Amino-acids and Peptides. Part XXXVI.[1] The Synthesis of Analogues of Bradykinin by the Picolyl Ester Method

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The picolyl ester method has been used to synthesise the following nine analogues and modifications of bradykinin: [5-β-cyclohexyl-L-alanine]-, [8-β-cyclohexyl-L-alanine]-, [5,8-bis-β-cyclohexyl-L-alanine]-, [1-L-homoarginine,5-β-cyclohexyl-L-alanine]-, [1-L-homoarginine]-, [1-L-valine]-, [1-L-valine,6-L-threonine]-, and [1-L-leucine,6-L-threonine]-bradykinin, and t-butoxycarbonylbradykinin. The first five analogues have, in the tests applied, biological activity similar to that of bradkyinin; the remainder have no significant activity.

PART XXXII[2] described the synthesis of bradykinin (I) by the picolyl ester method[3a-d] and it was there re-

1 2 3 4 5 6 7 8 9
Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg †
(I)

ported that prolonged hydrogenation of the protected bradykinin saturated the two phenylalanine rings, giving [5,8-bis-β-cyclohexyl-L-alanine]-bradykinin, which showed high bradykinin-like activity in the guinea-pig ileum test. This is at variance with the earlier conclusion,[4] based on the inactivity of analogues with aliphatic side-chains in positions 5 and 8, that aromatic residues are essential for activity. We report here first the synthesis of [5-β-cyclohexyl-L-alanine]-, [8-β-cyclohexyl-L-alanine]-, and [5,8-bis-β-cyclohexyl-L-alanine]-bradykinin by the picolyl ester method;[5] their synthesis by the solid-phase method is reported in the preceding paper.[6] The [8-β-cyclohexyl-L-alanine]-analogue has been described in a patent published while this work was proceeding.[7]

In previous syntheses by this method we have generally used active esters for coupling, but with sterically hindered components (an extreme case was the proline-2 to proline-3 coupling in the synthesis of bradykinin[2]) the final stages of the acylation can be very slow. The amino-component is customarily added as its bistrifluoroacetate, from which triethylamine liberates the free amino-component; under these conditions (*i.e.* in the presence of trifluoroacetate), we have found[8] that dicyclohexylcarbodi-imide can cause extensive trifluoroacetylation of the amino-component, especially when proline is involved. We were therefore pleased to find[8] that the coupling method of König and Geiger,[9] in which dicyclohexylcarbodi-imide is used with 1-hydroxybenzotriazole, reduces the extent of this side reaction to a very small amount, and this procedure was therefore used in the later stages of the synthesis of the [5-β-cyclohexyl-L-alanine]-bradykinin and throughout the subsequent syntheses.

The synthesis of [5-β-cyclohexyl-L-alanine]-bradykinin was performed stepwise from $N(\omega)$-nitro-L-arginine 4-picolyl ester; the t-butoxycarbonyl group was used for α-amino-group protection, except at the final step when benzyloxycarbonyl was more convenient, and serine was protected by formation of the benzyl ether. The reaction conditions and yields and properties of the protected intermediates are shown in Table 1. The protected di-, tri-, and tetra-peptides were isolated by extraction into aqueous citric acid. When using the citric acid separation careful choice of the organic solvent is necessary; the citrates of highly lipophilic protected peptide picolyl esters may partition unfavourably between aqueous citric acid and solvents such as ethyl acetate and dichloromethane, and in such cases ether, with or without light petroleum, may be appropriate; in most cases in the present work, a mixture of ethyl acetate and ether was suitable. In subsequent stages of this synthesis, the product was separated on Amberlyst-15 cation-exchange columns (saturated with 3-bromopyridine to prevent removal of t-butoxycarbonyl groups);[10] this procedure is more generally applicable. In every case, without further purification the protected intermediates had satisfactory elemental analyses and t.l.c. showed only traces of contaminants in at least three different solvents (loading 0·7—1 mg). The overall yield of protected nonapeptide [from $N(\omega)$-nitroarginine 4-picolyl ester dihydrobromide] was 37%. Hydrogenation (with palladium–charcoal) was stopped after 24 h in order to avoid saturation of the phenylalanine rings, and in consequence some nitroarginine residues remained; a simple fractionation on carboxymethylcellulose gave [5-β-cyclohexyl-L-alanine]-bradykinin triacetate hexahydrate [(XXXIII) in Table 5] in

† Abbreviations follow the I.U.P.A.C.–I.U.B. rules for the Abbreviated Designation of Amino-Acid Derivatives and Polypeptides (reprinted in the Chemical Society Specialist Periodical Report, 'Amino-acids, Peptides and Proteins,' ed. G. T. Young, The Chemical Society, London, 1970, vol. 2, p. 226). Cha = β-cyclohexylalanine; Har = homoarginine; Pic = 4-picolyl; DCCI = dicyclohexylcarbodi-imide, HOBt = 1-hydroxybenzotriazole. Amino-acids are of the L-configuration.

[1] Part XXXV, A. W. Williams and G. T. Young, *J.C.S. Perkin I*, 1972, 1194.

[2] D. J. Schafer, G. T. Young, D. F. Elliott, and R. Wade, *J. Chem. Soc.* (*C*), 1971, 46.

[3] (*a*) R. Camble, R. Garner, and G. T. Young, *Nature*, 1968, **217**, 247; (*b*) R. Garner and G. T. Young, *ibid.*, 1969, **222**, 177; (*c*) R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc.* (*C*), 1969, 1911; (*d*) R. Garner and G. T. Young, *ibid.*, 1971, 50.

[4] J. M. Stewart, *Fed. Proc.*, 1968, **27**, 63.

[5] Preliminary report, G. A. Fletcher and G. T. Young, Proc. 11th European Peptide Symposium, Vienna, 1971, eds. H. Nesvadba and J. Rudinger, North Holland, Amsterdam, in the press.

[6] D. F. Elliott, P. Moritz, and R. Wade, preceding paper.

[7] F. Schenck, A. Oberdorf, and G. Schmidt-Kastner, Ger.P. 1,298,997/1969.

[8] G. A. Fletcher, M. Löw, and G. T. Young, in preparation.

[9] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.

[10] J. Burton, G. A. Fletcher, and G. T. Young, *Chem. Comm.*, 1971, 1057; J. Burton and G. T. Young, *Israel J. Chem.*, 1972, 201.

92% yield (33% overall), which gave satisfactory results in elemental and amino-acid analyses, and was homogeneous on electrophoresis at pH 1·8 and 6·0, and on t.l.c. (*ca.* 1 mg loading).

