CARBOXAMIDE PROTECTION IN PEPTIDE SYNTHESIS

BY
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A Thesis submitted in partial fulfilment for the Degree of Master of Science in the University of Nairobi.

1988

This Thesis is my original work and has not been presented for a degree in any other University.

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This Thesis has been submitted for examination with our approval as University Supervisors.

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DEDICATION

To my father Mr. Z.N. Nyarango, mother, brothers and sisters.

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TABLE OF CONTENTS

PAGE

LIST OF TABLES

LIST OF ILLUSTRATIONS

ABSTRACT

In the first part the use of 4-methoxy-2--methylbenzyl and 4-methoxy-1-naphthalenemethyl as protecting groups for the amide side chain of glutamine and asparagine during peptide synthesis is demonstrated. The second part involves the hydration of some cyano containing model compounds (cyano group is an undesired functional group formed by the dehydration of the unprotected carboxamide group when N,N-dicyclohexylcarbodiimide is used as the coupling reagent in the course of peptide synthesis) under mild conditions to the corresponding carboxamide containing products.

4-Methoxy-2-methylbenzonitrile and 4-methoxy-1-naphthonitrile were reduced to 4-methoxy-2-methylbenzylamine and 4-methoxy-1-naphthalenemethylamine respectively using lithium aluminium hydride in ether. These groups were then used to prepare the carboxamide protected derivatives viz. Carbobenzoxy-N^{CA}-4-methoxy--2-methylbenzylleucinamide; carbobenzoxyleucyl-N^{CA}-4--methoxy-2-methylbenzylglycinamide; tert-butyloxycarbonylphenylalanyl-α-benzyl-N^{CA}-4-methoxy-2-methylbenzylasparaginate; tert-butyloxycarbonylglycyl--phenylalanyl-α-benzyl-N^{CA}-4-methoxy-2-methylbenzyl--asparaginate; carbobenzoxy-N^{CA}-4-methoxy-1-naphthal--enemethylleucinamide; carbobenzoxy-N^{CA}-4-methoxy-1-

-naphthalenemethylglycinamide; tert-butyloxycarbonyl-phenylalanyl-α-benzyl-N^{CA}-4-methoxy-1-naphthalene-methylasparaginate; and tert-butyloxycarbonylglycyl-phenylalanyl-α-benzyl-N^{CA}-4-methoxy-1-naphthalene-methylasparaginate. Cleavage of all protecting groups (i.e. Carboxamide, Carboxyl, and Amino) is carried out using boron trifluoride with acetic acid complex (BTFA) to obtain the fully deprotected products i.e. leucinamide; leucylglycinamide; phenylalanylasparagine and glycylphenylalanylasparagine.

In the second part, model studies on the hydration of nitrile to the amide using (i) hydrogen peroxide in a base (Radziszenwskis reaction) and (ii) 33% hydrogen bromide in glacial acetic acid were carried out. The nitrile model compounds used for the study include, benzonitrile; 4-methoxy-1--naphthonitrile; carbobenzoxy-β-cyanoalanine; carbobenzoxy-γ-cyano-α-aminobutyric acid; carbobenzoxy-β-cyanoalanylglycine methyl ester and carbobenzoxy-γ-cyano-α-aminobutylglycine methyl ester. Carbobenzoxyasparagine and carbobenzoxyglutamine were dehydrated to their corresponding cyano derivatives using N,N'-dicyclohexylcarbodiimide in pyridine at a temperature of 16°C. The synthesis of the cyano dipeptides was carried out using N-hydroxysuccinimide coupling procedure.

CHAPTER 1

INTRODUCTION

Amino acids can generally be classified as organic salts that contain amino (basic) and carboxyl (acidic) groups. They are non-volatile crystalline solids with unusually high melting points and low acidity and basicity constants. They are insoluble in non-polar solvents and are known to conduct electricity either in aqueous solution or in the molten state.

The amino acid (α-amino acids) whose amino and carboxyl groups are attached to the same carbon atom are the main constituents of the natural occurring peptides and proteins. Some of the common amino acids are listed in Table 1[1].

TABLE 1

SOME OF THE COMMON AMINO ACIDS

NAME	ABBREVIATION	STRUCTURE
Glycine	Gly	NH_2-CH_2-COOH
Alanine	Ala	$NH_2-\underset{\displaystyle CH_3}{\underset{\vert}{CH}}-COOH$
Aspartic acid	Asp	$NH_2-\underset{\displaystyle CH_2}{\underset{\vert}{\underset{\displaystyle COOH}{\underset{\vert}{CH}}}}-COOH$

Table 1 continued

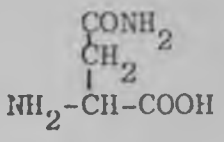
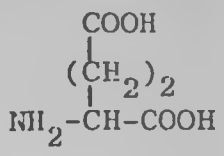
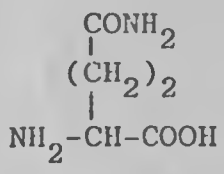
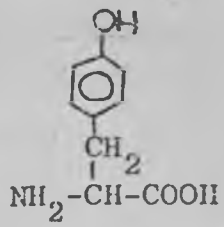
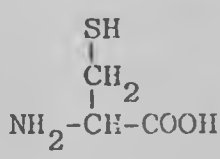
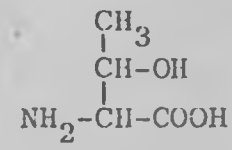
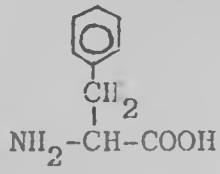
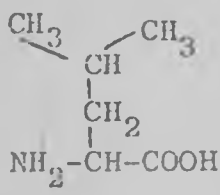
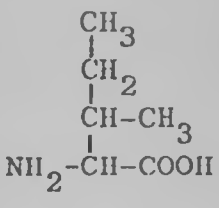
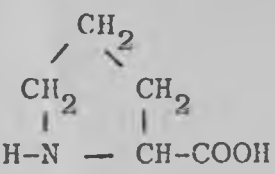
NAME	ABBREVIATION	STRUCTURE
Asparagine	Asn	
Glutamic acid	Glu	
Glutamine	Gln	
Tyrosine	Tyr	
Cysteine	Cys	
Threonine	Thr	
Phenylalanine	Phe	
Leucine	Leu	

Table 1 continued

NAME	ABBREVIATION	STRUCTURE
Isoleucine	Ile	
Proline	Pro	

Peptides are formed as a result of the reaction between amino and carboxyl groups of α-amino acids. The resultant amide bond (-NH-CO-) are referred to as peptide bonds. Depending on the number of amino acids involved in the peptide bond formation, a dipeptide (one peptide bond), a tripeptide (two peptide bonds) etc. can be obtained.[2] The molecules of peptides and of proteins consist of chains formed by amino acids linked to one another by peptide bonds.

Since amino acids contain at least one amino and one carboxyl group, they can take part in acylation reactions either as

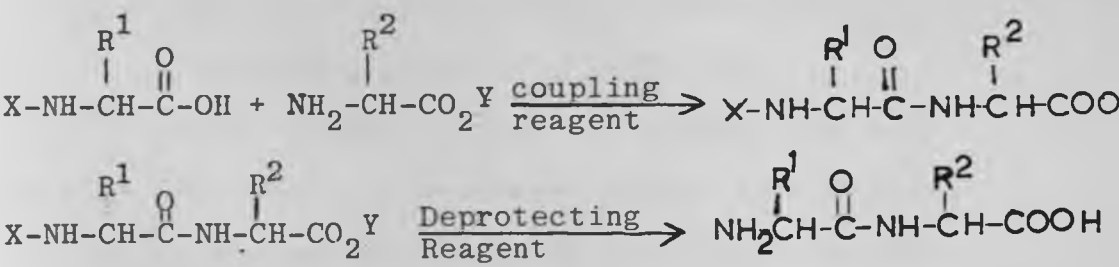
acylating reactants (carboxyl component) or

compounds to be acylated (amino component).

It is, therefore, important to allow only one of these two groups to be involved in peptide bond formation while the other is fully protected (see Scheme 1).

SCHEME 1

PEPTIDE BOND FORMATION



X- amino protecting group.

Y- Carboxyl protecting group.

R^1 and R^2 - non-reactive side chain .

Thus preventive precautions are taken to avoid the formation of a large number of undesired side compounds. The protecting groups must be of such a

nature as to allow their easy introduction to the particular amino acid and to be readily and selectively removed without destroying the labile peptide bond or destroying the optical activity of the peptide. Other reactive side-chains in various amino acids must also be protected in the course of peptide synthesis. These reactive side-chains include sulfhydryl; guanidino; indole ring in tryptophan; hydroxyl[3] and carboxamide groups.

1;1 Amino protecting groups.

1:1:1 Benzyloxycarbonyl ($C_6H_5CH_2-O-\overset{O}{\overset{\|}{C}}-$) and substituted benzyloxycarbonyls.

These groups are easy to prepare and are easily introduced in the amino acid. They protect an amino acid against unwanted acylation reactions and against racemization[4].

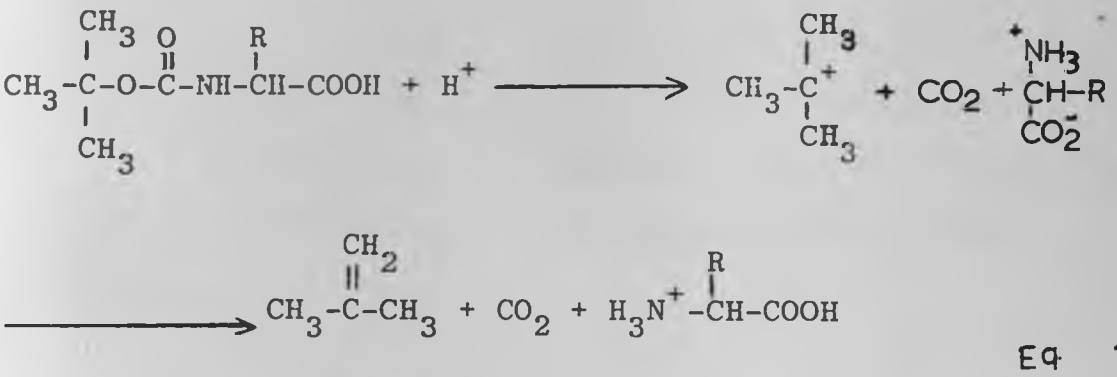
The benzyloxycarbonyl group is stable towards alkaline hydrolysis and normally it is removed from non-sulfur containing compounds by catalytic hydrogenation[5]. However hydrogenolysis fails with sulfur containing amino acids and peptides as sulfur poisons the catalyst. For sulfur containing compounds either hydrogen bromide in glacial acetic acid (33% HBr/CH_3COOH)[6] solution or trifluoroacetic acid/hydrogen bromide solution can be used. Other

removal methods includes reduction with sodium in liquid ammonia, aqueous hydrochloric acid[7]; hydrogen chloride in ethanol[8] or in chloroform[9(a),9(b)]; phosphonium iodide[10,11] in acetic acid. However by far hydrogenation and solvolysis using HBr/ACOH are the most widely used procedures. Examples of substituted groups used include 2,4-dichlorobenzyloxycarbonyl[12]; 2-bromobenzyloxycarbonyl[13]; p-nitrobenzyloxycarbonyl[14(a), 14(b)]; p-methoxybenzyloxycarbonyl[15(a),15(b)] and 3,5-dimethoxybenzyloxycarbonyl[16] groups.

1:1:2 tert-BUTYLOXYCARBONYL GROUP

This group is easily prepared and its amino acid derivatives can be conveniently prepared using tert-butyloxycarbonylazide.[17] It is stable towards catalytic hydrogenation, sodium in liquid ammonia, alkali and hydrazine.[18] However it's rapidly cleaved by mild acidic conditions i.e. HCl in benzene; HBr in acetic acid; trifluoroacetic acid (TFA) in different solvents i.e. CH_2Cl_2 and $CHCl_3$; BF_3 etherate in acetic acid[19] and liquid hydrogen fluoride.[20]

The cleavage reaction proceeds via the formation of t-butyl cation which are converted to isobutene by the elimination of a proton (equation 1).



Eq 1

The presence of tert-butyl cation intermediate may be a source of serious side reactions as it can undergo a substitution reaction at the indole of tryptophan[21] or the formation of sulfonium salts from the thio ether of methionine[22]. So as to suppress this reaction small quantity of carbonium ion scavangers such as anisole can be added.

Other aliphatic urethane groups with desirable properties i.e. different sensitivity to acids and ease of preparation have also been used. These groups include adamantyloxycarbonyl[23] and amyloxycarbonyl groups[24].

1:1:3 TRIPHENYLMETHYL GROUPS

Under normal circumstances monoalkylated amino groups can be acylated but the bulky trityl group

introduces enough steric hindrance so that the amino group is completely protected.[25] The steric hindrance offered by this group is so great that some coupling reactions do not succeed with certain of the trityl amino acids.

1:1:4 p-TOLUENESULFONYL GROUP

In aqueous media under comparatively mild alkaline conditions which are common in the coupling steps; many of the α-tosylamino acid chlorides undergo a characteristic decomposition yielding tosylamide, carbon dioxide and aldehydes. It is normally cleaved by sodium in liquid ammonia.[26]

1:2 CARBOXYL PROTECTING GROUPS

1:2:1 METHYL & ETHYL ESTERS

Esterification of the amino acids is normally achieved by the bubbling of HCl gas into a suspension of amino acid in absolute MeOH or EtOH.[27] However the most convenient method is the use of thionyl chloride[28]. The thionyl chloride is dropped into cold methanol or ethanol followed by the addition of the amino acid. To complete the reaction, the solution can either be left standing or maybe refluxed for a few minutes. So as to ease the esterification procedure, several

catalysts have been suggested i.e. p-toluene sulfonic acid[29] and ion-exchange resin[30]. A more recent procedure involves the azeotropic distillation of water[31].

These groups can be removed by alkaline hydrolysis in various solvents such as acetone, methanol and dioxane at room temperature or below room temperature.

However this method of removal is faced with problems. It may lead to hydrolysis of some sensitive amide bonds e.g. elimination of ammonia from the carboxamide groups of the side chains in asparagine and glutamine residues or cyclization of aspartyl peptides to aspartimide derivatives; lastly it may also lead to racemization.

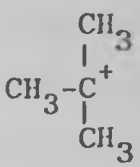
1:2:2 BENZYL & BENZHYDRYL ESTERS

The amino acid esters of these groups are most conveniently prepared from the alcohols with organic acids as catalysts and solvents which allow the azeotropic distillation of water.[32(a),32(b),32(c)] They are normally cleaved by catalytic hydrogenolysis; sodium in liquid ammonia; alkali hydrolysis and prolonged treatment with HBr/CH_3COOH[33]. Other substituted benzyl esters that have been used include p-nitrobenzyl[34] and p-methoxybenzyl[35] groups.

1:2:3 tert-BUTYL ESTER

This groups is generally prepared by acid catalyzed addition of isobutene to N-protected amino acids.[36] The catalyst frequently used include H_2SO_4 and p-toluene sulfonic acid.

The tert-butyl esters may be removed by treatment with HCl in organic solvents, aqueous hydrochloric acid,[37] trifluoroacetic acid and HBr/ACOH.[38] The ease of acid cleavage of this group can be attributed to the formation of the carbonium ion.



Other groups which have been used for the same purpose include phenacyl, phenyl and 4-picolyl esters.

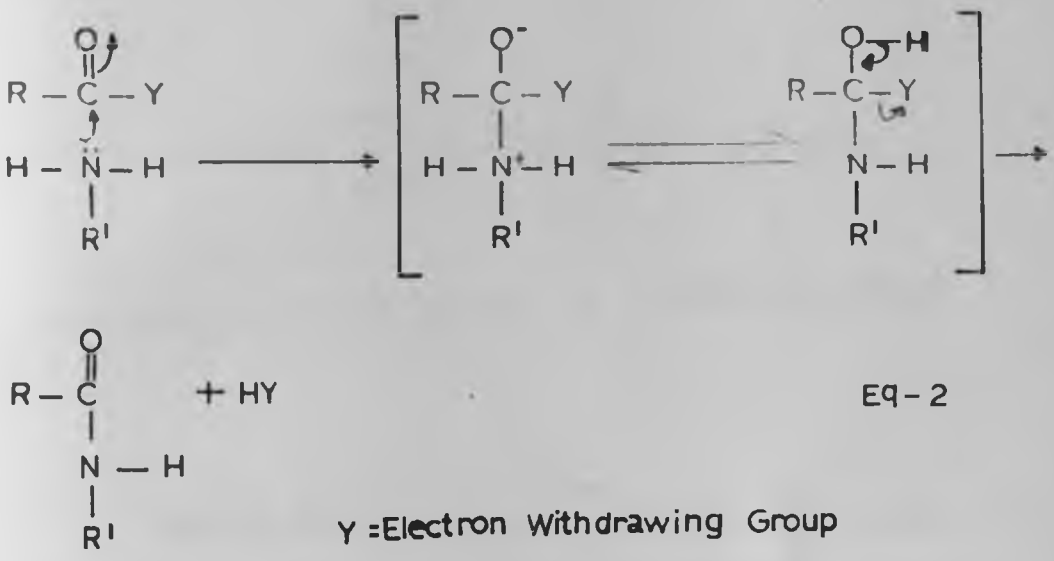
1:3 SULFURHYDRYL PROTECTING GROUPS

Protection of the thiol function of cysteine during peptide synthesis is considered to be essential because of the high nucleophilicity of the bivalent sulfur, which renders it a strong competitor with the amino group in acylation reaction and secondly is the ease of oxidation of sulfhydryl to disulfides.

The most popular protecting group is the S-benzyl group.[39] It is normally removed by sodium in liquid ammonia although side reactions have been observed for example fission of peptide bonds and desulfuration may take place. The removal by liquid HF requires longer times and might sometimes be incomplete.

1:4 COUPLING METHODS

During the formation of amide bonds, the principal reaction in the building of such chains is the acylation of the amino group of an amino acid by the carboxyl group of a second amino acid. So as to enhance the reaction, a reactive derivative of the carboxyl component should be present. This component should have the characteristic property of increasing the reactivity of the carboxyl carbon atom by increasing its electrophilicity. Therefore the already low concentration of electrons on this group is further decreased in the activated derivatives by the negative inductive effect of the activating substituent (equation 2).



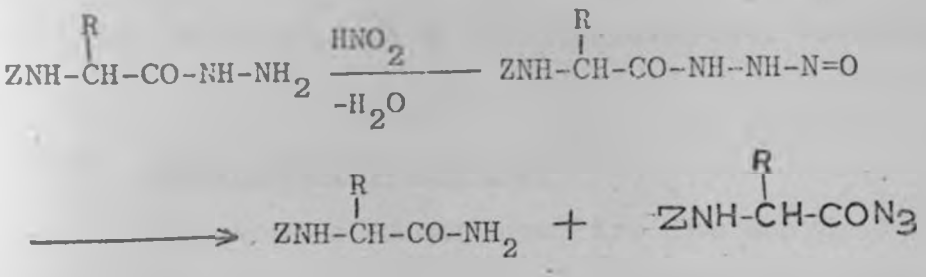
Eq-2

From the above example (equation 2) the electrophilic center easily allows the attack of the nucleophilic amino group with the formation of an intermediate product. Some of the earliest electron withdrawing groups used are the azides and chlorides.

1:4:1 AZIDE GROUP

They are synthesized by the hydrozinolysis of the acylamino acids to obtain the corresponding hydrazides, which are then converted to the azide with nitrous acid.[40]

This method yields peptides of high optical purity but side reaction are possible at the formation of azide step (equation 3).



Eq 3

High yields can be obtained when the azides are prepared using an organic nitrite at low temperature ($-25^{o}C$) in a homogenous and highly acidic solution.

1:4:2 ACID CHLORIDE METHOD

In this method the acylamino acids are treated with phosphorus penta or trichloride or thionyl chloride.[41] Elevated temperatures are normally required for this reaction but this may lead to serious side reactions.

Eq 4

On heating the already formed acid chloride decomposes to alkyl chloride and N-carboxyanhydrides (equation 4).

