Feedback Control in Organic Synthesis. A System for Solid Phase Peptide Synthesis with True Automation

Linda Cameron, Morten Meldal, and Robert C. Sheppard

MRC Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH, U.K.

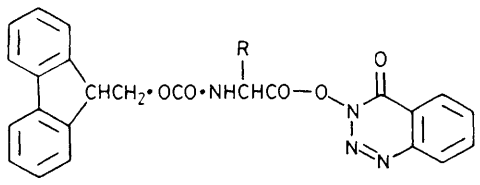
A technique for solid phase peptide synthesis using esters of 3,4-dihydro-3-hydroxy-4-oxobenzotriazine is described which permits real time monitoring of acylation reactions on the resin support with feedback control of the synthesis.

The development of solid phase techniques[1] has revolutionised peptide and oligonucleotide synthesis and has potential for other macromolecules. Commonly, the many liquid-transfer operations and reaction times are controlled by predefined computer or punched tape programs incorporated in automatic equipment. It has thus far not been possible to establish optimum reaction times and other parameters by automatically monitoring the progress of reactions as they proceed. The problem is an important one in peptide synthesis because reaction rates can vary very substantially as the peptide chain is extended,† both because of simple side chain steric factors and because of sequence-dependent aggregation effects within the resin matrix.[2,3] The latter are especially serious in view of their generally unpredictable onset, and are a common cause of failure in unmonitored syntheses. We report now an entirely new, non-invasive method for monitoring acylation reactions on the solid phase which permits for the first time true automation of peptide synthesis.

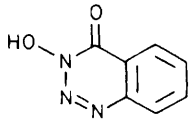
Recently[4] we described the use of Fmoc-amino acid esters (**1**) of 3,4-dihydro-3-hydroxy-4-oxobenzotriazine (**2**) in continuous flow, solid phase peptide synthesis. These esters are excellent acylating agents,[5] reacting cleanly with resin-bound amino groups with liberation of the hydroxy component (**2**). The latter is a sensitive indicator for unreacted amine, giving strong absorption in the visible region ($\lambda_{max.}$ 440 nm) on ionisation. Thus addition of reagent (**1**) to a primary or secondary amine-resin gives an immediate intense yellow colouration which fades as the reaction proceeds to completion. In the absence of dissolved base, the permeating solution remains colourless throughout. We have constructed a simple photometric system capable of measuring this colour intensity on columns of polydimethylacrylamide resin.[6] Essentially, light from a low voltage quartz–halogen source is focused onto a *ca.* 4 mm bed of the translucent resin support, and the diffused, transmitted light refocused through a 440 nm narrow band pass filter onto a phototransistor detector. A magnetically operated shutter mechanism limits exposure of the resin to the light source. The detector output is digitised, sampled, and processed directly by the controlling microprocessor. A typical real time plot of the fall in absorption to a steady baseline state as acylation proceeds is shown in Figure 1.

The fluctuations in the descending trace of Figure 1 are due to absorption by the recirculating acylating species (**1**) and unionised hydroxy component (**2**) in solution. They can be simply removed by averaging the data over a time corresponding approximately to the recirculation period. Figures 2—4

† This is apparently not the case in solid phase oligonucleotide synthesis where internucleotide bond formation occurs at similar rates regardless of the purine or pyrimidine base involved.



(1)



(2)

H—Val—Gln—Ala—Ala—Ile—Asp—Tyr—Ile—Asn—Gly—OH

(3)

H—Asp—Asp—Glu—Val—Asp—Val—Asp—Gly—Thr—Val—Glu—Glu—Asp—

—Glu—Gly—Lys—Ser—Tyr—Gly—OH

(4)

