Peptide Synthesis. Part 1. Preparation and Use of Polar Supports based on Poly(dimethylacrylamide)

By **Reza Arshady, Eric Atherton, Derek L. J. Clive,** and **Robert C. Sheppard,*** M.R.C. Laboratory of Molecular Biology, Hills Road, Cambridge CB2 2QH

Details are given of the preparation of two functionalised polar support materials based on poly(dimethylacrylamide). Their solvation and other properties make them particularly suitable for solid-phase synthesis of polar substances, particularly of peptides and oligonucleotides, and also for solid-phase peptide and protein sequencing. Procedures are described for solid-phase peptide synthesis and are illustrated by preparation of a decapeptide.

In 1972, investigations were begun in this laboratory into several aspects of solid-phase peptide synthesis. The principle [1,2] of stepwise assembly of a polypeptide chain anchored at its carboxy-terminus to an insoluble polymeric support is an attractive one, but has significant limitations. These arise largely from the need for near-quantitative reaction at every step in order to minimise accumulation of resin-bound impurities. The limits of the method are to a large extent set by the nearness to which quantitative reaction can be consistently achieved. In solution-phase peptide synthesis, optimisation of reaction conditions in terms of reaction media, protecting-group combinations, and deprotection and coupling reagents has been a constant goal. In solid-phase synthesis where analytical problems are more severe and the immediate effect of small changes in reaction conditions is less easily discernible, much less optimisation has taken place although the need is even greater. The vast majority of applications of solid-phase peptide synthesis have used techniques not greatly dissimilar from that introduced by Merrifield in 1964.[3] Our aims, therefore, have been to develop, through application of sound chemical principles, procedures less empirical than those of the early method. These studies have resulted, *inter alia*, in the design and synthesis of novel support systems more polar in character than polystyrene and thus more compatible in solvation properties with the growing peptide chain. Unlike polystyrene, these polymers are freely permeated and swollen by a wide range of polar organic solvents (and also by water). Their use allows both peptide-bond formation and deprotection reactions to be carried out in reaction media optimised for the reaction involved, rather than dictated by the characteristics of the resin support. A series of reversible linkage agents for attachment of the peptide to the solid support and cleavage under a variety of conditions have also been prepared, and new combinations of N^{α} and side-chain protecting groups devised. Together these developments constitute an efficient and versatile system of solid-phase synthesis in which all the reactions take place under exceptionally mild conditions.

The basis for much of our work was indicated in an early review lecture,[4] and a number of preliminary communications [5-9] and symposium accounts [10-15] have since been published. This series of papers presents the experimental basis for our results. They may be read in conjunction with a recent review article [16] which presents the general philosophy of our approach in greater detail. This present paper deals primarily with the preparation of novel solid support materials. It illustrates the application and value of polar polyamide supports by synthesis of a difficult decapeptide sequence. A concurrent series [17-20] reports application of the same solid supports to oligonucleotide synthesis. Their use in peptide and protein sequencing studies has also been described.[21]

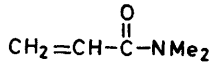
Reasons for our belief that polar support media freely permeated and solvated by dipolar aprotic solvents might be advantageous over the customary polystyrene resins have been given previously.[4,16] Essentially we felt that elimination of the solvation differences between the resin matrix and the attached peptide chains would minimise steric interference and entrainment phenomena, as well as providing opportunity for use of optimal polar solvent media in coupling and deprotection reactions. As far as we are aware, the only previous work on the application of polar supports in solid phase synthesis comprise an attempt by Anfinsen [22] to use modified synthetic polypeptides (with the objective of using enzymic reagents in aqueous solution), and synthesis of a pentapeptide on a carbohydrate support.[23] Exploration of carbohydrate-derived supports has also been mentioned by Merrifield.[24]

Results and Discussion

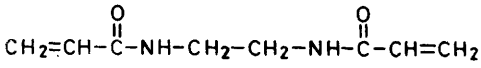
From the beginning,[4] our attention was directed towards polyamide supports for which solvation compatibility with attached peptides could be readily envisaged. Our first supports [10] were derived from commercial polyacrylamide modified by replacement of primary amide by dimethylamide groups so as to minimise internal hydrogen bonding. Possible use of polyacrylamide itself in solid-phase synthesis had, in fact, been mentioned previously by Inman and Dintzis,[25] but their suggestion was untenable because such resins are permeated only by good hydrogen-bond-forming solvents such as water. Modified polyacrylamide was freely permeated and swollen by dimethylformamide and other polar solvents (including water) and proved to be an effective support material in solid-phase synthesis.[10] Several peptide sequences were assembled on this resin,

including the simple test tetrapeptide Leu-Ala-Gly-Val, bradykinin, and the decapeptide sequence Boc-Val-Gln-Ala-Ala-Ile-Asp(OBzl)-Tyr(Bzl)-Ile-Asn-Gly (residues 65—74 of acyl carrier protein).[10] The last is apparently a particularly difficult sequence for which no satisfactory polystyrene-based synthesis had been reported at this time, despite substantial effort.[26] An undesirable structural feature of this modified commercial polyacrylamide resin, however, was its cross-linking by the formaldehyde-derived reagent, methylene bisacrylamide. This was expected to be appreciably labile under the acidic conditions used in then current deprotection strategies, and probably accounted for variability in the results obtained. Subsequent supports were therefore prepared in a more controlled and defined manner by copolymerisation of appropriate monomer mixtures.

For our first totally synthetic resin,[5] the monomer mixture was chosen so as to provide directly a stable cross-linked polymer appropriately functionalised for use in peptide synthesis without further major modification. Possible formation of reactive or sterically restricting (cross-linking) resin-bound side products during subsequent modification reactions was thus avoided. Opportunity was also taken to incorporate directly an internal reference amino-acid (β-alanine) within the resin matrix. Use of the amino-group of this amino-acid residue as the attachment point for the growing peptide chain enabled the degree of functionalisation of the polymer to be easily determined. Its presence also facilitated analytical operations during peptide synthesis. Thus the polymerisation mixture contained the basic structural monomer dimethyl acrylamide (1), the flexible but stable cross-linking agent



(1)



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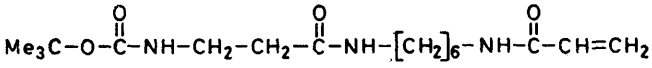
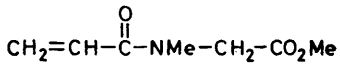


(3)

bis(acrylamido)ethane (2), and the internal reference and functionalising agent *N*-(*N*-t-butoxycarbonyl-β-alanyl)-*N'*-acryloylhexamethylenediamine (3). This last named monomer provided additionally a long and flexible spacer arm ensuring separation of the polymer and peptide chains. A suspension polymerisation technique was used, the immiscible solvents being water and 1,2-dichloroethane. Polymerisation was initiated with ammonium persulphate. A basic monomer : cross-linking agent : functionalising agent ratio of 100 : 12 : 7.3 provided a resin which swelled some ten times its dry bed volume in dimethylformamide and was functionalised with t-butoxycarbonylamino-groups to the extent of 0.3—0.4 milliequiv. g^{-1}. The polymer is simply converted into free amine by conventional acidic (HCl–AcOH) deprotection and neutralisation reactions.

