UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

CHEMISTRY

Master of Philosophy

Thermally Labile Linkers for Combinatorial Synthesis
by Seema Gupta

Combinatorial synthesis is a valuable new technique that enables large numbers of compounds to be prepared and tested for biological activity simultaneously. The approach involves the synthesis of compounds upon a solid support and hence a mild method is desirable for the cleavage of such compounds from the resin.

A new safety catch linker was developed that cleaved peptides from the solid support using thermolysis. Thermolysis offers a mild method of cleavage and this project incorporated the use of a sulfoxide safety catch linker which cleaved via a rearrangement mechanism.

The most important and novel result was the synthesis of a linker that can especially be used with tentagel (**18**) as well as polystyrene resins (aminomethyl resin (**16**) and chloromethyl resin) (**8**). The linker remained entirely intact throughout the assembly of amino acids by Fmoc chemistry. Upon oxidation with sodium metaperiodate, the linker was labile to heat and thus cleavage of the peptide occurred from the resin.

In conclusion, a novel thermally labile linker has been synthesised, that is of great use because the peptide attached to the linker can be released from tentagel in water for biological testing.

Preface

The research described in this thesis was carried out under the supervision of Dr. Mark Bradley at the University of Southampton between October 1994 and September 1995. No part of thesis has previously been submitted for a degree at this or any other University except where a specific acknowledgement has been made.

For my mum, dad and Praveen

Acknowledgements

I would like to thank my supervisor Dr. Mark Bradley for his support, guidance and enthusiasm throughout the project. I would also like to thank Dr. Richard Luke from Zeneca Pharmaceuticals for the CASE sponsorship.

I would also like to thank the following people for their help with analytical techniques:

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Finally I would like to thank all other members of the Wolfson Laboratory.

Abbreviations

AcOH	Acetic acid
Ala	Alanine
Ar	Aryl
BME	β-mercaptoethanol
^{t}Boc	tertiary Butyloxycarbonyl
^{t}Bu	tertiary Butyl
Bzl	Benzyl
$CDCl_3$	Chloroform-*d*
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEPT	Distortionless Enhancement by Polarisation Transfer
DIC	*N,N'*-Diisopropyl carbodiimide
DCC	*N,N'*-Dicyclohexylcarbodimide
DCU	Dicyclohexylurea
DIPEA	*N,N'*-Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N,N'*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DSB	4-(2,5-Dimethyl-4-methylsulfinylphenyl)-4-hydroxybutanoic acid
eqv	equivalents
EtOAc	Ethyl Acetate
EtOH	Ethanol
FAB MS	Fast Atom Bombardment Mass Spectrometry
Fmoc	9-Fluorenyl methyloxycarbonyl
Gly	Glycine
HAL	Hypersensitive Acid labile Linker
HMPB	4-Hydroxymethyl-3-methoxyphenoxy acetic acid
HOBt	1-Hydroxybenzotriazole
HPLC	High Pressure Liquid Chromatography
hr	hour
IR	Infra Red
	br, broad
	m, medium
	s, strong
	w, weak

Lys	Lysine
MBHA	*p*-Methylbenzyhydrylamide
Me	Methyl
mins	minutes
Mpt	Melting point
MS	Mass Spectrometry
Msob	4-Methylsulfinylbenzyl
Msz	4-Methylsulfinylbenzyloxycarbonyl
NMR	Nuclear Magnetic Resonance
	δ, chemical shift
	d, doublet
	dd, double doublet
	dt, double of triplets
	dq, double of quartets
	J, coupling constant (measured in Hertz)
	m, multiplet
	q, quartet
	s, singlet
	t, triplet
PAL	Peptide Amide Linker
PAM	Phenylacetamidomethyl
PEG	Polyethylene Glycol
Phe	Phenylalanine
ppm	parts per million
Rf	Retardation factor
Rink Acid	4-(2',4'-Dimethoxyphenyl-hydroxymethyl)-phenoxymethyl-polystyrene
Rink Amide	4-(2',4'-Dimethoxyphenyl-aminomethyl)-phenoxymethyl-polystyrene
SASRIN	Super Acid Sensitive Resin
SPPS	Solid Phase Peptide Synthesis
Ser	Serine
TFMSA	Trifluoromethanesulfonic acid
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Tlc	Thin layer chromatography
UV	Ultra Violet

Contents

Chapter 1
Introduction

1.1 Solid Phase Peptide Chemistry

Solid phase peptide chemistry or polymer supported synthesis, a development that has revolutionised peptide synthesis, was introduced by Bruce Merrifield in 1963[1]. The reactions used are the same as in orthodox synthesis but one of the reactants is attached to a polymer support.

The attraction of solid supports for peptide synthesis is that they provide the following[2]:

- *Facile separation* of all other reagents due to the resin's insolubility in the solvents used.

- *High yields* can be obtained, as excess reagents can be used to drive the reaction to completion, also side products (such as DCU) and other unwanted reaction products can be washed away.

- *No purification* needed or necessary.

- *Automation,* since the method involves the repetitive use of a small number of similar operations.

Thus, SPPS [3] provides many advantages over classical methods of synthesis in solution by shortening the time required for synthesis of the peptides, as impurities are washed away. This avoids using cumbersome-laborious techniques such as separation (columnn chromatography) and recrystallisation procedures.

The SPPS method can be described as follows [4]:

A *N*-α-derivatised amino acid is attached to an insoluble solid support (e.g Merrifield's support) through a linker. The amino acid can be attached via two methods, it may be attached directly to the linker already on the support, or first attached to the linker, with subsequent attachment of the amino acid-linker to the support.

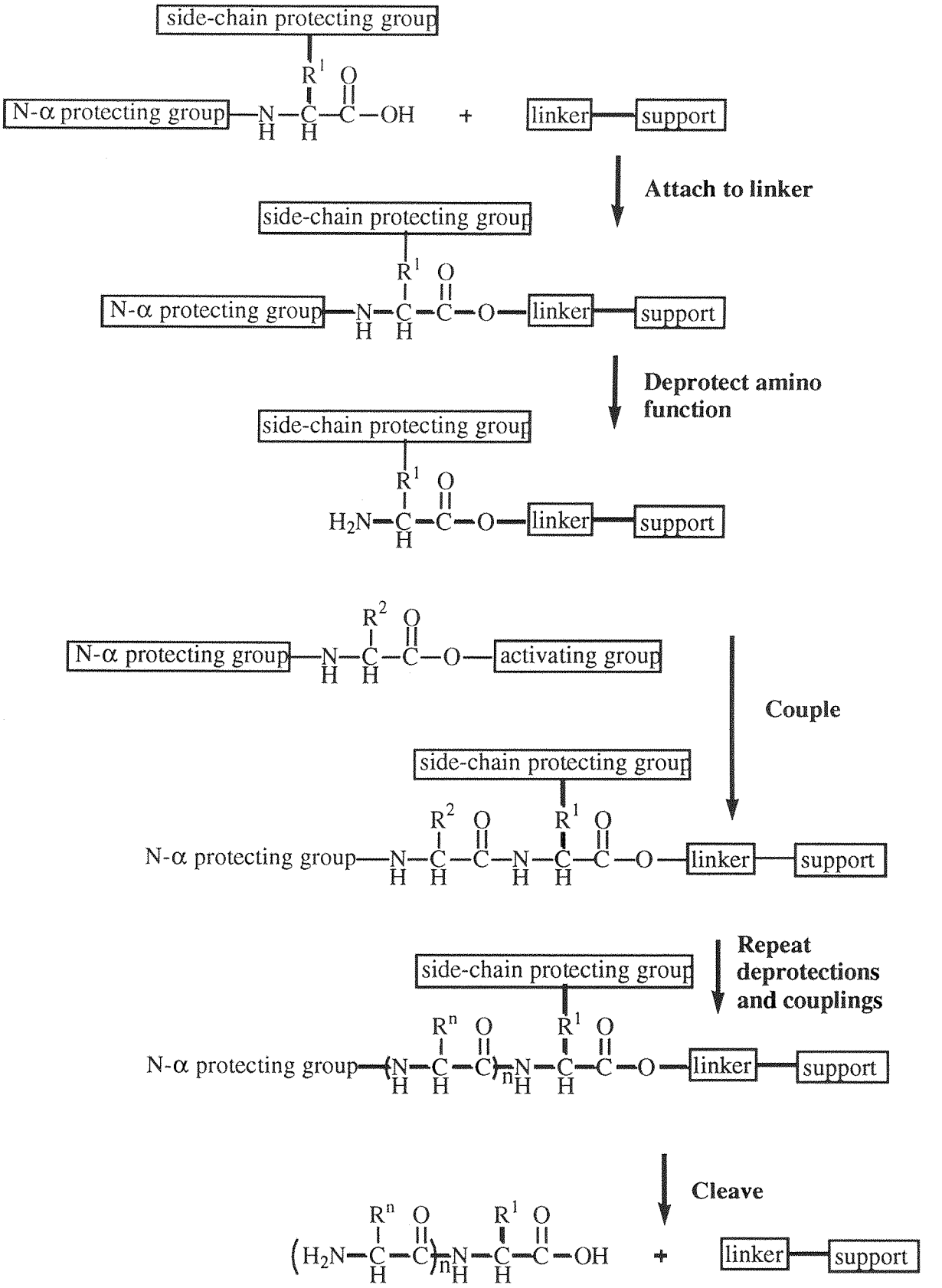
The amino protecting group is then removed and the amino acid-linker-support is washed thoroughly with solvent. The subsequent amino acid (which is already protected with an amino protecting group) is preactivated and then coupled to the amino acid-linker-support.

Preactivation of an amino acid normally occurs either in the form of a symmetrical anhydride or active ester; or directly (*in situ*) in the presence of an activator. After the coupling step the amino protected dipeptide is washed with solvent and the deprotection and coupling steps are repeated until the desired peptide is formed.

Finally the peptide-linker-support is cleaved to obtain the peptide as a free acid or amide depending on the linker used. The cleavage conditions may also remove the side chain protecting groups

The concept of SPPS is outlined schematically in **Scheme 1.1**.

The following section will concentrate on types of solid supports.



Scheme 1.1: Generalised approach to SPPS

1.2 Solid Supports

The first requirement of a solid support is that it has to be insoluble in all solvents used and it must have a stable physical form which allows filtration. It also has to contain a functional group to which the first protected amino acid can be linked by a covalent bond.

1.2.1 Merrifield Resin

This is the original resin used by Merrifield [1] and is most widely used due to its very low reactivity, its low cost and its excellent swelling properties in a range of solvents (except water and ethanol).

This polystyrene resin is non-polar as it consists predominantly of aromatic groups, in which some of the benzene rings are substituted by chloromethyl groups (**Fig 1.1**)

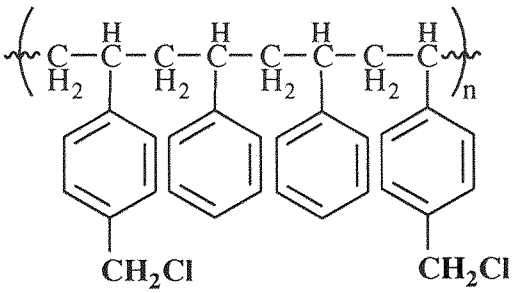


Fig 1.1: Merrifield resin (chloromethylated polystyrene)

The non-polarity of the resin, gives the resin its characteristic of being able to swell tremendously in non-polar solvents e.g dichloromethane, although poorly in polar solvents such as methanol. Solvation [5] plays an important part in SPPS (**fig 1.2**).

The dry beads of Merrifield's resin have a diameter of 10 to 400μm but can swell upto sixfold in volume with non-polar solvents.

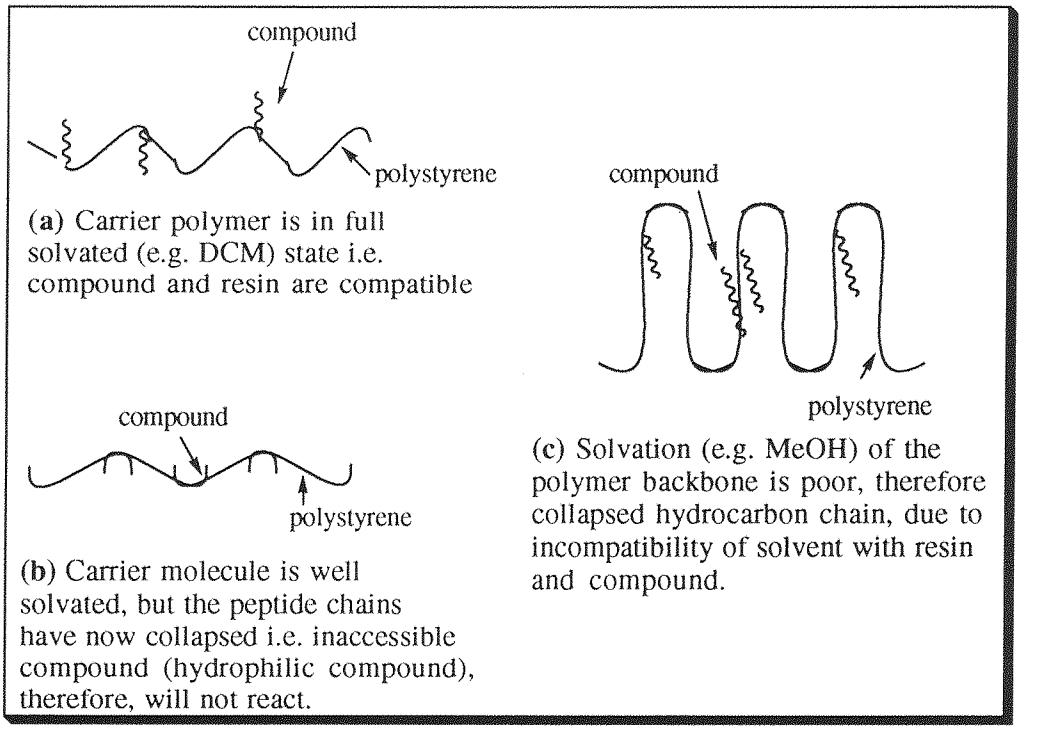


Fig 1.2: Solvation states of the peptide-polymer

Merrifield's resin has a porous gel-like structure which permits the penetration of reagents, particularly in the presence of swelling solvents. The resin is also completely hydrophobic in nature, whereas the growing peptide is much more hydrophilic. This contrast can induce a chain-folding effect in which the peptide satisfies its own hydrogen bonding demands rather than being solvated.

Polymerisation of styrene with the difunctional compound, divinylbenzene, leads to the resin containing crosslinked beads. The vinyl groups participate in the growth of separate chains and each chain can grow independently in different directions with the resulting polymer being three dimensional.

This crosslinking has a large effect on physical properties as it restricts the relative mobility of the polymer chains. Thus, Merrifield's resin is made with 1% divinylbenzene which provides the resin with its mechanical strength and insolubility.

Less soft divinylbenzene can also be used for crosslinking, Birr used as little as 0.5% divinylbenzene-styrene copolymers in a special reactor [6]. It is noteworthy to mention that beyond 1% divinylbenzene crosslinking inhibits bead swelling.

It is also possible to achieve high substitutions (i.e the degree of functionalisation of the resin) of up to 1.4-5mmol/g on Merrifield's resin, but steric congestion begins to be a problem (i.e. increased intermolecular aggregation) if the substitution is increased greater than this.

A modification of the Merrifield resin leads to the aminomethyl resin (**fig 1.3**). Aminomethyl resins are very important in solid phase peptide chemistry because they allow the introduction of a variety of linkers with different properties.

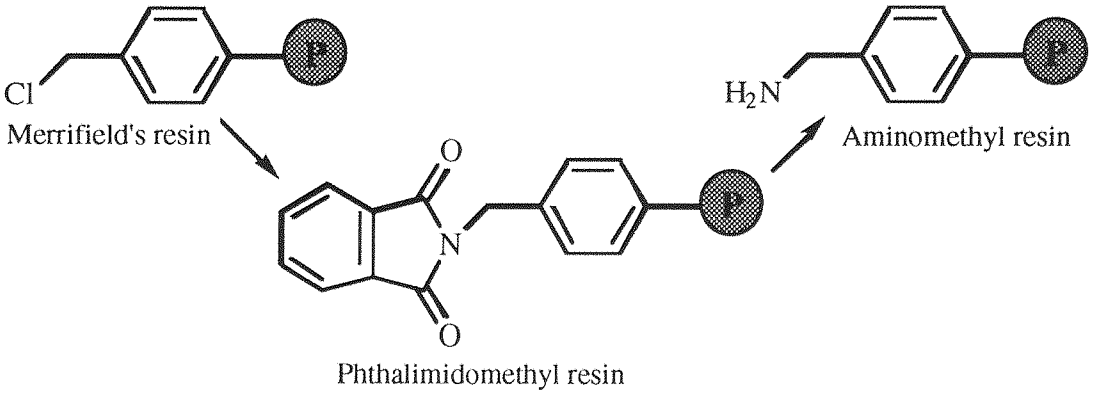


Fig 1.3: Preparation of Aminomethyl resin

Aminomethyl copoly-(styrene-1%-divinylbenzene) resins of high purity and in a variety of substitution levels have been synthesised [7], with up to 96% substitution of available benzene rings. In order to prepare such high loading resins, the synthesis of aminomethyl resins was improved by studying different catalysts for the preparation of the phthalimidomethyl-resin. The use of ferric chloride as catalyst, for the functionalisation of the polystyrene resin proved to be the best catalyst; as it provides high capacity and high purity functionalised resins that have good swelling and microscopic appearances.

1.2.2 Polydimethylacrylamide Resin

An additional polymer support explored [6,8] is based on the concept that the insoluble support and peptide chain should be of similar polarities, hence, the synthesis of hydrophilic peptides would be easier if a hydrophilic resin was used rather than a hydrophobic resin such as Merrifield's resin.

The polydimethylacrylamide resin is freely permeated[9] and is swollen by a wide range of polar organic solvents e.g 1g of resin will swell to 20ml in either dimethylformamide, dimethylacetamide or water.

These properties of the polydimethylacrylamide resin, make it more suitable for solid phase synthesis of polar substances, particularly of peptides and oligonucleotides, and also for solid phase peptide and protein sequencing.

1.2.3 Kieselguhr Resin

Another type of resin used is Kieselguhr [10], which is an inorganic polymer that is full of pores. The polymerisation of soft dimethylacrylamide gel [11] takes place inside the porous matrix.

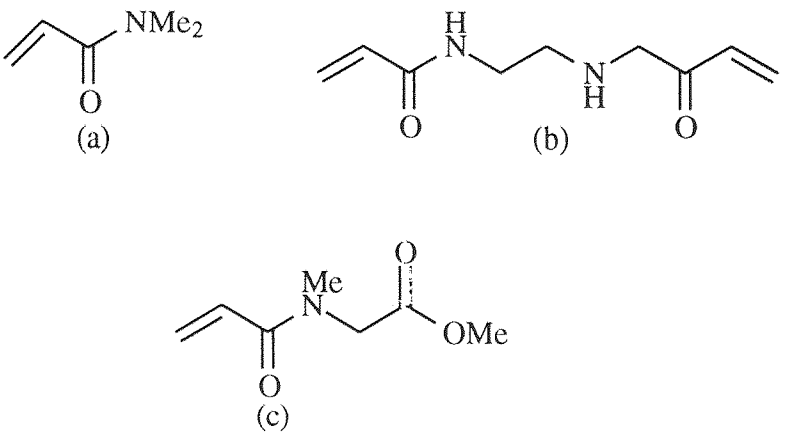


Fig 1.4: Polymerisation mixture inside pores of Kieselguhr

The pores of the support are very large when swollen (several thousand Ängstroms in diameter). In fact, the porosity of the support is that from the organic gel i.e. copolymerised from dimethylacrylamide (**fig 1.4a**), ethylene bisacrylamide (**fig 1.4b**), and acryloysarcosine methyl ester (**fig 1.4c**); but the mechanical properties are from the inorganic support (i.e. size, rigidity, strength).

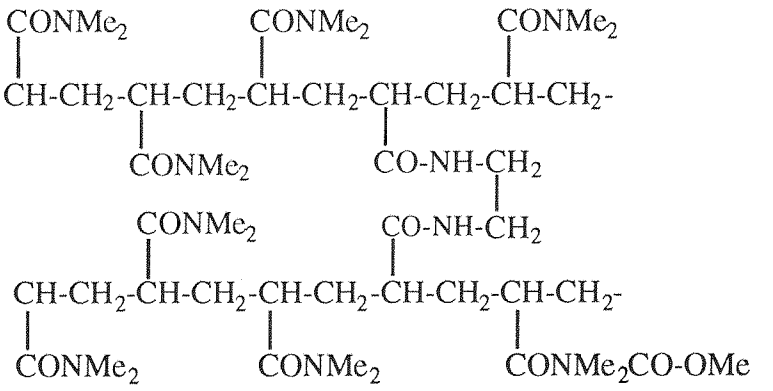


Fig 1.5: Co-polymerised product from (a), (b), & (c)

The main advantage of using such a resin is that it is solid, therefore easier to handle. However, there are a few disadvantages [12,14] with this support which could inhibit its wider use, especially in large scale synthesis. Firstly, the support has low levels of substitution, approximately 0.1-0.2 $mmol^{-1}g^{-1}$. Secondly, the resin should be handled with care due to its brittleness, hence, the physical stability is poor which could lead to the escape of the gel from the pores during solvation.

