Translated from *Uspekhi Khimii*, **46**, 852-877 (1977) U.D.C. 542.95 : 547.466

Methods of Synthesis of Peptides

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Chemical methods for the activation of the carboxy-groups of the aminoacid used to synthesise peptides are examined, and the possible reaction mechanisms are described. Examples of the synthesis of certain peptides are presented. The bibliography includes 151 references.

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I. INTRODUCTION

This review deals with the methods for the activation of the carboxy-groups of the *N*-acylaminoacids which may be used to form the peptide linkage. It includes data published up to 1964. The methods of synthesis of peptides published up to 1965 have been examined in detail in familiar monographs devoted to the chemistry of peptides[1,2]. The review is not concerned with the methods for the detection of the functional groups of aminoacids, the problems of the isolation and purification of peptides, and the methods for the determination of the purity of synthetic peptides.

II. ACTIVATED *N*-ACYLAMINOACID ESTERS

The activated esters of *N*-substituted aminoacids are extremely convenient for the synthesis of peptides. The possibility of the isolation, purification, and storage of the activated esters greatly facilitates peptide synthesis. Furthermore, there are wide possibilities for varying the properties of activated esters, in particular aryl esters, by selecting suitable substituents in the benzene ring.

One may state without exaggeration that *N*-hydroxysuccinimide esters have enjoyed the greatest popularity in recent years[3,4]. They have been used more frequently than *p*-nitrophenyl[5] and 2,4,5-trichlorophenyl esters[6]. The synthesis of *N*-hydroxysuccinimide esters presents no difficulties; they can be readily obtained with the aid of dicyclohexylcarbodiimide or mixed anhydrides[7], or from the *N*-hydroxysuccinimide esters of dichloroacetic[8] or trifluoroacetic[9] acids.

After the *N*-hydroxysuccinimide esters had been proposed for the synthesis of peptides, esters based on hydroxylamine derivatives began to attract attention because of their high reactivity, the ready availability of the reagents required, and the low degree of racemisation or its absence.

Esters derived from *NN*-dialkylhydroxylamines, in particular those derived from 1-hydroxypiperidine[10,11], which have been synthesised with the aid of carbodiimide or mixed anhydrides, have been proposed as acylating agents. The esters derived from *NN*-dialkylhydroxylamines exhibit a comparatively weak acylating capacity, but, in the presence of acetic acid as the catalyst, acylation proceeds at an appreciable rate at room temperature. The enhancement of reactivity can be explained by the protonation of the nitrogen atom of the hydroxylamine:

$$R{-}COONR'R'' \xrightarrow{H^+} R{-}COO\overset{+}{N}HR'R'' .$$

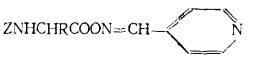
Certain small peptides have been synthesised with the aid of 1-piperidinyl esters[12-14], but their inadequate reactivities precluded their wide employment in peptide chemistry. Some studies have been devoted to esters derived from aldoximes and ketoximes[15-18]:

$$RCOON{=}C\begin{matrix}R'\\R''\end{matrix} ,$$

where R′ = alkyl or aryl and R″ = alkyl or hydrogen. The esters of ketoximes are stable crystalline substances with a fairly high reactivity in relation to amines. When they react with aminoacid esters or free aminoacids at room temperature, peptides are obtained in 50-90% yield. The degree of racemisation in the synthesis of Anderson's tripeptide was 20%,[15,16] but this hardly reflects the properties of the activated esters, since the ester derived from 3-nitroacetophenoxime, used to study the racemisation reaction, has been synthesised with the aid of carbodiimide from Z-Gly-L-Phe-OH. There is no doubt that the racemisation occurred mainly in the synthesis of the ester itself, which, incidentally, does not rule out the formation of a racemic product also when the activated ester combines with ethyl glycinate.

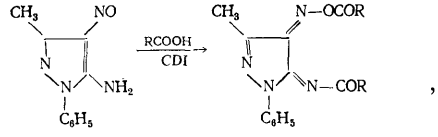
The synthesis of peptides with the aid of esters derived from ketoximes in the presence of acetic acid is more effective[17]. The addition of 1-1.5 moles of acetic acid increases the yield of peptides on average by 20-30%. Esters of ketoximes made it possible to synthesise serine and threonine peptides without protecting the hydroxy-group and also ω-nitroarginine peptides in 83-98% yield. The solubility of simple ketoximes in water facilitates the isolation and purification of peptides after the reaction. The method has been recommended for the stepwise synthesis of various peptides.

Interesting results have been obtained with esters derived from pyridine-4-aldoxime[18]:



In terms of their reactivity in the presence of acetic acid, esters derived from pyridine-4-aldoxime approach 2,4,6-trichlorophenyl esters and surpass *p*-nitrophenyl esters.

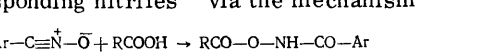
Using esters obtained from pyridine-4-aldoxime, certain peptides constituting fragments of angiotensin have been synthesised. An advantage of esters of pyridine-4-aldoxime is the ease of elimination of the reaction products and of the excess ester, since pyridine-4-aldoxime and the esters obtained from it readily dissolve in an acid medium. Esters derived from 5-amino-3-methyl-4-nitroso-1-phenylpyrazole can also be included among esters obtainable from oximes[19]:



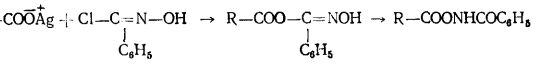
where CDI is carbodiimide.

They exhibit a high acylating activity and make it possible to synthesise peptides in high yields (90–95%) over a period not exceeding 1 h. Certain di-, tri-, and tetrapeptides have been synthesised with the aid of esters of this type. The Weygand[20] and Izumiya[21] tests showed that there is no racemisation at all in this reaction. The esters have yet another interesting feature: they are coloured red, while the reaction product (substituted 5-amino-4-nitrosopyrazole) is green, which makes it easier to follow the condensation reaction.

Hydroxamic acid esters have also attracted attention. A study on the synthesis of benzohydroxamic acid esters and the corresponding nitriles[22] via the mechanism



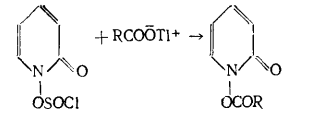
is of interest. The yield of esters in this reaction varies from 65 to 100%. Benzohydroxamic acid esters are fairly reactive and react with aminoacid esters at room temperature, giving peptides in yields not exceeding 75%. The low yields are caused by the Lossen rearrangement. Activated esters of the same type can be obtained also by the condensation of *N*-protected aminoacids with hydroxamic acids in the presence of carbodiimide[22] or by the reaction of the silver salts of *N*-protected aminoacids with the chloride of benzohydroxamic acid[23,24]; smaller yields are obtained from triethylammonium salts:



Although the majority of the esters are crystalline substances, they need not be isolated for peptide synthesis. The presence of acetic acid in the reaction of benzohydroxamic acid esters with amines appreciably accelerates the process. The authors believe[24] that the degree of racemisation does not exceed 0.1% for many peptides.

Esters based on *N*-hydroxyurethanes (for example, $HONHCOOC_2H_5$, which is both a hydroxamic acid and a carbamic acid ester) are likewise of interest. These esters have been synthesised with the aid of carbodiimide and mixed anhydrides in yields exceeding 90%.[25] A characteristic feature of these compounds is their appreciable resistance to the Lossen rearrangement. Esters based on *N*-hydroxyurethanes react readily both with aminoacid esters in an organic medium and with aminoacid salts in an aqueous medium. The yields of peptides are more than 90 and approximately 80% respectively. The use of *N*-hydroxyurethane as an additive in the carbodiimide synthesis of peptides lowers the degree of racemisation to 0–2% (Anderson's test). In contrast to *N*-hydroxysuccinimide[26], no side reactions were observed under these conditions, even if the mixture of *N*-hydroxyurethane with carbodiimide was kept at room temperature for many days[25].

Related but somewhat unusual esters are those derived from 1-hydroxy-2-pyridone[27,28]. They can be synthesised from acylaminoacid chlorides and 1-hydroxy-2-pyridone or from 2-oxo-1-pyridyl chlorosulphite and thallium salts of acylaminoacids[28]:

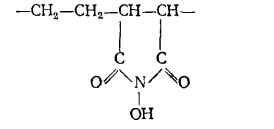


2-Oxo-1-pyridyl esters exhibit a high acylating activity, but, owing to their low stability and the difficulty of their synthesis, they have not so far found applications in the synthesis of peptides.

