UNIVERSITY OF SOUTHAMPTON

COMBINATORIAL CATALYSIS: THE DEVELOPMENT OF NEW SCREENS AND CATALYSTS FROM SPLIT AND MIX LIBRARIES.

by

Iain Lingard

Doctor of Philosophy

Department of Chemistry
Faculty of Science

September 2002

UNIVERSITY OF SOUTHAMPTON

ABSTRACT

FACULTY OF SCIENCE

DEPARTMENT OF CHEMISTRY

Doctor of Philosophy

COMBINATORIAL CATALYSIS. THE DEVELOPMENT OF NEW SCREENS AND CATALYSTS FROM SPLIT AND MIX LIBRARIES.

by Iain Lingard

A new method is described for the detection of active catalysts from libraries generated by combinatorial synthesis. The technique was reliant on bifunctional substitution of a resin with a common substrate and a library of potential catalysts. Site proximity effects allowed the interaction between these two species such that catalysis could occur. The substrate was chosen such that under reaction conditions, catalytic activity brought about a visual change, and thus allowed the identification of beads which contained active catalysts. Selection of positive beads, followed by identification of the supported library compound led to the discovery of novel catalysts.

Using this technique a library of 1000 potential tripeptide catalysts were screened for Diels Alder catalytic activity. A strong sequence correlation was found in the active catalysts which led to the synthesis of six arginine based catalysts which were found to give up to four fold rate accelerations.

A new mass spectrometry based method of peptide sequencing, suitable for single bead applications has been developed, based on the software extraction of isotopically labelled fragments.

A new mild acid labile amine protecting group is described.

Table of Contents.

Acknowledgements

I would like to express my gratitude to Mark Bradley, for his guidance, optimism and encouragement throughout this research. I couldn't have asked for a better supervisor. Thanks also to Jose Pastor, without whose hard work and dedication, the peptide sequencing method could not have been developed.

I was generously supported throughout my studies by Novartis Pharma. My thanks go to them and to Gurdip Bhalay.

All the members of the Bradley group, past and present, have made my time working here so enjoyable. There cannot be many research groups with such a friendly atmosphere and supportive environment, I will miss you all! Sean, Dave and Sonia, you were here with me from the start, I enjoyed learning with you all, and good luck in your new jobs! Katie, thanks for being both an excellent lab and housemate, you are always a ray of sunshine! Jeff, thanks for many delicious meals, and for organising such excellent holidays. Karin, James, Mizio and Sylvain, I'll remember the fantastic skating, skiing and sightseeing trips. Most of all, thank you Cristina, for all your kindness, support and encouragement, and the happiness you have given me, you have helped me more than you can possibly realise!

And of course, I wish to thank my family. Mum and Dad, thanks for your love and encouragement, I wouldn't have got here without you.

Abbreviations.

Ac	Acetyl
ϵAhx	6-Aminohexanoic acid
Aloc	Allyloxycarbonyl
Boc	tert-Butoxycarbonyl
br	broad (spectral peak)
Bz	Benzoyl
Cbz	Carboxybenzyl
d	doublet (NMR assignment)
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCM	Dichloromethane
Dde	1-(4,4-Dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl
DIC	*N,N'*-Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DVB	1,4-Divinylbenzene
EI	Electron impact (mass spectrometry)
ES	Electrospray (mass spectrometry)
EtOAc	Ethyl acetate
Fmoc	9-Fluorenylmethoxycarbonyl
Hdoc	*E,E*-2,4-Hexadienyloxycarbonyl
HOBt	1-Hydroxybenzotriazole
HMDS	1,1,1,3,3,3-Hexamethyldisilazane
Hsl	Homoserine lactone
LC-MS	Liquid chromatography - mass spectrometry
m	mutiplet (NMR assignment)
m/z	mass/charge ratio (mass spectrometry)
MALDI-TOF	Matrix assisted laser desorption/ionisation – time of flight mass

MAS-NMR	Magic angle spinning – nuclear magnetic resonance (spectroscopy)
MeCN	Acetonitrile
MeOH	Methanol
Mp	Melting point
MS	Mass spectrometry
NMM	*N*-Methylmorpholine
NMR	Nuclear magnetic resonance
Pbf	2,2,4,6,7-Pentamethyldihydrobenzofuran-5-sulfonyl
PEG	Polyethylene glycol
PS	Polystyrene
PyBOP	Benzotriazole-1-yloxytrispyrrolidinophosphonium hexafluorophosphate
Pyr	Pyridine
q	quartet (NMR assignment)
RT	Retention Time
RNA	Ribonucleic acid
RP-HPLC	Reverse phase – high pressure liquid chromatography
s	singlet (NMR assignment)
Su	Succinimide
t	triplet (NMR assignment)
TBAF	Tetrabutylammonium fluoride
Teoc	2-(Trimethylsilyl)ethoxycarbonyl
TFA	Trifluoroacetic acid
TG	TentaGel
THF	Tetrahydrofuran
TIPS	Triisopropylsilyl
TLC	Thin layer chromatography
Trt	Trityl (triphenylmethyl)
Ts	Tosyl
UV	Ultraviolet

Naturally occurring amino acids are denoted by their standard three letter codes.

Chapter 1. Introduction.

1.1. Combinatorial discovery of catalysts.

Since its introduction, combinatorial chemistry has been extensively applied to the discovery of new pharmaceutical agents, but it is only recently that the same techniques have been used for the discovery and development of new catalysts.[1,2,3,4] The drive for high throughput screening has empowered the pharmaceutical industry with the necessary techniques and equipment to identify biologically active compounds from huge numbers of potential structures. However, related techniques for use in catalyst discovery have not, until recently, advanced to the same level of sophistication.

Much of the research done in this field has involved the development of heterogeneous inorganic catalysts.[5,6] The research described in this thesis relates to the development of organic homogeneous catalysts and immobilised homogeneous catalysts and as such, heterogeneous catalysis will not be discussed in this introduction.

In the development of organic and organometallic homogeneous catalysts,[ψ] three approaches have been used, which are depicted in **Figure 1**.[7] Traditional organic synthesis is slow, but allows very accurate screening of compounds for catalytic activity as the exact structure and purity of the compound tested is known. Split and mix synthesis allows the very rapid generation of large numbers of compounds, but suffers from the difficulties in identifying active compounds from the screening of the library. Between the two extremes, parallel synthesis offers an acceleration in synthesis throughput, whilst still allowing the accurate identification of active catalysts.

[ψ] For the purposes of this thesis, and the research described, heterogenised catalysts, i.e. homogeneous catalysts which have been attached to a solid support making them insoluble in the reaction media, will be considered to be homogeneous in nature.

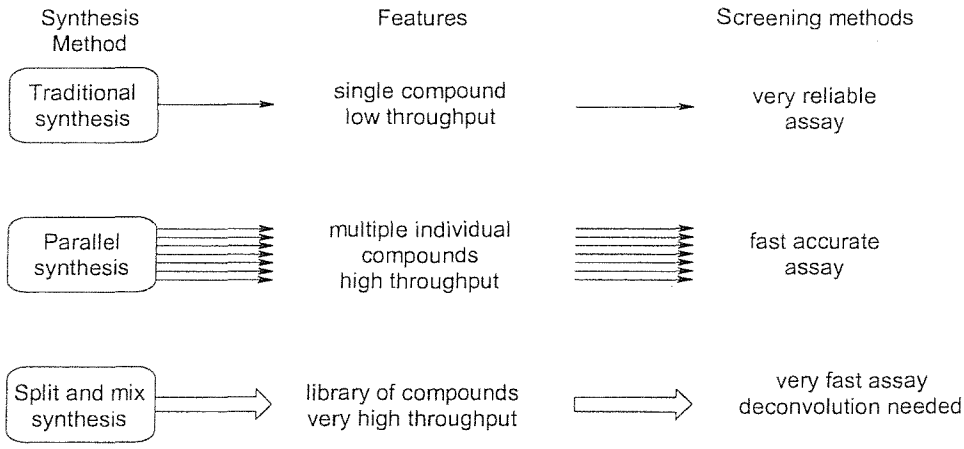


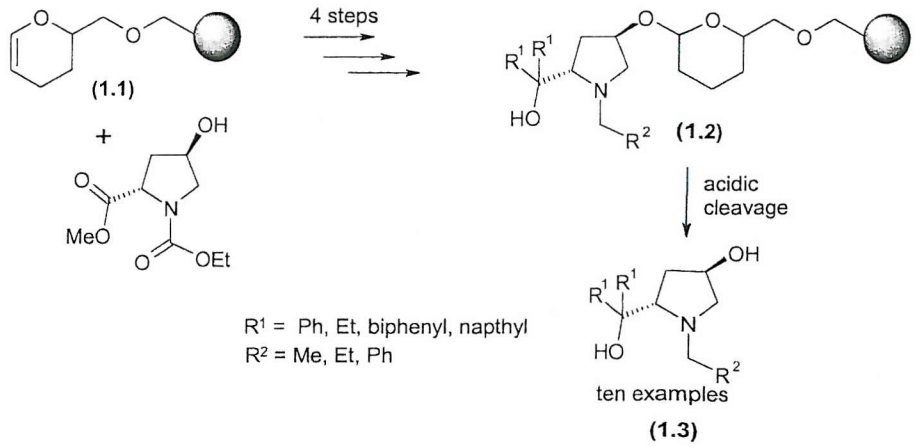
Figure 1. Three approaches to catalyst discovery.

When using combinatorial chemistry for the discovery of catalysts, the screening methodology is of great importance. In this introduction, a distinction will be made between examples of research where single compounds were screened one by one from a set synthesised by parallel methods, and examples where libraries are screened *en masse*.

1.2. Catalyst discovery by parallel synthesis and screening.

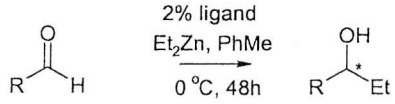
Asymmetric aldehyde alkylation.

In 1995, Ellman *et al.* reported the synthesis of a small library of substituted 2-pyrrolidinemethanol ligands on the solid phase **(1.2)**.[8] The library was synthesised on polystyrene substituted with the tetrahydropyranyl (THP) linker[9] **(1.1)** in four steps **(Scheme 1)**.



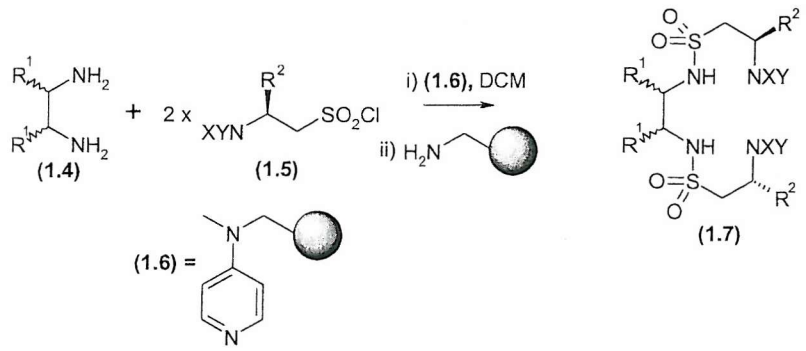
Scheme 1. Synthesis of 2-pyrrolidinemethanol ligands.

The compounds were then individually examined as ligands for the enantioselective addition of diethylzinc to aldehydes, both on the solid phase **(1.2)** and once cleaved from the resin **(1.3)** **(Scheme 2)**. The ligands were found to be active both in solution and on the solid phase, the solution phase ligands giving the best enantiomeric excesses. The most efficient ligand gave 100% conversion, with 93% ee, determined by chiral HPLC.



Scheme 2. Addition of diethylzinc to aldehydes.

Alternative ligands for this reaction have been synthesised in a parallel manner by Gennari and co-workers using solid supported reagents.[10] C_2 symmetric diamines **(1.4)** were reacted with an excess of sulfonyl chlorides **(1.5)** (reaction promoted by the addition of polymer supported DMAP **(1.6)**). Unreacted **(1.5)** was removed by the addition of polymer supported amine to give ligands **(1.7)** **(Scheme 3)**.

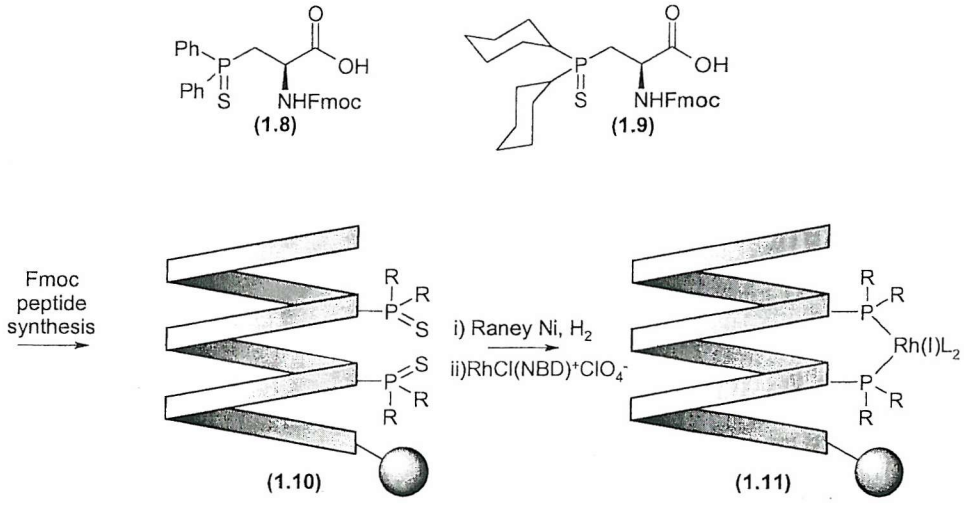


Scheme 3. Synthesis of C_2 symmetric ligands.

The compounds were investigated as ligands for the Ti(O*i*Pr)$_4$ catalysed insertion of diethylzinc onto a variety of aldehydes. Multiple substrates were investigated in one pot for each ligand, increasing the efficiency of the screening process in a method first introduced by Kagan.[11] Each product was separated by chiral HPLC analysis, allowing the stereoselectivity of each ligand for various substrates to be determined at the same time.

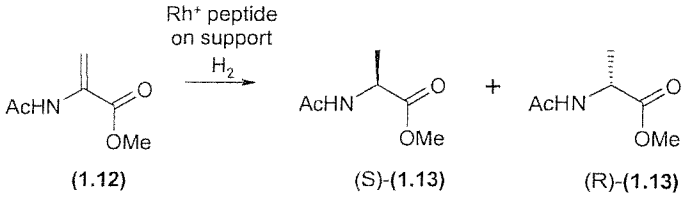
Phosphine containing peptides for catalytic hydrogenation.

Gilbertson and co-workers synthesised phosphine sulphide containing amino acids **(1.8)** and **(1.9),** and then incorporated these into a peptide which was designed to fold into an α-helix, with two phosphine groups on the same side **(1.10).**[12,13] The phosphine sulfides were then reduced, and rhodium was coordinated to the peptide **(1.11) (Scheme 4).**



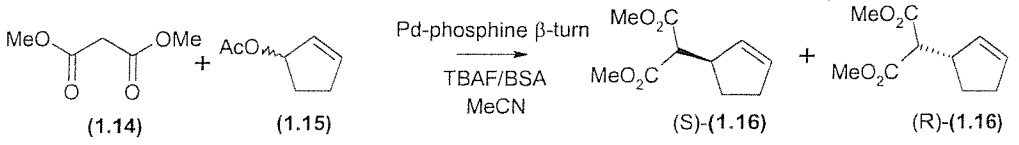
Scheme 4. Rhodium coordinating phosphine containing peptide α-helixes.

A library of 63 similar peptides, all containing two phosphine residues and coordinating rhodium was synthesised in parallel on polyethylene pins grafted with aminomethyl polystyrene[14] and examined for the ability to catalyse the asymmetric hydrogenation of dehydroalanine **(1.12)** to alanine **(1.13)** **(Scheme 5)**. The products for the reaction with each ligand were analysed by chiral GC and several of the peptides were found to catalyse enantioselective reduction (<20% ee).



Scheme 5. Rhodium catalysed hydrogenation.

More recently, the same group have used peptidic β-turns containing the phosphine residue **(1.8)** to coordinate palladium. A 96 member library of these compounds was then screened for enantioselective catalysis of the addition of dimethyl malonate **(1.14)** to cyclopentenyl acetate **(1.15)**, resulting in high enantiomeric excesses (up to 80% ee) of the product **(1.16)** **(Scheme 6)**.[15]

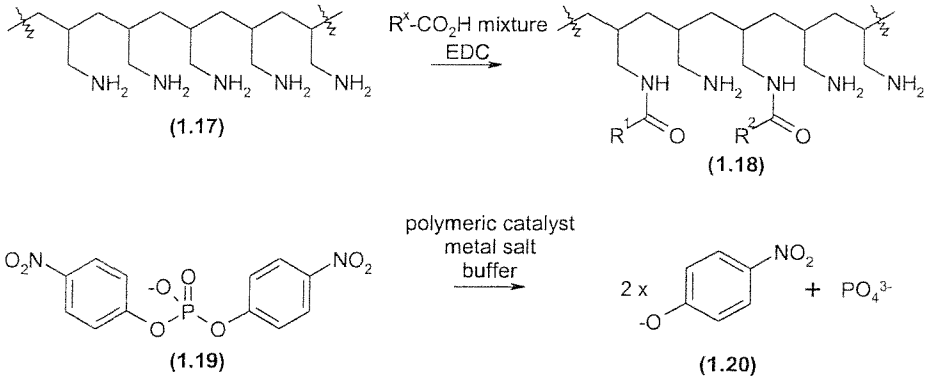


Scheme 6. Palladium catalysed enantioselective substitution.

Randomly generated polymeric catalysts.

In 1995 Menger and co-workers generated hundreds of samples of 'randomly' substituted polyallylamines by reaction of portions of polyallylamine **(1.17)** with known mixtures of functionalised carboxylic acids and a coupling reagent. Each portion therefore contained a mixture of compounds, with only the relative ratio of the substitutions known in each case **(1.18)**. For each substituted polymer generated, three different carboxylic acids were used from eight. To each compound mixture was added a metal salt, and then the complexes

were screened in parallel for phosphatase activity by monitoring at 400 nm the rate of cleavage of bis-4-nitrophenylphosphate **(1.19)** to give the chromophoric 4-nitrophenolate **(1.20)** **(Scheme 7)**.[16] Rate accelerations of up to 3000 fold relative to the uncatalysed reaction were observed.

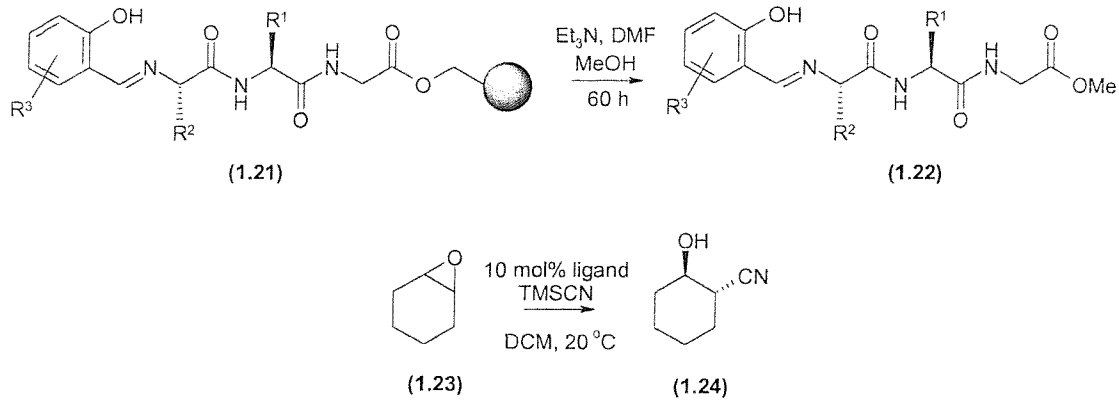


Scheme 7. Generation of polymeric catalysts for phosphate hydrolysis.

Similar methodologies were used by the same group for the discovery of reduction[17] and dehydration[18] catalysts.

Enantioselective addition of TMSCN to *meso* epoxides.

The addition of trimethylsilylcyanide (TMSCN) to epoxides to yield β-cyanohydrins is catalysed by titanium Schiff base complexes.[19] Snapper, Hoveyda and co-workers have developed an asymmetric version of the reaction using a dipeptide modified Schiff base as a ligand. Ligands of general structure **(1.21)** were synthesised via solid phase Fmoc peptide chemistry and capping of the *N*-terminus with substituted 2-hydroxybenzaldehydes. The ligands were cleaved from the resin **(1.22)** and their ability to catalyse the addition of TMSCN to cyclohexene oxide **(1.23)** in the presence of Ti(O*i*Pr)$_4$ to give cyanohydrin **(1.24)** with stereoselectivity was investigated **(Scheme 8)**. The ee for each ligand was determined by chiral GC analysis of the product from each reaction mixture.[20]



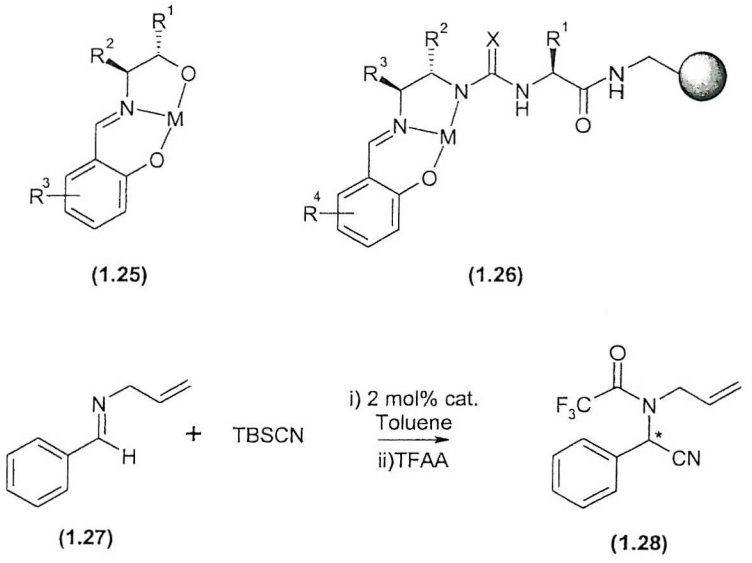
Scheme 8. Dipeptidic Schiff bases as ligands for the addition of TMSCN to *meso* epoxides.

2080 possible structures existed in the structure space designed by the selection of amino acids and benzaldehydes, but not all were synthesised. The identity of the most active compounds was determined by a positional scanning approach,[21] whereby one of the building blocks (amino acid 1 or 2, benzaldehyde) was modified on its own (R^1 first) to find the best functionality in that position. The next building block was then modified, and so on, and the most active ligand defined as that with the combination of the best individual building blocks. The optimised ligand was found to catalyse the reaction with as ee of 86%.

In a later paper, the group undertook a similar set of experiments with different epoxides, and screened the ligands on the solid phase.[22]

Catalysts for the asymmetric Strecker reaction.

The asymmetric hydrocyanation of imines (Strecker reaction) offers one of the most direct routes to chiral α-amino acids.[23] Jacobsen and Sigman have used parallel synthesis to investigate ligands based on known catalyst structures, a tridentate Schiff base complex **(1.25)**. Catalysts of general structure **(1.26)** were evaluated as catalysts for the addition of TMSCN to *N*-allyl-benzaldimine **(1.27)**. The yield and ee of the product **(1.28)** were analysed by chiral GC **(Scheme 9)**.[24]



Scheme 9. Catalysts for the asymmetric Strecker reaction.

The ligands were optimised using an iterative positional scanning approach, and a ligand was identified which catalysed the addition of HCN to imines with greater than 90% ee, even in the absence of any transition metal.

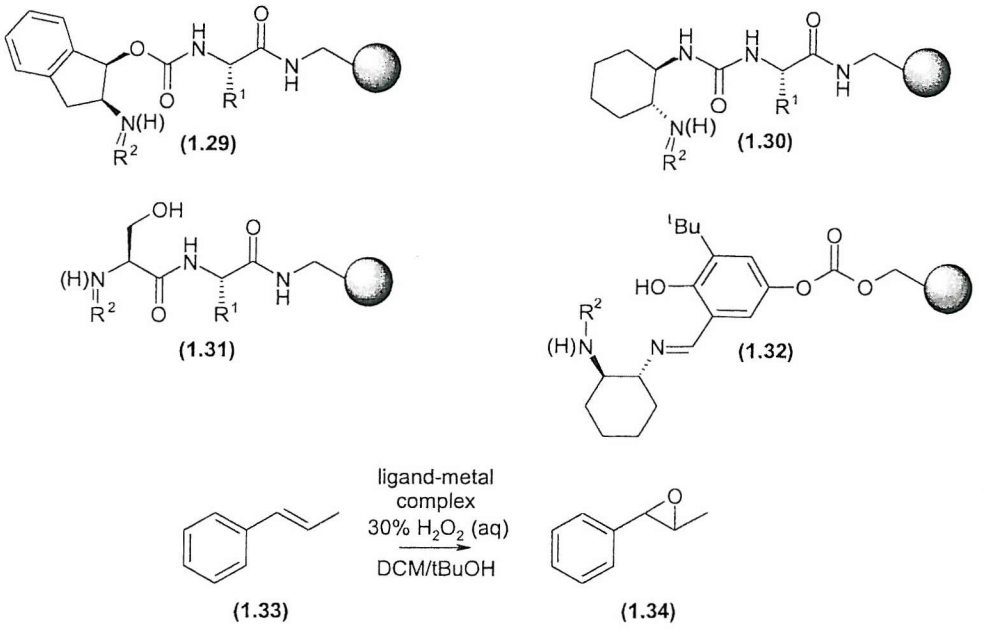
A later paper described further optimisation of catalysts for this reaction, and identified a catalyst which allowed the synthesis of an unnatural amino acid via the Strecker reaction in greater than 99% ee and 84% overall yield after recrystallisation.[25]

Snapper, Hoveyda and co-workers have also investigated this reaction, using the same ligand family described earlier for the addition of TMSCN to epoxides **(Scheme 8)**. The titanium catalysed addition of TMSCN to α,β-unsaturated imines led to the discovery of catalysts giving greater than 99 % ee.[26]

Alkene epoxidation catalysts.

Jacobsen and Francis investigated transition metal complexes of libraries of ligands with structures **(1.29) – (1.32)** for activity as alkene epoxidation catalysts.[27] 192 compounds were synthesised in parallel, and pools of all the catalysts were complexed with 30 different transition metal sources. These mixtures were then used to catalyse the oxidation by H_2O_2 of *E*-β-methylstyrene **(1.33)** to the epoxides **(1.34) (Scheme 10)**. Following the identification of $FeCl_2$ as the best metal source, using chiral GC, Fe(II) complexes of the

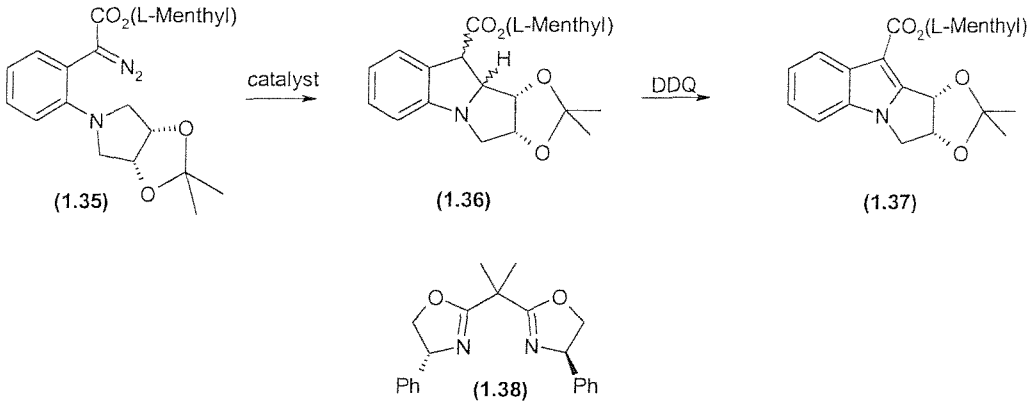
192 individual ligands, and a further 96 optimised ligands were evaluated and three complexes with poor enantioselectivity (ee = 20%) were identified.



Scheme 10. Ligands for alkene epoxidation.

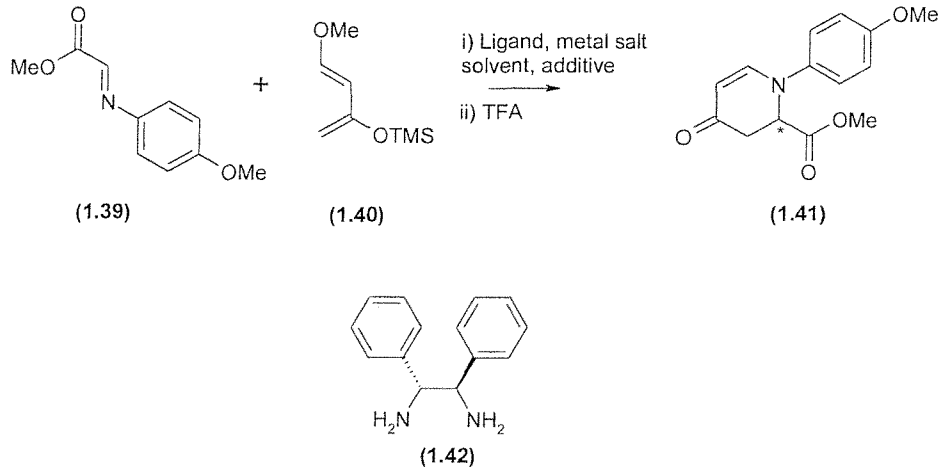
Parallel investigation of catalytic systems with known ligands.

An alternative use of parallel combinatorial techniques is the identification of more efficient catalytic systems from mixtures of known ligands, metals and solvents. One example of such an approach is the optimisation of catalyst systems for the diastereoselective insertion of carbenes generated by the decomposition of diazocarbonyl compounds described by Burgess *et al.*.[28] Four ligands in conjunction with seven metal sources and four solvents were used to generate 96 different combinations, and these were then tested for their ability to catalyse the decomposition of **(1.35)** to give the tricyclic compound **(1.36)** which was oxidised to **(1.37)** **(Scheme 11)**. HPLC was used to determine the diastereomeric excess (de) of compound **(1.37)** formed using each mixture. Ligand **(1.38)** in combination with $AgSbF_5$ in THF was found to give the best results (yield 75%, 56% de).



Scheme 11. Catalytic systems for a carbene insertion reaction.

Another example of this use of parallel techniques to evaluate catalyst systems was reported by Whiting *et al.*, who examined homochiral Lewis acids as asymmetric catalysts for the *aza*-Diels Alder reaction.[29,30] Three C_2-symmetric ligands, four metal salts, three solvents and two additives were used to generate a library of 144 different catalytic mixtures. The reaction investigated was between *N*-arylimine **(1.39)** with Danishefsky's diene **(1.40)**, followed by hydrolysis to give compound **(1.41)** **(Scheme 12)**. The results were obtained by chiral HPLC, and the most efficient system was identified as a combination of (*R,R*)-1,2-diphenylethylenediamine **(1.42)** and MgI_2 in acetonitrile with 2,6-lutidine as an additive, giving an ee of 97%.



Scheme 12. Asymmetric Lewis acid catalysed *aza* Diels Alder reaction.

In addition to the examples presented here, many groups have reported the use of high throughput analysis technologies such as capillary array electrophoresis,[31] electrospray mass spectrometry,[32] and CD-HPLC.[33] See reference 4 for a review of the use of these techniques in catalyst discovery.

1.3. Catalyst discovery by direct library screening.

Split and Mix Synthesis.

The most powerful method of generating large numbers of compounds is the split and mix synthesis technique first developed by Furka *et al.*,[34] and Lam *et al.*.[35] As shown in **Figure 2**, solid support resin is split, for example, into three equal portions, and each is reacted in a separate vessel with a different molecule of type A. The resulting product resins are the mixed together, and divided again into three portions, each now containing some resin substituted with A^1, some with A^2, and some with A^3. The coupling of three molecules of type B, now results in nine total compounds. Since each bead of resin could only have been in one pot at one time, each bead only contains one compound. Mixing and splitting of the resin again, followed by coupling with compounds of type C results in 27 compounds. Therefore 27 compounds (3^3) have been synthesised in only 9 synthetic steps (3 x 3). This collection of compounds is often referred to as a library. A general rule applies, that the total number of compounds formed (N) is equal to the number of building blocks used in each reaction step (n), raised to the power of the number of split and mix rounds (m). The number of reaction steps required (R), however, is equal to the number of building blocks used, multiplied by the number of rounds.

$$N = n^m \qquad\qquad R = nm$$

If we consider the split and mix synthesis of a library of peptides each with five residues, using all 20 naturally occurring amino acids, The number of compounds formed is 3.2 million, requiring 100 synthetic steps.

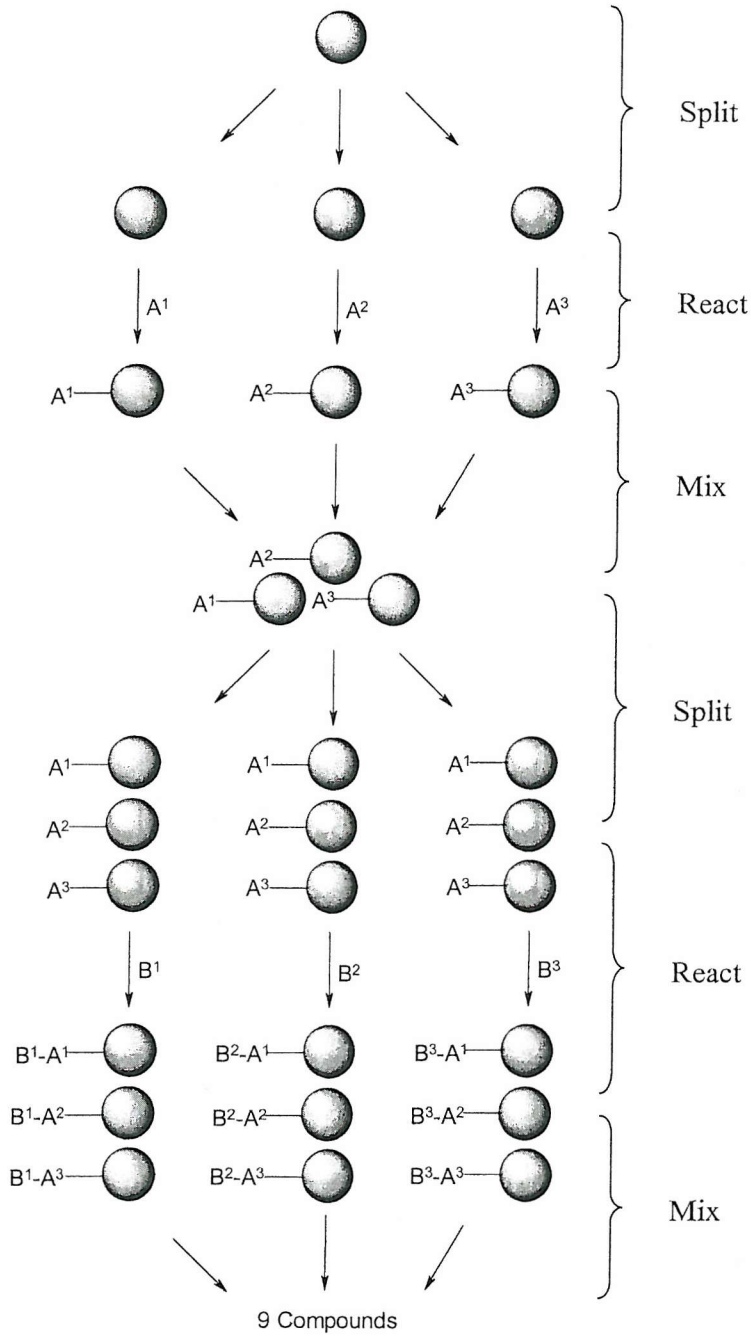
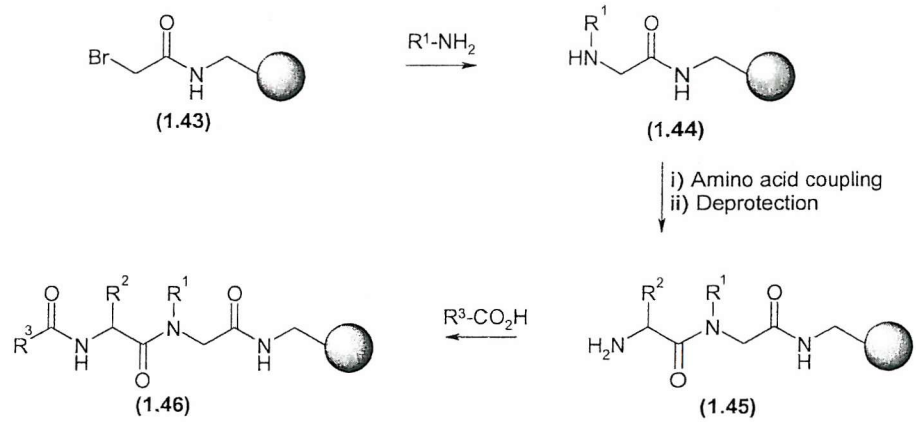


Figure 2. Split and mix synthesis method.

In the discovery of catalysts from split and mix libraries, the method used to identify active catalysts is often more complex than the synthesis of the library. Therefore, the examples presented in this field will be grouped by the catalysis detection method, rather than the catalysed reaction.

Infra-Red thermographic imaging.

Recent advances in photovoltaic infra red imaging, using cameras equipped with focal plane array detectors, have allowed the development of an ingenious method for the detection of active catalysts by thermographic imaging. Originally reported by Moates *et al.* for the investigation of ignition temperatures of a small library of metal doped aluminas,[36] the technique was used by Morken and Taylor as a technique for the detection of catalysis from a split and mix library.[37] A library of acyl transfer catalysts were synthesised by split and mix synthesis. Bromoacetyl resin **(1.43)** was substituted with 15 amines to give resin **(1.44)** which was then coupled with 14 amino acids (and a skip codon)[ψ] and deprotected to give resin **(1.45)**. A collection of 14 carboxylic acids (and a skip codon) were then coupled to the *N*-terminus to give a final library of compounds with general structure **(1.46)** **(Scheme 13)**. The use of 15 monomers in each position gave a theoretical library size of 3150 distinct compounds. The library was encoded with Still's tagging technique.[38]



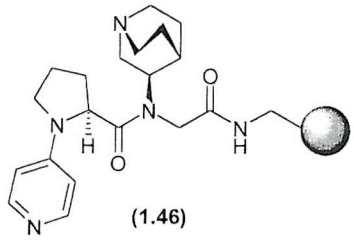
Scheme 13. Synthesis of a trimeric acyl transfer catalyst library.

A portion of the library (~7000 beads) was added to a mixture of ethanol, acetic anhydride and triethylamine in chloroform, and observed through an IR camera. The ability of the compounds to catalyse the acyl transfer reaction between acetic anhydride and ethanol was evaluated by thermal imaging. The reaction between these reagents is exothermic, therefore if a bead is functioning as a catalyst, faster reaction will be taking place and the

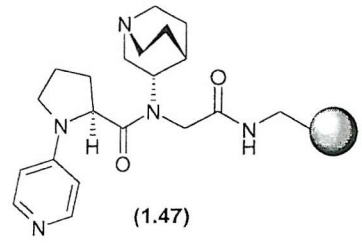
---

[ψ] A skip codon represents the omission of this synthetic step to a portion of the library.

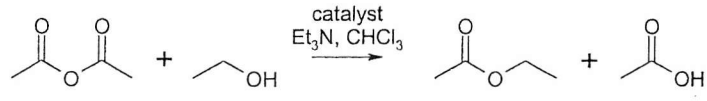
amount of heat produced in the vicinity of that bead will be increased. The observed result was that catalytically active beads appeared as 'hot spots' in the thermal image recorded by the camera. These beads were picked from the reaction medium and the structure of the catalysts supported on them determined from the tags present. Out of 23 beads selected, 21 contained either compound **(1.46)** or **(1.47)**. These were then resynthesised and their activity for the acyl transfer reaction analysed in greater detail. Both compounds were found to be active acyl transfer catalysts, compound **(1.46)** resulting in the highest increase in reaction rate **(Scheme 14)**.



(1.46)

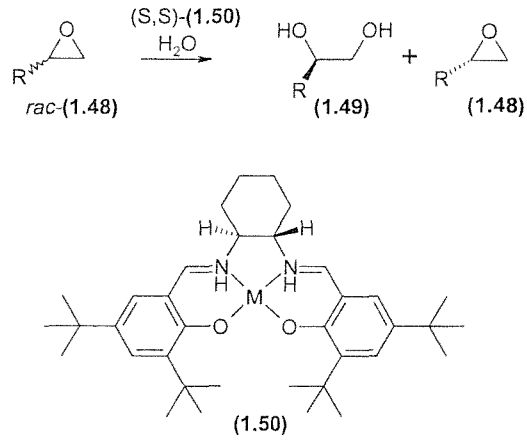


(1.47)



Scheme 14. Active acyl transfer catalysts identified by thermographic screening.

Reetz and co-workers described a method whereby this technology could be adapted to the investigation of catalytic chiral resolution.[39] The catalytic system investigated was the ring-opening hydrolysis of chiral epoxides **(1.48)** to give 1,2-diols **(1.49)**. The use of transition metal containing salen (*N,N'*-bis(salicylidene)ethylenediamine) catalysts **(1.50)** induces kinetic resolution of racemic epoxides by enantioselective ring-opening reactions, originally reported by Jacobsen *et al.* **(Scheme 15)**.[40,41]

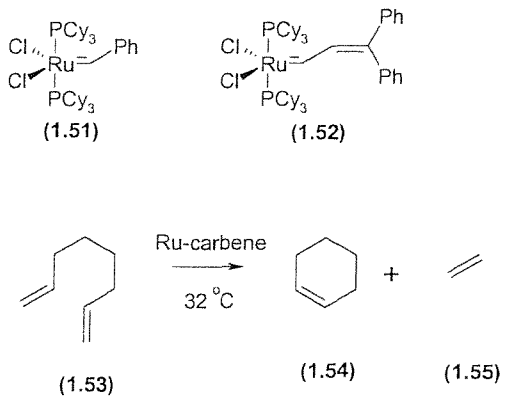


Scheme 15. Kinetic resolution of chiral epoxides.

The stereochemistry of the cyclohexyl ring in **(1.50)**, and the nature of the metal centre have profound effects on the efficiency and stereochemical outcome of the resolution. I.R. thermography was used to rapidly screen catalyst-metal combinations with a variety of chiral epoxides. For each combination three wells of a 96 well plate were used. In one well was placed the catalyst mixture and the racemic epoxide, in the other two wells the two enantiopure stereoisomers of the epoxides were added. The reaction was monitored through an I.R. camera. Due to the relative rates of reaction, different amounts of heat were evolved for each well. The well containing the isomer which was hydrolysed fastest by the ligand-metal combination evolved the most heat and was thus observed to be hottest by I.R. thermography. The well with the other enantiomer was observed to be coolest, and the well containing the racemate appeared to be between the other two. Thus the selectivity could be determined for each catalyst by which well was observed to be hottest, and the difference in temperature between the hottest and coolest well gave a representation of the level of selectivity. After the investigation of several catalyst-substrate mixtures, the results were found to be in line with those previously determined by bulk analysis.[42]

The same group later reported an interesting alternative use of I.R. thermography for catalyst discovery, the monitoring of endothermic reactions.[43] The ring-closing metathesis (RCM) reaction is known to be catalysed by ruthenium carbene complexes, such as the Grubbs precatalysts **(1.51)** and **(1.52)**.[44] It was not known at the start of the investigation whether the reaction was exothermic or endothermic, but I.R thermography determined

the latter to be the case. The ring-closing metathesis of 1,7-octadiene (**1.53**) to cyclohexene (**1.54**) and ethylene (**1.55**) was investigated with four ruthenium carbene catalysts in a 96 well plate, and the reactions monitored using an I.R. camera (**Scheme 16**).



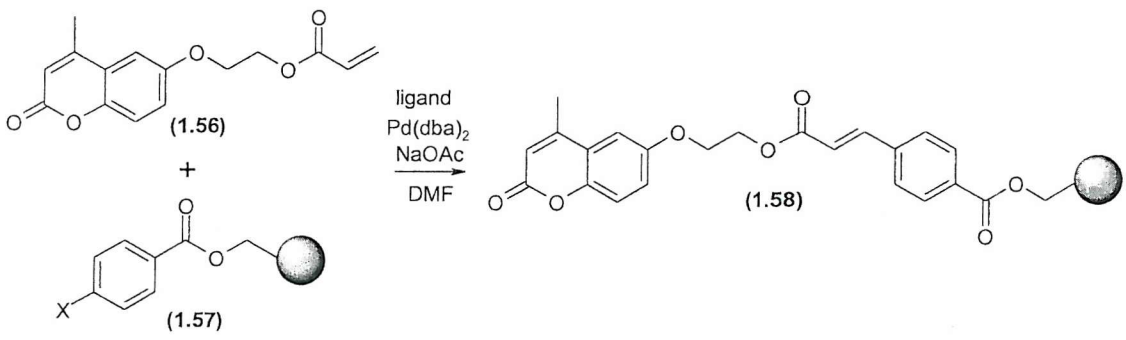
Scheme 16. Ring closing metathesis reaction.

All of the wells were observed to become cooler during the reaction, by comparison with a control well which contained octane in place of the diene. The level of cooling was found to be representative of the efficiency of the catalyst observed, by comparison with batch synthesis of the same reactions. It was not clear, however, how much the cooling was due to the endothermic nature of the reaction, and how much due to the cooling generated by the liberation of ethylene gas from the reaction mixture. The technique however gave a proportional indication of catalyst activity. Reactions with a variety of diene substrates were undertaken, and similar results observed.

An alternative to I.R. thermography was reported by Connolly and Sutherland. The use of a 96 well plate in conjunction with a 96 position array of thermistors allowed a more flexible method of reaction temperature monitoring, without the need to use I.R. transparent materials.[45] The transition metal-salen complex catalysed chiral resolution of epoxides, as described above, was again investigated using this technique, and the protocol was found to be a reliable method for monitoring catalysis.