The biggest problem with the resin, is of the blocking of frits when filtering (milligen synthesiser). This resin is also very expensive.

1.2.4 Polyhipe Resin

Polyhipe is a polydimethylacrylamide supported in (and chemically bonded to) macroporous polystyrene. Thus, like the Kieselguhr support, it is very porous. The Polyhipe resin actually overcomes the problems outlined for the Kieselguhr resin i.e. low levels of substitution and poor physical stability.

In contrast, the Polyhipe resin provides excellent flow and physical characteristics[12]. The Polyhipe resin is polymerised via vinyl condensation polymers (polystyrene co-divinylbenzenes) with a 90% pore volume within the structure.

The above is then functionalised to give amino groups on the scaffold (primary) polymer which are then reacted with ethene-oyl (acryloyl) chloride (this ensures chemical immobilization at the surface of each cell, the double bonds also take part in the following polymerisation) before the pores are treated with dimethylacryamide (during which the secondary gel matrix is formed). Polymerisation now gives a polymer chemically linked to the bead.

The resin now has the strength of polystyrene, the solvation ability of acrylamide and a covalent attachment of the acrylamide resin to the polystyrene. All of these factors contribute to a very stable and robust resin, which has good swelling properties and high substitution levels.

To summarise, the polyhipe resin has clear advantages over existing supports when used in continuous flow synthesis i.e excellent flow properties, reagent/solvent accessibility under low pressure. There is no loss of gel (unlike Kieselguhr resin), due to the chemical binding of the secondary matrix, even under exceptional solvent flow conditions.

1.2.5 Tentagel Resin (Rapp resin)

This resin was introduced in 1990, but is probably the most versatile and reliable resin available. The tentacle polymers [13] , also called PS-PEG copolymers, are useful supports in solid phase syntheses of peptides and oligonucleotides. The latter, provides one of the main uses for such supports i.e the synthesis of oligonucleotides, as polystyrene beads do not permit solvation by aqueous solvent co-mixtures.

The tentagel resin is based on a hydroxymethyl polystyrene (**fig 1.6**) but to this is grafted [14] polyethylene glycol (PEG, average size 3000Da).

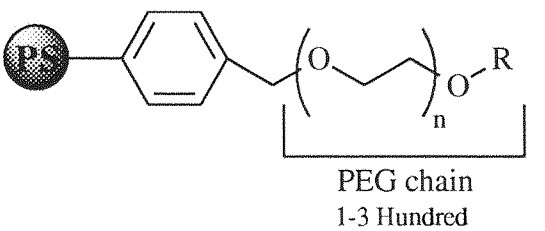


Fig 1.6: Graft copolymer from crosslinked PS and linear PEG

The free hydroxy termini allows functionalisation to the resin and incorporation of an insoluble support provides homogeneity due to the freedom of the PEG termini. 70% of the resin consists of PEG, thus the beads are mainly characteristic of PEG, although the resin does retain the physical attributes (structural stability) of the polystyrene bead. The beads come in various sizes and have good levels of substitution.

PEG provides a degree of polarity to the resin and thus the resin is solvated in virtually all solvents including water, with the exception of ether. The solvation is thus dominated by the PEG chain which leads to a degree of mobility of the peptides bound to them. Solubility in water provides a great advantage as screening will be easier, as the peptide can be released from tentagel in water for biological testing. For example, tentagel resin is essential when investigating the binding of a tethered compound to a target enzyme; as the protein necessitates aqueous conditions. Tentagel resin can swell in the buffer containing the enzyme, making it an excellent choice for solid phase screening.

Another property of PS-PEG copolymer that is seldomly seen in other polymers is that due to the solvation; the PS-PEG beads are stable to pressures up to 200 bars (the condition under which relatively high flow rates can be achieved), which encourages their use in continuous flow peptide synthesis. This is advantageous as it shortens the time for the coupling cycle.

Although tentagel can be seen as a breakthrough in resins, it is expensive.

1.3 Linkers [4,15]

The compound (the linker) that joins the peptide to the resin bead is an essential part of solid phase synthesis and combinatorial libraries, as the "linker' will determine both the method used for cleavage from the solid support and the terminal functionality of the peptide.

Thus, the linker is placed between the growing peptide and supporting resin (**fig 1.7**).

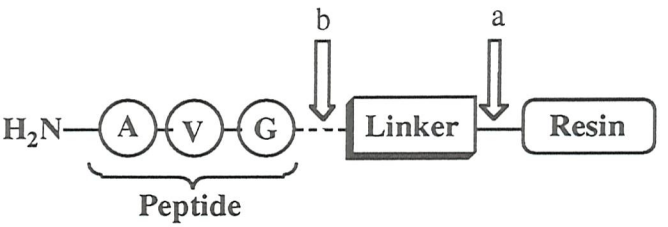


Fig 1.7: The linker is covalently attached to the resin and molecule of interest, however in order for synthesis to be undertaken the bond[a] between the resin and linker must be inert. The bond[b] between the linker and the peptide can be broken by selective cleavage.

Therefore, the linker has certain properties:

- It should be able to form derivatives of amino acid carboxy groups that can be easily attached to functional groups of the polymer support.

- It must be selectively cleaved to release the intact peptide.

- It must be resistant towards the reagents and solvents employed during chain elongation.

Bearing the above in mind, many linkers have been developed for anchoring and selectively removing large or small peptides from the solid polymer support with, and without the protecting groups remaining intact.

The linkers designed have been tailored to suit either the Fmoc (base lability) strategy or Boc (acid lability) strategy. However, other useful linkers have been synthesised in which peptides acids or amides can be cleaved via different methods i.e light (photolabile linkers), palladium , fluoride ions and finally a safety catch mechanism can be used to cleave a peptide. All the above will be discussed briefly in the following section.

1.3.1 Acid Labile Linkers

Peptides synthesised on specific benzyl type resins are usually removed from the solid support by acidolysis with a strong acid, commonly liquid hydrogen fluoride. However, this method is unsuitable for the preparation of protected peptide segments, as in both the Fmoc/^{t}Bu strategies and Boc/Bzl strategies, practically all of the protecting groups used are removed by HF treatment. The solid phase synthesis of

protected peptide segments therefore, must be undertaken, where the conditions for the acidolytic cleavage are as mild as possible.

1.3.1.1 Wang resin linker

The 'Wang [16,17] linker (4-hydroxymethylphenoxy linker) (**fig 1.8**) is the most widely utilised linker in Fmoc chemistry. It is used if the formation of a carboxylic acid is required. The alkoxybenzyl alcohol resin was developed by Wang, principally for the synthesis of free peptides. It is now finding wider application in stepwise solid phase synthesis, where mild acidic conditions for the final cleavage of the peptide from the solid support are necessary.

Peptides are attached to the benzyl alcohol linker through formation of an ester. The linker is prepared by reacting Merrifield resin with 4-hydroxybenzyl alcohol in the presence of $NaOCH_3$.

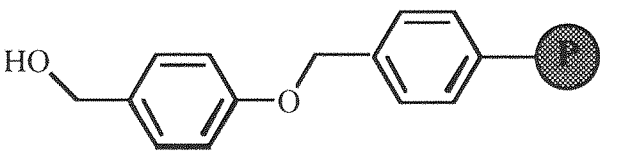


Fig 1.8: Wang resin linker

1.3.1.2 Rink acid linker

This linker [18](**fig 1.9**) is highly acid labile (i.e 0.2% TFA in DCM will cleave the peptide from resin), unfortunately it is so sensitive to acids, that premature cleavage from the resin occurs during coupling when using coupling catalysts such as HOBt. To overcome this drawback, the coupling solution is buffered with DIEA.

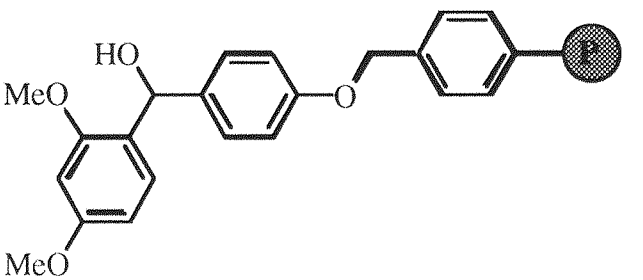


Fig 1.9: Rink acid linker

1.3.1.3 Rink amide linker

The Rink linker is useful for the formation of primary amides.

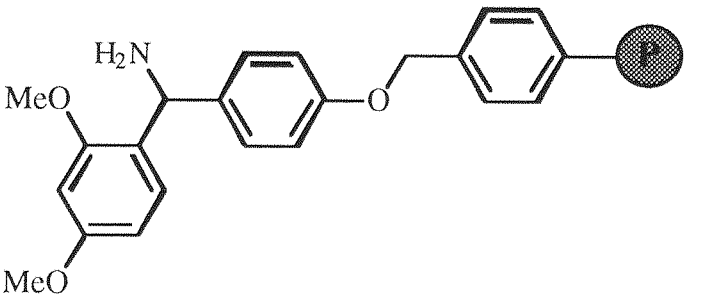


Fig 1.10: Rink amide linker

1.3.1.4 SASRIN Linker

The acronym SASRIN is short for super acid sensitive resin. This linker (**fig 1.11**) was described by Mergler [19,20]. It is incredibly acid labile as attached peptides can be removed with as little as 0.5-1% TFA in DCM. This is advantageous as this will allow the synthesis of fully *tert*-Butyl protected peptides. Due to the mild acidolytic conditions, large fully protected peptide fragments can also be cleaved in excellent yields. The Boc protecting group may also be used for N^{α} protection of amino acids, as the cleavage conditions are adequately mild enough to allow the Boc group to be maintained.

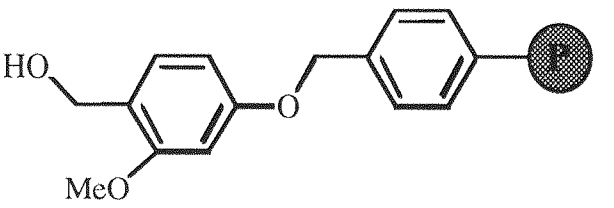


Fig 1.11: SASRIN linker

1.3.1.5 HMPB linker

The HMPB linker, 4-(4-hydroxy-methyl-3-methoxyphenoxybutyryl) (**fig 1.12**) was developed by Riniker and Sheppard. It is similar in structure to the SASRIN linker. The two extra CH_2 groups lessen the electron withdrawing effect of the carboxyl group at the ether oxygen, therefore, allowing it to operate as a more powerful electron donor; this then increases the linkers lability to acid i.e milder cleavage conditions e.g~1% TFA in DCM.

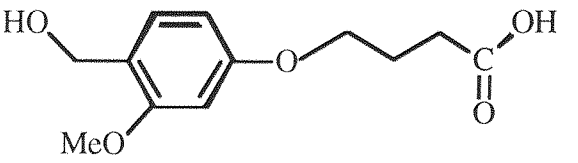


Fig 1.12 HMPB linker

1.3.1.6 PAM linker

The phenylacetamidomethyl (PAM) linker (achieved by attachment [21]of the carboxy end of the linker to the aminomethyl resin) is more resistant to acid, due to the increased stability of the benzyl ester conferred by the electron-withdawing *para*-carboxamidomethyl substituent.

The linker [22] can be cleaved by strong acids such as TFMSA, HF, or tetra-*n*-butylammonium fluoride (to give the free acid) or by sodium hydroxide (base cleavage) (**fig 1.13**).

The advantage of using the PAM linker is due to it's greater acid stability (compared to the hyroxymethyl-poly(styrene-*co*-divinylbenzene) resin) which will result in higher yields of large peptides by SPPS and not losses of peptide chains as with the latter resin.

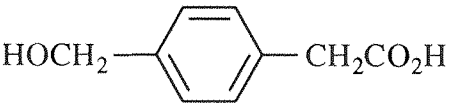


Fig 1.13 PAM linker

1.3.1.7 MBHA Linker

This linker (**fig 1.14**) is similar to the PAM linker in the sense that it requires a strong acid such as HF, for cleavage of the peptide. However, this linker will afford a peptide amide upon cleavage. The electron donating methyl group [23] in the *para* position aids the cleavage with acid.

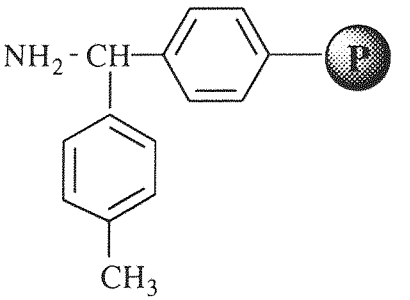


Fig 1.14: MBHA acid labile linker

1.3.1.8 PAL linker

The user friendly acronym PAL is short for peptide amide linker. The PAL linker [24] is useful for the synthesis of peptide *C*-terminal amides. A number of naturally occurring peptides, including oxytocin, secretin, apamin, calcitonin, thymosin, and several releasing hormones in the brain, are isolated as *C*-terminal peptide amides. This can be carried out under strong acidolysis (**fig 1.15**).

The conditions for cleavage from the linker for small model peptides are TFA/DCM/dimethyl sulfide in a ratio of 14:5:1 at 25°C for two hours; this will also remove *tert*-butyl side chain protecting groups.

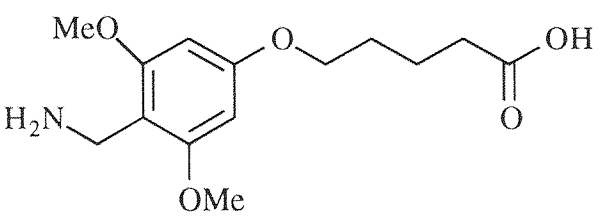


Fig 1.15: PAL linker

1.3.1.9 HAL linker

The HAL linker (**fig 1.16**) otherwise known as a hypersensitive acid-labile linker [25] has also been prepared and applied to solid-phase peptide synthesis using the Fmoc group for N^{α}-protection and *tert*-butyl ethers, esters and urethanes for side-chain protection.

It is structurally similar to the PAL linker, although upon cleavage it produces free acids. The linker can also be used with any amino-functionalised support and it can be cleaved with very dilute solutions of TFA in dichloromethane or even 10% acetic acid in dichloromethane, hence leaving *tert*-butyl-based side chain protecting groups intact.

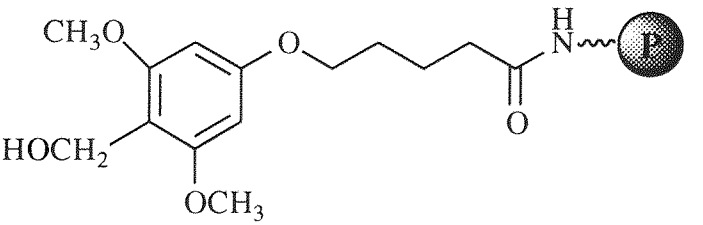


Fig 1.16: HAL linker

1.3.1.10 Silicon based linker

An alternative approach for linkage (opposed to linkage through a functionality already present in the desired target molecule) is linkage through a functional group that can be removed efficiently when required.

Plunkett and Ellman tested a silicon [26] based linkage (**fig 1.17**) strategy in the synthesis of solid phase benzodiazepine derivatives [27]. In this case, the silicon-aryl bond will be cleaved using acidic conditions such as anhydrous HF. The amino acid side chain protecting groups will be removed by treatment with TFA/dimethyl sulfide/water. This linker is useful for the synthesis of functionalised heterocyclic compounds.

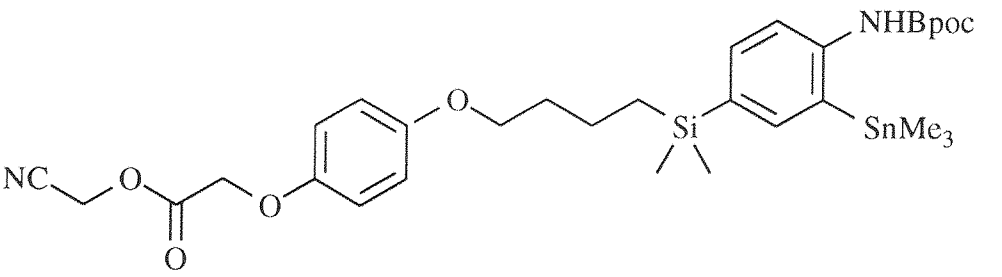


Fig 1.17: Silicon based linker

1.3.1.11 Thioester Resin Linkers

This linker [28] is compatible with Boc chemistry and generates peptide *C*-terminal thioacids by acidolytic cleavage with HF. The linker (**fig 1.18**) is useful for chemically ligating functional proteins as it acts as a reactive functionality on one of the peptide fractions to be reacted. Hence, it is of much use for large peptides (greater than 70 amino acids) as it overcomes the length limitations of SPPS.

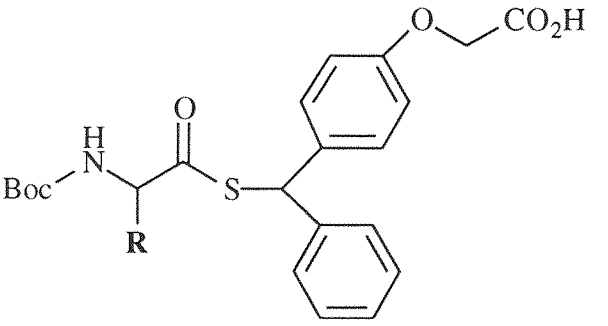


Fig 1.18: Thioester linker

1.3.1.12 Linkers based upon the dibenzoheptadienyl ring system

Efficient, versatile linkers[29] based upon the dibenzoheptadienyl ring system, have been developed for the synthesis of *C*-terminal primary (**fig 1.19**) and secondary amides and hydrazides. The linkers are compatible with N^{α}-Fmoc/*tert* Butyl strategy.

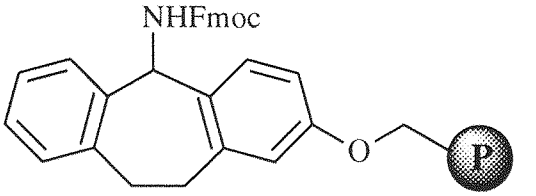


Fig 1.19

The synthesis of many peptides have synthesised with the above, such as Bombesin, and Big Gastrin.

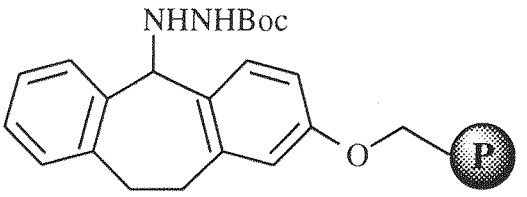


Fig 1.20

Examples of *C*-terminal hydrazides synthesised with **fig 1.20** are H-Leu-Ile-Phe-Ala-Gly-$NHNH_2$, and H-Leu-His-Leu-Val-Leu-Arg-Gly-Gly-$NHNH_2$.

Cleavage conditions of greater than 50% TFA in dichloromethane are required to give the *C*-terminal primary amides and hydrazides from both linkers.

1.3.2 Fluoride cleavage linkers-via cleavage of silicon substituted esters

Silicon based linkages can be cleaved by treatment with fluoride ions. Mullen and Barany [30] reported the use of the linker (**fig 1.21**). It can be cleaved cleanly under relatively mild conditions based on the lability to fluoridolysis of a silicon-oxygen bond incorporated into the linker. Thus, peptides were cleaved with tetrabutylammomium fluoride in DMF (in the presence of appropriate scavengers when required) to give the peptide acid.

The linker can be used in conjunction with Fmoc chemistry; but is not suitable for Boc chemistry due to the acidolysis required to remove the Boc group.

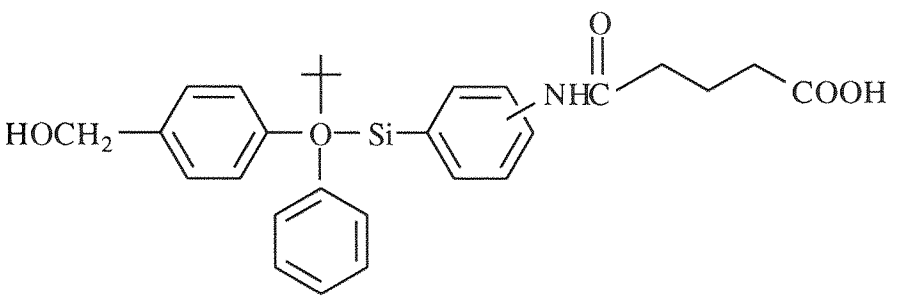


Fig 1.21: Fluoride labile linker

Ramage investigated the use of a silicon-containing linker (**fig 1.22**) for the synthesis of protected peptides.(including the synthesis of a protecting peptide segment corresponding to the 1-35 sequence of ubiquitin).

