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Advances in and Problems of Solid-phase Peptide Synthesis

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The present state of the solid-phase method of peptide synthesis is considered, its advantages and disadvantages are analysed, and the trends in and prospects of its further development are described. Attention has been concentrated on problems of improving polymeric carriers, of obtaining aminoacyl polymers, and also the problem of the formation of erroneous sequences in solid-phase peptide synthesis.
The bibliography includes 376 references.

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I. INTRODUCTION

In 1973 ten years elapsed since the publication of Merrifield's report [1] of the synthesis of peptides on an insoluble polymeric carrier. The method proposed, called the solid-phase method, has been widely used in the synthesis not only of peptides but also of a number of compounds of other classes. About 500 papers have already been published on the solid-phase synthesis of peptides, among them several reviews, the most complete of which are those of Merrifield [2] and Losse and Neubert [3] as well as the monograph of Stewart and Young [4], which has been translated into Russian.

The above reviews [2-4] cover the literature until the beginning of 1969, representing a survey of approximately 150 experimental papers. It is useful to review the literature from 1969 to the beginning of 1972. The available reviews of Marglin and Merrifield [5], Geiger [6], Wünsch [7], Waki and Izumiya [8], Kishida and Sakakibara [9,10], and Losse [11] deal only with separate aspects of solid-phase peptide synthesis. There is only one review in Russian, that of Vesa, which was published in 1968. Later investigations are described in the monograph of Stewart and Young [4] and the reviews of Merrifield [2] and Losse and Neubert [3]. We regard the present publication as a continuation of these reviews.

Abbreviations Adopted

The abbreviations used for the names of aminoacids are those recommended by the IUPAC–IUP Commission on Nomenclature [Biochemistry, **5**, 2485 (1966)].

AOC	= t-amyloxycarbonyl †;
BOC	= t-butyloxycarbonyl;
BMV	= *N*-(2-benzoyl-1-methylvinyl);
DVB	= *p*-divinylbenzene;
BPOC	= 2-(*p*-biphenylyl)isopropyloxycarbonyl;
DMSO	= dimethyl sulphoxide;
DME	= dimethylformamide;
DNP	= 2,4-dinitrophenyl;
DCHA	= dicyclohexylamine (or dicyclohexylammonium);
DCC	= *NN'*-dicyclohexylcarbodi-imide;
Z	= benzyloxycarbonyl;
CDI	= *NN'*-carbonyldi-imidazole;
MBZH	= di-(4-methoxyphenyl)methoxy-(4,4'-dimethoxybenzhydryl);
MZ	= *p*-methoxybenzyloxycarbonyl;
NPS	= *o*-nitrophenylsulphenyl;
THF	= tetrahydrofuran;
TFA	= trifluoroacetyl;
FOC	= furfuryloxycarbonyl;
Pht	= phthalyl;
P	= polymeric carrier.

†POC = cyclopentyloxycarbonyl is quoted in the IUPAC–IUB Report (Ed. of Translation).

II. POLYMERIC CARRIER

The polymeric carrier is the main component in solid-phase peptide synthesis. Clearly the selection of the carrier is extremely important for the success of the process.

The copolymer of styrene and 1–2% DVB proposed by Merrifield[1] is completely insoluble in the usual solvents but swells satisfactorily in methylene chloride, DMF, dioxan, and chloroform. This is extremely important, since it makes it possible to employ virtually the entire bulk of the polymeric bead for the reactions[13].

The above carrier[1,14] is still most frequently used. The same may be said of the "anchor" group with the aid of which the first aminoacid is added to the polymeric carrier: as can be seen from the data quoted in Table 1, the chloromethyl group clearly predominates.

Although in the vast majority of studies[15–227] the authors used the chloromethylated copolymer of styrene and 1–2% DVB, a considerable proportion of studies on solid-phase peptide synthesis in fact dealt with the modification of the "anchor" group. This is due to a number of difficulties involved in the preparation of the aminoacyl polymer by Merrifield's method (see the next section of the present review).

Table 1. Polymeric carriers.

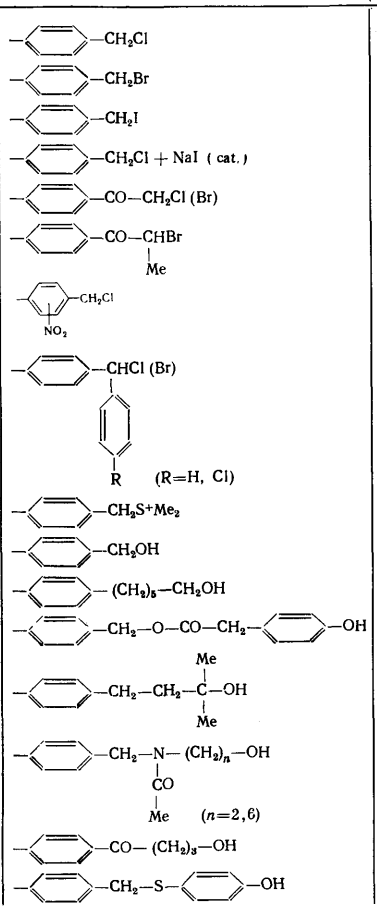
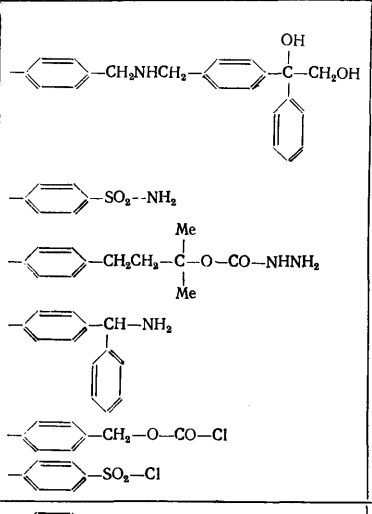
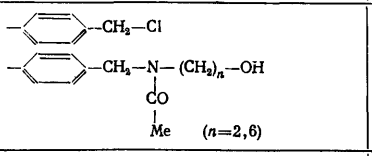
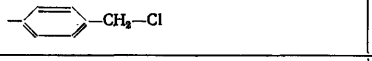
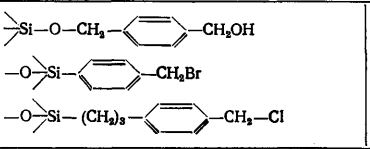
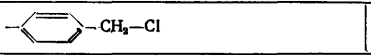
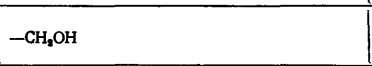
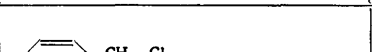
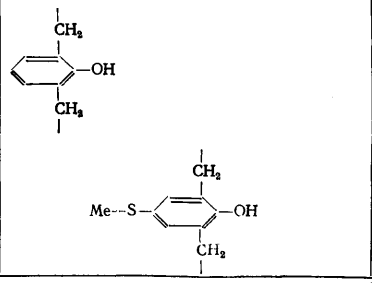
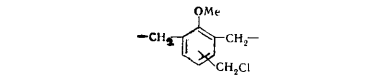
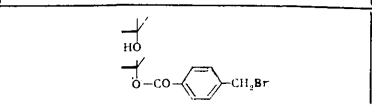
Type of polymer	Functional group	References
Gel copolymer of styrene and 1-2% DVB		13–105, 106[a], 107–227
		143, 228, 230–233
		228, 229
		229, 234–238
		239–245
		240–241
		1, 246–248
		249–251
		218, 252, 253
		65, 209, 233, 254–260
		261
		262
		263
		264–266
		264, 267
		268, 269

Table 1 (continued).

Type of polymer	Functional group	References
Gel copolymer of styrene and 1-2% DVB		270
		271
		263, 272
		273–282
		283[b], 284–287
		288, 289
Macroporous highly cross-linked styrene-DVB (up to 12%) copolymer		173, 199, 294–297
		296
Glass beads coated with a layer of polystyrene		53, 173, 199, 298, 299
Pellicular carrier		62, 300–302
		302, 303
		304
Polystyrene, mol.wt. 200000		305–309
Poly(ethylene glycol), mol. wt. 10000–20000		310–312
Graft copolymer of polychlorotrifluoroethylene and chloromethylstyrene		313
Polyphenol		314, 315
		269
Resitol		93, 115, 316
Sephadex LH-20 and G-25		317, 318
		317, 318

[a] Copolymer of styrene and 5% DVB.
[b] "Popcorn" type of copolymer of styrene and 0.5 DVB.

