The preparation of racemic and optically active beta-amino-acids and their utilisation in peptide formation.

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THE PREPARATION OF RACEMIC AND OPTICALLY ACTIVE β-AMINO-ACIDS AND THEIR UTILISATION IN PEPTIDE FORMATION

by

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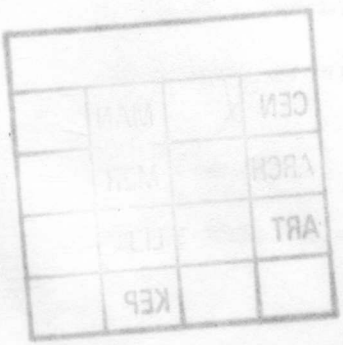
A thesis presented in part fulfilment of the requirements for the degree of Doctor of Philosophy of the Council for National Academic Awards.

Robert Gordon's Institute of Technology, Aberdeen.



January 1978

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SUMMARY

β-Amino acids are being isolated from biologically active peptide antibiotics with increasing frequency nowadays. Recognition of the importance of these amino acids, has led to their incorporation into synthetic peptide analogues and into polymers.

The main part of this thesis reports the successful preparation of linear and cyclic peptides of racemic and optically active β-aminobutyric acid.

β-Amino acid cyclisation studies by Barker[84] had led to the conclusion that oxazinones could only be obtained from saturated β-benzamido-acids containing quaternary β-cabon atoms, while other saturated β-benzamido-acids (e.g. β-aminobutyric acid) yield only symmetrical anhydrides. However, in this work oxazinones of both racemic and optically active β-aminobutyric acid were isolated, charaterised, and used as peptide coupling intermediates. A chiral oxazinone has thus been isolated for the first time, and used as a coupling intermediate[85] for the preparation of optically active peptides.

Transacylation[89], of β-benzamidobutyric acid was encountered, during treatment of this compound with acetic anhydride under reflux conditions. While the use of isobutyl chloroformate allowed obtention of oxazinone in high yield at temperatures below -10°, its use at temperatures around 0° led to esterification of oxazinone by isobutyl alcohol.

Arndt-Eistert Synthesis using N-tosyl derivatives led to the isolation of a chiral β-lactam during Wolff rearrangement in an inert solvent.

Elsewhere in this thesis, it has been demonstrated that the formation of cyclohexylamide[89] as a side-product during peptide coupling of β-amino-pivalic acid with the acid of DCCIis due to alkaline cleavage of N-acylurea.

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ERRATA

1. p iii Summary, line 3: read chloroformate.
2. p 13 para 3, line 5: read N-O-nitrophenyl-sulphenyl.
3. p 14a (44) read R = $NHNH_2$.
4. p 16 4.1, line 1: word spacing between amides and dash (-) δ-valerolactams.
5. p 17 para 2, line 3 ~~β~~ → -butyrolactams.
6. p 25a Fig 18: (72)-O~~M~~ → -OH
7. p 31a missing: contains structures 76, 77, 80, 81, 82 (available).
8. p 34a Fig 22
9. p 34b Fig 22a
10. p 42 para 1, line 12 read diethylphosphite
11. p 64 para 2, line 2: (70) should read (71)
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13. p 65 para 3, line 4: (68) should read (69)
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I N T R O D U C T I O N

Utilisation of cyclic amino acid and cyclic peptide intermediates in peptide synthesis

Several aspects of peptide synthesis have received attention in reviews and monographs, but cyclic intermediates employed in the formation of the peptide bond have not been similarly reviewed in a collective and comprehensive manner. This introduction intends to contribute towards filling this gap and has particular relevance in the context of the practical part of this thesis.

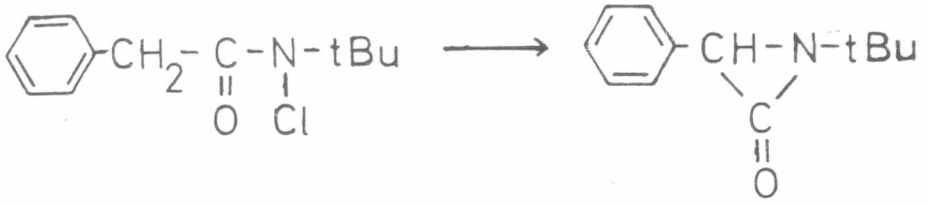
The application of a series of activated cyclic intermediates is discussed. They include: aziridinones, β-lactams, oxazolones, N-carboxyanhydrides, pyrrolidones, piperidones, oxazinones, and some larger ring-size lactams.

1. THREE-MEMBERED RING-SYSTEMS

1.1. Aziridinones. These are three-membered α-lactams; cyclic amides formed in principle by intramolecular elimination of water between a carboxyl and amino group.

As early as 1908, Leuchs[1] proposed an aziridinone structure for N-carboxy-α-amino acid anhydrides which rapidly loose carbon dioxide on heating to form polypeptides. Since then, aziridinones have been suggested as possible intermediates in peptide synthesis, though never having been isolated from either amino acid or peptide derivatives until 1962[2]. In 1961, in the course of investigations of the Favorskii-type rearrangement of N-t-butyl N-chloramide (1),

Fig.1



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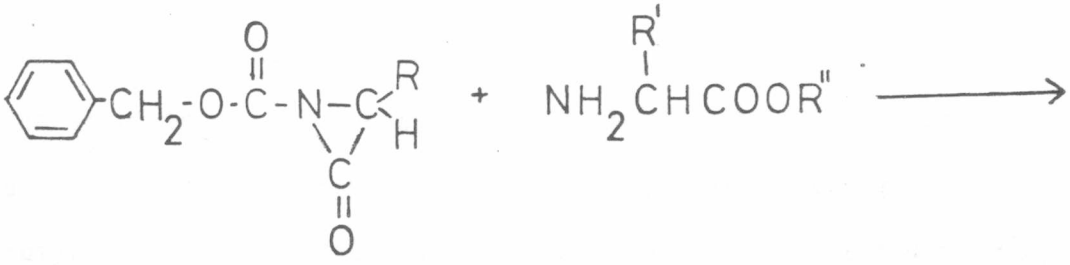

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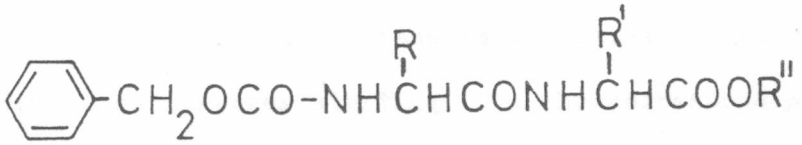

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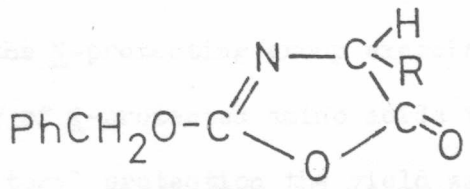

(R = $PhCH_2$)

(4)

Baumgarten[3] first detected an aziridinone as the intermediate. It could not be isolated in pure form but showed a carbonyl absorption band at 1847cm^{-1} in the infrared spectra. A year later, he succeeded in isolating N-t-butyl-3-phenyl aziridinone (2).

In subsequent years synthesis of other aziridinone derivatives was reported.[4-10] Typical published routes were by the dehydro-halogenation of either N-haloamides or α-haloamides. It was demonstrated that the N-substituent decisively influences the ease of ring formation and the stabilisation of the corresponding aziridinone. All the aziridinones synthesised to date have an N-substituent which is an electron-donating and sterically bulky alkyl group. Both N-t-butyl[3] and N-adamantyl[11] aziridinones have been isolated in pure form.

In 1973, Miyoshi[12] reported the synthesis of optically active N-acylated aziridinone derivatives starting from the corresponding N-benzyloxycarbonyl-L-amino acids by the use of phosgene, thionyl chloride, or phosporus oxychloride. The reaction was carried out in tetrahydrofuran at -20 to -30° using a stoichiometric quantity of triethylamine. Among other N-protecting groups were p-bromobenzyloxycarbonyl and p-chlorobenzyloxycarbonyl, which also yielded quantitavely the corresponding aziridinones. The author[12] also found that the nature of the N-protecting group exercised a marked influence on the ability of N-protected amino acids to convert to the aziridinone. Thus with tosyl protection the yield was diminished; with p-methoxybenzyloxycarbonyl protection, t-butoxycar-

bonyl and t-amyloxycarbonyl protection aziridinones could not be detected at all; with benzoyl and acetyl protection, oxazolones resulted; and with benzyl protection the corresponding N-benzyl-N-carboxyanhydride was produced[12]. Some of Miyoshi's N-acylated aziridinones were obtained in crystalline form. The retention of optical integrity by the aziridinones was demonstrated by hydrolysis to give the corresponding amino-acids (although the paper does not quote actual data). The structures of the cyclic intermediates were affirmed on the basis of spectroscopic evidence.

The aziridinones prepared by Miyoshi were found to be effective acylating reagents, thus he used them, either after isolation or *in situ*, to prepare peptides in reactions which proceeded with retention of configuration (Fig. 1.). Coupling *via* aziridinones appears to be a particularly effective way to overcome steric hindrance with some components. The preparation of the gastrin C-terminal tetrapeptide amide by this method has been described[13].

In a recent communication, Jones and Witty[14] disputed Miyoshi's assignment of structure (3). They base their argument on spectroscopic characterisation. The authors assert that the heterocycle in question is 2-benzyloxy-4-benzyloxazol-5-(4H)-one(4)[14], the first oxazolone derivative derived from a urethane-protected amino-acid.

2. FOUR-MEMBERED RING-SYSTEMS

2.1. β-Lactams (or 2-Azetidinones). The four-membered β-lactam ring system of compounds was not known until the beginning of the

present century. The first β-lactams were prepared accidentally by Staudinger and his co-workers[15], in the course of their investigations of the reaction of diphenylketene with Schiff's bases. After 1943, renewed interest in the synthesis and overall chemistry of β-lactams was given further impetus by the discovery that the natural penicillin[16] and cephalosporin[17] antibiotics contain the β-lactam ring in their structure. Over the years the chemistry and synthesis of β-lactams has received frequent attention in reviews.[18,19,20,21]

The carbonyl group of the β-lactam structure is reasonably activated (ν_{max} 1730-1780cm^{-1}), and hence has the potential to react with a nucleophilic entity such as an amino acid ester to yield a peptide.

For many years, however, β-lactams were not easily accessible. The simpler β-lactams which are both less stable and very reactive, have presented a greater problem to synthesise than the highly substituted β-lactams. Moreover, the configurational strain sustained in the β-lactam ring-structure is an important contributing factor to the difficulty in the formation of this ring system. As a general rule, the ease of formation of any lactam ring-system is determined, to a large extent, by ring size. Thus the γ- and δ-amino acids give lactams quantitatively on heating: these γ- and δ-lactams contain five and six-membered rings, and are also referred to as 2-pyrrolidones and 2-piperidones respectively.

During the past ten to fifteen years β-lactams have been used as monomers for the preparation of high molecular weight poly-β-amides

Fig. 2

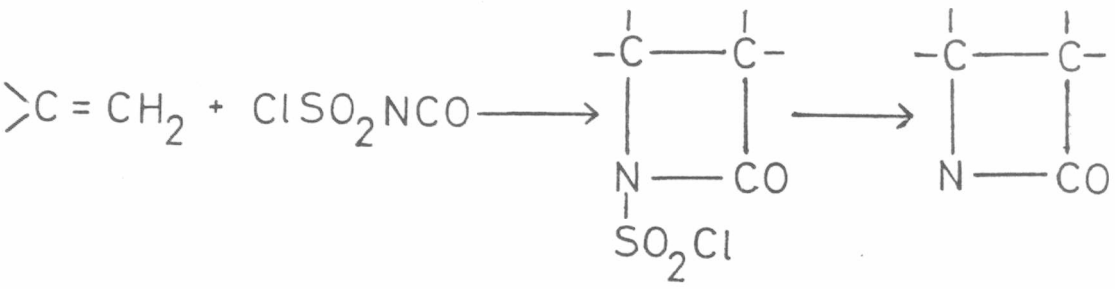

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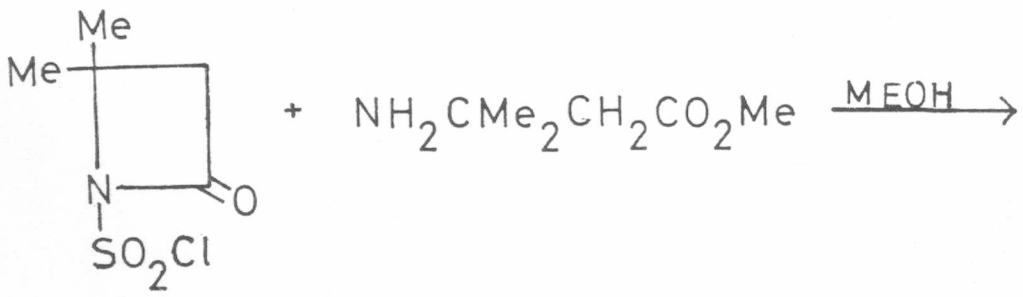
Fig. 3



(7) (8)

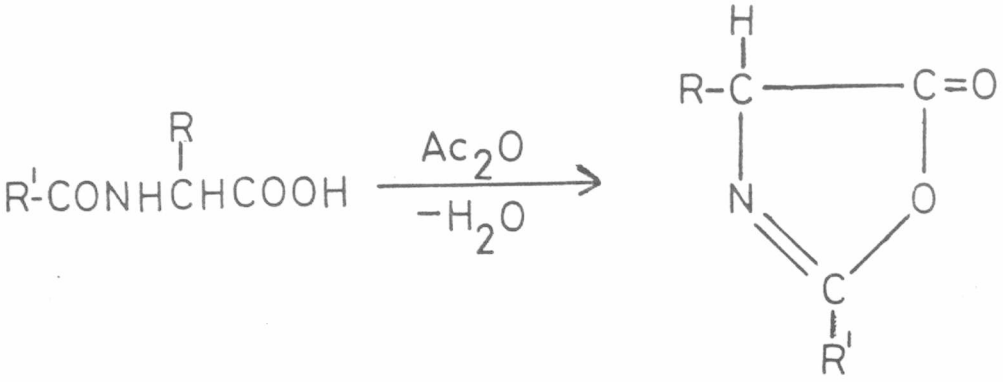
(9)

with fibre-forming properties. The utilisation of β-lactam derivatives in this way only became possible after the discovery, by Graf[23], of a novel and high-yield synthetic route to β-lactams *via* the reaction of chlorosulphonyl isocyanate with olefins (Fig. 2). The resulting *N*-chlorosulphonyl-β-lactams (5) are converted to the free β-lactams (6) of the sulphonyl chloride group by hydrolysis under weakly acidic conditions. The anionic polymerisation of β-lactams has afforded poly-β-amides containing up to 10000 units[24] in the chain.

The ability of the β-lactam ring to undergo aminolytic cleavage appears to be widely recognised in the literature, and yet there has evidently been little effort directed towards the exploitation of these activated intermediates for stepwise peptide synthesis.

The acylating potential of 2-azetidinones in peptide synthesis was recently investigated by Drey and his collaborators[25]. For instance *N*-chloro-sulphonyl-2-azetidinone (7) was treated with methyl β-amino-isovalerate (8) in methanol and triethylamine to give the dipeptide diester (9)[25] as an oil in reasonable yield (Fig. 3). The reaction demonstrated that the *N*-chlorosulphonyl-2-azetidinone (7) could be used as an intermediate in acylating the sterically hindered amino-ester (8). The same azetidinone was used with various *N*-protecting groups. The *N*-sulphenylated-2-azetidinone derivative was found to be a poor acylating agent. However, the tosyl group has been noted for its ability to activate both the δ- and γ- lactams to nucleophilic agents[26]. However the tosylazetidinone was insufficiently reactive to be used for peptide synthesis, even though aminolysis

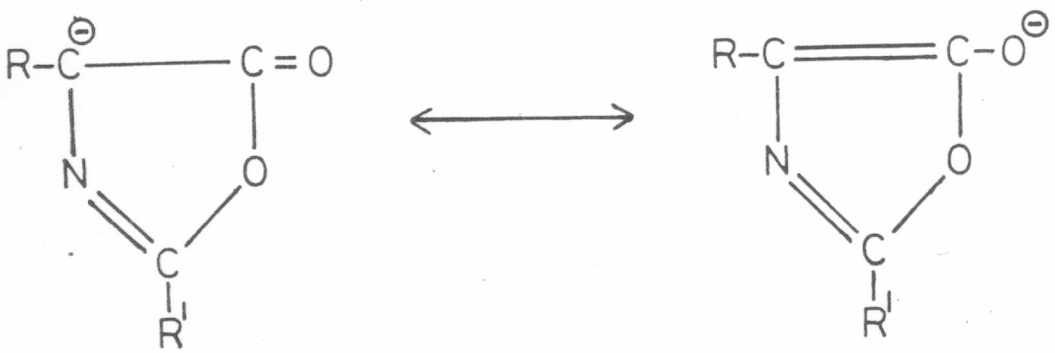
Fig. 4



(10)

(11) R^{I} = Ph, R = $PhCH_2$

Fig. 5



gave tosyl-β-isovaleranilide.

3. FIVE-MEMBERED RING-SYSTEMS

3.1. Oxazolones (Azlactones). As early as 1928, Bergmann and Zervas[27] postulated the formation of oxazolones to explain the racemisation observed during the acetylation of amino-acids in glacial acetic acid with more than an equivalent amount of acetic anhydride. Oxazolones are accessible from α-amino-acids by intramolecular elimination; it is possible to regard them as the inner anhydrides of the α-acylamino acids.

Acetic anhydride has been used predominantly as the cyclising reagent of choice (Fig. 4); other reagents used for this purpose include dicyclohexylcarbodi-imide (DCCI). The oxazolones (10) once formed, racemize readily by base-catalysed removal of proton from the asymmetric carbon, a process which is facilitated by resonance stabilisation of the resultant anion (Fig. 5).

It is well-known that N-substituted amino acids show a much smaller tendency to racemization than amino acids with primary amine functions. Acylated, N-substituted amino acids have no enolizable amide hydrogen and therefore the cyclisation reaction must be much slower, since no base catalysis is possible. The resulting compounds would be charged oxazolonium salts[28].

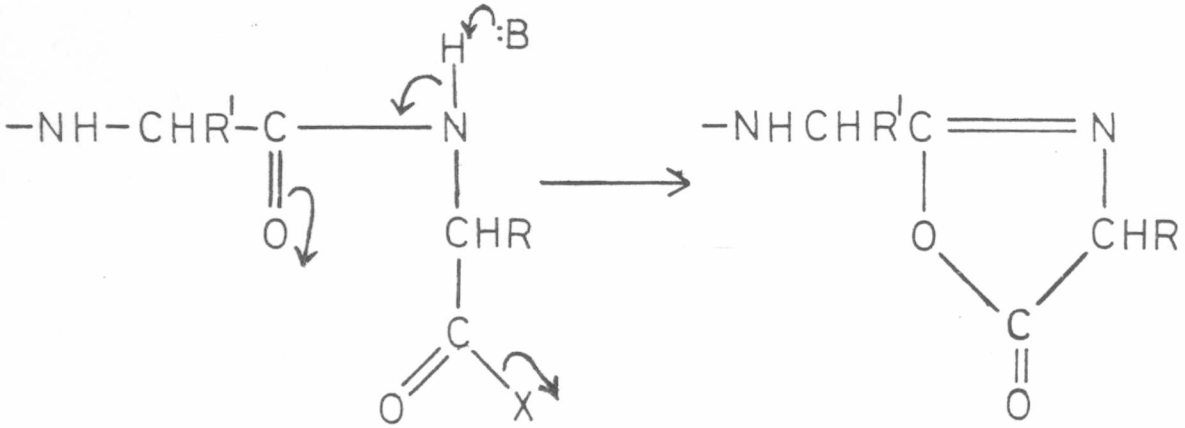
Early attempts[29] to isolate optically active oxazolones were unsuccessful. In a review article in 1946, Carter[30] described the racemization of oxazolones as an extremely facile process such that no optically active modifications could be isolated.

A great impetus in many laboratories to study the chemistry of oxazolones was provided by the incorrect proposition of a thiazolidine-oxazolone structure for penicillin[31].

Techniques for the synthesis of optically active oxazolones were developed as a result of efforts to confirm this idea. Thus in 1964 Goodman and Levine[32] reported the isolation of the first optically active oxazolone in the crystalline state, 2-phenyl-L-4-benzyl-oxazolone (11). Benzoyl-L-phenylalanine was allowed to react with acetic anhydride in dioxane. The reaction was followed polarimetrically, and it was observed that the rotation changed from positive to negative as the product formed. At the point of maximum negative rotation the solvent was removed and the oxazolone purified. This event provided a sound basis for the study of the factors that influence the racemisation of oxazolones.

Oxazolone formation, during N-acylation of α-amino acids or during activation of the N-acylated derivatives of these amino-acids in peptide coupling is, pre-eminently, a function of the nature of the N-protecting group as well as the mode of activation employed. The nature of the base and solvent polarity as well as side chain groups play an auxiliary role[33]. Thus the formation of an oxazolone intermediate is greatly enhanced when using amino-protecting groups such as the benzoyl, acetyl, trifluoro-acetyl, and formyl groups. Young and co-workers[34,35,36] found that the yields of L-peptide in the DCCI coupling of benzoyl, acetyl, and formyl-L-leucine with glycine ethyl ester in dichloromethane were 54%, 70%, and 94%, respectively. These

Fig.6

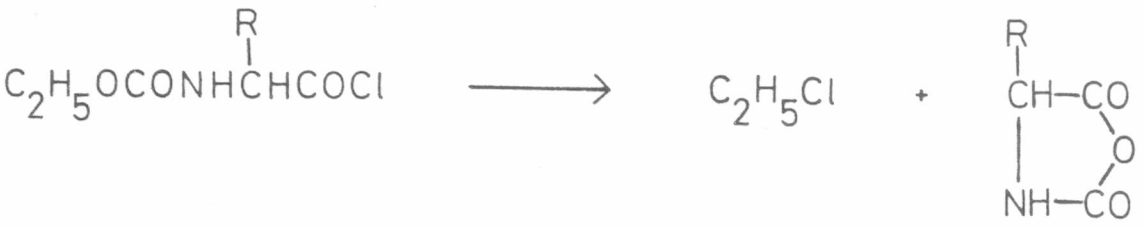


values must have been related to the relative ease of formation of the oxazolone intermediate.

From dipeptides oxazolone formation can take place under the driving influence of the carboxyl activating group and removal of proton by base (Fig. 6); this however is a less facile process than for acylated α-amino acids[37].

The carbonyl of the oxazolone system is highly activated, with a characteristic infrared absorption band at $1832cm^{-1}$. As a result they are sensitive to nucleophilic attack and are capable of undergoing aminolysis to yield peptides or amides. The preponderant limiting factor in the use of this method of activation is the problem of racemisation. It does not always follow, however, that peptide coupling *via* the oxazolone intermediate will give racemic products; whether racemisation occurs will depend on the ratio of the rates of racemisation and ring opening[37]. Early literature contains examples of the preparation of *N*-acetylated and *N*-benzoylated peptides by the oxazolone method[38,39,40]. Oxazolone derivatives prepared from amino-acids lacking a hydrogen atom at the α-position are sterically stable. Thus the oxazolone method of activation was used by Leplawy *et al.*[41] in 1960 to prepare peptides, in high yield, of the severely hindered amino acid, α-methylalanine. Further experiments along similar lines were carried out by Faust and Lange[42], Faust and Kleppel[43], and Diehl and Young[44]. Subsequently, oxazolone intermediates were used for the synthesis of peptides and polymers of some sterically hindered α,α-disubstituted amino acids by Jones and his associates[45]. In the same

Fig.7



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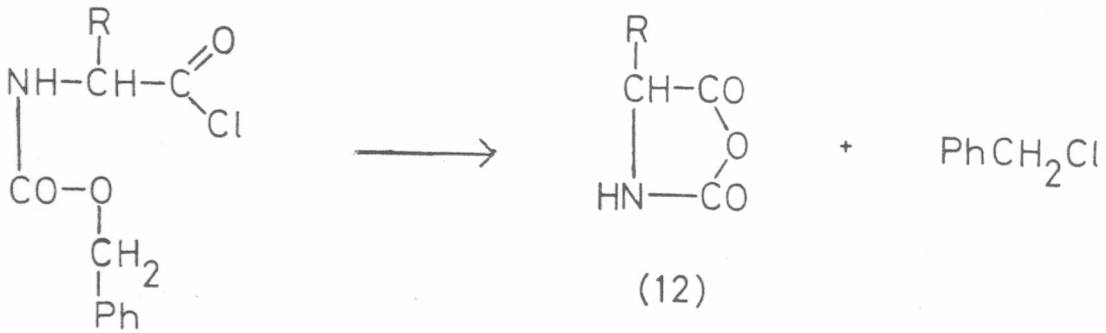
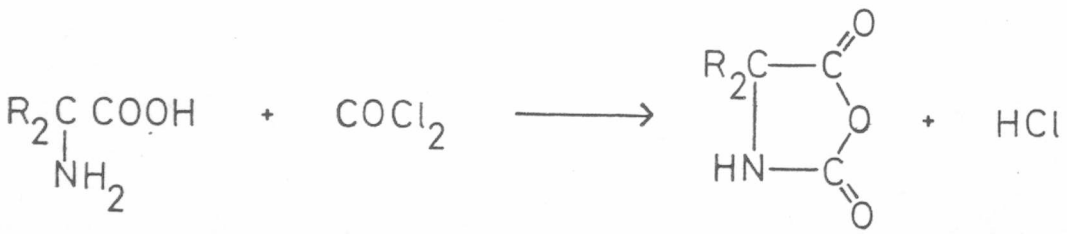


(12)

Fig.9



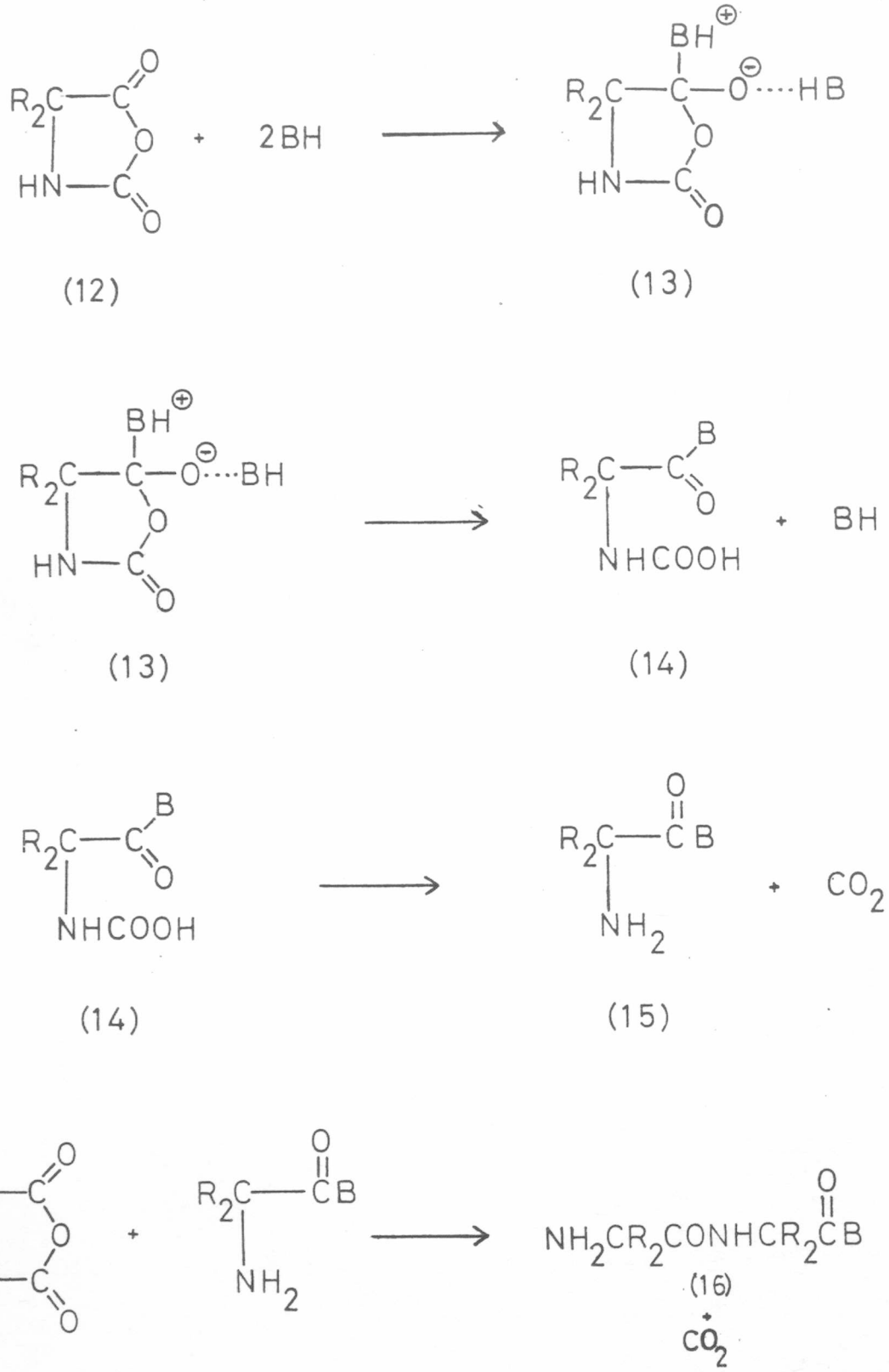
(12)

year Kenner et al.[46] extended the same principle to the preparation of peptides of the cycloalkane amino-acid, 1-aminocyclohexane-carboxylic acid.

3.2. N-Carboxyanhydrides (NCA's). **N-Carboxyahdrides or** 3-alkyloxazolidine-2, 5-diones (12) are also referred to as Leuchs' anhydrides[47,48]. Leuchs discovered the ready conversion of Fischer's carbethoxyamino acid chlorides, prepared by reaction of the N-carbethoxy derivative of an α-amino acid with thionyl chloride, to N-carboxy-amino acid anhydrides (12) through thermal elimination of alkylchlorides (Fig. 7). Similarly, NCA formation readily takes place during treatment of benzyloxycarbonylamino acids with thionyl chloride. The acid chloride formed under these conditions readily decomposes to liberate benzyl chloride and NCA (12) (Fig. 8). This reaction has sometimes been put to preparative use. The preferred method of preparation involves reacting the amino acid directly with phosgene (Fig. 9)[49,50].

NCA's can be considered a special type of mixed anhydrides. These cyclic compounds are highly activated and undergo attack by nucleophilic reagents including amino acid esters, the latter lead to the formation of peptides. They have been used widely as activated intermediates for the formation of polyamino acids[50], and occasionally for the synthesis of defined peptides. NCA's can be polymerised with a variety of catalysts, such as water, primary, secondary, and tertiary amines, and strong bases such as sodium methoxide. Base-catalysed polymerisation of NCA's[51] is one of the principal methods used in polyamino acid synthesis. The technique of base-initiated polymerisation

Fig. 10


R2C
C
O
HN
C
O
+ 2BH
BH⊕
O⊖····HB
(12)
(13)
O⊖····BH
+ BH
NHCOOH
(14)
B
+ CO2
NH2
(15)
+
NH2CR2CONHCR2CB
(16)
+
CO2

of NCA monomers was discovered as early as 1927[52]. However the first controlled polymerisation reaction yielding a high molecular weight polypeptide was not accomplished until 1947, when water was used to initiate the polymerization of D,L-phenyl-alanine-NCA and L-leucine-NCA yielding polymers with molecular weights of approximately 15000[53].

