Amino-acids and Peptides, Part 44.[1] Synthesis of Protected Peptides related to Sequence 23—30 of Bovine Insulin B-Chain

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Protected peptides related to the *C*-terminal octapeptide sequence (1) of the B-chain of bovine insulin, including those in which phenylalanine-24 and -25 have been replaced by alanine and by *N*-methylphenylalanine, have been synthesised by the picolyl ester method for use in the semi-synthesis of modified insulins. A further example of the usefulness of the piperidino-oxycarbonyl amino-protection group, cleaved selectively by sodium dithionite, is provided.

We describe here the synthesis of fragments having sequences related to the *C*-terminal portion of the bovine insulin B-chain (1),† prepared as part of a collaborative

23 24 25 26 27 28 29 30
Gly–Phe–Phe–Tyr–Thr–Pro–Lys–Ala
(1)

programme in which they would be used to replace the natural sequence by coupling with the suitably protected des-(**B 23—30**)-insulin, obtained by trypsin degradation of natural insulin.[2] In particular, besides a suitably protected derivative of the natural sequence (1) itself [compound (8) in the Table], phenylalanines-24 and -25 have been replaced by alanine separately and together [compounds (11), (13), and (15)], and also by *N*-methylphenylalanine [compounds (18), (20), and (22)], which, when incorporated in the insulin molecule, would prevent the important hydrogen-bonding between monomers because the B-chains in the associating monomers are in the form of anti-parallel β-pleated sheet at this point, with hydrogen-bonding between Phe-24 of one molecule and Tyr-26 of the other.[3] We have also prepared protected fragments with truncated sequences, ending at Pro-28 [compound (27)] and Tyr-26 [compound (30)].

The peptides were synthesised by the picolyl ester method,[4] using benzyl protection for the hydroxy-groups of tyrosine and threonine, and piperidino-oxycarbonyl[5] for the ε-amino-group of lysine. The scheme involved the subsequent selective removal of the lysine side-chain protection and its replacement by t-butoxycarbonyl; this was to be followed by hydrogenolysis to remove the benzyl and picolyl groups as shown in the Scheme for the natural sequence, with the possibility of esterification of the terminal carboxy-group by means of a suitable diazoalkane, if required.[6] In this way the fragment would be converted into a form suitable for use in semi-synthesis, having protection readily

† Abbreviations follow the I.U.P.A.C.-I.U.B. rules, reprinted in the Chemical Society Specialist Periodical Report 'Amino-acids, Peptides and Proteins' ed. G. T. Young, The Chemical Society, London, 1972, vol 4, p. 441. Pic = 4-picolyl; Pipoc = piperidino-oxycarbonyl; MePhe = *N*-methylphenylalanine; NSu = succinimido. Chiral amino-acids are of the L-configuration.

Synthesis of protected peptides related to insulin B 23—30

(2) Boc–Lys(Pipoc)–Ala–OPic
(3) Boc–Pro–Lys(Pipoc)–Ala–OPic
(4) Boc–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(5) Boc–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(6) Boc–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(7) Boc–Phe–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(8) Z–Gly–Phe–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(9) Boc–Ala–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(10) Boc–Phe–Ala–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(11) Z–Gly–Phe–Ala–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(12) Boc–Ala–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(13) Z–Gly–Ala–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(14) Boc–Ala–Ala–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(15) Z–Gly–Ala–Ala–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(16) Boc–MePhe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(17) Boc–Phe–MePhe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(18) Z–Gly–Phe–MePhe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(19) Boc–MePhe–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(20) Z–Gly–MePhe–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(21) Boc–MePhe–MePhe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(22) Z–Gly–MePhe–MePhe–Tyr(Bzl)–Thr(Bzl)–Pro–Lys(Pipoc)–Ala–OPic
(23) Boc–Thr(Bzl)–Pro–OPic
(24) Boc–Tyr(Bzl)–Thr(Bzl)–Pro–OPic
(25) Boc–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–OPic
(26) Boc–Phe–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–OPic
(27) Bpoc–Gly–Phe–Phe–Tyr(Bzl)–Thr(Bzl)–Pro–OPic
(28) Boc–Phe–Tyr(Bzl)–OPic
(29) Boc–Phe–Phe–Tyr(Bzl)–OPic
(30) Bpoc–Gly–Phe–Phe–Tyr(Bzl)–OPic