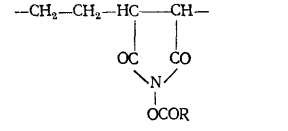
Despite the wide use of esters derived from *N*-hydroxysuccinamide in the synthesis of peptides, the search for new *N*-hydroxyimides of dibasic acids is being continued. Thus, the esters derived from *N*-hydroxyglutarimide have been synthesised and tested[29]. These esters, obtained with the aid of dicyclohexylcarbodiimide, are highly reactive, but do not have an appreciable advantage over esters derived from *N*-hydroxysuccinamide.

This can probably be also said of esters obtained from *N*-hydroxyphthalimide, first proposed in 1961[30] and recently used in the synthesis of certain peptides corresponding to the oxytocin sequence[31].

Activated esters obtained from polymers containing *N*-hydroxyimide groups are of particular interest. The copolymerisation of ethylene with maleic anhydride followed by treatment with hydroxylamine yielded a polymer containing units resembling *N*-hydroxysuccinimide:



Polymers of this type, which can be both soluble in organic solvents[32] and insoluble and cross-linked[33,34], can be obtained. An *N*-protected aminoacid RCOOH can be added with the aid of carbodiimide to the polymer, which gives rise to an activated polymeric ester:

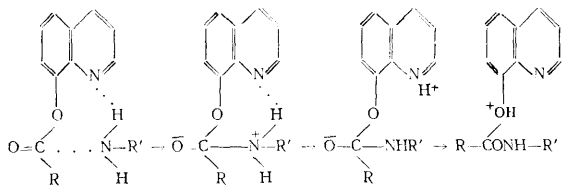


The latter can react with a solution of an amino-component in dimethylformamide. The yield of peptides in this reaction exceeds 90% as a rule if the activated ester is taken in a threefold excess. In the presence of equimolecular amounts of the reactants, the yield of peptide varies within wide limits (from 40 to 100%), depending on the nature of the aminoacid.

Polymeric activated esters are convenient because they obviate the need for the procedure involving the elimination of the excess of the activated ester and the ester component. At the same time they exhibit a high acylating activity, comparable to that of the usual esters obtained from *N*-hydroxysuccinimide. A heptapeptide corresponding to the 159–165 sequence of carboxypeptidase A (H–Ser–Pro–Cys–Ser–Glu–Thr–Tyr–OH) has been synthesised with the aid of polymeric activated esters in an overall yield of 59.5% before the elimination of protecting groups and 47.6% after their elimination[33]. The synthesis

of the octapeptide Boc–Met–Asp(OBzl)–Ser(Bzl)–Ser(Bzl)–Thr–Ser(Bzl)–Ala–Ala–OBzl has been achieved with an overall yield of 27%, the yields in the individual stages lying in the range 57–100%.[32]

Much attention has been devoted to activated esters whose reactivity can be accounted for by intramolecular base catalysis, in the first place esters derived from 8-hydroxyquinoline[35-39]. Kinetic studies on the aminolysis of these esters suggested the following reaction mechanism[36]:



This type of influence of nucleophiles adjoining the ester carbonyl group has also been noted in other examples, which will be discussed below.

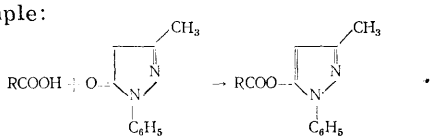
Esters are synthesised from 8-hydroxyquinolines either with the aid of carbodiimides or using mixed anhydrides, the yield being 70–90%. The esters formed by 8-hydroxyquinoline with the majority of benzyloxycarbonylamino-acids are stable crystalline substances, readily soluble in the usual organic solvents. The reaction with the amino-components proceeds at room temperature over a period of 4–20 h, the yields of peptides amounting to 75–97%. It is of interest that neither acetic acid nor heavy metal salts have any influence on the kinetics of the aminolysis reaction, which is consistent with the mechanism involving intramolecular catalysis[36]. Racemisation was not observed in the synthesis of Z–Leu–Phe–Val–OBu-t.[37] The reactivity of the esters derived from 5-chloro-8-hydroxyquinoline and 5,7-dichloro-8-hydroxyquinoline exceeds somewhat that of esters obtained from 8-hydroxyquinoline itself and they may be synthesised by the same methods[38]. Their suitability for the synthesis of peptides has been demonstrated in relation to a number of di-, tri-, tetra-, and penta-peptides, obtained in 65–97% yield. However, one should note that only comparatively simple aminoacids were used in all cases: glycine, alanine, valine, leucine, phenylalanine, and *S*-benzylcysteine.

In contrast to the esters obtained from 8-hydroxyquinoline, those of 3-hydroxy- and 6-hydroxyquinolines are appreciably less reactive, particularly the esters obtained from 6-hydroxyquinoline[39]. Apart from 8-hydroxyquinoline, 8-hydroxyquinaldine has been used to synthesise activated esters[40]. Furthermore, the copolymerisation of 8-benzyloxy-5-vinylquinaldine with divinylbenzene gave a polymer containing 8-hydroxyquinaldine groups. After the removal of the benzyl group, the copolymer was used to synthesise polymeric activated benzyloxycarbonylalanine esters[40]. Aminolysis by ethyl glycinate gave the dipeptide Z–Ala–Gly–OC_2H_5 in 87% yield.

The effect of the intramolecular base catalysis was manifested to a greater extent for esters based on 2-hydroxypyridine[41] and 2-mercaptopyridine[42]. Esters have been synthesised from 2-hydroxypyridine with the aid of carbodiimide in pyridine. In other solvents, 2-pyridyl esters could not be synthesised. The majority of 2-pyridyl esters of t-butoxycarbonylaminoacids are crystalline, readily soluble substances with a very high reactivity. According to kinetic measurements, the time of 50% reaction with amines is between 8 and 86 min. Under these conditions, *p*-nitrophenyl esters have a half-reaction time ranging from 540 to 1900 min. However, such a high reactivity has been observed only in non-polar solvents (chloroform, methylene chloride, etc.). In polar solvents, for example, in dimethylformamide, the activity of 2-pyridyl esters is sharply reduced, which has been explained by the inactivating influence of polar solvents on the intramolecular base catalysis[41].

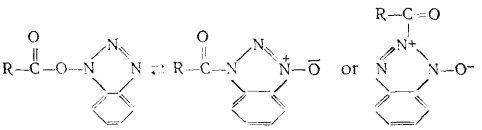
Pyridyl esters react readily with amino-components, forming peptides in 80–96% yield. They can be used to synthesise peptides on a polymeric carrier; thus oxytocin has been obtained in 70% yield using a fivefold excess of 2-pyridyl esters at each stage. Pyridyl esters react fairly readily with alcohols and hydroxyacids, so that they can be used to synthesise depsipeptides. 2-Pyridyl thio-esters, which can be readily obtained with the aid of carbodiimide, mixed anhydrides, or acid chlorides, have similar properties. They also readily react with amines and alcohols in non-polar solvents; on the other hand, in polar solvents (dimethylformamide, dimethyl sulphoxide) their reactivity falls. In contrast to 2-pyridyl esters, 3-pyridyl and 4-pyridyl esters of acylaminoacids are less reactive, which can be explained by the absence of intramolecular base catalysis. However, their reactivity is nevertheless fairly high, in particular, that of 3-pyridyl esters, which exceeds even the reactivity of *p*-nitrophenyl esters[43]. In contrast to 2-pyridyl esters, 3-pyridyl esters are very stable. Their reaction with amino-components gives rise to peptides in yields ranging from 50 to 97%.

All pyridyl esters have the advantage that the hydroxypyridines formed in the synthesis of peptides can be readily washed out of the reaction products. In a certain sense, the esters derived from the enolic form of substituted pyrazolone resemble 2-pyridyl esters[16]. They are readily obtained with the aid of carbodiimide or mixed anhydrides, for example:



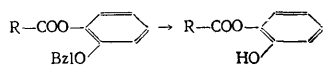
The esters obtained from the enolic form of pyrazolones have a high reactivity, similar to that of *p*-nitrophenyl esters, and readily react with amino-components, forming peptides in 70–80% yield. The esters synthesised from the pyrazolone enol can be used to synthesise peptides in an aqueous organic medium, using in the reaction aminoacid salts as the amino-component. The yields of peptides are in this case 60–73%. The degree of racemisation determined by Anderson's method proved to be 8.4%; admittedly, one must bear in mind that the activated ester Z–Gly–Phe–OPyr has been synthesised from the dipeptide Z–Gly–Phe–OH with the aid of dicyclohexylcarbodiimide, which might have involved some racemisation already in the stage involving the synthesis of the activated ester itself.