The syntheses of [8-β-cyclohexyl-L-alanine]-bradykinin and [5,8-bis-β-cyclohexyl-L-alanine]-bradykinin followed the same pattern, but isolation at every stage was performed by the Amberlyst-15 procedure. The reaction conditions and yields and properties of the intermediates are shown in Tables 2 and 3, respectively. Again, every intermediate gave satisfactory results in elemental analysis without further purification, and t.l.c. revealed only traces of contaminants; the overall yields of protected nonapeptide were 42 and 33%, respectively. Hydrogenation followed by fractionation on carboxymethylcellulose gave the triacetate pentahydrate [(XXXIV) in Table 5] (89% yield) and triacetate hexahydrate [(XXXV) in Table 5] (80% yield), respectively, and the same evidence of homogeneity was obtained as for the [5-β-cyclohexyl-L-alanine]-analogue. All three analogues containing β-cyclohexylalanine in place of phenylalanine had biological activity similar to that of bradykinin itself (isolated guinea pig ileum assay); it is clear that aromatic residues are not necessary for activity. This result illustrates the caution required in drawing conclusions from the effect of replacements in such a structure.

We have extended our investigation of the structural factors influencing biological activity by lengthening the side-chain in position 1 by one methylene group. The condensation of $N(\alpha)$-benzyloxycarbonyl-$N(\omega)$-L-homoarginine with the octapeptide 4-picolyl ester derived from the t-butoxycarbonyl derivative (VIII) (Table 1) gave the protected nonapeptide (XXIII) (Table 4); on hydrogenation this gave [1-L-homoarginine,5-β-cyclohexyl-L-alanine]-bradykinin [(XXXVI) in Table 5, where the properties and analysis are given]. The product had biological activity (isolated guinea pig ileum) similar to that of the arginine-1 analogue. We have also synthesised (following the preliminary work with D. J. Schafer [11]), by the same procedures, [1-L-homoarginine]-bradykinin itself. In this synthesis, only the protected hexapeptide and octapeptide 4-picolyl esters [(XXIV) and (XXV) in Table 4] were analysed, control at each of the other steps being effected by t.l.c. only. Hydrogenation and the usual purification gave [1-L-homoarginine]-bradykinin as the analytically pure triacetate octahydrate [(XXXVII) in Table 5]; again, the biological activity (guinea pig ileum and rat uterus) was similar to that of bradykinin. The hydrochloride of this compound has been reported,[12] but this salt was too hygroscopic for satisfactory analysis and the biological activity was not reported.

It has been shown that the free α-amino-group of arginine-1 is not essential for activity: 1-deamino-bradykinin has 22% of the activity of bradykinin [13] and N-acetyl-bradykinin has 50% of the activity (both in the rat uterus test).[4] By coupling $N(\alpha)$-t-butoxycarbonyl-$N(\omega)$-nitro-L-arginine to the octapeptide 4-picolyl ester derived by removal of the t-butoxycarbonyl group from compound (XXV) in Table 4, we prepared the protected nonapeptide (XXVII) (Table 4); hydrogenation and purification as usual gave t-butoxycarbonylbradykinin [(XXXVIII) in Table 5], which showed no significant biological activity (guinea pig ileum and rat uterus tests). It appears that although the free amino-group is not required, there is a limitation of space in this region.

[1-L-Valine,6-L-threonine]-bradykinin was isolated from the amphibian *Rana nigromaculata* Hallowell by Nakajima,[14] who reported an activity of about 1/5th of that of bradykinin in the isolated rat uterus assay. The replacement of serine-6 by threonine requires only one base change in the genetic code, [6-L-threonine]-bradykinin is known to be active,[15] and there is evidence that it is the natural plasma kinin of the turtle.[16] But the replacement of arginine-1 by valine would require two base changes, each intermediate being active, and as far as we are aware this would be the first example of substantial activity of an analogue without arginine in position 1. We have therefore synthesised [1-L-valine,6-L-threonine]-bradykinin and two possible evolutionary precursors, [1-L-valine]- and [1-L-leucine,6-L-threonine]-bradykinin. The same protected octapeptide 4-picolyl ester (XXV) (Table 4) as was used in the synthesis of [1-L-homoarginine]-bradykinin served for the synthesis of protected [1-L-valine]-bradykinin [(XXVIII) in Table 4] and the free nonapeptide diacetate [(XXXIX) in Table 5] was isolated as usual: it had no detectable activity at 400 times the amount in the rat uterus assay or at 10 times the amount in the guinea-pig ileum assay of a dose of bradykinin just sufficient to produce a response in each tissue. For the two analogues containing threonine, a further synthesis was carried through by the same methods, the protected tetrapeptide and octapeptide 4-picolyl esters being characterised [(XXIX) and (XXX) in Table 4]. The final coupling step gave the protected nonapeptide 4-picolyl esters [(XXXI) and (XXXII) in Table 4], and hydrogenation gave pure [1-L-valine,6-L-threonine]- and [1-L-leucine,6-L-threonine]-bradykinin [(XL) and (XLI) in Table 5]. In contrast to the finding of Nakajima, our synthetic [1-L-valine,6-L-threonine]-bradykinin showed no significant activity ($<0.12\%$ in the isolated rat uterus assay and $<10\%$ in the isolated guinea pig ileum assay). [1-L-Leucine,6-L-threonine]-bradykinin was also inactive.

We report also the synthesis of a fragment, $N(\alpha)$-benzyloxycarbonyl-$N(\omega)$-nitro-L-arginyl-L-prolyl-L-prolylglycine 4-picolyl ester, designed for the synthesis of bradykinin analogues by coupling through the acid

[11] D. J. Schafer, D.Phil. Thesis, Oxford, 1970.
[12] H. Arold and D. Stibenz, *J. prakt. Chem.*, 1970, **312**, 1161.
[13] W. H. Johnson, H. D. Law, and R. O. Studer, *Experientia*, 1969, **25**, 573; *J. Chem. Soc.* (*C*), 1971, 748.
[14] T. Nakajima, *Chem. and Pharm. Bull.* (*Japan*), 1968, **16**, 769.
[15] E. Schröder and R. Hempel, *Experientia*, 1964, **20**, 529.
[16] R. S. Dunn and A. M. Perks, *Experientia*, 1970, **26**, 1220.

hydrazide and azide, but preliminary experiments showed that substantial degradation of the nitroarginine residue occurred during hydrazinolysis and saponification; similar difficulty in the hydrazinolysis and saponification of the corresponding ethyl ester has been reported,[17] and the relatively simple syntheses of the protected nonapeptide caused us to abandon this route. The use of the azide method of coupling in conjunction with the picolyl ester procedure has been illustrated by the coupling of benzyloxycarbonyl-L-phenylalanyl-L-leucyl azide with glycine 4-picolyl ester, giving pure protected tripeptide 4-picolyl ester in 79% yield.

