Racemisation-Free Sequential Polypeptide Synthesis

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Summary The use of *o*-hydroxyphenyl esters for racemisation-free sequential polypeptide synthesis is reported.

THE most successful route to sequential polypeptides (I)[1] has been *via* peptide active esters (II)[2] as shown in Scheme 1.

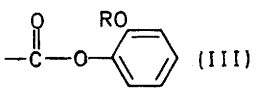
SCHEME 1.

X = Cl, Br *etc.*; AA = amino-acid residues; Y = *p*-nitrophenyl, pentachlorophenyl *etc.*

$$\underset{\text{(II)}}{HX\cdot AA_1\cdot AA_2\cdots AA_N\cdot OY} \xrightarrow[\text{Polar aprotic solvent}]{\text{Tertiary base}} \underset{\text{(I)}}{H\text{-}[\text{-}AA_1\cdot AA_2\cdots AA_N\text{-}]_n OH}$$

As Rydon has pointed out,[3] the requirements for an optically pure polymer are very stringent: even if only 1% of the condensation steps occur with concomitant racemisation, then after a degree of polymerization of 100 has been attained, *ca.* 39% of the resulting chains will have an inverted configuration at at least one residue. Judicious choice of conditions can reduce the risk of racemisation to very low levels, but the problem is exacerbated by the lack of accurate methods for detecting small amounts of racemate if any is formed. Clearly a procedure is needed which can be shown in model systems to be entirely free from the danger of racemisation.

(ii) The unreactive *o*-benzyloxyphenyl ester group (III; R = benzyl) can be used for carboxyl protection until activation is required, when acidolysis or hydrogenolysis provides a reactive *o*-hydroxyphenyl ester.[4]



(III)

Polyglycyl-L-prolyl-L-alanine (a collagen model) was synthesised as shown in Scheme 2 *via* the fully protected tripeptide derivative (IV), which, although not crystalline, was obtained in a pure condition: some of the properties of the polymer (purified by dialysis, lyophilisation, and drying to constant weight at 100°/0·1 mm.) are summarised in the Table, together with published data on a preparation of the

TABLE. *Properties of polyglycyl-L-prolyl-L-alanine*

	o-Hydroxyphenyl ester route	*p*-Nitrophenyl ester route (Data of Blout *et al.*[5a])
% Hydration, by weight	12·5[a]	< 1
Solubility in H_2O	Very sparingly soluble[b]	Soluble
$[\alpha]_{546}^{20}$	−426° (*c* 0·05, H_2O)[c]	−208° (*c* 0·08, H_2O)
Molecular weight (Archibald method)	$1{\cdot}2 \pm 0{\cdot}1 \times 10^4$	$1{\cdot}40 \pm 0{\cdot}05 \times 10^4$

[a] Calculated from results of elemental analysis. Apart from firmly held water, the polymer was pure, as judged by the C : N ratio (found 2·85, calculated for $C_{10}H_{15}N_3O_3$ 2·85), the n.m.r. spectrum (in $CF_3.CO_2H$, no impurities detected), and amino-acid analysis (Gly : Pro : Ala = 1·00 : 0·95 : 1·05).

[b] Solutions of concentration up to 0·1% in water could be obtained by heating at 50° for several hr. Soluble in acidic media.

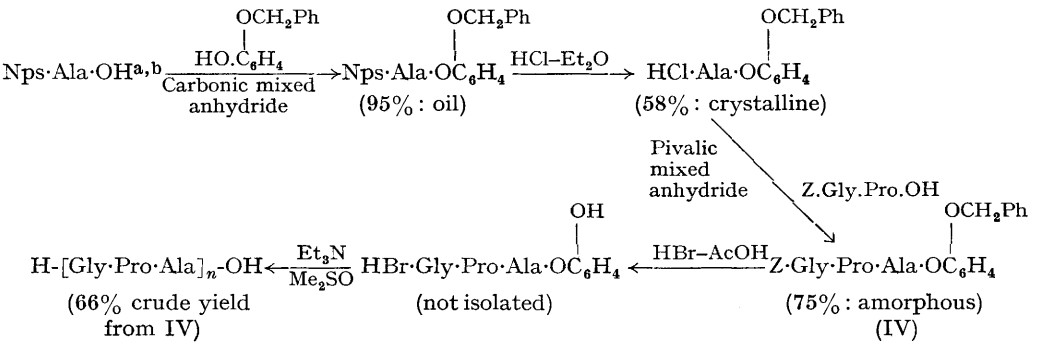
[c] Average of four separate determinations in the range −410 to −440°. Measurements were made after equilibration of the solutions at 20° for 24 hr.

