Amino-acids and Peptides. Part XXXVIII.[1] Use of 4-Picolyloxycarbonylhydrazides as 'Handles' to facilitate Peptide Synthesis. Synthesis of Fragments related to Porcine Gastric Inhibitory Polypeptide

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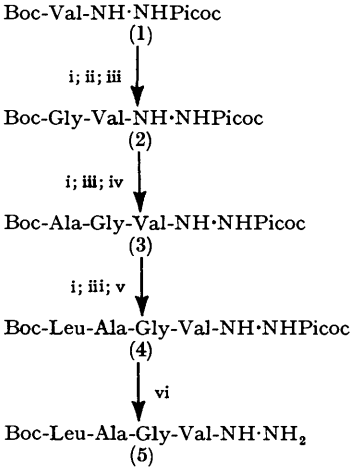
The use of the 4-picolyloxycarbonylhydrazide (4-pyridylmethoxycarbonylhydrazide) of the carboxy-terminal amino-acid provides both protection of the hydrazide group during peptide synthesis and a weakly basic 'handle' by which the product can readily be isolated after each coupling reaction. The procedure is illustrated by the synthesis of t-butoxycarbonyl-L-leucyl-L-alanylglycyl-L-valine hydrazide (5) and of the 4-picolyloxycarbonylhydrazides of a number of fragments related to the *N*-terminal sequence of porcine gastric inhibitory polypeptide. The 4-picolyloxycarbonyl group was removed by catalytic hydrogenolysis and by electrolytic reduction. The 4-picolyloxycarbonylhydrazides of eight t-butoxycarbonylamino-acids are described.

THE incorporation of the carboxy-terminal amino-acid residue as its 4-picolyl ester provides a weakly basic 'handle' by which, during synthesis, the growing peptide can readily be separated, by transfer to an acidic phase, from the non-basic reagents, co-products, and by-products, and this 'picolyl ester method' of peptide synthesis has been used successfully in the synthesis of many peptides of 8—9 residues in high overall yield.[2,3] Such a procedure has the advantages over the 'solid phase' method that reactions are carried out under mild conditions in solution; their progress can therefore be followed readily and their completion can be assured, and the product of each stage can be characterised and (if need be) further purified.

For larger peptides, the synthesis of fragments and their assembly by the acid azide method of coupling is an important route. 4-Picolyl esters of protected peptides can be converted smoothly into acid hydrazides,[2] but there are well-known advantages in the use of the carboxy-terminal amino-acid as its hydrazide, protected during synthesis by the benzyloxycarbonyl or t-butoxycarbonyl group, which is removed when the synthesis of the fragment is complete. In order to provide a 'handle' procedure in such a case, we have examined the use of 4-picolyloxycarbonylhydrazides.[4] The 4-picolyloxycarbonyl group has been used for the protection of amino-groups.[5]

4-Picolyloxycarbonylhydrazine is best obtained by the action of hydrazine hydrate on *p*-nitrophenyl 4-picolyl carbonate, the preparation of which was kindly communicated to us by Drs. R. F. Hirschmann and D. F. Veber.[6] The 4-picolyloxycarbonylhydrazine was isolated as its *p*-nitrophenol salt. t-Butoxycarbonylamino-acids were condensed with the hydrazine by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole,[7] and the 4-picolyloxycarbonylhydrazides of the t-butoxycarbonyl derivatives of L-valine (1), glycine, L-alanine, L-leucine, L-isoleucine, L-phenylalanine, β-benzyl L-aspartate, and *O*-benzyl-L-tyrosine are described here.

The use of such protected hydrazides was examined first by the synthesis of t-butoxycarbonyl-L-leucyl-L-alanyl-glycyl-L-valine hydrazide (5) (see Scheme). The



SCHEME * Reagents: i, $CF_3{\cdot}CO_2H$; ii, Boc-Gly; iii, dicyclohexylcarbodi-imide, 1-hydroxybenzotriazole; iv, Boc-Ala; v, Boc-Leu; vi, H_2–Pd or electrolytic reduction

* Abbreviations follow the I.U.P.A.C.–I.U.B. recommendations for symbols for Amino-Acid Derivatives and Peptides (1971) (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 (4-pyridylmethyl); Picoc = 4-picolyloxycarbonyl. Amino-acids are of the L-series.

product (2) of the first coupling reaction (using dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole) was isolated by extraction from ethyl acetate solution into aqueous citric acid,[8] and this simple separation afforded analytically and chromatographically pure material in 93% yield. In the use of protected hydrazides there is no risk of the formation of dioxopiperazines in the di- and tri-peptide stages of synthesis. The tripeptide derivative (3) was isolated similarly, but (as in the case of protected peptide 4-picolyl esters) with increasing size the solubility of the peptide 4-picolyloxycarbonylhydrazide in aqueous citric acid may be expected to decrease and at the tetrapeptide stage the product (4) was isolated by use of the cation-exchanger Amberlyst-15 (saturated

[1] Part XXXVII, G. A. Fletcher, M. Löw, and G. T. Young, *J.C.S. Perkin I*, 1973, 1162.
[2] R. Camble, R. Garner, and G. T. Young, *J. Chem. Soc.* (*C*), 1969, 1911.
[3] G. T. Young, in 'The Chemistry of Polypeptides,' ed. P. G. Katsoyannis, Plenum Press, New York, 1973, p. 43.
[4] Preliminary report, R. Macrae and G. T. Young, *J.C.S. Chem. Comm.*, 1974, 446.
[5] D. F. Veber, S. F. Brady, and R. Hirschmann, in 'Chemistry and Biology of Peptides,' ed. J. Meienhofer, Ann Arbor Science Publications, 1972, 315.
[6] R. F. Hirschmann and D. F. Veber, in preparation.
[7] W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 788.
[8] R. Garner and G. T. Young, *J. Chem. Soc.* (*C*), 1971, 50.

with 3-bromopyridine to avoid removal of the t-butoxycarbonyl group [9,10]); the 4-picolyloxycarbonylhydrazide (4) was absorbed by the resin from a solution in dimethylformamide and was eluted in the usual way by means of pyridine in dimethylformamide, giving analytically and chromatographically pure material in 89% yield. The 4-picolyloxycarbonyl group was removed by catalytic hydrogenolysis in 80% acetic acid and also by eletrolytic reduction at a mercury cathode,[11] giving t-butoxycarbonyl-L-leucyl-L-alanylglycyl-L-valine hydrazide (5), in yields of 88 and 77%, respectively.

