

# ORGANIC CHEMISTRY IN PEPTIDE SYNTHESIS

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## INTRODUCTION

There is a widely held impression among organic chemists that peptide synthesis is a craft or mystery with a symbolism, language, and ritual all its own, something quite remote from the interests of those working in other fields. On occasions such as this Congress, which bring together chemists interested in a great diversity of special subjects, it should be our aim to discuss matters of common interest rather than to present detailed and specialized results. It will therefore be the aim of this lecture—as the title already suggests—to touch on some of the general organic chemistry underlying the special methods of peptide synthesis, even though this has not been a major concern of the author's own work. Perhaps some points of interest to the non-specialist may emerge; and, conversely, we may come upon suggestions which might be worth developing for the practical purposes of peptide synthesis.

All the chemical problems of peptide synthesis revolve about a single, elementary reaction: the formation of an amide bond. Many methods have been developed for carrying out the synthetic reaction proper; but perhaps even more effort has been devoted to the complex of problems concerned with protecting, or blocking, those groups of the reactants—by the very nature of things, polyfunctional molecules—which are not to enter into reaction at a given stage<sup>1</sup>. We shall deal with both these aspects in turn, selecting certain particular methods for more detailed treatment.

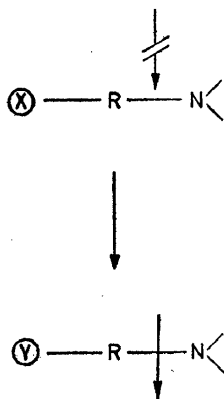
## AMINO GROUP PROTECTION

The fundamental requirements for a protecting group are obvious: such groups must be capable of selective introduction on the functional group to be protected, they must be stable to the conditions of the reaction or sequence of reactions through which protection is desired, and they must be removable under conditions which do not cause damage to the rest of the molecule. The extent of this problem can be appreciated when we recall that in addition to peptide links and the  $\alpha$ -amino and carboxyl groups, peptides of the common or protein amino-acids alone can carry additional amino, carboxyl and amide groups, alcoholic or phenolic hydroxyls, thiol, thioether, or disulphide groups, guanidino groups, and imidazole or indole rings; and as novel or "unnatural" amino-acids are introduced into peptide synthesis the problems correspondingly grow. In addition to the fundamental qualifications of a protecting group there are further considerations of a practical nature which may be important and, indeed, critical in actual

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use—for instance, availability of the reagents required, crystallinity of the derivatives, their solubility properties, and so on.

The conflicting requirements of stability and lability in protecting groups are most usually met by making the fission reaction, or its conditions, as specific as possible. Another tactical principle worth noting makes use of protecting groups which are themselves quite robust but can be modified, by a specific reaction, so as to labilize the bond linking the protecting group to the functional group on the amino-acids (*Figure 1*). This principle, which



*Figure 1.* The “safety-catch” principle

might be called the “safety-catch” principle, greatly extends the range of reactions which can be applied to the removal of protecting groups; some examples will be given later.

A surprising number of elements has been pressed into service in the design of amino protecting groups (*Figure 2*). The inclusion of iron in the Table

H										He
Li	Be	B	C	N	O	F				Ne
Na	Mg	Al	Si	P	S	Cl				Ar
K	Ca	Sc	Ti	V	Cr	Mo	Fe	Co	Ni	
Cu	Zn	Ga	Ge	As	Se	Br				Kr
Rb	Sr	Y	Zr	Nb	Mo	Tc	Ru	Rh	Pd	
Ag	Cd	In	Sn	Sb	Te	I				Xe
Cs	Ba	La	Hf	Ta	W	Re	Os	Ir	Pt	
Au	Hg	Tl	Pb	Bi	Po	At				Rn
Fr	Ra	Ac	Th	Pa	U					

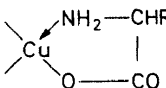
*Figure 2.* Elements used in amino-protecting groups (heavy shading) or in other capacities in peptide synthesis (light shading)

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is not a mistake: a ferrocene-containing protecting group (ferrocenyl-methyloxycarbonyl) has, in fact, been examined<sup>2</sup>, but rejected for practical reasons.

The element in the protecting group actually linked to the amino nitrogen can also be one of many (*Table 1*). The H—N combination signifies protection of the amino group by protonation—an attractive procedure, but

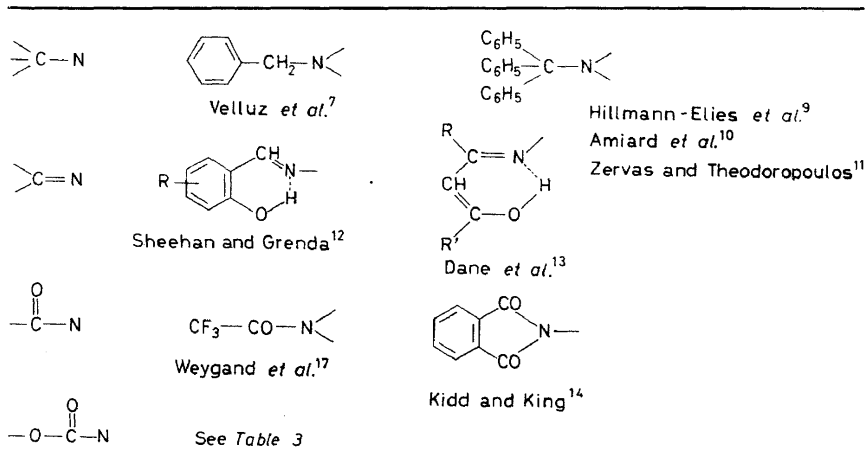
*Table 1.* Elements bonded to nitrogen in protecting groups

(H) — N	$\overset{+}{\text{N}}\text{H}_3\text{—}$	
(N) — N	$\overset{-}{\text{N}}=\overset{+}{\text{N}}=\text{N—}$	
(S) — N	$\text{Ar—SO}_2\text{—N} \begin{array}{l} \diagdown \\ \diagup \end{array}$ $(\text{C}_6\text{H}_5)_3\text{C—S—N} \begin{array}{l} \diagdown \\ \diagup \end{array}$	Fischer <sup>56</sup> ; Schönheimer <sup>57</sup> Stelakatos <sup>3</sup>
(Si) — N	$(\text{CH}_3)_3\text{Si—N} \begin{array}{l} \diagdown \\ \diagup \end{array}$	Birkofer <i>et al.</i> <sup>5</sup>
(P) — N	$(\text{C}_7\text{H}_7\text{O})_2\text{—PO—N} \begin{array}{l} \diagdown \\ \diagup \end{array}$	Zervas <i>et al.</i> <sup>4</sup>
(Cu) — N		Kurtz <sup>6</sup>
(C) — N		See <i>Table 2</i>

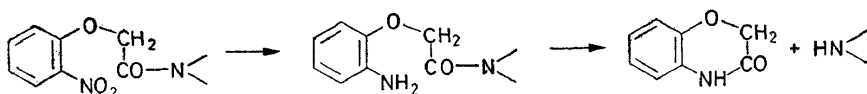
of limited applicability. The N—N bond is present in the azido group—an amino group protected, as it were, by nitrogenation. The arylsulphonyl grouping is a well-known example of an S—N linked protecting group; recently, aralkylsulphenamides have also shown promise<sup>3</sup>. Dibenzylphosphoramidates can be split by a two-stage procedure—hydrogenation and mild acid hydrolysis<sup>4</sup>. Trialkylsilazanes are very labile and can be regarded as protected amino groups only in the widest sense of the word<sup>5</sup>; but copper complex formation<sup>6</sup> is a widely used expedient for protecting suitably placed amino groups.

