Progress Towards the Solid Phase Generation of New Peptidomimetic-Based Libraries *via* 5(4*H*)-Oxazolone Intermediates

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A thesis submitted for the degree of Doctor of Philosophy **December 1999**

For Mum and Dad

Acknowledgments

My most sincere thanks go to Prof. Nick Turner for his support, encouragement and guidance throughout the course of this research. I would also like to extend my thanks to all the academic and technical staff for their advice and assistance. In particular John Millar and Wesley Kerr for permitting extensive use of the NMR for running gels, Donald Robertson and Patrick Hencher for the use of their IR spectrometer, Tim, Raymond, Derek and Kenny at the stores, Adam Staines for help with modelling studies and Sally Shirran for assistance with LC-MS analysis.

I would like to thank Dr Thijs Stock for his advice at the outset and I am indebted to Dr Ian Archer, Dr James Dowden and Dr Francois Alexandre for proof reading this thesis at such short-notice. Furthermore, I am very grateful to the ECPT for funding this research.

Finally, many thanks to the members of the Turner-Flitsch group for all the laughs, Michael for his love and support and especially my parents, for always being there.

Declaration

This thesis is submitted in part fulfilment of the requirement for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work 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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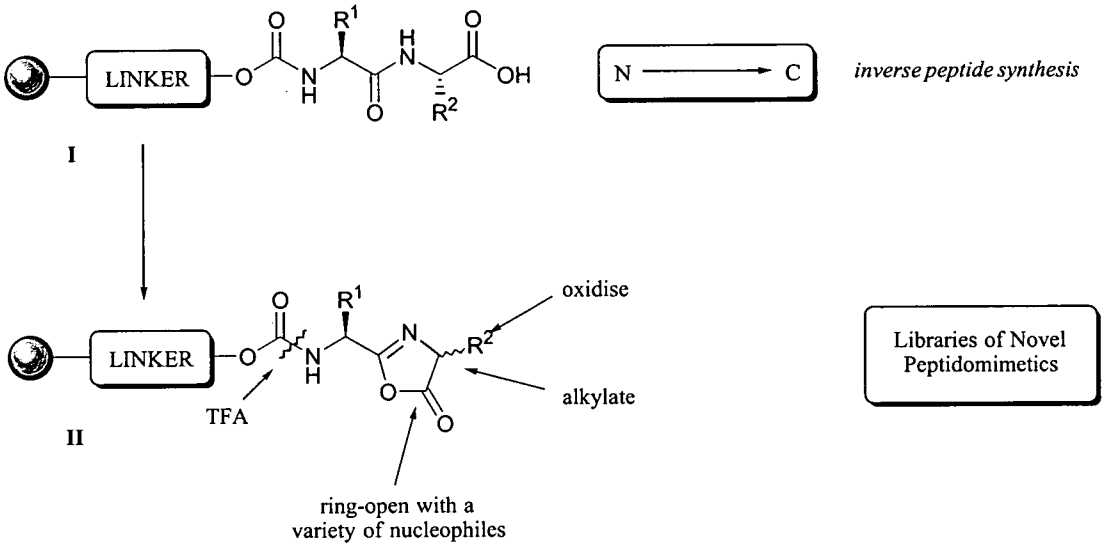
December 1999

Abstract

In recent years, much effort has been directed towards the development of new strategies for the efficient synthesis of libraries of peptidomimetics as potential therapeutic leads. The solid phase inverse synthesis of peptides (N→C) has only received a fraction of the attention given to the classical (C→N) solid phase assembly of peptides due to oxazolone-induced epimerisation. However, rather than trying to prevent its formation we have demonstrated how the peptide-derived 5(4*H*)-oxazolone can be used effectively as an ideal precursor to a range of peptidomimetics.



Scheme 1 *General strategy for solid phase peptidomimetic synthesis* via *5(4*H*)-oxazolones*

The development of a suitable strategy for the solid phase synthesis of inverse peptides is described and investigated as a valuable route into C-terminally modified peptides. This methodology is then applied to the construction of the polymer-supported 5(4*H*)-oxazolone **II** and various transformations thereof have been examined, often with solution phase comparisons.

Abbreviations

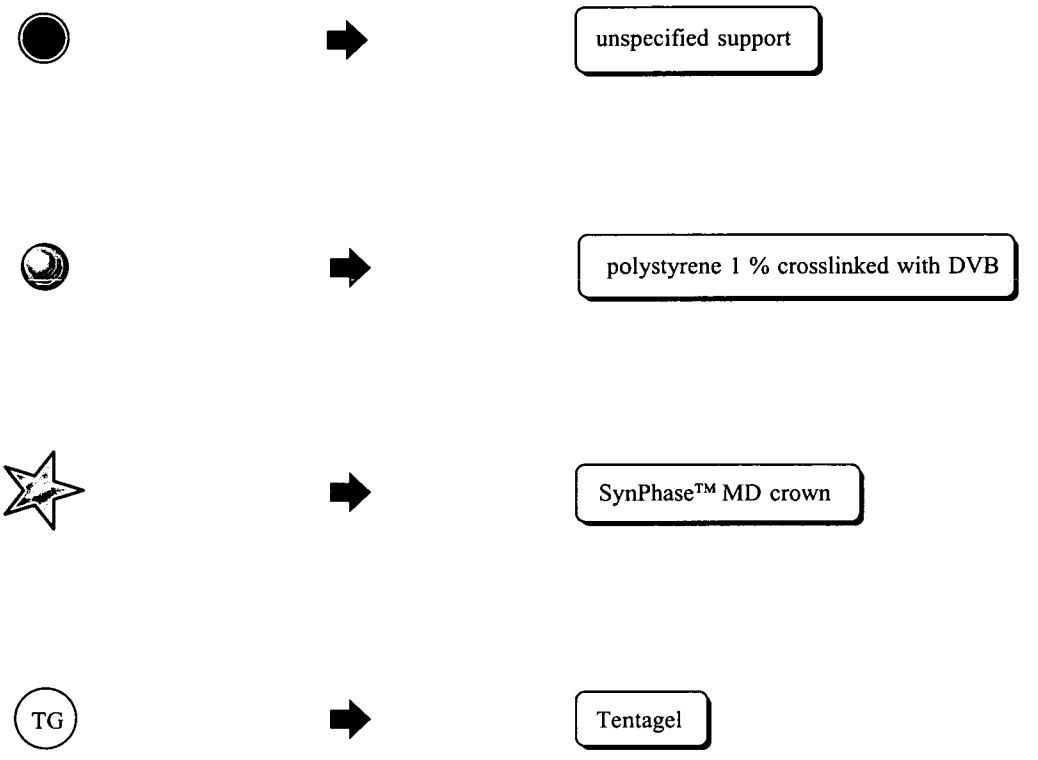
AA	amino acid residue
Abz	2-aminobenzoic acid
Acp	*cis*-2-aminocyclopentane carboxylic acid
Aib	aminoisobutyric acid
Ala	L-alanine
Aloc	allyloxycarbonyl
aq	aqueous
Ar	aryl
Arg	L-arginine
AW	reaction carried out by Andrew Wright
β-Ala	β-alanine
BEMP	2-*t*-butylimino-2-diethylamino-1,3-dimethylperhydro-1,3,2-diazaphosphorine
Boc	*N*-*tert*-butoxycarbonyl
BOP	benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate
bp	boiling point
br	broad
BSA	*N*,*O*-bis(trimethylsilyl)acetamide
BSTFA	bis(trimethylsilyl)trifluoroacetamide
Bz	benzyl
c	multiplet centre
Cbz	carboxybenzyl
$CDCl_3$	deuterochloroform
$CHCl_3$	chloroform
CT	Chiron Technologies
d	doublet
DCC	1,3-dicyclohexylcarbodiimide
DCM	dichloromethane
DCU	dicyclohexylurea
DDQ	2,3-dichloro-5,6-dicyano-1,4-benzoquinone
Ddz	α,α-dimethyl-3,5-dimethoxy-benzyloxycarbonyl
d.e.	diastereomeric excess
DIC	1,3-diisopropylcarbodiimide
DIPEA	*N*,*N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DME	dimethoxyethane (ethyleneglycol dimethyl ether)
DMF	*N*,*N*-dimethylformamide
DMSO	dimethylsulfoxide
DVB	divinylbenzene
ea	each
EI	electron impact
EDCI	1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride
ESMS	electrosray mass spectrometry
ether	diethyl ether

Et_3N	triethylamine
EtOAc	ethyl acetate
EtOH	ethanol
eq	equivalent
FAB	fast atom bombardment
Fmoc	9-fluorenylmethoxycarbonyl
Gly	glycine
HATU	*N*-[(dimethylamino)-1*H*-1,2,3-triazol[4,5-b]pyridin-1-ylmethylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide
HCl	hydrochloric acid
HMPA	4-(hydroxymethyl)-phenoxyacetyl aminomethyl (linker)
H_2O	water
HOAt	1-hydroxy-7-azabenzotriazole
HOBt	1-hydroxybenzotriazole hydrate
HPLC	high performance liquid chromatography
h	hour(s)
i	ill-defined due to second order effects
Iva	isovaline
IR	infra red
KBr	potassium bromide
KHMDS	potassium bis(trimethylsilyl) amide
KOBt	hydroxybenzotriazole potassium salt
LC-MS	liquid chromatography-mass spectrometry
Leu	L-leucine
lit.	literature value
Lys	lysine
m	multiplet
m	*meta*
MD	methacrylic acid/dimethylacrylamide graft polymer
Me	methyl
MeCN	acetonitrile
MeOH	methanol
min	minute(s)
mp	melting point
MS	mass spectroscopy
NaCl	sodium chloride
NaHMDS	sodium bis(trimethylsilyl) amide
Na_2SO_4	sodium sulfate (anhydrous)
NMM	*N*-methyl morpholine
NMP	1-methyl-2-pyrrolidinone
NMR	nuclear magnetic resonance
Nova	Novabiochem
o	*ortho*
OFm	9-fluorenemethyl
op	displayed on opposite page
p	*para*
Ph	phenyl
Phe	L-phenylalanine

PL	Polymer Laboratories
ppm	parts per million
PS	polystyrene
PyAOP	7-azabenzotriazol-1-yl-*N*-oxytris-pyrrolidino-phosphonium hexafluorophosphate
PyBroP	bromo-tris-pyrrolidino-phosphonium hexafluorophosphate
q	quartet
R_f	retention factor
R_t	retention time
RT	room temperature
Ser	L-serine
t	triplet
TBTU	2-(1*H*-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TFA	trifluoroacetic acid
THF	tetrahydrofuran
tlc	thin-layer chromatography
TNBS	2,4,6-trinitrobenzene sulfonic acid
Tr	trityl
TSA	*p*-toluene sulfonic acid
Tyr	L-tyrosine
UV	ultra violet
Val	L-valine
Wang	4-(hydroxymethyl)-phenoxymethyl (linker)

N.B. amino acids are assumed to be L-configuration unless otherwise stated

Key



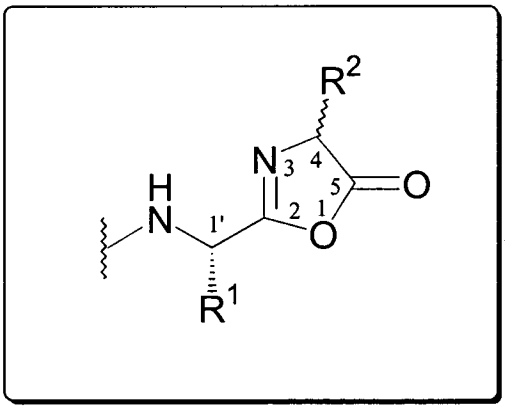


Figure 1 *Numbering system for dipeptide-derived 5(4*H*)-oxazolones*

Contents

1. Introduction

1.1 Solid Phase Chemistry

1.1.1 General Introduction

In 1963, Bruce Merrifield laid the foundations for what was to emerge as the solid phase and combinatorial chemistry revolution, by introducing a method for assembling peptides where the C-terminal amino acid residue was covalently bound to a polymer support.[1] This innovative technology made a dramatic impact on peptide chemistry, facilitating the stepwise construction of long chain polypeptides otherwise unattainable due to solubility and purification problems. Since then, solid phase synthesis has achieved wider recognition in areas such as oligonucleotide chemistry[2] and has more recently been applied to the synthesis of small molecule libraries.[3]

The main features which have contributed to the popularity of solid phase chemistry are well known. For instance, since the solid support is ultimately filtered, excess reagents can be used to drive the reaction to completion. Resin-bound intermediates are often more stable and easier to handle than their solution-phase counterparts and tedious purification protocols become obsolete, with product isolation achieved by simply washing away excess reagents and by-products. Finally, and perhaps most significantly, in terms of its application to combinatorial chemistry and library synthesis, the process is readily amenable to automation.

As with any technology, there are limitations. The resin beads themselves are often fragile and care must be taken with regard to agitation and thermal stability. Time and effort is required to develop the synthetic strategy, as solution phase chemistry does not always translate directly to solid phase, especially bearing in mind that the choice of linker and support will dictate the range of solvents and reagents tolerated. Extra steps are required for initial attachment and final cleavage of the compound

from the support. In addition, to ensure reactions reach completion, reaction times are often long and since excess reagents are employed, cost and toxicity should also be considered. Perhaps the most frustrating aspect of working on the solid phase, however, is the lack of effective procedures for on-resin analysis. There have in fact been numerous advances in this area over the last decade,[4-6] leading to a rapidly growing array of available techniques, but there are still many shortcomings and these will be exemplified throughout the course of this thesis.

1.1.2 Resins and Linkers

In solid phase synthesis the starting material requiring elaboration is anchored to the polymer support (resin) *via* a linker (Figure 2). These components are crucial to the success of the synthesis and must be chosen carefully, with respect to the synthetic conditions, when developing a solid phase strategy.[7]

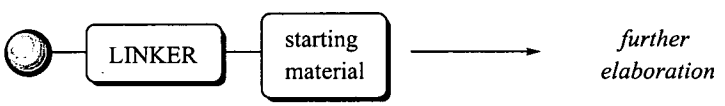


Figure 2 *General system for solid phase synthesis*

1.1.2.1 Resin

A great deal of research has been directed towards adapting existing chemical transformations for use in solid phase strategies,[8-12] however, it is important not only to consider the manipulations carried out on supported compounds, but also the nature of the support itself.[13] Fundamental issues, such as the accessibility of the functional sites to the solution phase reagents and the functionality required to attach the linker, together with mechanical stability and chemical compatibility, should be given particular attention.

The two polymer supports which feature primarily in the literature are polystyrene-divinylbenzene copolymer and those that combine polyethylene glycol with 1-2 % cross-linked polystyrene, although the desire to perform reactions under increasingly diverse conditions has led to the continuing development of alternative support

materials.[14,15] Polystyrene resins (Figure 3) are the least expensive supports available, as well as being relatively stable with generally high loading capacities. However, they are also poorly solvated in protic solvents and swelling is fundamental for reagent accessibility, with more than 99 % of the functional groups lying within the bead.[16] These limitations have provided the impetus for the development of alternative supports for use in, for example, aqueous systems.

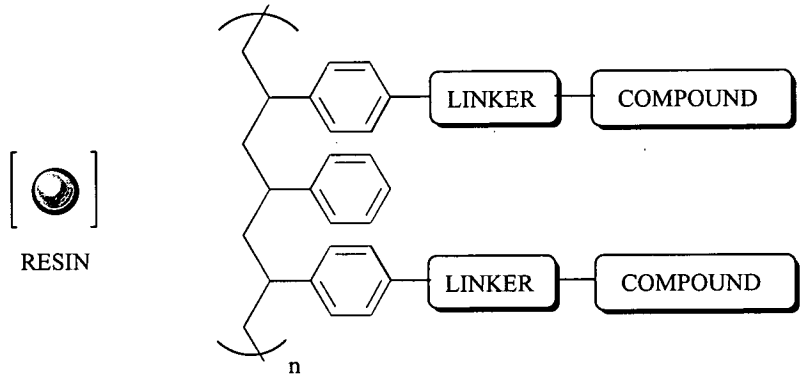


Figure 3 *Polystyrene as a solid support*

The research detailed in this thesis was carried out predominantly with functionalised polystyrene resin, however, SynPhase™ MD crowns (Figure 4) were also investigated.

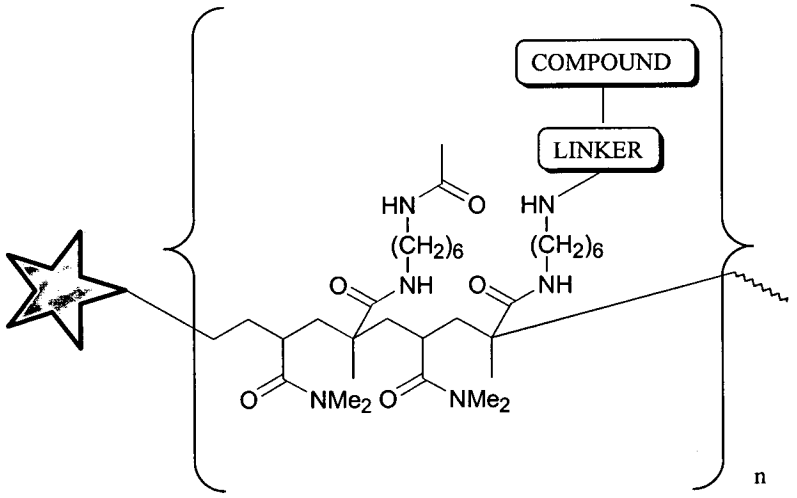


Figure 4 *Funtionalised SynPhase ™MD crown*

These crowns are principally surface supports comprising a derivatised graft of methacrylic acid-dimethylacrylamide copolymer on a polyethylene crown. This alternative support technology overcomes some of the limitations of conventional polymer resins as there is no requirement for swelling, individual weighing, excessive washing and filtration. Problems of impurities due to resin degradation[17] are also avoided.

1.1.2.2 Linkers

The linker exists as a cleavable handle with the purpose of tethering the starting compound to the support for synthetic manipulation, before finally releasing the desired product after completion of the synthesis. The choice of linker is critical to the solid phase process and there are many criteria which must be fulfilled. Essential prerequisites include stability to all the reagents and conditions employed during the synthesis, while maintaining the capacity for selective cleavage to deliver the intact target with correct terminal functionality. Attachment to the polymer and starting material should be facile and high yielding, with the linker molecule itself readily available at low cost. Some other desirable features include those which may facilitate reaction monitoring, asymmetric induction, sequential or partial release and the potential for reuse.

The first linkers were based on the benzyl ether/ester protecting group, the acid lability of which could be increased to the desired level by introducing *ortho* and *para* methoxy substituents on the aromatic ring.[18] In the same way, many of the current linkers are modified versions of solution phase protecting groups and are themselves considered bifunctional protecting groups which are often, but not necessarily, orthogonal to those employed to protect the resin-bound growing compound.

The linker imposes many of its own limitations to the synthetic strategy in terms of stability, cleavage and functionality thereafter. As a consequence, there has been a great interest in developing increasingly sophisticated linkers to overcome these limitations, presenting the solid phase chemist with an extensive arsenal to choose from.[19,20]

1.1.3 Combinatorial Chemistry

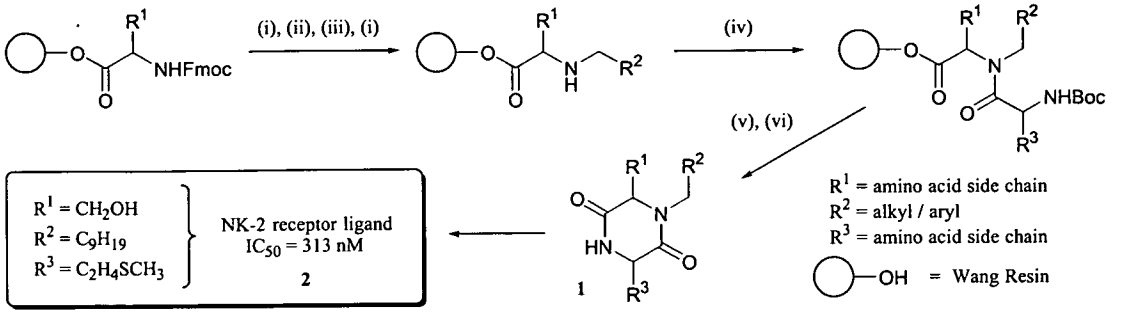
1.1.3.1 Introduction

Solid phase synthesis, when combined with combinatorial chemistry, has found one of its main applications in the field of drug discovery. With the advent of new high throughput screening technologies and advances in molecular biology and genome sequencing leading to the identification of more and more protein targets, the demand for diverse collections of compounds to screen against these targets has soared. The realisation emerged that combinatorial chemistry, defined by Gallop[21] as the "systematic and repetitive covalent connection of a set of different 'building blocks' of varying structures to each other to yield a large array of diverse molecular entities," together with solid phase synthesis, could hold the key for accelerated drug discovery. Thus united, solid phase synthesis and combinatorial chemistry have emerged as a very powerful tool for the identification of biologically active compounds with therapeutic value.[22-24]

1.1.3.2 Solid phase combinatorial chemistry as a tool for drug discovery

The significant impact that solid phase combinatorial chemistry has made on drug discovery to date, has not yet been fully evident due to the unavoidable time lag resulting from extensive clinical and toxicology trials of drug candidates. In addition, successful strategies and their subsequent screening results, tend to be withheld from the public domain until intellectual property has been secured. Consequently, it is only in recent years that industry has begun to disclose details of successful drug candidates, finally establishing combinatorial chemistry as a valuable tool for drug research.[25]

For example, Pfizer developed a solid phase strategy for the synthesis of diketopiperazines (Scheme 2).[26] They used a split and mix combinatorial methodology to generate a library of 1000 substituted analogues of type **1**. Screening and deconvolution of this library lead to the identification of a number of biologically active components which displayed an affinity for the neurokinin-2 receptor, *e.g.* **2**.



Scheme 2 *Pfizer diketopiperazine combinatorial library* - reagents and conditions: (i) mix and split (ii) piperidine, DMF (iii) R^2CHO, $NaBH(OAc)_3$, DCM, sonicate (iv) $BocHNCHR^3CO_2H$, PyBrOP, DIPEA, DCM (v) TFA (vi) toluene, Δ

1.1.3.3 Library generation

Library generation effectively falls into two categories: random and biased. Random libraries are generally used for lead identification by generating molecular diversity through the incorporation of a diverse range of functional groups to create novel compounds with no particular basis. Biased libraries, are mechanism or structure-based and may be employed for rational drug discovery, or lead optimisation, where rapid access to SAR (structure-activity relationship) information is required. Apart from the quantity of information and speed at which it can be acquired, the very nature of combinatorial chemistry makes one of its major benefits the relative ease of re-synthesising any compounds which are identified as hits. This is in direct contrast to natural products leads, many of which are highly complex structures incorporating a host of stereogenic centres and often only accessible through tedious multi-step syntheses. Preparing diverse analogues of these leads for SAR studies becomes, therefore, both costly and time-consuming.

The growing number of classical reactions which have been translated from solution to solid phase[8-12] and the range of automation available,[7] have together helped to establish solid phase combinatorial chemistry as a key tool in the drug discovery effort.[3,23]

1.1.3.4 Peptides

Much of the original combinatorial chemistry practised by the pharmaceutical industry originated from research based on the solid phase synthesis of peptides.[21,27] This is hardly surprising considering the pharmacological importance of peptides, the large and accessible variety of structurally diverse amino acid building blocks, together with the well-established coupling techniques available.

Peptides themselves have limited utility as bioavailable therapeutic agents due to their undesirable pharmacokinetics.[28] For instance, they generally display poor oral activity, possibly due to a lack of specific transport systems and high molecular weight. They are very susceptible to proteolytic degradation in the gastro-intestinal tract and serum and are rapidly excreted by the liver and kidneys.

All these factors render peptides unlikely candidates for pharmaceutical development. Instead, recent focus has centred on both non-oligomeric, small molecule compound libraries,[3,23] which will not be discussed any further here, and peptidomimetic research.

1.2 Peptidomimetics

1.2.1 Peptidomimetics in Drug Discovery

1.2.1.1 Background

Peptidomimetic research involves the discovery of peptide analogues that maintain the ability to bind to peptide receptors but which have greater bioavailability, biostability and possibly greater selectivity than endogenous or synthetic peptide ligands.

The distinct feature of peptides which is so advantageous is their ability to present an effectively infinite number of pharmacological profiles through the diverse range of acidic, basic, hydrophobic/hydrophilic, and aromatic side chains they can display.

Peptidomimetics (Figure 5) can be described as modified peptides which mimic the 3D array of side-chains of the key amino acids in the parent peptide *i.e.* peptides incorporating unnatural or pseudo amino acids and which can imitate or block the biological effect of their peptide prototypes at the receptor level. Potential advantages of such mimics over their peptide counterparts include an improved resistance to proteolysis, as well as increased lipophilicity and thus enhanced bioavailability. However, an ideal peptidomimetic must also display high receptor affinity and selectivity if it is to satisfy the required pharmacological criteria.

Furthermore, with the development of sophisticated crystallography and NMR techniques, peptidomimetic ligands of receptors or enzymes may be rationally designed to inhibit or enhance a specific activity, depending on the therapeutic requirement.

1.2.1.2 Categories and preparation

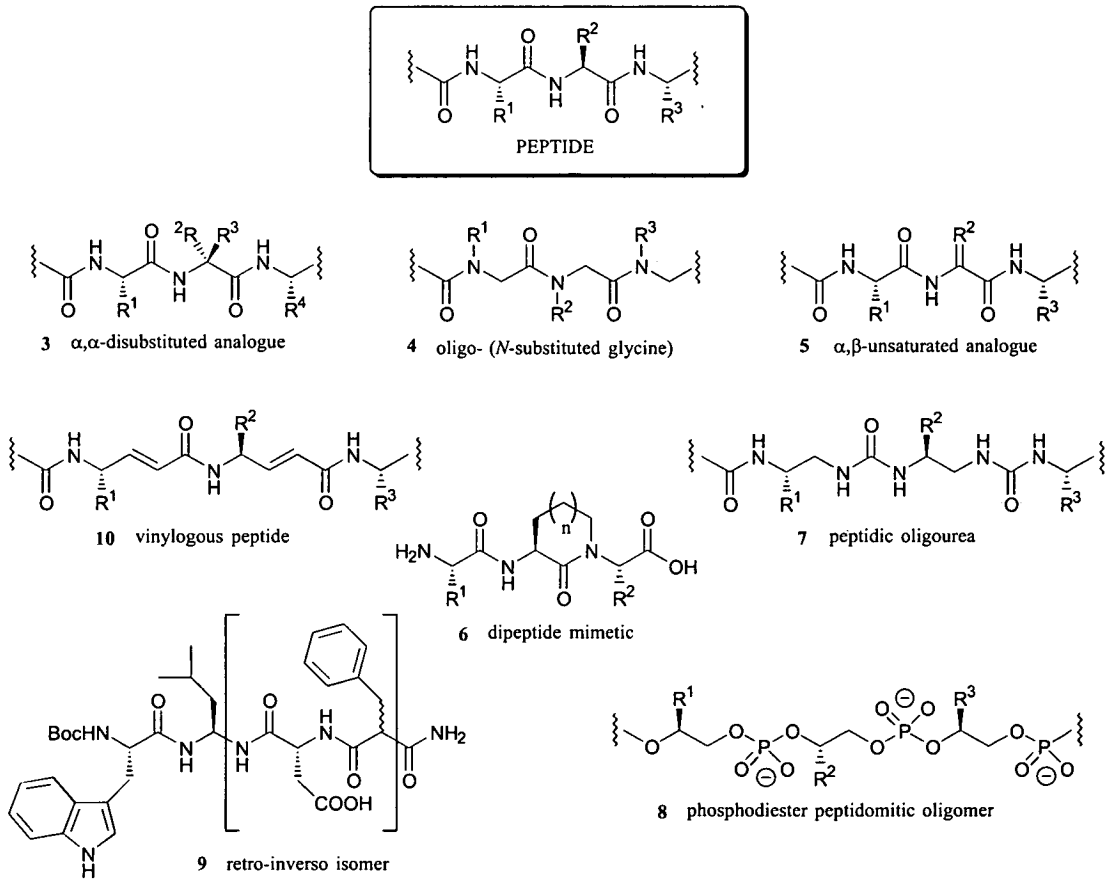


Figure 5 *Some examples of peptidomimetic structures*

Many methods of manipulating peptide structures so as to maintain their activity while improving their pharmacological profile, have been explored.[3,22,29,30] Some popular modifications include using amino acid analogues *e.g.* α,α-dialkylated (**3**) (**1.2.2**), N-alkylated[31] (**4**), α,β unsaturated (**5**) (**1.2.3**) as well as β- and D-amino acids.

Conformational restrictions can be incorporated into peptide structures to form peptidomimetics. Since these conformationally restricted derivatives[32] *e.g.* cyclic or bridging dipeptide analogues of the type **6**, are no longer recognised by peptidases they display improved metabolic stability.

Backbone modifications are also used as a method of achieving protease resistance and increasing *in vitro* half-life. There are many examples including the peptidic oligourea (**7**) which was the basis of a 160 member pilot library by Burgess,[33] the oligomeric mimetic with a phosphodiester backbone[34] (**8**), retro-inversomers[35,36] where D-amino acids are incorporated and the direction of the peptide chain reversed (**9**), as well as vinylogous peptides[37] (**10**) and isosteric replacements of the amide bond.

1.2.1.3 Peptidomimetics as therapeutic agents

Over the last thirty-five years or so, numerous biologically active peptides *e.g.* somatostatin, substance P, angiotensin II, enkephalin and cholecystokinin, have been discovered and their structures determined. These neurotransmitters, neuromodulators and hormones not only play a vital role in terms of their biological function but also serve as valuable templates for the development of new selective drugs with enhanced pharmacokinetics.[29] Veber describes how some of the peptidomimetic methodologies, described above, have been employed for generating modified versions of a number of native neuropeptides.[28]

In addition, there are many examples of successful peptidomimetic drug candidates, from both solution and solid phase syntheses, now appearing in the literature.[38-41]

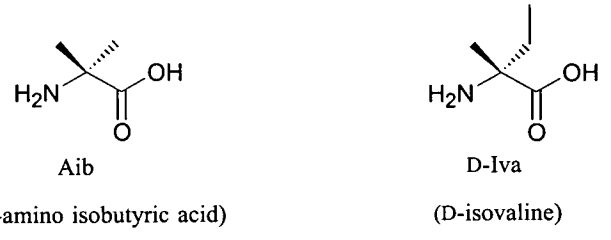
1.2.1.4 Closing remarks

Three of the methods for modifying peptides, described above (***1.2.1.2***), have been examined in more detail during the course of this research and discussed in Chapter 4. Brief introductions to these selected classes of peptidomimetics follow, primarily with respect to solid phase development.

1.2.2 Peptides Incorporating α,α-Dialkylated Amino Acids

1.2.2.1 Background

An example of a family of naturally occurring peptides in this category are the Peptaibols.[42] These amphipathic microbial peptide antibiotics contain an unusually high proportion of α,α-dialkylated amino acids, including Aib and D-Iva (Figure 6) as well as having a C-terminal amino alcohol and an acylated N-terminus.



Aib (α-amino isobutyric acid)

D-Iva (D-isovaline)

Figure 6 *α-α-Dialkylated amino acids from the naturally occurring peptaibols*

Incorporation of these hindered amino acid residues into the frameworks of the Peptaibols is thought to contribute to their ability to form *trans*-membrane helical ion channels.[42] Non-proteinogenic α-alkylated amino acids have a remarkable influence on the conformation of the small peptides into which they are incorporated. For instance, research has shown that peptide analogues of this type, which possess an α-methyl substituent, appear to induce 3_{10} and α-helical secondary structures,[43] whereas in derivatives where the two substituents are longer chain alkyls, more extended secondary conformations are favoured.[44] Thus, the restricted conformational flexibility imposed by the second α–C substituent in such

peptidomimetics can be exploited as a means to investigate enzyme mechanisms and may also serve to alter the biological activity of the peptide analogue.

Dialkylation at the α-C is also expected to increase resistance to enzyme degradation and thus improve biological half-life. For example, Yokum *et al.* reported the synthesis of a series antimicrobial α,α-dialkylated amino acid rich peptides with *in vivo* anti-brucellae activity.[45]

1.2.2.2 Synthesis

Although many examples of synthetic routes towards α,α-disubstituted amino acids, both racemic and enantiopure, have been reported,[46-52] incorporation of these units into peptide molecules has proven more troublesome.[53-55]

Peptides containing α,α-dialkylated-α-amino acids may be prepared by introducing the suitably protected C^{α} disubstituted amino acid at the appropriate stage during classical peptide synthesis. However, steric hindrance at the dialkylated carbon centre, along with the poor nucleophilicity displayed by the hindered amino component, serve to impede this process. Forcing conditions, such as elevated temperatures and long reaction times may be employed, but this tends to result in epimerisation of the C-terminal residue.[55]

Heimgartner[47,56] sought to overcome the problems by using 2,2-disubstituted-3-amino-2*H*-azirines as highly activated amino acid equivalents in the role of amino coupling element. The so-called azirine/oxazolone method is depicted on the following page (Figure 7).

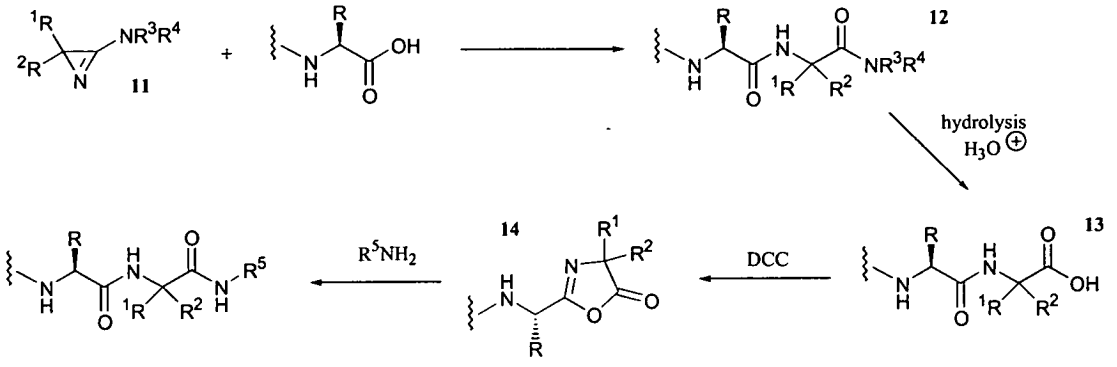
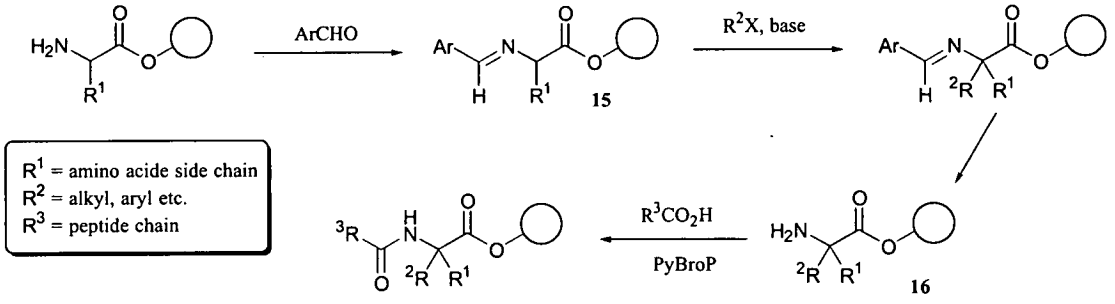


Figure 7 *Heimgartner's azirine/oxazolone method*

Coupling of the C-terminal peptide with the highly reactive 3-amino-2H-azirine (**11**) proceeded quantitatively at 0 - 25 °C. Very mild hydrolysis (3 M HCl, THF/H_2O, 25 °C), of the peptide amide **12** to the acid **13** could be achieved, thus avoiding oxazolone-induced epimerisation, provided *N*-methyl anilide (R_3 = Me, R_4 = Ph) was used. The ring strain of azirine derivative **11** provided sufficient activation to obviate the necessity for activation of the carboxy-terminus, which generally results in undesirable epimerisation (**1.3.1**). Activation to facilitate coupling of a second amino component was carried out *via* DCC-mediated cyclisation to the 5(4*H*) oxazolone (**14**). This activated peptide derivative (**1.3**) is amenable to aminolysis, yielding the desired dialkylated peptide derivative. Unfortunately, epimerisation of the activated oxazolone **14** was observed since long reaction times and elevated temperatures were required to facilitate coupling of the sterically hindered amino component. This problematic side reaction, which is discussed in greater detail in section **1.3.1**, however, may be suppressed by utilising a combination of additives such as HOBt and copper (II) chloride.[57,58] Further chain extension was then achieved by deprotection and coupling of another 2,2-disubstituted-3-amino-2*H*-azirine to finally yield the desired peptidomimetic. This method has since been extended to a number of other useful dialkylated peptide derivatives.[59,60]

The advantages of this strategy are evident, but its proficiency as an efficient solid phase route has yet to be investigated.

Most reported synthetic methods for the synthesis of α,α-dialkylated peptide analogues involve incorporation of individually modified amino acids during normal peptide synthesis. In contrast, O'Donnell and co-workers[61,62] have reported a procedure whereby the new side chains are built into the peptide backbone during synthesis, using a method for the formation of C-C bonds under mild conditions. They describe the use of Schiff base activated amino acids as a new procedure for the α-alkylation of terminal peptidic amino acid residues on solid phase.



Scheme 3 *O'Donnell's "di-UPS"*

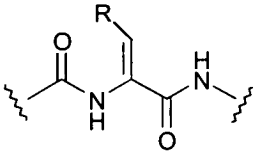
α-Alkylation of the resin-bound Schiff-base ester (**15**) was achieved using BEMP as a base with the desired electrophile (*e.g.* alkyl halide) at room temperature. Transimination with hydroxylamine furnished free amine **16**, followed by coupling or protection to extend or terminate the peptide chain. Final cleavage from the resin released the α,α-dialkylated peptide derivative in greater than 50 % yield. Although a number of **di**substituted peptide derivatives were prepared using this solid phase **u**nnatural **p**eptide **s**ynthesis procedure ("di-UPS"), a distinct drawback of this methodology is its lack of stereoselectivity, since it results in the formation of epimeric peptides.

1.2.3 Peptidomimetics Containing Dehydroamino Acid Residues

1.2.3.1 Background

Nature provides many examples of biologically active peptides containing dehydroamino acid residues (**17**).[63,64] Analogues of many peptides incorporating α,β-unsaturated residues, have been used to determine SARs through the modified

bioactivities they exhibit. Tentoxin (Figure 8), for instance, is a cyclic tetrapeptide which incorporates a Z-α,β-dehydro-*N*-methyl phenylalanine residue and displays phytotoxic properties. Produced by *Alternaria tenuis*, this biologically active peptide promotes chlorosis in the developing seedlings of a number of flowering plants and has thus attracted the attention of many research groups.[63] As a result, many synthetic routes to Tentoxin and its analogues have been reported,[65,66] addressing some of the problems associated with the synthesis of dehydropeptides (***1.2.3.2***).



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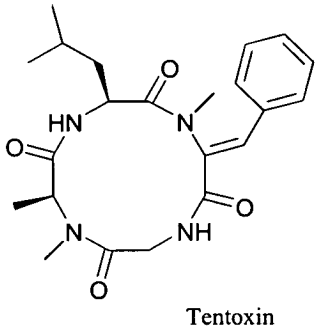


Tentoxin

Figure 8 *Peptide analogues incorporating dehydroamino acid residues*

Incorporation of an α,β-dehydroamino acid moiety into a peptide skeleton, has thus been shown to influence the reactivity and conformation of the resulting peptide analogue.[63]

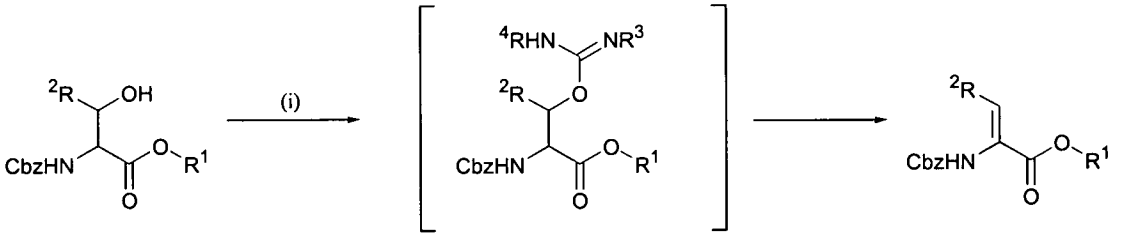
α,β-dehydroamino acids are also useful synthons in peptide chemistry, facilitating the preparation of α-keto acids, and various peptide amides. Furthermore, asymmetric hydrogenation of dehydropeptide analogues is an established method for the preparation of optically pure α-amino acids and peptides,[67] with examples of this now being reported on the solid phase.[68]

1.2.3.2 Synthesis

A wide array of approaches have been reported for the preparation of dehydroamino acids and peptides,[63,64,67] although very few have exploited solid phase synthesis as a means of accessing these analogues in a combinatorial fashion.[69,70]

The most popular route employed, which also mimics the biosynthetic process, is β-elimination, which can be performed on complete peptide amino acid residues, such

as serine and threonine, at the end of the synthesis. Since the presence of an α,β double bond in an amino acid residue leads to weak nucleophilicity of the enamine and poor reactivity of the carboxylic group, introducing the dehydro unit at the end of the synthesis circumvents the problems associated with coupling these unreactive dehydro derivatives. Many of the methods reported for the preparation of dehydroamino acids *via* β-elimination[63] are low yielding and involve the generation of unstable amino acid derivatives. Miller, however, was able to overcome these limitations by developing an isourea-mediated β-elimination process for the preparation of serine, cysteine and threonine derived dehydro amino acids (Scheme 4).[71]



Scheme 4 *Isourea-mediated β-elimination* - reagents and conditions: (i) $R^4N=C=NR^3$, CuCl

Stammer has also reported a very convenient means of accessing dehydroamino acids and peptides *via* 5(4*H*)-oxazolone intermediates.[72,73] This method, which involves the direct oxidation of the dipeptide-derived 5(4*H*)-oxazolone with DDQ, is discussed in greater detail along with its successful application to this research in section **4.2.1**.

1.2.4 C-Terminally Modified Peptides

1.2.4.1 Background

In solid phase peptide synthesis, the majority of linkers tether the growing peptide chain to the support *via* the C-terminal carbonyl as an ester or amide, releasing the free carboxyl group on cleavage. However, amongst the vast range of natural peptides and biologically active analogues, exist many examples of peptides with functionalised C-termini. For instance Neuropeptide Y (NPY) and FMRFamide both

contain C-terminal amides. NPY, one of the most abundant neurohormones in the mammalian central and peripheral nervous systems, is a 36-mer with a C-terminal tyrosinamide which has been confirmed as essential for its receptor affinity.[74] Likewise, the C-terminal amide of the molluscan regulatory tetrapeptide, FMRFamide, is an important structural requirement for its biological activity.[75] Furthermore, it has already been mentioned (***1.2.2.1***) that the naturally occurring peptide antibiotics, the Peptaibols, possess C-terminal amino alcohols. Some other functionalities which have been observed include alcohols, esters, ethers, aldehydes, *N*-alkylamides, *N,N*-dialkylamides, hydrazides, trifluoromethyl ketones, mercaptoalkylamides, thioamides and thioesters.[76] Head-to-tail cyclic peptides, possessing no carboxy-terminus as such, also fall into this category and their biological significance is also particularly evident.[77]

Introducing a different functional group at the C-terminus, may render a molecule less susceptible to carboxypeptidases. It may also improve the capacity for crossing various biological barriers and even enhance both solubility and substrate specificity. C-terminally modified peptides, therefore, constitute an important class of peptidomimetics which hold great potential for both mechanistic probes and pharmaceutical development. C-terminal peptide esters have also found application in peptide synthesis as substrates for enzyme-catalysed fragment coupling reactions.[78]

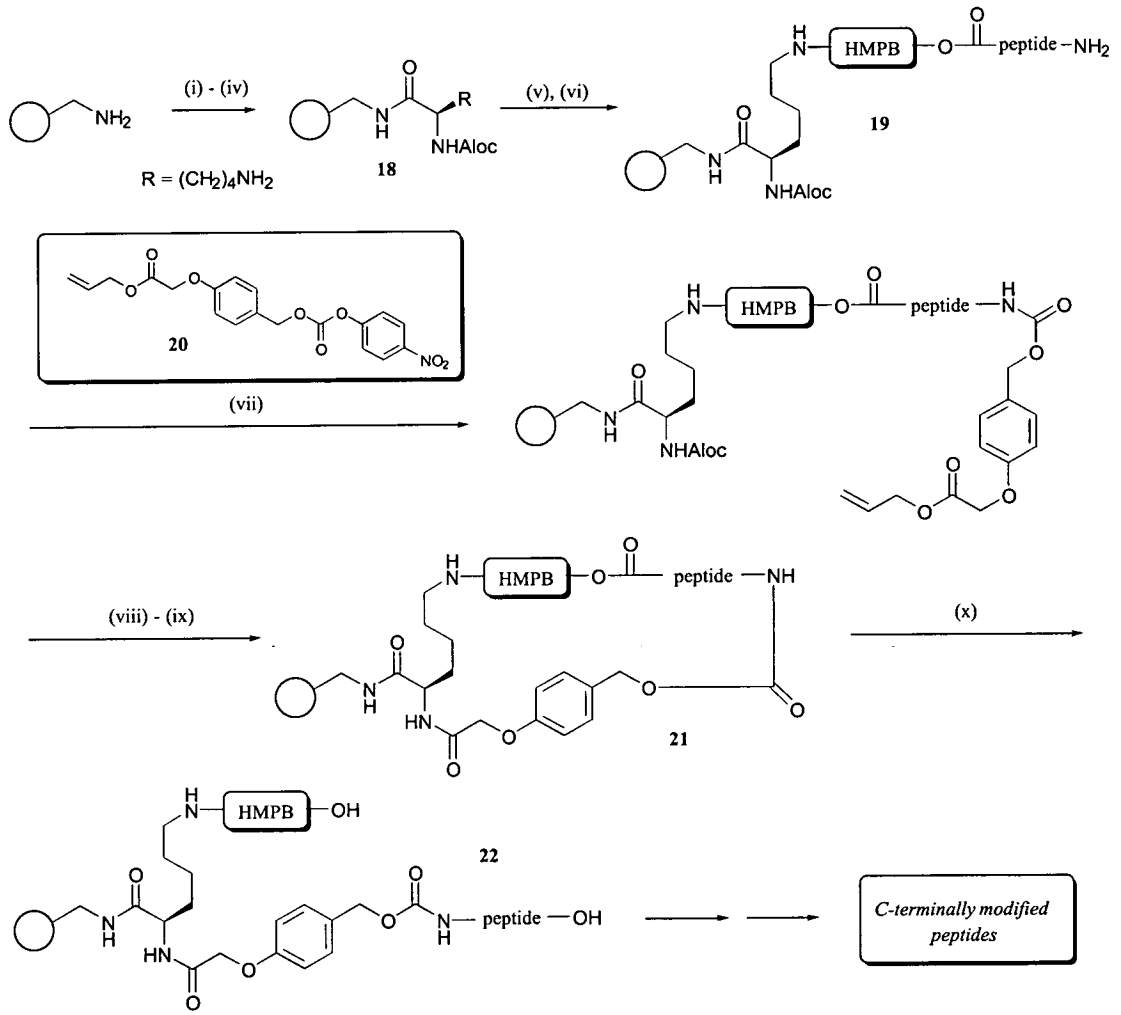
1.2.4.2 Synthesis

As a result of the growing interest in this field, many solid phase routes into C-terminally modified peptide analogues have been established. For example, a number of linkers have been developed to enable direct access to peptide aldehydes,[79] amides[80,81] and *p*-nitroanilides.[82] However, it would be more desirable to develop a system which would enable a large variety of functional groups to be incorporated at the C-terminus of the supported peptide, in a combinatorial manner. One obvious route would be to assemble peptides in the non-conventional direction (N-C), leaving the C-terminus exposed to be manipulated as required. Even though this method

risks oxazolone-induced epimerisation (**1.3**) it has been examined by a number of groups and will be discussed in more detail in Chapters 2 and 3.

Two other successful methods for introducing C-terminal modifications are Bradley's 'internal resin capture' approach[83] and Barany's 'backbone amide linker' (BAL) strategy,[84] both of which are described below.

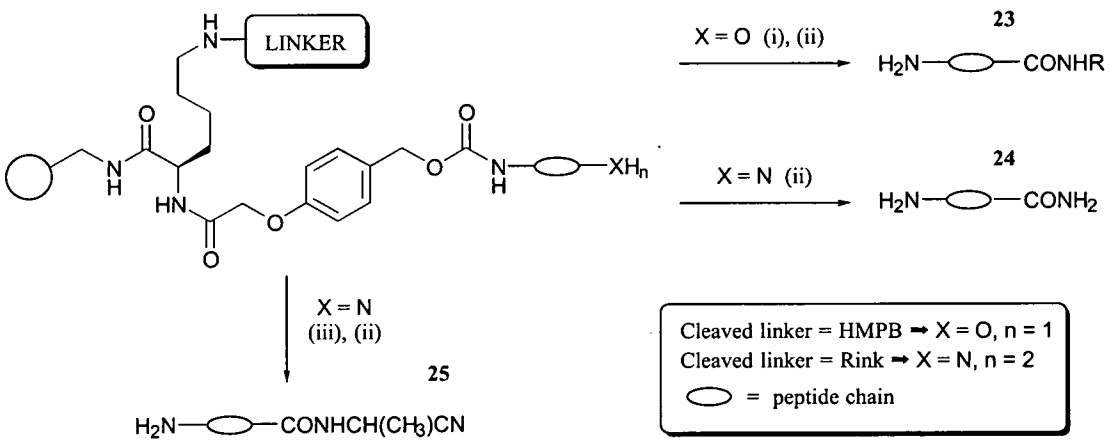
Bradley and co-workers devised an inversion/modification strategy applicable to a range of C-terminally modified peptides. The peptide chain was first assembled in the conventional (C-N) direction, thus exploiting all the benefits of this well-established methodology, but was subsequently inverted such that the peptide still remained anchored to the support (Scheme 5).



Scheme 5 Reagents and conditions: (i) Fmoc-Lys(Boc)-OH, DIC, HOBt (ii) 20 % piperidine, DMF (iii) diallylpyrocarbonate, Et_3N (iv) 25 % TFA, DCM (v) 4-(4-hydroxymethyl-3-methoxyphenoxy)-

butanoic acid (HMPB), DIC, HOBt, DCM (vi) Fmoc peptide chemistry (vii) **20**, pyridine, DMF (viii) $Pd(PPh_3)_4$, dimedone, DCM : THF (1:1) (degassed) (ix) PyBroP, DMAP, DIPEA, DCM (x) 1 % TFA, DCM

The basic strategy involved coupling 4-(4-hydroxymethyl-3-methoxyphenoxy)-butanoic acid to the ε-amino group of **18**, before assembling the desired peptide sequence using standard Fmoc peptide chemistry to give **19**. Carbonate **20** was then coupled and simultaneous deprotection of the allyl carbonate (Aloc) and allyl ester, followed by cyclisation, furnished the resin-bound cyclic peptide **21**. Treatment with 1 % TFA effected cleavage of the highly acid labile HMPB linker, exposing the C-terminus to yield the polymer supported inverted peptide **22**. The free carboxyl group was then activated, giving rise to slight oxazolone-mediated epimerisation (**1.3**), modified and cleaved using 95 % TFA to yield the desired C-terminally modified peptide (Scheme 6).



Scheme 6 Reagents and conditions: (i) RNH_2 (4 eq), PyBroP (2 eq), DIPEA (2 eq), DCM (ii) 95 % TFA, DCM (iii) cyanuric chloride, DMF

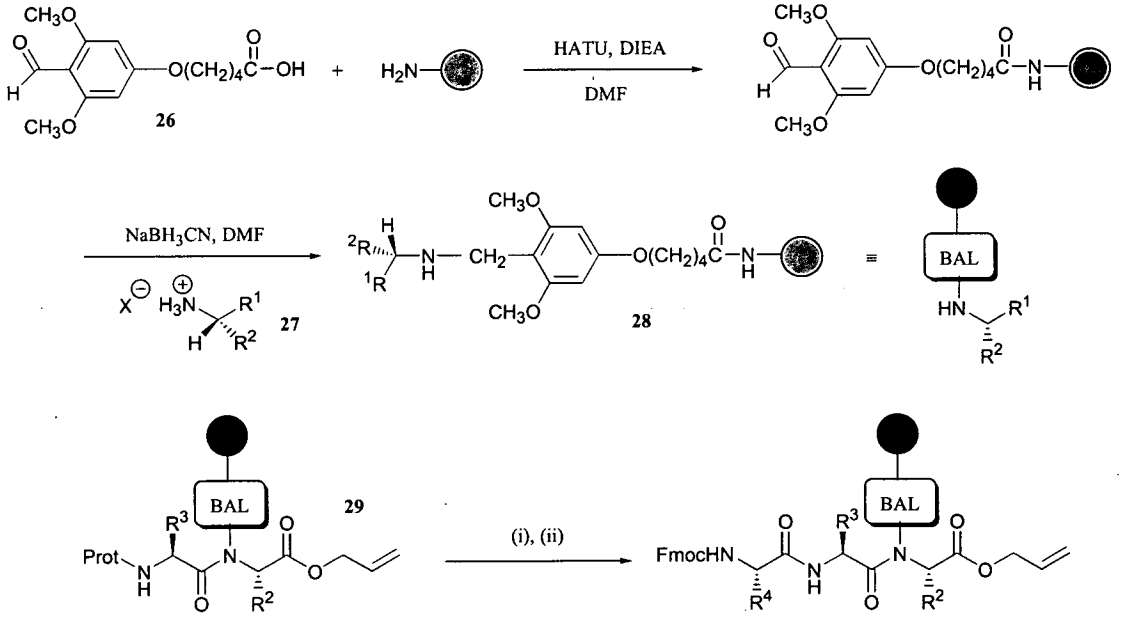
When the HMPB linker was employed, as previously shown (Scheme 5), the carboxyl group delivered on cleavage was activated and treated with amine to afford the corresponding amide **23** (Scheme 6). When the Fmoc-Rink linker (*p*-[α-(1-[9*H*-fluoren-9-yl]-methoxyformamido)-2,4-dimethoxybenzyl]-phenoxyacetic acid) was used in place of HMPB, acidic cleavage gave the primary amide **24**, or treatment with cyanuric chloride in DMF followed by cleavage furnished the peptide nitrile **25**.

A broad array of C-terminally modified peptide analogues were prepared using this methodology.[83]

A particularly attractive feature of the inversion method is that it is considered to be 'self purifying'. Only those peptides which undergo successful internal resin capture (cyclisation) to **21** (Scheme 5) will remain on the resin during treatment with 1 % TFA, therefore, any peptides or impurities attached to the support solely *via* the HMPB linker will being cleaved and washed away leaving the pure product bound to the support.

The alternative BAL approach adopted by Barany *et al.*[84,85] not only offers a general route to a wide range of C-terminally modified peptides, but may also be applied to the synthesis of cyclic peptides, modified amino sugars and small organic molecules.

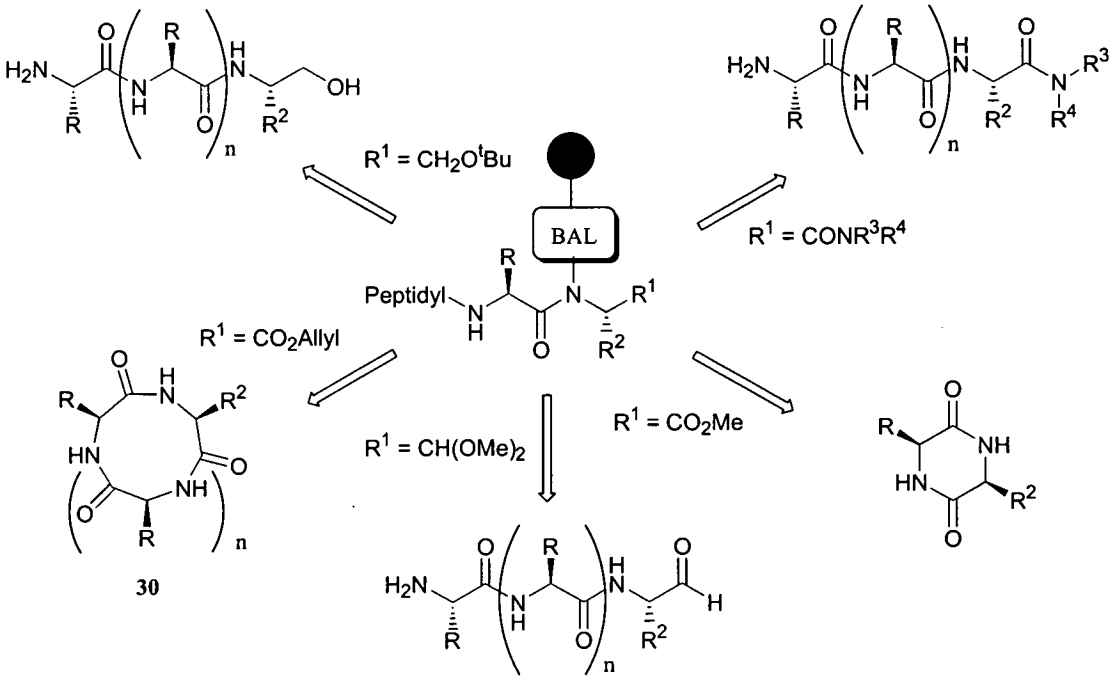
The basic crux of the strategy is attachment of analogues to the polymer support *via* an N^{α} backbone amine (Scheme 7), leaving both termini free for elongation or manipulation.



Scheme 7 Reagents and conditions: $R^1 = CO_2CH_2CH{=}CH$, R^2, R^3 & R^4 = amino acid side chain, X = Cl, CF_3CO_2 or $CH_3C_6H_5SO_3$, Prot = Tr or Ddz, (i) TFA/H_2O/DCM (2:1:97 Tr; 3:1:96 Ddz) (ii) FmocNHCHR^{4}CO$_2$H, PyAOP, DIPEA, DMF

The BAL handle (**28**) was prepared by coupling 5-(4-formyl-3,5-dimethoxyphenoxy)-valeric acid (PALdehyde) (**26**) with aminomethyl polystyrene or PEG, followed by a one-pot, on-resin reductive amination. Acylation of the secondary amine nucleophile was slower than with a primary amine, nonetheless, coupling of the second amino acid residue to yield **29** could be achieved using a number of coupling conditions. Standard C-N peptide elongation was then used to assemble the desired polypeptide chain, before cleavage from the resin. A number of C-terminal peptide analogues were prepared by this strategy[85] using different starting amino acid derivatives instead of **27** (Scheme 8).

For instance a peptide alcohol was prepared in high purity by coupling phenylalaninol *tert*-butyl ether to the BAL handle, before chain extension and cleavage. In the same way, a C-terminal aldehyde was synthesised by starting with glycinal dimethylacetal and Leu-enkephalin dimethylamide was obtained by coupling leucine dimethylamide hydrochloride salt as the first amino acid residue.



Scheme 8 *Some applications of the BAL handle approach*

Further useful applications of this approach, including the formation of head-to-tail cyclic peptides **30**, are illustrated in Scheme 8.

1.2.5 Closing Remarks

As illustrated, the term peptidomimetic covers a broad range of structurally diverse compound types. These different analogues have a variety of applications, but all bear the common potential for development as therapeutic agents. Although many sophisticated routes to each type of peptide analogue have been reported, there still remains a demand for an efficient, general, solid phase system, applicable to the combinatorial synthesis of a whole variety of peptidomimetics.

Thus, the aim of this research was to develop a solid phase system which would provide access to a wide range of peptidomimetic structures. The dipeptide-derived 5(4*H*)-oxazolone was selected as an ideal synthon.

1.3 5(4*H*)-Oxazolones

1.3.1 General Overview

5(4*H*)-Oxazolones†, of the general structure **31**, may be regarded as anhydrides of *N*-acyl amino acids. This class of heterocycle has received periodic bursts of interest since the preparation of the first unsaturated oxazolone (**32**) by Plöchl, over a century ago.[86] Erlenmeyer, after determining the correct structure of Plöchl's new heterocycle, was the first to establish unsaturated oxazolones, coined Erlenmeyer azlactones, as useful intermediates for the synthesis of α-keto and α-amino acids.[87] The late 1940s witnessed a revived interest in the chemistry of 5(4*H*)-oxazolones, when the thiazolidine-oxazolone structure was implicated, albeit erroneously, in the chemistry of penicillin. However, the research prompted by this proposal, together with more recent investigations, has served to establish the 5(4*H*)-oxazolone as a valuable synthon for a variety of transformations.[88-90]

Figure 9 *5(4H) oxazolone: general structures*

Not surprisingly, 5(4*H*)-oxazolones are not held in quite such high regard in the field of peptide chemistry, where they have been ascribed as the primary cause of racemisation during peptide synthesis.[91] In order for peptide coupling to take place, it is necessary to activate the carboxy component prior to aminolysis, and it is at this stage that the optical purity of the growing peptide chain is in danger of being compromised. Activated acylamino acids and peptides undergo base-catalysed cyclisation to 5(4*H*)-oxazolones as depicted in Figure 10. These oxazolones may be considered activated derivatives of peptides, and are thus susceptible to aminolysis with amino acid nucleophiles, ultimately leading to peptide chain elongation. However, 5(4*H*)-oxazolones also spontaneously racemise *via* the resonance stabilised pseudoaromatic anion **33** leading to undesirable epimeric peptide products. In contrast, when the acyl substituent of the activated carboxy component is an alkoxycarbonyl protecting group *e.g.* Boc, Cbz or Fmoc, racemisation is not only limited, but aminolysis occurs at a faster rate. Thus, solid phase peptides synthesised in the C-N direction using activated Cbz-protected carboxy components suffer little or no detectable racemisation. It is for this reason that inverse (N-C) peptide synthesis has been neglected in favour of C-N synthesis, since elongation in the inverse direction requires activation of the C-terminal residue which is not protected from oxazolone-mediated racemisation. Nonetheless, studies into the mechanism of this racemisation procedure have enabled the development of a range of additives which have different suppression effects on the rate of racemisation,[57,58] indicating that racemisation free N-C peptide coupling may become commonplace in the not too distant future.

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† also oxazolin-5(4*H*)-ones and archaically azlactones

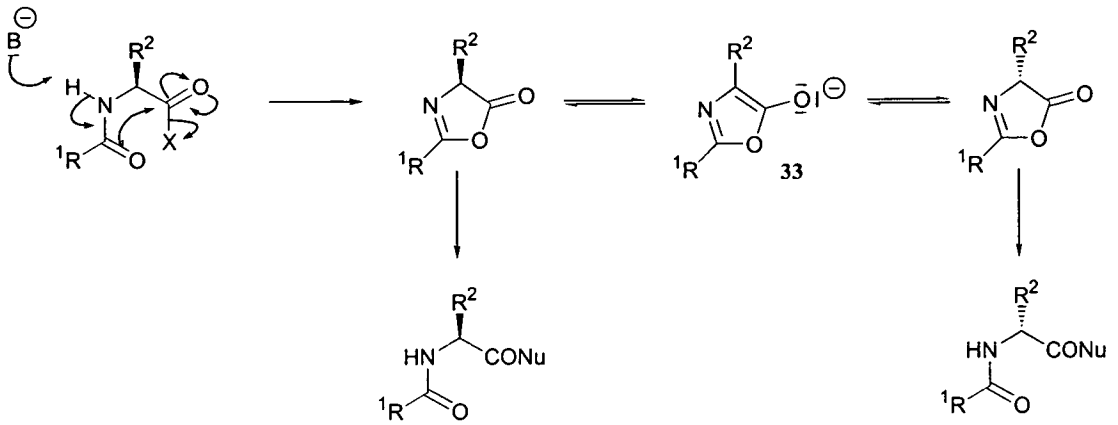


Figure 10 *Racemisation* via *5(4*H*)-oxazolones*

Although perceived as a limitation, the racemisation of 5(4*H*)-oxazolones, which proceeds at a faster rate than nucleophilic ring-opening, has facilitated their use as substrates for dynamic kinetic resolution. Indeed, there has been a recent wave of reports describing the use of 5(4*H*)-oxazolones as valuable precursors for the preparation of optically active α-amino acids. For instance, many groups have studied the enzymatic-catalysed enantioselective ring-opening of 2-phenyl 5(4*H*)-oxazolones as a route into optically active amino acid derivatives.[92-96] Other examples of the use of 5(4*H*)-oxazolones in this manner include, dynamic resolution using chiral catalysts,[97] crystallisation-induced resolutions[98] and asymmetric transformations catalysed by chiral ligand complexes.[99]

As already stated, 5(4*H*)-oxazolones are useful intermediates for the synthesis of a diverse range of compounds. Most of the research reported, however, focuses on the use of 2-phenyl 5(4*H*)-oxazolones, with various substituents at the C4 position, including aryl-and alkylidene unsaturated derivatives. Many of these transformations will be discussed further in Chapter 5, as potential areas for future research, however, the particular oxazolone derivative at the basis of this research is the dipeptide-derived 5(4*H*)-oxazolone (**34**). This intermediate may well prove a useful means of accessing a range of therapeutically desirable peptidomimetics.