The method has been investigated further by the synthesis of the protected tridecapeptide 4-picolyloxycarbonylhydrazide (6), having the sequence of residues 1—13 of

$$\overset{1}{\text{Boc-Tyr(Pic)}}\text{-}\overset{2}{\text{Ala}}\text{-}\overset{3}{\text{Glu(OPic)}}\text{-}\overset{4}{\text{Gly}}\text{-}\overset{5}{\text{Thr(Bzl)}}\text{-}\overset{6}{\text{Phe}}\text{-}\overset{7}{\text{Ile}}\text{-}\overset{8}{\text{Ser}}\text{-}$$
$$\overset{9}{\text{(Bzl)-Asp(OBzl)}}\text{-}\overset{10}{\text{Tyr(Pic)}}\text{-}\overset{11}{\text{Ser(Bzl)}}\text{-}\overset{12}{\text{Ile}}\text{-}\overset{13}{\text{Ala}}\text{-NH·NHPicoc}$$
(6)

porcine gastric inhibitory polypeptide.[12] A protected benzyloxycarbonylhydrazide of this tridecapeptide, having benzyl side-chain protection, has been described,[13] but the sparing solubility of the intermediates caused difficulty, and it was of interest to examine whether the use of the 4-picolyloxycarbonylhydrazide would be advantageous. The synthesis was stepwise, starting from L-alanine *N'*-4-picolyloxycarbonylhydrazide, and again using dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole for the coupling reactions. Table 1 gives details of the synthesis. The protected dipeptide hydrazide was isolated by extraction into aqueous citric acid, but in subsequent stages isolation was performed by means of Amberlyst-15. Use has been made earlier of 4-picolyl for the protection of the β-carboxy-group of aspartic acid, in a synthesis of [Val^5]-angiotensin-II,[8] and we report here its use analogously for glutamic acid. The use of *O*-4-picolyl-L-tyrosine has been recorded only briefly.[14] The object of the incorporation of further 4-picolyl groups, in the side-chains, was to increase the solubility of the higher protected peptides, which with the corresponding *O*-benzyl protection was still poor, and to increase the rate of absorption of the protected peptides by the Amberlyst resin; for the t-butoxycarbonyl heptapeptide 4-picolyloxycarbonylhydrazide of sequence 7—13 with only benzyl side-chain protection this rate was unacceptably low. In fact, there was no significant improvement in the solubility of the higher protected peptides in dimethylformamide in this case, but the absorption by the resin was facilitated. From the decapeptide (15) (Table 1) onwards the decreasing solubility in dimethylformamide made operations difficult. In these cases t.l.c. was unsatisfactory for the detection of small amounts of amino-component, and the completion of the coupling reaction was determined by a colorimetric assay of the amino-component in the reaction mixture, by a procedure developed for this purpose in earlier work with Dr. J. Burton; the assay was based on the 2,4,6-trinitrobenzenesulphonic acid–sulphite method for the quantitative determination of amino-groups in proteins, devised by Fields.[15]

This extension of the 'handle' procedure has been tested further in the synthesis of the 4-picolyloxycarbonylhydrazides of other fragments related to the sequence 1—13 of porcine gastric inhibitory polypeptide (see Table 2). In several of these syntheses serine, threonine, and tyrosine were used without side-chain protection, coupling in these cases being brought about by active esters. The yields, constants, and analyses of the products are shown in Table 2; the last compound in the Table has an inversion of the natural sequence. The 4-picolyloxycarbonylhydrazides (18) and (19) (Table 2) were hydrogenolysed to give the free hydrazides in good yield.

EXPERIMENTAL

The general instructions in Part XXXVI [10] apply with the following additions and modifications. Additional solvent systems for t.l.c. were: (J) acetonitrile–water (3 : 1), (K) butan-2-ol–aqueous 3% ammonium hydroxide (3 : 1), and (O) chloroform–methanol–water (55 : 40 : 10). Hydrazides were detected by the ferric chloride–potassium ferricyanide spray.[16] Samples for amino-acid analysis were hydrolysed by 6M-hydrochloric acid containing 1% phenol at 110 °C for 18—24 h. Peptides containing *O*-4-picolyltyrosine gave 0·1—0·2 mol. equiv. of tyrosine under these conditions; the unchanged ether is strongly adsorbed on the resin and requires sodium hydroxide for elution. When the phenol was omitted, *ca.* 0·5 mol. equiv. of tyrosine were obtained. The general procedures given in Part XXXVI [10] for synthesis by the picolyl ester method apply, with the following modifications. The time for the removal of the t-butoxycarbonyl group by trifluoroacetic acid was 5 min; confirmation of the completion of the removal was obtained by t.l.c. of the solution of the free amino-component. In the isolation procedure using citric acid, the organic layer containing product was washed with brine before drying over magnesium sulphate. In the isolation procedure using Amberlyst-15, the residue left after evaporation of the coupling solvent was taken up in ethyl acetate or dichloromethane, and triethylammonium trifluoroacetate and 1-hydroxybenzotriazole were removed by washing with 2M-sodium hydrogen carbonate; the solution was then washed with water and brine and dried. When the product was insoluble in water-immiscible solvents, the residue was triturated with 2M-sodium hydrogen carbonate and water, and dried at reduced pressure. Amberlyst-15 was in every case saturated overnight with 3-bromopyridine [20% (v/v) solution in ethyl acetate, in volume equal to that of the resin]. The time required for complete absorption of the coupling product on the resin varies (1—3 h in the work described here), and confirmation that absorp-

[9] J. Burton and G. T. Young, *Israel J. Chem.*, 1971, 201.
[10] G. A. Fletcher and G. T. Young, *J.C.S. Perkin I*, 1972, 1867.
[11] P. M. Scopes, K. B. Walshaw, M. Welford, and G. T. Young, *J. Chem. Soc.*, 1965, 782.
[12] J. C. Brown and J. R. Dryburgh, *Canad. J. Biochem.*, 1971, **49**, 867.
[13] R. Camble, R. Cotton, A. S. Dutta, J. J. Gormley, C. F Hayward, J. S. Morley, and M. J. Smithers, in 'Peptides 1972,' eds. H. Hanson and H.-D. Jakubke, North Holland, Amsterdam, 1973, p. 200.
[14] A. Gosden, D. Stevenson, and G. T. Young, *J.C.S. Chem. Comm.*, 1972, 1123.
[15] R. Fields, *Biochem. J.*, 1971, **124**, 581.
[16] K. Hofmann, R. Schmiechen, R. D. Wells, Y. Wolman, and N. Yanaihara, *J. Amer. Chem. Soc.*, 1965, **87**, 611.

