Translated from *Uspekhi Khimii*, **51** 1567 - 1578 (1982)

Protection of the Amino-group in Peptide Synthesis

S.V.Burov and M.P.Smirnova

N-Protecting groups most widely used in peptides synthesis are examined. The possible mechanisms of the elimination of the protecting groups in acid and alkaline media are described and the methods of synthesis of the reactants for the introduction of certain protecting groups are considered.
The bibliography includes 65 references.

CONTENTS

I. Introduction 902
II. Acid-labile protecting groups 902
III. Alkali-labile protecting groups 906

I. INTRODUCTION

Two main trends may be isolated in the development of peptide chemistry: the improvement of methods for the formation of the peptide bond and the development of new more convenient protecting groups. The advances made in this field in recent times made it possible to simplify greatly the synthesis and to increase its effectiveness.

This review deals with the present state of the problem of the utilisation of *N*-protecting groups in the synthesis of peptides by the classical and solid-phase methods.

Despite the availability of deblocking methods such as catalytic hydrogenation, electrochemical reduction, treatment with sodium in liquid ammonia, irradiation with ultraviolet light, etc., the majority of protecting groups can be classified as either acid-labile or alkali-labile. Subsequently, we shall adhere to this conventional subdivision, considering other methods for the removal of protecting groups only in those cases where they are of special interest. The traditional protecting groups are described only briefly and some of these are not considered at all since their properties have been frequently discussed in the literature[1-5].

II. ACID-LABILE PROTECTING GROUPS

The first acid-labile protecting group, which has found extensive practical applications and which has exerted a great influence on the development of peptide synthesis, was the benzyloxycarbonyl group proposed in 1932 by Bergmann and Zervas[6]. The simplicity of the preparation and the ready availability of benzyloxycarbonyl derivatives, their high resistance to racemisation, and the wide variety of deblocking methods (treatment with hydrogen bromide in acetic acid, treatment with sodium in liquid ammonia, catalytic hydrogenolysis, and irradiation with ultraviolet light) are the main causes of the popularity of this protecting group.

New methods for the removal of the benzyloxycarbonyl protecting group, which have many advantages compared with the traditional methods, have been developed comparatively recently.

In 1977, Jackson and Johnstone[7] proposed a hydrogenation procedure involving hydrogen transfer, which proved convenient in the reduction of aromatic nitro-compounds. In the same year it was used to remove a number of protecting groups including the benzyloxycarbonyl group in classical peptide synthesis[8] and subsequently also in synthesis by the solid-phase method[9]. It was found that in this case the removal of the protecting groups is much faster and in addition it is possible to avoid the formation of side products in the deblocking of nitroarginine-containing peptides.

In the initial version, the hydrogenation with hydrogen transfer reduced to refluxing for several hours a solution of the protected derivative in ethanol in the presence of cyclohexene and palladium on charcoal or of palladium black. However, the refluxing of the peptide solution, which is itself undesirable, becomes particularly risky in the presence of protecting groups such as the t-butoxycarbonyl and t-butyl ester groups in view of the possibility of their being split off.

It has been shown[10] that these difficulties can be overcome when cyclohexa-1,4-diene is used as the hydrogen donor. In this case the deblocking is achieved at room temperature in 1.5–2 h in the presence of palladium and charcoal, while the use of palladium black permits the complete elimination of the protecting group in 5 min. The method proved convenient also for derivatives of certain sulphur-containing aminoacids, in particular methionine.

Yet another version of the hydrogenation reaction with hydrogen transfer was proposed in 1979, in which provision is made for the employment of formic acid as the hydrogen donor[11]. The benzyloxycarbonyl group is removed by treatment with a 4.4% solution of formic acid in methanol in the presence of palladium black in an atmosphere of nitrogen or by passing a solution of the protected derivative in methanol, containing 4.4% of formic acid, through a column containing palladium black. The time taken to eliminate the benzyloxycarbonyl group does not exceed 10 min in most cases; in addition this hydrogenation method greatly reduces the time taken to eliminate the *O*-benzyl groups.

Thus hydrogenation with hydrogen transfer makes it possible to remove rapidly and effectively the benzyloxycarbonyl group and a number of other protecting groups.

The t-butoxycarbonyl group, the parent of acid-labile groups eliminated under mild conditions, plays a no less important role in peptide synthesis. As a result of the appearance of this group[12], the possibilities of both the classical and solid-phase methods of synthesis of peptide have been greatly expanded.

Either the Schanabel method[13] or the Schwyzer method[14], in which the amino-group is acylated by treatment with the corresponding azide, were used initially to introduce the protecting group. However, in subsequent years a tendency arose to search for more efficient methods and reactants for the synthesis of protected derivatives. Here one may mention the application of systems such as t-butoxycarbonyl azide–triethylamine[15], t-butoxycarbonyl azide–imidazole, and t-butoxycarbonyl azide–1,1,3,3-tetramethylguanidine-1,2,4-triazole[16]. In the last case the method is so effective that it makes it possible to obtain fully protected derivatives of insulin by the direct acylation of the natural hormone, the yield of the reaction product exceeding 90%.