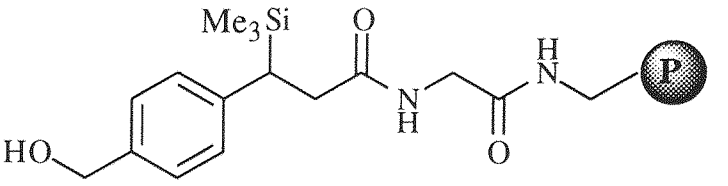


Fig 1.22: Ramage's silicon based linker

The Ramage linker-resin is compatible with the Fmoc/*tert*Butyl strategy, but is not compatible with the Boc/benzyl strategy.

1.3.3 Palladium cleavable linkers-via the catalysis of allyl esters

An allylic linker [31] has been used in the synthesis of protected peptide fragments on the aminomethyl polystyrene resin. The allyl or allyloxycarbonyl groups can be removed under conditions that are mild and specific. Detachment of the peptide takes place by a Pd-catalysed allyl transfer reaction using silylated amines.

Thus the advantages of using an allyl linker (**fig 1.23**) is that the cleavage conditions are compatible with Fmoc/*t*-Bu and Boc/bzl strategies; and because of the extremely mild cleavage conditions.

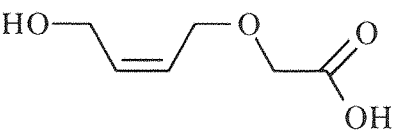


Fig 1.23: Palladium labile linker

1.3.4 Photolabile linkers

These should provide ideal linkages because of their compatibility with both Boc and Fmoc chemistry. Photolysis also offers a milder method of cleavage and provides a free carboxylic acid at the end *C*-terminus.

Rich and Gurwara [32,33] reported the use the photolabile linker illustrated in **fig 1.24**, where irradiation of the peptide resin in methanol at 350 nm gave cleavage of the peptide.

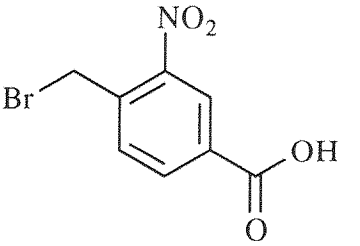


Fig 1.24: Photolabile linker

The above linker actually suffers several limitations i.e it is difficult to obtain high yields of methionine containing peptides, without contamination with methionine sulfoxide. The kinetics for the cleavage are also rather slow, ranging from 12 to 24 h.

It is noteworthy to mention that the above linker is not fully compatible with Fmoc/*t*-Bu chemistry as the peptide-linker bond is not very stable to treatment with piperidine. Thus, the synthesis of long peptides should be avoided when using the previously mentioned protecting strategy.

A new photolinker based on α-methylnitroveratrylamine [34] (**fig 1.25**) was designed for the release of peptide and small molecule amides from a solid support to aqueous environments. This linker manages to overcome all the difficulties of the previous linker and incorporates the Fmoc protecting group. Photolytic cleavage in pH 7.4 phosphate-buffered saline (PBS) containing 5% DMSO was performed by irradiating for 3h with 365 nm UV light.

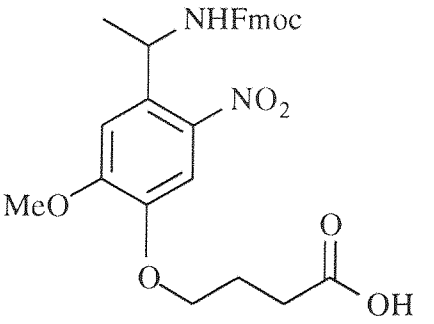


Fig 1.25: Photolabile linker

1.3.5 Base labile linkers

The utility of the linker [35] shown in **fig 1.26** was demonstrated by synthesis of leu-enkephalin and its [D-Ala]^2 analogue. The peptide is cleaved by a mixture of dioxane/methanol/0.1M NaOH (30:9:1).

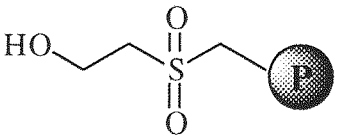


Fig 1.26: Base labile linker

Another linker synthesised is the CASET(2) [2-(4-carboxyphenylsulfonyl)-ethanol] [36]; it is very useful for the synthesis of protected oligopeptides on resin supports. Peptides can be cleaved rapidly from this linker (**fig 1.27**) by a base catalyzed β–elimination using 0.1 M NaOH as for **fig 1.26**. The linker is compatible with the Boc/Bzl strategy.

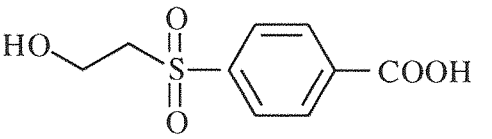


Fig 1.27: Base catalyzed β–elimination linker

The design of another base labile linker [37] belongs to the 9-hydromethylfluorene type of protecting groups, that can be cleaved by base through a β–elimination reaction. The linker (**fig 1.28**) is compatible with both the Boc/bzl and Boc/Fm based side chain protecting groups and is stable to DMF and DIEA; thus peptides on the growing chain are not prematurely lost. Cleavage conditions to give peptide acids are treatment with *N* -methylmorpholine in DMF.

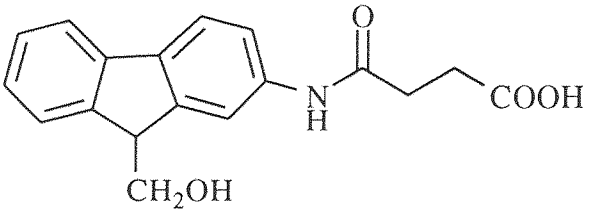


Fig 1.28: Base labile fluorene derived linker

The linkers discussed in the next section are of a special type , based upon the so called "safety catch principle[38].

1.3.6 Safety Catch Linkers

The safety catch principle in its narrower sense involves a deprotection process[39] based on conversion of a relatively stable group (this group must be entirely inert throughout all normal operations of the synthesis) into a labile and cleavable one e.g. a linker that cleaves only after oxidation or a base catalysed elimination.

The safety catch is **orthogonal** to all other protecting functionality's (**fig 1.29**).

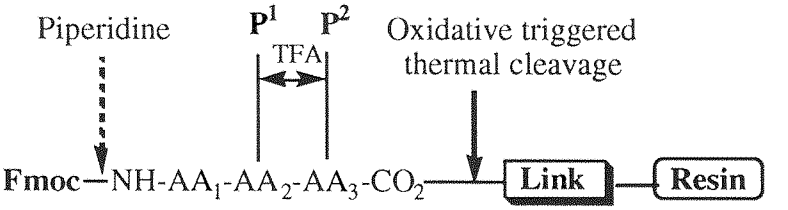


Fig 1.29.: Demonstrates a protection [40] scheme based on a combination of a base labile Fmoc group and base stable but semi-permanent protecting groups- acid labile P^1, P^2 and a thermally labile anchoring link.

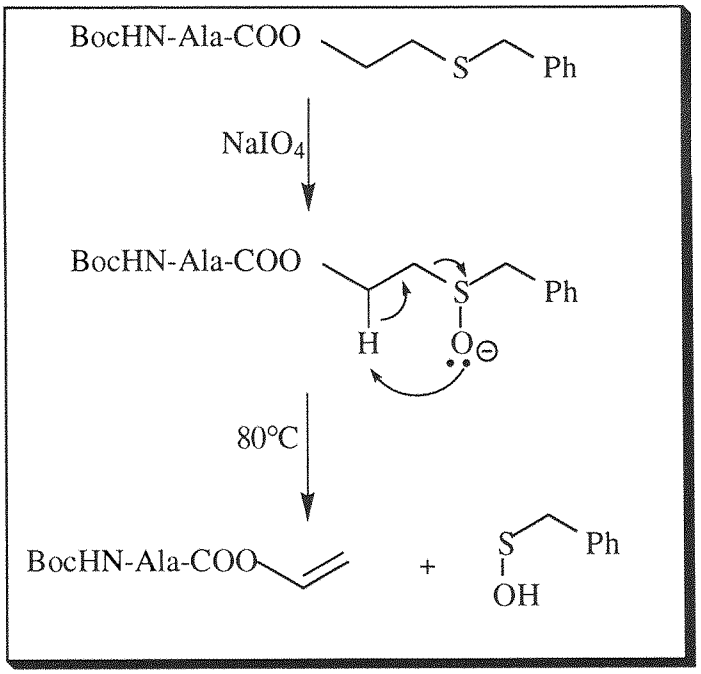
1.3.6.1 Sulfoxide-based safety catch linker

The first proposal for a safety catch linker is based on a sulfoxide rearrangement. Literature precedent [41] suggests that the reaction should proceed at a relatively low temperature with the compounds being liberated as their easily hydrolysed vinyl esters.

Thioethers are robust functionality's, suitable for a range of chemistries and readily accessible synthetically. Additionally the chemistry of the linker has no real requirement for the end group being a carboxylate and could therefore be utilised to give rise to a variety of different termini.

Oxidation of the thioether to the sulfoxide is a facile transformation for which many reagents have been employed [42]. These include hydrogen peroxide, oxone, sodium metaperiodate, nitric acid, chromic acid and *m*-chloroperbenzoic acid. The oxidation will provide the desired safety catch mechanism.

The pericyclic rearrangement is heat induced and should release the peptide. Sulfoxides undergo rearrangement on pyrolysis at about 80°C [43] (**Scheme 1.2**). The mechanism will follow a *syn* elimination. Temperatures for the rearrangement of phenyl sulfoxides are normally around 50°C , whereas those for the methyl sulfoxides are normally around 110°C [39].



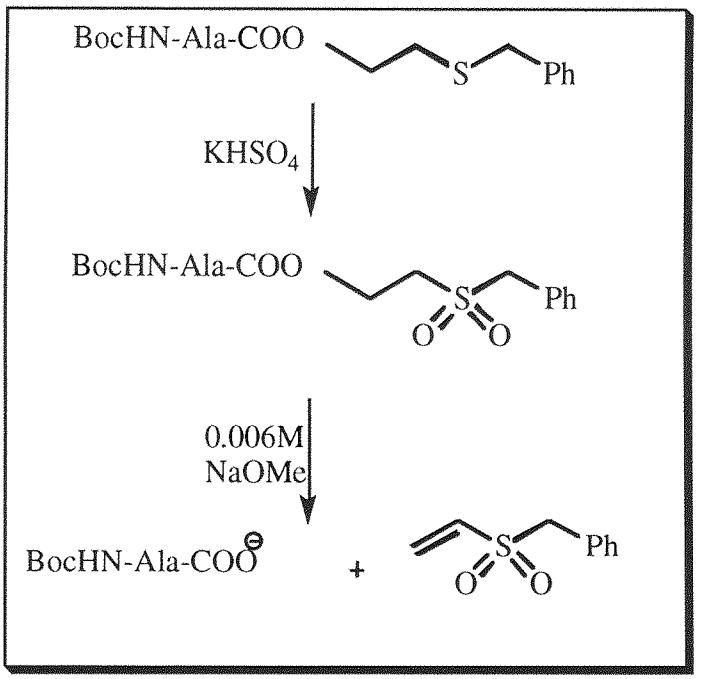
Scheme 1.2: Sulfoxide safety catch linker

The main intention of this study was to provide a method of cleavage from the resin that is under mild conditions; opposed to using noxious reagents and harsh biologically incompatible conditions. Thus, cleavage from the resin after chemical oxidation transforms the former stable thioether into a labile sulfoxide.

The chemistry can also be readily extended to sulfones which although not allowing thermal release would allow very mild base cleavage to take place.

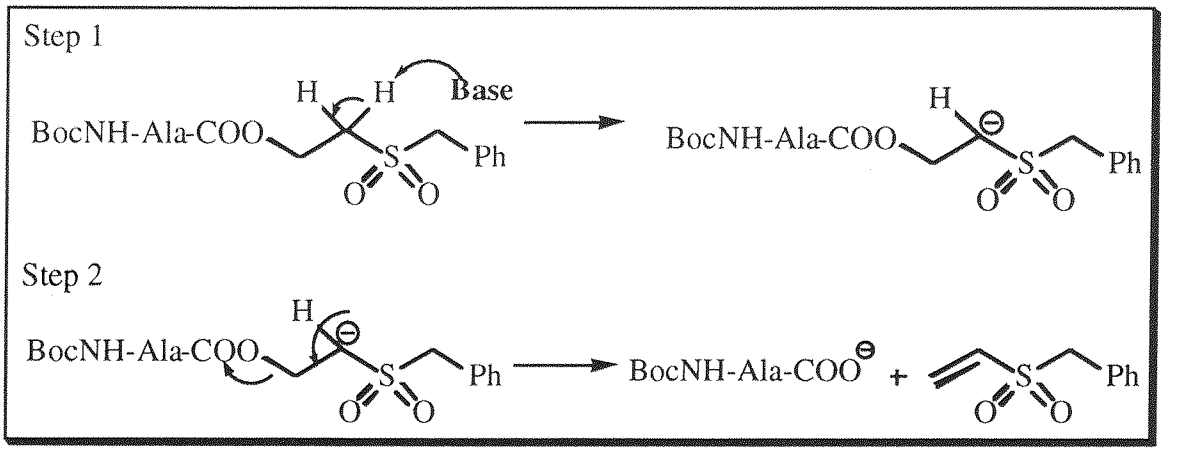
1.3.6.2 Sulfone-based safety catch linker

Most commonly, organic peracids have been used for the oxidation of sulfides to sulfones [44]. Although these peracids rapidly oxidise sulfides to sulfoxides at low temperatures (~-78°C), the higher temperatures often required for the sulfide to sulfone interconversion (0°C to room temperature) can cause oxidation of other functional groups. Hence, the use of the chemoselective oxidising agent potassium hydrogen persulfate (**Scheme 1.3**).



Scheme 1.3: Sulfone based safety catch linker

Sulfones with a β-hydrogen also undergo elimination on treatment with base [44]. The elimination of the sulfonyl group should proceed under relatively mild conditions due to the acidic β–hydrogen. The sulfone will undergo elimination *via* the *E1cB* mechanism and will stabilise the carboanionic character of the transition state (**Scheme 1.4**).



Scheme 1.4: *EIcB* mechanism for sulfone safety catch linker

1.3.6.3 Examples of other Safety Catch Linkers

Kenner [45] reported the use of the safety catch linker illustrated in **fig 1.30**. The peptide-resin bond is stable to basic hydrolysis conditions, but *N*-methylation with diazomethane leads to an *N*-methylated peptide-sulfonamide resin, from where protected peptides can be cleaved by mild basic hydrolysis or hydrazinolysis.

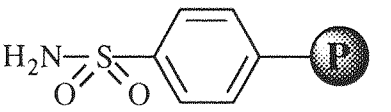


Fig 1.30: Kenner's safety catch linker

A new safety catch linker has also been used in solid phase peptide synthesis, for a reductive acidolysis final deprotection strategy[46].

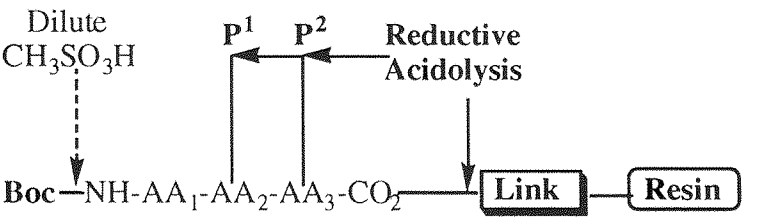
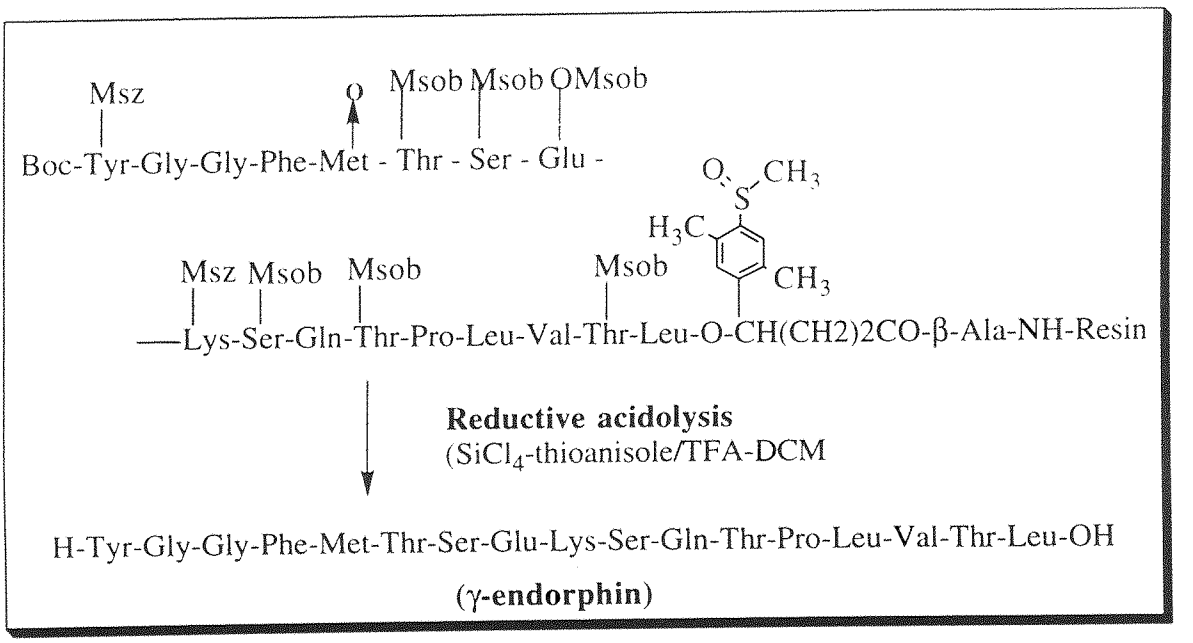


Fig 1.31

The reductive acidolysis takes place with $SiCl_4$, scavengers and TFA.

To illustrate the new strategy incorporating the safety catch linker, Kiso et *al* [46] synthesised γ-endorphin (H-Tyr-Gly-Gly-Phe-Met-Thr-Ser-Glu-Lys-Ser-Gln-Thr-Pro-Leu-Val-Thr-Leu-OH, **scheme 1.5**). Synthesis began from Boc-Leu-O-DSB-resin. The final protected peptide-resin was prepared using N^{α}-Boc -amino acids bearing reductive acidolytic-cleavable side chain protecting groups such as Tyr(Msz), Met(O), Thr(Msob), Ser(Msob), Glu(OMsob), and Lys(Msz). The protected γ-endorphin resin was deprotected and cleaved from the resin with $SiCl_4$ scavengers and TFA.



Scheme 1.5: Synthesis of γ-endorphin by a reductive acidolysis final deprotection strategy

Chapter 2
Results and Discussion

The aim of the project was to synthesise thermally labile linkers for combinatorial synthesis on the solid phase using a variety of resins. The long term goal was to cleave the peptides from the resin in water using heat.

2.1 Solution Phase Study

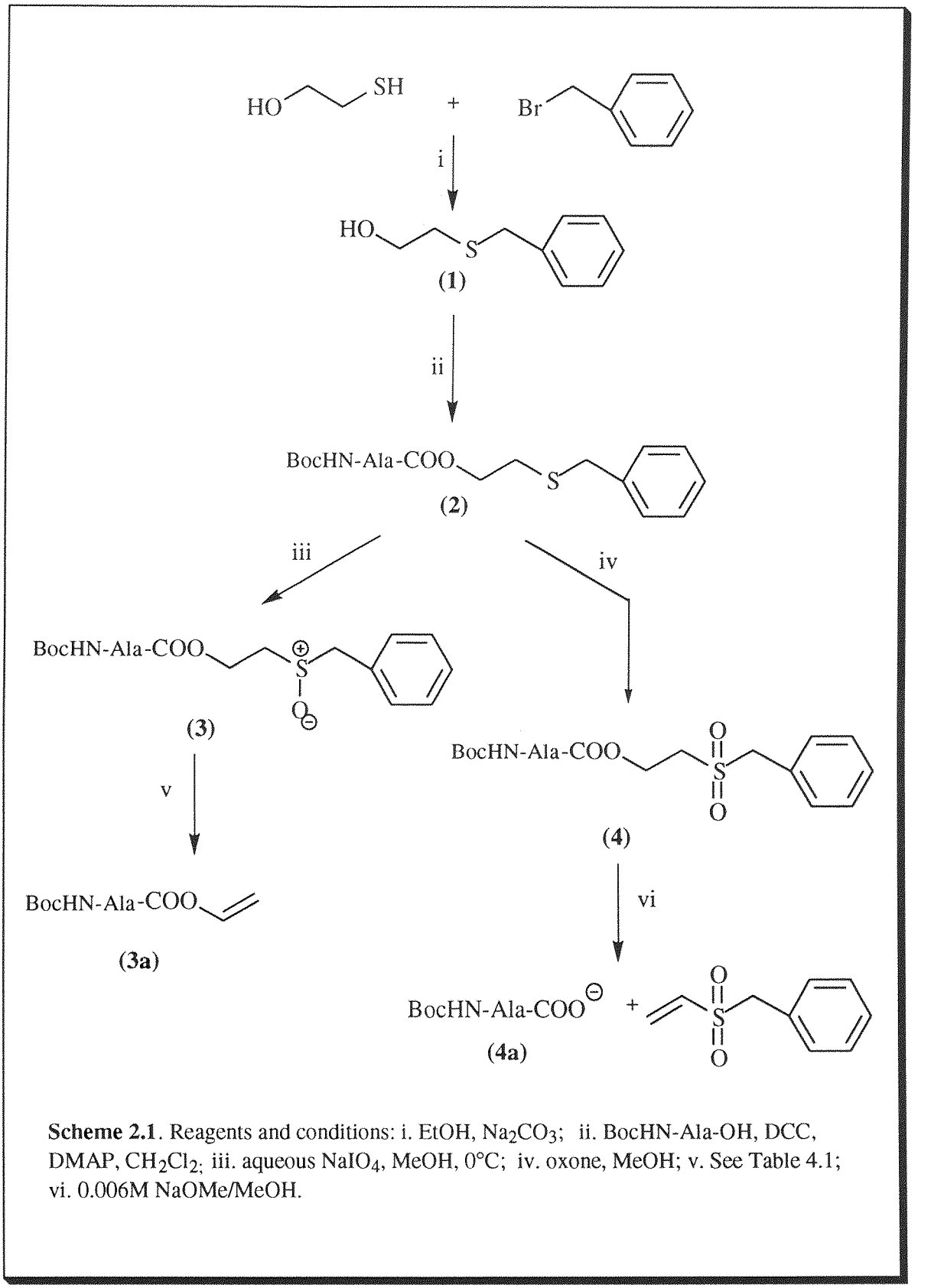
Investigation of the reaction by solution phase was conducted so that the course of the reaction could be followed by t.l.c, so that any intermediates and products detected could be analysed spectroscopically.