1.3.2 Dipeptide-Derived 5(4*H*)-Oxazolones

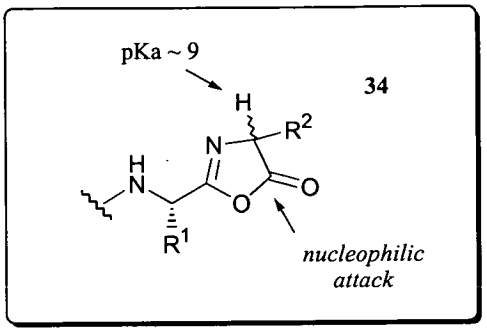


Figure 11 *Dipeptide-derived 5(4*H*)-oxazolone*

The dipeptide-derived oxazolone (**34**) can be readily prepared by dehydration of a dipeptide or peptide chain which is unprotected at the C-terminus. This reaction is usually achieved by the use of acetic anhydride or DCC. However, in terms of solid phase research the carbodiimide activation is preferable, since harsh conditions are not usually compatible with commonly used peptide linker systems.

The dipeptide-derived oxazolone was selected as a useful synthon for two main reasons. Firstly, deprotonation under mild conditions affords the enolate which is amenable to alkylation, thus providing a route into α,α-dialkylated amino acids and peptides (**1.2.2**). It was also predicted, that because the original oxazolone contains a stereogenic centre at C1', asymmetric induction may be observed during the alkylation step, with the success of this depending on the substituent R^1.

The second feature of the oxazolone to be exploited was its susceptibility to nucleophilic ring opening, which can be achieved using a number of nucleophiles, (**4.2.1**). Thus, after elaboration of the resin-bound oxazolone moiety, aminolysis can be effected with a range of nucleophiles, to afford an array of C-terminally modified peptide analogues.

As well as examining the potential for alkylating the 5(4*H*)-oxazolone, a further elaboration to be investigated was the DDQ-induced oxidation at the C4 position[72] as a means of accessing dehydropeptides (**1.2.3**). All these transformations, alkylation,

oxidation and ring-opening, will be discussed in greater detail in the relevant sections in Chapter 4.

1.4 Conclusions and Aim of Project

Given the significance of combinatorial solid phase chemistry and peptidomimetic structures in the drug discovery process, along with the beneficial features of the dipeptide-derived 5(4*H*)-oxazolone moiety, the aim of the research was to use the resin-bound oxazolone as a versatile precursor to numerous peptide analogues as depicted below (Figure 12).

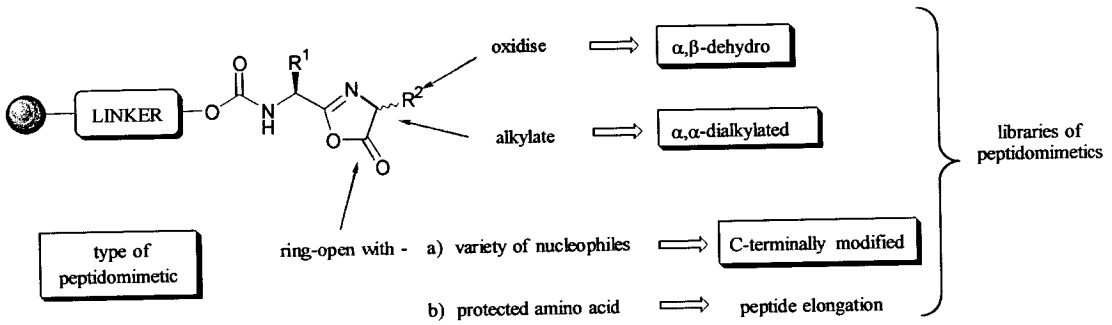


Figure 12 *General strategy - peptidomimetic libraries* via *supported 5(4*H*)-oxazolone intermediates*

2. Results and Discussion I

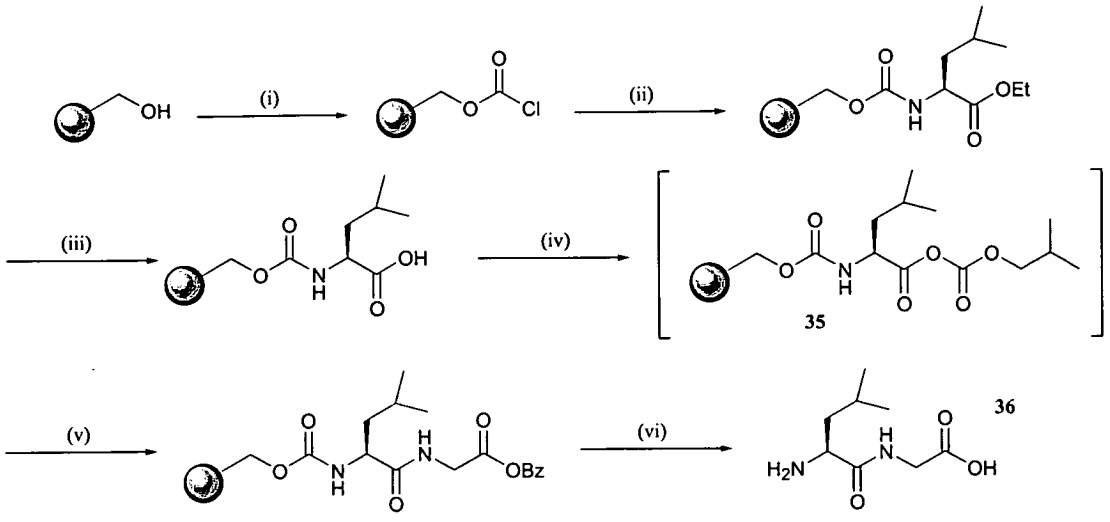
2.1 Inverse Peptide Synthesis

2.1.1 General Overview

As advanced in Chapter 1, C-terminally modified peptides hold a great deal of potential as therapeutically active peptidomimetics. Many of the strategies employed for the synthesis of these peptide analogues have already been outlined (***1.2.4.2***), but an alternative general approach, and perhaps the most obvious route, is *via* the direct assembly of peptides in the N-C direction. This method not only leaves the C-terminus free to be functionalised or capped as required, but can also be used to facilitate fragment coupling (the process of condensing partial sequences of the target peptide, rather than constructing the whole chain in a stepwise manner).

Inverse (N-C) peptide synthesis has been largely ignored in favour of the conventional C-N peptide elongation, due to the danger of oxazolone-mediated racemisation (Figure 10). Nevertheless, there have still been a number of attempts at developing suitable conditions for the N-C construction of peptides as a route into valuable C-terminally modified peptide analogues. A few examples are described below.

After Merrifield's seminal publication in 1963[1] of a radical new method for the synthesis of peptides covalently anchored to an insoluble support, Letsinger[100,101] was the first to investigate the feasibility of performing solid phase peptide synthesis in the N-C direction. He describes using "popcorn" polymer (polystyrene 0.5 % crosslinked with divinylbenzene) to synthesise leucylglycine (**36**) *via* the mixed anhydride method illustrated in Scheme 9.



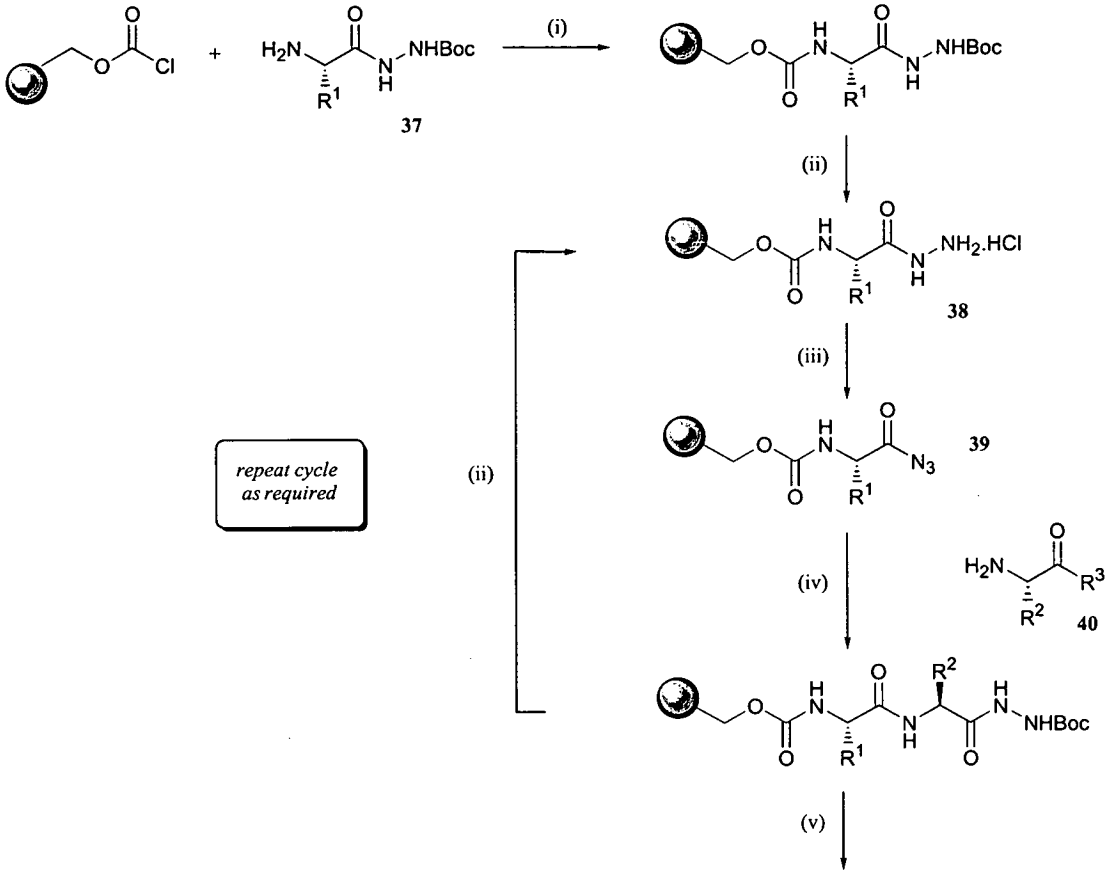
Scheme 9 Reagents and conditions: (i) phosgene, benzene (ii) HCl-Leu-OEt, Et_3N, DMF, 2.5 h (iii) 0.8 M NaOH, MeOH, acetone, 3 h (iv) isobutyl chloroformate, Et_3N, toluene (v) TSA-Gly-OBz, Et_3N, DMF, 2.5 days (vi) 15 % HBr in acetic acid

The leucine derivative was coupled to the activated support, deprotected and treated with isobutyl chloroformate to afford the carboxylic-carbonic anhydride **35**. Aminolysis with glycine benzyl ester *p*-toluenesulfonate, followed by cleavage and deprotection furnished the target dipeptide **36**.

This was the only reported instance of N-C directed solid phase peptide synthesis until seven years later when Merrifield and Felix[102] described the inverse synthesis of a tetrapeptide using the acyl azide method outlined in Scheme 10.

Recognising the potential of peptide synthesis in this unconventional direction as a route into C-terminally modified peptides, Merrifield's objective was to determine a method for the efficient, racemisation-free coupling of peptides. At this time, the azide method,[103] originally pioneered by Curtius at the turn of the century, was considered to be completely racemisation free. Indeed, although recent evidence suggests that racemisation does occur under certain conditions, it is still less prone than most other coupling methods available. Merrifield and Felix adopted the Rudinger and Honzl[104] approach to azide coupling, utilising amino acid Boc-hydrazides (1-aminoacyl-2-*tert*-butyloxycarbonylhydrazines) as protected amino

components that could be converted to reactive amino acid azides in two steps. The general solid phase strategy is outlined below (Scheme 10).



Scheme 10 Reagents and conditions: (i) Et_3N, $CHCl_3$ (ii) 4 M HCl-dioxane, 23 °C, 30 min (iii) *n*-butyl nitrite, THF, -30 °C, 1 h (iv) **40a** R^3 = NHNHBoc or **40b** R^3 = $OC(CH_3)_3$, THF, -30 then 0 then 25 °C (v) HBr-TFA or HF

The amino acid Boc-hydrazide (**37**) was first linked to the support and then deprotected to yield the hydrochloride salt of the resin-bound amino acid hydrazide (**38**). Conversion to the azide (**39**) was achieved by treatment with *n*-butylnitrite in THF, suitably activating it for coupling with the next amino acid Boc-hydrazide residue (**40a**). After this coupling procedure, the deprotection, activation and coupling steps were repeated, until the desired peptide chain had been constructed. Finally, the C-terminal residue was coupled as the *tert*-butyl ester (**40b**), before concomitant deprotection and cleavage from the resin using HBr in TFA. Merrifield and Felix demonstrated the versatility of this methodology by synthesising the tetrapeptide Leu-Ala-Gly-Val both in a stepwise manner and by fragment coupling

of the two dipeptide components. The latter strategy resulted in a higher yield of cleaved product, highlighting the advantages of fragment condensation over consecutive stepwise elongation.

The numerous side reactions associated with the use of peptide hydrazides and azides are well documented,[103] the most common being Curtius rearrangement to isocyanates, leading to urea containing peptides and secondly decomposition to amides, resulting in chain termination. Merrifield and Felix also studied the stability of the leucine azide and observed that although there was no evidence of rearrangement, there was some decomposition of the azide at 4 °C. These problems provide an explanation for why the "racemisation-free" acyl azide method has been superseded as a routine method for stepwise peptide synthesis, by other methods such as carbodiimide and phosphonium-mediated coupling. Indeed, in their closing remarks the two authors recommend that due to solvent limitations and poor yields, the azide approach should not be considered as "a general replacement for solid phase peptide synthesis", but rather be regarded as a "valuable supplement" to this field of chemistry.

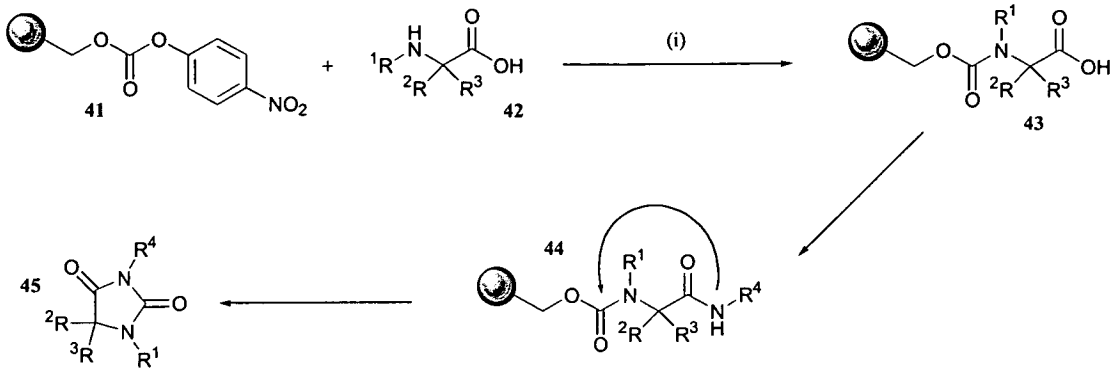
More recent work by Sharma *et al.*[105] utilises trialkoxy silyl esters and DIC/HOBt activation to perform inverse peptide syntheses, before *in situ* modification to peptide analogues *e.g.* alcohols. The basis of their strategy is the stability of the silyl derivatives between pH 4 and 8, rendering them immune to hydrolysis under the conditions of peptide bond formation, yet easily removed by either acidic or basic conditions. The peptides Leu-Ala-Gly-Val and leucine-enkephalin were synthesised in this way. Both were obtained in good yield and required minimal purification. Hydrophobic sequences were also synthesised using BOP as the activating agent, with good yields of high purity products. Furthermore, no protection of hydroxy-functionalised side-chains was required. The method was extended to fragment condensation and also to the preparation of some C-terminally modified peptides including analogues based on leucine-enkephalin. Racemisation, normally a major drawback in inverse peptide synthesis, was also considered, but careful examination of the leucine-enkephalin product indicated that racemisation had not occurred.

A completely different approach, adopted by Jakube *et al.*[106] exploits the reverse hydrolysis potential of proteases which are normally responsible for breaking down the very peptide chains chemists labour to synthesise. Polypeptides are synthesised in the N-C direction in the ribosome, but unfortunately ribosomal peptidyl transferase is only active *in vivo*, and therefore precludes its use in *in vitro* systems. Instead, Jakube and co-workers have demonstrated how the cysteine protease clostripain, along with other enzymes, can be used to synthesise a tetrapeptide (Lys-Tyr-Arg-Ser) comprised exclusively of unprotected trifunctional amino acids in high yield (62 %). The advantages of employing enzymes for such couplings are that reaction conditions are mild and free from racemisation and there is no need to employ side chain protection. There are also limitations associated with this method, however, including the restrictive specificity of proteases and the obvious risk of proteolytic degradation of the starting peptide or product.

An approach which appeared particularly promising as an efficient means of accessing inverse peptides on the solid support, was the method reported by Dressman *et al.*,[107] outlined in the following section (**2.1.2**).

2.1.2 Solid Phase Synthesis of Hydantoins

In 1996, Dressman and co-workers described a novel approach for the solid phase synthesis of hydantoin libraries. The main crux of their strategy involved N terminal linkage to a support, elongation in the N-C direction followed by base catalysed cyclisation, effecting simultaneous cleavage from the polymer support as depicted in Scheme 11.



Scheme 11 *Solid phase synthesis of hydantoins* - reagents and conditions (i) BSA, DMAP, DMF, RT, 48 h; MeOH (ii) DCC, HOBt, R^4NH_2, DMF, RT, 24 h (iii) Et_3N, MeOH, 55-88 °C, 48 h

A series of free amino acids (**42**) were dissolved in DMF with the aid of N,O-bis(trimethylsilyl)acetamide (BSA; 10 eq) and gentle heating. Coupling to the activated carbonate (**41**) was carried out in the presence of DMAP (2 eq) to afford the corresponding resin-bound amino acid derivative (**43**). After amide formation (**44**), the novel cyclisation/cleavage step was accomplished with excess Et_3N at elevated temperatures to afford the desired hydantoins (**45**). By utilising 20 amino acids and more than 80 primary amine building blocks, Dressman and co-workers were able to synthesise a library of 800 individual hydantoins for biological screening.

The feature of this strategy that seemed particularly attractive was the use of free amino acid residues, obviating the laborious synthesis of specifically protected amino acid building blocks. The authors also noted that a relatively diverse range of amino acids was tolerated by the chemistry.

Given the benefits and apparent suitability of the Dressman system, the decision was made to adopt this methodology as a means of accessing dipeptide-derived 5(4*H*)-oxazolones on the solid support.

2.2 Linker

2.2.1 Choice of Linker

As previously discussed (***1.1.2.2***) the choice of linker is paramount in determining the success of a solid phase synthetic strategy. Considerations include ease of attachment, stability during compound elaboration and facile selective cleavage to yield the correct terminal functionality.

The popular acid-labile alkoxybenzyl alcohol resin (Wang linker) (**46** - polystyrene-bound) was first introduced[18] for the synthesis of protected peptide fragments containing a free carboxyl group, *via* an ester linkage to the carboxy terminus. It has since been used to attach a wide range of functionalities, by first activating, then derivatising with a variety of nucleophiles.[19] The lability of acid-cleavable linkers depends on the relative stability of the cation formed on cleavage, compared to the protonated linker. The electron-donating *para*-oxygen on the Wang linker, stabilises the cleavage-induced cation, thus enabling mild acidic cleavage with 50 % TFA/DCM. The "Wang-type" linker (**47**) developed by Bradley for the solid phase synthesis of polyamines,[108-110] incorporates an amide bond adjacent to the benzylic ether. The author noted that attachment to the polymer *via* this functionality, rather than the benzylic ether of Wang, led to fewer side products, presumably a feature of the relative acid stabilities of these two moieties. Bradley's polystyrene-bound "Wang type" linker (**47**), denoted hereafter as PS-HMPA, since it can be derived from the reaction of 4-(**h**ydroxy**m**ethyl)-**p**henoxyacetic **a**cid with aminomethyl **p**olystyrene, has also been applied to libraries of amidines[111-113] and natural product analogues.[114]

Figure 13 *Comparison of Wang and "Wang-type" linker systems*

The HMPA linker (**47**) has also been reported by others[115,116] for use in peptide synthesis, where attachment is *via* an ester bond to the C-terminus of either a reference amino acid or the growing peptide chain. Amines, however, may be coupled to activated PS-HMPA *via* a urethane, which is stable to the conditions required for activation of the first residue and continued peptide synthesis. Initial anchoring to aminomethyl polystyrene resin can be achieved using standard peptide coupling conditions and on completion of the synthesis, the linker can be cleaved readily with 50 % TFA to deliver the desired amino terminus after *in situ* decarboxylation. In light of these many features, we opted for Bradley's PS-HMPA (**47**), to support the 5(4*H*)-oxazolone for further elaboration, as depicted in Figure 14.

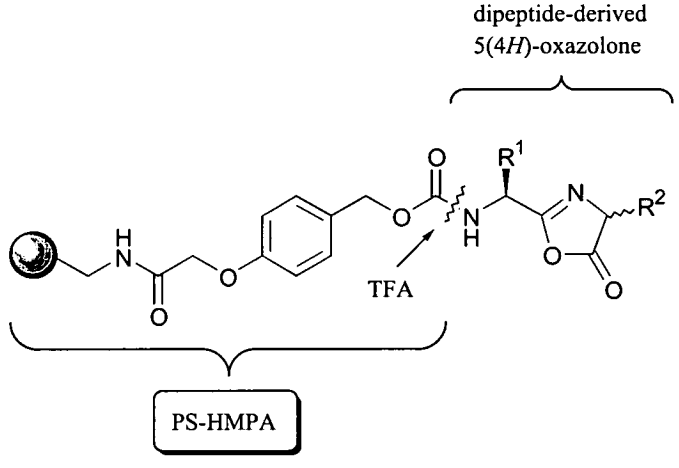


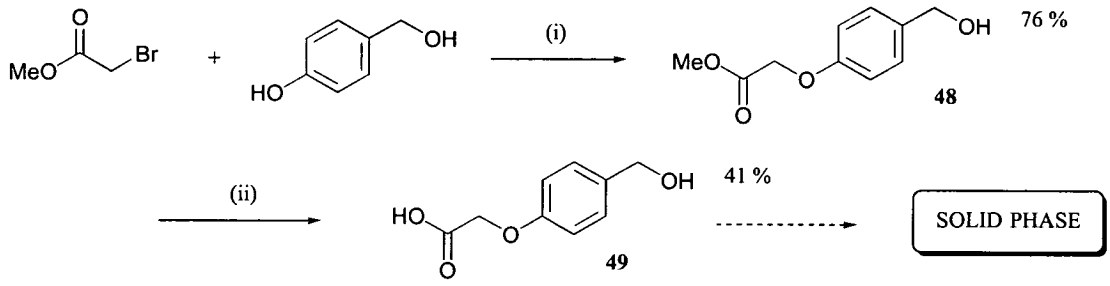
Figure 14 *Target polymer-supported 5(4*H*)-oxazolone, tethered* via *the HMPA linker*

An added attraction of this linker system, is its structural similarity to the Cbz protecting group, facilitating valid comparisons with solution phase investigations on Cbz-protected dipeptide-derived 5(4*H*)-oxazolones.

2.2.2 Preparation of Resin-Bound HMPA Linker

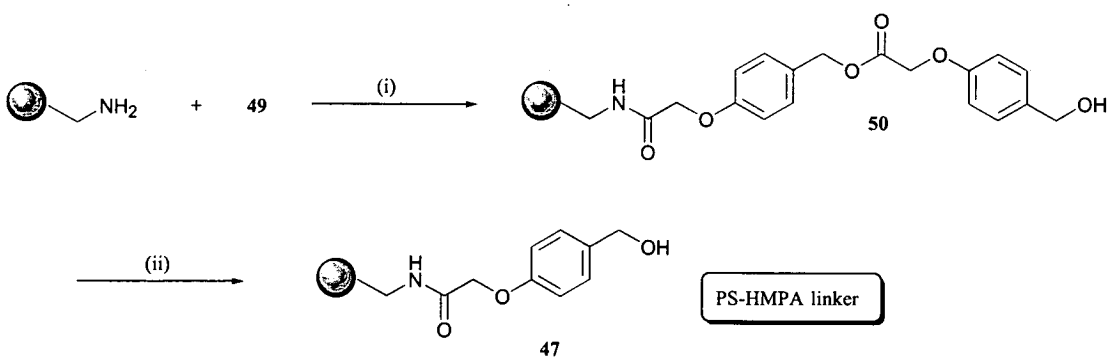
2.2.2.1 Synthesis of 4-(hydroxymethyl)phenoxyacetic acid (49)

4-(Hydroxymethyl)-phenoxyacetic acid (**49**) is commercially available, but expensive, so was instead prepared from 4-hydroxybenzyl alcohol and methyl bromoacetate in two steps, in an overall yield of 59 %. (Scheme 12).



Scheme 12 Reagents and conditions: (i) NaI, K_2CO_3, DMF (ii) 4 M LiOH (aq), THF

The formation of methyl 4-(hydroxymethyl)phenoxyacetic acid (**48**) proceeded selectively, with no evidence of the corresponding 4-(hydroxybenzyl)oxyacetic acid arising from nucleophilic attack by the methoxide anion. Alkaline hydrolysis, using lithium hydroxide monohydrate, gave the crude acid, which was recrystallised to afford **49** in 41 % yield. The poor yield at this stage is thought to be a feature of the work up (unoptimised), as the reaction itself appears to proceed smoothly by tlc.



Scheme 13 Reagents and conditions: (i) DIC, HOBt, DCM, DMF (ii) $N_2H_4.H_2O$, DMF

Standard peptide coupling reagents DIC and HOBt were used to couple **49** to aminomethylated polystyrene resin (Scheme 13). However, analysis of the resin sample by IR gave a very distinctive and unexpected ester stretch (ν_{max} 1757 cm^{-1}) indicating that the resin bound alcohol had reacted with excess activated acid to yield the ester **50**. This was confirmed by gel phase ^{13}C NMR analysis (Figure 15).

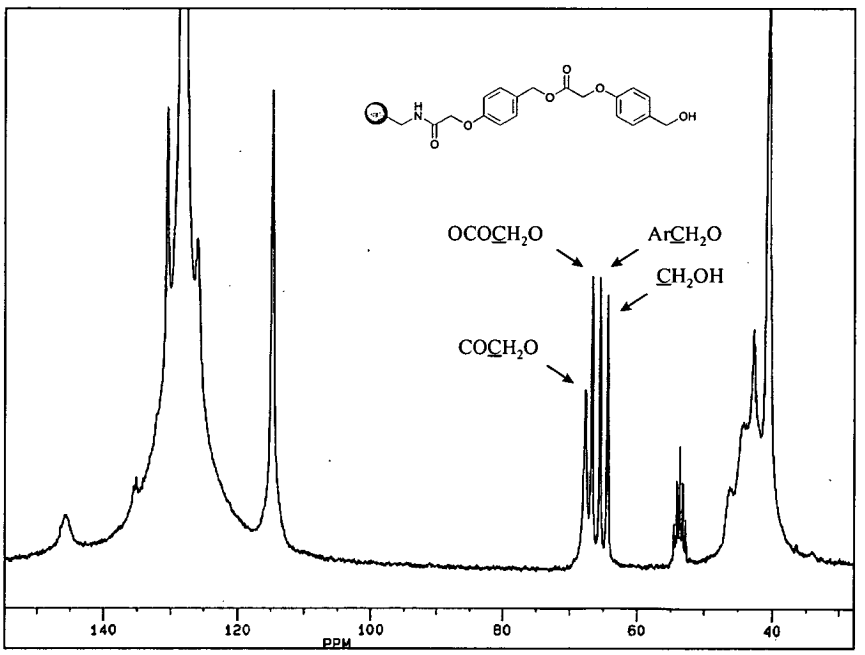


Figure 15 *Gel phase ^{13}C NMR of* ***50***

The resin-bound ester was hydrolysed using hydrazine monohydrate to give the desired PS-HMPA linker **47** in quantitative yield, confirmed by IR and gel phase ^{13}C NMR (Figure 16).

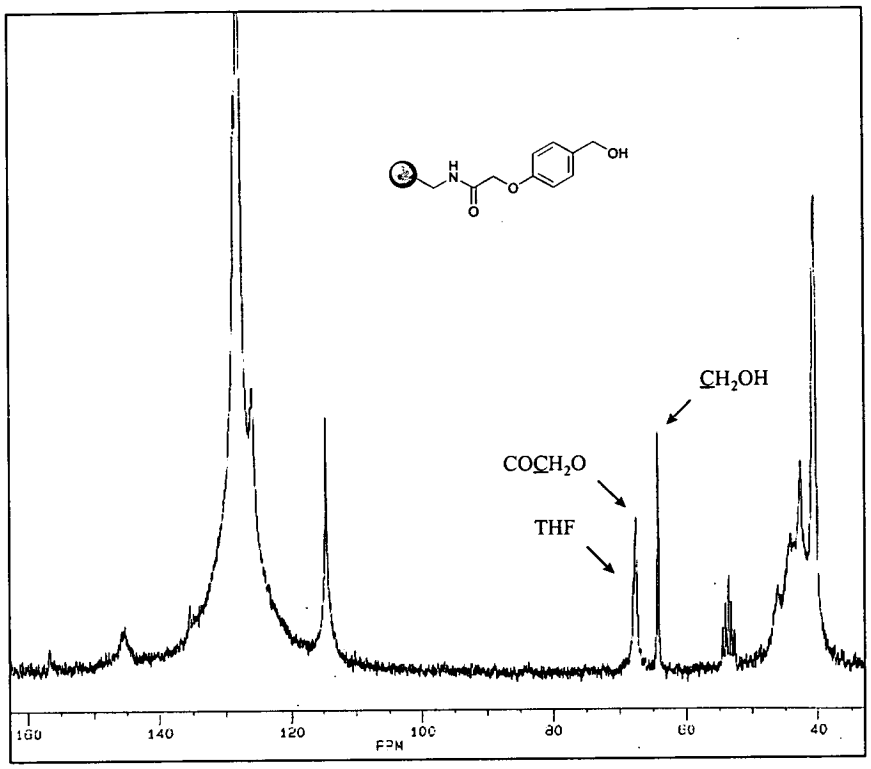
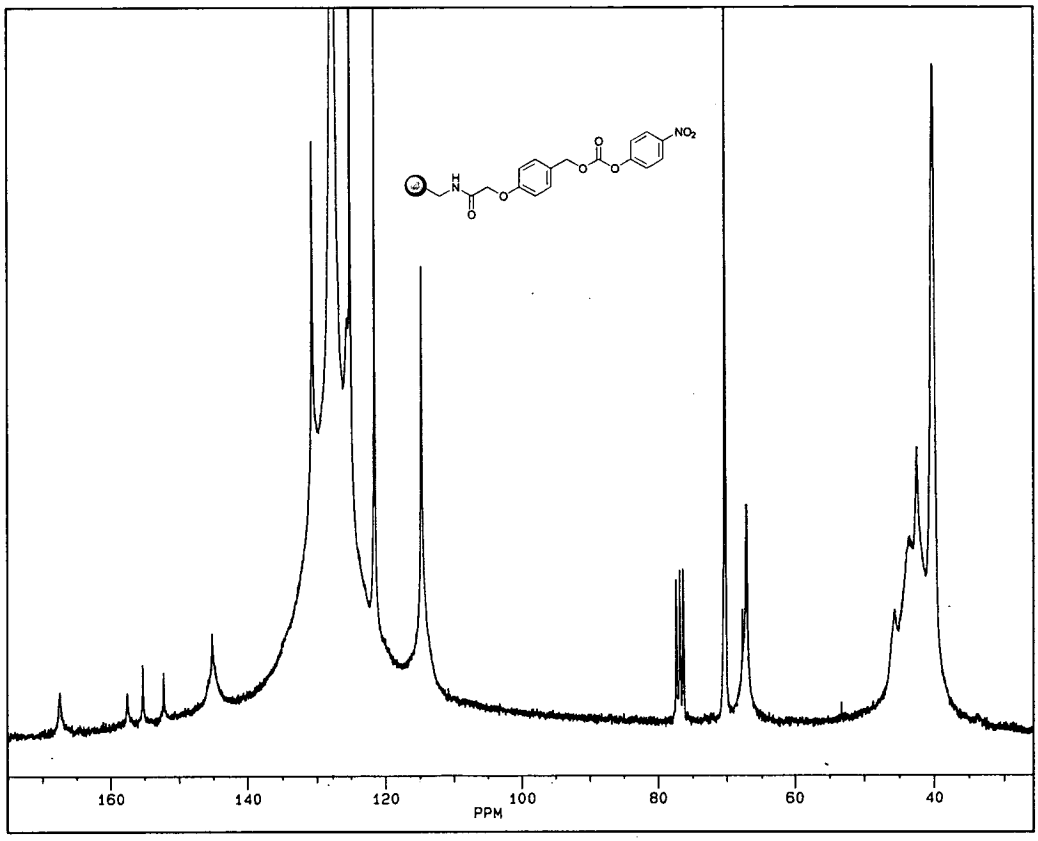


Figure 16 *Gel phase ^{13}C NMR of* ***47***

It is interesting to note that no mention of this side reaction was made by Bradley *et al.*, even though the same reaction conditions, with the exception of DMF, were employed.[112] It is possible, therefore, that if **50** was formed, then activation with *p*-nitrophenyl chloroformate would instead yield **51** (Figure 17). Further reaction would proceed as normal, with the only difference being release of the extra contaminant **52** on cleavage.



Spectrum 1 *Gel phase ^{13}C NMR of* ***53***

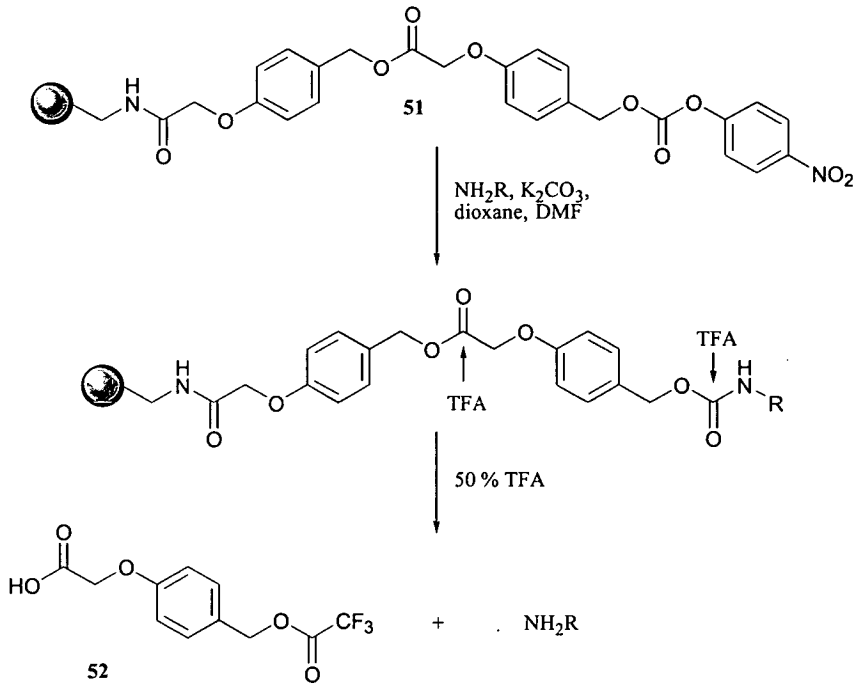
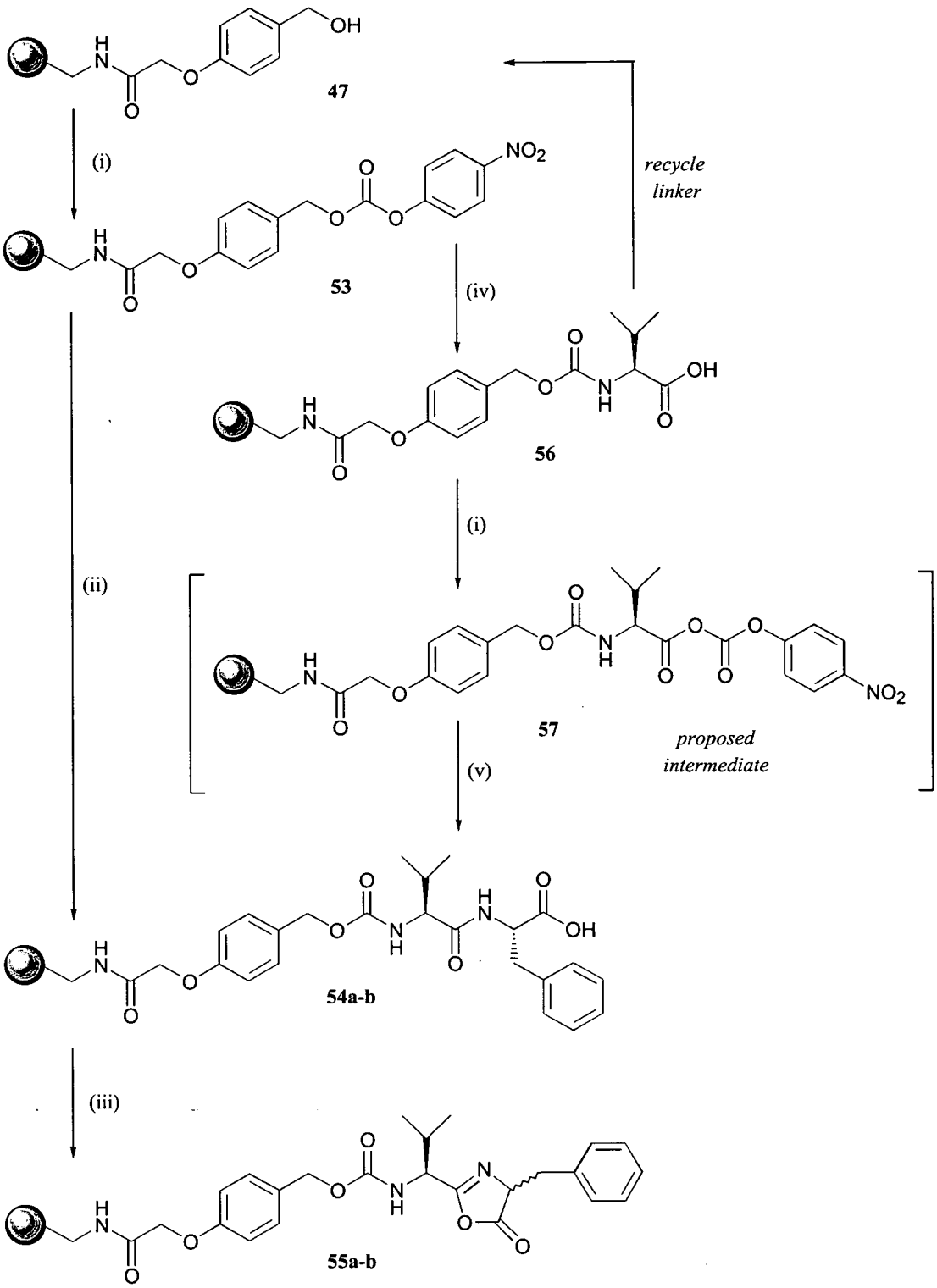


Figure 17 *Possible cleavage products if ester formation undetected*

2.3 Preparation of Supported 5(4*H*)-Oxazolones *via* 1st Strategy

The decision was made to synthesise the direct precursor of the target 5(4*H*)-oxazolone, by coupling a commercially available dipeptide, in order to confirm the viability of this approach before investigating a stepwise route. Therefore, in an analogous way to that used by Bradley,[112] PS-HMPA (**47**) was first activated by treatment with excess *p*-nitrophenyl chloroformate and pyridine in DMF to afford the reactive carbonate (**53**) (Spectrum 1)[op] (Scheme 14). At this stage it was possible to obtain an approximate loading of the activated linker using *p*-nitrophenol analysis (***6.3.4.2***), which detects the amount of *p*-nitrophenol released on treatment with 20 % piperidine in DMF. A loading of 1.0 mmol/g was calculated which indicates a yield, accounting for weight gain (***6.3.4.3***), of 100 %*[†] based on the loading quoted by the manufacturer of the aminomethyl polystyrene used.

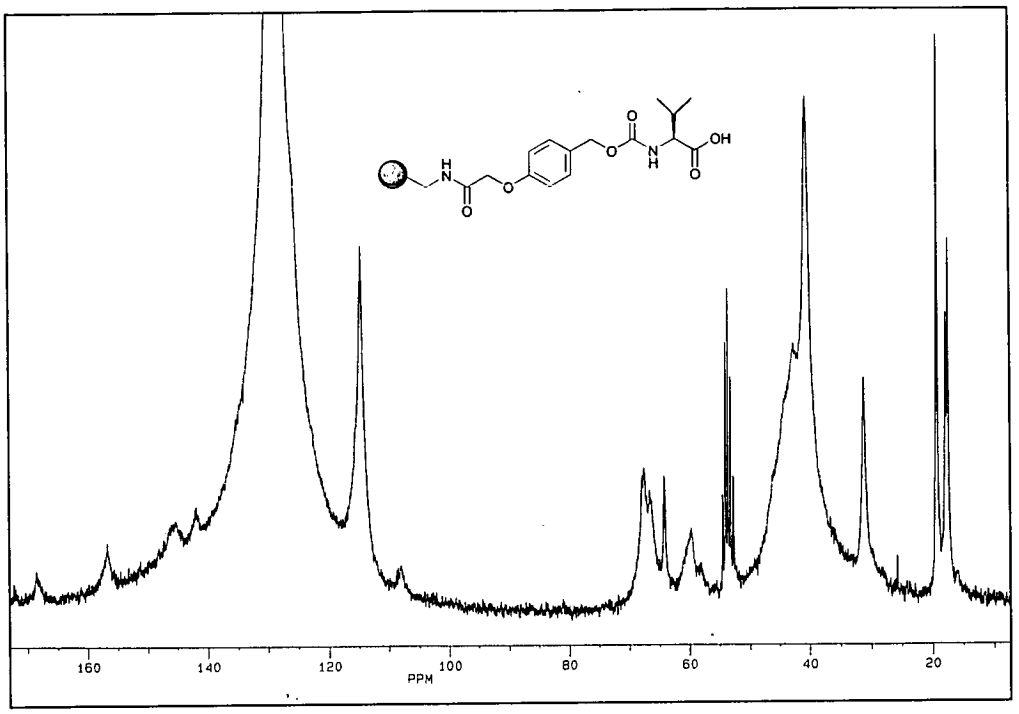
[†] see section ***6.3.4.3*** for explanation of loading notation



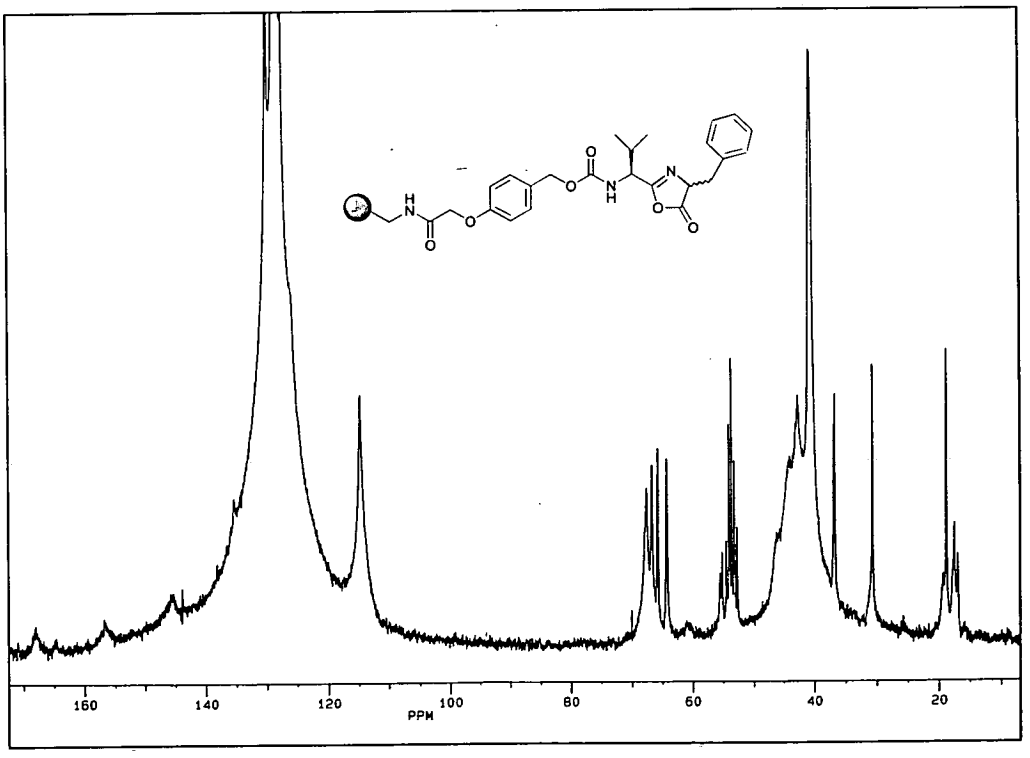
Scheme 14 Reagents and conditions: (i) *p*-nitrophenyl chloroformate, pyridine, DCM (ii) Val-Phe, BSA, DMAP, DMF (iii) EDCI, DCM (iv) L-valine, BSA, DMAP, DMF (v) L-phenylalanine, BSA, DMAP, DMF; **54/55a** derived from direct dipeptide coupling; **54/55b** derived from stepwise coupling

The unprotected dipeptide valinylphenylalanine (Val-Phe; 2 eq) was dissolved in DMF, using BSA (10 eq) and sonication at elevated temperatures, according to the method used by Dressman *et al.*.[107] Coupling was achieved by addition of the dipeptide solution to carbonate **53** (Scheme 14), which was pre-swollen in DMF, along with DMAP (4 eq), followed by agitation for 15 h. A yellow solution indicated successful displacement of the *p*-nitrophenol group and IR and gel-phase ^{13}C NMR analysis confirmed complete disappearance of the carbonate moiety. Acidic cleavage, followed by ES-MS verified the presence of the desired dipeptide (m/z 265 MH^+), however it was apparent from the number of other signals present in the mass spectrum and the poor quality of the NMR spectrum that the product was not pure. Nevertheless, the resin-bound dipeptide (**54a**) was treated with a solution of EDCI in DCM, promoting cyclisation to afford the target resin-bound oxazolone (**55a**). The success of this step was apparent from the distinctive stretch in the IR spectrum (ν_{max} 1825 cm^{-1}) corresponding to the oxazolone carbonyl. ES-MS analysis of resin-bound 5(4*H*)-oxazolones is not so informative, however, since acidic cleavage from the resin results in almost complete hydrolysis of the oxazolone back to the original dipeptide. Additional confirmation of the presence of the oxazolone was achieved by ring-opening with an amine nucleophile *e.g.* benzylamine and ESMS analysis of the resulting derivative (**6.10.8**).

Similarly, the stepwise coupling of two free amino acids was attempted, as this would facilitate preparation of a diverse series of dipeptide-derived oxazolones on the solid phase (Scheme 14). Since the *p*-nitrophenyl moiety had facilitated loading analysis during initial activation of the linker, it was hoped the activation of the first resin-bound amino acid (**56**) could be achieved *via* the mixed carboxylic-carbonic acid anhydride (**57**),[117] in a similar method to Letsinger[100] (Scheme 9). Once activated, nucleophilic attack would be directed towards the carbonyl of the original carboxy component, since the second carbonyl group suffers diminished reactivity due to resonance with the two flanking oxygen atoms. Aminolysis with a second amino acid residue would, therefore, yield the desired resin-bound peptide (**54b**), with release of *p*-nitrophenol.



Spectrum 2 *Gel phase ^{13}C NMR of* ***56***



Spectrum 3 *Gel phase ^{13}C NMR of* ***55b***

Thus, coupling of the free amino acid residue, L-valine, was achieved in a similar manner to the dipeptide (Scheme 14). On-resin analysis techniques, such as IR and gel-phase ^{13}C NMR, along with cleavage and ES-MS indicated successful coupling to yield **56**, but once again the purity of the product was a concern (Spectrum 2).[op] Activation was accomplished using the same conditions employed to activate the starting linker affording the proposed intermediate **57**. IR analysis, along with *p*-nitrophenol analysis (0.71 mmol/g, 82 %) confirmed the presence of the aryl-nitro group, but the gel phase ^{13}C NMR proved difficult to interpret due to poor resolution permitting assignment of only some diagnostic peaks with others remaining unidentified. In addition, ES-MS analysis of the cleavage product gave a mass ion corresponding to the active ester as opposed to the carboxylic-carbonic acid anhydride. This observation may well be due to decarboxylation during acidic cleavage followed by re-association of the amino acid and 4-nitrophenol. Solution phase mixed anhydrides are renowned for their poor stability and are rarely isolated. It is therefore likely that the resin-bound anhydride was obtained but that analysis of the solution cleavage products is futile, due to immediate decomposition.

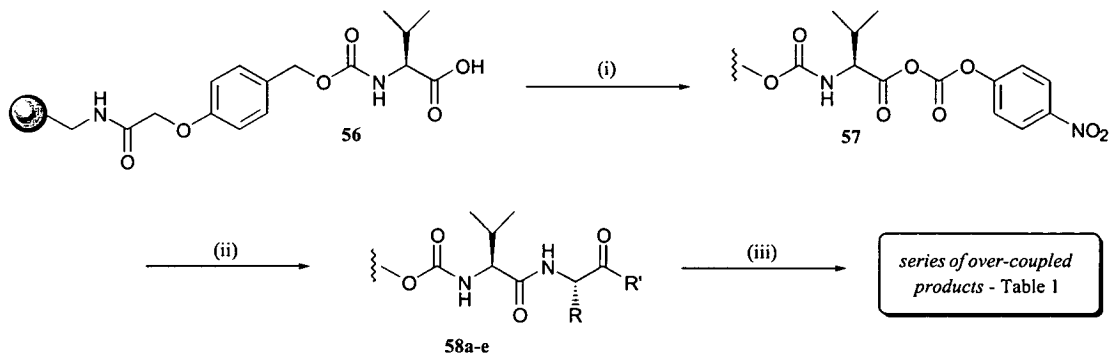
Coupling of the second free amino acid residue did, however, confirm that the resin had been successfully activated since the target resin-bound dipeptide was obtained, albeit, as indicated by IR, NMR and ES-MS, in an impure form. Cyclisation to the resin-bound 5(4*H*)-oxazolone (**55b**) was effected in the same manner as with **55a**, affording the desired product (Spectrum 3),[op] albeit impure.

It was also discovered at this stage, that the PS-HMPA linker (**47**) may be recycled after acidic cleavage, by simple hydrolysis of the cleavage-induced TFA ester to the benzyl alcohol, using hydrazine hydrate. The isolated resin bound HMPA linker may then be reactivated and used in the synthetic procedure with no apparent detrimental effect on reaction yields, although the starting loading is generally lower.

Although, the target resin-bound 5(4*H*)-oxazolone had indeed been successfully synthesised by this method, the evident low purity of the coupling products was of concern and was investigated as follows.

2.4 BSA-Assisted Coupling of Free Amino Acids

Resin-bound valine (**56**) was activated in the usual manner and treated with a series of free amino acids and the dipeptide Val-Phe, all of which had been dissolved in DMF using BSA as previously described.



Scheme 15 Reagents and conditions: (i) *p*-nitrophenyl chloroformate, pyridine, DCM (ii) nucleophile, BSA, DMAP, DMF (iii) TFA/DCM/H_2O (9:10:1)

After cleavage, all the products were analysed by ES-MS, which in each case indicated the main product to be the desired di/tri-peptide but with many higher oligomers present as significant impurities. The findings are tabulated on the following page (Table 1).

Product	Nucleophile	ES-MS of cleavage mixture			
58		*desired product*		*by-products*	
		m/z	peptide (MH^+)	*m/z*	peptide (MH^+)
a	Val	217	Val-Val	118	Val
				316	Val-Val-Val
b	Phe	265	Val-Phe	118	Val
				166	Phe
				217	Val-Val
				313	Phe-Phe
				364	Val-Val-Phe
				412	Val-Phe-Phe
c	Leu	231	Val-Leu	118	Val
				132	Leu
				217	Val-Val
				245	Leu-Leu
				330	Val-Val-Leu
				344	Val-Leu-Leu
d	Ser	205	Val-Ser	105[a]	Ser
				118	Val
				217	Val-Val
				279	Ser-Ser-Ser
				304	Val-Val-Ser
				391	Val-Val-Ser-Ser
e	Val-Phe	364	Val-Val-Phe	118	Val
				217	Val-Val
				265	Val-Phe
				463	Val-Val-Val-Phe
				511	Val-Phe-Val-Phe

Table 1 *Cleavage products from BSA-assisted coupling:* a) M^+ c.f. MH^+

These results indicate that the free amino acids are over-reacting at each coupling stage to yield, for instance Val-Val and Val-Phe-Phe. There is also evidence of incomplete coupling, where some amino acid residues are coupling directly to free active sites on the resin during the second cycle, as opposed to the activated resin-bound amino acid residue.

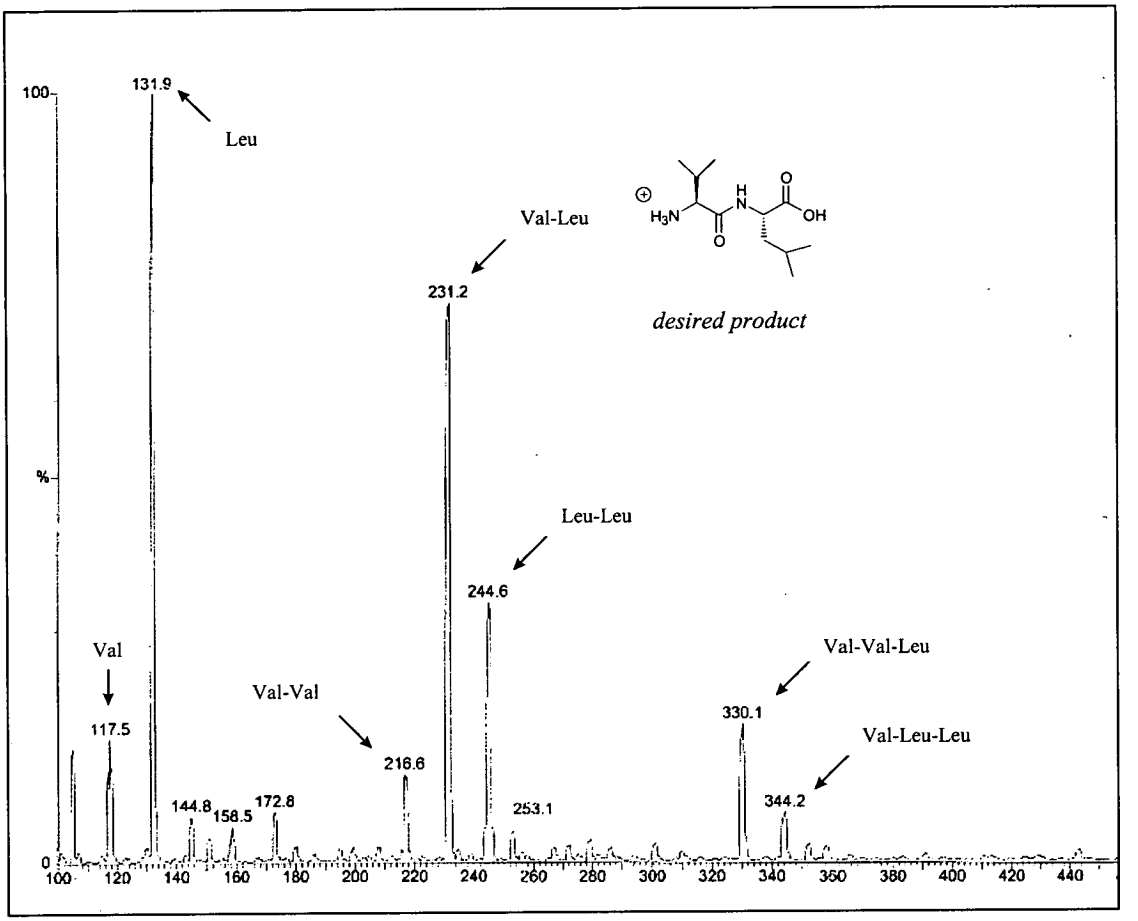


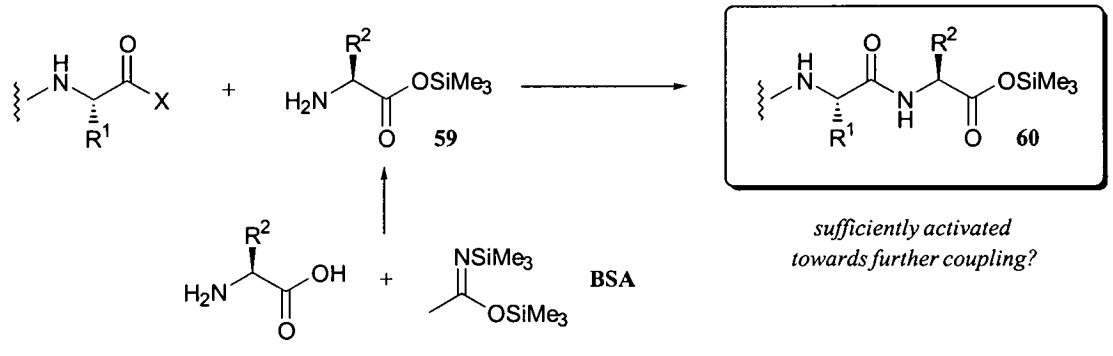
Figure 18 *Mass spectrum of* ***58c*** *illustrating over-coupled impurities*

In order for this to be taking place, some sort of activated amino acid derivative must be formed after the initial coupling, allowing aminolysis to afford the doubly- and triply-coupled products observed.

One possibility may involve the *p*-nitrophenol released on aminolysis, condensing with the coupled residue to yield an active ester, which could subsequently suffer aminolysis by the excess free amino acid in solution. This hypothesis, however,

seems unlikely, because although it is possible to prepare active esters of free amino acids *in situ*,[118] this procedure requires addition of an activating reagent, most commonly DCC.

Another proposal concerns the addition of a large excess of BSA, used to improve the poor solubility of the free amino acids. The BSA aids dissolution by silylating the carboxy terminus to yield the trimethyl silyl ester (**59**) and it is proposed that in the presence of DMAP, the coupled derivative (**60**) may be sufficiently activated to promote further aminolysis to yield the over-coupled products observed (Scheme 16).



Scheme 16 *Proposed role of BSA in amino acid coupling*

If this second proposal is indeed true, the reason why these extra couplings were not eluded to by Dressman *et al.*, can be explained by close examination of their hydantoin synthesis. The hydantoin strategy can be described as 'self-purifying', meaning that only those nucleophiles which undergo successful cyclisation to form hydantoins are simultaneously cleaved from the support, leaving any over- or under-coupled products bound to the resin. Cleavage products, thus obtained are consequently very pure. However, a high purity accompanied by a poor yield, implies a significant proportion of product not amenable to cyclisation-induced cleavage *i.e.* over- or under-coupled by-products. As predicted, the yields obtained *via* the Dressman system were often poor (12 - 76 %) relative to the purity of the cleavage products (> 90 % in most cases). This presents sound evidence that the

over- and under-coupling observed in this synthesis, may well be occurring during the hydantoin synthesis, since any impurities formed will not be released from the resin and hence remain undetected.

Léger and co-workers[119] attempted to use the BSA analogue, bistrimethylsilyl trifluoroacetamide (BSTFA), to enhance the solubility of an unprotected phenylalanine residue. They hoped to solvate the amino acid *via* the trimethylsilyl ester in order to prepare a library of dipeptides with C-terminal capping groups. However, although preparation of this derivative did facilitate coupling between the amino acid and resin-bound Boc equivalent, the yields obtained were very poor and an alternative procedure involving lithium salts was reported. Unfortunately, this method was also beset with its own limitations and did not appear to be suitable with regard to this work.

A number of other solvent systems were examined in an attempt to find suitable conditions for the dissolution of free amino acids, which would also be compatible with reaction conditions and resin swelling requirements, without success. Focus instead shifted to developing an alternative strategy where problems of poor solubility and over-coupling could be avoided.

2.5 Conclusions

2.5.1 Future Potential

Although these attempts to couple free amino acids in the inverse direction did not prove fruitful at the time of investigation, a modified solution phase synthetic route has since come to light, which may hold the key for future success in this area.

In a recent publication, Mitin and Ryadnov[120] describe how the problems of solubility and racemisation can be overcome, and subsequently demonstrate how free amino acids can be used effectively for the inverse synthesis of peptides in solution. The free amino acids residues were successfully dissolved in 3 M solutions of either $Ba(ClO_4)_2$, $Ca(ClO_4)_2$ or $Ca(NO_3)_2$ in DMF. They could then be coupled to the Cbz-

protected N terminal amino acid residue *via* EDCI (1.5 eq)-mediated activation in the presence of HOBt (1 eq) and $CuCl_2$ (0.5 eq). The combination of $CuCl_2$ and HOBt is reported to suppress completely oxazolone-mediated racemisation[58] during peptide synthesis using cardodiimide activation. A number of penta-peptides were synthesised in high yields (81 - 95 %) using this approach, which was also extended to fragment coupling.

Unfortunately, this appealing procedure was reported too late to be examined in the scope of this work, but certainly appears to hold potential for the inverse synthesis of optically pure peptides, especially if extended to the solid phase.

2.5.2 Change of Strategy

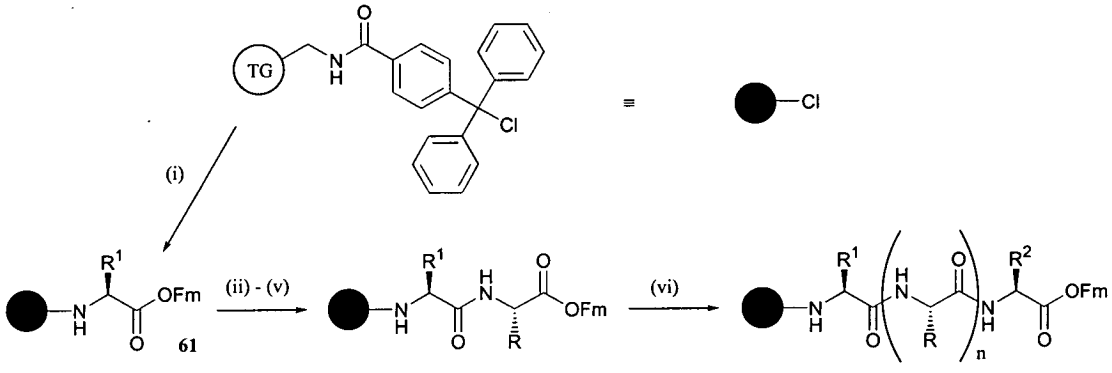
Considering the problems associated with the first strategy employed, the decision was made to adopt an entirely different approach using protecting groups in order to circumvent the problems of amino acid solubility and unwanted over-coupling. A paper by Bayer *et al.*[121] appeared to offer a promising alternative (**3.1.1**).

3. Results and Discussion II

3.1 Solid Phase N-C Peptide Synthesis - Successful Approach

3.1.1 Inverse Solid Phase Peptide Synthesis using 9-Fluorenylmethyl Esters

Among the various methods developed for the inverse synthesis of peptides (**2.1**) the strategy reported by Bayer and co-workers[121] seemed to provide a possible solution to the problems encountered during the previous approach (**2.3**).