1-Benzotriazolyl esters obtained with the aid of carbodiimide are exceptionally reactive[44]. For example, the reaction of the activated ester of benzyloxycarbonylphenylalanine with the ethyl ester of valine at 0°C leads to the formation of more than 90% of the peptide after only 5 min. It is of interest that 1-benzotriazolyl esters undergo a rearrangement on crystallisation from solution. They exist in solution in the form of *O*-esters and in the crystalline state in the form of *N*-acylbenzotriazole oxide:

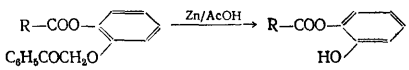


However only three 1-benzotriazolyl esters (BT) have been described hitherto: Z–Phe–OBT, Z–Gly–OBT, and Z–Thr–OBT. The high reactivity of 1-benzotriazolyl esters is apparently the reason why they are difficult to handle. 1-Hydroxybenzotriazole can probably be usefully employed as an additive in the carbodiimide synthesis, which will be discussed below.

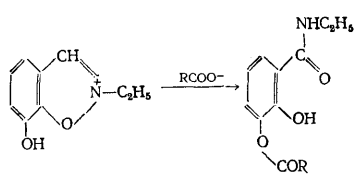
Activated esters of another type, whose enhanced reactivity can be explained by intramolecular catalysis, are those derived from catechol (*o*-hydroxyphenyl esters). They can be synthesised either from *o*-phenylene sulphite or, in a much higher yield, from *o*-benzyloxyphenol, by condensation with the aid of carbodiimide followed by the elimination of the benzyl group by hydrogenolysis or by treatment with hydrogen bromide in acetic acid[45]:



One can also begin with *o*-phenacyloxyphenol, since the phenacyl group is eliminated under milder conditions than the benzyl group[46]:



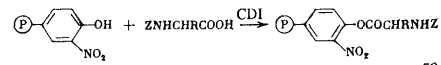
o-Hydroxyphenyl esters of acylaminoacids are fairly reactive. Thus the *o*-hydroxyphenyl ester of phthaloylglycine gives 91% of the peptide on reacting with ethyl glycinate at room temperature for 1 h.[45] For comparison, one may point out that phenyl phthaloylglycinate forms less than 1% of the peptide under the same conditions; the most important feature is that there is no racemisation at all when *o*-hydroxyphenyl esters are used. Because of this, *o*-hydroxyphenyl esters have been employed to synthesise polypeptides by the condensation of *o*-hydroxyphenyl esters of various tripeptides, for example, Lys–Ala–Ala[46,50]. 4,5-Dichloro-2-hydroxyphenyl esters are approximately three times as reactive as *o*-hydroxyphenyl esters, but they have not been used for the synthesis of peptides owing to the difficulty of obtaining them[45]. Esters containing the hydroxy-group in the *o*-position relative to the ester group are obtained ultimately by the reaction of the 2-ethyl-7-hydroxybenzoisoxazolium cation with acylaminoacids[51,52]:



(A number of intermediates are not indicated).

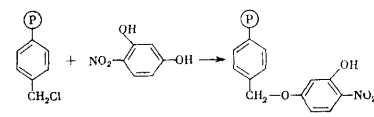
The activated esters formed are highly reactive. Thus the corresponding ester of benzyloxycarbonylglycine reacts with phenylalanine in aqueous sodium bicarbonate solution and forms 91% of benzyloxycarbonylglycylphenylalanine after 15 min. Peptide esters, in particular benzyloxycarbonylglycylphenylalanine, obtained from the 2-ethyl-7-hydroxybenzoisoxazolium cation, are very resistant to racemisation even under fairly severe conditions (12 h in 0.4 M solution of triethylamine in dimethylformamide). 2-Ethyl-7-hydroxybenzoisoxazolium fluoroborate is apparently one of the most interesting reagents for the synthesis of peptides[51]. Esters based on *o*-nitrophenol, which have been investigated in detail recently by Bodanszky and coworkers[53–56], are very promising. In contrast to *p*-nitrophenyl esters, *o*-nitrophenylesters are very reactive; the rate of their aminolysis depends less on the nature of the solvent, which can be accounted for by the intramolecular influence of the *o*-nitro-group. The high reactivity of these esters permitted their successful employment for the synthesis of protected oxytocin, the yield at each stage amounting to 80–95%.

Among other types of activated esters, much attention has been devoted to esters with a polymeric base. Their advantage is that the procedure involving the isolation of the peptide synthesised is greatly facilitated. This is particularly important for activated esters based on nitrophenols, as the latter are difficult to remove from peptides by hydrolysis. Polymeric phenols are obtained either by the copolymerisation of substituted styrenes with divinylbenzene[57–59], or by a reaction involving the polymer chain[60,61], or the phenol-formaldehyde condensation is used[62]. Activated polymeric esters of acylaminoacids are synthesised with the aid of carbodiimide[59,60]:

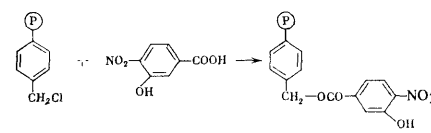


Using such esters, certain di- and tri-peptides[59] as well as bradykynin were synthesised in an overall yield of 39%. Naturally, the method can be used only in those cases where all the protected intermediate peptides are soluble in organic solvents.

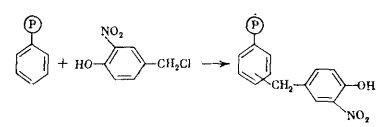
It was also found that the polymeric carrier obtained by the polymerisation of substituted styrene is not very strong mechanically, which leads to the fragmentation of polymer grains[60]. This necessitated the search for other methods whereby a polymeric carrier containing nitrophenol groups can be prepared. Chloromethylated cross-linked polystyrene was used for this purpose and nitroresocinol was attached to it in the presence of a base[61]:



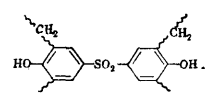
This polymeric carrier was mechanically strong and was used to synthesise various peptides. In another instance, 3-hydroxy-4-nitrobenzoic acid was attached to chloromethylated polystyrene[60]:



or 4-hydroxy-3-nitrobenzyl chloride was used, reacting with the styrene–divinylbenzene copolymer in the presence of aluminium chloride[63]:

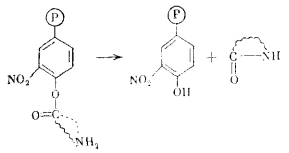


In all cases, activated esters were synthesised with the aid of carbodiimide and then, using 1.5 times the amount of the required polymeric activated ester, certain peptides were obtained, but, despite the excess of the activated ester, the yield was lower than in those cases where the synthesis was carried out in solution[60]. The polymeric activated ester, obtained on the basis of the condensation resin



had similar properties; the yield of peptides synthesised from it did not exceed 70%.[62]

Thus despite a number of useful properties, polymeric activated esters having the above structure are not appreciably advantageous compared with the analogous low-molecular-weight compounds. Their only advantage is perhaps in the case where they are used to cyclise peptides[64]. The peptide attached to polymeric nitrophenol can undergo only intermolecular cyclisation after the removal of the protecting group from the amino-group at the end of the chain; intermolecular condensation is then virtually ruled out:

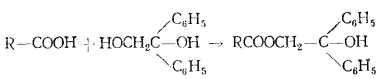


The yield of cyclopeptides was greater than in the analogous cases where a soluble activated ester is used.