It has been suggested quite recently that the above protecting group be introduced using di-t-butyl pyrocarbonate[17]. The application of this reagent makes it possible to simplify greatly the method of synthesis and to increase the availability of the corresponding derivatives. Subsequent studies showed that the method is suitable also in the case of certain other protecting groups[18].

According to the method proposed by Pozdnev[17,18], the synthesis of aminoacid derivatives reduced to their treatment with di-t-butyl pyrocarbonate in the isopropyl alcohol–water system in the presence of triethylamine for 30 min at room temperature. The reaction takes place with a high yield (~90%) and the products formed do not as a rule require further purification.

Since the introduction of the t-butoxycarbonyl protecting group, the development of the solid-phase peptide synthesis has in essence begun. The employment of the benzyloxycarbonyl protecting group enabled Merrifield to synthesise only short model peptides[19] because of the occurrence of a side reaction involving the branching of the peptide–polymer bond in the deblocking process at acid pH. At the same time in the case of the t-butoxycarbonyl protecting group, under conditions ensuring its virtually quantitative removal, the degree of cleavage of the *C*-terminal benzyl ester is 1–2%.[20] As a result of this, it was possible to achieve the stepwise synthesis of ribonuclease A (124 aminoacid residues) by the solid-phase method[21].

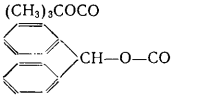
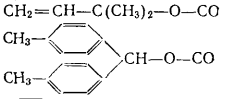
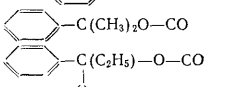
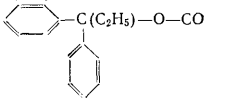
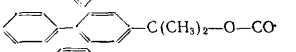
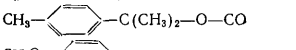
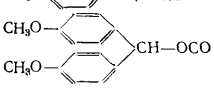
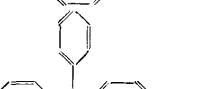
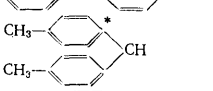
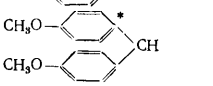
In the classical peptide synthesis the t-butoxycarbonyl protection of the α-amino-group in aminoacids is usually employed in combination with the following groups for the blocking of functional side groups in aminoacids: the nitro- or tosyl-group for the guanidino-group of arginine, the benzyloxycarbonyl group for the ε-amino-group of lysine, the benzyl group for the imidazole nitrogen of histidine and the sulphydryl group of cysteine, the benzyl ester groups for the carboxy-groups of glutamic and aspartic acid (and the *C*-terminal carboxy-group), and the benzyl ether groups for the hydroxy-groups of serine, threonine, and tyrosine.

Thus, after an appropriate choice of other protecting groups, the t-butoxycarbonyl group can be used successfully in the synthesis of peptides with a wide variety of aminoacid sequences by both classical and solid-phase methods.

Nevertheless, mention must be made of a series of specific difficulties arising in the employment of the protecting group. It is sufficient to note the problem of selectivity in the blocking of the α- and ε-amino-groups of lysine[22] and the t-butylation of the tryptophan-containing peptides, involving the removal of the t-butoxycarbonyl group[23–25]. It therefore became necessary to develop *N*-protecting groups more labile in acids than the t-butoxycarbonyl group and also groups which can be removed selectively in the presence of the t-butoxycarbonyl protecting group and the t-butyl ester groups used for the blocking of the functional side groups in aminoacids or the *C*-terminal carboxy-group.

The resistance of groups of this kind to the action of acids and the mechanisms of their elimination were investigated in detail in 1968.[26] It was shown in relation to various alkyl and urethane groups that the rate of their acidolytic cleavage depends on the stability of the carbonium ion formed and the acidity of the reaction medium. The rate of removal of certain protecting groups then proved to be 60000 times higher than that of the t-butoxycarbonyl group (Table 1). Unfortunately most of them are unsuitable for use in peptide synthesis owing to the instability of the azides or chloroformates used as the reagents for the introduction of the protecting group. The authors therefore decided to employ the diphenylisopropoxycarbonyl group[27], the rate of elimination of which is 3000 times higher than that of the t-butoxycarbonyl group. It has been suggested that this protecting group be introduced by the reaction of the corresponding azide or phenyl carbonate with aminoacid esters or salts. It proved possible to use dilute acetic acid and other weak acid reagents for the deblocking, which permits the retention of other acid-labile groups present in the growing peptide chain, particularly the t-butoxycarbonyl and t-butyl ester groups. By virtue of the large difference between the rates of elimination of the benzyloxycarbonyl and diphenylisopropoxycarbonyl protecting groups, it is then possible to solve the problem of selectivity for trifunctional aminoacids.

Table 1. The time of the 50% cleavage (τ_{50}) of $RHNCH_2COOY$ ($Y = C_2H_5$) with 80% CH_3COOH at 22–25 °C.[26]

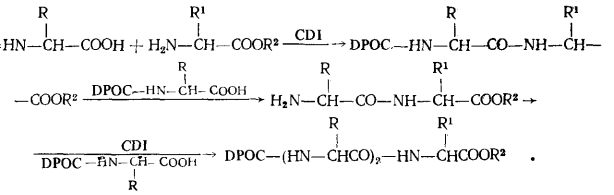
R	τ_{50}
$(CH_3)_3COCO$	58 days
	42 days
$CH_2=CH-C(CH_3)_2-O-CO$	14 h
	7 h
	2 h
	52 min
	30 min
	7 min
	1 min
	4 min
	28 days**
	~12 days

*Y = H.