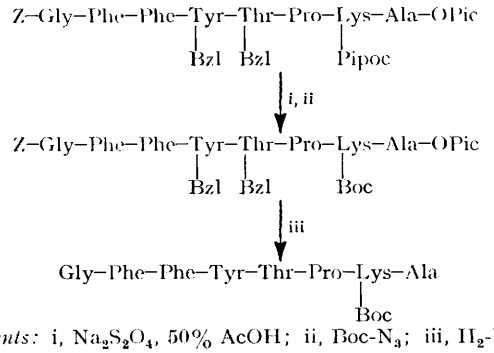
TABLE (*continued*)

Compound [a]	Amino-component [b] (mmol)	Acylating-component [c] (mmol)	Isolation [d]	Yield (%)	$[\alpha]_D^{20}$ (°) [e]	R_F (t.l.c.)
(2)	Ala–OPic, 2HBr (20.0)	Boc–Lys(Pipoc) [f] (24)	C	95	−16	0.59(E4); 0.47(A2)
(3)	Compound (2) (17.0)	Boc–Pro (24.4)	C	94.5	−65	0.43(E4); 0.55(A2)
(4)	Compound (3) (15.0)	Boc–Thr(Bzl) (18.0)	C	91	−42	0.44(E4); 0.52(A2)
(5)	Compound (4) (9.0)	Boc–Tyr(Bzl) (10.8)	C	96	−31	0.47(E4); 0.65(A2)
(6)	Compound (5) (2.0)	Boc–Phe (2.4)	A	90	−32	0.61(E4); 0.69(A2)
(7)	Compound (6) (1.0)	Boc–Phe (1.2)	A [g]	88	−39	0.66(E4); 0.67(A2)
(8)	Compound (7) (0.5)	Z–Gly (0.6)	A	90	−40	0.67(E4); 0.65(A2)
(9)	Compound (5) (2.0)	Boc–Ala (2.4)	A	87	−37	0.66(E4); 0.55(A2)
(10)	Compound (9) (0.85)	Boc–Phe (1.02)	A	83.5	−28	0.60(E4); 0.51(A2)
(11)	Compound (10) (0.65)	Z–Gly (0.78)	A	81	−29	0.46(E4); 0.65(A2)
(12)	Compound (6) (0.8)	Boc–Ala (0.96)	A	84	−46	0.65(E4); 0.53(A2)
(13)	Compound (12) (0.5)	Z–Gly (0.6)	A	84	−38.5	0.68(E4); 0.55(A2)
(14)	Compound (9) (0.85)	Boc–Ala (1.02)	A	81	−41	0.60(E4); 0.48(A2)
(15)	Compound (14) 0.57)	Z–Gly (0.68)	A	92	−29	0.48(E4); 0.46(A2)
(16)	Compound (5) (2.0)	Boc–MePhe [f] (2.4) [h]	A	85	−41	0.64(E4); 0.46(A2)
(17)	Compound (16) (0.4)	Boc–Phe (0.48)	A	86	−46	0.71(E4); 0.60(A2)
(18)	Compound (17) (0.3)	Z–Gly (0.33)	A	76	−52	0.68(E4); 0.62(A2)
(19)	Compound (6) (0.65)	Boc–MePhe [f] (0.72) [h]	A	84.5	−49	0.67(E4); 0.63(A2)
(20)	Compound (19) (0.50)	Z–Gly (0.55)	A	93	−49	0.64(E4); 0.57(A2)
(21)	Compound (16) (0.4)	Boc–MePhe [f] (0.48) [h]	A	83	−63	0.66(E4); 0.60(A2)
(22)	Compound (21) (0.17)	Z–Gly (0.19)	A	82	−52.5	0.65(E4); 0.54(A2)
(23)	Pro–OPic, 2HBr [i] (17.5)	Boc–Thr(Bzl) (21.0)	C	87	−28	0.65(E4); 0.62(A2)
(24)	Compound (23) (10.0)	Boc–Tyr(Bzl) (12.0)	A	87	−20	0.65(E4); 0.65(A2)
(25)	Compound (24) (5.0)	Boc–Phe (6.0)	A	92	−19.5	0.57(E4); 0.68(A2)
(26)	Compound (25) (4.5)	Boc–Phe (5.4)	A	88	−30	0.63(E4); 0.68(A2)
(27)	Compound (26) (1.0)	Bpoc–Gly–ONSu [j,k] (1.0)	A	81	−31	0.66(E4); 0.70(A2)
(28)	Boc–Tyr(Bzl)–OPic (10.0)	Boc–Phe (13.0) [l]	A	97.5	−13	0.64(E4); 0.73(A2)
(29)	Compound (28) (6.0)	Boc–Phe (7.8) [l]	A	83	−27	0.82(E4); 0.67(A2)
(30)	Compound (29) (1.0)	Bpoc–Gly–ONSu [j,m] (1.05)	A	46	−15	0.80(E4); 0.75(A2)

