The Synthesis of Solid-Supported Cyclohexan-1,3-dione (CHD) and its Applications as a Solid-Phase Reagent

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A thesis submitted for the degree of
Doctor of Philosophy

University of Edinburgh

October 2003
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ABSTRACT

Combinatorial chemistry has had a major impact on organic chemistry, enabling the synthesis of thousands of compounds in parallel, using state-of-the-art robotics for synthesis, purification and analysis. The benefits are most evident in the pharmaceutical industry where there is a high demand for novel compounds as drug candidates. Library synthesis relies greatly on the use of solid-supported reagents or scavenger resins which afford ease of purification and isolation of the required product.

The synthesis of multifunctional cyclohexane-1,3-dione (CHD) I on polystyrene resin, using microwave irradiation in a key step is described. CHD resin I was used for the 'capture and release' of a library of amides where both resin acylation and amide release were also accelerated using microwave irradiation. CHD resin was also used as an allyl-cation scavenger resin in the palladium-catalysed deprotection of O-Alloc-alcohols.

The preparation of enantiomerically pure compounds is an ongoing challenge for organic chemistry and is especially important in the preparation of drug candidates. CHD resin underwent lipase-catalysed 'capture and release', demonstrating an unusual reversal of selectivity. In each case the predominant enantiomer observed was the (R)-(−)-3-phenylbutyryl acyl group rather than the expected (S)-enantiomer,
despite the fact that the hydrolases under study showed exclusive selectivity for the (S)-enantiomer in the corresponding solution-phase hydrolysis reactions. The use of dimedone 1,3-enol esters for the screening of hydrolase activity by UV/Vis detection of copper-dimedone chelates is also described.
ACKNOWLEDGEMENTS

I would like to thank Professor Nicholas Turner for all his help, advice and enthusiasm over the last three years. My industrial supervisors, Morag Easson and Jason Tierney have both been amazingly helpful, always attending meetings equipped with an open mind and copious suggestions. I am grateful to Organon laboratories for giving me the chance to do a three month placement on site at Newhouse, Lanarkshire. They provided me with lab space, chemicals and resins and allowed me access to their analytical department and combichem equipment. Thanks to Dan Fletcher for running my never ending supply of MAS-probe NMR samples and for training me in use of the software and probe. I would also like to thank Ian Sadler for helping me run MAS-probe NMR samples back at Edinburgh University. I am grateful to Sabine Flitsch and Rein Ulijn for their invaluable advice on lipase-catalysed reactions on solid-phase.

A big thanks to the members of the Turner/Flitsch group who have made working with them a pleasure. They have kept me sane through excessive resin washing, HPLC traumas and sub-zero lab temperatures. Thanks to lab 25a for their constant banter and lab 25b/120 for a sane retreat from animal noises and tiger lab coats. Huge thanks go to my family and friends for always being there and putting up with my rantings on organic food and shampoo adverts. Finally thanks to James for all his support and love.
Abbreviations

Ala  Alanine (A)
Alloc  Allyloxycarbonyl
Arg  Arginine (R)
Asn  Asparagine (N)
Asp  Aspartic acid (D)
BAL  Backbone amide linker
Boc  tert-Butoxycarbonyl
BSA  Bovine serum albumin
br  Broad
Cbz  Carbobenzyloxy (also Z)
CHD  Cyclohexane-1,3-dione
CPG  Controlled pore glass
CVL  *Chromobacterium viscosum* lipase
Cys  Cysteine (C)
Dap  Diaminopropionic acid
DBU  1,8-Diazabicyclo[5.4.0]undec-7-ene
DCA  Dichloroacetic acid
DCC  Dicyclohexylcarbodiimide (also DCCI)
Dde  1-(4,4-Dimethyl-2,6-dioxocyclohexylidene)ethyl
Ddz  N*-2-(3,5-Dimethoxyphenyl)propyl[2]oxycarbonyl
d.e.  Diastereoisomeric excess
DIC  Diisopropylcarbodiimide
DIPEA  N,N-Diisopropylethylamine
DMAc  Dimethylacetamide
DMAP  4-Dimethylaminopyridine
DMPU  N,N'-Dimethylpropyleneurea
DMSO  Dimethylsulfoxide
EDC  1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide
E  Enantiomeric ratio
e.e.  Enantiomeric excess
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<td>ES⁻</td>
<td>Negative Ion Electrospray MS conditions</td>
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<tr>
<td>ES⁺</td>
<td>Positive Ion Electrospray MS conditions</td>
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<tr>
<td>Fmoc</td>
<td>9-Fluorenlymethoxycarbonyl</td>
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<tr>
<td>Gln</td>
<td>Glutamine (Q)</td>
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<tr>
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<tr>
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<td>Glycine (G)</td>
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<td>2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate</td>
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<tr>
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<td>HOBt</td>
<td>1-Hydroxybenzotriazole</td>
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<td>i</td>
<td>Ipso</td>
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<td>Ile</td>
<td>Isoleucine (I)</td>
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<tr>
<td>J</td>
<td>Coupling constant</td>
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<tr>
<td>LDA</td>
<td>Lithium diisopropylamine</td>
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<tr>
<td>Lys</td>
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<td>MAS</td>
<td>Magic angle spinning</td>
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<tr>
<td>Met</td>
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<td>MTP</td>
<td>Microtitre plate</td>
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<tr>
<td>NMM</td>
<td>N-Methylmorpholine</td>
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<td>NMP</td>
<td>N-Methyl pyrrolidone</td>
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<td>Porcine pancreas lipase</td>
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<td>Pro</td>
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<td>PyBOP</td>
<td>Ben佐triazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate</td>
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<td>Bromo-tris-pyrrolidino-phosphonium hexafluorophosphate</td>
</tr>
<tr>
<td>Ser</td>
<td>Serine (S)</td>
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<td>SPOS</td>
<td>Solid phase organic synthesis</td>
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<td>SPPS</td>
<td>Solid phase peptide synthesis</td>
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TBDMS  tert-Butyldimethylsilyl
TBTU  2-(1H-Benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate
TIPS  Triisopropylsilyl
TFA  Trifluoroacetic acid
Thr  Threonine (T)
TNBS  Trinitrobenzenesulfonic acid
Trp  Tryptophan (W)
$p$-TSA  para-Toluene sulfonic acid
Tyr  Tyrosine (Y)
Val  Valine (V)
$\delta$  Chemical shift

**Resin types**

The following resin bead nomenclature is used throughout this thesis:

- **AP**  Argopore macroporous resin
- **PS**  Argonaut polystyrene resin
- **PC**  Polymer labs $\text{PEGA}^{1900}$ resin
- **T**  Tentagel resin

Where resin type is not given, the resin bead may be a variety of structures or is not specified in the literature.
1. Background and Introduction

1.1 Solid-phase organic synthesis (SPOS)

Solid-phase organic synthesis (SPOS) is a technique in which compounds in a synthetic sequence are attached to polymer beads (traditionally polystyrene) in order to aid product purification. At the end of each reaction the polymer-bound product is removed from the reaction mixture by simple filtration and washing with a range of solvents. SPOS lends itself to combinatorial chemistry in which libraries of compounds are prepared in parallel either as mixtures or as individual compounds. The pharmaceutical industry has been a major driving force in the development of SPOS. Until approximately two decades ago leads (chemical starting points) were generated by the synthesis of individual compounds or by the screening of in-house libraries and natural products. It is estimated that the top ten pharmaceutical companies of the world must quadruple the number of new drugs launched per year in order to retain their competitive edge. The time taken to get a drug to market must be reduced and one way of achieving this is to increase the number of compounds synthesised for screening. Recent advances in biochemistry have enabled the isolation of a large number of new receptors and enzymes as molecular targets resulting in a medicinal chemistry bottleneck. The culmination of nearly two centuries of preparative chemistry has been the development of combinatorial chemistry and high throughput screening techniques and equipment, which has enabled the acceleration of drug discovery at reduced cost, time and effort.

1.1.1 Solid phase peptide synthesis (SPPS)

The origins of SPOS can be traced back to the work of Merrifield\(^1\) and Letsinger\(^2\) in the 1960's and the concept has since revolutionised chemical synthesis. Merrifield used a chlorinated co-polymer of styrene and divinylbenzene \(1\) (Merrifield resin) as a solid support upon which a peptide chain was synthesised by stepwise addition of protected amino acids (figure 1). At the end of each synthetic step the resin was washed to remove any excess reagents and by-products and peptide \(2\) was cleaved from the resin at the end of the synthesis. One advantage of SPPS over traditional solution phase methods is that an excess of reagent is used to drive the reaction to
completion leaving a product attached to an insoluble polymer bead which is filtered off and washed to remove any remaining reagents. Merrifield termed the phrase 'solid phase synthesis' and won a Nobel Prize in 1984 for his work. However, it was about 15 years before the concept of SPOS was to be used in combinatorial chemistry.

(i) Attach first N-protected aminoacid
(ii) Filter
(iii) N-deprotection

Figure 1: Merrifield's solid-phase peptide synthesis

1.1.2 Peptide libraries

An early example of chemically generated molecular diversity was the development of the 'one-bead, one-peptide' approach by Lam et al. in 1991.\(^3\) The technique involves the synthesis of large numbers of immobilised peptides using 'mix and split' methodology and subsequent testing while still attached to the bead. In the study nineteen naturally occurring amino acids (omitting cysteine to avoid complications with disulfide crosslinking) were used to prepare a possible 2,476,099 \((19^5)\) resin bound pentapeptides and tested to find six beads with activity comparable to the natural substrate. Active beads were physically removed by hand from the test mixture and the peptide sequence determined using a microsequencer.

Peptides, despite lending themselves very well to solid phase chemistry, do not generally make very good drugs due to their limited bio-availability and rapid physiological clearing times. For this reason the techniques developed for peptide
synthesis have been adapted to reactions other than amide couplings for use in combinatorial chemistry.

1.1.3 Combinatorial chemistry

The number of compounds within a combinatorial library must be limited so that it does not become too large. For example, there are approximately 7,000 commercially available phenols and therefore there are an immense number of potential compounds produced using these chemicals. For this reason library development is often combined with molecular modelling studies to limit the number of possible permutations. The quality of molecular diversity is also important. Chemical availability may limit library diversity and important compounds may be omitted from a library because the starting material is unavailable.

A major limitation of relying upon combinatorial libraries for lead optimisation is that the time taken to develop synthetic methodology may exceed that for a project timescale. Hence chemical leads may have been disregarded by the time a library is prepared. The chemistry is limited by the linkers used to join the molecule to the polymer resin and until relatively recently was greatly limited by the time taken to isolate and purify each compound upon cleavage from the resin. Recent advances in technology over the last few years have enabled easier library work up and purification allowing for the fast preparation of solution phase libraries. Liquid-liquid extraction (e.g. Myriad Allex) and high performance liquid chromatography (HPLC) purification (e.g. Biotage Flex) can be performed in parallel and the purity levels monitored using UV spectroscopy. Preparative LC-MS can be used to purify compounds, collecting fractions only when the correct m/z value is observed by mass spectroscopy.

The acceleration of compound synthesis, whilst easing the bottleneck within the chemistry department, may lead to a backlog elsewhere within a drug development programme, for example compound analysis by NMR and other spectroscopic methods. However, recent advances in flow NMR techniques allow high throughput analysis of compound libraries.

Within combinatorial libraries the purity levels may be compromised leading to more errors in screening results. An impurity may give an incorrect result and valuable
time may be wasted chasing a false lead. It is therefore important that any lead, once identified is re-synthesised and re-tested. Studies by Teague et al.\textsuperscript{4} indicate that the most valuable libraries for initial screening are those consisting of small (low Mr) polar molecules made via relatively simple one or two step syntheses rather than larger, more complex drug-like molecules. In this way a mM active lead can be found and its pharmacokinetics or affinity optimised using further libraries. If complexity is introduced too early in library generation, it may result in the oversight of potential leads.

New combinatorial chemistry techniques are of increasing value, both in expanding size and molecular diversity of compound banks and in allowing rapid optimisation of the activity and pharmacokinetics of interesting leads. If these new methodologies are teamed with medicinal chemistry and molecular modelling techniques the drug discovery process is dramatically reduced and the time taken to get a drug to market greatly diminished.

1.1.4 Disadvantages of SPOS

Despite the success of SPOS within industry, there are a number of drawbacks. It is not possible to monitor solid-phase reactions with most conventional solution phase techniques (e.g. TLC, MS or HPLC) due to the insoluble resin structure. Analysis of resins requires specialist techniques such as magic angle spinning NMR.\textsuperscript{5} Recent advances in the field of analytical SPOS, mean that there are now many more analytical methods available to the solid-phase organic chemist.\textsuperscript{6,7} Transferring a solution-phase reaction onto a polymer-bound substrate often requires a high degree of optimisation, which can be time consuming. Reaction times on solid-phase are often extended compared to the analogous solution-phase reaction. Polymer bound reaction times and yields are often altered dramatically by using specific solvents to allow swelling or changing the polymer backbone. Li et al. hypothesise that a resin bead can be viewed as another solvent phase in organic reactions.\textsuperscript{8} Attachment and removal of the required molecule onto a polymer support adds two additional steps to any synthetic route. There are therefore very few examples of convergent synthesis on solid-phase. Finally, the required molecule will contain a specific functional group when cleaved from the solid support unless a traceless linker is used.
1.1.5 Polymer aided solution-phase chemistry

A relatively new concept in organic chemistry is the use of solid supported reagents and scavenger resins in combination with solution-phase chemistry. This approach combines the purification advantages of solid-phase synthesis with the flexibility of solution-phase chemistry. Solid-phase reagents and scavengers are amenable to automated chemistry and allow analysis using conventional methods since the products remain in solution. More than one solid phase reagent can be used at any one time making it possible to combine mutually incompatible reagents, in theory enabling reactions which are otherwise impossible in solution phase. Solid-phase catalysts can easily be isolated from reaction mixtures and recycled to minimise costs or waste. Reagents are generally thought to be less toxic and safer when attached to a solid support.

Less optimisation of chemistry is required than with SPOS making solid-phase reagents and scavenger resins useful in both linear and convergent synthetic pathways. Ley et al. demonstrated the utility of these reagents in the synthesis of natural products (±)-oxomaritidine, (±)-epimaritidine and (±)-epibatidine. They also achieved the convergent synthesis of Sildenafil (Viagra) using polymer-supported reagents. Now that more solid-phase reagents and scavenger resins are commercially available, polymer assisted solution-phase chemistry is a technique available to all organic chemists.

1.2 Monitoring solid-phase reactions

Ideally a solid-phase reaction can be driven to completion using excesses of reagents. In reality this is not often the case and unlike solution phase chemistry any resin bound synthetic intermediate cannot be separated from unreacted starting material. The latter therefore accumulates towards the end of the synthesis. Any product cleaved from the resin in the final step may require further purification, somewhat defeating the purpose of SPOS.

For this reason on-bead analytical techniques are required to monitor the progress of solid-phase reactions. There are limited methods by which resin intermediates can be analysed and when no suitable analytical technique is available the only choice is to cleave and analyse the product released from the solid support. The 'cleave and
analyse' technique can be time consuming, an expensive waste of resin and is not ideal for situations where intermediates are not stable to the cleavage process.

One of the most useful tools for analysing the reaction progress on solid-phase is FT-IR,\textsuperscript{12} which is essentially the solid-phase equivalent of TLC. The advantages are that only a small quantity of resin is required, the cost of such instrumentation is low and a qualitative spectrum can be acquired in less than one minute. The technique is destructive however, and depends on the molecule under study having an analytically visible functional group (\textit{e.g.} a carbonyl group). Observing relative peak intensities or areas can give a qualitative estimate of reaction progress and whether synthesis has gone to completion.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure2.png}
\caption{Magic angle at which dipolar interactions are minimised}
\end{figure}

MAS-probe NMR\textsuperscript{5} is an extremely useful technique for analysis of solid-phase resins. The resin (approximately 5 mg) is placed in a zirconium rotor and swollen with a drop of deuterated solvent. The zirconium rotor is then placed in the MAS-probe and spun at the magic angle (\(\theta\)) of 54.7° where \(3\cos^2 \theta = 1\). At this angle dipolar interactions are minimised giving rise to clearer NMR peaks (figure 2). The rotor is spun at high speed to remove unhomogeneities in the sample. MAS-NMR spectra are normally quite broad but as the loading on the resin or the flexibility of the backbone is increased, peaks generally become sharper.\textsuperscript{13} \(^1\)H-MAS-NMR is an extremely powerful diagnostic tool for SPOS but can become complicated when studying more complex structures. For this reason it is often quite useful to add functionalities with clear diagnostic peaks (\textit{e.g.} the tert-butyl peak of a Boc group or the aromatic peaks of a Fmoc group) to enable clear analysis.

On large scale reactions the weight gain or loss can be measured to give an estimate of resin loading. When utilising smaller quantities, any weight change is often very small and therefore hard to detect. Residual solvent may be trapped inside the resin.
structure on drying affecting results. Resin beads tests, such as the chloranil test for secondary amines, can give a qualitative analysis of resin functionalities and have been widely used, particularly in SPPS.

Ideally the analysis of solid-phase reactions should be based on a mixture of techniques: resin bead tests, UV analysis of cleaved products (e.g. Fmoc UV analysis, see section 6.2), $^1$H-MAS-NMR and elemental analysis.

1.2.1 Loading values

The loading quoted ($L_{\text{quote}}$) by a company for a commercially available resin is often determined by either nitrogen analysis or using test reactions with model chromophores followed by cleavage and measurement of the amount recovered. Either way it is often necessary to calculate the maximum theoretical loading for a product of a resin reaction. When the molecular weight attached to a resin is increased the loading is decreased and vice-versa. The maximum theoretical loading ($L_{\text{max}}$) can be calculated using equation 1 (where $L_{\text{sm}}$ = loading of the starting material; $M_{\text{wt}}$ = molecular weight added to the resin).

$$L_{\text{max}} \text{ (mmol/g)} = \frac{L_{\text{sm}}}{1 + \frac{L_{\text{sm}} \times M_{\text{wt}}}{1000}}$$  \hspace{1cm} \text{equation 1}

When the loading of a predecessor is unknown it is possible to calculate backwards to get the calculated loading ($L_{\text{calc}}$) using equation 2.

$$L_{\text{calc}} \text{ (mmol/g)} = \frac{L_{\text{sm}}}{1 - \frac{L_{\text{sm}} \times M_{\text{wt}}}{1000}}$$  \hspace{1cm} \text{equation 2}

The increase or decrease in weight of a resin upon reaction can also be used to get an idea of the loading. The coupling efficiency can be calculated using equation 3.

$$\text{coupling efficiency} = \frac{\text{actual weight increase} \times 100}{\text{expected weight increase}}$$  \hspace{1cm} \text{equation 3}

[where, expected weight increase = $\frac{L_{\text{sm}} \times M_{\text{wt}} \times \text{initial weight of resin}}{1000}$]

The loading with respect to elemental analysis ($L_{\text{ea}}$) can be calculated according to equation 4 (where, $EA$ = percentage of element found by elemental analysis).

$$L_{\text{ea}} \text{ (mmol/g)} = \frac{10 \times EA}{M_{\text{wt}}}$$  \hspace{1cm} \text{equation 4}
1.2.2 Polymer Supports

Polymer supports can be categorised into four main types: Polystyrene, gel type, macroporous and poly(ethylene glycol) acrylamide (PEGA) resins. The properties of each are discussed in table 1.

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Polystyrene</th>
<th>Gel type</th>
<th>Macroporous</th>
<th>PEGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Structure</td>
<td>moderately flexible</td>
<td>polyethylene graft, very flexible</td>
<td>highly rigid open pore structure</td>
<td>highly flexible open pore structure</td>
</tr>
<tr>
<td>Commercial name</td>
<td>-</td>
<td>Tentagel</td>
<td>Argopore</td>
<td>-</td>
</tr>
<tr>
<td>Crosslinking</td>
<td>1-2 %</td>
<td>-</td>
<td>&gt; 8 %</td>
<td>-</td>
</tr>
<tr>
<td>Swelling</td>
<td>High swelling in DCM, DMF, THF</td>
<td>High swelling in most solvents</td>
<td>Low swelling in most solvents</td>
<td>High swelling, must be handled wet</td>
</tr>
<tr>
<td>Compatible Solvents</td>
<td>All but highly polar protic solvents (e.g. MeOH)</td>
<td>All but diethyl ether, hydrocarbons and certain alcohols</td>
<td>All solvents are compatible</td>
<td>Protic solvents including water</td>
</tr>
<tr>
<td>Suitability for IR</td>
<td>Good</td>
<td>Fair</td>
<td>Good</td>
<td>Poor</td>
</tr>
<tr>
<td>Suitability for NMR</td>
<td>Fair</td>
<td>Excellent because the polymer is highly flexible</td>
<td>Poor because the polymer is highly rigid</td>
<td>Poor because loading is low</td>
</tr>
<tr>
<td>Typical loadings / mmol/g</td>
<td>0.6-4.0</td>
<td>0.35-0.45</td>
<td>0.60-1.1</td>
<td>0.2</td>
</tr>
<tr>
<td>Specific advantages</td>
<td>High loading</td>
<td>Excellent for low temperature reactions</td>
<td>Easily washed and dried</td>
<td>Open structure accessible to enzymes</td>
</tr>
</tbody>
</table>

Table 1: Properties of the four basic types of polymer support
1.3 The history of scavenger resins

Scavenger resins, in the form of ion-exchange resins, have been available for many years. When using ion-exchange resins, compounds are removed from solution by ionic interaction with the resin. One of the first examples of covalent scavenging techniques in the literature was a paper by Eli Lilly chemists, Kaldor and Siegel in 1996. They introduced the concept of using solid supported nucleophiles and electrophiles to purify non-peptide small molecule libraries. They demonstrated proof of concept using the amine acylation reaction shown in scheme 1, removing excess acylating agent with aminomethylpolystyrene 3. NMR of the resultant mixture showed only the desired urea and none of the isocyanate starting material. Various scavenger resins were reported in the same paper, including immobilised amine, isocyanate, aldehyde and acid chloride functionalities.

\[
\begin{array}{c}
\text{Scheme 1: Scavenger resins – proof of concept}
\end{array}
\]

Five compounds, prepared by a two-step synthesis again by Kaldor and Siegel, is shown in scheme 2. Imine formation using an excess of primary amine ensured that the reaction went to completion. Conversion to secondary amine was then affected using solid-supported borohydride and excess primary amine scavenged using polymer-bound carbaldehyde 4. The secondary amines formed were reacted with a range of isocyanates to form a library of five ureas in high to quantitative yield with high HPLC purity. Excess isocyanate was scavenged using aminomethylpolystyrene 3.
Scheme 2: A representative example of the use of scavenger resins for library synthesis

The synthesis of polymeric reagents 5 to 7, starting from either Merrifield or aminomethylpolystyrene resin 3, was demonstrated in a paper by Booth and Hodges. They highlighted the utility of these reagents in the synthesis of ureas, thioureas, sulfonamides and amides, and in the two-step synthesis of pyrazole 8 shown in scheme 3 at 97% HPLC purity. In the first step, morpholine resin 5 was used to scavenge hydrochloric acid and isocyanate resin 6 was used to scavenge excess hydrazine. A cocktail of quenching reagents was used in the second step to remove both excess reagents and impurities. Morpholine resin 5 was used to remove hydrochloric acid, trisamine resin 7 to scavenge excess chloroformate and isocyanate resin 6 to remove amine impurities.
1.3.1 Complementary molecular reactivity and molecular reactivity

Work by Searle/Monsanto chemists (Parlow et al.) demonstrated the sequestering of both solution phase reagent and by-product using the same scavenging resin, a method they term ‘complementary molecular reactivity and molecular reactivity’ (CMR/R). In scheme 4, 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDC) was used to affect the Moffat oxidation of secondary alcohol 9 to ketone 10 using DMSO and catalytic dichloroacetic acid (DCA) in dichloromethane. In the process of the reaction, carbodiimide 11 was converted to urea 12, both of which were scavenged using a combination of acidic resin 13 and basic resin 14. The use of mutually incompatible resins was allowed due to polymer-bound site isolation. The refined CMR/R technique was used to prepare a libraries of approximately four hundred compounds, resulting in the identification of an active herbicide with a four-fold improvement in activity over the initial lead.
Scheme 4: Moffat oxidation using polymer assisted purification to simplify work-up

1.3.2 Further work in this area

Since 1998, the amount of published work in the area of scavenger resins has been immense, thus demonstrating the utility of this technique. For example, scheme 5 demonstrates the use of scavenger resins to prepare a library of 14 ureas in high yield and excellent purity (85-100 % by HPLC) by Raju et al.\textsuperscript{19}

Scheme 5: Urea library
Raju *et al.* used aminomethylpolystyrene resin 3 with trisamine resin 7 to scavenge excess nitophenyl carbamate, and used isocyanate resin 6 with chloroformate resin 15 to scavenge excess amine and the nitrophenol by-product.

