^APPLICATION OF TETRALINYLAMINES AS CARBOXAMIDE PROTECTING GROUPS IN PEPTIDE SYNTHESIS^

BY

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DECLARATION

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

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

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ABSTRACT

A study was carried out to convert 1-tetralone and some of its derivatives to the amines by Leuckart reaction. In this reaction, the ketones (1) 1-cetralone, (2) 5-methoxy-1 -tetra1one, (3) 6-methoxy-l-tetralone, (4) 7-methoxy-1-tetralone and (5) 5,7-dimethyl-l-tetralone were converted to the formyl derivatives. These formamides namely, (la) 1,2,3,4-tetrahydro-l-naphthvl, (2a) 5-methoxy--1, 2,3,4-tetrahydro-1-naphthy1, (3a) 6-methoxy--1,2,3,4-tetrahydro-1-naphthy1, (4a) 7-methoxy-1,2,3, 4-tetrahydro-l-naphthyl and (5a) 5,7-dimethy1--1,2,3,4-tetrahvdro-l-n3phthyl formamides were then hydrolyzed to their corresponding amines:- (lb) 1-aminotetralin , (2b) 1-amino-5-methoxytetra1in, (3b) 1-amino-G-methoxytetralin, (4) l-.omino-7--methoxytetralin, and (5b) l-amino-5,7-dimethyltetral This was carried out by both acidic (concentrated hydrochloric acid) and basic (1C% aqueous NaOH) conditions. The former gave yields ranging from 0% to 75%, while the latter gave yields ranging from 90% to 97%. Acid hydrolysis of 3a and 4a gave black-gummy compounds and no amines were obtained. This showed that hydrolysis of formyl derivatives under basic conditions gave better results and is recommended.

Suitability of these 1-tetralinyl groups as potential carboxamide protecting -for asparagine and glutamine side chain amide groups were investigated. The amines lb,2b,3b,4b and 5b were used as precursors to prepare the carboxamide protected derivatives namely, (6) Boc-Gln(1,2,3,4-tetrahydro--l-naphthyl)-a-OBzl, (7) Boc-Asn(1,2,3,4-tetrahydro--1-naphthyl)-B-OBzl, (8) Boc-Gln(5-methoxy--1,2,3,4-tetrahydro-l-napnthyl)-a-OBzl, (9) Boc-Asn-(5-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-B-OBzl, tlQ) Boc-Gln(6-methoxy-l,2,3,4-tetrahydro-l-napthy1)--a-OBzl, (11) Boc-Asn (6-methoxy-1,2,3,4-tetrahydro--1-naphthyl)-a-0Bzl, (12) Boc-G1n(7-methoxy-1,2,3,4--tetrahydro-l-naphthyl)-a-OBzl, (13) Boc-Asn--(7-methoxy-l,2,3,4-tetrahydro-l-naphthyl)-a-0Bzl, (14) Boc-Gln(5,7-dimethyl-l,2,3,4-tetrahydro-l--naphthyl)-a-0Bzl,(15) Boc-Asn(5,7-dimethyl-l,2,3,4--tetrahydro-l-naphthyl)-g-OBzl. The N,N'-dicyc--lohexylcarbodiimide/N-hydroxysuccinimide (DCC-HONSU) coupling methoO gave yields ranging from 40% to 86%. These carboxamide protected derivatives were subjected to cleavage studies in TFA-Ch^C^-anisole (50:46:2-v/v) . The protecting groups in glutamine derivatives (6, 6,10,12. and 14) were removed within 24hr. In the carboxamide protected derivatives of asparagine (7 , 9,11,13 andl5), the protecting groups in 9 and 11 were found too labile to be used during

peptide synthesis. The protecting groups in 7 , 13 and 15 were stable in the above deprotecting reagent upto 24hr. These derivatives (7 , 13 and 15)were therefore subjected to cleavage studies in boron trifluoride complex with acetic acid (BTFA). This completely removed the protecting . groups. The group in 7 was cleaved completely after 4hr, in 13 after 3hr and in 15 after 3hr. This showed that the groups 1,2,3,4-tetrahydro-1-naphthyl,7-methoxy--1,2,3,4-tetrahydro-l-naphthy1 and 5,7-dimethyl--1,2,3,4-tetrahydro-l-naphthyl were found to be potential carboxamide protecting groups due to their stability in TFA-CH_2Cl_2-anisole (50:48:2 v/v) and their easy cleavage by BTFA.

The carboxamide protected derivatives whose protecting groups were found promising (7.,13 and 15Jwere used in the synthesis to the dipeptides Boc-Phe-Asn(1,2,3,4-tetrahydro-1-naphthyl)-g-OBzl, Boc-Phe-Asn(7-methoxy-1,2,3,4-tetrahydro-1-naphthyl)--a-OBzl and Boc-Phe-Asn(5,7-dimethy1-1,2,3,4--tetrahydro-l-naphthyl)-g-OBzl. The carboxamide protecting groups in these dipeptides also behaved in the same way as in 7,13 and 15. These dipeptides were used in .synthesis to the tripeptides, Boc-Ile-Phe-Asn-(1,2,3,4-tetrahydro-1-naphthyl)-6-OBzl, Boc-I1e-Phe-Asn-(7-methoxy-1, 2, 3, 4-tetrahydro-l-naphthyl) -ct^CBzl and Boc-I1e-Phe-Asn(5,7-dimethyl-1,2,3,4-tetrahydro-1-naph-thyl)-B-OBzl.

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CHAPTER ONE

INTRODUCTION

There are 20 naturally occurring a-amino acids, which are the main building blocks of all peptides and proteins. Thece amino acids are called a-amino acids because they bear an amino and a carboxylic acid group on the same carbon atom.

Of the a-amino acids, only glycine does not possess a snecific rotation. The rest contain an asymmetric centre namely the a-carbon. This centre confers on the molecule the property of chirality which is necessary condition for optical activity. The a-amino acids are classified into acidic, neutral and basic. They differ from each other by having different side chain group (R). The naturally occurring amino acids are the L-isomers (except glycine.) and some are listed on Table I ($H_2NCH(R)CO_2H$).

TABLE I

SOME OF THE NATURALLY OCCURRING a-AMINO ACIDS

Name	Abbreviati ons	R
	Acidic a-amino acids	
L-Aspartic acid	Asp	$-CH_2co_2H$
L-Glutamic acid	Glu	$-(CH_2)_2CO_2H$

TABLE 1 continued

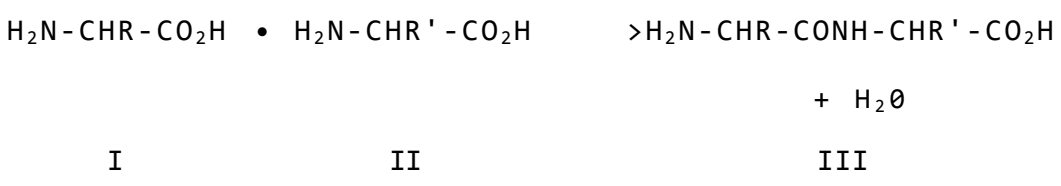
Name	Abbreviation	
	Neutral a-amino acids	
L-Cystine	(^ys (j:ys	-CH_2-S-S-CH_2CHINH"$_2$)CQ
L-Cysteine	Cys	-CH_2SH
L-Asparagine	Asn	-CH_2CONH_2
L-Phenylalanine'	Phe	-$CH_2C_6H_5$
L-Threonine*	Thr	-CH(OH)CH.
L-Serine	Ser	•CH_2OH
L-Glutamine	Gin	•$(CH_2)_2CONH_2$
(.-Methionine*	Met	•$(CH_2)_2SCH_3$
L-Tyrosine	Tyr	$CH_2C_6H_4OH$
Glycine	Gly	-H
L-Alanine	Ala	-CH.
L-Valine*	Val	•$CH(CH_3)_2$
L-Leucine*	Leu	•$CH_2CH(CH_3)_2$
L-Isoleucine	lie	•$CH(CH_3)CH_2CH_3$
	Basic a-amino acids	
L-Lysine	Lys	$(CH_2)_4NH_2$
L-Arginine	Arg	2 3 ,| 2 NH

'These are essential amino acids that arc needed in the diet to prevent a negative nitrogen balance.

In peptide synthesis involving preparation of larger molecules containing two or more amino acid residues joined together by a peptide bond, the carboxyl (-COOH)^" ° and amino group C-NH^)^' not participating in peptide bond formation and ether reactive functional groups must be protected. This protection of the amino acid minimises racemization, side products and increases its solubility. Since ami no acids contain at least one amino and one carboxy1 group they can take part in acylaticn reactions in two different ways: as acylating reactants (carboxyl components) or as the compound to be acylated (amino components). Thus if no preventive precautions are taken, a large number of undesired compounds will be formed in addition to the desired product as given in Scheme 1.

SCHEME 1

Coupling of two unprotected amino acids



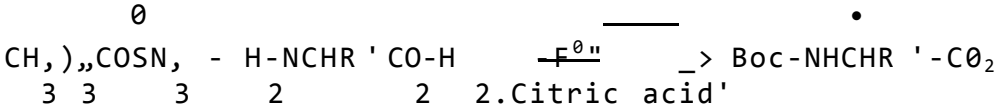
In addition to the required dipeptide III, carboxyl group of II can also couple with amino jjroup of I.

Two other dipeptides can also be formed by intermolecular coupling between two molecules of I and also of II. The coupling of I and II may yield not only the dipeptides but quite readily the cyclic dipeptides. The amino group may be protected by formyl , tosyl ' , phthaloyl ' , trifluoroacetyl, carbobenzoxy [1] [8] ' , tert-butyloxy-carbonyl[10"17], cyclopentyloxycarbony1, triphenyl-methyl(trityl) etc., while the carboxyl group may be protected by esterification using such groups as methyl[1,7], ethyl[1,7], benzyl[1-6,8], tert-butyl[18,19] etc. A series of reactions in Scheme 2 show how protection of amino and carboxyl groups in an a-amino acid may be carried out.

SCHEME 2

Protection of amino and carboxyl groups in amino acids.

(i) Amino group protection of one amino acid^^'*^



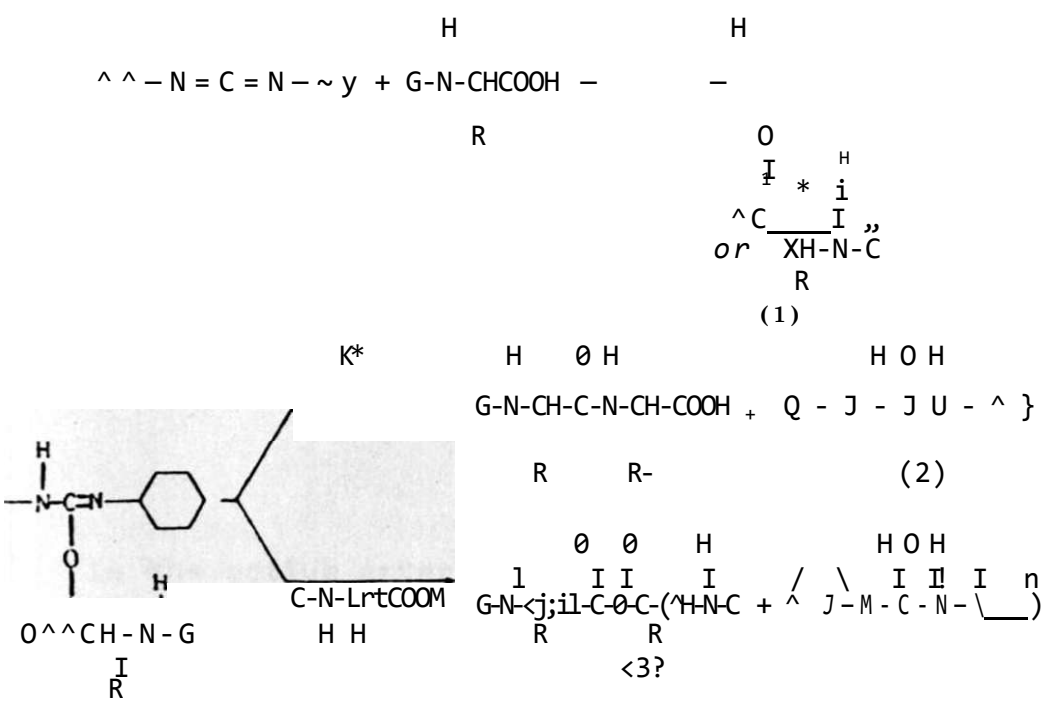
(ii) Carboxyl group protection in the other amino acid.[5,6]

$H_2NCHR"C0_2H$ • CGH_5CH_2OH ~~G^EAK~~ > $H_2NCHR"C0_2CH_2CGH_5$
base

In peptide bond formation, either carboxyl or amino group of the amino acid has to be activated, without which coupling is bound to be slow or not at all. Amino group activation is by isocyanates, phosphazo, phosphite amides etc. Carboxyl group activation is by acid chlorides, azides, mixed anhydrides, activated esters[2^ 22], carbodiimides[2^ etc. the latter are used as coupling agents in peptide synthesis. A coupling agent is a compound added to the mixture of carboxyl and amino components and its main function is usually to form an intermediate anhydride or active ester,- Bv far the most commonly used coupling agent is dicyclohexylcarbodiimide (OCC)[24,25]. The mechanism of the DCC-mediated peptide coupling was studied by D.F. Detar[26]
Addition of the- carboxyl component to the C=N double bond gives (l)j this is called an 0-acvlisourea (see Scheme 3). Intermediate (1) is not isolated as such but is allowed to undergo nucleophilic attack by the aijiino component. Intermediate (1J can also react with an additional mole of carboxyl component to form an anhydride (3), which can also acylate the amino component. One side reaction known to occur during the DCC coupling is the spontaneous rearrange-

SCHEME 3

Mechanism of the DCC-mediated peptide coupling

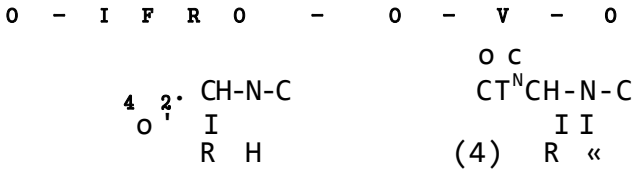


G represents amino protecting group.

roent of the O-acy1isourea (1) to the N-acylurea(4) (Scheme 4). This latter compound is unreactive towards amino acids. This rearrangement is minimized by working in methylene chloride or acetonitrile as so lvent?'[7]

SCHEME 4

Rearrangement of O-acy1isourea to N-acylurea



In the active ester method an N-protected amino acid is converted to an ester, which in general under mild conditions is a weaker acylating agent than is a mixed anhydride. It is found that ordinary methyl or ethyl esters react too slowly with amino acids to be practical. Groups on the alcohol part of the ester that tend to withdraw electrons should improve the susceptibility of the ester carbonyl to attack by a nucleophilic a-amino group. Schwyzer made use

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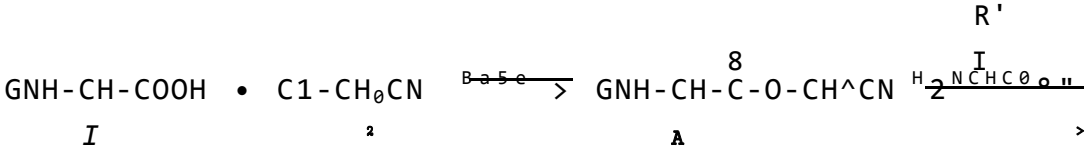
of cyanomethyl esters, where an electron-withdrawing nitrile group is in the alcohol part of the ester (Scheme 5).

SCHEME 5

Carboxyl Protection by cyanomethyl group.



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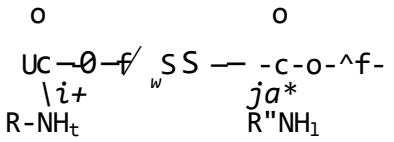
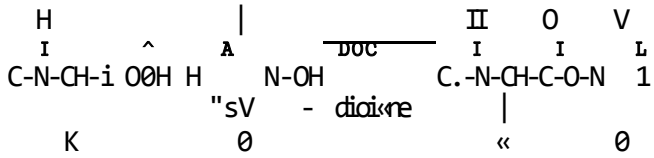
Later phenyl and nitrophenvl esters came into use. Here nucleophilic attack is encouraged by an increase in the electronic stability of the incipient phenoxide ion because of delocalization of negative charge onto an aromatic ring.