Compound	Found (%) C	H	N	Formula	Required (%) C	H	N	Amino-acid analysis Gly	Phe	Ala	Tyr	Thr	Pro	Lys	
(2)	58.1	7.8	13.0	$C_{26}H_{41}N_5O_7$	58.3	7.7	13.1			1.00				1.00	
(3)	58.05	8.0	12.9	$C_{31}H_{48}N_6O_6$	57.8	7.6	12.85			1.00			1.02	1.03	
(4)	61.4	7.7	11.65	$C_{42}H_{61}N_7O_{10}$	61.2	7.4	11.9			1.00		0.99	0.99	1.04	
(5)	64.4	7.2	10.2	$C_{58}H_{76}N_8O_{12}$	64.7	7.1	10.4			1.00	0.99	0.99	1.00	1.04	
(6)	64.7	7.0	10.2	$C_{67}H_{85}N_9O_{13},H_2O$	64.75	7.1	10.1		1.02	1.00	0.95	0.97	1.00	0.98	
(7)	65.8	7.1	10.3	$C_{76}H_{94}N_{10}O_{14},H_2O$	65.6	6.9	10.0		2.09	1.00	0.94	0.92	0.99	1.03	
(8)	64.8	6.6	10.6	$C_{81}H_{95}N_{11}O_{15}$	64.8	6.6	10.3	1.01	1.95	1.00	0.96	0.90	0.99	1.01	
(9)	63.5	7.4	10.8	$C_{61}H_{81}N_9O_{13}$	63.8	7.1	10.95			2.00	0.96	0.90	1.01	1.02	
(10)	64.2	7.1	10.7	$C_{70}H_{90}N_{10}O_{14}$	64.4	6.9	10.7		1.03	2.00	0.98	0.92	0.99	1.02	
(11)	63.8	6.45	11.2	$C_{75}H_{91}N_{11}O_{15},H_2O$	63.7	6.6	10.9	0.99	1.02	2.00	0.87	0.93	0.99	1.03	
(12)	64.3	7.1	10.7	$C_{70}H_{90}N_{10}O_{14}$	64.4	6.9	10.7		1.00	2.00	0.91	0.97	0.97	1.02	
(13)	63.9	6.6	11.2	$C_{75}H_{91}N_{11}O_{15},H_2O$	63.7	6.6	10.9	1.00	1.02	2.00	0.87	0.93	0.99	1.03	
(14)	63.5	7.1	11.6	$C_{64}H_{86}N_{10}O_{14}$	63.0	7.1	11.5			2.98	0.98	0.98	1.00	1.00	
(15)	61.55	6.7	11.6	$C_{69}H_{87}N_{11}O_{15},2H_2O$	61.5	6.8	11.4	0.98		2.89	0.98	0.98	1.00	1.00	
(16)	64.2	7.3	10.2	$C_{68}H_{87}N_9O_{13},2H_2O$	64.1	7.2	9.9			1.00	0.97	0.98	0.99	1.01	MePhe 1.00
(17)	65.3	7.2	10.1	$C_{77}H_{96}N_{10}O_{14},2H_2O$	65.05	7.1	9.85		0.99	1.00	0.98	0.99	0.99	0.99	MePhe 0.98
(18)	65.7	6.85	9.9	$C_{82}H_{97}N_{11}O_{15},2H_2O$	65.9	6.7	10.3	0.99	0.97	1.00	0.97	0.99	1.02	1.01	MePhe 1.00
(19)	65.2	6.9	9.9	$C_{77}H_{96}N_{10}O_{14},2H_2O$	65.05	7.1	9.85		0.98	1.00	1.00	0.98	0.99	1.01	MePhe 0.99
(20)	65.7	7.0	10.7	$C_{82}H_{97}N_{11}O_{15},H_2O$	65.9	6.7	10.3	1.00	0.98	1.00	0.99	0.99	0.98	0.99	MePhe 0.99
(21)	65.2	7.0	10.15	$C_{78}H_{98}N_{10}O_{14},2H_2O$	65.25	7.2	9.8			1.00	0.98	0.97	1.03	1.01	MePhe 1.99
(22)	65.2	7.2	9.6	$C_{83}H_{99}N_{11}O_{15},2H_2O$	65.3	6.8	10.1	0.98		1.00	0.96	1.00	0.98	1.01	MePhe 1.97
(23)	64.9	6.9	8.25	$C_{27}H_{35}N_3O_6$	65.2	7.1	8.45								
(24)	67.4	6.7	7.3	$C_{43}H_{50}N_4O_8,H_2O$	67.2	6.8	7.3								
(25)	69.2	6.2	7.6	$C_{52}H_{59}N_5O_9$	69.5	6.6	7.8		1.00		0.99	0.99	1.00		
(26)	69.0	6.5	7.8	$C_{61}H_{68}N_6O_{10},H_2O$	68.8	6.7	7.9		2.03		1.01	0.99	1.00		
(27)	71.4	6.25	7.75	$C_{74}H_{77}N_7O_{11}$	71.6	6.3	7.9	1.00	1.99		1.00	1.01	1.00		
(28)	70.95	6.6	6.9	$C_{36}H_{39}N_3O_6$	70.6	6.6	6.9								
(29)	68.4	6.4	7.1	$C_{45}H_{48}N_4O_7,2H_2O$	68.2	6.6	7.1								
(30)	71.55	6.3	7.2	$C_{58}H_{57}N_5O_8,H_2O$	71.7	6.1	7.2								