EXPERIMENTAL

Thin-layer chromatograms were run on unbaked Kieselgel $HF_{254/366}$ plates: R_F values refer to the following systems (proportions by volume): n-butanol–acetic acid–water mixtures (A1) 4 : 1 : 5 (upper layer), (A2) 10 : 1 : 3, and (A3) 4 : 1 : 1; (B1) n-butanol–pyridine–water (2 : 1 : 2; upper layer); (B2) as (B1) but in proportions 2 : 2 : 1; methanol–chloroform mixtures (E1) 1 : 1, (E2) 1 : 2, (E3) 1 : 4, (E4) 1 : 10, (E5) 1 : 15, (E6) 1 : 19; (F) propan-2-ol–pyridine–acetic acid–water (10 : 5 : 4 : 4); ethyl acetate–pyridine–acetic acid–water mixtures (G1) 60 : 20 : 6 : 11, (G2) 40 : 20 : 6 : 11, (G3) 120 : 20 : 6 : 11, (G4) 10 : 20 : 6 : 11; (H) n-butanol–pyridine–acetic acid–water (15 : 10 : 3 : 12); (L) phenol–water (4 : 1); (M) methanol–acetic acid–water (4 : 2 : 1); (N) propan-2-ol–60% aqueous ammonium hydroxide (100 : 44). Spots were detected by ninhydrin, by chlorine and starch–iodide, by u.v. illumination, and (when appropriate) by the Sakaguchi reagent. When testing products for purity, the loading of the plate was 0·7—1 mg. A Locarte high-voltage apparatus was used for electrophoresis, with Whatman 3MM paper; the loading was usually 0·3 mg. M.p.s were determined with a Kofler hot-stage apparatus. Evaporations were carried out with a rotary evaporator below 35° and solutions in organic solvents were dried over magnesium sulphate. Samples for amino-acid analysis were hydrolysed in 6N-hydrochloric acid for 16—24 h at 115° under vacuum, and analyses were performed on a Beckman 120 C or a Jeol JLC-5AH automatic analyser. The amino-acid contents of solutions were determined by the addition of known amounts of norleucine or (if the mixture contained leucine) alanine to the mixture. For use in the Amberlyst-15 isolation procedure, dimethylformamide was distilled from phthalic acid at 25 mmHg and used immediately.

General Synthetic Procedures.—' General Procedures ' referred to in the text are as follows.

(1) *Removal of the* N(α)-*t-butoxycarbonyl group* (' *deprotection step* '). The t-butoxycarbonyl derivative was dissolved in trifluoroacetic acid and after the stated time (*ca.* 1 h) at room temperature the solvent was evaporated off and the residue was triturated with ether and collected.

(2) *Liberation of the amino-component from the salt before coupling.* An excess (*ca.* 4 equiv.) of triethylamine was added to the solution of the salt (dihydrobromide or bistrifluoroacetate) in the coupling solvent. After 5—15 min the excess of triethylamine was removed on the rotary evaporator; when a high-boiling solvent (*e.g.* dimethylformamide) was used, evaporation was stopped when no amine was detected by a moist indicator paper held just above the liquid. When the solvent was more volatile, solvent lost during the removal of the excess of triethylamine was replaced before the start of the coupling reaction.

(3) *Coupling reactions.* These were continued until no amino-component could be detected by t.l.c. In the dicyclohexylcarbodi-imide–1-hydroxybenzotriazole method of König and Geiger,[9] the carboxy-component was allowed to react first with these reagents (for 1 h at 0° and 1 h at room temperature), according to the ' pre-activation ' procedure.

(4) *Isolation of protected peptide* 4-*picolyl esters.* (i) *By extraction into aqueous citric acid. t-Butoxycarbonyl-*O-*benzyl-*L-*seryl-*L-*prolyl-*L-*phenylalanyl-*N(ω)-*nitro-*L-*arginine* 4-*picolyl ester* [*compound* (IV), *Table* 1]. This is a typical case of a product isolated after a coupling reaction involving dicyclohexylcarbodi-imide. The reaction mixture (see Table 1 [solvent dimethylformamide (12 ml)] was diluted with ethyl acetate (15 ml) and stirred at 0° for 1 h. The dicyclohexylurea was filtered off and washed with ethyl acetate and the combined filtrates were evaporated. The residue was partitioned between 2N-citric acid (75 ml) and ethyl acetate–ether (1 : 1; 25 ml); the organic layer was extracted further with 2N-citric acid (3 × 25 ml) and the combined aqueous phases were washed with ether (2 × 50 ml) and neutralised with solid sodium hydrogen carbonate. The product was then extracted into ethyl acetate (3 × 50 ml). The extract was dried and evaporated, giving solid protected tetrapeptide ester (93%) with the constants shown in Table 1.

Compounds (II) and (III) of Table 1 were isolated by the procedure (partitioning between ethyl acetate and aqueous citric acid) described in Part XXXII [2] for the isolation of t-butoxycarbonyl-L-prolyl-L-phenylalanyl-*N*(ω)-nitro-L-arginine 4-picolyl ester.

(ii) *By use of Amberlyst-*15. (a) *For t-butoxycarbonyl-peptide* 4-*picolyl esters.* The resin was stored as the H^+ form under methanol (1·75 mequiv. per ml). A column of V ml of wet resin (15 ml for 1 mequiv. of 4-picolyl ester) was washed successively with $10V$ ml of methanol, $10V$ ml of ethyl acetate, $20V$ ml of 10% (v/v) 3-bromopyridine (redistilled and free from pyridine) in ethyl acetate, and finally $20V$ ml of ethyl acetate. In every case, the coupling reactions had been carried out with dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole in dimethylformamide, and the dicyclohexylurea and the dimethylformamide were removed as described for the citric acid procedure. The residue from the evaporation of the ethyl acetate solutions of the product was taken up in ethyl acetate (5—$10V$ ml) and the solution was washed with water (2 × $2V$ ml, to remove salts) and dried. The solution was then passed down the column and recycled nine times; the progress of absorption and the absence of product in the final filtrate were checked by t.l.c. of a sample of the eluate concentrated to 1/10th volume and loaded heavily. Non-basic co-products and by-products were then washed off the column by at least $10V$ ml of ethyl acetate. The product was eluted by at least $10V$ ml of a solution of pyridine in dimethylformamide [freed from amines by distillation from phthalic acid (25% v/v)]. The eluates containing product were evaporated to dryness and the residue was triturated with ether, or was precipitated from dichloromethane by ether, to remove traces of 3-bromopyridine. The product was dried at 30° and 1 mmHg for 24 h before analysis. In

[17] R. A. Boissonnas, St. Guttmann, and P.-A. Jacquenod, *Helv. Chim. Acta*, 1960, **43**, 1349.

some cases (noted in the Tables) additional product was recovered by combining the washes and repeating the absorption procedure. In general we prefer now to use pyridine for elution instead of the more basic triethylamine; 4-methylmorpholine and 2,4,6-collidine have also been used, but without advantage.

(b) *For* N-*benzyloxycarbonylpeptide* 4-*picolyl esters.* In these cases the Amberlyst-15 column was used as the H^+ form, the 3-bromopyridine being omitted.

Synthesis of Protected Nonapeptides of [*Cha*[5]]-, [*Cha*[8]]-, *and* [*Cha*[5,8]]-*Bradykinin:* N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-β-*cyclohexyl*-L-*alanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (*Protected* [5-β-*Cyclohexyl*-L-*alanine*]-*bradykinin*) (IX). N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-β-*cyclohexyl*-L-*alanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (*Protected* [8-β-*Cyclohexyl*-L-*alanine*]-*bradykinin*) (XVII). N(α)*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolyl*-β-*cyclohexyl*-L-*alanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-β-*cyclohexyl*-L-*alanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (*Protected* [5,8-*Bis*-β-*cyclohexyl*-L-*alanine*]-*bradykinin*) (XXII). Details of these syntheses are given in Tables 1, 2, and 3, respectively. The overall yields from *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide were 37, 42, and 33%, respectively. All the compounds in these Tables, except compounds (II)—(IV), are new.