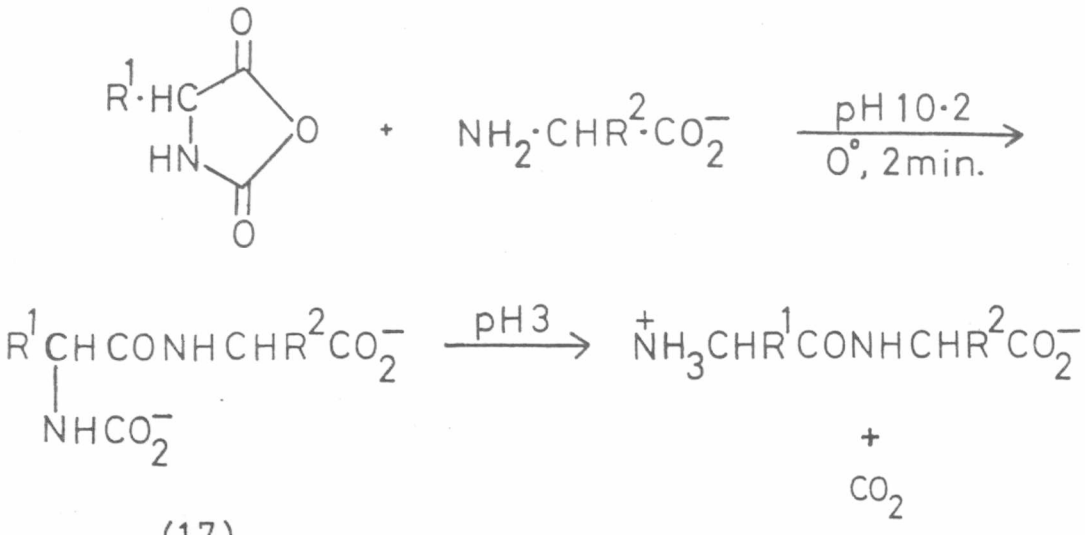
The mechanism of the initiation step of an NCA polymerisation reaction by base is determined by the amino acid involved and by the strength and stereochemistry of the base[54]. Addition of base to the carbonyl function of the monomer at the C_5-position of the oxazolidine-2,5-dione (12) ring leads to the formation of a transient intermediate (13), which first rearranges to a carbamic acid derivative (14) and then decarboxylates to give the starting amino acid (15). Nucleophilic attack by the amine at the NCA carbonyl carbon results in ring opening, proton transfer, and further loss of carbon dioxide (Fig.10). Propogation continues by reaction of the product (16) with more NCA.

Thirty years ago Bailey[55,56] carried out investigations into conditions leading to controlled peptide synthesis *via* NCA's. Hunt and du Vigneaud[57] reacted alanine-NCA with two equivalents of histidine methyl ester, and isolated the dipeptide in the form of a copper complex. Wessely *et al.*[58] also employed the NCA method in the preparation of di- and tripeptides of D,L-phenylalanine, albeit in low yield. Birkofer and Modic[59] succeeded in preparing *N*-carboxy-β-amino acid anhydrides corresponding to Leuchs' compound (*N*-carboxy-β-alanine anhydride), and in synthesizing poly-β-amides containing up to 100 amino-acid units. The α,α-disubstituted β-amino acids occupy a

special place, β-elimination, which can lead to loss of ammonia, or to cleavage of the polymer chain, is suppressed in these cases. Thus Lincoln[60] was able to convert β-aminopivalic acid (in the medium of cresol at 190°) into polyamides whose viscosities correspond to molecular weights of 5000 to 10000.

The possibility of controlled peptide synthesis in aqueous solution from NCA's was first investigated by Bartlett and Jones[61] in 1956. The NCA's of glycine, DL alanine and α-aminoisobutyric acid were reacted with water (in aqueous solution at 0°) and also with some amino acids and the reaction rate constants determined by measurement of the rate of evolution of carbon dioxide. The authors found that there were two competing reactions of importance to interfere with the clean production of a desired peptide. One of these was the reaction of the anhydride with hydroxyl ion, the other the reaction of the product peptide with anhydride to give the peptide with one more amino acid unit. After 1966 a series of papers were published by Hirschmann, Denkewalter and their collaborators describing the use of NCA's for controlled peptide synthesis, in aqueous media, without the isolation of intermediates. High yields of peptides were obtained[62] when NCA was added to an aqueous solution of an amino-acid or peptide under very carefully controlled conditions (Fig. 11 illustrates the preparation of a dipeptide). Detailed investigations of the mechanism of the reaction and of the optimum conditions for the preparation of dipeptides were subsequently described[63]. Control of the pH was vital because at pH values below ca. 10 the dipeptide carbamate (17) loses carbon dioxide giving the dipeptide at a rate

Fig.11

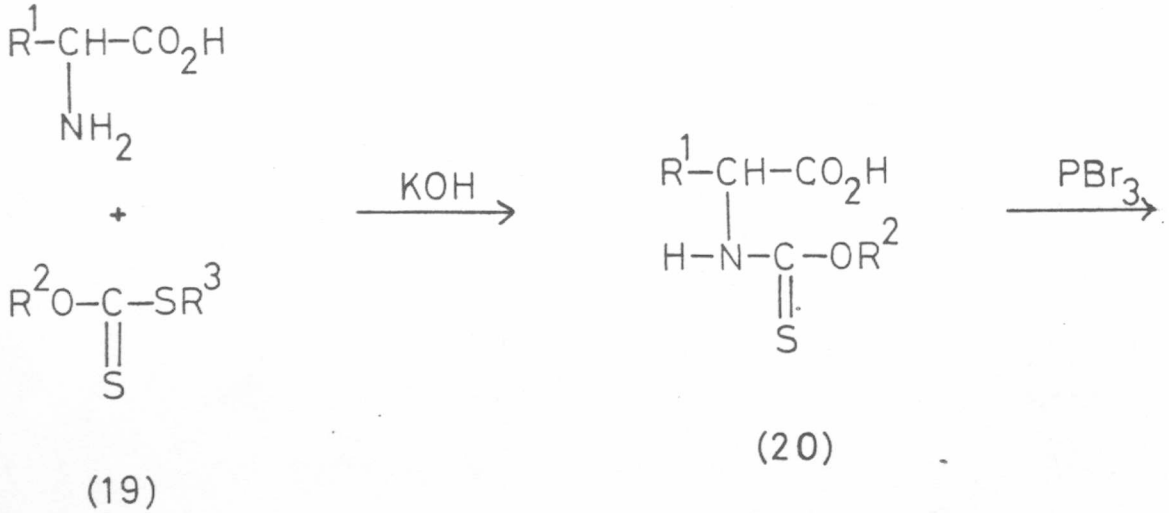


(17)

Fig. 12

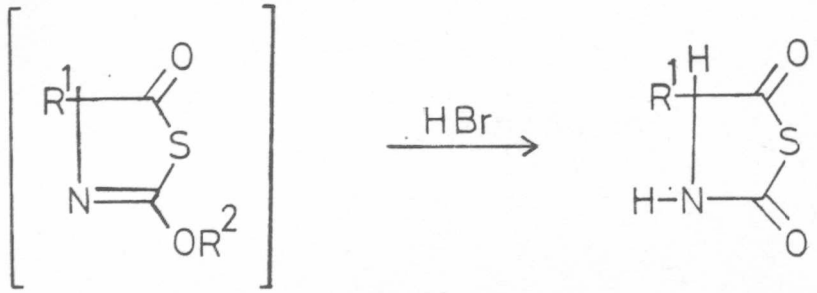
H·Trp-Met-Asp-Phe·NH_2

(18)



(19)

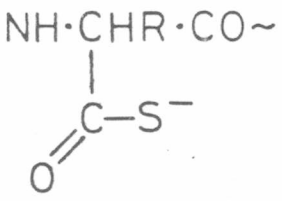
(20)



(21)

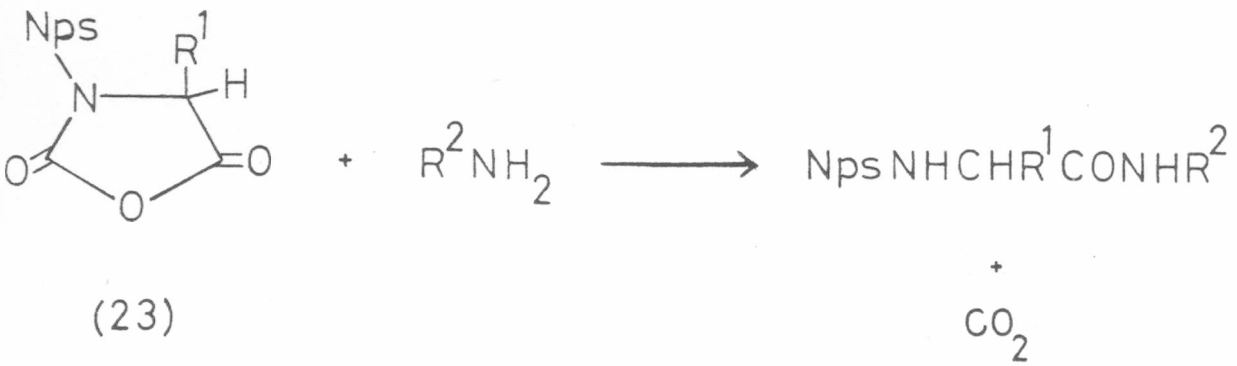
sufficient to cause significant competition with the original amino-component for the NCA; a situation of 'over-reaction'. On the other hand, if the pH is greater than ca. 10.5, hydrolysis of the NCA and other side reactions occur. Very rapid dissolution of the NCA is necessary to avoid 'over-reaction'. The reactions were free of racemisation. The product from one reaction of this type can be coupled directly with a second NCA without further manipulation and the process repeated several times. By this method oligopeptides may be prepared very rapidly without isolation of intermediates and using relatively easily prepared starting materials. A notable advantage with this method is that the side-chain functional groups which are usually protected in other methods of peptide synthesis can be left exposed without detrimental consequences. A demonstration of the feasibility of the method was the preparation of C-terminal tetra-peptide of gastrin (18)[64] in one hour, using phenylalanineamide as the amino component in the first step.

The sulphur analogues (21) of the NCA's, the 2,5-thiazolidinedi-ones (or N-thiocarboxy-anhydrides, NTA's)[64] can also be used in a manner similar to the NCA's. The method of choice for the preparation of NTA's (21) of high optical purity involves the cyclisation of N-(alkoxythiocarbonyl) amino acids (20) with phosphorus tribromide (Fig. 12). The thiocarbamate salts (22) which are produced are more stable than the corresponding carbamate salts, and over-reaction is therefore a less serious problem with these derivatives. Thus lower pH values can be used, with a consequent reduction in hydrolysis of the anhydride and other side-reactions induced by base. Because they



(22)

Fig. 13



(23)

are particularly prone to yield by-products as a consequence of conversion to isocyanates, the NCA's of histidine and glycine are not suitable, and in these cases the NTA's are preferred. Unfortunately the NTA's give a certain degree of racemized product[64]. The N-carboxyanhydride and the N-thiocarboxyanhydride methods were used in the preparation of the fragments required for the synthesis of ribonuclease-S-protein by Strachan, Hirschmann, et al[65].

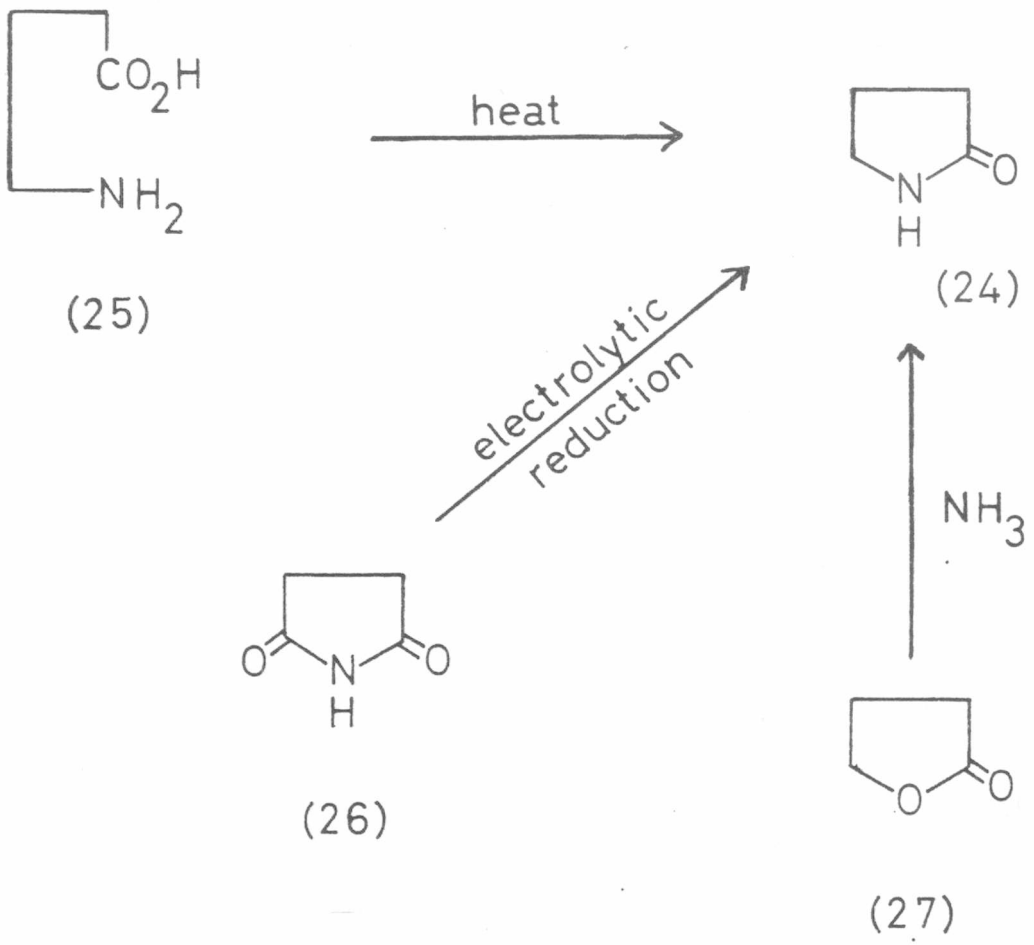
Stepwise controlled synthesis of a model tetrapeptide on a water-soluble poly-ethylenimine support by the NCA method has been investigated by Blecher and Pfaender[66]. The finished peptide was cleaved from the polymer by **enzymatic action.**

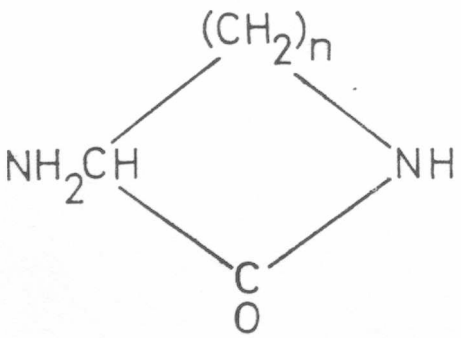
In order to overcome the general instability of NCA's in repetitive synthesis of peptides a number of N-protected NCA's have been investigated. NCA's can be quantitatively acylated with o-nitrophenylsulphenyl chloride in anhydrous solvents in the presence of triethylamine or N-methylmorpholine, yielding N-o-nitrosulphenyl-N-carboxyanhydrides (23)[67], which are crystalline, more stable than the parent NCA, and do not polymerize[67]. The derivatives (23) react with nucleophiles such as amines as shown in Fig. 13.

3.3. 2-Pyrrolidones. Pyrrolidones are cyclic amides γ-butyrolactams (24). They can be obtained by intramolecular thermal dehydration of γ-aminobutyric acids (25). They can also be obtained by electrolytic reduction of succinimides (26), or by the action of ammonia on butyrolactones (27) (Fig. 14)[68].

Ester hydrochlorides of lysine[69] and α,γ-di-aminobutyric acid[69,70]

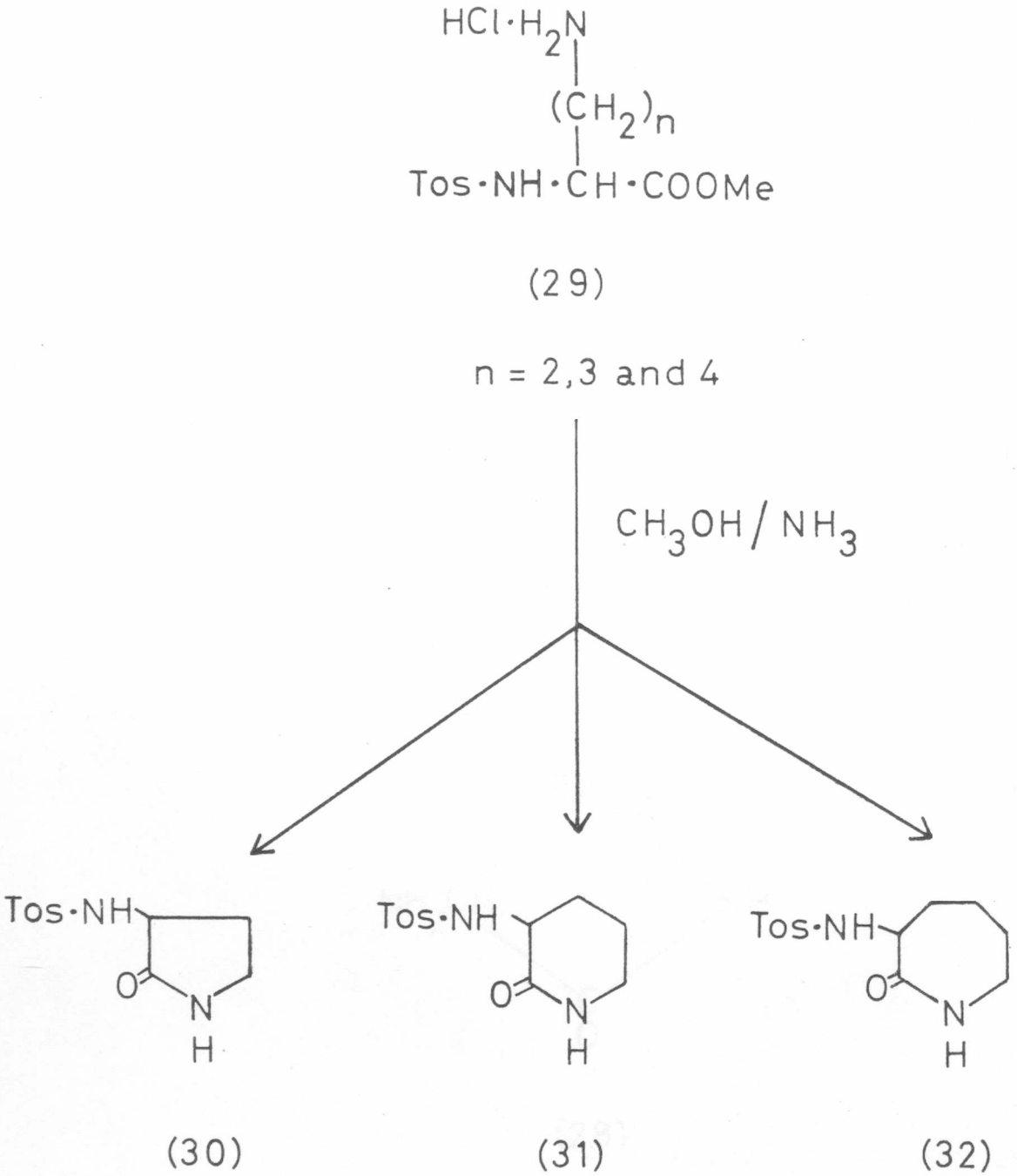
Fig.14


CO2H
NH2
heat
N
H
O
(24)
(25)
electrolytic
reduction
NH3
O
N
H
O
(26)
O
O
(27)


(CH2)n
NH2CH
NH
C
O

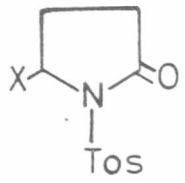
(28)

Fig. 15


HCl·H2N
(CH2)n
Tos·NH·CH·COOMe
(29)
n = 2,3 and 4
CH3OH / NH3
Tos·NH
O
N
H
(30)
Tos·NH
O
N
H
(31)
Tos·NH
O
N
H
(32)

in the presence of sodium alkoxides at room temperature readily cyclised to afford 3-amino lactams (28) (n=4 and 2 respectively). In addition, Rudinger[71] found that Curtius degradation of N-tosyl-L-glutamic acid β-hydrazide gave 3-tosylpyrrolid-2-one (30). In 1957, Barras and Elmore[72] observed that methyl ester hydrochlorides of α-N-tosyldiamino-acids (29) (n=2: α,γ-diaminobutyric acid; n=3: ornithine; n=4: lysine) may be cyclized on treatment with methanolic ammonia to form 3-tosyl-L-aminopyrrolid-2-one (30), 3-tosyl-L-aminopiperid-2-one (31), and 3-tosyl-L-aminohomopiperid-2-one (32) respectively. The yields (88,82 and 49% respectively) indicate that the seven-membered ring is less readily formed than the other two. A good yield of 3-tosylpiperid-2-one (31) also resulted from treatment of the ester hydrochloride (29; n=3) with triethylamine in methanol at room temperature. The three foregoing lactams were also obtained by the hydrogenolysis of the corresponding α-N-tosyl-ω-N-benzyloxycarbonyldiamino-carboxylic acid amides in methanol in the presence of acetic acid. The yields were quantitative for the 2-pyrrolidone (30) and the 2-piperidone (31), whereas only 7% of the 2-homopiperidone (32) was obtained, thereby emphasising the relative difficulty of closing the seven-membered ring.

Barras and Elmore[72] also characterised the three lactams that they isolated by means of infrared spectroscopy. The carbonyl absorption band for the pyrrolidone was found to be at 1698 cm^{-1}, while the two other cyclic systems were at 1658 cm^{-1}. Du Vigneaud and his collaborators[73], and then, independently in another laboratory, Rudinger[74] recognised that the cyclic derivative, 1-tosylpyrrolid-5-one-2-carboxylic acid (33) constitutes an activated intermediate



(33) X = COOH

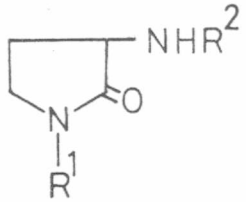
(34) X = H

$R^1NHCH_2CH_2CH_2CONHCH_2COOR^2$

(35) R^1 = Tos, $R^2 = C_2H_5$

(36) R^1 = Tos, R^2 = H

(37) $R^1 = R^2$ = H



(38) $R^1 = R^2$ = Z

(39) $R^1 = R^2$ = Tos

(40) R^1 = Tos, R^2 = Z

$R^1NHCH_2CH_2CH(NHR^2)CONHCH_2COR$

(41) $R^1 = R^2$ = Tos, $R = OC_2H_5$

(42) R^1 = Tos, R^2 = Z, $R = OC_2H_5$

(43) $R^1 = R^2$ = Z, $R = NH_2$

(44) $R^1 = R^2$ = Z, $R = N{\cdot}NH_2$

(45) $R^1 = R^2$ = Z, $R = OC_2H_5$

$Z = C_6H_5CH_2OCO-$

capable of undergoing nucleophilic attack by an amino ester to yield α- and γ-glutamyl peptides and peptides of glutamine. Later, Poduška and Rudinger[75] extended the same principle to the synthesis of derivatives and peptides of other amino acids, such as γ-aminobutyric acid and α,γ-diaminobutyric acid. For example γ-tosylaminobutyric acid was cyclised to N-tosylpyrrolidone (34) by the action of thionyl chloride, or sec-butyl chloroformate in the presence of tertiary base. Cyclisation in this instance therefore proceeded under standard conditions of the "mixed anhydride" method of peptide synthesis. Reaction of the lactam with glycine ethyl ester in boiling acetonitrile or without solvent, afforded γ-tosylaminobutyroylglycine ethyl ester (35). The authors have not stated the yield; however free dipeptide (37) was obtained by saponification of the ester (35) followed by treatment of the N-protected dipeptide acid (36) with sodium in liquid ammonia. Detosylation was also carried out by the action of hydrogen bromide in glacial acetic acid[76,77].

Dibenzyloxycarbonyl-α,γ-diaminobutyric acid was first converted to the cyclic pyrrolidone derivative (38) by Wilkinson[70] via the action of phosphorus pentachloride. Zaoral, Rudinger and Šorm[78] demonstrated that this derivative (38) undergoes aminolytic fission on treatment with ammonia, hydrazine, or glycine ethyl ester to yield the amide (43), hydrazide (44) of dibenzyloxy-carbonyldiaminobutyric acid, and dibenzyloxy-carbonyl-α,γ-diaminobutyroylglycine ethyl ester (45), respectively. Poduška and Rudinger[75] treated ditosyl-L-α,γ-diaminobutyric acid with thionyl chloride and obtained an optically active pyrrolidone derivative (39). This compound (39) reacted readily with

glycine ethyl ester to give a fully-protected dipeptide (41).

Different α- and γ-amino protecting groups were used[75] in order to enable selective liberation of one or other of these functional groups for further synthetic reactions as would be necessary in the synthesis of more complex peptides of α,γ-diaminobutyric acid. Hence α-N-benzyloxycarbonyl-γ-N-tosyl-L-α,γ-diaminobutyric acid was used as starting material for conversion to a pyrrolidone derivative (40) via the "carbonic mixed anhydride" route (using sec-butyl chloroformate in the presence of tertiary base). By reacting this γ-butyrolactam derivative (40) with glycine ethyl ester Poduška and Rudinger obtained the fully-protected dipeptide (42).

Pyrrolidone is a heterocycle that will give polymers by ring-opening reactions. The reaction is activated by alkaline catalysts, usually employed in conjunction with acylating agents such as carboxylic acid chlorides and anhydrides, isocyanates, and inorganic anhydrides such as phosphoric anhydride[79]. However, low temperatures are required. Above 60-80°, the polymer will revert to the monomer in the presence of catalyst. The ease of polymerisation, within a series of lactams of intermediate ring size, by this method of initiation is as follows: caprolactam ≥ pyrrolidone > piperidone[80]. Under these conditions, pyrrolidones have been polymerized into polyamides (nylon-4) having a sufficiently high molecular weight for fibre production.

4. SIX-MEMBERED RING-SYSTEMS

4.1. Piperidones. These are cyclic amides-δ-valerolactams. They are obtainable from δ-amino-carboxylic acids in principle by

elimination of water, albeit under vigorous conditions.

As has been noted above, piperidone derivatives (31) were also isolated in 82% yield by Barras and Elmore[72] during treatment of methyl ester hydrochlorides of α-N-tosyl-L-ornithine (29, n=3) with methanolic ammonia.

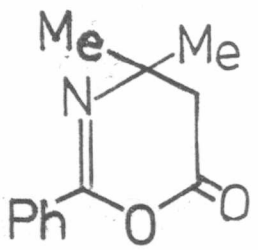
Piperidone derivatives are capable of being used as activated peptide coupling intermediates. As has been noted before, Rudinger[26] found that the reactivity of both the β-butyrolactams and δ-valerolactams was assisted by amino-protection with the tosyl group. Furthermore, Rudinger and his associates[81] found that aminolysis of the lactam ring is dependent upon the solvent. Acetonitrile and nitromethane were shown to be suitable, while dioxane, pyridine, diethyl phosphite, and dimethylformamide gave less satisfactory results. Piperidones have however not been extensively used as peptide coupling intermediates.

The parent compound has been polymerized anionically[82], in the presence of N-acetyl-α-piperidone (from piperidone and acetic anhydride[79]) to produce nylon-5.

4.2. Oxazinones. In the course of their endeavours to synthesize penicillin homologues Baker and Ollis[83] treated β-benzamidoisovaleric acid (46) with hot acetic anhydride and obtained a six-membered heterocyclic compound, the oxazinone derivative (47). This compound resembled the familiar oxazolones derived from the N-acyl-α-amino acids in reacting with water, ethanol, and aniline to give the parent acid and its ethyl ester and anilide respectively. Such oxazinone

$PhCONHCMe_2CH_2CO_2H$

(46)



(47)

(48) $NH_2CMe_2CH_2CO_2Me$

(49) $PhCONHCMe_2CH_2CONHCMe_2CH\ CO_2Me$

(50) $PhCONHCMe_2CH_2CONHCMe_2CH_2CO_2H$

derivatives are only obtainable from β-amino acids, but might also arise in principle by dehydration of, for example, N-acyl derivatives of aspartic acid, which are simultaneously α- and β- amino acids.

In 1954 Barker[84] sought to investigate whether the conversion of N-benzoylated β-amino acids to oxazinone derivatives was general. The competing reaction in this synthesis is that for symmetrical anhydride formation. Barker differentiated the anhydrides which he obtained by their lack of volatility[84] and by lower yields of anilide obtained with aniline at room temperature. According to Barker[84], saturated β-benzamido-acids containing quaternary β-carbon atoms are dehydrated by acetic anhydride to oxazinones; other saturated β-benzamido-acids yield symmetrical anhydrides.

Recently Lowbridge and Drey[85] investigated the preparation of peptides from β-amino acids. Attempts to prepare simple peptides from suitably amino- and carboxyl- terminal protected derivatives of β-aminoisovaleric acid failed using conventional coupling techniques. This was most probably attributable to steric hindrance at the amino function. The authors then investigated the use of oxazinone derivatives. Their approach was analogous to work carried out in Liverpool by Kenner and his collaborators[41,45,46] in which oxazolones were used to overcome the problem of steric hindrance in peptide synthesis. The oxazinone derivative (47) was thus prepared *via* the route previously prescribed by Baker and Ollis[71]. Aminolysis of the oxazinone (47) with the amino ester (48) under reflux conditions afforded the desired dipeptide (49) in good yield. The product was saponified to obtain the N-protected dipeptide acid (50) from which the 'dipeptide oxazinone'

Fig.16

was derived by the action of acetic anhydride. Aminolysis of the dipeptide oxazinone afforded a fully protected tripeptide in an overall yield of 50%[85]. Drey, Lowbridge and Ridge[86] later prepared additional dihydro-oxazinone derivatives to study the generality of the method. It was noticed[86] in the synthesis of the tripeptides that as the peptide length was extended, more severe reaction conditions were required. Deprotection of the finished peptide was carried out by saponification of the ester, followed by removal of the benzoyl group by electrolytic reduction[87]. The yields following N-deprotection were mediocre[86].

The possibility of substituting for the benzoyl group some other N-terminal acylprotecting groups which would be more easily removable was investigated[88,89]. The formyl group, removable by the use of methanolic hydrogen chloride, was used by Kenner's group [41,45,46] for preparation of their activated oxazolone derivatives. Ridge[89] prepared the formyl derivative of β-aminoisovaleric acid via the formylation method of Muramatsu et al.[90], obtaining the desired product in low yield. The derivative could not be converted to oxazinone.

Further investigations on the utility of the oxazinone method are reported in this thesis.

5. SEVEN-MEMBERED RING-SYSTEM. The seven-membered cyclic amides are generally referred to as the caprolactams. These compounds do not appear to have been exploited as intermediates for stepwise peptide synthesis. However, caprolactams polymerize readily at high temperatures with anionic initiators to give polycaproamide or nylon-6 (Fig. 16)[82]. The product is a tough horny material with a melting

point of about 215°. It may be fabricated to a tough film by pressing in a laboratory press at temperatures in excess of 215°, or it may be extruded from a spinneret in the form of a filament.

Caprolactam polymerization can also be initiated by catalytic amounts of water[91] at elevated temperatures. Introduction of water does not lead to hydrolysis of the caprolactam to the amino acid followed by a typical polycondensation reaction. The polymer grows by reaction of the chain end with additional cyclic monomer in a manner similar to that noted above for the polymerization of N-carboxy-anhydrides. Just as in the case of butyrolactams, the polymerization of caprolactams is very effectively catalysed by trace quantities of N-acyl lactams[79].

6. EIGHT-, AND NINE-MEMBERED LACTAMS[92]. The eight-membered ringed compounds, 7-heptanolactams or enantholactams, and the nine-membered ringed 8-octanolactams or capryl lactams are known.[92] Like caprolactam, enantholactam and the capryllactam can be polymerized[92] in reactions initiated by catalytic amounts of water at elevated temperatures. The relative rates of polymerization for these three lactams, which in fact are the most important monomers generally polymerized by this type of initiation are: caprolactam > enantholactam ≃ capryllactam.

The ten-, eleven-, and twelve-membered lactams are very difficult to prepare because of transannular crowding of hydrogen[92] atoms, so that these polyamides are prepared directly from the ω-amino acids.

Discussion of Experimental Results.