### 1.3.3 Impurity annihilation

Impurity annihilation is a phrase coined by Barrett *et al.* in which by products are polymerised *in situ* to afford easy purification by filtration. The procedure is exemplified by the condensation of acid chlorides and amines to form amides and an example of impurity annihilation is shown in scheme 6. Excess amine was removed by co-polymerisation with 1,4-phenylene diisocyanate and pentaethylenehexamine.

![Scheme 6: Impurity annihilation](image)

Barett *et al.* proceeded to use DNAD reagent 16 (figure 3) for chromatography-free parallel Mitsonobu reactions. Excess DNAD-H$_2$ was removed from the reaction by ring opening metathesis polymerisation (ROMP) using Grubbs catalyst 17 (figure 3).

![Figure 3: DNAD reagent 16 for Mitsonobu reactions and Grubbs catalyst 17 for its subsequent ROMP](image)

### 1.4 Novel developments in scavenger resin technology

One disadvantage of scavenger resins is that they can be expensive. Recent developments have lead to cheaper sequestering reagents such as nucleophilic...
scavenging isatoic anhydride resin 18, which has been prepared from Merrifield resin (the cheapest resin available), and isatoic anhydride as shown in scheme 7. Anhydride loading was determined at 3.2 mmol/g.

Scheme 7: Isatoic anhydride resin 18 and its synthesis

Low specificity is often observed for scavenger resins but new resins include acetoxyethyl methyl acrylate (AAEM) resin 19 which has been used to scavenge primary amines in the presence of secondary amines (figure 4). Only a limited number of solvents (DMF, THF) can be used with polystyrene resins but the use of macroporous resins allows the use of a wider range of solvents.

Figure 4: Acetoxyethyl methyl acrylate (AAEM) resin 19 and macroporous trisamine resin 20

Macroporous trisamine 20, synthesised from macroporous Merrifield resin, is now commercially available from Argonaut technologies. This resin shows increased scavenging because the large pore sizes make it easier for impurities to enter the internal resin structure. The resin does not rely upon solvent swelling to allow compounds into its internal structure. The loadings of scavenger resins are often poor. High loading ROMPGELE anhydride scavengers developed by Barrett et al. have been used to scavenge amines and hydrazines. Both ROMPGELE 21 and the carboxylic acid formed upon ring opening.
of the anhydride have been used to scavenge amines (scheme 8) giving an impressive effective loading of 10.8 mmol/g.

\[
\begin{align*}
\text{Scheme 8: High loading ROMPAGEL scavenger resins}
\end{align*}
\]

In a study by Marsh et al. dendritic high loading resins\textsuperscript{26} were prepared as scavenger resins. Dendritic wedges were grown upon Wang resin to prepare proton scavenger 22 and nucleophilic scavenger 23 (figure 5), both of which gave comparable efficiency to commercial resins at significantly lower concentration.

\[
\begin{align*}
\text{Figure 5: Dendritic resins}
\end{align*}
\]

Ley et al. discuss the potential advances in polymer bound reagents and scavenger resins in a large review of the topic.\textsuperscript{27} They propose the use of alternative support materials such as cellulose and mesoporous solids. They also suggest changing the format of such reagents so they are more applicable to automation and process chemistry, for example the use of scavenger frits or ‘tea bags’ containing solid-phase reagents.
1.5 **Background to the project**

The ideas discussed in this thesis arose from work done by Elizabeth Moir on a collaborative project with Professor Malcolm Walkinshaw at the University of Edinburgh. Computational screening of an array of molecules against the X-ray structure of the active site of cyclophilin indicated that 5,5-dimethyl-1,3-cyclohexanedione (dimedone) 24 (figure 6) showed weak but appreciable binding. Dimedone acts as a mimic for the natural ligand, cyclosporin, interacting with the cyclophillin protein in a similar way to the normal substrate. Elizabeth Moir prepared a range of compounds for screening against cyclophilin based on a dimedone core.

![Figure 6: Dimedone](image)

The versatile reactivity of the 1,3-diketone moiety of dimedone is well known, thus highlighting the multiple applications of a solid-supported analogue. Dimedone is known to be able to scavenge allyl cations in palladium catalysed allyl deprotection\textsuperscript{28,29} as well as reacting with amines to form \( \beta \)-ketoenamines.\textsuperscript{30} The latter reaction does not occur at room temperature therefore amine scavenging may be expected to occur more readily via the enol-mesylate.\textsuperscript{31} Dimedone rapidly reacts with aldehydes and is used as a diagnostic test for aldehydes versus ketones.\textsuperscript{32,33} We envisaged utilising solid-supported diketone 25 as a solid supported scavenger reagent for allyl cations, aldehydes and amines, as shown in scheme 9.
Scheme 9: Possible applications of solid-supported cyclohexanedione

Initial experiments by Elizabeth Moir showed that oxidative cleavage of the dimedone peptide conjugate 26 using ammonium molybdate, to regenerate native peptide 27, was possible in 53 % yield (scheme 10). This suggested that, an immobilised dimedone molecule could be used as a novel backbone amide linker (BAL) for use in the synthesis of amides and peptides. Further optimisation of this cleavage would be required for high yielding cleavage from the solid-phase.

Scheme 10: Oxidative cleavage

1.5.1 Initial project aims

(1) To develop the chemistry for preparation of solid-phase dimedone analogues in high yield.

(2) To test the applicability of these analogues as solid-phase reagents, scavenger resins and backbone amide linkers.
1.6 Backbone amide linkers

Backbone amide linkers (BAL) is a term coined by Barany et al., \(^{34,35}\) which describes the attachment of a resin directly to the amide bond. PALdehyde resin (scheme 11) was used for the solid phase synthesis of C-termini modified peptides such as peptidic aldehydes,\(^{36}\) esters, alcohols, anhydrides, dialkylamides and ethers. The C-termini was also eliminated to from cyclic peptides in the synthesis of biologically relevant peptide analogues. Such C-terminus modified peptides are useful synthetic targets, with potential applications in the study of enzyme mechanisms. Such analogues make attractive drug targets with decreased susceptibility to enzymatic degradation, improved ability to cross biological barriers (e.g. the blood-brain barrier), increased solubility or increased receptor binding and substrate specificity.

The BAL based on a tris(alkoxy)benzylamide structure shown in scheme 11a was joined via the nitrogen of the amide bond. A suitably protected amino acid residue (as either the free base or salt) was coupled to PALdehyde resin via on-resin reductive amination using sodium cyanoborohydride and acetic acid in methanol. Intermediate N-protection and deprotection steps were not required. N-acylation enables extension of the peptide chain from the carbon to the nitrogen terminus, to form a polypeptide with retention of stereochemistry. The peptide chain could also be grown from the C-terminus.

Acylation of the sterically hindered secondary amine proved to be quite difficult but was achieved using symmetric anhydrides in dichloromethane/DMF (9:1) in the absence of any base. If the Fmoc protected anhydride was used, Fmoc deprotection under basic conditions resulted in the spontaneous formation of diketopiperazines as shown in scheme 11b. Diketopiperazine formation is favoured by the use of an allylic amino acid protecting group, a sterically unhindered glycine residue and the BAL secondary amine. Barany et al. have used this side reaction to prepare diketopiperazines.\(^{37}\)
Scheme 11: (a) Attachment of an amino acid via reductive amination and (b) diketopiperazine formation

In order to prevent diketopiperazine formation the symmetric anhydride was protected using acid labile \( N^\omega-2-(3,5\text{-dimethoxyphenyl})\text{-propyloxy} \)carbonyl (Ddz) protecting groups. Upon amine deprotonation a quaternary ammonium ion was formed, thus preventing cyclisation and enabling further coupling with amino acids to form a polypeptide. The Ddz protecting group was favoured due to its superior coupling characteristics. The peptide chain was extended by successive acylation and \( N \)-deprotection steps as shown in scheme 12. The \textit{ortho}-methoxy substituents on BAL increased the linker’s acid lability, thus enabling cleavage using TFA in water (19:1).

Barany \textit{et al.} used BAL to synthesise H-Val-Tyr-Phe-Ala-O-Allyl as a single diastereoisomer.\textsuperscript{38} The chain was extended from both the \( N \)-terminus and the \( C \)-terminus via anchoring of the BAL through the alanine residue. While still attached to the BAL, the \( C \)-terminus allyl ester was removed using Pd(Ph\textsubscript{3})\textsubscript{4} in chloroform/acetic acid/NMM (37:2:1) and reacted with a variety of amino acid \( p \)-nitroanilides with minimal racemisation of the Ala residue.
BAL and related linkers have been used to prepare cyclic peptides, a specific example of which is cyclo-(Arg-o-Phe-Pro-Glu-Asn-Tyr-Glu-Ala-Ala), again prepared by Barany et al. This cyclic peptide was prepared in > 85% purity by analytical HPLC. On-resin cyclisation was carried out by activation of the C-terminus with PyAOP/acetic acid in the presence of DIEA with dichloromethane solvent. When the p-BAL based linker was replaced by o-BAL, not only was the synthesis greatly simplified, but reactivity was increased and the linker remained highly acid labile. Polymer supported dimedone provides an alternative to Barany’s BAL, being attached to the peptide backbone via a carbonyl group as opposed to a peptidic nitrogen. CHD linker would be both acid and base stable. Both linkers allow the growth of peptide chain in both the C- and N-directions.

1.7 Previous examples of solid-supported dimedone

1.7.1 Cyclohexanedione resin for the characterisation of chemical libraries by HPLC retention times

Cyclohexanedione resin 28 (prepared by Graf von Roedern) was used to demonstrate a technique by which library members were identified by their HPLC retention times. Twenty compounds were synthesised on solid phase from four...
aldehydes and five vinyl ethers then cleaved from the resin and analysed by HPLC (scheme 13). A computer algorithm was used to estimate the compound retention times based on the substitution pattern of the products. No information on the preparation of cyclohexanedione resin 28 is given as the paper is lacking in any synthetic details. The solid-support used for this synthesis is not described.

Scheme 13: Cyclohexanedione resin and its application in library synthesis

1.7.2 The Dde protecting group

The 1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) protecting group, based on dimedone developed by Bycroft is used for the orthogonal side chain protection of Fmoc-lysine (scheme 14).

Scheme 14: Dde protecting group for the side chain protection of Fmoc-lysine and the product obtained upon cleavage

The Dde group is selective for primary amines, is both acid and base stable and is removed using 2 % v/v hydrazine in DMF. Deprotection results in the formation of
3,6,6-trimethyl-4-oxo-4,5,6,7-tetrahydro-1H-indazole. The Dde group has been used extensively by Bycroft et al. for the synthesis of polyamines and to carry affinity tags such as biotin.

1.7.3 Dde based polyamine linker

Scheme 15 details the use of solid-supported dimedone for the synthesis of polyamines by Bycroft et al. In this instance the resin was bound through the C2 position to give immobilised linker 29.

Dimedone 24 was acylated with the mono-protected dicarboxylic acid shown, thus providing an anchoring point for the resin backbone. Linkers shorter than four carbons showed incomplete stability towards piperidine. Resistance was improved...
by increasing the length of the alkyl spacer. 2-Acylated dimerdone was then masked with an amine and deprotected to allow attachment to amino functionalised resin. Resin 29 was subsequently transaminated with diamines and extended to form polyamines which were released from the resin using hydrazine. The same linker was applied to the synthesis of oligosaccharides and was found to be stable to carbohydrate reaction conditions. Aminosugars were immobilised directly or sugars were attached to the resin via a p-aminobenzyl alcohol 'converter'. The linker was regenerated after use, by treatment with hydroxide.

1.7.4 Dde based peptide linkers

The slight instability displayed by Dde towards piperidine in DMF can be tolerated for most applications however, use of the Dde linker to prepare large peptides lead to compromised purity.

Scheme 16: Dde based peptide linkers

Dde N- to N'-migration from a side chain (or ω-amino group) to the ε-amino functionality of lysine has been reported, resulting in scrambling of the group within the peptide chain. This lead Bycroft et al. to investigate the base stability of a range
of Dde variants. The relevant acyl group was coupled to dimedone using DCC coupling conditions (scheme 16). The 2-acyl dimedone derivatives were further reacted with Fmoc-lysine to give a range of Fmoc-Lys(Ddx)-OH. These were coupled to chlorotrityl linked Merrifield resin and their base stability noted. Increased base stability was found with increasing chain length. When \( R = \text{Bu} \) (Ddiv) the optimal base stability was observed. Peptide synthesis from the Fmoc-lysine core allowed TFA cleavage of the chlorotrityl linker before or after Dde deprotection.

The Dde linker \((R = \text{Me})\) on Wang linked polystyrene resin was used by Gopalsamy et al. to prepare a library for screening as vitronectrin receptor inhibitors (scheme 17). \(^5\) N-Dde migration was observed when bulky or unreactive chloroformates or isocyanates were used so they switched to using the more stable Ddiv linker. The use of Wang linked resin allowed the library to be cleaved from the resin using TFA.

**Scheme 17: Synthesis of a peptide library using Dde and Ddiv based linkers**

Bycroft et al. used O-Dmab linker for the attachment of carboxy groups as shown in scheme 18. The linker relied on the known instability of 4-aminobenzyl esters. They demonstrated the utility of the linker in the synthesis of a model cyclic peptide. \(^5\) In a similar manner a O-Dmab linker analogue was used to prepare
Leucine-Enkaphalin (H-Tyr-Gly-Gly-Phe-Le-OH) and Angiotensin II (H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH) in high purity after hydrazine linker cleavage.\textsuperscript{52} The major disadvantage of this linker for SPPS was that the peptide chain could only be grown in one direction because one terminus of the linked amino acid was always involved in attachment to the resin backbone. Using the proposed cyclohexanedione linker the peptide chain would be grown in both directions, allowing for greater diversity and reduced complexity.

1.7.5 Primary amine linker for peptide synthesis

A resin bound cyclohexanedione was used by Bannwarth \textit{et al.}\textsuperscript{53} as a linker for primary amines (scheme 19). Birch reduction of 3,5-dimethoxybenzoic acid and methylolation with methyl iodide followed by hydrolysis gave diketone 32. This derivative was then acylated with acetic anhydride followed by DMAP catalysed $O$-to $C$- rearrangement. The methyl ester was converted to the free acid by reaction with lithium hydroxide to give ADCC linker 33. The application of the ADCC linker was demonstrated through reaction with L-phenylalanine methyl ester followed by attachment to polystyrene resin through the $C5$ position of cyclohexanedione. Successful cleavage of L-phenylalanine methyl ester using hydrazine resulted in the formation of indazole derived resin 34.

\begin{scheme}
\centering
\includegraphics[width=0.8\textwidth]{scheme18}
\caption{O-Dmab linker for attaching carboxy groups}
\end{scheme}
The Bannwarth method of resin attachment through the C5 cyclohexanedione position is similar to our proposed method for preparation of cyclohexanedione resin. The Bannwarth linker however, is C-acylated prior to attachment to the resin therefore blocking the C2-position. We proposed synthesising cyclohexanedione resin, with a free 1,3-dione system leaving it open to reaction with a range of functionalities. Lysine coupling to cyclohexanedione resin via the amino side chain would allow for peptide synthesis from both the N- and C-termini.

Scheme 19: Resin bound cyclohexanedione as a linker for primary amines
2. Results and Discussion – Synthesis of CHD resin

2.1 Retrosynthetic analysis

Previous work by Elizabeth Moir suggested the retrosynthetic analysis, detailed in scheme 20, for the synthesis of resin bound dimedone. The diketone moiety would be generated by hydrolysis of the corresponding bis-enol ether on solid phase, which in turn would be attached to the resin by an amide coupling with N-methylaminomethyl resin. The tertiary amide was chosen for increased amide stability, being less susceptible to hydrolysis and acylation in later stages of the synthesis. Acid functionalised bis-enol ether would be formed by the Birch reduction of 3,5-dimethoxybenzoic acid. Previous studies, again by Elizabeth Moir, found that the Birch reduction and subsequent alkylation of 3,5-dimethoxybenzoic acid with bromoethane (i.e. R = Et) gave higher yields than if iodomethane (i.e. R = Me) was used.

Scheme 20: Retrosynthetic analysis of cyclohexanedione resin

2.2 Birch reduction of 3,5-dimethoxybenzoic acid

3,5-Dimethoxybenzoic acid 35 was subjected to a Birch reduction and alkylation with bromoethane to give 1-ethyl-3,5-dimethoxycyclohexa-2,5-dienecarboxylic acid 36 (scheme 21). The large scale synthesis required a considerable amount of optimisation in order to prevent formation of by-products 37 and 38. Di-alkylated by-product 37 was formed if more than 1.1 equivalents of bromoethane were used. Methoxycyclohexenone by-product 38 was formed by partial hydrolysis if the
product was insufficiently chilled during the acidic work-up. The reaction was performed on large scale (20 g) in quantitative yield without the need for purification.

\[
\begin{align*}
\text{MeO} & \quad \text{OMe} & \quad \text{MeO} & \quad \text{OMe} \\
\text{O} & \quad \text{acid} & \quad \text{O} & \quad \text{OH} \\
\text{35} & \quad \text{(i), (ii), (iii)} & \quad \text{36} & \quad \text{37} & \quad \text{38}
\end{align*}
\]

Reagents and conditions: (i) NH₃(0), THF, Li, -78 °C; (ii) EtBr, 1 h, -78 °C o/n; (iii) 2M HCl, RT, 1 h (> 99 %).

Scheme 21: Birch reduction of 3,5-dimethoxybenzoic acid

Acid 36 was used for subsequent coupling reactions either to amines for solution-phase analogues, or directly coupled to the resin.

2.3 ‘Linker on a linker’ strategy; Argopore-MB resin

In general polystyrene resins require the use of non-protic solvents to swell the beads and expose reaction sites. Argonaut Technologies Argopore resins on the other hand, have highly cross-linked, rigid, and macroporous structures with internal reaction sites which are accessible without swelling with solvent. Reagents gain access to reaction sites via diffusion through the open pore structure rather than a swollen gel phase. A greater range of solvents are therefore compatible with Argopore resins and swelling is low and highly predictable. Loadings of Argopore resins are generally quite high and typically between 0.6 and 1.1 mmol/g. Larger pore sizes mean that resin washing is easier requiring less solvent. The resins dry more rapidly than lower crosslinked resins, especially under vacuo. However, the rigid resin structure reduces the suitability for analysis by gel phase or MAS-probe NMR.

A resin containing a linker was considered for the development of substituted cyclohexan-1,3-dione linkers. The linker allows the cleavage of resin bound analogues from resin at each stage of the synthesis for analysis by conventional
solution-phase techniques. In this way one linker can be used to refine the properties of another; \textit{i.e.} a 'linker on a linker' strategy.

![Diagram]

**Scheme 22:** Cleavage of the MB linker

We chose to use methoxybenzyl (MB) linker based on the Sasrin linker\textsuperscript{55} (scheme 22) containing electron withdrawing methoxy groups. The methoxy groups increase the acid lability of SASRIN compared to the Wang linker.\textsuperscript{56} The Wang linker is cleaved by 95 \% TFA in DCM whereas the MB linker is cleaved by 1-3 \% TFA in DCM.

### 2.3.1 Solution-phase analogues

![Synthesis diagram]

**Scheme 23:** Synthesis of benzyl methyl amide analogues

Methylamide solution phase analogues 39 and 40 were prepared for comparison with the product formed upon cleavage of bis-enol ether or cyclohexane-1,3-dione MB linked resins. Birch acid 36 was coupled to methylamine using uronium coupling agent 2(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium tetrafluoroborate (TBTU), triethylamine and hydroxybenzotriazole in DMF to form methylamide 39 (scheme
23). Bis-enol ether 39 was hydrolysed to diketone 40, observed predominantly in its keto form by $^1$H-NMR in chloroform.

### 2.3.2 Solid-phase synthesis on Argopore-MB resin

The strategy for development of cyclohexane-1,3-dione resins on Argopore-MB resin is outlined in scheme 24.

![Diagram](image_url)

**Reagents and conditions:** (i) MeNH$_2$·HCl, NaBH(OAc)$_3$, DMF, AcOH, RT, o/n; (ii) Fmoc-Gly-OH, TBTU, DIPEA, DMF, RT, o/n (double coupling); (iii) TFA/H$_2$O (95:5), 2h, RT (72 %); (iv) Acid 36, TBTU, HOBt/H$_2$O, Et$_3$N, DMF, RT, o/n (double coupling); (v) TFA/H$_2$O (95:5), 2h, RT (12 %).

**Scheme 24:** Synthesis on Argopore-MB resin

Formyl functionalised Argopore-MB resin 41 was converted to $N$-methylamino resin 42 by reductive amination$^{57}$ with methylvamine hydrochloride using sodium triacetoxyborohydride in 2 % acetic acid in DMF. The disappearance of the carbonyl peak in FT-IR was observed upon forming amino functionalised resin 42. The loading of methylamino resin 42 was determined by coupling with Fmoc-Glycine to form resin bound amino acid 43. A double coupling reaction was
performed in order to maximise the loading. Complete coupling was verified by disappearance of colouration with chloranil and bromophenol blue resin bead tests. The loading of resin bound amino acid 43 was determined by Fmoc-UV analysis to be 0.67 mmol/g. By calculating backwards the loading of 42 was found to be 0.82 mmol/g. Subsequent TFA cleavage of resin bound amino acid provided methylcarbamoylmethyl-carbamic acid 9H-fluoren-9-ylmethyl ester 44 in 72% yield with some contamination by methylamine due to incomplete Fmoc-Glycine coupling.

Acid 36 from the Birch reduction was double coupled to amino functionalised resin 42 using previously established conditions to form resin bound bis-enol ether 45. Bis-enol ether 45 was subsequently cleaved with TFA to yield methoxycyclohexenone 46 and diketone 40 after LC-MS purification, thus establishing successful coupling to the resin. Attempts at on bead hydrolysis, to give the required diketone resin using a wide variety of weakly acidic conditions, resulted in cleavage of the linker and hence a loss in resin loading. The analysis of Argopore resins by MAS-probe NMR was unsuccessful due to the rigid nature of the polymer backbone.

2.4 Synthesis on polystyrene resin

At this point work was transferred to polystyrene resin containing no linker in order to overcome the problems of analysis and linker lability associated with Argopore-MB linked resin.

2.4.1 Solution-phase analogues

In order to establish the conditions required for the preparation of cyclohexanedione resin and to provide standards for comparison with polystyrene based resins by NMR and FT-IR, solution-phase analogues were prepared with the polystyrene backbone replaced by a phenyl ring. The synthesis of cyclohexane-1,3-dione derivative 49 is shown in scheme 25. Benzyl-methyl-amide 47 was synthesised in high yield by the amide coupling of Birch acid 36 and N-methylbenzylamine using previously established TBTU conditions. Bis-enol ether 47 was subsequently hydrolysed to cyclohexane-1,3-dione 49 in quantitative yield using 2M HCl. The hydrolysis of bis-enol ether 47 proceeded via methoxycyclohexenone intermediate 48.
Methoxycyclohexenone 48 was isolated in moderate yield from aluminium chloride catalysed hydrolysis of bis-enol ether 47.

![Diagram](image)

**Reagents and conditions:** (i) N-methylbenzylamine, TBTU, HOBT.H₂O, Et₃N, DMF, 1 ½h (81 %); (ii) 2M HCl/THF (1:1) 4h (> 99 %); (iii) AlCl₃, DCM, RT, 4h (63 %).

**Scheme 25:** Synthesis of benzyl methyl amide derivatives

Dimedone 24 exists predominantly in the enol form in chloroform and methanol. Energy minimisation calculations indicated that cyclohexane-1,3-dione 49 exists as both electronically favoured enol form 49a and thermodynamically favoured keto form 49b (figure 7). Both of these forms were observed by proton and carbon NMR in chloroform subsequently complicating the spectra.

![Diagram](image)

**Figure 7:** Enol-keto tautomerism in cyclohexane-1,3-dione derivative 49

### 2.4.2 Solid-phase synthesis on polystyrene resin

Adaptation to polystyrene resin utilised a similar strategy to synthesis on Argopore-MB resin. Formylpolystyrene resin 50 was converted to N-methylaminomethyl polystyrene resin 51 by reductive amination with methylamine using sodium triacetoxyborohydride (scheme 26). Amino functionalised resin 51 was coupled to
Fmoc-Glycine to establish a Fmoc-UV resin loading of 0.97 mmol/g for amino acid coupled resin 52, and a calculated loading for amino functionalised resin 51 of 1.33 mmol/g. The coupling of Birch acid 36 with N-methylaminomethyl polystyrene resin 51 was optimised using the range of coupling conditions and reagents detailed in table 2. The optimum coupling conditions were found to be DIC in a DCM/DMF (1:1) mixture rotated at room temperature for 2 ½ hours to give resin bound bis-enol ether 54. A double coupling reaction was performed to ensure high resin loading.

Reagents and conditions: (i) MeNH₂·HCl, NaBH⁴(OAc)₃, DMF, AcOH, RT, o/n; (ii) Fmoc-Gly-OH, TBTU, DIPEA, DMF, RT, o/n; (iii) (AcO)₂O, DIPEA, DMAP, DMF, 2 ½ h; (iv) Acid 36, DIC, DCM/DMF (1:1), RT, 2 ½ h (double coupling); (v) TFA/H₂O/DMF (90:5:5), RT, 2 ½ h.