One of the active esters commonly used is N-hydroxy-succinimide developed by Anderson, Zimmerman, and Callahan[22]. The N-hydroxysuccinimide ester of an N-protected amino acid was synthesized as shown in Scheme 6.

SCHEME 6

N-Hycroxysuccinimid&_ ester cf N-protected amino acid.



In dipeptide synthesis, one has to go through the protection of amino group of the first amino acid before activating its carboxyl group. Thus if we protect the amino group of glycine with some reagent G-X, and activate the -COOH group with some reagent Y-G', then a synthesis of glycylalanine, would proceed as shown in Scheme 7,

SCHEME 7

Synthesis of glycyla Ianine

Protection $H_2N\text{-}CH_2\text{-}C\text{-}OH + G\text{-}X \longrightarrow GNH\text{-}CH_2\text{-}C\text{-}0H - HX$

Activation $GNH\text{-}CH_2\text{-}C\text{-}0H + Y\text{-}G' \longrightarrow GNH\text{-}CH_2\text{-}C\text{-}G' + Y\text{-}0$

Coupling $GNH\text{-}CH_2\text{-}C\text{-}G' + H_2N\text{-}CH\text{-}C\text{-}0Z$

CH_3

$GNH\text{-}CH_2C0NH(J:H\text{-}?\text{-}DZ + HG'$

CH_3

Deprotection $GNH\text{-}CH^ONHCH\text{-}C^\text{-}OZ \longrightarrow H_2N\text{-}CH_2C0NHJH\text{-}^OH$

CH_3 CH_3

G = amino protecting groupj Z = carboxyl protecting group.

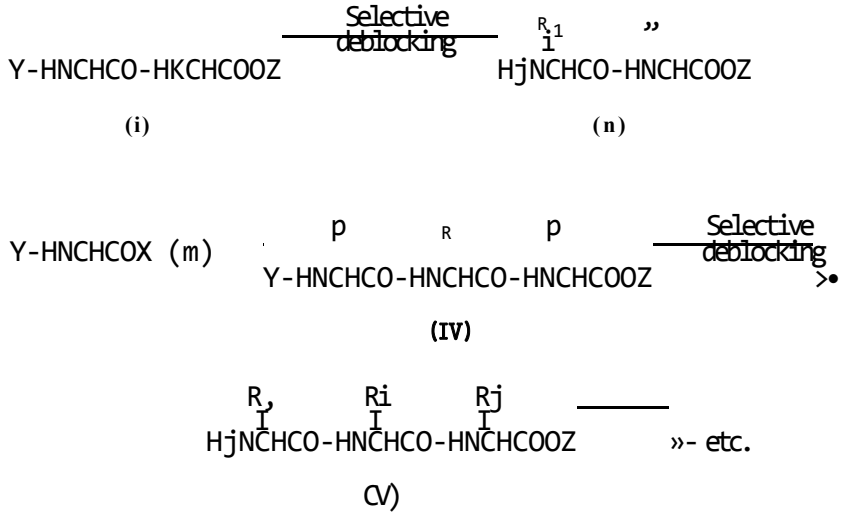
There are three general ways for the synthesis of a peptide chain: (a) stepwise elongation starting from the N-terminal amino acid, lb) stepwise elongation starting from the C-terminal amino acid, and (c) joining together small peptides with the proper partial sequences (fragment condensation). The three methods are shown in Schemes 8, 9 and 10 respectively.

(2) Stepwise elongation from the N-terminal amino acid (Scheme 6).

In this method selective unmasking of amino-protecting group of a peptide I affords an amino component II which in turn may be condensed with an activated acylamino acid III to give the corresponding protected peptide IV, which is selectively deblocked to give V, etc. Thus, elongation of the peptide chain by this approach involves the linking of one amino acid at a time to the N-terminal amino group of the growing peptide chain. Racemization is not a serious problem in this method, since activated acvl amino acids serve as carboxyl components.

SCHEME 8

Stepwise elongation from the N-terminal amino acid,



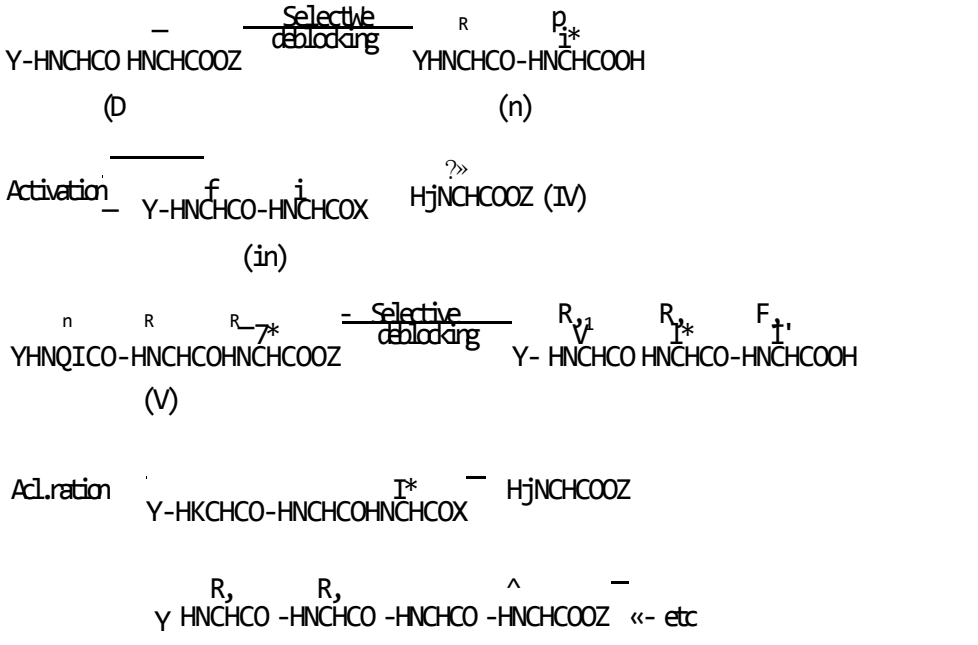
V = amino protecting groupi Z = carboxyl protecting group

(b) Stepwise elongation from the C-terminal amino acid (Scheme 9) .

In this approach the carboxyl protecting group(Z) of a peptide I is selectively removed, and the resulting new carboxyl component II is activated by e group (X). The activated peptide III subsequently reacts with an amino acid ester (or amino acid anion) IV to give the protected peptide V. In an analogous manner peptide V can be converted to VI, etc. Condensation of the acyl peptide carboxyl component II with the amino acid component IV can also be brought about by activating the amino group of the latter instead of the carboxyl group of the former. By either procedure elongation of the peptide chain is accomplished by linking one amino acid at a time to the carboxyl terminal amino acid residue of a given peptide chain.

SCHEME 9

Stepwise elongation from the C-terminal amino acid



(c) Fragment Condensation (Scheme 10)

This is elongation of the chain by the coupling of fragments. This mode need not imply an elongation in just one direction, but could start from the middle, for example, and build in both directions. This kind of coupling is the preferred method for large peptides.

SCHEME 10

Fragment condensation

GNHCHRCOG* + H^CHl*' COOZ ——> GNHCHRCONHCHR' COOZ

selective deblocking ^ GNHCHRCONHCHR' C00H —activate→ GNHCHRGONHCHR' COG'
C—tenninal

GNHCHR"C0G' + H_2NCHR'" COOZ —^ep protect→ N—terminal, H /NCHR* CONHaiR'" COOZ

GNHQIRCONHCHK[1] COG* • H^NQIR" C0NHCH«'W COOZ eking*

H_2NCHRC0NHaiRf CONH CH R*C0NHCHR"' COOH

G = amino protecting group* Z= corboxvl protecting group,

It should be obvious that not only the a-amino group of the N-terminal residue, but also certain side chain groups may require protection before coupling is to take place because many of them are also nucleophilic in nature. If R,R',R" etc. are

reactive groups such as -CH^CO^H, -Ch^-SH, $-(CH_2)_2C0_2H$, $-(CH_2)_4NH_2$, $-(CH_2)_2-SCH_3$,$-CH_2OH$, $-CH(CH_3)OH$, $-CH_2CgH_50H$, $-CH_2C0NH_2$, $-(CH_2)_2C0NH_2$ etc., they, too must be protected to prevent side reactions in the course of peptide synthesis.

Peptides constitute an important branch in chemistry because a lot of them possess biological activity. Some of the hormones important for normal body activities are made of peptides. These aut principally as transmitters of information and coordinators of the activities of the various tissues in the organism.

A selection of naturally occurring peptides are given in Table II. Most of these substances occur

TABLE II

Some of the naturally occurring peptides.

Peptide	Source	No. of amino acids
Vasopressin	Hypothalamus	9
Oxytocin	Posterior pituitary	9
Corticotropin	Anterior pituitary	39
Insulin	B-cells of pancrease	51

in man and with structural modifications in many other vertebrates as well. Both vasopressin and oxytocin are nonapeptides, similar in structure but yet different in function. They differ in amino acids at positions 3 and 8. Oxytocin causes stimulation

of milk release while vasopressin regulates the absorption of water at the distal renal tubule. All synthetic analogues of oxytocin in which other

1 2 3 4 5 6 7 8 9
C^s-Tyr-Ile-Gln-Asn-Cyjs-Pro-Leu-Gly-NH^ Oxytocin

1 2 3 4 5 6 7 8 9
Cys-Tyr-Phe-Gln-Asn-Cys-Pro-Arg-Gly-NH- Vasopressi

positions (3,4 and 8) have been altered show lower potency in contracting the mammalian uterus.

Studies on a number of biologically active peptides have shown that a number of these contain glutamine and/or asparagine amino acid residues, and other amino acids having amide groups at the carboxyl end of the peptide. In synthesizing these peptides containing carboxamide groups, it is important that the carboxamide side chain group be protected.

The amide groups of asparagine and glutamine undergo the following side reactions (eq 1-3) during peptide synthesis: (Scheme 11)[28,29]

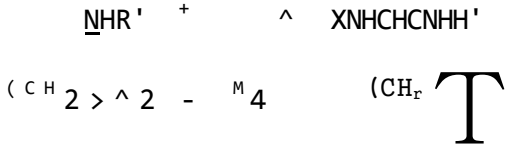
(1) deamination to the corresponding acidj

(2) formation of imides and subsequent hydrolysis [N-protected asparagine or glutamine enters (a), asparaginyl or glutaminvl peptides (b). In this case the loss of a proton by the action of alkali occurs in both position a and <J. The a site is more

SCHEME 11

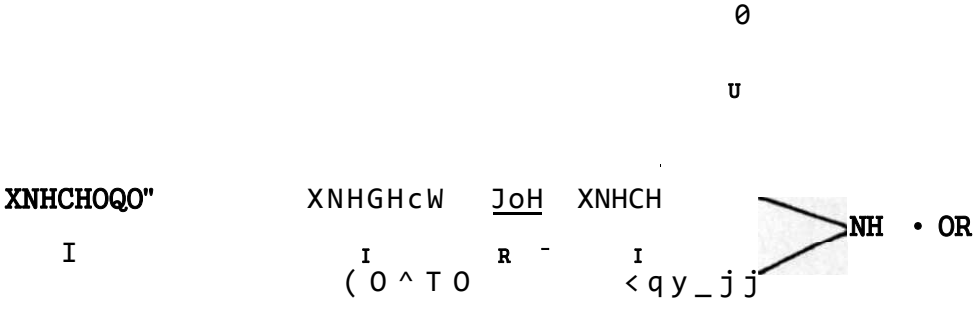
Side reactions of asparagine and glutamine amide groups

1) Deamination to the corresponding acids.



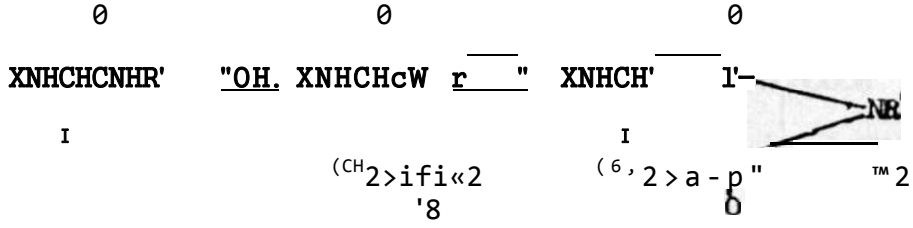
2) Formation of imides and subsequent hydrolysis

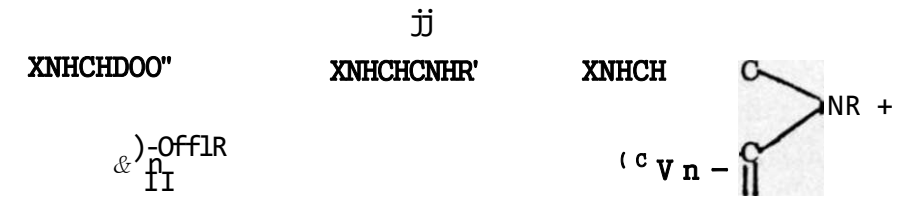
a) **N-protected asparagine or glatamine esters.**



b) **Asparaginyl or glutaminyl peptides.**

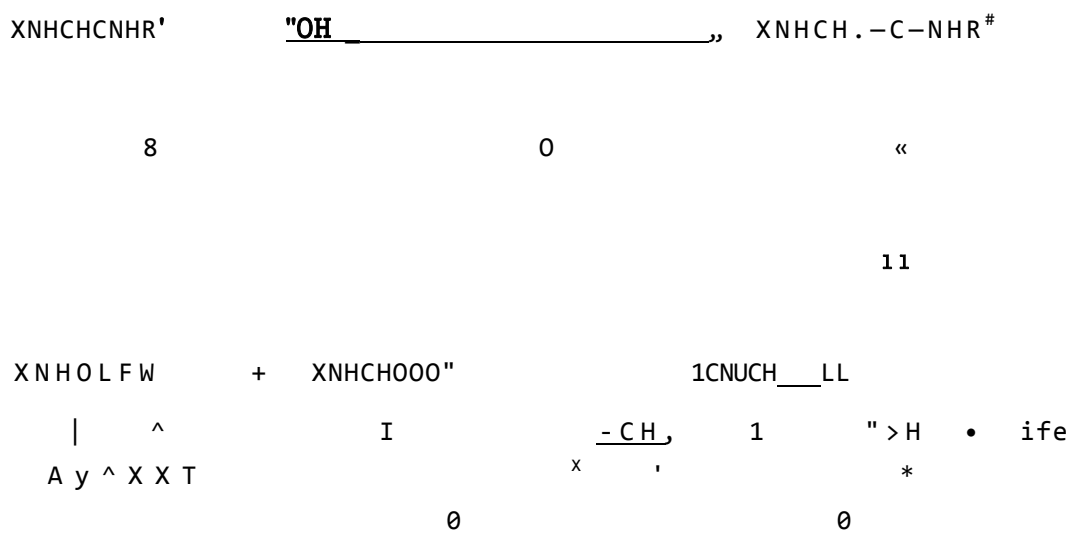
First Case: Initial abstraction of proton from peptide chain.



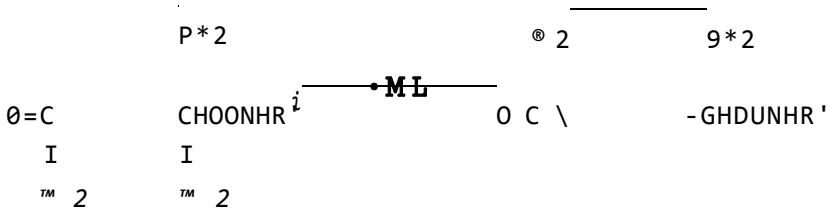


Scheme 11 Continued

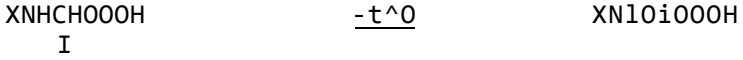
(c) Second case: Initial abstraction of proton from the side chain amide group.



3) Formation of pyroglutamyl peptides from glutaminyl peptides.



4) Dehydration



X = amino protecting group or peptide chain

n = 1, asparagine

n = 2, glutamine

R' = peptide chain

R = aryl or alkyl

reactive because of the greater e1ectrophy1ic strength of the a carbon atom as compared with that of the u> carbon atom. The subsequent release of the -NH^ group leads to formation of a and OJ isomeric peptides, though the latter is obtained in greater amount. Reaction at the w site causes cleavage of the peptide (c)]j (3) formation of pyroglutamyl derivatives from glutaminyl peptides* and (4) dehydration.

