Novel Linkers for Solid-phase Synthesis

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The University of Edinburgh

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This thesis is dedicated to John Watson Masson
Acknowledgements

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Declaration

This thesis is submitted in part fulfilment of the requirement for the degree of Doctor of Philosophy at the University of Edinburgh. Unless otherwise stated, the work is original, and has not been previously submitted, in whole or in part, for any degree at this, or any other university.
Abstract

One of the most critical factors in the success of a solid-phase synthesis is the choice of linker. The linker must be stable during synthesis then cleaved under conditions that do not damage the product. Ideally then the cleavage conditions should be both mild and selective. For this reason resin/linker systems such as 2 have been designed for cleavage by enzyme. 2 was synthesised using a variety of spacers and solid supports, starting from either soluble linker 1 or support-bound aldehyde 3. Activation of the ethylthiolate group of 2 and subsequent displacement by primary and secondary alcohols gave 4 in good yield. Both enzymatic and acidic cleavage of alcohol from 4 was investigated. As the acid stability of 4 could be modulated depending on the nature of the spacer it was possible to induce cleavage under very mild acidic conditions.

The utility of 2 was demonstrated by coupling of Fmoc serine methyl ester through the side-chain hydroxyl functionality and subsequent elaboration to a pentapeptide (H-SAVSS-OMe). This approach could find application in the synthesis of cyclic peptides and peptides with modified C-terminii.
### Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>Ac</td>
<td>acetyl</td>
</tr>
<tr>
<td>AMP</td>
<td>adenosine monophosphate</td>
</tr>
<tr>
<td>6-APA</td>
<td>6-aminopenicillanic acid</td>
</tr>
<tr>
<td>approx.</td>
<td>approximately</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>Av.</td>
<td>average</td>
</tr>
<tr>
<td>Boc</td>
<td>butoxycarbonyl</td>
</tr>
<tr>
<td>Bt</td>
<td>benzotriazole</td>
</tr>
<tr>
<td>Bu'</td>
<td>tertiary butyl</td>
</tr>
<tr>
<td>BTAC</td>
<td>benzyltrimethylammonium chloride</td>
</tr>
<tr>
<td>Bzl</td>
<td>benzyl</td>
</tr>
<tr>
<td>cat.</td>
<td>catalytic</td>
</tr>
<tr>
<td>CMP</td>
<td>cytidine monophosphate</td>
</tr>
<tr>
<td>CPG</td>
<td>controlled pore glass</td>
</tr>
<tr>
<td>CT</td>
<td>chymotrypsin</td>
</tr>
<tr>
<td>d</td>
<td>doublet</td>
</tr>
<tr>
<td>DBU</td>
<td>1,8-diazabicyclo[5.4.0]undec-7-ene</td>
</tr>
<tr>
<td>Dde</td>
<td>1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl</td>
</tr>
<tr>
<td>DEAD</td>
<td>diethylazodicarboxylate</td>
</tr>
<tr>
<td>DIC</td>
<td>diisopropylcarbodiimide</td>
</tr>
<tr>
<td>DIEA</td>
<td>diisopropylethylamine</td>
</tr>
<tr>
<td>DHP</td>
<td>dihydropyran</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-dimethylaminopyridine</td>
</tr>
<tr>
<td>DMF</td>
<td>$N,N$-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>dimethylsulphoxide</td>
</tr>
<tr>
<td>DTT</td>
<td>dithiothreitol</td>
</tr>
<tr>
<td>EEDQ</td>
<td>2-ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline</td>
</tr>
<tr>
<td>EI</td>
<td>electron impact</td>
</tr>
<tr>
<td>equiv.</td>
<td>equivalent(s)</td>
</tr>
<tr>
<td>ESI</td>
<td>electrospray ionisation</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
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<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>FAB</td>
<td>fast atom bombardment</td>
</tr>
<tr>
<td>Fmoc</td>
<td>9-fluorenylmethoxycarbonyl</td>
</tr>
<tr>
<td>Fmoc-aa-OH</td>
<td>Fmoc amino acid</td>
</tr>
<tr>
<td>Gal</td>
<td>galactose</td>
</tr>
<tr>
<td>GDP</td>
<td>guanosine diphosphate</td>
</tr>
<tr>
<td>Glc</td>
<td>glucose</td>
</tr>
<tr>
<td>GlcNAc</td>
<td>N-acetylglucosamine</td>
</tr>
<tr>
<td>h</td>
<td>hour(s)</td>
</tr>
<tr>
<td>HMP</td>
<td>4-hydroxymethylphenyl</td>
</tr>
<tr>
<td>HMBA</td>
<td>4-hydroxymethylbenzoic acid</td>
</tr>
<tr>
<td>HMPA</td>
<td>4-hydroxymethylphenoxyacetic acid</td>
</tr>
<tr>
<td>HOBt</td>
<td>1-hydroxybenzotriazole</td>
</tr>
<tr>
<td>HOct</td>
<td>ethyl 1-hydroxy-1H-1,2,3-triazole-4-carboxylate</td>
</tr>
<tr>
<td>HPLC</td>
<td>high performance liquid chromatography</td>
</tr>
<tr>
<td>IBX</td>
<td>o-iodoxybenzoic acid</td>
</tr>
<tr>
<td>IR</td>
<td>Infra-red</td>
</tr>
<tr>
<td>Lac</td>
<td>Lactose</td>
</tr>
<tr>
<td>m</td>
<td>multiplet</td>
</tr>
<tr>
<td>max.</td>
<td>maximum</td>
</tr>
<tr>
<td>mp</td>
<td>melting point</td>
</tr>
<tr>
<td>MS</td>
<td>mass spectrometry</td>
</tr>
<tr>
<td>Nbb</td>
<td>4-hydroxymethyl-3-nitrobenzamide</td>
</tr>
<tr>
<td>NeuAc</td>
<td>N-acetylneuraminate (sialic acid)</td>
</tr>
<tr>
<td>NIS</td>
<td>N-iodosuccinimide</td>
</tr>
<tr>
<td>NMM</td>
<td>N-methyl morpholine</td>
</tr>
<tr>
<td>NMR</td>
<td>nuclear magnetic resonance</td>
</tr>
<tr>
<td>PA</td>
<td>phenylacetic acid</td>
</tr>
<tr>
<td>PAM</td>
<td>phenylacetamide</td>
</tr>
<tr>
<td>PCC</td>
<td>pyridinium chlorochromate</td>
</tr>
<tr>
<td>PEG</td>
<td>polyethylene glycol</td>
</tr>
<tr>
<td>PPT</td>
<td>porcine pancreatic trypsin</td>
</tr>
<tr>
<td>PPTS</td>
<td>pyridinium p-toluenesulphonate</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------------------------------------</td>
</tr>
<tr>
<td>PTP</td>
<td>protein tyrosine phosphatase</td>
</tr>
<tr>
<td>q</td>
<td>quartet</td>
</tr>
<tr>
<td>quin.</td>
<td>quintet</td>
</tr>
<tr>
<td>ROESY</td>
<td>rotating-frame overhauser spectroscopy</td>
</tr>
<tr>
<td>RT</td>
<td>room temperature</td>
</tr>
<tr>
<td>s</td>
<td>singlet</td>
</tr>
<tr>
<td>SRF</td>
<td>serum response factor</td>
</tr>
<tr>
<td>t</td>
<td>triplet</td>
</tr>
<tr>
<td>TBAF</td>
<td>tetrabutylammonium fluoride</td>
</tr>
<tr>
<td>TBTU</td>
<td>2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate</td>
</tr>
<tr>
<td>TEA</td>
<td>triethylamine</td>
</tr>
<tr>
<td>TEMPO</td>
<td>2,2,6,6-tetramethylpiperidinyl-1-oxy</td>
</tr>
<tr>
<td>TFA</td>
<td>trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>tetrahydrofuran</td>
</tr>
<tr>
<td>THP</td>
<td>tetrahydropyran</td>
</tr>
<tr>
<td>TOCSY</td>
<td>total correlation spectroscopy</td>
</tr>
<tr>
<td>Trt</td>
<td>trityl</td>
</tr>
<tr>
<td>pTSA</td>
<td>p-toluenesulfonic acid</td>
</tr>
<tr>
<td>UDP</td>
<td>uridine diphosphate</td>
</tr>
</tbody>
</table>
In the introduction to this thesis a number of resins are discussed with reference to the literature. Where feasible attempts have been made to accurately describe the nature of the solid support. In some instances this has simply not been possible due to the limited information provided about the solid support in the original reference. The following symbols have been used as abbreviations for general types of solid supports.

This has been used to represent a linkage unspecified in the paper under discussion.

This symbol has been used to represent a solid support which has been unspecified in the paper under discussion or where a number of solid supports have been used

ArgoGel

Controlled pore glass

This is the symbol indicates a PEGA resin. Any additional structural information is given in the text.

Polyamide

Polystyrene
The resins which have been used in the experimental section of this thesis are illustrated below with appropriate abbreviations.

The amino PEGA resin used consisted of a co-polymer of bis-2-acrylamidoprop-1-yl polyethyleneglycol\textsubscript{800}, 2-acrylamidoprop-1-yl[2-aminoprop-1-yl]polyethylene glycol\textsubscript{800} and dimethylacrylamide.

PEGA resins with different functionality were all derived from this resin.

The polystyrene resins used were based on copoly(styrene-1\%divinylbenzene), for example carboxypolystyrene:

Where structures have been derived from formylpolystyrene, part of the polymer backbone has also been drawn to highlight the fact that there is an alkyl group para to the aldehyde as this is of particular relevance in the discussion.
formylpolystyrene

The NovaSyn® TG resins used were based on a composite of low cross-linked polystyrene and 3000-4000 molecular weight polyethylene glycol which had been terminally functionalised, for example NovaSyn® TG amino resin shown below. NovaSyn® TG carboxy resin and NovaSyn® TG acetal resin were prepared from the NovaSyn® TG amino resin.
Numbering

Occasionally, the same support-bound compound was prepared by more than one route. Where this was the case the support bound compounds have been given the same number followed by a letter to distinguish between them. It is important to acknowledge that the compound has been prepared by different routes because although the desired product is the same in each case any support bound impurities present may be different.
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1. INTRODUCTION

1.1 Solid-Phase Synthesis
Solid-phase synthesis entails the attachment of a substrate molecule to an insoluble polymeric support by means of a cleavable linker. Upon elaboration of the support-bound substrate the linker must remain stable. At the end of the synthesis the linker is cleaved to release the product from the solid support. The conditions which induce this cleavage should be sufficiently mild that the product is not damaged. This chemistry, was first used in 1963 for the solid-phase synthesis of peptides\(^1\) and has several advantages over solution synthesis. Purification protocols are reduced to a simple filtration of the resin-bound material. This allows the use of a large excess of reagents to drive reactions to completion as soluble reagents and by-products are easily washed away. Another important advantage associated with solid-phase synthesis it that it is easily amenable to automation. It is not surprising then that since its inception many different classes of compounds have been synthesised on solid support\(^2-4\). Before beginning such a synthesis consideration has to be given to the choice of linker.

1.2 Linkers Used for the Immobilisation of Alcohols and Phenols
Many biologically important compounds, such as prostaglandins\(^5\) and peptaibols\(^6\), contain a hydroxy functionality that could be used for attachment to a solid support in the same way as the carboxy functionality of amino acids is used in solid-phase peptide synthesis. To allow classical solid-phase synthesis of C-terminal peptide amino alcohols, N-protected amino alcohols have been esterified with succinic anhydride (Scheme 1). The resulting hemi-succinates were attached to the solid support (chloromethyl polystyrene) by the Cs-salt method\(^7\). Peptide synthesis was carried out using the classical Boc-TFA strategy to produce amino alcohol analogues of enkephalin and somatostatin. Release from the resin, with simultaneous side-chain deprotection was accomplished using anhydrous HF. The peptide hemi-succinate was easily cleaved by mild saponification to give the CH\(_2\)OH analogue.
This approach was simultaneously applied to the synthesis of a number of tetrapeptide alcohols by Swistok et al. Although in this instance the free alcohol was released from the support by hydrolysis using either NH₃/MeOH or excess hydrazine in DMF. A similar rational was used in the solid-phase synthesis of N-(alkoxyacyl)amino alcohols. However, instead of derivatising the amino alcohol, the amino TentaGel solid support was derivatised with glutaric acid. This was esterified with an amino alcohol and after synthetic manipulation NaOH hydrolysis was used to cleave the ester linkage. Ester linkages have also been formed directly to the solid support (acid chloride resin prepared from chloromethylpolystyrene). Cleavage took place on treatment with a 1:1 mixture of dioxane and concentrated ammonia. Attachment of alcohols to chloromethylpolystyrene itself resulted in an ether linkage traditionally cleaved by acidolysis or hydrogenolysis. More recently this type of linkage has been cleaved using tin tetrachloride.

There are also several examples where linkers used for the immobilisation of alcohols have been cleaved by acidic rather than basic hydrolysis. The Rink linker (4, Scheme 2) was used as an acid labile linker for the synthesis of peptide amides. Since then primary alcohols and a phenol functionality have been coupled to the chloride derivative (5, Scheme 2) under mild conditions though no examples of transformation carried out on the bound alcohols were described. The alcohols were released under relatively mild acidic conditions using 5% TFA in CH₂Cl₂.
Anchoring of amino acids to solid support through side chain functionality has been used in the solid-phase synthesis of cyclic peptides\textsuperscript{15}. In this case the aim was not the synthesis of a cyclic peptide but of a peptide with a modified C-terminus in addition to a urea functionality\textsuperscript{16}. The tyrosinol derivative 7 was coupled to the brominated Wang linker derivative 8 to give 9 (Scheme 3) which was then deprotected and coupled to amino functionalised resin (aminomethyl polystyrene bearing an amino PEG spacer).

*Scheme 2 Coupling alcohols to the Rink linker*
Scheme 3 Coupling of tyrosinol derivative and Wang linker derivative

Direct coupling of a phenol to Wang resin has been carried out using the Mitsunobu reaction (Scheme 4).

Scheme 4 Mitsunobu reaction on solid support

A trichloroacetimidate coupling method (Scheme 5) has also been used to couple primary, secondary and tertiary alcohols to Wang resin and polyethyleneglycol hydroxymethylphenyl resin (HMP-TentaGel) in good yield.
Scheme 5 Coupling to solid support using p-alkoxybenzyl trichloroacetimidates

Monoacylation of primary and secondary diols attached by this method was achieved in good yield. Previously, symmetrical diols had been monoprotected by trityl chloride resin 16 (Figure 1) allowing the remaining hydroxyl group to be selectively benzoylated 18. Again treatment with acid was used to effect cleavage. Trityl chloride resin has been used in several solid-phase applications requiring the immobilisation of alcohols and phenols including a one pot synthesis of isoxazolones 19. Various trityl based resins are now commercially available amongst which are trityl chloride resin 16 and 2-chlorotrityl resin 17 (Figure 1).

Figure 1 Trityl Based Linkers

2-Chlorotrityl resin 17 has been used in a solid-phase synthesis of peptaibols 6. Peptaibols are antibiotic peptides which contain a C-terminal amino alcohol. The resin was loaded with Fmoc amino alcohols and automated peptide synthesis was performed. Cleavage of the ether bond to release the product peptaibols from the solid support was accomplished by a cleavage cocktail containing 50% TFA. Although trityl based linkers have been used to immobilise various of nucleophiles
their acid sensitivity makes them only useful for a limited range of reactions. The phenylfluorenyl linker system (PhFl linker) was developed to have the broad applicability of the trityl group accompanied by increased acid stability\textsuperscript{20}. This linker was coupled to amino modified solid supports to give 18 (Scheme 6) which was activated by treatment with acetyl chloride. The chloride could then be displaced by an acid or an amine.

\[
\text{Scheme 6 The PhFl linker}
\]

Increased acid stability of this system over the trityl linker was observed however no example of alcohol coupling were given. Increased acid stability can be advantageous in some cases but substrates such as prostaglandins are delicate structures which are sensitive to acidic and basic conditions. In the solid-phase synthesis of a variety of prostaglandins the alcohol starting material was coupled to a dibutylsilyl chloride substituted resin\textsuperscript{5}. The core alcohol structures were elaborated then cleaved from the support by dilute HF/pyridine. This is one of the few cleavage reagents compatible with the $\beta$-hydroxy ketone of the E series of prostaglandins. Similarly, a diphenylsilyl chloride resin was used in the synthesis of fall army moth sex pheromone\textsuperscript{21}. Coupling of the alcohol to the solid support was performed in the presence of base and cleavage was effected by fluoridolysis using tetrabutylammonium fluoride (TBAF). A silyl ether based linker was also used for
the solid-phase synthesis of glycopeptides$^{22}$ attached to the solid support through the side chain hydroxyl of an amino acid. The product was released using CsF and AcOH in THF in the presence of 18-crown-6. A slightly different silyl ether based linker was used by Routledge et al.$^{23}$ $^{20}$ (Scheme 7). The hydroxyl functionality of the resin bound linker was activated by carbonyldiimidazole then coupled with an alcohol. Cleavage was effected with $\text{F}^-$ which attacks at the exo-silyl moiety resulting in alcohol release.

\[
\begin{align*}
\text{Me}_3\text{Si} & \quad \text{O} \quad \text{PS} \\
\text{OH} & \\
20 & \\
\downarrow & \text{carbonyldiimidazole} \\
\downarrow & \text{pyridine} \\
\downarrow & \text{CH}_2\text{Cl}_2 \\
\text{Me}_3\text{Si} & \quad \text{O} \quad \text{PS} \\
\text{O} & \text{N} \\
\text{II} & \\
21 & \\
\downarrow & \text{ROH} \\
\downarrow & \text{DBU} \\
\downarrow & \text{CH}_2\text{Cl}_2 \\
\text{Me}_3\text{Si} & \quad \text{O} \quad \text{PS} \\
\text{O} & \text{OR} \\
22 & \\
\end{align*}
\]

\textbf{Scheme 7} Silyl linker possessing an exo cleavage site

The ester and silyl ether linkages described so far are limited by the lability of the attachment functionality to nucleophilic or basic reagents. For this reason a base stable tetrahydropyran (THP) linkage was developed. Ellman et al.$^{24}$ designed a dihydropyran (DHP) functionalised resin 23 (Scheme 8). Acid catalysed attachment
of an alcohol gives a base stable THP linkage. Cleavage from the resin is accomplished by acid. Primary and secondary alcohols were coupled in good yield but tertiary alcohols gave poor loadings.

Scheme 8 Attachment of alcohols to DHP functionalised resin

This linkage has found several applications. It was used in the synthesis of a library of inhibitors of the protease cathepsin D based about the (hydroxyethyl)amine isostere\textsuperscript{25}. In an analogous approach a second linker based on the DHP protecting group was synthesised\textsuperscript{26}. This method differs from that of Ellman in that the alcohol was coupled to dihydropyran-2-carboxylic acid benzyl ester \textsuperscript{25} (Scheme 9) which was then deprotected and coupled to an amine resin.

Scheme 9 Use of the dihydropyran-2-carboxylic acid linker

It is easier to couple primary, secondary or even tertiary alcohols to this bifunctional linker than to Ellman's DHP system. The bifunctional linker was used in the solid-phase synthesis of the peptide alcohol octreotide, a metabolically stable somatostatin analogue\textsuperscript{26}. A second solid-phase synthesis of octreotide was carried out used \textit{p}-carboxybenzaldehyde to anchor Fmoc-threoninol to an amine resin (Figure 2) \textsuperscript{27}.  

8
The acetal linkage was stable during the stepwise elongation of the peptide which was released by a cleavage mixture containing 90% TFA. Oxidation in solution gave the disulphide bond of octreotide. Diols have been immobilised in an analogous fashion as 1,3-dioxane and 1,3-dioxolane acetals by reaction with Wang aldehyde resin\textsuperscript{28}. The linkage produced when an alcohol or phenol was coupled to the active carbonate resin 30 (Figure 3)\textsuperscript{29} was also cleaved by acid.

This linkage was compatible with the Boc/Bzl peptide synthesis strategy and a number of peptide alcohols were synthesised in demonstration of this fact. Cleavage and concomitant removal of Bzl side chain protection was effected using strong acid. The use such of harsh cleavage conditions can also be avoided by using linkers which are cleaved by photolysis. Coupling of alcohols or phenols to the active carbonate resin 31 (Figure 4) produced from 4-hydroxymethyl-3-nitrobenzamide
(Nbb) polystyrene resin (prepared from 4-hydroxymethyl-3-nitrobenzoic acid and 4-methylbenzhydrylamine resin) results in a linker which can be cleaved by photolysis.

![Chemical structure](image)

**Figure 4** *Active carbonate resin prepared from 4-hydroxymethyl-3-nitrobenzamide (Nbb) polystyrene resin*

Routledge *et al* used the latent photolabile linker 32 (Scheme 10) which was based on a dithane protected substituted benzoin.

![Chemical structures](image)

**Scheme 10** *Use of the dithiane protected 3-alkoxybenzoin photolabile linker*

Carboxyldiimidazole was used to activate the secondary hydroxyl of 32 to produce 33 and alcohol coupling then gave carbonate 34. Removal of the dithane protecting group from 34 followed by photolysis resulted in alcohol release.
Another linkage strategy which avoids the use of strong acid or base is that designed by Zheng et al. Support-bound linker 36 (Scheme 11) was esterified with N-Boc ethanolamine and peptide synthesis was carried out, during which the quinone moiety was stable. The peptides were liberated by reduction of the linkage with sodium hydrosulfite in THF/water.

![Scheme 11](image)

*Scheme 11 A redox sensitive linker system*

Another way to avoid the use of harsh cleavage conditions is the use of enzymes as cleavage agents. There are several examples of enzymes being used in reactions with
immobilised substrates (Section 1.3 and references therein) but only a few where they are used for the cleavage of linkers\textsuperscript{32-34} (Sections 1.4 and 1.5).

1.3 Enzymatic Reactions Involving Support-bound Substrates

There are many potential advantages in using enzymes for modification of resin-bound substrates such as specificity of reaction and mild reaction conditions. When carrying out enzymatic reactions as part of a solid-phase synthesis these advantages are combined with those inherent in solid-phase chemistry. In enzymatic systems that use complex mixtures of enzymes, cofactors and buffers working up reactions by filtration is particularly appealing. The main difficulty in performing enzymatic reactions on solid supports is limited enzymic accessibility to substrates bound to the insoluble polymer. The polystyrene based resins traditionally used in solid-phase synthesis have been shown to exclude enzymes completely\textsuperscript{35}. Ideally, the resin should be suited to both chemical and enzymatic transformations. It should be chemically and mechanically stable under a wide range of reaction conditions and compatible with the aqueous conditions used for enzymatic reactions. Resins suitable for solid-phase enzymatic synthesis are often also appropriate for use in solid-phase assays. In such assays the ligands remain covalently attached to the solid support. The assays may involve binding of the target to the ligand on solid support (binding assay) or detection of functional properties for example proteolytic or phosphorylation substrates (functional assay)\textsuperscript{36}. Therefore, solid-phase functional assays also involve enzymatic reactions on solid support. The types of resin that have been successfully used with enzymes fall into two broad categories, those where the resin swells to allow penetration (at least to some degree) by the enzyme, for example PEGA resins and those where reaction occurs at a functionalised surface, for example controlled pore glass (CPG).

1.3.1 Enzymatic Reactions on Non-Swelling Solid Supports

Slomczynska\textsuperscript{37} carried out a comparative study of the suitability of four solid supports for use in enzymatic synthesis. These supports were polyacrylamide, polyethyleneglycol-polystyrene, CPG and Kel-F-g-styrene. Polyacrylamide and polyethylene glycol cross linked polystyrene graft resin have functional sites
distributed throughout the polymer matrix (these types of resin are discussed in greater detail in Section 1.3.2). Both swell in organic and aqueous media. In contrast CPG based supports are porous, non-swellable, rigid matrices. Kel-F-g-styrene is a non cross-linked support which consists of an impermeable core surrounded by a mobile layer of linear polystyrene chains. α-Chymotrypsin and papain catalysed hydrolysis of substrates bound to each of the solid supports were selected as model reactions. The reaction conditions were derived from solution-phase experiments but the enzyme concentration and reaction time were increased. CPG was found to be the best support. The use of a spacer, (Gly)$_{10}$ and/or material with larger pore-size significantly improved yields. The hydrolysis was also dependant on the amount of miscible co-solvent used. CPG supports 1270 and 3000 were selected as carriers to study the enzymatic coupling of peptide segments catalysed by papain. The coupling yields were moderate (31-54%) but this did demonstrate that CPG could be used with enzymes. As the suitability of CPG for use with enzymes had been demonstrated and it is a solid support routinely used in the solid-phase synthesis of oligonucleotides it is not surprising that enzymes have been used in oligonucleotide synthesis on CPG. Penicillin G acylase was used to remove the phenylacetyl groups from an oligonucleotide bound to CPG$^{38}$. Support-bound protected oligonucleotide 39 (Scheme 12) was treated with enzyme to give immobilised deprotected oligonucleotide 40 (Scheme 12). The oligonucleotide was released from the support and enzymatic digestion followed by HPLC showed only deprotected deoxyguanosine and thymidine were present. When the enzyme cleavage step was omitted from this protocol protected nucleoside was observed.
An enzyme has also been used for the purification of CPG supported oligodeoxyribonucleotides\textsuperscript{39}. The last residue coupled to the support bound oligonucleotide possessed a 5'-hydroxyl protecting group (benzoyl) orthogonal to that used for the capping of truncated failure sequences (levulinoyl). The protecting group was removed from the failure sequences and the support treated with exonuclease spleen phosphodiesterase. This enzyme requires a free 5'-hydroxyl for recognition and cleavage of oligonucleotides and therefore specifically hydrolysed the failure sequences in the presence of the 5'-protected target. The procedure was carried out on long chain alkylamine CPG (500 Å). From analysis of the product released after treatment with enzyme it was observed that the phosphodiesterase was unable to hydrolyse fragments smaller than 10 bases. Increased reaction time or additional enzyme did not reduce the concentration of these fragments suggesting they were inaccessible to the enzyme.

Enzymes have also been used in oligonucleotide synthesis on long chain alkylamine CPG to extend the polymer from the 3' end by enzymatic ligation with another oligonucleotide or a polymerase and nucleotide triphosphates opposite a template. In some examples near quantitative yield was obtained for the ligation reaction\textsuperscript{40}.

CPG has been also been used in solid-phase oligosaccharide synthesis\textsuperscript{41}. The cesium salt of a disaccharide acceptor \textsuperscript{41} was attached to commercially available \textit{N}-iodoacetate aminopropyl CPG \textit{via} a nucleophilic displacement (Scheme 13).
Glycosylation of 43 using UDP-Gal and β-1,4-galactosyltransferase then CMP-NeuAc and α-2,3-sialyltransferase was accomplished in excellent yield (>98% for each step). However, when CPG was used as support for the chemical-enzymatic synthesis of the O-sialyl-LacNAc octapeptide\textsuperscript{42} 46 (Scheme 14) the results were less encouraging.
Scheme 14 Chemical-enzymatic synthesis of the O-sialyl-LacNac octapeptide 46 on solid support

The yield for the synthesis was 9% (based on the initial amino acid loading of the resin). This low yield was, at least in part, due to the poor yield for the chemical synthesis on CPG. This was perhaps not unexpected as CPG is not the optimal solid support for peptide synthesis. Like CPG, sepharose (a polysaccharide based support\textsuperscript{43}) has been used with galactosyl transferase enzyme. GlcNAc-β-hexanolamine was coupled to a Sepharose gel by CNBr activation which results in base labile bonds. Partially purified UDP galactosyltransferase was used for the preparation of Galβ(1→4)GlcNAc-β-hexanolamine. After 18h, approximately 85% of the theoretical transfer had taken place and the addition of more enzyme or UDP-Gal did not increase the extent of reaction. Treatment with 2M NaOH released 80% of the ligand\textsuperscript{44}. The glycoside thus produced was found to be identical to the product formed by the enzyme acting on GlcNAc-β-hexanolaolamine in solution. This indicated that the matrix did not affect the specificity of the enzyme. More recently
the solid-phase enzymatic synthesis of a sialyl-Lewis X tetrasaccharide has also been carried out on a sepharose matrix\textsuperscript{45}. Firstly, a model reaction was carried out using sepharose gels with different lengths of a PEG type spacer as it was thought that increased spacer length would improve access of the enzyme. 1,4-\(\beta\)-galactosylation of \(N\)-acetyl glucosamine using a galactosyl transferase enzyme was selected as the model reaction (Scheme 15).

\begin{center}
\textbf{Scheme 15} Enzymatic galactosylation of \(N\)-acetyl glucosamine on Sepharose
\end{center}

The yields for transfer ranged from 70\% using a 12 atom spacer to 98\% using a 71 atom spacer. The gel with the 71 atom linker was used in the synthesis of a sialyl-Lewis X tetrasaccharide (Scheme 16).
The enzymatic transformations were carried out with yields of 75% (not optimised) to 95% and the purified product was obtained in a yield of 57%. The importance of an appropriate spacer was also borne out in a study by Könnecke et al.\textsuperscript{46} using a silica support. A support-bound leucine was used as the nucleophile in protease mediated couplings. The coupling experiments were carried out using thermolysin, \( \alpha \)-chymotrypsin and papain with suitable carboxyl components. For this experiment aminopropylsilica was derivatised with a spacer bearing the necessary leucine (Leu-Aca-Leu-Aca-aminopropylsilica, where Aca is a 6-aminohexanoyl unit). The thermolysin mediated coupling was achieved in a yield of 60%. However, the yield for the \( \alpha \)-chymostrypsin coupling was only 20% and no product was observed for the papain coupling. \( \alpha \)-Chymostrypsin and papain both have esterase activity and the hydrolysis of the esters, which were used as the source of carboxyl component, resulted in acids which were not coupled to the amine on the time-scale of the
experiment. Similar experiments carried out using supports with shorter spacers were completely unsuccessful.

A hexaglycine spacer was selected to derivatise aminopropyl silica for use in glycopeptide synthesis. β-1,4-Galactosyltransferase catalysed sugar chain elongation of a support-bound glycopeptide acceptor was followed by α-2,3-sialyltransferase catalysed sialylation. The yields for the enzyme catalysed reactions were 55% and 65% respectively. These yields were determined by cleavage of the α-chymotrypsin sensitive linker (discussed in Section 1.4) used to anchor the glycopeptides to the support. As well as being employed in enzymatic synthesis a silica support was used in the solid-phase synthesis and identification of substrates for a protein tyrosine phosphatase (PTP) from a resin-bound library of phosphotyrosyl peptides in an on-bead functional assay.

A solid-phase phosphopeptide library was synthesised in which the tyrosine position was doped with 30% glycine to generate a sequencable tag (Scheme 17). The resin bound library was then subjected to on bead dephosphorylation with the chosen PTP then cleavage with α-chymotrypsin (CT) which is selective for those sequences which have undergone dephosphorylation. The free amino termini that resulted from the CT cleavage were then coupled to an amine reactive fluorophore or chromophore allowing the location of positive beads. The relevant peptides were then identified through sequencing of the encoding strand.
Scheme 17 On-bead identification of substrates for protein tyrosine phosphatases

This is a highly efficient example of an on-bead functional assay. However, despite this and other successes achieved with non-swelling resins the potential advantage of swelling resin over a surface is the homogeneous character of the resin interior allowing equal access to all substrate molecules.

1.3.2 Enzymatic Reactions Carried Out on Swellable Supports

One of the earliest attempts at polymer supported enzymatic synthesis was carried out by Zehavi et al\textsuperscript{48} using commercial aminoethyl substituted polyacrylamide beads. The polymer bound sugars 51, 52 and 53 acted as acceptors in galactosyltransferase catalysed reaction with UDP-\(\alpha\)-galactose as the donor under
the conditions of Nunez and Barker\textsuperscript{44}. \textbf{52} and \textbf{53} were N-acylated after attachment of the sugar/linker construct.

![Chemical structure of 51](image1)

![Chemical structure of 52](image2)

![Chemical structure of 53](image3)

**Figure 5** \textit{Polymer bound acceptors in galactosyltransferase reaction}

The yield for lactose produced by galactosyl transfer to \textbf{51} and \textbf{52} was 0.06\% and 0.36\% respectively. The yield of product for the galactosyl transfer to \textbf{53} was also very low (0.87\%). This general strategy, involving product release by photolysis, was used several times with other enzymes always with very low yields\textsuperscript{48-51}. A study was carried out to improve the accessibility of insoluble polyamide polymers in enzymatic reactions\textsuperscript{52}. In the model reaction 4-carboxy-2-nitrobenzyl $\beta$-D-glucopyranoside 54 (Figure 6) was coupled a variety of polyacrylamide beads. The accessibility of the glucose containing polymers in enzymatic glycosylations was then determined by using galactosyl transferase to prepare lactose.

