Pergamon

Tetrahedron 56 (2000) 3005–3011

TETRAHEDRON

Synthesis of Acyl Carrier Protein Fragment 65–74 on a Flexible Cross-linked Polystyrene Support: Comparison with Merrifield Resin

C. Arunan and V. N. Rajasekharan Pillai*

School of Chemical Sciences, Mahatma Gandhi University, Kottayam, Kerala 686-560, India

Received 27 September 1999; revised 14 December 1999; accepted 13 January 2000

Abstract—A novel 1,6-hexanediol diacrylate-cross-linked polystyrene support was prepared by suspension polymerization. The new support was used for synthesis of a difficult sequence-acyl carrier protein fragment 65–74 (ACP 65–74). The quantity and purity of peptide obtained from the new support were higher than when Merrifield resin was used. The reason for the high synthetic efficiency of the new support was found to be its ability to suppress the β-sheet formation of the growing peptide.

Introduction

Solid phase synthesis of peptides has witnessed dramatic progress since its inception.[1] Various polymer supports, new linkers, protection schemes and coupling methods have since made the technique simple and almost foolproof.

In spite of the improvements in the methodology of synthesis, understanding the chemical basis of various problems akin to the synthesis and the development of optimized conditions for synthesis; a group of peptide sequences have emerged as difficult sequences, which are resistant to easy synthesis by chemical methods. Two distinct classes of sequence related incomplete amino acylation have been reported: random and non-random.[2] Randomly difficult aminoacylations are due to sterically hindered reactions in which the side chain which contributes to the steric factors, and side chain protecting groups whose presence decreases the solubility of amino acid.[3] Sequence specific problems are some of the main problems in peptide synthesis and they are present in all methods of peptide synthesis. In solid-phase peptide synthesis (SPPS) the sequence specific problem is present irrespective of resin type.[4,5] Sequence specific problems have been found to be the result of the formation of β-sheet structure between pendant peptide chains.[6]

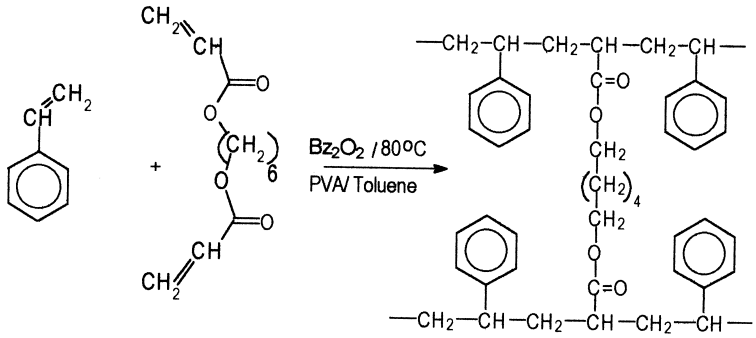
The cross-linked polymer becomes a highly swollen gel when it comes in contact with a *good solvent*. A dissolved linear polymer can be considered as an extended state of swelling of the cross-linked polymer. NMR studies have revealed that the segment mobility of 1% cross-linked polystyrene chain is comparable to linear polystyrene in solution.[7] However, in cross-linked polymers the dissolution tendency of the polymer is counter balanced by the elastic restraining force exerted by the cross-linking agent.[8] Thus, it can be expected that the solvation of polymer chain and pendant peptide chain would markedly decrease as the cross-linking increases. As β-sheet formation can bring additional cross-linking within the matrix, the synthesis of sequences favouring such secondary structures by SPPS will be difficult even if we use supports having high swelling characteristics. Studies have shown that the formation of β-sheet structure is accompanied by a drastic decrease in swelling and solvation of the peptidyl resin.[9–11] Thus, the main criteria in choosing a support for peptide synthesis is that it should be compatible with the peptide towards all the solvents used for the synthesis and capable of suppressing the β-sheet forming tendency of the growing peptide.

With this view in mind, we have developed a polystyrene based polymer support cross-linked with 2%, 1,6-hexanediol diacrylate (HDODA). The polymer was found to be more easily swollen in both polar and non-polar solvents compared to divinyl benzene based polystyrenes (Merrifield resin).[12] The applicability of the support for peptide synthesis was illustrated in the synthesis of a difficult sequence-acyl carrier protein fragment 65–74 (Val-Gln-Ala-Ala-Ile-Asp-Tyr-Ile-Asn-Gly). This protein fragment has been widely accepted as a model peptide for illustrating the efficiency of a reagent,[13–15] polymer support[16,17] or a

Keywords: β-sheet structure; ACP 65–74; aggregation; solid phase peptide synthesis.

