Novel Side Chain Protecting Groups For Solid Phase Peptide Synthesis.

Peter A. Longstaff.

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To My Parents
And Linda.

This thesis is submitted in part fulfillment of the requirements of the degree of Doctor of Philosophy in the University of Edinburgh. Unless otherwise stated the work described is original and has not been previously submitted in whole or in part at this or any other university.

University of Edinburgh.
September 1989.

Abstract.

This thesis broadly describes the principle of solid phase peptide synthesis and the most significant protecting groups that have been developed for use with this method. The work describes some of the inherent limitations and problems associated with these groups and defines the criteria for the design of a carboxyl protecting group that would not have these deficiencies.

These criteria are applied to the design of a novel side chain carboxyl protecting group and three new protecting groups are synthesised and evaluated in model stability studies.The 1-(4'-fluoro)-phenyl-2-methylprop-2-yl group was used to protect the side chain carboxyl function of aspartic acid. Following successful amine protection and stability studies the protecting group was used to prepare the penta-peptide H-IleLeuAspAsnIle-OH by the solid phase method.

An extension of the use of this protecting group to the side chain functions of lysine and glutamic acid was also investigated.

Contents

CHAPTER:1 INTRODUCTION. Page.

The Solid Phase Principle. 1.

The Support. 3.

Protection and Deprotection Strategies. 4.

1. Graded Acidolysis and the Tert. Butyloxycarbonyl Group.
2. Orthogonal Protection and the 9-Fluorenylmethoxycarbonyl (Fmoc) Group.

Side Chain Carboxyl Protection. 11.

1. The Need For Side Chain Carboxyl Protection.
2. Side Reactions that Occur with Protected Carboxyl Functions.

Acid Labile Aspartyl and Glutamyl Side Chain Protecting Groups. 17.

1. Tert. Butyl Esters.
2. Alkylation on Deprotection of Acid Labile Side Chain Protecting Groups and Recent Developments with this Type of Group.

Criteria for the Design of a Novel Side Chain Protecting Group for Aspartic and Glutamic Acid. 22.

1. Steric Hindrance.
2. Orthogonal Protection.
3. Carbocation Inactivation.
4. Protection and Deprotection Monitoring Label.
5. Economics.

CHAPTER:2 DISCUSSION.

The 3-Ethyl-pent-3-yl Group. 29.

The 1-(3'-Trifluoromethyl phenyl)-2-methyl prop-2-yl Group. 38.

The 1-(4'-Fluoro phenyl)-2-methyl propyl Group. 43.

The Side Chain Protection of Aspartic Acid. 47.

The Synthesis of a Peptide. 56.

The Side Chain Protection of Glutamic Acid. 58.

The Side Chain Protection of Lysine. 61.

Conclusion. 68.

CHAPTER:3 EXPERIMENTAL.

Notes. 72.

3-Ethyl pentan-3-ol (35). 74.

3-Ethyl pent-2-ene (34). 74.

Hydrocinnamic Acid t-Butyl Ester (40). 75.

Hydrocinnamic Acid 3-Ethyl-pent-3-yl Ester (38). 75.

3-(Trifluoromethyl phenyl)acetic Acid Methyl Ester (83). 76.

4-Fluorophenylacetic Acid Methyl Ester (85). 76.

1-(3'-Trifluoromethyl phenyl)-2-methyl propan-2-ol (45). 78.

CHAPTER:3 EXPERIMENTAL (CONT'D). Page.

1-(4'-Fluoro phenyl)-2-methyl propan-2-ol (52). 78.

3-(3'-Trifluoromethyl)phenyl-2-methyl pentan-3-ol (46). 81.

Hydrocinnamic acid 1-(3'-trifluoromethyl phenyl)-2-methyl-prop-2-yl ester (47). 82.

Hydrocinnamic acid 1-(4'-fluoro phenyl)-2-methyl prop-2-yl ester (53). 82.

Benzyl(N-benzyloxycarbonyl)-β-(1-(4'-fluoro phenyl)-2-methyl-prop-2-yl]-L-aspartate (57). 84.

L-Aspartic acid β-[1-(4'-fluoro phenyl)-2-methyl-prop-2-yl] ester (58). 86.

N-[9-Fluorenylmethyloxycarbonyl]-β-[1-(4'-fluoro phenyl)-2-methyl prop-2-yl]-L-aspartic acid (61). 87.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-β-[1-(4'-fluoro)-phenyl-2-methyl prop-2-yl]-L-aspartic acid cyclohexyl-ammonium salt (62). 89.

N-t-Butoxycarbonyl-β-[1-(4'fluoro phenyl)-2-methyl-prop-2-yl]-L-aspartic acid cyclohexylammonium salt (64). 90.

N-[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-β-[1-(4'-fluoro) phenyl-2-methyl prop-2-yl]-L-aspartyl glycine methyl ester (65). 92.

Deprotection of N-[2,2-bis(4-nitrophenyl)ethoxycarbonyl -[1-(4'-fluoro phenyl)-2-methyl prop-2-yl]-L-aspartyl glycine methyl ester (65) using 1,5-diazobicyclo[4,3,0,] non-5-ene (25). 93.

Sidechain deprotection of N-[2,2-bis(4-nitrophenyl)-ethoxycarbonyl]-β-[1-(4'-fluoro phenyl)-2-methyl prop-2-yl] -L-aspartylglycine methyl ester (65) using trifluoroacetic acid. 95.

The solid phase synthesis of H-IleLeuAspAsnIle-OH (68). 96.

N-Benzyloxycarbonyl-α-benzyl-γ-[1-(4'-fluoro)phenyl-2-methyl prop-2-yl]-L-glutamate (70). 96.

L-Glutamic acid γ-[1-(4'-fluoro phenyl)-2-methyl-prop-2-yl ester (71). 98.

Phenyl-1-(4'-fluoro phenyl)-2-methyl prop-2-yl carbonate (80). 98.

4-Nitrophenyl-1-(4'-fluoro phenyl)-2-methyl prop-2-yl carbonate (81). 98.

N^{ϵ}-[1-(4'-Fluoro phenyl)-2-methyl prop-2-yloxycarbonyl] -L-lysine (77). 100.

H.p.l.c. experiments to determine the relative stability of a series of esters of hydrocinnamic acid to base hydrolysis. 102.

H.p.l.c. experiments to determine the relative stability of a series of esters of hydrocinnamic acid to acid hydrolysis. 105.

REFERENCES. 107.

COURSES ATTENDED. 111.

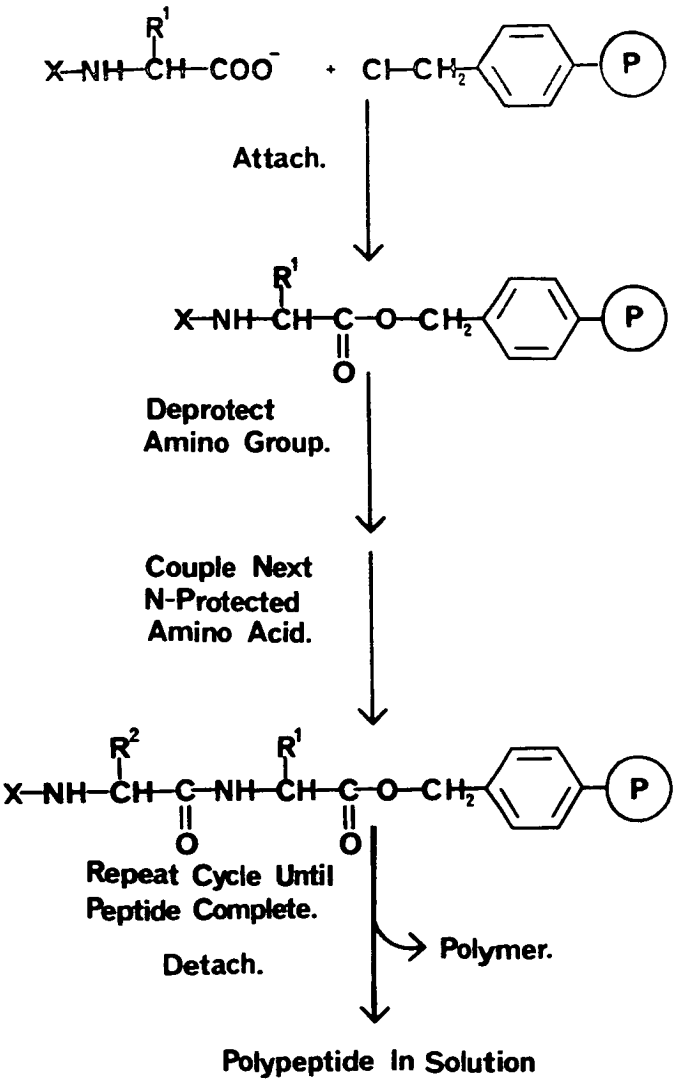
CHAPTER 1: INTRODUCTION

The Solid Phase Principle

The solution phase synthesis of large polypeptides is a very difficult and often impossible task. This is due to the steps of coupling, deprotection, isolation and purification of the intermediates not only being very time consuming and repetitive but also due to the problems of solubility of these intermediates in suitable solvents. The need for a new method of peptide synthesis that avoided these inherent problems was recognised by R.B. Merrifield who conceived and developed the technique of solid phase peptide synthesis[1,2]. For this work he was awarded the Nobel Prize in 1984[3]. The principle of solid phase peptide synthesis is the use of an insoluble polymeric support from which the peptide chain can be sequentially constructed. The general method is shown in Scheme I.

The main advantages of such a technique over solution phase methods are outlined below.

1. The multistep synthesis is greatly accelerated and simplified as it is possible to carry out all reactions in a single vessel thereby avoiding mechanical losses encountered during isolation and purification of intermediates.


Attach.
Deprotect
Amino Group.
Couple Next
N-Protected
Amino Acid.
Repeat Cycle Until
Peptide Complete.
Detach.
Polymer.
Polypeptide In Solution

Scheme I

2. High yields of final products can be obtained through the use of excess reagents to force the reactions to completion. The excess reagents can then be removed simply by filtration and washing.

3. The procedure can be automated.

Some of the main features of solid phase peptide synthesis are now discussed.

The Support

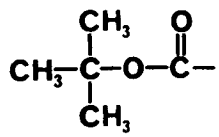
The design of the resin to which the growing peptide is attached is of prime importance if the technique of solid phase synthesis is to be successful. The support must not only be insoluble and rigid but must be capable of functionalisation to an acceptably high degree. These characteristics are found in cross-linked polymers which can be prepared in the form of beads which are insoluble, easily filtered and have the required rigidity to make mechanical manipulation possible. The polymer should, however, only be crosslinked to a small degree (0.5-2%) so that on contact with the reaction solvents swelling occurs thereby allowing access for the reagents to the interior of the beads where the majority of the functional groups occur. Access to these functional groups can be further increased by attaching them to the polymer via a spacer (handle) molecule (see later).

Swelling, in addition, also serves to accommodate the increasing mass of the growing peptide chain.

Protection and Deprotection Strategies

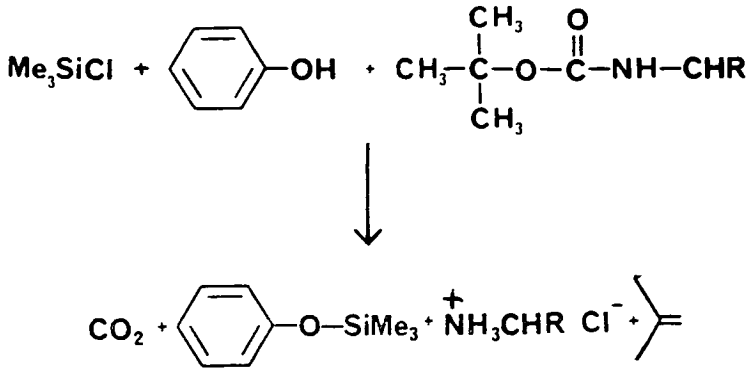
1. Graded Acidolysis and the Tert. Butyloxycarbonyl Group

Until recently the most extensively used protecting group for the α-amino function in solid phase peptide synthesis was the tert.butyloxycarbonyl (Boc) group[4] (1).



1.

The popularity of this protecting group arose because of its stability in basic conditions combined with desired lability in acidic media. The relative cheapness and ease of introduction of this group were also attractive features. The cleavage is usually carried out using 50% trifluoroacetic acid in methylene chloride or chlorotrimethylsilane-phenol recently developed by Merrifield et al.[5] (Scheme II).



Scheme II

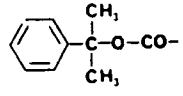
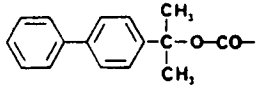
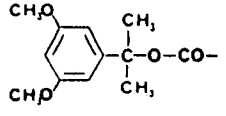
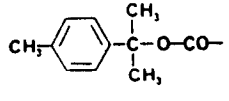
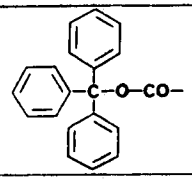
These conditions have negligible effect on the benzyl ester type of protection that is normally used for side chain protection when using Boc protection. This allows repeated deprotection of the α-amino function while retaining the semi-permanent protection of the side chains. Acidolytic deprotections can, however, cause problems when the acid lability of the Boc group and the peptide-resin link are not sufficiently different, leading to premature cleavage of the peptide from the resin. Another problem encountered with the Boc group (and most other acid labile protecting groups) is the modification of amino acid side chains in the peptide by carbocations generated during deprotection. Electrophilic aromatic substitution of the aromatic systems of tyrosine[6] and tryptophan[7,8] and the alkylation of the sulphur containing side chains of methionine[9] and cysteine can readily occur under these conditions, the addition of carbonium ion scavengers having little effect. Indeed these scavengers, for example anisole, have also been shown to be sources of alkyl cations which can lead to alkylation in the presence of strong acids[10].

Although the Boc group was used extensively it was recognised that the utilisation of more acid labile α-amino protection would be advantageous by allowing the use of more labile side chain protection and peptide-resin links thereby resulting in the peptide being subjected to milder acidolytic conditions.

One of the α-amino protecting groups introduced to satisfy this requirement was the 2-(4-biphenyl)-isopropyl-(2)-oxycarbonyl (Bpoc) group (Table 1 (2)), developed by Sieber and Iselin[11] which enabled Boc protection to be used for side chains due to the Bpoc group being 3000 times (see Table 1) more labile in acid conditions, specifically very dilute (2%) solutions of trifluoroacetic acid (TFA) in methylene chloride. The main disadvantages of the Bpoc group are carbonium ion induced side reactions and the unfortunate instability of the free Bpoc protected amino acids which can be overcome by storing them as the dicyclohexylammonium salts and liberating before use.

Some other protecting groups developed in a similar way were the 2-phenylpropyl-(2)-oxycarbonyl (Poc) group[12,13] (Table 1 (3)), the 2-(3,5-dimethoxy-phenyl)propyl-(2)-oxycarbonyl (Ddz) group[14] (Table 1 (4)) and the 2-(4-methylphenyl)propyl-(2)-oxycarbonyl (Mpc) group (Table 1 (5)). The acid labilities of these groups and the Bpoc group have been established relative to the Boc group and these results are reproduced in Table 1[11].

TABLE 1: 2 Aryl-isopropyloxycarbonyl Amino Protecting Groups

Protecting Group	Name	Abbrev.	*Relative Cleavage Rate
	2-Phenylpropyl-(2)-oxycarbonyl	Poc	700
	2-(4-Biphenyl)propyl-(2)-oxycarbonyl	Bpoc	3000
	2-(3,5-Dimethoxyphenyl)propyl-(2)-oxycarbonyl	Ddz	1400
	2-(4-Methylphenyl)propyl-(2)-oxycarbonyl	Mpc	11000
	Triphenylmethyl	Trt	21000

* Relative Cleavage Rate In 80% Acetic Acid At 22-25°C (Boc = 1)

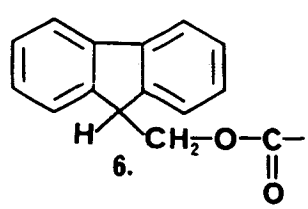
An illustration of the use of these very acid labile protecting groups is the synthesis of insulin[15].

The use of these groups has not, however, found general acceptance due to the expense involved in producing the protected amino acids. More importantly any protection scheme relying on differential kinetic stability of protecting groups cannot be totally efficient due to the formation of impurities caused by the cumulative effects of small amounts of cleavage of protected side chains per cycle in the solid phase synthesis.

2. Orthogonal Protection and the 9-Fluorenylmethoxy-carbonyl (Fmoc) Group

Orthogonal protection[16], the use of two or more protecting groups that can be removed independently, in any order, using different reactions and reagents, is used to avoid the problems associated with the graded acidolysis approach.

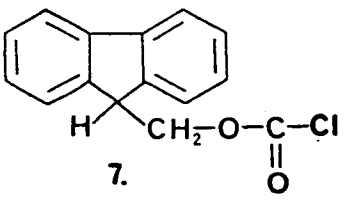
It was the introduction of β-eliminating protecting groups, particularly the 9-fluorenylmethoxy-carbonyl (Fmoc) group (6), that made this approach possible.



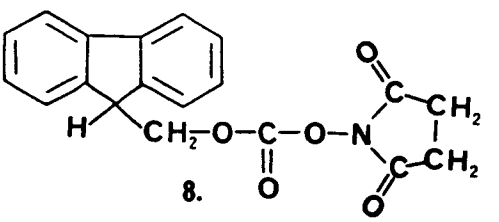
6.

This group was devised by Carpino and Han[17] in 1970, but found little use until Sheppard in 1981, after a study of available β-eliminating protecting groups[18] utilised the Fmoc group to synthesise little gastrin[19], a peptide that had proved inaccessible by conventional (Boc, benzyl ester) solid phase strategies.

Fmoc protected amino acids are easily prepared from 9-fluorenylmethylchloroformate[20] (7)or N-(9-fluorenylmethoxycarbonyloxy)succinimide[21,22] (8), in aqueous sodium carbonate solution to give solids that are stable at room temperature.

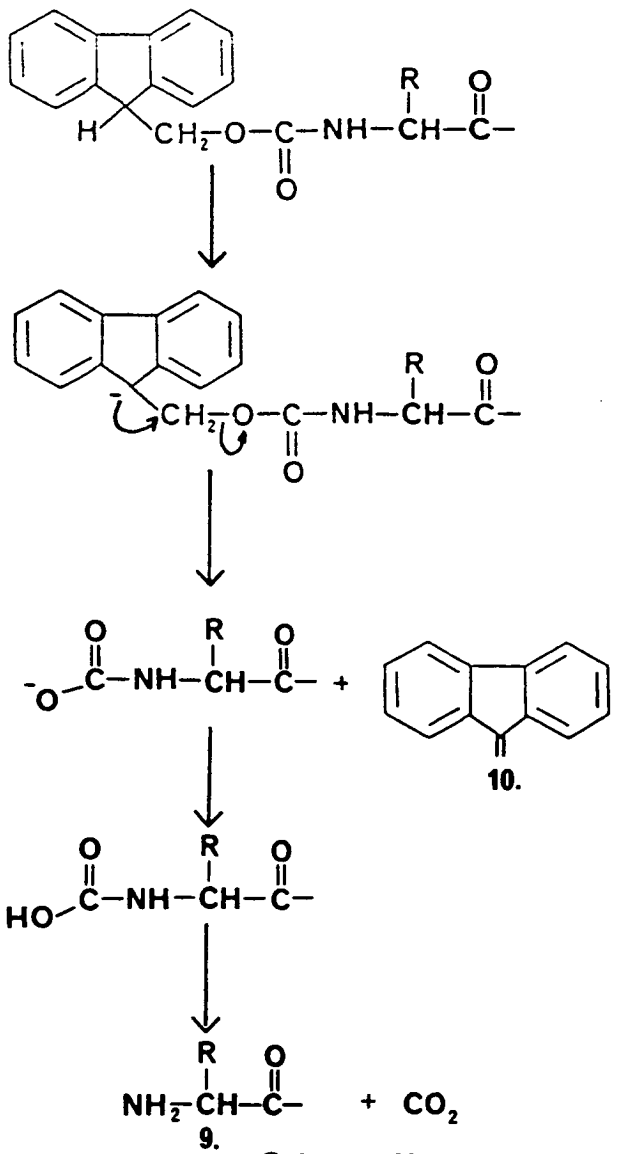


7.



8.

Cleavage of the Fmoc group is usually carried out using 20% piperidine in dimethylformamide solution to yield the free amino acid (9) and the olefin (10) (Scheme III)



10.

9.

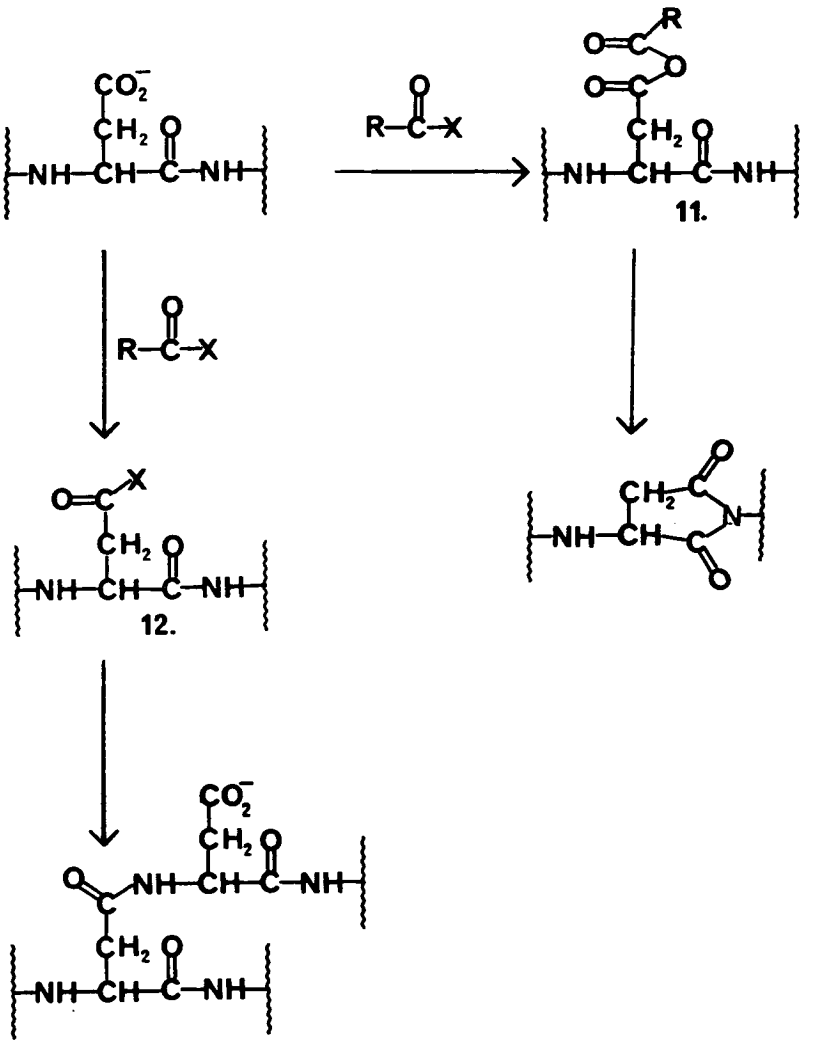
Scheme III

These basic nucleophilic conditions can, however, produce premature cleavage of peptides from Merrifield type resins. This has been overcome by the introduction of acid labile/base stable p-alkoxybenzyl alcohol resins by Wang[23] and the use of polyamide resins by Sheppard[24]. The latter used tert.butyl ester type side chain protection to synthesise the biologically active peptides (15 leucine) little gastrin[19], Substance P[18] and acyl carrier protein (65-74)[18].

Side Chain Carboxyl Protection

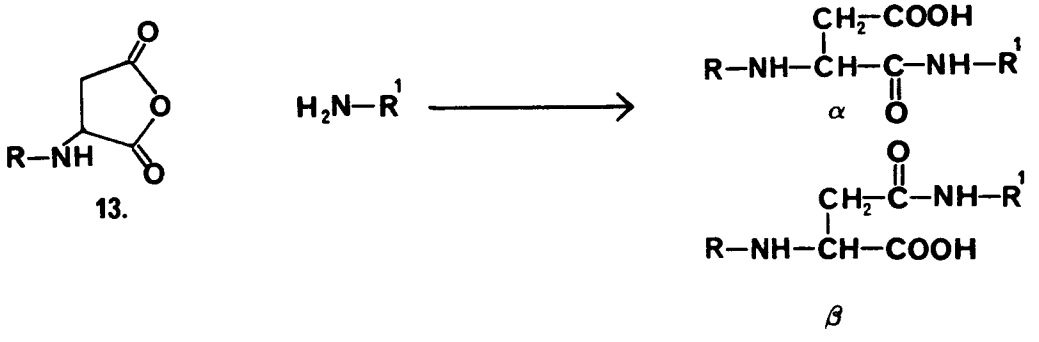
1. The Need for Side Chain Carboxyl Protection

Protection of the carboxyl side chains of aspartic and glutamic acid in peptide synthesis is considered obligatory as it firstly prevents any complications arising due to carboxylate counter ions, for example, trialkylammonium ions, that would be present with free side chains. Protection of these functions also prevents competition between the free carboxylate and the free -amino group for nucleophilic attack on the active esters used in solid phase peptide synthesis. If this competition occurs mixed anhydrides (11) and active esters (12) are formed. These then undergo side reactions that result in chain cyclisation[25] and branching[26] (Scheme IV).