TABLE 1

Synthesis of [Cha[5]]-bradykinin: protected peptide intermediates [a]

	Deprotection step			Coupling step							Found (%)				Required (Calc. [e]) (%)		
Compound [b]	Boc-derivative (mmol)	$CF_3 \cdot CO_2H$ (ml)	Time (min)	$Me_2N \cdot CHO$ (ml)	Acylating agents (mmol)	Time [c]	Isolation [d] procedure	Yield (%)	$[\alpha]_D^{20}$ (°)	R_F (t.l.c.)	C	H	N	Formula	C	H	N
(II) [f]	[g]			9	Boc-Phe-OTcp (7·0)	9 days	C	96	−11	A1, 0·53; E4, 0·30; G1, 0·83	55·7	6·35	17·2	$C_{26}H_{35}N_7O_7$	(56·0	6·3	17·5)
(III) [f]	2·50	3·0	60	10	Boc-Pro-OTcp (3·75)	4 days	C	97	−32	A1, 0·50; E4, 0·35; G1, 0·70	56·4	6·4	16·6	$C_{31}H_{42}N_8O_8$	(56·8	6·5	17·1)
(IV) [f]	3·94	6·0	30	12	Boc-Ser(Bzl) (4·74) DCCI (4·74) HOBt (4·74)	6 h	C	93	−36 (*c* 0·7)	A1, 0·63; E3, 0·76; E4, 0·50; G3, 0·64	58·3	6·3	14·5	$C_{41}H_{53}N_9O_{10},H_2O$	(58·0	6·5	14·85)
(V)	0·25	1·0	60	2·0	Boc-Cha (0·32) [h] DCCI (0·32) HOBt (0·32)	2 h	A	81	−57 (*c* 0·6)	A2, 0·50; E3, 0·77; E4, 0·53; G3, 0·78	59·6	6·85	13·6	$C_{50}H_{68}N_{10}O_{11},1{\cdot}5H_2O$	59·5	7·0	13·8
(VI)	0·19	1·1	75	1·3	Boc-Gly (0·25) DCCI (0·25) HOBt (0·25)	9 h	A	87	−44 (*c* 0·8, CH_2Cl_2)	A2, 0·54; E4, 0·50; G3, 0·70	58·45	6·6	14·0	$C_{52}H_{71}N_{11}O_{12},1{\cdot}5H_2O$	58·3	6·9	14·4
(VII)	0·15	0·9	60	1·5	Boc-Pro-OTcp (0·45)	4 days	A	80	−42 (*c* 0·9)	A2, 0·53; E4, 0·46; G3, 0·62	58·9	6·7	14·3	$C_{57}H_{78}N_{12}O_{13},1{\cdot}5H_2O$	58·7	6·95	14·4
VIII)	0·35	3·0	70	4·3	Boc-Pro (0·70) DCCI (0·70) HOBt (0·70)	90 min	A	96	−48	A2, 0·30; E4, 0·38; G1, 0·74; G3, 0·53	58·6	6·8	14·7	$C_{62}H_{85}N_{13}O_{14},2H_2O$	58·6	7·0	14·4
(IX)	0·20	1·5	60	1·5	Z-Arg(NO_2)-OPcp (0·60)	2 days	A(H^+)	82	−43 (*c* 0·4)	A2, 0·22; E4, 0·36; G3, 0·48	55·8	6·5	16·5	$C_{71}H_{94}N_{18}O_{17},3H_2O$	55·8	6·6	16·5

[a] The amino-component for each coupling step [except for compound (I), see note *g*] was obtained from the t-butoxycarbonyl derivative as shown under the heading 'deprotection step' and according to the general procedure described in the Experimental section. Yields are calculated on the amount of t-butoxycarbonyl derivative taken [except for compound (I), see note *g*] and are for product with the stated constants and analysis. Optical rotations were measured for solutions in dimethylformamide (*c* 1) unless otherwise stated. [b] The compounds are numbered as follows:

(II) Boc-Phe-Arg(NO_2)-OPic
(III) Boc-Pro-Phe-Arg(NO_2)-OPic
(IV) Boc-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic
(V) Boc-Cha-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic
(VI) Boc-Gly-Cha-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic
(VII) Boc-Pro-Gly-Cha-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic
(VIII) Boc-Pro-Pro-Gly-Cha-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic
(IX) Z-Arg(NO_2)-Pro-Pro-Gly-Cha-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic

All except compounds (II), (III), and (IV) (ref. 2) are new. [c] For reactions using dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole, the time given is that after the addition of the amino-component to the activated carboxy-component. [d] C = isolation by extraction into aqueous citric acid; A = isolation by means of Amberlyst-15 (saturated with 3-bromopyridine). A(H^+) = isolated by means of Amberlyst-15 (H^+). [e] Percentages in brackets are the calculated values for compounds previously reported. [f] Ref. 2. [g] For compound (II) the amino-component was *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide (5 mmol; ref. 3*c*) and the yield is calculated on this. [h] D. F. Elliott, P. Moritz, and R. Wade, preceding paper. [i] The washings from the Amberlyst column were recycled.

N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*homoarginyl*-L-*prolyl*-L-*prolyl*-β-*cyclohexyl*-L-*alanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (XXIII).—The protected octapeptide 4-picolyl ester (VIII) (Table 1) (0·134 mmol) was deprotected and coupled (DCCI–HOBt) in the usual way with *N*(α)-benzyloxycarbonyl-*N*(ω)-nitro-L-homoarginine [12] (0·2 mmol). The product in dichloromethane–ethyl acetate (2 : 1 v/v) was applied to an Amberlyst-15 (H^+) column, contaminants being washed off by dimethylformamide; the washes were reapplied to a second column to complete the recovery, giving *protected nonapeptide* 4-*picolyl ester* (XXIII) (85% yield; 37% overall); the constants and analysis are shown in Table 4.