The aims of the work discussed in this thesis were as follows:

(a) to examine in detail the circumstances of formation of the cyclohexylamide derivative (57) isolated in the course of preparation of peptides of β-aminopivalic acid via the agency of DCCI[89];

(b) to investigate problems associated with the utilisation of commercially available racemic β-aminobutyric acid in the preparation of linear and cyclic homopeptides;

(c) to prepare optically active β-aminobutyric acid, and using it as a building unit in the preparation of linear and cyclic homopeptide derivatives employing such peptide coupling techniques as would avoid or minimise racemisation;

(d) to ascertain the utility of the "oxazinone route" as a method of peptide coupling in the respect of optically active β-amino acids.

$NH_2CH_2CMe_2CO_2Et$

(51)

$NH_2CH_2CMe_2CO_2H$

(52)

$ZNHCH_2CMe_2CO_2H$

$Z = C_6H_5CH_2OCO-$

(53)

$ZNHCH_2CMe_2CONHCH_2CMe_2CO_2R$

(55) R = Et

(56) R = H

(a) DCCI Side Reaction ; The Formation of a Cyclohexylamide

Ridge[89] reported that a coupling reaction, mediated by DCCI, between β-benzyloxycarbonyl-aminopivalic acid (53)[89] and β-aminopivalic acid ethyl ester (51)[95], carried out under standard conditions resulted in a two-component mixture which at the time could not be successfully separated. The two components exhibited similar t.l.c. behaviour when ethyl acetate, chloroform, and chloroform-ethyl acetate were used as solvent systems; it was therefore assumed that both products were neutral substances. The peptide component was isolated as the acid (56)[89], after saponification. The organic extract of the alkaline solution afforded a neutral substance which analysed correctly for the cyclohexylamide derivative (57)[89], and gave cyclohexylamine on acid hydrolysis. From this result the origin of the cyclohexylamide (57) could only be conjectured[89]. In 1973, Fenwick reported the isolation of a cyclohexylamide derivative as a by-product during use of DCCI in the preparation of an aziridine derivative. He[96] attributed the formation of the amide to nucleophilic attack by the amine on the N-acylurea (NAU) derivative, a product resulting from rearrangement of O-acylisourea. The DCCI coupling reaction reported by Ridge[89] was repeated. In addition, a complementary experiment was carried out to isolate the suspected side-product of this reaction, namely, β-benzyloxycarbonyl urea (54). We have now shown that cyclohexylamide derivatives are alkaline cleavage products of N-acylureas.

Preparation of β-aminopivalic acid and derivatives. Preparation of β-aminopivalic acid involved hydrogenation of ethyl α-cyanoisobutyrate[93] in the presence of a catalyst, Raney nickel[94]. This method[95] gave β-aminopivalic acid ethyl ester (51)[95] in 50% yield; part of the ester was hydrolysed with hydrochloric acid under reflux conditions to afford

the amino acid (52) hydrochloride[96] in high yield.

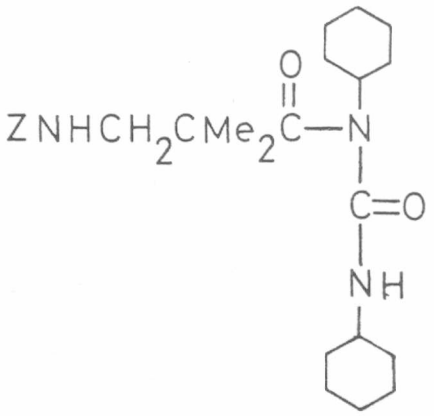
The acid (52) hydrochloride[96] was acylated under Schotten-Baumann conditions, according to the method of Bergmann and Zervas[97], to give β-benzyloxycarbonylaminopivalic acid (53). The identity of the product was confirmed by comparative spectroscopy[89]. A small portion of the N-protected amino acid (53) was converted into a dicyclohexylammonium salt[89] in 70% yield.

Isolation of the NAU derivative (54)

The factors that favour the formation of the N-acylurea (NAU) rearrangement product during the use of DCCI as a coupling agent in peptide synthesis are well documented[98,99,100]. NAU formation is favoured by high dilution, presence of an organic base, such as triethylamine, and carrying out the coupling reaction, using DCCI at temperatures well above 0°.

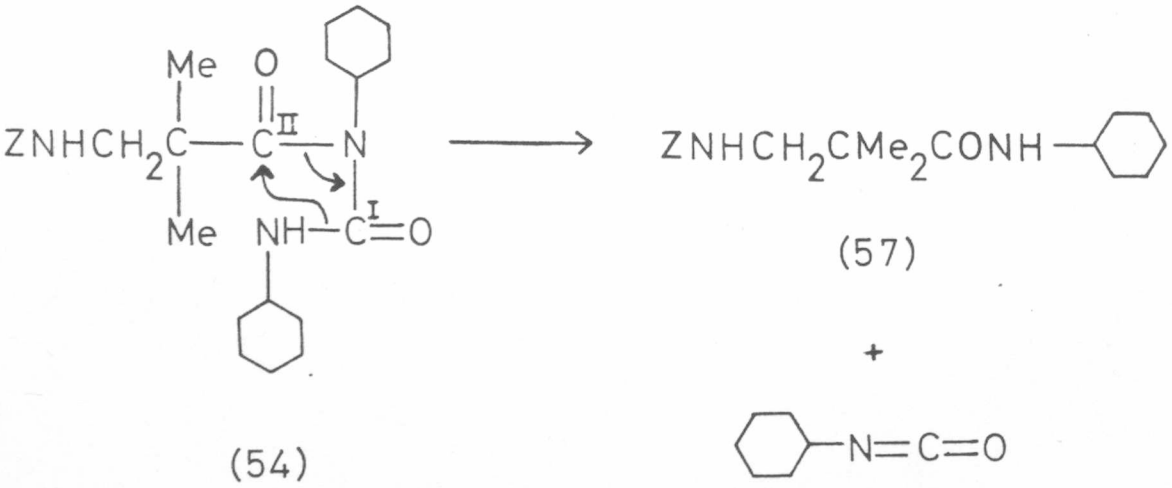
Thus an experiment directed towards the isolation of the NAU derivative of β-benzyloxycarbonylaminopivalic acid (54) was carried out by mixing equimolar quantities of N-protected amino acid (53) and DCCI in ethyl acetate. Excess triethylamine was also added to the reaction which was then allowed to stir at room temperature for 7 days, before 'work-up'. A product, obtained in 20% yield, was an oil; identified as the expected NAU derivative on the basis of spectroscopic examination. The material crystallized from light petroleum after prolonged refrigeration, and the assignment of the compound was confirmed by elemental analysis.

A considerably higher yield of the NAU derivative (58%), was realised when the same reaction was carried out at 40° over the same length of time as above.



(54)

Fig. 17



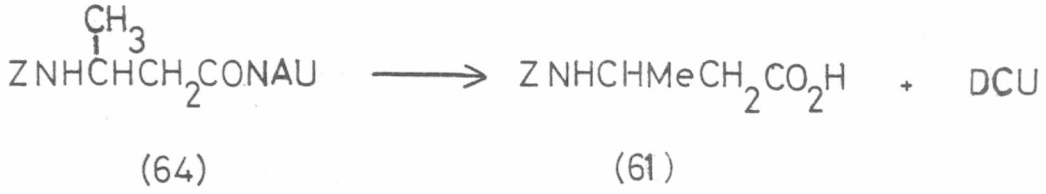
(54)

(57)

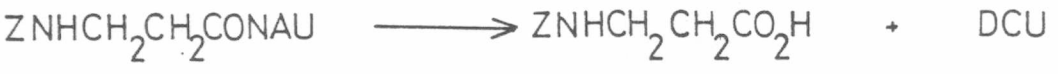
Coupling reactions via DCCI, and studies on NAU derivatives

The coupling reaction between β-benzyloxycarbonyl-aminopivalic acid (53) and β-aminopivalic acid ethyl ester (51) via DCCI was carried out under standard conditions. The neutral solution from the usual 'work-up' procedure revealed two spots on t.l.c. These ran closely together in chloroform and chloroform-ethyl acetate mixtures, but it was found that the components separated satisfactorily in benzene-ethyl acetate systems. This pointed to the possibility of achieving a complete separation of the two components by column chromatography. A silica column was prepared and eluted with a solvent mixture of benzene and ethyl acetate, which was varied by making progressive increases in ethyl acetate on the basis of t.l.c. monitoring of the eluate. In this way the faster-moving substance, which was found to be NAU (54), was obtained in a 52% yield; the slower-moving substance had all the characteristics of the fully-protected dipeptide (55), and this was recovered in a 37% yield (yields were calculated on material put onto the column). Both substances were recovered in a chromatographically pure state, the mixed portion accounted for the deficiency in the overall yield of products.

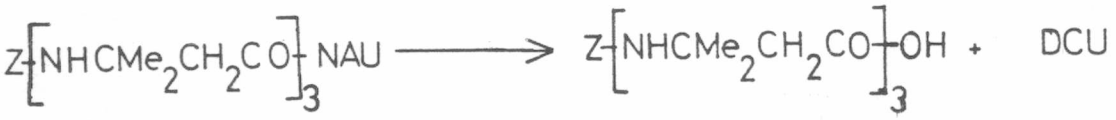
Treatment of the dipeptide ester (55) with alkali and work-up of the reaction according to standard procedure afforded a dipeptide acid (56) as the sole product of the reaction. The NAU derivative (54), isolated by an independent experiment mentioned above, was also treated with base separately and the product of the saponification shown to be a cyclohexyl-amide derivative (57). The second product of NAU cleavage by this pathway (Fig. 17) could be cyclohexylisocyanate, however attempts to demonstrate the presence of this compound by t.l.c. examination of the mother liquors



(64) (61)



(58)



(59) (60)

employing an authentic sample of the compound as an internal standard, produced negative results. Since cyclohexylisocyanate is a volatile compound, it is most probable that it was lost by evaporation either during the course of the reaction or during 'work-up'. Nevertheless, by this experiment the origin of cyclohexylamide (57) as a product of NAU (54) cleavage under basic conditions was unequivocally demonstrated.

It was decided to saponify other NAU derivatives at hand in order to investigate the generality of this reaction pathway. Base treatment of D L-β-benzyloxycarbonylaminobutyroyl NAU (64) yielded N, N' -dicyclohexylurea (DCU) and the corresponding N-protected amino acid (61)[103]; β-benzyloxycarbonylalanyl NAU (58)[88] gave DCU and β-benzyloxycarbonylalanine[101] and β-benzyloxycarbonylaminoisovaleryl-β-aminoisovaleryl-β-aminoisovaleryl NAU (59) afforded DCU and the N-protected peptide acid (60)[88].

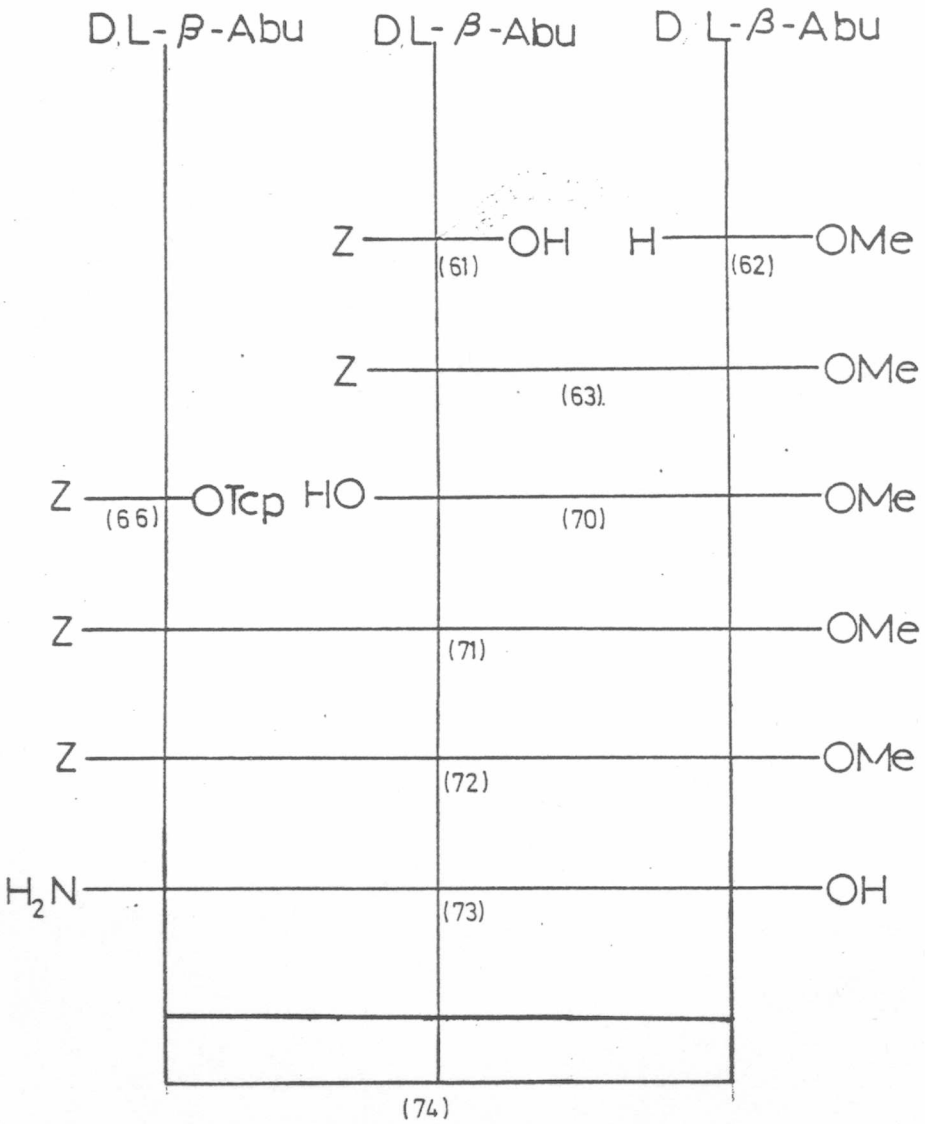
The two cleavage reactions can be rationalised in terms of steric hindrance at carbonyl (II) (Fig. 17), in the case of NAU (54), resulting in the hydroxyl ion being directed at carbonyl (I). The carbonyl (II), in the other NAU molecules considered, is relatively exposed and therefore preferential hydroxyl ion attack takes place at this point.

(b) Preparation of di- and tripeptides of D L-β-aminobutyric acid followed by cyclisation (Fig. 18).

The racemic 3-aminobutyric acid (D L-β-aminobutyric acid, β-Abu) used for the peptide synthetic work that follows (Fig. 18) was obtained commercially.

An infrared (i.r.) spectrum of the compound was recorded, the melting

FIG.:- 18


D.L-β-Abu
D.L-β-Abu
D.L-β-Abu
Z
OH
(61)
H
OMe
(62)
Z
OMe
(63)
Z
(66)
OTcp
HO
OMe
(70)
Z
OMe
(71)
Z
OMe
(72)
H2N
OH
(73)
(74)

point (m.p.) checked for correspondence with that given in the literature[102], and the homogeneity of the acid certified by thin layer chromatography (t.l.c.), whereupon single minhydrin positive spots were obtained in three different solvent systems.

Preparation of the amino- and carboxyl-protected derivatives.

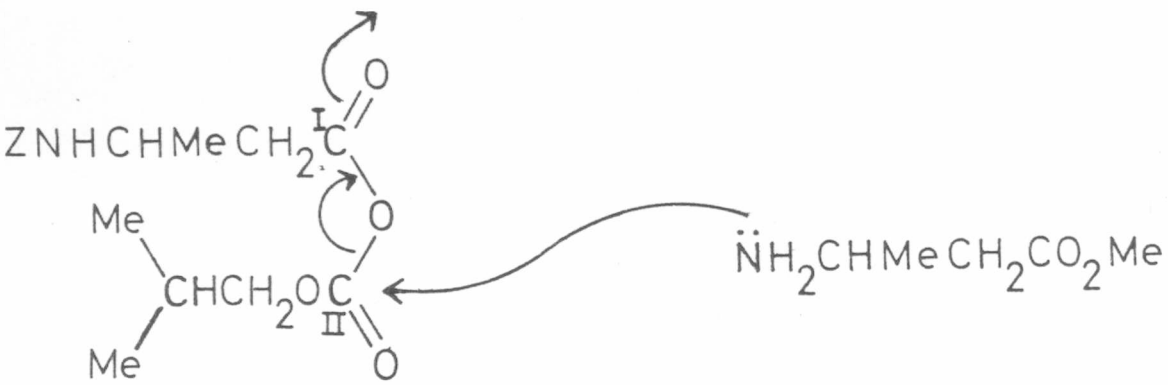
Benzyloxycarbonyl was the N-protecting group of choice in the peptide synthetic work described in this section. DL-β-Benzyloxycarbonylaminobutyric acid (61) was isolated by Birkofer and Modic[103] as a monohydrate in 84% yield. In this work, preparation of DL-β-benzyloxycarbonylaminobutyric acid (61)[103] gave yields of 64%. The derivative (61) has a marked capacity to solvate and needs prolonged drying in vacuo over phosphorus pentoxide.

Preparation of the methyl ester of DL-β-aminobutyric acid (62)[103] was carried out according to the method of Brenner and Huber[104], and of Curtius[105] alternatively.

Investigation of coupling methods.

Attempted preparation of dipeptide (63) by coupling DL-β-benzyloxycarbonylaminobutyric acid (61)[103] and the free methyl ester (62)[104,105] using DCCI yielded the NAU rearrangement product (64) in 70% yield. T.l.c. examination of the neutral solution indicated that this was the sole product of the coupling reaction. The experiment was repeated using DCCI in the presence of the catalytic additive, 1-hydroxybenzotriazole[106]. This led to isolation of the desired fully-protected dipeptide (63) in 37% yield; NAU (64) was also present as a side-product

Fig.19

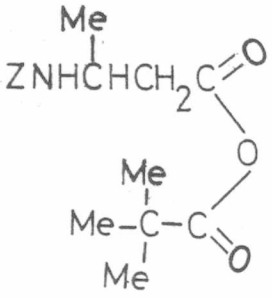


and was recovered in 12% yield, by crystallization from the mother liquors.

Coupling of the benzyloxycarbonyl derivative (61) with the amino ester (62) hydrochloride in the presence of triethylamine using the carbonic mixed anhydride (CMA) method resulted in the isolation of only 13% of the dipeptide (63). The product (63) was obtained by crystallization from the neutral solution of the reaction, which on t.l.c. examination had been found to contain two components. The dipeptide (63) corresponded with the slower-moving neutral component. No further work was done on the residue from the mother liquors. However, it is reasonable to surmise that another neutral species could have been formed by acylation taking an "unexpected route" so that attack by the amino ester (62) takes place at the alternative carbonyl (II) (Fig. 19) resulting in urethane formation[108, 109]; in this case a side-product which would be β-isobutyloxycarbonylaminobutyroyl methyl ester (Fig. 19).

Coupling via the CMA method was further investigated using a modification of the technique due to Applewhite and Nelson[110]. The method[110] involved "inverse addition" and an excess of chloroformate with a 5-10 min interval of activation; led to the dipeptide (63) being obtained in 29% yield. Repetition using "inverse addition" with zero activation time afforded only 11% of the dipeptide (63). Yet another modification of the CMA method was investigated, that is, the excess mixed anhydride method due to Tilak et al.,[111] which led to the isolation of the dipeptide in 50% yield.

Pivaloyl chloride proved useful, as a reagent, in the mixed anhydride

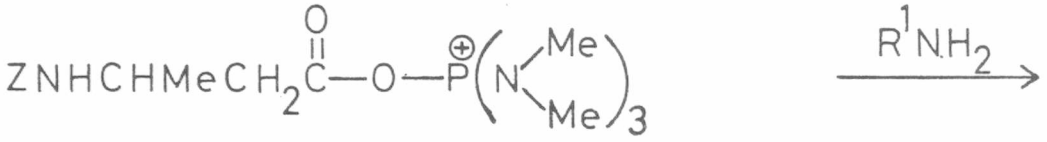


(65)

synthesis of α-benzyloxycarbonylaminoisobutyroyl-α-amino-isobutyric acid methyl ester, a compound not accessible by most current methods of peptide synthesis[112]. It was therefore considered worthwhile to investigate the efficacy of the mixed anhydride of pivalic acid with regard to peptide synthesis involving β-aminobutyric acid. Preparation of the pivalic acid mixed anhydride was carried out under the standard conditions of mixed anhydride synthesis. The anhydride (65) was isolated in 96% yield, in crystalline state, the infrared spectrum clearly showed the presence of an activated carbonyl group (ν max 1800, 1700 cm^{-1}). The compound gave correct results on elemental analysis. Coupling of the pivaloyl anhydride (65) with the amino ester (62) under reflux conditions afforded the dipeptide (63) in 60% yield.

The yields obtained by the coupling methods investigated so far were considered unsatisfactory. It was therefore decided to investigate the use of the active ester route. 2, 4, 5-Trichlorophenol, which had been used successfully with β-amino acids by previous workers[88, 89], became the reagent of choice. DL-β-Benzyloxycarbonylaminobutyroyl 2, 4, 5-trichlorophenyl active ester (66) was prepared *via* a DCCI[113] mediated reaction in pyridine at -15 to -10°. Low temperatures were maintained during the esterification process in order to suppress the formation of *N*-acylurea. Separation of DCU was facilitated by dilution of the reaction mixture with ether before filtration. The active ester (66) was isolated and characterised before use in the coupling reaction. Coupling was carried out in a concentrated solution (in view of the bimolecular nature of the reaction) of acetonitrile at 60° during a period of twenty-four hours. The yield (40%) was disappointing.

Fig. 20



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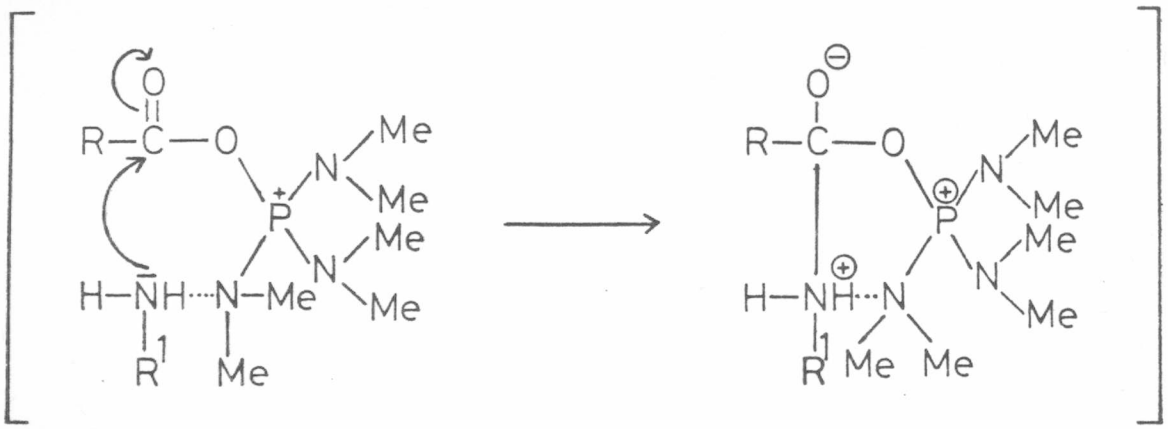


R = $ZNHCHMeCH_2-$

R^1 = $-CHMeCH_2CO_2Me$

(68)

$ZNHCHMeCH_2CONHCHMeCH_2CO_2H$

(69)

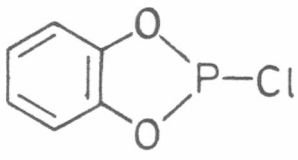
Carboxylic acids, such as acetic acid, are known to have a catalytic effect[114, 115] on aminolysis reactions involving active esters; however a yield of only 38% was realised when the active ester coupling reaction was repeated in the presence of acetic acid. Active ester coupling in hexamethylphosphoramide (HMPA) proceeded rapidly (10-15 min) and in relatively good yield (67%).

The facility of acylation experienced during use of HMPA as solvent can be visualised in terms of HMPA reacting with the active ester (66) to form a short-lived acyloxyphosphonium intermediate* (67); acylation can then proceed by direct attack (Fig. 20) of the amino component (62) at the activated carboxyl group with anchimeric assistance by a hydrogen-bonded cyclic transition state (68)[116].

An attempt to prepare a pivaloyl anhydride of N-protected dipeptide acid (69) failed due to lack of a suitable solvent. Coupling of N-protected dipeptide acid (69) with the amino component (62) via DCCI and 1-hydroxybenzotriazole in dimethylformamide afforded the fully-protected tripeptide (71), albeit in meagre yield (15%).

Free amino dipeptide ester (70) was prepared by catalytic (10% palladium on charcoal) hydrogenolysis of the fully-protected dipeptide (63). The oily product (70) was reacted in situ with the N-protected amino acid active ester (66) in HMPA to afford the fully-protected tripeptide (71) in 64% yield. Saponification of the tripeptide (71) to liberate the tripeptide acid (72) took longer than expected, some twenty-four hours. Subsequent experience showed that this process is facilitated by a higher water content (for example 30% aqueous alcohol) in the solvent system.

* Kenner and his associates[161] proposed phosphonium activated intermediates produced by the use of HMPA.



(75)

The free tripeptide (73) was obtained by catalytic hydrogenolysis of the N-protected tripeptide acid (72). The low yield (32%) of product (73) was partly attributed to incomplete saponification of (71). The pure free tripeptide (73) was high-melting, water soluble, and gave a pink colour with ninhydrin reagent.

Preparation of D L-cyclo-(tri-β-aminobutyroyl) (74).

Cyclisation involves the formation of a peptide bond between the amino and carboxyl group of the linear peptide (73). The activation required for this reaction was, in principle, not different from that required for the synthesis of a linear peptide. Unlike a typical peptide coupling reaction, cyclisation is a unimolecular process and therefore concentration independent. The reaction proceeds in high dilution (ten to a hundredfold) with concomitant suppression of polymerisation[117].

In the 'direct route' employed here the cyclisation reaction was effected with the aid of o-phenylene phosphorochloridite (75)[118, 119], a reagent that had been successfully used before in cyclisations involving peptides of β-amino acids[88, 89]. In this reaction the activation step was carried out in a rather concentrated (0.07M) solution of free linear tripeptide (73) with a 10% excess of o-phenylene phosphorochloridite in diethylphosphite at 0°. The resulting reaction mixture was then diluted (0.004M) with diethylphosphite, and triethylamine (to remove the hydrochloric acid formed during the reaction) added concomitantly, for the cyclisation step.

Some cyclo-tripeptide (74) separated from the reaction solution at room temperature; this product (74) represented a crude yield of 40%.

$PhCONHCHMeCH_2CO_2H$

(76)

$PhCONHCHMeCH_2COCl$

(77)

$PhCONHCHMeCH_2CO_2Et$

(80)

$PhCONHCHMeCH_2CONHCHMeCH_2CO_2Me$

(81)

$PhCONHCHMeCH_2CONHPh$

(82)

More cyclo-tripeptide (74) was obtained by evaporation of diethylphosphite and passing the residual material, dissolved in water, through acidic and basic ion-exchange resin columns. The overall yield of cyclo-tripeptide (74) after purification by sublimation was 32%. The structure of the cyclo-tripeptide (74) was confirmed by elemental analysis and mass spectrometry (Fig. 21).

The phenomenon of cyclodimerisation, that is, reaction of two molecules of the monomeric peptide with the formation of a cyclic dimer, has been shown to occur quite frequently with tripeptides and with pentapeptides[120, 121]. It was therefore essential to examine the low resolution mass spectrum (Fig. 21) of the cyclic product (74) to ascertain whether the cyclisation reaction was free of dimerisation products. However, the molecular ion at m/e 255 corresponded with that for the cyclo-tripeptide (74) of β-aminobutyric acid, there was no evidence of higher molecular weight fragments.

Investigation of isolability of the oxazinone derivative (79) of DL-β-aminobutyric acid and of its use as a peptide coupling intermediate[85, 86].

An attempt, by Barker[84], to cyclise the benzoyl derivative (76)[83] of DL-β-aminobutyric acid after treatment with acetic anhydride was reported[84] to have yielded only the symmetrical anhydride (78)[84]. The product (78) was non-crystalline, and the author's[84] criteria for differentiating the anhydride (78), from a possible oxazinone structure (79), were lack of volatility and lower yields of anilide (82).

However in the present programme it was presupposed that the possibility of effecting a cyclisation of the benzoyl derivative (76) to the corresponding oxazinone (79) still existed, using alternative conditions. The N-protected acid (76) was therefore treated with thionyl chloride, using carbon tetrachloride as a solvent, in an attempted cyclisation[41,89]. Neither the desired oxazinone (79) nor the symmetrical anhydride (78) was isolated. It was clear that the reaction had been considerably hampered by lack of homogeneity of the reaction mixture due to lack of solubility of the acid (76) in carbon tetrachloride. Repetition of the reaction with benzene as solvent afforded a crude liquid product (30%), the infrared spectrum of which exhibited the absence of the N-H absorption band at *ca.* 3300 cm^{-1} and a shift in the absorption band for the carbonyl group to higher frequency, 1795 cm^{-1}. Unfortunately, however, attempts to purify the product were of no avail. Nevertheless, retrospective comparison of infrared spectra, after isolation of the pure oxazinone (79), revealed that the crude product had contained the oxazinone (79).

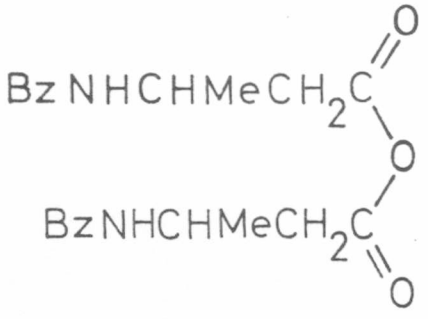
Cyclisation of the benzoyl derivative (76) *via* thionyl chloride using dichloromethane as the solvent resulted in a low yield of crystalline product with an activated carbonyl group absorption at 1815 cm^{-1}, and the N-H absorption at 3315 cm^{-1} retained. Retrospective comparison showed that the compound was the symmetrical anhydride (78).

It was believed that problems of purification and poor yield could be overcome by isolation of the acid chloride (77) intermediate in a pure state, with subsequent cyclisation.

The experiment[122] on the isolation of the acid chloride (77) afforded only 30% of the purified compound. The difficulty experienced in obtaining a good yield of acid chloride in this work was rather in accord with that of Gerrard and Thrush[123, 124]. It was found[123, 124] that the acid anhydride was the first product of the reaction, and it then reacted further with more thionyl chloride to give the acid chloride. The rate of formation of acid chloride[125] was reported, in general, to be comparatively slow and hence they[123,124,125] recommended that the reaction was suitable for the preparation of anhydrides.

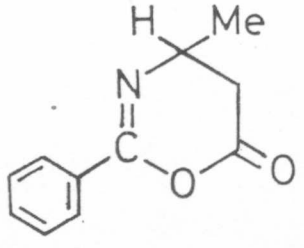
In view of the prevailing ambiguity over identification of the products of attempted cyclisation and in view of the noted[89] spectroscopic and structural similarities between anhydride and oxazinone derivatives, it was consequently decided to synthesise the anhydride (78) and fully characterise this for purposes of differentiation.