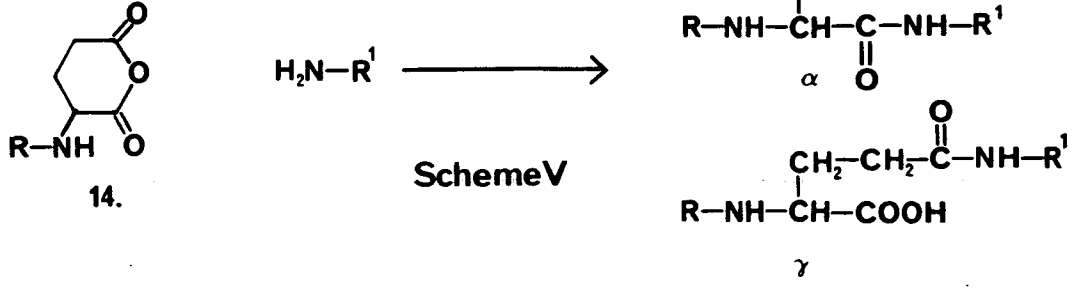


Scheme IV

Protection of carboxyl side chains prevents these reactions occurring and thus prevents the formation of the cyclic anhydrides (13) and (14) of aspartic and glutamic acid during activation of N-protected amino acids. These cyclic anhydrides are of no use in peptide synthesis because, on ring opening, α and β (or γ) peptides are formed (Scheme V).



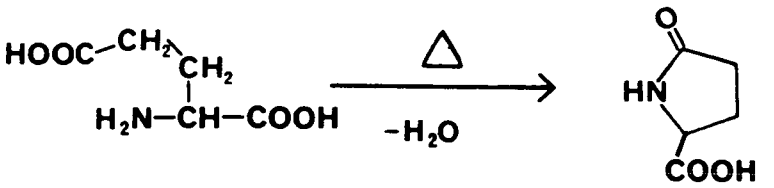
13.



14.

Scheme V

Cyclisation to pyroglutamic acid (15) (5-pyrrolidone-2-carboxylic acid) readily occurs with glutamic acid derivatives bearing unprotected γ-carboxyl functions. This cyclisation can take place on heating or on activation of the γ-carboxyl and can occur even with N-acyl derivatives.

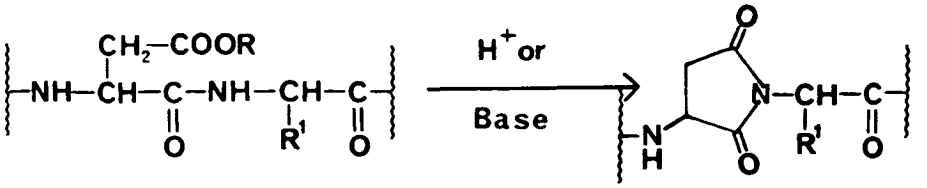


15.

King opening of (15) yields only glutamic acid and, therefore, does not result in side products during peptide synthesis. In the case, however, of free peptides with glutamic acid at the N-terminus cyclisation in acidic[32], basic[33] or neutral[34] conditions can occur thereby affecting the stability of the free peptide.

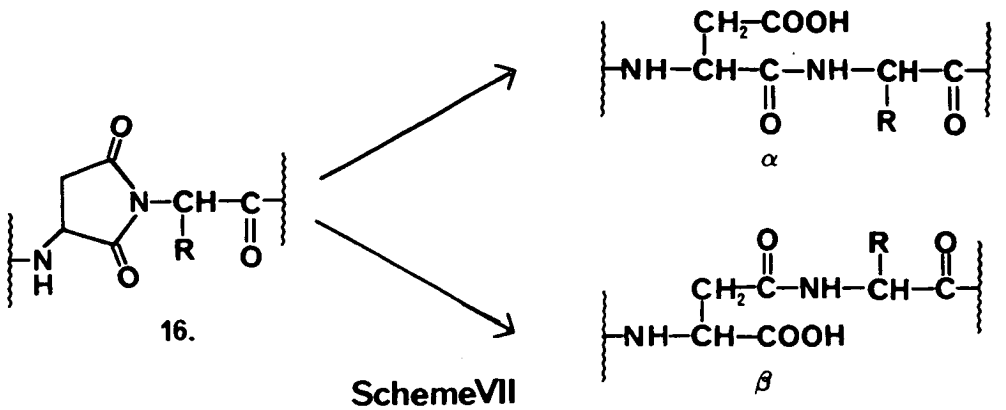
2. Side Reactions that Occur with Protected Carboxyl Functions

The side reactions that occur with side chain aspartyl and glutamyl residues are well documented[27]. The principle side reaction of the aspartyl residue is the formation of aspartimides (16) in acidic or basic media (Scheme VI). This was first observed by Battersby and Robinson in 1955[28].



SchemeVI 16.

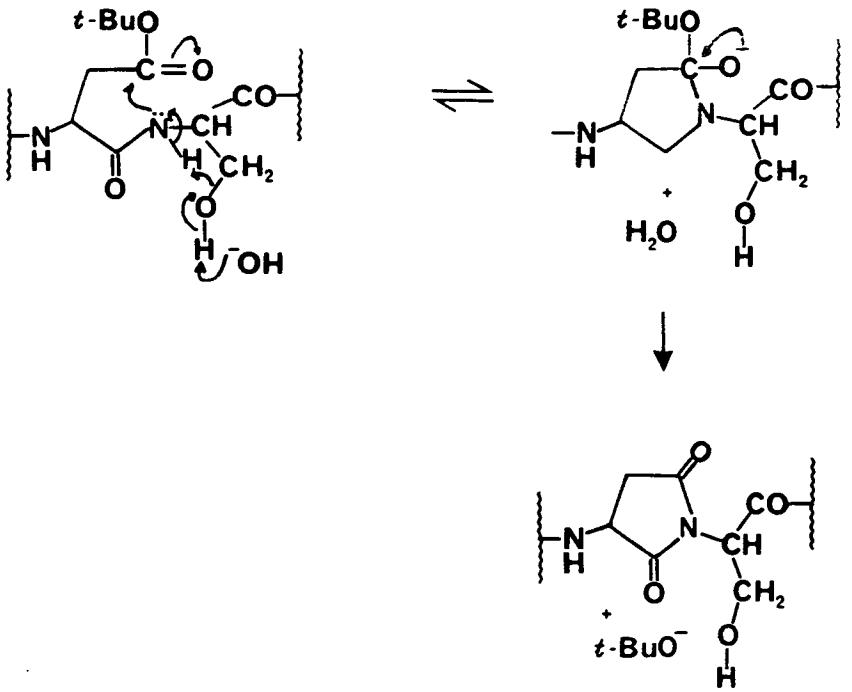
Opening of the aspartimide ring (16) can occur in both directions to give α and β aspartyl derivatives (Scheme VII).



SchemeVII

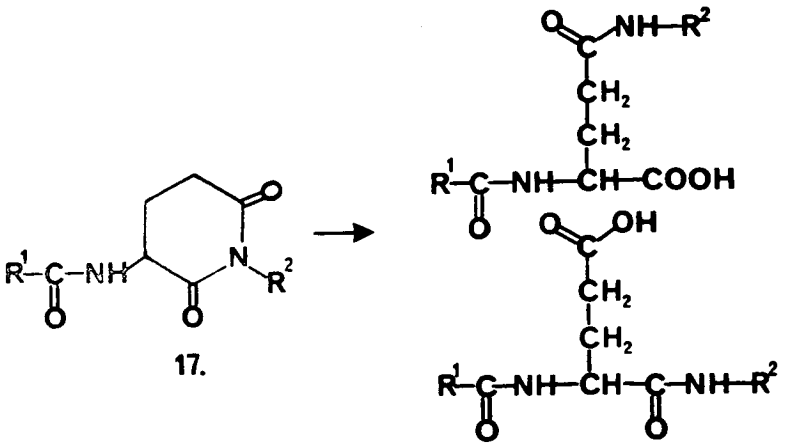
This rearrangement occurs to varying degrees with all carboxyl protecting groups, even t-butyl esters, which are normally considered to be resistant to base catalysed hydrolysis but do not possess similar resistance to intramolecular nucleophilic attack. Tertiary butyl esters due to steric bulk and electron donation do, however, show a greater resistance to this side reaction than, for example, benzyl esters. Studies have also shown that the ease of aspartimide formation is also sequence dependent. The presence of aspartyl glycine, aspartyl serine and aspartyl histidine residues promotes this reaction due presumably to favourable steric influences in the case

of glycine, an intra-molecular catalysis effect with histidine[29] and with serine possibly an intramolecular catalysed reaction with the following mechanism[30]. (Scheme VIII).



SchemeVIII

Ring closure is, however, hindered in aspartyl valine and aspartyl leucine sequences and in the presence of a negative charge in the side chain of the phenolic hydroxyl of tyrosine. Aspartimide formation has also recently been reported[31] to occur during hydrogenolytic removal of β-benzyl protecting groups in neutral conditions. The analogous formation of the glutarimide (17) also occurs[35,36], but less readily than the corresponding aspartimide. Glutarimides are, however, the likely intermediates in transpeptidation reactions[37] (Scheme IX).



17.

Scheme IX

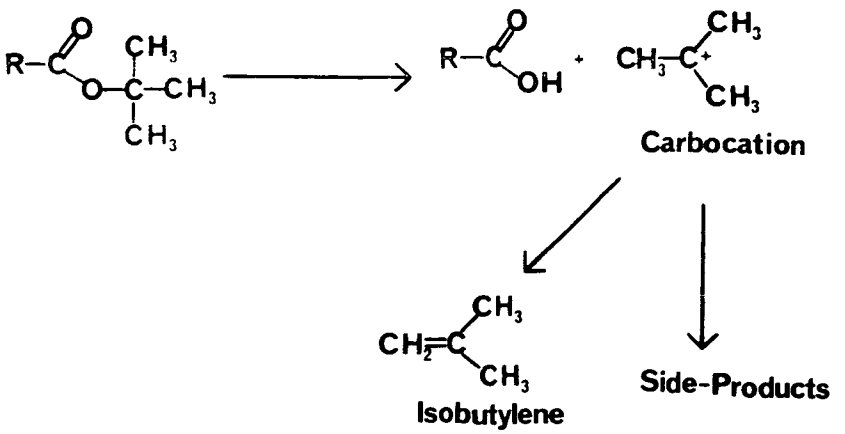
Acid Labile Aspartyl and Glutamyl Side Chain Protecting Groups

If a base labile N^{α}-amino protecting group, for example Fmoc (6), is to be used in solid phase peptide synthesis, then acid labile side chain protecting groups are required if an orthogonal protecting group approach is to be used. This approach avoids the use of harsh acidic or basic conditions, that could prove detrimental to the peptide.

1. Tert. Butyl Esters

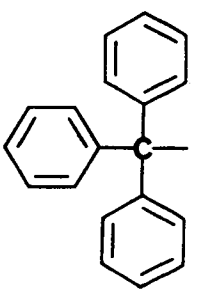
The most frequently used carboxyl protecting group for this approach is the t-butyl ester which is resistant to hydrogenolysis and, under normal conditions, ammonolysis and base catalysed hydrolysis. They are, however, susceptible to acid catalysed alkyl-oxygen fission where the t-butyl group is lost as isobutylene under mild acidic conditions, for

example, trifluoroacetic acid (TFA) at room temperature[38] or tosic acid in refluxing benzene[39] (Scheme X).



Scheme X

While t-butyl esters are acid labile they are sufficiently resistant to weak acids, for example, citric acid, to allow safe handling of intermediates protected by them. This stability is missing in compounds protected by, for example, the triphenylmethyl (trityl) group[40] (18) which is even cleaved by acetic acid at room temperature.



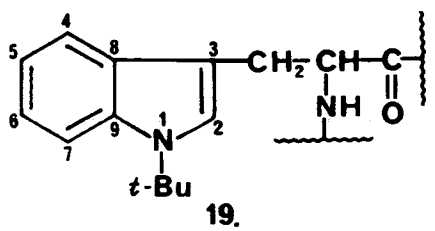
18.

There have been several attempts[41-44], at trying to cleave the Boc group (1) selectively in the presence of t-butyl esters. This, however, cannot be considered a reliable procedure, as with most graded acidolysis strategies.

2. Alkylation on Deprotection of Acid Labile Side Chain Protecting Groups and Recent Developments with this Type of Protecting Group

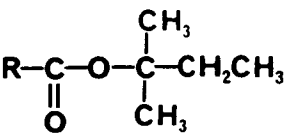
As mentioned earlier, a disadvantage associated with most acid labile protecting groups is the possible alkylation of amino acid side chains due to stable alkylcations generated during deprotection. The alkylation of tryptophan has, for example, been observed[8] with the principal product being the N^{In}-tert.butyl tryptophan derivative (19) but migration of the alkyl group from the nitrogen to positions 2, 5 and 7 can also occur.

The problems of alkylation and aspartimide formation have led to the introduction of new protecting groups which, it is hoped, will solve these problems.



19.

The 2,2-dimethylpropyl (tert-amyl) group (20) has, for example, been used to protect the acid functions of aspartic and glutamic acid in the preparation of protected fragments of lysozyme[45].

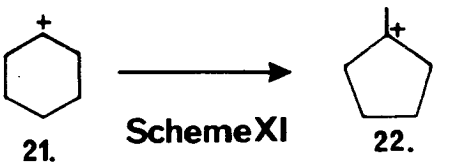


20.

The terminal tert-amyl esters are susceptible to cleavage by trypsin under special conditions, whereas the side chain esters can be cleaved by more general conditions of trifluoroacetic acid at 4°C for 40 minutes. This provides differentiation between the side chain and terminal carboxyl groups of a peptide but obviously has limited applications.

Another more promising approach has been the use of β-cyclopentyl[46], β-cyclohexyl[47], β-cycloheptyl, β-cyclooctyl[48] and β-menthyl[49] esters of aspartic acid to provide steric hindrance to intramolecular nucleophilic attack at the ester carbonyl by the amide nitrogen in acidic and basic media, thereby suppressing aspartimide formation. The problem of alkylation at amino acid side chains by carbocations on deprotection

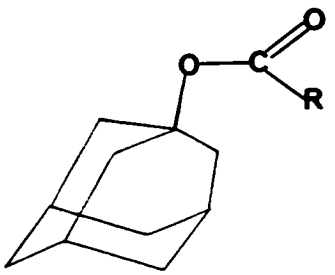
is also considered in the design of these protecting groups and is best illustrated by the cyclohexyl protecting group. This group, on deprotection, yields the cyclohexyl carbocation (21) that spontaneously rearranges, possibly within the solvent cage, to the more stable and hence less reactive methylcyclopentyl tertiary cation (22)[50] (Scheme XI).



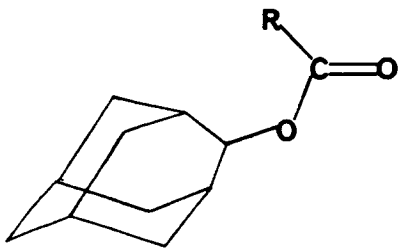
The cyclohexyl protecting group has been shown to give less aspartimide formation under basic conditions than the corresponding benzyl ester due to increased steric hindrance. The cleavage of the cyclohexyl group, however, requires the use of hydrogen fluoride, conditions which have proved harmful to peptide sequences. The cyclohexyl group while having the correct design features for a novel protecting group requires too harsh acidic conditions for removal.

A similar approach using β-1-adamantyl and β-2-adamantyl aspartates has recently been reported[51]. The 1-adamantyl group (23) is easily cleaved by trifluoroacetic acid whereas the 2-adamantyl group

(24) is stable to the above conditions but cleaved within 5 minutes by methanesulphonic acid (MSA)[51] at room temperature.



23.



24.

Both esters do not, however, show good resistance to base hydrolysis which is a prerequisite for an acid labile side chain protecting group. Studies, using model peptides, on aspartimide formation with these protecting groups do, however, show a significant improvement over benzyl ester protection but the instability of these protecting groups to basic conditions would, however, be the limiting factor in their use.

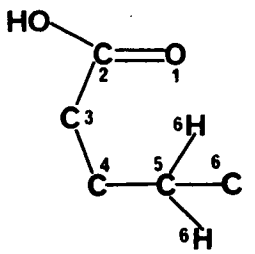
Criteria for the Design of a Novel Side Chain Protecting Group for Aspartic and Glutamic Acid

The problems associated with the aspartyl and glutamyl residues in peptide synthesis and the shortcomings of the currently used protecting groups mentioned previously indicate a need in peptide chemistry for an acid labile protecting group that can satisfy the following criteria.

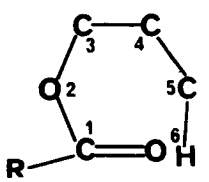
1. Steric Hindrance

The protecting group must provide considerable steric hindrance to inter and intramolecular attack of the carbonyl of the ester group to prevent base hydrolysis and aspartimide or glutarimide formation. When considering the design of a protecting group one has therefore to consider the work of Newman[53,54] who, after studying the rates of esterification of a series of carboxylic acids incorporating different degrees of steric hindrance, devised the following rule commonly called the "rule of six". This states:

> "In reactions involving addition to an unsaturated function containing a double bond, the greater the number of atoms in the six position the greater the steric effect".



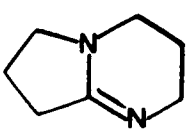
Although this rule was devised for sterically hindered acids we considered that it could also apply to the degree of hindrance in the six position of the alcoholic component of the ester which would be important in limiting nucleophilic attack at the carbonyl function of the ester. We choose the carbonyl carbon as position one.



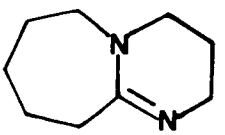
A potential protecting group should therefore provide this steric hindrance at the 6-position.

2. Orthogonal Deprotection Stability

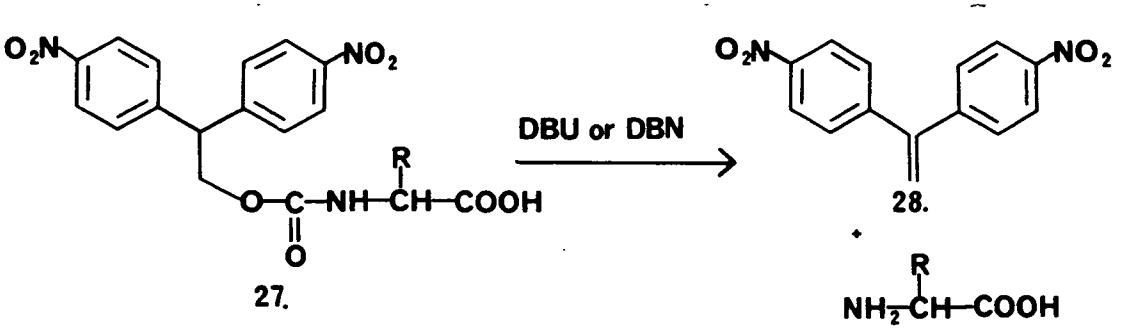
The protecting group must be completely stable to the other reagents used in an orthogonal protection scheme. It must, for example, be stable to secondary amines like piperidine in the concentrations used to cleave the Fmoc protecting group (6). Stability to diazobicyclo 4 3 0 non-5-ene (DBN) (25) and diazobicyclo 5.4.0 undec-7-ene (DBU) (26) is also essential if the N-(2,2-bis-(4-nitrophenyl)-ethoxycarbonyl (Bnpeoc) (27) group recently developed by Ramage et al.[55] is to be used (Scheme XII). The advantages of this group over Fmoc are currently under investigation.



DBN.
25.

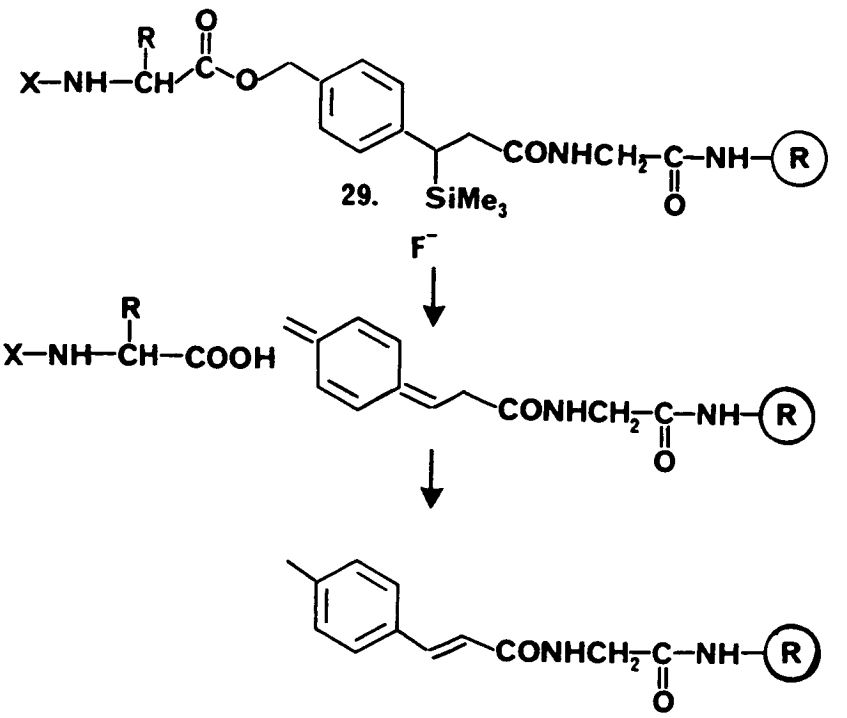


DBU.
26.



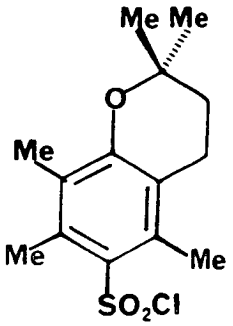
Scheme XII

The side chain protecting group must also be stable to the conditions used to cleave the fully protected peptide from the polymeric support. It is proposed to use the fluoride ion to cleave the linking agent (handle) (29) (Scheme XIII).

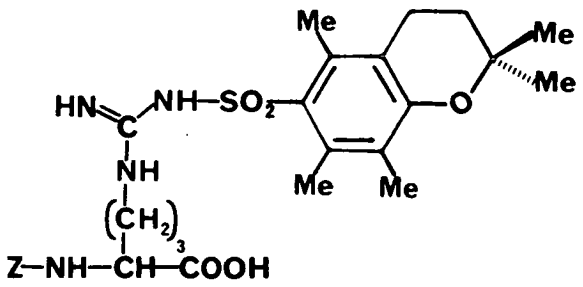


Scheme XIII

A novel side chain carboxyl protecting group must also be compatible with the protecting groups used for other side chain functions and must therefore have similar acid lability to these protecting groups. The recent introduction[55] of the 2,2,5,7,8-pentamethyl -chroman-6-sulphonyl chloride (Pmc) group (30) for protection of the side chain guanidino group of arginine (31) indicates that 50% trifluoroacetic acid/methylene chloride for a period of 1 hour is the desired condition for removal of the carboxyl protecting group.



30.



31.

3. Carbocation Inactivation

If the problems of alkylation of amino acid side chains on deprotection of acid labile protecting groups are to be avoided there must be some mechanism, considered during the design of the protecting group, whereby the carbocation produced can become deactivated before recapture by the reactive sites of the peptide. This approach was described earlier in the case of the cyclohexyl protecting group where the rearrangement of the secondary cyclohexyl carbocation (21) to the more stable and hence less reactive tertiary methylcyclopentyl cation (22) takes place precluding any alkylation of the peptide. (Scheme XI).

4. Protection and Deprotection Monitoring Label

The incorporation of a physical means by which the protection and deprotection of a protecting group can be followed should be considered important when designing a novel protecting group. Monitoring, by ultraviolet absorption, the production of the corresponding piperidine adduct of the alkene (10) or (28) produced during Fmoc and Bnpeoc deprotection is now carried out routinely in solid phase peptide synthesis and gives a very good indication of cycle efficiency. An n.m.r. label could also provide a means of following protections and deprotections and would be desirable for side chain functions.

5. Economics

One must also consider economics when designing a novel protecting group. It must be inexpensive, easy to synthesise, and yield derivatives that are non-toxic with ready solubility in organic media.

CHAPTER:2

Discussion

The 3–Ethyl pent–3–yl Group.

The 1–(3'–Trifluoromethyl phenyl)–2–methyl prop–2–yl Group.

The 1–(4'–Fluoro phenyl)–2–methyl prop–2–yl Group.

The Side Chain Protection of Aspartic Acid.

The Synthesis of a Peptide.

The Side Chain Protection of Glutamic Acid.

The Side Chain Protection of Lysine.

Conclusion.

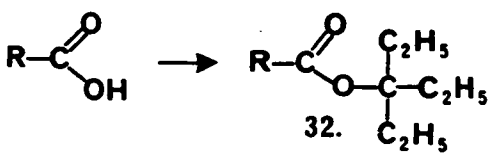
CHAPTER 2: DISCUSSION

The criteria for the design of a novel side chain carboxyl protecting group stated at the end of chapter 1 have, so far, not been fulfilled. This is an area in which significant advances must be made if the methodology of solid phase peptide synthesis is to be improved. This chapter describes the development of a novel protecting group that, it is hoped, will fulfil these criteria.