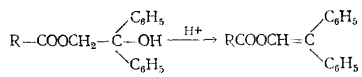
Esters of acylaminoacids and peptides based on 4-methylthiophenol have interesting properties[65-68]. Methylthiophenyl esters exhibit a very weak acylating activity and the methylthiophenyl group can be regarded as a protecting group. However, when the sulphide is oxidised to sulphone by hydrogen peroxide or peracids, the reactivity of the ester increases sharply and it can be used for the effective synthesis of peptides. The applicability of esters derived from methylthiophenol (MTP) has been demonstrated in relation to the synthesis of the heptapeptide Z–Lys(Z)–Leu–Lys(Z)–Ile–Ala–Lys(Z)–Val–OMTP, which was obtained in 73% yield by the condensation of the 4-methanesulphonylphenyl ester (MSO_2P) of the tripeptide with the methylthiophenyl ester of the tetrapeptide[66]:

$$\text{Z—Lys(Z)—Leu—Lys(Z)—OMSO}_2\text{P} + \text{H—Ile—Ala—Lys(Z)—Val—OMTP} \rightarrow$$
$$\rightarrow \text{Z—Lys(Z)—Leu—Lys(Z)—Ile—Ala—Lys(Z)—Val—OMTP.}$$

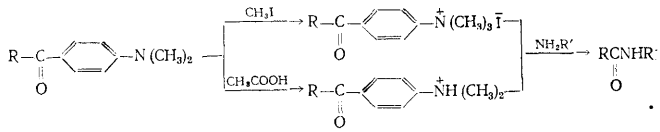
Unfortunately this entailed a partial loss of optical activity (approximately 5%). Furthermore, this method of activation, involving the use of strong oxidants (30% H_2O_2 or 85% *m*-chloroperbenzoic acid), is contraindicated for peptides containing methionine, cysteine, or cystine[68]. Other aminoacids are quite stable under the activation conditions, and their methylthiophenyl and methanesulphonylphenyl esters are stable crystalline substances. After the removal of the protecting groups, the salts of the aminoacid esters may be obtained. Methylthiophenyl esters constitute an example of esters with variable reactivity. Another example of esters of this kind is provided by those derived from 1,1-diphenylethylene glycol, the primary hydroxy-group of which can be readily esterified with the aid of thionyl chloride in pyridine:[69]



This ester is inactive, but, when it has been treated with trifluoroacetic acid, it readily splits off water and is converted into the activated 2,2-diphenylvinyl ester:



This ester reacts readily with the amino-component at room temperature, forming the peptide in 80% yield after 1.5 h. The malonic ester is introduced to bind the diphenylacetaldehyde produced. Using the 2,2-diphenylvinyl ester in the last stage of the synthesis of antamanide, it was possible to achieve cyclisation in 20% yield, which is a fair result compared with other methods[69]. Esters based on *p*-dimethylaminophenol, which may be obtained with the aid of dicyclohexylcarbodiimide or by the mixed anhydride method, have similar properties[70]. Dimethylaminophenyl esters are inert with respect to amines, but, on being protonated or treated with methyl iodide, they react readily with the amino-group, forming peptides:



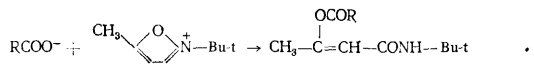
A number of di-, tri-, and tetra-peptides have been synthesised using dimethylaminophenyl esters in 50–98% yield[71].

Among activated esters of this type, the pentafluorophenyl esters, which can be synthesised either with the aid of carbodiimide or mixed anhydrides, or a complex of pentafluorophenol with dicyclohexylcarbodiimide, are of considerable interest[72]. The pentafluorophenyl esters exhibit an exceptional reactivity and permit the synthesis of peptides in yields exceeding as a rule 90%. Many peptides, including oxytocin, corticotropin, and antamanide, have been synthesised with the aid of pentafluorophenyl esters[73,74]. Presumably such esters will find extensive applications in peptide chemistry. Activated esters based on a phenol containing the sulphonamide group have been proposed[75]:

$$\text{ZNHCHRCOO—C}_6\text{H}_4\text{SO}_2\text{NHCOCH}_3$$

These esters have been synthesised with the aid of phosphoryl chloride in pyridine. Measurements of the rate of aminolysis have shown that their reactivity is equal to that of *p*-nitrophenyl esters. The yields of peptides were 65–90%; the *p*-acetylsulphamidophenol formed in the reaction can be readily removed by hydrolysis with an aqueous sodium bicarbonate solution. The addition of acetic acid to the reaction mixture has some catalytic effect.

The products of the interaction of Woodward's K-reagent with acylaminoacids can also be classified as activated esters[76]:



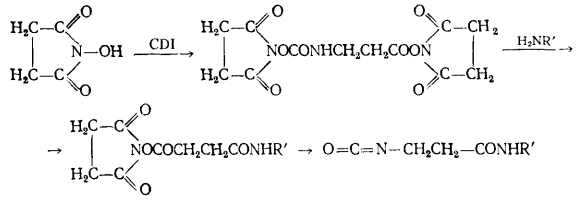
The substituted vinyl esters of *N*-protected aminoacids obtained in this way are stable crystalline substances and exhibit an appreciable acylating activity. For example, when the activated ester of benzyloxycarbonylglycine was allowed to react with benzylamine, the amide was obtained in 98% yield[76].

III. ACTIVATION OF THE CARBOXY-GROUP IN *N*-ACYLAMINOACIDS IN THE PRESENCE OF THE AMINO-COMPONENT

The methods for the activation of the carboxy-group in the presence of the amino-component have an undoubted advantage, since in this case the entire process is simplified and the probability of other processes (side reactions) is reduced. Although there are considerable difficulties in the selection of the reactants which interact selectively with the carboxy-group, efforts in this direction have not

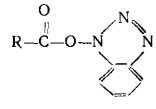
been unsuccessful. The most striking example in this field, which has played a decisive role in the development of the chemistry of peptides, is provided by various carbodiimides, in the first place by cyclohexylcarbodiimide. Studies on these lines are being successfully continued at the present time and very interesting results have been obtained in many instances. During the last five years, particular attention has been devoted to the modification of the carbodiimide synthesis by employing various additives which would tend to decrease the degree of racemisation and would eliminate side reactions. Interest in this type of modification of carbodiimide synthesis was aroused by reports concerning the favourable influence of the addition of *N*-hydroxysuccinimide in the synthesis of peptides with the aid of dicyclohexylcarbodiimide[77,78]. It was shown that the addition of one equivalent and more of *N*-hydroxysuccinimide not only prevents the formation of *N*-acylurea, but also suppresses racemisation almost completely. Some time later it was observed that, together with the favourable effect, the addition of *N*-hydroxysuccinimide can also give rise to an undesirable side process[26,79].

When *N*-hydroxysuccinimide reacts with carbodiimide, the product is the ester formed from *N*-hydroxysuccinimide and succinimido-oxycarbonyl-β-alanine, which can readily react with the amino-component and can undergo other changes:



This side process is particularly dangerous when the carboxy-component is only weakly reactive owing to steric hindrance (for example, valine and isoleucine)[80] and in some cases the addition of *N*-hydroxysuccinimide does not prevent racemisation (for example, up to 46% for Z-Pro-Val-Pro-OBu-t)[81].

All these findings stimulated the search for more effective additives to carbodiimide in order to reduce the degree of racemisation and suppress side reactions. A large number of hydroxy-compounds were tested and it was established that many of them react similarly to *N*-hydroxysuccinimide[82]. Hydroxytriazoles and hydroxytriazines proved to be particularly valuable, although the effect of the additive depends also on the structure of the peptide and the nature of the solvent. 1-Hydroxybenzotriazole is the most convenient substance. Since the effect depends only slightly on the structure of the substituted hydroxybenzotriazoles, it is useful to employ unsubstituted 1-hydroxybenzotriazole[44]. It is suggested that 1-acyloxybenzotriazole

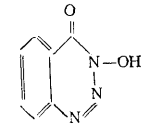


is a reaction intermediate which reacts subsequently with the amino-component forming the peptide. Certain 1-acyloxybenzotriazoles have been isolated and characterised.

Unfortunately, 1-hydroxybenzotriazole does not always rule out racemisation either. Thus, in the synthesis of Z-Pro-Val-Pro-OBu-t, it was found that the amount of racemic product is 9%.[83] However, in most cases racemisation was not observed, and, what is also important, the yields of peptides in the presence of added 1-hydroxybenzotriazole were higher than in the presence of *N*-hydroxysuccinimide.

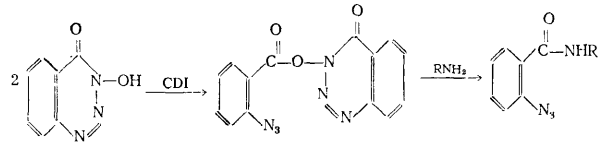
The addition of 1-hydroxybenzotriazole to carbodiimide makes it possible to add Boc-His(Bzl)-OH without racemisation[84]. Condensation with the aid of carbodiimide alone is accompanied in this instance by appreciable racemisation.

