Development Of New Methods For Peptide Synthesis

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This thesis is submitted in part fulfilment 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 for any degree at this or any other university.

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Finally I offer my thanks to all my friends and family.

This thesis is dedicated to

my parents, Lorna and Robert

Abstract

The preparation and evaluation of a number of carboxylic acid protecting groups is reported. The preparation of aspartic acid side chain esters of 9-anthracenylmethanol, 1-(9'-anthracenyl)-3-methylbutan-3-ol, 1-(4'-fluorophenyl)-2-methylpropan-2-ol, dibenzosuberol and the 4-methyl-2,6,7-trioxabicyclo[2.2.2]octyl-ortho ester are described.

The conversion of these into suitably protected N^{α}-Fmoc derivatives for the solid phase synthesis of peptides has been carried out and the comparison with established protection strategies is discussed.

Model compounds of these derivatives have been prepared which have enabled an evaluation to be carried out of the stability of these systems to acidic and basic hydrolysis.

Contents

Abbreviations

Acm	acetamidomethyl
Bnpeoc	2, 2-bis(4'-nitrophenyl)ethoxycarbonyl
Boc	tertiary butoxycarbonyl
d	doublet
DCC	N, N'-dicyclohexylcarbodiimide
DIC	N, N'-diisopropylcarbodiimide
DMAP	4-N, N-dimethylaminopyridine
DMF	N, N-dimethylformamide
FAB	fast atom bombardment
Fmoc	9-fluorenylmethoxycarbonyl
HOBt	1-hydroxy-1, 2, 3-benzotriazole
HOCt	1-hydroxy-4-ethoxycarbonyl-1, 2, 3-triazole
HPLC	high performance liquid chromatography
HRMS	high resolution mass spectrometry
IR	infrared
m	multiplet
NMR	nuclear magnetic resonance spectroscopy
PAM	phenylacetamidomethyl
Pmc	2, 2, 5, 7, 8-pentamethylchroman-6-sulphonyl
s	singlet
t	triplet
TFA	trifluoroacetic acid
TLC	thin layer chromatography
THF	tetrahydrofuran
trityl	triphenylmethyl
TsOH	4-toluenesulphonic acid
UV	ultraviolet
Z	benzyloxycarbonyl

ACTH adrenal corticotropin hormone

DIEA diisopropylethylamine

CHAPTER 1: INTRODUCTION

1.1 SOLID PHASE PEPTIDE SYNTHESIS

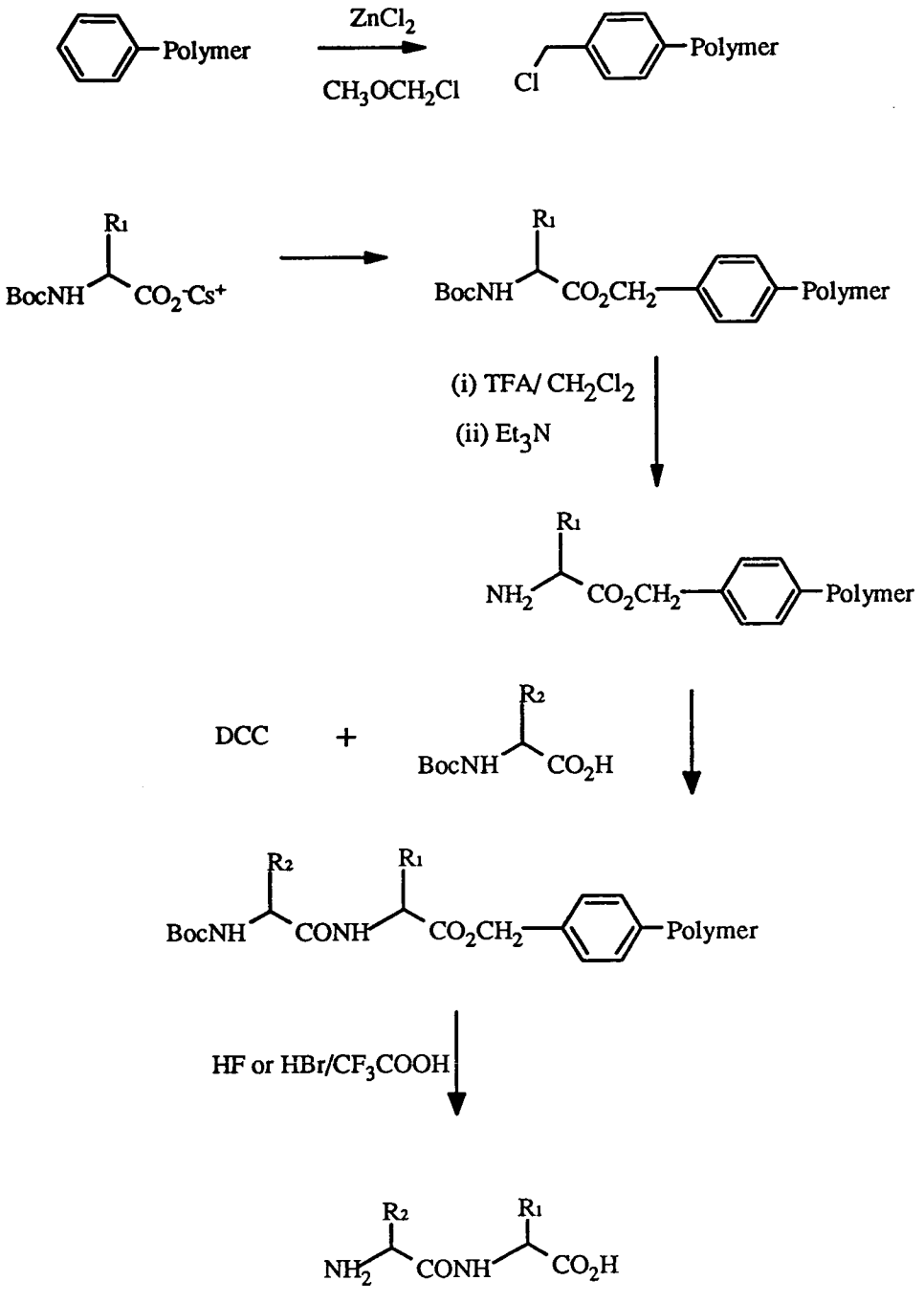
Solid phase peptide synthesis was introduced in 1963 by Merrifield[1] who developed this innovative methodology to overcome the problems associated with the synthesis of high molecular weight peptides in solution.

Peptide synthesis in solution is burdened by the need for isolation and purification at every chemical step. The rate of the acylation coupling reactions can often be very slow consequently making the syntheses very time-consuming and labour intensive. Also the larger the peptide becomes the greater the problems encountered in its solubility in organic solvents such as dichloromethane and DMF.

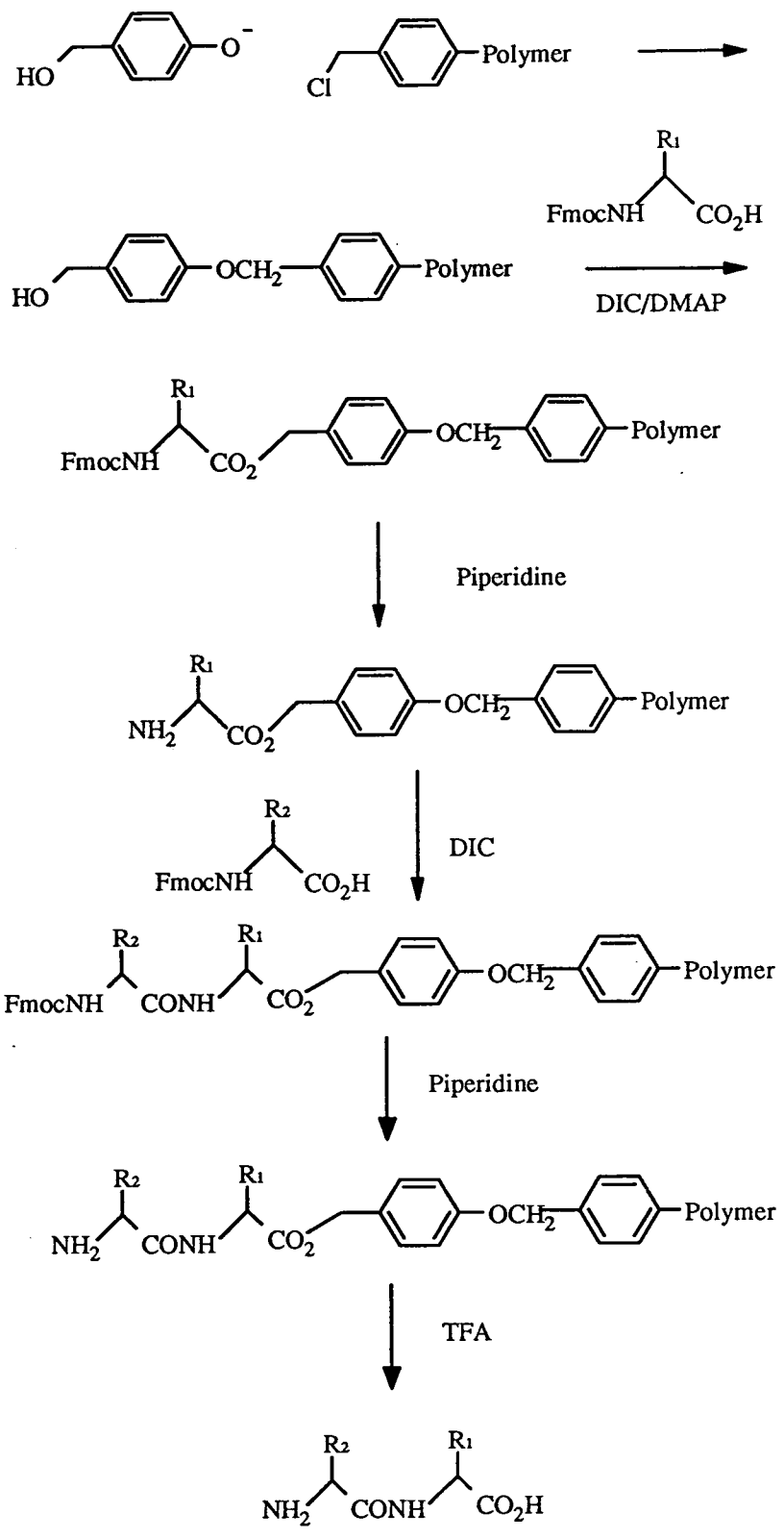
These are the problems that were addressed and overcome by Merrifield's procedure. This is generally summarised in scheme 1. The original idea of solid phase synthesis has been subject to a number of modifications. The original Boc[2] based methodology (Merrifield) is complemented by the Fmoc modified protocol as exemplified by scheme 2.

Because the synthesis is executed on an insoluble polymeric support the solubility problems are largely circumvented. Use of an excess of the acylating agent can enhance the coupling efficiency and maximise the yield. All the purification is carried out upon completion of the synthetic cycle, negating the need for recrystallisation after every step.

Finally the automation of solid phase peptide synthesis has contributed greatly to its current widespread application. This has made rapid synthesis of very high molecular weight compounds possible. However there still exists scope for methodological improvements.



Scheme 1: Merrifield solid phase peptide synthesis.



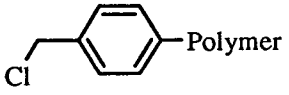
Scheme 2: Fmoc modified solid phase peptide synthesis

1.1.1 The insoluble support

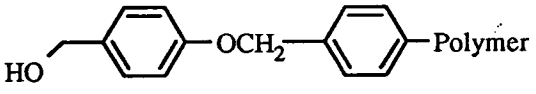
Merrifield's pioneering synthesis was carried out using a styrene-divinylbenzene copolymer which once chloromethylated could be functionalised with the first amino acid in the peptide sequence by esterification. It has been generally accepted that copolymerisation of styrene with 0.5-2% divinylbenzene results in the best physical properties. This firstly will allow swelling during solvation to occur and secondly maintain rigidity and insolubility thus allowing facile filtration. The swelling is necessary to facilitate the access of reactants and to maximise the useful physical space for the growing peptide chain.

1.1.2 The peptide-resin linkage

The peptide is attached to the polymer by esterification through a linker moiety. With the Merrifield resin (1) this can be achieved by reaction of the caesium salt of the protected amino acid with the alkyl halide. Alternatively the Wang[3] (*p*-benzyloxybenzyl alcohol) resin (2) contains a hydroxyl terminus and can be esterified with the symmetrical anhydride of the amino acid. This may be prepared using diisopropylcarbodiimide and DMAP (0.2 mol equivalent) and can be facilitated by sonication. This resin (4) possesses a degree of acid lability towards TFA that makes it particularly suitable for use in conjunction with Fmoc protection. Both linkers (1) and (2) afford the C-terminal carboxylic acid of the peptide product. Upon cleavage.



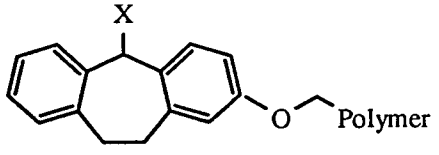
(1)



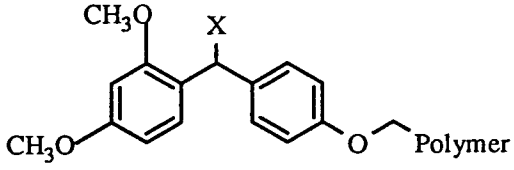
(2)

There has been prodigious effort expended in the development of novel linkers in solid phase chemistry and these have allowed the synthesis of peptide amides[4, 5] and peptide hydrazides. The compounds (3) and (5) are examples of linkers capable of producing peptide amides.

Alternative linkers have also given rise to a choice of reaction conditions for the construction of peptide acids. Thus the very acid labile Rink resin[5] has been applied in Fmoc chemistry and has found use in the preparation of protected fragments because its lability exceeds that of the side chain protecting groups. However significant losses were observed when it was used in conjunction with HOBt, which although being only slightly acidic is used repeatedly.

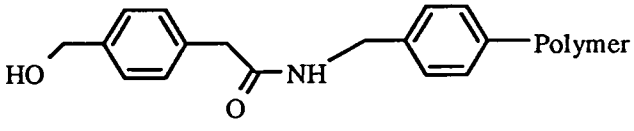


(3), X=NH_2; (4), X=OH



(5), X=NH_2; (6), X=OH

The phenylacetamidomethyl (PAM) resin[6, 7] has found application in the scheme using Boc chemistry. Premature loss of peptide from the resin when the Merrifield resin shown in Scheme 1 was used resulted in the need for a more acid stable support. The PAM linker (7) achieves this due to the inductive effect of the electron withdrawing group -CH_2CONH-.



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1.1.3 The requirement for protection in peptide synthesis

Selective protection of the reactive functional groups present in amino acids must be employed to achieve the preparation of the desired compound. Without this a number of possible couplings may occur, some of which are described in Figure 1 where R_1 and R_2 are simple alkyl groups.

The C-terminal amino acid requires protection of its carboxylate function and this is usually achieved by esterification. A variety of groups have been devised for this purpose including benzyl, phenyl[8] and trityl[9] esters. In the solid phase method protection is accomplished by esterification to the solid support.

Reversible protection of the N^{α}-amino group of the acylating component is mandatory. The N^{α}-protecting group must be amenable to quantitative removal under mild conditions that leave the other functional groups unaffected, especially any side chain protecting groups that are present during the synthesis.

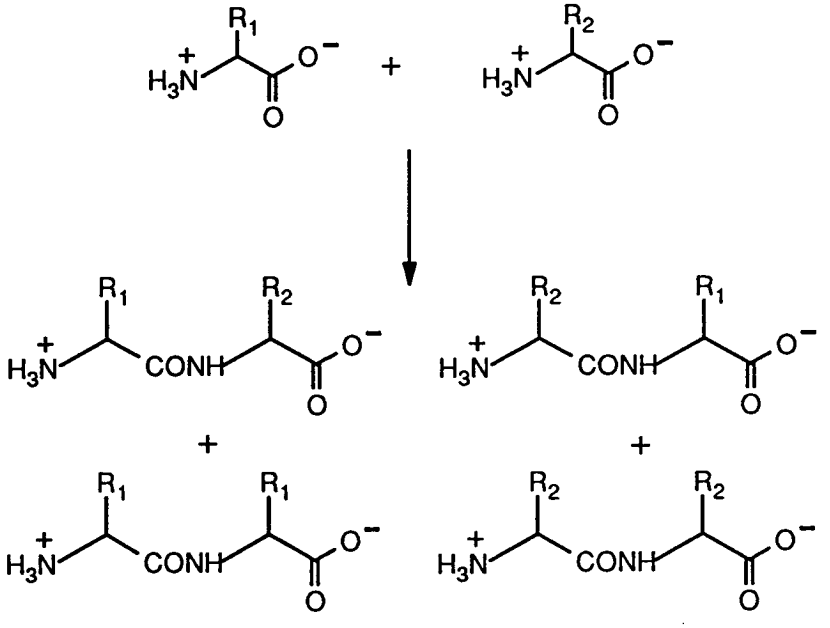
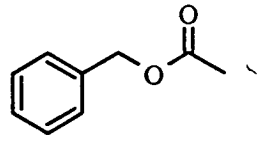


Figure 1: Peptide formation without protecting groups

1.1.4 Protecting group strategies in peptide chemistry: Temporary α-amino protection

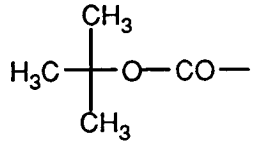
The first generally successful reversible amine protecting group for peptide synthesis was the benzyloxycarbonyl (Z) group (8) developed by Bergmann and Zervas[10].



(8)

This group is rapidly cleaved by catalytic hydrogenolysis under neutral conditions at room temperature. It can also be removed by acidolysis using HBr in acetic acid when hydrogenolysis proves inappropriate.

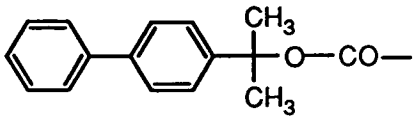
Although Merrifield initiated his studies using benzyloxycarbonyl protection this was soon to be replaced by the tertiary-butoxycarbonyl (Boc) group[11] (9) the acid lability of which greatly exceeds that of benzyloxycarbonyl.



(9)

Application of Boc (9) protection of the amino function allowed the use of milder conditions for the repeated acid deprotection steps involved and reduced the premature loss of unfinished peptide from the support. This protecting group can be cleaved using TFA in dichloromethane and can be selectively removed in the presence of benzyl based protection. This ease of acid lability is due to the stability of the tertiary butyl cation and this led to the development of even more acid labile protecting groups (Figure 2). The 2-(4'-biphenyl)propyl-2-oxycarbonyl (10), 2-

phenylpropyl-2-oxycarbonyl (11), 2-(3',5'-dimethoxyphenyl)propyl-2-oxycarbonyl (12) and trimethylphenyl (trityl) (13) protecting groups have subsequently been developed[12] based upon closely related stabilised cations.



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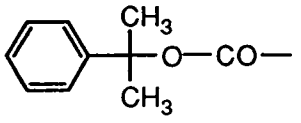

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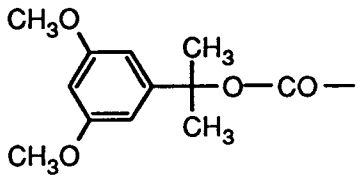

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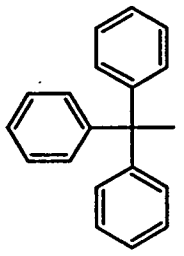


(13)

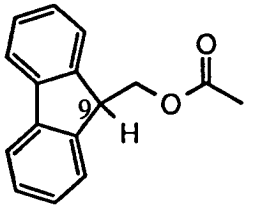
Figure 2: Some examples of very acid labile N^{α}-protecting groups

The protecting group Bpoc (10) was found to be cleaved 3000 times more rapidly than Boc[12]. In some of the above examples however the extreme sensitivity to acid causes them to be too unstable for most practical uses.

When one of these very acid labile N^{α} protecting groups (e.g. Boc or Bpoc) is employed they are compatible with benzyl based side chain protection. In this protocol a graded acidolysis deprotection procedure is employed where the amine protection can be removed by relatively mild acid (e.g. TFA) and the side chain protecting groups and the peptide-resin linkage are cleaved with HBr in TFA or HF alone.

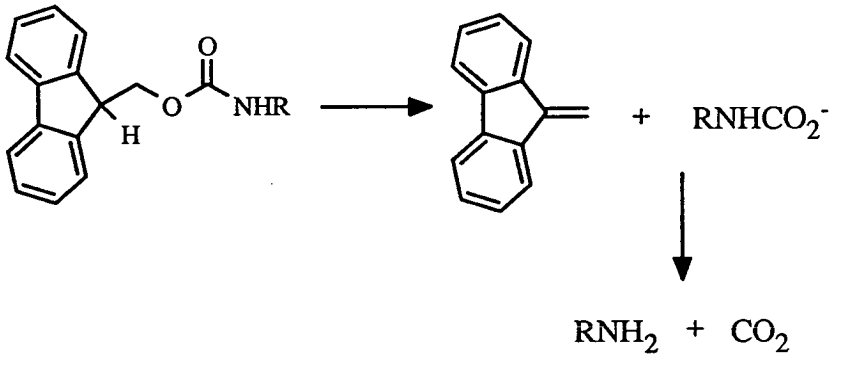
However, because such a procedure is not perfect and because some peptide sequences are peculiarly acid sensitive, the base labile fluorenylmethoxycarbonyl (Fmoc) protecting group (14) of Carpino[13, 14] has been adopted widely as N^{α} protecting group in solid phase peptide synthesis. In combination with acid labile

side chain protection Fmoc provides a clearly defined orthogonality. The Fmoc group can be removed using a secondary amine such as piperidine or morpholine. It has also been shown that piperidine and pyrrolidine can bring about complete deprotection of Fmoc within 5 minutes in dipolar aprotic solvents[16].



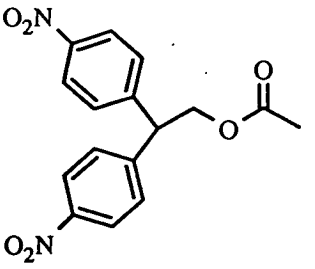
(14)

The proton at the 9 position is readily abstracted to form the fluorenyl anion which benefits from aromatic stabilisation. This is followed by elimination to the alkene in an E1cb mechanism releasing a carbamic acid which spontaneously decarboxylates to give the free amino compound.



Scheme 3: The deprotection of Fmoc N^{α}-protection

The base lability of this protecting group makes it ideal for use in conjunction with acid labile side chain protection eliminating any of the losses experienced under conditions of graded acidolysis. Subsequently other base labile protecting groups such as the 2, 2-bis(4'-nitrophenyl)ethoxycarbonyl (Bnpeoc) (15) group[15] have been introduced. Using this strategy the peptide products do not experience strongly acid conditions during the final deprotection protocol.



(15)

Many amino acids are polyfunctional and require protection at their side chains during the assembly of the polypeptide. These are generally referred to as permanent protecting groups. In Fmoc peptide chemistry the most common side chain protecting groups are acid labile and are often tertiary butyl based[16].

AMINO ACID	PROTECTING GROUP	REFERENCE
Asp/Glu	tertiary butyl ester	17, 18
Arg	Pmc	19
His	Trityl	20
Cys	Acm/ S^{t}Bu	21, 22
Ser/Thr/Tyr	tertiary butyl ether	23
Lys	Boc	24

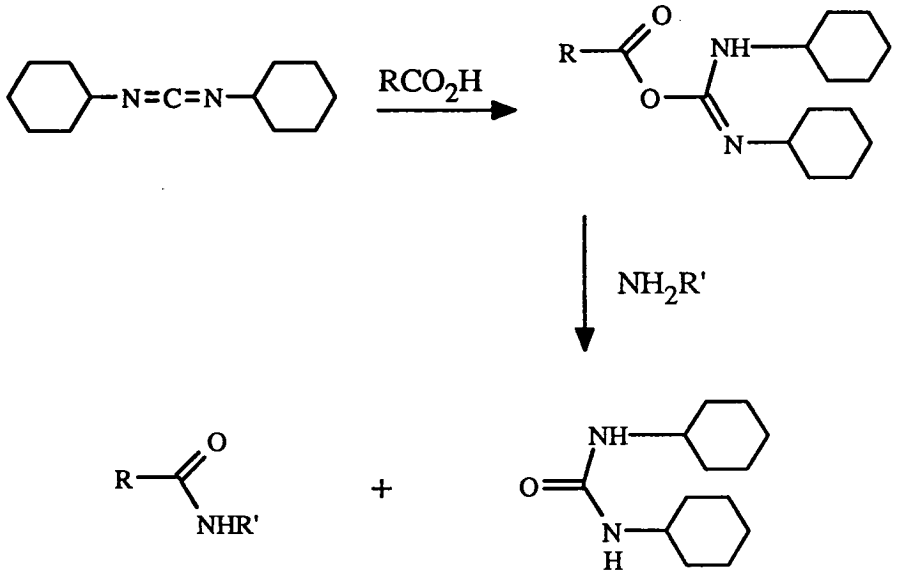
Table 1: Commonly used side chain protecting groups in Fmoc chemistry

1.1.5 Peptide bond formation

Coupling of amino acids is realized in practice by the activation of one carboxylic acid to attack by the amine of the next amino acid. A great variety of synthetic methods have been applied to the synthesis of amide bonds.

Dicyclohexylcarbodiimide[25] has most frequently been exploited for the synthesis of peptide bonds in which an O-acyl urea intermediate is the agent

responsible for the acylation to give the peptide and the urea as the by-product (Scheme 4), which can be removed from the resin by washing with methanol. The urea derived from diisopropylcarbodiimide is more readily soluble in dichloromethane therefore DIC is often preferred for solid phase peptide synthesis.



Scheme 4: Amide bond formation via DCC

If the activated species is not attacked by a nucleophile sufficiently quickly it may spontaneously rearrange to the N-acyl urea compound (Figure 3). These species are completely inert and the reaction is irreversible.

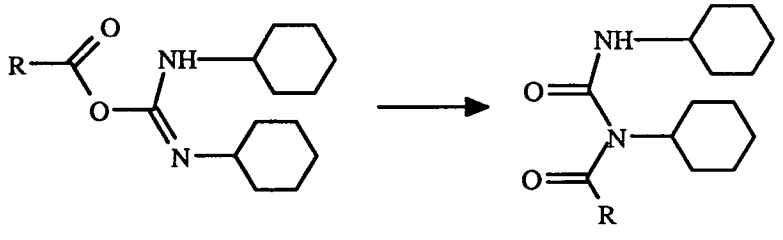


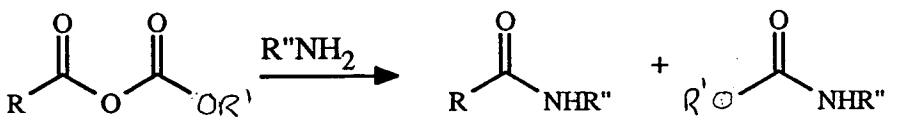
Figure 3: N-acylurea formation

Addition of DMAP[26], a powerful acylation catalyst, prevents this side reaction from occurring. It reacts very rapidly with the O-acyl urea resulting in formation of the activated intermediate (16) which can be attacked by the amino

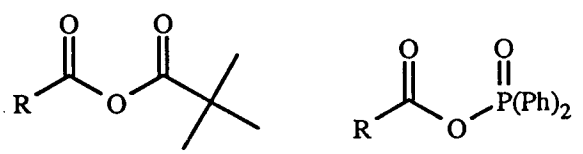
component resulting in peptide bond formation.

(16)

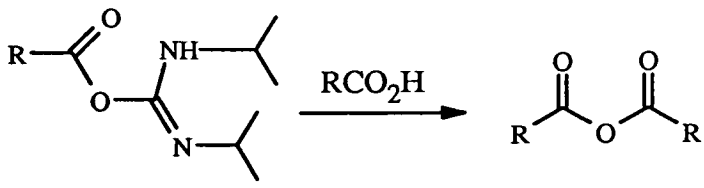
Preparation of acid anhydrides is a rapid and effective method for the activation of carboxylic acids. Mixed anhydrides[27] can be derived from the acid and the corresponding alkyl chloroformate but they have two possible carbonyl groups activated to nucleophilic attack. This results in a decrease of the chemical specificity unless great care is exercised in the choice of anhydride used.



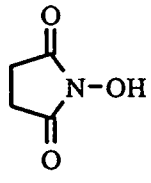
The very hindered pivalic anhydride[28] can be used to direct attack at the desired carbonyl while phosphinic anhydrides such as diphenylphosphinic anhydride[29] provide complete selectivity as the phosphorus centre is not attacked.



Alternatively symmetrical anhydrides have been frequently employed but these are wasteful because only half of the reagent is transferred. This can be very costly when expensive protected intermediates are involved. Symmetrical anhydrides can be prepared by reaction of the appropriate amino acid with 0.5 equivalents of carbodiimide. DCC may be used for this purpose but it is often found to be expedient to use DIC which gives rise to the more soluble urea.



Active esters do not suffer from the same drawbacks as anhydrides and can often be prepared in a stable form[30, 31] for use in automated synthesis or, alternatively, may be prepared *in situ* from the acid, hydroxy component and the carbodiimide. This approach generally inhibits rearrangement to the N-acyl urea. N-hydroxysuccinimide (18), pentafluorophenol (19) and N-hydroxybenzotriazole (20) represent some of the most utilised examples of this type possessing a range of reactivity towards aminolysis.



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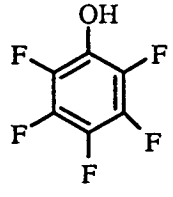

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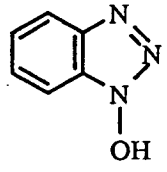
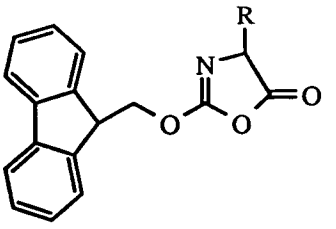


(20)

Classically acid azides[32] have been employed for the synthesis of peptides but they have not been adopted in automated solid phase synthesis. Azides are generally efficient acylating agents but require low reaction temperatures for their use in order to prevent rearrangements to isocyanates and subsequent urea formation.