* Corresponding author. Tel.: +91 481 594432; fax: +91 481 594432; e-mail: mgu@md2.vsnl.net.in

0040–4020/00/$ - see front matter
PII: S0040-4020(00)00055-7



Scheme 1. Copolymerization of styrene and 1,6-hexanediol diacrylate by suspension polymerization.

synthetic condition.[18] Although the synthesis of this decapeptide seems straightforward, early attempts to synthesize the peptide by standard procedures were either discouraging and ended with low yield and purity. The addition of asparagine, isoleucine and tyrosine gives a progressive decrease in the yield of peptide.[19] This may be due to the steric hindrance of the amino acids. But the conformation of the peptide is another important contributing factor to the low yield of peptide. The peptide undergoes strong internal association during the synthesis.[18] The deprotection of the 9th residue is followed by the formation of β-sheet structure between pendant peptide chains, eventually leading to decreased swelling and solvation of the peptidyl resin. Thus the diffusion of the last amino acid, Val, into the interior of the matrix is strongly restricted and the attachment of it to the resin-bound nonapeptide becomes very difficult. NMR studies of ACP resin (Merrifield) reveal that the segmental mobility of the polystyrene backbone is markedly decreased[20] indicating the presence of β-sheet formation.

In this paper, we describe the synthesis of ACP fragment (65–74) sequence using the new support, and a comparison has been made with Merrifield resin (divinylbenzene cross-linked polystyrene). The synthesis showed that the peptide was obtained in high yield and purity using the new support unlike the Merrifield support. The possible causes for the high synthetic efficiency in terms of the conformational behavior of the peptide have also been investigated by IR spectroscopy.

Results and Discussion

Polymer preparation

The copolymer of styrene and 1,6-hexanediol diacrylate was prepared by the suspension polymerization method. Dibenzoyl peroxide was used as the radical initiator (Scheme 1). The polymer was obtained in bead shape and the yield was nearly 90%. The incorporation of the cross-linking agent was confirmed by IR spectroscopy. IR (KBr): 1720, 1490 cm^{-1} (ester C=O), 2910, 2850 cm^{-1} (CH_2 str. of HDODA and polystyrene), 3020, 700 cm^{-1} (C–H of benzene). C H analysis: C-90.5% (91.09%) and H-7.2%(7.7%). Beads with sizes ranging 200–400 mesh size were used for the synthesis of peptide. The resin exhibited better swelling than Merrifield resin in solvents used for peptide synthesis (Table 1).

Table 1. Swelling characteristics (volume of solvent (mL) imbibed by 1 g of resin) of 2% HDODA-PS and Merrifield resin

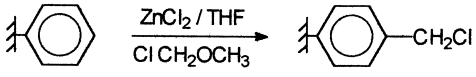
Solvent	Merrifield resin	HDODA-PS
DCM	5.9	10.8
NMP	6.2	8.6
DMF	3.6	4.0
THF	3.2	7.0
$CHCl_3$	8.0	9.0

Functionalization of the polymer support

A chloromethyl group was introduced on the benzene ring of the resin by Friedel Craft's reaction using chloromethyl methyl ether (CMME) and $ZnCl_2$[21] (Scheme 2). $ZnCl_2$ was suitable for controlling the chloromethylation reaction effectively. The chlorine capacity was estimated by the modified Volhard procedure.[22] The capacity was found to be 2.0 mmol of Cl/g. IR (KBr): 1420, 668 cm^{-1} (C–Cl).

Synthesis of ACP(65–74) fragment

The peptide was synthesised on HDODA-PS and 2% Merrifield resin (chlorine capacity 2.02 mmol/g). Boc glycine was covalently bound to both supports by cesium salt method. The extent of esterification was estimated by the picric acid method.[23] Both the supports gave almost same level of Boc glycine substitution. The amino capacity of HDODA-PS was 1.9 mmol of NH_2/g and that of Merrifield support was 1.85 mmol/g. However, this was achieved by increasing the time given for esterification reaction in the case of Merrifield resin. In the case of HDODA-PS, the esterification reaction was complete by 36 h, but 48 h had to be used for Merrifield resin to achieve the same substitution level. The initial progress of the reaction may be the same on both supports, but the final stage of the reaction in Merrifield system is largely suppressed by the steric effect caused by the poor swelling properties of the resin. In the case of HDODA-PS, all the functional sites are equally accessible for the reaction, because of the high swelling behaviour of the polymer.



Scheme 2. Chloromethylation of 2% HDODA-PS.