Further research led to an even more effective compound—3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine[82]:



Its addition to carbodiimide made it possible to obtain optically pure peptides even in those cases where neither 1-hydroxybenzotriazole nor *N*-hydroxysuccinimide (this is even less effective) were able to suppress racemisation completely. Thus, the degree of racemisation was 38% in the synthesis of Tfa-Pro-Val-Pro-OBu-t without any additive in the carbodiimide, in the presence of one mole of *N*-hydroxysuccinimide the degree of racemisation was 17%, the addition of one mole of 1-hydroxybenzotriazole resulted in a degree of racemisation of 3.4%, while the addition of one mole of 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine reduced the degree of racemisation to less than 1%.[82]

However, the yield of peptides in the presence of 3-hydroxy-4-oxo-3,4-dihydro-1,2,3-benzotriazine is less than in the presence of 1-hydroxybenzotriazole, although it is higher than in the presence of *N*-hydroxysuccinide. The reduction of the yield is the result of a side reaction involving carbodiimide[84]:

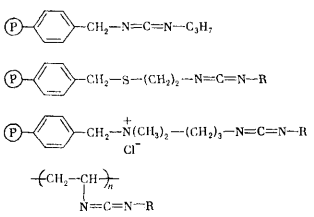


It is of interest that this type of side reaction occurs when the reaction mixture contains all the components at once. On the other hand, if the synthesis is carried out initially without the amino-component (pre-activation) and the latter is added only after a certain time, the side reaction is not observed and the peptides are obtained in very high yields. Unfortunately such pre-activation does not always guarantee the absence of racemisation[82].

The introduction of additives into carbodiimide has been very popular recently. Carbodiimide synthesis with added *N*-hydroxysuccinimide and 1-hydroxybenzotriazole competes successfully with azide coupling; its use permitted a number of complex peptide syntheses (glucagon[85], thyrocalcitonin[86], and secretin[87]).

As regards the carbodiimide synthesis itself, polymeric modifications of carbodiimide are of interest. In particular, the polymeric carbodiimide $-[-(CH_2)_6-N=C=N-]_n-$ has been obtained[88,89] and the use of an excess of this compound led to the synthesis of certain simple dipeptides in high yields: Z-Gly-Gly-OC_2H_5 (92%), Pht-Gly-Glu$(OBzl)_2$ (89%), and Z-Cys(SZ)-Gly-OBzl (93%). However, the

syntheses of more complex peptides have not been reported. Other polymeric carbodiimides have also been synthesised[90]:



However, only a few simple peptides have been synthesised with their aid.

In 1961 Wolman and coworkers proposed a method for the synthesis of peptides involving the oxidation of acylaminoacid hydrazides[91,92]:

$$R{-}CONHNH_2 + NH_2R' \xrightarrow{O} RCONHR' + N_2 \quad .$$

Later it was found in 1968 that acylaminoacids phenylhydrazides can be oxidised to phenylazoacylaminoacids, which are fairly stable crystalline compounds[93]:

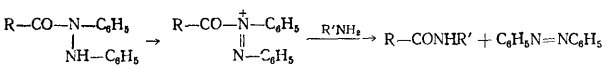
$$R{-}CONHNHC_6H_5 \xrightarrow{O} RCON{=}NC_6H_5 \quad .$$

Acylaminoacid phenylhydrazides have been synthesised from acylaminoacids and phenylhydrazines under the influence of papain:

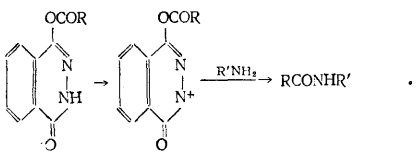
$$RCOOH + NH_2NHC_6H_5 \xrightarrow{\text{papain}} RCONHNHC_6H_5 \quad .$$

This novel procedure for the preparation of hydrazides gives fairly high yield of the product and is fairly general. The hydrazides have been oxidised by *N*-bromosuccinimide and lead tetra-acetate in 70–83% yield. Phenylazoacylaminoacids and peptides react very readily with the amino-group. Thus, in the reaction of Z–Gly–Phe–N=NC$_6$H$_5$ with H–Gly–Phe–NHNHC$_6$H$_5$, the degree of conversion was 90% after 8 min. The degree of racemisation in the synthesis of the tripeptide Z–Gly–Phe–Gly–OC$_2$H$_5$ varied from 0 to 4.5%, depending on the conditions of synthesis. The best result was obtained when redistilled ethyl glycinate was used as the amino-component.

In this type of peptide synthesis, it is possible to use acylaminoacid 1,2-diphenylhydrazides, which can be oxidised by *N*-bromosuccinimide or 1-chlorobenzotriazole to azonium salts, and these readily react with the amino-component, forming peptides[69]:



The yields of peptides amount to 70–80%. A similar result has been achieved with the aid of 4-acyloxyphthalazone[69]:



In both instances, the yield of peptides proved to be inadequate. The attempts to use these compounds for cyclisation in solution and on a polymeric carrier were unsuccessful.

A series of studies devoted to the use of phosphorus(III) compounds (phosphines and phosphites) in peptide synthesis is of particular interest. When these substances are used, unusual oxidation-reduction reactions take place during which the carboxy-group of the acylaminoacid is activated.

The reaction of a carboxylic acid, arenesulphenamide, and triphenylphosphine results in the formation of a carboxamide[94,95]. Heavy metal ions (Cu^{2+}, Hg^{2+}, etc.) are used to bind the thiophenol liberated:

$$(RCOO)_2Cu + ArSNHR' + (C_6H_5)_3P \rightarrow RCONHR' + (ArS)_2Cu + (C_6H_5)_3P{=}O \quad .$$

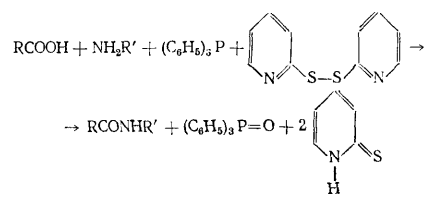
The arenesulphenamide can be replaced by a diaryl sulphide and an amine[96]:

$$RCOOH + (ArS)_2 + (C_6H_5)_3P + R'NH_2 \xrightarrow{Hg^{++}} RCONHR' + (C_6H_5)_3P{=}O + (ArS)_2Hg \quad .$$

For example, Z–Phe–Gly–OC$_2$H$_5$ has been synthesised by this method in 96% yield[94].

Unfortunately, in the synthesis of a number of other peptides difficulties were encountered in their isolation from the reaction mixture. It was found that it was difficult to separate peptides from triphenylphosphine oxide and it was therefore necessary to employ in all cases chromatographic separation on silica gel, which greatly complicated the procedure[97]. The study of racemisation in the synthesis of Bz–Leu–Gly–OC$_2$H$_5$ showed that a racemic peptide is formed in appreciable amounts (3–8%).[79,80] Certain unsaturated compounds, for example, 2,3-dihydropyran and butyl vinyl ether, have been used instead of heavy metal ions to bind thiophenol[98]. The yield of peptides was then 90–97%. In order to facilitate the purification procedure and to increase the yield of peptides, the water-soluble reagents $[(CH_3)_2NC_6H_4]_3P$ and $[\overset{+}{I}(CH_3)_3\overset{+}{N}C_6H_4S]_2$ have been proposed[99]. In the synthesis of peptides, this made it possible to employ an excess of reagents, which were then readily removed by washing the peptides with water. Various dipeptides containing virtually all the natural aminoacids have been synthesised using the water-soluble reagents. The yield of peptides was 70–98%.[99]

Di-2-pyridyl disulphide proved to be a convenient compound for the oxidation-reduction condensation[98,100]. The 2-mercaptopyridine formed in the reaction with triphenylphosphine isomerises to the stable thiono-form and does not give rise to undesirable reactions:



Various dipeptides have been synthesised in 82–93% yield using di-2-pyridyl disulphide. One must note that aminoacids containing hydroxy-groups (serine, threonine, and tyrosine) do not require the protection of the hydroxy-group and the yields of peptides are high (84% for Z–Ser–Gly–OC$_2$H$_5$, 83% for 2-Thr–Gly–OC$_2$H$_5$ and 93% for Z–Tyr–Gly–OC$_2$H$_5$). In the synthesis of peptides from Z–Gln–OH and Z–Asn–OH, the dehydration of the amide groups was not observed and the yields of peptides proved to be 85–86%. The octapeptide Z–Ala–Gly–Leu–Gly–Gly–Gly–Leu–Gly–NHNHBoc was synthesised in 70% yield. The employment of di-2-pyridyl disulphide permitted the successful application of this method in solid-phase synthesis[98,101].

