenzyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**7**), which span the amino acid sequence 17–23 of bovine pancreatic deoxyribonuclease A and contain a 2-acetamido-2-deoxy-D-glucose residue, were synthesized. On treatment with lithium hydroxide, the blocked glycohexapeptide **7** gave 2-acetamido-1-*N*-[*N*-(benzyloxycarbonyl)-L-aspart-1-oyl-(L-alanyl-L-threonyl-L-leucyl-L-alanyl-L-serine)-4-oyl]-2-deoxy-β-D-glucopyranosylamine.

INTRODUCTION

Recent evidence suggests that glycoproteins of the 2-acetamido-1-*N*-(L-aspart-4-oyl)-2-deoxy-β-D-glucopyranosylamine type are biosynthesized by a post-translational event in which glycosylation involves transfer of a large-sized oligosaccharide residue from an isoprenoid phosphate intermediate to an asparagine residue, followed by removal of D-glucose and D-mannose residues, and rebuilding of the terminal chains[1]. In order to elucidate the possible role played by the peptide sequence in the rebuilding of the chain, synthetic glycopeptides having the structure of known glycoproteins are of interest. Because of the complexity of the chemical structure of the oligosaccharides involved, advantage was taken of the availability of oligosaccharides

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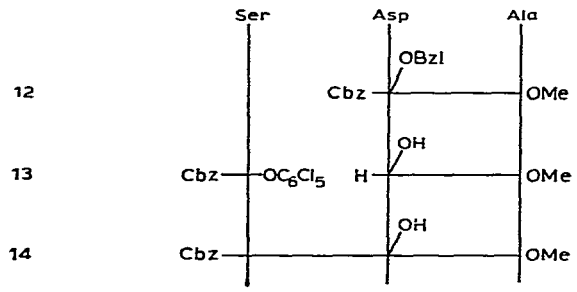
oyl]-2-deoxy-β-D-glucopyranosylamine (**6**) was attempted by coupling *N*-(benzyloxycarbonyl)-L-seryl-L-aspartyl-L-alanine methyl ester (**14**) with 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine[5,6] (**1**) but was unsuccessful.

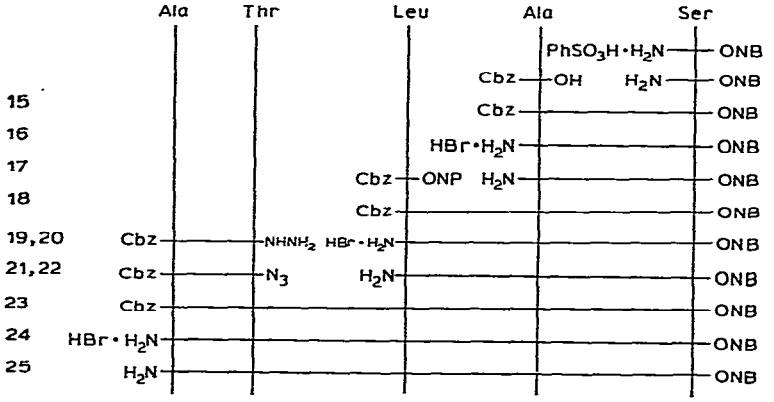
N-(Benzyloxycarbonyl)-L-seryl-L-aspartyl-L-alanine methyl ester (**14**) was synthesized by "left-hand" elongation of the dipeptide L-aspartyl-L-alanine methyl ester (**13**) with *N*-(benzyloxycarbonyl)-L-serine pentachlorophenyl ester[7], as shown



Scheme 1

in Scheme 1; the dipeptide **13** had been obtained by hydrogenolysis of the 4-benzyl ester of *N*-(benzyloxycarbonyl)-L-aspartyl-L-alanine methyl ester[17] (**12**). In an alternative route for the synthesis of **6**, *N*-(benzyloxycarbonyl)-L-serine pentachlorophenyl ester[7] was coupled with 2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[L-aspart-1-oyl-(L-alanine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**5**), which had been obtained from 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[4] (**4**) by treatment with hydrogen bromide in acetic acid, followed by triethylamine.

