# The Use of Mono-esters of Catechol in the Racemisation-free Synthesis of Sequential Polypeptides with Amino or Carboxy Side-chains

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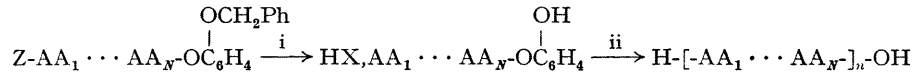
*Summary* A procedure for the racemisation-free synthesis of sequential polypeptides with amino or carboxy side-chains is described and exemplified by a synthesis of poly(lysylalanylalanine) trifluoroacetate.

IT has been shown[1,2] that simultaneous scission of the protecting groups of a benzyloxycarbonylpeptide 2-benzyloxyphenyl ester (I) followed by polymerisation of the resulting peptide 2-hydroxyphenyl ester salt (II) with triethylamine in dimethyl sulphoxide is a convenient and racemisation-free[1-3] route to sequential polypeptides (Scheme 1). In our preliminary work on this subject,[1,2] hydrogenolysis proved less satisfactory than acidolysis for the deprotection of (I) with the consequence that our preferred procedure was inapplicable to cases with functional



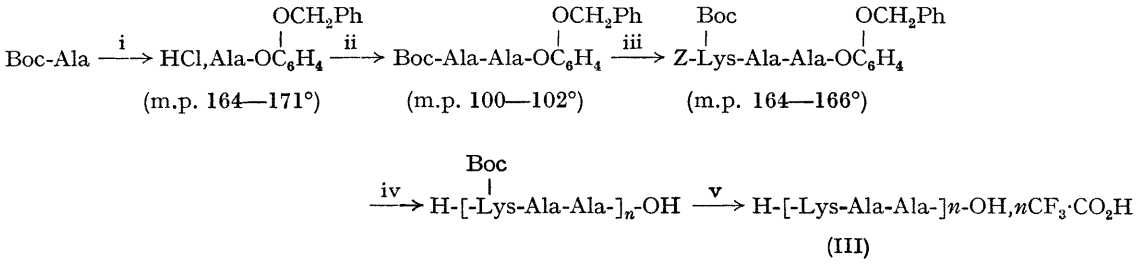

(I) (II)

i, HBr–AcOH or $H_2$–Pd; ii, $Et_3N$–$Me_2SO$

AA = an amino-acid residue; X = an anion; $-C_6H_4$(OCH$_2$Ph) = 2-benzyloxyphenyl; $-C_6H_4$(OH) = 2-hydroxyphenyl.

SCHEME 1

side-chains masked by means of protecting groups which could be removed under mild conditions after polymerisation. A recently published[3] modification of our method employs peptide esters of mono-*O*-phenacylcatechol: since the phenacyl group can be selectively removed (zinc–acetic acid) in the presence of protecting groups based on t-butyl of the applied material emerged at the void volume (molecular weight > *ca.* 30,000) and the remainder showed a broad distribution of molecular weight between *ca.* 30,000 and 1000. Minimum values of the molecular weight averages calculated[5] from the gel filtration results were: number average 6700, weight average 11,100.




(III)

i, $HOC_6H_4(OCH_2Ph)$, mixed carbonic anhydride, then HCl–EtOAc (yield 64%); ii, Boc-Ala-ONSu (88%); iii, 90% $CF_3 \cdot CO_2H$ then Z-Lys(Boc)-ONSu (79%); iv, $H_2$–Pd–AcOH then $Et_3N$–$Me_2SO$ (40%); v, 90% $CF_3 \cdot CO_2H$ then dialysis and lyophilisation (39%). (NSu = succinimido).

Abbreviations for amino-acids (all of which are L) and their use in the formulation of derivatives follow the relevant Tentative Rules of the I.U.P.A.C.–I.U.B. Commission on Biochemical Nomenclature which are reprinted in *Specialist Periodical Reports Amino-acids, Peptides, and Proteins*, 1970, **2**, ch. 5.

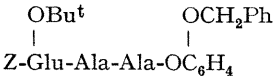
SCHEME 2

alcohol or benzyl alcohol, this stratagem can be used for the synthesis of sequential polypeptides having acid-labile side-chain protection.

We now ascribe our previous difficulties with the hydrogenolytic conversion of (I) into (II) to the presence of traces of catalyst poisons derived from sulphur-containing reagents involved in the preparations of (I). Subsequent experience has shown that, providing such reagents are avoided at all stages of the preparation of the derivatives (I), the hydrogenolysis proceeds smoothly and quantitatively. This improvement has enabled us to extend our method to the preparation of sequential polypeptides with amino or carboxy side-chains, and the synthesis of poly(lysylalanylalanine) trifluoroacetate (III) is outlined in Scheme 2 as an example. The polymer was obtained as a white fluffy powder which gave satisfactory elemental and amino-acid analyses and had spectroscopic properties (i.r., n.m.r.) as expected for (III). When it was subjected to gel filtration on a calibrated column of Biogel-P100 which was swollen and eluted with phenol–acetic acid–water (1 : 1 : 1),[4] *ca.* 5%

Poly(glutamylalanylalanine) has been similarly obtained *via* the fully protected monomer (IV).

A further matter relating to the generality of our procedure concerns the ease with which the crucial intermediates (I) can be purified. In the early phases of this work[1,2] we had some difficulties in this respect although we were never unsuccessful. In addition to the intermediates




(IV) m.p. 165—168°

described previously[2] and above, a range of derivatives (I) at the di-, tri-, and tetra-peptide levels containing, *inter alia*, residues of asparagine, glutamine, azetidine-2-carboxylic acid, pipecolic acid, *O*-t-butylserine, and ω-tosylarginine have since been prepared:[6] all were easily purified solids.

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[1] J. H. Jones, *Chem. Comm.*, 1969, 1436.
[2] R. D. Cowell and J. H. Jones, *J. Chem. Soc.* (*C*), 1971, 1082.
[3] Y. Trudelle, *Chem. Comm.*, 1971, 639.
[4] A. Pusztai and W. B. Watt, *Biochim. Biophys. Acta*, 1970, **214**, 463; we checked that the $V_E/V_0$ — log $M$ relationship for this system was linear, using a range of synthetic peptides and natural proteins which spanned the molecular weight distribution of our polymer.
[5] For an outline of the principles of the determination of molecular weight distributions and averages from gel filtration results, see H. Determann, 'Gel Chromatography,' Springer-Verlag, Berlin, 1968, p. 120, *et sequens*.
[6] R. Fairweather, D. R. Regester, and J. Walker, unpublished work.