**The degree of elimination was less than 0.1%.

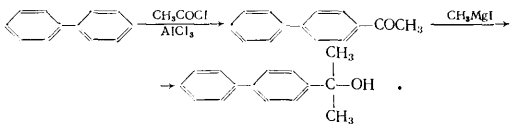
The disadvantages of the protecting group considered may include, together with the low availability of the initial reagents, also the unduly high acid lability, as a result of which the corresponding aminoacid derivative must be stored in the form of dicyclohexylammonium salts. Theoretically, there is even the possibility of removing the diphenylisopropoxycarbonyl (DPOC) protecting group in the course of the formation of the peptide bond under the influence of the COOH group of the carboxy-component[28], which can lead to the doubling of the aminoacid residue introduced in accordance

with the scheme†



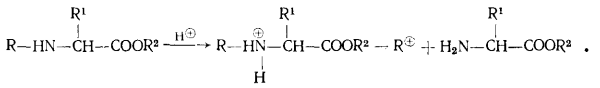
However, hitherto such cases have not been noted.

Since the diphenylisopropoxycarbonyl group is of undoubted interest from the standpoint of its employment in peptide synthesis, it is appropriate to consider the problem of the synthesis of the initial reagents for the introduction of this protecting group. In particular, the alcohol used as the starting compound in the synthesis of the corresponding azide or phenyl carbonate can be obtained as follows[29]:



We shall consider further the mechanism of the acid-catalysed elimination of alkyl and urethane protecting groups and the practical conclusions which follow from this.

In the case of alkyl groups, the cleavage under the influence of acids apparently proceeds via a mechanism involving the protonation of the nitrogen of the α-amino-group with the subsequent removal of the alkyl residue in the form of the corresponding carbonium ion:



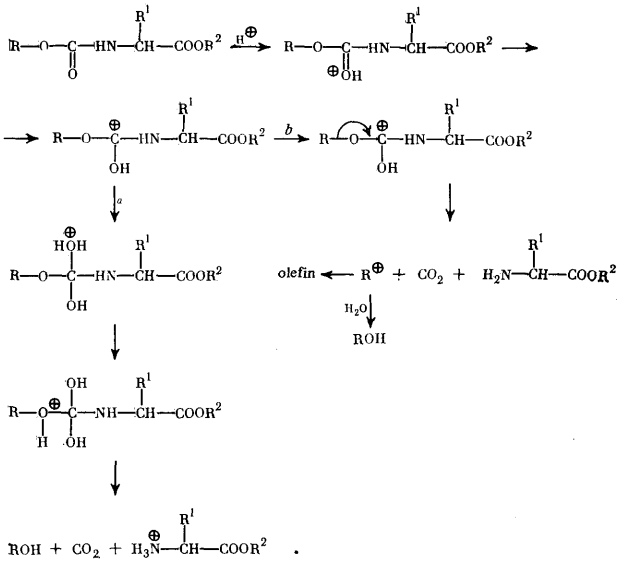
This is also indicated by the finding that, in the detritylation of peptides by treatment with an ethanol solution of hydrogen chloride under anhydrous conditions, ethyl trityl ether is formed in addition to the hydrochloride of the corresponding peptide, while in the presence of water triphenylmethanol is formed[30].

Evidently in this instance the rate of elimination of the protecting group is determined by the stability of the carbonium ion formed. Among the known groups of the type considered, the trityl group is therefore most readily removed by acids. Despite this, the trityl protecting group has not found extensive applications in peptide synthesis for the blocking of the α-amino-group owing to the steric hindrance which it generates and the associated difficulties in the synthesis of the corresponding derivatives. However, it is used to block the functional groups in the side chains of aminoacids (mainly the mercapto-groups of cysteine).

There is apparently a possibility of using protecting groups with a smaller number of phenyl moieties containing electron-donating substituents in the ring (Table 1), while the replacement of the phenyl moiety by the methyl moiety permits the stabilisation of the carbonium ion as a result of the formation of an olefin. However, one cannot rule out the possibility that in the latter case the problem may arise of the stability and reactivity of the halogeno-derivatives used for the introduction of such groups.

†CDI = dicyclohexylcarbodiimide.

In the acid cleavage of protecting groups of the urethane type, there is a possibility of two reaction mechanisms[31]. Structures related to the benzyloxycarbonyl group react via a bimolecular mechanism, while those related to the t-butoxycarbonyl group react via a unimolecular mechanism:



Hence it follows that groups of the first type are removed at a higher rate the greater the electron density in the benzene ring. Indeed, it has been shown[32] that the rate of cleavage of *para*-substituted derivatives of the benzyloxycarbonyl group by hydrogen bromide in acetic acid varies as follows:

$$CH_3O > C_6H_5 \sim CH_3 > H > F > Cl > NO_2 \quad .$$

In the case of groups of the second type there is a correlation between the rate of elimination of the protecting groups and the rate of solvolysis of the corresponding tosylates[33,34]. The results are presented in Table 2.