The protected pentapeptide *N*-[benzyloxycarbonyl)-L-alanyl-L-threonyl-L-leucyl-L-alanyl-L-serine *p*-nitrobenzyl ester (**23**) (sequence 19–23) was synthesized



ONP = $C_6H_5NO_2$-*p*

ONB = $CH_2C_6H_4NO_2$-*p*

Scheme 2

as shown in Scheme 2. For the synthesis of the "right-hand" tripeptide, *N*-(benzyloxycarbonyl)-L-leucyl-L-alanyl-L-serine *p*-nitrobenzyl ester (**18**), *N*-(benzyloxycarbonyl)-L-alanine, and L-serine *p*-nitrobenzyl ester (prepared by treating the benzenesulfonic salt of L-serine *p*-nitrobenzyl ester with triethylamine) were coupled in the presence of DCCI to give *N*-(benzyloxycarbonyl)-L-alanyl-L-serine *p*-nitrobenzyl ester (**15**), which was treated with 30% hydrogen bromide in acetic acid to give **16**. Neutralization of this compound with triethylamine gave the free base **17**, which was coupled with *N*-(benzyloxycarbonyl)-L-leucine *p*-nitrophenyl ester[18]. The resulting compound **18** was treated with 30% hydrogen bromide in acetic acid to give **20**, and then with triethylamine, to give L-leucyl-L-alanyl-L-serine *p*-nitrobenzyl ester (**22**).

Coupling of the "left-hand" component, namely, *N*-(benzyloxycarbonyl)-L-alanyl-L-threonine azide (**21**) [which had been obtained from *N*-(benzyloxycarbonyl)-L-alanyl-L-threonine hydrazide[4] (**19**) by treatment with nitrous acid], with **22** afforded **23**, the benzyloxycarbonyl group of which was removed with 30% hydrogen bromide in acetic acid to give the hydrobromide **24**. Neutralization with triethylamine gave **25**, which was coupled with **2** in the presence of Woodward's reagent K (WRK)[19].

EXPERIMENTAL

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, semimicro tubes with a Perkin–Elmer No. 141 polarimeter. I.r. spectra were recorded, for KBr disks, with a Perkin–Elmer spectrophotometer Model 237. Evaporations were performed *in vacuo*, the bath temperature being kept below 45°. The homogeneity of the compounds was verified by t.l.c. on precoated plates of Silica gel (Merck); the spots were detected by spraying the plates with 20% sulfuric acid and heating for a few min at 200°. Solvents (v/v) used were: 19:1 (*A*), 14:1 (*B*), and 4:1 (*C*) chloroform–ethanol; 14:1 (*D*), 4:1 (*E*), and 1:1 (*F*) chloroform–methanol; and (*G*) 4:1 pyridine–water. The amino acid composition of hydrolyzates of peptides and glycopeptides was determined either with a Beckman Spinco Model 117 amino acid analyzer (a.a.a.) or by g.l.c. of the *N*-trifluoroacetyl butyl esters with a Perkin–Elmer Model 900 gas chromatograph on a column of Tabsorb (Regis Chemical Co., Chicago, IL 60610) programmed for a rise of 4° per min from 75 to 225°. The peptides and glycopeptides were hydrolyzed by heating with constant-boiling hydrochloric acid (~5.8M) for 24 h at 108°, 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 (0.1 mL) of trifluoroacetic anhydride in dichloromethane for 1 h at 100°. The results are reported in molecular proportions relative to the Ser residue of the compounds. Discrepancies between the found and calculated results for Asp, Leu, and Thr residues had previously been reported[20,21]. The microanalyses were performed by Dr. W. Manser, Zurich, Switzerland.