The C—N bond combination still characterizes the majority of protecting groups (*Table 2*). One structural type here includes benzyl<sup>7</sup>, dibenzyl<sup>8</sup>, triphenylmethyl<sup>9-11</sup> and kindred groups; the stabilization of Schiff base structures by hydrogen bonding as in derivatives of *o*-hydroxyaldehydes<sup>12</sup> or  $\beta$ -dicarbonyl compounds<sup>13</sup> may bring these protecting groups, too, within the range of general usefulness. A second structural type (*Table 2*) covers compounds with carbonyl—nitrogen bonds: either acyl groups proper, or carbamic acid derivatives. The acyl groups include the well-known

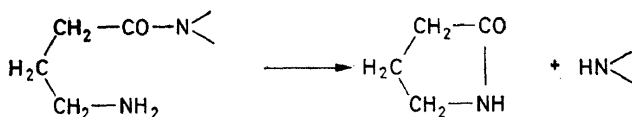
Table 2. Protecting groups attached to nitrogen through carbon



phthaloyl<sup>14-16</sup> as well as the trifluoroacetyl<sup>17</sup> and formyl<sup>18</sup> groups—both removable by solvolysis, as well *o*-nitrophenoxyacetyl<sup>19</sup> which is an interesting example of a group whose removal is based on steric rather than electronic reactivity factors, and also embodying a “safety-catch”: Reduction of the nitro group gives an amino group suitably placed for intramolecular aminolysis of the acyl-amino bond, laying free the amino group of the peptide<sup>19</sup> (Figure 3). This principle appears well worth developing: We have shown

Figure 3. Removal of the *o*-nitrophenoxyacetyl protecting group

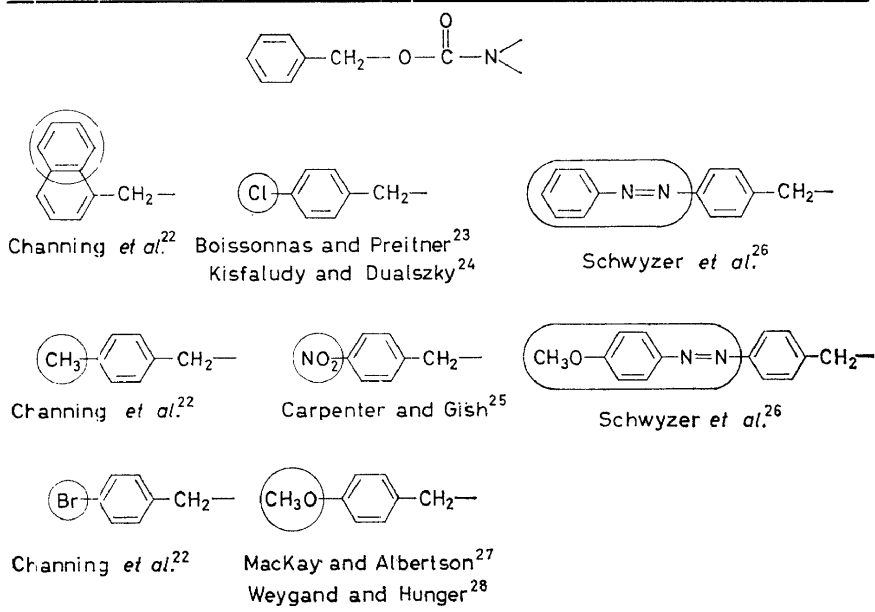
that even so simple a group as  $\gamma$ -aminobutyryl can be split off by the same mechanism under rather mild conditions<sup>20</sup> (Figure 4), and suitable modification, for instance, the introduction of a “safety-catch”, might well give a usable protecting group.

Figure 4. Fission of  $\gamma$ -aminobutyryl peptides

The most numerous, and most popular, class of protecting groups is based on the carbamic acid structure. The famous carbobenzyoxy group of Bergmann and Zervas<sup>21</sup> marked a breakthrough in peptide synthesis. In a way, it has triumphed over its own limitations by fathering a whole family of such urethane protecting groups (Table 3). First, it is true, the innovators did

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Table 3. Modified benzyloxycarbonyl protecting groups



not venture to leave the fundamental benzyl ester structure but introduced substituents primarily to modify the physical properties of the derivatives (crystallinity<sup>22-25</sup>, colour<sup>26</sup>). However, a number of alkyl carbamates was also found to qualify as protecting groups as well as some thiocarbamates (*Table 4*). All these protecting groups share this fundamental feature, that their removal involves formation of the carbamic acid as an intermediate; in fact, under alkaline conditions the carbamate salt may continue to function as a labile protecting group—for instance, preventing side reactions with participation of the amino groups which might otherwise occur. On the other hand, the ways in which the formation of the free carbamic acid is achieved vary greatly, both in technique and in mechanism. In principle, the fission of the carbamate might be facilitated either by increasing the electrophilic reactivity of the carbonyl group—this would lead to ready carbonyl-oxygen fission—or by making the C—O bond reactive and encouraging alkyl-oxygen fission (*Figure 5*). In practice, the first approach has a serious limitation since steric relations in acylpeptides involving  $\alpha$ -amino acids are necessarily such that the peptide nitrogen is in a favourable position for attack on the acyl carbonyl group, and any increase in the reactivity of this carbonyl group will facilitate hydantoin formation as well as the intermolecular hydrolytic reaction. An example is provided by the behaviour of thiocarbamate esters which, under neutral or alkaline conditions, tend to give the hydantoin rather than the carbamate salt<sup>33</sup> (*Figure 6*). Eventually, thiourethane derivatives joined the ranks of useful protecting groups after all when oxidation with peracids was found to liberate the amino group<sup>32</sup>. Further instances of oxidative removal of acyl groups are

Table 4. Other carbamate or thiocarbamate protecting groups

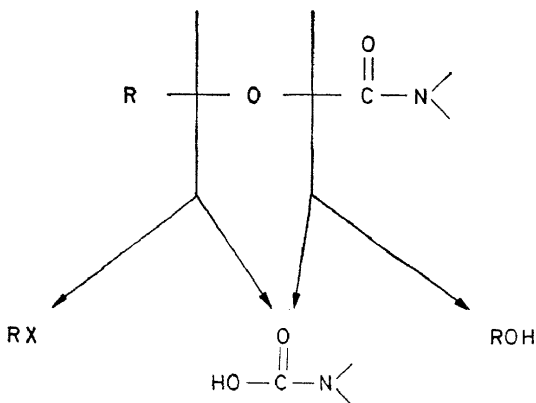
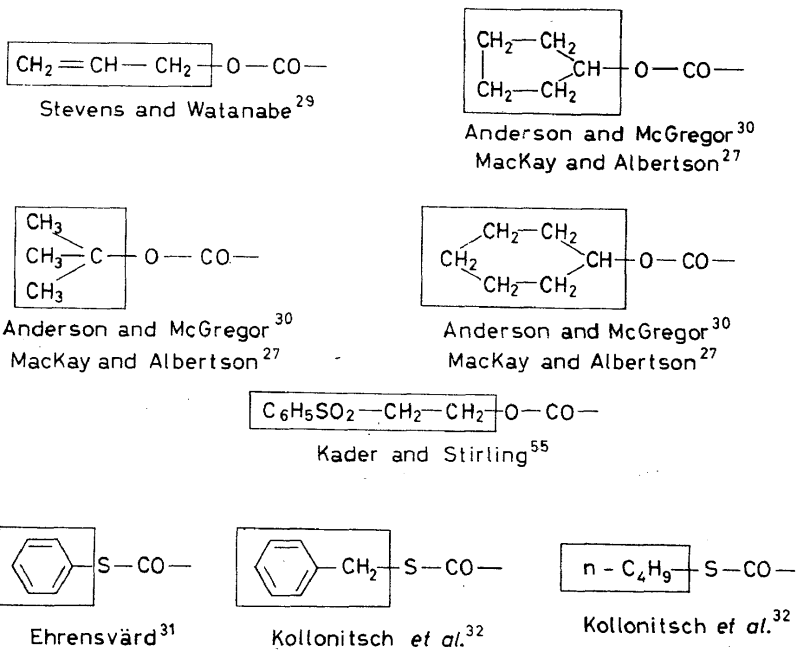


Figure 5. Modes of fission of carbamate protecting groups

provided by the formyl<sup>134</sup> and pyruvoyl<sup>135</sup> groups, and since here again the reaction presumably leads primarily to the carbamic acid (Figure 7) these acyl groups must also be regarded in this context as potential carbamic acids.

However, most successful urethane protecting groups rely on alkyl-oxygen bond fission of one kind or another for conversion to the carbamic acid. The

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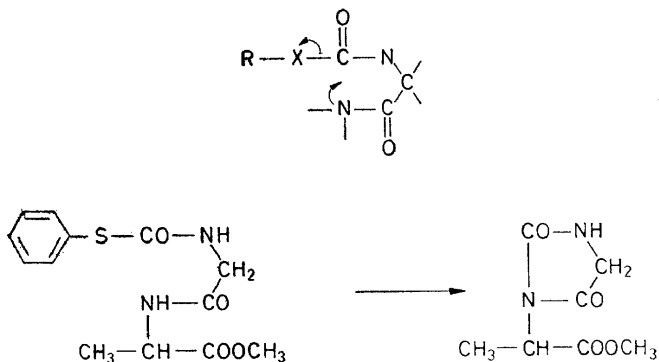


Figure 6. Hydantoin formation as a result of increasing carbonyl reactivity in carbamate protecting groups