A number of carboxamide protecting groups have been studied. These include P-methoxybenzvl,[30] 2,4-dimethoxybenzyl,[31] 4,4'-dimethoxybenzhydryl,[32] 2,4,6-trimethoxybenzyl, xanthyl,[34] 4-methoxy-?-methylbenzyl,[35] etc. Although these groups have been successfully used in preparing dipeptides, -only benzhydryl group has been used in preparing longer peptides, where several amino-deprotecting and peptide bond formation steps have been successfully carried out in the presence of carboxcimide groups fully protected with benzhydryl group.[3 G] Thus, a good protecting group prevents side reactions, racemization and increases solubility of the protected amide.

The first part of this project is the conversion of 1-tetralone[37] derivatives to 1-aminotetra1in derivatives by Leuckart reaction which involves

heating aldehyde or ketone in the presence of excess ammonium formate to give formamide which upon hydrolysis gives the corresponding primary amine.[38-42]

Crossley and Moore[41] have indicated that there are a number of experimental conditions which influence the yield of the desired amine. In order to study the effect of the reagent upon the yield of amine, the two scientists carried out a series of experiments on the condensation of formamide with 3-phenyl-2-butanone in which the source of preparation of the formamide was varied. The reactions were carried out using a ratio of five moles of reagent to one mole of ketone and the formyl derivative was hydrolyzed in concentrated hydrochloric acid by refluxing for eight hours. Table III shows the yield of N-formyl-3-phenylbutan-2-amine obtained by using the various reagents. Table III shows, that the use of formamide gave a lower yield of product than that obtained from ammonia and formic acid. The addition of formic acid to formamide increased the yield to that obtained from the ammonia and formic acid reagent.

They also found that the temperature at which the reaction was carried out had an effect on the yield. In order to determine the effect of temperature on the yield of product, three experiments

TABLE III

Effect of variation in the Leuckart reaction on the condensation with 3-nheny1-2-hutanone. Acid hydrolysis of formyl derivative.

Run	Leuckart reagent	Temp.°C	Time, hrs	Yield of amine;
1	Formamide	170-180	22	27
2	Formic acid and ammonia (5 moles each)	175-185	21	45
3	Formamide and formic acid (2.5 moles each)	170-180	22	47
4	Formamide and formic acid (2.5 moles each)	170-180	15	48
5	Formic acid and ammonia (5 moles each)	160-170	7.	50

were run on 3-phenyl-2-butanone, using the reagent from ammonia and formic acid in which the temperature of the reaction was varied, although all were heated for fifteen hours. The results are shown in Table IV. It is evident that the yield was influenced by

TABLE'IV

Effect of temperature on the Leuckart reaction with 3-phenyl-2-butano'ne. Time: Fifteen hours. Acid hydrolysis of formyl derivative.

Run	Temp.°C	Amine %
6	190-200	23
7	170-180	47
0	160-170	50

the temperature at which the condensation was carried out and in these experiments the yield was twice as much at 160-170 as at 190-200°.

The effect of varying the time of heating when carrying out the condensations at various temperatures is shown in Table V. A yield of 50% was obtained when the condensation was carried but

TABLE V

Fffect of time on the Lnuckart condensation with 3-phenyl-2-butanone. Temperature: 190-200°.

Run	T ime,hrs	Amine%
9	15	23
10	5	50
11	3	47
	Temperature:	160-170'
12	15	50
13	7	50
14	4	48

at 190-200° for five hours, while a yield of only 23% was obtained after heating for fifteen hours. The reaction may be heated for fifteen hours at 160-170° without a reduction in yield, although the same results were obtained by heating for as short a period as four hours.

At least two procedures have been described for the hydrolysis of the formyl derivative to the corresponding amine. In one, 30% sodium hydroxide solution was used and in the other concentrated hydrochloric acid was the hydrolytic agent. The effect of various concentrations of these two hydrolytic agents on purified N-formyl-3-pheny1-butan-2-amine is shown in Ta'ble VI. At the same time, the two made an extensive study of the variation

TABLE VI

Effect of time and reagents on the hydrolysis of N—formyl-3-phenylbutan-2-amine.

Run	Hydrolytic reagent	Time of hydro-lysis,hrs	Amine yield %
15	Concentrated hydrochloric acid	8	59'
16	10% hydrochloric acid	8	75
17	10% hydrochloric acid	1	67
18	30% sodium hydroxide	8	0
19	10% sodium hydroxide	8	71
20	6.6% " "	8	76

in time and experimental conditions on the hydrolysis of the formyl derivative from the reaction mixture

without subsequent purification. The best yield was obtained by hydrolyzing the formyl derivative directly in the reaction mixture with concentrated hydrochloric acid.

In .this project it is proposed to carry out a conversion of 1-tetralone and some of its derivatives to the amines by Leuckart reaction. In tnis reaction the ketones are converted to the formyl derivatives. Hydrolysis of these derivatives to the amines is carried out using 36% concentrated hydrochloric acid on the one hand and in the other 10% aqueous NaOH was the hydrolytic ac.ent,

The next stage is in using these amines (1-aminotetralin and its derivatives) as precursors in synthesizing the carboxamide protected derivatives of a-benzyl t^-butyloxycarbonylglutamate, a-benzyl t:-bu ty loxycarbonylgspartate and B-ben.:yl ^-butyl-oxyearbonylaspartate.

After successfully preparing carboxamide . protected glutamine and asparagine derivatives, stability of their protecting groups in TFA-

. CH_2Cl_2-anisole(50:48:2 v/v) are studied using thin-layer chromatography (TLC). The protectinp group(s) that is/are not cleaved -For upto 24hr is/are then subjected to boron trifluoride in acetic acid (BTFA). Generally a good carboxamide protecting

group should be stable in TFA-Ch^C^-anisole and HCl-dioxane[44] (reagents used for removal of amino-protecting groups) and should be readily removed with strong cleavage reagents like BTFA^'[4]^ and $HF_{\#}$® which are used for complete removal^of most of the protecting groups at the end of peptide synthesis. Complete removal of the car.boxamide - protecting group gives a free amide group. Optical activity[49] of the peptide or amino acid should be maintained during the introduction and removal of the protecting groups.

The protecting groups that are stable in TFA-CF^C^-anisole but cleaved when subjected to BTFA will be promising as carboxamide protecting groups. Some of these carboxamide protected derivatives whose protecting groups are nromising will be used to synthesize a few peptides for testing their suitability as protecting groups under peptide synthesis conditions.

CHAPTER TWO

RESULTS AND DISCUSSION

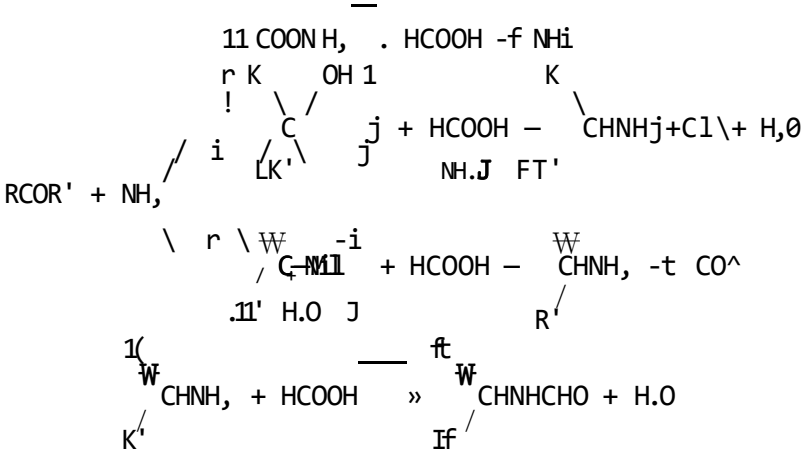
2.1 SYNTHESIS QF AMINES BY LEUCKART REACTION:

Although the exact mechanism has not been definitely established, the reaction can be explained in the following steps: (a) the ammonium formate dissociates into ammonia and formic acid at the temperature of the reactionj and (b) ammonia adds to the carbonyl group ur condenses to form the corresponding imine, (c) the formic acid then acts as a reducing agent to remove the hydroxyl or reduce the imino groupj and (d) if in excess, may form the formyl derivative which is subsequently hydrolyzed to the free amine. The other proposed mechanism is where formamide is the reactive agent (both depicted in Scheme 12).

SCHEME 12

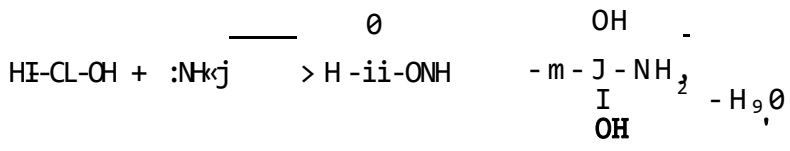
Leuckart reaction mechanism

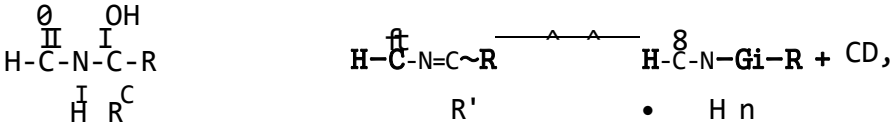
Case one:



Scheme 12 Continued

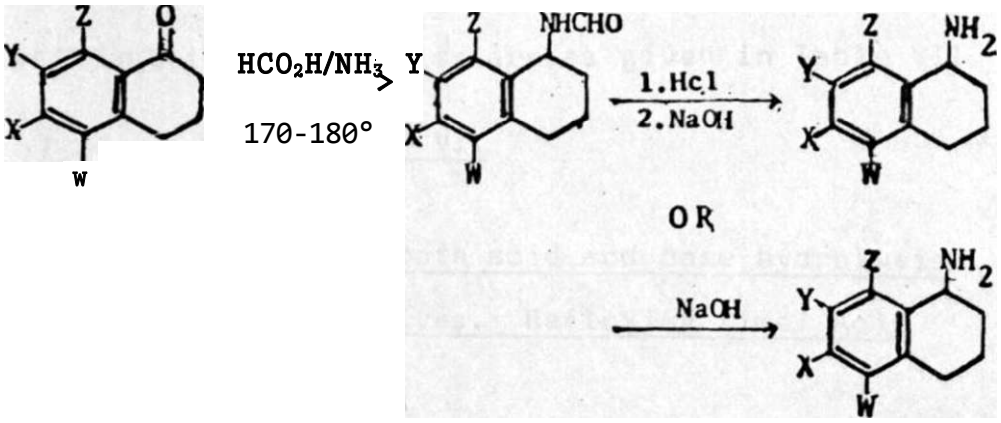
Case two:





SCHEME 13

2.1.1 CONVERSION OF 1-TETRALONE AND ITS DERIVATIVES TO 1-AHINOTETRALIN DERIVATIVES.[41]



1-S la-Sa lb-5b

	W	X	Y	Z
1,1a,1b	H	H	H	H
2,2a,2b	CH_3O-	H	H	H
3,3a,3b	H	CH_3O	H	H
4,4a,4b	H	H	CH_30	H
5,5a,5b	CH_3	H	CH_3	H

the naming of the compounds are as follows:-

1 = 1-Tetralone 1b = 1-Aminotetralin

2 = 5-Methoxy-l-tetralone 2b = l-Amino-5-methoxytetralin

3 = 6-Methoxy-l-tetralone 3b = l-Amino-6-methoxytetralin

4 = 7-Methoxy-l-tetralone 4b = 1-Amino-7-methoxytetra1in

5 = 5,7-Dimethyl-l-tetralone 5b = 1-Amino-5,7-dimethy1tetra1in

la = N-l,2,3,4-Tetrahydro-l-naphthyl formamide

2a = N-5-I^{v}lethoxy-l,2,3,4-tetrahydro-l-naphthyl formamide

3a = N-6-Methoxy-l,2,3,4-tetrahydro-1-naphthyl formamide

4a = N-7-Methoxy-l,2,3,4-tetrahydro-l-naphthyl formamide

5a = N-5,7-Dimethyl-l,2, 3,4-tetrahydro-1-naphthyl formamide

The compounds la-5a are the formyl derivatives and the hydrolysis was with 36% concentrated hydrochloric acid on the one hand and in the other 10% aqueous NaOH was the hydrolytic agent. The results are as given in Table VII.

TABLE VII

Amine synthesis from both acid and base hydrolysis of the formyl derivatives. Refluxing time: Acid 3hn Base 3hr.

Formyl der.	AcidtAmine %) (crude formyl der.)	Acid(Amine %) (Pure formyl der.)	B.ise (Amine %)
la	44	54	97

TABLE VII Continued

Formyl der.	AcidCAmine %) (Crude formyl der.)	Acid (Amine %) (Pure formyl der.)	Base (Amine %)
2a	60	75	95
3a	0	U	94
4a	0	0	96
5a	40	52	90

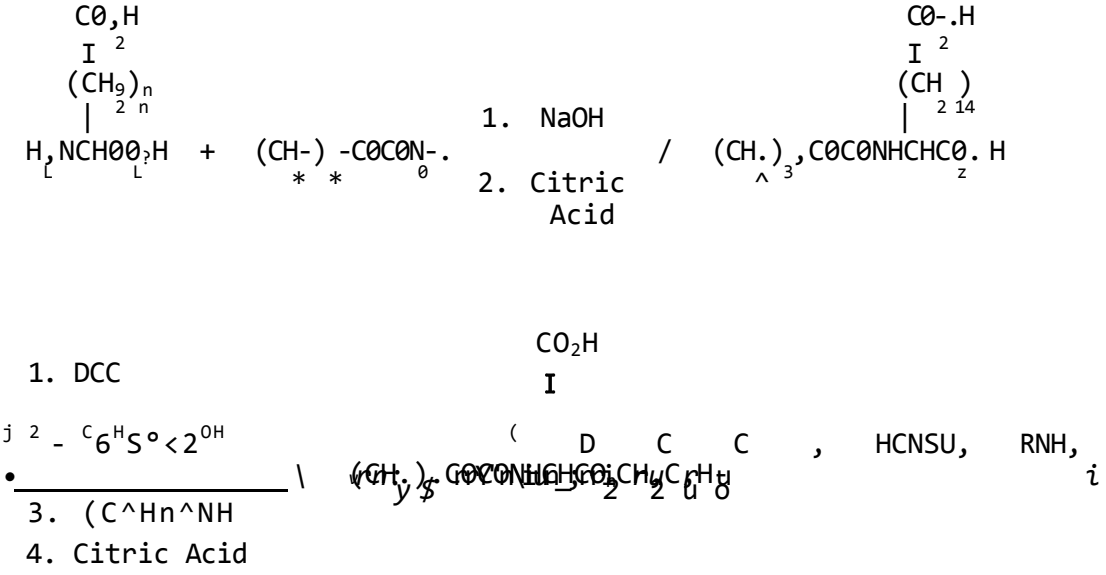
Acid hydrolysis of formyl derivatives were done in two ways. In case one, the crude formyl derivatives were refluxed directly with concentrated hydrochloric acid. In the other case, the already purified formyl derivatives were refluxed with the concentrated acid. The amine yields of the first case are based on the starting ketones while in case two the yields are based on the pure formyl derivatives. Base hydrolysis was by refluxing purified formyl derivatives with 10% aqueous NaOH. It can be concluded from the table that a higher yield of all the amines was obtained when 10% aqueous NaOH was the hydrolytic agent as compared to 36% concentrated hydrochloric acid. The amines were not obtained when acid hydrolysis of N-6-methoxy-1, 2,3,4-letrahydro-1-naphthyl formamide and N-7-methoxy-1, 2,3,4-tetrahydro-1-naphthyl formamide were done. The black-gummy compound that was obtained after hydrolysis could have been due to the ease of polymerization of their formyl derivatives.

2.2 SYNTHESIS OF CARBOXAMIDE PROTECTED ASPARAGINE AND GLUTAMINE DERIVATIVES[5,11]

Amines (RNH_2) will react with Boc-Asp-a-Q3z1 and Doc-Glu-a-OBzl in the presence of coupling reagents e.g. DCC to give a-benzyl tert-butyloxy-carbonyl-N^{CA}-R-asparaginate and a-benzyl tert-butyloxycarbonyl-N^{CA}-R-glutaminate respectiv-elv as shown in Scheme 14 (R=Carboxamide protecting group) The coupling gave yields ranging from 40% to 69%.

SCHEME 14

Synthesis of carboxamide protet-tud asparagine and glutami-ne derivatives.