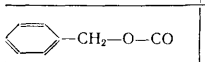
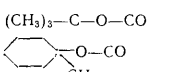
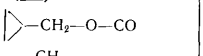
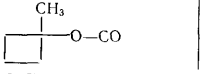
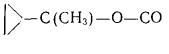
Their investigation enabled the authors[34] to propose a new protecting group—the 1-methylcyclobutoxycarbonyl group, which is stable in 50% acetic acid and is eliminated by treatment with trifluoroacetic acid in less than 30 min. The enhanced stability in relation to aqueous acetic acid, usually employed as the solvent in the purification of higher peptides, imparts to this group certain advantages compared with the t-butoxycarbonyl protecting group.

Comparison of the data in Tables 1 and 2 permits the following conclusions: (1) the rate of the acid-catalysed elimination increases sharply on passing from the bimolecular to the unimolecular mechanism; (2) the introduction of substituents promoting the delocalisation or decrease of the positive charge of the carbonium ion formed increases the rate of elimination; (3) the presence of a methyl group at the carbon atom with a positive charge also increases the rate of elimination as a result of the stabilisation of the carbonium ion by the formation of an olefin.

It is noteworthy that the formation of carbonium ions in the removal of protecting groups of the type considered is a source of extremely undesirable side reactions, such as the alkylation of the methionine and tryptophan residue[35], in the peptide synthesised. For example, Wünsch pointed out that deblocking with hydrogen fluoride in both classical and solid-phase synthesis favours the occurrence of side reactions

involving tryptophan derivatives even in the presence of added substances which reduce the extent of such reactions[36]. It has also been stated in a number of communications that the employment of additives such as mercaptoethanol, anisole, thioanisole, ethane-1,2-dithiol, scatole etc. by no means always guarantees the absence of side reactions[37-39].

Table 2. The 50% cleavage times (τ_{50}) of various *N*-protecting groups in the deblocking of phenylalanine derivatives[34].

Protecting group	τ_{50} (25° C, min)		Protecting group	τ_{50} (25° C, min)	
	CF_3COOH	HCOOH		CF_3COOH	HCOOH
	300	—		1*	4
	40	—		1*	2
	2	—		—	1.5

*Total cleavage time.

Returning now to the consideration of the acid-labile *N*-protecting groups, we shall consider those among them which have not found extensive applications in peptide synthesis but which may prove extremely valuable for this purpose by virtue of particular advantages.

Here we may mention in the first place the o-nitrobenzenesulphenyl protecting group[40], which has been used successfully in the synthesis of oxytocin analogues[41]. The corresponding protected derivatives are usually obtained with the aid of *o*-nitrobenzenesulphenyl chloride, although it has recently been suggested that *o*-nitrophenyl thiocyanate be used[42]. The latter is more stable on storage and reacts with aminoacids in the presence of silver nitrate at pH 6–9, giving rise to satisfactory yields. In 1979 Cipens and coworkers developed a new method for the synthesis of *o*-nitrobenzenesulphenyl chloride and *o*-nitrobenzenesulphenylaminoacids[43]. It was found that, when di-(2-nitrophenyl) sulphide is chlorinated with sulphuryl chloride in methylene chloride at 40–45 °C, it is possible to avoid the formation of impurities.

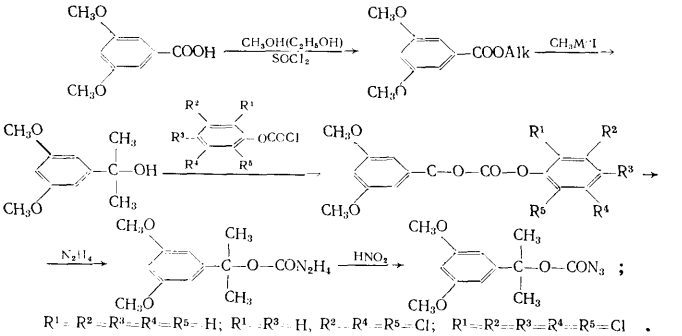
The *o*-nitrobenzenesulphenyl protecting group can be removed selectively in the presence of t-butyl esters with the aid of a solution of hydrogen chloride in diethyl ether.

It has been suggested[44] that the adamantyloxycarbonyl group be employed[44]. The protecting group is introduced with the aid of adamantyloxycarbonyl chloroformate, which can be obtained from the corresponding alcohol by treatment with an excess of phosgene in benzene solution in the presence of pyridine. The reaction with aminoacids takes place on cooling or at room temperature over a short period. The protecting group is removed by treatment with trifluoroacetic acid in the course of 15 min, adamantane forming a stable carbonium ion which can react with an anion from the surrounding medium but is incapable of losing a proton to form an olefin.

The authors[44] also noted that the adamantyloxycarbonyl derivatives are ninhydrin-positive on heating to 100 °C. It is likewise of interest that adamantyloxycarbonyl chloride can be used as the condensing agent in the synthesis by the method of mixed anhydrides, but this method of formation of the peptide bond is not of special value.