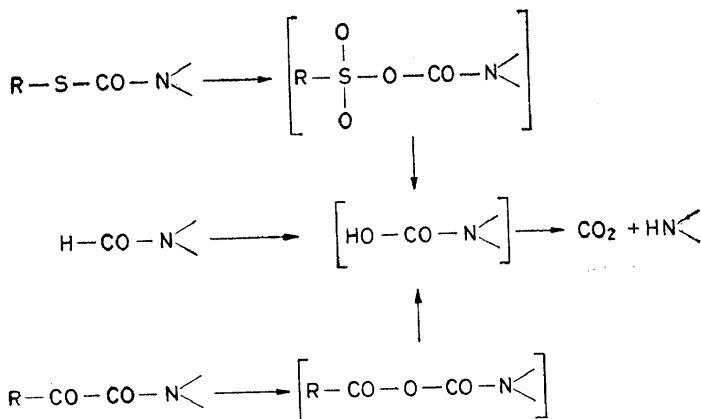
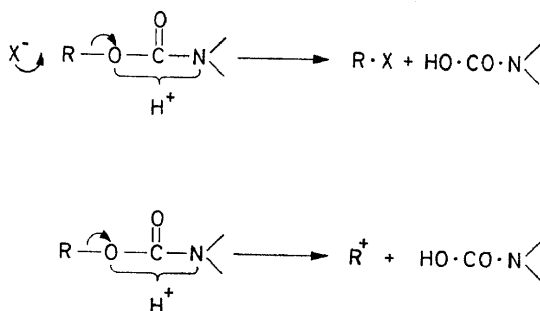


Figure 7. Oxidative removal of protecting groups

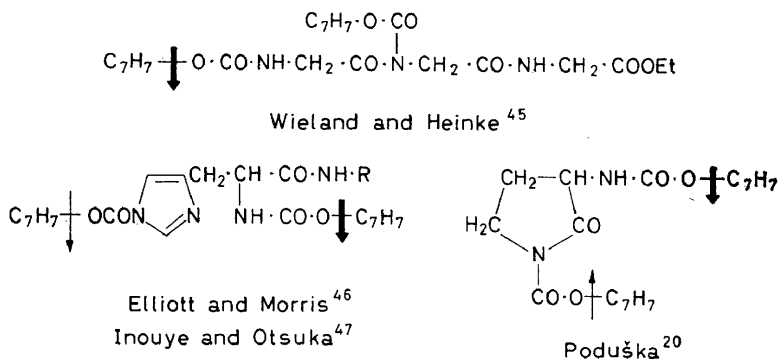
classical example is the hydrogenolysis of the benzyl-oxygen bond on which the original Bergmann-Zervas method was based<sup>21</sup>. All the substituted benzylurethanes are susceptible to this type of fission, as is the allyloxy-carbonyl group<sup>29</sup> though here saturation of the double bond competes. When the substituent does not complicate matters hydrogenolysis can be effected by reduction with sodium in liquid ammonia<sup>36</sup> as well as catalytically.

It then transpired that the benzyl-oxygen bond could also be split by acid-catalysed solvolysis. Hydrogen iodide<sup>37, 38</sup>, hydrogen bromide<sup>39-41</sup>, and—less universally—hydrogen chloride<sup>42, 43</sup> have been used, hydrogen bromide in glacial acetic acid<sup>39, 40</sup> being the most popular reagent. There is some doubt about the precise site of protonation in the urethane group<sup>44</sup>, and also about the actual mechanism of the carbon-oxygen bond fission (Figure 8). A decrease in the basicity of the nitrogen atom, for instance by

acylation or inclusion in a heterocyclic ring system, decreases the rate of fission with hydrogen bromide: The "normal" benzyloxycarbonyl group is preferentially removed from derivatives such as those shown in *Figure 9*. This type of evidence suggests that the nitrogen is the site of protonation<sup>44</sup>. As regards the carbonyl-oxygen fission step, the greater effectiveness of hydrogen bromide as compared with hydrogen chloride suggests participation of the anion but, on the other hand, in trifluoroacetic acid benzyl cations are certainly formed and, unless a scavenger is present, will make themselves felt by C-benylation<sup>48</sup>.



*Figure 8.* Possible modes of fission of carbamate protecting groups with acid

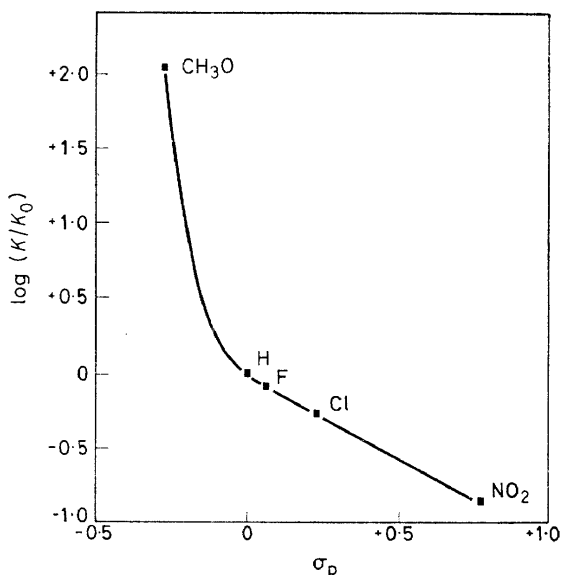


*Figure 9.* Differential removal of benzyloxycarbonyl groups

The effect of substituents in the benzyl group on the reaction rate might give added information on these points. Preparative experience has shown that a *p*-nitro substituent appreciably decreases the rate of fission of the benzyloxycarbonyl group with hydrogen bromide<sup>49, 50</sup> whereas a *p*-methoxy substituent greatly increases the rate<sup>27, 28</sup>. We have, with Dr K. Bláha<sup>51</sup>, made a rough quantitative study of the rate of fission of several substituted benzyloxycarbonyl groups from glycine with 0.75M hydrogen bromide in acetic acid; the rates were measured by following the evolution of carbon dioxide in a Warburg apparatus. A plot of the logarithms of the



rate constants against the Hammett  $\sigma_p$  values (*Figure 10*) shows that the reaction is of the "negative-rho" type, with good linearity except for the *p*-methoxy derivative which reacts very much more rapidly than it should. This relation suggests a change in reaction type between the benzyloxy-carbonyl and *p*-methoxybenzyloxy-carbonyl groups, perhaps from a bimolecular to a monomolecular mechanism of bond fission. Further work to check this is under way.



*Figure 10.* Hammett plot for rates of fission of *p*-substituted benzyloxy-carbonyl groups with 0.75M hydrogen bromide in glacial acetic acid at 25°

In the alkylcarbamate series we used the same technique to study the rate of fission of cyclopropyloxy- and cyclohexyloxy-carbonylglycine together with some additional derivatives which we prepared for the purpose and which we expected to show graded reactivities toward the hydrogen bromide reagent: the cyclohexyl carbamates with *cis*- and *trans*-2-methyl and *cis*- and *trans*-4-*t*-butyl substituents in the ring. The rates not only followed the expected order qualitatively but gave an excellent linear free energy relation (*Figure 11*) when plotted logarithmically against the rates of solvolysis of the corresponding cycloalkyl tosylates<sup>52, 53</sup>, showing that the two reactions have similar stereoelectronic requirements over this structural range and confirming that the cycloalkylcarbamate fission is a true solvolysis.

Studies of this kind, suitably extended, should be useful in predicting the behaviour of further carbamate esters and choosing blocking groups likely to be of use in peptide synthesis.

The reactions used for the removal of protecting groups derived from the carbamate structure thus include catalytic hydrogenation, chemical reduction, acid-catalysed substitution and elimination. The recent introduction

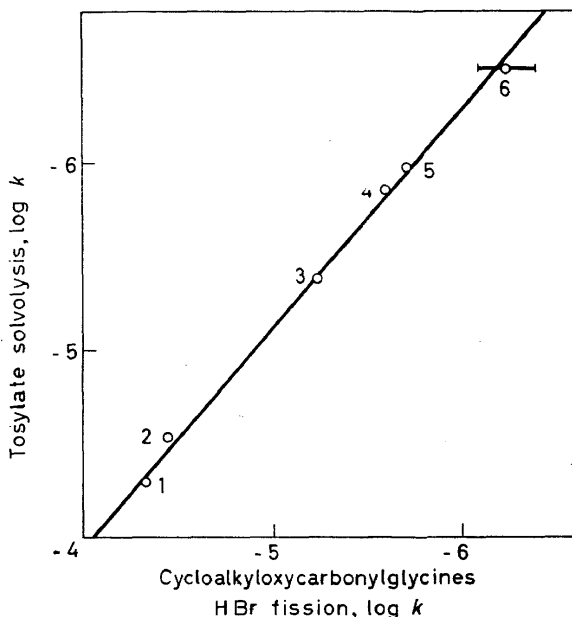


Figure 11. Logarithmic plot of the rate constants for fission of cycloalkyloxycarbonylglycines with 1.25M hydrogen bromide in glacial acetic acid at 25° against the rate constants for solvolysis of the corresponding cycloalkyl tosylates in ethanol at 50°; 1: cyclopentyl, 2: *cis*-2-methylcyclohexyl, 3: *cis*-4-*t*-butylcyclohexyl, 4: cyclohexyl, 5: *trans*-4-*t*-butylcyclohexyl, 6: *trans*-2-methylcyclohexyl

of photolysis (for instance of the benzyloxycarbonyl group itself)<sup>54</sup> and base-catalysed elimination (from a  $\beta$ -sulphonylethyl carbamate)<sup>55</sup> for alkyl-oxygen fission (Figure 12) shows that the potentialities of the urethane protecting group are not yet exhausted.