1:4:3 ACID ANHYDRIDE METHOD

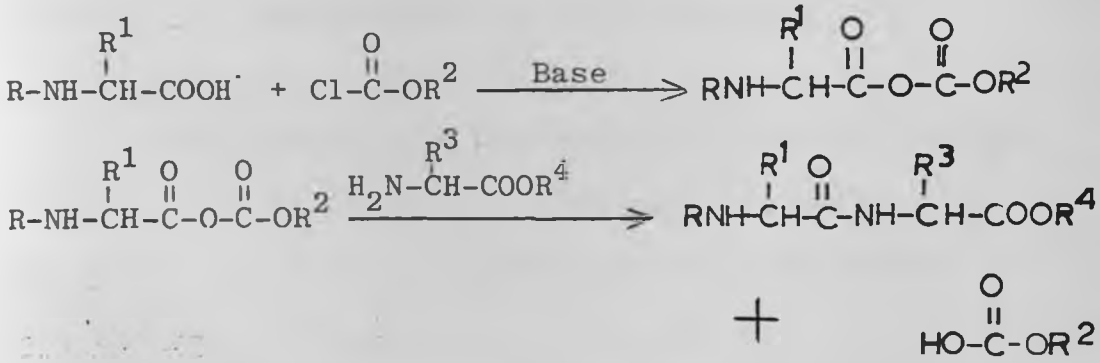
The most commonly used are the anhydrides of monoesters of carbonic acid and organic acids.[42]

1:4:3:1 a. Mixed anhydrides with monoesters of carbonic acid

The anhydrides are formed by the reaction of N-protected amino acids with various esters of chloroformic acid.(Scheme 2).[43]

SCHEME 2

USE OF MIXED ANHYDRIDES IN PEPTIDE SYNTHESIS

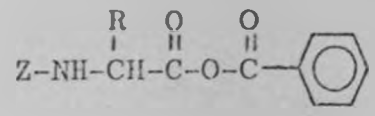


The condensation of the acyl amino acid and chloroformate requires only a few minutes at -5 to -10°C[44].

The main disadvantage of this method is that it is always accompanied by racemization.

1:4:3:2 b. Mixed anhydrides with organic acids[45,46]

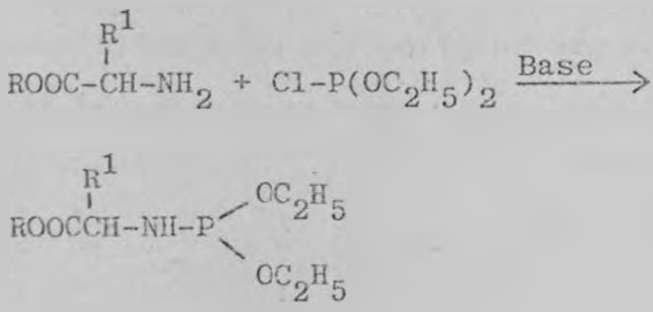
This method is besieged with low yield of desired product.



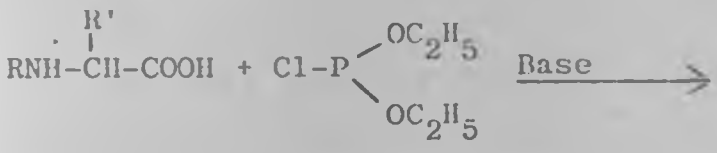
This can be attributed to the electron-withdrawing effect of the phenyl group which renders the carbonyl carbon of the benzoyl residue sufficiently electrophilic to compete in the acylation of an amino with the protected aminoacyl part of the molecule. Hence to obtain satisfactory results, an electron releasing group should be used.

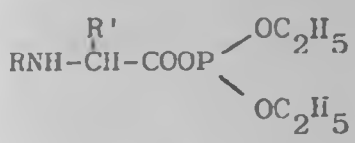
1:4:3:3 c. Mixed anhydrides with esters of phosphorus acid[47]

The ester chlorophosphites and pyrophosphites are used to form reactive amides with the amino components (equation 5) as well as reactive anhydrides with the carboxyl components (equation 6).

1. 

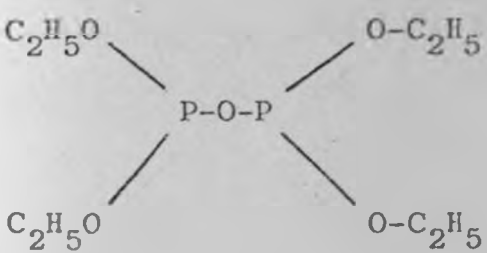
Eq 5

2. 



Eq 6

However the most widely used of the phosphites has been the tetraethyl pyrophosphite.



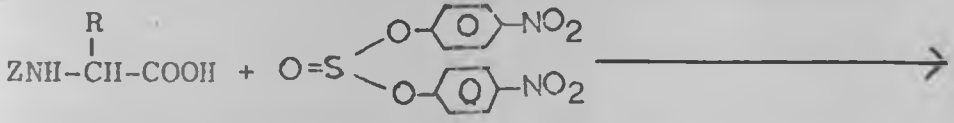
It has been used in the preparation of naturally occurring polypeptides.

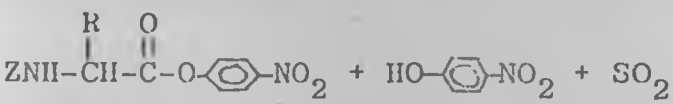
1:4:4 ACTIVATED ESTERS

Active esters are less reactive but highly selective in their mode of action. Transesterification is not observed under mild conditions. They can be crystallized from alcohols and are more resistant to hydrolysis as compared to the anhydrides.[48,49]

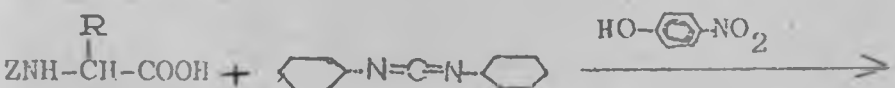
1:4:4:1 ARYL ESTERS

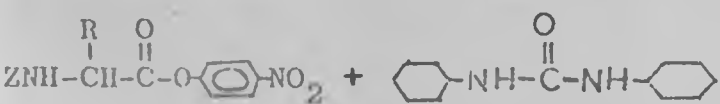
The most feasible method of preparation include exchange reactions between acylamino acids and diarylsulfites (equation 7a).[50] N,N'-Dicyclohexylcarbodiimide catalyst esterification with p-nitrophenyl is also possible (equation 7b).

(i) 



Eq 7a

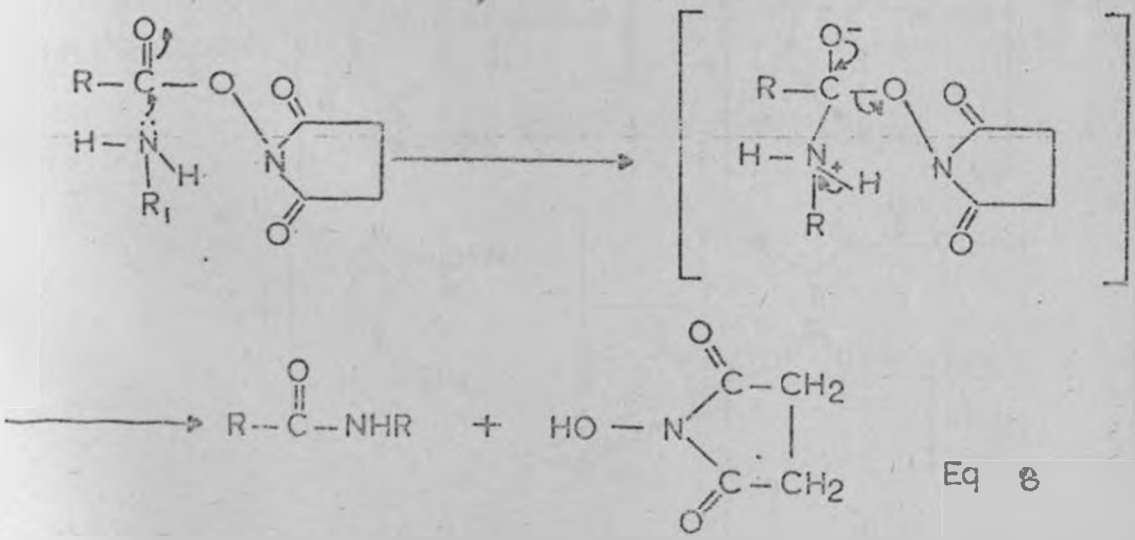
(ii) 



Eq 7b

1:4:4:2 N-HYDROXYSUCCINIMIDE ESTERS

The N-protected amino acid derivatives of this groups owe their reactivity towards nucleophiles to electron withdrawing effect (equation 8). The derivative of these groups are conveniently synthesized via the carbodiimide method and have proved the most useful.[51] They are stable, crystalline compounds. Reaction with amino and peptide esters is usually complete within 1hr. N-hydroxysuccinimide is water soluble and thus easily removed from the products. Other groups that have been successfully used include, 1-hydroxypiperidine esters[52] and derivatives of N-ethyl benzoxazolium salts.[53]



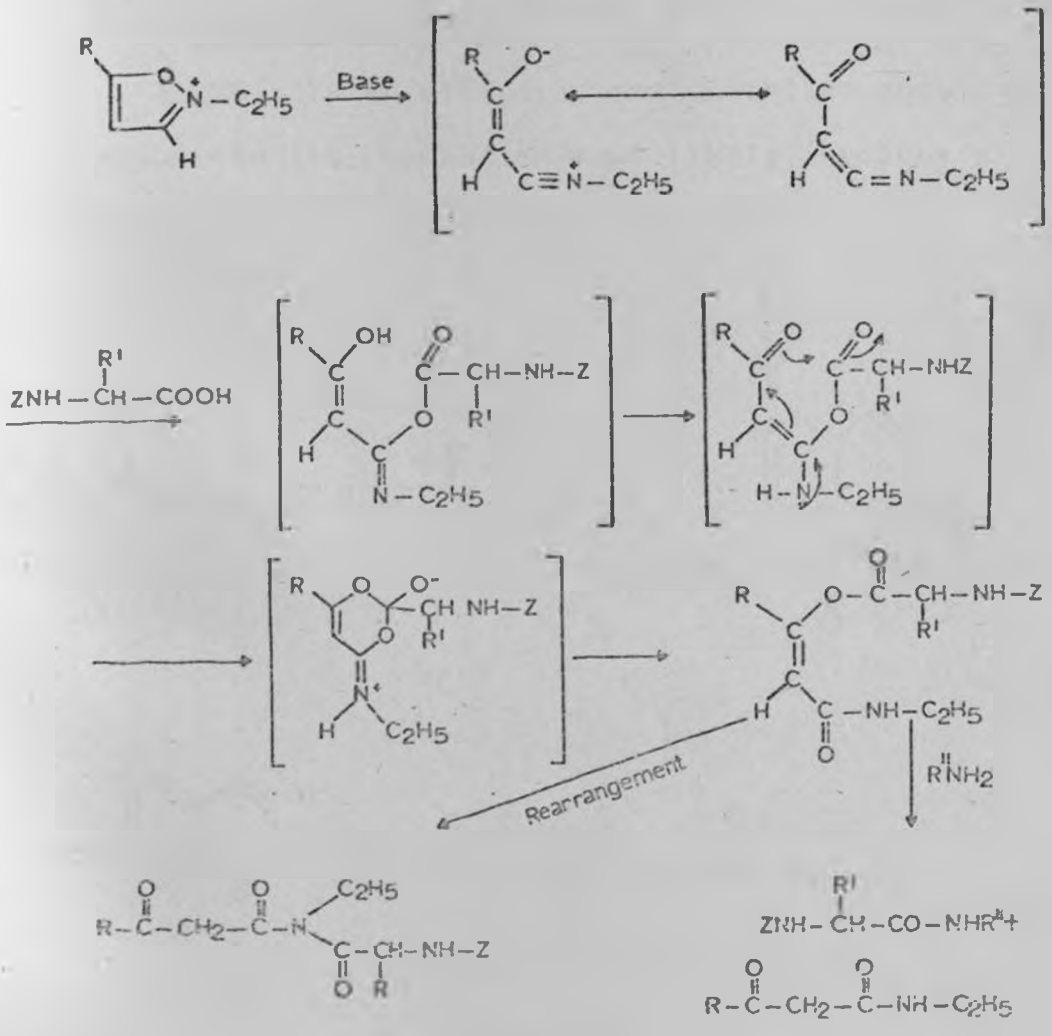
Eq 8

1:4:5 1. ISOXAZOLIUM DERIVATIVES

This compound is commercially available as N-ethyl-5-phenylisoxazolium-3-sulfonate. The mechanism of the reaction involves first ring-opening by proton abstration via a concerted reaction to form a ketoketene imine. The N-protected amino acid is then added (Scheme 3).[54,55]

SCHEME 3

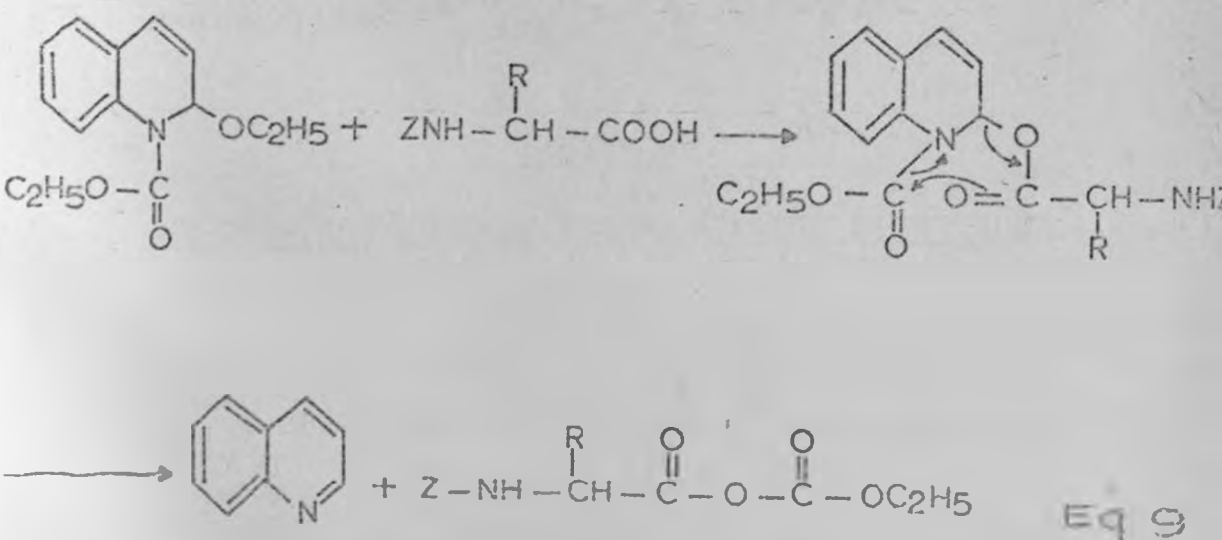
USE OF N-ETHYL-5-PHENYLISOXAZOLIUM-3-SULPHONATE IN PEPTIDE SYNTHESIS



This reaction gives high yield and most important, the carboxyl groups of serine, threonine and tyrosine can be activated without protection of the side chain hydroxyls. Its main disadvantages are the rearrangement of the enol ester to an imide and the limited choice of solvents. The most commonly used solvents are acetonitrile or nitromethane. There appears to be appreciable racemization in the activation of acyl peptides.

1:4:6 . 1-ETHOXYCARBONYL-2-ETHOXY-1,2-DIHYDRO-QUINOLINE (EEDQ)

This is an efficient and selective coupling reagent and its mechanism most likely involves a mixed anhydride intermediate (equation 9).



Eq 9

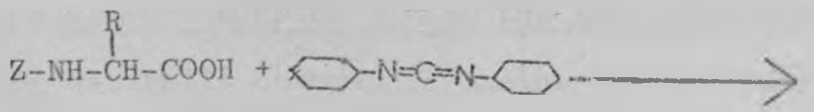
1:4:7 CARBODIIMIDES

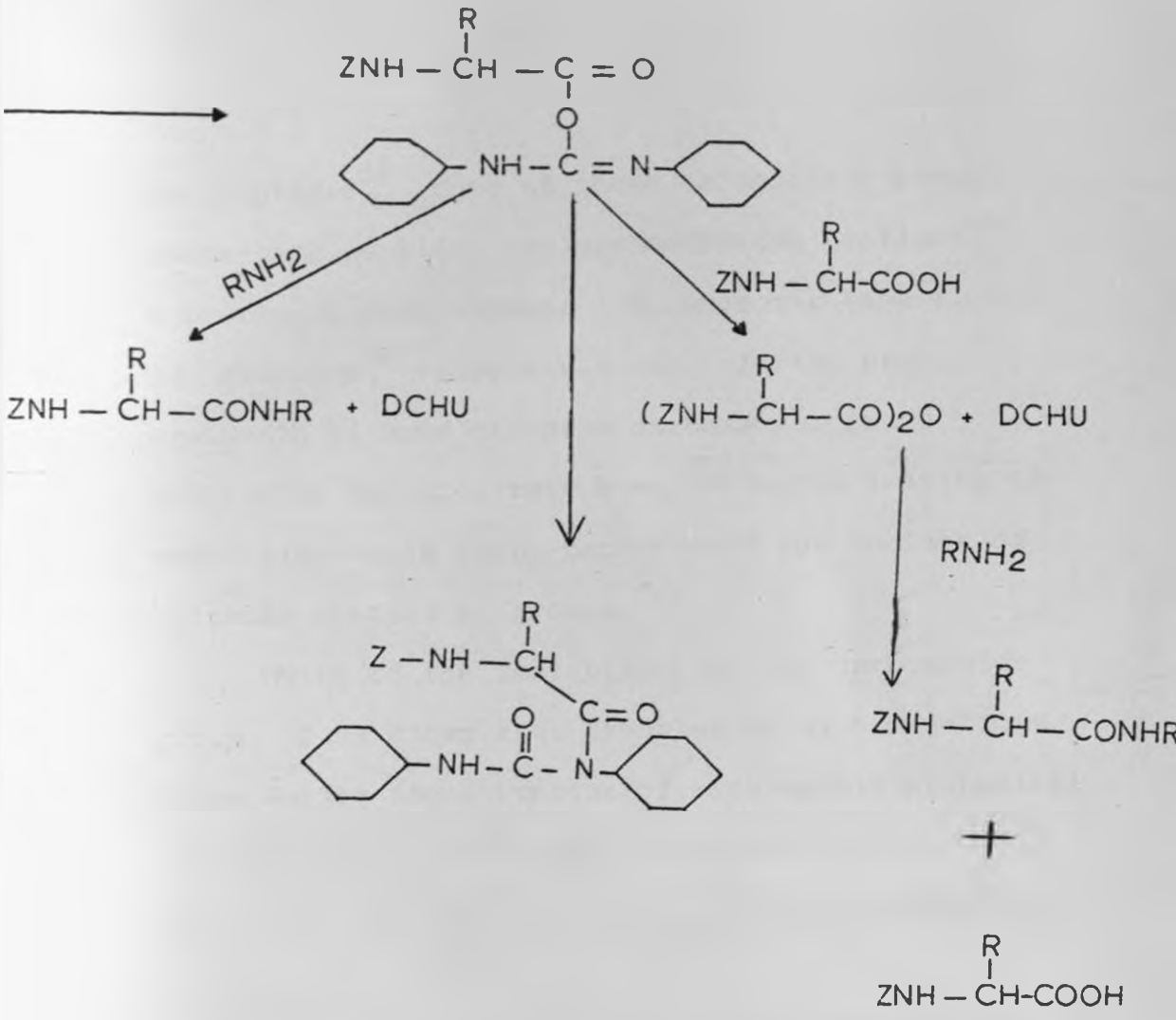
Dicyclohexylcarbodiimide (DCC) is the most popular reagent used. The mechanism involves the addition of the N-protected amino acid to the reagent to form a reactive intermediate an O-acylisourea. From here several reaction pathways are possible.
a. direct attack of the amino component on the reactive intermediate with the formation of the peptide bond, b. attack by the carboxyl component resulting in the formation of a symmetrical anhydride, which in turn acylates the amine, c. O→N acyl migration with the formation of an N-acylurea.

The formation of the urea by the last reaction is one of the limitation imposed on the use of this group as it is difficult to separate from the desired peptide. This reaction can be suppressed by the use of solvents like dichloromethane and acetonitrile.[56,57]

SCHEME 4

USE OF N,N'-DICYCLOHEXYLCARBODIIMIDE IN PEPTIDE SYNTHESIS





In order to reduce racemization encountered in the activation of acyl peptides with DCC and to reduce the amount of N-acylureas, N-hydroxy compounds such as N-hydroxysuccinimide can be incorporated.