The t-pentyloxycarbonyl protecting group was proposed in 1967.[45] In contrast to the t-butoxycarbonyl group, the corresponding chloroformate could be used to introduce it. The reaction is carried out with the aminoacid ester and the resulting derivative is hydrolysed or converted into a hydrazide. t-Pentyloxycarbonyl azide or di-t-pentyl pyrocarbonate can also be used as the reagents for the introduction of the protecting group. The group hardly differs in its properties from the t-butoxycarbonyl protecting group.

The possibility of using the 3,5-dimethoxy-α,α-dimethylbenzyloxycarbonyl protecting group has been investigated[46]. Subsequently this group was used widely in the synthesis of peptides by both classical and solid-phase methods[47,48]. A method of synthesis of 3,5-dimethoxy-α,α-dimethylbenzyloxycarbonyl azide and the corresponding mixed phenyl carbonates from 3,5-dimethoxybenzoic acids via the following scheme has been developed (the yields are not less than 80% in all the stages):



These reagents are introduced into the reaction with aminoacid salts and Triton B in anhydrous pyridine. The protecting group can be removed selectively in the presence of the t-butoxycarbonyl group and t-butyl esters by treatment with 1–5% trifluoroacetic acid in methylene chloride for 8–30 min. It was found that the rates of the acidolytic elimination of the t-butoxycarbonyl, 3,5-dimethoxy-α,α-dimethylbenzyloxycarbonyl, and 2-(*p*-biphenylyl)isopropoxycarbonyl groups are in the ratios 1 : 1400 : 3000. The proposed protecting group is also photolabile, which makes it possible to remove it by ultraviolet radiation. The protected derivatives are thermolabile and give a positive response to the ninhydrin test on heating to 100 °C. The use of the above group in the synthesis by the method of mixed anhydrides makes it possible to simplify greatly all the operations and in addition to avoid the side reactions noted in a number of instances for proline-containing peptides[49].

Among the latest developments in the application of acid-labile *N*-protecting groups, mention may be made of the 1,1-(1-adamantyl)-1-methylethoxycarbonyl protecting group[50]. The phenyl carbonate synthesised from adamantane-1-carboxylic acid is used as the reagent for the introduction of this group. The reaction with aminoacids is carried out in dimethylformamide at 50 °C. The deblocking is achieved by treatment with 3% trifluoroacetic acid in methylene chloride for 2–3 min.

Other acid-labile *N*-protecting groups have either not found extensive applications in peptide synthesis or are of purely theoretical interest.

In conclusion it should be noted that the protecting groups eliminated under mild acid conditions play an important role in the classical and particularly in the solid-phase peptide synthesis. Their application for the protection of the *N*-end in the synthesis of polypeptides permits the employment for the blocking of the functional groups in the aminoacid side

chains not only of comparatively stable groups such as the benzyloxycarbonyl groups but also of groups of the type of t-butoxycarbonyl and t-butyl esters. This simplifies both the operations in the course of the growing of the peptide chain and the deblocking stages.

III. ALKALI-LABILE PROTECTING GROUPS

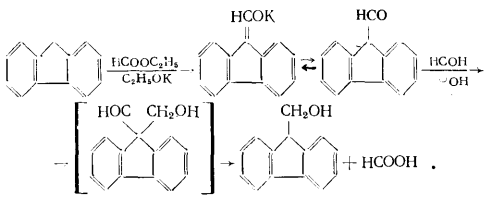
It is noteworthy that at the present time only a few alkali-labile groups of this type are used in peptide synthesis. However, the results show that the search for protecting groups eliminated under mild alkaline conditions constitutes an extremely promising development in peptide chemistry. Indeed, the appearance of such protecting groups would make it possible to avoid the difficulties arising in deblocking by acids of methionine- and tryptophan-containing peptides and their use in combination with the acid-labile protection for the functional groups in the side groups of aminoacids would greatly extend the scope of peptide synthesis.

The first step in this direction was the use of the trifluoroacetyl protecting group[51]. Trifluoroacetylaminoacids can be obtained by treating the aminoacid with trifluoroacetic anhydride in anhydrous trifluoroacetic acid or by the aminolysis of trifluoroacetate esters, while the protecting group can be eliminated by treatment with aqueous ammonia, sodium hydroxide, or barium hydroxide or by interaction with basic ion-exchange resins.

Unfortunately there is a possibility of a whole series of side reactions even in the course of the formation of the protected aminoacid derivatives and in addition under certain conditions rearrangements are observed with formation of azlactones, which leads to the racemisation of the protected aminoacid residue. Trifluoroacetyl derivatives are therefore used mainly only in gas-chromatographic analysis for the determination of the aminoacid sequence or of the degree of racemisation[52,53].

The 9-fluorenylmethoxycarbonyl protecting group, developed in 1970, is nowadays used to an increasing extent in peptide synthesis[54]. The authors introduced this group by means of the corresponding azide or chloroformate in aqueous dioxan in the presence of sodium bicarbonate or carbonate.

The starting compound for the synthesis of the reagents used to introduce the above protecting group is 9-fluorenylmethanol. The latter is a comparatively inaccessible compound but it can be readily obtained from fluorene in accordance with the following scheme[62]:



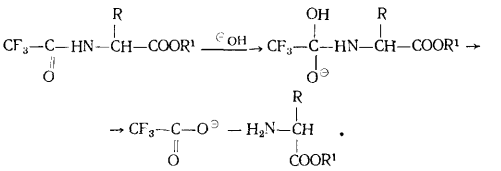
The yield of the final product was more than 67%. The intermediate 9-formylfluorene need not apparently be isolated in a pure form.