Fluorogenic Assays.

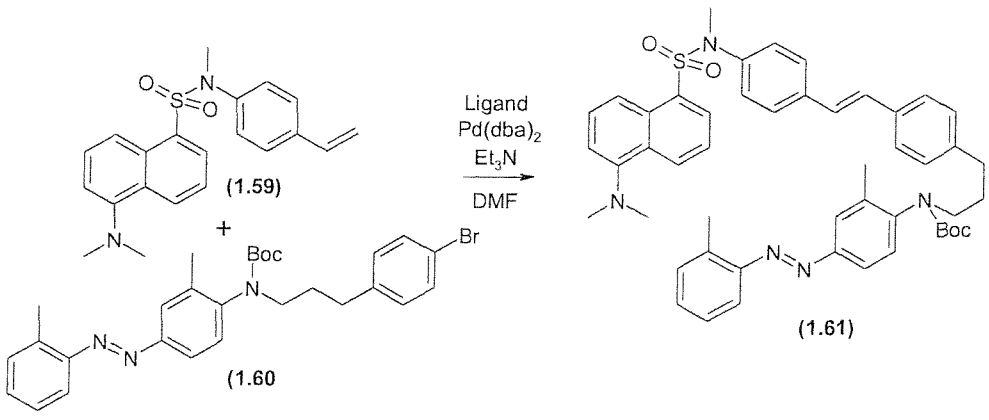
A fluorescent assay for high throughput catalyst analysis was first described by Hartwig *et al.* in 1999. The properties of a wide variety of phosphine ligands were investigated with application to the Heck reaction for activated and unactivated aryl halides.[46] The method of reaction monitoring was by Heck coupling of a fluorescent acrylate **(1.56)** to a solid supported aryl halide **(1.57)** resulting in the fluorescent compound bound to the solid phase **(1.58)** **(Scheme 17)**. Samples of resin **(1.57)** were therefore reacted with the acrylate in the presence of $Pd(dba)_2$ and the ligand for examination. After a fixed reaction time the resin was washed and the efficiency of the reaction judged by the fluorescence observed due to the coupling of the fluorophore to the beads. Comparisons of the observed results with GC based analysis of the same reactions were favourable. 45 ligands were examined using the technique, and different ligands were identified as giving the best activity for the aryl chloride or bromide.



Scheme 17. Heck coupling with a fluorescent acrylate.

The same group later used a fluorescence resonance energy transfer (FRET) approach to the screening of Heck reaction ligands.[47] FRET relies on the fact that when the emission band of a fluorescent molecule overlaps with the absorption band of another, and when the two chromophores are located within close proximity to each other (20-80 Å), the emitted photons from the fluorophore are immediately absorbed by the other chromophore (quencher). Thus when the fluorophore and quencher are bonded together, no fluorescence is observed. Therefore, dansyl styrene **(1.59)**, a molecule which exhibits strong fluorescence was reacted through a palladium catalysed Heck reaction with an arylbromide azo dye **(1.60)**, an efficient fluorescence quencher. The product molecule

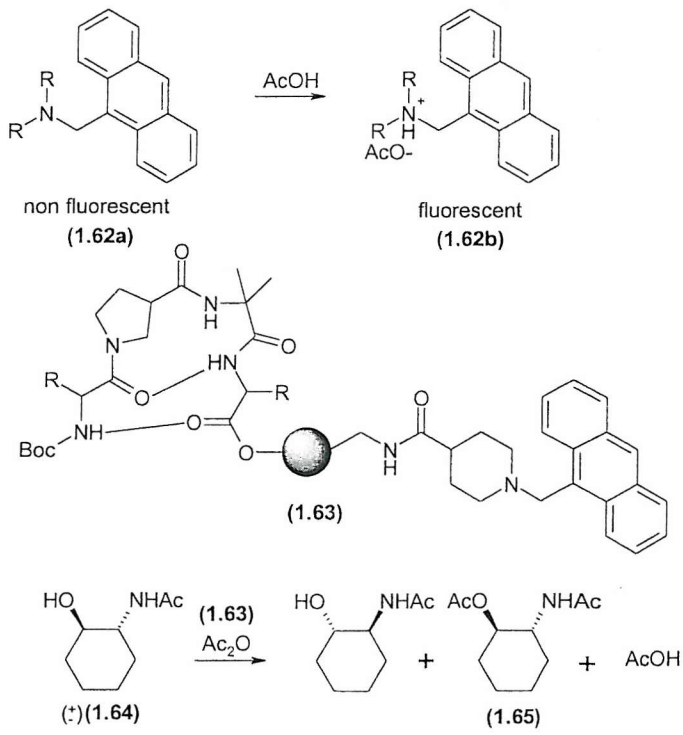
(1.61) exhibits very low fluorescence when compared with the starting material (**Scheme 18**).



Scheme 18. Heck reaction monitoring by FRET.

96 Phosphine ligands were evaluated in a 96 well plate by monitoring the disappearance of fluorescent activity as the reaction progressed. The best catalysts were then re-examined with a variety of different substrates.

Aminomethylanthracenes (**1.62**) are pH sensitive fluorophores which undergo photoinduced electron transfer in the free amine form (**1.62a**), but fluoresce when protonated (**1.62b**).[48] Copeland and Miller proposed the use of such indicators to detect the acid formed during catalytic acyl transfer using an anhydride.[49] Resin was thus partially substituted with an aminomethylanthracene moiety and the remaining resin sites were used to build one of four β-turn peptides (**1.63**) known to catalyse certain acyl transfer kinetic resolutions, such as the selective acylation of racemic *trans*-1,2-acetamidocyclohexanol (**1.64**) with acetic anhydride to give one enantiomer preferentially of the diacylated product (**1.65**) (**Scheme 19**).[50]



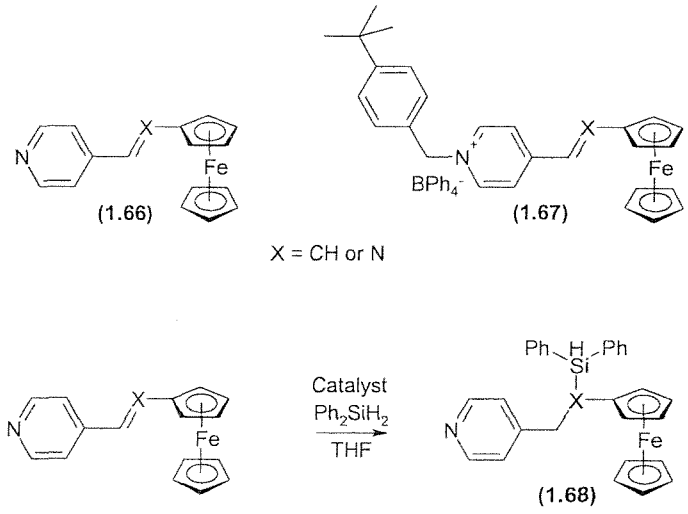
Scheme 19. A fluorescent assay for acyl transfer catalysts.

The four catalyst containing resin samples were mixed with control beads containing the sensor but no catalyst and added to the reaction mixture. The resin beads were easily identified by their pronounced fluorescence as a result of the acetic acid produced as a reaction by-product, whilst the control beads remained dark. The authors suggest that this method may be suitable for the screening of split and mix libraries using either fluorescence microscopy or fluorescence activated bead sorting.

The same sensor system was later incorporated into the matrix of a polymeric gel, and used for the investigation of a split and mix library of peptidic acyl transfer catalysts.[51] The beads were incorporated into the gel during its setting, and on addition of the acyl transfer reagents, active beads showed fluorescence, again as a result of the acetic acid produced. The rate of diffusion of the acetic acid was slow enough so that the fluorescence remained located within the active beads for considerable time. Beads showing high levels of fluorescence were picked and sequenced by the Edman method. The active hits were all found to contain a π-methylhistidine residue at the *N*-terminus, a feature already known to the researchers as being important for catalytic activity.[50]

Visual Assays.

The first example of a colourimetric assay for the combinatorial investigation of catalysis was reported in 1998 by Crabtree and co-workers.[52] Four ferrocenyl dyes of general structure **(1.66)** or **(1.67)** were used to monitor the activity of twelve hydrosilation catalysts. The dyes were dispensed into a 96 well plate, and one of the catalysts added to each solution. The addition of Ph_2SiH_2 caused the hydrosilation of the dyes in wells containing an active catalyst, resulting in loss of conjugation between the ferrocene and pyridine moieties, to compounds of structure **(1.68)**, resulting in disappearance of the dye colour **(Scheme 20)**.

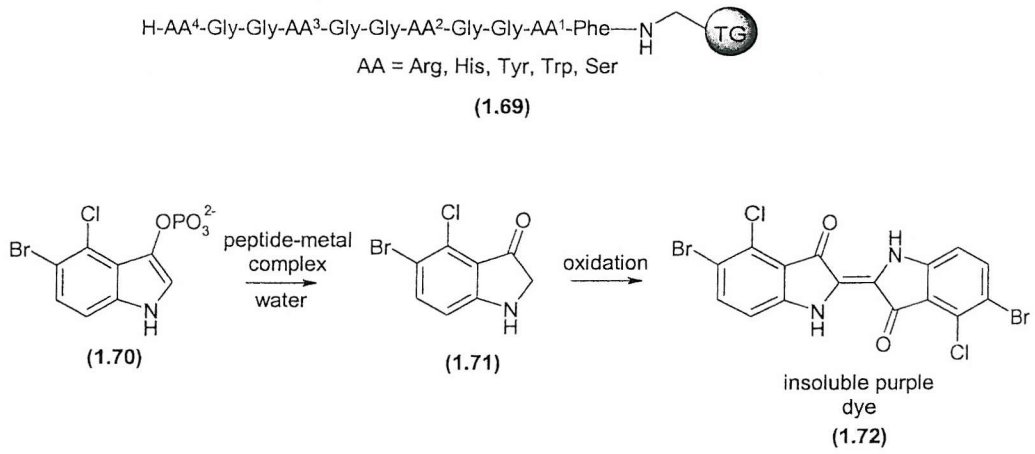


Scheme 20. Hydrosilation of ferrocene dyes.

Wilkinson's catalyst ($RhCl(PPh_3)_3$), which was already known as a hydrosilation catalyst,[53] and $Pd(Ar_2PC_6H_4CH_3)(OAc)_2$, a known catalyst for the Heck reaction,[54] were found to be the most active for this reaction.

The application of colourimetric catalyst screening to a split and mix library of peptide-zirconium complexes for phosphate hydrolysis was reported by Berkessel and Hérault.[55] A library of 625 peptides of general structure **(1.69)** were synthesised on TentaGel using the split and mix methodology. The library beads were then complexed with a variety of metal salts, and added to a solution of hydroxyindolyl phosphate **(1.70)** in buffer. Phosphate cleavage released the indoxyl derivative **(1.71)** which was oxidised in air to the highly

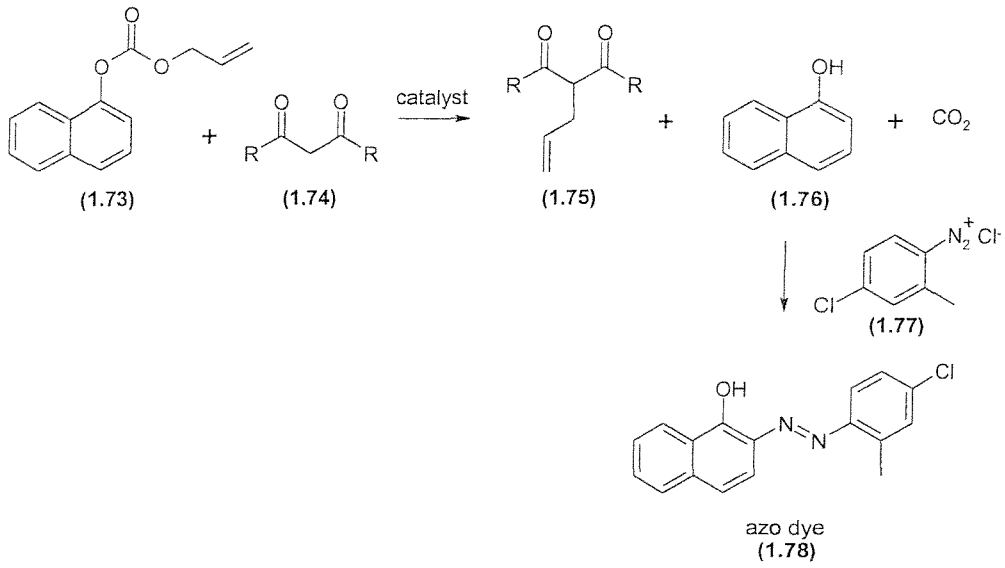
coloured and insoluble indigo dye **(1.72)** **(Scheme 21)**. The dye precipitates inside beads containing an active catalytic species.



Scheme 21. Phosphate hydrolysis catalysts.

Activity was found with the peptides in conjunction with Zr^{4+}, and the beads which exhibited the greatest colour change were selected and the peptide structure determined by Edman sequencing. The peptide H-Ser-Gly-Gly-His-Gly-Gly-Arg-Gly-Gly-His-Phe-OH was identified as the most active species, and from solution phase kinetic analysis was found to give five fold rate acceleration against the uncatalysed reaction.

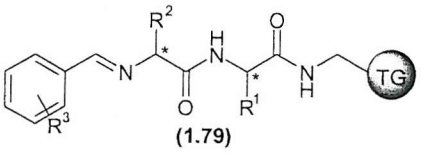
Lavastre and Morken used a colourimetric assay to examine metal-ligand combinations as allylic alkylation catalysts.[56] Catalytic transfer of an allyl group from 1-napthyl allyl carbonate **(1.73)** to a malonate **(1.74)** to give compound **(1.75)** released 1-napthol **(1.76)**. Reaction of **(1.76)** with Fast Red diazonium salt **(1.77)**[57] generated an azo dye **(1.78)** resulting in a highly coloured solution **(Scheme 22)**. 12 transition metals, and 8 ligands were mixed in a 96 well plate, and compounds **(1.73)**, **(1.74)** and **(1.77)** were added. The reaction was monitored by the observed colour change caused by the synthesis of dye **(1.78)**.



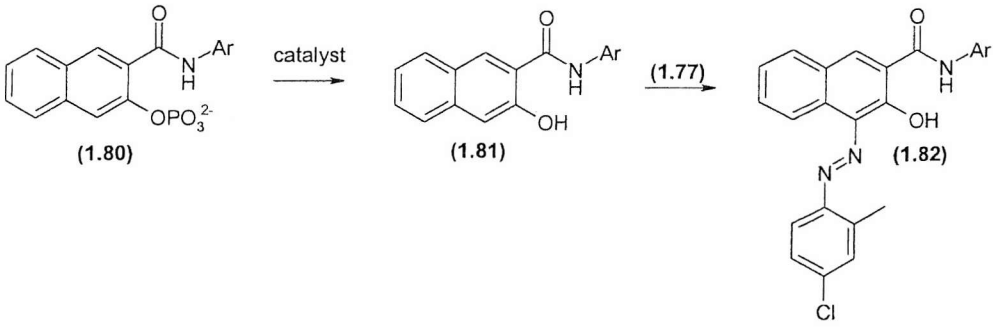
Scheme 22. Colourimetric detection of allyl transfer.

Several ligand-metal combinations led to active catalysis and therefore dye generation, including previously known palladium complexes for this reaction.[58] Two new complexes of iridium however were identified, and subsequent batch screening confirmed their activity.

A similar strategy was recently employed by the same group for the discovery of synthetic phosphatases from a solid supported split and mix library.[59] 3360 Dipeptidic Schiff base ligands of general structure **(1.79)**, incorporating 9 heteroaromatic unnatural amino acids in position 1, 22 natural and unnatural amino acids in position 2, and 46 benzaldehydes in position 3, were generated using split and mix synthesis on TentaGel resin, with Still's tagging strategy.[38] These were complexed with gadolinium (III) and investigated for phosphatase activity with phosphate **(1.80)** as the substrate. The addition of Fast Red **(1.77)** again allowed the detection of catalysis by reaction with the produced napthol **(1.81)** to generate azo dye **(1.82)** **(Scheme 23)**.



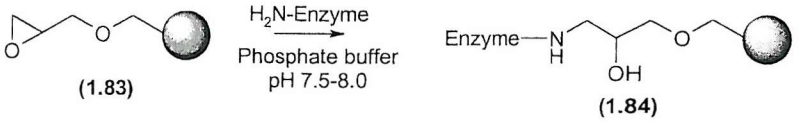
(1.79)



Scheme 23. Colourimetric assay for a synthetic phosphatase.

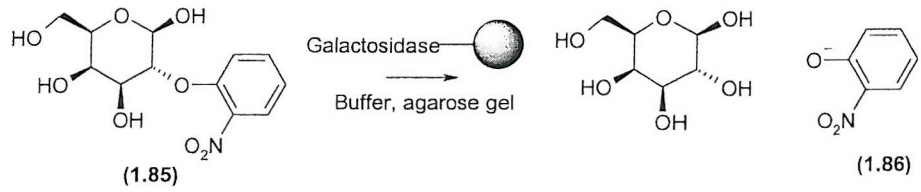
Two compounds were identified from the screen, which on further analysis were found to give 4 and 10 fold rate accelerations compared with the uncatalysed reaction.

One possible problem with some of the colourimetric strategies described above is the diffusion of the coloured species from the active bead, leading to loss of signal. The use of insoluble dyes which precipitate inside the bead is one solution, as described in the work of Berkessel and Hérault above.[55] An alternative strategy has been described by Davis and co-workers, relying on the isolation of the catalyst beads in a semi-solid gel.[60] The use of a low melting agarose gel containing an indicator for catalytic activity prevents the diffusion of the colour from active beads. The potential of the technique for the discovery of catalysts was examined by layering a mixture of enzyme substituted resin beads **(1.84)** (generated by the ring opening of epoxide resin **(1.83) (Scheme 24)**[61]) and control beads onto the surface of an agarose gel containing a colour changing substrate for the catalyst.



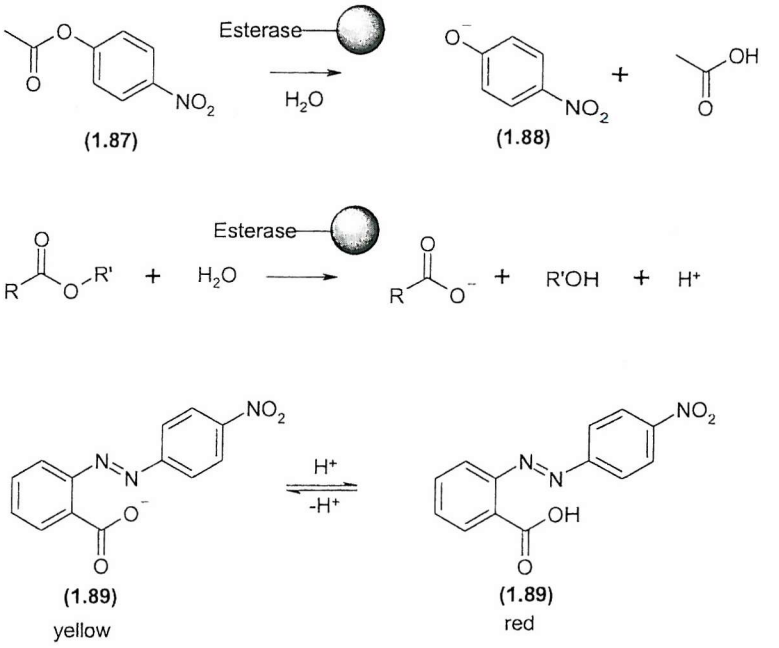
Scheme 24. Immobilisation of enzymes to the solid phase.

Two enzymes were used for the test screens. Firstly, immobilised galactosidase was used in conjunction with an agarose gel containing *o*-nitrophenyl-β-D-galactopyranoside **(1.85)**. Cleavage by the enzyme released the nitrophenolate **(1.86)** which labelled the enzyme containing beads with a yellow colour **(Scheme 25)**. No diffusion of the colour away from the active beads was observed.



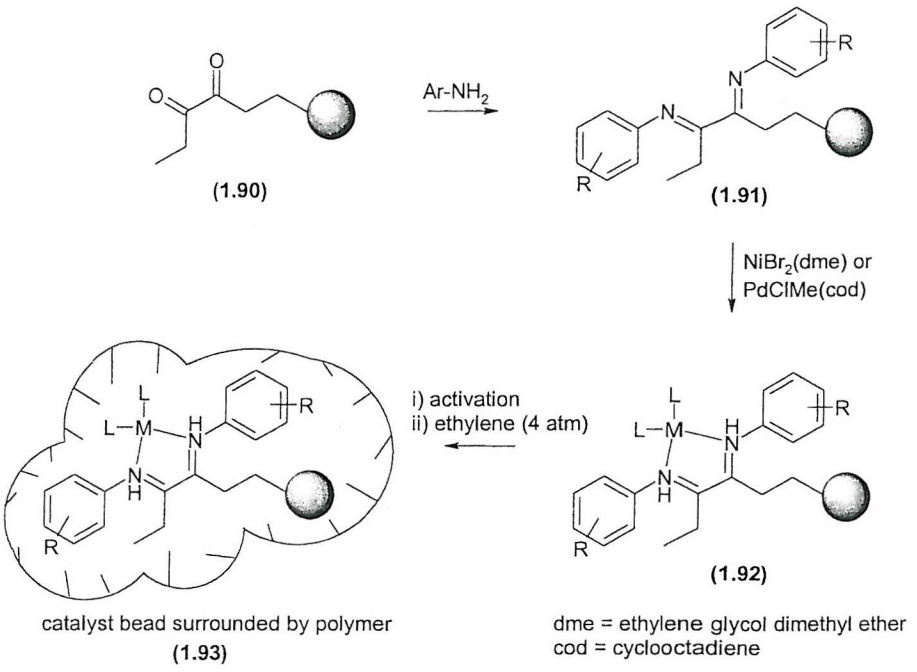
Scheme 25. Enzymatic hydrolysis of a galactopyranose.

In similar experiments, a resin immobilised esterase was used in two screening models. Firstly, the hydrolysis of 4-nitrophenyl acetate **(1.87)**, to yield the yellow nitrophenolate **(1.88)**, and secondly, the detection of a general ester hydrolysis by incorporating a pH indicator **(1.89)** into the agarose, allowing the detection of protons formed during the hydrolysis **(Scheme 26)**.



Scheme 26. Screens for esterase activity.

Powers, Murphy and co-workers at Symyx Technologies have developed an intriguing visual assay for the discovery of olefin polymerisation catalysts.[62,63] 48 Solid supported diimines **(1.91)** were prepared in parallel by the addition of various aromatic amines to diketone resin **(1.90)**, and complexed with $Ni^{(II)}$ or $Pd^{(II)}$ to generate 96 solid supported catalysts of general structure **(1.92)** these were screened for activity as ethylene polymerisation catalysts in a specially designed 96 well reactor block, and the produced polymers analysed by GPC **(Scheme 27)**. An observation made during the experiments was that the beads containing the most active catalysts grew in size due to the build up of polymer on the beads surface **(1.93)**. The researchers realised that this made an excellent opportunity to develop a visual assay. Thus, the catalysts were resynthesised, incorporating secondary amine tags, for analysis using Gallop's dansylated amine tagging strategy,[64] mixed, and screened by reaction with ethylene *en masse*. Five beads which had grown the most in size were selected and the structure obtained by identification of the tags. They were found to be catalysts which had given some of the best results in the first GPC based screen.

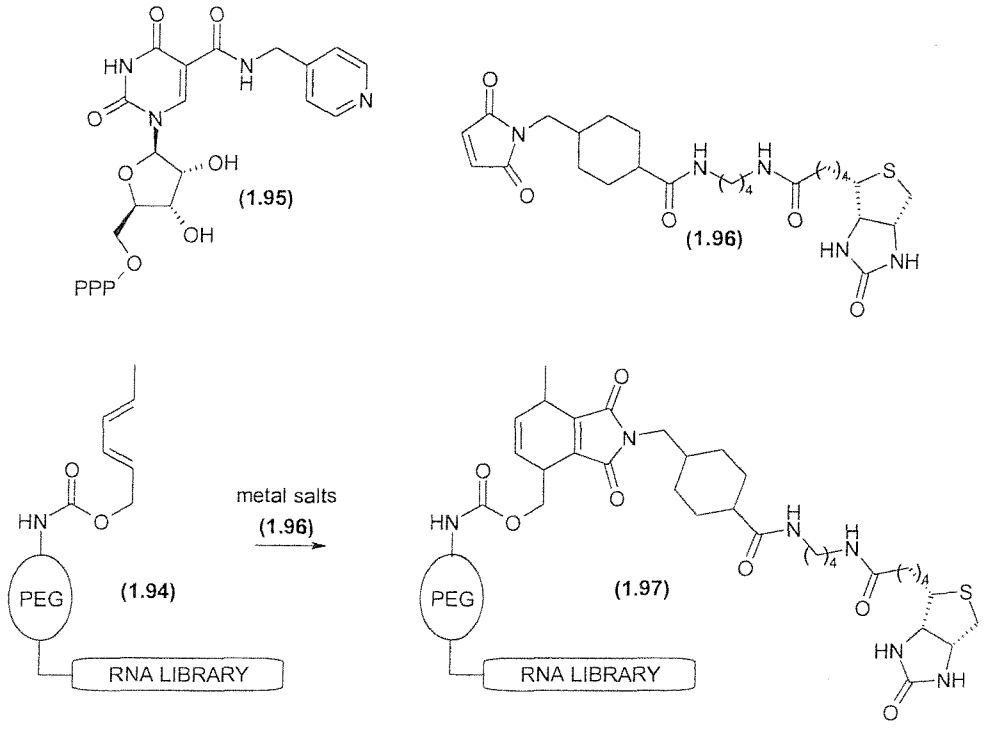


Scheme 27. Ethylene polymerisation catalysts.

Biologically based selection assays.

RNA based catalysts (artificial ribozymes) for the Diels Alder reaction have been the subject of extensive investigations by two groups over the last 5 years. Eaton *et al.*, of NeXstar Pharmaceuticals attached a diene via a PEG spacer to the 5' end of a library of $\sim 10^{14}$ 100 nucleotide RNA molecules **(1.94)**.[65] It was previously reported by Jäschke and Seelig of the University of Berlin that diene modified RNA strands could be labelled with maleimides using the Diels Alder reaction.[66] The library of RNA contained the pyridyl modified uridine nucleotide **(1.95)**, and this moiety aided the binding of the RNA strand to a variety of metal ions. These RNA-metal conjugates were reacted with biotinylated maleimide **(1.96)**, to give the product molecules **(1.97)** **(Scheme 28)**. It was found that certain RNA sequences self-catalysed the reaction, leading to reaction with the maleimide. Streptavidin binding of the biotin moiety was used to separate reacted RNA from the unreacted library members, and these were amplified by PCR and re-entered into the screening process. After 12 rounds of selection, the products were amplified and sequenced. 6 families of related RNAs were found in the selected strands, and one family was found to be present in 59% of the samples, giving a consensus sequence

(UUCUAACGCG). The catalytic activity was found to be copper (II) dependant, and also dependant on the presence of the modified uridine.

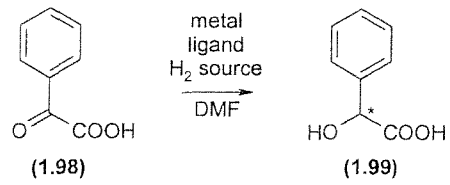


Scheme 28. Streptavidin binding assay for Diels Alder catalyst activity.

Jäschke and Seelig reported the discovery, using similar screening methodologies, of an RNA motif which catalysed the Diels Alder reaction using unmodified nucleosides in the absence of transition metals, and found that the selected RNA oligomer was able to catalyse the reaction of the two substrates when not bound to the RNA.[67]
Both groups have continued to contribute to this field of catalyst development, Eaton *et al.* investigated alternative modified uridine nucleosides,[68] and investigated secondary structures and substrate specificities of the active sequences.[69] Whilst Jäschke *et al.* investigated the stereospecificity and substrate dependence.[70,71]

The use of an immunoassay for the detection of enantioselective catalytic activity has been reported by Wagner *et al.*.[72] A competitive enzyme immunoassay[73] was used to identify ligand-metal combinations leading to high yields and enantioselectivities for the reduction

of 2-oxo-phenylacetic acid (**1.98**) by hydrogen transfer into mandelic acid (**1.99**) (**Scheme 29**).



(1.98) (1.99)

Scheme 29. Enantioselective reduction of an α-keto acid.

88 ligand-metal combinations were examined, and the reaction products of each split into two portions. These were added to two microtitre plates, containing a specific antibody to the product. In one plate the antibody was selected to bind the racemate of (**1.99**), the other plate one specific enantiomer. Before the addition of the reaction mixtures, an enzyme linked to compound (**1.99**) was added to each plate such that all the antibody binding sites in both plates were fully occupied. Addition of the product mixtures caused displacement of the enzyme from the antibody, such that it could be washed away. In the plate containing the racemate selective antibody, both enantiomers of the product caused enzyme displacement, whilst in the enantioselective plate, only one enantiomer displaced it. After washing the plates, a substrate for the enzyme was added, chosen because enzyme activity led to a colour change. Measuring the level of colour change allowed determination of the amount of enzyme present in each well, and therefore the amount of (**1.99**) that had displaced it. The yield of each reaction could therefore be determined from the racemic plate, and the ee from the difference between the two (**Figure 3**). The authors state that since antibodies can be raised to almost any compound, this assay has wide ranging applications in catalyst discovery.

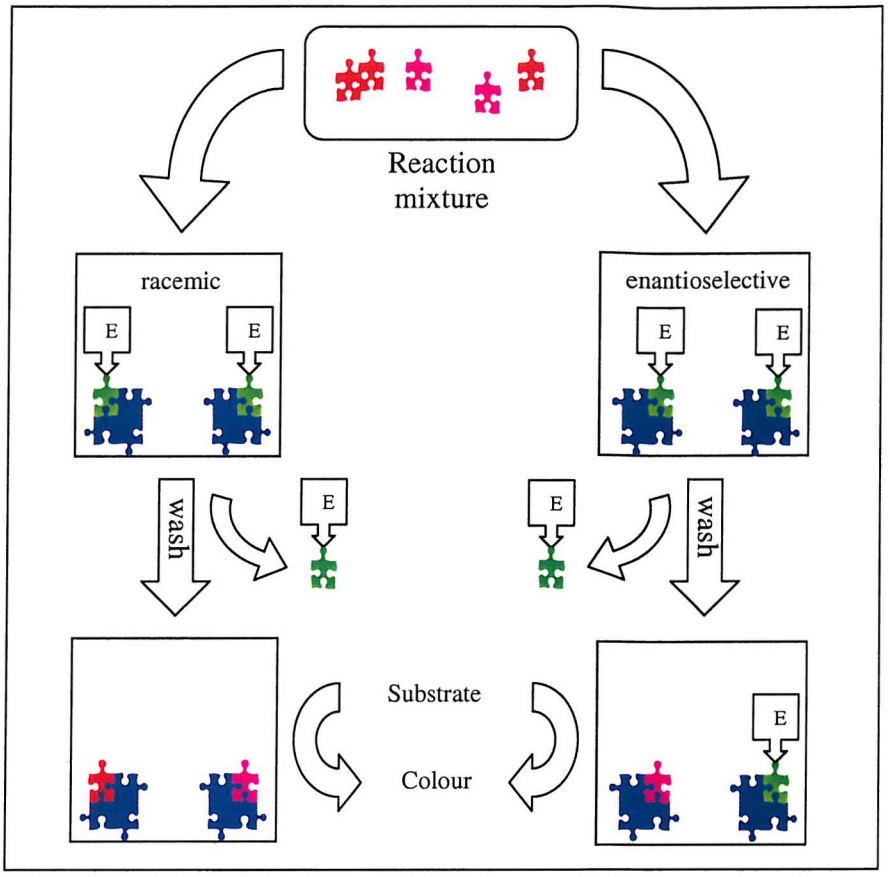


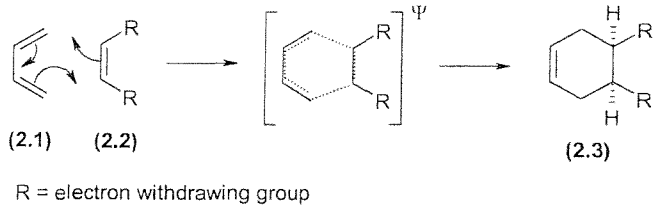
Figure 3. Competitive enzyme immunoassay.

The field of combinatorial catalysis is therefore a fast growing one, with many new developments and techniques being reported. The research reported is of great interest to both industry and academia and promises to be a rich seam of novel catalysts in the future.

Chapter 2. The discovery of Diels Alder catalysts from combinatorial peptide libraries.

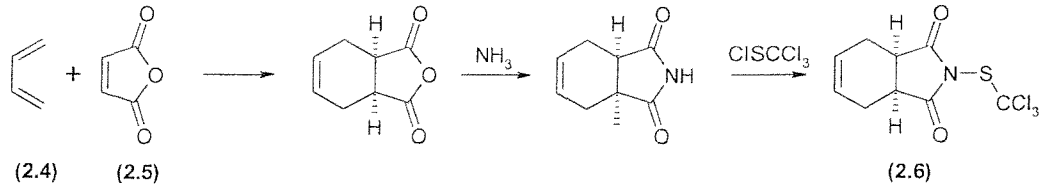
2.1. The Diels Alder reaction.

The Diels Alder reaction is the most famous, and one of the most useful pericyclic reactions. It was discovered in 1928 by Otto Diels and Kurt Alder,[74] work for which they were jointly awarded the Nobel Prize in 1950. The reaction is the cycloaddition of a diene **(2.1)** and a dienophile **(2.2)** to form a cyclohexene **(2.3)** **(Scheme 30)**, and is promoted by the use of an electron rich *cisoid* diene and a dienophile with an electron poor double bond (often due to a conjugated carbonyl group), or more rarely, by an electron poor diene and an electron rich dienophile.[75]



Scheme 30. Diels Alder reaction.

The reaction is one of the most powerful methods for making cyclohexene and cyclohexane containing compounds and has been employed in numerous important syntheses, for example the synthesis of the fungicide captan **(2.6)** from 1,3-butadiene **(2.4)** and maleic anhydride **(2.5)** **(Scheme 31)**.[76]



Scheme 31. Synthesis of captan.

Generally Diels Alder reactions are accomplished by heating the reagents together either neat or in a solvent, but the reaction can also be catalysed by the addition of a Lewis acid.[77] Catalysis is achieved by coordination of the electron withdrawing group of the dienophile, increasing its electropositivity and therefore lowering the energy of the LUMO of the dienophile's double bond. The effect of the catalyst on the frontier orbitals is depicted in **Figure 4**.[78]

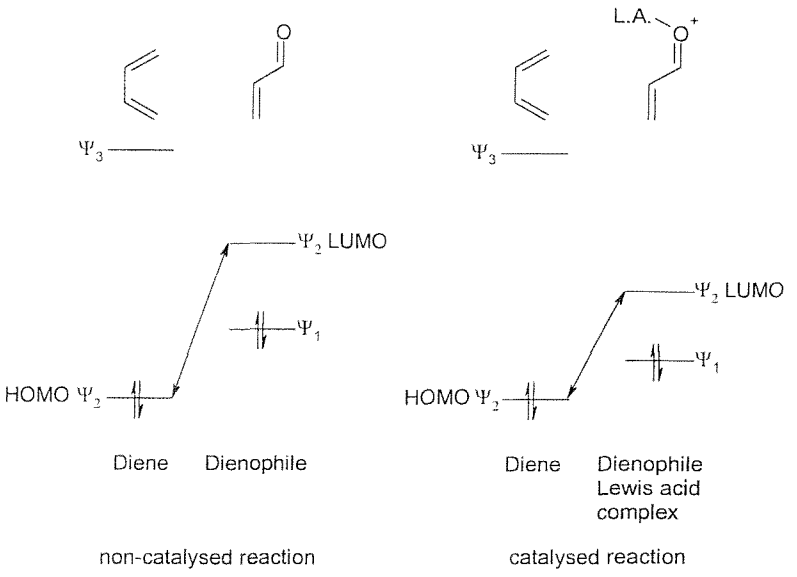
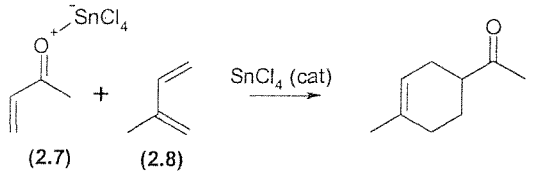


Figure 4. Effect of Lewis acid catalysis on the frontier orbitals of a Diels Alder reaction.

In **Scheme 32** the Lewis acid $SnCl_4$ coordinates the carbonyl group of methyl vinyl ketone **(2.7)** activating the double bond and allowing it to react with 2-methyl-1,3-butadiene **(2.8)** at room temperature, a reaction which otherwise would require heating in a sealed tube.[79]

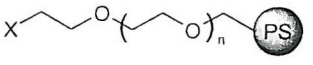


Scheme 32. Lewis acid catalysis of the Diels Alder reaction.

2.2. Same bead substrate-catalyst libraries.

The aim of this research was to develop a technique which would allow the generation and screening of on-bead catalyst libraries using combinatorial techniques. The key component of this strategy was to find a method that would allow the evaluation of catalytic efficiency of compounds attached to single beads in the presence of the rest of the library. This would allow a single screen to show which compounds were active and which were not, without the need to spatially separate the beads and test them individually.

TentaGel resin consists of polystyrene beads cross-linked (1-2%) with divinylbenzene, which have been modified by anionic graft polymerisation of ethylene oxide onto the resin and have the general structure shown in **Figure 5**.[80]



n ~ 50

X = NH_2, OH, SH, or Br

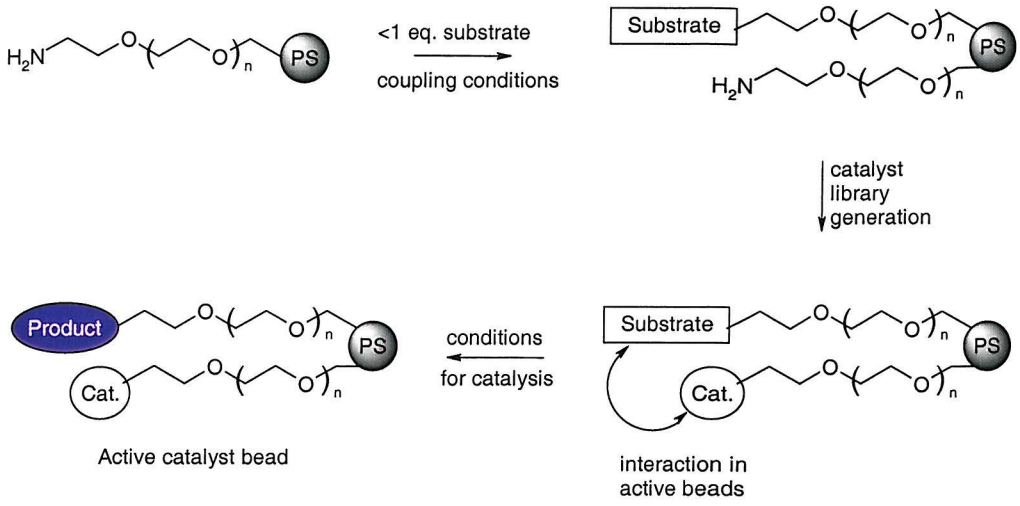
Figure 5. Structure of TentaGel resin.

The resin was originally developed for use in continuous flow synthesis as it has relatively uniform swelling properties in a variety of solvents, but it has other uses, for example it swells in polar solvents, and thus is often used instead of standard polystyrene resins where solvents such as methanol or water are required to carry out chemistries.

It has been shown by ^{13}C-NMR relaxation time studies conducted by Bayer,[80] that the mobility of compounds attached to TentaGel is similar to their mobility if attached to PEG chains in solution.

The catalysis detection technique proposed, relies on the ability of compounds bonded to the ends of the PEG chains on TentaGel to interact with each other within the same bead. By partially substituting aminoethyl TentaGel with a substrate for a catalytic reaction, and then using the remaining free sites on this resin to generate a library of catalysts, any catalytically active library members would be able to promote the reaction of substrate molecules mounted on the same bead by a site to site catalysis interaction **(Scheme 33)**. If the reaction being investigated was chosen such that the modification of the substrate

under catalysed conditions leads to a colour change, then beads containing active catalysts would display this colour change, allowing them to be selected from the library.



Scheme 33. Proposed method for the detection of catalytic activity.

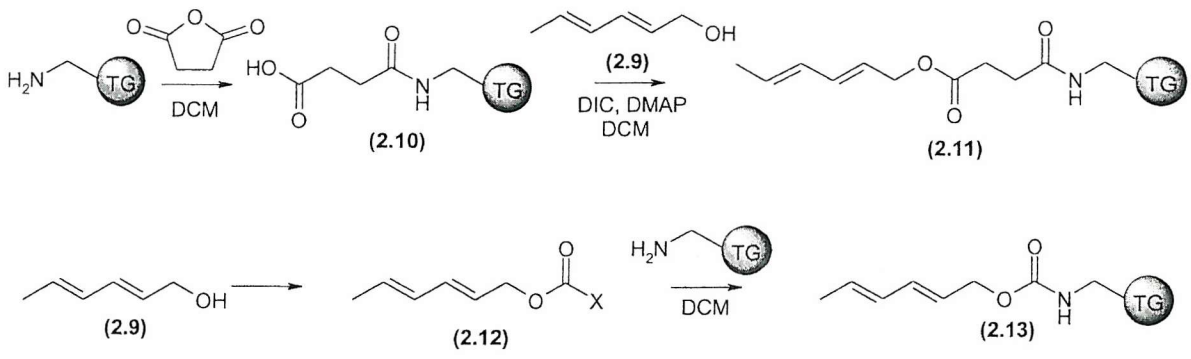
2.3. A screen for the detection of Diels Alder catalyst activity from a library of peptides.

It has already been shown that RNA strands can be used to catalyse the Diels Alder reaction (Chapter 1 page 28), in the presence of transition metals. It was hoped that a library of peptides might offer an alternative source of Diels Alder catalysts. Amino acids offer a wide variety of functionalities, such as basic, acidic, hydrophilic and hydrophobic sites, aromatic rings and hydrogen bond donors and acceptors which allow enzymes to catalyse an almost limitless range of reactions, far beyond the scope of human efforts in catalysis. Shorter peptide chains lack the detailed three dimensional structure of enzymes, but carry the same functional groups and therefore may be capable of catalysing the same reactions. In the case of the Diels Alder reaction, catalysis is generally by activation of the dienophile by a Lewis acid, a role that could perhaps be replaced by a hydrogen bond acceptor, which is in effect a weak Lewis acid. Several amino acid side chains have hydrogen bond acceptor functionalities, for example lysine (amine), serine (alcohol), glutamine (carboxamide) and others. These functionalities, together with other binding

interactions such as van der Waals forces, π stacking interactions and polar attractions could help to promote the reaction between the diene and dienophile, and lead to reaction catalysis. Therefore a method for screening a library of small peptides as Diels Alder catalysts was devised.

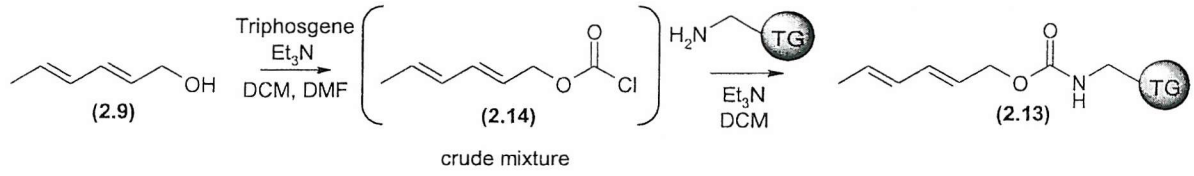
2.4. Substrates for combinatorial catalyst screening.

In order to obtain colourimetric detection of the Diels Alder reaction on resin, it seemed that one of the substrates (diene or dienophile) should be attached to the resin, and the other labelled with a dye. On reaction therefore, the dye would become covalently attached to the resin allowing product formation (and therefore the efficiency of the catalyst) to be observed. In both the work of Eaton *et al.* and Jäschke *et al.* described above, the diene was attached to the RNA strand, and the dienophile to the labelling moiety. It was therefore decided to use a similar arrangement. *E,E*-2,4-Hexadien-1-ol **(2.9)** was chosen as the diene, with the alcohol as the point of attachment to the resin. Two methods of resin attachment were possible. Firstly, the opening of succinic anhydride with the amino group of the TentaGel resin to give acid functionalised resin **(2.10)**, followed by esterification of the alcohol to give the diene functionalised resin **(2.11)**. Secondly, The activation of the alcohol as an active carbonate **(2.12)**, followed by displacement of the leaving group by the resin amine to give the diene functionalised resin **(2.13)** **(Scheme 34)**.



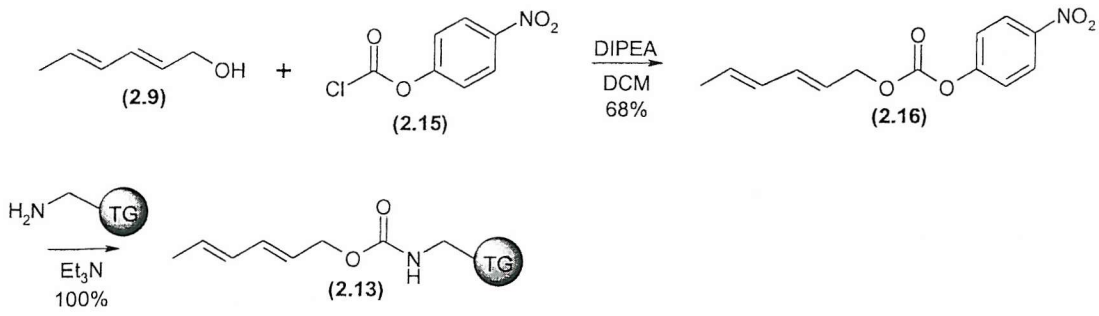
Scheme 34. Possible syntheses of diene substituted TentaGel resins.