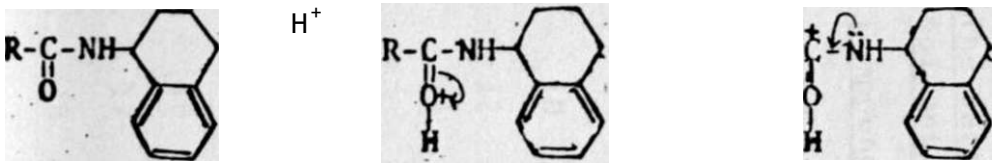
2.3 CLEAVAGE STUDIES OF THE CARBOXAMIDE PROTECTED DERIVATIVES OF GLUTAMINE AND ASPARAGINE IN TFA-CH_2Cl_2-anisole (50:48:2 v/v) AND SOME OF THEM IN BTFA-TFA.

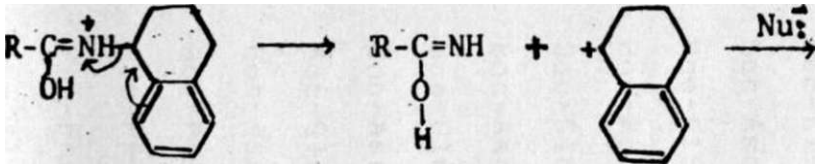
Both Boo and benzyl protecting groups are easily removed under mild conditions. The former is selectively removed with 50% TFA-CH_2C1_2 while the latter is selectively removed.by catalytic hydrogenation. The two groups are easily removed by HF and BTFA-TFA. In addition to removing these two protecting groups, they also remove carbobenzox trityl, tert-butyl, diphenylmethy1, xanthyl, 2,4-dimethoxybenzyl etc. They do not remove methyl ethyl esters, or affect peptide bonds. BTFA-TFA was tested to find whether it would remove some of the glutamine and asparagine carboxamide protecting groups. Anisole was used as a carbonium ion trap.

The proposed cleavage mechanism of the carboxamide protecting group in acid is given in Scheme 15.

SCHEME 15

Proposed cleavage mechanism of the carboxamide protecting group





R is $H_2NCH(CH_2)_n$- with n= 1 or 2
$C0_2H$

The carboxamide protected asparagine and glutamine derivatives treated with TFA-CH_2Cl_2-anisole (50:48:2 v/v) and BTFA-TFA are listed on Table VIII. The standard compounds that were used in cleavage components identification are shown in Table IX. The cleavage results for TFA-CH_2Cl_2-anisole and BTFA-TFA are given on Tables X and XI respectively.

TABLE VIII

The carboxamide protected asparagine and plutamine derivatives and their Rfs in solvent system F.

Compound No.	Compound	Rf
6	Boc-Gln(1,2,3,4-tetrahydro-l-naphthyl)-a-0Rzl	0.63
7	Boc-Asn(1, 2, 3,4-tetrahydro-1-naphthyl) -p-DBzl	0.89
8	Boc-Gln(5-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-a-G9zl	0.81
9	Boc-Asnt 5-methoxy -1,2, 3, 4-tetrahydro -1-naphthyl)-B"03zl	0.83-
10	Boc-Gln(6-methoxy-l,2,3,4-tetrahydro-l-nanhthyl)-a-03zl	0.78
11	Boc-Asn(6-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-a-D3zl	0.77
12	Boc-Gln(7-methoxy-1,2,3,4-tetrahydro-l-nanhthyl)-a-03zl	0.82
13	Bnc-Asn(7-methoxy-l,2,3,4-tetrahydro-1-naphthyl)-a-03zl	0.83
14	Boc-Gln(5,7-dimethyl-1,2,3,4 -tetrahydro-1-naphthy1)-a-OBzl	0.87
15	Boc-Asnt 5,7-dimethvl-l,2,3,4-tetrahydro-l-naphthyl)-6-0Bzl-	0.91
16	Boc-Phe-Asn (1 , 2, 3, 4-tetrahydro-1-naphthylB-OBzl	0.75
17	Boc-Phe-Asn(7-methoxy-1,2,3,4 -1etrahydro-1-naphthy1)-a-0Bzl	0.69
18	Boc-Phe-Asn(5,7-dimethyl-l,2,3,4-tetrahydro-l-nanhthyl)-B-0Bzl	0.76
19	Roc-Ile-Phe-Asn(l,2,3,4-tetrahydro-l-nanhthyl)-6-0Bzl-	0.73*
20	Boc-Ile-Phe-Asn(7-methoxv-l,2,3,4 -tetrahydro-1-naphthy1)-a-OBz1	0.33*
21	Boc-I le-Phe-Asn(5, 7-dimethyl - 1,?, 3, 4-tetrahydro - 1-naphthyl) - 6-HBzl	*0.-70**

'solvent syct cm (.'

TABLE IX

Standard compounds for cleavage components identification. Solvent system F was used.

Compound	
H-Gln(1,2,3,4-tetrahydro-l-naphthvl)-a-OBzl	0.51
H-Asn(1, 2, 3, 4-tetFahvdro-l-naphthvl) -fj-PBzl	0.64
H-Gln(5-methoxy-l,2,3,4-tetrahydro-1-naphthy1)-a-OBzl	0.52
H-Asn(5-methoxy-l,2,3,4-tetrahydro-1-naphthy1)-6-OBzl	0.59
H-Gin(6-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-a-OBzl	0.50
H-Asn(6-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-a-OBzl	0.55
H-Gln(7-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-a-OBzl	0.50
H-Asn(7-methoxy-l,2,3,4-tetrahydro-1-naphthvl)-a-OBzl	0.58
H-Gin(5, 7-dimethy1-1,2,3,4-tetrahydro-1-naphthy1)-a-OBzl	0.54
H-Asn(5, 7-dimethyl-l, 2, 3, 4-tetrahydro-1-naphthyl).-B-OBzl	0.60
a-Benzy1-L-glutaminate	0.18
L-glutamine	o.o6
B-Benzyl-L-isoasparaginate	0.13
L-Isoasparagine	0.05
a-Benzyl-L-asparaginate	0.17
•L-Asparagine	Q.Q5

TABLE X

TFA-CH-,C 1-,-anisole (50:48;2 v/v) cleavage studies of the carboxamide protecting groups of glutamine and asparagine. l=starting compoundj 2=derivative with only Boc-deprotectedi 3=derivative with both Boc and carboxamide protecting group deprotected or Boc and benzyl group deprotectedj 4=all the protecting groups deprotected. Solvent system F.

Compound	Cleavage duration, hr	Cleavage products (R^.) 1	2	3	4	Extent of protecting group removal (24hr)
6 '	0	0.83	-	-	-	Partial
	1	-	0.51			
	3	-	0.51	0.18	-	
	5	-	0.51	0.18	-	
	7	-	0.51	0.18	-	
	24	-	0.51	0.18	-	
7	0	0.89				No removal
	1	-	0.64			
	3	-	0.64			
	5	-	0.64	-		
	7	-	0.64			
	24	-	0.64	-		
0	0	0.81	-	-	-	Partial
	1	-	0.52			
	3	-	0.52			
	5	-	0.52			
	7	-	0.52	0.18	-	
	24	-	0.52	0.18	-	

TABLE X Continued

Compound	Cleavage duration. hr	Cleavage products (R,J 1	2	3	4	Extent of protecting group removal (24hr).
9	0	0.83	-	-		Partial
	1	-	0.59	0.13		
	3	-	0.59	0.13		
	5	-	0.59	0.13		
	7	-	0.59	0.13		
	24	—	0.59	0.13		
10	0	0.78	-			Complete removal
	1	-	0.50	0.18		
	3	-	0.50	0.18		
	5	-	0.50	0.18		
	7	-	0.50	0.18		
	24	—	-	0.18		
11	0	0.77			T	Complete removal
	1		0.58	0.17.		
	3		0.58	0.17		
	5		0.58	0.17		
	7		0.58	0.17		
	24			0.17		
12	0	0.82				Partial
	1	-	0.50			
	3	-	0.50			
	5	-	0.50			
	7	-	0.50			
	24	-	0.50	0.18		

TABLE XI Continued

Compound	Cleavage duration,	Cleavage products (R^) 1 2	3	4	Extent of protecting group removal (24hr).
13	0	0.83			No removal
	1		0.58		
	3		0.58		
	5		0.58		
	7		0.58		
	24		0.58		
14	0	0.87			Partial
	1		0.54	0.18	
	3		0.54	0.18	
	5		0.54	0.18	
	7		0.54	0.18	
	24		0.54	0.18	
15	0	0.91			No removal
	1		0.60		
	3		0.60		
	5		0.60		
	7		0.60		
	24		0.60		
16	0	0.75			No removal
	1		0.62		
	3		0.62		
	5		0.62		
	7		0.62		
	24		0.62		

TABLE XI Continued

Compound	Cleavage duration.	Cleavage products (R_f) 1	2	3	4	Extent of protecting group removal (24 hr).
17	0	0.69	-	-		No removal
	1	-	0.57	-		
	3	-	0.57	-		
	5	-	0.57	-		
	7	-	0.57	-		
	24	–	0.57			
18	0	0.76	-			No removal
	1	-	0.60	-		
	3	-	0.60	-		
	5	-	0.60	-		
	7	-	0.60	-		
	24	-	0.60	-		

TABLE XI

BTFA studies of carboxamide protecting groups of asparagine

Compound	Cleavage duration, hr	Cleavage products (R^) 1	2	3	4	Duration for complete removal of protecting group (hr)
7	0	0.89	-			4
	1	-	0.64	0.22		
	2	-		0.22	0.05	
	3	-	-	0.22	0.05	
	4	-			0.05	

TABLE XI Continued

Compound	Cleavage' duration, hr	Cleavage products (R_f)			Ourajion for^ of protectinc group (hrj
13	0	0.83	-		
	1	-	0.58	0.37	0.05
	2	-		0.17	0.05
	3	-			0.05
15	0	0.91			
	1			0.13	0.05
	2			0.13	0.05
	3				0.05
	0	0.75			
	1		0.62	0.12	
	2			0.12	
	3			0.12	0.02
	4				0.02
17	0	0.69			
	1		0.57	0.12	
	2			0.12	
	3				0.02
13	0	0.76			
	1			0.12	
	2			0.12	
					0.02

The case with which the carboxamide protecting group is removed will in theory aepeno cn the stabilization of the carbonium ion by either inductive or resonance effect of the substituent group on the 1-tetralinyl ^oup. The unsubstituted 1-tetralinyl group will give a secondary carbonium ion which is stabilized by the delocalization

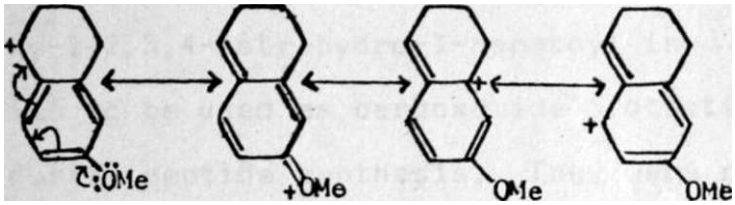
process and the inductive effect of the ring.When an electron donating group is substituted on positions 5,6,7 or 8 of the 1-tetralinyl group, the carbonium ion formed is more stable than the one formed from the unsubstituted 1-tetralinyl group. These groups would then be more easily removed under acidic medium than the unsubstituted 1-tetralinyl group. However, 1-tetralinyl groups with electron donating groups at positions 6 and 8 would be more readily cleaved than those with substituents at positions 5 and 7 as they would be respectively at para and ortho positions with respect to the carbon one where the amino group was attached in the original carboxamide protected compound. Thus after cleavage the delocalized structures will at one stage have the carbon bearing the positive charge attached to the electron conating group with an effective distribution of charge, while for positions 5 or 7 there is separation of charge in delocalized structures as shown in Scheme 16.

Cleavage studies on the carboxamide protected derivatives of glutamine (6,8,10,12 and 14) showed that all the protecting groups were removed by TFA-CH_2Cl_2-anisole (50:48:2 v/v) within 24 hrs. For the carboxamide protecting groups in asparagine

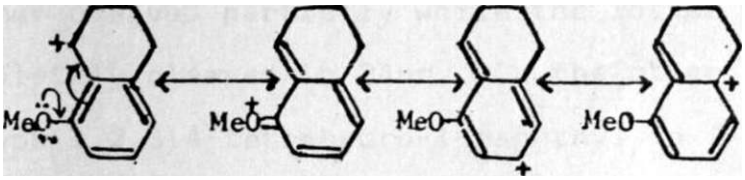
SCHEME 16

Resonance structures of 1-tetralinyl group with electron donating groups at positions 6,8,5 or 7

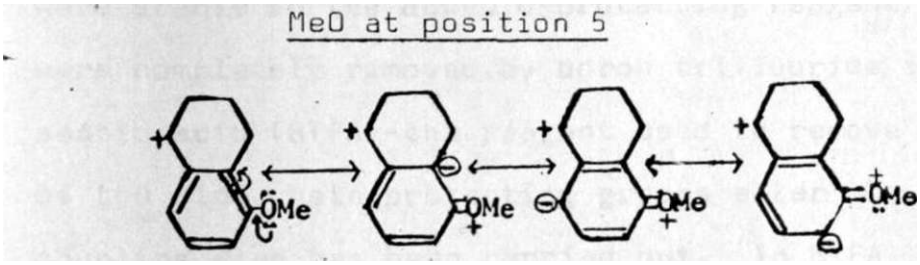
MeO at position 6



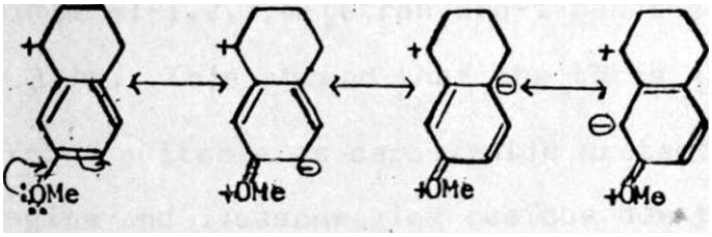
HeO at position 8



MeO at position 5



MeO at position 7



derivatives, it was found that the groups 5-methoxy-l,2,3,4-tetrahydro-l-naphthyl in 9 and 6-methoxy-l,2,3,4-tetrahydro-1-naphthyl in 11 were too labile to be used as carboxamide protecting groups during peptide synthesis. They, were readily removed by TFA-CH_2Cl_2-anisole (50:48:2 v/v) immediately before the next amino acid residue was coupled into the growing peptide chain, with the former cleaved partially while the latter was completely cleaved in 24hr. On the other hand the groups 1,2,3,4-tetrahydro-l-naphthyl in 7 , 7-methoxy-l,2,3,4-tetrahydro-1-naphthy1 in 13 and 5,7-dimethyl-l,2,3,4-tetrahydro-l-naphthyl in 15 were stable in the above deprotecting reagent but were completely removed by boron trifluoride in acetic acid (BTFA)-the reagent used to remove most of the side chain protecting groups after the final coupling step has been carried out. In BTFA, the group 1,2,3,4-tetrahydro-l-naphthy1 in 7 was completely removed after 4hr, 7-methoxy-1,2,3,4--tetrahydro-l-naphthyl in 13 after 3hr and 5,7-dimethyl-l,2,3,4-tetrahydro-1-naphthyl in 15 after 3 hr. This showed that the three groups were found suitable as carboxamide protecting in asparagine and isoasparagine residue due to their stability in TFA-CH_2Cl_2-anisole (50:48:2 v/v) but were readily cleaved by BTFA. Final products

were isoasparagine and asparagine. As mentioned earlier, 1-tetralinyl groups with electron donating groups at positions .6 and 8 would be expected to be more readily cleaved as carbonium ion formed will be more resonance stabilized. This explains why the 6-methoxy-1,2,3,4-tetrahydro-1-naphthy1 group was the most easily cleaved. The 5-methoxy-1,2,3,4--tetrahydro-l-naphthyl group whose carbonium ion is not stabilized by the methoxy group by resonance is not as easily cleaved as the former group.

1,2,3,4-tetrahydro-l-naphthyl shows marked stability in TFA-CH_2Cl_2-anisole (50:48:2 v/v) since the carbonium ion formed from the unsubstituted 1-tetralinyl group is less stable. 7-methoxy--1,2,3,4-tetrahydro-l-naphthyl and 5,7-dimethyl--1,2,3,4-tetrahydro-l-naphthyl also show marked stability because the delocalized structures after cleavage do not at any one stage have the carDon bearing the positive charge attached to the electron donating groups (only possible for positions 6 or 8). In BTFA, 7-methoxy-l,2,3,4-tetrahydro-l-naphthyl and 5,7-dimethyl-l,2,3,4-tetrahydro-l-naphthyl groups are slightly less stable as compared to the unsubstituted 1-tetralinyl group. This is because

the first two have electron donating groups on the ring and hence a slightly more stable carbonium ion is formed as compared to the carbonium ion formed by the unsubstituted 1-tetralinyl group.