Synthesis in the solution phase began with the alkylation of β-mercaptoethanol with benzyl bromide, which proceeded in 88% yield. This was followed by the esterification of (**1**) with a Boc-protected amino acid using DCC/DMAP, this was achieved in an excellent yield. It was subsequently oxidised to the sulfoxide [52] (**3**) or the sulfone [53] (**4**) by either sodium metaperiodate or the highly chemoselective oxidising agent potassium hydrogen persulfate (commercially sold as oxone) respectively (see **scheme 2.1**).

Thermal cleavage studies were carried out on the sulfoxide (see **Section 2.2**) and base catalysed elimination studies (see **Section 2.3**) on the sulfone.

2.2 Temperature study (in solution phase)

Attempted rearrangement of Boc-Ala-(-1-hydroxy-2-sulfoxo)-S-benzyl ethyl ester (**3**) to form the vinyl ester (**3a**) was performed by heating in DMF or THF. No reaction occurred at 40°C, so the temperature was gradually raised in ~10°C, increments up to a maximum of 80°C. Temperatures higher than this are unfeasible due to the thermal instability of the Boc group. Examination by proton NMR spectrometry after 3 hours



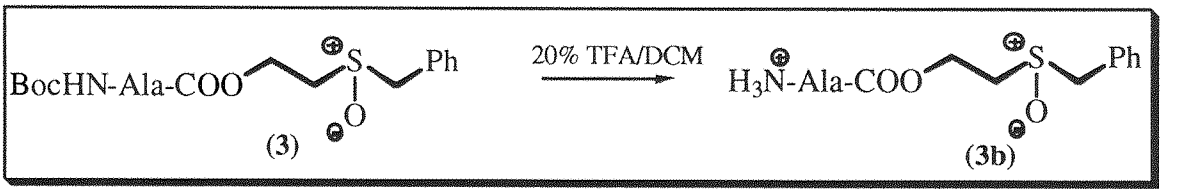
Scheme 2.1. Reagents and conditions: i. EtOH, Na_2CO_3; ii. BocHN-Ala-OH, DCC, DMAP, CH_2Cl_2; iii. aqueous $NaIO_4$, MeOH, 0°C; iv. oxone, MeOH; v. See Table 4.1; vi. 0.006M NaOMe/MeOH.

indicated complete recovery of starting material, therefore no reaction had taken place.

The above experiment was repeated with dioxane, however no change was observed to the starting sulfoxide. Thermolysis was then undertaken in THF, temperature was again increased in ~10°C increments. No change was seen to the sulfoxide at 30°C-60°C. After 72 hours at 65°C, the ^{1}H NMR spectrum indicated the complete loss of starting material as shown by the disappearance of the resonance's corresponding to the diastereoisomeric sulfoxide methylene group at 3.2 ppm. Tlc analysis showed the new product to have the same R_f value as the sulfone (**4, scheme 2.1**).

Due to oxidation of the sulfoxide (**3**) to the sulfone (**4**), the thermolysis was carried out under nitrogen atmosphere at reflux in THF. The proton NMR spectrum of the product showed a set of doublets at δ 5.4-5.7ppm, suggesting the presence of the required product (**3a**), however, the spectrum also indicated numerous impurities making further analysis of the spectrum difficult.

Boc-Ala-(-1-hydroxy-2-sulfoxo)-S-benzyl ethyl ester (**3**) was deprotected with 20% trifluoroacetic acid in dichloromethane to form the free peptide, (**3b, scheme 2.2**).



Scheme 2.2

Attempted rearrangement of the sulfoxide (**3b**) was then carried out at high temperatures by thermolysis in water (pH 7) at 100°C. The proton NMR of the product was complex, analysis indicated that some of the sulfoxide starting material was still present, but there was no sign of the required products.

2.3 Elimination Study (in solution phase)

Elimination of Boc-Ala-(-1-hydroxy-2-sulfonyl)-S-benzyl ethyl ester (**4**) to form Boc-alanine was performed with 0.006M sodium methoxide. Proton NMR showed the desired product (**4a**), thus showing the elimination to be successful.

2.4 Solid Phase Study

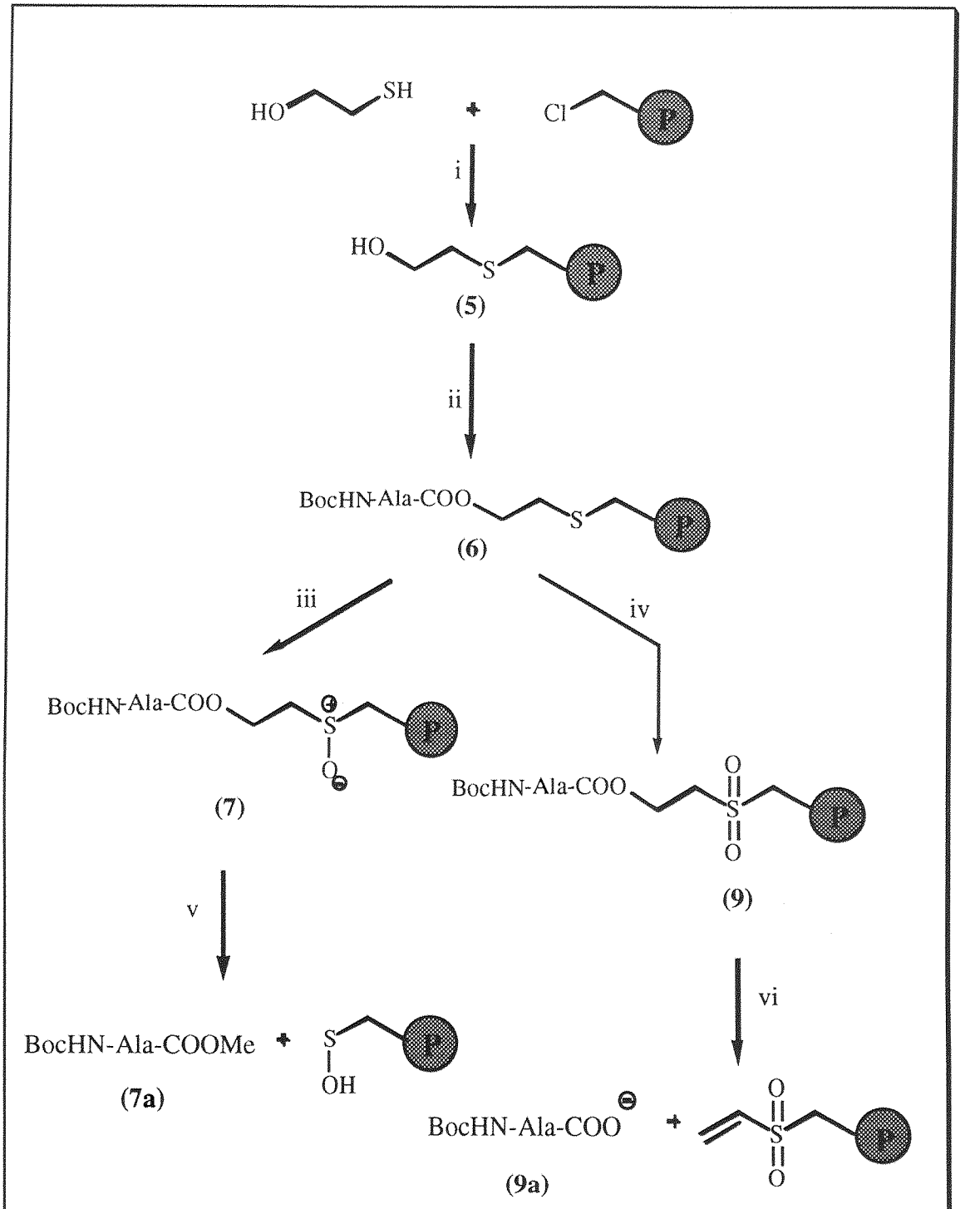
2.4.1 Coupling with Merrifield's resin

β–Mercaptoethanol was tethered to the chloromethylated polystyrene resin (substitution: 1.4 mmol/g) in preparation for solid phase peptide synthesis, using a catalytic amount of potassium iodide and sodium carbonate in DMF (see **scheme 2.3**).

This was followed by esterification with the first amino acid, Boc-Ala-OH, using DIC as the coupling reagent and DMAP as the catalyst. In order to achieve higher yields, the coupling reaction was repeated. The success of the reaction was estimated by calculation of weight increase (26%) and after deprotection, by the quantitative ninhydrin test [42](see **section 4.2**). The extent of coupling of alanine in this reaction was 29%.

The above analytical technique determined the concentration of free amino groups associated with the resin bound peptides by the reaction with ninhydrin. The observation of a blue colouration was a positive indication of the presence of free amino groups attached to the resin. UV detection at λ570nm then showed direct correlation to the amount of amine present, hence the extent of the coupling reaction.

Although the yield for the reaction was fairly low, we had an acceptable and sufficient amount of material to continue with the synthesis.



Scheme 2.3. Reagents and conditions: i. DMF, Na_2CO_3; ii. BocNH-Ala-OH, (2 eqv.), DIC (2 eqv.), DMAP (0.5 eqv.), CH_2Cl_2; iii. aqueous $NaIO_4$, MeOH/DCM, 0°C; iv. oxone, MeOH/DCM; v. Reflux, MeOH; vi. 0.006M NaOMe/MeOH.

Two small quantities (0.1g) of the derivatised resin, Boc-Ala-(1-hydroxy-2-mercapto) S-methyl polystyrene ethyl ester resin (**6**) were then treated individually; the first sample was treated with sodium metaperiodate and the latter with potassium hydrogen persulfate to give the sulfoxide, Boc-Ala-(1-hydroxy-2-sulfoxo) S-methyl ethyl ester polystyrene resin (**7**) and the sulfone, Boc-Ala-(1-hydroxy-2-sulfonyl) S-methyl ethyl ester polystyrene resin (**9**) respectively.

The sulfoxide (**7**) (pre-swollen in DCM) was then treated at a gentle reflux with methanol. The product was cleaved in excellent yield (100%), from the support as the methyl ester. ^{1}H-NMR confirmed the presence of Boc-alanine methyl ester. Tlc also confirmed that Boc-alanine had not been isolated as first thought, (as a result of hydrolysis of the Boc-alanine vinyl ester) as the R_f value was not concordant with authentic Boc-alanine, but was of higher value.

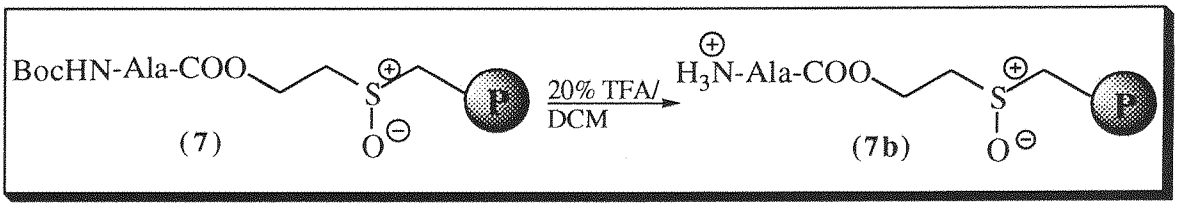
A sample of the sulfide (**6**) and sulfone (**9**) Merrifield resin derivatives of (**7**) were also treated with methanol in reflux (under nitrogen). Analysis by tlc showed no cleavage had occurred (**Table 2.1**).

In the scheme proposed in the introduction (see **scheme 1.2**), the cleavage products from Boc-Ala-(1-hydroxy-2-sulfoxo) S-benzyl ethyl ester resin (**3**) are the hydrolysed vinyl ester and benzyl sulfenic acid. The transformation that actually takes place is illustrated in **scheme 2.3**. The products are Boc-alanine methyl ester and sulfenic methylated resin.

Elimination of Boc-Ala-(1-hydroxy-2-sulfonyl)-S-methyl polystyrene ethyl ester resin (**9**) to form Boc-alanine was performed with 0.006M sodium methoxide. Proton NMR showed Boc-alanine and electrospray mass spectrometry showed a peak at 189 (the relative molecular mass of Boc-alanine), thus showing the elimination to be successful.

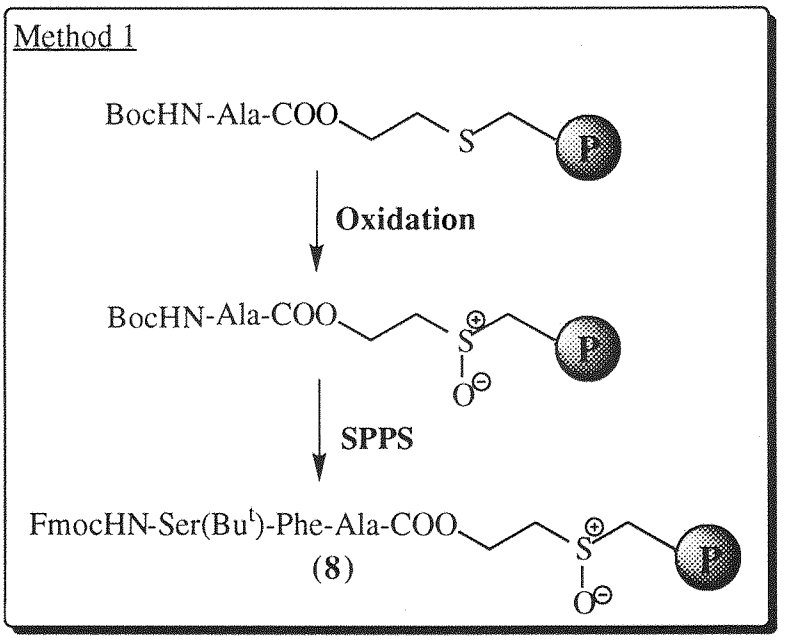
2.4.2 Effect of oxidation on peptide couplings

Boc-Ala-(1-hydroxy-2-sulfoxo) S-methyl polystyrene ethyl ester resin (**7**) was deprotected with 20% TFA in dichloromethane to obtain (**7b, scheme 2.4**). Fmoc protected amino acids were then added sequentially with HOBt and DIC, preforming the activated ester.



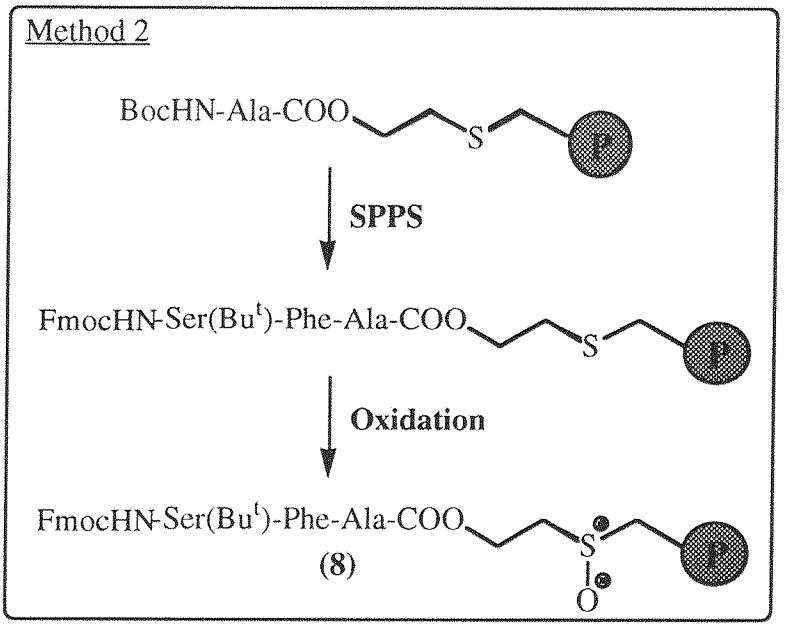
Scheme 2.4

The protected amino acids added were Fmoc-Phe-OH and Fmoc-Ser(ᵗBu)-OH.



Scheme 2.5

The synthesis of the tripeptide was undertaken via two methods. Firstly, (Method 1, **scheme 2.5**) esterification of Boc-Ala-OH to the resin , followed by oxidation of the sulfide to the sulfoxide and then assembly of the tripeptide was continued. Secondly (Method 2, **scheme 2.6**), the tripeptide was synthesised and then oxidation of the sulfide to the sulfoxide was carried out. The purpose of the study was to see if oxidation of the sulfide would effect subsequent amino acids couplings. The study proved that either **method 1** or **2** can be undertaken for the synthesis of Fmoc-Ser(ᵗBu)-Phe-Ala-(1-hydroxy-2-sulfoxo)-S-methyl polystyrene ethyl ester resin (**8**), as oxidation has no effect on the peptide couplings illustrated in **scheme2.6**.



Scheme 2.6

Thermolysis of the linker was then attempted by refluxing the pre-swollen resin (DCM) in methanol (under nitrogen). The reaction was monitored by tlc. Analysis by tlc 3h later showed no cleavage had occurred, so the reaction was left refluxing overnight. The tripeptide was successfully cleaved as a methyl ester (36%) from the solid support.

Analysis of the proton NMR spectra confirmed the presence of a tripeptide (**8a**) by the characteristic signals associated with the constituent amino acids. Further correlation was performed by a 2D (^{1}H-^{1}H) COSY experiment, from this, assignment of the structure of the tripeptide was unambiguously Fmoc-Ser(^{t}Bu)-Phe-Ala-methyl ester (**8a**).

High resolution mass spectrometry indicated a mass peak at 616 $[M+H]^+$ which corresponds to the cleavage product obtained, Fmoc-Ser(^{t}Bu)-Phe-Ala-methyl ester (**8a**).

MALDI-TOF MS (**fig 2.1**) data for the cleavage product Fmoc-Ser(^{t}Bu)-Phe-Ala-methyl ester (**8a**) was also obtained. The spectra was calibrated against bradykinin (1061.3). A peak at 637 was observed, indicative of the product (**8a**) incorporating a sodium ion. This peak was also seen by ES-MS.

Analysis of the product by analytical HPLC showed the product eluted as a single peak with a retention time of 23 mins (**fig 2.2**).

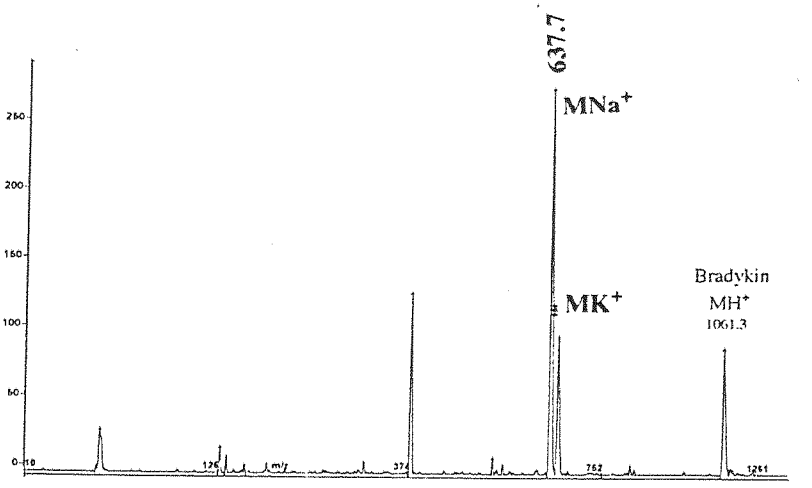


FIGURE 2.1

Analysis of tripeptide, FmocHN-Ser(^{t}Bu)-Phe-Ala methyl ester (**8a**), [M+Na], 637.7.