The influence of various additives on the degree of racemisation in the synthesis of Bz–Leu–Gly–OC$_2$H$_5$ by the oxidation-reduction condensation was investigated[102]:

$$(Bz{-}Leu{-}O)_2Cu^{II} + NPS{-}Gly{-}OC_2H_5 + (C_6H_5)_3P \rightarrow$$
$$\rightarrow Bz{-}Leu{-}Gly{-}OC_2H_5 + (NPS)_2Cu + (C_6H_5)_3P{=}O \quad .$$

Phenol, *p*-nitrophenol, 2,4-dinitrophenol, 2,4,6-trinitrophenol, *N*-hydroxysuccinimide, and trimethylacetic acid were also used as additives.

It was found that the content of the L-isomer increases from 57 to 80% as the pK of the additive is reduced from 9.89 to 0.38. However, *N*-hydroxysuccinimide deviates from this relation, the content of the L-isomer amounting to 84% for pK = 9.4. A study of the dependence of the degree of racemisation on the nature of the solvent and the structure of the mercury derivatives (added to bind thiophenol) showed that there is no clear-cut trend and the degree of racemisation remains considerable (5–63%).[103] Thus the oxidation-reduction condensation method can be used only for the stepwise growth of the peptide chain. An unusual version of the oxidation-reduction condensation is the method of synthesis of peptides using anodic oxidation for the regeneration of diaryl disulphides[104]. The thiophenol formed in the reaction is oxidised to diaryl disulphide, which is thus returned to the reaction:

$$RCO\bar{O} + NH_2R' + \overset{+}{P}R_3'' + ArSSAr \rightarrow RCONHR' + O{=}PR_3'' + 2ArSH$$

(anodic oxidation)

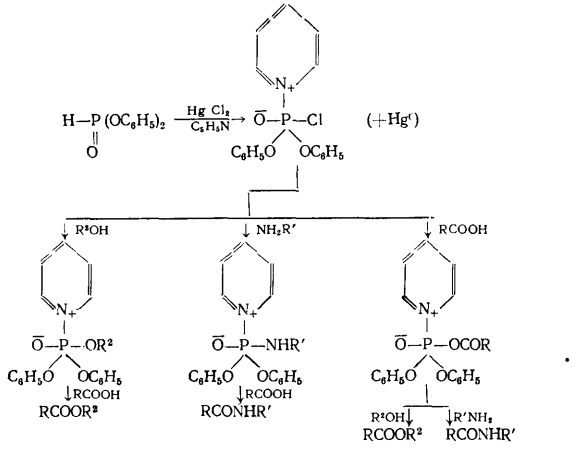
This process makes it possible to introduce the diaryl disulphide into the reaction in an amount which is much less than that corresponding to stoichiometry, and the probability of the formation of side products is thereby reduced.

The use of the oxidation-reduction reaction for the synthesis of various activated esters is of interest[105]:

$$RCOOH + HOR' + (C_6H_5)_3P + \text{(2-pyridyl disulphide)} \rightarrow RCOOR' + (C_6H_5)_3P{=}O + 2\ \text{(2-mercaptopyridine)}$$

Pentachlorophenyl, succinimide, and 8-quinolinyl esters have been synthesised by this procedure in 60–86% yield. This method permits the easy synthesis of activated asparagine, glutamine, and nitroarginine esters.

Another example of the oxidation-reduction reaction leading to the activation of the carboxy-group is that of diphenyl phosphite with mercury(II) salts in pyridine[106]. Using this reaction, it is possible to synthesise both acid amides and esters. The reaction is carried out in all cases in pyridine either at 45°C or on boiling and proceeds via the following mechanism:



The reaction has been used to synthesise Z–Gly–Gly–OC_2H_5 and Z–Gly–Gly–OH, which were obtained in 94 and 50% yields respectively.

An interesting example of the use of phosphorus(III) compounds in the synthesis of peptides is the method based on the reaction of phosphines and phosphorous acid amides with carbon tetrachloride or tetrabromide[107,108]:

$$R_3P + CX_4 \rightarrow \underset{X^-}{\overset{+}{R_3P}}{-}CX_3 \xrightarrow{R'COOH} \underset{X^-}{\overset{+}{R_3P}}{-}OCOR' \xrightarrow{R''NH_2} R'CONHR'' + R_3P{=}O\ ,$$

where

$$X{=}Cl,\ Br;\ R{=}CH_3{-}\text{(N-heterocycle)},\ C_6H_6,\ (CH_3)_2N,\ (C_2H_5)_2N\ .$$

The reaction is carried out at a reduced temperature (between −20° and 0°C) and the yield of peptides is 60–85%. A study of racemisation in the synthesis of Bz–Leu–Gly–OC_2H_5 showed that the amount of racemic products obtained is usually 2–8% and that the degree of racemisation is much higher (88%) only in the case of triphenylphosphine. This shows that the carboxy-group is not activated via the stage involving the formation of acid chlorides, although it is known that the acid chloride is formed readily in the reaction of a carboxylic acid, carbon tetrachloride, and phosphines[109]. When the carboxy- and amino-components are present simultaneously, the activated species is apparently an acyloxyphosphonium ion[107,110]. It has been suggested that triphenyl phosphite be used for the synthesis of peptides in the presence of imidazole[111]. The reaction proceeds via the mechanism

$$RCOOH + NH_2R' + (C_6H_5O)_3P \xrightarrow{\text{imidazole}} RCONHR' + (C_6H_5O)_2PH(=O) + C_6H_5OH\ .$$

Various peptides have been synthesised in high yields with the aid of triphenyl phosphite; the method has been used successfully in the synthesis of biologically active peptides (fragments of gastrin, corticotropin, etc.)[112]. Diphenyl phosphite, which makes it possible to synthesise peptides in 65–90% yields in the presence of pyridine, proved to be just as effective[113,114]. A similar effect was achieved also when diaryl sulphites were used instead of phosphites[115].

The employment of *o*-nitrobenzenesulphenyl derivatives of aminoacids for the synthesis of peptides is of interest. It has been found that aryl thioesters of acylaminoacids and peptides can react with salts of *o*-nitrobenzenesulphenylaminoacids, to form peptides[116]:

$$ZNHCHRCOSC_6H_5 + NBSNHCHR'CO\bar{O}\overset{+}{N}a \rightarrow ZNHCHRCONHCHR'CO\bar{O}\overset{+}{N}a + NBSSC_6H_5\ .$$

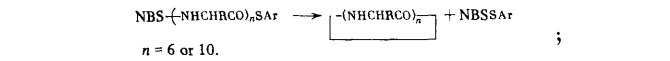
[where NBS is the *o*-nitrobenzenesulphenyl group]. The reaction requires fairly severe conditions: an aqueous methanol solution of the reactants was maintained for 6 h at 70°C and the yield of peptides was 56–76%. The attempts to use for this reaction the esters of *o*-nitrobenzenesulphenylaminoacids instead of the salts have not been successful. The method employing imidazole was more successful in this respect[117], which made it possible to reduce the reaction temperature to 45°C and to shorten the reaction time considerably (to 10–45 min):

$$ZNHCHRCOSAr + NBS(NHCHR'CO)_n OCH_3 \xrightarrow{\text{4 equiv. of imidazole}} ZNHCHRCO(NHCHR'CO)_n OCH_3 + NBSSAr,$$

where Ar = C_6H_5, $C_6H_4NO_2$, or $C_6H_4CH_3$ and n = 1–3.

The yield of peptides was about 90% and the degree of racemisation (Anderson's test) proved to be 4% for the *p*-nitrophenyl thioester. The method is convenient because it does not require the preliminary elimination of the protecting group from the amino-end of the peptide.

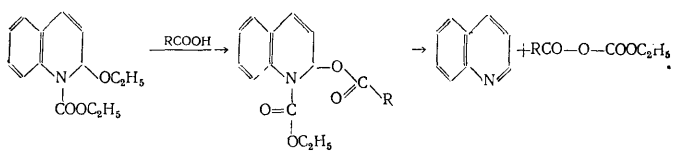
It was also tested in the cyclisation of certain hexa- and deca-peptides, and gave good results:



$n = 6$ or 10.