Synthesis of Protected Nonapeptides of [1-L-*Homoarginine*]-, *t-Butoxycarbonyl*-, [1-L-*Valine*]-, [1-L-*Valine*,6-L-*threonine*]-, *and* [1-L-*Leucine*,6-L-*threonine*]-*bradykinin.*—*General.* Each of these syntheses started from *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide and proceeded stepwise with t-butoxycarbonylamino-acids as far as the protected octapeptide 4-picolyl ester. The amino-component was liberated as described under 'General Procedures' and coupling was performed by means of dicyclohexylcarbodi-imide with 1-hydroxybenzotriazole (1·2—1·3 equiv. of each and of the carboxy-component, except when otherwise stated in Table 4). The protected di-, tri-, and tetra-peptide 4-picolyl esters were isolated by the citric acid method already

TABLE 2

Synthesis of [Cha[8]]-bradykinin: protected peptide intermediates [a]

Compound [b]	Deprotection step: Boc-derivative (mmol)	$CF_3 \cdot CO_2H$ (ml)	Time (min)	Coupling step: $Me_2N \cdot CHO$ (ml)	Carboxy-component [c] (mmol)	Time [d]	Yield [e] (%)	$[\alpha]_D^{20}$ (°)	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
(X)	*a*			9·0	Boc-Cha (2·7)	1 h	93	−16	A1, 0·56; E3, 0·64; E4, 0·31; G1, 0·79	55·3	7·3	17·15	$C_{26}H_{41}N_7O_7$	55·3	7·3	17·4
(XI)	1·67	3·0	80	6·0	Boc-Pro (2·34)	1 h	85	−36 [f]	A2, 0·38; E3, 0·67; E4, 0·52; G1, 0·70; G3, 0·65	54·5	7·05	16·5	$C_{31}H_{48}N_8O_8,H_2O$	54·8	7·4	16·6
(XII)	5·72	10·0	90	23·0	Boc-Ser(Bzl) (7·62)	1 h	84	−39 (*c* 1·2)	A2, 0·33; E3, 0·46; E4, 0·49; G1, 0·74; G3, 0·70	57·1	7·0	14·5	$C_{41}H_{59}N_9O_{10},H_2O$	57·4	7·1	14·7
(XIII)	0·385	2·5	60	1·3	Boc-Phe (0·46)	*g*	96 [h]	−40 (*c* 0·5)	A2, 0·48; E4, 0·42; G1, 0·84; G3, 0·71	60·4	6·8	13·7	$C_{50}H_{68}N_{10}O_{11},H_2O$	60·0	7·0	13·9
(XIV)	0·70	2·0	70	3·3	Boc-Gly (1·14)	*g*	96	−36 (*c* 0·5)	A2, 0·41; E4, 0·52; G1, 0·86; G3, 0·77	59·0	7·1	14·8	$C_{52}H_{71}N_{11}O_{12},H_2O$	58·8	7·00	14·6
(XV)	0·62	4·3	90	5·0	Boc-Pro (0·93)	1 h	94	−41 (*c* 0·7)	E3, 0·75; E4, 0·47; G1, 0·80; G3, 0·71; M, 0·76	58·2	7·0	14·3	$C_{57}H_{78}N_{12}O_{13},2H_2O$	58·4	7·0	14·3
(XVI)	0·12	0·7	60	2·0	Boc-Pro (0·18)	1 h	82	−50 (*c* 0·5)	A2, 0·27; E4, 0·35; G1, 0·70; G3, 0·60	57·6	6·6	14·35	$C_{62}H_{85}N_{13}O_{14},3H_2O$	57·6	7·0	14·2
(XVII)	0·11	1·3	80	2·0	Z-Arg(NO_2) (0·165)	*g*	89	−49 (*c* 0·3)	A2, 0·20; E4, 0·26; G3, 0·25	55·5	6·3	16·0	$C_{71}H_{94}N_{18}O_{17},3 \cdot 5H_2O$	55·4	6·6	16·35

[a] Note *a* of Table 1 applies, except that the amino-component for the preparation of compound (X) was *N*(ω)-nitroarginine 4-picolyl ester dihydrobromide (1·8 mmol) and the yield is calculated on this. [b] The compounds are numbered as follows:

(X) Boc-Cha-Arg(NO_2)-OPic
(XI) Boc-Pro-Cha-Arg(NO_2)-OPic
(XII) Boc-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XIII) Boc-Phe-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XIV) Boc-Gly-Phe-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XV) Boc-Pro-Gly-Phe-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XVI) Boc-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XVII) Z-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic

All are new compounds. [c] In each coupling step equimolar amounts of carboxy-component, dicyclohexylcarbodi-imide, and 1-hydroxybenzotriazole were used, with the 'pre-activation' procedure of König and Geiger.[9] [d] In each case t.l.c. showed that the reaction was complete within 1 h of the addition of the amino-component. [e] Isolation was performed by use of Amberlyst-15 (saturated with 3-bromopyridine), except in the case of compound (XVII), for which Amberlyst-15 (H^+) was used. [f] The optical rotation was measured on material from a separate preparation. [g] The reaction was complete within 1 h but the reaction mixture was left overnight. [h] The washes were recycled through the Amberlyst column.

TABLE 3

Synthesis of [Cha[5,8]]-bradykinin: protected peptide intermediates [a]

Compound [b]	Deprotection step: Boc-derivative (mmol)	$CF_3 \cdot CO_2H$ (ml)	Time (min)	Coupling step: $Me_2N \cdot CHO$ (ml)	Carboxy-component [c]	Yield [d] (%)	$[\alpha]_D^{20}$ (°)	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
(XVIII)	0·385 [e]	2·5	60	2·6	Boc-Cha (0·50)	88	−44 (*c* 0·8)	A2, 0·52; E4, 0·42; G3, 0·74; N, 0·68	59·2	7·2	13·6	$C_{50}H_{74}N_{10}O_{11},H_2O$	59·2	7·6	13·8
(XIX)	0·495	1·8	90	3·3	Boc-Gly (0·645)	89	−47 (*c* 0·5)	A2, 0·46; E4, 0·55; G3, 0·78	57·8	7·4	14·55	$C_{52}H_{77}N_{11}O_{12},2H_2O$	57·7	7·5	14·25
(XX)	0·172	1·5	30	1·7	Boc-Pro (0·223)	89	−45 (*c* 0·6)	A2, 0·40; E3, 0·80; E4, 0·43; G3, 0·68; M, 0·75	57·75	7·15	14·7	$C_{57}H_{84}N_{12}O_{13},2H_2O$	57·9	7·4	14·3
(XXI)	0·253	2·8	60	2·0	Boc-Pro (0·496)	81	−50 (*c* 0·35)	A2, 0·29; E4, 0·36; G1, 0·85; G3, 0·65	57·7	7·2	14·5	$C_{62}H_{91}N_{13}O_{14},3H_2O$	57·6	7·5	14·2
(XXII)	0·062	1·0	60	2·0	Z-Arg(NO_2) (0·093)	87	−51 (*c* 0·4)	A2, 0·26; E4, 0·26; G1, 0·50; G3, 0·25	55·2	6·5	15·9	$C_{71}H_{100}N_{18}O_{17},4H_2O$	55·1	6·9	16·2

[a] Note *a* in Table 1 applies. [b] The compounds are numbered as follows:

(XVIII) Boc-Cha-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XIX) Boc-Gly-Cha-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XX) Boc-Pro-Gly-Cha-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XXI) Boc-Pro-Pro-Gly-Cha-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic
(XXII) Z-Arg(NO_2)-Pro-Pro-Gly-Cha-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic

All are new compounds. [c] Note *c* of Table 2 applies. In each case t.l.c. showed that the reaction was complete within 1 h but except for compound (XXI) the reaction mixture was left overnight. [d] Isolation was performed by use of Amberlyst-15 (saturated with 3-bromopyridine), except in the case of compound (XXII), for which Amberlyst-15 (H^+) was used. [e] The Boc-derivative was compound (XII) of Table 2, Boc-Ser(Bzl)-Pro-Cha-Arg(NO_2)-OPic.

described, the protected penta-, hepta-, and octa-peptide 4-picolyl esters by Amberlyst-15 (buffered with 3-bromopyridine), and the benzyloxycarbonyl-nonapeptides by Amberlyst-15 (H^+); for the penta-, hexa-, and hepta-peptides, the solvent for application to the column was ethyl acetate, and for the octa- and nona-peptides, ethyl acetate–dichloromethane (1 : 1 v/v). At each step the purity of the isolated products was checked by t.l.c. and only those intermediates listed in Table 4 were analysed.