The preparation of DL-β-benzamidobutyroyl symmetrical anhydride (78) was carried out according to the method used by Barker. The resulting crude oily product was dissolved in ethyl acetate and examined by t.l.c. The product was found to be a mixture consisting of three components, all of them "activated" in so far as they reacted with the hydroxylamine/ferric chloride spraying reagent. Two components with higher R_f values were separated from the third by extraction with light petroleum (b.p. 60-80°). The residual material then crystallized to afford the symmetrical anhydride (78) in 65% yield. The two components in the petroleum wash were separated by column chromatography; spectroscopic evidence led to the recognition of the two by-products being the



Bz = C_6H_5CO-

(78)



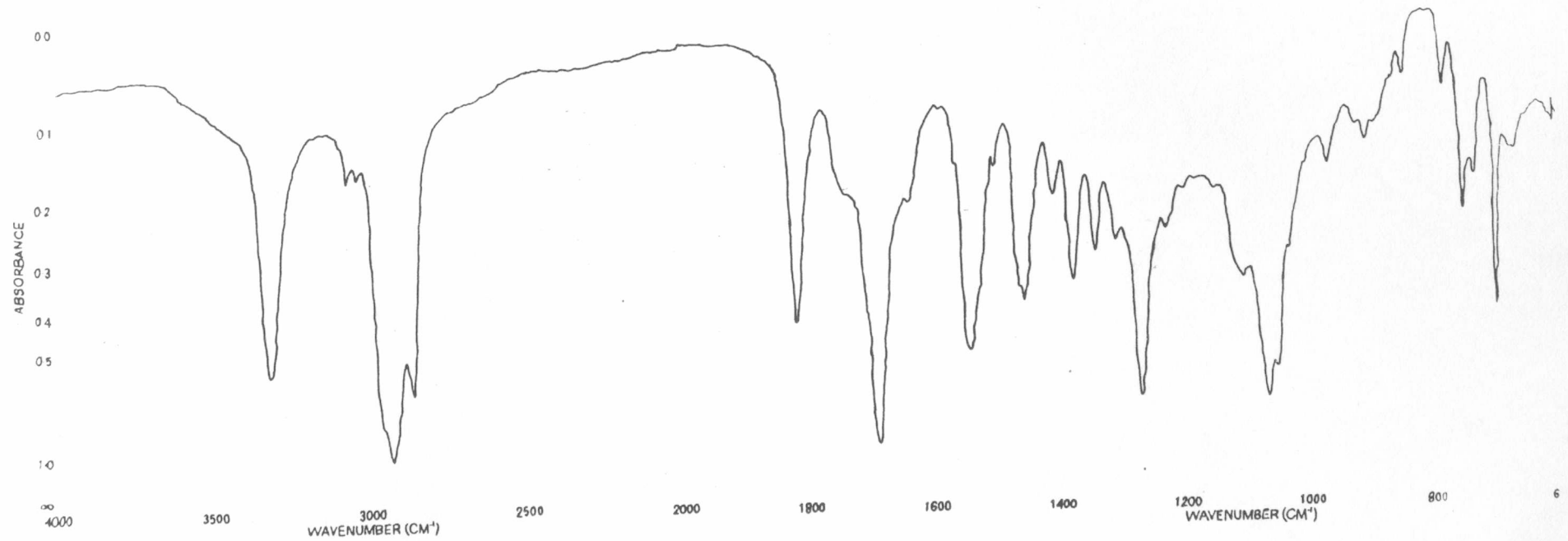
(79)

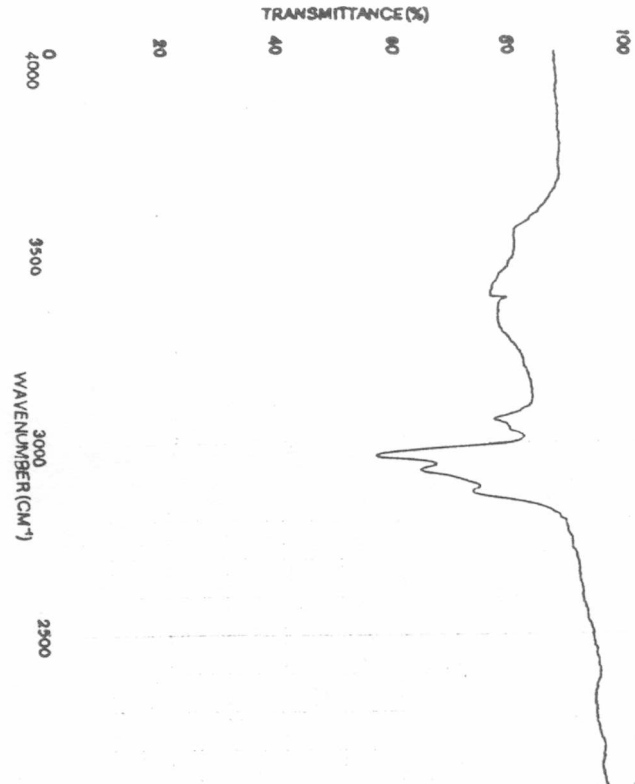
oxazinone derivative (79) and the ethyl ester (80) of DL-β-benzamidobutyric acid. The formation of the latter (80) must have been a consequence of the ethanol impurity, present in ethyl acetate, acting as a nucleophile in respect to the symmetrical anhydride (78) or oxazinone (79).

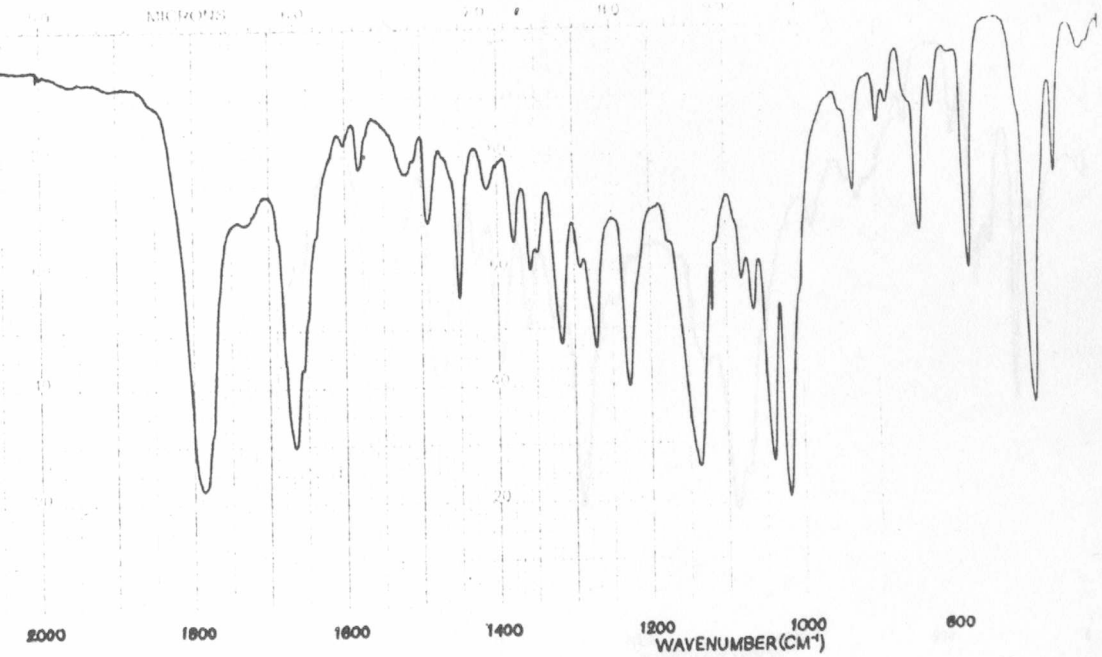
The reaction of DL-β-benzamidobutyric acid with acetic anhydride was also examined for its dependence on temperature. Treatment with acetic anhydride under reflux conditions for 20 min. afforded neither the symmetrical anhydride (78) nor the oxazinone (79), instead products were isolated which showed that cleavage of the benzoyl group had occurred to a considerable extent. The products isolated from the reaction were benzoic acid anhydride in 4% yield, benzoic acid in 21% yield, β-benzamidobutyric acid in 35%, β-acetamidobutyric acid in 7% yield, and β-aminobutyric acid in undetermined yield. Both the cleavage of the benzoyl group and the formation of the N-acetyl derivative may be ascribed to a transacylation reaction[89] *via* elimination of the benzoyl group as an acylium ion. The formation of benzoic acid probably took place, *inter alia*, *via* the benzoic anhydride in the presence of elements of water.

On the other hand, reaction of acetic anhydride and DL-β-benzamidobutyric acid at room temperature led to the isolation of the oxazinone (79) (alongside the anhydride (78)) in 7% yield. The yield of oxazinone increased to 13% when the reaction was carried out at higher dilution.

A high yield preparative route for the oxazinone (79) was provided by the CMA method, using freshly purified and dried tertiary base (triethylamine or N-methylmorpholine) at temperatures below -10°. The


0.0
0.1
0.2
0.3
0.4
0.5
1.0
∞
ABSORBANCE
4000
3500
3000
2500
2000
1800
1600
1400
1200
1000
800
6
WAVENUMBER (CM⁻¹)
WAVENUMBER (CM⁻¹)


TRANSMITTANCE (%)
100
80
60
40
20
0
4000
3500
3000
2500
WAVENUMBER (CM⁻¹)


2000
1800
1600
1400
1200
1000
800
WAVENUMBER(CM⁻¹)

oxazinone, obtained in 92% yield, was chromatographically pure and was used for spectroscopic characterisation (Fig.22a).

A study of the infrared spectra of the anhydride (78) (Fig. 22) and the oxazinone (78) (Fig.22a) was made. The infrared spectrum of the anhydride (78) is recognisable by the absorption band for N-H stretching at 3300 cm^{-1}, while the absorption band for one of its activated carbonyl groups is shifted to higher frequency, 1815 cm^{-1}, than in the case for the oxazinone (79). The anhydride features three absorption bands at frequencies above 1600 cm^{-1}, whereas the oxazinone (79) is characterised by only two strong bands at 1795 and 1670 cm^{-1}. The oxazinone exhibits some more strong absorption bands at 1110-1175 cm^{-1}, and a rather characteristic doublet at 1000-1050 cm^{-1}.

The low resolution mass spectrum (Fig. 23) for the oxazinone (79) gave a peak for the molecular ion at m/e 189, a value in accord with the molecular weight of the derivative (79). The base peak at m/e 105 corresponds to the benzoyl fragment and the other prominent peak at m/e 77 is the phenyl residue which results from the decay of the benzoyl group. The loss of carbon monoxide is discernible, albeit in low abundance, at m/e 161; and the peak at m/e 174 is attributed to loss of the methyl radical. Chemical studies that prove oxazinone structure are discussed in the later stages of this thesis (p.47).

The oxazinone (79) proved to be an effective acylating agent, reacting with aniline to afford the anilide (82) in 76% yield and with the amino ester (62) to give the benzoyl dipeptide methyl ester (81) in 66% yield.

(c) Preparation of optically active β-aminobutyric acid and its derivatives

The Arndt-Eistert[126] synthesis provides a method for the conversion of carboxylic acids into their homologues in three stages (Fig. 24). Some three decades ago, Balenović[127, 128] exploited this method for the preparation of optically active β-amino acids starting from the corresponding α-amino acids.

During use of this route, preservation of stereochemical integrity at the asymmetric carbon atom contained in the group R (Fig. 24) is of paramount importance. However, conversion of an amino acid into the N-protected acid derivative, or carboxyl activation of the same derivative might very well lead to racemisation at the asymmetric carbon atom *via* oxazolone formation. The intervention of racemisation at this stage is primarily dependent on the nature of the N-protecting group employed. The next stage at which the loss of optical activity may arise is during conversion of the diazoketone - Wolff rearrangement - in the presence of pertinent reagents to yield the homologated acid or its derivative (Fig. 24).

There are several mechanistic postulations for the Wolff rearrangement[129,130,131]. However, the generally accepted mechanism is one that assumes that elimination of nitrogen from the diazoketone results in the formation of a transitory carbene structure, characterised by an electron-defficient carbon, to which the migrating group R brings its electron pair (Fig.25).

The stereochemical effect of rearrangement, as it influences the configuration of the migrating group R, is a question relevant to all

Fig. 24

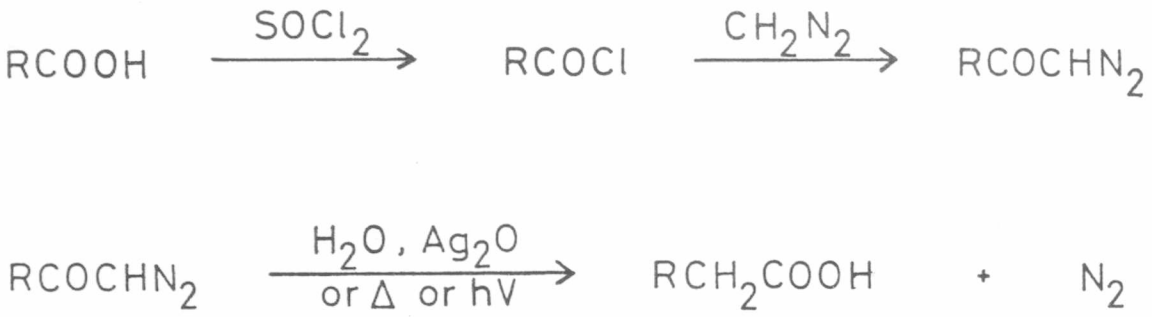


Fig. 25

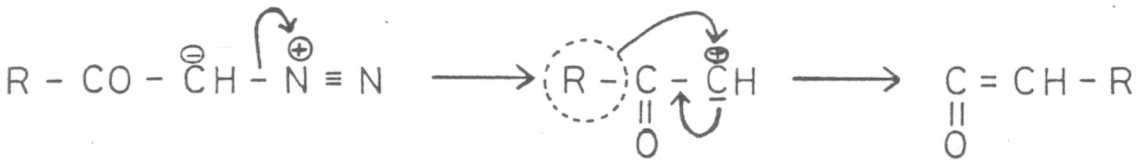
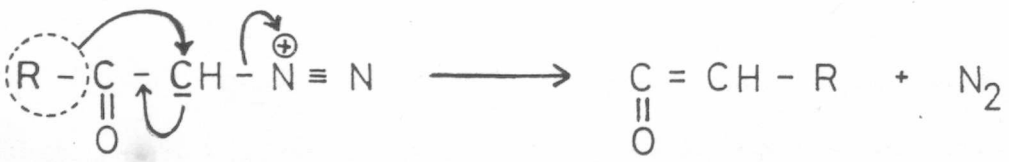


Fig. 26



rearrangements in which R is an alkyl or substituted alkyl group. Numerous investigations have demonstrated that the Wolff rearrangement proceeds with retention of configuration[132, 133]. Lane and Wallis[134], for instance, isolated a product of 99.5% optical purity. The high retentions of optical activity reported by various authors[131] strongly suggest that the migrating group R never leaves the system. The reaction, at least in some cases, must therefore be an intramolecular process in which the transfer of the R residue is synchronous with the elimination of nitrogen (Fig. 26).

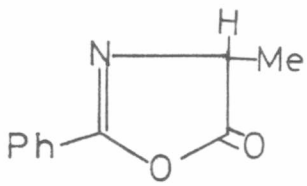
The Arndt-Eistert synthesis was therefore chosen as the route to optically active β-aminobutyric acid. In this work there also arose the question of selection of suitable N-protecting groups and a compatible method of carboxyl activation. Balenović *et al.*[128] used the phthaloyl group for N-protection and proceeded *via* the acid chloride method of activation. But the phthaloyl group is problematical in regard to the method of cleavage at the end of the synthesis[127,88,135].

Synthesis of benzoyl derivatives.

In the work described, the first N-protecting group to be tried was the benzoyl group. Success with the benzoyl group would have provided quick access to the oxazinone derivatives by cyclisation of the homologated acid. As has been noted in the introduction, oxazinones are useful peptide coupling intermediates[85,86]. Nevertheless, it was found that N-benzoylation of L-alanine under Schotten-Baumann conditions was prone to racemisation and that even when the desired optically active benzoyl derivative (83) had been obtained by acylation under pH-stat monitored

$PhCONHCHMeCO_2H$

(83)

$PhCONHCHMeCOCl \longrightarrow$ 

(84) (85)

$TosNHCHMeCO_2H$

(86)

$TosNHCHMeCOCHN_2$

(88)

$TosNHCHMeCH_2CONH_2 \xrightarrow{H^{\oplus}} TosNHCHMeCH_2CO_2H$

(89) (90)

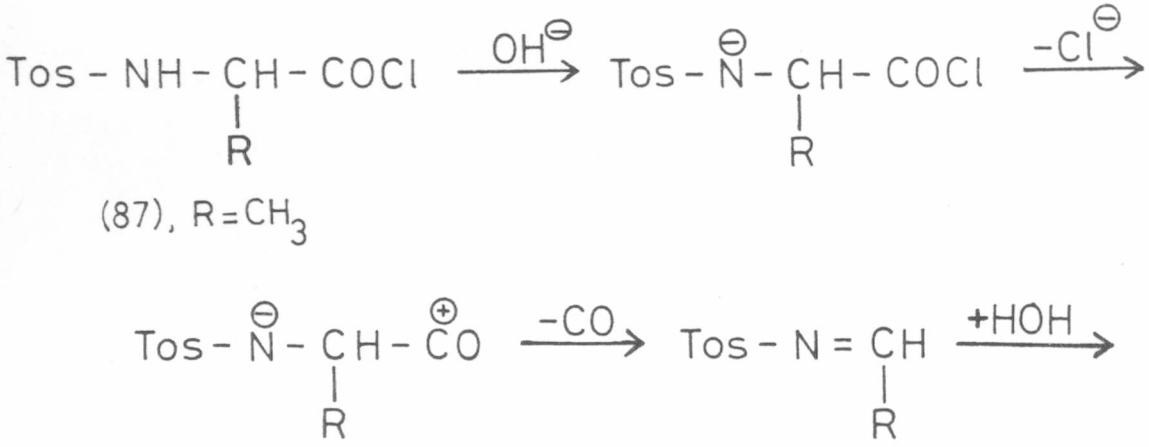
conditions, activation by the acid chloride method inevitably led to racemisation *via* the formation of an oxazolone. The oxazolone in the crude product was recognised by its characteristic infrared absorption band at 1832 cm^{-1}, in addition, a very low optical rotation was recorded for the crude product. This result was expected.

Synthesis of tosyl derivatives.

Introduction of the p-toluenesulphonyl (tosyl) group is known to be a process that is free of racemisation. Preparation of the *N*-tosyl derivative (86) of L-alanine proceeded smoothly in a yield of 78%. An attempt to isolate the *N*-tosyl L-alanyl chloride (87) failed since no method of purification could be found. This result was seemingly in accord with that of Wiley *et al*.[138] who reported that tosyl amino acid chloride derivatives were not amenable to purification by the usual means and thus converted them to amides. However, the crude product (87) had a satisfactory optical rotation.

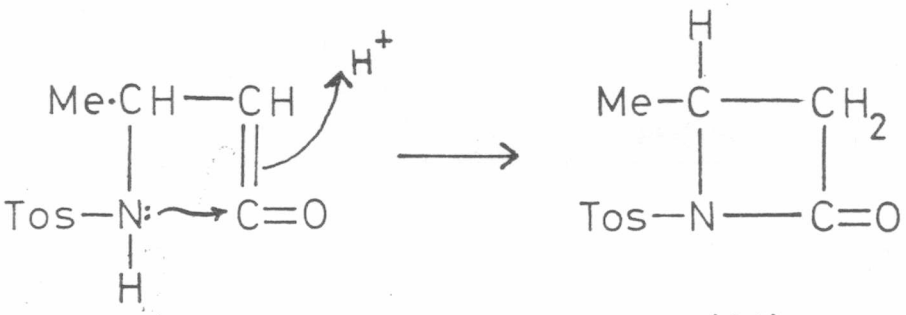
Interaction of the crude acid chloride (87) with excess diazomethane[139] yielded a two-component mixture, characterised by two t.l.c. bands of about equal size and equal intensity on visualisation with iodine vapour. The characteristic yellow colour of the faster-moving component led to the inference that the substance represented the desired diazoketone (88). T.l.c. investigations using an authentic sample of p-toluenesulphonamide as an internal standard demonstrated that the slower-moving component was in fact p-toluenesulphonamide. The latter was a side-product, estimated at about 50% of the overall yield, attributable to base-catalysed decomposition, a β-elimination reaction at the carbon-nitrogen

Fig. 27



$$Tos - NH_2 + RCHO$$

Fig. 28



(91)

bond whose mechanism was postulated by Beecham[141] and also by Wiley et al.[142] (Fig. 27).

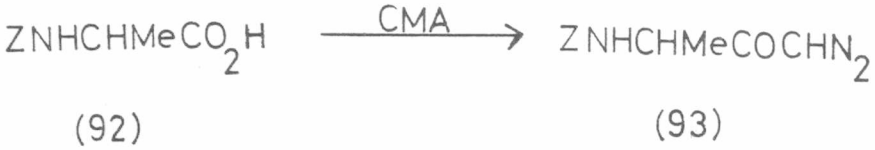
Further evidence for diazoketone (88) being one of the two components in the mixture was provided by the infrared spectrum which showed a characteristic absorption for the diazo group[150] at 2120 cm^{-1}.

The crude diazoketone (88) was dissolved in ethanolic ammonia solution to which aqueous silver nitrate was added to catalyse the Wolff rearrangement. The reaction furnished an optically active amide (89) in a yield of 45%. Acidic hydrolysis of the amide (89) to N-tosyl L-homoalanine (90) proceeded smoothly in 93% yield.

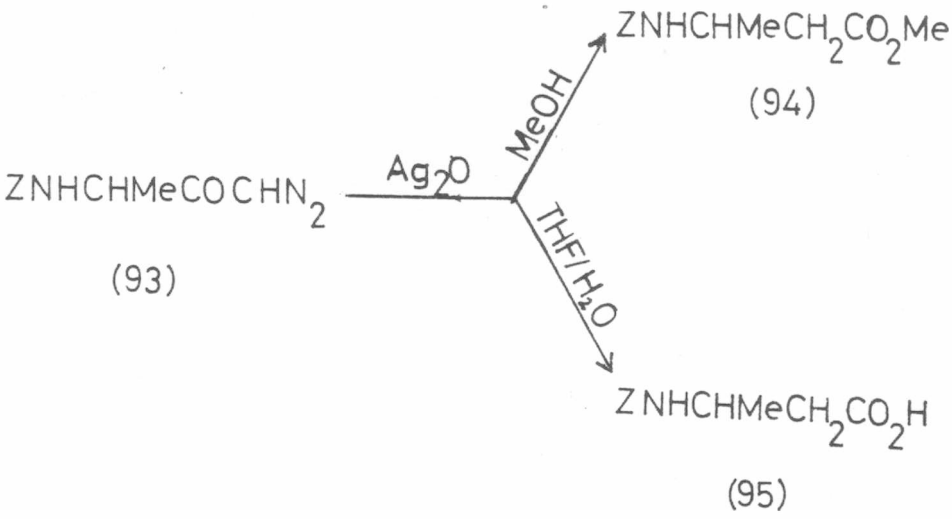
However, aqueous silver nitrate - catalysed Wolff rearrangement of N-tosyl-L-alanyl diazoketone in an inert solvent, tetrahydrofuran, resulted in the formation of an N-tosyl β-lactam (91). The formation of the product (91) was rationalised by intramolecular cyclisation of the hypothetical ketene intermediate (Fig. 28). The pure product (91) was obtained via a column chromatographic separation, the yield of β-lactam (91), calculated on the total amount of the two-component mixture placed on the column, was 21%. The identity of the structure (91) was confirmed by elemental analysis and by spectroscopic studies.

The mass spectrum for the azetidinone (91) exhibited the highest signal at m/e 240 ($M^+ + 1$), for the protonated molecular ion. This data was taken as additional evidence to confirm the molecular structure of the azetidin-2-one (91). Attempts to hydrolyse the compound (91) by acid hydrolysis under reflux conditions proved unsuccessful.

Failure to obtain clean products from reactions involving tosyl derivatives


ZNHCHMeCO2H
CMA
ZNHCHMeCOCHN2

(92) (93)


ZNHCHMeCH2CO2Me
MeOH
ZNHCHMeCOCHN2
Ag2O
THF/H2O
ZNHCHMeCH2CO2H

(94) (93) (95)

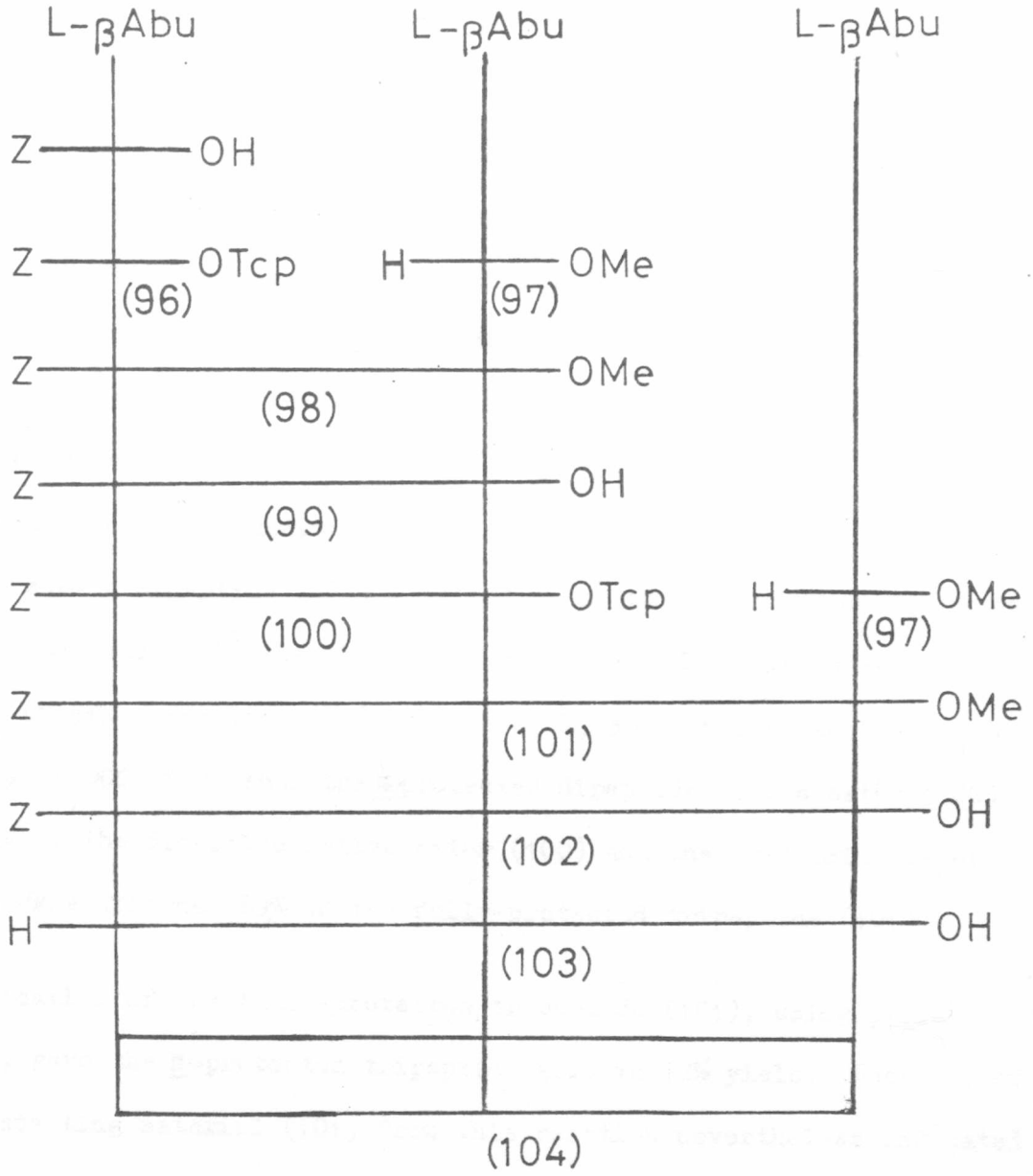
precluded their further usage. It was therefore decided to use an alternative amino-protecting group.

Synthesis of L-β-aminobutyric acid (106)[127] via benzyloxycarbonyl derivatives.

The benzyloxycarbonyl derivative (92)[144] was prepared in a yield of 75%. Penke et al.[145] isolated the diazoketone (93) in the form of an oil, in a 33% yield. In this programme, preparation of the diazoketone (93)[145] was carried out in a solvent system of ether and dichloromethane. Time intervals of 16-24 hours were allowed for diazoketone (93)[145] formation. The desired product (93)[145], whose identity was confirmed by elemental analysis, was obtained in crystalline form in 83% yield.

Wolff rearrangement of the diazoketone (93) in dry methanol, with silver benzoate in triethylamine[130] as catalyst, gave β-benzyloxycarbonyl-L-aminobutyric acid methyl ester (94) in a yield of 88%. Saponification of the ester (94) provided the N-protected acid (95) in 79% yield. The homologated acid (95) could also be isolated in 78% or 82% yield respectively by rearrangement of the diazoketone (93) in aqueous tetrahydrofuran or acetone using silver benzoate in triethylamine[130] as catalyst. Finally catalytic hydrogenolysis of the benzyloxycarbonyl group gave L-β-aminobutyric acid (106)[127]. The acid (106)[127] had a higher melting point than that recorded by Balenovic[127] and its specific rotation fell within the mean values given by Fischer et al.[147] and by Balenovic[127].

Fig. 29



$$Z\,NHCHMeCH_2CONHCHMeCH_2CONHCHMeCH_2\overset{O}{\overset{\|}{C}}-OTcp$$

(105)

Optically active peptides from benzyloxycarbonyl derivatives (Fig. 29)

β-Benzyloxycarbonyl-L-aminobutyric acid (95) was coupled with 2, 4, 5-trichlorophenol using DCCI affording the 2, 4, 5-trichlorophenyl derivative (96) in a yield of 92%. Catalytic hydrogenolysis of β-benzyloxycarbonyl-L-aminobutyric acid methyl ester (94) - especially purified by recrystallization from boiling light petroleum (60-80°) - gave L-β-aminobutyric acid methyl ester (97) in a yield of 94%; the oily product (97) was chromatographically homogenous and ninhydrin positive. Coupling between the active ester (96) and the free amino ester (97) in HMPA (under the same conditions as already described for the preparation of the racemic dipeptide (63)) resulted in a 71% yield of the dipeptide (98). This ester (98) was saponified giving the dipeptide acid (99) in a yield of 91%. Reaction of (99) with 2, 4, 5-trichlorophenol in the presence of DCCI furnished the N-protected dipeptide active ester (100). Coupling of the dipeptide active ester (100) and the free amino ester (97) in HMPA afforded 69% of the fully-protected tripeptide (101).

Saponification of the fully-protected tripeptide (101), using pyridine as solvent, gave the N-protected tripeptide acid in 72% yield. Recovery of 19% of starting material (101) from this reaction nevertheless indicated that a longer reaction time would have been beneficial.

Catalytic transfer hydrogenation[148] of the N-protected tripeptide acid (102), with cyclohexene and 10% palladium/charcoal, in methanol, under reflux for 20 min yielded the free linear tripeptide (103) in a yield of 85%.

PREPARATION OF CYCLO-(TRI-L-β-AMINOBUTYROYL) (104)

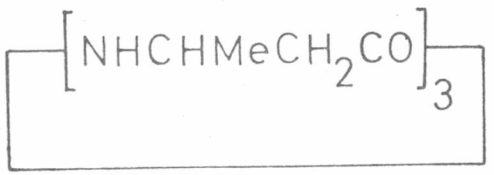
Cyclisation by the action of o-phenylene phosphorochloridite (75)[118,119].