1:5 CARBOXAMIDE GROUP OF ASPARAGINE AND GLUTAMINE

Many naturally occuring proteins and pentides have to date been isolated and found to contain asparagine and/or glutamine residues and others to contain carboxamide group at the carboxyl end of

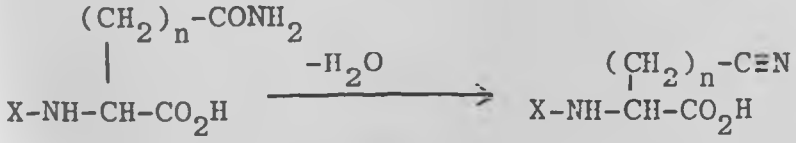
the peptides.[58] Some of these carboxamide group containing peptides include membranes peptides,[59] neurohypophysial hormone i.e. oxytocin (see figure 1), glucogen[60] vasopressin etc. In the past, synthesis of some of these carboxamide group containing peptides have been attempted leaving the amide side-chain group unprotected due to lack of suitable protecting groups.

Owing to the instability of the carboxamide group, it is known that troublesome side reactions occur during the formation of asparaginyl/glutaminyl peptide bonds. These reactions are most prevalent especially when DCC coupling group is incorporated.

Some of the side reaction initiated by DCC in the course of the synthesis of these peptides include (a) dehydration to the corresponding cyano derivatives; (b) transpeptidation, (c) deamination to carboxylic acid (d) and the formation of pyroglutamyl derivatives from glutaminyl peptides.

SCHEME 5

1:5:1 a. Dehydration to the corresponding[61] cyano derivatives



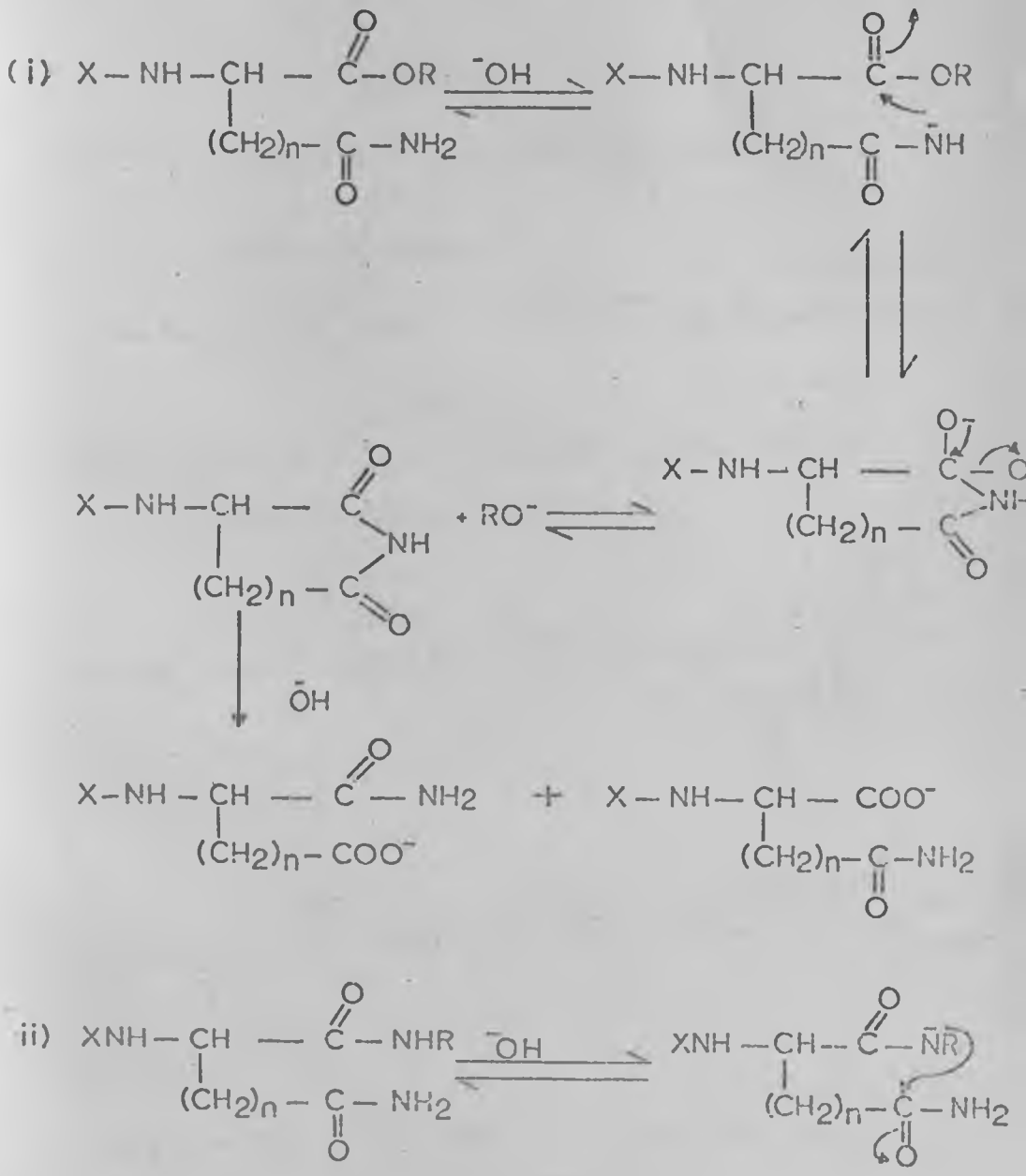
X = amino protecting group

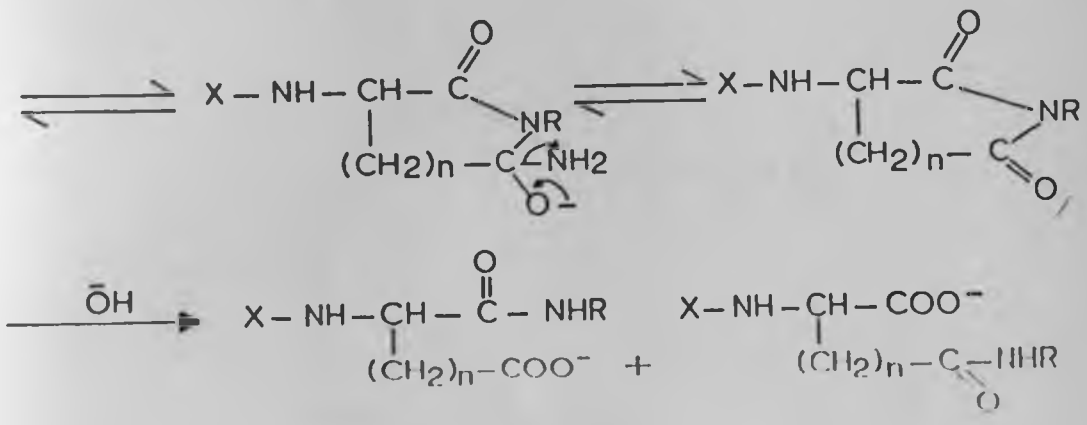
n = 1-asparagine

n = 2-glutamine

SCHEME 6

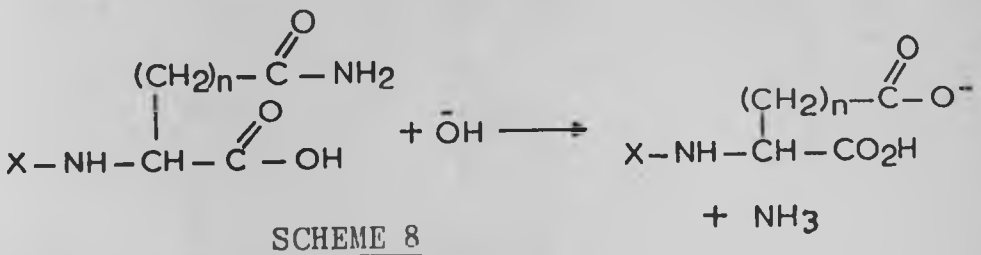
1:5:2 b. Transpeptidation[62]





SCHEME 7

1:5:3 c. Deamination to Carboxylic acid[63]



SCHEME 8

1:5:4 d. Formation of pyroglutamyl derivatives from glutaminyl peptides.

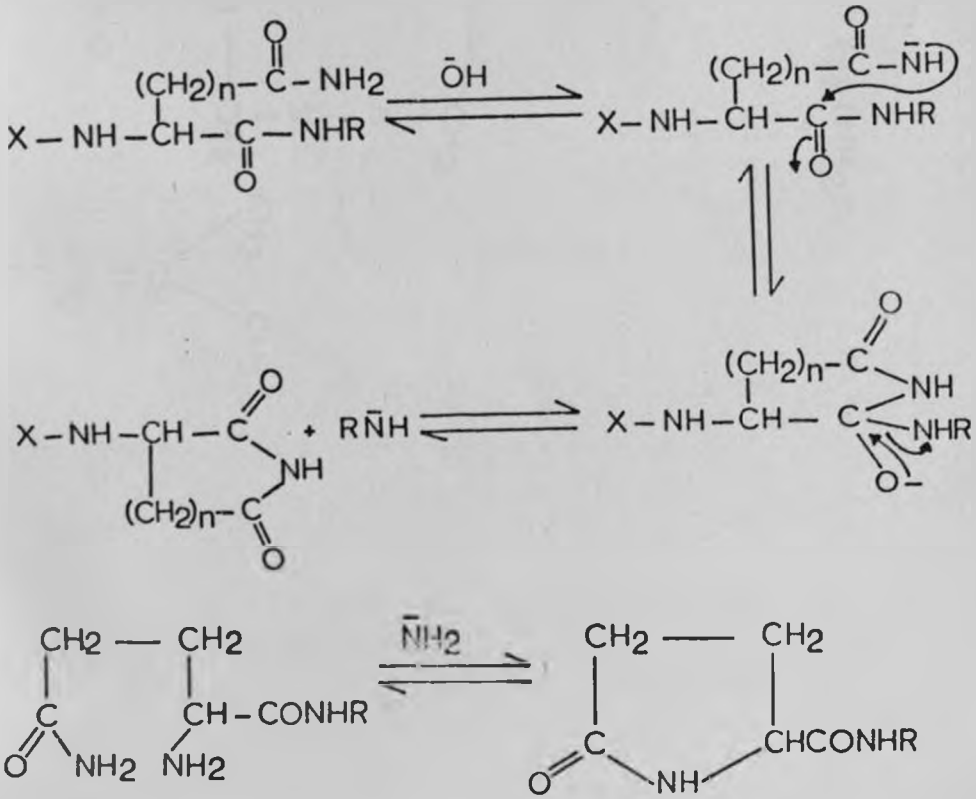
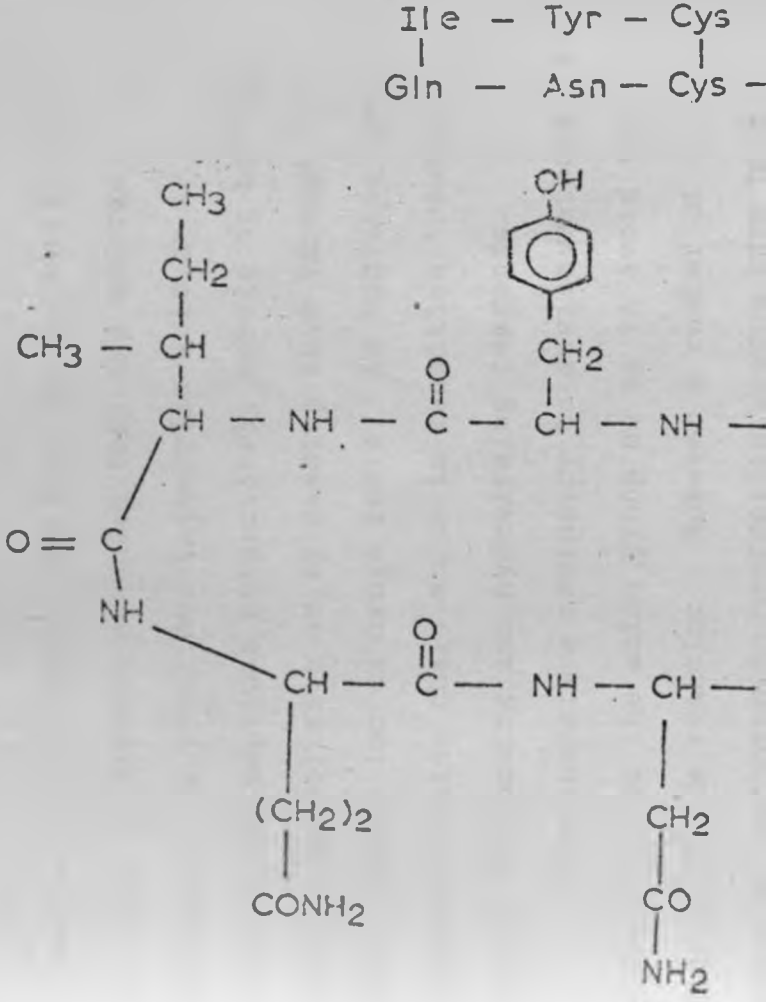
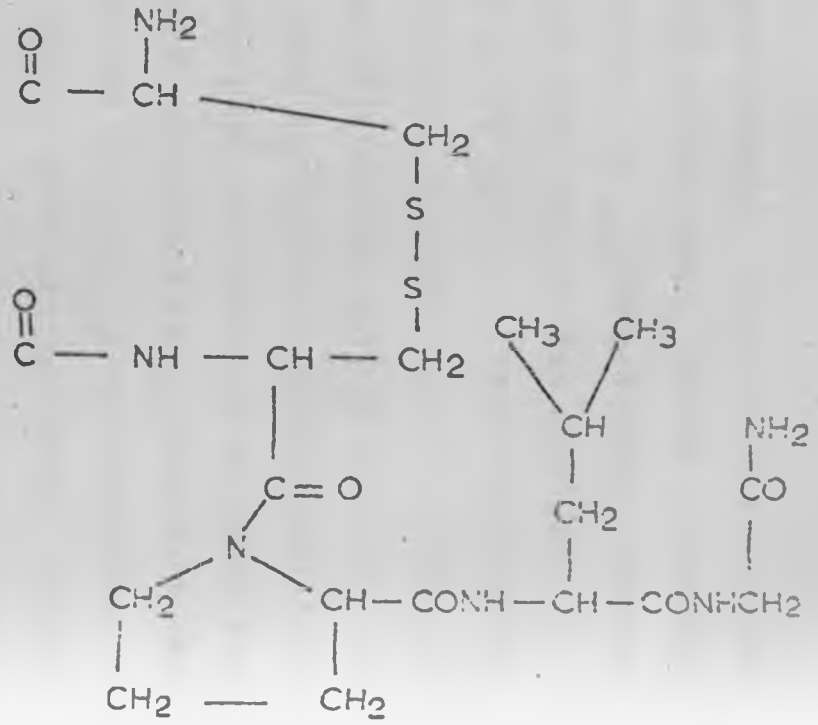


Figure 1.


Ile — Tyr — Cys
Gln — Asn — Cys —
CH3
CH2
CH3 — CH
CH — NH — C — CH — NH —
O
CH2
OH
O = C
NH
CH — C — NH — CH —
(CH2)2
CONH2
CH2
CO
NH2

STRUCTURE OF OXYTOCIN

- Pro — Leu — Gly(NH_2)



Glutamine or glutaminyl peptides are so labile that even in neutral solution they can undergo cyclization to pyrolidonecarboxylic acid or to pyroglutamyl peptides respectively merely by heating the aqueous solution or by passing them through a strong acidic ion exchange resin. In addition the unprotected side chain amide is sensitive towards various deblocking and hydrolysing reagents.

It's therefore necessary to devise methods of protection for the amide group so as to avoid the aforesaid side reaction. However a number of potential carboxamide protecting groups have been studied. These include bis(2,4-dimethoxybenzyl)[64], 2,4-dimethoxybenzyl[65], 2,4,6-trimethoxybenzyl,[66] p-methoxybenzyl[67] and benzhydryl groups. They have been found to be either too labile under acidic conditions generally associated with deprotection in the course of peptide synthesis or too strongly held that they are not completely removed with stronger deprotecting reagents at the end of peptide synthesis.

Cleavage studies have been carried out on 4-methoxy-2-methylbenzyl[68] and 4-methoxy-1-naphtha--lenemethyl groups[69] when coupled to N-protected amino acid derivatives. These groups have been

found to be stable in CF_3CO_2H/CH_2Cl_2(TFA) solution, a reagent normally used to remove tert-butyloxy-carbonyl a group that is commonly used to protect the amino group. They are readily cleaved using either HF or BTFA solution at the end of peptide synthesis.

The aim of this research project is to utilize these carboxamide protecting groups in peptide synthesis. First investigation involves the use of 4-methoxy-2-methylbenzyl and 4-methoxy-1-naphthalenemethyl groups as carboxamide protecting groups for peptide synthesis. This involves the synthesis of carboxamide protected amino acids, di and tripeptides using 4-methoxy-2-methylbenzyl and 4-methoxy-1-naphthalenemethyl groups and at the end of peptide synthesis they are fully deprotected using boron trifluoride acetic acid complex. In addition to removing carboxamide protecting groups this reagent also removes carboxyl and amino protecting groups to regenerate the fully deprotected amino acid, di and tripeptides.

The second investigation is to demonstrate how cyano group (an undesired functional group formed by dehydration of the unprotected carboxamide group in the course of peptide synthesis) can mildly be converted to carboxamide group. Several model

compounds containing cyano group were converted to tne amide group by Radziszewskis reaction (hydrogen peroxide in a base) and 33% Hydrobromic acid in acetic acid respectively.

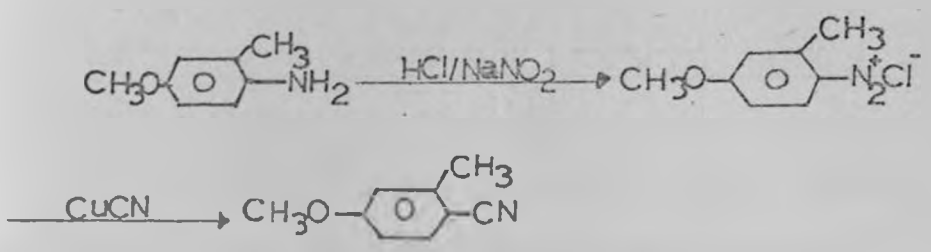
The success of this synthesis could mean that carboxamide containing peptides could be synthesized using either 4-methoxy-2-methylbenzyl or 4-methoxy-1--naphthalenemethyl group as carboxamide protecting groups and remove them at the end of peptide synthesis using boron trifluoride in acetic acid. This will enable chemists to prepare pure carboxamide group containing peptides in very high yields; also the conversion of the cyano group under mild conditions to the corresponding carboxamide group using both H_2O_2 and HBr/ACOH will enable peptide chemists to synthesize carboxamide containing peptides using the cyano as a carboxamide protecting group. Moreover, peptide chemists will stop worrying about a cyano group as in the end of peptide synthesis it could be converted back to the desired carboxamide group containing peptide.

CHAPTER 2

RESULTS AND DISCUSSION

2:1 SYNTHESIS OF AMINES

In order to obtain the corresponding nitrile, 4-methoxy-2-methylaniline is converted to the diazonium salt and then reacted with cuprous cyanide to obtain 4-methoxy-2-methylbenzonitrile (60% yield) as shown in equation 10.



Eq-

The amines used for the synthesis of the carboxamide protected amino acid derivatives were synthesized by the reduction of their corresponding nitriles using lithium aluminium hydride ($LiAlH_4$) in dry diethyl ether (Et_2O). $LiAlH_4$ being both convenient and powerful reducing agent, has been used extensively for the selective reduction of the nitriles (or any polar functional group) to their corresponding amines by several researchers with excellent yields.[70,71,72]

Using $LiAlH_4$ in dry Et_2O, 4-methoxy-2-methylbenzonitrile and 4-methoxy-1-naphthonitrile were reduced to 4-methoxy-2-methylbenzylamine and 4-methoxy-1-naphthalenemethylamine with yields of 70 and 75% respectively. The lower than expected yields may be attributed to the fact that the reaction was not carried out under inert atmosphere for example under nitrogen, and that the $CaCl_2$ used was not efficient in maintaining anhydrous conditions in the system (moisture decomposes the $LiAlH_4$).

2:2 SYNTHESIS OF CARBOXAMIDE PROTECTED AMINO ACID DERIVATIVES

Amines (RNH_2) in the presence of N,N'-dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (HONSU) or some other coupling reagents will react with N-protected amino acids to give the carboxamide protected derivatives i.e. coupling an amine (RNH_2) with α-benzyl tert-butyloxycarbonylaspartate in the presence of DCC-HONSU will give α-benzyl tert-butyloxycarbonyl-N^{CA}-R-asparaginate (where R is the carboxamide protecting group).