N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*homoarginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (*Protected* [1-L-*Homoarginine*]-*bradykinin*) (XXVI).—The protected hexapeptide 4-picolyl ester [(XXIV), Table 4] was isolated; properties and analysis are reported in Table 4. The next intermediate characterised was the protected octapeptide (XXV), and this and the *protected nonapeptide* 4-*picolyl ester* (XXVI) are described in that Table. The overall yield from *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide was 16%; some loss was incurred when the protected hexapeptide (XXIV) crystallised on the column during isolation.

t-Butoxycarbonyl-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (XXVI).—The protected octapeptide 4-picolyl ester (XXV) (Table 4) was deprotected as usual and coupled with t-butoxycarbonyl-*N*(ω)-nitro-L-arginine. The *protected nonapeptide* 4-*picolyl ester* [(XXVII) in Table 4] was isolated on Amberlyst-15 (buffered with 3-bromopyridine) {overall yield from *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide, 13%; see the previous comment on the yield of protected [Har[1]]-bradykinin}.

Benzyloxycarbonyl-L-*valyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (*Protected* [1-L-*Valine*]-*bradykinin*) (XXVIII).—The protected octapeptide 4-picolyl ester (XXV) (Table 4) was deprotected as usual and coupled with benzyloxycarbonyl-L-valine. The named *protected nonapeptide* [(XXVIII) in Table 4] was isolated on Amberlyst-15 (H^+) {overall yield from *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide, 14%; see the previous comment on the yield of protected [Har[1]]-bradykinin}.

Benzyloxycarbonyl-L-*valyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*threonyl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (*Protected* [1-L-*Valine*,6-L-*threonine*]-*bradykinin*) (XXXI).—This synthesis (from nitroarginine 4-picolyl ester) followed the same pattern as that for protected [Har[1]]-bradykinin, except that *N*-butoxycarbonyl-*O*-benzyl-L-threonine replaced the serine analogue, and the *protected tetrapeptide* 4-*picolyl ester* [(XXIX) in Table 4] and the *protected octapeptide* 4-*picolyl ester* [(XXX) in Table 4] were isolated and characterised. The final coupling was to benzyloxycarbonyl-L-valine, and the *protected nonapeptide* 4-*picolyl ester* [(XXXI) in Table 4] was isolated on Amberlyst-15 (H^+) [overall yield from *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide, 23%].

TABLE 4

Synthesis of [Har[1],Cha[5]]-, [Har[1]]-, [Val[1]]-, [Val[1],Thr[6]]-, and [Leu[1],Thr[6]]-bradykinins and t-butoxycarbonylbradykinin: protected intermediates [a]

No.	Compound	Acylating agents [b]	$[\alpha]_D^{20}$ (°) [c]	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (Calc.) (%) C	H	N
(XXIII)	Z-Har(NO_2)-Pro-Pro-Gly-Cha-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·5	−39 [d,e]	A2, 0·22; E4, 0·18; G1, 0·80; G3, 0·62	54·7	6·4	15·8	$C_{72}H_{98}N_{18}O_{17},5H_2O$	54·9	6·7	15·9
(XXIV)	Boc-Gly-Phe-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic [f]	1·3	−33 [d]	A2, 0·65; E4, 0·30; E6, 0·15; G1, 0·78	57·9	6·2	14·4	$C_{52}H_{65}N_{11}O_{12},2H_2O$	(58·3	6·15	14·4)
(XXV)	Boc-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·15	−65 [d]	A2, 0·49; E4, 0·36; E6, 0·24; G1, 0·70	58·7	6·5	14·4	$C_{62}H_{79}N_{13}O_{14},2H_2O$	(58·8	6·55	14·4)
(XXVI)	Z-Har(NO_2)-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·5		A2, 0·17; E4, 0·28; G1, 0·85; G3, 0·33	58·1	6·3	17·0	$C_{72}H_{90}N_{18}O_{17},H_2O$	57·9	6·1	16·8
(XXVII)	Boc-Arg(NO_2)-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·5		A2, 0·15; E4, 0·29; G1, 0·69; G3, 0·31	55·6	6·5	17·0	$C_{68}H_{90}N_{18}O_{17},2H_2O$	55·7	6·4	17·2
(XXVIII)	Z-Val-Pro-Pro-Gly-Phe-Ser(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·5		A2, 0·23; E4, 0·38; G1, 0·81; G3, 0·48	60·9	6·5	14·0	$C_{70}H_{86}N_{14}O_{15},H_2O$	60·7	6·4	14·2
(XXIX)	Boc-Thr(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·33	−30 [g]	A2, 0·60; E4, 0·54; G3, 0·62	56·65	6·4	14·4	$C_{41}H_{53}N_9O_{10},2H_2O$	56·8	6·6	14·5
(XXX)	Boc-Pro-Pro-Gly-Phe-Thr(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·5	−31 [g]	A2, 0·50; E4, 0·34; G1, 0·72	58·55	6·4	13·8	$C_{63}H_{81}N_{13}O_{14},2H_2O$	58·8	6·6	14·2
(XXXI)	Z-Val-Pro-Pro-Gly-Phe-Thr(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·35		A2, 0·24; E4, 0·41; G1, 0·81; G3, 0·41	60·3	6·5	14·3	$C_{71}H_{88}N_{14}O_{15},2H_2O$	60·3	6·5	14·0
(XXXII)	Z-Leu-Pro-Pro-Gly-Phe-Thr(Bzl)-Pro-Phe-Arg(NO_2)-OPic	1·35		A2, 0·24; E4, 0·41; G1·;0 80 , G3, 0·41	60·3	6·5	14·2	$C_{72}H_{90}N_{14}O_{15},2H_2O$	60·6	6·6	13·9

[a] Further details of the preparations are given in the Experimental section. The analyses are of the whole isolated product. All the compounds except (XXIV) and (XXV) (ref. 2) are new. [b] This column gives the molar proportions of the carboxy-component, dicyclohexylcarbodi-imide, and 1-hydroxybenzotriazole, relative to the amino-component, used in the last coupling step in the synthesis of the stated compound. [c] *c* 1 unless otherwise stated. [d] Solvent dimethylformamide. [e] *c* 0·4. [f] This product crystallised on the buffered Amberlyst-15 column when applied to the column in ethyl acetate solution; it was washed off the column by means of dichloromethane, which was evaporated off, and the isolation then proceeded normally using a mixture of ethyl acetate and dichloromethane (1 : 1 v/v) as solvent for application to the column. Some loss occurred. [g] Solvent dichloromethane.

