Solid Phase Peptide Synthesis using N_α-Fluorenylmethoxycarbonylamino Acid Pentafluorophenyl Esters

Eric Atherton and Robert C. Sheppard

Medical Research Council Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

Fmoc-amino acid pentafluorophenyl esters have been used successfully in the synthesis of a difficult decapeptide sequence using a polar reaction medium and resin support.

Traditionally,[1] solid phase peptide synthesis is carried out using t-butoxycarbonyl (Boc) amino acids activated *in situ* with an equivalent amount of dicyclohexylcarbodi-imide. A significant advance was the introduction of preformed Boc[2,3] and fluorenylmethoxycarbonyl (Fmoc) amino acid anhydrides[4,5] in both polystyrene[2,5] and polyamide-based[3,4] solid phase synthesis, avoiding contact of the reactive resin-bound amino group with the activating reagent. Acylation reactions are rapid, especially in polar media such as dimethylformamide.[6]

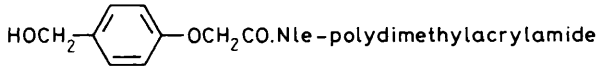
Activated esters (particularly *p*-nitrophenyl[7,8] or trichlorophenyl[9] derivatives) have also been used from time to time in solid phase synthesis but reaction rates may be low even in the presence of catalysts. Again polar reaction media are to be preferred.[7—9] This last consideration is particularly relevant in polyamide-based synthesis since this resin support is totally compatible with a wide range of aprotic polar organic solvents.

Recently we have sought alternative activated Fmoc-amino acid derivatives which would combine the high reaction rates and freedom from side reactions achievable with symmetrical anhydrides with the crystallinity, stability, and ease of handling of activated esters. Manual pre-activation† at each step in the synthesis would be avoided and mechanisation substantially simplified. We report now experiments in the polyamide solid phase series which suggest that Fmoc-amino acid pentafluorophenyl esters may be generally suitable when used in polar dimethylformamide solution in the presence of 1-hydroxybenzotriazole as catalyst.

For a test case we selected again[3,4] the very difficult acyl carrier protein 65—74 sequence (I).[11] Earlier attempts to assemble (I) using *p*-nitrophenyl esters in the presence of the catalyst 1-hydroxybenzotriazole were quite unsuccessful, although excellent syntheses were achieved using symmetrical anhydrides. Fmoc-amino acid pentafluorophenyl esters were prepared following the procedures of Kisfaludy and Schön.‡[12] The poly(dimethylacrylamide) resin[3] (II) was functionalised with an internal reference norleucine residue and with the acid-labile *p*-alkoxybenzyl alcohol linkage agent as usual.[4] Esterification of the C-terminal Fmoc-glycine residue utilised the preformed symmetric anhydride with dimethylaminopyridine catalysis.[4] The progress of all subsequent acylation reactions was monitored by sensitive ninhydrin[13] and trinitrobenzenesulphonic acid§[14] colour tests for residual amine, and by later amino acid analysis.

H.Val.Gln.Ala.Ala.Ile.Asp.Tyr.Ile.Asn.Gly.OH
1 10

(I)



(II)

Fmoc-asparagine pentafluorophenyl ester (five-fold excess in dimethylformamide) reacted completely with deprotected[4] glycyl resin at the time of the first colour test (25 min). The reaction was allowed to continue for a total of 50 min. Sterically hindered Fmoc-isoleucine pentafluorophenyl ester reacted with the resulting asparaginyl–glycyl resin much more sluggishly. Positive colour tests were obtained after 49 min. After 60 min 1-hydroxybenzotriazole catalyst was added. Only very faint colour tests were obtained after an additional 45 min and the reaction was terminated after a total reaction period of three hours. *O*-t-butyl-Fmoc-tyrosine pentafluorophenyl ester reacted completely within 25 min. This reactivity pattern was confirmed with the succeeding residues. Complete acylation was indicated at the first colour test for t-butyl

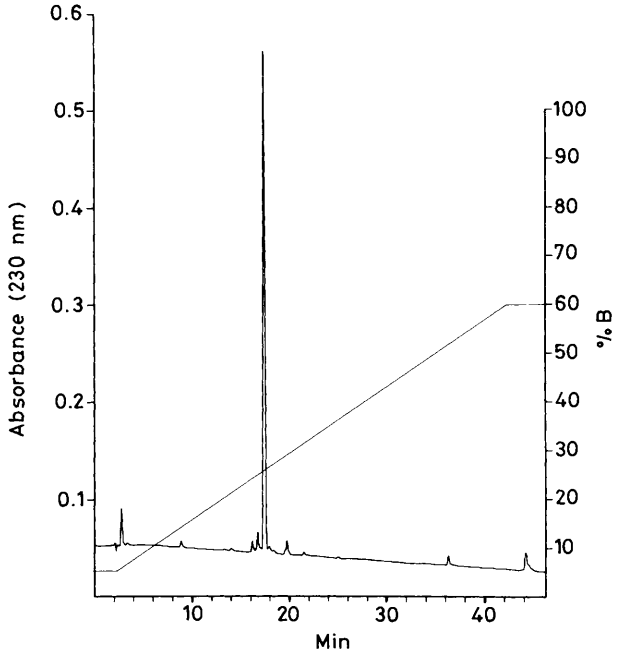


Figure 1. Analytical h.p.l.c. of total crude decapeptide on Aquapore RP-300. Reservoir A contained 0.1% aq. trifluoroacetic acid; B contained 90% acetonitrile, 10% A. After 2 min elution with 5% B, a linear gradient of 5—60% B was developed over 40 min at a flow rate of 1.5 ml/min. On μ-Bondapak C_{18}, reservoir A contained 0.01 M ammonium acetate, pH 4.5; B, 90% acetonitrile, 10% A. After 2 min at 15% B, a linear gradient of 15—35% over 40 min eluted the decapeptide at 11.5 min.