Having given this important class of derivatives their due we shall discuss a blocking group which has been something of a problem child—nevertheless, a favourite with some of us: the toluene-*p*-sulphonyl or tosyl

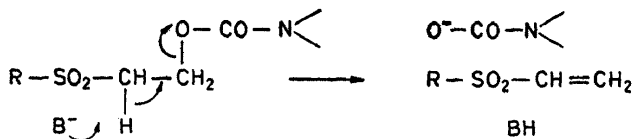


Figure 12. Alkaline fission of a  $\beta$ -tolylsulphonylethyl carbamate

group. This group, attached to amino nitrogen, is very stable to acid and alkaline hydrolysis, hydrazinolysis, and a range of other conditions encountered in peptide synthesis. Although it was known that it could be split off by reduction with phosphonium iodide<sup>56, 57</sup> or with sodium in liquid ammonia<sup>58</sup>, the tosyl group was slow to find general favour, probably because of the difficulties attending the isolation of the peptide from the

large amounts of inorganic matter present. The introduction of ion-exchangers appeared to offer an answer to this problem but somewhat to our surprise we found when we tried this approach some years ago that mere removal of sodium ions did not give salt-free products<sup>59</sup>. Though much work has been done on the sodium-liquid ammonia system little appears to have been recorded about the course of reaction with sulphonamides beyond the fact that the sulphur-containing moiety is reduced to mercaptan<sup>60</sup>. However, we found that our troubles were due to the presence of sulphate and sulphite ions in the reaction mixture<sup>59</sup> and we have also isolated toluene-*p*-sulphinic acid as a further reaction product<sup>61, 62</sup> (Figure 13). The sulphite

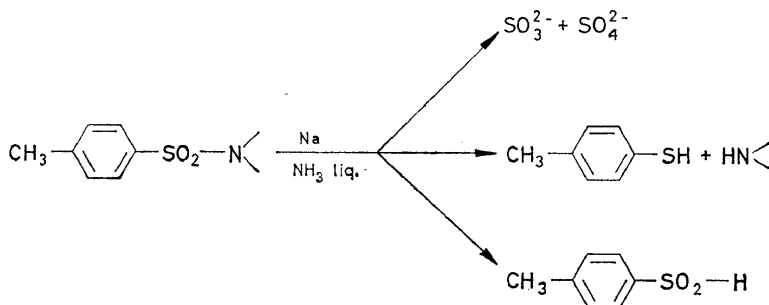


Figure 13. Reduction of tosylamides with sodium in liquid ammonia

presumably arises by the mode of reductive fission between carbon and sulphur described for sodium benzenesulphonate<sup>63</sup>, and the sulphate by atmospheric oxidation of the sulphite<sup>59</sup>. The sulphinate might arise by reductive fission of the sulphur-nitrogen bond (Figure 14a), but the possibility of an elimination mechanism (Figure 14b) cannot be ignored. In a recent study of this reaction, we have found<sup>64</sup> that under suitable conditions sulphinate may account for over 85 per cent of the sulphur-containing reaction products, without a corresponding degree of racemization in the amino-acid formed. Furthermore, toluene-*p*-sulphinic acid is formed in the reduction of tosyl- $\alpha$ -aminoisobutyric acid which lacks an  $\alpha$  hydrogen atom but not by treatment of tosylamino-acids with sodium amide in liquid ammonia. It therefore appears that the reductive mechanism accounts for most, if not all, of the sulphinate formed.

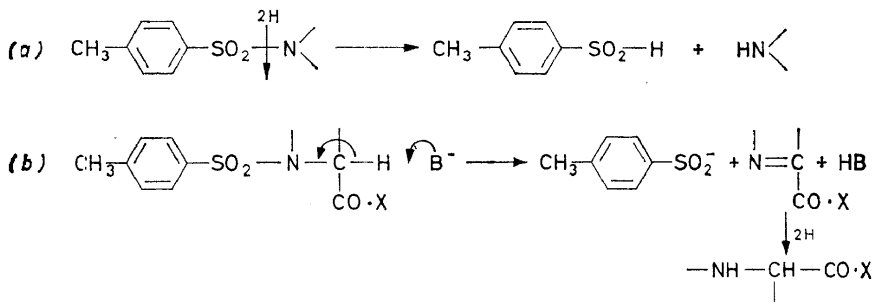
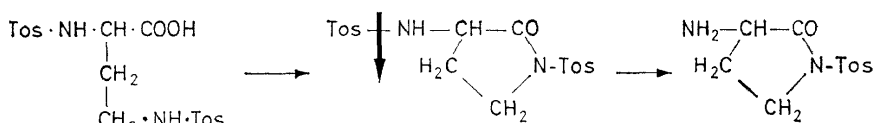


Figure 14. Possible modes of formation of sulphinic acid

Sulphonamides are also split by hydrogen bromide or iodide<sup>65-67</sup>; again, hydrogen bromide in acetic acid is a convenient reagent<sup>67</sup>. We introduced this technique into the peptide field and found that it can be applied under conditions which do not in general lead to peptide bond fission<sup>68</sup>. The reaction is known to be a reduction, giving aryl disulphide and bromine<sup>65</sup>. We have observed that acylation of the sulphonamide nitrogen stabilizes the tosyl group<sup>69</sup>. An interesting example is provided by the behaviour of ditosyl- $\alpha,\gamma$ -diaminobutyric acid with hydrogen bromide in acetic acid<sup>70</sup> (*Figure 15*): The reagent effects ring-closure, and in the



*Figure 15.* Reaction of ditosyl- $\alpha,\gamma$ -diaminobutyric acid with hydrogen bromide in acetic acid

resulting *N*-tosyl-lactam only the tosyl group bound to amino nitrogen is removed, the product being 3-amino-1-tosylpyrrolid-2-one, a useful intermediate in the synthesis of diaminobutyric acid peptides. We may conclude that the detosylation reaction is initiated by protonation of the nitrogen and continues, perhaps, through nucleophilic attack by bromide at the sulphur and reduction of the sulphonyl bromide.

Some distinctive—and, be it admitted, mainly negative—features of the tosyl protecting group are due to the mobility of the tosylamide proton. One example is the ready cyclization of the diaminobutyric acid derivative just mentioned. It is also notorious that tosylamino acids give very poor yields in peptide synthesis through mixed acid anhydrides. Together with Dr M. Zaoral we have investigated this reaction in the model system, tosylglycine with aniline as the amine component and *s*-butyl chloroformate as the reagent<sup>71</sup>. From the reaction mixture he isolated, by classical methods, no less than ten crystalline compounds, shown in *Table 5*. Without going into the precise genesis of these products it will be seen that, apart from what should be the “normal” reaction product and recovered starting materials, most of the products characteristically have an acylated tosyl-amino group, either by *s*-butyloxycarbonyl or by a tosylglycyl residue†. An answer to the practical problem was also found: With the sterically highly hindered trimethylacetyl chloride as the reagent, *N*-acylation is suppressed and good yields of peptides can be obtained<sup>72</sup>.

The fragmentation reaction undergone by tosylamino acid chlorides and azides in aqueous alkaline solution<sup>75</sup> (*Figure 16a*) which sets yet another limitation on their use is again initiated by dissociation of the tosylamino group proton; though it is interesting to note that a closely similar fragmentation (*Figure 16b*) has been observed with the uncharged carboxyl-activated derivatives of  $\alpha$ -dibenzylamino acids<sup>76</sup>.

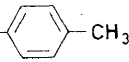

† The formation of such imide-type derivatives has also been observed in the case of benzyloxycarbonylglycine, though not to the same extent<sup>45,73,74</sup>.



methods actually in use basically involve activation of the carboxyl group by increasing carbonyl reactivity. An important exception is an approach recently developed by Brenner<sup>77</sup> which utilizes steric effects rather than polarization to achieve bond formation under mild conditions. However, we shall here be concerned with some methods of the conventional, "activated-carboxyl"<sup>77</sup> type.