Scheme 17 *Bayer's method for the inverse (N-C) peptide synthesis* - Reagents and conditions: (i) X-AA_1-OFm (X = TSA or TFA)†, DIPEA, DCM (ii) 20 % piperidine, DMF (iii) acetic acid, DCM (iv) HOBt, DIC, DMF or TBTU, NMM, DMF (v) HOBt-AA_2-OFm (vi) continued inverse peptide synthesis - R = amino acid side chain

Their approach, depicted in Scheme 17, involved the stepwise coupling of amino acid 9-fluorenylmethyl (Fm) esters to the activated C-terminus of the growing, polymer-bound, peptide chain. The first residue was tethered *via* its N-terminus to the polymer-supported trityl linker (**61**), before being subjected to a sequence of steps involving deprotection, neutralisation and activation. Amino acid derivatives were then coupled as their HOBt salts, with the Fm ester protection facilitating UV loading analysis in the same way as Fmoc protection (**3.1.2**).

† If amino acid HCl salts were employed, the yield of coupled product suffered a significant reduction

In classical peptide synthesis, the carboxy coupling component is in excess in solution, thus shifting the equilibrium in favour of coupling. However, during inverse synthesis, the activated C-terminal residue is the resin-bound component and in order for the coupling reaction to be high yielding, it is imperative that the activation step itself reaches virtual completion.

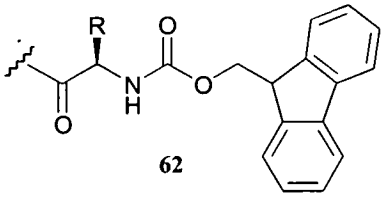
In an attempt to achieve the obligatory high yields, Bayer *et al.* examined two principle methods for C-terminal activation: HOBt/DIC and TBTU/NMM (Scheme 17). Of the two methods, TBTU/NMM was found to be more efficient, with only minimal by-product formation. However, unlike the more acidic HOBt activation, TBTU activation appeared to promote increased susceptibility to oxazolone-mediated racemisation (**1.3.1**).

Overall, the comprehensive study carried out by Bayer *et al.* provided a promising approach for the N-C synthesis of peptides as a route into resin-bound oxazolone derivatives. The protecting group strategy not only serves to prevent the undesirable multiple-coupling which had been a limitation of the first strategy employed (Chapter 2), but also facilitates loading analysis of polymer-supported intermediates. As a method for activation, the more efficient TBTU/NMM approach was selected over the HOBt/DIC protocol, since the normal disadvantage of oxazolone-induced racemisation was not perceived as a drawback, as the resin-bound oxazolone was ultimately the target synthon. Thus, the decision was made to examine this method on the PS-HMPA system as a viable strategy for solid phase N-C peptide and thus 5(4*H*)-oxazolone synthesis.

3.1.2 Preparation of Standard Amino Acid Building Blocks

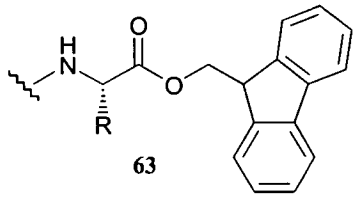
The 9-fluorenylmethyl (Fm) ester (**63**) has been reported a number of times in relation to side chain amino acid protection.[122,123] As a protecting group it is an extension of the extensively used solid phase N-terminal Fmoc protecting group (**62**) pioneered by Carpino *et al.*[124] (Figure 19). The ester displays the same advantageous features as the related Fmoc group, but enjoys far much less attention, doubtless as a consequence of the popularity of C-N directional peptide synthesis compared to N-C.

The main benefits exhibited by both these groups are orthogonality with other popular protecting groups *e.g.* Boc, together with facile cleavage that can be achieved using 20 % piperidine in DMF. Moreover, the UV active dibenzofulvene, which is released upon cleavage, can be used to quantify resin loadings (***6.3.4.1***). This method of loading analysis has been established as the one of primary methods for monitoring the success of classical solid phase peptide synthesis.



62

Fmoc
N-terminal protection

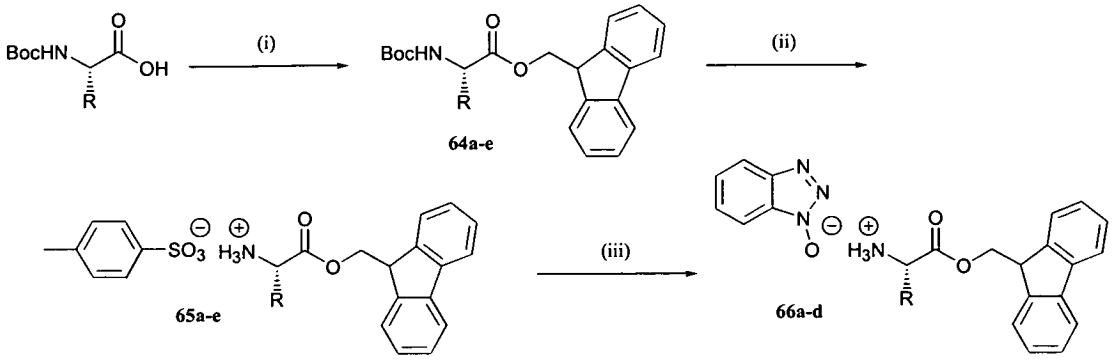


63

Fm ester
C-terminal protection

Figure 19 *9-Fluorenylmethyl derived protecting groups*

In order to prepare the required building blocks, selected Boc-amino acids were first protected as their Fm esters (**64a-e**) by condensation with 9-fluorenylmethanol in the presence of DMAP (Scheme 18).[121] The N-terminal Boc groups were cleaved by treatment with TFA. Addition of *p*-toluenesulfonic acid allowed *in situ* preparation of the TSA salts (**65a-e**), which could be isolated by simple trituration with ether.[125] Amino acid residues which were to be coupled directly to the activated resin were used as their TSA salts, however, amino acid residues required for subsequent couplings were converted to their HOBt derivatives (**66a-d**). In order to perform the salt exchange, the potassium salt of HOBt (KOBt) (**67**) was prepared by stirring equimolar amounts of HOBt hydrate and potassium hydroxide in absolute ethanol. Solvent removal afforded the desired salt **67**. The HOBt salts were then prepared by addition of KOBt to a solution of the amino acid TSA salt, resulting in precipitation of potassium tosylate. After filtration, the solution was concentrated and trituration with copious ether, in most cases, yielded the desired HOBt salt (**66a-d**) in moderate yield. Unfortunately, as a consequence of the poor solubility of the majority of the HOBt salts prepared, ^{1}H NMR analysis proved ineffective.



Scheme 18 Reactions and conditions: (i) 9-fluorenylmethanol, DCM, DMAP, 0 °C - RT (ii) TFA, TSA (iii) KOBt (**67**), MeOH; R = amino acid side-chain

TSA and HOBt salts of valine, phenylalanine, glycine, alanine and leucine Fm esters were prepared in good yields (Table 2). It was found that direct conversion to the TSA salt, without purification of intermediates, followed by simple trituration from ether was the most efficient protocol, as can be exemplified by the two yields quoted for the synthesis of TSA-Phe-OFm (**65a**, R = CH_2Ph) (Table 2).

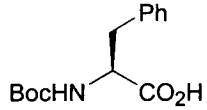
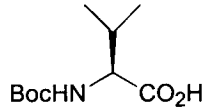
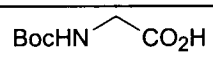
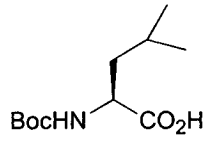
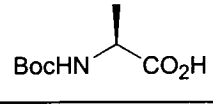
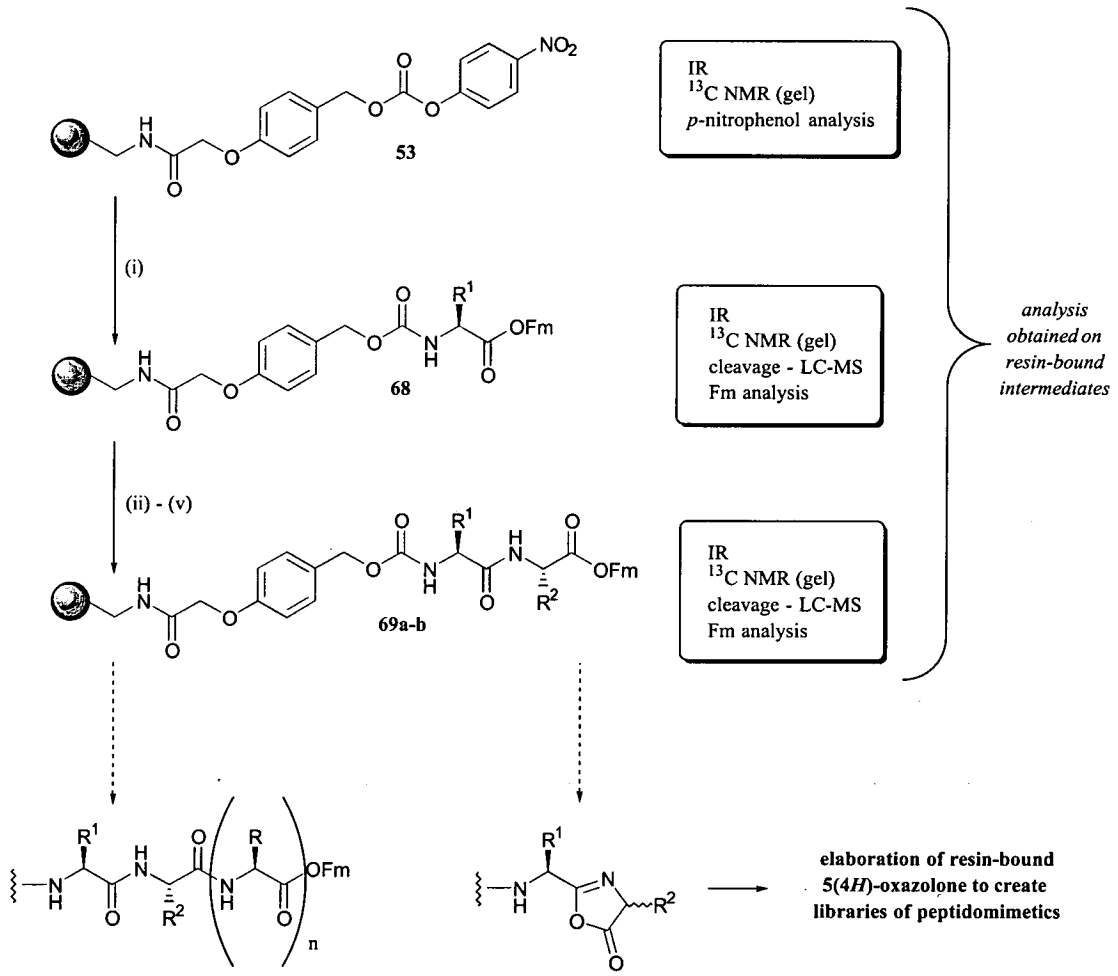
Entry	Amino acid building block (AA)	Boc-AA-OFm		TSA-AA-OFm		HOBt-AA-OFm	
		N°	% yield	N°	% yield	N°	% yield
1		**64a**	60 %	**65a**	96 % (58 %[a])	**66a**	86 %
			N/A[b]		91 %[a]		
2		**64b**	N/A[b]	**65b**	87 %[a]	**66b**	56 %
3		**64c**	N/A[b]	**65c**	96 %[a]	**66c**	N/A[c]
4		**64d**[AW]	N/A[b]	**65d**[AW]	78 %[a]	**66d**	17 %[d]
5		**64e**[AW]	N/A[b]	**65e**[AW]	99 %[a]	-	-

Table 2 *Synthesis of building blocks:* a) % yield over two steps b) crude product reacted on without purifying c) trituration unsuccessful, therefore reacted as crude solution in DMF d) trituration inefficient, therefore *in situ* exchange attempted during coupling

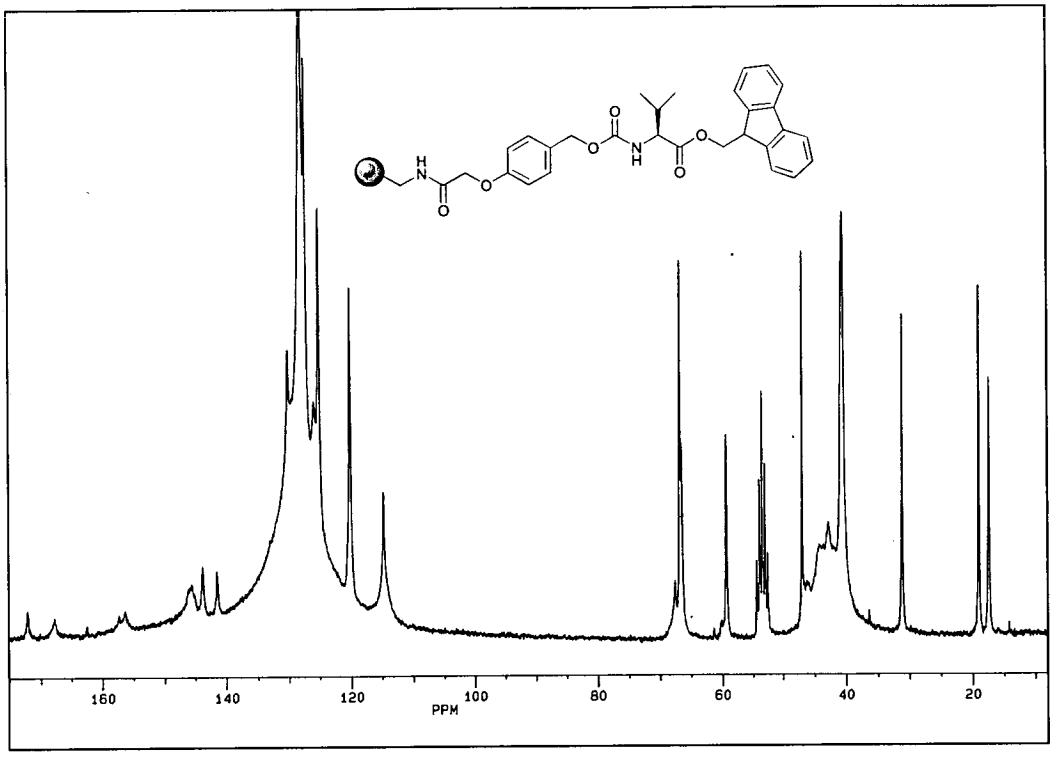
Glycine (R = H) and leucine (R = $CH_2CH(CH_3)_2$) posed the main problems with respect to HOBt salt formation. In each case trituration, after salt exchange, proved problematic. HOBt-Gly-OFm (**66c**) was therefore prepared in the usual manner, but reacted successfully as a crude solution in DMF. In contrast, *in situ* exchange of TSA-Leu-OFm (**65d**) to the corresponding HOBt salt (**66d**) was attempted during the coupling reaction, with only moderate success.

3.1.3 Application of Fm-Ester Strategy on PS-HMPA System

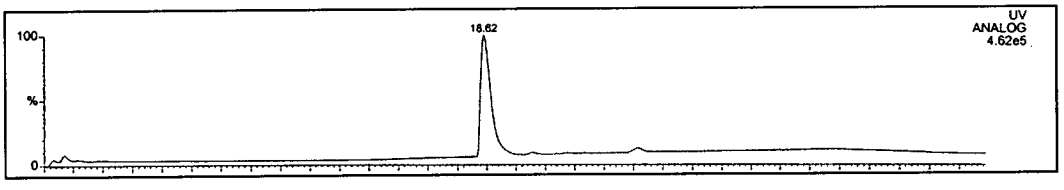


Scheme 19 Reactions and conditions: (i) TSA-AA_1-OFm, DMAP, DMF (ii) 20 % piperidine, DMF (iii) 3 % acetic acid, DCM (iv) TBTU, NMM, DMF (v) HOBt-AA_2-OFm (vi) EDCI, DCM; **69a** R^1 = $CH(CH_3)_2$, R^2 = CH_2Ph; **69b** R^1 = $CH(CH_3)_2$, R^2 = H

The first amino acid residue Fm ester derivative was coupled as its TSA salt to activated PS-HMPA resin (**53**) to afford **68** (Scheme 19). Deprotection,



Spectrum 4 *Gel phase ^{13}C NMR of* ***68***



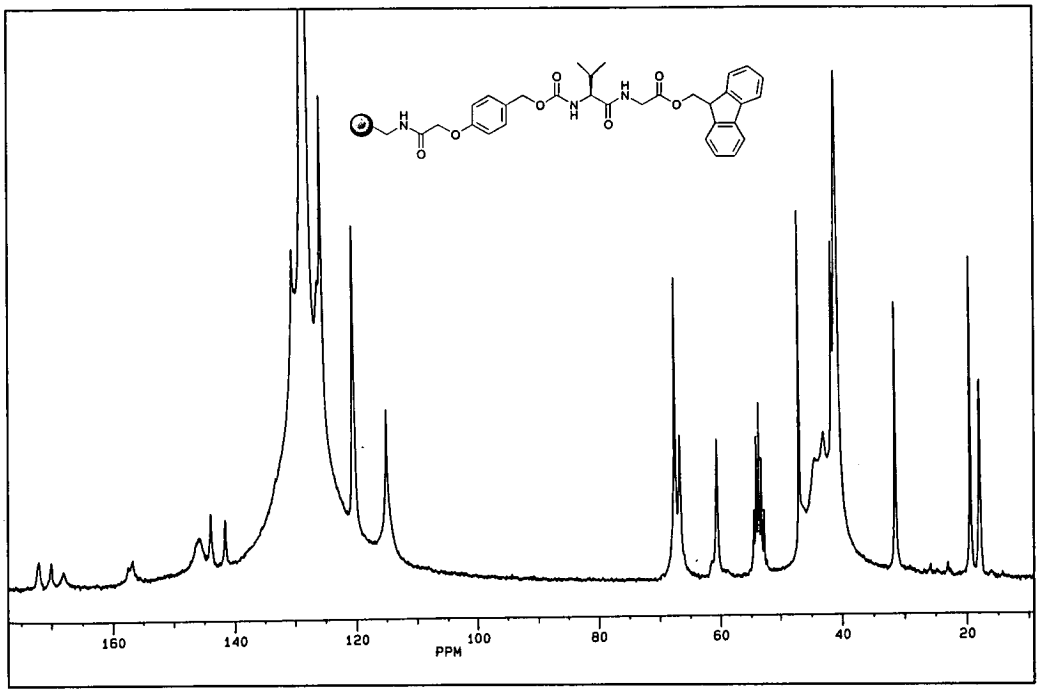
Spectrum 5 *LC-MS trace at 214 nm of* ***68*** *after cleavage*

neutralisation and activation were carried out as described by Bayer, before coupling of the second amino acid Fm ester as its HOBt salt to yield the corresponding dipeptide (**69**).

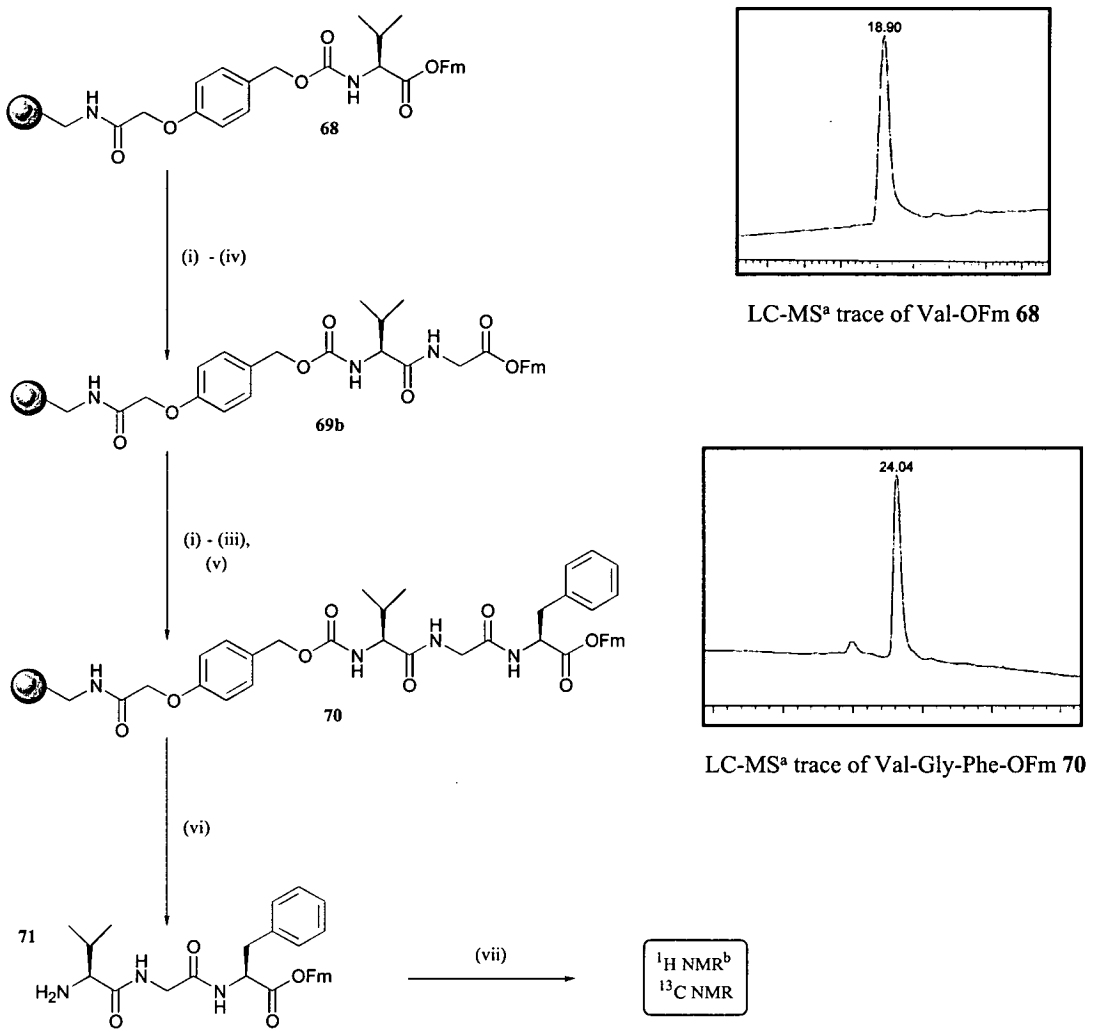
Resin-bound dipeptide Val-Phe-OFm (**69a**) was prepared in this way and unlike the attempts detailed in Chapter 2, no over-coupled by-products were observed. Instead, gel phase ^{13}C NMR analysis for both PS-HMPA-Val-OFm (**68**) (Spectrum 4)[op] and PS-HMPA-Val-Phe-OFm (**69a**), gave very high quality spectra, with no starting material being observed. Furthermore, LC-MS analysis of each intermediate indicated that both intermediates had been formed in high purity e.g. (Spectrum 5).[op]

At the dipeptide stage, two options for proceeding exist (Scheme 19). If the sequence of deprotection, neutralisation, activation and coupling of HOBt-AA-OFm is repeated, continued peptide elongation may be achieved. Alternatively, the dipeptide may serve as the precursor to the target resin-bound 5(4*H*)-oxazolone. This latter transformation is demonstrated and extended in the following chapter (**4.1.2**).

In order to demonstrate the applicability of this inverse system and confirm the purity of the products obtained, the tripeptide Val-Gly-Phe-OFm (**71**) was synthesised, cleaved from the resin and purified by semi-preparative HPLC (Scheme 20).



Spectrum 6 *Gel phase ^{13}C NMR of* ***69b***



Scheme 20 Reactions and conditions: (i) 20 % piperidine, DMF (ii) 3 % acetic acid, DCM (iii) TBTU, NMM, DMF (iv) HOBt-Gly-OFm, DMF (v) HOBt-Phe-OFm, DMF (vi) TFA, DCM, H_2O (9:10:1) (vii) preparative HPLC purification elution gradient (**6.4.3** Table 9) a) LC-MS elution gradient A (**6.4.2** Table 7) b) see Figure 20

The dipeptide Val-Gly-OFm (**69b**) was synthesised in the same manner as Val-Phe-OFm (**69a**), except as already mentioned, a crude solution of HOBt-Gly-OFm (**66c**) was employed. At this stage, rather than performing a cyclisation to yield the resin-bound 5(4*H*)-oxazolone, peptide synthesis was continued by coupling HOBt-Phe-OFm (**66a**) to afford the tripeptide **70**. Each solid phase intermediate up till this point had been fully and satisfactorily characterised using the on-resin techniques available eg Spectrum 6^{op}. In addition, an aliquot (4 - 6 mg) of each resin sample was cleaved and analysed by ESMS and LC-MS. However, in order to obtain a solution for ^{1}H NMR, a larger resin sample (70 mg) of the tripeptide (**70**) was cleaved and purified by semi-preparative HPLC (**6.4.3**). The purified tripeptide product was analysed by

^{1}H NMR to give the desired spectrum (Figure 20) as clear evidence of the success of this approach.

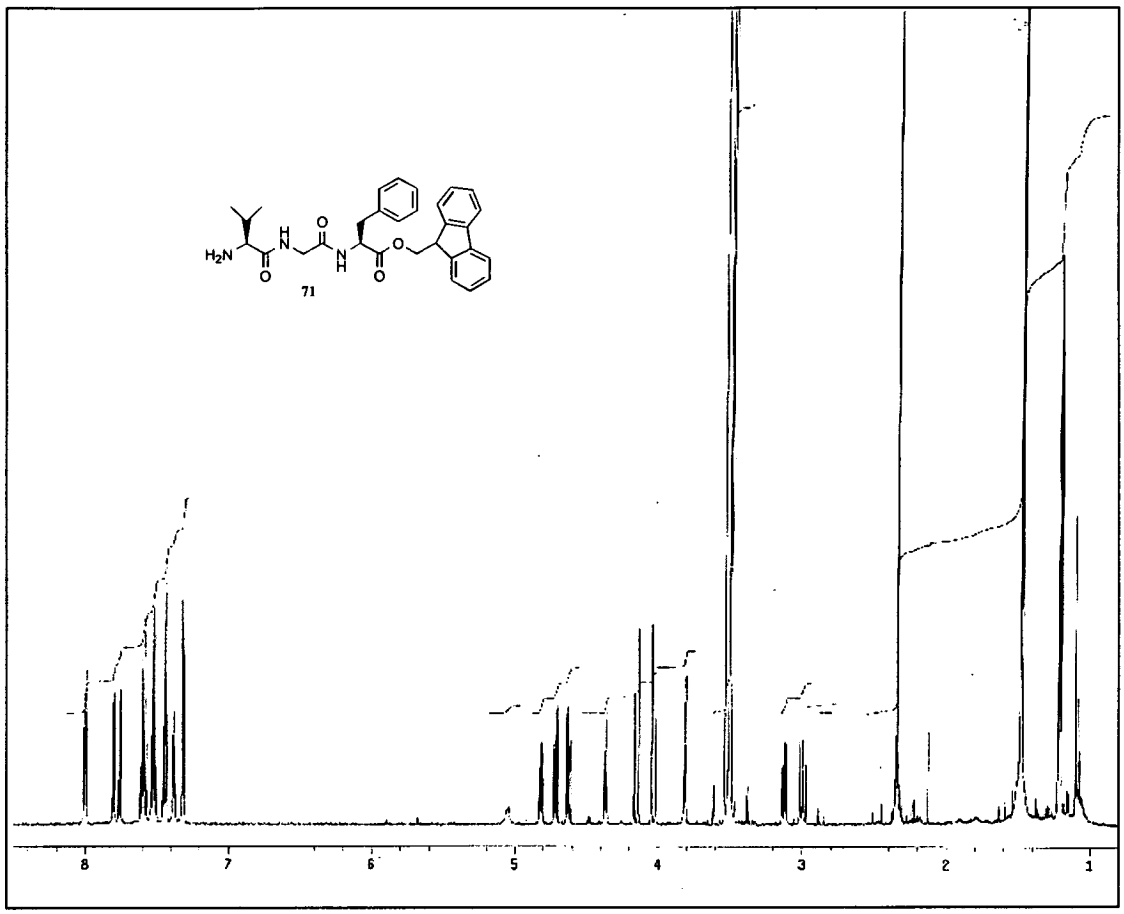
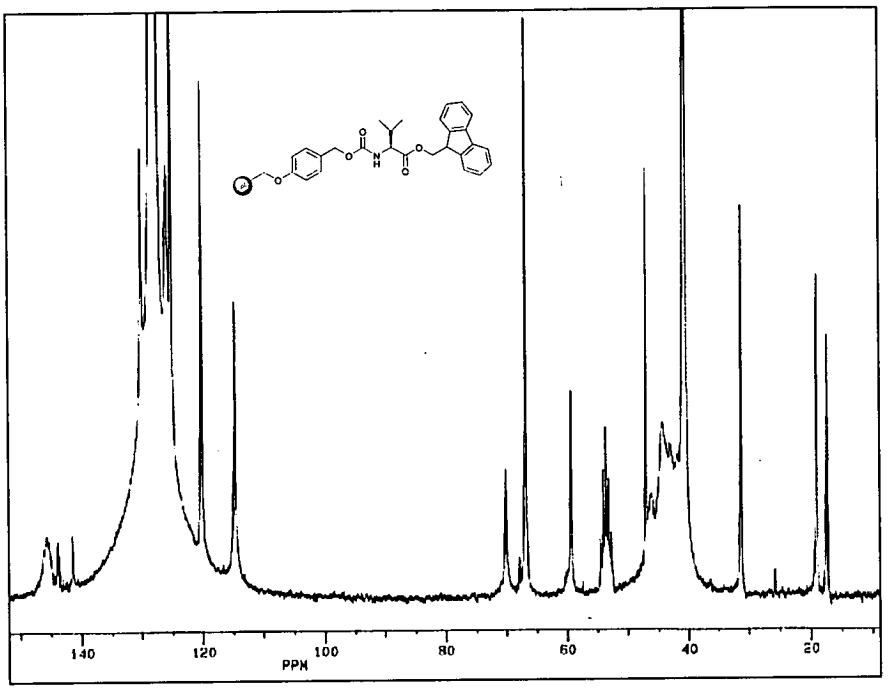


Figure 20 *^{1}H NMR spectrum of purified Val-Gly-Phe-OFm 71*

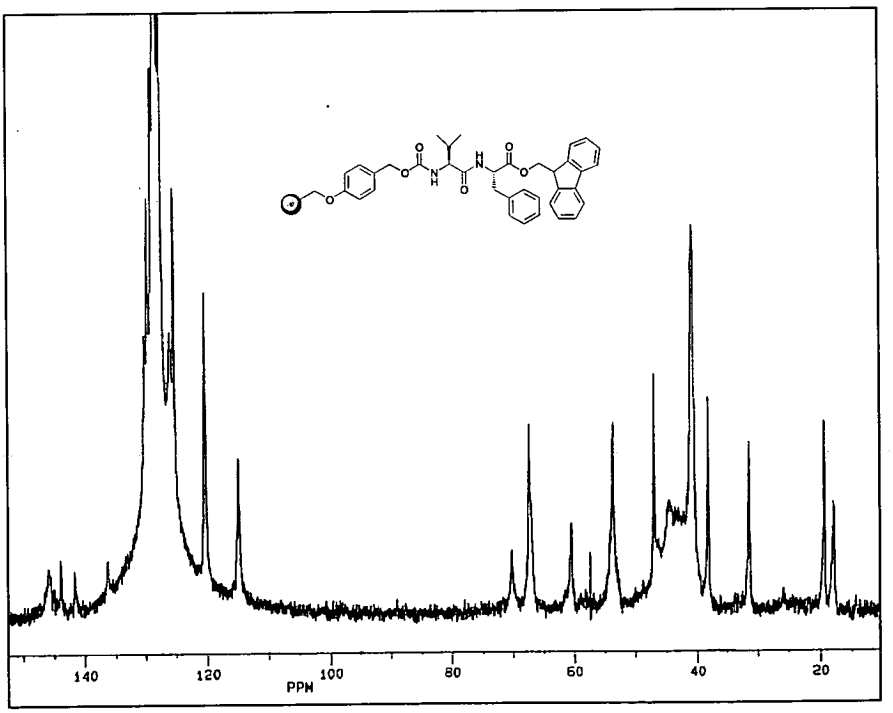
3.2 Linker Modification

3.2.1 Comparison of Wang and HMPA Linkers

In order to test the applicability and versatility of this inverse approach further, it was decided to synthesise a library of diverse tripeptides and C-terminally modified dipeptides (**3.3.3**). However when synthesising even the smallest library, cost and availability of starting materials are both important factors to be considered. It was with this in mind that the Wang linker was considered as an alternative to the HMPA linker, upon which all the previous investigations had been performed. The Wang resin (**46**) is commercially available and is also significantly cheaper than the PS-



Spectrum7 *Gel phase ^{13}C NMR of* ***73a***

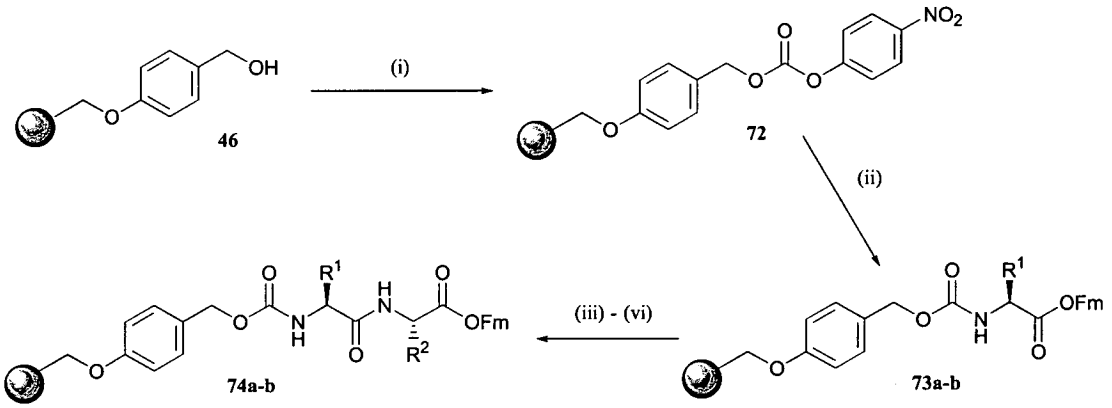


Spectrum 8 *Gel phase ^{13}C NMR of* ***74a***

HMPA (**47**) precursor, aminomethylated polystyrene resin, of comparable size and loading *e.g.* £26 and £34 respectively per 5 g (0.5 - 1.2 mmol/g)[Nova]. The decision was therefore made to adopt the PS-Wang linker system as a cheaper and faster alternative, especially since large quantities of resin would be required for the library synthesis and further studies.

3.2.2 Inverse N-C Approach Using the Wang Linker System

Thus PS-Wang (**46**) was activated in the same manner as the PS-HMPA system to yield the activated carbonate **72** in 100 %* yield (0.91 mmol/g) (Scheme 21). The dipeptides PS-Wang-Val-Phe-OFm (**74a**) and PS-Wang-Ala-Leu-OFm (**74b**) were prepared in order to confirm the applicability of the Wang system to the inverse strategy. Both dipeptides were prepared as depicted in Scheme 21, with thorough analysis of each intermediate confirming the expected successful translation of the inverse methodology between the two, highly similar linker systems (Spectra 7 & 8).[op]



Scheme 21 *Reagents and conditions:* (i) *p*-nitrophenyl chloroformate, pyridine, DCM (ii) TSA-AA_1-OFm, DMF (**73a** $R^1 = CH(CH_3)_2$, **73b** $R^1 = CH_3$) (iii) 20 % piperidine, DMF (iv) 3 % acetic acid, DCM (v) TBTU, NMM, DMF (vi) HOBt-AA_2-OFm (**74a** $R^1 = CH(CH_3)_2$, $R^2 = CH_2Ph$, **74b** $R^1 = CH_3$, $R^2 = CH_2CH(CH_3)_2$)

Essentially the only problem encountered was the coupling of Leu-OFm. Trituration of the HOBt salt **66d** had proved fruitless, so an *in situ* salt exchange during the coupling reaction was attempted, with only moderate success.

3.3 Scope of Inverse Approach

3.3.1 Combinatorial Approaches and Diversity

The different strategies for introducing molecular diversity to compound libraries are well documented.[3,21,27] In order to test the scope and applicability of this method for inverse peptide synthesis, a small library of peptide analogues of the general structure **75** (Figure 21), was prepared using a variety of building blocks in a parallel fashion (**3.3.3**).

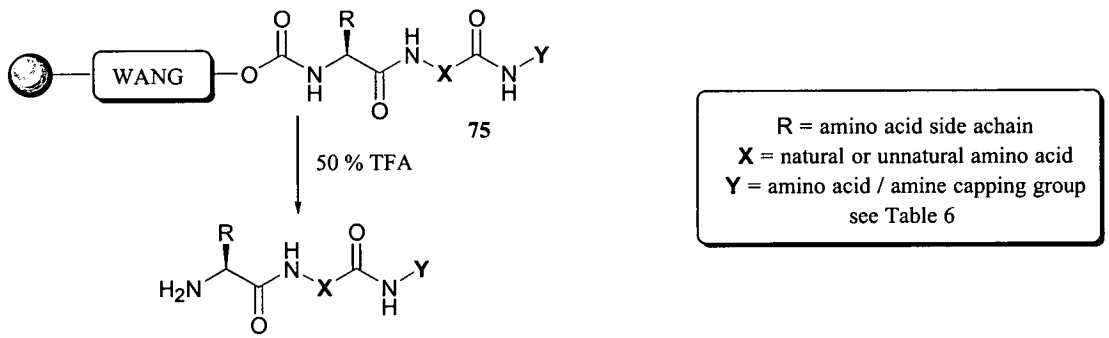


Figure 21 *General structure of library components*

3.3.2 Preparation of Library Building Blocks

A range of Fm ester protected building blocks (Table 3) were prepared using the method previously described (**3.1.2**). This series of natural and non-proteinogenic amino acids, were first protected as their Fm esters (**76a-d**), before deprotection of the N-termini and final exchange to the HOBt salts (**78a-d**). Once again, the Boc-AA-OFm derivatives (**76a-d**) were isolated directly as TSA salts (**77a-d**), however trituration was not feasible for some intermediates which simply formed oils. Crude TSA-Acp-OFm (**77b**) was carried through one stage further to the HOBt salt (**78b**) where trituration once again proved futile. This intermediate was therefore coupled successfully as a crude solution in DMF, although the resulting dipeptide was not as pure as the other resin-bound intermediates. Attempted derivatisation of anthranilic acid in this way also proved problematic, so Boc-Abz-OFm (**76d**) was instead

purified before deprotection to form the TSA salt (**77d**). The synthesis, analysis and subsequent coupling of these derivatives were also hampered by poor solubility.

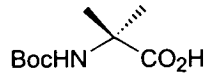
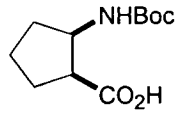
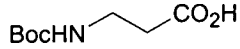
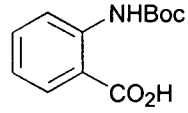
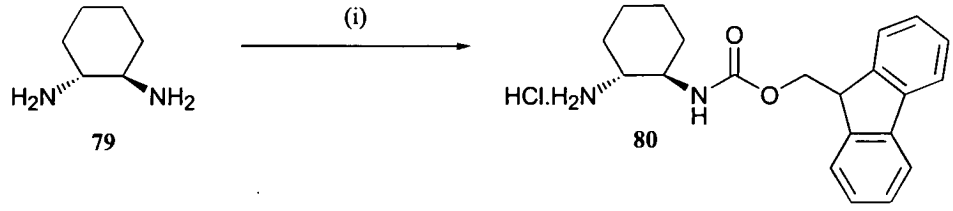
Entry	Amino acid building block (AA)	Boc-AA-OFm		TSA-AA-OFm		HOBt-AA-OFm	
		N°	% yield	N°	% yield	N°	% yield
1		**76a**	N/A[a]	**77a**	56 %[b]	**78a**	82 %
2		**76b**	N/A[a]	**77b**	N/A[c]	**78b**	N/A[d]
3		**76c**	N/A[a]	**77c**	87 %[b]	**78c**	87 %
4		**76d**	15 %	**77d**	86 % (13 %[b])	**78d**	N/A[e]

Table 3 *Synthesis of building blocks:* a) crude product reacted on without purifying b) % yield over two steps c) trituration unsuccessful, therefore carried directly through to HOBt salt d) trituration unsuccessful, therefore reacted as crude solution in DMF e) trituration unsuccessful, therefore *in situ* exchange attempted during coupling

These difficulties serve to highlight the problem of trying to implement generic procedures for structurally diverse building blocks. Commercially available building blocks are therefore an advantage, but in turn, restrict the diversity which can be incorporated.

The C_2-symmetric diamine (**79**) (Scheme 22) was also selected as a potential building block. It was hoped that incorporation of this unit into the peptide chain would provide access to Jacobsen and Katsuki-type ligands[126] for asymmetric catalysis (**3.3.4**). Mono-protection of the symmetrical diamine was deemed necessary, in order to prevent both amino groups reacting with activated resin-bound C-terminal residues, as this would probably result in undesirable cross-linking of the resin.



Scheme 22 *Mono-protection of symmetrical diamine* - reagents and conditions: (i) 9-fluorenylmethyl chloroformate, diethyl ether, 0 °C

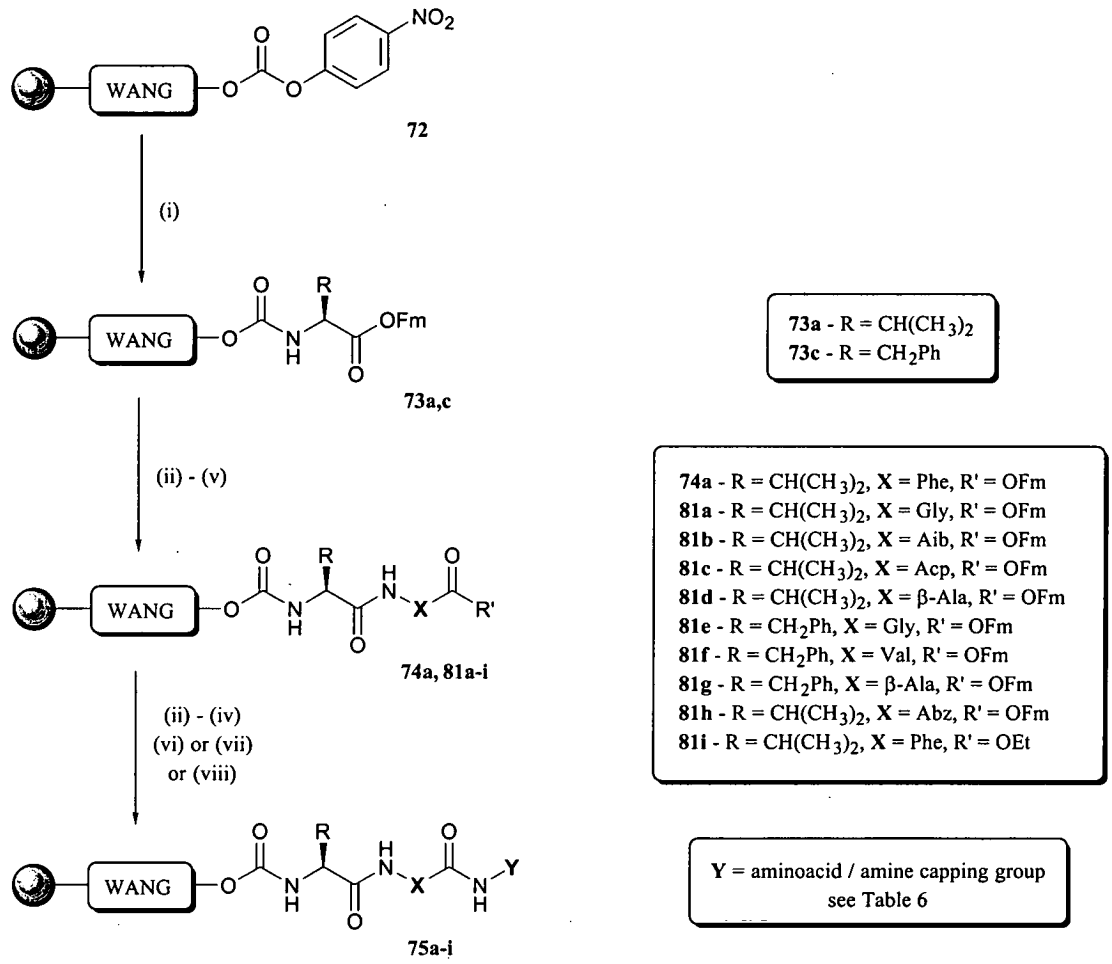
A solution of the diamine (**79**) was added drop-wise to a cooled solution of 9-fluorenylmethyl chloroformate in ether. It was hoped that **80** would precipitate before the second amino group could attack a further molecule of chloroformate, but that if a second displacement did take place, the di-protected amine would remain in solution.

Slow addition of the diamine did result in the immediate formation of a white precipitate, which was filtered and dried. Unfortunately, the white solid proved insoluble in all deuterated solvents available. However, ^{1}H and ^{13}C NMR analysis of a suspension of the salt in d^6 DMSO did indicate that the desired product had formed, but that it was possible that some of the unwanted dichloride species was also present. In hindsight, a better method for minimising formation of the dichloride species, could be reverse addition of the chloroformate to an excess solution of the diamine. However, since the starting material was relatively expensive, it was used as a library building block in its crude form, as it was hoped that the solubility of the mono-protected species would be sufficient to allow some diffusion into the resin's reactive sites with the reaction being driven to completion by the resulting equilibrium. By the same analysis, it was predicted that the highly insoluble dichloride salt would remain as a suspension and no reaction would take place. The observed results are discussed in Section **3.3.3**.

3.3.3 Library Synthesis

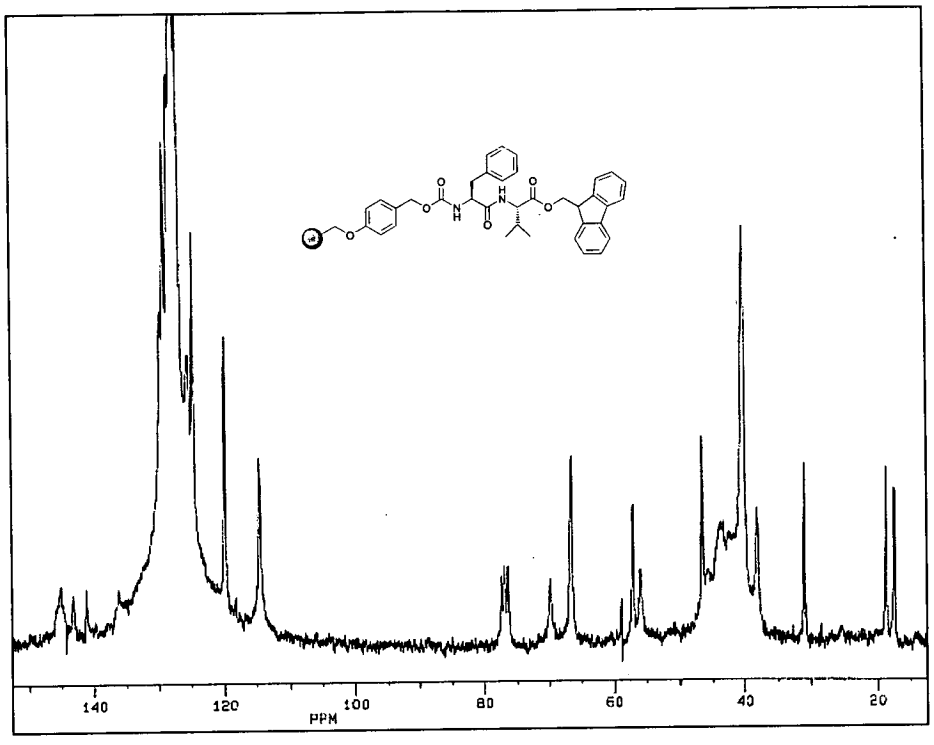
Therefore, in order to examine the scope of the synthesis, a library consisting of both natural and unnatural tripeptides as well as C-terminally modified dipeptides was prepared using the set of building blocks described previously (**3.3.2**). The activated

polystyrene-bound Wang resin (**72**) was divided into two portions and derivatised as shown (Scheme 23). The N-terminal amino acid residues, Val-OFm and Phe-OFm, were coupled as their TSA salts using DMAP and DMF to give **73a** and **73c**, each with a calculated loadings of 0.45 mmol/g. Full on-resin analysis, followed by cleavage and ESMS confirmed that both couplings had proceeded successfully, leading to single peaks in the LC-MS.

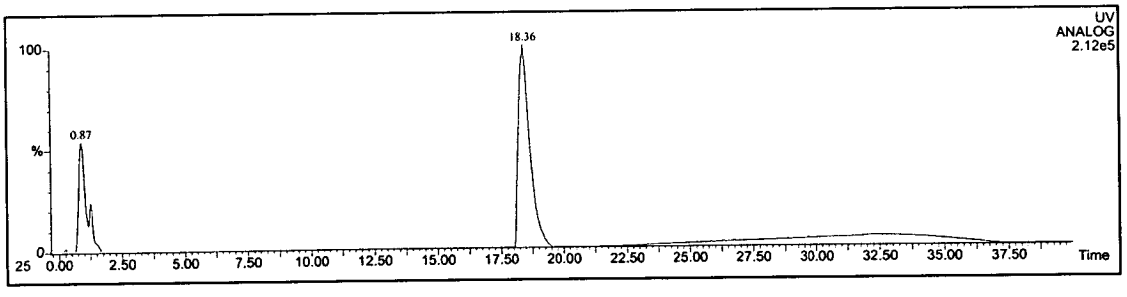


Scheme 23 *Library Synthesis* - reagents and conditions: (i) TSA-Val-OFm or TSA-Phe-OFm, DMAP, DMF (ii) 20 % piperidine, DMF (iii) 3 % acetic acid, DCM (iv) TBTU, NMM (v) HOBt-**X**-OFm, DMF (vi) HOBt-AA_1-OFm, DMF (vii) amine, HOBt.H_2O, DMF (viii) TSA-AA_2-OFm or HCl-AA_2-OFm, HOBt.H_2O, Et_3N, DMF

The two supported amino acid esters (**73a** & **73c**) were then divided into 300 mg portions. Each sample was deprotected, neutralised, activated and derivatised



Spectrum 9 *Gel phase ^{13}C NMR of* ***81f***



Spectrum 10 *LC-MS trace at 214 nm of* ***81g*** *after cleavage*

according to the protocol detailed in Table 4, to yield a variety of supported dipeptides (Table 4).

Step	Reagent / solvent	Duration, repetition & volume[a]
deprotection	20 % piperidine in DMF	3 x 15 min / 10 ml
wash	DMF	3 x 10 ml
neutralisation	3 % acetic acid in DCM	4 x 5 min / 12 ml
wash	DMF	3 x 10 ml
activation	TBTU (8 eq), NMM (8 eq), DMF	≥40 min / 15 ml
wash	DMF	3 x 10 ml
coupling of amino acid or capping amine	b) HOBt-AA-OFm (6 eq), DMF or c) HOBt.H_2O (6 eq), amine (6 eq), DMF or d) X-AA-OFm, HOBt.H_2O, Et_3N (6 eq ea), DMF or e) HCl-Phe-OEt, HOBt.H_2O, Et_3N (6 eq ea), DMF	≥15 h / 15 ml
wash	standard wash protocol (**6.2.4**)	-

Table 4 *Protocol for inverse N-C library synthesis - coupling of second and subsequent building blocks to resin bound protected peptide* **73a** & **73c** - a) ml/300 mg resin b) building block = amino acid (AA) (**6.8.3**) c) building block = amine (RNH_2) (***6.9.14.2***) d) building block = amino acid (AA) where exchange to HOBt salt has not been possible (**6.9.11**), X = TSA or HCl e) ethyl ester derivative employed to prepare component suitable for fragment coupling

Each supported product was analysed by IR, gel phase ^{13}C NMR and Fm analysis, before acidic cleavage and subsequent LC-MS analysis. The spectra obtained in every case, apart from **81h**, confirmed successful conversion to the desired product. The clarity of the spectra, coupled with the single peaks observed on the LC-MS traces, indicated that the couplings had proceeded very cleanly and in high yield (Spectra 9 & 10).[op] Loadings in each case were high, further indicating that the couplings had proceeded efficiently.

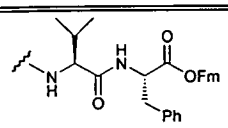
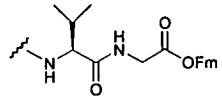
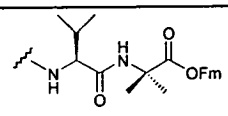
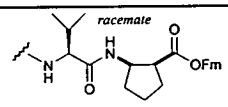
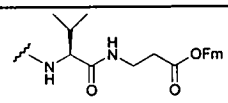
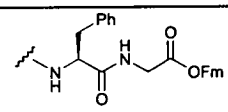
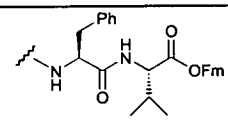
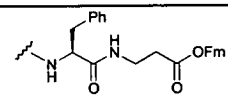
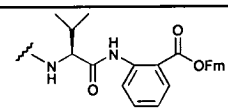
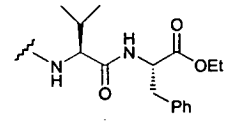
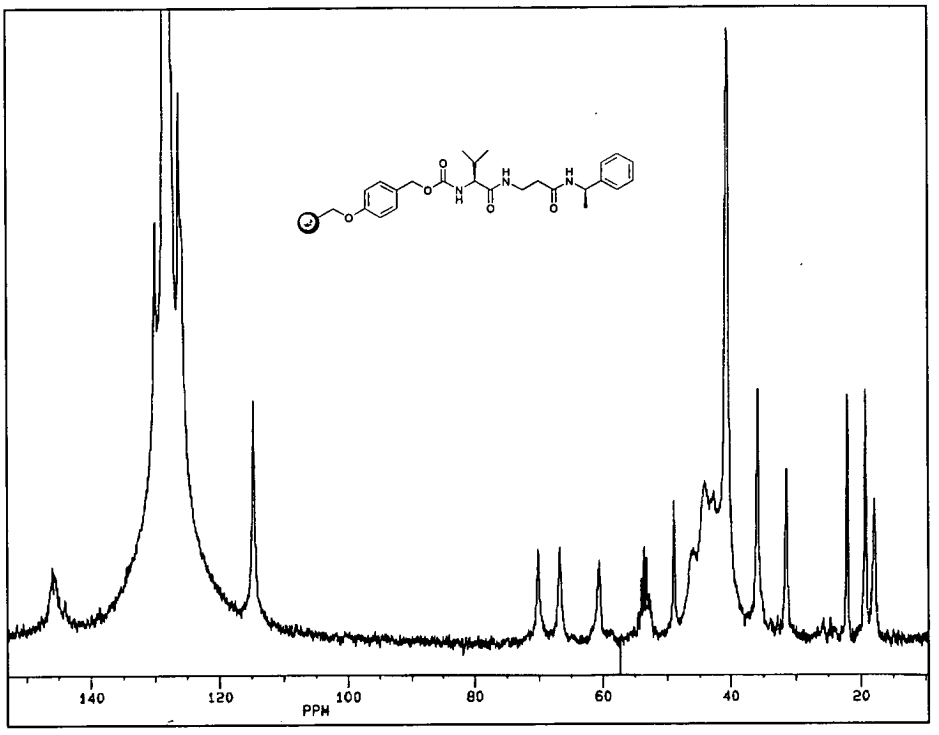
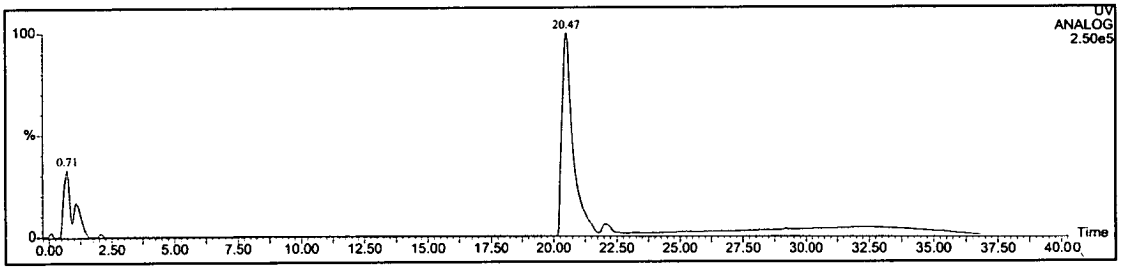
N°	Resin PS-HMPA	Peptide	ESMS[a] MH^+	LC-MS[b] R_t min	Fm[c] mmol/g	IR	NMR	Purity %[d]
74a	-Val-OFm		443	21.1	0.57	✓	✓	A
81a	-Val-OFm		353	9.1	0.54	✓	✓	A
81b	-Val-OFm		381	16.9	0.43	✓	✓	A
81c	-Val-OFm		407	18.5[e] 19.2[e]	0.49	✓	✓	A
81d	-Val-OFm		367	12.0	0.65	✓	✓	A
81e	-Phe-OFm		401	17.8	0.43	✓	✓	A
81f	-Phe-OFm		443	20.7	0.42	✓	✓	A
81g	-Phe-OFm		415	18.4	0.37	✓	✓	A
81h	-Val-OFm		✖	✖	✖[f]	?	✖	-
81i	-Val-OFm		293	18.6[f]	N/A	✓	✓	A

Table 5 *Data for resin-bound peptides formed from coupling of second amino acid building block:* a) ES-MS after cleavage with TFA:DCM:H_2O (9:10:1) (**6.4.1**) b) LC-MS gradient A as detailed in section **6.4.2** c) after treatment with 20 % piperidine in DMF (***6.3.4.1***) d) estimated from LC-MS UV trace: A = 80-100 % e) one of two *cis* diastereomers f) UV spectrum did not give standard dibenzofulvene curve g) LC-MS gradient B as detailed in section **6.4.2**.

The failure of the anthranilic acid derivative to couple successfully is likely to be related to the difficulties encountered when preparing the HOBt derivative **78d** (**3.3.2**) and the poor nucleophilicity of the aromatic amine. TSA-Abz-OFm (**77d**) was eventually employed in the coupling reaction and an *in situ* salt exchange was



Spectrum 11 *Gel phase ^{13}C NMR of* ***75e***



Spectrum 12 *LC-MS trace at 214 nm of* ***75b*** *after cleavage*

attempted using Et_3N and HOBt hydrate. IR analysis of the resin-bound product displayed an absorbance at ν_{max} 1817 cm^{-1}, indicating that the resin was probably still in its activated form, with no visible evidence of coupling having taken place. It is possible, therefore that either the nucleophilicity of the aromatic amino acid was not sufficient to promote coupling under these conditions, or the *in situ* exchange failed, or indeed that the failure was due to combination of both these factors.

This library of resin-bound dipeptides was then deprotected, neutralised and activated as before. Once activated each supported dipeptide was treated with a nucleophile (Table 4). Those compounds treated with amino acid building blocks resulted in continued peptide elongation, others were C-terminally 'capped' with a variety of amines (Table 6). During the activation step, those compounds containing a chiral C-terminal amino acid residue (**74a**, **81f**) became susceptible to oxazolone-mediated racemisation, with the resulting tripeptides (**75a** & **75g**) being obtained as mixtures of diastereomers that were distinguishable by LC-MS.

As before, each resin-bound product was analysed by IR, gel phase ^{13}C NMR, and where applicable, Fm analysis, before acidic cleavage followed by ESMS or LC-MS. There was, however, some evidence of incomplete neutralisation, with peaks in some of the NMR spectra corresponding to the piperidinium salt. Nevertheless, the NMR spectra coupled with the ESMS and LC-MS data obtained, gave clear indication that the main product, in each case was the target peptide or peptidomimetic, with only trace impurities observable (Spectra 11 & 12).[op]

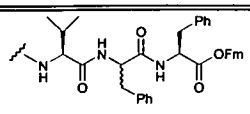
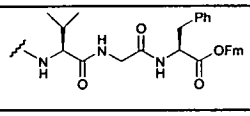
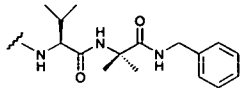
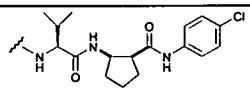
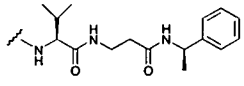
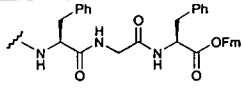
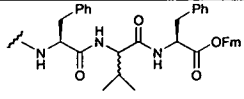
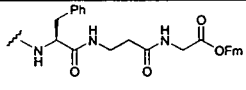
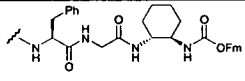
N°	Initial resin	Peptide/C-terminally modified analogue	ESMS[a] MH^+	LC-MS R_t min	Fm[b] mmol/g	IR	^{13}C gel NMR	Purity %[c]
75a	**74a**		590	25.6[d] 26.3[d]	0.35	✓	✓	B
75b	**81a**		500	20.5[d]	0.34	✓	✓	A
75c	**81b**		292	11.4[ef]	N/A	✓	✓	B
75d	**81c**		338[g] 340[h]	20.7[ef]	N/A	✓	✓	C
75e	**81d**		292	15.4[e]	N/A	✓	✓	B
75f	**81e**		548	25.1[d]	0.24	✓	✓	A
75g	**81f**		590	26.4[d] 27.1[d]	0.41	✓	✓	C
75h	**81g**		472	19.4[d]	0.33	✓	✓	A
75i	**81e**		514	19.2[d]	0.04	✓	✖	-

Table 6 *Data for resin-bound peptides formed from coupling of third building block:* a) ES-MS after cleavage with TFA:DCM:H_2O (9:10:1) (**6.4.1**) b) after treatment with 20 % piperidine/DMF (***6.3.4.1***) c) estimated from LC-MS UV trace: A = 80-100 %; B = 60-80 %; C = 40-60 % d) LC-MS gradient A as detailed in section **6.4.2** e) LC-MS gradient B as detailed in section **6.4.2** f) broad peak from two diastereomers g) MH^+/Cl^{35} h) MH^+/Cl^{37}

The only derivative which did not appear to undergo successful coupling, in this instance was **75i**. Although a weak product signal was observed by ESMS, no meaningful information could be derived from the gel phase ^{13}C NMR. Fmoc analysis confirmed the presence of the Fmoc group and it was concluded that the coupling had occurred to yield **75i**, but only to a very slight extent. Rather than the reactivity of the amine posing the problem, it is likely that lack of solubility and purity of the building block are to blame for the poor coupling observed. Although sonication at elevated temperature and addition of triethylamine were employed in

an attempt to aid dissolution of the amine salt, the reaction was finally carried out as a milky suspension. It is therefore hardly surprising that only a trace amount of the desired peptide analogue (**75i**) was detected. One way to overcome the poor solubility in DMF could have been to dissolve the amino salt in TFA and induce formation of the TSA salt before converting to the HOBt salt. Time restrictions, however, prevented this course of action from being investigated.

Nevertheless, small peaks corresponding to deletion peptides *i.e.* those arising from incomplete coupling of the second amino acid residue, were detected in a few of the library members. However, LC-MS indicated that these impurities were minor, with the target product being the major peak. Product purity was estimated to be greater than 85 % for the majority of library members prepared using the Fm methodology.

3.3.4 Conclusions

Overall, it has been demonstrated that Bayer's approach for the inverse synthesis of peptides may be applied to the PS-HMPA and PS-Wang systems. Moreover the method is amenable to a wide range of building blocks, potentially leading to a vast array of C-terminally modified peptides, for applications in binding studies and as possible therapeutic agents. In addition, loading analysis is facilitated by the UV active Fm ester protecting group, however other esters may instead be employed to afford analogues, such as Val-Phe-OEt **81i**. These analogues may find use as components for the convergent syntheses of long chain peptides, using the fragment condensation approach.[127]

Although the advantages of this method are clear, a number of problems have also been noted. For instance, building block preparation is a costly, time-consuming and often complex process, especially if a large set of diverse units is required. Racemisation also poses a significant problem if long chain polypeptide analogues are desired. However, results describing conditions which can effect complete suppression of the racemisation process[58] indicate that oxazolone-induced racemisation will become a problem of the past. Therefore, assuming that complete suppression of racemisation can be achieved, the solid phase inverse synthesis

described may provide a long-awaited solution to the problem of direct access to C-terminally modified peptide analogues.