The selection of the R-group studied as a potential carboxamide protecting group is mainly determined by: (a) what is already known about the substituent effect on the benzyl group; (b) by the

results of other R-groups already studied; (c) the nature of the carbonium ion that is formed during hydrolysis. It is well known that electron-donating groups attached to the benzyl group at para and ortho position are expected to increase the possibility of the R-group being cleaved under acidic hydrolysis conditions. The groups that have been extensively studied have one or more methoxy and methyl groups, or both the methoxy and methyl groups substituted on the benzyl group. Both groups are electron donating.

4-Methoxy-2-methylbenzyl(4-MeO-2-meBzl) and 4-methoxy-1-naphthalenemethyl(4-MeO-1-NM) carboxamide protected amino acid derivatives are crystalline products and are easily obtained by reacting the amine with the corresponding N-protected amino acids using DCC-HONSU as the coupling reagents. The resultant percent yields are presented in Table 2.

TABLE 2

CARBOXAMIDE PROTECTED AMINO ACID DERIVATIVES

Compound	% yield
Boc-Gly-NH(4-MeO-2-MeBzl)	62.6
Z-Leu-NH(4-MeO-2-MeBzl)	50
Boc-Asn(4-MeO-2-MeBzl)-OBzl	91.7
Boc-Gly-NH(4-MeO-1-NM)	68.9
Boc-Asn(4-MeO-1-NM)-OBzl	67.6

The DCC-HONSU coupling method gave yields ranging from 50 to 91.7%.

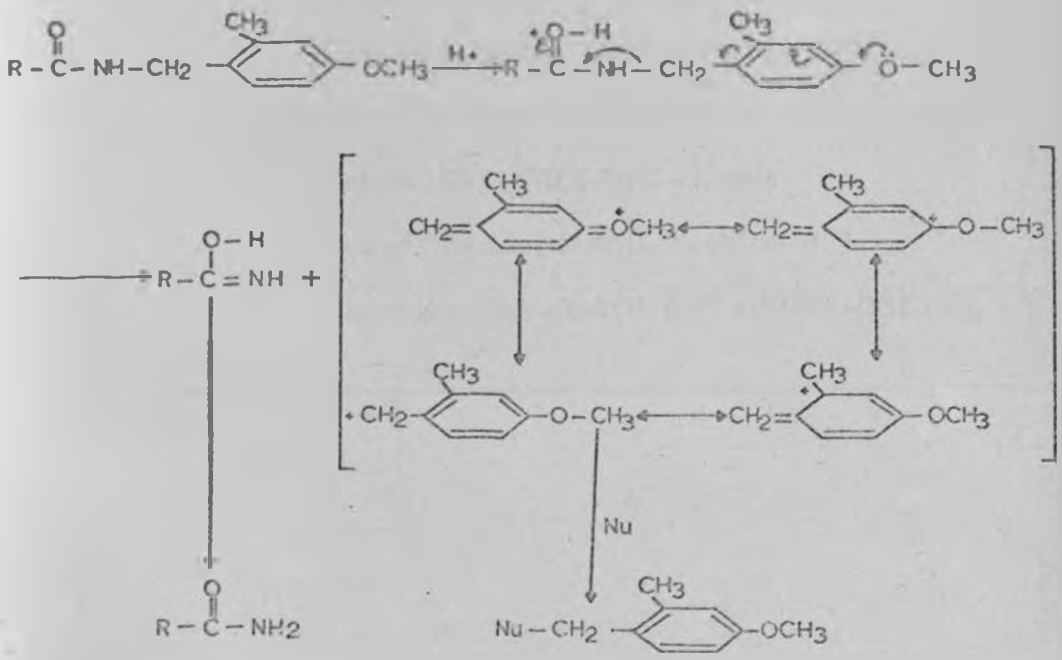
2.3 CLEAVAGE STUDIES OF THE CARBOXAMIDE PROTECTED AMINO ACID DERIVATIVES IN BTFA-TFA.

tert-Butyloxycarbonyl(Boc) and carbobenzoxy(Z) groups were used as amino protecting groups owing to the ease with which they are removed under mild acidic conditions. Boc and Z groups can be selectively removed in the presence of Bzl group using 50% TFA-CH_2Cl_2 and 33% HBr-ACOH respectively. The Bzl group is removed using catalytic hydrogenation in the presence of Boc-group.[73] However, simultaneous removal of Z and Bzl groups takes place under catalytic hydrogenation. At the end of peptide synthesis, all

the protecting groups (Boc, Z and Bzl) can be removed by HF or BTFA. In addition to removing these three groups, HF and BTFA also removes the following protecting groups; amino-protecting groups:- trityl or any other acid labile protecting groups that are used; carboxyl-protecting groups:- tert-butyl groups; carboxamide-protecting groups:- 2,4-dimethoxybenzyl; 2,4,6-trimethylbenzyl groups. The proposed mechanism for the cleavage of the carboxamide protecting group in acid is demonstrated by the cleavage of 4-methoxy-2-methylbenzyl group in Scheme 9.

SCHEME 9

CLEAVAGE MECHANISM OF 4-METHOXY-2-METHYLBENZYL GROUP



R= part of an amino acid or any peptide chain.

Nu= Nucleophile.

For the naphthyl group, more resonance structures are possible. The carboxamide protected amino, dipeptide and tripeptides subjected to cleavage studies are presented in Table 3 and the results of the cleavage studies are presented in Table 5.

TABLE 3

CARBOXAMIDE PROTECTED AMINO ACID, DIPEPTIDE AND TRIPEPTIDES SUBJECTED TO CLEAVAGE STUDIES.

Compound No.	Compound
I	Z-Leu-NH(4-MeO-2-meBzl)
II	Z-Leu-Gly-NH(4-MeO-2-meBzl)
III	Boc-Phe-Asn(4-MeO-2-meBzl)-OBzl
IV	Boc-Gly-Phe-Asn(4-MeO-2-meBzl)-OBzl
V	Z-Leu-Gly-NH(4-MeO-1-NM)
VI	Boc-Phe-Asn(4-MeO-1-NM)-OBzl
VII	Boc-Gly-Phe-Asn(4-MeO-1-NM)-OBzl

TABLE 4

THIN LAYER CHROMATOGRAPHY FOR STANDARD COMPOUNDS IN SOLVENT SYSTEM B

Compound	R_f
I	0.6
Leu-NH_2	0.01
II	0.56
Leu-Gly-NH_2	0.04
III	0.73
H-Phe-Asn(4-MeO-2-meBzl)-OBzl	0.49
Phe-Asn	0.06
IV	0.84
H-Gly-Phe-Asn(4-MeO-2-meBzl)-OBzl	0.65
Gly-Phe-Asn	0.04
V	0.53
VI	0.844
H-Phe-Asn(4-MeO-1-NM)-OBzl	0.55
VII	0.76
H-Gly-Phe-Asn(4-MeO-1-NM)-OBzl	0.48

TABLE 5

BTFA-TFA CLEAVAGE OF THE CARBOXAMIDE PROTECTING GROUPS (1=STARTING COMPOUND)

Compound	Cleavage duration (hrs)	Cleavage products(R_f) 1	2	3	4	Duration of protecting group removal (hrs)
I	0	0.6	-	-	-	5hrs
	1	0.59	0.35	-	-	
	2	0.59	0.35	0.01	-	
	3	-	0.34	0.01		
	5	-	-	0.01		
	7	-	-	0.01		
II	0	0.56	-	-	-	5hrs
	1	0.56	0.35	-	-	
	2	0.54	0.31	0.04	-	
	3	-	0.31	0.04	-	
	5	-	-	0.03	-	
	7	-	-	0.04		
III	0	0.73	-	-	-	5hrs
	1	-	0.49	0.12	0.06	
	2	-	-	0.12	0.06	
	3	-	-	0.11	0.05	
	5	-	-	-	0.06	
	7	-	-	-	0.06	
IV	0	0.83	-	-	-	5hrs
	1	-	0.6	0.2	0.04	
	2	-	-	0.2	0.03	
	3	-	-	0.21	0.03	
	5	-	-	-	0.03	
	7	-	-	-	0.03	
V	0	0.53	-	-	-	5hrs
	1	0.53	0.42	0.038		
	2	-	0.43	0.041		
	3	-	0.43	0.04		
	5	-	-	0.04		
	7	-	-	0.04		

TABLE 5 Continued

Compound	Cleavage duration (hrs)	Cleavage products(R_f) 1	2	3	4	Duration of protecting group removal (hrs)
VI	0	0.844	-	-		3hrs
	1	-	0.55	0.2	0.038	
	2	-	-	0.205	0.04	
	3	-	-	-	0.04	
	5	-	-	-	0.04	
	7	-	-	-	0.042	
VII	0	0.78	-	-	-	3hrs
	1	-	0.493	0.23	0.046	
	2	-	-	0.22	0.047	
	3	-	-	-	0.045	
	5	-	-	-	0.040	
	7	-	-	-	0.044	

The ease with which the carboxamide protecting groups are removed depends on the stability of the resultant carbonium ion formed. Stabilization can either be due to resonance or by inductive effect of the substituent groups. Substitution at position ortho or para with electron donating groups makes the resultant primary carbonium ion of the benzyl and naphthyl groups particularly stable hence easily cleaved off under mild acidic condition. However this group i.e. 4-methoxy-2-methylbenzyl and 4-methoxy-1-naphthalenemethyl groups have been found to be stable in 50% TFA-CH_2Cl_2 a reagent used for the removal of the common amino protecting groups.

Cleavage studies using BTFA-TFA on 4-methoxy-2-methylbenzyl and 4-methoxy-1-naphthalenemethyl groups has been demonstrated. Cleavage studies on the 4-methoxy-2-methyl-

benzyl protected derivatives i.e. Z-Leu-NH(4-MeO-2-meBzl(I), Z-Leu-Gly-NH(4-MeO-2-meBzl)(II), Boc-Phe-Asn(4-MeO-2-meBzl)-OBzl)(III), and Boc-Gly-Phe-Asn(4-MeO-2-meBzl)-OBzl(IV) show that cleavage is complete after 5hrs. For compound I and II the Z group is completely removed after 3hrs.

For the 4-methoxy-1-naphthalenemethyl protected derivatives i.e. Z-leu-Gly-NH(4-MeO-1-NM)(V), Boc-Phe-Asn(4-MeO-1-NM)-OBzl(VI) and Boc-Gly-Phe-Asn (4-MeO-1-NM)-OBzl(VII), cleavage takes 3hrs apart from (V) where cleavage took 5hrs. The difference in cleavage time between 4-methoxy-2-methylbenzyl and 4-methoxy-1-naphthalenemethyl groups may be attributed to the fact that 4-methoxy-1-naphthalenemethyl group forms a more stable carbonium ion and also due to the fact that it is bulky as compared to 4-methoxy-2-methylbenzyl group hence it is easier to cleave.

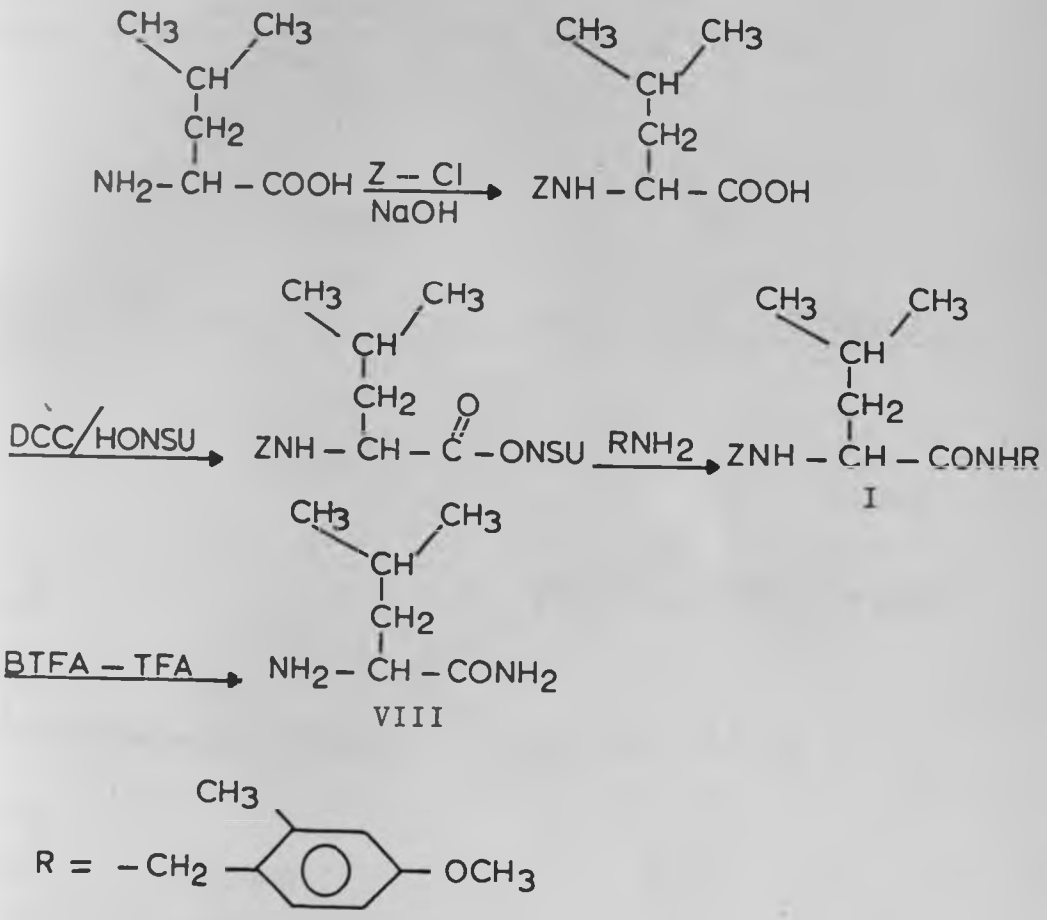
2:4 APPLICATION OF CARBOXAMIDE PROTECTED DERIVATIVES IN PEPTIDE SYNTHESIS

The advantages offered by the use of carboxamide protected asparagine and glutamine include: prevention of the formation of pyroglutamyl peptides from glutaminyl peptides; prevention of dehydration of carboxamide; prevention of the formation of imides;

prevention of deamination and finally, it increases the solubility of peptides so protected in organic solvents. The use of 4-methoxy-2-methylbenzyl and 4-methoxy-1-naphthalenemethyl groups as carboxamide protecting groups in classical peptide synthesis has been demonstrated by the synthesis of a few asparaginyl containing derivatives. Since these groups are stable in TFA, the Boc-group can be selectively removed. The peptide synthesis was done by deprotecting Boc group of the carboxamide protected derivatives, followed by coupling with N-hydroxysuccinimide ester of N-protected amino acids as shown in Scheme 10, 11 and 12.

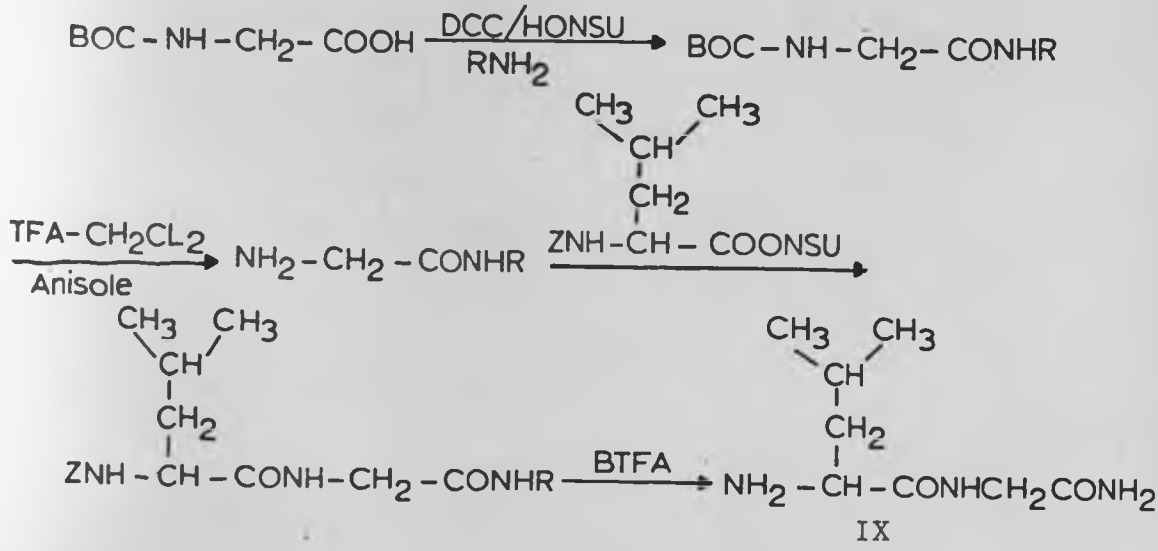
SCHEME 10

SYNTHESIS OF LEUCINAMIDE



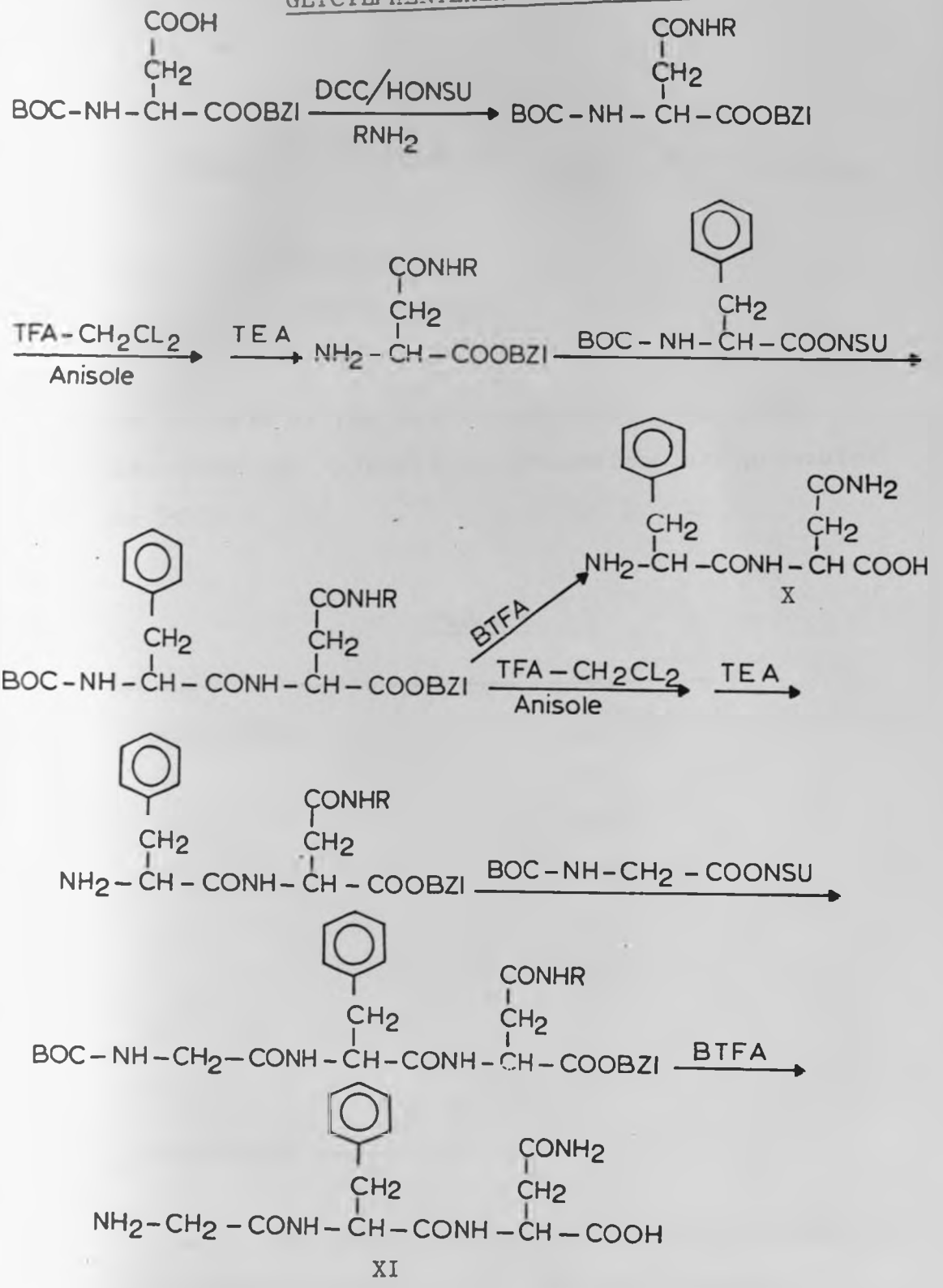
SCHEME 11

SYNTHESIS OF LEUCYLGLYCINAMIDE

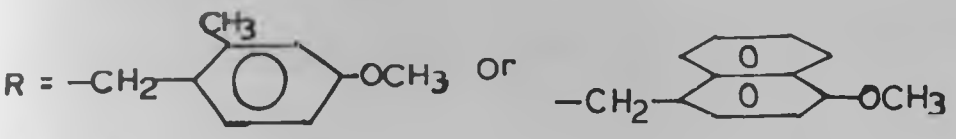


SCHEME 12

SYNTHESIS OF PHENYLALANYLASPARAGINE AND GLYCYLPHENYLALANYLASPARAGINE



Z - Carbobenzoxy



TEA - Triethylamine

Boc - tert-butyloxycarbonyl

The % yield of the fully protected amino acid, dipeptide and tripeptides synthesized are presented in Table 6