Table 2. Protocol for the synthesis of ACP 65–74

Step	Reagent	No/time (min)
1.	DCM	5×2
2.	30% TFA in DCM	1×30
3.	DCM	5×2
4.	5% DIEA in DCM-NMP	1×5
5.	5% DIEA in NMP	1×5
6.	NMP	3×2
7.	2.5 mequiv. of active ester of amino acid	1×45
	DMSO (15%)	1×15
	DIEA (3.8 mequiv.)	1×5
8.	MeOH-DCM (33:67 V/V)	5×5
9.	Kaiser test—if positive repeat step 6–8	
10.	DCM	5×2

The first amino acid substitution level on both supports was kept constant with a view to keeping the overall statistical distribution of the growing peptide chain the same on both the supports. This is very important when we consider the influence of aggregation of peptide. It may be argued that the aggregation can occur even at low loading[24] and hence there is no significance in keeping the same substitution level. But there is no other choice than keeping the functional group capacity constant when comparing two polymer supports. It was assumed that the distribution of the functional groups on both the supports is the same.

Subsequent amino acids were attached to the resin by the activated ester method using DCC/HOBt in NMP. Boc group was removed by 30% TFA in DCM, followed by neutralization with 5% DIEA in NMP–DCM mixture and 5% DIEA in NMP. The coupling was performed in NMP, which can disrupt the β-sheet formation.[25] DMSO was added at the end of the reaction, which is a good β-sheet structure destabilizing agent.[18] The presence of DIEA enhances the reaction rate by increasing the pH of the medium. The coupling reaction was followed by the Kaiser test. The general protocol adopted for the synthesis is shown in Table 2. Most of the coupling was completed in the first coupling itself in the case of HDODA-PS. However, a second coupling was used to ensure completion. However, more couplings had to be used in order to obtain a negative Kaiser test in the case of the Merrifield system. The number of couplings given for both supports until a negative Kaiser test was obtained is shown in Fig. 1. In the case of Merrifield resin, difficulty in aminoacylation was observed at many stages. However couplings were continued until a negative Kaiser test was obtained, without resorting to capping.

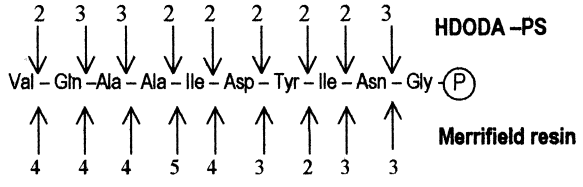


Figure 1. Number of couplings employed for the complete aminoacylation for different amino acids in the synthesis of ACP 65–74 using Merrifield and 1,6-hexanediol-diacrylate cross-linked polystyrene resin. The numbers indicate how many times the coupling had to be repeated until it offered a negative ninhydrin test.

Table 3. Amino acid analysis of ACP 65–74 bound to HDODA-PS and Merrifield resin

Amino acid	Actual value	HDODA-PS	Merrifield resin
Ala	2	2.07	3.37
Asp	2	2.04	1.47
Glu	1	0.83	0.48
Gly	1	1.00	1.00
Ile	2	1.97	2.20
Tyr	1	0.98	0.85
Val	1	0.61	0.51

When all the amino acids were incorporated, the peptidyl resin was subjected to amino acid analysis. The values are given in Table 3. The amino acid analysis of the peptidyl resin is an indication of the homogeneity of the growing peptide chain. The amino acid values of the peptide grown on HDODA-PS agrees well with actual value where as the amino acid analysis data of the peptide bound to Merrifield resin indicate the presence of impure peptides.

The peptide was cleaved from both the supports using neat TFA in the presence of thioanisole, *m*-cresol and ethanedithiol. The yield of crude peptide obtained from HDODA-PS was 82% and from Merrifield system 55%, calculated on the basis of first amino acid substitution. Both the peptides were dissolved in methanol and loaded in an analytical RP column. The profiles are shown in Fig. 2. From the HPLC profiles it is clear that the peptide obtained from HDODA-PS was highly homogeneous.

The formation of deletion peptides in the case of Merrifield system though the coupling reactions were employed until a negative Kaiser test was obtained, may be explained as follows. The DVB-cross-linked resin is not appreciably swellable in the Kaiser test solution, therefore only those groups that are very accessible to the reagent will respond to the test. However, in the highly functionalized resin most of the functional groups are placed inside the polymer network and these are not easily accessible to the reagent because of poor swelling and steric factors. The swellability further decreases, when the peptide undergoes internal aggregation via β-sheet structure.[26] In the case of HDODA-PS, the greater swelling and flexible nature of cross-linker allows easy permeation of the reagents into the interior of the matrix hence the chances of leaving any amino groups unattacked by ninhydrin is slim compared to the DVB-cross-linked support.