As described earlier the t-butyl group is now the most frequently used protecting group for amino acid carboxyl functions in solid phase peptide synthesis and is the protecting group to which any newly introduced protecting group would be compared.

The 3-Ethyl⌒pent-3-yl Group

It was initially decided to study the suitability and utility of the 3-ethyl⌒pent-3-yl group for carboxyl protection. That is, the protection of an acid as the 3-ethyl⌒pent-3-yl ester (32) (Scheme XIV).

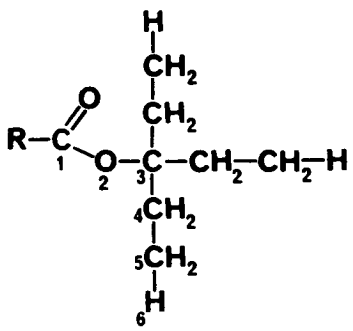


32.

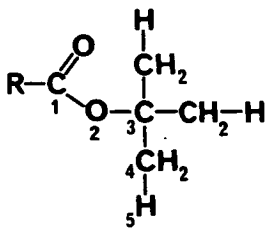
Acid Protected Acid

Scheme XIV

The reasons for studying this group stem from the previously mentioned criteria for a protecting group (chapter 1). Firstly, if the "rule of six" concerning steric hindrance is considered, the 3-ethyl -pent-3-yl group will present considerably more steric hindrance to inter and intra-molecular nucleophilic attack at the ester carbonyl function than the t-butyl group due to the "six numbers" being 9 and 0 respectively.

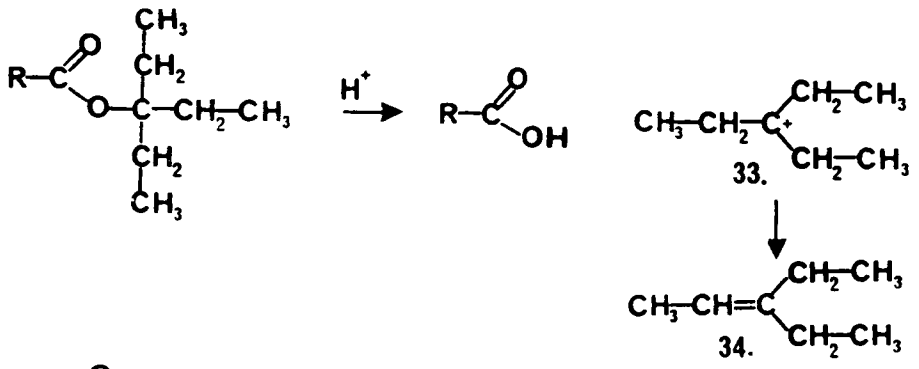


Six Number — 9

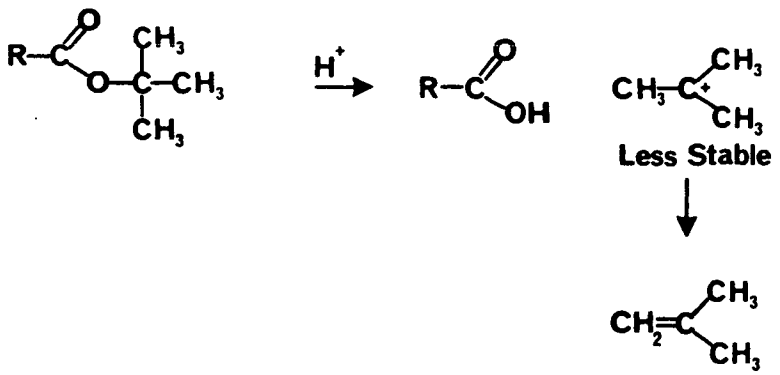


Six Number — 0

This, in the context of solid phase peptide synthesis, would prevent base hydrolysis during orthogonal deprotection and aspartimide (or glutarimide) formation in acid, basic or neutral conditions. The cleavage of the 3-ethyl-pent-3-yl group would also be expected to be more facile than that of the t-butyl group in acidic conditions. This would be due to further stabilisation of the carbocation intermediate (32) by increased electron donation and the formation of a more stable tri-substituted olefin (34) by loss of a proton from this carbocation (Scheme XV).



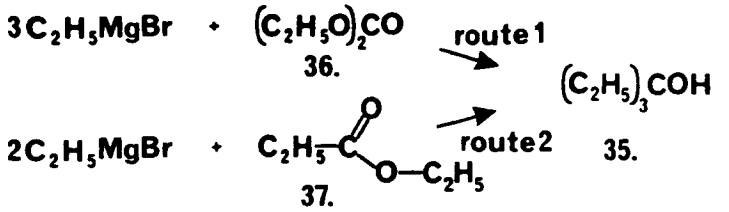
Compare



Scheme XV

It was expected that the formation of this olefin (34) on deprotection would occur very rapidly, within the solvent cage, thereby avoiding the possibility of carbocation induced side reactions occurring.

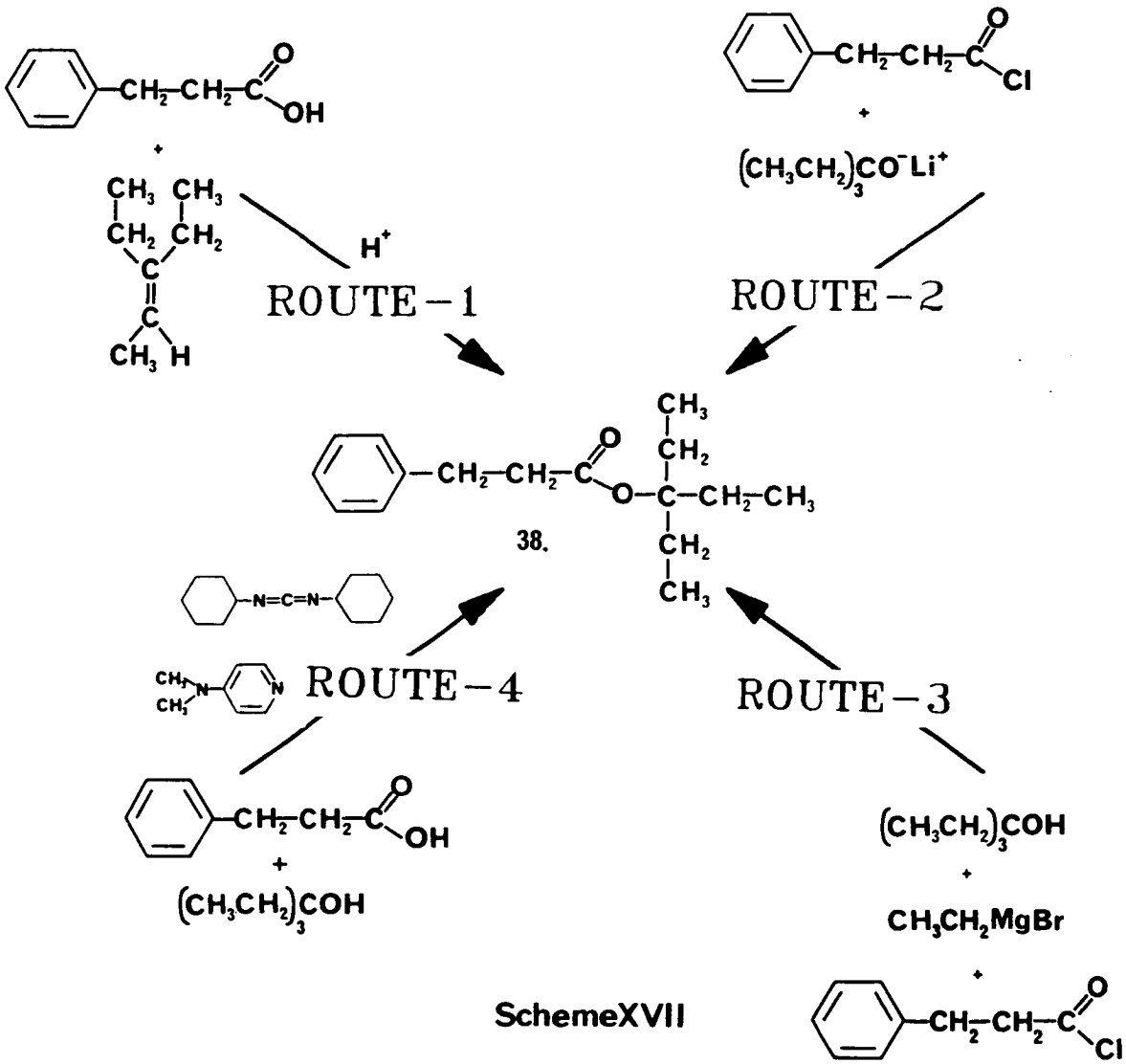
It was therefore decided to synthesise a quantity of 3-ethyl pentan-3-ol[56,57] (35) and then study the protection and deprotection of a simple carboxylic acid in order to assess the suitability of the 3-ethyl pent-3-yl group as a protecting group. Two routes to 3-ethyl pentan-3-ol (35) were studied (Scheme XVI).

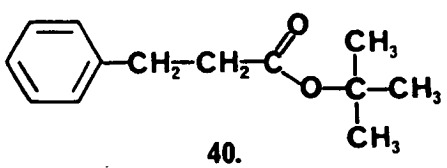


Scheme XVI

Both of these routes afforded the alcohol as expected. Difficulty was, however, found in purifying the alcohol made by route 1 as diethyl carbonate (36) and the desired alcohol have similar boiling points (126°C and 137°C respectively) which made purification by distillation difficult. This problem was not encountered with route 2 which gave a high yield of alcohol (80%) from ethyl propionate (37).

Having successfully isolated the pure alcohol it was decided to prepare some of the corresponding olefin 3-ethyl-pent-2-ene[58] (34). This was easily done by distilling the alcohol slowly in the presence of iodine after which the distillate was washed with sodium thiosulphate solution, water and finally fractionated over sodium to yield the olefin in good yield (70%).


ROUTE−1
ROUTE−2
ROUTE−3
ROUTE−4
38.
SchemeXVII


40.

The following routes to the 3-ethyl pent-3-yl ester (38) of hydrocinnamic acid (3-phenylpropionic acid), a simple carboxylic acid, were studied (Scheme XVII).

Route 1

This route is analogous to the method usually employed for the preparation of t-butyl esters using isobutylene and an acid catalyst usually p-toluene-sulphonic acid or sulphuric acid[59]. These acids were used with the alkene 3-ethyl pent-2-ene which resulted in the formation of a polymeric substance in the case of sulphuric acid and the desired ester (37), in small yield (3%), using p-toluenesulphonic acid.

Route 2

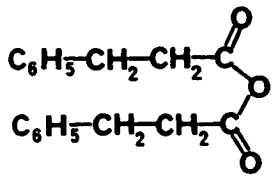
This method, introduced by Kaiser and Woodruff[60] uses the reaction between a lithium alkoxide and an acid chloride to form an ester. This method has the advantage that no acidic products, which could decompose the ester, are formed and hence this method is suitable for the preparation of very acid labile esters derived from tertiary alcohols or other alcohols which readily undergo acid catalysed rearrangements. This reaction consequently proved an excellent method for producing the t-butyl (40) and 3-ethyl pent-3-yl (38) esters of hydrocinnamic acid in yields greater than 65%. These esters were purified by distillation in vacuo.

Route 3

This reaction relies on the Grignard reagent acting as a base by removing a proton from the alcohol (35) thus forming an alkoxide ion which can undergo a nucleophilic reaction with the acid chloride used. This reaction proved successful in forming the desired ester (38) though in lower yield (50%) than in route 2 above.

Route 4

This is a common, and usually successful method of forming esters[61,62]. Unfortunately, in this circumstance, it proved unsuccessful due to the acid being more nucleophilic than the sterically hindered alcohol thus forming the symmetrical anhydride (39) rather than the desired ester.



39.

It is interesting to note that various attempts to purify the 3-ethyl pent-3-yl ester by silica flash chromatography proved unsuccessful due to the slightly acidic nature of silica. This acidity completely hydrolysed the ester thus illustrating the extreme acid lability of this compound. The t-butyl ester (40) was, by comparison, unaffected by silica thus

showing it to be less acid labile as expected. Both esters were, however, preserved when neutral alumina was used for purification showing that hydrolysis of the 3-ethyl^pent-3-yl ester was probably due to the acidity of the silica used.

It was decided to attempt to compare the stability of the methyl, t-butyl and 3-ethyl^pent-3-yl esters of hydrocinnamic acid to base hydrolysis by following the reactions by h.p.l.c. This, however, resulted in a number of problems, the main one being that the 3-ethyl^pent-3-yl ester was completely cleaved on the slightly acidic silica h.p.l.c columns used. The methyl and t-butyl esters were stable on the silica h.p.l.c columns, again illustrating the greater acid lability of the 3-ethyl pent-3-yl group. This problem was eventually solved by using a graphitic carbon packing newly developed by Knox[69].

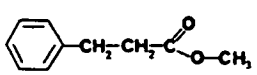
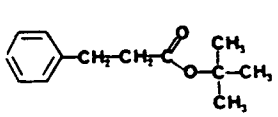
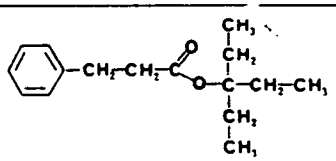
Difficulties were also encountered in finding a suitable solvent system that produced different retention times for each of the three esters and hydrocinnamic acid. This was solved by using a 1:1 mixture of acetonitrile/ammonium acetate (0.1M) solution for the methyl and t-butyl esters using the silica h.p.l.c columns and a 100% solution of acetonitrile for the 3-ethyl^pent-3-yl ester using the

graphitic carbon column. The base hydrolysis was performed in a 1:1 dioxan/sodium hydroxide solution.

It was also necessary to find a way of quenching the reaction after a period of time so that the degree of hydrolysis could be determined accurately. A potassium orthophosphate/sodium hydroxide buffer solution (pH7) was used for this purpose.

A technical problem that had to be overcome was the amount of base that was required to be present for the hydrolysis to go to completion. By performing a number of experiments using 1.0, 1.2 and 1.5 equivalents of base it was found that the amount of ester and acid remained constant. When 2.0 equivalents of base were used the hydrolysis proceeded to completion. The following qualitative results were then obtained (Table 2).

TABLE 2: Ester Hydrolysis Studies.

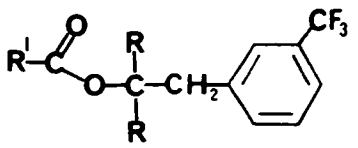
ESTER	BASE HYDROLYSIS (2 EQUIV. OF NaOH.)	ACID HYDROLYSIS (TFA/DCM SOLN.)
	COMPLETE HYDROLYSIS 20 MINUTES	-----
	25% HYDROLYSIS 24 HOURS	50% TFA INSTANTANEOUS 20% TFA 15 MINS
	STABLE > 4 DAYS	20% TFA INSTANTANEOUS

These results show the relative stabilities of the three esters studied to base hydrolysis. The 3-ethyl^pent-3-yl ester (38) therefore shows greater stability in basic conditions than the t-butyl ester (40) reflecting the increased steric hindrance to nucleophilic attack at the carbonyl group of the ester as desired.

A study of the relative stability of the t-butyl and 3-ethyl^pent-3-yl esters in acidic conditions was also attempted (Table 2). The t-butyl ester was cleaved instantly in 50% trifluoroacetic acid/methylene chloride solution and within 15 minutes in 20% trifluoroacetic acid/methylene chloride solution. The cleavage of the 3-ethyl^pent-3-yl ester was extremely facile with the result that the time for complete cleavage was too small to measure by the technique being used. Although possessing the desired base stability the 3-ethyl^pent-3-yl group was, however, considered too acid labile to be of practical application in solid phase peptide synthesis for side chain carboxyl protection.

The 1-(3'-Trifluoromethyl^phenyl)-2-methyl^prop-2-yl Group

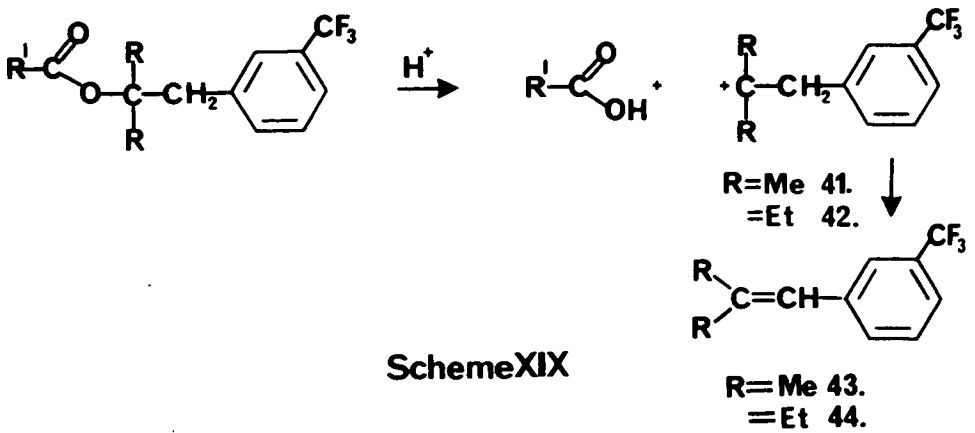
As a result of these findings it was decided to study acids protected in the following form (Scheme XVIII).



R=Me,Et

SchemeXVIII

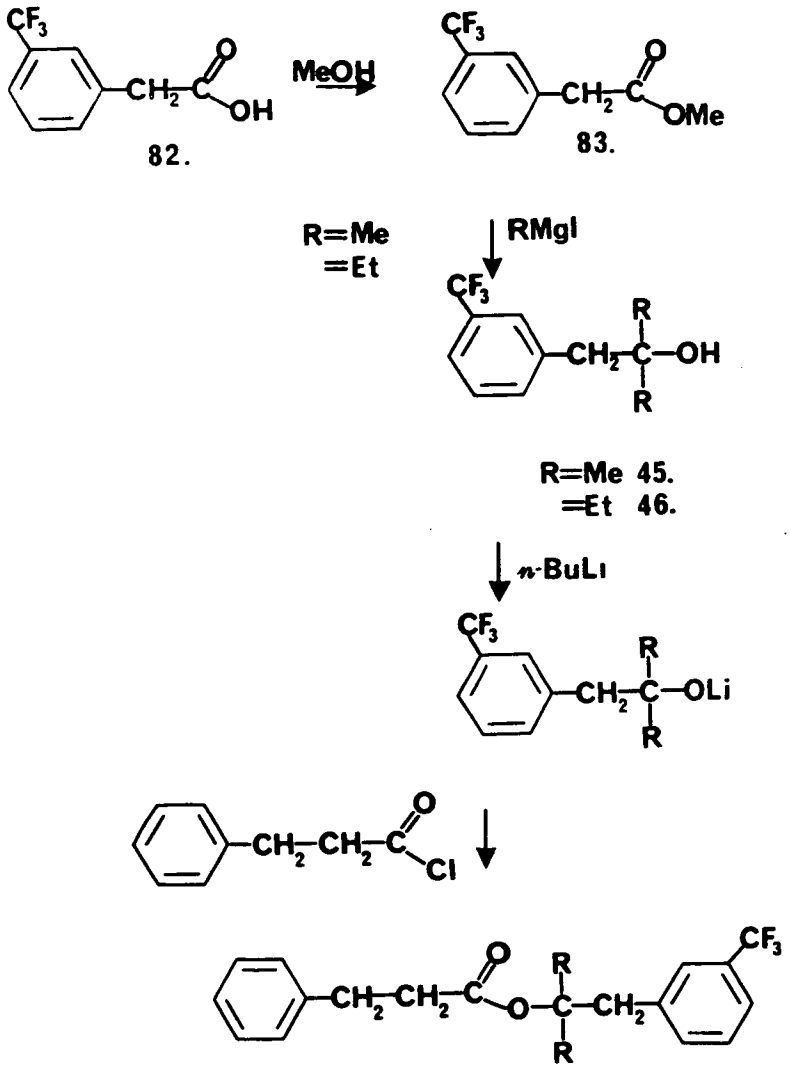
The reasons for considering such esters to be possible suitable protecting groups are similar to those of the 3-ethyl pent-3-yl group in that such esters will present a large steric hindrance to nucleophilic attack at the carbonyl group of the ester. On acidolytic deprotection a self-quenching carbocation, (41) or (42), could be produced, this quenching being the result of the formation of a trisubstituted styrene, (43) or (44). (Scheme XIX).



SchemeXIX

A ^{19}F n.m.r label was also incorporated into this protecting group thereby enabling protections and deprotections to be monitored easily.

The derivatives (47),(48) of hydrocinnamic acid were made by the following route (Scheme XX).

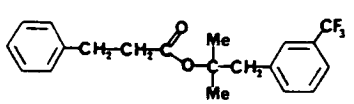
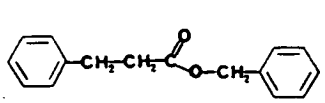
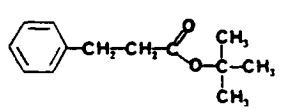

CF3
CH2
C
O
OH
MeOH
82.
OMe
83.
R=Me
=Et
RMgl
R
45.
=Et 46.
n-BuLi
OLi
Cl
47.
=Et 48.

Scheme XX

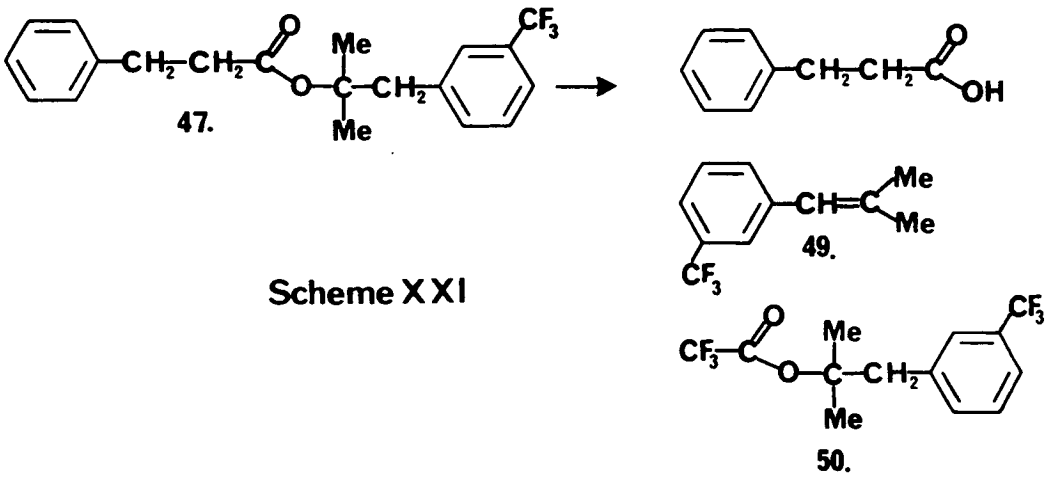
When R=Et the formation of the alcohol (46) presented no major problems and proceeded in reasonable yield (60%). The formation of the corresponding ester (48) did, however, prove more difficult and afforded the desired ester in poor yield (20%) due, presumably, to the high steric bulk of the tertiary alcohol. The case where R=Me was, however, more promising and resulted in the formation of the alcohol (45), a white, low melting solid, and the corresponding ester (47) in good yield (80% and 70% respectively).

The stability of this protecting group to acid and base hydrolysis was then studied using h.p.l.c to monitor the reaction as before. These results are summarised below (Table 3) and compared to the cleavage times for the t-butyl and benzyl esters of hydrocinnamic acid under the same conditions.

TABLE 3: Ester Hydrolysis Studies.

ESTER	BASE HYDROLYSIS (2 EQUIV. OF NaOH.)	ACID HYDROLYSIS (TFA/DCM SOLN.)
	25% HYDROLYSIS 5 DAYS	90% TFA 1 HOUR 50% TFA 4 HOURS
	COMPLETE HYDROLYSIS 1 HOUR	STABLE > 3 DAYS
	25% HYDROLYSIS 24 HOURS	50% TFA INSTANTANEOUS 20% TFA 15 MINS

These results show that the 1-(3'-trifluoromethyl^phenyl)-2-methyl^prop-2-yl ester (47) is significantly more stable to acid and base hydrolysis than the corresponding t-butyl ester (40). The products of deprotection were then established by synthesis and comparison using h.p.l.c. to be the styrene (49), the trifluoroacetate (50) and hydrocinnamic acid (Scheme XXI).

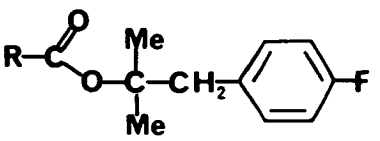


Scheme XXI

Deprotection to form the trisubstituted styrene (49) via the carbocation (41) had, therefore, occurred as expected. The trifluoroacetate (50) presumably results from reaction of this carbocation with trifluoroacetic acid.