[a] All compounds are new. [b] Except in the preparations of compounds (2) and (23), the amino-component was prepared by the action of trifluoroacetic acid on the stated t-butoxycarbonyl derivative. [c] Unless otherwise stated, coupling was by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole in tetrahydrofuran solution. [d] C = Citric acid procedure, A = Amberlyst procedure. In the latter procedure the solvent for application to the resin was ethyl acetate except in the isolation of compounds (7), (12), (28), and (29), when dichloromethane was used, compounds (8), (10), (13), (15), and (21) when chloroform was used, and compound (20), for which a mixture of ethyl acetate and dichloromethane (2 : 1) was used. [e] Optical rotations were measured in chloroform (*c* 1), excepting compounds (17), (18), (20), (21), (22), and (26), for which *c* = 0.2—0.6. [f] Liberated from the dicyclohexylammonium salt by 0.7M-citric acid and extracted into ethyl acetate. [g] The product gelled in ethyl acetate and was applied to the Amberlyst in dichloromethane solution. [h] Coupling was through the mixed anhydride prepared from pivaloyl chloride and *N*-methylmorpholine in tetrahydrofuran at −20 °C, as described for the preparation of t-butoxycarbonyl-*N*-methylphenylalanyl-*N*-methylphenylalanine methyl ester. [i] The coupling solvent was tetrahydrofuran–dimethylformamide (4 : 1). The ester hydrobromide was added to the acylating mixture at 0 °C, followed by *NN*-di-isopropylethylamine. [j] J. E. Schwertner, H. Berndt, H-G. Gielen, and H. Zahn, *Annalen*, 1975, 581. [k] The coupling (in tetrahydrofuran solution) was complete in 2 h at 0 °C. The same product was obtained in 71% yield by coupling Bpoc–Gly by means of dicylohexylcarbodi-imide (without 1-hydroxybenzotriazole). [l] The solvent for the coupling reaction was dimethylformamide. [m] The coupling (in dimethylformamide solution) was complete in 40 min.

removable under mild acid conditions. This sequence of reactions, which worked well in the test case, provides a further example of the usefulness of the piperidino-oxycarbonyl group for amino-protection, being stable to acid but rapidly cleaved under very mild conditions



Reagents: i, $Na_2S_2O_4$, 50% AcOH; ii, Boc-N_3; iii, H_2-Pd/C

SCHEME

by sodium dithionite.[4,7] We describe here our preferred route for the preparation of N^{α}-t-butoxycarbonyl-N^{ϵ}-piperidino-oxycarbonyl-L-lysine. In the preparation of the truncated fragments the *N*-terminal residue was incorporated as the acid-labile *N*-2-(biphenyl-2-yl)isopropoxycarbonyl derivative, and it was found that protected peptide picolyl esters having this acid-sensitive group can still be isolated by use of Amberlyst-15, buffered with 3-bromopyridine. For the highly hindered coupling of t-butoxycarbonyl-*N*-methylphenylalanine the mixed pivalic anhydride [8] was used.