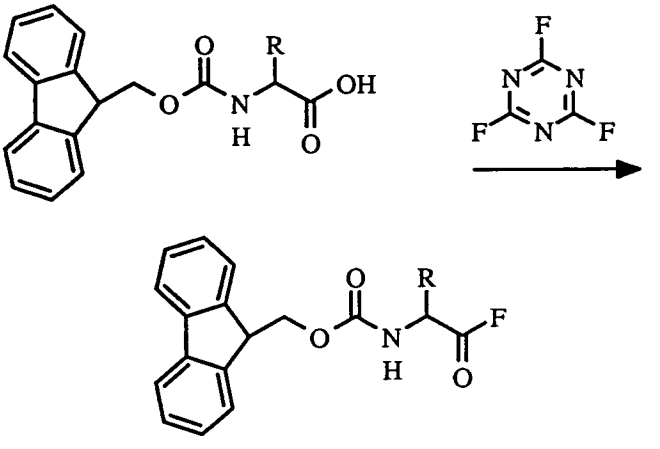
Carpino has proposed that Fmoc amino acid chlorides[33] be extended to solid phase peptide synthesis. Their tendency to form oxazolones (21) in the presence of tertiary organic bases results in a sluggish reactivity[34] and they have not been widely adopted.



(21)

Their application in conjunction with tertiary butyl based side-chain protection has been unsuccessful, due to traces of HCl that are inevitably present.

To overcome this limitation Fmoc amino acid fluorides[35] can be used for the direct amination in the presence of an amine base without oxazolone formation. They can be prepared from the Fmoc amino acid and cyanuric fluoride to yield crystalline derivatives.



(22)

Scheme 5: The preparation of an Fmoc amino acid fluoride

These intermediates are compatible with Boc and tertiary butyl protected intermediates and are not prone to racemisation. They are stable under aqueous conditions and do not react well with neutral oxygen nucleophiles in contrast to acid chlorides, however they are unsuitable intermediates for the preparation of active esters.

Upon completion of an activation/coupling cycle in solid phase peptide synthesis, before deprotection of the N^{α}-terminal Fmoc group is effected an efficient "capping" of any unreacted amine from the previous residue is required in order to prevent the formation of deletion peptides. This can be done by acetylation using a solution of acetic anhydride, diisopropylethylamine, and N-hydroxybenzotriazole[36].

1.1.6 Deprotection of Fmoc

The deprotection of Fmoc using 20% piperidine in DMF leads to the formation of an adduct between the dibenzofulvene produced and the base, this has also been observed with morpholine.

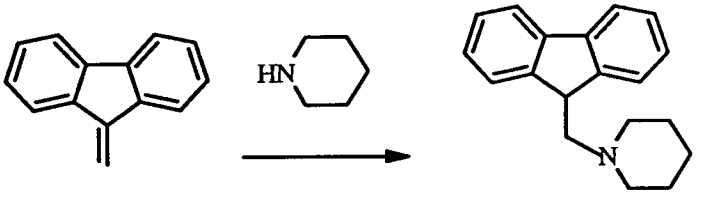
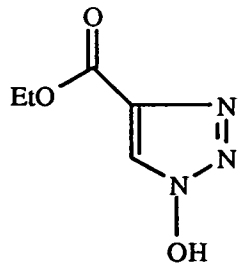


Figure 4: The formation of 9-piperidinylmethylfluorene

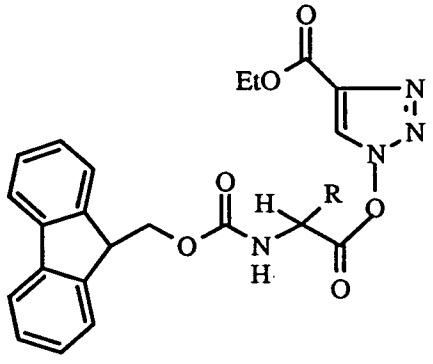
This gives rise to a UV spectrum with a maximum at 302 nm[37]. At this point the extinction coefficient of residual fulvene in the mixture is relatively low and the measurement of the spectrum at this wavelength can be used to calculate the efficiency of deprotection and give an indication of the coupling efficiency. Such a monitoring system has been employed in this laboratory.

The extension of the monitoring to the coupling steps to provide immediate feedback, and thus control of the synthesis, has recently been realised through the use of the triazole (23) 1-hydroxy-4-ethoxycarbonyl-1,2,3-triazole (HOCt) for the activation of Fmoc amino acids.



(23)

Substituting the fused phenyl ring of HOBt with an ethyl ester group simplifies the UV spectrum of the active ester (24) formed between this triazole and an Fmoc amino acid.



(24)

The ultra violet spectrum of the active ester is measured at 302 nm before and after the coupling step. This is compared to a solution of a standard (fluorene) to deduce the amount of Fmoc ester which has been incorporated[38]. This can be used to provide immediate information and offers the opportunity for recoupling of the residue before deprotection is carried out and it is too late.

1.1.7 Final deprotection

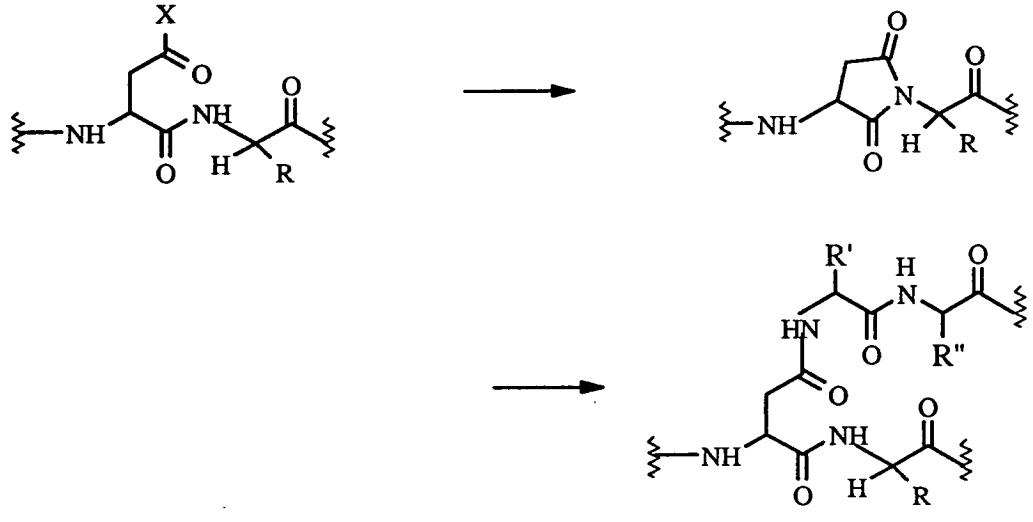
Upon completion of the synthesis, acid catalysed solvolysis is used to liberate the peptide from the resin and remove the side chain protecting groups. This can be effected by the action of 95% aqueous TFA together with the appropriate scavengers. An alternative hard acid deprotection[39] has been proposed by using

bromotrimethylsilane in TFA and reducing the contact time to around 20 minutes. The bromotrimethylsilane when dissolved in TFA is a source of hard acid, the silyl group acting as a surrogate for the proton of HBr. In studies using Boc chemistry where benzyl ester protection of the side chain of aspartic acid is favoured this procedure was found to minimise the acid catalysed cyclisation to aspartimide peptides.

1.2 SIDE REACTIONS IN PEPTIDE SYNTHESIS[40]

1.2.1 Unprotected carboxyl function

The β-carboxyl of aspartic acid requires to be protected during the assembly of the peptide. In the absence of protection during activation the aspartyl β-carboxylate can behave as a nucleophile resulting in complications[41]. Thus during activation by anhydride or active ester the unprotected β-carboxylic acid may combine with the activating reagent resulting in the activation of the β-aspartyl carboxylic acid to nucleophilic attack.



X = Activating species (e.g. ester or anhydride)

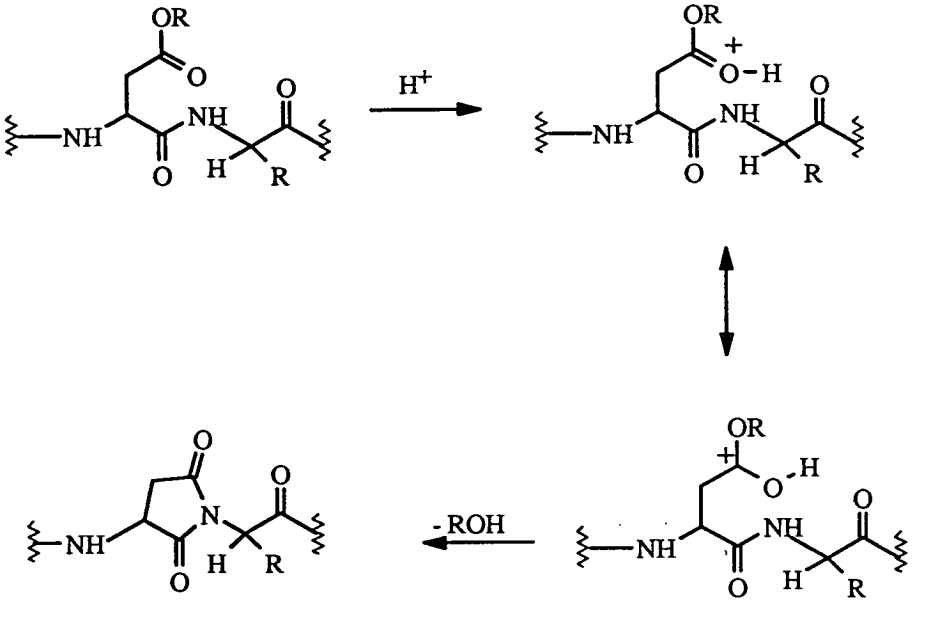
Figure 5: Side reactions in unprotected aspartyl peptides

This activated species (Figure 5) may react with another peptide chain on the

resin to cause branching or it can be attacked by the adjacent amide nitrogen to give the cyclic imide. The cyclisation to aspartimide peptides is an ongoing problem in solid phase peptide synthesis. However aspartimide formation is not only a problem observed in synthesis. The pH-dependant cyclisation[42] of unprotected aspartyl peptides has been reported to occur during storage in aqueous solution. The phenomenon is aggravated at low pH.

1.2.2 Protected carboxyl function

The protection of aspartic acid as a carboxylic ester is not without problems. Cyclisation to aspartimide peptides has been observed in a number of protected examples and can occur by either acidic (Figure 6) or basic catalysis (Figure 7), conditions that are prevalent throughout solid phase peptide synthesis methodology. In Boc chemistry repeated TFA/triethylamine (or DIEA) treatment is used to remove semi-permanent amine protection and regenerate the free amine. In Fmoc solid phase peptide synthesis piperidine is used for Fmoc deprotection and TFA is employed for final deprotection and liberation of the peptide from the resin.



(17)

Figure 6: Acid catalysed aspartimide formation

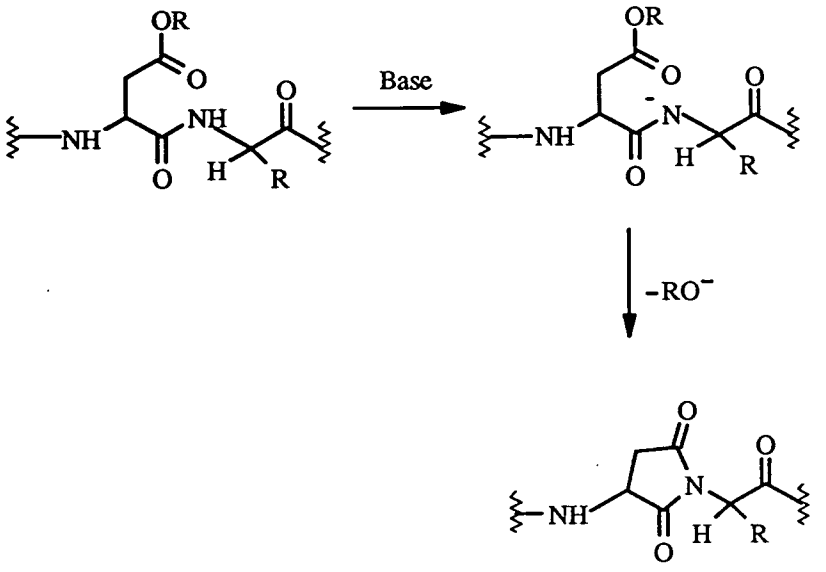


Figure 7: Base catalysed aspartimide formation

1.2.3 The chemistry of the aspartimide peptide

Upon formation of this cyclic imide the peptide may then be subject to nucleophilic attack and consequently ring opening. Alkaline hydrolysis e.g. sodium hydroxide[43] will result in the regeneration of the peptide but ring opening to the β-peptide prevails under these conditions. Prolonged exposure to piperidine or pyrrolidine, conditions that prevail in Fmoc chemistry ring opening occurs[44, 45]. The amide results and once more attack is predominantly at the α-carboxyl (Figure 8).

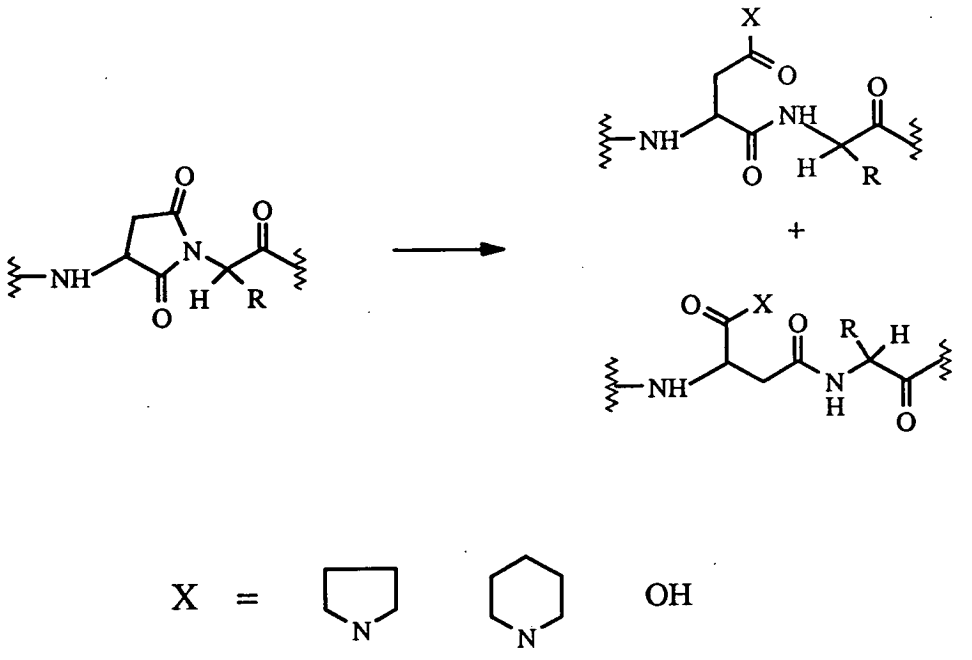
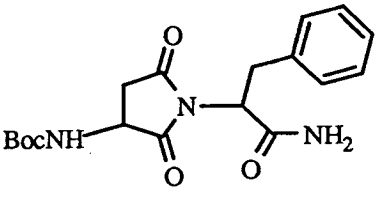


Figure 8: Products resulting from nucleophilic attack upon the aspartimide ring

In the aspartimide ring racemisation of the aspartyl residue may occur when exposed to nucleophilic bases[46]. Studies carried out on aspartyl-phenylalanine amide sequences have revealed the presence of an epimerised mixture of α and β aspartyl peptides resulting from the action of piperidine on the aspartimide compound (25).



(25)

The formation of aspartimide compounds in peptide synthesis is particularly prevalent when glycine[47] is the residue adjacent to the aspartic acid. Cyclisation has been observed in aspartyl-serine[48] sequences when serine has not been protected. The mechanism has been rationalised by intramolecular catalysis involving base promoted removal of the serine hydroxyl proton followed by abstraction of the proton from the amide and ring closure[49] (Figure 9)

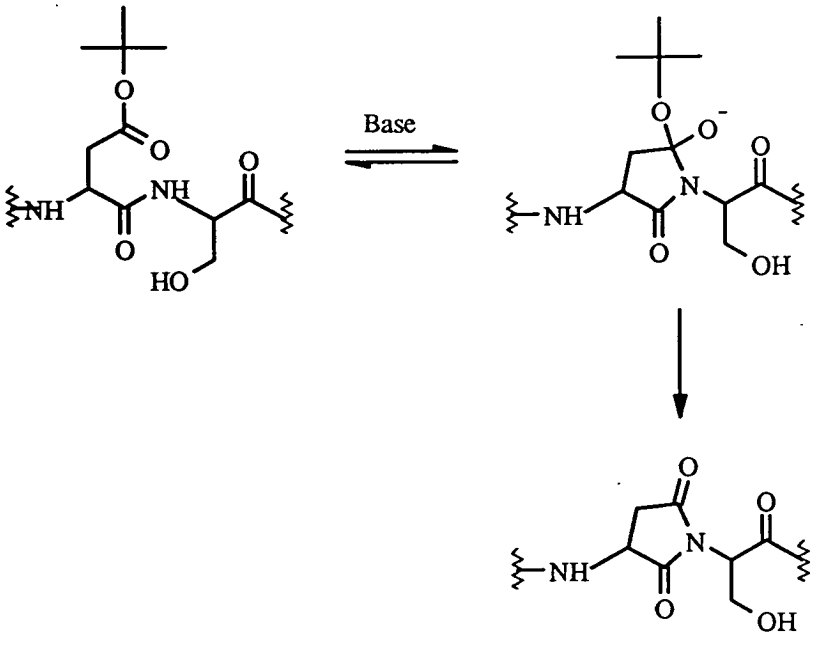


Figure 9: Aspartimide formation in aspartyl-serine peptides

The cyclic nature of the intermediate was deduced from the results of the aminolysis of (26) where the α and β asparaginyl peptides (Figure 10) were the products obtained[50].

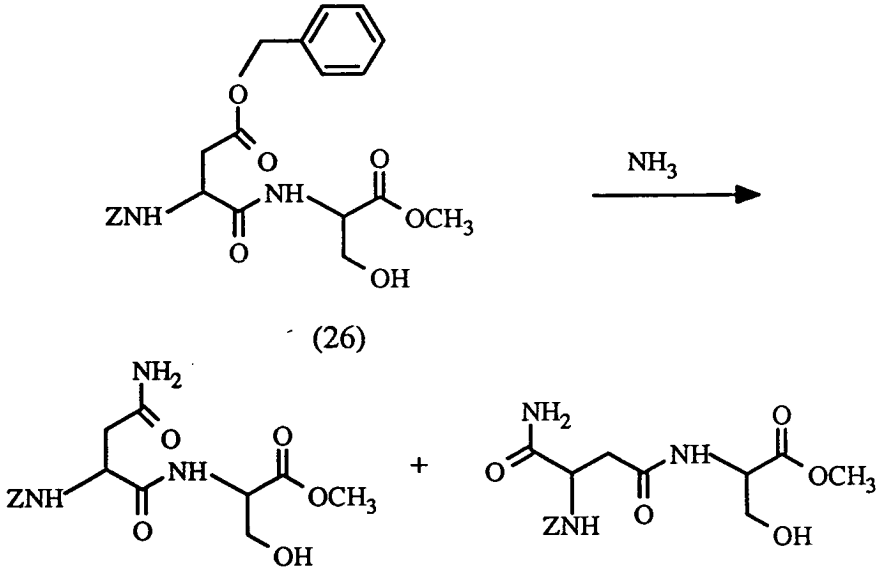


Figure 10: The formation of isomeric asparagine peptides

When histidine[51] is the amino acid following the aspartyl residue a similar intramolecular catalysis brought about by the histidine imidazole has been reported.

Aspartyl-isoleucine and aspartyl-valine are relatively unaffected presumably due to steric hindrance.

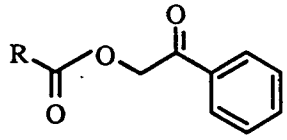
Asparagine derivatives are also known to undergo α to β rearrangement under basic conditions via a cyclic imide intermediate[52]. The structure (17) may therefore be derived from peptides possessing an asparaginyl-glycine bond. These derivatives can be cyclised under weakly alkaline conditions[53] to give the succinimide (aspartimide) peptide and ammonia. This reaction was responsible for the erroneous structure determination of porcine ACTH. The chemical synthesis by Schwyzer and Sieber[54] of this 39 amino acid hormone led to the correction of this error. Asparagine replaced the aspartate residue and the glutamine was replaced by a glutamic acid in the correct sequence[55].

1.3 PROTECTING GROUPS FOR ASPARTIC ACID

Benzyl esters were the first to be applied to β aspartyl protection and still find favour in Boc chemistry. Tertiary butyl esters[16] are used throughout Fmoc methodology and both protecting groups possess shortcomings. Repeated application of acid during Boc based peptide synthesis causes cyclisation to occur with the expulsion of benzyl alcohol. The tertiary butyl ester although regarded as stable to nucleophiles does not totally suppress aspartimide formation[56]. The instability of this group to hydroxide has been noted[57]. This group is still the most commonly used aspartate protecting group particularly in Fmoc chemistry. When difficult sequences are involved this is not acceptable[58, 59].

With Boc N^{α} protection in solid phase peptide synthesis cyclohexyl esters[60] (28) have been employed. This has been shown to suppress the formation of aspartimide during HF cleavage of peptides. During exposure to triethylamine it was shown to give 14% imide after 24 hours, this was compared to benzyl ester

protection which produced 100% cyclic compound under the same conditions. This would appear to make the cyclohexyl ester unsuitable for Fmoc synthesis. Furthermore the studies of Pedroso *et al*[56] showed that upon treatment with 20% piperidine in DMF that the cyclohexyl ester gave 67% imide compared to 100% with benzyl and 11% when tertiary butyl protection was examined. This study also showed that under HF treatment the cyclohexyl ester provided 6% imide.



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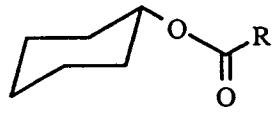
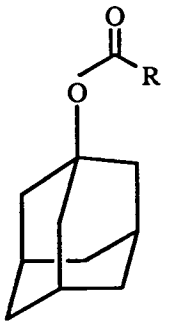


(28)

The use of phenacyl esters (28) has been applied to Boc chemistry with some success by Merrifield[61]. They may be deprotected using thiophenoxide prior to HF global deprotection. This technique led to the observation of 2.4% aspartimide upon cleavage attributed to acid catalysed ring closure acting upon the free carboxylate. Bodansky[62] noted that the phenacyl group may actually have moderately activating properties as it could be described as a methyl ester possessing an electron withdrawing benzoyl substituent. This could then favour base catalysed nucleophilic attack leading to cyclisation. Its instability to nucleophiles as first noted would lead to reservations about its utility for the suppression of aspartimide formation under the basic conditions prevalent in Fmoc chemistry.

The use of the adamantyl[63] esters (29) and (30) has also been examined with respect to aspartimide formation. Both of the isomers are acid labile to differing degrees. The 1-adamantyl (29) system based on a tertiary alkyl group can be removed in TFA but is resistant to 7M HCl in dioxane. The 2-adamantyl (30) isomer can be cleaved rapidly by methanesulphonic acid but is resistant to the conditions that cleave the 1-adamantyl ester. Both were shown to be resistant to basic conditions; aqueous sodium carbonate and 55% piperidine left both adamantyl esters

unaffected. No example of their use in Fmoc peptide chemistry has been reported but an examination[64] of their compatibility with Boc protection has been evaluated. In studies on a model aspartyl-serine peptide between 0-3% of aspartimide was observed under coupling conditions and 1-4% during HF cleavage.



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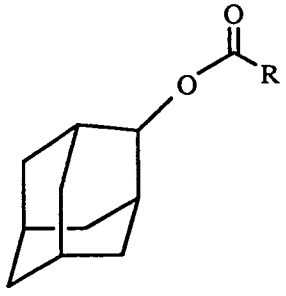
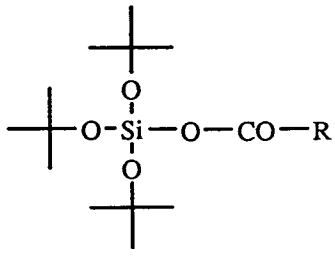


(30)

In the development of silyl esters having improved stability over trimethylsilyl the tri-(tert-butoxy)silyl[65] esters (31) were prepared. The reagent used in their preparation is available from the reaction between trichlorosilane and tertiary butanol in the presence of pyridine and are easily incorporated into the side chain of aspartic acid. Despite their steric bulk they were found not to impede coupling reactions. They are cleaved almost instantaneously by 50% TFA but so far have not been applied to a synthesis involving an aspartimide sequence nor is any example reported of their use in Fmoc solid phase peptide synthesis.



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1.4 REQUIREMENTS FOR A NOVEL PROTECTING GROUP

The design of a novel protecting group for peptide synthesis is restricted by the need for compatibility with established procedures. Thus some of the points that require consideration are now discussed.

1.4.1 Orthogonal deprotection

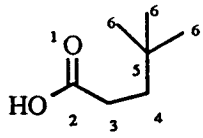
An orthogonal deprotection strategy is defined[66] as being one in which a set of independent classes of protecting group exist, where any one class of protecting group may be removed in any order in the presence of all the others without affecting them.

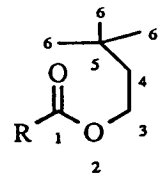
Thus a novel side chain protecting group for use with Fmoc must be completely stable to attack by bases such as piperidine which are used throughout the synthetic cycle for the deprotection of Fmoc. Therefore an acid labile protecting group that will be rapidly deprotected by TFA compatible with existing side chain protection and resin cleavage is the objective.

1.4.2 Steric hindrance

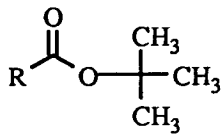
In order to realise the goal of a derivative that will prevent the formation of aspartimide peptides a sterically hindered ester was sought. In carrying out this aim it has proved expedient to quantify the steric nature of the substrate by consideration of its degree of substitution. Following observations and experimental evidence reported by Newman[67] with regard to substitution reactions of carboxylic acids an empirical rule was proposed;

"In reactions involving addition to an unsaturated function the greater the number of atoms in the 6 position the greater will be the steric effect"

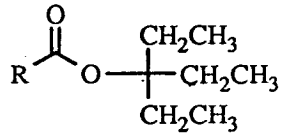




The original numbering for acids has been modified to begin from the carbonyl carbon, the position of nucleophilic attack upon the ester. This rule of six was described by Longstaff[68] and can be best exemplified by the comparison of the 3-ethylpent-3-yl group and the tertiary butyl group, to represent the extremes of the possibilities.



Six number = 0



Six number = 9

The attempted application of this group to solid phase peptide synthesis was not possible due to its considerable acid lability. However it did exhibit an impressive resistance to basic hydrolysis as predicted by the rule of six.

1.4.3 Monitoring

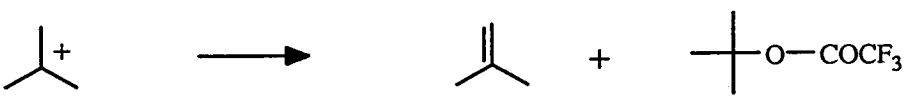
In designing an improved protecting strategy the opportunity arises to to incorporate a physical label at the same time. A chemical tag that could easily be detected by simple physical or spectroscopic means would be desirable in order that the side reactions, which constitute the major problem in aspartic acid containing peptides, may be monitored. The simplest label would be one that was amenable to UV analysis which would allow *in situ* monitoring of chain assembly and deprotection stages to follow the course of the synthesis.

1.4.4 By-products

The problem of side reactions caused by the formation of activated cationic

species during deprotection is a frequently encountered problem in peptide synthesis. Alkylation of certain amino acid residues (e.g. Trp[69], Tyr[70] and Met[71]) results from acidolysis. This has previously been addressed in the development of side chain protection strategies e.g in the cyclohexyl ester of Merrifield[60] whereby the secondary cyclohexyl cation was observed to undergo rearrangement to the relatively stable methylcyclopentyl cation.

However it would be advantageous if the cation formed upon deprotection yielded a neutral species immediately upon cleavage. One of the products formed upon removal of the tertiary butyl based groups is isobutylene however it has been reported that tertiary butyl trifluoroacetate also produced upon cleavage can provide a reservoir of strongly alkylating tertiary butyl cations[72].



1.4.5 Solubility

The solubility of a novel derivative must come within the specifications of the synthetic protocol with respect to solid phase synthesis in order to avoid damage to the machine components. In addition increased concentration of reagents will improve the effectiveness of the reagent during the coupling steps. A solubility in DMF of 0.25 mol l^{-1} is required for compatibility with the automated peptide synthesiser.

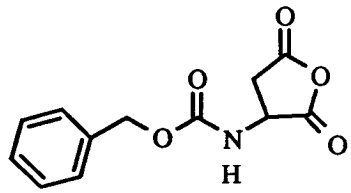
1.4.6 Economic viability

It would be advantageous if the protecting group could be prepared simply from relatively inexpensive and readily available starting materials. The protecting group must be cleaved quantitatively upon completion of the synthesis to maximise the recovery of product. It is important that the incorporation of the group into an appropriate amino acid derivative and its coupling during the assembly of a polypeptide are efficient in a field of chemistry where excesses of reagents are commonly used to effect close to quantitative yields.

CHAPTER 2: DISCUSSION

2.1 STRATEGIES FOR THE PROTECTION OF ASPARTIC ACID

When protecting the side chain functionality of an amino acid the initial protection of the α-amino and α-carboxyl functions is of the utmost importance.



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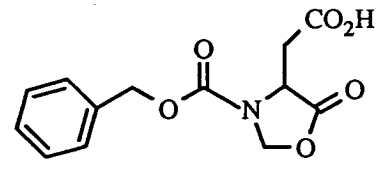

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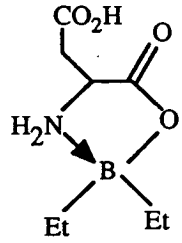

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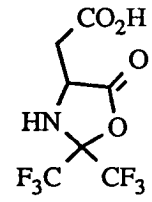

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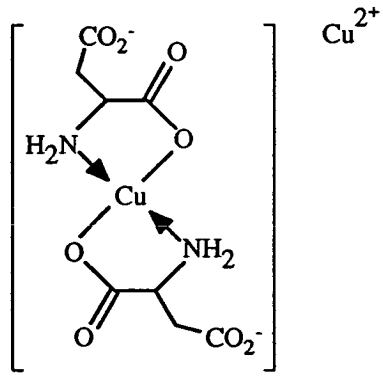


(36)

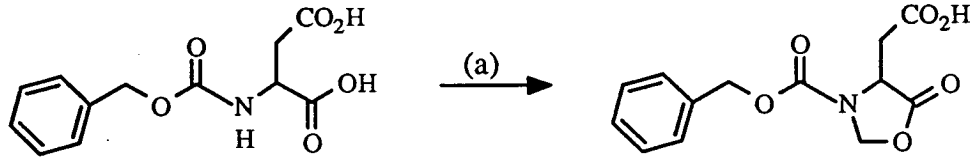
Figure 11: Options for the protection of aspartic acid

Examples of specific esterification of the side chain of aspartic acid are limited to certain very well worked out examples whose experimental details are not usually

found to be generally applicable (for example; tertiary butyl[73], benzyl[74] and cyclohexyl[75] esters).