The suggestion[4] that *o*-hydroxyphenyl esters (III; R = H) might be especially useful in this field was based on two reasons.

(i) Sensitive racemisation tests show that the esters (III; R = H) undergo coupling without racemisation in simple oligopeptide preparations. This finding was attributed to intramolecular general base catalysis, which accelerates aminolysis but not oxazolone formation (the principal cause of racemisation).[4]

same polymer obtained by Blout *et al.*[5a] by the *p*-nitrophenyl ester polymerisation method. It seems probable that some racemisation intervened in the *p*-nitrophenyl ester synthesis, as the *o*-hydroxyphenyl ester product is for the synthesis of poly-L-prolyl-L-alanylglycine[6] entirely precluded racemisation, since the *C*-terminal residue of the polymerising unit was glycine: their preparation of poly-L-prolyl-L-alanylglycine[6] has almost the same specific rotation

SCHEME 2.



[a] Abbreviated designations for amino-acid residues (all of which are L) and their mode of use are as recommended in I.U.P.A.C. Information Bulletin No. 25, 1966.

[b] Nps = *o*-nitrophenylsulphenyl; Z = benzyloxycarbonyl; C_6H_4 (OCH$_2$Ph) = *o*-benzyloxyphenyl; C_6H_4 (OH) = *o*-hydroxyphenyl.

optically pure (see below): the differences between the two materials described in the Table are an indication of the extent to which small numbers of racemic residues can affect the properties of a sequential polypeptide, since Blout and his colleagues[5a] showed by enzymic methods that the amount of racemisation incurred in their synthesis was very small.

While this work was in progress Professor E. R. Blout informed the author that he and his associates had synthesised poly-L-prolyl-L-alanylglycine,[6] which has the same repeating amino-acid sequence as polyglycyl-L-prolyl-L-alanine except at the termini. The strategy used by them as the polyglycyl-L-prolyl-L-alanine reported here, and the circular dichroism spectra of the two preparations are essentially identical. This comparison, which is in order because the molecular weights of the two preparations are almost the same, shows unequivocally that racemisation did not occur in the present synthesis.

I thank Professor E. R. Blout for the ultracentrifuge molecular weight determination and for allowing reference to be made to unpublished results obtained in his laboratory.

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[1] For recent references to the synthesis and study of sequential polypeptides see "Amino-acids, Peptides, and Proteins," Specialist Periodical Report, the Chemical Society, London, 1969, vol. 1, p. 143 and 200.
[2] D. F. DeTar, in "Peptides," eds. H. C. Beyerman, A. van de Linde, and W. Maassen van den Brink, North Holland Publishing Co., Amsterdam, 1967, p. 125 and references cited therein.
[3] H. N. Rydon, Lecture delivered at the Chemical Society Anniversary Meeting, Exeter, 1967.
[4] J. H. Jones and G. T. Young, *J. Chem. Soc.* (*C*), 1968, 436.
[5] (a) S. M. Bloom, S. K. Dasgupta, R. P. Patel, and E. R. Blout, *J. Amer. Chem. Soc.*, 1966, **88**, 2035; (b) P. J. Oriel and E. R. Blout, *ibid.*, p. 2041.
[6] F. R. Brown, tert., G. P. Lorenzi, and E. R. Blout, to be published.