IR investigation of peptidyl resin

The use of IR spectroscopy in the investigation of the conformation of the peptide bound to cross-linked polymer has been widely exploited by many workers.[6,24,26–30] We have used this technique to gain an insight into how the conformation of growing peptide when bound to our resin is different to that when it is bound to Merrifield's resin. The peptidyl resins of Boc deprotected nonapeptide (65–73) and protected decapeptide of both the supports were subjected to IR investigation by the procedure described by Narita.[27] The Amide I region was observed to give an insight into the conformation behaviour of peptide in the solid state. It has

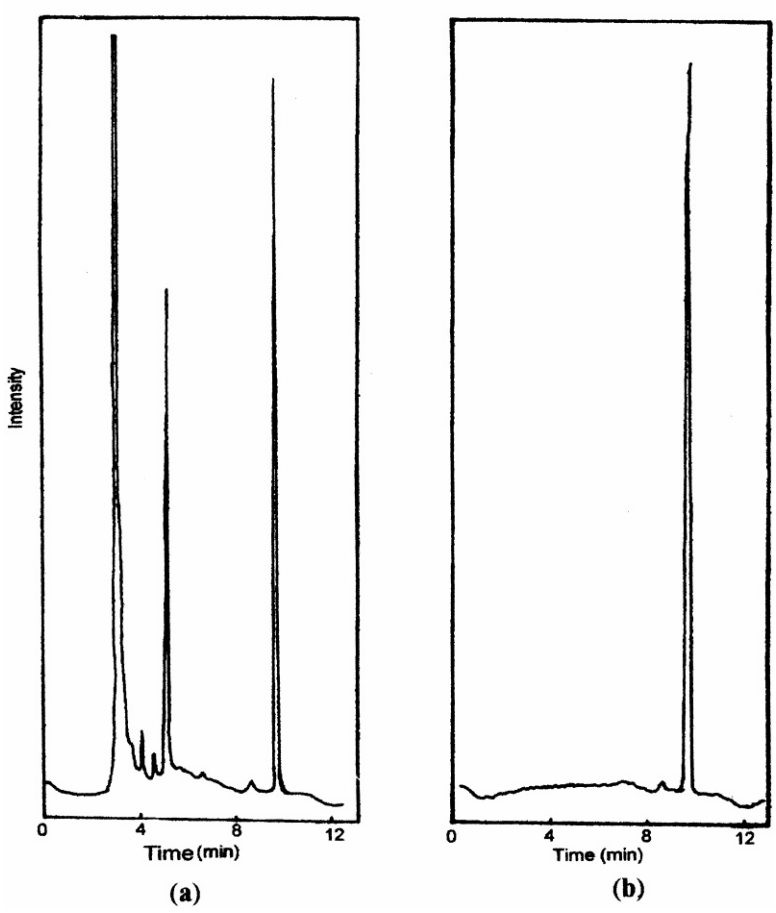


Figure 2. HPLC traces of ACP fragment 65–74: (a) peptide obtained from Merrifield's support; (b) Peptide obtained from HDODA-PS; conditions: rpc C18, 5 μ, 4×250 mm, chart speed 2 mm/min. Solvent system: A, water (containing 0.1% TFA), B, CH_3CN (containing 0.1% TFA), gradient used—solvent B: 0–100% in 45 min, 100% for 15 min, 100–0% in 5 min. Flow rate: 0.5 mL/min detection at 220 nm (UV).

been reported that the aggregation commences at the deprotection of 9th residue.[16] Figs. 3 and 4 show the amide I region of Boc removed nonapeptidyl resin and protected decapeptidyl resins respectively. The deprotected nonapeptide bound HDODA-PS showed a peak at 1662 cm^{-1}, which was assigned, to the random conformation of the peptide (Fig. 3b). The same peptide when bound to the Merrifield support showed a peak at 1642 cm^{-1} which was assigned to a β-sheet structure (Fig. 3a). The decapeptide when bound to the new support existed in the random state in the solid state whereas it assumed a β-sheet structure in the Merrifield system (Fig. 4). Thus the formation of β-sheet structure of the peptide when bound to Merrifield system could be the reason for the incomplete aminoacylation. As the random structure is more prone to solvation, the amino acylation reaction on the new support is fairly easy. The role of 1,6-hexanediaol diacrylate in the destabilization of β-sheet was also investigated IR spectrophotometrically. For this, a tripeptide AAG that was reported to have a tendency to form β-sheets[29] was bound on to the new support by the standard methodology. The C═O stretching peak of the free polymer was slightly shifted from 1720 to 1710 cm^{-1} and broadened (Fig. 5). This shows that a few of the C═O groups of the ester in the cross-linker are involved in the peptide-polymer interaction. The cross-linker interacts with the NH of peptide at different site in the solid state and thus the tendency of the peptide to acquire a β-sheet structure is suppressed. During coupling when NMP and DMSO were added, the peptides in the random structure were easily solvated. In the case of the Merrifield system, however, the peptide assumed this β-sheet structure, and once it was formed, disruption by NMP or DMSO becomes very difficult. Thus, in conclusion 1,6-hexanediol diacrylate cross-linked polystyrene is an efficient support for the synthesis of difficult sequences.