Additionally, provided the synthesis of the mono-protected diamine **80** can be optimised and the unit successfully incorporated into the growing peptide chain, this particular class of building block could lead to the development of libraries of diverse metal-salen complexes for asymmetric catalysis (Figure 22).[126] By protecting one of the primary amines with the Fmoc group, the cyclic unit may then be incorporated into the peptide chain *via* the second amino group (**82**). Therefore, once the Fmoc group has been removed, peptide elongation, this time in the classical C-N direction, may be continued to yield folded peptides for use as metal ligands (**83**).

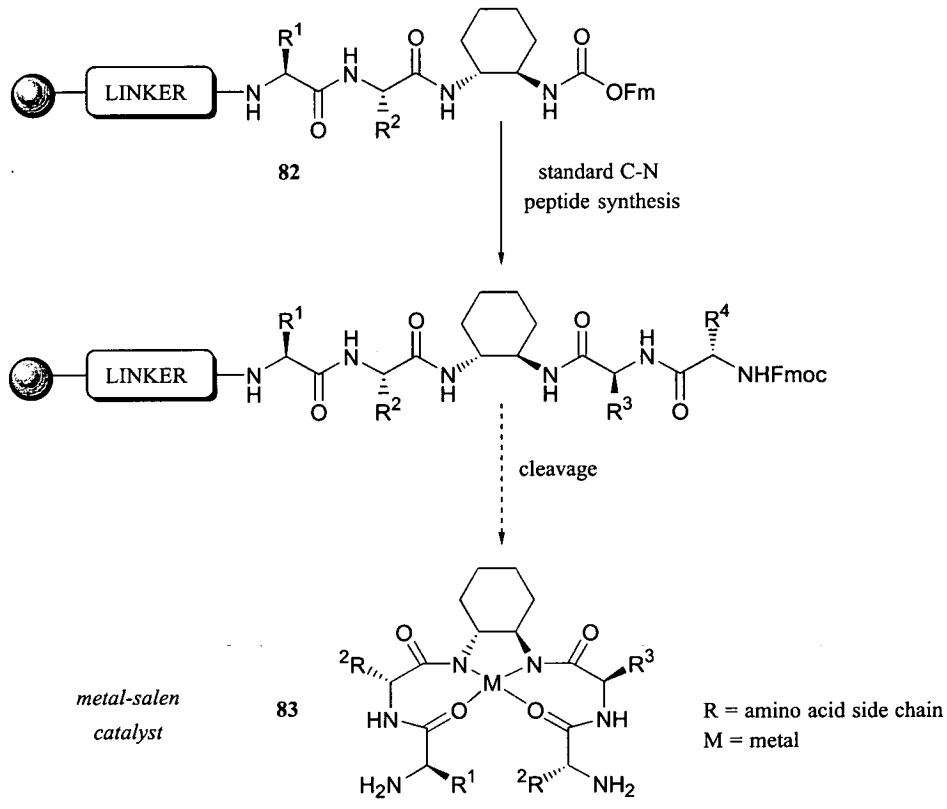


Figure 22 *Application of library products - preparation of metal-salen catalyst*

However, in the context of this research, inverse synthesis is employed primarily as route into the dipeptide-derived 5(4*H*)-oxazolone targets. Oxazolone formation is

therefore necessary and not something to be avoided, with subsequent suppression of the racemisation process being the desirable option.

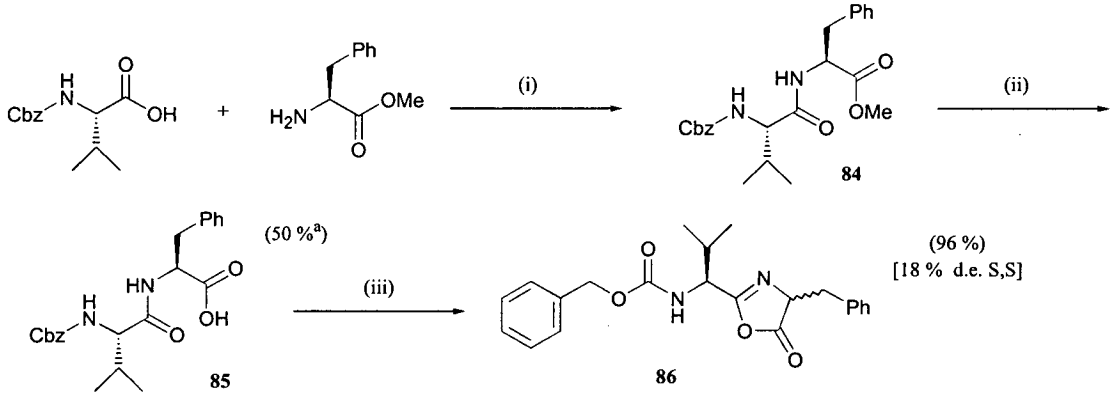
Having established a successful route for the inverse synthesis of peptides, it then remained to apply this to the preparation of the resin-bound dipeptide-derived 5(4*H*)-oxazolone and examine its possible transformations (Chapter 4).

4. Results and Discussion III

4.1 5(4*H*)-Oxazolones - Synthesis

4.1.1 Solution Phase

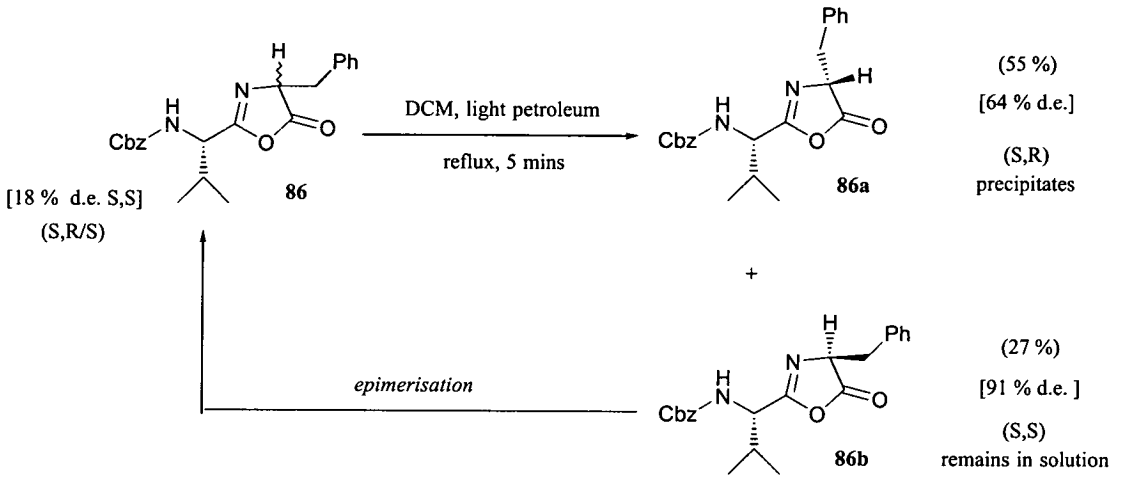
When developing new solid phase strategies and attempting novel transformations on supported intermediates, it is often very useful to perform parallel reactions in solution. Not only can solution phase conditions be optimised before testing on the supported derivatives, but the analysis obtained on pure solution phase samples can serve to assist the characterisation of on-resin analogues. Accordingly, those transformations to be investigated in terms of the solid phase 5(4*H*)-oxazolone, were first carried out in solution and are described in the relevant sections.



Scheme 24 *Solution synthesis of a dipeptide-derived 5(4*H*)-oxazolone **86*** - reagents and conditions: (i) DCC, DCM, 0 °C - RT (ii) LiOH, THF, H_2O (iii) EDCI, DCM; a) yield over two steps

The Cbz-protected 5(4*H*)-oxazolone (**86**) was successfully prepared in a crude yield of 96 % using standard peptide coupling procedures. Initial DCC-mediated coupling of N-protected valine and carboxy-protected phenylalanine afforded enantiopure **84** in a crude yield of 99 %. Since it proved difficult to remove DCC and DCU impurities at this stage, the crude dipeptide ester was used directly in the hydrolysis step. Recrystallisation from chloroform furnished the pure dipeptide (**85**) in an overall yield of 50 %. DCC was found to be unsuitable for the subsequent cyclisation

reaction, since its use necessitated purification by column chromatography, leading to silica-induced decomposition. Instead, treatment with the water-soluble carbodiimide EDCI, promoted dehydration and cyclisation to the target 5(4*H*)-oxazolone (**86**), which was obtained as a mixture of diastereomers [d.e. 18 % (*S*,*S*)].

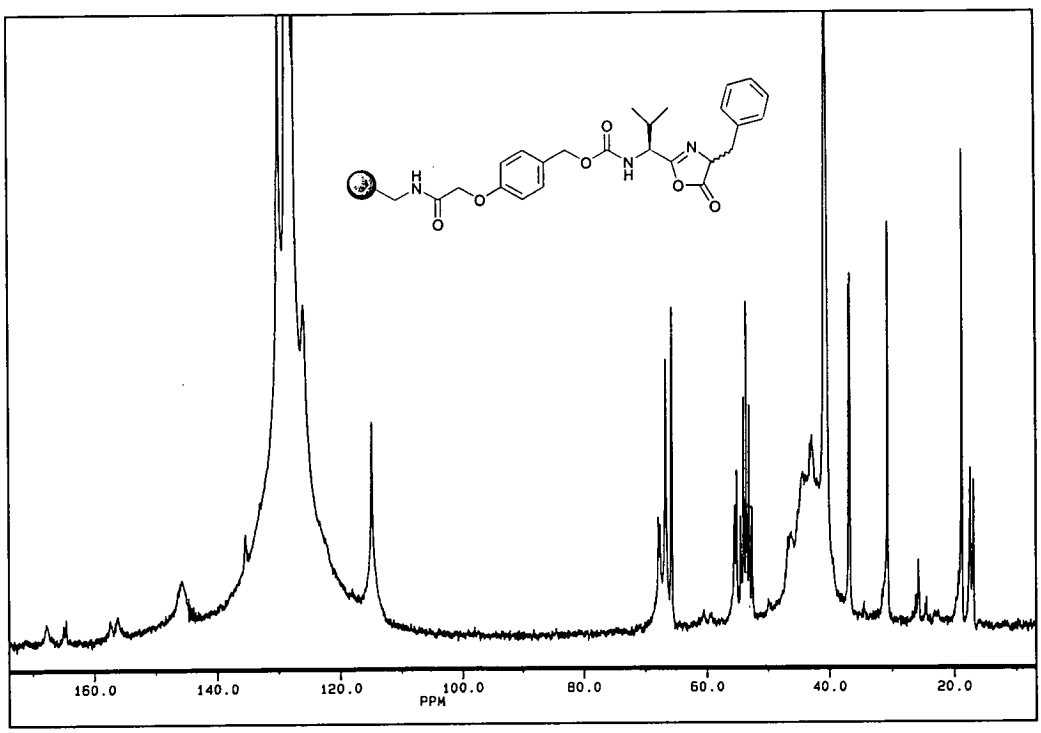


Scheme 25 *Crystallisation-induced dynamic resolution*

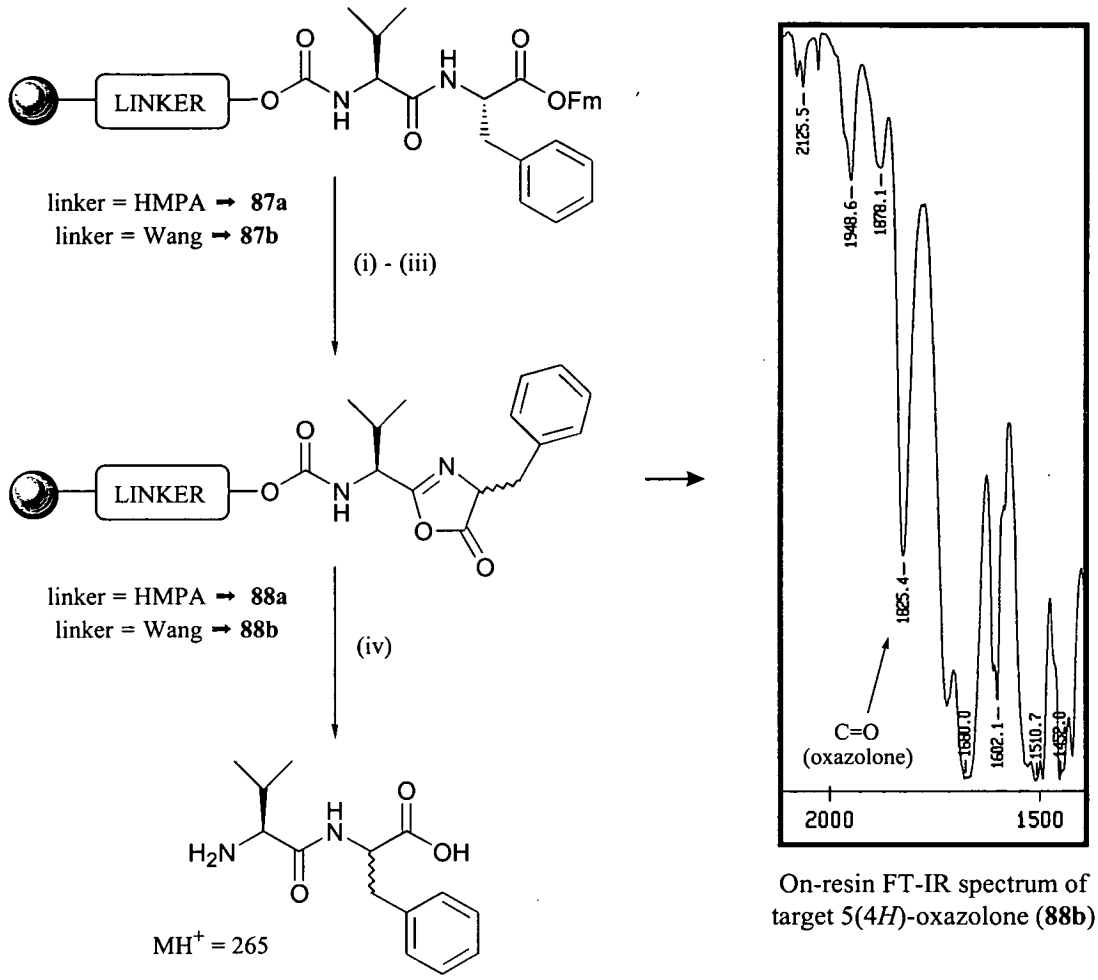
At this point it is worth noting that the separation of the two diastereomers of **86** can be facilitated by employing crystallisation-induced dynamic resolution[98] (Scheme 25). This effect was achieved by simply refluxing the 5(4*H*) oxazolone in DCM/light petroleum (1:8) for 5 min. On cooling, the (*S*,*R*) diastereomer (**86a**) precipitated out of solution whereas the (*S*,*S*) diastereomer (**86b**) was isolated on concentration of the mother liquor. It was also discovered that addition of one drop of Et_3N could induce epimerisation and thus facilitate the conversion of **86b** to **86a**, but not *vice versa*. As the *R* configuration is favoured, this method of resolution could prove a particularly convenient way to access unnatural D-amino acid derivatives.

4.1.2 Solid Phase

The target polymer supported 5(4*H*)-oxazolone (**88**) may be easily obtained from the resin-bound dipeptide precursor (**87**) as shown in Scheme 26.



Spectrum 13 *Gel phase ^{13}C NMR of* ***88a***



On-resin FT-IR spectrum of target 5(4*H*)-oxazolone (**88b**)

Scheme 26 *Solid phase synthesis of target 5(4*H*)-oxazolone* - reagents and conditions: (i) 20 % piperidine, DMF (ii) 3 % acetic acid, DCM (iii) EDCI, DCM (iv) TFA, DCM, H_2O (9:10:1)

Deprotection and neutralisation of the dipeptide derivatives **87a** and **87b** were conducted as if preparing to continue with the inverse peptide synthesis (Chapter 3). Analogous to the solution synthesis, cyclisation to the 5(4*H*)-oxazolones **88a** and **88b** was then promoted by treatment with EDCI. IR analysis gave the distinctive oxazolone carbonyl stretch (ν_{max} = 1825 cm^{-1}) as illustrated in Scheme 26 and gel-phase ^{13}C NMR analysis confirmed the presence of each oxazolone (**88a-b**) as a mixture of diastereomers (Spectrum 13).[op] ESMS analysis gave the mass ions corresponding to the hydrolysis products ($MH^+ + H_2O$), since the acidic cleavage conditions induce ring-opening of the heterocycle to its peptide precursor.

Since oxazolones may be considered as activated ester derivatives of dipeptides, a further method for confirming their presence, is ring-opening with a nucleophile. If successful, this serves to confirm that the resin bound intermediate is indeed in an activated form, as well as demonstrating one of the many useful applications of 5(4*H*)-oxazolones *i.e.* as precursors to C-terminally modified peptide analogues.

4.2 5(4*H*)-Oxazolones - Transformations

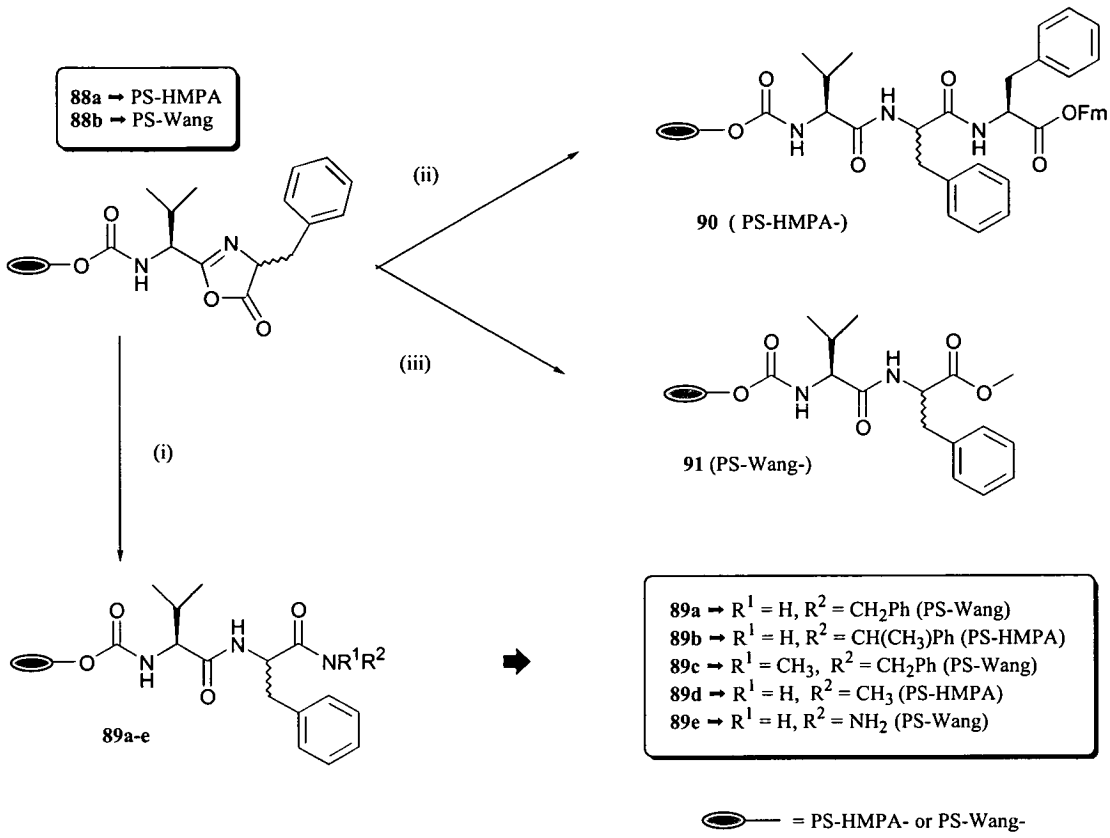
4.2.1 Ring-Opening Reactions

4.2.1.1 General Introduction

As mentioned above, 5(4*H*)-oxazolones may be considered as activated ester derivatives of amino acids or peptides and thus are highly susceptible to nucleophilic ring opening.[87,88] Moreover oxazolone derivatives which are unsaturated[128] and even dialkylated[56,129] at the C4 position can been readily cleaved with amine nucleophiles. A variety of ring-opening reactions were examined on the solid phase derivatives (**88a-b**), in order to demonstrate the potential for the preparation of C-terminally modified peptide analogues and also the ability to continue peptide synthesis after elaboration of the core fragment.

4.2.1.2 Solid phase ring-opening reactions

The polymer-supported 5(4*H*)-oxazolone intermediates **88a** and **88b** were subjected to a series of nucleophilic ring opening reactions using a variety of substituted amines to afford a series of peptide amides (Scheme 27).



Scheme 27 *Ring-opening of resin-bound 5(4H)-oxazolone* - reagents and conditions: (i) NHR^1R^2, DMAP, NMP (ii) TSA-Phe-OFm, DMAP, DMF (iii) Et_3N, MeOH, DMF

The general procedure involved addition of the amine nucleophile (3 eq) and catalytic DMAP to a suspension of the resin which was pre-swollen in NMP. After agitating for at least 15 h, the resin was filtered and washed according to the standard procedure and analysed in the usual manner. A series of C-terminally modified peptides (**89a-e**) were obtained using this procedure, demonstrating the variety of amines tolerated (Table 7).

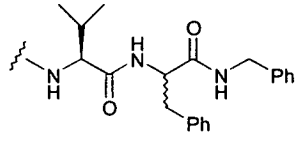
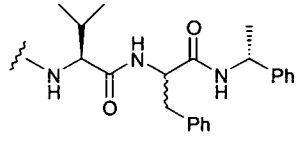
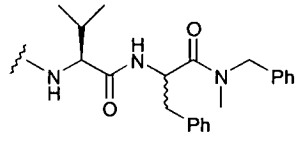
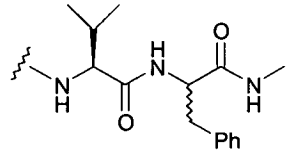
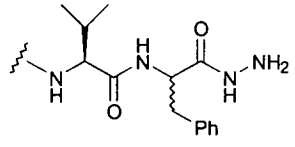
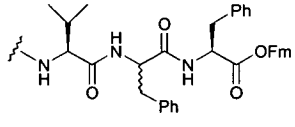
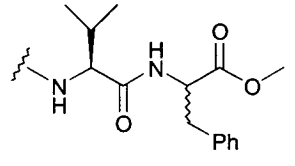
N°	S.M.	Nucleophile	Ring-opened product	ESMS[a] (M + H)	LC-MS R_t min	IR	^{13}C gel NMR
89a	**88b**	H_2N Ph		354	5.5[b] 8.3[b]	✓	✓
89b	**88a**	H_2N Ph		368	20.8[c] 21.6[c]	✓	✓
89c	**88b**	N H Ph		368	22.4[c] 23.2[c]	✓	✓
89d	**88a**	$HCl.H_2NCH_3$		278	1.2[b] 1.3[b]	✓	✓
89e	**88b**	$N_2H_4.H_2O$		279	-	✓	✓
90	**88a**	HOBt-Phe-OFm		591	26.1[b] 26.7[b]	✓	-[d]
91	**88b**	CH_3OH		279	17.9[c] 19.2[c]	✓	✓

Table 7 *Data for products from ring-opening of 5(4H)-oxazolone* a) ES-MS after cleavage with TFA:DCM:H_2O (9:10:1) (**6.4.1**) b) LC-MS gradient A as detailed in section **6.4.2** c) LC-MS gradient B as detailed in section **6.4.2** d) analysis not possible due to small sample

Ring-opening reactions were also accomplished using an amino acid and an alcohol as the nucleophilic components (Scheme 27). The Fm ester protected amino acid was reacted as its TSA salt and the desired resin-bound tripeptide **90** (Table 7) was obtained in 61 % yield (0.23 mmol/g). Attempts to effect methanolysis of **88b** in the presence of catalytic sodium methoxide failed. However, successful alcoholysis was

achieved by adding catalytic Et_3N to a suspension of **88b** in DMF/MeOH (1:1)[†] to afford the peptide ester (**91**). These results demonstrate the further application of this ring-opening methodology as a means of accessing C-terminally modified peptides, or indeed facilitating the continuation of peptide synthesis following some other elaboration of the oxazolone moiety.

4.2.1.3 Conclusions

The ring-opening of the resin-bound 5(4*H*)-oxazolone has been demonstrated using methanol, an amino acid and a series of amine nucleophiles. The wide variety of nucleophiles tolerated by this system indicates that the ring-opening of 5(4*H*)-oxazolones may be extended to the synthesis of a whole range of C-terminally modified peptide analogues.

There is, unfortunately, a clear disadvantage of this method as an independent route to C-terminally modified peptides. All the peptide derivatives prepared using this method were obtained as mixtures of diastereomers, due to racemisation of the 5(4*H*)-oxazolone starting material. It is hoped that the use of additives,[57,58] as already discussed, may provide a solution to this problem by suppressing the racemisation of the oxazolone species. Until complete suppression has been achieved, however, this particular method of obtaining peptide analogues should be restricted to the use of peptides incorporating amino acid residues at the C-terminal position which are either achiral, or lack an α-hydrogen. Nevertheless, the nucleophilic ring-opening of oxazolone species does remain a valuable means of continuing peptide synthesis after modification of this moiety in some other manner. Some of these alternative elaborations are discussed in the following sections.

Some further applications could include stereoselective ring-opening using a chiral DMAP-type catalyst[97] or enzyme catalysed ring-opening. Indeed, many examples of the latter have been reported as routes into enantiopure α-amino acids.[92-94,96,130] The polystyrene resin, used as the support in all the research described so far, is unlikely

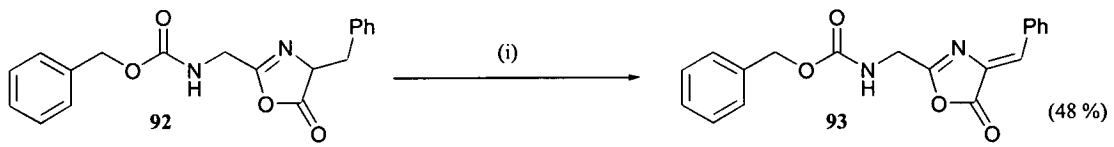
[†] DMF was required to swell the resin

to be compatible with the conditions required for enzyme reaction. Therefore, a preliminary investigation into the possibility of using surface supports, such as SynPhase™ crowns, has been conducted and is discussed in Chapter 5.

4.2.2 Oxidation

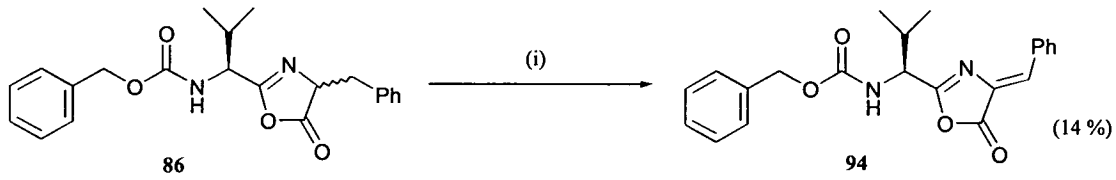
4.2.2.1 Solution DDQ Oxidation

In 1978 Konno and Stammer published the first of many papers describing the use of 2,3-dichloro-5,6-dicyano-1,4-benzoquinone[131] (DDQ) for the solution oxidation of dipeptide-derived oxazolones.[72,73,128,132] Among the number of dehydrogenating agents examined by the authors, DDQ was found to give the highest yield when used in combination with catalytic 2,4,6-collidine in DME for 24 h. Stammer and co-workers demonstrated the oxidation of oxazolone **92**, derived from Cbz-Gly-Phe-OH, using these optimum conditions to afford **93** in 48 % yield (Scheme 28). It was also shown that, as in the Erlenmeyer azlactone synthesis, the double bond generated had the *Z* configuration.



Scheme 28 *Stammer's oxazolone oxidation* - reagents and conditions: (i) DDQ, 2,4,6-collidine, DME, 24 h

It was hoped that the oxidation described by *Stammer et al.* could be applied to solid phase dipeptide derived oxazolones, to provide a means of accessing libraries of dehydropeptides. In order to obtain a solution analogue for comparisons with solid phase products the unsaturated oxazolone **94** was prepared according to Stammer's optimum conditions (Scheme 29).



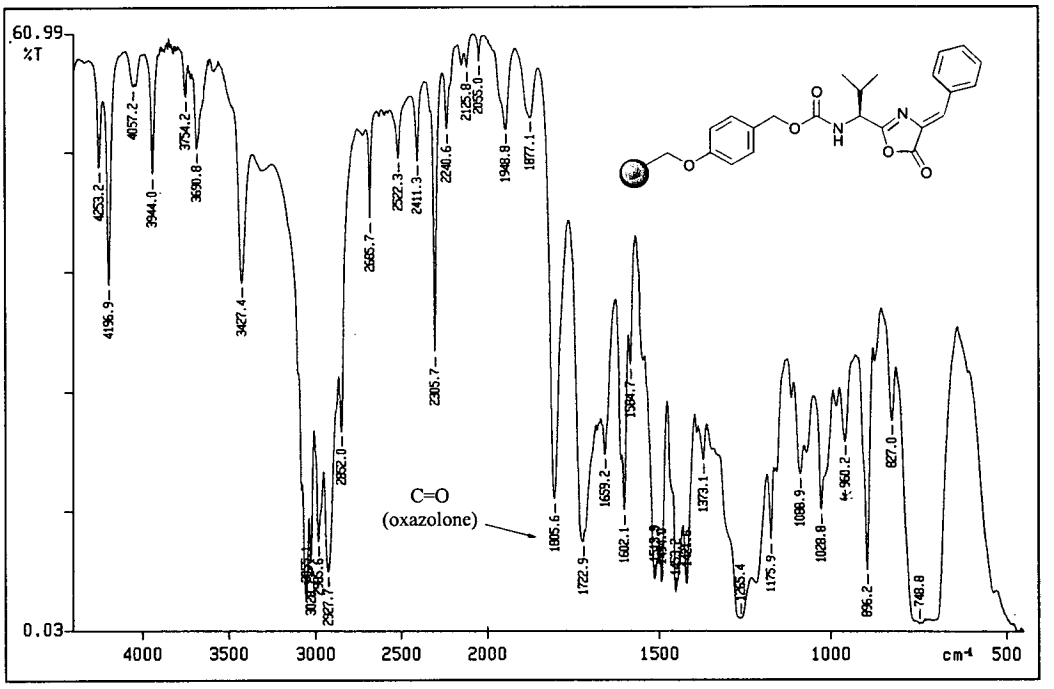
86 94

Scheme 29 *Solution-phase oxidation* - reagents and conditions: (i) DDQ, 2,4,6-collidine, DME, 24 h

A solution of the N-protected dipeptide-derived 5(4*H*)-oxazolone (**86**) in anhydrous DME, was treated with DDQ and 2,4,6-collidine to give a deep red solution. After stirring for 24 h under an inert atmosphere, the resulting red precipitate was filtered off and the filtrate concentrated to afford a dark red oil. The target unsaturated oxazolone (**94**) was purified using column chromatography and isolated as a white solid which readily decomposed when stored at room temperature.

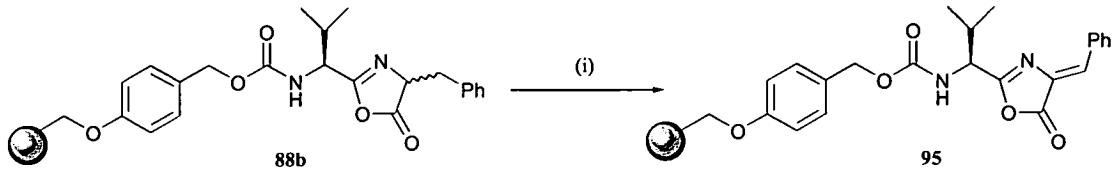
The unsaturated oxazolone is clearly identified not only by the lack of the C-4 proton in the ^{1}H NMR spectrum, but also by the presence of a multiplet (δ_H 8.03-8.11 ppm) corresponding to the two *ortho* protons on the α,β-unsaturated aromatic ring. Furthermore, the vinyl benzylic proton appears in the aromatic region (δ_H 7.21 ppm) and the oxazolone carbonyl stretch in the IR suffers a shift of approximately 20-30 cm^{-1} (ν_{max} 1799 cm^{-1}). High resolution mass spectrometry further confirmed successful oxidation, albeit in a poor yield. Since a single product was obtained, the double bond was assigned *Z* configuration, in line with the results described by Stammer.[73]

The poor stability and low yield of the product are obviously the main disadvantages of this, otherwise very convenient, reaction. The low yield is thought to be a feature of the poor stability of the product. However, oxidation performed on resin-bound intermediates obviates the need for a purification step, with product isolation being achieved by simple washing. In addition, resin-bound intermediates are often more stable than their solution phase counterparts and solid phase combinatorial chemistry could also be exploited for the preparation of libraries of diverse dehydropeptide analogues.



Spectrum 14 *IR spectrum of* ***95***

4.2.2.2 Solid Phase DDQ Oxidation



Scheme 30 *Solid-phase DDQ oxidation* - reagents and conditions: (i) DDQ, DME

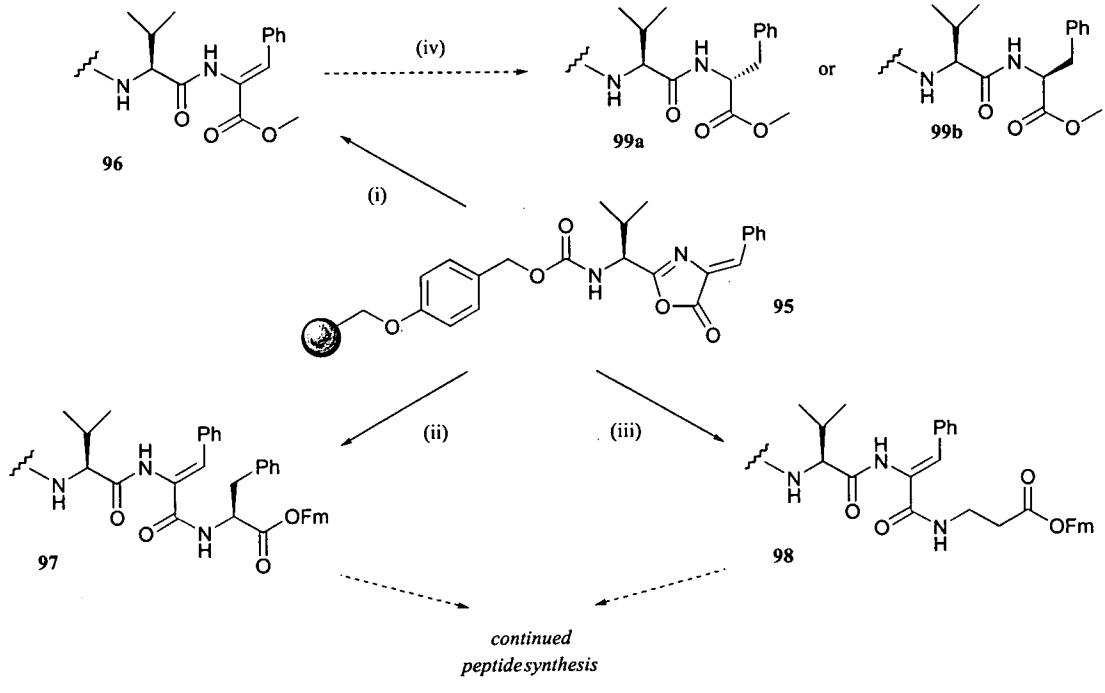
The resin-bound 5(4*H*)-oxazolone (**88b**) was pre-swollen in DME before treatment with an excess of DDQ. Addition of 2,4,6-collidine to the reaction slurry caused an instant colour change, but the resulting products gave complex spectra, indicating a mixture of products, so it was decided to omit this reagent and use DDQ on its own.[66] The resulting slurry was agitated for 15 h before being filtered and washed according to standard procedure. On-resin IR (ν_{max} = 1805 cm^{-1} - C=O oxazolone) (Spectrum 14)[op] and ^{13}C NMR analysis confirmed successful oxidation to the unsaturated derivative **95**, although the broadness and complexity of the NMR spectrum indicated incomplete reaction. It was found that if this product was subjected to the original reaction conditions for a second time, a cleaner ^{13}C NMR spectrum was obtained. Even this spectrum was reasonably broad, possibly due to DDQ and DDQH impurities lingering in the rusty-coloured resin. Cleavage with TFA/DCM/H_2O (9:10:1) followed by ESMS gave peaks corresponding to the hydrolysed product ($MH^+ + H_2O$) and the hydrated and hydrolysed product ($MH^+ + 2$ x H_2O). When anhydrous cleavage conditions were employed (50% TFA in DCM) the actual product mass ion (MH^+) was observed along with the hydrolysis product ($MH^+ + H_2O$). The oxazolone starting material however, was also present to a minor extent in each sample, indicating that even repeated subjection to excess reagents for prolonged reaction times, although improved the yield, was not sufficient to drive the reaction to completion.

In systems where the Wang linker is used to tether alcohols to a polymer support, the benzylic ether linkage has been found to be susceptible to oxidative cleavage by DDQ. Indeed, several publications have reported the use of DDQ as a mild

alternative cleavage reagent for ether-linked systems.[133-136] However, since the target compounds were observed on-resin and in the final cleavage mixture, even after prolonged reaction times, it was assumed that the urethane linked system was not susceptible to oxidative cleavage, presumably due to the resonance stabilisation of this functionality.

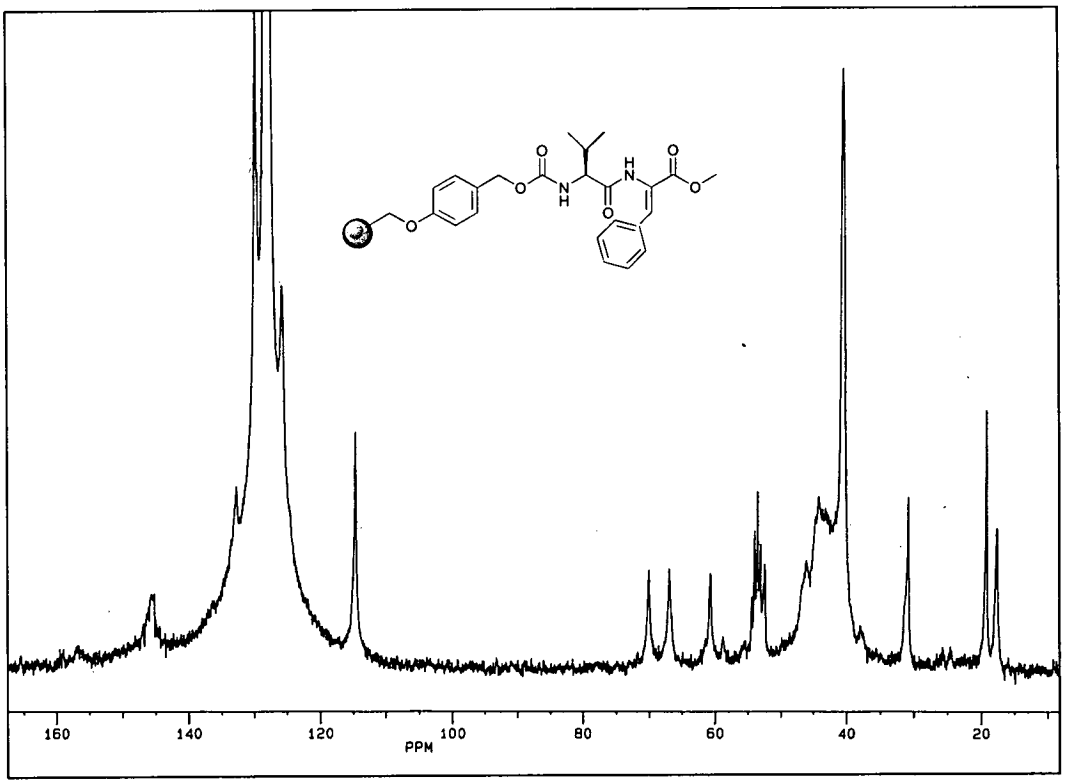
4.2.2.3 Further Manipulations

A number of ring-opening reactions were performed on the polymer-bound unsaturated oxazolone derivative (**95**) (Scheme 31). The reactions described, further verify the success of the oxidation to yield the solid phase dehydro derivative, as well as demonstrating some of the potential applications of this transformation.



Scheme 31 *Further manipulations of α,β-unsaturated resin-bound 5(4H)-oxazolone* - reagents and conditions: (i) Et_3N, MeOH, DMF (ii) TSA-Phe-OFm, DMAP, NMP (iii) TSA-β-Ala-OFm, DMAP, NMP (iv) H_2, Rh-L*: [Rh(diPAMP)(norbornadiene)]BF_4 ➡ (*S,S*); [Rh(Ph-CAPP)(norbornadiene)]BF_4 ➡ (*S,R*)[68]

Ring-opening reactions were carried out according to the procedures employed for the saturated oxazolone analogues (***4.2.1.2***). Thus, the resin-bound dehydro oxazolone **95** was swollen in a mixture of MeOH and DMF (1:1) before addition of



Spectrum 15 *Gel phase ^{13}C NMR of* ***96***

catalytic Et_3N. Over-night agitation was followed by filtration, washing and drying in the usual manner. Analysis of the resin by IR and gel phase ^{13}C NMR, indicated successful alcoholysis of the unsaturated oxazolone to afford the target dipeptide ester **96** (Spectrum 15).[op] Acidic cleavage, followed by ESMS gave the mass ion corresponding to Val-Δ^zPhe-OMe, giving further evidence of successful ring-opening. LC-MS gave a single major peak corresponding to the dehydropeptide ester (**96**), with trace impurities possibly arising from DDQ-derived contaminants.

Ring-openings were also conducted using amino acids as the nucleophilic components, demonstrating the application of this method for the synthesis of dehydropeptide analogues. In a similar way to the reaction of the saturated derivative (**88b**), the α,β-unsaturated oxazolone (**95**) was suspended in NMP and treated with the TSA salt of the Fm ester protected amino acid and catalytic DMAP. Usual work up gave the corresponding tripeptide incorporating a dehydro-phenylalanine unit. The two amino acid derivatives employed were TSA-Phe-OFm (**65a**) and TSA-β-Ala-OFm (**77c**), furnishing the tripeptides Val-Δ^zPhe-Phe-OFm (**97**) and Val-Δ^zPhe-β-Ala-OFm (**98**) respectively. LC-MS analysis of both cleavage products give a single peak on the LC trace with the correct corresponding mass ion. Not only did this confirm that the starting material had been successfully oxidised, as the tripeptide obtained would be two mass units greater and a mixture of diastereomers, but also that one configuration is favoured for the unsaturated unit. As previously mentioned, this is in line with the results of others[66] and it is therefore highly likely to be the Z isomer.

4.2.2.4 Conclusions and Applications

Ring-opening of the resin-bound α,β-unsaturated 5(4*H*)-oxazolone (**95**) has been demonstrated using different nucleophiles. Not only has this served to confirm the success of the oxidation reaction, but lends support to the proposal that 5(4*H*)-oxazolone derivatives are useful precursors to a number of desirable peptide analogues. For instance, alcoholysis of the unsaturated oxazolone has been shown to afford the corresponding dehydropeptide ester. Although useful in their own right,

these derivatives could also be selectively reduced[68] to afford either the L,L (**99a**) or L,D (**99b**) peptide ester as depicted (Scheme 31).

Ring-opening with amino acids has also been demonstrated. If applied in a combinatorial manner, this methodology could used to prepare a diverse range of dehydropeptide analogues (Scheme 31).

Furthermore, by applying this methodology to polymer-supported oxazolone intermediates, the problems of poor stability and yield have been addressed. For example, unlike the solution analogues, the unsaturated supported intermediates appear to be stable at room temperature for prolonged periods of time. In addition, by driving the reaction through repeated treatment with excess reagents, yields have been significantly improved, although still require further optimisation.

Unsaturated oxazolone intermediates also exist as potential precursors to a number of other valuable compound classes. Some examples of these further applications will be mentioned briefly in Chapter 5.

4.2.3 Alkylation

4.2.3.1 Solution Phase Investigation

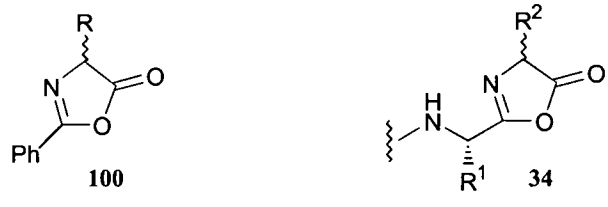
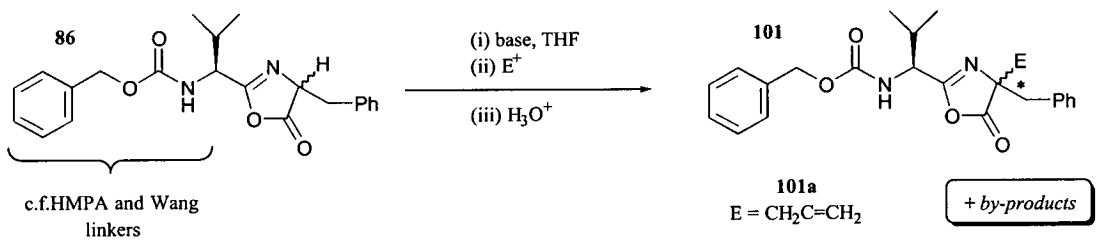


Figure 23 *General structures for alkylation studies*

Many studies have been carried out on the alkylation of 2-phenyl 5(4*H*)-oxazolones (**100**) with varying degrees of success.[129,137-139] From this research it is also known that the C4 alkylation of oxazolone derivatives lacking a phenyl substituent at C2, proceed less efficiently. Therefore, in order to establish the optimum conditions for

successful alkylation at the C4 position of dipeptide-derived 5(4*H*)-oxazolone intermediates (**34**), previous work[140] explored the effects of a number of variables on the yield and diastereoselectivity of this reaction. A range of electrophiles, bases, additives and temperatures were examined and some conclusions which can be drawn from the results obtained, are summarised below:

- an inverse relationship exists between d.e. and yield.
- it is possible to alkylate the dipeptide derived oxazolones in yields up to 85 % and d.e.s up to 81 %, but not concomitantly.
- moderate d.e.s can probably be attributed to the distance of the chiral auxiliary from the C4 centre at which asymmetric induction has to take place. Also, the pendant arm side-chain at C1' is able to rotate freely so that R^1 (Figure 23) can lie above or below the plain.
- addition of metal salts in most cases results in an increase in d.e. due to chelation control, but decrease in yield as the reaction proceeds less cleanly due to high sensitivity to temperature and stoichiometry.
- the low reactivity of the pseudo-aromatic carbanion can be partially blamed for the low yields and complex mixtures formed.



Scheme 32 *Alkylation of solution phase 5(4*H*)-oxazolone*

In order to attempt the alkylation on the solid phase oxazolone derivative **88a**, it was first necessary to prepare solution derivatives for comparison and also attempt to optimise the conditions further with respect to the specific oxazolone derivative involved. Conditions were selected as a basis for the extended optimisation by considering the oxazolone derivative upon which all the solid phase studies had been based, along with the necessity for employing conditions which would be compatible

with the resin and linker system. The conditions which appeared to fit these criteria and which provided the best compromise of yield and diastereoselectivity were either sodium or potassium bis(trimethylsilyl) amide, with a reactive electrophile such as allyl bromide in THF at 0 °C. A number of alkylations were performed using these conditions, but the desired product had to be isolated from a mixture of by-products and thus initial yields of **101a** were very poor (~ 16 %).

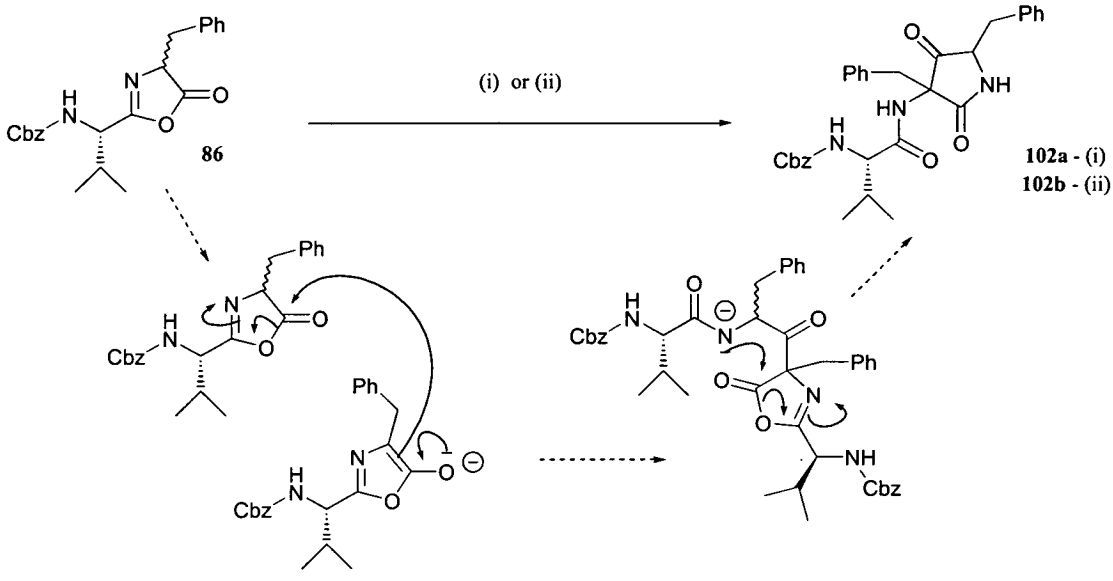
In an attempt to optimise the conditions further, the by-products formed during the alkylation were examined in more detail. In this way, it was hoped that by identifying the main side reactions, modifications to the conditions or procedure could be implemented so as to prevent their formation and thus enhance the yield of the target **101**. As described below (***4.2.3.2***), such scrutiny lead to the identification of one of the major side reactions as a dimerisation process. As a result, conditions were altered to hinder this process by performing the reverse addition of **86** to a solution of the electrophile and base. In this way, as soon as the oxazolone was added, it was deprotonated and the resulting enolate immediately quenched with the electrophile, before any self-condensation could occur. The reaction mixtures used were also relatively dilute to further impede the unwanted dimerisation.

As a consequence **101a** was prepared in 52 % yield with a 55 % d.e. of the (*S,R*) product. Although still far from ideal, as previously-mentioned, it is known that the C4 alkylation of oxazolones which lack a phenyl substituent at the C2 position proceed less efficiently. It is also possible that the dialkylated product is susceptible to decomposition on silica giving rise to the poor yields observed.

4.2.3.2 Dimerisation

As mentioned, during the various attempts at the solution phase alkylation of oxazolone **86**, the formation of several recurring by-products was observed. Once isolated, analysis by ^{1}H NMR and mass spectrometry indicated that the major by-product was an example of a Rügheimer dimer.[141,142] Although analogues of this dimer (**102**), with a core pyrrolidine-2,4-dione structure, have been mentioned

several times in the literature,[143-148] they have received very little attention as molecules in their own right *i.e.* potential applications have not been examined.



Scheme 33 *Dimerisation of the 5(4H)-oxazolone* - reagents and conditions: (i) NaHMDS, THF, 0 °C - RT, concentrated (ii) as for (i) but highly dilute

The dimerisation in this system is believed to arise due to the susceptibility of the oxazolone to nucleophilic attack. Deprotonation of one molecule of the oxazolone results in formation of the enolate, which is then free to attack the carbonyl function of another oxazolone molecule, initiating the rearrangement that finally gives rise to the dimer **102** (Scheme 33).

In order to confirm the proposed structure, the reaction conditions were altered in such a way as to favour the dimerisation process and thus isolate enough material for further analysis. Therefore, base was added drop-wise to a concentrated solution of the oxazolone to encourage self condensation. Pyrrolidine-2,4-dione analogue **102a** was obtained as a 2:1 mixture of diastereomers (Scheme 33). Once again high resolution mass spectroscopy gave the mass ion corresponding to the dimer structure, but the ^{1}H NMR spectrum proved difficult to interpret due to its complexity. The reaction was therefore repeated using a very dilute solution of oxazolone **86** and the resulting product **102b** was isolated as a single diastereomer corresponding to the major isomer of **102a**.

The NMR data from both compounds (**102a-b**) combined with data from COSY and NOE experiments (Figure 25) allowed full characterisation of the major and minor diastereomers. Unfortunately the critical NOE information which would have allowed determination of the relative cross-ring geometry was not present. Furthermore, all attempts to obtain a crystal structure of **102a-b** or analogues thereof failed, due to the tendency of these compounds to form fine needles unsuitable for crystallographic studies. Interestingly, work carried out by Leban and Colson on the base dimerisation of urethane-protected amino acid *N*-carboxanhydrides (UNCAS),[149] involved the isolation of **102**. The crystal structure of an analogue lead to the geometry of **102** being assigned *Z*, however, differences in the synthesis and solvent for NMR studies prevented direct comparison.

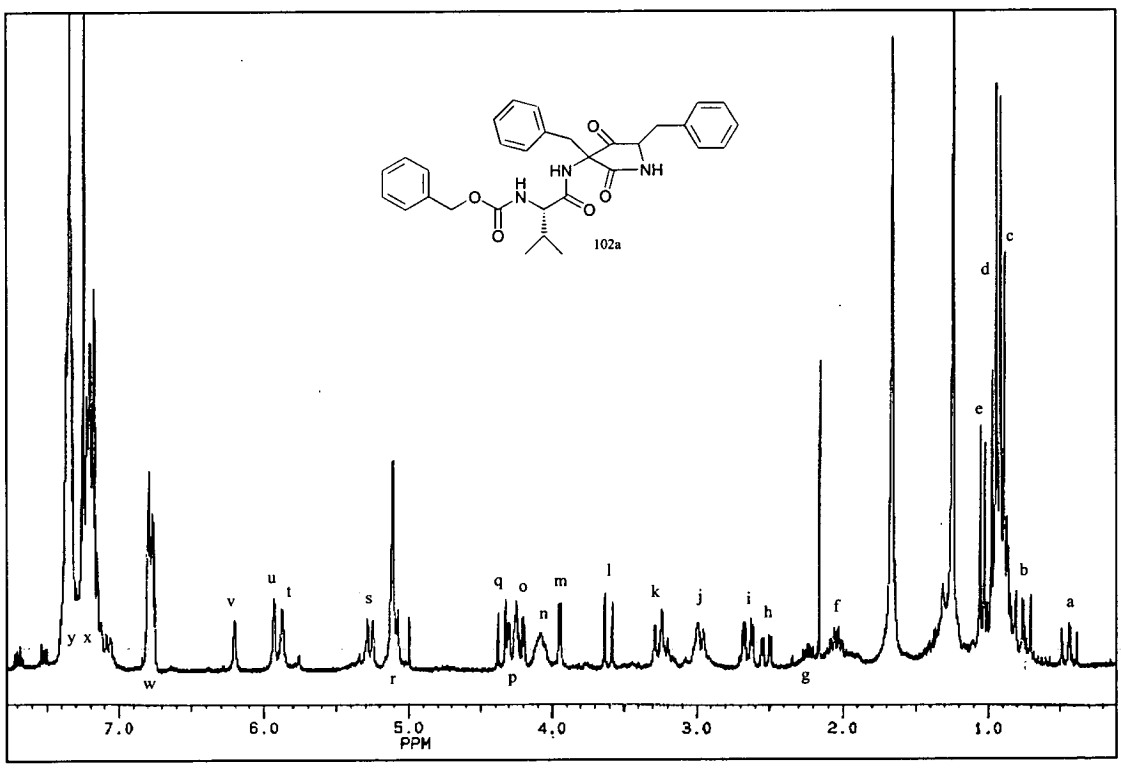


Figure 24 *^{1}HNMR spectrum of **102a** (2:1 mixture of diastereomers)*

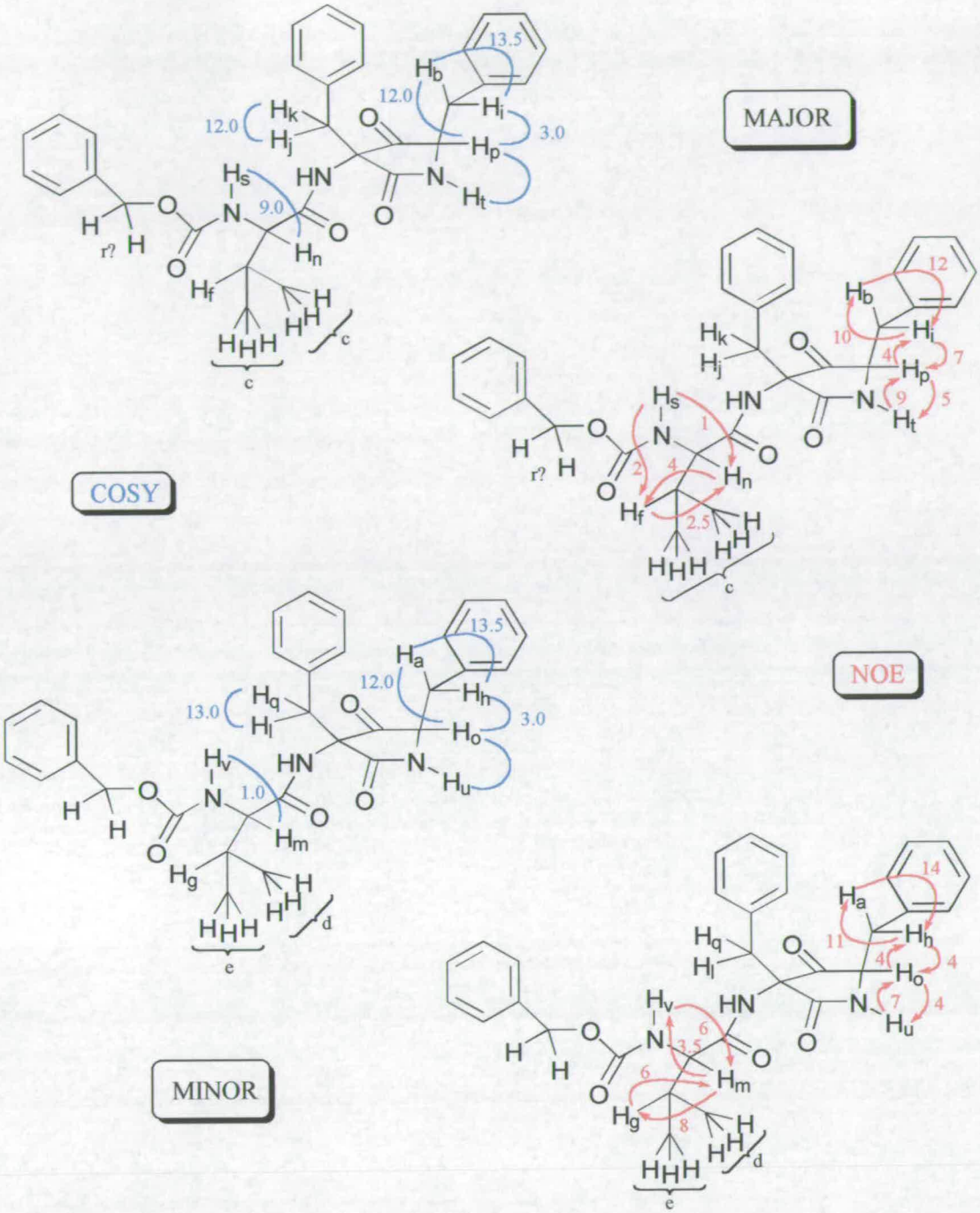


Figure 25 *Maps of NOE and COSY data from analysis on both diastereomers of* ***102***

An interesting feature of each diastereomer of **102a** displayed by the ^{1}H NMR is the presence of a single, very highly shielded benzylic proton signal (*e.g.* major diastereomer δ_H 0.71 ppm). It is likely that this shielding is due to the proton being orientated over one or sandwiched between two of the aromatic rings present in the dimer. The neighbouring geminal proton, however, has a vastly different chemical

shift (δ_H 4.28 ppm), indicating that although attached to the same carbon, the two protons are witnessing very different magnetic environments. Preliminary modelling studies were carried out on the four possible stereoisomers[†], in an attempt to investigate the basic feasibility of this prediction. An example of one of the models obtained is shown (Figure 26) and demonstrates how, in theory, it is possible for these two neighbouring protons to witness the environments described. This hypothesis is supported further by the potential presence of a hydrogen bond (2.09 Å) which may serve to hold this core conformation in place.

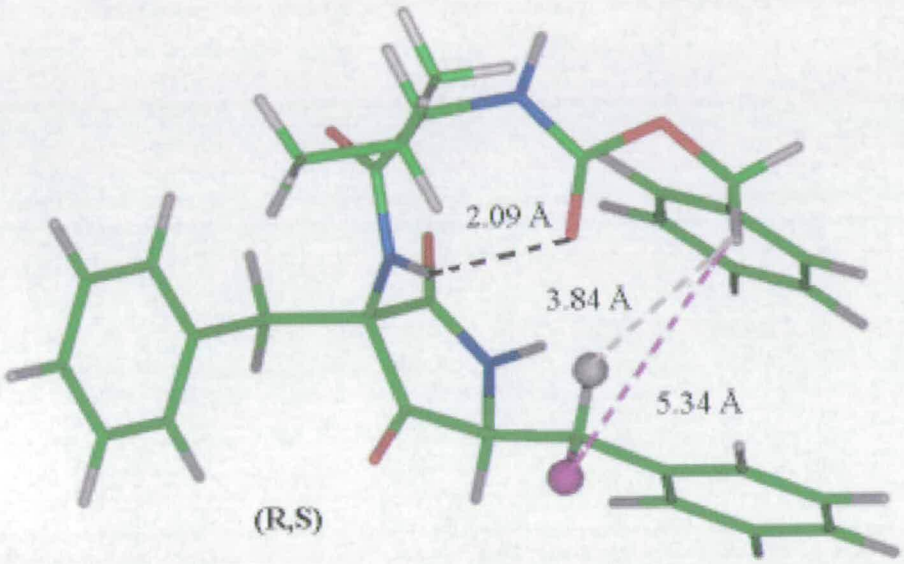


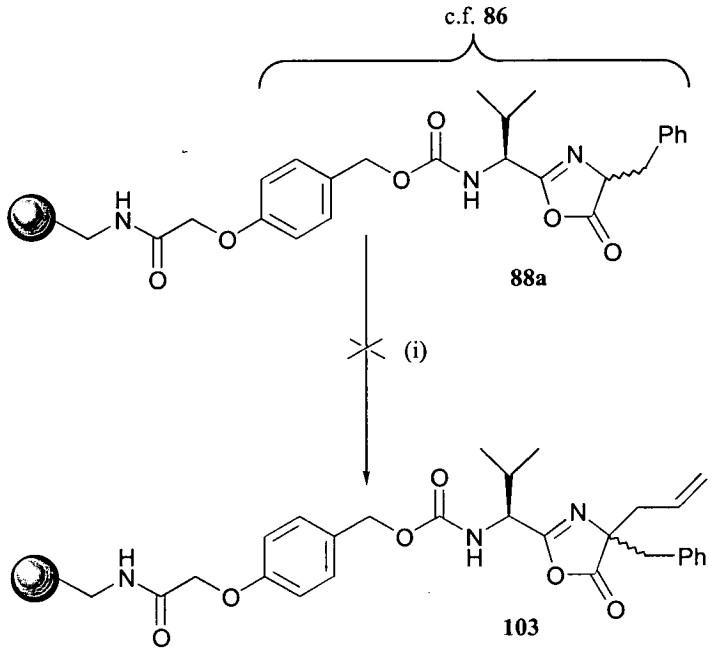
Figure 26 *Model of potential conformation of (*R,S*) diastereomer of* ***102***

A potential application of this reaction could be to adapt the dimerisation to the solid phase. With one oxazolone component tethered to the resin, it may be possible to perform controlled dimerisations, where the solution phase oxazolone is designed to introduce further diversity, thus facilitating the library synthesis of Rügheimer-type structures for screening.

[†] only the valine stereochemistry is fixed

4.2.3.3 Alkylations on Solid Phase - Preliminary Results

Given the moderate success of oxazolone alkylations in the solution phase, the same conditions were employed in an attempt to achieve better yields on supported intermediates. For instance, it was expected that yields could be improved further since dimerisation would be considerably less likely to occur between two oxazolone fragments bound to the same support. It was also hoped that the well-documented 'polymer advantage'[13] of employing excess reagents to drive reactions to completion would also help to improve the yield.



