Extension of the "Handle" Method of Peptide Synthesis: the Use of 4-Picolyloxycarbonylhydrazides

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Summary The 4-picolyloxycarbonylhydrazide of a carboxy-terminal amino-acid [*e.g.* (I)] provides a weakly basic 'handle' by which the coupling product can readily be isolated at each stage of peptide synthesis; then hydrogenolysis of the 4-picolyl group yields the hydrazide required for fragment coupling.

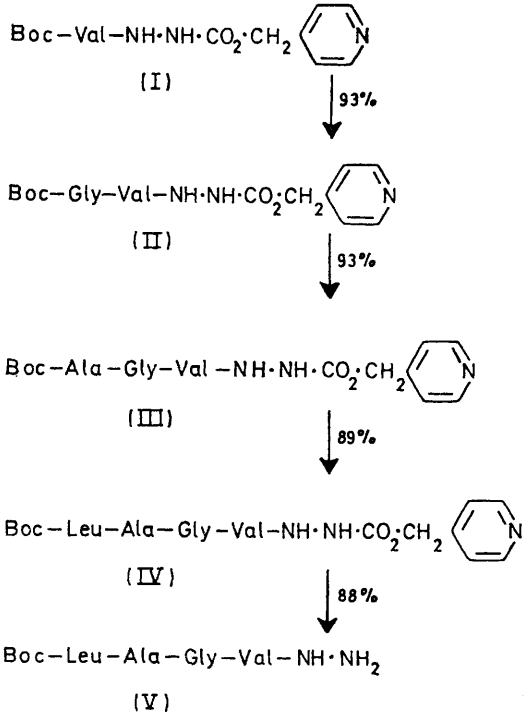
THE picolyl ester method of peptide synthesis[1] provides a 'handle' by which the product is removed into an acidic phase after each coupling step and so is readily purified. We now report that the synthesis of the hydrazides of protected fragments, for further coupling by the azide route, can be facilitated using the 4-picolyloxycarbonylhydrazide of the carboxy-terminal amino-acid.

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

(VI)

Pic = 4-picolyl, Picoc = 4-picolyloxycarbonyl.

4-Picolyloxycarbonylhydrazide was first prepared[2] by the reaction of 4-picolyl alcohol with 2,4,5-trichlorophenyl chloroformate to give 4-picolyl 2,4,5-trichlorophenyl carbonate, which with hydrazine hydrate gave the hydrazide. A better route is from 4-picolyl *p*-nitrophenyl carbonate, prepared by the reaction of bis-*p*-nitrophenyl carbonate with 4-picolyl alcohol and *N*-methylmorpholine in dichloromethane at room temperature. Drs. R. F. Hirschmann and D. F. Veber kindly informed us of this preparation.[3] With hydrazine hydrate in methanol at 0° (10 min) 4-picolyl *p*-nitrophenyl carbonate gave nearly quantitatively 4-picolyloxycarbonylhydrazide as its *p*-nitrophenol salt (m.p. 93·5—95°). t-Butoxycarbonyl-L-valine was condensed with the hydrazide by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole in dimethylformamide, and the resulting *N*-t-butoxycarbonyl-L-valyl-



SCHEME

Boc = t-butoxycarbonyl. (II) m.p. 137—139°, $[\alpha]_D^{20}$ − 13°; (III) m.p. 221·5—223°, $[\alpha]_D^{20}$ − 20°; (IV) hemi-hydrate, m.p. 140—5°, $[\alpha]_D^{20}$ − 15°; (V) hemi-hydrate, m.p. 202—205°, $[\alpha]_D^{20}$ − 13°. Optical rotations are in dimethylformamide (*c* 1). All compounds are new and had satisfactory elemental analyses.

N'-4-picolyloxycarbonylhydrazide [(I), 83% yield; amorphous, characterised by elemental analysis and n.m.r.] was used in the synthesis shown in the Scheme. At each step, the t-butoxycarbonyl group was removed by means of trifluoroacetic acid, and the appropriate t-butoxycarbonylamino-acid was then condensed with the amino-component by means of dicyclohexylcarbodi-imide and 1-hydroxybenzotriazole in dimethylformamide. The protected di- and tri-peptide hydrazides were isolated by extraction into aqueous citric acid, and the protected tetrapeptide hydrazide was isolated on the acidic ion-exchanger Amberlyst-15 (saturated with 3-bromopyridine).[4] In each case the product obtained was pure (elemental analysis and t.l.c.).

The procedure has been used successfully for the synthesis of other protected peptide 4-picolyloxycarbonylhydrazides including (VI), having the sequence 1-13 of porcine gastric-inhibitory polypeptide;[5] in the first step isolation was by means of aqueous citric acid, and in subsequent steps by means of Amberlyst-15 (saturated with 3-bromopyridine).

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