tion is complete must be obtained in each case by t.l.c. of a concentrated sample of the supernatant liquor. It is undesirable to leave the product on the resin for longer periods. Optical rotations were determined for solutions in dimethylformamide (*c* 1) unless otherwise stated.

p-*Nitrophenyl* 4-*Picolyl Carbonate*.—The procedure is essentially that of Hirshemann and Veber.[6] A solution of 4-picolyl alcohol (4-pyridylmethanol) (19·6 g, 180 mmol; azeotropically dried with benzene) in dichloromethane (100 ml) was added over 30 min to a solution of bis-*p*-nitrophenyl carbonate [17] (45 g, 150 mmol) in dichloromethane (500 ml), and a solution of *N*-methylmorpholine (15·2 g, 150 mmol) in dichloromethane (50 ml) was then added slowly. After 3 days the solution was washed with water (2 × 300 ml), 0·1M-sulphuric acid (500 ml), saturated aqueous sodium hydrogen carbonate (4 × 500 ml), and water (500 ml), and then dried and evaporated. The residue was recrystallised from ethyl acetate at 40°, giving carbonate (15·0 g, 36·5%) of m.p. 101—104°; R_F 0·60 (E4), 0·61 (A2) (Found: C, 56·7; H, 3·7; N. 10·0. Calc for $C_{13}H_{10}N_2O_5$: C, 56·9; H, 3·7; N, 10·2%) Addition of light petroleum to the mother liquor gave more product as the *p*-nitrophenol salt (20· 7 g, 33·5%), m.p. 112—115°; R_F 0·60 and 0·45(*p*-, nitrophenol) (E4), 0·49 and 0·29 (acetone–light petroleum, 1 : 1).

4-*Picolyloxycarbonylhydrazine* p-*Nitrophenol Salt*.—p-Nitrophenyl 4-picolyl carbonate (8·94 g, 35 mmol) was suspended in methanol (100 ml) and a solution of hydrazine hydrate (1·75 g, 35 mmol) in methanol at 0° was added. T.l.c. (solvent E4) showed the reaction to be complete after 10 min. The solvent was evaporated off and the residue was triturated with toluene and with light petroleum, giving the *salt* as a yellow solid (10·0 g, 95%), m.p. 93·5—95°, R_F 0·50 (*p*-nitrophenol), 0·22 (E4); 0·80, 0·47 (J); 0·51, 0·10 (acetone–light petroleum, 1 : 1); $\nu_{max.}$ ($CHCl_3$) 1732 and 1703 cm^{-1}; τ [$CDCl_3$–$(CD_3)_2SO$] 1·47 (2H, pyridyl 2- and 6-protons), 1·96 (2H, phenyl 3- and 5-protons), 2·74 (2H, pyridyl 3- and 5-protons), 3·14 (2H, phenyl 2- and 6-protons), 4·39 (4H, N*H*·NH_2 and O*H*), and 4·85 (2H, CH_2O) (Found: C, 50·9; H, 4·8; N, 18·2 $C_{13}H_{14}N_4O_3$ requires C, 51·0; H, 4·6; N, 18·3%)

*t-Butoxycarbonyl-*L*-valine* N′-4-*Picolyloxycarbonylhydrazide* (1).—Dicyclohexylcarbodi-imide (2·48 g, 12 mmol) was added to a stirred solution of t-butoxycarbonyl-L-valine (2·60 g, 12 mmol) and 1-hydroxybenzotriazole (1·62 g, 12 mmol) in dimethylformamide (20 ml). The mixture was stirred at 0° for 30 min and then at room temperature for 1 h, 4-Picolyloxycarbonylhydrazine *p*-nitrophenol salt (3·06 g 10 mmol) and triethylamine (1·01 g, 10 mmol) were added and the solution was stirred at room temperature overnight. Ethyl acetate (20 ml) was added, the mixture was filtered, and the filtrate was evaporated The residue was dissolved in ethyl acetate–ether (1 : 1; 40 ml) and the product was extracted into 0·7M-citric acid (4 × 30 ml); the aqueous phase was washed with ether (4 × 20 ml) and then made alkaline with solid sodium hydrogen carbonate. The product was extracted into ethyl acetate (4 × 20 ml) and the solution was dried and evaporated. Trituration of the residue with light petroleum gave the *hydrazide* as an amorphous solid (3·02 g, 83%), $[\alpha]_D^{20}$ −18°; R_F 0·40 (E4), 0·83 (G3), 0·50 (A2), 0·76 (J); $\nu_{max.}$ ($CHCl_3$) 1755 and 1690 cm^{-1}; τ ($CDCl_3$) 0·89 (1H, N′H), 1·47 (2H, pyridyl 2- and 6-protons), 2·32 (1H, NH), 2·79 (2H, pyridyl 3- and 5-protons), 4·62 (1H, CH·N*H*), 4·86 (2H, CH_2O), 5·90 (1H, C*H*·NH), 7·90 (1H, C*H*Me_2), 8·60 (9H, Me_3), and 9·05 (6H, CH*Me*$_2$) (Found: C, 55·7; H, 7·2; N, 15·0 $C_{17}H_{26}N_4O_5$ requires C, 55·5; H 7·1; N, 15·3%)