2-Acetamido-3,4,6-tri-O-*acetyl-1*-N-[N-(*benzyloxycarbonyl*-L-*seryl*)-L-*aspart*-

1-oyl-(p-*nitrobenzyl ester*)-*4-oyl*]-*2-deoxy-β*-D-*glucopyranosylamine* (**3**). — (*a*). To a solution of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosylamine[5,6] (**1**), obtained by the hydrogenation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[22] in the presence of platinum oxide (30 mg) in ethanol (50 mL), was added *N*-(benzyloxycarbonyl)-L-seryl-L-aspartic 1-*p*-nitrobenzyl ester (**9**; 245 mg) in benzene–ethanol (10 mL), followed by[17] EEDQ (130 mg), and the mixture was stirred for 24 h at room temperature. The solvents were evaporated, water (10 mL) was added, and the solid was filtered off, washed successively with M hydrochloric acid, water, 1% sodium hydrogencarbonate solution, and water, dried, and recrystallized from *N,N*-dimethylformamide–ethanol (yield 170 mg, 41%), m.p. 244–245° (dec.), $[\alpha]_D^{22}$ +3.7° (*c* 0.9, *N,N*-dimethylformamide); t.l.c. (*A*): R_F 0.3; ν_{max}^{KBr} 3325 (NH), 1725 (OAc), 1675 (Cbz group CO), and 1650–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{36}H_{43}N_5O_{17}$: C, 52.88; H, 5.30; N, 8.56; O, 33.27; Asp, 1.00; Ser, 1.00. Found: C, 52.76; H, 5.42; N, 8.65; O, 33.34; Asp, 0.71; Ser, 1.00 (g.l.c.).

(*b*). Compound **1**, obtained by hydrogenation of 2-acetamido-3,4,6-tri-*O*-acetyl-2-deoxy-β-D-glucopyranosyl azide[22] (300 mg) by a procedure similar to that described for method (*a*), was dissolved in *N,N*-dimethylformamide (10 mL). To this solution was added **9** (245 mg), followed by DCCI (105 mg). The mixture was stirred for 1 h at 0°, and overnight at room temperature. After addition of a few drops of acetic acid and stirring for an additional 5 min, *N,N*-dicyclohexylurea was filtered off. The filtrate was evaporated to dryness, and the residue was washed successively with M hydrochloric acid, water, 1% sodium hydrogencarbonate solution, and water, and recrystallized from *N,N*-dimethylformamide–ethanol, to give 110 mg (27%), m.p. 261–262° (dec.); the i.r. spectrum and R_F value in t.l.c. were identical with those of the product prepared by method (*a*).

2-Acetamido-3,4,6-tri-O-*acetyl-1*-N-[N-*benzyloxycarbonyl*-L-*seryl*)-L-*aspart-1-oyl*-(L-*alanine methyl ester*)-*4-oyl*]-*2-deoxy-β*-D-*glucopyranosylamine* (**6**). — Compound[+] **4** (130 mg) in 19:1 (v/v) acetic acid–water (20 mL) was hydrogenated in the presence of 5% palladium-on-charcoal (20 mg). After filtration, and evaporation of the filtrate, the residue {2-acetamido-3,4,6-tri-*O*-acetyl-1-*N*-[L-aspart-1-oyl-(L-alanine methyl ester)-4-oyl]-2-deoxy-β-D-glucopyranosylamine (**5**)} was dissolved in *N,N*-dimethylformamide (5 mL), *N*-(benzyloxycarbonyl)-L-serine pentachlorophenyl ester[7] (97 mg) was added, and the mixture was stirred for 2 days at room temperature. The *N,N*-dimethylformamide was evaporated, and the residue was washed successively with M hydrochloric acid and water, dried, and recrystallized from hot acetonitrile, to give 60 mg (45%) of **6**, m.p. 261–262° (dec.), $[\alpha]_D^{21}$ +4.6° (*c* 1.3, *N,N*-dimethylformamide); t.l.c. (*A*): R_F 0.15; ν_{max}^{KBr} 3300 (NH), 1730 (OAc), 1660 (Cbz group CO), and 1560–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{33}H_{45}N_5O_{16}$: C, 51.62; H, 5.91; N, 9.12; O, 33.35; Ala, 1.00; Asp, 1.00; Ser, 1.00. Found: C, 51.53; H, 5.82; N, 9.01; O, 33.13; Ala, 0.93; Asp, 0.98; Ser, 1.00 (g.l.c.).