Essentially, derivatives of the type R·CO—X are required where X is electron-attracting. Again, the atom linked to the carbonyl may be oxygen, nitrogen, chlorine, sulphur, or selenium. In all but the last of the examples shown in *Table 6* activation is achieved by the inductive effect of the substituent. However, the richest group of activated intermediates is of the

Table 6

CO — Cl		
CO — O	— O·CH <sub>2</sub> ·C≡N	Schwyzler <i>et al.</i> <sup>78</sup>
	— O·SO <sub>2</sub> <sup>-</sup>	Kenner <i>et al.</i> <sup>79</sup>
	— O·P(OEt) <sub>2</sub>	Cramer and Gärtner <sup>80</sup>
CO — N	— N(CH <sub>3</sub> )·SO <sub>2</sub> —  —CH <sub>3</sub>	} Wieland and Henning <sup>81</sup> Poduška and Rudinger <sup>82</sup>
CO — S	— S·C <sub>2</sub> H <sub>5</sub>	
CO — Se	— Se— 	Jakubke <sup>84</sup>

type R·CO—O—X=Y where mesomeric effects are of major importance. This covers active esters of the nitrophenyl type<sup>88, 89</sup>, the adducts with ethoxyacetylene<sup>87</sup>, carbodiimide<sup>95</sup>, and cyanamide<sup>96</sup> and a host of others (see *Table 7*). The rather less numerous intermediates with CO—N bonds

Table 7

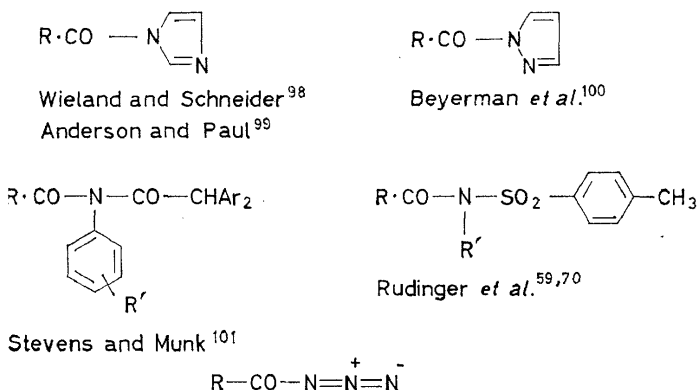
R·CO—O—CH=CH <sub>2</sub> Weygand and Steglich <sup>85</sup>	R·CO—O— 	R·CO—O— 
	Wieland and Sehring <sup>90</sup>	Sheehan and Hess <sup>95</sup>
R·CO—O—C≡CH Bodanszky <sup>86</sup>	R·CO—O— 	R·CO—O— 
	Boissonas <sup>92</sup>	Losse and Weddige <sup>96</sup>
R·CO—O— 	Wieland and Bernhard <sup>93</sup>	
Bodanszky <i>et al.</i> <sup>88</sup> Iselin <i>et al.</i> <sup>89</sup>	Vaughan and Osato <sup>91</sup>	
R·CO—O— 	R·CO—O— 	R·CO—O—N=C 
Arens <i>et al.</i> <sup>87</sup>	Goldschmidt and Wick <sup>94</sup>	Pless <sup>97</sup>

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include the acylazoles (acylimidazoles<sup>98, 99</sup>, acylpyrazoles<sup>100</sup>), ketene imine adducts<sup>101</sup>, and acid azides (Table 8).

The aminolysis of acid azides is one of the oldest methods of peptide synthesis, dating back to Curtius; yet azides are probably most generally known in organic chemistry for another reaction—the rearrangement

Table 8



bearing the same author's name. Indeed, this rearrangement can be an awkward side reaction in peptide synthesis. According to circumstances it may lead under preparative conditions to unsymmetrical or symmetrical ureas or, in the presence of suitably placed functional substituents in the acyl group, to cyclic derivatives<sup>102</sup> (Figure 17). The best known case is that of benzyloxycarbonylserine azide which affords benzyloxycarbonylamino-oxazolidone<sup>103</sup> (Figure 18). Preparative experience indicates that the

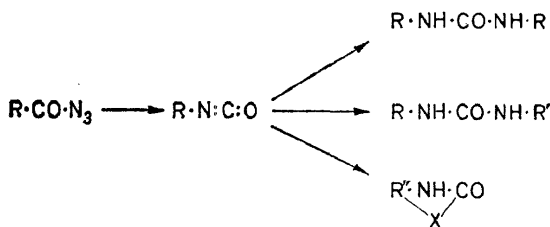


Figure 17. Possible results of Curtius rearrangement

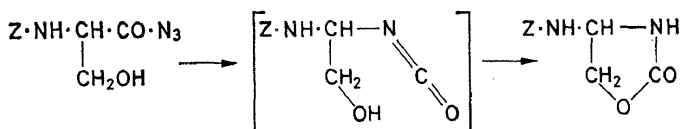
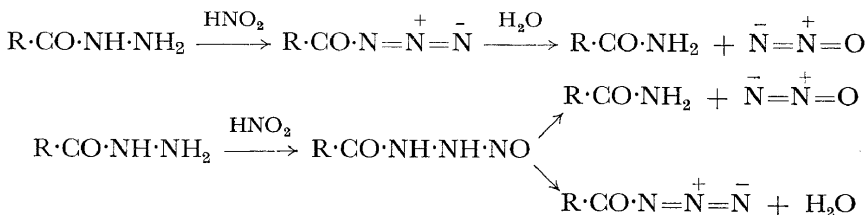


Figure 18. Curtius rearrangement of benzyloxycarbonylserine azide

hydroxyl group not merely attacks the isocyanate as it is formed but accelerates the rearrangement itself: The serine azide rearranges appreciably faster than analogous derivatives without the functional group.

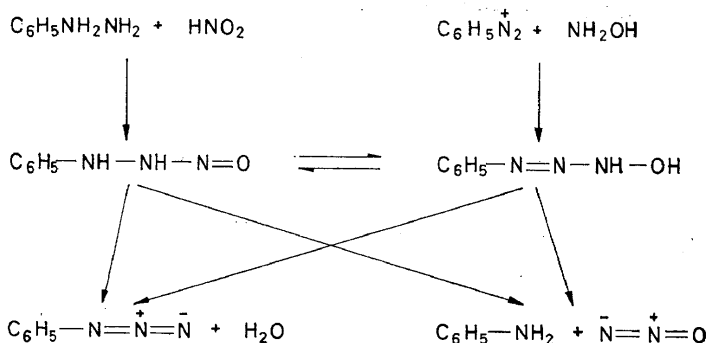
Nor is the Curtius rearrangement the only side reaction we have to contend with. In the preparation of azides from hydrazides, the formation of diacylhydrazines<sup>104</sup> has sometimes been noted; nitration in the tyrosine nucleus<sup>105</sup> and nitrosation of the indole ring in tryptophan<sup>106</sup> may occur; and, as we have found, thioesters may be converted to the sulphoxides<sup>107</sup>. However, a side reaction which has been even more bothersome, and certainly less expected, than the Curtius rearrangement has been the formation of the amide corresponding to the initial hydrazide as a byproduct<sup>107</sup>. Two possibilities had been considered for the genesis of the amide<sup>108</sup>: one, that it arises from the azide by a hydrolytic reaction, the other, that it is formed from an intermediate, presumably a nitrosohydrazide, by elimination of nitrous oxide, whereas the azide is formed from the same intermediate by loss of water (*Figure 19*). Although nitrosation might occur either at the  $\alpha$  or the  $\beta$  nitrogen atom the linear arrangement of the three nitrogen atoms



*Figure 19.* Possible modes of amide formation

in the azide, and of the two nitrogen atoms and the oxygen in nitrous oxide, requires that both of these compounds be formed from the  $\beta$ -nitrosohydrazide.

Evidence from analogy favours the occurrence of a common intermediate. For instance, the reaction of phenylhydrazine with nitrous acid may give rise to aniline and nitrous oxide or to phenyl azide, and it has been concluded that the  $\beta$ -nitrosohydrazine functions as an intermediate in both reaction paths<sup>109</sup> (*Figure 20*). Again, the coupling of a diazonium salt with



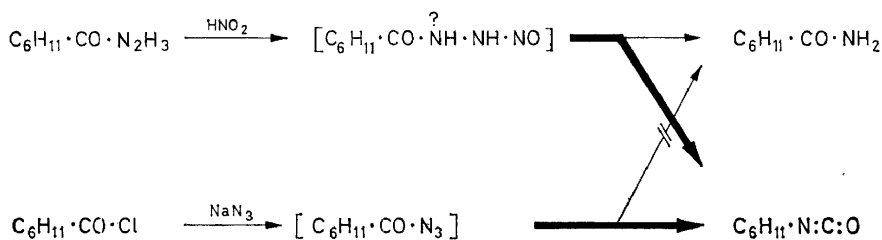
*Figure 20.* Alternate reaction paths in the decomposition of  $\beta$ -nitrosophenylhydrazine and coupling of hydroxylamine with phenyldiazonium ion



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hydroxylamine, which should in the first instance give a hydroxytriazeno tautomeric with the nitrosohydrazine, eventually gives both sets of products<sup>110</sup>. Moreover, the reaction conditions shown to determine the pathway of the nitrosohydrazine decomposition<sup>109</sup>—acidity, temperature, and solvent—have also been found to affect the degree of amide formation in the azide synthesis<sup>107</sup>.