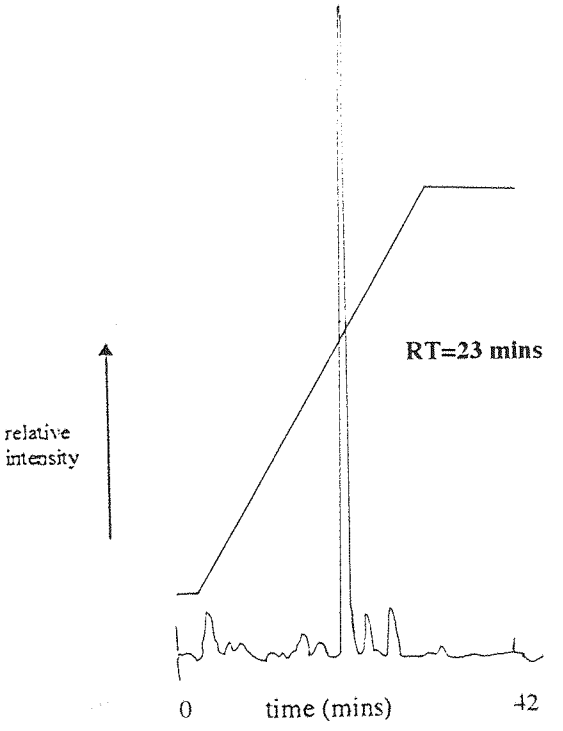


FIGURE 2.2

Analytical HPLC elution profile of peptide (**8a**) after cleavage by refluxing in methanol.

2.4.3 Coupling with Aminomethyl resin

Bromoacetamidomethyl resin (**13**) was prepared from the Merrifield resin as shown in **Scheme 2.7**. The starting Merrifield resin was treated with potassium phthalimide in DMF to yield phthalimidomethyl resin (**10**) which was subjected to hydrazinolysis to convert the phthalimide group to an amine, giving the aminomethyl resin (**11**).

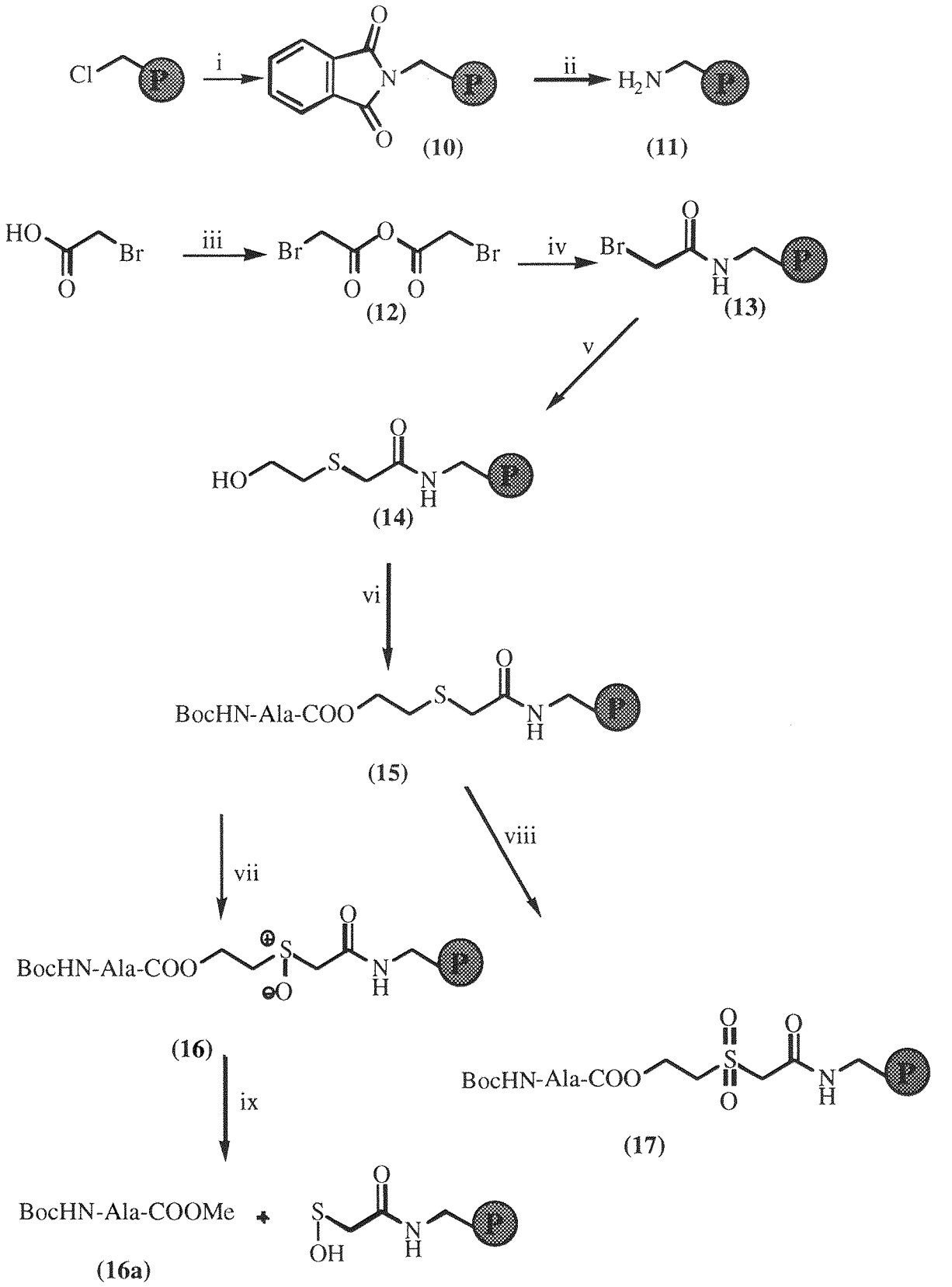
In preparation for the solid phase peptide synthesis, the aminomethyl resin (**11**) was treated with bromoacetic anhydride (**12**) which was prepared from bromoacetic acid and DCC under a dry atmosphere to give the bromoacetamidomethyl resin (**13**).

Alkylation of bromoacetamidomethyl resin (**13**) was carried out with excess β-mercaptoethanol and a small quantity of sodium carbonate in DMF. The reaction was left stirring overnight, to ensure it reached completion. This is one of the main problems of solid phase peptide synthesis i.e blind reactions , due to lack of analytical techniques. However, the success of this reaction was vital for the next step of the synthesis; the esterification with Boc-alanine. Hence, the necessity for large excesses of reagents to drive the reaction to completion.

The esterification was carried out by DMAP and DIC. The success of the reaction was monitored by quantitative ninhydrin test, which indicated a loading of 64% of alanine to the derivatised resin, (1-hydroxy-2-mercapto)-S-acetamidomethyl ethyl ester (**14**). The coupling reaction was repeated in order to achieve maximum loading of alanine on the resin.

Oxidation of the esterified resin, Boc-Ala-(1-hydroxy-2-mercapto)-S-acetamidomethyl ethyl ester (**15**) with sodium periodate gave the sulfoxide, Boc-Ala-(1-hydroxy-2-sulfoxo)-S-acetamidomethyl ethyl ester resin (**16**) and oxidation with potassium hydrogen persulfate gave the sulfone, Boc-Ala-(1-hydroxy-2-sulfono)-S-acetamidomethyl ethyl ester (**17**).

Infra red spectroscopy was used (by making up a KBr disc) to analyse the sulfoxide (**16**), (from the oxidation of the sulfide on the aminomethyl resin) and compare it with the starting sulfide (**15**). Unfortunately infra red spectroscopy of resin bound compounds was inconclusive in most cases because of the high background.



Scheme 2.7. Reagents and conditions: i. KI, DMF, Potassium phthalimide, 100°C, 5h; ii. EtOH reflux, N_2H_4, 7h; iii. DCC, CH_2Cl_2; iv Aminomethyl resin (**10**), DMF, r.t, 3h; v. DMF, BME; vi. BocHN-Ala-OH (2eqv.), DIC (2 eqv.), DMAP (0.5 eqv.), CH_2Cl_2; vii. aqueous $NaIO_4$, MeOH/DCM, 0°C; viii. oxone, MeOH/DCM; ix. Reflux, MeOH.

Solid phase synthesis precludes the use of traditional analytical techniques such IR and NMR. Consequently a recently reported analytical technique was used and will be discussed in the next section.

2.4.4 Characterisation study using Rink amide resin

The analytical method used to monitor the solid phase reaction was a matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry [47].

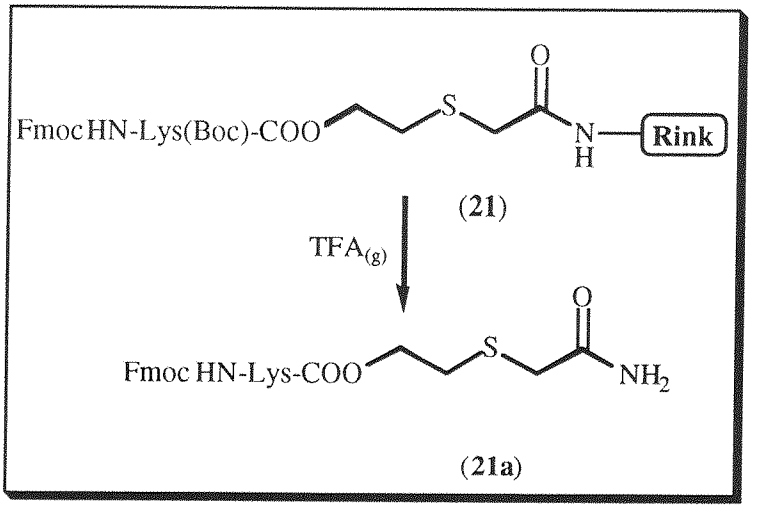
The purpose of involving the Rink amide resin in the synthesis, were for characterisation purposes. Reactions on peptides attached to resin by the acid labile Rink linker are released as primary amides when directly monitored from a single bead using this technique.

Bradley [48] reported this analytical method for the analysis of new solid phase reactions, as it is material efficient and time efficient. The method also makes use of an extensive range of established peptide type linkers, besides the Rink linker (e.g HMPB and Wang) and creates a collection of various termini (amines, carboxylates and homoserine lactones).

Thus, solid phase peptide synthesis was undertaken using a sample of (**12**) with the Rink amide resin, using, the same methodology as used previously with the aminomethyl and tentagel resin. Esterification of the resin was undertaken by treatment with Fmoc-Lys(Boc)-OH, in place of Boc-alanine.

Fmoc-Lys(Boc)-OH was chosen as the amino acid, opposed to Boc-alanine due to the mass of the Fmoc group.

Hence, MALDI-TOF MS data (**fig 2.3**) was obtained directly from the resin, by treating a single bead of (**21**) with gaseous TFA for 30 mins to cleave the peptide *in situ*. (**21a, scheme 2.8**).



Scheme 2.8

The cleavage product (**21a**) was obtained from Fmoc-Lys(Boc)-(1-hydroxy-2-mercapto) S-acetamidomethyl ethyl ester Rink resin (**21**) and was identified by a weak mass peak at 484 (**fig 2.3**). Subsequently this product was oxidised, but only the sodiated species (523) observed in the MALDI-TOF MS data. It is assumed that complete oxidation has taken place, as there is no evidence of the starting material (**21**) on the spectra, thus supporting the hypothesis that oxidation with sodium periodate was successful.

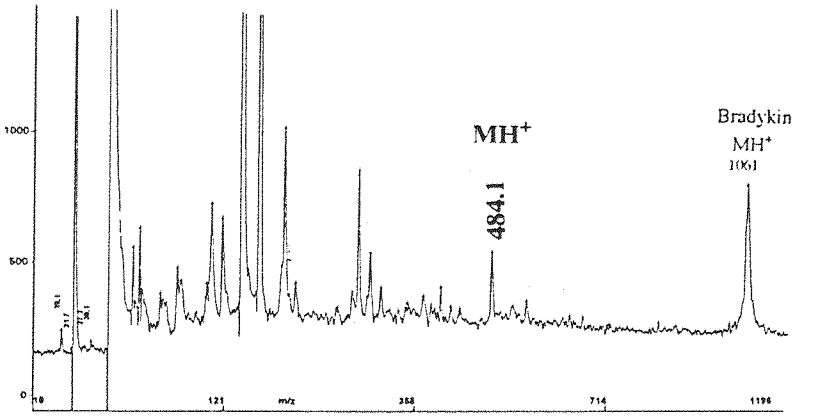


FIGURE 2.3
TFA vapour cleavage and analysis of peptide, FmocHN-Lys(Boc)-(1 hydroxy-2-mercapto)-S-acetyl amide (**21**), [M+H], 484.1.

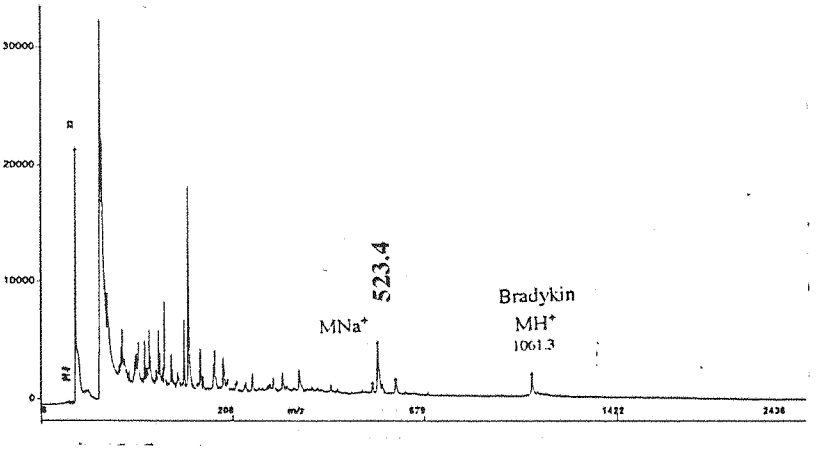
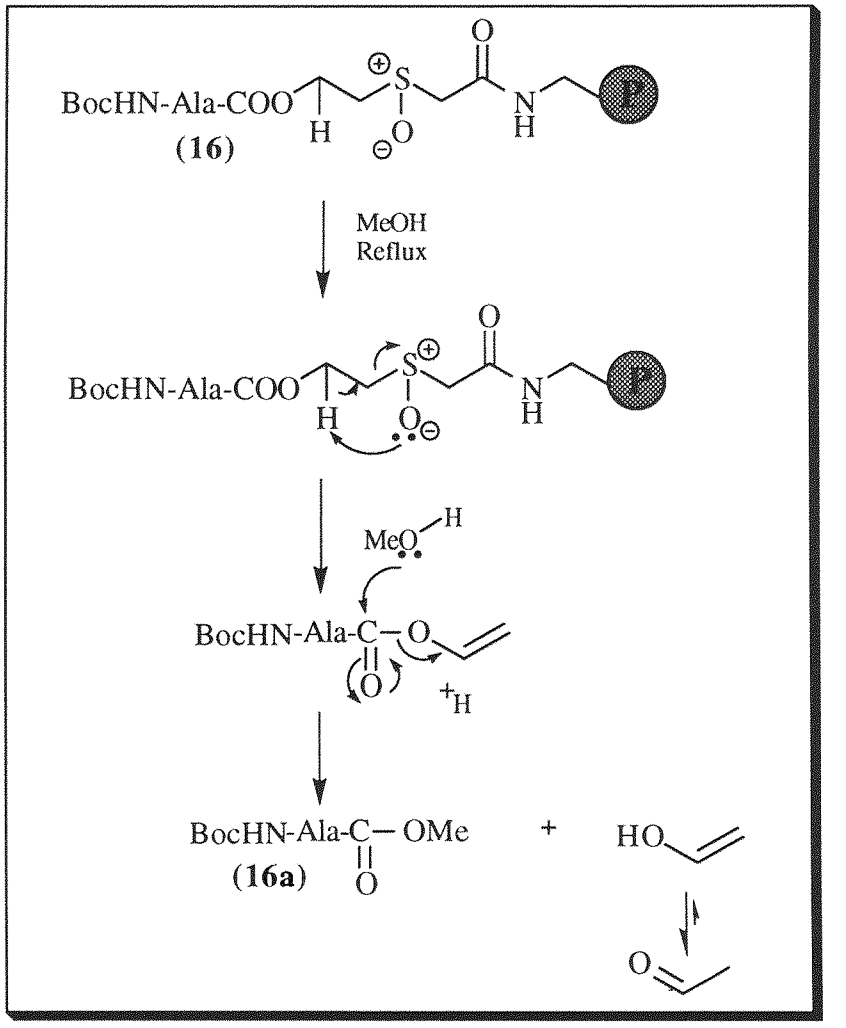


FIGURE 2.4
TFA vapour cleavage and analysis of peptide, FmocHN-Lys(Boc)-(1 hydroxy-2-sulfoxo)-S-acetyl amide (**22**), [M+Na], 523.4.

2.4.5 Thermolysis Study

A sample of the sulfide (**15**) and sulfone (**17**) aminomethyl resin derivatives of (**16**) were also treated with methanol at reflux (under nitrogen). Analysis by tlc showed no cleavage had occurred (**Table 2.1**).

In view of these results, cleavage of the peptide from the resin must have taken place via a rearrangement mechanism and not by methanolysis. If methanolysis took place, cleavage products from the sulfone (**17**) and sulfide (**15**) would be expected, this is not the case. A postulation is that the sulfoxide rearranges to form the reactive vinyl ester as an intermediate, which is then attacked by methanol, hence resulting in the methyl ester as the product (see **scheme 2.9**).



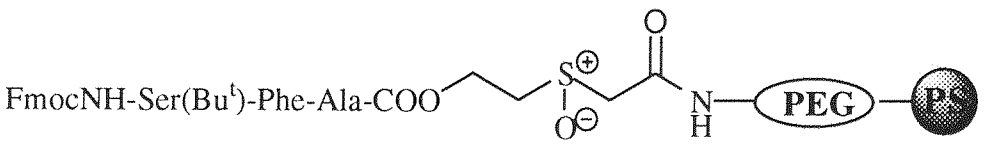
Scheme 2.9

The advantage, of using the aminomethyl resin, was that the time for thermolysis of the linker (2h) was considerably lower than what it was for the linker attached to the chloromethyl resin (5h).

2.4.6 Coupling with amino functionalised tentagel resin

Solid phase peptide synthesis was undertaken using a sample of (**12**) with tentagel, using, the same methodology as used previously with the aminomethyl resin.

The Fmoc protected tripeptide (**18**) coupled to tentagel, was heated to reflux in methanol to see if cleavage would occur on a resin that would swell in water. Thermolysis of the linker was successful according to the Rf value displayed on tlc, which was concordant to the Rf value of the tripeptide methyl ester cleaved from the Merrifield resin.



(**18**)

However, RP HPLC showed a number of products to be present, and proton NMR showed the tripeptide to be present, but accompanied with impurities.

Advantage of the fact that the above resin swells in water was taken, by deprotecting the above protected tripeptide with 20% piperidine in DMF, to obtain the free amine. The deprotected tripeptide was now heated in reflux in water. Cleavage of the deprotected tripeptide was successful.

Mass spectrometry indicated a peak at 379 which corresponds to the cleavage product obtained. However, proton NMR showed more peaks than expected. Analytical HPLC also showed impurities in the sample (see **fig 2.5**).

A sample of the sulfide and sulfone (**20**) amino functionalised tentagel resin derivatives of (**18**) were also treated with methanol (for the protected tripeptide) and in water (for the free tripeptide) under reflux (under nitrogen). Analysis by tlc showed no cleavage had occurred (**Table 2.1**).

Elimination of the protected tripeptide FmocSer(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfonyl)-

S-acetamidomethyl ethyl ester tenta gel resin (**20**) to form FmocSer(^{t}Bu)-Phe-Alanine was performed with 0.006M sodium methoxide. Electrospray mass spectrometry showed FmocSer(^{t}Bu)-Phe-Alanine, with the correct peak at 601, thus showing the elimination to be successful.