These syntheses provide further examples of the value of the picolyl ester ' handle ' procedure, in the synthesis of the seven protected octapeptides. Yields of *ca.* 90% of analytically pure product were obtained for the addition of each amino-acid residue, the overall yields (calculated on the *C*-terminal residue) varying between 44 and 56%.

EXPERIMENTAL

T.l.c. was on unbaked Kieselgel HF 254/366; solvent (A2) was n-butanol–acetic acid–water (10 : 1 : 3, by volume) and solvent (E4) was methanol–chloroform (1 : 9). Evaporation was by rotary evaporator at reduced pressure below 35 °C. Samples for amino-acid analysis (JEOL JLC-5AH analyser) were hydrolysed in 6 M-hydrochloric acid with added phenol for 18—24 h at 110 °C. M.p.s were determined with a Kofler hot-stage apparatus, and optical rotations on a Perkin-Elmer 141 automatic polarimeter (1-dm cell). Solutions in organic solvents were dried over magnesium sulphate or sodium sulphate. Electrophoresis was on a Michl apparatus at 60—80 V cm^{-1}, on Whatman No. 1 or 3 MC paper, using pyridine–acetic acid–water (25 : 1 : 225) buffer (pH 6.5) and 2% formic acid, 8% acetic acid in water (pH 1.9).

N^{α}-t-*Butoxycarbonyl*-N^{ϵ}-*piperidino-oxycarbonyl*-L-*lysine Dicyclohexylammonium Salt*.—To N^{α}-t-butoxycarbonyl-L-lysine [9] (23.5 g, 0.095 5 mol) suspended in dimethylformamide (100 ml) was added 1,1,3,3-tetramethylguanidine (12.5 ml, 0.095 5 mol) and then piperidino-2,4,5-trichlorophenyl carbonate [5] (45.1 g, 0.143 mol) slowly during 1.5 h. After 4 h the solution was evaporated; the residue was dissolved in chloroform (200 ml) and washed with 0.7M-citric acid (3 × 200 ml) and with brine and then evaporated. The residue was dissolved in sodium hydroxide (0.6M; 200 ml) and washed with dichloromethane (3 × 200 ml). The aqueous layer at 0 °C was brought to pH 3.5 by the addition of solid citric acid and the liberated oil was extracted into ethyl acetate (3 × 200 ml). The combined extracts were washed with water and then brine and dried ($MgSO_4$) and evaporated. T.l.c. of the residual foam showed a single ninhydrin-positive spot together with a trace of trichlorophenol. The foam was therefore dissolved in ether and a solution of dicyclohexylamine (19 ml, 0.095 5 mol) in ether was added. Cooling to −20 °C gave a white solid which was recrystallised from propan-2-ol, giving *salt* (35.9 g, 68%) of m.p. 135—136 °C, $[\alpha]_D^{20}$ +8° (*c* 1.07 in methanol) (Found: C, 63.0; H, 9.9; N, 9.75. $C_{29}H_{54}N_4O_6$ requires C, 62.8; H, 9.75; N, 9.9).

L-*Alanine* 4-*Picolyl Ester Dihydrobromide*.—*N*-Benzyloxycarbonyl-L-alanine 4-picolyl ester [4] (9.0 g, 2.86 mmol) was dissolved in acetic acid (15 ml) and a solution of hydrogen bromide in acetic acid (45% w/v; 45 ml) was added slowly. After 1 h ether was added and the precipitated product was recrystallised from aqueous acetone, giving the *salt* (9.07 g, 96.5%), m.p. 165—167 °C; $[\alpha]_D^{20}$ +2° (*c* 1.0 in $Me_2N{\cdot}CHO$) (Found: C, 31.5; H, 3.9; Br, 46.5; N, 8.0. $C_9H_{14}Br_2N_2O_2$ requires C, 31.6; H, 4.1; Br, 46.8; N, 8.2%).