The following analogues were prepared similarly: *t-butoxycarbonyl-*L*-alanine* N′-4-*picolyloxycarbonylhydrazide* (50%), m.p. 173—175° (from acetone), $[\alpha]_D^{20}$ −23°; R_F 0·23 (E4), 0·71 (G3), 0·44 (A2), 0·64 (J) (Found: C, 53·2; H, 6·5; N, 16·4 $C_{15}H_{22}N_4O_5$ requires C, 53·3; H, 6·5; N, 16·6%); *t-butoxycarbonyl-*L*-leucine* N′-4-*picolyloxycarbonylhydrazide* (92%), m.p. 136·5—137·5°, $[\alpha]_D^{20}$ −21°; R_F 0·39 (E4), 0·82 (G3), 0·41 (A2) (Found: C, 56·6; H, 7·5; N, 14·9. $C_{18}H_{28}N_4O_5$ requires C, 56·8; H, 7·4; N. 14·7%); *t-butoxycarbonyl-*L*-phenylalanine* N′-4-*picolyloxycarbonylhydrazide* (92%), m.p. 177—178°, $[\alpha]_D^{20}$ −5°, R_F 0·39 (E4), 0·82 (G3), 0·41 (A2) (Found: C, 60·9; H, 6·1; N, 13·6. $C_{21}H_{26}N_4O_5$ requires C, 60·9; H, 6·3; N, 13·6%); *t-butoxycarbonylglycine* N′-4-*picolyloxycarbonylhydrazine* (81%), m.p. 163·5—165°; R_F 0·1 (E4), 0·73 (L), 0·63 (G3) (Found: C, 51·7; H, 6·5; N, 17·1. $C_{14}H_{20}N_4O_5$ requires C, 51·9, H, 6·2; N, 17·3%); *t-butoxycarbonyl-*O*-benzyl-*L*-tyrosine* N′-4-*picolyloxycarbonylhydrazide* (94%), m.p. 188—190°, $[\alpha]_D^{20}$ 0°, $[\alpha]_{365}^{20}$ +18°; R_F 0·30 (E4), 0·79 (G3), 0·58 (A2) (Found: C, 64·5; H, 6·2; N, 10·9. $C_{28}H_{32}N_4O_6$ requires C, 64·6; H, 6·2; N, 10·8%); *t-butoxycarbonyl-*β*-benzyl-*L*-aspartate* N′-4-*picolyloxycarbonylhydrazide* (87%), m.p. 168—170°, $[\alpha]_D^{20}$ −17°; R_F 0·43 (E4), 0·73 (G3), 0·50 (A2) (Found: C, 58·5; H, 5·9; N, 11·8. $C_{23}H_{28}N_4O_7$ requires C, 58·6; H, 5·9; N, 11·9%); *t-butoxycarbonyl-*L*-isoleucine* N′-4-*picolyloxycarbonylhydrazide* (95%), amorphous, $[\alpha]_D^{20}$ −21°; R_F 0·39 (E4), 0·61 (A2), 0·82 (G3) (Found: C, 56·7; H, 7·5; N, 14·4. $C_{18}H_{26}N_4O_5$ requires C, 56·9; H, 7·4; N, 14·7%).

L-*Alanine* N′-4-*Picolyloxycarbonylhydrazide Bistrifluoroacetate Hydrate*.—The t-butoxycarbonyl derivative was dissolved in trifluoroacetic acid, and after 25 min at room temperature the solution was evaporated; the residue was triturated with ether, giving the *hydrazide* (97·5%) as a hygroscopic solid, $[\alpha]_D^{20}$ +7° (*c* 1·1 in H_2O); R_F 0·1 (A2), 0·17 (K) (Found: C, 34·1; H, 3·8; N, 11·3. $C_{10}H_{14}N_4O_3$,-$2CF_3{\cdot}CO_2H,H_2O$ requires C, 34·0; H, 3·6; N, 11·3%).

Removal of the 4-*Picolyloxycarbonyl Group from t-Butoxycarbonyl-*L*-alanine* N′-4-*Picolyloxycarbonylhydrazide*.—(A) *By hydrogenolysis*. The protected hydrazide (100 mg, 0·31 mmol) in 50% aqueous dimethylformamide was hydrogenolysed over palladium–charcoal (10%; 60 mg). After 1 h the solution was filtered (Celite 545) and evaporated; the residue was recrystallised from ether, giving the hydrazide (46·5 mg, 73%), m.p. 94·5—96° (lit.,[18] 96°), $[\alpha]_D^{20}$ −25° (*c* 0·4 in MeOH), R_F 0·32 (E4), 0·70 (G3). Hydrogenolysis in methanol or in 50% aqueous acetic acid gave the same product, identical with that obtained by the action of hydrazine hydrate on t-butoxycarbonyl-L-alanine methyl ester in methanol.

(B) *By electrolytic reduction*. The protected hydrazide (100 mg, 0·31 mmol) in M-sulphuric acid (4 ml) at 0° was reduced at a mercury cathode (current 150 mA) in the apparatus described in ref. 11. After 1·5 h the solution was diluted with methanol (5 ml) and passed down a column of Amberlite IR-45 resin (OH^-). The column was washed with 50% aqueous methanol (50 ml) and the eluates were evaporated; the residue contained 4-methylpiperidine and a small amount of material from which the t-butoxycarbonyl group had been removed, and the product was purified by chromatography on silica gel, with chloroform and then 10%

[17] R. Glatthard and M. Matter, *Helv. Chim. Acta*, 1963, **46**, 795.
[18] G. I. Tesser and R. J. F. Nivard, *Rec. Trav. chim.*, 1964, **83**, 53.

methanol in chloroform as eluants. Evaporation of the appropriate fractions and recrystallisation from ether gave needles of t-butoxycarbonyl-L-alanine hydrazide (40 mg, 70%), m.p. 96—97°; $[\alpha]_D^{20}$ −27° (*c* 1 in MeOH).

t-Butoxycarbonylglycyl-L-valine N'-4-picolyloxycarbonylhydrazide (2).—t-Butoxycarbonyl-L-valine *N'*-4-picolyloxycarbonylhydrazide (2·50 g, 6·5 mmol) was dissolved in trifluoroacetic acid (15 ml) at room temperature. After 5 min the solution was evaporated and the residue was triturated with ether. The resulting solid trifluoroacetate salt was dissolved in dimethylformamide (10 ml) and triethylamine (2·62 g, 26 mmol) was added; after 10 min the excess of triethylamine was removed by evaporation, leaving a solution of amino-component. Dicyclohexylcarbodi-imide (1·68 g, 8·0 mmol) was added to a solution of t-butoxycarbonylglycine (1·40 g, 8·0 mmol) and 1-hydroxybenzotriazole (1·05 g, 8·0 mmol) in dimethylformamide (10 ml) at 0°. This acylating mixture was left for 30 min at 0 °C and 1 h at room temperature, and the solution of amino-component was then added. The condensation was complete in 1 h (t.l.c. with solvent E4), and the product was isolated by use of aqueous citric acid as described for t-butoxycarbonyl-L-valine *N'*-4-picolyloxycarbonylhydrazide, yielding the *protected dipeptide hydrazide* (2·58 g, 93%), m.p. 137—139°, $[\alpha]_D^{20}$ −13°; R_F 0·23 (E4), 0·70 (G3), 0·42 (A2), 0·65 (K) (Found: C, 53·7; H, 6·9; N, 16·6. $C_{19}H_{29}N_5O_6$ requires C, 53·9; H, 6·9; N, 16·5%).