2-Acetamido-3,4,6-tri-O-*acetyl-1*-N-[N-(*benzyloxycarbonyl*)-L-*aspart-1-oyl*-(L-

alanyl-L-*threonyl*-L-*leucyl*-L-*alanyl*-L-*serine* p-*nitrobenzyl ester*)-*4-oyl*]-*2-deoxy*-β-D-*glucopyranosylamine* (**7**). — To a solution of WRK[19] (63 mg) in acetonitrile (10 mL) at 0° were added **2** (149 mg) and 4-methylmorpholine (25 μL), and the mixture was stirred for 65 min at room temperature (until the suspension had clarified). To a solution of **23** (182 mg) in acetic acid (1.5 mL) was added 30% hydrogen bromide in acetic acid (1.5 mL) at room temperature. After 1 h, the acids were removed *in vacuo* at room temperature, to give the hydrobromide of L-alanyl-L-threonyl-L-leucyl-L-alanyl-L-serine *p*-nitrobenzyl ester (**24**), which was washed thoroughly with ether (anhydrous), and dried in the presence of sodium hydroxide pellets. The dried pentapeptide **24** was dissolved in acetonitrile (10 mL) and treated with 4-methylmorpholine (25 μL), to give a solution of L-alanyl-L-threonyl-L-leucyl-L-alanyl-L-serine *p*-nitrobenzyl ester (**25**) which was added to the solution containing **2** and WRK. The mixture was stirred for 24 h at room temperature, the solvent was evaporated, and the residue was washed successively with water, M hydrochloric acid, water, 1% sodium hydrogencarbonate, and water, and dried. Recrystallization from ethanol gave 42 mg (14%) of **7**, m.p. 233–236° (dec.), $[\alpha]_D^{20}$ +1.6° (*c* 0.48, *N*,*N*-dimethylformamide); t.l.c.: R_F 0.6 (*B*), 0.56 (*D*); ν_{max}^{KBr} 3325 (NH), 1730 (OAc), 1655 (Cbz group CO), and 1700–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{52}H_{71}N_9O_{22}$: C, 53.25; H, 6.10; N, 10.73; O, 29.97; Ala, 2.00; Asp, 1.00; Leu, 1.00; Ser, 1.00; Thr, 1.00. Found: C, 53.02; H, 6.06; N, 10.64; O, 29.86; Ala, 2.00; Asp, 1.40; Leu, 1.02; Ser, 1.00; Thr, 0.87 (a.a.a.).

2-Acetamido-1-N-[N-(*benzyloxycarbonyl*)-L-*aspart-1-oyl*-(L-*alanyl*-L-*threonyl*-L-*leucyl*-L-*alanyl*-L-*serine*)-*4-oyl*]-*2-deoxy*-β-D-*glucopyranosylamine* (**8**). — A suspension of compound **7** (118 mg) in 0.1M lithium hydroxide (4 mL) was stirred for 2 h at room temperature, deionized by treatment with Dowex 50 (H^+) cation-exchange resin, filtered, and the filtrate evaporated. The amorphous residue was purified by dissolution in ethanol, and precipitation with acetonitrile. Attempts to crystallize it were unsuccessful (yield 72 mg, 79%), m.p. 137–140° (mobile, with dec., shrinking at 102°), $[\alpha]_D^{20}$ −16° (*c* 1.2, water); R_F 0.84 (*G*), 0.77 (*F*); ν_{max}^{KBr} 3300 (NH), 1630 (Cbz group CO), and 1700–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{39}H_{60}N_8O_{17} \cdot 6.5\ H_2O$: C, 45.47; H, 7.14; N, 10.88; Ala, 2.00; Asp, 1.00; Leu, 1.00; Ser, 1.00; Thr, 1.00. Found: C, 45.14; H, 6.25; N, 10.95; Ala, 1.93; Asp, 0.97; Leu, 1.31; Ser, 1.00; Thr, 0.60 (a.a.a.).

