Active Esters and Resins in Peptide Synthesis: the Role of Steric Hindrance

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Summary In solid-phase peptide synthesis, because of the steric hindrance caused by the polymeric support the choice of activating groups should be directed by experiments carried out on the resin and not by the relative reactivities measured in solution.

THE significance of steric hindrance is not unknown in peptide synthesis; *e.g.*, the use of the easily removable triphenylmethyl (trityl) amino-protecting group is greatly limited by its bulkiness, yet it can be applied for the protection of the amino-group of glycine, where at least the side-chain of the amino-acid does not increase the hindrance. Concerns about steric hindrance were raised by the introduction of solid-phase peptide synthesis,[1,2] especially since Merrifield reported[1] that active esters cannot be used with his technique. The error in this observation could be demonstrated[3] by the application of *p*-nitrophenyl esters[4,5] and it became obvious that active esters are not only applicable in the solid-phase approach, but in the case of certain amino-acids, particularly asparagine and glutamine, only by the use of active esters can a single product be obtained, while with condensing agents such as dicyclohexylcarbodi-imide[6] formation of nitrile derivatives[7] from the carboxamides results in undesirable mixtures. An additional advantage of active esters is derived from the conveniently simple determination of the leaving component (*e.g.*, *p*-nitrophenol): monitoring of the acylation reaction by u.v. spectra of the filtrates provides evidence for the completion of the reaction.[8]

The exclusive use of *p*-nitrophenyl esters for acylation in solid-phase peptide synthesis was adopted with good results by some laboratories.[9,10] On the other hand, unsatisfactory rates[11] requiring the addition of catalysts were reported in connection with otherwise highly reactive esters, and in one case[12] complete lack of success was found when acylamino-acid esters of hydroxysuccinimide[13] were applied. This discrepancy prompted a comparison of the reaction rates of different active esters toward nucleophiles which are attached to a polymeric support.

The results of these comparisons are summarized in the Table, which clearly demonstrates the not unexpected[14] differences in reaction rates of different active esters and the influence of the side-chain of the nucleophile. However, the hindrance by the matrix of the polymeric support is a factor sufficient to change even the order of relative reactivities; *e.g.*, the pentachlorophenyl[15] ester of t-butyloxycarbonyl-L-leucine which in *solution* reacts by an order of magnitude faster than the corresponding *p*-nitrophenyl

Half reaction times (min. active esters of t-butyloxycarbonyl-L-leucine

Nucleophile	–ONO	–ONM	–ONP	–PCP
Benzylamine ..	3	120	15	<1
Gly–*O*–Bu^t ..	12	500	60	4
Val–*O*–Bu^t ..	90	10,000	840	60
Gly–resin	150	—	840	1100
Val–resin	600	—	2400	1800

The reactions were carried out in ethyl acetate at room temperature with 0·02M concentration of the reactants. Rates were measured by the u.v. absorption of the liberated phenols. *Abbreviations*: –ONO: *o*-nitrophenyl ester; –ONM: *m*-nitrophenyl ester; –ONP: *p*-nitrophenyl ester; –PCP: pentachlorophenyl ester.

ester, is only about as active as the latter when the nucleophile (the amino-group of glycine) is attached to the Merrifield resin. Furthermore, while in solution the *o*-nitrophenyl ester of protected leucine is less reactive (*e.g.*, towards glycine t-butyl ester) than the pentachlorophenyl ester of the same acid, with glycyl resin the same *o*-nitrophenyl ester reacts faster than the pentachlorophenyl ester.

In addition to the combined hindering effect of the activating groups, amino-acid side-chains and the resin-matrix, the influence of the growing peptide chain has also to be considered. The observations here presented suggest that in solid-phase synthesis with active esters the choice of activating groups should not be directed by relative reactivities measured in solution. More valid information can be obtained from acylation reactions carried out on the resin to be used in the actual synthesis.

(*Received, August 25th*, 1969; *Com.* 1291.)

[1] R. B. Merrifield, *J. Amer. Chem. Soc.*, 1963, **85**, 2149.
[2] R. L. Letsinger and M. J. Kornet, *J. Amer. Chem. Soc.*, 1963, **85**, 3045.
[3] M. Bodanszky and J. T. Sheehan, *Chem. and Ind.*, 1964, 1423.
[4] M. Bodanszky, *Nature*, 1955, **175**, 685.
[5] M. Bodanszky, *Ann. New York Acad. Sci.*, 1960, **88**, 655.
[6] J. C. Sheehan and G. P. Hess, *J. Amer. Chem. Soc.*, 1955, **77**, 1067.
[7] D. T. Gish, P. G. Katsoyannis, G. P. Hess, and R. J. Stedman, *J. Amer. Chem. Soc.*, 1956, **78**, 5954; C. Ressler, *ibid.*, 5956.
[8] M. Bodanszky and J. T. Sheehan, *Chem. and Ind.*, 1966, 1597.
[9] S. Hörnle, *Z. physiol. Chem.*, 1967, **348**, 1355.
[10] V. Weber, S. Hörnle, G. Grieser, K. H. Herzog, and G. Weitzel, *Z. physiol. Chem.*, 1967, **348**, 1715.
[11] H. C. Beyerman, C. A. M. Boers-Boonekamp and H. Maassen Van Den Brink-Zimmermannova, *Rec. Trav. chim.*, 1968, **87**, 257.
[12] H. Klostermeyer, *Chem. Ber.*, 1968, **101**, 2823.
[13] G. W. Anderson, J. E. Zimmerman, and F. Callahan, *J. Amer. Chem. Soc.*, 1963, **85**, 3039.
[14] V. Gut and J. Rudinger, "Peptides 1968" (Proceedings of the Ninth European Peptide Symposium), North Holland, Amsterdam, 1968, p. 185.
[15] G. Kupryszewski and M. Kaczmarek, *Roczniki Chem.*, 1961, **35**, 935.