To obtain more direct evidence we reinvestigated, together with Dr J. Honzl<sup>107</sup>, the reaction of cyclohexanecarbohydrazide with nitrous acid<sup>111</sup>. Though it did not prove possible to characterize the unstable material we suspect of being the nitrosohydrazide we did find that, over a range of reaction conditions, some amide was always present in the product. When, on the other hand, the azide was prepared by reaction of the acid chloride with sodium azide and in turn exposed to analogous conditions, no amide could be detected (*Figure 21*). We therefore believe that the amide is, in fact,



*Figure 21.* Amide formation in the preparation of cyclohexanecarbonyl azide by different methods

formed by a reaction path alternative to azide formation. We also found conditions under which amide formation from benzyloxycarbonyl-*S*-benzylcysteine hydrazide, a particularly difficult compound in this respect, could be suppressed entirely. These are based on the use of nitrosyl chloride or a nitrite ester with hydrogen chloride in an anhydrous solvent, rather than aqueous sodium nitrite, for preparation of the azide<sup>107</sup>.

At best, the azide synthesis with its formidable array of possible side reactions remains a difficult procedure. If in spite of this it still is one of the most widely used synthetic methods this is due to the fact that for all its vices it has one overriding virtue: even under the most stringent conditions it does not cause racemization of the activated amino-acid residue<sup>112, 113</sup> and it can therefore be confidently used for joining together peptide units. In this respect the method is unique and it is an intriguing question just why this is so.

The mechanism still most commonly accepted to explain racemization of carboxyl-activated acylamino acids or peptides postulates the intermediate formation of an oxazolone, by intramolecular attack of oxygen at the activated carbonyl<sup>114</sup> (*Figure 22*). It has been suggested<sup>115</sup> that the varying degree of racemization observed with different methods of carboxyl activation under otherwise comparable conditions reflects the relative reactivity of the carbonyl toward oxygen nucleophiles and nitrogen nucleophiles. However, it is difficult to understand the absolute discrimination in the given instance. Goodman has pointed out<sup>116</sup> that the explanation should be sought

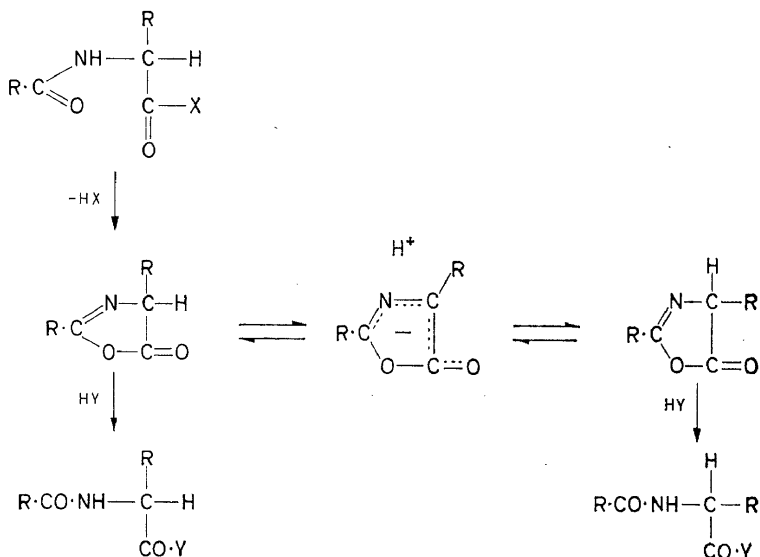


Figure 22. Mechanism of racemization through azlactone formation

in the properties of the azide group itself rather than its effect on the carbonyl. If, for instance, the amino component became attached to the azide group in some way subsequent reaction with the carbonyl group might be of the sterically facilitated type. Thus full, covalent addition to the terminal

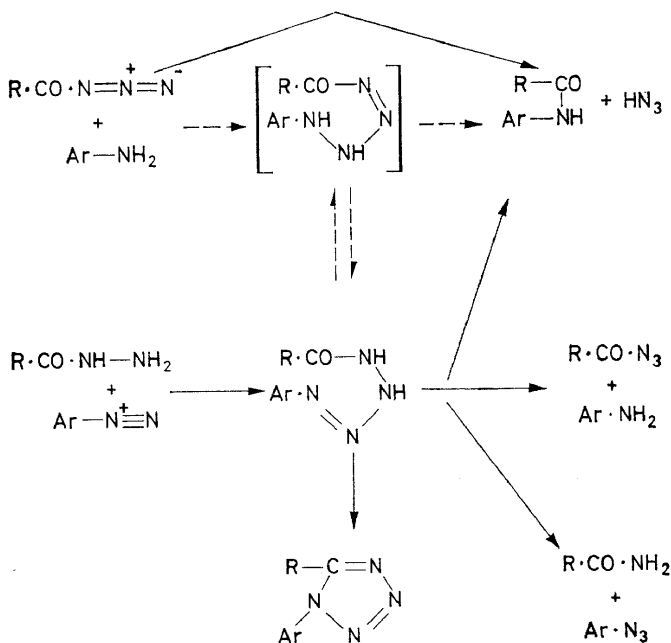
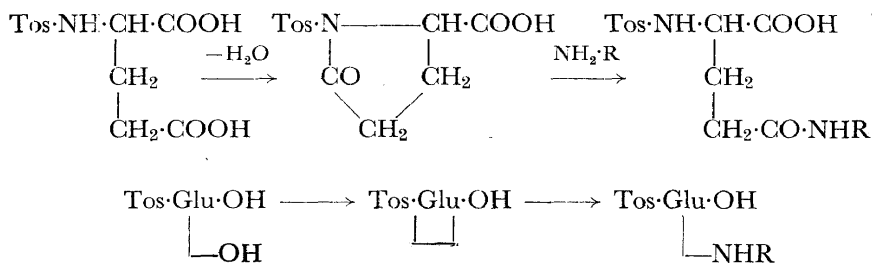


Figure 23. A hypothetical addition mechanism in the aminolysis of azides, and the known reactions of arylazohydrazides

N=N bond would give a tetrazene in which the amino group should be suitably placed for attack on the carbonyl (*Figure 23*). To test this possibility Dr. K. Jošt in my laboratory examined the properties of the tetrazenes (azohydrazides) formed from benzyloxycarbonylglycine hydrazide and several aryldiazonium compounds. The known<sup>117, 118</sup> reactions of such azohydrazides are shown in the lower part of *Figure 23*; from the decomposition of our tetrazenes, with or without solvents, we isolated fair yields of the anilides—a product not before recorded; but occurrence of the aryl azide and other by-products which are not observed in the synthesis of the same anilides from preformed azide make the tetrazene a very unlikely intermediate in the azide coupling. Though, then, this simplest hypothesis has proved incorrect it still remains likely that a, possibly more subtle, interaction of the amine component with the azide group will provide the key to the special features of the azide synthesis.

From the oldest, and the most universal, nitrogen-bonded activating group we shall pass to one of the newest, and most specific—but one which nevertheless offers some points of chemical interest. In the course of work on tosylglutamic acid, it was found that the lactam formed by ring-closure of the  $\gamma$ -carboxyl to the tosylamino group readily undergoes aminolysis<sup>59, 69, 119–121</sup> (*Figure 24*). The inductive effect of the arylsulphonyl



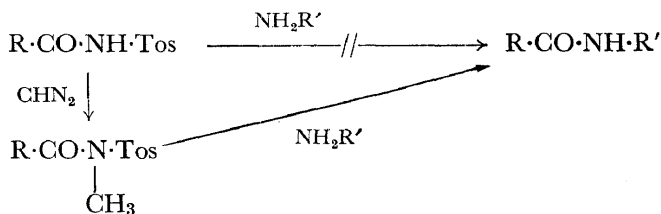
*Figure 24.* Formation and aminolysis of 1-tosylpyroglutamic acid

group, transmitted through the nitrogen, is sufficiently strong to put the carbonyl group into the "activated carboxyl" class. Tosylpyroglutamic acid has, indeed, proved a versatile intermediate in the synthesis of peptides of glutamic acid and of glutamine<sup>59, 69, 119–122</sup>. The preparation of a naturally occurring peptide<sup>123, 124</sup>,  $\gamma$ -glutamyl-S-methylcysteine, may serve by way of a single example<sup>122, 125</sup> (*Figure 25*). The homologous *N*-tosylpiperidone has also been prepared and exploited in the synthesis of  $\alpha$ -aminoadipic acid peptides<sup>126, 127</sup>.