Details of the preparation and use of this polymer are provided in the Experimental section but further discussion is not given here because in our view it has to some extent been superseded by a more recent resin. From the preparative point of view this second polymer presents a number of advantages. The β-alanine resin described above is derived by copolymerisation of monomers of widely different character. In particular, the functionalising agent (3) is only sparingly soluble in the two-phase polymerisation medium, and its inclusion in the monomer mixture increases the tendency for production of amorphous as opposed to beaded resin. Better results were obtained in this connection by use of a vibromix agitator rather than simple stirring (see Experimental section). Production of beaded resin with this particular momomer mixture was always a problem and could be achieved only on a small scale. Nevertheless, it should be emphasised that the amorphous polymer is chemically equally satisfactory in peptide (and oligonucleotide) synthesis, and in our hands has not presented any serious manipulative problems (*cf.* refs. 27 and 28). Extended syntheses carried out with its aid include β-endorphin (31 residues, four times) [6,8] and assemblies of pancreatic trypsin inhibitor (58 residues, twice).[12,29] The new resin described below is readily and reproducibly obtained in a beaded form. The functionalising agent (3) was also difficult to prepare, as was the somewhat simpler *N*-trifluoroacetyl-*N'*-acryloylhexamethylenediamine which was also investigated as a potential functionalising agent in polymer preparation. A new synthesis of monosubstituted derivatives of αω-diamines has recently been described [30] and may aid in the solution of this particular problem. Very recently, the hydrochloride salt of monoacryloylhexamethylenediamine has also been used directly for the preparation of amino-functionalised poly(dimethylacrylamide) [27] and poly(acryloylpyrrolidine).[28]

In the new resin, these problems were overcome by replacement of (3) by the simpler and readily prepared acryloyl derivative (4) of sarcosine methyl ester.[9] The



(4)

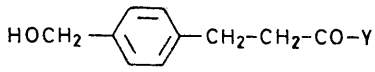
resulting polymer thus carried methoxycarbonyl groups rather than (protected) primary amines. Although this necessitates departure from the principle of minimum post-polymerisation modification discussed above, conversion of these methoxycarbonyl groups into amino-functions proved straightforward (see below). An

additional, possibly important feature of the functionalising monomer (4) is its close structural similarity to dimethylacrylamide (1). Although reactivity ratios for the various monomers have not been determined by us, the close similarity of (1) and (4) suggest comparable reactivity and hence likelihood of random distribution of (4) throughout the resin matrix. Clumping of functional groups may therefore be less likely than with mixtures of structurally more dissimilar monomers.

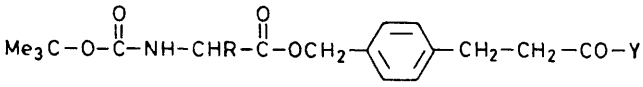
The polymer is easily obtained in a finely beaded form using the suspension polymerisation technique described in the Experimental section. The degree of functionality is now measured by the sarcosine content. However, the ninhydrin colour yield of this secondary amino-acid is particularly low, and as an analytical aid during peptide synthesis, more accurate results are obtained by incorporating a second internal reference before addition of the reversible linkage agent and elaboration of the peptide chain. This technique was adopted generally and is illustrated below. Variation of the content of (4) in the polymerisation mixture has thus far enabled resins functionalised to the extent of 0.13—1.0 milliequiv. g^{-1} to be prepared without difficulty. One gram of these methoxycarbonyl functionalised resins (dry volume *ca.* 2.2 ml) occupy a volume of 20—21 ml when swollen with a wide range of organic solvents (dichloromethane, dimethylformamide, pyridine, methanol) useful for peptide and oligonucleotide synthesis. In dioxan the swollen volume is only 10 ml. In the fully swollen state, both resins are physically stable and withstand agitation for many days in a Merrifield-style [31] glass reaction vessel without appreciable loss. In the collapsed, more granular form, the resins are more friable.

The sarcosine-containing polymer is readily functionalised with primary amino-groups by treatment with an excess of ethylenediamine. Reaction can be followed by loss of i.r. absorption at 1 745 cm^{-1} (methyl ester). Routinely the conversion is achieved by shaking with neat diamine overnight at room temperature. These mild conditions are to be contrasted with those (heating at 175 °C in ethylene glycol) necessary for similar functionalisation of commercial poly(acryloylmorpholine) (Enzacryl K2).[32] Originally a largely aqueous washing procedure was used for removing excess of ethylene diamine,[9] but this is now more easily achieved with organic solvents (Experimental section).

The primary amino-groups provide appropriate linkage points for the growing peptide chain. This particular reactive function was chosen because of the ease of efficient attachment through amide bond formation. and because of the flexibility it confers. Thus by using various simply designed and prepared intermediary linkage agents, peptide-resin bonds may be formed of varying stability and cleavage by a wide range of reagent types. This provides the basis of a versatile system of solid-phase synthesis in which protecting-group combinations may be easily altered. As mentioned above, additional internal reference-spacer amino-acid may be interposed between the resin matrix and linkage agent. The range of linking agents thus far employed has been listed elsewhere; [16] here we are concerned only with the simple benzyl alcohol derivative (5) which provides a peptide–resin benzyl ester bond comparable to that of conventional polystyrene-based solid-phase synthesis.



(5) Y = OH
(7) Y = $OC_6H_2Cl_3$-2,4,5



(6) Y = OH
(8) Y = $OC_6H_2Cl_3$-2,4,5

The linkage agent (5) is simply prepared by chloromethylation of β-phenylpropionic acid followed by iodide-ion-catalysed aqueous hydrolysis. It may be attached to the amino-functionalised resin either alone or already esterified to the first protected amino-acid [as in (6)]. Initially we considered [5] the latter procedure particularly favourable because it avoids formation of the benzyl ester linkage on the solid phase. Ester bonds are more difficult to establish quantitatively than are amides, and it was advantageous to carry out this step beforehand in free solution with isolation and purification of the resulting benzyl ester. A similar conclusion was evidently reached later by Merrifield and his colleagues [33] in connection with modified polystyrene resins. This approach was used in the work described here,[5] but the introduction of 4-dimethylaminopyridine as a particularly effective catalyst for ester formation under mild conditions [34] now enables separate introduction of the linkage agent and first protected amino-acid (see, *e.g.* refs. 7, 8, and 9).

Efficient and unambiguous acylation of (5) requires protection of the carboxy-group. To avoid lengthy synthetic procedures involving successive protection and deprotection steps (*cf.* ref. 33). it was appropriate to use, as protecting group Y, an ester stable to conditions for acylation of the benzyl alcohol function, but sufficiently reactive to undergo subsequent aminolysis by resin-bound amine. 2,4,5-Trichlorophenyl ester derivatives proved suitable. Formation of the ester derivative (7) was straightforward using dicyclohexylcarbodi-imide as the condensing agent, and this was in turn smoothly acylated by pre-formed symmetrical anhydrides of a range of t-butoxycarbonylamino-acids. The glycyl, alanyl, phenylalanyl, and γ-benzylglutamyl (8; R = H, Me, CH_2Ph, and $CH_2CH_2CO_2CH_2Ph$) have thus far been prepared.