Attempts have been made to employ bromomethylated and iodomethylated styrene–DVB copolymers. However, such carriers can be prepared only in two stages‡. Thus Tilak[228] initially condensed the chloromethylated polymer with a carboxylic acid and then decomposed the resulting ester linkages with a solution of hydrogen bromide or iodide in acetic acid. The comparatively rare employment of such carriers, particularly of the iodomethylated polymer[143,229-233], is probably in fact due to the difficulties in their preparation. Therefore the method of the intermediate formation of iodomethylpolystyrenes by the reaction of chloromethylated derivatives of *N*-protected aminoacids in the presence of sodium iodide as the catalyst[234] is extremely attractive. Subsequently this method was successfully applied in a number of other laboratories[229,235-238].

Weygand[239] and, independently, Mizoguchi[240,241] suggested that a halogenoacetyl polymer be used. It improves the conditions governing the addition of the first aminoacid to the polymeric carrier but precludes the employment of acid reagents for the separation of the peptide from the carrier. The alkaline reagents used for this purpose[239-243] increase the risk of racemisation of the peptide. For this reason, halogenoacetyl polymers are usually employed to synthesise amides, hydrazides, and esters of protected peptides[240,241,244,245]. The same may be said also of the α-bromopropionyl polymer proposed by Mizoguchi and coworkers[240,241].

The polymeric carriers examined suffer from the same disadvantages as the chloromethylated Merrifield copolymer nitrated in the aromatic rings[1]. The enhanced resistance of the ester linkage of the peptide to acid reagents makes the nitrated carrier more suitable for the synthesis of protected amides and hydrazides of peptides[246]. Thus, using ammonia or amines for the separation of peptides from the polymeric carrier, Takashima et al.[247,248] obtained a number of oxytocin analogues.

The benzhydryl polymer proposed by Southard and coworkers[249-251] permits the employment of mild acid reagents for the separation of the peptide from the carrier but requires the use in the synthesis of extremely acid-labile protective groups, which also involves certain difficulties.

An interesting version of the synthesis of aminoacyl polymers without using condensing agents and tertiary amines was proposed by Dorman and coworkers[252]. The dimethyl(arylmethyl)sulphonium polymer employed by these workers was subsequently used to synthesise a number of biologically active peptides[218,253].

The polymeric carriers in which a hydroxyl forms part of the "anchor" group are of undoubted interest. This is primarily the hydroxymethylated polymer of styrene proposed by Bodanszky and Sheehan[254], which has been frequently used in solid-phase peptide synthesis despite a number of important disadvantages[65,209,233,250-260]. The hydroxyhexamethylene polymer proposed by Bayer et al.[261], the *p*-hydroxyphenylacetoxy polymer investigated by Blake and Li[262], and the t-hydroxyalkyl polymer of Wang and Merrifield[263] belong to the same group of carriers. The hydroxyalkylamino-polymer, developed by Tilak and Hollinden[264], is also suitable; it can be used successfully in combination with the Z-protected α-amino groups of aminoacids. The hydroxybutyryl polymer has similar properties[264,267]. The impossibility of using acid reagents in the stage involving the removal of the peptide from the carrier must be regarded as a disadvantage of these carriers.

Many of the hydroxyl-containing carriers make it possible to convert the peptidyl polymer into a polymeric activated peptide ester at the end of the synthesis, which leads to many possibilities in its subsequent use (see Section VII). This is primarily the polymer with hydroxyphenyl sulphide groups proposed by Marshall and Liener[268] and independently by Flannigan and Marshall (G.R. Marshall)[269]. The oxidation of the sulphide group to the corresponding sulphone leads to the formation of an activated peptide ester. A carrier with analogous properties was obtained by Wieland et al.[270] by the reaction of 4'-aminomethyl-2,2-diphenylmethanediol with a chloromethylated carrier. It can be converted by dehydration into an activated vinyl ester of the peptide. The polymeric carrier containing the sulphonamide group has similar properties[271]. *N*-Alkylation of the sulphonamide group facilitates the alkaline hydrolysis, ammonolysis, and hydryzinolysis of the "anchor" linkage.

Special carriers whose "anchor" sections already contain the appropriate functional groups are used to synthesise the amides and hydrazides of peptides under the conditions of the acid cleavage of peptidyl polymers. These are the t-alkyloxycarbonylhydrazide polymer of Wang and Merrifield[263,272] and the benzhydrylamino-polymer proposed by Pietta and Marshall[273]. Monahan, Rivier, and coworkers[274-279] as well as other investigators[280-282] frequently used the benzhydrylamino-polymer to obtain a number of biologically active peptide amides.

Together with polymeric carriers whose functional groups are capable of interacting with the carboxyl of the combined aminoacid, carriers permitting the synthesis of peptides from the terminal NH_2 group are sometimes used. Thus Letsinger and Kornet[283] proposed a carrier of the alkyloxycarbonyl chloride type capable of interacting with the α-amino-group of an aminoacid. However, the suggested approach has not been widely used in solid-phase synthesis owing to the risk of racemisation in the activation of the carboxy-group of the peptide. Nevertheless a carrier of the alkyloxycarbonyl chloride type has been used effectively to add to a polymeric carrier peptides containing ω-amino-groups in diaminoacid residues[284-286]. The peptidyl polymers thus obtained can be cyclised directly on the carrier[284,285].

A novel method for solid-phase peptide synthesis from the terminal NH_2 group was developed by Felix and Merrifield[287], who suggested that aminoacid BOC-hydrazides can be used for the synthesis. The activation of the carboxyl of the peptide being synthesised by the azide method virtually rules out the possibility of racemisation in the peptide formation stage. Dahlmans and coworkers[288,289] also suggested that a polymeric carrier containing sulphonyl chloride groups be used for the synthesis of peptides from the terminal NH_2 group, the aminoacid residues being introduced into the chain in the form of their t-butyl esters. However, like the entire approach of this kind, this method has not so far become widely used.

‡There exists a little known but extremely simple method of synthesising iodomethylated styrene polymers and copolymers by treating the chloromethylated polymer with NaI [see V. V. Korshak, S. V. Rogozhin, and V. A. Davankov, Izv. Akad. Nauk SSSR, Ser. Khim., 1912 (1965); Vysokomol. Soed., 8, 1686 (1966)] (Ed. of Translation).

Not only the anchor group of the carrier but also the physicochemical properties of the polymeric matrix itself are important for the solid-phase synthesis. The polymeric carrier proposed by Merrifield ensures the diffusion of the dissolved reactants within the grains as a result of swelling in many organic solvents, which permits the employment of the entire bulk of the polymeric bead and at the same time its mechanical strength is fairly high. On the other hand, it is not free from many disadvantages. Studies of the structure of styrene–DVB copolymers are being continued [290–293]. The variation of the swelling of the gel polymeric carrier as a function of the chemical nature of the solvent and also the inadequate dimensions of the cells of the polymeric network complicate the diffusion process. To improve the diffusion of the solutes into the polymeric grain and to stabilise the polymer structure, some investigators suggest the employment of macroporous rigidly cross-linked carriers based on polystyrene. The advantages of the latter were noted a long time ago [294], but their practical application began later [173,199,295–297]. A comparative assessment of different carriers [173] suggests that macroporous carriers are extremely promising.

A higher rate of diffusion of solutions into the carrier beads can be achieved by employing fine-mesh polymers, but such carriers are inconvenient to handle. Therefore Bayer et al. [53] suggested that glass beads coated by a thin layer of polymers be used as the carrier. The possibility of employing such hybrid carriers was also demonstrated by other investigators [173,199,298,299]. The disadvantages of these carriers are their low specific capacity and the possibility of the gradual disruption of the outer organic layer, which leads to an appreciable loss of the peptide synthesised. The latter disadvantage is to a large extent eliminated when the anchor organic molecules are fixed on the glass beads by covalent bonds [60,300–304]. According to the authors, such pellicular carriers can be used for column solid-phase peptide synthesis.