Benzyloxycarbonyl-L-*leucyl*-L-*prolyl*-L-*prolylglycyl*-L-*phenylalanyl*-O-*benzyl*-L-*threonyl*-L-*prolyl*-L-*phenylalanyl*-N(ω)-*nitro*-L-*arginine* 4-*Picolyl Ester* (*Protected* [1-L-*Leucine*, 6-L-*threonine*]-*bradykinin*) (XXXII).—The protected octapeptide (XXX) (Table 4) was deprotected and coupled with benzyloxycarbonyl-L-leucine and the *protected nonapeptide*-4-*picolyl ester* [(XXXII), Table 4] was isolated on Amberlyst-15 (H^+) [overall yield from *N*(ω)-nitro-L-arginine 4-picolyl ester dihydrobromide, 23%].

Hydrogenation of Protected Nonapeptides and Isolation of the Analogues of Bradykinin. L-*Arginyl*-L-*prolyl*-L-*prolylglycyl*-β-*cyclohexyl*-L-*alanyl*-L-*seryl*-L-*prolyl*-L-*phenylalanyl*-L-*arginine* ([5-β-*Cyclohexyl*-L-*alanine*]-*bradykinin*) (XXXIII).—The protected nonapeptide [compound (IX) in Table 1] (45·7 mg) in 80% aqueous acetic acid (4·0 ml) was hydrogenated over palladium–charcoal (10%; 46 mg) for 24 h. The catalyst was filtered off (Celite) and the combined filtrate and washings were evaporated to dryness. The residue was dissolved in triethylammonium acetate buffer (pH 5·0,

0·05M; 10 ml) and fractionated on a carboxymethylcellulose column ($NHEt_3^+$), with gradient elution [6] to pH 7·0, 0·60M buffer (6 ml fractions). Electrophoresis at pH 1·8 indicated that tubes 10—12 contained small amounts of peptides with nitroarginine intact; tubes 30—35 contained product, and evaporation and lyophilisation gave [5-β-cyclohexyl-L-alanine]-bradykinin [(XXXIII) in Table 5, where the yield, constants, and analysis are recorded].

from the salt before coupling gave product contaminated with considerable amounts of the 2,5-dioxopiperazine.

(ii) *t-Butoxycarbonyl-L-prolyl-L-prolylglycine 4-picolyl ester.* t-Butoxycarbonyl-L-prolylglycine 4-picolyl ester (1·0 mmol) was deprotected by the general procedure and to a solution of the resulting bistrifluoroacetate in dimethylformamide (1·0 ml) were added t-butoxycarbonyl-L-proline 2,4,5-trichlorophenyl ester (4·0 mmol) and triethylamine (0·9 ml).

TABLE 5

Analogues of bradykinin [a]

Compound	1 2 3 4 5 6 7 8 9	Yield (%)	$[\alpha]_D^{20}$ (°)	$[M]_D^{20}$	R_F (t.l.c.)	$E_{Arg}^{1.8}$	$E_{Arg}^{6.0(6.4)}$
(XXXIII)	Arg-Pro-Pro-Gly-Cha-Ser-Pro-Phe-Arg [Cha[5]]-bradykinin	92	−69	−855°	G4, 0·80; H, 0·77; A2, 0·07	0·67	0·60
(XXXIV)	Arg-Pro-Pro-Gly-Phe-Ser-Pro-Cha-Arg [Cha[8]]-bradykinin	89	−71 (*c* 0·5)	−887	A2, 0·07; B2, 0·03; H, 0·73; G4, 0·77	0·68	0·60
(XXXV)	Arg-Pro-Pro-Gly-Cha-Ser-Pro-Cha-Arg [Cha[5,8]]-bradykinin	80	−72	−902	A2, 0·07; B2, 0·03; H, 0·77; G4, 0·81	0·67	0·60
(XXXVI)	Har-Pro-Pro-Gly-Cha-Ser-Pro-Phe-Arg [Har[1],Cha[5]]-bradykinin	73	−106	−1335	A2, 0·04; G4, 0·72; H, 0·80	0·66	0·60
(XXXVII)	Har-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg [Har[1]]-bradykinin	76	−84	−1048	A2, 0·03; G4, 0·72; H, 0·43	0·70	0·60 (6·4)
(XXXVIII)	Boc-Arg-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg Boc-bradykinin	80	−97	−1183	A2, 0·10; G4, 0·90; H, 0·50	0·57	0·52 (6·4)
(XXXIX)	Val-Pro-Pro-Gly-Phe-Ser-Pro-Phe-Arg [Val[1]]-bradykinin	74	−93	−986	A2, 0·06; G4, 0·80; H, 0·47	0·55	0·43 (6·4)
(XL)	Val-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg [Val[1],Thr[6]]-bradykinin	78	−96	−1036	A2, 0·08; G4, 0·80; H, 0·50	0·63	0·47 (6·4)
(XLI)	Leu-Pro-Pro-Gly-Phe-Thr-Pro-Phe-Arg [Leu[1],Thr[6]]-bradykinin	77	−94	−1026	A2, 0·08; G4, 0·80; H, 0·49	0·62	0·46 (6·4)

									Found (%)				Required (%)		
	Pro	Arg	Orn + Lys	Gly	Phe	Ser	Cha		C	H	N	Formula	C	H	N
(XXXIII)	3·00	1·99	0·02	1·04	1·03	0·89	1·04		50·0	7·3	15·2	$C_{50}H_{79}N_{15}O_{11},3CH_3\cdot CO_2H,6H_2O$	49·8	7·2	15·5
(XXXIV)	3·00	2·02	0·01	1·03	1·04	0·86	1·03		50·0	7·3	15·8	$C_{50}H_{79}N_{15}O_{11},3CH_3\cdot CO_2H,5H_2O$	50·0	7·1	15·7
(XXXV)	3·00	2·00	0·03	1·05	—	0·89	1·99		49·0	7·8	15·4	$C_{50}H_{85}N_{15}O_{11},3CH_3\cdot CO_2H,6H_2O$	49·3	8·0	15·4
(XXXVI)	3·00	0·98	0·04	1·05	1·00	0·90	1·01	Har, 1·05	48·4	7·5	14·9	$C_{51}H_{87}N_{15}O_{11},3CH_3\cdot CO_2H,8H_2O$	48·5	7·8	15·0
(XXXVII)	3·00	0·94	0·03	1·02	1·95	0·88		Har, 1·05	49·3	7·1	14·8	$C_{51}H_{75}N_{15}O_{11},3CH_3\cdot CO_2H,8H_2O$	49·5	7·4	15·2
(XXXVIII)	3·00	0·95	0·02	1·01	1·96	0·87		Val, 0·98	50·0	7·1	13·3	$C_{49}H_{70}N_{12}O_{11},2CH_3\cdot CO_2H,8H_2O$	50·1	7·4	13·25
(XXXIX)	3·00	1·95	0·03	1·02	1·97	0·86			50·15	7·1	14·5	$C_{55}H_{81}N_{15}O_{13},2CH_3\cdot CO_2H,7H_2O$	50·15	7·4	14·8
(XL)	3·00	0·96	0·03	0·98	1·97			Val, 1·01; Thr, 0·89	52·7	7·5	13·7	$C_{50}H_{72}N_{12}O_{11},2CH_3\cdot CO_2H,5H_2O$	52·8	7·3	13·7
(XLI)	3·00	0·98	0·02	1·00	1·96			Leu, 1·01; Thr, 0·93	53·7	7·6	13·55	$C_{51}H_{74}N_{12}O_{11},2CH_3\cdot CO_2H,4H_2O$	53·8	7·4	13·8