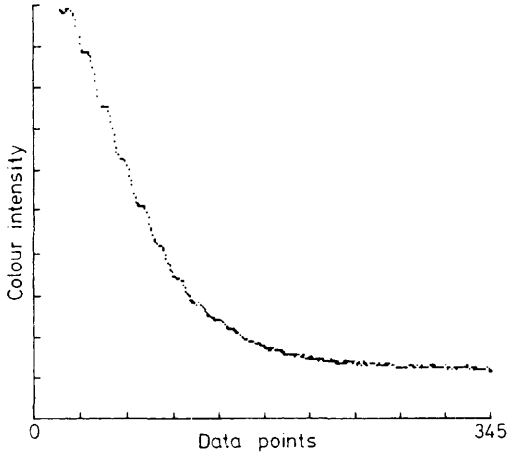


Figure 1. Plot of resin colour intensity *versus* time for a model coupling of Fmoc·Val·ODhbt with isoleucyl-resin. 345 Readings were collected at 12 s intervals. The analogue data were digitised and plotted directly.

show results obtained during actual peptide synthesis using the Fmoc-polyamide continuous flow procedure.[6] The data are now plotted to show sample addition (Figure 2,a), fall in absorption as acylation proceeds (b), and the differences between successive readings (c). These last are plotted on the very much expanded vertical scale of −5 to +5, with the horizontal delimiters shown set at −1 and +1. In these early experiments, acylation was arbitrarily considered complete when 5 successive difference readings lay between these limits. The acylation step was automatically terminated after an additional (arbitrary) 10 min period.

The system was tested initially by solid phase synthesis of the difficult acyl carrier protein decapeptide sequence (**3**)

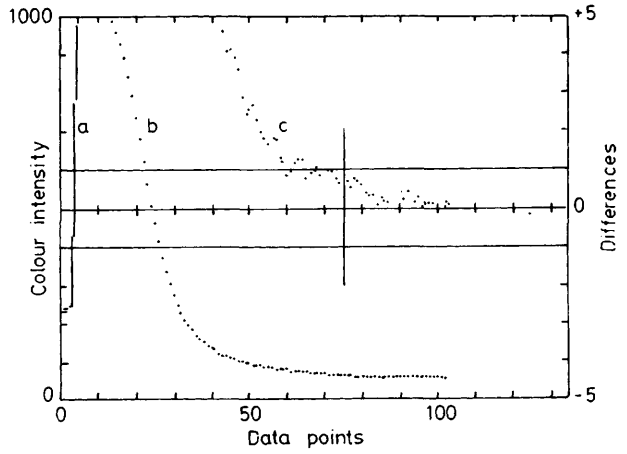


Figure 2. Coupling of Fmoc·Asn·ODhbt with glycyl-resin in the synthesis of acyl carrier protein decapeptide residues 65—74 (**3**). The vertical line marks the computer-determined end point. For details see text.

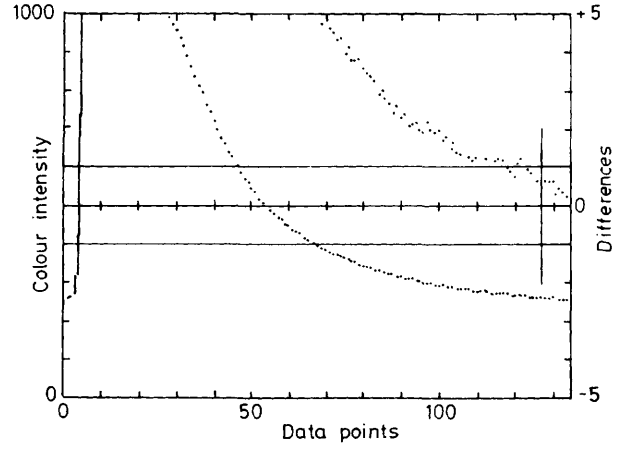


Figure 3. Coupling of Fmoc·Tyr(Bu^t)·ODhbt with asparaginylglycyl-resin. For details see text.

