The Use of Polyhexamethylenecarbodi-imide, an Insoluble Condensing Agent, in Peptide Synthesis

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RECENTLY peptide and polypeptide synthesis on a polymer support has been developed with striking success. In Merrifield "solid-phase peptide synthesis" the growing polypeptide chain is bound to an insoluble polymer while the *N*-blocked amino-acid is in solution.[1] A "reverse Merrifield" method has been developed lately in which the active ester of the *N*-blocked amino-acid is bound to the insoluble polymer, the free peptide ester being in solution.[2,3]

The carbodi-imides are well-known condensing agents in peptide synthesis.[4] Various polymers containing the carbodi-imide functional group in their backbone chain have been known for some time as polymers with film- and fibre-forming capabilities.[5]

We report the use of an insoluble polycarbodi-imide as a condensing agent in peptide synthesis. The condensing agent is bound to an insoluble carrier while the acylamino-acid, as well as the growing peptide chain, is in solution. Among various polycarbodi-imides which have been tested, best results were obtained using polyhexamethylenecarbodi-imide (I).

$$-[CH_2]_6\cdot N=C=N-\{-[CH_2]_6\cdot N=C=N-\}_n-[CH_2]_6- \quad (I)$$

The polymer (I) was obtained by the catalytic decarboxylation of 1,6-di-isocyanate hexane using 3-methyl-1-phenyl-3-phospholene 1-oxide as a catalyst,[6,7] and dry *N*-methyl-2-pyrrolidone as a solvent. The resulting polymer was treated with ethanol to block terminal isocyanate groups present, filtered off, ground, and fractionated by extraction with boiling methylene chloride in order to remove any low-molecular-weight compounds. The product was then treated with acetyl *N*-hydroxysuccinimide[8] which acetylates any free amino-group which might have been formed during the polymerization.

Z–Gly–Gly–OEt was obtained by suspending 20 mmole of compound (I) in methylene chloride containing Z–Gly–OH (2·5 mmole), HCl–Gly–OEt

(2·5 mmole), and E_3N (2·5 mmole), with stirring for 12 hr. The polymer was removed by filtration and washed with methylene chloride; the organic solvent was then removed *in vacuo*. The residue was dissolved in wet ethyl acetate and washed with 1N-HCl, 5% $NaHCO_3$, and water. On evaporation, a crystalline product remained, yield 92%, m.p. 79—80° (reported[9] m.p. 82—83°). Analogously Pht–Gly–L-Glu–$(OBz)_2$ was obtained from Pht–Gly–OH and HCl–L-Glu–$(OBz)_2$; yield 89%, m.p. 92—94°, $[\alpha]_D^{25}$ −16·8 [*c* 2·0 (EtOH)] {reported[10] m.p. 90—93°, $[\alpha]_D^{23}$ − 17·1 [*c* 2·0 (EtOH)]}, *NS*-di-Z–L-Cys–Gly–OBz was obtained from *NS*-di-Z–L-Cys–OH and HCl–Gly–OBz; yield 93%, m.p. 116—118°, $[\alpha]_D^{25}$ − 44·8 [*c* 2·0 (dimethylformamide)] {reported[11] m.p. 118—119°, $[\alpha]_D^{25}$ − 45·5 [*c* 2·0 (DMF)]}, and acetyl *N*-hydroxysuccinimide from acetic acid and *N*-hydroxysuccinimide; yield 82%; m.p. 129—130° (reported[8] m.p. 130°).

Work is under way to use this reagent in the synthesis of various low- and high-molecular-weight peptides as well as in the synthesis of nucleotides and polynucleotides.

All compounds reported in this Communication gave satisfactory nitrogen analyses.

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