Examples of suitably protected intermediates (Figure 11) include the N^{α}-protected cyclic anhydride[76] (32) which can be directly esterified but a mixture of α and β isomers will usually be obtained (although the α isomer usually predominates as the α carboxyl is the more electrophilic position). Purification relies upon selective crystallisation of the appropriate amine salt[77] which will not always give satisfactory results especially with N^{α}-Fmoc protection.

The oxazolidinone derivatives, cleaved under hydrogenolytic conditions, offer a more reliable control of regiochemistry but they also have their drawbacks. The compound (35) can be prepared form the amino acid using hexafluoroacetone[78] however this has not found popularity due to the difficulties involved with the handling of hexafluoroacetone. The attempted preparation of the oxazolidinone derivative[79] (33) was unsuccessful under the conditions set out in Scheme 6 and was not pursued further.



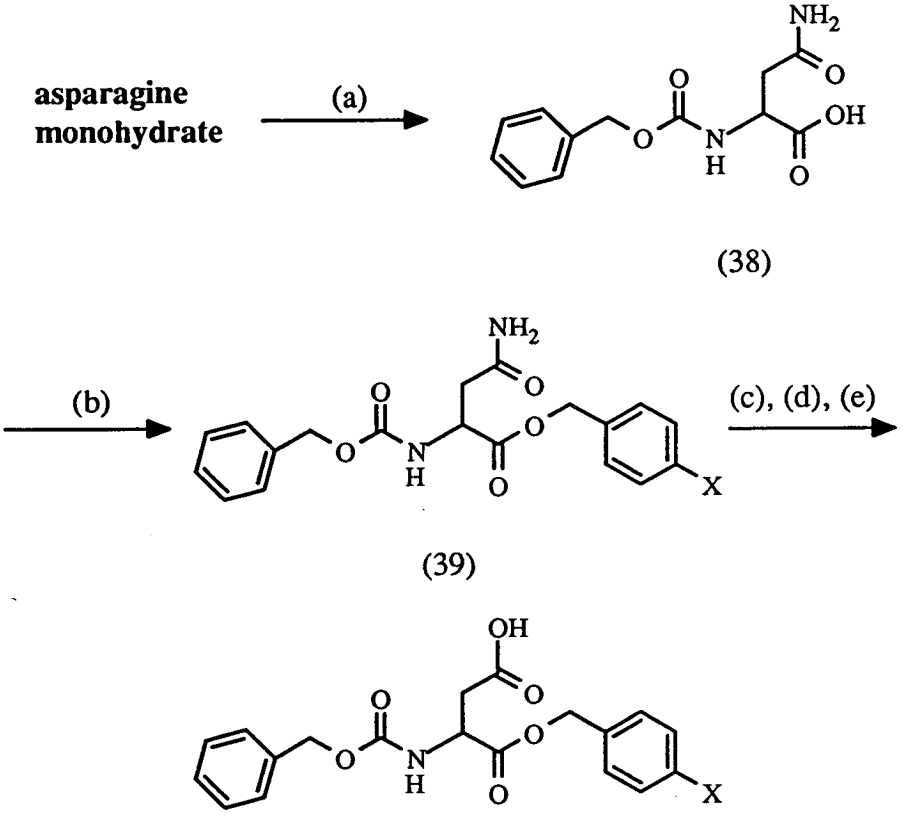
(33)

(a) parformaldehyde, acetic anhydride, thionyl chloride, acetic acid, 100°C, 4h.

Scheme 6: Route to of 3-benzyloxycarbonyl-5-oxo-4-oxazolidineacetic acid

The boroxazolidinone[80] (34) offers an alternative to the above strategies as it is cleaved using anhydrous hydrogen chloride to regenerate the amino acid as its salt, however these conditions were thought to be too harsh for use in conjunction with acid sensitive derivatives.

The aspartic acid copper complex copper salt[81] (36) is easily prepared in almost quantitative yield, is exceedingly stable to prolonged storage and is easy to use. Its use has so far been limited to reaction with relatively simple, primary alkyl halides only.



(a) benzylchloroformate, sodium hydrogen carbonate; (b) dicyclohexylamine, benzyl bromide or 4- nitrobenzyl bromide; (c) nitrosylsulphuric acid, glacial acetic acid; (d) cyclohexylamine; (e) potassium hydrogen sulphate.

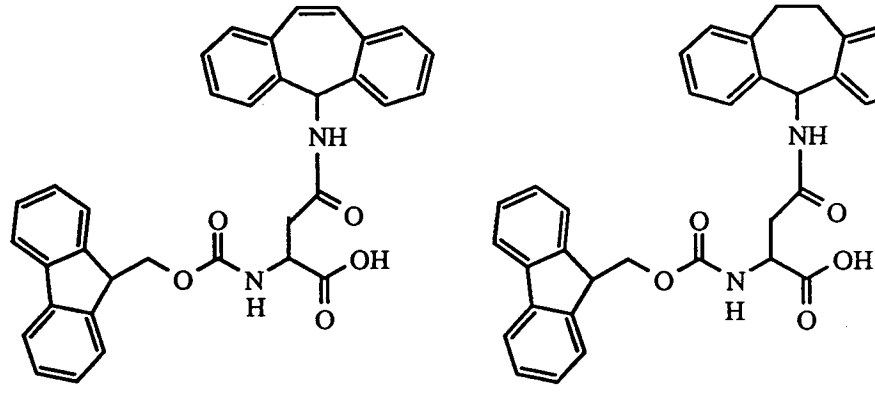
Scheme 7: The preparation of N^{α}-benzyloxycarbonyl aspartic acid α benzyl ester

Scheme 7 summarises a reliable, general protection of the α-amine and α-acid functions of aspartic acid. The benzyloxycarbonylaspartic acid α-benzyl esters (40) and (41) can be prepared from asparagine by exploiting the amide of the amino acid as a protecting group for the β acid functionality. Benzyloxycarbonylasparagine

(38) was converted to its benzyl ester (39) by alkylating the ammonium salt with the appropriate halide. The amide can then be converted to a carboxylic acid by the action of nitrosylsulphuric acid[82] in glacial acetic acid.

2.2 DIBENZOSUBERYL DERIVATIVES

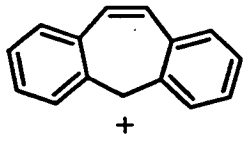
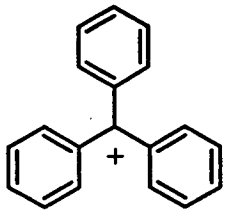
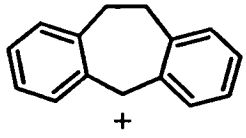
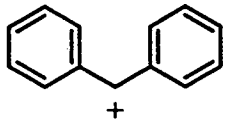
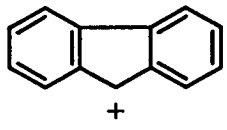
The alcohol dibenzosuberol (10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5-ol)[83] (43) has been the subject of much interest since it's synthesis in 1950. Pless[84] carried out a study of the relative stabilities of the cations summarised in Figure 13 and established the use of the dibenzosuberyl group as an acid-labile side chain protecting group for a number of amino acids. The dibenzosuberyl group was applied to the protection of the sulfhydryl group of cysteine, the hydroxyl group of serine and the ε-amino group of lysine. It was also used for α-amino and α-carboxyl protection



(41) (42)

Figure 12: Dibenzosuberenyl and dibenzosuberyl protected asparagine derivatives

The Fmoc and Bnpeoc derivatives of asparagine and glutamine protected by the dibenzosuberyl and dibenzosuberenyl groups have been synthesised for use in solid phase peptide synthesis[85]. They have been evaluated in comparison with trityl and methoxybenzhydryl protection but have not been widely adopted.

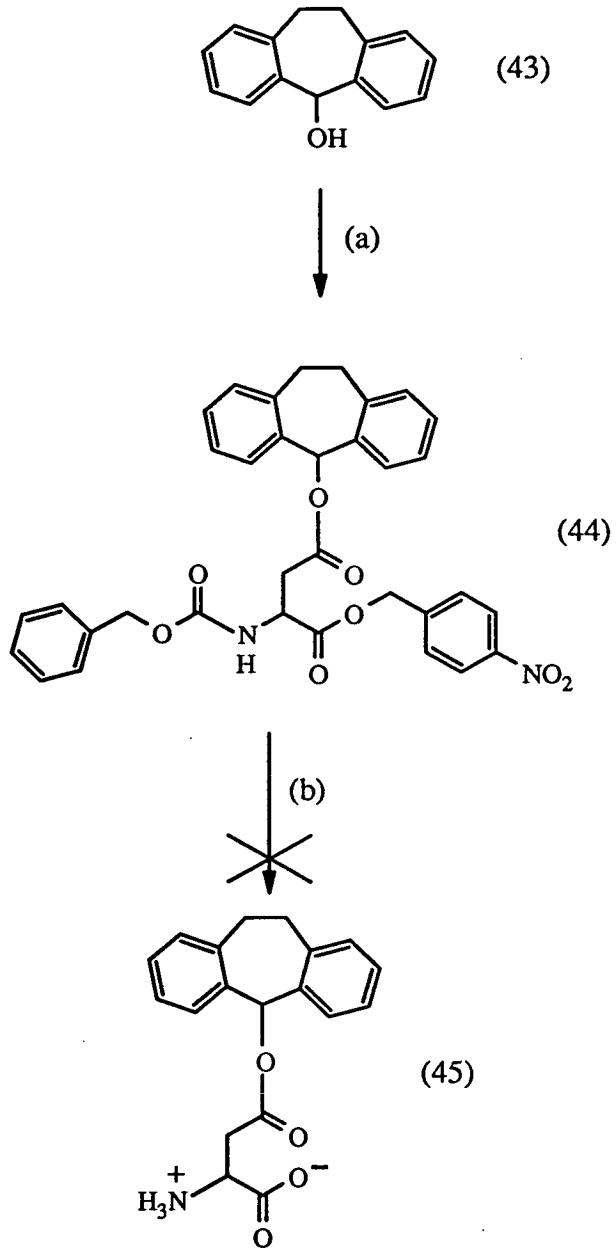
Cation	pK_a
	-3.7
	-6.6
	-8.0
	-13.3
	-14.0

$$ROH + H^+ \rightleftharpoons R^+ + H_2O$$

$$K_a = [R^+]\,[H_2O] / [ROH]\,[H^+]$$

Figure 13: Stability of a series of related cations[84]

2.2.1 The attempted preparation of aspartic acid β-dibenzosuberyl ester



(a) DCC, DMAP, (40), CH_2Cl_2; (b) H_2, Pd/C, methanol.

Scheme 8: The attempted preparation of aspartic acid β-dibenzosuberyl ester

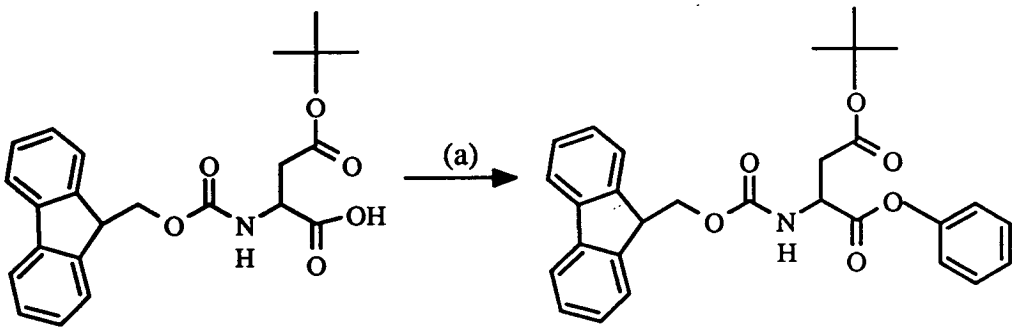
The aim then was to extend this strategy to the side chain protection of aspartic acid through the preparation of N^{α}-fluorenylmethoxycarbonylaspartic acid-β-dibenzosuberyl ester (50). The dibenzosuberyl moiety was successfully introduced

via DCC/DMAP[86] mediated coupling of the aspartate derivative (40) to the alcohol as shown in Scheme 8. Upon hydrogenolysis to remove the amine and α-carboxyl protecting groups none of the desired β-ester (45) could be detected. Instead aspartic acid and dibenzosuberane were obtained as a result of complete hydrogenolysis of the protecting groups. Closer inspection reveals that the C-5 position in the dibenzosuberyl ring may be regarded as a substituted benzylic derivative and this proved not to be resistant to catalytic hydrogenolysis.

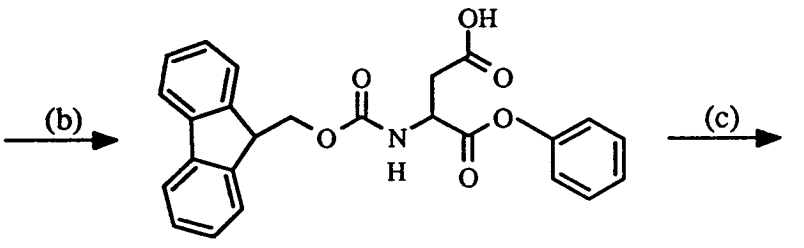
The aspartic acid copper complex (36) has been applied to the preparation of side chain esters using a number of primary alkyl halides but there is no reported example of its alkylation with a secondary alkyl halide. This was briefly examined but no reaction was observed between 5-chlorodibenzosuberane and the copper complex under literature conditions[81].

2.2.2 The attempted preparation of N^{α}-fluorenylmethoxycarbonylaspartic acid β-dibenzosuberyl ester

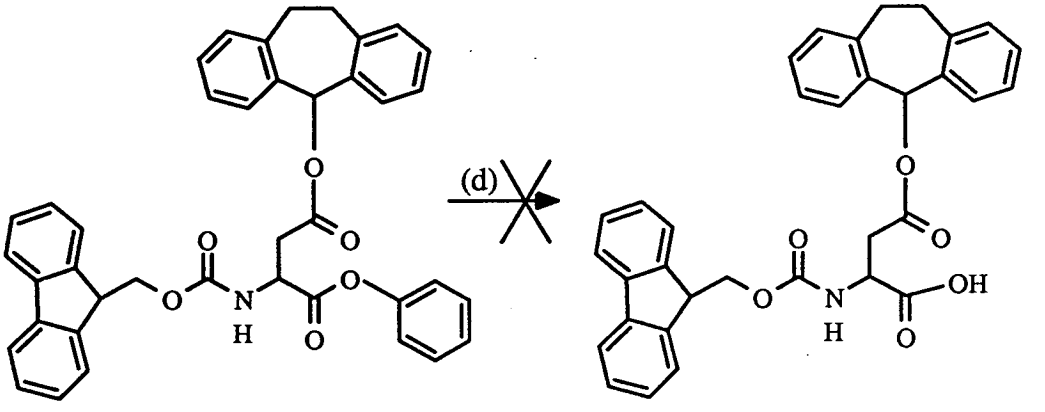
An alternative route to the target compound was devised (Scheme 9) utilising a base labile ester for temporary α-carboxyl protection. By using the commercially available starting material (46) differentiation between the α and β functionalities is already supplied. The phenyl ester of the derivative (46) was prepared by the method of Ramage[87]. This type of protecting group can be rapidly removed using dilute alkaline solution containing a catalytic quantity of hydrogen peroxide. The peroxide anion is an α-effect[88] nucleophile. The enhanced nucleophilicity of the peroxide anion is rationalised by repulsion between the non-bonding electrons of the oxygen adjacent to the oxygen atom bearing the negative charge. The intermediate percarboxylic acid produced will be rapidly hydrolysed to the carboxylic acid regenerating peroxide in the process.



(46) (47)



(48)



(49) (50)

(a) DCC, pyridine, phenol; (b) TFA, CH_2Cl_2 (c) DCC, DMAP, (43),(d) sodium hydroxide, hydrogen peroxide, water.

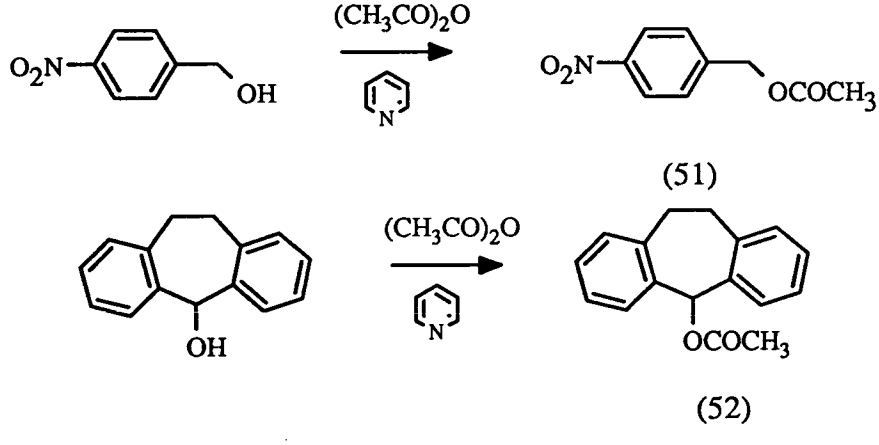
Scheme 9: The attempted preparation of N^{α}-fluorenylmethoxycarbonylaspartic acid -β-dibenzosuberyl ester

The tertiary butyl ester was smoothly removed in trifluoroacetic acid to give the α-protected carboxylic acid (48). This was esterified using dibenzosuberol and

DCC/DMAP. Unfortunately and surprisingly, selective hydrolysis of the phenyl ester could not be achieved. TLC indicated that a mixture of products was produced and upon aqueous work up no single product could be isolated. TLC and NMR evidence indicated that significant competitive hydrolysis of the dibenzosuberyl ester was occurring under these conditions.

2.2.3 Stability Studies

This observed instability to base had not been reported previously. A comparative study of the base lability of the acetate esters of 4-nitrobenzyl alcohol (51) and dibenzosuberol (52) was carried out (Scheme 10). It was expedient to use the nitrobenzyl ester because the chemical shifts of the benzylic protons would allow differentiation between the product and starting material. In the alcohol the benzylic protons are found at δ_H=4.8 and at δ_H=5.2 in the corresponding ester. The base lability of nitrobenzyl esters is well documented.



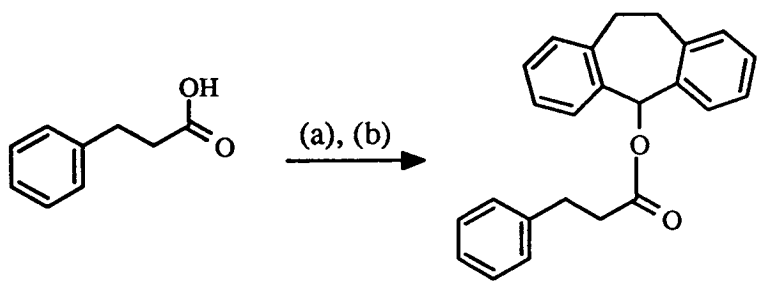
Scheme 10: The preparation of model acetate esters

The hydrolysis of the respective esters was monitored by ^1H NMR and quantified by inspection of the integrals. While it can be seen that the *para*-nitrobenzyl ester (51) was much more labile the dibenzosuberyl acetate (52) was still hydrolysed at a significant rate.

Time (min)	1	30	120
% (51)	100	-	-
% (52)	64	33	17

Table 2: NMR study of ester hydrolysis

Hydrocinnamate esters are useful as model compounds for protecting group stability studies in HPLC analysis. The dibenzosuberyl hydrocinnamate (53) was prepared via the acid chloride as shown by Scheme 11.



(53)

(a) oxalyl chloride; (b) pyridine, dichloromethane, dibenzosuberol.

Scheme 11: The preparation of dibenzosuberyl hydrocinnamate

Aqueous alkaline hydrolysis was used as an indicator of the general stability of the compound to nucleophiles. The stability to alkali of this hydrocinnamate was studied by reverse phase HPLC. This showed that after 3 hours 50% hydrolysis had occurred which is consistent with the NMR results.

2.2.4 Concluding remarks

It has been shown that from synthetic and physical studies a number of structurally different dibenzosuberyl esters are relatively susceptible to hydrolysis by alkali. Their specific incorporation into the side-chain of aspartic acid proved

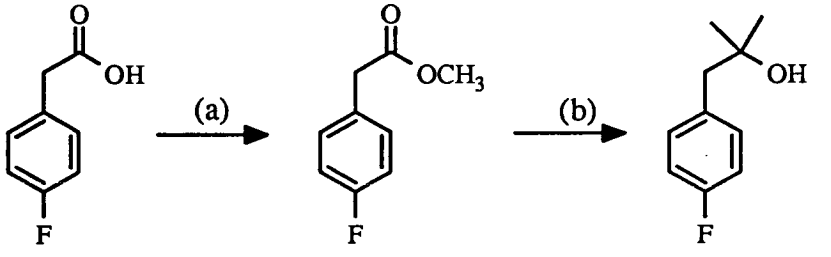
fruitless and the properties of the derivatives isolated were not encouraging. Thus it was concluded that these compounds would not satisfy the necessary criteria for an aspartic acid side chain protecting group.

2.3 THE DERIVATIVES OF 1-(4'-FLUOROPHENYL)-2-METHYLPROPAN-2-OL

This protecting group had originally been described by Longstaff[68], who applied it successfully to the synthesis of a pentapeptide Ile.Leu.Asp.Asn.Ile. Encouraged by the preliminary findings the protecting group was prepared in order that its use may be extended to the synthesis of a peptide that is susceptible to aspartimide formation.

2.3.1 Synthesis of 1-(4'-fluorophenyl)-2-methylpropan-2-ol

The methyl ester (55) can be obtained in high yield from 4-fluorophenylacetic acid. Reaction of this with the Grignard reagent methylmagnesium iodide yields the alcohol (56) (Scheme 12).

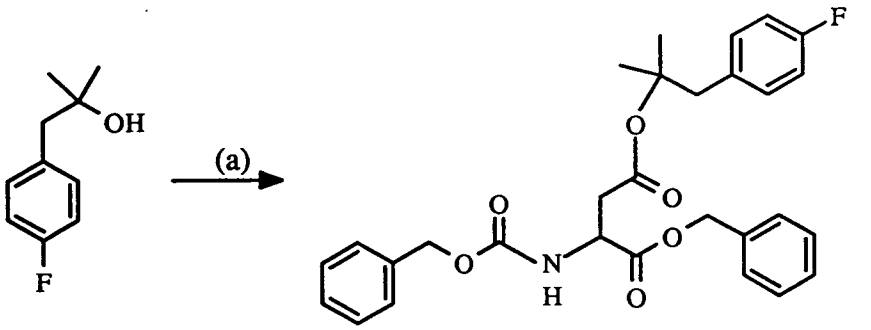


(54) (55) (56)

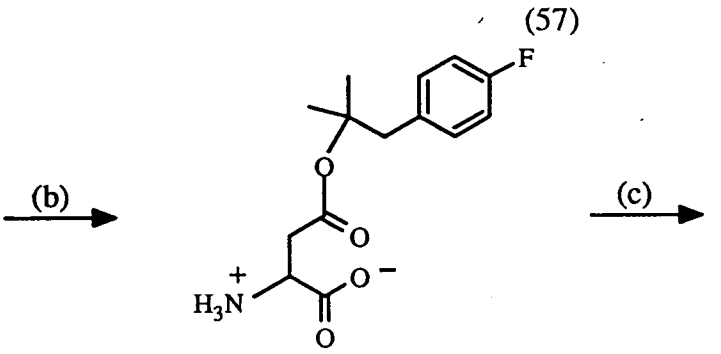
(a) methanol, concentrated sulphuric acid; (b) magnesium, iodomethane.

Scheme 12: The preparation of 1-(4'-fluorophenyl)-2-methylpropan-2-ol

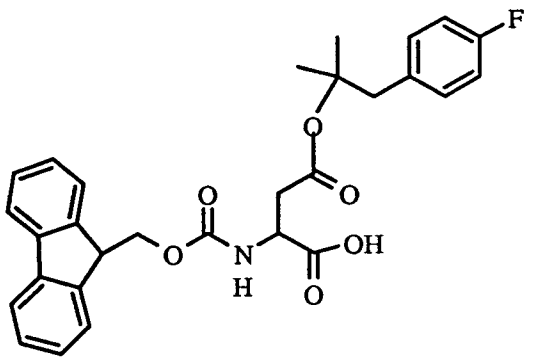
2.3.2 Synthesis of N^{α}-9-fluorenylmethoxycarbonylaspartic acid-β-1-(4'-fluorophenyl)-2-methylprop-2-yl ester



(57)



(58)



(59)

(a) (40), DCC, DMAP; (b) H_2, Pd/C, methanol; (c) 9-fluorenylmethyl-succinimidyl carbonate, triethylamine.

Scheme 13: Preparation of N^{α}-9-fluorenylmethoxycarbonylaspartic acid-β-1-(4'-fluorophenyl)-2-methylprop-2-yl ester

The alcohol (56) could be coupled to the protected acid (40) using DCC/DMAP (Scheme 13) to give the derivative (57) as a low melting solid after column chromatography. Catalytic hydrogenolysis gave the amino acid ester (58)

which was converted to the Fmoc protected compound (59) for use in solid phase peptide synthesis.

The fluorophenyl ring was designed as a probe for use in analysing the aspartimide reaction by ^{19}F NMR. The volume and the relative involatility of DMF means the effluent cannot easily be analysed by NMR. A relatively small quantity of protecting group (56) was lost as a result of aspartimide formation making the NMR experiment too insensitive. It is not possible to distinguish between ring closure brought about by acid or base treatment through the use of a ^{19}F NMR label.

2.4 ANTHRACENYL DERIVATIVES

Monitoring of the synthesis by ultraviolet (UV) spectrophotometry in a way similar to the system used during Fmoc deprotection would allow observation of the cyclisation *in situ*. The ultraviolet spectrum of the organic phase after acid cleavage can be used to determine the approximate quantity of UV tag that was still intact at this point. A suitable UV chromophore was required that would be distinct from the dibenzofulvene-piperidine adduct at 302 nm and sufficiently intense to provide a sensitive probe.

The UV/visible spectrum of anthracene[89] (Figure 15) fulfilled these criteria and the ring could be functionalised without perturbing the chromophore. Typically anthracene has maximum wavelength values at 347, 365 and 385 nm and its extinction coefficient at 300 nm is approximately 500 $dm^3mol^{-1}cm^{-1}$, while the extinction coefficient of the fulvene-piperidine adduct at 300 nm is 9200 $dm^3mol^{-1}cm^{-1}$ (i.e. it is about 5% of that of the adduct).

The reactivity of the anthracene ring is towards electrophilic substitution at the 9 and 10 positions. Therefore although 1 and 2 substituted compounds are known the 9 substituted derivatives are more easily accessible and were chosen for this

project.

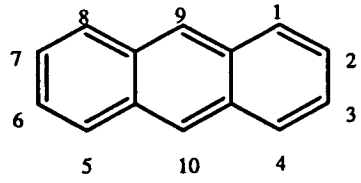


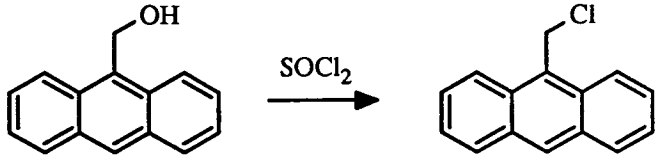
Figure 15: The numbering in the anthracene ring

2.4.1 The esters of 9-anthracenylmethanol

It has been demonstrated on a peptide containing an aspartyl-glycine sequence that benzyl ester protection of aspartic acid leads to the formation of the cyclic aspartimide compound. The anthracenylmethyl group can be regarded as a substituted benzyl group and possesses a chemical similarity, e.g rapid hydrogenolysis under catalytic conditions and similar sensitivity to basic hydrolysis. This group was studied as an α carboxyl protecting group in 1965 by Stewart[90] and in 1973 Kornblum[91] demonstrated that it was sensitive to nucleophiles such as mercaptans. It was intended that this system be used to demonstrate the practicability of the monitoring.

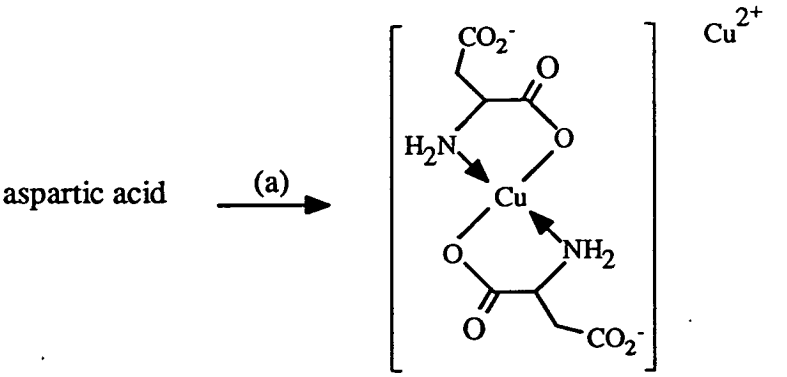
2.4.2 The synthesis of N^{α}-9-fluorenylmethoxycarbonylaspartic acid-β-9-anthracenylmethyl ester

The anthracene derivative 9-chloromethylanthracene was required in large quantities and was prepared from the alcohol (60) and thionyl chloride at ambient temperature over a period of 12 to 24 hours.