Experimental

General

All side chain protected Boc amino acids (L) were procured from Peninsula Company, CA, USA. Simple amino acids (L) (Merck, Germany) were Boc protected following literature procedures.[31] Boc Carbazate, HOBt, DCC and NMP were obtained from Sigma Chemial Company, USA. HDODA, TFA, DIEA, DMSO, Cs_2CO_3, thioanisole, *m*-cresol, ethanedithiol, TEA and styrene were obtained from Aldrich Chemical Company, WI, USA. All solvents were of commercial grade and purified before their use. IR spectra of the polymer samples were taken using a Shimadzu IR spectrophotometer (IR 470). Amino acid analyses were performed on LKB 4151 ALPHA PLUS

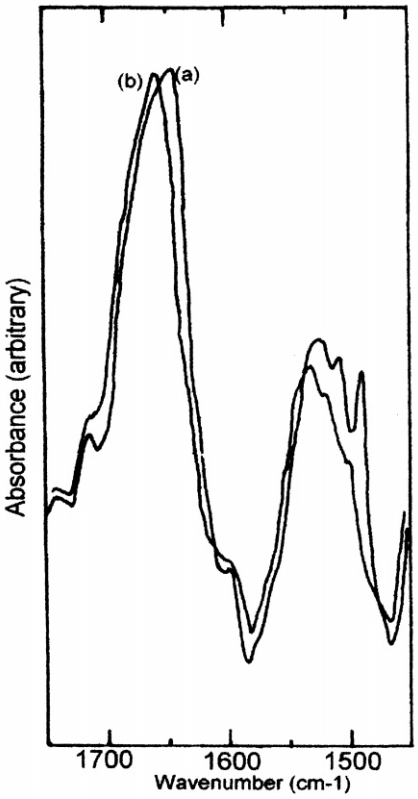


Figure 3. Amide I region of deprotected noapeptide: (a) peptide bound to Merrifield resin; (b) peptide bound to HDODA-PS.

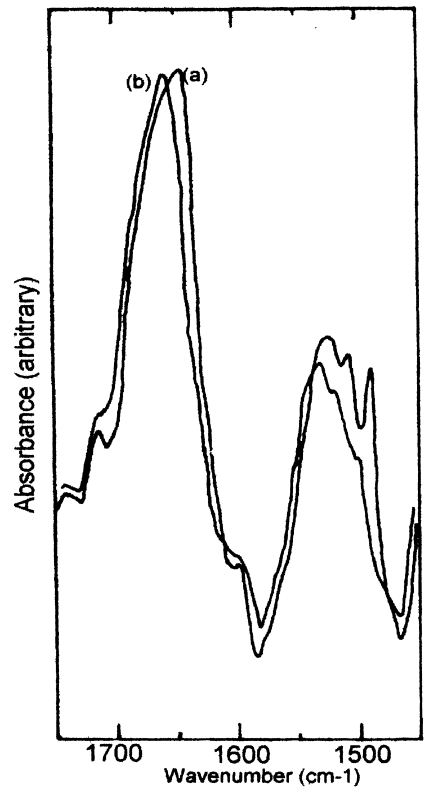


Figure 4. Amide I region of protected decapeptide: (a) peptide bound to Merrifield resin; (b) peptide bound to HDODA-PS.

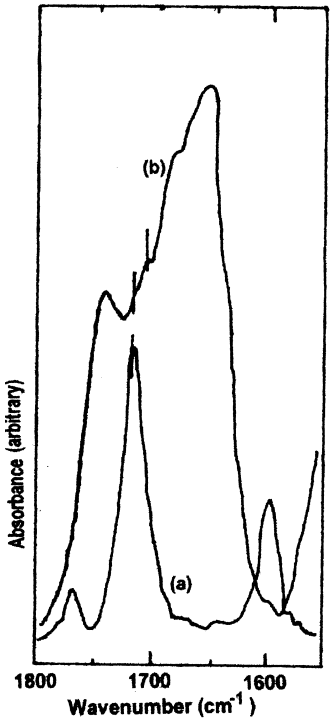


Figure 5. IR spectra of the the carbonyl region of (a) the HDODA-PS and (b) AAG bound HDODA-PS.

amino acid analyzer using ninhydrin detection. Optical density was measured using Shimadzu UV/Vis spectrophotometer (UV 160A) at 358 nm. HPLC analysis was conducted using pharmacia LKB Pep RPC 5/5 system using C18 RP column and binary gradient solvent system (water and acetonitrile containing 0.1% TFA as the solvents). The flow rate of the solvent was 0.5 mL/min and detection was at 214 nm.