![Chemical structure of 54](image4)

**Figure 6** \textit{4-Carboxy-2-nitrobenzyl $\beta$-D-glucopyranoside}
It was found that the length of spacer used had a dramatic effect on the enzymatic reaction. The loading of acceptor and swelling properties of the resins also appeared to affect the yield in some instances. This methodology was finally used to prepare lactose in an isolated yield of 51%. Polyacrylamide resins are permeated only by good hydrogen bond forming solvents such as water. Polyacrylamide modified by replacement of the primary amide by dimethylamide groups was freely permeated and swollen by dimethylformamide and other polar solvents including water. The modified commercial resin was expected to be appreciably labile under acidic conditions. To avoid this undesirable feature of the modified commercial polyacrylamide a resin was prepared by polymerisation of dimethylacrylamide, cross linking agent bis(acrylamido)ethane and acryloyl sarcosine methyl ester. This polymerisation was also carried out within the pores of rigid macroporous inorganic particles (Kieselguhr) to produce a support which, after derivatisation with ethylene diamine, was suitable for continuous flow solid-phase peptide synthesis. Meldal et al. compared this Kieselguhr supported polydimethylacrylamide with a PEGA resin in the synthesis and on-resin enzyme assay of a library of fluorescence quenched protease substrates. The PEGA resin was a copolymer of bis-2-acrylamidoprop-1-yl polyethylene glycol, monoacrylamidoprop-1-yl polyethylene glycol and N,N-dimethylacrylamide. Excellent mechanical stability and good swelling in aqueous buffer were observed. The permeability of the PEGA resin was studied using substilisin carlsberg (27 KDa) on a good model fluorescence quenched substrate covalently linked to the resin. Quantitative cleavage was observed after 24 hours suggesting that the enzyme could permeate the resin. Similar results were obtained for the Keiselguhr supported resin. A library of internally quenched fluorogenic substrates was synthesised on each type of resin and incubated with substilisin carlsberg. Cleavage of a peptide sequence by the enzyme caused the bead to fluoresce. On a given fluorescent bead a proportion of the substrate had been cleaved. Sequencing then gave the substrate structure and from the ratio of residues in the cleaved and uncleaved sequence it was possible to generate a semi-quantitative estimation of enzymatic conversion. When the results obtained using this method were compared with those obtained in solution studies there was some inconsistency. In spite of these differences, the ranking of substrates in solution and on solid-phase
was essentially the same. This suggests that although the solid support had some influence on the assay it did not render it invalid. This library was also subjected to treatment with cruzipain (57KDa). Hydrolysis occurred only on the surface of some of the beads. A second library was synthesised on a PEGA resin with better swelling properties to provide information on the substrate specificity of cruzipain. PEGA resin prepared by copolymerisation of bis-acrylamido-PEGA and partially acyloylated diaminoPEG and N, N-dimethylacrylamide was used in an ingenious extension of this concept. A library of potential subtilisin carlsberg inhibitors was synthesised on PEGA beads bearing an optimised fluorescence quenched substrate. In order to accomplish this, a proportion of the amine functional groups the PEGA resin beads were reacted with 4-hydroxymethylbenzoic acid and the remainder with the first amino acid of the fluorescence quenched substrate. Due to the selectivity of active esters for amino nucleophiles the substrate was synthesised in the presence of the free hydroxyl group (Scheme 18) then acylated to prevent sequencing. The hydroxyl function was esterified with the first amino acid and a peptide library was prepared by the split synthesis approach.

\[
\text{NH}_2 \quad \text{Fmoc-aa-OH (0.5 equiv.)} \quad \text{4-hydroxymethylbenzoic acid (1.5 equiv.)}
\]

\[
\text{NH}_2 \quad \text{PEG}
\]

\[
\text{H} \quad \text{NHaaFmoc}
\]

Scheme 18 Derivatisation of resin for synthesis of inhibitor library and fluorogenic substrate

The resultant beads were incubated with enzyme. If the library member did not inhibit the enzyme the fluorescence quenched substrate could be cleaved and the bead would fluoresce. Some of the non-fluorescent beads were collected and sequenced by Edmann degradation to give the structure of inhibitors. In all the beads some cleavage product was found indicating that the enzyme reaction was not fully inhibited in the interior of the PEGA resin. Following the same strategy a library of
inhibitors for cruzipain\textsuperscript{61} was synthesised on beads bearing a fluorescence quenched substrate. The PEGA resin used in this instance was obtained from the polymerisation of mono and bis acrylamido-PEG\textsubscript{1900} and acrylamide\textsuperscript{62}. When illuminated beads were sliced, the observed fluorescence indicated that the large cruzipain (57 KDa) had limited access to the interior of the bead. In order to develop selective enzyme inhibitors for matrix metalloprotease MMP-9 which exists in forms of approximately 67/83 KDa\textsuperscript{63} it was necessary to increase the pore size of PEGA supports. This lead to the development of long chain cross linked PEGA\textsubscript{4000-8000}. Partially acryloylated bis-amino-PEG\textsubscript{1900, 4000, 6000 or 8000} was co-polymerised with acrylamide. A fluorescence quenched peptide library was synthesised on PEGA\textsubscript{4000} and incubated with activated MMP-9\textsuperscript{63}. Substrates which were highly selective for MMP-9 were identified but differences were observed between the solid-phase assay and solution studies. In addition to the assays described above, a PEGA resin was used in a solid-phase chemo-enzymatic synthesis of glycopeptides\textsuperscript{35}. This PEGA resin was obtained by the co-polymerisation of mono and diacryloylated polyethylene glycol with acrylamide. A small glycopeptide was assembled on the resin by chemical synthesis. This support-bound glycopeptide acted as acceptor in a \(\beta-(1\rightarrow4)\)-galctosyltransferase catalysed reaction with UDP-Gal. The enzyme reaction was \(>95\%\) complete after 48 hours during which time the enzyme was remarkably stable. This stability suggested that PEGA resin may be suitable for enzyme assays even with unstable proteases which would loose activity in contact with silica or polystyrene\textsuperscript{59}. Using a more highly cross-linked PEGA resin the enzymatic reaction was only 50\% complete after 72 hours. This suggested that the increased cross-linking restricted the access of the enzyme.

The suitability of TentaGel and ArgoGel for on-bead functional screening has been compared with that of a PEGA resin\textsuperscript{64}. TentaGel is the trade mark name for a polystyrene polyethylene glycol graft co-polymer. Graft co-polymers with PEG chains of 3000 KDa are about 70\% PEG and about 30\% polystyrene thus the properties of these polymers are mainly determined by the PEG portion \textsuperscript{65}. ArgoGel also consists of a cross-linked polystyrene backbone grafted with polyethylene glycol. A fluorescently labelled substrate for papain (23 KDa) was synthesised on each of the resins (Figure 7).
The three sets of beads were incubated with enzyme. Treatment of the dansyl-peptide-PEGA beads with enzyme resulted in a significant reduction in fluorescence indicating that cleavage had occurred. Edman sequencing confirmed the major site of cleavage (FG-L, Figure 7) as expected. A second cleavage site, consistent with literature observation, was also detected (LG-G). The other beads appeared unaffected by prolonged treatment with papain when visualised by fluorescence microscopy although partial cleavage was detected by release of the dansyl fluorophore. Edman sequencing of the ArgoGel beads revealed complex mixture of peptide fragments suggesting that the ArgoGel resin had affected the specificity of papain. PEGA resin was therefore chosen as the solid support for the synthesis of library of fluorescent peptide substrates for papain. This library was used in an on-bead assay to investigate the substrate specificity of papain. The general observations were consistent with literature results.

The low level of hydrolysis on TentaGel was in keeping with the findings of Vagner et al. TentaGel beads bearing a short N°-protected peptide substrate were treated with an appropriate enzyme. The enzyme cleaves the peptide only at the enzyme accessible surface sites (shaving) thus differentiating between the sites accessible to enzyme and those not. Synthesis using orthogonal chemistries for peptide assembly on the surface and in the interior allows generation of two structures on the same...
bead. The surface structure for interaction with a receptor and the interior structure for coding. The interior peptide should not interact with the receptor (Scheme 19).

Scheme 19 Strategy for enzyme mediated spatial segregation

For example, treatment of beads bearing a peptide sequence which binds anti-β-endorphin (170 KDa) with α-chymotrypsin abolishes the binding activity. Chymotryptic shaving saturates at 2-2.5% of the total sites compared to elastase or pepsin which can access 10-15% of the sites. However, shaving with elastase or pepsin to 2-2.5% fails to cleave all the sites that may later interact with the target endorphin suggesting that the shaving process is dynamic. It is interesting that chymotrypsin and elastase both with molecular weight 22 KDa shave different percentages (2-2.5% cf. 10-15%). Obviously, factors other than the molecular weight of the enzyme play a part in determining the accessibility of sites. The extent of shaving depends on how good the substrate is for the shaving enzyme. The enzyme must shave off the surface peptide to the extent where insufficient remains for binding to the receptor. How much this is depends on the affinity of the peptide for the receptor. What is required is a good substrate for shaving and appropriate affinity of the macromolecular receptor for the substrate. The successful application of this approach was demonstrated in the synthesis and screening of a peptide encoded peptide library. The low level of enzyme penetration was however contrary to the results obtained in an investigation using confocal scanning laser microscopy. Untreated TentaGel, acylated TentaGel (controls for the identification of non-specific binding) and TentaGel bearing a trypsin inhibitor peptide were incubated with fluorescently labelled porcine pancreatic trypsin (PPT) and examined by confocal scanning laser microscopy. In this study it was found that the fluorescently
labelled PPT (23.5 KDa) could penetrate the core of 90 μm TentaGel beads. The bead size may have been a significant factor as 130 μm beads were used in the work of Vagner et al. TentaGel beads (90 μm) were also used in an on-bead assay for the discovery of linear substrate motifs of protein kinases. Random pentapeptide and hexapeptide libraries were synthesised on TentaGel and the beads were incubated with cyclic AMP dependant protein kinase and [γ-32P]ATP. 32P labelled beads were isolated for microsequencing. The substrate motif identified was identical to that previously reported in the literature. This assay method was later used to identify a novel peptide substrate for a protein tyrosine kinase.

It is apparent that enzyme reactions involving resin bound substrates can be successfully carried out in both solid-phase enzymatic synthesis and on-bead functional assays. The most decisive factor in the success of these reactions appears to be the choice of resin. Different resins are suited to different applications as described above. Direct comparison cannot be made between the resins used in different studies as they may differ in composition or have been derivatised with spacers, factors which can dramatically affect their suitability. In the case of solid-phase functional assays there are concerns that the specificity of enzymes could be perturbed and for this reason, solution assays are often carried out for confirmation of results.

1.4 Enzyme Cleavable Linkers

In the first example of an enzyme-cleavable linker, Elmore et al carried out peptide synthesis on resin-linker system (Figure 8). Release from the support (a polyacrylamide) by digestion with calf-spleen phosphodiesterase resulted in peptide esters. Any residual phosphate ester on the product could be removed by treatment with alkaline phosphomonoesterase and the ester itself could be cleaved by HBr in acetic acid (33% w/v).
Resin-bound linker 59 (Figure 9) was also designed for cleavage by an enzyme in this case α-chymotrypsin which is selective for the phenylalanyl ester bond. The utility of this approach was demonstrated in the solid-phase synthesis of a glycopeptide which involved both chemical peptide synthesis and enzyme catalysed sugar chain elongation (discussed in Section 1.3.1). As with the phosphodiesterase cleavage part of the enzyme recognition site (phenylalanine) was retained in the product. The phenylalanine residue could be removed from the product enzymatically.

The peptide bond hydrolysed by α-chymotrypsin is within the polypeptide chain. For this reason α-chymotrypsin is termed an endopeptidase. When the susceptible peptide linkage is at the amino or carboxy terminus of a protein the enzyme is termed an exopeptidase. If a linker was designed for cleavage by an exo-enzyme no part of the enzyme recognition site would be retained in the products after release from the support. Penicillin acylase is an inexpensive, commercially available example of an enzyme which could be used to cleave a linker in this manner.

1.5 Penicillin Acylase (Penicillin Amidase) Cleavable Exo-Linker
Penicillin acylases (also called penicillin amidase) are grouped into three classes based on their specificity\(^70\). Penicillin G acylase which preferentially hydrolyses
penicillin G, penicillin V acylase which preferentially hydrolyses penicillin V and ampicillin acylase which specifically hydrolyses ampicillin. Penicillin G acylase can be purchased both in solution and immobilised on a solid support such as Eupergit. Immobilised penicillin-G acylase has been used in the industrial production of 6-aminopenicillanic acid (6-APA), an important intermediate in the synthesis of semi-synthetic β-lactam antibiotics, from penicillin-G\textsuperscript{71} (Scheme 20)

\[ \text{Penicillin G} \xrightarrow{\text{Penicillin Acylase}} 6\text{-APA} \]

\textbf{Scheme 20 Production of 6-APA}

Penicillin acylase selectively hydrolyses esters and amides of phenylacetic acid. The enzyme accepts a wide range of structures in the amine or alcohol component but shows increased specificity with regard to the structure of the acyl group accepting only minor changes\textsuperscript{72}. The reactions catalysed by penicillin acylases are reversible. At acidic or neutral pH (4.0-7.0) acylation reactions are catalysed whilst at alkaline pH (7.5-8.5) hydrolysis takes place\textsuperscript{70}. Both reactions have been employed in the penicillin acylase catalysed protection and deprotection of amino groups during solution phase peptide synthesis\textsuperscript{73}. Penicillin acylase is enantioselective in its treatment of beta-lactams, amides and esters and has been used in an enzymatic resolution of 62 (Figure 10) an intermediate in the synthesis of the antibiotic Loracarbef\textsuperscript{74}.

\[ \text{Figure 10 An intermediate in the synthesis of Loracarbef} \]
Penicillin amidase from *E. coli* is an 80 KDa heterodimer. The N-terminal serine has been shown to be involved in catalysis\(^75\) and a catalytic mechanism for amide cleavage proposed (Scheme 21).

\[ \text{Scheme 21 Proposed catalytic mechanism of penicillin amidase} \]

The crystal structure of the enzyme complexed with phenylacetic acid shows the phenyl moiety points into a mainly hydrophobic pocket which is lined with aromatic residues and hydrophobic side-chains. This complementary fit explains the specificity of the enzyme towards the phenyl moiety on a broad range of substrates. A linker cleaved by this enzyme should therefore possess this recognition structure. In addition the linker must have a suitable functionality for attachment to the solid support and a point for the attachment of an alcohol (ROH, Figure 11). This type of structure may be accessed through chemistry developed by Katritzky *et al*\(^76\).
The three component condensation reaction between an aldehyde, an amide and benzotriazole produces N-(1-benzotriazol-1-ylalkyl)amides 66\(^7\) (Scheme 22). This reaction is believed to proceed via a hydroxyalkyl benzotriazole intermediate 65 which then reacts with the amide (Scheme 22).

\[
\begin{align*}
R\text{CHO} + & \quad \text{64} \\
\rightarrow & \quad \text{65} \\
\overset{R'C\text{ONH}_2}{\rightarrow} & \quad \text{66} \\
\end{align*}
\]

Scheme 22 Proposed mechanism for adduct formation

\(N\)-(1-Benzotriazol-1-ylalkyl)amides 66 undergo reaction with sodium alkoxides to produce \(N\)-(\(\alpha\)-alkoxyalkyl)amides\(^8\) 67 (Scheme 23).

\[
\begin{align*}
R'\text{ONa} \quad \text{66} \\
\rightarrow & \quad \text{67} \\
\end{align*}
\]

Scheme 23 Synthesis of \(N\)-(\(\alpha\)-alkoxyalkyl)amides

This could be a viable method for the attachment of alcohols to the proposed linker however this approach would require the generation of the sodium salt of the alcohol.
to be attached. This could be avoided by the preparation of an N-acylhemithioaminal 70 (Scheme 24).^{79}

Scheme 24 Synthesis of N-acylhemithioaminals

After coupling the linker to the solid support the thiol group could be activated for displacement by a weak nucleophile such as an alcohol. The only other requirement is that the structure of the aldehyde used in the initial three component condensation reaction provides access to a suitable functionality for tethering the linker to the solid support. Aldehyde 69 (Scheme 25) meets this requirement.

Scheme 25 Synthesis of linker 73

The synthetic plan described in Scheme 25 would give access to linker 73 which has been designed to meet all the requirement for a penicillin amidase cleavable exo-linker.
After this strategy was conceived a paper was published also describing an enzyme cleavable exo-linker\textsuperscript{34} 74 (Figure 12). This linker was designed so that the enzyme (a lipase) would cleave the linker acetate group to produce a phenolate anion which would then spontaneously fragment to release the product.

\[
\begin{align*}
\text{OAc} & \quad \text{TG} \\
\text{RX} & \quad X = \text{NH, O, CR}_2
\end{align*}
\]

\textbf{Figure 12} Lipase cleavable exo-linker

1.6 Project Aim

In the first instance the aim was the development of linker 73 for which there was an existing synthetic route\textsuperscript{80}. This linker appeared to have low acid and base stability but a systematic study had not yet been undertaken so this was a priority. The fact that this linker had shown signs of being both acid and base labile was also the motive for the design of a linker without an aromatic spacer. The synthesis of linker 73 was lengthy and time-consuming and the aim was to obtain an superior synthetic procedure to access a more stable linker. Comparison of the synthesis, stability and enzyme cleavage of the two types of linker would then be used to assess which linker would be most suitable for application to a solid-phase synthesis.
2. RESULTS AND DISCUSSION - LINKERS SYNTHESISED IN SOLUTION

2.1 Development and Study of Linker 73
For initial studies linker 73 was synthesised by the previously developed route\textsuperscript{80} involving synthesis of the linker in solution. The linker was subsequently coupled to solid support. This is described in the following chapter together with procedures for determining the loading of linker 73 on solid support. The coupling of alcohols to the support-bound linker was then investigated.

2.1.1 Synthesis of Linker 73 and Subsequent Coupling to Solid Supports
Linker 73 was prepared in four steps from carboxybenzaldehyde 77 and 3-azidoaminopropane 76 (Scheme 26). 3-Azido-aminopropane 76 was first obtained by nucleophilic displacement of 3-chloropropylamine hydrochloride 75 using sodium azide (Scheme 26). Reaction of amine 76 with the acid chloride, prepared from carboxybenzaldehyde 77 by treatment with thionyl chloride, gave aldehyde 69. Heating aldehyde 69 with equimolar amounts of phenylacetamide and benzotriazole in toluene under Dean-Stark conditions, produced the adduct 71. Adduct 71 was laborious to purify by column chromatography resulting in a bottle-neck in the synthesis. Treatment of 71 with ethylthiolate sodium salt gave the thioethyl derivative 72 in excellent yield. The reduction of the azido group was then accomplished using triphenylphosphine and water to give the desired amine 73.
Scheme 26 Reagents and conditions: (i) NaN₃, H₂O, reflux, 16 h (ii) SOCl₂, THF, reflux, 1 h (iii) NH₂(CH₂)₃N₃, TEA, THF, RT, 15 min. (iv) PhCH₂CONH₂, benzotriazole, toluene, reflux under Dean-Stark conditions, 23 h (v) EtSNa, THF, RT, 1 h (vi) PPh₃IH₂O, THF, RT, 4 days. * No yield is available for this step though yields in the range 40-60% have been obtained by other group members.

In initial experiments, the linker 73 was coupled to oxirane acrylic beads (78, Scheme 27). This solid support was selected as it had previously been used for the immobilisation of penicillin amidase⁸¹. However, the coupling resulted in undesirable reactive functionality (secondary amine and hydroxyl) on the solid support. For this reason an alternative support was sought.
NovaSyn® TG carboxy resin (carboxy TG resin, TG-CO₂H) is a composite of cross-linked polystyrene and 3000-4000 molecular weight polyethylene glycol which has been terminally carboxy functionalised. This resin swells well in water and was chosen because PEG-PS resins had been successfully used with enzymes (Section 1.3.2). Linker 73 was coupled to carboxy TG resin 80 (loading 260 µmol/g) using 2-(1H-benzotriazol-1-yl)-1,1,3,4-tetramethyluronium tetrafluoroborate (TBTU), conditions previously applied by Boehm⁸², to give 81a (Scheme 28).

These coupling conditions resulted in linker loadings determined to be in the range 42-50% by overnight treatment of the resin with 2M HCl at room temperature (the development of this method of loading determination is discussed below in Section 36).
2.1.2). As six fold excess of linker 73 was used in this coupling protocol an attempt was made to recycle the coupling mixture. The liquors from a coupling reaction were retained and used to treat fresh resin under coupling conditions. This resin was then subjected to acidic cleavage conditions (2M HCl at room temperature overnight) and subsequent HPLC analysis of the cleavage mixture indicated no linker had been coupled.

As the yields for the coupling of linker 73 to carboxy TG had been low and the coupling mixture could not be reused the product of the coupling reaction was more closely examined. The IR spectrum of linker 73 on carboxy TG (81a) showed an absorbance corresponding to an amide carbonyl, as expected from the coupling of the linker. However, as the coupling had not gone to completion one would have expected to see an absorbance corresponding to residual acid functionality, known to be at 1732 cm\(^{-1}\) from the IR spectrum of carboxy TG resin itself. Instead another absorbance at 1705 cm\(^{-1}\) was observed. This suggested that an impurity was forming on the surface of the resin. In an attempt to identify this unknown carboxy TG resin was treated with the coupling reagents under coupling conditions but in the absence of linker. The IR spectrum of the resultant resin indicated the presence of the absorbance at 1705 cm\(^{-1}\). Gel Phase \(^{13}\)C NMR was also employed in an bid to provide more information however the spectrum was inconclusive. The combination of low yields for the coupling of linker 73 to carboxy TG resin and the formation of an unknown support-bound impurity during the coupling procedure led to an alternative procedure being adopted.

Coupling of linker 73 to carboxy TG using diisopropyl carbodiimide (DIC, Scheme 29) was accomplished in a yield of 79% (190 \(\mu\)mol/g, as determined by acidic cleavage (TFA:CH\(_2\)Cl\(_2\):H\(_2\)O, 9:10:1) and subsequent HPLC of the phenylacetamide/phenylacetic acid released). That different acidic cleavage conditions were used to determine the loading is irrelevant as both methods were believed to result in complete cleavage of linker from the solid support as discussed in Section 2.1.2. The coupling mixture from this reaction was successfully retained and reused with fresh resin to achieve a linker loading of 190 \(\mu\)mol/g (79%) again determined by acidic cleavage (TFA:CH\(_2\)Cl\(_2\):H\(_2\)O, 9:10:1) and subsequent HPLC of
the phenylacetamide/phenylacetic acid released (Section 2.1.2). In each case no absorbance at 1705 cm\(^{-1}\) was observed in the IR spectrum of the resultant resin.

![Chemical structure](image)

**Scheme 29** Reagents and Conditions: (i) DIC, HOBt, DIEA, DMF, 16-24 h, RT

The resin bound linker 81b was examined by \(^{13}\)C gel phase NMR and an excellent spectrum obtained (Figure 13). The SCH\(_2\)CH\(_3\) and SCH\(_2\) signals can clearly be seen at 14.5 ppm and 25.4 ppm respectively. The PhCH\(_2\) signal can be seen at 43.0 ppm and the CH at 55.4 ppm. Seven other signals between 29.3 and 69.6 account for the remaining CH\(_2\) groups from the linker itself and the resin. There are five aromatic CH\(_{ar}\) signals (126.6, 126.9, 127.4, 128.5 and 129.1) and three aromatic C\(_{ar}\) signals (134.1, 135.4 and 142.6) as might be expected. The four signals ascribed to carbonyl carbons were observed at 166.7, 170.4, 172.3 and 173.1 ppm.
Figure 13 $^{13}$C Gel phase NMR spectrum of linker 73 on TG (81b)
Having found appropriate conditions for the coupling of linker 73 to carboxy TG resin these conditions were then applied to the coupling of linker 73 to carboxy PEGA resin. PEGA resins also swell in aqueous and organic media and has been successfully used with enzymes (Section 1.3.2). As carboxy PEGA resin 84 was not freely commercially available it was obtained by derivatisation of commercial amino PEGA 83 using succinic anhydride (Scheme 30).

![Scheme 30](image)

**Scheme 30 Reagents and conditions:** (i) DMAP, Pyridine, DMF, RT, overnight

The coupling of linker 73 to carboxy PEGA (Scheme 31) was achieved in a yield of 97% (340 μmol/g) from 83 as determined by acidic cleavage (TFA:CH₂Cl₂:H₂O, 9:10:1) and subsequent HPLC quantification of the phenylacetamide/phenylacetic acid produced (Section 2.1.2). This also confirms the successful preparation of 84. Reuse of this coupling mixture with fresh resin produced material which was also found to have a loading of 340 μmol/g.

![Scheme 31](image)

**Scheme 31 Reagents and conditions:** (i) DIC, HOBt, DIEA, DMF, RT, 16-24 h

TG and PEGA resins were selected as potentially suitable for use with enzymes. However, as linker 73 can be cleaved under acidic conditions it was also coupled to carboxypolystyrene resin 86 (Scheme 32). Linker 73 was coupled to the acid fluoride prepared from carboxy polystyrene (Scheme 32).
Scheme 32 Reagents and conditions: (i) cyanuric fluoride, pyridine, anhydrous CH$_2$Cl$_2$, RT, 3 h (ii) linker 73, DIEA, DMF

Cleavage of the linker from the solid-support (88a) using TFA:CH$_2$Cl$_2$:H$_2$O (9:10:1) for 1 hour indicated incomplete coupling. There was also a discrepancy between duplicate cleavages (380 µmol/g and 450 µmol/g as compared to a maximum theory yield of 740 µmol/g). The observed yield for the coupling may have been reduced by insufficient cleavage time. Treatment with TFA:CH$_2$Cl$_2$:H$_2$O (9:10:1) for 1 hour had been expected to result in complete cleavage$^{83}$. Later this was suspected not to be the case. The reaction time for the preparation of the acid fluoride had been reduced compared to the original protocol$^{83}$ and this may also have contributed to the low yield. It is also possible that the coupling could have been reduced due to the presence of ethanol in the reaction solvent (CH$_2$Cl$_2$) used in acid fluoride preparation (Scheme 32). Ethanol (0.5%) was used to stabilise the dichloromethane and it seems possible that it may have been further concentrated by repeated distillation. Any ethanol present could have resulted in the formation of 89 though no obvious ester carbonyl absorbance was observed in the IR spectrum of the product.
It is possible that the actual coupling yield was higher than the observed value. However, the coupling of linker 73 to carboxypolystyrene 86 was more successfully carried out using DIC, HOBt and DIEA in DMF (Scheme 33).

Scheme 33 Reagents and conditions: (i) DIC, HOBt, DIEA, DMF, RT, 16-24 h

The yield for this reaction was 99% (840 µmol/g) and reuse of coupling mixture resulted in 92% coupling (770 µmol/g). In each case the yields were determined by acidic cleavage (TFA:CH₂Cl₂:H₂O, 9:10:1) and subsequent HPLC quantification of the phenylacetamide/phenylacetic acid produced (Section 2.1.2). The ¹³C gel phase carbon NMR spectrum of linker 73 on polystyrene (88b) showed the diagnostic SCH₂CH₃ and SCH₂ peaks at 14.8 and 25.8 ppm respectively (Figure 14) however it was of poorer quality than the ¹³C gel phase carbon NMR spectrum of the corresponding TG compound 81b (Figure 13).
Figure 14 $^{13}$C Gel phase NMR spectrum of linker 73 on Polystyrene (88b)
2.1.2 Determination of the Loading of Linker 73 on Solid Support

For these solid phase studies it was important to develop reliable and accurate protocols for the determination of the loading of linker 73 onto resin. Cleavage to release phenylacetamide/phenylacetic acid was selected as this would result in a UV active probe which could be monitored by HPLC.

Cleavage of linker 73 with aqueous acid produced phenylacetamide 70, which was then partially hydrolysed to give phenylacetic acid 90 (Scheme 34).

\[
\text{Scheme 34 Reagents and conditions: (i) MeOH:3M HCl (1:2), RT, 16-48 h}
\]

As expected treatment of linker 73 on carboxy TG (81a) with aqueous acid also resulted in the production of phenylacetamide and phenylacetic acid (Scheme 35).

\[
\text{Scheme 35 Reagents and conditions: (i) 2M HCl, RT, 16-24 h}
\]

In order to determine the loading of linker 73 on carboxy TG by acidic cleavage and HPLC determination of the phenylacetamide and phenylacetic acid produced it was necessary to find conditions that would result in complete cleavage. Solution studies
to ascertain appropriate cleavage conditions were taken as a starting point and 2M HCl was selected as possible cleavage agent. Linker 73 was insoluble in 2M HCl therefore it was dissolved in a mixture of 3M HCl:MeOH (2:1). Overnight reaction at room temperature resulted in complete cleavage of linker 73. A control reaction was also carried out. As linker 73 did not dissolve completely in H₂O:MeOH (2:1) it was dissolved in H₂O:MeOH (1:1). Overnight reaction at room temperature resulted in cleavage of 10-20%. After coupling to carboxy TG linker 73 was more stable as evidenced by the fact that treatment of 81a with MeOH:H₂O (1:1) overnight at room temperature resulted in <2% cleavage from the resin. This indicated that though solution studies are valuable as a starting point direct comparison between solution and solid-phase cannot be drawn.

The linker 73 on carboxy TG (81a) was treated with aqueous 2M HCl overnight at room temperature and the phenylacetic acid and phenylacetamide produced used to determine the loading. Treatment of the same resin with 5M HCl and subsequent HPLC of the cleavage mixture indicated the same loading. It was therefore assumed that cleavage with 2M HCl overnight at room temperature resulted in complete cleavage of the linker from carboxy TG and this method could be routinely used to calculate the loading of linker.

Linker 73 could also be cleaved under strong basic conditions. Treatment of linker 73 on carboxy TG (81a) with 2M aqueous NaOH overnight at room temperature resulted in the production of phenylacetic acid (Scheme 36).

Scheme 36 Reagents and conditions: (i) 2M NaOH, RT, 16-24 h

HPLC determination of the phenylacetic acid indicated the same loading as that determined using 2M HCl cleavage. It was concluded that this method also resulted in complete cleavage of the linker from the resin and could also be used to determine the loading of linker 73 on carboxy TG.

However, neither method was appropriate for cleavage of linker 73 from polystyrene as this resin doesn’t swell under aqueous conditions. Treatment with
TFA:CH₂Cl₂:H₂O (9:10:1) overnight at room temperature was therefore used to determine the loading of linker 73 on polystyrene. The experiments that allow the assumption that this method will also result in full cleavage of this type of structure are discussed in Section 3.1.1. This method was also deemed compatible with TG and PEGA resins as both swell under these conditions.

2.1.3 Stability of Linker 73 on Carboxy TG (81a)

The acid stability of linker 73 on carboxy TG (81a) was assessed by treatment with various concentrations of acid for 24 h at room temperature (Table 1) and determining the extent of cleavage by HPLC. The results clearly illustrated that linker 73 is very acid labile. For example, treatment with 1M HCl at room temperature results in almost complete cleavage of the linker and even 0.1M HCl results in 10% cleavage. The base stability of 81a was also examined by treatment with varying concentrations of NaOH for 24 h at room temperature. Again HPLC analysis was used to determine the extent of cleavage. It was obvious that linker 73 on carboxy TG is also very base labile (Table 2) as almost complete cleavage was achieved with 0.1M NaOH.

<table>
<thead>
<tr>
<th>Conditions</th>
<th>% Cleavage of 81a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5M HCl</td>
<td>100</td>
</tr>
<tr>
<td>2M HCl</td>
<td>100</td>
</tr>
<tr>
<td>1M HCl</td>
<td>80</td>
</tr>
<tr>
<td>0.5M HCl</td>
<td>40</td>
</tr>
<tr>
<td>0.25M HCl</td>
<td>20</td>
</tr>
<tr>
<td>0.1M HCl</td>
<td>10</td>
</tr>
<tr>
<td>10% TFA</td>
<td>80</td>
</tr>
<tr>
<td>H₂O</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 1 Acid stability of 81a

<table>
<thead>
<tr>
<th>Conditions</th>
<th>% Cleavage of 81a</th>
</tr>
</thead>
<tbody>
<tr>
<td>5M NaOH</td>
<td>90</td>
</tr>
<tr>
<td>2M NaOH</td>
<td>100</td>
</tr>
<tr>
<td>1M NaOH</td>
<td>100</td>
</tr>
<tr>
<td>0.5M NaOH</td>
<td>100</td>
</tr>
<tr>
<td>0.25M NaOH</td>
<td>100</td>
</tr>
<tr>
<td>0.1M NaOH</td>
<td>90</td>
</tr>
<tr>
<td>H₂O</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2 Base stability of 81a
Treatment of 81a with aqueous NaOH was expected to produce phenylacetic acid. However, at lower concentrations of aqueous NaOH phenylacetamide was observed in addition to as phenylacetic acid (Table 3).

<table>
<thead>
<tr>
<th>Base</th>
<th>phenylacetic acid</th>
<th>phenylacetamide</th>
</tr>
</thead>
<tbody>
<tr>
<td>5M NaOH</td>
<td>106 µmol/g</td>
<td>0 µmol/g</td>
</tr>
<tr>
<td>2M NaOH</td>
<td>129 µmol/g</td>
<td>0 µmol/g</td>
</tr>
<tr>
<td>1M NaOH</td>
<td>118 µmol/g</td>
<td>16 µmol/g</td>
</tr>
<tr>
<td>0.5M NaOH</td>
<td>117 µmol/g</td>
<td>31 µmol/g</td>
</tr>
<tr>
<td>0.25M NaOH</td>
<td>72 µmol/g</td>
<td>62 µmol/g</td>
</tr>
<tr>
<td>0.1M NaOH</td>
<td>26 µmol/g</td>
<td>79 µmol/g</td>
</tr>
</tbody>
</table>

Table 3 Aqueous NaOH cleavage of 81a

It was possible that this cleavage proceeded by abstraction of a proton which lead to imine formation and subsequent hydrolysis afforded phenylacetamide which was then itself hydrolysed by the aqueous NaOH to give phenylacetic acid (Scheme 37). When examining the chemical stability of linker 73 on carboxy TG (81a) the question arose of its stability during storage. 81a was found to be sufficiently stable that storage at 4 °C for three months had no effect on the loading as determined in each case by 2M HCl cleavage and subsequent HPLC.
Scheme 37 Possible mechanism for the cleavage of linker 73 from carboxy TG by aqueous NaOH

2.1.4 Coupling of Alcohols to Linker 73 on Solid-Support

2-Phenylpropan-1-ol and 1-phenylpropan-2-ol were selected as a model alcohols for coupling because of their immediate availability and favourable HPLC properties. These alcohols were coupled to 81a in yields of 73-82% using N-iodosuccinimide and a catalytic amount of triflic acid in anhydrous CH₂Cl₂ (Scheme 38).
As a method for determination of the potential loading of primary alcohol by a less time consuming means than cleavage and HPLC, Fmoc-Ser-OMe was coupled to linker 73 on carboxy TG (81b, Scheme 39) using the conditions previously developed for coupling of alcohols. The Fmoc protecting group was then cleaved using 20% piperidine in DMF. UV determination of the piperidine-dibenzofulvene adduct produced on cleavage (Scheme 39) indicated an alcohol loading of 140 μmol/g (78% from 81b).
Scheme 39 Reagents and conditions: (i) Fmoc-Ser-OMe, NIS, triflic acid (cat.), CH₂Cl₂, 4 Å molecular sieves, RT, 16-24 h (ii) 20% piperidine in DMF, RT, 10 min. in ultrasonic bath

In the same way, Fmoc-Ser-OMe was coupled to linker 73 on PEGA to produce 98 (Section 7.11.7). Subsequent cleavage and UV analysis indicated a loading of 270 μmol Fmoc/g (87% from 85). Fmoc-Ser-OMe was coupled to linker 73 on polystyrene (88a*) to give 99a (Section 7.11.8) and a loading of 600 μmol Fmoc/g (87% from 88a) was determined.