The carbamate moiety in the second example was considered to be more suitable. Formation of the chloroformate **(2.14)** by the reaction of the alcohol with triphosgene and triethylamine in DCM, with the addition of a drop DMF as a catalyst was found to be messy, and purification of the product difficult due to the instability of the chloroformate to silica based chromatography. Reaction of an excess of the crude reaction mixture with TentaGel S NH_2 gave 100 % substituted resin **(2.13)** **(Scheme 35)**, however, in order to use this system for catalyst detection, it was necessary to obtain resin which was partially substituted with the diene. The inability to purify the chloroformate therefore meant that it would not be possible to know the amount of compound present in order to calculate the substitution of the resin. Therefore this method was not suitable for the synthesis of TentaGel partially substituted with diene.



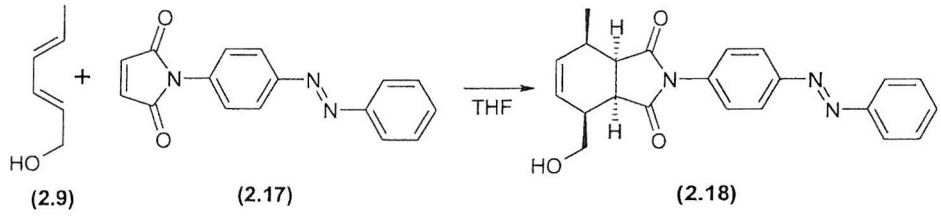
Scheme 35. Synthesis of diene substituted TentaGel via chloroformate.

An alternative to the chloroformate for the synthesis of carbamates is the 4-nitrophenyl carbonate **(2.16)**. This was readily synthesised by treatment of the alcohol with 4-nitrophenylchloroformate **(2.15)** with DIPEA as the base. The compound was found to be substantially more stable to chromatography on silica gel than the chloroformate **(2.14)** and was therefore successfully purified. Reaction of an excess of the compound with TentaGel S NH_2 in the presence of triethylamine again successfully resulted in 100 % substituted resin **(2.13)** **(Scheme 36)**.



Scheme 36. Synthesis of diene substituted TentaGel via 4-nitrophenylorthoformate.

Having successfully identified a suitable diene for the reaction and developed a method for the attachment to the solid phase, a dienophile needed to be found which met the requirements for catalyst detection, i.e. that it was coloured. Maleimides generally make excellent dienophiles for the Diels Alder reaction due to the electron withdrawing nature of the two carbonyl groups conjugated to the double bond, so it seemed that the coloured maleimide 4-phenylazomaleinanil **(2.17)** would be a suitable compound to use as the labelling dienophile. Its reaction with compound **(2.9)** was investigated **(Scheme 37)**[Ψ], and the half life of the reaction at ~5 mM concentration was determined by HPLC to be approximately 12 hours. This made the reaction suitable for catalyst discovery as with such a slow reaction, any catalysis should be easily observable.

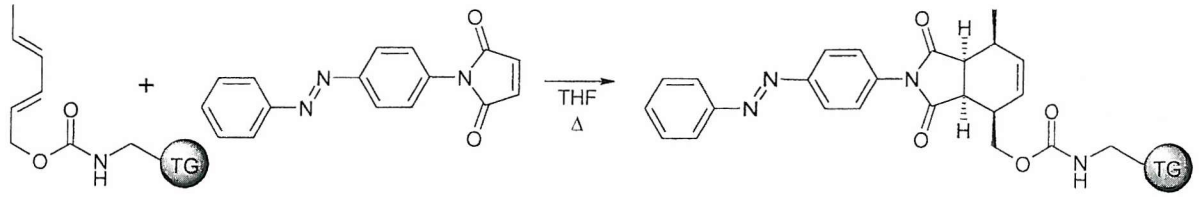


Scheme 37. Solution phase Diels Alder reaction.

Therefore, resin **(2.13)** was heated at 60 °C with compound **(2.17)** in THF for 16 hours, and washed repeatedly with a wide variety of solvents. The resin remained orange in

[Ψ] The stereochemistry of **(2.18)** could not be assigned, and so has been assumed to be the normally observed endo product.

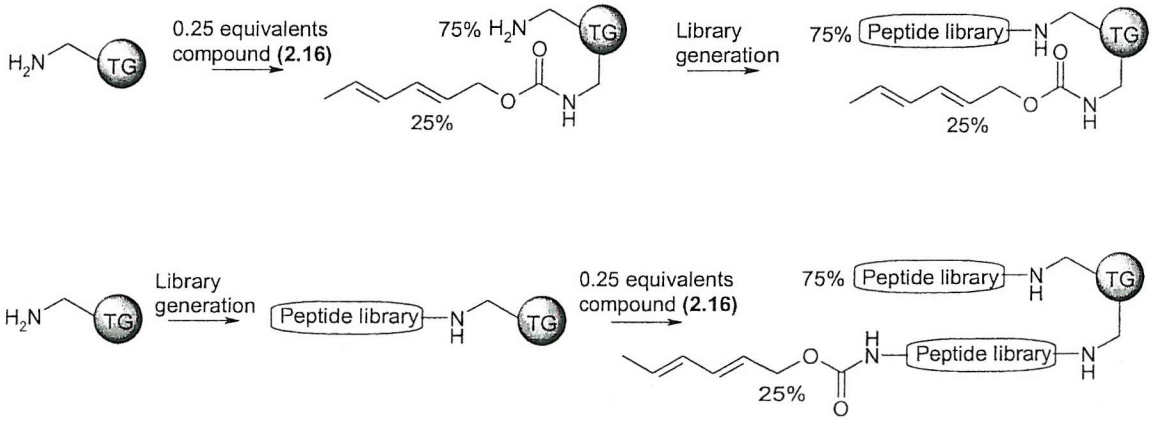
colour, suggesting that the dye had indeed been covalently bound to the resin through a Diels Alder reaction **(Scheme 38)**.



Scheme 38. Solid phase Diels Alder reaction.

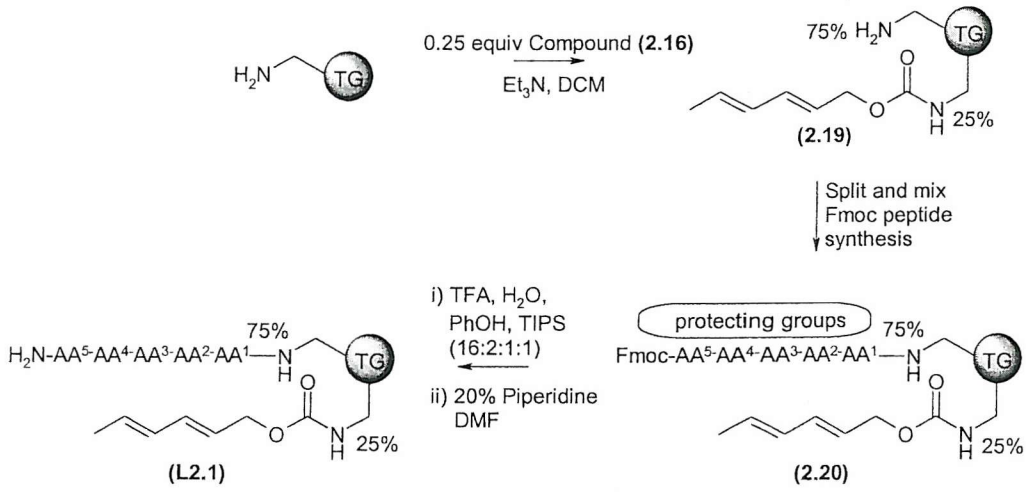
2.5. Synthesis of peptide libraries for screening as Diels Alder catalysts.

Two types of library were investigated for the discovery of Diels Alder catalysts. In the first the resin was partially substituted with diene, and then the remaining free resin sites were used to generate the peptide library. In the second library, the unsubstituted resin was used for the library generation, and then the *N*-termini of the peptides were capped with the diene **(Scheme 39)**.



Scheme 39. Design of two catalyst libraries.

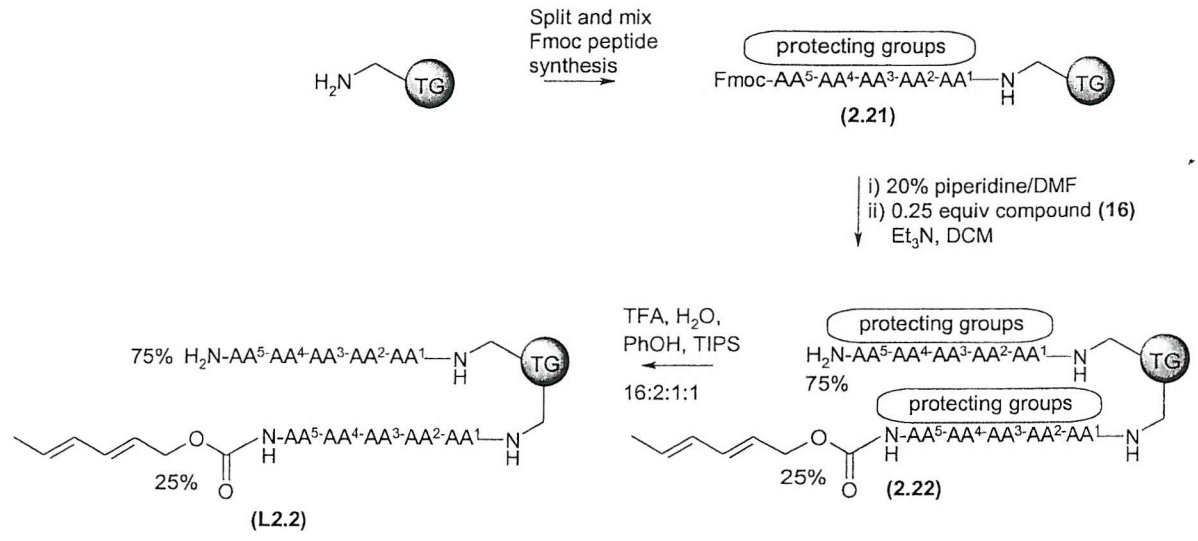
TentaGel S NH_2 resin was therefore reacted with 0.25 equivalents of compound **(2.16)** in the presence of triethylamine, and the reaction monitored by TLC to confirm that all the available reagent had reacted with the resin, such that the resin was 25 % substituted with diene. The remaining free sites on resin **(2.19)** were then used for the generation of split and mix library of pentapeptides by Fmoc peptide synthesis. Ten Fmoc amino acids were used for the generation of the library, these were: Fmoc-Phe-OH, Fmoc-Ala-OH, Fmoc-Ser(O^{t}Bu) -OH, Fmoc-Gly-OH, Fmoc-Arg(Pbf) -OH, Fmoc-Trp-OH, Fmoc-Val-OH, Fmoc-Glu(O^{t}Bu) -OH, Fmoc-Leu-OH, Fmoc-Gln-OH. The amino acids were chosen to represent a variety of acidic, basic, polar and hydrophobic residues in order to generate structurally varied peptides to evaluate as catalysts. Lysine and cysteine were excluded from the selection to avoid possible Michael additions to the maleimide, giving rise to false positive catalyst identification. The library contained a possible 100,000 different peptides, and was synthesised on a scale such that a 20 fold excess of resin beads was used (~ 2 million), and therefore all possible structures should be present. After completion of the library synthesis **(2.20)**, the side chain protecting groups were removed by treatment with TFA/H_2O/PhOH/ TIPS,[81] and the Fmoc group was removed from the *N*-terminus to give library **(L2.1) (Scheme 40)**.



Scheme 40. Synthesis of library **(L2.1)**.

A second pentapeptide library was synthesised directly onto TentaGel S NH_2 resin following an identical protocol to that used for the synthesis of library **(L2.1)**. After the

completion of the synthesis **(2.21)**, the Fmoc group was removed from the *N*-terminus and the resulting amino groups were 25 % capped with diene by reaction with compound **(2.16)** to give resin **(2.22)**. The side chain protecting groups were then removed as before to give library **(L2.2)** **(Scheme 41)**. All the terminal amines were not capped for two reasons. Firstly, so that each bead in library **(L2.2)** would contain as much diene as a bead from library **(L2.1)**, and thus should pick up an equal amount of dye if equally active. Secondly, so that active beads could be picked and analysed by Edman sequencing, a protocol which requires a free *N*-terminus. Edman sequencing is discussed in greater detail later in this thesis (Chapter 4, page 73).

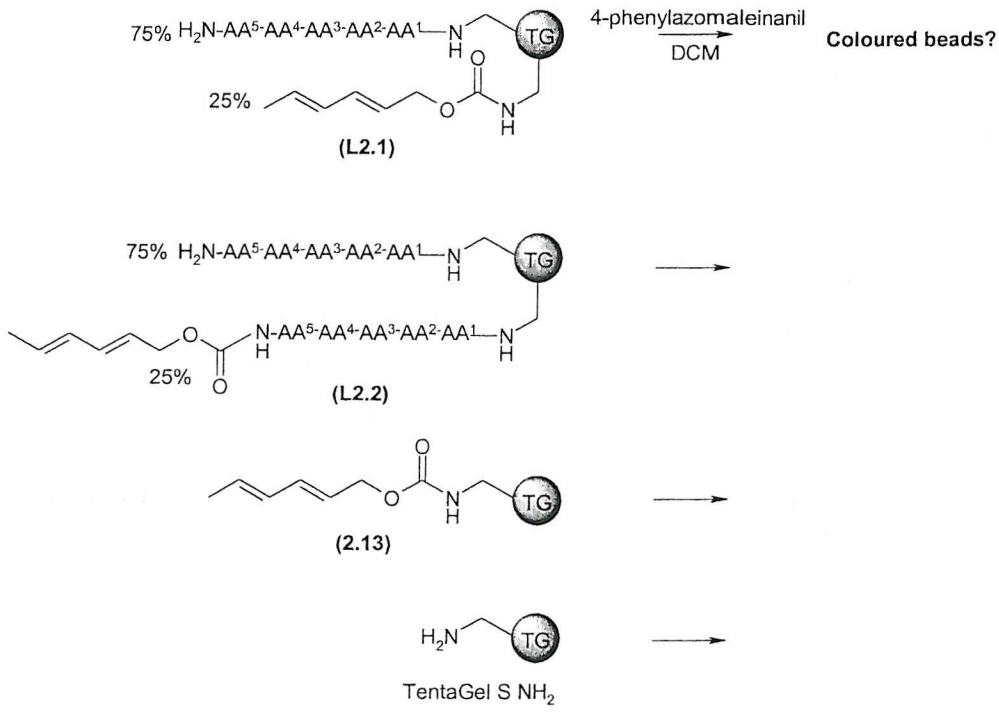


Scheme 41. Synthesis of library **(L2.2)**.

Following the synthesis of the libraries, experiments to determine suitable conditions for the catalyst screening experiments were carried out. Diene substituted TentaGel resin **(2.13)** was reacted with 0.5 equivalents of 4-phenylazomaleinanil **(2.17)** in DMF, DCM, THF and benzene. The reactions were monitored under a microscope and the uptake of the dye by the resin beads assessed. The reaction in DCM was found to be the fastest, resulting in the greatest uptake of dye by the beads after a set period of time. The sample reacted in DMF also showed significant uptake, with the other two solvents giving much slower reaction.

2.6. Screening of the peptide libraries.

10 mg samples of each of libraries **(L2.1)** and **(L2.2)** were swollen in DCM and 0.1 equivalents of 4-phenylazomaleinanil (relative to diene loading) were added to each reaction. Samples of resin **(2.13)** and unsubstituted TentaGel S NH_2 resin were treated under the same conditions to act as controls for the screening experiment **(Scheme 42)**.



Scheme 42. Screening of peptide libraries.

After 24 hours no uptake of the dye was observed for the unsubstituted TentaGel or either of the library samples. Resin **(2.13)** however showed uptake of the dye as had been observed previously. The observed result that neither of the library samples showed any dye uptake was rather a puzzling one, as even if the pentapeptides were inactive as Diels Alder catalysts, some uptake should have occurred due to the uncatalysed background reaction as observed for the diene substituted resin sample. One possible explanation to the observed result was that the diene was not present in the library samples. It had been considered that the diene carbamate functionality would be stable to all the reaction

conditions, as it had similar structure to the Aloc protecting group which is stable to the conditions used in the library synthesis **(Figure 6)**.[82]

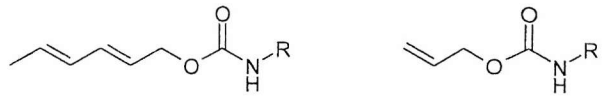


Figure 6. Structure of diene carbamate vs. Aloc group.

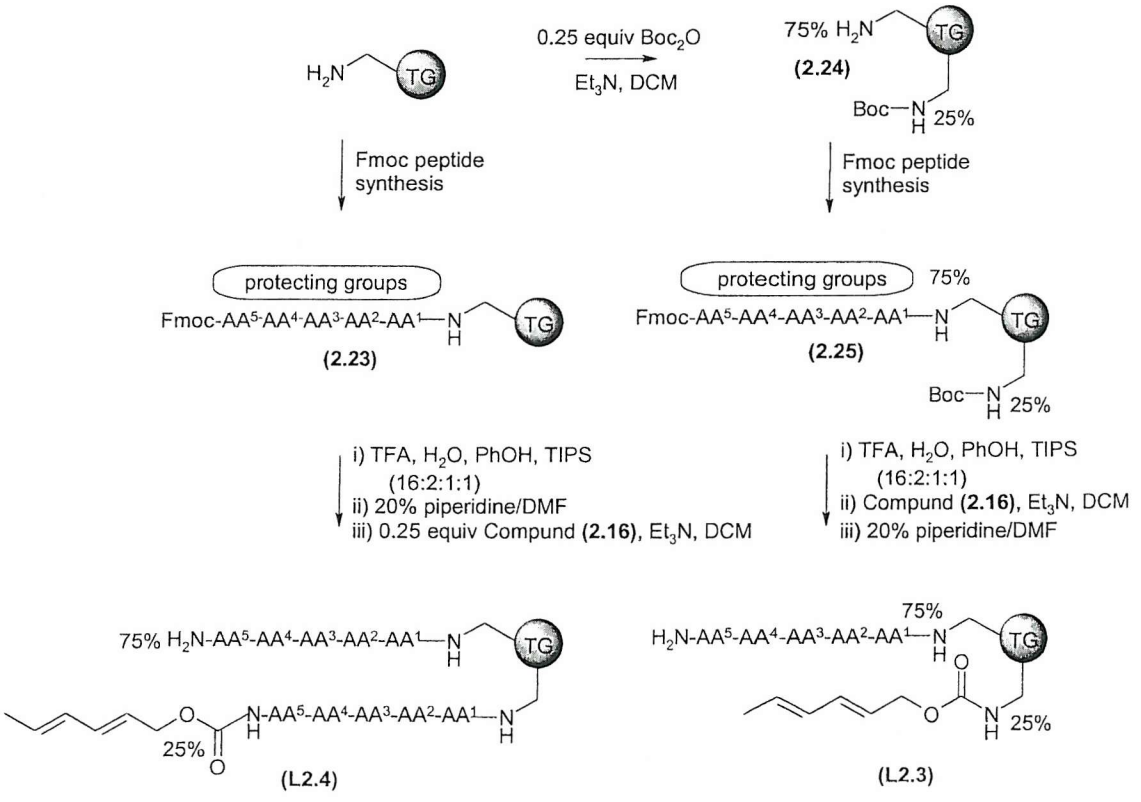
It was decided however to confirm that the group would not be removed by any of the reagents used during the library synthesis. Samples of diene substituted resin **(2.13)** were treated with 20% piperidine in DMF, DIC and HOBt in DMF/DCM and TFA/H_2O/PhOH/TIPS, and then tested for free amine groups using the ninhydrin test.[83] The functionality appeared to be stable to piperidine, and to the coupling conditions, but treatment with TFA resulted in the removal of the group liberating the free amine **(Scheme 43)**.

Scheme 43. Cleavage of the diene by treatment with TFA.

Further experiments showed that the functionality was particularly sensitive to acidic conditions, for example treatment with 1% TFA in DCM was sufficient to remove the group. More detailed examinations of the stability and lability of this functionality, hereafter named the *E,E*-2,4-hexadienyloxycarbonyl group, and investigations into its use as a amine protecting group can be found in Chapter 5 of this thesis.

2.7. Resynthesis and screening of pentapeptide libraries.

Following the discovery of the lability of the hexadienyloxycarbonyl group, an alternative library synthesis strategy was devised. For the first library, TentaGel S NH_2 resin was 25% capped with a Boc group,[84] then library synthesis was carried out using the split and mix methodology. Removal of the side chain protecting groups by treatment with TFA/H_2O/PhOH/TIPS as before also removed the Boc group, releasing the remaining amino groups on the resin, which were then capped with diene by treatment with compound **(2.16)**. Finally, removal of the *N*-terminal Fmoc group gave library **(L2.3)**. Library **(L2.4)** was synthesised in a manner similar to library **(L2.2)**, but with the removal of side chain protection taking place before the addition of the diene **(Scheme 44)**.



Scheme 44. Synthesis of libraries **(L2.3)** and **(L2.4)**.

Libraries **(L2.3)** and **(L2.4)** were then screened for activity, again with resin **(2.13)**, and unsubstituted TentaGel as controls. The uptake of the dye by the library beads was monitored under a microscope, and photographs were taken at intervals for subsequent analysis. After 24 hours significant colour change was observed for some of the resin beads. **Figure 7** is a photograph taken during the screen of a bead considered to have a high uptake of the dye. It can be seen that the dye has caused colouration to all the beads and to the solution in which they are suspended, but that the bead encircled has a darker hue, indicating that more dye has been absorbed.

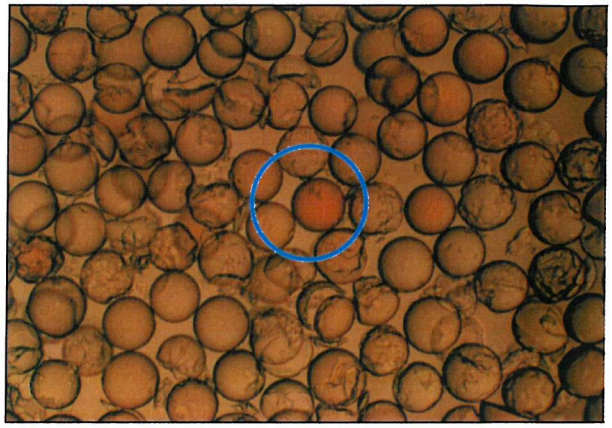


Figure 7. Active bead photographed from the screening of library **(L2.3)** (x40).

Several beads from both samples displayed similar levels of dye uptake, indicating that both libraries were equally useful for catalyst uptake. The control samples of resin **(2.13)**, and unsubstituted TentaGel showed no increased uptake, the level of colouration was uniform across the samples due to the diffusion of the dissolved dye into the beads. Following the success of this initial screen, the experiment was repeated several times, and for each library sample, the best two or three beads were photographed for later analysis and picked from the suspension. **Figure 8** shows examples of beads which were judged to be active and picked from both libraries.

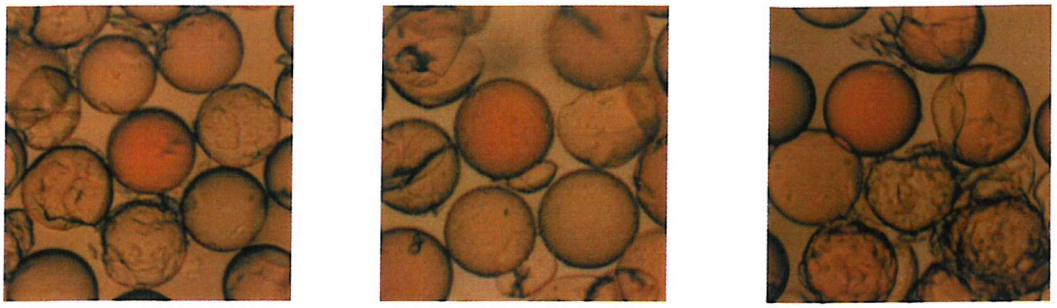
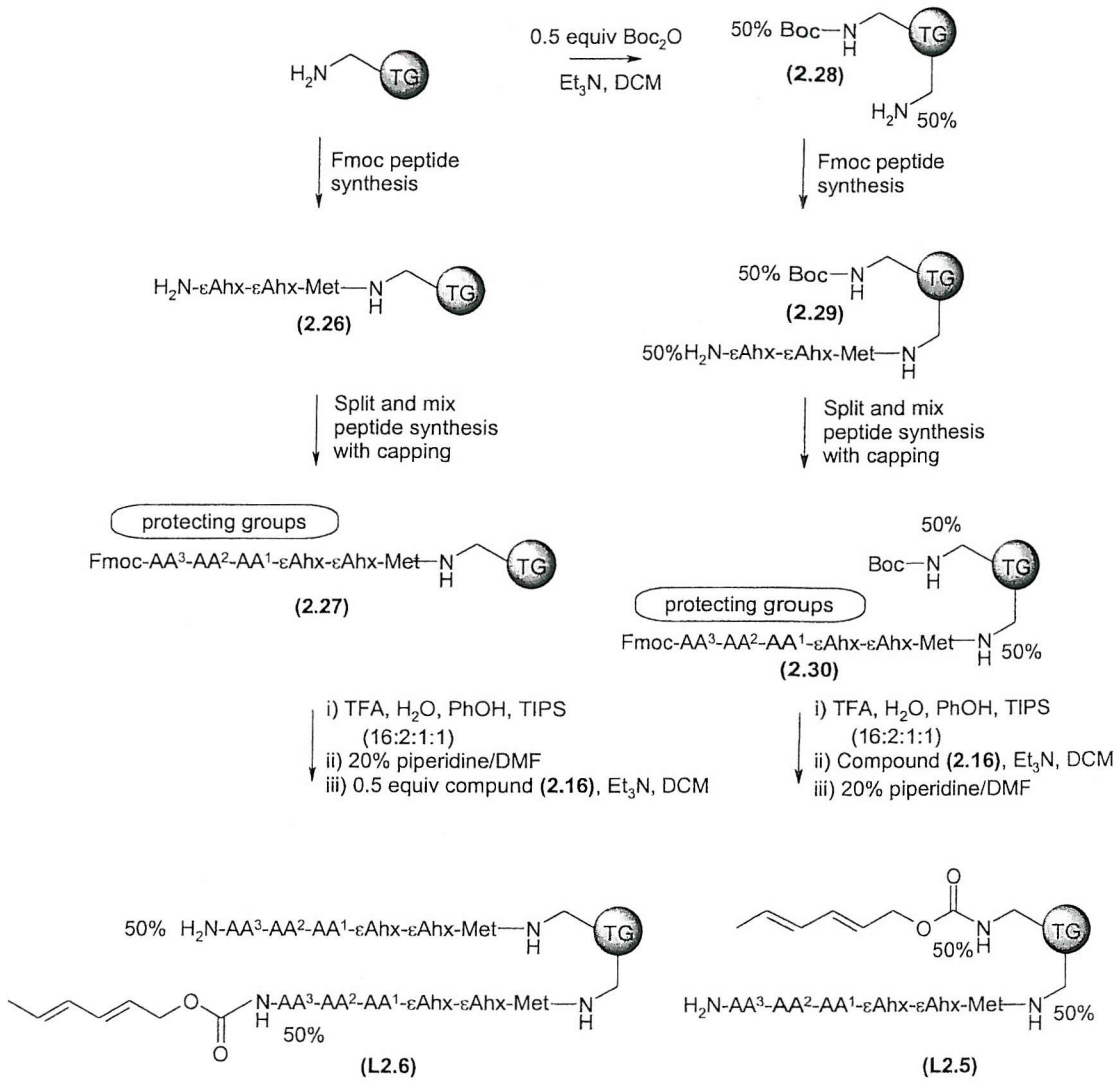


Figure 8. Examples of active beads from libraries **(L2.3)** and **(L2.4)** (x 100).

In total ten beads were picked from each library and the best three from each were sent away for Edman sequencing. Unfortunately the organisation used was unable to sequence the beads, and no other facility was at this time available at reasonable cost. Therefore an alternative method for the sequencing of active beads was needed.

2.8. Synthesis of libraries incorporating isotopically labelled capped ladders for peptide sequencing.

Chapter 4 of this thesis describes the development of a new method for the sequencing of peptides selected from combinatorial libraries. The method is mass spectrometry based, and relies on the partial capping of the peptide library after each coupling with a 1:1 mixture of acetic acid and D_3 acetic acid, and requires the use of a linker-spacer construct between the resin and the peptide library. Therefore new libraries incorporating a methionine residue as the linker, and two 6-aminohexanoic acid residues as spacer units were synthesised. The libraries consisted of only tripeptides this time, to ease the sequencing of any active beads, and the ratio of library to diene was changed to 1:1. Libraries **(L2.5)** and **(L2.6)** were therefore synthesised in a similar manner to that used for the previous libraries, incorporating the linker-spacer construct, and isotopic labelling **(Scheme 45)**.

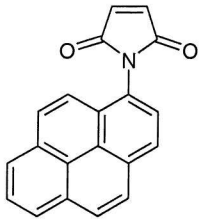


Scheme 45. Synthesis of libraries **(L2.5)** and **(L2.6)** incorporating isotopic labelled capping.

2.9. Screening of Diels Alder catalyst libraries with a fluorescent dienophile.

It was felt that the differentiation between active and inactive beads observed during the earlier screening experiments was not as good as had been hoped, and therefore an alternative dye should be used. The use of a fluorescent dye should offer a more sensitive screen, resulting in better differentiation between active and inactive beads.

N-(1-pyrenyl)maleimide (**2.31**) is a commercially available fluorescent maleimide with an adsorption maximum at ~340 nm, and emission at ~390-420 nm.



N-(1-Pyrenyl)maleimide (**2.31**)

Samples from libraries (**L2.5**) and (**L2.6**) were therefore screened replacing the dye used with compound (**2.31**). The reaction was monitored through a microscope, with illumination through a type A fluorescence filter, which allows excitation between 340 and 360 nm, and suppresses emission below 420 nm. Unfortunately some beads in the samples were found to be fluorescent at the observed wavelengths in the absence of the dye. **Figure** 9 shows photographs of fluorescent samples from library (**L2.5**), both in the presence and absence of the pyrene dye (**2.31**).

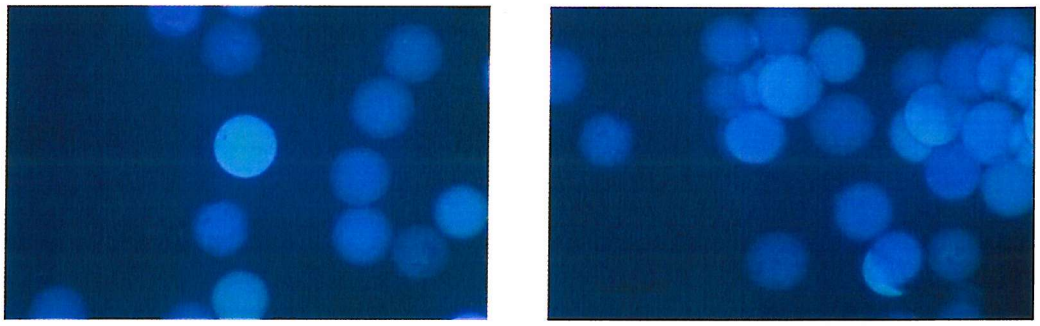
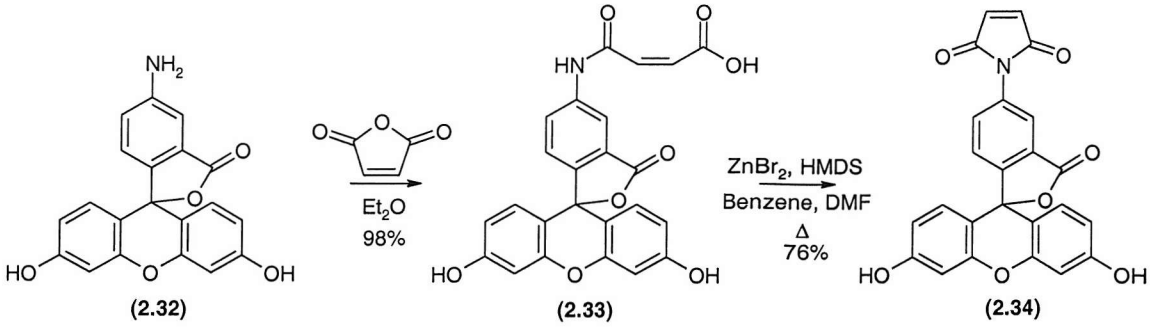


Figure 9. (a) "Active" bead observed in screening of library (**L2.5**). (b) Control (no dye) sample of library (**L2.5**) under identical viewing conditions (x 100).

The library was examined at other wavelengths in the absence of dye, and was found not to fluoresce when observed through an I3 filter. This filter allows excitation between 450 and 490 nm, and suppresses emission below 515 nm. This makes it suitable for use with fluorescein based dyes, which are excited at ~490 nm, and emit at ~520 nm. Thus attempts were made to synthesise a fluorescein based maleimide.

5-Aminofluorescein **(2.32)** was therefore reacted with maleic anhydride in diethyl ether to give 3-(fluorescien-5-ylcarbamoyl)-acrylic acid **(2.33)** in good yield. The ring closing of the maleimide was found to be difficult however. Treatment with DCC was unsuccessful, as was a commonly used dehydration with NaOAc in acetic anhydride.[85] The cyclisation was eventually achieved by the use of the Lewis acid $ZnBr_2$, and HMDS in refluxing benzene to give the required *N*-fluorescein-5-yl maleimide **(2.34)** **(Scheme 46)**.[86]



Scheme 46. Synthesis of *N*-fluorescein-5-yl maleimide.

Libraries **(L2.5)** and **(L2.6)** were again screened for Diels Alder catalytic activity by treatment of a sample from each library with 0.01 equivalents of compound **(2.34)** in DMF. Almost immediately several beads from each library were observed to be significantly fluorescent. **Figure 10** shows an active bead under both fluorescent and normal viewing conditions. It can be clearly seen that the bead has taken up the dye to a far greater level than its neighbours.

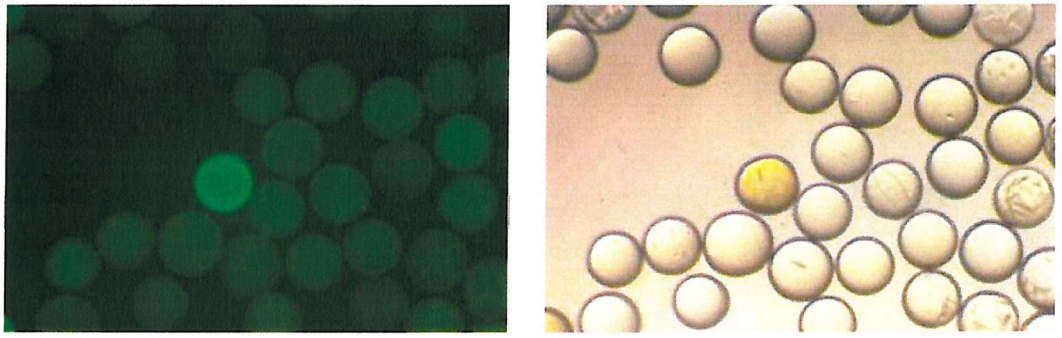


Figure 10. (a) Active bead from library **(L2.6)** viewed under U.V. light through an I3 filter cube. (b) the same bead viewed under normal light conditions (x 100).

Diene substituted TentaGel and unsubstituted TentaGel were again used as controls for the reaction. The unsubstituted TentaGel showed no uptake of the dye, while the diene substituted resin showed no fluorescence under the library screening conditions, but a low level of fluorescence was observed after 24 hour treatment with 0.1 equivalents of compound **(2.34)** as shown in **Figure 11**.

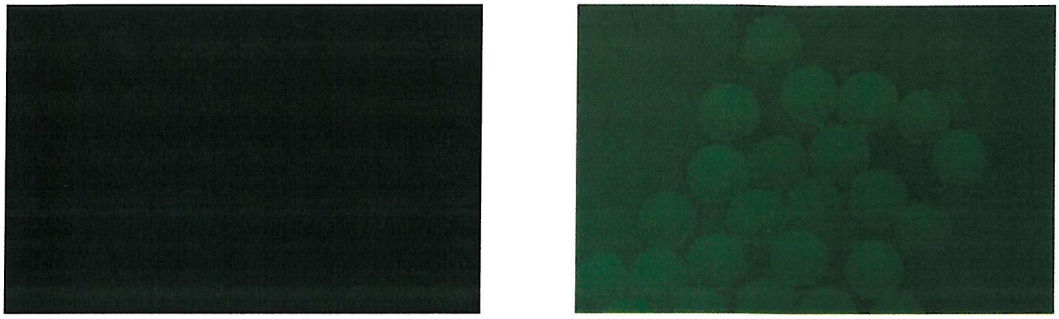
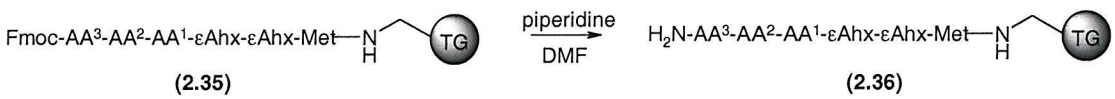


Figure 11. (a) Diene substituted resin **(2.13)**, treated with 0.01 equivalents **(2.34)** for 2 hours. (b) After 24 hours with 0.1 equivalents of compound **(2.34)** (x 100).

In addition to the two controls used previously, a sample of resin **(2.35)** (resin **(2.29)** after side chain protecting group removal) was used as a third control to ensure that the uptake of the dye was due to a Diels Alder reaction with the resin supported diene, and not merely a binding interaction between the dye and the peptides in the libraries. No fluorescence was observed for this control under the library screening conditions. Treatment of resin **(2.35)** with piperidine to remove the Fmoc group and give a free *N* terminus **(2.36)** **(Scheme 47)** and re-examination under the screening conditions, however, led to the development of a low level of fluorescence by some of the beads in the sample, as can be seen in **Figure 12(b)**.



(2.35) **(2.36)**

Scheme 47. Peptide library resins used as diene free controls.

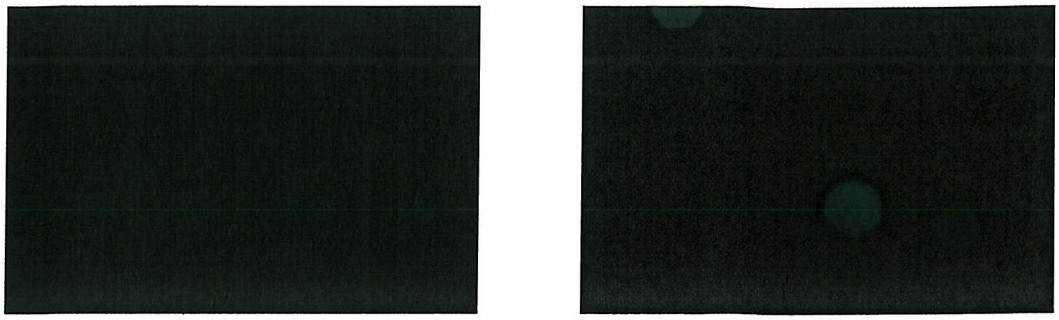
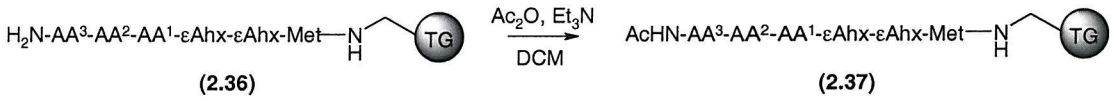


Figure 12. (a) Resin (**2.35**) under library screening conditions. (b) Resin (**2.36**) under library screening conditions (x 100).

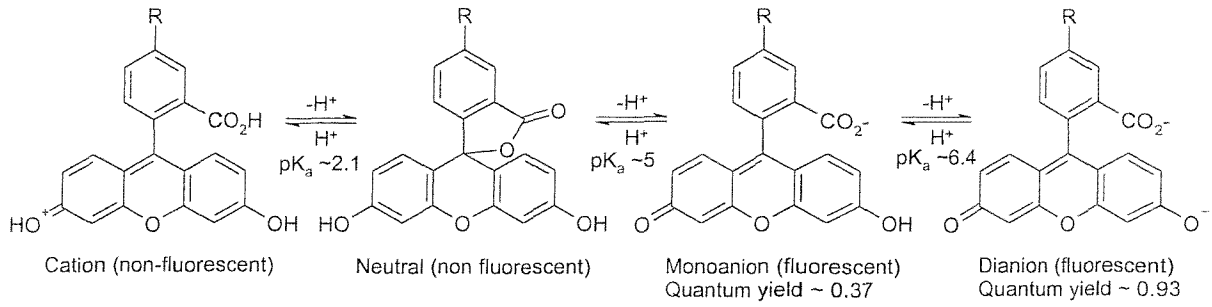
What was the cause of this dye uptake? Did it suggest that the observed uptake for libraries (**L2.5**) and (**L2.6**) were not due to a Diels Alder reaction after all? It was first postulated that amino groups at the *N*-terminus of the peptides had reacted with the maleimide *via* a Michael addition, although the uptake was limited to some beads, and all the beads had free amino residues. In order to confirm that this was not the reason however, resin (**2.36**) was exhaustively acetylated by treatment with and excess of acetic anhydride and triethylamine to give resin (**2.37**) (**Scheme 48**).



Scheme 48. Acetylation of control resin (**2.43**).

Examination of this resin under the screening conditions gave identical results to those observed for resin (**2.36**). In comparison with the results observed for the screening of libraries (**L2.5**) and (**L2.6**), the level of fluorescence was low, but it was felt to be important to identify the cause of the change in dye uptake from resin (**2.35**) (no uptake) to resins (**2.36**) and (**2.37**). One possible answer was protonation of basic sites in the peptide structure. Resin (**2.35**) had been treated with TFA in order to remove the side chain protecting groups prior to its use as a screening control, whilst resin (**2.36**) had been treated with a base (piperidine) prior to its use. In order to confirm this theory, resins (**2.36**) and (**2.37**) were treated with TFA, washed and then reacted for two hours with 0.01 equivalents of dye (**2.34**). Examination of the resins under U.V. illumination led to no

observed fluorescence. Base was then added to both resins to ensure that any dye present was in the fluorescent (dianionic) form[87,88] **(Scheme 49)** and again no fluorescence was observed.



Scheme 49. Ionic equilibria of fluorescein derivatives.

Having shown that non Diels Alder binding of the dye could be prevented by treatment of the resin with TFA prior to screening, it was necessary to ensure that libraries **(L2.5)** and **(L2.6)** would still show fluorescence under these conditions. It had already been noted that the diene moiety was sensitive to treatment with TFA **(Scheme 43)**, so experiments were undertaken to quantify how much acid could be used without removing this group. Treatment with 0.1 % TFA in DCM for one minute was found to prevent the non Diels Alder binding of the dye by the peptide, whilst being mild enough to leave the diene moiety intact. Based on these results a new screening protocol was developed, whereby samples of libraries **(L2.5)** and **(L2.6)**, resin **(2.36)**, resin **(2.13)**, and unsubstituted TentaGel were treated with 0.1 % TFA in DCM for one minute, washed several times with DCM, and then allowed to react with 0.1 equivalents of compound **(2.34)** in either DCM or DMF for two hours. The resins were then washed with a variety of solvents, including dilute aqueous acid and base, suspended in DMF and examined as before under U.V. illumination through an I3 fluorescence filter. None of the controls showed any fluorescence, while both libraries **(L2.5)** and **(L2.6)** showed significant numbers of fluorescent beads in the samples that had been reacted in both solvents. Ten beads were selected, photographed and picked from the samples of each library in each solvent, examples of which are shown in **Figure 13**.

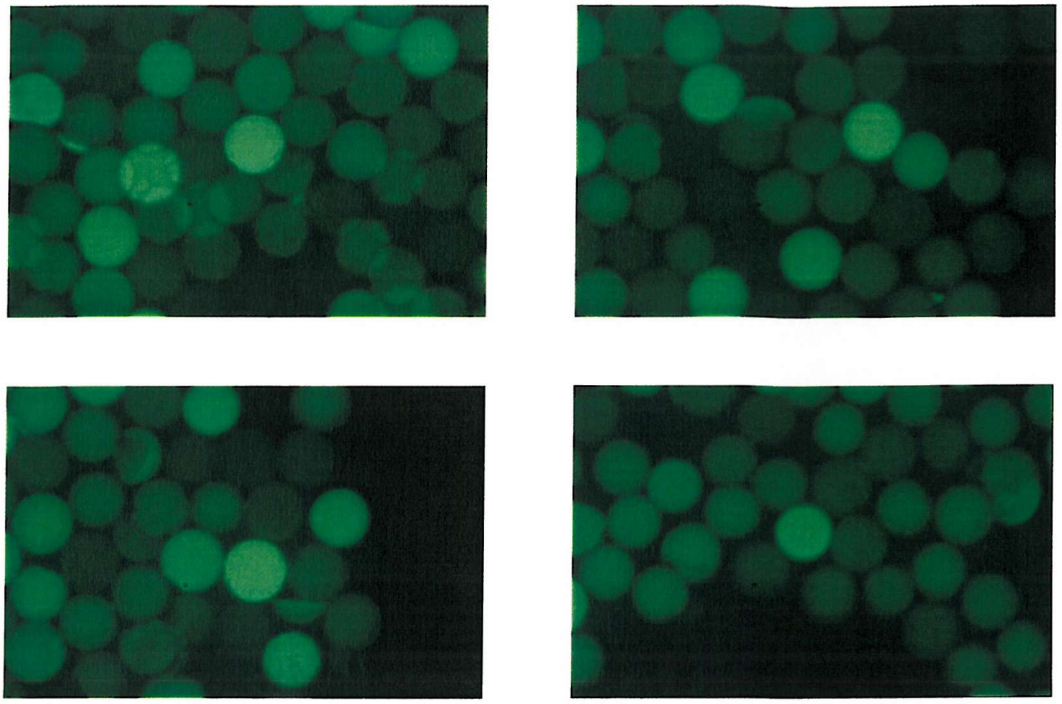


Figure 13. Examples of active beads picked from libraries **(L2.5)** and **(L2.6)** (x 100).