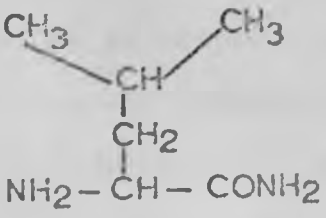
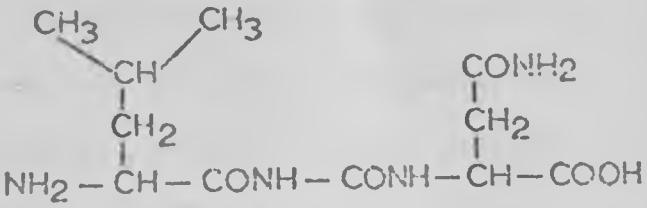
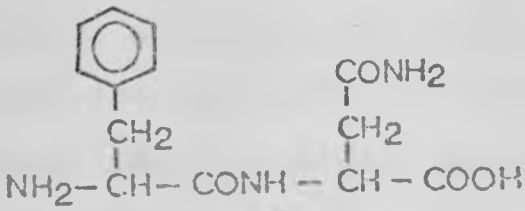
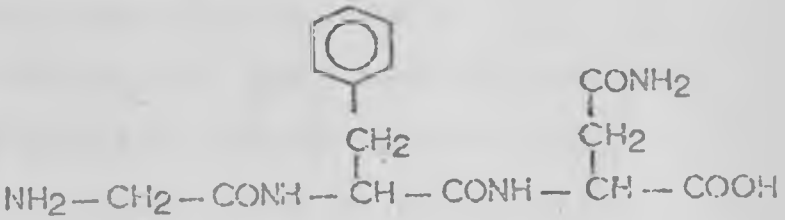
TABLE 6

Compound No.	% Yield
I	50
II	40
III	48.6
IV	68.8
V	64.5
VI	48
VII	75.4

The fully protected derivatives are then deprotected using BTFA-TFA solution at the end of peptide synthesis to obtain the fully deprotected products

TABLE 7

FULLY DEPROTECTED AMIDES, DIPEPTIDES AND TRIPEPTIDES

COMPOUND	
VIII	
IX	
X	
XI	

shown in Table 7. These products are dissolved in a suitable solvent and passed through a column of amberlite

IRA-400 in the OH^- form to remove excess BTFA-TFA.

The yield obtained of compound VIII, IX, X and XI from the cleavage of 4-methoxy-2-methylbenzyl group is 70%, 88.8%, 95% and 66.3% respectively and the cleavage of 4-methoxy-1-naphthalenemethyl group gives 93.4%, 67% and 73.6% of compounds IX, X and XI respectively.

Therefore, it can be concluded that these two groups are good carboxamide protecting groups as they are stable in 50% TFA-CH_2Cl_2 and are easily cleaved off at the end of peptide synthesis by BTFA-TFA solution. The synthesis of Boc-Gly-Phe-Asn(4-MeO-2-me Bzl)-OBzl(IV) and Boc-Gly-Phe-Asn(4-MeO-1-NM)-OBzl(VII) in relatively good yields seems to indicate that a bulky carboxamide protecting group such as 4-methoxy-1-naphthalenemethyl group does not sterically hinder or dramatically slow down the peptide bond formation step. The hydrogenation of Boc-Asn(4-MeO--2-MeBzl)-OBzl and Boc-Asn(4-MeO-1-NM)-OBzl using 5% palladium on activated carbon catalysis gave a high yield of the pure acids.

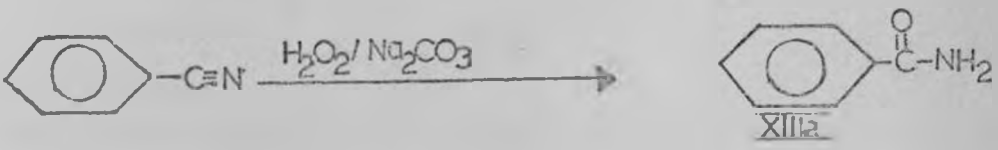
2:5 HYDRATION OF CYANO TO CARBOXAMIDE GROUP DURING PEPTIDE SYNTHESIS.

During peptide synthesis especially when N,N'-dicyclohexylcarbodiimide (DCC) is used as the coupling reagent, the carboxamide group of asparagine and/or glutamine if unprotected may undergo a dehydration reaction to the corresponding cyano derivative with yields of up to 50% under the conditions of various peptide synthesis (Scheme 5).

The cyano derivatives are of interest in peptide synthesis owing to the possibility of hydration back to the carboxamide group at the end of peptide synthesis using mild conditions. This is equivalent to using the cyano group as a carboxamide protecting group hence at the end we obtain pure asparaginyl or glutaminyl peptides. The hydration can either take place under basic (Hydrogen peroxide) in a base or by acidic (hydrogen bromide/Acetic acid) conditions.

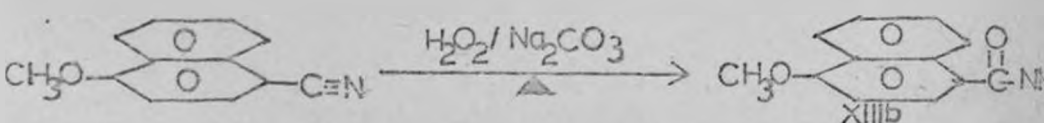
Acidic hydration may be carried out under conditions routinely used in peptide synthesis for the removal of the carbobenzoxy protecting group in the Ben Ishai procedure.

The conversion of nitriles to the carboxamide groups has been demonstrated using several nitrile containing model compounds both in acidic and basic hydration conditions to obtain the corresponding carboxamide groups. The sequence of reactions that have been carried out are shown in equation 11, 12 and scheme 13).

i) 

XII(a)

Eq

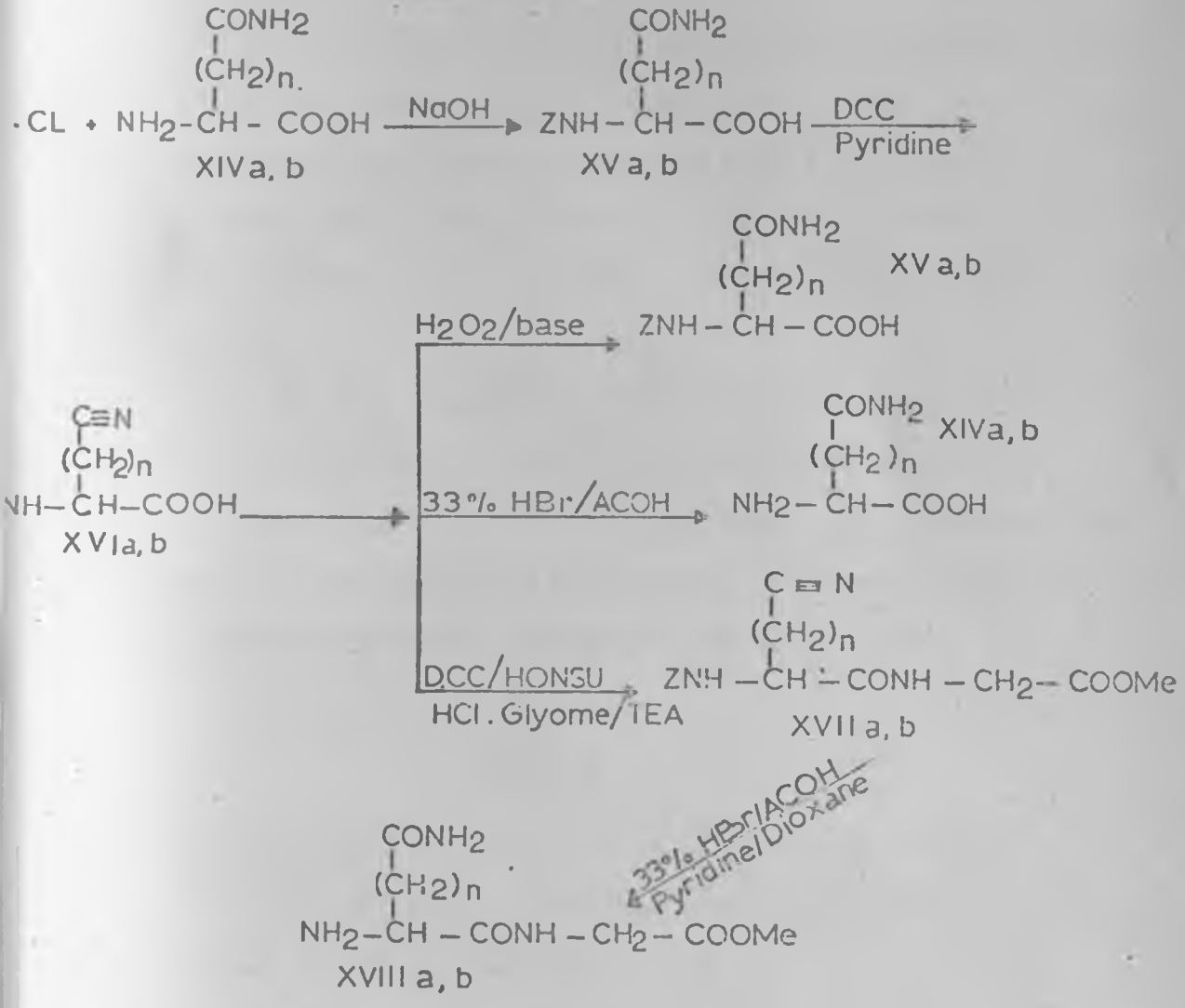
ii) 

XII(b)

Eq 1

SCHEME 13

SYNTHESIS AND CONVERSION OF NITRILES TO AMIDES



a, n = 1, asparagine & asparagine derivatives
b, n = 2, glutamine & glutamine derivatives
TEA = Triethylamine

The nitrile model compounds used for this hydration are benzonitrile (XII (a)); 4-methoxy-1-naphthonitrile (XII(b)); carbobenzoxy-β-cyanoalanine (XVIa); carbobenzoxy-γ-cyano-α-aminobutyric acid (XVIb); carbobenzoxy-β-cyanoalanylglycine methyl ester (XVIIa) and carbobenzoxy-γ-cyano-α-aminobutylglycine ester (XVIIb).

The dehydration of amides is readily effected by treatment with N,N'-dicyclohexylcarbodiimide in pyridine under very mild conditions. The carbodiimides are powerful dehydrating agents. The results of the conversions are presented in Tables 8 and 9.

TABLE S

RESULTS OF THE CONVERSION OF THE NITRILE MODEL COMPOUNDS TO THEIR CORRESPONDING CARBOXAMIDE GROUPS USING H_2O_2/Na_2CO_3.

Compound	Yield
XIIIa	80%
XIIIb	47%
XVa	99%
XVb	69.5%

TABLE 9

RESULTS OF THE CONVERSION OF THE NITRILE MODEL COMPOUNDS TO THEIR CORRESPONDING CARBOXAMIDE GROUPS USING 33% HBr/ACOH.

Compound	Yield
XIIIa	no reaction
XIIIb	no reaction
XVa	89%
XVb	93%
XVIIIa	83.3%
XVIIIb	77.8%

The conversion of benzonitrile (XIIa) and 4-methoxy-1-naphthonitrile (XIIb) to the corresponding benzamide (XIIIa) and 4-methoxy-1-naphthalamide (XIIIb) proceeds with yields of 80% and 47% respectively. The conversion of 4-methoxy-1-naphthonitrile only proceeds at high temperature 60^{o}C and (H_2O_2) hydrogen peroxide concentration of 10% as compared to benzonitrile which proceeds at room temperature and hydrogen peroxide concentration of 6%. This difference may be due to the distribution of the positive charge throughout the system of the 4-methoxy-1-naphthonitrile group during protonation and this suppresses attack by the

peroxide ion (HOO^-). This distribution is further encouraged by the presence of the methoxy group (which is electron donating) at the para position which stabilizes the positive ion formed.

The conversion of carbobenzoxy-β-cyanoalanine (XVIa) and carbobenzoxy-γ-cyano-α-aminobutyric acid (XVIb) to carbobenzoxy asparagine (XVa) and carbobenzoxy-glutamine (XVa) proceeds with yields of 99% and 69.5% respectively. The hydration reaction proceeds and has no effect on the Z-group.

Under acidic hydration (Table 9), the conversion of benzonitrile (XIIa) and 4-methoxy-1-naphthonitrile (XIIb) to their corresponding amides does not appear to take place when conditions suitable for peptide synthesis are used (the nitrile peak at 2200 cm^{-1}-IR spectra is still present). However the hydration of carbobenzoxy-β-cyanoalanine (XVIa); carbobenzoxy-γ-cyano-α-aminobutyric acid (XVIb); carbobenzoxy-β-cyanoalanylglycine methyl ester (XVIIa) and carbobenzoxy-γ-cyano-α-butylglycine methyl ester (XVIIb) to asparagine (XIVa); glutamine (XIVb); asparaginylglycine methyl ester (XVIIIa) and glutaminylglycine methyl ester (XVIIIb) respectively proceeds with yields of 89%, 93%, 83.3% and 77.3% respectively. The 33% HBr/ACOH solution apart from hydrating the nitrile to the carboxamide also cleaves

of the Z group so that in the end we have the fully deprotected peptide.

This therefore means that the presence of the cyano group as a side product during peptide synthesis when DCC is used as the coupling reagent need not bother chemists as it can be converted to the carboxamide group under mild conditions using H_2O_2/base (with retention of Z-group) or 33% HBr/ACOH (with cleavage of Z-group at the end of peptide synthesis.

CHAPTER 3

EXPERIMENTAL SECTION

All melting points were determined in capillaries on a Gallenkamp melting point apparatus and are uncorrected. Infrared spectra were done on Pye-unicam infrared spectrophotometer model SP3-300 and absorptions were reported in wave numbers. Proton Nuclear Magnetic Resonance (^{1}HNMR) spectra were done on Perkin Elmer Spectrometer Model R12B (60MHz). Thin layer chromatography was done on silica gel coated plates (silica gel 60, layer thickness ≈ 0.2mm) in the following solvent systems: A, chloroform:ethylacetate (1:1); B, chloroform: methanol:acetic acid (85:10:5); C, dichloromethane: benzene: ethyl acetate (10:10:5); D, 1-butanol: acetic acid: H_2O:pyridine (15:20:2:3); E, benzene: ethyl alcohol: pet ether (40-60) (5:14:1); F, chloroform: dichloromethane: ethyl acetate (10:5:10). Spots were revealed by ninhydrin in acetone and iodine vapour.

Ion exchange resin Amberlite IRA-400 in the $^-$OH form was used for purification of the fully deprotected products. $(CH_3)_4Si$(TMS) is used as the standard for NMR and the chemical shifts are reported in δ values (i.e. ppm downfield from TMS as standard of reference). All the amino acids except glycine used in synthesis were of the L-Configuration.

3:1 · SYNTHESIS OF AMINES

3:1:1 SYNTHESIS OF 4-METHOXY-2-METHYLBENZONITRILE

a. A solution of copper sulfate pentahydrate (28.09g, 112.5 mmol) and sodium chloride (7.865g, 126 mmol) in 90 ml of hot water was prepared in a one litre 3-necked round-bottomed flask. The flask was fitted with a mechanical stirrer and an alkaline solution of sodium metabisulfate (5.51g, 29 mmol) and sodium hydroxide (1.32g, 49.5 mmol) in 40ml of water was added during a five minutes period. The mixture was allowed to cool to room temperature and washed by decantation with cold water, the cuprous chloride was obtained as a white powder. The cuprous chloride was suspended in 80 ml of cold water and the flask was fitted with a mechanical stirrer. A solution of 98% sodium cyanide (14.34g, 292.5 mmol) in about 25ml of water was added in portions and the mixture was stirred whereupon the cuprous chloride entered into solution with considerable evolution of heat. The mixture was then cooled by surrounding the flask with ice-cold water. While the cuprous cyanide was cooling, 4 methoxy-2-methylaniline (12.5g, 900 mmol) was mixed in a 500 ml 3-necked round-bottomed flask, with 21.7 ml of commercial 28% hydrochloric acid (sp. gr. 1.18) and enough cracked ice (about 90g) was added to bring the temperature of the mixture to $0^{o}C$. A solution of sodium nitrite (6.35g, 91.3 mmol) in 36 ml

of water was added with stirring to the resulting suspension of the amine hydrochloride. The temperature being kept at 0-5^{o}C by the addition of cracked ice.. The addition of the nitrite occupied about 15 min. At the end of the operation, the mixture showed a distinct and permanent reaction for free nitrous acid on testing with moist starch-iodide paper (colour change from white to blue). The mixture was then cautiously neutralized by the addition of dry sodium carbonate with stirring. At the end point the red litmus changed to blue.

b. The cold cuprous cyanide solution was chilled to 0-5^{o}C by the addition of ice and 25 ml of toluene was poured on the surface. To this mixture was slowly added the cold neutralized diazonium solution with vigorous stirring, temperature was maintained between 0-5^{o}C by the occasional addition of ice. As soon as the diazonium solution came into contact with the coprous cyanide a dark yellow oily precipitate was formed which at once began to give off nitrogen, the resulting nitrile was taken up by the toluene as soon as it is formed. After all the diazonium solution had been added, the temperature was held between 0-5^{o}C for 30 min. longer and then allowed to rise to room temperature. The flask was heated on a water-bath and warmed to 50^{o}C without stirring. The

mixture was then allowed to cool and then it was transferred to a one litre bolt-head flask and distilled in a current of steam until no more oil passed over. The solvent (toluene) was removed on a rotary evaporator in vacuo to yield a dark brown oil (8gm, 60%); TLC, solvent system A, Rf= 0.86; IR spectrum (Neat, γmax): CH, 3000 cm^{-1} and 2900 cm^{-1}; -C≡N, 2200 cm^{-1}; ^{1}HNMR spectrum (CCl_4): δ7.42 (s,1H), δ6.8 (d,2H) due to aromatic protons; δ3.6 (s,3H) due to the methoxy protons and δ2.25 (s,3H) due to the methyl protons.

3:1:2 4-METHOXY-2-METHYLBENZYLAMINE

In a one litre 3-necked round bottomed flask containing a magnetic stirrer, reflux condenser and a dropping funnel, were placed 200 ml of anhydrous diethyl ether (Et_2O) and lithium aluminium hydride ($LiAlH_4$) (8.51g, 224 mmol). All openings were protected from atmospheric moisture until completion of the reaction by calcium chloride ($CaCl_2$) tubes. A solution of 4-methoxy-2-methylbenzonitrile (8g, 56 mmol) in 300 ml of anhydrous Et_2O was added dropwise for 2 hrs while refluxing gently. The solution was refluxed for a period of 24hrs. The general hydrolysis procedure for destroying the unreacted $LIALH_4$ complex was used. The solution was

cooled to 0°C in an ice-water bath and with vigorous stirring 14.9 ml of water were added, followed by 11.2 ml of 20% aqueous sodium hydroxide (NaOH) and 52.2 ml of water were added in succession. The granular inorganic precipitate was filtered off and washed with three 100 ml portions of anhydrous Et_2O and the filtrate and washings were combined. The Et_2O solution was washed with three 37.4 ml portions of 20% HCl. The acidic solution was heated for 20 min. and washed with two 18.7 ml portions of warm benzene. Benzene 37.4 ml was added to the acidic solution, the mixture was cooled to 0°C in ice-water bath and aqueous 4N NaOH was added dropwise with stirring until the solution was made alkaline. The benzene layer was separated by extraction and the aqueous layer was washed with three 60ml portions of benzene. The benzene portions were combined and dried over anhydrous sodium sulphate. The drying agent was filtered off with suction at the pump. The solvent was removed at the rotary evaporator in vacuo at 30°C to obtain the product (5.75g, 70%). TLC, solvent system A, Rf= 0.8; IR spectrum (Neat, γmax): primary amine due to ($-NH_2$ group) with peaks at 3290 cm^{-1} and 3210 cm^{-1}; ^{1}HNMR spectrum (DMSO-d_6): δ7.04 (s,1H) and δ6.52 (d,2H) for the aromatic protons; δ3.58 (s,3H) for the methoxy protons; δ3.51 (s,2H) for the methylene protons; δ2.12 (s,3H) for the methyl protons.