* 87a was obtained by reaction of the acid fluoride prepared from carboxy polystyrene with linker 73. This material was prepared by James Dowden83
2.1.5 Summary

Linker 73 could be synthesised in 4 steps from carboxybenzaldehyde in a yield of 20-27% overall. The linker could be coupled to various solid-supports (TG, PEGA and polystyrene) in excellent yield (79-99%) using DIC/HOBt. This coupling procedure was a great improvement on the TBTU based protocol used previously. The support bound linker was labile under acidic conditions. 80% cleavage was observed on treatment with 10% TFA for 24 hours at room temperature. Base lability was also observed with complete cleavage being induced using 0.25M NaOH for 24 hours at room temperature. A primary alcohol (Fmoc-Ser-OMe) was coupled to linker 73 on each of the aforementioned resins in good yield (78-87%). This work provides an excellent basis for extension to new and improved linkers of this type.

2.2 Model Reactions - Testing the Suitability of an Aliphatic Aldehyde for Linker Synthesis

To prepare a more acid stable, enzyme labile linker the aromatic aldehyde 69 used in the synthesis of linker 73 was to be replaced by an aliphatic aldehyde. It was postulated that the cationic intermediate formed during acidic cleavage would be less stable in the absence of conjugation to the benzene ring. To ensure that an aliphatic aldehyde could be used in the linker synthesis in a similar way to an aromatic aldehyde model reactions were carried out (Scheme 40). Phenylacetaldehyde 100 was selected for the model reaction because of its immediate availability and reaction with phenylacetamide and benzotriazole gave the desired product 101 in a yield of 39%. The benzotriazole group was successfully displaced using ethylthiolate sodium salt to give 102 in very good yield (91%).

\[
\begin{align*}
100 & \xrightarrow{(i)} \text{PhCH}_2\text{CONH}_2, \text{ benzotriazole, toluene, reflux under Dean-Stark conditions, 9 h} \\
101 & \xrightarrow{(ii)} \text{EtSNa, THF, RT, 2.5 h}
\end{align*}
\]

Scheme 40 Reagents and conditions: (i) \text{PhCH}_2\text{CONH}_2, \text{ benzotriazole, toluene, reflux under Dean-Stark conditions, 9 h} (ii) \text{EtSNa, THF, RT, 2.5 h}
2.3 Progress Towards Synthesis of Linker 103

After the success of the model reactions (Section 2.2) compound 103 was chosen as a target because the corresponding aldehyde intermediate required could potentially be accessed from commercial starting material.

Using the same approach to linker 103 as had been previously applied to linker 73 would have required the reaction of 3-azidopropanal with phenylacetamide and benzotriazole in toluene at reflux. The low molecular weight of the azide component was prohibitive and an alternative using an N-protected 3-aminopropanal was sought. Thus 3-azido-1,1-diethoxypropane 105 was prepared from commercially available 3-chloro-1,1-diethoxypropane 104 by nucleophilic displacement using sodium azide. The azido group was reduced using triphenylphosphine and water to give 3,3-diethoxypropylamine 106 (Scheme 41).

Before deprotection of the acetal of 106 the amine functionality was protected using 2,2,2-trichloroethylchloroformate (Scheme 42). The trichloroethoxycarbonyl protecting group was selected as it was expected to be stable in the pH range 1-12\textsuperscript{84} and removable by zinc in aqueous THF (pH 5.5-7.2) or zinc in ethanol at reflux\textsuperscript{85}. It was necessary to protect the amine prior to deprotection of the acetal as this increased the molecular weight of the aldehyde and avoided the risk of polymerisation. Also a free amine would interfere in the reaction between the aldehyde, benzotriazole and phenylacetamide as it would be more nucleophilic than phenylacetamide. The protected amine 107 was found to decompose when an attempt to purify it by column chromatography was made though the crude \textit{\textsuperscript{1}H} NMR indicated that the product was sufficiently pure for progression to the next step. The
acetal 107 was deprotected using trifluoroacetic acid to give the aldehyde in a yield of 50% from 3,3-diethoxy-1-propylamine 106 (Scheme 42).

Scheme 42 Reagents and conditions: (i) 2,2,2-trichloroethylchloroformate, TEA, CH₂Cl₂, 0 °C-RT, 45 min. (ii) 50% TFA, CHCl₃, 0 °C-RT, 2.5 h

Purification of this unstable product by column chromatography contributed to the poor yield for this deprotection step consequently it was also used without purification. Reaction between aldehyde 108 (crude), benzotriazole and phenylacetamide appeared to give the 1:1:1 adduct 109 (Scheme 43). The identity of this compound could not be confirmed through characterisation as it could not be purified by column chromatography due decomposition. The yield of what appeared to be crude adduct 109, from pure 3,3-diethoxy-1-propylamine 106 was 66%. This was further reduced to 40% after purification by precipitation. The next step in the reaction sequence was to displace the benzotriazole group using the ethylthiolate anion. Although an attempt was made at displacement ¹H NMR, however, revealed that the desired product was not present in any appreciable quantity.
Scheme 43 Reagents and conditions: (i) PHCH₂CONH₂, benzotriazole, toluene, reflux under Dean-Stark conditions, 5 h

2.4 Synthesis and Study of Linker 110

In view of the difficulties experienced with the synthesis of compound 103 discussed in Section 2.3 an approach was sought which would avoid the use of protecting groups. It was proposed that reaction of benzotriazole with phenylacetamide and an aliphatic aldehyde bearing a terminal bromine would produce a useful intermediate, provided that the bromide could then be selectively displaced using sodium azide. 4-Bromobutanal 113 (Scheme 44) was selected as this could be prepared from THF and would ultimately result in the synthesis of linker 110

2.4.1 Synthesis of Linker 110

4-Bromobutanal 113 was prepared in one of two ways in each case starting with the ring opening of THF using boron tribromide. In the first instance the product was obtained by direct oxidation of the borate ester using PCC (Scheme 44).
In the second case transesterification of the borate ester using methanol gave 4-bromobutanol 114 and subsequent TEMPO mediated oxidation gave the desired 4-bromobutanal 113 (Scheme 45) in comparable yield overall (39% cf. 46%). Neither approach was amenable to scale up much beyond that described in the experimental section and both are rather involved procedures to obtain a simple starting material.

As an alternative, appropriate conditions for the deprotection of commercially available 2-(3-bromopropyl)-5,5-dimethyl-[1,3]dioxane 115 were sought which would have provided access to 4-bromobutanal. Several unsuccessful attempts were made to deprotect 115. Literature precedent suggested it was necessary to convert this protecting group first to a dimethyl acetal, from which the desired aldehyde could then be released (Scheme 46).
The reaction to form 4-bromo-1,1-dimethoxybutane 116 did not go to completion and a prolonged reaction time led to formation of side-products. On a small scale 116 was obtained in low yield with recovery of starting material. However, on a larger scale no 116 was obtained and 91% of the starting material was recovered. In a similar approach an attempt was then made to prepare the 1,3-dioxolane 117 (Scheme 47).

On small scale 117 was obtained in reasonable yield (60 mg, 58%). Again on a larger scale this was less successful. The conditions which gave the best ratio (by $^1$H NMR) of 117:115 (7:1) on a small scale resulted in a poorer ratio of 4.5:6 when applied to a larger scale reaction. Small scale deprotections of the 5-membered acetal 117 were carried out in the presence of the six membered acetal 115. Conditions were established where the appearance of an aldehyde proton peak was observed along with the disappearance of the CH signal from the 5-membered acetal 117 in the $^1$H NMR spectrum. It was intended that the five-membered acetal 117 would be deprotected in the presence of the 6-membered acetal and the aldehyde obtained by
distillation. This separation was however unsuccessful so a mixture of 115 and 113 was used without purification in the next step (Scheme 48).

Scheme 48 Reagents and conditions: PhCH₂CONH₂, benzotriazole, toluene, reflux under Dean-Stark conditions, 2.5 h

A complex mixture was obtained and it was decided that it would be more efficient to revert to one of the methods previously used to obtain the aldehyde 113. The adduct 118 formed between 4-bromobutanal 113, benzotriazole and phenylacetamide (Scheme 49) was obtained in a maximum yield of 52% though the yield obtained for this reaction was routinely much lower than this due, at least in part, to the repeated column chromatography that was required. Recrystallisation also proved unsuccessful. Treatment of adduct 118 with sodium azide did result in selective displacement of the bromide to give azide 119. In the next step nucleophilic displacement of the benzotriazole group by the ethylthiolate anion gave the thioethyl derivative 120. The azido group was subsequently reduced using triphenylphosphine and water to give the desired product 110. The extensive time and effort required to obtain pure adduct 118 combined with poor yields at this step are serious drawbacks in the synthesis of larger quantities of linker 110.
Scheme 49 Reagents and conditions: (i) PHCH₂CONH₂, benzotriazole, toluene, reflux under Dean-Stark conditions, 2 h (ii) NaN₃, DMF, RT, 5 h (iii) EtSNa, THF, RT, 2.5 h (iv) PPh₃/H₂O, THF, RT, overnight.

2.4.2 Enzymatic Hydrolysis of Linker 110

As linker 110 was designed to be more acid stable than linker 73, it was essential to ensure that it was a substrate for penicillin amidase. This was most easily done by treatment of linker 110 in solution with immobilised penicillin amidase (Scheme 50). The phenylacetic acid produced indicated 60% cleavage had been effected clearly demonstrating that linker 110 was a substrate for the enzyme.

Scheme 50 Reagents and conditions: (i) immobilised penicillin amidase, phosphate buffer (25 mmolar, pH 7.8), RT, 16-24 h
2.4.3 Coupling of Linker 110 to Carboxy TG Resin

Linker 110 was coupled to carboxy TG resin using TBTU\textsuperscript{82}. The yield varied from 46% to quantitative (110-250 µmol/g) as determined by acidic cleavage (5-6M HCl) and HPLC. As for the coupling of linker 73 to carboxy TG using this method an absorbance in the IR spectrum at 1705 cm\textsuperscript{-1} indicated an impurity formed on the surface of the resin.

![Diagram](Diagram)

Scheme 51 Reagents and conditions: (i) HOBt, TBTU, DIEA, DMF, RT, 16-24 h

As this coupling method used a six fold excess of linker 110 the liquors from the coupling were retained and used to treat fresh carboxy TG resin under coupling conditions. No coupling was observed as determined by acidic (5-6M HCl) cleavage and HPLC. As the yields for coupling of linker 110 to TG-CO\textsubscript{2}H were variable (46% to quantitative) it was decided to derivatise linker 110 to give an acid (122, Scheme 52) and couple this to amine resin. This would also increase the length of the spacer unit. It was possible to derivatise linker 110 using succinic anhydride in the presence of DMAP and pyridine in DMF to give the corresponding acid. The desired product 122 was obtained in a low yield (43%) most likely due to repeated purification to remove residual succinic anhydride. This low yield, together with the low yield incurred in the synthesis of linker 110 itself was prohibitive in pursuing linker 122.
As another alternative to coupling linker 110 itself to the support azide 119 was reduced to give 123 (Scheme 53). It was proposed that this compound could be coupled to the solid support and the displacement of the benzotriazole group by the ethylthiolate anion carried out on resin. The reduction was successful giving the desired amine 123 in a yield of 62%. This approach was not pursued but superseded by that described in Section 3.2.

2.4.4 Determination of the Loading of Linker 110 on Carboxy TG
As for linker 73, cleavage of linker 110 with aqueous acid produced phenylacetamide, which was then partially hydrolysed under aqueous conditions to give phenylacetic acid 90 (Scheme 54). Linker 110 was treated with 2M HCl overnight at room temperature. HPLC analysis indicated a peak corresponding to residual linker 110. When this was repeated using 5M HCl this peak was no longer observed. This suggested that 5M HCl was an appropriate starting point from which to establish suitable conditions for cleavage of linker 110 from carboxy TG.
Treatment of linker 110 coupled to carboxy TG (121) with 5M HCl for 16-24 h as expected also produced phenylacetamide and phenylacetic acid (Scheme 55). HPLC of the cleavage mixture indicated loadings ranging from 46%-quantitative. As quantitative yield was obtained in one instance it was assumed that this cleavage method does induce complete cleavage and was therefore a suitable means of determining the loading.

2.4.5 Stability of Linker 110 on Carboxy TG (121)
Linker 110 was designed to be more acid stable than linker 73. To verify that this was the case linkers 73 and 110 were coupled to carboxy TG resin to give 81a and 121 respectively. Subsequently each was treated with varying concentrations of aqueous HCl or 10% TFA.
The results (Table 4) clearly illustrate that 121 is the more stable. For example, when each of the resins was treated with 10% TFA at room temperature overnight 80% of 81a was cleaved as compared to only 30% of 121.

<table>
<thead>
<tr>
<th>Acid</th>
<th>% 81a Cleaved</th>
<th>% 121 Cleaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>5M HCl</td>
<td>100</td>
<td>80</td>
</tr>
<tr>
<td>2M HCl</td>
<td>100</td>
<td>90</td>
</tr>
<tr>
<td>1M HCl</td>
<td>80</td>
<td>40</td>
</tr>
<tr>
<td>0.5M HCl</td>
<td>40</td>
<td>20</td>
</tr>
<tr>
<td>0.25M HCl</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td>0.1M HCl</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>H2O</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10% TFA</td>
<td>80</td>
<td>30</td>
</tr>
</tbody>
</table>

Table 4 Relative acid stability of 81a and 121

This may be even more clearly demonstrated by treating 81a and 121 with 0.5M HCl and monitoring the cleavage of each with time (Table 5, Table 6, Graph 1).
<table>
<thead>
<tr>
<th>Time/h</th>
<th>% Cleavage of 81a</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>19</td>
<td>38</td>
</tr>
<tr>
<td>24</td>
<td>50</td>
</tr>
<tr>
<td>28</td>
<td>54</td>
</tr>
<tr>
<td>44.5</td>
<td>81</td>
</tr>
<tr>
<td>48</td>
<td>67</td>
</tr>
</tbody>
</table>

Table 5 Cleavage of 81a using 0.5M HCl

<table>
<thead>
<tr>
<th>Time/h</th>
<th>% Cleavage of 121</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>25.5</td>
<td>17</td>
</tr>
<tr>
<td>48</td>
<td>34</td>
</tr>
<tr>
<td>51</td>
<td>51</td>
</tr>
</tbody>
</table>

Table 6 Cleavage of 121 using 0.5M HCl

The base stability of linker 110 was also examined by treatment of 121 with varying concentrations of aqueous NaOH (Table 7). Treatment of 121 with 5M NaOH or 1M NaOH resulted in 30% cleavage.

Graph 1 Plot of initial cleavage of 81a and 121 by 0.5M HCl with time
<table>
<thead>
<tr>
<th>Base</th>
<th>% Cleavage of 121</th>
<th>% Cleavage of 121 (repeat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5M NaOH</td>
<td>30</td>
<td>20</td>
</tr>
<tr>
<td>2M NaOH</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>1M NaOH</td>
<td>30</td>
<td>25</td>
</tr>
<tr>
<td>0.5M NaOH</td>
<td>25</td>
<td>25</td>
</tr>
<tr>
<td>H₂O</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 7 Base stability of linker 110 on carboxy TG (121)

121 was also found to be sufficiently stable that storage at 4 °C for 6 weeks had no effect on the loading as determined in each case by acidic (5-6M HCl) cleavage and subsequent HPLC.

2.4.6 Coupling of Alcohols to Linker 110 on Carboxy TG (121)

An attempt was made to couple 2-phenylpropan-1-ol to resin 121 (loading 200 μmol/g) using NIS in CH₂Cl₂ at room temperature overnight. After the coupling reaction a single acidic cleavage (5M HCl) at room temperature overnight was used to estimate the loading of linker and alcohol. The loading of linker was found to be reduced to 70 μmol/g and the loading of the alcohol was 30 μmol/g. The displacement was repeated using freshly distilled CH₂Cl₂ and loading of linker remained unchanged (200 μmol/g), though only ca.10% coupling of the alcohol was observed. Using both freshly distilled CH₂Cl₂ and a catalytic amount of triflic acid also resulted in the linker loading remaining unchanged and ca.10% coupling of the alcohol.
As the coupling of 2-phenylpropan-1-ol to resin 121 appeared to be unsuccessful an attempt was made to couple 2-phenylpropan-1-ol to the azide precursor 120 of linker 110 (Scheme 57). Linker 110 itself was not used for this purpose as the amine could act as a nucleophile competing with the alcohol in the displacement reaction. The desired product 125 was obtained in 82% yield. This suggested that the coupling of the alcohol to the solid support may not have been the problem. In fact, it seemed more likely that the problem lay with the severity of the subsequent acidic cleavage conditions used in the analysis of the alcohol product. These conditions may have caused 2-phenylpropan-1-ol to eliminate to give 2-phenylpropene. This could result in apparent coupling yields lower than those obtained.

Scheme 56 Reagents and conditions: (i) 2-phenylpropan-1-ol, NIS, triflic acid (cat.), 4 Å molecular sieves, CH₂Cl₂, RT, 16-24 h

Scheme 57 Reagents and conditions: (i) 2-phenylpropan-1-ol, NIS, CH₂Cl₂, 4Å molecular sieves, 0 °C-RT, 1 h
Linker 110 was synthesised from 4-bromobutanal in a maximum overall yield of 21% and coupled to carboxy TG resin. It was also found to be more acid and base stable than linker 73. Though the coupling of a primary alcohol to linker 110 on carboxy TG appeared to be unsuccessful it seems more likely that the problem was the cleavage conditions employed rather than the coupling. Although linker 110 had interesting properties, the extensive purification required during the synthesis of 110 and the low overall yield were prohibitive to its general use.

2.5 Other Approaches to the Synthesis of Aliphatic Linkers
As the synthesis of 110 was not amenable to scale-up alternative syntheses of linkers from aliphatic aldehydes were sought. It was thought that the commercially available dimethyl acetal 126 could be deprotected to give 3-bromopropanal 127 which would undergo reaction with phenylacetamide and benzotriazole in the same way as 4-bromobutanal. This was expected to produce 128 and the same chemistry used for the synthesis of linker 110 could then be used to generate its homologue 103 (Scheme 58).

Treatment of the dimethylacetal 126 with 50% TFA resulted in a mixture of the desired aldehyde, starting material and aldehyde trimer. This mixture was subjected
to reaction with phenylacetamide and benzotriazole in toluene at reflux under Dean-Stark conditions. 129 was isolated from a complex mixture in a yield of 29% from benzotriazole.

\[
\begin{align*}
\text{129}
\end{align*}
\]

It was likely that the elimination of HBr from 3-bromopropanal took place prior to the adduct formation as compound 118 (Scheme 49) was isolated when starting with 4-bromobutanal suggesting that the bromine is not easily displaced by benzotriazole after adduct formation. Michael addition of benzotriazole to either the unsaturated aldehyde or to the corresponding adduct would give rise to the isolated product 129. An attempt was also made to carry out an \textit{in situ} deprotection of acetal 126 by reaction with phenylacetamide, benzotriazole and a catalytic amount pTSA in toluene at reflux under Dean-Stark conditions. The only compound isolated from the resultant mixture appeared to be impure 129 though this did suggest that \textit{in situ} deprotection of the acetal did occur. Another commercially available protected aldehyde with appropriate functionality for attachment to solid support was 3,3-diethoxypropionic acid ethyl ester (132, Scheme 59). As adduct 130 had previously been successfully synthesised\textsuperscript{88} there was little concern about deprotection of the acid functionality prior to adduct formation.

\[
\begin{align*}
\text{130}
\end{align*}
\]

Deprotection of the acetal 132, adduct formation and displacement of the benzotriazole group was expected to furnish linker 127.
The ester 132 was hydrolysed to the corresponding acid 133. Since there appeared to be some evidence that \textit{in situ} deprotection of an acetal was possible under the conditions of adduct formation this was attempted using 133 (Scheme 59). This result was a multi-component mixture.

![Scheme 59](image)

\textbf{Scheme 59} \textit{Reagents and conditions}: (i) THF, MeOH, 1M NaOH, RT, 2 h (ii) benzotriazole, phenylacetamide, pTSA, toluene, reflux under Dean-Stark conditions, 1 h

The only compound isolated from the mixture was 135, in a yield of 3.5%.
3. RESULTS AND DISCUSSION - GENERATION OF LINKERS ON SOLID SUPPORT

Although both linkers 73 and 110 demonstrated much potential their preparation on large scale was prohibitively time consuming and attempts to synthesise related linkers were unsuccessful. An obvious alternative approach was to construct the linker on solid support thus taking full benefit of the advantages of solid-phase chemistry.

3.1 Resin/linker Systems Designed for Acidic Cleavage

As linker 73 could be cleaved under mild acidic conditions it was not always necessary to use an enzyme compatible resin. Polystyrene resin was therefore selected as it was available with higher loading of appropriate functional groups than the enzyme compatible resins investigated previously (TG and PEGA).

In order to make a variety of linkers based on the motif 136 a range of support-bound aromatic aldehydes was required. Reaction of these aldehydes with benzotriazole and phenylacetamide was expected to give support-bound benzotriazole adducts. Subsequent displacement of the benzotriazole group using the ethyithiolate anion would then result in the desired resin/linker systems. As the objective was cleavage under mild acid conditions a support-bound aliphatic aldehyde was not required because this would result in a more acid stable structure.

3.1.1 Adduct Formation and Determination of Loading

Of the support-bound aldehydes used for adduct formation formyl polystyrene 137 was commercially available.
It was necessary to synthesise the other aldehydes. Displacement of the chloride from chloromethyl polystyrene 139 using 4-hydroxybenzaldehyde 138 produced a support-bound aromatic aldehyde 140 with an oxygen at the para position (Scheme 60). Elemental analysis of 140 indicated <0.1% chlorine suggesting that the displacement had been successful.

Scheme 60 Reagents and conditions: (i) NaH, NaI, DMF, 60 °C, 24 h

The appearance of an aldehyde absorbance at 1694 cm⁻¹ in the IR spectrum of the product was also observed (Figure 15).
Figure 15 a. IR of chloromethylpolystyrene resin 139 b. IR of resin-bound aldehyde 140

Acylation of aminomethyl polystyrene 141 with carboxybenzaldehyde 77 gave a resin bound aromatic aldehyde with an amide group in the para position 142 (Scheme 61). Again the appearance of an aldehyde absorbance in the IR spectrum of the product was observed (Figure 16).
Scheme 61. Reagents and conditions: 1. EEDQ, THF, 48 h, RT

Figure 16a. IR of aminomethyl poly(styrene resin 141) b. IR of resin-bound aldehyde 142
Reaction of each of the support-bound aldehydes 137, 140 and 142 with phenylacetamide and benzotriazole in toluene under Dean-Stark conditions produced the resin bound adducts 146, 147 and 143 respectively (Scheme 64 and Scheme 65). 147 was predicted to be less acid stable than 146 due to the electron donating oxygen para to the acetal cleavage site. Conversely, 143 with its electron withdrawing amide group in the para position was predicted to be more acid stable. As 143 was expected to be the most stable any acidic cleavage conditions which resulted in complete cleavage of this adduct should also induce complete cleavage of the other adducts. Treatment of 143 for 19 h with TFA:CH₂Cl₂:H₂O (9:10:1) produced phenylacetic acid, phenylacetamide and benzotriazole (Scheme 62) which were quantified by HPLC (Figure 17, retention time 4.3 min., 18.6 min. and 21.4 min. respectively). Treating the same resin again with TFA:CH₂Cl₂:H₂O (9:10:1) for a further 22 h resulted in production of a negligible quantity of benzotriazole and <5% phenylacetamide. On this basis it was assumed that complete cleavage had occurred.

Scheme 62 Reagents and conditions: (i) TFA:CH₂Cl₂:H₂O (9:10:1), overnight, RT
Figure 17 HPLC trace from cleavage of resin bound adduct 143

Using this method 143 was found to have a benzotriazole, and hence adduct, loading of 340 μmol/g (46% from aminomethyl polystyrene). The phenylacetamide and phenylacetic acid indicated a phenylacetamide loading of 600 μmol/g. This suggested that instead of forming the desired 1:1:1 adduct that some 2:1 phenylacetamide:aldehyde resin adduct 145 has been formed.

This in conjunction with the fact that no residual aldehyde was observed in the 13C gel carbon spectrum also suggested that the initial acylation of aminomethyl-polystyrene did not proceed to completion as only 470 μmol/g of 143 + 145 (340 +
(600-340)/2) µmol/g) was produced when a maximum loading of 740 µmol/g of 143 + 145 could be produced. Cleavage and analysis of 146 also indicated a degree of over-reaction. The loading of benzotriazole was 520 µmol/g as compared to a phenylacetamide loading of 620 µmol/g. IR and 13C gel carbon NMR indicated no residual aldehyde. A benzotriazole loading of 520 µmol/g is quantitative based on manufacturers loading of aldehyde (570 µmol/g).

Over-reaction was not considered a significant hindrance as the 2:1 adduct 145 was expected to be more stable than the desired adduct itself and should not participate in later steps. The only consideration was the concomitant reduction in yield of the active linker. In an bid to avoid over-reaction formation of adduct 147 was monitored by IR.

The reaction was stopped when there appeared to be no further change in the aldehyde carbonyl region of the IR spectrum. A small peak corresponding to residual aldehyde was observed in the 13C gel carbon spectrum of the final product. This illustrated that though this type of IR spectroscopy (CH2Cl2 squash, Section 6.3.3) gives some information about the extent of reaction it cannot be relied upon absolutely. Acidic cleavage of 147 produced the expected benzotriazole (450 µmol/g, 62% from chloromethyl polystyrene), phenylacetamide and small amount of
phenylacetic acid. Another peak was also observed which was so close in retention time to phenylacetamide as to be not completely resolved (the phenylacetamide was not therefore quantified). The compound responsible for this peak was identified as 4-hydroxybenzaldehyde. It was not surprising that under the acidic cleavage conditions the ether bond was also cleaved (Scheme 63).

**Scheme 63** Reagents and conditions: (i) TFA:CH$_2$Cl$_2$:H$_2$O (9:10:1), overnight, RT

<table>
<thead>
<tr>
<th>Commercial starting resin</th>
<th>Adduct Formed</th>
<th>Loading of active adduct/(μmol/g)</th>
<th>% Yield from commercial resin</th>
</tr>
</thead>
<tbody>
<tr>
<td>137</td>
<td>146</td>
<td>520</td>
<td>quantitative</td>
</tr>
<tr>
<td>139</td>
<td>147</td>
<td>450</td>
<td>62%</td>
</tr>
<tr>
<td>141</td>
<td>143</td>
<td>340</td>
<td>46%</td>
</tr>
</tbody>
</table>

Table 8 Loading of support-bound adducts
The yields for the adduct formation from commercial starting materials are summarised in Table 8.

Scheme 64 Reagents and conditions: (i) PhCH₂CONH₂, benzotriazole, pTSA (cat.), toluene, reflux under Dean-Stark conditions, 18 h (ii) NaSEt, THF, RT, overnight (iii) Fmoc-Ser-OMe, NIS, triflic acid (cat.), 4 Å molecular sieves, CH₂Cl₂, RT, overnight (iv) 1-phenylpropan-2-ol, NIS, triflic acid (cat.), 4 Å molecular sieves, CH₂Cl₂, RT, overnight (v) 2-phenylpropan-1-ol, NIS, triflic acid (cat.), 4 Å molecular sieves, CH₂Cl₂, RT, overnight
Scheme 65 *Reagents and conditions:* (i) PhCH₂CONH₂, benzotriazole, pTSA (cat.), toluene, reflux under Dean-Stark conditions, for \( x = \text{CONH} \) \( T = 18 \) h, \( x = \text{O} \) \( T = 4.5 \) h (ii) NaSEt, THF, RT, overnight (iii) Fmoc-Ser-OMe, NIS, triflic acid (cat.), 4 Å molecular sieves, CH₂Cl₂, RT, overnight

3.1.2 Displacement of the Benzotriazole Group Using Sodium Ethyllithiate and Subsequent Alcohol Coupling

Benzotriazole was displaced from resins 146, 147 and 143 using sodium ethyllithiate to give 148, 151 and 152 respectively (Scheme 64 and Scheme 65). Sulfur elemental analysis indicated that this reaction had proceeded in excellent yield in each case (Table 9) and characteristic \( \text{SCH}_2\text{CH}_3 \) and \( \text{SCH}_2 \) peaks were observed in the \( ^{13}\text{C} \) gel phase carbon NMR spectra (Figure 18, Figure 19, Figure 20) at 15 and 26 ppm respectively.
<table>
<thead>
<tr>
<th>SEt adduct</th>
<th>Loading/ (µmol/g)</th>
<th>% Yield from benzotriazole adduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>148</td>
<td>490</td>
<td>91</td>
</tr>
<tr>
<td>151</td>
<td>510</td>
<td>111</td>
</tr>
<tr>
<td>152</td>
<td>360</td>
<td>103</td>
</tr>
</tbody>
</table>

Table 9 Yields for displacement of benzotriazole from solid support by sodium ethylthiolate

Figure 18 $^{13}$C Gel phase NMR spectrum of 148
Activation of the thioethyl group of each of these resins (148, 151 and 152) using N-iodosuccinimide (NIS) and a catalytic amount of triflic acid then allowed displacement by Fmoc-Ser-OMe to give 149, 153 and 154 (Scheme 64 and Scheme
65). The loading of Fmoc-Ser-OMe was determined by Fmoc cleavage and UV analysis (Table 10).

<table>
<thead>
<tr>
<th>Resin</th>
<th>Loading of Fmoc-Ser-OMe/(μmol/g)</th>
<th>% Yield from benzotriazole adduct</th>
</tr>
</thead>
<tbody>
<tr>
<td>149</td>
<td>300</td>
<td>67</td>
</tr>
<tr>
<td>153</td>
<td>400</td>
<td>98</td>
</tr>
<tr>
<td>154</td>
<td>290</td>
<td>91</td>
</tr>
</tbody>
</table>

Table 10 Yields for the displacement of ethylthiolate by Fmoc-Ser-OMe

Displacement of the thioethyl group of resin 148 using 2-phenylpropan-1-ol (Scheme 64) also appeared to be successful as two CH₃ peaks, arising from the existence of 150 as diastereomers could clearly be seen in the ¹³C gel spectrum of this resin. When an attempt was made to couple 1-phenylpropan-2-ol to resin 148 to give 144 (Scheme 64) the CH₃ peaks could barely be seen in the ¹³C gel spectrum. Though not quantitative this did suggest that the coupling of the secondary alcohol was less successful.

3.1.3 Acid Stability

The relative acid stability of linkers 73 and 110 was adequately assessed by examining the linkers themselves on solid support. A more synthetically useful picture of the acid stability of the resin/linker systems generated on solid support was obtained by examining the acid stability after coupling of an alcohol, namely Fmoc-Ser-OMe. Applying the same rational as for the benzotriazole adducts 153 was predicted to be least acid stable due to the electron donating oxygen para to the acetal cleavage site and 154 with it’s electron withdrawing amide group in the para position was predicted to be most acid stable. 154 was also expected to be directly comparable, in terms of acid stability, to the previously prepared 99a.
The resins were treated with acetic acid:CH₂Cl₂:H₂O (27:68:5) at room temperature for a given time. When the cleavage time was 15 min., the results (Table 11) clearly indicated that system 153 was least stable and systems 154 and 99a were of comparable stability.

<table>
<thead>
<tr>
<th>Resin</th>
<th>Loading*/ (µmol/g)</th>
<th>Cleavage for 15 min./(µmol/g)</th>
<th>% Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>99a</td>
<td>600</td>
<td>10</td>
<td>2</td>
</tr>
<tr>
<td>149</td>
<td>280</td>
<td>50</td>
<td>18</td>
</tr>
<tr>
<td>153</td>
<td>400</td>
<td>170</td>
<td>43</td>
</tr>
<tr>
<td>154</td>
<td>270</td>
<td>10</td>
<td>4</td>
</tr>
</tbody>
</table>

* loading determined by cleavage of Fmoc from the solid support (Section 6.3.2)

Table 11 Acid stability of resin/linker systems

Results obtained from cleavage over longer periods of time (1h or 20 h, Table 12) were also largely in agreement with this prediction. The only deviation from the predicted trend was that when the cleavage time was 20 h a larger proportion of 149 than 153 was cleaved. Interestingly these were samples which required an additional dilution prior to HPLC injection. This suggested that the process of diluting the sample may have introduced error.
<table>
<thead>
<tr>
<th>Resin</th>
<th>loading*/μmol/g</th>
<th>Cleavage for 1 h/μmol/g</th>
<th>% Cleavage</th>
<th>Cleavage for 20 h/μmol/g</th>
<th>% Cleavage</th>
</tr>
</thead>
<tbody>
<tr>
<td>99a</td>
<td>600</td>
<td>30</td>
<td>5</td>
<td>270</td>
<td>45</td>
</tr>
<tr>
<td>149</td>
<td>280</td>
<td>130</td>
<td>46</td>
<td>300*</td>
<td>100*</td>
</tr>
<tr>
<td>153</td>
<td>400</td>
<td>300*</td>
<td>75*</td>
<td>320*</td>
<td>80*</td>
</tr>
<tr>
<td>154</td>
<td>270</td>
<td>20</td>
<td>7</td>
<td>140</td>
<td>50</td>
</tr>
</tbody>
</table>

* additional dilution of HPLC mixture was necessary before injection
* loading determined by cleavage of Fmoc from the solid support (Section 6.3.2)

Table 12 Acid stability of resin/linker systems

3.1.4 Conclusions
A range of resin/linker systems has been efficiently generated on the solid support. A primary alcohol (Fmoc-Ser-OMe) has been coupled to each of these systems in good (67%) to excellent (98%) yield. The acid stability of these systems was found to be modulated depending on the nature of the support-bound aldehyde starting material. A resin/linker system with comparable acid stability to that observed for linker 73 on polystyrene was amongst those synthesised. The ease of synthesis of these systems should make their use preferable to the use of analogous linkers in solution.

