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AN OPTICALLY PURE SEQUENCE PEPTIDE, POLY.GLY.GLY.PHE.

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Efficient methods have recently been described for the synthesis of sequence peptide polymers based on the polymerization of "active" esters eq. 1 (1-3).* The generality of the polymerization with respect

(1) HBr.H.Asp(OCH$_3$).Ser(H).Gly.ONP $\xrightarrow{\text{Base}}$ Poly.Asp(OCH$_3$).Ser(H).-Gly.

to functional side chains has been demonstrated. Furthermore, a number of polymers have been reported in which the monomer had a potentially racemizable C-terminal residue, but the optical purity of the incorporated C-terminal residues has not been rigorously investigated. We now report an example of the use of the "active" ester technique for preparing a polymer from HBr.H.Gly.Gly.Phe.ONP and have carried out studies on both the intermediates and on the polymer which show that little or no racemization of the sensitive phenylalanine group has occurred.

The central problem here is that azlactone formation occurs readily upon treating "active" esters with bases, including nucleophiles.

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* In the convention used here the amino acid abbreviation always stands for the residue: Gly = -HNCH$_2$CO-, Ser = -HNCH(CH$_2$OH)CO-. This makes it possible to designate explicitly and unambiguously the precise derivative being discussed. ONP = p-O$_2$NC$_6$H$_4$O-. DCC is dicyclohexylcarbodiimide. HOPCP is pentachlorophenol.

The resulting azlactones are good acylating agents, so that acylation of the nucleophile is not prevented, but the product is partly or extensively racemized. For example, hydrolysis of Bz.Phe.ONP has been shown to proceed through azlactone and this in turn was shown to give racemic Bz.Phe.OH (4, 5).

The polymerization reaction can be carried out under a variety of conditions which may be expected to lead to differing extents of direct polymerization and of indirect polymerization through azlactone. We chose sodium p-nitrophenoxide as base in dimethylsulfoxide since this recipe had given good results in other sensitive cases.* We selected HBr.H.Gly.Gly.Phe.ONP as the example: the phenylalanine group is easily racemized and it was important to use a "monomer" with a single optically active center in order to be able to establish reliably the extent of racemization during polymerization.

Assay of peptides and of polymers for optical purity is neither easy nor routine. However, hydrolytic techniques have been developed for aspartic acid, serine, glutamic acid, and phenylalanine which are accurate to about 2-3% per determination. These error estimates summarize our experience with a very large number of samples with a variety of structures. (1b) Hydrolysis of peptides containing glycine along with aspartic acid, glutamic acid, or serine is best carried at about 100°. Phenylalanine containing peptides require more drastic conditions: 10-15 hrs. at 120° in a sealed tube with 5N HCl. Longer times gave gradual loss of activity. At 100° the results were erratic; both high and low values were observed depending on the peptide.

Rotations of phenylalanine were read in a mixture of one part of conc. hydrochloric acid and three parts of dimethylformamide and were taken within 20-25 min. after preparation. The advantage of this relatively unstable solvent is a high and reproducible rotation value for phenylalanine: $[M]_D^{25}$ + 89.6 with a per reading standard deviation of 2.

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*The use of the polyfunctional catalysts of Beyerman may prove advantageous (7).

Where applicable enzymic hydrolysis may provide a rigorous test of optical purity (8).

Application of these new techniques to each of the key intermediates in the synthesis (eqs. 2-4) shows that all were optically pure. Molar rotations at 25° and 589 mμ of the intermediates (all L) were as follows: Z.Phe.ONP, - 61 (c 2 in ethyl acetate); HBr.H.Phe.ONP + 140 (c 2 in CH_3OH); Z.Gly.Gly.Phe.ONP, - 1.4 ± 2 (c 2 in $CHCl_3$); HBr.H.Gly.Phe.ONP - 129 (c 2 in CH_3OH); poly.Gly.Gly.Phe. + 67° (c 2 in dichloroacetic acid, corrected for 95% purity). All the above intermediates upon hydrolysis as described above showed a molar rotation for phenylalanine of 89.6 ± 1.5, average of two determinations on each. Upon hydrolysis, the polymer showed a phenylalanine rotation of 86.5. Although 4% low, this value is within permissible limits based on combined errors of rotation and of estimates of polymer purity. A high degree of optical integrity is clearly within reach of present experimental methods, even with an optically active C-terminal group.*

In the synthesis of "monomer" we have found it advisable to start from the carboxyl end using the p-nitrophenyl group as a blocking group (eqs. 2, 3). (Other active esters may be used in this sequence of reactions.) Conversion of a C-terminal amino acid to its ester according to

(2) $\underset{\underline{1}}{\text{Z.Phe.ONP}} + \text{HBr} \xrightarrow{\text{HOAc}} \underset{\underline{2}}{\text{HBr.H.Phe.ONP}}$

(3) $\text{Z.Gly.Gly.OH} + \text{DCC} + \underline{2} \xrightarrow{(C_2H_5)_3N} \underset{\underline{3}}{\text{Z.Gly.Gly.Phe.ONP}}$

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*Kovacs et al (3) prepared this polymer using the pentachlorophenyl ester method. However, the evidence cited is not adequate to show whether there was loss of activity during the polymerization step. No rotation value is given for the polymer, and the optical purity of a key intermediate, Z.Gly.Gly.Phe.OPCP, is in question (see below). The discrepancy may have arisen in part from the fact that the reported assay technique (6N HCl, reflux) is similar to one we have found to give erratic results.

eq. 5, on the other hand, gives partly racemized products (5). The mechanisms of these reactions and the presently known scope and limitations have been discussed (5).

(4) 3 + HBr $\xrightarrow{HOAc}$ HBr.H.Gly.Gly.Phe.ONP

(5) Z.Gly.Asp.(OCH_3).OH + HONP + DCC ⟶ largely racemic ester.

If the racemization is not too extensive and if the solubility properties of the optically active ester are favorable, then it may be possible to isolate optically pure product. However, the occurrence of racemization is sufficiently well demonstrated in a number of such reactions that optical purity must be carefully examined.* We call specific attention to this problem because it has come to our notice that certain commerical sequence polymers may have been made using such steps.

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*Kovacs et al (3) prepared Z.Gly.Gly.Phe.OPCP by a route similar to eq. 5 and report $[\alpha]_{589}^{25}$ - 10°, m.p. 112. This sample is extensively racemized. We have prepared L-Z.Gly.Gly.Phe.OPCP by the alternate sequence similar to eqs. 2-4 using instead diisopropylcarbodiimide as coupling reagent (eq. 3) and find a m.p. of 149-150°, $[\alpha]_{589}^{25}$ - 30 (c, .05 $CHCl_3$). This sample of L-ester was not very soluble in chloroform. In our hands Kovacs' method of synthesis gave a series of ester fractions which were rather difficult to separate from DCU. We examined four samples having correct C,H,N analyses and the expected NMR with no DCU peaks. These showed rotations of - 7.0, - 11.3, -16.9 and 25.1 and various melting points in the 120-150° range. These were hydrolyzed and the rotations of the resulting phenylalanine measured. A plot of the ester rotation vs the phenylalanine rotation gave a straight line with a 0,0 intercept and indicated that rotation for the pure ester is $[\alpha]_{589}^{25}$ -32 + 2° (c, 1 in $CHCl_3$) in agreement with that which we found directly. The ester product from the coupling of Z.Gly.Gly.Phe.OH + HOPCP + DCC appears to be about 30-40%D and 70-60%L. These results are comparable to those we have observed in other systems with p-nitrophenol (1b, 5).

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