The value of synthetic polyamide supports in peptide synthesis was initially demonstrated for the β-alanine-containing * resin by synthesis of the previously mentioned decapeptide sequences comprising residues 65—74 of acyl carrier protein. This was thought to be a particularly appropriate test case since the serious difficulties encountered during previous synthetic attempts on polystyrene had been ascribed to solvation phenomena within the resin matrix.[26] This was the main problem that polyamide resins were designed to minimise. In order that the results obtained should as far as possible reflect the properties of the new resin matrix-solvent combination, rather conventional methodology was adopted at this stage regarding other features of the system.† Thus t-butoxycarbonylamino-acids were used throughout and functional side-chains were protected as benzyl ester (aspartic acid) or 2,6-dichlorobenzyl ether (tyrosine)[35] derivatives. Anhydrous hydrogen chloride in acetic acid was used for deprotection at intermediary stages, and liquid hydrogen fluoride for final cleavage of all protecting groups, including the benzyl ester resin linkage. Coupling reactions, apart from those for asparagine and glutamine residues, were carried out using pre-formed symmetrical anhydrides.[36] This is a departure from the conventional *in situ* dicyclohexylcarbodi-imide activation employed by Marshall and his colleagues,[26] but the latter is inappropriate in the polar resin–polar solvent context. Activation of acylamino-acids by carbodi-imide in *e.g.* dimethylformamide is excessively slow as judged by dicyclohexylurea precipitation, and $O \longrightarrow N$ migration of the intermediate *O*-acylurea is favoured.[37] Prior activation with 0.5 equiv. of carbodi-imide in the preferred solvent methylene chloride was therefore employed, with isolation of the resulting symmetrical anhydride by filtration from precipitated urea, evaporation of the filtrate, and dissolution in dimethylformamide before addition to the resin. Other reasons for favouring symmetrical anhydrides as acylating agents in solid-phase synthesis have been given elsewhere.[16] Our belief that minimisation of chain-terminating side-reactions is to be aided by rapid acylation of free amino-groups has encouraged the use of large excesses of acylating species. In this respect, the extreme swelling of poly(dimethylacrylamide) by *e.g.* dimethylformamide (*ca.* 20—21 ml g^{-1}) compared with polystyrene (*ca.* 6—8 ml g^{-1}) is disadvantageous, since higher absolute amounts of acylating agent are required for equivalent concentration. This argument neglects possible partition effects[38] of acylating agent between the resin matrix and solvent domains. The 3—4-fold molar excesses normally employed[26] in conventional polystyrene-based syntheses may be equivalent to more than ten-fold in the more swollen polyamide case. More economic use of activated amino-acid derivatives may be obtained using more highly functionalised polyamide resins. This aspect is under current investigation. In the present work we have generally employed six-fold molar excesses of acylating species which gives very rapid reaction under the favourable polar reaction conditions in dimethylformamide. It is probable that high efficiency in acylation can be achieved in most instances using lower molecular proportions of activated amino-acid. On the other hand, repeated acylation reactions which are not infrequently required in conventional polystyrene-based synthesis have rarely proved necessary on polyamide resins, and not at all in the present work.

Asparagine and glutamine were introduced as *p*-nitrophenyl esters. Following the reports of König and Geiger[39] that active ester aminolysis is substantially accelerated in dimethylformamide solution by 1-hydroxybenzotriazole (in less polar tetrahydrofuran it has a retarding effect), our practice has been to add this catalyst to the acylation mixture and sometimes also to a preceding wash cycle.

All reactions (apart from symmetrical anhydride formation from t-butoxycarbonylamino-acids) were carried out in a modified Beckman 990 Peptide Synthesizer. As designed, this machine uses a stirred, Teflon or Teflon-lined glass reaction cup with a large unwetted Teflon surface above the liquid level. Using relatively involatile dimethylformamide as the main solvent, we observed accumulation of a white crystalline deposit on these upper surfaces, presumably the acetate or hydrochloride salt of the volatile tertiary amine used in the neutralisation step. The reaction cup was therefore replaced by a conventional glass reaction vessel,[31] totally wetted internally by rotation through *ca.* 180° in a wrist action shaker. This substitution eliminated the possibility of amine salt contaminating the reaction mixture during the acylation step. Some minor ancillary modifications to the Synthesizer were necessary. Filling of the glass reaction vessel was ensured by timed application of reduced pressure, initiated by opening of either transfer valve. A nitrogen feed line was installed in each metering vessel activated by opening of the appropriate transfer valve. This precluded ingress of air into the reaction vessel should reduced pressure be applied after filling was complete.

Assembly of the decapeptide sequence was straightforward. An additional internal reference-spacer ‡ amino-acid (Boc-leucine) was first added to the β-alanyl resin followed by the Boc-glycyl linkage agent (8; R = H). The symmetrical anhydride and activated ester coupling procedure used for these and subsequent steps are detailed in the Experimental section. The intermediate Boc-leucyl-β-alanyl resin was deprotected using hydrogen chloride in acetic acid; washing of the resin with t-amyl alcohol was used to avoid the exothermic mixing of dimethylformamide and acetic acid. The

* Use of the sarcosine-containing resin is illustrated with a different range of protecting groups in the following paper.

† Alternative procedures designed to take fuller advantage of the polyamide support system and also to generally milden the reaction conditions in solid-phase synthesis have been reported briefly,[7,8,16] and are discussed in detail in the following paper.

‡ The value of internal reference amino-acids as analytical aids in solid-phase synthesis has recently been re-stressed by Matsueda.[40]

following nine amino-acid residues were incorporated using the same general procedures.

Completion of reaction at each coupling step was verified by qualitative ninhydrin tests,[41] and samples were withdrawn for amino-acid analysis at each stage. In contrast to the results previously obtained on a polystyrene support,[26] uniformly high (>90%) analysis figures were obtained throughout (Figure 1). Amino-acid analysis of resin-bound peptides is notoriously unreliable (see, *e.g.* ref. 42); in the present case collapse of the support to insoluble and impermeable polyacrylic acid before complete amino-acid release is a potential source of error. After cleavage of the peptide from the resin with liquid hydrogen fluoride, the total peptide fraction gave good analytical figures for the decapeptide structure. A single purification by ion-exchange chromatography on diethylaminoethyl cellulose (Figure 2) gave apparently pure material as judged by amino-acid analysis, single spots on paper electrophoresis and on thin layer chromatography, and repeated ion-exchange chromatography. The overall yield was 48%.* Decapeptide from a later preparation (by Mr. B. J. Williams) gave at the same stage of purification the h.p.l.c. profile shown in Figure 3.