The photographs of the selected beads were post-analysed to attempt to obtain values for the intensity of the fluorescence in each. The photographs were first converted to 24 bit greyscale format, and then professional image analysis software (Image Pro Plus) was used to calculate the average 'whiteness intensity' of the active bead in each image. The results of these analyses are depicted in **Figure 14**, and confirm two of the earlier estimations. Firstly, that samples from both libraries took up the dye to similar extents, suggesting that both were equally suited to the detection of catalysts. Secondly, that DCM was a better solvent for the reaction, leading to higher levels of dye uptake.

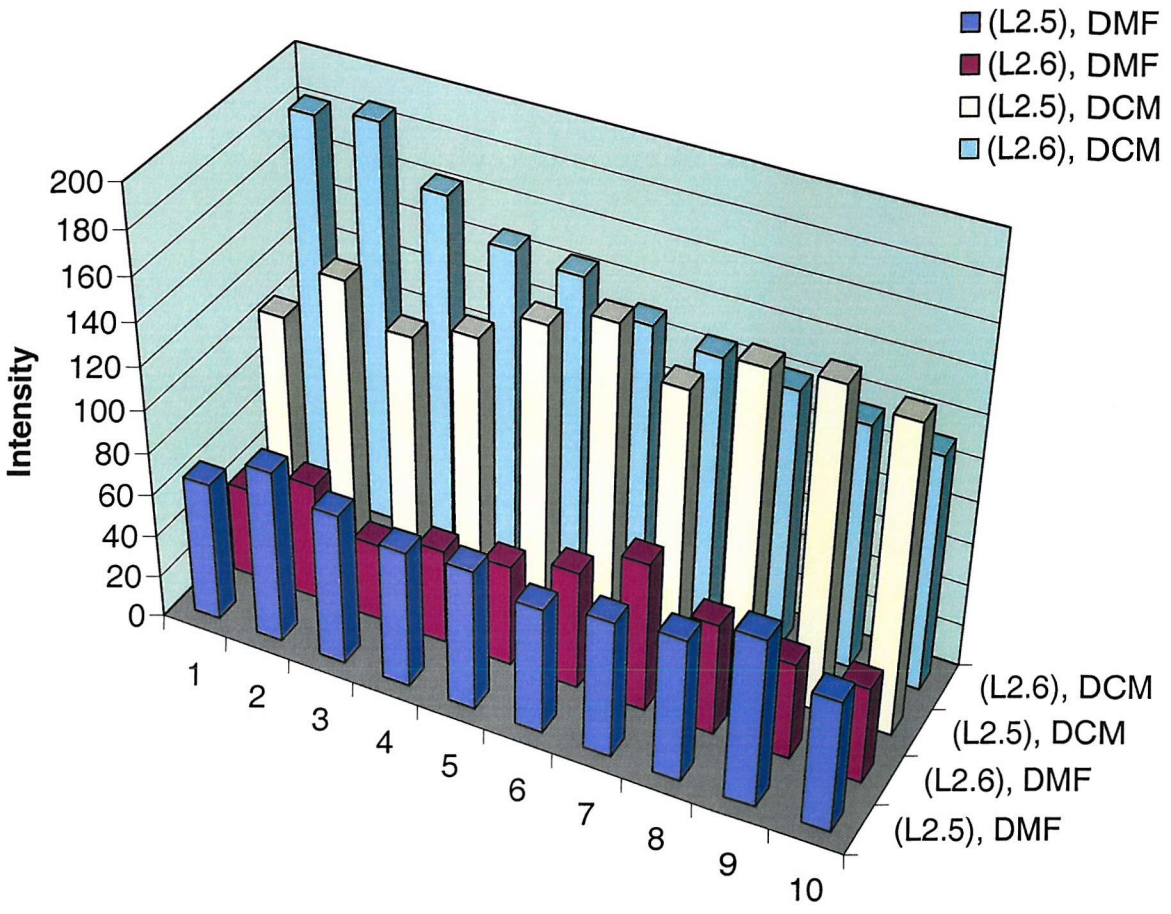


Figure 14. Fluorescence intensities of beads picked from libraries **(L2.5)** and **(L2.6).**

2.10. Sequencing of active beads selected from libraries (L2.5) and (L2.6).

Five active beads were picked from each sample (twenty total) and were sent for Edman sequencing, as a new facility had become available within the University. Of these, fifteen sequences were obtained, the other five being lost during sample preparation. **Table 1** shows the results of the sequencing.

Table 1. Sequencing results of active catalyst beads.

Sample	Fluorescence intensity	AA^3	AA^2	AA^1
(L2.5), DMF, 2	80	Phe	Arg	Arg
(L2.5), DMF, 5	65	Gln	Arg	Arg
(L2.5), DMF, 6	58	Ser	Gly	Arg
(L2.6), DMF, 5	48	Phe	Arg	Arg
(L2.6), DMF, 6	56	Val	Leu	Arg
(L2.6), DMF, 7	71	Arg	Val	Arg
(L2.6), DMF, 8	53	Ala	Arg	Gln
(L2.5), DCM, 4	126	Arg	Gln	Arg
(L2.5), DCM, 5	141	Gly	Arg	Arg
(L2.5), DCM, 6	151	Arg	Arg	Arg
(L2.5), DCM, 10	146	Leu	Arg	Arg
(L2.6), DCM, 1	178	Ala	Arg	Arg
(L2.6), DCM, 2	183	Arg	Leu	Leu
(L2.6), DCM, 4	143	Gln	Val	Arg
(L2.6), DCM, 5	140	Arg	Ala	Ala

As can be clearly seen from the table, all the samples contained the residue arginine (highlighted). The high degree of correlation between the samples from the two libraries

supports the screening technique as a detection method for catalysis, as the uptake of the dye was clearly dependent on the peptide sequence, and not a random occurrence. Following these results, arginine based peptides were examined in greater detail as catalysts for the Diels Alder reaction. These experiments and results are described in Chapter 3.

2.11. Conclusions.

A new method has been developed for the detection of catalytic activity from combinatorial libraries. The technique involves the partial substitution of TentaGel synthesis resin with a suitable substrate for the catalysis, and the generation of a library of potential catalysts on the remaining reaction sites. Site to site interaction between the substrate and library members allows catalytic interactions within each bead, and the modification of the substrate is detected by a colour change. In the research accomplished, a fluorescent dienophile was used to label beads from libraries of short peptides which showed catalytic activity for the Diels Alder reaction, by reaction with a diene substituted onto the same resin. Beads selected from these libraries were sequenced and a high degree of correlation between the sequences was observed. The residue arginine was especially prolific in the sequences obtained, and thus arginine based peptides will be investigated as Diels Alder catalysts.

The technique should be adaptable to the detection of catalytic activity for any reaction in which a colour change can be observed. The use of dye labelled substrates should, therefore, allow the modification of almost any reaction to investigation using this protocol.

Chapter 3. Synthesis and kinetic studies of arginine based Diels Alder catalysts.

3.1. Introduction.

From the catalyst screening experiments carried out and detailed in Chapter 2, it was discovered that the presence of arginine appeared to be an important factor for catalysis. Out of fifteen sequences obtained, all contained at least one arginine residue, and ten contained two, while one of the sequences was probably[Ψ] returned as Arg-Arg-Arg (see Chapter 2). Strong evidence thus existed that the amino acid arginine has a crucial role in the activity of these catalysts.

Six arginine based peptides as potential catalysts were therefore prepared and their activity examined through a series of solution phase experiments. Three of these catalysts were homogeneous, i.e. soluble in the reaction media, while the other three were solid supported or heterogenised. All the catalysts had the general structure Arg_n-ϵAhx-ϵAhx-X where n = 1, 2 or 3 for each set, and X = NH_2 for the homogeneous catalysts, and Met-TentaGel for the solid supported catalysts, analogous to the handle used in the solid phase screen.

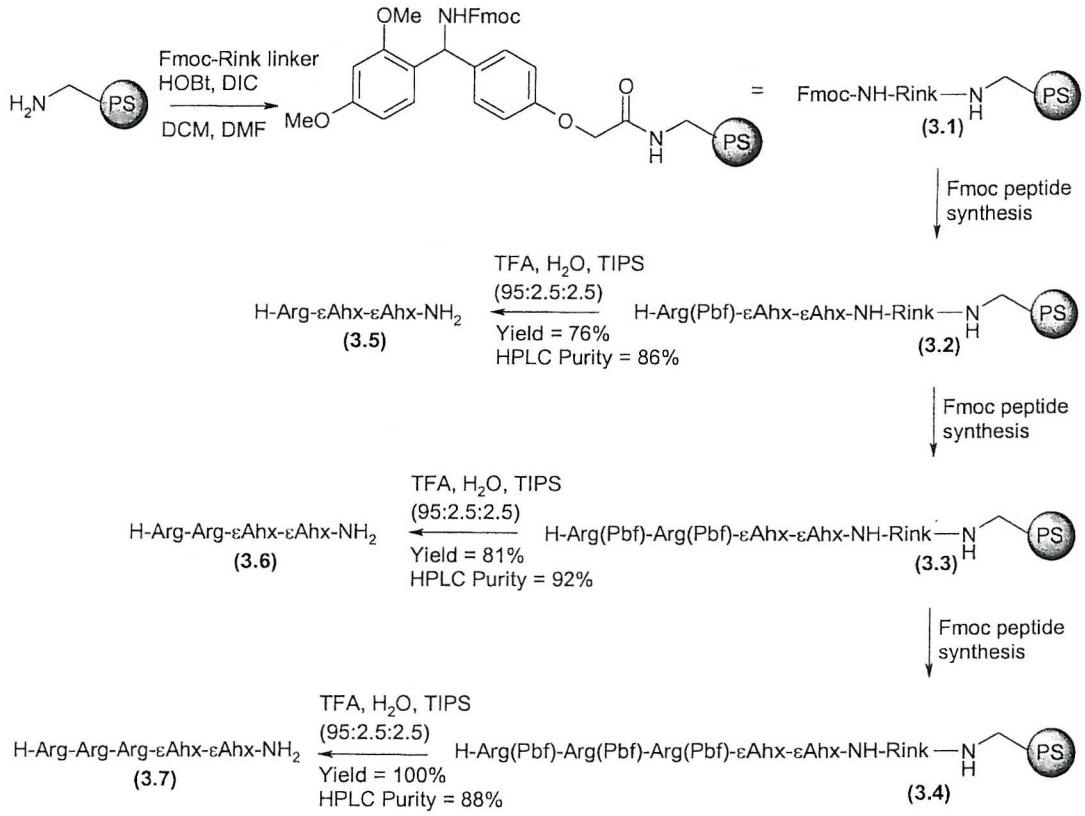
3.2. Synthesis of arginine based Diels Alder catalysts.

The homogeneous catalysts **(3.5-7)** were synthesised by solid phase peptide synthesis on aminomethyl polystyrene resin (2% cross linked with DVB) using the Fmoc strategy with a Rink amide linker,[89] which is cleaved with TFA to give carboxamides **(Scheme 50)**. The arginine groups were introduced with their side chains protected with the 2,2,4,6,7-pentamethyldihydrobenzofuran-5-sulfonyl (Pbf) group,[90] and the cleavage of the peptides as peptide amides was achieved by treatment with TFA/water/TIPS (95:2.5:2.5) which on

---

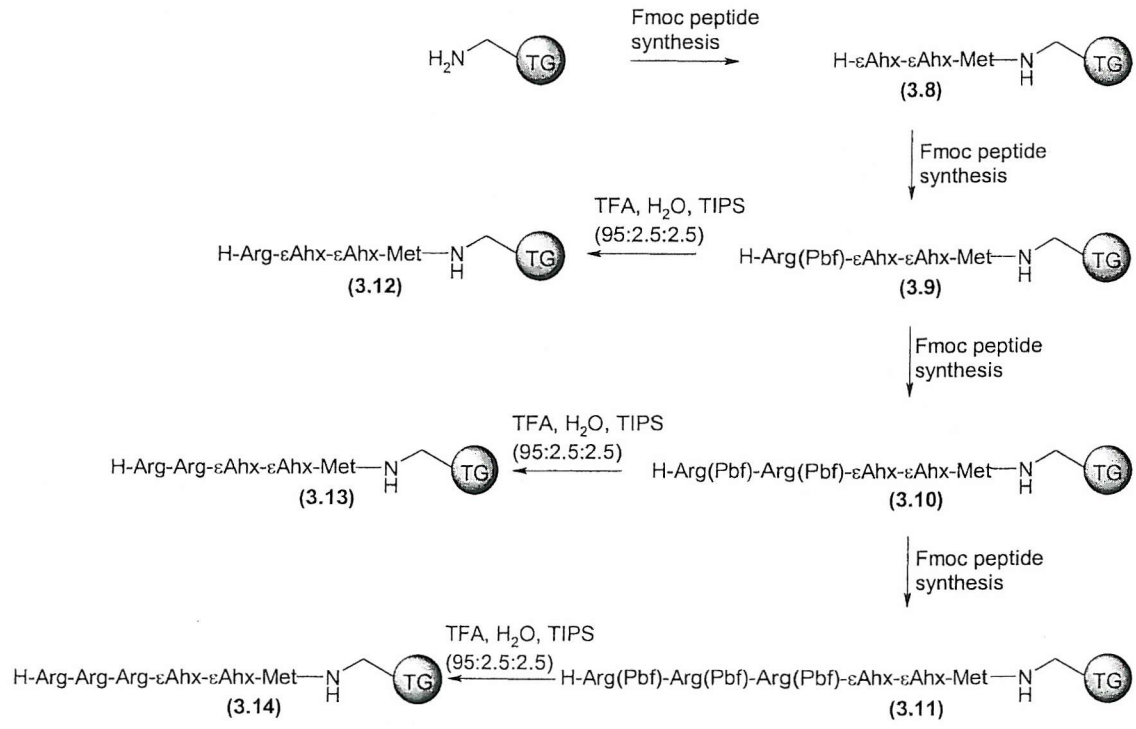
[Ψ] Sequencing of sample 6 from the screening in DCM of library **(L2.5)** returned Arg-Arg-Arg (see **Table 1**). There were, however, unusual signal strength differences between subsequent residues in the sequencing of this sample.

extended treatment also removes the Pbf groups.[91] The peptides were recovered in excellent yield and purity by precipitation from cold diethyl ether.



Scheme 50. Synthesis of homogeneous arginine based Diels Alder catalysts.

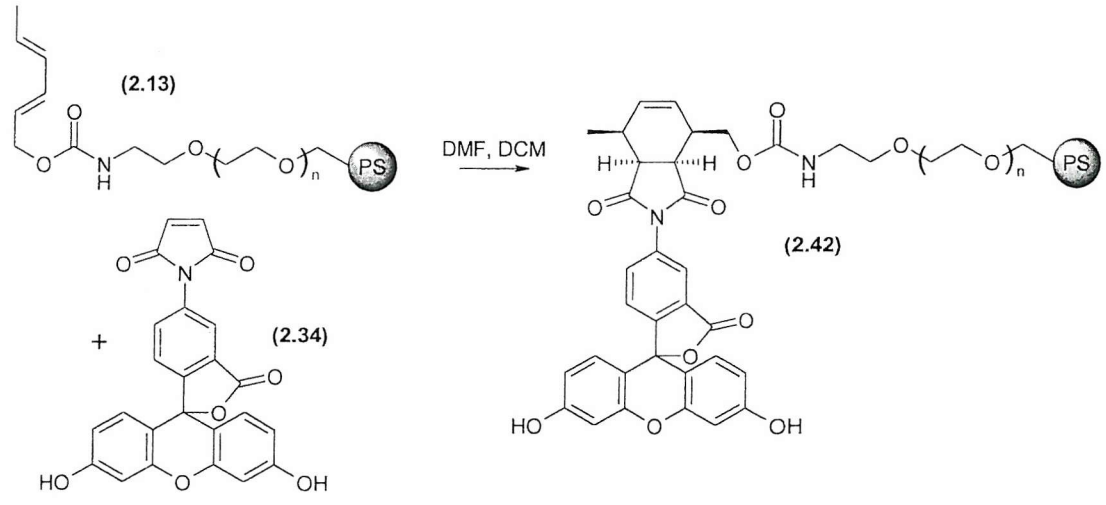
The solid supported catalysts **(3.12-14)** were synthesised in a similar fashion on TentaGel S NH_2 resin using the amino acid methionine in place of the Rink linker. Treatment with TFA/water/TIPS again removed the side chain protecting groups **(Scheme 51)**.



Scheme 51. Synthesis of solid supported arginine based Diels Alder catalysts.

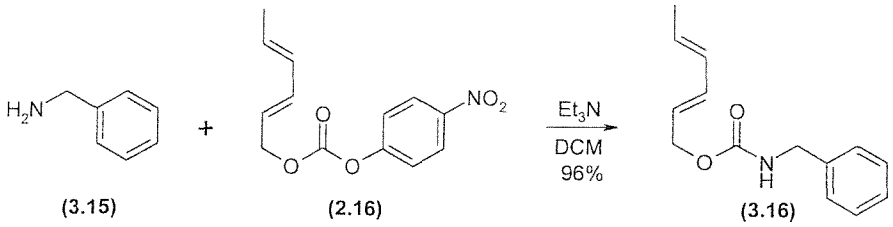
3.3. Kinetics studies of arginine based Diels Alder catalysts.

In order to test the efficacy of the catalysts it was first necessary to find a solution phase reaction which was similar to that used in the solid phase screening experiments **(Scheme 52)**.



Scheme 52. Reaction used in the solid phase screening experiments.

It was decided to use hexadienyloxycarbonyl benzylamine **(3.16)** as the diene for the reaction. The compound was readily synthesised in a manner similar to that used for the diene functionalisation of TentaGel, by the reaction of benzylamine **(3.15)** with *E,E*-2,4-hexadienyl-(4-nitrophenyl) carbonate **(2.16)** in the presence of triethylamine **(Scheme 53)**.



(3.15) (2.16) (3.16)

Scheme 53. Synthesis of a solution phase diene analogue.

The reactivity of compound **(3.16)** with *N*-(5-fluoresceinyl)maleimide **(2.34)** was then examined. During solid phase screening experiments, the modified fluorescein was dissolved at very low concentrations in DMF and added to the library sample which had been pre-swollen in DCM. This methodology was found to be impractical for a solution phase experiment as the solubility of the dye in this system was so low that massive solvent volumes were required to dissolve enough of the dye to obtain a reasonable amount of the product. A number of alternative solvent systems were tried, but in most cases the dye was found to have very low solubility. The compound was found to be soluble in basic water (pH ~ 10) but it was felt that the basicity of the solvent might have an overriding effect on any catalysis of the reaction. Moderate solubility in DMSO was observed, but it was observed that the compound was unstable in DMSO solution and degraded over time, while an odour of DMS was produced, suggesting that the dye had been oxidised by the DMSO in some manner. HPLC traces of this degradation over time are shown in **Figure 15** (DMSO peak has been removed for clarity).

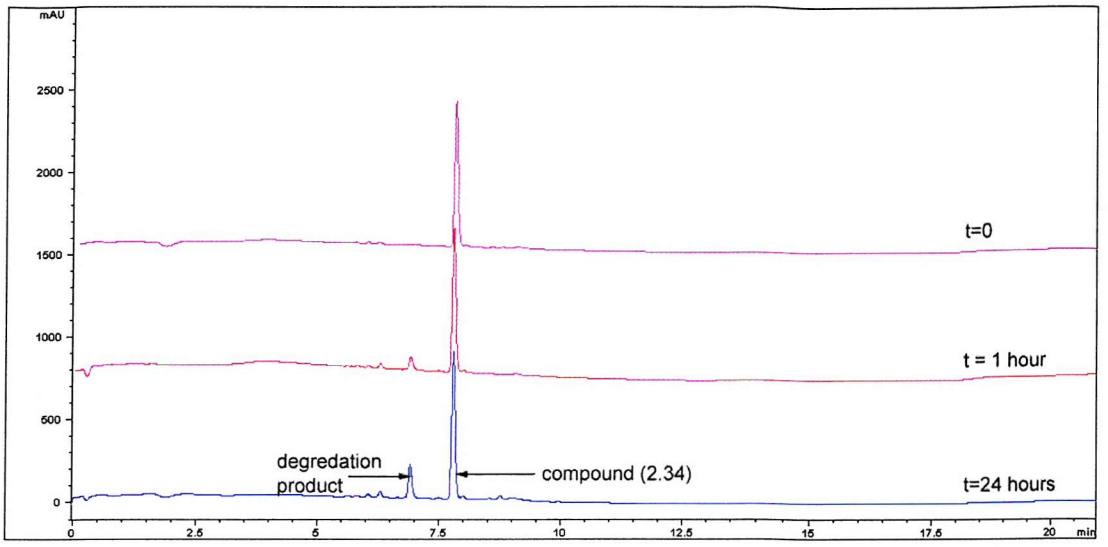
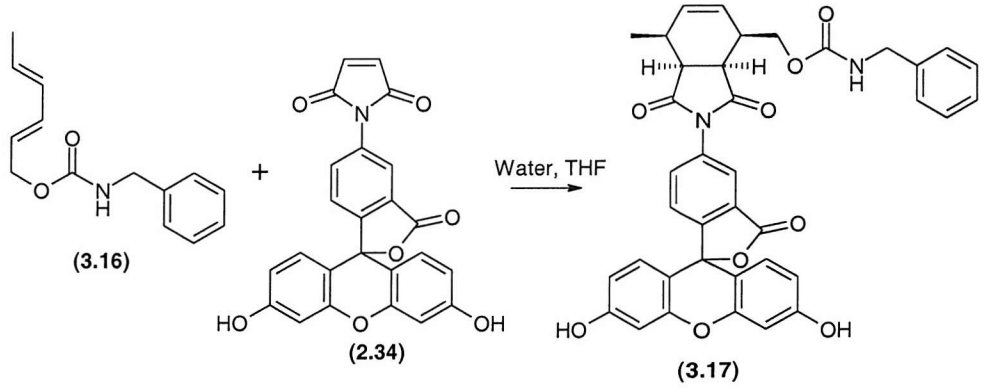


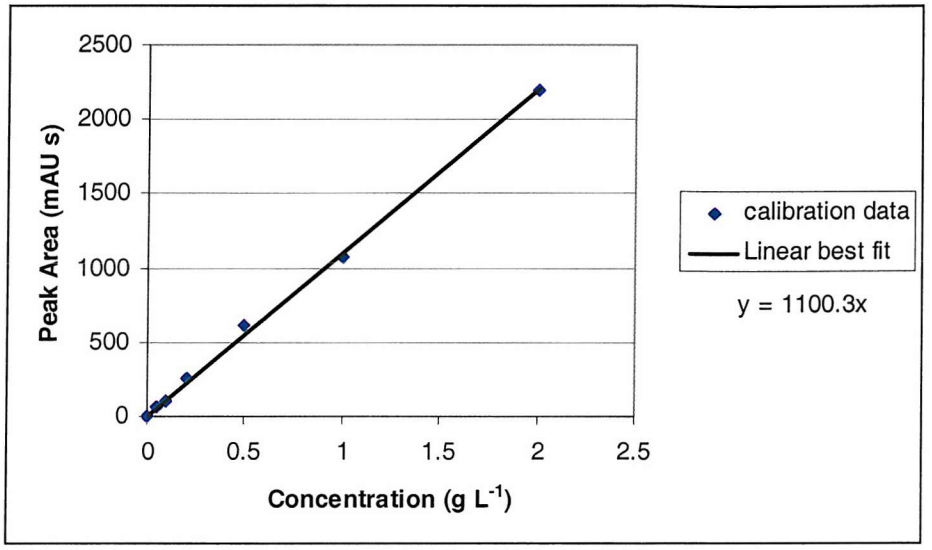
Figure 15. Degradation of compound (**2.34**) in DMSO.

In a 4:3 mixture of water/THF, both the dye and the diene were found to be moderately soluble and fairly stable, although some degradation of the dye over time was still observed. These were the only conditions, however, that gave satisfactory levels of solubility and thus these were used for the solution phase experiments. The Diels Alder reaction between *E,E*-2,4-hexadienyloxycarbonyl benzylamine and *N*-(fluorescein-5-yl)maleimide to form benzyl (2-(5-fluoresceinyl)-7-methyl-1,3-dioxo-3a,4,7,7a-tetrahydro-isoindol-4-ylmethyl) carbamate (**3.17**) was observed to be fairly slow (Error! Reference source not found.). An equimolar mixture of the reagents, each at 3.5 mM concentration in water/THF, was allowed to react at room temperature and aliquots were taken at regular intervals and analysed by RP-HPLC.



Scheme 54. Solution phase Diels Alder reaction.

The amount of product formed was determined by the integrated absorbance (peak area) at 220 nm, using a calibration curve generated by measuring the peak area formed by the injection of known concentration solutions. **Graph 1** shows the peak area plotted against concentration of the product, and a line of best fit has been generated. The plot shows the expected linear relationship between peak area and compound concentration, and allows the conversion of peak area measurements from experimental samples into the absolute amounts of compound **(3.17)** formed.



Graph 1. Calibration of Diels Alder product HPLC analysis.

The complete disappearance of *N*-(5-fluoreceinyl)maleimide was observed after approximately 3 days, however not all had undergone the Diels Alder reaction, as degradation of compound **(2.34)** was still observed as a competing reaction **(Figure 16)**.

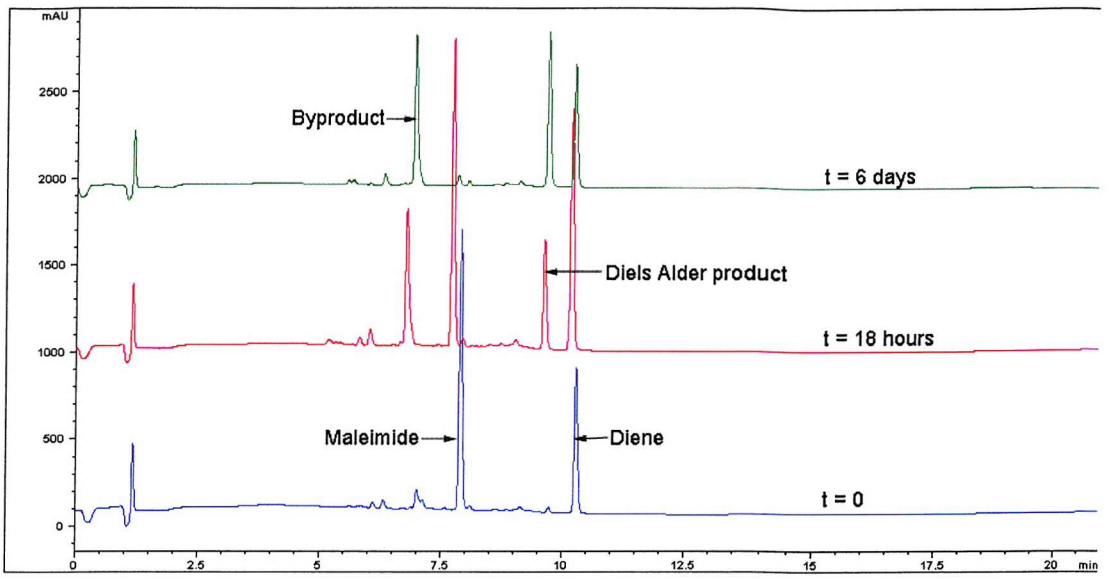
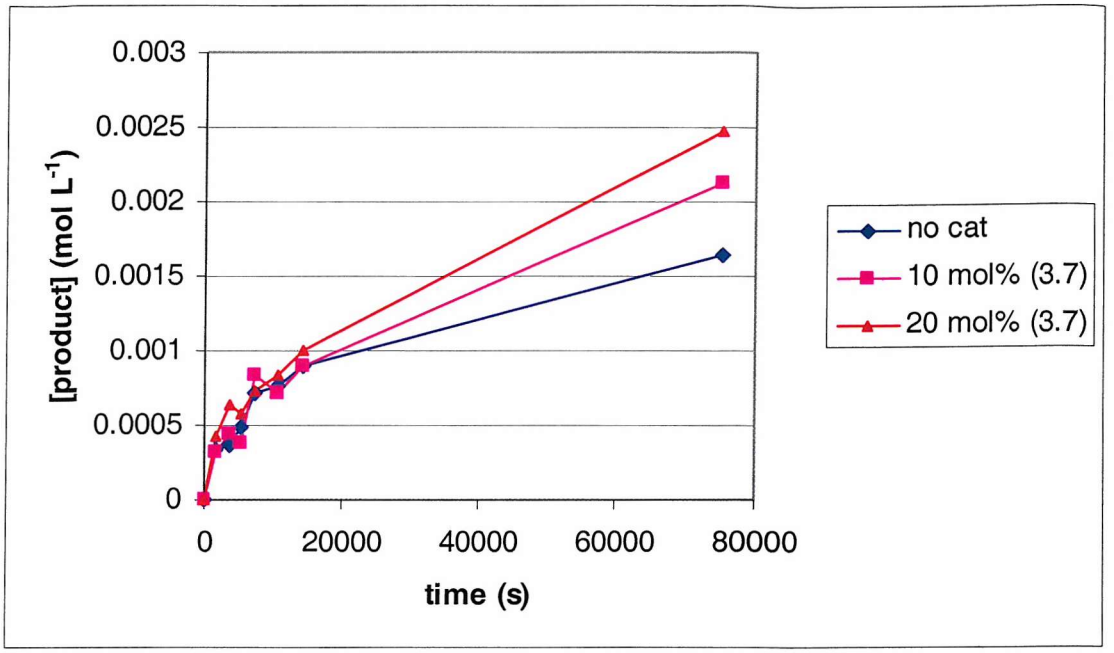


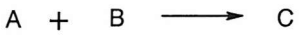
Figure 16. Overlaid HPLC traces for Diels Alder reaction profile.

Having established a method for monitoring the reaction, experiments were carried out to investigate the catalytic activity of the compounds identified from the catalyst library screening. In the first experiment an equimolar solution of *N*-(5-fluoresceinyl)maleimide and hexadienyloxycarbonyl benzylamine was split into three equal portions. The first portion was allowed to react without catalyst (control), to the second was added 10 mol% of the triple arginine based solution phase catalyst (**3.7**), and to the third, 20 mol% of the same catalyst was added. Aliquots were taken at regular intervals and immediately diluted with water and analysed by RP-HPLC. The results of this experiment are shown in **Graph 2**.



Graph 2. Catalysis of Diels Alder reaction by **(3.7)** at two concentrations.

It can clearly be seen from the graph that the introduction of the triple arginine compound **(3.7)** to the reaction mixture had a catalytic effect on the reaction. In order to quantify the efficiency of the catalyst it was necessary to determine the rate constants for the three reactions. The Diels Alder reaction follows a bimolecular mechanism, i.e. the rate determining step involves two molecules, and therefore follows second order kinetics. For the general bimolecular reaction shown in **Scheme 55**, the rate of the reaction is defined by **Equation 1**, where k is the rate constant for the reaction, and $[A]$ and $[B]$ are the concentrations of reagents A and B respectively.



Scheme 55. General bimolecular reaction.

$$Rate = k[A][B]$$

Equation 1.

Since both starting materials A and B are in equal concentration at the start of the reaction, and are consumed at the same rate, $[B]$ can be replaced in the equation by $[A]$. The rate of

reaction is defined as the change in concentration of starting material with respect to time, giving an alternative form shown in **Equation 2.**

$$-\frac{d[A]}{dt} = k[A]^2$$

Equation 2.

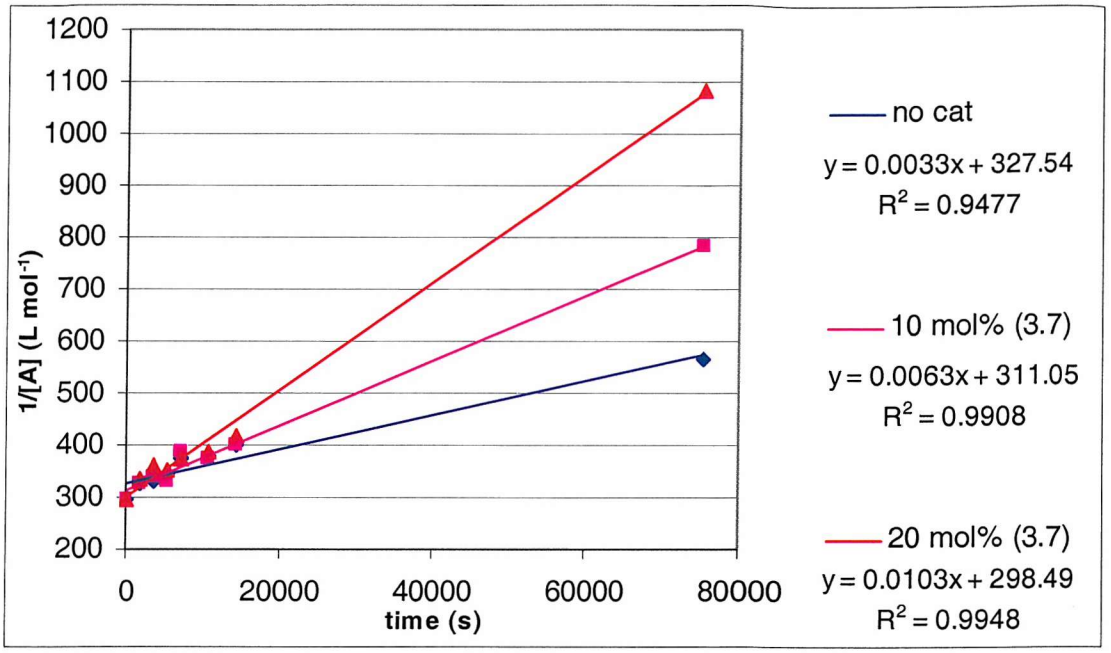
Rearranging and integrating both sides of the equation gives the integrated rate law **(Equation 3)**,[92] where $[A]_0$ is the concentration of A at t = 0.

$$\frac{1}{[A]} = kt + \frac{1}{[A]_0}$$

Equation 3.

From this equation it can be seen that for a second order reaction, a plot of $1/[A]$ against t will give a straight line of gradient k. In the experiments conducted, however, the concentration of product rather than starting material was measured, thus values of [A] were calculated by subtracting the concentration of product from the initial concentration $[A]_0$.

For each of the three experiments $1/[A]$ was plotted against t, and a linear line of best fit generated using linear regression. The units of t have been changed to seconds (s) so that the rate coefficient k has the standard units (L mol^{-1} s^{-1} for a second order reaction). The results are shown in **Graph 3** along with the equations of the best fit lines from which the values of k for each reaction can be determined.



Graph 3. Second order plot of reaction data for 10 and 20 mol% compound (**3.7**).

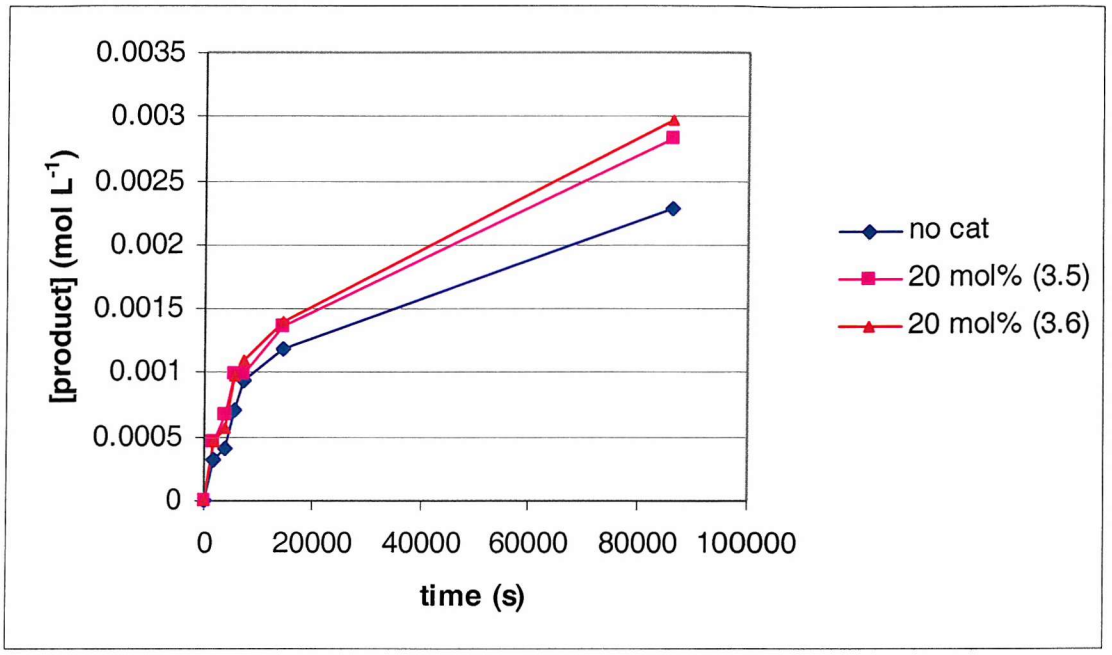
From these graphs it can be seen that there is a good fit to second order kinetics, as shown by the good R^2 values for the lines of best fit. The values for k, determined from the gradient, show rate accelerations for both catalysed reactions over the uncatalysed reaction as shown in **Table 2**. The observed rate accelerations of 1.9 and 3.1 fold for 10 and 20 mol% respectively were not as high as had been hoped, however the results indicated that compound (**3.7**) was indeed a catalyst for the Diels Alder reaction.

Table 2. Observed rate accelerations from compound (**3.7**).

Catalyst	Rate coefficient (k) (L mol^{-1} s^{-1})	Rate acceleration (k_{cat}/k_{uncat})
None	0.0033	1
10 mol% (**3.7**)	0.0063	1.9
20 mol% (**3.7**)	0.0103	3.1

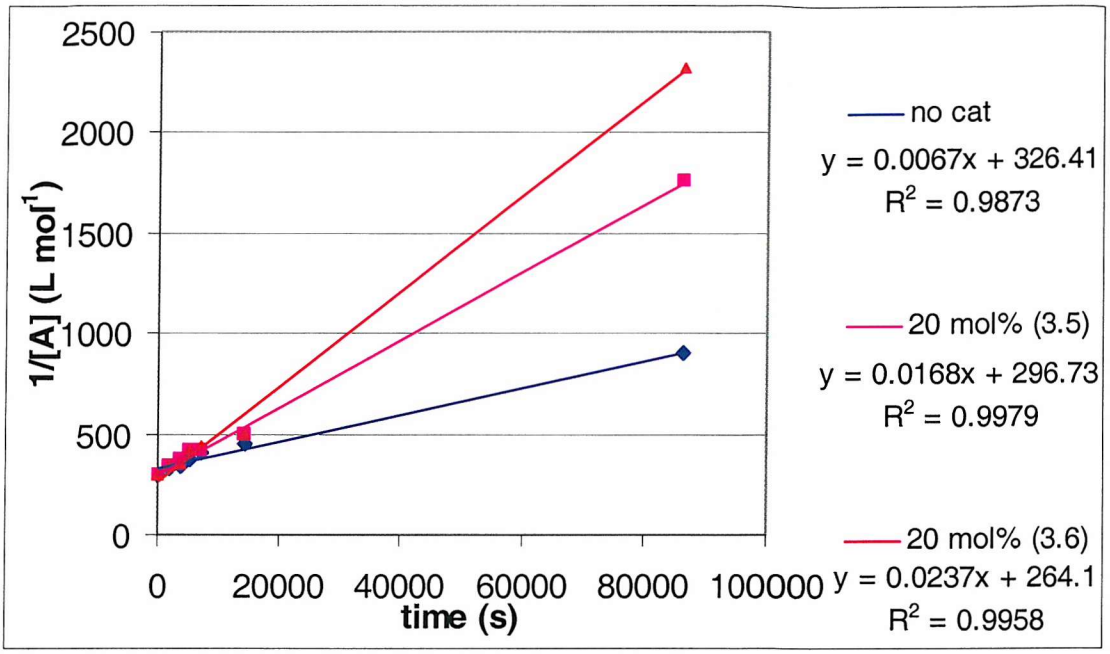
Following these results it was decided to analyse the efficiency of the other catalysts at 20 mol% in order to increase the observed rate accelerations. The reaction was therefore

repeated, replacing compound (**3.7**) with the single and double arginine based homogeneous catalysts (**3.5 & 3.6**) at 20 mol%. The same reaction conditions were employed and samples were taken and analysed by the same technique. A plot of the concentration of product against time for the three experiments is shown in **Graph 4**.



Graph 4. Catalysis of Diels Alder reaction by (**3.5 & 3.6**).

From this graph it can be seen that both these compounds also have catalytic activity. Replotting the data to obtain second order rate constants gives the values of *k* shown in **Graph 5**.



Graph 5. Second order plot of reaction data for catalyst **(3.5 & 3.6)**.

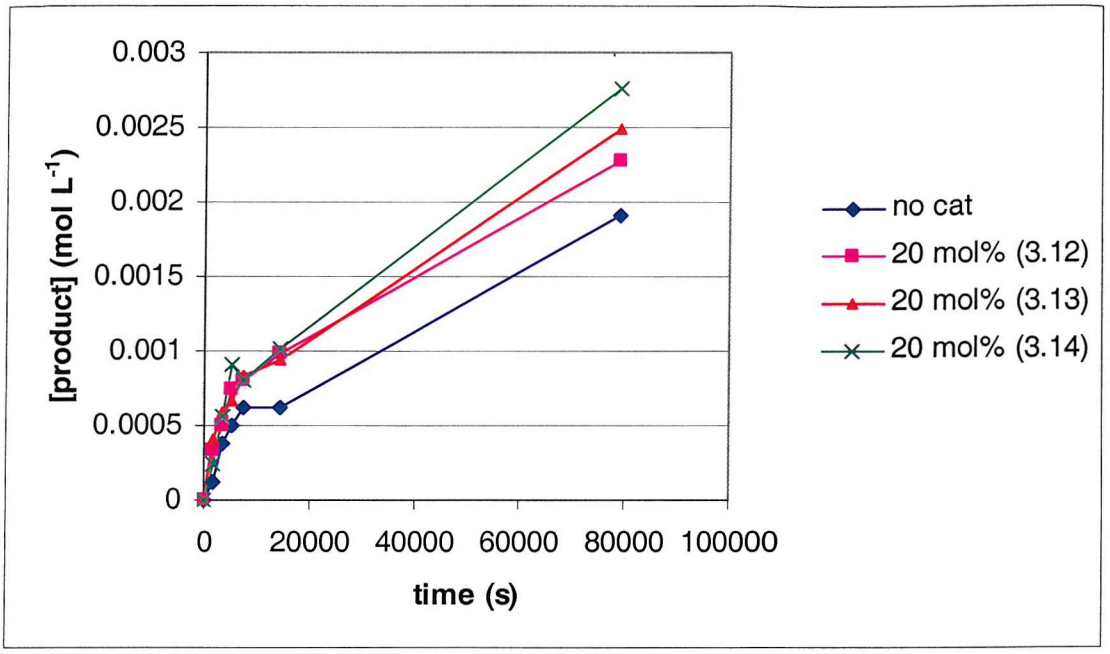
Table 3 shows the observed rate accelerations for catalysis of the Diels Alder reaction by compounds **(3.5 & 3.6)**. Again the rate accelerations are relatively low (2.5 and 3.5 respectively) but indicate that these compounds have catalytic activity.

Table 3. Observed rate accelerations from compounds **(3.5 & 3.6)**.

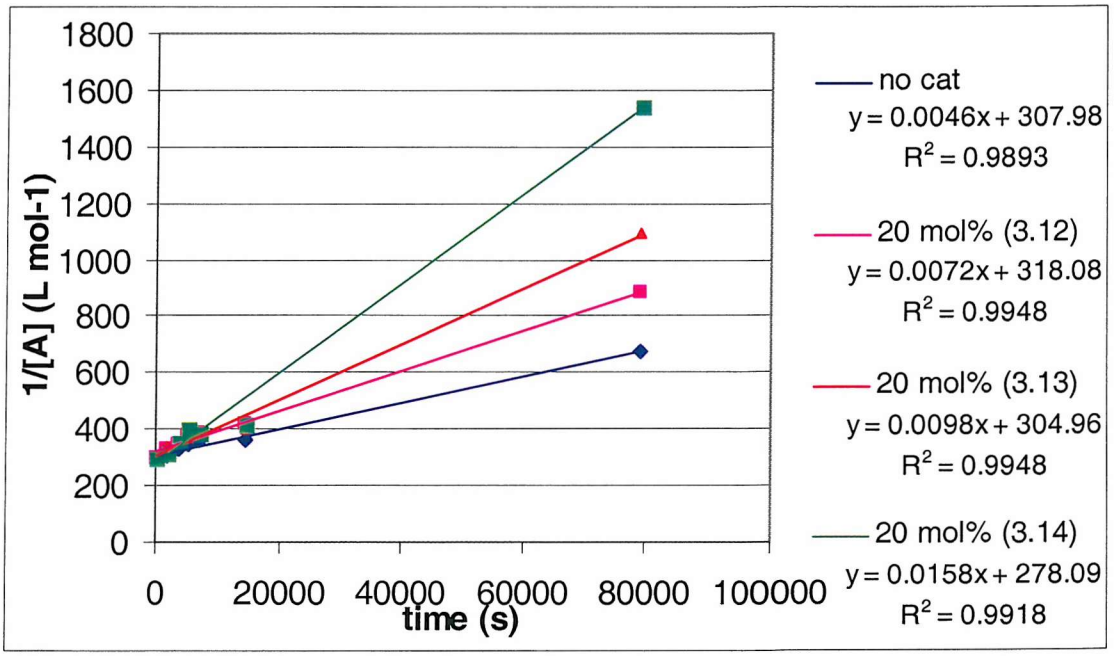
Catalyst	Rate coefficient (k) (L mol^{-1} s^{-1})	Rate acceleration (k_{cat}/k_{uncat})
None	0.0067	1
20 mol% **(3.5)**	0.0168	2.5
20 mol% **(3.6)**	0.0237	3.5

The solid supported catalyst candidates **(3.12-3.14)** were tested under identical conditions, shaking the vessels to keep the catalyst resin suspended in the reactions medium. **Graph 6** shows concentration of product formed plotted against time, and again shows that the reactions containing the solid supported compounds proceed faster than the control reaction, therefore these compounds do act as catalysts. Again, second order plots of the

data for the control reaction and the three catalysed reactions enabled values of *k* to be determined (**Graph 7**).