Scheme 26: Synthesis on Argopore-MB resin

In SPOS, unreacted resin sites are often ‘capped’ to prevent side reactions in latter stages of the synthesis. Unreacted N-methylamino sites on coupled resin 54 were acylated using acetic anhydride in DMF with DIPEA and a catalytic amount of DMAP. However, the ¹⁵N-MAS-NMR spectra of capped resin 55 showed no
significant change from the un-capped resin 54. The $^1$H-MAS-NMR and FT-IR spectra for control amide resin 53 were obtained for comparison. The FT-IR of control resin 53 contained a carbonyl peak at 1656 cm$^{-1}$ and a $^1$H-MAS-NMR a broad methyl peak at 2.8 ppm. The spectra for capped resin 55 showed neither of the peaks observed for control resin 53 thus establishing that capping of resin 54 was unnecessary.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Conditions*</th>
<th>$^1$H-MAS NMR</th>
<th>FT-IR</th>
<th>Chloranil Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DIC, DMF/DCM (1:1)</td>
<td>good</td>
<td>good</td>
<td>colourless</td>
</tr>
<tr>
<td>2</td>
<td>DIC, HOBt, DMF/DCM (1:1)</td>
<td>contains impurities</td>
<td>extra peaks</td>
<td>pale green</td>
</tr>
<tr>
<td>3</td>
<td>PyBOP, DIPEA, DMF</td>
<td>contains impurities</td>
<td>extra peaks</td>
<td>pale green</td>
</tr>
<tr>
<td>4</td>
<td>PyBrOP, DIPEA, DMF</td>
<td>peaks quite small</td>
<td>good</td>
<td>pale green</td>
</tr>
<tr>
<td>5</td>
<td>TBTU, HOBt, Et$_3$N, DMF</td>
<td>peaks quite small</td>
<td>extra peaks</td>
<td>pale green</td>
</tr>
<tr>
<td>6</td>
<td>PyBOP, DIPEA, DMAP, DMF</td>
<td>contains impurities</td>
<td>extra peaks</td>
<td>pale green</td>
</tr>
<tr>
<td>7</td>
<td>PyBrOP, DIPEA, DMAP, DMF</td>
<td>contains impurities</td>
<td>extra peaks</td>
<td>pale green</td>
</tr>
<tr>
<td>8</td>
<td>TBTU, NMM, DMF</td>
<td>contains impurities</td>
<td>extra peaks</td>
<td>pale green</td>
</tr>
<tr>
<td>9</td>
<td>DIC, HOAt, DMF/DCM (1:1)</td>
<td>contains impurities</td>
<td>extra peaks</td>
<td>pale green</td>
</tr>
<tr>
<td>10</td>
<td>HBTU, NMM, DMF</td>
<td>good</td>
<td>good</td>
<td>pale green</td>
</tr>
</tbody>
</table>

*5eq coupling agent, 10 eq base and 0.5 eq DMAP where applicable

Table 2: Optimisation of coupling conditions

Hydrolysis of bis-enol ether 54 to form the required cyclohexane-1,3-dione resin 57 was unsuccessful, despite attempting a large range of acid conditions (including lewis acids), basic conditions and temperatures profiles. In each case we observed a methoxy peak at 3.7 ppm in the $^1$H-MAS-NMR indicating incomplete hydrolysis to methoxycyclohexenone resin 56 (figure 8). Encouragingly, hydrolysis using TFA resulted in a formation of a TFA enol ester peak at 1781 cm$^{-1}$ in the FT-IR indicating some hydrolysis to cyclohexanedione resin was occurring.
Figure 8: $^1$H-MAS-NMR for resin samples (a) formylpoystyrene resin 50; (b) amino functionalised resin 51; (c) bis-enol ether resin 54 and (d) methoxycyclohexenone resin 56
2.5 Investigations into methoxycyclohexenone reactivity

Scheme 27 details the synthesis of solution-phase analogue 3-methoxy-5,5-dimethyl-cyclohex-2-enone 58, prepared from dimeredone 24 using trimethylsilylchloride and DIPEA in methanol. The reduction in yield is due to the low boiling point (and hence high volatility) of the product. Basic conditions were required to push the reaction to completion and prevent acidic hydrolysis of the product back to dimeredone.

\[
\begin{align*}
\text{O} & \hspace{1cm} \text{OH} \hspace{1cm} \text{O} \hspace{1cm} \text{OMe} \\
24 & \hspace{1cm} \hspace{1cm} \hspace{1cm} \hspace{1cm} \hspace{1cm} 58 \hspace{1cm} \hspace{1cm} \hspace{1cm} \hspace{1cm} 59 \\
\text{TEST HYDROLYSIS CONDITIONS} \\
(i) & \hspace{1cm} \text{TMS-Cl, DIPEA, MeOH, RT, o/n (80 %); (ii) Mg, PhBr, THF, RT, 1h (50 %); (iii) (COCl)_, DMF, DCM, 0 °C→RT, o/n (86 %).}
\end{align*}
\]

Reagents and conditions: (i) TMS-Cl, DIPEA, MeOH, RT, o/n (80 %); (ii) Mg, PhBr, THF, RT, 1h (50 %); (iii) (COCl)2, DMF, DCM, 0 °C→RT, o/n (86 %).

Scheme 27: Synthesis of methoxycyclohexenone 58 and derivatives

Dimeredone 24 can be thought of as a vinylogous acid (pKₐ = 5.22) and methoxycyclohexenone 58 as a vinylogous ester. Dixon et al. 59 showed that vinylogous ester 58 undergoes hydrolysis at measurable rates between pH 2 and 4.5 and again between pH 10 and 11. Hydrolysis reactions nearer neutrality were too slow to be easily monitored.

3-Methoxy-5,5-dimethyl-cyclohex-2-enone 58 was subjected to a range of hydrolysis conditions. All the conditions identified for solution-phase hydrolysis showed incomplete hydrolysis on the resin. The failure of resin hydrolysis may be due to the inaccessibility of protic reagents into the internal structure of the bead.

We subsequently investigated the reactivity of the methoxycyclohexenone functionality with a view to using methoxycyclohexenone resin 56 as a solid-phase reagent. Methoxycyclohexenone 58 reacted with phenylmagnesium bromide (prepared in situ from the reaction of phenylbromide and magnesium) to yield
phenylcyclohexenone 59 in moderate yield after purification (scheme 27). When the corresponding reaction was performed on solid-phase no apparent change in $^1$H-MAS-NMR or FT-IR was observed. Methoxycyclohexenone 58 was reacted with oxalyl chloride in DCM at 0 °C containing a catalytic amount of DMF to form chlorocyclohexenone 60 in high yield after distillation. The corresponding reaction was performed on solid-phase as shown in scheme 28 to yield resin bound chlorocyclohexenone 60. The elemental analysis of chlorocyclohexenone 60 gave a chlorine loading of 1.33 mmol/g and hence a calculated resin loading of 1.34 mmol/g for methoxycyclohexenone resin 56. Resin 61 was washed extensively with a range of solvents to ensure that the resins contained no impurities before elemental analysis. The final resin wash was with non-chlorinated solvents such as ether and the resins dried at 40 °C in a vacuum oven. The resin was analysed by MAS-probe NMR to ensure than no DCM was present in the samples submitted for chlorine elemental analysis.

\[
\begin{align*}
\text{56} & \quad \text{L}_{\text{calc}} = 1.34 \ \text{mmol/g} \\
\text{61} & \quad \text{L}_{\text{calc}} = 1.33 \ \text{mmol/g}
\end{align*}
\]

Reagents and conditions: (COCl)$_2$, DMF(cat), DCM, RT, o/n.

Scheme 28: Calculation of methoxycyclohexenone resin 56 loading

2.6 Adaptation to macroporous Argopore resin

In order to solve the problem of incompatibility of the polystyrene resin with aqueous and protic reagents, synthesis was transferred to macroporous support. Macroporous resins do not require swelling to access reaction sites allowing reagents into the internal surface area by diffusion. As discussed in the background and introduction chapter, improved scavenging was observed with trisamine resin attached to macroporous resin, compared to polystyrene resin with lower crosslinking. Macroporous formylpolystyrene resin is not commercially available so $N$-methyl aminomethyl polystyrene resin 63 was prepared from macroporous Merrifield resin 72. Amination with methylamine in DMF (scheme 29) yielded
beads showing a positive chloranil test. \(N\)-Methyl aminomethylpolystyrene resin 63 was coupled to Fmoc-Glycine using TBTU and DIPEA in DMF. Fmoc-coupled resin 64 was analysed by Fmoc-UV analysis to give a loading of 0.55 mmol/g and calculating backwards a loading of 0.65 mmol/g for amino resin 63. Birch acid 36 was coupled to \(N\)-methyaminomethyl macroporous resin 63 to yield bis-enol ether resin 65 using the previously established coupling conditions. Subsequent attempts at hydrolysis to yield CHD resin 66 resulted in incomplete reaction. This approach was abandoned due to difficulty in analysing macroporous resins by 'H-MAS-NMR and FT-IR.

![Chemical structure of resin coupling](image)

**Reagents and conditions:** (i) MeNH₂·HCl, DIPEA, DMF, RT, o/n; (ii) Fmoc-Gly-OH, TBTU, DIPEA, DMF, RT, o/n; (iii) Acid 36, DIC, DCM/DMF (1:1), RT, 2 \(\frac{1}{2}\)h (double coupling); (iv) TFA/H₂O/DMF (90:5:5), RT, 2 \(\frac{1}{2}\)h.

**Scheme 29:** Adaptation to macroporous Argopore resin

### 2.7 Spacers for solid-phase synthesis

#### 2.7.1 Rationale for using a spacer

Resin backbone flexibility and hence compatibility with protic reagents has been increased by adding spacers to prepare a more gel-like resin. For example, commercially available PEG resin contains ethylene glycol chains of varying or uniform length that are grafted to a polystyrene backbone. At the termini of these chains, the desired linkers are attached and chemistry is conducted as normal. The advantage of incorporating such a spacer moiety into a resin is that long chains extend the reactive sites further into solution and so may allow reactions to occur as
if in a solution environment. It is not as necessary to swell the resin in order to expose the reactive sites; therefore there are a greater range of compatible solvents. The increased flexibility of the polymer chains allow for clearer MAS-probe NMR and reaction kinetics are improved due to the ease of diffusion of reagents to reaction sites.

![Figure 9: Amino-PEG spacer used in HIV protease synthesis](image)

There are many examples in which the incorporation or modification of a spacer moiety has enhanced or indeed facilitated subsequent reactions. Bradley et al. demonstrated the advantages of incorporating a PEG spacer for the solid-phase synthesis of HIV protease inhibitors. Alcohol to aldehyde oxidation by sulphur trioxide-pyridine in anhydrous DMSO in the presence of triethylamine failed when the molecule was attached to a simple aminomethyl polystyrene resin. By incorporating an amino-PEG spacer between the linker and the solid support (figure 9), the steric hindrance in the region around the primary alcohol group was reduced and the reaction occurred. The authors attributed the reaction enhancement to the increased solvation of the solid-phase support.

Kobayashi et al. reported the synthesis of 5-(4'-chloromethylphenyl) pentylpolystyrene (CMPP), a new resin that allowed Lewis acid-catalysed reactions to occur in better yields than with Merrifield or WANG resin. The CMPP linker incorporates a non heteroatomic spacer group consisting only of an alkyl chain which can be readily prepared from commercially available starting materials (scheme 30). We therefore proposed that if a spacer was added to bis-enol ether polystyrene resin, the enol ether functionality would be extended further into solution, thus allowing easier access to solvents and reagents.
Scheme 30: Synthesis of CMPP resin

2.7.2 Linker synthesis

Project student Adele Mclean carried out the following spacer synthesis shown in scheme 31. 4-(Fmoc-methylamino)-butyric acid 68 was prepared in large scale and reasonable yield from the reaction of 4-(methylamino)butyric acid with fluorenyl methylchloroformate in sodium carbonate solution at pH 8-9.

The Fmoc protecting group was chosen to allow resin loading determination by Fmoc-UV analysis. On resin piperidine removal of an Fmoc group is a quick and well documented procedure. Optimisation of coupling conditions for amide coupling to N-methylaminomethyl polystyrene resin 51 revealed high resin loading with carbodiimide coupling reagent EDC.HCl, hydroxybenzotriazole and DIPEA in DMF. Fmoc-UV analysis of resultant resin 69 showed a resin loading of 0.64 mmol/g and interestingly showed much clearer MAS-probe NMR signals that the corresponding non-spacer resin. The Fmoc group was removed by five minute reaction with 20 % piperidine in DMF to yield amino functionalised resin 70 with a calculated resin loading of 0.75 mmol/g. Amino resin 70 was coupled to Birch acid 36 using previously established coupling conditions to give bis-enol ether 71. Subsequent hydrolysis of bis-enol ether 71 resulted in formation of methoxycyclohexenone resin 72 with no apparent diketone formation. No significant improvement in hydrolysis was observed despite the attachment of a five atom spacer. Further studies would include an even longer spacer arm possibly containing ethylene glycol groups in a similar manner to Bradley et al.63
Reagents and conditions: (i) 10 % Na₂CO₃ (aq), Fmoc-Cl, 1,4-dioxane, 0 °C, 3h (76 %); (ii) N-methylaminomethyl polystyrene resin 51, EDC.HCl, HOBt.H₂O, DIPEA, DMF (double coupling); (iii) 2 % piperidine in DMF, RT, 5 mins; (iv) Acid 36, DIC, DCM/DMF (1:1), RT, 2 ½h (double coupling); (v) TFA/H₂O/DMF (90:5:5), RT, 2 ½h.

Scheme 31: Spacer synthesis

2.8 Alternative protecting groups

2.8.1 Silyl enol ethers

Scheme 32 details an alternative protection protocol employed to increase enol ether lability towards hydrolysis. Silyl enol ethers were chosen on account of their high lability, ease of synthesis and the literature precedence for Birch reduction of silyloxybenzoic esters by Hamilton et al. The Birch reduction of benzoic esters is
an excellent alternative to the Birch reduction of benzoic acids because the subsequent work-up does not require strongly acidic conditions, the solubility of the benzoic esters is improved over the acids and the products are generally more stable. 3,5-Dihydroxybenzoic acid 73 was methylated by refluxing in methanol and concentrated sulfuric acid overnight to give methyl 3,5-dihydroxybenzoate 74 in quantitative yield. Methyl 3,5-dihydroxybenzoate 74 was silyl protected using tert-butyl(dimethyl)silyl chloride (TBDMS-Cl) or triisopropylsilyl chloride (TIPS-Cl) and imidazole in DMF to give methyl-disilyloxybenzoates 75 and 76 respectively in high yields. Direct protection of dihydroxybenzoic acid 73 yielded a mixture of products.

Reagents and Conditions: (i) c. H₂SO₄, MeOH, reflux, 4 h (> 99 %); (ii) R-Cl, imidazole, DMF, RT, 2h (75 > 99 %, 76 89 %); (iii) NH₃, THF, Li, -78 °C; (iv) EtBr, 1h, -78 °C aq; (v) 2M HCl, RT, 1h (77 43 %, 78 17 %); (vi) LiOH·H₂O, 1,4-dioxane/H₂O, RT, 2h.

Scheme 32: Orthogonal protection of 3,5-dihydroxybenzoic acid to silyloxybenzoic esters and subsequent Birch reduction

We performed the Birch reduction of TBDMS and TIPS protected methyl-disilyloxybenzoates 75 and 76 in ammonia with lithium, sec-butanol followed by bromoethane to give bis-enol ethers 77 and 78 respectively in moderate to low yield after purification. Despite the low yields the work ups were much easier than that of acid 36 and the products were much more stable to chromatography on silica.
Lithium hydroxide hydrolysis of TBDMS-protected methyl-benzoate 77 in 1,4-dioxane did not yield the required acid 79. The silyl enol ether functionalities of ester 77 were rapidly hydrolysed to give diketone 80. The TIPS protecting group was expected to show increased base stability over TBDMS however, TIPS-protected methyl-benzoate 78 showed silyl enol ether hydrolysis when treated with aqueous lithium hydroxide in 1,4-dioxane. Attempted aluminium chloride catalysed coupling of methyl benzoate 77 to N-methylbenzylamine (according to a procedure developed by Barn et al.) resulted in no amide formation. Work was abandoned at this point since Birch reductions of silyl enol ether derivatives proved more complex than previously thought. Reactions were lower yielding than expected and the silyl protecting groups appeared to be unstable to methyl ester hydrolysis conditions.

2.8.2 β-Diketone protecting groups

Bis-enol ether 36 was hydrolysed using 2M HCl/THF (1:1) to give diketone 80 in high yield after trituration (scheme 33). Direct coupling of acid 80 to either N-methylbenzylamine or benzyl alcohol directly was unsuccessful due to the high reactivity of the diketone and hence the formation of a large number of by-products. Scheme 12 shows how the enol form of β-diketone 80 was protected using a variety of alcohols at reflux with a catalytic quantity of p-toluene sulfonic acid. The alcohols were used as solvents for β-diketone 80 and no other solvent is required. Alcohols were chosen that form stable carbocations upon enol ether hydrolysis facilitate β-diketone protection.

The reaction of β-diketone 80 with propan-2-ol, sec-butanol and allyl alcohol to produce enol ethers 81 to 83 occurred in reasonably high yields with little or no purification. Reaction of tert-butanol with β-diketone 80 however, gave a variety of products by TLC and the corresponding enol ether was not isolated. This was a disappointment because the tert-butyl enol ether would cleave to yield a stable tert-butyl carbocation which in turn would act as a driving force for hydrolysis on solid-phase.

Solid-phase enol ethers 84 to 86 were prepared by amide coupling between N-methylaminomethyl polystyrene resin 51 and enol ethers 81 to 83 respectively, using the standard coupling conditions. Solid-phase enol ethers 84 to 86 were seen clearly
by MAS-probe $^1$H-NMR and FT-IR and showed negative chloranil tests indicating complete coupling.

Reagents and Conditions: (i) 2M HCl/THF (1:1), RT, 4h (75 %); (ii) ROH, p-TSA.H$_2$O, reflux, 5h (81 88 %; 82 > 99 %; 83 76 %); (iii) N-methylaminomethyl polystyrene resin 51, DIC, DCM/DMF (1:1), RT, 2 1/2 h (double coupling); (iv) TFA/H$_2$O/DMF (95:5:5), RT, 2 1/2 h; (v) RhCl(PPh$_3$)$_3$, DABCO, THF/EtOH (1:1), RT, 24h; (vi) acidic hydrolysis.

Scheme 33: Synthesis of enol 80 and subsequent alcoholic protection

Iso-propyloxy and sec-butyloxy enol ester resins 84 and 85 were subjected to TFA hydrolysis however; residual alkyloxy peaks were observed in the $^1$H-MAS-NMR indicating incomplete hydrolysis in each case. Corey et al. showed that allyl ethers can be isomerised under neutral aprotic conditions to 1-propenyl ethers using Wilkinson's rhodium catalyst and the resultant 1-propenyl can be cleaved at pH 2. Allyl isomerisation was attempted on solid phase derivative 86 to yield 1-propenyl derivative 87 but the catalyst did not appear to affect any change in shift of the allylic
protons in $^1$H-MAS-NMR. Insoluble catalyst impurities remained in the resin even after extensive washing.

2.9 C4-Acylation and alkylation

The commonly used synthesis of dimerdone 24 is via Michael addition of the enolate of diethyl malonate to 4-methylpent-3-en-2-one 88 followed by Robinson annulation, ester hydrolysis and decarboxylation$^{72}$ (scheme 34). Ester 89 was isolated in moderate yield before saponification and decarboxylation and subsequently hydrolysed with lithium hydroxide to acid 90 again in moderate yield. Attempted direct coupling to N-methylaminomethyl polystyrene resin 51 resulted in a mixture of products by $^1$H-MAS-NMR due to the high lability of the unprotected diketone functionality.

Reagents and Conditions: (i) NaOEt, diethylmalonate, EtOH, reflux, 45 mins (75 %); (ii) LiOH.H$_2$O, 1,4-dioxane/H$_2$O, 80 °C, 6 h (79 %); (iii) N-methylaminomethyl polystyrene 51, DIC, DCM/DMF (1:1), RT, 2 ½h.

Scheme 34: Synthesis of C4-acylated derivatives

In a similar manner to above, the lithium enolate of dimerdone 24 was coupled to Merrifield resin 1 to form C4-alkylated dimerdone resin 91 (scheme 35). Diketone formation was evident in the FT-IT of resin 91. The resin was coupled to Fmoc-Glycine in the presence of DMAP to affect O- to C- rearrangement, yielding C2-acylated resin 93. The loading was determined by Fmoc-UV analysis to be 0.23 mmol/g for resin 93, which gave a calculated resin loading of 0.25 mmol/g for resin
Resin cross-linking to form C2- and C4-alkylated resin 92 may explain the low resin loading. In order to avoid cross-linking the lithium enolate of methoxycyclohexenone derivative 58 was coupled to Merrifield resin 1 to form C4-alkylated methoxycyclohexenone resin 94. However, hydrolysis of resin 94 with TFA resulted in incomplete hydrolysis similar to previous attempts at hydrolysis on polystyrene resin.

Reagents and Conditions: (i) LDA, DMU, THF, -78 C, 1h; (ii) Merrifield resin 1, Nal, RT, 0/n; (iii) Fmoc-Gly-OH, DMAP, DMF, DIC, RT, 0/n; (iv) TEA/H2O/DME (90:5:5), RT, 21/2h.

Scheme 35: C4-attachment to the resin

2.10 High loading and dendritic resins

In order to clearly analyse hydrolysis of resin bound enol ethers we adapted the existing chemistry onto higher loading resin. Higher loading resins would not only provided us with clearer FT-IR data but would give sharper peaks by MAS-probe NMR. Higher loading resins were expected to show superior scavenging ability over lower loading resins. Improvement in scavenging ability was observed by Marsh et al. in the preparation of dendritic resins.

2.10.1 Higher loading resins via coupling to branched amino acids

N-Methylaminomethyl polystyrene resin 51, prepared from the reductive amination of formylpolystyrene (see section 2.4.2), was coupled with Fmoc-Lysine(Boc)-OH using DIC to give lysine based linker 95 (scheme 36). Theoretically, lysine-based
linker 95 had double the loading of the starting resin 51. The Fmoc-UV analysis of amino acid coupled resin 95 was found to be 0.59 mmol/g, giving a calculated amino loading for resin 96 of 1.96 mmol/g. Lysine linked resin 95 was not prepared because concern that the linker would not be cost effective made the synthesis unfeasible. Both Fmoc-lysine(Boc)-OH and Fmoc-lysine(Fmoc)-OH are expensive reagents to buy commercially.

![Scheme 36: Lysine linker synthesis](image)

Reagents and Conditions: (I) Fmoc-Lys(Boc)-OH, DIC, DCM/DMF (1:1), RT, 2½ h; (ii) 20% piperidine in DMF; (iii) TFA/DCM (1:1).

2.10.2 Higher loading commercially available resins

High loading Merrifield resin 1 was reacted with methylamine hydrochloride in basic DMF solution (scheme 37) to prepare high loading N-methylaminomethyl polystyrene resin 97.

![Scheme 37: Attempted preparation of high loading amino resin 97](image)

Reagents and Conditions: (i) MeNH₂.HCl, DIPEA, DMF, RT, o/n; (ii) Fmoc-Gly-OH, TBTU, DIPEA, DMF, RT, 2½ h (double coupling).

When resin 97 was coupled to Fmoc-glycine using uronium coupling agent TBTU and subjected to a Fmoc-UV analysis, the loading of resin 99 was low at 0.30 mmol/g. We suspect that loading of N-methylaminomethyl resin 97 was reduced due
to higher levels of cross-linking to form tertiary amine resin 98. Resin 97 was much more ‘crunchy’ in texture than the original Merrifield resin indicative of crosslinking. A weighed sample of crosslinked resin 98 in DMF, swelled to a lesser extent than the same amount of un-crosslinked resin 1.

2.10.3 Dendritic trisamine resin

Commercially available trisamine resin 100 was coupled to Fmoc-glycine to yield Fmoc-Glycine coupled resin 101 (scheme 44). Resin 101 showed a loading of 1.42 mmol/g after Fmoc-UV analysis giving an amino loading of 2.35 mmol/g for resin 100. This resin was coupled with Birch acid 36 using the standard coupling procedure to give dendritic bis-enol ether 102. Solid-phase bis-enol ether 102 was hydrolysed at room temperature using TFA/H₂O/DMF (90:5:5) to yield a mixture of methoxycyclohexenone and cyclohexanedione (CHD) resin 103.