3.2 Resin/linker Systems Designed for Enzyme Cleavage
As before (Section 2.1.1) resins TG and PEGA were selected for potential enzyme cleavage. In order to synthesise analogous resin/linker systems to 151 and 152 (Section 3.1.2) support-bound aldehydes 155, 156, 157 and 158 were required.
3.2.1 Preparation of Aldehydes

155 was synthesised by displacement of the bromide from Bromo TG 159 (Scheme 66). This reaction was deemed to have been successful as elemental analysis indicated <10% residual bromine.

![Scheme 66 Reagents and conditions: (i) Bu₄NI, NaH (60% in oil), DMF, RT for 24 h then 60°C for 24 h](image)

An attempt was made to prepare the corresponding aldehyde on PEGA resin by means of a Mitsunobu reaction starting with HMPA PEGA (160, Scheme 67).

![Scheme 67 Reagents and conditions: (i) PPh₃, DEAD, THF, 0°C to RT, 18 h](image)

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This reaction was judged, on the basis of the IR spectrum, to have been unsuccessful and the resin, assumed to still be 160, was then subjected to IBX oxidation (Scheme 68).

\[ \text{Scheme 68 Reagents and conditions: (i) IBX, DMSO, RT, 3 days} \]

\[ \text{13C Gel carbon NMR later showed the presence of an aldehyde carbonyl in the spectrum of the resin prior to IBX oxidation. This suggested that the Mitsunobu reaction had been, at least to some extent, successful and that the resin bound aldehyde 156 used in adduct preparation may in fact have been either 161 or mixture of 161 and 162.} \]

\[ \text{IBX oxidation was also used for the preparation of resin bound aldehyde 158a from HMBA PEGA 163 (Scheme 69).} \]
Scheme 69 Reagents and conditions: (i) IBX, DMSO, RT, 18 h

Alternatively, 158b was prepared by reaction of amino PEGA 83 with carboxybenzaldehyde 77 (Scheme 70).

Scheme 70 Reagents and conditions: (i) EEDQ, THF, RT, 16-48 h

In the same way resin bound aldehyde 157 was prepared from amino TG resin 163 (Scheme 70).

As these aldehydes were to be used to make resin/linker systems for enzymatic cleavage a support-bound aliphatic aldehyde would also be useful as additional acid stability may be an advantage. TG acetal resin 164b was commercially available and easily deprotected to give aldehyde 165 (Scheme 71). Adduct formation from this aldehyde would produce a system analogous to the coupling of linker 110 onto carboxy TG (121).

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b The structure of this resin was described by 2 different sources as 4,4-diethoxybutyramido TG and 6,6-diethoxyhexamido TG. I have shown the structure as 4,4-diethoxybutyramido TG.
Scheme 71 Reagents and conditions: (i) 5M HCl, H₂O, acetone, reflux, 1 h.

For all these resins the presence of the aldehyde was confirmed by IR spectroscopy though the loading was not quantified. 155, 156, 158a, 158b, 157 and 165 were each progressed through benzotriazole adduct formation, displacement of the benzotriazole group by the ethylthiolate anion and alcohol (Fmoc-Ser-OMe) coupling as discussed in Sections 3.1.1 and 3.1.2 for polystyrene bound aldehydes.

3.2.2 Synthesis of Resin/linker Systems and Alcohol Coupling

Reaction of aldehyde resin 155 with phenylacetamide and benzotriazole resulted in adduct resin 166 (Scheme 72). This was found, by acidic cleavage, to have a phenylacetamide loading of 90-100 μmol/g and a benzotriazole loading of 120 μmol/g (46% from Bromo TG). It was unexpected that the benzotriazole loading was greater than the phenylacetamide loading. The discrepancy may have been partly due to the benzotriazole displacing residual bromide from the resin to give 169.

No 4-hydroxybenzaldehyde was observed in the cleavage mixture indicating the ether bond was not cleaved under the conditions of loading determination as it had been when the resin was polystyrene. As the loading of benzotriazole was so low it was possible that the adduct decomposed during the reaction. Displacement of the benzotriazole group by sodium ethylthiolate gave the desired support-bound linker 167 for which sulfur elemental analysis indicated a loading of 110 μmol/g (92%). Fmoc-Ser-OMe was then coupled to give an alcohol loading of 100 μmol/g. This corresponded to a yield of 75% from benzotriazole adduct 166 but compared poorly with the maximum theoretical loading of Fmoc-Ser-OMe (250 μmol/g).
In the same way adduct 170 (Scheme 73) was formed from aldehyde resin 156 and subjected to acidic cleavage. HPLC of the cleavage mixture indicated that the benzotriazole loading was 190 µmol/g while the phenylacetamide loading was 230 µmol/g. As for adduct 166 (Scheme 72) no 4-hydroxybenzaldehyde was observed in the mixture. The percentage yield of benzotriazole adduct from commercial resin could not be calculated as the full structure of the aldehyde input was not known (Section 3.2.1). Displacement of the benzotriazole group with sodium ethylthiolate resulted in 171. This resin was determined to have a loading of 70 µmol/g by sulfur elemental analysis and Fmoc-Ser-OMe was then coupled to give 172 (alcohol loading 70 µmol/g, 39% from 170).
Scheme 73 Reagents and conditions: (i) PhCH$_2$CONH$_2$, benzotriazole, pTSA (cat.), toluene, reflux under Dean-Stark conditions, 18h (ii) NaSEt, THF, RT, 16-24 h (iii) Fmoc-Ser-OMe, NIS, triflic acid (cat.), 4 Å molecular sieves, CH$_2$Cl$_2$, room temp, 16-24 h

The $^{13}$C gel phase carbon NMR spectra of 170 and 171 did not contain any aldehyde carbonyl resonance however the spectrum of 172 did. It may have been that the aldehyde was regenerated under the conditions of the activation and displacement of the thioethyl group or that 172 was unstable on storage. This was not observed for any other resin and may have been related to the nature of the starting aldehyde 156 (Section 3.2.1).

Following the same procedure reaction of phenylacetamide and benzotriazole with aldehyde 158a produced resin 173a (Scheme 74) with 100 μmol/g benzotriazole (27% from HMBA PEGA) and 130 μmol/g phenylacetamide. Progression to the corresponding thioethyl compound resulted in resin 174a for which the sulfur elemental analysis was very low (20 μmol/g). However, Fmoc-Ser-OMe was coupled to this resin to give an alcohol loading of 100 μmol/g (quantitative from 173a) suggesting that the sulfur elemental analysis was erroneously low. Unexpectedly low results were sometimes observed for sulfur elemental analysis when the resin used was TG or PEGA rather than polystyrene. The overall yield to the Fmoc compound 175a from HMBA PEGA was 32%.
As an alternative to beginning this synthesis with the oxidation of HMPA PEGA aldehyde resin 158b was prepared by the acylation of amino PEGA resin with carboxybenzaldehyde (Section 3.2.1). 158a and 158b were both PEGA resins bearing the same type of aldehyde. However, as the aldehyde was generated in a different way in each case they may have differed in loading and also in what else besides the aldehyde was on the resin. The adduct 173b formed from aldehyde 158b (Scheme 75) was found to contain 100 μmol/g benzotriazole (29% from PEGA-NH₂) and 140 μmol/g phenylacetamide. Sulfur elemental analysis of the thioethyl compound 174b produced from adduct 173b was very low (<0.1%). However, when this resin was subjected to Fmoc-Ser-OMe coupling Fmoc analysis of the product 175b indicated a loading of 100 μmol/g (quantitative from 173b). The overall yield from PEGA-NH₂ was 31% comparable to that observed from HMBA PEGA (32%).
Scheme 75 Reagents and conditions: (i) PhCH₂CONH₂, benzotriazole, pTSA (cat.), toluene, reflux under Dean-Stark conditions, 14.5 h (ii) NaSEt, THF, RT, overnight (iii) Fmoc-Ser-OMe, NIS, triflic acid (cat.), 4 Å molecular sieves, CH₂Cl₂, RT, overnight.

Aldehyde 157 was prepared as the TG analogue of PEGA aldehydes 158a and 158b and it was used to generate adduct 176 (Scheme 76). The benzotriazole loading was 100 µmol/g (30% from carboxy TG resin) and the phenylacetamide loading 130 µmol/g. Sulfur analysis of the thioethyl compound 177 prepared from this adduct was very low (<0.1%). However, after subjecting this resin to Fmoc-Ser-OMe coupling conditions an alcohol loading of 100 µmol/g was observed. This corresponds to a yield of 91% from 176 (29% from carboxy TG resin).
Scheme 76 Reagents and conditions: (i) PhCH₂CONH₂, benzotriazole, cat. pTSA, toluene, reflux under Dean-Stark conditions, 21.5 h (iii) NaSEt, THF, RT, 16-24 h (iv) Fmoc-Ser-OMe, NIS, cat. triflic acid, 4 Å molecular sieves, CH₂Cl₂, RT, overnight.

As for the resin bound aromatic aldehydes described above aliphatic aldehyde 165 underwent reaction with benzotriazole and phenylacetamide to produce the support-bound adduct 179 (Scheme 77). This reaction was also monitored by IR spectroscopy nevertheless, as before, this proved an unsatisfactory way of determining whether the reaction had gone to completion. The loading of benzotriazole was found to be 110 µmol/g, (42% from the acetal resin) and the phenylacetamide loading was found to be 100 µmol/g. Displacement of benzotriazole to give the thioethyl compound resulted in resin for which the sulfur elemental analysis was again very low (<0.1%). However, the Fmoc-Ser-OMe was coupled to a loading of 40 µmol/g, (36% from 179, 16% from 4,4-diethoxybutyramido TG). This was very significant as it added credence to the suggestion (Section 2.4.6) that the coupling of alcohol 2-phenylpropan-1-ol to linker 110 on carboxy TG (121) had not been unsuccessful but that the cleavage conditions used to assess the extent of coupling were indeed overly aggressive.
Scheme 77 Reagents and conditions: (i) PhCH₂CONH₂, benzotriazole, toluene, reflux under Dean-Stark conditions, 8 h (ii) NaSEt, THF, RT, 16-24 h (iii) NIS, CH₂Cl₂, triflic acid (cat.), 4 Å molecular sieves, RT, 16-24 h

Resin 180 was also treated with secondary alcohol 1-phenylpropan-2-ol under alcohol coupling conditions (Scheme 78).

Scheme 78 Reagents and conditions: (i) 1-phenylpropan-2-ol, NIS, CH₂Cl₂, triflic acid (cat.), 4 Å molecular sieves, RT, 16-24 h

The success of this coupling was measured by cleavage of the resultant resin 182 with 2M HCl overnight at room temperature. 1-phenylpropan-2-ol corresponding to a loading of 50μmol/g (45%) was observed by in the cleavage mixture by HPLC. This also suggests that the coupling of alcohol 2-phenylpropan-1-ol to linker 110 on carboxy TG (121, Section 2.4.6) had not been unsuccessful but that the cleavage conditions used to assess the extent of coupling were overly aggressive.
3.2.3 Preliminary Enzyme Studies

Previously, when attempts had been made to carry out enzymatic cleavage of linker 73 from carboxy TG the work-up procedure consisted of decanting the phosphate buffer solution from the resin, acidifying this solution to denature the enzyme and then obtaining phenylacetic acid by extraction of this mixture. Preliminary studies into the enzymatic cleavage of alcohols from solid support were also carried out using this method. However, owing to the limited amount of material available this procedure had to be carried out on very small scale. On such a scale the error due to mechanical loss was undoubtedly very significant. Time constraints also meant that this method could not be validated or optimised. Resins 168, 172, 175a, 175b, 178 and 181 were subjected to penicillin amidase solution and the reactions treated as described above. Resins 95b and 98 arising from the coupling of linker 73 to carboxy TG and PEGA respectively followed by Fmoc-Ser-OMe coupling were also subjected to these conditions for comparison. In each case less than 10% Fmoc-Ser-OMe was recovered. Control reactions were performed in which the resin was treated with phosphate buffer alone. These also resulted in a recovery of Fmoc-Ser-OMe of <10%. For the reasons discussed above it was difficult to draw a conclusion from these results.

3.2.4 Conclusions

A range of resin/linker systems designed for enzymatic cleavage was efficiently generated on the solid support. A primary alcohol (Fmoc-Ser-OMe) has been coupled to each of these systems. The development of an enzyme cleavage protocol was begun but a satisfactory procedure has not yet been developed.
4. RESULTS AND DISCUSSION - PEPTIDE SYNTHES

Pentapeptide 183 was selected as the target compound to demonstrate the application of linker 73 in synthesis. This compound is based on a glycopeptide fragment 184 from serum response factor (SRF)\textsuperscript{89}. SRF is a member of the MADS-box family of transcription factors and is of significant biological importance\textsuperscript{90}.

![Figure 21 Target peptide and glycopeptide fragment from SRF](image)

Solid-phase peptide synthesis is generally carried out by coupling the carboxyl functionality of the first amino acid to the solid support through a linker. Subsequent deprotection/ amino acid activation and coupling cycles are then carried out (Scheme 79). At the end of the synthesis the linker and protecting groups are cleaved to give the desired peptide\textsuperscript{91}.

The present linker system allowed an alternative approach by coupling through side-chain hydroxyl groups. When carrying out synthesis of the target compound 183 using linker 73 on polystyrene (88a/b) the first amino acid (Fmoc-Ser-OMe) would be coupled through the side-chain hydroxy functionality rather than the carboxyl group. After the coupling of the first amino acid peptide synthesis would be carried out in the usual fashion.
4.1 Preliminary work - Manual Synthesis of Peptide 183

Linker 73 had been coupled to carboxy polystyrene using the acid fluoride coupling method discussed in section 2.1.1. Fmoc-Ser-OMe was coupled to this material using the conditions previously developed for alcohol coupling (Section 2.1.4) to give 99a (Scheme 80). The Fmoc protecting group was removed using piperidine in DMF to give 185a and the next amino acid (Fmoc-Ser(Trt)-OH) coupled using standard peptide coupling reagents (TBTU, HOBT and NMM in DMF) to give 186a.
Scheme 80  **Reagents and conditions:** (i) 20% piperidine in DMF, RT, 25 min. (ii) Fmoc-Ser(Trt)-OH, TBTU, HOBt, NMM, DMF, RT, overnight, 6 h (double coupling)

The deprotection/activation and coupling cycle shown in Scheme 80 was repeated for Fmoc-Val-OH then Fmoc-Ala-OH and finally Fmoc-Ser(Bu')-OH in turn to produce 187a.

After each coupling step a portion of the resin was subjected to treatment with TFA:DCM:H₂O (9:10:1). In addition to effecting release from the solid support these conditions also removed the side-chain tertiary-butyl and trityl protecting groups but
not the N⁰-protecting group (Fmoc). Analysis of the cleavage products by electrospray mass spectrometry indicated the presence of the desired MNa⁺ ion in each case. Impurity peaks were also observed, most notably MNa⁺-87 was seen in later samples which suggested that the first amide bond forming reaction may have been incomplete.

In order to obtain more material this synthesis was repeated. The batch of linker 73 on polystyrene used was prepared using DIC/HOBt coupling (88b, Section 2.1.1). Fmoc-Ser-OMe was coupled to this material using the conditions previously developed for alcohol coupling (Section 2.1.4) to give 99b (Scheme 81). The deprotection step was carried out using 20% piperidine in DMF and the activation and coupling step was carried out using the appropriate amino acid with TBTU, HOBt and NMM in DMF (Scheme 81) to produce 187b.

![Scheme 81](image)

Scheme 81 Reagents and conditions: (i) 20% piperidine in DMF, RT, 25 min. (ii) Fmoc-aa-OH, TBTU, HOBt, NMM, DMF, RT, 6h, overnight (double coupling)

At the end of the synthesis acidic cleavage using TFA:DCM:H₂O (9:10:1) and analysis of the cleavage mixture by mass spectrometry indicated a very small peak at 708 corresponding to MNa⁺ and a peak corresponding to MNa⁺-87. This suggested that this synthesis had been less successful. Further attempts at this synthesis were
limited by the availability of linker 73. For this reason resin/linker systems generated on solid support were examined.

In the first instance the synthesis was also carried out using resin/linker system 148 as it was available in large quantities due to the commercial nature of the starting material (formyl polystyrene).

Fmoc-Ser-OMe was coupled onto 148 using the standard alcohol coupling protocol (Section 3.1.2) to give 149 (Scheme 82). The deprotection of 149 was effected using 20% piperidine in DMF and subsequent coupling of Fmoc-Ser(Trt)-OH was carried out using TBTU, HOBt and NMM in DMF to produce 188. Removal of the Fmoc protecting group from 188 using piperidine in DMF and UV analysis of the deprotection solution indicated that the coupling had been unsuccessful (<25%). The reason for the failure of this coupling reaction is not yet known although the absence of a spacer in this structure and/or limited chemical stability of this resin linker construct may have been responsible.
Scheme 82 Reagents and conditions: (i) 20% piperidine in DMF, RT, 25 min., RT (ii) Fmoc-Ser(Trt)-OH, TBTU, HOBt, NMM, DMF, RT, 6 h, overnight (double coupling)

The support generated system 152 was also easily accessible. This system would be expected to have similar properties to linker 73 on polystyrene (88a/b).

Fmoc-Ser-OMe was coupled to resin 152 using the standard alcohol coupling procedure (Section 3.1.2). The Fmoc group was removed using piperidine in DMF and Fmoc-Ser(Trt)-OH coupled using coupled using TBTU, HOBt and NMM in
DMF. This deprotection/activation and coupling cycle was repeated for Fmoc-Val-OH then Fmoc-Ala-OH and finally Fmoc-Ser(Bu')-OH in turn to produce 189a.

Treatment of 189a with TFA:DCM:H2O (9:10:1) and analysis of the products released by mass spectrometry revealed the MNa⁺ peak at 708. The MH⁺-87 peak was not significant though other peaks were present. Peptide synthesis using 152 was most attractive because of the ease of synthesis of the resin/linker system and the encouraging mass spectral data.

4.2 Automated Synthesis of Pentapeptide 183 using Resin/linker System 152
Fmoc-Ser-OMe was coupled to the resin/linker system 152 to produce 154 (Section 3.1.2) in yields of 84% to 91% (270 µmol Fmoc/g to 290 µmol Fmoc/g). 154 was then used in the automated synthesis of pentapeptide 183. The resin was introduced to the peptide synthesiser and a capping step was carried out (acetic anhydride/DIEA/HOBt in DMF/Dioxane). Although strictly speaking not necessary in this case due to the mode of a attachment of the first amino acid this capping procedure was part of the synthesiser’s program. The Fmoc protecting group was removed using piperidine in DMF/Dioxane. The next amino acid was then coupled using ethyl 1-hydroxy-1H-1,2,3-triazole-4-carboxylate 190 (HOCt)⁹² and DIC in DMF/Dioxane.

This capping/deprotection/coupling procedure was repeated with each of the Fmoc amino acids until the Fmoc-protected pentapeptide had been assembled (Scheme 83).
Scheme 83 Reagents and conditions: (i) acetic anhydride, DIEA, HOBT, DMF:dioxane (1:1), RT, 10 min. (ii) 20% piperidine in DMF dioxane (1:1), RT, 6 min., 20% piperidine in DMF dioxane (1:1), RT, 1.5 min. (iii) Fmoc-aa-OH, HOCl, DIC, DMF:dioxane (1:1), RT, 30 min.

A small portion of the N^{\text{Fmoc}}-protected pentapeptide was cleaved from the solid support 189b using TFA:CH$_2$Cl$_2$:H$_2$O (9:10:1) and the cleavage products examined by mass spectrometry (Figure 22). The mass spectrum clearly shows the desired MNa$^+$ ion at 708.
The Fmoc protecting group was removed from the resin-bound peptide 189b by treatment with piperidine in DMF. The peptide 183 was then cleaved from the solid-support by overnight treatment with TFA:CH₂Cl₂:H₂O (9:10:1) (Scheme 84). The crude peptide was slurried in ether and the product was examined by mass spectrometry (Figure 23). MH⁺ (464) and MNa⁺ (486) peaks were seen indicating the presence of the desired product. The regeneration of a support-bound aldehyde was observed in the IR spectrum of the resin after cleavage.
Scheme 84 Reagents and conditions: 20% piperidine in DMF, RT, 30 min. (ii) TFA:DCM:H₂O (9:10:1), RT, 19.5 h

Figure 23 Mass spectrum of pentapeptide 183 prior to preparative HPLC
The peptide was purified by preparative HPLC and examined by analytical HPLC (Figure 24).

![Analytical HPLC trace of pentapeptide 183 after purification](image)

**Figure 24 Analytical HPLC trace of pentapeptide 183 after purification**

The product was examined by 'H NMR (Figure 25). The NMR peaks were assigned with the aid of 2-dimensional spectra (Appendix I). The product appeared acceptably pure with the exception of an unidentified impurity was present with resonances at 1.67, 1.78 and 3.12 ppm.

4.2.1 Conclusions

The support generated resin/linker system had been successfully used in the synthesis of the target pentapeptide 183.
Figure 25 $^1H$ NMR spectrum of purified peptide 183
5. SUGGESTIONS FOR FUTURE WORK

The generation of linkers on solid support was very successful (Section 3) and the synthesis of a peptide using one of these resin/linker systems (152) was also promising (Section 4.2)

\[
\text{Ph} - \text{H} \quad \text{N} \quad \text{S} \quad \text{O} \quad \text{N} \quad \text{Me} \quad \text{PS}
\]

However, the enzymatic cleavage has not been investigated in sufficient depth to draw valuable conclusions (Section 3.2.3). Therefore, several avenues are open for further investigation.

Resin/linker system 152 could be used to synthesise peptides with a modified C-terminus, for example C-terminal peptide amino alcohols such as the amino alcohol analogues of enkephalin and somatostatin synthesised by Neugebauer and Escher\(^7\). Equally, it could be applied to the synthesis of cyclic peptides, for example, the cyclic peptide synthesised by Wirkus-Romanowska \textit{et al}\(^{93}\) 191 could be attached to the solid-support through the serine hydroxyl as was demonstrated in the synthesis of pentapeptide 183 (Section 4.2)

\[
\text{Cys-Val-Gln-Ser-Tyr-Val-Pro-Leu-Phe-Pro-Cys}
\]

As regards the enzymatic cleavage future work should focus on method development for product recovery from the cleavage mixture. When an appropriate method has been established the evaluation of the enzymatic cleavage could be effectively carried out. It is apparent from the literature (Section 1.3 and references therein) that a range of solid supports could prove suitable although it may be necessary to derivatise the resin with an appropriate spacer. Amongst the most promising candidate resins is a PEGA resin prepared for the access of matrix metalloprotease MMP-9\(^{63}\) which is of comparable molecular weight to penicillin amidase.
6. EXPERIMENTAL - GENERAL

6.1 Equipment and Reagents

'\textsuperscript{1}H and '\textsuperscript{13}C NMR spectra were recorded on Bruker AC200, Bruker AC250, Varian Gemini 200 and Varian INOVA 600 MHz instruments.

Electron impact (EI) mass spectrometry was carried out on a Finnigan 4500 or a Finnigan 4600 instrument and fast atom bombardment (FAB) mass spectrometry was performed using a Kratos MS50TC instrument.

Electrospray (ESI) mass spectrometry was carried out on a Micromass Platform II.

Elemental analysis was performed using a Perkin-Elmer 2400 CHN Elemental Analyser.

Elemental analysis of resin bound compounds was carried out by Medac Ltd.

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

Infra-red spectroscopy was carried out on a Biorad FTS-7 instrument or a Perkin Elmer Paragon 1000.

An Applied Biosystems 430A Peptide Synthesiser was employed for automated peptide synthesis.

The 360 °C blood rotator was a Stuart Scientific SB1 and was used to agitate samples at room temperature.

IR cards refers to 3M (type 61) disposable IR cards.

Analytical TLC was carried out on Merck aluminium backed plates coated with silica gel 60F\textsubscript{254}. Ninhydrin, p-anisaldehyde and ammonium molybdate dips, iodine staining and UV light (254 nm) were used for visualisation.

All reagents and solvents were standard laboratory grade and used as supplied unless otherwise stated. Where a solvent has been described as dry either it was purchased as anhydrous grade or was distilled prior to use. CH\textsubscript{2}Cl\textsubscript{2} was distilled from calcium hydride and THF (pre-dried over sodium wire) was distilled from sodium benzophenone ketal. Where petroleum ether is stated in the text petroleum ether b.p. 40-65 °C was used. Penicillin amidase enzyme was purchased from Sigma as a solution in 0.1 M phosphate buffer pH 7.5 (76 mg protein/ml, 23 units/mg of protein). Immobilised penicillin amidase refers to penicillin amidase attached to
microporous oxirane acrylic beads (lyophilised powder containing approx. 50% glucose as stabiliser, 108 units/g of solid) also purchased from Sigma.

6.2 High Performance Liquid Chromatography (HPLC)
The HPLC system used, for all applications other than those involving peptide 183, consisted of a Waters 486 Tunable Absorbance Detector and a Waters 600E Pump and Controller together with the Waters Millennium Chromatography manager. The column employed was a Phenomenex Sphereclone RP-18 (5 μm particle size) with dimensions 25cm × 4.6 mm i.d. A Spherisorb ODS 2 (5 μm particle size) pre-column of length 1 cm was also used. Samples were injected via a 20 μl loop and a flow rate of 1 ml/min was used for elution. A wavelength of 215 nm was used for sample detection. Initially, phenylacetic acid, phenylacetamide, 2-phenylpropan-1-ol and 1-phenylpropan-2-ol were monitored using gradient elution (System 1 or 1a, Table 13).

<table>
<thead>
<tr>
<th>Time</th>
<th>% phosphate buffer (25mM, pH 6.5)</th>
<th>% Acetonitrile</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>15</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td>20</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>30</td>
<td>60</td>
<td>40</td>
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<tr>
<td>40</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>50</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>55</td>
<td>100</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 13 System 1 and 1a HPLC Gradients (system 1 had a 50 min. run time whereas 1a had the full 55 min.)

In the case of gradient elution three standards were run for phenylacetamide and phenylacetic acid and a calibration curve constructed for each. For the alcohols only a single standard was injected. This was later replaced by an isocratic system.
System no. | Compound | Isocratic System
---|---|---
2 | phenylacetic acid and phenylacetamide | 0.1% TFA:MeCN (70:30)
3 | 2-phenylpropan-1-ol | 0.1% TFA:MeCN (70:30)
4 | 1-phenylpropan-2-ol | 0.1% TFA:MeCN (70:30)
5 | benzotriazole | phosphate buffer (25 mM, pH 6.5):MeCN (92:8)
6 | Fmoc-Ser-OMe | 0.1% TFA:MeCN (60:40)

### Table 14 Isocratic HPLC systems

When using the isocratic systems three standards were run for each compound and a calibration curve constructed or a standard concordancy test was carried out.

The equipment used for semi-preperative HPLC consisted of a Gilson 805 Nanometric Module, a Gilson 811c Dynamic Mixer, a Gilson 305 Pump, a Gilson 306 Pump and a Gilson 119 UV/Vis Detector. The column used was an ABI Aquapore RP18 with dimensions 25cm × 10 mm i.d. The solvent system used is described in Table 15.

<table>
<thead>
<tr>
<th>Time/min.</th>
<th>% (MeCN+0.1% TFA)</th>
<th>% (H2O+0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-10</td>
<td>10-40</td>
<td>90-60</td>
</tr>
<tr>
<td>10-32</td>
<td>40-75</td>
<td>60-25</td>
</tr>
<tr>
<td>33-41</td>
<td>70-90</td>
<td>30-10</td>
</tr>
<tr>
<td>33-41</td>
<td>90</td>
<td>10</td>
</tr>
<tr>
<td>41-43</td>
<td>90-10</td>
<td>10-90</td>
</tr>
</tbody>
</table>

### Table 15 HPLC system used for purification of peptide 183

The equipment used for analytical HPLC of the purified peptide consisted of two Applied Biosystems 1406A solvent delivery systems, an Applied Biosystems 1480 injector/mixer and an Applied Biosystems 1783 detector/controller. The column used
was a Vydac C18, 250 mm x 4.6 mm (5 µm particle size). Peptide 183 was eluted using the gradient given in Table 16.

<table>
<thead>
<tr>
<th>Time/min.</th>
<th>% (MeCN + 0.1% TFA)</th>
<th>% (H₂O + 0.1% TFA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-2</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>2-30</td>
<td>0-40</td>
<td>100-60</td>
</tr>
<tr>
<td>30-32</td>
<td>40-90</td>
<td>60-10</td>
</tr>
<tr>
<td>32-34</td>
<td>90-0</td>
<td>10-100</td>
</tr>
<tr>
<td>34-36</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 16 HPLC system used for analytical HPLC of purified peptide 183

6.3 General Protocols

6.3.1 Resin Washing Protocol
A typical wash cycle for 20-50mg of resin consists of THF (3ml x 2), DMF (3ml x 2), DMF:methanol (1:1) (3ml x 2), DMF (3ml x 2), THF (3ml x 2) and finally CH₂Cl₂ (3ml x 2).

6.3.2 Fmoc Analysis
Dry resin (2-10 mg) was accurately weighed in to a 5 or 10 ml volumetric flask. Freshly prepared 20% piperidine in DMF solution was added up to the mark and the mixture sonicated for 10 min.. The supernatant was transferred to a UV cell and the absorbance at λ=301nm recorded. The loading was then calculated using Equation 1 derived from the Beer-Lambert Law using the extinction coefficient quoted by Sabatier et al\textsuperscript{94}. 

Equation 1: \( L = \frac{A \times V}{7.8 \times m} \)

L- loading in mmol Fmoc/g resin
A- absorbance at λ=301nm
V- volume in ml, m=mass of resin in mg
6.3.3 IR Spectroscopy
Where an IR has been taken of the resin this has been done by swelling the resin in CH$_2$Cl$_2$ between NaCl plates.

6.4 Nomenclature of Resin Bound Compounds
When naming compounds in accordance with IUPAC recommendations it is necessary to determine the characteristic group to be cited as a suffix or as functional class name.

For example, in the case of 110, the amide functional group takes precedence over the thioether and amine functional groups. The amide, therefore is the characteristic group and the other groups are substituents which are described using prefixes in alphabetical order. Hence, 110 is called N-[4-amino-1-(ethylsulfanyl)but-1-yl]-2-phenylacetamide

![Image of molecule 110]

When naming resin bound compounds the resin has been considered to be the functional class name. For example, 4-[Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl]phenoxyethyl polystyrene (147).

![Image of molecule 147]

6.5 Gel Phase $^{13}$C NMR
Gel Phase $^{13}$C NMR was carried out by swelling the resin in CD$_2$Cl$_2$. Where $^{13}$C NMR data is quoted in the experimental section diagnostic peaks have been assigned sometimes with the aid of relevant solution spectra. These peaks are observed in the presence of other peaks which were not assigned and may be due to the resin,
impurities generated by reaction on solid support or the desired compound itself. This is unavoidable as the separation of resin bound compounds is impossible.

6.6 Calculation of Yields for Support-Bound Compounds
When calculating the maximum theory yield for a resin bound compound a correction has been made for the weight increase or loss incurred during the reaction.

\[
\text{Ph-} \quad \begin{array}{c}
\text{H} \\
\text{N} \\
\text{NH}_2
\end{array} - \text{N} \quad \begin{array}{c}
\text{SEt} \\
\text{PS}
\end{array} + \quad \begin{array}{c}
\text{HO} \\
\text{PS}
\end{array} \rightarrow \quad \begin{array}{c}
\text{Ph} \\
\text{H} \\
\text{N} \\
\text{N}
\end{array} - \begin{array}{c}
\text{N} \\
\text{SEt}
\end{array} \quad \begin{array}{c}
\text{O} \\
\text{O}
\end{array} \quad \text{PS}
\]

Scheme 85 Coupling of Linker 73 to PS-CO₂H

For example, in the case of coupling linker 73 to carboxypolystyrene (Scheme 85) the loading of functional groups on the carboxypolystyrene was 1.24 mmol/g. If complete coupling was achieved 1g of resin would increase in weight by \((1.24 \times 10^{-3} \times (\text{MWt of linker 73} - 18)) = 0.46 \text{ g}\). The maximum loading of linker would therefore be \(1.24 \times 10^{-3}/1.46 = 0.85 \times 10^{-3} \text{ mol/g}\). Cleavage from the resin indicated a loading of 840 μmol/g. The percentage yield was calculated to be \((840/850 \times 100) = 99\%\).