Synthesis of 2% cross-linked HDODA-PS

Styrene (10 mL) was destabilized using 1% NaOH solution (3×15 mL). It was then washed with water (3×10 mL) and dried over anhydrous calcium chloride. A 1% solution of PVA (Average mol. wt. 75,000) in water (110 mL) was prepared and kept stirred in a polymerization vessel at 80°C with a constant flow of N_2 through the solution. A mixture of 1,6-hexanediol diacrylate (0.45 mL, 2 mol%), styrene (11.33 mL, 98 mol%), toluene (2.31 mL, 20 vol% of monomer ratio) as diluent, and dibenzoyl peroxide (600 mg) was prepared and added to the PVA solution. The stirring was continued for 8 h. The polymer beads were collected by filtration through a sintered disc (G3) and washed thoroughly with hot water (3×20 mL, 3 min.) to remove PVA, acetone (20 mL×3, 3 min.) and methanol (20 mL×3, 3 min.). The polymer was then Soxhlet extracted using acetone, DCM, and methanol to remove linear polymers. Yield: 9 g. The polymer beads were sieved. Beads of 200–400 mesh sizes were used for peptide synthesis.

Chloromethylation of the polymer support

Anhydrous $ZnCl_2$ (1.5 g) was placed in an Erlenmeyer flask. Conc. HCl (3 drops) and water (5 drops) were added. The

solution was heated until the solid was completely dissolved. Heating was continued until a solid mass of $ZnCl_2$ was left which was melted on further heating. When it became a mobile liquid the flask was kept in a desiccator, and allowed to cool. The solid was dissolved in THF (10 mL) and kept sealed.

The dry resin beads (2 g) were kept in DCM (20 mL) in a R.B. flask. After half an hour CMME[32] (12 mL) and a solution of $ZnCl_2$ (0.4 mL) in THF was added to the swollen resin. The mixture was kept at 50°C for 5 h. The resin was filtered, washed with THF (20 mL×3, 3 min), THF/4N HCl (20 mL×3, 3 min.) THF-water (1:1 v/v)(20 mL×3, 3 min.), water (20 mL×3, 3 min.) and finally with methanol. The resin was dried under vacuum.

Estimation of the extent of chloromethylation

To the chloromethylated resin (200 mg) was added 5 mL of pyridine and the mixture kept at 110°C for 5 h. The mixture was quantitatively transferred with acetic acid-water (1:1 v/v 30 mL) and diluted with water (25 mL). Conc. HNO_3 (7 mL) and $AgNO_3$ (0.1 N, 10 mL) were added to this solution and titrated against standard ammonium thiocyanate solution (0.1 N) using ferric alum as indicator. A blank was also performed. Chlorine capacity: 2.0 mmol/g.

Attachment of Boc Gly on HDODA-PS

To 0.19 g of Boc Gly (1.2 mmol) in ethanol (5 mL) a saturated solution of cesium carbonate was added until the pH of the solution reached 7.0. The solution was stirred for 2 h. The ethanol was evaporated under reduced pressure. Water was removed by azeotropic distillation with dry benzene. The white powder of the cesium salt of Boc Glycine was dried under vacuum in the presence of P_2O_5. The salt was dissolved in NMP (2 mL), and to this, 0.3 g of chloromethylated resin (Capacity 2.0 mmol/g) was added. The mixture was kept at 50°C for 36 h. The resin was filtered, washed with NMP (5 mL×3, 3 min.), NMP–water (5 mL×3, 3 min.), NMP (5 mL×3, 3 min.) NMP–water mixture (1:1 v/v) (5 mL×3,3 min.), NMP (5 mL×3, 3 min.), DCM (5 mL×3, 3 min.) and methanol (5 mL×3, 3 min.). The resin was kept in a vacuum desiccator containing P_2O_5. Weight of the resin: 370 mg. The substitution level: 1.9 mmol of Glycine/g of the resin (by picric acid method). For the entire synthesis 100 mg of Boc Glycine resin (0.19 mmol of NH_2) was used.

Attachment of Boc Gly on Merrifield resin

Boc Glycine (1.2 mmol, 0.19 g) was dissolved in ethanol (5 mL). The pH of the solution was brought to 7 by the slow addition of saturated solution of cesium carbonate. The solution was stirred for 2 h. The solution was rotary evaporated to remove ethanol and water. The white powder of cesium salt of Boc-Gly was dissolved in NMP (2 mL). Merrifield's resin (2% cross-linked 200–400 mesh, 0.3 g, 2.02 mmol of Cl/g of the resin) was added to the solution. The mixture was kept at 50°C for 48 h in an oil bath. The resin was filtered washed with NMP (5 mL×3, 3 min.), NMP–water mixture (5 mL×3, 3 min.), NMP (5 mL×3, 3 min.), NMP–water mixture (1:1 v/v) (5 mL×3,3 min.), NMP (5 mL×3, 3 min.), DCM (5 mL×3, 3 min.) and methanol (5 mL×3, 3 min.). The resin was kept in a vacuum desiccator containing P_2O_5. Weight of the amino acid resin: 350 mg. 100 mg of Boc Glycine resin (0.185 mmol of NH_2) was used for the synthesis of the peptide.