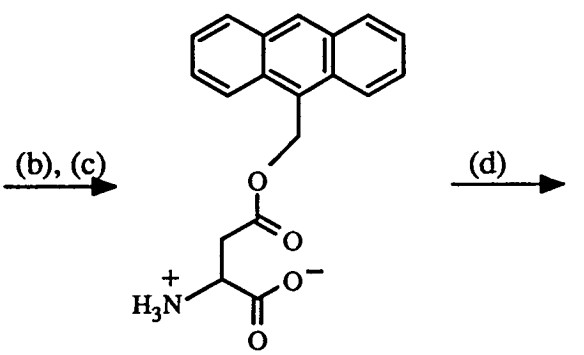


(60) (61)

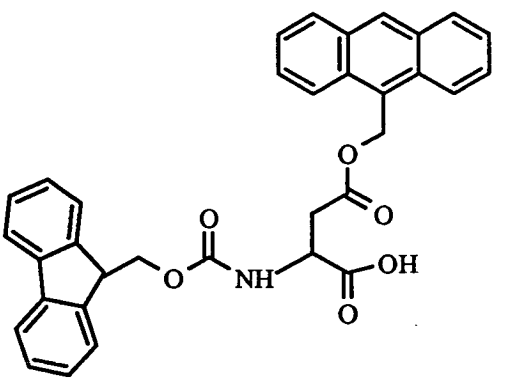
Figure 16: The preparation of 9-chloromethylanthracene



(36)



(63)



(64)

(a) copper (II) acetate, water; (b) 9-chloromethylanthracene, tetramethylguanidine; (c) ethylenediamine tetraacetic acid disodium salt; (d) 9-fluorenylmethyl succcinimidyl carbonate, triethylamine.

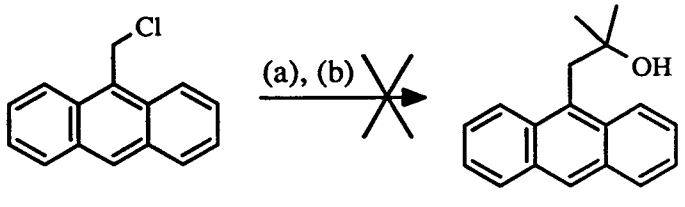
Scheme 14: The preparation of N^{α}-fluorenylmethoxycarbonylaspartic acid-β-9-anthracenylmethyl ester

Combination of the copper complex (36), aspartic acid and

tetramethylguanidine in the ratio of 1:2:4 provided the copper aspartate complex tetramethylguanidinium salt[81]. One mole of the original complex (36) generates two moles of the aspartic acid copper complex tetramethylguanidinium salt as the copper displaced by the tertamethylguanidine is sequestered by the aspartic acid. The alkylation of this salt with 9-chloromethylanthracene could be achieved more efficiently than if the copper salt itself was used for the reaction. The product of this reaction was decomposed using the sodium salt of ethylenediaminetetraacetic acid, without intermediate purification, to obtain the amino acid ester (63) in a yield of 40%. This compound proved difficult to recrystalise due to its very poor solubility characteristics. The incorporation[92] of the Fmoc amine protection proved inefficient presumably because of steric hindrance. Prolonged reaction times in this case are discouraged on account of the basicity of the reaction conditions, which may cause deprotection. Therefore, under these circumstances a yield of 42% of (64) was found to be acceptable for the purposes of this study (Scheme 14).

2.4.3 The synthesis of 1-(9'-anthracenyl)-2-methylpropan-2-ol

In order to model the tertiary butyl type of protecting group the compound (65) was prepared. Initial attempts to use the Grignard reagent anthracenylmethyl magnesium chloride and combine it with acetone failed (Scheme 15). The Grignard reagent could not be prepared in either anhydrous THF or diethyl ether.

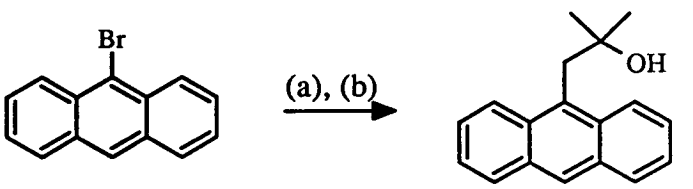


(65)

(a) magnesium, ether or THF; (b) acetone.

Scheme 15: The attempted preparation of 1-(9'-anthracenyl)-2-methylpropan-2-ol

By taking 9-bromoanthracene with n-butyllithium the aryl lithium[93] species could be formed and this reacted smoothly with isobutylene oxide with isolated yields of up to 85% (Scheme 16).



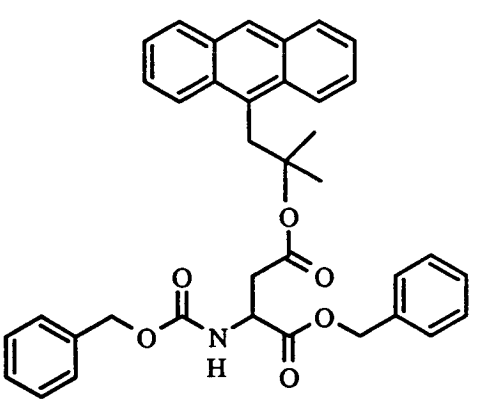
(80) (65)

(a) n-butyllithium, THF, -78°C; (b) isobutylene oxide.

Scheme 16: The preparation of 1-(9'-anthracenyl)-2-methylpropan-2-ol

2.4.4 The chemistry of 1-(9'-anthracenyl)-2-methylpropan-2-ol

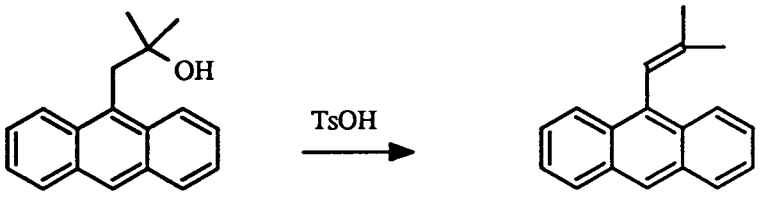
Initial studies of the esterification of the alcohol were centred around the established DCC/DMAP methodology in order to prepare (66) but this was unsuccessful. Repeated attempts with these reagents using different solvent conditions (DMF, dichloromethane and ethyl acetate) never provided any of the required product. Diisopropylcarbodiimide was used in place of the more sterically hindered DCC without success.



(66)

Tertiary esters can be prepared from the corresponding alkene under

conditions of acid catalysis[17, 18]. Dehydration of the alcohol in TFA/dichloromethane gave a mixture of products that could not be purified. The alcohol (65) was converted to the alkene (66) by refluxing with 4-toluenesulphonic acid to give the product shown in Scheme 17 as deduced from its NMR spectra. There was no reaction observed between this alkene and the compound (40) in the presence of 4-toluenesulphonic acid.

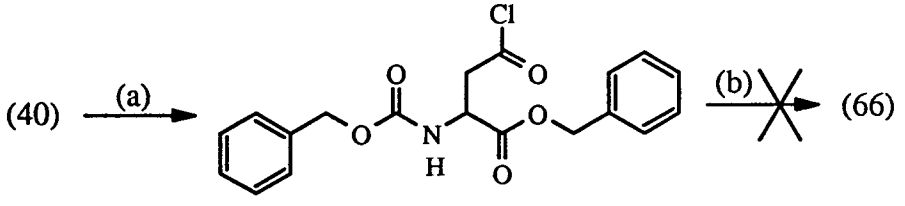


(67)

Scheme 17: Dehydration of 1-(9'-anthracenyl)-2-methylpropan-2-ol

A number of small scale experiments followed by TLC were carried out to further probe the reactivity of the alcohol. These are described below.

Oxalyl chloride was used to prepare the acid chloride of the aspartate compound (40) and this was characterised by the presence in the infrared of the bands at approximately 1790 cm^{-1} and the absence of absorptions between 2800-3400 cm^{-1} typical of hydroxyl groups.



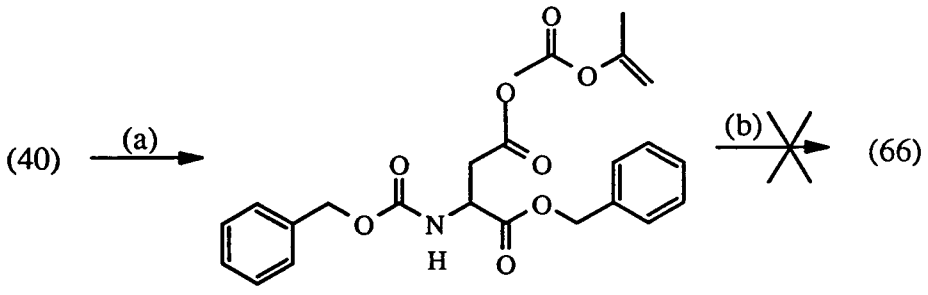
(68)

(a) oxalyl chloride, reflux; (b) (65), base.

Scheme 18: The attempted preparation of N^{α}-benzyloxycarbonylaspartic acid-α-benzyl-β-1-(9'-anthracenyl)-2-methylprop-2-yl ester

The alkoxide of the alcohol (65) was prepared using n-butyllithium or sodium hydride but none of the intended ester (66) could be detected upon addition of the acyl halide (68).

Activating the acid as a mixed anhydride was attempted with isopropenyl chloroformate[94] and triethylamine. This material (69) was used without characterisation in combination with the alcohol, triethylamine and DMAP. No reaction was observed under these conditions (Scheme 19).

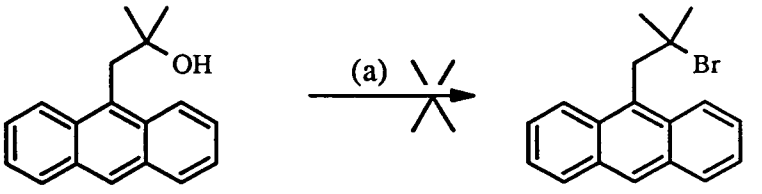


(69)

(a) triethylamine, DMAP, isopropenyl chloroformate, THF; (b) (65).

Scheme 19: The attempted preparation of N^{α}-benzyloxycarbonylaspartic acid-α-benzyl-β-1-(9'-anthracenyl)-2-methylprop-2-yl ester

Tertiary alkyl bromides can be prepared by a number of methods. It was intended that the halide (70) could be prepared then combined with a suitable carboxylate salt to provide a tertiary ester. HBr in acetic acid containing 0.2 equivalents of lithium bromide at 0°C[95] has been used to convert tertiary butyl alcohol to tertiary butyl bromide efficiently with high level of purity. The alcohol (65) could not however be converted to the bromide (70) under the conditions employed (Scheme 20). NMR and infrared spectroscopy indicated that the starting material remained unchanged.

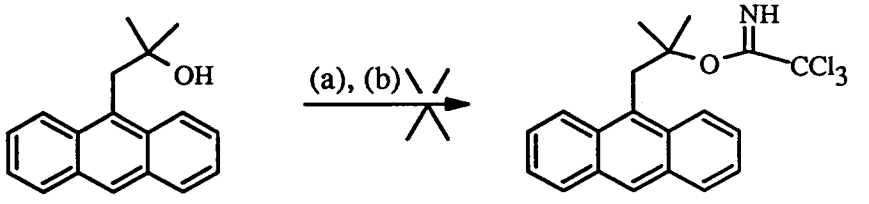


(70)

(a) 48% HBr, LiBr (0.75 eq.).

Scheme 20: The attempted preparation of 1-(9'-anthracenyl)-2-methyl-2-bromopropane

The reaction between trichloroimidates and carboxylic acids in the presence of boron trifluoride diethyl etherate[96] has been used in the preparation of tertiary esters. This was applied to this system but the preparation of the trichloroimidate (71) (Scheme 21) was not successful and the starting alcohol was recovered unchanged.

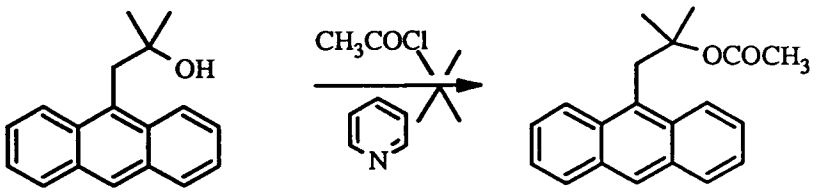


(71)

(a) sodium hydride (0.1 eq.), dry ether; (b) trichloroacetonitrile, 60 minutes.

Scheme 21: The attempted preparation of 1-(9'-anthracenyl)-2-methylprop-2-yl trichloroimidate

The alcohol (65) could not be converted even to its acetate ester (Scheme 22) with pyridine and acetyl chloride. Extractive work up of portions over a period of two days gave starting material alone.



(72)

Scheme 22: The attempted preparation of 1-(9'-anthracenyl)-2-methylprop-2-yl acetate

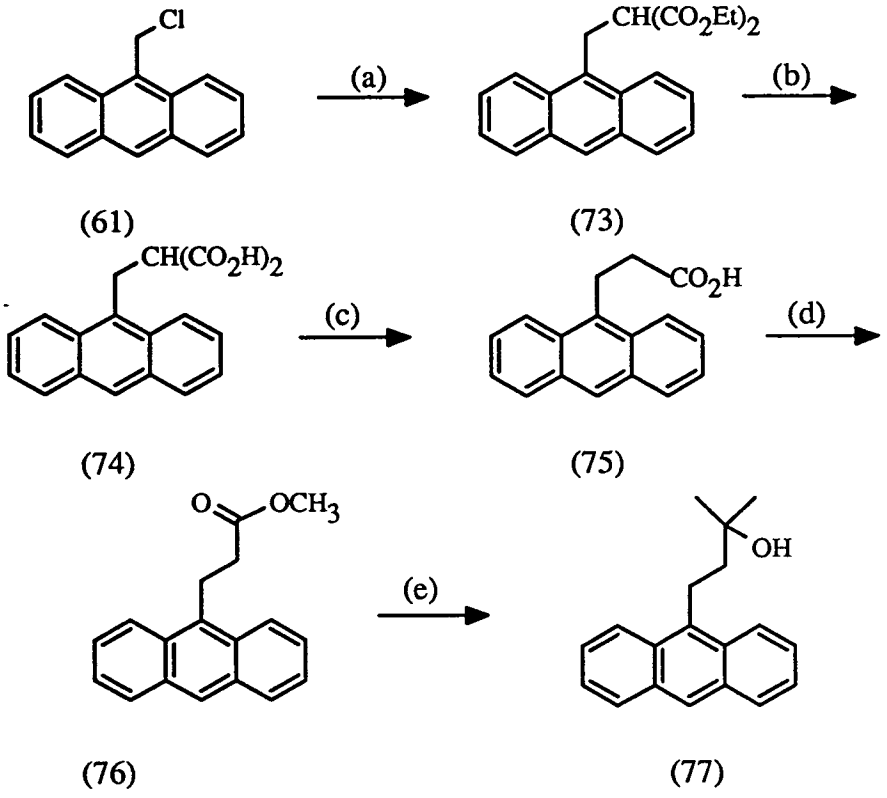
Thus it was concluded that the steric hindrance caused by the protons at C-1 and C-8 must be preventing interaction of the reagents[97].

2.4.5 The synthesis of 1-(9'-anthracenyl)-3-methylbutan-3-ol

An attempt was made to relieve the steric hindrance caused by the H-1 and H-8 protons by extension of the side chain. The derivative (77) was synthesised in order to overcome the problems due to steric hindrance.

9-Chloromethylanthracene was combined with diethyl malonate and sodium hydride to give the product (73) as described in Scheme 23. The hydrolysis of the diethyl ester was achieved by both acid and base catalysed hydrolysis to provide quantities of the diacid (74). The yield with sodium hydroxide in aqueous acetone was superior and more reproducible than when concentrated hydrochloric acid in dioxane was used. The malonic acid derivative (74) proved to be thermally stable to a temperature of greater than 200°C. Simply heating to this temperature under an inert nitrogen atmosphere caused degradation of the molecule and no useful material was isolated. Using refluxing tetrahydronaphthalene (b.p. 207°C) the decarboxylation could be executed in 1.5 hours but with varying yields and unreliable recovery of acid. The methyl ester (76) was obtained from the acid catalysed esterification in methanol of the carboxylic acid (75) and was then combined with the Grignard reagent methyl magnesium iodide to give the alcohol

(77). This was accomplished in satisfactory yield and the products were obtained without chromatographic purification.



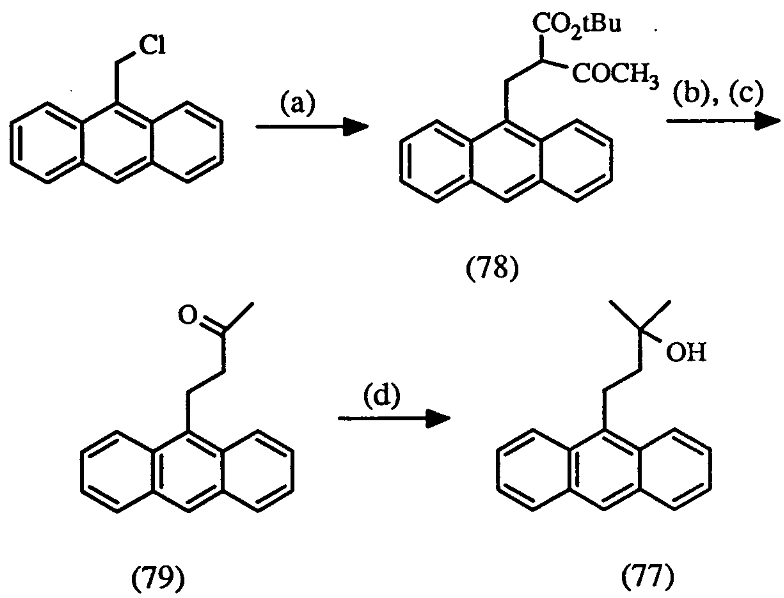
(a) sodium hydride, diethyl malonate; (b) sodium hydroxide or hydrochloric acid; (c) tetrahydronaphthalene, reflux; (d) methanol, conc. sulphuric acid; (e) magnesium, iodomethane.

Scheme 23: The preparation of 1-(9'-anthracenyl)-3-methylbutan-3-ol

The preparation of the alcohol was simplified by replacing the malonic ester with the β-keto ester (78). β-Keto acids are in general less thermally stable than their analogous diacids[98].

The preparation of the β-keto ester (78) from the reaction of 9-chloromethylanthracene with tertiary butylacetoacetate (Scheme 24) and sodium hydride was carried out in THF at reflux since no reaction could be detected at room temperature. This compound (78) was used without purification, its NMR indicating

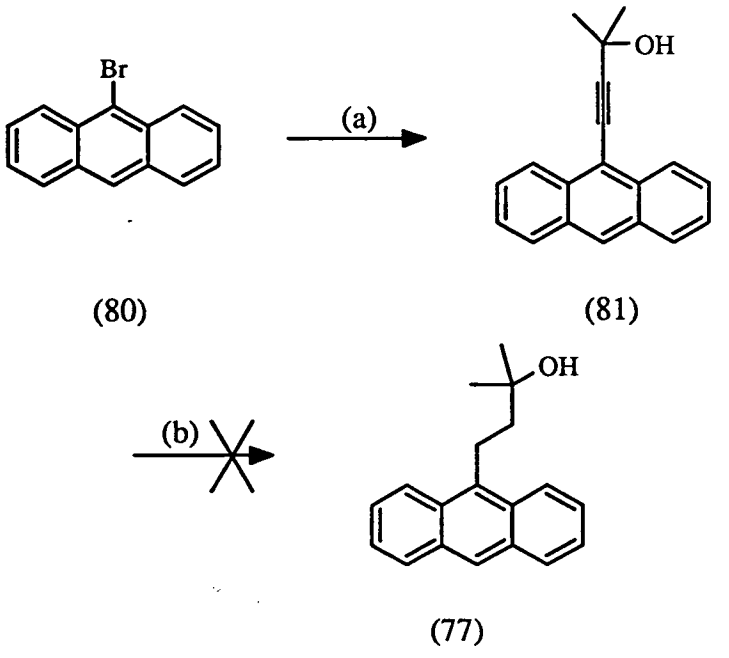
that a high degree of conversion was obtained. The acid was obtained by the action of anhydrous TFA and was then decarboxylated in refluxing toluene. Purification of the ketone (79) over silica resulted in approximately 70% yield based on 9-chloromethylanthracene. The Grignard reaction between methyl magnesium iodide and the ketone (79) to give the alcohol (77) was lower yielding than when the methyl ester (76) was the substrate (Scheme 23). Overall the second scheme proved simpler and more efficient.



(a) sodium hydride, tertiary butylacetoacetate, (b) TFA, dichloromethane, (c) toluene, 110°C, (d) magnesium, iodomethane.

Scheme 24: The preparation of 1-(9'-anthracenyl)-3-methylbutan-3-ol

Further simplification of the synthesis of this compound (77) by preparation of the alkyne (81) was envisaged (Scheme 25). This could be reduced to the alcohol by catalytic hydrogenation. The palladium (0) catalysed coupling of 9-bromoanthracene and 2-methylbut-3-yn-2-ol was carried out by analogy with the method of Lau *et al.*[99] with variable yields of product (81) obtained.



(a) triphenylphosphine, palladium (II) acetate, triethylamine, 2-methylbut-3-yn-2-ol
(b) hydrogen, Pd/C (See Table 3).

Scheme 25: The attempted preparation of 1-(9'-anthracenyl)-3-methylbutan-3-ol

The compound (81) was identified by accurate mass spectrometry and by the presence of the hydroxyl in its infra-red spectrum. This was confirmed by the presence of an exchangeable proton at 1.8 ppm although the alkyne absorption could not be detected by infrared nor were the alkyne quaternary carbons observed in the NMR.

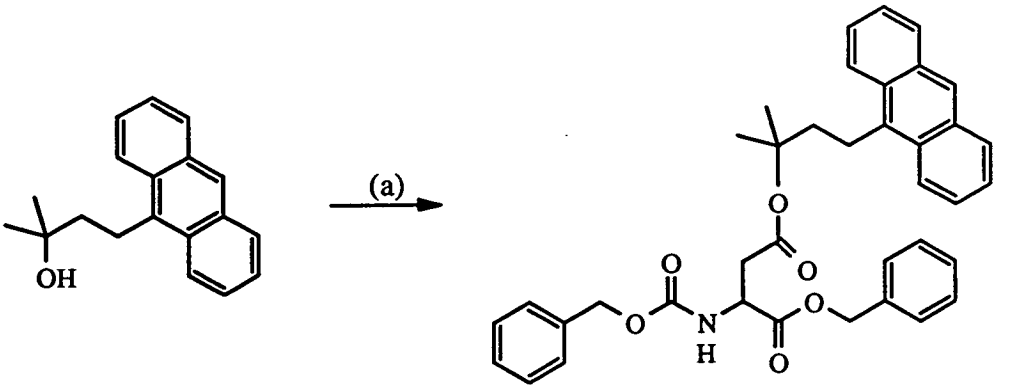
Repeated attempts to reduce the triple bond through catalytic hydrogenation (10% palladium on charcoal) at ambient temperature and atmospheric pressure were unsuccessful. Increasing the pressure and varying the temperature (Table 3) did not provide the desired alcohol. TLC and ^{1}H NMR evidence indicated that the compound (81) had decomposed.

solvent	pressure	temperature	time
methanol	3 atm	ambient temp.	16 hours
methanol/ethyl acetate	3.5 atm	30°C	6 hours
ethyl acetate	2 atm	ambient temp.	4 hours

Table 3: Conditions for the attempted reduction of (81)

2.4.6 The synthesis of N^{α}-9-fluorenylmethoxycarbonylaspartic acid-β–1(9'-anthracenyl)-3-methylbut-3-yl ester

The efficient coupling of the alcohol (77) to the aspartyl derivative (40) was achieved by using an excess of the acid (40) because this acid was regarded as the more expendable component (Scheme 26). Additionally this acid could be removed by extraction with sodium carbonate or sodium hydrogen carbonate upon completion of the coupling. Acceptable yields of the ester were obtained using a threefold excess of acid (40) and dicyclohexylcarbodiimide (a 1:1 ratio of acid to carbodiimide was used to prevent the formation of the symmetrical anhydride). The crude ester was carried through to the hydrogenolysis stage because intermediate purification was impossible. The R_f of the ester and alcohol on TLC in a range of solvent systems was found to be almost identical hence a chromatographic separation were not feasible. An overall yield of side chain protected amino acid from these two steps of 80%, with respect to the alcohol, was obtained after removal of the amine and α-carboxyl protection by catalytic hydrogenation. Introduction of Fmoc using fluorenylmethylsuccinimidyl carbonate with triethylamine[92] was achieved with diminished yield relative to previous systems (e.g. 1-(4'-fluorophenyl)-2-methylprop-2-yl ester) presumably due to the steric hindrance of the side chain ester.



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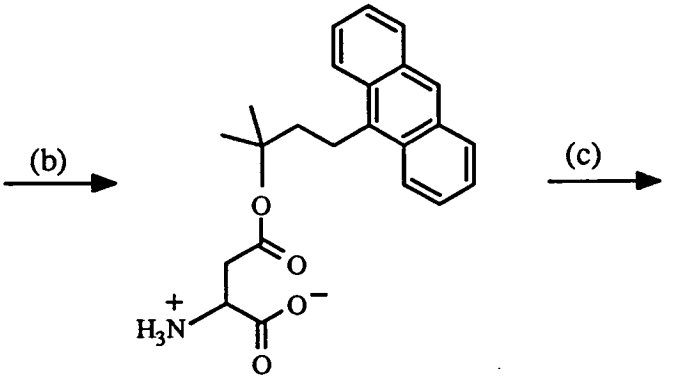

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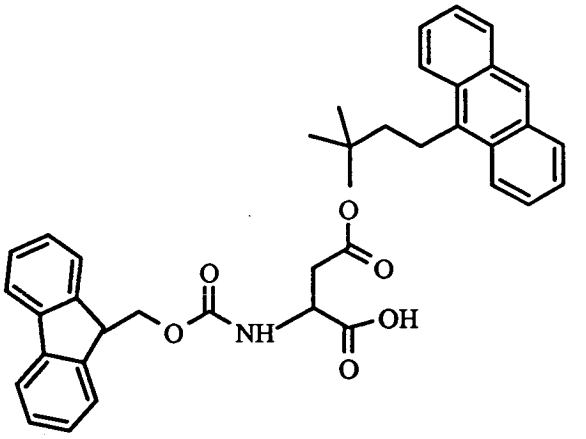


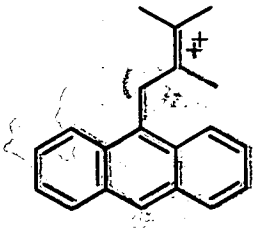
(84)

(a) DCC, DMAP, (40), dichloromethane; (b) H_2, Pd/C, THF/ methanol (3:7), (c) 9-fluorenylmethyl-succinimidyl carbonate, triethylamine.

Scheme 26: Preparation of N^{α}-fluorenylmethoxycarbonylaspartic acid β-1-(9'-anthracenyl)-3-methylbut-3-yl ester

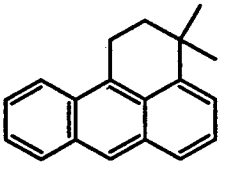
2.4.7 Deprotection studies

The fate of the cation (85) under conditions of peptide deprotection was investigated.



(85)

This species will be produced initially during acidolysis and it would be advantageous if it could scavenge itself decomposing to neutral non alkylating product. Because the side chains of some amino acids[69, 70, 71] are sensitive to alkylation, scavengers such as ethanedithiol are used during final deblocking and resin cleavage.



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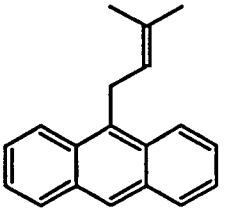


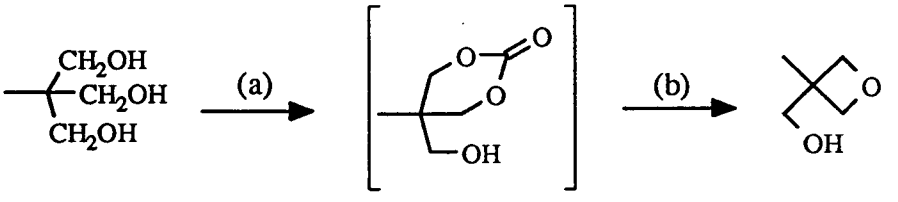
(87)

It was hoped that the cation would be susceptible to intramolecular alkylation to form (86) thus rendering itself unreactive. The alcohol was stirred with 70% TFA and TLC showed that a mixture of products was formed. Similarly with toluenesulphonic acid in refluxing toluene a complex mixture was observed. High resolution mass spectrometry found a peak for $C_{19}H_{19}$ [i.e. (86) or (87)] but analysis of the ^1H NMR spectrum indicated that a mixture of materials was present. HPLC analysis of the TFA cleavage of the hydrocinnamate ester (95) also resulted in the formation of a mixture of products. Further examination of these materials (e.g. by

GC/MS) was not undertaken.

2.5 THE PREPARATION OF ORTHO ESTER DERIVATIVES

The protection of the side chain of aspartic acid as an ortho ester[100] was also investigated. Their acid lability appeared to be quite within the specifications of the Fmoc peptide synthesis procedures and additionally it was thought probable that the products of acidolysis would be non-alkylating. The stability of these compounds to nucleophiles such as Grignard reagents seemed to indicate that they would be more than adequate for protection against intramolecular attack from an amide during assembly of a peptide. This type of protection has to date enjoyed limited application to amino acid chemistry, although their compatibility with urethane type protecting groups has been demonstrated[101].

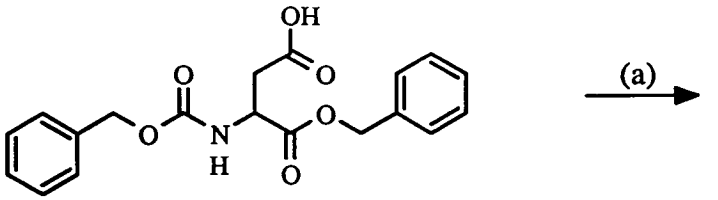


(88) (89) (90)

(a) potassium hydroxide, diethyl carbonate, (b) pyrolysis [220°C, 50 mmHg].

Scheme 27: The synthesis of 3-methyl-3-hydroxymethyloxetane

3-Methyl-3-hydroxymethyloxetane (90) was prepared from the triol (88) *via* the pyrolysis of the intermediate carbonate ester (89) by the method of Pattison[102] (Scheme 27).