t-Butoxycarbonyl-L-alanylglycyl-L-valine N'-4-Picolyloxycarbonylhydrazide (3).—This was prepared from the protected hydrazide (2) (2·1 g, 5·0 mmol) and t-butoxycarbonyl-L-alanine as described for compound (2), and isolated in the same way by use of aqueous citric acid; the yield of *protected tripeptide hydrazide* (3) was 2·30 g (93%); m.p. 221·5—223°; $[\alpha]_D^{20}$ −20°; R_F 0·08 (E4), 0·63 (G3), 0·32 (A2), 0·63 (J) (Found: C, 53·3; H, 6·7; N, 17·0. $C_{22}H_{34}N_6O_7$ requires C, 53·4; H, 6·9; N, 17·0%).

t-Butoxycarbonyl-L-leucyl-L-alanylglycyl-L-valine N'-4-Picolyloxycarbonylhydrazide (4).—This was prepared from the protected hydrazide (3) (1·70 g, 3·45 mmol) and t-butoxycarbonyl-L-leucine (dried in ethyl acetate solution over $MgSO_4$) as described for the hydrazide (2), except that an additional 1·0 mmol of acylating reagents was added after 4 h, the reaction then being complete after 1 h more. The product was isolated as follows. The mixture was filtered, the filtrate was evaporated, and the solid residue was triturated with 2M-sodium hydrogen carbonate (to remove 1-hydroxybenzotriazole and triethylammonium trifluoroacetate) and then with water, and dried under vacuum. The residue was dissolved in dimethylformamide (50 ml) and the solution was shaken with Amberlyst-15 resin (saturated with 3-bromopyridine; 50 ml) for 1 h. The resin was transferred to a column, which was washed with ethyl acetate (150 ml) and then eluted with pyridine (20%) in dimethylformamide (450 ml). Evaporation of the eluate and trituration of the residue with ether gave the *protected tetrapeptide hydrazide* (4) (1·81 g, 89%), m.p. 140—145°, $[\alpha]_D^{20}$ −15°; R_F 0·25 (E4), 0·66 (G3), 0·51 (A2), 0·63 (J) (Found: C, 54·7; H, 7·5; N, 15·6. $C_{28}H_{45}N_7O_8,0{\cdot}5H_2O$ requires C, 54·5; H, 7·5; N, 15·6%).

t-Butoxycarbonyl-L-leucyl-L-alanylglycyl-L-valine hydrazide (5). (A) *By hydrogenation.* The protected hydrazide (4) (240 mg, 0·40 mmol) in 80% acetic acid (8 ml) was hydrogenolysed over palladium–charcoal (10%; 150 mg). After 2·5 h the solution was filtered (Celite 545) and evaporated. The residue was left in a vacuum desiccator over potassium hydroxide and phosphoric oxide to remove 4-methylpiperidine acetate, giving the *hydrazide* (5) (164 mg, 88%), m.p. 198—201°, $[\alpha]_D^{20}$ −13°; R_F 0·21 (E4), 0·67 (G3), 0·65 (A2), 0·67 (J), 0·72 (L) (Found: C, 52·1; H, 8·9; N, 17·4. $C_{21}H_{40}N_6O_6,0{\cdot}5H_2O$ requires C, 52·4; H, 8·5; N, 17·5%); Leu, 0·95; Ala, *1·00*; Gly, 0·98; Val, 0·97.

(B) *By electrolytic reduction.* A solution of the protected hydrazide (4) (50 mg, 0·083 mmol) in 0·05M-sulphuric acid (4 ml) was reduced at a mercury cathode (current 30 mA) as described for the electrolytic reduction of t-butoxycarbonyl-L-alanine *N'*-4-picolyloxycarbonylhydrazide. After 1 h the solution was passed down a column (1 × 10 cm) of Amberlite IR-45 resin (OH^-) and the product was washed through with 50% aqueous methanol (50 ml). Evaporation yielded an oil which t.l.c. showed to contain the required hydrazide and a small amount of tetrapeptide hydrazide from which the t-butoxycarbonyl group had been removed. Preparative layer chromatography with 10% methanol in chloroform and elution of the band of R_F 0·21 with 10% dimethylformamide in methanol gave the hydrazide (5) (29 mg, 77%), m.p. 195—198°, $[\alpha]_D^{20}$ −11·5° (*c* 0·5 in $Me_2N{\cdot}CHO$); R_F 0·21 (E4), 0·67 (G3), 0·65 (A2).

Further Examples of the Use of 4-Picolyloxycarbonylhydrazides: Synthesis of Fragments related to Sequence 1—13 of Porcine Gastric Inhibitory Polypeptide.—t-Butoxycarbonyl-O-4-picolyl-L-tyrosyl-L-alanyl-γ-4-picolyl-L-glutamylglycyl-O-benzyl-L-threonyl-L-phenylalanyl-L-isoleucyl-O-benzyl-L-seryl-β-benzyl-L-aspartyl-O-4-picolyl-L-tyrosyl-O-benzyl-L-seryl-L-isoleucyl-L-alanine N'-4-picolyloxycarbonylhydrazide (6) (*Table* 1); *t-Butoxycarbonyl-L-threonyl-L-phenylalanyl-L-isoleucyl-O-benzyl-L-seryl-β-benzyl-L-aspartate N'-4-picolyloxycarbonylhydrazide, t-butoxycarbonyl-O-benzyl-L-tyrosyl-L-alanyl-γ-benzyl-L-glutamylglycine N'-4-picolyloxycarbonylhydrazide* (18), *t-butoxycarbonyl-L-threonyl-L-phenylalanyl-L-isoleucine N'-4-picolyloxycarbonylhydrazide, t-butoxycarbonyl-L-seryl-β-benzyl-L-aspartyl-L-tyrosyl-O-benzyl-L-seryl-L-isoleucyl-L-alanine N'-4-picolyloxycarbonylhydrazide, and t-butoxycarbonyl-L-isoleucyl-O-benzyl-L-seryl-L-alanine N'-4-picolyloxycarbonylhydrazide* (19) (*Table* 2). Synthesis was stepwise in each case: the appropriate t-butoxycarbonylamino-acid, dicyclohexylcarbodi-imide, and 1-hydroxybenzotriazole were used (each in 1·2—1·5 molar proportions, relative to the amino-component) in the 'pre-activation' procedure [7] [as described above for compound (2)] with dimethylformamide as solvent. In some cases (noted in the Tables) active esters were used for coupling. When the acyl component had 4-picolyl side-chain protection the minimum excess of acylating agents was used, because of the greater difficulty of removing excess of the acyl component by the normal isolation procedures. The amino-component was prepared from the appropriate t-butoxycarbonyl derivative (described in this paper) as described above for compound (2). Isolation was carried out by the Amberlyst procedure [described above for compound (4)] except where noted in the Tables. The completion of each reaction and isolation step was monitored by t.l.c., but some larger coupling products were too insoluble for the reliable detection of small amounts of amino-component in this way, and the completion of the coupling reaction was then determined by the colorimetric assay described below.