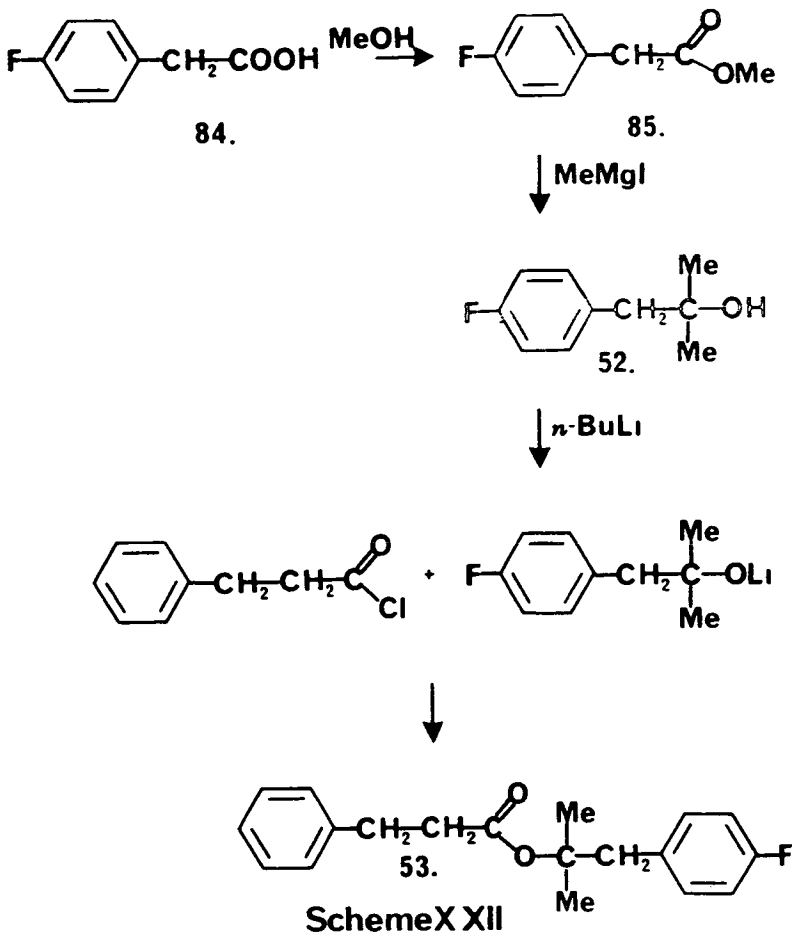
The 1-(4'-Fluorophenyl)-2-methylprop-2-yl Group

Although the trifluoromethylphenyl-derived protecting group (41) possessed the required stability in basic media it was thought to be too acid stable to be used with the 2,2,5,7,8-pentamethylchroman-6-sulphonyl chloride (Pmc) arginine side chain protecting group (30) which is cleaved in 50% trifluoroacetic acid/methylene chloride solution in 1 hour[55]. In order to increase the acid lability, while retaining the base stability, protected acids of the following type (51) were studied.



51.

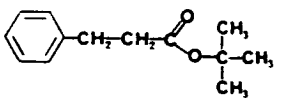
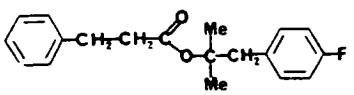
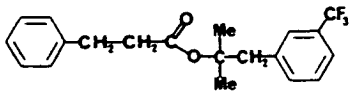
The corresponding alcohol (52) and hydrocinnamate (53) were prepared in good yield (75% and 60%) as before (Scheme XXII).



SchemeX XII

The stability of this ester to acidic and basic conditions was then studied using h.p.l.c. as before. The following, very favourable, results were obtained. (Table 4). The results for the t-butyl ester and trifluoromethylphenyl analogues are also presented for comparison.

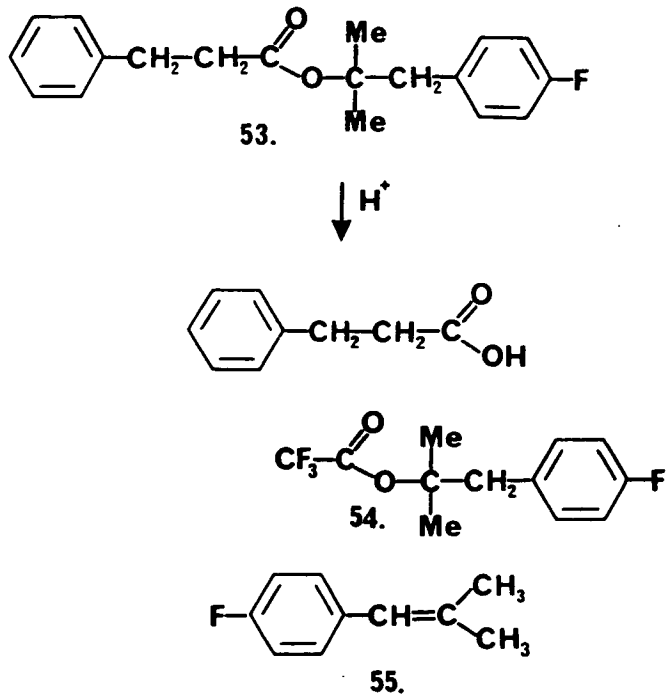
TABLE 4: Ester Hydrolysis Studies.

ESTER	BASE HYDROLYSIS (2 EQUIV. OF NaOH.)	ACID HYDROLYSIS (TFA/DCM SOLN.)
	25% HYDROLYSIS 24 HOURS	50% TFA INSTANTANEOUS 20% TFA 15 MINS
	25% HYDROLYSIS 5 DAYS	50% TFA 15 MINS 20% TFA 30 MINS
	25% HYDROLYSIS 5 DAYS	90% TFA 1 HOUR 50% TFA 4 HOURS

These results show that the 1-(4'-fluorophenyl)-2-methylprop-2-yl ester (53) is more acid stable than the corresponding t-butyl ester but less acid stable than the 1-(3'-trifluoromethylphenyl)-2-methyl prop-2-yl analogue (47). This degree of acid lability combined with good stability to base hydrolysis and the presence of a ^{19}F n.m.r. label indicate that the 1-(4'-fluorophenyl)-2-methylprop-2-yl group (51) has the desired characteristics to be a useful side chain carboxyl protecting group for solid phase peptide synthesis. The ester was also subjected to tetra-butylammonium fluoride (TBAF, 1 equivalent) in methylene chloride, a reagent which is

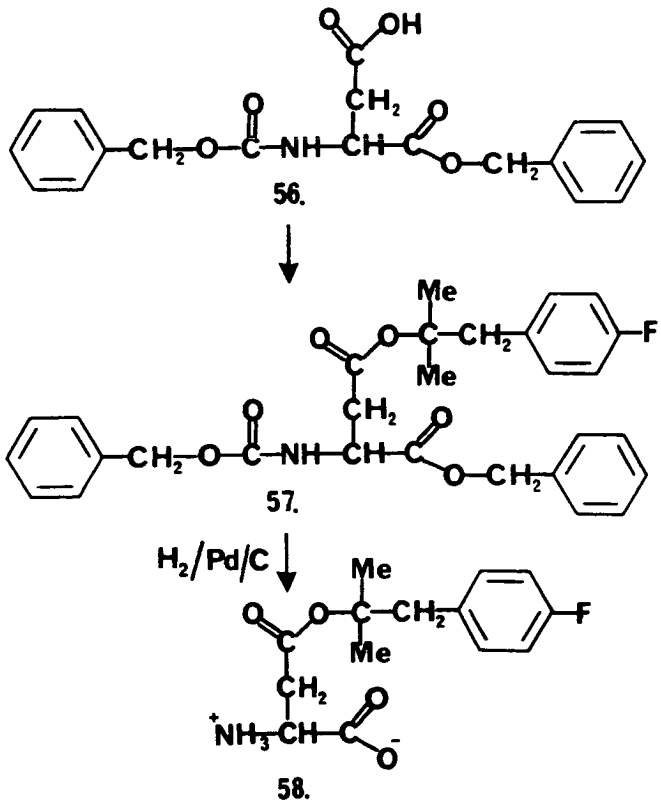
used in peptide synthesis for cleavage of the peptide from the polymeric support. The ester was unaffected by these conditions over a period of 17 hours in solution as required.

The products on removal of this protecting group were again the corresponding trifluoroacetate (54), the trisubstituted styrene (55) and hydrocinnamic acid (Scheme XXIII).



Scheme XXIII

It was therefore decided to apply this protecting group directly to carboxyl side chains of amino acids by studying the protection of benzyl N^{α}-Z-L-aspartate[63] (56). Hydrogenolysis of the fully protected amino acid (57) would then yield the free, side chain protected, aspartic acid (Scheme XXIV).

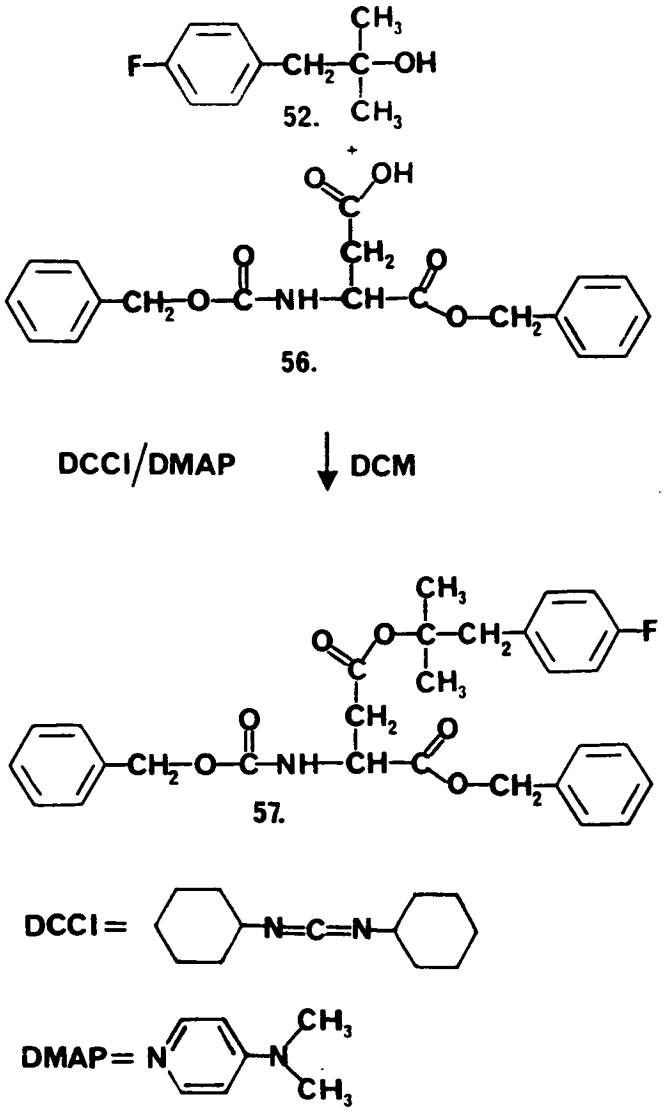


Scheme XXIV

The formation of the tertiary ester side chain of (56) proved to be a very difficult reaction presumably due to steric contraints. A number of reactions were attempted involving the acid chloride

side chain or diphenylphosphinyl (DPP) mixed anhydride of (56) and the alcohol or lithium salt of the alcohol (52). A dicyclohexylcarbodiimide (DCCI)/ dimethylaminopyridine (DMAP) reaction[62] at room temperature was also carried out. All these experiments, however, resulted in starting materials being recovered.

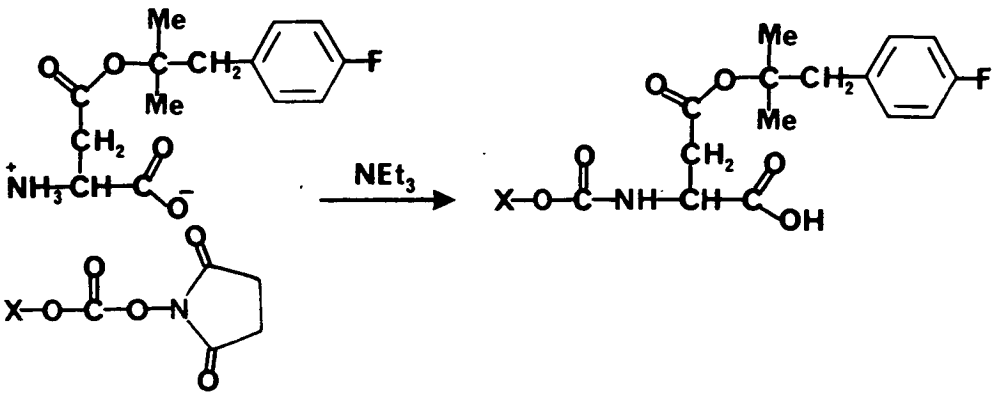
As a final attempt the DCCI/DMAP reaction was repeated but stirred at 4°C for 3 days (Scheme XXV). The desired ester (57) was isolated (in 55% yield) after purification by silica flash chromatography. This purification, however, proved difficult due to the alcohol (52) and ester (57) behaving similarly on t.l.c and during flash column chromatography. It was therefore found more practical to use the ester in crude form for the next stage which involves removal of the benzyloxycarbonyl (Z) and benzyl ester protecting groups by hydrogenolysis (Scheme XXIV). This reaction was carried out in 2% water/methanol solution using 10% palladium on charcoal (10% w/w). The removal of the Z and benzyl ester groups proved very facile and was completed (by t.l.c.) within one hour. The free amino acid (58) was then isolated by removal of the catalyst by filtration, concentrating to half volume, then adding diethyl ether until a white jelly-like solid precipitated out of solution.

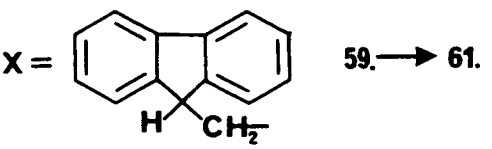


Scheme XXV

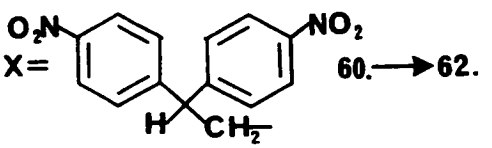
This was then filtered and dried in vacuo to yield the pure amino acid (58) in good yield (72%).

The N^{α}-9-fluorenylmethyloxycarbonyl (Fmoc) and N^{α}-2,2-bis(4'-nitrophenyl)ethoxycarbonyl (Bnpeoc) derivatives (61) and (62) of this amino acid were then prepared from the corresponding succinimidyl carbonates (59), (60) using triethylamine in a dioxan/water solution[64] (Scheme XXVI).





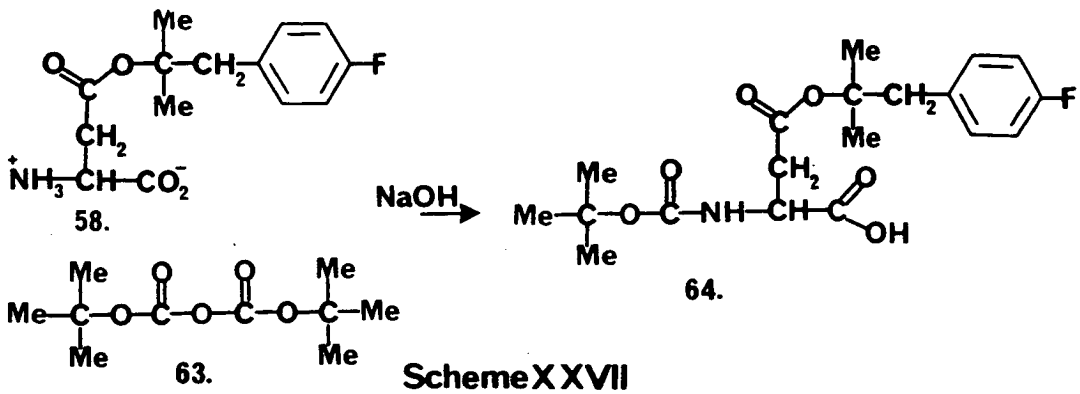
59. → 61.



60. → 62.

Scheme XXVI

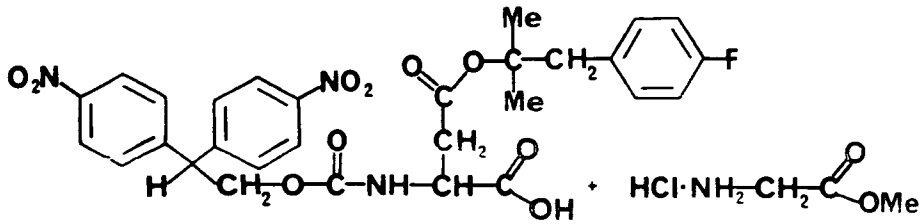
The Bnpeoc derivative (62) was easily purified by formation of the corresponding cyclohexylammonium salt whereas recrystallisation from chloroform/petrol (40/60) afforded the pure Fmoc derivative (61). Both compounds were obtained in good yield (86% and 75% respectively). The N^{α}-t-butyloxycarbonyl derivative (64) of (58) was also prepared using di-tert butyl pyrocarbonate (63) in aqueous sodium hydroxide/dioxan solution[65]. (Scheme XXVII).



Scheme XXVII

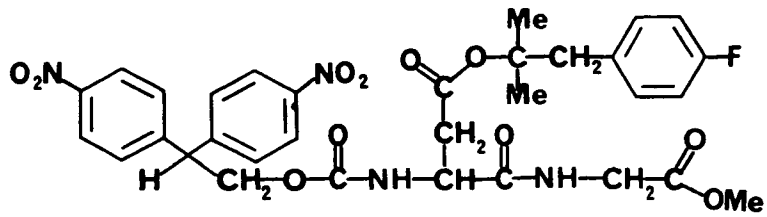
Purification of (64) was achieved by formation of the corresponding cyclohexylammonium salt in good yield (69%).

The Bnpeoc derivative (62) was then coupled with glycine methyl ester hydrochloride using dicyclohexyl-carbodimide (DCCI) and triethylamine in tetrahydro-furan/acetone solution to afford the dipeptide (65) in good yield (82%) after purification by silica flash chromatography. (Scheme XXVIII)



62.

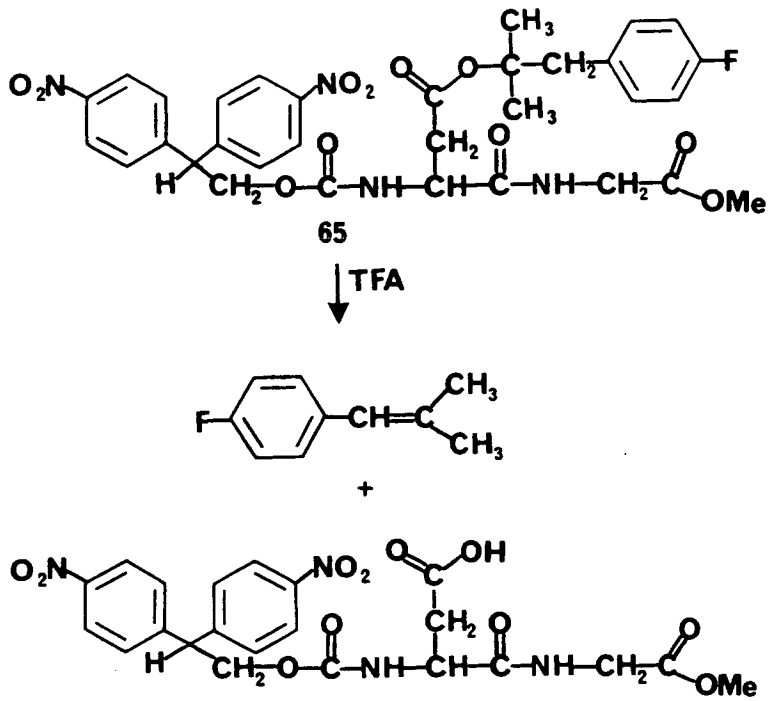
DCCI/TEA



65.

SchemeXXVIII

The dipeptide (65) was then treated with trifluoroacetic acid and the cleavage monitored by h.p.l.c. as before. (Scheme XXIX) The following results were obtained (Table 5).



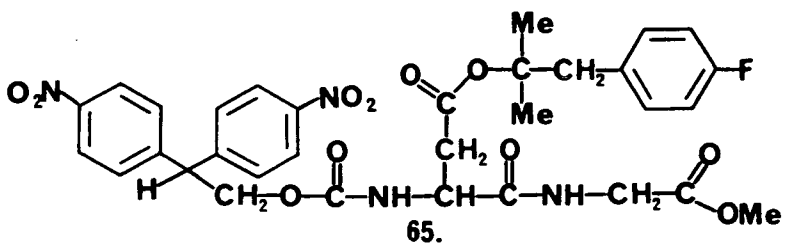
Scheme XXIX

TABLE 5: Peptide Cleavage Studies.

CONDITIONS	CLEAVAGE TIME
50% TFA. 45% DCM. 5% H_2O	1 HOUR
95% TFA. 5% H_2O	30 MINUTES

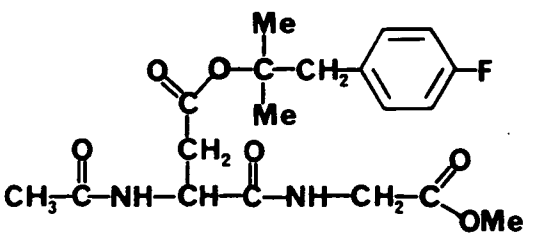
The side chain protection was therefore selectively cleaved in the presence of the Bnpeoc group. The rate of cleavage was somewhat slower than observed in the model studies (Table 4) but was considered acceptable for the purpose of solid phase peptide synthesis.

Selective cleavage of the Bnpeoc group of (65) using 1,5 diazobicyclo 4 3 0 non-5-ene (DBN) (25) in the presence of the side chain protection was also achieved. The products, isolated by preparative t.l.c were 1,1-bis(4-nitrophenyl)ethene (28) and the N-acetyl derivative (66) formed, in situ, by reaction of the free amine produced with acetic anhydride in the presence of pyridine. The selective cleavage occurred in good yield (89%) (Scheme XXX). No loss of the side chain carboxyl protecting group could be detected thus aspartimide formation was not favoured.

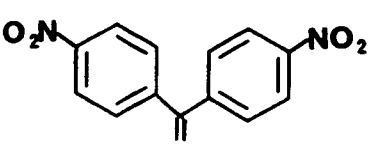


65.

1. DBN/ACOH
2. AC_2O/Pyridine



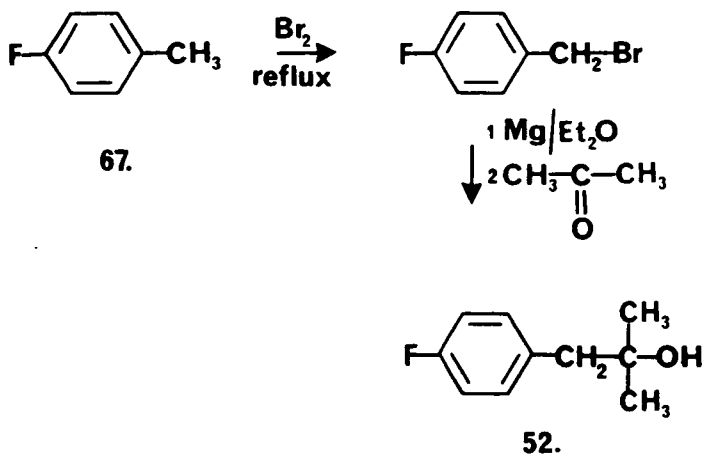
66.



28.

Scheme XXX

These results show that the 1-(4'-fluoro^phenyl)-2-methyl^prop-2-yl protecting group (51) has the desired degrees of acid lability and base stability to be of use in an orthogonal solid phase peptide synthetic approach. The design of the protecting group also incorporates steric features, discussed in chapter 1, that should suppress the common side reactions of alkylation and cyclisation with aspartyl and glutamyl residues. The incorporation of an n.m.r label to monitor the protections and deprotections has also been achieved by the incorporation of a fluorine atom. In order to improve the economic viability of this protecting group an alternative and potentially less expensive route to 1-(4'-fluoro^phenyl)-2-methyl propan-2-ol (52) was investigated (Scheme XXXI).



Scheme XXXI

This route was completed in an overall yield of 72% starting from inexpensive 4-fluorotoluene (67) (£7.10/100g) and using excess acetone and magnesium metal to complete the synthesis. The alcohol (52) could therefore be made on a large scale very economically.

The Synthesis of a Peptide

The application of the 1-(4'-fluoro phenyl)-2-methyl prop-2-yl protecting group to the synthesis of the comparatively simple pentapeptide Ile Leu Asp Asn Ile (68) by solid phase peptide synthesis was then studied.

H-Ile-Leu-Asp-Asn-Ile-OH

68.