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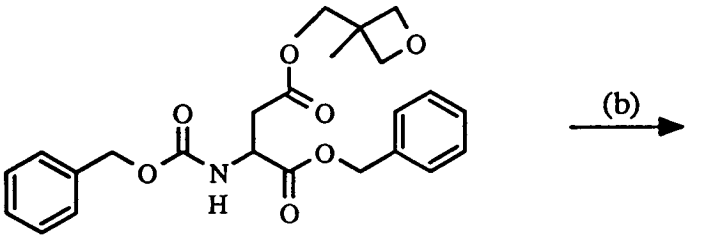

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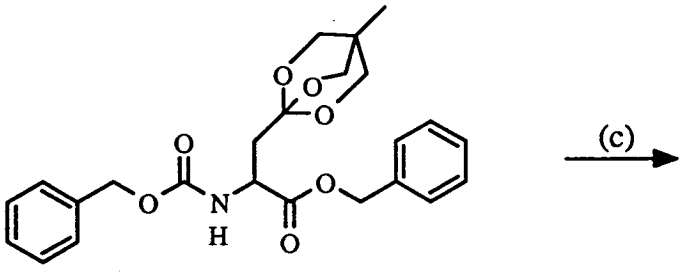
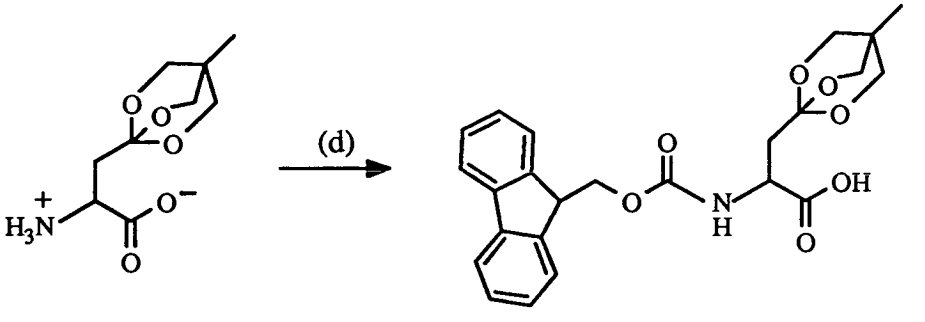


(92)



(93) (94)

(a) DCC, DMAP, 3-methyl hydroxymethyl oxetane ;(b) boron trifluoride diethyl etherate, triethylamine; (c) H_2, Pd/C, methanol; (d) 9-fluorenylmethyl-succinimidyl carbonate

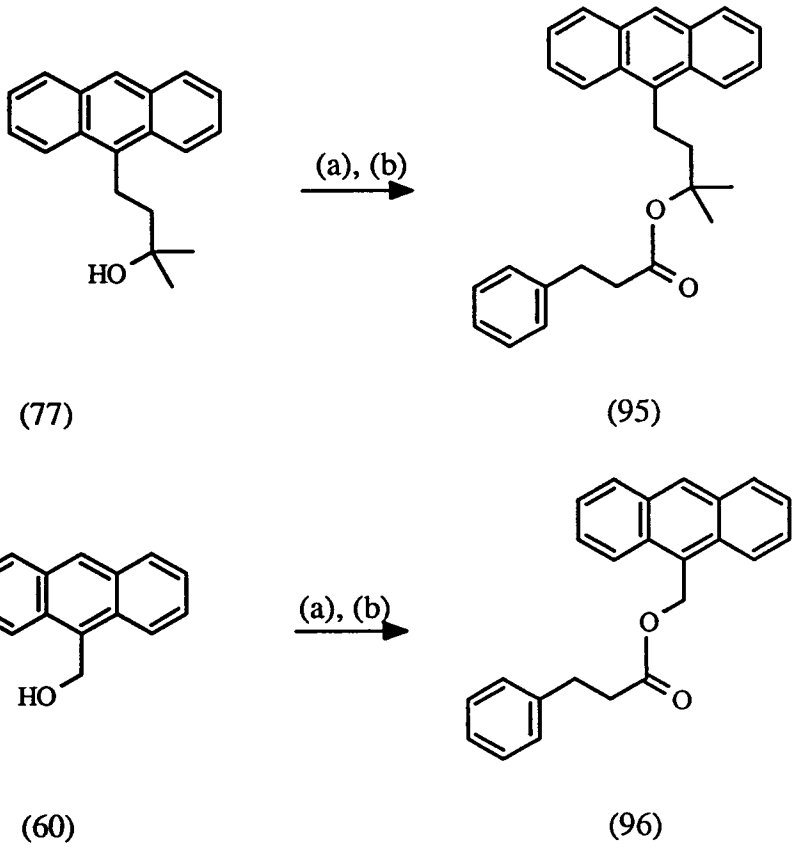
Scheme 28: The preparation of N^{α}-fluorenylmethoxycarbonyl-2-amino-3-(4'-methyl-2',6',7'-trioxabicyclo[2.2.2]octyl)-propionic acid

Esterification of (90) was achieved by carbodiimide coupling (Scheme 28) before the ester (91) was elaborated using the elegant Lewis acid-catalysed rearrangement of Corey[103]. Upon cleavage of the temporary benzyl and benzyloxycarbonyl protection by hydrogenolysis the Fmoc protection was introduced as for the other amino acids using fluorenylmethylsuccinimidyl carbonate in dioxane to provide the derivative (94) for use in solid phase peptide synthesis.

2.6 STABILITY STUDIES ON MODEL ESTERS

2.6.1 Synthesis of 9-anthracenyl hydrocinnamate esters

The two protecting groups based on the anthracenyl alcohols (60) and (77) were esterified with hydrocinnamic acid using DCC/DMAP coupling as shown in Scheme 29.



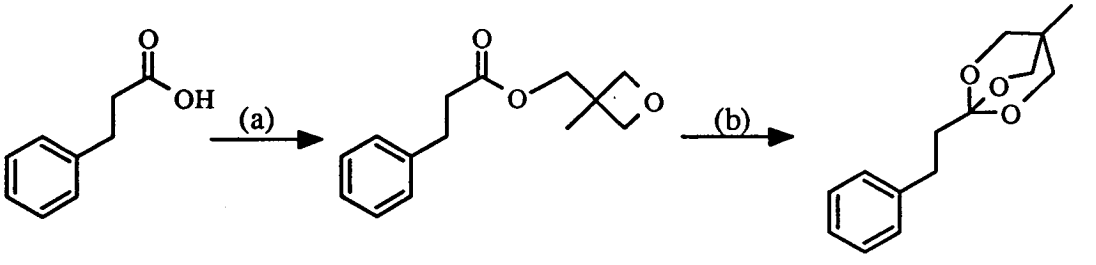
(a) DCC, DMAP; (b) hydrocinnamic acid.

Scheme 29: Preparation of model hydrocinnamate esters

The compounds (95) and (96) were obtained in satisfactory yield and were extensively purified by recrystallisation and column chromatography for the purposes of the HPLC study.

2.6.2 Synthesis of hydrocinnamoyl-3-methyl-3-hydroxymethyl-2,6,7-trioxabicyclo[2.2.2]octane

The hydrocinnamate ester of the alcohol (90) was prepared using DCC and DMAP then converted to the ortho ester derivative (98) using the boron trifluoride Lewis acid catalysed rearrangement as described in Scheme 30.



(97) (98)

(a) DCC, DMAP, (90); (b) boron trifluoride diethyl etherate, triethylamine.

Scheme 30: The preparation of phenyl ethyl -4-methyl-2,6,7-trioxabicyclo[2.2.2]octane

After purification of (98) it was found that its storage at room temperature was not feasible as it was found to readily decompose. The hygroscopic nature of this derivative was surprising as the derivative (92) was stable at room temperature for several months Therefore the stability studies were carried out using compound (92).

2.6.3 Alkaline hydrolysis studies

In the search for a protecting group that is orthogonal to Fmoc the stability to alkali of the hydrocinnamate derivatives was evaluated (Chapter 3.3), with the

exception of the example noted previously. This also provided an indication of the resistance of the ester towards nucleophilic attack.Thus the ease of hydrolysis exhibited by the 9-anthracenylmethyl and dibenzosuberyl esters indicated that they were inappropriate candidates for this purpose.

The tertiary 1-(9'-anthracenyl)-3-methylbut-3-yl and 1-(4'-fluorophenyl)-2-methylprop-2-yl esters have a pronounced stability to alkaline hydrolysis. The order of stability 1-(9'-anthracenyl)-3-methylbut-3-yl greater than 1-(4'-fluorophenyl)-2-methylprop-2-yl greater than tertiary butyl was observed but none were as effective as the 3-ethylpent-3-yl group[68].

Hydrocinnamate	Reaction time	Degree of hydrolysis
tertiary butyl[68]	24 hours	25%
3-ethylpent-3-yl[68]	4 days	0%
benzyl[68]	1 hour	100%
1-(4'-fluorophenyl)-2-methylprop-2-yl[68]	5 days	25%
dibenzosuberyl	3 hours	50%
9-anthracenylmethyl	3 hours	55%
1-(9'-anthracenyl)-3-methylbut-3-yl	6 days	10%

Table 4: Studies on the alkaline hydrolysis of some model esters

2.6.4 Acidolysis studies

In conjunction with the stability to basic conditions a novel protecting group requires to be rapidly cleaved in the presence of TFA. Table 5 contains the data for the acidolysis in TFA for all the esters (Chapter 3.3).

When the derivatives with poor base stability are eliminated from consideration the order of lability was found to be tertiary butyl greater than 1-(4'-fluorophenyl)-2-methylprop-2-yl greater than 1-(9'-anthracenyl)-3-methylbut-3-yl.

Hydrocinnamate	Concentration TFA/dichloromethane	Time for complete acidolysis
tertiary butyl[68]	50% 20%	instantaneous 15 min
3-ethylpent-3-yl[68]	20%	instantaneous
benzyl[68]	90%	3 days
1-(4'-fluorophenyl)-2-methylprop-2-yl[68]	50% 20%	15 min 30 min
dibenzosuberyl	20% 50%	30 min instantaneous
9-anthracenylmethyl	20%	instantaneous
1-(9'-anthracenyl)-3-methylbut-3-yl	50% 90%	3 hours 15 min

Table 5: Studies on the acidolysis of some model esters

Only the 1-(9'-anthracenyl)-3-methylbut-3-yl group fails to meet this criterion as it has been shown to have a pronounced stability at concentrations of TFA that bring about instantaneous or nearly instantaneous cleavage of the other

examples.

2.7 SOLID PHASE PEPTIDE SYNTHESIS

The hexapeptide Val.Lys.Asp.Gly.Tyr.Ile has been the subject of much investigation due to its pronounced tendency to undergo cyclic aspartimide formation. For this reason it has been adopted as a test peptide for the evaluation of a series of novel protecting groups.

2.7.1 Synthesis of Val.Lys.Asp.Gly.Tyr.Ile

The peptide was prepared by the Fmoc N^{α}-protecting strategy on an ABI automated peptide synthesiser using acid labile Wang resin and base labile Fmoc N^{α}-amine protection in conjunction with acid labile side chain protecting groups (Chapter 3.4) for the examination of the following protecting groups. The conditions used for Fmoc deprotection (20% (v/v) piperidine in DMF) and final acidolysis (aqueous TFA and scavengers) have been consistent throughout to enable comparisons to be made.

2.7.2 Tertiary butyl ester protection[44]

- the use of N^{α}-fluorenylmethoxycarbonylaspartic acid β-tertiary butyl ester

When the hexapeptide was synthesised with tertiary butyl side chain protection 13% aspartimide peptide was found. Only when piperidine was replaced by pyrrolidine for the deprotection of Fmoc was any ring opened compound observed. In this case it was the pyrrolidine amide compound.

2.7.3 1-(4'-Fluorophenyl)-2-methylprop-2-yl ester protection

- the use of N^{α}-fluorenylmethoxycarbonylaspartic acid β-1-(4'-fluorophenyl)-2-methylprop-2-yl ester

When this protecting group was used for the synthesis of the hexapeptide the results were comparable to the tertiary butyl group with respect to aspartimide formation. HPLC showed that the crude cleaved material contained 15% of the aspartimide compound. This was purified by preparative HPLC and characterised by high resolution FAB mass spectrometry and amino acid analysis.

2.7.4 9-Anthracenylmethyl ester protection

- the use of N^{α}-fluorenylmethoxycarbonylaspartic acid β-9-anthracenylmethyl ester

A sample of 9-anthracenylmethanol in DMF was passed through the flow cell as a standard for calibration. From this it was found that as little as 1% loss of protecting group per deprotection cycle could be detected. The use of the anthracenylmethyl ester in the synthesis led to the isolation of the cyclisation product only. This was seen by detection of the deprotection eluent at 365 nm during the deprotection washes, by passing a diluted sample through an ultraviolet flow cell immediately after piperidine treatment. HPLC analysis was consistent with the U.V. observations and high resolution mass spectrometry confirmed that this was the aspartimide compound. It is clear from this that no ring opening is occurring under conditions of acid hydrolysis (i.e. upon liberation of the peptide from the resin and side chain deprotection). In this experiment no ring opened product (e.g. piperidine amide) was detected in contrast to other reports. Since there was no evidence of ring opening during acid or base treatment all the peptide isolated in subsequent experiments with the other protecting groups must have been the α isomer.

2.7.5 1-(9'-Anthracenyl)-3-methylbut-3-yl ester protection

- the use of N^{α}-fluorenylmethoxycarbonylaspartic acid β-1-(9'-anthracenyl)-3-methylbut-3-yl ester

Ultraviolet monitoring of the valine, lysine and aspartic acid deprotection cycles did not detect any material absorbing at 365 nm. This indicated that less than 6% cyclisation occurred during the assembly of the sequence and that the protecting group appeared to be stable to the aminolysis by the amide of glycine under base catalysed conditions.

However the peptide produced by TFA cleavage was analysed by HPLC and 38.5% of the mixture was found to be aspartimide compound. The rate of acidolysis of this group in model studies is much slower than that for tertiary butyl and it would seem reasonable to assume that it is the persistance of the protonated intermediate (Figure 7, p 19) that allowed the cyclisation to occur.

It was decided to examine the effect of using bromotrimethylsilane deprotection conditions. This resulted in diminution of the degree of aspartimide obtained. This may be due to the shorter deprotection time utilised in this technique rather than any change in the acidolysis mechanism. In this deprotection strategy 75% peptide was obtained as opposed to 61.5% in the aqueous TFA cleavage.

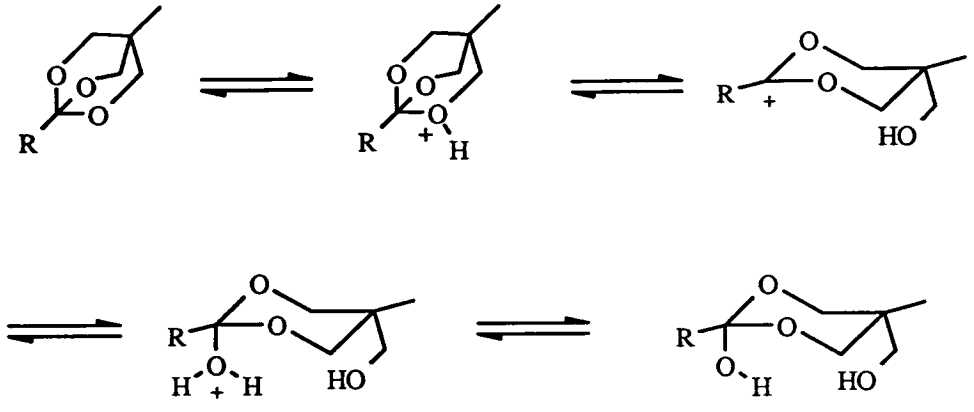
2.7.6 4-Methyl-2,6,7-trioxabicyclo[2.2.2]octane

- the use of N^{α}-fluorenylmethoxycarbonyl-2-amino-3-(4'-methyl-2',6',7')-trioxabicyclo[2.2.2]octylpropionic acid

The evaluation of this protecting group in solid phase peptide synthesis was once more carried out upon the hexapeptide Val.Lys.Asp.Gly.Tyr.Ile. Analytical HPLC of the cleaved peptide indicated the 100% formation of the aspartimide peptide, which was confirmed by high resolution mass spectrometry.

The acid catalysed hydrolysis of ortho esters[104, 105] has been studied in some detail and is reported as being dependant upon rate determining addition of water to a carbocation formed rapidly in acidic solution. Therefore hydrolysis could be

brought about as a result of the repetition of the steps described in Scheme 17.



Scheme 17: The mechanism for ortho ester hydrolysis

As addition of water is rate determining for the hydrolysis then the competing nucleophilic attack bringing about ring closure may become problematic if it were sufficiently rapid. Therefore the formation of intermediates of the kind shown in Figure 17 may be responsible for cyclisation.

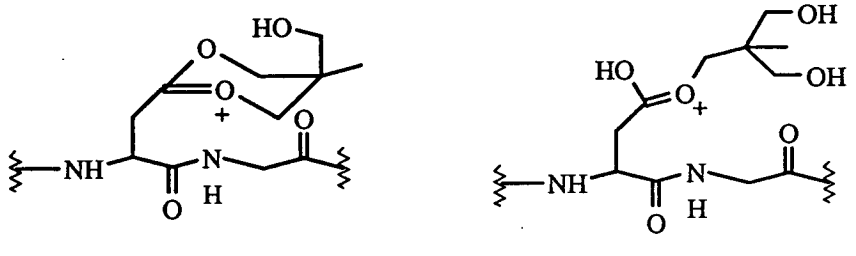


Figure 17: Potential intermediates in the deprotection of an ortho ester protected peptide

protecting group	% aspartimide	% peptide
tert-butyl[41]	13	87
1-(4'-fluorophenyl)-2-methylprop-2-yl	15	85
9-anthracenylmethyl	100	0
1-(9'-anthracenyl)-3-methylbut-3-yl	38.5	61.5
3-methyl-2,6,7-trioxabicyclo[2.2.2]octyl	100	0

Table 6: Observed proportion of peptide:aspartimide with respect to each protecting group

2.7.7 Concluding remarks

A comparative study has been carried out in which the relative utility for the protection of aspartic acid of a number of esters has been examined.

The esters based upon the dibenzosuberyl group and the 9-anthracenylmethyl group are unstable to conditions of basic hydrolysis and experimental evidence in the example of the 9-anthracenylmethyl group has shown that it is unsuitable for N^{α}-Fmoc peptide synthesis and by analogy it is argued that this will also be true for the dibenzosuberyl group.

The use of ortho ester protection is attractive because of the stated resistance to nucleophilic attack but examination of its application to the synthesis of a small peptide has shown that the sole product of the synthesis is the aspartimide peptide. It is reasonable to assume that this is a result of the acidolysis conditions.

The sterically hindered tertiary esters based on the 1-(4'-fluorophenyl)-2-

methylprop-2-yl and 1-(9'-anthracenyl)-3-methylbut-3-yl groups afford protection against the base catalysed intramolecular cyclisation and have potential for monitoring the progress of the synthesis. Unfortunately in the example of 1-(4'-fluorophenyl)-2-methylprop-2-yl it has not been possible to confirm at which point cyclisation occurs.

From a consideration of the stability profiles of 1-(9'-anthracenyl)-3-methylbut-3-yl group and 4-methyl-2,6,7-trioxabicyclo[2.2.2]octyl, group and UV analysis in the case of the former groups, the significance of the acid catalysed cyclisation cannot be overlooked and appears to play a major role in the tertiary hindered examples.

Based upon observations on the 1-(9'-anthracenyl)-3-methylbut-3-yl group this study has shown that steric hindrance obtained in a tertiary butyl like system appears to be the key to preventing base catalysed cyclisation during Fmoc deprotection.

The combination of solid phase peptide synthesis, UV data on the 1-(9'-anthracenyl)-3-methylbut-3-yl group and the alkaline hydrolysis data showed that tertiary esters are sufficiently hindered to prevent aspartimide formation during peptide synthesis. This suggests that it should be possible to design an acid labile protecting group that is sterically hindered for use in the protection of the aspartic acid side chain.

CHAPTER 3: EXPERIMENTAL

3.1 NOTES

Melting points were recorded in open capillaries on a Büchi 510 melting point apparatus and are quoted uncorrected. Optical rotations were measured in a 10 cm cell using a AA 1000 polarimeter (Optical Activity Ltd.). Ultra-violet spectra were recorded on a Cary 210 spectrophotometer. Infra-red spectra were recorded on a Perkin-Elmer 281 or Bio-Rad FTS-7 (FT-IR) spectrophotometer. ^{1}H NMR spectra were recorded on Brüker WP 80 (80 MHz), WP 200 (200 MHz) and WH 360 (360 MHz) spectrometers with tetramethylsilane (TMS) as external standard ($\delta_H = 0.00$). ^{13}C NMR spectra were recorded on Brüker WP 200 (50 MHz) and WH 360 (90 MHz) with TMS as external standard ($\delta_C = 0.00$). ^{19}F NMR spectra were recorded on a Brüker WP 80 (75 MHz). Thin Layer Chromatography (TLC) was performed on aluminium sheets precoated with silica gel $60F_{254}$ in the following solvent systems;

(A) 9/1, chloroform/methanol

(B) 75/13/12, n-butanol/formic acid/water

(C) 3/1/1, n-butanol/acetic acid/water

(D) 15/10/6/3, n-butanol/pyridine/water/acetic acid

(E) dichloromethane

(F) 1/1, dichloromethane/ethyl acetate

(G) 1/1, hexane/diethyl ether

(H) 4/1, hexane/ethyl acetate

and were visualised with UV at 254 nm, iodine, Mary's spray (4,4'-bis-[dimethylamino]-benzhydrol) for carboxylic acids, ninhydrin for amino acids and 5% c.H_2SO_4/methanol followed by charring. Mass spectra were recorded on a

Kratos MS50TC. Elemental analyses were carried out on a Carlo Erba model 1106 or Perkin-Elmer 2400 CHN elemental analyser. The following solvents were dried from the reagents given in parentheses, dichloromethane (calcium hydride), diethyl ether (sodium wire), tetrahydrofuran (sodium wire), ethyl acetate was distilled, DMF and dioxane were peptide synthesis grade (Rathburn Chemicals). Amino acids are unless otherwise stated of the L configuration and were purchased from the SAS group of companies and protected derivatives from Novabiochem. Amino acid analysis was carried out on LKB 4151A alpha plus amino acid analyser after hydrolysis in a sealed tube with constant boiling hydrochloric acid at 110°C for 20 hours. Applied Biosystems equipment was used for HPLC (783A absorbance detector, 2 x 400 solvent delivery systems and a 1480A injection/mixer or equivalent) and eluted with water/0.1% TFA (solvent A) and acetonitrile/0.1% TFA (solvent B) using the gradients and columns described in the text. Detection of the eluants was at 214 nm or 365 nm.

3.2 EXPERIMENTAL

N^{α}-Benzyloxycarbonylasparagine (38)

Benzylchloroformate (50 ml, 0.35 mol) was added to a stirred solution of asparagine monohydrate (52.5 g, 0.35 mol) at 0°C in 1M sodium hydrogen carbonate (700 ml). After 24 hours, ensuring the solution was still alkaline, the excess benzyl chloroformate was removed by washing with diethyl ether. Upon acidification of the aqueous layer with concentrated hydrochloric acid the *title compound* (70 g, 75%) precipitated as a white solid which was dried *in vacuo* at 40°C, m.p. 165-167°C (lit.[106], 163°C); $[\alpha]_D^{22}$ +8.0° (c 1.0, glacial acetic acid) (lit.[10], +9.6°); ν_{max}(nujol)/cm^{-1} 3410 and 3340 (NH), 1700 (urethane CO), 1645 and 1525 (amide CO); δ_H(200 MHz, $(CD_3)_2SO$) 2.38-2.61 (2H, m, βCH_2), 4.31-4.39 (1H, m, αCH), 5.02 (2H, s, benzylic CH_2), 6.93 (1H, br. m, NH), 7.35 (5H, s, aromatic CH).

N^{α}-Benzyloxycarbonylasparagine benzyl ester (39)

N^{α}-Benzyloxycarbonylasparagine (70 g, 0.26 mol) was dissolved in DMF (500 ml) containing dicyclohexylamine (48 ml, 0.24 mol) and warmed to 75°C then benzyl bromide (28 ml, 0.24 mol) was added. After 20 minutes the solution was filtered then poured into ice (1000 ml) and extracted with ethyl acetate (2 x 300 ml). The organic phase was washed with 1M sodium hydrogen carbonate (500 ml), water (500 ml), dried ($MgSO_4$) and evaporated. The *title compound* (55 g, 59%) was obtained as a white solid from diethyl ether, m.p. 128-131°C (lit.[82], 131-132°C); $[\alpha]_D^{22}$ -11.5° (c 2.5, DMF), (lit.[82], -12.9°); δ_H(80 MHz, $(CD_3)_2SO$) 2.63-2.68 (2H, m, βCH_2), 4.35-4.62 (1H, m, αCH), 5.03-5.11 (2 x 2H, s, benzylic CH_2), 7.33 (10H, s, aromatic CH), 7.70-7.80 (2H, br. d, amide NH_2).

N^{α}-Benzyloxycarbonyl aspartic acid α–benzyl ester cyclohexylammonium salt

N^{α}-Benzyloxycarbonylasparagine benzyl ester (27.6 g, 77 mmol) was dissolved in glacial acetic acid (400 ml) and nitrosyl sulphuric acid (15g, 120 mmol) was added in 4 portions at intervals of several minutes while the temperature was maintained below 25°C. Ethyl acetate (100 ml) was added before heating at reflux to remove nitrogen oxides. The solvent was removed *in vacuo* and the residue poured into a large volume of water then extracted with ethyl acetate (2 x 250 ml). The organic phase was washed with water (200 ml), brine (200 ml) and dried ($MgSO_4$). Upon removal of the solvent under reduced pressure cyclohexylamine (8.9 ml, 78 mmol) in diethyl ether (200 ml) was added and the cooled solution gave a precipitate of the *title compound* (23.3 g, 69%) which was filtered and washed copiously with ether, m.p. 118°C (lit.[107], 126°C).

N^{α}-Benzyloxycarbonyl aspartic acid α-benzyl ester (40)

N^{α}-Benzyloxycarbonyl aspartic acid α-benzyl ester cyclohexylammonium salt (30 g, 66 mmol) was partitioned between 2M potassium hydrogen sulphate (500 ml) and ethyl acetate (500 ml) then stirred for 2 hours. The organic phase was separated, the aqueous phase extracted with ethyl acetate (2 x 250 ml) then the combined organic solutions were washed with brine (500 ml) and dried ($MgSO_4$). Upon removal of the solvent *in vacuo* the solid obtained was recrystallised from ether/light petroleum(40-60) to give *title compound* (15.15 g, 65%) as a white solid, m.p. 79-81°C (lit.[108], 82-83°C); $[\alpha]_D^{22}$ -11.4° (c 1.2, glacial acetic acid) (lit.[109], -9.3°C); TLC- (A) R_f 0.4; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3432 (NH), 1740 (ester CO), 1512 (amide II); δ_H(200 MHz, $CDCl_3$) 2.92-3.06 (2H, m, βCH_2), 4.68-4.73 (1H, m, αCH), 5.12 (2H, s, benzylic CH_2), 5.18 (2H, s, benzylic CH_2), 5.93-5.98 (1H, br. d, NH), 7.32 (5H, s, aromatic), 7.34 (5H, s, aromatic).

N^{α}-Benzyloxycarbonyl-α-benzyl-β-aspartyl chloride (68)

N^{α}-Benzyloxycarbonyl aspartic acid α-benzyl ester (180 mg, 0.5 mmol) was dissolved in oxalyl chloride (10 ml) then heated at reflux for 1 hour, until the evolution of gas ceased. This was then cooled and evaporated then evaporated three times from dry, distilled dichloromethane to give the *title compound* (200 mg) as an oil, $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3423 (NH), 1789 (CO acyl halide), 1725 (CO ester), 1509 (amide II).

N^{α}-Benzyloxycarbonyl aspartic acid-β-dibenzosuberyl-α-4-nitrobenzyl ester (44)

To a solution of N^{α}-benzyloxycarbonylaspartic acid-α-4-nitrobenzyl ester (4 g, 10 mmol) and dibenzosuberol (2.2 g, 10 mmol) were dissolved in distilled dichloromethane (30 ml) and stirred at 0° C. Dicyclohexylcarbodiimide (2 g, 10 mmol) and a catalytic amount of DMAP were then added. After stirring for 3 days at room temperature the solution was filtered and evaporated. The crude material was dissolved in diethyl ether and washed with 10% sodium carbonate (100ml), dried over magnesium sulphate and evaporated. A solid was obtained by trituration with ether/cyclohexane then recrystallised from ethyl acetate/n-hexane to give the *title compound* (2.5 g, 42%) as an off-white solid, m.p. 113-114°C (Found: C, 68.4; H, 5.2; N, 4.9. $C_{34}H_{30}N_2O_8$ requires C, 68.7; H, 5.2; N, 4.9%); $[\alpha]_D^{22}$ +4.8 (c 1.0, $CHCl_3$); TLC- R_f (A) 0.82, R_f (E) 0.5; $\nu_{max}(CHCl_3)/cm^{-1}$ 3420 (NH), 1760 and 1725 (CO), 1610 (aromatic), 1500 (amide CO); δ_H(80 MHz, $CDCl_3$) 2.92-3.45 (6H, m, β-CH_2, Dbs CH_2-CH_2), 4.57-4.80 (1H, m, αCH), 5.07 (4H, s, 2 x benzylic CH_2), 5.53-5.75 (1H, br. d, NH), 6.86 (1H, s, 5CH Dbs), 7.11-7.38 (15H, m, aromatic CH), 8.02 and 8.13 (2 x 1H, s, aromatic CH); δ_C(50 MHz, $CDCl_3$) 31.75 (CH_2, Dbs), 36.21 (βCH_2), 49.98 (αCH), 65.17, 66.51 (benzylic CH_2), 79.29 (5CH Dbs), 123.09, 125.68, 127.51, 127.65, 127.98, 128.45, 129.02, 129.89 (aromatic CH), 135.62, 139.48 (quaternary Dbs), 155.50, 169.27, 169.88 (CO quaternary); m/z (FAB) 595

(MH+); HRMS: Found 595.20808. $C_{34}H_{31}N_2O_8$ requires 595.20802.