2.4 APPLICATION OF CARBOXAMIDE PROTECTED DERIVATIVES IN PEPTIDE SYNTHESIS.

The carboxamide protected derivatives whose protecting groups were found promising (7,13 and 15) were used in peptide synthesis. This was done by deprotecting Boc groups of the carboxamide protected derivatives, followed by coupling with N-hydroxysuccinimide ester of N-protected amino acid (Scheme 17). The dipeptide carboxamide protecting groups 1,2,3,4-tetrahydro-1-naphthyl in 16, 7-methoxy-1,2,3,4-tetrahydro-1-naphthy1 in 17 and 5,7-dimethyl- 1,2,3,4-tetrahydro-1-naphthy1 in 18 were stable in TFA-CH_2C1_2~aniso1e (50:48:2 v/v) within 24hr. In BTFA, the group in 16 was completely cleaved after 4hr, in 17 after 3hr and in 18 after 3hr. These protecting groups were therefore found promising due to their stability in TFA-CH_2Cl_2-anisole (50:48:2 v/v) and their easy cleavage by BTFA. These dipeptides were used in

synthesizing the tripeptideS 19, 20 and 21- respectively (Scheme 18). The dipeptides yield ranged from 75% to 90% while tripeptides were from 70% tu 70%.

SCHEME 17

Oipeptide synthesis from carboxamide protected derivative

Boc-Asn(R)-a-OBzl —(1.TFA, 2.Triethvlami'nc)→ H-Asn(R)-a-OBzl

→ Boc-Phe-Asn(R)-a-ORz1

SCHEME 18

Tripeptide synthesis from the fully protected dipeptide

^(Dipep^Se)$^{3"0"0821}$ —(1 TFA, ~~^'riethylamin^~~)→ H-Phe-Asn(R)-a-OBz1

~~Boc-I1e-ONSu^~~ → $_{B}$oc-Ile-Phe-Asn(R)-a-OBzl (Tripeptide)

R= Carboxamide protecting group in 7, 13 or 15.

CHAPTER THREE

3.1 GENERAL EXPERIMENTAL SECTION:

Ascending thin-layer chromatograms (TLC) were run on silica gel G(60) with solvent systems benzene, Aj chloroform, Bj chloroform-ethyl acetate (3:1 v/v), Cj chloroform-acetone (1:1 v/v), Oj diethyl ether, E» chloroform-methanol-glacial acetic acid (85:10:5 v/v), F. Cleavage solvent H was trifluoro-acetic acid-dichloromethane-anisole (50:48:2 v/v). Melting points (uncorrected) were determined in capillary tubes in a Gallenkamp melting point apparatus. Spots were revealed with iodine vapour and ninhvdrin solution (0.2g ninhydrin in 100ml of acetone). Acid hydrolyzates of peptides were prepared using 6N hydrochloric acid (110°, 16hr). Nuclear magnetic resonance spectra (NMR) were measured using a Perkin-Elmer R12B(60MHz). Infrared snectra (IR) were measured using a Pye-Unicam SP3-300 infrared spectrophotometer.

3.2 SYNTHESIS OF 1-TETRALONE[37]

In a 500-rnl round-bottomed flask, fitted with a reflux condenser carrying at the top a tube leading to a gas absorption trap, were placed 32.8g (0.2 mol) of y-phenylbutyric acid and 32g (0.27 mol) of thionyl chloride. The mixture was carefully heated on a steam

bath until the acid was melted, and then the reaction was allowed to proceed without the application of external heat. After twenty five to thirty minutes hydrogen chloride was no longer evolved and the mixture was warmed on the steam bath for ten minutes. The flask was then connected to the water pump, evacuated, and heated for ten minutes on the steam bath and finally for two or three minutes over a small flame in order to remove the excess thionyl chloride. The acid chloride thus obtained was a nearly colourless liquid and needed no further purification. The flask was cooled, 175ml of carbon disulfide was added and the solution cooled in an ice-bath. 30g (0.23 mol) of aluminium chloride was added rapidly in one lot, and the flask was immediately connected to the reflux condenser. After a few minutes, the rapid evolution of hydrogen chloride ceases and the mixture was slowly warmed to the boiling point on the steam bath. After heating and shaking the mixture for ten minutes the reaction was complete. The reaction mixture was cooled to 0°C, and the aluminium chloride complex was decomposed by the careful addition, with shaking, of lOOg of ice. 25ml of concentrated hydrochloric acid was added and the mixture transfered to a 2-1 round-bottomed flask and steam-distilled. The carbon disulfide distilled

first, then there was a definite break in the distillation, after which the reaction product comes over completely in about 2-1 of the next distillate. The oil was separated, and the water was extracted three times with 100-ml portions of benzene. The oil and extracts were combined, solvent removed, and the residue distilled at reduced pressure: wt. 18.lg (62%)j b.p. 113-116°/6mm. TLC, solvent system E, R^- = 0.93. IR spectrum (neat, vmax): aromatic proton stretch, 3030 cm ^j C=0 group, 1680 cm"[1]. NMR spectrum ($CC1_4$) : 67.25 (m, 4H) for the aromatic protonsj 62.'1 (m, 2H) , 62.5 (m,2H) and 62.1 (m,2H) for the tetralinyl methylene protons.

3.3 SYNTHESIS OF FORMYL DERIVATIVES[41]

3.3.1 N-6-METH0XY-l,2,3,4-TETRAHYORO-1-NAPHTHYL FORMAMIDE,

To a three-necked flask equipped with a dropping-funnel, thermometer and down-directed condenser, was added with care 5.17g (85.1 mmol) of 28% ammonia and 4.35g (85.1 mmol) of 90% formic acid. The temperature of the solution was raised to 160°C by distilling out water, and 3g (17 mmol) of 6-methoxy-l-tetralone in chloroform was added for ten minutes. The temperature was maintained at

170–180°C for two hours and any ketone which distilled was returned to the flask at intervals. On cooling a brown mass was formed which on analysis by TLC was found to contain four compounds of R_F = 0.97, 0.80, 0.38 and 0.06 with C as solvent system. The compounds were isolated by column chromatography using the same solvent system as for TLC. Compound with R^ 0.38 was found to be the formyl derivative: wt. 1.2g (34%)i m.p. 77-78°C. TLC, solvent system C, R_f= 0.38. IR spectrum (KBr, vmax): NH, 3250 cm ^j C=0 group, 1635 cm ^i aromatic proton stretch, 3040 cm ^. NMR spactrum($C0Cl_3$): 68.2 (rn,lH) for the aldehydic proton; 67.2 (m,lH), 66.7 (m,2H) for the aromatic protons > 65.4 (signal,1H) for CONH protonj 63.8(s,3H) for the methoxy protons; 62.8 (m,3H) for tetralinyl protons on C-l, C-4; 6l.93(m,4HJ for tetralinyl methylene protons on C-2, C-3.

3.3.2 N-7-METHOXY-1,2,3,4-TETRAHYDPO-1-NAPHTHYL FORMAMIDE.

This formyl derivative was synthesized from 3g (17 mmol) of 7-methoxy-1-tetralone, 5.17g (85.1 mmol) of 28% ammonia, 4.35g (85.1 mmol) of 90% formic acid in the same manner as described

for N-6-methoxy-l,2,3,4-tetrahydro-l-naphthyl formamide: wt. 1.32g (37.8%), m.p. 78-79 C. TLC, solvent system C, 0.42. IR spectrum (KBr, vmax): NH, 3270 cm"[1], C=0 group, 1635 cm"[1]. NMR spectrum (CDC 13J: 68.22 (m,lH) for the aldehydic proton* 66.82 (m,3H) for the aromatic protons, 65.15 (signal,1H) for CONH proton, 63.77 (s,3H) for the methoxy protons, 62.66 (m,3H) for tetralinyl protons on C-l, C-4, 61.85 (m,4H) fur tetralinyl methylene protons on C-2, C-3.

3.3.3 N-5,7-PIMETHYL-l,2,3,4-TETRAHY0RD-l-NAPHTHYL FORMAMIDE.

This formyl derivative was synthesized from 3g (17 mmol) of 5,7-dimethyl-l-tetralone, 5.17g (85.1 mmol) of 28% amrncnia, 4.35g (85.1 mmol) of 90% formic acid in the same manner as described for N-6-methoxy-l,2,3,4-tetrahydro-l-naphthyl formamide: wt. 1.56g (45.1%), m.p. 106-107°C. TLC, solvent system C, R^= 0.54. IR spectrum (Klir, vmax): NH, 3240 cm"[1], C=0 group, 1630 cm"[1]. NMR spectrum (CDC 1^): 68.15 (m,lH) for the aldehydic proton, 66.92 (s,2H) for the aromatic protons, 65.1 (signal,1H) for CONH proton, 62.55 fm,3H) for tetralinyl protons on C-l, C-4, 62. 5 (s,3H),

52.17 (s,3H) for ths two methyl groups cn the ring; 61.85 (m,4h) for tetralinyl methylene protons on C-2, C-3.

3.3.4 N-5-METHOXY-1,2,3,4-TETRAHYDRQ-1-NAFHTHYL FORMAMIOE.

This formyl derivative was synthesized from 3g (17 mmol) of 5-methoxy-l-tetralons, 5.17g (85.1 mmol) of 28% ammonia, 4.35g (85.1 mmol) of 90% formic acid in the same manner as described far N-6-methoxy-1,2,3,4-tetrahydro-1-naphthyl fcrmarr.ide: wt. 1.4g (40%), m.p. 141-142°C. TLC, solvent system C, R_f= 0.48. IR spectrum (KEr, virsax): MH, 3270 cm"[1]) C=0 group, 1640 cm"[1]. NI^R soectrum C CDC1^) s <58.16 (m,lH) for the aldehydic proton; <56.9 (m,3H) fcr the aromatic protcnsj £5.1 (signal,1H) for CONH protonj 63.8 (s,3H) for the methoxy protons; 62.6 (m,3H). for tetralinyl protons on C-l, C-4; 51.85 (m,4H) for tetralinyl methylene protons on C-2, C-3.

3.3.5 N-1,2,3,4-TETRAHY0R0-1-NAPHTHYL FORMAMIOE.

This formyl derivative was synthesized from 4g (27.4 mmol) of 1-tetralone, 8.31g (136.8 mmol) of 28% ammonia, 6.99g (136.8 mmol) of 90% formic

acid in the same manner as described for N-6-methoxy-l,2,3,4-tetrahydro-l-naphthvl formamide: wt. 2.49g (52%), m.p. 74-76°C. TLC, solvent system C, R_p= 0.51. IR spectrum (nujol, vmax): NH, 3260 cm^{-1}, C=0 group, 1620 cm"^{1}. NMR spectrum ($CDC1_3$): 68.15 (m,lH) for the aldehydic proton, 57.19 (m,4H) for the aromatic protons, 65.25 (signal, 1H) for CONH proton, 62.75 (m,3H) for tetralinyl protons on C-l, C-4, 61.9 (m,4H) for tetralinyl methylene protons on C-2, C-3.

3.4 SYNTHESIS OF AMINES[41]

3.4.1 1,2,3,4-TETRAHYDR0-1-NAPHTHYLAMINE-(1-AMINQTETRALIN).

To a three-necked flask equipped with a dropping-funnel, thermometer, and down-directed condenser, was added with care 8.31g (136.8 mmo1) of 28% ammonia and 6.99g (136.8 mmolJ of 90% formic acid. The temperature of the solution was raised to 160°C by distilling out water, ami 4g (27.4 mmol) of 1-tetralone was added at one time using a dropping-funnel. The temperature wa: maintained at 170-180°C for two hours and any ketone which distilled was returned to the flask at intervals. The formyl derivative was hydrolyzed in the reaction

mixture by refluxing for three hours with 10ml of 36% concentrated hydrochloric acid. After standing overnight, a white crystalline solid was formed. The mixture was diluted with 50ml of water and extracted with 10ml of benzene, to remove water-insoluble material. The aqueous solution was treated with 10ml of 45% sodium hydroxide solution and the oil thus produced extracted with 20ml of benzene. The benzene solution was washed throe times with 10ml of w.iter each time, dried with anhydrous sodium sulphate and the benzene removed by distillation under reduced pressure. The residue gave 1.77g (44%) of product, b.p. 246-247°C/714 mm. TLC, solvent system A, R_f= 0.06. IR spectrum (neat, vmax):IMH stretching vibration for primary amine, 3310 cm ^{1} and 3220 cm aromatic proton stretch, 3020 cm ^{1}j methylene stretches, 2880' cm"^{1} and 2860 cm"^{1}. NMR spectrum (CDCl-j): 67.2 (m,4H) for the aromatic protonsj 63.9 (m, ?H) for the -IMH^ protonsj 62.8 (m,3H) for the tetralinyl protons on C-l, C-4, 61.8 (m,4H) for tetralinyl methylene protons on C-2, C-3.

3.4.2 5-HETHOXY-l,2,3,4-TETRAHYORO-1-NAPHTHY-LAMINE (l-AMINO-5-METHQXYTETRALIN).

This amine was synthesized from 3g (17 mmol) of 5-methoxy-1-tetralone, 5.17g (85.1 mmol) of 28% ammonia, 4.35g (85.1 mmol) of 90% formic acid in the same manner as described for the synthesis of 1-aminotetralin. The amine salt was readily soluble in warm water. Crystallization of the amine was done by adding petroleum ether (b.p. 40-60®), which gave a pale-yellow precipitate: wt. 1.79p (59.5%)j m.p. 109-111°C. TLC, solvent system A, R_f=0.06. IR spectrum (KBr, vmax): NH stretching vibrations for primary amine, 3330 cm ^{1} and 3330 cm. ^{1}j aromatic proton stretch, 3050 cm"^{1}) methyl stretches 2980 cm ^{1} and 2930 cm ^{1} j methylene stretches, 2860 cm ^{1} and 2820 cm"^{1}. NMR spectrum (CDClg): 67.07 (m,3H) for the aromatic protonsi 64.00 (m,2H) for the -l\IH$_2$ protonsj 63,82 (s,3H) for the methoxy protonsj 62.77 (m,3H) for the tetralinyl protons on C-l, C-4, 62.12 (rn,4H) for tetralinyl methylene protons on C-2, C-3.

3.4.3 5,7-DIMETHYL-1,2,3,4-TETRAHYDR0-1-NAPHTHYLAMINE (l-ANINQ-5,7-0IMETHYLTETRALIN).

This amine was synthesized from 2g (11.5 mmol) of 5,7-dimethyl-1-tetralone, 3.48g (57.4 mmol) of 20% ammonia, 2.64g (57.4 mmol) of 90% formic acid in the same manner as described for the synthesis of 1-aminotetralin. The amine salt was readily soluble in warm water. Crystallization of the amine was done by adding petroleum ether (b.p. 40-60°), that gave a white precipitate: wt. 0.8g (39.8%), m.p. 106-107°C. TLC, solvent system B, 0.06. IK spectrum (K Br, vmax): NH stretching vibrations for primary amine, 3320 cm ^ and 3300 cm aromatic proton stretch, 3040 cm^{-1}. NMR spectrum ($CDC1_3$): 67.1 (m,1H), 66.9 (m,lH) for the aromatic protonsj 64.0 (m,2H) for the -NH^ protonsj 62.6 (m,3H) for the tetralinyl protons on C-l, C-4, 62.3 (s,3H), 62.2 (s,3H) for the two methyl groups on the ringj 61.9 (m,4H) for tetralinyl methylene protons on C-2, C-3.

3.4.4 ATTEMPTED SYNTHESIS OF 6-METHOXY-1,2,3,4--TETRAHYORO-1-NAPHTHYLAMINE (1-AMINO-6--METHOXYTETRALIN) AND 7-METHOXY- 1,2,3,4--TETRAHYDRO-1-NAPHTHYLAMINE (1-AMINO-7-METHOXYTETRALIN).

The attempted synthesis of the amines were from 3g (17mmol) of 6-methoxy-1-tetralone or 7-methoxy-1-tetralone, 5.17g (85.1 mmol) of 28% ammonia, 4.35g (85.1 mmol) of 90% formic acid in the same manner as described for the synthesis of 1-aminotetralin. Acid hydrolyses of the formyl derivatives gave black-gummy compounds. The amines were not obtained.