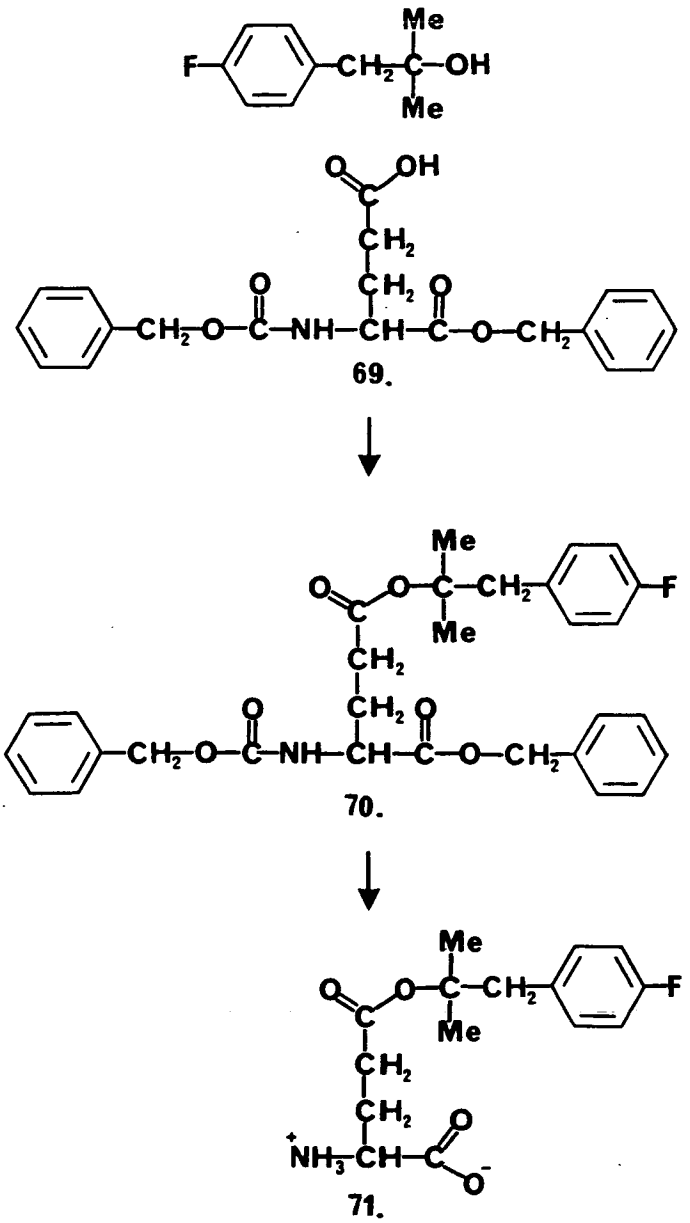
This peptide was chosen as a target because of its obvious lack of complicating side chain functionalisation; however, it did incorporate hindered Fmoc amino acid derivatives. The synthesis was carried out on an Applied Biosystems peptide synthesiser (Model 430A) utilising Fmoc methodology which was monitored, during deprotection, by the ultra violet absorbance of the corresponding piperidine adduct of (10) produced as a by-product. The synthesis appeared, by this monitoring, to progress very well due to a constant level of UV absorbance per

deprotection being observed. The resultant crude peptide was then obtained in good yield (273mg, 93%) after cleavage from the p-alkoxybenzyl (Wang) resin with trifluoroacetic acid (95%) in methylene chloride. This treatment would also have removed the novel aspartyl side chain protection (51) used in the synthesis. A portion (45mg) of the peptide was then passed down a Sephadex G10 gel filtration column using acetic acid (1M) as eluent. Detection of the eluted peptide was achieved by the use of a polarimeter. The peptide (23mg) was then recovered by freeze drying the appropriate collected fractions. The excellent purity of the peptide (68) was indicated by amino acid analysis, mass spectrometry (F.A.B), proton n.m.r (360 MHz) and t.l.c. The ^{19}F n.m.r (80 MHz) indicated no fluorine present in the sample indicating the complete removal of the aspartyl side chain protecting group as expected.

The above result indicates that the 1-(4'-fluoro phenyl)-2-methyl prop-2-yl protecting group is of use in the solid phase synthesis of peptides. Unfortunately the application of this protecting group to the synthesis of other, more complex, peptides has not yet been studied. This will be pursued in the near future.

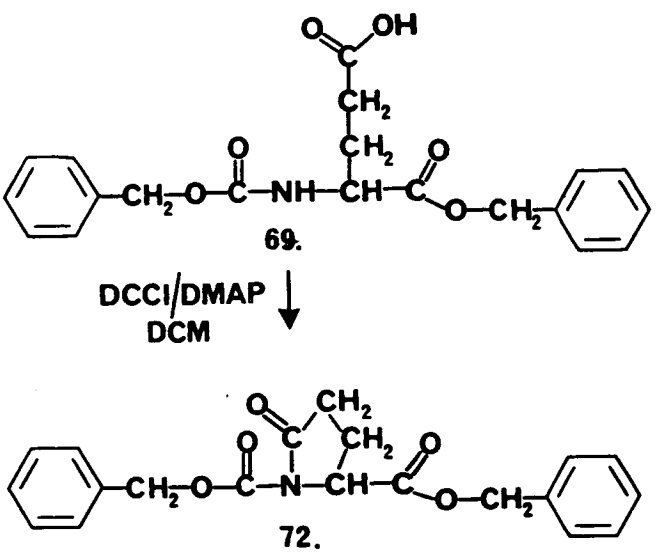
The Side Chain Protection of Glutamic Acid

The next step, following the synthesis of the peptide mentioned above was to study the analogous protection of the glutamic acid side chain using the 1-(4'-fluorophenyl)-2-methylprop-2-yl protecting group (51) (Scheme XXXII).



Scheme XXXII

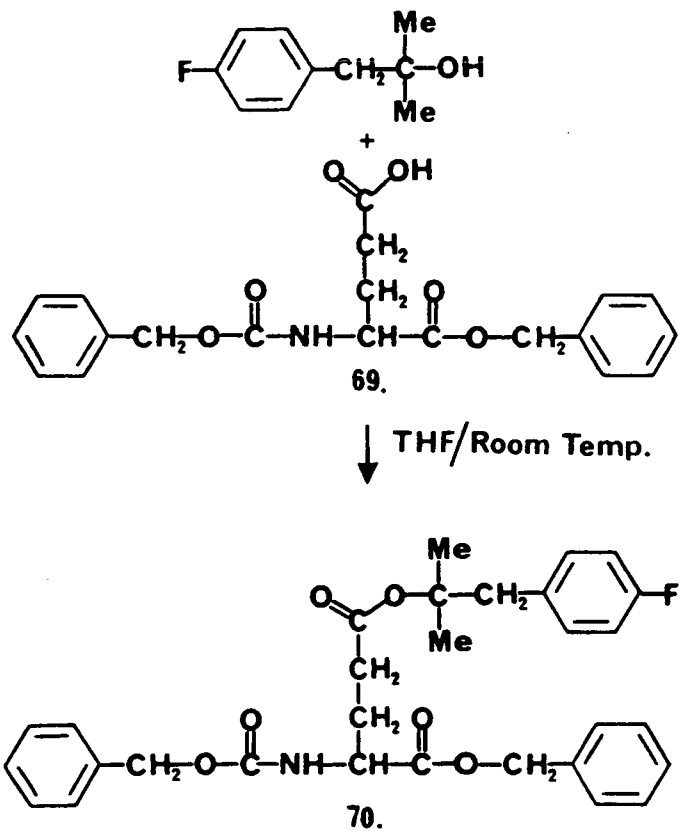
The protection of (69)[66] was first attempted using dicyclohexylcarbodiimide/dimethylaminopyridine in dichloromethane at 4°C, conditions that had proved successful for the analogous aspartic acid protection (Scheme XXIV). Unfortunately, however, the formation of the pyroglutamic acid derivative (72) proved to be a serious competing reaction under these conditions (Scheme XXXIII).



Scheme XXXIII

Indeed, with methylene chloride as solvent the pyroglutamic acid derivative (72) was formed in up to 40% yield. The white crystalline solid (72) was easily obtained by triturating the crude reaction mixture with ethyl acetate and cooling overnight.

The use of tetrahydrofuran (THF) as solvent for this reaction did however suppress the formation of (72) to an extent that 15% of this side product was obtained when the reaction was stirred for 24 hours at room temperature. This was thought to be acceptable considering the large steric bulk of the alcohol (52) used in the esterification. (Scheme XXXIV).

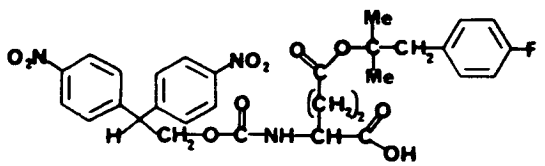


Scheme XXXIV

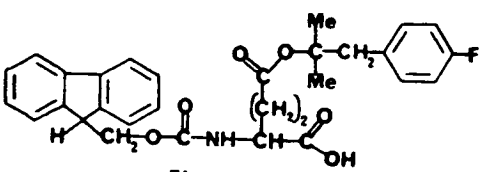
The separation of the fully protected amino acid (70) from any unreacted alcohol (52) again, as with aspartic acid, proved very difficult by conventional chromatographic means due to these two compounds

having similar properties on silica t.l.c. Removal of most of the alcohol (52) from the protected amino acid (70) was, however, achieved by washing the crude reaction mixture with warm petroleum ether (b.p 60-80°C) to give in moderate yield (65%) a soft white solid which by t.l.c. appeared to be predominantly the desired ester (70). The hydrogenolysis of this compound, in aqueous methanol (2%), resulted in the initial formation of a clear gum which on trituration with petroleum ether (b.p 60-80°C) afforded a white solid which appeared by t.l.c (ninhydrin positive), n.m.r and mass spec. to be the desired, side chain protected, glutamic acid (71).

The synthesis of the corresponding Bnpeoc (73) and Fmoc (74) derivatives of (71) would be the next natural extension of this work.



73.

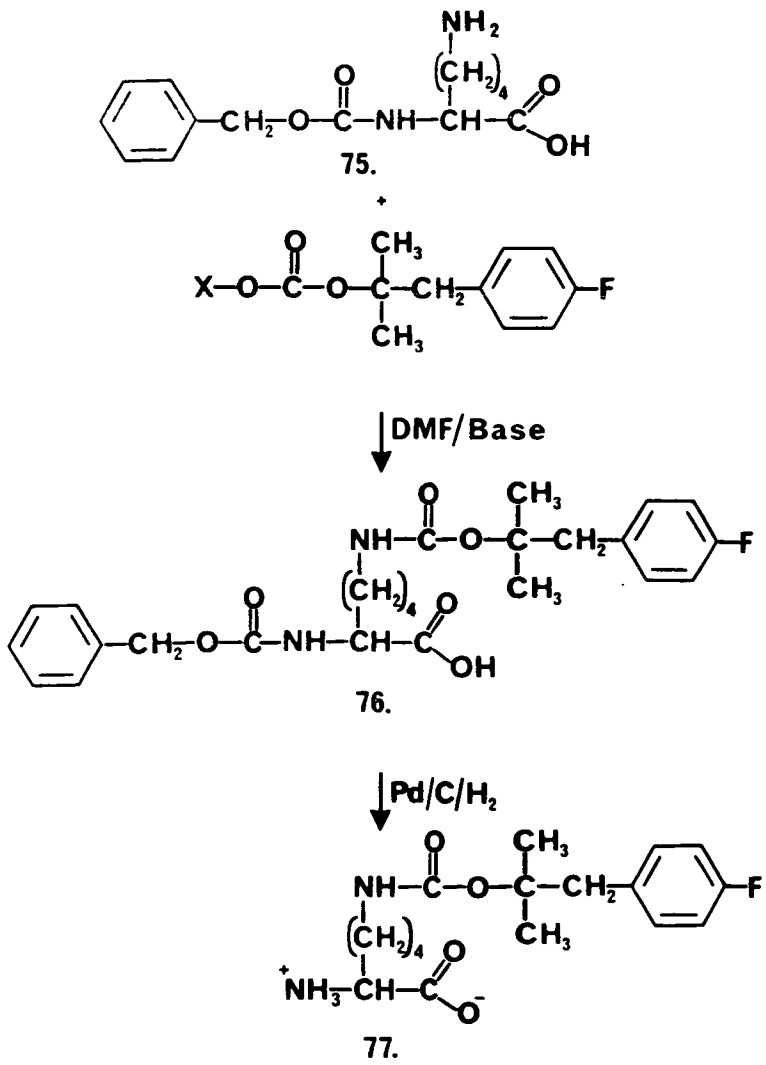


74.

The Side Chain Protection of Lysine

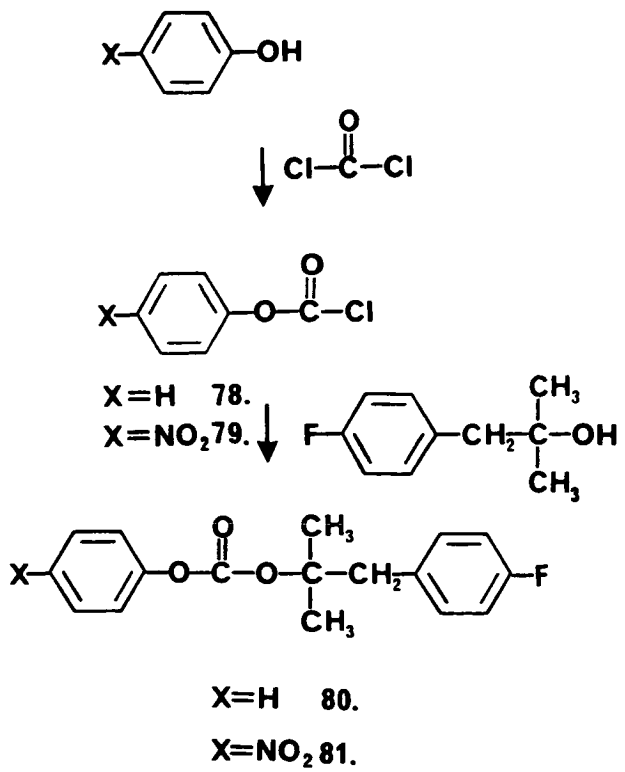
It was also considered of interest to study the suitability of the 1-(4'-fluorophenyl)-2-methylprop-2-yl group (51) for protection of the amino side chain of lysine. The protection of N^{α}-benzyloxycarbonyl-L-lysine[67] (75) was therefore studied which,

after hydrogenolysis, should then yield the desired, side chain protected, amino acid (77) (Scheme XXXV).



Scheme XXXV

The mixed carbonates (80) and (81) were therefore prepared, in basic conditions, from 1-(4'-fluoro phenyl)-2-methyl propan-2-ol (52) and the corresponding phenyl chloroformate (78) or 4-nitro-phenylchloroformate (79) (Scheme XXXVI).



Scheme XXXVI

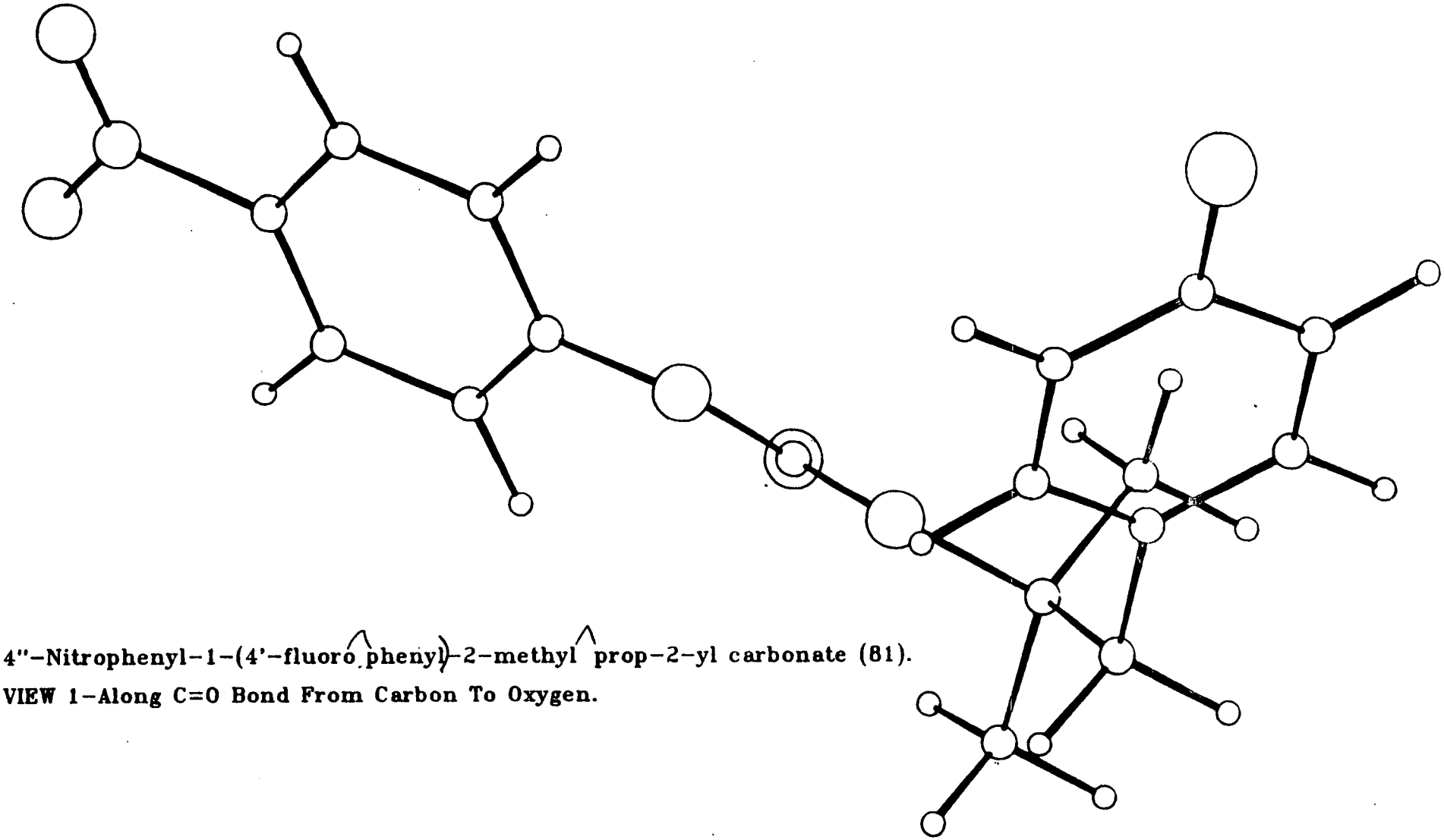
The protection of Z-lysine (75) using these carbonates (Scheme XXXV) was then studied.

The reaction using the phenylchloroformate (78) was performed at 60° in DMF using benzyltrimethyl ammonium hydroxide (Triton B) as base. It was observed, by t.l.c, that the carbonate (80) disappeared completely after stirring at this temperature for 3 days. The analogous reaction using the 4-nitrophenyl carbonate (81) proceeded more rapidly as would be expected and was complete, as judged by t.l.c, within 4 hours.

The crude products (76) from these reactions did, however, prove very difficult to purify due to

the presence of the corresponding phenol in the reaction mixture. Consequently, the crude reaction mixture was subjected to hydrogenolysis which afforded the desired product (77), indicated by n.m.r. (^{1}H,^{19}F) and mass spec., which was isolated in moderate yield (45%). Unfortunately, lack of time did not permit further work in this area.

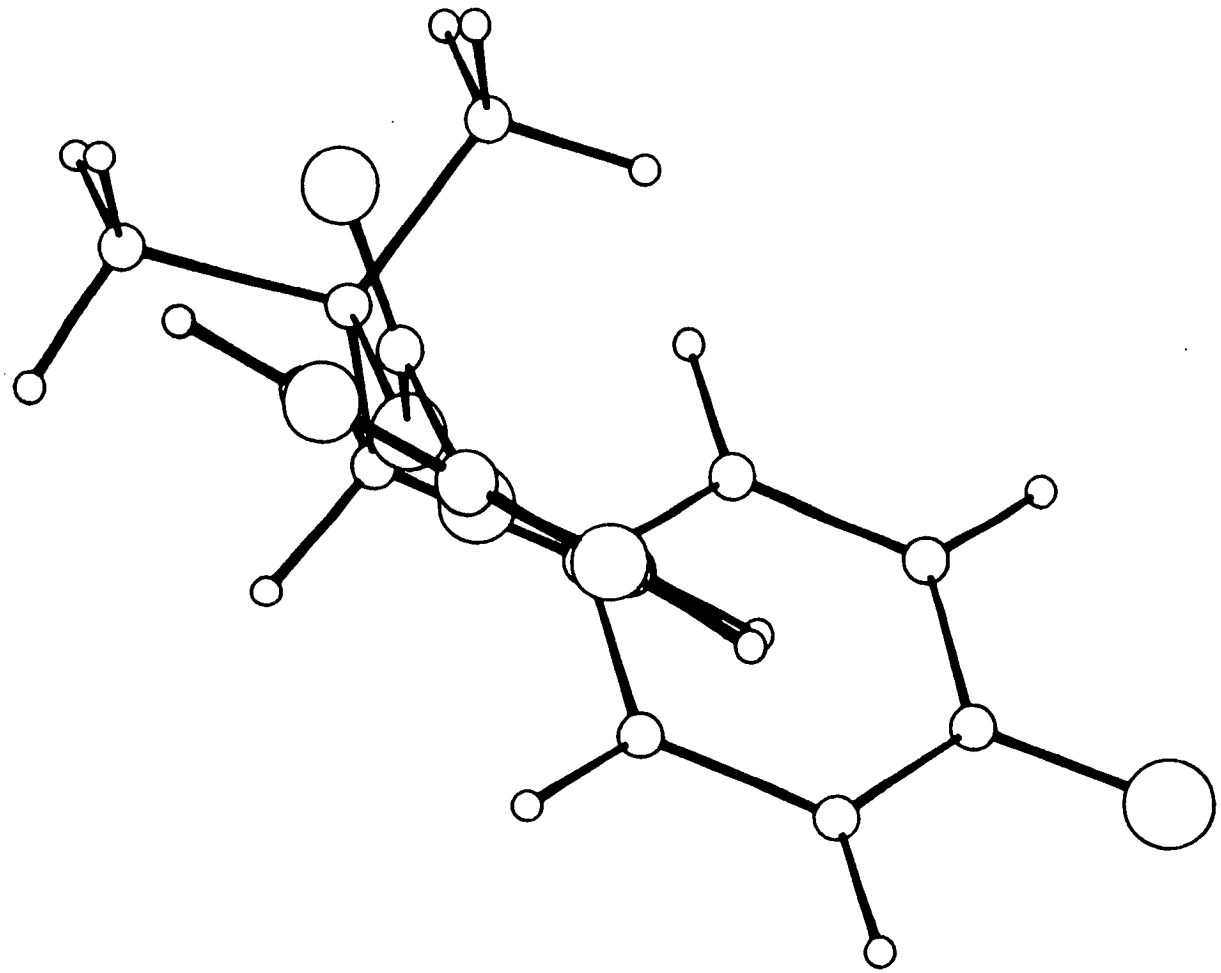
The synthesis of the crystalline mixed carbonate (81) did, however, present the opportunity for a crystal structure of a compound containing the 1-(4'fluoro phenyl)-2-methyl prop-2-yl group (51) to be determined. From this it was hoped to confirm the great steric crowding of the carbonyl group in this molecule as would be expected from application of the "rule of six" discussed earlier (page 23). The following crystallographic views (1,2 and 3) of the molecule illustrate the possible steric hindrance imposed by the 1-(4'-fluoro phenyl)-2-methyl prop-2-yl group to nucleophilic attack at the carbonyl group. These crystal structures can, of course, only be used to indicate the conformation of the molecule in the solid phase. They do, however, indicate likely interactions and conformations that may occur in solution and hence indicate the possible reactivity/stability of the molecule in this phase. It is difficult to draw any further conclusions from these crystal structures.



4''-Nitrophenyl-1-(4'-fluorophenyl)-2-methylprop-2-yl carbonate (81).

VIEW 1-Along C=O Bond From Carbon To Oxygen.

4''-Nitrophenyl-1-(4'-fluoro phenyl)-2-methyl prop-2-yl carbonate (81).

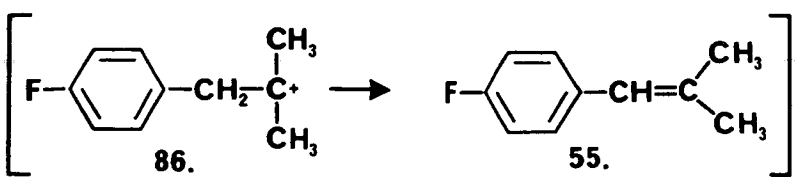


4''-Nitrophenyl-1-(4'-fluorophenyl)-2-methylprop-2-yl carbonate (81).
VIEW 3-Down The Plane Of The NO_2 Group.

Conclusion

This chapter has described the various stages of development of a novel protecting group, the 1-(4'-fluorophenyl)-2-methylprop-2-yl group (51), that has demonstrated great potential as an improvement on current protecting groups for side chain carboxyl protection in solid phase peptide synthesis. This group, due to its steric properties, has been shown to possess excellent stability to base hydrolysis in model compounds and consequently would be highly resistant to both inter and intra-molecular nucleophilic attack thereby precluding the possibility of base hydrolysis and amino acid cyclisation during peptide synthesis. The group does, however, possess the required degree of acid lability to enable it to be compatible with the 2,2,5,7,8-pentamethylchroman-6-sulphonyl (Pmc) group (30) which is used for arginine side chain protection (31).

The deprotection of the 1-(4'-fluorophenyl)-2-methylprop-2-yl group also occurs to give a very stable tri-substituted styrene (55), via the carbocation (86).



86. 55.

The formation of this product is clearly preferable to that of side products that can result from the alkylation of amino acid side chains by carbocations.

The protecting group also incorporates a ^{19}F n.m.r label that enables the protection and deprotection of the amino acid side chains to be followed easily. This label should also assist purification of peptide sequences when non-acidic techniques (Scheme XIII) are used to cleave the peptide from the solid support because, under these conditions, the amino acid side chain would remain fully protected.