3:1:3 4-METHOXY-1-NAPHTHALENEMETHYLAMINE

This compound was prepared from 4-methoxy-1--naphthonitrile (4.2g, 2.32 mmol) and $LiALH_4$ (4g, 100 mmol) in the same manner as described for the synthesis of 4-methoxy-2-methylbenzylamine. The oily yellow residue obtained was dissolved in 10 ml benzene and cooled to $0^{o}C$. To this solution was added 10 ml of concentrated HCl. The yellow precipitate was filtered off, washed with three 30 ml portions of benzene and the product was dried *in vacuo* to obtain the product (3.677g, 75.8%); m.p.>$200^{o}C$ (Lit. 215).[69] TLC, solvent system, CH_2Cl_2:$CHCl_3$:CH_3OH (1:1:1), Rf= 0.59; IR spectrum (KBr, γmax):Broad peak (HCl) with maximum at 3440 cm^{-1} due to NH stretching vibration; NMR (CF_3COOH): δ8.1 and δ6.4 (m,6H) for the substituted naphthyl group protons; δ4.4 (s,2H) for the methylene protons; δ4.4 (s,2H) for the methylene protons; δ3.6 (s,3H) for the methoxy protons.

3:2 SYNTHESIS OF CARBOXAMIDE PROTECTED AMINO ACID DERIVATIVES.

3:2:1 tert-BUTYLOXYCARBONYL-N^{CA}-4-METHOXY-2-METHYL-BENZYLGLYCINAMIDE

A stirred mixture of Boc-glycine (1g, 5.7 mmol) and N-hydroxysuccinimide (0.78g, 6.75 mmol) in 6 ml of dichloromethane (CH_2Cl_2) was cooled to $-5^{o}C$. To this mixture was added N,N'-dicyclohexylcarbodiimide

(DCC) . (1.36g, 6.6 mmol) in 20 ml of CH_2Cl_2 and the mixture was stirred at -5°C for 50 mins. A solution of 4-methoxy-2-methylbenzylamine (0.87g, 5.7 mmol) was added and the mixture was stirred at -5°C for an additional 50 mins. Then the mixture was stirred at room temperature for 24hrs. Acetic acid (0.15ml) was added. The mixture was stirred for 15 mins. and the dicyclohexylurea (DCHU) was filtered off and washed with three 6 ml portions of CH_2Cl_2. The solvents were removed on a rotary evaporator in vacuo and the residue was dissolved in 9ml of CH_2Cl_2. Some insoluble crystals were filtered off. Chloroform (14 ml) was added to the filtrate, the solution was washed with three 18 ml portions of 5% aqueous citric acid, 24 ml portions of 5% sodium bicarbonate ($NaHCO_3$) and five 30 ml portions of deionized water. The organic layer was dried over anhydrous sodium sulphate (Na_2SO_4) and the solvents were removed on a rotary evaporator in vacuo. The semi-solid is dissolved in 12 ml of hot ethyl acetate (EtOAC) cooled to room temperature and filtered off. To the filterate a solution of 48 ml of petroleum ether (40-60°C) was added dropwise and the mixture was kept at 0-5°C overnight. The precipitate was filtered off, washed with three 9 ml portions of petroleum ether (40-60°C) - EtOAC (4:1) and dried in vacuo to obtain the product (11g, 62.6%); m.p.

117-118°C; TLC,solvent system A,Rf= 0.53; IR spectrum (KBr, max):NH, 3360 cm^{-1}; -C=O groups 1820 cm^{-1}, 1730 cm^{-1} and 1690 cm^{-1}; NMR ($CDCl_3$): δ7.3 (s,1H), δ6.73 (d,2H) for the aromatic protons; δ6.6 (br s,1H) for the -NH proton; δ4.35 (s;2H) for the -CONH-$\underline{CH_2}$-CO protons; δ3.75 (s,3H) for the methoxy protons; δ3.74 (s,2H) for the methylene protons (attached to the aromatic system); δ2.5 (s,3H) for the methyl protons and δ1.4 (s,9H) for the $(CH_3)_3$-C protons of the Boc-group.

3:2:2 SYNTHESIS OF CARBOBENZOXYLEUCINE

A solution of leucine (10g, 76.2 mmol) in aqueous 4N NaOH (19 ml) was chilled to about 5°C. A total of 23 ml of 4N NaOH and carbobenzoxychloride (Z-Cl) (14.3g, 84 mmol) was added alternatively in about five equal portions over 30 mins. with vigorous stirring and cooling in an ice-bath. The reaction mixture was kept at 0-4°C overnight. The solution was extracted with Et_2O (25 ml) to remove excess Z-Cl. The aqueous fraction was acidified slowly with 5N HCl (to approximately PH 1) with cooling in ice-bath. A white thick oil precipitated. After further cooling in ice-bath the oil was extracted with EtOAc. A suspension was filtered off from EtOAc and washed with Et_2O to yield white crystalline

compound (unreacted leucine). The clear EtOAc layer was dried with anhydrous magnesium sulphate ($MgSO_4$). EtOAc was evaporated off at the rotary evaporator *in vacuo* to yield a yellow syrup. (15.819g, 78%). TLC, solvent system A, Rf = 0.3; solvent system D, Rf = 0.8; IR spectrum (KBr pellet γmax):- NH, 3400 cm^{-1}; -C=O, 1720 cm^{-1}, and 1660 cm^{-1}; NMR spectrum (DMSO-d_6): δ7.35 (s,5H) for aromatic protons; δ6.7 (Br s,1H) for the NH protons; δ5.01 (s,2H) for O-CH_2-Ar protons; δ4.5 (m,1H) for the methine protons; δ4 (d,2H) for the methylene protons; δ0.85 (d,6H) for the two methyl group protons of leucine.

3:2:3 CARBOBENZOXY-N^{CA}-4-METHOXY-2-METHYL BENZYLLEUCINAMIDE (I)

This compound was synthesized from carbobenzoxyleucine (1g, 3.77 mmol) N-hydroxysuccinimide (0.477g, 4.14 mmol); N,N'-dicyclohexylcarbodiimide (0.850g, 4.14 mmol)and 4-methoxy-2-methylbenzylamine (0.57g, 3.77 mmol) in the same manner as described for the synthesis of tert-butyloxycarbonyl-N^{CA}-4--methoxy-2-methylbenzylglycinamide to obtain the product (0.746g, 50%); m.p. 118-119°C; TLC, solvents system A, Rf= 0.65; IR spectrum (KBr pellet, γmax): NH, 3300 cm^{-1}; -C=O 1660 cm^{-1}, 1630 cm^{-1}; NMR ($CDCl_3$):

δ7.4 (s,5H), δ7.2 (s,1H), δ6.4 (s,2H) for the aromatic protons; δ5.1 (s,2H) for the -CO-$\underline{CH_2}$-Ar protons; δ4.35 (s,2H) for the -CONH-$\underline{CH_2}$-Ar; δ3.8 (s,3H) for the methoxy protons; δ2.3 (s,3H) for the methyl protons; δ1.65 (d,2H) for the methylene protons of leucine; δ1 (d,6H) for the $(\underline{CH_3})_2$-CH protons.

3:2:4 α-BENZYL-tert-BUTYLOXYCARBONYL-N^{CA}-4-METHOXY-2-METHYLBENZYLASPARAGINATE.

This compound is synthesized from Boc-aspartic acid-α-benzyl ester (1g, 3.1 mmol); N-hydroxysuccinimide (0.357g, 3.1 mmol); DCC (0.64g, 3.1 mmol) and 4-methoxy-2-methylbenzylamine (0.467g, 3.1 mmol) in the same manner as described for the synthesis of tert-butyloxycarbonyl-N^{CA}-4-methoxy-2-methylglycinamide to obtain the product (1.3g, 91.7%); m.p. 197-198°C; TLC, solvent system A, Rf = 0.82; I.R spectrum (KBr pellet, γmax): NH, 3300 cm^{-1}; -C=O, 1730 cm^{-1} and 1650 cm^{-1}; NMR ($CDCl_3$): δ7.4 (s,5H), δ7.1 (d,2H), δ6.8 (s,2H) due to the aromatic protons; δ5.25 (s,5H) for the -O-$\underline{CH_2}$-Ar; δ4.4 (d,2H) for the C-$\underline{CH_2}$-CONH-; δ2.35 (s,3H) for the methyl protons and δ1.5 (s,9H) due to the $(CH_3)_3C$- protons.

3:2:5 tert-BUTYLOXYCARBONYL-N^{CA}-4-METHOXY-1-NAPHTHALENEMETHYLGLYCINAMIDE

A stirred mixture of (1g, 5.7 mmol) of tert-butyloxycarbonylglycine and N-hydroxysuccinimide (0.78g, 6.75 mmol) in 6ml of CH_2Cl_2 was cooled to -5^oC. To this mixture was added DCC (1.36g, 6.6 mmol) in 20 ml of CH_2Cl_2 and the mixture was stirred at -5^oC for 50 minutes. A solution of 4-methoxy-1-naphthalenemethylamine (1.3g, 6.95 mmol) in 6ml CH_2Cl_2 was added and the mixture was stirred at -5^oC for an additional 50 minutes. The mixture was stirred at room temperature for 24 hrs. Acetic acid (0.15 ml) was added, the mixture was stirred for 15 minutes and DCHU was filtered off and washed with three 6 ml portions of CH_2Cl_2. The solvents were removed on a rotary evaporator in vacuo and the residue was dissolved in 9ml of CH_2Cl_2. Some insoluble crystals were filtered off. Chloroform (14 ml) was added to the filtrate. The solution was washed with three 18 ml portions of 5% aqueous citric acid, 24 ml portions of deionized water, the organic layer was dried over anhydrous Na_2SO_4 and the solvents were removed on a rotary evaporator in vacuo. The semi-solid was dissolved in 12ml hot EtOAc cooled to room temperature and filtered off.

To the filterate a solution of 48 ml of petroleum ether (bp. 40-60^{o}C) was added dropwise and the mixture was kept at 0-5^{o}C overnight. The precipitate was filtered off washed with 9 ml portions of petroleum ether (bp. 40-60^{o}C): EtOAc (4:1) and dried in vacuo to give the product (1.35g, 68.9%); m.p. 118-119^{o}C; TLC . solvent system A, Rf = 0.58; I.R. spectrum (KBr pellet, γmax): -NH 3400 cm^{-1}; -C=O, 1720 cm^{-1} and 1670 cm^{-1}; NMR spectrum ($CDCl_3$): δ8.3, δ6.6 (br m, 6H) for the aromatic protons; δ4.8 (s,2H) for the -CONH-CH_2-CO- protons; δ3.9 (s,3H) for the methoxy protons; δ3.7 (s,2H) for the -CONH-CH_2-Ar protons; δ1.3 (s,9H) for the three methyl group protons of the $(CH_3)_3$-C group.

3:2:6 α-BENZYL-tert-BUTYLOXYCARBONYL-N^{CA}-4-METHOXY-1-NAPHTHALENEMETHYLASPARAGINATE.

This compound was synthesized from tert-butyloxycarbonylaspartic acid-α-benzyl ester (1g, 3.1 mmol), N-hydroxysuccinimide (0.3g, 3.1 mmol) DCC (0.64g, 3.1 mmol) and 4-methoxy-1-naphthalene-methylamine (0.58g, 3.4 mmol) in the same manner as described for the synthesis of tert-butyloxycarbonyl-N^{CA}-4-methoxy-1-naphthalene methylglycinamide to obtain crystalline produced (1.0316g, 67.6%); m.p. 98-100^{o}C; (Lit. 100-102^{o}C)[69] TLC, solvent system A, Rf= 0.763; Rf (chloroform)= 0.15; IR spectrum (KBr

pellet, γmax): NH, 3300 cm^{-1}; -C=O; 1720 cm^{-1} and 1670 cm^{-1} NMR spectrum ($CDCl_3$): δ8.4, δ6.5 (6H) for the aromatic protons of the substituted naphthyl group merged at δ7.3 (s,5H) with the phenyl group protons; δ5.2 (s,2H) for O-$\underline{CH_2}$-Ar protons; δ4.7 (s,2H) for C-$\underline{CH_2}$-CONH- protons; δ3.95 (s,2H) for the -CONH-$\underline{CH_2}$-Ar protons; δ1.35 (s,9H) for $(CH_3)_3$-C- protons.

3:3 APPLICATION OF CARBOXAMIDE PROTECTED DERIVATIVES IN PEPTIDE SYNTHESIS

SYNTHESIS OF ACTIVATED ESTERS & DIPEPTIDES

3:3:1 CARBOBENZOXYLEUCINE-N-HYDROXYSUCCINIMIDO ESTER

To a solution of carbobenzoxyleucine (2g, 7.6 mmol) and N-hydroxysuccinimide (HONSU) (0.87g, 7.54 mmol) in 10 ml of dioxane was added N,N'-dicyclohexylcarbodiimide (DCC) (1.7g, 8.3 mmol). The formed dicyclohexylurea (DCHU) was filtered off and washed with a small quantity of dioxane. The filterate was concentrated in vacuo to yield a yellow oil which was triturated with 3 portions of anhydrous Et_2O. Some anhydrous Et_2O was added and 0.08g of crystalline material was filtered off. The filtrate was evaporated at the rotor evaporator

to yield a yellow crystalline product (2.17g, 79.6%) m.p. 114-116°C (Lit., m.p. 116-117°C)[73]; TLC, solvent system A, Rf=0.65; solvent system D, Rf=0.4; I.R. spectrum (KBr pellet, γmax): NH, 3320 cm^{-1}; -C=O, 1820 cm^{-1}, 1740 cm^{-1} and 1680 cm^{-1}; NMR (DMSO-d_6): δ7.35 (s,5H) for the aromatic protons; δ5.2 (s,2H) for the Ar-$\underline{CH_2}$-O-CO- protons; δ3.2 (t,1H) for the methine proton attached to the carbonyl group; δ2.8 (s,4H) for the -$\underline{CH_2}$-$\underline{CH_2}$- protons of the ester group; δ2.6 (s,1H) for the methine proton and δ0.95 (d,6H) for the two methyl group protons $(CH_3)_2$-CH-.

3:3:2 <u>tert</u>-BUTYLOXYCARBONYLPHENYLALANINE-N-HYDROXYSUCCINIMIDO ESTER

<u>tert</u>-Butyloxycarbonylphenylalanine (1g, 3.4 mmol) N-hydroxysuccinimide (3.4 mmol,0.391g) were mutually combined and dissolved in 10 ml anhydrous dimethoxyethane (DME) at 0°C. DCC (0.903g, 4.4 mmol) was added with stirring and the solution was kept at 0-5°C for 24 hrs. The DCHU formed was filtered off and the solvent evaporated to dryness in an open dish. The crystalline product obtained was recrystallized from isopropyl alcohol: Diisopropyl ether (1:1) to obtain the pure product (1.08g, 75%); m.p. 151-152°C (Lit. 152-153°C)[73]; TLC, solvent system

B,Rf=0.33; IR spectrum (KBr pellet, γmax): NH, 3340 cm^{-1}; -C=O, 1830 cm^{-1}, 1740 cm^{-1} and 1680 cm^{-1}; NMR spectrum ($CDCl_3$): δ7.3 (s,5H) for the aromatic protons; δ5(t,1H) for the methine proton; δ2.5 (d,2H) for the methylene group protons attached to the benzene ring; δ2.8 (s,4H) for the $-\underline{CH_2}-\underline{CH_2}-$ protons of the esters group; δ1.4 (s,9H) for the $(CH_3)_3C-$ protons.

3:3:3 tert-BUTYLOXYCARBONYLGLYCINE-N-HYDROXYSUCCINIMIDO ESTER

N,N'-dicyclohexylcarbodiimide (1.29g, 6.27 mmol) was added to a solution of tert-butyloxycarbonylglycine (1g, 5.7 mmol) and N-hydroxysuccinimide (0.657g, 5.7 mmol) in 1.4 dioxane 10 ml with stirring. The reaction mixture was kept in the refrigerator overnight. The formed N,N'-dicyclohexylurea was filtered off and washed with a small quantity of dioxane. The filtrate was concentrated *in vacuo* to yield a thick oil which was then triturated with three portions of Et_2O to yield white crystalline material (1.105g, 64.7%); m.p. 165-167°C (Lit. 168-170°C)[73]; TLC, solvent system A Rf= 0.64; IR spectrum (Nujol moll, γmax): NH, 3300 cm^{-1}; -C=O 1800 cm^{-1} (HONSU), 1740 cm^{-1}; NMR spectrum ($CDCl_3$): δ4.3 (s,2H) for the methylene protons; δ2.8 (s,4H) for the $-\underline{CH_2}-\underline{CH_2}-$ protons of the HONSU; δ1.5

(s,9H) for the $(CH_3)_3C$- protons.

3:3:4 CARBOBENZOXYLEUCYL-N^{CA}-4-METHOXY-2-METHYL-BENZYLGLYCINAMIDE (II)

A solution of tert-butyloxycarbonyl-N^{CA}-4-methoxy-2-methylbenzylglycinamide (0.6g, 1.93 mmol) in 12.5 ml of trifluoroacetic acid (TFA) and 1 ml anisole was stirred for 25 minutes at room temperature. The solvent was removed on a rotary evaporator in vacuo at 25°C-30°C. The residue was triturated with dry Et_2O to give white crystalline compound. The crystalline salt was dissolved in 5 ml of dimethylformamide (DMF) and the PH was adjusted to 7 with triethylamine (TEA). To this solution was added carbobenzoxyleucine-N-hydroxy-succinimido ester (0.7g, 1.93 mmol). The solution was stirred at room temperature for 14 hrs and the reaction was monitored by TLC with solvent system A. At the end of the reaction 25ml of EtOAc was added and the solution was washed with three 40ml portions of 5% aqueous citric acid, two 40ml portions of deionized water. The organic portion was dried over anhydrous sodium sulphate. The product is further purified using column chromatography to obtain the crystalline product (0.345g, 40%) m.p. 118-119°C;

TLC, with solvent system B, Rf=0.68; I.R spectrum (KBr pellet, γmax): NH, 3300 cm^{-1}; -C=O, 1780 cm^{-1}, 1760 cm^{-1}; NMR ($CDCl_3$): δ7.3 (s,5H), δ7 (s,1H) and δ6.5 (d,2H) for the aromatic protons; δ5.1 (s,1H) for the methine proton attached to the carbonyl group; 4.95 (s,2H) for the Ar-$\underline{CH_2}$-CO- protons; δ4.3 (d,2H) for the -NH-$\underline{CH_2}$-CO- protons; δ3.9 (d,2H) for the -CONH-$\underline{CH_2}$-Ar protons; δ3.7 (s,2H) for the methoxy protons; δ1.4 (broads, 2H) for $(CH_3)_2CH-\underline{CH_2}$-CO- protons and δ0.9 (s,6H) for the $(\underline{CH_3})_2$-CH- protons.

3:3:5 <u>tert</u>-BUTYLOXYCARBONYLPHENYLALANYL-α-BENZYL-N^{CA}-4-METHOXY-2-METHYLBENZYLASPARAGINATE (III)

This compound was synthesized from <u>tert</u>-butyloxycarbonyl-N^{CA}-4-methoxy-2-methylasparaginate (0.641g, 1.4 mmol) and tert-butyloxycarbonyl-phenylalanine-N-hydroxysuccinimido ester, in the same manner as described for the synthesis of carbobenzoxyleucyl-N^{CA}-4-methoxy-2-methylbenzylglycinamide to obtain a crystalline product (0.4g, 48.6%) m.p. 146-147°C; TLC, solvent system A, Rf=0.82, solvent system E, Rf=0.8 and solvent system F, Rf=0.8; IR spectrum (KBr pellet, γmax): NH 3300 cm^{-1}; -C=O, 1730 cm^{-1}, 1680 cm^{-1} and 1645 cm^{-1}; NMR spectrum ($CDCl_3$): δ7.2 (d,11H), δ6.7 (s,2H) for the aromatic protons; δ5.1 (s,2H) for the O-$\underline{CH_2}$-Ar

protons; δ4.2 (d,2H) for the -C-$\underline{CH_2}$-CO- protons; δ3.7 (s,3H) for the methoxy protons; δ3.2 (d,2H) for the C-$\underline{CH_2}$-Ar protons; δ2.75 (s,2H) for the N-CONH-$\underline{CH_2}$-Ar protons; δ2.25 (s,3H) for the methyl protons attached to the aromatic ring; δ1.35 (s,9H) for the $(CH_3)_3C$- protons.