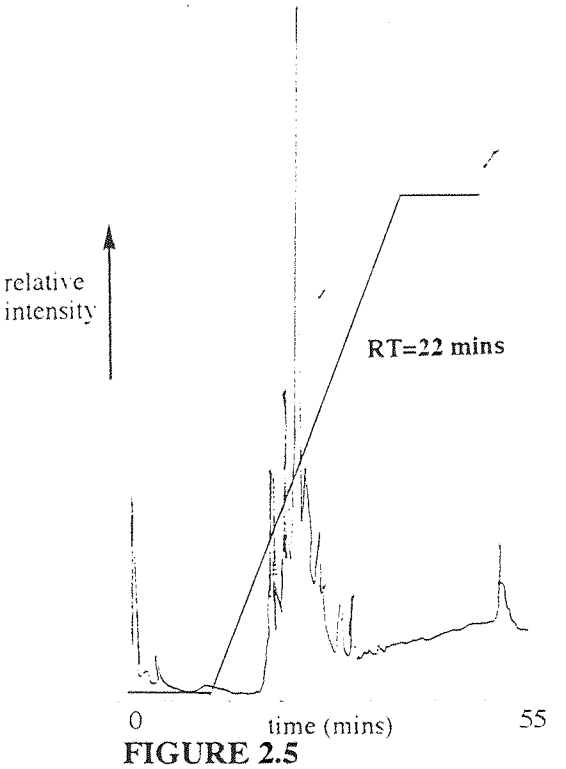
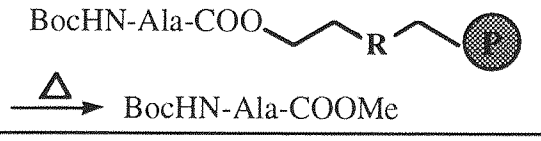
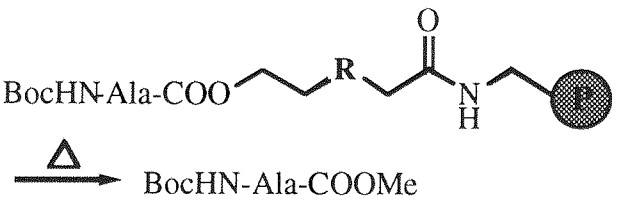
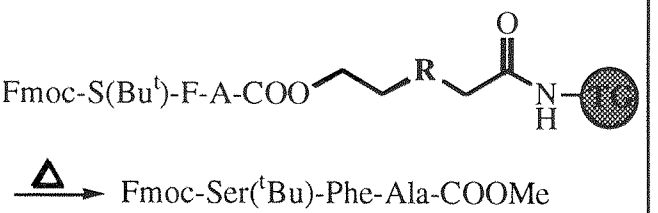
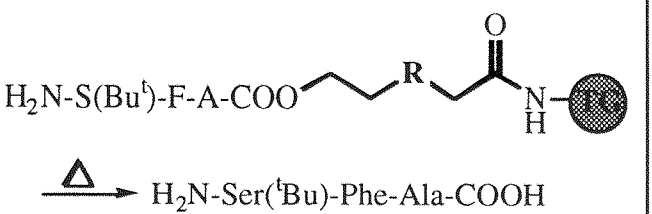


FIGURE 2.5
Analytical HPLC elution profile of the product cleaved from peptide (**19**) by refluxing in water.

The compounds shown in **Table 2.1** summarise the data obtained from the control studies.

Table 2.1

Reaction	Product detection by tlc		
	Sulfide R=S	**Sulfoxide R=SO**	**Sulfone $R=SO_2$**
i[a] 	×	✓	×
ii[a] 	×	✓	×
iii[a] 	×	✓	×
iv[b] 	×	✓	×

[a] This reaction was refluxed in methanol

[b] This reaction was refluxed in distilled water

P= Polystyrene

TG=Tentagel Resin

Chapter 3
Conclusion

3.1 Solution Phase

Thermolysis studies undertaken in the solid phase proved difficult with the solvents chosen, dioxane,tetrahydrofuran, and dimethylformamide. If more time was permitted, thermolysis reactions undertaken in methanol would be of great interest. Unfortunately clean data of thermolysis products in the solution phase were not obtained.

However, the elimination of Boc protected alanine from of Boc-Ala-(1-hydroxy-2-sulfonyl)-S-benzyl-ethyl ester (**4**) was a success with sodium methoxide (0.006M). Further work in this area should be directed at using weaker, bio-compatible bases such as sodium hydrogen carbonate.

3.2 Solid Phase

Reactions carried out in the solid phase on various resins were successful, this included both the thermolysis studies and the elimination studies.

Thermolysis reactions were tried with single amino acid attached to sulfoxo derivatised resins and tripeptides attached to sulfoxo derivatised resins. The resins used were polystyrene (aminomethyl resin, chloromethyl resin) and finally tentagel resin.

The cleavage products obtained from the thermolysis studies undertaken with methanol, were always the methyl ester peptides or amino acids and not the vinyl ester peptides. However, when using water as the solvent, the deprotected tripeptide on the tentagel resin did produce the acid.

Control thermolysis studies undertaken with mercapto and sulfonyl derivatised resins, indicated that the work carried out on the sulfoxo derivatised resins was solely due to the electrocyclic mechanism induced by the sulfoxide oxygen, and not by methanolysis.

The inclusion of the Rink resin in the project was of great importance, as it confirmed that the sulfoxide had been made on the resin. Monitoring reactions on solid phase during the course of the project provided great difficulties, as no methods of analysis were available for the alkylation of BME to the resin and the oxidation reactions. This was overcome by leaving the reactions for longer time periods and using excesses of reagents.

Elimination reactions undertaken on the resin were successful. Again, there was doubt to whether the oxidation of the sulfide to the sulfone on the resin was complete; although long reaction times and excesses of the potassium hydrogen persulfate oxidising reagent were utilised to drive the reaction to completion.

Elimination of the Fmoc protected tripeptide (**20**) on the tentagel resin was carried out successfully with 0.006M sodium methoxide, to give the product.

The most important and novel result is the synthesis of a linker that can especially be used with tentagel (**18**) as well as polystyrene resins (aminomethyl resin (**16**) and chloromethyl resin) (**8**). The linker also remains entirely intact throughout the assembly of amino acids by Fmoc chemistry. Upon oxidation with sodium metaperiodate, the linker is labile to heat and thus cleavage of the peptide occurs from the resin.

In conclusion, a novel thermally labile linker has been synthesised, that is of great use because of its solubility in water, as the peptide attached to the linker can be released from tentagel in water for biological testing.

Chapter 4
Experimental

4.1 General Procedures:

NMR spectra were recorded on Bruker AC 300 spectrometer operating at 300 MHz and 75 MHz for proton and carbon NMR respectively. Peak assignments were performed by DEPT experiments for ^{13}C NMR.

T.l.c. analysis was performed on DC-Alufolien Kieselgel 60 F_{254} (25 Folien 2 x 4 cm-schrichtdiche 0.2 mm), developed with either 0.3% ninhydrin in 1-butanol and 3% AcOH (to reveal the presence of free amines), followed by heating for a few minutes unless otherwise stated; or compounds were developed with bromo cresol green (to reveal the presence of carboxylic acids). Compounds were also visualised under short wavelength ultra violet light at 254nm. Column chromatography was performed on machereynagel silica gel 60 (40-63 micrometers).

Mass spectra were recorded on a VG-250 SE double focusing mass spectrometer in fast atom bombardment (FAB) (70 eV) mode. Electrospray mass spectra were recorded on a VG Platfom, using 10µl loop injections of sample to MeCN@ 20µl/min. HPLC delivery system, HP 1050. Cone voltage 25V, ES capillary 3.5 kV

MALDI-TOF spectrum were recorded on a GSG FOCUS Benchtop II linear- Laser desorption time of flight mass spectrometer.

IR spectra were recorded as liquid films (unless stated otherwise) between NaCl plates on a Perkin Elmer 1600 Fourier Transform spectrometer.

HPLC was carried out using a Waters 600 Multi-solvent Delivery System, equipped with a C-18 ODS column (size 25cm x 4.6mm) and using a controlled gradient (see individual experimental for gradient conditions) of 0.1% TFA/MeCN and 0.1% TFA/H_2O as eluting solvent. UV absorbance at 270nm (unless stated otherwise) was measured.

Melting points were determined using a Gallenkamp melting point apparatus, and are uncorrected.

Commercial materials were used without further purification.

4.2 General methods:

Ninhydrin quantitative analysis [49]***-The test for free amines:***

Samples for quantitative ninhydrin analysis were prepared as described:- 2-5 mg dry resin was weighed into a 10 x 75 mm test tube. Reagent A (6 drops) and reagent B (2 drops) were added, mixed well and placed in an oil bath set at 100°C for 10 min. To another test tube only the reagents were added as a control. The tubes were then placed in cold water and 60% ethanol (2ml) was added and mixed thoroughly. The solutions were then filtered through a Pasteur pipette containing a tight plug of wool, washed twice with 0.5M Et_4NCl (0.5ml) and diluted to 25ml with 60% ethanol. UV/VIS spectroscopy monitoring of the reagent blank was at 570 nm. This was measured against the absorbance of the resin filtrates. Incorporation of this absorbance into equation 1 provides information on the degree of substitution of the resin (i.e. the level of free amine).

EQUATION 1:

$$\mu mol/g = A_{570} \times V(ml) / \varepsilon'_{570} \times W\ (mg) \times 10^6$$
$$(\varepsilon' = 1.5 \times 10^4)$$

Reagent A

Solution 1- Reagent grade phenol (40g, 0.43mols) was mixed with absolute ethanol (10ml). The mixture was warmed until dissolved. IWT TMD-8 ion exchange resin (4g) was added and the mixture stirred for 45 mins then filtered.

Solution 2- KCN (65mg, 1mmol) was dissolved in water (100ml). The KCN solution (2ml) was then diluted to 100ml with pyridine (freshly distilled from ninhydrin). IWT TMD-8 ion exchange resin (4g) was added, stirred for 45 min and filtered. Solutions 1 and 2 were mixed.

Reagent B

Ninhydrin (2.5g, 14mmol) was dissolved in absolute ethanol (50 ml). The solution was stoppered, stored in the dark under nitrogen.

Method A: N-terminal Fmoc deprotection

Fmoc deprotection was carried out by treating the protected resin (0.1g) with 20% piperidine in DMF (15ml) for 20min. The resin was then filtered, washed with DMF (4 x 10ml) and then treated again with 20% piperidine in DMF for a furthur 5 min. The resin was filtered and washed with DMF(1 x 10ml), DCM(2 x 10ml) and finally MeOH(1 x 5ml).

Method B: N-terminal Boc deprotection

Boc deprotection was carried out by treating the protected resin (0.1g) with 20% TFA in DCM (10ml) for 20min. This was repeated for a further 5 min The resin was then filtered, neutralised with neat triethylamine (3x 10ml) and then washed thoroughly with DMF (4 x 10ml), DCM(3x 10ml) and finally MeOH(1 x 5ml).

Method C: Solid phase peptide synthesis coupling conditions

A Fmoc amino acid (2 eqv) and HOBt (1 eqv) were stirred into a minimal amount of DMF for 10 min. DIC (2 eqv) was added and stirring continued for a further 10 min. This mixture was added to the free amine (**8**) (pre-swollen in DMF) i.e after deprotecting the Boc group by method B. The mixture was stirred at room temperature for 2h or longer to effect coupling (shown by a negative ninhydrin test).

Method D: Oxidation [50]of Sulfide to Sulfoxide on Solid Phase

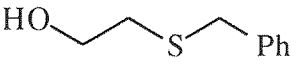
A solution of sodium metaperiodate (2eqv) in water (5ml) was added dropwise to the vigorously stirred ice-cold solution of (**6, 15 & 21**) (0.1g,0.14 mmol) in dichloromethane (5ml) and methanol (3ml) at 0°C. The reaction mixture was stirred overnight at room temperature. The resin was then filtered, washed with DMF (4 x 10ml), DCM(3x 10ml), water (3 x 10ml) and finally MeOH(1 x 5ml) and dried *in vacuo.*

Method E: Oxidation [51] of Sulfide to Sulfone on Solid Phase

To a stirred solution of (**6, 15 &18**) (0.1g, 0.14 mmol) in dichloromethane (5ml) and methanol (3ml) at 0°C (ice/water bath) was added a solution of 49.5% potassium hydrogen persulfate (3 eqv) in water (5ml). The reaction (pH 2-3) was stirred at room temperature for 2 days. The resin was then filtered, washed with DMF (4 x 10ml), DCM(3x 10ml), water (3 x 10ml) and finally MeOH(1 x 5ml) and dried *in vacuo.*

4.3 Solution Phase Chemistry

Preparation of S-Benzyl-mercaptoethanol (1)



Benzyl bromide (2.18g, 1.5 ml, 1.3 mmol) was dissolved in ethanol (10ml) containing β-mercaptoethanol (1g, 0.87ml, 1.3 mmol). The reaction was stirred at room temperature for 3.5 hr. Sodium carbonate (~50mg) was added to the reaction. After 1 hr, the reaction mixture was extracted with EtOAc (3 x 25ml) and the pooled organic extracts were washed with water (25ml) and brine(25ml). The separated organic layer was dried over magnesium sulfate and then filtered and evaporated *in vacuo.* The product was purified by column chromatography on silica gel (EtOAc:Petroleum Ether /1:4) to give a pale yellow oil.

Yield 1.89g (88%)

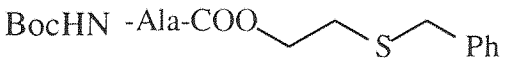
Tlc: (EtOAc:Pet Ether) (80:20) $R_f = 0.51$ (visualised by UV)

δ_H (300 MHz, $CDCl_3$) 2.29 (s, 1H, $CH_2O\underline{H}$); 2.58 (t, 2H, $SC\underline{H}_2$, *J*6 Hz), 3.62(t, 2H, $OC\underline{H}_2$, *J*6 Hz); 3.68 (s, 2H, $C\underline{H}_2Ph$); 7.24 (m, 5H, Ph)

δ_C (75 MHz, $CDCl_3$) 34.44 ($\underline{C}H_2S$); 35.90 ($\underline{C}H_2Ph$), 60.41 ($\underline{C}H_2O$); 127.36 (*para*-Aryl-$\underline{C}$H); 128.79 (*meta*-Aryl-$\underline{C}$H); 129.03 (*ortho*-Aryl$\underline{C}$H); 138.23 (*ipso*-Aryl$\underline{C}$);

δ_C (75 MHz, $CDCl_3$, DEPT analysis): 34.44 ($\underline{C}H_2S$); 35.90 ($\underline{C}H_2Ph$), 60.41 ($\underline{C}H_2O$); 127.36 (*para*-Aryl$\underline{C}$H); 128.79 (*meta*-Aryl$\underline{C}$H); 129.03 (*ortho*-Aryl$\underline{C}$H)

Preparation of Boc-Ala-(1-hydroxy-2-mercapto)-S-benzyl-ethyl ester (2)



S-Benzyl-mercaptoethanol (**2**) (0.51g, 3.03 mmol) was dissolved in dichloromethane (10ml) containing DMAP (37mg, 0.30 mmol, 0.1 eqv) to which Boc-Ala-OH (0.57g, 3.03 mmol) dissolved in dichloromethane (10ml) was added. This was followed by the addition of *N'N*-dicyclohexylcarbodiimide (0.62g, 3.03 mmol) and stirred at room temperature. After 3 hr, a furthur equivalent of Boc-Ala-OH (0.57g, 3.03 mmol) was added. After 5 hr, the mixture was filtrated. A mixture of EtOAc (25 ml) and a saturated solution of sodium hydrogen carbonate (25ml) were then added to the residue and the separated organic phase was extracted with a 10% solution of citric acid (25ml), with saturated sodium hydrogen carbonate (25ml) and water (25ml). The organic solution was dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo*. The product was purified by column chromatography on silica gel (EtOAc:Petroleum Ether 30:70) to give a yellow oil.

Yield 0.8g (78%)

Tlc: (EtOAc:Pet Ether:AcOH) (20:80:1) $R_f = 0.42$ (positive ninhydrin test)

IR (NaCl): 3361.3 (b, NH stretch), 2977.0 (s, CH stretch); 1736.8 and 1713.4 (s, ester and carbamate stretch); 1514.0 (m, NH bend); 1453.8 (m, CH deformations); 1163.0 (s, SCH_2 stretch).

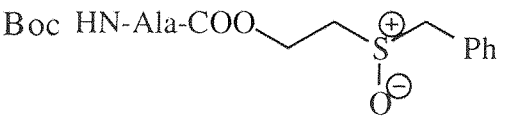
M/Z (FAB), 249 $[M+H\text{-benzyl}]^+$ 5%, 215 $[M+H\text{-mercapto benzyl}]^+$ 10%

δ_H (300 MHz, $CDCl_3$) 1.34 (d, 3H, Ala-C<u>H</u>$_3$, *J*7); 1.40 (s, 9H, $C_4\underline{H}_9$); 2.58 (t, 2H, $SC\underline{H}_2$, *J*7); 3.69 (s, 2H, $C\underline{H}_2Ph$); 4.18 (m, 3H, Ala-αC<u>H</u>, $OC\underline{H}_2$); 5.23 (d, 1H, Boc-N<u>H</u>, *J* 7); 7.27 (m, 5H, $C_6\underline{H}_5$).

δ_C (75 MHz, $CDCl_3$) 18.67 ($\underline{C}H_3$), 28.23 (($\underline{C}H_3$)C), 29.53 ($\underline{C}H_2S$); 36.42 ($\underline{C}H_2Ph$); 49.34 (Ala-α<u>C</u>H); 63.83 ($\underline{C}H_2O$); 79.90 (CH_3)<u>C</u>), 127.31 (*para*-Aryl<u>C</u>H); 128.71 (*meta*-Aryl<u>C</u>H); 129.02 (*ortho*-Aryl<u>C</u>H); 138.02 (*ipso*-Aryl<u>C</u>); 155.24 (urethane); 173.31 (ester <u>C</u>OO).

δ_C (75 MHz, $CDCl_3$, DEPT analysis): 18.67 ($\underline{C}H_3$), 28.24 (($\underline{C}H_3$)C), 29.52 ($\underline{C}H_2S$); 36.42 ($\underline{C}H_2Ph$); 49.35 (Ala-α<u>C</u>H); 63.83 ($\underline{C}H_2O$); 127.31 (*para*-Aryl<u>C</u>H); 128.71 (*meta*-Aryl<u>C</u>H); 129.02 (*ortho*-Aryl<u>C</u>H)

Preparation of Boc-Ala-(1-hydroxy-2-sulfoxo)-S-benzyl-ethyl ester (3)[5]



A solution of sodium metaperiodate (1.02g, 4.78 mmol) in water (10 ml) was added dropwise to a vigorously stirred ice-cold solution of (**2**) (1.62g, 4.78 mmol) in methanol (20 ml). The reaction mixture was stirred for an additional 3.5 hr. The precipitated iodate was filtered off and washed with methanol, and the combined filtrates were concentrated to 10ml and then extracted with chloroform (7x15ml). The combined organic extracts were washed with water (100ml) dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo*. The product was purified by column chromatography on silica gel (EtOAc:Petroleum Ether /70:30) to give a pale yellow oil.

Yield 0.18g (11%)

Tlc: (EtOAc:Pet Ether:AcOH) (70:30:1) $R_f = 0.73$ (positive ninhydrin test)

IR (NaCl): 3346.1 (b, NH stretch), 2942.9 (s, CH stretch); 1746.8 and 1703.2 (s, ester and carbamate stretch); 1517.9 (m, NH bend); 1452.6 (m, CH deformations); 1163 (m, CO stretch); 1065.7 (m, sulfoxide stretch).

M/Z(FAB), 356 $[M+H]^+$,42%; 256 $[M\text{-}Boc]^+$,100%;

HR, ARGON FAB,(MNOBA matrix): 356 $[M+H]^+$, $C_{17}H_{25}O_5NS$ Calc. 356.1532, Found 356.1546.

δ_H (300 MHz, $CDCl_3$) 1.34 (d, 3H, Ala-C<u>H</u>$_3$, *J* 7); 1.41 (s, 9H, $C_4\underline{H}_9$); 2.86 (m, 2H,SC<u>H</u>$_2$), 3.69 (s, 2H, C<u>H</u>$_2$Ph); 4.26 (m, 1H, Ala-αC<u>H</u>,) 4.47 (m, 2H, OC<u>H</u>$_2$); 5.27 (d 1H, Boc-N<u>H</u>, *J* 7); 7.31(m, 5H, $C_6\underline{H}_5$).

δ_C (75 MHz, $CDCl_3$) 18.42 (<u>C</u>H$_3$); 28.45 ((<u>C</u>H$_3$)C); 49.73 (Ala-α<u>C</u>H); 49.97 (<u>C</u>H$_2$S); 58.13 (<u>C</u>H$_2$Ph); 58.57 (<u>C</u>H$_2$O); 80.06 (CH$_3$)<u>C</u>); 128.72 (*para*-Aryl<u>C</u>H); 129.19 (*meta*-Aryl<u>C</u>H); 129.44 (*ipso*-Aryl<u>C</u>); 130.28 (*ortho*-Aryl<u>C</u>H); 155.29 (urethane), 173.18 (ester <u>C</u>OO).

δ_C (75 MHz, $CDCl_3$, DEPT analysis): 18.43 (<u>C</u>H$_3$); 28.46 ((<u>C</u>H$_3$)C); 49.73 (Ala-α<u>C</u>H); 49.97 (<u>C</u>H$_2$S); 58.13 (<u>C</u>H$_2$Ph); 58.57 (<u>C</u>H$_2$O); 128.72 (*para*-Aryl<u>C</u>H); 129.19 (*meta*-Aryl<u>C</u>H); 130.28 (*ortho*-Aryl<u>C</u>H)

Thermolysis of Boc-Ala-(1-hydroxy-2-sulfoxo)-S-benzyl-ethyl ester (3a)

The thermolysis of Boc-Ala-(1-hydroxy-2-sulfoxo)-S-benzyl ethyl ester was carried out by heating in various solvents at temperatures between 30-100°C (**Table 4.1**). The reaction was monitored by t.l.c.