The protected aminoacids or peptides are deblocked by treatment with liquid ammonia, ethanolamine, morpholine, or similar bases. It has recently been suggested that piperidine bound to polystyrene[55] or other polymeric bases[56] be used for this purpose. According to Carpino and Han[54], the 9-fluorenylmethoxycarbonyl group is stable under the conditions where it is treated with hydrogen bromide or chloride in organic solvents and trifluoroacetic acid and also under hydrogenolysis conditions. However, a special study of the hydrogenation of protected derivatives in methanol in the presence of small amounts of acetic acid and 10% of palladium on charcoal showed that the side products are formed in this instance[57]. The authors explained the observed discrepancy in the results by the different qualities of the catalysts employed: a partially poisoned catalyst can be inactive in relation to the derivative considered.

In the classical peptide synthesis the 9-fluorenylmethoxycarbonyl protecting group has been used in the successive growing of the peptide chain by the method of activated esters[58] and also for the blocking of the α-amino-group in trifunctional aminoacids in combination with the t-butoxycarbonyl group and the t-butyl ester[59] groups for the protection of the functional side groups. Side reactions, leading to the dimerisation of the aminoacid residue introduced, have been noted in a few instances under these conditions.

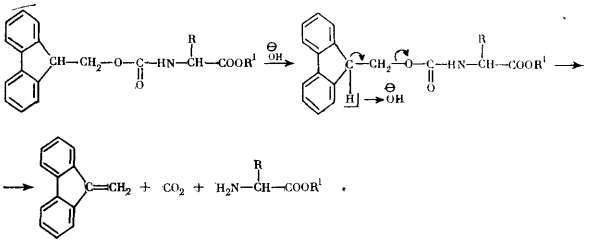
The possibilities for the employment of the derivatives under consideration in the solid-phase peptide synthesis have been investigated in detail[60,61].

In the study of the mechanisms of the elimination of alkali-labile groups, certain formal analogies can be drawn with the mechanisms of the elimination of the acid-labile protecting groups. Thus protecting groups related to the trifluoroacetyl group are removed in accordance with the following scheme:



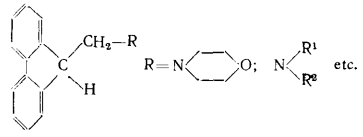
The above reaction equation resembles formally the acid-catalysed elimination of an alkyl group and in both cases the protecting group is removed without fragmentation and with formation of the corresponding ion. In the instance considered the reaction rate is evidently determined by the fractional positive charge on the carbon atom of the carbonyl group.

Unique ideas concerning the mechanism of the removal of the 9-fluorenylmethoxycarbonyl protecting group have not so far been developed. The nature of the solvent is apparently significant in this instance. In aprotic solvents the process mechanism has not been established, while in water, methanol, and t-butyl alcohol it is close to the carbanionic mechanism[56]. To a first approximation, the elimination of the 9-fluorenylmethoxycarbonyl group can be represented by the equation



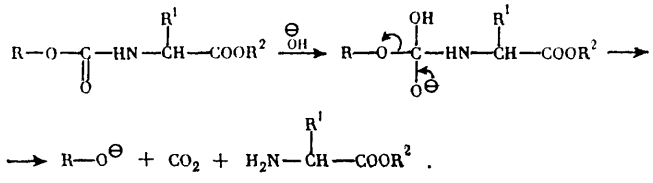
The character of this reaction is similar to that of the elimination of protecting groups of the benzyloxycarbonyl type—the group fragments in both instances and the process is not

accompanied by the formation of ions having an independent existence. In this instance the rate of cleavage is determined by the stability of the intermediate carbanion. The stabilisation of the carbanion derived from the 9-fluorenylmethoxycarbonyl protecting group is achieved by β-elimination, leading to the formation of dibenzofulvene. Depending on the reaction conditions and the reagents used in deblocking, an adduct with dibenzofulvene of the type



can be formed as a side product.

Continuing the comparison of the mechanisms of the elimination of the acid- and alkali-labile protecting groups, one may postulate the existence of a third pathway leading to the removal of the alkali-labile groups. The reaction must then proceed via a fragmentation stage with formation of independently existing ions, as happens in the case of the t-butoxycarbonyl group. The mechanism of this process can be represented by the equation



Evidently the rate of this reaction depends on the stability of the anion formed and hence on the electronic effects of the substituent R. The possibility of the operation of this mechanism is confirmed indirectly by the finding that, on passing from the benzyloxycarbonyl group to the phenoxy- and naphthoxy-carbonyl and related protecting groups, which might show a greater tendency towards the formation of anions on fragmentation, an increase of resistance to the action of hydrogen bromide in acetic acid is observed. The elimination under the influence of this reagent can be carried out only under severe conditions leading to the rupture of the peptide bond[63]. Apparently the introduction into the benzene ring of substituents promoting the stabilisation of the anion formed would make it possible to obtain alkali-labile protecting groups of the type considered. At the present time such *N*-protecting groups are not used in peptide synthesis.