Colorimetric Determination of the Completion of a Coupling Reaction, by Use of Trinitrobenzenesulphonic Acid (with J. Burton).—The method is based on that of Fields [14] for the assay of amino-groups. Solution A contains 1·0M-2,4,6-trinitrobenzenesulphonic acid in 0·1M-sodium sulphite, and

solution B is 0·25M-di-isopropylethylamine in dimethylformamide–water (9 : 1). Solution A (0·1 ml) was mixed with solution B (9·9 ml) and an equal volume of the mixture was added to each of two 10 mm cuvettes; the absorption spectrum was again recorded, as a standard; the absorption rose to a maximum within 3—10 min. A sample of the coupling solution (*ca.* 50 μl) was added to a fresh portion of the mixed assay solution in the sample cuvette, an equal

TABLE 1

Synthesis of Boc-Tyr(Pic)-Ala-Glu(OPic)-Gly-Thr(Bzl)-Phe-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc

Compound [a]	Yield [b] (%)	M.p. (°C)	$[\alpha]_D^{20}$ (°) [c]	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
(7)	85 [d]	163—165	−24·5	E4, 0·31 G3, 0·76	55·7	7·3	15·5	$C_{21}H_{35}N_5O_6$	55·9	7·3	15·5
(8)	95	187—190	−17	E4, 0·38 G3, 0·70 A2, 0·52	59·3	7·3	13·2	$C_{31}H_{44}N_6O_8$	59·2	7·1	13·4
(9)	88	170—172	−6	E4, 0·30 G3, 0·60 A2, 0·22	61·8	6·8	12·6	$C_{46}H_{58}N_8O_{10},0·5H_2O$	61·9	6·6	12·6
(10)	88	185—187·5	−20	E4, 0·33 A2, 0·41	62·5	6·4	11·4	$C_{57}H_{69}N_9O_{13},0·5H_2O$	62·4	6·4	11·5
(11)	88	194—196	−22	E4, 0·30 G3, 0·68	62·4	6·4	11·3	$C_{67}H_{80}N_{10}O_{15},H_2O$	62·7	6·4	10·9
(12)	87	210—215	−19	E4, 0·31 A2, 0·42	62·8	6·5	11·3	$C_{73}H_{91}N_{11}O_{16},H_2O$	62·8	6·7	11·0
(13)	76 [e]	265—268 (decomp.)	−15	E4, 0·35 A2, 0·54	64·1	6·7	10·8	$C_{82}H_{100}N_{12}O_{17},H_2O$	63·8	6·6	10·5
(14)	92	252—257 (decomp.)	−18	H, 0·88 K, 0·63	62·7	6·5	10·1	$C_{93}H_{113}N_{13}O_{19},3H_2O$	63·0	6·7	10·3
(15) [f]	86	261—267 (decomp.)	−8	J, 0·67	63·0	6·6	11·1	$C_{95}H_{116}N_{14}O_{20},2H_2O$	63·1	6·6	10·8
(16) [g]	83	245—252 (decomp.)	*h*	J, 0·67	61·9	6·5	11·3	$C_{106}H_{128}N_{16}O_{23},3H_2O$	62·1	6·5	11·0
(17) [i]	81	241—249 (decomp.)	−14	J, 0·67 L, 0·85	61·6	6·5	11·4	$C_{109}H_{133}N_{17}O_{24},3H_2O$	61·8	6·6	11·3
(6) [j]	81	245—251 (decomp.)	−22	A1, 0·54 L, 0·95	62·1	6·3	11·4	$C_{124}H_{147}N_{19}O_{26},4H_2O$	62·3	6·5	11·2

[a] The compounds, all of which are new, are numbered as follows:

(7) Boc-Ile-Ala-NH·NHPicoc
(8) Boc-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(9) Boc-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(10) Boc-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(11) Boc-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(12) Boc-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(13) Boc-Phe-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(14) Boc-Thr(Bzl)-Phe-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(15) Boc-Gly-Thr(Bzl)-Phe-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(16) Boc-Glu(OPic)-Gly-Thr(Bzl)-Phe-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(17) Boc-Ala-Glu(OPic)-Gly-Thr(Bzl)-Phe-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc
(6) Boc-Tyr(Pic)-Ala-Glu(OPic)-Gly-Thr(Bzl)-Phe-Ile-Ser(Bzl)-Asp(OBzl)-Tyr(Pic)-Ser(Bzl)-Ile-Ala-NH·NHPicoc