N$^{\alpha}$-9-Fluorenylmethoxycarbonylaspartic acid-α-phenyl-β-tertiary butyl ester (47)

N$^{\alpha}$-9-Fluorenylmethoxycarbonylaspartic acid-β-tertiary butyl ester (4.1 g, 0.01 mol) and phenol (0.94 g, 0.01 mol) were dissolved in distilled dichloromethane (50 ml) and cooled to 0°C. Dicyclohexylcarbodiimide (2.0 g, 0.01mol) and two drops of pyridine were added. After stirring for 5 days at room temperature, the solution was filtered then evaporated and dissolved in ethyl acetate (100 ml), washed with sodium hydrogen carbonate (2 x 100 ml), citric acid (100 ml) and water (100 ml). The solution was dried ($MgSO_4$) then evaporated to yield the *title compound* (3.48 g, 72%) upon recrystallisation from ethanol, m.p. 133-136°C (Found: C, 70.9; H, 6.35; N, 3.3. $C_{29}H_{29}NO_6$ requires C, 71.4; H, 6.0; N, 2.9%); $[\alpha]_D^{22}$ +26.0° (c 0.5, CH_2Cl_2); TLC- R_f (E) 0.26, R_f (F) 0.77; λ_{max}(MeOH)/nm 291, 280 and 256 (ε/dm^{3}mol^{-1}cm^{-1} 5268, 2946 and 17411); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3431 (NH), 3067 and 2979 (CH), 1764 (ester CO), 1722 (urethane CO), 1593 (aromatic), 1510 (amide II); δ_H(200 MHz, $CDCl_3$) 1.50 (9H, s, $C(CH_3)_3$), 2.82-3.18 (2H, m, βCH_2), 4.22-4.53 (3H, m, Fmoc CH_2, Fmoc 9-CH), 4.80-4.88 (1H, m, αCH), 5.92 (1H, d, NH), 7.11-7.82 (13H, m, aromatic); δ_C(90 MHz, $CDCl_3$) 27.90 (^{t}Bu CH), 37.82 (βCH_2), 46.92 (αCH), 50.56 (Fmoc CH), 67.18 (Fmoc CH_2), 81.97 (^{t}Bu quaternary), 119.82, 121.13, 124.93, 124.98, 126.00, 126.91, 127.56, 129.33 (aromatic CH), 141.11, 143.51, 143.66, 150.50 (aromatic quaternary) 155.84, 169.57, 169.96, (CO quaternary); m/z (FAB) 488 (MH+); HRMS: Found 488.20727. $C_{29}H_{30}NO_6$ requires 488.20730.

N$^{\alpha}$-9-Fluorenylmethoxycarbonylaspartic acid-α-phenyl ester (48)

N$^{\alpha}$-9-Fluorenylmethoxycarbonylaspartic acid-α-phenyl-β-tertiary butyl ester (3.1 g, 6.3 mmol) was dissolved in distilled dichloromethane (10 ml) and stirred for 3 hours

at room temperature with trifluoroacetic acid (2 ml). Upon removal of the solvent under reduced pressure and trituration with ether the *title compound* (2.23 g, 81%) was obtained as a white powder, m.p. 171-173°C (Found: C, 69.15; H, 5.0; N, 3.3. $C_{25}H_{21}NO_6$ requires C, 69.6; H, 4.9; N, 3.25%); $[\alpha]_D^{22}$ -37.6° (c 0.5, DMF); TLC R_f (A) 0.43 R_f (B) 0.82; λ_{max}(MeOH)/nm 396, 288 and 265 (ε/dm^{3}mol^{-1}cm^{-1} 6552, 5862 and 22414); ν_{max}($CHCl_3$)/cm^{-1} 3431 (NH), 3067, 3032 (CH), 1766 (ester CO), 1721 (urethane CO), 1594 (aromatic), 1509 (amide II); δ_H(200 MHz, $(CD_3)_2SO$) 2.59-3.06 (2H, m, βCH_2), 4.25-4.4 (3H, m, Fmoc CH_2, Fmoc CH), 4.59-4.70 (1H, m, αCH), 6.77 (1H, d, NH), 7.06-8.10 (13H, m, aromatic); δ_C(90 MHz, $(CD_3)_2SO$) 34.88 (βCH), 46.76 (αCH), 50.57 (Fmoc CH_2), 115.41, 118.98, 120.27, 121.81, 125.15, 125.23, 125.41, 127.24, 127.83, 129.52 (aromatic CH), 140.95, 143.77, 143.94 (aromatic quaternary), 157.50, 170.00, 172.31 (CO quaternary); m/z (FAB) 432 (MH^+); HRMS: Found 432.14468. $C_{25}H_{22}NO_6$ requires 432.14470.

<u>N^{α}-9-Fluorenylmethoxycarbonylaspartic acid-α-phenyl-β-dibenzosuberyl ester (49)</u>

N^{α}-9-Fluorenylmethoxycarbonylaspartic acid-α-phenyl ester (2 g, 4.6 mmol) and dibenzosuberol (1 g, 4.7 mmol) were dissolved in distilled dichloromethane (50 ml) and cooled to 0°C then dicyclohexylcarbodiimide (0.98 g, 4.7 mmol) and DMAP (50 mg) were added. After 5 days stirring at room temperature the solution was filtered, evaporated and dissolved in ethyl acetate (150 ml). This was washed with 1M sodium hydrogen carbonate (2 x 100 ml) and water (100 ml) then dried over magnesium sulphate. Upon removal of approximately two thirds of the volume of the solvent *in vacuo* light petroleum(40-60) was added to precipitate the *title compound* (1.97 g, 68%) as a white solid upon recrystalisation from ethanol, m.p. 135-137°C (Found; C, 75.9; H, 5.5; N, 2.4. $C_{40}H_{33}NO_6$ requires C, 77.0; H, 5.3; N, 2.25%); $[\alpha]_D^{22}$ +6.8° (c 0.5, CH_2Cl_2); TLC- R_f (F) 0.76, R_f (A) 0.98; λ_{max}(MeOH)/nm 300, 289 and 265 (ε/dm^{3}mol^{-1}cm^{-1} 57291, 46875 and 204167);

$\nu_{max}(CHCl_3)/cm^{-1}$ 3430 (NH), 3067 and 3032 (CH), 1769 (CO ester), 1601 (aromatic), 1508 (amide II); δ_H(200 MHz, $CDCl_3$) 2.97-3.73 (6H, m, βCH_2, Dbs CH_2CH_2), 4.17-4.47 (3H, m, Fmoc CH_2, Fmoc CH), 4.82-4.91 (1H, m, αCH), 5.86 (1H, d, NH), 6.8-7.76 (21H, m, Dbs 5H, aromatic); δ_C(90 MHz, $CDCl_3$) 32.12 and 32.28 (Dbs CH_2CH_2), 36.99 (βCH_2), 46.89 (αCH), 50.37 (Fmoc CH), 67.20 (Fmoc CH_2), 80.47 (Dbs 5CH), 119.80, 121.08, 124.97, 125.94, 126.01, 126.09, 126.92, 127.55, 128.84, 128.90, 129.25, 129.93, 130.22, 130.22, 130.29, (aromatic CH), 135.67, 135.83, 140.00, 140.16, 141.10, 143.50, 143.60, 150.17 (aromatic quaternary), 155.17, 169.21, 169.74 (CO quaternary); m/z (FAB) 624 (MH^+); HRMS: Found 624.23863. $C_{40}H_{34}NO_6$ requires 624.23859.

Dibenzosuberyl acetate (52)

Dibenzosuberol (2.1 g, 10 mmol) was dissolved in distilled acetic anhydride (3 ml) and pyridine (5 ml) then stirred at room temperature for one day. The solution was exhaustively evaporated and water (15 ml) was added. The aqueous phase was extracted with diethyl ether (2 x 25 ml) then the organic phase was dried ($MgSO_4$) evaporated and triturated with light petroleum (40-60) to yield the *title compound* (1.72 g, 68%), m.p. 85-87°C (lit.[110], 87°C), TLC- R_f (E) 0.55, R_f (F) 0.71: $\nu_{max}(CHCl_3)/cm^{-1}$ 3032 (CH), 1730 (CO); δ_H(80 MHz, $CDCl_3$) 2.08 (3H, s, CH_3), 2.84-3.76 (4H, m, CH_2-CH_2), 6.93 (1H, s, 5-CH), 7.08-7.50 (8H, m, aromatic CH).

4-Nitrobenzyl acetate (51)

4-Nitrobenzyl alcohol (4 g, 26 mmol) was dissolved in acetic anhydride (5 ml) and pyridine (5 ml) then stirred overnight. The solution was then poured into water (50 ml) and extracted with diethyl ether (2 x 50 ml). The combined organic phase was evaporated to provide the *title compound* (4.1 g, 78%), m.p. 75°C (Lit.[111], 74-77°C); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3030, 2890, 2990 (CH), 1760 (ester CO); δ_H(80 MHz,

$(CD_3)_2CO$), 2.12 (3H, s, CH_3), 5.26 (2H, s, CH_2), 7.68 (2H, d ^3J 8.9 Hz, aromatic H_2 and H_6), 8.26 (2H, d ^3J 8.8 Hz, H_3 and H_5).

Dibenzosuberyl hydrocinnamate (53)

Hydrocinnamic acid (3.7 g, 25 mmol) was dissolved in dry benzene (40 ml) containing oxalyl chloride (8 ml) and refluxed for 1 hour, cooled then evaporated. The residue was then evaporated twice more from dry dichloromethane before dissolving in dichloromethane (40 ml) to which pyridine (10 ml) and dibenzosuberol (5.25g, 25 mmol) were added. After refluxing for 1 hour the cooled solution was diluted with dichloromethane (50 ml) then washed with 2M hydrochloric acid (100 ml), 2M sodium hydroxide (100 ml) and dried ($MgSO_4$). Removal of the solvent *in vacuo* and trituration with n-hexane then recrystallisation from n-hexane gave the *title compound* (6.8 g, 80%), m.p. 72°C (Found: C, 84.25; H, 6.65. $C_{24}H_{22}O_2$ requires C, 84.2; H, 6.4%); TLC- R_f (E) 0.61, R_f (A) 0.82; $\lambda_{max}(CH_2Cl_2)$/nm 266 (ε/$dm^3mol^{-1}cm^{-1}$ 986); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3060, 2987 and 2945 (CH), 1740 (CO), 1604 (aromatic); δ_H(200 MHz,$CDCl_3$) 2.62-2.73 (2H, m, CH_2), 2.98-3.06 (4H, m, CH_2), 3.46-3.58 (2H, m, CH_2), 7.05-7.45 (12H, m, aromatic); δ_C(90 MHz, $CDCl_3$) 30.64 (CH_2CO), 32.10 (dibenzosuberyl CH_2CH_2), 36.00 (benzylic CH_2), 78.79 (CH), 125.88, 127.95, 128.18, 128.45, 129.46 and 130.03 (aromatic CH), 136.33 and 139.80 (dibenzosuberyl quaternary), 140.03 (hydrocinnamoyl quaternary), 171.40 (CO); m/z (FAB) 342 (M^+); HRMS: Found 342.16199. $C_{24}H_{22}O_2$ requires 342.16197.

4-Fluorophenylacetic acid methyl ester (55)

4-Fluorophenylacetic acid (82.75 g, 0.53 mol) was dissolved in methanol (500 ml) containing concentrated sulphuric acid (5 ml) then heated at reflux for 8 hours. The cooled solution was poured into water (1000 ml) then extracted twice with ethyl

acetate (250 ml). The combined organic phase was washed with 1M sodium hydrogen carbonate (250 ml) and brine (250 ml) then dried (Na_2SO_4) and evaporated to yield an oil that upon distillation gave the *title compound* (44.7 g, 80%) as a clear liquid, b.p. 80°C/0.9 mmHg (lit.[68], 59°C/0.2 mmHg); ν_{max}(liquid film)/cm^{-1} 3030 and 2980 (CH), 1755 (CO), 1610 (aromatic); δ_H(200 MHz, $CDCl_3$) 3.58 (2H, s, CH_2) 3.67 (3H, s, CH_3) 6.93-7.28 (4H, m, aromatic).

1-(4'-Fluorophenyl)-2-methylpropan-2-ol (56)

To a suspension of magnesium turnings (27 g, 1.1 mol) in diethyl ether (30 ml) stirred under a stream of nitrogen was added iodomethane (15.6 ml, 1.1 mol) in diethyl ether (100 ml) with cooling. 4-Fluorophenylacetic acid methyl ester (79 g, 0.47 mol) in diethyl ether (100 ml) was slowly added. After stirring at room temperature for one day the solution was poured into 2M ammonium chloride/ice slurry (1 litre) then extracted with approximately 500 ml of diethyl ether. The organic solution was washed with water (250 ml) and brine (250 ml) then dried over sodium sulphate and evaporated. The *title compound* (67.8 g, 85%) was obtained upon vacuum distillation as a clear liquid, b.p. 74°C/0.4 mmHg (lit.[68], 68°C/0.6 mmHg); ν_{max}($CHCl_3$) /cm^{-1} 3600 (free OH), 3460 (br., H-bonded OH), 2960 and 2810 (CH), 1610 (aromatic); δ_H(80 MHz, $CDCl_3$) 1.17 (6H, s, $(CH_3)_2$), 1.67 (1H, s, OH), 2.69 (2H, s, CH_2) 6.82-7.23 (4H, m, aromatic).

N^{α}-Benzyloxycarbonylaspartic acid-α-benzyl-β-(4'-fluorophenyl)-2-methylprop-2-yl ester (57)

N^{α}-Benzyloxycarbonylaspartic acid-α-benzyl ester (10 g, 28 mmol) and 1-(4'-fluorophenyl)-2-methylpropan-2-ol (4.2 g, 25 mmol) were dissolved in distilled dichloromethane (100 ml) and cooled to 0°C. Dicyclohexylcarbodiimide (6.2 g, 30 mmol) and DMAP (300 mg) were added. After 3 days the solution was filtered and

the solvent removed *in vacuo*. The residue was dissolved in ethyl acetate (250 ml) and filtered once more then washed with 10% sodium carbonate (200 ml), dried ($MgSO_4$) and evaporated to give an oil that was purified by flash chromatography over silica gel, eluted with 20% ethyl acetate in hexane. Precipitation from diethyl ether using hexane then recrystallisation from pentane gave the *title compound* (7.3 g, 57%), m.p. 50°C (Found: C, 68.0; H, 5.9; N, 2.7. $C_{29}H_{30}FNO_6$ requires C, 68.6; H, 5.9; N, 2.7%); $[\alpha]_D^{22}$ -8.8° (c 0.5, CH_2Cl_2); TLC- R_f (H) 0.27, R_f (A) 0.84; $\lambda_{max}(CH_2Cl_2)$/nm 258, 266 and 271 (ε/$dm^3mol^{-1}cm^{-1}$ 980, 1081 and 737); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3445 (NH), 3033, 2981 and 2945 (CH), 1729 (ester CO), 1604 (aromatic), 1509 (amide II); δ_H(200 MHz, $CDCl_3$) 1.37 (6H, s, $(CH_3)_2$), 2.71-2.94 (2H, m, βCH_2), 2.97 (2H, s, fluorophenyl CH_2), 4.60-4.69 (1H, m, αCH), 5.13 and 5.18 (4H, s, benzyl and benzyloxycarbonyl CH_2), 5.79 (1H, d, NH), 6.88-7.11 (4H, m, fluorophenyl aromatic), 7.34 (10H, s, benzyl and benzyloxycarbonyl aromatic); δ_F(75 MHz, $CDCl_3$) -120.19; δ_C(50 MHz, $CDCl_3$) 25.37 and 25.44 (2 x CH_2), 37.34 (βCH_2), 44.82 (CH_2-$(CH_3)_2$), 50.22 (αCH), 66.63, 66.97 (benzylic CH_2), 82.95 (quaternary $C(CH_3)_2$), 114.47 (d, $^3J_{CF}$ 21.1 Hz, C_2, C_6) 127.71, 127.80, 127.99 and 128.15 (benzyl and benzyloxy aromatic CH), 131.46 (d, $^2J_{CF}$ 7.5 Hz, aromatic C_3, C_5), 134.90 and 135.85 (benzylic CH_2), 155.65 (fluorophenyl C_1) 161.36 (d, $^1J_{CF}$ 224 Hz, fluorophenyl C_4), 169.69, 170.38 (CO); m/z (FAB) 508 (MH^+); HRMS: Found 508.21354. $C_{29}H_{31}FNO_6$ requires 508.21352.

Aspartic acid-β-1–(-4'-fluorophenyl)-2-methylprop-2-yl ester (58)

N^{α}-Benzyloxycarbonyaspartic acid-α-benzyl-β-1-(4'-fluorophenyl)-2-methylprop-2-yl ester (5.3 g, 0.01 mol) was dissolved in aqueous methanol (98% v/v, 50 ml) and stirred with 10% palladium on charcoal (500 mg) for 90 minutes under an atmosphere of hydrogen. The solution was filtered through celite to remove the catalyst then the solvent was removed *in vacuo*. Trituration with ether then treatment

of the mother liquors with n-hexane gave a solid which was recrystallised from methanol/ether to give the *title compound* (2.5 g, 88%) as a white solid, m.p. 215°C (Lit.[68], 169-172°C); (Found: C, 60.2; H, 6.9; N, 5.0. $C_{14}H_{18}NFO_4$ requires C, 59.35; H, 6.4, N, 5.0%); $[\alpha]_D^{22}$ +1.8° (c 0.5, DMF); TLC- R_f (B) 0.9, R_f (C) 0.4; δ_H(200 MHz, CD_3OH) 1.55 (6H, s, $(CH_3)_2$), 2.80-3.09 (2H m, βCH_2), 3.15 (2H, s, *C*H-$(CH_3)_2$), 3.88-3.94 (1H, m, αCH), 7.05-7.35 (4H, m, aromatic); m/z (FAB) 284 (MH^+); HRMS: Found 284.12981. $C_{14}H_{19}FNO_4$ requires 284.12980.

<u>N^{α}-9-Fluorenylmethoxycarbonylaspartic acid-β-(4'-fluorophenyl)-2-methylprop-2-yl ester (59)</u>

Aspartic acid-β-1–(4'-fluorophenyl)-2-methylprop-2-yl ester (1 g, 3.5 mmol) in water (5 ml) containing triethylamine (0.6 ml, 5.8 mmol) was stirred with 9-fluorenylmethyl-succinimidyl carbonate (1.2 g, 3.5 mmol) for 24 hours. This was then poured into water (25 ml) and acidified with 2M potassium hydrogen sulphate to approximately pH 3 then extracted with ethyl acetate (250 ml). The organic phase was washed with water (100 ml), dried ($MgSO_4$) and evaporated to give a gum which was dissolved in boiling toluene and cooled overnight to yield the *title compound* (1.4 g, 76%) as a white solid, m.p. 115-117°C (Found: C, 68.7; H, 5.7; N, 2.8. $C_{29}H_{28}FNO_6$ requires C, 68.9; H 5.6; N, 2.8%); $[\alpha]_D^{22}$ -1.2° (c 1.0, EtOH); TLC- R_f (A) 0.26; $\lambda_{max}(CH_2Cl_2)$/nm 302, 290 and 268 (ε/$dm^3mol^{-1}cm^{-1}$ 5254, 6102 and 22557); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3440 (NH), 2850 (CH), 1760 (urethane CO), 1719 (ester CO), 1605 (aromatic), 1510 (amide II); δ_H(200 MHz, $CDCl_3$) 1.40 and 1.44 (6H, s, $(CH_3)_2$), 2.81-3.10 (4H, m, βCH_2, CH_2-$(CH_3)_2$), 4.19-4.45 (3H, m, Fmoc CH_2 and CH), 4.62-4.67 (1H, m, αCH), 5.78 (1H, d, NH), 6.94-7.77 (12H, m, aromatic); δ_C(50 MHz, $CDCl_3$) 25.70 (2 x CH_3), 37.37 (βCH_2), 45.12 (CH_2-$(CH_3)_2$), 46.82 (αCH), 50.08 (Fmoc CH), 67.26 (Fmoc CH_2), 83.68 (CH_2-*C*-$(CH)_3)_2$-O), 114.73 (d, $^3J_{CF}$ 20.9 Hz, C_2, C_6), 119.81, 124.92, 126.91, 127.57

(Fmoc aromatic CH), 131.64 (d, $^2J_{CF}$ 7.8 Hz, C_3, C_5), 141.09, 143.41, 143.57 (Fmoc quaternary), 155.99 (fluorophenyl C_1), 161.61 (d, $^1J_{CF}$ 244 Hz, C_4), 170.25 and 170.86 (CO); m/z (FAB) 506 (MH^+); HRMS: Found 506.19788. $C_{29}H_{29}FNO_6$ requires 506.19787.

L-Aspartic acid copper (II) complex copper (II) salt octahydrate (62)

To a solution of aspartic acid (26.6 g, 200 mmol) in water (1000 ml) at 70°C was added a solution of copper(II)acetate (41.2 g, 206 mmol) in water (750 ml) over a period of 1 to 2 hours. This was left to stand for 2 days then filtered and washed with water, ethanol and ether to yield a blue solid which was then used without further characterisation (52.1 g, 98%).

9-Chloromethylanthracene (61)

9-Anthracenylmethanol (15.0 g, 72 mmol) was dissolved in dry benzene (100 ml) containing thionyl chloride (1.2 eq.,6.0 ml, 86 mmol) then heated at reflux for 4 hours. The cooled solution was evaporated exhaustively to provide a crude yellow solid. This was then triturated and washed with cold diethyl ether to provide the *title compound* (11 g, 68%), m.p.132-135°C (Lit.[112], 141-142.5°C); δ_H(200 MHz, $CDCl_3$) 5.55 (2H, s, CH_2),7.46-7.65 (4H, m, aromatic H_2,H_3,H_6 and H_7), 7.97-8.01 (2H, m, aromatic H_1 and H_8), 8.27-8.31 (2H, m, aromatic H_4 and H_5), 8.41 (1H, s, aromatic H_{10}).

Aspartic acid-β-9-anthracenylmethyl ester (63)

L-Aspartic acid copper (II) complex copper (II) salt octahydrate (2.98 g, 5.6 mmol) and aspartic acid (1.5g, 11.2 mmol) were dissolved in DMF (10 ml) and water (1.6 ml) then tetramethylguanidine (2.8 ml, 22.4 mmol) was added and the solution stirred for 2 hours at room temperature. 9-Chloromethylanthracene (5.35 g, 24

mmol) was added and the reaction left overnight then acetone was added to give a fine precipitate which was filtered then resuspended in water and ground with a glass rod to break up the solid before filtering once more. This solid was then suspended in a solution containing ethylenediaminetetraacetic acid (3.2 g, 11 mmol) and sodium hydrogen carbonate (1.8 g, 22 mmol) in water (25 ml) and then shaken vigorously for 1 hour. Upon dilution with water (30 ml approx.) the *title compound* (3 g, 40%) precipitated as a yellow solid which was washed copiously with ether, m.p.175°C; (Found: C, 66.0; H, 5.4; N, 3.7. $C_{19}H_{17}NO_4$ requires C, 70.6; H 5.3; N, 4.3%); $[\alpha]_D^{22}$ -32.5° (c 0.04, DMF); TLC- R_f(C) 0.63, R_f(B) 0.84; $\lambda_{max}(CHCl_3)$/nm 365, 386 and 410 (ε/dm^{3}mol^{-1}cm^{-1} 3426, 4074 and 4074); ν_{max}(bromoform mull)/cm^{-1} 3392 (br., ammonium), 2933 (CH), 1703 (carboxyl CO), 1633 (amino acid I); δ_H(80 MHz, $(CD_3)_2SO$) 2.07 (2H, m, βCH_2), 6.13 (2H, m, Ar-CH_2) 7.55-8.71 (9H, m, aromatic); m/z (FAB) 323 (M^+); HRMS: Found 323.11577. $C_{19}H_{17}NO_4$ requires 323.11575.

N^{α}-9-Fluorenylmethyloxycarbonylaspartic acid-β-9-anthracenylmethyl ester (64)

9-Fluorenylmethyl-succinimidyl carbonate (3.1 g, 9.2 mmol) and aspartic acid-β-9-anthracenylmethyl ester (2.5 g, 7.7 mmol) were stirred in 60% aqueous dioxane (60 ml) containing triethylamine (1.6 ml, 11 mmol) for 1 day at room temperature. Water (100 ml) was added and the solution was acidified to pH 3 with 2M potassium hydrogen sulphate then extracted with ethyl acetate (3 x 250 ml). The combined organic solutions were washed with water (100 ml), brine (100 ml) and dried ($MgSO_4$) then evaporated under reduced pressure to yield the *title compound* (1.8 g, 42%) as a solid which was recrystallised from chloroform/light petroleum(60-80), m.p.150-155°C (Found: C, 71.0; H, 4.95; N, 2.3. $C_{34}H_{27}NO_6$ requires C, 74.8; H, 4.95; N, 2.3%); $[\alpha]_D^{22}$ -9.5°(c 2.0, DMF); TLC- R_f (A) 0.12, R_f (B) 0.87; λ_{max}(MeOH)/nm 288, 300, 347, 365 and 385 (ε/dm^{3}mol^{-1}cm^{-1} 4148, 4258, 3057,

4585 and 4367); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3426 (NH), 1730 (CO urethane), 1610 (aromatic), 1512 (amide II); δ_H(80 MHz, $CDCl_3$) 2.75-3.92 (2H, m, βCH_2), 3.95-4.82 (4H, m, αCH, Fmoc CH and CH_2), 5.95 (2H, br. s, anthracenyl CH_2), 5.5-5.75 (1H, m, NH), 6.93-8.30 (17H, m, aromatic); δ_C(50 MHz, $(CD_3)_2SO$) 37.10 (βCH), 46.79 (αCH), 51.89 (Fmoc CH), 58.65 (anthracenyl CH_2), 65.75 (Fmoc CH_2), 120.26, 125.39, 125.96, 126.46, 126.87, 127.24, 127.77, 129.05 (aromatic CH), 130.64, 131.01 and 131.61 (anthracenyl quaternary), 143.98 and 140.85 (Fmoc quaternary), 155.90 (ester CO), 171.16 (urethane CO), 173.82 (acid CO); m/z (FAB) 545 (M^+); HRMS: Found 545.18381. $C_{34}H_{27}NO_6$ requires 545.18382.

9-Anthracenylmethyl hydrocinnamate (96)

9-Anthracenylmethanol (4.1 g, 20 mmol) and hydrocinnamic acid (3.7 g, 25 mmol) dissolved in distilled dichloromethane (100 ml) and cooled to 0°C were combined with DCC (5.1 g, 25 mmol) and DMAP (100 mg) then stirred for 2 days at room temperature. The solution was filtered to remove dicyclohexylurea then the solvent was removed *in vacuo*. The residue thus obtained was dissolved in ethyl acetate (250 ml) then washed with sodium carbonate (10%, 200 ml), dried over magnesium sulphate and the ethyl acetate removed *in vacuo*. The *title compound* (4.4 g, 65%) was obtained as a solid from diethyl ether/light petroleum(60-80), m.p. 78°C (Found: C, 84.5; H, 6.1. $C_{24}H_{20}O_2$ requires C, 84.7; H, 5.9%); TLC- R_f (A) 0.83, R_f (E) 0.74; λ_{max}(MeOH)/nm 384, 364, 346 and 330 (ε/dm^{3}mol^{-1}cm^{-1} 7672, 8354, 5541 and 2728); ν_{max}(CH_2Cl_2)/cm^{-1} 3033 and 2936 (CH), 1734 (CO); δ_H(200 MHz, $CDCl_3$) 2.62-2.70 (2H, m, CH_2CO), 2.91-2.99 (2H, m, benzylic CH_2), 6.13 (2H, m, anthracenyl CH_2), 7.10-7.30 (4H, m, anthracenyl aromatic), 7.39-7.60 (5H, m, hydrocinnamyl aromatic), 8.00-8.05 (2H, m, anthracenyl aromatic), 8.25-8.29 (2H, m, anthracenyl aromatic), 8.50 (1H, s, anthracenyl H_{10}); δ_C(90 MHz, $CDCl_3$) 30.72 (CH_2CO), 35.56 (hydrocinnamate benzylic CH_2), 58.55 (anthracenyl CH_2), 123.68, 124.83, 125.97, 126.37, 128.02, 128.84, 128.93 (aromatic) 130.76, 131.08 (anthracenyl quaternary), 140.06 (hydrocinnamyl quaternary) 172.84 (CO); m/z (FAB) 341 (MH^+); HRMS: Found 341.15415. $C_{24}H_{21}O_2$ requires 341.15414.

1-(9'-Anthracenyl)-2-methylpropan-2-ol (65)

To a solution of 9-bromoanthracene (5.2 g, 0.02 mol) in dry, distilled THF (20 ml) stirred under nitrogen and cooled to -78°C was added *n*-butyllithium (1.6 moldm^{-3}, 12.5 ml, 0.02 mol). Upon complete addition of the base, isobutylene oxide (1.8 ml, 0.02 mol) was added and the mixture was allowed to warm to room temperature.

After three hours the reaction was poured into 2M ammonium chloride/ice slurry (100 ml) then extracted with diethyl ether (2 x 200 ml), dried ($MgSO_4$) and the solvent removed *in vacuo*. After trituration with n-hexane and recrystallisation from ethanol/water the *title compound* (2 g, 40%) was obtained as a pale grey solid, m.p. 112-113°C (Found: C, 85.8; H, 7.1. $C_{18}H_{18}O$ requires C, 86.4; H, 7.2%); TLC- R_f (A) 0.72 R_f (H) 0.29; λ_{max}(MeOH)/nm 347, 365 and 385 (ε/$dm^3mol^{-1}cm^{-1}$ 6477, 10625 and 10454); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3587 (OH), 2987 (CH), 1621 (aromatic); δ_H (80 MHz, $CDCl_3$) 1.33 (6H, s, $(CH_3)_2$), 1.38 (1H, s, OH), 3.91 (2H, s, CH_2) 7.23-7.53 (4H, m, aromatic H_2, H_3, H_6, H_7), 7.93-8.06 (2H, m, aromatic H_1, H_8), 8.32-8.45 (3H, m, aromatic H_4, H_5, H_{10}); δ_C(90 MHz, $CDCl_3$) 30.82 ($(CH_3)_2$), 39.75 (CH_2), 73.20 (COH), 124.02, 124.53, 125.15, 126.02, 128.19 (aromatic CH), 129.55, 130.51, 130.72 (aromatic quaternary); m/z (FAB) 250 (M^+); HRMS: Found 250.13574. $C_{18}H_{18}O$ requires 250.13576.