A number of studies devoted to the search for new alkali-labile protecting groups and to the expansion of the applications of the already existing groups have been published in recent years. As early as 1970, the possibility of modifying the 9-fluorenylmethoxycarbonyl group in order to increase or decrease its sensitivity to bases was pointed out[54].

In 1978 Merrifield suggested that the 2-sulpho-9-fluorenylmethoxycarbonyl protecting group be used in the elimination of side products with a shortened aminoacid sequence from the peptides synthesised by the solid-phase method[64]. The 2-sulpho-9-fluorenylmethoxycarbonyl group is attached to the amino-group of the peptidylpolymer and the resulting derivative and other elimination products are separated on diethylaminoethyl-cellulose and deblocked. The protecting group is introduced using the corresponding chloroformate or the *p*-nitrophenyl ester of the aminoacid. The deblocking is carried out by treatment with aqueous morpholine or piperidine.

The possibility of using the *p*-substituted benzyloxycarbonyl group as an alkali-labile protecting group has been investigated recently[65]:

$$R-COO-C_6H_3(R^1)-CH_2-OCO-$$

$R = CH_3$, $R^1 = H$; $R = (CH_3)_2CH$, $R^1 = H$; $R = OC_2H_5$, $R^1 = H$; $R = (CH_3)_2CH-O$, $R^1 = H$; $R = C_2H_5S$, $R^1 = H$; $R = (CH_3)_2CH-NH$; $R^1 = H$; $R = (CH_3)_2N$, $R^1 = H$; $R = (CH_3)_2CH-O$, $R^1 = Cl$.

It was shown that groups of this type are more stable in trifluoroacetic acid than the benzyloxycarbonyl protecting group and can be removed selectively in the presence of the latter and the t-butoxycarbonyl group by treatment with H_2O_2 in ammonia.

Thus, despite the fact that the use of alkali-labile *N*-protecting groups in peptide synthesis is so far still fairly limited, the data obtained hitherto show that this trend will have a significant influence on its development.

REFERENCES

1. J.P.Greenstein and M.Winitz, "Chemistry of Amino Acids and Peptides" (Translated into Russian), Izd. Mir, Moscow, 1965.
2. F.Schröder and K.Lubke, "The Peptides" (Translated into Russian), Izd.Mir, Moscow, 1967.
3. R.Boissonnas, in "Uspekhi Organicheskoi Khimii" (Advances in Organic Chemistry), Izd.Mir, Moscow, 1966, Vol.3, p.158.
4. J.F.McOmie (Editor), "Protective Groups in Organic Chemistry" (Translated into Russian), Izd.Mir, Moscow, 1976.
5. P.M.Hardy, Chem. and Ind., 1979, p.617.
6. M.Bergmann and L.Zervas, Ber., 1932, 65, 1192.
7. A.E.Jackson and R.A.W.Johnstone, Synthesis, 1976, 685.
8. A.Gattadchattli and S.M.Kadlebal, J.Chem.Soc., Perkin Trans.I, 1977, 490.
9. K.A.Shabbir and K.M.Sivanandaiah, Synthesis, 1978, 750.
10. A.M.Felix, E.P.Heimer, T.J.Lambros, C.Tzougraki, and J.Meienhofer, J.Org.Chem., 1978, 43, 4194.
11. E.A.Babiker, M.Gattadahalli, G.P.Royer, and G.E.Means, J.Org.Chem., 1979, 44, 3442.
12. F.C.McKay and N.F.Albertson, J.Amer.Chem.Soc., 1957, 79, 4686.
13. E.Schanabel, Ann.Chem., 1967, 702, 188.
14. R.Schwyzer, P.Sieber, and H.Kappeler, Helv.Chim. Acta, 1959, 42, 2622.
15. K.A.Shabbir and K.M.Sivanandaiah, Indian J.Chem., 1977, 80.
16. D.Keglevic, S.Vuksan, B.Mulac, B.Ladesic, S.Iskric, and S.Kveder, Croat.Chem.Acta, 1978, 51, 167.
17. V.F.Pozdnev, Khim.Prirodn.Soed., 1979, 543.
18. V.F.Pozdnev, Bioorg.Khim., 1978, 4, 1273.
19. R.B.Merrifield, J.Amer.Chem.Soc., 1963, 85, 2149.
20. R.B.Merrifield, Endeavour, 1965, 23, 3.
21. B.Gutte and R.B.Merrifield, J.Amer.Chem.Soc., 1969, 91, 501.
22. M.Bodanszky and M.A.Ondetti, "Peptide Synthesis", Intersci., New York, 1966.
23. E.Wünsch, E.Jueger, L.Kisfaludy, and M.Low, Angew.Chem., 1977, 89, 330.
24. M.Low, L.Kisfaludy, and P.Sohar, Z.physiol.Chem., 1978, 359, 1643.

