Solid-phase Peptide Synthesis without Acidolysis: the Synthesis of Thymosin α_1 on a New Benzhydrylamine Resin

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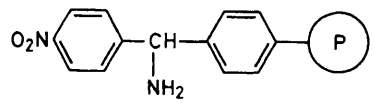
Summary The use of base-labile N^{α}-fluoren-9-ylmethoxycarbonyl-substituted amino-acids, benzyl-based side-chain protecting groups, and a *p*-nitrobenzhydrylamine resin enabled solid-phase synthesis of thymosin α_1 to be carried out under non-acidic, exceptionally mild conditions even in the final deprotecting step, which was performed by catalytic transfer hydrogenolysis with cyclohexa-1,4-diene.

EFFORTS to devise milder conditions for solid-phase peptide synthesis[1] have been continuously made over the last ten years. The repetitive acidolytic removal of N^{α}-temporary protecting groups [*e.g.*, t-butoxycarbonyl and 1-(biphenyl-4-yl)-1-methylethoxycarbonyl] has been replaced, in a few laboratories, by non-hydrolytic base removal of N^{α}-fluoren-9-ylmethoxycarbonyl (Fmoc) groups[2] while a *p*-alkoxybenzyl peptide-to-resin linkage[3] was employed in order to perform the final detachment from the solid support, as well as cleavage of t-butyl-based side-chain protecting groups, by treatment with CF_3CO_2H. At the same time, several authors[4] reported that peptides can be released from benzyl-type ester resins by hydrogenolysis, thus offering an alternative to the acidolytic cleavage of peptide-to-resin bonds. I now describe a new solid-phase synthesis of thymosin α_1[5] (**1**), an acetyl octacosapeptide from the calf thymus gland, during which, for the first time,† no treatment with acid was employed, final cleavage of the peptide from a substituted benzhydrylamine resin being carried out by catalytic transfer hydrogenolysis.

Ac-Ser-Asp-Ala-Ala-Val-Asp-Thr-Ser-Ser-Glu-Ile-Thr-Thr-Lys-Asp-Leu-Lys-Glu-Lys-Lys-Glu-Val-Val-Glu-Glu-Ala-Glu-Asn-OH

(**1**)

The solid support (**2**) (0·41 mmol g^{-1}) was prepared by Friedel–Crafts acylation of copoly(styrene–1% divinylbenzene) with *p*-nitrobenzoyl chloride, followed by essentially the same procedure described for the synthesis of benzhydrylamine resin.[6] Peptide synthesis was commenced by coupling N^{α}-Fmoc-aspartic acid α-benzyl ester‡ to resin (**2**) in the presence of dicyclohexylcarbodi-imide (DCC); the resin loading was 0·27 mmol g^{-1}. The aspartyl resin (740 mg, 0·20 mmol) was transferred to the glass reaction vessel of a manual apparatus, acetylated with acetic anhydride to block all amino-groups on the resin which had not reacted, and submitted to a programme[7] for stepwise synthesis from the carboxy-terminus. The Fmoc group, employed throughout the synthesis for temporary N^{α} protection, was removed by treatment with 55% piperidine in dimethylformamide (DMF), and the protected amino-acids were twice coupled to the growing peptide resin as their immediately preformed symmetrical anhydrides[8] (4-fold excess per coupling). Completeness of coupling reactions§ was monitored both by the ninhydrin colour test[9] and the fluorometric method,[10] and the progress of the chain assembly was checked at intervals by total acid hydrolyses[11] of intermediate peptide resins with subsequent amino-acid analyses. The following groups were employed for side-chain protection: Ser and Thr, benzyl ether; Asp and Glu, benzyl ester; Lys, benzyloxycarbonyl. After coupling of the last serine residue, the Fmoc group was removed as usual, and the peptide resin acetylated with acetic anhydride in the presence of pyridine.