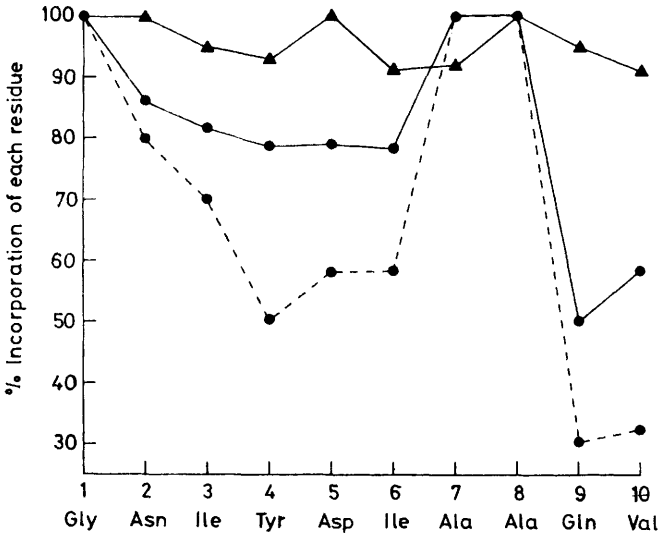


FIGURE 1 Amino-acid incorporation during assembly of acyl carrier protein residues 65—74. (●) on polystyrene; (——) by an essentially standard procedure; [26] (– – – –) using double acylation and other modifications;[26] (▲) present work on poly-(dimethylacrylamide)

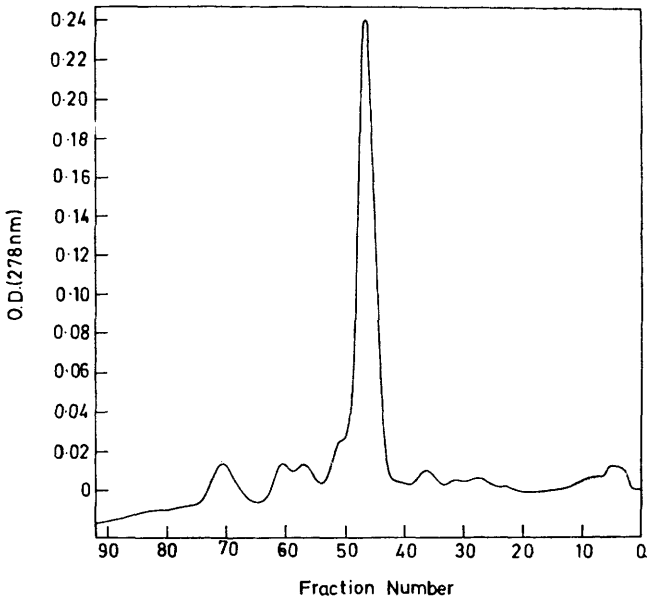


FIGURE 2 Chromatography of crude synthetic acyl carrier protein residues 65—74 on diethylaminocellulose DE52

We conclude that polar polyamide supports provide useful alternatives to polystyrene in solid-phase peptide synthesis. The most recent sarcosine-containing polymer is easily prepared in a finely beaded form from readily accessible starting materials. The excellent permeation of the resin by a wide range of polar organic solvents allows peptide bond formation to be carried out in a medium (dimethylformamide, dimethylacetamide, or other polar organic media) in which both the peptide and polymer are likely to be freely solvated and which is at the same time chemically suitable for the reaction processes involved. Functionalisation of the resin with primary amino-groups confers considerable flexibility and versatility by allowing easy attachment of a range of reversible linkage agents.[16] The polar character of the support makes it also useful in areas where polystyrene

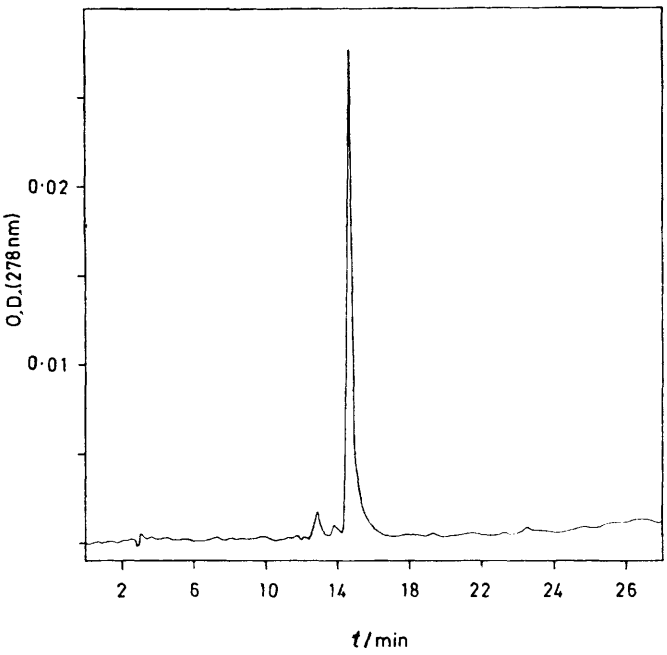


FIGURE 3 H.p.l.c. of decapeptide after single ion-exchange purification. Partisil ODS column, linear gradient of 5—60% MeCN in 0.01M NH_4OAc, pH 4.5 over 40 min, flow rate 1.5 ml min^{-1}

* This figure is lowered by inefficiencies in the ion-exchange chromatographic procedure in use at the time. Thus re-chromatography of the isolated decapeptide gave a recovery of 55%, although only a single peak was eluted from the column.

has in the past been less well suited, *e.g.* solid-phase oligonucleotide synthesis [17–20] and protein sequencing.[21] Other applications can be envisaged.

EXPERIMENTAL

Dimethylacrylamide was prepared according to the method of Ratchford and Fisher.[43] It was stored in the refrigerator in the presence of hydroquinone and distilled *in vacuo* (b.p. 71—72 °C at 12 mmHg) immediately before use.

NN′-*Bisacryloylethylenediamine*.—Anhydrous sodium acetate (217 g, 2.6 mol) and 1,2-diaminoethane (72 g, 1.2 mol) were added to anhydrous chloroform (1.2 l) and cooled to 0 °C in an ice-bath. Distilled acryloyl chloride (218 g, 2.4 mol) was added dropwise during 1 h to the stirred mixture which was then allowed to warm to 10 °C. Hydroquinone (2 g) was added, and the mixture refluxed for 1 h and filtered hot. The filtrate was allowed to cool and the crystalline product was collected, dissolved in hot chloroform (2 l) and the mixture filtered. On cooling the filtrate the product crystallised and was collected, dried over calcium chloride, and stored in the dark; yield 162 g (80%), m.p. 141—143 °C. A sample (40 g) recrystallised from chloroform gave 31 g, m.p. 143—144 °C (lit.,[29] m.p. 144—145 °C); t.l.c. (silica) in ethyl acetate–acetone (1 : 1), R_F 0.25 (single spot). An alternative general procedure has recently been described.[44]

N-(*t-Butoxycarbonyl-β-alanyl*)*hexamethylenediamine*.— t-Butoxycarbonyl-β-alanine (18.9 g, 100 mmol) and *N*-hydroxysuccinimide (11.5 g, 100 mmol) were dissolved in dimethoxyethane (200 ml) and the solution cooled to 0 °C. Dicyclohexylcarbodi-imide (22.7 g, 110 mmol) was added, and the mixture was then kept for 5 h at 0 °C and overnight at 4 °C before being filtered and the precipitated urea washed with dimethoxyethane (60 ml). The combined filtrate and washings were added dropwise to a cooled (4 °C) solution of hexamethylenediamine (34.8 g, 300 mmol) in dimethoxyethane (100 ml), and the mixture kept for 2 h at 0 °C and 3 h at room temperature. Filtration and evaporation of the filtrate gave a light yellow oil to which was added ice (500 g) and potassium hydrogensulphate (94 g) to pH 2. The mixture was filtered and the filtrate washed twice with ethyl acetate (2 × 100 ml). The aqueous solution was brought to pH 12 with 6N sodium hydroxide and extracted with chloroform (4 × 100 ml). The combined extracts were dried (Na_2SO_4) and evaporated to give an almost colourless oil (18.3 g, 64%), R_F in butanol–acetic acid–water (4 : 1 : 1) (silica) 0.35, with minor impurities at 0.07 and 0.25. The product was used in the following preparation without further purification.