![Scheme 38: Synthesis of dendritic cyclohexanedione resin 103](image)

Reagents and Conditions: (i) Fmoc-Gly-OH, TBTU, DIPEA, DMF, RT, 2½h (double coupling); (ii) Acid 36, DIC, DCM/DMF (1:1), RT, 2½h (double coupling); (iii) TFA/H₂O/DMF (90:5:5), RT, 2½h.

A large TFA enol ester peak was observed at 1781 cm⁻¹ in the FT-IR hydrolysed resin 103 indicating a reasonable degree of cyclohexanedione functionality. The main advantage of using high loading resin became apparent at this point as the ¹H-
MAS-probe NMR of resins 102 and 103 were much clearer than previously prepared lower loading resins. We obtained a $^{13}$C-MAS-probe NMR for resin 103, the first time a carbon-NMR had been clear enough to interpret for resins of this functionality.

Resin 103 was reacted with oxalyl chloride and catalytic DMF to give vinylogous acid chloride resin 104. Elemental analysis of chlorine content gave an elemental analysis loading of 2.75 mmol/g for resin 104 and a calculated loading of 2.78 mmol/g for resin 103.

2.11 Microwave-assisted organic chemistry

Microwave-assisted organic synthesis has received a substantial amount of attention over the last few years. This increased interest is due, not only the availability of commercial microwave equipment (e.g. CEM Corp., USA, www.cem.com; and Personal Chemistry, Sweden, www.personalchemistry.com) but to the drive towards solvent free conditions and shorter reaction times. A number of papers have been published advocating the advantages of using of single mode microwave irradiation at 2.45 GHz to heat and speed up reactions. Conventional oil bath heating relies on transferring heat to the reaction vessel followed by heating of the reaction mixture itself. In a microwave intended for organic synthesis, microwave radiation travels through the walls of the reaction vessel and is directed onto the reactants and solvent.

2.11.1 Microwave dielectric heating

The electric field component of a microwave is responsible for dielectric heating. Dielectric heating occurs via two major mechanisms, the first of which is dipolar polarization. Polar molecules attempt to align themselves with the oscillating electric field of a microwave (figure 10) but under microwave irradiation molecules do not have time to precisely follow the field. The discrepancy between the orientation of the field and the dipole results in energy loss from the dipole by molecular friction and collisions.

The second mechanism by which the electric field component interacts with the sample is by conduction. Ions within a solution move through the mixture under the influence of an electric field and the increased collision rate results in the conversion
of kinetic energy to heat. This mechanism is a much more efficient method of heat
generation than dipolar polarization.

\[ \text{\textbf{Figure 10:} Dipolar molecules attempting to align themselves with an oscillating electric field} \]

2.11.2 Choice of solvent

The choice of solvent for solid-phase microwave chemistry is absolutely critical. The solvent must both be able to swell the resin and be thermally stable at the elevated temperatures reached under microwave irradiation. The solvent must also have good interactions with microwaves and have a high boiling point to avoid high-pressure reactions. Some solvents (e.g. methanol), which are known to have poor swelling properties at room temperature, may change in polarity under microwave irradiation. The ability of a solvent to interact with microwaves can be quantified using the loss tangent (tan\(\delta\)), which relates the dielectric constant of the solvent to the efficiency with which the solvent converts any absorbed energy into heat. Table 3 shows the loss tangent and boiling point for a range of solvents commonly used in SPOS.\(^7\)

<table>
<thead>
<tr>
<th>Solvent</th>
<th>tan(\delta)</th>
<th>bpt / °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMF</td>
<td>0.161</td>
<td>153</td>
</tr>
<tr>
<td>THF</td>
<td>0.047</td>
<td>65</td>
</tr>
<tr>
<td>DCM</td>
<td>0.042</td>
<td>40</td>
</tr>
<tr>
<td>MeOH</td>
<td>0.659</td>
<td>65</td>
</tr>
<tr>
<td>Water</td>
<td>0.123</td>
<td>100</td>
</tr>
</tbody>
</table>

\[ \text{\textbf{Table 3:} Loss tangent (tan}\delta\text{) and boiling point for a range of solvents commonly used in SPOS} \]
A solvent that can absorb a high quantity of microwave irradiation will have a tanδ value > 0.1. Non-polar solvents (such as dichloromethane) are transparent to microwaves, therefore allowing for the possibility of ‘specific microwave effects’.

In contrast when using polar solvents (with high tanδ values) any specific effects are masked by solvent absorption and reaction rates are comparable to rates observed with classical heating at the same temperature. If required, the absorbing capacity of a solvent may be increased by using polar or ionic reactants or by adding a small amount of an ionic liquid. A trial and error approach is always recommended, as reactivity in specific solvents is difficult to predict.

In the past, rate accelerations observed in microwave-assisted organic chemistry have been attributed to a specific microwave effect. The boiling points of solvents have been increased by up to 26 °C by a phenomenon known as the superheating effect.

There has been much debate about the source of rate enhancement in microwave-assisted reactions. A considerable number of reports of microwave chemistry are based on inaccurate comparisons with classical conditions that do not allow clear conclusions to be drawn. Some believe that any increase in reaction rate can be ascribed to different temperature regimes only accessible by microwave dielectric heating whereas other reports describe a ‘specific microwave effect’.

2.11.3 Microwave-assisted SPOS

The heterogeneous reaction conditions and extended reaction times often associated with SPOS can lead to problems such as non-linear kinetic behaviour, solvation issues and degradation of the polymer support. Any technique that can address these issues by reducing reactions times is of considerable use to the chemical research industry and in particular in high-throughput synthesis. There are no reported examples of using microwaves to assist multi-step solid-phase or solution-phase library production to date.

The first example of the use of solid-phase in conjunction with microwaves is in the preparation of peptides by Yu et al. They observed a 2-4 fold rate increase in amide couplings especially for hindered side chain amino acids. The reactions were performed in a conventional multi-mode microwave oven.
Scheme 39: Microwave-assisted solid-phase (a) Suzuki and Stille reactions; (b) aryl/heteroaryl C- to N-cross couplings; (c) urea formation; (d) Ugi 4CC; (e) Knoevenagel couplings and (f) hydantoin synthesis
In 1996 Larhed *et al.* 82 used a single-mode microwave to perform both the Suzuki and Stille reactions on Tentagel resin, shown in scheme 39a. They noted minimal degradation of the solid support and quote high yields of TFA cleaved products. The same laboratory used microwave-assisted palladium catalysed coupling to convert solid phase iodides to tetrazoles, and quoted high yields of aryl tetrazoles after cleavage from the resin. 83 This work was followed by that of Combs *et al.* 84 who used a 1000 W domestic oven to affect the aryl/heteroaryl C- to N- cross coupling reaction shown in scheme 39b. The reaction gave low yields of approximately 30% TFA cleaved benzimidazole after 48 hours at room temperature but showed an increase in yield to 56% after 3 \times 10\text{ seconds at full power.}

A kinetic study undertaken by Yu *et al.* 85 demonstrated that the rate of urea formation (scheme 39c) was dramatically increased using microwave irradiation. By monitoring FT-IR peak intensity they were able to show that at room temperature reactions times of up to 200 minutes are reduced to at most 12 minutes in the microwave.

Ugi four-component condensations (4CC) are known to proceed rapidly in solution phase. Hoel *et al.* 86 observed reaction times from 24 hours to several days when the reaction was undertaken on solid-phase. Amino resin on PEG-grafted polystyrene could undergo Ugi-4CC reactions, in less than five minutes of microwave irradiation at 60 W with a range of aldehydes, acids and isocyanides (scheme 39d) to give 18 α-acylamino amides after TFA cleavage. The reaction was performed in a mixture of dichloromethane and methanol that both swelled the resin and absorbed microwave energy.

Kuster *et al.* 87 prepared resin bound nitroalkanes by a microwave-assisted Knoevenagel condensation with a range of aldehydes as shown in scheme 39e. Substrates were subjected to high pressure promoted Diels-Alder cycloadditions to prepare a range of bicyclic products. Kurth *et al.* prepared hydantions in low yield by barium hydroxide catalysed microwave promoted cyclisation and concomittal cleavage from the resin backbone (scheme 39f). 88 The yields were comparable to the optimised yields observed by conventional heating but reaction times were greatly reduced to minutes rather than days.
None of the above examples give details on the temperature or pressure reached in the microwave reactions, presumably due to the lack of suitable instrumentation. The lack of such data causes potential difficulties in replication. Modern microwave machines for organic synthesis are often equipped with a probe for monitoring the temperature and pressure of reactions. Two microwave cavity designs are currently commercially available. The multimode cavity is similar to that of a domestic microwave oven with the entering microwaves reflected by the walls of the cavity and eventually forming a pattern of standing waves. This leads to the formation of hot and cold spots inside the cavity and reaction mixture. For this reason many companies have chosen to manufacture single-mode machines where the electromagnetic radiation is focussed through evenly spaces slits in the cavity wall thus enabling even heating throughout the sample.

Kappe et al.\textsuperscript{89} studied the rate of coupling of benzoic acid to Wang resin (scheme 40a) using microwave irradiation at a range of temperatures to speed up the rate of acylation. They monitored the acylation levels using FT-IR and by TFA mediated linker cleavage and found that reaction times were reduced from days at room temperature to minutes using microwave irradiation. They preferred the use of a symmetric anhydride (method B) rather than a three component O-acylisourea protocol (method A) in which decomposition to the N-acylurea occurred. 1-Methyl-2-pyrrolidone (NMP) was chosen instead of the originally recommended DMF because of its increased thermal stability, high tanδ value and high boiling point (203 °C) compared to DMF (153 °C). Polystyrene resins showed superior swelling characteristics in NMP over DMF. The low volatility of NMP allowed reactions to be carried out in unsealed vessels. Kappe et al.\textsuperscript{90} went on to show that esterifications of chlorofunctionalised Wang and Merrifield resins using a range of carboxylic acids (including sterically hindered aromatic acids) was accelerated using microwave irradiation (scheme 40b). They also demonstrated the cleavage of Merrifield resin using TFA/DCM (1:1) under microwave irradiation at 500 W for 30 minutes.
Scheme 40: Microwave-assisted esterifications

An extremely detailed study also by Kappe et al. details the synthesis of a polymer bound enone using microwave-assisted chemistry\(^\text{91}\) (Scheme 40c). The synthesis involved acylation of a hydroxy-functionalised resin with a variety of β-ketoesters followed by Knoevenagel condensation with a range of aldehyde building blocks. For both steps they analysed the kinetics of reaction by monitoring the FT-IR carbonyl peak areas, calculating a rate constant for both steps. Kappe et al. examined polymer beads under the microscope and found that neither the physical appearance nor the swelling of the beads changed upon microwave irradiation. They attributed the dramatic rate increases observed, to the rapid ‘in-core’ heating of the solvent, and not to ‘non-thermal’ microwave effects as previously postulated.

Microwave assisted reactions are not confined to polymer beads, and Scharn et al. demonstrated the use of cellulose membranes to synthesise a spatially addressed parallel library of 1,3,5-triazines via a microwave assisted nucleophilic substitution
reaction (scheme 41). The first nucleophilic substitution reaction was complete at room temperature but harsher conditions were required to achieve substitution of the last chlorine atom. The reaction times and yields were greatly improved using microwave irradiation for the latter substitution.

Scheme 41: Microwave assisted 1,3,5-triazine synthesis on cellulose membranes

When considering solid-phase one must remember that the mixture is heterogeneous, giving rise to a large temperature difference between the resin bead and the surrounding solvent. The temperature measured in solid-phase reactions is most likely to be the average temperature of the reaction mixture. Temperatures measured in SPOS using microwave synthesisers are therefore not a clear indication of the temperature profile and may lead to irreproducibility. Higher reactivity has been seen for resins heated in the microwave than under conventional heating but this might be just due to inaccuracy of measuring the temperature at the actual reaction site inside the bead.

2.11.4 Polymer-aided solution-phase chemistry using microwaves

The current drive towards polymer aided solution phase chemistry is mirrored in the number of publications using solid-phase reagents in conjunction with microwave irradiation. Ley et al. used microwave-assisted organic chemistry, solid-supported reagents and scavenger resins in their synthesis of Viagra to demonstrate the utility of these new techniques.
Solid-phase methylating agent 105 for the methylation of carboxylic acids has been prepared from commercially available carbodiimide resin by Crosignani et al.\textsuperscript{93} under microwave irradiation (scheme 42). The yield of the reaction was greatly increased using microwave irradiation. Subsequent methylation of acids using methylating reagent 105 was performed at room temperature.

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\text{PS} \begin{array}{c}
N=C=N \\
\text{MeOH, } \alpha, 135 \degree C \\
70 \text{ mins}
\end{array} \begin{array}{c}
\text{PS} \begin{array}{c}
N=C=N \\
105
\end{array}
\end{array}};
\node (b) at (3,0) {\begin{array}{c}
\text{HN—NH} \\
{\begin{array}{c}
N—N
\end{array}}
\end{array}};
\node (c) at (5,0) {\begin{array}{c}
\text{HN—NH} \\
{\begin{array}{c}
N—N
\end{array}}
\end{array}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 42: Synthesis of methylating agent 105 using microwave irradiation}

\textsuperscript{94} Ohberg and Westman demonstrated this technique using polymer supported (PS-DMAP) 106 in the solution phase synthesis of thiohydantoins using microwave heating (scheme 43a).

\begin{center}
\begin{tikzpicture}
\node (a) at (0,0) {\begin{array}{c}
\text{R}^1\text{N—COOH} \\
\text{PS—N=C=N} \end{array}};
\node (b) at (3,0) {\begin{array}{c}
\text{R}^1N—C=S \\
\text{MeCN, } \alpha, 180 \degree C, 5 \text{ mins}
\end{array} \begin{array}{c}
\text{PS} \begin{array}{c}
\text{HN—NH} \\
\text{N—N}
\end{array}
\end{array}};
\node (c) at (6,0) {\begin{array}{c}
\text{R}^1\text{N—COOH} \\
\text{PS—N=C=N} \end{array}};
\end{tikzpicture}
\end{center}

\textbf{Scheme 43: Microwave-promoted and polymer aided solution phase chemistry}
Polystyrene bound DMAP 106 gave slightly lower yields than triethylamine but gave a cleaner reaction mixture and made purification easier. In this example the temperature of reaction was monitored using an IR-sensor. Scheme 43b details the use of polymer supported Burgess reagent 107 or PS-BEMP 108 to affect the microwave-promoted cyclisation to 1,3,4-oxadiazoles by Brain and Brunton.95 Similar yields were noted for both thermal and microwave heating but the later case the reaction times were greatly reduced.

A paper by Jacob Westman96 from Personal Chemistry demonstrates the development of a one pot Wittig reaction using polymer supported triphenylphosphine 109, promoted by microwave dielectric heating (scheme 43c). They observed purities greater than 98 % over a range of 15 unsaturated products. Unusually they choose to use methanol as a solvent, which is known to have bad swelling properties with polystyrene resin. Under microwave conditions however, methanol becomes less polar thus swelling the resin to a higher degree.

Microwave irradiation has been used by Ley et al.97 to decrease reaction times for thionation of secondary and tertiary amides using a polymer supported Lawesson's reagent 110 (scheme 44). Thionation showed a marked acceleration after 10-15 minutes irradiation in toluene. A small amount of ionic liquid (1-ethyl-3-methyl-1H-imidazolium hexafluorophosphate) was added to the toluene to improve the adsorption and dissipation of microwave energy. The advantages of using an ionic liquid are that they are relatively inert, stable to high temperatures, have negligible vapour pressure and dissolve to an appreciable amount in organic solvents.

![Scheme 44: Microwave-promoted thionation using a polymer supported Lawesson's reagent 110](image)

**2.12 Microwave assisted synthesis of cyclohexanedione resin**

Given the considerable literature precedent for improving the yields of solid-phase reactions using microwave irradiation we attempted enol ether hydrolysis in a CEM
Discover microwave. Reaction conditions were monitored using a black body radiation sensor to measure temperature, and a pressure probe inserted through the septum of the reaction tube. A variety of conditions for hydrolysis of resin bound methoxycyclohexenone 56 to cyclohexane-1,3-dione (CHD) resin 57 were tested and are shown in table 4. The disappearance of enol ether methoxy proton resonances in \( ^1 \text{H}-\text{MAS-NMR} \) was used to monitor hydrolysis progress. FT-IR was not useful in monitoring this reaction as the carbonyl peaks were broad and not very distinct.

![Scheme 45: Linker cleavage under microwave assisted acidic hydrolysis](image)

When highly acidic conditions were used (table 4, entries 1 and 2) microwave irradiation caused linker cleavage resulting in formation of amide 40 (scheme 45). Amide 80 was isolated from the resin washings after resin hydrolysis and quantified to give an estimate of the loading lost (L_{lost}) (table 4). When methoxycyclohexenone resin 56 was heated in high acid concentration the resin underwent a colour change to give a red resin due to cation formation, again indicative of linker cleavage. The red colour dispersed once the resin was washed with methanol and the cation was quenched.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Acid concentration TFA/H(_2)O/DMF</th>
<th>Microwave conditions</th>
<th>( L_{\text{lost}} / \text{mmol/g} )</th>
<th>Presence of OMe peak in ( ^1 \text{H}-\text{MAS-NMR} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>90:5:5</td>
<td>110 °C (50 W) 5 mins</td>
<td>0.41</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>90:5:5</td>
<td>110 °C (50 W) 1 min</td>
<td>0.39</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>1:1:1</td>
<td>110 °C (50 W) 5 mins</td>
<td>negligible</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>1:1:1</td>
<td>110 °C (50 W) 10 mins</td>
<td>negligible</td>
<td>No</td>
</tr>
</tbody>
</table>

**Table 4: Hydrolysis conditions for cyclohexenone formation**

When lower acid concentrations were used (table 4, entries 3 and 4) a longer reaction time was required to ensure complete enol ether hydrolysis. After five minutes of
irradiation enol ether protons were still seen in the MAS-probe NMR of the resin however, the peak disappeared upon ten minutes of irradiation.

Direct hydrolysis of bis-enol ether 54 gave a mixture of products by $^1$H-MAS-NMR. The optimum conditions for hydrolysis were found to be; preliminary room temperature hydrolysis to methoxycyclohexenone 56 followed by microwave hydrolysis to diketone 57. When solid phase enol ester hydrolysis occurred, a peak at 1781 cm$^{-1}$ in the FT-IR was observed corresponding to a TFA enol ester peak for resin 111. This peak was removed upon washing the resin with a nucleophilic amine (*e.g.* $^6$butylamine) to regenerate the required diketone 57 (scheme 46a). The $^1$H-MAS-NMR of CHD 57 was not very clear with broadened lines presumably due to enol-keto tautomerism.

![Scheme 46](image)

**Reagents and Conditions:** (i) TFA/H$_2$O/DMF (1:1:1), θ, 110 °C (50 W) 10 mins; (ii) $^6$BuNH$_2$ wash; (iii) neat TFA, RT, 5 mins (> 99 %).

**Scheme 46:** TFA enol ester formation (a) on resin with removal and (b) in solution

Solution phase dimedone TFA enol ester 112 was prepared by treatment of dimedone 24 with neat TFA (scheme 46b). The product of this reaction showed a TFA enol ester peak at 1783 cm$^{-1}$ matching the peak observed in the corresponding solid-phase reaction. The product rapidly decomposed to dimedone 24 on standing at room temperature.

Aqueous 1M FeCl$_3$.6H$_2$O solution has been used to identify 1,3-enol functionalities in solution. When a few beads of CHD resin 57 were swollen in DMF the treated with 1M FeCl$_3$.6H$_2$O (aq) solution a brown colour due to iron complexation was observed after standing overnight. Resin bound bis-enol ether 54 and
methoxycyclohexenone 56 showed no significant change in colouration when subjected to the same conditions. When CHD resin 57 was tested with 2,4-DNP reagent, we observed no significant colour difference compared to 2,4-DNP treated bis-enol ether 54.

Hydrolysis of dendritic methoxycyclohexenone resin 103 under the established microwave conditions resulted in resin decomposition (scheme 47). The texture of dendritic resin 103 was altered once heated in the microwave and the $^1$H-MAS-NMR spectrum lacked the characteristic linker ethyl peaks. We propose that the tertiary amine functionality contained within the dendritic linker was not stable to microwave irradiation.

Reagents and Conditions: TFA/H$_2$O/ DMF (1:1:1), $\delta$, 110 °C (50 W) 10 mins, $^9$BuNH$_2$ wash.

Scheme 47: Hydrolysis of dendritic methoxycyclohexenone

### 2.13 Summary

In summary we have demonstrated the synthesis of solid-supported cyclohexane-1,3-dione (CHD) on polystyrene resin using microwave irradiation. The synthesis of high loading dendritic cyclohexanedione resin is described, although in compromised yield. The following chapters demonstrate the applications of CHD resin as a solid-phase reagent and scavenger resin.
3. Results and Discussion – Applications of CHD resin

3.1 Screening for scavenging ability

In order to establish the reactivity of CHD resin 57 we undertook the following screen using the Bohdan Neptune Synthesiser. Resin bound CHD 57 was shaken overnight with each of the substrates shown in table 5. Standard 55.5 mM solutions in dichloromethane were prepared using the Bohdan Neptune and the substrates dispensed to the resin in Bohdan Mini Blocks using the robotic arm. The resin was shaken overnight using a mini block shaker then the resin filtered and washed three times with DCM. The reactions were repeated to ensure reactions had gone to completion and the resins were washed again three times with DCM.

<table>
<thead>
<tr>
<th>Aldehydes</th>
<th>Secondary</th>
<th>Acids</th>
<th>Hydrazines</th>
</tr>
</thead>
<tbody>
<tr>
<td><img src="image" alt="Aldehydes" /></td>
<td><img src="image" alt="Secondary" /></td>
<td><img src="image" alt="Acids" /></td>
<td><img src="image" alt="Hydrazines" /></td>
</tr>
<tr>
<td>Ketones</td>
<td>Anilines</td>
<td>Alkyl Halides</td>
<td>α,β-Unsaturated</td>
</tr>
<tr>
<td><img src="image" alt="Ketones" /></td>
<td><img src="image" alt="Anilines" /></td>
<td><img src="image" alt="Alkyl Halides" /></td>
<td><img src="image" alt="α,β-Unsaturated" /></td>
</tr>
<tr>
<td>Primary Amines</td>
<td>Acid Chlorides</td>
<td>Malonates</td>
<td></td>
</tr>
<tr>
<td><img src="image" alt="Primary Amines" /></td>
<td><img src="image" alt="Acid Chlorides" /></td>
<td><img src="image" alt="Malonates" /></td>
<td></td>
</tr>
</tbody>
</table>

Table 5: Substrates used to screen the scavenging ability of CHD resin 57

The resin samples were analysed by MAS-probe NMR and gave one interesting result; the reaction between CHD resin 57 and acid chlorides to yield enol esters 114a and 114b (scheme 48a). The 1H-MAS-NMR clearly showed enolic protons
(-5.7 ppm), alkyl protons of the tert-butyl ester (1.2 ppm) or aromatic protons of the phenyl ester (-8.1 ppm).

![Reaction Scheme]

Reagents and Conditions: (i) RCOCl, DCM, RT, o/n; (ii) PhCOCl, DMF, RT, 4h (72%).

Scheme 48: Enol ester formation (a) on resin and (b) in solution

The tert-butyl and phenyl ester carbonyls were clearly seen in the FT-IR spectra of resins 114a and 114b at 1716 and 1713 cm⁻¹ respectively. For solution-phase comparison, dimedone enol ester 115 was prepared in high yield after purification, by reaction of dimedone 24 with benzoyl chloride (scheme 48b). The product showed a distinct enolic proton peak in the ¹H-NMR spectrum at 5.99 ppm and an enol ester peak at 1739 cm⁻¹ in the FT-IR. Prompted by these promising results we envisaged using CHD resin as a capture and release reagent for the transfer of acyl groups.

3.1.1 Elucidation of acylation mechanism

At this stage we were unsure whether direct acylation of methoxycyclohexenone resin 56 was possible, thus avoiding an additional hydrolysis step to form CHD resin 57. Solution phase studies revealed that methoxycyclohexenone 58 reacted slowly with benzoyl chloride to form dimedone enol ester 115 however, in low yield (23 %) after purification. We needed to establish whether methoxycyclohexenone 58 underwent initial hydrolysis to dimedone 24 followed by acylation, or was reacting directly to yield enol ester 115 (scheme 49).
**Scheme 49:** Two possible mechanisms for acylation

Deuterated methoxycyclohexenone \( D6-58 \), containing chemically distinguished methylene groups, was used as a mechanistic probe to observe the regioselectivity of enol ester formation. The lithium kinetic enolate of dimesone was prepared by deprotonation of methoxycyclohexenone 58 with LDA\(^{99} \) and subsequently reacted with D\(_2\)O to form deuterated methoxycyclohexenone \( D6-58 \) (scheme 50).

**Reagents and Conditions:** (i) Diisopropylamine, \(^6\)BuLi, DMPU, THF, -78 °C, 1h; (ii) D\(_2\)O, RT, o/n (100 %); (iii) PhCOCl, DCM, RT, 2 days (20 %).