1-(9'-Anthracenyl)-2-methylprop-2-ene (67)

1-(9'-Anthracenyl)-2-methylpropan-2-ol (500 mg, 2 mmol) was dissolved in benzene with 4-toluenesulphonic acid and heated at reflux for 3 hours .The cooled solution was then washed with 10% sodium carbonate (2 x 50 ml) and brine (100 ml) then dried ($MgSO_4$). This was then evaporated to yield the *title compound* (420 mg, 90%) as a yellow oil, TLC- R_f (A) 0.83, R_f (E) 0.78; $\lambda_{max}(CH_2Cl_2)$/nm 352, 370 and 290 (ε not calculated); δ_H(200 MHz, $CDCl_3$) 1.50 (3H, d, ^4J 1.5 Hz, Z-CH_3), 2.25 (3H, d, ^4J 1.2 Hz, E-CH_3), 6.87 (1H, m, CH), 7.25- 7.58 (4H, m, aromatic), 8.02-8.27 (4H, m, aromatic), 8.43 (1H, s, aromatic H_{10}); δ_C(50 MHz, $CDCl_3$) 19.75 and 25.42 (CH_3), 120.89 (CH), 124.87, 125.53, 126.41, 128.00, 128.45 (aromatic CH), 129.68, 131.31, 133.33, 138.69 (quaternary); m/z (FAB) 232 (M^+); HRMS: Found 232.12521. $C_{18}H_{16}$ requires 232.12519.

Diethyl-2-(9'-anthracenylmethyl)malonate (73)

Diethyl malonate (1.8 ml, 12 mmol) was added dropwise to a suspension of 50% sodium hydride (570 mg, 12 mmol) in dry THF (50 ml) cooled in an ice/water bath. To this was added 9-choloromethylanthracene (2.5 g, 11 mmol) in THF then the reaction was stirred for 18 hours. The solvent was partially evaporated then the solution diluted with water (200 ml) and the pH was adjusted to between 3 and 5 with 2M potassium hydrogen sulphate before extracting with ethyl acetate (300 ml). This solution was dried over magnesium sulphate then evaporated to yield, upon trituration with cyclohexane, the *title compound* (1.85 g, 48%) as a yellow solid, m.p. 68-71°C; TLC- R_f (A) 0.77, R_f (G) 0.47; $\lambda_{max}(CH_2Cl_2)$/nm 368, 388 and 407 (ε/$dm^3mol^{-1}cm^{-1}$ 5639, 7194 and 6222); $\nu_{max}(CHCl_3)/cm^{-1}$ 3060 and 2980 (CH) 1760 (CO); δ_H(80 MHz, $CDCl_3$) 1.03 (6H, t, ^3J 7.1 Hz, CH_3) 3.83-4.34 (7H, m, benzylic CH_2, CH, 2 x CH_2 ethyl) 7.32-8.36 (9H, m, aromatic); δ_C(90 MHz, $CDCl_3$) 13.64 (CH_3), 61.41 (CH_2), 123.98, 124.72, 125.78, 126.89 and 129.16 (aromatic CH), 129.77, 128.33 and 131.30 (aromatic quaternary), 169.10 (CO); m/z (FAB) 350 (M^+); HRMS: Found 350.15180. $C_{22}H_{22}O_4$ requires 350.15180.

2-(9'-Anthracenylmethyl)-malonic acid (74)

Method (A)

Diethyl-2-(9'-anthracenylmethyl)-malonate (200 mg, 570 mmol) was dissolved in acetone (40 ml) and sodium hydroxide (60 mg) in water (10 ml) was added. This was heated at reflux for 2 hours then diluted with 2M sodium hydroxide (100 ml) and washed with diethyl ether (100 ml). The aqueous layer was then acidified with concentrated hydrochloric acid and extracted with toluene (2 x 100 ml), dried ($MgSO_4$) and evaporated to give upon trituration with n-hexane the *title compound* (132 mg, 92%), m.p. 205°C (decomp) (lit.[113], 209-210°C); δ_H(200 MHz, $(CD_3)_2CO$)

3.92 (1H, t, ^3J 7.3 Hz, CH), 4.31 (2H, d, ^3J 7.3 Hz, CH_2), 7.46-7.61 (4H, m, aromatic H_2, H_3, H_6 ,H_7), 8.06-8.11 (2H, m, aromatic H_1, H_8), 8,41-8.51 (3H, m, aromatic H_4, H_5, H_{10}).

Method (B)

Diethyl-2-(9'-anthracenylmethyl)-malonate (1 g, 2.8 mmol) in dioxane (10 ml) containing concentrated hydrochloric acid (5 ml) and water (5 ml) was heated at reflux for 4 hours. The cooled solution was made basic with 2M NaOH then washed with ether (2 x 100 ml). The aqueous layer was acidified with concentrated hydrochloric acid and repeatedly extracted with ethyl acetate, dried over magnesium sulphate and evaporated. Trituration with hexane provided the *title compound* (560 mg, 68%). Analytical data was consistent with that found in method (A).

1-(9'-Anthracenyl)-propionic acid (75)

2-(9'-Anthracenylmethyl)-malonic acid (200 mg, 0.68 mmol) was dissolved in tetrahydronaphthalene (20 ml) and heated at reflux until evolution of carbon dioxide ceased (90 min) then extracted with 6M sodium hydroxide (200 ml). The aqueous phase was washed with pentane (2 x 100 ml), acidified with concentrated hydrochloric acid and extracted with ethyl acetate (2 x 75 ml). The combined organic phase was dried ($MgSO_4$) and the solvent removed *in vacuo*. The resultant oil was triturated with hexane to give the *title compound* (100 mg, 59%), m.p. 186-189°C (lit.[114], 194°C); TLC R_f (A) 0.64, R_f (B) 0.9; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3495 (OH), 2800-3300 (br OH), 3066, 2989 (CH), 1712 (CO); δ_H(200 MHz, $(CD_3)_2SO$) 2.62 (2H, t, ^3J 8.32 Hz, CH), 3.86 (2H, t, ^3J 7.99 Hz, CH), 7.48-7.62 (4H, m, aromatic H_2, H_3, H_6, H_7), 8.07-8.12 and 8.29-8.51 (4H, m, aromatic (H_1 and H_8),(H_4 and H_5)), 8.58 (1H, s, aromatic H_{10}). 12.36 (1H, br. s, COOH).

1-(9'-Anthracenyl)-propionic acid methyl ester (76)

1-(9'-Anthracenyl)-propionic acid (12.7 g, 0.05 mol) was dissolved in methanol (250 ml) containing concentrated sulphuric acid (3 ml) then heated at reflux for 18 hours. The cooled solution was poured into water (700 ml) then extracted with ethyl acetate (2 x 250 ml). The combined organic layers were then washed with 10% sodium hydrogen carbonate (300 ml), dried ($MgSO_4$) and evaporated to yield a yellow solid. Recrystallisation from ethanol/water gave the *title compound* (11 g, 85%), m.p. 68-70°C (Found; C, 80.8; H, 6.1. $C_{18}H_{16}O_2$ requires C, 81.8; H, 6.1%); TLC- R_f (E) 0.6, R_f (G) 0.47; $\lambda_{max}(CH_2Cl_2)$/nm 333, 349, 368 and 388 (ε/$dm^3mol^{-1}cm^{-1}$ 2160, 4560, 7320 and 6960); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3072 and 2950 (CH), 1731 (CO), 1601 (aromatic); δ_H(200 MHz, $CDCl_3$) 2.75-2.83 (2H, m, CH_2CO), 3.74 (3H, s, CH_3), 3.77-4.01 (2H, m, benzylic CH_2), 7.47-7.58 (4H, m, aromatic H_2, H_3, H_6, H_7), 7.95-8.03 (2H, m, aromatic H_1, H_8), 8.24-8.36 (3H, m, aromatic H_4, H_5, H_{10}); δ_C(90 MHz, $CDCl_3$) 23.07 (CH_2CO), 34.68 (benzylic CH_2), 51.56 (OCH_3), 123.64, 124.69, 125.71, 126.19, 129.11 (aromatic CH), 129.24, 131.35, 132.09 (aromatic quaternary), 173.26 (CO); m/z (FAB) 264 (M^+); HRMS: Found 264.11508. $C_{18}H_{16}O_2$ requires 264.11503.

3-(9'-Anthracenylmethyl)-tertiary butylacetoacetate (78)

Sodium hydride (80 %, 1.2 g, 39 mmol) was suspended in dry THF and stirred at 0°C. To this was added tertiary butylacetoacetate (6.3 ml, 38 ml) and 9-chloromethylanthracene (8.8 g, 38.8 mmol), then the reaction was heated at reflux for 3 hours, poured into ice/water and the pH adjusted to pH 6. This was extracted with ethyl acetate and the organic solution washed with brine and dried ($MgSO_4$). Evaporation of the solvent under reduced pressure yielded the *title compound* (10 g, 74%) as an oil which was used without further purification, TLC- R_f (E) 0.43; $\nu_{max}(CHCl_3)$/cm^{-1} 3030, 2985 and 2935 (CH), 1733 (ester CO), 1712 (ketone CO); δ_H(360 MHz, $CDCl_3$) 1.23 (9H, s, $C(CH_3)_3$), 2.08 (3H, s, $COCH_3$), 3.91 (1H, t, ^3J 7.0 Hz, CH), 4.14-4.24 (2H, m, CH_2), 7.42-7.53 (4H, m, aromatic H_2, H_3, H_6, H_7),

7.96-8.02 (2H, m, H_1, H_8), 8.23-8.35 (3H, m, H_4, H_5, H_{10}); m/z (FAB) 348 (M^+); HRMS: Found 348.17255. $C_{23}H_{24}O_3$ requires 348.17253.

1-(9'-Anthracenyl)-butan-3-one (79)

3-(9'-Anthracenylmethyl)-tertiary butylacetoacetate (10 g, 28.7 mmol) was dissolved in dichloromethane (50 ml) and then stirred with trifluoroacetic acid (50 ml) for 2 hours. This was evaporated and then evaporated twice more from dichloromethane alone. The solid thus obtained was dissolved in toluene and refluxed for 2 hours (until evolution of gas ceased) then evaporated to give an oil purified by dry flash chromatography eluting with n-hexane/dichloromethane then dichloromethane to give the *title compound* (6.5 g, 90%), m.p. 83-86°C (Found: C, 86.0; H, 6.3. $C_{18}H_{16}O$ requires C, 87.0, H, 6.5%); TLC- R_f (E) 0.55, R_f (H) 0.3; $\lambda_{max}(CHCl_3)$/nm 390, 370 and 351 (ε/$dm^3mol^{-1}cm^{-1}$ 8944, 9316 and 5155); $\nu_{max}(CHCl_3)$/cm^{-1} 3067, 2989 (CH), 1715 (CO), 1602 (aromatic); δ_H(360 MHz, $CDCl_3$) 2.18 (3H, s, CH_3), 2.88-2.92 (2H, m, CH_2CO), 3.87-3.91 (2H, m, benzylic CH_2), 7.43-7.53 (4H, m, aromatic H_2, H_3, H_6, H_7), 7.99-8.01 and 8.19-8.21 (4H, m, aromatic H_1 and H_2, H_4 and H_5), 8.34 (1H, s, aromatic H_{10}); δ_C(90 MHz, $CDCl_3$), 21.23 (CH_2CO), 29.64 (CH_3), 44.11 (benzylic CH_2) 123.58, 124.60, 125.53, 125.81, 129.03 (aromatic CH), 131.24, 132.77 (aromatic quaternary), 207.70 (CO); m/z (FAB) 248 (M^+); HRMS: Found 248.12013. $C_{18}H_{16}O$ requires 248.12011.

1-(9'-Anthracenyl)-3-methylbutan-3-ol (77)

Method (A)

Iodomethane (3.4 g, 24 mmol) in dry THF (10 ml) was added to a stirred suspension of magnesium turnings (590 mg, 24 mmol) in dry ether over 10 minutes to form the Grignard reagent as a greyish suspension. To this was added 1-(9'-anthracenyl)-butan-3-one (5 g, 20 mmol) in THF (80 ml) then the mixture was refluxed for 4

hours and cooled. The solution was then poured into a cold solution of 1M ammonium chloride and extracted with ethyl acetate (3 x 200 ml). The combined organic phase was washed with water (200 ml), brine (200 ml), dried over magnesium sulphate and evaporated to produce an oil that was purified over silica gel eluting with dichloromethane to yield the *title compound* (2.8 g, 53%), m.p. 99-101°C (Found: C, 85.15; H, 8.0 $C_{19}H_{20}O$ requires C, 86.3; H, 7.6%); TLC- R_f (A) 0.56, R_f (E) 0.2; $\lambda_{max}(CH_2Cl_2)$/nm 334, 351, 369 and 390 (ε/dm^{3}mol^{-1}cm^{-1} 1416, 3009, 4779 and 4603); $\nu_{max}(CH_2Cl_2)$/cm^{-1} 3605 (OH), 3014, 2931 and 2854 (CH); δ_H(200 MHz, $CDCl_3$) 1.46 (6H, s, $C(CH_3)_3$), 1.89-2.16 (2H, m, $CH_2C(CH_3)_3$) 3.66-3.78 (2H, m, benzylic CH_2), 7.22-7.55 (4H, m, aromatic H_2, H_3, H_6, H_7), 7.94-8.02 (2H, m, aromatic H_1, H_8), 8.24-8.33 (3H, m, aromatic H_4, H_5, H_{10}); δ_C(50 MHz, $CDCl_3$) 22.45 ($CH_2C(CH_3)_3$), 29.03 (2 x CH_3), 44.31 (benzylic CH_2), 70.97 (COH), 123.91, 124.58, 125.31, 125.40, 129.02 (aromatic CH), 129.16, 131.39 and 134.41 (quaternary aromatic); m/z (FAB) 264 (M^+); HRMS: Found 264.15140. $C_{19}H_{20}O$ requires 264.15141.

Method (B)

Iodomethane (6 ml, 96 mmol) was added to magnesium turnings (2.3 g, 96 mmol) stirred in dry ether under nitrogen. Upon formation of the Grignard reagent 1-(-9'-anthracenyl)-propionic acid methyl ester (10.5 g, 0.04 mol) in ether was added and the reaction left for 24 hours at room temperature then refluxed for 1.5 hours, cooled and added to a cold solution of 1M ammonium chloride. After extracting with diethyl ether (2 x 200 ml), drying of the solution ($MgSO_4$), removal of the solvent *in vacuo* and trituration with hexane gave a solid. Recrystallisation from ethanol/water gave the *title compound* (8 g, 75%) as a pale yellow solid. Analytical data was consistent with that found in method (A).

1-(9'-Anthracenyl)-3-methylbut-1-yn-3-ol (81)

A solution containing 9-bromoanthracene (5 g, 19.5 mmol), triphenylphosphine (1 g, 3.9 mmol, 0.2 eq.), 2-methylbut-3-yn-2-ol (3 ml, 29 mmol) and palladium (II) acetate (50 mg, 1.9 mmol, 0.1 eq.) in triethylamine (100 ml) was heated at reflux for 15 hours. The triethylammonium hydrobromide that precipitated was removed by filtration and then the solvent was removed under reduced pressure. The oil obtained was dissolved in ether then washed with 1M sodium hydrogen carbonate (200 ml), 2M hydrochloric acid (200 ml), dried ($MgSO_4$) and evaporated. The crude oil was purified by dry flash chromatography eluting with dichloromethane and the collected fractions crystallised upon evaporation to give the *title compound* (2.1 g, 41%) as a red solid, m.p.155-158°C (Found: C, 85.2; H, 6.4. $C_{19}H_{16}O$ requires C, 87.7; H, 6.2%); TLC- R_f (A) 0.8, R_f (E) 0.14; λ_{max}(MeOH) 364, 384 and 406 (ε/$dm^3mol^{-1}cm^{-1}$ 9509, 9816 and 10123); $\nu_{max}(CHCl_3)/cm^{-1}$ 3598 (OH), 3068, 3021, 2979, 2933 and 2872 (CH); δ_H(200 MHz, $CDCl_3$) 1.82 (6H, s, 2 x CH_3), 1.84 (1H, s, OH), 7.41-7.62 (4H, m, aromatic), 7.95-8.22 (4H, m, aromatic), 8.39 (1H, s, aromatic H_{10}); δ_C(50 MHz, $CDCl_3$) 31.49 (2 x CH_3), 71.51 ($(CH_3)_2COH$), 121.11, 123.88, 124.57, 126.47, 127.00, 127.23, 127.37, 127.86, 130.10 (aromatic CH), 134.07, 138.16, 148.98 (aromatic quaternary), (alkyne quaternary not observed); m/z (FAB) 261 (MH^+); HRMS: Found 260.12010. $C_{19}H_{16}O$ requires 260.12011.

<u>N^{α}-Benzyloxycarbonylaspartic acid-α-benzyl-β-1-(9'-anthracenyl)-3-methylprop-3-yl ester (82)</u>

N^{α}-Benzyloxycarbonylaspartic acid-α-benzyl ester (7.8 g, 22 mmol) and 1-(9'-anthracenyl)-3-methylbutan-3-ol (2.2 g, 8 mmol) were dissolved in distilled dichloromethane (100 ml) and cooled to 0°C before the addition of DCC (4.7 g, 23 mmol) and DMAP (100 mg). The reaction mixture was maintained at 4°C for 4 days then filtered and evaporated. The residue was dissolved in ethyl acetate (200 ml) and washed with 10% sodium carbonate (200 ml), water (100 ml) and brine (200 ml)

then dried ($MgSO_4$) and evaporated to yield the *title compound* as a crude oil (10g crude) that was used without purification, δ_H(200 MHz, $CDCl_3$) 1.60 (6H, s, CH_3 x 2), 2.11-2.19 (2H, m, $CH_2C(CH_3)_2$), 2.89-3.21 (2H, m, βCH_2), 3.51-3.61 (2H, m, anthracenyl CH_2-Ar), 4.71-4.75 (1H, m, αCH), 5.09 (2H, s, benzylic CH_2), 5.19 (2H, s, benzylic CH_2), 5.92 (1H, dr. d., NH), 7.27 (10H, s, phenyl x 2), 7.36-7.54 (4H, m, anthracenyl), 7.97-8.02 (2H, m, anthracenyl), 8.34 (1H, s, anthracenyl H_{10}); m/z (FAB) 603 (M^+).

Aspartic acid-β-1-(9'-anthracenyl)-3-methylbut-3-yl ester (83)

The crude N^{α}-benzyloxycarbonylaspartic acid-α-benzyl-β-1-(9'-anthracenyl)-3-methylprop-3-yl ester (10 g) was dissolved in THF/methanol (3:7, 100 ml) and cooled to 0°C before addition of 10% palladium on charcoal (2 g). This was stirred under hydrogen for 24 hours. The catalyst was removed by filtration through celite and the solvent was removed *in vacuo*. The *title compound* was obtained as a yellow solid upon trituration with ether 2.4 g (80 % based on 1-(9-anthracenyl)-3-methylbutan-3-ol), m.p.125-130°C (decomposed); $[\alpha]_D^{22}$ +1.2°(c 0.5 DMF); TLC-R_f (C) 0.54, R_f (B) 0.68; λ_{max}(MeOH)/nm 348, 365 and 385 (ε/$dm^3mol^{-1}cm^{-1}$ 8046, 11494 and 11494); ν_{max}(bromoform mull)/cm^{-1} 3394 (br.,m.), 2933, 2853 (CH), 1701 (carboxyl CO), 1637 (amino acid I); δ_H(80 MHz, $(CD_3)_2SO$) 1.12 (6H, s, CH_3), 1.62-1.84 (2H, m, CH_2CO), 2.50-2.59 (2H, m, βCH_2), 3.85-4.00 (2H, m, Ar-CH_2), 7.17-8.48 (9H, m, aromatic); m/z (FAB) 380 (MH^+); HRMS: Found 380.18621. $C_{23}H_{26}NO_4$ requires 380.18617.

N^{α}-9-Fluorenylmethoxycarbonylaspartic acid-β-1(9'-anthracenyl)-3-methylbut-3-yl ester (84)

Aspartic acid-β-1-(9'-anthracenyl)-3-methylbut-3-yl ester was dissolved in water (25 ml). Triethylamine (800 µl, 5.7 mmol) then 9-fluorenylmethyl-succinimidyl

carbonate (2.7g, 8 mmol) in dioxane were added. After 3 hours at room temperature water (200 ml) was added and 2M potassium hydrogen sulphate was added to bring the solution to approximately pH 3. This was extracted with chloroform (2 x 150 ml) and this was washed with water (2 x 200 ml), dried ($MgSO_4$) then evaporated. Purification by dry column flash chromatography eluted with ether and ether/ethyl acetate gave the *title compound* (2g, 65 %), m.p. 136-140°C (Found: C, 69.8; H, 6.5; N, 3.9. $C_{38}H_{34}NO_6$ requires C, 75.85; H, 5.85; N, 2.3%); $[\alpha]_D^{22}$ -4.2° (c 1.2, DMF); TLC- R_f (A) 0.19, R_f (C) 0.88; $\lambda_{max}(CHCl_3)$/nm 290, 300, 351, 370 and 390 (ε/$dm^3mol^{-1}cm^{-1}$ 10635, 10952, 1349, 2222, 2063); $\nu_{max}(CHCl_3)/cm^{-1}$ 3408 (NH), 3032, 2938 and 2858 (CH), 1716 (CO), 1606 (aromatic), 1513 (amide II); δ_H(200 MHz, $CDCl_3$, 55°C) 1.21 (3H, s, CH_3), 1.50 (3H, s, CH_3), 2.76-2.95 (4H, m, $COCH_2$, CH_2), 3.48-3.98 (1H, m, αCH), 4.05-4.78 (5H, m, Ar-CH_2, Fmoc CH_2, Fmoc CH), 6.34 (1H, br. s, NH), 7.11-8.19 (17H, m, aromatic); δ_C(50 MHz, $(CD_3)_2SO$) 24.56 ($COCH_2$), 25.71 (CH_3), 46.81 (CH_2CH_2), 49.65 (αCH), 52.91 (βCH_2), 59.89 (Fmoc CH), 65.62 (Fmoc CH_2), 81.81 ($C(CH_3)_2$), 120.16-129.05 (aromatic CH), 131.28, 134.72 (anthracenyl quaternary), 140.82, 144.03 (Fmoc quaternary), 155.75, 168.38, 170.77 (CO); m/z (FAB) 601 (MH^+); HRMS: Found 601.24648. $C_{38}H_{35}NO_6$ requires 601.24642.

Hydrocinnamic acid 1-(9'-anthracenyl)-3-methylbut-3-yl ester (95)

1-(9'-Anthracenyl)-3-methylbutan-3-ol (2 g, 7.6 mmol) and hydrocinnamic acid (3.3 g, 22 mmol) were dissolved in distilled dichloromethane (50 ml) and stirred at 0°C. DCC (4.5 g, 22 mmol) and a trace of DMAP were added and the reaction stirred at 4°C for 5 days. The solution was filtered and evaporated then the residue was applied directly to a dry flash column. Elution with dichloromethane then trituration with hexane gave the *title compound* (1.7 g, 56%), m.p. 94-96°C; TLC- R_f (F) 0.83, R_f(E) 0.65; $\lambda_{max}(CHCl_3)$/nm 349, 367 and 388 (ε/dm^{3}mol^{-1}cm^{-1} 980, 1617 and 1519); $\nu_{max}(CHCl_3)$/cm^{-1} 3031, 2934 and 2856 (CH), 1719 (CO); δ_H(200 MHz, $CDCl_3$) 1.65 (6H, s, CH_3 x 2), 2.09-2.18 (2H, m, $CH_2C(CH_3)_2$), 2.66-2.78 (2H, m, CH_2CO), 2.95-3.09 (2H, m, CH_2-Ar hydrocinnamoyl), 3.54-3.63 (2H, m, CH_2-Ar anthracenyl), 7.13-7.34 (4H, m, anthracenyl aromatic), 7.41-7.55 (5H, m, hydrocinnamyl aromatic), 7.98-8.03 (2H, m, anthracenyl aromatic), 8.19-8.24 (2H, m, anthracenyl aromatic), 8.34 (1H, s, anthracenyl H_{10}); δ_C(90 MHz, $CDCl_3$) 22.14 ($CH_2C(CH_3)_2$), 25.27 (CH_3 x 2), 31.13 (CH_2CO hydrocinnamyl), 37.01 (CH_2-Ar hydrocinnamyl), 42.18 (CH_2-Ar anthracenyl), 82.19 ($C(CH_3)_2$), 123.83, 124.65, 125.44, 125.63, 126.07, 128.17, 128.33, 129.08 (aromatic), 129.27, 131.44, 133.82 (anthracenyl quaternary), 140.43 (hydrocinnamyl quaternary), 172.22 (CO); m/z (FAB) 396 (M^+); HRMS: Found 396.20893. $C_{28}H_{28}O_2$ requires 392.20892.

3-Methyl-3-hydroxymethyloxetane (90)

1,1,1-*Tris*(hydroxymethyl)ethane (120 g, 1 mole) and diethyl carbonate (118 g, 1 mole) and a solution of ethanolic potassium hydroxide (1 g in 10 ml) were heated at reflux under nitrogen for twenty minutes then approximately 2 moles of ethanol was distilled at atmospheric pressure. The remainder of the material was pyrolysed at a temperature of approximately 200-220°C and a pressure of 50-100 mmHg to

produce a distillate boiling between 120-140°C from which the *title compound* (35 g, 34%) was then obtained by redistillation, b.p 40°C/22 mmHg (Lit.[96], 80°C/40 mmHg); $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3621 (free OH), 3440 (H-bonded OH); δ_H(200 MHz, $CDCl_3$) 1.12 (3H, s, CH_3) 3.03 (1H, t, ^3J 4.7 Hz, OH), 3.62 (2H, d, ^3J 4.3 Hz, CH_2 (exo)), 4.34 (2H, d, ^2J 5.8 Hz, CH_2 (endo)), 4.48 (2H, ^2J 5.8 Hz, CH_2 (endo)).

N$^{\alpha}$-Benzyloxycarbonylaspartic acid-α-benzyl-β-3-methyl-3-hydroxymethyloxetane ester (91)

To a solution of N$^{\alpha}$-benzyloxylcarbonylaspartic acid α-benzyl ester (8.2 g, 23 mmol) and 3-methyl-3-hydroxymethyloxetane (3 g, 30 mmol) in distilled dichloromethane (70 ml) stirred at 0°C was added dicyclohexylcarbodiimide (5.4 g, 26 mmol) and a trace of DMAP. This was stirred for 2 days at 4°C, filtered and evaporated then dissolved in approximately 150 ml of ethyl acetate. This was washed with 10% sodium carbonate (2 x 100 ml), water (100 ml) and dried over $MgSO_4$. Upon evaporation an oil was obtained and purified over silica gel eluted with chloroform and chloroform/methanol to yield the *title compound* (8.7g, 86%) as a clear oil, $[\alpha]_D^{22}$ +13.9° (c 1.0, $CHCl_3$); TLC- R_f (A) 0.68, R_f (E) 0.24; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3432 (NH), 2960 and 2882 (CH), 1736 (ester CO), 1611 (aromatic), 1508 (amide II); δ_H(360 MHz, $CDCl_3$) 1.23 (3H, s, CH_3), 2.93-3.07 (2H, m, βCH_2), 3.96-4.15 (2H, m, oxetane exo CH), 4.32-4.40 (4H, m, oxetane endo CH), 4.69 (1H, m, αCH) 5.16, 5.18 (4H, s, CH_2 benzylic), 6.0 (1H, d, NH), 7.33 (10H, s, aromatic); δ_C (50 MHz, $CDCl_3$) 20.64 (CH_3), 36.50 (βCH_2), 38.68 (oxetane quaternary), 50.26 (αCH), 67.33 (oxetane exo CH_2) 66.80 and 68.75 (oxetane endo CH_2), 79.05 (2 x benzylic CH_2), 127.79, 127.93, 128.14, 128.28 and 128.36 (aromatic CH), 134.90 and 135.95 (aromatic quaternary), 155.79, 170.26 and 170.45 (CO quaternary); m/z (FAB) 442 (MH^+); HRMS: Found 442.1870. $C_{24}H_{28}NO_7$ requires 442.18656.

N^{α}-Benzyloxycarbonyl-2-amino-3-(4'-methyl-2,6,7-trioxabicyclo[2.2.2]octyl) propionic acid (92)

N^{α}-Benzyloxycarbonylaspartic acid-α-benzyl-β-3-methyl-3-hydroxymethyloxetane ester (8.3 g, 18.8 mmol) was dissolved in freshly distilled dichloromethane (50 ml), cooled to 0°C and stirred under a stream of nitrogen. Freshly distilled boron trifluoride diethyl etherate (600 µl, 5 mmol) was added and the reaction stirred for 2 hours at 0°C. The solution was concentrated then cooled and triethylamine (2.7 ml, 20 mmol) was added. Ether was then added to precipitate the amine/Lewis acid complex. After filtration of this solid the solvent was removed *in vaccuo* then the *title compound* (6.6 g, 78%) was obtained by crystallisation from diethyl ether and washed with n-hexane, m.p. 98-100°C (Found C, 65.4; H, 6.2; N, 3.2. $C_{24}H_{27}NO_7$ requires C, 65.3; H, 6.2; N, 3.2%); $[\alpha]_D^{22}$ -2.1° (c 3.0, $CHCl_3$); TLC- R_f (A) 0.81; $\nu_{max}(CHCl_3)/cm^{-1}$ 3429 (NH), 3032, 2939 and 2883 (CH), 1722 (ester CO), 1509 (amide II); δ_H(200 MHz, $CDCl_3$) 0.72 (3H, s, CH_3) 2.03-2.44 (2H, m, CH_2), 3.76 (6H, s, ortho ester 3 x CH_2), 4.50-4.54 (1H, m, αCH), 5.11 and 5.14 (4H, s, benzylic CH_2), 6.08 (1H, d, NH), 7.34 (10H, s, aromatic); δ_C(50 MHz, $CDCl_3$) 14.07 (CH_3), 29.94 (ortho ester *C*-CH_3 quaternary), 37.00 (βCH_2), 50.13 (αCH), 66.59 (2 x benzylic CH_2), 72.07 (ortho ester CH_2), 107.81 (ortho ester C-O quaternary), 127.84, 127.95, 128.06, 128.20 (aromatic CH), 135.54 and 135.89 (aromatic quaternary), 155.84. 170.18 and 171.04 (CO quaternary); m/z (FAB) 442 (MH^+); HRMS: Found 442.18659. $C_{24}H_{28}NO_7$ requires 442.18656.