Antamanide was synthesised in this way in 30% yield[118]. It is noteworthy that this reaction occurs in pyridine or in tetrahydrofuran, while in dimethylformamide undesirable side reactions predominate. This limitation narrows the region where the reaction can be applied.

A very interesting development of the method of mixed anhydrides has been a series of studies on the application of 1,2-dihydroquinoline derivatives in the synthesis of peptides[119-122]. *N*-Ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, which can be readily obtained from ethyl chloroformate and quinoline, is a fairly stable compound and does not react with amines; however, its reaction with carboxylic acids readily gives rise to mixed anhydrides:



Since the reaction is carried out in the presence of an amine, the mixed anhydride reacts with the latter immediately, forming an amide (peptide). Various peptides have been synthesised in yields exceeding 90% using *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. The degree of racemisation was determined in the synthesis of ethyl benzoyl-leucylglycinate (Young test), which is a very sensitive peptide; however, the racemic product was not detected. In a study of the racemisation by a more accurate method[120], only a minimal degree of racemisation was detected (approximately 0.2%). Such unusual behaviour of the mixed anhydride can be accounted for by the fact that, as soon as it is produced, it immediately reacts with the amino-component, so that its lifetime is very short and side processes do not occur.

A polymeric modification of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline has been described[121]. The polymer was obtained by the copolymerisation of 6-isopropenylquinoline with styrene, and was then converted into the active reactant by reaction with ethyl chloroformate and ethanol. The polymeric reactant makes it possible to synthesise peptides easily and rapidly in yields of 84-92%. After the reaction, the polymer can be again converted into the active reactant.

N-Isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline has been recommended for use in ordinary solid-phase synthesis of peptides (Merrifield's method)[122]. This reagent is just as effective as *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline. Certain peptides containing serine, threonine, and asparaginine have been obtained in yields of 80-95%. The octapeptide has been synthesised from Z(OCH$_3$)-Asp(OBzl)-Phe-Cys(Bzl)-Leu-Glu(OBzl)-Pro-OH and H-Gly-Ala-O-polymer (fourfold excess) as an example of the use of this reagent in the solid-phase synthesis. The latter was carried out over a period of 48 h and the yield was nearly quantitative. In order to determine the degree of racemisation, the dipeptide Ac-Ile-Gly-OCH$_3$ was used[123], the degree of racemisation found amounting to 4.1%. In the synthesis of the tripeptide Z-Gly-Ala-Leu-OBzl with the aid of *N*-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, the degree of racemisation was only 0.2%.[120]

The ynamines $R-C\equiv C-NR_2'$ have been suggested for the stepwise synthesis of peptides[124,125]:

$$RCOOH + NH_2R' + R''-C\equiv C-NR_2''' \rightarrow RCONHR' + R''CH_2CONR_2''' .$$

The reaction is carried out at 40°C in tetrahydrofuran, the yields of certain dipeptides amounting to 83-99%. However, the attempts to synthesise Z-Leu-Phe-Val-OCH$_3$ gave a poor yield of the tripeptide (25%) and the degree of racemisation was high, varying from 17 to 27% depending on the reaction temperature. It appears that ynamines must be used with caution in the synthesis of peptides. They proved to be much more effective in the preparation of symmetrical anhydrides used subsequently to synthesise peptides[124].

Diphenylphosphoryl azide[127], which can be readily obtained from diphenylphosphoryl chloride and sodium azide in acetone, proved to be a very interesting reagent for the synthesis of peptides. It is a stable non-explosive liquid, which can be distilled at a reduced pressure. When it is allowed to react with a solution containing carboxy- and amino-components, peptides are obtained at 0°C easily and in high yields:

$$RCOOH + NH_2R' + N_3PO(OC_6H_5)_2 \rightarrow RCONHR' .$$

It is suggested that the carboxylic acid azide RCON$_3$ is an intermediate. This is confirmed by the fact that, when a carboxylic acid, diphenylphosphoryl azide, and alcohols are heated, the corresponding urethanes are obtained:

$$RCON_3 \rightarrow RN=C=O \xrightarrow{R'OH} RNHCOOR' .$$

Diphenylphosphoryl azide is a universal reagent permitting the synthesis of peptides containing virtually any amino-acids in high yields. The following peptides have been synthesised with its aid (the yields are indicated in brackets):

Z—Asn—Phe—OCH$_3$ (80%); Z—Gln—Ser—OCH$_3$ (80%);
Z—Val—Tyr—OCH$_3$ (69%); Z—Thr—Phe—OCH$_3$ (72.5%);
Z—Met—Gly—OC$_2$H$_5$ (90%); Boc—Trp—Gly—OC$_2$H$_5$ (89.5%);
Z—Arg(NO$_2$)—Leu—OCH$_3$ (83%); Z—His—Leu—OCH$_3$ (95%).
Z—Leu—Leu—Val—Phe—OCH$_3$ (87%);

Racemisation was not observed in the synthesis of the tetrapeptide Z-Leu-Leu-Val-Phe-OCH$_3$, but Young's test showed that the degree of racemisation is about 5%.

IV. OTHER METHODS FOR THE ACTIVATION OF THE CARBOXY-GROUP

Mixed anhydrides obtained from the esters of chlorocarbonic acids are very important in the synthesis of peptides. Despite the fact the method of mixed anhydrides was developed at the beginning of the 1950s, interest in this procedure has still not weakened; new studies in which new versions of the method are proposed are published fairly frequently.

A new method for the preparation of mixed anhydrides, based on the use of stable *N*-alkoxycarbonyl-*NNN*-trialkylammonium salts, is of interest[128]. Certain salts of this type are fairly stable and can be used to form mixed *N*-acylaminoacid anhydrides, for example:

$$BF_4^-R_3\overset{+}{N}-\underset{\underset{O}{\|}}{C}OC_4H_9 + ZNHCHR'COOH \rightarrow ZNHCHR'CO-O-CO-OC_4H_9 .$$

Certain di- and tri-peptides have been synthesised in this way in yields of 70-97%.

Another example of a new method of synthesis of mixed anhydrides involves the use of dihydroquinoline derivatives, which were considered in Section III.[119-122]

Among the methodological developments devoted to the use of mixed anhydrides, special attention should be given to the method based on the use of a large excess of the mixed anhydride[129]. This approach permits the synthesis of peptides in a nearly quantitative yield and makes it possible to carry out the process without the isolation and purification of the intermediate peptides. The method has been successfully applied in the synthesis of a fragment of the growth hormone[128], secretin[129], and other peptides.

Despite the fact that the mixed anhydrides with certain carboxylic and alkylcarbonic acids were introduced into peptide chemistry long ago and have gained general recognition by virtue of their high reactivity and the simplicity of their synthesis, the search for new types of mixed anhydrides is still being continued. The necessity for such search probably arises due to the instability of mixed anhydrides and the side reactions which occur in many instances. Admittedly the number of new types of mixed anhydrides is not all that large and they can hardly compete with the generally recognised mixed anhydrides.

One of such new mixed anhydrides proposed is the compound obtained by the reaction of toluene-*p*-sulphonic acid anhydride with hexamethylphosphoramide[131]:

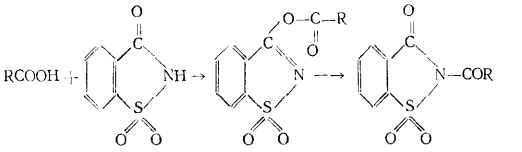
$$[(CH_2)_2N]_3PO + Tos_2O \rightarrow [(CH_3)_2N]_3\overset{+}{P}-O-\overset{+}{P}[N(CH_3)_2]_3 \cdot 2Tos\bar{O} \rightarrow$$
$$\xrightarrow{RCO\bar{O}} RCO-O-\overset{+}{P}[N(CH_3)_2]_3\,Tos\bar{O} \xrightarrow{R'NH_2} RCONHR' + [(CH_3)_2N]_3PO \quad .$$

The resulting acyloxytris(dimethylamino)phosphonium tosylate is fairly stable in solution and rapidly reacts with the amino-component, forming peptides in a high yield for the majority of aminoacids (81–96%). Aliphatic aminoacids containing hydroxy-groups (serine and threonine) cannot be used in this reaction without protecting the hydroxy-group. Histidine also gives unsatisfactory results. However, the absence of racemisation (less than 1%) is of interest; it can probably be accounted for by the impossibility of the formation of the oxazolone ring owing to the steric hindrance caused by the dimethylamino-groups. At the same time, the presence in solution of chloride or bromide ions may promote the appearance of the racemic product, which was noted previously[132].