6.7 Safety Notice
Please note “many compounds of both organic and inorganic derivation, which contain the azide function, are unstable or explosive under appropriate conditions of initiation”\(^{95}\). Appropriate precautions should be taken when handling such compounds. Organic azides synthesised in this project were reduced using PPh₃/H₂O in THF prior to disposal. IBX has been reported to detonate\(^{96}\), again appropriate precautions should be taken.
7. EXPERIMENTAL - SOLUTION SYNTHESIS OF LINKERS

7.1 Preparation of \( N-1-(4-(3\text{-Aminopropylcarbamoyl})phenyl(ethylsulphanyl)-methyl)-2-phenylacetamide, \) Linker 73 (Scheme 26)

7.1.1 3-Azidopropylamine\(^{97}\) \(76\)

Sodium azide (23.0 g, 354 mmol, 2.30 equiv.) was added to a solution of 3-chloropropylamine hydrochloride (75, 20.0 g, 154 mmol) in distilled water (76 ml). The mixture was heated under reflux for 16 h then cooled to 0°C. Diethyl ether (80 ml) was added followed by potassium hydroxide pellets (10.4 g, 185 mmol, 1.20 equiv.), whilst maintaining the temperature below 10 °C. The aqueous layer was separated and extracted with diethyl ether (20 ml x 2), saturated with sodium chloride, then extracted again with diethyl ether (20 ml x 2). The combined organic extracts were dried (\( \text{Na}_2\text{SO}_4 \)), filtered and the solvent removed \textit{in vacuo} to give a pale yellow oil (9.79 g) which was purified by distillation (5.63 g, 37%). \( \delta_H (\text{CDCl}_3; 200 \text{ MHz}) 1.25 (2H, s, \text{NH}_2), 1.65 (2H, \text{quin, } J 6.8, \text{CH}_2\text{CH}_2\text{CH}_2), 2.72 (2H, t, J 6.8, \text{NH}_2\text{CH}_2), 3.30 (2H, t, J 6.8, \text{CH}_2\text{N}_3), \) Ref. \(^{97}\) \( \delta_H (\text{CDCl}_3; 80 \text{ MHz}) 1.46 (2H, s), 1.75 (2H, \text{quin, } J 6.8), 2.80 (2H, t, J 6.8), 3.37 (2H, t, J 6.7). \)

7.1.2 \( N-(3\text{-Azidopropyl})-4\text{-formylbenzamide }69\)

Thionyl chloride (11.7 ml, 19.1 g, 161 mmol, 3.00 equiv.) was added to a solution of carboxybenzaldehyde \( 77 \) (9.00 g, 60.0 mmol, 1.12 equiv.) in THF (90 ml) under nitrogen. The mixture was heated at reflux until all the starting material had dissolved then for a further 1 h before cooling to room temperature. The solvent was removed \textit{in vacuo}, the flask was refilled with nitrogen and dry THF (60 ml) was added. This solution was transferred dropwise under nitrogen pressure to a flask
containing 3-azidopropylamine (76, 5.34 g, 53.4 mmol) and triethylamine (7.40 ml, 5.40 g, 53.4 mmol, 1.00 equiv.) in THF (72 ml) at 0 °C. The resultant mixture was stirred at room temperature for 15 min. Water (70 ml) and chloroform (20 ml) were added and the mixture separated. The aqueous layer was washed with chloroform (20 ml x 2), saturated with sodium chloride and washed with chloroform (20 ml x 2). The combined organic solutions were dried (Na₂SO₄) and the solvent removed in vacuo to give a yellow oil which crystallised as a white solid (7.72 g, 62%). Rₑ 0.42 [petroleum ether:ethyl acetate (2:1)]; δₑ (CDCl₃; 250 MHz) 1.90 (2H, quin., J 6.5, CH₂CH₂CH₂), 3.44 (2H, t, J 6.5, CH₂N₃), 3.55 (2H, q, J 6.5 NHCH₂), 6.74 (1H, broad s, NH), 7.90 (4H, s, CH₆), 10.04 (1H, s, CHO); (Ref. 98  δₑ (CDCl₃; 250 MHz) 1.85 (2H, dd, J 7.0), 3.39 (2H, t, J 6.5), 3.51 (2H, dd, J 6.0), 6.78 (1H, broad s), 7.85 (4H, s), 10.00 (1H, s))

7.1.3 N-[[4-(3-Azidopropylcarbamoyl)phenyl]benzotriazol-1-yl)methyl]-2-phenylacetamide 71

A mixture of aldehyde 69 (6.50 g, 28.0 mmol, 1.00 equiv.), phenylacetamide (3.78 g, 28.0 mmol, 1.00 equiv.) and benzotriazole (3.34g, 28.0 mmol, 1.00 equiv) in dry toluene (100 ml) was heated under reflux for 23 h in Dean-Stark apparatus. The mixture was allowed to cool and the toluene removed in vacuo. The crude product was obtained by trituration from diethyl ether (note no yield can be quoted as some of the crude material was given to another member of the group. This reaction has been carried out by several other group members with yields of 40-60%). A portion of the crude material was purified by column chromatography [silica, petroleum ether:ethyl acetate (1:2)] and the product, a white solid, was obtained in pure form by filtration of the appropriate fractions. Rₑ 0.43 [ethyl acetate:petroleum ether (2:1)]; (Found: C, 63.94; H, 5.37; N, 23.88%. Requires: C, 63.94; H, 5.16; N, 23.92%); δₑ
(CDCl₃; 250 MHz) 1.84 (2H, quin., J 6.6 CH₂CH₂CH₂), 3.36 (2H, t, J 6.6, CH₂N₃), 3.46 (2H, pseudo-quartet, J 7.0, NHCH₂), 3.67 (1H, d, J 15.4, PhCH₂), 3.69 (1H, d, J 15.4, PhCH₂), 6.91 (1H, t, J 5.8 CH₂N₃), 7.07-8.20 (15 H, m, CH₃, CHNH₃). Alternatively, crude product was slurried in ethyl acetate. Petroleum ether was added resulting in a fine precipitate and clear supernatant. The precipitate was filtered off and purified by column chromatography (ethyl acetate:petroleum ether (3:2) or CH₂Cl₂:MeOH (98:2)) to give a white solid. Rf 0.24 [petroleum ether:ethyl acetate (2:1)]; νmax (IR card)/cm⁻¹ 3271 (NH amide), 2097 (N₃), 1639 (amide I), 1538 (amide II); δH (CDCl₃; 250 MHz), 1.86 (2H, quin., J 6.6 CH₂CH₂CH₂), 3.40 (2H, t, J 6.6, CH₂N₃), 3.46 (2H, quartet, J 6.4, NHCH₂), 3.67 (2H, s, PhCH₂), 6.58 (1H, t, J 5.8 CH₂N₃), 7.11-8.07 (15 H, m, CH₃, CHNH₃). δC (CDCl₃; 63 MHz), 28.5, 37.8 (CH₂), 43.1 (PhCH₂), 49.5 (CH₂), 63.8 (CH), 109.6, 119.9, 124.5, 126.5, 127.5, 127.5, 128.1, 129.0, 129.2 (CH₃), 132.6, 133.6. 135.0, 139.2, 145.5 (C₆H₅), 166.7, 170.9 (CO); m/z (FAB) 469 (0.7%, MH⁺); (Found: MH⁺, 469.2089. C₂₅H₂₄N₈O₂ requires MH⁺, 469.2101).

7.1.4 N-([[4-(3-Azidopropylcarbamoyl)phenyl]ethylsulphonyl]methyl)-2-phenylacetamide 72

71 (1.82 g, 3.88 mmol) and technical grade ethylthiolate sodium salt (0.72 g, 8.56 mmol, 2.21 equiv.) were stirred together in THF (40 ml) for 2 h at RT CHCl₃ (50 ml) and H₂O (50 ml) were added and the mixture separated. The aqueous layer was extracted with CHCl₃ (50 ml x 3). The combined organic extracts were washed with H₂O (50 ml) which was back-extracted with CHCl₃ (25 ml). The combined organic solutions were dried (Na₂SO₄) and the solvent removed in vacuo to give a pale yellow solid (1.33 g, 83%). Rf 0.39 [petroleum ether:ethyl acetate (1:2)]; νmax (CD₃OD, IR card)/cm⁻¹ 3290 (NH), 2096 (N₃), 1639 (amide I), 1542 (amide II); δH (CDCl₃; 250 MHz) 1.23 (3H, t, J 7.4, CH₃), 1.88 (2H, quin, J 6.6, CH₂CH₂CH₂), 2.51 (2H, m, SCH₂), 3.42 (2H, t, J 6.6, CH₂N₃), 3.52 (2H, q, J 6.6, CH₂NH), 3.63 (2H, s,
7.1.5 72 (2.44 g, 5.94 mmol) was dissolved in THF (50 ml) and water (1.67 ml, 92.8 mmol, 15.6 equiv.). PPh₃ (1.87 g, 7.12 mmol, 1.20 equiv.) was added and the mixture stirred at room temperature for 4 days. The solvent was removed in vacuo and the mixture triturated with diethyl ether. The product, a white solid, was obtained in a yield of 2.00 g (87%). A small portion was triturated with ether a second time to obtain material for melting point analysis. mp 121.3-123.3 °C; Rₚ base-line [CH₂Cl₂:MeOH (9:1)]; νₘₐₓ (CD₃OD, IR card)/cm⁻¹ 3285 (NH), 1640 (amide I), 1542 (amide II); δₜ (CD₃OD; 250 MHz) 1.31 (3H, t, J 7.4, CH₃), 3.74 (2H, quin, J 6.8, CH₂CH₂CH₂), 2.59 (1H, dq, J 13.0, 7.4, SCH₂), 2.67 (1H, dq, J 13.0, J 7.4, SCH₂), 2.83 (2H, t, J 7.0 CH₂NH₂), 3.53 (2H, t, J 6.8, CONHCH₂), 3.67 (1H, d, J 16.0, PhCH₂), 3.70 (1H, d, J 16.2, PhCH₂), 6.33 (1H, s, CH), 7.32-7.43 (5H, m, C₆H₅), 7.59-7.62 (2H, m, CH₉), 7.84-7.89 (2H, m, CH₉); δₐ (CDCl₃; 63 MHz) 13.1 (CH₃), 24.4 (SCH₂), 30.9, 36.2, 37.7 (CH₂), 41.8 (PhCH₂), 54.6 (CH), 126.1, 126.6, 127.7, 128.1 (CH₉), 133.2, 135.0, 142.7 (C₉), 171.5, 168.0 (CO) m/z (FAB) 386 (94%, MH⁺), 91 (100%, [PhCH₂⁺⁺); (Found: MH⁺, 386.1902. C₂₁H₂₇N₃O₂S requires MH⁺, 386.1906).
7.2 Synthesis of \(N\)-[1-(Ethylsulphanyl)-2-phenylethyl]-2-phenylacetamide 102
(Scheme 40)

7.2.1 \(N\)-[1-(Benzotriazol-1-yl)-2-phenylethyl]-2-phenylacetamide 101

\[
\begin{array}{c}
\text{Ph} \\
\text{N} \\
\text{H} \\
\text{Ph} \\
\end{array}
\]

Phenylacetamide (1.12 g, 8.29 mmol, 1.00 equiv.), benzotriazole (0.99 g, 8.31 mmol, 1.00 equiv.) and phenylacetaldehyde 100 (0.97 ml, 1.00 g, 8.32 mmol, 1.00 equiv.) were dissolved in dry toluene (30 ml) in a Dean-Stark apparatus. The mixture was heated under reflux for 9 h then cooled to room temperature. The toluene was removed in vacuo to give an oil. This was purified by column chromatography [silica, petroleum ether:ethyl acetate (1:1)] to yield a pale yellow solid (1.18 g, 39%). mp 117-118 °C; \(R_f\) 0.34 [petroleum ether:ethyl acetate (2:1)]; \(\nu_{\text{max}}\) (\(\text{CH}_2\text{Cl}_2\), IR card)/cm\(^{-1}\) 3269 (broad NH, amide), 1660 (amide I), 1538 (amide II); \(\delta_{\text{H}}\) (CDCl\(_3\); 250 MHz), 3.43 (1H, d, \(J = 16.0\), PhCH\(_2\)CO), 3.48 (1H, d, \(J = 16.0\), PhCH\(_3\)CO), 3.55 (1H, dd, \(J = 14.0, 7.5\), PhCH\(_2\)CH), 3.60 (1H, dd, \(J = 14.0, 7.5\), PhCH\(_3\)CH), 6.65 (1H, d, \(J = 9.0\), NH), 6.87 (1H, dt, \(J = 9.0, 7.5\), CH\(_{\alpha}\)), 6.97-8.00 (14H, m, CH\(_{\alpha}\)); \(\delta_{\text{C}}\) (CDCl\(_3\); 63 MHz), 40.3, 43.1 (PhCH\(_2\)CH, PhCH\(_2\)CO), 62.8 (CH), 110.0, 119.4, 124.1, 127.2, 126.7, 128.6, 129.0, 129.2 (CH\(_{\alpha}\)), 132.8, 133.5, 134.5, 145.3 (C\(_{\alpha}\)), 170.7 (CO); \(m/z\) (FAB) 357 (8.6%, MH\(^+\)), 238 (22%, MH\(^+\)-benzotriazole), 91 (39%, [PhCH\(_2\)\(^+\)]\(^+\)); (Found: MH\(^+\), 357.1713. C\(_{20}\)H\(_{20}\)N\(_4\)O requires MH\(^+\), 357.1715).

7.2.2 \(N\)-[1-(Ethylsulphanyl)-2-phenylethyl]-2-phenylacetamide 102

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\text{N} \\
\text{S} \\
\text{Ph} \\
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101 (103 mg, 0.29 mmol) dissolved in dry THF (1 ml) was added to a suspension of technical grade ethylthiolate sodium salt (53 mg, 0.63 mmol, 2.17equiv.) in dry THF (4 ml) under nitrogen. The mixture was stirred at room temperature for 2.5 h.
Distilled water (5 ml) was added and the mixture was separated. The aqueous layer was extracted with chloroform (5 ml x 3). The organic extracts were combined and the solvent removed in vacuo to give a pale yellow solid requiring no further purification (78 mg, 90%). mp 108-110 °C; Rf 0.80 [Ethyl acetate:petroleum ether (2:1)]; νmax (CHCl3, IR card)/cm⁻¹ 3281 (NH), 1651 (amide I), 1535 (amide II); δH (CDCl3; 250 MHz) 1.19 (3H, t, J 7.4, CH3), 2.49 (2H, m, SCH₂), 2.83 (1H, dd, J 14.0, J 6.5, CHCH₃Ph), 2.98 (1H, dd, J 14.0, J 5.7, CHCH₂Ph), 3.50 (2H, s, PhCH₂CO), 5.45 (2H, broad m, CH, NH, reduces to 1H, t, J 6.4, CH, after D₂O shake) 7.01-7.35 (10H, m, CH₃); δC (CDCl₃; 63 MHz) 14.7 (CH₃), 24.8 (SCH₂), 41.6, 43.7 (CHCH₂Ph, PhCH₂CO), 53.9 (CH), 134.2, 136.1 (C₆H₅), 126.8, 127.4, 128.3, 129.0, 129.3 (CH₃), 170.2 (CO); m/z (FAB) 300 (19%, MH⁺), 238 (100%, [M-SEt]⁺), 208 (16.7%, [M-PhCH₂]⁺), 165 (17%, [M-PhCH₂CONH]⁺), 120 (65%, [PhCH₂CO]⁺), 91 (87%, [PhCH₂]⁺); (Found: MH⁺, 300.1417. C₁₈H₂₁N₂O.S requires MH⁺, 300.1422).

7.3 Partial Synthesis of N-[3-Amino-1-(ethylsulfanyl)prop-1-yl]-2-phenylacetamide 103 (Scheme 41, Scheme 42, Scheme 43)

7.3.1 3-Azido-1,1-diethoxypropane 105

Sodium azide (3.70 g, 56.7 mmol, 1.89 equiv.) was added to DMF (24 ml) and warmed slightly. 3-Chloro-1,1-diethoxypropane 104 (5.00 g, 30.0 mmol) was added and the mixture was stirred at 60 °C for 23 h. The mixture was cooled to room temperature then distilled water (25 ml) and diethyl ether (50 ml) were added. The mixture was separated and the aqueous phase washed with diethyl ether (50 ml x 2, 25 ml x 2). The combined organic extracts were washed with saturated ammonium chloride solution (25 ml x 4), distilled water (50 ml), dried over MgSO₄ and concentrated in vacuo to give a pale yellow liquid (4.62 g, 89%). Rf 0.65 [petroleum ether:ethyl acetate (4:1)]; νmax (CHCl₃)/cm⁻¹ 2100 (N₃); δH (CDCl₃; 200 MHz) 1.20 (6H, td, J 7.0, 0.8, OCH₂CH₃ x 2), 1.84 (2H, td, J 6.8, 5.6, CHCH₃), 3.37 (2H, t, J
6.8, \( CH_2N_3 \), 3.57 (4H, \( m, CH_2O \)), 4.56 (1H, \( t, J 5.6, CHF \)); \( \delta_C (CDCl_3; 63 MHz) 15.2 \) (CH), 33.0, 47.6, 61.7 (CH₂), 300.3 (CH).

7.3.2 3,3-Diethoxypropylamine 106

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\text{NH}_2
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A mixture of 3-azido-1,1-diethoxypropane (105, 4.44 g, 25.6 mmol) triphenylphosphine (8.18 g, 31.2 mmol, 1.22 equiv.) and water (5 ml, 0.28 mol, 11.2 equiv.) in THF (45 ml) was stirred overnight at room temperature. The solution was dried using MgSO₄ and the THF was removed by distillation in vacuo. Diethyl ether was added and a precipitate formed which was removed by filtration then slurried in a small volume of diethyl ether. The slurry was filtered and the combined diethyl ether solutions concentrated in vacuo before purification by column chromatography [silica, gradient elution from CH₂Cl₂ to CH₂Cl₂:methanol (8:2)] to give a yellow liquid (2.04 g, 54%). \( R_f \) baseline [CH₂Cl₂:methanol (9:1)]: \( \delta_H (CDCl_3; 200 MHz) 1.18 \) (6H, \( t, J 7.0, OCH_2CH_3 \times 2 \)), 1.64 (2H, \( s, NH_2 \)), 1.75 (2H, \( td, J 6.8, 5.6, CHCH_2 \)), 2.77 (2H, \( t, J 6.8, CH_2NH_2 \)), 3.48 (2H, \( dq, J 9.6, 7.0, OCH_2CH_3 \times 2 \)), 3.63 (2H, \( dq, J 9.6, 7.0, OCH_2CH_3 \times 2 \)), 4.57 (1H, \( t, J 5.6, CH \)); \( \delta_C (CDCl_3; 63 MHz) 15.2 \) (CH₃), 37.1, 38.0, 61.1 (CH₂), 101.6 (CH); (Ref. 99 \( \delta_H (CDCl_3; 200 MHz) 1.21 \) (6H, \( t, J 7.1 \)), 1.58 (2H, s), 1.78 (2H, q), 2.81 (2H, t), 3.53 and 3.66 (m, 4H), 4.62 (1H, t)).

7.3.3 Preparation of 2,2,2-Trichloroethyl-N-(3,3-dimethoxypropyl)carbamate 107

3,3-diethoxypropylamine 106 (1.97 g, 13.4 mmol) was dissolved in CH₂Cl₂ (40 ml). Triethylamine (2 ml) was added and the mixture was cooled to 0°C. 2,2,2-Trichloroethyl chloroformate (2.20 ml, 3.40 g, 16.0 mmol, 1.19 equiv.) was added dropwise and the mixture stirred at room temperature for 45 min. Diethyl ether (50 ml) and water (50 ml) were added and mixture was separated. The aqueous layer was extracted with diethyl ether (25 ml). The combined organic solutions were washed
with distilled water (50 ml x 2) and each wash was back-extracted with diethyl ether (25 ml). The combined diethyl ether solutions were concentrated in vacuo to give a yellow liquid which was used without purification in the next step. Rf 0.54 [ethyl acetate:petroleum ether (1:2)]; δH (CDCl3; 250 MHz) 1.21 (6H, t, J 7.1, CH3CH3 x 2), 1.85 (2H, td, J 6.2, 5.2, CHCH3), 2.52 (2H, q, J 7.2, CH2NH), 3.43 (2H, m, OCH2 x 2), 3.67 (2H, m, OCH3 x 2), 4.57 (1H, t, J 5.2, CH), 4.70 (2H, s, CH2OCCl3), 5.53 (1H, broad, NH).

7.3.4 Preparation of N-(2,2,2-Trichloroethoxycarbonyl)-3-aminopropanal 108

107 (from 3,3-diethoxypropylamine 106, 1.97 g, 13.4 mmol) was dissolved in chloroform (60 ml). The solution was cooled to 0°C and 50% trifluoroacetic acid (30 ml) was added slowly. The reaction mixture was stirred at room temperature for 2.5 h then chloroform (50 ml) and distilled water (50 ml) were added. The mixture was separated and the aqueous layer extracted with chloroform (20 ml). The combined organic solutions were washed with distilled water (50 ml), saturated bicarbonate solution (50 ml) and distilled water (50 ml x 3). Each wash was back-extracted with chloroform. The combined organic solutions were dried (MgSO4) and the solvent removed in vacuo to give a viscous yellow liquid (4.23 g) which was used without further purification in the next step. This material was also prepared on a smaller scale and purified by column chromatography [silica, ethyl acetate:petroleum ether (1:2)] in a yield of 0.42 g (50%) from 3,3-diethoxypropylamine 106. Rf 0.23 [ethyl acetate:petroleum ether (1:2)]; δH (CDCl3, 250 MHz) 2.77 (2H, t, J 5.8, CH2CHO), 3.52 (2H, q, J 5.8, CH2NH), 4.69 (2H, s, CH2CCl3), 5.43 (1H, broad, NH), 9.81 (1H, s, CHO); δC (CDCl3, 63 MHz) 34.5, 43.7, 74.4 (CH2), 95.3 (CCl3), 154.41 (NHCO), 200.85 (CHO); m/z (FAB) 250 (42%, [C6H535Cl37ClNO3]+ ), 248 (44%, [C6H535Cl37ClNO3]+ ), 206 (40%, [250-CH2CHO]+), 204 (38%, [248-CH2CHO]+), 100 (92%, [M-CCl3CH2O]+); (Found (El): M+, 246.9577. C6H535Cl37ClNO3 requires M+, 246.9570), (Found: M+, 248.9546. C6H535Cl37ClNO3 requires M+, 248.9540).
7.3.5 Attempted Preparation of \( N-[1-(\text{Benzotriazol-1-yl})-N-2,2,2\text{-trichloroethoxycarbonyl-3-aminopropyl}]-2\text{-phenylacetamide} \) 109

![Chemical Structure](image)

Aldehyde 108 (2.44 g, crude, 9.82 mmol) was dissolved in dry toluene (84 ml) in a Dean-Stark apparatus under nitrogen. Benzotriazole (1.17 g, 9.83 mmol, 1.00 equiv) and phenylacetamide (1.33 g, 9.85 mmol, 1.00 equiv.) were added and the mixture was heated under reflux for 5 h then allowed to cool. The toluene was removed \textit{in vacuo} and petroleum ether added to afford a gum. Trituration with diethyl ether then gave a powdery solid which was obtained by filtration. The solid was dissolved in chloroform (100 ml) to give a solution which was washed with a saturated solution of sodium carbonate (30 ml x 3) then concentrated \textit{in vacuo} to a pale yellow/orange solid (2.46 g). Crude product (0.96 g) was dissolved in a minimum volume of warm ethyl acetate. Petroleum ether was added to give a precipitate (still impure) which was then obtained by filtration (0.59 g, 40% from 3,3-diethoxy-1-propylamine 106). R$_f$ 0.51 [petroleum ether:ethyl acetate (1:2)]; \( \nu_{\text{max}} \) (CH$_2$Cl$_2$, IR card)/cm$^{-1}$ 3349 (broad, NH), 1724 (CO, urethane), 1660 (CO, amide I), 1538 (amide II); \( \delta_{\text{H}} \) (CDCl$_3$; 250 MHz) 2.61 (2H, m, CH$_2$NH), 3.23 (2H, m, CHCH$_2$), 3.56 (*PhCH$_2$), 4.68 (*CH$_2$Cl$_3$), 5.36 (*NH), 6.80 (1H, m, CHN), 6.99 (1H, d, \( J \) 9.3, NHCH), 7.14-8.03 (*CH$_m$); \( m/z \) (FAB) 486 (6.5%, MH$^+$ for C$_{20}$H$_{20}$Cl$_2$N$_5$O$_3$), 484 (1.8%, MH$^+$ for C$_{20}$H$_{20}$Cl$_3$N$_5$O$_3$). (Found: MH$^+$, 484.0720. C$_{20}$H$_{20}$Cl$_3$N$_5$O$_3$ requires MH$^+$, 484.0710). * these signals were partially obscured by impurity peaks so integration was uncertain.
7.4 Preparation of \( N-[1-(4\text{-Amino}-1-\text{-(ethylsulfanyl)}\text{-but-1-yl})\text{-2-phenylacetamide}} \) 110 and Related Linkers 122 and 123 (Scheme 44, Scheme 45, Scheme 46, Scheme 47, Scheme 49, Scheme 52 and Scheme 53)

### 7.4.1 Preparation of 4-Bromobutanal\(^{100} \) 113

![Image of 4-Bromobutanal](image)

THF (4.90 ml, 4.31g, 59.8 mmol) was added dropwise to a stirred solution of BBr\(_3\) (1.92 ml, 5.09g, 20.3 mmol, 0.34 equiv.) in anhydrous CH\(_2\)Cl\(_2\) (25 ml) at 0 °C under nitrogen. The mixture was heated under reflux for 1 h. After cooling to room temperature the solution was added by nitrogen pressure transfer to a stirred suspension of pyridinium chlorochromate (17.30 g, 80.3 mmol, 1.34 equiv.) in dry CH\(_2\)Cl\(_2\) (100 ml). The mixture was heated under reflux for 1 h then cooled to room temperature and diethyl ether (100 ml) was added. This mixture was filtered through a layer of celite and a layer of silica. The resulting filtrate was concentrated \textit{in vacuo} to a green liquid which was purified by Kugelrohr distillation yielding a colourless oil (4.15 g, 46%). \( R_f \) 0.67 [ethyl acetate:petroleum ether (1:1)]; \( \nu_{\text{max}} \) (CHCl\(_3\))/cm\(^{-1}\) 1726 (CO); \( \delta_{\text{H}} \) (CDCl\(_3\); 200 MHz) 2.07 (2H, quin. d, \( J \) 6.0, 0.8, CH\(_2\)CH\(_2\)CH\(_2\)), 2.57 (2H, td, \( J \) 6.4, 1.0, CH\(_2\)CHO), 3.37 (2H, \( J \) 6.4, CH\(_2\)Br), 9.70 (1H, d, \( J \) 1.0, OH); \( \delta_{\text{C}} \) (CDCl\(_3\); 63 MHz) 24.6, 32.6, 41.9 (CH\(_2\)), 200.7 (CHO); (Ref.\(^{101} \), \( \delta_{\text{H}} \) (CDCl\(_3\); 300 MHz) 2.17 (2H, apparent quintet, \( J \) 6.6), 2.67 (2H, \( J \) 6.6), 3.45 (2H, \( J \) 6.4), 9.80 (1H, s)).

### 7.4.2 Preparation of 4-Bromobutanol\(^{100} \) 114

![Image of 4-Bromobutanol](image)

THF (4.90 ml, 4.31 g, 59.8 mmol) was added dropwise to a stirred solution of BBr\(_3\) (1.92 ml, 5.09g, 20.3 mmol, 0.4 equiv.) in anhydrous CH\(_2\)Cl\(_2\) (25 ml) at 0 °C under nitrogen. The mixture was heated under reflux for 1 h. The solvent was removed \textit{in vacuo} and the residue was heated under reflux with anhydrous MeOH (10 ml) for 1 h. The volatile materials, methanol and methyl borate were removed under reduced
pressure. The product was obtained after purification by distillation in a yield of 6.35 g (69%). R\text{f} 0.67 \text{[ethyl acetate:petroleum ether (1:1)]}; \nu_{\text{max}} (\text{CH}_2\text{Cl}_2, \text{IR card})/\text{cm}^{-1} 3325 \text{[broad, OH]}; \delta_{\text{h}} (\text{CD}_3\text{OD}; 200 \text{MHz}) 1.75 (2\text{H, m, CH}_2\text{CH}_2\text{Br}), 2.02 (2\text{H, m, CH}_3\text{CH}_2\text{OH}), 3.57 (2\text{H, t, J 6.6, CH}_2\text{Br}), 3.67 (2\text{H, t, J 6.4, CH}_2\text{OH}); \delta_{\text{c}} (\text{CDCl}_3; 63 \text{MHz}) 29.0, 30.8, 33.5 (\text{CH}_2), 61.8 (\text{CHO}); m/z (\text{EI}) 154 (1.4\%, C_4H_9O^{81}\text{Br}^+), 152 (1.4\%, C_4H_9O^{79}\text{Br}^+), 136 (66\%, C_4H_9O^{81}\text{Br}^+\text{-H}_2\text{O}), 134 (65\%, C_4H_9O^{79}\text{Br}^+\text{-H}_2\text{O}), 73 (51\%, [M-Br]^+); (\text{Found: } M^+, 151.9838. C_4H_9O^{81}\text{Br} \text{requires } M^+, 151.9837, \text{Found: } M^+, 153.9820. C_4H_9O^{81}\text{Br} \text{requires } M^+, 153.9818).

7.4.3 Oxidation of 4-Bromobutanol 114 to 4-Bromobutanal 113

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TEMPO\textsuperscript{86} (45 mg, 0.29 mmol, 0.01 equiv.) was added to a solution of 4-bromobutanol 114 (4.50 g, 29.4 mmol) in CH\textsubscript{2}Cl\textsubscript{2} (90 ml) followed by a saturated aqueous solution of sodium bicarbonate (45 ml) containing sodium bromide (302 mg, 2.93 mmol, 0.10 equiv.) and BTAC (273 mg, 1.47 mmol, 0.05 equiv.). To this cooled (ice bath) and well stirred mixture a solution of sodium hypochlorite (1.12M, 33.6 ml, 37.6 mmol, 1.28 equiv.), saturated sodium bicarbonate (30 ml) and brine (60 ml) was added dropwise over 1 h. The reaction mixture was stirred at 0°C for 45 min. then at room temperature for 20 min.. The phases were separated and the aqueous layer was extracted with CH\textsubscript{2}Cl\textsubscript{2} (100 ml x 3). The combined organic solutions were washed with saturated sodium bicarbonate (100 ml) and brine (100 ml), each wash was back-extracted with CH\textsubscript{2}Cl\textsubscript{2} (100 ml). The combined organic solutions were dried over anhydrous sodium sulphate. The CH\textsubscript{2}Cl\textsubscript{2} was removed under reduced pressure and the product purified by distillation (3.07 g, 69%). Analysis refer to Section 7.4.1 There was evidence of an impurity in this material though it appeared to be <10 mol\%. This compound also has a tendency to trimerise. \delta_{\text{h}} (\text{CDCl}_3; 200 \text{MHz}) 1.82 (6\text{H, m, CHCH}_2), 1.99 (6\text{H, m, CH}_2\text{CH}_2\text{Br}), 3.43 (6\text{H, t, J 6.6, CH}_2\text{Br}), 4.91 (3\text{H, t, CH, J 4.8}); \delta_{\text{c}} (\text{CDCl}_3; 63 \text{MHz}) 36.6, 32.6, 33.3 (\text{CH}_2), 100.3 (\text{CH}).
7.4.4 Preparation of 4-Bromo-1,1-dimethoxybutane 116

115 (0.2 ml, 240 mg, 1.05 mmol) was added to pTSA (12 mg, 0.06 mmol, 0.06 equiv.) in methanol (12 ml). The mixture was heated at reflux for one h and stirred at room temperature for 2 days. Saturated sodium bicarbonate solution (5 ml) was added and the mixture extracted with CH$_2$Cl$_2$ (10 ml x 3). The CH$_2$Cl$_2$ solution was washed with water (10 ml) then dried (MgSO$_4$) before being concentrated in vacuo.

The product was purified by column chromatography [silica, gradient elution beginning with petroleum ether:ethyl acetate (39:1)] to give the product, a clear liquid, in a yield of 40 mg, 39% (based on recovered starting material) and recovered starting material (100 mg). $R_f$ 0.65 [ethyl acetate:petroleum ether (1:1)]; $\delta_h$ (CDCl$_3$; 200 MHz) 1.73 (2H, m, CH$_2$), 1.91 (2H, m, CH$_2$), 3.30 (6H, s, CH$_3$ x 2), 3.42 (2H, t, J 6.4, CH$_2$Br) 4.37 (1H, t, J 5.4, CH); $\delta_c$ (CDCl$_3$; 63 MHz) 27.8, 30.9, 33.4 (CH$_2$), 42.7 (CH$_3$), 100.7 (CH). (Ref. 102, $\delta_h$ (CDCl$_3$, 60 MHz) 1.80 (4H, m), 3.27 (6H, s), 3.37 (2H, t, J 6) 4.33 (1H, t, J 5)).