**Scheme 50:** Elucidation of methoxycyclohexenone acylation using deuterated analogues

The compound observed upon deuteration showed a significant reduction in C6 proton peak height in \(^1\)H-NMR indicating C6 substitution. A small amount of C4 peak reduction was observed indicating multiple-deuteration. When deuterated methoxycyclohexenone \( D6-58 \) was subjected to acylation using benzoyl chloride, enol esters \( D4-115 \) and \( D6-115 \) were isolated in a 50:50 mixture by NMR (note that the numbering of ring carbons differs for compounds 58 and 115). An equal
reduction in both C4 and C6 peak height in 1H-NMR was observed, indicating that label scrambling had occurred. The reaction therefore cannot be concerted and therefore proceeded via dimedone 24 to give mixed product deuteration. The fact that methoxycyclohexenone 58 must undergo hydrolysis before acylation may account for the poor yield when methoxycyclohexenone 58 is subjected to acylation with benzoyl chloride. Interestingly the acylation of methoxycyclohexenone resin 56 was monitored by FT-IR and the appearance of an enol ester peak was clearly seen upon reaction with acid chlorides. We suspect that once bis-enol ether resin 54 was reacted with TFA a mixture of hydrolysed and un-hydrolysed sites were formed. The acylation we observed can therefore be attributed to the acylation of diketone sites only. In order to maximise the level of acylation the resin must therefore be completely hydrolysed to the diketone form.

3.2 Acyl and sulfonyl ‘capture and release’ reagents

A 'capture and release' reagent is classified a functionalised polymer which allows the trapping of a small molecule as an activated polymer intermediate. This activated intermediate can be washed with a range of solvents to ensure high purity then subjected to a second transformation with a new reagent to release the product into solution. This review will focus on acyl and sulfonyl transfer protocols using suitably functionalised resins.

Polymer supported dimethylaminopridine 106 (PS-DMAP) has been used for the synthesis of amides and mixed anhydrides via the corresponding 1-substituted pyridinium salts (scheme 51a). PS-DMAP has also been used for the synthesis of sulfonamides by reaction of PS-DMAP with sulfonyl chlorides. 1-Substituted pyridinium salts were released using anilines but give poor yields with secondary amines.

Polymer supported hydroxybenzotriazole 116 (PS-HOBt) was first introduced as a reagent for the acylation of N-nucleophiles by Pop et al. Tertiary aliphatic amides were prepared by this method, but the release reaction proceeded in low yields when anilines were used. Pop et al. attached the HOBr portion to the solid phase using an electron withdrawing sulfonamide linker to enhance reactivity. In additional work
by Dendrinos and Kalvretenos, PS-HOBt 116 was used for the transfer of protecting groups (Fmoc, Cbz and Boc)\textsuperscript{105} and the synthesis of N-hydroxysuccinimide esters\textsuperscript{106} (scheme 51b).

Scheme 51: Acyl transfer reagents (a) PS-DMAP 106 and (b) PS-HOBt 116

In a similar manner acyl groups have been immobilized onto polymer supported carbodiimide 117 (PS-EDC) (table 6) and released by amines to form amides\textsuperscript{107}, thiols to form thio esters\textsuperscript{108} and sulfonamides to form acyl-sulfonamides\textsuperscript{109}. The reagents were added to PS-EDC 117 in one pot with the resin acting more as a solid-phase coupling reagent.

Scheme 52: PS-TBD 118 as a triflate or nonaflate transfer reagent

Polymer supported 1,5,7-triazabicyclo[4.4.0]dec-5-ene 118 (PS-TBD)\textsuperscript{110} has been used as a solid-supported base that ionically captures aromatic phenols and reacts
with 4-nitrophenol triflate/nonaflate to produce aromatic triflates and nonaflates in solution (scheme 52).

Adamczyk et al. used polymer supported \textit{N}-hydroxy-succinimide\textsuperscript{119} (PS-NHS) (table 6) to prepare an amide library. They were able to use secondary amines but did not attempt to use anilines of any kind.

Polymer supported oxime \textbf{120} has been used to prepare unsymmetrical ureas\textsuperscript{112} by the two methods outlined in scheme 53. PS-Oxime \textbf{120} was reacted with an isocyanate to give intermediate oxime carbamate \textbf{121}, followed by release from the solid support by amine thermolysis. For less available or hard to prepare isocyanates, oxime carbamate resin \textbf{121} was generated by reaction with phosgene followed by the corresponding amine. The optimum temperature for thermolytic cleavage in toluene was found to depend upon the intermediate oxime carbamate used. Excess amine was removed using Dowex-sulfonic acid resin.

Scheme 53: ‘Capture and release’ of ureas using polymer supported oxime \textbf{120}

Kim et al.\textsuperscript{113} were able to acylate heterocyclic amines of reduced nucleophilicity using polymeric active esters of 4-hydroxy-3-nitrobenzophenone \textbf{122} (scheme 54). The electron-deficient phenol group allowed for intermediate anchoring of an acid chloride according to work pioneered by Cohen \textit{et al.} in 1982\textsuperscript{114} to yield acylated resin \textbf{123}.

Kin \textit{et al.} were not able to push acylation reactions to completion even after overnight heating at 60 °C so used an excess of amine to ensure high reactivity.
Unreacted amine was scavenged using Amberlite resin. Unfortunately this resin also scavenged amides 124 so they demonstrated an alternative acylation method using polymer supported 2-tert-butylamino-2-diethylamino-1,3-dimethyl-perhydro-1,3,2-diazaphosporine (PS-BEMP) 108 to furnish amides 124 in high yields and purity.

Scheme 54: Release using heterocyclic amines of reduced nucleophilicity

Polymer supported tetrafluorophenol 126 (PS-TFP) (developed by Salvino et al.) has been used to capture acids using an amide coupling (scheme 55) Resin bound activated TFP-esters were released using amines to give amides in solution phase. Resin bound sulfonate esters were prepared by reaction of PS-TFP with sulfonyl chlorides and were released using amines to give sulfonamides. A para-amide group provided additional electron withdrawal alongside the electron withdrawing fluorine groups. The loading of resin bound TFP esters was determined by $^{19}$F-NMR.

Scheme 55: PS-TFP for the ‘capture and release’ of (a) amides and (b) sulfonamides
### 3.2.1 Summary of ‘capture and release’ reagents

<table>
<thead>
<tr>
<th>No.</th>
<th>Resin</th>
<th>Structure</th>
<th>Substrates prepared</th>
<th>Nucleophile Specificity</th>
<th>Recycle</th>
</tr>
</thead>
<tbody>
<tr>
<td>106*</td>
<td>PS-DMAP</td>
<td><img src="image1.png" alt="Structure" /></td>
<td>Amides, carbamates, sulfonamides</td>
<td>Aliphatic and substituted benzylamines/anilines. Not secondary amines</td>
<td>yes</td>
</tr>
<tr>
<td>116*</td>
<td>PS-HOBt</td>
<td><img src="image2.png" alt="Structure" /></td>
<td>Amides, carbamates, N-hydroxy-succinimides</td>
<td>Low yields with anilines especially with electron-withdrawing substituents</td>
<td>yes</td>
</tr>
<tr>
<td>117*</td>
<td>PS-EDC</td>
<td><img src="image3.png" alt="Structure" /></td>
<td>Amides, thio esters and acyl-sulfonamides</td>
<td>Not anilines but can use secondary amines. Thiols and primary sulfonamides</td>
<td>no</td>
</tr>
<tr>
<td>118*</td>
<td>PS-TBD</td>
<td><img src="image4.png" alt="Structure" /></td>
<td>Aryl triflates and nonaflates</td>
<td>Substituted phenols</td>
<td>yes</td>
</tr>
<tr>
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<td>PS-NHS</td>
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<td>Amides</td>
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<td>yes</td>
</tr>
<tr>
<td>120</td>
<td>PS-Oxime</td>
<td><img src="image6.png" alt="Structure" /></td>
<td>Ureas</td>
<td>Secondary amines and anilines</td>
<td>yes</td>
</tr>
<tr>
<td>122</td>
<td>PS-Nitrophe nol</td>
<td><img src="image7.png" alt="Structure" /></td>
<td>Amides</td>
<td>Specifically less nucleophilic heterocyclic amines</td>
<td>yes</td>
</tr>
<tr>
<td>126*</td>
<td>PS-TFP</td>
<td><img src="image8.png" alt="Structure" /></td>
<td>Amides and sulfonamides</td>
<td>Secondary amines and anilines</td>
<td>yes</td>
</tr>
</tbody>
</table>

* = commercially available

Table 6: ‘Capture and release’ reagents
3.3 ‘Capture and release’ of amides using CHD resin

Having surveyed the literature for acyl group ‘capture and release’ reagents, it became apparent that cyclohexanedione (CHD) resin 57 was a novel target for such purpose with the added advantage of multifunctionality, able to fulfil a range of roles. Further investigation into acylation/release conditions and loading levels were required in order to optimise the ‘capture and release’ process detailed in scheme 56. An accurate value of resin loading was required to avoid the presence of residual amine in the amide product upon release.

![Scheme 56: Overview of the ‘capture and release’ process](image)

### 3.3.1 Optimisation of acylation conditions

The reaction of CHD resin 57 with benzoyl chloride (scheme 48a) was used to test a range of acylation conditions. The screen revealed that $O$-acylation was promoted under acidic conditions in dichloromethane. We reasoned that the presence of acid prevents base catalysed migration of the acyl group from $O$- to $C$-.$^1$ Non-polar dichloromethane seems an unusual choice for microwave reactions but appeared to give the best results with minimal resin decomposition. The reagents in the reaction mixture absorbed enough microwave irradiation to allow the reaction to proceed.

The capture of a benzoyl group onto the solid phase was accompanied by the appearance of an enol ester carbonyl peak at 1738 cm$^{-1}$ in FT-IR. In order to gain further insight into the kinetics of acylation we decided to monitor the reaction using FT-IR. By observing the ratio of carbonyl (1738 cm$^{-1}$) to carbon-carbon double bond (1632 cm$^{-1}$) peak intensities ($\nu_{C=O}/\nu_{C=C}$) in FT-IR (figure 12b) the level of acylation...
was determined. High levels of acylation were achieved using microwave-assisted heating compared to lower acylation levels at room temperature (figure 12a).

![Figure 12: (a) Plot of peak intensity against time for the conversion 57→114b; (b) FT-IR spectrum showing relative C=O and C=C intensity](image)

Optimum acylation conditions are shown in scheme 57. To ensure maximum acylation, CHD resin 57 was subjected to a double microwave acylation for ten minutes each time. If CHD resin was acylated under reflux in an oil bath at 110 °C lower levels of acylation were obtained than if microwave irradiation was used. When the resin beads were heated in the microwave the centre of the beads are likely to be considerably hotter than the temperature measured by the black body irradiation thermometer in the microwave cavity. The temperature measured was an average temperature for the reaction mixture rather than an actual temperature of the internal area of the bead. For this reason higher levels of acylation were observed than using conventional heating. Further studies using microwave irradiation on solid-phase beads is underway in Professor Andrew Harrison’s group at Edinburgh University.

Enol ester 114b was washed with non nucleophilic base (DIPEA) to remove residual acetic acid from the resin structure. Residual acetic acid was shown to form acetyl amides in the release step and found to contaminate the required amide product. Acylation of hydrolysed sites on dendritic resin 103 under microwave irradiation
resulted in resin decomposition thus provided further evidence that the tertiary amine linker was unstable to microwave heating.

\[
\begin{align*}
\text{O} & \quad \text{OH} \\
\text{O} & \quad \phantom{\text{OH}} \\
\text{N} & \quad \text{PS} \\
\rightarrow \quad \text{(i)} & \quad \text{(ii)} \\
\text{O} & \quad \text{OH} \\
\text{O} & \quad \phantom{\text{OH}} \\
\text{N} & \quad \text{PS} \\
\end{align*}
\]

**Scheme 57:** Optimum acylation conditions

### 3.3.2 Determination of resin loading

CHD resin 57 was coupled to 3,5-dichlorobenzoyl chloride to give chloro-substituted enol ester resin 114c (scheme 58). This resin was analysed by elemental analysis and shown to have a resin loading of 0.65 mmol/g. This gave a calculated resin loading of 0.73 mmol/g for CHD resin 57.

\[
\text{57} \quad \text{L}_{\text{calc}} = 0.73 \text{ mmol/g} \\
\text{114c} \quad \text{L}_{\text{EA}} = 0.65 \text{ mmol/g}
\]

**Reagents and Conditions:** 3,5-DichloroArOCl (5 eq), MeCO\textsubscript{2}H (5 eq), DCM, \(\omega\), 110 °C (300 W) 10 mins \(\times\) 2.

**Scheme 58:** Determination of resin loading

### 3.3.3 Optimisation of release conditions

We have successfully demonstrated that the release of amide into solution can also be accelerated by microwave heating. The release process was monitored using FT-IR of the model reaction shown in scheme 59. Kinetic studies of the release reaction revealed residual enol ester functionalities on the resin unless excess amine was
present (figure 13). Graph 13 does not show the value $\nu_{C-O}/\nu_{C-C} = 0$ because no baseline correction of the FT-IR spectra was performed. The lowest possible value of $\nu_{C-O}/\nu_{C-C}$ was therefore 0.45.

Figure 13: Plot of peak intensity against time for the conversion $114b\rightarrow57$

Higher release levels were observed when the resin mixture was bombarded by continuous microwave irradiation whilst being continuously cooled. A special feature of the CEM Discover microwave allows continuous cooling of samples enabling higher power levels over the time-course of the reaction. The reaction occurred at high yield with minimal decomposition products in methanol. Methanol does not swell polystyrene resins at room temperature due to its high polarity. The polarity of methanol decreases at elevated temperatures under microwave irradiation, allowing swelling of the resin.

Excess amine was scavenged from the reaction mixture using Dowex-50WX sulfonic acid resin. Some reduction of yield was observed upon scavenging of amine with Dowex-50WX. This resulted in lowering of the resin loading with respect to 'capture and release' from 0.65 to 0.2 mmol/g. Optimum conditions for release are shown in scheme 59 yielding benzylamide $127a$ in 98 % yield by weight and 100 % purity by LC-MS at 220 nm. Upon second use of CHD resin 57, amide $127a$ was obtained in 63 % yield (by weight) and > 99 % purity (by LCMS at 220 nm) thus demonstrating the ability to recycle CHD resin 57.
3.3.4 Library synthesis of amides by ‘capture and release’

CHD resin 57 was used to prepare a library of amides shown in table 7 in varying purity and yields according to the protocol detailed in scheme 60. A CEM Explorer Carousel with robotic arm enabled overnight serial synthesis of a number of reactions in sealed microwave tubes.

Reagents and Conditions: (i) R'COCl (5 eq), MeCO₂H (5 eq), DCM, 0, 110 °C (300 W) 10 mins x 2; (ii) 10 % DIPEA in DMF wash; (iii) R²R'NH (20 eq), MeOH, 0, 125 °C (200 W) 30 mins; (iv) Dowex-50WX resin, 1h, RT.

Scheme 60: General protocol for the synthesis of a library of amides

The amide release reaction required work-up with Dowex-50WX resin which was added directly to the reaction mixture before manual filtration into test tubes. This was followed by evaporation of solvent on the Christ Solvent Evaporator. The yield
of product was determined by accurate determination of weight. Amides 127a-127p
were analysed by LC-MS at 220 nm using an acetonitrile (0.1 % TFA)/water (0.1 %
TFA) gradient over 30 minutes on a C18 Luna Phenomenex column. Aromatic enol
esters generally gave higher yields and purities of their corresponding amides than
aliphatic enol esters. Aniline gave lower yields overall, presumably due to reduced
amine nucleophilicity.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Structure</th>
<th>No.</th>
<th>Yield (%)(^a)</th>
<th>Purity (%)(^b)</th>
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<tr>
<td>1</td>
<td><img src="127a" alt="Structure" /></td>
<td>127a</td>
<td>98 63(^c)</td>
<td>100 100(^c)</td>
</tr>
<tr>
<td>2</td>
<td><img src="127b" alt="Structure" /></td>
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</tr>
<tr>
<td>3</td>
<td><img src="127c" alt="Structure" /></td>
<td>127c</td>
<td>91</td>
<td>86</td>
</tr>
<tr>
<td>4</td>
<td><img src="127d" alt="Structure" /></td>
<td>127d</td>
<td>88</td>
<td>99</td>
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<td>72</td>
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<td>127g</td>
<td>62</td>
<td>85</td>
</tr>
<tr>
<td>8</td>
<td><img src="127h" alt="Structure" /></td>
<td>127h</td>
<td>63</td>
<td>97</td>
</tr>
</tbody>
</table>

\(^a\) yields by weight; \(^b\) purity by LC-MS at 220 nm; \(^c\) resin recycling

**Table 7**: Capture and release of amides 127a-127h
<table>
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<tr>
<th></th>
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<th>100</th>
<th>77</th>
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</thead>
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<tr>
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<td>127j</td>
<td>28</td>
<td>96</td>
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<td>11</td>
<td><img src="image3.png" alt="Chemical Structure" /></td>
<td>127k</td>
<td>46</td>
<td>19</td>
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<tr>
<td>12</td>
<td><img src="image4.png" alt="Chemical Structure" /></td>
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<td>91</td>
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<td><img src="image5.png" alt="Chemical Structure" /></td>
<td>127m</td>
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<td>127n</td>
<td>61</td>
<td>49</td>
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<td>127p</td>
<td>55</td>
<td>39</td>
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</table>

*a* yields by weight; *b* purity by LC-MS at 220 nm

Table 7 (cont.): Capture and release of amides 127i-127p

### 3.4 CHD as an electrophilic and nucleophilic scavenger resin

Dimedone 24 reacts with acetone at reflux in a Knoevenagel type reaction to form diketone 128 shown in scheme 61. Dimedone exists primarily in the enol form as shown and reacts with aldehydes to form a dimer as shown in scheme 61. Dimedone has been used as a reagent for the differential characterisation and separation of aldehydes and ketones as it readily yields dimers of the form 129 with aldehydes, but not ketones, from a mixture of the two.
To test the idea that CHD resin 57 could scavenge electrophiles, solution phase diketone 49 was reacted with benzaldehyde in dichloromethane (scheme 62). The reaction proceeded at a slow rate but after four days showed near complete formation of dimer 130 that was isolated by preparative-HPLC. The reduction in yield was due to loss of product upon purification. This is sufficient to suggest that the solid supported analogue could act as a scavenger for aldehydes although the dimer is unlikely to be formed in the solid phase due to polymer bound site isolation. Diketone 49 was reacted with benzylamine in dichloromethane (scheme 62) to yield enamine 131, which was isolated by preparative-HPLC. Formation of this product at room temperature suggests that the solid supported analogue could act as a scavenger for amines.

To determine the amount of substrate scavenged by CHD resin 57, $^1$H-NMR was utilised to give a quantitative measurement of scavenging ability in deuterated solvent. A small amount of acetonitrile was used as an internal standard. Three equivalents of CHD resin 57 were shaken with benzaldehyde in deuterated DCM and the $^1$H-NMR spectra of the mixture measured before and after scavenging. Initially, CHD resin 57 appeared to scavenge benzaldehyde from solution however, the $^1$H-MAS-NMR spectrum of the resultant resin showed no significant change in resin structure indicating that the benzaldehyde may be being retained inside the resin porous structure. A similar test was performed with benzylamine but insufficient scavenging was observed. The mini-block screen undertaken in section 3.1 further confirmed the hypothesis that scavenging of amines and aldehydes with CHD resin 57 was not feasible.
Reagents and Conditions: (a) PhCHO, DCM, 4 days, RT (43 %); (b) PhCH$_2$NH$_2$, DCM, o/n, RT (47 %).

Scheme 62: Test solution phase scavenging reaction

3.5 CHD as an allyl cation scavenger resin

Dimedone 24 has been extensively used to scavenge allyl cations in the palladium-catalysed deprotection of N-Alloc-carbamates$^{28,29}$ (figure 14). The allyl-cations react with the nucleophilic 1,3-diketone moiety of dimedone thus preventing the formation of allylamine by-products.

Figure 14: Pd-catalysed catalytic cycle for N-Alloc deprotection
3.5.1 Deprotection of N-Alloc carbamates

In order to further analyse the deprotection of N-Alloc carbamates, a model system was established. N-Alloc deprotection of N-Alloc-benzylamine 133 was performed in the presence of excess dimedone 24 (scheme 63). N-Alloc-benzylamine 133 was prepared in moderate yield by the reaction of benzylamine 132 with allyl chlorofomate in pyridine/DCM solution according to the method described by Fader et al. N-Alloc-benzylamine 133 was reacted overnight with 5 mol % of tetrakis(triphenylphosphine)palladium (0) in the presence of excess dimedone 24 but the only product observed from the reaction mixture was the benzyl-enamine of dimedone 134. Interestingly, significantly cleaner reactions were observed when commercially available tetrakis(triphenylphosphine)palladium (0) was used rather than its preparation in situ from palladium acetate and triphenylphosphine.

Reagents and Conditions: (i) Alloc-Cl, py/DCM (1:1), RT, 2h; (ii) Pd(PPh₃)₄ (5 mol %), dimedone 24 (5eq), THF, RT, 30 mins; (iii) Alloc-Cl, py, THF RT, 2h (> 99 %); (iv) Pd(PPh₃)₄ (5 mol %), dimedone 24 (5eq), THF, RT, 30 mins (95 %)

Scheme 63: Alloc-protection and subsequent palladium-catalysed deprotection in solution

Due to the lack of proton resonances in the NMR spectrum of benzylamine we chose another substrate that would give a more diagnostic NMR upon deprotection. N-Alloc-alanine ethyl ester 136 was prepared in high yield by the reaction of the hydrochloride salt of alanine ethyl ester 135 with allylchlorofomate in pyridine/DCM solution (scheme 63). When N-Alloc-alanine ethyl ester 136 was reacted with 5 mol
% of tetrakis(triphenylphosphine)palladium (0) in the presence of excess dimedone 24 the only product observed from the reaction mixture was the dimedone enamine 137.

3.5.2 Deprotection of O-Alloc carbonates

Since Alloc-carbamates release amines that undergo reaction with dimedone, we decided to switch to using Alloc-carbonates. The deprotection of Alloc-carbonates would give less nucleophilic alcohol products which would not react with dimedone. In this instance, dimedone 24 prevents the formation of allyl alcohol by-products. O-Alloc-benzyl alcohol 139 was prepared in quantitative yield by the reaction of benzyl alcohol 138 with allylchlorofomate and pyridine in THF solution (scheme 63) according to the method described by Corey et al.118 When O-Alloc-benzyl alcohol 139 was reacted with 5 mol % of tetrakis(triphenylphosphine) palladium (0) in the presence of excess dimedone 24, benzyl alcohol 138 was obtained in near quantitative yield. Benzyl alcohol 138 was not nucleophilic enough to react with dimedone. Di-allylated dimedone 140 was isolated in 40 % yield.

3.6 Allyl cation scavenging using CHD resin

The deprotection of O-Alloc benzyl alcohol 139 was attempted using CHD resin 57 to scavenge allyl cations. The reaction was subjected to the standard conditions developed above and was complete in just 30 minutes to give benzyl alcohol 138 in low yield (scheme 64). Analysis of allylated-resin 141 by MAS-probe NMR showed allyl resonances at 2.5, 5.1 and 5.7 ppm corresponding to allyl protons. Benzyl alcohol 138 isolated from the reaction mixture was contaminated with allyl benzyl ether 142 formed by the direct reaction of allyl cations with benzyl alcohol 138. We propose that the allyl cations reacted with benzyl alcohol before they had time to diffuse into the resin structure of solid-phase CHD 57. In order to slow the reaction down and allow time for reagent diffusion into the resin structure, the concentration of catalyst was reduced to 0.1 mol %. In doing so, the reaction was only complete after overnight stirring. The yield of benzyl alcohol 138 was increased to 41 % but the sample still contained allyl benzyl ether 142. The sample therefore required purification by column chromatography.
Reagents and Conditions: CHD-PS resin 57: Pd(PPh₃)₄ (5 mol %), THF, RT, 30 mins (41 %); CHD-dendritic resin 103: Pd(PPh₃)₄ (0.1 mol %), THF, RT, o/n (87 %).

Scheme 64: Palladium-catalysed deprotection of O-Alloc benzyl alcohol using resin scavengers

The rate of scavenging was increased when dendritic resin 103 was used in place of CHD resin 57. The increased loading of dendritic resin 103 enabled faster allylation scavenging. The deprotection of O-Alloc benzyl alcohol 139 using 0.1 mol % of Pd(PPh₃)₄ catalyst in an overnight reaction is detailed in scheme 64. Benzyl alcohol 138 was obtained in 87 % yield with minimal formation of allyl benzyl ether 142 by-product thus eliminating the need for purification by column chromatography. Allylated dendritic resin 143 showed clear allyl resonances at 2.8, 5.1 and 5.8 ppm corresponding to allyl protons.