2-Amino propionic acid-3-methyl-2,6,7-trioxabicyclo[2.2.2]octane (93)

N^{α}-Benzyloxycarbonyl-2-amino-3-(4'-methyl-2,6,7-trioxabicyclo[2.2.2]octyl propionic acid (3 g, 6.8 mmol) was dissolved in methanol (50 ml) and cooled to 0°C. Palladium on charcoal (10%, 300 mg) was added and the solution warmed to room

temperature. This was stirred under an atmosphere of hydrogen for 90 minutes. The catalyst was removed by filtration through celite and the solvent removed *in vacuo*. Trituration with ether produced the *title compound* (1.4 g, 95%) as a crystalline solid, m.p. 160°C (decomp.); TLC- R_f (C) 0.13, R_f (B) 0.44; ν_{max}(KBr) 3376 (broad), 1737 (carboxylate), 1268 (CO ortho ester); δ_H(200 MHz, CD_3OD) 0.91 (3H, s, CH_3), 2.05-2.55 (2H, m, βCH_2), 3.85 (1H, d of d, ^3J 11.2 Hz, ^3J 2.4 Hz, αCH), 4.04 (6H, s, CH_2 x 3); δ_C(50 MHz, CD_3OD) 12.28 (CH_3), 29.29 (quaternary CH_3-*C*), 36.38 (βCH_2), 71.67 (CH_2 x 3), 106.91 (quaternary βCH_2*C*), 171.74 (CO); m/z (FAB) 218 (MH^+); HRMS: Found 218.10285. $C_9H_{16}NO_7$ requires 218.10284.

N$^{\alpha}$-9-Fluorenylmethoxycarbonyl-2-amino-3-(4'-methyl-2,6,7-trioxabicyclo[2.2.2]octyl) propionic acid (94)

A solution of 2-amino propionic acid-trioxabicycl[2.2.2]octane (400 mg, 1.8 mmol) in water (10 ml) containing triethylamine (260 µl, 1.9 mmol) was stirred together with a solution of 9-fluorenylmethyl-succinimidyl carbonate (570 mg, 1.7 mmol) in dioxane (10 ml) for 4 hours. This was diluted with water (100 ml) then acidified with aqueous citric acid (20% w/v) and extracted with ethyl acetate (2 x 200 ml). The organic phase was washed with water (2 x 200 ml) and dried ($MgSO_4$). A white foam was obtained upon evaporation which was crystallised from a solution of ether and n-hexane to give the *title compound* (500 mg, 67%), m.p. 88-92°C (Found: C, 64.1; H, 6.6; N, 3.5. $C_{24}H_{25}NO_7$ requires C, 65.6; H, 5.7; N, 3.2%); $[\alpha]_D^{22}$ -1.1° (c 1.0, DMF); TLC- R_f (C) 0.65, R_f (B) 0.8; λ_{max}($CHCl_3$)/nm 301, 290 (ε/$dm^3mol^{-1}cm^{-1}$ 4559, 3868); ν_{max}(CH_2Cl_2)/cm^{-1} 3432 (NH), 3054, 2986 (CH), 1730 (CO), 1606 (aromatic), 1509 (amide II), 1262 (CO, ortho ester); δ_H(200 MHz, $CDCl_3$) 2.55-3.05 (3H, s, CH_3), 2.55-3.05 (2H, m, βCH_2), 3.49 (6H, s, CH_2 x 3), 3.97-4.63 (3H, m, Fmoc CH and Fmoc CH_2), 6.35 (1H, d, NH), 7.20-7.78 (8H, m, aromatic); δ_C(90 MHz, $(CD_3)_2SO$); 16.64 (CH_3), 36.28 (βCH_2), 38.36(*C*CH_3), 46.82 (Fmoc

CH) 50.62 (αCH), 63.72 (3 x CH_2), 66.81 (Fmoc CH_2), 120.34, 125.47, 127.33 and 127.90 (aromatic), 140.94 and 144.00 (aromatic quaternary), 156.13 (ortho ester quaternary CO) 170.40 (urethane CO), 172.74 (acid CO); m/z (FAB) 440 (MH^+); HRMS: Found 440.17090. $C_{24}H_{26}NO_7$ requires 440.17091.

Hydrocinnamic acid 3-methyl-3-hydroxymethyloxetane ester (97)

Hydrocinnamic acid (3 g, 20 mmol) and 3-methyl-3-hydroxymethyloxetane (2 g, 19.6 mmol) were dissolved in distilled dichloromethane and cooled to 0°C before the addition of DCC (4.1 g, 20 mmol) and DMAP (50 mg). After stirring for 2 days at 4°C the solution was filtered and the solvent evaporated . The residue was dissolved in ethyl acetate (100 ml) and washed with sodium hydrogen carbonate (2 x 75 ml) and dried ($MgSO_4$). The bulk of the solvent was removed *in vacuo* and then passed through a shallow pad of basic alumina which was washed with small portions of dichloromethane. The collected organic washings were evaporated to give the *title compound* (3.9 g, 85%) as a clear oil, TLC- R_f (E) 0.2, R_f (A) 0.79; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3067, 3030, 2952 and 2882 (CH), 1736 (CO), 1602 (aromatic); δ_H(200 MHz, $CDCl_3$) 1.24 (3H, s, CH_3), 2.62-2.71 (2H, m, CH_2CH_2CO), 2.91-2.99 (2H, m, benzylic CH_2),4.12 (2H, s, oxetane CH exo), 4.30 (2H, d, ^2J 6.0 Hz, oxetane CH endo), 4.42 (2H, d, ^2J 5.9 Hz, oxetane CH endo) 7.12-7.31 (5H, m, aromatic); δ_C(90 MHz, $CDCl_3$) 20.80 (CH_3), 30.65 (CH_2CO), 35.39 (benzylic CH_2), 38.71 (oxetane quaternary), 68.38 (CH_2 exo), 79.20 (CH_2 endo), 126.04 (aromatic C_4), 127.95 and 128.23 (aromatic CH), 139.99 (aromatic quaternary), 172.60 (CO); m/z (FAB) 235 (MH^+); HRMS: Found 235.13342. $C_{14}H_{19}O_3$ requires 235.13341.

Hydrocinnamyl-4-methyl-2,6,7-trioxabicyclo[2.2.2]octane (98)

Hydrocinnamic acid 3-methyl-3-hydroxymethyloxetane ester (3.5 g, 15 mmol) was dissolved in distilled dichloromethane and cooled to 0°C then distilled boron

trifluoride diethyl etherate (460 μl, 3.75 mmol) was added and the solution maintained at 0°C for 3 hours. Triethylamine (2 ml,15 mmol) was added followed by ether (300 ml) and the precipitate filtered. Removal of the solvent *in vacuo* affords the *title compound* (2.5 g, 71%) as a white solid, m.p. 109-111°C; $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3055, 2971, 2937 and 2879 (CH), 1605 (aromatic), 1258 (CO stretch); δ_H(200 MHz, $CDCl_3$) 0.81 (3H, s, CH_3), 1.95-2.06 (2H, m, CH_2-CO), 2.74-2.99 (2H, m, benzylic CH_2), 3.93 (6H, s, 3 x CH_2 ortho ester), 7.13-7.32 (5H, m, aromatic); δ_C(90 MHz, $CDCl_3$) 14.22 (CH_3), 29.34 (CH_2CO), 29.95 (CCH_3), 38.26 (benzylic CH_2), 72.31 (CH_2 x 3), 108.35 (CO), 125.48 (C_4 aromatic), 128.1 (CH aromatic) 141.61 (CO); m/z (FAB) 235 (MH^+); HRMS: Found 235.13342 $C_{14}H_{19}O_3$ requires 235.13341.

3.3 STABILITY STUDIES

NMR Stability measurement

Comparison of the acetate esters of dibenzosuberol and 4-nitrobenzyl alcohol

Two solutions were prepared;

A 4-nitrobenzyl acetate (51) (3.9 mg, 20.5 μmol) in hexadeuteroacetone (0.5 ml)

B Dibenzosuberyl acetate (52) (5.0 mg, 20.5 μmol) in hexadeuteroacetone (0.5 ml)

in standard NMR tubes.

To each was added NaOH in D_2O (0.2 ml, 0.125 M, 1 eq.) The NMR spectra were recorded at 200 MHz . The benzylic protons of (51) were used as diagnostic δ_H 5.26 (acetate) and 4.79 (alcohol). The 5-H resonance in (52) were used δ_H 6.90 (acetate) and 6.17 (alcohol). Integration of these peaks provided a measure of the degree of hydrolysis.

HPLC Stability measurement

Using a C_4 RP 300 Aquapore butyl 7 micron (4.6 x 100 mm) column with a gradient A 95%, B 5% to A 10%, B 90% over 25 minutes the following retention times were measured;

Hydrocinnamic acid	7.9 minutes
1-(9'-Anthracenyl)-3-methylbut-3-yl hydrocinnamate	20.8 minutes
9-Anthracenylmethyl hydrocinnamate	18.9 minutes
Dibenzosuberyl hydrocinnamate	19.1 minutes

Stability of hydrocinnamate esters to acidolysis

To a solution of TFA in dichloromethane (10 ml) (20, 50 or 90%) was added the ester (20 mg). Over a period of time 1 ml portions were removed and evaporated

then dissolved in 1 ml of acetonitrile for HPLC analysis (25 µl injection) until acidolysis was complete.

Stability of hydrocinnamate esters to alkaline hydrolysis

The ester (2 mmol) was dissolved in dioxane (5 ml) then sodium hydroxide (4 mmol) dissolved in water (5 ml) was added. Aliquots of 0.5 ml were removed at intervals and quenched using pH 7 phosphate buffer (25 ml) then 25 µl injections were quantified using and automatic integrator attached to the HPLC detector.

TLC Stability measurement

Stability of 4-methyl-2,6,7-trioxabicyclo[2.2.2]octyl derivative

N-benzyloxycarbonyl-2-amino-3-(4'-methyl-2,6,7-trioxabicyclo[2.2.2]octyl)-propionic acid (20 mg) was dissolved with stirring in 95% aqueous trifluoroacetic acid (10 ml) and examined by TLC to monitor the progress of the reaction. Plates were run against a standard sample of starting material and free acid and eluted with 90% chloroform/10% methanol.

Benzyloxycarbonyl aspartic acid benzyl ester R_f 0.4.

N-benzyloxycarbonyl-2-amino-3-(4'-methyl-2,6,7-trioxabicyclo[2.2.2]octyl)-propionic acid R_f 0.68.

Total hydrolysis of the ortho ester was observed within 1 minute.

3.4 SOLID PHASE PEPTIDE SYNTHESIS

Solid phase peptide synthesis was carried out on an Applied Biosystems 430A automated peptide synthesiser using standard cycles as summarised below;

1. Removal of N^{α}-Fmoc protecting group; 20% piperidine/DMF (9 ml), (3 min. and 1 min.). An aliquot is taken and diluted for UV monitoring (302 nm) to assess coupling efficiency.

2. Washing of resin; DMF/dioxane (1:1), nine times.

3. Activation of protected amino acid; First coupling Fmoc amino acid (1 mmol) in DMF/dioxane (6 ml, 2:1), with DIC (2 ml, 0.25 $moldm^{-3}$ in dioxane) for 15 min.. Second coupling Fmoc amino acid (0.5 mmol) in DMF (2 ml) with HOBt (2 ml, 0.25 $moldm^{-3}$ in dioxane) then DIC (2 ml, 0.25 $moldm^{-3}$ in dioxane) for 20 min..

4. Coupling of each activated species for 30 min..

5. Washing of resin, 5 times between each coupling (DMF/dioxane, 1;1).

6. Capping of unreacted amine functionality, A: 1 ml 0.5 $moldm^{-3}$ acetic anhydride in DMF, 1 ml 0.5 $moldm^{-3}$ pyridine in DMF then diluted with DMF (6 ml) for 3 min. then 6 min.. B: 0.5 $moldm^{-3}$ of acetic anhydride in DMF/0.125 $moldm^{-3}$ of diisopropylethylamine in DMF/0.2% HOBt(w/v) in DMF, 10 ml of reagent for 10 min.

7. Washing of resin DMF/dioxane (1:1) 7 times.

Val-Lys-Asp-Gly-Tyr-Ile

This peptide was prepared using the cycles summarised except where otherwise indicated. Four syntheses were executed using Boc side chain protection for lysine,

tert-butyl protection for tyrosine and the protecting groups stated for aspartic acid;

Fmoc-Ile-OH was dissolved in DMF with DIC (0.5 eq.) and a trace of DMAP to give the symmetrical anhydride and sonicated for 30 min. with Wang resin (0.85 mmol substitution). The resin was then filtered and washed with DMF, dichloromethane and ether. The loading is measured by U.V.

1, 9-Anthracenylmethyl

0.55 mmol/g Fmoc-Ile-R (456 mg, 0.25 mmol) was used as starting material for the synthesis. Standard double coupling was used with the exception of glycine which was coupled as it's symmetrical anhydride (2 eq.). The capping protocol used was A.

2, 1-(4'-Fluorophenyl)-2-methylprop-2-yl

0.55 mmol/g Fmoc-Ile-R (456 mg, 0.25 mmol) was used with coupling as 1 except 4 equivalents of glycine symmetrical anhydride was used. Capping protocol A was used.

3, 1-(9'-Anthracenyl)-2-methylbut-2-yl

0.39 mmol/g Fmoc-Ile-R (256 mg, 0.1 mmol) was used and the Fmoc amino acids were coupled as HOBt esters, double coupled (2 eq per coupling) and glycine as it's symmetrical anhydride (4 eq.).Capping protocol B was used.

4, 4'-Methyl-2,6,7-trioxabicyclo[2.2.2]octyl

0.39 mmol/g Fmoc-Ile-R (256 mg, 0.1 mmol) used with the synthesis conditions as for 3.

Peptide cleavage conditions

A. Aqueous TFA cleavage

The peptide was liberated from the resin and the protecting groups removed in TFA/water/anisole (93:4:0.3) for 3 hours. The resin was removed by filtration then the solvent removed *in vacuo* before trituration with ether. The ether was decanted and the peptide washed with ether dissolved in aqueous acetic acid (10 %) and lyophilised. Crude cleaved material was analysed by reversed phase HPLC with an integrator attached to calculate proportions of products. Purification for the purpose of characterisation was by reversed phase HPLC. (Vydac C_{18} 10 micron (25 x 250 mm) Gradient: 5% B to 45% B {24 min}, 45% B to 95% B {26 min}, 95% B to 5% B {28 min}.

TMSBr/TFA cleavage

A solution containing ethanedithiol (0.5 ml), *m*-cresol (0.1 ml), thioanisole (1.2 ml) and TFA (7.5 ml) was cooled to 0°C and the resin bound peptide added. Bromotrimethylsilane (1.3 ml) was added and the reaction stirred at 0°C for 15 minutes under nitrogen. This was then partitioned between 20% acetic acid (v/v) and diethyl ether. The aqueous phase was then washed several times with ether and the lyophilised.

Typical analytical data

Peptide:

HPLC: Vydac C_{18} 5 micron (4 x 250 mm) reverse phase column, R_t 18.8 min.(5% B to 45% B {24 min}, 45% B to 95% B {26 min}, 95% B to 5% B {28 min}); m/z (FAB) 694 (MH^+); HRMS: Found 694.37759. $C_{32}H_{52}N_7O_{10}$ requires 694.37759;

Amino acid analysis, Val 0.99 (1.00); Lys 1.07 (1.00); Asp 1.10 (1.00), Gly 0.97 (1.00); Tyr 0.85 (1.00), Ile 0.95 (1.00).

Aspartimide:

HPLC: Vydac C_{18} 5 micron (4 x 250 mm) reverse phase column, R_t 19.2 min.(5% B to 45% B {24 min}, 45% B to 95% B {26 min}, 95% B to 5% B {28 min}); m/z (FAB) 676 (MH^+); HRMS: Found 676.36696 $C_{32}H_{50}N_7O_9$ requires 676.36697; Amino acid analysis, Val 1.05 (1.00); Lys 0.97 (1.00); Asx 1.01(1.00), Gly 1.01 (1.00); Tyr 0.93 (1.00), Ile 1.02 (1.00).

REFERENCES

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

2. R. B. Merrifield, *J. Am. Chem. Soc.*, 1964, **86**, 1385.

3. S. S. Wang, *J. Am. Chem. Soc.*, 1973, **95**, 1328.

4. R. Ramage, S. L. Irving and C. McInnes, *Tetrahedron Lett.*, 1993, **34**, 6599.

5. H. Rink, *Tetrahedron Lett.*, 1987, **28**, 3787.

6. A. R. Mitchell, B. W. Erickson, M. N. Ryabtsev, R. S. Hodges and R. B. Merrifield, *J. Am. Chem. Soc.*, 1976, **98**, 7357.

7. A. R. Mitchell, S. B. H. Kent, M. Engelhard and R. B. Merrifield, *J. Org. Chem.*, 1978, **43**, 2845.

8. G. W. Kenner and J. H. Seely, *J. Am Chem. Soc.*, 1972, **94**, 3259.

9. G. C. Stelakatos, A. Paganou and L. Zervas, *J. Chem. Soc. (C)*, 1966, 1191.

10. M. Bergmann and L. Zervas, *Ber. Dtsh. Chem. Ges.*, 1932, **65**, 1192.

11. F. C. McKay and N. F. Albertson, *J. Am. Chem. Soc.*, 1957, **79**, 4686.

12. P. Sieber and B. Iselin, *Helv. Chim. Acta*, 1968, **51**, 614.

13. L. A. Carpino and G.Y. Han, *J. Am. Chem. Soc.*, 1970, **92**, 5748.

14. L. A. Carpino and G. Y. Han, *J. Org. Chem.*, 1972, **37**, 3404.

15. R. Ramage, A. J. Blake, M. R. Florence, T. Gray, G. Raphy and P. Roach, *Tetrahedron*, 1991, **47**, 8001.

16. C. Chang, M. Waki, M. Ahmad, J. Meienhofer, E. O. Lundell and J. D. Haug, *Int. J. Pept. Prot. Res.*, 1980, **15**, 59.

17. R. Schwyzer and H. Dietrich, *Helv. Chim. Acta.*, 1961, **44**, 2003.

18. E. Schroder and E. Klieger, *Liebigs Ann. Chem.*, 1964, **673**, 196.

19. J. Green, O. M. Ogunjobi, R. Ramage, A. S. J. Stewart, S. McCurdy and R. Noble, *Tetrahedron Lett.*, 1988, **29**, 4341.

20. P. Sieber and B. Riniker, *Tetrahedron Lett.*, 1987, **28**, 6031.

21. D. F. Veber, J. D. Milkowski, S. Varga, R. G. Denkewalter and R. Hirschman, *J. Am. Chem. Soc.*, 1972, **94**, 5456.

22. A. Chimiak, *"The Peptides 1962"* (G. T. Young Ed.) p37, Pergamon, Oxford, (1963).

23. E. Wunsch and J. Jentsch, *Chem. Ber.*, 1964, **97**, 2590.

24. R. Schwyzer and W. Rittel, *Helv. Chim. Acta.*, 1961, **44**, 159.

25. J. C. Sheehan and G. P. Hess, *J. Am. Chem. Soc.*, 1955, **77**, 1067.

26. G. Holfe, W. Steglich and H. Vorbruggen, *Ang. Chem. Int. Ed. Eng.*, 1978, **17**, 569.

27. J. R. Vaughan and R. L. Osato, *J. Am. Chem. Soc.*, 1952, **74**, 676.

28. J. Wieland, W. Kern and R. Sehring, *Liebigs Ann. Chem.*,1950, **569**, 117

29. T. Gray, Ph. D. Thesis, University of Edinburgh, 1989.

30. L. Kisfauldy and I. Schon, *Synthesis*, 1983, 325.

31. I. Schon and L. Kisfauldy, *Synthesis*, 1986, 303.

32. T. Curtius, *Ber. Dtsh. Chem. Ges.*, 1902, **35**, 3226.

34. L. A. Carpino, H. G. Chao, M. Beyermann and M. Bienert, *J. Org. Chem.*, 1991, **56**, 2635.

33. L. A. Carpino, B. J. Cohen, K. E. Stevens, Y. Sadat-Aalaee, J. Tien and D. C. Langridge, *J. Org. Chem.*, 1986, **51**, 3732.

35. L. A. Carpino, E. M. E. Mansour and Y. Sadat-Aalaee, *J. Org Chem.*, 1991, **56**, 2611.

36. K. Shaw, personal communication

37. K. M. Otteson, R. L. Noble, P. D. Hoeprich, K. T. Shaw and R. Ramage, *Proceedings of the 13th American Peptide Symposium 1993*, in press.

38. A. Davidson, Ph. D. Thesis, University of Edinburgh, 1993.

39. H. Yajima, N. Fujii, S. Funakoshi, T. Watanabe, E. Muryama and A. Otaka, *Tetrahedron*, 1988, **44**, 805.

40. M. Bodansky and J. Martinez, *Synthesis*, 1981, 333.

41. M. Bodansky and S. Natarajan, *J. Org. Chem.*,1975, **40**, 2495; S. Natarajan, and M. Bodansky, *J. Org. Chem.*,1976, **41**, 1269.

42. S. Capasso, L. Mazzarella, F. Sica, A. Zagari and S. Salvadori, *J. Chem. Soc. Chem. Comm.*, 1992, 918.

43. A. R. Battersby and J. C. Robinson, *J. Chem. Soc.*, 1955, 259.

44. G. Cotton, Ph. D. Thesis, University of Edinburgh, 1994

45. R. Dolling, M Beyermann, J. Kernchen, E. Krause, P. Franke, M. Brudel and M. Bienert, *Third International Symposium "Innovation and Perspectives in Solid Phase Synthesis"* 1993 (in Press).

46. I. Schon, T. Szirtes, A. Rill, G. Balogh, Z. Vadasz, J. Seprodi, I. Teplan, N. Chino, K. Y. Kumogaye and S. Sakakibara, *J. Chem. Soc. Perkin Trans. 1*, 1991, 3212.

47. M. A. Ondetti, A. Deer, J. T. Sheehan, J. Pluscee and O. Kocy, *Biochemistry*, 1968, **7**, 4069.

48. S. A. Bernhard, A. Berger, J. H. Carter, E. Katachalski, M. Sela and Y. Shalitin, *J. Am. Chem. Soc.*,1962, **84**, 2421.

49. R. Schwyzer, B. Iselin, H. Kappeler, B. Riniker, W. Rittel and H. Zuber, *Helv. Chim. Acta.*, 1963, **46**, 1975.

50. R. W. Hanson. and N. H. Ryden, *J. Chem. Soc.*,1964, 837.

51. T. Baba, H. Sugiyama and S. Seto, *Chem. Pharm. Bull.*,1973, **21**, 207.

52. E. Sondheimer and R. W. Holley, *J. Am. Chem. Soc.*, 1954, **76**, 2467.

53. E. E. Haley and B. J. Corcoran, *Biochemistry*, 1967, **6**, 2668.

54. R. Schwyzer and P. Sieber, *Helv. Chim. Acta*, 1966, **49**, 134.

55. B. Riniker, P. Sieber and W. Rittel, *Nature New Biol.*, 1972, **235**, 114.

56. E. Nicolas, E. Pedroso and E. Giralt, *Tetrahedron Lett.*, 1989, **30**, 497.

57. J. E. Baldwin, M. G. Moloney and M. North, *Tetrahedron*, 1989, **45**, 6319.

58. Y. Kiso, H. Itoh, S. Tanaka, T. Kimuraa and K. Akaji, *Tetrahedron Lett.*, 1993, **34**, 7599.

59. S. S. Wang, C. C. Yang, I. D. Kinlesha, M. Sonenberg and R. B. Merrifield, *Int. J. Pept. Prot. Res.*, 1973, **6**, 103.

60. J. P. Tam, T. Wong, M. W. Riemen, F. Tjoeng and R. B. Merrifield, *Tetrahedron Lett.*, 1979, 4033.

61. C. C. Yang and R. B. Merrifield, *J. Org. Chem.*, 1976, **41**, 1032.

62. M. Bodansky and J. Martinez, *J. Org. Chem.*, 1978, **43**, 3071.

63. Y. Okada, S. Iguci and K. Kawasaki. *J. Chem. Soc. Chem. Comm.*, 1987, 1533.

64. Y. Okada and S. Iguci, *J. Chem. Soc. Perkin Trans.1*, 1988, 2129.

65. W. Gruszecki, M. Gruszecki and H. Bradaczek, *Proceeding of the Twenty-first European Peptide Symposium 1990*, (E. Giralt and D. Andreu Ed.), 1991 p27, ESCOM Science publishers B. V.

66. G. Barany and R. B. Merrifield, *J. Am. Chem. Soc.*, 1977, **99**, 7363.

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

68. P. Longstaff, Ph. D. Thesis, University of Edinburgh, 1989.

69.E. Wunsch, E. Jaeger, L. Kisfauldy and M. Low, *Ang. Chem. Int. Ed. Eng.*, 1977, **16**, 317.

70.B. W. Erickson and R. B. Merrifield, *J. Am. Chem.Soc.*, 1973, **95**, 3750.

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

72. S. F. Brady, R. Hirshmann and D. F. Veber, *J. Org. Chem.*, 1977, **42**,143.

73. G. Lajoie, A. Crivici and J. G. Adamson, *Synthesis*, 1990, 571.

74. L. Benoiton, *Can. J. Chem.*, 1962, **40**, 570.

75. G. K. Toth and B. Penke, *Synthesis*, 1992, 361.

76. W. D. John and G. T. Young, *J. Chem. Soc.*, 1954, 2870.

77. E. Wunsch and A. Zwick, *Z. Physiol. Chem.*, 1963, **333**, 108.

78. K. Burger, M. Rudolph and H. Neuhauser, *Liebigs Ann. Chem.*, 1991, 1365.

79. M. Itoh, *Chem. Pharm. Bull.*,1969, **17**, 1679.

80. G. H. L. Nefkens and B. Zwanenburg, *Tetrahedron*, 1983, **39**, 2995.

81. W. A. R. Van Heeswijk, M. J. D. Eenink and T. Feijen, *Synthesis*, 1982, 744.

82. G. H. L. Nefkens and R. J. F. Nirvard, *Recl. Trav. Chim. Pays-Bas*,1965, **84**, 1315.

83. W. Treibs and H. Klinkhammer, *Chem. Ber.*,1951, **84**, 671.

84. J. Pless, *Helv. Chim. Acta*, 1976, **59**, 499.

85. D. Maclean, Ph. D. Thesis, University of Edinburgh, 1991.

86. M. K. Dhaon, R. K. Olsen, K. Ramasamy, *J. Org. Chem.*,1982, **47**,1962.

87. I. J. Galpin, P. M. Hardy, G. W Kenner, J. R. McDermott, R. Ramage, J. H. Seely and R. G. Tyson, *Tetrahedron*, 1979, **35**, 2577.

88. P. Jencks and M. Gilchrist, *J. Am. Chem. Soc.*, 1968, **90**, 2622.

89. R. A. Friedel and M. Orchin, *Ultraviolet Spectra of Aromatic Compounds*, J. Wiley and Sons, Inc. New York 1951, p 388.

90. F. H. C. Stewart, *Aust. J. Chem.*,1965, **18**, 1699.

91. N. Kornblum and A. Scott, *J. Am. Chem. Soc.*, 1974, **96**, 590.

92. R. E. Shute and D. H. Rich, *Synthesis*, 1987, 346.

93. H. -D. Becker, L. Hansen and K. Andersson, *J. Org. Chem.*, 1986, **51**, 2956.

94. P. Jouin, B. Castro, C. Zaggef, A. Pantaloni, J. P. Senet, S. Lecolier and G. Sennyey, *TetrahedronLett.*, 1987, **28**, 1661.

95. H. Masada and Y. Murotoni, *Bull Chem. Soc. Jpn.*, 1980, **53**, 1181.

96. H. P. Wessel, T Luersen and D. R. Bundle, *J. Chem. Soc. Perkin Trans. 1*, 1985, 2247.

97. J. Trotter, *Can. J. Chem.*, 1959, **37**, 351.

98. D. S. Noyce and M. A. Metesiel, *Org. Prep. Proced. Int.*, 1982, **14**, 249.

99. W. B. Austin, N. Bilow, W. J. Kelleghan and K. S. Y. Lau, *J. Org. Chem.*, 1981, **46**, 2280.

100. R. H. DeWolfe, *Synthesis*, 1974, 153.

101. M. A. Blaskovich and G. Lajoie, *J. Am. Chem. Soc.*, 1993, **115**, 5021; M. A. Blaskovich and G. Lajoie, *Tetrahedron Lett.*, 1993, **34**, 3837.

102. D.B. Pattison, *J. Am. Chem. Soc.*, 1957, **79**, 3455.

103. E. J. Corey and N. Raju, *Tetrahedron Lett.*, 1983, **24**, 5571.

104. O. Bouab, G. Lamaty and C. Moreau, *J. Chem. Soc. Chem. Comm.*, 1978, 678.

105. R. H. DeWolfe and J. L. Jensen, *J. Am. Chem. Soc.*, 1963, **85**,3264.

106. R. A. Boissonas, St. Guttman, P. -A. Jaquenoud and J. P. Waller, *Helv. Chim. Acta.*, 1955, **38**, 1491.

107. P. Roach, Ph. D. Thesis, University of Edinburgh, 1990.

108. G. H. L. Nefkens and R. J. F. Nirvard, *Recl. Trav. Chim. Pays-Bas*,1964, **83**, 199.

109. K. Hofmann, W. Haas, M. J. Smithers and G. Zanetti, *J. Am. Chem. Soc.*, 1965, **87**, 631.

110. V. Mychajlyszyn and M. Protiva, *Collect. Czech. Chem Commun.*, 1959, **24**, 3955.

111. W. W. Hartman and E. J. Rahrs, *Org. Synth.*, 1944, **24**, 79.

112. W. T. Hunter, J. S. Buck, F. W. Gubitz and C. H. Bolen, *J. Org. Chem.*, 1956, **21**, 1512.

113. F. H. C. Stewart, *Aust. J. Chem.*,1960, **13**, 478.

114. G. H. Daub and W. C. Doyle, *J. Am. Chem. Soc.*, 1952, **74**, 4449.

Courses attended

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Medicinal chemistry (Prof R. Baker MS&D)

Discovery and pharmacology of Zoladex (ICI Pharmaceuticals)

Advances in the synthesis and activity of agrochemicals (Schering Agrochemicals)

Topics in organic chemistry (various speakers, University of Edinburgh)

Symposium on protein engineering (Royal Society of Edinburgh)

NMR spectroscopy (Dr I. H. Sadler and Dr J. Parkinson, University of Edinburgh)