N-*Methyl*-L-*phenylalanine Methyl Ester Hydrochloride*.—*N*-Methyl-L-phenylalanine [10] (1.54 g, 0.86 mmol) was added to a solution of thionyl chloride (0.70 ml, 0.946 mmol) and methanol (10 ml) at −8 °C.[11] The solution was refluxed for 5 h and then evaporated; recrystallisation of the residue from methanol gave the *amino-ester hydrochloride* (1.51 g, 76%), m.p. 85–87 °C, $[\alpha]_D^{20}$ +59° (*c* 1.0 in $CHCl_3$) (Found: C, 57.2; H, 7.1; N, 6.25. $C_{11}H_{16}ClNO_2$ requires C, 57.5; H, 7.0; N, 6.1).

N-t-*Butoxycarbonyl*-N-*methyl*-L-*phenylalanyl*-N-*methyl*-L-*phenylalanine Methyl Ester*.—This compound was prepared in order to determine whether the mixed pivalic anhydride method, under the conditions to be used in the synthesis of the fragments, resulted in racemisation. To a solution of *N*-butoxycarbonyl-*N*-methyl-L-phenylalanine (180 mg, 1.05 mmol, liberated from its dicyclohexylammonium salt [12] by means of 0.7M-citric acid and ethyl acetate) in tetrahydrofuran (5 ml) at −20 °C was slowly added *N*-methylmorpholine (0.118 ml, 1.05 mmol) and then pivaloyl chloride (0.105 ml, 1.05 mmol). After 10 min a mixture of *N*-methylmorpholine (0.111 ml) and *N*-methyl-L-phenylalanine methyl ester hydrochloride (231 mg, 1.0 mmol) in tetrahydrofuran (5 ml) at −10 °C was added. The temperature was allowed to rise and next day the solution was evaporated; the residue was dissolved in ethyl acetate and the solution was washed with aqueous citric acid, aqueous sodium hydrogencarbonate, water, and brine, and dried. Evaporation gave the *protected dipeptide* (400 mg, 87%) as an oil, R_F 0.78 (E4), $[\alpha]_D^{20}$ −30° (*c* 1.0 in $CHCl_3$) (Found: C, 66.5; H, 7.1; N, 5.7. $C_{26}H_{34}N_2O_5,H_2O$ requires C, 66.1; H, 7.2; N, 5.9%). A portion of the product was hydrolysed in 6M-hydrochloric acid at 110 °C for 24 h. Evaporation gave a white solid of $[\alpha]_D^{20}$ +47.0° (*c* 0.5 in 1M-NaOH); a sample of *N*-t-butoxycarbonyl-*N*-methyl-L-phenylalanine hydrolysed similarly gave product of $[\alpha]_D^{20}$ +48.1° (*c* 0.7 in 1M-NaOH).

N-t-*Butoxycarbonyl*-O-*benzyl*-L-*tyrosine* 4-*Picolyl Ester*.—Dicyclohexylcarbodi-imide (5.56 g, 2.7 mmol) was added during 2 h to *N*-t-butoxycarbonyl-*O*-benzyl-L-

tyrosine (10.0 g, 2.7 mmol) and 4-picolyl alcohol (2.18 g, 2.0 mmol) in dichloromethane at −10 °C. The temperature was allowed to rise and next day ethyl acetate was added and the solution at 0 °C was filtered and then evaporated. The residue was dissolved in ethyl acetate and the solution was washed (sodium hydrogencarbonate, brine) and dried, and then applied to Amberlyst-15 resin (3-bromopyridinium *form*; 300 ml). After 1 h the resin was washed with ethyl acetate and the product was eluted with 0.5M pyridine in ethyl acetate. Evaporation of the eluate and trituration of the residue with light petroleum gave a solid which was recrystallised from ethyl acetate–light petroleum, giving the *ester* (6.2 g, 67%), m.p. 78—80 °C, $[\alpha]_D^{20}$ −18.5° (*c* 1.1 in $Me_2N{\cdot}CHO$); R_F 0.74 (A2), 0.93 (G3) (Found: C, 70.25; H, 6.6; N, 6.1. $C_{27}H_{30}N_2O_5$ requires C, 70.1; H, 6.5; N, 6.1%).