(**2**)

Cleavage of the peptide-to-*p*-nitrobenzhydrylamine resin bond, as well as removal of side-chain protecting groups, was carried out by catalytic transfer hydrogenation with cyclohexa-1,4-diene in the presence of palladium black generated *in situ*.[11] Previous hydrogenolyses of acetylglycyl-, acetylvalyl-, and acetyl-α-aspartyl-*p*-nitrobenzhydrylamine resins had afforded the corresponding acetyl amino-acid amides in good yields (81—88%) provided that a large excess of catalyst was employed and repeated (two to three) hydrogenations were performed. Accordingly, the acetyloctacosapeptide resin was extensively washed with EtOH, CH_2Cl_2, and DMF, dried to constant weight (1·03 g), and suspended in dry DMF. Palladium(II) acetate (1·0 g) was then added and the hydrogenation with cyclohexa-1,4-diene (10 ml) performed as previously described;[4d] hydrogenation was repeated twice again with fresh catalyst, and the peptide isolated (65 mg). The cleavage yield was 84% as determined by titration with HCl and nitrogen analysis on the recovered resin after hydrolysis. The crude material was then purified on a DEAE-Sephadex A-25 column (0·05 M Tris-HCl buffer, pH 8·0, linear gradient of NaCl

† Previously described solid-phase syntheses of thymosin α_1 (S. S. Wang, R. Makofske, A. Bach, and R. B. Merrifield, *Int. J. Pept. Protein Res.*, 1980, **15**, 1; T. W. Wong and R. B. Merrifield, *Biochemistry*, 1980, **19**, 3233) employed CF_3CO_2H for N^{α}-Boc (Boc = t-butoxycarbonyl) deprotection and anhydrous HF for the final cleavage of the peptide-to-resin bond and side-chain protecting groups.

‡ N^{α}-Fmoc amino-acids were prepared according to the procedures reported in the literature (L. A. Carpino and G. Y. Han, *J. Am. Chem. Soc.*, 1970, **92**, 5748; *J. Org. Chem.*, 1972, **37**, 3404; *ibid.*, 1973, **38**, 4218; C.-D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E. O. Lundell, and J. D. Haug, *Int. J. Pept. Protein Res.*, 1980, **15**, 59) from fluoren-9-ylmethyl chloroformate and the corresponding amino-acids with suitable side-chain protection.

§ If necessary, the peptide resin was coupled three times by the same procedure.

0—0·4 M) followed by filtration on Bio-Gel P6 (1% AcOH) to give thymosin α_1 as an amorphous white powder (38 mg; 22% overall yield¶ based on starting aspartyl resin). The amino-acid composition was: Asp 4·12 (calc. 4), Thr 2·84 (3), Ser 2·89 (3), Glu 6·08 (6), Ala 3·04 (3), Val 2·85 (3), Ile 0·89 (1), Leu 1·00 (1), Lys 4·12 (4) [48 h hydrolysis in 6 N HCl at 110 °C; longer hydrolysis (96 h) gave Val 3·05, Ile 0·98]. The product gave a single spot an acrylamide gel isoelectric focusing (pH 3·5—9·5) and on t.l.c. (silica gel Merck 60 F-254; R_f 0·18 in n-butanol–pyridine–acetic acid–water 5:5:2:4 v/v); $[\alpha]_D^{25}$ −112·5° (*c* 0·1, H_2O). Biological properties of the synthetic thymosin α_1 will be reported separately.

These results suggest that catalytic transfer hydrogenation with cyclohexa-1,4-diene as hydrogen source, although limited by the presence of sulphur-containing amino-acid residues,** could be a useful alternative to the final acidolytic cleavage of peptide-amide-to-resin bonds, and in conjunction with the Fmoc group forms an exceptionally mild strategy for solid-phase synthesis of peptide amides or of peptides possessing CO_2H-terminal asparagine or glutamine residues.

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¶ Uncorrected for loss due to coupling monitoring and amino-acid analyses during the synthesis.

** Although literature reports (A. E. Jackson and R. A. W. Johnstone, *Synthesis*, 1977, 685; G. M. Anantharamaiah and K. M. Sivanandaiah, *J. Chem. Soc., Perkin Trans.* 1, 1977, 490; refs. 4d and 11) on the inhibition of catalytic transfer hydrogenation by sulphur-containing amino-acids are conflicting, it was possible to cleave acetylmethionine-amide from the *p*-nitrobenzhydrylamine resin (**2**) in 64% yield (R. Colombo, unpublished results).

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