Scheme 34 *Attempted alkylation of supported oxazolone intermediate* - reagents and conditions: (i) KHMDS, allyl bromide, THF

The supported oxazolone (**88a**) was suspended in anhydrous THF, before addition of freshly-distilled allyl bromide (3 eq). A solution of KHMDS in THF (3 eq) was added drop-wise and the resin slurry agitated under an inert atmosphere for 16 h. After filtering, washing and drying, analysis of the resin indicated that the reaction had been unsuccessful. The distinctive oxazolone carbonyl stretch was no longer evident, indicating that either ring-opening or some other transformation had occurred. However, analysis by ESMS gave no signal corresponding to the dialkylated target molecule (**103**) or the starting material. Indeed, no major peak, as such, was observed and none of the other signals identified. Gel phase ^{13}C NMR

analysis was also poorly resolved and too broad to derive any meaningful data, indicating that the conditions employed were probably not compatible with the resin.

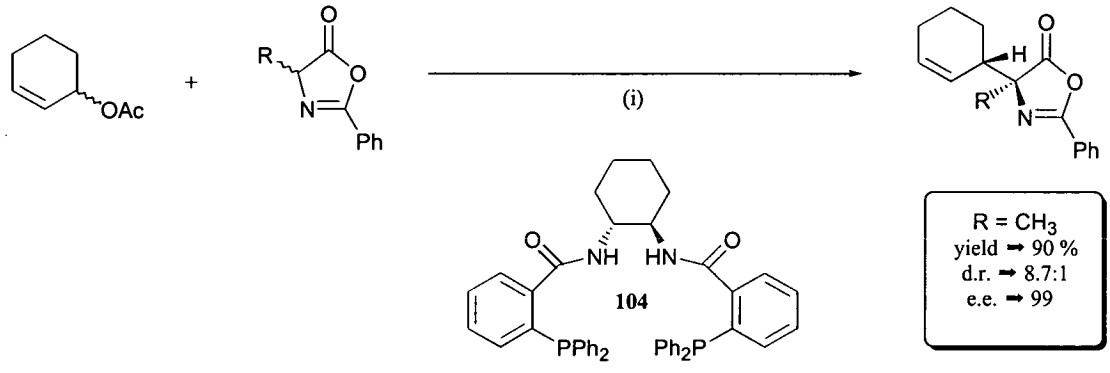
4.2.3.4 Conclusions

Although the alkylation of the polystyrene-bound oxazolone was not successful in this instance, it merely demonstrates how conditions which prove effective for solution phase reactions do not always translate successfully to solid phase systems. For instance, it is possible that the hindered base was unable to gain adequate access to the functional sites within the resin, to cause deprotonation and subsequent alkylation. It is also feasible that the base itself was not entirely compatible with the polymer support employed. Furthermore, since purification is not possible it is essential that solid phase reactions are high yielding. Therefore, if the other by-products observed in the solution study were still favoured, the resin-bound product could have been crude to the extent that any successful alkylation products were obscured.

A possible route around these problems would be to use an alternative support, which might prove compatible with the reagents and did not require diffusion of the reagents into buried reactive sites. With this in mind, a preliminary investigation into the feasibility of employing SynPhase™ MD crowns was conducted and is described in more detail in Chapter 5.

Another potential strategy which has yet to be proved viable on solid supported intermediates, is the strategy described by Trost *et al.*.[150] By using the chiral ligand **104** and a precatalyst palladium complex **105**, Trost was able to demonstrate the asymmetric alkylation of 2-phenyl substituted oxazolones as depicted in (Scheme 35). Impressive yields, diastereomeric ratios (d.r.) and enantiomeric excesses (of the major and minor diastereomers) (e.e.) were obtained for a variety of R substituents. Time did not permit examination of this methodology with respect to the polystyrene-bound dipeptide-derived oxazolone system. Moreover, the difference in reactivity between 2-phenyl and dipeptide derived oxazolones has already been

described, indicating that direct transfer between the two systems may not prove altogether straight forward.



Scheme 35 *Asymmetric alkylation of 2-phenyl 5(4H)-oxazolones - reagents and conditions:* (i) $[(\eta^3\text{-}C_3H_5PdCl)_2]$ (**105**), Et_3N, MeCN

5. Future Work

5.1 5(4*H*)-Oxazolones and Enzymes

5.1.1 Introduction

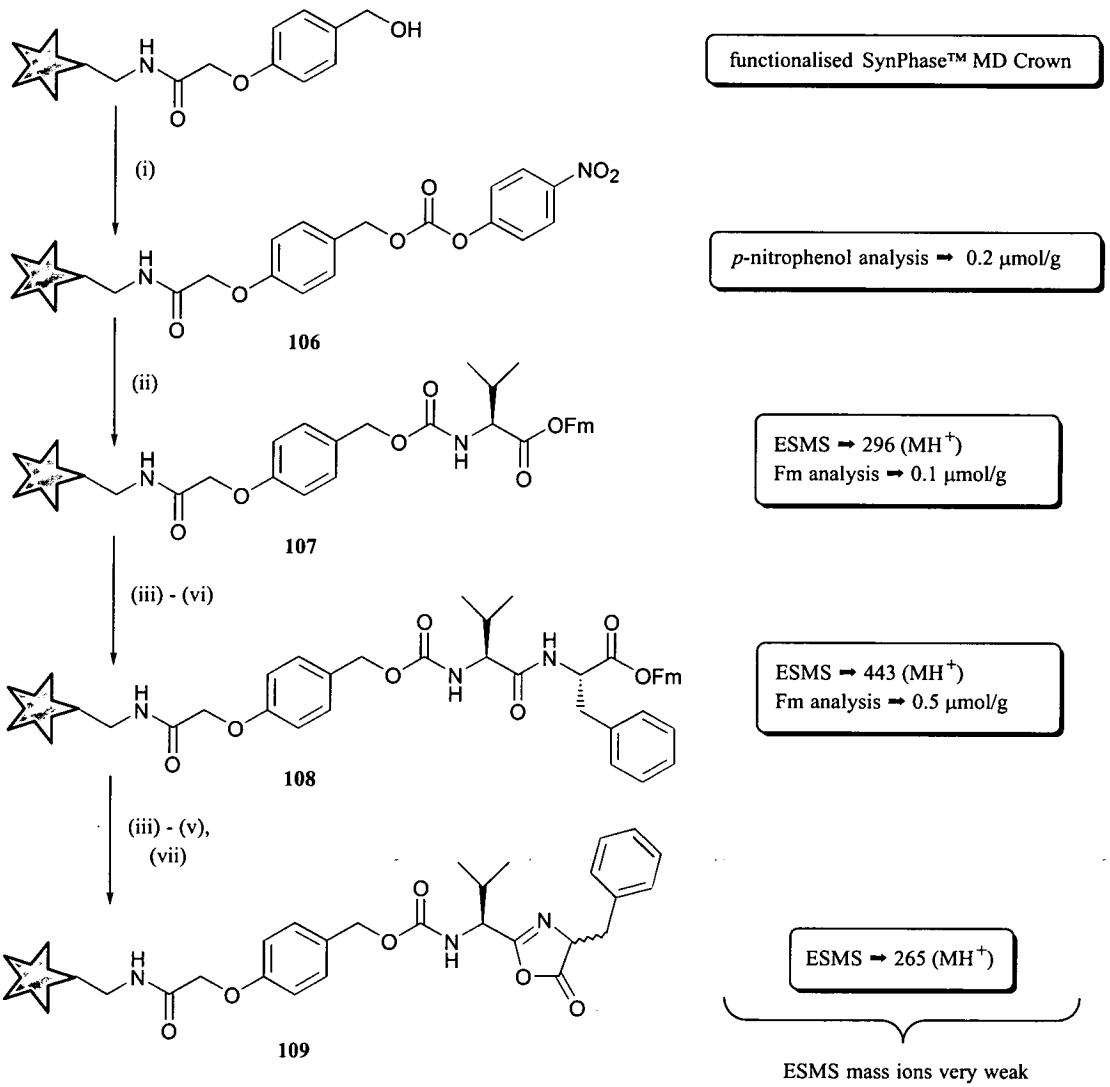
5(4*H*)-oxazolones have found utility as substrates for a number of enzyme-catalysed transformations. For instance the enzyme catalysed dynamic resolution of these units as a route into optically active α-amino acid derivatives has already been described (**1.3.1**). Furthermore, 5(4*H*)-oxazolones have been employed as activated acyl donors for α-chymotrypsin[151] and papain[152] catalysed peptide fragment condensations.

The advantages of employing enzymes to catalyse such reactions include high regio- and stereoselectivity, mild reaction conditions, lack of racemisation and minimal side chain protection. It would therefore be very useful if these enzyme-catalysed transformations could also be applied to 5(4*H*)-oxazolone derivatives bound to a polymer support.

The polystyrene beads which were used as the support material throughout the investigations described, are unlikely to be suitable for transformations involving enzymes. For instance, not only is it unlikely that the enzymes would be able to gain access to the buried functional sites, but the polymer does not swell in the aqueous systems required for many enzyme-catalysed reactions. It was therefore necessary to consider using a different support, which would both allow enzyme access and be compatible with aqueous systems. The previously described (***1.1.2.1***) SynPhase™ MD crowns appeared to meet the required criteria and were selected for some preliminary research.

5.1.2 Preliminary Results Using SynPhase™ Crowns

The inverse synthesis of the dipeptide-derived 5(4*H*)-oxazolone was carried out in much the same way as it had been for the polystyrene bound derivative (Scheme 36). The crowns however, being surface supports, are much lower loading and require only very gentle agitation. Moreover, in order to fully submerge the crowns and still maintain a concentration suitable for the reaction a ten fold excess of reagents was employed.



Scheme 36 *N-C synthesis of 5(4*H*)-oxazolone using SynPhase ™crowns* - reagents and conditions: (i) *p*-nitrophenyl chloroformate, pyridine, DCM (ii) 20 % piperidine, DMF (iii) 3 % acetic acid, DCM (iv) TBTU, NMM, DMF (v) HOBt-Val-OFm, DMF (vi) HOBt-Phe-OFm, DMF (vii) EDCI, DCM

The principal difficulty encountered using the crowns was the lack of analysis available. As non-swelling surface supports, on resin gel phase ^{13}C NMR and IR analysis were not feasible and any destructive analysis *e.g.* cleavage followed by ESMS, required sacrificing an entire crown.

In order to confirm the success of each step, sufficient crowns were withheld for ESMS and quantitative (*p*-nitophenol or Fm) analysis. The low loadings (1.8 μmole/g starting loading) of the crowns meant that analysis was very weak and mass spectroscopy signals were often difficult to see.

Although it would appear from the, albeit weak, ESMS data and loading analyses that each supported derivative **106-109** was prepared, the lack of more convincing data precluded any further transformations from being examined. It is possible that the weak signals are a feature of the altered washing protocol recommended by the manufacturers. By washing with only MeOH and DCM, rather than a wider variety of solvents, it is possible that there is insufficient removal of the impurities and excess reagents, resulting in swamping of the target signals. Further examination of this supported system is clearly necessary before its scope can be properly explored.

Provided the problem with analysis of the crowns can be overcome, these supports appear particularly promising for facilitating enzyme-catalysed reactions on supported oxazolone derivatives. It is also possible that employing such surface supports for the alkylation and dimerisation reactions described in section **4.2.3**, might help overcome problems such as compatibility and functional site access for hindered bases.

5.2 Other Applications

The elegance of the resin-bound 5(4*H*)-oxazolone fragment is that its application is not merely limited to acting as a precursor for the preparation of the derivatives described. Its versatility can be reflected in the vast scope of reactions for which it is

potentially the basis. Figure 27 represents some of the many diverse transformations with marked potential for future investigation.

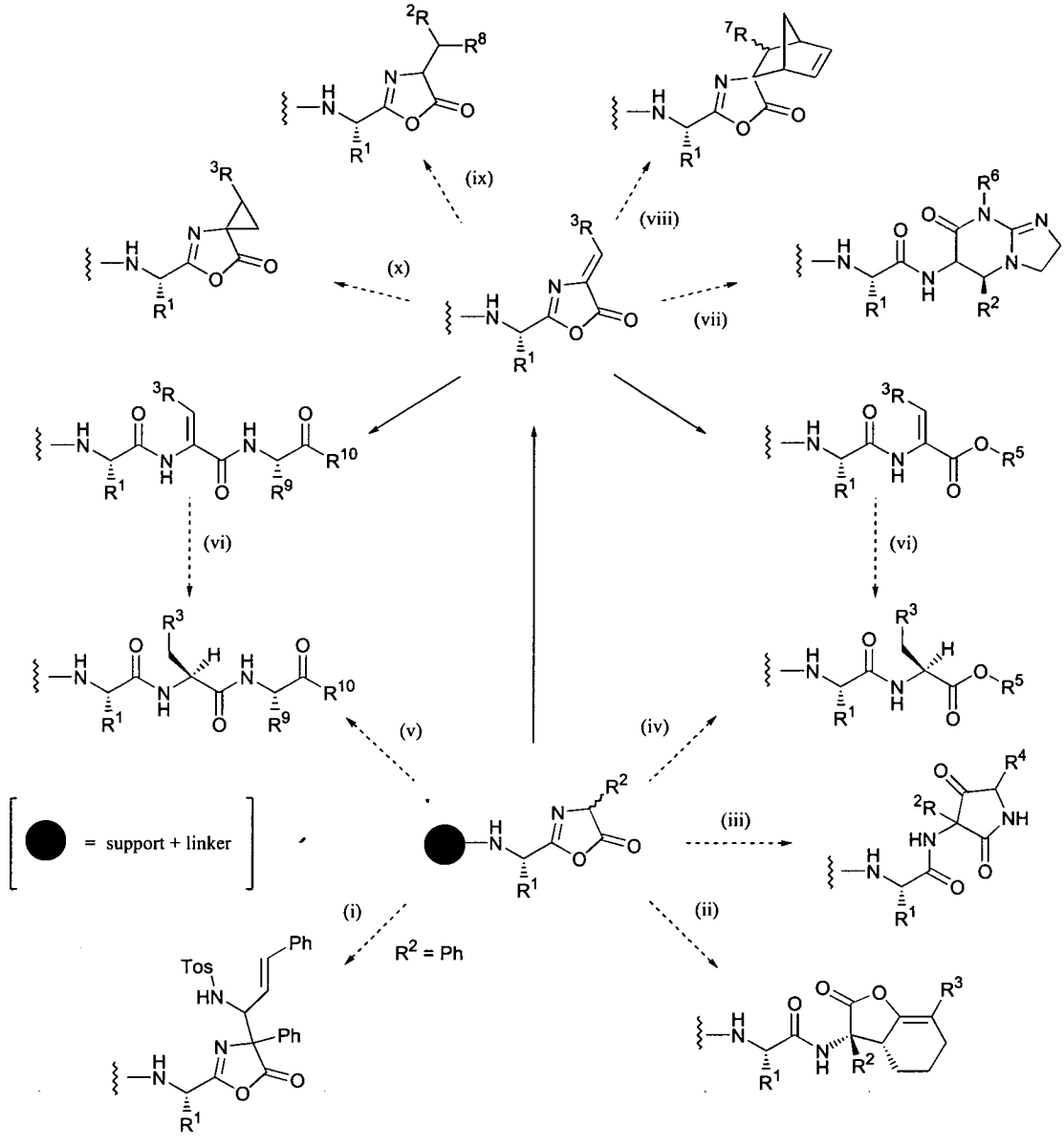


Figure 27 *Potential of the resin-bound 5(4*H*)-oxazolone* - references: (i) Michael addition[153] (ii) tamdem alkylation and translactonisation[154] (iii) dimerisation (***4.2.3.2***) (iv) enzyme-catalysed alcoholysis[96] (v) enzyme-catalysed condensation[151,152] (vi) asymmetric reduction[68] (vii) ring transformation of Michael adduct[155] (viii) Diels-Alder[156] (ix) conjugate addition of Grignard reagent[157] (x) asymmetric cyclopropanation[158]

6. Experimental

6.1 General Experimental

^{1}H and ^{13}C NMR spectra were recorded on Bruker AC250, Bruker WH 360 or Varian UNITY INOVA 600 instruments. Chemical shifts (δ_H, δ_C) of signals are reported in ppm, and coupling constants (*J*) in Hertz (Hz). Residual protic solvent present in the deuterated sample was used as internal standard in NMR spectra, *e.g.* C*H*Cl_3 in $CDCl_3$. When products were obtained as a mixture of diastereomers, "**M**" and "**m**" denote signals corresponding to the major and minor diastereomers respectively.

Electron impact (EI) nominal mass spectra were recorded on a Finnigan 4600 mass spectrometer. EI high resolution and fast atom bombardment (FAB) accurate and nominal mass spectra were recorded on a Kratos MS5OTC instrument.

IR absorption spectroscopy was performed on a Perkin-Elmer FT-IR Paragon 1000 spectrophotometer, and frequencies (ν) measured in wave numbers (cm^{-1}). Samples were measured on disposable IR cards (Type 61 3M, polyethylene, 19 mm aperture), as thin films, KBr discs or Nujol mulls. Resin-bound samples were swollen in DCM and compressed between NaCl plates.

Melting points were measured on a Gallenkamp melting point apparatus and are uncorrected.

Optical rotations were performed on an AA-1000 polarimeter from Optical Activity Ltd (measurements being made at the sodium D-line). Concentrations are given in g/100 ml and $[\alpha]_D^{20}$ are quoted in 10^{-1} deg cm^2 g^{-1}.

Elemental analysis (CHN) was performed on a Perkin Elmer 2400 CHN Elemental Analyser.

Possible low energy conformations of **102** were explored using Insight II™ from Biosym with energy minimsations performed using a Discover™ forcefield (Biosym).

Flash chromatography employed silica gel 60H (0.04-0.063 mm, 230-400 mesh) (Merck 9385) and analytical tlc was carried out on Merck aluminium-backed plates coated with silica gel 60 F_{254}, 0.25 mm. Visualisation techniques include UV fluorescence (254 nm), and ammonium molybdate, ninhydrin and permanganate dips.

All reactions with air- or moisture-sensitive reactants or solvents were carried out in oven- or flame-dried glassware under a positive pressure of dry argon. THF was distilled under argon from sodium, with benzophenone ketyl as indicator, and dichloromethane was distilled from calcium hydride shortly before use. All other solvents described as anhydrous have been purchased as "anhydrous-grade". Where DMF was required for reactions on solid phase or qualitative analytical tests, "peptide synthesis grade", purchased from Rathburns, was employed. Solvents and reagents used for quantitative resin analysis were "spectrophotometric grade", and the H_2O and MeCN used for LC-MS and HPLC were "HPLC grade" and filtered prior to use. Allyl bromide was purified prior to use by distillation and 2,4,6-collidine was distilled and stored over calcium oxide. All other reactants and solvents were "reagent-grade" and used as supplied unless otherwise stated. Where light petroleum has been stated in the text, petroleum ether (bp 40 - 65 °C) has been used.

6.2 General Experimental - Solid Phase

6.2.1 Nomenclature of Resin-Bound Compounds

When naming resin-bound compounds, the resin has been considered to be the functional class name in accordance with IUPAC recommendations and the compound anchored to the resin is described using prefixes. Hence, **47** is called 4-(hydroxymethyl)-phenoxyacetyl aminomethyl polystyrene.

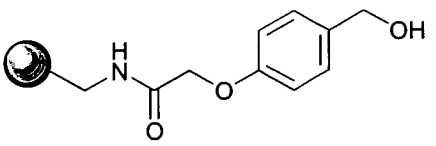


Figure 28 *Example of supported compound (47)*

When resin-bound amino acids and peptides are described, the non-IUPAC names valine, phenylalanine etc. have been employed for clarity. Also, the traditional method for naming peptides, on- or off-resin, which have been synthesised in the classical C-N direction, is from N terminus to C terminus. Therefore, in order to achieve consistent nomenclature for peptides cleaved from the resin, resin-bound peptides synthesised in the inverse (N-C) direction are, nevertheless, named according to this standard method. Thus, the peptide/peptidomimetic chain is systematically named (N-C) and included in the full compound name as a prefix to the parent resin *e.g.* **69a** is given the name 4-(N^{α}-methoxycarbonylvalylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxyacetyl aminomethyl polystyrene.

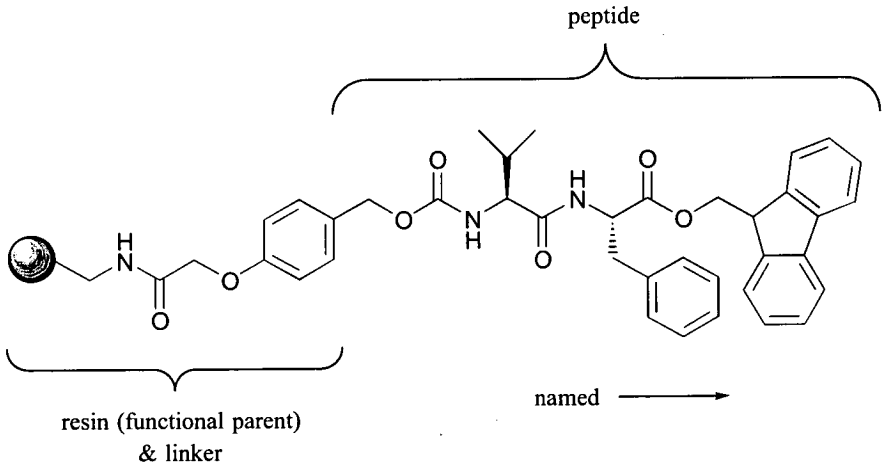


Figure 29 *Example of a polymer-supported inverse peptide analogue (69a)*

6.2.2 Resins/Crowns

The resins employed were aminomethylated polystyrene resin purchased from Novabiochem (Nova) HL 38-75 μm copoly-(styrene-1 % DVB) and Polymer Laboratories (PL) 75-150 copoly-(styrene-1 % DVB), or PL-Wang resin 75-150 μm

copoly-(styrene-1 % DVB). Preliminary studies were carried out on I-Series SynPhase™ methylacrylic acid/dimethylacrylamide graft polymer (MD) crowns, purchased from Chiron Technologies (CT).

6.2.3 Solid Phase Reactions

Solid phase reactions were carried out in either 3, 10 or 25 ml plastic isolute SPE filtration columns with top caps, luer tip caps and 20 μm porosity frits, purchased from Jones Chromatography and spun on a blood rotator to effect thorough mixing of the resin and reagents. Large scale reactions (>1.5 g resin) were carried out in standard glass round bottom flasks and a New Brunswick Scientific Gyrotory® water bath shaker was used to provide suitable agitation. In general, reactions were carried out using a minimum three-fold excess of reagents for polystyrene resins and a minimum ten-fold excess when reacting SynPhase™ MD crowns. On the whole, reactions were allowed to proceed for >15 h before filtering and washing as described below.

6.2.4 Washing Protocol

Once the solid phase reaction was deemed complete, the polystyrene resin was filtered and washed according to the following procedure: THF (x 2); DMF (x 2); DMF/MeOH (1:1) (x 2); DMF (x 2); THF (x 2); DCM (x 2). Plastic pipettes were used to provide agitation during the washing steps in order to avoid loss of the polystyrene resin which sticks to glass. When using isolute tubes a VacMaster™ 10 wash station was employed, otherwise a standard sinter funnel and water pump were used. The resin was then dried to constant weight under vacuum before analysing using the following methods (**6.3** and **6.4**). SynPhase™ MD crowns were washed with MeOH (x 3) and DCM (x 1) before drying and analysing as above.

6.3 On-Resin Analysis of Polymer-Supported Intermediates

6.3.1 IR Spectroscopy

Samples were prepared by taking a pre-washed and dried aliquot of resin (~5-10 mg), swelling in DCM and pressing between a pair of NaCl plates. The broad C-Cl stretch from the DCM masks any diagnostic peaks below ν_{max} ~820 cm^{-1}, but signals in the carbonyl region are generally very apparent.

6.3.2 Gel Phase ^{13}C NMR Spectroscopy

Samples were prepared by loading dry resin into an NMR tube (~1-1.5 cm depth) and adding CD_2Cl_2 dropwise until the resin had swollen fully[†]. Due to the broad nature of gel phase spectra, quaternary carbons are often too weak or broad to be observed clearly. Also, the polymer backbone itself gives two very broad peaks in the benzylic and aromatic regions of the spectrum, often obscuring any weaker signals. Therefore, where gel phase NMR data has been quoted in the experimental section, peaks have been assigned as far as possible, often with the aid of relevant solution spectra. Where atoms are not assigned, it is assumed that the corresponding peaks have either been obscured or are too weak to assign confidently.

6.3.3 Qualitative Detection of Resin-Bound Free Amines

6.3.3.1 Kaiser test[159]

A small sample of washed and dried resin (~2 mg) was treated with two drops each of reagents (A), (B) and (C). The resin suspension was then heated (~100 °C) for 2-5 min and any colour change noted. A dark blue/black colour indicated the presence of free amine, whereas a straw brown colour verified complete coupling. Due to the sensitivity of the test (5 μmole/g free amine) if a pale blue colour developed after 5 min it was assumed that the reaction had reached virtual completion, nevertheless, the test was always used in conjunction with the TNBS test (***6.3.3.2***).

[†] Care must be taken to avoid any air bubbles or foreign bodies (as unable to filter) which may give rise to distortions in the NMR spectrum.

Reagents: (A) ninhydrin (500 mg) in EtOH (10 ml); (B) phenol (80 g) in EtOH (20 ml); (C) 0.001 M potassium cyanide (2ml) diluted to 100 ml with pyridine.

6.3.3.2 TNBS test[160]

A small portion of washed and dried resin was treated with one drop each of solutions (C) and (D). Red polystyrene beads, viewed under a microscope, indicated the presence of free amine.

Reagents: (C) 10 % DIPEA in DMF; (D) 1 % TNBS in DMF.

6.3.4 Determination of Loading

6.3.4.1 Fmoc/Fm analysis

A minimum of two samples of dry resin (4-10 mg) were weighed directly into 10 ml volumetric flasks and made up to the volumetric level with a solution of 20 % piperidine in DMF. The samples were sonicated at room temperature for 4 h, before recording the UV spectrum of the supernatant between 280 and 320 nm. Where absorbances with a λ_{max} >1 were measured, the sample was diluted with blank 20 % piperidine in DMF, until an absorbance of λ_{max} <1 was achieved in accordance with the Beer-Lambert Law.

The formula below was used to calculate the resin loading:

$$\textbf{mmol/g} = (\textbf{A} \times \textbf{v}) / (\varepsilon \times 10^{-3} \times \textbf{wt})$$

[**A** - absorbance @ 301 nm (λ_{max}); **v** - volume (ml); **wt** - weight of resin (mg); ε - extinction coefficient of *N*-(9-fluorenylmethyl)piperidine[161] (ε 301 nm = 7800 M^{-1} cm^{-1})]

6.3.4.2 p-*Nitrophenol analysis*

One blank, three standards and two samples were prepared as follows:

Blank DMF (1 ml) was added to 20 % piperidine in DMF (1 ml) and the solution stirred for 1 h. The solution was diluted to 10 ml with MeOH, 200 µl withdrawn and diluted to 5 ml with H_2O.

Standard *p*-nitrophenol (4-6 mg) was dissolved in DMF (2 ml) and 20 % piperidine in DMF (2 ml) added. The bright yellow solution was agitated for 1 h before being diluted to 25 ml with MeOH, 250 μl withdrawn and diluted to 10 ml with H_2O.

Sample a sample of dry resin (4-6 mg) was suspended in DMF (1 ml). Addition of 20 % piperidine in DMF (1 ml) was followed by agitation for 1 h and the resultant bright yellow slurry diluted to 10 ml with MeOH, 200 μl withdrawn and diluted to 5 ml with H_2O.

Using the blank as a reference, the UV spectrum of each supernatant was recorded between 200 and 600 nm. The approximate resin loading was determined using the formula below:

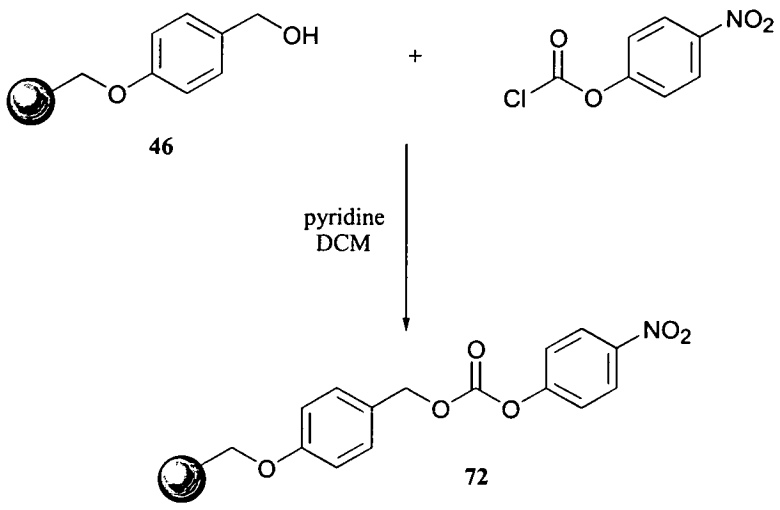
$$\textbf{mmol/g} = [\mathbf{A}_{sample} \times \mathbf{wt}_{std} \times 1000]/[\mathbf{wt}_{sample} \times \mathbf{A}_{std} \times 4 \times 139.1]$$

[A - absorbance @ 400 nm; wt - weight of resin/*p*-nitrophenol (mg)]

Due to the errors incurred from weighing small samples and carrying out repeated dilutions, the loadings obtained from both methods (***6.3.4.1*** & ***6.3.4.2***) are only considered to be accurate to ~ ± 0.05 mmol/g and are merely intended to give an indication of the success of the reaction. In terms of starting loadings for reactions, although the loading of the resin to be derivatised was calculated where possible, in order to account for inconsistencies, reagents were often added with respect to the maximum loading (manufacturer). Where yields are quoted, however, the calculated loading of the precursor has been used to determine the theoretical maximum loading of the product (***6.3.4.3***).

6.3.4.3 Calculation of yields based on loading analysis

When determining the yield of a solid phase reaction, the weight gain/loss during the reaction must be accounted for when calculating the maximum theoretical yield.



Scheme 37 *Activation of PS-Wang (46)*

For instance during the activation of Wang resin **46** (Scheme 37), there is a net weight gain of 165.11 g/mole functionality. Therefore the increase in weight of 1 g of starting material (0.9 mmol/g) after activation would be (0.9 x 10^{-3} x 165.11) = 0.15 g. Therefore, the maximum possible loading of the activated resin can be calculated as (0.90 / 1.15) = 0.78 mmol/g. The loading of **72** was determined to be 0.90 mmol/g by *p*-nitrophenol analysis (***6.3.4.2***). The percentage yield was therefore calculated to be (0.90 / 0.78 x 100) = 100 %*,[†] thus exemplifying the inaccuracy of the loading analysis method. Therefore, as with the loadings, yields are only included to give a rough indication of the reaction concerned, with corroborating evidence of yield and purity being obtained from gel phase ^{13}C NMR and LC-MS analysis.

6.4 Product Release and Subsequent Analysis

6.4.1 General Procedure for Acidic Cleavage

An aliquot of dry resin (~4-6 mg) was treated with a solution of TFA/DCM/H_2O (9:10:1) (300 µl) for 1 h. The resin was filtered using a glass pipette and cotton-wool, washed with DCM and the filtrate and washings combined and concentrated.

[†] Calculated yields of greater than 100 % (e.g. 115% **72**) are denoted by an asterix.

6.4.2 ES-MS/LC-MS

After cleavage (**6.4.1.**) the crude product was dissolved in a solution of MeCN/H_2O (1:1) with 0.01 % TFA. An aliquot (100 - 500 µl depending on expected yield) was withdrawn and diluted to 1 ml with the original solution. Analysis by ES-MS was carried out on a Micromass™ Platform II spectrometer with MassLynx Version 2.3, Build 5 software. When required, LC-MS analysis was carried out using a Waters™ 2690 Separations Module and a Waters™ 486 Tunable absorbance Detector measuring at 215 nm. A Phenomenex Luna 3 µ C18(2) 50 x 1.00 mm column was used as the stationary phase eluting with H_2O/MeCN at a flow rate of 0.1 ml min^{-1}. Retention times (R_t) are quoted in minutes. Two gradient elutions were employed (Tables 7 and 8):

Time (min)	H_2O	MeCN
0	80 %	20 %
5	80 %	20 %
25	40 %	60 %
30	80 %	20 %
40	80 %	20 %

Table 8 *LC-MS gradient A*

Time (min)	H_2O	MeCN
0	95 %	5 %
5	95 %	5 %
35	5 %	95 %
40	5 %	95 %
45	95 %	5 %
60	95 %	5 %

Table 9 *LC-MS gradient B*

6.4.3 Semi-Preparative HPLC

HPLC analysis was carried out on large-scale cleavage products, using a Waters™ 600 Controller and Pump, a Waters™ 486 Tunable absorbance Detector measuring at 214 nm and Millenium software. A Phenomenex Sphereclone 5 µ ODS(2) 250 x 10.0

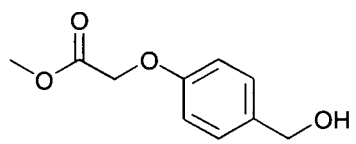
mm column was used as the stationary phase eluting with a H_2O/MeCN gradient (Table 9) at a flow rate of 5 ml min^{-1}. Retention times (R_t) are quoted in minutes.

Time (min)	H_2O	MeCN
0	30 %	70 %
2	30 %	70 %
12	10 %	90 %
13	10 %	90 %
13.5	30 %	70 %
15	30 %	70 %

Table 10 *HPLC gradient*

6.5 Synthesis of Resin-Bound HMPA Linker (PS-HMPA) (47)

6.5.1 Methyl-4-(hydroxymethyl) phenoxyacetate (48)

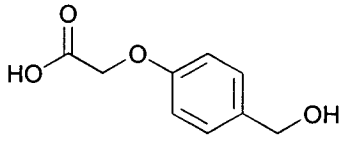


Anhydrous potassium carbonate (5.00 g, 0.04 mole) and sodium iodide (1.20 g, 8.0 mmol) were suspended in anhydrous DMF (10 ml). To this solution was added a solution of 4-hydroxybenzyl alcohol (5.00 g, 0.04 mmol) in DMF (30 ml), dropwise (exothermic), with stirring. Methyl bromoacetate (4.25 ml, 0.05 mmol) was added dropwise and the suspension left to stir under an inert atmosphere (>15 h). The reaction was quenched with H_2O (100 ml) and saturated ammonium chloride (100 ml). The product was taken up with chloroform (3 x 200 ml) and the combined extracts washed alternately with saturated sodium thiosulfate (2 x 400 ml), H_2O (2 x 400 ml) and brine (2 x 400 ml). After drying (Na_2SO_4), the solvent was removed under reduced pressure to yield the crude product as a yellow oil. Purification by column chromatography, eluting with EtOAc/light petroleum (2:3), furnished the title compound as a white solid (5.95 g, 76 %).

R_f (EtOAc/light petroleum, 1:2) 0.19; Mp 46-47 °C; (Found: C, 61.55; H, 6.13; $C_{10}H_{12}O_4$ requires C, 61.22; H, 6.16); ν_{max} (thin film)/cm^{-1} 3252 (OH), 1770 (C=O), 1614, 1587 & 1515 (aromatic C=C), 842, 820 or 810 (*p*-disubstituted Ar-H); δ_H (250

MHz, $CDCl_3$) 2.04 (1H, br s, O*H*), 3.77 (3H, s, OCH_3), 4.57 (2H, s, CH_2OH), 4.60 (2H, s, COCH_2O), 6.82 (2H, d, *J* 9.0, 2 x *m*-Ar*H*CH_2OH), 7.26 (2H, d, *J* 9.0, 2 x *o*-Ar*H*CH_2OH); δ_C (63 MHz, $CDCl_3$) 52.1 (OCH_3), 64.6 (*C*H_2OH), 65.1 (CO*C*H_2O), 114.4 (2 x *m*-*Ar*HCH_2OH), 128.5 (2 x *o*-*Ar*HCH_2OH), 134.2 (*Ar*CH_2OH), 157.1 (*Ar*OCH_2), 169.3 (*C*OOCH_3); Found (FAB) M^+ 196.07369, $C_{10}H_{12}O_4$ requires 196.07356.

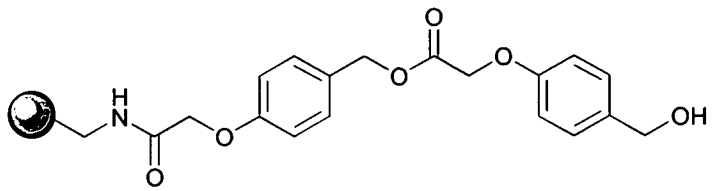
6.5.2 4-(Hydroxymethyl)-phenoxyacetic acid 49



To a solution of ester **48** (2.00 g, 10.20 mmol) in THF (150 ml), was added a 4 M solution of lithium hydroxide monohydrate (26 ml, 0.10 mole) and the resulting solution stirred at room temperature until tlc analysis (EtOAc/light petroleum, 1:2) indicated disappearance of starting material (30 min). The solution was acidified by addition of 2 M HCl and the product extracted with EtOAc (3 x 300 ml). The combined organic extracts were washed with brine (2 x 300 ml), dried (Na_2SO_4) and concentrated. The crude solid was recrystallised from minimum boiling EtOAc, to furnish the title compound as white crystals (0.77 g, 41 %).

Mp 110-111°C, Lit.[116] 110.5-112°C; (Found: C, 59.60; H, 5.56; $C_9H_{10}O_4$ requires C, 59.34; H, 5.53); ν_{max} (KBr)/cm^{-1} 3539 & 3379br (alcohol & acid OH), 1718 (C=O), 1610, 1588 & 1514 (aromatic C=C), 856, 841 or 825 (*p*-disubstituted Ar-H); δ_H (250 MHz, CD_3OD) 4.62 (2H, s, CH_2OD), 4.73 (2H, s, CH_2OAr), 7.00 (2H, d, *J* 9.0, 2 x *m*-Ar*H*CH_2OD), 7.37 (2H, d, *J* 9.0, 2 x *o*-Ar*H*CH_2OD); δ_C (63 MHz, CD_3OD) 62.9 (*C*H_2OD), 64.0 (CO*C*H_2O), 113.6 (2 x *m*-*Ar*HCH_2OD), 127.7 (2 x *o*-*Ar*HCH_2OD), 133.7 (*Ar*CH_2OD), 156.9 (*Ar*OCH_2), 170.8 (CH_2*C*OOD); Found (FAB) M^+ 182.05775, $C_9H_{10}O_4$ requires 182.05791.

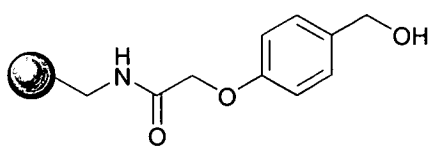
6.5.3 4-[4'-(Hydroxymethyl)-phenoxyacetoxymethyl]-phenoxyacetyl aminomethyl polystyrene 50



Typical procedure[111] - a solution of 4-(hydroxymethyl)-phenoxyacetic acid (**49**) (1.64 g, 9.0 mmol), HOBt (1.22 g, 9.0 mmol) and DIC (1.41 ml, 9.0 mmol) in DCM/DMF (1:1, 40 ml) was added to a suspension of aminomethylated polystyrene resin (1.0 mmol/g^{Nova} - 4.50 g, 4.5 mmol) in DCM (100 ml). The reaction was agitated at room temperature (>15 hours). A small sample (20 mg) of resin was withdrawn and washed with EtOH (x 3), dried and analysed for the presence of free amine using both the Kaiser and TNBS tests (***6.3.3.1*** *&* ***6.3.3.2***). Upon observation of a negative result[†], the full resin sample was filtered, washed according to standard protocol and dried to a constant weight under vaccuum.

ν_{max} (DCM)/cm^{-1} 3601 & 3428 (OH & NH), 1757 (ester C=O), 1679 (amide I C=O), 1612, 1602 (aromatic C=C), 1512 (amide II NH), 1493 (aromatic C=C), 829 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 42.9 (*C*H_2NH), 64.5 (*C*H_2OH), 65.4 (*C*H_2OCO), 66.7 (OCO*C*H_2O), 67.6 (NHCO*C*H_2O), 114.6 & 114.7 (2 x *m*-*Ar*HCH$_2$O), 128.2 (*Ar*CH$_2$OCO obscured by broad resin signal), 128.6 (2 x *o*-*Ar*HCH$_2$OH), 130.4 (2 x *o*-*Ar*HCH$_2$OCO), 134.9 (*Ar*CH$_2$OH), 157.3 & 158.2 (2 x *Ar*OCH$_2$), 168.9 (2 x *C*OCH$_2$O).

6.5.4 4-(Hydroxymethyl)-phenoxyacetyl aminomethyl polystyrene 47



Typical procedure - resin-bound ester (**50**) (assume ≤1.0 mmol/g - 6.22 g, ≤6.22 mmol) was suspended in DMF (60 ml). Hydrazine monohydrate (15.1 ml, 0.31

[†] where samples tested positive for free amine further equivalents of reagents were added and reaction times increased.

mole) was added to the slurry and the reaction mixture agitated at room temperature (>15 hours). A small portion (20 mg) of resin was withdrawn, washed: THF (x 2); DMF(x 2); DMF/MeOH (1:1) (x 2); DMF/H_2O (1:1) (x 2); DMF/MeOH (1:1) (x 2); DMF(x 2); THF (x2); DCM (x 2); and analysed by IR. Disappearance of the strong ester stretch (ν_{max} ~1757 cm^{-1}) indicated complete hydrolysis. After filtration the full resin sample was washed as above and dried to constant weight.

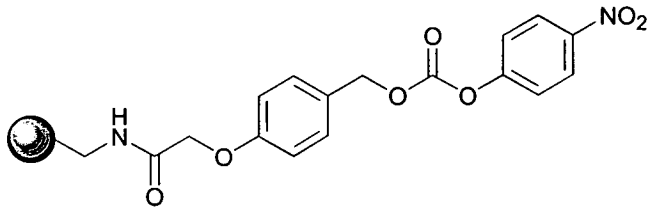
ν_{max} (DCM)/cm^{-1} 3603 & 3427 (OH), 1679 (amide I C=O), 1602br (aromatic C=C), 1532 (amide II NH), 1510 (aromatic C=C), 830 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 42.7 (CH_2NH), 64.3 (CH_2OH), 67.6 ($NHCOCH_2O$), 114.7 (2 x *m*-$ArHCH_2O$), 128.7 (2 x *o*-$ArHCH_2OH$), 135.5 ($ArCH_2OH$), 156.7 ($ArOCH_2$), 168.2 ($COCH_2O$).

6.6 Preparation of Resin-Bound 5(4*H*)-oxazolones 55a-b Using Free Amino Acids

6.6.1 General Procedure for Activation of Linkers 47[111] & 46

A solution of *p*-nitrophenyl chloroformate (3 eq) in dry DCM, was added to a suspension of the resin-bound linker (1 eq) in dry DCM. Anhydrous pyridine (3 eq) was added and the reaction agitated under an atmosphere of argon (>15 h). An aliquot (~20 mg) of resin was withdrawn, washed according to the standard protocol, dried and analysed by IR. Upon observation of a strong carbonate stretch (ν_{max} ~1767 cm^{-1}) the entire sample was filtered and washed thoroughly in the usual manner.

6.6.2 4-[Hydroxymethyl-(4'-nitrophenylcarbonate)]-phenoxyacetyl aminomethyl polystyrene 53

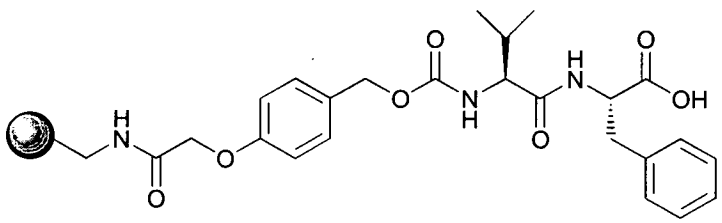


The general procedure (**6.6.1**) detailed above, was followed using *p*-nitrophenyl chloroformate (2.78 g, 13.8 mmol) in DCM (40 ml), PS-HMPA (**47**) (assume ≤1.0

mmol/g - 4.6 g, ≤4.6 mmol) in DCM (20 ml) and anhydrous pyridine (1.21 ml, 13.8 mmol). A strong carbonate stretch (ν_{max} 1767 cm^{-1}) present in the IR spectrum of the small aliquot of resin indicated success and the entire sample was filtered and washed thoroughly. Gel phase ^{13}C NMR indicated complete conversion to the desired resin-bound carbonate (**53**).

Loading 1.0 mmol/g (*p*-nitro), 100 %*; ν_{max} (DCM)/cm^{-1} 3430 (NH), 1767 (carbonate C=O), 1681 (amide I C=O), 1596 (aromatic C=C), 1529 (asymmetric NO_2), 1513 (amide II NH), 1493 (aromatic C=C), 1349 (symmetric NO_2), 864 (*p*-disubstituted *p*-nitro Ar-H), 834 (*p*-disubstituted Ar-H); δ_C (63 MHz, $CDCl_3$, gel) 42.5 (CH_2NH), 67.2 (CH_2OAr), 70.3 (CH_2OCO), 114.7 (2 x *m*-$ArHCH_2O$), 121.5 (2 x *m*-$ArHNO_2$), 125.1 (2 x *o*-$ArHNO_2$), 130.6 (2 x *o*-$ArHCH_2O$), 145.1 ($ArNO_2$-obscured by broad polymer peak), 152.2 ($ArOCO$), 155.2 ($ArOCH_2$), 157.5 (OCOO), 167.4 (NHCO).

6.6.3 4-(N^{α}-Methoxycarbonylvalylphenylalanine)-phenoxyacetyl aminomethyl polystyrene 54a - *via* direct dipeptide coupling

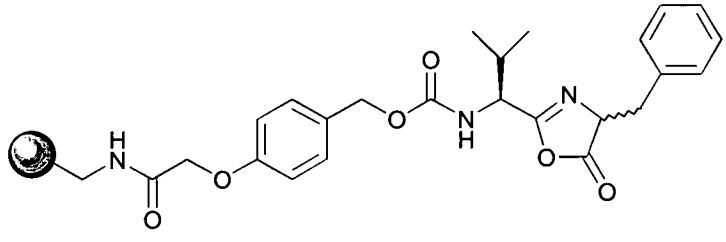


Valinylphenylalanine (250 mg, 0.95 mmol) was dissolved in DMF (10 ml) using BSA (0.59 ml, 2.40 mmol) and sonication (≤60 °C).[107] This solution was added to activated resin (**53**) (~1.0 mmol/g - 240 mg, ~0.24 mmol) followed by a solution of DMAP (59 mg, 0.48 mmol) in DMF (0.5 ml). The resin suspension was spun at room temperature (>15 h). The resin was filtered and washed according to the standard protocol and dried under vacuum. IR analysis indicated successful coupling due to the disappearance of the distinctive carbonate stretch (ν_{max} 1767cm^{-1}). Gel phase ^{13}C NMR analysis gave a very broad spectrum, which proved difficult to interpret but indicated that the desired product was indeed present but impure.

ν_{max} (DCM)/cm^{-1} 3425 & 3321 (2° amide & urethane NH), 1682br (urethane, acid & 2 x amide I C=O), 1602br (aromatic C=C), 1504br (2 x amide II NH & aromatic

C=C), 830 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 17.9 ($CH(C^AH_3C^BH_3)$), 19.2 (($CH(C^AH_3C^BH_3)$), 31.1 ($CH(CH_3)_2$), 40.7 (CH_2Ph obscured by broad polymer signal), 53.6 ($CHCH_2Ph$ obscured by solvent), ~61br ($CHCH(CH_3)_2$), 64.3 (unknown), 67.5br ($COCH_2O$ & CH_2OCONH), 114.7 (2 x *m-Ar*HCH_2O), 128.2br (all other aromatics obscured by broad polymer signal); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 265 (MH^+), 287 (MNa^+).

6.6.4 (1'*S*, 4''*RS*)-4-[*N*-Methoxycarbonyl-2'-methyl-1'-(4''-benzyl-5''-oxo-4'',5''-dihydro-oxazol-2''-yl)-aminopropyl]-phenoxyacetyl aminomethyl polystyrene 55a - derived from dipeptide coupling

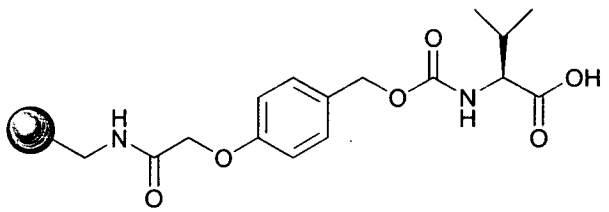


Resin-bound dipeptide (**54a**) (assume ≤1 mmol/g - 164 mg, ≤0.16 mmol) was allowed to swell in DCM (8 ml), before addition of EDCI (92 mg, 0.48 mmol). The resulting suspension was spun at room temperature (15 h). The resin was filtered and washed according to the standard protocol, before drying under vacuum. Analysis by IR confirmed the presence of the desired oxazolone (ν_{max} 1824cm^{-1}). Gel phase ^{13}C NMR analysis indicated the title compound was present as a mixture of diastereomers, but extra peaks and the general broad quality of the spectrum suggested that the product was not pure.

ν_{max} (DCM)/cm^{-1} 3427 (2° amide & urethane NH), 1825 (oxazolone C=O), 1722 (urethane C=O), 1680 (amide I C=O), 1602 (aromatic C=C), 1510 (amide II NH), 1494 (aromatic C=C), 828 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 16.9 & 17.5 ($CH(C^AH_3C^BH_3)$), 18.8 & 19.2 (($CH(C^AH_3C^BH_3)$), 30.6 (2 x $CH(CH_3)_2$), 36.7 & 36.8 (2 x CH_2Ph), 55.1 & 55.5 (2 x $CHCH_2Ph$), 64.3 (unknown), 65.7 & 65.8 (2 x $CHCH(CH_3)_2$), 66.7 ($COCH_2O$), 67.2 & 67.6 (2 x CH_2OCONH), 114.7 (2 x *m-Ar*HCH_2O), 127.4 (*Ar*H Phe), 128.6 (2 x *Ar*H Phe), 129.8 (2 x *Ar*H Phe), 135.3 (*ipso-*

C Phe); Cleavage[†] 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 247 (MH^+), 265 (MH^+ + H_2O), 287 (MNa^+ + H_2O).

6.6.5 4-(N^{α}-Methoxycarbonylvaline)-phenoxyacetyl aminomethyl polystyrene 56

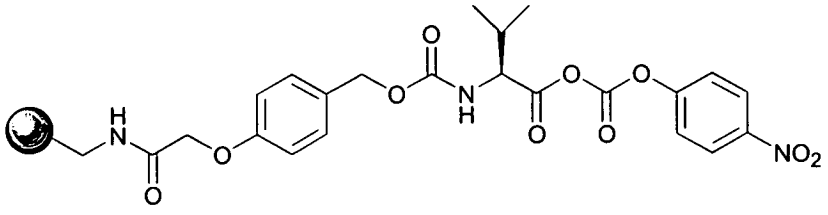


L-Valine (1.87 g, 16 mmol) was dissolved in DMF (115 ml) with the help of BSA (9.89 ml, 40 mmol) and sonication at elevated temperature (≤60 °C).[107] After adding activated resin **53** (~1.0 mmol/g - 4.0 g, ~4.0 mmol) along with a solution of DMAP (0.98 g, 8.0 mmol) in DMF (5 ml), the suspension was agitated at room temperature (>15 h). A small sample of resin was withdrawn from the bulk reaction, washed according to standard protocol, dried and analysed by IR. Disappearance of the distinctive carbonate stretch (ν_{max} 1767cm^{-1}) indicated successful coupling, allowing the main resin sample to be filtered and washed accordingly. After drying, analysis by IR, gel phase ^{13}C NMR and after cleavage, ES-MS indicated the presence of the desired product. However, the poor quality of the spectra obtained implied that it was not pure.

ν_{max} (DCM)/cm^{-1} 3424 & 3329 (2° amide & urethane NH), 1731br (urethane & acid C=O), 1673br (amide I C=O), 1602 (aromatic C=C), 1504br (2 x amide II NH & aromatic C=C), 827 (*p*-disubstituted Ar-H); δ_C (63 MHz, $CDCl_3$, gel) 17.5 or 17.9 ($CH(C^AH_3C^BH_3)$), 19.3 ($(CH(C^AH_3C^BH_3)$), 31.2 ($CH(CH_3)_2$), 59.8br ($CHCH(CH_3)_2$), 64.2 (unknown), 66.6 ($COCH_2O$), 67.5 (CH_2OCONH), 114.7 (2 x *m-Ar*HCH_2O), 134.2 (2 x *o-Ar*HCH_2O), 156.8br (OCONH), 168.5br ($NHCOCH_2O$), 174.9br (COOH); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 118 (MH^+).

[†] treatment with TFA results in almost complete hydrolysis of the 5(*4H*)-oxazolone

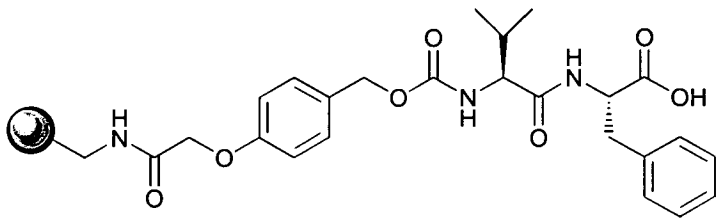
6.6.6 4-[N^{α}-Methoxycarbonylvalyloxycarboxy-4'-(nitrophenoxy)]-phenoxyacetyl aminomethyl polystyrene 57 - proposed activated intermediate for stepwise coupling



A solution of *p*-nitrophenyl chloroformate (1.08 g, 5.37 mmol) in anhydrous DCM (18 ml), was added to a suspension of **56** (assume ≤1.0 mmol/g - 1.79 g, ≤1.79 mmol) in dry DCM (6 ml). Anhydrous pyridine (0.43 ml, 5.37 mmol) was added slowly (exothermic) and the reaction agitated at room temperature, under argon (>15 h). An aliquot of resin was removed and washed according to the standard protocol. Analysis, by IR, of the dried sample indicated the presence of a nitro group and strong carbonyl stretches, accordingly the bulk reaction was drained, washed and dried to constant weight. Analysis by gel phase ^{13}C NMR resulted in a broad spectrum, which proved difficult to interpret, however, diagnostic peaks have been selected and the proposed assignments detailed below.

Loading 0.71 mmol/g (*p*-nitro), 82%; ν_{max} (DCM)/cm^{-1} 3429 (2° amide & urethane NH), 1767 & 1723 (mixed anhydride of carbonic acid & urethane C=O), 1681 (amide I C=O), 1614 & 1602 (aromatic C=C), 1524 (asymmetric NO_2), 1513 (amide II NH), 1493 (aromatic C=C), 1348 (symmetric NO_2), 864 (*p*-disubstituted *p*-nitro Ar-H), 832 (*p*-disubstituted Ar-H); δ_C (63 MHz, $CDCl_3$, gel) 17.5 & 17.8 (2 x $CH(C^AH_3C^BH_3)$), 18.8 & 19.1 (2 x $CH(C^AH_3C^BH_3)$), 31.0br ($CH(CH_3)_2$), 42.6 (CH_2NH), 46.1 (unknown), 55.6, 59.7 or 60.5 ($CHCH(CH_3)_2$), 66.7 (unknown), 67.5 ($COCH_2O$), 70.6 (CH_2OCONH), 114.8 (2 x *m*-$ArHCH_2O$), 116.0 & 122.0 (unknown), 122.5 (2 x *m*-$ArHNO_2$), 125.3 (2 x $ArHNO_2$), 130.1 (2 x *o*-$ArHCH_2O$), 141.9br ($ArNO_2$); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 239 (MH^+-CO_2).

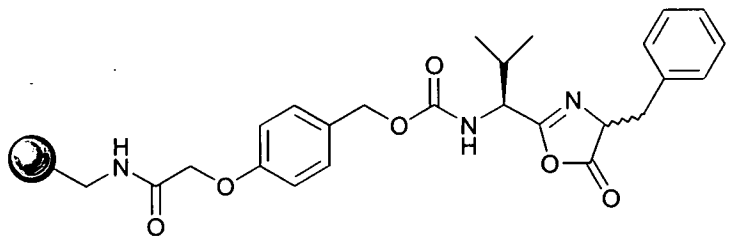
6.6.7 4-(N^{α}-Methoxycarbonylvalylphenyalanine)-phenoxyacetyl aminomethyl polystyrene 54b - *via* stepwise coupling



L-Phenylalanine (344 mg, 2.08 mmol) was dissolved in DMF (10 ml), with the help of BSA (1.29 ml, 5.20 mmol) and sonication (3 h).[107] PS-HMPA-Val (**56**) (~0.6 mmol/g - 860 mg, ~0.52 mmol) was allowed to swell in this solution before addition of DMAP (127 mg, 1.04 mmol) and the resulting suspension was shaken at room temperature (>15 h) . The yellow reaction solution was drained off and the resin washed according to the standard protocol. After drying, analysis by gel phase ^{13}C NMR, IR and, after cleavage, ES-MS did indicate the presence of the title compound. However, the quality of spectra obtained suggested that **54b** was present as the major product in a mixture of several by products leading to some difficulties in interpretation and assignment. ^{13}C NMR peaks have been assigned as far as possible.

ν_{max} (DCM)/cm^{-1} 3426 & 3334 (2° amide & urethane NH), 1681br (urethane, acid & 2 x amide I C=O), 1614 & 1602 (aromatic C=C), 1504br (2 x amide II NH & aromatic C=C), 823 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 17.9 ($CH(C^AH_3C^BH_3)$), 19.3 (($CH(C^AH_3C^BH_3)$), 31.2 ($CH(CH_3)_2$), 40.7 (CH_2Ph partially obscured by broad polymer signal), 53.6 ($CHCH_2Ph$ obscured by solvent), 60.7br ($CHCH(CH_3)_2$), 64.3 (unknown), 66.6 ($COCH_2O$), 67.6 (CH_2OCONH), 114.8 (2 x *m*-$ArHCH_2O$), 128.5 & 129.7 (5 x ArH Phe obscured by broad polymer signal), 134.2 (2 x *o*-$ArHCH_2O$), 156.4br (OCONH), 174.4br (COOH); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 265 (MH^+), 287 (MNa^+).

6.6.8 (1'*S*, 4''*RS*)-4-[*N*-Methoxycarbonyl-1'-(4''-benzyl-5''-oxo-4'',5''-dihydro-oxazol-2''-yl)-2'-methyl-aminopropyl]-phenoxyacetyl aminomethyl polystyrene 55b - derived from stepwise coupled dipeptide



PS-HMPA-Val-Phe (**54b**) (assume ≤0.6 mmol/g - 0.54 g, ≤0.33 mmol) was allowed to swell in DCM (4 ml), before addition of EDCI (0.19 g, 0.99 mmol). The reaction was spun at room temperature (>15 h). A small sample of resin was withdrawn, washed according to standard lab protocol and dried. Analysis by IR showed a stretch at ν_{max} 1824cm^{-1}, indicative of the 5(4*H*)-oxazolone carbonyl group. The remaining resin was filtered, washed according to general procedure and dried. Subsequent analysis indicated that the desired product had indeed been formed, however, ^{13}C NMR spectral data was of poor quality due to the impurity of the final product and was too broad to derive any meaningful data.

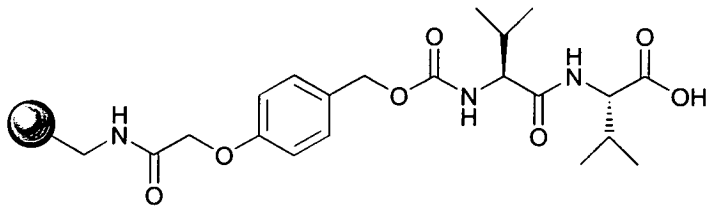
ν_{max} (DCM)/cm^{-1} 3427 (2° amide & urethane NH), 1824 (oxazolone C=O), 1716 (urethane C=O), 1682 (amide I C=O), 1602 (aromatic C=C), 1530 & 1511 (2 x amide II NH), 1494 (aromatic C=C), 827 (*p*-disubstituted Ar-H); Cleavage[†] 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 265 ($MH^+ + H_2O$).

6.6.9 General procedure for BSA-assisted coupling of free amino acids 58a-d[107]

Unprotected L-amino acid/dipeptide (4 eq) was dissolved in DMF (4 ml), with the help of BSA (10 eq) and sonication (≤60°C). PS-HMPA-Val (**56**) (~ 0.7 mmol/g, 1 eq) was allowed to swell in this solution before addition of a solution of DMAP (4 eq) in DMF (1 ml). The reaction was spun at room temperature (>15 h), before being filtered and washed according to standard protocol.

[†] treatment with TFA results in almost complete hydrolysis of the 5(*4H*)-oxazolone

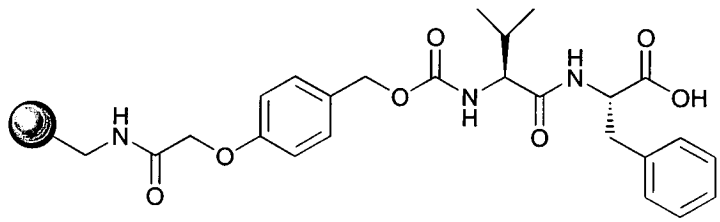
6.6.10 4-(N^{α}-Methoxycarbonylvalylvaline)-phenoxyacetyl aminomethyl polystyrene 58a



The general procedure outlined above (**6.6.9**) with L-valine (66 mg, 0.56 mmol) and a further portion of DMF (1 ml) to aid dissolution.

ν_{max} (DCM)/cm^{-1} 3427 & 3316 (2 x amide & urethane NH), 1682br & 1652br (urethane, acid & 2 x amide I C=O), 1602br (aromatic C=C), 1504br (2 x amide II & aromatic C=C); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 217 (MH^+); other products tabulated in results and discussion (Table 1).

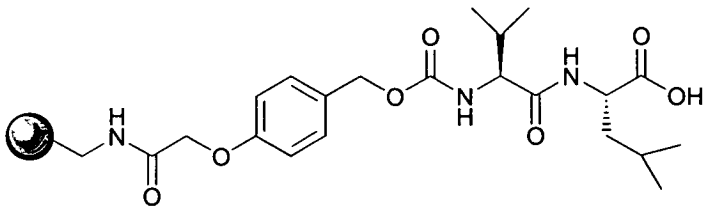
6.6.11 4-(N^{α}-Methoxycarbonylvalylphenylalanine)-phenoxyacetyl aminomethyl polystyrene 58b



The general procedure detailed previously (**6.6.9**) with L-phenylalanine (93 mg, 0.56 mmol).

ν_{max} (DCM)/cm^{-1} 3426 & 3317 (2 x amide & urethane NH), 1682br (urethane, acid & 2 x amide I C=O), 1602br (aromatic C=C), 1493br (2 x amide II & aromatic C=C); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 265 (MH^+); other products tabulated in results and discussion (Table 1).

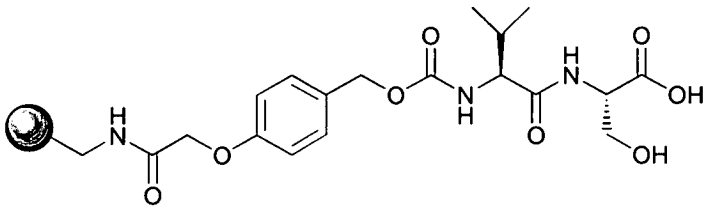
6.6.12 4-(N^{α}-Methoxycarbonylvalylleucine)-phenoxyacetyl aminomethyl polystyrene 58c



The general procedure outlined previously (**6.6.9**) with L-leucine (37 mg, 0.28 mmol).

ν_{max} (DCM)/cm^{-1} 3427 & 3316 (2 x amide & urethane NH), ~1680br & 1652br (urethane, acid & 2 x amide I C=O), 1602br (aromatic C=C), 1504br (2 x amide II & aromatic C=C); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 231 (MH^+); other products tabulated in results and discussion (Table 1).