[b] Yields are calculated on the amount of t-butoxycarbonyl derivative of the amino-component, *e.g.* on Boc-Ala-NH·NHPicoc for compound (7), and refer to product with the stated constants and analysis. [c] Optical rotations were measured for solutions in dimethylformamide (*c* 0·5). [d] Isolated by the citric acid method. [e] Product was adsorbed on the dicyclohexylurea, which was further extracted with dimethylformamide, but some loss occurred. [f] The protected peptide (15) did not dissolve completely in trifluoroacetic acid but deprotection appeared to be complete in 10 min. In the coupling reaction, 1·05 molar proportions of acylating agents to amino-components were used. T.l.c. could not clearly determine the completion of the coupling reaction; absence of amino-component was confirmed by the colorimetric assay described in the Experimental section. Found: Gly, 0·98; Thr, 0·89; Phe, 0·95; Ile, 1·96; Ser, 1·84; Asp, 1·02; Tyr, 0·17; Ala *1·00*. [g] The completion of the coupling reaction was determined by colorimetric assay. Found: Glu, 0·91; Gly, 1·00; Thr, 0·91; Phe, 0·97; Ile, 1·96; Ser, 1·90; Asp, 1·04; Tyr, 0·16; Ala *1·00*. [h] The compound was insufficiently soluble. [i] The liberated amino-component formed a gel in the dimethylformamide, and additional solvent was added to form a solution; completion of the coupling reaction was determined by colorimetric assay. The product itself formed a viscous solution in dimethylformamide and absorption on the Amberlyst-15 was slow; not all of the product had been transferred to the resin in 3 h, and the first washings of the column contained product which was recovered by a second treatment with fresh resin. Found: Ala, *2·00*, Glu, 0·93; Gly, 1·00; Thr, 0·94; Phe, 1·00; Ile, 2·06; Ser, 1·84; Asp, 1·08; Tyr, 0·16. [j] The reaction mixture [from 830 mg of compound (17) in 10 ml of dimethylformamide] using 1·05 molar proportions of acylating agents to amino-component became very viscous, and coupling required 16 h, as determined by colorimetric assay. The product was only slightly soluble in dimethylformamide, and it was isolated with some loss by reducing the volume of the reaction mixture and precipitating with 2M-sodium hydrogen carbonate, giving 1·04 g of crude product. This mixture (500 mg) was dissolved overnight in dimethylformamide (100 ml) and shaken with Amberlyst-15 (saturated with 3-bromopyridine) (3 h); the resin was washed with dimethylformamide and elution as usual gave protected hydrazide (370 mg) with the constants and analysis shown in the Table. Found: Tyr, 0·26; Ala, *2·00*; Glu, 0·89; Gly, 1·00; Thr, 0·92; Phe, 0·97; Ile, 1·96; Ser, 1·88; Asp, 1·08.

trum between 600 and 400 nm was recorded on a Unicam SP 800 spectrophotometer, to provide a baseline. A sample of a solution of the amino-component, containing *ca.* 10^{-7} mol (*ca.* 1—10 μl) was added to one cuvette and the volume of the coupling solvent was added to the reference cuvette, and the absorption was recorded. This assay can be used in the presence of dicyclohexylcarbodi-imide. In the coupling reaction to prepare protected decapeptide (15),

TABLE 2

Synthesis of 4-Picolyloxycarbonylhydrazides of other fragments related to a sequence 1—13 of porcine gastric inhibitory polypeptide

Compound [a]	Yield (%) [b]	M.p. (°C)	$[\alpha]_D^{20}$ (°) [c]	R_F (t.l.c.)	Found (%) C	H	N	Formula	Required (%) C	H	N
Boc-Ser(Bzl)-Asp(OBzl)-NH·NHPicoc	92 [d]	64—70	−18	E4, 0·39 G3, 0·82 A2, 0·71	60·9	6·3	10·5	$C_{33}H_{39}N_5O_9$	61·0	6·0	10·8
Boc-Ile-Ser(Bzl)-Asp(OBzl)-NH·NHPicoc	95 [e]	126—130	−18	E4, 0·45 G3, 0·79 A2, 0·57	60·8	6·6	11·1	$C_{39}H_{50}N_6O_{10}$,0·5 H_2O	60·7	6·6	10·9
Boc-Phe-Ile-Ser(Bzl)-Asp(OBzl)-NH·NHPicoc	88 [f]	165—168	−14	E4, 0·53 G3, 0·85 J, 0·65	63·0	6·7	10·7	$C_{48}H_{59}N_7O_{11}$	63·3	6·5	10·8
Boc-Thr-Phe-Ile-Ser(Bzl)-Asp(OBzl)-NH·NHPicoc	95 [g]	202—205 (decomp.)	−13	E4, 0·30 O, 0·95	60·8	6·6	11·2	$C_{52}H_{66}N_8O_{13}$,H_2O	60·7	6·6	10·9
Boc-Glu(OBzl)-Gly-NH·NHPicoc	50 [h]	93—96	−5	E4, 0·27 J, 0·67 O, 0·90	56·5	6·3	12·5	$C_{16}H_{33}N_5O_8$,0·5H_2O	56·5	6·2	12·7
Boc-Ala-Glu(OBzl)-Gly-NH·NHPicoc	93·5 [i]	117—119	−13	E4, 0·19 J, 0·67 O, 0·87	56·6	5·9	13·5	$C_{29}H_{38}N_6O_9$	56·7	6·6	13·7
Boc-Tyr(Bzl)-Ala-Glu(OBzl)-Gly-NH·NHPicoc (18)	97 [j]	166—168	−3	E4, 0·19 K, 0·69 O, 0·88	60·6	6·2	11·3	$C_{45}H_{53}N_7O_{11}$,1·5H_2O	60·4	6·3	11·0
Boc-Phe-Ile-NH·NHPicoc	86 [k]	136—138	−20	E4, 0·29 J, 0·67 K, 0·68	61·5	7·0	13·1	$C_{27}H_{37}N_5O_6$	61·5	7·0	13·3
Boc-Thr-Phe-Ile-NH·NHPicoc	97	185—190	−19	E4, 0·29 J, 0·67 K, 0·63	58·4	7·0	13·7	$C_{30}H_{42}N_6O_8$	58·6	6·8	13·7
Boc-Tyr-Ser(Bzl)-Ile-Ala-NH·NHPicoc	88·5 [m]	174—178	−9	E4, 0·20 J, 0·67 O, 0·87	59·8	6·6	11·9	$C_{40}H_{53}N_7O_{10}$,0·5H_2O	60·0	6·8	12·2
Boc-Asp(OBzl)-Tyr-Ser(Bzl)-Ala-NH·NHPicoc	90 [n]	179—182	−16	E4, 0·14 0·23 (trace) J, 0·67	60·6	6·6	11·3	$C_{51}H_{64}N_8O_{13}$,H_2O	60·3	6·5	11·4
Boc-Ser-Asp(OBzl)-Tyr-Ser(Bzl)-Ile-Ala-NH·NHPicoc	89·5 [o]	196—200 (decomp.)	−15	E4, 0·22 J, 0·67 O, 0·90	59·3	6·5	11·7	$C_{54}H_{69}N_9O_{15}$,0·5H_2O	59·3	6·4	11·7
Boc-Ser(Bzl)-Ala-NH·NHPicoc	90·5 [p]	68—72	−8	E4, 0·35 G3, 0·79	57·1	6·6	12·9	$C_{25}H_{33}N_5O_7$,0·5H_2O	57·3	6·5	13·3
Boc-Ile-Ser(Bzl)-Ala-NH·NHPicoc (19)	89·5 [p]	151—153	−14	E4, 0·23 G3, 0·75	59·1	7·0	13·3	$C_{31}H_{44}N_6O_8$	59·2	7·1	13·4