3:3:6 <u>CARBOBENZOXYLEUCYL-N^{CA}-4-METHOXY-1-NAPHTHALENEMETHYLGLYCINAMIDE</u>(V)

Solution of <u>tert</u>-butyloxycarbonyl-N^{CA}-4-methoxy-1-naphthalenemethylglycinamide (0.39g, 1.13 mmol) in 12.5ml of TFA and 1 ml of anisole was stirred for 25 minutes at room temperature and the solvent was removed on a rotor evaporator <u>in vacuo</u> at 25-30^{o}C. The residue was recrystallized from Et_2O to give a white crystalline product. The crystalline salt was dissolved in 5ml of DMF and the PH was adjusted to 7 using triethylamine. To this solution was added carbobenzoxyleucine-N-hydroxysuccinimide ester (0.4g, 1.13 mmol). The solution was stirred at room temperature for 14hrs. At the end of the reaction 25ml of ethylacetate was added and the solution was washed with three 40ml portions of aqueous $NaHCO_3$ and five 30ml portions of deionized water. The organic layer was dried over anhydrous Na_2SO_4. Further purification is

carried out using column chromatography (solvent system chloroform: ethyl acetate (1:1)) to obtain the pure product (0.25g, 64.5%); m.p. 116-118°C; TLC, solvent system B, Rf=0.6; I.R spectrum (KBr pellet, γmax): NH, 3340 cm^{-1}; NMR ($CDCl_3$): δ8.3, δ6.5 (m,11H) for the aromatic protons; δ4.85 (s,2H) for the -O-$\underline{CH_2}$-Ar protons; δ4.65 (d,2H) for the -CONH-$\underline{CH_2}$-CO- protons; δ3.85 (s,3H) for the methoxy protons; δ3.65 (s,2H) for the CONH-$\underline{CH_2}$-Ar protons and δ0.8 (d,6H) for the two methyl group protons $(\underline{CH_3})_2CH$-.

3:3:7 <u>tert</u>-BUTYLOXYCARBONYLPHENYLALANYL-α-BENZYL-N^{CA}-4-METHOXY-1-NAPHTHALENE METHYLASPARAGINATE

This compound was synthesized from <u>tert</u>-butyloxycarbonyl-N^{CA}-4-methoxy-1-naphthalenemethyl-asparaginate (0.675g, 1.37 mmol) and <u>tert</u>-butyloxy-carbonylphenylalanine-N-hydroxysuccinimido ester (0.49g, 1.37 mmol) in the same manner as described for the synthesis of carbobenzoxyleucyl-N^{CA}-4-methoxy--1-naphthalenemethylglycinamide to obtain the crystalline product (0.405g, 48%); m.p. 140-141°C: TLC, solvent system B, Rf=0.533; solvent system D, Rf=0.91; IR spectrum (KBr γmax): NH, 3300 cm^{-1}; -C=O, 1730 cm^{-1}, 1640 cm^{-1}; NMR ($CDCl_3$): δ8.4, δ6.5 (m,16H) three merged peaks of the two phenyl groups

and the naphthyl protons; δ5.2 (s,2H) for O-$\underline{CH_2}$-Ph protons; δ4.8 (d,2H) for -C-$\underline{CH_2}$-CO- protons; δ4 (s,3H) for the methoxy protons; δ3.8 (d,2H) for the CO-NH-$\underline{CH_2}$-Ar protons; δ1.4 (d,2H) for the methylene protons (-C-$\underline{CH_2}$-Ar) and δ0.9 (s,9H) for the $(CH_3)_3C$- protons.

3:4 SYNTHESIS OF TRIPEPTIDES

3:4:1 tert-BUTYLOXYCARBONYLGLYCYLPHENYALANYL-α-BENZYL-N^{CA}-4-METHOXYBENZYLASPARAGINATE(IV)

This compound was synthesized from tert-butyloxycarbonylphenylalanyl-α-benzyl-N^{CA}-4-methoxy--2-methylbenzylasparaginate (0.2g, 3.39 mmol) and tert-butyloxycarbonylglycine-N-hydroxysuccinimido ester (0.92g, 3.39 mmol) in the same manner as described for the synthesis of tert-butyloxycarbonylphenylalanyl -α-benzyl-N^{CA}-4-methoxy-2-methylbenzylasparaginate to obtain the product (0.15g, 68.8%); m.p. 194-195°C; TLC, solvent system A, Rf=0.294; solvent system B, Rf=0.77; IR spectrum (KBr pellet, νmax): NH, 3300 cm^{-1}; -C=O, 1700 cm^{-1} and 1630 cm^{-1}; NMR spectrum (DMSO-d_6): δ7.3 (d,11H); δ6.75 (s,2H) for the aromatic protons; δ5.1 (s,2H) for the -O-$\underline{CH_2}$-Ar protons; δ4.5 (s,2H) for the -CONH-$\underline{CH_2}$-CO protons; δ4.1 (s,2H) for CONH-$\underline{CH_2}$-Ar protons; δ3.65 (s,3H) for the methoxy protons. δ3.1 (d,2H) -C-$\underline{CH_2}$-CO- protons; δ2.6 (d,2H) for C-$\underline{CH_2}$-Ar protons; δ2.2 (s,3H) for the methyl

protons that are attached to the aromatic system; δ1.3 (s,9H) for the methyl protons of $(CH_3)_3C$-.

3:4:2 tert-BUTYLOXYCARBONYLGLYCYLPHENYALANYL--α-BENZYL-N^{CA}-4-METHOXY-1-NAPHTHALENEMETHYL-ASPARAGINATE (VII)

This compound is synthesized from tert-butyloxycarbonylphenylalanyl-N^{CA}-4-methoxy-1-naphthalenemethylasparaginate (0.25g, 0.4 mmol) and tert-butyloxycarbonylglycine-N-hydroxysuccinimido ester (0.11g, 0.4 mmol) in the same manner as described for the synthesis of carbobenzoxyleucyl-N^{CA}-4-methoxy-1-naphthalenemethylglycinamide to obtain the pure product (0.153g, 75.4%); m.p. 137-138°C; TLC,solvent system A,Rf=0.34; solvent system B,Rf=0.7; IR (KBr pellet, γmax): NH, 3260 cm^{-1}; -C=O, 1730 cm^{-1} and 1620 cm^{-1}: NMR spectrum ($CDCl_3$ solvent): δ8, δ6.3 (m,16H) for the two phenyl and the naphthyl groups; δ5.15 (s,2H) for O-$\underline{CH_2}$-Ph protons; δ4.7 (s,2H) for -CONH-$\underline{CH_2}$ protons; δ4.1 (d,2H) for the -CONH-$\underline{CH_2}$-Ar protons; δ3.6 (s,3H) for the methoxy protons; δ3.1 (d,2H) for -C-$\underline{CH_2}$-CO protons; δ2.5 for C-$\underline{CH_2}$-Ar protons; δ1.3 (s,9H) for the $(CH_3)_3C$- protons.

3:5 BORON TRIS(TRIFLUOROACETATE) (BTFA) CLEAVAGE OF CARBOXAMIDE PROTECTED AMINO ACID DERIVATIVES.

3:5:1 CLEAVAGE STUDIES OF CARBOBENZOXY-N^{CA}-4-METHOXY-2-METHYLBENZYLLEUCINAMIDE IN BORON TRIFLUORIDE COMPLEX WITH ACETIC ACID (36% BF_3, $BF_3.2CH_2COOH$).

Small quantities of carbobenzoxy-N^{CA}-4-methoxy--2-methylbenzylleucinamide (I) was dissolved in 0.5ml TFA, 1ml BTFA was added. The mixture were stirred and TLCed at 1hr, 2hrs, 3hrs, 5hrs and 7hrs respectively. The solvent system used was chloroform: methanol: Acetic acid (85:10:5). Detecting agent used was ninhydrin in acetone.

The same procedure is carried out for the following compounds, carbobenzoxyleucyl-N^{CA}-4-methoxy--2-methylbenzylglycinamide (II); tert-butyloxycarbonyl phenylalanyl-α-benzyl-N^{CA}-4-methoxy-2-methyl benzylasparaginate (III); tert-bubyloxycarbonylglyl-phenylalanyl-α-benzyl-N^{CA}-4-methoxy-2-methylbenzyl asparaginate (IV); carbobenzoxyleucyl-N^{CA}-4-methoxy-1-naphthalenemethylglycinamide (V); tert-butyloxycarbonyl-phenylalanyl-α-benzyl-N^{CA}-4-methoxy-1-naphthalene-methylasparaginate (VI); tert-butyloxycarbonylglycyl-phenylalanyl-α-benzyl-N^{CA}-4-methoxy-1-naphthalenemethyl-asparaginate (VII).

3:6 CLEAVAGE OF CARBOXYL PROTECTING GROUP (α-BENZYL) BY HYDROGENOLYSIS

3:6:1 tert-BUTYLOXYCARBONYL-N^{CA}-4-METHOXY-2-METHYLBENZYLASPARAGINE

tert-Butyloxycarbonyl-α-benzyl-N^{CA}-4-methoxy-2-methylbenzylasparaginate(0.639g,1.4 mmol) was dissolved in 50ml of methanol with mechanical stirring. To this was added 0.5g of 5% Pd/c in portions (care must be taken to avoid igniting the reaction mixture). Hydrogen gas was bubbled through the reaction mixture at room temperature and normal pressure. Portions of the reaction mixture were withdrawn after 30 minutes, 1hr, 1½hrs and 2hrs respectively and TLCed in the solvent system ethyl acetate: chloroform (1:1). At the end of the reaction the catalyst was filtered off and washed well with methanol. The methanol was stripped off from the filtrate at the rotary evaporator and the residue is vacuum dried to yield a pale yellow crystalline product (0.83g, 98.7%); m.p. 145-146°C. TLC, solvent system A, Rf=0.37; IR spectrum (KBr pellet, γmax): Broad peak from 3700 cm^{-1} to 2500 cm^{-1} for the carboxyl group OH^- stretching vibration; NH, 3300 cm^{-1}; -C=O, 1670 cm^{-1} and 1620 cm^{-1}; NMR ($CDCl_3$): δ7.3 (s,1H), δ6.7 (d,2H) for the aromatic protons; δ4.3 (d,2H) for the

C-$\underline{CH_2}$-CO- protons; δ3.8 (d,2H) for the -CONH-$\underline{CH_2}$-Ar protons; δ3.65 (s,3H) for the methoxy protons; δ1.35 (s,9H) for the $(CH_3)_3$-C protons.

3:6:2 tert-BUTYLOXYCARBONYL-N^{CA}-4-METHOXY-1-NAPHTHALENEASPARAGINE

This compound is synthesized by the hydrogenolysis of tert-butyloxycarbonyl-α-benzyl-N^{CA}-4-methoxy-1-naphthaleneasparaginate (0.639g, 1.4 mmol) in the same manner as described for tert-butyloxycarbonyl-α-benzyl-N^{CA}-4-methoxy-2-methylbenzyl-asparaginate to obtain a yellow crystalline product (0.68g, 80.6%); m.p. 125-126°C; (Lit.125).[69] TLC, solvent system A, Rf= 0.36; IR spectrum (KBr,γmax): Broad peak from 3700 cm^{-1} to 3100 cm^{-1} for the OH stretchin vibration of the carboxyl group; -C=O, 1670 cm^{-1} and 1620 cm^{-1}; NMR spectrum ($CDCl_3$): δ8.3, δ6.5 (m,6H) for the aromatic protons; δ4.3 (d,2H) for the C-$\underline{CH_2}$-CO- protons; δ3.8 (s,3H) for the methoxy protons; δ2.8 (d,2H) for the CONH-$\underline{CH_2}$-Ar protons; and δ1.35 (s,9H) for $(CH_3)_3$-C protons.

3:7 SYNTHESIS OF FULLY DEPROTECTED AMIDES, DIPEPTIDES AND TRIPEPTIDES.

3:7:1 LEUCINAMIDE (VIII)

Carbobenzoxy-N^{CA}-4-methoxy-2-methylbenzyl leucinamide (0.4g, 1 mmol) was dissolved in 1ml TFA and 2ml of BTFA was added. The mixture was stirred for 5hrs. Dry Et_2O(50ml) was added and the precipitate was filtered off and dissolved in 10ml of methanol and chromatographed on amberlite IRA-400 in the ^-OH form. The methanol solvent was removed at the rotor evaporator in vacuo and the residue is recrystallized from ethanol to obtain the product (0.11g, 70%) m.p. 104-106°C; TLC, solvent system D, Rf=0.37; I.R (KBr pellet γmax):-NH_2 (doublet) 3340 cm^{-1} and 3270 cm^{-1}; -C=O, 1660 cm^{-1}. NMR (DMSO-d_6): δ5.5 (m,1H) for the methine protons; δ1.65 for the methylene protons; δ1 (d,6H) for the two methyl group and protons.

3:7:2 LEUCYLGLYCINAMIDE (IX)

(a) This compound was synthesized from carbobenzoxyleucyl-N^{CA}-4-methoxy-2-methylbenzylglycinamide (0.2g, 0.439 mmol); TFA 0.5ml, BTFA 1ml in the same manner as described for the synthesis of leucinamide. The product is recrystallized from ethanol to obtain the final product (0.073g, 88.8%).
(b) It is also synthesized from carbobenzoxyleucyl-N^{CA}-4-methoxy-1-naphthalenemethylglycinamide (0.2g, 41 mmol) in the same manner as described for

leucinamide to obtain the product (0.07g, 93.4%). M.p. 95-97^{o}C; TLC, solvent system D, Rf= 0.13; IR spectrum (KBr pellet, γmax): -NH_2, and NH broad peak between 3345 cm^{-1} and 3280 cm^{-1}; -C=O, 1670 cm^{-1}, 1660 cm^{-1}; NMR (CD_3COOD): δ5.1 (t,1H) for the methine proton next to the carbonyl group; 4.3 (d,2H) for the -CONH-$\underline{CH_2}$-CO; δ1.5 (d,2H) for the methylene protons of leucyl group; δ0.9 (d,6H) for the two methyl group protons.

3:7:3 PHENYLALANYLASPARAGINE(X)

(a) This compound was synthesized from tert-butyloxycarbonylphenylalanyl-α-benzyl-N^{CA}-4-methoxy-2-methylbenzylasparaginate (0.2g, 0.34 mmol) 1 ml TFA and 2ml BTFA in the same manner as described for the synthesis of leucinamide. The product is recrystallized from EtOAc: Pet ether (1:1) to obtain the pure product (0.09g, 95%).

(b) It was also synthesized by the use of tert-butyloxycarbonylphenylalanyl-N^{CA}-4-methoxy-1-naphthalenemethylasparaginate-α-benzyl ester (0.2g, 31.28 mmol) as described for the synthesis of leucinamide but stirring is carried out for 3hrs. It is recrystallized from ethyl acetate: Petroleum ether (40-60) (4:1) to obtain the crystalline product (0.059g, 67%); m.p. 180-189^{o}C; TLC, solvent system D, Rf= 0.57; IR spectrum (KBr pellet, γmax): -C=O, 1680 cm^{-1},

1640 cm^{-1}; NMR (DMSO-d_6): δ7.25 (s,5H) for aromatic protons; δ4.2 (d,2H) for C-$\underline{CH_2}$-$CONH_2$ group protons; δ3.1 (d,2H) for the C-$\underline{CH_2}$-Ar protons.

3:7:4 GLYCYLPHENYLALANYLASPARAGINE (XI)

(a) This compound was synthesized from tert-butyloxycarbonylglycylphenyalanyl-α-benzyl-N^{CA}-4-methoxy-2-methylbenzylasparaginate (0.11g, 1.702 mmol) in the same manner as described for the synthesis of leucinamide to obtain the product (0.038g, 66.3%).

(b) It's also synthesized from tert-butyloxy-carbonylglycylphenylalanyl-α-benzyl-N^{CA}-4-methoxy-1-naphthalenemethylasparaginate (0.13g, 1.9 mmol) in the same manner as described for the synthesis of leucinamide but stirring is carried out for 3hrs to obtain the crystalline product (0.047g, 73.6%); m.p. >200°C, solvent system D, Rf=0.12; IR spectrum (KBr pellet, γmax): NH (doublet), 3400 cm^{-1} and 3350 cm^{-1}; -C=O, 1720 cm^{-1}, 1695 cm^{-1} and 1675 cm^{-1}; NMR spectrum (DMSO-d_6): δ7.4 (s,5H) for the aromatic protons; δ4.5 (d,2H) for the C-$\underline{CH_2}$-$CONH_2$ protons; δ2.7 (d,2H) for the C-$\underline{CH_2}$-Ar protons.

3:8 CONVERSION OF NITRILES TO AMIDES

3:8:1 CONVERSION OF BENZONITRILE (XIIa) TO BENZAMIDE (XIIIa) USING HYDROGEN PEROXIDE[74]

Benzonitrile (5g, 49.5 mmol) was added to 50ml of 6% solution of hydrogen peroxide (H_2O_2)

and the mixture was made slightly alkaline with 16% sodium carbonate (Na_2CO_3). The mixture was stirred and the temperature maintained at 25°C for 4hrs. The amide separates out as white flakes. The stirring was interrupted and the mixture surrounded by ice and salt whereupon more of the amide separated out. It is then filtered off, washed with cold H_2O, dried over H_2SO_4 for 4hrs and crystallized from absolute ethyl alcohol. The reaction is then repeated but stirred for 24hrs. The pure product was obtained as white crystalline compound 4hrs (2.07g, 53.6%); 24hrs (wt. 3.10g, 80%); m.p. 127-129°C (Lit. m.p. 129-130°C);[75] TLC, solvent system A, Rf= 0.17; solvent system B, Rf=0.66; IR spectrum (KBr, γmax): $-NH_2$, 3400 cm^{-1}, 3230 cm^{-1}; -C=O, 1650 cm^{-1}; NMR spectrum (CF_3COOH solvent): δ7.5 multiplet for the aromatic protons.

3:8:2 CONVERSION OF 4-METHOXY-1-NAPHTHONITRILE(XIIb) TO 4-METHOXY-1-NAPHTHALAMIDE(XIIIb) USING HYDROGEN PEROXIDE.

This compound was converted using 4-methoxy--1-naphthonitrile (0.155g, 0.85 mmol) and 50ml 10% solution of H_2O_2 in the same manner as described for the conversion of benzonitrile. (No reaction occurs at 25°C and H_2O_2 concentration of 6%). The mixture was stirred at 65°C for 24hrs during which time the

amide separates out as white flakes. The product is recrystallized from ethyl acetate to obtain the pure product (0.0805g, 47%); m.p. >200°C; TLC, solvent system C,Rf=0.33; IR spectrum (KBr pellet, γmax): $-NH_2$ 3350 cm^{-1} and 3250 cm^{-1} (doublet); -C=O, 1680 cm^{-1}; NMR spectrum (DMSO-d_6): δ8.3, δ7, δ6.1 (m,6H) for the substituted aromatic protons; δ3.4 (s,3H) for the methoxy protons.

3:8:3 SYNTHESIS OF CARBOBENZOXYASPARAGINE[76] (XVa)

Sodium hydroxide [2M] and carbobenzoxychloride (0.723g, 4.24 mmol) were added in portions within 90 minutes with vigorous stirring to a 16% aqueous solution of asparagine (6g, 4 mmol). The PH was maintained throughout at approximately 8 and the temperature was kept below 25°C. When the base was no longer readily consumed it's addition was discontinued and stirring was continued for another 1hr. The reaction mixture were worked up by the extraction with Et_2O followed by acidification to PH 1 and it was filtered and recrystallised from methanol. The compound was obtained as white crystalline product. (7.11g, 62.5%); m.p. 165-166°C (Lit. 165°C)[76]; TLC, solvent system B, Rf= 0.5; IR spectrum (KBr pellet, γmax): $-NH_2$, 3400 cm^{-1} and 3320 cm^{-1}; -C=O, 1735 cm^{-1}, 1690 cm^{-1} and 1640 cm^{-1}; NMR spectrum (DMSO-d_6): δ7.25

(s,5H) for the aromatic protons; δ 6.8 (br s,1H) -NH proton; δ 4.95 (s,2H) for O-$\underline{CH_2}$-Ar protons; δ 4.3 (m,1H) for the methine protons; δ 2.4 (d,2H) for the C-$\underline{CH_2}$-CO protons.