Table 4.1

METHOD	TIME (hr)	Temperature (°C)	Solvent	R_f[a](solvent)[b]
A	0.5	30	DMF	0.73
A	1	40	"	0.73
A	1.5	50	"	0.73
A	2	60	"	0.73
A	2.5	70	"	0.73
A	3	80	"	0.73
A	3	80	DIOXANE	0.73
A	3	30-60	THF	0.73
A	72	65	THF	0.67
C	72	65	THF	0.46,0.60,0.73 0.80
B	2	100	WATER	0.46[c]

METHOD A:Boc-Ala-(1-hydroxy-2-sulfoxo)-S-benzyl-ethyl ester (5mg, 0.014 mmol) was dissolved in solvent and the solution heated.

METHOD B: Boc-Ala-(1-hydroxy-2-sulfoxo)-S-benzyl-ethyl ester (5mg, 0.014 mmol) was dissolved in 20% TFA in dichloromethane, filtered, dried *in vacuo* and then heated to reflux in water.

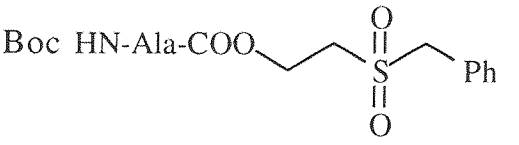
METHOD C: Boc-Ala-(1-hydroxy-2-sulfoxo)-S-benzyl-ethyl ester (5mg, 0.014 mmol) was placed in a small reaction vessel under a positive pressure of nitrogen and heated in an oil bath at 65°C with periodic monitoring by t.l.c.

[a] Boc-Ala-(1-hydroxy-2-sulfoxo)-S-benzyl-ethyl ester R_f=0.73

[b](EtOAc:Pet Ether:AcOH) 80:20:1

[c](1-BuOH:NH_3) 70:30

Preparation of Boc-Ala-(1-hydroxy-2-sulfonyl)-S-benzyl-ethyl ester (4)



To a stirred solution of (**2**) (0.20g, 0.59 mmol) in methanol (10 ml) at 0°C was added a solution of potassium hydrogen persulfate (0.13g, 0.88 mmol) in water (5ml). The resulting slurry was stirred for 4h at room temperature, diluted with water, and extracted with chloroform (3x 10ml). The combined organic extracts were washed with water and brine, dried over anhydrous sodium sulfate, filtered and evaporated to dryness *in vacuo*. The product was purified by column chromatography on silica gel (EtOAc:Petroleum Ether /70:30) to give a white crystalline solid.

Yield 0.128g (58%), Mpt: 90-92°C

Tlc: (EtOAc:Pet Ether:AcOH) (70:30:1) $R_f = 0.67$ (positive ninhydrin test)

IR (NaCl, Nujol mull): 3365.8 (b, NH stretch); 2921.2 (s, CH stretch); 1757.9 and 1688.0 (s, carbamate stretch 2x C=O stretch); 1518.9 (m, NH bend); 1454.5 (m, CH deformations); 1363.2 and 1113.2 (s, SO_2 stretch).

M/Z(FAB), 372 $[M+H]^+$, 10%; 272 $[M\text{-}Boc]^+$,100%

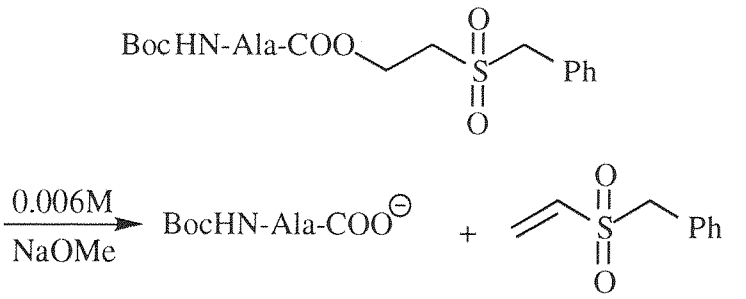
HR, ARGON FAB,(MNOBA matrix): 372 $[M+H]^+$, $C_{17}H_{25}O_6NS$ Calc. 372.1480, Found 372.1472.

δ_H (300 MHz, $CDCl_3$) 1.34 (d, 3H, Ala-C<u>H</u>$_3$, *J* 7); 1.41 (s, 9H, C_4<u>H</u>$_9$); 3.20 (ddd, 2H, SC<u>H</u>$_2$), 4.28 (s, 2H, C<u>H</u>$_2$Ph); 4.32 (m, 1H, Ala-αC<u>H</u>); 4.47 (m, 2H, OC<u>H</u>$_2$); 5.16 (d 1H, Boc-N<u>H</u>, *J* 7); 7.38(m, 5H, C_6<u>H</u>$_5$).

δ_C (75 MHz, $CDCl_3$) 18.25 (<u>C</u>H$_3$); 28.44 ((<u>C</u>H$_3$)C); 49.37 (Ala-α<u>C</u>H); 50.26 (<u>C</u>H$_2$S); 58.39 (<u>C</u>H$_2$Ph); 60.82 (<u>C</u>H$_2$O);80.32 (CH$_3$)<u>C</u>), 127.55 (*ipso*-Aryl<u>C</u>); 129.24 (*meta*-Aryl<u>C</u>H); 129.35 (*para*-Aryl<u>C</u>H); 131.05 (*ortho*-Aryl<u>C</u>H); 155.34 (urethane); 173.04 (ester <u>C</u>OO).

δ_C (75 MHz, $CDCl_3$, DEPT analysis): 18.25 (<u>C</u>H$_3$); 28.45 ((<u>C</u>H$_3$)C); 49.38 (Ala-α<u>C</u>H); 50.26 (<u>C</u>H$_2$S); 58.39 (<u>C</u>H$_2$Ph); 60.82 (<u>C</u>H$_2$O); 129.24 (*meta*-Aryl<u>C</u>H); 129.35 (*para*-Aryl<u>C</u>H); 131.06 (*ortho*-Aryl<u>C</u>H).

Elimination of Boc-Ala-(1-hydroxy-2-sulfonyl)-S-benzyl-ethyl ester (4a)



The title sulfone (7mg, 0.018 mmol) was dissolved in 0.006M sodium methoxide in methanol (3ml) and the solution was stirred at room temperature for 3 hr. The solution was diluted with water (10ml), acidified to pH 3 with 2M potassium hydrogen sulfate and then extracted with EtOAc (3x10ml). The combined organic extracts were washed with brine (10ml), dried over sodium sulfate and the solvent removed *in vacuo* to give a white crystalline solid.

Yield 1.3mg (7%)

Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) $R_f = 0.60$

δ_H (300 MHz, $CDCl_3$) 1.45 (d, 3H, Ala-C<u>H</u>$_3$, *J*7); 1.46 (s, 9H, C_4<u>H</u>$_9$); 4.35 (m, 1H, Ala-α<u>CH</u>);5.06 (d, 1H, Boc-N<u>H</u>, *J* 7).

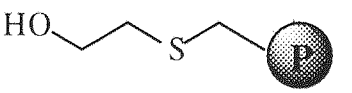
M/Z(ES), 190 $[M+H]^+$, 15%; 401 $[2M+ Na]^+$,100%

HR, ARGON FAB,(MNOBA matrix): 190 $[M+H]^+$, $C_8H_{16}O_4N$ Calc. 190.1079, Found 190.1076.

4.4 Solid Phase Peptide Chemistry

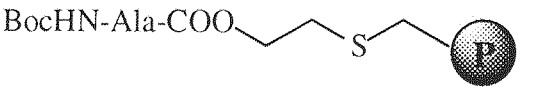
All resin suspensions were filtered and washed thoroughly with (unless stated otherwise) DMF (2x 10 ml), DCM (2x 10ml) methanol (1x 10ml).

Preparation of 1-hydroxy-2-mercapto S-methyl ethyl ester polystyrene resin (5)



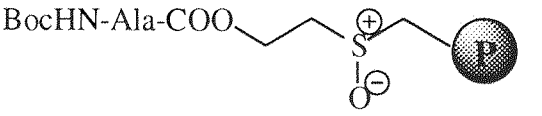
Chloromethylated polymer (Merrifield resin) (1g, 1.4 mmol), anhydrous KI (~30mg) and sodium carbonate (15mg, 0.14 mmol, 0.1 eqv) were added to a solution of β-mercaptoethanol (5.46g, 70 mmol, 50 eqv) in DMF (11 ml). The reaction was stirred for 48 hr. The resin was filtered, washed and dried *in vacuo*.

Preparation of Boc-Ala-(1-hydroxy-2-mercapto) s-methyl ethyl ester polystyrene resin (6)



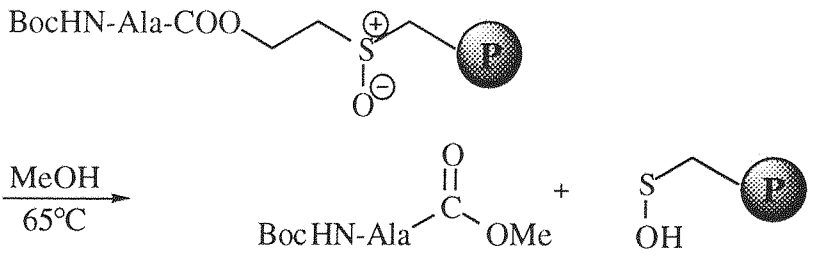
Boc-Ala-OH (0.52g, 2.8mmol, 2 eqv), HOBt (0.18g, 1.4mmol,) and DMAP (80mg, 0.7mmol, 0.5 eqv) were dissolved in a minimum amount of DCM and stirred for 10 min at room temperature. DIC (0.35g, 2.8 mmol, 2 eqv) was added and stirring was continued for a further 20 min. This mixture was added to the derivatised resin (**5**) and agitated under nitrogen pressure at room temperature for 72 hr to effect coupling. The resin was then filtered and washed with dichloromethane (6 x5ml).
The esterification was repeated with the same excess of reagents.
After deprotection of the Boc group, substitution was measured by a quantitative ninhydrin test, which indicated 29% coupling of alanine.

Preparation of Boc-Ala-(1-hydroxy-2-sulfoxo) S-methyl ethyl ester polystyrene resin (7)



See Method D for Oxidation conditions

Thermolysis of Boc-Ala-(1-hydroxy-2-sulfoxo) S-methyl ethyl ester polystyrene resin (7a)



The title resin (50mg) (pre-swollen in DCM) was suspended in MeOH (7ml) and heated at reflux, under nitrogen for 5h. The solution was monitored periodically by t.l.c. The resin was filtered and washed with MeOH(2 x 10ml). The filtrate was then collected and dried *in vacuo*.

Yield 4.7mg (100%)

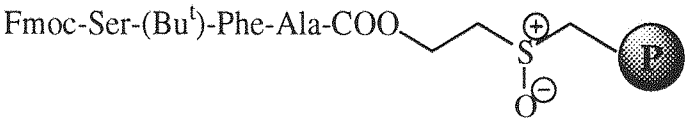
Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) $R_f = 0.66$ (ninhydrin active)

M/Z (ES), $([M+H]^+$ submitted

HR, ARGON FAB,(MNOBA matrix): submitted

δ_H (300 MHz, $CDCl_3$) 1.37 (d, 3H, Ala-C<u>H</u>$_3$, *J*7); 1.46 (s, 9H, C$_4$<u>H</u>$_9$); 3.75 (s, 3H, OC<u>H</u>$_3$); 4.32 (m, 1H, Ala-αC<u>H</u>); 5.00 (m, 1H, Boc-N<u>H</u>)

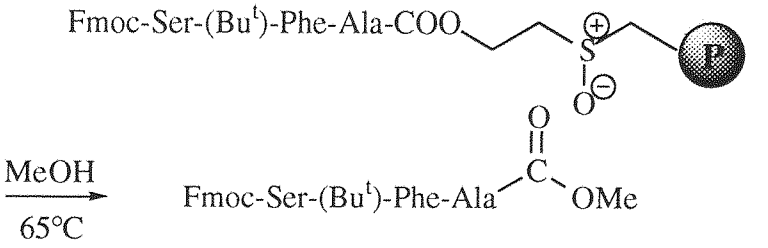
Preparation of Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfoxo) S-methyl ethyl ester polystyrene resin (8)



Boc-Ala-(1-hydroxy-2-sulfoxo) S-methyl ethyl ester polystyrene resin (7) was deprotected using method B and the subsequent Fmoc amino acids were coupled via method C. Coupling was confirmed by a negative (colourless) ninhydrin test.

After deprotection of the Fmoc group from Serine, the resin substitution was measured by a quantitative ninhydrin test, which indicated 0.46mmol NH_2/g.

Thermolysis of Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfoxo) S-methyl ethyl ester polystyrene resin (8a)



The title resin (0.1g) (pre-swollen in DCM) in MeOH (10ml) was heated to reflux under nitrogen. The solution was monitored periodically by t.l.c analysis. The reaction was left refluxing overnight. The resin was filtered and washed with MeOH (2 x 10ml). The filtrate was then collected and dried *in vacuo*. The product was analysed by reverse phase HPLC and was found to contain predominantly one compound which eluted after 33 mins. Elution conditions, 0min: 50% A, 50% B; 2min: 50% A, 50% B; 32min: 100% A, 0% B; 37min: 100% A, 0% B; 42min: 50% A, 50% B; (A= 0.1%TFA/MeCN and B= 0.1%TFA/H_2O).

Yield 8.6mg (30%)

Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) $R_f = 0.73$ (UV active)

IR (NaCl): 3361.3 (b, NH stretch), 2977.0 (s, CH stretch); 1736.8 and 1713.4 (s, ester and carbamate stretch); 1514.0 (m, NH bend); 1453.8 (m, CH deformations); 1163.0 (s, SCH_2 stretch).

M/Z (ES), 616.4 $[M+H]^+$, 5%; 638.4 $[M+Na]^+$,100%

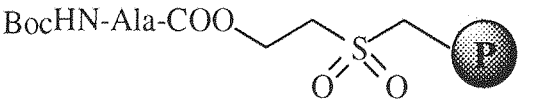
HR, ARGON FAB,(MNOBA matrix): 616 $[M+H]^+$, $C_{35}H_{41}O_7N_3$ Calc. 616.3032, Found 616.3826.

MALDI-TOF MS (DHB matrix): 177[M+9-fluorenyl methoxy]$^+$; 193.4[M+9 fluorenyl methoxycarbonyl]$^+$; 376.8[M+Ser(^{t}Bu)-Phe-Ala-COOMe]$^+$; 637.7$[M+Na]^+$; 654.5$[M+K]^+$

δ_H (300 MHz, $CDCl_3$) 1.37 (d, 3H, Ala-C<u>H</u>$_3$, *J*7); 1.45 (s, 9H, C_4<u>H</u>$_9$); 3.01-3.24 (dd, 2H, Phe-C<u>H</u>$_2$, *J*6,14); 3.39 (t, 1H,*J*8) and 3.75 (m, 1H) of Ser(^{t}Bu)-C<u>H</u>$_2$O; 3.71 (s, 3H, OC<u>H</u>$_3$); 4.21 (m, 2H, Fmoc C<u>H</u> & Ser(^{t}Bu)-αC<u>H</u>,*J* 7); 4.37 (d, 2H, Fmoc C<u>H</u>$_2$O, *J*8); 4.52 (m, 1H, Ala-αC<u>H</u>);. 4.72 (q, 1H, Phe-αC<u>H</u>,*J* 6); 5.71 (d, 1H, Ser(^{t}Bu)-N<u>H</u>,*J* 6); 6.56 (d, 1H, Ala-N<u>H</u>,*J* 8); 7.03 (d, 1H, Phe-N<u>H</u>,*J* 7); 7.27-7.44 (m, 5H, Phe-C<u>H</u>); 7.41 (t, 1H, Fmoc-C<u>H</u>,*J* 7); 7.60 (d, 1H, Fmoc-C<u>H</u>,*J* 7); 7.79 (d, 1H, Fmoc-C<u>H</u>,*J* 8).

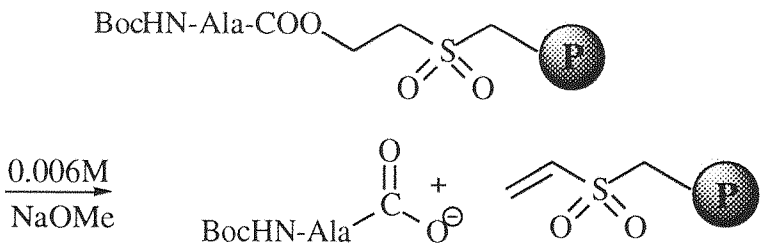
δ_C (75 MHz, $CDCl_3$) 18.44 ($\underline{C}H_3$-Ala), 22.87 ($\underline{C}H_3)_3$, *t ert*-Bu), 27.52 ($\underline{C}H_2$Phe); 47.35 (Ala-α$\underline{C}$H); 48.36 ($\underline{C}$H-Fmoc); 52.60 (O$\underline{C}H_3$-Ala); 54.45 (Phe-α$\underline{C}$H), 55.01 (Ser-α$\underline{C}$H), 61.80 ($\underline{C}H_2$-Fmoc); 67.45 ($\underline{C}H_2$O-Ser); 76.55 $(CH_3)_3\underline{C}$-*tert*-Bu); 120.20-128.91 ($\underline{C}_6H_5$, aromatics from Fmoc); 129.56 (Aryl-$\underline{C}$H-Fmoc); 136.43 (Aryl-$\underline{C}$H-Phe); 141.51 (Aryl-$\underline{C}$H-Fmoc); 143.92 (urethane), 170.12 (Phe-$\underline{C}$ONH); 170.24 (Ser-$\underline{C}$ONH); 172.89(Ala methyl ester $\underline{C}$OO).

Preparation of Boc-Ala-(1-hydroxy-2-sulfonyl) S-methyl ethyl ester polystyrene resin (9)



See Method E for Oxidation conditions

Elimination of Boc-Ala-(1-hydroxy-2-sulfonyl)-S-methyl ethyl ester polystyrene resin (9a)



The title sulfone (7mg, 0.018 mmol) pre-swollen in DCM (3ml) was suspended in 0.006M sodium methoxide in methanol (3ml). The solution was stirred at room temperature for 3 hr. The resin was then filtered, washed with water (5ml) and the resulting filtrate was diluted with water (5ml), acidified to pH 3 with 2M potassium hydrogen sulfate and extracted with EtOAc (3x10ml). The combined organic extracts were washed with brine (10ml), dried over Na_2SO_4 and the solvent removed *in vacuo* to give a white crystalline solid.

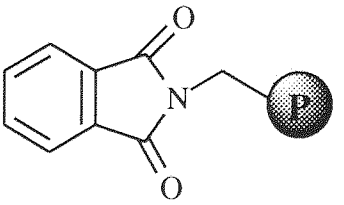
Yield 0.5mg (93%)

Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) $R_f = 0.69$ (stain: bromocresol green[52])

δ_H (300 MHz, $CDCl_3$) 1.34 (d, 3H, Ala-$\underline{C}H_3$,*J* 7); 1.41 (s, 9H, $C_4\underline{H}_9$); 4.26 (m, 1H, Ala-α$\underline{C}$H); 5.27 (m, 1H, Boc-N$\underline{H}$).

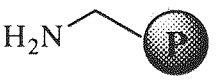
M/Z (ES), 190.2 $[M+H]^+$,10%, 401.2 $[2M+Na]^+$, 100%
M/Z(FAB), 372 $[M+H]^+$, 10%; 272 $[M\text{-}Boc]^+$,100%
HR, ARGON FAB,(MNOBA matrix): 190 $[M+H]^+$, $C_8H_{16}O_4N$ Calc. 190.1079, Found 190.1070.

Phthalimide Resin (10)



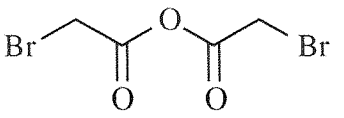
Merrifield resin, (substitution 0.7mmol/g), (10g, 7mmol) was reacted with potassium phthalimide (3.26g, 17.5 mmol, 2.5 eqv) and a catalytic amount of potassium iodide (ca~10mg) were heated at 100°C in DMF (100 ml) for 6hr. The resin was filtered and washed with DMF (1x 40ml), ethanol (1x 40ml), ether (1x 40ml) and then subsequently dried *in vacuo.*
Yield 10.53g

Aminomethyl Resin (11)[53]



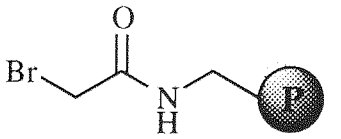
Phthalimidomethyl resin (4.38g, 0.7 mmol phthalimide/g) was heated under reflux in ethanol (80ml) containing hydrazine monohydrate (4.5ml) for 6hr under a nitrogen atmosphere. The resin was filtered and washed with methanol (3x40ml), DMF (1x 40ml), ethanol (1x40ml), ether (1x40ml) and dried *in vacuo* to give the aminomethyl resin.