Graph 6. Catalysis of Diels Alder reaction by solid supported compounds (**3.12-3.14**).



Graph 7. Second order plot of reaction data for solid supported catalysts (**3.12-3.14**).

The calculated values for *k* for each experiment and the observed rate accelerations are shown in **Table 4**. The solid supported catalysts **(3.12-3.14)** show similar levels of activity to their solution phase analogues. This is an encouraging result as it suggests that the transfer of catalyst structure from the compounds selected from a solid supported library to a solution phase analogue with slightly different structure, does not lead to a large change in activity and therefore the solid supported library is a useful model for solution phase catalysts.

Table 4. Observed rate accelerations from compounds **(3.12-3.14)**.

Catalyst	Rate coefficient (*k*) (L mol^{-1} s^{-1})	Rate acceleration (k_{cat}/k_{uncat})
None	0.0046	1
20 mol% **(3.12)**	0.0072	1.6
20 mol% **(3.13)**	0.0098	2.1
20 mol% **(3.14)**	0.0158	3.4

3.4. Conclusions.

Analysis of the catalysis of the Diels Alder reaction by the arginine containing peptides **(3.5-3.7, 3.12-3.14)** shows that these compounds do indeed act as catalysts for the reaction of *N*-(5-fluoresceinyl)maleimide **(2.34)** with hexadienyloxycarbonyl benzylamine **(3.16)**. The rate accelerations were between 1.6 and 3.5 depending on the catalyst structure, the compounds containing a greater number of arginine residues giving the higher rate accelerations. This low level of catalysis is somewhat at odds with the high level of selectivity observed in the catalyst screening experiments, where 'active' beads showed levels of fluorescence much greater than the 'background' beads. One might expect that lower levels of catalytic activity observed from these kinetic experiments would have led to much lower differentiation between catalytically active and inactive beads.
Three possible solutions to the discrepancy between these results spring to mind. Firstly, that the limiting conditions applied to the catalyst library screen, where the diene was in vast excess compared to the fluorescent dienophile **(2.34)**, leads to an exaggeration of the catalytic effect, i.e. that a small catalytic acceleration leads to a large difference in the

amount of fluorescent dienophile taken up by an active bead. This explanation is plausible as long as the Diels Alder reaction is essentially irreversible as would be expected in this case. In an irreversible reaction a relatively small rate acceleration will allow the active beads to rapidly consume all the available dienophile, leading to a similar visual result to that which would be observed for much higher rate accelerations. With a reversible reaction however, the small rate difference between the reaction rates in active and inactive beads would lead to a rapid equilibration of the dienophile across the whole library, and therefore a loss of selectivity. This might not be observed in a screen where larger differences in the rate of reactions existed, and the uncatalysed reaction was so slow that this equilibration did not occur.

A second possible explanation is that a non-reactive binding interaction between the 'active' peptides selected by the screen acted to increase the local concentration of the dienophile within the beads, leading to a higher rate of Diels Alder reaction within these beads. This would not in fact be catalysis of the reaction, as the increase in dienophile uptake by an 'active' bead would not be due to a modification of the reaction rate constant *k*, as observed in a catalytic rate acceleration, but rather due to increased concentration of starting material. It was shown in a control reaction that no permanent binding interaction between the peptides and dienophile occurred, as a washing step removed all the dye from a sample of the library which had not been substituted with diene, but even a weak binding interaction could lead to a local concentration difference and therefore different reaction rates within different beads. The kinetic analysis has however shown that these arginine containing compounds are catalysts for the Diels Alder reaction, therefore if this explanation is correct, the observed fluorescence difference must be due to a combination of both a binding and catalytic effect.

The third explanation for the discrepancy between the observed fluorescence difference between active and inactive beads and the rate accelerations generated by the selected catalysts, lies in the difference in the substrates used for the screening reaction and the kinetics experiments. During experiments involving the selection of catalytically active RNA strands for the Diels Alder reaction, as described in the introduction to Chapter 2, both Jäschke *et al.*[71] and Tarasow *et al.*[68] found that even small variations in the diene or dienophile from the ones used in the selection experiments resulted in a large drop in catalytic activity. The multiple rounds of catalyst selection and elaboration used in these

groups' experiments led to this high level of substrate specificity however, and it seems unlikely that this level of substrate dependence would be observed from the single round of selection that identified these arginine based catalysts.

In conclusion a new method for the discovery of catalysts from combinatorial libraries, which relies on site to site substrate-catalyst interaction within flexible PEG based TentaGel resin, has been used to examine a split and mix library of small peptides for activity as Diels Alder catalysts. Peptide sequences obtained from beads which the screen had labelled as active, were found to have a high degree of correlation, and the amino acid arginine was found to be common to all the sequences obtained. Based on the results of this screen, six arginine containing peptides, three of them attached to a solid support, of similar structure to the general structure of the library. Compounds were synthesised using Fmoc solid phase peptide synthesis and kinetic experiments were undertaken to determine the level of activity of these compounds as Diels Alder catalysts. All of the compounds were found to be catalysts for the Diels Alder reaction between *N*-(5-fluoresceinyl)maleimide **(2.34)** and hexadienyloxycarbonyl benzylamine **(3.16)**, although the rate accelerations observed were not as high as had been hoped.

These results show that the technique described for the discovery of new catalysts from large split and mix libraries could be a valuable new method in the combinatorial discovery and development of catalysts. The technique can be readily adapted to any catalytic bond forming reaction, as long as one substrate can be immobilised onto a solid support and the other labelled with a fluorescent dye. It has also been demonstrated that peptides can act as catalysts for the Diels Alder reaction in the absence of transition metals.

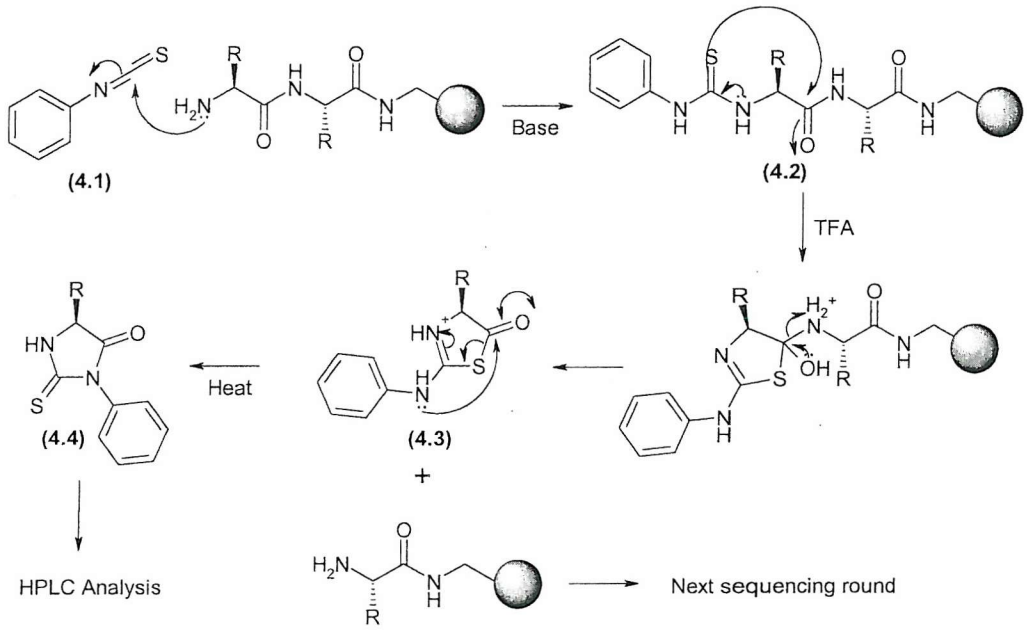
Chapter 4. Ion-Extraction Ladder Sequencing of Peptides.

Note: The work described in this chapter was done in collaboration with Jose J Pastor of the University of Barcelona. The research presented in this chapter has been submitted for publication in the Journal of Combinatorial Chemistry.

4.1 Introduction to peptide sequencing.

Since the introduction of combinatorial 'split and mix' solid phase synthesis by Furka *et al.* in 1991,[34] peptide libraries have been important for the discovery of active peptides for a variety of biological applications. Early developments included the discovery of ligands to a monoclonal antibody against β-endorphin,[35] and peptide based antimicrobials.[93] The use of combinatorial chemistry has certain drawbacks however, especially the identification of compounds. Once a bead substituted with a compound of interest has been selected from a library due to its desired biological activity or binding affinity, it is necessary to obtain the identity of this active molecule. In the case of peptides this is traditionally achieved by Edman sequencing. Developed in the 1950s by Pehr Edman, the technique was originally developed for the sequencing of small peptide fragments resulting from enzymatic digests of proteins, in order to obtain their primary structure. As a result it was originally a solution phase technique, however the first step of the process involves the immobilisation of the peptide onto a solid support, such as a modified glass disc or membrane. In the case of peptides from solid supported combinatorial libraries, this immobilisation step is not required, as the resin bead acts as the solid support for the sequencing. Treatment of the immobilised peptide with excess phenylisothiocyanate **(4.1)** under basic conditions causes the formation of a thiourea **(4.2)** with the amino acid at the *N*- terminus **(Scheme 56)**. The excess phenylisothiocyanate is removed by washing, and treatment with TFA leads to cyclisation onto the amide carbonyl, and hydrolytic cleavage to give the thiazolinone derivative of the terminal amino acid **(4.3)**, leaving the remainder of the peptide attached to the solid phase. The thiazolinone is then incubated under acidic conditions, and rearranges to the phenylthiohydantoin amino acid derivative (PTH-amino

acid) (**4.4**). The amino acid is identified by HPLC analysis of this PTH-amino acid, as these have very distinct and well defined retention times (**Figure 17**). As the reaction of the *N*-terminal amino acid with phenylisothiocyanate only occurs under basic conditions, and the cyclisation/cleavage step under acidic conditions, only the terminal amino acid is cleaved. The remaining peptide is then subjected to repeated reaction cycles, cleaving and identifying one amino acid from the *N*-terminus each round.



Scheme 56. Mechanism of Edman peptide sequencing.

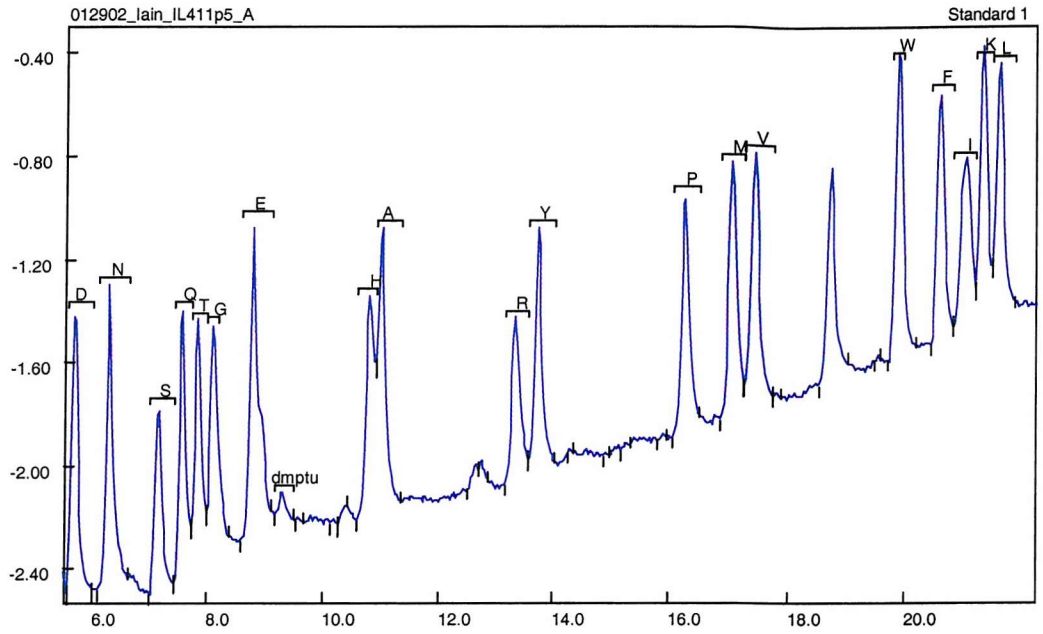


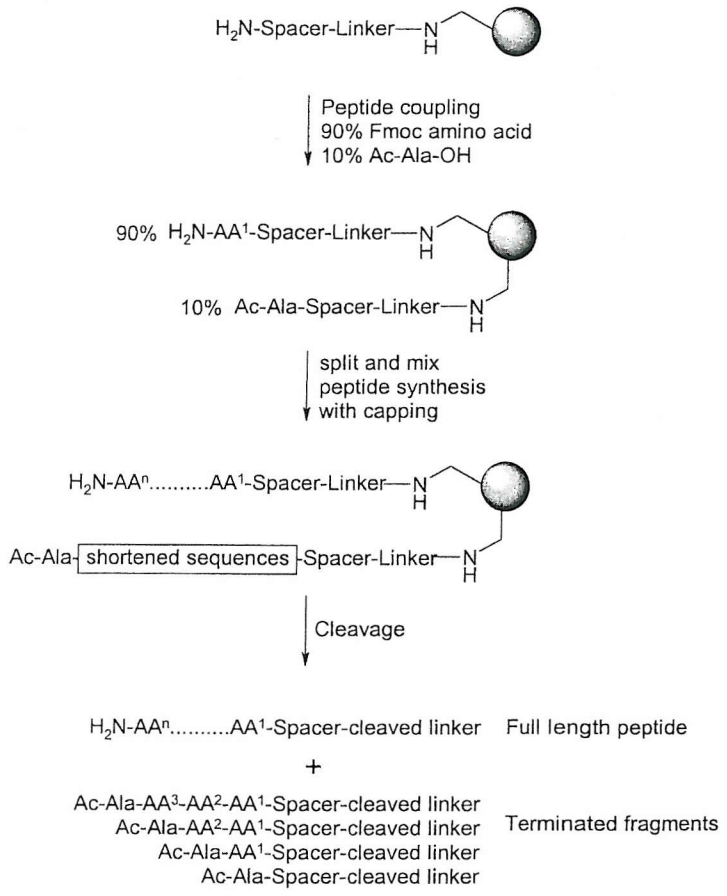
Figure 17. HPLC trace of the 20 naturally occurring PTH-amino acids.

Edman sequencing is an excellent method for the accurate determination of peptide composition, and has been optimized and automated with great success, but nevertheless has several drawbacks. Firstly, the chromatography has been optimized for the analysis of PTH derivatives of the 20 naturally occurring amino acids, and is therefore not suitable for the sequencing of peptides containing modified residues. Secondly, automated sequencers are expensive and their running costs are relatively high, therefore sequencing is often contracted out at a per residue cost and can be uneconomic for large numbers of samples. Thirdly, it is time consuming as the reaction-cleavage-conversion-analysis takes approximately two hours for each residue, sequencing of longer peptides therefore takes many hours.

In order to obtain sequences from the potentially large numbers of samples generated by a combinatorial library screening, alternative strategies have been developed. Positional encoding, for example, allows a database to be generated from which the structure can be obtained from the location of the target within a defined spatial array. This methodology is demonstrated by pin synthesis,[94] string synthesis.[95] Chemical encoding is carried out by the tagging of the solid phase with reporter groups, for example halogenated tags,[38] which can be cleaved from the beads and read using electron capture gas chromatography. The

presence of certain tags gives the synthetic route and therefore the structure of the compound attached to the bead. Recently electronic encoding has become available through the utilization of radio frequency transponders, whose individual codes can be read by a computer with the required peripheral device and a database can be used to determine the sequence.[96,97] This technology can be used only in conjunction with systems for the containment of resin aliquots such as 'teabags'[98] or microkans.[99]

An elegant solution to the problem of peptide sequencing was reported by Youngquist and Keough[100, 101] with the introduction of the concept of ladder sequencing. In this method a peptide library was generated on a defined peptide linker and spacer using 'split and mix' synthesis. During each coupling reaction however, a small amount of *N*-acetyl-alanine was mixed with each of the Fmoc amino acids used for the coupling (except for the final residue), capping a small percentage of the growing peptide in each library member. At the end of the synthesis, each bead carried the full length peptide plus a family of truncated peptides caused by chain termination **(Scheme 57)**.



Scheme 57. Synthesis of a peptide ladder for sequencing by mass spectrometry.

Once active beads had been selected and picked from the library, the peptide was cleaved from the resin along with the capped fragments and the mixture analysed by matrix assisted laser desorption/ionisation time-of-flight (MALDI-TOF) to give a 'ladder' of peaks. The peptide sequence could then be determined from the mass difference between adjacent peaks from the *N*-terminus (full length peptide) at high mass to the shortest ladder fragment (spacer and cleaved linker only) at low mass, each peak representing one of the truncated library members. A graphical representation of a spectrum generated by this method is shown in **Figure 18**.

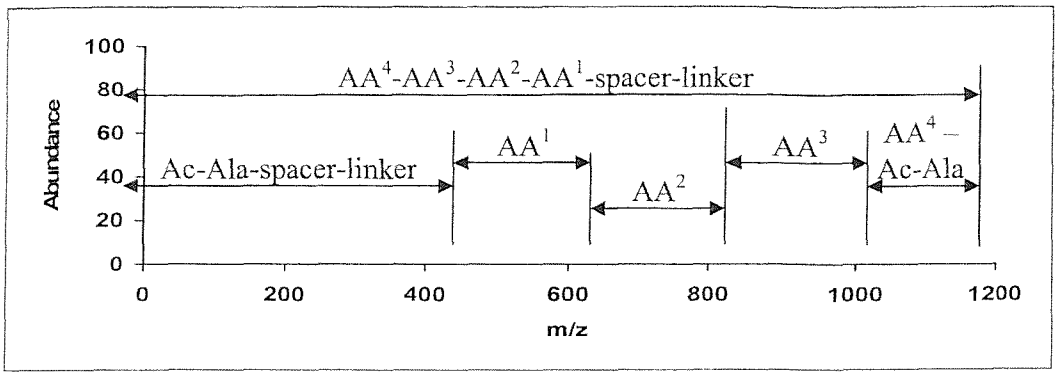
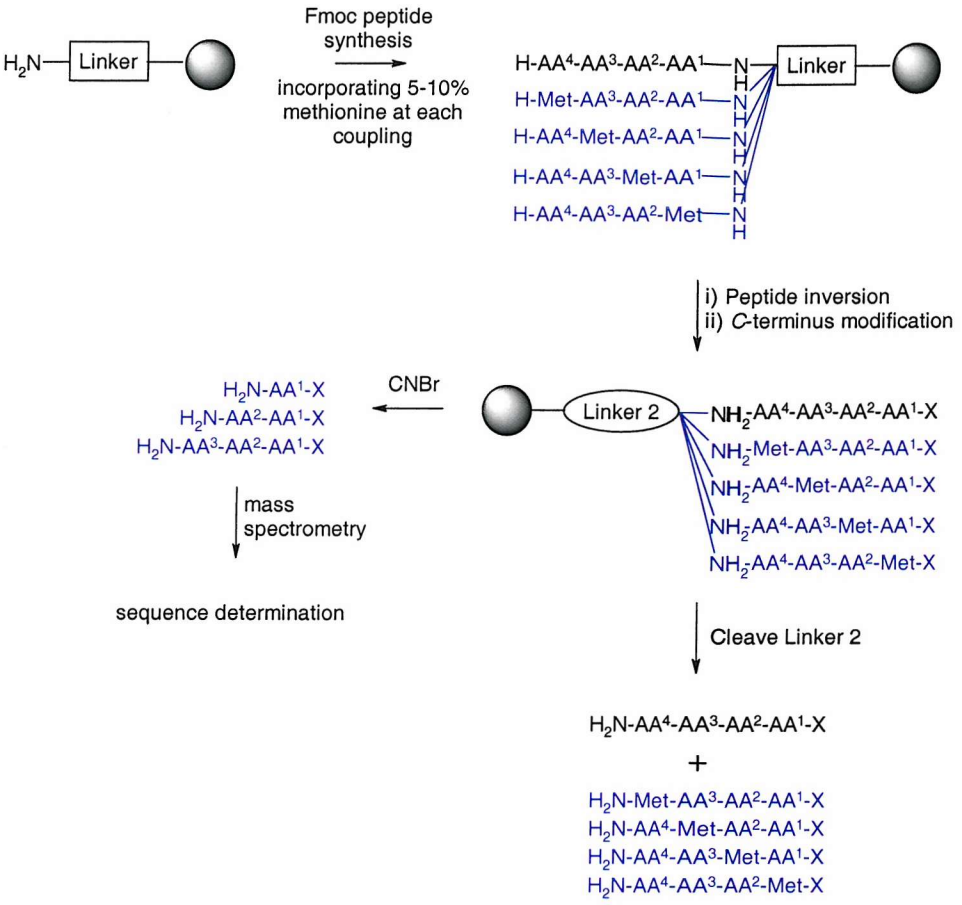


Figure 18. Stylised representation of a peptide ladder sequence obtained from MALDI-TOF mass spectroscopy of a peptide ladder generated by Youngquist and Keough's method.

Modification of this technique has been reported by several groups. For example, instead of capping the growing peptide chain, Bradley *et al.* partially incorporated the amino acid methionine in a similar fashion.[102] Methionine can be selectively cleaved in the presence of other amino acids by treatment with cyanogen bromide,[103] thus creating the family of chain shortened fragments required for ladder sequencing. This method was chosen instead of the capping described previously, as the peptide was inverted on the solid phase *via* a cyclisation/cleavage strategy, exchanging the attachment point from the *C*-terminus to the *N*-terminus such that the *C*-terminus could be modified prior to cleavage.[104] In order to achieve this it was essential that the full length peptide and the methionine encoded peptides were all cyclised. A capping strategy could not have been employed as this would have blocked the *N*-terminus of the ladder fragments preventing cyclisation **(Scheme 58)**.

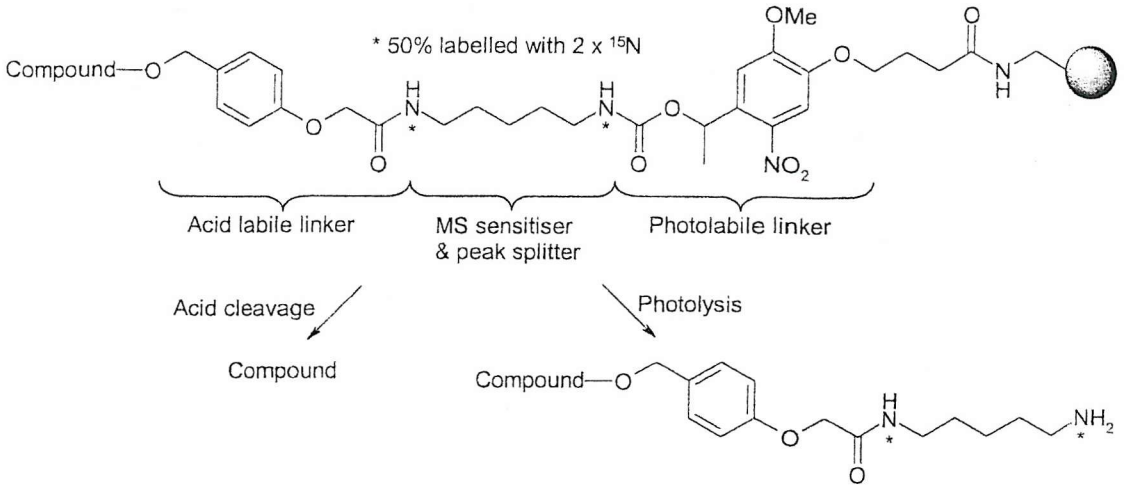


Scheme 58. Synthesis of inverted peptides incorporating methionine for ladder sequencing.

A recent paper by Griesinger *et al.* describes the use of dual capping groups and software based analysis of molecular ion redundancy to enable the MS based sequencing of defined peptide libraries without the need for capping at every synthesis step.[105]

4.2. Isotopic enhancement for improved mass spectrometry analysis.

Mass spectrometry has become the method of choice for the analysis of compounds selected from one bead one compound libraries due to its high sensitivity, which allows analysis of even the very small amounts of compound obtained from the cleavage of a single bead (typically a few hundred pmoles). This level of sensitivity is not always enough however, particularly when the synthesis or cleavage has led to significant amounts of by products in the sample. Three groups of scientists at Glaxo Wellcome have developed an excellent method for avoiding the difficulties involved in the analysis of mass spectrometry samples from single bead cleavages. Initially reported by Geysen *et al.*,[106] isotopic peak splitting involves the incorporation of a 1:1 ratio of two stable isotopes (initially reported with $^{14}N/^{15}N$) into the analyte molecule. The mass spectrum will then display a doublet for this compound, allowing it to be distinguished from the background and impurity peaks in the spectrum. Building on this work, and the work of Carrasco *et al.*, in which a dual linker strategy incorporating a quaternary ammonium group as a MS sensitiser was developed,[107] McKeown and co-workers developed dual linker analytical constructs containing isotopic peak splitting labels for monitoring and analysing reactions and compounds on the solid phase. The strategy involves the use of two orthogonal linkers, one photolabile, and the other acid cleavable, with an isotopically labelled mass sensitiser between the two **(Scheme 59)**.[108] Treatment of the resin with acid releases the synthesised compound into solution for screening in the classical way, while U.V. irradiation cleaves the photolabile linker releasing the compound attached to the mass sensitiser. The analysis of this compound is thus aided by the presence of a primary amine to enhance ionisation, and a isotopic peak splitter to aid the observation of the signal against any impurities and spectral noise.



Scheme 59. A photolabile analytical construct.

Several other examples of analytical constructs making use of this strategy have been developed, with a wide range of linkers,[109,110,111] and including other features such as chromophores.[112] The use of peptides containing two peak splitting isotopic labels (one at each terminus) has allowed the use of this technique for identifying protease specificity.[113] An elaboration of this peak splitting strategy has been developed by Lane and Pipe, in a protocol they entitle 'accurate isotopic difference analysis'.[114,115] The technique involves the use of isotopically labelled tags, which are used to label compounds on the solid support. On cleavage, the compound is released from the resin with attached tags incorporating a peak splitting isotopic label. Software can then be used to extract from the mass spectrum only the peaks which have a partner peak at approximately equal intensity with an accurate mass difference which matches the isotopic label.

4.3. An improved method for peptide sequencing from combinatorial libraries.

It was proposed that Youngquist and Keough's method for the sequencing of peptides from combinatorial libraries[100,101] could be enhanced by combination with Lane and Pipe's isotopic labelling and data analysis,[114,115] whereby the incorporation of an isotopic label into the capping group used for ladder generation, would allow data reduction software to

select the peptide ladder peaks from the background noise and impurities, therefore obtaining an excellent signal to noise ratio (**Figure 19**).

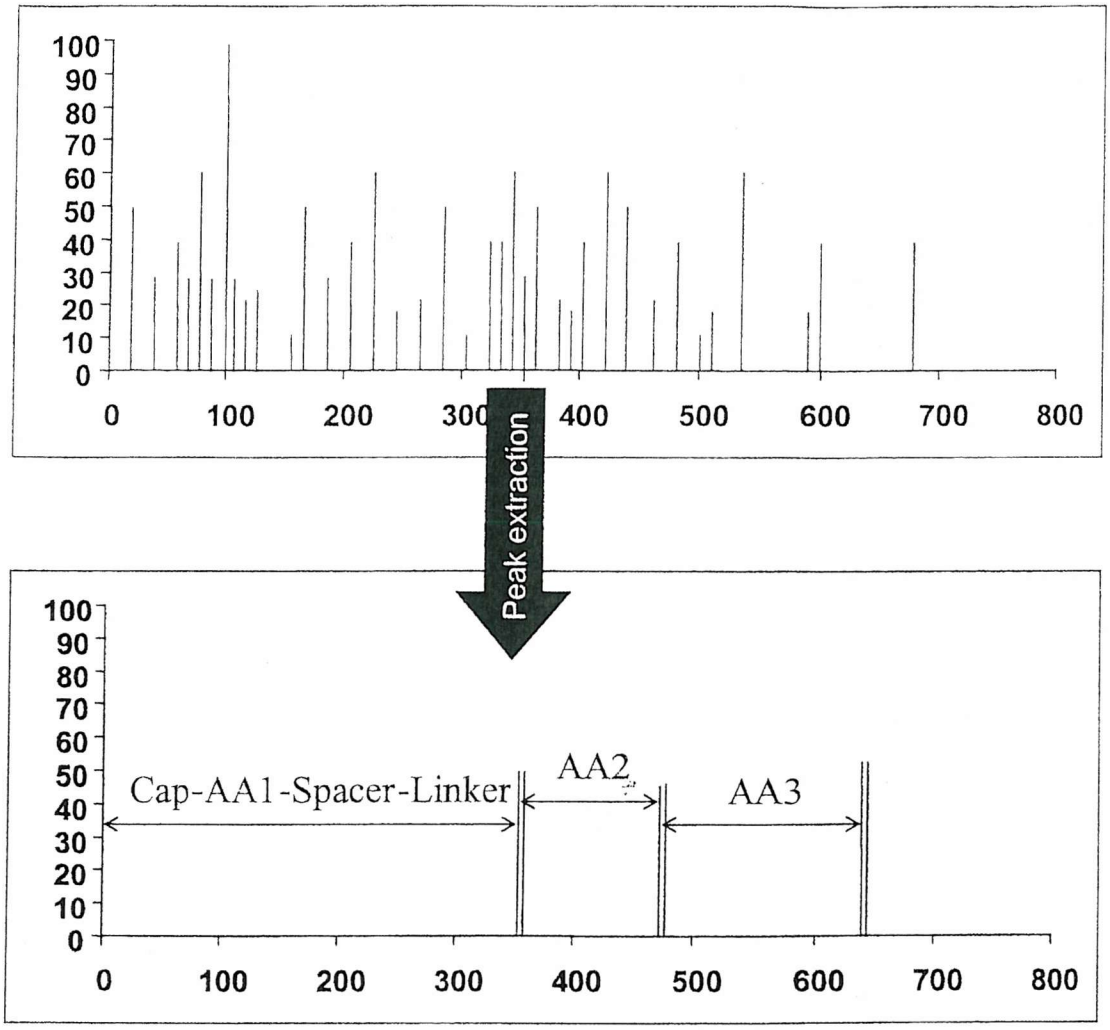
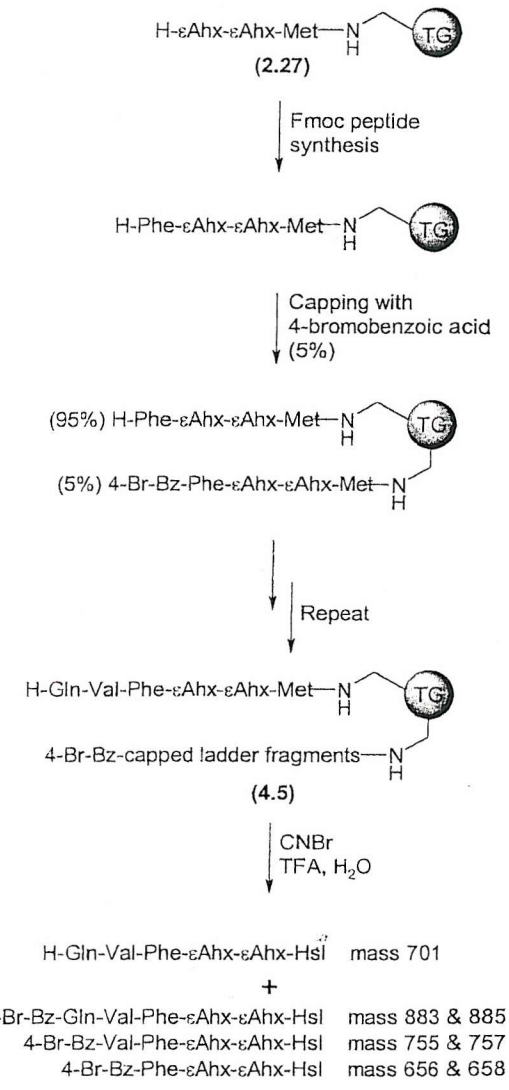


Figure 19. Stylised representation of ladder sequencing with incorporated isotopic labelling.

4.4. Synthesis and analysis of test peptides.

In order to test the methodology it was necessary to synthesise a test peptide incorporating an isotopically labelled capping group. As in Youngquist's work,[100] it was decided to use the amino acid methionine as the linker, which was cleaved by treatment with cyanogen bromide under acidic conditions. A spacer unit of two 6-aminohexanoic acid residues (ϵAhx) was added so that the smallest cleaved peptide ladder fragments would have a mass out of the noisy low mass region of the spectrum. The capping group chosen was 4-bromobenzoic acid, as bromine displays a natural isotope ratio of 1:1 $^{79}Br/^{81}Br$. Synthesis of a test peptide of sequence H-Gln-Val-Phe-OH was thus performed on the linker spacer unit described, with 5% capping of the growing peptide chain with 4-bromobenzoic acid, DIC and HOBt after removal of the Fmoc group to give resin **(4.5) (Scheme 60)**.



Scheme 60. Synthesis of a bromobenzoic acid capped test peptide ladder.

The resin was then treated with cyanogen bromide in TFA/water to cleave the peptide and capped ladder. LC-MS analysis of the cleavage mixture showed that the four compounds were present in the cleavage mixture, and the bromine isotope pattern was observed **(Figure 20)**.

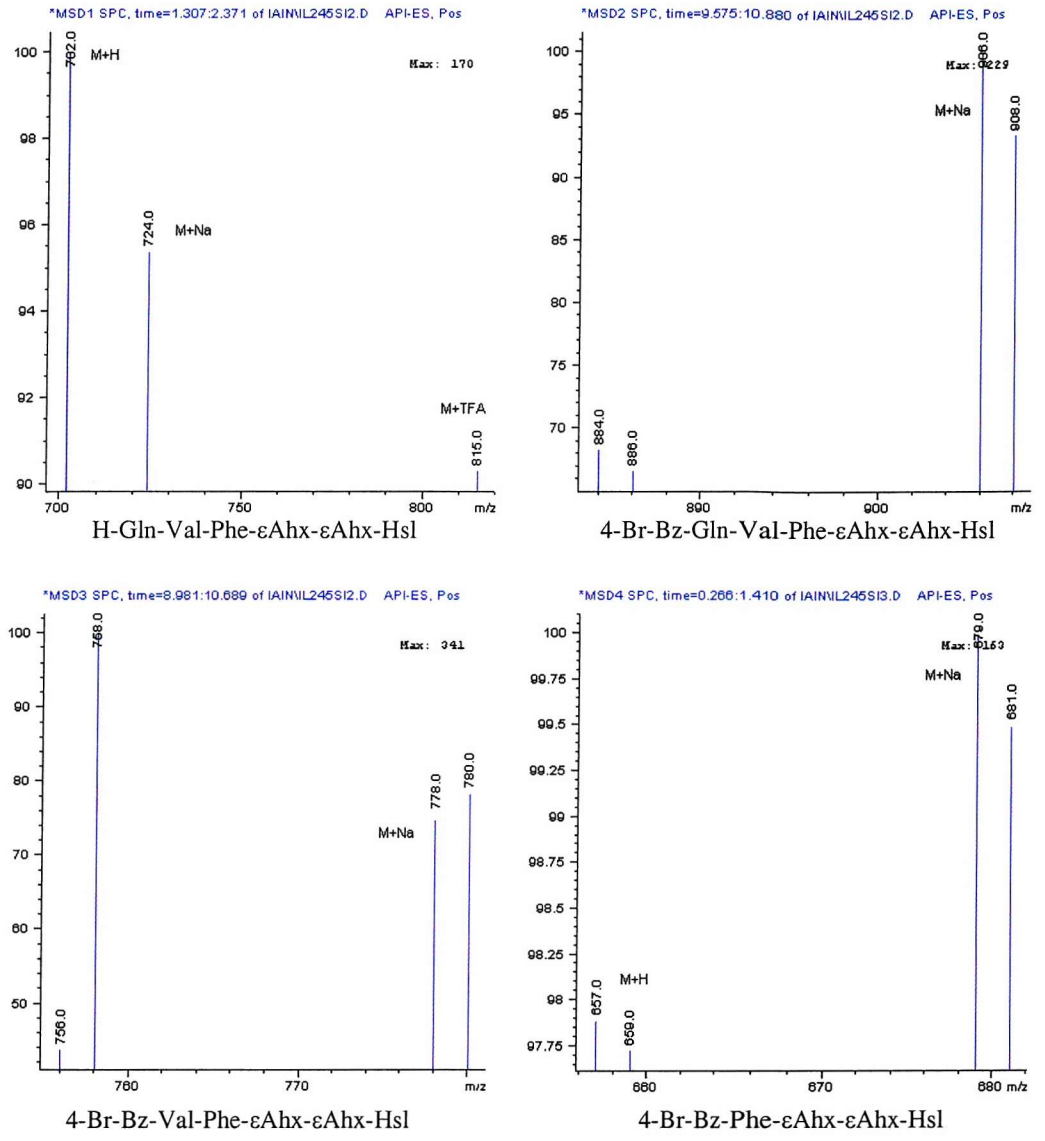


Figure 20. LC-MS spectra of the four cleavage products.

Attempts to analyse the cleavage mixture by electrospray mass spectrometry followed by software peak selection however failed. It was not possible to separate the labelled peaks from the background in the spectrum, as applying the peak selection criteria resulted in a large number of peaks against which the peptide sequence could not be determined. The probable cause of this failure was the use of bromine as an isotopic label in conjunction with cyanogen bromide (a brominating agent) as the cleavage reagent. It would seem that most of the impurities resulting from the cleavage were themselves brominated, and thus not removed from the spectrum by isotopic peak selection. In light of these findings, it

was decided to change the capping group and attempt the synthesis and analysis again. In place of 4-bromobenzoic acid, a 1:1 mixture of acetic acid and D_3-acetic acid was used to cap the growing peptide chain. The peptide was synthesised with the same sequence as before (H-Gln-Val-Phe-εAhx-εAhx-Met-TentaGel), and the cleavage products analysed by electrospray mass spectrometry. MassLynx v3.2 was used to extract the peaks containing isotopic labels by a cluster analysis function, the criteria of which are displayed in **Figure 21.**

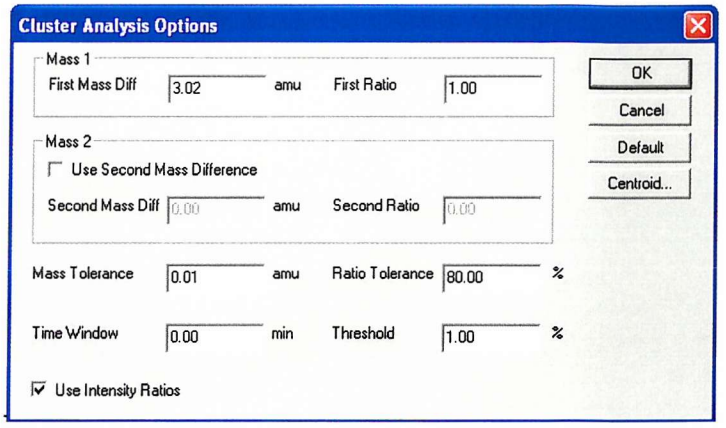


Figure 21. MassLynx cluster analysis criteria.

Cleavage mixtures from several single bead cleavages were analysed and the cluster analysis performed. **Figure 22** is a representative example in which only one of the ladder peak pairs was observed. The peaks extracted by the cluster analysis were mass 656 and 659, which matched the expected mass for Ac-Val-Phe-εAhx-εAhx-Hse $[M+Na]^+$ (Note: the homoserine lactone (Hsl) formed on cleavage has been hydrolysed to give homoserine (Hse)).

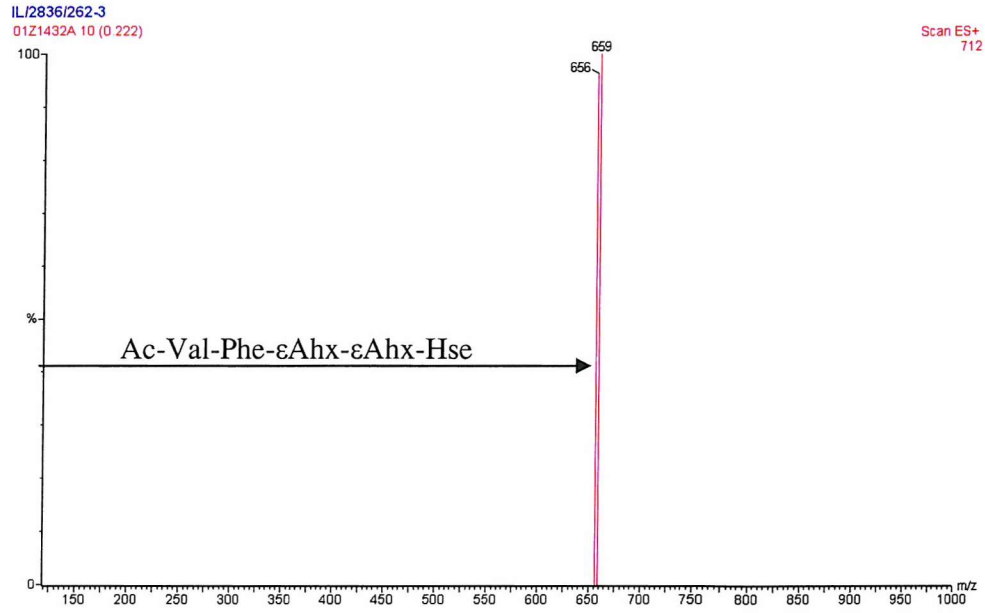
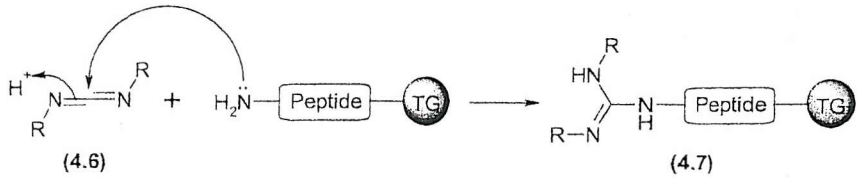


Figure 22. Spectra generated by cluster analysis of cleavage products.

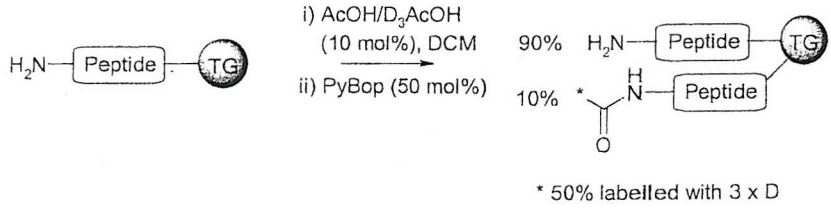
Spectra taken of the cleavage mixtures from other cleavages gave similar results, showing only one of the three expected ladder fragments. There were two possible explanations for these observations. Firstly, that 5% capping was not a high enough level, to give satisfactory results, and secondly, that during the capping step, the mixture (which was only enough to cap 5% of the available reaction sites) was not distributed evenly through the resin, and therefore only some of the beads had their peptides capped at each round whilst others escaped capping altogether. Both of these problems could be solved with a change to the experimental procedure, thus the level of capping was increased to 10%, and the mixture of acetic acid and D_3 acetic acid was added to the resin and allowed to equilibrate through the whole reaction mixture before the coupling agents (DIC and HOBt) were added. A resynthesis of the test peptide following this adapted protocol was performed and beads were cleaved and analysed as before. Similar results were observed, although many of the spectra now displayed two of the three expected pairs of peaks after cluster analysis. An alternative procedure replacing the mixture of acetic acids with a 1:1 mixture of acetic anhydride and D_6 acetic anhydride, followed by the addition also failed to give satisfactory results. It was therefore postulated that all of the acetic acid or acetic anhydride was not reacting with the peptide chain, perhaps due to hydrolysis of the active ester or anhydride in the reaction medium. In solid phase synthesis, reactions are often

driven to completion by the use of excess reagents. This approach cannot be directly applied in this case, for obvious reasons, but an alternative strategy was devised for the capping, whereby the acetic acids mixture (10 mol%) was allowed to equilibrate through the reaction mixture, followed by the addition of an excess of coupling reagent (50 mol%), thus ensuring that all the acetic acid reacted with the *N*-terminus of the peptide chain. The use of a carbodiimide coupling reagent **(4.6)** such as DIC however would lead to a detrimental side reaction, whereby the excess reagent would react with the amine to form a guanidine **(4.7)** at the *N*-terminus, blocking further reaction **(Scheme 61)**.[116,117,118]



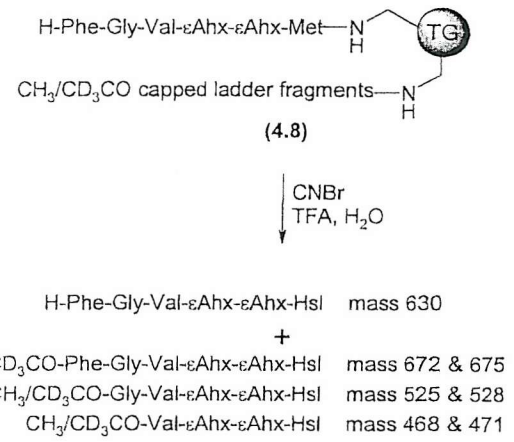
Scheme 61. Formation of a guanidine side product.

Phosphonium based coupling regents however do not undergo this side reaction however, and can thus be used in excess.[119] Therefore the phosphonium salt coupling agent PyBop[120] was used to drive the capping reaction to completion **(Scheme 62)**.



Scheme 62. Revised capping strategy.

Using this revised synthesis strategy, the test peptide H-Phe-Gly-Val-ϵAhx-ϵAhx-Met-TentaGel **(4.8)** was synthesised, and single bead cleavages were performed **(Scheme 63)**.