N-(*Benzyloxycarbonyl*)-L-*seryl*-L-*aspartic 1*-*p*-*nitrobenzyl ester* (**9**). — A solution of L-aspartic 1-*p*-nitrobenzyl ester[9] (350 mg) in *N*,*N*-dimethylformamide (1 mL) was treated with triethylamine (0.14 mL); *N*-(benzyloxycarbonyl)-L-serine pentachlorophenyl ester[7] (480 mg) in *N*,*N*-dimethylformamide (15 mL) was added, and the mixture was stirred for 48 h at room temperature. The *N*,*N*-dimethylformamide was evaporated, the residue was dissolved in ethyl acetate, and the solution was washed successively with M hydrochloric acid and water, dried (sodium sulfate), and evaporated. The syrupy residue was triturated with ether, to yield a solid (350 mg) which was recrystallized from ethanol–ether, to give needles (yield 335 mg, 68%), m.p. 132–133° (shrinking at 73°), $[\alpha]_D^{21}$ −19° (*c* 0.42, *N*,*N*-dimethylformamide);

t.l.c. (*E*): R_F 0.56; ν_{max}^{KBr} 3325 (NH), 1630 (Cbz group CO), and 1725–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{22}H_{23}N_3O_{10}$: C, 53.98; H, 4.74; N, 8.58; O, 32.69; Asp, 1.00; Ser, 1.00. Found: C, 53.68; H, 4.85; N, 8.48; O, 32.56; Asp, 0.86; Ser, 1.00 (g.l.c.).

N-(*Benzyloxycarbonyl*)-L-*seryl*-L-*aspartic diethyl ester* (**10**). — Sodium nitrite (800 mg) was added under vigorous stirring to a solution of *N*-(benzyloxycarbonyl)-L-serine hydrazide[15] (2.5 g) in acetic acid (5 mL), conc. hydrochloric acid (3 mL), and water (20 mL) at 0°. The azide, which separated as an oil, was extracted with cold ethyl acetate, and the extract was washed thoroughly with a cold solution of sodium hydrogencarbonate, and dried (sodium sulfate). The azide solution was filtered into a solution of diethyl aspartate prepared from the hydrochloride of diethyl L-aspartate[16] (2.3 g) in *N*,*N*-dimethylformamide (10 mL) and triethylamine (1.4 mL). The mixture was kept for 24 h at 6°, and 4 h at room temperature, and then the solvents were evaporated. The solid residue was washed successively with M hydrochloric acid, water, and 1% sodium hydrogencarbonate solution, and recrystallized from isopropyl alcohol–hexane as needles (yield 2.2 g, 53%), m.p. 56–57° (dec.), $[\alpha]_D^{21}$ −15° (*c* 0.47, *N*,*N*-dimethylformamide); t.l.c. (*A*): R_F 0.5; ν_{max}^{KBr} 3280 (NH), 1650 (Cbz group CO), and 1725–1600 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{19}H_{26}N_2O_8$: C, 55.60; H, 6.39; N, 6.83; O, 31.19; Asp, 1.00; Ser, 1.00. Found: C, 55.54; H, 6.37; N, 6.80; O, 31.76; Asp, 0.80; Ser, 1.00 (g.l.c.).