3.4.5 6-METH0XY-1,2,3,4-TETRAHYDR0-1-NAPHTHYLAMINE C1-AMINO-6-METHQXYTETRALIN) .

0.6g (2.9 mmol) of N-6-methoxy-1,2,3,4--tetrahydro-1-naphthyl formamide was hydrolyzed with 25ml of 10% sodium hydroxide solution under reflux at 140°C for three hours. The amine obtained was extracted with 10ml of chloroform. The solution was dried with anhydrous sodium sulphate and solvent

removed in vacuo. The residue was dissolved in ethyl acetate and petroleum ether (b,p. 40-60°) added. A yellow-orange precipitate was formed: wt. 0.49g (94.2%), m.p. 97-98°C. TLC, solvent system A, 0.06. IR spectrum (KBr, vmax): NH stretching vibrations for primary amine, 3400 cm^{1} and 3380 cm^{1}, aromatic proton stretch, 3040 cm^{1}. NMR spectrum $CCDC1_3$) : 67.3 (m,lH), 66.75 (m,2H) for the aromatic protons, 63.87 (m,2H) for the -NH^ protcns, 63.82 (s,3H) for the methoxy protons, 62.77 (m,3H) for tetralinyl proton:; on C-l, C-4, 61.86 (m,4H) for tetralinyl methylene protons on C-2, C-3.

3.4.6 7-i^{y}IETH0XY-l,2,3,4-TETRAHYDRQ-l-NAPHTHYLAMINE (l-AMINO-7-METHOXYTETRALIN).

This amine was synthesized from 0.6g (2.9 mmol) of N-7-methoxy-1,2,3,4-tetrahydro-1 -naphthy1 formamide in the same manner as di.-scribed for the synthesis of 6-methoxytetra1 in: wt. 0.5g (96.2%), m.p. 83-85°C. TLC, solvent system A,R_f= 0.07. IR spectrum (KBr, vmax): NH stretching vibrations for primary amine, 3390 cm"^{1} and i370 cm^{-1}. NMR spectrum ($CDC1_3$): 67.37 (s,lH), 67.0 (m,2H) for the aromatic protons, 64.0 (m,2H) for the -NH^

protons» 63.79 (s,3H) for the methoxy protonsj 62.70 (m,3H) for tetralinyl protons on C-l, C-4» 61.85 (m,4H) for tetralinyl methylene protons on C-2, C-3.

3.5 SYNTHESIS OF t-BUTYLOXYCARBONVL-L-PHENY-LALAMINE (Boc-Phe-OH')[17]

A mixture of 1.65g (0.01 mol) of L-pheny-lalanine, 3.59g (0.015 mol) of t-butyl p-nitrophenyl-carbonate, 2,65g (0.025 mol) of sodium carbonate, 15ml of t-butyl alcohol and 10ml of water was refluxed by steam-bath for thirty minutes. Two liquid layers persisted but all solids dissolved during this period. The condenser was removed and the mixture was concentrated by an air stream during ten minutes heating to remove t-butyl alcohol. Sodium p-nitrophenolate dihydrate crystallized and was collected after cooling and washed w.ith 7ml of water in three portions* the filterate was adjusted to pH 5 to 6 by dilute hydrochloric acid and extracted with two 20ml portions of ether to remove any remaining t-butyl p-nitrophenylcarbonate and p-nitrophenol. The aqueous portion was adjusted to pH about 1 and the t-butyloxycarbony1-L-pheny-lalanine was extracted into three 10ml portions of

anhydrous ether. After evaporation of the ether, the solid residue was recrysta11ized from 60ml of petroleum ether (b.p. 40-60°) plus 4rnl of ethyl acetate: wt. 1.4g (52.6%), m.p. 78-00° (Lit. 79-80°, Anderson, McGregor, 1957). TLC, solvent system B, R_p= 0.6. IR spectrum (nujol, vmax) : NH, 3260 cm"[1]! C=0 groups, 1700 cm"[1] and 1630 cm"[1]. NMR spectrum (CDCl.^): 67 .25 (s,5H) for the aromatic protons, 64.9 (signal,1H) for C0NH proton, 64.5 (signal,1H) for Phe methine, 63.1 (m,2H) for the C-CH_2~Ph protons, 1.38 (s,9H) for the (CH^C protons.

3.6 SYNTHESIS OF t-BUTYLOXYCARBONYL-L-ISOLEUCINE (Boc-Ile-OH)[17]

A mixture of 6.55g (0.05 mol) of L-iso1eucine, 14.95g (0.063 mol) of t-butyl p-nitrophenyl-carbonate, 2.28g (0.057 mol) of sodium hydroxide, 100ml of t-butyl alcohol and 50ml of water was refluxed by steam-bath for 30 minute:). Two liquid layers persisted but all solids dissolved during this period. The condenser was removed and the mixture was concentrated by an air stream during ten minutes heating to remove t-butyl alcohol. Sodium p-nitrophenolate dihydrate crystallized and was collected after cooling and washed with 35ml of

water in three portions; the filtjrate was adjusted to pH 5 to 6 by dilute hydrochloric acid and extracted with two 100ml portions of ether to remove any remaining t-butyl p-nitrophenylcarbonate and p-nitrcpneno1. The aqueous portion was then aajusteo to pH about 1 and the t-butyloxycarbonyl-L--isoleucine was extracted into three 5Gml portions of anhydrous ether. After evaporation of the ether, the solid residue was recrystal1ized from acetone-water: wt. l.Oflg (9%) (Lit. 96%, Schnabel, 1967), m.p. 49-55°. TLC, solvent system 3, R_i.= 0.61.

IR spectrum (nujol, vmax): NH, 3340 cm C=C groups, 1705 cm^{-1} and 1660 cm^{-1}. NMR spectrum (CDC1-,): 55.15 (signal,1H) for C0NH proton, 64.3 (signal,1H) for lie methine, 51.0 Crn.cH) for the two methyls of lie, 61.5 (s, 9H) for $(CH_3)_qC$ protons.

3.7 SYNTHESIS OF N-HYOROXYSUCCINIMIDE ESTERS[2]^

3.7.1 N-HYDROXYSUCCINIMIDE ESTER QF t-SUTYLQXY-CARBDNYL-L-PHENYLALANINE (Boc-Phe-OMS'J) .

3utyloxycarbony1-L-phenylalanine (1.Og, 3.98 mmol) and N-nydroxysuccinimide (0.45g, 3.99mmol) were mutually dissolved in 10ml o+ anhydrous dimethoxyethane (DME) at 0°C. Then dicvclohsxyl-carbodiimide (0CC) (0.903g, 3.98 mmol - 10%) was dissolved with stirring and the solution kept at 0-5°C for a period of twenty four hours.

The dicyclohe*ylurea which formed was separated by filteration and the filterate evaporated to dryness in an open dish leaving a crystalline residue of 1.44g of crude product. Two successive recrysta11izations from isopropyl-alcohol-diisopropyl ether gave the pure product: wt. l.Ofig (75%), m.p. 151-152° (Lit. 152-153°, Anderson et al. 1954). TLC, solvent sys'tem B, R_f = 0.33. IR spectrum (nujol, vmax): NH, 3360 cm C*0 groups, 1610 cm"^{1}, 1720 cm^{-1} and 1690 cm"^{1}. NMR spectrum (CDClg): 67.3 (s,5H) for the aromatic protons; 64.9 (m,2H) for CONH and Phe methine protons; 63.28 (m,2H) for the C-CH_2~Ph protons; 62.83 (s,4H) for the succinimido methylene protons; 61.4 (s,9H) for .(CH^tC protons.

3.7.2 N-HYDROXYSUCCINIMIDE ESTER OF t-BUTYLOXY-CARBONYL-L-ISOLEUCINE (Boc-Ile-ONSU).

Butyloxycarbonyl-L-isoleucine (0.79g., 3.42 mmol) and N-hydroxysuccinimide (0.39g, 3.42 mmol) were mutually dissolved in 10ml of anhydrous dimethoxyethane at 0°C. Then dicyclohexylcarbodiimide (0.78g, 3.42 mmol + 10%) was dissolved with stirring and the solution kept at 0-5°C for a period of twenty four hours.

The dicyclohexy1 urea which formed was separated by filteration and the filterate evaporated to dryness

in an open dish leaving a crystalline residue of 0.92g of crude product. Two successive recrystallizations from diisopropyl ether gave the nure product 0.63g (58%), m.p. 91-92° (Lit. 92-93°, Anderson et al. 1954). TLC, solvent system B, 0.31. IR spectrum (nujol, vmax) : IMH, 3350 cm" C=0 groups, 1805 cm"[1], 1720 cm"[1] and 1670 cm"[1]. NMR spectrum C CDC1$_3$) : 64.96 (signal,1H) for CONH proton, 64.55 (signal,1H) for the lie methine, 62.81 (s,4H) for the succinimido methylene protons 61.44 (s,9H) for (C H ^ C protons, 61.04 (m,6H) for the two methyls of H e.

3.3 SYNTHESIS OF tert-BUTYLOXYCARBONYLASPARTIC ACIO[11]

This compound was prepared by the pH-stat method (Schnabel, 19G7). To a stirred solution of 26.5g (0.2 mol) of aspartic acid in deionised water dioxane was added 4N sodium hydroxide until pH 9 was reached. N-tert-Butyloxycarbonyl azide (31.5g, 0.22 mol) was added and pH maintained at 10.2 by continuous addition of 4N sodium hydroxide. The Boc-Asp was extracted with ethyl acetate. Crystall zation was by ethyl acetate-petroleum ether (b.p. .40-60°) which gave a white precipitate: wt. 32.Sg (70%), m.p. 114-115° (Lit.114-116°, Anderson et al. 1567).

3.9 SYNTHESIS OF b-BENZYL l-aUTYLOXYCARBONYL-ASPARTATE (Boc-Asp-a-OBzl)[5]

This compound was synthesized in the same manner as described in the literature (Hruby et al., 1973). 11.75g (0.05 mol) of N-tert-butyloxy-carbonylaspartic acid (prepared by the pH-s'tat method of Schnabel), was converted to tert-buty1-oxycarbonylaspartic acid anhydride by reacting with 11.38g (0.055 mol) of dicyclohexylcarbodiimide (DCC), and the powdered product was. stirred with 5.4g (0.05 mol) of anhydrous benzyl alcohol for about 24 hours. The crude oil was reacted with 13.65g (0.075 mol) of dicyclohexylainine (DCHA) to give Boc-Asp-a-OBzl DCHA salt (m.p.140-142°, Lit 141-142, Hruby et al., 1973). The free Boc-Asp-a-OBzl was regenerated from the salt with 20% aqueous citric acid and recrysta11izea from ethano1-water at 2°: wt. 12.12g (75%:; -n.p. 99-101° (Lit. 99.5-10l[c], Hruby et al., 1973i. TLC, solvent system 5, R^= 0.64. IR spectrum (nujol, vmax) : NH, i3G0 cm C=0 groups, 1725 err"[1] and 1640 cm"[1]. NMR spectrum CCDC 1^) : 67.35 (s,5H) for the aromatic prorons; 65.65 (signal, 1H) for C0NH proton:.; 65.22 (s,2H) for the -O-CH^-Ph protons; o4.6 (signal, 1H) for Asp methinej 63.0 (m,2Hj for the Asp methylene- protons; 61.48 (s,9H) for $(CH_3)_3C$ protons.

3.10 SYNTHESIS OF CARBOXAMIDE PROTECTED ASPARAGINE AMD GLUTAHINE DERIVATIVES[5]

PA

3.10.1 A-BENZYL t-5UTYLOXYCAF.SONYL-N -1,2, J,4--TETRAHyDRO-l-NAPHTHYLGLUTAniNATE (Boc-Gln-(1,2,3,4-TETRAHYPRO-1-NAPHTHYi)-ct-QBzl).

A stirred mixture of 0.5g (1.5 mmol) of a-benzyl _t-butyloxycarbanylglutamate and 0.26g (2.25 mmol) of N-hydroxysuccinimide in 3ml of dichloromethane was cooled to -5°C. To this mixture was added 0.34g (1.65 mmol) of dicyclohexyl-carbodiimide in 5ml of dichloromethane, and the mixture was stirred at -5°C for 50 minutes. A solution of 0.24g (1.65 mmol) of 1,2,3,4-tetrahydro--1-naphthylamine was added and the mixture was stirred %at -5°C for an additional 50 minutes and at room temperature for 24hr. Acetic acid (O.Q75ml) was added, the mixture was stirred for 15 minutes, and the dicyclohexylurea was filtered off and washed with three 3ml portions of dichloromethane. The solvents were removed on a rotary evaporator in vacuo and the residue was dissolved in 4.5ml of dichloromethane. Some insoluble crystals were filtered off. Chloroform (7ml) was added to the filtsrate, the solution was washed with three Sml portions of 5\ aqueous citric acid, three 12ml portions of 5\ aqueous sodium bicarbonate, and"five 15ml portions of deionised water. The organic layer was dried over

anhydrous sodium sulphate and the solvents were removed on a rotary evaporator in v<jcuo. The semi-solid was dissolved in 6ml of hot ethyl acetate, cooled to room temperature and filtered off. To the filterate a solution of 24ml of petroleum ether (b.p. 40-60°C) was added dropwise, and the mixture kept at 0-5°C overnight. The precipitate was filtered, washed with three 4.5ml portions 'of petroleum ether (b.p. 40-60°C) - ethyl acetate (4:1) and dried vacuo to give the product: wt. 0.31g (5%) j m.p. 82-33°. TLC, solvent system C, R_f= 0.80. IR spectrum (nujol, vrnax): NH, 3310 cm C=0 groups, 1740 cm^{-1}, 1680' cm^{-1} and 1630 cm^{-1}. NMR spectrum ($CDC1_3$): 67.45 (s,5H) for the phenyl group; 67.3 (m,4H) for the aisubstituted phenyl group; 56.0 (signal,1H), 65.4 (signal,1H) for CONH protons; 6L.25 (s,2H) for the -0-CH_2"Ph protonu; 64.3 (signal,1H) for Gin methine; 62.85 (m,3H) for tetralinyl protons on C-l, C-4, 62.28 (m,4H) fcr Gin methylene protons; 61.96 (m,4H) for tetralinyl methylene protons on C-2, C-3; 61.52 (s,9H) for (CH^.)„C protons.

rA

3.10.2 B-BENZYL t-BUTYLOXYCARBONYL-N-1,2,3,4--TETRAHYDRO-1-NAPHTHYLISOASPARAGINATE (Boc-Asn(1,2,3,4-tetrahydro-1-naphthyl) --e-OBzl).

This compound was prepared from 0.485g (1.5 mmol) of 6"benzyl _t-butyloxycarbonyiaspartate (Boc-Asp-3-OBzl) and 0.24g (1.65 mmol) of 1,2,3,4-1etrahydro-1-naphthylamine in the sarr.e manner as described for the preparation of Boc-Gln(1,2,3,4-tetrahydro-1-naphthyl)-a-OBzl: wt. 6.6g (09%); m.p. 124-125°. TLC, solvent system C, R_f= 0.93. IR spectrum (KBr, vmax): NH, 3230 cm"^{1}; C-=0 groups, 1730 cm"^{1}, 1680 cm"^{1} and 1645 cm"^{1}. NMR soectrum (CDC1.J: 67.35 (s,5H) for the phenyl group; 57.15 (m,4H) for the disubstituted phenyl group, 66.7 (signal, 1H), 65.6 (signal,1H) for C0r;H protons; 65.^{1}5 (s,2H) for the -0-CH_2"Ph protons,-64.5 (signal.1H) for Asn methinsi OI.8 (,T,4H) for the tetralinyl methylene protons on C-2, C-3; 61.34 (s, SH) for (CH-J^C protons.