As mentioned in chapter 1, another factor that has to be considered with potential protecting groups is the economics of production of the protecting group and ease of introduction of the group into the synthetic scheme. 1-(4'-Fluoro phenyl)-2-methyl propan-2-ol (52) can be made easily and inexpensively from common reagents (Scheme XXXI) and thus could be made easily on a large scale. The compound is easily purified by distillation and has been used for the protection of the side chain of aspartic acid in good yield (Scheme XXIV). The isolation of this derivative (58) required no complex purification techniques and was achieved by simply precipitating the desired compound out of solution with the addition of diethyl ether.

Subsequent derivatisation (Scheme XXVI) and use of the product (61) in solid phase peptide synthesis presented few problems.

This work has also explored the use of other protecting groups namely the 3-ethyl pent-3-yl and 1-(3'-trifluoromethyl phenyl)-2-methyl prop-2-yl groups (Schemes XIV and XVIII). These groups, although found unsuitable for the particular approach used for solid phase synthesis in this project, could be of use with other strategies or, indeed, other branches of organic chemistry.

This research project has answered the main questions it was originally intended to address. It has, as with most research projects, revealed many new unanswered questions.

CHAPTER:3

Experimental.

Notes.

Preparations.

CHAPTER 3: EXPERIMENTAL

Notes

All amino acids were purchased from the SAS group of companies and were used as supplied. Melting points were recorded on a Reichert 7905 melting point apparatus and are uncorrected. Optical rotations were measured on a AA 1000 polarimeter (Optical Activity Ltd) using a 10 cm cell. Infra-red spectra were measured on a Perkin Elmer 781 spectrophotometer in dichloromethane unless otherwise stated. Ultraviolet (U.V.) spectra were recorded in dry, distilled dichloromethane on a SP8-400 spectrophotometer. Mass spectra and high resolution mass spectra (H.R.M.S) were recorded on a Kratos MS50TC spectrometer. High performance liquid chromatography was carried out using a Waters system using two 6000A pumps, a U6K model injector, a 660 automatic gradient controller and a Waters UV detector (Model 441) operating on 254 or 229 nm. H.p.l.c. solvent systems were as quoted in the text. Amino acid analyses were carried out on an LKB 4150 alpha amino acid analyser following sealed tube hydrolysis in constant boiling hydrochloric acid at 110°C for 18 hours. Elemental analyses were carried out on a Carl Erba elemental analyser model 1106. ^{1}H n.m.r spectra were recorded on either Bruker WP 80 (80 MHz), WP 200 (200 MHz) or WH 360 (360 MHz) machines in the solvent indicated using tetramethylsilane (TMS) as the external standard (δ = 0.00). ^{19}F n.m.r. spectra (broad band ^{1}H decoupled)

were recorded on a Bruker WP 80 machine operating at 75.4 MHz and ^{13}C n.m.r spectra recorded on either a Bruker WP 200 machine operating at 50.3 MHz or a Bruker WH 360 machine operating at 90 MHz. Samples were dissolved in the solvent indicated and chemical shifts were measured relative to TMS assigned at zero. All solvents were distilled before use and the following were dried using the reagents given in parentheses; acetonitrile (calcium hydride), chloroform (phosphorous pentoxide), dichloromethane (calcium hydride), tetrahydrofuran (sodium benzophenone), dioxan (sodium benzophenone), diethyl ether (sodium wire), toluene (sodium wire), N,N-dimethylformamide (calcium hydride), acetone (4A molecular sieves) and methanol (magnesium-iodine). Thin layer chromatography (t.l.c) was carried out on plastic sheets precoated with silica gel 60GF-254 (Merck 5735) in the following systems.

A 100% Petroleum ether (b.p. 40-60°C).

B 95% Petroleum ether/5% ethyl acetate.

C 50% Petroleum ether/50% dichloromethane.

D 70% Petroleum ether/30% diethyl ether.

E 50% Petroleum ether/50% diethyl ether

F 100% Dichloromethane.

G 80% Petroleum ether/20% diethyl ether.

H 80% Petroleum ether/20% ethyl acetate.

I 60% n-Butanol/20% water/20% acetic acid.

J 20% Methanol/80% chloroform.

K 10% Methanol/90% chloroform.

3-Ethyl pentan-3-ol (35)

To a solution of the Grignard reagent prepared from freshly distilled ethyl bromide (109 g, 1 mol.) and magnesium metal (24.30 g, 1 mol.) in anhydrous diethyl ether (500 ml) was added slowly, with stirring, dried ethyl propionate (40.85 g, 0.40 mol.) in an equal volume of diethyl ether (50 ml). After stirring at room temperature overnight the reaction mixture was heated on a water bath at 60°C for 1 hour. The mixture was then decomposed by adding to a mixture of water, ice and excess dilute hydrochloric acid (2M). This solution was then extracted with diethyl ether (2 x 150 ml). The combined extracts were then washed with saturated sodium hydrogen carbonate solution (2 x 100 ml), distilled water (3 x 100 ml) before drying over anhydrous sodium sulphate. The solution was then filtered and the solvent removed in vacuo to yield a clear liquid. This was then distilled and the fraction boiling at 139°C (1 atm) collected (37.2 g, 80%) (lit.[56,57] b.p.139°C, 1 atm). The viscous liquid had a penetrating camphor-like smell. Vmax 3610cm^{-1} (OH); δH ($CDCl_3$, 80MHz) 1.39 (6H,q $^3J_{CH2-CH3}$ 7.5Hz, CH_2's), 0.81 (9H, t,$^3J_{CH2-CH3}$ 7.4 Hz, CH_3's).

3-Ethyl pent-2-ene (34)

3-Ethyl pentan-3-ol (35) (19.2 g, 0.165 mol.) was distilled slowly in the presence of iodine (0.1 g). The distillate was then washed with sodium

thiosulphate solution (2 x 10 ml, 0.05M), and distilled water (2 x 10 ml), then dried over anhydrous calcium chloride. The liquid was then filtered and twice distilled from sodium metal to yield the desired olefin (11.32 g, 70%) b.p 95-97°C (1 atm) (lit.[58] b.p 95-97°C, 1 atm).

Hydrocinnamic acid t-butyl ester (40)

Hydrocinnamic acid 3-ethyl pent-3-yl ester (38)

To a solution of t-butanol (3.70 g, 0.05 mol.) or 3-ethyl pentan-3-ol (5.81 g, 0.05 mol.) in dry tetrahydrofuran (75 ml) was added, under dry nitrogen, during several minutes, n-butyl lithium in hexane (39 ml; 1.4M, 0.055 mol.). After 30 minutes, to the resulting yellow solution was added, during 5 minutes, by dropwise addition, a solution of hydrocinnamoyl chloride (9.30 g,0.55 mol.)in tetrahydrofuran (50 ml). The resulting solution was brought to reflux for 1 hour, cooled to 0°C using ice, and hydrolysed by the addition of distilled water (100 ml). This was then extracted with three portions (3 x 50 ml) of diethyl ether and the combined organic phases washed with distilled water (3 x 50 ml) and dried over anhydrous sodium sulphate. The solution was then filtered and concentrated in vacuo to yield a clear liquid which was purified by vacuum distillation.

Hydrocinnamic acid t-butyl ester (40), (7.21 g, 70%); b.p 119-121°C (7 mm) (lit[60] 120-122°C (7 mm)) t.l.c-A Rf 0.64; Vmax (CH_2Cl_2) 1720cm^{-1} (ester CO); δ_H

($CDCl_3$, 80MHz), 7.25 (5H, m, Ar-H), 2.94-2.55 (4H, br. m, CH_2's), 1.44 (9H, s, CH_3's).

Hydrocinnamic acid 3-ethyl-pent-3-yl ester; (8.06 g, 65%); b.p 110°C (0.2 mm); (Found: C, 77.0; H, 9.9; $C_{16}H_{24}O_2$ requires C, 77.4, H,9.7%) t.l.c-B Rf 0.73 (alumina); Vmax (CH_2Cl_2) 1730 cm^{-1} (ester C=O); δ_H ($CDCl_3$, 80 MHz), 7.25 (5H, m, Ar-H), 3.00-2.48 (4H, br.m, CH_2's) 1.83 (6H,q $^3J_{CH2-CH3}$ 7.6 Hz, ethyl CH_2'S) 0.80 (9H,t,$^3J_{CH2-CH3}$ 7.2Hz, ethyl CH_3's). δc ($CDCl_3$, 360 MHz), 170.80 (ester C=O), 140.78 (aromatic C_1) 128.36 (aromatic C_2,C_6),128.19 (aromatic C_3,C_5), 126.00 (aromatic C_4), 88.29 (O-C(CH_2CH_3)$_3$), 36.78(-CH_2-CO),31.10(-CH_2-CH_2-CO-),26.79(O-C(CH_2CH_3)$_3$) 7.48 (CH_3's); m/z (FAB) 249, 207, 163, 151, 133; H.R.M.S. 249.18545 $C_{16}H_{25}O_2$ (MH^+) requires 249.18544.

3-(Trifluoromethyl phenyl)acetic acid methyl ester(83)
4-Fluorophenylacetic acid methyl ester (85)

3-(Trifluoromethyl phenyl) acetic acid (82) (10.2 g, 0.05 mol.) or 4-fluorophenyl acetic acid (84) (7.71 g, 0.05 mol.) was dissolved in methanol (125 ml) after which concentrated sulphuric acid (98%, 3 ml) was slowly added to the solution with stirring. The reaction mixture was then heated under reflux for 8 hours. After this period the solution was added to distilled water (300 ml) which was then extracted with ethyl acetate (2 x 100 ml). The combined extracts were then washed with saturated sodium hydrogen carbonate solution (2 x 100 ml), distilled water (2 x

100 ml), brine (100 ml) and finally dried over anhydrous sodium sulphate. The solution was then filtered and the solvent removed in vacuo to yield a clear liquid. This crude product was then distilled under reduced pressure using a small bore, 15cm long, vigreux column.

3-Trifluoromethyl phenyl acetic acid methyl ester. (10.25g, 94%), b.p 62°C (0.1mm); (Found: C, 55.2; H,4.1% calc. for $C_{10}H_9F_3O_2$: C,55.0; H,4.2%); t.l.c-C Rf 0.34; Vmax (CH_2Cl_2) 1740 cm^{-1} (ester C=O); δ_H ($CDCl_3$, 80 MHz) 7.50 (4H, m, Ar-H), 3.71 (3H, s, methyl ester), 3.68 (2H, s, CH_2); δ_F ($CDCl_3$, 80 MHz) -62.4; δ_c ($CDCl_3$, 200 MHz) 170.93 (C=O), 135.00, 130.42 (aromatic quaternaries), 132.61, 128.80, 125.90, 123.73 (aromatic C-H), 124.00 (q, $^1J_{C-F}$ 272Hz, CF_3), 51.68 (methyl ester), 40.40 (CH_2); m/z (EI) 218, 159, 59,32. H.R.M.S. 218.0556, $C_{10}H_9F_3O_2$ requires 218.1731.

4-Fluorophenylacetic acid methyl ester. (7.57 g, 90%), b.p 58°C (0.2 mm Hg); (Found: C, 64.5; H, 5.46% $C_9H_9FO_2$ requires C, 64.3; H, 5.4%); t.l.c-D Rf 0.36; Vmax (CH_2Cl_2) 1740 cm^{-1} (C=O); δ_H ($CDCl_3$, 80 MHz) 7.35-6.87 (4H, br.m, Ar-H), 3.70 (3H,s, methyl ester), 3.60 (2H, s, CH_2); δ_F ($CDCl_3$, 80 MHz).
δc ($CDCl_3$, 200 MHz) 171.60 (C=O), 161.80 (d, $^1J_{C-F}$ 245.6 Hz, C-F), 130.56 (d, $^2J_{C-F}$ 7.7Hz, aromatic C_3, C_5), 129.44 (quaternary C_1), 115.14 (d, $^3J_{C-F}$ 21.5 Hz, aromatic C_2, C_6), 51.78 (OCH_3), 39.97 (CH_2); m/z 169, 149 137 H.R.M.S. 169.06650 $C_9H_{10}O_2F_1$ (M^+) requires 169.006648.

1-(3'-Trifluoromethyl phenyl)-2-methyl propan-2-ol (45)

1-(4'-Fluoro phenyl)-2-methyl propan-2-ol (52)

To the Grignard reagent prepared from methyl iodide (10.22 g, 0.072 mol.) and magnesium metal (1.75 g, 0.072 mol.) in diethyl ether (85 ml) was added dropwise, under nitrogen, and with stirring either 3-trifluoromethyl phenylacetic acid methyl ester (83) (6.54g, 0.03 mol) or 4-fluorophenylacetic acid methyl ester (85) (5.04 g, 0.03 mol) in diethyl ether (30ml). The reaction was stirred overnight and then heated under reflux for 1 hour, resulting in a light grey solution. This was then added to iced saturated ammonium chloride solution (300 ml) with vigorous stirring and the organic phase was separated. The aqueous phase was then extracted with two portions of diethyl ether (2 x 100 ml). The combined extracts were then washed with saturated sodium hydrogen carbonate solution (2 x 100 ml), water (3 x 100 ml), and brine (100 ml) and finally dried over anhydrous sodium sulphate. The solution was then filtered and concentrated *in vacuo* to yield either a white solid (45) which was recrystallised from petrol (40/60) and dried *in vacuo* or a clear viscous liquid (52) which was purified by distillation under reduced pressure using a small bore vigreux column.

1-(3'-Trifluoromethyl phenyl)-2-methyl propan-2-ol (45) (5.23 g, 80%); mp 52-56°C; (Found: C, 60.8; H, 6.0; $C_{11}H_{13}F_3O$ requires C,60.6; H, 6.0%); t.l.c -E Rf 0.19;

Vmax (CH_2Cl_2) 3600 cm^{-1} (OH); λmax (CH_2Cl_2) 265 (ε 595); δ_H ($CDCl_3$, 200 MHz), 7.51-7.39 (4H, m, Ar-H), 2.80 (2H, s, CH_2), 1.49 (1H, br.s, OH), 1.22 (6H, s, CH_3's); δ_F ($CDCl_3$, 80 MHz) -63.60; δ_c ($CDCl_3$, 360 MH_z), 138.87 (quaternary C_1), 133.71 (C_4), 130.10 (q, $^2J_{C-F}$ 32 Hz, quaternary C_3), 128.73 (C_2), 126.90 (d, $^4J_{C-F}$ 3.0 Hz, C_5) 124.10 (q, $^1J_{C-F}$ 272 Hz, CF_3), 122.94 (d, $^5J_{C-F}$ 3.4 Hz, C_6), 70.67 (-C(CH_3)$_2$OH), 49.18 (CH_2), 28.76 (CH_3'S); m/z (FAB) 218, 203, 159. H.R.M.S 218.1228 calc. for $C_{11}H_{13}F_3O$ 218.2173.

1-(4'-Fluoro phenyl)-2-methyl propan-2-ol (52) (3.78 g, 75%); b.p 68°C (0.6 mm); (Found: C, 71.3; H, 7.9 $C_{10}H_{13}FO$ requires C, 71.4; H, 7.8%); t.l.c-D Rf 0.31; Vmax (CH_2Cl_2) 3600 cm^{-1} (OH); λmax (CH_2Cl_2) 266 (ε 648); δ_H ($CDCl_3$, 80 MH_z) 7.26-6.85 (4H, m, Ar-H), 2.72 (2H, s, CH_2), 1.42 (1H, br.s, OH), 1.20 (6H,s, CH_3's); δ_F ($CDCl_3$, 80 MHz) -116.61; δ_c ($CDCl_3$, 200 MH_z) 161.59 (d, $^1J_{F-C}$ 244.2 Hz, C_4-F), 133.52 (C_1), 131.62 (d, $^2J_{F-CH}$ 8.0 Hz, C_3,C_5), 114.59 (d, $^3J_{F-CH}$ 21.3 Hz, C_2,C_6), 70.46 (-C(CH_3)$_2$OH), 48.72 (CH_2), 28.88 (CH_3's); m/z (FAB) 168, 153, 83 H.R.M.S. 168.0951 $C_{10}H_{13}FO$ requires 168.0952.

<u>Alternative Preparation of 1-(4'fluoro phenyl)-2-methyl propan-2-ol (52)</u>

4-Fluorotoluene (67) (20.0 g, 0.18 mol) was brominated by volatilising bromine (30.0 g, 0.10 mol) in a slow stream of dry air and passing the vapour into the boiling 4-fluorotoluene. The solution was

then heated under reflux for 1 hour and allowed to cool. The oil was then dissolved in a large excess of diethyl ether (300 ml) which was then washed with distilled water (3 x 300 ml) and sodium hydroxide solution (300 ml, 0.1 M). Finally the organic phase was washed with sodium bisulphate solution (300 ml, 0.1 M) to remove any 4-fluorobenzaldehyde and dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo afforded a clear viscous oil which was distilled to give 4-fluorobenzyl bromide (30.6 g, 90%), b.p. 85°C (15 mm), (lit.[68] b.p 85°C, 15 mm).

The 4-fluorobenzyl bromide (10.0 g, lit 0.053 mol.) was dissolved in diethyl ether (40 ml). This was added slowly and dropwise to magnesium turnings (1.28 g, 0.052 mol.) stirred in diethyl ether (25 ml). The reaction required gentle heating to initiate and was seen to turn progressively more green in colour. After addition of all the 4-fluorobenzyl bromide heating was continued for a further 15 minutes after which excess dry, distilled acetone (7.4 ml, 0.1 mol.) was added slowly with stirring. After complete addition the solution was again heated gently and allowed to cool. Work-up for this Grignard reaction was as previously described with the desired alcohol (52) being purified by distillation in vacuo, b.p 68°C (0.6 mm). Overall yield from 4-fluorotoluene was 72% with analytical data consistent with previously stated.

3-(3'-Trifluoromethyl phenyl)-2-methyl pentan-3-ol (46)

Under nitrogen, with stirring, ethyl bromide (1.31 g, 0.012 mol.) in diethyl ether (10 ml) was added slowly to magnesium turnings (0.29 g, 0.012 mol.) in diethyl ether (35ml). This resulted in the reaction reaching reflux, the temperature was controlled by cooling with ice. The solution become brown/grey in colour and was stirred at room temperature for a further hour after all the ethyl bromide had been added. 3-(Trifluoromethyl phenyl) acetic acid methyl ester (83) (1.09 g, 0.005 mol.) in diethyl ether (10 ml) was then added gradually to the stirred solution again resulting in some heat being released. The reaction was then stirred overnight. After this period the solution, which had become slightly green in colour, was refluxed for 30 minutes to ensure complete reaction. The reaction mixture was then added, with vigorous stirring, to a mixture of dilute hydrochloric acid (2M; 50 ml), distilled water (250 ml) and crushed ice. The organic phase was then separated. The aqueous layer was further extracted with two portions (2 x 100 ml) of diethyl ether. The combined extracts were then washed with a solution of saturated sodium hydrogencarbonate (2 x 50 ml), distilled water (2 x 50 ml), brine (50 ml) and dried over anhydrous sodium sulphate. On filtering and evaporation *in vacuo* a clear viscous liquid (1.120 g) was obtained.

This was then purified by silica flash chromatography using dichloromethane/petrol (b.p 40-60°C) as eluent. This gave the title compound (46) (0.735g, 60%); (Found: C, 63.7; H, 6.9 $C_{13}H_{17}F_3O$ requires C, 63.4; H, 7.0%); t.l.c-C Rf 0.27; Vmax 3600 cm^{-1} (OH); λmax (CH_2Cl_2) 265 (ε601); δ_H ($CDCl_3$, 80 MHz) 7.85-7.44 (4H,m,Ar-H), 2.80 (2H,s, phenylmethyl CH_2), 1.62-1.33 (5H, br.s, q, $^3J_{CH2-CH3}$ 5.9 Hz, pentyl CH_2's and OH), 0.93 (6H, t, $^3J_{CH2-CH3}$ 6.7 Hz, CH_3's); δ_F ($CDCl_3$, 80 MHz) -62.26; δ_C ($CDCl_3$, 200 MH_z), 140.28 (quaternary C_1) 135.38 (C_4), 132.5 (q, $^2J_{C-F}$ 30 Hz, quaternary C_3), 129.72 (C_2), 128.53 (d, $^4J_{C-F}$ 3.7 Hz, C_5) 125.50 (q, $^1J_{C-F}$ 270 Hz, CF_3), 123.05 (C_6), 76.05 (-C(C_2H_5)$_2$-OH), 45.96 (Ar-CH_2-), 31.94 (-C(CH_2CH_3)$_2$-OH) 9.14 (-C(CH_2CH_3)$_2$-OH) m/z (FAB) 245, 229, 217, 187, 173, 159. H.R.M.S. 245.11532 $C_{13}H_{16}F_3O$ requires 245.11531.

Hydrocinnamic acid 1-(3'-trifluoromethyl phenyl)-2-methyl prop-2-yl ester (47)

Hydrocinnamic acid 1-(4'-fluoro phenyl)-2-methyl prop-2-yl ester (53)

1-(3'-Trifluoromethyl phenyl)-2-methyl propan-2-ol (45) (1.09 g, 0.005 mol.) or 1-(4'-fluoro phenyl)-2-methyl propan-2-ol (52) (0.84 g, 0.005 mol.) was dissolved in anhydrous tetrahydrofuran (15 ml) under dry nitrogen. To this was added, with stirring, n-butyllithium (3.67 ml; 1.2M, 5.5 mmol.) in hexane. The solution was seen to turn dark red in colour

indicating the formation of the lithium salt. The solution was stirred for a further 30 minutes after which hydrocinnamoyl chloride (0.924g, 5.5 mmol.) in THF (20 ml) was added dropwise. The red colour disappeared and the solution was heated under reflux for 3 hours. After this period distilled water (50ml) was added and the solution was extracted with ethyl acetate (2 x 50 ml). The combined extracts were then washed with saturated sodium hydrogen carbonate solution (2 x 50 ml), distilled water (2 x 50 ml) and brine (50 ml) and finally dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo gave a clear liquid which was purified by chromatography on an alumina (grade 2) column using gradient elution with petrol (40/60)/diethyl ether.

Hydrocinnamic acid 1-(3'-trifluoromethyl phenyl)-2-methyl prop-2-yl ester (47) (1.23 g, 70%); (Found: C, 68.6; H, 6.3. calc. for $C_{20}H_{21}F_3O_2$; C, 68.6; H, 6.0%); t.l.c-F Rf 0.75; Vmax (CH_2Cl_2) 1725 cm^{-1} (C=O); λmax (CH_2Cl_2) 254 (ε 769); δ_H ($CDCl_3$, 200 MHz), 7.55-7.18 (9H, br.m, Ar-H), 3.09 (2H, s, $OC(CH_3)_2\underline{CH_2}$-), 2.97-2.53 (4H, br.m, $PhC\underline{H_2}C\underline{H_2}$-), 1.46 (6H, s, $-OC(C\underline{H_3})_2CH_2$-); δ_F ($CDCl_3$, 80 MHz), -67.25; δ_C ($CDCl_3$, 200 MHz) 172.12 (carbonyl), 140.47 (phenyl C_1), 138.10 (CF_3-Ar, C_1), 133.74 (CF_3-Ar, C_4), 130.30 (q, $^2J_{C-F}$ 31.5 Hz, CF_3-Ar, C_3) 128.30 (phenyl C_2, C_6), 128.19 (phenyl C_3, C_5), 127.13 (CF_3-Ar, C_2), 126.06 (CF_3-Ar, C_5), 125.99 (phenyl C_4), 123.20 (CF_3-Ar, C_6) 81.48 ($O\underline{C}(CH_3)_2$-),

46.34 ($O-C(CH_3)_2CH_2-$), 36.93 ($PhCH_2CH_2-$), 30.92 ($Ph\underline{C}H_2CH_2-$), 25.81 (CH_3's); $\underline{C}F_3$ was undistinguished; m/z (FAB) 350, 311, 241; H.R.M.S. (FAB) 350.14935. calc. for $C_{20}H_{21}F_3O_2$ requires 350.14934.

Hydrocinnamic acid 1-(4'-fluoro phenyl)-2-methyl prop-2-yl ester (53) (0.86 g, 60%) (Found: C, 77.18; H, 7.26 calc. for $C_{19}H_{21}O_2F$ C, 76.91; H, 6.99%.); t.l.c-F Rf 0.70; Vmax (CH_2Cl_2) 1730 cm^{-1} (C=O); λmax (CH_2Cl_2) 257 (ε 760); δ_H ($CDCl_3$,80 MHz), 7.30-6.93 (9H, br.m, Ar-$\underline{H}$), 3.01 (2H,s,$OC(CH_3)_2C\underline{H}_2-$), 2.96-2.55 (4H, br.m, $PhC\underline{H}_2C\underline{H}_2-$), 1.42 (6H,s,$-OC(C\underline{H}_3)_2CH_2-$); δ_F ($CDCl_3$, 80 MHz) -116.45; δc ($CDCl_3$, 200 MHz), 172.10 (carbonyl), 161.53 (d, $^1J_{C-F}$ 244.0 Hz, F-Ar C_4) 140.44 (phenyl C_1), 132.67 (d, $^4J_{C-F}$ 3.3 Hz, F-Ar C_1), 131.68 (d, $^2J_{C-F}$ 7.76 Hz, F-Ar C_3, C_5) 128.21 (phenyl C_2,C_3, C_5,C_6), 126.0 (phenyl C_4), 114.52 (d, $^3J_{C-F}$ 21.3 Hz, F-Ar C_2,C_6), 81.74 ($O\underline{C}(CH_3)_2-$),45.42 ($O-C(CH_3)_2\ \underline{C}H_2-$), 36.85 ($PhCH_2\underline{C}H_2-$), 30.79 ($Ph\underline{C}H_2CH_2-$), 25.67 ($CH_3$'s); m/z (FAB) 301, 283, 259, 189; H.R.M.S (FAB) 301.16037 calc. for $C_{19}H_{22}O_2F$ 301.16037.