7.4.5 Preparation of 2-(3-Bromopropyl)-[1,3]dioxolane 117

115 (0.1 ml 120 mg, 0.51 mmol), pTSA (18 mg, 0.09 mmol, 0.18 equiv.) and ethylene glycol (10 ml) was heated to 70 °C for 2 h then cooled to room temperature. Water (20 ml) and CH$_2$Cl$_2$ (30 ml) were added. The phases were separated and the organic layer washed with saturated sodium bicarbonate (20 ml) then water (30 ml). The organic solution was concentrated in vacuo then purified by column chromatography [silica, gradient elution beginning with ethyl acetate:petroleum ether (1:50)] to give the product (60 mg, 58%). $R_f$ [ethyl acetate:petroleum ether (1:1)]; $\delta_h$ (CD$_3$OD; 200 MHz) 1.77 (2H, m, CH$_2$CHBr$_2$), 1.94 (2H, m, CHCH$_2$), 3.47 (2H, t, J 6.6, CH$_2$Br), 3.82 (2H, m, OCH$_2$ x 2), 3.93 (2H, m, OCH$_2$ x 2), 4.83 (1H, t, J 4.4, CH); $\delta_c$ (CD$_3$OD; 63 MHz) 26.6, 31.4, 32.3, 32.3, 64.0 (CH$_3$), 102.9 (CH);
7.4.6 Preparation of N-[1-(Benzotriazol-1-yl)-4-bromobut-1-yl]-2-phenylacetamide

4-Bromobutanal 113 (3.34 g, 22.1 mmol, 1.00 equiv.), benzotriazole (2.63 g, 22.1 mmol, 1.00 equiv.) and phenylacetamide (2.99 g, 22.1 mmol, 1.00 equiv.) were dissolved in dry toluene (190 ml) in a Dean-Stark apparatus and heated under reflux for 2 h. The toluene solution was cooled to room temperature and then washed with saturated sodium carbonate solution (70 ml x 3). Each wash was back-extracted with toluene (50 ml). The combined organic solutions were dried (MgSO₄) and the solvent was removed in vacuo. The product was purified by column chromatography [silica, petroleum ether:ethyl acetate (2:1)] to give a white solid (4.77 g, 52%). mp 109.6-110.0 °C; Rf 0.45 [petroleum ether:ethyl acetate (1:1)]; (Found C, 55.80%; H, 4.94%; N, 14.80%). Requires C, 55.82%; H, 4.94%; N, 14.47%); νmax (CH₂Cl₂, IR card)/cm⁻¹ 3271 (broad, NH), 1662 (amide I), 1538 (amide II); δ H (CDCl₃; 250 MHz) 1.76 (2H, m, CH₂CH₂Br), 2.48 (2H, m, CHCH₂), 3.34 (m, 2H, CH₂Br), 3.52 (1H, d, J 16.0, PhCHₐ), 3.58 (1H, d, J 16.0, PhCH₉), 6.59 (1H, d, J 9.4, NH), 6.71 (1H, m, CH), 7.13-8.05 (9H, m, CHₐ); δ C (CDCl₃; 63 MHz) 28.2, 32.0, 32.4, (CH₂), 43.0 (PhCH₂), 61.2 (CH), 110.2, 119.4, 124.4, 127.3, 127.8(4), 128.8(1), 109.0 (CHₐ), 132.6, 133.8, 145.3 (Cₐ), 171.1 (CO); m/z (FAB) 389 (42%, [C₁₈H₂₉BrN₄O⁺]), (24%. [C₁₈H₂₀⁷⁹BrN₄O⁺]), 270 (91%, [C₁₈H₂₀⁸¹BrN₄O⁺]-benzotriazole), 268 (63%, [C₁₈H₂₀⁷⁹BrN₄O⁺]-benzotriazole), 120 (90%, [PhCH₂CO⁺]); (Found: MH⁺ 389.0788, C₁₈H₁₉⁸¹BrN₂O requires MH⁺ 389.0801, Found: MH⁺ 387.0788. C₁₈H₁₉⁷⁹BrN₂O requires MH⁺ 389.0801).
7.4.7 Preparation of N-[4-Azido-1-(benzotriazol-1-yl)but-1-yl]-2-phenylacetamide

A mixture of 118 (1.00g, 2.58 mmol) and sodium azide (0.34 g, 5.23 mmol, 2.03 equiv.) in DMF (4 ml) was stirred at room temperature for 5 h. Chloroform (30 ml) and water were added and the mixture separated. The aqueous layer was extracted with chloroform (10 ml). The combined organic solutions were washed with saturated ammonium chloride solution (30 ml x 3), water (30 ml), dried over MgSO₄ and concentrated in vacuo. The product was purified by column chromatography (silica, petroleum ether:ethyl acetate (1:1) to give a white solid (0.74 g, 82%). Rf 0.42 [ethyl acetate:petroleum ether (1:1)]; νmax (CH₂Cl₂, IR card)/cm⁻¹ 3272 (broad, NH), 2099 (N₃), 1662 (amide I), 1538 (amide II); δH (CDCl₃; 200 MHz) 1.46 (2H, m, CH₂CH₂CH₂), 2.41 (2H, m, CHCH₂), 3.24 (2H, m, CH₂N₃), 3.47 (1H, d, J 15.8, PhCHₐ), 3.52 (1H, d, J 15.8, PhCHₐ), 6.69 (2H, m, CH, NH), 7.07-7.98 (9H, m, CHₐ); δc (CDCl₃; 63MHz), 24.7, 31.0 (CH₂), 42.9 (PhCH₂), 50.3 (CH₂), 61.6 (CH), 110.2, 119.3, 124.4, 127.3, 127.8, 128.7, 129.0 (CHₐ), 132.7, 133.8, 145.2 (Cₐ), 171.2 (CO); (Found: MH⁺, 350.1721. C₁₈H₁₉N₇O requires MH⁺, 350.1729).

7.4.8 Preparation of N-[4-Azido-1-(ethylsulfanyl)but-1-yl]-2-phenylacetamide

A mixture of 119 (300 mg, 0.68 mmol) and technical grade ethylthiolate sodium salt (140 mg, 1.66 mmol, 2.44 equiv.) in dry THF (15 ml) was stirred at room temperature under nitrogen for 2.5 h. Chloroform (50 ml) and distilled water (30 ml) were added. The mixture was separated and the aqueous phase extracted with chloroform (10 ml). The organic layer was washed with water (30 ml x 2) each time back-extracting the aqueous layer with chloroform (20 ml). The combined organic
extracts were dried (MgSO₄) and the solvent removed in vacuo. The product was purified by column chromatography [silica, petroleum ether:ethyl acetate (2:1)] to give a pale yellow solid (167 mg, 67%). Rₜ 0.48 [ethyl acetate:petroleum ether (1:2)]; vₜₘₜₜ (CH₂Cl₂, IR card)/cm⁻¹ 3277 (broad, NH), 2098 (N₃), 1644 (amide I), 1538 (amide II); δₜ (CDCl₃; 200 MHz) 1.14 (3H, t, J 7.5, SCH₂CH₃), 1.55 (4H, m, CH₂CH₂CH₂N₃), 2.40 (2H, m, SCH₂), 3.19 (2H, t, J 6.2, CH₂N₃), 3.53 (2H, s, PhCH₂), 5.10 (1H, m, CH), 5.37 (1H, d, J 9.3, NH), 7.17-7.36 (5H, m, CHₚ); δₜ (CDCl₃; 63 MHz) 14.8 (CH₃), 24.6, 25.5, 33.0 (CH₂), 43.9 (PhCH₂), 50.6 (CH₂), 53.0 (CH), 127.5, 129.0, 129.1(CHₚ), 134.3 (Cₚ) 170.4 (CO); m/z (FAB) 293 (3.4%, MH⁺), 250 (13%, [M-N₃]⁺), 231 (36%, [M-SEt]⁺); (Found: MH⁺ 293.1442. C₁₄H₂₀N₄O₅S requires MH⁺ 293.1436).

7.4.9 Preparation of N-[1-(4-Amino-1-(ethylsulfanyl)but-1-yl]-2-phenylacetamide

A mixture of azide 120 (130 mg, 0.44 mmol), triphenylphosphine (140 mg, 0.54 mmol, 1.23 equiv.) and distilled water (0.1 ml, 5.56 mmol, 12.6 equiv) in THF (5 ml) was stirred at room temperature overnight. The solvent was removed in vacuo and the material purified by column chromatography [silica, CH₂Cl₂:methanol (9:1) then methanol] to give an oil (88 mg, 74%). mp 118-120 °C; Rₜ baseline [Ethyl acetate:petroleum ether (1:1)]; vₜₘₜₜ (IR card)/cm⁻¹ 3271 (NH₂, NH), 1642 (amide I), 1536 (amide II); δₜ (CDCl₃; 250 MHz) 1.16 (3H, t, J 7.4, CH₃), 1.64 (4H, m, CH₂CH₂CH₂NH₂), 2.44 (2H, m, SCH₂), 2.70 (2H, m, CH₃NH₂), 3.02 (broad s, NH₂), 3.59 (2H, s, PhCH₂), 5.15 (1H, m, CH), 6.40 (1H, d, J 9.8, NH), 7.28-7.36 (5H, m, CHₚ); δₜ (CDCl₃; 63 MHz) 14.8 (CH₃), 24.6, 28.4, 33.2, 40.9, 43.7 (CH₂), 52.2 (CH), 127.3, 128.9, 129.2 (CHₚ), 134.6 (Cₚ), 170.6 (CO); m/z (FAB) 267 (52%, MH⁺); (Found: MH⁺ 267.1542. C₁₄H₂₀N₂O₅S requires MH⁺ 267.1531).
7.4.10 Preparation of [1-Ethylsulfanyl-N]- (2-phenylacetyl)-1,4-diaminobutyl]-N4-oxobutanoic acid 122

110 (122 mg, 460 mmol), succinic anhydride (236 mg, 2.36 mmol, 5.13 equiv.), DMAP (292 mg, 2.39 mmol, 5.28 equiv.), pyridine (12 drops) and DMF (5 ml) were stirred together at room temperature under nitrogen for 4.5 h. Water (25 ml) and ethyl acetate (50 ml) were added and the mixture stirred at room temperature for a further 1 h. The aqueous layer was separated and back-extracted with ethyl acetate (10 ml). The combined ethyl acetate solutions were washed with dilute HCl (25 ml x 3). Each aqueous wash was back-extracted with ethyl acetate (10 ml). The combined organic solutions were washed with water until the aqueous layer was pH neutral. The ethyl acetate solution was dried (MgSO₄) and the solvent removed in vacuo. The mixture was dissolved in ethyl acetate (50 ml) and stirred with water (25 ml) for 3 h. The aqueous layer was separated and back-extracted with ethyl acetate (10 ml). The combined organic solutions were dried (MgSO₄) and the solvent removed in vacuo. The mixture was dissolved in ethyl acetate (50 ml) again and stirred with water (25 ml) for 3 h. The aqueous layer was separated and back-extracted with ethyl acetate (10 ml). The combined organic solutions were dried (MgSO₄) and the solvent removed in vacuo to give a white solid (73 mg, 43%). mp 127.5-128.5 °C; R_f 0.60 [reverse phase plates, methanol:CH₂Cl₂ (1:9)]; ν_max (CH₂Cl₂/CH₃OH IR card)/cm⁻¹ 3284 (broad NH, OH), 1721 (CO acid), 1646 (amide I), 1544 (amide II); δ_H (CD₃OD; 360 MHz) 1.24 (3H, t, J 7.4, CH), 1.64 (2H, m, CH₂CH₂NH), 1.78 (2H, m, CHCH₂), 2.47 (1H, dq, J 12.8, 7.4, SCH_a), 2.53 (2H, pseudo-triplet, 2nd order pattern, CH₂CO₂H), 2.59 (1H, dq, J 12.8, 7.4, SCH_b), 2.67 (2H, pseudo-triplet, 2nd order pattern, NHCOCH₂), 3.24 (2H, t, J 6.8, CH₂NHCO), 3.53 (1H, d, J 13.8, PhCH₂), 3.69 (1H, d, J 13.8, PhCH₂), 5.17 (1H, dd, J 8.2, 6.0, CH), 7.31-7.41 (5H, m, CH₄); δ_C (CDCl₃; 91 MHz ) 13.4 (CH₃), 24.1, 25.6, 28.5, 29.7, 32.0, 37.9, 42.0 (CH₂), 52.2 (CH), 126.0, 127.7, 128.1 (CH₆), 135.2 (C₆), 171.8, 172.1, 174.4, (CO); m/z (FAB) 389 (46%, [MNa⁺]), 91 (100%, PhCH₂⁺); (Found: MNa⁺ 389.1504. C₁₆H₂₆N₂O₄S requires MNa⁺ 389.1511).
7.4.11  \( N-[4\text{-Amino-1-(benzotriazol-1-yl)}\text{-but-1-yl}]\text{-2-phenylacetamide} \) 123

119 (100 mg, 0.29 mmol) and PPh\(_3\) (90 mg, 0.34 mmol, 1.17 equiv.) were dissolved in THF (3 ml) and water (0.1 ml, 5.56 mmol, 19.1 equiv.) and stirred overnight at room temperature. The solvent was removed \textit{in vacuo} and the product purified by column chromatography [silica, CH\(_2\)Cl\(_2\):methanol (9:1) then 100% methanol] to give an pale orange waxy solid (59 mg, 62%). mp 44-46 °C; \( R_f \) baseline [methanol:CH\(_2\)Cl\(_2\) (1:9)]; \( \nu_{\text{max}} \) (CDCl\(_3\), IR card)/cm\(^{-1}\) 3368 (broad, NH, NH\(_2\)), 1665 (amide I), 1549 (amide II); \( \delta_{\text{H}} \) (CDCl\(_3)/D_2O; 250 MHz) 1.40 (2H, m, CH\(_2\)CH\(_2\)CH\(_2\)), 2.36 (2H, q, J 7.6, CHCH\(_2\)), 2.64 (2H, t, J 6.4, CH\(_2\)NH\(_2\)), 3.50 (2H, s, PhCH\(_2\)), 6.67 (1H, t, J 7.0, CH), 7.12-8.00 (9H, m, CH\(_{\text{ar}}\)); \( \delta_{\text{C}} \) (CDCl\(_3\); 63 MHz) 28.2, 31.4, 40.7, 42.9 (CH\(_2\)), 62.0 (CH), 110.3, 119.3, 124.2, 127.1, 127.6 128.7, 129.1 (CH\(_{\text{ar}}\)), 132.7, 134.1, 145.3 (C\(_{\text{ar}}\)), 171.1 (CO); m/z (FAB) 324 (5.3% MH\(^+\)), 205 (17%, MH\(^+\)-benzotriazole), 91 (100%, PhCH\(_2\))\(^+\); (Found: MH\(^+\) 324.1815. C\(_{18}\)H\(_{21}\)N\(_5\)O requires MH\(^+\) 324.1824).

7.5 Attempted Synthesis of \( N-[1-(\text{Benzotriazol-1-yl)}\text{-3-bromoprop-1-yl}]\text{-2-phenylacetamide} \) 128 (Scheme 58)

128
7.5.1 Synthesis of \(N-[1-(Benzotriazol-1-yl)-3-(benzotriazol-1-yl)prop-1-yl]-2-phenylacetamide\) 129

3,3-Dimethoxybromopropane (126, 1.94 g, 10.6 mmol) was dissolved in \(CH_2Cl_2\) (40 ml). The mixture was cooled to 0 °C. 50% TFA was added dropwise to the mixture which was stirred with ice-bath cooling for 6 h. Water (70 ml) was added to the reaction and the mixture separated. The aqueous layer was back-extracted with \(CH_2Cl_2\) (30 ml). The organic layer was washed with water (70 ml) until the pH of the aqueous layer was neutral. Each time the aqueous layer was back-extracted with \(CH_2Cl_2\) (30 ml). The combined organic solutions were dried (\(Na_2SO_4\)) and the solvent removed \textit{in vacuo}. The product was redissolved in \(CH_2Cl_2\) (40 ml). The mixture was cooled to 0 °C. 50% TFA was added dropwise to the mixture which was stirred with ice-bath cooling for 6 h. Water (70 ml) was added to the reaction and the mixture separated. The aqueous layer was back-extracted with \(CH_2Cl_2\) (30 ml). The organic layer was washed with water (70 ml) until the pH of the aqueous layer was neutral. Each time the aqueous layer was back-extracted with \(CH_2Cl_2\) (30 ml). The combined organic solutions were dried (\(Na_2SO_4\)) and the solvent removed \textit{in vacuo}, to give, by \(^1\text{H NMR}\), a 7:2.3:2.5 mixture of aldehyde:trimer:starting material (0.53 g). This mixture, phenylacetamide (0.53g, 3.92 mmol.) and benzotriazole (0.46 g, 3.86 mmol) was heated under Dean-Stark conditions in toluene (10 ml) for 1 h. The toluene was removed to give a gum which was dissolved in \(CH_2Cl_2\) (100 ml). This solution was washed with saturated \(Na_2CO_3\) (50 ml x 3). Each time the aqueous layer was back-extracted with \(CH_2Cl_2\) (30 ml). The combined \(CH_2Cl_2\) solutions were dried (\(MgSO_4\)) and the solvent removed \textit{in vacuo}. Purification by column chromatography [silica, ethyl acetate:petroleum ether (1:1)] gave 129 in a yield of 0.23 g (29% based on benzotriazole). \(R_f\) 0.19 [ethyl acetate:petroleum ether (1:1)]; \(\nu_{\text{max}}\) (nujol)/cm\(^{-1}\) 3252, 3207 (broad, NH), 1693 (amide I), 1534 (amide II); \(\delta\)\(^{(\text{H})}\) ((CD\(_3\))\(_2\)CO; 200 MHz) 3.23 (2H, m, CH\(_2\)), 3.54 (1H, d, J 14.6, PhCH\(_2\)) 3.60 (1H,
d, J 14.6, PhCH₆), 4.87 (2H, t, J 7.2, CH₂Bt), 6.82 (1H, dt, J 8.8, 7.6, CH), 7.16-7.25 (5H, m, CH₆), 7.35-7.53 (4H, m, CH₂ar), 7.63-7.68 (1H, m, CH₆), 7.80-7.85 (1H, m, CH₂ar), 7.96-8.03 (2H, m, CH₂ar), 8.74 (1H, J 8.6, NH) δC (CDCl₃; 63 MHz ) 32.6, 41.4, 43.2, (CH₂), 59.61 (CH), 109.1, 110.3, 118.3, 118.6, 122.9, 123.1, 125.7, 126.3, 126.4, 127.4, 128.3 (CH₂ar), 131.9, 132.2, 134.5, 144.9 (C₁ar), 169.8 (CO); m/z (FAB) 412 (1.8% MH⁺), 293 (21%, MH⁺-benzotriazole), 136 (56%, PhCHCONH₃⁺) 91 (100%, PhCH₂⁺); (Found: MH⁺ 412.1868. C₂₃H₂₁N₂O requires MH⁺ 412.1886).

7.6 Attempted synthesis of 3-(Benzotrizol-1-yl)-N-(2-phenylacetyl)-3-aminopropanoic acid 134 (Scheme 59)

![Chemical structure]

7.6.1 Synthesis of 3,3-Diethoxypropanoic Acid 133

3,3-Diethoxypropanoic acid ethyl ester 132 (1.35g, 7.01 mmol) was dissolved in THF:MeOH (3:1, 40 ml). 1M NaOH (17 ml) was added dropwise and the mixture stirred at room temperature for 2 h. The volatile solvents were removed and the residue acidified with 1M citric acid then saturated with sodium chloride. The mixture was extracted with CH₂Cl₂ (30 ml x 5). The organic solution was dried (MgSO₄) and the solvent removed in vacuo to give a pale yellow viscous liquid in a yield of 1.08 g (96%). Rₙ 0.19 [ethyl acetate:acetic acid (99:1)]; νmax (CH₂Cl₂, IR card)/cm⁻¹ 3074 (broad, OH), 1715 (CO); δH (CDCl₃; 200 MHz) 1.16 (6H, t, J 7.0, CH₃ x 2), 2.66 (2H, d, J 5.8, CHCH₂), 3.52 (2H, dq, J 9.4, 7.0, CH₂CH₂ x 2), 3.64 (2H, dq, J 9.4, 7.0, CH₂CH₂ x 2), 4.91 (1H, t, J 5.8, CHCH₂), 10.40 (1H, t, J 5.8, CHCH₂), 10.40 (1H, broad s, OH); δC (CDCl₃; 63 MHz ) 14.9 (CH₃), 39.5 (CH₂), 61.8 (CH₂CH₂), 99.1 (CH), 175.1 (CO); m/z (FAB) 164 (8.1%, MH⁺), 117 (100%, MH⁺-EtOH); (Found: MH⁺ 163.0969. C₁₃H₁₄O₄ requires MH⁺ 163.0970).
3,3-Diethoxypropionic acid 133 (0.40 g, 2.47 x 10 mmol), benzotriazole (0.30 g, 2.52 mmol, 1.02 equiv.), phenylacetamide (0.33 g, 2.44 mmol, 0.99 equiv.) and pTSA (13 mg, 0.07 mmol, 0.03 equiv.) were heated under Dean-Stark conditions in toluene (10 ml) for 1 h. The solvent was removed in vacuo and the residue dissolved in CH₂Cl₂ (30 ml). Saturated Na₂CO₃ (20 ml) was added and the aqueous layer was extracted with CH₂Cl₂ (20 ml x 2) then acidified with 1M citric acid and saturated with NaCl. The aqueous layer was extracted twice more with CH₂Cl₂ (20 ml x 2). The combined organic solutions were dried (MgSO₄) and the solvent removed in vacuo. Purification by column chromatography [silica, ethyl acetate:petroleum ether (1:2) + 1% acetic acid] gave 135 in a yield of 59 mg (3.5%). mp 108-109 °C; R₇0.60
[ethyl acetate:petroleum ether (1:1) + 1% acetic acid]; νₘₐₓ (CDCl₃, IR card)/cm⁻¹: 3400-3200 (broad, OH), 1732 (CO); δₜ (CDCl₃; 200 MHz) 1.12 (3H, t, J 7.0, CH₃), 3.16 (1H, dd, J 16.2, 5.0 CH₃CO₂H), 3.44 (1H, dd, J 16.4, 8.0, CH₂CO₂H), 3.34 (1H, dq, J 9.4, 7.0, CH₃CH₂), 3.57 (1H, dq, J 9.4, 7.0, CH₃CH₂), 5.03 (1H, dd, J 8.0, 5.0, CHCH₂), 7.45 (2H, m, CH₉), 7.77 (1H, m, CH₉), 8.08 (1H, m, CH₉); δ₂ (CDCl₃; 63 MHz), 14.4 (CH₃), 39.8, 65.0 (CH₂), 85.9 (CH), 110.6, 119.9, 124.6, 127.9 (CH₉), 131.6, 145.9 (C₉), 172.9 (CO); m/z (FAB) 236 (53%, MH⁺), 120 (100%, H⁺+benzotriazole) (Found: 236.1043. C₁₁H₁₃N₃O₃ requires MH⁺ 236.10354).
7.7 Cleavage of Linkers in Solution

7.7.1 Cleavage of \( N-(\{4-(3\text{-Aminopropylcarbamoyl})\text{phenyl}\text{ethylsulphanyl}\}-\text{methyl})-2\text{-phenylacetamide} \) 73 in Solution using Aqueous HCl

\[
\text{Ph} \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{S}}{\text{Et}} \quad \overset{\text{NH}}{\text{H}}
\]

73 (2 mg) was weighed into each of three vials. To the first MeOH (300 µl) and H\(_2\)O (300 µl) was added (blank). MeOH (150 µl) and 3M HCl (300 µl) were added to each of the other two. These vials were placed in the blood rotator overnight. The HCl solutions were neutralised using 2M NaOH and H\(_2\)O was added to the blank so that all samples were of similar volumes. The samples were then analysed by HPLC (Section 6.2, system 1a). Acidic cleavage was quantitative in each case. 10-20% hydrolysis observed in the blank.

7.7.2 Cleavage of \( N-(\{4-(3\text{-Aminopropylcarbamoyl})\text{phenyl}\text{ethylsulphanyl}\}-\text{methyl})-2\text{-phenylacetamide} \) 73 in Solution using TFA:CH\(_2\)Cl\(_2\):H\(_2\)O (9:10:1)

\[
\text{Ph} \quad \overset{\text{O}}{\text{N}} \quad \overset{\text{S}}{\text{Et}} \quad \overset{\text{NH}}{\text{H}}
\]

73 (2 mg) was weighed into each of three vials. To the first MeOH:CH\(_2\)Cl\(_2\):H\(_2\)O (9:10:1, 300 µl) was added. TFA:CH\(_2\)Cl\(_2\):H\(_2\)O (9:10:1, 300 µl) was added to each of the other two. These vials were placed in the blood rotator for 3 h. The solvent was removed \textit{in vacuo} and methanol (4 ml) was added to each of the samples which were then analysed by HPLC (Section 6.2, system 1a). The acidic cleavages produced phenylacetamide indicating quantitative cleavage in one case and in the other <5% linker remained. 30% cleavage was observed in the blank.
7.7.3 Cleavage of N-[1-(4-Amino-1-(ethylsulfanyl)but-1-yl]-2-phenylacetamide 110 in Solution Using HCl

110 (2 mg) was weighed into each of three vials. H₂O (400 μl) was added to one (blank) and 2M HCl (400 μl) was added to each of the other two. The vials were placed in the blood rotator overnight at room temperature. The HCl solutions were neutralised using 2M NaOH. H₂O was added to the blank so that all samples were of similar volume. The samples were then analysed by HPLC (Section 6.2, system 1). Linker 110 remained in the neutralised samples. This experiment was repeated using 5M HCl and 5M NaOH. No 110 remained in the neutralised samples as determined by HPLC (Section 6.2, system 1a).

7.7.4 Cleavage of N-[1-(4-Amino-1-(ethylsulfanyl)but-1-yl]-2-phenylacetamide 110 in Solution Using Immobilised Penicillin Amidase

110 (2 mg) was added to each of three vials followed by 25 mM phosphate buffer (pH 7.8, 1.54 ml). Immobilised penicillin amidase (462 mg, 50 units) was then added to two of the three vials and the samples were placed in the blood rotator overnight. An aliquot was removed from each sample and examined by HPLC (Section 6.2, system 1). 60% hydrolysis was observed for each of the enzymatic cleavages. No hydrolysis was observed in the blank.

7.8 Coupling of Linkers to Solid Supports

7.8.1 Preparation of 4-[[Ethylsulphanyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81a82

Linker 73 (5.4-6.1 equiv.), TG-CO₂H (80, 60-100 mg, 1 equiv.), TBTU (11.1-12.4 equiv.), HOBt (11.4-12.7 equiv.), DMF (3-4 ml) and DIEA (10.7-12.4 equiv.) were added to a vial which was placed in the blood-rotator for 16-48 h. The resin was filtered off and the filtrate retained. The resin was washed according to the standard protocol and dried to a constant weight in the vacuum oven at ambient temperature.
δ_C (CD_2Cl_2; 63 MHz) 30.1, 30.8, 30.6, 69.8 (CH_2), 172.7 (CO); ν_max (CH_2Cl_2)/cm^{-1} 1732 (CO acid), 1669 (amide CO).

ν_max (CH_2Cl_2)/cm^{-1}, 1705 (unknown), 1658 (amide CO). Cleavage and HPLC (Section 7.9.1) indicated loadings of 110-120 μmol/g (42-50% coupling).

7.8.2 Reuse of Coupling Mixture from Section 7.8.1
The filtrate from Section 7.8.1 was added to TG-CO_2H (<1 equiv.). The mixture was placed in the blood-rotator overnight. The resin was filtered off, washed according to the standard protocol and dried to a constant weight in the vacuum oven at ambient temperature. Cleavage and HPLC (Section 7.9.1) indicated no coupling.

7.8.3 Treating Carboxy TG Resin with the Coupling Reagents In the Absence of Linker 73
TG-CO_2H (80, 100 mg, 260 μmol), TBTU (104 mg, 324 μmol, 12.5 equiv.), HOBt (41 mg, 300 μmol, 11.5 equiv.), DIEA (39.0 mg, 299 μmol, 11.5 equiv.) and DMF (4 ml) were added to a vial which was placed in the blood-rotator overnight. The resin was filtered off, washed according to the standard protocol and dried to a constant weight in the vacuum oven at ambient temperature. ν_max (CH_2Cl_2)/cm^{-1} 1705, 1667, 1642, 1150 (unknown).
7.8.4 Stability of 4-[[Ethylsulphonyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81a on Storage

Resin 81a with a loading of 110 μmol/g as determined by cleavage and HPLC (Section 7.9.1) was stored in a refrigerator for 3 months. The loading after this period was determined by cleavage and HPLC (Section 7.9.1) to be 110 μmol/g.

7.8.5 Treatment of 4-[[Ethylsulphonyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81a with Methanol:H₂O (1:1)

Resin (2 mg) was added to each of two vials. Methanol:H₂O (1:1, 300 μl) was added to each. The vials were placed on the blood rotator for 24 h. Methanol:H₂O (1:1, 300 μl) was then added to each. An aliquot was removed and centrifuged for approx. 1 min. The supernatant was examined by HPLC (Section 6.2, system 1a). Negligable cleavage was observed.

7.8.6 Preparation of [4-Ethylsulfanyl-N-(2-phenylacetyl)-4-amino]butylamido TG 121

![Diagram](attachment:diagram.png)

Linker 110 (6.0-6.1 equiv.), TG-CO₂H (80, 50-110 mg, 1 equiv.), TBTU (11.9-13.1 equiv.), HOBt (12.3-15.1 equiv.), DMF (2-4 ml) and DIEA (9.4-11.1 equiv.) were added to a vial which was placed in the blood-rotator overnight. The resin was filtered off and the filtrate retained. The resin was washed according to the standard protocol and dried to a constant weight in the vacuum oven at ambient temperature. δC (CD₂Cl₂; 62.9 MHz), 15.0 (1C, CH₃), 24.4 (SCH₂), 26.6, 31.5, 31.7, 32.9, 38.7, 39.4, 43.4 (PhCH₂), 69.8 (CH₂), 28.7, 31.2 (CH₂, weak intensity, impurity), 53.3 (1C, CH), 127.0 128.7, 129.3 (CH₃) 170.6 (CO, amide), νmax (CH₂Cl₂/cm⁻¹), 1664 (amide CO). Cleavage and HPLC (Section 7.9.2) indicated loadings of 110-250 μmol/g (46%-quantitative).
7.8.7 Reuse of Coupling Mixture from Section 7.8.6
The filtrate from Section 7.8.6 was used to treat fresh TG-CO\textsubscript{2}H 80. The mixture was placed in the blood-rotator overnight. The resin was filtered off, washed according to the standard protocol and dried to a constant weight in the vacuum oven at ambient temperature. Cleavage and HPLC (Section 7.9.2) indicated that the coupling had failed.

7.8.8 Stability of [4-Ethylsulfanyl-N-(2-phenylacetyl)-4-amino]butylamido TG 121 on Storage
Resin 121 with a loading of 180 μmol/g as determined by cleavage and HPLC (Section 7.9.2) was stored in a refrigerator for 6 weeks. The loading after this period of time was determined to by cleavage and HPLC (Section 7.9.2) to be 200 μmol/g.

7.8.9 Preparation of PEGA-CO\textsubscript{2}H 84

\[
\begin{align*}
\text{HO} & \quad \text{PEG} \\
\text{N} & \\
\text{84}
\end{align*}
\]

PEGA-NH\textsubscript{2} 83 (0.45 g, max. loading 0.4 mmol/g, max. 180 μmol), succinic anhydride (0.21 g, 2.08 mmol, 11.6 equiv.), DMAP (68 mg, 550 μmol, 3.1 equiv.) and pyridine (0.4 ml) were added together with DMF (5 ml). The mixture was agitated on the blood rotator overnight then filtered, washed according to the standard protocol and dried to a constant weight in a vacuum oven at ambient temperature. v\textsubscript{max} (CH\textsubscript{2}Cl\textsubscript{2}/cm\textsuperscript{-1}) 1732 (acid CO), 1667-1634 (CO amide).

7.8.10 General Procedure for Coupling of N-([4-(3-Aminopropyl-carbamoyl)phenyl]-ethysulphanyl)methyl)-2-phenylacetamide to Carboxy TG 80, PEGA 84 or PS 86 Using DIC
Resin (pre-washed according to the standard protocol and dried to a constant weight under vacuum at ambient temperature, 116-178 mg), linker (5.9-6.4 equiv.), HOBt (12.9-13.6 equiv.) and DMF (0.5-1.0 ml) were added together followed by DIC (12.9-13.1 equiv.) and DIEA (13.4-14.1 equiv.). The mixture was placed on the
blood-rotator overnight. The resin was filtered off and the filtrate retained. The resin was washed with DMF (5 ml x 2) then according to the standard wash protocol or simply according to the standard wash protocol and dried to a constant weight in the vacuum oven at ambient temperature.

7.8.11 General Procedure for Re-use of Coupling Mixture from 7.8.10
The filtrate from Section 7.8.10 was added to appropriate fresh resin (<1 equiv.). The mixture was placed on the blood-rotator overnight. The resin was filtered off, washed according to the standard wash protocol and dried to a constant weight in the vacuum oven at ambient temperature.

7.8.12 Preparation of 4-{[Ethylsulphanyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81b Using DIC

This was carried out as described in Section 7.8.10. \( \nu_{\text{max}} \) (CH\(_2\)Cl\(_2\)) cm\(^{-1}\) 1673-1642 (CO amide); \( \delta_c \) (CD\(_2\)Cl\(_2\); 63 MHz) 14.5 (CH\(_3\)), 25.4 (SCH\(_2\)), 29.3, 31.3, 31.5, 36.1, 36.3, 39.2 (CH\(_2\)), 43.0 (PhCH\(_2\)), 55.4 (CH), 69.6 (CH\(_3\)), 126.6, 126.9, 127.4, 128.5, 129.1 (CH\(_ar\)), 134.1, 135.4, 142.6, (C\(_ar\)), 166.7, 170.4, 172.3, 173.1, (CO amide). Cleavage and HPLC (Section 7.9.3) indicated a linker loading of 190 \( \mu \text{mol/g} \) (79% coupling).