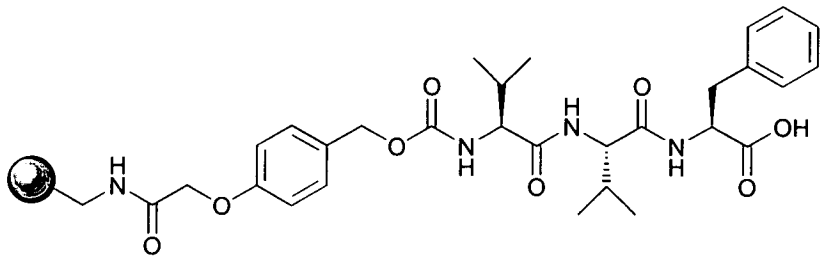
6.6.13 4-(N^{α}-Methoxycarbonylvalylserine)-phenoxyacetyl aminomethyl polystyrene 58d



The general procedure outlined above (**6.6.9**) with L-serine (29 mg, 0.28 mmol).

ν_{max} (DCM)/cm^{-1} 3427 & 3317br (2 x amide & urethane NH & OH), 1682br & 1651br (urethane, acid & 2 x amide I C=O), 1603br (aromatic C=C), ~1500br (2 x amide II & aromatic C=C); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 205 (MH^+); other products tabulated in results and discussion (Table 1).

6.6.14 4-(N^{α}-Methoxycarbonylvalylvalylphenylalanine)-phenoxyacetyl aminomethyl polystyrene 58e



The general procedure detailed previously (**6.6.9**) with valinylphenylalanine (71 mg, 0.27 mmol).

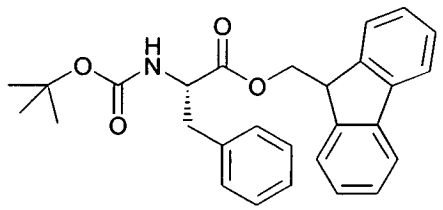
ν_{max} (DCM)/cm^{-1} 3426 & 3316 (3 x 2° amide & urethane NH), ~1680br & 1651 br (urethane, acid & 3 x amide I C=O), 1602br (aromatic C=C), 1504br (3 x amide II & aromatic C=C); Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 364 (MH^+); other products tabulated in results and discussion (Table 1).

6.7 Preparation of Building Blocks for Inverse Peptide Synthesis using OFm Ester Methodology

6.7.1 General procedure for OFm ester protection of Boc-amino acid derivatives[121]

Boc-amino acid derivative (1 eq), 9-fluorenylmethanol (2 eq) and catalytic DMAP (1 %) were dissolved in anhydrous DCM (3-5 ml/mmol) and cooled (ice/H_2O bath). DIC (2 eq) was added dropwise, with stirring to the cooled solution. The reaction was continued until complete conversion of starting amino acid, as indicated by tlc. The white urea precipitate was then filtered off and washed with DCM. The filtrate and washings were combined and washed consecutively with H_2O (x 2), saturated sodium bicarbonate (x 2), and 10 % citric acid (x 2), then alternately with H_2O (x 2) and brine (x 2). The organic layer was separated, dried (Na_2SO_4), filtered and concentrated. The crude product was then carried straight through to the TSA salt (**65a-e**) unless otherwise stated.

6.7.2 9-Fluorenylmethyl N^{α}-(*tert*-butoxycarbonyl)-phenylalaninate 64a



6.7.2.1

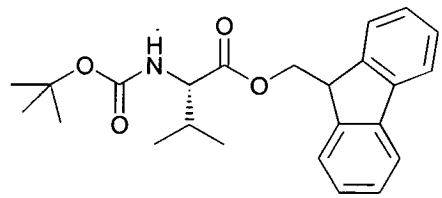
The general procedure outlined above (**6.7.1**) was followed using Boc-phenylalanine (2.50 g, 11.5 mmol), 9-fluorenylmethanol (4.51 g, 23 mmol), DMAP (12 mg, 0.1 mmol), DCM (40 ml) and DIC (3.60 ml, 23 mmol). The crude product was purified by column chromatography, eluting with hexane/EtOAc (5:1). Recrystallisation of the combined fractions with hot hexane/EtOAc (5:1) furnished the title compound as white crystals (3.05 g, 60%).

R_f (Hexane:EtOAc, 5:1) 0.18; Mp 126-127 °C, Lit.[162] 126-127 °C; (Found: C, 76.01; H, 6.50; N, 3.24; $C_{28}H_{29}NO_4$ requires C, 75.82; H, 6.59; N, 3.16); $[\alpha]_D^{20}$ -6 (c 1.00, $CHCl_3$), Lit.[125] $[\alpha]_D^{20}$ -6 (c 0.84, $CHCl_3$); ν_{max} (thin film)/cm^{-1} 3364br (NH), 1742 (ester C=O), 1712 (urethane C=O), 1605 (aromatic C=C), 1498br (amide II & aromatic C=C), 758 & 701 (monosubstituted Ar-H), 740 (*o*-disubstituted Ar-H); δ_H (250 MHz, $CDCl_3$) 1.46 (9H, s, $C(CH_3)_3$), 3.02 (1H, dd, *J* 14.0, 6.5, $CHCH_AH_BPh$), 3.10 (1H, dd, *J* 14.0, 6.5, $CHCH_AH_BPh$), 4.16 (1H, t, *J* 7.0, OCH_2CH), 4.44 (2H, d, *J* 7.0, OCH_2CH), 4.70 (1H, br dt, *J* 8.0, 6.5, $NHCHCH_2Ph$), 5.06 (1H, br d, *J* 8.5, $NHCHCH_2$), 7.12-7.15 (2H, m, Ar*H*), 7.21-7.36 (5H, m, Ar*H*), 7.43 (2H, t, *J* 7.5, Ar*H*), 7.53-7.58 (2H, m, Ar*H*), 7.78 (2H, d, *J* 7.5, Ar*H*); δ_C (63 MHz, $CDCl_3$) 28.1 ($C(CH_3)_3$), 38.1 ($CHCH_2Ph$), 46.5 (OCH_2CH), 54.3 ($CHCH_2Ph$), 66.9 (OCH_2CH), 79.8 ($OC(CH_3)_3$), 119.8 (2 x *Ar*H OFm), 124.8 (*Ar*H OFm), 124.9 (*Ar*H OFm), 126.9 (*Ar*H Phe), 127.0 (2 x *Ar*H OFm), 127.7 (2 x *Ar*H OFm), 128.4 (2 x *Ar*H Phe), 129.1 (2 x *Ar*H Phe), 135.8 (*ipso-C* Phe), 141.1 (2 x C^4 OFm), 143.2 (C^4 OFm), 143.3 (C^4 OFm), 154.9 (*O*CONH), 171.7 (*C*OO); Found (FAB) MH^+ 444.21701, $C_{28}H_{30}NO_4$ requires 444.21748.

6.7.2.2

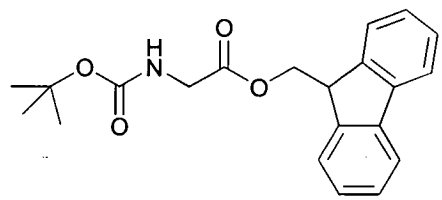
Improved yield, over two steps, when the general procedure outlined above (**6.7.1**) with Boc-phenylalanine (6.10 g, 23 mmol), 9-fluorenylmethanol (9.03 g, 46 mmol), DMAP (24 mg, 0.2 mmol), DCM (80 ml) and DIC (7.20 ml, 46 mmol), was followed and the crude product deprotected and carried through to the TSA salt (**65a**) without any further purification.

6.7.3 9-Fluorenylmethyl N^{α}-(*tert*-butoxycarbonyl)-valinate 64b



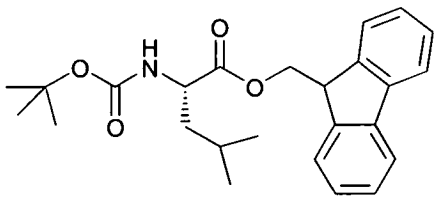
The general procedure outlined above (**6.7.1**) was followed with Boc-valine (5.0 g, 23 mmol), 9-fluorenylmethanol (9.03 g, 46 mmol), DMAP (24 mg, 0.2 mmol), DCM (80 ml) and DIC (7.20 ml, 46 mmol), to furnish the title compound as a yellow/white solid. The crude product was deprotected and carried through to the TSA salt (**65b**) without any further purification.

6.7.4 9-Fluorenylmethyl N^{α}-(*tert*-butoxycarbonyl)-glycinate 64c



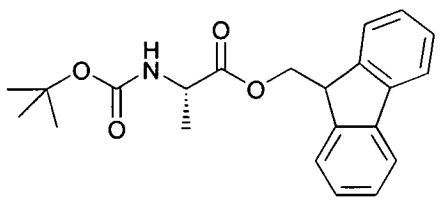
The general procedure outlined above (**6.7.1.**) with Boc-glycine (4.03 g, 23 mmol), 9-fluorenylmethanol (9.03 g, 46 mmol), DMAP (24 mg, 0.2 mmol), DCM (80 ml) and DIC (7.20 ml, 46 mmol), was followed to furnish the crude title compound as a yellow/white solid. The crude product was deprotected and carried through to the TSA salt (**65c**) without any further purification.

6.7.5 9-Fluorenylmethyl N^{α}-(*tert*-butoxycarbonyl)-leucinate 64d[AW]



The general procedure outlined above (**6.7.1**) was followed with Boc-leucine (2.03 g, 8.7 mmol), 9-fluorenylmethanol (3.41 g, 17.4 mmol), DMAP (10 mg, 0.01 mmol), and DIC (2.73 ml, 17.4 mmol), to furnish the crude title compound which was deprotected and carried through to the TSA salt (**65d**) (without any further purification.

6.7.6 9-Fluorenylmethyl N^{α}-(*tert*-butoxycarbonyl)-alaninate[AW] 64e

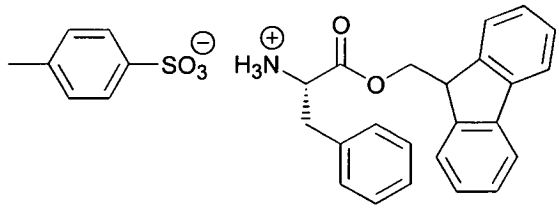


The general procedure outlined above (**6.7.1**) was followed with Boc-alanine (1.97 g, 10.4 mmol), 9-fluorenylmethanol (4.08 g, 20.8 mmol), DMAP (12 mg, 0.10 mmol), and DIC (3.25 ml, 20.8 mmol), to furnish the crude title compound which was deprotected and carried through to the TSA salt (**65e**) without any further purification.

6.7.7 General procedure for preparation of ammonium salts of amino acid 9-fluorenylmethyl ester tosylates[125]

The crude, or otherwise, Boc-amino acid fluorenylmethyl ester (1 eq) was treated with TFA (~1-4 ml/mmol) with stirring for 15-20 min. *p*-Toluene sulfonic acid (TSA) (1.15 eq) was added along with, where necessary, extra TFA to aid dissolution. After stirring for a further 5 min, the TFA was removed under reduced pressure and the resulting oil triturated with copious ether to yield the desired salt.

6.7.8 9-Fluorenylmethyl N^{α}-(tosylate)-phenylalaninate 65a



6.7.8.1

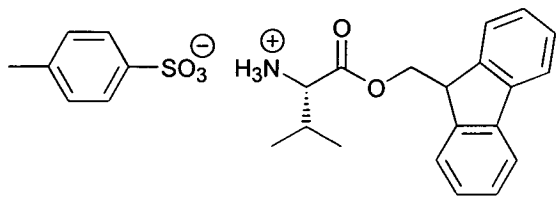
The general procedure outlined above (**6.7.7**) was followed using purified Boc-Phe-OFm (**64a**) (2.92 g, 6.58 mmol), TFA (25 ml) and TSA (1.44 g, 7.57 mmol), to afford the title compound as a white solid (3.25 g, 96 %).

6.7.8.2

The general procedure outlined above (**6.7.7**) was followed using crude Boc-Phe-OFm (**64a**) (~23 mmol), TFA (25 ml) and TSA (5.03 g, 26.45 mmol), to afford the title compound as a white solid (10.85 g, 91 % over two steps).

Mp 199-201 °C, Lit.[125] 210-212°C; (Found: C, 69.53; H, 5.67; N, 2.81; $C_{30}H_{29}NO_5S$ requires C, 69.88; H, 5.67; N, 2.72); $[\alpha]_D^{20}$ -41 (c 1.00, MeOH); ν_{max} (KBr)/cm^{-1} 1740 (ester C=O), 1609br (aromatic C=C), 1209br & 1036 (SO_3^-), 814 (*p*-disubstituted Ar-H), 759, 740, 699, & 681 (mono- & *o*-di-substituted Ar-H); δ_H (250 MHz, DMSO d^6) 2.27 (3H, s, CH_3Ar), 2.97^c (2H, m, CHC$H_A$$H_B$Ph), 4.14 (1H, bt, *J* 6.5, OCH_2CH), 4.43-4.41 (2H, m, OCH_AH_BCH & $CHCH_2$Ph), 4.59 (1H, dd, *J* 10.5, 6.0, OCH_AH_BCH), 7.09-7.16 (4H, m, Ar*H*), 7.21-7.63 (11H, m, Ar*H*), 7.86-7.90 (2H, m, Ar*H* OFm), 8.51 (3H, bs, NH_3^+); δ_C (63 MHz, DMSO d^6) 20.9 (CH_3Ar), 36.0 ($CHCH_2$Ph), 46.1 (OCH_2CH), 53.3 ($CHCH_2$Ph), 67.1 (OCH_2CH), 120.3 (2 x *Ar*H OFm), 125.2 (*Ar*H OFm), 125.4 (*Ar*H OFm), 125.7 (2 x *Ar*H TSA), 127.3 (*Ar*H Phe), 127.4 (*Ar*H OFm), 127.5 (*Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H TSA), 128.8 (2 x *Ar*H Phe), 129.5 (2 x *Ar*H Phe), 134.6 (*ipso-C* Phe), 138.2 ($ArCH_3$), 140.9 (2 x C^4 OFm), 143.2 (C^4 OFm), 143.4 (C^4 OFm), 145.3 ($ArSO_3^-$), 169.3 ($COOCH_2$); Found (FAB) MH^+ 344.16555, $C_{23}H_{22}NO_2$ requires 344.16505.

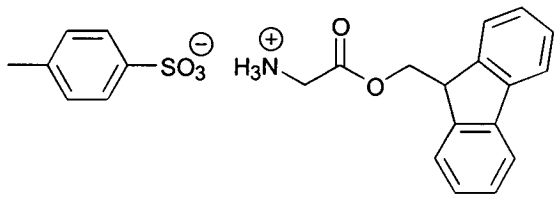
6.7.9 9-Fluorenylmethyl N^{α}-(tosylate)-valinate 65b



The general procedure outlined above (**6.7.7**) was followed using crude Boc-Val-OFm (**64b**) (~23 mmol), TFA (25 ml) and TSA (5.03 g, 26.45 mmol), to afford the title compound as a white solid (9.40 g, 87 % over two steps).

Mp 172-174°C, Lit.[121] 179-180°C; $[\alpha]_D^{20}$ -14 (c 1.00, MeOH); ν_{max} (KBr)/cm^{-1} 3447 (NH), 1749 (ester C=O), 1617br (aromatic C=C), 1187br & 1036 (SO_3^-), 1011, 813 (*p*-disubstituted Ar-H), 755, 741, 683, & 676 (mono- & *o*-di-substituted Ar-H); δ_H (250 MHz, DMSO d^6) 0.55 (3H, d, *J* 7.0, CH(C^A*H*$_3$C^{B}H$_3$)), 0.63 (3H, d, *J* 7.0, CH(C^{A}H$_3$C^B*H*$_3$)), 1.80-1.88 (1H, m, C*H*(CH$_3$)$_2$), 2.27 (3H, s, C*H*$_3$Ar), 3.75 (1H, bs, C*H*CH(CH$_3$)$_2$), 4.32 (1H, t, *J* 5.0, OCH$_2$C*H*), 4.60 (1H, dd, *J* 11.0, 5.0, OC*H*$_A$H$_B$CH), 4.94 (1H, dd, *J* 11.0, 5.0, OCH$_A$*H*$_B$CH), 7.12 (2H, d^i, *J* ~8, Ar*H* TSA), 7.29-7.45 (4H, m, Ar*H* OFm), 7.50 (2H, d^i, *J* ~8, Ar*H* TSA), 7.67-7.74 (2H, m, Ar*H* OFm), 7.88 (2H, ddd, *J* 6.5, 2.5, 1.0, Ar*H* OFm), 8.29 (3H, bs, NH_3^+); δ_C (63 MHz, DMSO d^6) 17.1 (CH(*C*^{A}H$_3$C^{B}H$_3$)), 17.9 (CH(C^{A}H$_3$*C*^{B}H$_3$)), 20.9 (*C*H$_3$Ar), 29.2 (CH*C*H(CH$_3$)$_2$), 46.4 (OCH$_2$*C*H), 57.2 (*C*HCH(CH$_3$)$_2$), 66.4 (O*C*H$_2$CH), 120.3 (2 x *Ar*H OFm), 125.0 (*Ar*H OFm), 125.1 (*Ar*H OFm), 125.7 (2 x *Ar*H TSA), 127.3 (2 x *Ar*H OFm), 127.9 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H TSA), 138.1 (*Ar*CH$_3$), 141.1 (*C*^{4} OFm), 141.2 (*C*^{4} OFm), 143.4 (*C*^{4} OFm), 143.6 (*C*^{4} OFm), 145.4 (*Ar*SO$_3^-$), 169.1 (*C*OO); Found (FAB) MH$^+$ 296.16375, $C_{19}H_{22}NO_2$ requires 296.16505.

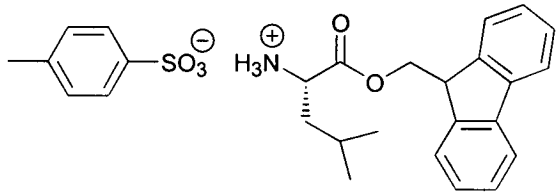
6.7.10 9-Fluorenylmethyl N^{α}-(tosylate)-glycinate 65c



The general procedure outlined above (**6.7.7**) was followed using crude Boc-Gly-OFm (**64c**) (~23 mmol), TFA (25 ml) and TSA (5.03 g, 26.45 mmol), to afford the title compound as a white solid (9.35 g, 96 % over two steps).

Mp 147-150 °C; ν_{max} (KBr)/cm^{-1} 1760 (ester C=O), 1597br (aromatic C=C), 1216, 1183 & 1033 (SO_3^-), 822 (*p*-disubstituted Ar-H), 759 & 744 (*o*-disubstituted Ar-H); δ_H (250 MHz, DMSO d^6) 2.26 (3H, s, C*H*$_3$Ar), 3.92 (2H, bs, C*H*$_2$COO), 4.29 (1H, t, *J* 7.0, OCH$_2$C*H*), 4.49 (1H, d, *J* 7.0, OC*H*$_2$CH), 7.11 (2H, d^i, *J* ~8, Ar*H* TSA), 7.31-7.46 (4H, m, Ar*H* OFm), 7.53 (2H, d^i, *J* ~8, *Ar*H TSA), 7.71 (2H, br d, *J* 7.5, Ar*H* OFm), 7.90 (2H, br d, *J* 7.0, Ar*H* OFm); 8.32 (3H, bs, N*H*$_3^+$); δ_C (63 MHz, DMSO d^6) 20.9 (*C*H$_3$Ar), 39.9 (*C*H$_2$COO), 46.1 (OCH$_2$*C*H), 67.0 (O*C*H$_2$CH), 120.4 (2 x *Ar*H OFm), 125.4 (2 x *Ar*H OFm), 125.7 (2 x *Ar*H TSA), 127.4 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H TSA), 138.1 (*Ar*CH$_3$), 140.9 (2 x *C*^{4} OFm), 143.4 (2 x *C*^{4} OFm), 145.3 (*Ar*SO$_3^-$), 168.0 (*C*OO); Found (FAB) MH$^+$ 254.11848, $C_{16}H_{16}NO_2$ requires 254.11810.

6.7.11 9-Fluorenylmethyl N^{α}-(tosylate)-leucinate^{AW} 65d

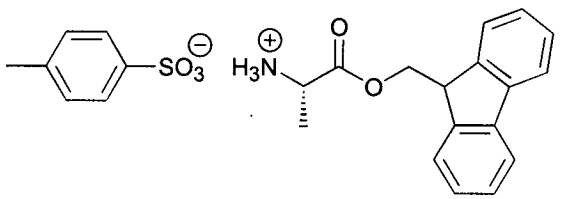


The general procedure outlined above (**6.7.7**) was followed using crude Boc-Leu-OFm (**64d**) (~8.7 mmol), TFA (20 ml) and TSA (2.15 g, 11.3 mmol), to afford the title compound as an off-white solid (3.25 g, 78 % over two steps).

Mp 195-196°C (decomposes), Lit.[121] 206-208°C; (Found: C, 67.25; H, 6.39; N, 2.79; $C_{27}H_{31}NO_5S$ requires C, 67.34; H, 6.49; N, 2.91); ν_{max} (KBr)/cm^{-1} 1750 (ester C=O), 1224, 1189 & 1038 (SO_3^-), 814 (*p*-disubstituted Ar-H), 758 & 733 (*o*-disubstituted Ar-H); δ_H (250 MHz, DMSO d^6) 0.53 (3H, d, *J* 5.0, CH(C^A*H*$_3$C^{B}H$_3$)), 0.61 (3H, d, *J* 5.0, CH(C^{A}H$_3$C^B*H*$_3$)), 1.08-1.31 (3H, m, C*H*$_2$C*H*(CH$_3$)$_2$), 2.27 (3H, s, C*H*$_3$Ar), 3.59-3.84 (1H, m, obscured by H_2O in DMSO, C*H*CH$_2$CH(CH$_3$)$_2$), 4.31 (1H, bs, OCH$_2$C*H*), 4.62 (1H, dd, *J* 11.0, 4.5 OC*H*$_A$H$_B$CH), 4.95 (1H, dd, *J* 11.0, 4.5, OCH$_A$*H*$_B$CH), 7.12 (2H, br d, *J* 8.0, Ar*H* TSA), 7.29-7.44 (4H, m, Ar*H* OFm), 7.50

(2H, br d, *J* 8.0, *Ar*H TSA), 7.65 (1H, br d, *J* 7.0, Ar*H* OFm), 7.71 (1H, br d, *J* 7.0, Ar*H* OFm), 7.88 (2H, br d, *J* 7.5, Ar*H* OFm); 8.25 (3H, bs, NH_3^+); δ_C (63 MHz, DMSO d^6) 20.9 (CH_3Ar), 21.6 ($CH(C^AH_3C^BH_3)$), 22.3 ($CH(C^AH_3C^BH_3)$), 23.4 ($CH(CH_3)_2$), 39.1 ($CH_2CH(CH_3)_2$), 46.5 (OCH_2CH), 50.5 ($CHCH_2CH(CH_3)_2$), 66.3 (OCH_2CH), 120.3 (2 x *Ar*H OFm), 124.9 (2 x *Ar*H OFm), 125.6 (2 x *Ar*H TSA), 127.2 (*Ar*H OFm), 127.3 (*Ar*H OFm), 127.9 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H TSA), 138.1 ($ArCH_3$) 141.1 (C^4 OFm), 141.2 (C^4 OFm), 143.4 (C^4 OFm), 143.6 (C^4 OFm), 145.4 ($ArSO_3^-$), 170.2 (*C*OO); Found (FAB) MH^+ 482.20052, $C_{27}H_{32}NO_5S$ requires 482.20012.

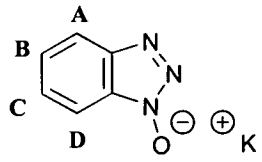
6.7.12 9-Fluorenylmethyl N^{α}-(tosylate)-alaninate[AW] 65e



The general procedure outlined above (**6.7.7**) was followed using crude Boc-Ala-OFm (**64e**) (~10.4 mmol), TFA (20 ml) and TSA (3.67 g, 19.32 mmol), to afford the title compound as a buff solid (4.56 g, 99 % over two steps).

Mp 186-191°C (decomposes), Lit.[121] 198-199°C; ν_{max} (KBr)/cm^{-1} 1751 (ester C=O), 1214, 1163 & 1033 (SO_3^-), 817 (*p*-disubstituted Ar-H), 767 & 742 (*o*-disubstituted Ar-H); δ_H (250 MHz, DMSO d^6) 1.14 (3H, d, *J* 7.0, $CHCH_3$), 2.27 (3H, s, CH_3Ar), 4.05[c] (1H, m, $CHCH_3$), 4.31 (1H, t, *J* 6.0, OCH_2CH), 4.50 (1H, dd, *J* 10.5, 6.0, OCH_AH_BCH), 4.73 (1H, dd, *J* 10.5, 6.0, OCH_AH_BCH), 7.12 (2H, dd, *J* 8.5, 0.5, Ar*H* TSA), 7.30-7.45 (4H, m, Ar*H* OFm), 7.52 (2H, d[i], *J* ~8, *Ar*H TSA), 7.65-7.72 (2H, m, Ar*H* OFm), 7.90 (2H, br d, *J* 7.5, Ar*H* OFm), 8.33 (3H, bs, NH_3^+); δ_C (63 MHz, DMSO d^6) 15.6 ($CHCH_3$), 20.9 (CH_3Ar), 46.4 (OCH_2CH), 48.0 ($CHCH_3$), 66.7 (OCH_2CH), 120.3 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 125.7 (2 x *Ar*H TSA), 127.4 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H TSA), 138.2 ($ArCH_3$), 141.0 (2 x C^4 OFm), 143.3 (C^4 OFm), 143.6 (C^4 OFm), 145.3 ($ArSO_3^-$), 170.2 (*C*OO); Found (FAB) MH^+ 268.13316, $C_{17}H_{18}NO_2$ requires 268.13375.

6.7.13 Potassium oxy-(benzotriazol-1-yl) (KOBt) 67



Typical procedure - HOBt hydrate (≤15% H_2O) (12.69 g, 0.08 mole) was dissolved in absolute EtOH (750 ml). Potassium hydroxide pellets (4.48 g, 0.08 mmol) were added and stirring continued until complete dissolution was observed (~20 min). The solvent was removed *in vacuo* to afford the title compound as a white solid (13.54 g, 98 % yield).

(Found: C, 42.02; H, 2.39; N, 24.56; $C_6H_4N_2OK$ requires C, 41.60; H, 2.33; N, 24.26); ν_{max} (KBr)/cm^{-1} 3056 (C-H Ar), 1611 (C=C Ar), 1449 (N-N), 1403 & 1383 (N=N), 784, 762 & 725 (*o*-disubstituted Ar-H); δ_H (250 MHz, DMSO d^6) 7.01[c] (2H, m, 2 x Ar*H* **B** & **C**), 7.55[c] (2H, m, 2 x Ar*H* **A** & **D**); δ_C (63 MHz, DMSO d^6) 112.2, 117.9, 121.0 & 122.2 (4 x *Ar*H), 127.3 & 143.0 (2 x *Ar*H); Found (FAB) MH^+ 174.00670, $C_6H_5N_3OK$ requires 174.00679.

6.7.14 General procedure for exchange of amino acid TSA salts to HOBt salts

KOBt (**67**) was dissolved in absolute methanol and added slowly to an equimolar solution of the ammonium salt of the-amino acid fluorenylmethyl ester tosylate, dissolved in absolute methanol. Precipitation of the potassium tosylate salt was facilitated by addition of diethyl ether. The salt was filtered and washed with ether, and the filtrate and washings combined and concentrated to dryness. When possible, trituration with copious ether furnished the desired HOBt salt which was filtered and dried, otherwise the crude product was used without any further purification.

6.7.15 9-Fluorenylmethyl N^{α}-oxy-(benzotriazol-1'-yl)-phenylalaninate 66a

The general procedure outlined above (**6.7.14**) was followed using TSA-Phe-OFm (**65a**) (2.06 g, 4.0 mmol) and KOBt (0.69 g, 4.0 mmol) in methanol (80 ml). Trituration with copious ether furnished the desired salt as a chalky white solid (1.64 g, 86 %).

6.7.16 9-Fluorenylmethyl N^{α}-oxy-(benzotriazol-1'-yl)-valinate 66b

The general procedure outlined above (**6.7.14**) was followed using TSA-Val-OFm (**65b**) (0.5 g, 1.07 mmol) and KOBt (0.18 g, 1.07 mmol) in methanol (20 ml). Trituration with copious ether furnished the desired salt as a chalky white solid (0.26 g, 56 %).

6.7.17 9-Fluorenylmethyl N^{α}-oxy-(benzotriazol-1'-yl)-glycinate 66c

The general procedure outlined above (**6.7.14**) was followed using TSA-Gly-OFm (**65c**) (1.70 g, 4.0 mmol) and KOBt (0.69 g, 4.0 mmol) in methanol (100 ml). Attempted trituration with copious ether lead to a sticky gum. On standing in ether the gum became more brittle and some solid salt was isolated, but in general the salt was freshly prepared and used directly, without trituration, as a solution in DMF.

6.7.18 9-Fluorenylmethyl N^{α}-oxy-(benzotriazol-1'-yl)-leucinate 66d

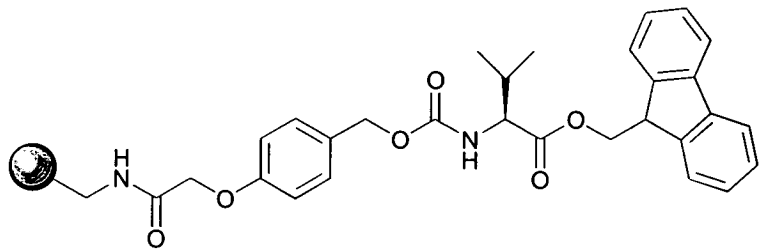
The general procedure outlined above (**6.7.14**) was followed using a suspension of TSA-Leu-OFm (**65d**) (450 mg, 1.0 mmol) in methanol (65 ml) and KOBt (173 mg, 1.0 mmol). After filtration of the potassium salt, concentration, followed by attempted trituration and standing in ether furnished an insoluble white solid (66 mg, 17 %). It was concluded that this was not an efficient method for the preparation of this particular salt and an *in situ* exchange was attempted instead (**6.8.13**).

6.8 Solid Phase Inverse Peptide Synthesis using Fm Ester Methodology

6.8.1 General procedure for coupling of first amino acid

Resin-bound activated carbonate (1 eq) was swollen in DMF before addition of a solution of the ammonium salt of the amino acid fluorenylmethyl ester tosylate (3 eq) in DMF. A catalytic portion of DMAP (~1-10 %) was added to the resin slurry, along with NMM (3 eq). The reaction was spun at room temperature for >15 h before filtering, washing, according to standard protocol, and drying under vacuum.

6.8.2 4-(N^{α}-Methoxycarbonylvalyloxymethylfluorenyl-9'-yl)-phenoxyacetyl aminomethyl polystyrene 68a



The general procedure outlined above (**6.8.1**) was followed using resin-bound carbonate (**53**) (assume ≤0.9 mmol/g- 0.60 g, 0.54 mmol) in DMF (4 ml), with TSA-Val-OFm (**65b**) (0.76 g, 1.62 mmol) in DMF (6 ml), catalytic DMAP and NMM (178 μl, 1.62 mmol). Analysis indicated successful coupling to furnish the title compound.

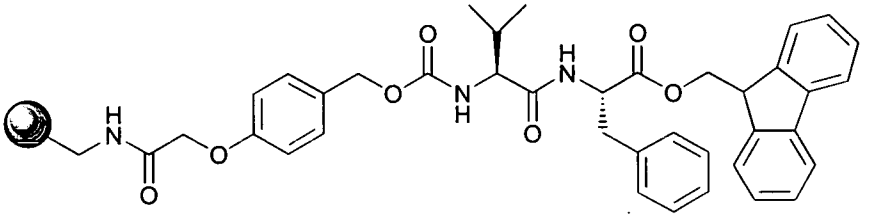
0.58 mmol/g (Fm), 95 %; ν_{max} (DCM)/cm^{-1} 3429 (NH), 1721 (ester & urethane C=O), 1677 (amide I C=O), 1602br (aromatic C=C), 1510 (amide II & urethane NH), 1493 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.4 (CH($C^AH_3C^BH_3$)), 19.0 (CH($C^AH_3C^BH_3$)), 31.1 (*C*H(CH_3)$_2$), 42.8 (*C*H_2NH), 47.0 (O*C*H_2CH), 59.4 (*C*HCH(CH_3)$_2$), 66.5 (*C*H_2OCONH), 66.8 (O*C*H_2CH), 67.6 (*C*H_2OAr), 114.8 (2 x *m*-*Ar*HCH_2O), 120.1 (2 x *Ar*H OFm), 125.1 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 127.9 (2 x *Ar*H OFm), 130.1 (2 x *o*-*Ar*HCH_2O), 141.4 (2 x C^4 OFm), 143.7 (2 x C^4 OFm), 156.3 & 157.3 (*Ar*OCH_2 & O*C*ONH), 172.1 (*C*OOCH_2); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 18.6 min; *m/z* (ES-MS) 296 (MH^+).

6.8.3 General procedure for coupling of second or subsequent amino acid

Resin-bound amino acid fluorenylmethyl ester (1 eq) was deprotected with 20 % piperidine in DMF (3 x 10 ml/300 mg resin; 15 min ea), washed (DMF x 3) and neutralised with 3 % acetic acid in DCM (4 x 12 ml/300 mg resin; 5 min ea). After washing (DMF x 3) the resin was treated with a solution of TBTU (8 eq) and NMM (8 eq) in DMF (15 ml/300 mg resin) for ≥40 min. The activated resin was filtered and washed (DMF x 3), before finally swelling in a solution of the HOBt ammonium salt of the amino acid fluorenylmethyl ester (6 eq) in DMF (15 ml/300 mg resin).

The suspension was spun at ambient temperature (>15 h) before filtering, washing according to the standard protocol, and drying under vacuum.

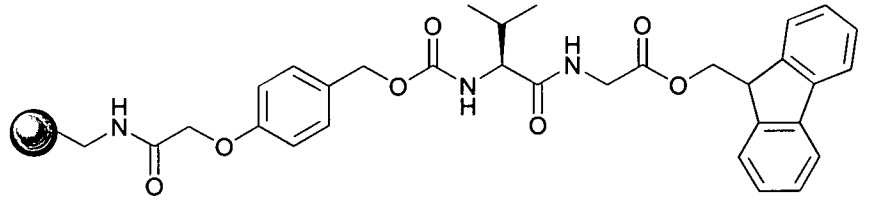
6.8.4 4-(N^{α}-Methoxycarbonylvalylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxyacetyl aminomethyl polystyrene 69a



The general procedure outlined above (**6.8.3**) was followed using PS-HMPA-Val-OFm (**68a**) (assume ≤0.9 mmol/g, 450 mg, 0.41 mmol), TBTU (1.04 g, 3.24 mmol), NMM (0.36 ml, 3.24 mmol) and HOBt-Phe-OFm (**66a**). Analysis of the isolated resin confirmed the presence of the title compound as a single diastereomer.

0.57 mmol/g (Fm), 100 %*; ν_{max} (DCM)/cm^{-1} 3426 & 3325br (2° amide & urethane NH), 1722br (ester & urethane C=O), 1674 (amide I C=O), 1602 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.7 (CH($C^AH_3C^BH_3$)), 19.2 (CH($C^AH_3C^BH_3$)), 31.2 (*C*H(CH_3)$_2$), 38.0 (CH*C*H$_2$Ph), 46.9 (O*C*H$_2$CH), 53.6 (*C*HCH$_2$Ph), 60.5 (*C*HCH(CH_3)$_2$), 66.6 (*C*H$_2$OCONH), 67.3br (O*C*H$_2$CH & *C*H$_2$OAr), 114.8 (2 x *m-Ar*HCH$_2$O), 120.2 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.4 (2 x *Ar*H OFm), 128.1 (2 x *Ar*H OFm), 128.7 (2 x *Ar*H Phe), 129.5 (2 x *Ar*H Phe), 130.0 (2 x *o-Ar*HCH$_2$O), 136.2 (*ipso-C* Phe), 141.5 (2 x C^4 OFm), 143.8 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 20.4 min (single diastereomer); *m/z* (ES-MS) 443 (MH$^+$), 465 (MNa$^+$).

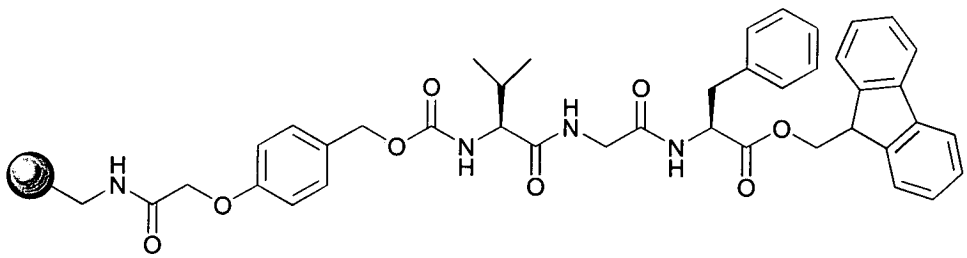
6.8.5 4-(N^{α}-methoxycarbonylvalylglycyloxymethylfluorenyl-9'-yl)-phenoxyacetyl aminomethyl polystyrene 64b



The general procedure outlined above (**6.8.3**) was followed using resin-bound HMPA-Val-OFm (**68a**) (≤0.3 mmol/g - 300 mg, 0.09 mmol), TBTU (231 mg, 0.72 mmol), NMM (79 μl, 0.72 mmol) and a partial suspension† of HOBt-Gly-OFm (**66c**) (210 mg, 0.54 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the glycine salt to furnish the title compound.

ν_{max} (DCM)/cm^{-1} 3427 & 3332br (2° amide & urethane NH), 1748 (ester C=O) 1716 (urethane C=O), 1674br (2 x amide I C=O), 1602 (aromatic C=C), 1512br (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$, gel) 18.3 (CH($C^AH_3C^BH_3$)), 19.9 (CH($C^AH_3C^BH_3$)), 31.9 (*C*H(CH_3)$_2$), 41.9 (NH*C*H$_2$CO), 47.3 (O*C*H$_2$CH), 60.7 (*C*HCH(CH_3)$_2$), 66.8 (*C*H$_2$OCONH), 67.7 (O*C*H$_2$CH & *C*H$_2$OAr), 114.7 (2 x *m-Ar*HCH$_2$O), 120.2 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.4 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 130.0 (2 x *o-Ar*HCH$_2$O), 141.4 (2 x *C*^{4} OFm), 143.8 (2 x *C*^{4} OFm), 156.6 & 156.7 (*Ar*OCH$_2$ & OCONH), 167.9 & 169.9 (2 x *C*ONH), 172.0 (CH$_2$*C*OO); Cleavage 9:10:1 TFA/DCM/H$_2$O: LC-MS^A R_t 19.6 min; *m/z* (ES-MS) 353 (MH$^+$).

6.8.6 4-(N^{α}-Methoxycarbonylvalylglycylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxyacetyl aminomethyl polystyrene 70



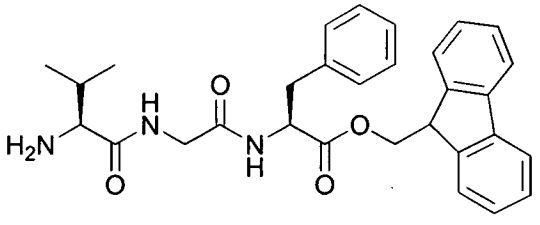
The general procedure outlined above (**6.8.3**) was followed using PS-HMPA-Val-Gly-OFm (**69b**) (~0.6 mmol/g - 215 mg, 0.13 mmol), TBTU (331 mg, 1.03 mmol), NMM (1.13 ml, 1.03 mmol) and a solution of HOBt-Phe-OFm (**66a**) (368 mg, 0.77 mmol) in DMF (10 ml). Analysis confirmed successful coupling to furnish the desired resin-bound tripeptide (**70**) as a single diastereomer.

ν_{max} (DCM)/cm^{-1} 3426 & 3325br (3 x 2° amide & urethane NH), 1715br (ester & urethane C=O), 1682br (3 x amide I C=O), 1602br (aromatic C=C), 1504br (3 x

† poor solubility leading to incomplete dissolution

amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.8 ($CH(C^AH_3C^BH_3)$), 19.3 ($CH(C^AH_3C^BH_3)$), 31.3 ($CH(CH_3)_2$), 37.8 ($CHCH_2Ph$), 40.6 ($NHCH_2CO$ partially obscured by broad polymer backbone), 46.8 (OCH_2CH), 53.8 ($CHCH_2Ph$), 60.4 ($CHCH(CH_3)_2$), 66.5 (OCH_2CH & CH_2OCONH), 67.2 (CH_2OAr), 114.8 (2 x *m-Ar*HCH_2O), 120.1 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.4 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.6 (2 x *Ar*H Phe), 129.4 (2 x *Ar*H Phe & 2 x *o-Ar*HCH_2O), 136.3 (*ipso-C* Phe), 141.4 (2 x C^4 OFm), 143.8(2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 24.0 min (single diastereomer); *m/z* (ES-MS) 500 (MH^+), 522 (MNa^+).

6.8.7 9-Fluorenylmethyl valylglycylphenylalaninate 71



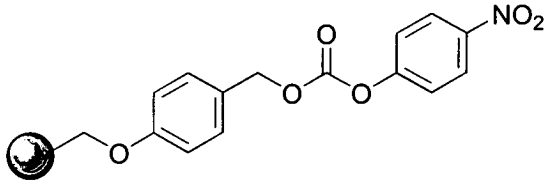
PS-HMPA-Val-Gly-Phe-OFm (**70**) (50 mg) was treated with a solution of TFA/DCM/H_20 (9:10:1) (5 ml) for 2 h. The resin was washed (DCM x 3) and the filtrate and washings combined and concentrated to give crude **71** (11 mg). The sample was purified by semi-preparatory HPLC, but during the purification procedure became contaminated with copper sulfate leading to paramagnetic distortion in the ^{1}H NMR spectrum. All remaining **70** (~70 mg) was cleaved under identical conditions and the crude product obtained (~17 mg) was purified in the same manner and the isolated tripeptide (7 mg) successfully analysed by ^{1}H NMR.

δ_H (600 MHz, CD_3OD) 1.21 (3H, d, *J* 6.84, $CH(C^AH_3C^BH_3)$), 1.22 (3H, d, *J* 6.84, $CH(C^AH_3C^BH_3)$), 2.33-2.36 (1H, m, $CH(CH_3)_2$), 3.00 (1H, dd, *J* 13.89, 8.66, CH_AH_BPh), 3.12 (1H, dd, *J* 13.89, 5.77, $CHCH_AH_BPh$), 3.81 (1H, d, *J* 5.98 $CHCH(CH_3)_2$), 4.03 (1H, d, *J* 16.78, $NHCH_AH_BCO$), 4.15 (1H, d, *J* 16.78, $NHCH_AH_BCO$), 4.37 (1H, br t, *J* 6.20, OCH_2CH), 4.62 (1H, dd, *J* 10.79, 6.30, OCH_AH_BCH), 4.72 (1H, dd, *J* 10.79, 6.30, OCH_AH_BCH), 4.82 (1H, dd, *J* 8.55, 5.77, $CHCH_2Ph$), 7.31 (2H, br d, *J* 7.16, Ar*H* Phe), 7.38[c] (1H, m, Ar*H* Phe), 7.43-7.46 (2H, m, Ar*H* Phe), 7.50-7.54 (2H, m, Ar*H* OFm), 7.57-7.61 (2H, m, Ar*H* OFm), 7.76 (1H,

d, *J* 7.37, Ar*H* OFm), 7.80 (1H, d, *J* 7.48, Ar*H* OFm), 8.00[c] (2H, overlapping doublets, Ar*H* OFm); δ_C (63 MHz, CD_3OD) 15.9 ($CH(C^AH_3C^BH_3)$), 16.9 ($CH(C^AH_3C^BH_3)$), 29.3 ($CH(CH_3)_2$), 36.1 ($CHCH_2Ph$), 41.6 ($NHCH_2O$), ~46 (OCH_2CH masked by strong solvent signal), 53.3 ($CHCH_2Ph$), 57.6 ($CHCH(CH_3)_2$), 66.0 (OCH_2CH), 119.1 (2 x *Ar*H OFm), 124.1 & 124.2 (2 x *Ar*HOFm), 125.3, 126.4, 127.0. 127.7 & 128.3 (4 x *Ar*H OFm & 5 x *Ar*H Phe), 135.5 (*ipso-C* Phe), 141.0 (2 x C^4 OFm), 142.2 (2 x C^4 OFm); *m/z* (ES-MS) 500 (MH^+), 522 (MNa^+).

6.8.8 Continuation of Fm ester mediated solid phase inverse peptide synthesis using 4-(hydroxymethyl)-phenoxymethyl polystyrene (Wang Resin)

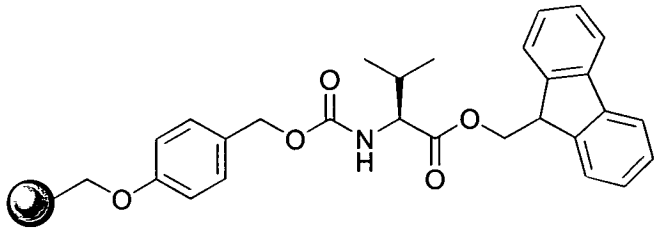
6.8.9 4-Hydroxymethyl-(4'-nitrophenylcarbonate)-phenoxymethyl polystyrene 72



The general procedure previously described (**6.6.1**) was followed using PS-Wang (0.9 mmol/g^{PL}, 6.0 g, 5.4 mmol) in DCM (20 ml), *p*-nitrophenyl chloroformate (3.27g, 16.2 mmol) in DCM (60 ml) and anhydrous pyridine (1.31 ml. 16.2 mmol). A strong carbonate stretch (ν_{max} 1766 cm^{-1}) present in the IR spectrum of the aliquot of resin indicated success and the entire sample was filtered and washed thoroughly. Gel phase ^{13}C NMR indicated complete conversion to the desired resin-bound carbonate (**72**).

0.91 mmol/g (*p*-nitro), 100 %*; ν_{max} (CH_2Cl_2)/cm^{-1} 1766 (carbonate C=O), 1602, 1584 (aromatic C=C), 1528 (asymmetric NO_2), 1493 (aromatic C=C), 1349 (symmetric NO_2), 864 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 70.2 (CH_2OAr), 71.0 (CH_2OCO), 115.0 (2 x *m-Ar*HCH_2O), 122.0 (2 x *m-Ar*HNO_2), 125.4 (2 x *o-Ar*HNO_2), 130.8 (2 x *o-Ar*HCH_2O), 145.5 (*Ar*NO_2-obscured by broad polymer peak), 152.6 (*Ar*OCO), 155.8 (*Ar*OCH_2), 159.7 ($OCOO$).

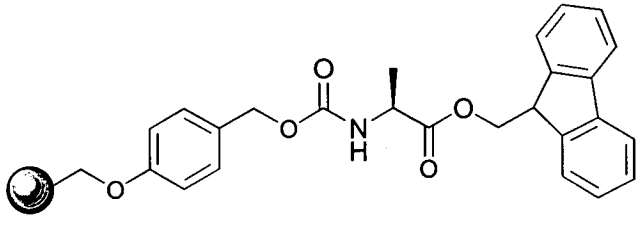
6.8.10 4-(N^{α}-Methoxycarbonylvalyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 73a



The general procedure previously described (**6.8.1**) was followed using resin-bound carbonate (**72**) (assume ≤0.90 mmol/g - 1.26 g, ≤1.13 mmol) in DMF (5 ml), with TSA-Val-OFm (**65b**) (1.59 g, 3.40 mmol) in DMF (10 ml), catalytic DMAP (1.5 mg, 0.01 mmol) and NMM (0.37 ml, 3.40 mmol). Analysis indicated successful coupling to furnish the title compound.

0.63 mmol/g (Fm), 79 %; ν_{max} (CH_2Cl_2)/cm^{-1} 3433 (NH), 1723br (ester & urethane C=O), 1602 & 1585 (aromatic C=C), 1514 (amide II urethane), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.3 (CH($C^AH_3C^BH_3$)), 19.0 (CH($C^AH_3C^BH_3$)), 31.2 ($CH(CH_3)_2$), 47.0 (OCH_2CH), 59.3 ($CHCH(CH_3)_2$), 66.8br (OCH_2CH & CH_2OCONH), 70.1 (CH_2OAr), 114.8 (2 x *m*-$ArHCH_2O$), 120.1 (2 x *Ar*H OFm), 125.1 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 130.0 (2 x *o*-$ArHCH_2O$), 141.5 (2 x C^4 OFm), 143.8 (2 x C^4 OFm), 156.4 (OCONH), 172.1 ($COOCH_2$); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 18.7; *m/z* (ES-MS) 296 (MH^+).

6.8.11 4-(N^{α}-Methoxycarbonylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 73b

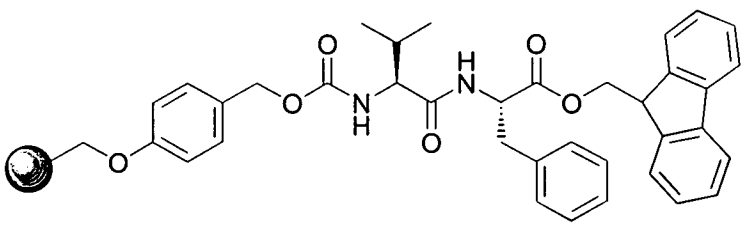


The general procedure previously described (**6.8.1.**) was followed using activated Wang resin (**72**) (assume ≤0.9 mmol/g - 240 mg, ≤0.22 mmol), TSA-Ala-OFm (**65e**)

(0.29 g, 0.66 mmol), catalytic DMAP (1 %) and NMM (73 μl, 0.66 mmol). Analysis indicated successful coupling to furnish the title compound.

0.64 mmol/g (Fm), 82 %; ν_{max} (CH_2Cl_2) 3434 (NH), 1716br (ester & urethane C=O), 1602 & 1584 (aromatic C=C), 1514 (amide II NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 18.3 ($CHCH_3$), 47.0 (OCH_2CH), 49.9 ($CHCH_3$), 67.0 (OCH_2CH & CH_2OCONH), 70.1 (CH_2OAr), 114.7 (2 x *m-Ar*HCH_2O), 120.1 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 130.0 (2 x *o-Ar*HCH_2O Phe), 141.5 (2 x C^4 OFm), 143.6 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 4.4 min; *m/z* (ES-MS) 268 (MH^+).

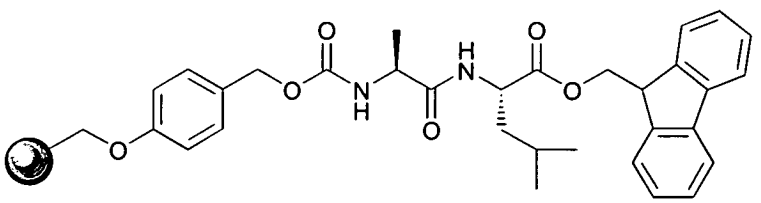
6.8.12 4-(N^{α}-Methoxycarbonylvalylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 74a



The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Val-OFm (**73a**) (assume ≤0.9 mmol/g - 750 mg, 0.68 mmol), TBTU (1.73 g, 5.4 mmol), NMM (0.59 ml, 5.4 mmol) and a solution of HOBt-Phe-OFm (**66a**) (1.94 mg, 4.05 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the phenylalanine ester to furnish the title compound as a single diastereomer.

0.57 mmol/g, 98 % (Fm); ν_{max} (CH_2Cl_2)/cm^{-1} 3422 & 3323br (2° amide & urethane NH), 1723br (ester & urethane C=O), 1681 (amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.8 ($CH(C^AH_3C^BH_3)$), 19.3 ($CH(C^AH_3C^BH_3)$), 31.3 ($CH(CH_3)_2$), 38.1 ($CHCH_2Ph$), 47.0 (OCH_2CH), 53.6 ($CHCH_2Ph$), 60.5 ($CHCH(CH_3)_2$), 67.4br (OCH_2CH & CH_2OCONH), 70.2 (CH_2OAr), 114.9 (2 x *m-Ar*HCH_2O), 120.3 (2 x *Ar*H OFm), 125.3 (2 x *Ar*H OFm), 127.5 (2 x *Ar*H OFm), 128.1 (2 x *Ar*H OFm), 128.8 (2 x *Ar*H Phe), 129.6 (2 x *Ar*H Phe), 130.1 (2 x *o-Ar*HCH_2O), 136.2 (*ipso-C* Phe), 141.6 (2 x C^4 OFm), 143.8 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 21.2 min (single diastereomer); *m/z* (ES-MS) 443 (MH^+), 465 (MNa^+).

6.8.13 4-(N^{α}-Methoxycarbonylalanylleucyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 74b

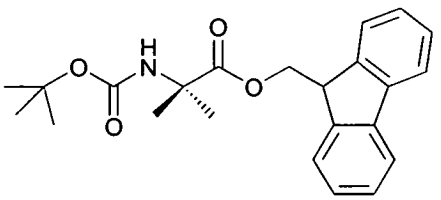


The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Ala-OFm (**73b**) (assume ≤0.9 mmol/g - 200 mg, 0.18 mmol), TBTU (462 mg, 1.44 mmol) and NMM (0.16 ml, 1.44 mmol). Since exchanging to the HOBt salt in the case of TSA-Leu-OFm had not been successful, an *in situ* exchange was attempted. Therefore, the activated resin was added to a solution of TSA-Leu-OFm (**65d**) (486 mg, 1.08 mmol), HOBt hydrate (≤15% H_2O) (286 mg, 1.80 mmol w.r.t. HOBt) and DMAP (22 mg, 0.18 mmol) in DMF (12 ml). Analysis confirmed that coupling of the leucine salt to furnish the title compound as a single diastereomer had taken place but that the resulting product was not pure.

0.18 mmol/g (Fm), 30 %; ν_{max} (CH_2Cl_2) 3423 (NH), 1715br (ester & urethane C=O), 1645 (amide II), 1602 (aromatic C=C), 1513 (amide II urethane NH), 1493 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 18.5 ($CHCH_3$), 21.8 ($CH(C^AH_3C^BH_3)$), 22.7 ($CH(C^AH_3C^BH_3)$), 24.8 ($CH(CH_3)_2$), 40.6 ($CH_2CH(CH_3)_2$ obscured by broad polymer signal), 47.0 (OCH_2CH), 49.7 ($CHCH_3$), 50.9 ($CHCH_2CH(CH_3)_2$) 66.7 (OCH_2CH & CH_2OCONH), 70.1 (CH_2OAr), 114.7 (2 x *m*-*Ar*HCH_2O), 120.1 (2 x *Ar*H OFm), 125.1 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 127.9 (2 x *Ar*H OFm), 129.9 (2 x *o*-*Ar*HCH_2O); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 18.1 min (single diastereomer); *m/z* (ES-MS) 381 (MH^+), 403 (MNa^+).

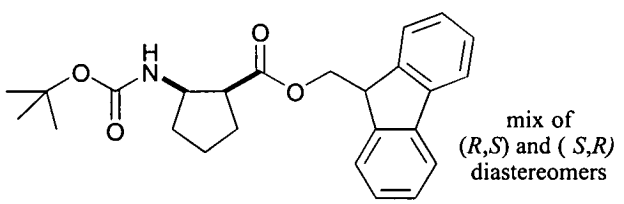
6.9 Preparation of Library Building Blocks

6.9.1 9'-Fluorenylmethyl N^{α}-(*tert*-butoxycarbonyl)-2-methylalaninate 76a



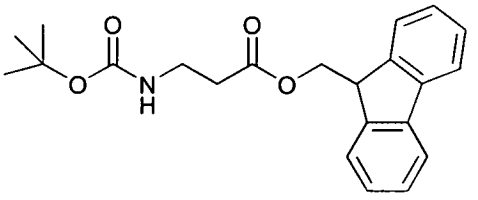
The general procedure outlined above (**6.7.1**) was followed with Boc-2-methylalanine (0.51g, 2.5 mmol), 9-fluorenylmethanol (0.98 g, 5.0 mmol), DMAP (4 mg, 0.03 mmol), DCM (10 ml) and DIC (0.78 ml, 5.0 mmol), to furnish the crude title compound as a yellow/white crumbly solid. The crude product was deprotected and carried through to the TSA salt (**77a**) without any further purification.

6.9.2 9'-Fluorenylmethyl *cis*-2-(*tert*-butoxycarbonylamino)-1-cyclopentanoate 76b



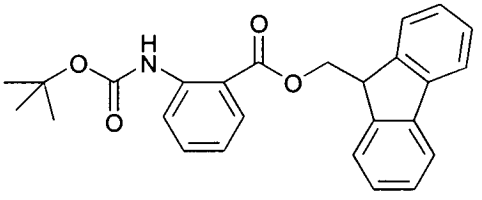
The general procedure outlined above (**6.7.1**) was followed with *cis*-2-(*tert*-butoxycarbonylamino)-1-cyclopentanoic acid (0.23g, 1.0 mmol), 9-fluorenylmethanol (0.39 g, 2.0 mmol), DMAP (2 mg, 0.01 mmol), DCM (5 ml) and DIC (0.31 ml, 2.0 mmol), to furnish the crude title compound as a yellow/white solid. The crude product was deprotected and carried through to the TSA salt (**77b**) without any further purification.

6.9.3 9-Fluorenylmethyl N^{α}-(*tert*-butoxycarbonyl)-β-alaninate 76c



The general procedure outlined above (**6.7.1**) was followed with Boc-β-alanine (5.0 g, 23 mmol), 9-fluorenylmethanol (9.03 g, 46 mmol), DMAP (24 mg, 0.2 mmol), DCM (80 ml) and DIC (7.20 ml, 46 mmol), to furnish the crude title compound as a yellow/white oily solid. The crude product was deprotected and carried through to the TSA salt (**77c**) without any further purification.

6.9.4 9-Fluorenylmethyl *N*-(*tert*-butoxycarbonyl) anthranilate 76d

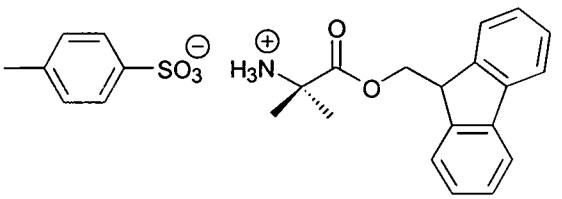


The general procedure outlined above (**6.7.1**) was followed using Boc-anthranilic acid (0.95 g, 4.0 mmol), 9-fluorenylmethanol (1.57 g, 8.0 mmol), DMAP (5 mg, 0.04 mmol), DCM (20 ml) and DIC (1.25 ml, 8.0 mmol). The crude product was purified by column chromatography, eluting with light petroleum/EtOAc (9:1) to furnish the title compound as a white solid (60 mg, 15%).

Rf (Hexane:EtOAc. 9:1) 0.27; ν_{max} (thin film)/cm^{-1} 3318 (NH), 2972 (aromatic C-H), 1732 (ester C=O), 1694 (urethane (C=O), 1603 & 1588 (aromatic C=C), 1524br (amide II & aromatic C=C), 754 & 741 (*o*-disubstituted Ar-H); δ_H (250 MHz, $CDCl_3$) 1.52 (9H, s, C(C*H*$_3$)$_3$), 4.38 (1H, t, *J* 7.0, OCH$_2$C*H*), 4.60 (2H, d, *J* 7.0, OC*H*$_2$CH), 7.05 (1H, ddd, *J* 8.0, 7.5, 1.0, Ar*H* Abz), 7.32 (2H, dt, *J* 7.5, 1.5, Ar*H* OFm), 7.42 (2H, ddt, *J* 7.5, 1.5, 0.5, Ar*H* OFm), 7.54[c] (2H, m, Ar*H* Abz), 7.63[c] (2H, m, Ar*H* OFm), 7.79 (2H, d[i], *J* ~ 7.5, Ar*H* OFm), 8.05 (1H, ddd, *J* 8.0, 1.5, 0.5, *Ar*H Abz), 8.47 (1H, dd, *J* 8.5, 1.0, *Ar*H Abz); δ_C (63 MHz, $CDCl_3$) 28.2 (C(*C*H$_3$)$_3$), 46.7 (OCH$_2$*C*H), 67.1 (O*C*H$_2$CH), 80.5 (O*C*(CH$_3$)$_3$), 114.1 (*Ar*COO), 118.8 (*Ar*H Abz), 120.0 (2 x *Ar*H OFm), 121.2 (*Ar*H Abz), 124.9 (2 x *Ar*H OFm), 127.1 (2 x *Ar*H

OFm), 127.8 (2 x *Ar*H OFm), 130.6 (*Ar*H Abz), 134.6 (*Ar*H Abz), 141.2 (2 x C^4 OFm), 142.4 (*Ar*NH), 143.5 (2 x C^4 OFm), 167.9 (*C*OO); Found (FAB) MH^+ 416.18495, $C_{26}H_{26}NO_4$ requires 416.18618.

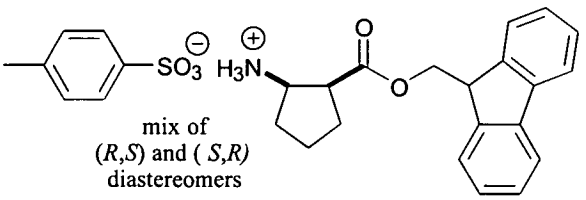
6.9.5 9'-Fluorenylmethyl N^{α}-(tosylate)-2-methylalaninate 77a



The general procedure (**6.7.7**) was followed using crude Boc-Aib-OFm (**76a**) (~2.5 mmol), TFA (10 ml) and TSA (548 mg, 2.88 mmol), to afford the title compound as an off-white solid (664 mg, 56 % over two steps).

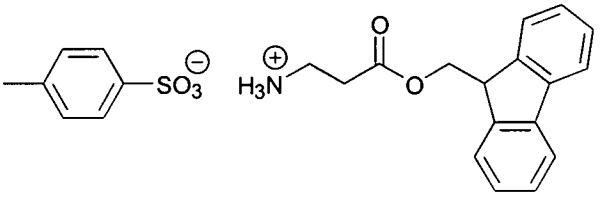
Mp 217-220 °C (decomposes); ν_{max} (KBr)/cm^{-1} 1746 (ester C=O), 1192, 1174 & 1037 (SO_3^-), 815 (*p*-disubstituted Ar-H), 764 & 742 (*o*-disubstituted Ar-H); δ_H (250 MHz, DMSO d^6) 1.24 (6H, s, C(C*H*$_3$)$_2$), 2.27 (3H, s, C*H*$_3$Ar), 4.33 (1H, t, *J* 5.5, OCH$_2$C*H*), 4.63 (2H, d, *J* 5.5, OC*H*$_2$CH), 7.12 (2H, d, *J* 8.0, Ar*H* TSA), 7.26-7.45 (4H, m, Ar*H* OFm), 7.52 (2H, d, *J* 8.0, Ar*H* TSA), 7.68 (2H, d, J 7.5, Ar*H* OFm), 7.89 (2H, d, *J* ~7, Ar*H* OFm), 8.43 (3H, bs, N*H*$_3^+$); δ_C (63 MHz, DMSO d^6) 20.9 (*C*H$_3$Ar), 23.2 (C(*C*H$_3$)$_2$), 46.4 (OCH$_2$*C*H), 56.0 (*C*(CH$_3$)$_2$), 67.1 (O*C*H$_2$CH), 120.3 (2 x *Ar*H OFm), 125.1 (2 x *Ar*H OFm), 125.6 (2 x *Ar*H TSA), 127.4 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H TSA), 138.1 (*Ar*CH$_3$), 141.0 (2 x C^4 OFm), 143.4 (2 x C^4 OFm), 145.3 (*Ar*SO$_3^-$), 171.6 (*C*OO); Found (FAB) MH^+ 282.15011, $C_{18}H_{20}NO_2$ requires 282.14940.

6.9.6 9'-Fluorenylmethyl *cis*-2-(ammonium tosylate)-1-cyclopentanoate 77b



The general procedure (**6.7.7**) was followed using crude Boc-Acp-OFm (**76b**) (~1.0 mmol), TFA (5 ml) and TSA (219 mg, 1.15 mmol). Unfortunately several attempts at trituration with ether were unsuccessful leading only to gums. The crude product was, therefore, taken straight through to the HOBt salt (**78b**) where it was hoped trituration would prove more successful.