<u>Benzyl(N^{α}-benzyloxycarbonyl)-β-(1-(4'-fluoro phenyl)-2-methyl prop-2-yl)-L-aspartate (57)</u>

N-Benzyloxycarbonyl-benzyl-L-aspartate (56) (3.563 g, 0.01 mol.), 1-(4'-fluoro phenyl)-2-methyl propan-2-ol (52) (1.677 g, 0.01 mol.) and 4-dimethyl-amino pyridine (0.122 g, 0.001 mol) was dissolved in dichloromethane (25 ml) and cooled to 0°C. To this solution, over a period of 2 minutes, was added, with

stirring, dicyclohexylcarbodiimide (2.500 g, 0.012 mol). The reaction was stirred for a further 5 minutes at 0°C then at 4°C for 3 days. After this period the cloudy solution was filtered and the solvent removed in vacuo. The residue was then redissolved in ethyl acetate (50 ml), cooled and filtered through celite to remove any remaining dicyclohexylurea. The filtrate was then washed with saturated sodium hydrogen carbonate solution (2 x 50 ml), distilled water (2 x 50 ml) and dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo afforded the crude product, a viscous oil (5.35 g), which was usually used in this form the next stage. The fully protected amino acid could, however, be purified by silica flash chromatography using ethyl acetate/petrol (40/60) as the eluent. Separation of the protected amino acid and alcohol starting material did, however, prove difficult, consequently reducing the overall yield. (2.79 g, 55%); (Found: C, 66.5, H, 5.85; N, 2.74 calc. for $C_{29}H_{30}FNO_6$ C, 68.6; H, 6.0; N, 2.8%); t.l.c-H Rf 0.36; Vmax 3430 cm^{-1} (NH), 1720 cm^{-1} (broad, C=O's); $[\alpha]_D^{25}$ -3.3; λmax (CH_2Cl_2) 265 (ε 1056); δ_H ($CDCl_3$, 200 MHz), 7.34 (10H,m,benzyl CH's), 7.00 (4H, m, F-Ar-H's), 5.80 (1H, br.s, NH), 5.18 (2H, s, benzyloxy CH_2), 5.13 (2H, s, benzyl CH_2), 4.66 (1H, br.m, CH),3.03-2.70 (4H, br.m, CH_2 and $OC(CH_3)_2C\underline{H}_2$),1.26 (6H, s, $OC(C\underline{H}_3)_2CH_2$); δ_F ($CDCl_3$, 360 MH_z) 170.58 (α C=O),169.91 (βC=O),

161.66 (d, $^{1}J_{F-C}$ 245 Hz, C-F), 155.86 (urethane C=O), 136.05 (benzyl C_1), 135.10 (benzyloxy C_1) 132.38 (F-Ar C_1), 131.67 (d, $^{2}J_{F-CH}$ 8.7 Hz, F-Ar C_3,C_5), 128.40 (benzyl CH's), 114.73 (d, $^{3}J_{F-CH}$ 21.1 Hz F-Ar C_2, C_6), 83.35 ($O\underline{C}(CH_3)_2CH_2$) 67.31, 66.97 (benzyloxy, benzyl CH_2's),50.46 (αCH),45.21 (β CH_2), 37.64 ($OC(CH_3)_2\underline{C}H_2$-) 25.73, 25.66 ($OC(\underline{C}H_3)_2CH_2$-); m/z (FAB) 508; H.R.M.S. 508.21354, $C_{29}H_{31}FNO_6$ (MH^+) requires 508.21352.

L-Aspartic acid β-(1-(4'-fluoro phenyl)-2-methyl-prop 2-yl) ester (58)

N^{α}-Benzyloxycarbonyl-β-(1-(4'-fluoro phenyl)-2-methyl-prop-2-yl)-L-aspartate (57) (2.25 g, 0.004 mol.) was dissolved in 2% water/methanol solution (20 ml) and cooled to 0°C. Palladium on charcoal catalyst (5%; 0.2 g) was then added slowly and the solution stirred at room temperature under an atmosphere of hydrogen. The hydrogenolysis was followed by t.l.c and was complete in 1 hour. The solution was then filtered through celite and concentrated in vacuo to half volume. Diethyl ether (approximately 350 ml) was then added gradually until a colourless jelly-like suspension formed. This was filtered and dried in vacuo overnight to yield a white solid (0.80 g, 64%). The filtrate was evaporated to dryness and the residue was redissolved in the minimum amount of methanol to which ethyl acetate was then added to obtain a further quantity of the same product (65 mg; total yield 72%), m.p 169-172 °C; (Found: C, 58.9; H, 6.4; N, 4.9; F,

6.89 calc for $C_{14}H_{18}FNO_4$; C, 59.3; H, 6.4; N, 4.9; F, 6.7%; $[\alpha]_D^{25}$ + 5.4; t.l.c-I Rf 0.2; Vmax (KBr Disc) 1730 cm^{-1} (ester C=O); λmax (CH_2Cl_2 265 (ε 635); δ_H (CD_3OD, 200 H_z) 7.31 (2H, overlapping d of d, $^3J_{F-CH}$ 5.5 Hz, $^3J_{F-CH}$ 8.8 Hz, meta CH's), 7.15 (2H, overlapping d of d, $^4J_{F-CH}$ 8.8Hz, $^3J_{CH-CH}$ 8.8Hz, ortho CH's), 3.90 (1H, d of d, $^3J_{CH-CH_2}$ 8.0Hz, 4.0Hz, CH), 3.16 (2H, s, $OC(CH_3)_2$ $\underline{C}H_2$-), 3.04 (1H, d of d, $^2J_{gemCH_2}$ 18Hz, $^3J_{CH-CH_2}$, geminal CH_2), 2.86 (1H, d of d, $^2J_{gemCH_2}$ 18Hz, $^3J_{CH-CH_2}$ 4 Hz, geminal CH_2), 1.56 (6H, s, $OC(\underline{C}H_3)_2CH_2$-); δ_F (CD_3OD, 80MH_z) -116.1; δc (CD_3OD, 200MH_z) 170.63, 170.10 (C=O's), C_4 quaternary not observed, 132.30 (quaternary C_1), 131.25 (d, $^2J_{C-F}$ 7.6Hz, C_3, C_5), 113.63 (d, $^3J_{C-F}$ 21.4 Hz, C_2, C_6), 82.97 ($O\underline{C}(CH_3)CH_2$-), 50.44 (αCH), 44.62 (βCH_2), 34.92 ($OC(CH_3)_2CH_2$), 24.19 ($OC(\underline{C}H_3)_2CH_2$-); m/z (FAB) 284, H.R.M.S. 284.12981, $C_{14}H_{19}FNO_4$ (MH^+) requires 284.12980.

<u>N^{α}-[9-Fluorenylmethyloxycarbonyl]-β-[1-4'-fluoro phenyl) -2-methyl prop-2-yl]-L-aspartic acid (61)</u>

To a stirred suspension of β-[1-(4'-fluoro phenyl) -2-methyl prop-2-yl]-L-aspartate (58) (1.101 g, 3.890 mmol) in distilled water (5 ml) was added a solution of triethylamine (0.60 g, 5.8 mmol) in dioxan (2 ml). To the resultant solution was added solid 9-Fluorenyl-methyl succinimidyl carbonate (1.31 g, 3.89 mmol). The solution was stirred at room temperature overnight, diluted with water (20 ml), acidified to pH

2.5 with saturated potassium hydrogensulphate solution and extracted with ethyl acetate (2 x 50 ml). The combined organic extracts were washed with water (3 x 50 ml) and dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo afforded a white/ yellow solid (2.134 g). This was recrystallised from chloroform/petrol (b.p 60-80°c) to give the desired product. (1.47 g, 75%); (Found: C, 68.8; H, 5.64; N, 2.89. calc for $C_{29}H_{28}FNO_6$ C,68.90; H, 5.58; N,2.77%); $[\alpha]^{25}$ =-3.3 t.l.c-K Rf 0.50; Vmax 3425cm^{-1} (NH), 1730cm^{-1} (C=O's) λmax(CH_2Cl_2) 266 (ε 16917); δ_H(CD_3COCD_3, 200 Mz), 7.88-7.00 (12H, br.m, Fmoc, Phenyl Ar-H's), 4.65 (1H, m, CH), 4.35 (3H,m,Fmoc CH,CH_2), 3.10 (2H,s, F-Ar-$C\underline{H}_2$-), 2.85 (2H,m,β CH_2), 1.45 (6H,s,OC($C\underline{H}_3$)$_2$-); δ_F($CDCl_3$, 8 MHz), -116.00; δ_c(CD_3COCD_3, 200 MHz), 170.95, 168.98, 155.17 (C=O's), 160.94 (d,'J_{C-F} 242.8 Hz, C_4-F), 143.35, 140.42 (Fmoc quaternary's), 132.54 (F-Ar C_1) 131.43 (d, $^2J_{F-CH}$ 7.8 Hz, F-Ar C_3,C_5), 126.87, 126.27, 124.46 119.14 (Fmoc CH_1s), 113.76 (d, $^3J_{F-CH}$ 21.1 Hz, F-Ar C_2,C_6), 81.64 (O$\underline{C}$(CH_3)$_2$$CH_2$-), 65.72 (Fmoc CH_2), 49.79 (Fmoc CH), 46.30 (Asp CH), 44.11 (Asp β CH_2), 36.75 (F-Ar-$\underline{C}H_2$-), 24.47 (CH_3's); m/z (FAB) 506, 490, 471, 179; H.R.M.S (FAB) 506.1978 (M^+) requires 506.19787.

N[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-β-[1-(4'-fluorophenyl)-2-methyl prop-2-yl]-L-aspartic acid cyclohexylammonium salt (62)

To a stirred suspension of L-aspartic acid β-[1-(4'-fluorophenyl)-2-methyl prop-2-yl] ester (58) (0.682 g, 2.4 mmol) in distilled water (2 ml) was added a solution of triethylamine (0.282 g, 2.7 mmol) in dioxan (2 ml). To the resultant solution was added solid 2,2-bis(4-nitrophenyl)ethyl-N-succinimidyl carbonate (60) (1.034 g, 2.4 mmol). The solution was stirred at room temperature overnight, then diluted with distilled water (10 ml), acidified to pH 2.5 with saturated potassium hydrogen sulphate solution and extracted into ethyl acetate (3 x 25 ml). The combined organic extracts were washed with water (3 x 25 ml) and dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo afforded a clear oil which was dissolved in the minimum amount of diethyl ether (40 ml). Cyclohexylamine (0.239 g, 0.0024 mol) in diethyl ether (5 ml) was then added to the cooled solution. A white precipitate formed, this being filtered off after 2 hours stirring at room temperature. The white solid was dried in vacuo overnight (1.45 g,86%),m.p 117-118°C; (Found: C,60.10; H, 5.86; N, 7.83; F, 2.53; $C_{35}H_{41}FN_4O_{10}$ requires C, 60.34; H, 5.93; N 8.04; F, 2.73%); $[\alpha]^{25}_D$=+1.50; t.l.c

-J Rf 0.33; Vmax (CH_2Cl_2) 1730cm^{-1} (C=O's), 1525cm^{-1}, 1350cm^{-1} (NO_2); λmax (CH_2Cl_2) 273(ε 16061); δ_H (CD_3OD, 200 MHz), 8.22 (4H, br.d, ^3J 8.5 Hz, nitrophenyl meta CH's), 7.64 (4H, br.d, ^3J 8.19 Hz, nitrophenyl ortho CH's), 7.22 (2H, complex, meta F-Ar-H), 7.00 (2H, complex, ortho F-Ar-H),4.79 (3H, br.m, Bnpeoc CH,CH_2), 4.36 (1H,m, CH), 3.07 (2H,s, F-Ar-CH_2-), 2.85 (2H,m, βCH_2), 2.25-1.00 (16H, br.m,CHA protons, OC(CH_3)$_2CH_2$); δ_c(CD_3OD, 200 MHz), 175.54, 170.50, 155.54 (C=O's), 160.91(d,'J_{F-C} 243.0Hz, C-F),147.21 (Bnpeoc C_4-NO_2's), 146.35 (Bnpeoc C_1's), 132.48 (F-Ar C_1), 131.26 (d, $^2J_{F-CH}$ 7.71 Hz,F-Ar C_3,C_5), 128.74 (Bnpeoc C_3's,C_5's), 122.90 (Bnpeoc C_2's,C_6's), 133.64 (d, $^3J_{F-CH}$ 21.32 Hz, F-Ar C_2,C_6), 81.66 (OC(CH_3)$_2CH_2$-), 65.11 (Bnpeoc CH_2) 52.35 (Asp αCH), 49.48 (Bnpeoc CH_2) 49.05 (Asp β CH_2), 24.31, 23.95, 23.45 (CHA CH_2's); m/z (FAB) 697, 517, 448, 440, 430; H.M.R.S. (FAB) 697.28844 $C_{35}H_{42}N_4O_{10}$ F(MH^+) requires 697.28847.

N-t-Butoxycarbonyl-β-[1-(4'-fluoro phenyl)-2-methyl-prop-2-yl]-L-aspartic acid cyclohexylammonium salt (64)

L-Aspartic acid β-[1-(4'fluoro phenyl)-2-methyl-prop-2-yl] ester (58) (0.535 g, 1.9 mmol) was dissolved in sodium hydroxide solution (10 ml, 0.2 M) at room temperature after which dioxan (10 ml) was added. Di-tert-butyl pyrocarbonate (0.411 g, 1.9 mmol) in dioxan (3 ml) was then added slowly with

stirring and the solution stirred for a further 2 hours. After this period the solution was concentrated in vacuo and washed with two portions of diethyl ether (2 x 10 ml). Ethyl acetate (50 ml) was then added and the solution acidified with dilute potassium hydrogen sulphate solution to pH 2.5. The organic phase was washed with distilled water (2 x 50 ml), brine (50 ml) and dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo resulted in a colourless oil being obtained. This was dissolved in diethyl ether (50 ml) and cyclohexylamine (0.186 g, 1.9 mmol) was added slowly at 0°C. The solution was stirred overnight after which the white precipitate formed was filtered, washed with diethyl ether and dried in vacuo at room temperature (0.628 g, 69%), m.p 137°C, (Found: C,62.2;H,8.3;N,5.9;$C_{25}H_{39}FN_2O_6$ requires C,62.2;H;8.2;N,5.8%); t.l.c-J Rf 0.40; Vmax 3430 (NH) 1720 cm^{-1} (broad; C=O) ($[\alpha]^{25}_D$+16.0) δH ($CDCl_3$, 200 MHz) 7.25-7.08 (2H,m,Ar-H) 6.98-6.89' (2H,m,Ar-H), 5.61 (1H, br.m, NH), 4.10 (1H,m,αCH), 2.96-2.76 (4H,br.m,βCH_2, $OC(CH_3)_2CH_2$-),2.00-1.57 (6H,br.m, CHA), 1.50-1.14 (20H,br.m, Boc CH_3's, CHA, O-$C(CH_3)_2$ CH_2-); δ_F ($CDCl_3$, 80 MHz)-116.49; δ_C ($CDCl_3$, 200 MHz) 176.13, 171.02, 155.09 (C=O's),161.80 (d,$^1J_{F-C}$ 245 Hz, F-Ar C_3,C_5), 114.37 (d, $^3J_{F-CH}$ 20.9 Hz, F-Ar C_2,C_6), 81.74 ($OC(CH_3)_2CH_2$-), 52.02 (Asp CH),49.81 (CHA, CH),45.37 (βCH_2),38.33 ($OC(CH_3)_2CH_2$-), 30.67 (CHA, CH_2), 28.05 ($OC(CH_3)_2CH_2$-), 25.32 (Boc CH_3's), 24.42, 24.16 (CHA, CH_2's). m/z (FAB) 483, 405; H.R.M.S. (FAB) 483.28700 $C_{25}H_{40}O_6N_2F_1$ (MH^+) requires 483.28702.

N[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-β-(1-(4'-fluorophenyl)-2-methylprop-2-yl]-L-aspartyl glycine methyl ester (65)

To a solution of N[2,2-bis(4-nitrophenyl)ethoxy-carbonyl]-β-[1-(4'-fluorophenyl)-2-methylprop-2-yl]-aspartic acid (62) (0.203 g, 0.36 mmol) in tetrahydrofuran/acetone (1:1; 5 ml), dicyclohexylcarbodiimide (0.089 g, 0.433 mmol) was added at 0°C. After 30 minutes stirring glycine methyl ester hydrochloride (0.045 g, 0.356 mmol) and triethylamine (0.036 g, 0.36 mmol) in tetrahydrofuran/acetonitrile (1:1; 5 ml) was added and the solution stirred at 4°C overnight. After this period the precipitate of dicyclohexylurea was removed by filtration and the solvent evaporated to dryness. The residue was then dissolved in ethyl acetate (20 ml) and a very small amount of acetic acid was added to decompose excess dicyclohexyl-carbodiimide. The precipitate was filtered off and the solvent again removed. The resulting oil (243 mg) was applied to a silica flash column using chloroform as eluent. This gave the pure dipeptide (0.198 g, 82%) m.p 52-53°C (Found: C,57.0;H,5.0;N,8.3;F,2.8; $C_{32}H_{33}FN_4O_{11}$ requires C,57.4;H,4.9;N,8.4;F,4.1%); $[\alpha]_D^{25}$ -4.0; t.l.c-K Rf 0.46; Vmax (CH_2Cl_2), 3430 cm^{-1} (NH),1730 cm^{-1} (broad;C=O); λmax (CH_2Cl_2)272 (ε 20040); δ_H ($CDCl_3$, 200 MHz), 8.18(4H, m, Bnpeoc Ar-H), 7.38(4H, m,Bnpeoc Ar-H), 7.11-6.90(5H, br.m, F Ar-H's, Gly NH), 5.83 (1H,br.d, $^3J_{NH-CH}$ 8.0 Hz, Asp NH), 4.60 (4H,

br.m, Bnpeoc CH,CH_2, Asp CH), 3.98 (2H,d,$^3J_{NH-CH2}$ 5.0 Hz, Gly CH_2), 3.73 (3H,s, methyl ester), 3.0-2.6 (4H, br.m, Asp βCH_2, $OC(CH_3)_2\underline{CH}_2-$), 1.41, 1.37 (6H, 2xS, $OC(\underline{CH}_3)_2CH_2-$); δ_F ($CDCl_3$, 80 MHz) -116.11; δ_C ($CDCl_3$, 80 MHz) 170.92, 170.19, 169.69, 155.25 (C=O's), 161.80 (d, $^1J_{F-C}$ 245 Hz, C-F), 147.07 (Bnpeoc C_4 quaternary aromatic, 146.61 (Bnpeoc C1 quaternary aromatic), 132.29 (d, $^4J_{F-C}$ 3.0 Hz, C_1 quaternary), 131.62 (d, $^2J_{F-CH}$ 7.9 Hz, C_3,C_5), 129.02 (Bnpeoc C_3,C_5), 124.00 (Bnpeoc C_2,C_6), 114.74 (d, $^3J_{F-CH}$ 21.1 Hz, C_2,C_6), 83.63 ($-O\underline{C}(CH_3)_2CH_2-$), 66.07 (Bnpeoc CH_2), 52.29 (methyl ester CH_3), 50.67 (asp α CH), 49.57 (Bnpeoc CH), 45.34 (Asp β CH_2), 41.10 (Gly CH_2), 37.07 ($OC(CH_3)_2CH_2-$), 25.60 ($OC(\underline{CH}_3)_2CH_2-$) m/z (FAB) 669, 535, 519. H.R.M.S 669.22082 $C_{32}H_{34}FN_4O_{11}$ (MH^+) requires 669.22079.

<u>Deprotection of N-[2,2-bis(4-nitrophenyl)ethoxy-carbonyl]-β-[1-(4'-fluoro phenyl)-2-methyl prop-2-yl]-L-aspartylglycine methyl ester (65) using 1,5-diazo-bicyclo [4,3,0] non-5-ene (25)</u>

N[2,2-Bis(4-Nitrophenyl)ethoxycarbonyl]-β-[1-(4'-fluoro phenyl)-2-methyl prop-2-yl]-L-aspartylglycine methyl ester (65) (0.080 g, 0.122 mmol) was dissolved in dimethylformamide (3 ml) to which 1,5-diazobicyclo [4,3,0] non-5-ene (25) (0.037 g, 0.244 mmol) and acetic acid (0.015 g', 0.244 mol) was added. The solution was stirred for 30 minutes after which acetic anhydride (0.024 g, 0.244 mmol) and pyridine (0.039 g,

0.488 mmol) was added. The solution was then stirred for 1 hour and the solvent was removed in vacuo. The residue was then taken up in ethyl acetate (20 ml), washed with saturated sodium hydrogen carbonate solution and dried over anhydrous sodium sulphate. The crude products (two spots by t.l.c) were purified by prep. t.l.c. The products obtained were N-acetyl-β-[1-(4'-fluorophenyl-2-methyl-prop-2-yl-L-aspartyl-glycine methyl ester (66), a colourless oil, and 1,1-bis(4-nitrophenyl)ethene 28) a yellow/white crystalline solid.

N-Acetyl-β-[1-(4'-fluorophenyl)-2-methyl prop-2-yl-L-aspartylgylcine methyl ester (66) (0.043 g, 89%), colourless oil, t.l.c-K Rf 0.5; Vmax 3420 cm^{-1} (NH), 1755, 1720, 1690 cm^{-1} (C=O's); $[\alpha]^{25}_{D}$=+4.2; δ_H ($CDCl_3$, 80 MHz) 7.24-6.82 (5H,m,Ar-H), 4.83 (1H, br.m, CH), 3.98 (2H,d, $^3J_{NH-CH}$ 5.5, Gly CH_2), 3.70 (3H,s, ester CH_3), 3.00 (2H,s,F-Ar-CH_2-), 2.79-2.47 (2H, complex, Asp β CH_2), 2.01 (3H,s,acetyl CH_3),1.42,1.40 (6H,2xs, $OC(CH_3)_2CH_2$-); δ_F($CDCl_3$, 80 MHz) -116.22; δ_C;($CDCl_3$, 200 MHz), 171.29, 170.72,170.26,169.67 (C=O's); 161.74 (d, $^1J_{C-F}$ 244.8 Hz, C_4-F), 132.46 (d, $^4J_{C-F}$ 3.2 Hz, F-Ar C_1), 131.78 (d, $^2J_{CH-F}$ 7.8 Hz, F-Ar C_3,C_5), 114.78 (d, $^3J_{CH-F}$ 21.1 Hz, F-Ar C_2,C_6), 83.52 ($OC(CH_3)CH_2$-), 52.19 (methyl ester CH_3),49.10 (Asp CH),45.58 (Asp β CH_2), 41.17 (Gly CH_2),36.68 ($OC(CH_3)_2CH_2$-),25.67 ($OC(CH_3)_2CH_2$-), 23.02 (acetyl CH_3); m/z (FAB) 397,247, H.R.M.S 397.17749 $C_{19}H_{26}FN_2O_6(MH^+)$ requires 397.17747.

1,1-bis(4-nitrophenyl)ethene (28) (0.032 g, 100%); m.p 175°C (Lit[63] 175-176°C); t.l.c-K; Rf 0.8; δ_H($CDCl_3$, 200 MHz) 8.20 (4H,m,Ar-H), 7.46 (4H,m,Ar-H), 5.76(2H,s,C=C$\underline{H}_2$); δ_C($CDCl_3$, 200 MHz) 147.58, 146.29 (quaternary C_1's,C_4's),146.52 ($\underline{C}$=CH_2),128.75 (aromatic C_3's,C_5's),123.68 (aromatic C_2's,C_6's),119.82 (C=$\underline{C}H_2$).

Side Chain deprotection of N-[2,2-bis(4-nitrophenyl)-ethoxycarbonyl]-β-[1-(4'-fluoro phenyl)-2-methyl prop-2-yl]-L-aspartylglycine methyl ester (65) using trifluoroacetic acid

N[2,2-Bis(4-nitrophenyl)ethoxycarbonyl]-β-[1-(4'-fluoro phenyl)-2-methyl prop-2-yl]-L-aspartylglycine methyl ester (65) (10 mg, 1.5 mmol) was dissolved in methylene chloride (2.25 ml) to which was added, with stirring, trifluoroacetic acid (2.5 ml) and distilled water (0.25 ml). A 1 ml aliquot was then withdrawn and evaportated to dryness in vacuo. The residue from this evaporative was then dissolved in acetonitrile (1 ml) and 15 μl was injected onto the h.p.l.c. column (spherisorb ODS-2). The degree of ester cleavage could then be judged by the change in size of the ester peak on the h.p.l.c. trace. The following results were obtained.