The effectiveness of the interaction of the polymeric carrier with reactant solutions is low owing to the presence of a heterogeneous system. Here the advantages of the solid phase synthesis give rise to a significant disadvantage. A way out of this difficulty can be found by combining the possibilities of the solid-phase synthetic method with the synthesis of peptides in solution. For this purpose, Shemyakin, Ovchinnikov et al. [305] developed a method of peptide synthesis on a soluble polymeric carrier. It was suggested that polystyrene ($M = 2 \times 10^5$), soluble in organic solvents and insoluble in water, be employed. This makes it possible to carry out the peptide synthesis in a homogeneous medium and the peptidyl polymer can be purified in a heterogeneous medium. However, under these conditions there are losses of the peptidyl polymer in its precipitation from solution and also certain other complications. Nevertheless a number of biologically active peptides, including gramicidin S could be synthesised by this method [306–309].

The proposed approach is now being developed by the group of Bayer and coworkers [310–312] using poly(ethylene glycol) ($M = 1 \times 10^4$–2×10^4) as the carrier. The authors use ultracentrifugation and ultrafiltration through membranes to purify the intermediates.

Apart from poly(ethylene glycol), which differs in its nature from the traditional carriers based on polystyrene, attempts have been made to use other polymers in the solid-phase synthesis, in particular graft polychlorotrifluoroethylene, chloromethylated polystyrene [313], various polyphenols [269,314,315], and also resitol methylated in the phenolic hydroxyl and chloromethylated in the aromatic ring [93,115,316]. Polymers based on dextrans [2], for example Sephadex LH-20 and G-25, were also tried for this purpose [317,318].

III. THE ADDITION OF THE FIRST AMINOACID TO THE POLYMERIC CARRIER

The method of adding *N*-protected aminoacid to the chloromethylated polymeric carrier in organic solvents in the presence of tertiary amines is most commonly used. To reduce the possibility of the formation of ionogenic groups on the carrier as a result of the interaction of the tertiary amine with the chloromethyl polymer via the mechanism

$$Et_3N + Cl{-}CH_2{-}\textcircled{P} \longrightarrow Cl^- \cdot Et_3N^+{-}CH_2{-}\textcircled{P},$$

it is suggested that triethylamine be replaced by other bases, for example by ethyldi-isopropylamine [28]. An even greater effect is achieved when quaternary tetramethylammonium bases, proposed for this purpose by Loffet [133,152], are used. A definite effect is also observed on changing the solvent in which the ester is formed. t-Butyl alcohol is most promising for this purpose [207,208].

The standard methods of preparation of aminoacyl polymers presuppose interaction between the components at high temperatures (50–80°C), which is not always acceptable. Thus Marglin [176] observed a number of side effects under these conditions and suggested that they can be eliminated by synthesising aminoacyl polymers in DMF with triethylamine in the course of 24 h at 25°C. The results were somewhat unexpected, since hitherto DMF could be used at this stage only at 50–75°C. [23,220]

Since the nature and conditions of the interaction of the *N*-protected aminoacid with the polymeric carrier are determined primarily by the nature of the "anchor" group of the latter, clearly all the above considerations are applicable also to all other carriers containing the chloroethyl group in the aromatic ring.

N-Protected aminoacids add to bromo- and iodomethylated carriers under milder conditions. The reaction is usually carried out with triethylamine in DMF at 20 to 30°C in the course of 36–72 h. [228,230–233] Dicyclohexylamine is sometimes used instead of triethylamine [231,232]. A *C*-terminal aminoacid is added to a chloromethylated carrier in the presence of sodium iodide as the catalyst under still milder conditions [234,236–238].

Analogous conditions are required for addition also in the case of halogenoacetylated carriers. Ethyl acetate, methylene chloride, or DMF are usually employed as solvents [239–245] and the bases are normally triethylamine or ethyldi-isopropylamine [239,242].

In conjunction with the benzhydryl polymer, Southard and coworkers [249,250] used DCHA salts of BMV-aminoacids and other *N*-protected aminoacids for the preparation of aminoacyl polymers. The reaction was carried out by refluxing in chloroform for 12 h [249] or in methylene chloride at 20°C for 4 days [250].

Using a dimethyl(arylmethylene)sulphonium polymer, an aminoacyl polymer was obtained under very mild conditions without a tertiary amine [252]:

$$Me_2S + Cl{-}CH_2{-}\textcircled{P} \longrightarrow Cl^- \cdot Me_2S^+{-}CH_2{-}\textcircled{P} \xrightarrow{HCO_3^-}$$

$$\longrightarrow HCO_3^- \cdot Me_2S^+{-}CH_2{-}\textcircled{P} \xrightarrow[2.\ \text{drying}\ 3.\ \text{heating}]{1.\ \text{BOC-aminoacid}}$$

$$\longrightarrow BOC{-}NH{-}CHR{-}COO{-}CH_2{-}\textcircled{P} + CO_2 + Me_2S\ .$$

The yield of reaction product is then 87–96%, [218,253] while with chloromethylated carriers it does not as a rule exceed 25–30%.

Condensing agents such as CDI and DCC are used for the addition of the first aminoacid to carriers whose "anchor" group incorporates a hydroxyl. It is then necessary to block the remaining free hydroxy-groups when condensing agents are used in the peptide formation stage, which is not always very convenient.

Toluene-*p*-sulphonyl chloride is sometimes used as the condensing agent in the stage involving the formation of the aminoacyl polymer [263,265,266].

Condensing agents are also employed for the addition of the first aminoacid to carriers whose "anchor" groups contain an amino- or a hydrazino-group. DCC is most frequently used for this purpose [263,273–282]. The corresponding 2,4,5-trichlorophenyl esters of aminoacids have been used with the sulphonamide carrier [271].

In the synthesis of peptides from the terminal NH_2 group different aminoacids are added to the polymeric carrier in the form of an ester, amide, or another involving the carboxy-group in the presence of trimethylamine [283–289].

An interesting modification of the addition of the aminoacid to the carrier involves a functional side group. Thus Glass et al. [226] used the imadazole ring of histidine for the interaction with a fluorodinitrophenyl group of the polymeric carrier.

In those cases where the carrier pore size changes in the course of the synthesis, the aminoacids can add to sections of the polymer which subsequently become appreciably inaccessible to chemical attack by other reagents [13]. As a result of screening by the carrier, these aminoacids do not enter into peptide formation reactions, which leads to the appearance of erroneous sequences. The amount of such inaccessible residues can reach 40%. [319]

One of the ways of overcoming these disadvantages is the addition to the polymeric carrier of short peptides, incapable of passing into the very small carrier pores, instead of the individual aminoacids. Attempts were made to employ for this purpose polymers with modified functional groups, in particular halogenoacetyl [239,242] and bromomethyl [230–232]. It proved possible to use a chloromethylated carrier only in the reaction with depsipeptides on refluxing in ethyl acetate [320,331] or with the simple dipeptide BOC-Gly-Gly-OH. [227] We believe that the employment of sodium iodide in conjunction with a chloromethyl polymer is very promising. It is then possible to add to the carrier di-, tetra-, and penta-peptides in amounts of the order of 0.12–0.32 millimole g^{-1}. [237,238]

IV. PROTECTION AND DEBLOCKING OF α-AMINO-GROUPS

The benzyloxycarbonyl protecting group, employed initially by Merrifield [1], proved to be relatively unsuitable under the conditions of solid-phase synthesis [4]. Therefore its application is limited if one disregards studies where Z-aminoacids were used to introduce the *N*-terminal residue of the peptide being synthesised. Under there conditions, the benzyloxycarbonyl group is removed in the acid separation of the peptide from the carrier or leads to more stable derivatives when the peptidyl polymer is subjected to ammonolysis, hydrazinolysis, or transesterification.