Scheme 63. Cleavage products of test peptide.

Electrospray mass spectrometry of the cleavage products gave all three expected peaks after cluster analysis, but the signals were found to be clearer when matrix assisted laser desorption/ionization time-of-flight (MALDI-TOF) spectrometry was used instead. An example of a MALDI-TOF spectrum of a single bead cleavage before and after cluster analysis are shown in **Figure 23**. In the unprocessed spectrum **(Figure 23a)**, the background noise was so high that it was impossible to deduce the sequence, and the full length peptide peak (653 $[M+Na]^+$) was not easily recognisable. Once cluster analysis had been performed however **(Figure 23b)**, the only peaks observed in the spectra were those due to the isotopically labelled peptide ladder, and the sequence could be easily read from the mass difference between the adjacent pairs of peaks, from the *N*-terminus at highest mass, to the amino acid attached to the spacer-linker construct at low mass.

The peaks observed were 695 (Ac-Phe-Gly-Val-εAhx-εAhx-Hsl [M+Na]), 548 (Ac-Gly-Val-εAhx-εAhx-Hsl [M+Na]), and 491 (Ac-Val-εAhx-εAhx-Hsl [M+Na]), returning the sequence as H-Phe-Gly-Val-εAhx-εAhx-Hsl as expected.

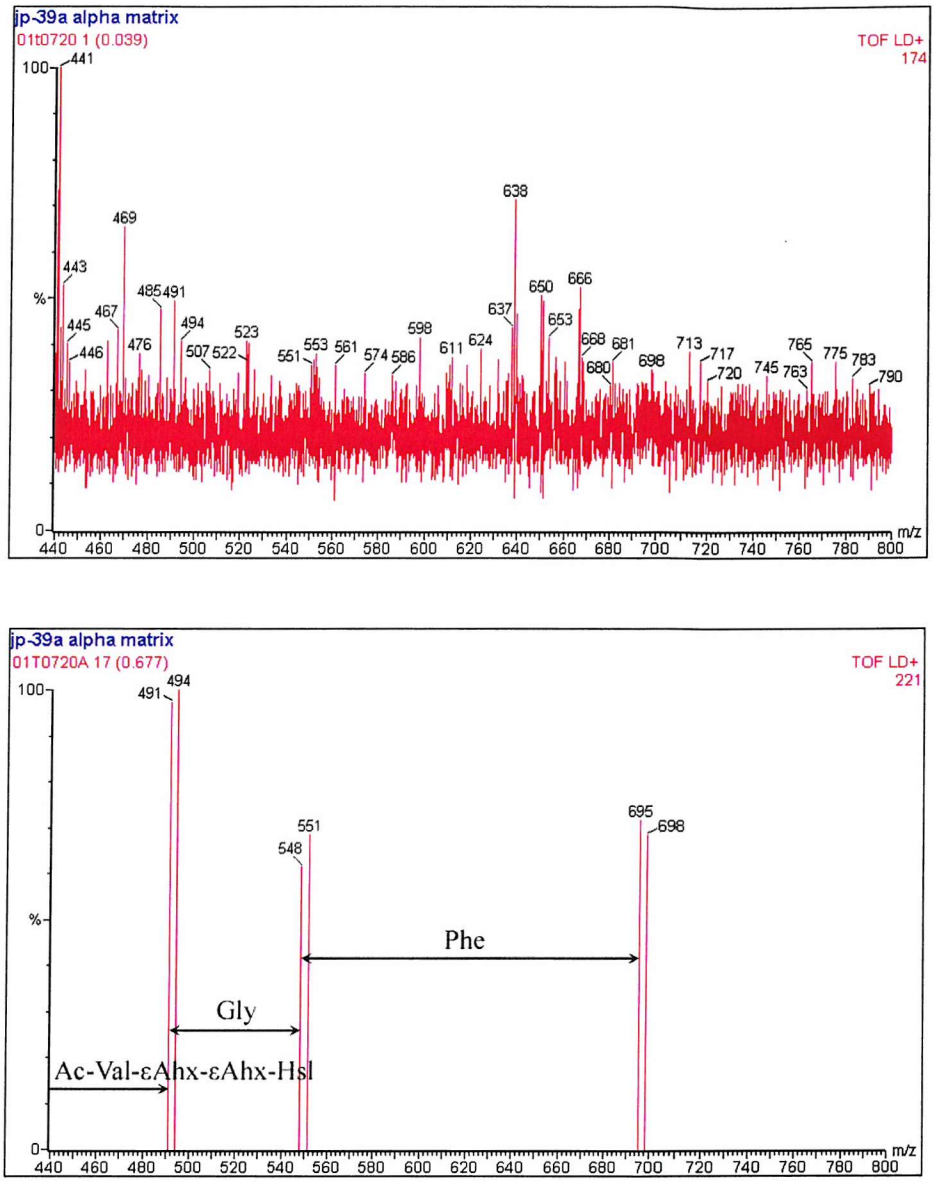


Figure 23. (a). Raw MALDI-TOF trace of cleaved test peptide and its capped ladder fragments; (b). Reprocessed MALDI-TOF spectrum of cleaved test peptide showing the ladder peaks for each amino acid.

4.5. Synthesis of combinatorial peptide libraries, and sequencing of single beads.

The successful sequencing of the test peptide by isotopic peak extraction showed the viability of the technique, and so three small libraries of compounds were synthesised using the capping strategy in order to confirm the technique with a wider range of amino acids, and with beads whose sequence was not known prior to the mass spectrometric analysis. The libraries were generated by the split and mix synthesis method of Furka, [121] on the spacer linker system –εAhx-εAhx-εAhx-Met-. The addition of an extra aminohexanoic acid unit into the spacer was to move the low mass ladder fragments further from the noise in the low range of a MALDI-TOF spectrum.

Library 1 (L4.1). A library of tripeptides was prepared *via* a split and mix synthesis strategy. In the first cycle the amino acids used were Val, Phe and Ala, in the second cycle Asn, Ser(O^{t}Bu) or Gln, and in the third Asp(O^{t}Bu), Tyr(O^{t}Bu) and Trp(Boc) to give 27 compounds. Single beads were randomly selected from the library, cleaved and sequenced to give the data shown in **Figure 24**.

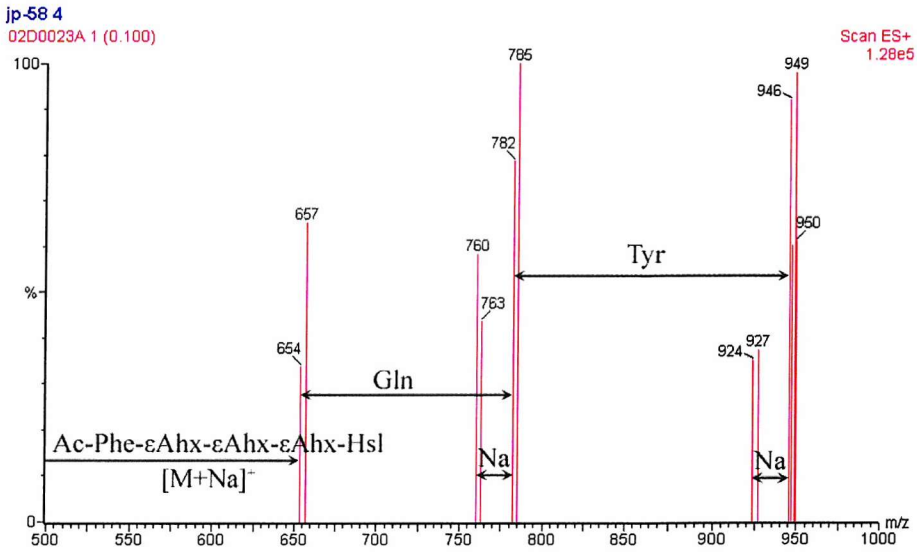


Figure 24. Sample bead from **(L4.1)**, processed and assigned. The sequence of the peptide is Tyr-Gln-Phe.

30 beads were cleaved and analysed from **(L4.1)**, and all were unambiguously sequenced, including a sample which was found to contain the sequence from two bead cleavages. Both sequences were successfully assigned.

Library 2 (L4.2). The more difficult residues Arg(Pmc), Trp(Boc) or Asn were incorporated at each of the three coupling cycles, generating a 27 member library constructed solely from these three amino acids. Again beads were randomly selected from the library, cleaved and sequenced. An example of a sequence obtained from this library is shown in **Figure 25**.

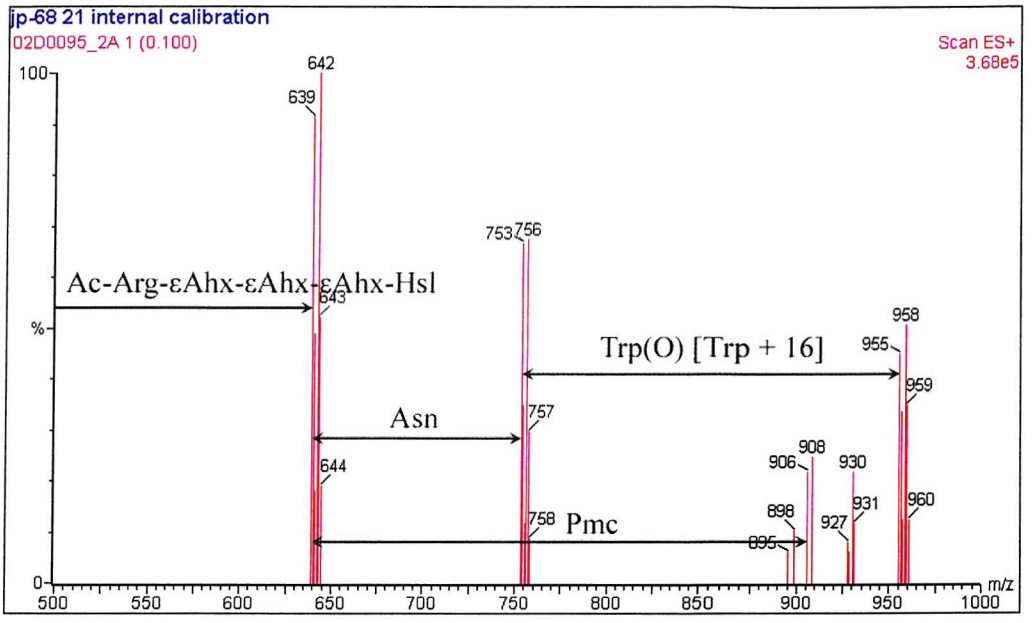


Figure 25. Sample bead taken from **(L4.2)**, processed and assigned. The sequence is Trp-Asn-Arg.

27 beads were cleaved from **(L4.2)** of which 23 were successfully sequenced. As can be seen in **Figure 25** above, the Pmc protecting group was often not completely removed from the arginine residues.

Library 3 (L4.3). Capping with two labelled capping groups was investigated to look at distinguishing residues of similar or identical mass. Therefore the sequence X-Gly-Val-εAhx-εAhx-εAhx-Met-TentaGel was synthesized, where X was one of the following amino acids Lys(Boc), Gln, Leu, Ile, Asp(O^{t}Bu), Asn. After coupling one member of each indistinguishable pair (Lys, Leu or Asp) or (Ile, Gln or Asn) 10% capping was carried out with a 1:1 mixture of either acetic /D_3-acetic acid or benzoic/D_5-benzoic acid. Thus on

sequencing members of this library two separate cluster analyses were run, one as before with a mass difference of 3.02 Daltons and the second with a mass difference of 5.03 Daltons (5D – 5H = 5.03). If the first cluster analysis only identifies two of the amino acids, the third must be identified from the second analysis.

Single beads were again randomly selected from the library, cleaved and sequenced. A sample sequence obtained by a combination of both cluster analyses is shown in **Figure 26**.

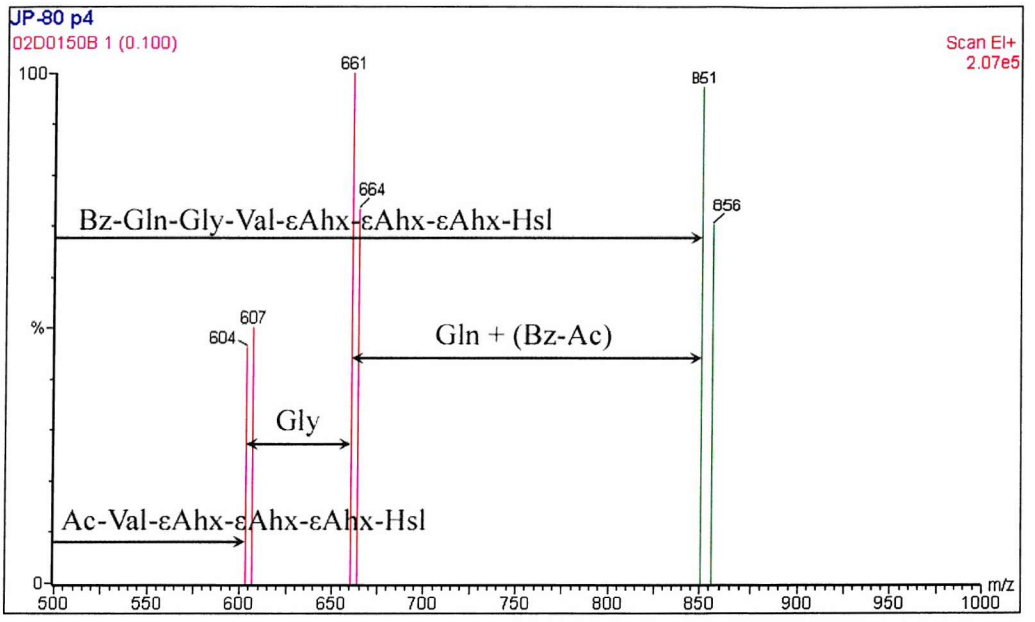


Figure 26. Sample bead from (**L4.3**), combination of two cluster analyses. Red peaks show analysis with a mass difference of 3.02. Only the first two amino acids are seen in the processed spectrum. The terminal amino acid therefore must be labelled with benzoic acid. Green peaks show analysis with a mass difference of 5.03. This gives the identity of the terminal amino acid as Gln, therefore the total sequence is Gln-Gly-Val.

Library (**L4.3**) consisted of only six members, so it was not necessary to analyse as many samples to get a representative picture of the sequencing success. Therefore eight samples were selected cleaved and analysed, all were successfully sequenced.

Overall all three libraries were sequenced with excellent success rates, as shown in **Table 5**.

Table 5. Results obtained in the sequencing of the three peptide libraries.

Library	L4.1	L4.2	L4.3
Library size	27	27	6
Samples analyzed	30	27	8
Sequences obtained	30	23	8
% Sequencing success	**100**	**85**	**100**

Several peptide modifications were observed during the screening of the three libraries. Arg and Ser were often observed in both the protected and unprotected forms, and Trp was often observed as the *N*-oxide. This oxidation of tryptophan is a commonly observed modification during MALDI analysis as reported by Youngquist *et al.*.[101]

4.6. Conclusions.

An isotope labelling chain termination strategy has been developed which provides peptide ladders suitable for MALDI-TOF MS analysis. The data generated can be ion extracted and cluster analyzed to give an excellent signal to noise ratio in a rapid and efficient method for the sequencing of peptides selected from split and mix combinatorial peptide libraries. The advantages over other methods of peptide sequencing are principally those of time, cost and reliability. Using a 100 well sample plate to introduce cleaved samples into the MALDI spectrometer allows the samples to be analyzed at a rate of approximately two minutes per sample. Data reduction and ladder analysis is also easy and quick, leading to a total sequencing time of approximately 5 minutes per sample. In comparison, Edman sequencing of peptides takes typically 1-2 hours per residue and is expensive. No specialist equipment is required for the protocol, which can be performed on a standard laboratory MALDI-TOF spectrometer with industry standard MS analysis software. The method is also highly reliable. Of 65 peptides analysed, 61 were unambiguously sequenced, a success rate of 94%. Although used in these examples for the sequencing of peptides, the protocol should be readily adaptable to the sequencing of libraries of any oligomeric molecules, and therefore has a useful place in the combinatorial methodology.

Chapter 5. Development of hexadienyloxycarbonyl (Hdoc) as a new acid cleavable protecting group for amines.

5.1. Introduction.

Protecting groups are an integral part of organic synthesis in all its forms. They are molecular fragments which can be added selectively to a molecule in order to mask a functional group, such that the group becomes inert to chemistries which would normally modify it. Good protecting groups must also be selectively removable in order to recover the unmasked functional group.

Kocieński has defined seven features which are preferable for a protecting group:[122]

1. It should be cheap or readily available.
2. The protecting group should be easily and efficiently introduced.
3. It should be easy to characterise and avoid such complications as the creation of new stereogenic centres.
4. It should be stable to the widest possible range of reaction and work-up conditions.
5. It should be stable to the widest possible range of techniques for separation and purification, such as chromatography.
6. It should be removed selectively and efficiently under highly specific conditions.
7. The by-products of the deprotection should be easily separated from the substrate.

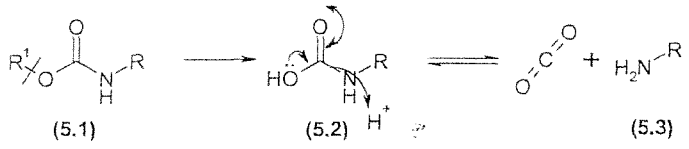
The combination of features 4, 5 and 6 give rise to the principle of orthogonality, that is, that a protecting group can be selectively removed using conditions which do not cause the removal of other (orthogonal) protecting groups. Also, *vice versa*, the group should be stable to the conditions used to remove these other groups.[123,124]

5.2. Amino protecting groups.

The amino group is a key functionality in organic chemistry, and because of its reactivity is one that frequently requires protection. It is an excellent nucleophile, as a result unprotected amines react faster than some other nucleophiles such as alcohols, leading to difficulties in chemoselectivity.

5.3. Carbamates.

Many of the commonly used amine protecting groups belong to the carbamate family (also known as urethanes). This functionality consists of an alkyloxycarbonyl group attached to the amine (**5.1**). Cleavage is accomplished by breaking the *O*-alkyl bond to release the carbamic acid (**5.2**) (or its anion), which spontaneously decomposes to give the free amine (**5.3**) plus carbon dioxide (**Scheme 64**).

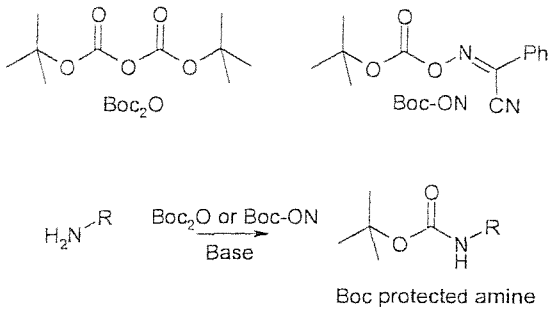


Scheme 64. Cleavage of carbamate based protecting groups.

Many different carbamate protecting groups exist, some of the most commonly used ones are outlined below.

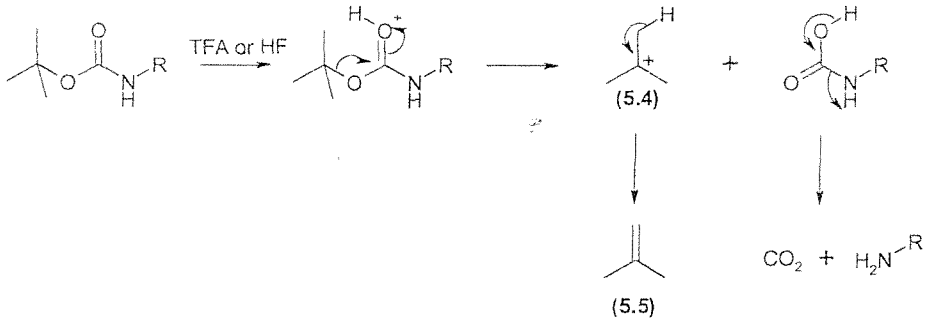
The Boc Group.

The *tert*-butoxycarbonyl group, which is commonly abbreviated to Boc, is a particularly useful amine protecting group due to its stability to a wide range of conditions.[84] It is inert to basic hydrolysis, hydrogenation, and nucleophilic attack, and is formed by the reaction of primary or secondary amines with di-*tert*-butyldicarbonate (Boc_2O) or 2-(*tert*-butoxycarbonyloxyimino)-2-phenylacetonitrile ('Boc-ON') in the presence of base (**Scheme 65**).



Scheme 65. Boc protection of amines.

Cleavage is accomplished by reaction with acids such as TFA and HF and occurs through protonation of the carbonyl group, which promotes the breakage of the tertiary C-O bond to yield the carbamic acid and a *tert*-butyl cation **(5.4) (Scheme 66)**. Tertiary cations such as this are stabilised by the inductive nature of the alkyl groups, and it is this stability that drives the reaction. Abstraction of a proton from one of the methyl groups leads to the formation of isobutylene **(5.5)** as one of several possible end by-products.

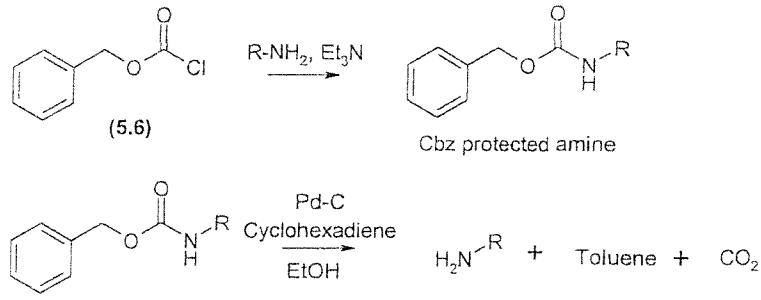


Scheme 66. Cleavage of the Boc group.

The Cbz (Z) group.

The benzyloxycarbonyl (abbreviated Cbz or Z) is one of the oldest amino protecting groups, introduced in 1932 by Bergman and Zervas,[125] and was a key point in the development of peptide chemistry. The group can be cleaved by hydrogenolysis, dissolving metal reduction and strong acidolysis. Protection is by treatment of the amine with readily available benzyl chloroformate **(5.6)** in the presence of base **(Scheme 67)**. Of the many different methods for the removal of the group, probably the most useful is

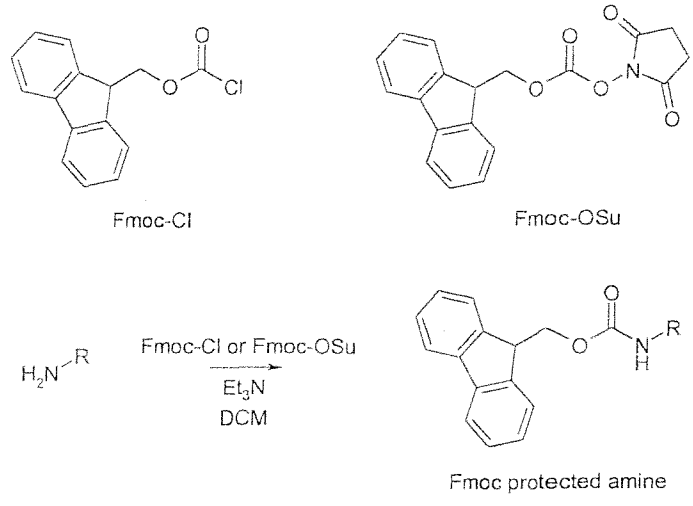
catalytic hydrogenolysis, either with gaseous hydrogen or hydrogen donors such as cyclohexadiene as shown in the example.



Scheme 67. Protection of amines with Cbz group and subsequent cleavage.

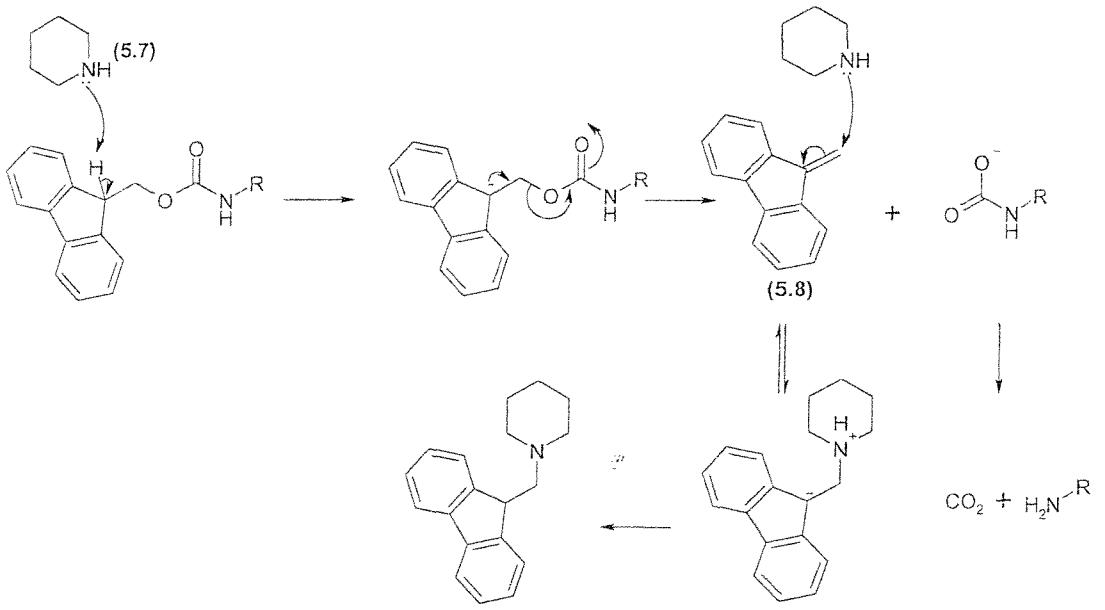
The Fmoc group.

The 9-fluorenylmethoxycarbonyl or Fmoc group was first reported by the Carpino group in 1972.[126] Despite its relatively high cost, it has become one of the most widely used protecting groups, especially in peptide synthesis. This is due to its excellent acid stability which makes it orthogonal to *tert*-butyl side chain protection, and the ease of its cleavage which is by treatment with 20% piperidine in DMF, or 5% DBU in DCM. The group is formed by reaction of the amine with 9-fluorenylmethylchloroformate (Fmoc-Cl) or *N*-(9-florenylmethoxcarbonyloxy)succinimide (Fmoc-OSu) in the presence of base (**Scheme 68**).



Scheme 68. Fmoc protection of amines.

Cleavage of the Fmoc group is accomplished by the base promoted abstraction of the proton at the tertiary centre to give a stabilised cyclopentadienyl anion, allowing E1cB elimination of the carbamic acid, leaving the fulvene (**5.8**) as the by-product. In **Scheme 69**, piperidine (**5.7**) is the base used to affect cleavage. The fulvene is moderately susceptible to nucleophilic attack, which can cause problems if the released amine reacts with it to form an uncleavable secondary amine. Under reaction conditions however, piperidine is used in vast excess, and so reacts with the fulvene first, preventing this problematic side reaction.

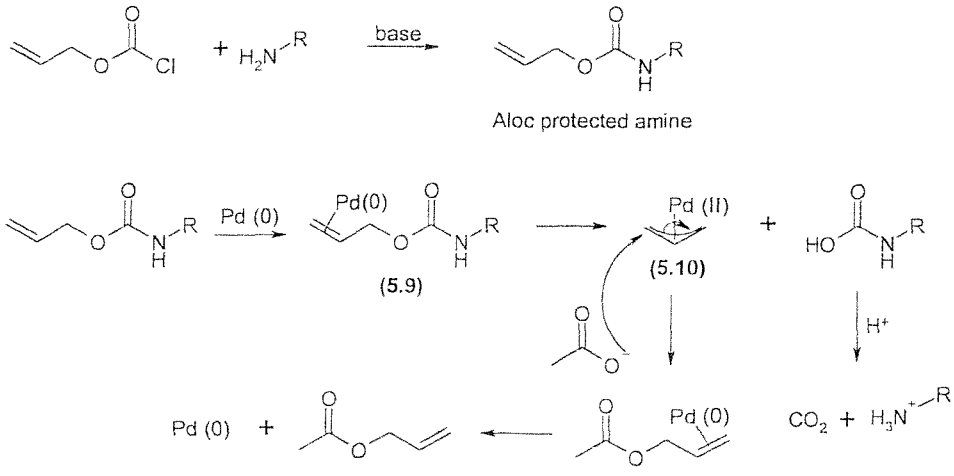


Scheme 69. Cleavage of the Fmoc group

The Aloc group.

The allyloxycarbonyl (Aloc) group is an important amine protecting group due to the selectivity and neutrality of its cleavage which is by palladium (0) catalysed allyl transfer.[82] The group is introduced in a manner similar to the other carbamates, by the use of the chloroformate, and is stable to a wide range of conditions including acids and bases. Cleavage is effected by treatment of the compound with stoichiometric or catalytic palladium (0), which coordinates to the allyl group to give an η^1 complex (**5.9**), which then rearranges to give an η^3 allyl complex (**5.10**), releasing the carbamic acid. An allyl acceptor must be added to the cleavage mixture. This is often a weak acid such as

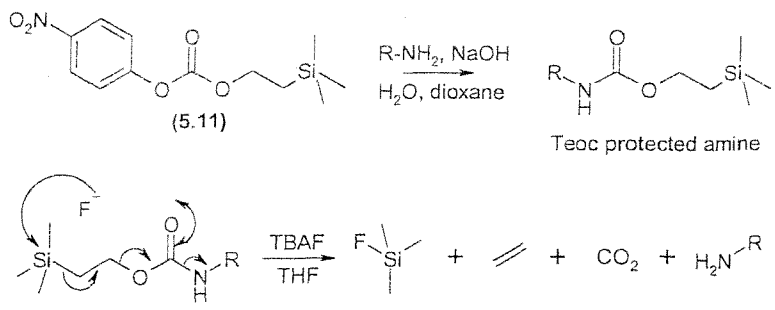
dimedone[127] or acetic acid[128] and has two functions. Firstly it acts as a nucleophile, displacing the palladium from the allyl complex, freeing it to react with another Aloc moiety. Secondly, as an acid it causes protonation of the released amine, preventing it from nucleophilic attack on the palladium allyl complex **(Scheme 70)**.



Scheme 70. Protection of amines with Aloc group and subsequent cleavage.

The Teoc group.

Another invention of the Carpino group,[129] the 2-(trimethylsilyl)ethoxycarbonyl (Teoc) group makes use of the high selectivity of fluoride ions for silicon. Formation is usually by treatment of the amine with 4-nitrophenyl-2-(trimethylsilyl)ethyl carbonate **(5.11)** in the presence of base **(Scheme 71)** as the chloroformate is unstable and must be freshly prepared in a hazardous reaction involving phosgene. Cleavage of the Teoc group is usually by treatment with TBAF in THF, [130] and occurs by fluoride attack on the silicon leading to β-elimination of the carbamic acid which decarboxylates as previously described.



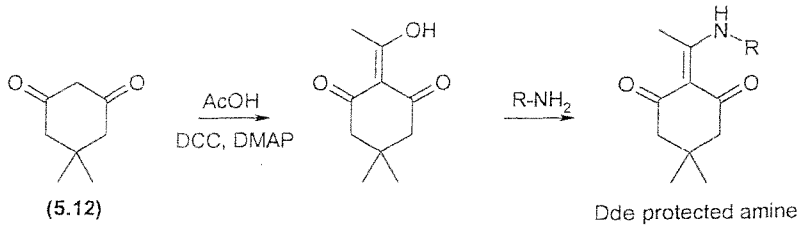
Scheme 71. Teoc protection of amines and subsequent cleavage.

5.4. Other amino protecting groups.

In addition to carbamate protecting groups, a number of other functionalities can be used for the protection of amines, some of which are introduced below.

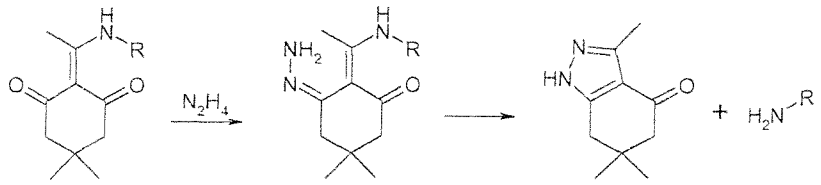
The Dde group.

The 1-(4,4-dimethyl-2,6-dioxocyclohex-1-ylidene)ethyl or Dde group is a relatively recent addition to the repertoire of amine protecting groups.[131] It is stable to both acidic and basic conditions, and is therefore orthogonal to both the Boc and Fmoc group. Synthesis is by the treatment of dimedone **(5.12)** with acetic acid in the presence of DCC and DMAP, followed by the addition of the amine **(Scheme 72)**.



Scheme 72. Dde protection of amines.

Cleavage of the Dde group is achieved by treatment with hydrazine, which is an excellent nucleophile and first attacks one of the carbonyl groups, cyclising to free the amine and give the pyrazole **(5.13)** as the end product **(Scheme 73)**.



(5.13)

Scheme 73. Hydrazine mediated cleavage of the Dde group.

The trityl group.

The triphenylmethyl or trityl group is more commonly used for the protection of alcohols[132] and thiols,[133] but can also be used as an amino protecting group. The group is cleaved by treatment with acid, but the triphenylmethyl cation is so stabilised by the resonance effects of the three phenyl groups, that cleavage can be effected under mildly acidic conditions such as 1% TFA in DCM, or acetic acid.

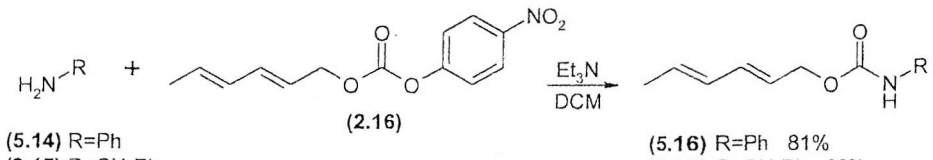
5.5. Hexadienyloxycarbonyl (Hdoc) as an amino protecting group.

It was observed during the synthesis of libraries of potential Diels Alder catalysts (see Chapter 2.6, page 43) that treatment of hexadienyloxycarbonyl amino TentaGel resin (**2.13**) with TFA in DCM regenerated the free amino resin (**Scheme 74**).

(2.13)

Scheme 74. Cleavage of hexadienyloxycarbonyl substituted resin.

It was therefore decided to investigate the possibility of using this functionality as an amine protecting group. To be useful as a protecting group it was important that it fulfilled the requirements listed above, therefore aniline (**5.14**), benzylamine (**3.15**) and 3-phenylpropylamine (**5.15**) were reacted with one equivalent of compound (**2.16**) in DCM in the presence of one equivalent of triethylamine (**Scheme 75**). These three compounds were chosen to represent amines in three different electronic environments, all containing an aromatic ring to aid analysis by RP-HPLC with detection by absorbance at 254nm.



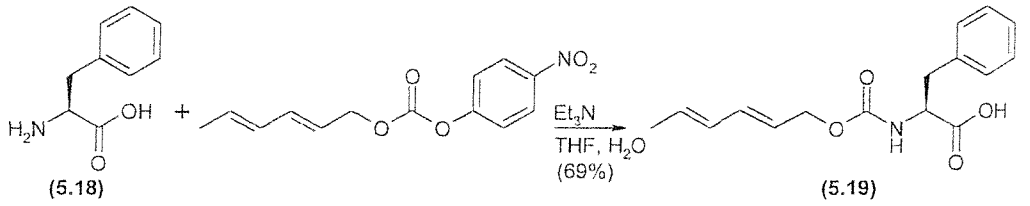
(**5.14**) R=Ph
(**3.15**) R=CH_2Ph
(**5.15**) R=$CH_2CH_2CH_2Ph$

(**5.16**) R=Ph 81%
(**3.16**) R=CH_2Ph 96%
(**5.17**) R=$CH_2CH_2CH_2Ph$ 94%

Scheme 75. Synthesis of Hdoc protected amines.

The synthesis of the three compounds (**5.16, 3.16, 5.17**) proceeded easily and in good yield, and the products were purified by a simple extraction. It was also hoped that the group would be suitable as an amino acid protecting group, so the protection of phenylalanine (**5.18**) was also achieved (**Scheme 76**). It was found that the reaction

between phenylalanine and compound **(2.16)** could be achieved by the addition of two equivalents of triethylamine in a 1:1 mixture of THF and water.



Scheme 76. Synthesis of Hdoc-Phe-OH.

Having successfully synthesised the four Hdoc protected amines **(3.15, 5.16, 5.17, 5.19)**, the stability of the Hdoc group was investigated under variety of conditions, chosen because they were either standard conditions for the removal of common protecting groups, or could be expected to be used regularly in organic synthesis. Four different concentrations of TFA in DCM were chosen; 95 % (c.f. Boc cleavage),[84Error! Bookmark not defined.] 10 % (c.f. Rink linker cleavage),[89] 1 % (c.f. trityl cleavage),[134] and 0.1 %. Other acidic conditions selected were 10 % acetic acid in DCM, 2M aqueous HCl, and 4M HCl/dioxane. Basic conditions examined were 20 % piperidine in DMF (c.f. Fmoc cleavage),[126] 5 % DBU in DCM and 2M aqueous NaOH. Other conditions tested were palladium (0) (c.f. Aloc and allyl ester cleavage),[128] 1M I_2 in DMF, 5 % N_2H_4 in DMF (c.f. Dde cleavage),[131] 1M $NaBH_4$ in THF, 1M TBAF in THF (c.f. Teoc and silyl protecting group cleavage),[130] heating at 100 °C in DMF, and exposure to UV radiation. In each case, a small aliquot of Hdoc-phenylalanine **(5.19)** was treated with the prescribed reagents, and the disappearance of starting material and appearance of deprotected phenylalanine were monitored by TLC. After 16 hours the mixture was analysed by RP-HPLC to confirm the stability or lability of the protecting group to these conditions. The results of these studies are shown in **Table 6**.

It was found that the Hdoc group could be readily cleaved from the amino acid with a variety of acidic conditions, including diluted solutions of TFA in DCM (entries 1-6). In each of these cases, a simple aqueous extraction returned the deprotected phenylalanine in pure form. The moiety was found to be stable to most of the other conditions investigated, but RP-HPLC analysis showed that the group had reacted with iodine causing a non-

cleavage modification to the molecule (entry 12). Treatment of this modified compound with 10 % TFA in DCM then failed to liberate the phenylalanine.

Table 6. Stability of the Hdoc group to a variety of conditions.

	Cleavage conditions	TLC				HPLC 16 hours	Conclusion
		10 min	1 hour	2 hours	16 hours		
1	95 % TFA/DCM	Cleaved	Cleaved	Cleaved	Cleaved	Cleaved	Cleaved
2	10 % TFA/DCM	Cleaved	Cleaved	Cleaved	Cleaved	Cleaved	Cleaved
3	1 % TFA/DCM	Cleaved	Cleaved	Cleaved	Cleaved	Cleaved	Cleaved
4	0.1 % TFA/DCM	Stable	Stable	Partially cleaved	Partially cleaved	Partially cleaved	Slowly cleaved
5	4M HCl/dioxane	Stable	Partially cleaved	Partially cleaved	Partially cleaved	Cleaved	Slowly cleaved
6	2M HCl/H_2O	Stable	Stable	Stable	Cleaved	Mainly cleaved	Slowly cleaved
7	10 % AcOH/DCM	Stable	Stable	Stable	Stable	Stable	Stable
8	20 % pip/DMF	Stable	Stable	Stable	Stable	Stable	Stable
9	5 % DBU/DCM	Stable	Stable	Stable	Stable	Stable	Stable
10	$Pd(PPh_3)_4$ in $CHCl_3$/AcOH/NMM (37:2:1)	Stable	Stable	Stable	Stable	Stable	Stable
11	2M $NaOH/H_2O$	Stable	Stable	Stable	Stable	Stable	Stable
12	1M I_2 in DMF	Modified	Modified	Modified	Modified	Modified	Modified
13	5 % H_4N_2/DMF	Stable	Stable	Stable	Stable	Stable	Stable
14	100 °C /DMF	Stable	Stable	Stable	Stable	Stable	Stable
15	1M $NaBH_4$ in THF	Stable	Stable	Stable	Stable	Stable	Stable
16	U.V. light/DCM	Stable	Stable	Stable	Stable	Stable	Stable
17	1M TBAF in THF	Stable	Stable	Stable	Stable	Stable	Stable

In order to confirm the cleavage by TFA/DCM solutions, a sample of Hdoc-Phe-OH was dissolved in 1% TFA in $CDCl_3$ and the reaction was monitored by ^1H NMR. After 24 hours, complete removal of the Hdoc group had occurred, and the phenylalanine resonances could be easily observed in the spectrum. The spectra is shown overleaf (**Figure 27**), overlaid with an authentic sample of H-Phe-OH for comparison. It can be seen that the by-product of the reaction is not easily identifiable, and is a mixture of compounds.

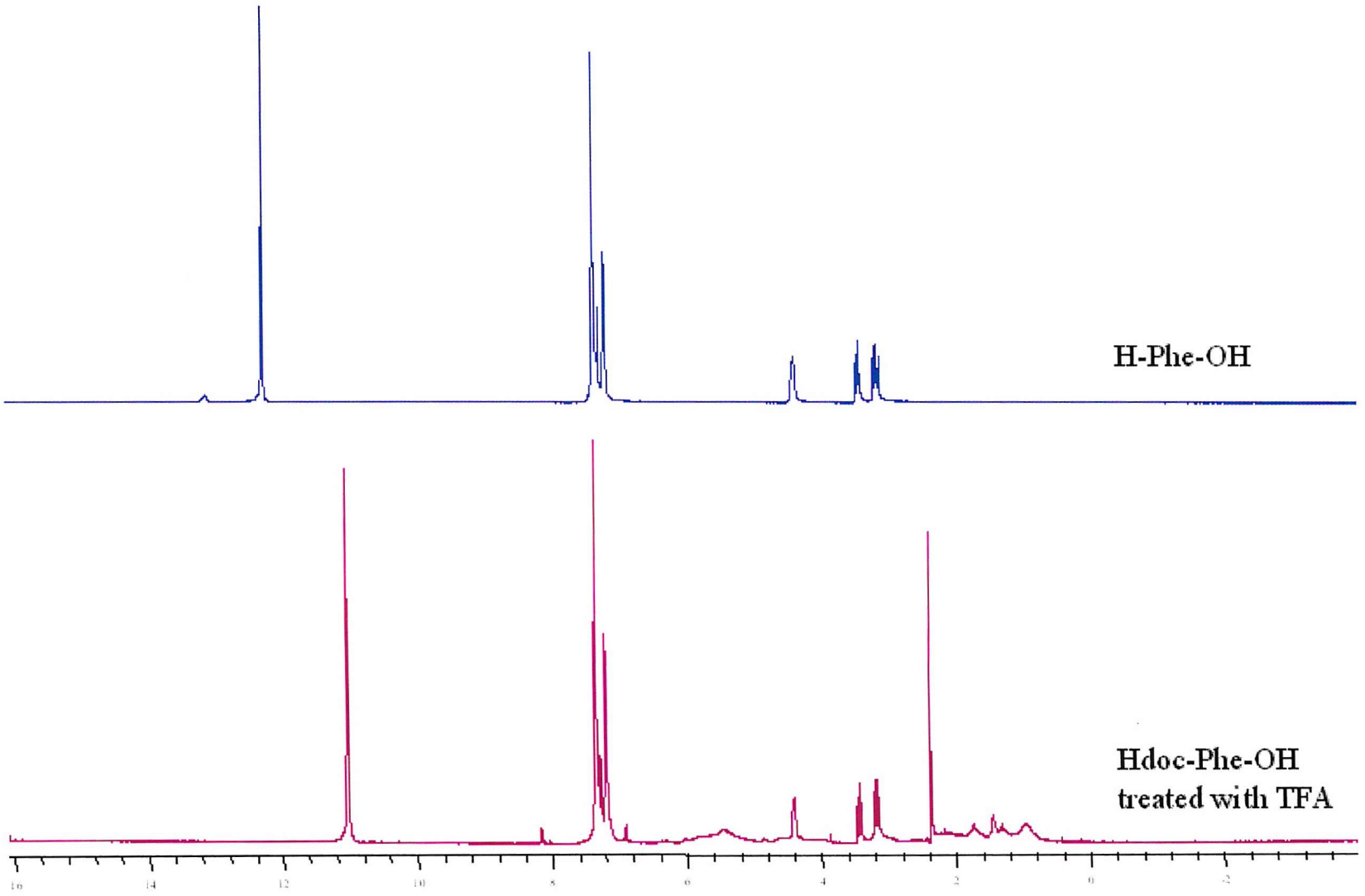
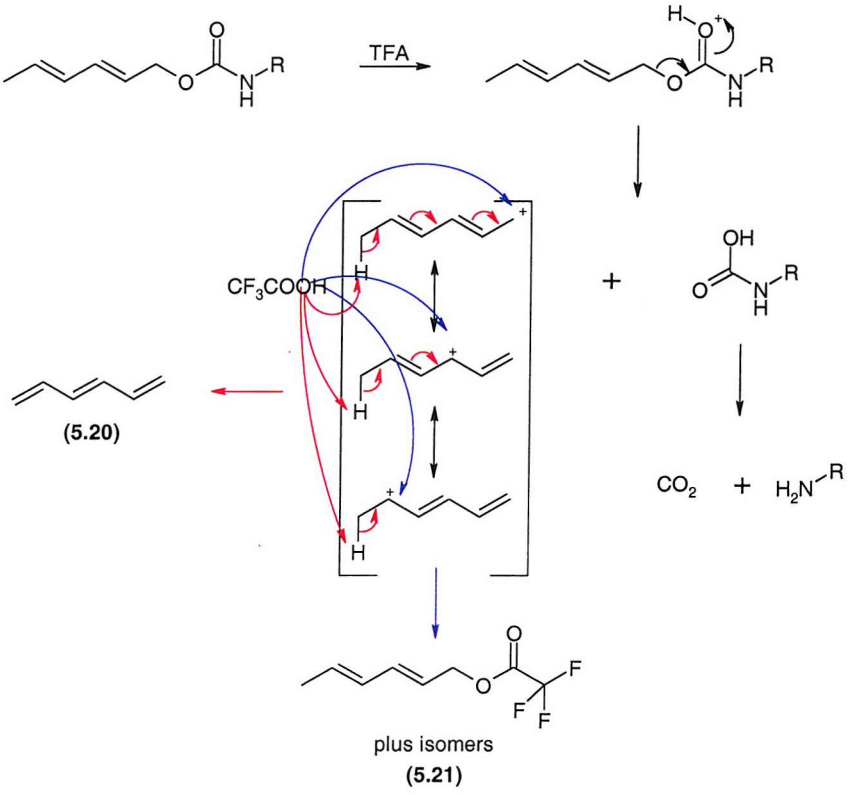


Figure 27. Overlaid ^{1}H NMR spectra of cleaved Hdoc-Phe-OH and authentic H-Phe-OH.