N-(*t-Butoxycarbonyl-β-alanyl*)-N′-*acryloylhexamethylenediamine*.—Acryloyl chloride (3.56 g, 39 mmol) was dissolved in methylene choride (70 ml) and added dropwise to a stirred and cooled (0 °C) solution of *N*-(t-butoxycarbonyl-β-alanyl)hexamethylenediamine (5.29 g, 18.4 mmol) and triethylamine (3.78 g, 37 mmol) in methylene chloride (65 ml). The reaction mixture was stirred for 4 h at room temperature, washed with water, dried (Na_2SO_4), and evaporated to give an oil (5 g) which crystallised from nitromethane (yield 2.74 g). After recrystallisation from the same solvent, the *product* (2.47 g, 39%) had m.p. 123—125 °C (Found: C, 60.0; H, 9.1; N, 12.05. $C_{17}H_{31}N_3O_4$ requires C, 59.78; H, 9.15; N, 12.31%).

Copoly[*dimethylacrylamide–bisacryloylethylenediamine–N-(t-butoxycarbonyl-β-alanyl)-N′-acryloylhexamethylenediamine*].—(*a*) Cellulose acetate butyrate (Eastman Kodak, 17% butyryl, ASTM visc. 15, 33.1 g) was added to dichloroethane (AnalaR, 490 ml) contained in a multineck 2-l reaction vessel (Quickfit FR2LF) and stirred until dissolved. The flask was placed in a water bath at 46 °C and a solution of *N*-(t-butoxycarbonyl-β-alanyl)-*N*′-acryloylhexamethylenediamine (11.94 g, 34 mmol) in dichloroethane (520 ml) added. The flask was purged with oxygen-free nitrogen for 15 min. Ammonium persulphate (5.75 g) was added to a solution of dimethylacrylamide (54.7 g, 552 mmol), and bisacryloylethylenediamine (9.97 g, 59 mmol) in warm water and the whole added to the reaction flask. Stirring using a four-bladed, 8.7-cm diameter propeller was maintained at 490—520 r.p.m. for 5.25 h, and the mixture then left overnight. Next morning the polymer was collected with acetone and thoroughly washed by stirring several times alternately with water and then acetone. Fines were removed by decantation of an aqueous acetone supernatant after the bulk of the resin had settled. The yield of white, largely amorphous vacuum-dried polymer was 60 g (Found: β-Ala, 0.37 milliequiv. g^{-1}).

(*b*) *N*-(t-Butoxycarbonyl-β-alanyl)-*N*′-acryloylhexamethylenediamine (1.5 g, 0.43 mmol) dissolved in dimethylformamide (10 ml) was added to a cooled (0 °C) solution of cellulose acetate butyrate (6.0 g) in dichloroethane (150 ml). The mixture was agitated vigorously (Vibromixer type El, Chemie Apparateban, Zurich) and purged with nitrogen. A solution of bisacrylolyethylenediamine (1.1 g 6.5 mmol) in water (50 ml) was added followed by dimethylacrylamide (5.5 g, 55.5 mmol) and water (50 ml). The mixture was warmed in a thermostat to 52 °C and after 10 min ammonium persulphate (0.4 g) dissolved in water (10 ml) was added. Nitrogen flow and agitation was continued for 5 h with replacement of evaporated dichloroethane (50 ml) after 90 min. The polymer was collected in a porosity 2 sintered filter funnel and washed successively with dimethylformamide, acetone, water, acetone, dimethylformamide, acetone, and ether. The dried (P_2O_5) resin (6.8 g) consisted of nicely formed, regular spheres. (Found: β-Ala, 0.26 milliequiv. g^{-1}).

Sarcosine Methyl Ester Hydrochloride.—Methanol (400 ml) was cooled and stirred in an ice–salt bath and thionyl chloride (32.2 ml) added dropwise, followed by sarcosine (36 g, 0.4 mol) over 15 min. The mixture was stirred in the cold for *ca.* 20 min, allowed to warm to room temperature during 2 h, and then refluxed for 2 h. After cooling the methanol was evaporated and the residual solid recrystallised from methanol–ether to give the crystalline methyl ester hydrochloride (51 g, 90%), m.p. 121.5—122.5 °C.

Acryloylsarcosine Methyl Ester.—Sarcosine methyl ester hydrochloride (30.9 g, 0.22 mol) was dissolved in chloroform (280 ml) and cooled with stirring in an ice–salt bath. Triethylamine (61.9 ml, 44.75 g, 0.44 mol) was added dropwise during 10 min followed by a solution of acryloyl chloride (18.0 ml, 19.9 g, 0.22 mol) in chloroform (150 ml) during 1.5 h. The cooling bath was then removed and the reaction mixture stirred overnight. Evaporation of the chloroform gave a white solid which was triturated with ether (400 ml) and filtered. Hydroquinone (*ca.* 10 mg) was added to the filtrate which was then evaporated and the residual oil distilled *in vacuo* into a receiving flask containing a further 10 mg of hydroquinone. Acryloylsarcosine methyl ester (23 g, 66%) had b.p. 96 °C/0.4 mmHg; δ 3.18 and 3.32 (asymmetrical, 3 H, –CONMe– showing *cis-trans*

isomerism), 3.84 (s, 3 H, $-CO_2Me$), 4.38 (s, 2 H, $-CH_2-$), and 5.8—7.2 (3 H, CH_2=CHCO−).

Copoly(dimethylacrylamide–bisacryoylethylenediamine–acryloylsarcosine methyl ester).—Typically, cellulose acetate butyrate (12.5 g) was completely dissolved in dichloroethane (300 ml) and placed in a cylindrical fluted polymerisation vessel [45] fitted with a stirrer and nitrogen inlet and maintained at 50 ± 1 °C in a thermostatically controlled water bath. The solution was stirred at 450 ± 20 r.p.m. and flushed with N_2 for 10 min before the monomer mixture, consisting of dimethylacrylamide (15 g, 1.52 mmol) acryloylsarcosine methyl ester (1.25 g, 7.96 mmol), and bisacryloylethylenediamine (1.75 g, 10.4 mmol), diluted with cooled (5 °C) dimethylformamide–water (1 : 2), (150 ml) and mixed well with ammonium persulphate (2.25 g), was added. Polymerisation was allowed to continue under a very slow stream of nitrogen for *ca.* 15 h when the mixture was cooled, diluted with acetone–water (1 : 1), stirred until a homogeneous suspension was obtained, and filtered. The recovered polymer was washed and fine particles removed by stirring and decantation using acetone–water (1 : 2) (3 × 1 l) and then acetone (3—4 × 500 ml). The polymer was finally washed with ether (2 × 500 ml), collected by filtration, and dried (P_2O_5) *in vacuo*; yield *ca.* 15 g of completely beaded resin (Found: Sar, 0.35 milliequiv. g^{-1}).

Reaction of the Sarcosine Polymer with Ethylenediamine.—This conversion was usually carried out in the Peptide Synthesiser immediately prior to peptide synthesis. The foregoing resin (1 g) was suspended in ethylenediamine (redistilled, 32 ml) and shaken overnight at room temperature. Next morning the reaction vessel was drained and the resin washed automatically with dimethylformamide or dimethylacetamide (15 × 2 min), 10% ethyldi-isopropylamine in dimethylformamide or dimethylacetamide (3 × 2 min), and dimethylformamide or dimethylacetamide (5 × 2 min).