3.7 CHD as a backbone amide linker

Backbone amide linkers and previous examples of solid-supported dimeredone analogues were discussed in chapter 1. We envisaged using solid-supported CHD 57 as a linker for the immobilisation of the carbonyl group of amides as detailed in scheme 65. Elizabeth Moir experienced difficulties in affecting O- to C- acylation on CHD resin due to the inaccessibility of resin sites, so it became important to find a method for efficient rearrangement or to affect C-acylation directly.
Scheme 65: Overview of CHD resin as a backbone amide linker

3.7.1 O- to C- rearrangement

The synthesis of O-acylated cyclohexane-1,3-diones and their subsequent O to C catalysed rearrangement has been described by Lakhvich et al. Enol esters of dimer were prepared by acylation of dimer with acid chlorides in the presence of pyridine. O- to C- rearrangement was affected using DMAP or aluminium chloride lewis acid catalysis. Using $^{13}$C-NMR they showed that the predominant tautomer of 2-acyldimer analogues contains an exocyclic C=O double bond and endocyclic enol form. The structure was stabilised by hydrogen bonding and was confirmed by X-ray crystal studies of benzoilidimerone. The synthesis of O- and C-acetyl dimer analogues is shown in scheme 66. Enol ester was prepared by reaction of dimer 24 with acetyl chloride in the presence of pyridine in quantitative yield and then subjected to cyanide catalysed rearrangement with acetone cyanohydrin to yield 2-acetyl dimerone in low yield. Higher yields were observed if dimer 24 was acylated with acetic anhydride in the presence of pyridine and DMAP added to catalyse in situ O- to C- rearrangement. The rearrangement was easily followed by TLC however; the reaction took three days for completion. This did not bode well for the solid-phase reaction which needed to be high yielding to avoid cross-contamination upon oxidative cleavage at the end of the synthesis.
Reagents and Conditions: (i) AcCl, py, DCM, RT, 1h (> 99 %); (ii) Et₃N, Me₂C(OH)(CN), MeCN, RT, o/n (31 %); (iii) Ac₂O, py, DMAP, RT, 3 days (87 %); (iv) Fmoc-Gly-OH, EDC.HCl, DMAP, DMF, RT, 1 ½h (40 %).

Scheme 66: Synthesis of O- and C-acetyl dimedone

Fmoc-glycine coupled dimedone 146 was prepared by coupling dimedone 24 with Fmoc-glycine using EDC coupling agent in the presence of DMAP to affect in situ O- to C- rearrangement. The ¹H-NMR and FT-IR spectral properties were used for comparison with solid-phase analogues.

The corresponding synthesis of C-acylated CHD derivatives is shown in scheme 67. A large range of conditions to C-acylate CHD resin 57 were attempted, including a variety of acylating agents, solvents and rearrangement conditions. The formation of C-acylated CHD 147 was monitored by the disappearance of the C=O enol ester peak at 1738 cm⁻¹ in the FT-IR. We also monitored the formation of an enolic OH peak at approximately 18 ppm and an exocyclic acetyl peak at 2.7 ppm in the ¹H-MAS-NMR. By comparison, solid-phase enol ester 114g showed a clear enol ester acetyl peak at 2.2 ppm and an enolic proton peak at 6.1 ppm. Complexation of dicarbonyl moieties with TBAF to fix the diketone in the enol form, has been shown previously to enhance C-acylation¹²⁵ however, complexation of CHD resin 57 with TBAF showed no increase in yield of C-acetylated CHD resin 147. The optimum conditions for acylation were found to be reaction of CHD resin 57 with acetyl chloride in the presence of DIPEA and acetone cyanohydrin for three days. The yield of the reaction was not improved upon using microwave irradiation. These conditions, despite being the most highly yielding, still resulted in retention of a
small amount of $O$-acetyl CHD 114g. Regardless of this fact $C$-acetyl CHD resin
147 was taken on for further synthesis.

![Diagram of chemical reactions](image_url)

**Reagents and Conditions:** (i) AcCl, DIPEA, $\text{Me}_2\text{C(CN)}\text{(OH)}$, DMF/MeCN (1:1), RT, 3 days; (ii) Fmoc-Gly-OH, DIC, DCM/DMF, DMAP, RT, $o/n \times 2$.

**Scheme 67:** Synthesis of $O$- and $C$-acylated CHD analogues

The loading of CHD resin 57 was calculated at 0.58 mmol/g (closely matching the value obtained elemental analysis) by coupling to Fmoc-glycine using DIC coupling agent in a DCM/DMF mixture in the presence of DMAP. The loading of Fmoc-glycine coupled resin 148a was determined at 0.50 mmol/g. The amount of $O$- versus $C$- acylation was not of consequence in this instance as the UV-Fmoc analysis did not specifically require either one analogue or the other.

### 3.7.2 Direct $C$-acylation via enamine formation

The direct $C$-acylation of enamines was used in the synthesis of $C$-acylated dimeredone analogues by Chu et al. Enamine formation blocks the enol position of dimeredone 24 thus directing acylation to the $C2$ position. Isobutyl enamine analogue 149 was prepared by the reaction of CHD resin 57 with isobutylamine in toluene under microwave irradiation (scheme 68). Solid-phase enamine 149 showed distinct NH stretches at 3426 and 3319 cm$^{-1}$ in the FT-IR spectrum and isobutyl proton resonances at 0.9 and 1.9 ppm in the MAS-probe NMR spectrum of the resin. When
Enamine formation was attempted under Dean Stark conditions the resin appeared to decompose and gave inconclusive spectral data by FT-IR and $^1$H-MAS-NMR.

Reagents and Conditions: (i) $\text{BuNH}_2$, PhMe, $\omega$, 145 °C (300 W) 10 mins; (ii) Fmoc-Gly-OH, EDC.HCl, DMF, RT, o/n x 2; (iii) 2M HCl, RT, 4h.

Scheme 68: Isobutylamine formation and subsequent C-acylation

Enamine 149 was coupled to Fmoc-glycine using EDC coupling agent and the resultant resin rotated in 2M HCl for four hours to remove enamine functionalities. Fmoc-glycine coupled resin 148b showed residual isobutyl peaks in $^1$H-MAS-NMR indicating incomplete enamine removal. The resin loading by UV-Fmoc analysis was found to be relatively low at 0.22 mmol/g so this resin was not utilised in further amino acid coupling reactions.

3.7.3 Dde-Amino acid solution phase analogues

In order to provide spectral comparisons for amino acid coupled resins the following solution phase analogues 150-152 were prepared (scheme 69). 2-Acetyl dimedone 145 was reacted with D/L-alanine ethyl ester 135 in the presence of DIPEA in DCM to give Dde-alanine ethyl ester 150 in quantitative yield. The key FT-IR spectral detail was an ester carbonyl stretch at 1738 cm$^{-1}$. 2-Acetyl dimedone 145 was reacted with L-Fmoc-Dap-OH in DMF in the absence of DIPEA to give Dde-(N-Fmoc-Dap)-OH 151 in quantitative yield. The reaction was performed in the absence of base to prevent Fmoc-deprotection of the cyclohexylidene product. The key FT-IR spectral detail in this case was a combined acid and carboxylate carbonyl stretch at 1721 cm$^{-1}$. Both Dde-amino acid analogues 150 and 151 contained diagnostic peaks in the $^1$H-NMR which were used to identify their solid-phase counterparts.
Reagents and Conditions: (i) D/L-Alanine ethyl ester 135, DIPEA, DCM, RT, 24h (> 99 %); (ii) L-Fmoc-Dap-OH, DMF, RT, 24h (> 99 %); (iii) NH₂NH₂, THF, RT, 1 ½h (88 %).

Scheme 69: Synthesis of solution-phase analogues

2-Acetyl dimedone 145 was also reacted with hydrazine in THF to form tetrahydroindazolone 152 in high yield. This compound was used as a solution-phase analogue for cleavage of solid-phase cyclohexylidene derivatives with hydrazine (see next section).

3.7.4 D/L-Alanine ethyl ester coupling on solid-phase

2-Acetyl CHD resin 147 was reacted with D/L-alanine ethyl ester 135 in the presence of DIPEA to form Dde-alanine analogue 153 (scheme 70). The attachment of amino acid to the resin was confirmed by monitoring ethyl ester peaks in the ¹H-MAS-NMR of resin 153. Solid-phase Dde-alanine analogue 153 was subjected to hydrazine cleavage as shown in scheme 70. There was no evidence for tetrahydroindazolone 154 formation by ¹H-MAS-NMR and alanine ethyl ester 135 was not observed in the resin washings. Mass spectrometry of the resin washings showed a peak at m/z = 75 [M+H]⁺ corresponding to hydrazone 155 formed from the cleavage of residual enol ester functionalities. The lack of alanine ethyl ester 135
possibly reflects the need for more forcing conditions. Time constraints prevented further optimisation of hydrazine cleavage.

Reagents and Conditions: (i) D/L-Alanine ethyl ester 135, DIPEA, DCM, RT, o/n; (ii) \( \text{NH}_2\text{NH}_2 \), DMF, RT. o/n; (iii) \( (\text{NH}_4)_6\text{Mo}_7\text{O}_{24}.4\text{H}_2\text{O} \), \( \text{H}_2\text{O}_2 \), AcOH, \( \theta \), 110 °C (50 W), 10 mins.

Scheme 70: Alanine ethyl ester 135 coupling on solid-phase

More promisingly, when Dde-alanine analogue 153 was subjected to molybdate catalysed cleavage using conditions previously established by Elizabeth Moir under microwave irradiation, 2-acetylamino-propionic acid methyl ester 156 was observed by mass spectrometry at m/z = 182 [M+Na]. Despite the unclean \(^1\)H-NMR spectrum of the released compound, distinct product peaks were identified. Again, time constraints prevented further optimisation of molybdate cleavage.

3.7.5 \( \text{l-Fmoc-Dap-OH} \) coupling on solid-phase

Scheme 71 details the synthesis of diaminopropionic acid (Dap) analogues on CHD resin. The advantage of using Dap for subsequent synthesis was the possibility of growing the amino acid chain in two directions. The peptide chain could then be removed from the resin using hydrazine.
Reagents and Conditions: (i) L-Fmoc-Dap-OH, DMF, RT, o/n; (ii) 20 % piperidine in DMF, RT, 2h; (iii) L-Boc-Ala-OH, TBTU, DIPEA, DMF, RT, o/n.

Scheme 71: Fmoc-Dap-OH coupling on solid-phase

2-Acetyl CHD resin 147 was reacted with L-Fmoc-Dap-OH, in the absence of base to yield Dde-Dap analogue 157 (scheme 71). The loading of Dde-Dap resin 157 was determined at 0.22 mmol/g by UV-Fmoc analysis. The resin was then subjected to piperidine catalysed Fmoc cleavage to give amino functionalised resin 158 that showed positive Kaiser, chloranil and TNBS colorimetric bead tests. Amino resin 158 was coupled to L-Boc-Ala using TBTU coupling agent in the presence of DIPEA. The resin showed complete coupling by chloranil and Kaiser colorimetric bead tests.

The $^1$H-MAS-NMR spectra of Dap-derived resins 157-159 were quite complicated but Boc-Ala coupled resin 159 showed a distinct tert-butyl peak at 1.5 ppm. Resin analysis to accurately assess resin coupling was too complicated to pursue this work.
any further. The concept however, was promising and may provide a starting point for further investigations.

3.8 Summary and further work

We have demonstrated the application of CHD resin as a capture and release reagent for the synthesis of amides and as an allyl scavenger resin. Investigations into the use of CHD as a backbone amide linker were promising but complicated and may require further work. A possible further application of 2-acetyl CHD resin is as a hydrazine scavenger resin as shown in scheme 72a. CHD related analogue shown in scheme 72b could be used as a safety catch linker, spontaneously releasing the required amine upon hydrazine deprotection.

Scheme 72: (a) Hydrazine scavenging and (b) potential safety catch linker based upon 2-acylated CHD resin
4. Results and Discussion - Hydrolase catalysed reactions on solid-phase

At this point in the project it became apparent that the use of dimedone-1,3-enol esters as novel substrates for lipase-catalysed reactions would be an interesting area for further investigation. We envisaged carrying out lipase-catalysed resolution of a racemic ester on CHD resin (scheme 73). The main advantage of performing this reaction on solid-phase would be the ease of purification as the products could easily be separated by simple filtration.

Reeve et al. demonstrated the preparative scale resolution of 3-phenylbutyric acid via lipase-catalysed hydrolysis of the corresponding methyl ester using a range of lipases.\textsuperscript{127} *Chromobacterium viscosum* lipase (CVL) was found to be highly selective for the (S)-(+) 3-phenylbutyryl acyl group. Both enantiomers of 3-phenylbutyric acid are commercially available so this acyl group was chosen for solid-phase studies. Comparison with work by Reeve et al. would allow direct comparison between enantioselectivities observed for methyl esters versus dimedone-1,3-enol esters.

Herein follows a brief introduction to lipase-catalysed reactions, focussing especially on examples on solid-phase, before discussing our work in this area.

4.1 Lipases in organic synthesis\textsuperscript{128-130}

Lipases have been widely used in organic synthesis due to their broad substrate specificity whilst maintaining high regio- and stereoselectivity. In nature they catalyse the hydrolysis of water insoluble long chain triglycerides. They are stable under adverse conditions including high temperatures and, possibly most usefully,
the use of organic media. Lipases have been used in both hydrolysis reactions in aqueous solvents or for transesterification reactions in anhydrous organic media. One of the main advantages of lipase catalysis is that co-enzymes or co-factors are not required.

4.1.1 Structural features of lipases

The 3D structure of lipases was first reported by Brady et al.\textsuperscript{131} in 1990 based on X-ray crystallography studies. The crystal structure of \textit{Chromobacterium viscosum} lipase, the focus of the work in the latter part of this chapter, was discussed by Lang et al.\textsuperscript{132} The main features of lipase 3D-structure are:

(a) A $\alpha/\beta$-hydrolase fold consisting of a hydrophobic sheet covered by $\alpha$-helices on both sides.

(b) An active site located on a $\beta$-sheet containing a catalytic triad of serine, histidine and aspartate/glutamate characteristic of all lipases.

(c) An oxyanion hole able to form hydrogen bonds and hence stabilise transition state intermediates.

(d) An $\alpha$-helix ‘lid’ which covers the active site and ensures that it is not exposed to solvent.

The mechanism of lipase-catalysed esterification or hydrolysis is shown in figure 15. The mechanism involves the formation of two tetrahedral intermediates, the first formed by nucleophilic attack of the serine residue of the catalytic triad onto the substrate. The tetrahedral intermediate loses water ($R^2 = H$) or an alcohol ($R^2 \neq H$) to give an acyl enzyme complex that is either attacked by water ($R^3 = H$) for hydrolysis or an alcohol ($R^3 \neq H$) for acylation. A second tetrahedral intermediate is formed that disassociates from the enzyme to give an ester or acid thus regenerating the lipase in its native form. Both of the tetrahedral intermediates involved in the mechanism are stabilised by hydrogen bonds to the oxyanion hole.

In nature, lipases act at an oil-water interface where a conformational change can occur opening the lid and allowing access to the active site. \textit{In vitro} lipases act at an aqueous-organic interface in a similar manner and show considerable rate increase when an interface is present. This therefore allows the use of bi-phasic mixtures of solvents in lipase catalysed reactions. Esterases are used to catalyse hydrolysis and
transesterification reactions similar to lipases. Esterases have similar 3D structure to lipases, but are deficient in an active site 'lid' and are consequently less tolerant of solvent mixtures.

Water can acts as a competitive nucleophile in transesterification reactions and therefore transesterification reactions should be performed under anhydrous conditions. Acylation reactions in pure organic media have been shown to give reduced yields and rates of reaction because the lid is thought to remain predominantly closed. The reduction in activity could also be due to changes in the pH of un-buffered organic solutions and changes to the substrate solvation.

![Figure 15: Mechanism of lipase catalysed hydrolysis (R^3 = H) or acylation (R^3 ≠ H)](image)

The structure of lipases has been shown to be similar in both water and hydrophobic solvents and binding of solvent molecules other than water has been observed. Rates of reaction and enantioselectivity have been altered by changing the solvent in a process called 'solvent engineering'.

The method of preparation of the lipase is important and can greatly affect its reactivity. The pH of the aqueous solution the enzyme was last in solution with, as
well as the method by which water was removed, can both influence the enzyme activity. Immobilised enzymes can aid recovery and re-use of the biocatalyst and there are a range of different enzyme supports available.

4.1.2 Monitoring the progress of lipase-catalysed resolutions

The rationale for performing a lipase-catalysed resolution is detailed in scheme 74. The racemic alcohol (scheme 74a) or acid (scheme 74b) can undergo transesterification with either an acylating agent or alcohol respectively. One enantiomer is a substrate for the lipase and will react and the other enantiomer is left unreacted. In this way, the products of the reaction can be easily separated by chromatography or re-crystallisation.

For both reactions the opposite and much faster, hydrolysis reaction can also be performed. In this instance the reaction is performed in water containing a small quantity of organic solvent (typically 10 %) to aid dissolution of the substrate and products.

The progress of any given lipase-catalysed reaction is monitored using two main factors. The enantiomeric excess of either the starting material or product gives a measure of the enantioselectivity of the reaction. High enantioselectivity is normally required for a successful lipase catalysed resolution. The enantiomeric excess (e.e.) of enantiomer A is calculated for example from HPLC data according to equation 5 where $P_A$ = peak area for enantiomer A and $P_B$ = peak area for enantiomer B.

\[
e.e.(\%) = \frac{(P_B - P_A)}{(P_A + P_B)} \times 100
\]  

**Scheme 74**: Rationale of lipase-catalysed resolutions of chiral (a) alcohols and (b) acids

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\[
e.e.(\%) = \frac{(P_B - P_A)}{(P_A + P_B)} \times 100
\]  

**equation 5**
The second parameter for monitoring lipase-catalysed reactions is the percentage conversion (c) which in turn leads to the yield of the reaction. Care must be taken to not confuse the percentage conversion (c), yield and isolated yield of the reaction. Reactions of this type can be quantified using the dimensionless 'enantiomeric ratio' ($E$)\textsuperscript{133} which represents a range of e.e.'s at a range of percentage conversions for any given reaction. The $E$ value of a reaction can be calculated according to equations 6-8 where $c =$ percentage conversion, e.e$_s =$ enantiomeric excess of the starting material and e.e$_p =$ enantiomeric excess of the product.

\[
E = \frac{\ln[(1-c)(1-e.e_s)]}{\ln[(1-c)(1+e.e_s)]} \quad \text{equation 6}
\]

\[
E = \frac{\ln[(1-c)(1+e.e_p)]}{\ln[(1-c)(1-e.e_p)]} \quad \text{equation 7}
\]

\[
E = \frac{\ln[(1-e.e_s)/(1+e.e_s/e.e_p)]}{\ln[(1+e.e_s)/(1+e.e_s/e.e_p)]} \quad \text{equation 8}
\]

Equations 6 and 7 give reliable results except at very low and very high extents of conversion where accurate measurements are restricted by errors derived from sample manipulation. The use of equation 8 is therefore preferred requiring the measurement of e.e. values only. Optical purity values are relative quantities in contrast to the conversion which is an absolute value. $E$ values less than 15 are unacceptable, $E$ values between 15 and 30 are moderate to good, and above 30 are excellent for use in organic synthesis.

### 4.1.3 The Kazlauskas rule

The chiral preference of lipases has been explained using the Kazlauskas rule, which predicts which enantiomer of a secondary alcohol reacts faster with respect to acylation.\textsuperscript{134,135} The favoured enantiomer is shown in figure 16 with the hydroxyl group pointing out of the plane of the paper where 'M' represents a medium sized substituent and 'L' represents a large substituent. The Kazlauskas rule applies in 90% of cases. A large percentage of the cases that do not follow the rule contain medium and large substituents that are not different 'enough' in size.
4.1.4 Activated acyl donors

For transesterification reactions, an activated acyl donor can be used to push the reaction equilibrium towards the required acylation. Suitable acyl donors include oximes, vinyl esters and anhydrides (figure 17).

Oximes have been shown to react faster than simple esters and enol esters, but a non-volatile oxime by-product is formed which may cause purification problems. The most popular choice of acyl donor is vinyl esters, which give virtually irreversible acylation forming volatile acetaldehyde as a by-product. Acetaldehyde has been shown in some cases to inactivate the enzyme by imine formation with lysine residues. The latter case, the use of anhydrides, is also considered irreversible but releases carboxylic acids that may decrease the $E$ of the reaction.

4.1.5 Solvent engineering

One of the most important fields of research in the biocatalysis arena is enhancing the specificity of enzymes by either modifying the enzyme itself (protein engineering) or by modifying the reaction conditions (solvent engineering). Gubicza and Szakács-Schmidt have provided an excellent review of the effects of reaction
conditions on the enantioselectivity and progress of a model esterification of 2-substituted propionic acids using Candida cylindracea lipase. The water content of the solvent used for such a reaction is very important and they observed a considerable improvement in yield when utilising an optimum water concentration of between 0.2 and 0.35 %. The activity of the enzyme increased linearly with decreasing solvent hydrophobicity of the solvent. Highly hydrophobic solvents were found to strip the enzyme of the water essential to its activity but low hydrophobicity solvents made the enzyme less rigid and therefore less enantioselective. For this reason a solvent mixture was required to see the optimum activity and enantioselectivity.

The water which covers the enzyme plays an important role in maintaining the enzyme flexibility. Addition of water mimics such as ethylene glycol made the enzyme more rigid and therefore more enantioselective.

4.2 Enzyme-catalysed reactions on solid-phase

4.2.1 Enzyme accessibility and solid supports

There are relatively few examples of solid-phase enzyme catalysed chemistry mainly due to the lack of understanding about enzyme accessibility into the internal structure of resin beads. An investigation by Bradley et al. into enzyme accessibility and solid supports\textsuperscript{137} using confocal Raman spectroscopy highlighted the confusion in the area. They found that the degree of enzyme accessibility was highly dependant on the enzyme molecular weight and the degree of flexibility of the resin structure. High conversions were consistently observed with controlled pore glass (CPG). None of the enzymes investigated could enter the polymer matrices of Tentagel (polyethylene grafted onto a polystyrene backbone) resin. Poly(ethylene glycol) acrylamide with an average molecular mass of 1900 Da (PEGA\textsubscript{1900}) was only compatible with low molecular weight enzymes (Mwt < 35 kDa). The synthesis of PEGA resins and their use in enzymatic assays was discussed by Renil et al.\textsuperscript{138} Reetz et al. showed that by using silicon based (Sol-gel) matrices containing a high degree of hydrophobic alkyl chains, the enzyme accessibility was dramatically improved\textsuperscript{139}. On changing from Si(OMe)\textsubscript{4} to PrSi(OMe)\textsubscript{3} matrices the relative enzyme activity was increased from 5 to 110 %.
4.2.2 Enzyme cleavable linkers for SP synthesis

Enzyme cleavable linkers are attractive targets for synthesis because they require mild conditions for cleavage. Enzyme cleavable linkers show good stereo-, region- and chemoselectivity. The first reported use of an enzyme cleavable linker was cleavage of a polyacrylamide bound phosphate SPPS linker, using calf spleen phosphodiesterase demonstrated by Elmore et al.\textsuperscript{140} Cleavage yields were improved by increasing the incubation time from one to seven days.

The 4-acyloxy-3-carboxybenzyloxy linker developed by Waldmann et al. and shown in scheme 75, was cleaved under mild conditions with a lipase or esterase to yield the product in reasonable yield despite the use of Tentagel resin.\textsuperscript{141} The mild conditions ensured that the attached molecule was not chemically altered during cleavage. The substrate specificity of the enzyme guarantees that only the intended ester is cleaved.

\begin{center}
\begin{tikzpicture}
\node[draw,rectangle,align=center] at (0,0) {
\begin{tabular}{c}
\textbf{Enzyme labile bond} \\
\end{tabular}
};
\node[draw,rectangle,align=center] at (2,0) {
\begin{tabular}{c}
\textbf{Enzyme initiated fragmentation} \\
\end{tabular}
};
\end{tikzpicture}
\end{center}

\textbf{Scheme 75:} Hydrolase cleavable 4-acyloxy-3-carboxybenzyloxy linker

A penicillin amidase cleavable linker developed by Flitsch et al. is shown in scheme 76.\textsuperscript{142} Penicillin amidase is a commercially available and widely used enzyme that is selective for the phenylacetyl group. Linker cleavage is initiated by hydrolysis of the phenylacetamide moiety generating a hemiaminal which fragments in the aqueous medium to release the alcohol from the resin. The linker contained a spacer fragment to aid enzyme accessibility to the reaction sites on the resin. High cleavage yields were observed for both polystyrene and Tentagel resins. The phenylacetyl group has
been used as a protecting group for oligonucleotides attached to CPG and cleaved by penicillin G acylase under similarly mild conditions.\textsuperscript{143}

\begin{equation}
\text{Penicillin amidase} \quad \text{catalysed OR hydrolysis}
\end{equation}

\textbf{Scheme 76: Penicillin amidase cleavable linker}

Waldmann \textit{et al.} developed an enzyme-labile safety catch linker for synthesis on a soluble polymer and the concept is shown in scheme 77. They observed minimal penicillin G acylase cleavage when Tentagel, CPG and PEGA supports were used but observed much higher levels of cleavage on using soluble polyethylene glycol conjugate with an average molecular mass of 6000 Da (POE\textsubscript{6000}).\textsuperscript{144}

\begin{equation}
\text{Target molecule} \quad \text{Acylase labile bond} \quad \text{MeO} \quad \text{NH}_{2} \quad \text{HX} - \text{Target molecule}
\end{equation}

\textbf{Scheme 77: Enzyme-labile safety-catch linker}

\subsection{4.2.3 Chemo-enzymatic synthesis of glycopeptides}

In an early example of solid-phase enzymatic synthesis, Meldal \textit{et al.} reported the use of bovine $\beta$-(1$\rightarrow$4)-galactosyl transferase for enzymatic glycosylation on PEGA\textsubscript{1900} resin.\textsuperscript{145} They observed complete reaction after 48 hours. Interestingly when the enzyme was pre-equilibrated with the resin for three days at 4 °C before
addition of the second sugar moiety, the yield was greatly improved. They therefore attributed the extended reaction times to the time required for the PEGA chains to fold around the enzyme.