3:8:4 SYNTHESIS OF CARBOBENZOXY-β-CYANOALANINE (XVIa)

A solution of carbobenzoxyasparagine (3g, 1.13 mmol) in 15ml of redistilled pyridine was maintained between 16-20°C and to this a solution of N,N'-dicyclohexylcarbodiimide (DCC) (2.44g, 11.8 mmol) in 7.6ml of pyridine was added in portions with stirring over 30 minutes. After 3hrs the precipitated N,N'-dicyclohexylurea (DCHU) was filtered off and the filterate was concentrated in vacuo to a small volume. The mixture was again filtered and the filtrate was concentrated to a thick syrup. Dilution with water caused the separation of some solids which were filtered off after 1hr in the cold. The filtrate was then acidified with 6N HCl, with the separation of white crystals. The product was recrystallized from dry ethylene dichloride. The pure product was obtained as white crystalline material. (1.59g, 50.6%); m.p. 129-130°C (Lit. 130-135°C)[76]; TLC, solvent system B, Rf=0.7; IR spectrum (KBr pellet, γmax): NH-, 3200 cm^{-1}; -C≡N, 2260 cm^{-1}; -C=O 1730 cm^{-1}, 1640 cm^{-1}. NMR spectrum (DMSO-d_6):

δ7.37 (s,5H) aromatic; δ5 (s,2H) for O-$\underline{CH_2}$-Ar protons; δ4.3 (t,1H) for the methine protons; δ2.88 (d,2H) for -C-$\underline{CH_2}$-C=N protons.

3:8:5 CONVERSION OF CARBOBENZOXY-β-CYANOALANINE (XVIa) TO CARBOBENZOXYASPARAGINE (XVa) USING HYDROGEN PEROXIDE.

Carbobenzoxy-β-cyanoalanine (0.782g, 3.15 mmol) was added to 50ml of 7.5% solution of H_2O_2 and the mixture made slightly alkaline with 10% Na_2CO_3. The mixture was stirred to ensure intimate mixture of the substance. The temperature is maintained at 25°C for 4hrs. The solution is then acidified to PH 1 with 6N HCl. The amide then separates out and it is filtered and recrystallized from methanol to obtain the product (0.834g, 99%); m.p. 160-162°C; TLC, solvent system B, Rf=0.55. IR spectrum (KBr pellet, γmax): $-NH_2$, 3400 cm^{-1} and 3320 cm^{-1}; -C=O, 1730 cm^{-1}, 1680 cm^{-1} and 1620 cm^{-1}; NMR spectrum (DMSO-d_6): δ7.25 (s,5H) for the phenyl protons; δ6.75 (br s,1H) for -NH protons; δ4.9 (s,2H) for O-$\underline{CH_2}$-Ph protons; δ2.3 (d,2H).

3:8:6 CONVERSION OF CARBOBENZOXY-β-CYANOALANINE (XVIa) TO ASPARAGINE (XIVa) USING 33% HYDROGEN BROMIDE IN ACETIC ACID.[77]

Hydrogen bromide (33%) in glacial acetic acid (1.4g, 3.35 mmol) was added to carbobenzoxy-β-cyanoalanine (1g, 427 mmol). All the openings were closed using calcium chloride tubes to trap water vapour. The mixture was allowed to stand at room temperature with occassional shaking until the evolution of carbon dioxide ceased after about 20 minutes. Dry ether (20ml) was then added and the reaction flask was kept in the refrigerator for about 4hrs. The solid hydrobromide which separates out was filtered, washed with dry ether and dried *in vacuo*. The compound is obtained as a white crystalline material. The salt is dissolved in the minimum amount of water, excess pyridine was added and the amino acid was precipitated with absolute alcohol. It is filtered; washed and dried *in vacuo* over sulphuric acid for 48 hrs to obtain the product. (0.507g, 89%); m.p. >200°C; (Lit. m.p. 235°C)[78]; TLC, solvent system B, Rf= 0.15; IR spectrum (KBr pellet, γmax): $-NH_3^+$ 3130 cm^{-1} and 3030 cm^{-1}; -C=O, 1680 cm^{-1} and 1610 cm^{-1}; NMR spectrum (D_2O): δ3.9 (1H) for the methine protons; δ2.9 (d,2H) for the methylene protons;

3:8:7 SYNTHESIS OF CARBOBENZOXY-β-CYANO ALANYLGLYCINE METHYL ESTER (XVIIa).

Finally powdered carbobenzoxy-β-cyanoalanine (0.5g, 2.05 mmol) in dioxane(15ml)was added to N-hydroxysuccinimide (0.257g, 2.22 mmol) and the solution was cooled to 10°C and N,N'-dicyclohexyl-carbodiimide (0.457g, 2.22 mmol) was added. The solution was stirred for 20 minutes and glycine methyl ester hydrochloride (0.229g, 2.05 mmol) in triethylamine (0.285 ml) and dimethylformamide (7ml) was added. The solution was then stirred at room temperature for 24hrs. The solvents were removed at the rotor evaporator. The crystalline compound was re-crystallized from ethanol to obtain the product (0.434g, 66.8%); m.p. 128-129°C; TLC, solvent system B,Rf=0.65; solvent system C,Rf=0.41; I.R spectrum (KBr pellet, γmax): -NH, 3300 cm^{-1} and 3260 cm^{-1}; -C≡N, 2100 cm^{-1} (very intense); -C=O 1735 cm^{-1}, 1735 cm^{-1} and 1680 cm^{-1}; NMR spectrum ($CDCl_3$): δ7.4 (s,5H) for the aromatic protons; δ5.2 (s,2H) for the O-$\underline{CH_2}$-Ar protons; δ4.1 (s,2H) for NH-$\underline{CH_2}$-CO protons: δ3.8 (s,3H) for the methyl protons of the ester group.

3:8:8 CONVERSION OF CARBOBENZOXY-β-CYANO ALANYLGLYCINE METHYL ESTER (XVIIa) TO ASPARAGINYLGLYCINE METHYL ESTER (XVIIIa) USING 33% HYDROGEN BROMIDE IN ACETIC ACID.

The conversion was carried out using 33% hydrogen bromide in acetic acid (1.67ml) and carbobenzoxy-β-cyanoalanine (1g, 4.27 mmol) in the same manner as described for the conversion of carbobenzoxy-β-cyanoalanylglycine methyl ester to asparagine. The semi solid that separates out is dissolved in the minimum amount of water, pyridine (excess) was added and the product was precipitated using dioxane (0.25g, 84.3%); m.p. 174-178°C; TLC, solvent system B, Rf=0.1; IR spectrum (KBr pellet, γmax): -NH, 3400 cm^{-1} and 3290 cm^{-1}; -C=O, 1745 cm^{-1}, 1680 cm^{-1} and 1620 cm^{-1}; NMR spectrum (DMSO-d_6): δ4.7 (t,1H) for the methine protons; δ4.3 (s,2H) for the NH-$\underline{CH_2}$-CO protons; δ3.8 (s,3H) for the methyl protons of the ester group and δ2.9 (d,2H) for C-$\underline{CH_2}$-CO- protons.

3:8:9 SYNTHESIS OF CARBOBENZOXYGLUTAMINE (XVb).

This compound was synthesized from glutamine (5.5g, 4 mmol) in the same manner as described for carbobenzoxyasparagine. The crude product is recrystallized from water to obtain the pure product.

(7.9g, 69.5%); m.p. 135-136°C (Lit. 137°C)[76]; TLC, solvent system B,Rf=0.5; IR spectrum (KBr pellet, γmax): $-NH_2$, 3435 cm^{-1}, 3400 cm^{-1}; -NH 3320 cm^{-1}; -C=O, 1620 cm^{-1};NMR spectrum (DMSO-d_6): δ7.3 (s,5H), for aromatic protons δ6.65 (bro s,1H) for -NH protons; δ4.95 (s,2H) for O-$\underline{CH_2}$-Ph protons; δ2 (br,4H) for the two -$\underline{CH_2}$-$\underline{CH_2}$- group protons.

3:8:10 SYNTHESIS OF CARBOBENZOXY-γ-CYANO-α-AMINO BUTYRIC ACID (XVIb)

This compound is synthesized from carbobenzoxyglutamine (3.46g, 1.23 mmol) in 9ml of pyridine, N,N-dicyclohexylcarbodiimide (3g, 15 mmol) in 4.5ml of pyridine in the same manner as described in the synthesis of carbobenzoxy-β-cyanoalanine. On acidification a thick yellow oil was obtained which was extracted into ethyl acetate. The extract was dried over magnesium sulfate ($MgSO_4$) and was concentrated in vacuo. The residue were further dried by azeotroping with anhydrous benzene to obtain the product (2.03g, 63%); b.p.>200°C; TLC,solvent system B,Rf=0.5; IR spectrum (Neat, γmax): -NH, 3330 cm^{-1}; -C≡N, 2240 cm^{-1} (very sharp); -C=O, 1690 cm^{-1}; NMR spectrum (CCl_4): δ7.35 (s,5H) for the aromatic protons; δ4.95 (s,2H) for the O-CH_2-Ar protons; δ2.3 (broad,4H) for the -$\underline{CH_2}$-$\underline{CH_2}$- protons.

3:8:11 CONVERSION OF CARBOBENZOXY-γ-CYANO-α-AMINO BUTYRIC ACID TO CARBOBENZOXY GLUTAMINE (XVb).

This conversion is carried out using carbobenzoxy-γ-cyano-α-aminobutyric acid (0.63g, 2.4 mmol) and 40ml of 7.5% H_2O_2 in the same manner as described for the conversion of carbobenzoxy-β-cyanoalanine. The product was crystallized from water to obtain the pure product (0.467, 69.5%); m.p. 135-138°C; TLC, solvent system B, Rf= 0.15; IR spectrum (KBr pellet, γmax): -OH, broad peak between 3600 cm^{-1} to 2700 cm^{-1} with maximum at 3430 cm^{-1} and 3400 cm^{-1}; $-NH_2-$ 3430 cm^{-1} and 3400 cm^{-1}; -C=O, 1690 cm^{-1}, 1645 cm^{-1} and 1620 cm^{-1}; NMR spectrum (DMSO-d_6): δ7.3 (s,5H) for the aromatic protons; δ6.65 (br,1H) for NH protons; δ4.95 (s,2H) for the O-CH_2-Ph protons δ2(br 4H) for the -CH_2-CH_2-protons.

3:8:12 CONVERSION OF CARBOBENZOXY-γ-CYANO-α-AMINO BUTYRIC ACID (XVIIa) TO GLUTAMINE (XIVb) USING 33% HYDROGEN BROMIDE IN GLYCIAL ACETIC ACID (33% HBr/ACOH).

This conversion is carried out using carbobenzoxy-γ-cyano-α-aminobutyric acid (1g, 3.8 mmol) and 33% HBr/ACOH (4ml) in the same manner as described for the conversion of carbobenzoxy-β-cyanoalanine to asparagine. Fine crystalline product is obtained. (6.52g, 93.7%); m.p. 178-180°C

(185°C decomp)[78]; TLC, solvent system B, Rf= 0.14; IR spectrum (KBr pellet, γmax): 3150 cm^{-1} and 3050 cm^{-1} due to $-NH_3^+$, -C=O: 1650 cm^{-1} and 1620 cm^{-1}; NMR spectrum (D_2O): δ3.8 (1H) for the methine protons; δ2.4 (br s,4H) for the $-CH_2-CH_2-$ protons.

3:8:13 SYNTHESIS OF CARBOBENZOXY-γ-CYANO-α-AMINOBUTYLGLYCINE METHYL ESTER (XVIIb)

This compound was prepared from carbobenzoxy-γ-cyano-α-aminobutyric acid (1g, 3.81 mmol), N-hydroxysuccinimide (0.43g, 3.81 mmol) in 20ml 1,4-dioxane, DCC (0.865g, 4.19 mmol) and glycine methyl ester hydrochloride in 2ml triethylamine and 5ml DMF in the same manner as described for the synthesis of carbobenzoxy-β-cyanoalanylglycine methyl ester. Crystalline product was obtained. (0.405g, 32%) m.p. 89-90°C; TLC,solvent system B, Rf=0.7; IR spectrum (KBr pellet, γmax): $-NH_2$ 3400 cm^{-1}, -C=N 2100 cm^{-1} (very sharp);-C=O, 1700 cm^{-1} and 1650 cm^{-1}; NMR spectrum ($CDCl_3$): δ7(s,5H) for the aromatic protons; δ5.2 (s,2H) for $-O-\underline{CH_2}-Ar$ protons; δ4.1 (s ,2H) for $NH-\underline{CH_2}-CO$ protons; δ3.8 (s,3H) for the methyl ester protons ($-O-CH_3$); δ2.2 (br 4H) for the $-\underline{CH_2}-\underline{CH_2}-$ protons.

3:8:14 CONVERSION OF CARBOBENZOXY-γ-CYANO-α-AMINO BUTYLGLYCINE METHYL ESTER (XVIIb) TO GLUTAMINYLGLYCINE METHYL ESTER (XVIIIb) USING 33% HYDROGEN BROMIDE IN ACETIC ACID.

This conversion is carried out using carbobenzoxy-γ-cyano-α-aminobutylglycine methyl ester (1g, 3.03 mmol) and 33% hydrobromide in acetic acid (2.1 mmol) in the same manner as described for the conversion of carbobenzoxy-β-cyanoalanylglycine methyl ester. Crystalline product was obtained. (0.64g, 77.8%); m.p. 135-136°C; TLC, solvent system B, Rf=0.05; IR spectrum (KBr pellet, γmax): $-NH_2$, 3400 cm^{-1} and 3300 cm^{-1} (doublet); -C=O, 1740 cm^{-1}; NMR spectrum (DMSO-d_6): δ4.1 (s,2H) for NH-$\underline{CH_2}$-CO protons; δ3.75 (s,3H) for the methyl protons of the ester group (-O-CH_3); δ2.2 (br 4H) for the -CH_2-CH_2- protons.

3:8:15 ATTEMPTED CONVERSION OF BENZONITRILE (XIIa) TO BENZAMIDE USING 33% HBr IN ACETIC ACID.

33% Hydrogen bromide in glacial acetic acid (4ml) was added to benzonitrile (5g, 49.4 mmol). All the openings were closed using calcium chloride tubes. The mixture was allowed to stand at room temperature with occassional shaking for 20 minutes. Dry ether (20ml) was added and the reaction flask

was kept in the refrigerator for about 4hrs. No crystalline product was formed. A thick oil product was formed (4.5g, 90%) b.p. 186-187^{o}C (Lit. b.p. of benzonitrile 188^{o}C),[79] IR spectrum (Neat) sharp peak at 2210 cm^{-1} due to C-C≡N stretching vibration (very intense). NMR (CCl_4 solvent) sharp singlet at δ7.37 for the aromatic protons.

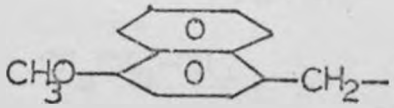
3:8:16 ATTEMPTED CONVERSION OF 4-METHOXY-1-NAPHTHALENE (XIIb) TO 4-METHOXY-1-NAPHTHALAMIDE.

4-Methoxy-1-naphthonitrile (0.155g, 0.85 mmol) was subjected to the same conditions as described in the attempted conversion of benzonitrile to benzamide. The ether solution is evaporated to obtain a crystalline product (0.137g, 88%), m.p. 99-100^{o}C (Lit m.p. of 4-methoxy-1-naphthonitrile 100-102^{o}C);[78] IR spectrum (KBr pellet), sharp peak at 2200 cm^{-1} due to -C≡N stretching vibration. NMR ($CDCl_3$, solvent) δ8.1 and δ6.4 (m,6H) for the substituted naphthyl group protons; δ3.6 (s,3H) for the methoxy protons.

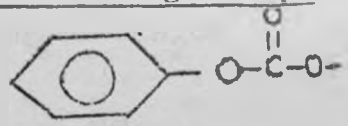
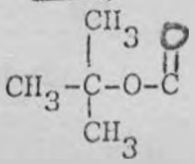
APPENDIX

LIST OF ABBREVIATIONS

Carboxamide Protecting Groups

Name	Formula	Abbreviation
4-Methoxy-2-methylbenzyl		4-MeO-2-meBzl
4-Methoxy-1-naphthalene		4-MeO-1-NM

Amino Protecting Groups

Benzyloxycarbonyl		Z
tert-butyloxycarbonyl		Boc

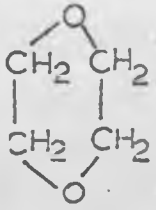
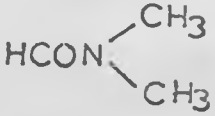
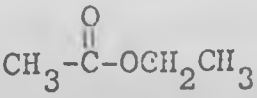
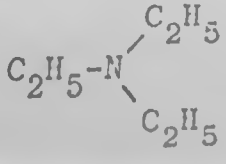
Carboxyl Activating and Protecting Groups

		-OBzl

List of Abbreviations continued

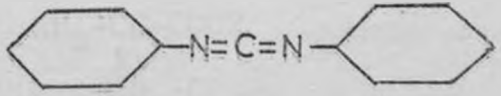
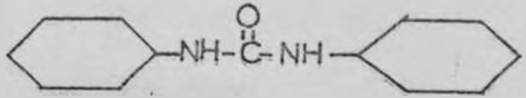
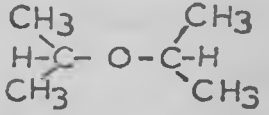
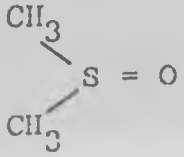
Name
1,4-Dioxane
N,N'-Dimethylformamide
Ethanol
Ethylacetate
Hydrogen fluoride
Methanol
Triethylamine

Other Chemicals

Formula	Abbreviation
	
	DMF
CH_3CH_2-OH	EtOH
	EtOAC
HF	
CH_3OH	MeOH
	Et_3N
TF COOH	TFA

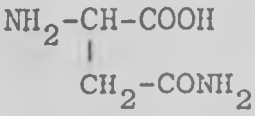
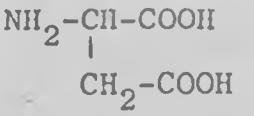
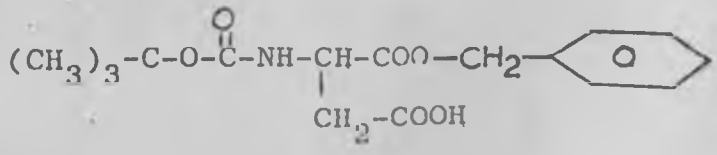
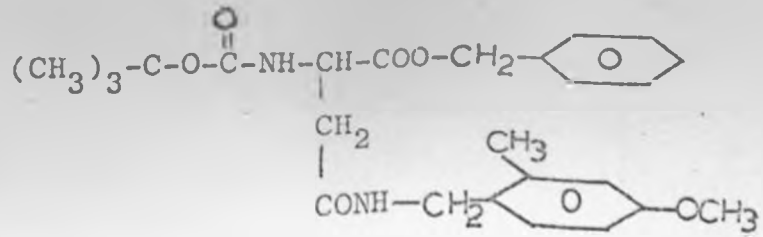
List of Abberviations continued

Other Chemicals

Name	Formula	Abbreviation
Acetic acid	CH_3COOH	ACOH
Boron tristrifluoroacetate	$(CF_3COO)_3B$	BTFA
Dicyclohexylcarbodiimide		DCC
Dicyclohexylurea		DCHU
Dichloromethane	CH_2Cl_2	-
Diethyl ether	$CH_3CH_2-O-CH_2-CH_3$	Et_2O
Diisopropyl ether		i-Pr_2O
Dimethyl sulfoxide		DMSO
1,2-Dimethoxyethane	$CH_3O-CH_2-CH_2-OCH_3$	1,2-DME

Aspartic Acid and It's Derivatives

The 4-methoxy-2-methylbenzyl group is used to illustrate how to write the name, formula and abbreviation of the carboxamide-protected amino acid.

Name	Formula	Abbreviation
Asparagine		Asn
Aspartic acid		Asp
α-Benzyl tert-butyloxycarbonylaspartate		Boc-Asp(α-Bzl)
α-Benzyl tert-butyloxycarbonyl-N^{CA}-4-methoxybenzylasparaginate		Boc-Asn(4-MeO-2-meBz)OBzl

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