Synthesis of Protected Peptides: General Procedures.—These follow the general description given in Part 42,[13] with some modifications. When tetrahydrofuran was to be the solvent for the coupling reaction the amino-component was liberated from its trifluoroacetate in cold chloroform by the addition of an excess of triethylamine; the solution was evaporated and the residue was then dissolved in the coupling solvent. When dimethylformamide was to be the coupling solvent an excess of triethylamine was added to a solution of the trifluoroacetate in this solvent and the excess of triethylamine was then removed by a short period of evaporation (prolonged evaporation leads to loss of triethylamine from triethylammonium trifluoroacetate). When coupling to amino-ester or dipeptide ester, the trifluoroacetate salt of the amino-component was dissolved in the coupling solvent, the acylating agent was added, and then a slight excess of di-isopropylethylamine. Dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole were used in the ' pre-activation ' procedure [14] (1 h at 0 °C and 1 h at room temperature). In the citric acid isolation procedure the organic solvent was ethyl acetate–ether (1 : 1 by volume); in the Amberlyst-15 isolation procedure the resin was saturated with 3-bromopyridine as usual, and the solvent for application to the resin (*ca.* 1 h) and for washing was normally ethyl acetate; the product was eluted by cooled pyridine in dimethylformamide. Details of each synthesis are given in the Table, in which any further modifications in the general procedure are noted.

N^{α}-*Benzyloxycarbonylglycyl*-L-*phenylalanyl*-L-*phenylalanyl*-O-*benzyl*-L-*tyrosyl*-O-*benzyl*-L-*threonyl*-L-*prolyl*-N^{ϵ}-*t-butoxycarbonyl*-L-*lysyl*-L-*alanine* 4-*Picolyl Ester*.—To a solution of protected octapeptide (8) (200 mg, 0.14 mmol) in 80% acetic acid (2 ml) was added sodium dithionite (50 mg, 0.26 mmol). T.l.c. (solvent E4) showed that after 20 min at room temperature the N^{ϵ}-piperidino-oxycarbonyl group has been completely removed. The solution was neutralised by sodium hydrogencarbonate and then evaporated; the residue was triturated with water, dried, and dissolved in dimethylformamide (10 ml). The pH was brought to 9.8 by addition of triethylamine, and t-butoxycarbonyl azide (0.4 ml, 0.28 mmol) was added. Next day the solution was evaporated and the residue was triturated with ether and taken up in chloroform. The solution was washed (water, sodium hydrogencarbonate, brine) and dried, and then taken up on Amberlyst-15 (3-bromopyridinium form; 4 ml) during 1 h. The resin was washed with chloroform and the product was eluted with cooled 40% pyridine in dimethylformamide (100 ml). Evaporation of the eluate left an oil which after trituration with ether gave *protected octapeptide* (145 mg, 72%) as a white solid, $[\alpha]_D^{20}$ −33° (*c* 1.0 in $CHCl_3$); R_F 0.66 (E4), 0.54 (A2) (Found: C, 65.6; H, 6.55; N, 9.4. $C_{81}H_{96}N_{10}O_{15}{\cdot}2H_2O$ requires C, 65.2; H, 6.7; N, 9.5%. Found after acid hydrolysis: Gly, 1.01; Phe, 1.98; Tyr, 0.98; Thr, 1.00; Pro, 1.00; Lys, 0.99; Ala, 1.00).

Glycyl-L-*phenylalanyl*-L-*phenylalanyl*-L-*tyrosyl*-L-*threonyl*-L-*prolyl*-N^{ϵ}-*t-butoxycarbonyl*-L-*lysyl*-L-*alanine Acetate*.—The protected octapeptide described above was hydrogenolysed in 50% acetic acid over palladium–charcoal in the usual way during 30 h. After trituration with water the *octapeptide* remained as a white solid (86% yield); electrophoresis showed a single spot $E_{Arg}^{1.9}$ 0.30 and $E_{Arg}^{6.5}$ 0.01 (Found: C, 58.7; H, 6.7; N, 11.7. $C_{52}H_{71}N_9O_{13}{\cdot}CH_3CO_2H{\cdot}H_2O$ requires C, 58.5; H, 7.0; N, 11.35. Found after acid hydrolysis: Gly, 1.01; Phe, 1.97; Tyr, 0.98; Thr, 0.99; Pro, 0.99; Lys, 0.99; Ala, 1.00).

We thank S.R.C. for a studentship held by J. G. Warnke and for financial assistance, Mr. N. N. Petter for technical assistance, and Dr. R. E. Offord and Dr. H. T. Storey for helpful discussions.

[0/249 *Received*, 12*th February*, 1980]

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