Bromoacetic anhydride (12)[54]



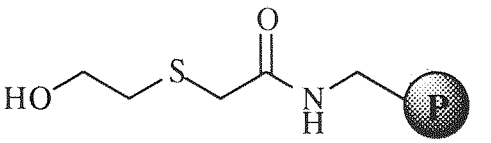
A solution of DCC (0.37g, 1.8 mmol) in dichloromethane (8ml) was added to a solution of bromoacetic acid (0.5g, 3.6 mmol) in dichloromethane (12ml) and the mixture was stirred for 30 min at room temperature. The resulting insoluble DCU was removed by filtration and the filtrate evaporated in a dry atmosphere to yield the title compound. The product was found to contain a trace of residual bromoacetic acid, but was used in the next reaction without purification.
Yield 0.72g (73%)
δ_H (300 MHz, $CDCl_3$) 3.99 (s,4H, $(BrCH_2CO)_2O$)

Bromoacetamidomethyl resin (13)



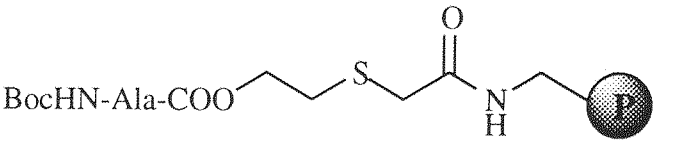
Aminomethyl resin (1.0g, 0.7mmol NH_2/g) and bromoacetic anhydride (0.39g, 1.8mmol) were stirred slowly in DMF (25ml) at room temperature for 3hr. The reaction was monitored by a ninhydrin test, the lack of a purple colouration indicating the reaction endpoint. The resin was collected by filtration and washed with dichloromethane (5x30ml) before drying *in vacuo.*

Preparation of (1-hydroxy-2-mercapto)-S-acetamidomethyl ethyl alcohol resin (14)



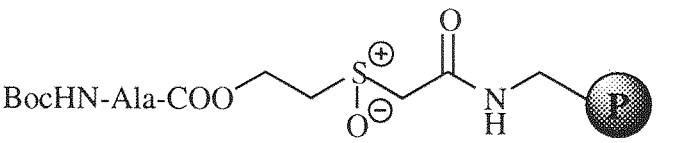
β-mercaptoethanol (0.54g, 7mmol, 10 eqv) was dissolved in DMF (5ml) and Na_2SO_4 (50mg) was added. This mixture was added to the derivatised aminomethyl resin (**13**), pre-swollen in DMF, and swirled at room temperature overnight. The resin was then filtered, washed and dried *in vacuo.*

Preparation of Boc-Ala-(1-hydroxy-2-mercapto)-S-acetamidomethyl ethyl ester resin (15)



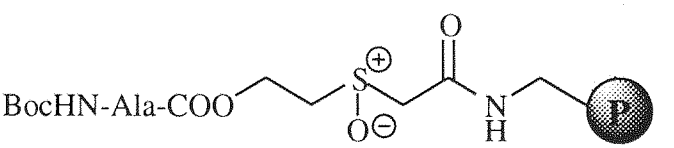
Boc-Ala-OH (0.26g, 1.4mmol, 2 eqv) and DMAP (42mg, 0.34mmol, 0.5 eqv) were dissolved in a minimum amount of DCM and stirred for 10 min at room temperature. DIC (0.17g, 1.4 mmol, 2 eqv) was added and stirring was continued for a further 20 min. This mixture was added to the derivatised aminomethyl resin (**14**), pre-swollen in DCM and stirred at room temperature for 4h to effect coupling. The resin was then filtered and washed with dichloromethane (6 x5ml).
After deprotection of the Boc group, substitution was measured by a quantitative ninhydrin test, which indicated 63.9% coupling of alanine i.e 0.39mmol NH_2/g.

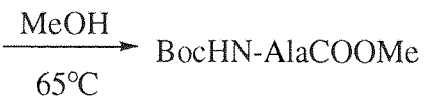
Preparation of Boc-Ala-(1-hydroxy-2-sulfoxo)-S-acetamidomethyl ethyl ester resin (16)



See Method D for Oxidation conditions

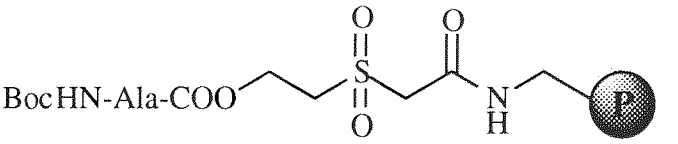
Thermolysis of Boc-Ala-(1-hydroxy-2-sulfoxo)-S-acetamidomethyl ethyl ester resin (16a)





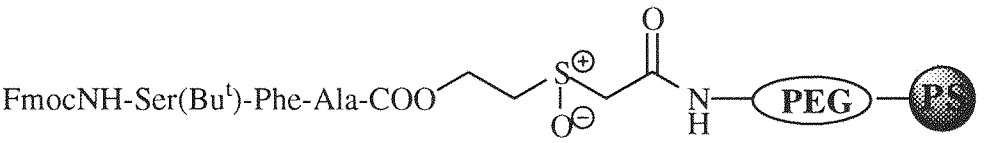
Boc-Ala-(1-hydroxy-2-sulfoxo)-S-acetamidomethyl ethyl ester resin (**16**) (74.6mg) was refluxed in methanol (10ml) under nitrogen, for 2h. The resin was filtered and washed with MeOH(2 x 10ml). The filtrate was then collected and dried *in vacuo*.
Yield 6.7mg (100%) (containing some water)
Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) $R_f = 0.66$ (ninhydrin active)
δ_H (300 MHz, $CDCl_3$) 1.37 (d, 3H, Ala-C$\underline{H}_3$,*J* 7); 1.45 (s, 9H, $C_4\underline{H}_9$); 3.75 (s, 3H, OC$\underline{H}_3$); 4.35 (m, 1H, Ala-αC$\underline{H}$); 5.30 (m 1H,N$\underline{H}$).

Preparation of Boc-Ala-(1-hydroxy-2-sulfonyl)-S-acetamidomethyl ethyl ester resin
(17)



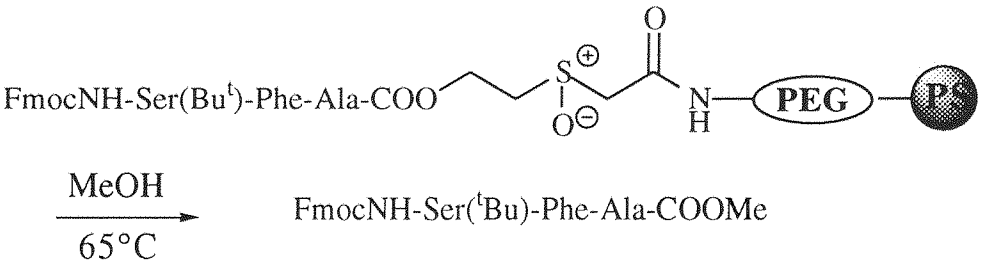
See Method E for oxidation conditions.

Preparation of Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfoxo) S-acetamidomethyl ethyl ester tentagel resin (18)



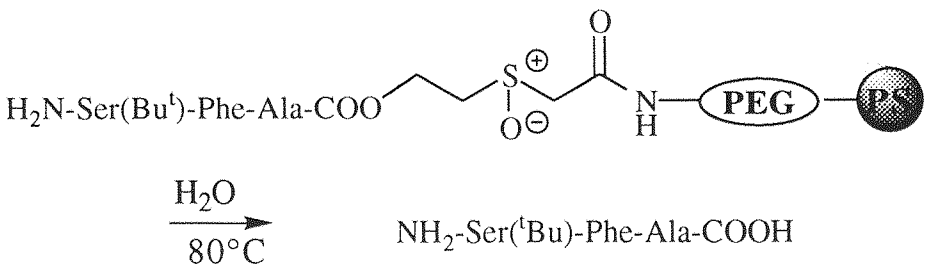
Reactions (**12**) - (**15**) were repeated (using an amino functionalised tentagel resin in place of the aminomethyl resin). Formation of the tripeptide was carried out via Method C. Subsequent oxidation to the sulfoxide was undertaken via Method D. After deprotection of the Fmoc group, substitution was measured by a quantitative ninhydrin test, 0.11mmol NH_2/g.

Thermolysis of Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfoxo) S-acetamidomethyl ethyl ester tentagel resin (18a)



Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfoxo) S-acetamidomethyl ethyl ester tentagel resin (**18**) (50mg) was refluxed in methanol (10ml) under nitrogen.
Yield 1.5mg (44%)
Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) R_f = 0.67 (UV active)
δ_H (360 MHz, $CDCl_3$) 1.37 (d, 3H, Ala-C$\underline{H}_3$, *J*7); 1.45 (s, 9H, $C_4\underline{H}_9$); 3.2-3.4 (dd, 2H, Phe-C$\underline{H}_2$, *J*6,14); 3.39 (m, 1H) and 3.75 (m, 1H) of Ser(^{t}Bu)-C$\underline{H}_2$O; 3.71 (s, 3H, OC$\underline{H}_3$); 4.21 (m, 2H, Fmoc C$\underline{H}$ & Ser(^{t}Bu)-αC$\underline{H}$,*J* 7); 4.37 (m, 2H, Fmoc C$\underline{H}_2$O); 4.52 (m, 1H, Ala-αC$\underline{H}$);. 4.72 (m, 1H, Phe-αC$\underline{H}$); 5.71 (d, 1H, Ser(^{t}Bu)-N$\underline{H}$,*J* 6); 6.00 (d, 1H, Ala-N$\underline{H}$,*J* 8); 7.03 (d, 1H, Phe-N$\underline{H}$,*J* 7); 7.27-7.44 (m, 5H, Phe-C$\underline{H}$); 7.41 (m, 1H, Fmoc-C$\underline{H}$); 7.60 (m, 1H, Fmoc-C$\underline{H}$); 7.79 (m, 1H, Fmoc-C$\underline{H}$).
HPLC analysis showed a number of compounds to be present.
Elution conditions:0min: 50% A, 50% B; 2min: 50% A, 50% B; 32min: 100% A, 0% B; 37min: 100% A, 0% B; 42min: 50% A, 50% B, where A= 0.1%TFA/MeCN and B= 0.1%TFA/H_2O.

Thermolysis of H_2N-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfoxo) S-acetamidomethyl ethyl ester tentagel resin (19)



(**19**) (100mg) was refluxed in distilled water (20ml) (pH7) under nitrogen for 20h. The crude, freeze dried peptide was dissolved in D_2O for analysis.

Yield 6.4mg (still containing some water)

Tlc: (1-BuOH:NH_3) (70:30) $R_f = 0.48$ (ninhydrin active)

δ_H (300 MHz, D_2O) 1.17 (d, 3H, Ala-C$\underline{H}_3$, *J*7); 1.30 (s, 9H, $C_4\underline{H}_9$);3.15-3.30 (dd, 2H, Phe-C$\underline{H}_2$, *J*7,18); 3.40 (t, 1H,*J*5) and 3.77 (m, 1H) of Ser(^{t}Bu)-C$\underline{H}_2$O; 3.71 (s, 3H, COO$\underline{H}$); 4.05 (m, 1H, Ser(^{t}Bu)-αC$\underline{H}$); 4.52 (m, 1H, Ala-αC$\underline{H}$);. 4.82 (m, 1H, Phe-αC$\underline{H}$); 7.27-7.44 (m, 5H, Phe-C$\underline{H}$)

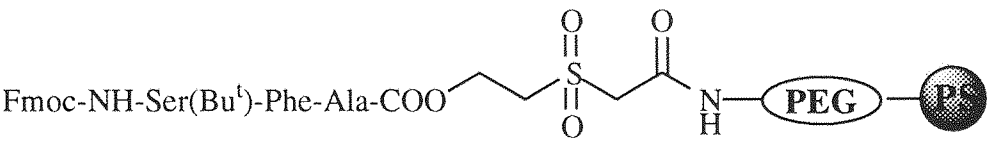
HR, ARGON FAB,(MNOBA matrix): 379 $[M+H]^+$, $C_{19}H_{29}O_5N$ Calc. 380.2185, Found 379.4262.

HPLC analysis showed a number of compounds to be present.

Elution conditions:0min: 0% A, 100% B; 10min: 0% A, 100% B; 35min: 100% A, 0% B; 45min: 100% A, 0% B; 55min: 0% A, 100% B, where A= 0.1%TFA/MeCN and B= 0.1%TFA/H_2O).

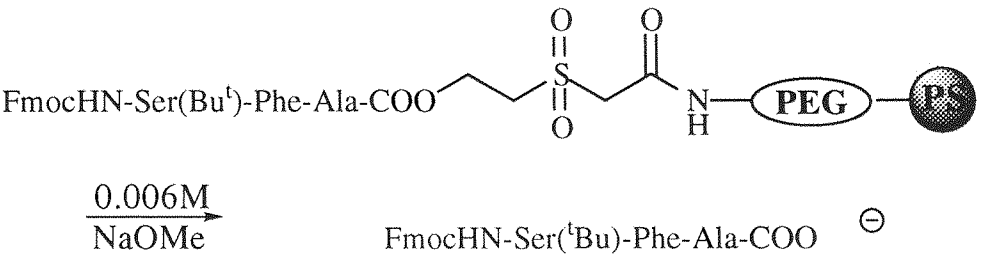
UV absorbance at 220nm

Preparation of Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfonyl) S-acetamidomethyl ethyl ester tentagel resin (20)



See Method E for oxidation conditions.

Elimination of Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-sulfonyl) S-acetamidomethyl ethyl ester tentagel resin (20a)



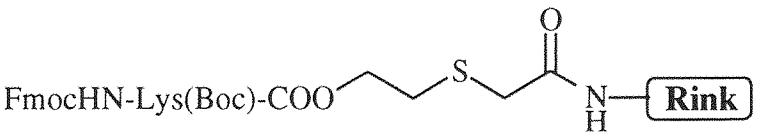
The title sulfone (23mg) pre-swollen in DCM (3ml) was suspended in 0.006M sodium methoxide in methanol (3ml). The solution was stirred at room temperature for 3 hr. The resin was then filtered, washed with water (5ml) and the resulting filtrate was diluted with water (5ml), acidified to pH 3 with 2M potassium hydrogen sulfate and extracted with EtOAc (3x10ml). The combined organic extracts were washed with brine (10ml), dried over sodium sulfate and the solvent removed *in vacuo* to give a white crystalline solid.

Yield 0.5mg (34%)

Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) $R_f = 0.66$

HR, ARGON FAB,(MNOBA matrix): 601 $[M]^+$, $C_{34}H_{39}O_7N_3$ Calc. 601.1372, Found 601.1335.

Preparation of Fmoc-Lys(Boc)-(1-hydroxy-2-mercapto) S-acetamidomethyl ethyl ester Rink resin (21)

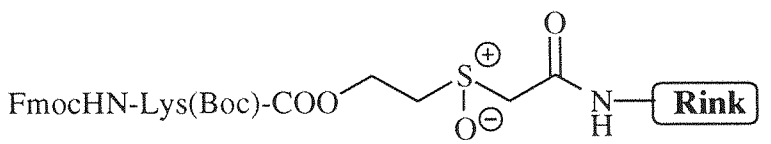


Reactions (12) - (14) were repeated (using the Rink amide resin in place of the aminomethyl resin). Esterification was followed as in (**15**), but using Fmoc-Lys(boc)-OH, in place of Boc-Ala -OH.

MALDI-TOF MS (DHB matrix): 222.3 $[M(Fmoc)+H]^+$; 484.1$[M+H]^+$;

After deprotection of the Fmoc group, substitution was measured by a quantitative ninhydrin test, 0.09mmol NH_2/g (40% coupling)

Preparation of Fmoc-Lys(Boc)-(1-hydroxy-2-sulfoxo) S-acetamidomethyl ethyl ester Rink resin (22)



See Method D for oxidation conditions.

MALDI-TOF MS (DHB matrix): 523.4$[M+Na]^+$; 537.8$[M+K]^+$

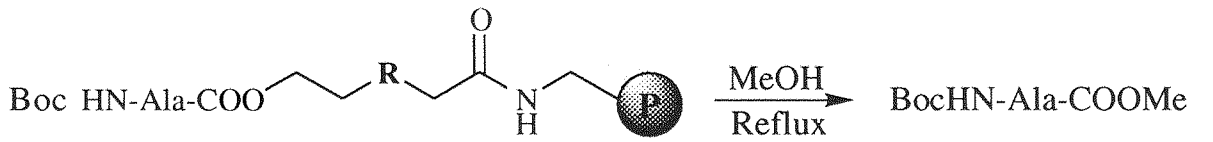
4.5 Control studies

4.5.1 Using Merrifield's resin

R=S, **R**^{1}=SO, **R**^{2}=SO_2

Boc-Ala-(1-hydroxy-2-mercapto) S-methyl polystyrene ethyl ester resin (**R**) (**6**) (50mg), sulfoxo (**R**^{1}) (**7a**) (50mg), and sulfone (**9**) (**R**^{2}) (50mg), were suspended in MeOH (7ml) and heated to reflux for 18h under nitrogen. The solutions were monitored periodically by t.l.c analysis. The resins were filtered and washed with MeOH (2 x 10ml). The filtrates were then collected and dried *in vacuo.*
Yield for (**R**) No material was recovered
Yield for (**R**^{1}) 4.7mg
Yield for (**R**^{2}) No material was recovered

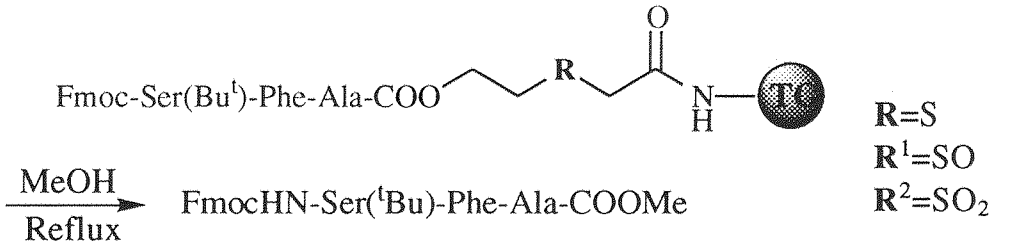
4.5.2 Using Aminomethyl resin



R=S, **R**^{1}=SO, **R**^{2}=SO_2

Boc-Ala-(1-hydroxy-2-mercapto)-S-acetamidomethyl ethyl ester resin (**R**) (**15**) (74.6mg), sulfoxo (**R**^{1}) (**16a**) (74.6mg), and sulfone (**R**^{2}) (**17**) (74.6mg), were suspended in MeOH (15ml) and heated to reflux for 2h under nitrogen. The solutions were monitored periodically by t.l.c analysis. The resins were filtered and washed with MeOH (2 x 10ml). The filtrates were then collected and dried *in vacuo.*
Yield for (**R**) No material was recovered
Yield for (**R**^{1}) 6.7mg
Yield for (**R**^{2}) No material was recovered

4.5.3 Using Tentagel resin



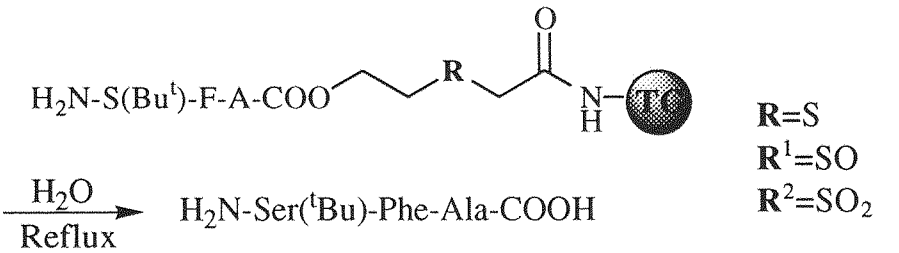
Fmoc-Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-mercapto) S-acetamidomethyl ethyl ester tentagel resin (**R**) (50mg), sulfoxo (**R^1**) (**18a**) (50mg), and sulfone (**R^2**) (**20a**) (50mg), were suspended in MeOH (10ml) and heated to reflux for 16h under nitrogen. The solutions were monitored periodically by t.l.c analysis. The resins were filtered and washed with MeOH (2 x 10ml). The filtrates were then collected and dried *in vacuo*.

Yield for (**R**) No material was recovered

Yield for (**R^1**) 1.5mg, Tlc: (EtOAc:Pet Ether:AcOH) (80:20:1) $R_f = 0.67$ (UVactive)

Yield for (**R^2**) No material was recovered

4.5.4 Tentagel resin (water study)



The above resin, Ser(^{t}Bu)-Phe-Ala-(1-hydroxy-2-mercapto) S-acetamidomethyl ethyl ester tentagel resin (**R**) (100mg), sulfoxo (**R^1**) (**19**) (100mg), and sulfone (**R^2**) (100mg), were suspended in distilled water (20ml) and heated to reflux for 20h under nitrogen. The solutions were monitored periodically by t.l.c analysis. The resins were filtered and washed with H_2O (2 x 10ml). The filtrates were then collected and dried *in vacuo*.

Yield for (**R**) No material was recovered

Yield for (**R^1**) 6.4mg, Tlc: (1-BuOH:NH3) 70:30 $R_f = 0.48$ (Ninhydrin active)

Yield for (**R^2**) No material was recovered

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