Conditions	Cleavage time
50% TFA/45% DCM/5% H_2O	Total hydrolysis 1hr
90% TFA/5% H_2O	Total hydrolysis 30 mins

The Solid Phase Synthesis of H-Ile Leu Asp Asn Ile-OH(68)

This peptide was prepared as described in the discussion chapter pages 56-57. The following information was, obtained on the limited quantity(23 mg) available, t.l.c-I Rf 0.5; amino acid analysis Asp: Ile: Leu, 2.07:1.98:0.97 requires 2:2:1; δ_H(D_2O,360 MHz), 4.81-4.75 (2H,complex,Asp CH, 4.42 (1H,m,Leu αCH), 4.35 (1H,d,$^3J_{CH-CH}$ 5.8 Hz, Ile α CH), 3.96 (1H,d,$^3J_{CH-CH}$ 5.5 Hz, IleαCH), 3.08-2.76 (4H,complex, Asp β CH_2, Asn β CH_2),2.00 (2H,br.m, Ile β CH's),1.62(3H,br complex, Leu β CH_2, CH),1.45 (2H, br m, Ile CH_2), 1.28(2H, br m, Ile) 0.95 (18H, br complex, Ile, Leu CH_3's); m/z (FAB) 587, 571, 457, 342, H.R.M.S. 587.34044, $C_{26}H_{47}N_6O_9$ (MH^+) requires 587.34043.

N$^{\alpha}$-Benzyloxycarbonyl-benzyl-γ-(1-(4'fluoro phenyl)-2-methyl prop-2-yl)-L-glutamate (70)

N-Benzyloxycarbonyl-benzyl-L-glutamate (69) (7.55 g, 0.02 mol.), 1-(4'fluoro phenyl)-2-methyl propan-2-ol (52) (3.43 g, 0.02 mol.) and 4-dimethyl-amino pyridine (DMAP) (0.24 g, 0.002 mol.) was dissolved in dry distilled tetrahydrofuran (10 ml) and cooled to 0°C. To this solution was added dicyclohexyl -carbodimide (DCC1) (5.00 g, 0.024 mol.). The reaction was then stirred for 24 hours at room temperature. After this period the solution was filtered and the solvent removed in vacuo. The residue was then redissolved in ethyl acetate (50 ml),

cooled to 0°C and filtered through celite to remove any remaining dicyclohexylurea. The filtrate was then washed with saturated sodium hydrogen carbonate (2 x 50 ml), and distilled water (2 x 50 ml), and dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo afforded a viscous clear yellow oil (13.5 g). This oil was then dissolved in the minimum amount of hot ethyl acetate and petroleum ether (b.p 40-60°C) added until the solution remained cloudy. The solution was allowed to cool to room temperature and then left overnight at -15°C. This procedure afforded a white crystalline precipitate, N-benzyloxycarbonyl-benzyl-L-pyroglutamic acid (72) which was removed by filtration. The removal of all the pyroglutamic acid was verified by the absence of a signal at 1800 cm^{-1} in the infra-red spectrum of the crude reaction mixture. The remaining clear viscous oil was then repeatedly washed with warm petroleum ether (b.p 60-80°C) to yield a soft white solid which was not purified further.

N-Benzyloxycarbonyl-benzyl-γ-(1-(4'-fluoro phenyl)-2-methyl prop-2-yl)-L-glutamate (70) (6.78 g, 65%); t.l.c-H Rf 0.7; Vmax 1730 cm^{-1} (broad; C=O's); m/z (FAB) 520, 488, 451; H.R.M.S 520.21349, $C_{30}H_{31}FNO_6$ $(M-1)^+$ requires 520.21352.

L-Glutamic acid γ-[1-(4'-fluoro phenyl)-2-methyl prop-2-yl ester (71)

N-Benzyloxycarbonyl-benzyl-γ-[1-(4'fluoro phenyl)-2-methyl prop-2-yl-L-glutamate (70) (1.61 g, 3.1 mmol.) was dissolved in 2% water/methanol solution (20 ml) and cooled to 0°C. Palladium on charcoal catalyst (5%; 160 mg) was added slowly and the solution stirred at room temperature under an atmosphere of hydrogen. The reaction was followed by t.l.c and was judged to be completed within 90 minutes. The solution was then filtered through celite and concentrated in vacuo to afford a clear sticky gum which on trituration with petroleum ether (b.p 60-80°C) afforded a white solid. The compound was characterised as follows:- (0.586 g, 96%); m.p 158-164°C; $[\alpha]_D^{25}$ = +5.2; t.l.c-I Rf 0.5; m/z (FAB), 298, 229, 148; H.R.M.S. 298.14544 $C_{15}H_{21}FNO_4$ (MH^+) requires 298.14545.

Phenyl-1-(4'fluoro phenyl-2-methyl prop-2-yl carbonate (80) 4-Nitrophenyl-1-(4'-fluoro phenyl)-2-methyl prop-2-yl carbonate (81)

A solution of 1-(4'-fluoro phenyl-2-methyl propan-2-ol (52) (3.36 g, 0.021 mol.) and pyridine (2.36 g, 0.03 mol.) in dichloromethane (30 ml) was stirred and cooled to -5°C. Phenyl chloroformate (78) (3.28 g, 0.021 mol.) or 4-nitrophenyl chloroformate (79) (4.23 g, 0.021 mmol). was then added slowly over 30 minutes maintaining the temperature at -5°C. The solution was then stirred at 4°C overnight. After

this period the solution was filtered to remove any precipitated pyridine hydrochloride and the filtrate was washed with distilled water (2 x 50 ml), 5% potassium hydrogen sulphate solution (2 x 50 ml), distilled water (2 x 50 ml) and dried over anhydrous sodium sulphate. Filtration and evaporation <u>in vacuo</u> afforded a yellow/white crystalline solid, which in the case of (80) was recrystallised from petroleum ether (b.p 60-80°C) or in the case of (81) from ethanol/water. Phenyl-1-(4'-fluoro phenyl)-2-methyl-prop-2-yl carbonate (80) (4.05 g, 70%); m.p 65°C; (Found: C, 70.6; H, 6.0; F, 7.0; calc. for $C_{17}H_{17}O_3F$; C, 70.8; H, 5.9; F, 6.6%); t.l.c -G Rf 0.31; Vmax (CH_2Cl_2) 1755 cm^{-1} (C=O); λmax (CH_2Cl_2) 264 (ε 1056); δ_H ($CDCl_3$, 200 MHz), 7.39 (2H, complex, <u>meta</u> F-Ar-<u>H</u>), 7.17 (5H, m, Ph-<u>H</u>), 7.00 (2H, complex, <u>ortho</u> F-Ar-<u>H</u>), 3.10 (2H, s,-OC(CH_3)$_2$C<u>H</u>$_2$-), 1.53 (6H, s,-OC(C<u>H</u>$_3$)$_2$$CH_2$); δ_F ($CDCl_3$, 80 MHz) -116.06; δ_C ($CDCl_3$, 200 MHz), 161.80 (d, $^1J_{C-F}$ 245.0 Hz, C_4-F), 151.66 (C=O), 150.91 (F-Ar C_1 quaternary), 132.12 (phenyl <u>ipso</u> quaternary), 131.70 (d, $^2J_{F-C}$ 8.0 Hz C_3,C_5), 129.24, 125.65, 121.07 (phenyl CH's), 114.8 (d, $^3J_{F-C}$ 21.2 Hz, C_2,C_6), 81.66 (-O<u>C</u> (CH_3)$_2$$CH_2$),45.11 (-OC($CH_3$)$_2$<u>C</u>$H_2$),25.27 (-C(<u>C</u>H$_3$)$_2$-$CH_2$); m/z (FAB) 289 H.R.M.S. 289.12399 $C_{17}H_{18}FO_3$ (MH^+) requires 289.12399.

4-Nitrophenyl-1-(4'-fluoro phenyl)-2-methyl prop-2-yl carbonate (81) (4.33 g, 65%); m.p 55-57°C; (Found: C, 61.1; H, 4.7; F, 6.1; N, 4.2 calc. for $C_{17}H_{16}FNO_6$; C, 61.3; H 4.8; F, 5.7; N, 4.2%); t.l.c -G Rf 0.19; Vmax (CH_2Cl_2) 1762 cm^{-1} (C=O), 1525, 1350 cm^{-1} (NO_2); λmax (CH_2Cl_2) 270 (ε 8658); δ_H ($CDCl_3$, 200 MHz), 8.27 (2H, overlapping d of d, ^3J 9.3 Hz, ^5J 3.2 Hz, nitrophenyl meta CH's), 7.35 (2H, overlapping d of d ^3J 9.3 Hz, ^5J 3.2 Hz, nitrophenyl ortho CH's), 7.17 (2H, complex, meta F-Ar-H's), 7.00 (2H, complex, ortho F-Ar-H's), 3.10 (2H, s, $-OC(CH_3)_2C\underline{H}_2$), 1.54 (6H, s, $-OC(C\underline{H}_3)_2CH_2$); δ_F ($CDCl_3$, 80 MHz) -115.69; δ_C ($CDCl_3$, 200 MHz), 161.70 (d, $^1J_{C-F}$ 245.1 Hz, C_4-F), 155.40 (C=O), 154.63 (NO_2-C), 150.27 (F-Ar-C_1 quaternary), 144.97 (NO_2-Ar C_1 quaternary), 131.71 (d, $^2J_{F-CH}$ 7.8 Hz, C_3,C_5), 125.05, 121.79 (NO_2-Ar CH's), 114.91 (d, $^3J_{F-CH}$ 21.1 Hz, C_2,C_6), 85.93 ($-O\underline{C}(CH_3)_2CH_2-$), 45.16 ($-OC(CH_3)\underline{C}H_2-$), 25.08 ($-OC(\underline{C}H_3)CH_2-$); m/z (FAB) 334, 292, 262, 214 H.R.M.S. 334.10908 $C_{17}H_{17}FNO_5$ (MH^+) requires 334.10906.

<u>N^{ε}-[1-(4'-fluoro phenyl)-2-methyl prop-2-yloxycarbonyl]-L-lysine (77)</u>

4-Nitrophenyl-1-(4'-fluoro phenyl)-2-methyl prop-2-yl carbonate (81) (0.157 g, 0.47 mmol.), triethylamine (0.10 g, 0.94 mmol.) and N^{α}-benzyloxycarbonyl-L-lysine (0.132 g, 0.47 mmol.) were dissolved in dimethyl-formamide (5 ml) and heated at 60°C, with stirring,

for 4 hours. The reaction was followed during this period by t.l.c. which indicated the gradual disappearance of (81) and the formation of nitrophenol as would be expected (Scheme XXXV). The solvent was then removed in vacuo and the residue was dissolved in ethyl acetate/water sodium (1:1, 200 ml). This solution was then acidified, using 5% citric acid solution to pH 2.5 and the organic extracts were washed with distilled water (2 x 20 ml), brine (2 x 20 ml) and finally dried over anhydrous sodium sulphate. Filtration and evaporation in vacuo afforded a viscous clear yellow oil (0.23 g). This oil was then dissolved in a 2% H_2O/MeOH solution (5 ml), cooled to 0°C and 10% palladium on charcoal catalyst added slowly. The solution was then stirred for 3 hours under an atmosphere of hydrogen after which the solution was filtered through celite and concentrated to half volume. Addition of diethyl ether resulted in the formation of a white jelly-like suspension which was isolated by filtration and afforded the crude product (45 mg). Evaporation of the filtrate in vacuo followed by redissolution in ethyl acetate and the addition of diethyl ether gave a further quantity (27 mg) of product. (72 mg, 45%); (Found: C, 59.0; H, 7.5; N, 8.6; $C_{17}H_{25}FN_2O_4$ requires; C, 60.0; H, 7.4; N, 8.2%); t.l.c -J Rf 0.1; δ_H (CD_3CO_2D, 200 MHz), 7.20 (2H, complex, meta Ar-H's), 7.01 (2H, complex, ortho Ar-H's), 4.08 (1H, complex, α CH), 3.57-3.0 (4H,

complex, $OC(CH_3)_2\underline{CH_2}$, Lys β CH_2), 2.04 (6H, complex, γ, δ, ε CH_2's), 1.41 (6H, s, CH_3's); δ_F (CD_3COOD, 80 MHz), -118.45; m/z (FAB) 341,297,191; H.R.M.S. 341.18765 $C_{17}H_{26}F_1N_2O_4$ (MH^+) requires 341.18765.

H.p.l.c experiments to determine the relative stability of a series of esters of hydrocinnamic acid to base hydrolysis

The appropriate ester (4.30 mmol.) was dissolved in freshly distilled dioxan (10 ml). To this was added, with rapid stirring, 10 ml of aqueous sodium hydroxide solution of the desired molarity to give the required base excess. Using a 2 ml pipette a portion of the reaction mixture was immediately withdrawn and added to 25 ml of a potassium orthophosphate/sodium hydroxide buffer solution (pH 7). An accurate stopwatch was started as soon as the 2 ml aliguot was added. 25 μl of this buffered solution was then injected directly onto the h.p.l.c column and the relative proportions of the ester and acid determined using the peak areas calculated by an automatic integrator. Using the stopwatch, which indicated the length of time the reaction had been in progress since the first sample, further samples were withdrawn and treated as above. The change in the ratio of ester to acid in the reaction mixture was therefore studied with respect to time.

As mentioned above the 3-ethyl pent-3-yl ester proved to be unstable on h.p.l.c. silica and was therefore studied using a graphitic carbon column. The following results were obtained using 2 molar equivalents of aqueous sodium hydroxide.

Hydrocinnamic acid - methyl, t-butyl (40) and benzyl esters

Column Used	ODS5-HL-132
Amount Injected	0.025 ml
Sensitivity	0.02
Eluent	50% acetonitrile/50% water buffered with ammonium acetate (0.2 M)
Gradient	Isocratic

Compound	Retention time (minutes)
Hydrocinnamic acid	2.4
Hydrocinnamic acid methyl ester	7.6
Hydrocinnamic acid t-butyl ester	27.5
Hydrocinnamic acid benzyl ester	25.1

Hydrocinnamic acid methyl ester	- complete hydrolysis in 20 minutes using 2 equivalents of NaOH.
Hydrocinnamic acid t-butyl ester	- 25% hydrolysis in 24 hours using 2 equivalents of NaOH.
Hydrocinnamic acid benzyl ester	- complete hydrolysis within 1 hour using 2 equivalents NaOH.

Hydrocinnamic acid 3-ethyl pent-3-yl ester

Column used	-	graphitic carbon
Amount Injected	-	0.025 ml
Sensitivity		0.02
Eluent		100% acetonitrile
Gradient		Isocratic

Compound	Retention time (minutes)
Hydrocinnamic acid	3.1
Hydrocinnamic 3-ethyl pent-3-yl ester	4.6

Hydrocinnamic 3-ethyl pent-3-yl ester underwent no base hydrolysis after 4 days in solution with 2 molar equivalents of sodium hydroxide.

Hydrocinnamic acid 1-(3'-trifluoromethyl phenyl)-2-methyl-prop-2-yl ester (47)

Hydrocinnamic acid 1-(4'-fluoro phenyl)-2-methyl prop-2-yl ester (53)

Column Used	ODS5-HL-135
Amount Injected	0.025 ml
Sensitivity	0.02
Eluent	80% acetonitrile/20% 0.2M ammonium acetate
Gradient	Isocratic

Compound	Retention time (minutes)
Hydrocinnamic acid	2.8
Hydrocinnamic acid 1-(3'trifluoromethyl phenyl)-2-methyl-prop-2-yl ester	6.5
Hydrocinnamic acid 1-(4'-fluoro-phenyl)-2-methyl prop-2-yl ester	6.2

Both esters showed 25% hydrolysis in 5 days with 2 molar equivalents of sodium hydroxide.

H.p.l.c experiments to determine the relative stability of a series of esters of hydrocinnamic acid to acid hydrolysis

The appropriate ester (1.80 mmol.) was dissolved in dry distilled methylene chloride (5 ml). To this was added, with stirring, the required amount of trifluoroacetic acid and a 1 ml aliquot immediately withdrawn from the solution. This portion was then evaporated to dryness in vacuo and the residue dissolved in acetonitrile (5 ml). This sample (0.025 ml) was then injected onto the h.p.l.c. column and the relative proportions of the ester and acid determined using peak areas calculated by an automatic integrator. This procedure was repeated again after a period of time. The same h.p.l.c. column and solvent systems were used as in the base hydrolysis experiment. The following results were obtained: (Table 6).

TABLE 6: Acid Stability Studies.

Hydrocinnamic Acid Ester	Acid Hydrolysis Conditions.	Observed Stability.
t-butyl ester (40).	50% TFA/DCM	Hydrolysis Instantaneous
3-ethyl pent-3-yl ester (38).	20% TFA/DCM	Hydrolysis Instantaneous
benzyl ester	50% TFA/DCM	Stable > 3 Days.
1-(3'-trifluoromethyl phenyl) -2-methyl prop-2-yl ester (47).	50% TFA/DCM	Total Hydrolysis 4 Hours.
	90% TFA/DCM	Total Hydrolysis 1 Hour.
1-(4'-fluoro phenyl)- 2-methyl prop-2-yl ester (53).	50% TFA/DCM	Total Hydrolysis 15 Minutes.
	20% TFA/DCM	Total Hydrolysis 30 Minutes.

REFERENCES

1. R.B.Merrifield, J. Am. Chem. Soc., 1963, 85, 2149.

2. R.B. Merrifield, Biochemistry, 1964, 3, 1385.

3. R.B. Merrifield, Angew. Chem. Int. Ed. Engl., 1985, 24 799.

4. F.C. McKay, N.F. Albertson, J. Am. Chem. Soc., 1957, 79, 4686.

5. E. Kaiser, J.P. Tam, T.M. Kubiak, R.B Merrifield, Tetrahedron Lett., 1988, 29, 303.

6. B.F. Lundt, N.L. Johansen, J Markussen, Int. J. Peptide Protein Res., 1979, 14, 344.

7. Y. Masui, N Chino, S. Sakakibara, Bull. Chem. Soc. JPN, 1980, 53, 464.

8. E. Jaeger, P. Thamm, S. Knof, E. Wunsch, Z. Physiol. Chem., 1978, 359, 1617.

9. R.L. Noble, D.Yamashiro, C.H. Li, J.Am. Chem. Soc., 1976, 98 2324.

10. H. Irie, N. Fujii, H. Ogawa, H Yajima, J. Chem. Soc., Chem. Commun., 1976, 992.

11. P. Sieber, B. Iselin, Helv. Chim. Acta, 1968, 51, 614.

12. U.Ragnarsson, S.M.Karlsson B.E.B. Sandberg, J. Org. Chem, 1974, 39 3837.

13. U. Ragnarsson, S.M. Karlsson, V. Hamberg Int. J. Peptide Protein Res., 1975, 7 307.

14. C.Birr, W. Lochinger, G. Stahnke, P. Lang, Leibigs Ann. Chem., 1972, 763, 162.

15. B Kamber, B. Riniker, P.Sieber, W. Rittel, Helv. Chim. Acta, 1976, 59, 2830.

16. G. Barany, R.B. Merrifield, J. Am. Chem. Soc., 1977, 99, 7363.

17. L.A. Carpino, G.Y. Han, J. Org. Chem., 1972, 37, 3404.

18. E. Atherton, C.J. Logan, R.C. Sheppard, J. Chem. Soc., Perkin Trans. I, 1981, 538.

19. E. Brown, R.C. Sheppard, B.J. Williams, J. Chem. Soc., Perkin Trans. I, 1983, 1161.

20. C.D. Chang, M Waki, M. Ahmad, J. Meienhofer, E.O. Lundell, J.D. Mauy. Int. J. Peptide Protein Res., 1980, 15, 59.

21. A Paquet, Can. J. Chem., 1982, 60, 978.

22. P Korenaar, B. Van Dijk, J.M. Peeters, B.J Raaben, P.J. Hans, M. Adams, G.I. Tesser, Int. J. Peptide Protein Res., 1986, 27, 398.

23. Su-Sun Wang, J. Am. Chem. Soc., 1973, 95, 1328.

24. R Arshady, E. Atherton, D.L.J. Clive, R.C. Sheppard, J. Chem. Soc., Perkin Trans. I, 1981, 529.

25. M. Bodanszky, S. Natarajan, J. Org. Chem, 1975, 40, 2495.

26. S. Natarajan, M. Bodanszky, J. Org. Chem., 1976, 41, 1269.

27. M. Bodanszky, J Martinez, Synthesis, 1981, 332.

28. A.R. Battersby, J.C. Robinson, J. Chem. Soc., 1955, 259.

29. T. Baba, H. Sugiyama, S. Seto, Chem. Pharm. Bull., 1973, 21, 207.

30. R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel, H. Zuber, Helv. Chim. Acta., 1963, 222 1975.

31. G. Perseo, R. Forino, M. Galantino, B. Gioia, V. Malatesta, R. De Castiglione, Int. J. Peptide Protein Res., 1986, 27, 51.

32. G.M. Bonora, C. Toniolo, A. Fontana, C. Di Bello E Scoffone, Biopolymers, 1974 13 157.

33. A.J. Hubert, R. Buyle, B. Hargitay, Helv. Chim. Acta, 1963, 46, 1429.

34. H. Wilson, R.K. Cannan, J. Biol. Chem., 1937, 119, 309.

35. A.R. Battersby, J.C. Robinson, J. Chem. Soc., 1956, 2076.

36. D.W. Clayton, G.W. Kenner, R.C.Sheppard, J. Chem. Soc., 1956, 371.

37. D.W. Clayton, G.W. Kenner, Chem. and Ind., 1953, 1205.

38. R. Schwyzer, H. Kappeler, Helv. Chim. Acta, 1961, 44, 1991.

39. G.S. Fonken, W.S. Johnson, J. Am. Chem. Soc., 1952, 74, 831.

40. G.C. Stelakatos, A Paganou, L Zervas, J. Chem. Soc., (C), 1966, 1192.

41. R.G. Hiskey, L.M. Beacham, V.G. Matl, J. Org Chem., 1972 37, 2472.

42. C.J. Gray, A.M Khoujah, Tetrahedron Lett., 1969, 31, 2647.

43. H Vorbruggen, K. Krolikiewicz, Angew. Chem. Int. Ed. Engl, 1975, 14, 818.

44. J. Goodacre, R.J. Ponsford, I. Stirling, Tetrahedron Lett., 1975, 42, 3609

45. A.R. Rees, R.E. Offord, Biochem. J., 1976, 159, 467.

46. J.S. Blake, Int J. Peptide Protein Res., 1979, 13, 418.

47. J.P. Tam, T-W Wong, M.W. Riemen, F-S. Tjoeng, R.B. Merrifield, Tetrahedron Lett., 1979, 4033.

48. N. Fujii, M. Nomizu, S. Futaki, A. Otaka, S. Funakoshi, K. Akaji, K. Watanabe, H. Yajima, Chem. Pharm. Bull., 1986, 34, 864.

49. H. Yajima, S. Futaki, A. Otaka, T. Yamashita, S. Funakoshi, K. Bessho, N. Fujii, Ibid, 1986, 34, 4356.

50. G.A. Olah, J. Lukas, J. Am. Chem. Soc., 1968, 90 933.

51. Y.Okada, S. Iguchi, K. Kawasaki, J. Chem. Soc., Chem. Commun., 1987, 1533.

52. H. Yajima, Y. Kiso, H. Ogawa, N. Fujii, H. Irie Chem. Pharm. Bull., 1975, 23, 1164.

53. M.S. Newman, J. Am. Chem. Soc., 1950, 72, 4783.

54. K.L. Leoning, A.B. Garrett, M.S. Newman, J. Am. Chem. Soc., 1952, 74, 3929.

55. R. Ramage, J. Green, M.R. Florence, Proc. 10th Amer. Peptide. Symp. 157.

56. W.W Moyer, C.S. Marvel, Org.Synth., 1931 XI, 98.

57. Schreiner, J. Prakt. Chem., 1910, 82, 295.

58. G. Edgar, G. Calingaert, R.E. Marker, J. Am. Chem. Soc., 1929, 51, 1483.

59. A.L. McCloskey, G.S. Fonken, Org. Synth., 1954, 34, 26.

60. E.M. Kaiser, R.A. Woodruff, J. Org. Chem., 1970, 35, 1198.

61. E. Vowinkel, Chem. Ber., 1967, 100, 16.

62. B.Neises, W. Steglich, Angew. Chem. Int. Ed. Engl., 1978, 17, 522.

63. G.H.L Nefkens, R.J.F Nivard, Recueil, 1965, 84, 1315.

64. R.E. Shute, D.H. Rich, Synthesis, 1987, 346.

65. O. Keller, W.E. Keller, G. van Look, G. Wersin, Org. Synth., 1984, 63, 160.

66. G.H.L. Nefkens, R.J.F Nivard, Recueil, 1964, 83, 199.

67. J.W. Scott, D Parker, D.R. Parrish Synth. Commun., 1981, 11(4), 303.

68. Beil., 5(2), 238.

69. Graphitic carbon h.p.l.c packing has been developed by Professor J. Knox of Edinbrugh University Chemistry Department.

Courses Attended

Organic research seminars (various speakers).

Current topics in Organic Chemistry (various lecturers, University of Edinburgh).

X-Ray Crystallography, (Dr O Kennard *et al.*, University of Cambridge).

N.M.R. Spectroscopy (Dr I.H Sadler, University of Edinburgh).

Mass Spectrometry (Professor K.R. Jennings, University of Warwick).

Medicinal Chemistry (various speakers, ICI and Beecham Pharmaceuticals).

Medicinal Chemistry (Professor P.G. Sammes, Brunel University).

Cell Biology (Dr J. Philips, University of Edinburgh Department of Biochemistry).

Mass Spectroscopy (Dr G Elliott and Dr A. Ashcroft, Kratos).

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