4.2.4 On-bead screening for enzyme specificity

Lowe et al. performed an evaluation on resins for on-bead screening by studying the specificity of papain using PEGA, Tentagel and Argogel supported peptides shown in figure 18. Argogel is a polyethylene grafted polymer on a polystyrene backbone which shows higher swelling in water than Tentagel. The terminal amino acid was capped with a dansyl fluorophore which showed high fluorescence and enabled the distinction between cleaved (non-fluorescent) and uncleaved (fluorescent) polymer beads. Papain is a 23 kDa proteinase enzyme which is specific for the Phe-Gly-Leu fragment shown. The phenylalanine fragment binds to the papain active site and directs protein hydrolysis to the Gly-Leu bond. Only low levels of cleavage were observed with Tentagel and Argogel resins indicating that Tentagel and Argogel restrict enzyme access to the beads. When either of these resins was used the cleavage appeared to occur non-specifically suggesting that the resin structure affected the specificity of papain.

![Figure 18: PEGA, Tentagel and Argogel supported peptides for papain cleavage](image)

Quantitative cleavage of the PEGA-bound substrate indicated that the interior of the beads is freely accessible to papain. The sites of cleavage corresponded with those observed in solution indicating that the resin structure did not interfere with the site specificity of the enzyme. They went on to investigate the subsite specificity of papain and chymotrypsin using peptide libraries based on the above principal.
4.2.5 Fluorescence resonance energy transfer (FRET) screening

An alternative to the screening method detailed in the previous section is fluorescence resonance energy transfer (FRET) screening in which the peptide chain is flanked by a fluorescence donor and a fluorescence acceptor. In the uncleaved state the bead does not fluoresce because any fluorescence is quenched. However, when the peptide is cleaved the bead is able to fluoresce and can be identified. Rossé et al. used FRET for the on-bead screening of substrates for novel proteases using Lucifer yellow and dabsyl flanked PEGA\textsubscript{1900} supported peptides shown in figure 19.\textsuperscript{147} The peptides were synthesised by split and mix synthesis and the beads incubated with the protease. The fluorescent beads were subsequently picked out and sequenced to give the sequence specificity of the protease.

![Figure 19: FRET screening for protease sequence specificity](image)

FRET screening of a one bead, two compound library by Meldal et al. showed how this technology could be applied to inhibitor screening.\textsuperscript{148} One peptide containing the amino acid chain under study and one peptide containing a FRET amino acid chain were attached to each PEGA\textsubscript{1900} bead and the beads incubated with cruzipain. The peptides competed for the active sites of the enzyme and any darker beads picked from the incubation mixture. Darker beads indicated that the inhibitor rather than the FRET peptide chain was interacting with the enzyme.

4.2.6 Enzymatic resolutions on solid-phase

To our knowledge there are only two examples of solid-phase enzymatic resolutions. The first example is the enzyme catalysed resolution of 2,2-dimethyl-3-(2,2-disubstituted vinyl)-cyclopropane carboxylic acids by Nanda et al.\textsuperscript{149} and shown in scheme 78. Porcine pancreatic lipase (PPL) affected cleavage of the (R)-(+)‐acyl
group from polystyrene linked Tentagel, Merrifield and Wang resins. Higher yields were observed with Tentagel resins however, the yields quoted in the paper do not add up to 100 %. In one instance the authors quote an enantiomeric yield over 50 %. The (S)-(−)-acyl group was cleaved from the resin however no details of the cleavage method were given. Compounds containing two halogen groups provided higher rates of hydrolysis than the compound containing a gem-dimethyl group.

\[
\text{Scheme 78: PPL catalysed kinetic resolution of chiral acids}
\]

Within the Turner/Flitsch group there is considerable precedent for enzymatic reactions on solid-phase. Rein Ulijn et al.\textsuperscript{150} have shown that high levels of thermolysin catalysed amino acid couplings to PEGA supported phenylalanine can be achieved if high concentrations of amino acids are used. The reactions are performed in bulk aqueous media with no organic solvent. They propose that the hydrophobic acylating agent can be found at high concentration inside the resin bead therefore driving the reaction to completion.

Rein Ulijn and Nicola Bisek\textsuperscript{151} carried out the enzymatic resolution of Fmoc-amino acids using an acylation and hydrolysis strategy on solid-support shown in scheme 79. Thermolysin catalysed acylation of PEGA\textsubscript{1900} supported and Wang linked phenylalanine was achieved at low dilution in phosphate buffer to yield Fmoc-L-Phe-L-Phe and Fmoc-L-Nle-L-Phe on the resin. The resultant dipeptides were cleaved from the resin using TFA with d.e. of 99 % by chiral HPLC. In this case a ten fold excess of racemic amino acid was used to push the reaction to completion. If Fmoc-D-Phe or Nle were used negligible acylation was observed in either case.
The reverse hydrolysis reaction was performed at high dilution to enable diffusion of the product from the resin structure and push the equilibrium towards cleavage. In this way the complementary L,D-dipeptide was retained on the resin and Fmoc-L-amino acids with 99 % e.e. collected from the filtrate.

Scheme 79: Thermolysin catalysed acylation and hydrolysis on solid-phase

Rein Ulijn et al have also demonstrated the use of two-photon microscopy to observe dansyl loading on PEGA1900 beads. They observed a homogeneous distribution of dansyl groups on each bead and have since shown using this method in unpublished work that full resin bead penetration of thermolysin takes approximately one hour. The PEG chains may restrict enzyme diffusion into the interior of the bead. Considerably lower rates of reaction were found for a thermolysin catalysed reaction compared to an analogous solution-phase reaction. Recent published results by Rein Ulijn et al. showed that the yields of solid-phase enzymatic reactions may be improved by using charged PEGA supports. The yield of penicillin G amidase catalysed cleavage was improved from approximately 10 to 50 % using a positively charged PEGA matrix. The charged resin not only
showed improved swelling but showed rapid diffusion of the oppositely charged amidase molecules.

4.3 Factors to consider when performing enzyme catalysed reactions on solid phase

Rein Ulijn et al. discussed the three main factors they used to explain the shift in equilibrium when one substrate for an enzymatic reaction is immobilised. The results were obtained from studies on thermolysin catalysed solid-phase peptide synthesis shown in scheme 79 however, the factors discussed below should be generally applicable to other enzyme catalysed reactions.

4.3.1 The effect of excess reagent on equilibrium position

One of the main advantages of using solid-phase chemistry is that excess reagents can be washed away from the products at the end of the synthesis. This means that a large excess of reagents can be used to push the equilibrium of a solid-phase reaction towards synthesis. Yields of solid-phase peptide synthesis were much higher than their corresponding solution-phase counterparts despite the use of excess reagents in both cases, so this alone cannot explain the phenomenon.

4.3.2 The removal of hydrophobic groups from aqueous solution

The PEGA1900 microenvironment is more hydrophobic than the surrounding bulk aqueous solution. Peptide synthesis was higher yielding when more hydrophobic enzyme substrates were used, suggesting that the hydrophobic amino acids are present at higher concentration in the PEGA1900 microenvironment than in the bulk solution. The high concentration at the reaction site acts as a driving force for the reaction and pushes the equilibrium towards synthesis. Further work in this area includes the design of tailor made hydrophobic protecting groups which may increase the yield of amino acid coupling reactions. The substrate hydrophobicity was considered to be the most important factor contributing to the increase in yield.
4.3.3 The effect of suppressed amine ionisation on the equilibrium position

Protonation of the amino functionalised resin shown in scheme 79 may well be significantly suppressed compared to the amino group of a soluble phenylalanine derivative and therefore peptide hydrolysis inhibited. This may be due to reduced pH of the PEGA1900 microenvironment due to the high concentration of acid components. If this is the case there will be an equilibrium shift towards peptide synthesis and hence an increase in reaction yields. This should not pose a problem in buffered solution and should only be the case at low pH's.

4.4 Dimedone-1,3-enol esters as hydrolase substrates

4.4.1 Solution phase dimedone-1,3-enol esters as hydrolase substrates

We were unsure whether dimedone-1,3-enol esters would be substrates for hydrolases and initially set out to test this hypothesis. Racemic 3-phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R/S)-162 was synthesised in high yield by treatment of racemic 3-phenylbutyric acid (R/S)-160 with cyanuric fluoride\(^{155}\) to yield acid fluoride (R/S)-161. This was followed by direct coupling to dimedone 24 in DCM containing DIPEA (scheme 80) to yield enol ester (R/S)-162.

![Scheme 80: Synthesis of dimedone-1,3-enol ester (R/S)-162](image)

**Reagents and Conditions:** (i) (CFN)\(_3\), py, DCM, 0 °C to RT, 1h (93 %); (ii) dimedone 24, DIPEA, DCM, RT, 2h (90 %).

To establish whether the dimedone containing ester (R/S)-162 was a suitable substrate for hydrolase-catalyzed reactions, 17 different lipases and esterases were screened for activity (scheme 81). To our knowledge this is the first example of the use of dimedone-1,3-enol esters as substrates for hydrolase-catalysed reactions.
The percentage conversion, after overnight reaction, and the enantiomeric ratio ($E$) of the reaction (at lower than 50% conversion) were monitored using reverse (C18 column) and normal phase HPLC (chiral-ODH column) respectively (table 8 and figure 21). Hydrolases showed ($S$)-selectivity if selectivity was observed at all. High levels of conversion were observed with *Chromobacterium viscosum* lipase (CVL, table 8, entry 1), *Candida lipolytica* esterase (CLE, table 8, entry 10) and *Mucor miehei* esterase (MME, table 8, entry 12). Minimal hydrolysis of the substrate was observed in the absence of lipase. The enantiomeric ratios ($E$) observed were lower than those observed by Reeve *et al.* in the resolution of 3-phenyl butyric acid$^{127}$ (final column of table 8) but generally followed a similar trend.

![Reaction scheme](image)

**Reagents and Conditions:** Hydrolase, 10% MeCN/0.1M KP buffer (pH = 7.0), RT, 16h.

**Scheme 81:** Screen for hydrolase activity

![Graph](image)

**Figure 20:** Plot of e.e. of ($S$)-acid, e.e. of ($R$)-enol ester and % conversion for the CVL-catalysed hydrolysis of 1,3-enol ester ($R$/S)-162
<table>
<thead>
<tr>
<th>Entry</th>
<th>Hydrolase</th>
<th>Abb$^a$</th>
<th>% Conv</th>
<th>$E$</th>
<th>$E_{\text{ref}}^{127}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chromobacterium viscosum lipase</td>
<td>CVL</td>
<td>&gt;99</td>
<td>88</td>
<td>&gt; 153</td>
</tr>
<tr>
<td>2</td>
<td>Pseudomonas cepacia lipase</td>
<td>PCL</td>
<td>54</td>
<td>6.3</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Porcine pancreatic lipase</td>
<td>PPL</td>
<td>69</td>
<td>5.4</td>
<td>-</td>
</tr>
<tr>
<td>4</td>
<td>Candida antarctica B lipase</td>
<td>CAL-B</td>
<td>10</td>
<td>1.3</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Pseudomonas fluorescens lipase</td>
<td>PFL</td>
<td>51</td>
<td>8.1</td>
<td>&gt; 34</td>
</tr>
<tr>
<td>6</td>
<td>Rhizopus arrhizus lipase</td>
<td>RAL</td>
<td>60</td>
<td>12</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Candida lipolytica lipase</td>
<td>CLL</td>
<td>50</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Penicillium roqueforti lipase</td>
<td>PRL</td>
<td>8.7</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Mucor javanicus lipase</td>
<td>MJL</td>
<td>28</td>
<td>1.3</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Candida lipolytica esterase</td>
<td>CLE</td>
<td>&gt;99</td>
<td>1.2</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Acetylcholine esterase</td>
<td>AChE</td>
<td>12</td>
<td>1.4</td>
<td>-</td>
</tr>
<tr>
<td>12</td>
<td>Mucor miehei esterase</td>
<td>MME</td>
<td>&gt;99</td>
<td>4.1</td>
<td>-</td>
</tr>
<tr>
<td>13</td>
<td>Bacillus species esterase</td>
<td>BSpE</td>
<td>18</td>
<td>1.8</td>
<td>-</td>
</tr>
<tr>
<td>14</td>
<td>Thermoanaerobium brockii esterase</td>
<td>TBE</td>
<td>10</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>15</td>
<td>Saccharomyces cerevisiae esterase</td>
<td>SCE</td>
<td>7.6</td>
<td>1.1</td>
<td>-</td>
</tr>
<tr>
<td>16</td>
<td>Bacillus sterothromphillus esterase</td>
<td>BSE</td>
<td>5.6</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>17</td>
<td>Bacillus thermoglycosidasius esterase</td>
<td>BTE</td>
<td>8.4</td>
<td>1.0</td>
<td>-</td>
</tr>
<tr>
<td>18</td>
<td>Control (no lipase)</td>
<td>-</td>
<td>3.1</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 8: Hydrolase catalysed hydrolysis of dimedone-1,3-enol ester ($R$/$S$)-162

![Graphical representation of % conversion and $E$ for hydrolase screening](image)

**Figure 21:** Graphical representation of % conversion and $E$ for hydrolase screening
The highest selectivity ($E = 88$) was observed with CVL which showed complete hydrolysis of the (S)-enol ester 162 after 2 hours. The time course of CVL-catalysed hydrolysis of dimedone-1,3-enol ester ($R/S$)-162 is shown in figure 20. (S)-acid 160 remains at high e.e. for the first couple of hours after which point hydrolysis of (R)-enol ester 162 occurs decreasing the e.e. slightly. The e.e. of residual (R)-enol ester 162 increases over the time course of the reaction to $> 99$ % e.e. as (S)-enol ester 162 is consumed.

In order to assess the relative activity of dimedone esters, the corresponding methyl ($R/S$)-163 and vinyl esters ($R/S$)-164 were prepared (scheme 82) according to procedures developed by Hirose et al. Refluxing 3-phenylbutyric acid ($R/S$)-160 in methanol containing concentrated sulfuric acid provided methyl 3-phenylbutanoate ($R/S$)-163 in quantitative yield. The reaction was repeated with (R)-3-phenylbutyric acid (R)-160 to form methyl 3-phenylbutanoate (R)-163.

Reaction of 3-phenylbutyric acid ($R/S$)-160 with mercuric acetate in vinyl acetate followed by heating at reflux with a catalytic amount of concentrated sulfuric acid yielded vinyl-phenylbutanoate ($R/S$)-164 in 64 % yield. Interestingly when the latter reaction was repeated with separate enantiomers of 3-phenylbutyric acid ($R/S$)-160, the mixtures were heated in the microwave at 145 °C (200 W) rather than in an oil bath. The reaction times were shortened to just five minutes and although the yields were slightly less (57 % and 48 % for R and S enantiomers respectively) the reactions were considerably simplified.

![Scheme 82: Synthesis of methyl ($R/S$)-163 and vinyl esters ($R/S$)-164](image)

**Reagents and Conditions:** (i) $c$.H$_2$SO$_4$, MeOH, reflux, 2 ½h ($> 99$ %); (ii) Hg(OAc)$_2$, vinyl acetate, RT, 30 mins; (iii) c.H$_2$SO$_4$, reflux, 4h (64 %).

**Scheme 82:** Synthesis of methyl ($R/S$)-163 and vinyl esters ($R/S$)-164
Under identical conditions to those used for dimedone-1,3-enol esters, (S)-163 and (S)-164 were shown to undergo complete conversion using CVL after 24 hours and 15 minutes respectively. This revealed that 1,3-enol esters possess reactivity less than their corresponding vinyl esters but more than simple alkyl esters.

In summary we have successfully demonstrated that dimedone-1,3-enol ester (R/S)-162 is a substrate for CVL-catalysed reactions displaying high enantioselectivity.

4.4.2 Synthesis of polymer supported CHD on PEGA_{1900} resin

In order to allow complete compatibility with enzymes cyclohexanedicone resin 167 was prepared on PEGA_{1900} support according to scheme 83. Amino substituted PEGA_{1900} resin 165 was coupled with 1-ethyl-3,5-dimethoxycyclohexa-2,5-diene-carboxylic acid 36 using previously established coupling conditions. Bis-enol ether 166 was hydrolysed to CHD resin 167 using a TFA/H_{2}O/DMF (90:5:5) mixture at room temperature. The MAS-probe NMR and FT-IR data for this resin was not conclusive but by analogy to previous work the resin was considered satisfactory for further work.

Reagents and Conditions: (i) Acid 36, DIC, DCM/DMF (1:1), RT, 2 ½ h x 2; (ii) TFA/H_{2}O/DMF (90:5:5), RT, 2 ½ h.

Scheme 83: Synthesis of PEGA_{1900} CHD resin 167

The resin loading of amino substituted PEGA_{1900} resin 165 was quoted at 0.2 mmol/g. The structure of PEGA resin is quite fragile and will crack if dried. For this reason the resin was stored and handled under methanol and not dried in the vacuum oven.

4.4.3 Hydrolysis of solid supported 1,3-enol esters

The synthesis of racemic solid-supported 1,3-enol ester (R/S)-169 is shown in scheme 84. Racemic 3-phenylbutyryl chloride (R/S)-168 was synthesised in reasonable yield after distillation from the reaction of 3-phenylbutyric acid (R/S)-160
with oxalyl chloride in DCM containing a catalytic amount of DMF. Acid chloride (R/S)-168 was then reacted directly with PEGA_{1900} supported CHD (R/S)-167 at room temperature in DCM to yield racemic solid-supported 1,3-enol ester (R/S)-167. An enol carbonyl peak in the FT-IR indicated that the coupling had occurred. Attempted coupling using 3-phenylbutyryl fluoride (R/S)-161 showed no apparent enol ester peaks in the FT-IR.

Reagents and Conditions: (i) (COCl)$_2$, DCM, DMF (cat), 0 °C, 3h (79 %) (ii) Acid chloride (R/S)-168, DCM, RT; (iii) 0.1 M NaOH, RT, 1h (standard HPLC peak area, 100 %); (iv) CVL, 10 % MeCN/0.1M KP$_4$ buffer (pH = 7.0), RT, 1h (2 %).

Scheme 84: Synthesis of resin bound 1,3-enol ester (R/S)-169, subsequent standard release and attempted CVL-catalysed hydrolysis

Racemic solid-supported 1,3-enol ester (R/S)-169 was subjected to standard release using sodium hydroxide at room temperature. The amount of racemic 3-phenylbutyric acid (R/S)-160 released from the resin was used to give a standard peak area by HPLC for subsequent lipase catalysed reactions.
Attempted CVL-catalysed hydrolysis of racemic 1,3-enol ester (R/S)-169 in a 10% acetonitrile/buffer mixture at room temperature for one hour resulted in low (2%) cleavage of (S)-3-phenylbutyric acid 160 with an e.e. of 51%. Longer reaction times resulted in no significant increase in yield. The 2% yield observed implies that the lipase is only interacting with the outer surface area of the bead and is unable to reach the inner sites on the resin.

Literature precedence indicates that the rate, and therefore yields, of enzyme catalysed reactions can be improved by subjecting the enzymatic mixture to microwave irradiation. Parker et al. found that by irradiating a hydrated lipase suspended in organic media with microwaves at 50 °C, the reaction rate was enhanced by two to three fold over classical heating. Due to our past success with improving yields of solid-phase reactions using microwave irradiation, we decided to attempt microwave heating of the CVL-resin mixture but no increase in reaction yield or e.e. was observed.

Previous studies by Rein Ulijn et al. showed that the yields of thermolysin catalysed hydrolysis can be improved by performing the reaction under high dilution. In this way the unfavourable equilibrium can be shifted towards resin cleavage. Time restraints have meant that the CVL-catalysed hydrolysis of 1,3-enol ester (R/S)-169 at high dilution has not been investigated. We decided at this point to investigate the more favourable corresponding acylation of CHD resin 167 using an excess of acylating agent to drive the reaction to completion.

4.4.4 CVL-catalysed transesterification on solid-phase

PEGA_{1900} supported CHD 167 was subjected to CVL catalyzed acylation using (+)-methyl 3-phenylbutanoate (R/S)-163 and (+)-vinyl 3-phenylbutanoate (R/S)-164 (scheme 85) to yield resin-bound 1,3-enol ester 169. Ten equivalents of acylating agent were used to ensure high conversions and the reaction performed in aqueous potassium phosphate buffer at neutral pH in the absence of organic solvent. The hydrophobic acylating agent was therefore present at reactions sites on the PEGA_{1900} beads in high concentration. After overnight reaction, the resin was extensively washed, followed by acyl group release from the resin using sodium hydroxide to give 3-phenylbutyric acid 160. Acid 160 was analyzed by HPLC (Chiracel-ODH
column) to determine the yield and enantiomeric excess (e.e.) of the reaction. Reaction yields were calculated by comparison of HPLC peak areas with standard sodium hydroxide release of racemic 1,3-enol ester (R/S)-169 (scheme 84). The results are shown in table 9 and reveal an unusual reversal of specificity. In each case the predominant enantiomer observed was the (R)-acid 160 rather than the expected (S)-acid despite the known preference for CVL to catalyze hydrolysis of the (S)-enantiomer in solution.

![Diagram of chemical reaction]

Reagents and Conditions: (i) Ester, hydrolase, 0.1M KP$_i$ buffer (pH = 7.0), RT, 16h; (ii) 0.1M NaOH, RT, 1h.

Scheme 85: On resin ‘capture and release’ of 3-phenylbutyric acid

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ester</th>
<th>Lipase</th>
<th>Product</th>
<th>Yield</th>
<th>e.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(R/S)-163</td>
<td>CVL</td>
<td>(R)-160</td>
<td>74</td>
<td>65</td>
</tr>
<tr>
<td>2</td>
<td>(R/S)-164</td>
<td>CVL</td>
<td>(R)-160</td>
<td>38</td>
<td>&gt;99</td>
</tr>
<tr>
<td>3</td>
<td>(R/S)-164</td>
<td>PCL</td>
<td>(R)-160</td>
<td>77</td>
<td>59</td>
</tr>
<tr>
<td>4</td>
<td>(R/S)-164</td>
<td>PPL</td>
<td>(R)-160</td>
<td>83</td>
<td>62</td>
</tr>
<tr>
<td>5</td>
<td>(R/S)-164</td>
<td>CVL$^a$</td>
<td>(R)-160</td>
<td>78</td>
<td>&gt;99</td>
</tr>
<tr>
<td>6</td>
<td>(R/S)-164</td>
<td>CVL$^b$</td>
<td>(R)-160</td>
<td>86</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

$^a$double acylation, $^b$triple acylation

Table 9: Lipase catalyzed resolution by ‘capture and release’

Use of the methyl ester (R/S)-163 (table 9, entry 1) resulted in a reasonable yield of (R)-acid 160 with modest e.e. whereas the more reactive vinyl ester (R/S)-164 (table 9, entry 2) gave a lower yield but excellent e.e. Concerns that the reversal in
selectivity may be due to contaminant esterases present in the commercially available CVL lead us to perform the same reaction using alternative hydrolases. The same reversal of enantioselectivity was obtained with *Pseudomonas cepacia* lipase (PCL) and porcine pancreatic lipase (PPL), both of which showed (S)-selectivity in the HPLC screen of dimedone-1,3-enol ester (R/S)-162. Subjecting the resin to a second acylation reaction after washing, resulted in an increased yield (78 %) (table 9, entry 5) and was further enhanced to 86 % with a triple acylation (table 9, entry 6). After one round of capture and release the resin capture and release of 3-phenylbutyric acid was repeated to recycle the resin. A reduced yield of (R)-acid 160 of 18 % and an e.e. of 87 % were obtained. The resin was not amenable to recycling but given further optimisation these results may be improved.