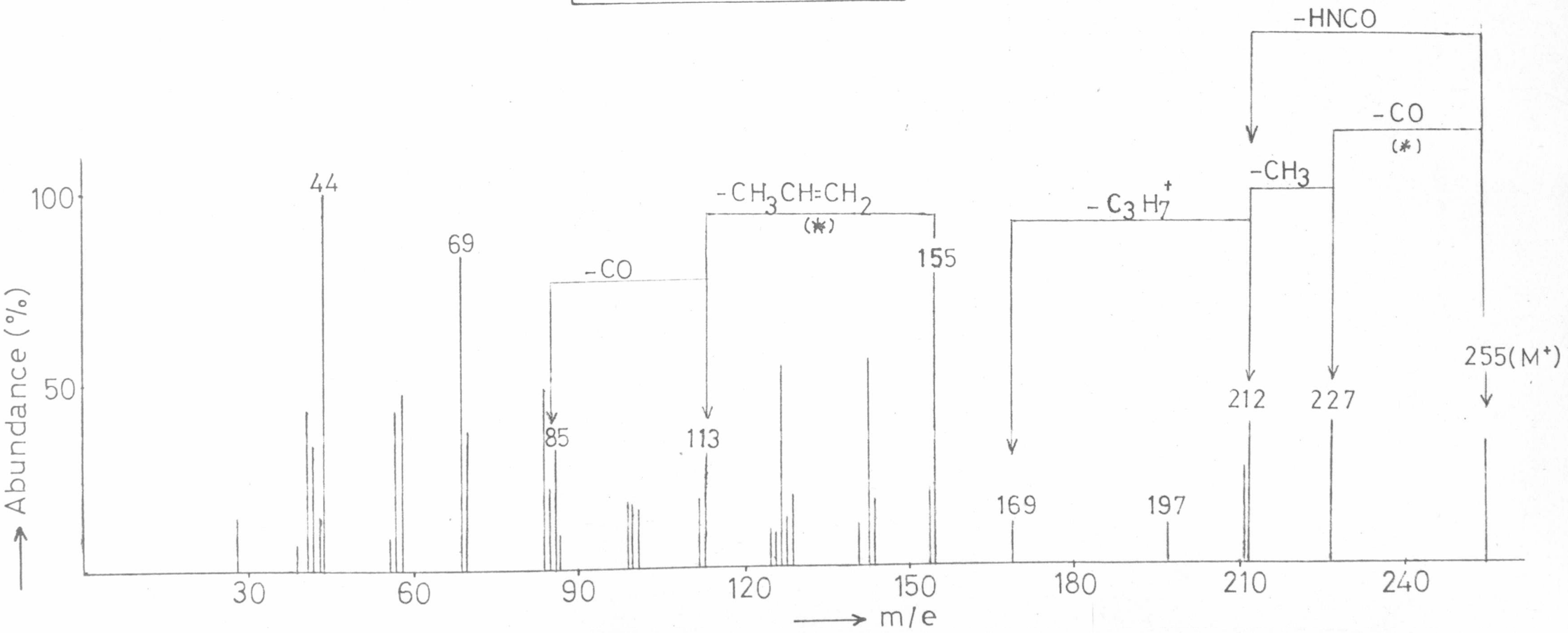
Attempted cyclisation of the optically active free linear tripeptide (103) through the agency of o-phenylene phosphorochloridite (75)[118,119], did not afford the desired optically active cyclic peptide (104), instead, starting material (103) was recovered. Due to the fact that only a very short period of homogeneity had been achieved during reaction, it was felt that insufficient solubility of the linear peptide (103) was responsible for this observation. Thus the reaction was repeated with an eightfold excess of diethylphosphite. In the standard procedure, the reaction is completed by heating under reflux for 15 min; for this experiment the system was heated under reflux until homogeneity was attained, and for a further 15 min thereafter. The crude cyclo-tripeptide (104) was recovered in 40% yield after separation from diethyphosphite, additionally, a further 8% of product (104) was obtained by recrystallization of the residue after evaporation of the solvent. Ion-exchange resin chromatography was inapplicable in this case because of the insolubility of the L-cyclo-tripeptide (104) in water*. Thus purification was carried out either by recrystallization from glacial acetic acid-ether, or by vacuum sublimation.

The molecular weight of the cyclic peptide (104), ascertained by mass spectrometry, gave a signal for the molecular ion at m/e 255 (M^+); there was no evidence for cyclodimerisation (Fig. 21).

* This is in contrast to the DL-cyclo-tripeptide (74), which is water-soluble. The solubility behaviour of the L-cyclo-tripeptide (104) may possibly be ascribed to its sterical homogeneity having resulted in a more rigid structure through stacking to form hydrogen-bonded cyclindrical aggregates[162]. Solvation by water is thus effectively hindered.

Fig. 21


[NHCHMeCH2CO]3


-HNCO
-CO
(*)
-CH3
-C3H7+
-CH3CH=CH2
(*)
-CO
44
69
155
85
113
169
197
212
227
255(M+)
100
50
Abundance (%)
30
60
90
120
150
180
210
240
m/e

Cyclisation via the active ester method[149].

The N-protected tripeptide acid (102) was converted to the 2, 4, 5-trichlorophenyl ester (105) through the agency of DCCI in pyridine. However, the yield (56%) was lower than had been experienced with all the previous active ester derivatives. Isolation of the product (105) had been rendered difficult by contamination with DCU, both compounds having similar melting points and solubilities. In consequence, difficulty was also encountered with elemental analysis. The peptide active ester (105) was deprotected by catalytic hydrogenolysis in a solvent system of methanol and a twofold equivalent of hydrochloric acid. Evaporation yielded the free amino peptide active ester hydrochloride (106), subsequently used in situ in the cyclisation reaction.

Cyclisation was effected under conditions of high dilution, in a solvent system of dimethylformamide and pyridine. Cyclo-(tri-L-β-aminobutyroyl) (104) was isolated by removal of solvent and trituration of the residue with water and aqueous methanol. Recrystallization of the triturated material from a glacial acetic acid-ether system provided the pure cyclic peptide (104) in 24% yield.

Mass spectral fragmentation data on the cyclo-tripeptide (104), (Fig.21).

Low resolution mass spectrometry on the cyclo-tripeptide (104) confirmed the structure and in addition revealed some interesting features in the cracking pattern.

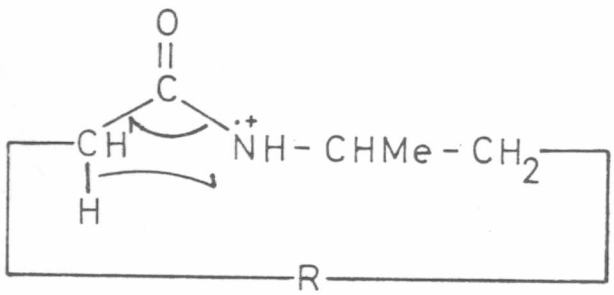
The molecular ion could be visualised as resulting from a ring opening process, which can take several pathways, inter alia a hydrogen transfer[158]

Fig.33

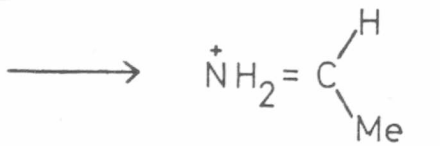
NH–CH Me–CH_2–CO–NH–CH Me–CH_2–CO–NH–CH Me–CH_2–CO^+ ± H

		42	56	84	99	112	127	141	169		197			(-1)
	28	43	57	85	100	113	128					213	227	255	(0)
		44	58	86	101		129	143							(+1)
				197	169	154	141	126	112	84	69	56	41		(-1)
255		227				155		127	113	85	70	57	42	28	(0)
			213				143	128		86		58	43		(+1)

Fig.34



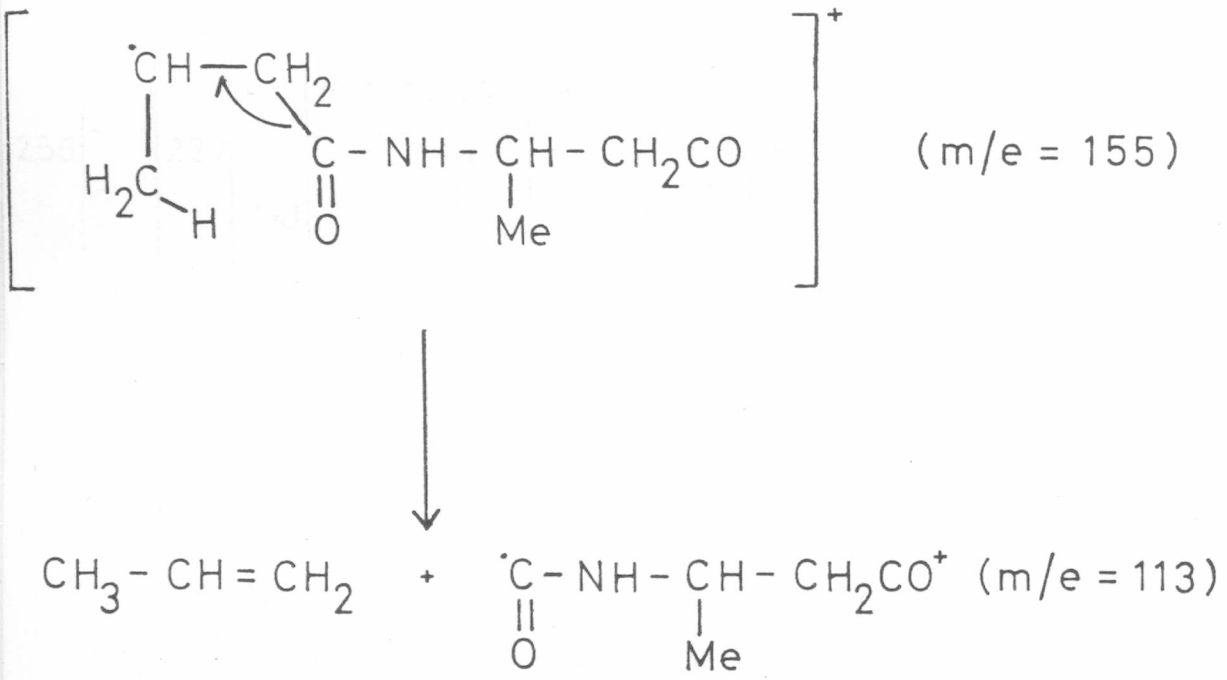
R = remainder of chain



(m/e = 44)

(117)

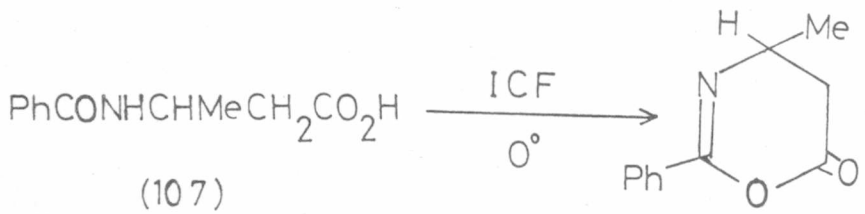
Fig.35



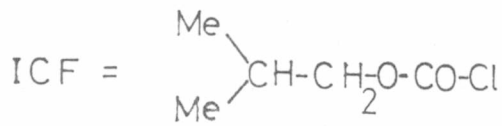
reaction (Fig. 34) followed by elimination of an amine fragment (117). The observation by Millard[158] of initial loss of an amine fragment being followed by loss of fragments containing respectively one less hydrogen atom and one more hydrogen atom - giving the socalled ±1 series - appears to be borne out in this example (Fig. 33). The base peak at m/e could be accounted for by the amine fragment (117).

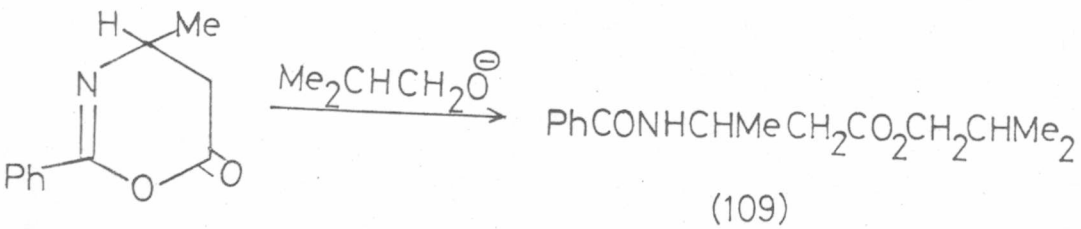
It also appears that the open-chain ion may undergo fragmentation from both termini (Fig. 33), with more complete fragmentation being attained from the N-terminal. The metastable peak at m/e 202 provided evidence for decomposition of the open-chain molecular ion (m/e 255) to the fragment m/e 227. Another metastable at m/e 81.5 corresponds to the decay of the fragment of m/e 155 directly to that of m/e 113 (Fig. 35), loss of carbon monoxide from this fragment (m/e 113) would result in another fragment at m/e 85.

Fragmentation by decarbonylation would appear to be a recurrent feature with the mass spectra of the cyclic peptides of β-amino acids[88, 89].



(107) (108)





(108) (109)

(d) Optically active peptides prepared via oxazinone intermediates (Fig. 30)

Benzoylation of L-β-aminobutyric acid (106)[127] proceeded smoothly affording a satisfactory yield (68%) of the optically active benzoyl derivative (107). Racemisation via oxazolone formation was not a possibility for this reaction, thus leaving only an alternative mechanism by direct ionisation of the β-hydrogen atom, which is a much less facile process.

The CMA method of carboxyl activation, which had been employed earlier furnishing high yields of racemic oxazinone (79), was the method of choice for the synthesis of the optically active oxazinone derivative (108). Cyclisation of the benzoyl derivative (107) yielded 98% of the optically active oxazinone (108), which exhibited a fairly high optical rotation.

There were no significant differences between the film infrared spectra for both the optically active and racemic oxazinones (Fig. 21). In addition, the mass spectrum, used to confirm the molecular weight, gave a signal for the molecular ion at m/e 189 (M^+), and a fragmentation pattern similar to that obtained for the racemic oxazinone (Fig. 23).

It was found that esterification of the benzoyl derivative (107) with isobutyl alcohol, rather than cyclisation, became the dominant process when the reaction was carried out at 0°. A preparation of oxazinone (108) using ice (0°) instead of dry-ice in acetone resulted in the isolation of isobutyl β-benzoyl-L-aminobutyrate (109) as the major product in a yield of 95%; the desired oxazinone (108) was the minor product, with a yield of 0.5%. This unexpected product (109), assigned primarily on the basis of proton n.m.r. examination, probably arose by action of isobutyl alcohol on the oxazinone (108), Fig.31.

Fig. 23

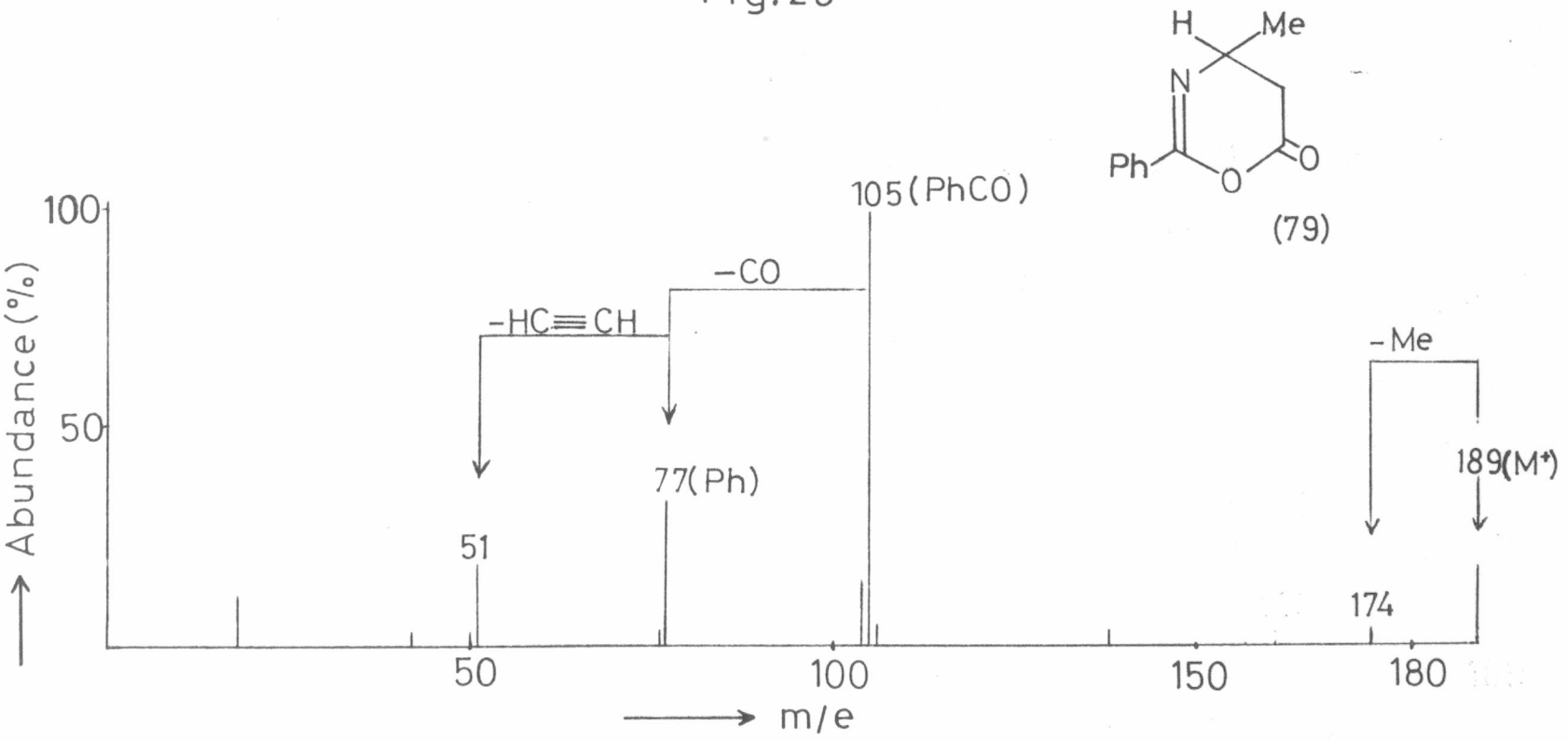
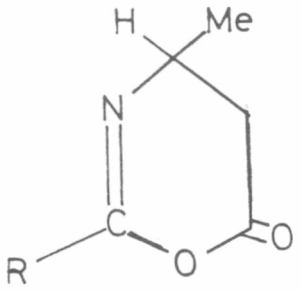
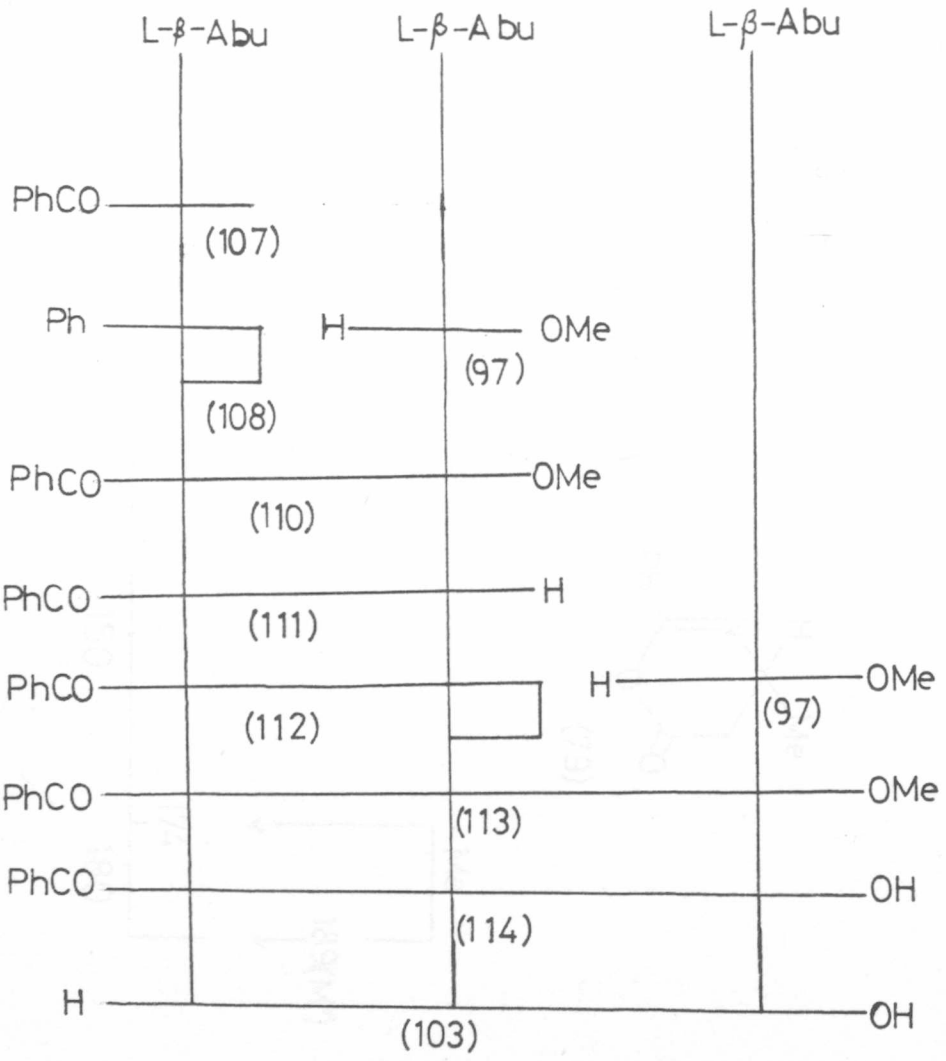

H
Me
N
Ph
O
O
(79)
100
50
Abundance (%)
105 (PhCO)
-CO
-HC≡CH
-Me
189 (M+)
77 (Ph)
51
174
50
100
150
180
m/e

Fig. 30



(108) R = Ph

(112) R = $PhCONHCHMeCH_2$



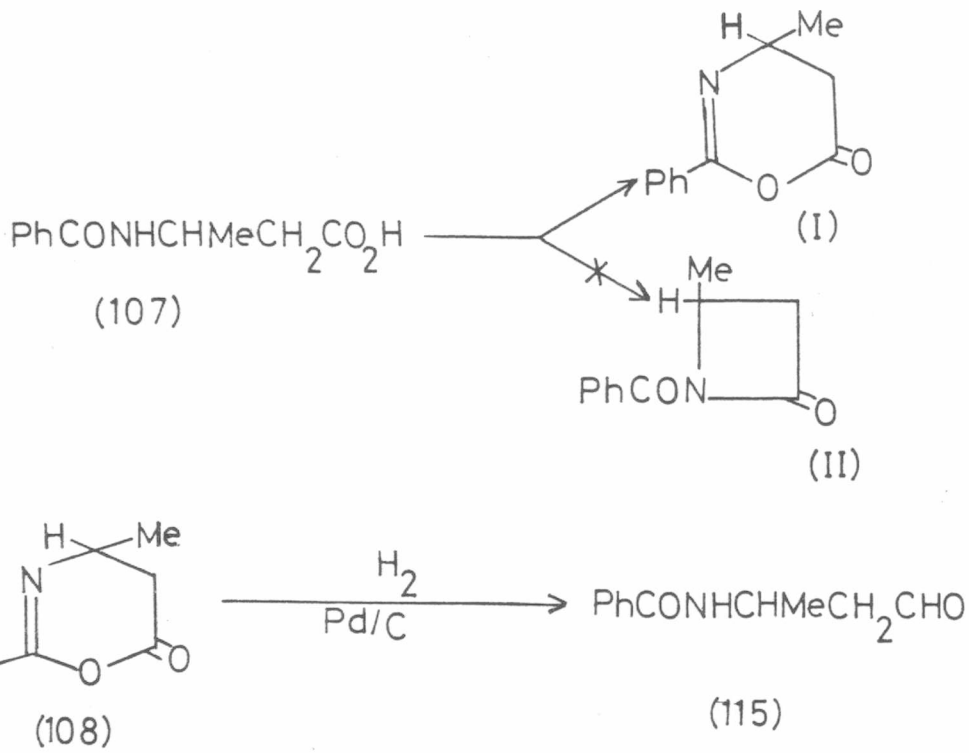
Acylation of methyl L-β-aminobutyrate with the oxazinone (108) proceeded smoothly and yielded the optically active fully-protected dipeptide (110).

Saponification of the dipeptide ester (110) afforded the N-protected dipeptide acid (111) in 79% yield. Cyclisation of the acid (111) via the CMA method, using acetonitrile as solvent, led to the isolation of the peptide oxazinone (112) in 82% yield . The product (112) was crystalline, high melting, and yet susceptible to hydrolysis. The peptide oxazinone (112) analysed correctly and its molecular weight was confirmed by mass spectrometry (Fig. 32) which gave the parent ion at m/e 274 (M^+).

Treatment of the peptide oxazinone (112) with methyl L-β-aminobutyrate in acetonitrile provided the fully-protected tripeptide (113) in 72% yield. The product (113) had a satisfactory optical rotation and was characterised by elemental analysis. The ester (113) was saponified in aqueous pyridine, and the resulting N-protected tripeptide acid (114) isolated in a 75% yield. The benzoyl group was removed by electrolytic reduction[25, 87] using a mercury cathode, a shielded platinum anode, and tetramethylammonium bromide dissolved in methanol as the electrolyte. Electrolysis was carried out at constant current (0.08A) and variable potential, using a Potentiostat Type TR 40/3A. The reaction was worked up by evaporation of methanol and changing the solvent to water. The aqueous solution was then desalted by electrodialysis, using ion-exchange membranes, on the Shandon Electrodialyser and Desalter[151]. After the current had dropped to a low steady state the solution was removed from the electrodialyser and evaporated to dryness. The product was found to be the chromatographically pure free tripeptide (103), 41%. The authenticity of the product (103) was certified by t.l.c. and by mass spectrometry.

Establishing suitable conditions for the electrolytic debenzoylation reaction to take place was a painstaking process.

Fig. 36


PhCONHCHMeCH2CO2H
(107)
H
Me
N
Ph
O
O
(I)
Me
H
PhCON
O
(II)
H
Me
N
Ph
O
O
(108)
H2
Pd/C
PhCONHCHMeCH2CHO
(115)
O2N
PhCONHCHMeCH2CH=N-NH
NO2
(116)

Proof of oxazinone structure (108) by catalytic hydrogenation studies.[89, 152]

In principle, cyclisation of the benzoyl derivative (107) may give rise to compounds of two general types (I, II); one pathway leading to oxazin-6-one (I) formation and the other to the acylazetidin-2-one structure (II), fig.36

In the course of studies aimed at clearing the ambiguity that existed in the chemical literature [153,154,155] over the matter of differentiation between acylazetidin-2-ones and the corresponding isomeric oxazin-6-ones,it was found that catalytic hydrogenation of an azetidinone structure did not effect any change whereas oxazinones were converted to aldehydes.[89, 152]

In this programme the optically active oxazinone (108) was taken up in dichloromethane and treated with hydrogen in the presence of 10% palladium on charcoal. Within seven hours the oxazinone (108) had disappeared and 'work-up' of the reaction, which included partitioning of the reaction solution between ethyl acetate and base to remove any trace of starting material (108) and possible symmetrical anhydride, gave the corresponding aldehyde (115) in 68% yield. The product (115) was positive to Schiff's test, exhibited an aldehydic proton resonance in the n.m.r. spectrum, was optically active, and analysed correctly for the elements. Reaction of the aldehyde (115) with 2, 4-dinitrophenyl hydrazine led to the isolation of the hydrazone derivative (116) in 32% yield.

Suggestions on extension of this work.

Isolation of a chiral β-lactam reported in this thesis (p.39) provides a new and simple route to this class of important compounds. The method deserves further investigation with a view to increasing the yields. Efforts could probably include the use of alternative catalytic systems which enable work to be carried on in anhydrous conditions and/or the use of alternative protecting groups.

In this thesis it has been demonstrated that oxazinones offer considerable scope in the synthesis of optically active peptides. However, further work needs to be carried out to find alternative protecting groups that will both afford oxazinones and be more amenable to selective cleavage than the benzoyl group.

Work is already in progress elsewhere to determine the three-dimensional structure of cyclo-tripeptides by n.m.r. and x-ray techniques. These studies may contribute towards elucidating the differences in solubility between racemic and optically active cyclo-tripeptides.

EXPERIMENTAL SECTION

New compounds are underlined when first mentioned. Melting points (m.p.) were determined either on a Gallenkamp apparatus or on an Electrothermal Melting Point Apparatus and are uncorrected, as are the boiling points (b.p.).

Infra-red spectra (i.r.) were recorded on liquids and solids (the latter as nujol mulls) between sodium chloride or potassium bromide discs using a Perkin Elmer 137 or 577 spectrophotometer.

^{1}HN.m.r. spectra (60 MHz) were taken using either a Varian A60 or the Perkin-Elmer R12B instrument, using deuterated solvents. Mass spectra (MS) were obtained either from an AEI MS30 instrument or from the PCMU Service at Harwell. Optical rotations were measured on a Bellingham & Stanley polarimeter.

Solvents were purified and dried before use[159]. Commercial diethyl phosphite was distilled at reduced pressure, the fraction used had b.p. 48-50°/2 mmHg, n_D^{20} 1.4100 (lit.[160] b.p. 50°/2 mmHg, n_D^{20} 1.4101). Petrol refers to the fraction of b.p. 60-80°.

In general, neutral products were isolated by washing solutions in ethyl acetate successively with 0.5M-hydrochloric acid, 0.5M-sodium hydroxide solution, and water, followed by drying, using anhydrous magnesium sulphate, and evaporation under reduced pressure using a rotary evaporator.

Thin layer chromatography (t.l.c.) on Merck Kieselgel G (thickness, 0.25 mm) employed the following solvent systems (v/v):

A	ethyl acetate	
B	chloroform	
C	chloroform: ethyl acetate	(96:4)
D	benzene: ethyl acetate	(92:8)
E	benzene: ethyl acetate	(84:16)
F	benzene: ethyl acetate	(68:32)
G	chloroform: cyclohexane: acetic acid	(40:10:5)
H	n-propanol: water	(1:1)
I	ethyl acetate: acetone	(50:10)
J	n-butanol: acetic acid: water	(40:10:10)
K	butan-2-ol: 3% soln. of ammonia	(100:44)
L	n-butanol: acetic acid: water	(40:10:50)

Unless otherwise stated, spots in t.l.c. work were detected by iodine vapour.

EXPERIMENTS DIRECTED TOWARDS INVESTIGATION OF THE PATHWAY TO FORMATION OF CYCLOHEXYLAMIDE (57)[89] DURING PEPTIDE FORMATION THROUGH THE AGENCY OF N, N'-DICYCLOHEXYLCARBODI-IMIDE (DCCI).

Preparation of β-aminopivaloyl ethyl ester (51)[95] α-Cyano-isobutyrate[93] (94.8g, 0.68 moles) in absolute ethanol (50 ml) was hydrogenated in the autoclave (40 atm, 80°) in the presence of Raney nickel[94] (approx 90g) suspended in absolute ethanol (150 ml). After 3h the catalyst was filtered off, the alcohol evaporated, and the residue distilled under reduced pressure to yield the desired product (48.6g, 49.8%), b.p. 59-61°/7.5 - 8 mmHg. (Lit.[95] 65-67°/12 mm).

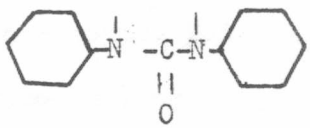
Preparation of β-aminopivalic acid hydrochloride (52)[96]. A solution of the foregoing ester (14.5g, 0.10 moles) was treated with hydrochloric acid (100 ml, 6M) and heated for 3h at 200° over a water bath. Removal of solvent and trituration of the solid residue with acetone yielded the acid hydrochloride[96] (14.2g, 92.6%), m.p. 149-151°(Lit.[96] m.p.149-151.5°).

Preparation of β-benzylocycarbonylaminopivalic acid (53). Solutions of benzyl chloroformate (8.1g, 6.75 ml, 47.5 mmoles) in acetone and sodium hydroxide (23.8 ml, 2M) were added concurrently during 1h to a stirred and cooled (0°) solution of the acid hydrochloride (52) (6.6g, 42.9 mmoles) in sodium hydroxide (21.5 ml, 2M) and acetone (10 ml). The reaction was allowed to stir overnight. Acetone was then removed on the rotavaporator, the aqueous phase extracted with ether, acidified (5 M-hydrochloric acid) to congo red, and extracted with ethyl acetate. The organic phase was washed with saturated sodium chloride solution, dried and evaporated to give a colourless oil (5.2g, 53.3%), R_{fG} 0.82.

Preparation of β-benzyloxycarbonyl aminopivalic acid (53)[89] dicyclohexylammonium salt. Dicyclohexylamine (0.48g, 2.64 mmoles) was added to a stirred and cooled (0°) solution of the foregoing derivative (53) (0.6g, 2.4 mmoles) in ethyl acetate. The reaction was stirred for 1h at room temperature followed by removal of solvent and recrystallization of the residue from ethyl acetate-petrol to yield a salt (0.7g, 69.6%), m.p. 142-145° (raised to 149-151° on further recrystallization). (Lit.[89] m.p. 153.5-154°).