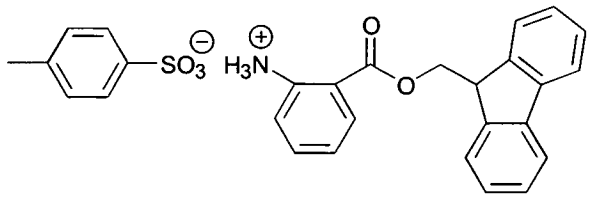
6.9.7 9-Fluorenylmethyl N^{α}-(tosylate)-β-alaninate 77c



The general procedure (**6.7.7**) was followed using crude Boc-β-Ala-OFm (**76c**) (~21 mmol), TFA (50 ml) and TSA (4.59 g, 24.15 mmol). Solubility of the crude ester proved a real problem, so the reaction was carried out as a suspension. Concentration and trituration, did, however, furnish the title compound as a pale yellow solid (8.00 g, 87 % over two steps), of very poor solubility.

Mp 158-160 °C; (Found: C, 65.87; H, 5.77; N, 3.05; $C_{24}H_{25}NO_5S$ requires C, 65.59; H, 5.73; N, 3.19); ν_{max} (KBr)/cm^{-1} 1736 (ester C=O), 1223, 1185 & 1036 (SO_3^-), 814 (*p*-disubstituted Ar-H), 760 & 741 (*o*-disubstituted Ar-H); δ_H (250 MHz, suspension in DMSO d^6) 2.27 (3H, s, CH_3Ar), 2.71 (2H, t, *J* 7.0, $COCH_2CH_2$), 3.01[c] (2H, m, $COCH_2CH_2$), 4.29 (1H, t, *J* 6.5, OCH_2CH), 4.42 (2H, d, *J* 6.5, OCH_2CH), 7.13 (2H, dd, *J* 8.5, 0.5, Ar*H* TSA), 7.34 (2H, dt, *J* 7.5, 1.5, Ar*H* OFm), 7.40-7.46 (2H, m, Ar*H* OFm), 7.53 (2H, d[i], *J* ~8, *Ar*H TSA), 7.66 (2H, d, *J* 7.5, Ar*H* OFm), 7.81 (3H, bs, NH_3^+), 7.90 (2H, br d, *J* 7.0, Ar*H* OFm); δ_C (63 MHz, DMSO d^6) 20.9 (CH_3Ar), 31.5 & 34.9 ($(CH_2)_2$COO), 46.3 (OCH_2CH), 66.1 (OCH_2CH), 120.4 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 125.6 (2 x *Ar*H TSA), 127.4 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.4 (2 x *Ar*H TSA), 138.2 ($ArCH_3$) 140.9 (2 x C^4 OFm), 143.7 (2 x C^4 OFm), 145.3 ($ArSO_3^-$), 170.4 (COO); Found (FAB) MH^+ 440.15431, $C_{24}H_{26}NO_5S$ requires 440.15317.

6.9.8 9'-Fluorenylmethyl 2-(ammonium tosylate) benzoate 77d



The general procedure (**6.7.7**) was followed using purified Boc-Abz-OFm (**76d**) (458 mg, 1.10 mmol), TFA (5 ml) and TSA (241 mg, 1.27 mmol), to afford the title compound as a buff solid (461 mg, 86 %).

Mp 173-175°C (decomposes); ν_{max} (KBr)/cm^{-1} 1702 (ester C=O), 1602 & 1578 (aromatic C=C), 1224, 1177& 1033 (SO_3^-), 814 (*p*-disubstituted Ar-H), 755 & 741 (*o*-disubstituted Ar-H); δ_H (360 MHz, DMSO d^6) 2.29 (3H, s, C*H*$_3$Ar), 4.43 (1H, t, *J* 6.0, OCH$_2$C*H*), 4.62 (2H, d, *J* 6.5, OC*H*$_2$CH), 6.62 (1H, ddd, *J* 8.0, 7.0, 1.0, Ar*H* Abz), 6.84 (1H, dd, *J* 8.5, 1.0, Ar*H* Abz), 7.13 (2H, dd, *J* 8.5, 0.5, Ar*H* TSA), 7.29 (1H, ddd, *J* 8.5, 7.0, 1.5, Ar*H* Abz), 7.34 (2H, dt, *J* 7.5, 1.0, Ar*H* OFm), 7.42 (2H, br t *J* 7.5, Ar*H* OFm), 7.50 (2H, d^i, *J* ~8, Ar*H* TSA), 7.61 (1H, dd, *J* 8.0, 1.5, Ar*H* Abz), 7.69 (2H, d, *J* 7.5, Ar*H* OFm), 7.92 (2H, d, *J* 7.5, Ar*H* OFm); δ_C (63 MHz, DMSO d^6) 21.7 (*C*H$_3$Ar), 47.3 (OCH$_2$*C*H), 66.5 (O*C*H$_2$CH), 110.9 (*Ar*COO), 117.3 (*Ar*H Abz), 118.4 (*Ar*H Abz), 121.1 (2 x *Ar*H OFm), 125.8 (2 x *Ar*H OFm), 126.4 (2 x *Ar*H TSA), 128.1 (2 x *Ar*H OFm), 128.6 (2 x *Ar*H OFm), 129.1 (2 x *Ar*H TSA), 131.3 (*Ar*H Abz), 135.1 (*Ar*H Abz), 138.8 (*Ar*CH$_3$), 141.7 (2 x *C*^{4} OFm), 144.7 (2 x *C*^{4} OFm), 146.1 (*Ar*SO$_3^-$), 167.8 (*C*OO); Found (FAB) MH$^+$ 488.15222, $C_{28}H_{26}NO_5S$ requires 488.15317.

6.9.9 9'-Fluorenylmethyl N^{α}-oxy-(benzotriazol-1''-yl)-2-methylalaninate 78a

The general procedure outlined above (**6.7.14**) was followed using TSA-Aib-OFm (**77a**) (0.5 g, 1.05 mmol) and KOBt (182 mg, 1.05 mmol) in methanol (20 ml). Trituration with copious ether furnished the desired salt as a chalky white solid (0.36 g, 82 %).

6.9.10 9'-Fluorenylmethyl *cis*-2-[ammonium-oxy-(benzotriazol-1''-yl)]-1-cyclopentanoate 78b

The general procedure outlined above (**6.7.14**) was followed using crude TSA-Acp-OFm (**77b**) (assume ≤1.0 mmol) and KOBt (173 mg, 1.0 mmol) in methanol (10 ml). Attempts at trituration with copious ether caused the product to oil out, so the crude product was used directly as a solution in DMF.

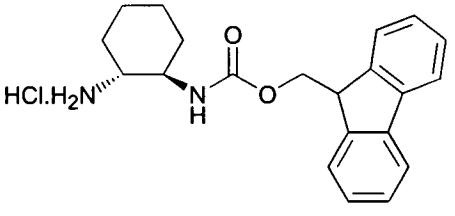
6.9.11 9-Fluorenylmethyl N^{α}-oxy-(benzotriazol-1'-yl)-β-alaninate 78c

The general procedure outlined above (**6.7.14**) was followed using a suspension† of TSA-β-Ala-OFm (**77c**) (2.0 g, 4.55 mmol) in MeOH (150 ml) and KOBt (0.79 g, 4.55 mmol). The final precipitate was filtered off and the filtrate concentrated and triturated with copious ether to afford the desired salt as an off-white solid (1.60 g, 87 %).

6.9.12 9-Fluorenylmethyl N^{α}-oxy-(benzotriazol-1'-yl)-anthranilate 78d

Small scale attempt at salt exchange *via* the general method (**6.7.14**) gave no appreciable amount of product. Instead the salt exchange was attempted *in situ* (**6.9.12**).

6.9.13 (1*R*,2*R*)-1-*N*-(9'-Fluorenylmethoxycarbonyl)-2-(ammonium chloride)-aminocyclohexane 80



A solution of (1*R*,2*R*)-(-)-1,2-diaminocyclohexane (0.50 g, 4.38 mmol) in diethyl ether (100 ml) was added dropwise, with stirring, to a cooled (ice/H_2O bath) solution of 9-fluorenylmethyl chloroformate (1.29 g, 5.04 mmol) in diethyl ether (100 ml) (~1

h).[163] The resulting white precipitate was filtered and dried (1.43 g). Unfortunately, the solid proved to be insoluble in all solvents suitable for NMR analysis. ^{1}H and ^{13}C NMR analysis of a suspension in DMSO d^6 proved too broad to assign accurately but resembled the spectrum predicted for a mixture of the dichloride salt and the desired mono-protected product.

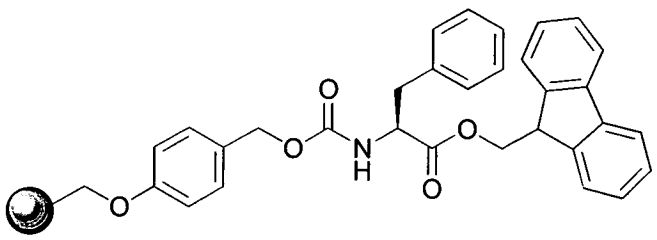
ν_{max} (KBr)/cm^{-1} 3393 (urethane NH), 3306 (ammonium NH), 2939br (saturated C-H), 1679 (urethane C=O), 1602 (aromatic C=C), 1541br (amide II & ammonium NH & aromatic C=C), 1498 (aromatic C=C), 759 & 737 (*o*-disubstituted Ar-H); Found (FAB) MH^+ 337.19176, $C_{21}H_{25}N_2O_2$ requires 337.19160.

6.10 Library Synthesis

6.10.1 4-(N^{α}-Methoxycarbonylvalyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 73a

The general procedure previously described (**6.8.1.**) was followed using activated Wang resin (**72**) (assume ≤0.9 mmol/g - 3.0 g, ≤2.7 mmol) in DMF (30 ml), with TSA-Val-OFm (**65b**) (3.79 g, 8.1 mmol) in DMF (15 ml), catalytic DMAP (33 mg, 0.27 mmol) and NMM (0.89 ml, 8.1 mmol). Analysis (see **6.8.10** for IR and gel phase ^{13}C NMR data) indicated successful coupling to furnish the title compound.
0.45 mmol/g (Fm), 57 %; Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 18.7 min; *m/z* (ES-MS) 296 (MH^+).

6.10.2 4-(N^{α}-methoxycarbonylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 73c

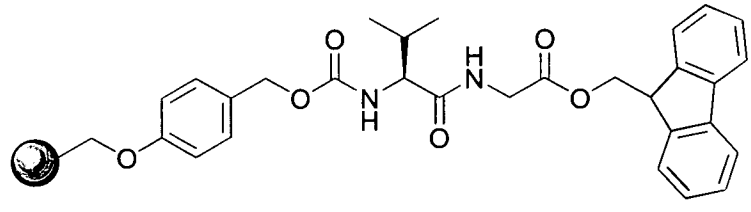


† was unable to find any solvent in which this salt would dissolve.

The general procedure previously described (**6.8.1**) was followed using activated Wang resin (**72**) (assume ≤0.9 mmol/g - 2.0 g, ≤1.8 mmol) in DMF (10 ml), with TSA-Phe-OFm (**65a**) (2.78 g, 5.4 mmol) catalytic DMAP (22 mg, 0.18 mmol) and NMM (0.59 ml, 5.4 mmol) in DMF (10 ml). Analysis indicated successful coupling to furnish the title compound.

0.45 mmol/g (Fm), 59 %; ν_{max} (DCM)/cm^{-1} 3427 (NH), 1722br (ester & urethane C=O), 1602 & 1584 (aromatic C=C), 1513 (amide II urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 38.1 (CH*C*H$_2$Ph), 46.9 (O*C*H$_2$CH), 55.2 (*C*HCH$_2$Ph), 66.8 (*C*H$_2$OCO) 67.2 (O*C*H$_2$CH), 70.1 (*C*H$_2$OAr), 114.7 (2 x *m*-*Ar*HCH$_2$O), 120.1 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.7 (2 x *Ar*H Phe), 129.4 (2 x *Ar*H Phe), 130.0 (2 x *o*-*Ar*HCH$_2$O), 136.2 (*ipso-C* Phe), 141.4 (2 x C^4 OFm), 143.8 (2 x C^4 OFm), 155.9 (O*C*ONH), 171.7 (*C*OOCH$_2$); Cleavage 9:10:1 TFA/DCM/H$_2$O: LC-MS^A R_t 21.4 min; *m/z* (ES-MS) 344 (MH$^+$), $C_{23}H_{22}NO_2$ requires 344.16505.

6.10.3 4-(N^{α}-Methoxycarbonylvalylglycyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 81a



The general procedure outlined above (**6.8.3**) was followed using resin-bound Wang-Val-OFm (**73a**) (~0.45 mmol/g - 300 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 µl, 1.08 mmol) and a solution of crude HOBt-Gly-OFm (**66c**) (~ 0.84 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the protected amino acid to furnish the title compound.

0.54 mmol/g, 100 %* (Fm); ν_{max} (DCM)/cm^{-1} 3425 & 3332br (2° amide & urethane NH), 1748 (ester C=O) 1716 (urethane C=O), 1681 (amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$, gel) 17.7 (CH(*C*^{A}H$_3$C^{B}H$_3$)), 19.1 (CH(C^{A}H$_3$*C*^{B}H$_3$)), 30.9 (*C*H(CH$_3$)$_2$), 41.1 (NH*C*H$_2$CO), 46.4 (O*C*H$_2$CH), 60.1 (*C*HCH(CH$_3$)$_2$), 66.8 (*C*H$_2$OCONH), 67.2

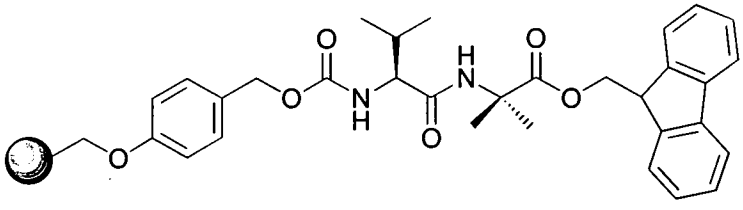
(OCH_2CH), 69.9 (CH_2OAr), 114.6 (2 x *m*-$ArHCH_2O$), 119.9 (2 x ArH OFm), 124.8 (2 x ArH OFm), 127.0 (2 x ArH OFm), 127.8 (2 x ArH OFm), 129.8 (2 x *o*-$ArHCH_2O$), 141.1 (2 x C^4 OFm), 143.2 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 9.1 min; *m/z* (ES-MS) 353 (MH^+), 375 (MNa^+).

6.10.4 4-(N^{α}-Methoxycarbonylvalylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 74a

The general procedure previously described (**6.8.3**) was followed using PS-Wang-Val-OFm (**73a**) (~0.45 mmol/g - 300 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 µl, 1.08 mmol) and a solution of HOBt-Phe-OFm (**66a**) (402 mg, 0.84 mmol) in DMF (15 ml). Analysis (see **6.8.12** for IR and gel phase ^{13}C NMR data) confirmed successful coupling of the protected amino acid to furnish the title compound as a single diastereomer.

0.57 mmol/g, 100 %* (Fm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 21.1 min (single diastereomer); *m/z* (ES-MS) 443 (MH^+), 465 (MNa^+).

6.10.5 4-(N^{α}-Methoxycarbonylvalyl-2'-methylalanyloxymethylfluorenyl-9''-yl)-phenoxymethyl polystyrene 81b

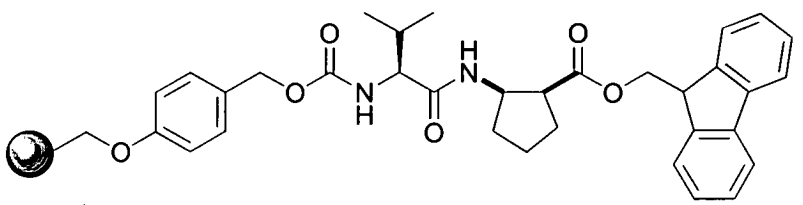


The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Val-OFm (**73a**) (~0.45 mmol/g - 300 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 µl, 1.08 mmol) and a solution of HOBt-Aib-OFm (**78a**) (350 mg, 0.84 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the dialkylated amino acid ester to furnish the title compound.

0.43 mmol/g (Fm), 88 %; ν_{max} (DCM)/cm^{-1} 3427 & 3330br (2° amide & urethane NH), 1716br (ester & urethane C=O), 1674 (amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$,

gel) 17.7 (CH($C^{A}H_3C^{B}H_3$)), 18.9 (CH($C^{A}H_3C^{B}H_3$)), 22.4 (NHC($C^{A}H_3C^{B}H_3$)), 24.4 (NHC($C^{A}H_3C^{B}H_3$)), 31.0 ($CH(CH_3)_2$), 46.8 (OCH_2CH), 56.3 ($NHC(CH_3)_2$), 60.0 ($CHCH(CH_3)_2$), 66.8br (OCH_2CH & CH_2OCONH), 69.8 (CH_2OAr), 114.5 (2 x *m*-$ArHCH_2O$), 119.8 (2 x *Ar*H OFm), 124.8 (2 x *Ar*H OFm), 127.0 (2 x *Ar*H OFm), 127.6 (2 x *Ar*H OFm), 129.7 (2 x *o*-$ArHCH_2O$), 141.1 (2 x C^{4} OFm), 143.4 (2 x C^{4} OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^{A} R_t 16.9 min; *m/z* (ES-MS) 381 (MH^+), 403 (MNa^+).

6.10.6 4-(N^{α}-Methoxycarbonylvalyl-*cis*-(amino-(2'-carboxymethylfluorenyl-9''-yl)-cyclopentyl)-phenoxymethyl polystyrene 81c

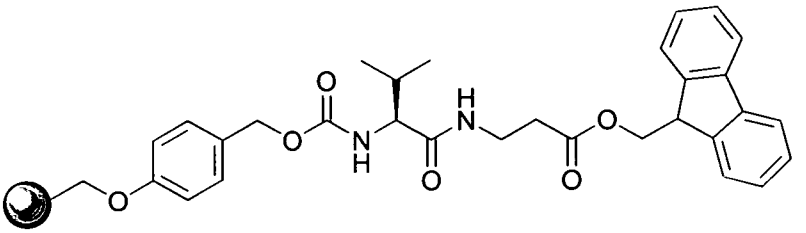


mixture of (R,S) &(S,R) diasteromers

The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Val-OFm (**73a**) (~0.45 mmol/g - 300 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 µl, 1.08 mmol) and a solution of crude HOBt-Acp-OFm (**78b**) (~ 0.84 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the cyclic amino acid ester to furnish the title compound as a mixture of *cis* diastereomers.

0.49 mmol/g (Fm), 100 %*; ν_{max} (DCM)/cm^{-1} 3420 & 3330br (2° amide & urethane NH), 1723br (ester & urethane C=O), 1674 (amide I C=O), 1602 & 1584 (aromatic C=C), 1513br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$, gel) 17.5 & 17.6 (2 x CH($C^{A}H_3C^{B}H_3$)), 19.1 (2 x CH($C^{A}H_3C^{B}H_3$)), 21.9 ($NHCHCH_2CH_2$), 28.3 (CH_2CH_2CHCO), 31.0 ($CH(CH_3)_2$), 31.5 ($NHCHCH_2CH_2$), 46.3 (NHCHCHCO), 46.7 (OCH_2CH), 51.9 (NHCHCHCO), 60.2br ($CHCH(CH_3)_2$), 66.2 (CH_2OCONH), 66.6 (OCH_2CH), 69.9 (CH_2OAr), 114.6 (2 x *m*-$ArHCH_2O$), 119.9 (2 x *Ar*H OFm), 124.8 (2 x *Ar*H OFm), 127.1 (2 x *Ar*H OFm), 127.8 (2 x *Ar*H OFm), 129.8 (2 x *o*-$ArHCH_2O$); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^{A} R_t 18.5 & 19.2 min (two *cis* diastereomers); *m/z* (ES-MS) 407 (MH^+), 429 (MNa^+).

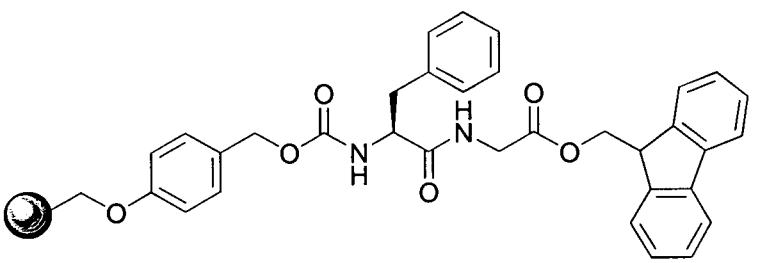
6.10.7 4-(N^{α}-Methoxycarbonylvalyl-β-alanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 81d



The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Val-OFm (**73a**) (~0.45 mmol/g - 300 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 μl, 1.08 mmol) and a partial suspension of HOBt-β-Ala-OFm (**78c**) (338 mg, 0.84 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the β-amino acid ester to furnish the title compound.

0.65 mmol/g (Fm), 100 %*; ν_{max} (DCM)/cm^{-1} 3429 & 3333br (2° amide & urethane NH), 1732br (ester & urethane C=O), 1674 (amide I C=O), 1602 & 1584 (aromatic C=C), 1513br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 18.1 (CH($C^{A}H_3C^{B}H_3$)), 19.5 (CH($C^{A}H_3C^{B}H_3$)), 31.6 ($CH(CH_3)_2$), 34.4 & 35.4 ($NHCH_2CH_2CO$), 47.2 (OCH_2CH), 60.8 ($CHCH(CH_3)_2$), 66.8 (OCH_2CH & CH_2OCONH), 70.5 (CH_2OAr), 115.0 (2 x *m-Ar*HCH_2O), 120.5 (2 x *Ar*H OFm), 125.5 (2 x *Ar*H OFm), 127.7 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H OFm), 130.3 (2 x *o-Ar*HCH_2O), 141.7 (2 x C^4 OFm), 144.2 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 12.0 min; *m/z* (ES-MS) 367 (MH^+), 389 (MNa^+).

6.10.8 4-(N^{α}-Methoxycarbonylphenylalanylglycyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 81e

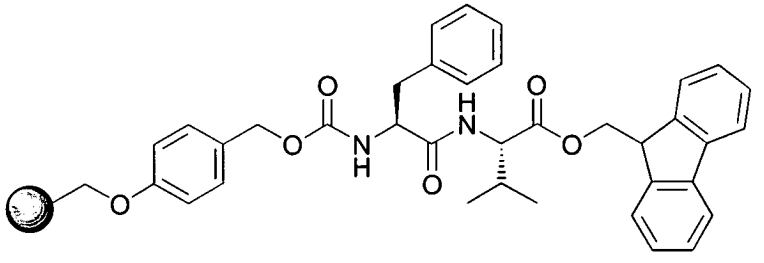


The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Phe-OFm (**73c**) (~0.45 mmol/g - 600 mg, 0.28 mmol), TBTU (694 mg, 2.16 mmol),

NMM (238 μl, 2.16 mmol) and a solution of crude HOBt-Gly-OFm (**78c**) (~ 1.68 mmol) in DMF (30 ml). Analysis confirmed successful coupling of the glycine ester to furnish the title compound.

0.43 mmol/g (Fm), 98 %; ν_{max} (DCM)/cm^{-1} 3421 & 3332br (2° amide & urethane NH), 1716br (ester & urethane C=O), 1682 (amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$, gel) 38.1 (CH*C*H$_2$Ph), 41.2 (NH*C*H$_2$CO), 46.3 (O*C*H$_2$CH), 55.9 (*C*HCH$_2$Ph), 67.1 (O*C*H$_2$CH & *C*H$_2$OCONH), 69.9 (*C*H$_2$OAr), 114.5 (2 x *m-Ar*HCH$_2$O), 119.9 (2 x *Ar*H OFm), 124.8 (2 x *Ar*H OFm), 127.0 (2 x *Ar*H OFm), 127.7 (2 x *Ar*H OFm), 128.4 (2 x *Ar*H Phe), 129.1 (2 x *Ar*H Phe), 129.7 (2 x *o-Ar*HCH$_2$O), 136.1 (*ipso-C* Phe), 141.1 (2 x C^4 OFm), 143.2 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H$_2$O: LC-MS^A R_t 17.8 min; *m/z* (ES-MS) 401 (MH$^+$), 423 (MNa$^+$).

6.10.9 4-(N^{α}-Methoxycarbonylphenylalanylvalyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 81f

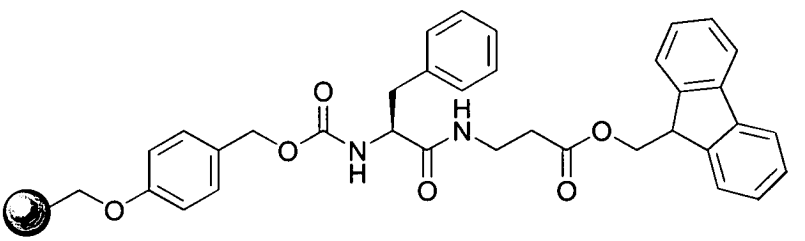


The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Phe-OFm (**73c**) (~0.45 mmol/g - 300 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 μl, 1.08 mmol) and a solution of HOBt-Val-OFm (**66b**) (362 mg, 0.84 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the valine ester to furnish the title compound as a single diastereomer.

0.42 mmol/g (Fm), 98 %; ν_{max} (DCM)/cm^{-1} 3420 & 3322br (2° amide & urethane NH), 1716br (ester & urethane C=O), 1682 (amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$, gel) 17.4 (CH(*C*^{A}H$_3$*C*^{B}H$_3$)), 18.6 (CH(C^{A}H$_3$*C*^{B}H$_3$)), 30.9 (*C*H(CH$_3$)$_2$), 38.1 (CH*C*H$_2$Ph), 46.6 (O*C*H$_2$CH), 56.0 (*C*HCH$_2$Ph), 57.2 (*C*HCH(CH$_3$)$_2$), 66.6 (O*C*H$_2$CH & *C*H$_2$OCONH), 69.8 (*C*H$_2$OAr), 114.5 (2 x *m-Ar*HCH$_2$O), 119.9 (2 x *Ar*H OFm),

124.7 (2 x *Ar*H OFm), 127.0 (2 x *Ar*H OFm), 127.7 (2 x *Ar*H OFm), 128.4 (2 x *Ar*H Phe), 129.1 (2 x *Ar*H Phe), 129.7 (2 x *o*-*Ar*HCH_2O), 136.1 (*ipso*-*C* Phe), 141.1 (2 x C^4 OFm), 143.2 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 20.7 min (single diastereomer); *m/z* (ES-MS) 443 (MH^+), 465 (MNa^+).

6.10.10 4-(N^{α}-methoxycarbonylphenylalanyl-β-alanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 81g



The general procedure outlined above (**6.8.3**) was followed using PS-Wang-Phe-OFm (**73c**) (~0.45 mmol/g - 300 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 µl, 1.08 mmol) and a partial suspension of HOBt-β-Ala-OFm (**78c**) (338 mg, 0.84 mmol) in DMF (15 ml). Analysis confirmed successful coupling of the glycine salt to furnish the title compound.

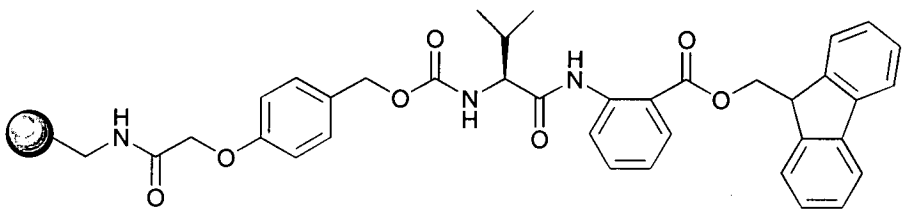
0.37 mmol/g (Fm), 86 %; ν_{max} (DCM)/cm^{-1} 3425 & 3328br (2° amide & urethane NH), 1723br (ester & urethane C=O), 1678 (amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$, gel) 33.5 & 34.5 (NH*C*H_2*C*H_2CO), 38.7 (CH*C*H_2Ph), 46.5 (O*C*H_2CH), 56.1 (*C*HCH_2Ph), 66.2 (O*C*H_2CH), 66.6 (*C*H_2OCONH), 69.8 (*C*H_2OAr), 114.5 (2 x *m*-*Ar*HCH_2O), 119.9 (2 x *Ar*H OFm), 124.7 (2 x *Ar*H OFm), 127.0 (2 x *Ar*H OFm), 127.7 (2 x *Ar*H OFm), 128.3 (2 x *Ar*H Phe), 129.0 (2 x *Ar*H Phe), 129.7 (2 x *o*-*Ar*HCH_2O), 136.2 (*ipso*-*C* Phe), 141.1 (2 x C^4 OFm), 143.4 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 18.4 min; *m/z* (ES-MS) 415 (MH^+), 437 (MNa^+).

6.10.11 General procedure for coupling of second or subsequent amino acid where exchange to HOBt salt has not been possible

Resin-bound amino acid fluorenylmethyl ester (1 eq) was deprotected with 20 % piperidine in DMF (3 x 10 ml/300 mg resin; 15 min ea), washed (DMF x 3) and

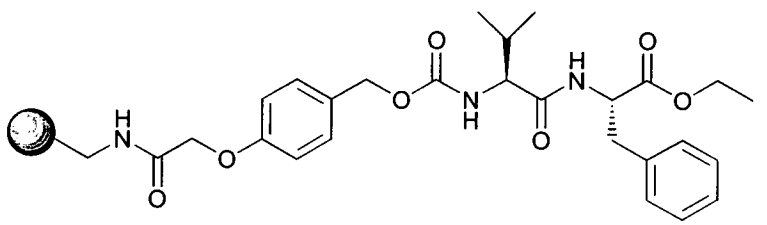
neutralised with 3 % acetic acid in DCM (4 x 12 ml/300 mg resin; 5 min ea). After washing (DMF x 3) the resin was treated with a solution of TBTU (8 eq) and NMM (8 eq) in DMF (15 ml/300 mg resin) for ≥40 min. The activated resin was filtered and washed (DMF x 3), before finally swelling in a solution/suspension of the amino acid fluorenylmethyl ester tosylate or hydrochloride salt (6 eq), HOBt hydrate (6 eq w.r.t. HOBt) and Et_3N (6 eq) in DMF (15 ml/300 mg resin). The suspension was spun at ambient temperature (>15 h) before filtering, washing according to the standard protocol and drying under vacuum.

6.10.12 4-(N^{α}-Methoxycarbonylvalylamino-(2'-carboxymethylfluorenyl-9''-yl)-benzyl)-phenoxymethyl polystyrene 81h - attempted synthesis



The general procedure detailed above (**6.10.11**) was followed using PS-Wang-Val-OFm (**73a**) (assume ≤0.9 mmol/g - 150 mg, 0.14 mmol), TBTU (347 mg, 1.08 mmol), NMM (119 µl, 1.08 mmol) and a solution of TSA-Abz-OFm (**77d**) (395 mg, 0.81 mmol), Et_3N (113 µl, 0.81 mmol) and HOBt hydrate (≤15% H_2O) (129 mg, 0.81 mmol w.r.t. HOBt) in DMF (8 ml). Initial analysis by acidic cleavage and ES-MS did not display the expected mass ion. Also, IR analysis indicated that the resin could still be in an activated form due to the presence of a strong stretch ν_{max} 1817 cm^{-1}, with the coupling of the amino acid not occurring to any detectable extent.

6.10.13 4-(N^{α}-methoxycarbonylvalylphenylalanyloxyethyl)-phenoxymethyl polystyrene 81i



The general procedure outlined above (**6.10.11**) was followed using PS-Wang-Val-OFm (**73a**) (assume ≤0.9 mmol/g - 300 mg, 0.27 mmol), TBTU (694 mg, 2.16

mmol), NMM (237 μl, 2.16 mmol) and a solution of L-phenylalanine ethyl ester hydrochloride (372 mg, 1.62 mmol), Et_3N (226 μl, 1.62 mmol) and HOBt hydrate (≤15% H_2O) (258 mg, 1.62 mmol w.r.t. HOBt) in DMF (15 ml). Analysis confirmed successful coupling of the ethyl ester to furnish the title compound as a single diastereomer.

ν_{max} (DCM)/cm^{-1} 3423 & 3328br (2° amide & urethane NH), 1732br (ester & urethane C=O), 1682 (amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C), 826 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 14.3 (OCH_2CH_3), 17.9 ($CH(C^AH_3C^BH_3)$), 19.3 ($CH(C^AH_3C^BH_3)$), 31.4 ($CH(CH_3)_2$), 38.2 ($CHCH_2Ph$), 53.6 ($CHCH_2Ph$), 60.6 ($CHCH(CH_3)_2$), 61.8 (OCH_2CH_3), 67.0 (CH_2OCONH), 70.3 (CH_2OAr), 114.9 (2 x *m*-$ArHCH_2O$), 127.3 (*Ar*H Phe), 128.8 (2 x *Ar*H Phe), 129.7 (2 x *Ar*H Phe), 130.1 (2 x *o*-$ArHCH_2O$), 136.5 (*ipso-C* Phe), 156.7 (OCONH), 171.2br (CONH & COO); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^B R_t 18.6 min (single diastereomer); *m/z* (ES-MS) 293 (MH^+).

6.10.14 General procedures for coupling of third building block into library

6.10.14.1 Building block = HOBt salt of amino acid

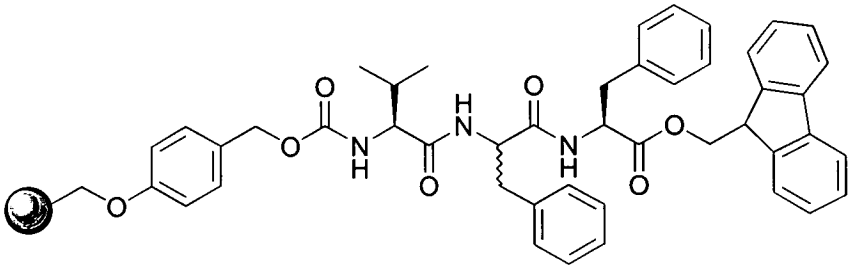
Resin-bound dipeptide fluorenylmethyl ester (assume ≤0.45 mmol/g, 200 mg, 0.09 mmol) was deprotected with 20 % piperidine in DMF (3 x 6 ml; 15 min ea), washed (DMF x 3) and neutralised with 3 % acetic acid in DCM (4 x 8 ml; 5 min ea). After washing (DMF x 3) the resin was treated with a solution of TBTU (231 mg,·0.72 mmol) and NMM (79 μl, 0.72 mmol) in DMF (10 ml) for ≥40 min. The activated resin was filtered and washed (DMF x 3), before finally swelling in a solution of the HOBt ammonium salt of the amino acid fluorenylmethyl ester (0.54 mmol) in DMF (10 ml). The suspension was spun at ambient temperature (>15 h) before filtering, washing according to the standard protocol and drying under vacuum.

6.10.14.2 Building block = amine

Resin-bound dipeptide fluorenylmethyl ester (assume ≤0.45 mmol/g, 200 mg, 0.09 mmol) was deprotected with 20 % piperidine in DMF (3 x 6 ml; 15 min ea), washed

(DMF x 3) and neutralised with 3 % acetic acid in DCM (4 x 8 ml; 5 min ea). After washing (DMF x 3) the resin was treated with a solution of TBTU (231 mg, 0.72 mmol) and NMM (79 µl, 0.72 mmol) in DMF (10 ml) for ≥40 min. The activated resin was filtered and washed (DMF x 3), before finally swelling in a solution of amine (0.54 mmol), HOBt hydrate (0.54 mmol w.r.t. HOBt) in DMF (10 ml). The suspension was spun at ambient temperature (>15 h) before filtering, washing according to the standard protocol, and drying under vacuum.

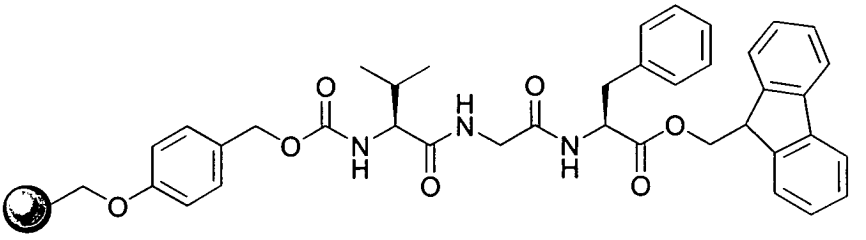
6.10.15 4-(N^{α}-Methoxycarbonylvalyl-D,L-phenylalanylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 75a



The general procedure previously described (***6.10.14.1***) was followed using PS-Wang-Val-Phe-OFm (**74a**) and a solution of HOBt-Phe-OFm (**66a**) (258 mg, 0.54 mmol) in DMF (10 ml). Analysis confirmed successful coupling of the amino acid ester to furnish the title compound as a mixture of diastereomers.

0.35 mmol/g (Fm), 61 %; ν_{max} (DCM)/cm^{-1} 3417 & 3307br (2° amide & urethane NH), 1715br (ester & urethane C=O), 1694 & 1668 (2 x amide I C=O), 1602 & 1585 (aromatic C=C), 1514br & 1509 (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.9 (CH($C^{A}H_3C^{B}H_3$)), 19.2 (CH($C^{A}H_3C^{B}H_3$)), 31.1 (*C*H(CH_3)$_2$), 38.0 (2 x CH*C*H_2Ph), 46.8 (O*C*H_2CH), 54.0br (2 x *C*HCH$_2$Ph), 60.6 (*C*HCH(CH_3)$_2$), 67.2 (O*C*H_2CH & *C*H_2OCONH), 70.1 (*C*H_2OAr), 114.7 (2 x *m*-*Ar*HCH$_2$O), 120.1 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.4 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.6 (4 x *Ar*H Phe), 129.4br (4 x *Ar*H Phe & 2 x *o*-*Ar*HCH$_2$O), 136.2br (2 x *ipso-C* Phe), 141.4 (2 x C^{4} OFm), 143.8 (2 x C^{4} OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^{A} R_t 25.6 & 26.3 min (two diastereomers); *m/z* (ES-MS) 590 (MH^+), 612 (MNa^+).

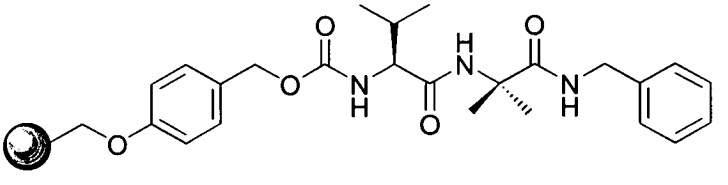
6.10.16 4-(N^{α}-Methoxycarbonylvalylglycylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 75b



The general procedure outlined above (***6.10.14.1***) was followed using PS-Wang-Val-Gly-OFm (**81a**) and a solution of HOBt-Phe-OFm (**66a**) (258 mg, 0.54 mmol) in DMF (10 ml). Analysis confirmed successful coupling of the ester protected amino acid to furnish the title compound as a single diastereomer.

0.34 mmol/g (Fm), 68 %; ν_{max} (DCM)/cm^{-1} 3419 & 3321br (2° amide & urethane NH), 1715br (ester & urethane C=O), 1668 (2 x amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.8 (CH(*C*^{A}H$_3$C^{B}H$_3$)), 19.2 (CH(C^{A}H$_3$*C*^{B}H$_3$)), 31.1 (*C*H(CH$_3$)$_2$), 37.8 (CH*C*H$_2$Ph), 42.9 (NH*C*H$_2$O), 46.7 (O*C*H$_2$CH), 53.6 (*C*HCH$_2$Ph), 60.6 (*C*HCH(CH$_3$)$_2$), 67.1 (O*C*H$_2$CH & *C*H$_2$OCONH), 70.0 (*C*H$_2$OAr), 114.6 (2 x *m*-*Ar*HCH$_2$O), 120.0 (2 x *Ar*H OFm), 125.1 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 127.9 (2 x *Ar*H OFm), 128.6 (2 x *Ar*H Phe), 129.3 (2 x *Ar*H Phe), 129.9 (2 x *o*-*Ar*HCH$_2$O), 136.2 (*ipso-C* Phe), 141.3 (2 x *C*^{4} OFm), 143.6 (2 x *C*^{4} OFm); Cleavage 9:10:1 TFA/DCM/H$_2$O: LC-MS^{A} R_t 20.5 min (single diastereomer); *m/z* (ES-MS) 500 (MH$^+$), 522 (MNa$^+$).

6.10.17 4-(N^{α}-Methoxycarbonylvalyl-(2'-methyl)-alanylaminobenzyl)-phenoxymethyl polystyrene 75c

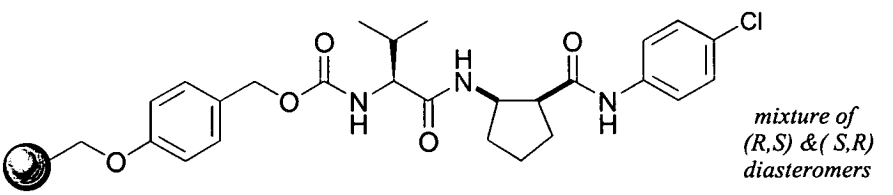


The general procedure outlined above (***6.10.14.2***) was followed using resin-bound dipeptide (**81b**) up until the neutralisation step, where the volatility of the DCM caused the top of the spinning isolute tube to fly off with complete loss of the resin sample. The reaction was therefore repeated using freshly prepared PS-Wang-Val-

Aib-OFm (assume 0.9 mmol/g, 200 mg, 0.18 mmol). The new sample of resin was deprotected with 20 % piperidine in DMF (3 x 6 ml; 15 min ea), washed (DMF x 3) and neutralised with 3 % acetic acid in DCM (4 x 8 ml; 5 min ea). After washing (DMF x 3) the resin was treated with a solution of TBTU (462 mg, 1.44 mmol) and NMM (158 µl, 1.44 mmol) in DMF (10 ml) for ≥40 min. The activated resin was filtered and washed (DMF x 3), before finally swelling in a solution of benzylamine (118 µl, 1.08 mmol) and HOBt hydrate (172 mg, 1.08 mmol w.r.t. HOBt) in DMF (10 ml). The suspension was spun at ambient temperature (>15 h) before filtering, washing according to the standard protocol, and drying under vacuum. Analysis confirmed successful coupling of benzylamine to the bulky dipeptide to afford the title compound.

ν_{max} (DCM)/cm^{-1} 3429 & ~3370br (2 x 2° amide & urethane NH), 1715 (urethane C=O), 1684 & 1681 (2 x amide I C=O), 1602 & 1584 (aromatic C=C), 1514br & 1505 (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 18.4 (CH($C^AH_3C^BH_3$)), 19.2 (CH($C^AH_3C^BH_3$)), 24.6 (NHC($C^AH_3C^BH_3$)), 26.3 (NHC($C^AH_3C^BH_3$)), 30.6 (*C*H(CH_3)$_2$), 43.4 (NH*C*H$_2$Ph), 57.4 (NH*C*(CH_3)$_2$), 61.8 (*C*HCH(CH_3)$_2$), 66.9 (*C*H$_2$OCONH), 70.1 (*C*H$_2$OAr), 114.5 (2 x *m*-*Ar*HCH$_2$O), 128.1br (5 x *Ar*HCH$_2$NH - masked by polymer aromatics), 130.0 (2 x *o*-*Ar*HCH$_2$O), 145.2 (*Ar*CH$_2$NH - masked by resin signal); Cleavage 9:10:1 TFA/DCM/H$_2$O: LC-MS[B] R_t 11.4 min; *m/z* (ES-MS) 292 (MH^+).

6.10.18 4-(*N*$^{\alpha}$-Methoxycarbonylvalyl-*cis*-{amino-[2'-carboxyamino-(4''-chloro)-phenyl]-cyclopentyl}-phenoxymethyl polystyrene 75d



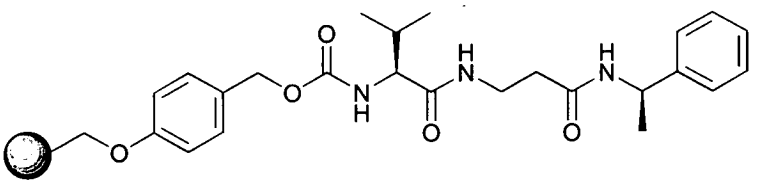
mixture of (R,S) &(S,R) diasteromers

The general procedure outlined above (***6.10.14.2***) was followed using PS-Wang-Val-Acp-OFm (**81c**) and a solution of *p*-chloro-aniline (69 mg, 0.54 mmol) and HOBt hydrate (86 mg, 0.54 mmol w.r.t. HOBt) in DMF (10 ml). Analysis confirmed successful coupling of the aromatic amine to furnish the title compound as a single

diastereomer. Due to the mixture of diastereomers the gel phase ^{13}C NMR was fairly broad and complex, however proposed assignments are detailed below.

ν_{max} (DCM)/cm^{-1} 3420 & 3314br (2° amide & urethane NH), 1715 (urethane C=O), 1668 & 1652 (2 x amide I C=O), 1602 & 1584 (aromatic C=C), 1514br & 1504 (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.5 (2 x CH($C^AH_3C^BH_3$)), 19.2 (2 x CH($C^AH_3C^BH_3$)), 22.4 & 23.1 (2 x NHCHCH$_2$CH$_2$), 28.4br (2 x COCHCH$_2$CH$_2$), 31.3br & 33.2br (2 x CH(CH$_3$)$_2$ & 2 x NHCHCH$_2$CH$_2$), 46.1 & 46.7 (2 x COCHCH$_2$CH$_2$), 51.8 & 53.2 (2 x NHCHCH$_2$CH$_2$), 60.4br (2 x CHCH(CH$_3$)$_2$), 66.8 (CH$_2$OCONH), 70.1 (CH$_2$OAr), 114.7 (2 x *m*-*Ar*HCH$_2$O), 128.1br (*p*-chloroaniline obscured), 129.9 (2 x *o*-*Ar*HCH$_2$O), 171.9br (2 x CONH); Cleavage 9:10:1 TFA/DCM/H$_2$O: LC-MS^B R_t 21.0 min (two *cis* diastereomers); *m/z* (ES-MS) 338 (MH$^+$/Cl^{35}), 340 (MH$^+$/Cl^{37}), 360 (MNa$^+$/Cl^{35}), 362 (MNa$^+$/Cl^{37}).

6.10.19 (1'*R*)-4-(*N*$^{\alpha}$-Methoxycarbonylvalyl-β-alanylamino-(1'-phenyl)-ethyl)-phenoxymethyl polystyrene 75e

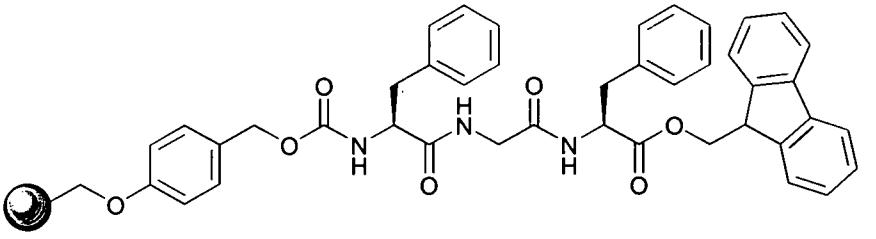


The general procedure outlined above (**6.10.14.2**) was followed using PS-Wang-Val-β-Ala-OFm (**81d**) and a solution of (*R*)-(+)-*α*-methylbenzylamine (70 µl, 0.54 mmol) and HOBt hydrate (86 mg, 0.54 mmol w.r.t. HOBt) in DMF (10 ml). Analysis confirmed successful coupling of the chiral amine to furnish the title compound as a single diastereomer.

ν_{max} (DCM)/cm^{-1} 3428 & 3299br (2° amide & urethane NH), 1714 (urethane C=O), 1668 & 1654 (2 x amide I C=O), 1602 & 1584 (aromatic C=C), 1538 & 1514 (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.9 (CH($C^AH_3C^BH_3$)), 19.3 (CH($C^AH_3C^BH_3$)), 22.1 (CHCH$_3$), 31.4 (CH(CH$_3$)$_2$), 35.8 (one or both of NHCH$_2$CH$_2$CO, one perhaps masked by polymer benzylics, 40.6br), 49.0 (NHCHCH$_3$), 60.6 (CHCH(CH$_3$)$_2$), 66.7 (CH$_2$OCONH), 70.1 (CH$_2$OAr), 114.7 (2 x *m*-*Ar*HCH$_2$O), 128.1 (2 x *Ar*HCH), 128.6 (2 x *Ar*HCH), 129.9 (2 x *o*-*Ar*HCH$_2$O),

170.5 (*C*ONH), 171.9 (*C*ONH); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[B] R_t 15.9 min (single diastereomer); *m/z* (ES-MS) 292 (MH^+), 314 (MNa^+).

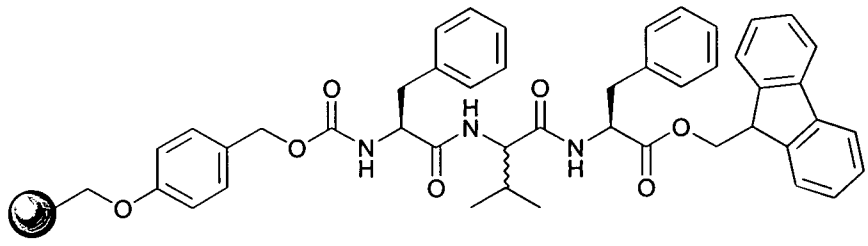
6.10.20 4-(N^{α}-Methoxycarbonylphenylalanylglycylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 75f



The general procedure previously described (***6.10.14.1***) was followed using PS-Wang-Phe-Gly-OFm (**81e**) and a solution of HOBt-Phe-OFm (**66a**) (258 mg, 0.54 mmol) in DMF (10 ml). Analysis confirmed successful coupling of the amino acid ester to furnish the title compound as a single diastereomer.

0.24 mmol/g (Fm), 60 %; ν_{max} (DCM)/cm^{-1} 3418 & 3324s (2 x 2° amide & urethane NH), 1715br (ester & urethane C=O), 1684 & 1681 (2 x amide I C=O), 1602 & 1585 (aromatic C=C), 1514 & 1505 (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, $CDCl_3$, gel) 37.7br (2 x $CHCH_2Ph$), 43.1 ($NHCH_2CO$), 46.8 (OCH_2CH), 53.6 ($OCOCHCH_2Ph$), 56.4 ($NHCOCHCH_2Ph$), 67.2 (OCH_2CH & CH_2OCONH), 70.0 (CH_2OAr), 114.7 (2 x *m-Ar*HCH_2O), 120.1 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.6 (4 x *Ar*H Phe), 129.4br (4 x *Ar*H Phe & 2 x *o-Ar*HCH_2O), 141.4 (2 x C^4 OFm), 143.6 (2 x C^4 OFm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 25.1 min (single diastereomer); *m/z* (ES-MS) 548 (MH^+), 570 (MNa^+), 586 (MK^+).

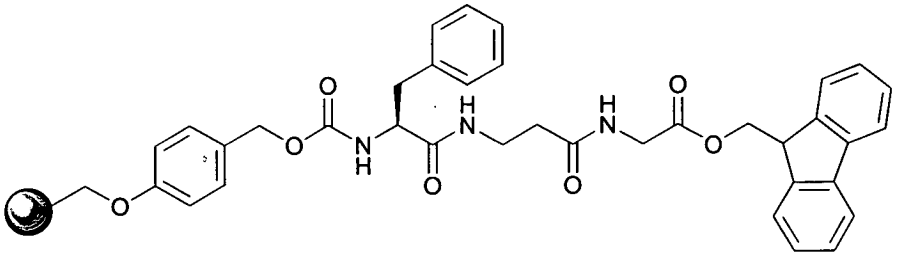
6.10.21 4-(N^{α}-Methoxycarbonylphenylalanyl-D,L-valylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 75g



The general procedure previously described (***6.10.14.1***) was followed using resin-bound dipeptide (**81f**) (assume 0.45 mmol/g, 140 mg, 0.06 mmol) and a solution of HOBt-Phe-OFm (**66a**) (182 mg, 0.38 mmol) in DMF (7 ml). Analysis confirmed successful coupling of the amino acid ester to furnish the title compound as a mixture of diastereomers.

0.41 mmol/g (Fm), 100 %*; ν_{max} (DCM)/cm^{-1} 3418 & 3307br (2° amide & urethane NH), 1738 (ester), 1715 (urethane C=O), 1684 & 1681 (2 x amide I C=O), 1602 & 1585 (aromatic C=C), 1515 & 1505 (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.5 & 18.0 (2 x CH($C^AH_3C^BH_3$)), 19.1 (2 x CH($C^AH_3C^BH_3$)), 31.2 (2 x $CH(CH_3)_2$), 38.0 (2 x $CHCH_2Ph$), 46.8 (OCH_2CH), 53.6 ($OCOCHCH_2Ph$), 56.1 ($NHCOCHCH_2Ph$), 58.3 (2 x $CHCH(CH_3)_2$), 67.2 (OCH_2CH & CH_2OCONH), 70.1 (CH_2OAr), 114.7 (2 x *m-Ar*HCH_2O), 120.1 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 127.3 (2 x *Ar*H OFm), 128.0 (2 x *Ar*H OFm), 128.6 (4 x *Ar*H Phe), 129.4br (4 x *Ar*H Phe & 2 x *o-Ar*HCH_2O), 136.4br (2 x *ipso-C* Phe), 141.4 (2 x C^4 OFm), 143.7 (2 x C^4 OFm), 171.6br (2 x *C*ONH & *C*OO); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 26.4 & 27.1 min (two diastereomers); *m/z* (ES-MS) 590 (MH^+), 612 (MNa^+).

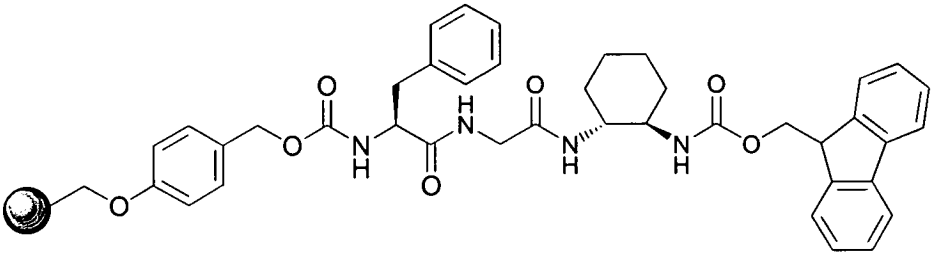
6.10.22 4-(N^{α}-Methoxycarbonylphenylalanyl-β-alanylglycyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 75h



The general procedure previously described (***6.10.14.1***) was followed using PS-Wang-Phe-β-Ala-OFm (**81g**) and a solution of crude HOBt-Gly-OFm (**66c**) (~ 0.54 mmol) in DMF (10 ml). Analysis confirmed successful coupling of the amino acid ester to furnish the title compound.

0.33 mmol/g, 92 % (Fm); ν_{max} (DCM)/cm^{-1} 3420 & 3344br (2 x 2° amide & urethane NH), 1715br (ester & urethane C=O), 1682 & 1652 (2 x amide I C=O), 1602 & 1584 (aromatic C=C), 1514br & 1505 (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 36.0br ($NHCH_2CH_2CO$), 38.5 ($CHCH_2Ph$), 41.4 ($NHCH_2CO$), 46.7 (OCH_2CH), 56.6 ($CHCH_2Ph$), 66.8 (CH_2OCONH), 67.3 (OCH_2CH), 70.1 (CH_2OAr), 114.7 (2 x *m-Ar*HCH_2O), 120.1 (2 x *Ar*H OFm), 125.2 (2 x *Ar*H OFm), 128.0br (4 x *Ar*H OFm & 2 x *Ar*H Phe), 129.6br (2 x *Ar*H Phe & (2 x *o-Ar*HCH_2O), 137.0 (*ipso-C* Phe), 141.3 (2 x C^4 OFm), 143.6 (2 x C^4 OFm), 172.1br (2 x *C*ONH & *C*OO); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 19.4 min; *m/z* (ES-MS) 472 (MH^+), 494 (MNa^+).

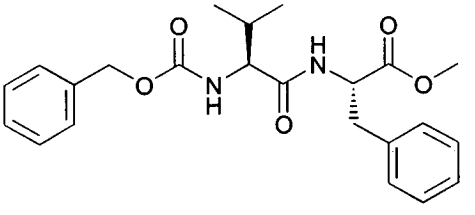
6.10.23 (1'*R*,2'*R*)-4-{N^{α}-Methoxycarbonylphenylalanylglycylamino-[1'-(2'-aminocarboxymethylfluorenyl-9''-yl)-cyclohexyl]}-phenoxymethyl polystyrene 75i



The general procedure detailed above (**6.10.11**) was followed using PS-Wang-Phe-Gly-OFm (**81e**) (assume ≤0.45 mmol/g - 60 mg, 0.03 mmol), TBTU (69 mg, 0.22 mmol), NMM (24 μl, 0.22 mmol) and a milky suspension of crude 1-(9'-fluorenylmethoxycarbonyl)-2-(hydrochloride)-(1*R*,2*R*)-1,2-diaminocyclohexane (**80**) (60 mg, ~0.16 mmol), Et_3N (23 μl, 0.16 mmol) and HOBt hydrate (≤15% H_2O) (26 mg, 0.16 mmol w.r.t. HOBt) in DMF (6 ml). After spinning for 16 h, the still opaque reaction mixture was drained away and the resin washed and dried in the usual manner. IR analysis indicated that there was no activated complex left and after cleavage ES-MS indicated that there was a trace presence of the desired product. The sample was too small and weak for meaningful gel phase ^{13}C NMR analysis.
0.04 mmol/g, 10 % (Fmoc); ν_{max} (DCM)/cm^{-1} 3416 & 3319br (2 x 2° amide & 2 x urethane NH), 1716br (2 x urethane C=O), 1682br (2 x amide I C=O), 1602 & 1584 (aromatic C=C), 1513br (2 x amide II & 2 x urethane NH), 1494 (aromatic C=C), 826 (*p*-disubstituted Ar-H); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 19.2 min (weak); *m/z* (ES-MS) 541 (MH^+), 319 (MH^+- Fmoc).

6.11 Transformations of the Dipeptide-Derived 5(4*H*)-Oxazolone

6.11.1 Methyl *N*-(benzyloxycarbonyl)-valinylphenylalaninate 84

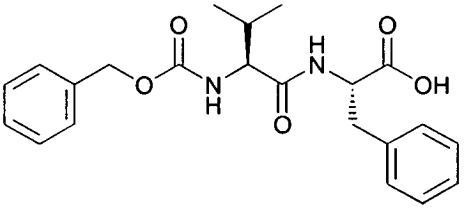


Typical procedure - salt-free phenylalanine methyl ester was prepared by dissolving L-phenylalanine methyl ester hydrochloride (9.06 g, 42 mmol) in sodium hydroxide solution (1.0 M, 60 ml), and extracting with DCM (3 x 60 ml). After drying (Na_2SO_4), the combined organic extracts of phenylalanine methyl ester were filtered directly into a solution of carbobenzyloxy-L-valine (10.05 g, 40 mmol) in DCM (60 ml) and the resulting solution cooled to 0 °C. DCC (8.25 g, 40 mmol) was then added, immediately giving rise to the formation of a white precipitate. After stirring

at 0°C for 1 h the reaction was allowed to warm to room temperature and left stirring for a further 16 h. The white precipitate of DCU was removed by filtration and the solution was washed with H_2O (3 x 80 ml), 2 M hydrochloric acid (80 ml) and saturated $NaHCO_3$ solution (80 ml). After drying (Na_2SO_4), the solution was filtered and the solvent removed under reduced pressure to afford the crude dipeptide **84** as a white gummy solid (16.41 g, 99 %). ^1H NMR indicated **84** was present as a single diastereomer.

ν_{max} (thin film)/cm^{-1} 3298br (NH), 1743 (ester C=O), 1692 (urethane & amide I), 1536 (amide II & aromatic C=C); δ_H (250 MHz, $CDCl_3$) 0.85-0.99 (6H, m, CH(C*H*$_3$)$_2$), 2.03-2.13 (1H, m, C*H*(CH$_3$)$_2$), 3.08-3.12 (2H, m, CHC*H*$_2$Ph), 3.71 (3H, s, OC*H*$_3$), 3.94-4.00 (1H, m, C*H*CH(CH$_3$)$_2$), 4.86 (1H, dt, *J* 8.0, 6.0, C*H*CH$_2$Ph), 5.10 (2H, s br, OC*H*$_2$Ph), 5.30-5.35 (1H, m, OCON*H*), 6.27 (1H, d, *J* 8.0, CON*H*), 7.06-7.09 (2H, m, Ar-*H*), 7.21-7.35 (8H, m, Ar-*H*); δ_C (63 MHz, $CDCl_3$) 17.5 (CH(*C*^{A}H$_3$C^{B}H$_3$), 19.0 (CH(C^{A}H$_3$*C*^{B}H$_3$), 30.9 (*C*H(C^{A}H$_3$C^{B}H$_3$), 37.8 (CH*C*H$_2$Ph), 52.2 (O*C*H$_3$), 53.0 (*C*HCH$_2$Ph), 60.2 (*C*HCH(CH$_3$)$_2$), 127.9, 128.0, 128.1, 128.4, 128.5 & 129.1 (10 x *Ar*-H), 135.4 & 136.1 (2 x *Ar*CH$_2$), 156.2 (O*C*ONH), 170.7 & 171.5 (CH*C*ONH & *C*OO); m/z (EI) 413 (M^+).

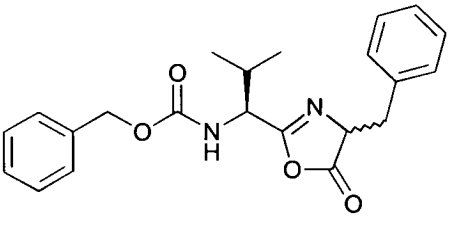
6.11.2 *N*-(Benzyloxycarbonyl)-valinylphenylalanine 85



To a solution of crude **84** (6.45 g, 15.6 mmol) suspended in THF/water (1:1) (90 ml) was added lithium hydroxide monohydrate (1.31 g, 31.2 mmol). The resulting mixture was allowed to stir at room temperature for 2.5 h before acidification with 2 M HCl (15 ml) and extraction with DCM (3 x 100 ml). The combined organic extracts were dried (Na_2SO_4) and concentrated under reduced pressure to afford crude **85** as a yellow solid (5.35 g). Recrystallisation from $CHCl_3$ (*ca* 30 ml) furnished pure **85** as a white powder (3.10 g, 50 %).

Mp 175-176 °C, Lit.[164] 167-168 °C; $[\alpha]_D^{20}$ -11.4 (c 0.5, MeOH), Lit.[164] -13.3 (c 1.0, MeOH); ν_{max} (Nujol) 3337 (OH), 1733 (C=O urethane), 1632 (carboxyl C=O), 1522 (amide II); δ_H (250 MHz, DMSO d^6) 0.80 (3H, d, *J* 6.5, CH($C^AH_3C^BH_3$)), 0.81 (3H, d, *J* 6.5, CH($C^AH_3C^BH_3$)), 1.85-1.99 (1H, m, C*H*(CH_3)$_2$), 2.90 (1H, dd, *J* 14.0, 9.0, CHC$H_A$$H_B$Ph), 3.06 (1H, dd, *J* 14.0, 5.0, CHC$H_A$$H_B$Ph), 3.90 (1H, dd, *J* 9.0, 7.5, C*H*CH(CH_3)$_2$), 4.41-4.50 (1H, m, C*H*C$H_A$$H_B$Ph), 5.03 (2H, s, OC$H_2$Ph), 7.14-7.28 (6H, m, Ar-*H* & CON*H*), 7.30-7.40 (5H, m, Ar-*H*), 8.19 (1H, d, *J* 7.5, OCON*H*), 12.72 (1H, br s, O*H*); δ_C (63 MHz, DMSO d^6) 18.2 (CH($C^AH_3C^BH_3$)), 19.2 (CH($C^AH_3C^BH_3$)), 30.6 (*C*H($C^AH_3C^BH_3$)), 36.9 (CH*C*H_2Ph), 53.5 (*C*HCH_2Ph), 60.2 (*C*HCH($C^AH_3C^BH_3$)), 65.5 (O*C*H_2Ph), 126.6 (*p-Ar*-H), 127.8 (*Ar*-H), 127.9 (*p-Ar*-H), 128.3 (2 x *Ar*-H), 128.5 (2 x *Ar*-H), 129.3 (2 x *Ar*-H), 137.2, 137.6, (2 x *Ar*CH_2), 156.1 (O*C*ONH), 171.3, 173.0 (2 x *C*O); Found (EI) 398.18444 (M^+), $C_{22}H_{26}O_5N_2$ requires 398.18417.