3.10.3 8-BENZYL t-BUTYLOXYCARBONYL-N-S-METHOXY--1,2,3,^-TETRAHYDRQ-l-NAPHTHYLISnASPARAniNATE (Boc-Asn(5-methoxy-l,2,3,4-tetrahydro-l-naphthy1)-B-OBzl)

This compound was prepared from 0.485g (1.5 mmol) of B-benzyl _t-butyloxycarbonylaspartate (Boc-Asp-0-OBzl) and 0.29g (1.65 mmol) of 5-methoxy--1,2,3,4-tetrahydro-1-naphthylamine in the same manner as described for the preparation of Boc-Gln(1,2,3,4-tetrahydro-1-naphthy1)-a-CBzl: wt. 0.52g (72%), m.p. 60-62°. TLC, solvent system C, R.p= 0.93. IR spectrum (nujol, vmax): NH, 3310 cm ^{1}, C=0 groups, 1720 cm"^{1}, 1600 cm"^{1} and 1645 cm"^{1}. NMR spectrum (CDC1_3): 67.33 (s,5H) for the phenyl group, 66.9 (m,3H) for the trisubstitutad phenyl group, 65.65 (signal,1H), 64.9 (signal,1H) for C0NH protons, 65.12 (s,2H) for the -D-C_H_2~Ph protons, 64.6 (signal,1H) for Asn methine, 63.79 (s,3H) for the methoxy protons, 61.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3, 61.33 (s,9H) for (CH-J,C protons.

3.10.4 g-BENZYL t-BUTYLOXYCARBONYL.-N^{uA}-5-METHOXY--1,2,3,4-TETRAHYDR0-1-NAPH1HYLGLUTAMINATE (Boc-Gln(5-methoxy-1,2,3,4-tetrahydro-l--naphthy1)-a-OBzl)

This compound was prepared from 0.5g (1.5 mmol) of a-benzyl _t-butyloxycarbonylglutama te (Boc-Glu-a-OBzl) and 0.29g (1.65.mmol) of 5-methoxy-1,2,3,4-tetrahydro-1-naphthylamine in the same manner as described for the preparation of Boc-Gln(1,2,3,4-tetrahydro-l-naphthyl)-a-OBzl: wt. 0.54g (73%); m.p. 104-105°. TLC, solvent system C, R_p= 0.83. IR spectrum (nujol, vmax): NH, 3320 cm"^{1}; C=0 groups, 1743 cm"^{1}, L6S0 cm"^{1} and 1635 cm"^{1}. NMR spectrum CCDC1_3): 67.33 (s,5n) for the phenyl group; 66.95 (m,3H) for the trisub-stituted phenyl group; 65.9 (signal,1H), 65.2 (signal,1H) for C0NH protons; 65.1/ (s,2Hi for the -0-CH_2-Ph protons; 64.25 (signal,1H) for Gin methine; 63.8 (s,3H) for the methoxy proton ,; 61.82 (m,4H) for the tetralinyl methylene protons on C-2, C-3; 51.4 (s, 3H ' for (CH_)-,C protons.

3.10.5 g-BENZYL t-BUTYLOXYCAR30NYL-N^{rA}-S-METHQXY--1,2,3,4-TETRAHYDRQ-1-NAPHTHYLASPARAGINATE (Boc-Asn(6-methoxy-1,2,3,4-tetrahydro-1--naohthyl)-a-03zl)

This compouno was prepared from 0.4S5g (1.5 mmol) of a-benzyl £-butyloxycarbcnylaspartate (Boc-Asp-a-OBzl) and 0.29g (1.65 mmol) of 6-methoxy-1,2,3,4-tetrahydro-l-naphthylamine in the same manner as described for the preparation of Boc-Gln(l,2,3,4-tetrahydro-l-naphthyl)-a-OBzl: wt. 0.56g C 78%)j m.p. 75-76°. TLC, solvent system C, R_f= 0.73. IR spectrum (KBr, vmax): NH, 3320 cm"^{1}; C=0 groups, 1730 cm"^{1}, 16B0 cm^{-1} and 153C cm"^{1}. NMR spectrum (C0C1,): 57.36 (s,5H) tor the phenyl group; 57.1 (m,lH), 6c.62 (m,2H) for the trisubstit ted phenyl group, 55.85 (signal,1H), 55.05 (signal, for C0NH protons; 55.2 (s,2H) for the -G-CH.-Ph protons; 54 .45 (signal,1H) for Asn methi.ne; 53.77 (s,3H) for the methcxy protons; 51.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3, 51.4 Cs,9H) for (CHg) $_c$ p$_{ro}$tons.

3.10.6 g-BENZYL t-BL)TYL0XYCARB0NYL-N$^{PA}_{u}$-6-METH0XY--1,2,3,4-TETRAHY0R0-1-NAPHTHYLGLUTAMINATE (Boc-Gln(6-methoxy-1,2,3,4-tetrahydro-1-naphthyl)-g-0Bzl)

This compound was prepared from 0.5g (1.5 mmol) of a-benzyl _t-butyloxycarbonylglutamate (Boc-GIu--a-OBzl) and 0.29g (1.65 mmol) of 6-rnethoxy-1,2,3,4--tetrahydro-1-naphthylamine in the same manner as described for the preparation of Boc-Gln(1,2,3,4--tetrahydro-l-naphthyl)-a-OBzl: wt. 0.56g (76%), m.p. 91-92°. TLC, solvent system C, R_f= 0.67. IR spectrum (KBr, vmax): iMH, 3310 cm^{1}, C=0 groups, 1730 cm^{-1}, 1680 cm"^{1} and 1630 cm"^{1}. NMR spectrum (C0C1$_3$): 67.34 (s,5H) for the phenyl group; 67.1 (m,lH), 65.7 (m,2H) for the trisubstitutea phenyl group, 65.8 (signal,1H) 65.0 (signal,1H) for CCflH protons, 65.19 (s,2H) for the -0-DH^-Ph protons, 64.25 (signal,1H) for Gin methine, 63.77 (s,3H) for tha methoxy protons, 61.32 (n,4H) fcr the cetralinyl methylene protons on C-2, C-3, 51.4 s,9H) fcr fChi) Q protons.

.3.10.7 8-BENZYL t-BUTYLQXYCARBONYL-N$^{r\,A}$-7-riETHQXY--1,2,3,4-TETRAHY0R0-1-NAPHTHYLISOASPARAGINATE (Boc-Asn(7-methoxy-1,2,3,4-tetrahydro-1--naphthyl)-B-OBzl)

This compound was prepared from 0.485g (1.5 mmol) of B-bsnzyl _t-butyloxycarhonvlaspartate (Boc-Asp-B-OBzl) and 0.29g (1.65 mmol) of 7-methoxy--1,2,3,4-tetrahydro-1-naphthylamine in the same manner as described for the preparraon of Boc-Gln-(l,2,3,4-tetrahydro-l-naphthyl)-a-0Bzl: wt. 0.4g (56%); m.p. 136-137°. TLC, solvent system C, R_f= 0.90. IR spectrum (KBr, vmax): NH, 3310 cm^{-1}; C=0 groups, 1720 cm^{-1}, 1670 cm^{-1} and 1635 cm^{-1}. NMR spectrum CCDC1$_3$): 67.38 (s,5H) for the phenyl group, 66.85 (m,3H) for the trisubstituted phenyl group; 55.55 (signal,1H), 65.0 (signal,1H) for C0NH protons; 55.2 (s,2H) for the -0-CH_2-Ph protons; 6^.55 (signal,1H) for Asn methine; S3.81 (s,3H) for the methoxy protons; 61.85 (m,4H) for che tetralinyl methylene protons on C-2, C-3; 61.46 (s,9H) for (CH$_3$)$_3$C protons.

3.10.8 g-BENZYL t-BUTYLQXYCARBONYL-N^{PA}-7-METHQXY--1,2,3,4-TETRAHYORO-1-NAPHTHYLASPARAGINATE (Boc-Asn(7-methoxy-l,2.3,4-tetrahydro-l--naphthyl)-g-OBzl)

This compound was prepared from 0.485g (1.5 mmol) of g-benzyl t_-butyloxycarbonylaspartate CBoc-Asp-a-OBzl) and 0.29g (1.65 mmol) of 7-methoxy--1, 2,3,4-tetrahydro-1-naphthylamine in the same manner as described for the preparation of Boc-Gin(1,2,3,4--tetrahydro-l-naphthyl)-a-OBzl: wt. 0.45g (62.5%), m.P. 85-86°. TLC, solvent system C, R = 0.75. IR spectrum (KBr, vmax): NH, 3310 cm"^{1}; C=0 groups, 1730 cm"^{1}, 1680 cm"^{1} and 1630 cm"^{1}. NMR spectrum ($CDC1_3$)s 67.34 (s,5H) for the phenyl group; 66.78 (m,3H) for the trisubstituted phenyl group, 55.33 (signal,1H), 65.1 (signal,1H) forCONH protons; 65.19 (s,2H) for the .-0-CH_2-Ph protons; 64. 55 (signal,1H) for Asn methine; 63.74 (s,3H) for the methoxy protons; 61.82 (m,4H> for the tetralinyl methylene protons on C-2, C-3; 61.4 (s,9H) for (CH) C protons.

3.10.9 G-BENZYL t-BUTYLOXYCARBONYL-N^{rA}-7-METHOXY--1,2,3,4-TETRAHYORO-1-NAPHTHYLGLUTAMINATE (Boc-Gln(7-methoxy-l,2,3.4-tetrahydro-1--naphthyl)-a-OBzl)

This compound was prepared from 0.5g CI.5 mmol) of a-benzyl _t-butyloxycarbonylglutamate-(Boc-Glu-a-OBzl) and 0.29g (1.65 mmol) of 7-methoxy-l,2,3,4-tetrahydro-l-naphthylamine in the same manner as described for the preparation of 3oc-Gln(1,2,3,4-tetrahydro-1-naphthyl)-a-OBzl: wt. 0.44g C60%I, m.p. 145-146°. TLC, solvent system C, R^= 0.66. IR spectrum (KBr, vmax): MH, 3320 cm ^{x}, C=0 groups, 1720 cm *, 1680 cm ^ and 1620 cm NMR spectrum (C0C1.J : 67.32 (s, 5H) for the phenyl group? 66.8 (m,3H) for the trisubstituted phenyl group 65.75 (signal,1H), 64.95 (signal,1H) for CONH protons? 65.17 (s,2H) for the -0-CH_2"Ph protons, 64.3 (signal, 1H) for Gin methine, 63.75 (s,3H) for the methoxy protons; 61.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3j 61.4 (s,9H) for $(Ch_3)_3$ protons.

3.10.10 B-BENZYL t-BUTYLOXYCARBONYL-N^{PA}-5,7-DIMETHYL-1,2,3,4-TETRAHYDRO-1-NAPHTHYLISOASPARAGINATE (Boc-Asn(5,7-dirnethy1-1,2,3,4--tetrahydro-1-naphthyl)-B-0Bzl).

This compound was prepared from 0.485,? (1.5 mmol) of 6-benzyl t^-butyloxycarbonylaspartate (Boc-Asp-g-OBzl) and 0.29g (1.65 mmol) of 5,7-dimethy1--1,2,3,4-tetrahydro-1-naphthylamine in the same manner as described for the preparation of Boc-Gln(1,2,3,4-tetrahydro-1-naphthyl)-a-OBzl: wt. 0.29g (40%); m.p. 143-144°. TLC, solvent system C, R_f= 0.94. IR spectrum (nujol, vmax): NH, 3300 cm C=0 groups, 1714 cm"^{1}, 1672 cm"^{1} and 1633 cm"^{1}. NMR spectrum ($CDC1_3$): 67.36 (s,5H) for the phenyl group; 66.95 (s,2H) for the tetrasubscituted phenyl group,-<56.5 (signal,1H), 65.6 (signal,1H) for C0NH protons; 65.19 (s,2H) for the -0-CH_2"Ph protons; 64.5 (signal, 1H) for Asn methine; 62.27 (s,3H), 62.2 (s,3H) for the two methyl groups on the ring; 61.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3; 61.41 (s, 9H) for (CHo)_ protons.

3.10.11 a-BENZYL t-BUTYLOXYCARBONYL-N^{PA}-5,7-DIMETHYL-1,2,3.4-TETRAHY0R0-1-NAPHTHYLGLUTAMINATE (Boc-GlnC 5, 7-dimethy1-j2,3,4-tetrahydro-l-naohthyl)-g-OBzl).

This compouna was prepared from 0.5g (1.5 rr,mol) of a-benzyl t_-butyloxycarbonylglutamate (Boc-Glu-a-OBzl) and 0.29g (1.65 mmol) of 5, 7-dimethy1-i,2,3,4-tetrahydro-l-naphthylamine in the same manner as described for the preparation of Boc-GIn(1,2,3,4-tetrahydro-1--naphthyl)-a-OBzl: wt. 0.4g (54%), m.p. 110-111°. TLC, solvent system C, R_f= 0.84. IR spectrum (nujol, vmax): NH, 3265 cm^{-1}, C=0 groups, 1720 cm^{-1}, 1570 cm^{-1} and 1620 cm^{-1}. NMR spectrum (CDC1-): 57.3 (s,5H) for the phenyl group, 56.87 (s,2H) for the tetra-substituted phenyl group, 55.9 (signal,1H), 65.3 (signal,1H) for CONH protons, 65.13 (s,2H) for -0-CH_2-Ph protons, 64.3 (signal,1H) for Gin methine, 62.2 (d,ltiH) for the two methyl groups and Gin methylene protons, 61.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3, 61.38 (s,9H) for ,(CH_).C protons.

3.10.11 a-BENZYL t-BUTYLOXYCARBONYL-IM[PA]-5,7-OIMETHYL-1,2,3,4-TETRAHY0R0-1-NAPHTHYLGLUTAMINATE (Boc-Gln(5,7-dimethy1-j 2,3,4-tetrahydro-l-naphthyl)-g-OBzl) .

This compouna was prepared from 0.5g (1.5 mmol) of a-benzyl t-butyloxycarbonylglutamate (Boc-Glu-a-OBzl) and 0.29g (1.65 mmol) of 5,7-dimethy1-1,2,3,4-tetrahydro-l-naphthylamine in the same manner as described for the preparation of Boc-Gln(1,2,3,4-tetrahydro-1--naphthyl)-a-08zl: wt. 0.4g (54%), m.p. 110-111°. TLC, solvent system C, R_f= 0.34. IR spectrum (nujol, vmax): NH, 3285 cm"[1], C=0 groups, 1720 cm [1], 1570 cm [1] end 1620 cm"[1]. NMR spectrum CCDC1_3) : 57.3 (s,5H) for the phenyl group, 56.87 (s,2H) for the tetra-substituted phenyl group, 65.9 (signal,1H), 65.3 (signal,1H) for C0NH protons, 65.13 (s,2H) for -0-CH_2-Ph protons, 64.3 (signal,1H) for Gin methine, 62.2 (d,lGH) for the two methyl groups and Gin methylene protons, 61.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3, 61.38 (s,9H) for (CH_)_C protons.

3.11 SYNTHESIS OF •IPEPTIDES?[6]

3.11.1 Boc-Phe-Asn(1,2,3,4-TETRAHYORO-1-NAPHTHYL)--g-OBzl.

A solution of Boc-Asn(1,2,3,4-tetrahydro-1--naphthyl)-B-OBzl (0.4g, 0.88 mmol) in 6ml trifluoroacetic acid and 0.5ml anisole was stirred for 25 minutes at room temperature. TFA was then removed on a rotary evaporator jin vacuo at 25-30°C. 4ml of dimethylformamide was then added to Lhc above solution and pH was adjusted to 7_ with triethylamine. To this solution was added Boc-Phe-ONSU (0.32g, 0.83 mmol) and solution was stirred for 14 hours at room temperature. Ethyl acetate (20ml) was added and the solution washed with three 20ml portions of 5% aqueous citric acid, two 20ml portions of 5% aqueous sodium bicarbonate and five 15ml portions of deionised water. The organic portion was dried over anhydrous sodium sulphate. The solvent was removed on a rotary evaporator in vacuo: wt. 0.40g (75.5";): m.p. 151-152°. TLC, solvent system C, R^= 0.76. IR spectrum (nujol, vmax): NH, 3280 cm"[1], C=0 groups, 1720 cm'[1], 1680 cm"[1] and 1625 cm"[1]. NMR spectrum (CDC1,): 67.36 (s,5h), 67.2(s,5H) for the two phenyl groups; 67.1 (s,4H) for the disubstituted phenyl group, 65.12 (s,2H) for the -0-CH=;Ph protons; 63 .05 (m,2H)

for the C-Ch[$_2$-Ph protons, 61.8 (m,4H) fur the tetralinyl methylene protons on C-2, C-i, 61.22 (s,9H) for (CH_3).-C protons.