Isolation of β-benzyloxycarbonylaminopivalic acid N-acylurea (NAU) (54). DCCI (0.21g, 1.0 mmoles) and triethylamine (0.15g, 0.2 ml, 1.5 mmoles) were added to a stirred solution of β-benzyloxycarbonylaminopivalic acid (0.25g, 1.0 mmoles) in ethyl acetate at room temperature. The reaction was allowed to stir at room temperature for 7 days during which it was continually monitored by t.l.c. for the disappearance of the acid. The precipitate, dicyclohexylurea (DCU) (174 mg, 76.2%), m.p. 228-230°, was filtered off and the filtrate worked up for the neutral. Evaporation to dryness gave an oily product (150 mg), R_{fD} 0.1 (very feint), 0.25 (prominent), which crystallized from boiling petrol to yield a compound identified by spectroscopy as the N-acylurea derivative (54) (90 mg, 19.7%), m.p. 56-60° (raised to 77-80° on recrystallization), R_{fD} 0.25, R_{fE} 0.48, R_{fF} 0.83. No quantitative yield of the slower running component could be recovered from the mother liquors. ν max (nujol) : 3150, 2850, 1725, 1625, 1500, 1450 cm^{-1}.

^{1}H N.m.r. (C D Cl_3)

τ (ppm)	multiplicity	protons	assignment
2.75	singlet	5	phenyl
2.8-2.9	broad signal	1	-NH-C(=O)-N-
4.6	broad singlet	1	-NH-
4.95	singlet	2	Ph-CH_2-O-
6.70	singlet)	2	β-methylene
6.82	singlet)		-NH-CH_2-CMe_2-
8.80	singlet superimposed on multiplet	6	α-gemdimethyl -$C(CH_3)_2$-CO-
8.2-9.7	broad multiplet	22	

The same synthesis was repeated at a higher temperature, 40°, resulting in a considerably higher yield (58%) of NAU (54). (Found: C, 68.5, H, 8.7; N, 9.3. $C_{26}H_{39}N_3O_4$ requires C, 68.24; H, 8.50; N, 9.19%).

Preparation of β-benzyloxycarbonylaminopivaloyl-β-aminopivalic acid ethyl ester (55). DCCI (2.52g, 12.2 mmoles) in ethyl acetate (40 ml) was gradually added to a stirred and cooled (0°) solution of β-benzyl-oxycarbonyl-aminopivalic acid (2.74g, 11.0 mmoles) and β-amino-pivalic acid ethyl ester (1.76g, 12.2 mmoles) in ethyl acetate (160 ml). The reaction was stirred for 5 min at 0° and then set aside for two days

at room temperature. DCU (1.09g, 39%), m.p. 230-232°, was filtered off, washed with a small volume of solvent; and the filtrate washed neutral, dried, filtered and evaporated to give an oil (4.8g). The product was found to be a mixture of two components, R_{fB} 0.30, 0.40; R_{fD} 0.28, 0.45; R_{fF} 0.41, 0.81.

Separation of the two components by silica gel column chromatography. Kieselgel Art 7734, product of Merck, taken in the proportion of 1 part of substrate to 120 parts of Kieselgel, was packed into a column which was then loaded with 1g of substrate. The column was eluted with a mixed solvent system of benzene and ethyl acetate. Progressive increases in ethyl acetate (8%, 16%, 32%, 100%) were made either after 5 bed volumes of eluate had been collected or on the basis of t.l.c. monitoring. The faster running component, NAU, was separated from the column in a yield of 0.52g, 52% (yield calculated on material put on to the column), the oily material was crystallized from boiling petrol, m.p. 57-60° (raised to 77-80° on recrystallization), R_{fE} 0.48. The slower running material, assumed to be fully-protected dipeptide (55), was recovered in a chromatographically pure state in a yield of 0.37g, 37%, and crystallized on long storage with petrol, m.p. 41-44°.

Isolation of β-benzyloxycarbonylaminopivaloyl-β-aminopivalic acid (56)[89]

Ethyl ester dipeptide (55) (350mg, 0.930 mmoles) in methanol (5 ml) was cooled (0°) and treated with sodium hydroxide (1 ml, 1N). The reaction was stirred overnight, the completion of saponification being certified by t.l.c. Methanol was evaporated, the aqueous phase extracted with ether, acidified (hydrochloric acid, 2N) to congo red,

and extracted with ethyl acetate. Evaporation of the organic phase yielded a dipeptide acid (56) (220mg, 68%), m.p. 99-101° (raised to 103-105° on recrystallization from ethyl acetate-petrol). (Ridge[89], reported m.p. 105.5-106.5°).

Preparation of β-benzyloxycarbonylaminopivalic acid cyclohexylamide (57)[89] via hydrolysis of the NAU derivative (54). β-Benzyloxycarbonylaminopivalic acid N-acylurea (54) (0.3g, 0.65 mmoles) in methanol (10 ml) was treated with sodium hydroxide (0.7 ml, 1N) at 0°. The reaction was stirred at room temperature for 6h during which it was monitored by t.l.c. Methanol was evaporated and the aqueous phase extracted with ethyl acetate. The organic phase was then washed with water, dried, and evaporated to dryness. Recrystallization of the residue from ethyl acetate-petrol yielded cyclohexylamide (57) (110mg, 59%), m.p. 91-93° (raised to 94-96° on further recrystallization), R_{fc} 0.40. (Ridge[89] reported m.p. 95-96°). Attempts to demonstrate the presence of cyclohexylisocyanate in the mother liquors by t.l.c. using an authentic sample of the compound as an internal standard yielded negative results.

BASE HYDROLYSIS OF OTHER NAU DERIVATIVES OF β-AMINO ACIDS

Base hydrolysis of DL-β-benzyloxycarbonylaminobutyroyl N-acylurea (63). To a cooled (0°) solution of the title compound (100mg, 0.22 mmoles) in aqueous methanol (10 ml, 20%) was added an equivalent of sodium hydroxide solution (0.25 ml, 1N), in a dropwise manner, with stirring. Work up of the reaction involved filtration of the precipitate, DCU (30mg, 60.8%), m.p. 226-228° (identity of compound confirmed by i.r. spectroscopy), followed by evaporation of methanol, neutralisation

(2N-hydrochloric acid) of the aqueous phase, extraction with ethyl acetate, then drying, filtering and evaporating the organic phase to dryness. Recrystallization of the residue from ethyl acetate-petrol yielded β-benzyloxycarbonylaminobutyric acid (6)) (44mg, 84.6%), m.p. 123-125°, R_{fG}0.8 (Lit.[103] m.p.129-130°(H_2O))

Base hydrolysis of β-benzyloxycarbonylalanyl NAU (58)[88, 101].

The experimental details are as described in the preceding experiment. The title NAU derivative (58) (60mg, 0.14 mmoles) in aqueous methanol (6 ml, 25%) was treated with sodium hydroxide solution (0.14 ml, 1N) at 0°. The reaction was stirred for 20h. The filtered solid was shown to be DCU by i.r. spectroscopy, m.p. 228-230°. T.l.c. examination of the organic layer revealed a single spot, R_{fA}0.0; R_{fG}0.73; R_{fJ}0.78. Spraying with bromo-cresol green resulted in yellow spots, indicating that the component present had a free carboxylic group, in this case β-benzyloxycarbonylalanine[102].

Base hydrolysis of β-benzyloxycarbonylaminoisovaleryl-β-aminoisovaleryl -β-aminoisovaleryl N-acylurea (59)[88]. The title N-acylurea (5mg) in aqueous methanol (5 ml, 20%) was treated with a few drops of sodium hydroxide solution (1N) and left to stand at room temperature with occasional shaking. The precipitated solid was identified as DCU (m.p. 228-230°) by i.r. spectroscopy. The filtrate was treated with a few drops of hydrochloric acid (1N) to neutral. T.l.c. on the filtrate (R_{fG}0.58) coincided with that for the corresponding N-protected tripeptide acid (60)[88] and gave a yellow spot on spraying with bromo-cresol green.

SYNTHESIS OF AMINO-ACID AND PEPTIDE DERIVATIVES OF RACEMIC β-AMINO-BUTYRIC ACID (Fig. 18)

Preparation of DL-β-benzyloxycarbonylaminobutyric acid (61)[103].

To a stirred and cooled (0°) solution of β-aminobutyric acid (15g, 0.145 moles) in sodium hydroxide (145.5 ml, N) benzyl chloroformate (27.8g, 23.4 ml, 0.163 moles) and sodium hydroxide (87 ml, 2N) were added simultaneously during 90 min. Stirring was continued overnight, and finally the reaction mixture was extracted with ether, and the aqueous phase acidified (hydrochloric acid 5N) to congo red to give the benzyloxycarbonyl derivative (21.9g, 63.8%), m.p. 124-128° (raised to 127-128° on recrystallization from ethyl acetate-petrol), R_{fH} 0.78. (Birkofer and Modic[103] reported m.p. 129-130° (H_2O)).

Preparation of DL-β-aminobutyric acid methyl ester (62)[103] hydrochloride. Thionyl chloride (10.9g, 0.1 moles) was added dropwise with stirring to ice/salt cooled (-10°) dry methanol (50 ml) followed by addition of β-aminobutyric acid (10g, 0.097 moles). The reaction was heated under reflux for 3h after which the excess thionyl chloride was evaporated to leave behind an oily residue (ν max 2850, 2000, 1750, 1600, 1500, 1375 cm^{-1}). Toluene was added to the residue, the solution swirled, and the toluene evaporated. The procedure was repeated three times. The ester hydrochloride (15.36g, 69.0%), m.p. 123-125°, R_{fC} 0.49, crystallized from ethyl acetate after nearly three months of refrigeration. (Found: C, 39.2; H, 7.8; N, 9.3; Cl, 23.4. $C_5H_{12}NO_2Cl$ requires C, 39.10; H, 7.80; N, 9.12; Cl, 23.08%).

The preparation was repeated using the method of passing hydrogen

chloride gas into a stirred and cooled (0°) suspension of β-amino-butyric acid (10g, 0.097 moles) in dry methanol (60.8g, 77 ml, 1.9 moles) until attainment of homogeneity. The desired product was obtained in 70.3% yield.

Isolation and characterisation of methyl DL-β-aminobutyrate (62)

A saturated solution of ammonia in chloroform (25 ml) (prepared by transferring liquid ammonia from a cylinder to a flask standing in an icebath (5°), and then allowing the ammonia to distil (5-20°) from the flask into chloroform in a dryice-acetone bath (-15 to -25°) , was shaken with the ester (62) hydrochloride (15.36g, 0.1 moles) and precipitated ammonium chloride (5g, 94%) filtered off. Evaporation of the filtrate yielded a residual yellowish liquid, assumed to be an amine, (6.9g, 61.6%-calculated on the amino acid), purified by vacuum distillation to give a colourless amino-ester (5.4g, 47.5%), b.p. 36-38°/9.5 mmHg. (Birkofer and Modic[103] reported b.p. 60°/18 mmHg).

Attempted preparation of D L-β-benzyloxycarbonylamino-butyroyl-β-aminobutyric acid methyl ester (63).

(i) Coupling via DCCI. A solution of DCCI (1.86g, 9.0 mmoles) in acetonitrile (10 ml) was added gradually to a stirred and cooled (0°) solution of D L-β-benzyloxycarbonylaminobutyric acid (2.13g, 9 mmoles) and the amino ester (62) (1.16g, 10 mmoles) in acetonitrile (50 ml). The reaction was stirred for 30 min at 0° and for 6h at room temperature. Acetonitrile was evaporated, the residue taken up in ethyl acetate, and worked up for the neutral. The solution was concentrated and then examined by t.l.c. R_{fC} 0.38, R_{fF} 0.55. Evaporation

to dryness gave DL-β-benzyloxycarbonylaminobutyric acid N-acylurea (64). (2.8g, 70.2%), m.p. 138-142° (raised to 143-145° on recrystallization from ethyl acetate-petrol). (Found: C, 67.69; H, 8.35; N, 9.67. $C_{25}H_{37}N_3O_4$ requires C, 67.69; H, 8.40; N, 9.47%).

(ii) Coupling via DCCI and 1-hydroxybenzotriazole[106]. DCCI (2.9g, 14 mmoles), 1-hydroxybenzotriazole (1.73g, 12.8 mmoles) and DL-β-benzyloxycarbonylaminobutyric acid (3.2g, 12.8 mmoles) were dissolved in acetonitrile (60 ml) with stirring and cooling (0°) for 1h and then for another 1h at 10°. To this solution was then added a solution of D L-β-aminobutyric acid methyl ester hydrochloride (2.3g, 15 mmoles) and triethylamine (2.2g, 16 mmoles) in acetonitrile (15 ml). The reaction was stirred at 10° for 1h and then at room temperature overnight. DCU (1.4g, 42.5%; m.p. 224-226°) was filtered off after change of solvent to ethyl acetate. The filtrate was worked up for the neutral in the usual way. T.l.c. of the washed solution showed two spots, R_{fC} 0.15, 0.38. The two components were separated by fractional crystallization. The component corresponding to R_{fC} 0.38 was shown to be N-acylurea derivative (64) (0.7g, 11.7%, calculated on the benzyloxycarbonyl derivative), m.p. 142-145°. The slower running spot was the fully-protected dipeptide (63) isolated in a yield of 1.6g, 37.2%, m.p. 96-100° (raised to 109-110° on further recrystallization from ethyl acetate-petrol). (Found: C, 60.57; H, 7.00; N, 8.26. $C_{17}H_{24}N_2O_5$ requires C, 60.70; H, 7.19; N, 8.33%).

(iii) Coupling via the carbonic mixed anhydride (CMA) route. To a cooled (-20 to -15°) solution of D L-β-benzyloxycarbonylaminobutyric

acid (4.65g, 19.6 mmoles) in ethyl acetate (80 ml), triethylamine (2.13g, 2.96 ml, 21.0 mmoles) and isobutylchloroformate (2.87g, 2.76ml, 21.0 mmoles) were added. After 3 min a precooled (-15°) solution of the amino ester (62) hydrochloride (3g, 19.6 mmoles) and triethylamine (2.13g, 2.96 ml, 21.0 mmoles) in ethyl acetate (15 ml) was added and the overall mixture stirred for 10 min at -5° and then for 2h at 0°. The reaction was stirred for a further 2h at room temperature and then filtered and washed as usual. The neutral solution revealed two spots on t.l.c. R_{fC}0.15, 0.49; R_{fE}0.42, 0.88. Fully protected dipeptide (63) (0.86g, 13.1%), m.p. 106-108°, was isolated by crystallization from ethyl acetate-petrol. The residual oil recovered after evaporation of the mother liquors could not be crystallized and the second component of the mixture was not isolated.

(iv) Coupling via CMA route using "Inverse Addition"[108]. A solution of isobutylchloroformate (1.15g, 8.44 mmoles) in ethyl acetate (10 ml) was stirred and cooled to -15°. To this was added dropwise during 3 min a previously cooled (-15°) solution of triethylamine (0.43g, 4.22 mmoles) and DL-β-benzyloxycarbonylaminobutyric acid (1g, 4.22 mmoles) in ethyl acetate (25 ml). The mixture was stirred at -15° for 8 min (activation time), then a solution of the amino ester (62) (1.48g, 12.66 mmoles) in ethyl acetate (20 ml) was added, after which the mixture was allowed to warm up to room temperature and stirring continued overnight. Working up the reaction in the usual way yielded an oily product, R_{fC}0.12, 0.68, 0.83. The oil was triturated with petrol (3 x 10 ml) and the residual material taken up in ether. Chromatographically pure (R_{fC}0.12) fully-protected dipeptide (63) (0.4g, 28.6%; m.p. 106-108°)

crystallized out of solution.

(v) Coupling via CMA route using "Inverse Addition" with a 20 min. activation time. This coupling reaction was conducted on the same scale and in the same manner as the foregoing, (iv), with the difference that first, the solution of the N-protected amino acid (61) and triethylamine in ethyl acetate was added to the solution of isobutylchloroformate in ethyl acetate during a period of 20 min; and second, the amino component was added to the carboxyl component immediately (20 min. activation time). A three component mixture was obtained as in the preceding experiment and the technique of extraction by trituration with petrol, followed by crystallization from ether-petrol yielded 0.15g, 10.7% of the desired product (62).

(vi) Coupling via excess CMA route[109]. To a stirred and cooled (-15°) solution of DL-β-benzyloxycarbonylamino-butyric acid (1g, 4.22 mmoles) in ethyl acetate (20 ml), an equivalent of N-methylmorpholine (0.43g, 4.22 mmoles) was added. To this mixture, at -15°, was added isobutylchloroformate (0.54g, 3.36 mmoles) gradually, during 3 min. The reaction was allowed to stir for 10 min and then a pre-cooled (-15°) solution of amino ester (62) (0.31g, 2.64 mmoles) in ethyl acetate (10 ml) was added. The reaction was stirred for 30 min at -15°, allowed to rise to room temperature, and then stirred for 2h. The reaction was filtered and then washed according to the standard procedure. The desired product was isolated by crystallization from ethyl acetate-petrol in yield of 0.44g, 50%, m.p. 104-106° (raised to 108-110°).

Preparation of pivalic acid mixed anhydride (65). Pivaloyl chloride (0.56g, 0.6 ml, 4.64 mmoles) was added to a stirred and cooled (-5°) solution of N-protected amino-acid (61) (1g, 4.22 mmoles) and triethylamine (0.47g, 4.64 mmoles) in ethyl acetate (20 ml). The reaction was stirred at -5° for 2h, at room temperature for 1h, filtered, and the filtrate evaporated. Toluene (20 ml) was added, and the evaporation repeated. This procedure was repeated 3 times, yielding DL-β-benzyloxycarbonylaminobutyric acid pivaloyl anhydride (1.37g, 96.5%), m.p. 70-74 (solvated) (raised to 103-105° on recrystallization from ethyl acetate-petrol), ν max (nujol) 3300, 2900, 1800, 1700, 1525, 1250, 1090, 1020, 690 cm^{-1}. (Found: C, 67.56; H, 7.21; N, 4.43. $C_{17}H_{23}N\ O_5$ requires C, 67.40; H, 7.44; N, 4.53%).

(vii) Coupling with pivalic acid mixed anhydride. The foregoing anhydride (65) (1.30g, 3.86 mmoles) in ethyl acetate (20 ml) was added gradually to a solution of the amino ester (62) (0.495g, 4.22 mmoles) in ethyl acetate (10 ml) under reflux. Heating under reflux was continued for 3h. Evaporation of solvent yielded an oily residue which was taken up in ethyl acetate and washed in the usual way. Evaporation of the washed solution yielded fully protected dipeptide (63) (0.84g, 59.6%) m.p. 96-100° (raised to 110-112° on recrystallization from ethyl acetate-petrol), R_{fA} 0.6.

Preparation of D L-β-benzyloxycarbonylaminobutyric acid 2, 4, 5-trichlorophenyl ester (66). A solution of DCCI (6.3g, 30.39 mmoles) in pyridine (10 ml) was added gradually to a stirred and cooled (-5°) solution of N-protected amino acid (60) (6g, 25.3 mmoles) and 2, 4, 5-

trichlorophenol (5.97g, 30.39 mmoles) in pyridine (12 ml). The reaction was stirred for 3h at -5°, set aside for 30h at 0°, and then diluted with ethyl acetate (120 ml) and left to stand for 1h at 0°. The solid material, DCU (4.65g, 68.9%, m.p. 226-228°) was filtered off and the filtrate evaporated to dryness. The oily residue was dissolved in ether and more DCU (1.9g, 28.3%; m.p. 226-228°) was recovered. Ether was evaporated and the residue, an oil, triturated with petrol to yield the desired 2, 4, 5-trichlorophenyl ester derivative (66) (9.8g, 93.0%), m.p. 64-67° (raised to 71-72° on recrystallization from boiling petrol). (Found: C, 51.74; H, 4.05, N, 3.31; $C_{18}H_{16}N\ O_4Cl_3$ requires C, 51.89; H, 3.87; N, 3.36%).

(viii) Coupling via 2, 4, 5-trichlorophenyl ester derivative (66) in acetronitrile. A solution of the active ester (66) (3g, 7.2 mmoles) in acetonitrile (5 ml) was added gradually to a stirred solution of the amino ester (62) hydrochloride (1.22g, 7.92 mmoles) and triethylamine (0.8g, 1.1 ml, 7.92 mmoles) in acetonitrile (10) at room temperature. The reaction was stirred at room temperature, under t.l.c. monitoring for 6h and then set aside overnight at 60°. The active ester continued to be detectable by t.l.c., R_{fB}0.88 (prominent), after the reaction had been kept at 60° for 24h. Acetonitrile was evaporated, replaced with ethyl acetate and the hydrochloride salt filtered. The reaction was worked up for the neutral in the usual way, the neutral solution evaporated to dryness, and the residue triturated with ether to yield the fully-protected dipeptide (63) (0.97g, 40.2%), m.p. 107-109°.

The reaction was repeated on the same scale and under the same conditions but with the one difference that acetic acid (0.452g, 7.20 mmoles)

had been added on an equimolar scale as the active ester. The yield was 0.91g, 37.6%.

(ix) Coupling via 2, 4, 5-trichlorophenyl ester derivative (66) in hexamethylphosphoramide (HMPA). A solution of the amino ester (62) hydrochloride (1.22g, 7.92 mmoles) and triethylamine (0.8g, 1.1 ml, 7.92 mmoles) in ethyl acetate (10 ml) was added to a stirred solution of the active ester (66) (3g, 7.2 mmoles) in HMPA (10) at room temperature. The reaction soon exothermed (10-15 min) and solidified. The precipitate, shown to be fully-protected dipeptide (63) (1.28g, 53.3%; m.p. 106-108°) was filtered off and washed with ethyl acetate. The combined filtrate was concentrated and the concentrated solution kept for 2h at 80-100° and then allowed to cool to room temperature. The reaction was then partitioned between ethyl acetate and a vast excess of water (450 ml). The organic layer was dried in the usual way, and evaporated to yield more dipeptide (63) (0.320g, 13.3%; m.p. 105-108°), giving an overall yield of 66.6%.

EXPERIMENTS DIRECTED TOWARDS SYNTHESIS OF DL-β-BENZYLOXYCARBONYL-AMINOBUTYROYL-β-AMINOBUTYROYL-β-AMINOBUTYRIC ACID METHYL ESTER (70).

Preparation of DL-β-benzyloxycarbonylaminobutyroyl-β-aminobutyric acid (69). A solution of the fully-protected dipeptide (63) (5g, 14.88 mmoles) in aqueous methanol (25 ml, 30%) was cooled (0°) and a solution of sodium hydroxide (15 ml, N) gradually added with stirring. The reaction was stirred overnight, and t.l.c. (R_{fA} 0.0) testified to the absence of starting material. Methanol was evaporated, the aqueous solution extracted with ethyl acetate, acidified (5N

hydrochloric acid) to congo red, and set aside at room temperature for 1h. The precipitate was filtered off, and recrystallized from ethyl acetate to yield the N-protected dipeptide acid (69) (3.52g, 73.4%) m.p. 186-190° (191-193° after further recrystallization). (Found: C, 59.50; H, 6.64; N, 9.01. $C_{16}H_{22}N_2O_5$ requires C, 59.61; H, 6.88; N, 8.79%).

Attempted preparation of DL-β-benzyloxycarbonylaminobutyroyl-β-aminobutyric acid pivaloyl anhydride. Pivaloyl chloride (0.41g, 0.45ml, 3.41 mmoles) was added to a stirred and cooled (-5°) suspension of N-protected dipeptide acid (69) (1g, 3.10 mmoles) and triethylamine (0.34g, 0.5 ml, 3.41 mmoles) in a mixture of ethyl acetate (40 ml) and dimethylformamide (5 ml). The reaction was stirred at -5° for 2h and at room temperature overnight. The solid material was filtered off, and the filtrate evaporated to dryness. The residue was triturated with toluene, which was again evaporated. The washing procedure with toluene was repeated 3 times. The final residual material (0.72g, 72%, calculated on dipeptide acid) was shown to be, N-protected dipeptide acid (69).

Preparation of DL-β-benzyloxycarbonylaminobutyroyl-β-aminobutyroyl-β-aminobutyric acid methyl ester (70) via DCCI and 1-hydroxybenzotriazole. DCCI (0.7g, 3.42 mmoles), 1-hydroxybenzotriazole (0.42g, 3.11 mmoles) and N-protected dipeptide acid (68) (1g, 3.11 mmoles) were dissolved in dimethyformamide (20 ml) with stirring and cooling at 0° for 1h and then for another 1h at 10°. To this solution was then added a solution of the amino ester (62) hydrochloride (0.53g, 3.42

mmoles) and triethylamine (0.34g, 0.5 ml, 3.42 mmoles) in dimethylformamide (10 ml). The reaction was stirred at 10° for 1h and then at room temperature for 12h. Solvent was evaporated, and ethyl acetate (100 ml) substituted. DCU (0.13g, 17.1%; m.p. 220-224°) was filtered off and the filtrate worked up for the neutral. Evaporation of the neutral solution yielded fully protected tripeptide (70) (0.196g, 15.1%), m.p. 179-182° (187-189° after recrystallization from methanol-ether), R_{fI} 0.35. (Found: C, 59.54; H, 7.29; N, 9.69. $C_{21}H_{31}N_3O_6$ requires C, 59.84; H, 7.41; 9.97%).

Preparation of D L-β-aminobutyroyl-β-aminobutyric acid methyl ester (71). A solution of the fully-protected dipeptide (63) (11.2g, 36.0 mmoles) in methanol (90 ml) was purged with nitrogen gas and 10% palladium on charcoal catalyst (1.1g) added. Hydrogen gas was passed through the mixture until evolution of carbon dioxide ceased (6h) (tested with barium hydroxide). The catalyst was removed by filtration and the filtrate evaporated to afford an oil (6.5g, 95.6%), R_J 0.43 (not colouring with ninhydrin). The new substance was presumed to be the title compound (71).

Preparation of D L-β-benzyloxycarbonylaminobutyroyl-β-aminobutyroyl-β-aminobutyric acid methyl ester (69) via the active ester route in HMPA. The experimental details are as described for the preparation of the dipeptide (63) via the active ester (66) route.

The free amino dipeptide ester (71) (6.5g, 34.4 mmoles) in HMPA (6 ml), brought forward in situ from the foregoing reaction, was added to a stirred solution of the N-protected amino-acid active ester (66) (12g,

28.8mmoles) in HMPA (15 ml) at room temperature. The reaction exothermed and solidified in 20-30 min. The desired product was isolated according to the procedure detailed above. Recrystallization from methanol-ether yielded the fully-protected tripeptide (70) (7.7g, 63.6%), m.p. 185-187°.

Preparation of DL-β-benzyloxycarbonylaminobutyroyl-β-aminobutyroyl-β-aminobutyric acid (72). A solution of the fully-protected tripeptide (70) (4g, 9.49 mmoles) in aqueous methanol (85 ml, 20%) was cooled (5°) and sodium hydroxide solution (10.5 ml), 1N) added in three portions during 6 min. The mixture was stirred at room temperature for 24h, during which it was monitored by t.l.c. Methanol was evaporated, the aqueous phase extracted with ethyl acetate, and acidified (2N-hydrochloric acid) to congo red. The precipitate was filtered off, washed with ice-cold water, and recrystallized from methanol-ether to give the N-protected tripeptide acid (72) (2.65g, 68.8%), m.p. 210-213°, (230-232° on further recrystallization). (Found: C, 58.87; H, 7.39; N, 10.27. $C_{20}H_{29}N_3O_6$ requires C, 58.96; H, 7.2; N, 10.3%).

Preparation of DL-β-aminobutyroyl-β-aminobutyroyl-β-aminobutyric acid (73). The foregoing N-protected tripeptide acid (72) (2.4g, 5.88 mmoles) was dissolved in methanol (130 ml) and hydrogenolysed over 10% palladium-charcoal (0.3g) during 6h. Filtration and evaporation yielded a mixture of solid and oil, R_f 0.32 (ninhydrin positive), 0.66 (ninhydrin positive). Spraying with bromo-cresol green and hydroxylamine/ferric chloride solutions showed that the faster moving spot represented free amino tripeptide methyl ester. Free tripeptide

(73) (0.5g, 32%), m.p. 240-241°, was isolated by recrystallization (twice) from methanol. (Found: C, 53.06; H, 8.28; N, 15.44. $C_{12}H_{23}N_3O_4$ requires C, 52.73; H, 8.48; N, 15.37%).

Preparation of DL-cyclo-(tri-β-aminobutyroyl) (74). A solution of the free tripeptide (73) (100 mg, 0.35 mmoles) in diethyl phosphite (5 ml) was cooled to 0° and o-phenylene phosphorochloridite (67.2mg, 0.385 mmoles) added gradually with stirring. After 5 min the solution was diluted with diethyl phosphite (75 ml) and triethylamine (70.8mg, 0.1 ml, 0.70 mmoles) added. The mixture was stirred overnight at room temperature and then heated under reflux for 15 min. The crude product (40 mg, 40%), m.p.> 260°, precipitated from the cool solution was filtered off. The product was examined by t.l.c., R_{fH}0.67; R_{fJ}0.45 (ninhydrin negative in both instances). Sublimation (220-230°/ 0.25 mmHg) of the crude yielded a fine crystalline cyclo-tripeptide (74) (20 mg, 20%) m.p. > 300°. The highest signal for this compound in the mass spectrum (Fig. 21) was at m/e 255 (M^+). (Found: C, 56.50; H, 8.20; N, 16.33. $C_{12}H_{21}N_3O_3$ requires C, 56.45; H, 8.29; N, 16.46%).

The diethyl phosphite filtrate was evaporated and the residue desalted with ion exchange resins as follows. The residue (20 mg) obtained after evaporation of diethyl phosphite was dissolved in water (6 ml) and the aqueous solution passed through columns of Dowex 50-X8 (H^+ form, 20g) and De-acidite FF-IP (OH^- form 30g) ion resins. The eluate was concentrated and examined by t.l.c., R_{fH}0.67; R_{fJ}0.45. After complete evaporation of solvent (water), the residue (20 mg) was sublimed under the same conditions as detailed above. The sublimate (II)(10 mg) was identical to the first in terms of i.r. spectroscopy and t.l.c.

behaviour. Overall yield was 30 mg, 32%.

EXPERIMENTS DIRECTED TOWARDS CYCLISATION OF D L-β-BENZAMIDOBUTYRIC ACID (76)[83] TO THE RACEMIC 4, 5- DIHYDRO-4-METHYL-1, 3-OXAZIN-6-ONE (79)

Preparation of D L-β-benzamidobutyric acid (76)[83]. Benzoyl chloride (16.3g, 13.6 ml, 0.116 moles) was added, in three portions, to a stirred and cooled (0°) solution of β-aminobutyric acid (10g, 0.097 moles) in sodium hydroxide (106.7 ml, 2N) during 15 min. The reaction was stirred at room temperature for 5h after which it was extracted with ether and then made acid (conc. hydrochloric acid) to congo red. The precipitate was filtered off, washed with ice water, and then with ether. Recrystallization from ethanol-water gave the benzoyl derivative (14.9g, 74.4%), m.p. 148-150° (151-152° after recrystallization from ethyl acetate-petrol), R_{fG} 0.44 (Baker and Ollis[83] reported m.p. 151.5°).

Attempted oxazinone synthesis via the acid chloride derivative cyclised in situ. Thionyl chloride (0.6g, 0.4 ml, 5 mmoles) in dry carbon tetrachloride (5 ml) was added dropwise to a stirred and cooled (0°) heterogeneous mixture of β-benzamidobutyric acid (0.52g, 2.5 mmoles) and triethylamine (0.5g, 5 mmoles) in dry carbon tetrachloride (20 ml). Stirring was continued at 0° for 10 min and at room temperature for 1h. The dark-coloured precipitate (0.92g) was filtered off and because of its excess weight (100% triethylamine hydrochloride = 0.68g) the solid in the filter funnel was washed with water and the undissolved portion found to be starting material (0.18g, 34.6%). The filtrate was evaporated down to yield a dark-coloured residue that could not be purified and was therefore not characterised.

The experiment was repeated using N-methyl morpholine as tertiary base. There was no improvement to the result.

Attempted synthesis using thionyl chloride and triethylamine in benzene. Thionyl chloride (1.72g, 1.1 ml, 14.5 mmoles) in dry benzene (5 ml) was added gradually to β-benzamidobutyric acid (2g, 9.7 mmoles) and triethylamine (1.8g, 14.6 mmoles), with stirring and cooling (0°). The reaction was stirred at 0° for 15 min, allowed to come to room temperature, and then heated under reflux over an oil bath at 80° for 4h. After cooling to room temperature the reaction was filtered and the filtrate evaporated to yield a dark-coloured residue. The residue was triturated with carbon tetrachloride (20 ml) and the precipitated triethylamine hydrochloride filtered off. The filtrate was evaporated and the resulting crude residue (0.69, 30% - calculated on the benzoyl derivative (76) examined by i.r. spectroscopy: ν max (film) 1795, 1675, 1230, 1140, 1040, 1020, 700 cm^{-1}. The crude material could not be further characterised as attempts at purification by vacuum distillation failed.