Z-Aminoacids can be used in combination with a hydroxyalkylamino-polymer [264–266,296] and a hydroxybutyryl polymer [264,267]. The benzyloxycarbonyl group is then removed with a 30% solution of hydrogen bromide in acetic acid without loss of peptide and carrier. Z-Aminoacids are also suitable for peptide synthesis on a polymer of the sulphonamide type [271]. The polymeric carrier based on poly(ethylene glycol) [310,312] has an interesting property, i.e. the benzyloxycarbonyl group can be removed by hydrogenation, which could not be done when insoluble carriers were used.

Nevertheless the main substituent used to protect the α-amino group in the synthesis of peptides on a polymeric carrier continues to be the BOC group (Table 2). The availability and stability of BOC-aminoacids, the possibility of removing the BOC group by mild acid reagents, and many other advantages make it virtually irreplaceable at the present stage of development of solid-phase peptide synthesis.

The BOC-group is usually removed with a 1 *N* solution of hydrogen chloride in acetic acid [14], despite the possible risk of acetylation of the peptide amino-groups by trace amounts of acetic acid in the last stage of peptide formation. However, careful neutralisation and washing reduce this risk to a minimum.

A 4 *N* solution of hydrogen chloride in dioxan, proposed for the first time by Stewart and Woolley [322], is beginning to be increasingly employed for the elimination of the BOC group. Dioxan promotes the swelling of the polymer, which improves the efficiency of the deblocking. This is important, since the incomplete removal of the *N*-protecting group can be one of the causes of the omission of aminoacid residues from the chain [169].

Trifluoroacetic acid [242,323] or its 50% solution in ethylene chloride were proposed in 1966–1967 for the removal of the BOC group. The methyl chloride solution was used by Gutte and Merrifield [324] in the synthesis of ribonuclease A. In addition trifluoroacetic acid prevents the formation of the pyroglutamic acid residue from the BOC-glutamine residue at the instant of the removal of the BOC group, which happens when a 1 N hydrogen chloride solution in acetic acid is employed [325]. A 4 *N* solution of hydrogen chloride in dioxan can also be used for this purpose. The present authors successfully applied this procedure in the solid-phase synthesis of a number of fragments of the insulin A-chain [126].

A mixture of standard reagents with mercaptoethanol is used to remove the BOC-group from the tryptophan residue. The possibility of employing for this purpose a 20% solution of mercaptoethanesulphonic acid in acetic acid [162] and also of a 0.1 *N* solution of hydrogen chloride in formic acid [221] has been demonstrated.

Special studies have been made on problems of the removal of the BOC group [60,97]. The possibility of employing at this stage hydrogen chloride solutions in propionic acid [97], in a methylene chloride–DMSO mixture [134], and in methanol [206] was investigated. Chou et al. [134] obtained interesting results demonstrating the dependence of the degree of removal of the BOC group on the chain length of the peptide synthesised, the nature of the deblocked aminoacid residue, and its location in the peptide chain, which the authors believe to be the most important factor.

The AOC group, whose chemical properties are similar to those of the BOC group, was employed in the solid-phase synthesis for the first time by Inukai et al. [314]. It will probably be used on a wider scale with increase in the manufacture of AOC-aminoacids.

The NPS group, which is readily removed by solutions of hydrogen chloride in an inert solvent [328] and also by thioacetamide in a mixture of acetic acid and methanol [327], was introduced almost simultaneously into the solid-phase

Table 2. N^{α}-protecting groups and methods for their removal in solid-phase peptide synthesis.

Protecting group	Methods of deblocking	References
Z	30% HBr in AcOH	264–267,271,296
	Hydrogenation over Pd/C	310,312
BOC	1 *N* HCl in AcOH, 30 min	16,17,19,22,29,32–34,38,39,43,44,47,48,52–55,57,59,60,62–64,67,70–72,74,75[a],76,78,81–83,86,89,90,94,95,97,98,100,102,104,106,108,111,112,116–119,122[b],123–125,127,131,132,137–145,153,155,156,159,161,165,166,170,172,174,177,178,184,188,195,198,201–203,205,214–217,220,221,224,225,233,235,247,248,253,255,257,261,269,273,297,300,301,308
	1,2–1.5 *N* HCl in AcOH, 30 min	80,96,107,135,175,240,241,312
	4 *N* HCl in dioxan, 30 min	24,30,31,36,37,41,42,45,49,51,60,65,66,73,79,85,87,92,105,110,113,129,126,146,147,157,160,179,180,182,200,212,222,236,238,280,299[c],310,311,313
	1 *N* HCl in dioxan, 30–45 min	21[d],88,161,231
	2 *N* HCl in dioxan, 20–30 min	284–286
	5.6 *N* HCl in dioxan	134
	30% HCl in dioxan, 20 min	50
	CF_3COOH, 30–40 min	46,74,226,242,253,271,278,317,318
	50% CF_3COOH in CH_2Cl_2, 15–30 min	42,60,84,97,128,129,134,148,157,165,167,168,190,191,193,194[e],196,197,202,209[f],211,213[g],218,223,233,258–260,262,268,275–277,281,282
	50% CF_3COOH in chloroform	101,103,183[h]
	0.8 *N* HCl in EtCOOH	97
	5.35 *N* HCl in 1:1 DMSO–CH_2Cl_2 mixture	134
	1 *N* HCl in MeOH, 30–40 min	206
BOC in the presence of Trp residue	20% $HSCH_2CH_2SO_3H$ in AcOH, 30 min	162
	0.1 *N* HCl in HCOOH	221
AOC	10% HCl in MeOH or dioxan, 1–2 h	241,245,315
	1 *N* HCl in AcOH, 40 min	28,241,244
	50% CF_3COOH in CH_2Cl_2	121
NPS	0.1–1 *N* HCl in organic solvent, 10–20 min	71,99,131,222,235,284,285
BPOC	0.5–1.5% CF_3COOH in CH_2Cl_2, 10–15 min	42,263,272
	0.05–0.1 *N* HCl in chloroform, 5–10 min	191,197,262
	Glacial AcOH, 8 h	156,159
BMV	Aqueous 0.4 *N* HCl in THF, 30 min	249
	1 *N* toluene-*p*-sulphonic acid in THF	250
FOC	1 *N* HCl in AcOH or dioxan	115,330
MZ	1 *N* HCl in AcOH, 30 min	149
	50% CF_3COOH in CH_2Cl_2, 30 min	232
Pht	N_2H_4.AcOH in DMF, 24 h	263
Me_3Si	1% AcOH in ethanol	309

[a] 0.5 *N* HCl in AcOH.
[b] 15 min instead of 30 min.
[c] 78 s instead of 30 min.
[d] 1:1 dioxan–CH_2Cl_2 mixture.
[e] 12.5–25% CF_3COOH in CH_2Cl_2.
[f] 75 min instead of 15–30 min.
[g] 4:1 CF_3COOH–CH_2Cl_2 mixture.
[h] 25% CF_3COOH in chloroform.

synthesis in three laboratories[326–328]. The group is extremely useful in many special cases owing to its high lability[71,99,131,222,235,284,285].

The BPOC group, proposed by Sieber and Iselin[329], is even more labile. It is two thousand times more sensitive than the BOC group to acid cleavage and can be removed with 75% trichloroacetic acid in 1.5 h,[329] with 0.5–1.5% solution of trifluoroacetic acid in methylene chloride[42,263,272], with 0.05–0.1 *N* hydrogen chloride solution in chloroform in 8–10 min,[191,197,262] and also with glacial acetic acid in 8 h.[156,159]

Mention should also be made of the BMV group, proposed by Southard and coworkers[249,250] in conjunction with a benzhydryl polymer. It can be removed with 0.4 *N* aqueous hydrogen chloride solution in THF or with 1 *N* solution of toluene-*p*-sulphonic acid in the same solvent. The FOC group[115,330] and MZ group[149,232], whose stabilities are similar to those of the BOC group, are also used in solid-phase peptide synthesis. The employment of the Pht group in conjunction with the hydroxy-t-alkylpolymer[263] and also of the trimethylsylyl group[309], which is suitable for the synthesis of peptides using aminoacid-*N*-carboxy-anhydrides, has been reported.