N-(*Benzyloxycarbonyl*)-L-*seryl*-L-*aspartic acid* (**11**). — Compound **10** (1.0 g) was added to a solution of sodium hydroxide (200 mg) in water (10 mL) and methanol (10 mL), and the mixture was stirred for 45 min at room temperature to give a clear solution which was acidified with 2M hydrochloric acid (2.5 mL); methanol was removed by evaporation, and the oil obtained was extracted into ethyl acetate. The extract was washed with water, dried (sodium sulfate), and evaporated. The residual oil crystallized from ethyl acetate–benzene (yield 250 mg, 29%), m.p. 146–147° (softened at 142°), $[\alpha]_D^{18}$ +3.1° (*c* 1.38, *N*,*N*-dimethylformamide); t.l.c. (*F*): R_F 0.11; ν_{max}^{KBr} 3350 (NH), 1635 (Cbz group CO), and 1725–1510 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{15}H_{18}N_2O_8$: C, 50.86; H, 5.12; N, 7.91; O, 36.12; Asp, 1.00; Ser, 1.00. Found: C, 50.97; H, 5.24; N, 7.87; O, 36.04; Asp, 1.07; Ser, 1.00 (g.l.c.).

N-(*Benzyloxycarbonyl*)-L-*seryl*-L-*aspartyl*-L-*alanine methyl ester* (**14**). — Compound[17] **12** (225 mg) in 3:1 (v/v) 1,4-dioxane–water (50 mL) was hydrogenated at a pressure of 1.35 bar in a Parr hydrogenator in the presence of 5% palladium-on-charcoal for 4 h at room temperature. The suspension was filtered, and the filtrate was evaporated. The resulting L-aspartyl-L-alanine methyl ester (**13**) was dissolved in *N*,*N*-dimethylformamide (5 mL), treated with *N*-(benzyloxycarbonyl)-L-serine pentachlorophenyl ester (240 mg), and the mixture stirred for 8 h at room temperature. *N*,*N*-Dimethylformamide was removed by evaporation, the residue was dissolved in ethyl acetate, and the solution was washed successively with M hydrochloric acid and water, dried (sodium sulfate), and evaporated, to yield **14** as an oil that crystallized from cold methanol–ether (yield 38 mg, 18%), m.p. 99–100° (softens at 84°), $[\alpha]_D^{24}$

−14° (*c* 0.71, *N,N*-dimethylformamide); t.l.c. (*C*): R_F 0.3; ν_{max}^{KBr} 3275 (NH), 1625 (Cbz group CO), and 1725–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{19}H_{25}N_3O_9$: C, 51.92; H, 5.73; N, 9.56; O, 32.78. Found: C, 51.90; H, 5.79; N, 9.38; O, 32.64.

N-(*Benzyloxycarbonyl*)-L-*alanyl*-L-*serine*-p-*nitrobenzyl ester* (**15**). — To a solution of *N*-(benzyloxycarbonyl)-L-alanine (1.17 g) in *N,N*-dimethylformamide (25 mL) were added a solution of L-serine *p*-nitrobenzyl ester benzenesulfonate[9] (1.99 g) in *N,N*-dimethylformamide (5 mL) containing triethylamine (0.7 mL) and then DCCI (1.05 g). The mixture was stirred for 2 h at 0°, kept overnight at room temperature, and, after adding a few drops of acetic acid and stirring for a few min, *N,N*-dicyclohexylurea was filtered off. The filtrate was diluted with water, and extracted with ethyl acetate. The extract was successively washed with M hydrochloric acid, water, 1% sodium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated. The residue crystallized from ethanol as needles (yield 1.8 g, 81%), m.p. 164–166°, $[\alpha]_D^{22}$ −4.1° (*c* 0.89, *N,N*-dimethylformamide); t.l.c. (*A*): R_F 0.5; ν_{max}^{KBr} 3450–3300 (NH, OH), 1660 (Cbz group CO), and 1740–1550 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{21}H_{23}N_3O_8$: C, 56.61; H, 5.20; N, 9.43; O, 28.73; Ala, 1.00; Ser, 1.00. Found: C, 56.61; H, 5.23; N, 9.41; O, 28.92; Ala, 1.03; Ser, 1.00 (g.l.c.).