3-(p-*Hydroxymethylphenyl)propionic acid* (5).—3-(*p*-Chloromethylphenyl)propionic acid [46] (28 g, 0.14 mol) was heated in boiling water (600 ml) containing sodium iodide (1.5 g) for 15 min. On cooling an oil formed, which crystallised on scratching. After standing for several hours at 5 °C, the *hydroxymethyl derivative* (21.5 g, 85%), m.p. 144—146 °C, was collected. Recrystallisation from water gave m.p. 148—150 °C (softening at 130 °C) (Found: C, 66.45; H, 6.75. $C_{10}H_{12}O_3$ requires C, 66.65; H, 6.71%).

2,4,5-*Trichlorophenyl* 3-(p-*Hydroxymethylphenyl)propionate* (7).—Dicyclohexylcarbodi-imide (24 g, 0.116 mol) dissolved in dimethylformamide (20 ml) was added dropwise during 10 min to a stirred and cooled (0 °C) solution of the foregoing carboxylic acid (5) (20 g, 0.11 mol) and 2,4,5-trichlorophenol (23 g, 0.12 mol) in dimethylformamide (60 ml). The mixture was allowed to warm to room temperature and stirred overnight. Dicyclohexylurea was removed by filtration and the filtrate evaporated to an oil which soon crystallised. Recrystallisation from ethyl acetate–light petroleum gave the *trichlorophenyl ester* (25.7 g, 65%), m.p. 85—89 °C, raised to 94—96 °C on recrystallisation from propan-2-ol (Found: C, 53.75; H, 3.7; Cl, 29.8. $C_{16}H_{13}Cl_3O_3$ requires C, 53.44; H, 3.64; Cl, 29.57%).

3-[p-(N-*t-Butoxycarbonylglycyloxymethyl)phenyl]propionic Acid* 2,4,5-*Trichlorophenyl Ester* (8; R = H).—Dicyclohexylcarbodi-imide (3.0 g, 14.5 mmol) dissolved in dichloromethane (15 ml) was added to a solution of t-butoxycarbonylglycine (5.25 g, 30 mmol) in dichloromethane (20 ml). The mixture was stirred at room temperature for 15 min, filtered, and a solution of the foregoing trichlorophenyl ester (7) (4.86 g, 13.5 mmol) in dichloromethane (40 ml), and then pyridine (1.23 ml), added to the filtrate. After 3 h at room temperature the reaction mixture was evaporated, the oily residue washed in ethyl acetate with 10% aqueous citric acid (2×), water (2×), ice-cold saturated aqueous sodium hydrogencarbonate (2×), water (2×), and brine, dried (Na_2SO_4), and concentrated to small volume. Precipitated dicyclohexylurea was filtered off and the filtrate evaporated to an oil which crystallised on trituration with light petroleum. The *t-butoxycarbonylglycine derivative* (5.6 g, 75%) had m.p. 106—107 °C, raised to 106—108 °C on recrystallisation from ether–light petroleum (Found: C, 53.45; H, 4.55; N, 2.6; Cl, 20.78. $C_{23}H_{24}Cl_3NO_6$ requires C, 53.45; H, 4.68; N, 2.71; Cl, 20.58%).

3-[p-N-*t-Butoxycarbonyl-*L*-alanyloxymethyl)phenyl]propionic Acid* 2,4,5-*Trichlorophenyl Ester* (8; R = Me).—This *alanine derivative* (81%) was prepared in a similar manner to the foregoing and had m.p. 87—88 °C (softening at 60 °C), m.p. 87—88 °C after recrystallisation from propan-2-ol, $[\alpha]_D^{18}$ −23.75° (*c* 1 in MeOH) (Found: C, 54.0; H, 4.95; N, 2.45. $C_{24}H_{26}Cl_3NO_6$ requires C, 54.30; H, 4.94; N, 2.64%).

3-[p-(N-*t-Butoxycarbonyl-*L*-phenylalanyloxymethyl)-phenyl]propionic Acid* 2,4,5-*Trichlorophenyl Ester* (8; R = CH_2Ph).—The *phenylalanine derivative* (69%) had m.p. 73—75 °C, raised on recrystallisation from propan-2-ol to 79—80 °C, $[\alpha]_D^{18}$ −7.8° (*c* 1 in dimethylformamide) (Found: C, 59.4; H, 4.9; N, 2.15; Cl, 17.5. $C_{30}H_{30}Cl_3NO_6$ requires C, 59.37; H, 4.98; N, 2.31; Cl, 17.52%).

3-[p-(N-*t-Butoxycarbonyl-γ-benzyl-*L*-glutamyloxymethyl)-phenyl]propionic Acid* 2,4,5-*Trichlorophenyl Ester* (8, R = $CH_2CH_2CO_2CH_2Ph$).—The *γ-benzylglutamyl derivative* (69%) had m.p. 81—83 °C, raised to 84—85 °C on recrystallisation from ether–light petroleum, $[\alpha]_D^{18}$ −10.6° (*c* 1 in dimethylformamide) (Found: C, 58.7; H, 5.05; N, 2.05. $C_{33}H_{34}Cl_3NO_8$ requires C, 58.37; H, 5.05; N, 2.06%).

Solid-phase Synthesis of Acyl Carrier Protein Residues 65—74.—Throughout the synthesis, every precaution was taken to maintain the highest purity of all solvents and reactants. AnalaR dimethylformamide was fractionally redistilled at 15-mmHg pressure through a 12-in fractionation column packed with Fenske gauze rings. A fore fraction was collected until no change in vapour temperature occurred between total reflux and total distillation. This vacuum distillation was run continuously while the synthesis was in progress. Dichloromethane was freshly distilled from phosphorous pentaoxide, and acetic acid from chromic oxide. Hydrogen chloride in acetic acid was prepared by bubbling dry hydrogen chloride into acetic acid until the weight increase corresponded to *ca.* 1.5M. The strength of this reagent declined to *ca.* 1M on standing attached to the solid-phase synthesiser. Dicyclohexylcarbodi-imide was redistilled *in vacuo*. t-Butoxycarbonyl-amino-acids were checked for identity and purity by m.p., $[\alpha]_D$, and t.l.c. (silica) in chloroform–methanol–acetic acid (85 : 10 : 5). Boc-Leu, Boc-Ile, Boc-Asp(OBzl), Boc-Ala, and Boc-Val were commercial samples (Fluka); Boc-Asn-ONp,[47] Boc-Gln-ONp,[48] and Boc-Tyr(Bzl-Cl_2) [35] were prepared by literature methods. All steps in the synthesis except symmetrical anhydride formation were carried out using a Beckman 990 Peptide Synthesiser, modified to incorporate an all-glass reaction vessel as described in the

text. Blockage of the glass sinter usually occurred once or twice in the initial stages using amorphous poly(dimethylacrylamide) resins; the vessel was then replaced and cleaned in chromic acid. Later steps then proceeded without difficulty.