In selecting the N^{α}-protecting group, investigators devoted special attention to the deblocking stage[99].

V. PROTECTION AND DEBLOCKING OF THE FUNCTIONAL GROUPS IN THE SIDE CHAINS OF TRIFUNCTIONAL AMINOACIDS

Fairly convenient protecting groups, satisfying the requirements of the solid-phase method, which in general are the same as the requirements of the classical method for the synthesis of peptides, are now available for all trifunctional aminoacids.

Arginine. Nitroarginine[18,22,24,36,37,42,44,51,52,54,59,69,88,95,120,124,128,129,131,139,140,142,144,145,148,160,161,165,168,175,186,191,193,200,201,206,211,215,216,218,220,223,225,233,250,263,268,275–278,280,281] is usually employed with subsequent hydrogenation of the peptide when separated from the carrier. When the peptidyl polymer is treated with anhydrous hydrogen fluoride, this stage can be eliminated. For peptides containing cysteine and methionine, N^G-tosylarginine is usually employed[70,71,76,92,107,121,172,179,191,194,197,212,262].

Aspartic and glutamic acids. The ω-carboxy-groups of these aminoacids are successfully protected by the formation of benzyl esters[19–21,24,27,32,36,37,42,44,51,55,57,59,76,78,88,94,124,127–129,142,143,145,148,161,167,172,175,188,190,197,198,206,220,231,233,250,263,268,282], which are readily cleaved on treatment of the peptidyl polymer with acid. t-Butyl esters, which are more labile in an acid medium but are resistant to hydrazinolysis, are sometimes used[95,100,102,151,156,159,205,209,238,262]. In order to keep the ω-carboxy-group protected even after the separation of the peptide from the polymeric carrier by acid treatment, nitrobenzyl esters of the aminoacids are employed[90,113,126,194].

Asparagine and glutamine. The *p*-nitrophenyl esters of the BOC derivatives of asparagine and glutamine are usually employed to introduce their residues into the peptide, since the use of DCHA leads to the formation of the corresponding nitriles. On the other hand, if DCHA has to be used, the amide groups of asparagine and glutamine must be protected, for example by the MBZH group, which can be removed by refluxing with trifluoroacetic acid[131].

Histidine. The N^{Im}-benzyl group is most often used to protect the histidine imidazole ring. It can be removed either by catalytic dehydrogenation or by sodium in liquid ammonia[21,33,37,44,59,76,80,81,82,95,96,100,102,117,120,121,137,139,140,142,157,161,169,172,175,188,190,192,194,198,201,211,212,215,216,225,233,262,263]. The N^{Im}-group of histidine can be deblocked simultaneously with the separation of the peptide from the polymeric carrier when N^{Im}-tosylhistidine is employed[183,186,200,277,278].

The DNP group, which can be removed by treatment with thiols at pH 8 and room temperature, must be regarded as the most convenient protecting group for the histidine side chain[47,63,124,146,153,165,181,196,206,281,282,332]. The possibility of removing this group under the conditions of alkaline hydrolysis, aminolysis, and hydrazinolysis of peptidyl polymers has also been demonstrated[181]. The choice of the protecting group for the histidine imidazole ring has been the subject of a special examination by Losse[331,332].

Lysine. The benzyloxycarbonyl group, which can be readily removed by the acid cleavage of the peptidyl polymer but is entirely stable in combination with the N^{α}-BOC protecting group, is usually employed to protect the N^{ϵ}-amino-group of lysine[16,29-31,33,44,47,49,50,63,76,87,88,102,124,127,128,129,144,145,148,151,153,155,156,159,161,172,176,182,188,194,196,197,206,220,221,231,253,275,280,282]. There are, however, data demonstrating the partial elimination of the N^{ϵ}-Z group when the N^{α}-BOC group is removed. In this respect the *p*-Br-Z protecting group is more reliable[213]. The tosyl group[40,92,114,117,179,194,203,262], which can be removed by treatment with sodium in liquid ammonia, and also the TMA group[24,36,37,45,51,296], which is eliminated by treatment with 1 *N* piperidine solution, are sometimes used to protect the lysine side chain.

Methionine. Methionine is usually used without protecting the functional group in the side chain, but special protecting agents must be used in the stage involving the separation of the peptide from the carrier (which is not always successful). For this reason BOC-Met(O)-OH is sometimes employed to synthesise methionine-containing peptides[124,128,145].

Ornithine. As in the case of lysine, the amino-group of ornithine in the side chain is protected by the benzyloxycarbonyl[275,284,285] or tosyl[93] group; MZ-Orn(p-NO_2-Z)-OH has also been used[149].

Serine and threonine. Simple benzyl esters[21,24,33,36-38,51,52,76,92,94,104,112,124,128,129,132,143-145,148,151,153,156,159,161,168,172,174,176,186-188,193,194,196-198,200,206,212,213,215,216,220,223,241,253,262,268,276-287,282], which are cleaved under the conditions of the acid treatment of the peptidyl polymers, are normally used to protect the hydroxy-groups of serine and threonine. When activated esters are used in the peptide formation stage, serine and threonine are introduced into the peptide chain without protecting the hydroxyl. The employment of Weygand's protecting group (1-benzyloxycarbonylamino-2,2,2-trifluoroethyl) for the serine and threonine side chains has been reported[38].

Tyrosine. The tyrosine hydroxy-group is usually protected in the form of the benzyl ether[20,24,32,33,36,39-41,43,44,47,48,51,57,59,63,74,76,80,92,104,110,112,114,120,121,124,126,128,137-140,142,143-145,155,156,159,167,170,172,174,175,184,186,188,193,197,200,203,205-208,210,212,213,233,235,237,238,240,241,243,247,248,262,263,275-278,282]. The latter is readily cleaved by hydrogen bromide in trifluoroacetic acid or by hydrogenation, but, in contrast to the analogous serine and threonine derivatives, it is relatively stable in anhydrous hydrogen fluoride, which is not very convenient. It is suggested that the *p*-methoxybenzyl and *m*-bromobenzyl groups are more suitable in this respect[213].

Cysteine. In most cases the benzyl group, which can be removed from the cysteine residue only with sodium in liquid ammonia under rigorously controlled conditions (owing to the possibility of side reactions), is used to protect the SH functional group of cysteine[33,39,41,43,44,48,70,71,74,76,79,81,104,107,110,112,128,129,131,138,143,155,167,170,172,174,184,194,198,203,236-238,247,248,255].

The employment of anhydrous hydrogen fluoride can also be made difficult by N → S acyl migration. It has been suggested that *S*-*p*-methoxybenzylcysteine be used to prevent this[69,81,145,151,220,282].

Sometimes the SH group of cysteine is protected with the trityl[15,81] and benzyloxycarbonyl[235] groups, but at the present time two approaches are of greatest interest for the synthesis of crysteine peptides. These are in the first place the employment of different protecting alkylthio-groups (for example, the ethylthio-group), proposed by Weber and coworkers[20,32,57,150]. These groups are removed by sulphitolysis. The second approach to the solution of the problem is based on the suggestion of Lunkenheimer and Zahn[61,111] that bis-BOC-cysteine be used for the peptide synthesis, which eliminates the need for special protection of the SH group. Both approaches still require further investigation, like the problem of the protection of the SH group as a whole.

The studies of Zahn and coworkers[81] and of Weber and coworkers[144,150] have been devoted to these problems.

VI. METHODS FOR THE FORMATION OF THE PEPTIDE BOND

The peptide formation stage is of key importance in peptide synthesis, which is particularly true of the solid-phase method. The absence of intermediate stages involving the purification of the peptide synthesised necessitates the attainment of 100% yield in this stage.

DCC remains the most common condensing agent (Table 3). All the protein aminoacids (except asparagine and glutamine) can be successfully introduced with the aid of this agent into the peptide chain joined to the polymeric carrier. Difficulties are sometimes encountered in the introduction of valine and isoleucine residues into the peptide chain[167] owing to their steric structure. The structure of the polymeric carrier also has a definite influence on the degree of the peptide formation reaction[199].