Estimation of Boc Glycine substitution: picric acid method

2 mg of resin after the removal of Boc, was treated with 0.1 M picric acid (in DCM). The excess unbound picric acid was removed by washing with DCM (3 mL×5, 2 min.). The resin was treated with 10% TEA (in DCM) (1 mL) and the free picric acid was collected. The resin was washed with 95% ethanol. The resin was washed again with 10% TEA (1 mL). All the washings were collected, diluted to 15 mL and its optical density (OD) at 358 nm was noted. From the OD the degree of substitution was calculated knowing that ϵ_{max} for picrate-TEA complex in ethanol at 358 nm=14,500. Substitution level: 1.85 mmol of glycine/g of Merrifield resin and 1.90 mmol/g of HDODA-PS.

Removal of Boc group: general procedure

The polymer was treated with 30% TFA (in DCM) for 30 min. The TFA solution was filtered and the resin was washed with DCM. This was then treated with 5% DIEA in DCM (5 min.) and 5% DIEA in NMP–DCM mixture (1:1 v/v) to yield the free amino acid resin or free peptidyl resin.

Coupling of individual amino acids

The pre-formed activated ester method was used for the coupling of amino acids to the free peptidyl resin. The activated ester was prepared using 2.5 mmol each of DCC, HOBt and amino acid in NMP (4 mL) for 1 mmol of glycine resin. The DCU formed was filtered off and the solution was added to the free peptidyl resin. The resin was shaken for 45 min. in a silinized glass peptide synthesiser. At the end of 45 min, DMSO (0.75 mL) was added to the mixture, and after 15 min 0.14 mL of DIEA was also added. The total volume of the solution was kept at 5 mL for effective coupling reaction. The solution was filtered and the resin was washed with MeOH and DCM mixture (33:67 v/v) to get rid of DCU and then with DCM:NMP mixture. The effectiveness of acylation was checked by the Kaiser test. The protocol adopted for the assembly of one amino acid is shown in Table 1. Weight of peptidyl resin: Merrifield; 190 mg, HDODA-PS; 270 mg.

Cleavage of peptide: general procedure

80 mg of ACP-resin was treated with neat TFA (10 mL), thioanisole (0.1 mL), *m*-cresol (0.1 mL). The mixture was stirred at room temperature for 48 h. TFA solution was collected and the TFA was removed under reduced pressure. On adding cold ether, the peptide was precipitated. It was washed several times with cold ether and dried. Yield: 45 mg (from HDODA-PS); 25 mg (from Merrifield resin). Amino acid analysis of pure peptide: Val 0.62 (1), Ala

2.1(2) Glu 0.9(1) Ile 1.95(2), Asp 2.1(2), Tyr 0.99(1), Gly 1(1).

Assembly of AAG on HDODA-PS

The assembly of the tripeptide was performed exactly as in the case of the ACP 65–74. The tripeptidyl resin was used for the IR conformational studies.

Abbreviations

Abbreviations for amino acids and peptide structure follow the recommendations of the IUPAC- IUB (Commission on Biochemical Nomenclature, *J. Biol. Chem.,* **1971**, *247*, 997). Other abbreviations used are: Boc: *tert*-Butyloxycarbonyl, Bzl: Benzyl, CMME: Chloromethyl methyl ether, DCC: Dicyclohexyl carbodiimide, DCU: Dicyclohexylurea, DCM: Dichloromethane, DIEA: *N*,*N*-Diisopropylethylamine, DMSO: Dimethyl sulfoxide, DMF: *N*,*N*′-Dimethylformamide, HDODA-PS: 1,6-hexanediol diacrylate cross-linked polystyrene, HOBt: 1-hydroxybenzotriazole, HPLC: High performance liquid chromatography, NMP: 1-methyl-2-pyrrolidone, OD: Optical Density, TEA: Triethylamine,