3.11.2 Boc-Phe-Asr. t'7-HETHOXY-1,2,3 ,4-TETRAHYDRO-1 - -NAPHTHYL)-6-OSzl.

This compound was prepared fron 0.39g (0.81 mmol) of Boc-Asn(7-methoxy-1,2,3,4 - terra hydro - 1 - -naphthyl)-g-OBzl and 0.2Sg (0.81 mmol) of Boc-Phe-ONSU in the same manner as described for the preparation of Boc-Phe-Asn(1,2,3,4-tetrahydro-1-naphthyl)-B-OBzl: wt. 0.44g (86%), m.p. 164-165°. TLC, solvent system C, R_f= 0.72. IR spectrum (KBr, vmax): NH, 3290 cm"^{1}, C=0 groups, 1720 cm'^{1}, 1680 cm"^{1} and 1G30 cm"^{1}. NMR spectrum ($CDC1_3$); 67.34 (s,5H), 67.19 (s,5H) for the two phenyl groups, 66.8 (m,3H) for the trisubstituted phenyl group, 65.12 (s,2h) for -O-Ch^-Pn protons, 63.69 (s,3H) for the methoxy protons, 63.0 (m,2H) for -C-CH_2-Ph protons, 61.75 (m,4H) for the tetralinyl methylene protons on C-2, C-3, 61.18 (i,,9H) for (CH-.) _C protons.

3.11.3 Boc-Phe-Asn(7-NETHQXY-1,2,3,4-TETRAHYDRO-l--NAPHTHYL)-a-OBzl.

This compound was prepared from 0.39g (0.81 mmol) of Boc-Asn(7-methoxy-1,2,3,4-tetrahydro--1-naphthyl)-a-OBzl and 0.29g (0.81 mmol) of Boc-Phe-ONSU in the same manner as Oescrideo for the preparation of Boc-Phe-Asn(1,2,3,4-1etrahydro-1--naphthyl)-6-0Bzl: wt. 0.46g (90.2%), m.p. 165-166°. TLC, solvent system C, R^.= 0.59. IR spectrum (KBr, vmax): NH, 3290 cm"^{1}) C=0 groups, 1735 cm"^{1}, 1680 cm"^{1} and 1630 cm"^{1}. NMR spectrum (CDC1_3): 57.37 (s,5H), 67.22 (s,5H) for the two phenyl groups, 66.8 (m,3H) for the trisubstituted phenyl group, 65.22 (s,2H) for the -0-CH_2~Ph prot'ns, 63.7 (s,3H) for the methoxy protons, 61.75 (m,4h) for the tetralinyl methylene protons on C-2, C-3, 61.37 (s,9H) for v'$CH_3)_3$C protons.

3.11.4 Boc-Phe-Asn(5,7-DIMETHYL-1,2,3,4-TETRAHY0RQ-1--NAPHTHVL)-B-OBzl.

This compound was prepared irom 0.3Sg (0.81 mmol) of Boc-Asn(5,7-dimethy1-1, 2, 3, 4--tetrahydro-i-r.aphthyi)-g-QBzl and 0.29g (0.81 mmol) of Boc-Phe-ONSU in the same manner as oescribeo for

the preparation of Boc-Phe-Asn(1,2,3,4-tetrahydro-1--naphthyl)-B-OBzl: wt. 0.4g (79%), m.p. 149-150°. TLC, solvent system C, 0.77. IR spectrum (nujol, vmax): NH, 3300 cm ^{1}, C=0 groups, 1730 cm 1685 cm"^{1} and 1635 cm^{-1}. NMR spectrum (COCI3O: 67.38 (s,5H), 67.22(s,5H) for the two phenyl groups, 66.87 (s,2H) for the tetrasubstituted phenyl group, 65.16 (s,2H) for -0-CH_2"Ph protons, 63.1 (m,2H) for the -C-CH^-Pn protons, 62.21 (merged s,5H) for the two methyl groups on the ring, 61.83 (m,4H) for the tetralinyl methylene protons on C-2, C-3, 61.23 (s,9H) for $(CH_3)_3C$ protons.

3.12 SYNTHESIS OF TRIPEPTIDES[JD]

3.12.1 Boc-Ile-Phe-Asn(1,2,3,4-TETPAHYDRG-1--NAPHTHYL)-B-OBzl.

A solution of Boc-Phe-Asn(1,2,3,4-1etrahydro-l-naphthyl)-6-QBzl (0.15g, 0.25 mmol) in 3ml TFA and 0.25ml of anisole was stirred at room temperature for 25 minutes. The solvent was removed on a rotary evaporator in vacuo at room temperature. The oil residue was treateo twice with 10ml portions of ethyl ether, and the ether was evaporatea to dryness each time. The white solid was oisEolv/ed in 10ml of dichloromethane and the pH was adjusted to 7

with triethylamine. Boc-Ile-ONSU (O.OSg, 0.25 mmol) was added, and the mixture was stirred for 35 minutes at room temperature. The mixture was filtered and the solvent removed in vacuo on a rotary evaoorator at room temperature. TLC of the crude product with solvent system C gave three components with 0.73, 0.35 and 0. Isolation of the components was by column chromatography and componentth 0.73 was found to be the tripeptide. Solvent mixture containing the tripeptide was removed in vacuo on a rotary evaporator at room temperature. "ne residue was dissolved in 4nl of ethyl acetate anc 20ml of petroleum ether (b.p. 40-60°) was added and placed in the freezer overnight. Tne precipitate was filtered off and dried in vacuo to give a white crystalline prooouct: wt. 0.14g (78°o), m.p. 203-204°. TLC, solvent system C, 0.73. IR spectrum (KBr, vmax): NH, 3260 cm^{-1},-C=0 groups, 1725 cm^{-1}, 1680 cm^{-1} and 1625 cm^{-1}. NMR spectrum ($CDC1_3$): ⟨57.33 (s,5H), 67.15 (s,5H) for the two phenyl groups, 67.08 (s,4H) for the disubstituted phenyl group, 65.12 (s,2H) for the -0-CH_9-Ph protons, 63.27 (n,2H) for the -C-CH_2-Ph protons, 61.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3, 61.35 (s,9H) for $(CH_3)_3C$ protons, 60.75 (m,6H) for the two methyl groups of lie.

3.12.2 Boc-Ile-Phe-Asn(7-METHOXY-1,2,3,4-TETRA-HYDRO-l-NAPHTHYL)-a-OBzl.

This compound was prepared from 0.16g (0.25 mmol) of Boc-Phe-Asn(7-methoxy-l,2,3,4--tetrahydro-l-naphthyl)-a-QBzl and O.GSg (0.25 mmol) of Boc-Ile-QNSU in the same manner as described for the preparation of Boc-I1e-Phe-Asn(1,2,3,4-tetra--hydro-l-naphthyl)-g-OBzl: white crystalline compound, wt. 0.15g (78%), m.p. 214-216°. TLC, solvent system C, R_f= 0.33, 0, 0.83. IR spectrum (KBr, vmax): NH, 3270 cm^{-1}, C=G groups, 1730 cm^{-1}, 1680 cm"[1] and 1530 cm"[1]. NMR spectrum (C•$C1_3$): 67.36 (s,5H), 67.23 (s,5H) for the two phenyl groups, 65.75 (m,3H) for the trisubstituted phenyl group, c 5. Z (s,2H) for the -O-CH^Ph protons, 63 .75 (s,3H) for the methoxy prctons, 61.8 (m,4H) for the tetralinyl methylene protons on C-2, C-3; 51.44 (s,9H) for $(CH_3)_3C$ protons, 50.9 (m,EH) fcr the two methyl groups of lie.

3.12.3 Boc -11 e-Pne-Asr.f 5,7-DIMETHYL-1,2,3, 4-TETRA-HYDRQ-1-NAPHTHYL) - 3-JBzl.

This compounc was prepared from 0.15g (0.25 mmol) of 3oc-Phe-Asn(5,7-dimethy1-1,2,3,4--tetrahydro-l-naphthyl)-3-QBzl and 0.08g (0.25 mmol)

of Boc-Ile-ONSU in the same manner as described for the preparation of Boc-Ile-Phe-Asn(1,2, 3,4--tetrahydro-l-naphthyl)-B-OBzl: white crystalline compound, wt. 0.13g (70%), m.p. 202-203. TLC, solvent system C, R_p= 0.70. IR spectrum (KBr, vmax): NH, 3260 cm"^{1}, C=0 groups, 1720 cm"^{1}, 1670 cm"^{1} and 1625 cm"^{1}. NMR spectrum ($CDC1_3$): 67 .35 (s,5H), 67.17 (s,5H) for the two phenyl groups, 66.83 (s,2H) for the tetrasubstituted phenyl group, 65.15 (s,2H) for the -O-CH^'Ph protons, 62.17 (merged s,6H) for the two methyl groups on the ring, 61.64 (m,4H) for the tetralinyl methylene protons on C-2, C-3;' cl.23 (s,9H) for $(CH_3)_qC$ protons, 60. 75 (r.,6H) for the two methyl groups of He.

3.13 CLEAVAGE STUDIES QF THE CARBOXAMIOE PROTECTED DERIVATIVES IN TRIFLUOROACETIC ACI0-0ICHL0--ROMETHANE-AiMISOLE (50:48:2 v/v).

Small quantities of the carboxamide protected glutaminate, asparaginate and isoasparaginate derivatives were placed in separate vessels, 2ml of cleavage solvent H, was added to each vessel and the mixtures were stirred at room temperature for 24hr. Samples under cleavage treatment were thin-layer

chromatographed at lhr, 3hr, 5hr, 7hr and 24hr. The solvent system was chloroform-methanol-glacial acetic acid (85:10:5 v/v). Detecting reagent was ninhydrin in acetone.

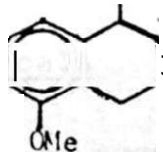
3.14 CLEAVAGE STUDIES OF Boc-Asn(1,2,3,4--tetrahydro-l-naphthyD-B-OBzl, Boc-Asnt 5,7--dimethyl-1,2,3,4-tetrahydro-1-naphthyl)--B-OBzl, Boc-Asn(7-methoxy-l,2,3,4-tetra--hydro-l-naphthyl)-a-OBzl and their dipeotides IN BORON TRIFLUORIDE COMPLEX WITH ACETIC ACID (36% BF-, ; BF_n . 2AC0H) .

Small quantities of the compounds were dissolved in 0.5ml TFA and 1.0ml of BTFA was adaed . The mixtures were stirred and thin-layer chrornatc-graphed at lhr, 2hr,3hr and 4hr. Solvent system was chloroform-methanol-glacial acetic acid (85:10:5 v/v). Detecting reagent was ninhydrin in acetone.

APPENDIX

LIST OF ABBREVIATIONS

Carboxamide Protecting Croups

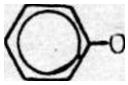
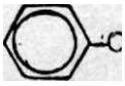
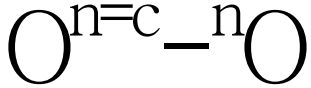
Name	Formula	Abbreviation
1,2,3,4-Tatrahydro-l-naphthyl		1.2.3,4-TH-l-NT
5-Mathoxy-l,2,3,4-tatrahydro-l-naphthyl		5-Meo-1,2.3,4-TH-1-NT
6-Nethoxy-l,2,3,4-tetrahydro-l-naphthyl		6-Meo-l,2,3,4-TH-l-NT
7-Mathoxy-l,2,3,4-tetrahydro-l-naphthyl'		7-Neo-1,2,3,4-*Thl*-1-NT
5,7-Oimathyl-l,2,3,4-tatrahydro-l-naphthyl		5,7-Me_2-1, 2,3,4-TH-1-NT

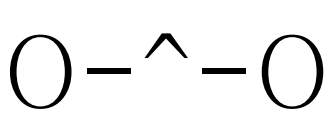
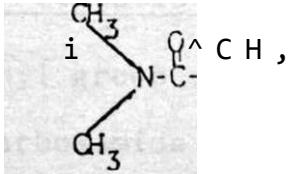
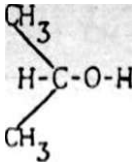
Aminw Prot^otlnfi Groups

Car-boxy 1 A c tiv.iting and Protecting Gro ups

Name	Formula	Ahbreviation
Ben zy loxy		OB z 1
N-Hydroxysuccin imido		QNSu

Qther Chemicals

Acetic Acid	CH_3COOH	HOAc
An i so 1 e		
Benzyl Alcohol		BzlfJH
Boron tristrifluoroacatatG	$(rF_3con)_3B$	BTFA
Dieyclohexylamine		DCHA
Olcyc lohflxylcarbod i irnidra		ncc

Name	Fornula	Abbreuiot: .in
Dicyclohexylurea		OCHU
Diethyl ether	CH_3-CH_2-O-C:H_2-CH_3	Et-,0
Oiisopropyl ether		i-pr_2o
N,N-D ime thy1formamide		DMF
E thano1	CH_3-CH_2-OH	EtOH
Ethyl Acetate	CH_-C-0-CH--CH	EtOAc
Hydrogen Fluoride	HF	HF
Isopropyl alcohol		i-PrOH

Name	Formula	Abbrev intion
t-Butyl p-nitrophenylcarbonate	$(CH_3)_3$ C - 0 - C t - H u - P - N P C	
Tetrahydrofuran	R ⁰ ^	
Trifluoroacetic Acid	CF_3C00H	TFA

Derivatives of Glutamic and Aspartic Acids

The 1, 2, 3 ,4 - te trahyd ro - 1 -naph thy 1 group is used t.o illustrate how to write the name, formular, and abbreviation of the carboxamide protected amino acid.

Name .	Formula '	Abbrev iat ion
a-Benzyl t-butyloxy-carbonylaspartate	0 (CH_3)$_3$C-0-C-NH-jTH-COOCH^CgH_5 CH^-COOH	Boc-Asp-a-OSz1
a-Benzyl t-butyloxy-carbonylglutamate	n (CH.,)$_2$"C00H $(CH_3)_3$C-0-U-NH-CH-COOCH^CgH_5	Boc-G1u-a-09z1

Name	Formula	Abbreviation
a-Benzyl t-butyloxy-carbonyl-N-1,2,3,4-tstra-hydro-l-naphthylasparaginate	$(CH_3)_3C\text{-}O\text{-}C\text{-}NH\text{-}CH(CH_2CONH\text{-}C_{10}H_7)COOCH_2C_6H_5$	Boc-Asn(1,2,3,4-TH-1-NT)n-GBzl
ct-Bsnzyl t-butyloxy-carbonyl-N -1,2,3,4-tetrahy• dro-l-naplithylglutaminate	$(CH_3)_3C\text{-}O\text{-}C\text{-}NH\text{-}CH(CH_2CH_2CONH\text{-}C_{10}H_7)CO\text{-}CH_2\text{-}C_6H_5$	Boc-Gln(l,2,3,4-TH-l-NT)a-OBzl
N-bert-Buty1oxyc*a*rbony1a*s*• partic Acid	$(CH_3)_3C\text{-}O\text{-}C\text{-}NH\text{-}CH(CH_2COOH)\text{-}COOH$	Doc-Asp

Name

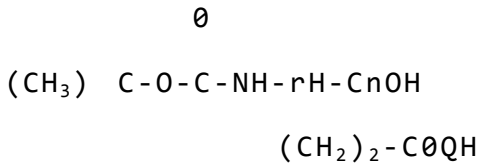
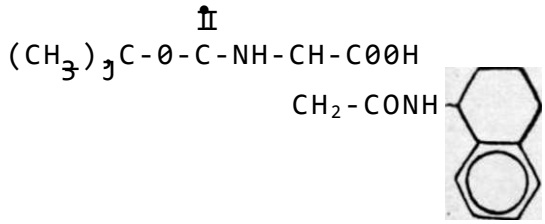
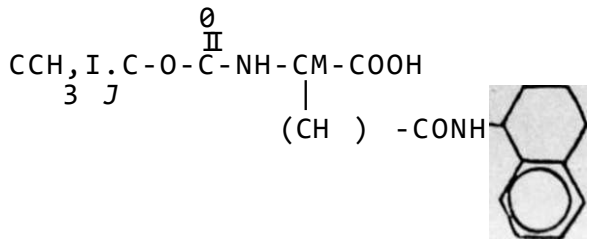
N-tert-Butyloxycarbonylglutamic Acid

PA

N-tert-Butyloxycarbonyl-N - 1,2,3,4-tetrahydro-l-naphthyl 'asparag ins

CA

'N-tert-Butyloxycarbonyl-N 1,2,3,4-tstrahydro-l-naphthyl glu tamine

Formula	Abbrev ia tion
	Boc-Glu
	Hoc-Asn(1,2,3,4-TH-l-NT)
	Qoc-Gln(l,2,3,4-TH-l-NT)

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