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RACEMIZATION DURING USE OF POLYMERIC SUPPORTS IN PEPTIDE SYNTHESES

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Recently, a great deal of interest has been shown in the use of polymeric reagents for the automation of peptide synthesis using a solid-phase reaction system. The resin reported by Merrifield has already been widely used (1,2), and several newer resins seem to have even more potential, since they would allow purification of the intermediate products during an extended synthesis (3-7). Most of these newer polymers fall into two categories: those that are used as typical coupling reagents, for example the EEDQ-based resin poly-Q (3), and those that are used in an active ester type synthesis, for example 4-OH-3-NO_2-polystyrene (4-6) and poly-HOSu (7). Using the racemization test reported by Izumiya et al (8), we have measured the racemization caused by the use of each of these three polymeric reagents, and compared it with that found with some standard monomeric reagents. For two of the resins, the extent of racemization was of an acceptably low level, but for the third, the presence

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Abbreviations: DCC, dicyclohexylcarbodiimide; DMF, dimethyl formamide; DME, 1,2-dimethoxyethane; HOSu, N-hydroxysuccinimide; EEDQ, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline; HOAc, acetic acid; TEA, triethylamine; Bzl, benzyl.

of considerable racemization suggested that this resin could only be used within certain restrictions.

The reaction schemes used for the racemization tests are shown in Figure 1.

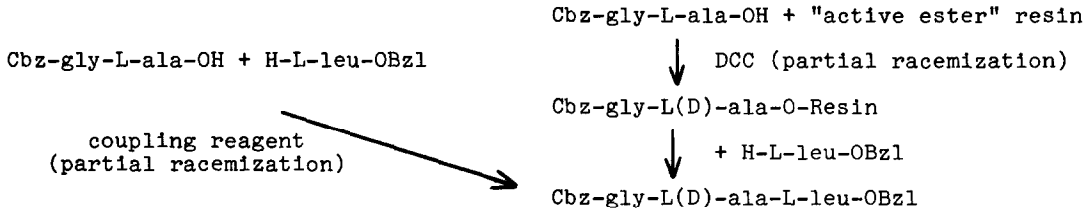

Figure 1

After coupling, the partially racemized product was hydrogenated to give the free tripeptide, and the relative amounts of the two diastereoisomers were determined as described by Izumiya (8). The results obtained are summarized in Table 1.

Racemization via oxazolone formation is essentially a first order reaction with respect to the reacting species, since it concerns only one amino acid unit. On the other hand, peptide bond formation is second order with respect to the reactants, and therefore, the relative rates of these two processes is concentration dependent. In order to give an internally consistent set of results, all reactions (except that with DCC alone) were done using the same initial concentrations of reactants. The effect of changing the concentration of reactants can be seen by comparing the racemization obtained in our experiment with DCC/HOSu (2.8%) with that reported by Izumiya for the same coupling agent (0.0%). These values reflect the four-fold decrease in initial concentrations in our experiments. The larger solvent volumes, and hence lower reactant concentrations, used for our experiments were dictated by the physical properties of the resins used.

Table 1

	Temp. (°C)	Time (hrs.)	% yield	% gly-D-ala-leu in free tripeptide
DCC[a)]	0	20	>95	24.6
DCC/HOSu[b)]	0	40	96	2.8
poly-HOSu/DCC[c)]	22	22	73	6.4
4-OH-3-NO_2-polystyrene/DCC[c)]	22	20	49	37.4
EEDQ[d)]	22	17	94	6.0
poly-Q[d)]	22	22	72	6.0

Experimental Conditions: L-leucine benzyl ester was prepared from the p-toluene sulfonate salt (100 mg) and TEA (35µl) by equilibration for 0.5 h. This was then coupled with:

a) Cbz-gly-L-ala (71.9 mg) in DMF (1.9 ml).

b) Cbz-gly-L-ala (71.9 mg) and HOSu (1.8 equiv.) in DME (10 ml).

c) Cbz-gly-L-ala-loaded resin[1] in DME (10 ml).

d) Cbz-gly-L-ala (71.9 mg) in DME (10 ml).

All coupling reagents were present in 1.1 mole equiv. The yields of Cbz-gly-L(D)-ala-leu-OBzl are based on H-L-leu-OBzl. Neutral products were isolated as usual, converted to the free tripeptide and analyzed by published procedures (9). Racemic gly-D,L-ala-L-Leu prepared from gly-D,L-ala was used as a standard.

[1]Resins were loaded and assayed by published procedures (3-6). Loading of 4-OH-3-NO_2-polystyrene was unsuccessful in DMF, but successful in acetonitrile-DMF (30:1).

Discussion of Results:

As shown by Izumiya, DCC/HOSu gave considerably less racemization than the other monomeric coupling agents examined, while DCC alone, even with a relatively high concentration of reactants, gave too much racemization to be tolerated in any synthetic scheme.

Comparison of the "active ester" resins indicated that while the use of poly-HOSu gave a moderate amount of racemization (6.4%), use of 4-OH-3-NO_2-polystyrene gave an almost entirely racemic product. Use of this latter support in coupling reactions would be limited to linking either fragments with glycine or proline as the C-terminal amino acid, or single amino acids where a urethane blocking group was employed (9). The synthesis, in good yield, of fully-active bradykinin in a single step backing-off type of synthesis

employing urethane blocking groups shows that this resin can be used to advantage within these limitations (6).

Use of the solid-phase coupling reagent, poly-Q, resulted in no more racemization than obtained with its monomeric counterpart, EEDQ, which is now finding wide use in peptide synthesis. Again, as with the "active ester" resins, use of urethane blocking groups in a backing-off procedure might entirely eliminate the formation of the undesired diastereoisomer.

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