25. O.Hiroshi, S.Takashi, I.Hiroshi, and Y.Haruaki, Chem.Pharm.Bull.(Tokyo), 1978, **26**, 3144.
26. P.Sieber and B.Iselin, Helv.Chim.Acta, 1968, **51**, 644.
27. P.Sieber and B.Iselin, Helv.Chim.Acta, 1968, **51**, 622.
28. G.P.Katsoyannis, in "Khimiya Polipeptidov" (The Chemistry of Polypeptides) (Translated into Russian), Izd.Mir, Moscow, 1977, p.378.
29. H.C.Gull and E.E.Turner, J.Chem.Soc., 1929, **1**, 498.
30. L.Zervas and D.M.Theodoropoulos, J.Amer.Chem.Soc., 1958, **78**, 1359.
31. R.Hirschman, Intra-Sci.Chem.Rep., 1971, **5**, 203.
32. K.Blaha and J.Rudinger, Coll.Czech.Chem.Comm., 1965, **30**, 585.
33. K.Blaha and J.Rudinger, Coll.Czech.Chem.Comm., 1965, **30**, 599.
34. D.G.Veber, S.F.Brady, and R.Hirschmann, "Proc.III Amer.Peptide Symp.Ann Arbor", Ann Arbor Publ., 1972.
35. B.F.Lundt, N.L.Johansen, et al., Int.J.Peptide Protein Res., 1978, **12**, 258.
36. E.Wünsch, in "Gut Hormones", Churchill Livingstone, Edinburgh, London and New York, 1978, p.28.
37. L.Kisfaludy, M.Low, I.Schon, and M.Sajgo, "Tezisy IV Vsesoyuznogo Simpoziuma po Khimii Belkov i Peptidov" (Abstracts of Reports at the IVth All-Union Symposium on the Chemistry of Proteins and Peptides), Minsk, 1977, p.54.
38. M.Low and L.Kisfaludy, Z.physiol.Chem., 1979, **360**, 13.
39. Yu.B.Alakhow, A.A.Kiryushkin, et al., J.Org. Chem., 1970, **35**, 3291.
40. J.Goerdeler and A.Holst, Angew.Chem., 1959, **71**, 775.
41. J.Hlavacek, T.Barth, K.Bláhar, and K.Jost, Coll. Czech.Chem.Comm., 1979, **44**, 275.
42. J.Savrda and D.H.Veyrat, J.Chem.Soc.(C), 1970, 2180.
43. R.Vegners, L.K.Polevaya, I.Vosekalna, I.Grinsteine, and G.Cipens, Izv.Akad.Nauk Latv.SSR, Ser.Khim., 1979, 103.
44. W.L.Haas, E.V.Krumkalns, and K.Gerzon, J.Amer. Chem.Soc., 1966, **88**, 1988.
45. Japanese P.1447532 (1967); Chem.Abs., 1968, **68**, 30064.
46. C.Birr, W.Lochinger, G.Stahnke, and P.Lang, Ann. Chem., 1972, **163**, 162.
47. C.Birr, M.Nassal, and R.Pipkorn, Int.J.Peptide Protein Res., 1979, **13**, 287.
48. C.Birr, in "Peptides 1972", North-Holland Publ.Co., Amsterdam, 1973, p.72.
49. H.C.Beyerman, T.Rammeloo, I.Renirl, I.Syrier, and A.Van Zoon, Rec.I.Royal Netherlands Chem. Soc., 1976, **95**, 143.
50. H.Kalbacher and W.Voelter, Angew.Chem., 1978, **90**, 998.
51. F.Weygand and E.Csendes, Angew.Chem., 1952, **64**, 136.
52. T.Devenyi and J.Gergely, "Aminoacids, Peptides, and Proteins" (Translated into Russian), Izd.Mir, Moscow, 1976, p.294.
53. F.Weygand, A.Prox, L.Schmidhammer, and W.Konig, Z.Naturforsch., 1963, **18**, 93.
54. L.A.Carpino and G.Y.Han, J.Amer.Chem.Soc., 1970, **92**, 5748.
55. L.A.Carpino, I.R.Williams, and A.Lopusinski, Chem. Comm., 1978, 450.
56. R.Arshady, E.Altherton, and R.C.Sheppard, Tetrahedron Letters, 1979, 1521.
57. J.Martines, J.C.Tolle, and M.Bodanszky, J.Org. Chem., 1979, **44**, 3596.
58. A.Bodanszky, M.Bodanszky, N.Chandramouli, et al., J.Org.Chem., 1980, **45**, 72.
59. C.-D.Chang, M.Waki, M.Ahmad, J.Meienhofer, et al., Int.J.Peptide Protein Res., 1980, **15**, 59.
60. E.Atherton, H.Fox, D.Harkiss, C.J.Logan, et al., Chem.Comm., 1978, 537.
61. E.Atherton, H.Fox, D.Harkiss, and R.C.Sheppard, Chem.Comm., 1978, 539.
62. A.Murray and D.L.Williams, in "Sintezy Organicheskikh Soedinenii s Izotopami Ugleroda" (The Syntheses of Organic Compounds with Carbon Isotopes) (Translated into Russiam), Inostr.Lit., Moscow, 1962, Part II, p.32.
63. R.A.Boissonnas and G.Preitner, Helv.Chim.Acta, 1953, **36**, 875.
64. R.B.Merrifield and A.E.Bach, J.Org.Chem., 1978, **43**, 4808.
65. G.Le Corre, E.Guibe-Jampel, and M.Wakselman, Tetrahedron, 1978, **34**, 3105.

Leningrad State University