which contains normal and sterically hindered residues, as well as one step at which massive hindrance due to internal aggregation occurs. Two-fold excesses of Fmoc-amino acid esters (**1**) were used for each peptide bond-forming step except the last (see below). The controlling microcomputer was programmed to collect a maximum of 135 readings at 20 s intervals. Figure 3 is the record for formation of the first peptide bond (asparagine to glycine). The end point was detected automatically at 23.2 min. The following sterically hindered isoleucine residue (Figure 3) was slower at 40 min, as expected. Successive residues gave end points at 19.4 min (Tyr), 17.4 min (Asp), 35.8 min (Ile), 17.8 min (Ala), 17.8 min (Ala), and 21.8 min (Gln). The last acylation step (valine to glutamine) is known from previous experience to be exceptionally slow owing largely to internal aggregation within the resin matrix. A four-fold excess of the activated ester was used. It was clearly incomplete (Figure 4) at the end of the time set for data collection (45 min). The count-down was suspended manually‡ and the reaction allowed to proceed

‡ This now occurs automatically. In the absence of operator intervention, acylation continues for 999 min if the end point is not reached during the data collection period. Data collection has also been extended to 250 readings at 30 s intervals.

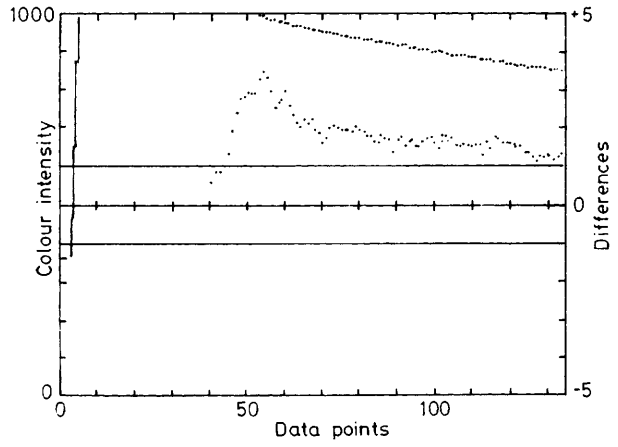


Figure 4. The final step (coupling of Fmoc·Val·ODhbt to *N*-terminal glutamine) in the assembly of (**3**). For details see text.

overnight. After deprotection and cleavage (92%) from the resin, the crude decapeptide (found: Val, 0.93; Glu, 0.99; Ala, 1.92; Ile, 1.64; Asp, 2.01; Tyr, 0.93; Gly 1.00) obtained was of good quality with h.p.l.c. profile comparable to that of previous, manually controlled syntheses. An equally satisfactory synthesis of the nineteen residue part sequence (**4**) of the calcium binding protein endoplasmin has since been achieved in similar manner.

We have previously shown that deprotection reactions in solid phase peptide synthesis may be simply monitored for completion.[6] The experiments on the acylation step described above now complete the basis for true automation of solid phase synthesis operated under continuous flow conditions. No additional reagents, resin removal, or other interference with the synthetic procedure is required. The data are produced, displayed to the operator and computer-interpreted as the reactions are proceeding and at a time when remedial action can be taken automatically or manually if required. There can be substantial speeding of synthesis, but the main importance lies in the detection of particularly slow steps which with preset reaction times could otherwise cause the synthesis to fail.

Received, 28th October 1986; Com. 1542

References

1 For a review see: G. Barany and R. B. Merrifield, in 'The Peptides,' eds. E. Gross and J. Meinenhofer, Academic Press, New York, 1980, vol. 2, p. 3.
2 S. B. Kent, in 'Peptides Structure and Function,' Proceedings of the 9th American Peptide Symposium, Toronto, 1985, Pierce Chemical Co., Rockford, 1985, p. 407.
3 E. Atherton and R. C. Sheppard, in ref. 2, p. 415.
4 E. Atherton, L. Cameron, M. Meldal, and R. C. Sheppard, *J. Chem. Soc., Chem. Commun.*, 1986, 1763.
5 W. König and R. Geiger, *Chem. Ber.*, 1970, **103**, 2034.
6 A. Dryland and R. C. Sheppard, *J. Chem. Soc., Perkin Trans. 1*, 1986, 125.