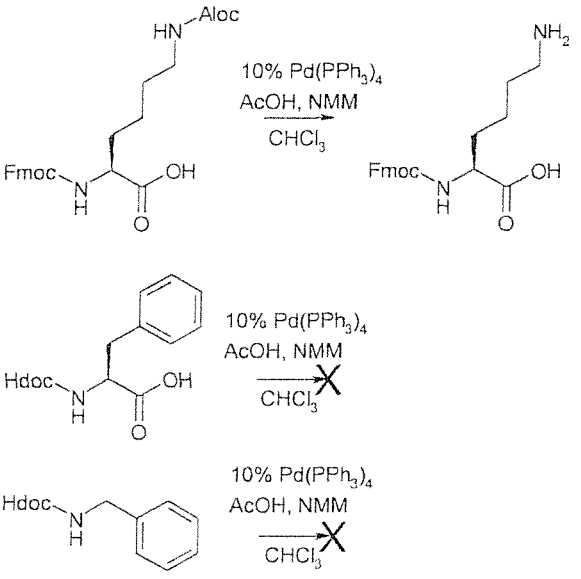
The mild acid lability of the group can be explained by resonance stabilisation of the hexadienyl cation across the conjugated diene system, as shown in **Scheme 77**. It was postulated that proton abstraction from any of the resonance structures would lead to the formation of 1,3,5-hexatriene **(5.20)** as the by product, however GC-MS analysis of the cleavage products suggested that the cation is quenched by reaction with TFA, yielding a mixture of hexadienyltrifluoroacetates **(5.21)**.



Scheme 77. Proposed cleavage mechanism.

The non-cleavage of the group by palladium (0) catalysis (entry 9 in **Table 6**) was somewhat of a surprise due to the group similarity to the Aloc group. In order to confirm that the group was not cleaved or modified by reaction with palladium, larger samples of Hdoc-phenylalanine and Hdoc-benzylamine were reacted with 10 mol % $Pd(PPh_3)_4$ in a mixture of $CHCl_3$/AcOH/NMM (37:2:1). Fmoc-Lys(Aloc)-OH was treated under identical conditions to confirm that these conditions were suitable for the cleavage of the Aloc group **(Scheme 78)**. After 24 hours reaction Fmoc-Lys-OH had precipitated from the solution, and LC-MS confirmed that the Aloc group had been completely removed.

Similar analysis of the reactions involving Hdoc protected amines, however, showed no cleavage of the protecting group. This was confirmed by aqueous workup, which recovered both the Hdoc protected amines and NMR analysis, which showed that the Hdoc groups were still intact.



Scheme 78. Palladium mediated cleavages.

5.6. Conclusions.

The 2,4-*E,E*-hexadienyloxycarbonyl or Hdoc group has been shown to be a mild acid cleavable protecting group for amines and amino acids. It is stable to a wide variety of conditions, including many that are used for the removal of other protecting groups. The group showed unexpected stability to cleavage by palladium (0) and may be a useful alternative to the Aloc group in strategies where the use of palladium would be detrimental. It is cleaved under similar conditions to the trityl group, which it may replace in strategies where the latter's steric bulk may cause synthetic difficulties.

Chapter 6. Experimental Section.

6.1. General Information.

The following solvents were used anhydrous and were prepared as follows:

DCM. Distilled from CaH_2 under N_2 immediately prior to use.

THF. Distilled from sodium/benzophenone under N_2 immediately prior to use.

Benzene. Distilled from CaH_2 under N_2 immediately prior to use.

Solution phase reactions were monitored by TLC (Merck silica gel aluminium backed 60 F_{254} plates) and visualised by UV fluorescence (254 nm). Peptide couplings were monitored on resin beads using the Kaiser ninhydrin test.[83] Flash chromatography was performed on a silica column using Keiselgel 60 230-400 mesh as supplied by *Merck* according to the method of W. C. Still *et. al.*[135].

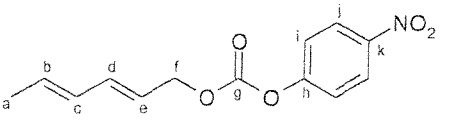
^{1}H NMR were recorded at 300 MHz on a Bruker AC-300 FT instrument, or at 400 MHz on a Bruker DPX-400 FT instrument. Chemical shifts (δ_H) are quoted in ppm relative to TMS with the residual solvent peak as the internal reference. Coupling constants (J) are quoted to the nearest whole Hertz. COSY 2 dimensional spectra were used to aid the assignment of complex molecules. **MAS-NMR** were recorded at 400 MHz on a Bruker DPX-400 FT instrument equipped with a HR-MAS 4mm probe samples were spun at 5000 Hz. **^{13}C NMR** were recorded at 75 or 101 MHz as above. HMQC and HMBC 2 dimensional spectra were used to aid the assignment of complex molecules. **Mass spectra.** Electrospray spectra were recorded as acetonitrile solutions introduced to a Fisons VG platform through a Hewlett Packard 1050 HPLC system. Mass Lynx v3.2 (Micromass Ltd., Manchester, UK) was used to analyse the results. Low resolution (**m/z**) are quoted with only molecular ions (M+H) and major peaks with percentage intensities of the base peak. MALDI-TOF spectra were recorded on a Thermobioanalysis Dynamo system using α-cyano-4-hydroxycinnamic acid as the sample matrix, and terfenadine and renin substrate tetradecapeptide as internal and external calibrants. **Infra red spectra** were recorded either neat or as a crushed resin sample on a Bio-Rad FTS-135 Fourier transform spectrometer equipped with a 'Golden Gate' attenuated total reflection (ATR) sample

stage. Only selected absorbtions ($\nu_{max.}$) are quoted. **Melting points** were determined on an Electrothermal hot stage apparatus and are uncorrected. **Microscopy.** Samples were viewed on a Leica DM-IL inverted transmission microscope at 40 and 100 × magnification. Fluorescence observations were made on the same microscope with a mercury vapour discharge lamp as the light source, and a type A or type I3 filter cube in the optical path. Images were recorded using a Nikon D1 digital SLR camera attached directly to the microscope, and have not been modified electronically. **HPLC.** Reverse phase HPLC analyses were performed on an Agilent Technologies 1100 modular HPLC system with detection by U.V. absorbance, or by evaporative light scattering detection using a Polymer Labs PLS-1000 detector. Solvent A: 0.1 % TFA in water. Solvent B. 0.042 % TFA in MeCN. Method 1: Supelco Discovery 50 mm x 2.1 mm 5 μm C18 column, eluted at 1 mL/min with a gradient of 10 % B, 90 % A to 90 % B, 10 % A over 3 minutes, followed by 2 minutes isocratic at 90 % B, 10 % A. Method 2. Phenomenex Prodigy 150 x 4.6 mm 5 μm (ODS3) C18 column, eluted at 0.5 mL/min with a gradient of 10 % B, 90 % A to 90 % B, 10 % A over 10 minutes, followed by 5 minutes isocratic at 90 % B, 10 % A. Semi-preparative HPLC separations were performed on an Agilent Technologies 1100 modular HPLC equipped with automated fraction collection triggered by absorbance at 254 nm. Method 3. Phenomenex Prodigy 250 × 10 mm 5 μm (ODS3) C18 column, eluted at 2.5 mL/min with a gradient of 100 % A to 100 % B over 20 minutes, followed by 5 minutes isocratic at 100 % B.

6.2. Experimental to Chapter 2.

E,E-2,4-hexadienyl-(4-nitrophenyl) carbonate (**2.16**).

Reaction of E,E-*2,4-hexadien-1-ol with* p-*nitrophenylchloroformate.*



2,4-*E,E*,-Hexadien-1-ol (10 g, 98 mmol.) was dissolved in DCM (50 mL) and *p*-nitrophenylchloroformate (21 g, 105 mmol.) was added. DIPEA (18.3 mL, 105 mmol.) was added dropwise with cooling, and the solution was stirred for 16 hours under N_2. The solvent was removed under vacuum and the residue was purified by flash chromatography on silica gel eluted with 5% EtOAc in hexane. The product was recrystallised from hexane/EtOAc to give yellowish/white needles. (17.4 g, 68%).

m.p. 94 - 96 °C

HPLC (Method 1) purity 100% (254 nm) RT 4.41 minutes.

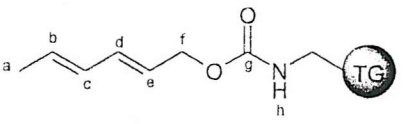
δ_H (400 MHz, $CDCl_3$), 1.70-1.72 (d, *J* 7, 3H, H-a), 4.69-4.71 (d, *J* 7, 2H, H-f), 5.58-5.65 (dq, *J* 7 & 12, 1H, H-b), 5.71-5.80 (dt, *J* 7 & 15, 1H, H-e), 5.98-6.05 (dd, *J* 10 & 12, 1H, H-c), 6.25-6.32 (dd, *J* 10 & 15, 1H, H-d), 7.29-7.32 (d, *J* 9, 2H, H-i), 8.19-8.21 (d, *J* 9, 2H, H-j).

δ_C (101 MHz, $CDCl_3$), 18.6 (C-a), 70.2 (C-f), 122.0 (C-b), 122.2 (C-i), 125.7 (C-j), 130.5 (C-c), 133.2 (C-e), 137.4 (C-d), 145.8 (C-k), 152.8 (C-g), 156.0 (C-h).

ν_{max} (Neat solid) 1747, 1282, 1224.

E,E-2,4-Hexadienyloxycarbonyl aminoethyl TentaGel **(2.13).**

Reaction of E,E-*2,4-hexadienyl-(4-nitrophenyl) carbonate with TentaGel S* NH_2.



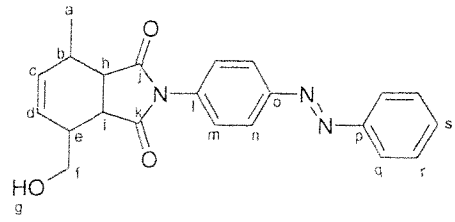
TentaGel S NH_2 (0.27 mmol./g, 500 mg, 0.135 mmol.) was swelled in DCM (5 mL), and compound **(2.16)** (130 mg, 0.5 mmol.) was added to the suspension followed by Et_3N (70 μL, 0.5 mmol.). The mixture was shaken for 16 hours, after which time a ninhydrin test showed that the reaction had gone to completion. The resin was filtered off, washed with DMF (5 mL × 3), DCM (5 mL × 3) MeOH (5 mL × 3) and diethyl ether (5 mL × 3) and then dried overnight in a vacuum oven.

δ_H (MAS-NMR, 400 MHz, $CDCl_3$) (presaturation to remove PEG signals) 6.75-7.2 (br m, polystyrene), 6.30-6.70 (br, m, polystyrene), 6.18-6.28 (dd, *J* 12 & 13, H-c), 6.01-6.10 (dd, *J* 12 & 12, H-d), 5.68-5.80 (m, H-b), 5.58-5.68 (m, H-e), 4.51-4.65 (d, *J* 5, H-f), 1.70-1.81 (d, *J* 7, H-a).

δ_C (Gel phase, C_6D_6) 157.0 (C-g), 134.4 (C-c), 131.7 (C-d), 130.5 (C-e), 126.3 (C-b), 65.4 (C-f), 18.5 (C-a).

4-Hydroxymethyl-7-methyl-2-(4-phenylazo-phenyl)-3a,4,7,7a-tetrahydro-isoindole-1,3-dione **(2.18)**.

Reaction of E,E-*2,4-hexadien-1-ol with 4-phenylazomaleinanil*



2,4 -*trans, trans*-Hexadiene-1-ol (125 mg, 2.5 mmol.) and *p*-phenylazomaleinanil (355 mg, 2.5 mmol.) were dissolved in THF (10 mL.) and stirred at 60 °C for 36 hours under N_2. The resulting solution was concentrated under vacuum and the residue was recrystallised from EtOAc by the addition of diethyl ether. The product was obtained as an orange solid (418 mg, 88%).

m.p. 124-125 °C.

HPLC (Method 1) purity 92 % (254 nm), RT 3.91 minutes.

δ_H (400 MHz, $CDCl_3$) 10.57 (br. s, 1H, H-g), 7.77-7.79 (d, 2H, *J* 9, H-n), 7.74-7.76 (d, 2H, *J* 7, H-q), 7.65-7.67 (d, 2H, *J* 9, H-m), 7.34-7.37 (t, 2H, *J* 7, H-r), 7.28-7.31 (t, 1H, *J* 7, H-s), 5.86-5.89 (dd, 1H, *J* 3 & 10, H-d), 5.12-5.44 (d, 1H, *J* 10, H-c), 4.31-4.35 (t, 1H, *J* 7, H-f), 4.11-4.14 (d, 1H, *J* 9, H-f), 3.22-3.24 (dd, 1H, *J* 4 & 7, H-i), 3.04-3.15 (m, 2H, H-e & H-h), 2.68-2.78 (m, 1H, H-b), 1.01 (d, *J* 8, 3H, H-a).

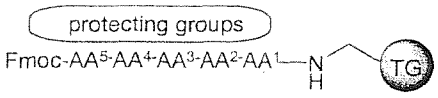
δ_C (101 MHz, $CDCl_3$) 180.8 (C-k), 171.2 (C-j), 153.2 (C-p), 149.5 (C-o), 141.3 (C-l), 136.7 (C-d), 131.1 (C-s), 129.5 (C-r), 124.5 (C-c), 124.4 (C-n), 123.1 (C-q), 120.5 (C-m), 73.0 (C-f), 47.6 (C-h) 39.4 (C-i), 38.3 (C-e), 32.0 (C-b), 17.1 (C-a).

m/z (ES+) 376 $[M+H]^+$ (30%), 580 (100%).

ν_{max} (Neat solid) 1758, 1693, 1598, 1532.

Protected library of 100,000 pentapeptides on TentaGel resin **(2.23)**.[35]

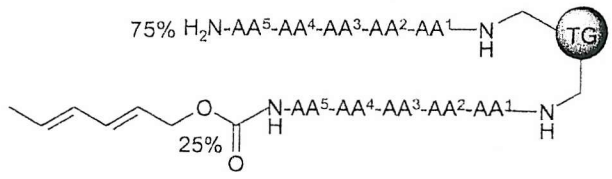
Split and mix synthesis of pentapeptides on TentaGel resin.



TentaGel S NH_2 resin (2 g, 0.29 mmol./g, 0.58 mmol.) was split into ten equal portions (10 x 0.058 mmol.), and each was allowed to swell in DCM (1 mL). Meanwhile, each of the following amino acids: Fmoc-Phe-OH (58 mg, 0.15 mmol.), Fmoc-Ala-OH (47 mg, 0.15 mmol.), Fmoc-Ser(O*t*Bu)-OH (58 mg, 0.15 mmol.), Fmoc-Gly-OH (45 mg, 0.15 mmol.), Fmoc-Arg(Pbf)-OH (97 mg, 0.15 mmol.) Fmoc-Trp-OH (64 mg, 0.15 mmol.), Fmoc-Val-OH (51 mg, 0.15 mmol.), Fmoc-Glu(O*t*Bu)-OH (64 mg, 0.15 mmol.) Fmoc-Leu-OH (53 mg, 0.15 mmol.) Fmoc-Gln-OH (55 mg, 0.15 mmol.) were dissolved in DMF (0.5 mL) and HOBt (20 mg, 0.15 mmol.) was added to each solution. DIC (20 μL, 0.15 mmol.) was then added to each solution and they were allowed to react for 10 minutes. One amino acid containing solution was then added to each of the resin suspensions and the mixtures shaken for 2 hours. A qualitative ninhydrin test[79] was performed to ensure that each coupling had gone to completion. The resins were filtered off and washed with DMF (3 x 1 mL), and DCM (3 x 1 mL). The ten resins were then pooled together, suspended in DMF (10 mL) and shaken thoroughly for 2 minutes. The resin was then filtered, suspended in 20% piperidine in DMF and shaken for 5 minutes, then filtered, washed with DMF (3 x 10 mL) and resuspended in 20% piperidine in DMF (10 mL). After 15 minutes further shaking, the resin was filtered, washed with DMF (3 x 5 mL), DCM (3 x 5 mL), and MeOH (3 x 5 mL), then suspended in DMF (10 mL) ready to be split into aliquots again. This split-coupling-mix-deprotect cycle was repeated four times. After mixing the resin aliquots for the last time however the resin was not reacted with 20% piperidine in DMF, but rather washed with DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL) and Et_2O (3 x 5 mL) then dried under vacuum.

Deprotected library of 100,000 pentapeptides on TentaGel, 25% hexadienyloxycarbonyl substituted at the *N*-terminus (**L2.4**).[81]

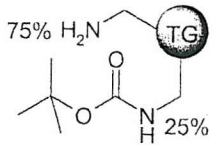
*Side chain deprotection, Fmoc group removal and diene capping on resin (**2.24**).*



To resin (**2.24**) (0.58 mmol. initial loading) was added TFA (8 mL), water (1 mL), TIPS (0.5 mL) and phenol (0.5 g), and the suspension was shaken for 2 hours. The resin was filtered off, washed with TFA (2 x 5 mL), DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL) and Et_2O (3 x 5 mL), then suspended in 20% piperidine in DMF (10 mL) and shaken for 5 minutes, then filtered, washed with DMF (3 x 10 mL) and resuspended in 20% piperidine in DMF (10 mL). After 15 minutes further shaking, the resin was filtered, washed with DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL) and Et_2O (3 x 5 mL), then suspended in DCM (10 mL). Compound (**2.16**) (40 mg, 0.15 mmol) was added to the suspension, followed by Et_3N (20 μL, 0.15 mmol.). After 2 hours shaking, TLC of the solution confirmed that no (**2.16**) remained. The resin was filtered off and washed with DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL) and Et_2O (3 x 5 mL), then dried under vacuum.

25% (*tert*-Butoxycarbonyl)-aminoethyl TentaGel (**2.24**).

*Partial protection of TentaGel S NH_2 resin by reaction with di-*tert*-butyldicarbonate.*

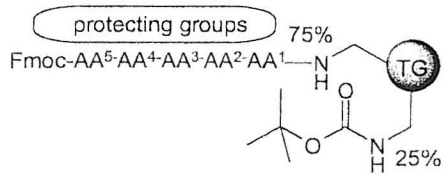


TentaGel S NH_2 resin (2 g, 0.29 mmol./g, 0.58 mmol.), was suspended in DCM (10 mL) and allowed to swell for 5 min. Di-*tert*-butyldicarbonate (35 mg, 0.145 mmol.) was then added, followed by Et_3N (20 μL, 0.15 mmol.) The resin was shaken for 2 hours, after

which time a TLC of the reaction solution showed that no di-*tert*-butyldicarbonate remained, then filtered off. The resin was washed with DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL) and Et_2O (3 x 5 mL) then dried under vacuum.

Protected library of 100,000 pentapeptides on TentaGel partially protected with a Boc group (**2.25**).[35]

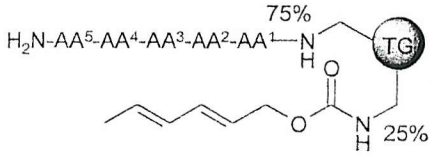
Split and mix synthesis of pentapeptides on resin ***(2.24)***.



Resin (**2.24**) (0.58 mmol. initial loading) was treated in an identical procedure to that described for the preparation of resin (**2.23**).

Deprotected library of 100,000 pentapeptides on TentaGel, 25% hexadienyloxycarbonyl substituted at the TentaGel amine (**L2.3**).[81]

Side chain deprotection, diene capping and Fmoc group removal on resin ***(2.25)***.



To resin (**2.25**) (0.58 mmol. initial loading) was added TFA (8 mL), water (1 mL), TIPS (0.5 mL) and phenol (0.5 g), and the suspension was shaken for 2 hours. The resin was filtered off, washed with TFA (2 x 5 mL), DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL) and Et_2O (3 x 5 mL), then re-suspended in DCM (10 mL). Compound (**2.16**) (80 mg, 0.3 mmol) was added to the suspension, followed by Et_3N (40 μL, 0.3 mmol.). After 2 hours shaking, a ninhydrin test confirmed that the reaction had gone to completion. The

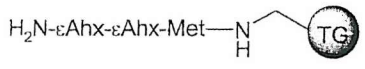
resin was filtered off and washed with DMF (3 x 5 mL), DCM (3 x 5 mL), MeOH (3 x 5 mL), then suspended in 20% piperidine in DMF (10 mL) and shaken for 5 minutes, then filtered, washed with DMF (3 x 10 mL) and resuspended in 20% piperidine in DMF (10 mL). After 15 minutes further shaking, the resin was filtered, washed with DMF (3 x 5 mL), DCM (3 x 5 mL), and MeOH (3 x 5 mL), and Et_2O (3 x 5 mL), then dried under vacuum.

Screening of samples from libraries **(L2.3)** and **(L2.4)** with 4-phenylazomaleinanil.

A 10 mg sample of each of libraries **(L2.3)**, **(L2.4)**, (750 nmol. diene substitution) resin **(2.13)**, and TentaGel S NH_2 were placed in a small petri dish. DCM (0.2 mL) containing 4-phenylazomaleinanil (20 μg, 75 nmol.) was added and lids sealed on to prevent evaporation of the solvent. The samples were viewed under a microscope and photographs were taken at regular intervals. After 24 hours, some resin beads from **(L2.3)** and **(L2.4)** were judged to be more coloured than those surrounding them. These beads were photographed and picked from the solutions using a micropipette. The samples containing resin **(2.13)** and TentaGel S NH_2 showed no increase in dye uptake. The screening experiment was repeated three times, and each time the most coloured three or four beads were photographed and picked from the **(L2.3)** and **(L2.4)** samples.

H_2N–εAhx-εAhx-Met-TentaGel **(2.26)**.[136]

Synthesis of a tripeptide on TentaGel resin.

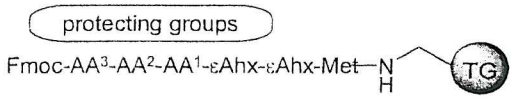


TentaGel S NH_2 resin (2g, 0.29 mmol/g, 0.58 mmol.) was allowed to swell in DCM (10 mL). Meanwhile, Fmoc-Met-OH (1.11g. 3 mmol.) and HOBt (0.41g, 3 mmol.) were dissolved in DMF (5 mL) and DIC (0.47 mL, 3 mmol.) was added. The solution was allowed to react for 10 minutes, and was then added to the resin suspension. After 2 hours shaking, a ninhydrin test showed that the reaction had gone to completion. The resin was

filtered off and washed with DMF (3 x 1 mL), and DCM (3 x 1 mL). The resin was then suspended in 20% piperidine in DMF and shaken for 5 minutes, then filtered, washed with DMF (3 x 10 mL) and resuspended in 20% piperidine in DMF (10 mL). After 15 minutes further shaking, the resin was filtered, washed with DMF (3 x 5 mL), DCM (3 x 5 mL), and MeOH (3 x 5 mL), Et_2O (3 x 5 mL) and dried under vacuum. The coupling reaction and Fmoc cleavage were repeated twice using Fmoc-εAhx-OH (1.06 g, 3 mmol.) in place of Fmoc-Met-OH.

Protected library of 1,000 tripeptides on εAhx-εAhx-Met-TentaGel resin (**2.27**).[35]

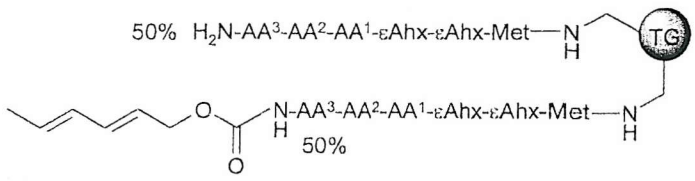
*Split and mix synthesis of tripeptides on resin (**2.26**).*



Resin (**2.26**) (0.58 mmol. initial loading) was treated with three rounds of split and mix peptide synthesis, as described for the preparation of resin (**2.23**). After the cleavage of the Fmoc group in each cycle, the resin was treated with a mixture of AcOH/D_3-AcOH 1:1 (1.8 mg, 0.03 mmol.), HOBt (4 mg, 0.03 mmol.) and DIC (4 μL, 0.03 mmol.) and shaken thoroughly for 1 hour. The resin was then washed with DMF (3 x 5 mL) and DCM (3 x 5mL) before suspension in DMF (10 mL) ready for splitting.

Deprotected library of 1,000 tripeptides on TentaGel, 50% hexadienyloxycarbonyl substituted at the *N*-terminus **(L2.6)**.[81]

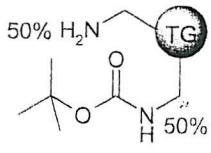
Side chain deprotection, Fmoc group removal and diene capping on resin ***(2.27)***.



Resin **(2.27)** was treated in a manner identical to that described for the preparation of library **(L2.4)**.

50% (*tert*-Butoxycarbonyl)-aminoethyl TentaGel **(2.28)**.

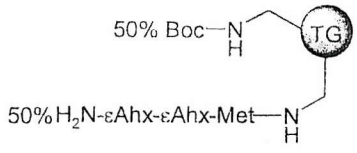
Partial protection of TentaGel S NH_2 *resin by reaction with* di-*tert*-butyldicarbonate *carbonate*.



TentaGel S NH_2 resin (2g, 0.29 mmol/g, 5.9 mmol.) was treated with di-*tert*-butyldicarbonate (70 mg, 0.29 mmol.) and Et_3N (40 μL, 0.3 mmol.) in a manner identical to that described for the preparation of resin **(2.23)**.

H_2N–εAhx-εAhx-Met-TentaGel (TentaGel 50% protected with a Boc group) **(2.29)**.[136]

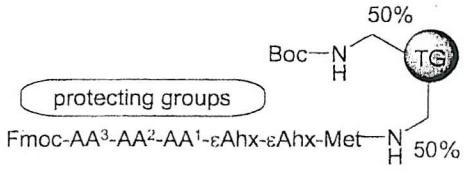
Synthesis of a tripeptide on resin ***(2.28)***.



Resin (**2.28**) (0.58 mmol. initial loading) was treated in a manner identical to that used for the synthesis of resin (**2.26**).

Protected library of 1,000 tripeptides on TentaGel partially protected with a Boc group (**2.30**).[35]

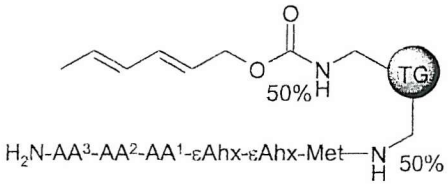
*Split and mix synthesis of tripeptides on resin (**2.29**).*



Resin (**2.29**) was treated in a manner identical to that described for the preparation of resin (**2.27**).

Deprotected library of 1,000 tripeptides on TentaGel, 50% hexadienyloxycarbonyl substituted at the TentaGel amine (**L2.5**).[81]

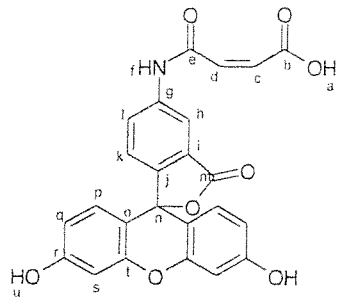
*Side chain deprotection, diene capping and Fmoc group removal on resin (**2.30**).*



Resin (**2.30**) was treated in a manner identical to that described for the preparation of library (**L2.3**).

3-(Fluorescein-5-ylcarbamoyl)-acrylic acid (**2.33**).[86]

Reaction of 5-aminofluorescein with maleic anhydride.



5-Aminofluorescein (1.05 g, 3.02 mmol.) and maleic anhydride (0.295 g, 3.02 mmol.) were dissolved in AcOH (50 mL) and stirred for 16 hours. The precipitated product was filtered off and washed with EtOAc (3 x 30 mL). The product was then dried under vacuum to give an yellow solid (1.26 g, 94%).

M.p >250 °C.

HPLC (Method 1) Purity 89% (ELSD) RT 2.87 minutes.

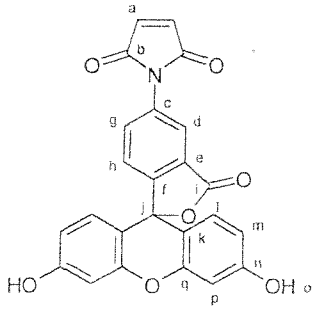
δ_H (300 MHz, DMSO-d_6) 12.0-13.0 (br s, 1H, H-a), 10.76 (s, 1H, H-f), 10.02-10.22 (br s, 2H, H-u), 8.35 (d, *J* 1, 1H, H-h), 7.82-7.89 (dd *J* 1 & 8 H-l), 7.20-7.28 (d, *J* 8, 1H, H-k), 6.68 (d, *J* 1, 2H, H-s), 6.33-6.62 (m, 6H, H-c, d, p, q).

m/z (ES-) 444 $[M-H]^-$ (17%),175 (100%), 159 (95%)

ν_{max} (Neat solid) 1704, 1681, 1580, 1535, 1460.

N-Fluorescein-5-yl maleimide **(2.34)**.[86]

Ring closing dehydration of 3-(fluorescein-5-ylcarbamoyl)-acrylic acid.



Compound **(2.33)** (200 mg, 0.45 mmol.) was dissolved in dry DMF (6 mL) and freshly distilled benzene (50 mL). HMDS (450 μL, 1.8 mmol) and $ZnCl_2$ (150 mg, 0.9 mmol) were added, and the mixture was refluxed under nitrogen for 4 hours. After cooling the

mixture was filtered and the filtrate concentrated under vacuum. The addition of 50 mL of iced water caused the product to precipitate. The product was filtered off and washed with distilled water (3 x 30 mL), then dried under vacuum to give an orange solid (148 mg, 77%).

m.p. >250 °C.

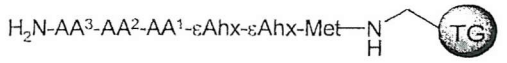
HPLC (Method 2) Purity 93% (ELSD) RT 7.88 minutes.

δ_H (300 MHz, DMSO-d_6) 9.85-10.15 (br s, 2H, H-o), 7.98 (s, 1H, H-d), 7.69-7.78 (d, *J* 8, H, H-g), 7.38-7.45 (d, *J* 8, 1H, H-h), 7.27 (s, 2H, H-a), 6.42-6.71 (m, 6H, H-l, m, p).

m/z (ES+) 428 $[M+H]^+$ (100 %).

Deprotected library of 1,000 tripeptides on TentaGel **(2.36)**.[81]

Removal of side chain protection and Fmoc group from resin ***(2.27)***.



Resin **(2.27)** (100 mg) was treated with TFA (0.8 mL), water (0.1 mL), TIPS (0.1 mL) and phenol (0.1 g), and the suspension was shaken for 2 hours. The resin was filtered off, washed with TFA (2 x 1 mL), DMF (3 x 1 mL), DCM (3 x 1 mL), MeOH (3 x 1 mL) and Et_2O (3 x 1 mL), then suspended in 20% piperidine in DMF (1 mL) and shaken for 5 minutes, then filtered, washed with DMF (3 x 1 mL) and resuspended in 20% piperidine in DMF (1 mL). After 15 minutes further shaking, the resin was filtered, washed with DMF (3 x 1 mL), DCM (3 x 1 mL), and MeOH (3 x 1 mL), and Et_2O (3 x 1 mL), then dried under vacuum.

Screening of libraries **(L2.5)** and **(L2.6)** with *N*-(fluorescein-5-yl) maleimide.

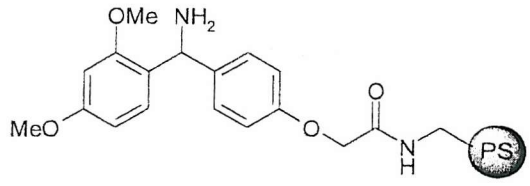
Two 10 mg samples of each of **(L2.5)**, **(L2.6)**, (1.5 μmol. diene substitution) **(2.36)**, **(2.13)**, and TentaGel S NH_2 were suspended in 0.1% TFA in DCM (0.5 mL) for 1 minute. The resins were then filtered off and immediately washed with DCM (3 x 0.5 mL). DCM or DMF (0.2 mL) containing compound **(2.34)** (65 μg, 150 nmol.) was added to each

sample, and the resins were shaken for two hours. The samples were then filtered off, and washed with DMF (3 x 0.5 mL), DCM (3 x 0.5 mL), MeOH (3 x 0.5 mL), 0.1M HCl (3 x 0.5 mL), 0.1 M Na_2CO_3 (3 x 0.5 mL), water (3 x 0.5 mL) and MeOH (3 x 0.5 mL) then placed in a Petri dish and DMF (0.2 mL) was added. The samples were viewed under a microscope with illumination from a mercury vapour lamp through an I3 fluorescence filter cube. Some resin beads from **(L2.5)** and **(L2.6)** showed considerable fluorescence. These beads were photographed and picked from the solutions using a micropipette. The samples containing resins **(2.36)**, **(2.13)** and TentaGel S NH_2 showed no increase in fluorescence. The screening experiment was repeated three times, and each time the most fluorescent three or four beads were photographed and picked from the **(L2.5)** and **(L2.6)** samples.

6.3. Experimental to Chapter 3.

H_2N-Rink AM-Polystyrene (3.1).[136, 89]

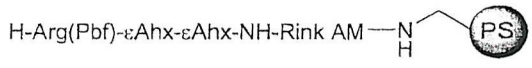
Coupling of the Rink AM linker onto polystyrene resin.



Aminomethyl polystyrene resin (200-400 mesh, 1.13 mmol/g, 0.5 g, 0.57 mmol.) was substituted with Fmoc Rink AM linker and the Fmoc group removed by the procedure described for the preparation of resin **(2.26)**.

H_2N-Arg(Pbf)-εAhx-εAhx-Rink AM-Polystyrene (3.2).[136]

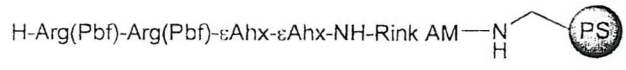
Fmoc peptide synthesis on resin (3.1).



Resin **(3.1)** (0.57 mmol) was substituted with Fmoc-εAhx-OH, Fmoc-εAhx-OH, and Fmoc-Arg(Pbf)-OH by the procedure described for the preparation of resin **(2.26)**. One third of the product resin was separated for cleavage to give **(3.5)**, the rest was carried through to the next reaction.

H_2N-Arg(Pbf)-Arg(Pbf)-εAhx-εAhx-Rink AM-Polystyrene (3.3).[136]

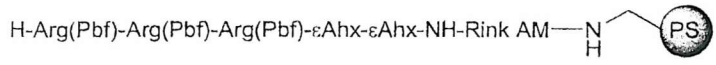
Fmoc peptide synthesis on resin (3.2).



Resin (**3.2**) (0.38 mmol) was substituted with Fmoc-Arg(Pbf)-OH, by the procedure described for the preparation of resin (**2.26**). Half of the product resin was separated for cleavage to give (**3.6**), the rest was carried through to the next reaction.

H_2N-Arg(Pbf)-Arg(Pbf)-Arg(Pbf)-εAhx-εAhx-Rink AM-Polystyrene (**3.4**).[136]

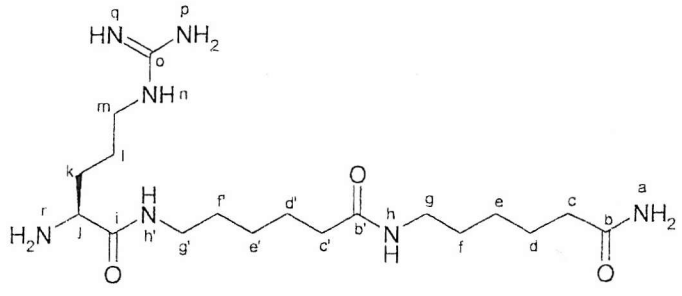
*Fmoc peptide synthesis on resin (**3.3**).*



Resin (**3.3**) (0.19 mmol) was substituted with Fmoc-Arg(Pbf)-OH, by an identical procedure to that described for the synthesis of resin (**2.26**).

H-Arg-ϵAhx-ϵAhx-NH_2 (**3.5**).[91]

*TFA mediated cleavage of H-Arg(Pbf)-ϵAhx-ϵAhx-Rink AM-polystyrene (**3.2**).*



H-Arg(Pbf)-ϵAhx-ϵAhx-Rink AM-polystyrene (**3.2**) (0.19 mmol) was washed with DMF (3 × 5 mL), AcOH (3 × 5 mL), DCM (3 × 5 mL) and MeOH (3 × 5 mL), then dried under vacuum overnight. The resin was then treated with a mixture of TFA, H_2O and TIPS (95:2.5:2.5) (3 mL) for 2 hours, and the resulting peptide solution was collected by vacuum filtration. The resin was washed with a further 3 mL of TFA followed by DCM (2 × 3 mL), and the combined solutions were allowed to react for 16 hours, then concentrated under vacuum. The addition of ice cold diethyl ether (10 mL) lead to precipitation of the

peptide which was isolated by filtration, washed with cold ether (2 × 10 mL), dissolved in water and then lyophilised to give the product as an off white gum (60 mg, 76%).

HPLC (Method S5OD), Purity 86% (ELSD), RT 0.98 min.

δ_H (300 MHz, D_2O) 3.71-3.82 (t, *J* 7, 1H, H-j), 3.15-3.23 (m, 2H, H-m), 3.08-3.15 (t, *J* 7, H-g), 2.98-3.07 (t, *J* 7, H-g'), 2.03-2.19 (m, 4H. H-c, c'), 1.69-1.8 (m, 2H, H-k), 1.54-1.63 (m, 2H, H-l), 1.39-1.53 (m, 4H, H-d, d'), 1.28-1.38 (t, *J* 7, 4H-f, f'), 1.09-1.21 (m, 4H, H-e, e').

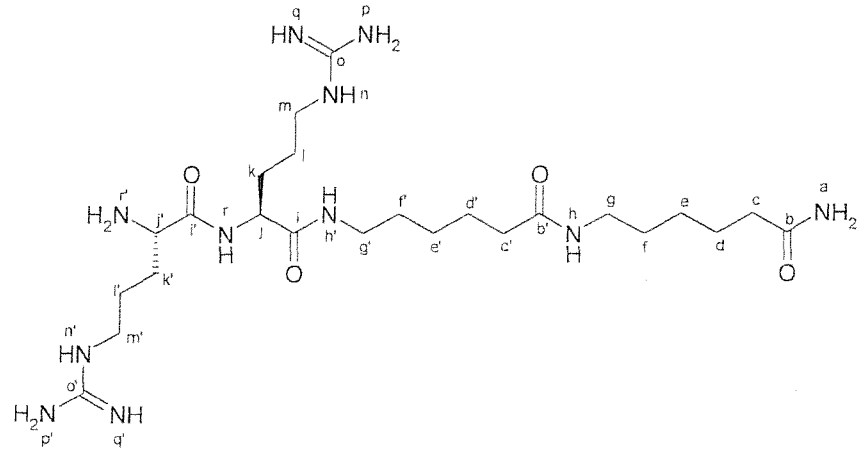
δ_C (76 MHz, D_2O) 180.7 (C-b), 177.1 (C-b'), 172.6 (C-i), 157.4 (C-o), 54.2 (C-j), 41.1 (C-m), 39.6 (C-g), 36.4 (C-c), 35.9 (C-c'), 29.2 (C-k), 26.1 (C-e, e'), 25.9 (C-f, f') 25.5 (C-d*), 25.4 (C-d*), 23.9 (C-l).

m/z (ES+) 400.2 (20%) $[M+H]^+$, 200.8 (25%) $[M+2H]^{2+}$, 126.9 (100%).

High resolution (ES+) $C_{18}H_{37}N_7O_3$, calculated 400.3036 $[M+H]^+$, found 400.3036.

H-Arg-Arg-ϵAhx-ϵAhx-NH_2 (3.6).[91]

TFA mediated cleavage of H-Arg(Pbf)-Arg(Pbf)-ϵAhx-ϵAhx-Rink AM-polystyrene (3.3).



H-Arg(Pbf)-Arg(Pbf)-ϵAhx-ϵAhx-Rink AM-polystyrene (**3.3**) (0.19 mmol) was treated in a manner identical to that used for the cleavage of H-Arg-ϵAhx-ϵAhx-NH_2.

The product was obtained as a fluffy white solid (90 mg, 81%)

* The use of an asterisk denotes that it was not possible to determine which residue this carbon was located, i.e. d* denotes that the signal is either d or d'.

M.p. 158-160 °C

HPLC (Method 1), Purity 92% (ELSD), RT 1.06 min.

δ_H (300 MHz, D_2O) 4.05-4.14 (t, *J* 7, 1H, H-j), 3.89-98 (t, *J* 7, 1H, H-j'), 2.90-3.14 (m, 8H, H-g, g', m, m'), 2.07-2.14 (t, *J* 7, 2H, H-c), 2.00-2.07 (t, *J* 7, 2H, H-c'), 1.71-1.83, (m, 2H, H-k'), 1.59-1.70 (m, 2H, H-k), 1.48-1.57 (m, 4H, H-l. l'), 1.40-1.48 (m, 4H, H-d, d'), 1.27-1.49 (t, *J* 7, 4H, H-f, f'), 1.05-1.23 (m, 4H, H-e, e').

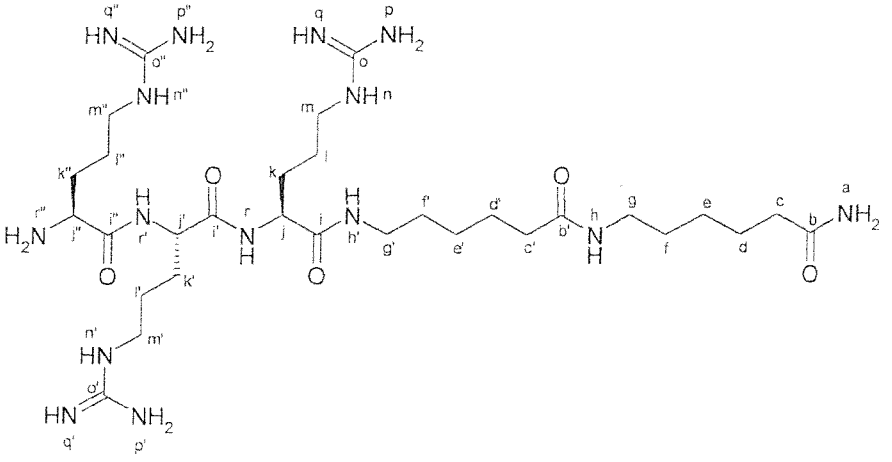
δ_C (76 MHz, D_2O) 180.9 (C-b), 177.5 (C-b'), 173.7 (C-i), 170.2 (C-i'), 157.6 (C-o, o'), 54.9 (C-j), 53.3 (C-j'), 41.3 (C-m*), 41.2 (C-m*), 40.0 (C-g*), 39.9 (C-g*), 36.5 (C-c), 35.7 (C-c'), 28.9 (C-k*), 28.8 (C-k*), 26.3 (C-e, e'), 25.75 (C-f, f') 25.8 (C-d*), 25.6 (C-d*), 25.2 (C-l*), 24.2 (C-l*).

m/z (ES+) 556.1 (10%) $[M+H]^+$, 278.8 (100%) $[M+2H]^{2+}$.

High resolution (ES+) $C_{24}H_{49}N_{11}O_4$, calculated 556.4047 $[M+H]^+$, found 556.4053.

H-Arg-Arg-Arg-ϵAhx-ϵAhx-NH_2 **(3.7)**.[91]

TFA mediated cleavage of H-Arg(Pbf)-Arg(Pbf)-Arg(Pbf)-ϵAhx-ϵAhx-Rink AM-polystyrene (3.4).



H-Arg(Pbf)-Arg(Pbf)-Arg(Pbf)-ϵAhx-ϵAhx-Rink AM-polystyrene **(3.4)** (0.19 mmol) was treated in a manner identical to that used for the cleavage of H-Arg-ϵAhx-ϵAhx-NH_2. The product was obtained as a fluffy white solid (143 mg, 100%)

M.p. 165 – 167 °C

HPLC (Method 1), Purity 88% (ELSD), RT 1.12 min.

δ_H (400 MHz, D_2O) 4.08-4.13 (t, *J* 7, 1H, H-j), 3.93-3.98 (t, *J* 7, 1H,. H-j'), 3.82-3.97 (t, *J* 7, 1H, H-j''), 2.83-3.01 (m, 10H, H-g, g', m, m', m''), 1.98-2.02 (t, *J* 7, 2H, H-c), 1.93-1.97 (t, *J* 7, 2H, H-c') 1.66-1.73 (m, 2H, H-k''), 1.49-1.63 (m, 4H, H-k, k'), 1.36-1.48 (m, 6H, H-l, l', l''), 1.29-1.35 (q, *J* 7, 4H, H-d, d'), 1.19-1.28 (m, 4H, H-f, f'), 0.98-1.11 (overlapping quintets, *J* 7 & 7, 4H, H-e, e').

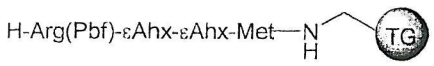
δ_C (101 MHz, D_2O) 180.5 (C-b), 177.1 (C-b'), 173.6 (C-i*), 173.5 (C-i*), 170.0 (C-i''), 157.2 (C-o, o', o''), 54.3 (C-j), 54.0 (C-j'), 52.9 (C-j''), 40.95 (C-m*), 40.9 (C-m*), 40.8 (C-m*), 39.53 (C-g*), 39.48 (C-g*), 36.1 (C-c), 35.3 (C-c'), 28.6 (C-k*), 28.5 (C-k*), 28.4 (C-f*), 28.39 (C-f*), 25.9 (C-l, l'), 25.86 (C-k''), 25.4 (C-e*), 25.2 (C-e*), 24.9 (C-d*), 24.7 (C-d*), 23.8 (C-l'').

m/z. (ES+) 712.5 (1%) $[M+H]^+$, 356.8 (100%) $[M+2H]^{2+}$, 238.3 (83%) $[M+3H]^{3+}$.