Attempted synthesis using thionyl chloride and triethylamine in dichloromethane. Thionyl chloride (1.72g, 1.1 ml, 14.5 mmoles) was gradually added to a stirred and cooled (0°) solution of β-benzamidobutyric acid (2g, 9.7 mmoles) and triethylamine (1.8g, 14.6 mmoles) in dichloromethane (20 ml). The reaction was stirred at 0° for 20 min. and at room temperature for 2h. The work up procedure was as in the foregoing experiment except that ether was used for trituration. The crude residue was distilled in the heating block, 127-130°/ 2-2.25 mmHg

the oily distillate (210mg, 10.5%) crystallized on standing, m.p. 86-90° (110-112° after recrystallization from ethyl acetate-petrol). The product was also examined by i.r. spectroscopy: ν_{max} (nujol) 3315, 1812, 1731, 1631, 1550 cm^{-1}.

Isolation of D L-β-benzamidobutyric acid chloride (77) using the method of Dorn[123]. Thionyl chloride (1.5g, 0.92 ml, 11.6 mmoles) was dropped onto D L-β-benzamidobutyric acid (2g, 9.7 mmoles). The mixture was stirred at room temperature, until it became homogeneous and the exothermic effect subsided. The reaction was then heated under reflux on a water-bath at 60-80° until the evolution of sulphur dioxide and hydrogen chloride gases ceased. Excess thionyl chloride was distilled off, at water-pump vacuum, and the crude residue (2.36g, 108.3%) examined by i.r. and n.m.r. Results of spectroscopic analysis at this stage conveyed the impression that the product had a reasonable degree of purity. Purification was carried out by distillation on a heating block at 75-80°/ 2 mmHg.

The resulting oily product (77) (0.65g, 29.8%) crystallized under refrigeration but melted at room temperature, ν_{max} (film): 3300 cm^{-1} (N - H), 1800 cm^{-1} (C = O), 1660 cm^{-1} (C = O, amide). Found: C, 58.5; H, 5.7; N, 6.5; Cl (sample decomposed). $C_{11}H_{12}NO_2Cl$ requires C, 58.5; H, 5.4; N, 6.2; Cl, 15.7%).

Treatment of D L-β-benzamidobutyric acid (76)[83] with acetic anhydride at 100° according to Barker[84]. Acetic anhydride (22g, 20 ml, 215 mmoles) and D L-β-benzamidobutyric acid (2g, 9.7 mmoles) were kept at 100° for 20 min. Removal of acetic anhydride at water-pump vacuum

afforded an oil. The oil was taken up in ethyl acetate and examined by t.l.c., R_{fE}0.05, 0.38, 0.85, (all three spots gave a reaction on spraying with hydroxylamine and ferric chloride solutions). The faster moving component was the first to react with the spraying reagents, followed by the slowest-moving.

Isolation of D L-β-benzamidobutyroyl symmetrical anhydride (78)[84].
The overall product of the "Barker experiment" was triturated several times with petrol and the combined petrol washes preserved for further work up. The extracted residual material crystallized from ether-petrol to afford symmetrical anhydride (78) (1.29g, 64.5% - calculated on β-benzamidobutyric acid), m.p. 86-89° (raised to 110-112° on recrystallization from ethyl acetate-petrol), R_{fE}0.05; R_{fA}0.35. The i.r. spectrum of the compound (78) was studied (Fig. 22). (Found: C, 66.5; H, 6.3; N, 7.3. $C_{22}H_{24}N_2O_5$ requires C, 66.7; H, 6.1; N, 7.1%).

Isolation of the minor components of the "Barker experiment".
Silica gel, taken in the ratio of 1 part of substrate to 100 parts of silica, was packed into a column which was then loaded with 500 mg of the residue obtained by evaporation of solvent from the petrol extract R_{fE}0.38, 0.85. The column was eluted with a mixed benzene-ethyl acetate solvent system, progressive increases in ethyl acetate (8%, 16%, 32%) being made on the basis of t.l.c. monitoring of the effluent.

D L-4, 5-Dihydro-4-methyl-1, 3-oxazin-6-one (79). The first component (108 mg, 21.6% - calculated on material put on the column) to run off the column in chromatographically pure state (R_{fD}0.68, R_{fE}0.85) was a mobile oily substance, ν max (film): 1795, 1675, 1230, 1140, 1040, 1020,

700 cm^{-1}. N.m.r. spectrum was consistent with the oxazinone structure (79) (108 mg, 5.4% - calculated on β-benzamidobutyric acid). Low resolution mass spectrometry (Fig. 23) gave m/e 189 (M^+), for precise mass on molecular ion. The derivative (79) was further characterised by i.r. spectroscopy (Fig.22a). (Found: C, 70.1; H, 6.2; N, 7.3. $C_{11}H_{11}N\ O_2$ requires C, 69.8; H. 5.86; N, 7.4%).

DL-β-benzamidobutyric acid ethyl ester (80). The second minor component (75 mg, 15% - calculated on material put on the column), obtained from the column in chromatographically pure state (R_{fE}0.38) was a viscous oil which crystallized from petrol, m.p. 42-44°. T.l.c. behaviour (ability to react with hydroxylamine and ferric chloride spraying reagents) and spectroscopic examination led to assignment of the compound to DL-β-benzamidobutyric acid ethyl ester (80)[83] (75 mg, 3.8% - calculated on β-benzamidobutyric acid). The identity of the compound was confirmed by direct synthesis as detailed below.

Preparation of DL-β-benzamidobutyric acid ethyl ester (80).[83]

β-Benzamidobutyric acid (1g, 4.8 mmoles) was dissolved in absolute ethyl alcohol (50 ml) to which 10% boron trifluoride etherate solution had been added. The reaction mixture was heated under reflux at 80° for 8h followed by removal of ethanol at reduced pressure. Boron trifluoride could not be separated from the desired product (80) at water-pump vacuum but the ester (80) was successfully isolated by distillation on the heating block (95-100°/ 2 mmHg) in chromatographically pure state (R_{fE}0.38) in a yield of 0.56g, 49.6%, m.p. 36-39° (raised to 44-46° on recrystallization from boiling petrol) (Baker and Ollis[83] gave m.p. 47.5°).

Treatment of DL-β-benzamidobutyric acid (76) with acetic anhydride under reflux conditions. Acetic anhydride (21.6g, 20 ml, 0.212 moles) and DL-β-benzamidobutyric acid (2g, 0.0097 moles) were heated under reflux at 140° for 20 min. Evaporation of acetic anhydride yielded a viscous oil that could not be meaningfully characterised by spectroscopic methods. The oil was examined by t.l.c., R_{fE} 0.04, 0.28, 0.4, 0.86. The oily product (2g) was divided into two parts: one portion (1g) for column chromatography and the other portion (1g) for base-ethyl acetate partitioning.

Column Chromatographic separation of reaction components. Separation was carried out using a 1:100 sample to silica gel ratio, with an ethyl acetate/benzene solvent gradient of 8%, 16% and 32%.

The first pure component (R_{fE} 0.86) to be eluted gave a spot that did not react with bromo-cresol green or with ninhydrin; but darkened when sprayed with hydroxylamine and ferric chloride solutions. I.r. and n.m.r. spectroscopy finally led to the realisation that the compound was benzoic acid anhydride (38 mg, 3.8%) I.r. and n.m.r. spectra of an authentic sample were run to confirm the result. The compound crystallized on standing in the refrigerator, m.p. 40-42° (m.p. 41-42°, authentic sample).

Benzoic acid. Further elution led to the recovery of benzoic acid (215 mg, 21.3%), m.p. 105-108° (raised to 121-123° on recrystallization from cyclohexane), R_{fE} 0.28.

DL-β-Benzamidobutyric acid. Washing the column in the last stages with ethyl acetate and then with acetone resulted in the recovery of DL-β-benzamidobutyric acid (0.18g, 18%) m.p. 152-154°; further confirmed by i.r. spectroscopy.

Attempted recovery of other acidic components by base-ethyl acetate partitioning

DL-β-Acetamidobutyric acid. The portion of material (1g) that was kept aside from above was partitioned between a cooled (0°) solution of sodium carbonate (5%) and ethyl acetate. The organic layer was dried (Mg S O_4) and evaporated to yield an oil (220 mg, 22.0%). The aqueous layers were combined, acidified (hydrochloric acid, 5N) to congo red, and extracted with ethyl acetate. Drying and evaporation of the organic layer afforded a residue which was triturated with ether. β-Benzamidobutyric acid separated and was recovered in a yield of 0.35g, 35%. The filtrate was evaporated to yield an oil (68 mg, 6.8%) which showed a single spot on t.l.c. R_{fJ}0.75, R_{fL}0.77. On the basis of spectroscopic and t.l.c. comparisons made between this product and a prepared sample (see below) of DL-β-acetamidobutyric acid, this acidic component was assigned as DL-β-acetamidobutyric acid.

DL-β-Aminobutyric acid. The aqueous layer resulting from the foregoing 'work up' was neutralised to about pH 7, concentrated and then examined by t.l.c., whereupon the presence of β-aminobutyric acid was detected; an authentic sample of the acid was used as an internal standard in all the solvent systems employed: R_{fJ}0.32, R_{fL}0.36, R_{fH}0.5.

Attempted N-acetylation of β-aminobutyric acid. Acetyl chloride (0.9g, 0.82 ml, 11.7 mmoles) was added to a stirred and cooled (5°) solution of β-aminobutyric acid (1.0g, 4.85 mmoles) in pyridine (3 ml). A considerable liberation of heat, during which the mixture turned red, was observed. The reaction was set aside overnight at room temperature after which it was partitioned between ethyl acetate and a solution of

hydrochloric acid (1N). The organic layer was shaken with sodium hydroxide solution (0.5N), the aqueous layer made acid (hydrochloric acid, 2N) to congo red, and extracted with ethyl acetate. The organic layer was dried and evaporated to afford an oily residue (0.34g, 26.2% - calculated on β-aminobutyric acid). T.l.c. showed that the residue was a composition of several species. No attempt was made to separate and identify the components.

N-acetylation of DL-β-Aminobutyric acid under Schotten-Baumann conditions. Acetyl chloride (0.8g, 0.73 ml, 9.8 mmoles) was added to a stirred and cooled (0°) solution of β-aminobutyric acid (1g) in a solution of sodium hydroxide (20 ml, 1N). The reaction was stirred at room temperature for 4h. The product was isolated by acidification (hydrochloric acid, 2N) to congo red, followed by extraction with ethyl acetate, drying of the organic layer and evaporating to dryness. The oily residue (0.42g, 32.3% - calculated on β-aminobutyric acid) gave a single spot on t.l.c., R_{fJ} 0.75, R_{fL} 0.77. Spraying the spots with bromo-cresol green resulted in a yellow coloration. Spectroscopic examination pointed to the product being DL-β-acetamidobutyric acid.

β-Acetamidobutyroyl cyclohexylammonium salt. Cyclohexylamine was added dropwise to an occasionally hand-swirled and occasionally ice-cooled solution of DL-β-acetamidobutyric acid (0.4g) in ethyl acetate (5 ml). There was an exotherm and an almost immediate precipitation of the crystalline product (0.36g, 54% - calculated on β-acetamidobutyric acid), m.p. 160-163° (raised to 169-171°, on recrystallization from ethyl acetate). (Found: C, 58.9; H, 10.2; N, 11.8. $C_{12}H_{24}N_2O_3$ requires C, 58.9; H, 9.9; N, 11.47%).

Treatment of DL-β-benzamidobutyric acid (76) with acetic anhydride at room temperature. The benzoyl derivative (76) (2g, 9.7 mmoles) and acetic anhydride (21.6g, 20 ml, 215.0 mmoles) were stirred together in the form of a heterogeneous mixture at room temperature until such time that the system had become homogeneous. Acetic anhydride was then evaporated under vacuum at a water-bath temperature of 50-60°. The oily residue was extracted several times with petrol and the combined petrol extracts evaporated down to yield the oxazinone (79) (145 mg, 7.25%).

The reaction was repeated under the same conditions as above except that 250 ml of acetic anhydride was used. The yield of oxazinone increased to 13.1%. The second component crystallized to give symmetrical anhydride (78) in each case.

Preparation of DL-β-benzamidobutyranilide (82) via the symmetrical anhydride (78). A solution of aniline (129.5 mg, 1.38 mmoles) in ether (10 ml) was added to a solution of the anhydride (78) (500 mg, 1.26 mmoles) in dichloromethane (10 ml). The addition was accompanied by an exotherm, almost immediately followed by precipitation of anilide (82) (262 mg, 74.8% - calculated on symmetrical anhydride), m.p. 169-172° (raised to 185-186° on recrystallization from ethyl acetate). (Baker and Ollis[83] gave m.p. 190°).

Preparation of oxazinone (79) via CMA. Isobutylchloroformate (1.59g, 1.52 ml, 11.64 mmoles) in dichloromethane (2 ml) was added gradually to β-benzamidobutyric acid (2g, 9.7 mmoles) and triethylamine (1.2g, 1.66ml, 11.64 mmoles) in dichloromethane at -15 to -10°. The reaction was stirred at this temperature for 15 min, at 0° for 10 min, and at room

temperature for another 15 min. Dichloromethane was evaporated and the solvent changed to petrol, thus allowing insoluble triethylamine hydrochloride to separate and be filtered off. Evaporation of the filtrate yielded pure oxazinone (79) (1.66g, 91.6%) in the form of an oil, (Fig. 21).

Preparation of DL-β-benzamidobutyranilide (82)[83] using oxazinone (79). A solution of aniline (35.2 mg, 0.38 mmoles) in dry ether (5 ml) was added to a solution of the dihydro-oxazinone derivative (79) (60 mg, 0.32 mmoles) in dry ether (8 ml). The mixture was hand-swirled and then allowed to stand at room temperature whereupon the anilide (82) (68 mg, 76.2%), m.p. 181-183° (raised to 185-186° on recrystallization from ethyl acetate) separated and was isolated in chromatographically pure state, R_{fE} 0.27. (Baker and Ollis[83] reported m.p. 190°).

Preparation of DL-β-benzamidobutyroyl-β-aminobutyric acid methyl ester (81) via oxazinone (79). A solution of methyl β-aminobutyrate (0.68g, 5.8 mmoles) in dichloromethane (4 ml) was added to the oxazinone derivative (79) (1.1g, 5.8 mmoles) in ether (4 ml) at room temperature. There was noticeable heating up of the mixture during hand-swirling, quickly followed by separation of crystalline dipeptide (81) (1.18g, 65.6%) from solution. Solvent was evaporated and the solid product (81), m.p. 126-129°, recrystallized from ethyl acetate-petrol, 133-135°, R_{fC} 0.35. (Found: C, 62.9; H, 7.0; N, 9.4. $C_{16}H_{22}N_2O_4$ requires C, 62.7; H, 7.2; N, 9.1%).

PREPARATION OF OPTICALLY ACTIVE 3-AMINOBUTYRIC ACID AND ITS DERIVATIVES

Attempted preparation of N-benzoyl L-homoalanine.

Attempted preparation of N-benzoyl L-alanine (83)[136]. Benzoyl chloride (26.2g, 21.7 ml, 0.19 moles) and a solution of sodium hydroxide (170 ml, 1N) were added simultaneously to a cooled (0°) and stirred solution of L-alanine (15g, 0.17 moles) in sodium hydroxide (170 ml, 1N). Stirring was continued for the next 10h during which the pH range was maintained approx. between 7.5 and 8.5. The mixture was then extracted with ether and acidified (5M-hydrochloric acid) to congo red. A white crystalline material precipitated, which was filtered off, washed with water, and recrystallized from ethyl acetate-petrol to give the benzoyl derivative (23.7g, 72.9%), m.p. 161-163° (165-166° on recrystallization), $[\alpha]_D^{20}$ 0.0 (c 9.3 in H_2O + 1 eq KOH). (Fischer[136] reported m.p. 165-166° for the racemate).

pH-controlled preparation of N-benzoyl L-alanine (83)[136]. Benzoyl chloride (15.5g, 13.0 ml, 0.12 moles) was added to a cooled (0°) and stirred solution of L-alanine (10g, 0.112 moles) in sodium hydroxide solution (56.5 ml, 2N) simultaneously with addition of base at a rate that the pH did not rise above 10. Stirring was continued for 8h under the above-stated pH conditions and then overnight. The reaction was then extracted with ether, the aqueous phase made acid (5M hydrochloric acid) to congo red, when a precipitate formed. The precipitate was filtered off, washed with water, and recrystallized from ethyl acetate and petrol to afford the benzoyl derivative (83) (14.4g, 66.4%), m.p. 147-149° (150-152α on recrystallization), $[\alpha]_D^{20}$ + 37.0° (c 9.3 in water + 1 eq KOH) (Fischer[136] reported m.p. 150-151°, $[\alpha]_D^{20}$ + 37.1° (c 9.3 in

H_2O + 1 eq KOH).

Attempted isolation of N-benzoyl-L-alanyl chloride (84). Benzoyl L-alanine (2g, 10.4 mmoles) was added to a system of dry benzene (10 ml) and thionyl chloride at room temperature. The reaction mixture was heated under reflux for 2½h during which it progressively darkened. Benzene and excess thionyl chloride were evaporated to give a crude oily product which, from its i.r. absorption at 1832 and 1645 cm^{-1}, with concomitant disappearance of the N-H absorption band, was assumed to contain some of the corresponding oxazolone (85) $[\alpha]_D^{20}$ + 0.15° (c 1 in ether). The product could not be purified by vacuum distillation.

Preparation of N-tosyl L-homo-alanine (90).

Preparation of N-tosyl L-alanine (86)[137]. Tosyl chloride (117.4g, 0.62 moles) dissolved in ethyl alcohol (75 ml) was added gradually and simultaneously with a solution of sodium hydroxide (154.5ml, 4N) to L-alanine (50g, 0.56 moles) in sodium hydroxide (140 ml, 4N) under vigorous stirring and internal cooling with ice to keep the temperature below 50°. Stirring was continued for 6h during which the pH was kept between 8 and 9 by means of a pH-meter. Ethanol was removed at reduced pressure and the aqueous phase treated with charcoal and filtered through a hyflo filterbed followed by acidification (conc. hydrochloric acid) to a pH of 1-2.5. A white crystalline substance separated out of solution on standing overnight, was filtered off, washed with water, and recrystallized from ethyl acetate and petrol to furnish the desired product (86) (107g, 78.1%), m.p. 131-133° (134-136° on further recrystallization), $[\alpha]_D^{20}$ -6.6° (c 1 in ethanol). (Fischer and Lipschitz[137] reported m.p. 134-135°, and $[\alpha]_D^{20}$ -6.8° (in ethanol)).

N-tosyl-L-alanyl chloride (87). N-tosyl-L-alanine (24.3g, 0.1 moles) was added to a system of dry benzene (100 ml) and thionyl chloride (32.0g, 0.27 moles). The system was heated under reflux for 2½h during which it progressively darkened. The mixture was evaporated down to dryness, the residue treated with dry benzene (3 X 50 ml), and the evaporation repeated each time in order to remove excess thionyl chloride. The oily residue solidified on being kept in an evacuated desiccator over paraffin wax and phosphorus pentoxide; giving a crude yield of 26.17g, 100.1%, (ν_{max} 570, 670, 820, 912, 1092, 1155, 1330, 1600, 1788 cm^{-1}), $[\alpha]_D^{20}$ -4.4 (c 1 in benzene). The compound was not amenable to purification by the usual techniques, vacuum distillation resulting in elimination and formation of p-toluenesulphonamide.

Attempted isolation of N-tosyl-L-alanyl diazoketone (88). A crude sample of N-tosyl-L-alanyl chloride (12g, 45.9 mmoles) in absolute ether (150ml) was added to a stirred and cooled (-5 to 0°) ethereal solution of diazomethane[139] (approx. 5X excess, prepared from N-methyl-N-nitroso-urea[140]) and kept for 30 min at -5 to 0° and then for a further 2h at 0°. Evaporation of the solution yielded a crude yellow oil (12.3g), which was shown to be a two-component mixture, R_{fB} 0.25, 0.48, R_{fC} 0.34, 0.58; ν_{max} (film) 3290, 2120, 1740, 1640, 1365, 1340, 1165 cm^{-1}.

N-tosyl-L-homoalanineamide (89). The overall product, unpurified diazoketone (12.3g) from the foregoing experiment was taken up in ethyl alcohol (75 ml) and then, together with conc. ammonia (160 ml, d = 0.88) and aqueous silver nitrate (18 ml, 10%) heated under reflux for 3h during which there was noticeable evolution of nitrogen from the reaction system as it progressively darkened. The solution was allowed

to cool to room temperature and decolouring charcoal added. The mixture was once again heated under reflux for 10 min and then filtered hot under suction through a hyflo filterbed. Ethanol was evaporated off the filtrate and crystalline material separated from the aqueous phase on standing at room temperature. Filtration, drying and recrystallization from ethanol-ether gave the amide (89) (5.3g, 44.8% - calculated on (86) m.p. 169-172° (raised to 174-176° on recrystallization), $[\alpha]_D^{20}$ + 2° (c 1 in ethanol). (Found: C, 51.5; H, 6.6; N, 11.1; S, 12.7. $C_{11}H_{16}N_2O_3S$ requires C, 51.54; H, 6.30; N, 10.90; S, 12.50%).

N-tosyl-L-homoalanine (90). The foregoing amide (2g, 7.8 mmoles) was suspended in hydrochloric acid (80 ml, 3N) and heated under reflux for 4h. The solution that resulted was allowed to stand overnight at room temperature. The acidic aqueous phase was extracted with ethyl acetate, the organic layer washed with water, dried and concentrated to yield the N-protected homologated acid (90) (1.85g, 92.8%), m.p. 119-121° (raised to 130-132° on recrystallization from ethyl acetate), $[\alpha]_D^{20}$ -7 (c 1 in ethanol). (Found C, 51.1; H, 6.1; N, 5.4; S, 12.4. $C_{11}H_{15}N\ O_4S$ requires C, 51.13; H, 5.87; N, 5.44; S, 12.46%.

Isolation of N-tosyl β-lactam (4-methyl-1-tosyl-azetidin-2-one (91)). The two-component system obtained in the experiment where crude N-tosyl-L-alanyl chloride was reacted with diazomethane was shown, on the basis of comparative t.l.c., to consist of N-tosyl-L-alanyl diazoketone, R_{fB}0.48, R_{fC}0.58; and p-toluenesulphonamide, R_{fB}0.25, R_{fC}0.34. An aqueous solution of silver nitrate (10 ml, 10%) was added to a solution of the two-component system (10g) in tetrahydrofuran (40 ml) and the overall

mixture heated under reflux overnight. Tetrahydrofuran was evaporated at reduced pressure and the residue, which showed complete absence of the $-\overset{+}{N}\equiv N$ absorption band (ν max 2120 cm^{-1})[150] in the i.r. spectrum, was re-examined by t.l.c., R_{fB} 0.25, 0.62 (the front-running spot reacted, colouring brown, on spraying with hydroxylamine and ferric chloride solutions). The mixture was separated on a silica gel column, employing chloroform as eluting solvent, to furnish chromatographically pure β-lactam (0.84g, 21% - calculated on material loaded on to the column), m.p. 77-79 (78-79°) on recrystallization from boiling petrol), R_{fB} 0.62. ν max (nujol) 1828, 1600, 1455, 1345, 1160, 1135, 670, 605 cm^{-1}. $[\alpha]_D^{20}$ + 80° (c 1 in chloroform).

^{1}HN.m.r. ($CDCl_3$)

τ (ppm)	multiplicity	protons	assignment
2.1 - 2.7	quartet	4	aromatic
5.0 - 5.35	multiplet	1	$-CH_2-\overset{1}{C}\underline{H}-CH_3$
5.35 - 5.6	doublet	2	$-\overset{1}{C}H-C\underline{H}_2-\overset{1}{C}=O$
7.57	singlet	3	$C\underline{H}_3-Ph$
8.45	doublet	3	$C\underline{H}_3-\overset{1}{C}H-$

The highest signal obtained for the lactam (91) the mass spectrum was at m/e 240(M^++1). The compound (91) did not hydrolyse on treatment with 5N-hydrochloric acid under reflux in 5h. (Found: C, 55.2; H, 5.3; N, 5.7; S, 13.4 $C_{11}H_{13}NO_3S$ requires C, 55.21; H, 5.48; N, 5.85; S, 13.40%).

PREPARATION OF OPTICALLY ACTIVE PEPTIDES USING BENZYLOXYCARBONYL DERIVATIVES OF L-β-AMINOBUTYRIC ACID (106)

Preparation of N-benzyloxycarbonyl-L-alanine (92). L-alanine (40g, 0.45 moles) was treated with benzyl chloroformate (83.6g, 70 ml, 0.49 moles) in the usual way, to yield the desired product (92) (75g, 75%), m.p. 83-85° (raised to 84-86° on recrystallization from ether-petrol), $[\alpha]_D^{22}$ -13.5 (c 2 in glac. acetic acid). (Bergmann and Zervas reported m.p. 87°, $[\alpha]_D^{27}$ -13.9 (c 2 in acetic acid)0.

Preparation of N-benzyloxycarbonyl-L-alanyl diazoketone (93)[145] via the CMA route[145, 146]. Isobutylchloroformate (15.02g, 15 ml, 0.11 moles) was added to a stirred and cooled(dry-ice-acetone bath at -15 to -5°) solution of N-benzyloxycarbonyl-L-alanine (22.4g, 0.1 moles) and N-methyl-morpholine (11.1g, 12.3 ml, 0.11 moles) in dichloromethane (100 ml). The precipitate, N-methylmorpholine hydrochloride (11.6, 84.4%), was filtered off, and to the filtrate a dried (potassium hydroxide pellets) ethereal solution of excess diazomethane (prepared from 40g of N-methyl-N-nitrosourea[140]) was added. The reaction system was stirred for 30 min at -5 to 0° and subsequently kept in the cold (0°) for 24h. The solvent was removed at reduced pressure and the residue crystallized from methanol and ether to yield benzyloxycarbonyl-L-alanyl diazoketone (93)[145] (20.6g, 83.2%), m.p. 74-77° (raised to 89-91° on recrystallization from ether-petrol), $[\alpha]_D^{20}$ -16.1° (c 1 in ethyl acetate). (Penke et al.[145] isolated the diazoketone (93) in the form of an oil). (Found: C, 58.4; H, 5.6; N, 16.7 $C_{12}H_{13}N_3O_3$ requires C, 58.29; H, 5.30; N, 16.99%).

Preparation of β-benzyloxycarbonyl-L-aminobutyric acid methyl ester (94). Benzyloxycarbonyl-L-alanine diazoketone (20g, 80.9 mmoles) prepared by the foregoing reaction was dissolved in dry methanol (140 ml). Several drops of silver benzoate (1g) dissolved in triethylamine[130] (9.1g, 11.6 ml) were added to the stirred solution fitted with a condenser at room temperature. There was an appreciable exotherm of the reaction system as it turned dark and evolution of nitrogen took place. A few more drops of catalyst[130] were added to the stirred reaction followed by addition of decolourizing charcoal and then filtration through a hyflo filterbed under suction. The filtrate was evaporated to dryness, the residue taken up in ethyl acetate and then worked up for the neutral, dried and evaporated to dryness. The residue crystallized on trituration with petrol to yield the N-protected amino acid ester (94) (17.9g, 88.2%), m.p. 46-49° (raised to 55-56° on recrystallization from boiling petrol), $[\alpha]_D^{20}$ -50° (c 1 in methanol). (Found: C, 62.4; H, 6.9; N, 5.3. $C_{13}H_{17}N\ O_4$ requires C, 62.14; H, 6.82; N, 5.57%).

Isolation of β-benzyloxycarbonyl-L-aminobutyric acid (95) by an indirect route from diazoketone. A solution of the afore-mentioned ester (94) (5g, 19.9 mmoles) in aqueous methanol (40 ml, 30%) was cooled to 0° and a solution of sodium hydroxide (10.9 ml, 2N) added gradually. The mixture was stirred at room temperature for 6h. Methanol was evaporated and the aqueous solution extracted with ether, made acid (2M-hydrochloric acid) to congo red, and extracted with ethyl acetate. The organic phase was washed with a concentrated solution of sodium chloride, dried, and evaporated to dryness. The residue was recrystallized from ether-petrol to give the desired N-protected acid (95) (3.6g, 77.8%),

m.p. 105-107° (raised to 109-110° on recrystallization), $[\alpha]_D^{20}$ -26.0° (c 1 in chloroform). (Found: C, 60.8; H, 6.4; N, 5.8. $C_{12}H_{15}N\ O_4$ requires C, 60.75; H, 6.37; N, 5.90).

Preparation of β-benzyloxycarbonyl-L-aminobutyric acid (95) by a direct route from diazoketone (93). Benzyloxycarbonyl-L-alanyl diazoketone (16g, 64.7 mmoles) was dissolved in aqueous tetrahydrofuran (41 ml, 35%) with stirring at room temperature. Silver benzoate (1g) dissolved in triethylamine[130] (11.6 ml) was added dropwise to the gently stirred solution of diazoketone. The addition of catalyst was followed, after 12 min, by considerable warming up of the reaction coupled with effervescence. When the reaction cooled to room temperature, more catalyst was added and the reaction stirred vigorously. The dark-coloured solution was filtered through filter aid under suction and the filter-cake washed with boiling ethyl acetate (80 ml). The organic solvents were removed on the rotavapor and the dark-coloured aqueous solution neutralised (40 ml, 2N NaOH soln.), after which decolouring charcoal was added. A clear solution obtained on filtration was made acid (conc. hydrochloric acid) to congo red, extracted with ethyl acetate, and the organic phase treated as in the foregoing experiment. The recrystallized residue yielded β-benzyloxycarbonyl-L-aminobutyric acid (12.2g, 79.7%), m.p. 108-110°, $[\alpha]_D^{20}$ -26° (c 1 in chloroform).

The same synthesis was also carried out using aqueous acetone as solvent under analogous conditions as above, giving a yield of 82%.

Preparation of β-benzyloxycarbonyl-L-aminobutyroyl 2, 4, 5-trichlorophenyl ester (96). DCCI (25.2g, 121.5 mmoles) was added to a stirred and cooled (-15 to -10°) solution of β-benzyloxycarbonyl-L-aminobutyric acid (24g, 101.16 mmoles) and 2, 4, 5-trichlorophenol (23.88g, 121.4 mmoles) in pyridine (60 ml). The overall system was stirred for 1h, the temperature not being allowed to rise above -5°, and then kept for 24h at 0°. The mixture was diluted with ether (150 ml) and left at 0° for 1h. The precipitate, DCU (28.4g, 93.6%; m.p. 228-230°), was filtered off and the filtrate evaporated to dryness. The residue was triturated with ether and more DCU (0.8g, 2.6%) removed. The filtrate was concentrated and the desired trichlorophenyl derivative (96) (38.6g 91.6%), m.p. 77-80° (raised to 92-94° on recrystallization from boiling petrol), crystallized from ether-petrol, $[\alpha]_D^{20}$ -17.0° (c 1 in chloroform) (Found: C, 52.2; H, 3.6; N, 3.5; Cl, 25.3. $C_{18}H_{16}N\ O_4Cl_3$ requires C, 51.89; H, 3.87; N, 3.36; Cl, 25.52%).

Isolation of methyl L-β-aminobutyrate (97). A solution of the N-protected amino acid ester (94) (12g, 47.75 mmoles) in methanol (36 ml) was hydrogenated in the presence of 10% palladium on charcoal (1.2g) for 6h. The catalyst was filtered off and the filtrate evaporated to give the crude oily product (97) (5.26g, 93.9%), R_{fJ} 0.47, R_{fK} 0.34 (ninhydrin + ve in both cases).