6.11.3 (1*S*,4'*RS*) 1-(Benzyloxycarbonylamino)-1-(4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-2-methyl propane 86



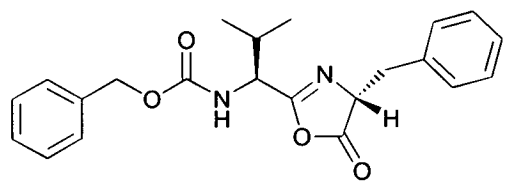
To a cooled (0 °C) suspension of **85** (2.32 g, 5.8 mmol) in DCM (70 ml), was added EDCI (1.23 g, 6.4 mmol). After stirring for 45 min a colourless solution had formed and after a total of 17 h stirring†, the reaction mixture was washed with H_2O, HCl (2 M), water, saturated sodium bicarbonate solution, and finally H_2O (70 ml ea). The organic phase was then dried (Na_2SO_4) and concentrated to afford **86** as a white solid (2.11 g, 96 %) and mixture of (*S,R*) and (*S,S*) diastereomers. ^{1}H NMR indicated an 18% diastereomeric excess w.r.t. (*S,S*).

δ_H (200 MHz, $CDCl_3$) 0.50 (3H, d, *J* 7.0, CH($C^AH_3C^BH_3$), **m**), 0.75 (3H, d, *J* 7.0, CH($C^AH_3C^BH_3$), **M**), 0.77 (6H, d, *J* 7.0, CH($C^AH_3C^BH_3$), **M** & **m**), 1.87-1.98 (2H, m,

† tlc analysis indicated full conversion after *ca* 3 h, but time restraints prevented immediate work-up.

C*H*$(CH_3)_2$, **M & m**), 3.05-4.34 (4H, m, CHC*H*$_2$Ph, **M & m**), 4.34-4.51 (4H, m, C*H*CH_2Ph, C*H*$CH(CH_3)_2$, **M & m**), 5.03-5.21 (6H, m, OC*H*$_2$Ph, N*H*, **M & m**), 7.12-7.28 (10H, m, Ar-*H*, **M & m**), 7.31-7.45 (10H, m, Ar-*H*, **M & m**); δ_C (63 MHz, $CDCl_3$) 16.6 (CH(*C*^{A}H$_3$C^{B}H$_3$), **m**), 17.2 (CH(*C*^{A}H$_3$C^{B}H$_3$), **M**), 18.4 (CH(C^{A}H$_3$*C*^{B}H$_3$), **m**), 18.5 (CH(C^{A}H$_3$*C*^{B}H$_3$), **M**), 30.4 (*C*H$(CH_3)_2$, **m**), 30.5 (*C*H$(CH_3)_2$, **M**), 36.2 (CH*C*H_2Ph, **m**), 36.5 (CH*C*H_2Ph, **M**), 54.7 (*C*HCH_2Ph, **m**), 55.0 (*C*HCH_2Ph, **M**), 65.3 (*C*H$CH(CH_3)_2$, **m**), 65.4 (*C*H$CH(CH_3)_2$, **M**), 67.0 (CH_2OCONH, **m**), 67.1 (CH_2OCONH, **M**), 127.2 (2 x *p-Ar*-H, **M & m**), 127.9 (2 x *Ar*-H, **M**), 128.0 (2 x *Ar*-H, **m**), 128.1 (2 x *p-Ar*-H, **M & m**), 128.3 (2 x *Ar*-H, **M**), 128.4 (6 x *Ar*-H, **M & m**), 129.5 (4 x *Ar*-H, **M & m**), 134.5 (*Ar*CH_2CH, **m**), 134.6 (*Ar*CH_2CH, **M**), 135.9 (*Ar*CH_2O, **M**), 136.0 (*Ar*CH_2O, **m**), 155.9 (2 x OCONH, **M & m**), 164.3 (*C*=N, **M**), 164.7 (*C*=N, **m**), 176.9 (2 x CH*C*OO, **M & m**); *m/z* (EI) 380 (M^+).

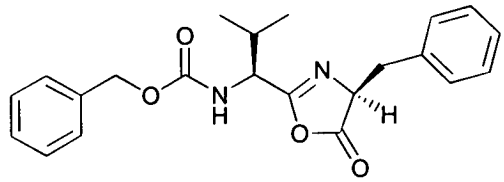
6.11.3.1 (1S,4'R) 1-(Benzyloxycarbonylamino)-1-(4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-2-methyl propane 86a



The solid (**86**) was recrystallised from boiling CH_2Cl_2/light petroleum (1:8), cooled and filtered to afford a white solid **86a** (1.22 g, 55 %): 64 % d.e. w.r.t. (*S,R*).

R_f (Light petroleum:EtOAc, 2:1) 0.30; Mp 116.5-117.8 °C; ν_{max} (KBr)/cm^{-1} 1829 (oxazolone C=O), 1716 (urethane C=O), 1674 (oxazolone C=N), 1558 (amide II); δ_H (250 MHz, $CDCl_3$) 0.53 (3H, d, *J* 6.5, CH(C^A*H*$_3$C^{B}H$_3$)), 0.76 (3H, d, *J* 6.5, CH(C^{A}H$_3$C^B*H*$_3$)), 1.86-1.96 (1H, m, C*H*$(CH_3)_2$), 3.16 (1H, dd, *J* 14.0, 5.5, CHC*H*$_A$H$_B$Ph), 3.26 (1H, dd, *J* 14.0, 5.0, CHCH$_A$*H*$_B$Ph), 4.41-4.47 (2H, m, C*H*CH_2Ph & C*H*$CH(CH_3)_2$), 5.07 (1H, d, *J* 12.0, OC*H*$_A$H$_B$Ph), 5.15 (1H, d, *J* 12.0, OCH$_A$*H*$_B$Ph), 5.27 (1H, d, *J* 9.0, N*H*), 7.14-7.27 (5H, m, Ar-*H*), 7.40 (5H, bs, Ar-*H*); δ_C (63 MHz, $CDCl_3$) 16.6 (CH(*C*^{A}H$_3$C^{B}H$_3$)), 18.4 (CH(C^{A}H$_3$*C*^{B}H$_3$)), 30.4 (*C*H$(CH_3)_2$), 36.2 (CH*C*H_2Ph), 54.7 (*C*HCH_2Ph), 65.3 (*C*H$CH(CH_3)_2$), 67.1 (OCH_2Ph), 127.2, 128.0, 128.1, 128.4 & 129.5 (10 x *Ar*-H), 134.5 (CH_2*Ph*), 135.9 (CH_2*Ph*), 155.9 (O*C*ONH), 164.7 (*C*=N), 176.9 (*C*OO); Found (EI) 380.17365 (M^+), $C_{22}H_{24}N_2O_4$ requires 380.17361.

6.11.3.2 (1S,4'S) 1-(Benzyloxycarbonylamino)-1-(4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-2-methyl propane 86b



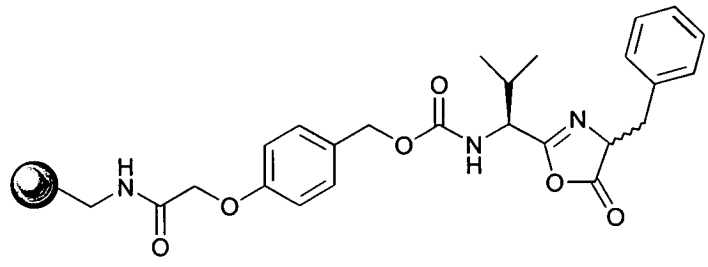
Subsequent concentration of the mother liquor yielded **86b** as a white powder (0.59 g, 27 %): 91 % d.e. w.r.t. (*S*,*S*).

R_f (Light petroleum:EtOAc, 2:1) 0.34; ν_{max} (KBr)/cm^{-1} 1830 (oxazolone C=O), 1717 (urethane C=O), 1654 (oxazolone C=N), 1540 (amide II); δ_H (250 MHz, $CDCl_3$) 0.77 (6H, d, *J* 7.0, $CH(C^A\mathit{H}_3C^B\mathit{H}_3)$), 1.93-1.98 (1H, m, $C\mathit{H}(CH_3)_2$), 3.11 (1H, dd, *J* 14.0, 6.0, $CHC\mathit{H}_A\mathit{H}_B$Ph), 3.25 (1H, dd, *J* 14.0, 5.0, $CHCH_A\mathit{H}_B$Ph), 4.38 (1H, dd, *J* 9.5, 5.5, $C\mathit{H}CH(CH_3)_2$), 4.47-4.51 (1H, m, $C\mathit{H}CH_2$Ph), 5.12 (2H, s, $OC\mathit{H}_2$Ph), 5.12-5.18 (1H, m, N*H*), 7.12-7.25 (5H, m, Ar-*H*), 7.36 (5H, bs, Ar-*H*); δ_C (63 MHz, DEPT, $CDCl_3$) 17.1 ($CH(\mathit{C}^AH_3C^BH_3)$), 18.5 ($CH(C^AH_3\mathit{C}^BH_3)$), 30.5 ($\mathit{C}H(CH_3)_2$), 36.5 ($CH\mathit{C}H_2$Ph), 55.0 ($\mathit{C}HCH_2$Ph), 65.4 ($\mathit{C}HCH(CH_3)_2$), 67.1 ($O\mathit{C}H_2$Ph), 127.2, 128.0, 128.1, 128.3, 128.4, & 129.5 (10 x *Ar*-H), 134.5 ($CHCH_2\mathit{Ph}$), 135.9 ($OCH_2\mathit{Ph}$), 155.8 ($O\mathit{C}ONH$), 164.3 (C=N), 176.9 ($\mathit{C}OOCH$); Found (EI) M^+ 380.17285, $C_{22}H_{24}N_2O_4$ requires 380.17361.

6.11.4 General procedure for synthesis of dipeptide-derived resin-bound 5(4*H*)-oxazolones 88a-b

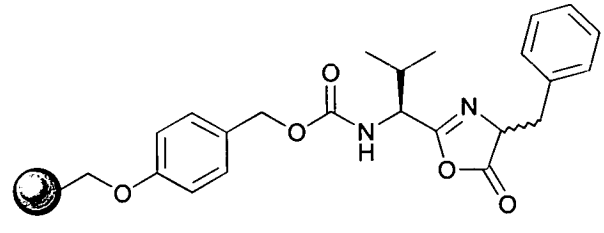
Resin-bound fluorenylmethyl ester-protected dipeptide (1 eq) was treated with 20 % piperidine in DMF (x 3; 15 min ea), washed (DMF x 3) and neutralised with 3 % acetic acid in DCM (x 4; 5 min ea). After washing this time with DCM (x 3), the deprotected resin was treated with EDCI (3 eq) in DCM (~15 ml/g resin) and spun at room temperature (>15 h). The resin slurry was filtered, washed according to the standard protocol and dried under vacuum.

6.11.5 (1'*S*,4''*RS*)-4-[*N*-Methoxycarbonyl-2'-methyl--1'-(4''-benzyl-5''-oxo-4'',5''-dihydro-oxazol-2''-yl)-aminopropyl]-phenoxyacetyl aminomethyl polystyrene 88a



The general procedure outlined above (**6.11.4**), was followed using PS-HMPA-Val-Phe-OFm (**69a**) (assume ≤0.9 mmol/g - 400 mg, 0.36 mmol) with EDCI (206 mg, 1.08 mmol). Analysis confirmed that cyclisation to the 5(4*H*)-oxazolone had occurred yielding the title compound as a mixture of (*S*,*S*) and (*S*,*R*) diastereomers. ν_{max} (DCM)/cm^{-1} 3428 (2° amide & urethane NH), 1823 (oxazolone C=O), 1722 (urethane C=O), 1673br (oxazolone C=N & amide I C=O), 1602 (aromatic C=C), 1510 (amide II NH), 1492 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 16.9 & 17.4 (2 x CH($C^AH_3C^BH_3$)), 18.7 (2 x CH($C^AH_3C^BH_3$)), 30.7 (2 x *C*H(CH_3)$_2$), 36.7 & 36.9 (2 x *C*H$_2$Ph), 55.1 & 55.5 (2 x *C*HCH$_2$Ph), 65.7 & 65.8 (2 x *C*HCH(CH_3)$_2$), 66.7 (*C*H$_2$OCONH), 67.6 (*C*H$_2$OAr), 114.8 (2 x *m*-*Ar*HCH$_2$O), 127.4 (*Ar*H Phe), 128.6 (2 x *Ar*H Phe), 129.8 (2 x *Ar*H Phe), 135.3 (*ipso*-*C* Phe); Cleavage[†] 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 265 ($MH^+ + H_2O$).

6.11.6 (1'*S*,4''*RS*)-4-[*N*-Methoxycarbonyl-2'-methyl-1'-(4''-benzyl-5''-oxo-4'',5''-dihydro-oxazol-2''-yl)-aminopropyl]-phenoxymethyl polystyrene 88b



The general procedure outlined above (**6.11.4**), was followed using PS-Wang-Val-Phe-OFm (**74a**) (assume ≤0.9 mmol/g - 700 mg, 0.63 mmol) with EDCI (361 mg,

[†] treatment with TFA results in hydrolysis of the 5(4*H*)-oxazolone

1.89 mmol). Analysis confirmed that cyclisation to the 5(4*H*)-oxazolone had occurred yielding the title compound as a mixture of (*S*,*S*) and (*S*,*R*) diastereomers. ν_{max} (DCM)/cm^{-1} 3429 (urethane NH), 1826 (oxazolone C=O), 1722 (urethane C=O), 1673br (oxazolone C=N & amide I C=O), 1602 & 1585 (aromatic C=C), 1513 (amide II NH), 1493 (aromatic C=C), 826 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 16.8 & 17.4 (2 x CH($C^AH_3C^BH_3$)), 18.7 (2 x CH($C^AH_3C^BH_3$)), 30.7 (2 x $CH(CH_3)_2$), 36.7 & 36.9 (2 x CH_2Ph), 55.0 & 55.4 (2 x $CHCH_2$Ph), 65.7 & 65.8 (2 x $CHCH(CH_3)_2$), 67.0 (CH_2OCONH), 70.1 (CH_2OAr), 114.8 (2 x *m*-*Ar*HCH_2O), 127.4 (*Ar*H Phe), 128.6 (2 x *Ar*H Phe), 129.8 (2 x *Ar*H Phe), 130.1 (2 x *o*-*Ar*CH_2O), 135.4 (*ipso*-*C* Phe), 177.4 (2 x CH*C*OO); Cleavage† 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 265 ($MH^+ + H_2O$).

6.11.7 General procedure for nucleophilic ring-opening of resin-bound 5(4*H*)-oxazolones 88a-b

6.11.7.1 Amines

To a slurry of the polymer-supported 5(4*H*)-oxazolone (1 eq) in NMP (1 ml/50 mg resin), was added the nucleophile (3 eq) along with DMAP (0.5 eq). The resulting suspension was agitated at room temperature (>15 h), before filtering, washing according to the standard protocol and drying.

6.11.7.2 Amino acid salts

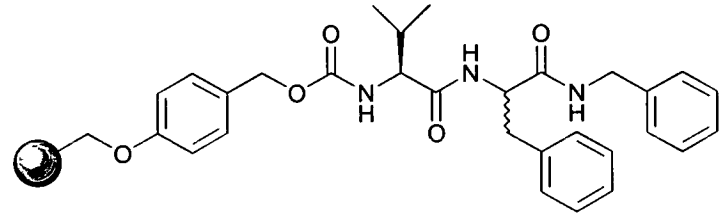
Polymer-supported 5(4*H*)-oxazolone (1 eq) was suspended in a solution of the ammonium salt of the amino acid fluorenylmethyl ester tosylate (3 eq) and DMAP (3.5 eq) in DMF/NMP (1 ml/40 mg resin). The resin slurry was spun at room temperature (>15 h), before being filtered, washed according to the standard protocol and dried.

6.11.7.3 Alcohols

Resin-bound 5(4*H*)oxazolone (1 eq) was suspended in a mixture of MeOH/DMF (1:1) (1 ml/20 mg resin). Triethylamine (0.5 eq) was added and the reaction rotated

at room temperature (>15 h), before filtering, washing according to the standard protocol and drying.

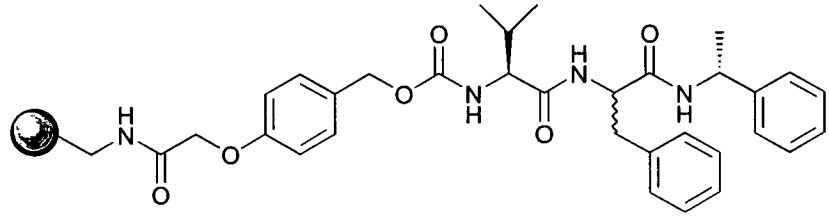
6.11.8 4-(N^{α}-Methoxycarbonylvalyl-D,L-phenylalanylaminobenzyl)-phenoxymethyl polystyrene 89a



The general procedure detailed above (***6.11.7.1***) was followed using resin-bound oxazolone **88b** (assume ≤0.45 mmol/g, 100 mg, 0.05 mmol), benzylamine (14.8 μl, 0.14 mmol) and DMAP (~2.5 mg, 0.02 mmol). Analysis indicated successful ring-opening to furnish the title compound as a mixture of diastereomers.

ν_{max} (DCM)/cm^{-1} 3424 & 3298br (2° amide & urethane NH), 1716 (urethane C=O), 1674 & 1651 (2 x amide I C=O), 1602br (aromatic C=C), 1549br (2 x amide II & urethane NH), 1494br (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 18.2br (2 x CH($C^AH_3C^BH_3$)), 19.1br (2 x CH($C^AH_3C^BH_3$)), 31.5 (2 x $CH(CH_3)_2$), ~40 (2 x $CHCH_2Ph$ - masked by polymer backbone), ~54br (2 x $CHCH_2Ph$ - obscured by solvent), ~61br (2 x $CHCH(CH_3)_2$), 66.8 (CH_2OCONH), 70.1 (CH_2OAr), 114.7 (2 x *m-Ar*HCH_2O), 128.2br (all other *Ar*H obscured by polymer back-bone); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 5.5br & 8.3br min (two diastereomers); *m/z* (ES-MS) 354 (MH^+), 376 (MNa^+).

6.11.9 (1'*R*)-4-[N^{α}-Methoxycarbonylvalyl-D,L-phenylalanylamino-1'-(phenyl)-ethyl]-phenoxyacetyl aminomethyl polystyrene 89b

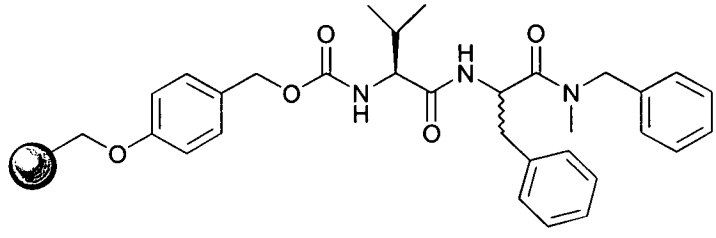


The general procedure detailed above (***6.11.7.1***) was followed using resin-bound oxazolone **88a** (assume ≤0.9 mmol/g, 120 mg, 0.11 mmol), (*R*)-(+)-α-

methylbenzylamine (42 μl, 0.32 mmol) and DMAP (~6.5 mg, 0.05 mmol). Analysis indicated successful ring-opening to afford the desired compound as a mixture of diastereomers.

ν_{max} (DCM)/cm^{-1} 3425 & 3304br (2° amide & urethane NH), 1711 (urethane C=O), 1677br (3 x amide I C=O), 1602 & 1581 (aromatic C=C), 1509br (3 x amide II & urethane NH), 1493 (aromatic C=C), 828 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 18.2br (2 x CH($C^AH_3C^BH_3$)), 19.1br (2 x CH($C^AH_3C^BH_3$)), 22.1br (2 x $CHCH_3$), 31.1 & 31.5 (2 x $CH(CH_3)_2$), 38.4 (2 x $CHCH_2Ph$ - partially obscured by polymer backbone), 48.9 ($NHCHCH_3$), 53.2br (2 x $CHCH_2Ph$ - partially obscured by solvent), 61.2br (2 x $CHCH(CH_3)_2$), 66.4 (CH_2OCONH), 67.6 (CH_2OAr), 114.7 (2 x *m*-$ArHCH_2O$), 128.2br & 128.5br (10 x ArH Phe - obscured by polymer back-bone), 129.5 (2 x *o*-$ArHCH_2O$), 137.1br (2 x *ipso*-*C*); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^B R_t 20.8 & 21.6 min (two diastereomers); *m/z* (ES-MS) 368 (MH^+).

6.11.10 4-[N^{α}-Methoxycarbonylvalyl-D,L-phenylalanyl-*N*-(methyl)-aminobenzyl]-phenoxymethyl polystyrene 89c

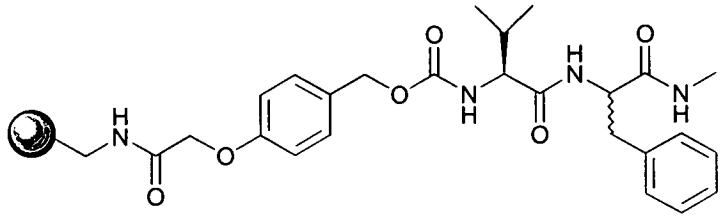


The general procedure detailed above (***6.11.7.1***) was followed using resin-bound oxazolone **88b** (assume ≤0.45mmol/g, 100 mg, 0.05 mmol), *N*-methylbenzylamine (17.6 μl, 0.14 mmol) and DMAP (~2.5 mg, 0.02 mmol). Analysis indicated successful ring-opening by the secondary amine to afford the desired compound as a mixture of diastereomers.

ν_{max} (DCM)/cm^{-1} 3417 & 3302br (2° amide & urethane NH), 1716br (ester & urethane C=O), 1684 & 1644br (2 x amide I C=O), 1602 & 1583 (aromatic C=C), 1514br (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.7 (CH($C^AH_3C^BH_3$)), 19.3 (CH($C^AH_3C^BH_3$)), 31.5 ($CH(CH_3)_2$), 39.3 (2 x $CHCH_2Ph$), 50.4 & 51.3 (NCH_3CH_2Ph), 53.2br (2 x $CHCH_2Ph$ - partially obscured

by solvent), 60.3 (*C*HCH(CH_3)$_2$), 66.8 (*C*H_2OCONH), 70.2 (*C*H_2OAr), 114.7 (2 x *m*-*Ar*HCH_2O), 128.1br (10 x *Ar*H Phe - obscured by polymer back-bone), 129.7 (2 x *o*-*Ar*HCH_2O), 136.5br (2 x *ipso-C*); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[B] R_t 22.4 & 23.2 min (two diastereomers); *m/z* (ES-MS) 368 (MH^+), 390 (MNa^+).

6.11.11 4-(N^{α}-Methoxycarbonylvalyl-D,L-phenylalanylaminomethyl)-phenoxyacetyl aminomethyl polystyrene 89d

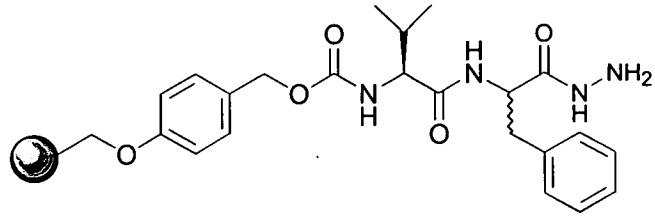


The general procedure detailed above (***6.11.7.1***) was followed using resin-bound oxazolone **88a** (assume ≤0.5mmol/g, 60 mg, 0.03 mmol), methylamine hydrochloride salt (6 mg, 0.09 mmol) as a suspension in NMP (1.5 ml) and stoichiometric† DMAP (11 mg, 0.09 mmol) to mop up the acid released. Analysis indicated successful ring-opening by the primary amine to afford the desired compound as a mixture of diastereomers.

ν_{max} (DCM)/cm^{-1} 3427 & 3315br (3 x amide & urethane NH), 1714 (urethane C=O), 1682br & 1652 (3 x amide I C=O), 1602br (aromatic C=C), 1513br (3 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.7 (CH(*C*^{A}H_3C^BH_3)), 19.3 (CH(C^AH_3*C*^{B}H_3)), 31.5 (*C*H(CH_3)$_2$), 39.3 (2 x CH*C*H_2Ph), 50.4 & 51.3 (N*C*H_3*C*H_2Ph), 53.2br (2 x *C*HCH_2Ph - partially obscured by solvent), 60.3 (*C*HCH(CH_3)$_2$), 66.8 (*C*H_2OCONH), 70.2 (*C*H_2OAr), 114.7 (2 x *m*-*Ar*HCH_2O), 128.1br (10 x *Ar*H Phe - obscured by polymer back-bone), 129.7 (2 x *o*-*Ar*HCH_2O), 136.5br (2 x *ipso-C*); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 1.2 & 1.3 min (two diastereomers); *m/z* (ES-MS) 278 (MH^+), 300 (MNa^+).

† w.r.t. methylamine

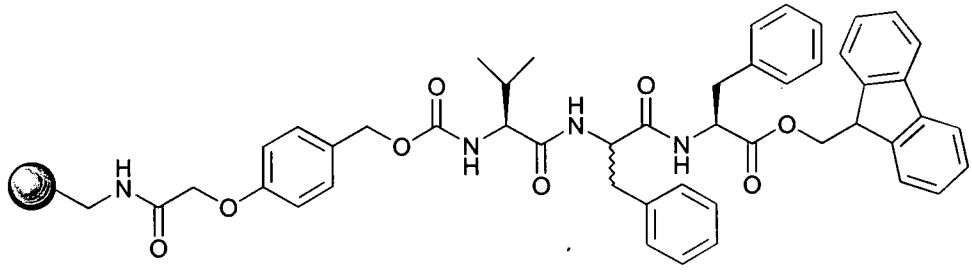
6.11.12 4-(N^{α}-Methoxycarbonylvalyl-D,L-phenylalanylhydrazino)-phenoxymethyl polystyrene 89e



The general procedure detailed above (***6.11.7.1***) was followed using resin-bound oxazolone **88b** (assume ≤0.45 mmol/g, 100 mg, 0.05 mmol), hydrazine hydrate (≤55 % H_2O) (9.34 μl, 0.14 mmol) and DMAP (~2.5 mg, 0.02 mmol). Analysis indicated successful ring-opening the desired compound as a mixture of diastereomers.

ν_{max} (DCM)/cm^{-1} 3420 & 3293 (2° amide, urethane and hydrazine NH), 1714 (urethane C=O), 1674 & 1652 (2 x amide I C=O), 1602 & 1584 (aromatic C=C), 1514br (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 18.0br (2 x CH($C^AH_3C^BH_3$)), 19.2br (2 x CH($C^AH_3C^BH_3$)), 31.5 (2 x *C*H(CH_3)$_2$), 38.5 & 38.7 (2 x CH*C*H_2Ph - partially obscured by polymer backbone), 53.2br (2 x *C*HCH_2Ph - partially masked by solvent), 60.7br (2 x *C*HCH(CH_3)$_2$), 66.8 (*C*H_2OCONH), 70.1 (*C*H_2OAr), 114.8 (2 x *m-Ar*HCH_2O), 128.2br (all other *Ar*-H obscured by polymer back-bone), 137.0br (*ipso-C* Phe); Cleavage 9:10:1 TFA/DCM/H_2O; *m/z* (ES-MS) 279 (MH^+), 301 (MNa^+).

6.11.13 4-(N^{α}-Methoxycarbonylvalyl-D,L-phenylalanylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxyacetyl aminomethyl polystyrene 90

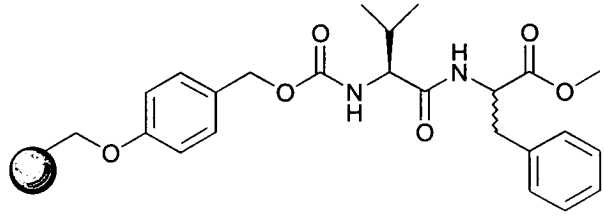


The general procedure detailed above (***6.11.7.2***) was followed using resin-bound oxazolone **88a** (assume ≤0.40 mmol/g, 49 mg, 0.02 mmol) with TSA-Phe-OFm

(**65a**) (31 mg, 0.06 mmol) and DMAP (9 mg, 0.07 mmol) in DMF (1 ml). Analysis indicated successful ring-opening to yield the desired tripeptide as a mixture of diastereomers. Gel phase ^{13}C NMR analysis was not feasible due to the small sample size.

Loading 0.23 mmol/g (Fm), 61 %; ν_{max} (DCM)/cm^{-1} 3424 & 3306br (2 x amide & urethane NH), 1732 & 1715 (ester & urethane C=O), 1694br & 1651br (2 x amide I C=O), 1601br (aromatic C=C), 1538, 1519 & 1504br (2 x amide II & urethane NH & aromatic C=C); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 26.1 & 26.7 min (two diastereomers); *m/z* (ES-MS) 591 (MH^+), 613 (MNa^+).

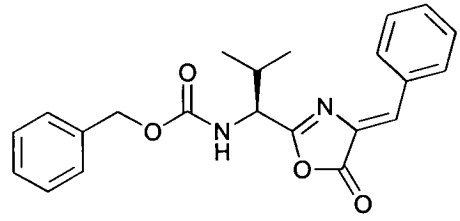
6.11.14 4-(N^{α}-Methoxycarbonylvalyl-D,L-phenylalanyloxymethyl)-phenoxymethyl polystyrene 91



The general procedure previously described (***6.11.7.3***) was followed using resin-bound oxazolone **88b** (assume ≤0.45 mmol/g, 150 mg, 0.07 mmol) and triethylamine (5 µl, 0.04 mmol) in MeOH/DMF (1:1) (6 ml). Analysis indicated successful ring-opening by the alcohol to afford the title compound as a mixture of diastereomers.

ν_{max} (DCM)/cm^{-1} 3422 & 3324br (2° amide & urethane NH), 1716br (ester & urethane C=O), 1679 (amide I C=O), 1602 & 1585 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C), 826 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 17.3 & 17.7 (2 x CH($C^AH_3C^BH_3$)), 19.1 & 19.3 (2 x CH($C^AH_3C^BH_3$)), 31.2 (2 x *C*H(CH_3)$_2$), 38.1 (2 x CH*C*H_2Ph), 52.4 (O*C*H_3), 53.4 (2 x *C*HCH$_2$Ph), 60.4 (2 x *C*HCH(CH_3)$_2$), 66.9 (*C*H_2OCONH), 70.2 (*C*H_2OAr), 114.8 (2 x *m-Ar*HCH$_2$O), 127.2 (*Ar*H Phe), 128.7 (2 x *Ar*H Phe), 129.4 (2 x *Ar*H Phe), 130.0 (2 x *o-Ar*HCH$_2$O), 136.3 (*ipso-C* Phe); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^B R_t 18.2 & 19.5 min (two diastereomers); *m/z* (ES-MS) 279 (MH^+).

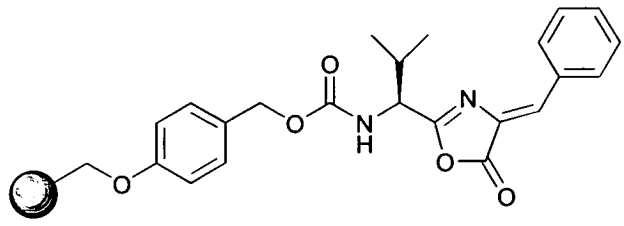
6.11.15 (1*S*)-1-(Benzyloxycarbonylamino)-1-(4'-benzylidene-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-2-methyl propane 94



A solution of DDQ (170 mg, 0.75 mmol) in dry dimethoxyethane (2 ml) was added to a solution of (1*S*,4'*RS*)-1-(Benzyloxycarbonylamino)-1-(4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-2-methyl propane (**86**) (285 mg, 0.75 mmol) in dry dimethoxyethane (4 ml). 2,4,6-Collidine (99 μl, 0.75 mmol) was added to the red solution, turning it deep red/black and the resulting solution stirred, under argon, at room temperature for 24 h. The red precipitate was filtered off and the filtrate concentrated to a dark red oil (516 mg). Column chromatography eluting with light petroleum/ether (5:1), furnished the title compound as a white solid (41 mg, 14 %), which decomposed rapidly when stored at room temperature.

R_f (Light petroleum:ether, 5:1) 0.12; (Found: C, 69.77; H, 5.70; N, 7.34; $C_{24}H_{29}NO_4$ requires C, 69.83 H, 5.86; N 7.40); $[\alpha]_D^{20}$ -41 (c 0.50, MeOH); ν_{max} (KBr)/cm^{-1} 3309 (urethane NH), 1799 (oxazolone C=O), 1784, 1695 (urethane C=O), 1687 & 1658 (amide I C=O, alkene C=C, oxazolone C=N), 1539 (amide II), 768 & 688 (mono-substituted Ar-H); δ_H (250 MHz, $CDCl_3$) 0.98 (3H, d, *J* 7.0, CH(C^A*H*$_3$C^{B}H$_3$)), 1.05 (3H, d, *J* 7.0, CH(C^{A}H$_3$C^B*H*$_3$)), 2.24-2.32 (1H, m, CHC*H*(CH$_3$)$_2$), 4.74 (1H, dd, *J* 9.5, 5.5, NHC*H*CH(CH$_3$)$_2$), 5.15 (2H, s, OC*H*$_2$Ph), 5.40 (1H, d, *J* 9.5, N*H*), 7.21 (1H, s, C=C*H*Ph), 7.29-7.45 (8H, m, Ar-*H*), 8.03-8.11 (2H, m, 2 x *o*-Ar-*H*CH=C); δ_C (63 MHz, $CDCl_3$) 17.4 (CH(C^{A}H$_3$C^{B}H$_3$)), 18.8 (CH(C^{A}H$_3$C^{B}H$_3$)), 31.3 (CH*C*H(CH$_3$)$_2$), 55.2 (*C*HCH(CH$_3$)$_2$), 67.2 (O*C*H$_2$Ph), 128.0, 128.2, 128.5, 128.8, 131.4, 132.4 & 133.1 (10 x *Ar*-H & C=*C*HPh), 132.8 (*C*=CHPh), 135.9 (OCH$_2$*Ph*), 155.9 (O*C*ONH), 167.0 (*C*=N), 176.9 (*C*OOC=N); Found (FAB) MH$^+$ 379.16579, $C_{22}H_{23}N_2O_4$ requires 379.16578.

6.11.16 (1'*S*)-4-[*N*-Methoxycarbonyl-2'-methyl-1'-(4''-benzylidene-5''-oxo-4'',5''-dihydro-oxazol-2''-yl)-aminopropyl]-phenoxymethyl polystyrene 95

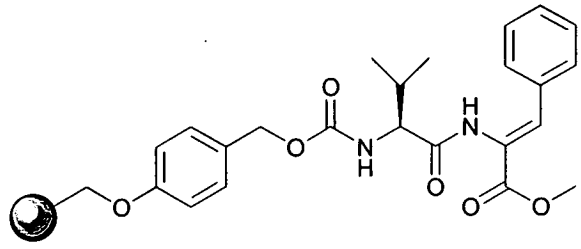


Typical procedure - resin-bound 5(4*H*)-oxazolone (**88b**) (assume ≤0.9 mmol, 500 mg, 0.45 mmol), was allowed to swell in dimethoxyethane (5 ml) before being treated with a solution of DDQ (306 mg, 1.35 mmol) in dimethoxyethane (3 ml). The resin slurry was spun at room temperature (15 h), before being washed according to the standard protocol and then resubjected to the original reaction conditions. After reacting for a further 15 h the resin was filtered, washed as before and dried. Analysis indicated almost complete conversion to the title compound, which appeared to be a single geometric isomer. Traces of unoxidised starting material were detected by ES-MS and also hindered interpretation of the gel phase ^{13}C NMR spectrum.

ν_{max} (DCM)/cm^{-1} 3427 (2° amide & urethane NH), 1805 (oxazolone C=O), 1722 (urethane C=O), 1660br (amide I C=O, alkene C=C, oxazolone C=N), 1602 & 1584 (aromatic C=C), 1514 (amide II), 1493 (aromatic C=C), 827 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 17.6 (CH($C^AH_3C^BH_3$)), 19.0 (CH($C^AH_3C^BH_3$)), 31.4 (2 x $CH(CH_3)_2$), 55.7 ($CHCH(CH_3)_2$), 67.1 (CH_2OCONH), 70.1 (CH_2OAr), 114.8 (2 x *m*-$ArHCH_2O$), 128.2 & 129.0 (4 x Ar-*H*), 130.0 (2 x *o*-$ArHCH_2O$), 131.6 & 132.6 (*Ar*-H & C=*C*HPh); Cleavage† 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 263 ($MH^+ + H_2O$), 281 ($MH^+ + 2 \times H_2O$); Cleavage 1:1 TFA/DCM: *m/z* (ES-MS) 245 (MH^+), 263 ($MH^+ + H_2O$).

† treatment with TFA results in almost hydrolysis of the 5(*4H*)-oxazolone

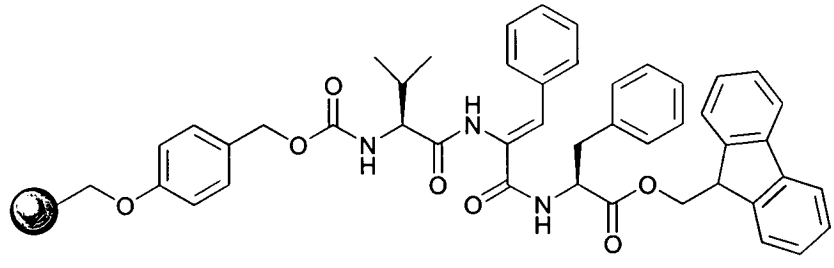
6.11.17 4-[N^{α}-methoxycarbonylvalyl-(*Z*)-α,β-dehydro-phenylalanyloxymethyl]-phenoxymethyl polystyrene 96



The general procedure previously described for 5(4*H*)-oxazolones (***6.11.7.3***), was followed using resin-bound 4-arylidene 5(4*H*)-oxazolone (**95**) (assume ≤0.45 mmol/g, 82 mg, 0.04 mmol) and triethylamine (2.6 µl, 0.02 mmol) in MeOH/DMF (1:1) (4 ml). Analysis indicated successful ring-opening by the alcohol to afford the title compound as a single stereoisomer.

ν_{max} (DCM)/cm^{-1} 3418 & 3307br (2° amide & urethane NH), 1721 & 1741br (ester & urethane & amide I C=O), 1612 (alkene C=C), 1602 & 1584 (aromatic C=C), 1514br (amide II & urethane NH), 1494 (aromatic C=C), 827 (*p*-disubstituted Ar-H); δ_C (63 MHz, CD_2Cl_2, gel) 17.7 (CH($C^AH_3C^BH_3$)), 19.2 (CH($C^AH_3C^BH_3$)), 30.8 (*C*H(CH_3)$_2$), 52.6 (O*C*H$_3$), 60.7 (*C*HCH(CH_3)$_2$), 67.0 (*C*H$_2$OCONH), 70.1 (*C*H$_2$OAr), 114.7 (2 x *m-Ar*HCH$_2$O), 128.1 (ArH Phe & C=*C*HPh obscured by polymer backbone), 128.7 (2 x *Ar*H Phe), 129.6 (2 x *Ar*H Phe), 130.0 (2 x *o-Ar*HCH$_2$O), 132.8 (*ipso-C* Phe); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^B R_t 16.9 min (single diastereomer); *m/z* (ES-MS) 277 (MH$^+$).

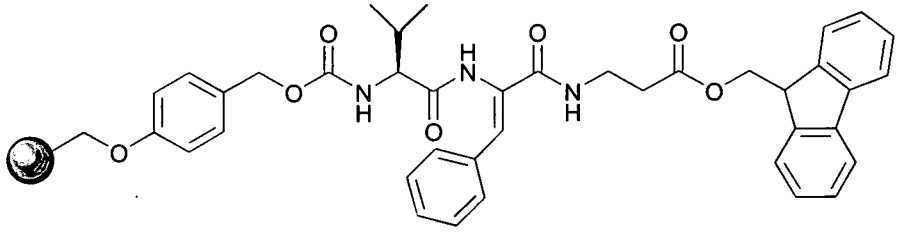
6.11.18 4-(*N*$^{\alpha}$-methoxycarbonylvalyl-(*Z*)-α,β-dehydro-phenylalanylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 97



The general procedure detailed above (***6.11.7.2***) was followed using resin-bound 4-arylidene 5(4*H*)-oxazolone **95** (assume ≤0.5 mmol/g, 80 mg, 0.04 mmol) with TSA-Phe-OFm (**65a**) (62 mg, 0.12 mmol) and DMAP (17 mg, 0.14 mmol) in NMP (2 ml). Analysis indicated successful ring-opening to yield the desired tripeptide as a single diastereomer. Gel phase ^{13}C NMR analysis was of poor quality due to sample size and too broad to interpret successfully.

Loading 0.16 mmol/g (Fm); ν_{max} (DCM)/cm^{-1} 3427 & 3317br (2° amide & urethane NH), 1714br (ester & urethane C=O), 1682br (3 x amide I C=O and alkene C=C), 1602br (aromatic C=C), 1513br (3 x amide II & urethane NH), 1494 (aromatic C=C); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^A R_t 26.9 min (single diastereomer); *m/z* (ES-MS) 588 (MH^+), 610 (MNa^+).

6.11.19 4-(*N*$^{\alpha}$-methoxycarbonylvalyl-(*Z*)-α,β-dehydro-phenylalanyl-β-alanyloxymethylfluorenyl-9'-yl)-phenoxymethyl polystyrene 98

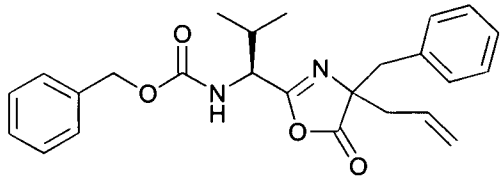


Resin-bound 4-arylidene 5(4*H*)-oxazolone **95** (assume ≤0.9 mmol/g, 150 mg, 0.14 mmol) was suspended in a suspension of TSA-β-Ala-OFm (**77c**) (178 mg, 0.41 mmol), NMM (45 μl, 0.41 mmol) and DMAP (2.5 mg, 0.02 mmol) in NMP (2 ml). The resin slurry was spun at room temperature (>15 h), before being filtered, washed according to standard protocol and dried. Analysis confirmed successful ring-opening to yield the desired dehydro-tripeptide as a single diastereomer. However, the gel phase ^{13}C NMR spectrum obtained was very weak and of fairly poor quality, indicating that the reaction had not gone to completion.

0.09 mmol/g (Fm); ν_{max} (DCM)/cm^{-1} 3423 & 3324br (2 x amide & urethane NH), 1716br (ester & urethane C=O), 1694br (2 x amide I C=O & alkene C=C), 1602 & 1585 (aromatic C=C), 1514br (2 x amide II & urethane NH), 1494 (aromatic C=C); δ_C (63 MHz, CD_2Cl_2, gel) 17.6 (CH($C^AH_3C^BH_3$)), 19.2 (CH($C^AH_3C^BH_3$)), 31.1

(*C*H(CH$_3$)$_2$), 33.9 & 35.8 (NHCH$_2$*C*H$_2$CO), 46.9 (OCH$_2$CH), 60.1 (*C*HCH(CH$_3$)$_2$), 67.2 (OCH$_2$CH & *C*H$_2$OCONH), 70.1 (*C*H$_2$OAr), 114.8 (2 x *m-Ar*HCH$_2$O), 120.1 (2 x *Ar*H OFm), 128.1br (6 x *Ar*H OFm, 3 x *Ar*H Phe and C=*C*H masked by broad polymer backbone), 129.5 (2 x *Ar*H Phe), 130.0 (2 x *o-Ar*HCH$_2$O); Cleavage 9:10:1 TFA/DCM/H$_2$O: LC-MS^A R$_t$ 20.0 min (single diastereomer); *m/z* (ES-MS) 512 (MH$^+$), 534 (MNa$^+$).

6.11.20 (1*S*,4'*RS*)-1-(Benzyloxycarbonylamino)-2-methyl-1-[4'-(prop-2''-enyl)-4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl] propane 101

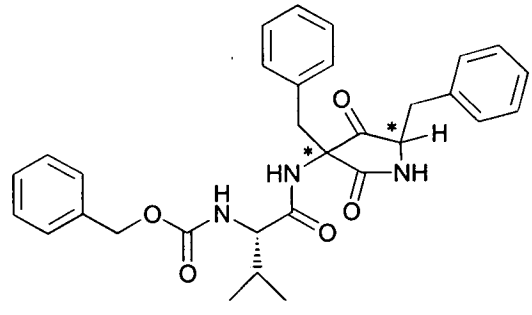


Typical procedure - to the reaction vessel containing anhydrous THF (20 ml), was added freshly-distilled allyl bromide (130 µl, 1.5 mmol) and NaHMDS (0.6 M in toluene, 1.23 ml, 0.74 mmol) before cooling to 0 °C. A solution of (1*S*,4'*RS*)-1-(benzyloxycarbonylamino)-1-(4'-benzyl-5'-oxo-4',5'-dihydro-oxazol-2'-yl)-2-methyl propane (**86**) (0.23 g, 0.6 mmol) in THF (10 ml) was added dropwise with stirring, resulting in a yellow solution and formation of a white precipitate. The reaction was allowed to warm to 7 °C with constant stirring and the progress monitored by tlc. After 100 min no starting material remained, and the reaction was quenched with saturated ammonium chloride solution (20 ml) followed by water (25 ml). The aqueous phase was separated and extracted with EtOAc (3 x 75 ml) and the combined organic extracts dried (Na$_2$SO$_4$) and concentrated. The resulting yellow oil was then redissolved in CHCl$_3$ and finally concentrated to afford a yellow oil (0.28 g). Purification by flash-column chromatography with EtOAc/light petroleum (1:3) as eluent, was carried out using a minimum amount of silica gel to avoid decomposition of the product. **101** was obtained as a pale yellow oil (0.13 g, 52 %). ^{1}H NMR indicated a 55 % diastereomeric excess of the (*S,R*) product.

R$_f$ (EtOAc:light petroleum, 1:4), 0.48); ν_{max} (thin film)/cm^{-1} 1819 (oxazolone C=O), 1728 (urethane C=O), 1674 (oxazolone C=N), 1519 (amide II); δ_H (250 MHz, CDCl$_3$) 0.50 (3H, d, *J* 7.0, CH(C^A*H*$_3$C^{B}H$_3$), **M**), 0.68 (3H, d, *J* 7.0, CH(C^{A}H$_3$C^B*H*$_3$),

M), 0.75 (6H, d, *J* 7.0, CH(C^A*H*$_3$$C^B$*H*$_3$), **m**), 1.77-1.85 (2H, m, C*H*(CH_3)$_2$, **M & m**), 2.61 (4H, br d, *J* 7.5, C*H*$_2$CH=CH_2, **M & m**), 3.06 (1H, d, *J* 13.5, C*H*$_A$$H_B$Ph, **M**), 3.14 (1H, d, *J* 13.5, CH_A*H*$_B$Ph, **M**), 3.04-3.17 (2H, m, C*H*$_2$Ph, **m**), 4.23-4.29 (1H, m, C*H*CH(CH_3)$_2$, **m**), 4.35 (1H, dd, *J* 9.5, 6.0, C*H*CH(C^AH$_3$$C^BH_3$), **M**), 5.06-5.18 (10H, m, OC*H*$_2$Ph, CH=C*H*$_2$ & N*H*, **M & m**), 5.51-5.65 (2H, m, CH_2C*H*=CH_2, **M & m**), 7.08-7.23 (10H, m, Ar-*H*, **M & m**), 7.32-7.37 (10H, m, Ar-*H*, **M & m**); δ_C (63 MHz, $CDCl_3$) 16.9 (CH(C^AH$_3$$C^BH_3$), **M**), 17.0 (CH($C^AH_3$$C^BH_3$), **m**), 18.5 (2 x CH($C^AH_3$$C^BH_3$), **M & m**), 30.3 (*C*H(CH_3)$_2$, **m**), 30.5 (*C*H(CH_3)$_2$, **M**), 40.9 (*C*H$_2$CH=CH_2, **M**), 41.4 (*C*H$_2$CH=CH_2, **m**), 42.4 (C*C*H$_2$Ph, **M**), 42.7 (C*C*H$_2$Ph, **m**), 54.8 (2 x C*H*CH(CH_3)$_2$, **M & m**), 67.0 (2 x O*C*H$_2$Ph, **M & m**), 73.9 (*C*COO, **M**), 74.0 (*C*COO, **m**), 77.1 (2 x CH_2*C*H=CH_2, **M & m**), 120.7 (CH_2CH=*C*H$_2$, **m**), 120.8 (CH_2CH=*C*H$_2$, **M**), 127.2, 127.3, 128.0, 128.1, 128.2, 128.4, 130.0, 130.1 & 130.4 (20 x *Ar*-H, **M & m**), 134.1 (2 x CH_2*Ar*, **M & m**), 136.0 (2 x CH_2*Ar*, **M & m**), 155.7 (2 x O*C*ONH, **M & m**), 163.0 (2 x N=*C*O, **M & m**), 178.5 (2 x *C*OO, **M & m**); Found (EI) M^+ 420.20659, $C_{25}H_{28}N_2O_4$ requires 420.20491.

6.11.21 N^{α}-(Benzyloxycarbonyl)-valinyl-*N*-(3,5-dibenzyl-2,4-dioxo-pyrrolidinyl-3-yl) amide 102a



To a cooled (0 °C) solution of 5(4*H*)-oxazolone **86** (0.46 g, 1.2 mmol) in anhydrous THF (5 ml) was added NaHMDS (0.6 M in toluene, 2.47 ml, 1.48 mmol), dropwise with stirring. The reaction was monitored by tlc (toluene/EtOAc, 2:1) and after 5 h the reaction was quenched with saturated ammonium chloride solution (3.5 ml) and H_2O (3.5 ml). The aqueous phase was separated and extracted with EtOAc (3 x 15 ml) and the combined organic extracts were dried (Na_2SO_4) and concentrated. After redissolving in $CHCl_3$ the solvent was removed under reduced pressure to yield a

pale yellow solid (0.45 g). The product was purified by flash-column chromatography on silica gel with toluene/EtOAc (2:1) as eluent, and along with a number of other by-products, **102a** was isolated as a white solid (152 mg, 24 %). ^{1}H NMR indicated a (2:1) ratio of diastereomers.

R_f (Toluene:EtOAc, 2:1), 0.16); ν_{max} (thin film)/cm^{-1} 3273 (NH), 1774 (ketone C=O), 1716br (urethane, lactam & amide I C=O), 1604 (aromatic C=C), 1538 (amide II & urethane NH), 1498 (aromatic C=C), 751 & 700 (mono-disubstituted Ar-H); δ_H (250 MHz, $CDCl_3$) 0.43 (1H, dd, *J* 13.5, 12.0, CHC*H*$_A$H$_B$Ph, **m**), 0.75 (1H, dd, *J* 13.5, 12.0, CHC*H*$_A$H$_B$Ph, **M**), 0.90 (3H, d, *J* 7.0, CH(C^A*H*$_3$C^{B}H$_3$), **M**), 0.93 (3H, d, *J* 7.0, CH(C^{A}H$_3$C^B*H*$_3$), **M**), 0.96 (3H, d, *J* 7.0, CH(C^A*H*$_3$C^{B}H$_3$), **m**), 1.04 (3H, d, *J* 7.0, CH(C^{A}H$_3$C^B*H*$_3$), **m**), 2.00-2.10 (1H, m, CHC*H*(CH$_3$)$_2$, **M**), 2.23 (1H, dt, *J* 7.0, 3.5, C*H*(CH$_3$)$_2$, **m**), 2.58 (1H, dd, *J* 13.5, 3.5, CHCH$_A$*H*$_B$Ph, **m**), 2.65 (1H, dd, *J* 13.5, 3.5, CHCH$_A$*H*$_B$Ph, **M**), 2.95-3.10 (1H, br d, C^{4}C*H*$_A$H$_B$Ph, **M**), 3.26 (1H, br d, *J* 12.0, C^{4}CH$_A$*H*$_B$Ph, **M**), 3.61 (1H, d, *J* 13.0, C^{4}C*H*$_A$H$_B$Ph, **m**), 3.94 (1H, dd, *J* 3.5, 1.0, C*H*CH(CH$_3$)$_2$, **m**), 4.08 (1H, m, C*H*CH(CH$_3$)$_2$, **M**), 4.22[i] (1H, br dd, *J* ~12, 3.0, C*H*CH$_A$H$_B$Ph, **m**), 4.28 (1H, dd, *J* 12.0, 2.5, C*H*CH$_A$H$_B$Ph, **M**), 4.35 (1H, d, *J* 13.0, C^{4}CH$_A$*H*$_B$, **m**), 5.11 (2H, br s, OC*H*$_2$Ph, **M**), 5.26 (1H, d, *J* 9.0, N*H*CHCH(CH$_3$)$_2$, **M**), 5.87 (1H, br s, N*H*CHCH$_A$H$_B$Ph, **M**), 5.93 (1H, br s, N*H*CHCH$_A$H$_B$Ph, **m**), 6.20 (1H, br s, N*H*CHCH(CH$_3$)$_2$, **m**), 6.75-6.80 (4H, m, Ar*H*, **m** & **M**), 7.06-7.41 (28H, m, 26 x Ar*H* & 2 x CHCON*H*C^4, **m** & **M**); Found (FAB) MH$^+$ 528.24937, $C_{31}H_{34}N_3O_5$ requires 528.24985.

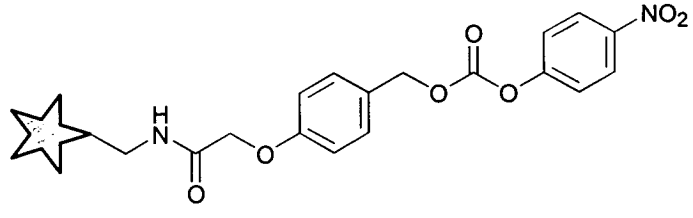
6.11.22 *N*$^{\alpha}$-(Benzyloxycarbonyl)-valinyl-*N*-(3,5-dibenzyl-2,4-dioxo-pyrrolidinyl-3-yl) amide 102b

To a cooled (0°C) solution of 5(4*H*)-oxazolone **86** (1.0 g, 2.60 mmol) in anhydrous THF (120 ml), was added NaHMDS, 0.6 M in toluene (5.2 ml, 3.12 mmol), dropwise over 20 min. The solution turned yellow and was allowed to stir (0°C - room temperature) for a total of 5 h. The reaction was quenched by pouring into saturated ammonium chloride solution (80 ml), H_2O (80 ml) was added and the product extracted with EtOAc (3 x 120 ml). The combined organic extracts were dried (Na_2SO_4), concentrated, dissolved in $CHCl_3$, to aid removal of EtOAc, and

concentrated to furnish a pale yellow foam (1.03 g). Purification by flash column chromatography eluting with toluene/EtOAc (2:1) furnished the title compound as a white solid (266 mg, 19 %). ^{1}H NMR confirmed that it was a single diastereomer. R_f (toluene:EtOAc, 2:1), 0.24); ν_{max} (thin film)/cm^{-1} 3282 (NH), 1777 (ketone C=O), 1710br (urethane, lactam & amide I C=O), 1606 & 1585 (aromatic C=C), 1534 (amide II & urethane NH), 1498 (aromatic C=C), 749 & 700 (mono-disubstituted Ar-H); δ_H (250 MHz, $CDCl_3$) 0.72 (1H, dd, *J* 13.5, 11.5, CHCH_AH$_B$Ph), 0.91 (3H, d, *J* 7.0, CH(C^AH_3C^{B}H$_3$), 0.93 (3H, d, *J* 7.0, CH(C^{A}H$_3$C^BH_3)), 2.01-2.11 (1H, m, CH(CH$_3$)$_2$), 2.64 (1H, dd, *J* 13.5, 3.5, CHCH$_A$$H_B$Ph), 2.95-3.02 (1H, br d, CCH_AH$_B$Ph), 3.29 (1H, br d, *J* ~12, CCH$_A$$H_B$Ph,), 4.15 (1H, br dd, *J* 9.0, 7.0, NHC*H*CH(CH$_3$)$_2$), 4.29 (1H, dd, *J* 11.5, 3.5, C*H*CH$_A$H$_B$Ph), 5.08 (1H, d, *J* 11.5, OCH_AH$_B$Ph), 5.14 (1H, d, *J* 11.5, OCH$_A$$H_B$Ph), 5.36 (1H, d, *J* 9.0, N*H*CHCH(CH$_3$)$_2$), 5.92 (1H, br s, N*H*CHCH$_A$H$_B$Ph), 6.77[i] (2H, dd, *J* ~8, ~2, 2 x Ar*H*), 7.04-7.40 (13H, m, 13 x Ar*H*), 7.62 (1H, s, CHC^{4}N*H*CO); δ_C (63 MHz, $CDCl_3$) 17.9 (CH(*C*^{A}H$_3$C^{B}H$_3$)), 18.8 (CH(C^{A}H$_3$*C*^{B}H$_3$)), 31.6 (*C*H(CH$_3$)$_2$), 35.5 (*C*H$_2$Ph), 38.5 (*C*H$_2$Ph), 58.7 (*C*HCH$_2$Ph), 63.2 (C*H*CH(CH$_3$)$_2$), 63.8 (NH*C*^{4}CH$_2$Ph), 67.0 (O*C*H$_2$Ph), 125.1, 126.8, 127.8, 127.9, 128.0, 128.3, 128.5, 128.6, 128.7, 128.8, 130.0 & 130.9 (15 x *Ar*-H), 132.2 (C^4*C*OCH), 135.9 (CH$_2$*Ph*), 136.1 (CH$_2$*P*h), 136.8 (OCH$_2$*Ph*), 156.3 (O*C*ONH), 171.4 (*C*ONH), 171.6 (*C*ONH); *m/z* (APCi) 528 (MH$^+$).

6.12 Preliminary Studies on SynPhase™ MD Crowns

6.12.1 4-[Hydroxymethyl-(4'-nitrophenylcarbonate)]-phenoxyacetamido SynPhase MD 106

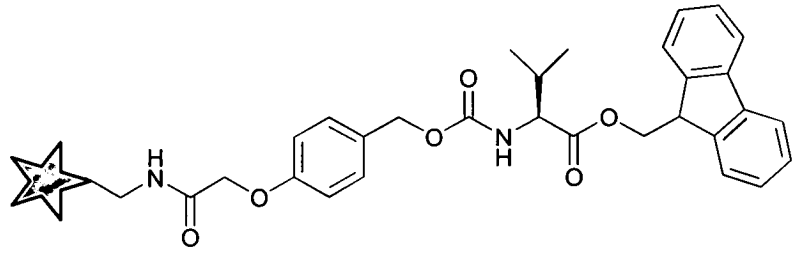


4-(Hydroxymethyl)-phenoxyacetamido SynPhase MD crowns (1.8 μmole/crown^{CT} - 30 crowns, 54 μmole) were suspended in dry DCM (10 ml) before addition of a

solution of *p*-nitrophenyl chloroformate (109 mg, 0.54 mmole). Anhydrous pyridine (44 µl, 0.54 mmole) was added and the suspension of crowns agitated very gently at room temperature, under argon. After 40 h the crowns were washed according to standard laboratory prototcol and dried. Two crowns were retained for *p*-nitrophenyl analysis and the remaining crowns reacted on (**6.12.2**).

Loading 0.2 µmole/g (*p*-nitro).

6.12.2 4-(N^{α}-Methoxycarbonylvalyloxymethylfluorenyl-9'-yl)-phenoxyacetamido SynPhase MD 107

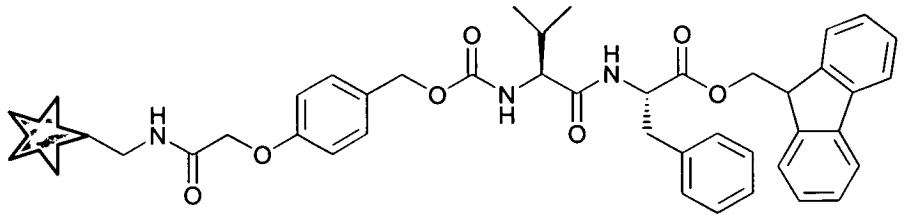


4-[Hydroxymethyl-(4'-nitrophenylcarbonate)]-phenoxyacetamido SynPhase MD crowns (**106**) (assume ≤1.8 µmole/crown - 28 crowns, 50.4 µmole) were suspended in a solution of TSA-Val-OFm (**65b**) (236 mg, 0.50 mmole) in DMF[†] (20 ml). A catalytic portion of DMAP (~10 %) was added along with NMM (55 µl, 0.50 mmole) and the suspension of crowns was shaken very gently at room temperature (12 h). The crowns were washed with MeOH (x 3) and DCM (x 1) and dried. One crown was cleaved, one retained for loading analysis and the remaining crowns reacted on (**6.12.3**).

Loading 0.1 µmol/g (Fm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS[A] R_t 9.2 min (broad and weak); *m/z* (ES-MS) 296 (MH^+).

[†] it was necessary to use a large volume of DMF due to poor solubitility of the valine salt.

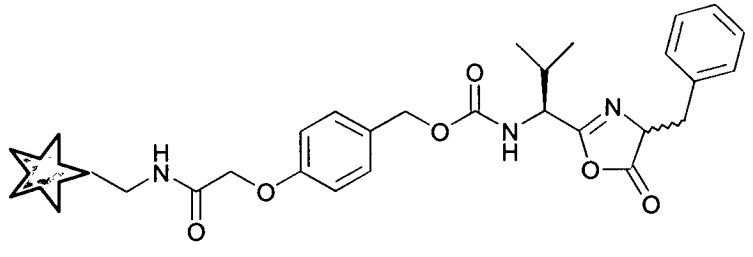
6.12.3 4-(N^{α}-Methoxycarbonylvalylphenylalanyloxymethylfluorenyl-9'-yl)-phenoxyacetamido SynPhase MD 108



Val-OFm-bound SynPhase MD crowns (**107**) (assume ≤1.8 μmole/crown - 26 crowns, 46.8 μmole) were deprotected with a solution of 20 % piperidine in DMF (3 x 20 ml; 15 min ea), before washing (MeOH x 3) and (DCM x 1). The crowns were then treated with 3 % acetic acid in DCM (4 x 20 ml; 5 min ea), washed with MeOH (x 3) and DCM (x 1) before suspending in a solution of TBTU (150 mg, 0.47 mmol) and NMM (51 μl, 0.47 mmol) in DMF (16 ml). After shaking for 1 h, the crowns were washed with MeOH (x 2) and finally suspended in a solution of HOBt-Phe-OFm (**66a**) (224 mg, 0.47 mmol) in DMF (16 ml) and agitated at room temperature for 12 h. The crowns were washed with MeOH (x 3) and DCM (x 1) and dried under vacuum. Two crowns were cleaved and analysed by LC-MS which indicated the coupling had occurred, but only gave a very weak signal. Fm analysis was carried out on a further two crowns also indicating a low yield of the product.

Loading 0.5 μmol/g (Fm); Cleavage 9:10:1 TFA/DCM/H_2O: LC-MS^{A} R_t 21.4 min (very weak); *m/z* (ES-MS) 443 (MH^+).

6.12.4 (1'*S*)-4-[*N*-Methoxycarbonyl-2'-methyl-1'-(4''-benzylidene-5''-oxo-4'',5''-dihydro-oxazol-2''-yl)-aminopropyl]-phenoxyacetamido SynPhase MD 109



Val-Phe-OFm-bound SynPhase MD crowns (**108**) (assume ≤1.8 μmole/crown - 12 crowns, 21.6 μmole) were deprotected with a solution of 20 % piperidine in DMF (3 x 10 ml; 15 min ea), before washing (MeOH x 3) and (DCM x 1). The crowns were

then treated with 3 % acetic acid in DCM (4 x 10 ml; 5 min ea), washed with MeOH (x 3) and DCM (x 1) before suspending in a solution of EDCI (41 mg, 0.22 mmol) in DCM (10 ml). After shaking (>24 h), the crowns were washed with MeOH (x 3) and DCM (x 1) and dried under vacuum. Acidic cleavage of two crowns, followed by ES-MS analysis only showed a very faint trace of the mass ion of the hydrolysed 5(4*H*)-oxazolone, indicating a very poor yield at this stage if at all.

Cleavage 9:10:1 TFA/DCM/H_2O: *m/z* (ES-MS) 265 ($MH^+ + H_2O$).

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