[a] All compounds are new. [b,c] Notes *b* and *c* of Table 1 apply. [d] Isolated by means of citric acid; the product was extracted into dichloromethane. [e] The residue from evaporation of the coupling solution was taken up in dichloromethane; the solution was washed with 2M-sodium hydrogen carbonate and then water, dried, and applied to Amberlyst-15 (1 h). [f] The product was applied to Amberlyst-15 in dimethylformamide–dichloromethane (1 : 3) (1·5 h). [g] Coupling was carried out by use of t-butoxycarbonyl-L-threonine pentachlorophenyl ester. The product was applied to Amberlyst-15 in dimethylformamide–dichloromethane (1 : 1) (2 h) Found: Thr, 0·95; Phe, 1·03; Ile, *1·00*; Ser, 0·90; Asp, 1·03. [h] Amino-component was liberated from the trifluoroacetate by means of di-isopropylethylamine. The product, isolated by extraction into citric acid, contained a contaminant (R_F 0·40 in E4) which was removed by chromatography of a chloroform solution on silica-gel (elution with 10% propan-2-ol in chloroform); some loss occurred at this stage. [i] Coupling was carried out by use of t-butoxycarbonyl-L-alanine pentachlorophenyl ester (1·5 equiv.) in dimethylformamide (24 h). The product was applied to Amberlyst-15 in dichloromethane solution (1 h). [j] Coupling was carried out by use of t-butoxycarbonyl-*O*-benzyl-L-tyrosine pentachlorophenyl ester (1·3 equiv.) in dimethylformamide (48 h). The product was applied to Amberlyst-15 in dichloromethane (1·5 h). Found: Tyr; 1·03; Ala *1·00*; Glu, 1·01; Gly, 1·02. [k] The product was isolated by the citric acid method. [l] Coupling was carried out by use of t-butoxycarbonyl-L-threonine pentachlorophenyl ester in dimethylformamide (4 h). The product was applied to Amberlyst-15 in dichloromethane–dimethylformamide (1 : 1) (2·5 h). Found: Thr, 0·97; Phe, *1·00*; Ile, 0·92. [m] The amino-component was prepared from t-butoxycarbonyl-*O*-benzyl-L-seryl-L-isoleucyl-L-alanine *N'*-4-picolyloxycarbonylhydrazine [compound (8) in Table 1]. Coupling was carried out with t-butoxycarbonyl-L-tyrosine pentachlorophenyl ester (1·2 equiv.) in dimethylformamide (48 h). The product was applied to Amberlyst-15 in dichloromethane solution (1 h). Found: Tyr, 0·97; Ser, 0·91; Ile, 0·94; Ala, *1·00*. [n] Coupling was performed with t-butoxycarbonyl-β-benzyl-L-aspartic acid pentachlorophenyl ester (1·05 equiv.) in dimethylformamide (4 h). Found: Asp, 1·04; Tyr, 1·06; Ser, 0·94; Ile, 1·01; Ala, *1·00*. [o] Coupling was carried with t-butoxycarbonyl-L-serine pentachlorophenyl ester (1·05 equiv.) (16 h). Found: Ser, 1·90; Asp, 1·05; Tyr, 1·05; Ile, 1·04; Ala, *1·00*. [p] Isolation was performed by the citric acid method; the product was extracted into ethyl acetate.

3 nmol of the nonapeptide amino-component could be detected, and by taking 50 μl of the reaction mixture, 0·15% of unchanged amino-component could be found.

t-Butoxycarbonyl-L-tyrosyl-L-alanyl-α-glutamylglycine Hydrazide.—The 4-picolyloxycarbonylhydrazide (18) (Table 2) (500 mg) was hydrogenolysed in a mixture of 50% aqueous acetic acid (20 ml) and dimethylformamide (5 ml) over palladium–charcoal (10%; 300 mg) for 18 h. The mixture was filtered, the filtrate was evaporated, and the co-product *N*-methylpiperidine acetate was removed on a column of Sephadex LH-20 in dimethylformamide. Evaporation gave *hydrazide* (300 mg, 94%), m.p. 155—158°; $[\alpha]_D^{20}$ −4° (*c* 0·5 in $Me_2N{\cdot}CHO$); R_F 0·60 (L), 0·24 (K), 0·90 (G4) (Found: C, 50·7; H, 6·7; N, 14·7. $C_{24}H_{36}N_6O_9,H_2O$ requires C, 50·5; H, 6·7; N, 14·7%); Tyr, 0·95; Ala, *1·00*; Glu, 1·00; Gly, 0·99.

t-Butoxycarbonyl-L-isoleucyl-O-benzyl-L-seryl-L-alanine Hydrazide.—A solution of the 4-picolyloxycarbonylhydrazide (19) (Table 2) (150 mg) in 50% acetic acid (10 ml) was hydrogenolysed over palladium–charcoal (10%; 50 mg) for 10 h. The mixture was filtered and the filtrate was evaporated, and 4-methylpiperidine acetate was removed from the residue on a silica column, with 4% methanol in chloroform as solvent, giving the *hydrazide* (74·5 mg, 81%), m.p. 195—198° (decomp.), $[\alpha]_D^{20}$ −3° (*c* 0·5 in $Me_2N{\cdot}CHO$); R_F 0·43 (E4), 0·73 (G3), 0·81 (H) (Found: C, 47·7; H, 8·6; N, 16·4. $C_{17}H_{35}N_5O_6,1{\cdot}5H_2O$ requires C, 47·4; H, 8·4; N, 16·3%).

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