Preparation of β-benzyloxycarbonyl-L-aminobutyroyl-L-β-aminobutyric acid methyl ester (98). Methyl L-β-aminobutyrate (5.26g, 44.95 mmoles) dissolved in hexamethyl-phosphoramide (5 ml) was added to a stirred solution of 2, 4, 5-trichlorophenyl derivative (96) (17.5g, 41.99 mmoles) in HMPA (25 ml) at room temperature. The reaction exothermed and

solidified within 12 min. The resulting heterogeneous system had water (50 ml) added onto it and the solid material was then filtered off and recrystallized from ethyl acetate to yield fully-protected dipeptide (98) (7.6g, 53.5%), m.p. 139-141° (raised to 143-145° on recrystallization), R_{fA} 0.65, $[\alpha]_D^{20}$ -5.6° (c 5 in acetone). The recovered mother liquors were further diluted with water (500 ml), and then extracted with ethyl acetate (3 X 80 ml). The organic layer was dried and concentrated to a small volume (25 ml), the presence of starting materials in this solution were detected by t.l.c. The solution was warmed (approx 50°) under reflux for 24h, evaporated to dryness and the residue triturated with ether to yield a second crop of product (98) (2.4g, 17.3%), m.p. 138-140°, resulting in a final yield from the reaction of 70.8%. Further recrystallization from ethyl acetate-petrol gave a sample of m.p. 144-145°. (Found: C, 60.8; H, 7.3; N, 8.3. $C_{17}H_{24}N_2O_5$ requires C, 60.7; H, 7.19; N, 8.33%).

Isolation of β-benzyloxycarbonyl-L-aminobutyroyl-L-β-aminobutyric acid (99). A solution of fully-protected dipeptide (98) (10g, 29.7 mmoles) in aqueous methanol (80 ml, 25%) was cooled to 0° and then treated with sodium hydroxide solution (32.7 ml, 1N). The reaction was left to stir at room temperature and then worked up according to the procedure previously detailed. The N-protected dipeptide acid (99) (8.6g, 90.5%) was recrystallized from acetone-ethyl acetate, m.p. 170-173° (raised to 174-176° by further recrystallization), $[\alpha]_D^{20}$ +10.4 (c 2 in dimethylformamide), R_{fG} 0.2. (Found: C, 59.6; H, 6.9; N, 8.9. $C_{16}H_{22}N_2O_5$ requires C, 59.62; H, 6.88; N, 8.79%).

Preparation of β-benzyloxycarbonyl-L-aminobutyroyl-L-β-aminobutyric acid 2, 4, 5-trichlorophenyl ester (100). DCCI (5.6g, 27.0 mmoles) was added to a stirred solution of N-protected dipeptide acid (99) (8g, 25 mmoles) and 2, 4, 5-trichlorophenol (5.3g, 27.0 mmoles) in pyridine (180 ml) at -10 to -5°. The reaction was stirred for 1h at -10 to -5° and then let to stand for 48h at 0°. The heterogeneous system obtained was diluted with ethyl acetate (180 ml) and then left to stand at 0° for 2h after which the solid, DCU (4.8g, 80%; m.p. 226-229°), was filtered off. The filtrate was evaporated to dryness and the residue dissolved in ethyl acetate under reflux. The solution was cooled to room temperature and the first crystals to separate from solution were found to be DCU (0.63g, 11.2%), m.p. 224-226° (confirmed by i.r. spectroscopy). The filtrate was concentrated and examined by t.l.c., R_{fc} 0.0, 0.43. The solution crystallized to yield the crude 2, 4, 5-trichlorophenyl derivative (100) (11.4g, 84.4%), m.p. 155-158° (raised to 163-165° on further recrystallization), $[\alpha]_D^{20}$ -20° (c 0.25 in methanol), R_{fc} 0.43. (Found: C, 52.73; H, 4.64; N, 5.78; Cl, 21.40. $C_{22}H_{23}N_2O_5Cl_3$ requires C, 52.66; H, 4.62; N, 5.58; Cl, 21.19%).

Preparation of β-benzyloxycarbonyl-L-aminobutyroyl-L-β-aminobutyroyl-L-β-aminobutyric acid methyl ester (101). Methyl L-β-aminobutyrate (2.6g, 22 mmoles) dissolved in HMPA (5 ml) was added to a stirred solution of the trichlorophenyl derivative (100) (10g, 19.9 mmoles) in HMPA (25 ml). The reaction was stirred at room temperature, and within 25 min it had solidified. Ehyl acetate (25 ml) was added to the reaction which was then warmed up, kept at 80-90° for 1h, allowed to cool to room temperature, more ethyl acetate (50 ml) added, and filtered. The separated solid was

washed, in the filter funnel, with ice-cooled ethyl acetate and then recrystallized from methanol to give the fully-protected tripeptide (101) (5.2g, 62%) m.p. 203-205° (raised to 204-205° on further recrystallization), $[\alpha]_D^{20}$ +26.6° (c 0.3 in methanol), R_{fI} 0.46. The mother liquors were mixed with excess water (500 ml) and extracted with ethyl acetate (3 X 60 ml). The organic layer was concentrated to a smaller volume and then kept under reflux (60-78°) overnight). More ethyl acetate was added and the solution was then worked up for the neutral in the usual way, resulting in the recovery of more tripeptide (101) (0.6g). The overall yield from the reaction was 69.2%. (Found: C, 59.83; H, 7.39; N, 9.83. $C_{21}H_{31}N_3O_6$ requires C, 59.84; H, 7.4; N, 9.97%).

Isolation of β-benzyloxycarbonyl-L-aminobutyroyl-L-β-aminobutyroyl-L-β-aminobutyric acid (102). The foregoing ester (101) (5g, 11.9 mmoles) was dissolved in aqueous pyridine (60 ml, 35%) and then treated with sodium hydroxide (6.5 ml, 2N) at room temperature. The reaction mixture was stirred for 8h during which it was monitored by t.l.c. for the disappearance of the ester (101). Pyridine was evaporated, and water added to complete the dissolution of the residual material. The alkaline aqueous phase was then extracted with ethyl acetate after which it was made acid (5M hydrochloric acid) to congo red and left overnight at room temperature. The precipitate (1.2g, R_{fG} 0.18, was filtered off and the filtrate extracted with ethyl acetate. Evaporation of the ethyl acetate solution furnished more product (R_{fG} 0.18) which was then combined with the first portion and recrystallized from methanol-ether to give N-protected tripeptide acid (102) (2.6g, 72.2%), m.p. 222-224°. Some

unhydrolysed ester (101) was recovered in a yield of 19.4%. Recrystallization of (102) from methanol-ether raised the m.p. to 223-225°, $[\alpha]_D^{20}$ +5.2° (c 2 in dimethylformamide). (Found: C, 58.91; H.7.04; N, 10.48. $C_{20}H_{29}N_3O_6$ requires C, 58.96; H, 7.17; N, 10.31%).

Isolation of L-β-aminobutyroyl-L-β-aminobutyroyl-L-β-aminobutyric acid (103) via the Catalytic Transfer Hydrogenation[148] method. A solution of N-protected tripeptide acid (102) (2.0g, 4.9 mmoles) from the foregoing reaction was dissolved in methanol (180 mol) and then mixed with cyclohexene (0.75g, 0.9 ml, 9.2 mmoles) and 10% palladium on charcoal catalyst (0.5g). The overall system was gently heated under reflux for 20 min, filtered, the catalyst washed with boiling methanol (100 ml), and the overall filtrate concentrated to give the free linear tripeptide (103) (1.1g, 84.6%), m.p. 268-270° (m.p. unchanged on recrystallization from methanol), $[\alpha]_D^{20}$ -9.0° (c 1 in water), R_{fJ} 0.32. (Found: C, 52.62; H, 8.30; N, 15.35. $C_{12}H_{23}N_3O_4$ requires C, 52.73; H, 8.48; N, 15.37%).

Attempted preparation of cyclo-(tri-L-β-aminobutyroyl) (104) via the agency of o-phenylene phosphorochloridite (75)[118, 119]. A suspension of free tripeptide (103) (100 mg, 0.37 mmoles) in diethyl phosphite (10 ml) was cooled to 0° and o-phenylene phosphorochloridite (75) (71mg, 0.407 mmoles) added with stirring. After 5 min the solution was diluted to 80 ml, with diethyl phosphite, and triethylamine (74.8 mg, 0.1 ml, 0.74 mmoles) added. The heterogenous system was stirred at room temperature for 1h and then heated under reflux for 30 min during which the system became homogeneous for a few seconds and then quickly reverted to the heterogeneous state. The precipitate (92 mg, 92%), m.p> 280°

was filtered off and examined by t.l.c., R_{fJ} 0.32, R_{fL} 0.36, and by i.r. spectroscopy; both techniques indicated that the recovered material was free linear tripeptide (103). Part of the recovered material (20 mg) in water (5 ml) was loaded on to an ion-exchange resin column of Dowex 50 - x 8 (H^+ form, 20 g) and eluted with water. Evaporation of the first eluate (100 ml) yielded no residue.

Preparation of cyclo-(tri-L-β-aminobutyroyl) (104) by the use of o-phenylene phosphorochloridite (75) (modified procedure). A suspension of free tripeptide (103) (100 mg, 0.37 mmoles) in diethyl phosphite (80 ml) was cooled to 0° and o-phenylene phosphorochloridite (75) (71 mg, 0.407 mmoles) added with stirring. After 5 min triethylamine (74.8mg, 0.1 ml, 0.74 mmoles) was added and the heterogeneous system stirred at room temperature for 1h. The heterogenous system was gently heated under reflux until a homogeneous state was attained (after 36 min) and further heating continued, on the homogeneous phase, for the next 15 min. The reaction was allowed to cool to room temperature, whereupon solid material simultaneously separated from solution, and then set aside overnight. The precipitate (40 mg, 40%), m.p.> 340°, was filtered off and purified by sublimation, 240-245°/2.10^{-3} mmHg, to yield a fine crystalline product (104) (33 mg, 33%), m.p.> 340°, R_{fJ} 0.24, R_{fK} 0.42, $[\alpha]_D^{20}$ + 15.0° (c 1 in glac. acetic acid). The molecular weight of the compound was checked by MS, which gave the highest signal at m/e 255 (M^+), (Fig. 21). (Found: C, 56.47; H, 8.44; N, 16.5. $C_{12}H_{21}N_3O_3$ requires C, 56.45; H, 8.29; N, 16.46%).

The diethyl phosphite filtrate was evaporated to dryness and the residue

washed with water, then with 50% aqueous methanol and finally with methanol. The dried crystalline residual material (8 mg, 8%), m.p.> 340°, was soluble in glacial acetic acid, R_{fJ} 0.24; R_{fK} 0.42. The material was further purified by recrystallization from a glacial acetic acid-ether system to yield a product (5 mg) identical with the analysed cyclic peptide (104) in terms of i.r. spectroscopy, mass spectrometry and t.l.c. behaviour. The overall yield of crude product (104) from the cyclisation reaction was 48%.

Preparation of β-benzyloxycarbonyl-L-aminobutyroyl-L-β-aminobutyroyl-L-β-aminobutyric acid 2, 4, 5-trichlorophenyl ester (105). N-protected tripeptide acid (102) (0.4g, 0.98 mmoles), 2, 4, 5-trichlorophenol (0.21g, 1.1 mmoles), and DCCI (0.23g, 1.1 mmoles) in pyridine (40 ml) were reacted together and worked up as in other similar experiments described earlier. The N-protected tripeptide active ester (105) was isolated in a yield of 0.322g, 56%, m.p. 192-194° (raised to 196-198° on recrystallization from methanol-ether). (Found: C, 53.1; H, 5.3; N, 7.0; Cl, 18.3. $C_{26}H_{31}N_3O_6Cl_3$ requires C, 53.12; H, 5.31; N, 7.15; Cl, 18.09%).

Preparation of cyclo-(tri-L-β-aminobutyroyl) (104) via the peptide active ester route[149]. A solution of N-benzyloxycarbonyl tripeptide active ester (105) (250 mg, 0.43 mmoles) in methanol (50 ml) and concentrated hydrochloric acid (0.1 ml) was purged with nitrogen gas and 10% palladium on charcoal (50 mg) added. Hydrogen was passed through the mixture until evolution of carbon dioxide ceased (tested with barium hydroxide). The solution was filtered through hyflo filter bed and the filtrate evaporated to afford a white solid residue. This

product, assumed to represent the tripeptide 2, 4, 5-trichlorophenyl ester hydrochloride (106) (186 mg, 89.4%), was used for further reaction in situ. Thus the ester hydrochloride (106) in dimethylformamide (20ml) was added dropwise under vigorous stirring to pyridine (80 ml) at 115° over a period of 2h, and stirred for another 1h. The solution was evaporated, and the residue triturated with water, then with 50% aqueous methanol and finally with methanol. The solid product (104) (23 mg, 23.7%), m.p. > 340°, R_{fJ} 0.24; R_{fK} 0.42, was recrystallized from glacial acetic acid-ether and had identical characteristics as the previously isolated cyclo-(tri-L-β-aminobutyroyl) (104).

SYNTHESIS AND UTILISATION OF OPTICALLY ACTIVE OXAZINONE DERIVATIVES

Isolation of L-β-aminobutyric acid (106)[127]. β-Benzyloxycarbonyl-L-aminobutyric acid (20 g, 84.3 mmoles) was dissolved in methanol (50 ml) and N-deprotected via the catalytic transfer hydrogenation method[148] detailed previously. The free amino acid (8.4 g, 97.6%) was recrystallized from methanol, m.p. 210-213° (raised to 218-219° on further recrystallization); $[\alpha]_D^{20}$ +43° (c 1 in methanol) and $[\alpha]_D^{18}$ +36.46° (c 0.48 in water), R_{fJ} 0.36. (Fischer[147] reported m.p. 210-212°, $[\alpha]_D$ +35.3 (absolute value); and Balenović[127] gave m.p. 212°, $[\alpha]_D^{18}$ +38.8° ± 1° (c 0.48 in water), and $[\alpha]_D^{19}$ +37.07 ± 1° (c 6.0 in water). (Found: C, 46.5; H, 8.8; N, 13.7. $C_4H_9NO_2$ calculated for C, 46.59; H, 8.8; N, 13.58%).

Preparation of β-benzoyl-L-aminobutyric acid (107). Benzoyl chloride (8.9 g, 7.4 ml, 0.064 moles) was added in three portions to a stirred and cooled (5°) solution of L-β-aminobutyric acid (6 g, 0.058 moles) in sodium hydroxide (60 ml, 2N). The solution was stirred for 10 min at 5°,

after completion of addition of benzoyl chloride, and then for 6h at room temperature. The reaction mixture was extracted with ether and the aqueous phase made acid (conc. hydrochloric acid) to congo red. The precipitate was filtered off, dried, and recrystallized from ethyl acetate and petrol to yield the benzoyl derivative (107) (8.6 g, 68.2%), m.p. 143-145° (raised to 144-146° on further recrystallization); $[\alpha]_D^{20}$ +24.2° (c 3 in acetone), R_{fG} 0.40 (Found: C, 63.7; H, 6.3; N, 6.9. $C_{11}H_{13}N\ O_3$ requires C, 63.75; H, 6.32; N, 6.76%).

Preparation of(+)-2-phenyl-1, 3-oxazin-6-one (108). A solution of β-benzoyl-L-aminobutyric acid (3 g, 14.55 mmoles) and N-methylmorpholine (1.8 g, 17.4 mmoles) in dichloromethane (60 ml) was added in three portions to a stirred and cooled (-15 to -10°) solution of isobutyl-chloroformate (2.46 g, 2.36 ml, 18.0 mmoles) in dichloromethane (10 ml). The reaction was kept stirring for 15 min at -15 to -10° and for another 15 min at room temperature and monitored for the disappearance of the acid by t.l.c. Dichloromethane was evaporated, the residue triturated with petrol (2 X 25 ml), and the insoluble hydrochloride (2.36 g, 98.7%) removed by filtration. The filtrate was evaporated down to yield the oily oxazinone derivative 108 (2.64g, 97.8%), 80°/0.15 mmHg; ν max (film) 1795, 1675, 1230, 1140, 1040, 1020, 700 (cm^{-1}). N.m.r. spectrum was found to be consistent with the oxazinone, and MS (Fig. 23) gave the highest peak at m/e 189 (M^+). $[\alpha]_D^{20}$ +26.8° (c 1 in ether). (Found: C, 69.79; H, 5.75; N, 7.05. $C_{11}H_{11}N\ O_2$ requires C, 69.83; H, 5.86; N, 7.40%).

Isolation of isobutyl β-benzoyl-L-aminobutyrate (109). Isobutylchloro-formate (0.82 g, 0.79 ml, 6.0 mmoles) in dichloromethane (5 ml) was

added, gradually, to a solution of β-benzoyl-L-aminobutyric acid (1 g, 4.85 mmoles) and triethylamine (0.6g, 0.43 ml, 5.8 mmoles) in dichloromethane (20 ml) at 0°. The reaction was stirred for 15 min at 0° and for another 15 min at room temperature, and monitored by t.l.c. for the disappearance of the starting material. Dichloromethane was evaporated and replaced by ether. The precipitated hydrochloride salt was removed by filtration and the filtrate evaporated down to give an oil which crystallised from boiling petrol to afford an isobutyl ester (109) (1.2 g, 95.3%), m.p. 65-68° (raised to 73-74° on recrystallization), $[\alpha]_D^{20}$ +24.2° (c 1 in chloroform). (Found: C, 68.3; H, 8.0; N, 5.4. $C_{15}H_{21}N\ O_3$ requires C, 68.42; H, 8.0; N, 5.32%).

The combined filtrates from this reaction were evaporated down to yield an oil, which was shown by i.r. spectroscopy to be the oxazinone derivative (108) (5 mg, 0.5%).

Preparation of β-benzoyl-L-aminobutyroyl-L-β-aminobutyric acid methyl ester (110) via the "oxazinone route". Methyl L-β-aminobutyrate (0.7g, 6.0 mmoles) in diethyl ether (10 ml) was added to a solution of the oxazinone derivative (108) (1 g, 5.3 mmoles) in diethyl ether (10 ml) at room temperature. There was a noticeable and immediate exotherm, crystalline material separated out of solution and was filtered off to yield a fully-protected dipeptide (110) (1.5 g, 94%), m.p. 171-174° (raised 172-174° on recrystallization from ethyl acetate), $[\alpha]_D^{20}$ +6.65° (c 1 in methanol). The mother liquors were evaporated down to dryness and the residue was triturated with petrol to yield more product (88mg, 5.9%), m.p. 131-133°, $[\alpha]_D^{20}$ 3.0° (c 0.5 in methanol). Recrystallization

of the second crop of material resulted in material identical to the first crop being obtained (45 mg, 3.0%), m.p. 172-174°, $[\alpha]_D^{20}$ +6.65° (c 1 in methanol). The overall yield of product (110) was 97%. (Found: C, 62.6; H, 7.0; N, 9.3. $C_{16}H_{22}N_2O_4$ requires C, 62.7; H, 7.2; N, 9.1%).

Isolation of β-benzoyl-L-aminobutyroyl-L-β-aminobutyric acid (111). The dipeptide ester (110) (3.5 g, 11.4 mmoles) from the foregoing reaction was taken up in aqueous methanol (65 ml, 25%) and saponified with sodium hydroxide (6.3 ml, 2N) in the usual way. N-protected dipeptide acid (111) was isolated in a yield of 2.6 g, 78.8%, m.p. 192-194° (raised to 193-195° on recrystallization from methanol-ether), $[\alpha]_D^{20}$ -7.1° (c 1 in dimethylformamide), R_{fG} 0.35. (Found: C, 61.5; H, 6.7; N, 9.8. $C_{15}H_{20}N_2O_4$ requires C, 61.63; H, 6.90; N, 9.58%).

Preparation of β-benzoyl-L-aminobutyroyl-2-oxazinone (112). Isobutyl-chloroformate (0.614 g, 0.6 ml, 4.5 mmoles) in acetonitrile (10 ml) was added in two portions to a stirred and cooled (-15 to -10°) solution of N-benzoyl dipeptide acid (111) (1.2 g, 4.10 mmoles) and triethylamine (0.45 g, 0.6 ml, 4.5 mmoles) in acetonitrile (45 ml). The reaction was stirred for 15 min at room temperature and monitored for the disappearance of the acid by t.l.c. Acetonitrile was evaporated and replaced by ether so that the hydrochloride salt precipitated and was removed by filtration. The filtrate was examined by i.r., ν max 1785 cm^{-1} (C=O), and thereafter evaporated down to give an oil, which soon crystallized in vacuo over phosphorus pentoxide to yield the desired peptide oxazinone (112) (0.92 g, 82.1%), m.p. 179-181° (raised to 183-185° on

recrystallization from acetonitrile), $[\alpha]_D^{20}$ +7.6° (c 1 in acetone), MS (Fig. 32) gave M^+ 274. (Found: C, 65.90; H, 6.76; N, 10.05. $C_{15}H_{18}N_2O_3$ requires C, 65.68; H, 6.61; N, 10.21%).

Preparation of β-benzoyl-L-aminobutyroyl-L-β-aminobutyroyl-L-β-aminobutyric acid methyl ester (113) via the peptide oxazinone (112). A solution of methyl L-β-aminobutyrate (0.28 g, 2.4 mmoles) in acetonitrile (5 ml) was added to a solution of the peptide oxazinone (112) (0.6 g, 2.18 mmoles) in acetonitrile (20 ml) at room temperature. The reaction was heated under reflux for 20 min, allowed to cool to room temperature, and then examined by t.l.c. (R_{fA} 0.01, 0.49). Solvent was evaporated and the solid residue triturated with ether and filtered off to yield the fully-protected tripeptide (113) (0.62 g, 72.0%), m.p. 233-235° (raised to 237-239° on recrystallization from methanol), $[\alpha]_D^{20}$ +14.0° (0.5 in dimethylformamide). (Found: C, 61.26; H, 7.49; N, 10.69. $C_{20}H_{29}N_3O_5$ requires C, 61.36; H, 7.47; N, 10.73%).

Isolation of β-benzoyl-L-aminobutyroyl-L-β-aminobutyroyl-L-β-aminobutyric acid (114). Sodium hydroxide (1.12 ml, 2N) was added during 5 min to a stirred and cooled (5°) solution of fully-protected tripeptide (113) (0.8 g, 2.04 mmoles) in aqueous pyridine (40 ml, 20%). The solution was stirred for 10h at room temperature after which solvent was evaporated and the solid residue taken up in water and extracted with ethyl acetate. Evaporation of the organic layer to dryness yielded some unhydrolysed ester (113) (45 mg, 5.6%). The aqueous phase was treated in the usual way resulting in the isolation of N-protected tripeptide acid (114) (0.58 g, 75.3%), m.p. 339° (m.p. unchanged on

recrystallization from methanol), $[\alpha]_D^{20}$ -5.4 (c 0.4 in dimethylformamide). (Found: C, 60.2; H, 7.2; N, 10.8. $C_{19}H_{27}N_3O_5$ requires C, 60.46; H, 7.21; N, 11.13%).

Isolation of L-β-aminobutyroyl-L-β-aminobutyroyl-L-β-aminobutyric acid (103) via debenzoylation[25, 150] of (114). A solution of the foregoing benzoyl derivative (114) (0.180 g, 0.47 mmoles) and tetramethylammonium bromide (536 mg, 3.5 mmoles) in methanol (25 ml) was electrolysed between a mercury cathode and a shielded platinum anode. Electrolysis was carried for 45-60 min at constant current (0.08A) and variable potential using a Potentiostat Type TR 40/3A. The end-point for the reaction was detected by t.l.c. and by liberation of hydrogen at the cathode. The reaction solution was then evaporated to dryness, the residue taken up in water, and the insoluble material, which was shown to be the undebenzoylated tripeptide (114) (approx. 30 mg, 17%), separated by filtration. The aqueous solution was desalted by electrodialysis, using cation and anion-exchange membranes on the Shandon Electrodialyser and Desalter after Wood[151]. The process was monitored by a drop of the current to a steady-state value (0.6 - 0.08A). The experiment was terminated after 2h. Evaporation of the solution to dryness yielded a clean product of the free linear tripeptide (103) (54 mg, 41.4%), m.p. 263-265° (free tripeptide (103) isolated earlier by debenzyloxycarbonylation gave m.p. 268-270°)(M.S. gave m/e 274 (M^+)).

CATALYTIC HYDROGENATION OF(+)-2-PHENYL-1,3-OXAZIN-6-ONE (108)

Isolation of N-benzoyl-L-β-aminobutyraldehyde (115). A solution of the oxazinone (108) (1.6 g, 8.47 mmoles) in dry dichloromethane (40 ml) was

purged with nitrogen and 10% palladium on charcoal (15 mg) added. Hydrogen gas was passed through the mixture until the oxazinone spot was shown to have disappeared by t.l.c. (7h). The crude product (R_{fA} 0.14, 0.78; R_{fB} 0.0, 0.48) was partitioned between 5% sodium bicarbonate solution and ethyl acetate, the organic layer dried and evaporated to yield a chromatographically pure (R_{fA} 78; R_{fB} 0.48) oil which crystallized from petrol to give the aldehyde (115) (1.1 g, 67.9%), m.p. 46-47°, $[\alpha]_D^{19}$ 2.4° (c 1 in dichloromethane). The product (115) gave a positive Schiff's test. ν max (film) 3300, 2950, 1715, 1625, 1520, 1280, 700cm^{-1}.

'H N.m.r. (DMSO)

τ (ppm)	multiplicity	protons	assignment
0.22	singlet	1	$-CH_2-CH=O$
1.8 - 2.8	multiplet	6	Ph-CONH
5.2 - 5.8	multiplet	1	$-NH-\underset{CH_3}{CH}-CH_2$
7.2 - 7.5	doublet	2	$>CH-CH_2-CHO$
8.6 - 8.9	doublet	3	$>CH-CH_3$

(Found: C, 68.9; H, 6.8; N, 7.0. $C_{11}H_{13}N\,O_2$ requires C, 69.09; H, 6.85; N, 7.32%).

Preparation of N-benzoyl-L-β-aminobutyraldehyde 2, 4-dinitrophenyl-hydrazone (116). The foregoing aldehyde (115) (0.5 g, 2.6 mmoles) was dissolved in the minimum of ethanol and then added to a warmed reagent solution of 2, 4-dinitrophenyl hydrazine (0.5 g, 2.6 mmoles) dissolved in ethanol (15 ml) and conc. hydrochloric acid. On cooling an orange crystalline product separated from solution and was filtered off. The product was recrystallized from ethanol-ether to afford the dinitrophenyl

hydrazone derivative (116) (0.3g, 32%), m.p. 175-177°, R_{fB} 0.36, R_{fA} 0.73. (Found: C, 54.6; H, 4.6; N, 18.5. $C_{17}H_{17}N_5O_5$ requires C, 54.98; H, 4.61; N, 18.86%).

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POSTGRADUATE STUDIES

In addition to relevant meetings and seminars, the following courses were attended:

"Peptide Synthesis", a lecture series given by Dr. C. N. C. Drey at Polytechnic of the South Bank, London.

"Interpretation of i.r. spectra", a course of lectures given by Dr. G. Youngson at Robert Gordon's Institute of Technology, Aberdeen.

"Nuclear Magnetic Resonance Spectroscopy", lectures by Dr. M. Fraser, Robert Gordon's Institute of Technology, Aberdeen.

POSTSCRIPT

Designation of structure (91) - discussed on p 39 and isolated as reported on p 82 in this thesis - has now been altered from 2-azetidinone (91) to 3-azetidinone (91*). The reassignment has been made on the basis of the sum total of recently obtained spectroscopic evidence.

In the i.r. spectrum, ν_{max}C=O is 1820cm^{-1}. In normal 2-azetidinones, ν_{max}C=O is in the ~1730-1760cm^{-1} region. In 3-azetidinones, however, ν_{max}C=O stretches fall within the ~1800-1820cm^{-1} region (see Experientia, 1970, 26, 1188; Chem. Pharm. Bull., 1973, 21, 288).

Information has been acquired from the 60 and 100 MHz ^{1}H n.m.r. spectra and from the ^{13}C n.m.r. spectrum. The chemical shifts for the CH_2 and CH protons (~δ4.6, 4.8) and carbons (δ69.7, 81.1) are too low for the 2-azetidinone (see the n.m.r. for 4-methyl-1-α-methyl benzyl azetidin-2-one, Tetrahedron, 1974, 30, 39). The ^{13}C chemical shift for the carbonyl carbon (δ197.0) is also too low for the azetidinone structure, literature values place it within the 166.4-175.3 p.p.m. range (see J.C.S. Perkin II, 1977, 1749).

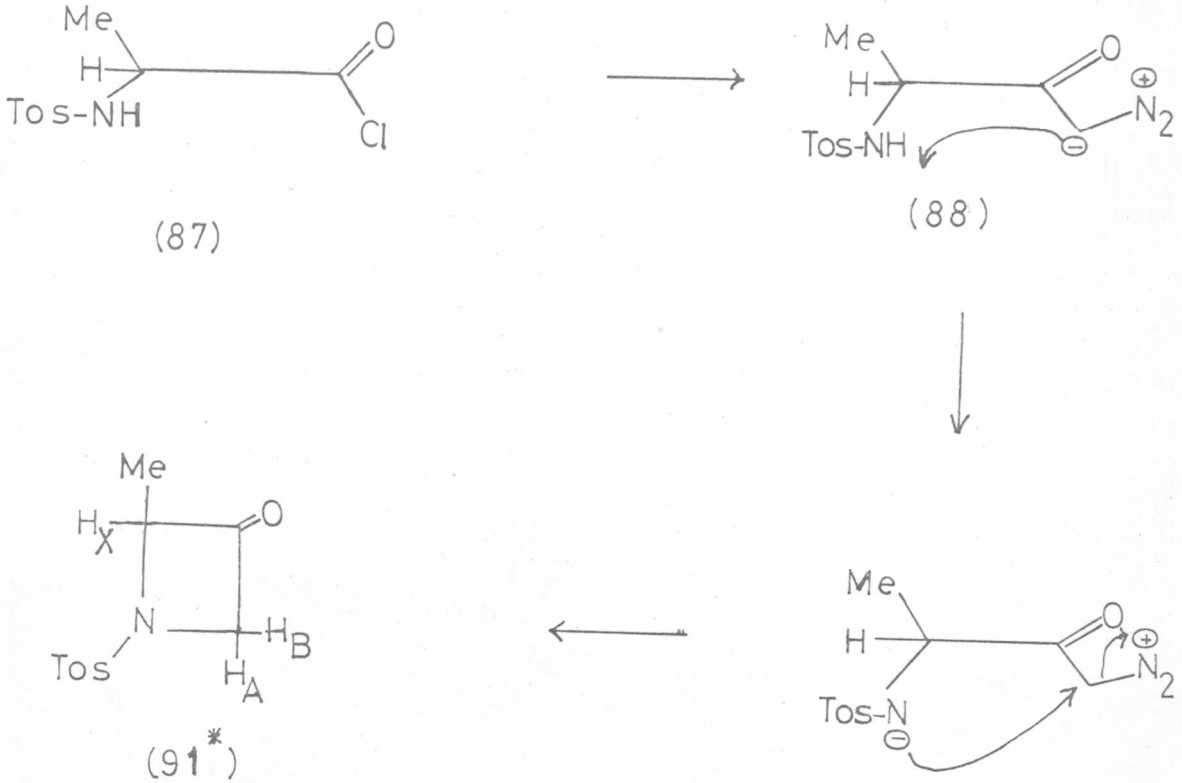
The coupling constants between the CH_2 and CH protons (J_{HH} ~ 1.2, 2.5) are too small for the azetidinone structure (see Tetrahedron, 1968, 24, 1275) where the expected values are $^3J_{cis}$ ~ 6Hz, and $^3J_{trans}$ ~ 2.5Hz.

The 100MHz ^{1}H n.m.r. spectrum resolves to what is evidently an AB part of an ABX coupling pattern, arising from long range transannular coupling between CH_2 protons (A and B) and the CH proton (X) (see 91*), which is in its turn coupled to the methyl protons.

From a chemical behavioural point of view, the inability of the compound (91*) to undergo hydrolysis accords with the structure not being the 2-azetidinone.

Fig 28* illustrates the proposed mechanistic route of 3-azetidinone (91*) formation.

Fig. 28*


Me
H
Tos-NH
O
Cl
(87)
Me
H
Tos-NH
O
⊕
N2
⊖
(88)
Me
H
Tos-N
⊖
O
⊕
N2
Me
H
X
O
N
Tos
HB
HA
(91*)