High resolution (ES+) $C_{30}H_{61}N_{15}O_5$, calculated 238.1731 $[M+3H]^+$, found 238.1727.

H-Arg(Pbf)-εAhx-εAhx-Met-TentaGel **(3.9)**.[136]

Fmoc peptide synthesis on resin ***(2.26)***.



Resin **(2.26)** (0.045 mmol.) was substituted with Fmoc-Arg(Pbf)-OH and the Fmoc group removed by the procedure described for the preparation of **(2.26)**. One third of the resin was separated for protecting group cleavage to give **(3.12)**, the rest was carried through to the next reaction.

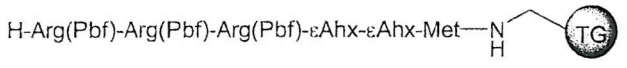
H-Arg(Pbf)-Arg(Pbf)-εAhx-εAhx-Met-TentaGel **(3.10)**.[136]

Fmoc peptide synthesis on resin ***(3.9)***.

Resin **(3.9)** (0.03 mmol.) was substituted with Fmoc-Arg(Pbf)-OH and the Fmoc group removed by the procedure described for the preparation of **(2.26)**. Half of the resin was separated for protecting group cleavage to give **(3.13)**, the rest was carried through to the next reaction.

H-Arg(Pbf)-Arg(Pbf)-Arg(Pbf)-εAhx-εAhx-Met-TentaGel **(3.11)**.[136]

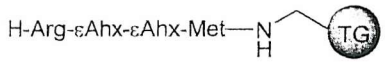
*Fmoc peptide synthesis on resin **(3.10)**.*



Resin **(3.10)** (0.015 mmol.) was substituted with Fmoc-Arg(Pbf)-OH and the Fmoc group removed by the procedure described for the preparation of **(2.26)**.

H-Arg-εAhx-εAhx-Met-TentaGel **(3.12)**.[91]

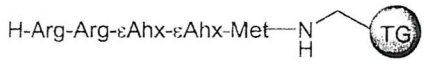
*Removal of side chain protection from resin **(3.9)**.*



Resin **(3.9)** (0.015 mmol.) was treated with a mixture of TFA, H_2O and TIPS (95:2.5:2.5) (1 mL) for 16 hours, filtered off and washed with DMF (3 x 1 mL), DCM (3 x 1 mL), MeOH (3 x 1 mL), 0.1 M Na_2CO_3 (3 x 1 mL), water (3 x 1 mL) MeOH (3 x 1 mL) and Et_2O (3 x 1 mL), then dried under vacuum.

H-Arg-Arg-εAhx-εAhx-Met-TentaGel **(3.13)**.[91]

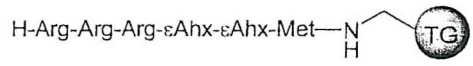
*Removal of side chain protection from resin **(3.10)**.*



Resin (**3.10**) (0.015 mmol.) was treated in an identical procedure to that described for the preparation of resin (**3.12**).

H-Arg-Arg-Arg-εAhx-εAhx-Met-TentaGel (**3.14**).[91]

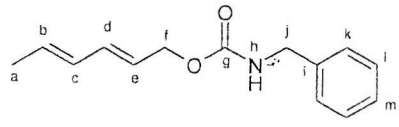
*Removal of side chain protection from resin (**3.11**).*



Resin (**3.11**) (0.015 mmol.) was treated in an identical procedure to that described for the preparation of resin (**3.12**).

E,E-2,4-Hexadienyl-(*N*-benzyl) carbamate (**3.15**).

Reaction of E,E-*2,4-hexadienyl-(4-nitrophenyl) carbonate (**2.16**) with benzylamine.*



Benzylamine (0.2 mL, 1.9 mmol) and *E,E*-2,4-hexadienyl-(4-nitrophenyl) carbonate (**2.16**) (0.5 g, 1.9 mmol) were dissolved in DCM (10 mL) under N_2. Et_3N (0.27 mL, 1.9 mmol) was added drop wise, upon which the solution turned yellow. The mixture was stirred for 2 hours and then the solvent removed under vacuum. The residue was dissolved in water and extracted with DCM three times. The combined organic extracts were washed with water and brine, dried over $MgSO_4$ and the solvent removed to give a yellowish solid (0.42 g 96%).

M.p. 37 - 39 °C

HPLC (Method 1) purity 96% (220 nm), RT 4.09 minutes.

δ_H (300 MHz, $CDCl_3$) 7.19-7.32 (m, 5H, H-k, l, m), 6.10-6.22 (dd *J* 10 & 15, 1H, H-c), 5.92-6.03 (dd, *J* 10 & 14, 1H, H-d), 5.62-5.71 (dq, *J* 15 & 8, 1H, H-b), 5.49-5.61 (dt, *J* 14

& 7, 1H, H-e), 4.95-5.04 (br s, 1H, H-h), 4.49-4.56 (d, *J* 7, 2H, H-i), 4.25-4.33 (d, *J* 7, 2H, H-f), 1.13-1.21 (d, *J* 8, 3H, H-a).

δ_C (75 MHz, $CDCl_3$) 156.6 (C-g), 138.7 (C-j), 134.7 (C-c), 131.3 (C-d), 130.6 (C-Ar), 128.8 (C-Ar), 127.7 (C-Ar), 126.3 (C-e), 124.4 (C-b), 65.7 (C-i), 45.2 (C-f), 18.3 (C-a).

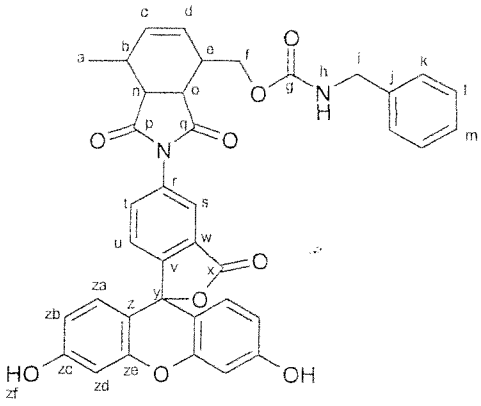
m/z (ES+) 254.1 (100%) $[M+Na]^+$.

High resolution (ES+) $C_{16}H_{21}NO_2$, calculated 254.1151 $[M+Na]^+$, found 254.1149.

ν_{max} (Neat solid) 3308, 1691, 1540, 1283.

Benzyl (2-(5-fluoresceinyl)-7-methyl-1,3-dioxo-3a,4,7,7a-tetrahydro-isoindol-4-ylmethyl) carbamate (3.17).

Diels Alder reaction of Hdoc-benzylamine with N-*fluorescein-5-yl maleimide (2.34).*



Compounds **(2.34)** (100 mg, 0.24 mmol.) and **(3.16)** (55 mg, 0.24) were dissolved in a 4:3 mixture of Water/THF (50 mL) and allowed to react for 6 days. The solvent was removed under vacuum and the residue purified by semi-preparative HPLC (Method 3). The fractions containing the product were combined and lyophilised to give the product as an orange solid (47 mg, 30%).

M.p. >250 °C

HPLC (Method 2) purity 100% (254 nm), RT 9.62 minutes.

δ_H (400 MHz, DMSO-d_6) 9.97-10.60 (br s, 2H, H-zf), 7.96 (s, 1H, H-s), 7.84-7.87 (t, *J* 6, 1H, H-h), 7.67-7.69 (d, *J* 8, 1H, H-t), 7.47-7.49 (d, *J* 8, 1H, H-u), 7.32-7.44 (m, 5H, H-k, l,

m) 6.80 (s, 2H, H-zd), 6.67-6.72 (m, 4H, H-za, zb), 5.93-6.02 (dd, *J* 16 & 9, 2H, H-c, d),4.55-4.65 (m, 2H, H-f) 4.30-4.32 (d, *J* 6, 2H, H-i), 3.61-3.67 (t, *J* 7, 1H, H-o), 3.34-3.42 (t, *J* 7, 1H, H-n), 2.76-2.84 (m, 1H, H-e), 2.64-2.71 (m, 1H, H-b), 1.48-1.51 (d, *J* 8, 3H, H-l).

δ_C (101 MHz, DMSO-d_6) 176.9 (C-p or q), 176.8 (C-p or q), 160.2 (C-x), 152.4 (C-g), 140.4 (Ar-quat) 135.3 (C-c or d)), 134.5 (C-t), 129.5 (C-za), 128.8 (C-u), 127.6 (C-c or d)), 127.3 (Ar), 125.3 (Ar), 123.2 (C-s), 113.2 (C-zb), 102.8 (C-zd), 64.8 (C-f), 45.4 (C-n), 44.3 (C-i), 43.7 (C-o), 36.3 (C-b), 31.9 (C-e), 17.1 (C-a).

m/z (ES+) 659 $[M+H]^+$ (25%), 681 $[M+Na]^+$ (15%), 130 (100%).

High resolution (ES+) $C_{38}H_{30}N_2O_9$ calculated 659.2024 $[M+H]^+$ found 659.2041.

ν_{max} (Neat solid) 1698, 1589, 1453, 1380, 1179, 1112.

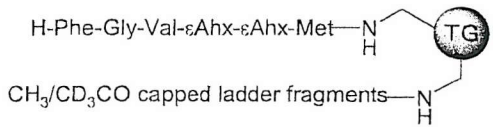
Kinetic Diels Alder experiments.

For each experiment compounds **(2.34)** (5 mg, 0.012 mmol.) and **(3.16)** (3 mg, 0.012 mmol.) were dissolved in water/THF 4:3 (3.5 mL), and catalyst (0, 10 or 20 mol%) was added. The mixtures were sonicated for 1 minute and then allowed to react. At regular intervals, a 20 μL sample was taken from the solution, diluted with 1 mL water, and a 20 μL injection was analysed by HPLC (Method 2). The amount of compound **(3.17)** formed was monitored by the appearance of a peak with RT 9.62 minutes. The concentration of product was calculated using a calibration curve by measuring the integrated peak area at 254 nm.

6.4. Experimental to Chapter 4.

H-Phe-Gly-Val-εAhx-εAhx-Met-TentaGel, 10% capped at each residue with AcOH/D_3-AcOH (**4.8**).[136]

Peptide synthesis with isotopic capping.



Resin (**2.26**) (0.135 mmol.) was substituted sequentially with Fmoc-Val-OH, Fmoc-Gly-OH and Fmoc-Phe-OH using the method described for the preparation of resin (**2.26**). After each coupling-deprotection step, partial capping was realized by shaking the resin with 0.1 equivalents of a 1:1 mixture of AcOH/D_3-AcOH, followed by the addition of 0.5 equivalents of PyBop and DIPEA for 45 min. The resin was then thoroughly washed with DMF (3 x 5 mL), DCM (5 x 5 mL) and methanol (3 x 5 mL), dried under vacuum and stored at -10 °C.

Single bead cleavage protocol for mass spectrometry sequencing.[100]

Single beads were picked randomly with a micropipette under 50 × magnification and placed into 100 μL capacity glass vial inserts inside 500 μL microcentrifuge tubes. 20 μL of a 20 mg/mL solution of cyanogen bromide in 1:1 H_2O/TFA was added to each and the tubes were sealed, sonicated for 5 minutes and then allowed to react in the dark for 24 h. The samples were then centrifuged under vacuum (3000 rpm, 50 °C) until dry. 10 μL of HPLC grade MeCN was added and the samples were sonicated for 5 min. then centrifuged at 5,000 rpm for 5 min.

MALDI sample preparation.[100]

2 μL of the cleaved peptide solution was applied to the MALDI sample plate and allowed to dry. A further 1 μL of sample and 1 μL of a saturated solution of α-cyano-4-hydroxycinnamic acid in 1:1 MeCN/H_2O containing 0.1% TFA were then applied on top, thoroughly mixed and allowed to dry.

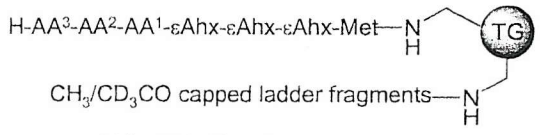
MALDI-TOF mass spectrometry and ladder sequencing.

All spectra were recorded in the positive ion mode on a Dynamo MALDI-TOF spectrometer. Spectra were obtained by summing multiple laser shots. Terfenadine (m/z 472.32 [M+H]), and Renin substrate tetradecapeptide (Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-His-Leu-Leu-Val-Tyr-Ser (m/z 1760 [M+H])) were used as external and internal calibrants. Peptide ladder fragments were generally observed as $M+Na^+$ adducts of the lactone.

Sequences were obtained by analysis of raw spectra using MassLynx v3.2 (Micromass Ltd., Manchester, UK). The cluster analysis function was used with the following parameters: First mass difference 3.02 or 5.03, first ratio 1:1, mass tolerance 0.1, ratio tolerance 80%, threshold 0.1-1%.

Mass spectrometry sequencing library 1 **(L4.1)**.[137]

Split and mix synthesis with isotopic capping.



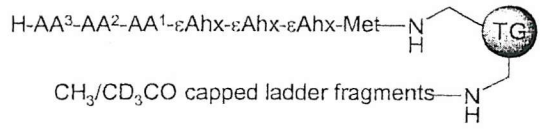
AA^1 = Val, Phe, Ala
AA^2 = Asn, Ser, Gln
AA^3 = Asp, Tyr, Trp

TentaGel S NH_2 (0.3g, 0.1 mmol.) resin was first substituted with the spacer-linker construct ϵAhx-ϵAhx-ϵAhx-Met using the method described for the preparation of resin **(2.26)**. Split and mix synthesis was then used to generate a library of 27 tripeptides using the coupling-deprotection-capping strategy described for the preparation of resin **(4.8)**.

The amino acid used in the first coupling was Fmoc-Val-OH, Fmoc-Phe-OH or Fmoc-Ala-OH, in the second position; Fmoc-As-OH, Fmoc-Ser(O^{t}Bu)-OH, or Fmoc-Gln-OH, and in the third position; Fmoc-Asp(O^{t}Bu)-OH, Fmoc-Try(O^{t}Bu)-OH or Fmoc-Trp(Boc)-OH. Side chain protecting groups were removed by treatment for 1 hour with a mixture of TFA (1.6 mL), water (0.2 mL), TIPS (0.1 mL) and phenol (100 mg). The resin was then thoroughly washed with DCM (5 × 5 mL) and methanol (3 × 5 mL), dried under vacuum and stored at -10 °C.

Mass spectrometry sequencing library 1 **(L4.2).**[137]

Split and mix synthesis with isotopic capping.

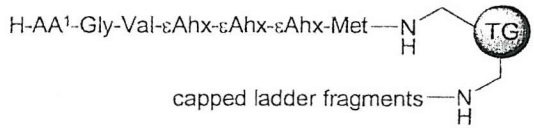


AA^1, AA^2, AA^3 = Arg, Trp or Asn

A 27 member tripeptide library was synthesized using the method described for the preparation of library **(L4.1)** using the amino acids Fmoc-Arg(Pac)-OH, Fmoc-Trp(Boc)-OH or Fmoc-As-OH in each of the three coupling cycles.

Mass spectrometry sequencing library **(L4.3).**[137]

Split and mix synthesis with isotopic capping.



AA^1 = Lys, Gln, Leu, Ile, Asp, Asn

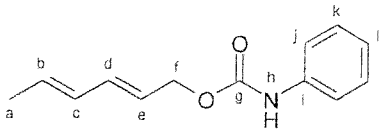
TentaGel S NH_2 (0.3g, 0.1 mmol.) resin was first substituted with the peptide-spacer-linker construct Gly-Val-ϵAhx-ϵAhx-ϵAhx-Met using the method described for the preparation of library **(L4.1)**, then split into six portions and each coupled with one of the

following amino acids: Fmoc-Lys(Boc)-OH, Fmoc-Leu-OH, Fmoc-Asp(O^{t}Bu)-OH, Fmoc-Ile-OH, Fmoc-Gln-OH, Fmoc-Asn-OH. The portions substituted with Lys, Leu or Asp were then partially capped with AcOH/D_3-AcOH as (**4.8**), the portions substituted with Ile, Gln or Asn were capped by the same method with a 1:1 mixture of benzoic acid/D_5 benzoic acid. The portions were then mixed and side chain deprotection was carried out as above.

6.5. Experimental to Chapter 5.

E,E-2,4-Hexadienyl-(*N*-phenyl) carbamate (**5.16**).

Reaction of E,E-*2,4-hexadienyl-(4-nitrophenyl) carbonate* ***(2.16)*** *with aniline.*



Aniline (0.17 mL, 1.9 mmol) and *E,E*-2,4-hexadienyl-(4-nitrophenyl) carbonate (**2.16**) (0.5 g, 1.9 mmol) were dissolved in DCM (10 mL) under N_2. Et_3N (0.27 mL, 1.9 mmol) was added drop wise, upon which the solution turned yellow. The mixture was stirred overnight and then the solvent removed under vacuum. The residue was purified by flash chromatography on silica gel, eluted with 5% EtOAc in hexane. The product was obtained as a pale yellow oil (0.335 g, 81%).

HPLC (Method 1) purity 92% (254 nm) RT 3.02 minutes.

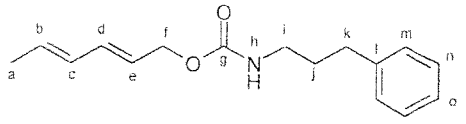
δ_H (300 MHz, $CDCl_3$) 7.05-7.13 (t, *J* 8, 2H, H-k), 6.59-6.67 (t, *J* 8, 1H, H-l), 6.52-6.58 (d, *J* 8, 2H, H-j), 6.09-6.19 (dd, *J* 14 & 10, 1H, H-c), 5.91-6.02 (dd, *J* 14 & 15, 1H, H-d), 5.53-5.68 (m, 2H, H-b, e), 3.66-3.72 (d, *J* 7, 2H, H-f), 1.63-1.70 (d, *J* 7, 3H, H-a).

δ_C (101 MHz, $CDCl_3$) 149.8 (C-g), 138.9 (C-i), 133.7 (C-c), 132.4 (C-d), 130.9 (C-k), 130.7 (C-l), 129.2 (C-j), 119.1 (C-b), 114.6 (C-e), 47.8 (C-f), 19.6 (C-a).

ν_{max} (Neat solid) 3407, 1587, 1503, 991.

E,E-2,4-Hexadienyl-(*N*-3-phenylpropyl) carbamate (**5.17**).

Reaction of E,E-*2,4-hexadienyl-(4-nitrophenyl) carbonate* ***(2.16)*** *with 3-phenylpropylamine.*



3-Phenylpropylamine (0.27 mL, 1.9 mmol) and *E,E*-2,4-hexadienyl-(4-nitrophenyl) carbonate **(2.16)** (0.5 g, 1.9 mmol) were dissolved in DCM (10 mL) under N_2. Et_3N (0.27 mL, 1.9 mmol) was added drop wise, upon which the solution turned yellow. The mixture was stirred for 2 hours and then the solvent removed under vacuum. The residue was dissolved in water and extracted with DCM three times. The combined organic extracts were washed with water and brine, dried over $MgSO_4$ and the solvent removed to afford a yellowish solid (0.462 g, 94%).

M.p. 40 - 42 °C

HPLC (Method 1) purity 90% (254 nm) RT 4.36 minutes.

δ_H (300 MHz, $CDCl_3$) 7.09-7.27 (m, 5H, H-m, n, o), 6.12-6.25 (dd, *J* 15 & 10, 1H, H-c), 5.92-6.04 (dd, *J* 14 & 10, 1H, H-d), 5.62-5.73 (dq, *J* 15 & 7, 1H, H-b), 5.48-5.62 (dt, *J* 14 & 7, 1H, H-e) 4.60-4.69 (br s, 1H, H-h), 4.43-4.51 (d, *J* 7, 2H, H-f), 3.07-3.19 (dt, *J* 7 & 8, 2H, H-i), 2.52-2.61 (t, *J* 8, 2H, H-k), 1.70-1.82 (tt, *J* 8 & 8, 2H, H-j), 1.65-1.72 (d, *J* 7, 3H, H-a).

δ_C (75 MHz, $CDCl_3$) 156.8 (C-g), 141.4 (C-l), 134.6 (C-c), 131.2 (C-d), 130.6 (C-Ar), 128.6 (C-Ar), 128.5 (C-Ar), 126.1 (C-e), 124.5 (C-b), 65.5 (C-f), 40.7 (C-h), 33.1 (C-k), 31.7 (C-j), 18.3 (C-a).

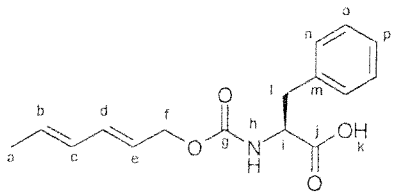
m/z (ES+) 260 $[M+H]^+$ (3%), 158 (100%)

High resolution (ES+) $C_{14}H_{17}NO_2$, calculated 282.1464 $[M+Na]^+$, found 282.1462.

ν_{max} (Neat solid) 3319, 1718, 1687, 1550, 1263.

<u>*N-(E,E*-2,4-Hexadienyloxycarbonyl)-phenylalanine **(5.19).**</u>

Reaction of E,E-*2,4-hexadienyl-(4-nitrophenyl) carbonate with phenylalanine.*



Phenylalanine (1 g, 6 mmol) and *E,E*-2,4-hexadienyl-(4-nitrophenyl) carbonate **(2.16)** (1.6 g, 6 mmol) were dissolved in 1:1 THF/H_2O (100 mL) and Et_3N (1.66 mL, 12 mmol) was

added drop wise with cooling. The mixture was stirred overnight, then concentrated under vacuum. The residue was dissolved in 1M $NaHCO_{3\,(aq)}$ (20 mL) and washed with EtOAc (3 × 15 mL). The aqueous layer was then acidified to pH 6 with 10% aqueous citric acid, and extracted with EtOAc (3 × 15 mL). The organic extracts were combined and washed with 5% aqueous citric acid solution and brine, dried over $MgSO_4$ and the solvent removed to give the product as a translucent gum. (1.19 g, 69%).

M.p. 53 - 54.5 °C

HPLC (Method 1) Purity 100% (ELSD) RT 3.65 minutes

δ_H (300 MHz, DMSO) 12.53-12.94 (br s, 1H, H-k), 7.52-7.61 (d, *J* 8, 1H, H-h), 7.17-7.29 (m, 5H, H-n, o, p). 6.12-6.23 (dd, *J* 10 & 15, 1H, H-c), 5.99-6.11 (dd, *J* 10 & 14, 1H, H-d), 5.64-5.78 (dq, *J* 15 & 7, 1H, H-b), 5.50-5.63 (dt, *J* 14, 6, 1H, H-e), 4.31-4.48 (m, 2H, H-f), 4.08-4.19 (m, 1H, H-i), 3.00-3.12 (dd, *J* 4 & 13, 1H, H-l^a), 2.74-2.88 (dd, *J* 9 & 13, 1H, H-l^b), 1.68-1.77 (d, *J* 7, 3H, H-a).

δ_C (101 MHz, $CDCl_3$) 175.6 (C-j), 156.4 (C-g), 136.2 (C-m), 135.1 (C-c), 131.6 (C-d), 130.8 (C-Ar), 129.7 C-Ar), 129.0 (C-Ar), 127.5 (C-e), 124.3 (C-b), 66.2 (C-f), 55.1 (C-i), 38.2 (C-l), 18.5 (C-a).

m/z (ES-) 288 (25%) $[M-H]^-$, 190.1 (100%).

High resolution (ES+) $C_{16}H_{19}NO_4$, calculated 312.1206 $[M+Na]^+$, found 312.1203.

ν_{max} (Neat solid) 3319, 1691, 1524, 1200.

Procedure for investigation of Hdoc lability.

A 10 mg sample of Hdoc-Phe-OH **(5.19)** was added to 1 mL of each of the following mixtures:

1. 95% TFA in DCM.
2. 10% TFA in DCM.
3. 1% TFA in DCM.
4. 0.1 % TFA in DCM.
5. 4M HCl in dioxane.
6. 2M HCl in water.

7. 10% AcOH in DCM

8. 20% piperidine in DMF.

9. 5% DBU in DCM.

10. 1 eq. $Pd(PPh_3)_4$ in $CHCl_3$/AcOH/NMM (37:2:1).

11. 2M NaOH in water.

12. 1M I_2 in DMF.

13. 5% N_2H_4 in DMF.

14. 100 °C heating in DMF

15. 1M $NaBH_4$ in THF

16. U.V. irradiation in DCM.

17. 1M TBAF in THF.

The reactions were monitored by TLC after 10 min, 1 hour, 2 hours and 16 hours. After 16 hours a 20 μL sample of each solution was diluted with MeCN (0.5 mL) and a 20 μL injection was analysed by HPLC (method 1) to determine the amount of Hdoc-Phe-OH that remained (RT 3.65 minutes), and the amount of H-Phe-OH (RT 1.69 minutes) formed. Samples 1, 2 and 3 were found to contain exclusively H-Phe-OH, samples 4, 5 and 6 were found to contain a mixture of the two compounds, and sample 12 was found to contain neither compound. The remaining samples were found to contain exclusively Hdoc-Phe-OH.

The identity of the cleaved compound was confirmed as H-Phe-OH by evaporation of sample 2, and comparison of the residue with an authentic sample by ^{1}H-NMR.

References.

[1] Hagemeyer, A.; Jandeleit, B.; Liu, Y., Poojary, D. M., Turner, H. W.; Volpe, A. F.; Weinberg, W. H. *Applied Catalysis A: General*, **2001**, *221*, 23-43.

[2] Borman, S. *Chem. Eng. News* **1996**, *74*, 4(45), 37-39.

[3] Gennari, C.; Nestler, H. P.; Piarulli, U.; Salom, B., *Liebigs Ann./Recueil,* **1997**, 637-647.

[4] Reetz, M. T. *Angew. Chem. Int. Ed.* **2001**, *40*, 284-310.

[5] For a review see: Jandeleit, B.; Schaefer, D. J.; Powers, T. S.; Turner, H. W.; Weinberg, W. H. *Angew. Chem. Int. Ed.* **1999**, *38*, 2494-2532.

[6] For a review see: Senkan, S. *Angew. Chem. Int. Ed.* **2001**, *40*, 312-329.

[7] Figure adapted from reference 1.

[8] Liu, G.; Ellman, J. A. *J. Org. Chem.* **1995**, *60*, 7712-3.

[9] Thompson, L. A.; Ellman, J. A. *Tetrahedron Lett.* **1994**, *35*, 9333-9336.

[10] Gennari, C.; Ceccarelli, S.; Piarulli, U.; Montalbetti, C. A. G. N; Jackson, R. F. W. *J. Org. Chem.* **1998**, *63*, 5312-5313.

[11] Gao, X.; Kagan, H. B. *Chirality*, **1998**, *10*, 120-124.

[12] Gilbertson, S. R.; Chen, G.; McLoughlin M. *J. Am. Chem. Soc.* **1994**, *116*, 4481-4482

[13] Gilbertson, S. R.; Wang, X. *J. Org. Chem.* **1996**, *61*, 434-435.

[14] Bray, A. M.; Maefi, N. J.; Geysen, H. M. *Tetrahedron Lett.* **1990**, *31*, 5811-5814.

[15] Gilbertson, S. R.; Collibee, S. E.; Agarkov, A. *J. Am. Chem. Soc.* **2000**, 6522-6523.

[16] Menger, F. M.; Eliseev, A. V.; Migulin, V. A. *J. Org. Chem.* **1995**, *60*, 6666-6667.

[17] Menger, F, M.; West, C. A.; Ding, J. *Chem. Comm.* **1997**, 633-634.

[18] Menger, F. M.; Ding, J.; Barragan, V. *J. Org. Chem.* **1998**, *63*, 7578-7579.

[19] Hayashi, M.; Tamura, M.; Oguni, N. *Synlett.* **1992**, 663-664.

[20] Cole, B. M.; Shimizu, K. D.; Krueger, C. A.; Harrity, J. P. A.; Snapper, M. L.; Hoveyda, A. H., *Angew. Chem. Int. Ed.* **1996**, *35*, 1668-1671.

[21] Dooley, C. T.; Houghten, R. A.; *Life Sciences* **1993**, *52*, 1509-1517.

[22] Shimizu, K. D.; Cole, B. M.; Krueger, C. A.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H., *Angew. Chem. Int. Ed.* **1997**, *36*, 1704-1707.

[23] For a review see Duthaler, R. O. *Tetrahedron* **1994**, *38*, 1539-1650.

[24] Sigman, M. S.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1998**, *120*, 4901-4902.

[25] Sigman, M. S.; Vachal, P.; Jacobsen, E. N. *Angew. Chem. Int. Ed.* **2000**, *39*, 1279-1281.

[26] Porter, J. R.; Wirschun, W. G.; Kuntz, K. W.; Snapper, M. L.; Hoveyda, A. H. *J. Am. Chem. Soc.* **2000**, *122*, 2657-2658.

[27] Francis M. B.; Jacobsen, E. N. *Angew. Chem. Int. Ed.* **1999**, *38*, 937-941.

[28] Burgess, K.; Lim, H.-J.; Porte, A. M.; Sulikowski, G. A. *Angew. Chem. Int. Ed.* **1996**, *35*, 220-222.

[29] Bromidge, S.; Wilson, P. C.; Whiting, A. *Tetrahedron Lett.* **1998**, *29*, 8905-8908.

[30] Whiting, A. *Chem. Br.* **1999**, 31-34.

[31] Reetz, M. T.; Kühling, K. H.; Deege, A.; Hinrichs, H.; Belder, D. *Angew. Chem. Int. Ed.* **2000**, *39*, 3891-3893.

[32] Reetz, M. T.; Becker, M. H.; Klein, H. W.; Stökigt, D. *Angew. Chem. Int. Ed.* **1999**, *38*, 1758-1761.

[33] Mikami, K.; Angelaud, R.; Ding, K.; Ishii, A.; Tanaka, A.; Sawada, N.; Kudo, K.; Senda, M. *Chem. Eur. J.* **2001**, *7*, 730-737.

[34] Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Pept. Prot. Res.* **1991**, *37*, 487-493.

[35] Lam, K. S.; Salmon, S. E.; Hersh, E. M.; Hruby, V. J.; Kazmiersky, W. M.; Knapp, R. J. *Nature*, **1991**, *354*, 82-84.

[36] Moates, F. C.; Somani, M.; Annamalai, J.; Richardson, J. T.; Luss, D.; Wilson, R. C. *Ind. Eng. Chem. Res.* **1996**, *35*, 4801-4803.

[37] Taylor, S. J.; Morken, J. P. *Science*, **1998**, *280*, 267-280.

[38] Ohlmeyer, M. H. J.; Swanson, R. N.; Dillard, L. W.; Reader, J. C.; Asouline, G.; Kobayashi, R.; Wigler, M.; Still, W. C. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 10922-10926.

[39] Reetz, M. T.; Becker, M. H.; Kühling, K. M.; Holzwarth, A. *Angew. Chem. Int. Ed.* **1998**, *37*, 2647-2650.

[40] Larrow, J. F.; Schaus, S. E.; Jacobsen, E. N. *J. Am. Chem. Soc.* **1996**, *118*, 7420-7421.

[41] Schaus, S. E.; Jacobsen, E. N. *Tetrahedron Lett.* **1996**, *37*, 7937-7940.

[42] Tokunaga, M.; Larrow, J. F.; Kakiuchi, F.; Jacobsen, E. N. *Science* **1997**, *277*, 936-938.

[43] Reetz, M. T.; Becker, M. H.; Liebl, M.; Fürstner, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 1236-1239.

[44] For a review see: Grubbs, R. H.; Chang, S. *Tetrahedron* **1998**, *54*, 4413-4450.

[45] Connolly, A. R.; Sutherland, J. D. *Angew. Chem. Int. Ed.* **2000**, *39*, 4268-4271.

[46] Shaughnessy, K. H.; Kim, P.; Hartwig, J. F. *J. Am. Chem. Soc.* **1999**, *121*, 2123-2132.

[47] Stambuli, J. P.; Stauffer, S. R.; Shaughnessy, K. H.; Hartwig, J. F. *J. Am. Chem. Soc.* **2001**, *123*, 2677-2678.

[48] For a review see: da Silva, A. P.; Gunaratne, H. Q. N.; Gunnlaugsson, T.; Huxley, A. J. M.; McCoy, C. P.; Rademacher, J. T.; Rice, T. E.; *Chem. Rev.*, **1997**, *97*, 1515-1566.

[49] Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **1999**, *121*, 4306-4307.

[50] Miller, S. J.; Copeland, G. T.; Papaioannou, N.; Horstmann, T. E.; Ruel, E. M.; *J. Am. Chem. Soc.* **1998**, *120*, 1629-1630.

[51] Harris, R. F.; Nation, A. J.; Copeland, G. T.; Miller, S. J. *J. Am. Chem. Soc.* **2000**, *122*, 11270-11271.

[52] Cooper, A. C.; McAlexander, L. H.; Lee, D-H.; Torres, M. T.; Crabtree, R. H. *J. Am. Chem. Soc.* **1998**, *120*, 9971-2.

[53] Barber, D. E.; Lu, Z.; Richardson, T.; Crabtree, R. H. *Inorg. Chem.* **1992**, *31*, 4709-4711.

[54] Hermann, W. A.; Brossmer, C.; Reisinger, C.-P.; Riermeier, T. H.; Ofele, K.; Beller, M. *Chem. Eur. J.* **1997**, *3*, 1357-1364.

[55] Berkessel, A.; Hérault, D. A. *Angew. Chem. Int. Ed.* **1999**, *38*, 102-105.

[56] Lavastre, O.; Morken, J. P. *Angew. Chem. Int. Ed.* **1999**, *38*, 3163-3165.

[57] Zollinger, H. *Diazo Chemistry I: Aromatic and Heteroaromatic Compounds.*, VCH, Weinheim, **1994**.

[58] Trost, B. M.; Van Vranken, D. L. *Chem. Rev.* **1996**, *96*, 295-422 and references therein.

[59] Danek, S. C.; Queffelec, J.; Morken, J. P. *Chem. Comm.* **2002**, 528-529.

[60] Müller, M.; Mathers, T. W.; Davis, A. P. *Angew. Chem. Int. Ed.*, **2001**, *40*, 3813-3815.

[61] Laumen, K.; Reimerdes, E. H.; Schneider, M.; Gorisch, H. *Tetrahedron Lett.* **1985**, *26*, 407-410.

[62] Boussie, T. R.; Coutard, C.; Turner, H. W.; Murphy, V.; Powers, T. S. *Angew. Chem. Int. Ed.* **1998**, *37*, 3272-3275.

[63] Boussie, T. R.; Murphy, V.; Hall, K. A.; Coutard, C.; Dales, C.; Petro, M.; Carlson, E.; Turner, H. W.; Powers, T. S. *Tetrahedron* **1999**, *55*, 11699-11710.

[64] Maclean D.; Schullek, J. R.; Murphy, M. M.; Ni, Z. J.; Gordon, E. M.; Gallop M. A. *Proc. Natl. Acad. Sci USA*, **1997**, *94*, 9805-9810.

[65] Tarasow, T. M.; Tarasow, S. L.; Eaton, B. E. *Nature*, **1997**, *389*, 54-57.

[66] Seelig, B.; Jäschke, A. *Tetrahedron Lett.* **1997**, *38*, 7729-7732.

[67] Seelig, B.; Jäschke, A. *Chem. Biol.* **1999**, *6*, 167-176.

[68] Tarasow, T. M.; Tarasow, S. L.; Tu, C.; Kellogg, E.; Eaton, B. E. *J. Am. Chem. Soc.* **1999**, *121*, 3614-3617.

[69] Tarasow, T. M.; Tarasow, S. L.; Eaton, B. E. *J. Am. Chem. Soc.* **2000**, *122*, 1015-1021.

[70] Seelig, B.; Keiper, S.; Stuhlmann, F.; Jäschke, A. *Angew. Chem. Int. Ed.* **2000**, *39*, 4576-4579.

[71] Stuhlmann, F.; Jäschke, A. *J. Am. Chem. Soc.* **2002**, *124*, 3238-3244.

[72] Taran, F.; Gauchet, C.; Mohar, B.; Meunier, S.; Valleix, A.; Renard, P. Y.; Créminon, C.; Grassi, J.; Wagner, A.; Mioskowski, C. *Angew. Chem. Int. Ed.* **2002**, *41*, 124-127.

[73] Taran, F.; Renard, P. Y.; Créminon, C.; Valleix, A.; Frobert, Y.; Pradelles, P.; Grassi, J.; Mioskowski, C. *Tetrahedron Lett.* **1999**, *40*, 1891-1894.

[74] Diels, O.; Alder, K. *Justus Liebigs Ann. Chem.* **1928**, 98.

[75] Sauer, J. *Angew. Chem.* **1967**, *79*, 76-82.

[76] Patent; Esso Research Eng. Co., US 2856410, **1955**.

[77] Yates, P. Eaton, P. *J. Am. Chem. Soc.* **1960**, *82*, 4436-4437.

[78] Diagram adapted from: Carruthers, W. *Some modern methods of Organic Synthesis* (3rd Ed.) Cambridge University Rress: Cambridge, 1987, ISBN 0521311179; p 222.

[79] Kreiser, W.; Haumesser, W.; Thomas, A. F. *Helv. Chim. Acta* **1974**, *57*, 164-167.

[80] Bayer, E., *Angew. Chem. Int. Ed.* **1991**, *30*, 113-129.

[81] Fields, C. G.; Fields, G. B., *Tetrahedron Lett.*, **1993**, *34*, 6661-6664.

[82] Kunz, H.; Unverzagt, C. *Angew. Chem. Int. Ed.* **1984**, *23*, 436-437.

[83] Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I., *Anal. Biochem.*, **1970**, *34*, 595-598.

[84] McKay, F. C.; Albertson, N. F. *J. Am. Chem. Soc.* **1957**, *79*, 4686-4690.

[85] El-Merzabani, M. M.; Sakurai, Y. *Chem. Pharm. Bull.* **1972**, *20*, 2459-2462.

[86] Reddy, P. Y.; Kondo, S. K.; Fujita, S.; Toru, T. *Synthesis* **1998**, 999-1002.

[87] Leonhardt, H.; Gordon, L.; Livingston R. *J. Phys. Chem.* **1971**, *75*, 245-249.

[88] Martin, M. M.; Lindqvist, L. *J. Luminescence* 1975, *10*, 381-390.

[89] Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787-3790.

[90] Carpino, L. A.; Shroff, H.; Triolo, S. A.; Mansour, E-S. M. E.; Wenschuh, H.; Albericio, F. *Tetrahedron Lett.* **1993**, *34*, 7829-32.

[91] White, P.; Dörner, B.; Steinauer, R. *Synthesis Notes, Novabiochem Catalog*; Novabiochem: Nottingham, 2002; pp 3.14-3.19.

[92] A more detailed justification for the derivation of the equations used can be found in the following text: Atkins, P. W. *Physical Chemistry* (5th Ed.) Oxford University Press: Oxford, 1994; ISBN 0-19-269042-6; pp 864-874.

[93] Houghten, R. A.; Dinh, K. T.; Burchin, D. E.; Blondell, S. E. In *Techniques in Protein Chemistry IV*; Amgeletti, R. H., Ed.; Academic Press: San Diego, CA, 1993; pp 249-256.

[94] Geysen, M. H.; Meloen, R. N.; Barleling, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1984**, *81*, 3998-4002.

[95] Furka, A,; Christensen, J. W.; Healy, E.; Tanner, H. R.; Saneii, H. *J. Comb. Chem.* **2000**, *2*, 220-223.

[96] Moran, E. J.; Sarshar, S.; Cargill, J. F.; Shahbaz, M. M.; Lio, A.; Mjalli, A. M. M.; Armstrong, R. W. *J. Am. Chem. Soc.* **1995**, *117*, 10787-10788.

[97] Nicolaou, K. C.; Xiao, X.–Y.; Parandoosh, Z.; Senyei, A.; Nova, M. P. *Angew. Chem. Int. Ed.* **1995**, *34*, 2289-2291.

[98] Houghten, R. A. *Proc. Natl. Acad. Sci. U.S.A.*, **1985**, *82*, 5151-5135.

[99] A system for the packaging of resin into porous polypropylene "cans" for larger scale solid phase synthesis has been developed and is marketed by IRORI.

[100] Youngquist, R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *Rapid Commun. Mass Spectrom.* **1994**, *8*, 77-81.

[101] Youngquist, R. S.; Fuentes, G. R.; Lacey, M. P.; Keough, T. *J. Am. Chem. Soc.* **1995**, *117*, 3900-3906.

[102] Davies, M.; Bradley, M. *Tetrahedron Lett.* **1997**, *38*, 8565-8568.

[103] Hancock, W. S.; Marshall, G. R. *J. Am. Chem. Soc.* **1975**, *97*, 7488-7489.

[104] Davies, M.; Bradley, M. *Angew. Chem. Int. Ed.* **1997**, *36*, 1097-1099.

[105] Hoffmann, C.; Blechschmidt, D.; Krüger, R.; Karas, M.; Griesinger, C. *J. Comb. Chem.* **2002**, *4*, 79-86.

[106] Geysen, H. M.; Wagner, C. D.; Bodnar, W. M., Markworth, C. J.; Parke, G. J.; Schoenen, F. J.; Wagner, D. S.; Kinder, D. S. *Chem. Biol.* **1996**, *3*, 679-688.

[107] Carrasco, M. R.; Fitzgerald, M. C.; Oda, Y.; Kent, B. H. *Tetrahedron Lett.* **1997**, *38*, 6331-6334.

[108] McKeown, S. C.; Watson, S. P.; Carr, R. A. E.; Marshall, P. *Tetrahedron Lett.* **1999**, *40*, 2407-2410.

[109] Murray, P. J.; Kay, C.; Sciciński, J. J.; McKeown, S. C.; Watson, S. P.; Carr, R. A. E. *Tetrahedron Lett.* **1999**, *40*, 5609-5612.

[110] Lorthioir, O.; McKeown, S. C.; Parr, N. J.; Washington, M.; Watson, S. P. *Tetrahedron Lett.* **2000**, *41*, 8609-8613.

[111] Lorthioir, O.; Carr, R. A. E.; Congreve, M. S.; Geysen, H. M.; Kay, C.; Marshall, P.; McKeown, S. C.; Parr, N. J.; Sciciński, J. J.; Watson, S. P. *Anal. Chem.* **2001**, *73*, 963-970.

[112] Williams, G. M.; Carr, R. A. E.; Congreve, M. S.; Kay, C.; McKeown, S. C.; Murray, P. J.; Sciciński, J. J.; Watson, S. P. *Angew. Chem. Int. Ed.* **2000**, *39*, 3293-3296.

[113] McKeown, S. C; Heudi, O.; Kay, C.; Marshall, P. *Rapid Commun. Mass Spectrom.* **2002**, *16*, 1054-1058.

[114] Lane, S. J.; Pipe, A. *Rapid Commun. Mass Spectrom.* **1999**, *13*, 798-814.

[115] Lane, S. J.; Pipe, A. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 782-793.

[116] Gausepohl, H,; Pieles, U.; Frank, R. W. In *Proceedings of the 12th American Peptide Symposium,* Smith, J. A.; Rivier, J. E. Eds., ESCOM, Leiden, 1992, pp. 523-524.

[117] Story, S. C.; Aldrich, J. V. *Int. J. Pept. Protein. Res.* **1994**, *43*, 292-296.

[118] Arttamangkul, S.; Arbogast, B.; Barofsky, D.; Aldrich, J. V. *Lett. Pept. Sci.* **1996**, *3*, 357-370.

[119] Albericio, F.; Cases, M.; Alsina, J.; Triolo, S. A.; Carpino, L. A.; Kates, S. A. *Tetrahedron Lett.* **1997**, *38*, 4853-4856.

[120] Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205-208.

[121] Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Peptide Protein Res.* **1991**, *37*, 487-493.

[122] Kocieński, P. J. *Protecting Groups*; Thieme: Stuttgart; New York, 1986; pp 2-3.

[123] Barany, G.; Merrifield, R. B. *J. Am. Chem. Soc.* **1977**, *99*, 7363-7365.

[124] Barany, G.; Albericio, F. *J. Am. Chem. Soc.* **1985**, *107*, 4936-42.

[125] Bergman, M.; Zervas, L. *Ber. Dtsch. Chem. Ges.* **1932**, *65*, 1192.

[126] Carpino, L. A.; Han, G. Y. *J. Org. Chem.* **1972**, *37*, 3404-3409.

[127] Kunz, H.; Unverzagt, C. *Angew. Chem. Int. Ed.* **1988**, *27*, 1697-99.

[128] Lee, J.; Griffin, J. H.; Nicas, T. I. *J. Org. Chem.* **1996**, *61*, 3983-3986.

[129] Carpino, L. A.; Tsao, J. –H.; Ringsdorf, H.; Fell, E.; Heltrich, G. *J. Chem. Soc., Chem. Commun.* **1978**, 358-359.

[130] Carpino, L. A.; Sau, A. C. *J. Chem. Soc., Chem. Commun.* **1979**, 514-515.

[131] Bycroft, B. W.; Chan, W. C.; Chhabra, S. R.; Hone, N. D. *J. Chem. Soc., Chem. Commun.* **1993**, 778-779.

[132] Khorana, H. G. *Pure Appl. Chem.* **1968**, *17*, 349-381.

[133] Hiskey, R. G. *J. Org. Chem.* **1966**, *31*, 1188-1195.

[134] Barlos, K.; Gatos, D.; Koutsogianni, S.; Schäfer, W.; Stavropoulos, G.; Wenqing, Y. *Tetrahedron Lett.* **1991**, *32*, 471-474.

[135] Still, W. C.; Khan, M.; Mitra, M. *J. Org. Chem.*, **1978**, *43*, 2923.

[136] Chan, W.; White, P. *Solid Phase Peptide Synthesis;* Oxford University Press: Oxford, UK, 1999.

[137] The synthesis of this library was performed by Jose J Pastor.