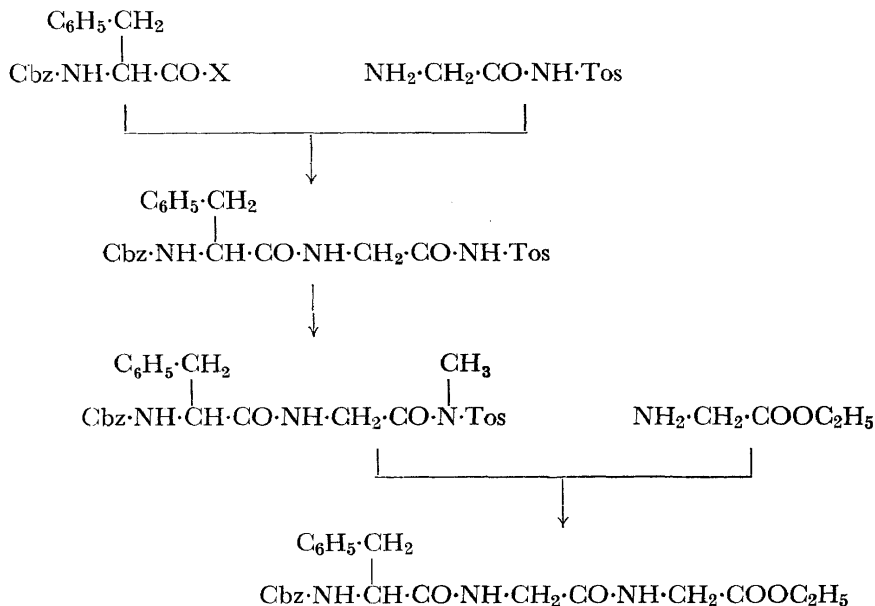
This work naturally directed attention to the analogous acyclic derivatives, that is, the *N*-tosylamides of protected amino-acids<sup>128</sup>. These can be made by acylation of toluene-*p*-sulphonamide<sup>81</sup> or from the free carboxylic acids by the action of tosyl isocyanate<sup>82</sup> (*Figure 26*). Aminolysis of these compounds proved quite difficult, obviously because in the presence of base the NH group dissociates ( $pK$  about 2.5)<sup>81</sup> and the negative charge sharply decreases the cationoid reactivity of the neighbouring carbonyl group. In the tosyllactams and related derivatives with tertiary nitrogen this difficulty does not arise and, indeed, the *N*-methyl derivatives of the acyltosylamides



treatment with diazomethane<sup>81, 82</sup> we have here a system in which activation of the carboxyl group can be achieved by mere methylation with diazomethane. An example of a model synthesis based on these relations<sup>82</sup> is shown in *Figure 28*.



*Figure 27.* Activation of *N*-acyltoslamides by methylation



*Figure 28.* Use of *N*-acyltoslamide intermediates in the synthesis of a tripeptide

On the whole, the use of this type of activation is likely to remain confined to intermediates with the *N*-tosyl-lactam structure where the tosyl group serves the dual purpose of amino-group protection and carboxyl activation. In addition to glutamic and  $\alpha$ -aminoadipic acid, ornithine and  $\alpha,\gamma$ -diaminobutyric acid contain carboxyl and amino groups suitably spaced for forming lactam structures and both have yielded intermediates of this kind<sup>70, 129, 130</sup>. In fact, as we have shown with Dr K. Poduška, *N* $\gamma$ -tosyl derivatives of diaminobutyric acid cyclize to the tosyl-lactams so readily that their formation successfully competes with peptide bond formation by most methods<sup>70, 129</sup>.

Thus, *N*<sup>α</sup>-benzyloxycarbonyl-*N*<sup>γ</sup>-tosyl-L- $\alpha,\gamma$ -diaminobutyric acid on treatment with *s*-butyl chloroformate and base smoothly gives the pyrrolidone<sup>70</sup>. Ring opening with, for instance, threonine methyl ester then gives the dipeptide ester<sup>70</sup> (Figure 29). The use of two different protecting groups on the diaminobutyric acid permits selective exposure of one amino group—in this instance, the  $\alpha$ -amino group—by hydrogenation. The peptide chain can then be extended at this position. Coupling with the protected dipeptide

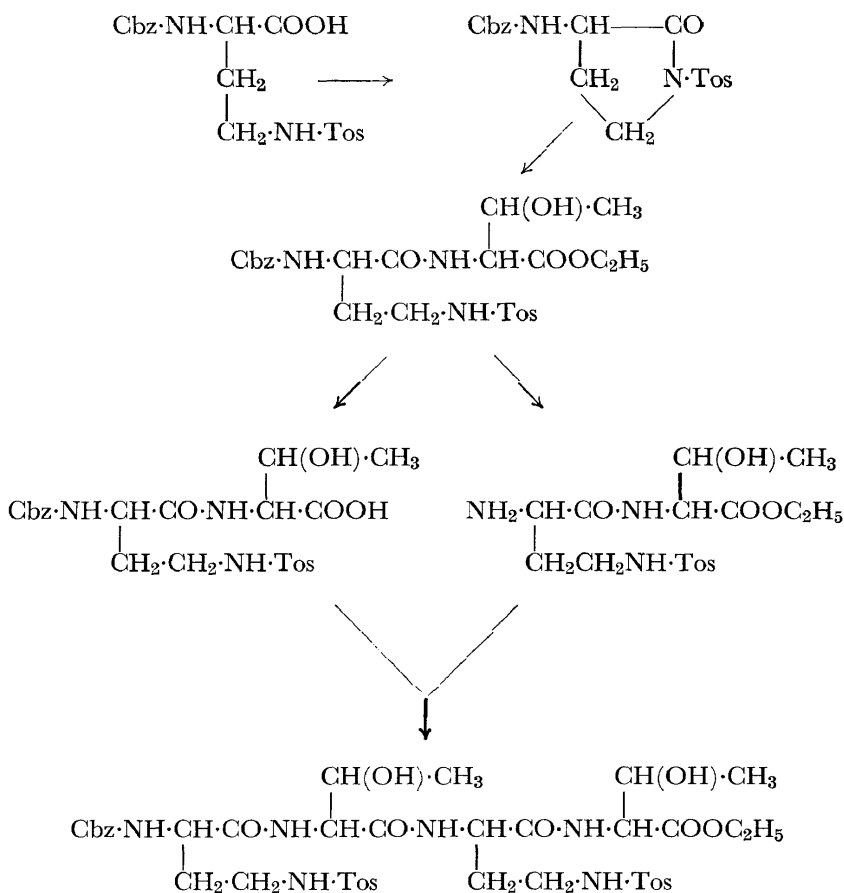


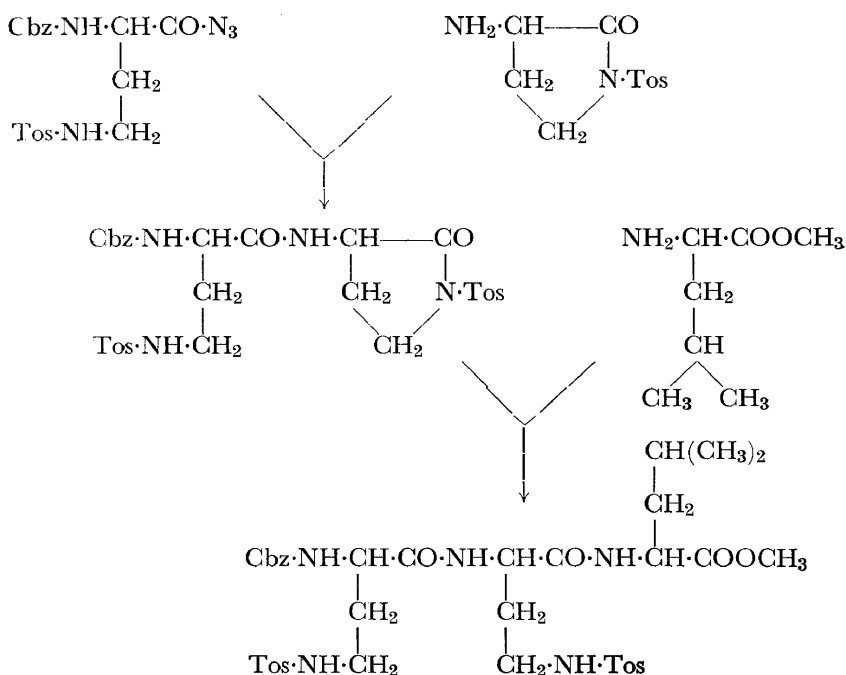
Figure 29. Synthesis of a tetrapeptide sequence from circulin A

obtained from the same intermediate by alkaline hydrolysis, using dicyclohexylcarbodi-imide, gives a tetrapeptide derivative with the sequence, diaminobutyryl-threonyl-diaminobutyryl-threonine<sup>82</sup> (Figure 29).