Symmetrical anhydride preparation was commenced during step seven (neutralisation) of the solid-phase synthesis cycle (see below). To the t-butoxycarbonyl-amino-acid (4 mmol, 12 equiv. g^{-1} of resin containing 0.33 milliequiv. g^{-1} amino-groups) dissolved in the minimum volume of freshly distilled dichloromethane was added a solution of dicyclohexylcarbodi-imide (0.41 g, 2 mmol), also in the minimum volume of dichloromethane. After 10 min at room temperature, dicyclohexylurea was removed by filtration, the filtrate evaporated and the residue dissolved in dimethylformamide * (15 ml) immediately prior to addition to the solid-phase synthesis reaction vessel.

The standard solid-phase synthetic cycle contained the following steps: (1) $EtCMe_2OH$, 5 × 2 min; (2) AcOH, 5 × 2 min; (3) 1N HCl–AcOH, 1 × 5 min, 1 × 25 min; (4) AcOH, 5 × 2 min; (5) $EtCMe_2OH$, 5 × 2 min; (6) DMF,* 10 × 2 min; (7) 10% NEt_3–DMF,* 3 × 2 min; (8) DMF, 5 × 2 min; (9) coupling, six-fold excess of t-butoxycarbonyl-amino-acid anhydride, DMF, 1 × 120 min; and (10) DMF, 5 × 2 min. For activated ester couplings, steps 9 and 10 were replaced by: (9a) six-fold excess of 1-hydroxybenzotriazole, DMF, 1 × 5 min; (10a) DMF, 3 × 2 min; (11) coupling, six-fold excess of t-butoxycarbonyl-amino-acid *p*-nitrophenyl ester, six-fold excess of 1-hydroxybenzotriazole, DMF, 1 × 240 min, and (12) DMF, 5 × 2 min. All additions to the reaction vessel were performed automatically except at steps (9) and (11) when the synthesiser was halted for manual addition of the freshly prepared symmetrical anhydride or activated ester solution. Samples were removed at each cycle for reaction with ninhydrin after step 8 and at intervals during the acylation reaction (step 9 or 11). They were washed well on a small sinter funnel successively with 10% triethylamine–dimethylformamide,* dimethylformamide, and ether. The qualitative ninhydrin reaction of Kaiser *et al.*[41] was used for assessment of completion of acylation; the sample taken at step 8 provided a reference blank, and after introduction of the leucine, glycine, tyrosine, alanine(1), and valine residues, was also used for amino-acid analysis. These samples were washed as described above, dried *in vacuo*, and *ca.* 5 mg hydrolysed in distilled constant-boiling hydrochloric acid (1 ml) containing a trace of phenol for 18 h at 110 °C. The hydrolysate was evaporated, partitioned between citrate buffer (pH 2) and chloroform, and an appropriate aliquot of the aqueous layer subjected to amino-acid analysis (JEOL 5AH analyser). Results are presented in Figure 1.

Solid-phase synthesis was commenced with 20 ml of DMF-swollen resin (*ca.* 0.95 g) of the Boc–β-alanine resin (Found; β-Ala, 0.36 milliequiv. g^{-1}). The following amino-acid derivatives were added sequentially using the procedures described above: (*a*) Boc-leucine (internal reference–spacer amino-acid); (*b*) Boc-glycyl linkage agent (8; R = H); (*c*) Boc-asparagine *p*-nitrophenyl ester; (*d*) Boc-isoleucine; (*e*) Boc-tyrosine 2,6-dichlorobenzyl ether; (*f*) Boc-β-benzyl aspartate; (*g*) Boc-isoleucine; (*h*) Boc-alanine; (*i*) Boc-alanine; (*j*) Boc-glutamine *p*-nitrophenyl ester; and (*k*) Boc-valine. Negative ninhydrin reactions were obtained in all cases at completion of the acylation period (2 h), and usually at the time of the first test (10—15 min). At the completion of the synthesis 1.27 g of peptide-resin was obtained (theory *ca.* 1.50 g, disregarding the 23 analytical samples removed) (Found: Gly, 0.19 mmol g^{-1}; Leu : Gly, 1.14, 1.13, 1.11, corresponding to retention of 89% of the peptide chains, 1.4% loss from the resin per synthetic cycle). A sample of the dried resin (190 mg) was treated with dry (CoF_3) distilled liquid hydrogen fluoride (10 ml) and anisole (0.3 ml) in a Toho Kasei reaction apparatus, initially at −78 °C warming to 0 °C during 1 h. The hydrogen fluoride was evaporated *in vacuo*, the solid residue dried (KOH and P_2O_5) overnight, and partitioned between 0.1M ammonium hydroxide and ethyl acetate. The organic phase was washed twice with additional aqueous ammonia, and the combined aqueous extracts evaporated yielding 40.8 mg, peptide content 33.3 μmol (Found; Asp, 2.11, Glu, 0.99; Gly, 1.10; Ala, 1.99; Val, 0.96; Ile, 1.96; Tyr, 0.90). Part (15.6 μmol) was chromatographed on a column (30 cm × 1.5 cm diameter) of diethylaminoethylcellulose DE52 using a linear gradient of 0.01—0.5M ammonium hydrogencarbonate, pH 8.1, flow rate 1 ml min^{-1}, 6-ml fractions collected with continuous monitoring of the effluent at 278 nm (Figure 2). Fractions 41—49 were combined and evaporated to give 7.48 μmol (48%) (Found: Asp, 2.06; Glu, 1.02; Gly, 1.02; Ala, 1.97; Val, 0.97; Ile, 1.95; Tyr, 1.02); paper electrophoresis at 3 kV and pH 6.5 gave a single spot, R_{Asp} = 0.19; tlc (silica) R_F 0.11 (n-BuOH–AcOH–H_2O, 66 : 12 : 26); R_F = 0.67 (n-BuOH–pyridine–AcOH–H_2O, 90 : 80 : 60 : 72). Re-chromatography of an aliquot (1.5 μmol) on the same ion-exchange column gave a single peak containing 0.82 μmol (55% recovery) (Found; Asp, 1.98; Glu, 1.03; Gly, 1.03; Ala, 1.98; Val, 1.00; Ile, 1.92; Tyr, 1.06).

* Our current practice favours the use of dimethylacetamide in place of dimethylformamide,[5] and ethyl di-isopropylamine in place of triethylamine.

We are grateful to Mr. M. Caviezel for his skilful participation in certain of the resin experiments, to Professor A. Ledwith for helpful discussions and a gift of the polymerisation vessel, and to the late Professor G. W. Kenner for much advice and encouragement.