[a] Optical rotations were measured on solutions in water [*c* 0·6—0·7 (anhydrous salt)] unless otherwise stated; $[M]_D^{20} = [\alpha]_D^{20} \times \frac{\text{mol. wt.}}{100}$. $E_{Arg}^{1.8}$ = migration on electrophoresis at pH 1·8 relative to that of arginine (loading *ca.* 0·3 mg). For t.l.c. the loading was 0·7—1·0 mg. In every case only one spot was detected by ninhydrin and Sakaguchi reagents. The yields are calculated on the protected nonapeptide taken, and include the hydrogenation and purification (carboxymethylcellulose) stages, giving product of the stated constants and analysis. Compounds (XXXIII), (XXXIV), and (XXXV) are also reported in the preceding paper. All other compounds are new.

[8-β-*Cyclohexyl*-L-*alanine*]-*bradykinin* (XXXIV), [5,8-*Bis*-β-*cyclohexyl*-L-*alanine*]-*bradykinin* (XXXV), [1-L-*Homoarginine*-5-β-*cyclohexyl*-L-*alanine*]-*bradykinin* (XXXVI), [1-L-*Homoarginine*]-*bradykinin* (XXXVII), *t-Butoxycarbonylbradykinin* (XXXVIII), [1-L-*Valine*]-*bradykinin* (XXXIX), [1-L-*Valine*,6-L-*threonine*]-*bradykinin* (XL), [1-L-*Leucine*,6-L-*threonine*]-*bradykinin* (XLI).—These were prepared from the corresponding protected nonapeptides (see Table 4) by hydrogenation as described for [5-β-cyclohexyl-L-alanine]-bradykinin; the yields, constants, and analyses are given in Table 5.

Synthesis of N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycine 4-Picolyl Ester*.—(i) *t-Butoxycarbonyl*-L-*prolylglycine 4-picolyl ester.* t-Butoxycarbonyl-L-proline 2,4,5-trichlorophenyl ester (11·8 mmol), glycine 4-picolyl ester dihydrobromide (9·1 mmol), and di-isopropylethylamine (18·8 mmol) were mixed in ethyl acetate (18 ml), and next day the *protected dipeptide ester* (8·9 mmol, 98%) was isolated by the citric acid procedure; m.p. 84—86°; $[\alpha]_D^{20}$ −57° (*c* 1 in EtOAc); R_F 0·38 (A1), 0·70 (E1), 0·61 (E3), and 0·40 (E4) (Found: C, 59·3; H, 7·05; N, 11·6. $C_{18}H_{25}N_3O_5$ requires C, 59·5; H, 6·9; N, 11·6%). Other preparations in which the amino-component was liberated

After 5 min the excess of triethylamine was removed by evaporation. After 4 weeks the product was isolated by the citric acid procedure, giving *protected tripeptide ester* (0·69 mmol, 69%) as an oil, $[\alpha]_D^{20}$ −76° (*c* 0·9 in EtOAc) (Found: C, 58·3; H, 7·3; N, 11·5. $C_{23}H_{32}N_4O_6,H_2O$ requires C, 58·0; H, 7·1; N, 11·7%); R_F 0·65 (E3), 0·51 (E4), and 0·81 (G1). Other preparations using dicyclohexylcarbodi-imide with 1-hydroxybenzotriazole for coupling gave product contaminated by t-butoxycarbonylproline 4-picolyl ester (identified by t.l.c. comparison); apparently the dipeptide ester rapidly gives the dioxopiperazine and 4-pyridylmethanol, which is then esterified with t-butoxycarbonylproline by the coupling reagents.

(iii) N(α)-*Benzyloxycarbonyl*-N(ω)-*nitro*-L-*arginyl*-L-*prolyl*-L-*prolylglycine 4-picolyl ester.* t-Butoxycarbonyl-L-prolyl-L-prolylglycine 4-picolyl ester (4·65 mmol) was deprotected by means of trifluoroacetic acid and the dipeptide ester was liberated by means of triethylamine in the usual way; the acylating agent was *N*(α)-benzyloxycarbonyl-*N*(ω)-nitro-L-arginine pentachlorophenyl ester (13·95 mmol). After overnight reaction the product was isolated by the Amberlyst-15 (H^+) procedure; it was necessary to wash the column thoroughly with dimethylformamide to remove

δ-lactam from the adsorbed product, and the washings were then recycled on the resin. The *protected tetrapeptide ester* (0·44 mmol, 60%) had $[\alpha]_D^{20}$ −61° (*c* 0·6 in $Me_2N \cdot CHO$), R_F 0·22 (A2), 0·20 (E4), 0·33 (G3), and 0·42 (M), with traces of contaminant at 0·30 (A2), 0·27 (E4), and 0·40 (G3) (Found: C, 54·2; H, 6·1; N, 17·7. $C_{32}H_{41}N_9O_9,H_2O$ requires C, 54·0; H, 6·0; N, 17·6%).

Use of the Acid Azide Method of Coupling in Conjunction with the Picolyl Ester Procedure. Synthesis of Benzyloxycarbonyl-L-phenylalanyl-L-leucylglycine 4-Picolyl Ester.—To a suspension of benzyloxycarbonyl-L-phenylalanyl-L-leucine hydrazide [18] (0·852 g, 2·0 mmol) in dimethylformamide (2 ml) was added 3·5N-hydrogen chloride in dioxan (1·4 ml; 5 mmol). The mixture was cooled to −20° and n-butyl nitrite (0·57 ml, 5 mmol) was added. After 30 min at −20°, the solution was neutralised (to pH 6—7) by dropwise addition of triethylamine, and glycine 4-picolyl ester, liberated from its dihydrobromide (1·33 mmol), in dimethylformamide (2·0 ml), was added. After 12 h at −20° and 12 h at room temperature, the mixture was worked up by the citric acid procedure, giving *protected tripeptide ester* (0·58 g, 79%), $[\alpha]_D^{20}$ −16° (*c* 1·3 in $Me_2N \cdot CHO$), R_F 0·45 (E4), 0·80 (G1), 0·76 (G3), and 0·50 (A2) (Found: C, 66·3; H, 6·7; N, 9·95. $C_{31}H_{36}N_4O_5,H_2O$ requires C, 66·15; H, 6·8; N, 10·0%); amino-acid analysis Leu 1·00, Gly 1·01, Phe 0·97.

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