For introducing diaminobutyric acid into the central position of a tripeptide, 3-amino-1-tosylpyrrolid-2-one is a convenient intermediate; this

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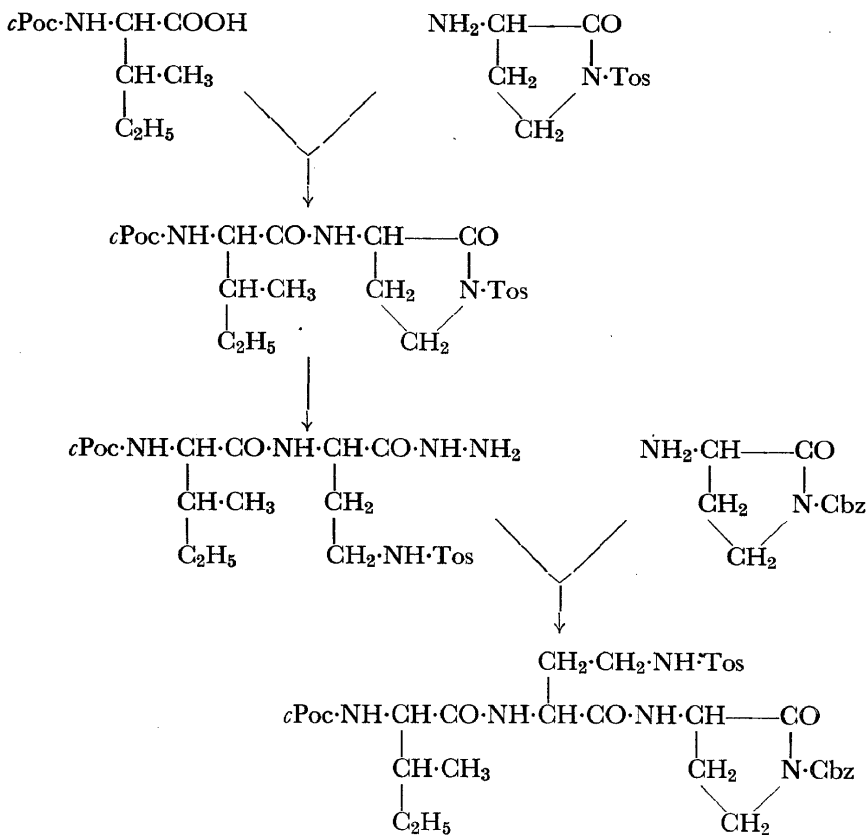
compound, as has already been noted, is formed from ditosyl- $\alpha,\gamma$ -diaminobutyric acid with hydrogen bromide in acetic acid<sup>70</sup>. It can be acylated at the amino group—by another protected diaminobutyric acid residue if desired: here the lactam method cannot be used, but the azide procedure gives satisfactory results provided the coupling is carried out in weakly acidic solution to suppress ionization of the tosylamide grouping<sup>70</sup>. The dipeptide-lactam can be converted to a protected tripeptide by aminolysis, *e.g.*, with *D*-leucine methyl ester (*Figure 30*).



*Figure 30.* Synthesis of a tripeptide sequence from circulin A

Similarly the 3-amino-1-tosylpyrrolidone can be acylated with cyclohexyloxycarbonylisoleucine. Here the use of pyrophosphite in diethylphosphite—a coupling method proceeding in acidic solution—prevents self-condensation of the lactam component. Hydrazinolysis gives the dipeptide hydrazide which is attached to another lactam intermediate, this time 3-amino-1-benzyloxycarbonylpyrrolid-2-one, by the azide synthesis (*Figure 31*). The tripeptide intermediate resulting after alkaline hydrolysis contains three different amino protecting groups. The benzyloxycarbonyl group can

be selectively removed by hydrogenation or, because of its greater reactivity in solvolysis, by mild treatment with hydrogen bromide in acetic acid. It is replaced by pelargonyl, the carboxyl group is esterified and the cyclopentyl-oxycarbonyl group now removed by somewhat more vigorous treatment with hydrogen bromide in acetic acid (*Figure 32*).



*Figure 31.* Synthesis of a differentially protected tripeptide

We then proceeded to attach the threonine-containing tetrapeptide by an azide synthesis—avoiding the possibility of racemization—to the fragment diaminobutyryl-diaminobutyryl-D-leucine and the resulting heptapeptide, again by an azide synthesis, to the tripeptide containing the fatty acid residue (*Figure 32*). Hydrazinolysis followed by removal of the benzyl-oxycarbonyl group gave a decapeptide hydrazide which, after conversion to the azide, cyclized. The resulting cyclodecapeptide (*Figure 33*) is related to the polypeptide antibiotic of the polymyxin group, circulin A (see ref. 131); and although the synthesis, as shown in *Figure 33*, would at first sight hardly arouse the interest of most organic chemists I hope I have been able



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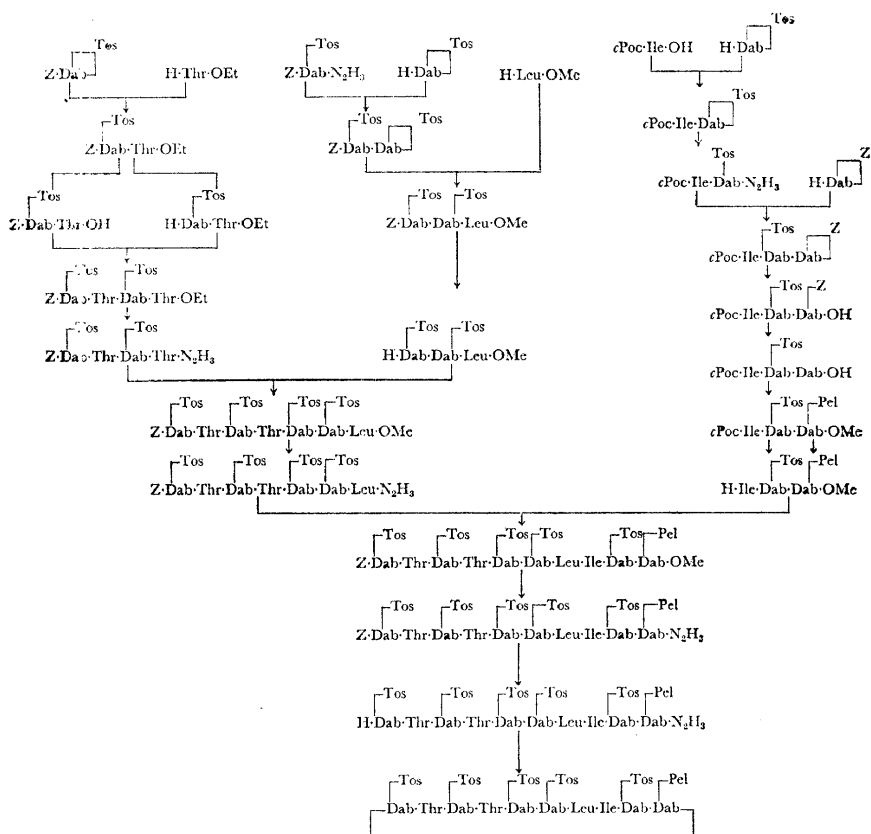


Figure 32. Synthesis of a protected cyclodecapeptide related to circulin A; general scheme

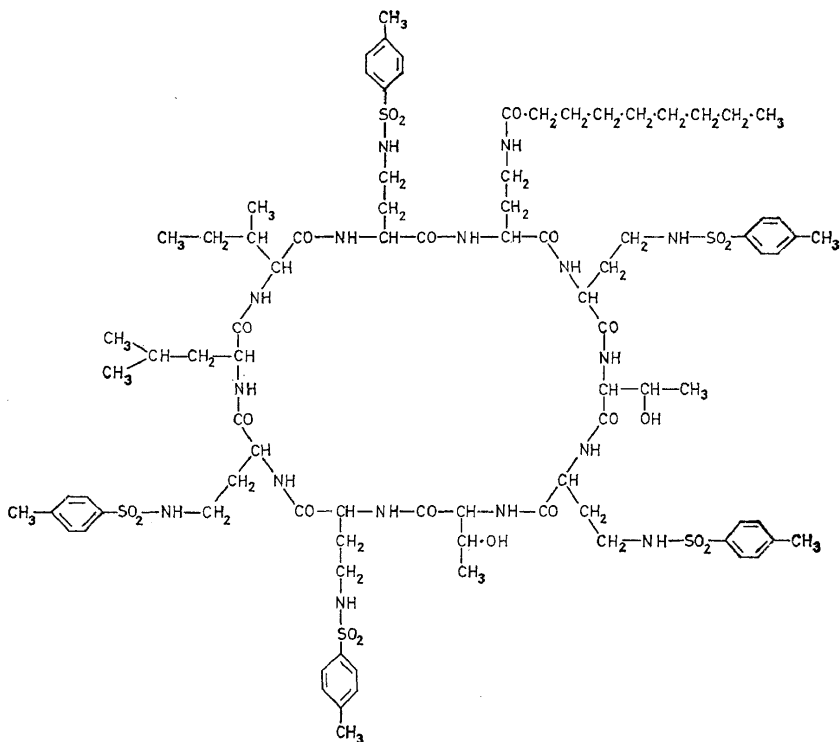


Figure 33. Protected cyclodecapeptide related to circulin A

to demonstrate, in this instance, that such syntheses are based, after all, on sound, generally comprehensible, and often interesting organic chemistry.

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