[0/986 *Received*, 25*th June*, 1980]

REFERENCES

[1] R. B. Merrifield, *Fed. Proc. Fed. Amer. Soc. Exp. Biol.*, 1962, **21**, 412; R. B. Merrifield, *J. Amer. Chem. Soc.*, 1963, **85**, 2149.
[2] For recent reviews, see (*a*) G. Barany and R. B. Merrifield, in 'The Peptides', eds. E. Gross and J. Meienhofer, Academic Press, New York, 1980, vol. 2, p. 3; (*b*) B. W. Erickson and R. B. Merrifield in 'The Proteins,' eds. H. Neurath, R. L. Hill, and C-L. Boder, Academic Press, New York, 3rd edn., 1976, vol. 1 pp. 257—590; (*c*) J. Meienhofer in 'Hormonal Proteins and Peptides II,' ed. C. H. Li, 1973, Academic Press, New York, pp. 46—267.
[3] R. B. Merrifield, *Biochemistry*, 1964, **3**, 1385.
[4] R. C. Sheppard, in 'Peptides 1971,' ed. H. Nesvadba, North Holland Publishing Co., Amsterdam, 1973, Proceedings 11th European Peptide Symposium, pp. 111—125.
[5] E. Atherton, D. L. J. Clive, and R. C. Sheppard, *J. Amer. Chem. Soc.*, 1975, **97**, 6585.
[6] E. Atherton, M. Caviezel, H. Over, and R. C. Sheppard, *J.C.S. Chem. Comm.*, 1977, 819.
[7] E. Atherton, H. Fox, D. Harkiss, C. J. Logan, R. C. Sheppard, and B. J. Williams, *J.C.S. Chem. Comm.*, 1978, 539.
[8] E. Atherton, H. Fox, D. Harkiss, and R. C. Sheppard, *J.C.S. Chem. Comm.*, 1978, 540.
[9] R. Arshady, E. Atherton, M. J. Gait, K. Lee, and R. C. Sheppard, *J.C.S. Chem. Comm.*, 1979, 425.
[10] E. Atherton and R. C. Sheppard, in 'Peptides 1974,' ed. Y. Wolman, Proceedings 13th European Peptide Symposium, Wiley, New York, 1975, pp. 123—127.

[11] E. Atherton, D. L. J. Clive, D. A. East, and R. C. Sheppard, in ' Peptides 1976,' ed. A. Loffet, Proceedings 14th European Peptide Symposium, Edition de la Universite de Bruxelles, 1976, p. 291.
[12] R. C. Sheppard, in ' Molecular Endocrinology,' eds. I. Macintyre and M. Szelke, Elsevier–North Holland, Amsterdam, 1977, pp. 43—56.
[13] E. Atherton and R. C. Sheppard, in ' Peptides,' eds. M. Goodman and J. Meienhofer, Proc. 5th American Peptide Symposium, Halstead Press, New York, 1977, p. 503.
[14] E. Atherton, R. C. Sheppard, and B. J. Williams, in ' Peptides 1978,' Proceedings 15th European Peptide Symposium, eds. I. Z. Siemion and G. Kupryszewski, Wroclaw University Press, Wroclaw, Poland, 1979, p. 207.
[15] E. Atherton, D. Jarvis, G. P. Priestley, R. C. Sheppard, and B. J. Williams, in ' Peptides, Structure and Biological Function,' eds. E. Gross and J. Meienhofer, Proceedings 6th American Peptide Symposium, Pierce Chem. Co., Illinois 1980, p. 361.
[16] E. Atherton, M. J. Gait, R. C. Sheppard, and B. J. Williams, *Bioorg. Chem.*, 1979, **8**, 370.
[17] M. J. Gait and R. C. Sheppard, *Nucleic Acids Res.*, 1977, **4**, 1135.
[18] M. J. Gait and R. C. Sheppard, *Nucleic Acids Res.*, 1977, **4**, 4391.
[19] M. J. Gait and R. C. Sheppard, *Nucleic Acids Res.*, 1979, **6**, 1259.
[20] M. J. Gait, M. Singh, R. C. Sheppard, M. D. Edge, A. R. Greene, G. R. Heathcliffe, T. C. Atkinson, C. R. Newton, and A. F. Markham, *Nucleic Acids Res.*, 1980, **7**, 1081.
[21] E. Atherton, J. Bridgen, and R. C. Sheppard, *FEBS Letters*, 1976, **64**, 173.
[22] C. B. Anfinsen, *Pure Appl. Chem.*, 1968, **17**, 461.
[23] G. P. Vlasov and A. Y. Bilibin, *Izvest. Akad. Nauk S.S.S.R., Ser. Khim.*, 1969, 1400.
[24] R. B. Merrifield, *Adv. Enzymol.*, 1969, **32**, 221.
[25] J. K. Inman and H. M. Dintzis, *Biochemistry*, 1969, **8**, 4074.
[26] W. S. Hancock, D. J. Prescott, P. R. Vagelos, and G. R. Marshall, *J. Org. Chem.*, 1973, **38**, 774.
[27] G. L. Stahl, C. W. Smith, and R. Walter, *J. Org. Chem.*, 1979, **44**, 3424.
[28] G. L. Stahl, R. Walter, and C. W. Smith, *J. Amer. Chem. Soc.*, 1979, **101**, 5383.
[29] E. Atherton, unpublished results.
[30] G. L. Stahl, R. Walter, and C. W. Smith, *J. Org. Chem.*, 1978, **43**, 2285.
[31] J. M. Stewart and J. D. Young, ' Solid Phase Peptide Synthesis,' Freeman, San Francisco, 1969, p. 65.
[32] C. K. Narang, K. Brunfeldt, and K. E. Norris, *Tetrahedron Letters*, 1977, 1819.
[33] R. B. Mitchell, S. B. H. Kent, M. Engelhard, and R. B. Merrifield, *J. Org. Chem.*, 1978, **43**, 2845.
[34] J. K. Chang, M. Shimizu, and S.-S. Wang, *J. Org. Chem.*, 1970, **41**, 3255.
[35] B. W. Erickson and R. B. Merrified, *J. Amer. Chem. Soc.*, 1973, **95**, 3750.
[36] F. Flor, C. Birr, and T. Wieland, *Annalen*, 1973, 1601; H. Hagenmeier and F. Hartmut, *Z. physiol. Chem.*, 1972, **353**, 1973.
[37] D. F. de Tar and R. Silverstein, *J. Amer. Chem. Soc.*, 1966, **88**, 1013, 1020.
[38] V. Gut and J. Rudinger, in ' Peptides 1968,' Proceedings 9th European Peptide Symposium, ed. E. Bricas, North Holland Publishing Co., Amsterdam, 1968, p. 185.
[39] W. König and R. Geiger, *Chem. Ber.*, 1972, **105**, 343; 1973, **106**, 3626.
[40] G. R. Matsueda and S. A. Gaehde in ' The Peptides,' Proceedings 6th American Peptide Symposium, Pierce Publishing Co. Illinois, 1980, p. 353.
[41] E. Kaiser, R. L. Colescott, C. D. Bossinger, and P. I. Cook, *Analyt. Biochem.*, 1970, **34**, 595.
[42] J. M. Stewart, C. Rena, G. R. Matsueda, and K. Harris, in ' Peptides 1976,' Proceedings 14th European Peptide Symposium, ed. A. Loffet, Editions de la Universite de Bruxelles, 1976, p. 285.
[43] W. P. Ratchford and C. H. Fisher, *J. Amer. Chem. Soc.*, 1947, **69**, 1913.
[44] J. A. Helpern and J. T. Sparrow, in the press.
[45] R. Arshady, G. W. Kenner, and A. Ledwith, *J. Polymer Sci.*, 1974, **12**, 2017.
[46] M. N. Bogdanov, *J. Gen. Chem. U.S.S.R.*, 1958, **28**, 1670; E. Felkenstein, *Ann. Chim.* (France), 1957, **2**, 587; L. Denivelle and E. Felkenstein, *Bull. Soc. chim. France*, 1957, 630.
[47] G. R. Marshall and R. B. Merrifield, *Biochemistry*, 1965, **4**, 2349.
[48] H. C. Beyerman, C. A. M. Boers-Boonekamp, and H. M. van den Brink-Zimmermannova, *Rec. Trav. chim.* 1968, **87**, 257.