Tetrahedron Letters No.21, pp. 1787-1790, 1970. Pergamon Press. Printed in Great Britain.

A COMPARATIVE STUDY OF TERMINATING AGENTS FOR USE IN SOLID-PHASE PEPTIDE SYNTHESIS

Lowell D. Markley and Linneaus C. Dorman
Edgar C. Britton Research Laboratory, The Dow Chemical Company, Midland, Michigan 48640

(Received in USA 27 January 1970; received in UK for publication 6 April 1970)

In an early publication, Merrifield[1] described the use of a terminating agent - a mixture of acetic anhydride and triethylamine in DMF - in the solid-phase synthesis of H-Leu-Ala-Gly-Val-OH. The terminating agent was used to block any N-terminal amino groups which had not reacted in the coupling steps. Blake and Li[2] also employed this procedure to assure complete acylation of the N-terminal amino group in the solid-phase synthesis of a heptapeptide. They reported that the acetylation procedure consumed 97% of the free amine during the reaction time employed. Recently Wieland[3] reported the use of 3-nitrophthalic anhydride as a terminating agent.

A terminating agent for solid-phase peptide synthesis application should eliminate the formation of difficultly separable peptide impurities without adversly affecting the desired peptide being synthesized. Ideally, the terminating agent must (a) have a low steric requirement for reaction at difficultly accessible resin sites, (b) have a high reactivity towards the amino group being terminated, yet no affect on the peptide bond or other functionality on the peptide being synthesized, (c) form a covalent bond with the amino group being terminated that will be stable to all of the reaction conditions used in the synthetic procedure, (d) cause no racemization of the peptide being synthesized, (e) be stable at room temperature while stored in a suitable solvent, e.g. methylene chloride or dimethylformamide, and (f) facilitate separation of terminated amino acid or peptide fragments from the desired peptide being synthesized.

A large number of amine reagents were examined. After preliminary experiments and evaluation of these in light of the criteria set forth above, there remained two reagents worthy of further study. These reagents were methanesulfonyl chloride-triethylamine, with *in situ* formation of sulfene,[4] and N-acetylimidazole.[5] Acetic anhydride-triethylamine, previously reported, was utilized as a basis for comparison.

The solid-phase synthesis of the tetrapeptide, H-Leu-Ala-Gly-Val-OH, was chosen for the model

TABLE I

Solid-Phase Synthesis of H-Leu-Ala-Gly-Val-OH with the Use of Terminating Agents

Terminating Agent	Time	% H-Val-OH	% H-Gly-Val-OH[c]	% H-Ala-Gly-Val-OH	% H-Leu-Ala-Gly-Val-OH	% Yield of Tetrapeptide[a]
No terminating agent 80% Amino acid + DCCI	--	22	16.7	8.6	52	38%[b]
No terminating agent Two fold excess amino acid + DCCI	--	--	--	8.2	91.8	66%
10 Fold excess $(CH_3CO)_2O$ + Et_3N 80% Amino acid + DCCI	40 min	1.00	2.89	1.67	94.4	42%
10 Fold excess CH_3SO_2Cl + Et_3N	40 min	0.77	--	2.2	97	40%
80% Amino acid + DCCI	60 min	--	--	2.2	97.8	48%
10 Fold excess N-acetylimidazole 80% Amino acid + DCCI	45 min	--	--	1.5	98.5	44%

[a]The yield is based on the amount of starting BOC-Valine resin ester and the amount of tetrapeptide formed as determined with an automatic amino acid analyzer (see text).

[b]Two minor peaks, possibly attributable to other peptide fragments, were also observed.

[c]H-Ala-Val-OH elutes with H-Gly-Val-OH. Its presence in the mixtures was not confirmed.

system for the evaluation of these three terminating agents. The syntheses were conducted utilizing 1 mmole of BOC-Valine-OCH_2  (~ 1 mmole Valine/gram). The coupling reactions were run for 2 hours using dicyclohexylcarbodiimide as the coupling agent in methylene chloride. Intentionally, only 0.8 equivalent of amino acids and DCCI were used so that considerable amounts of uncoupled amino acids would remain after each coupling step. Following each coupling step, the peptide-resin was washed with 1 ml of triethylamine dissolved in 15 ml of methylene chloride and then with methylene chloride (three 15-ml portions). The peptide-resin was then treated with 10 mmoles (a ten-fold excess with respect to the BOC-valine resin ester) of the terminating agents dissolved in methylene chloride for the length of time designated in Table I. Following the synthesis, the tetrapeptide was cleaved from its resin support with hydrogen bromide in trifluoroacetic acid.[1] The crude peptide mixture remained after removal of volatile acids in vacuo was subjected to analysis by an automatic amino acid analyzer which was adapted for analysis of peptides of this model system.

As shown in Table I, N-acetylimidazole exhibited the most efficient termination of the three termination reagents studied. To further test the terminating agents, sulfene and N-acetylimidazole, the tripeptide resin BOC-Ala-Gly-Val-OCH_2  was prepared and treated with 80% of the equivalent amount of leucine and DCCI. The termination reactions were again run using a ten-fold excess of terminating agent in methylene chloride. As shown in Table II, the best results were again obtained with N-acetylimidazole, where less than 1% of unreacted tripeptide was found after running the termination reaction for 1 hour. On the basis of this study, N-acetylimidazole is concluded to be a very effective terminating agent in the solid-phase synthesis of peptides.

The authors would like to acknowledge the very helpful discussions and suggestions of Professor M. Calvin. We also wish to thank Messrs. Mapes, Contino and Frantz of the Special Services Laboratory of The Dow Chemical Company for their help and cooperation in performing the peptide analyses.

References

1. R. B. Merrifield, J. Am. Chem. Soc., 85, 2149 (1963).
2. J. Blake and C. H. Li, J. Am. Chem. Soc., 90, 5882 (1968).
3. T. Wieland, C. Birr and H. Wissenbach, Angew. Chem. internat. Edit., 8, 764 (1969).
4. W. E. Truce, R. W. Campbell and J. R. Norell, J. Am. Chem. Soc., 86, 288 (1964).
5. H. A. Staab, Angew. Chem. internat. Edit., 1, 351 (1962).
6. L. C. Dorman, Tetrahedron Letters, 2319 (1969).

TABLE II

Coupling of BOC-Leu-OH and H-Ala-Gly-Val-OCH_2-⟨O⟩-Ⓡ in the Evaluation of Sulfene and N-Acetylimidazole as Terminating Agents

	Time	% H-Ala-Gly-Val-OH	% H-Leu-Ala-Gly-Val-OH	% Termination as Shown by Pyr·HCl[a]
No terminating agent	--	43%	57%	54%
80% Amino acid + DCCI				
10 Fold excess CH_3SO_2Cl + Et_3N	60 min	4.5	95.5	94.3
80% Amino acid + DCCI				
10 Fold excess N-acetylimidazole	30 min	3.1	96.9	94.3
80% Amino acid + DCCI	60 min	0.9	99.1	> 98

[a]Non-destructive method for determination of completeness of termination reaction, see Ref. 6.