The nature of the solvent is likewise very important; it must be of relatively low polarity. Therefore methylene chloride, sometimes with small amounts of added DMF, is usually employed in the condensation stage. Under these conditions, a 2.5-3-fold excess of the reactants is used, the reaction being carried out in the course of 2-4 h with stirring in a minimal volume of the solvent. Sometimes the condensation with DCC is performed in other organic solvents, in particular in chloroform[101,103,160,250] or THF.[30,31,49,50,87] The method of activated esters has been frequently attempted in the solid-phase synthesis, but without success. Thus Bodanszky and Sheehan[246,254] investigated the employment of *p*-nitrophenyl esters of aminoacids. However, despite a number of advantages compared with the carbodi-imide method, this procedure is as a whole more laborious, since it requires a longer

condensation time and the preliminary synthesis of amino-acid derivatives. Nevertheless many important peptides, including the insulin A-chain and its analogues, have been obtained using *p*-nitrophenyl esters[333,334]. Beyerman et al.[335] investigated the catalytic effect of 1,2,4-triazole when activated esters are used. This approach was employed also by a number of other investigators[116,117,161,215,216], despite the claim of Ragnarsson et al.[136] that 1,2,4-triazole has no catalytic effect.

Table 3. Methods for peptide formation in solid phase synthesis.

Method	Condensation conditions	References
Dicyclohexylcarbodi-imide	CH_2Cl_2, 25°C, 2-4 h	17,19,21,24,25,28,29,32—48,51,53—55,57,59—61,63,65,69—71,73,74,76—83,85,86,88—94,96,97,99,104,107,108,110,112,114—118,120—124,126—129,131,132,135,137—146,148,149,153—157,159,161,163,164,166,167,169,171,172,174,175,177—179,182—184,186,188,190—193,196—201,205—207,209—216,218,220,222,223,225,227,233,235,236,238,240,247—249,253,255—257,259—263,268,269,272,273,275—277,280—282,286,289,296,297,299—301,303,304,312,313
	CH_2Cl_2-DMF, 25°, 2-4 h	97,111,113,244
	DMF, 25°, 12-14 h	16,95,96,100,102,105[a],119,127,147,231[b],245,278,308
	$CHCl_3$, 25°, 2 h	101,103,160,250[c]
	THF, 20° (before swelling), 20° (12 h)	30,31,49,50,87
Activated *p*-nitro-phenylesters	DMF, 25°, 8-24 h	54,84,107,118,127,156,159,167,172,180,235,300,301,308[d],317,318
	with 1,2,4-triazole in DMF, 25°, 12-24 h	116,117,136,161,203[e],215,216,255
N-hydroxysuccinimide esters		44,70,106,107,192,317,318
	with 1,2,4-triazole	116
	N-hydroxysuccinimide + DCHA in DMF, 25°, 8-12 h	78,117,127,242,284,285
Pentachlorophenylesters	DMF, 25°, 12 h	52,56,95,100,102
	with *N*-methylmorpholine in DMF, 25°, 20 h	226
Trichlorophenylesters	DMF-CH_2Cl_2	67,69,111
	with 1,2,4-triazole in DMF, 25°, 20-65 h	157
	with 2,6-lutidine in DMF, 25°, 24 h	271
2-Pyridyl esters	CH_2Cl_2, 25°, 12 h	170
Mixed anhydrides with isobutyl chloro-carbonate	DMF	27,310—312
With *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydro-quinoline	CH_2Cl_2, 25°, 2-12 h	168
Symmetrical anhydrides	CH_2Cl_2, 25°, 2 h	158,207
N-Carboxyanhydrides	DMF, 25°, 24 h	16
	$CHCl_3$, 25°, 1.5 h	309
Water-soluble carbo-di-imide	H_2O	310

[a] 5 h. [b] 72 h. [c] 12 h. [d] $CHCl_3$ instead of DMF. [e] 1-Hydroxybenzotriazole instead of 1,2,4-triazole.

N-Hydroxysuccinimide esters, used for the first time by Shemyakin, Ovchinnikov, and coworkers[305] in combination with a soluble carrier, are being increasingly applied in the peptide formation stage. This method has proved suitable also for insoluble polymers[44,70,106,107,116,192,318]. The esters are frequently obtained directly at the instant of condensation, using *N*-hydroxysuccinimide and DCC.[78,117,127,242,284,285]

Instances where trichloro- and pentachloro-phenyl esters were employed (Table 3), sometimes with added 1,2,4-triazole[157], *N*-methylmorpholine[226], and 2,6-lutidine[271], are also known. The use of 2-pyridyl esters has also been reported[170]. Some of the methods enumerated above have been compared by Bodanszky and Bath[56].

Among other procedures for the formation of the peptide bond, mention should be made of the method of mixed anhydrides with isobutyl chlorocarbonate, which has been used for the synthesis both from the *N*-end[283] and from the *C*-end[27,310-312]. The employment of the mixed anhydride of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline and a BOC-aminoacid has also been reported[168]. The possibility of employing symmetrical anhydrides of BOC-aminoacids[158,207] and aminoacid *N*-carboxyanhydrides is likewise possible[16]. Admittedly in this case it is still impossible to control exactly the number of combined aminoacid residues, which limits the applicability of this method. The possibility of the interaction of *N*-carboxyanhydrides with *N*-trimethylsilyl derivatives of aminoacids joined to a non-cross-linked polystyrene has been demonstrated[309].

When poly(ethylene glycol) is used in the solid-phase synthesis, the peptide chain may be grown using the water-soluble carbodi-imide[310].

VII. THE PROBLEM OF THE FORMATION OF ERRONEOUS SEQUENCES IN SOLID-PHASE PEPTIDE SYNTHESIS

It is noteworthy that none of the methods of peptide formation discussed above can guarantee 100% condensation. The situation is increasingly complicated with increasing growth of the chain being synthesised. To ensure an adequate degree of condensation, it is necessary to employ an increasing excess of the aminoacids. Thus in the synthesis of the 104-member analogue of the apoprotein of cytochrome c from horse heart, a 30–70-fold excess of BOC-aminoacids was used in the last stages[44,88]. The yields are somewhat improved, but the fundamental difficulties are not eliminated, by repeated condensations[104,112,121,148,169,198,207,209,211,233,259,260]. All this leads to the omission of individual aminoacid residues from the chain. Bayer, Hagenmaier, and coworkers[53,62,86] devoted much attention to the problem of the formation of erroneous sequences associated with the omission of aminoacid residues due to the incomplete occurrence of the deblocking and peptide formation stages. They estimated not only qualitatively but also quantitatively the possible related peptide impurities and suggested a number of methods for detecting such side reactions, in particular methods based on mass spectroscopy and ^{19}F NMR[132,302,312]. Baas et al.[336] actually proposed a special formula for the quantitative estimation of possible impurities, taking into account the yield at each peptide formation stage.

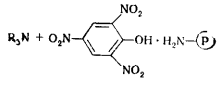
The foregoing considerations obviously demonstrate the importance of increasing the yield in the condensation stage and the necessity for careful and clear-cut monitoring of this stage as well as the stage involving the removal of N^{α}-protecting groups. There are a number of methods for following these processes. The most traditional are quantitative hydrolysis of the peptidyl polymers with subsequent aminoacid analysis[1], the separation of the intermediate peptides from the polymeric carrier and analysis of their homogeneity by various procedures, and spectrophotometric determination of any one reaction

product whose amount is proportional to the yield at the condensation or deblocking stages. For example, it is possible to determine the *p*-nitrophenol formed when one uses *p*-nitrophenyl esters of aminoacids [254,308,333], *o*-nitrophenylsulphenyl chloride, where NPS-aminoacids are employed [284,285], or benzoylacetone when the BMV protecting group is used [250].

The dansylation or dinitrophenylation method is sometimes employed to determine free amino-groups [73,185,215,216,312], but these procedures are laborious, which hinders their wide-scale employment.