The absence of racemisation permitted the use of this method for the coupling of large peptide fragments. The hexapeptide Z–Ile–Gly–Val– –Gly–Ala–Pro–NH_2 (the double dash indicates the junction of the fragments), the octapeptide Z–Gly–Leu–Leu–Gly–Gly– –Phe–Ala–Gly–OCH_3, and the octapeptide Z–Thr(t-But)–Ala– –Ile–Gly–Val–Gly–Ala–Pro–NH_2 have been obtained in yields of 84, 86, and 81% respectively. The reaction is carried out in hexamethylphosphoramide, which is an effective solvent for various peptides. Mixed anhydrides based on boric acid[133] have not been found to be quite so effective, for example:

$$RCO\bar{O} + ClB(OCH_3)_2 \rightarrow RCOOB(OCH_3)_2 \xrightarrow{R'NH_2} RCONHR' \quad .$$

By allowing the sodium salt of benzoylphenylalanine to remain in contact with chlorodimethoxyborane for 18 h at room temperature and then introducing freshly distilled ethyl glycinate into the mixture, the peptide Bz–L–Phe–Gly–OC_2H_5 was obtained in 31% yield. The analogous reaction at 55°C led to the formation of the racemate. The mixed anhydride acyloxydimethoxyborane undergoes a number of reactions, for example it gives rise to triacyloxyborane $(RCOO)_3B$, the symmetrical anhydride RCOOCOR, and other products. All these factors are responsible for the low yields of peptides[134]. A novel application of saccharin is its use in the synthesis of mixed anhydrides with acylaminoacids[135]:



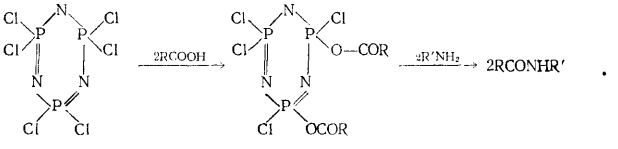
The mixed anhydrides are obtained with the aid of carbodiimide or thionyl chloride in 74–95% yield. They are readily crystallisable, highly fusible compounds with a considerable stability on storage. At the same time, they are very reactive, reacting readily with the amino-components at 0°C and forming peptides in 86–95% yield. They can be employed to synthesise peptides in an aqueous medium, using solutions of aminoacids and peptides in aqueous potassium bicarbonate. The yields of peptides are 63–95%, and the saccharin liberated in the reaction can be readily washed away with water.

The use of acylguanidine for the synthesis of peptides is of interest[136]. Acylguanidines are obtained in satisfactory yields by the reaction of methyl esters of acylaminoacids with free guanidine. Acylguanidines are crystalline compounds with fairly high melting points (150–190°C) and are soluble in polar solvents. They react fairly effectively with amines, forming amides (peptides); the protonation of guanidine is postulated in this process:

$$RCO-\overset{NH}{\overset{\|}{N}}H-C-NH_2 \xrightarrow{H^+} R-CO-NH-\overset{NH_2^+}{\overset{\|}{C}}-NH_2 \xrightarrow{NH_2R'} RCONHR' + \overset{NH_2^+}{\overset{\|}{C}}(NH_2)_2 \quad .$$

The reaction is carried out in an aqueous or aqueous organic medium at room temperature at pH 8. The yields of dipeptides are 68–90%.

The comparatively low yields of peptides and complete racemisation were noted when polyphosphoric acid esters $(C_2H_5O_3P)_n$ were used[137]. Peptides were synthesised by refluxing a solution in $CHCl_3$ or CH_3CN; their yield did not exceed 75% and the peptides of comparatively simple aminoacids were obtained: glycine, alanine, phenylalanine, and leucine. The method can be hardly used to synthesise even small peptides owing to the unduly severe condensation conditions. The application in the synthesis of peptides of hexachlorocyclotriphosphatriazine, which can react with two molecules of the acylaminoacid to form an unusual mixed anhydride, has been described[138]:



The symmetrical anhydrides of Boc-aminoacids, obtained with the aid of ynamines at 0°C[126]:

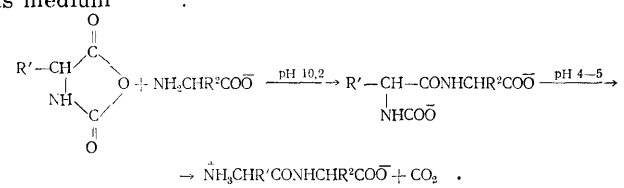
$$2R-COOH + CH_3C{\equiv}C-N(C_2H_5)_2 \rightarrow RCOOCOR + CH_3CH_2CON(C_2H_5)_2 \quad ,$$

or with the aid of phosgene at −40°C,[136,137] proved to be very effective. The reaction of the symmetrical anhydrides with the amino-component terminates after 10–30 min. All these factors make it possible to achieve a single peptide coupling after each 2–3 h in a yield exceeding 90% at each stage. The excess of the anhydride (an

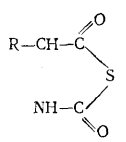
excess by a factor of 1.5 is used) and the Boc-aminoacid formed in the reaction can be readily regenerated after washing with a solution of sodium bicarbonate. Using symmetrical anhydrides, it has been possible to synthesise rapidly and in a satisfactory yield the nonapeptide Boc–Leu–Ile–Ile–Leu–Val–Pro–Pro–Phe–Phe–OBzl without isolating and identifying the intermediates[126].

The symmetrical anhydrides of t-butoxycarbonylaminoacids proved to be convenient reagents for the synthesis of peptides on a polymeric carrier[141]. The use of anhydrides ensures a high yield at each stage. An advantage of this synthesis is also that a comparatively simple regeneration of the excess anhydride is possible. A linear decapeptide, corresponding to antamanide, has been synthesised in 96% yield using symmetrical anhydrides.

The employment of internal anhydrides–*N*-carboxyanhydrides, which can be readily obtained from aminoacids by treatment with phosgene[142], proved to be very productive in the synthesis of peptides. Until recently, *N*-carboxyanhydrides were used mainly to obtain polypeptides and statistical copolypeptides. It was found that, under strictly controlled conditions, *N*-carboxyanhydrides can be used for the stepwise synthesis of peptides in an aqueous medium[142-145]:



For effective synthesis, it is necessary to maintain the pH within a narrow range, since at pH < 10 the carbamate of the dipeptide already loses CO_2, and the resulting dipeptide may react with the *N*-carboxyanhydride to form a side product. On the other hand, at pH > 10.5 the *N*-carboxyanhydride hydrolyses and other side reactions take place. When the pH is maintained rigorously in the range 10.2–10.5, peptides are synthesised at a very high rate. It is important that, among all the aminoacids, only lycine and cysteine require the protection of side groups. The C-terminal tetrapeptide gastrin H–Trp–Met–Asp–Phe–NH_2 has been synthesised in 1 h with the aid of *N*-carboxyanhydrides. For certain aminoacids (for example, histidine and glycine), it is useful to use thio-analogues of *N*-carboxyanhydrides (thiazolidine-2,5-diones)[146,147]:



In this case, the peptide synthesis can be carried out at lower pH values. The *N*-carboxyanhydride method can be conveniently used to synthesise peptide fragments consisting of 4–8 aminoacid residues. It has been applied successfully in the synthesis of ribonuclease fragments[148].

A disadvantage of this method is the low stability of the *N*-carboxyanhydrides, which cannot be synthesised before their immediate requirement and stored for a long time. Furthermore, serious difficulties are encountered in the synthesis of certain *N*-carboxyanhydrides and their thio-analogues. Efforts have been made recently to overcome these difficulties. It was found that *N*-carboxyanhydrides with a substituent at the nitrogen atom are much more stable than the unsubstituted *N*-carboxyanhydrides; they do not polymerise and at the same time react readily with the amino-components, forming *N*-protected peptides. The following protecting groups have been used: NBS[149,150], benzyl, 2,4-dimethoxybenzyl, 2,4,6-trimethoxybenzyl, diphenylmethyl, bis-(4-methoxyphenyl)methyl, and 9-xanthenyl[151]. Protected *N*-carboxyanhydrides have been synthesised either from unprotected *N*-carboxyanhydrides[149] or from protected aminoacids and phosgene[151]. Certain small peptides have been synthesised with the aid of protected *N*-carboxyanhydrides both in solution and on an insoluble polymeric carrier, the yield of peptides being very high in certain instances[150].

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