N-(*Benzyloxycarbonyl*)-L-*leucyl*-L-*alanyl*-L-*serine* p-*nitrobenzyl ester* (**18**). — Compound **16** was obtained by treating **15** (1.0 g) in acetic acid (5 mL) with 30% hydrogen bromide in acetic acid (5 mL) for 1 h at room temperature, and subsequently precipitating with anhydrous ether. To a solution of **16** in *N,N*-dimethylformamide (5 mL) containing triethylamine (0.32 mL) was added *N*-(benzyloxycarbonyl)-L-leucine *p*-nitrophenyl ester[18] (860 mg) in chloroform (15 mL). The mixture was stirred for 24 h at room temperature, the solvents were evaporated, the residue was dissolved in ethyl acetate, and the solution was washed successively with M hydrochloric acid and water, dried (sodium sulfate), and evaporated. The residue crystallized from ethanol (yield 0.65 g, 52%), m.p. 109–110° (softened at 76°), $[\alpha]_D^{21}$ −17.0° (*c* 0.88, *N,N*-dimethylformamide); t.l.c. (*A*): R_F 0.58; ν_{max}^{KBr} 3300 (NH), 1630 (Cbz group CO), and 1730–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{27}H_{34}N_4O_9$: C, 58.06; H, 6.14; N, 10.03; Ala, 1.00; Leu, 1.00; Ser, 1.00. Found: C, 57.95; H, 5.98; N, 10.02; Ala, 0.99; Leu, 0.96; Ser, 1.00 (g.l.c.).

N-(*Benzyloxycarbonyl*)-L-*alanyl*-L-*threonyl*-L-*leucyl*-L-*alanyl*-L-*serine p-nitrobenzyl ester* (**23**). — A solution of **19** (ref. 4; 170 mg) in water (1.5 mL), acetic acid (0.15 mL), and concentrated hydrochloric acid (0.05 mL) was cooled to 0° and treated with sodium nitrite (35 mg). The syrupy azide (**21**) was extracted into cold ethyl acetate (20 mL), and the extract was washed with a cold, saturated solution of sodium hydrogencarbonate, and dried (sodium sulfate), A solution of **22** was prepared from *N*-(benzyloxycarbonyl)-L-leucyl-L-alanyl-L-serine *p*-nitrobenzyl ester (**18**; 280 mg) in acetic acid (1.5 mL) by treatment with 30% hydrogen bromide in acetic acid (1.5 mL) for 1 h at room temperature, followed by removal of the acids at room temperature *in vacuo*, and addition of triethylamine (70 μL) in *N,N*-dimethyl-

formamide (1 mL). This solution was added to the solution of the azide (**21**) in ethyl acetate, and the mixture was kept for 24 h at 6°, and then for 24 h at room temperature. The solvents were evaporated, and the residue was dissolved in chloroform. The solution was successively washed with M hydrochloric acid, water, 1% sodium hydrogencarbonate, and water, dried (sodium sulfate), and evaporated, to give an amorphous material (250 mg) which crystallized from acetonitrile (yield 175 mg, 47%), m.p. 192–193° (dec.) (softened at 186°), $[\alpha]_D^{19}$ −15.5° (*c* 1.23, *N*,*N*-dimethylformamide); t.l.c. (*D*): R_F 0.6, ν_{max}^{KBr} 3290 (NH), 1625 (Cbz group CO), and 1740–1525 cm^{-1} (peptide Amide I).

Anal. Calc. for $C_{34}H_{46}N_6O_{12}$: C, 55.88; H, 6.35; N, 11.50; O, 26.27; Ala, 2.00; Leu, 1.00; Ser, 1.00; Thr, 1.00. Found: C, 55.74; H, 6.22; N, 11.50; O, 26.72; Ala, 2.08; Leu, 0.95; Ser, 1.00; Thr, 0.98 (a.a.a.).

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