The extent of the condensation reaction has also been analysed by determining the chloride ions in the triethylamine hydrochloride formed in the stage involving the neutralisation of the aminopeptidyl polymer (Table 4). However, it is then impossible to establish whether the reduced content of chloride ions is due to the incomplete condensation reaction or whether it is due to the incomplete deblocking of the α-amino-groups.

Table 4. Methods for the determination of free amino-groups in solid-phase peptide synthesis.

Reagent	References
Dansyl chloride or DNP chloride + H_2N–(P)	73,185,215,216,312
Et_3N + HCl · H_2N–(P)	29,38,70,81,90,94,107,108,111,113,142,159,253,284,285
Et_3N + HX · H_2N–(P) (HX from Py.HX) (X = Cl or Br)	35,62,67,78,86,132,151,164,198,199,253
R_3N + 	258—260
2-Hydroxy-1-naphthaldehyde + H_2N–(P)	60,77,84,99,129,191,257
2-Amino-1-naphthaldehyde + H_2N–(P)	223
Ninhydrin + H_2N–(P)	78,134,168,169,200,203,211,226,277,278,280—282,310,312,313
Bromocresol Purple (indicator)	337
Potentiometric titration with $HClO_4$ + AcOH	58,68,195,214

Dorman's method [35], in which the reaction mixture is treated with pyridine hydrohalide after the peptide formation stage in order to form the hydrohalides of the amino-groups which failed to condense, is more convenient in this respect. (The BOC group of the aminoacid introduced remains unaffected under these conditions.) The resulting salt of the aminoacyl polymer is treated with triethylamine, washed, and the filtrates are analysed for chloride ions [62,67,78,86,132,151,164,198,199,253]. Instead of pyridine hydrohalide, one can use, for example, picric acid [258–260]. The picrate formed is decomposed by a suitable amine and the solution is analysed spectrophotometrically for picric acid.

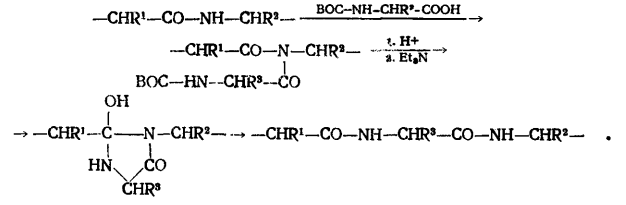
There are a number of other qualitative and quantitative methods whereby free amino-groups can be estimated. These include the use of 2-hydroxy-1-naphthaldehyde [60,77,84,99,129,191,257], 2-amino-1-naphthaldehyde [223], the Bromocresol Purple indicator [337], and also ninhydrin (Table 4).

Some of the methods quoted for estimating free amino-groups have been assessed in special investigations [164,338]. We believe that the procedure which is fastest, most accurate, and, what is most important, susceptible to automation is that proposed by Brunfeldt and coworkers [58,68,195,214], involving the potentiometric titration of the free amino-groups with perchloric acid in the presence of acetic acid.

Together with the problem of determining the extent of the condensation reaction, the problem of the blocking of the amino-groups which failed to enter into the peptide formation reaction merits examination. Thus, after each condensation stage, Merrifield [1] acetylated with acetic anhydride the amino-groups which failed to react, a procedure which is frequently employed also at the present time [62,86,91,92,116,117,139,148,179,197,198,212,215,216,260,281,313]. *N*-Acetylimidazole is also used for this purpose [91,282].

In order to create large differences between the acid-base properties of the required peptide and the side products, it is convenient to employ 3-nitrophthalic [55,177,203,207] and 3-sulphopropionic [131] anhydrides as blocking agents. The derivatives of shorter peptides formed under these conditions contain an additional acid group which facilitates their separation from the desired peptide.

Whereas the problem of monitoring the solid phase synthesis has to some extent been solved, that of increasing the yield of the peptide formation stage still remains entirely open. The duplication of the condensation stages and the employment of a large excess of *N*-protecting aminoacids do not provide a solution. Moreover, a large excess of BOC-aminoacids can lead to an additional number of erroneous sequences owing to the incorporation of the aminoacid residues within the peptide chain being synthesised [46], in accordance with the following mechanism:



It is inconvenient to employ an excess of *N*-acylated aminoacids also for purely economical reasons, since their regeneration is relatively ineffective and requires further development [339].

From this point of view, the most interesting method of economical solid-phase peptide synthesis is that of Esko and Karlsson [77], which is based on the sorption of the BOC-aminoacid by the aminoacyl polymer. The amount of sorbed reactant is sufficient to carry out the peptide formation stage and the excess BOC-aminoacid can be removed from the reaction vessel even before adding the condensing agent. The sorption phenomenon was used by Elliott et al. [218] in the synthesis of bradykinin analogues.

The low efficiency of the condensation reaction is probably related to the fact that, as the chain being synthesised grows in length, there is a possibility that it may roll up on the carrier into a disordered coil, which leads to the screening of the *N*-terminal amino-group. Hydrogen bonds can play a significant role in this process. This hypothesis is confirmed by the fact that the addition of urea, which dissociates hydrogen bonds, to the reaction mixture results in an increase of the rate and degree of deblocking of the BOC groups [134] and also in an increase of the yield in the peptide formation stage [97,148,282].

We believe that one of the possible methods of preventing the coiling of the peptide chain may be the additional (segmental) fixation of the individual sections of the growing peptide chain on the carrier as a result of the interaction with the latter of the functional side groups of the peptide [236].

Nevertheless the problem of the solid-phase synthesis of long peptide chains remains open. At the present stage the most rational procedure in this respect involves a combination of the possibilities of the solid-phase method (the synthesis of short chains) and the classical procedure for joining them together in solution. This approach has been called the solid phase-fragment method.

Furthermore, one should bear in mind the possibility of the block version of peptide synthesis on a carrier. This method was investigated for the first time by Weygand and Ragnarsson [340] and in greater detail by Omenn and Anfinsen [341]. Subsequently block synthesis in different versions was used also in other laboratories [96,102,117,127,147,215,227,231,232,257,297]. However, block condensation on a polymeric carrier involves a number of specific difficulties which so far restrict the application of this method in practice.

VIII. METHODS FOR THE SEPARATION OF PEPTIDES FROM THE POLYMERIC CARRIER

The commonest method for the separation of peptides from the polymeric carrier involves the use of hydrogen bromide in trifluoroacetic acid (Table 5). A number of protecting groups, other than the *S*- and N^{Im}-benzyl, N^{G}-nitro-, tosyl, and *O*-*p*-nitrobenzyl groups, are removed at the same time. A 1:1 mixture of trifluoroacetic acid and methylene chloride has been used recently fairly frequently as a solvent in this process [65,77,128,168,176,179,188,194], which increases the swellability of the polymer.

When the peptide does not contain serine and threonine residues, it is possible to separate it from the carrier by treatment with hydrogen bromide in acetic acid [116,260,284,285,301]. To protect the residues of certain other amino-acids (cysteine, methionine, and tyrosine) from the alkylating action of the benzyl bromide formed in the reaction, protecting agents such as anisole, ethyl methyl sulphide, or methionine are added to the reaction mixture.

Peptides are being increasingly separated from the polymeric carrier by treatment with anhydrous hydrogen fluoride, which was used for this purpose for the first time by Lenard and Robinson [342]. All protecting groups, other than the N^{Im}-benzyl group, are also removed at the same time.

Among other acid agents, one must mention the use of a 50% solution of trifluoroacetic acid in methylene chloride and a solution of boron trifluoride in ether, which are used in special cases (Table 5). The employment of hydrogen bromide in trifluoroacetic acid for a long period (more than 1h) can lead to partial degradation of the peptide, particularly when the chain contains proline residues [116,343,344].

Alkaline agents, for example a solution of sodium hydroxide in aqueous alcohols, are also used in the stage involving the cleavage of the peptidyl polymer [1,30,31,49,50,87]. Water–dioxan or water–alcohol–dioxan solutions of sodium hydroxide are likewise employed [25,171,238,240,241,310–312]. The use of sodium methoxide [312] and sodium thiophenoxide [241,242] has been described.