7.8.13 Reuse of Coupling Mixture from the Preparation of 4-{[Ethylsulphanyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81b Using DIC
This was carried out as described in Section 7.8.11. \( \nu_{\text{max}} \) (CH\(_2\)Cl\(_2\)) cm\(^{-1}\) 1668-1638 (CO amide); \( \delta_c \) (CD\(_2\)Cl\(_2\); 63 MHz) 14.5 (CH\(_3\)), 25.4 (SCH\(_2\)), 29.4, 31.3, 31.5, 36.0, 36.1, 36.3, 39.2 (CH\(_2\)), 43.0 (PhCH\(_2\)), 55.4 (CH), ca. 70 (CH\(_2\), unresolved from backbone CH\(_3\)), 126.6, 126.9, 127.4, 128.5, 129.1 (CH\(_ar\)), 134.2, 135.4, 142.6, (C\(_ar\)), 166.7, 170.4, 172.1, 173.0, (CO amide). HPLC (Section 7.9.3) indicated a linker loading of 190 \( \mu \text{mol/g} \) (79% coupling).
7.8.14 Preparation of 4-[(Ethylsulphanil-N-(2-phenylacetil)]aminomethyl]-benzamidopropyiamido PEGA 85 Using DIC

85 was prepared from PEGA-CO₂H 84 using the procedure given in Section 7.8.10. ν_max (CH₂Cl₂/cm⁻¹) 1732 (residual CO acid), 1673-1617 (CO amide); δ_c (CD₂Cl₂; 63 MHz) 14.6 (CH₃), 25.5 (SCH₂), 29.5, 31.7, 36.1, 43.2, (CH₂); 55.5 (CH), 129.2, 128.7, 127.4, 129.4 (CH₆), 134.2, 135.4, 142.6 (C₆), 166.6, 170.3, 171.5, 173.2 (CO). Cleavage and HPLC (Section 7.9.3) indicated a loading of 340 μmol/g, 97% coupling.

7.8.15 Reuse of Coupling Mixture from the Preparation of 4-[(Ethylsulphanil-N-(2-phenylacetil)]aminomethyl]-benzamidopropyiamido PEGA 85 Using DIC

This was carried out as in Section 7.8.11 using PEGA-CO₂H resin (<1 equiv.). ν_max (CH₂Cl₂/cm⁻¹) 1732 (residual CO acid), 1673-1615 (CO amide). Cleavage and HPLC (Section 7.9.3) indicated a loading of 340 μmol/g, 97% coupling.

7.8.16 Preparation of 4-[(Ethylsulphanil-N-(2-phenylacetil)]aminomethyl]-benzamidopropyiamido Polystyrene 88b Using DIC

88b was prepared using the procedure given in Section 7.8.10. ν_max (CH₂Cl₂/cm⁻¹) broad absorbance in the carbonyl region; δ_c (CD₂Cl₂; 63 MHz) 14.8 (CH₃), 25.8 (SCH₂). Cleavage and HPLC (Section 7.9.3) indicated a loading of 840 μmol/g, 99% coupling.
7.8.17 Reuse of Coupling Mixture from the Preparation of 4-[[Ethylsulphonyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido Polystyrene 88b Using DIC

This was carried out as in Section 7.8.11. \( v_{\text{max}} \) (CH\(_2\)Cl\(_2)/\text{cm}^{-1} \) 1716-1674 (CO amide); Cleavage and HPLC (Section 7.9.3) indicated a loading of 770 \( \mu \text{mol/g} \), 92% coupling.

7.9 Cleavage of Linkers on Solid Support for HPLC Analysis

7.9.1 Cleavage of 4-[[Ethylsulphonyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81a

\[ \text{Ph} \quad \text{Et} \quad \text{NH} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{TG} \]

Resin (2 mg) was added to two vials followed by HCl or NaOH (300 \( \mu \text{l} \)). The vials were placed on the blood rotator for 16-24 h. The cleavage mixtures were neutralised with HCl or NaOH of an appropriate concentration. An aliquot was removed and centrifuged for approx. 1 min. The supernatant was examined by HPLC (Section 6.2, system 1a).

7.9.2 Cleavage of [4-Ethylsulfanyl-N-(2-phenylacetyl)-4-amino]butylamido TG 121

\[ \text{Ph} \quad \text{Et} \quad \text{NH} \quad \text{N} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{H} \quad \text{N} \quad \text{H} \quad \text{O} \quad \text{O} \quad \text{N} \quad \text{TG} \]

As Section 7.9.1 except replacing 2M HCl and 2M NaOH with 5-6M HCl and 5M NaOH.
7.9.3 Cleavage of 4-[[Ethylsulphonyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81b, PEGA 85 or Polystyrene 88b Using CH$_2$Cl$_2$:TFA:H$_2$O (9:10:1)

CH$_2$Cl$_2$:TFA:H$_2$O (9:10:1) was added to resin (2 mg). This was repeated. CH$_2$Cl$_2$:H$_2$O (9:1) was added to resin (2 mg) and anhydrous CH$_2$Cl$_2$ was added to resin (ca. 2 mg). The mixtures were placed on the blood rotator overnight (minimum 20 h). In each case the resin was filtered off and washed with CH$_2$Cl$_2$ (5 ml). The filtrate was evaporated to dryness and the residue dissolved 0.1% TFA:MeCN (70:30, 0.6ml) and examined by HPLC (Section 6.2, system 2).

7.10 Determination of Acid or Base Stability of 4-[[Ethylsulphonyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81a and (4-Ethylsulfanyl-N-(2-phenylacetyl)-4-amino)butylamido TG 121

7.10.1 Determination of Acid or Base Stability of 4-[[Ethylsulphonyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81a

Resin (2 mg) was weighed into a vial. Acid or NaOH (300 µl) of a given concentration was added to each of the vials which were then placed on the blood rotator for 24 h. The sample was neutralised using HCl or NaOH of appropriate concentration. An aliquot was removed and centrifuged for approx. 1 min. The supernatant was examined by HPLC (Section 6.2, system 1a).
7.10.2 Determination of Acid or Base Stability of [4-Ethylsulfanyl-N-(2-phenylacetyl)-4-amino]butylamido TG 121

This was carried out as Section 7.10.1

7.10.3 Measurement of Acid Stability of 4-[[Ethylsulphanyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido TG 81a with Time

Resin (81a, 2 mg) was weighed into a number of vials. 0.5M HCl (300 µl) was added to each of the vials. The vials were placed on the blood rotator. After given time interval one of the samples was neutralised using 0.5M NaOH. An aliquot was removed and centrifuged for approx. 1 min. The supernatant was examined by HPLC (Section 6.2, system 1a).

7.10.4 Measurement of Acid Stability of [4-Ethylsulfanyl-N-(2-phenylacetyl)-4-amino]butylamido TG 121 with Time

This was carried out as Section 7.10.3.

7.11 Coupling of Alcohols

7.11.1 Preparation of Ni-[4-Azido-1-(2-phenylprop-1-yloxy)butyl]-2-phenylacetamide 125

![Chemical Structure](image)

120 (60 mg, 0.21 mmol) was dissolved in dry CH$_2$Cl$_2$ (2 ml). 4 Å molecular sieves were added and the mixture cooled to 0 °C. 2-Phenylpropan-1-ol (30 µl, 29 mg, 0.21 mmol, 1.00 equiv.) was added followed by NIS (69 mg, 0.31 mmol, 1.48 equiv.) and the mixture stirred at room temperature for 1 h. CH$_2$Cl$_2$ (4 ml) and water (4 ml) were added followed by sodium thiosulphate which was added until the solution decolourised. The mixture was separated and the aqueous layer extracted with CH$_2$Cl$_2$ (2 ml). The organic layer was washed with water (5 ml x 2) each time back extracting the aqueous layer with CH$_2$Cl$_2$ (2 ml). The combined organic solutions
were dried (Na₂SO₄) and the solvent removed in vacuo to and the product was purified by column chromatography (silica, ethyl acetate:petroleum ether (1:3)) to give a colourless oil (61 mg, 82%). Rₚ 0.56 [Ethyl acetate:petroleum ether (1:1)]; νmax (CH₂Cl₂, IR card/cm⁻¹ 3295 (broad, NH), 2096 (N≡), 1653 (amide I), 1539 (amide II); δH (CDCl₃, 200 MHz) 1.19 (3H, m, CH₃), 1.50 (4H, m, CHCH₂CH₂), 2.90 (1H, m, PhCH), 3.13 (2H, m, CH₂N₃), 3.52 (4H, m, PhCH(CH₃)CH₂, PhCH₂), 5.43 (0.5H, d, J 10.0, NH), 5.46 (0.5H, d, J 9.6, NH), 7.10-7.40 (10H, m, CHar); δC (CDCl₃; 63 MHz ) 18.0 (CH₃), 18.2 (CH₃), 24.1 24.2, 32.4, 32.5 (CH₂), 39.6, 39.7 (CH), 43.7, 43.8, 50.6, 50.7, 73.7, 73.8 (CH₂), 79.3, 79.3 (CH), 126.2, 127.1, 127.2, 127.3, 128.1, 129.0, 129.1, 129.1 (CHar), 134.2(8), 134.3(1), 143.9(8), 144.0(3), (C₆), 170.9 (CO); m/z (FAB) 367 (93.4%, MH⁺), 289 (38.4%, MH⁺-C₆H₆), 231 (35.7%, PhCH(CH₃)CH₂OH), 119 (93.8%, PhCH₂CO⁺), (Found: 367.2137, MH⁺ requires 367.2134).

7.11.2 Coupling of 2-Phenylpropan-1-ol to 4-{{Ethylsulphanyl-N-(2-phenylacetyl)}aminomethyl}benzamidopropylamido TG 81a

NIS (228 mg) was added to dry CH₂Cl₂ (9.2 ml) followed by triflic acid (3 µl) (note the NIS does not go completely into solution). This mixture was allowed to stand for 30 min. Resin (81a, linker loading 110 µmol/g, 18.2 mg, 2.00 µmol) dry CH₂Cl₂ (1.5 ml), 2-phenylpropan-1-ol (8.4 mg, 62 µmol, 31 equiv.) and 4 Å molecular sieves added to a tube which was placed on the blood rotator for 30 min. The NIS mixture (80 µl, approx. 4 equiv.) was added to the tube. The tube was placed on the blood rotator overnight. The resin was filtered off and CH₂Cl₂ added until the resin floated to the top and could be removed by pipette from the molecular sieves. The resin was washed according to the standard wash protocol and dried to a constant weight in the
vacuum oven at ambient temperature. Cleavage and HPLC (Section 7.9.1) indicated an alcohol loading of 90 μmol/g, 82%.

### 7.11.3 Coupling of 1-Phenylpropan-2-ol to 4-{[Ethylsulphanyl-N-(2-phenylacetyl)]aminomethyl}-benzamidopropylamido TG 81a

![](image1)

NIS (228 mg) was added to dry CH₂Cl₂ (9.2 ml) followed by triflic acid (3 μl) (note the NIS does not go completely into solution). This mixture was allowed to stand for 30 min. Resin (81, linker loading 110 μmol/g, 13.7 mg, 1.51 μmol) dry CH₂Cl₂ (1.5 ml), 1-phenylpropan-2-ol (14.1 mg, 1.04 μmol, 68.9 equiv.) and 4 Å molecular sieves were added to a tube which was placed on the blood rotator for 30 min. The NIS mixture (60 μl, approx. 4 equiv.) was added to the tube. This tube was placed on the blood rotator overnight. The resin was filtered off and CH₂Cl₂ added until the resin floated to the top and could be removed by pipette from the molecular sieves. The resin was washed according to the standard wash protocol and dried to a constant weight in the vacuum oven at ambient temperature. Cleavage and HPLC (as Section 7.9.1) indicated an alcohol loading of 80 μmol/g, 73%.

### 7.11.4 Attempted Coupling of 2-Phenylpropan-1-ol to [4-Ethylsulfanyl-N-(2-phenylacetyl)-4-amino]butylamido TG 121

![](image2)

NIS (228 mg) was added to dry CH₂Cl₂ (9.2 ml) followed by triflic acid (3 μl) (note the NIS does not go completely into solution). This mixture was allowed to stand for 30 min. Resin (81, linker loading 110 μmol/g, 13.7 mg, 1.51 μmol) dry CH₂Cl₂ (1.5 ml), 1-phenylpropan-2-ol (14.1 mg, 1.04 μmol, 68.9 equiv.) and 4 Å molecular sieves were added to a tube which was placed on the blood rotator for 30 min. The NIS mixture (60 μl, approx. 4 equiv.) was added to the tube. This tube was placed on the blood rotator overnight. The resin was filtered off and CH₂Cl₂ added until the resin floated to the top and could be removed by pipette from the molecular sieves. The resin was washed according to the standard wash protocol and dried to a constant weight in the vacuum oven at ambient temperature. Cleavage and HPLC (as Section 7.9.1) indicated an alcohol loading of 80 μmol/g, 73%.
NIS (228 mg) was added to dry CH$_2$Cl$_2$ (9.2 ml) followed by triflic acid (3 µl) (note the NIS does not go completely into solution). This mixture was allowed to stand for 30 min. Resin (121, linker loading 110 µmol/g, 30.0 mg, 3.30 µmol), dry CH$_2$Cl$_2$ (3 ml), 2-phenylpropan-1-ol (9.6 mg, 70 µmoles, 21 equiv.) and 4 Å molecular sieves were added to a tube which was placed on the blood rotator for 30 min. The NIS mixture (130 µl, approx. 4 equiv.) was added to the tube. This tube was placed on the blood rotator overnight. The resin was filtered off and CH$_2$Cl$_2$ added until the resin floated to the top and could be removed by pipette from the molecular sieves. The resin was washed according to the standard wash protocol and dried to a constant weight in the vacuum oven at ambient temperature Cleavage and HPLC (as Section 7.9.2) indicated negligible coupling.

7.11.5 Coupling of Fmoc-Ser-OMe to 4-([Ethylsulphanyl-N-(2-phenylacetyl)]-aminomethyl]benzamidopropylamido Carboxy TG 81b, PEGA 85 or Polystyrene 88a

NIS (230 mg) was added to dry CH$_2$Cl$_2$ (10.0 ml) followed by triflic acid (3 µl) (note the NIS does not go completely into solution). This mixture was allowed to stand for 30 min. Resin (61-104 mg), dry CH$_2$Cl$_2$ (1.0 ml), Fmoc-Ser-OMe (10-10.4 equiv.) and 4 Å molecular sieves were added to a tube which was placed on the blood rotator overnight. The resin was filtered off and CH$_2$Cl$_2$ added until the resin floated to the top and could be removed by pipette from the molecular sieves. The resin was washed according to the standard wash protocol and dried to a constant weight in the vacuum oven at ambient temperature.
7.11.6 Coupling of Fmoc-Ser-OMe to 4-\{[Ethylsulphonyl-N-(2-phenylacetyl)]-aminomethyl\}-benzamidopropylamido TG 81b

![Chemical structure](image)

UV 140 \(\mu\)mol Fmoc/g (78% from 81b); \(\nu_{\text{max}}\) (CH\(_2\)Cl\(_2\))/cm\(^{-1}\) 1753-1704 (CO ester, CO urethane), 1681-1634 (CO amide); \(\delta_c\) (CD\(_2\)Cl\(_2\); 63 MHz) 43.0 (PhCH\(_2\)) 47.1 (CH Fmoc and/or OCH\(_3\)), 66.8 (CH\(_2\) Fmoc), 119.9 (CH\(_{ar}\) Fmoc).

7.11.7 Coupling of Fmoc-Ser-OMe to 4-\{[Ethylsulphonyl-N-(2-phenylacetyl)]-aminomethyl\}-benzamidopropylamido PEGA 85

![Chemical structure](image)

Refer to Section 7.11.5.

UV 270 \(\mu\)mol Fmoc/g (87% from 85); \(\nu_{\text{max}}\) (CH\(_2\)Cl\(_2\))/cm\(^{-1}\) 1749 (CO ester), 1725 (CO urethane), 1672-1622 (CO amide); \(\delta_c\) (CD\(_2\)Cl\(_2\); 63 MHz) 43.2 (PhCH\(_2\)) 47.1 (CH Fmoc and/or OCH\(_3\)), 66.9 (CH\(_2\) Fmoc), 119.9, (CH\(_{ar}\)) Fmoc

7.11.8 Coupling of Fmoc-Ser-OMe to 4-\{[Ethylsulphonyl-N-(2-phenylacetyl)]-aminomethyl\}-benzamidopropylamido Polystyrene 88a

![Chemical structure](image)
Refer to Section 7.11.5. UV 600 μmol Fmoc/g (87% from 88a); $\nu_{\text{max}}$ (CH$_2$Cl)$_2$/cm$^{-1}$
broad absorbance in the carbonyl region.
8. EXPERIMENTAL - GENERATION OF LINKERS ON SOLID SUPPORT

Compounds 140, 142, 143, 147, 146, 155, 156, 158a, 166, 170 and 173a were synthesised by Dr Anne Routledge. All analysis and further work was carried by the author.

8.1.1 Acylation of Amino Resins with 4-Formylbenzoic Acid

Amino resin (0.40-1.00 g) was swollen in THF (15 ml/g). 4-carboxybenzaldehyde (3.0-3.7 equiv.) was added followed by portion-wise addition of EEDQ (3.0 equiv.). The mixture was agitated at room temperature for 16-48 h (note 142 was prepared under an inert atmosphere). In the case of filtered and washed with DMF (10 ml x 3), THF (10 ml x 3) and CH$_2$Cl$_2$ (10 ml x 3) and dried to a constant weight in the vacuum oven at ambient temperature.

8.1.2 Formation of Resin Bound Benzotriazole Adducts

The aldehyde resin (0.2-3.0 g) was swollen with toluene (13-40 ml per gram of resin). Benzotriazole (10.0 equiv.), phenylacetamide (9.0-10.0 equiv.) and p-toluenesulphonic acid (0.1-0.5 equiv.) were added and the mixture heated under reflux using a Dean-Stark apparatus for time T (Table 17). The resin was filtered off and washed with DMF (x 3), THF (x 3) and CH$_2$Cl$_2$ (x 3).
<table>
<thead>
<tr>
<th>Aldehyde resin</th>
<th>Resin Bound Benzotriazole Adduct</th>
<th>time T/h</th>
</tr>
</thead>
<tbody>
<tr>
<td>140</td>
<td>147</td>
<td>4.5</td>
</tr>
<tr>
<td>155</td>
<td>166</td>
<td>18</td>
</tr>
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<td>156</td>
<td>170</td>
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<td>18</td>
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<tr>
<td>157</td>
<td>176</td>
<td>21.5</td>
</tr>
<tr>
<td>158a</td>
<td>173a</td>
<td>18</td>
</tr>
<tr>
<td>158b</td>
<td>173b</td>
<td>14.5</td>
</tr>
<tr>
<td>137</td>
<td>146</td>
<td>18</td>
</tr>
<tr>
<td>165</td>
<td>179</td>
<td>8</td>
</tr>
</tbody>
</table>

Table 17 Reaction Conditions for the Formation of Resin Bound Benzotriazole Adducts

8.1.3 Determination of Benzotriazole Adduct Loading

CH$_2$Cl$_2$:TFA:H$_2$O (9:10:1, 300 µl) was added to resin (2 mg). This was repeated. The mixtures were placed on the blood rotator overnight (minimum 19 h) at ambient temperature. In each case the resin was filtered off and washed with CH$_2$Cl$_2$ (5 ml). The filtrate was evaporated to dryness and the residue dissolved in 0.25 mM phosphate buffer (pH 6.5) or 0.25 mM phosphate buffer (pH 6.5):MeCN (92:8) then examined by HPLC (Section 6.2, system 5).

8.1.4 Repeat Cleavage of Benzotriazole Adduct 143

Resin 143 was treated as described in Section 8.1.3. When the resin which had been treated with CH$_2$Cl$_2$:TFA:H$_2$O (9:10:1) was filtered off it was retained and treated for a second time with CH$_2$Cl$_2$:TFA:H$_2$O (9:10:1, 300 µl) as before. Subsequent HPLC (Section 6.2, system 5) indicated no further release of benzotriazole.

8.1.5 Repeat Cleavage of Benzotriazole Adduct 176

The procedure described in Section 8.1.4. was repeated using adduct 179. The second cleavage resulted in negligible benzotriazole release.
8.1.6 Displacement of the Benzotriazole Group from the Support-bound Adducts Using Sodium Ethyliothiolate To Give Support-bound Linkers

THF (1 ml per 100 mg of resin) was added to benzotriazole adduct resin and sodium ethyliothiolate (technical grade, 5.9-10.7 equiv.) in a tube which was placed on the blood rotator overnight. The following day the resin was filtered off, washed twice with water then according to the standard wash protocol and dried to a constant weight in the vacuum oven at ambient temperature.

8.1.7 Displacement of the Thioethyl Group of the Support-bound Linkers Using Fmoc-Ser-OMe

Anhydrous CH$_2$Cl$_2$ (10 ml) was added to NIS (224-231 mg) followed by triflic acid (3 µl) (note the NIS does not go completely into solution). This mixture was allowed to stand for 30 min. Meanwhile, anhydrous CH$_2$Cl$_2$ (0.8-2.0 ml) and 4 Å molecular sieves were added to resin (82-255 mg) and Fmoc-Ser-OMe (9.9-11.6 equiv.). This mixture was placed on the blood rotator for 30 min. An aliquot of the NIS/triflic acid/CH$_2$Cl$_2$ solution (corresponding to approx. 4 equiv. of NIS) was then added and the mixture placed on the blood rotator overnight. The resin was filtered off and washed according to the standard wash protocol then dried to a constant weight in the vacuum oven.

8.1.8 Synthesis of Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl Polystyrene

146 was synthesised from formyl polystyrene 137 according to Section 8.1.2. HPLC 1. Bt 530 µmol/g, PA+PAM 650 µmol/g, 2. Bt 510 µmol/g, PA+PAM 590 µmol/g (Av. Bt 520 µmol/g, 104% from formyl polystyrene 137, Av. PA+PAM 620
µmol/g), \( \nu_{\text{max}} (\text{CH}_2\text{Cl}_2)/\text{cm}^{-1} 1683 \) (CO amide). Note when treated overnight with DCM:H_2O (9:1) 130 µmol/g of benzotriazole was released.

8.1.9 Synthesis of Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl Polystyrene 148

148 was synthesised from 146 according to Section 8.1.6. Sulfur elemental analysis 1.56% (loading 490 µmol/g, 91% from 146); \( \nu_{\text{max}} (\text{CH}_2\text{Cl}_2)/\text{cm}^{-1} 1683 \) (CO amide); \( \delta_c (\text{CD}_2\text{Cl}_2; 63 \text{ MHz}); 14.9 (\text{CH}_3), 25.5 (\text{SCH}_2) \).

8.1.10 Displacement of the Thioethyl Group of Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl Polystyrene 148 using Fmoc-Ser-OMe

149 was synthesised from 148 according to Section 8.1.7. UV 300 µmol Fmoc/g (67% from 146, overall yield 67% from formyl polystyrene); \( \nu_{\text{max}} (\text{CH}_2\text{Cl}_2)/\text{cm}^{-1} 1750 \) (CO ester), 1722 (CO urethane), 1680 (CO amide); \( \delta_c (\text{CD}_2\text{Cl}_2; 63 \text{ MHz}); 47.4 (\text{CH Fmoc and/or OCH}_3), 54.9 (\text{CH}), 67.3 (\text{CH}_2 \text{ Fmoc}) \).
8.1.11 Displacement of the Thioethyl Group of Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl Polystyrene 148 using 2-Phenylpropan-1-ol

150 was synthesised from 148 according to Section 8.1.7. replacing Fmoc-Ser-OMe with 2-phenylpropan-1-ol (9.8 equiv.). \( \nu_{\text{max}} (\text{CH}_2\text{Cl}_2)/\text{cm}^{-1} 676 \) (CO amide); \( \delta_c (\text{CD}_2\text{Cl}_2; 63 \text{ MHz}) 18.3, 18.6 \) (CH).  

8.1.12 Attempted Displacement of the Thioethyl Group of Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl Polystyrene 148 using 1-Phenylpropan-2-ol

144 was synthesised from 148 according to Section 8.1.7. replacing Fmoc-Ser-OMe with 1-phenylpropan-2-ol (9.9 equiv.). \( \nu_{\text{max}} (\text{CH}_2\text{Cl}_2)/\text{cm}^{-1} 1676 \) (CO amide); \( \delta_c (\text{CD}_2\text{Cl}_2; 63 \text{ MHz}) \) two very small peaks just above noise at ca. 20 ppm.  

8.1.13 Synthesis of p-Formylphenoxymethyl Polystyrene 140

Chloromethylpolystyrene (2.00 g, 1.92 mmol) was swollen in anhydrous DMF (20 ml). 4-Hydroxybenzaldehyde (703 mg, 5.76 mmol, 3.00 equiv.) and anhydrous NaI
(29 mg, 0.192 mmol, 0.10 equiv.) were added. NaH (60% dispersion in oil, 230 mg, 5.76 mmol, 3.00 equiv.) was then added portion wise. After evolution of hydrogen had ceased the mixture was heated at 60 °C under an inert atmosphere with intermittent agitation for 24 h. The resin was filtered and washed with DMF (10 ml x 3), DMF:H₂O (1:1, 10 ml), DMF (10 ml), THF (10 ml x 3), CH₂Cl₂ (10 ml x 3). Chlorine elemental analysis <0.1%; ν max (CH₂Cl₂)/cm⁻¹ 2743 (CH aldehyde), 1694 (CO aldehyde); δ c (CD₂Cl₂; 63 MHz) 115.2 (CH₂), 190.6 (CO).

8.1.14 Synthesis of 4-[Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl]phenoxy-methyl Polystyrene 147

147 was synthesised from 140 as described in Section 8.1.2. HPLC 1. Bt 430 μmol/g 2. Bt 460 μmol/g (Av. Bt 450 μmol/g, 62% from chloromethylpolystyrene) (peak in the HPLC trace which co-elutes with an authentic sample of 4-hydroxybenzaldehyde therefore phenylacetamide could not be quantified); ν max (CH₂Cl₂)/cm⁻¹ 1682 (CO amide); δ c (CD₂Cl₂; 63 MHz) 43.0 (PhCH₂), 115.1 (CH₂), 190.8 (residual CO aldehyde). Note when treated overnight with DCM:H₂O (9:1) 30 μmol/g of benzotriazole was released.
8.1.15 Synthesis of 4-[Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl]phenoxy-methyl Polystyrene 151

151 was synthesised from 147 as described in Section 8.1.6. Sulfur elemental analysis 1.63% (loading of 510 μmol/g, 111% from 147); $v_{\text{max}}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1674 (CO amide); $\delta_C$ (CD$_2$Cl$_2$; 63 MHz) 14.9 (CH$_3$), 25.7 (SCH$_2$), 43.7 (PhCH$_2$) 55.6 (CH), 190.7 (CO, residual aldehyde).

8.1.16 Displacement of the Thioethyl Group of 4-[Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl]phenoxy-methyl Polystyrene 151 with Fmoc-Ser-OMe

153 was synthesised from 151 as described in Section 8.1.7. UV 400 μmol Fmoc/g (98% from 147, overall yield from chloromethylpolystyrene (139) 62%); $v_{\text{max}}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1750 (CO ester), 1722 (CO urethane), 1682 (CO amide); $\delta_C$ (CD$_2$Cl$_2$; 63 MHz) 47.3 (CH Fmoc and/or OCH$_3$), 54.9 (CH) 67.2 (CH$_2$ Fmoc).

8.1.17 Synthesis of p-Formylbenzamidomethyl Polystyrene 142
142 was synthesised from aminomethyl polystyrene 141 according to Section 8.1.1. $\nu_{\text{max}}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 2738 (CH aldehyde), 1705 (CO aldehyde) and 1669-1647 (CO amide); $\delta_{\text{C}}$ (CD$_2$Cl$_2$; 63 MHz) 191.7 (CO aldehyde).

8.1.18 Synthesis of 4-[Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl]-benzamidomethyl Polystyrene 143

![Diagram of 143]

143 was synthesised from 142 as described in Section 8.1.2 (note this reaction boiled dry overnight). HPLC 1. Bt 330 µmol/g, PA+PAM 560 µmol/g, 2. Bt 350 µmol/g, PA+PAM 650 µmol/g (Av. Bt 340 µmol/g, 46% from aminomethyl polystyrene 141, Av. PA+PAM 610 µmol/g), $\nu_{\text{max}}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1668 (broad, amide CO).

8.1.19 Synthesis of 4-[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]-benzamidomethyl Polystyrene 152

![Diagram of 152]

152 was synthesised from 143 as described in Section 8.1.6. Sulfur elemental analysis 1.15% (loading of 360 µmol/g, 103% from 143); $\nu_{\text{max}}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1671-1638 (broad, CO amide); $\delta_{\text{C}}$ (CD$_2$Cl$_2$; 63 MHz) 14.8 (CH$_3$), 25.7 (SCH$_2$).
8.1.20 Displacement of the Thioethyl Group of 4-[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]benzamidomethyl Polystyrene 152 with Fmoc-Ser-OMe

154 was synthesised from 152 as described in Section 8.1.7. UV 290 μmol Fmoc/g (91% from 152, 46% overall yield from aminomethyl polystyrene 141); ν_max (CH₂Cl₂)/cm⁻¹ 1652 (broad, CO amide), 1722 (broad, CO urethane), 1749 (CO ester); δ_C (CD₂Cl₂; 63 MHz) 47.3 (CH Fmoc and/or OCH₃), 67.2 (CH₂ Fmoc), 120.1 (CH₉).  

8.1.21 Synthesis of p-Formylphenoxy TG 155

TG-Br 159 (1.00 g, 0.28 mmol) was swollen in DMF (20 ml). 4-Hydroxybenzaldehyde (171 mg, 1.40 mmol, 5.00 equiv.), Bu₄NI (10 mg) and NaH (60% dispersion in oil, 56 mg, 1.40 mmol, 5.00 equiv.) were added. The reaction was agitated overnight at room temperature then overnight at 60 °C. The resin was filtered and washed with DMF (10 ml × 3), H₂O (10 ml), DMF (10 ml), THF (10 ml × 3) and CH₂Cl₂ (10 ml × 3). Bromine elemental analysis 0.14% (<10% residual bromine); ν_max (CH₂Cl₂)/cm⁻¹ 1694 (CO aldehyde); δ_C (CD₂Cl₂; 63 MHz) 114.7 (CH₉), 130.1 131.8, 163.8 (C₉), 190.4 (CO).
8.1.22 Synthesis of 4-[Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl]phenoxy 

TG 166

166 was synthesised from 155 as described in Section 8.1.2. HPLC 1. Bt 120 μmol/g, PAM 100 μmol/g, 2. Bt 120 μmol/g, PAM 90 μmol/g (Av. Bt 120 μmol/g, 46% from TG-Br 159, Av. PAM 100 μmol/g); \( \nu_{\text{max}} \) (CH\(_2\)Cl\(_2\)) cm\(^{-1}\) 1682 (CO amide); \( \delta_c \) (CD\(_2\)Cl\(_2\); 63 MHz) 42.8 (PhCH\(_2\)), 110.6 (benzotriazole CH\(_{ar}\)), 114.7 (CH\(_{ar}\)). Note when treated overnight with DCM:H\(_2\)O (9:1) 40 μmol/g of benzotriazole was released.

8.1.23 Synthesis of 4-[Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl]phenoxy TG 167

167 was synthesised from 166 as described in Section 8.1.6. Sulfur elemental analysis 0.5% (loading of 110 μmol/g, 92% from 166); \( \nu_{\text{max}} \) (CH\(_2\)Cl\(_2\)) cm\(^{-1}\) 1667-1633 (CO amide); \( \delta_c \) (CD\(_2\)Cl\(_2\); 63 MHz) 14.7 (CH\(_3\)), 25.5 (SCH\(_2\)), 43.4 (PhCH\(_2\)), 55.4 (CH), 114.5 (CH\(_{ar}\)).
8.1.24 Displacement of the Thioethyl Group of 4-[(Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl]phenoxy TG 167 with Fmoc-Ser-OMe

168 was synthesised from 167 as described in Section 8.1.7. UV 90 µmol Fmoc/g (75% from 166, overall yield 36% from bromo TG 159); \( \nu_{\text{max}} (\mathrm{CH}_2\mathrm{Cl}_2)/\text{cm}^{-1} \) 1749 (CO ester), 1722 (CO urethane), 1675 (CO amide); \( \delta_{\text{C}} (\mathrm{CD}_2\mathrm{Cl}_2; 63 \text{ MHz}) \) 43.1 (PhCH\(_2\)), 47.1 (CH Fmoc and/or OCH\(_3\)), 114.7 (CH\(_{\text{ar}}\)) 119.8 (CH\(_{\text{ar}}\) Fmoc).

8.1.25 Attempted Synthesis of p-(p-Formylphenoxy)methylphenoxyphosphorylamido PEGA 161

HMPA PEGA resin (160, 0.36 mmol/g, 0.50g, 0.18 mmol) was swollen in anhydrous THF (30 ml). PPh\(_3\) (141 mg, 0.54 mmol, 3.00 equiv. was added to the mixture under argon and stirred at 0°C. 4-Hydroxybenzaldehyde (66 mg, 0.54 mmol, 3.00 equiv.) was added followed by dropwise addition of DEAD (85 ml, 94 mg, 0.54 mmol, 3.00 equiv.). The mixture was allowed to warm up to room temperature then agitated overnight. The resin was filtered and washed with THF (x 3), DMF (x 3), THF (x 3) and CH\(_2\)Cl\(_2\) (x 3) then dried to a constant weight in the vacuum oven at ambient temperature. \( \delta_{\text{C}} (\mathrm{CD}_2\mathrm{Cl}_2; 63 \text{ MHz}) \) 190.6 (CO aldehyde); \( \nu_{\text{max}} (\mathrm{CH}_2\mathrm{Cl}_2)/\text{cm}^{-1} \) broad absorbance 1663-1628.
8.1.26 Preparation of Resin 156

\[
\text{IBX (250 mg, 0.89 mmol, 5 equiv.) was dissolved in DMSO (5 ml) and added to 161 (0.36 mmol/g, 0.50 g, 0.18 mmol). The mixture was agitated over 3 days. The resin was filtered off and washed with DMF (x3), THF (x3), CH}_2\text{Cl}_2 \text{ (x 3) then dried to a constant weight in the vacuum oven. } v_{\text{max}} \text{ (CH}_2\text{Cl}_2)/\text{cm}^{-1} \text{ broad absorbance 1674-1601.}
\]

8.1.27 Synthesis of Resin 170

\[
\text{170 was synthesised from 156 as described in Section 8.1.2. HPLC 1. Bt 200 \mu\text{mol/g PA+PAM 240 \mu\text{mol/g, 2. Bt 190 \mu\text{mol/g PA+PAM 230 \mu\text{mol/g (Av. Bt 190 \mu\text{mol/g, 58% from HMPA PEGA 160, Av. PAM 230 \mu\text{mol/g), } v_{\text{max}} \text{ (CH}_2\text{Cl}_2)/\text{cm}^{-1} 1656-1631}}}
\]
(broad absorption, CO amide); δ C (CD₂Cl₂; 63 MHz) 110.2 (benzotriazole CH₃). Note when treated overnight with DCM:H₂O (9:1) 20 μmol/g of benzotriazole was released.