† Storage of pre-prepared symmetrical anhydrides has been suggested (ref. 10) but not widely adopted.

‡ M.p.s were generally in good agreement with those reported (ref. 12) although t.l.c. examination showed in most cases some contamination with dicyclohexylurea and with the starting Fmoc-amino acid. Improved preparative procedures are being sought.

§ In our hands the sensitivity of the test is much increased if the reagent is freshly dissolved in dimethylformamide containing 10% di-isopropylethylamine.

aspartate-6 (10 min), alanine-4 (13 min), and alanine-3 (5 min). Isoleucine-5 again required catalysis by hydroxybenzotriazole which was added after 43 min and gave complete reaction after an additional 50 min. An anomalous result was obtained after addition of Fmoc-glutamine-2 pentafluorophenyl ester with only occasional resin beads giving positive colour tests for residual amine in a generally colourless bulk background. Some shrinkage of the resin also occurred at this stage. Reaction was complete 35 min after the addition of catalyst. The terminal valine residue was nearly complete after 25 min and was left overnight without catalysis.

The completed decapeptide was cleaved from the resin with 95% trifluoroacetic acid. Residual resin analysis showed cleavage to be 96% complete. No significant peptide was lost from the resin during the course of the synthesis. The isolated yield of crude decapeptide determined by quantitative amino acid analysis was 91% (Found: Gly, 1.00; Asp, 1.89; Ile, 1.80; Tyr, 0.93; Ala, 1.90; Gln, 0.98; Val, 0.97). H.p.l.c. of this unpurified product is shown in Figure 1. The retention time of the principal peak is identical with that of previous preparations. A second h.p.l.c. system gave a very similar result.

We conclude that Fmoc-amino acid pentafluorophenyl esters may be valuable alternatives to symmetrical anhydrides for solid phase peptide synthesis. Their crystallinity and apparent stability greatly simplify and speed the conduct of solid phase synthesis. Reaction rates are substantially accelerated by the catalyst 1-hydroxybenzotriazole and appear likely to be adequate for most sequences. The high purity (>90%) of the crude reaction product obtained above shows that serious side reactions are not induced by the reagent. In contrast, earlier experiments[15] using reactive Fmoc-amino acid *N*-hydroxysuccinimide esters gave quite unsatisfactory results, presumably due to side reactions introduced by the hydroxy component.

Received, 27th September 1984; Com. 1369

References

1 G. Barany and R. B. Merrifield, in 'The Peptides,' eds. E. Gross and J. Meienhofer, Academic Press, New York, 1980, vol. 2, p. 3.
2 F. Flor, C. Birr, and T. Wieland, *Liebigs Ann. Chem.*, 1973, 1601; H. Hagenmeier and F. Hartmut, *Z. Physiol. Chem.*, 1972, **353**, 1973.
3 E. Atherton, D. L. Clive, and R. C. Sheppard, *J. Am. Chem. Soc.*, 1975, 97, 6585; R. Arshady, E. Atherton, D. L. J. Clive, and R. C. Sheppard, *J. Chem. Soc., Perkin Trans 1*, 1981, 529.
4 E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, and B. J. Williams, *J. Chem. Soc., Chem. Commun.*, 1978, 537; E. Atherton, C. J. Logan, and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1981, 538.
5 C.-D. Chang and J. Meienhofer, *Int. J. Pept. Protein Res.*, 1978, **11**, 246.
6 S. B. H. Kent and R. B. Merrifield, in 'Peptides 1980,' (Proceedings of the 16th European Symposium) ed. K. Brunfeldt, Hensinger, Denmark, p. 328.
7 M. Bodanszky and J. T. Sheehan, *Chem. Ind., (London)*, 1964, 1423.
8 S. A. Kahn and K. M. Sivanandaiah, *Tetrahedron Lett.*, 1976, 199.
9 S. Sivanandaiah and S. Gurusiddappa, *Synthesis*, 1981, 365.
10 E. P.Heimer, C.-D. Chang, T. Lambos, and J. Meienhofer, *Int. J. Pept. Protein Res.*, 1981, **18**, 237.
11 W. S. Hancock, D. J. Prescott, P. R. Vagelos, and G. R. Marshall, *J. Org. Chem.*, 1973, **38**, 774.
12 L. Kisfaludy and I. Schön, *Synthesis*, 1983, 325.
13 E. T. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Anal. Biochem.*, 1970, **34**, 595.
14 W. S. Hancock and J. E. Battersby, *Anal. Biochem.*, 1976, **71**, 261.
15 E. Brown and R. C. Sheppard, unpublished results.