In order to obtain peptide amides, peptidyl polymers are treated with ammonia under different conditions

Table 5. Methods for the separation of peptides from the polymeric carrier.

Reagent	Reaction conditions	References
Acid reagents		
HBr in CF_3COOH	25°, 60–90 min	17,19,21,27[a],32,34,35,37,38,40,45–47,52–55,57,59,63,74,76,79–83,86,90,91,93–95,100,102,106,111,114–117,119,120,122,126,130,132,135,137,139–143,148,153,154,158,161,166,170,172,175,176,180,190,196,201,207,208,210,211,215,216,218[b],224,225,227,233,235,238,241,253,256,257,260,286,287,299,301,303,304
HBr in 1:1 CF_3COOH–CH_2Cl_2 mixture	20–25°, 60–90 min	65,77,128,168,176,179,188,194
HBr in AcOH (40–60% soln.)	20–25°, 20–60 min	116,260,284,285,301
HF (liq.)	0°, 30–60 min	24,28,36,37,42,44[c],51,71,77,85,88,98,113,121,124,127–129,145,154,160,167,169,176,182,183,186,191,194,197,198,206,213[c],216,220,221,223,231,232[c],273–282,297,313
50% CF_3COOH in CH_2Cl_2 or $CHCl_3$	25°, 30 min	249,250,263,272
0.03 *N* BF_3 in ether + AcOH–$CHCl_3$	—	250
Alkaline reagents		
1–2 *N* NaOH in ROH (R = Me or Et)	25°, 30–60 min	30,31,40,49,50,87,114,116[d],243,296,315
0.5 *N* NaOH in dioxan	25°, 6 h	240,241
2–4 *N* NaOH in dioxan	20–25°, ~1 h	238,261
2 *N* NaOH in dioxan–ethanol mixture	—	25,171
Aqueous 0.05 *N* NaOH	—	310–312
1 M MeONa in MeOH	—	312
~10% PhSNa in DMF	20–25°, 5–10 h	241,242
Ammonolysis		
Sat. NH_3 in ROH (R = Me or Et)	0–4°, 12–24 h	29,39,43,48,69,71,73[e],104[f],112[f],123[g],138,144,146[h],147h,155[f],176,178,184,194,240,241,244,245,248,255
NH_3(liq.)	—	29
NH_3(liq.) in DMF	~20°, 5–10 days in sealed tube	156,159,174,205,209,261
Sat. NH_3 in 1:1 DMF–ROH mixture (R = Me or Et)	~20°, 24–72 h	41,105,107,110,192,193,200[i]
$MeNH_2$ or Me_2NH in MeOH	0–4°, 20–24 h	203,247
Hydrazinolysis		
N_2H_4 or $N_2H_4.H_2O$ in ROH (R = Me or Et)	20–25°, 3–15 h	42,118[j],240,241
N_2H_4 or $N_2H_4.H_2O$ in DMF	20–25°, 1–3 days	118,126,149,212,221
Transesterification		
MeOH–Et_3N or other tertiary amine	20–25°, 16–48 h	18,64[k],132,157,163,170,177,222,241,255
MeOH–Et_3N or other tertiary amine in DMF	~20°, 18–48 h	92[l],107,159
MeOH–anion exchanger	20–25°, 2 h	33,64

[a] 3–6 min. [b] At 5°C. [c] 1.5 h. [d] 1% NaOH. [e] Sat. at −20°, 48 h at 5°C. [f] 2.5 h at −10°C, 18 h at 22°C. [g] 70 h. [h] Sat. at 0°C, 48 h at 20°C. [i] 20°, 4:1 dioxan–MeOH mixture. [j] 48 h. [k] MeOH, EtOH, i-PrOH + various tertiary amines. [l] 22 h at 42°C.

(Table 5). The possibility of interchain aminolysis, which may lead to the occurrence of erroneous sequences, has been reported [227]. Intrachain aminolysis is sometimes also observed, particularly in the dipeptide stage, with formation of dioxopiperazines. This process is facilitated when the *C*-terminal aminoacid is proline or an *N*-methylated aminoacid [202,214,233,259,260].

Hydrazine or hydrazine hydrate is frequently used to separate the peptide from the carrier, particularly in the synthesis of fragments for subsequent azide block condensation in solution (Table 5).

The transesterification method in the presence of tertiary amines or a strongly basic anion-exchange resin has been applied quite recently in the stage involving the cleavage of the peptidyl polymer (Table 5). In this connection there is a risk of partial loss of the peptide from the polymeric carrier when the peptidyl polymer is washed with alcohol after the neutralisation stage [64] and also of the conversion of ω-benzyl esters into ethyl or methyl esters [345].

A comparative assessment of the various methods for the separation of peptides from the polymeric carrier has been carried out [346] and the effect of various solvents on the extent of separation of the peptide has been investigated [98].

Hydrogen bromide in trifluoroacetic acid in the case of a polymer of the alkyloxycarbonyl chloride type [287] and alkaline agents or a 36% solution of hydrogen bromide in acetic acid in the case of a polymer treated with sulphonyl chloride [289] are used to separate peptides attached to the carrier via the *N*-terminal amino-group.

Peptides joined to the carrier via a side chain amino-group are separated under similar conditions [284-286].

As already mentioned in Section II, there are many carriers whereby the peptidyl polymer can be converted at the end of the synthesis into an activated polymeric ester [268-271]. This ester can react with any amino-component, for example with the ester of the *C*-terminal aminoacid [268] or with the deblocked α-amino-group of the peptide synthesised (cyclisation on the carrier) [269]. A similar situation arises also when the hydrazides and 4-carboxyphenylhydrazides are used in the stage involving the formation of the aminoacyl polymer [108]. The above derivatives can be activated also at the end of the synthesis.

IX. AUTOMATION OF SOLID PHASE PEPTIDE SYNTHESIS

The absence of non-standard operations in the solid-phase synthesis makes it possible to mechanise and automate the entire process. The first automatic synthesisers were reported as early as 1965–1966. [347,348] A discussion of the design characteristics of such automatic apparatus can probably serve as the subject of a separate review and therefore we shall confine ourselves here to the examination of the principal trends in the construction of apparatus of this kind.

An interesting version of an automatic synthesiser with a perforated tape programming unit was proposed by Brunfeldt and coworkers [349-351], who also suggested a special apparatus whereby one can monitor automatically the synthesis by potentiometric titration [68,214]. A number of studies have been made on the development and improvement of individual units of the automatic device. Thus, a fundamentally new design for the reaction vessel was developed by Birr and Lechinger [166,352]. They made the reactor in the form of a rotor with porous walls, which made it possible to separate the solvent from the carrier by centrifugal filtration. The systems for the metering and supply of solutions, the methods of stirring the reaction components in the vessel, etc. are also being improved [211,219,353-356].

It is still difficult to specify the optimal version of the automatic synthesiser. However, it is already clear that such apparatus will become available to many investigators in the immediate future.

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Summarising the foregoing one can conclude that solid-phase peptide synthesis is in a state of intense development. While having many important advantages over classical methods of synthesis, it is not free from disadvantages which limit its practical applications. In assessing its present state and the prospects for its development, both undue enthusiasm and excessive skepticism would be inappropriate. A sorber, critical approach to this problem is particularly necessary. However, the data examined above show that many imperfections of the solid-phase method are typical growing pains, which are normally cured eventually. This is shown particularly by the recent publications on the improvement of the conditions for peptide formation [357], on the development of new protecting groups for trifunctional aminoacid residues [358], and on the application of modified polymeric carriers [359]. The methods for the addition of the first aminoacid to the polymeric carrier are also being improved [360,361] and the solid-phase-fragment method of synthesising peptides is being successfully developed [362].

The above trends have been reflected also in certain very recent publications [363-376] which appeared during the preparation of this review for the press.

All this leads to the hope that the existing versions of solid-phase peptide synthesis will be rapidly improved and that the serious disadvantages from which the method still suffers will be overcome.

On the whole, the solid-phase method can be regarded as an extremely promising procedure for peptide synthesis.

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