8.1.28 Synthesis of Resin 171

![Resin 171](image)

171 was synthesised from 170 as described in Section 8.1.6. Sulfur elemental analysis 0.22% (loading 70 μmol/g); ν max (CH₂Cl₂)/cm⁻¹ 1668-1634 (CO amide); δ C (CD₂Cl₂; 63 MHz) 14.6 (CH₃), 25.5 (SCH₂).

8.1.29 Displacement of the Thioethyl Group Resin 171 with Fmoc-Ser-OMe

![Resin 172](image)

172 was synthesised from 171 as described in Section 8.1.7. UV 70 μmol Fmoc/g (39% from 170, quantitative from 171, overall yield 23% from HMPA PEGA 160); ν max (CH₂Cl₂)/cm⁻¹ 1749 (CO ester), 1723 (CO urethane), 1663-1634 (CO amide); δ C (CD₂Cl₂; 63 MHz) 47.2 (CH Fmoc and/or OCH₃), 120.0 (CHₓ Fmoc), 190.6 (reappearance of CO aldehyde).

8.1.30 Synthesis of p-Formylbenzamido PEGA 158a

![Resin 158a](image)

H MBA PEGA 163 (0.50 g, maximum loading 0.4 mmol/g, maximum 0.2 mmol) was swollen in DMSO (anhydrous, 10 ml). IBX (112 mg, 0.4 mmol, 2 equiv.) was added
and the mixture shaken overnight at ambient temperature. The resin was filtered and washed with DMF (10 ml x 3), THF (10 ml x 3) and CH₂Cl₂ (10 ml x 3) then dried to a constant weight at ambient temperature in the vacuum oven. IBX (260 mg, 0.93 mmol,) was dissolved in DMSO (5 ml) and added to the resin. The mixture agitated for 3 days. The resin was filtered and washed with DMF (10 ml x 3), THF (10 ml x 3) and CH₂Cl₂ (10 ml x 3). υ max (CH₂Cl₂)/cm⁻¹ 1704 (CO aldehyde), 1666-1615 (broad absorbance, CO amide).

8.1.31 Synthesis of 4-[Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl]-benzamido PEGA 173a

![173a](image)

173a was synthesised from 158a as described in Section 8.1.2. HPLC 1. Bt 110 μmol/g, PAM 140 μmol/g, 2. Bt 100 μmol/g, PAM 120 μmol/g (Av. Bt 100 μmol/g, 27% from HMBA PEGA 163, Av. PA+PAM 130 μmol/g), υ max (CH₂Cl₂)/cm⁻¹ 1659-1639 (CO amide); δC (CD₂Cl₂; 63 MHz) 14.6 (CH₃), 25.5 (SCH₂), 43.3 (PhCH₂), 55.5 (CH).

8.1.32 Synthesis of 4-[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]benzamido PEGA 174a

![174a](image)

174a was synthesised from 173a as described in Section 8.1.6. Sulfur elemental analysis 0.06% (loading 20 μmol/g); υ max (CH₂Cl₂)/cm⁻¹ 1673-1615 (CO, amide); δC (CD₂Cl₂; 63 MHz) 14.6 (CH₃), 25.5 (SCH₂), 43.3 (PhCH₂), 55.5 (CH).
8.1.33 Displacement of the Thioethyl Group of 4-[Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl]benzamido PEGA 174a Using Fmoc-Ser-OMe

175a was synthesised from 174a as described in Section 8.1.7. UV 110 μmol Fmoc/g (quantitative from 173a, overall yield 32% from HMBA PEGA 163); $\nu_{max}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1748 (CO ester), 1722 (CO urethane), 1661-1634 (CO amide); $\delta_C$ (CD$_2$Cl$_2$; 63 MHz) 47.2 (CH Fmoc and/or OCH$_3$), 119.9 (CH$_{ar}$ Fmoc).

8.1.34 Synthesis of p-Formylbenzamido PEGA 158b

158b was synthesised from amino PEGA 83 as described in Section 8.1.1. $\nu_{max}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1706 (CO aldehyde), 1660-1634 (CO amide); $\delta_C$ (CD$_2$Cl$_2$; 63 MHz) 127.9, 129.6 (CH$_{ar}$), 191.7 (CO aldehyde).

8.1.35 Synthesis of 4-[Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl]-benzamido PEGA 173b
173b was synthesised from 158b as described in Section 8.1.2. HPLC 1. Bt 100 μmol/g, PA+PAM 140 μmol/g, 2. Bt 100 μmol/g, PA+PAM 140 μmol/g (Bt Av. 100 μmol/g, 29% from amino PEGA 83, Av. PA+PAM 140 μmol/g), νmax (CH₂Cl₂)/cm⁻¹ 1662-1639 (CO amide); δC (CD₂Cl₂; 63 MHz) 110.3 (benzotriazole CH₃).  

8.1.36 Synthesis of 4-[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]benzamido PEGA 174b

![](image)

174b was synthesised from 173b according to Section 8.1.6. Sulfur elemental analysis <0.1%; νmax (CH₂Cl₂)/cm⁻¹ 1673-1615 (CO, amide); δC (CD₂Cl₂; 63 MHz) 14.6 (CH₃), 25.6 (SCH₂), 55.5 (CH).  

8.1.37 Displacement of the Thioethyl Group of 4-[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]benzamido PEGA 174b using Fmoc-Ser-OMe

![](image)

175b was synthesised from 174b according to Section 8.1.7. UV 100 μmol Fmoc/g (quantitative from 173b, overall yield 31% from amino PEGA 83); νmax (CH₂Cl₂)/cm⁻¹ 1753-1736 (CO ester), 1723-1698 (CO urethane), 1665-1614 (CO amide); δC (CD₂Cl₂; 63 MHz), 47.2 (CH Fmoc and/or OCH₃), 120.0 (CH₃ Fmoc).
8.1.38 Synthesis of p-Formylbenzamido TG 157

![Diagram of 157](image)

157 was synthesised from amino TG 163 as described in Section 8.1.1. \( \nu_{\text{max}} \) (CDCl\(_3\))/cm\(^{-1}\) 1705 (CO aldehyde), 1661 (CO amide); \( \delta \) (CDCl\(_3\); 63 MHz) 128.0, 129.5 (CH\(_\text{ar}\)), 166.2 (CO amide), 191.8 (CO aldehyde).

8.1.39 Synthesis of 4-[Benzotriazol-1-yl-N-(2-phenylacetyl)aminomethyl]-benzamido TG 176

![Diagram of 176](image)

176 was synthesised from 157 as described in Section 8.1.2. HPLC 1. Bt 100 \( \mu \text{mol/g} \), PA+PAM 100 \( \mu \text{mol/g} \), 2. Bt 100 \( \mu \text{mol/g} \), PA+PAM 110 \( \mu \text{mol/g} \) (Av. Bt 100 \( \mu \text{mol/g} \), from aminomethyl TG 163, PA+PAM 110 \( \mu \text{mol/g} \)), \( \nu_{\text{max}} \) (CDCl\(_3\))/cm\(^{-1}\) broad absorbance 1713-1659; \( \delta \) (CDCl\(_3\); 63 MHz) 110.2 (benzotriazole CH\(_\text{ar}\)).

8.1.40 Synthesis of 4-[Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl]benzamido TG 177

![Diagram of 177](image)

177 was synthesised from 176 as described in Section 8.1.6. Sulfur elemental analysis <0.1%; \( \nu_{\text{max}} \) (CDCl\(_3\))/cm\(^{-1}\) 1660 (CO amide); \( \delta \) (CDCl\(_3\); 63 MHz) 14.6 (CH\(_3\)), 25.5 (SCH\(_2\)), 43.3 (PhCH\(_2\)), 55.5 (CH).
8.1.41 Displacement of the Thioethyl Group of 4-[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]benzamido TG 177 with Fmoc-Ser-OMe

178 was synthesised from 177 as described in Section 8.1.7. UV 100 μmol Fmoc/g (91% from 176, overall yield 29% from amino TG 163); $\nu_{\text{max}}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1754-1645 (broad, CO); $\delta_\text{C}$ (CD$_2$Cl$_2$; 63 MHz) 47.2 (CH Fmoc and/or OCH$_3$), 119.9 (CH$_{ar}$ Fmoc).

8.1.42 Synthesis of 4-Oxobutyramido TG 165

TG acetal resin' 164 (0.50 g, 0.28 mmol/g, 0.14 mmol), acetone (6 ml), H$_2$O (2 ml) and HCl (5 M, 20 μl) was heated at reflux for 1 h. The resin was filtered off, washed with diethyl ether (5 ml x 5) and dried to constant weight in the vacuum oven. $\nu_{\text{max}}$ (CH$_2$Cl$_2$)/cm$^{-1}$ 1663 (CO amide), 1722 (CO aldehyde); $\delta_\text{C}$ (CD$_2$Cl$_2$; 63 MHz) 172.2 (CO, amide), 202.6 (CO, aldehyde).

---

The structure of this resin was described by 2 different sources as 4,4-diethoxybutyramido TG and 6,6-diethoxyhexamido TG. I have shown the structure as 4,4-diethoxybutyramido TG.
8.1.43 Synthesis of [3-Benzotriazol-1-yl-N-(2-phenylacetyl)-3-aminopropyl]amido TG 179

179 was prepared from 165 according to Section 8.1.2. HPLC 1. Bt 120 μmol/g, PA+PAM 110 μmol/g, 2. Bt 100 μmol/g, PA+PAM 90 μmol/g (Bt Av. 110 μmol/g, 42% from diethyl acetal resin 164), PA+PAM Av. 100 μmol/g, \( \nu_{\text{max}} \) (CH\(_2\)Cl\(_2\))/cm\(^{-1}\) 1660 (CO amide); \( \delta \) (CD\(_2\)Cl\(_2\); 63 MHz) 110.8 (benzotriazole CH\(_{ar}\))

8.1.44 Synthesis of [3-Ethylsulphanyl-N-(2-phenylacetyl)-3-aminopropyl]amido TG 180

180 was prepared from 179 according to Section 8.1.6. Sulfur elemental analysis <0.1%; \( \nu_{\text{max}} \) (CH\(_2\)Cl\(_2\))/cm\(^{-1}\) 1666 (CO, amide); \( \delta \) (CD\(_2\)Cl\(_2\); 63 MHz) 14.9 (CH\(_3\)), 43.5 (PhCH\(_2\)) 127.0, 128.7, 129.2 (CH\(_{ar}\)), 135.4 (C\(_{ar}\)), 170.1, 172.5 (CO).

8.1.45 Displacement of the Thioethyl Group of [3-Ethylsulphanyl-N-(2-phenylacetyl)-3-aminopropyl]amido TG 180 using Fmoc-Ser-OMe

181 was synthesised from 180 according to the procedure described in Section 8.1.7. UV 40 μmol Fmoc/g, 36% from 179, 16% from 4,4-diethoxybutyramido TG 164; \( \nu_{\text{max}} \) (CH\(_2\)Cl\(_2\))/cm\(^{-1}\) 1754-1714 (CO ester, CO urethane), 1681-1634 (CO amide); \( \delta \)
\( \nu_{\text{max}} (\text{CH}_2\text{Cl}_2)/\text{cm}^{-1} \) 1754-1714 (CO ester, CO urethane), 1681-1634 (CO amide); \( \delta_c \) (CD\(\_\)Cl\(\_\)Cl; 63 MHz) 47.1 (CH Fmoc and/or OCH\(\_\)), 66.8 (CH\(\_\) Fmoc), 119.9 (CH\(\_\) ar Fmoc).

8.1.46 Displacement of the Thioethyl Group of [3-Ethylsulphanyl-N-(2-phenylacetyl)-3-aminopropyl]amido TG 180 using 1-Phenylpropan-2-ol

182 was synthesised from 180 according to Section 8.1.7. replacing Fmoc-Ser-OMe with 1-phenylpropan-2-ol (10.3 equiv.). Cleavage and HPLC (as Section 7.9.1 except using HPLC system 4) indicated a loading of 50 \( \mu \text{mol alcohol/g}, 45\% \).
9. EXPERIMENTAL - ACID STABILITY STUDIES

9.1 Acid Cleavage
Resin (2-4 mg) was accurately weighed out into vials. Acetic acid:CH₂Cl₂:H₂O (27:68:5, 300 µl) was added to each and the mixture placed on the blood rotator for 15 minutes, 1 h or 20 h. The resin was filtered off and washed with CH₂Cl₂ (5 ml). The filtrate was evaporated to dryness and the residue dissolved 0.1% TFA:MeCN (60:40, 0.6ml) and examined by HPLC (Section 6.2, system 6).

<table>
<thead>
<tr>
<th>Resin</th>
<th>loading*/µmol/g</th>
<th>Cleavage after 15 min./µmol/g</th>
<th>Cleavage after 1 h /µmol/g</th>
<th>Cleavage after 20 h/µmol/g</th>
</tr>
</thead>
<tbody>
<tr>
<td>153</td>
<td>400</td>
<td>170</td>
<td>300*</td>
<td>320*</td>
</tr>
<tr>
<td>149</td>
<td>280</td>
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<td>140</td>
</tr>
<tr>
<td>99a</td>
<td>600</td>
<td>10</td>
<td>30</td>
<td>270</td>
</tr>
</tbody>
</table>

* additional dilution of HPLC mixture was necessary before injection
* loading as determined by Fmoc cleavage (Section 6.3.2)

Table 18 Acid Cleavage Studies
10. EXPERIMENTAL - ENZYME CLEAVAGE STUDIES

10.1 Enzyme Cleavage

Penicillin amidase solution (700 µl) was added to resin (2-3.5 mg) and the mixture placed on the blood rotator for 16 h. The mixture was extracted with (CH$_2$Cl$_2$) (5 ml x 2). The organic layer was evaporated *in vacuo* and the residue dissolved in 0.1% TFA:MeCN (60:40) and examined by HPLC (Section 6.2, system 6).

<table>
<thead>
<tr>
<th>Resin</th>
<th>% Recovery of Fmoc-Ser-OMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>95b</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>98</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>168</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>172</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>178</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>175a</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>175b</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>181</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

**Table 19** Enzyme Cleavages

<table>
<thead>
<tr>
<th>Resin</th>
<th>% Recovery of Fmoc-Ser-OMe</th>
</tr>
</thead>
<tbody>
<tr>
<td>95b</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>98</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>168</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>172</td>
<td>ca. 10%</td>
</tr>
<tr>
<td>178</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>175a</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>175b</td>
<td>&lt;10%</td>
</tr>
<tr>
<td>181</td>
<td>&lt;10%</td>
</tr>
</tbody>
</table>

**Table 20** Enzyme Cleavage Blanks

10.2 Control

Phosphate buffer (0.1 M, pH 7.5, 700 µl) was added to resin (2-3.5 mg) and the mixture placed on the blood rotator for 16 h. The mixture was extracted with CH$_2$Cl$_2$ (5 ml x 2). The organic layer was evaporated *in vacuo* and the residue dissolved in 0.1% TFA:MeCN (60:40) and examined by HPLC (Section 6.2, system 6).
11. EXPERIMENTAL - PEPTIDE SYNTHESIS

11.1 Manual Peptide Synthesis - General Procedures

11.1.1 Removal of Fmoc Protecting Group and Amide Bond Forming Reaction
Resin (corresponding to 65 mg - 192 mg of the Fmoc-Ser-OMe resin) was treated with 20% piperidine in DMF (4 ml) for 30 min. The resin was filtered off and the filtrate retained. The resin was washed according to the standard wash protocol. The filtrate (30 μl-60 μl) could be diluted to 5 ml in a volumetric flask and the UV absorbance measured. Resin (corresponding to 65 mg - 192 mg of the Fmoc-Ser-OMe resin), Fmoc-amino acid (2.9-5.2 equiv.), TBTU (4.4-6.2 equiv.), HOBt (4.6-8.0 equiv.), DMF (3-6 ml) and NMM (2.9-3.1 equiv.) were added to a reaction vessel which was placed on the blood rotator for 6 h. The resin was filtered off and washed with DMF (5-10 ml x 2) then CH₂Cl₂ (5-10 ml x 2). Fresh reagents were added to the resin and the vessel placed on the blood rotator overnight (16 h). The resin was filtered off and washed according to the standard protocol.

11.1.2 Cleavage and Mass Spectrometry
Resin (ca. 3 mg) was treated with TFA:CH₂Cl₂:H₂O (9:10:1, 300 μl) for 3 - 18.5 h. The supernatant was removed and the resin washed with CH₂Cl₂ (1-5 ml). The CH₂Cl₂ solution was evaporated to dryness and the residue examined by electrospray mass spectrometry.
11.2 Attempted Manual Synthesis of Pentapeptide 183 on Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl Polystyrene 148

11.2.1 Coupling of Fmoc-Ser-OMe to Ethylsulphanyl-N-(2-phenylacetyl)aminomethyl Polystyrene 148 to Produce 149

Refer to Section 8.1.7. UV 300 µmol/g.

11.2.2 Attempted Peptide Synthesis

149 was deprotected as described in Section 11.1.1. Fmoc-Ser(Trt)-OH was then coupled as (refer to Section 11.1.1). Deprotection of the resulting resin, as described in Section 11.1.1, and UV analysis indicated 24% coupling.

11.3 First Manual Synthesis of Pentapeptide on 4-[[Ethylsulphanyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido Polystyrene (88a)

11.3.1 Coupling of Fmoc-Ser-OMe to 4-[[Ethylsulphanyl-N-(2-phenylacetyl)]aminomethyl]-benzamidopropylamido Polystyrene 88a to Produce 99a

Refer to Section 8.1.7.
11.3.2 Peptide Synthesis

99a was deprotected as described in Section 11.1.1 and Fmoc-Ser(Trt)-OH coupled as described in Section 11.1.1. This deprotection/coupling protocol was repeated with each of the following amino acid Fmoc-Val-OH, Fmoc-Ala-OH and Fmoc-Ser(tBu)-OH. Except that the first coupling of a given amino acid was overnight in duration and the second (double) coupling was for 6 h. The Fmoc group was not removed after the final coupling. m/z (ESI) 708 (MNa⁺), 621 (MNa⁺⁻87).

11.4 Second Manual Synthesis of Pentapeptide on 4-[[Ethylsulphanyl-N-(2-phenylacetetyl)aminomethyl]-benzamidopropylamido Polystyrene (88b)

11.4.1 Coupling of Fmoc-Ser-OMe to 4-[[Ethylsulphanyl-N-(2-phenylacetetyl)aminomethyl]-benzamidopropylamido Polystyrene 88b to Produce 99b

Refer to Section 8.1.7.

11.4.2 Peptide Synthesis

99b was deprotected as described in Section 11.1.1 and Fmoc-Ser(Trt)-OH coupled as described in Section 11.1.1. This deprotection/coupling protocol was repeated with each of the following amino acid Fmoc-Val-OH, Fmoc-Ala-OH and Fmoc-Ser(tBu)-OH. The Fmoc group was not removed after the final coupling. UV 130 μmol Fmoc/g, m/z (ESI) 708 (MNa⁺), 621 (MNa⁺⁻87).
11.5 Manual Synthesis of Pentapeptide using Resin Bound System 4-[Ethylsulphanil-N-(2-phenylacetyl)aminomethyl]benzamidomethyl polystyrene

11.5.1 Coupling of Fmoc-Ser-OMe to 4-[Ethylsulphanil-N-(2-phenylacetyl)aminomethyl]benzamidomethyl polystyrene 152 to Produce 154

Refer to Section 8.1.7. UV 290 μmol Fmoc/g,

11.5.2 Peptide Synthesis

154 was deprotected as described in Section 11.1.1 and Fmoc-Ser(Trt)-OH coupled as described in Section 11.1.1. This deprotection/coupling protocol was repeated with each of the following amino acid Fmoc-Val-OH Fmoc-Ala-OH and Fmoc-Ser(Bu)-OH. The Fmoc group was not removed after the final coupling. UV 100 μmol Fmoc/g, m/z (ESI) 708 (MNa⁺), 686 (MH⁺).
11.6 Automated Synthesis of Pentapeptide using Resin Bound System 4-\[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]benzamidomethyl Polystyrene

11.6.1 Coupling of Fmoc-Ser-OMe to 4-\[Ethylsulphonyl-N-(2-phenylacetyl)aminomethyl]benzamidomethyl polystyrene 152 to Produce 154

Refer to Section 8.1.7.

11.6.2 Capping Step
The resin was vortexed with acetic anhydride (0.5 M), DIEA (0.125 M) and HOBt (0.015 M) in DMF:Dioxane (1:1, 10 ml) for 10 min. It was then washed with DMF:Dioxane (1:1, 10 ml x 6).

11.6.3 Deprotection Step
The resin was vortexed with 20% piperidine in DMF:Dioxane (1:1, 10 ml) for 6 min., washed with DMF:Dioxane (1:1, 10 ml x 4) then treated again with 20% piperidine in DMF:Dioxane (1:1, 10 ml) for a further 1.5 min.. The resin was washed with DMF:Dioxane (1:1, 10 ml x 10).

11.6.4 Coupling Step
DMF (2 ml) was added to the amino acid (1 mmol) followed by HOCt in DMF (0.25 M, 4 ml). This mixture was then added to dioxane (2ml) and DIC in dioxane (0.25 M, 4 ml). After 5 min. this solution was added to the resin. The mixture was agitated for 30 min. then the resin was filtered and washed with DMF:Dioxane (1:1, 10 ml x 6).
11.6.5 Cleavage and Mass Spectrometry

Refer to section 11.1.2

11.6.6 Peptide Synthesis

Resin 154 (140 mg) was subjected to the capping step (Section 11.6.2). Removal of the Fmoc protecting group was carried out as described in Section 11.6.3. Fmoc-Ser(Trt)-OH was then coupled according to Section 11.6.4. This capping/deprotection coupling sequence was repeated with Fmoc-Val, Fmoc-Ala and Fmoc-Ser(tBu)-OH. The Fmoc group was not removed after the final coupling step. m/z (ESI) 708 (MNa⁺), 686 (MH⁺).

11.6.7 Final Fmoc Removal (Manual) from 189a and Cleavage of the Peptide 183 from the Resin

20% piperidine in DMF (4 ml) was added to resin bound peptide (119.2 mg). The mixture was placed on the blood rotator for 30 min. The resin was filtered off and washed according to the wash protocol described in Section 6.3.1. The resin (105.1 mg) was then treated with TFA:CH₂Cl₂:H₂O (9:10:1, 1 ml). This cleavage mixture was placed on the blood-rotator overnight (19.5 h). The resin was filtered off and washed with CH₂Cl₂ (12 ml). The filtrate was evaporated to dryness to give a solid suspended in a yellow oil. m/z (ESI) 464 (MH⁺).

11.6.8 Purification of Peptide 183

m/z (ESI) 464 (MH⁺).
The crude peptide was slurried in ether (1 ml) the slurry was centrifuged and the supernatant decanted. This was repeated a further 3 times. The solid thus obtained was dissolved in MeCN:H₂O (1:1, 1 ml) then lyophilised overnight to give white solid (12 mg). This material was further purified by semi-preparative HPLC (Section 6.2). Purified peptide was subjected to analytical HPLC as described in (Section 6.2) and shown to be predominately one peak. δc (MeOD₃; 600 MHz) 0.93 (Val CH₃), 0.96 (Val CH₃), 1.41 (Ala CH₃), 2.12 (Val CH(CH₃)₂), 3.74 (CO₂CH₃), 3.83 (Ser⁴ CH₂OH, Ser ⁵ CH₂OH), 3.93 (Ser ⁵ CH₂OH), 3.98 (Ser¹ CH₆OH), 4.04 (Ser ¹ CH₆OH), 4.21 (Val α-CH), 4.41 (Ala α-CH), 4.49 (Ser ⁴ α-CH), 4.53 (Ser ⁵ α-CH), 7.96 (Val NH), 8.05 (Ser ⁴ NH), 8.05 (Ser ⁴ NH), 8.14 (Ser ⁵ NH), 8.71 (Ala NH). Impurity peaks at 1.67 (m), 1.78 (m) and 3.12 (broad).


80. J. F. Pilard, .
83. J. Dowden, *Unpublished Results*.
98. I. Burgess, *Unpublished results*.
13. APPENDIX I - TWO-DIMENSIONAL NMR SPECTRA
Figure 26 TOCSY Spectrum of peptide 183
Figure 27 ROSEY Spectrum of peptide 183
14. APPENDIX II - PUBLICATION

A novel linker for the attachment of alcohols to solid supports

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Abstract

A novel linker for the attachment of alcohols on solid support is described. This linker can be cleaved either enzymatically using penicillin amidase, or by very mild acid hydrolysis using 10% TFA. © 1998 Elsevier Science Ltd. All rights reserved.

Keywords: supported reactions; enzymes; alcohols, carbohydrates.

The recent surge of interest in solid-phase organic synthesis has resulted in the need for new solid-phase methodologies, in particular new linkers for functional groups other than amines and carboxylic acids [1]. Enzyme-cleavable linkers are particularly attractive because cleavage might be achieved under mild, neutral and aqueous conditions. Two reports have demonstrated that enzymes can be used to cleave molecules from solid supports using a phosphodiesterase [2] and an endopeptidase (chymotrypsin) [3]. A major drawback of both methods is that the compound released from the solid support retains part of the recognition site of the enzyme, i.e. a phosphate ester [2] and a peptide with C-terminal phenylalanine residues [3]. Although both 'tags' could in principle subsequently be cleaved with phosphatases or peptidases respectively, this would introduce several additional synthetic steps into the reaction sequences. Here we describe the design and synthesis of a more general linker, which can be cleaved either with penicillin amidase (EC 3.5.1.11), a commercially available and widely used enzyme [4] or by the use of dilute TFA, thereby complementing the existing range of alcohol linkers.

Penicillin amidase is known to catalyse the hydrolysis of a wide range of amines protected as the corresponding phenylacetyl derivatives and has also been used in peptide synthesis for the cleavage of cysteine protecting groups [5]. Thus, in order to incorporate the enzyme recognition site, the linker 1 was designed as shown in Scheme 1, in which -OR

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represents the alcohol group. It was envisaged that cleavage would be initiated by hydrolysis of the phenylacetamide moiety, generating the hemiaminal 2 which should easily fragment in aqueous medium releasing the alcohol ROH.

\[
\text{PhCN} \quad \text{Soaper} \quad \xrightarrow{\text{penicillin amidase}} \quad \xrightarrow{\text{H}_2\text{O}} \quad \text{ROH}
\]

Scheme 1

An activated form of the linker was accessible using methodology developed by Katritzky et al. [6-8]. The benzotriazole derivative 4 was prepared in 67% yield by refluxing aldehyde 3 with benzotriazole and phenylacetamide in a Dean-Stark apparatus (Scheme 2). Although the benzotriazole is a good leaving group, and can be replaced by strong nucleophiles, we have found that the thioethyl group is more convenient for our purposes. The latter can be activated with a thiophilic reagent, such as N-iodosuccinimide, and is then susceptible to displacement by relatively poor nucleophiles such as secondary amines. The thioethyl derivative 5 was easily obtained in excellent yield by reaction of 4 with sodium ethanethiolate followed by reduction of the azide group to afford the amine 5.

Scheme 2

Amine 5 was then coupled to a variety of solid supports in reasonable to excellent yields (Scheme 3). A number of Tentagel® and PEGA® resins were investigated because of their compatibility with aqueous reaction conditions and because they had been reported to be suitable for enzyme-catalysed reactions [9]. Coupling yields were between 75% (TentaGel®-COOH) and 100% (PEGA®-COOH). Polystyrene was also successfully derivatised in quantitative yield by activating the resin as the acylfluoride and should be useful when the

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1Typical procedure for the coupling of the linker 5 to solid supports (for Tentagel and PEGA resins): A mixture of the resin (100 mg; loading 0.2 mmol/g), the linker 5 (50 mg, 0.13 mmol), TBTU (77 mg, 0.24 mmol), HOBt (32 mg, 0.24 mmol) and N,N-diisopropylethylamine (31 mg, 0.24 mmol) in DMF (2 ml) was shaken for 16 h at 25 °C. The resin was washed twice with 10 ml aliquots of THF, DMF:MeOH (1:1), DMF:THF and CHCl₃, and dried.

2Typical procedure for the coupling of the linker 5 to solid supports (for polystyrene resin): Cyanuric fluoride (0.837 g, 6.2 mmol) was added (with caution) to a suspension of carboxypolystyrene resin (1.00 g; loading 1.24 mmol/g), and pyridine (0.220 ml, 2.48 mmol) in dichloromethane (3 ml) and the mixture was agitated on a blood rotator for 16 h at 25 °C. The resin was washed with 10 ml aliquots of THF, DMF, THF and CH₂Cl₂, and dried in a vacuum oven (vapour 1805 cm³). N,N-Diisopropylethyamine (0.432 ml, 2.48 mmol) was added to a suspension of the linker 5 (2.40 g, 6.23 mmol) and the acyl fluoride resin (1.00 g, 1.24 mmol/g) in DMF (5 ml) and was agitated on a blood rotator for 16 h at 25 °C. The resin was washed twice with 10 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF:THF and CH₂Cl₂, and dried.
linker is used for non-aqueous chemistry, since the loading is generally higher (1.24 mmol/g).

![Diagram](image)

**Scheme 3**

(i) 5 (6.5 eq), 2-(1H-1H-2H-3H-tetrahydro-1H-benzo[d]imidazol-1-yl)-1.1.3.3-tetramethyluronium tetrafluoroborate (12 eq), HOBt (12 eq), DIEA (12 eq), DMF, 16h, 25°C; (ii) 2M NaOH; (iii) 2M HCl; (iv) TFA:DCM:H2O 9:10:1; (v) penicillin amidase.

The loading values of the linker on the resin were determined by hydrolytic cleavage of the phenylacetamide group using either strong acid or base and quantitative determination by HPLC of the amount of phenylacetamide or phenylacetic acid released, respectively. These loading values were then used to determine the efficiency of cleavage of the linker using penicillin amidase. It was found that penicillin amidase was indeed able to effect cleavage, although the yield of enzyme cleavage was strongly dependent on the resin used, ranging from 20% for PEGA to a maximum yield of 50% for Tentagel. We are currently attempting to improve these yields by varying the spacer arm of the linker and also by using tailor-made resins that are more compatible with enzyme catalysed reactions. In view of the susceptibility of the linker to cleavage under mild acid conditions (aqueous TFA), this protocol was used in subsequent experiments involving attachment of a range of alcohols.

The thioethyl group in 6 was activated by treatment with N-iodosuccinimide followed by displacement with a variety of alcohols 7 to 11 (Scheme 4). Fmoc-protected serine methyl ester was found to couple in excellent yield to the polystyrene-linker and could also be cleaved quantitatively, as judged by HPLC analysis after acid release. Fmoc analysis of the resin bound 7 was in good agreement with the values derived from cleavage reactions. Secondary alcohols 8 and 9 gave good yields of coupling. These could possibly be improved by double-coupling methods, since analysis of phenylacetamide loading revealed that some of the linker had remained intact on the resin.

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1. Chemical cleavage of the linker from solid support and determination of the loading: (for Tentagel and PEGA resins): A suspension of the resin (2 mg) in 2M HCl (300 μl) or 2M NaOH (300 μl) was shaken for 16h at 25 °C. An aliquot of the supernatant solution was neutralised and analysed by HPLC by comparison with standard solutions.

2. Enzymatic cleavage of the linker from solid support: A solution of penicillin amidase (700 units) in 0.1M potassium phosphate buffer (pH 7.5; 0.4 ml) was added to the resin (2 mg) and the mixture was shaken for 16h at 25 °C. The solution was removed, treated with 2M HCl (0.5 ml) and extracted with CH₂Cl₂ (2 x 5 ml). The combined organic layers were concentrated, the remaining residue was dissolved in 300 μl methanol and analysed by HPLC.

3. Typical procedure for the coupling of alcohols to 6: A suspension of the resin bound linker 6 (50 mg, 0.05 mmol) in CH₂Cl₂:TFA:H₂O (10:9:1 v/v, 4ml) was agitated for 3h. An aliquot (300 μl) was removed, concentrated under reduced pressure, dissolved into the same volume of MeOH and analysed by HPLC.

4. In the case of alcohol 8, 10 and 11 4μmol of triflic acid was added and the suspension agitated for a further 16h. The resin was washed with twice with 3 ml aliquots of THF, DMF, DMF:MeOH (1:1), DMF, THF, CH₂Cl₂ and was separated from molecular sieves by decantation.
Since we were particularly interested in applying the linker to solid phase carbohydrate synthesis, the protected glucosides 10 and 11 were coupled to linker 6. Yields of coupling were markedly improved to 75% and 30% respectively, by adding catalytic amounts of triflic acid (0.125 eq) to the NIS solution.

Scheme 4

In summary, we have developed an efficient synthesis of the linker 6 and have shown that a variety of alcohols can be coupled under mild conditions. The linker can be cleaved by penicillin amidase, although yields are currently restricted to 50%, or alternatively by quantitative mild acid cleavage. Both attachment to, and cleavage from, the linker can be achieved under much milder conditions than methodology based on dihydropryran-functionalised resins [11]. It should therefore be particularly useful for more acid labile compounds, such as carbohydrates and acid-labile protecting groups.

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References and Notes