References

1. Merrifield, R. B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.
2. Meister, S. M.; Kent, S. B. H. *Peptides: Structure and Function: Proceedings of the 8th American Peptide Symposium*; Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co: Rockford, IL, 1984, pp 103–106.
3. Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*; 2nd ed.; 1984, 46.
4. Kent, S. B. H. *Ann. Rev. Biochem.* **1988**, *57*, 959.
5. Atherton, E.; Sheppard, R. C. *Peptides: Structure and Function, Proceedings of the 9th American Peptide Symposium*; Deber, C. M., Hruby, V. J., Kopple, K. D., Eds.; Pierce Chemical Co: Rockford, IL, 1985, pp 415–418.
6. Narita, M.; Ogura, T.; Sato, K.; Honda, S. *Bull. Chem. Soc. Jpn.* **1986**, *59*, 2433.
7. Live, D. H.; Kent, S. B. H. *Elastomers and Rubber Elasticity*; In *ACS Symposium Series*, Mark, J. E., Lal, J., Eds.; American Chemical Society: Washington D.C, 1982; 193, pp 501–515.
8. Milton deL, R. C.; Adams, P. A. *J. Am. Chem. Soc.* **1990**, *112*, 6039.
9. Milton deL, R. C.; Wormald, P. J.; Brandt, W.; Millar, R. P. *J. Biol. Chem.* **1986**, *261*, 16990.
10. Live, D. H.; Kent, S. B. H. *Peptides: Structure and Function*; In *Proceedings of the 8th American Peptide Symposium*, Hruby, V. J., Rich, D. H., Eds.; Pierce Chemical Co: Rockford, IL, 1984, pp 65–68.
11. Ford, W. T.; Balakrishnan, T. *Macromolecules* **1981**, *14*, 284.
12. Unpublished results from this laboratory.
13. Fuller, W. D.; Cohen, M. P.; Shabankareh, M.; Blair, R. K. *J. Am. Chem. Soc.* **1990**, *112*, 7414–7416.
14. Wenschuh, H.; Beyerman, M.; Krause, E.; Brudel, M.; Winter, R.; Schumann, M.; Carpino, L. A.; Bienert, M. *J. Org. Chem.* **1994**, *59*, 3275–3280.
15. Carpino, L. A.; Chao, H. G. *J. Org. Chem.* **1991**, *56*, 2635–2642.
16. Meldal, M. *Tetrahedron Lett.* **1992**, *33*, 3077–3080.
17. Atherton, E.; Fox, H.; Harkiss, D.; Logan, C-J.; Sheppard, R. C.; Williams, B. J. *J. Chem. Soc., Chem Commun.* **1978**, 537–539.
18. Hyde, C.; Johnson, T.; Sheppard, R. C. *J. Chem. Soc., Chem. Commun.* **1992**, 1573–1575.
19. Hancock, W. S.; Prescott, D. J.; Vagelos, P. R.; Marshall, G. R. *J. Org. Chem.* **1973**, *38*, 774–781.
20. Deber, C. M.; Lutek, M. K.; Heimer, E. P.; Felix, A. M. *Pept. Res.* **1989**, *2*, 184–188.
21. Fienberg, R. S.; Merrifield, R. B. *Tetrahedron* **1974**, *30*, 3209–3212.
22. Vogel's *Text Book of Quantitative Inorganic Analysis*, 4th ed.; Longman Group Ltd, Essex, England, 1978, p 342.
23. Stewart, J. M.; Young, J. D. *Solid Phase Peptide Synthesis*, 2nd ed.; Pierce Chemical Co: Rockford, IL, 1984.
24. Narita, M.; Tomotake, Y.; Isokawa, S.; Matsuzawa, T.; Miyauchi, T. *Macromolecules* **1984**, *17*, 1903–1906.
25. Hendrix, J. C.; Jarret, J. T.; Anisfeld, S. T.; Lansbury Jr, P. T. *J. Org. Chem.* **1992**, *57*, 3414–3420.
26. Narita, M.; Isokawa, S.; Tomotake, Y.; Nagasawa, S. *Polymer J.* **1983**, *15*, 25–28.
27. Narita, M.; Isokawa, S.; Matsuzawa, T.; Miyauchi, T. *Macromolecules* **1985**, *18*, 1363–1366.
28. Narita, M.; Ishikawa, K.; Chen, J-Y.; Kim, Y. *Int. J. Peptide Protein Res.* **1984**, *24*, 580–587.
29. Narita, M.; Lee, J-S.; Hayashi, S.; Yamazaki, Y.; Hitomi, M. *Bull. Chem. Soc. Jpn.* **1993**, *66*, 494–499.
30. Oh Uchi, S. K.; Yang, J. Y.; Lee, J. S.; Murakawa, Y.; Narita, M. *Polym. J.* **1996**, *28*, 1033–1038.
31. Schnabel, E. *Liebig, Ann. Chem.* **1967**, *702*, 188.
32. Caution! Suspected carcinogen. CMME is prepared by passing dry HCl through a mixture of formaldehyde (66 mL) and methanol (33 mL) kept at 0°C. The oily layer formed after 2 h collected, dried using $CaCl_2$ and used without further purification.