4.4.5 *Investigations into the reversal of enantioselectivity*

In order to probe this unusual reversal of selectivity, the acylation reactions were repeated using enantiomerically enriched acyl donors (synthesised according to scheme 82). When (R)-vinyl ester 164 (e.e. = 96 %) was used, the (R)-acid 160 was obtained in high yield and e.e. (table 10, entry 1). However, switching to the enantiomerically enriched (S)-vinyl ester 164 (e.e. = 92 %) also gave the (R)-acid 160 in low yield but high e.e. (table 10, entry 2). The lipase selectively catalyses acylation with the minor contaminant (R)-enantiomer in the presence of excess (S)-enantiomer.

<table>
<thead>
<tr>
<th>Entry</th>
<th>Ester</th>
<th>Lipase</th>
<th>Product</th>
<th>Yield</th>
<th>e.e.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>(R)-164</td>
<td>CVL</td>
<td>(R)-160</td>
<td>92</td>
<td>&gt;99</td>
</tr>
<tr>
<td>2</td>
<td>(S)-164</td>
<td>CVL</td>
<td>(R)-160</td>
<td>7</td>
<td>&gt;99</td>
</tr>
</tbody>
</table>

Table 10: Lipase catalyzed resolution by ‘capture and release’ with enantiomerically enriched acylating agents

The reaction in the absence of lipase showed negligible levels of 3-phenylbutyric acid 160 by HPLC, eliminating the possibility of background reaction between CHD resin 167 and vinyl ester 164. Similarly, the corresponding solution-phase reaction between dimedone 24 and either vinyl ester (R/S)-164 or 3-phenylbutyric acid (R/S)-160 showed no evidence of conversion.
Previous studies in our laboratories have shown that diffusion of enzymes into PEGA$_{1900}$ resin occurs over a period of approximately one hour. When CVL was allowed to pre-equilibrate with the resin for one hour before addition of vinyl ester (R/S)-164, the e.e. of the liberated (R)-acid 160 decreased to 90 %. Under these conditions the (S)-vinyl ester 164 presumably undergoes a small degree of transesterification at the reaction site, prior to hydrolysis. Longer pre-equilibration times did not lead to further reductions in e.e. By contrast, when methyl ester 163 was pre-reacted with CVL for one hour, to allow for (S)-methyl ester hydrolysis before addition of the resin, the e.e. of the reaction increased to > 99 %.

![Figure 22: Solid-phase time course for transesterification of CHD resin 167 with methyl ester (R/S)-163.](image)

The time course of acylation with methyl ester (R/S)-163 was monitored using four samples of CHID resin 167 rotated at room temperature for 1, 2, 4 and 8 hours respectively. The time course experiment was performed with methyl ester 163 rather than vinyl ester 164 because the (S)-enantiomer of the latter is too rapidly hydrolysed to observe even low level (S)-acylation. A gradual increase in e.e. of (R)-acid 160 release was observed after sodium hydroxide cleavage (figure 22). Transesterification was complete after the eight hour period. The lower e.e. observed after one or two hours is indicative of acylation by (S)-methyl ester 163 before subsequent solution-phase CVL hydrolysis to the unreactive (S)-acid 160.
4.4.6 **Explanation of the observed reversal of selectivity**

Parallel kinetic resolution is a technique that has been utilised to overcome one of the fundamental limitations of kinetic resolutions.\(^{158}\) A decrease in e.e. is often observed at conversions close to 50 % due to the increase in relative concentration, and therefore the relative reaction rate, of the less reactive enantiomer in a kinetic resolution. To overcome this problem the slower reacting enantiomer can be removed from the reaction mixture by a parallel reaction thus maintaining the 1:1 ratio of enantiomers. The enantiomer with slow reactivity in the kinetic resolution should ideally be more reactive towards the parallel reaction. The competing reactions should ideally occur at a similar rate therefore delivering two products with substantially improved e.e. closer to the theoretical 50 % yield.

The concept of parallel kinetic resolution is clearly demonstrated in an example by Rakels *et al.*\(^{159}\) and shown in scheme 86. Methyl-2-chloropropionate \(^{170}\) underwent simultaneous hydrolysis and aminolysis in a *Candida cylindracea* lipase-catalysed parallel kinetic resolution in the presence of \(n\)-butylamine in buffer saturated solvents.

\[\text{MeO} \quad \text{Cl} \quad \text{Me} \]
\[\text{(R)-170} \quad \text{lipase} \quad \text{HO} \quad \text{Me} \quad \text{Cl} \]
\[\text{HYDROLYSIS} \quad \text{(R)-171} \quad \text{MeO} \quad \text{Cl} \quad \text{Me} \]
\[\text{AMINOLYSIS} \quad \text{(S)-172} \quad \text{MeO} \quad \text{Cl} \quad \text{Me} \]

**Scheme 86:** Parallel kinetic resolution of methyl-2-chloropropionate

(R)-Methyl-2-chloropropionate \(^{170}\) underwent lipase-catalysed hydrolysis to form (R)-2-chloropropionic acid \(^{171}\) whereas (S)-methyl-2-chloropropionate \(^{170}\) underwent parallel aminolysis to form (S)-2-chloropropionamide \(^{172}\). The selectivity observed by Rakels *et al.* was consistent with the result of *Candida cylindracea* lipase-catalysed transesterification of 2-chloropropionic acid \(^{171}\) by Klibanov *et al.*\(^ {160}\) in an early example of the technique (scheme 87a). However, when *Candida cylindracea* lipase-catalysed aminolysis of methyl-2-chloropropionate
170 was performed by Gotor et al.\textsuperscript{161} a reversal of selectivity was observed (scheme 87b). There is no comment in the paper concerning the parallel hydrolysis of (R)-methyl-2-chloropropionate 170 to unreactive (R)-2-chloropropionic acid 171. The reaction was performed in anhydrous organic solvents but large excesses of lipase were used thus providing a small quantity of water in the form of water of crystallisation. No attempt to isolate reaction by-products was mentioned in the paper.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{scheme_87.png}
\caption{(a) Transesterification of 2-chloropropionic acid 171 and (b) aminolysis of methyl-2-chloropropionate 170 showing a reversal of selectivity}
\end{figure}

The reversal of specificity we observed can be rationalized by considering the competing enzymatic hydrolysis of vinyl ester acylating agent in an enzyme-catalyzed parallel resolution analogous to that reported by Rakels et al.\textsuperscript{159} (scheme 88). (S)-Vinyl ester 164 undergoes rapid formation of an acyl-enzyme intermediate with the lipase followed by rapid hydrolysis to form (S)-acid 160. Solution phase hydrolysis of vinyl ester 164 revealed that the (S)-enantiomer is completely hydrolyzed in less than 15 minutes under these conditions. The corresponding hydrolysis of the (R)-enantiomer is much slower than that of the (S)-enantiomer allowing the enzyme intermediate to undergo transesterification to form the resin bound (R)-enol ester 169. The difference in observed e.e. for transesterification with methyl versus vinyl ester (table 9, entries 1 and 2) can be explained by considering the faster rate of hydrolysis of vinyl versus methyl ester.
An alternative scenario involves both (R)- and (S)-acylation followed by rapid (S)-hydrolysis from the resin. This mechanism cannot be ruled out completely but seems unlikely in light of the low levels of CVL-catalysed hydrolysis of racemic enol ester \((R/S)-169\), observed in section 4.4.3.

![Scheme 88: Explanation of reversed enantiospecificity](image)

### 4.4.7 Alternative nucleophiles for resin release

When ammonia was used for release of resin bound 1,3-enol ester 169 instead of sodium hydroxide, (R)-3-phenylbutyramide 175 was released from the resin in 32% yield with e.e. > 99% (scheme 89). The yield, calculated by standard release of racemic 1,3-enol ester 169 using ammonia in methanol, was comparable to that observed for the release of 3-phenylbutyric acid under analogous conditions. Reaction between vinyl ester 164 or 3-phenylbutyric acid 160 and ammonia does not occur at room temperature under these reaction conditions thus proving that CVL-catalysed acylation of CHD resin 167 was occurring.
Reagents and Conditions: (i) vinyl ester (R/S)-164, CVL, 0.1M KP, buffer (pH = 7.0), RT, 16h; (ii) 7N NH₃ in MeOH, RT, 1h.

**Scheme 89:** Resin release of primary amide (R)-175 using ammonia in methanol

HPLC standard (R/S)-3-phenylbutyramide 175 was prepared in 75 % yield by nucleophilic displacement of the 3-phenylbutyryl acyl group from (R/S)-enol ester 162 as shown in scheme 90a. The corresponding (R)-3-phenylbutyramide 175 standard was prepared in quantitative yield by the reaction of (R)-3-phenylbutyric acid 160 with isobutylchloroformate to form the mixed anhydride, followed by reaction with ammonia gas at 0 °C for 10 minutes (scheme 90b) in a method developed by Fort et al.¹⁶²

**Scheme 90:** Synthesis of primary amide standards (R/S)-175 and (R)-175

Reagents and Conditions: (i) 7N NH₃ in MeOH, MeOH, 0 °C, 3h (75 %); (ii) Et₃N, 'BuOCl, 0 °C, 15 mins; (iii) NH₃(9), 0 °C, 10 mins (> 99 %).
The solution phase dimedone-1,3-enol ester 162 studied to this point contained a gem-dimethyl substituent in the C5-position. When considering enol esters attached to CHD resin however, the C5 position is a chiral centre and diastereoisomers pairs of compounds are formed. The chiral centre at C5 may affect the reactivity of such systems so, in order to study this hypothesis C5-modified solution phase analogues (5R, 3S)-178 and (5R, 3S)-178 were synthesised (scheme 91).

**Reagents and Conditions:** (i) PhCH₂NH₂, TBTU, HOBT.H₂O, Et₃N, DMF, RT, 1 1/2h (78 %); (ii) 12M HCl, THF, RT, 2h (95 %); (iii) (S)-acid fluoride 161, DIPEA, DCM, RT, 1h (61 %); (iv) CVL, 10 % MeCN/0.1M KP₀ buffer (pH = 7.0), RT, monitor time course.

**Scheme 91:** Synthesis of C5 modified analogue 178 and subsequent CVL-catalysed time course hydrolysis

Birch acid 36 was coupled to benzylamine using TBTU uronium reagent and HOBT in DMF containing triethylamine to yield bis-enol ether 176 in 78 % yield. Bis-enol
ether 176 was subjected to acid hydrolysis in concentrated hydrochloric acid to give diketone 177 in quantitative yield. Diketone 177 was then coupled to (S)-3-phenylbutyryl fluoride 161 in DCM containing DIPEA to yield a mixture of C5-modified solution phase analogues (5R, 3S)-178 and (5S, 3S)-178 after column chromatography.

The diastereoisomeric mixture of C5 modified analogue 178 was easily separated by column chromatography on a chiral column with long retention times of 56 and 71 minutes. The mixture was found to be completely racemic at C5 with a negligible diastereoisomeric ratio (d.e.).

The time course of CVL catalysed hydrolysis was monitored over 5 hours but no appreciable difference in hydrolysis rate for the different enantiomers was observed thus establishing that the chiral centre at C5 plays no part in the rate of enzymatic reaction at C3.

4.5 Summary and further work

In summary we have shown that dimedone-1,3-enol esters are substrates for hydrolase-catalysed reactions with reactivity between the corresponding methyl and vinyl esters. Dimedone-1,3-enol esters were synthesised in two steps from the acid and dimedone 24 via the acid fluoride. CVL was identified as having high conversion and enantioselectivity for 3-phenylbutyric enol ester of dimedone (R/S)-162 and was used to affect transesterification of the 3-phenylbutyric acyl group on to CHD PEGA1900 supported resin 167. An unusual reversal of specificity was observed. In each case the predominant enantiomer observed was (R)-(−)-3-phenylbutyric acid (R)-160 (e.e. > 99 %) rather than the expected (S)-acid 160, despite the fact that CVL showed exclusive selectivity for the (S)-enantiomer in the corresponding solution-phase hydrolysis reactions.

Further work in this area includes optimisation of the release of the chiral acyl group from the resin by ammonia. The yield for this step was quite low possibly due to the use of methanol as solvent which does not swell the resin to its maximum amount. Investigations into nucleophilic cleavage from the resin include the use of methoxide to provide esters or amines to provide secondary and tertiary amides (scheme 92).
Scheme 92: Potential nucleophilic cleavage from the resin by alternative nucleophiles to yield esters, secondary amides and tertiary amides.

A further interesting investigation would be to find an (R)-selective hydrolase which would yield (S)-acyl groups under similar resin conditions.
5. Results and Discussion – Novel screening methodologies

In the previous chapter we demonstrated that dimedone-1,3-enol esters are substrates for hydrolase catalysed reactions. We proposed using dimedone-1,3-enol esters for the screening (ideally colorimetric) of hydrolase activity by detecting dimedone 24 formed upon hydrolysis (scheme 93). The advantage of using dimedone-1,3-enol esters over more reactive p-nitrophenol and vinyl esters, is that they are more stable and therefore less likely to give false positive results in screening. What follows is a review of techniques currently in use for the high-throughput screening for enzyme, and in particular hydrolase, activity.

Scheme 93: Proposed screening for hydrolase activity by dimedone detection

5.1 Review of high-throughput screening methodologies for enzyme activity

The development of chiral catalysts for the enantioselective synthesis of chiral compounds is of great academic interest. Currently efficient enantioselective catalysts include transition metal complexes such as rhodium-DIPAMP complexes to catalyse asymmetric hydrogenations, used in the large scale synthesis of L-DOPA. An alternative to chemocatalysis is biocatalysis, but nature imposes a problem of limited substrate specificity. Site-directed mutagenesis has been used to improve enzyme activity and selectivity but this method cannot be used in all cases as it requires detailed knowledge of the 3D-structure of the enzyme and its catalytic
mechanism. In vitro evolution has been extensively used to improve activity and selectivity of a wide number of biocatalysts.

5.1.1 UV/Vis-based systems

Reetz et al. showed how p-nitrophenol esters can be used to screen for lipase activity with a library of *Pseudomonas aeruginosa* lipase mutants.\textsuperscript{166,167} The appearance of yellow coloured p-nitrophenoxide can be used to monitor the test reaction shown in scheme 94 by measuring the absorbance at 401 nm as a function of time. The mature form of *Pseudomonas aeruginosa* lipase catalyses the hydrolysis of the test substrate, however with a marginal degree of enantioselectivity ($E = 1$). Using the error prone polymerase chain reaction (epPCR) the lipase gene was subjected to random mutagenesis (with a mutation frequency of one or two amino acid changes per lipase molecule) to produce a library of 5414 members. After four rounds of mutagenesis the enantioselectivity was impressively improved from $E = 1$ to 11.3.

![Scheme 94: p-Nitrophenoxide screening for lipase activity](image)

The p-nitrophenol screening method has been used to identify ‘hits’ by monitoring the rates of $(R)$- and $(S)$-substrates screened independently. The ratio of the initial rates of hydrolysis of each enantiomer does not give the enantiomeric ratio because it ignores the substrate competition for the enzyme active site. The enantioselectivity of an enzyme is the ratio of the specificity constants, $k_{cat}/K_M$ for the enantiomers. The Michaelis-Menton constant, $K_M$ is (roughly) an inverse measure of the affinity or strength of binding between the enzyme and substrate. The lower the $K_M$ the greater the affinity of the substrate for the enzyme and the lower the concentration of substrate required to achieve a given rate. Janes and Kazlauskas found that when $(R)$- and $(S)$-enantiomers were treated separately differences in $K_M$ values over and underestimated $E$ by as much as 70 %. They developed the Quick-$E$ method to

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simulate the competitive conditions of enzymatic processes,\textsuperscript{168} the principal of which is shown in scheme 95. To reintroduce competition they introduced resorufin tetradecanoate as a reference compound and measured the initial rates of hydrolysis of the mixture. The ratio of these rates gave the selectivity of the hydrolase for the (S)-compound shown. Repeating this procedure for the (R)-substrate gave the selectivity for the other enantiomer thus allowing the calculation of the overall enantioselectivity of the reaction. The major disadvantage of this method is that pure enantiomers of the substrate are required.

\begin{center}
\includegraphics[width=\textwidth]{scheme.png}
\end{center}

\textbf{Scheme 95:} The Quick-E method for accurate $E$ calculation

One major disadvantage of using $p$-nitrophenol esters is their artificial reactivity which can lead to the identification of false positives in screening methods using $p$-nitrophenoxide chromophores. An alternative developed by Kalauskas \textit{et al.}\textsuperscript{169} used a $p$-nitrophenol indicator in buffer to measure the change in acidity upon hydrolysis of less reactive alkyl esters. Rapid-screening of hydrolases using a bromothymol blue indicator was demonstrated by Bornscheuer \textit{et al.}\textsuperscript{170} A blue to yellow indicator colour change was used to identify active enzymes in preliminary screening. Bornscheuer \textit{et al.} monitored the production of acetic acid (formed upon hydrolysis of acetates) using a cascade of enzymatic reactions to form NADH which was monitored at 340 nm.\textsuperscript{171}

\subsection{Fluorescent based systems}

Fluorescent based systems are much more sensitive that UV/Vis-based systems and therefore can be used to detect very low concentrations. A versatile periodate coupled fluorogenic assay for screening hydrolytic enzymes developed by Reymond \textit{et al.} is detailed in scheme 96.\textsuperscript{172} Compounds 179 (where X = O, NH), formed upon
enzymatic cleavage of the corresponding esters, amides, epoxides and bisphosphates, were oxidised to aldehyde 180 using periodate which in turn underwent β-elimination with bovine serum albumin (BSA) to form fluorescent umbelliferone 181. The time dependent increase in fluorescence was monitored for each of the enzymatic reactions.

Scheme 96: Umbelliferone-based screening methodology

Assaying microbial enzymes in crude cultures is greatly simplified if the use of a secondary oxidant is avoided. For this purpose, cyanohydrin esters 182 (scheme 97a) were developed which do not require the use of periodate for umbelliferone 181 formation.173 Cyanohydrin 183 formed upon hydrolysis spontaneously eliminates to form aldehyde 180 and hence umbelliferone 181 upon BSA catalysed β-elimination. The use of any secondary reaction was avoided by screening with acyloxy methyl esters 184, the products of which spontaneously release umbelliferone upon rearrangement of hydroxymethyl ether 185 (scheme 97b).174 Low level artificial reactivity was observed with both of these substrates due to their increased reactivity.
Copeland and Miller have demonstrated the use of acetic acid sensor 186 in acyl transfer reactions, with applications in biocatalysis screening (scheme 98). An extension of their work in which they attached fluorophore 186 to polystyrene beads alongside the catalyst under study, was used for real-time screening of one-bead/one compound combinatorial libraries. The beads were exposed to the reaction conditions and the relative fluorescence measured by fluorescence microscopy. The rates of reaction determined by this method were found to correlate with those determined by standard techniques.

High-throughput determination of hydrolase activity in transesterification reactions in organic solvent were monitored by reaction of acetaldehyde by-product with 4-hydrazino-7-nitro-2,1,3-benzoxadiazole 187 (NBD-H) (scheme 99). The hydrazone formed was highly fluorescent and was detected even at very low concentrations. By determining the standard curve for hydrazone concentration versus the relative fluorescence, the percentage conversion for a range of enzymes was determined. No e.e. evaluation was undertaken and there is no comment in the paper on the time taken for the fluorescent tag to react with acetaldehyde. NBD-H is
an expensive reagent, and although used in very small quantities in this example, would not be the reagent of choice for screening of larger numbers of enzymes.

Scheme 99: Detection of acetaldehyde by reaction with NBD-H 187

Green fluorescent chemosensor, calcein 188 was used by Reynmond et al. for the detection of amino acids formed upon protease cleavage of peptides (scheme 100). When chelated to copper, calcein 188 was non-fluorescent. When displaced from the metal centre by amino acids calcein 188 was highly fluorescent in solution. Monitoring the formation of green fluorescent colour gave a quantitative analysis of protease activity.

Scheme 100: Amino acid assay using copper-ligand complexes

5.1.3 IR-Thermographic assays

Reetz et al. introduced the use of IR-thermographic detection for the screening of enantioselective catalysts in 1998. They monitored the heat output of lipase-catalysed esterification of 1-phenylethanol with vinyl acetate using an infrared camera. By treating individual enantiomers in different wells of the microtitre plate (MTP) and observing heat output they showed the lipase preference for (R) over (S)-1-phenylethanol. This technique was also used in conjunction with heterogeneous mixtures containing solid-supported enzymes.
5.1.4 Circular dichroism assays
Separation of enantiomers is possible using HPLC on chiral columns but the process is lengthy and the columns expensive. One alternative to this method is the reverse phase separation of chiral starting materials and products followed by the determination of e.e. of these enantiomeric mixtures by circular dichroism.\(^{179}\) The absorbance, and hence the concentration of the solution was measured by UV.

5.1.5 Mass-spectrometric assays
The detection of pseudoenantiomers by electrospray ionisation mass spectrometry (ESI-MS) was demonstrated by Reetz et al.\(^{80}\) The advantage of this rapid technique is that chromatographic separation of pseudoenantiomers or products was not required. Pseudoenantiomers 189 and 190, differing in mass by three mass units, were subjected to lipase-catalysed hydrolysis and the products of the reaction detected by ESI-MS (scheme 101). The ratio $[M]^-/[M+3]^+$ gave an estimate of the e.e. value which closely matched the e.e. determined by gas chromatography (GC).

\[
\begin{align*}
\text{Ph} & \text{Me} + \text{Ph} & \text{Me} \\
\text{OAc} & \text{OAc[D}_3\text{]} \\
189 & M = 164 & 190 & M+3 = 167 \\
\text{Enzymatic hydrolysis} & \text{OH} & \text{AcOH} & \text{Ac[D}_3\text{]OH} \\
\text{M' = 60} & \text{M'+3 = 63}
\end{align*}
\]

Scheme 101: ESI-MS screening

The drawbacks of this technique are that isotopically labelled substrates must be prepared and the results may be tainted by secondary isotope effects.

5.1.6 Capillary array electrophoresis assays
Capillary array electrophoresis (CAE) has enabled the super-high throughput screening of enantioselective catalysts.\(^{181}\) Six bundles of 16 capillaries were used to analyse compounds from a 96 well-MTP. If the electrolyte used contained chiral selectors such as cyclodextrin derivatives then e.e. determination was possible. Enantiomeric separation of chiral compounds on glass microchips using CE assays has also been utilised.
5.1.7 The competitive cat-ELISA assay

The enzyme-linked immunosorbent assay (ELISA) can be used to obtain a single point measurement of product formation in high-throughput format. The ideal setup for monitoring reaction kinetics involves continuous measurement of product increase. The competitive cat-ELISA assay (developed by Reymond et al.)\textsuperscript{82} was used to measure product formation in test reactions releasing hapten. At the beginning of the reaction the antibody sensor contains products with quenched fluorescent tags (figure 23). Competitive displacement from the antibody by product molecules from the enzymatic reaction, releases the fluorescent tagged molecules into solution, where the fluorescence can be measured. The advantage of this assay is that the substrates are non-activated and therefore give less false positives. The disadvantage is that the sensor equilibration is rate limiting at high catalyst or substrate concentration.

![Diagram](image)

**Figure 23:** Principal of the competitive cat-ELISA assay

5.2 Development of a colorimetric assay based on dimedone

A well known colorimetric test for enols is the formation of green/blue complexes with copper II acetate.\textsuperscript{98} On addition of copper II acetate the enol-form of dimedone complexed with copper to give a bright green colour in DMSO solution. The proposed structure of this adduct is shown in figure 24. The structure is a Jahn-Teller distorted $d^7$ octahedral complex with longer axial bonds to complexed DMSO and two bidentate dimedone ligands. Complex 191 showed $\lambda_{\text{max}}$ of 417 nm with $\varepsilon = 84 \text{ M}^{-1} \text{ cm}^{-1}$ corresponding to a d-d transition. Spectroscopy in the near infrared showed no other distinct peaks compared to a copper II acetate blank in DMSO.
Both the copper II dimedone and copper II acetate complexes showed a large absorbance at approximately 720 nm in the red colour region. The copper II dimedone complex was green because it absorbed a larger amount of blue radiation (~ 420 nm) than the copper II acetate complex. $^1$H-NMR of dimedone in $d_6$-DMSO/$D_2$O (3:1) showed 100 % of the enol form reflecting the highly enolisable dimedone structure. Copper II dimedone complexes are reported to be unstable due to the fixed geometry of dimedone compared to non-cyclic enols. We propose that the copper II dimedone complex 191 was stabilised in solution by axial DMSO ligands.

![Diagram](image)

**Figure 24: Proposed structure of green copper II dimedone complex in DMSO**

Alternatives to copper based colorimetric testing include the reaction of dimedone with iron III chloride solution to form a dark brown colour. Initial experiments showed that iron III chloride was not compatible with lipase catalysed hydrolysis and seemed to inhibit the required reaction. Secondary reaction of dimedone with chromophore or fluorophore containing hydrazines and aldehydes was deemed too slow to be of use for screening. The reaction between dimedone and copper was chosen for further studies because the formation of coloured product was instantaneous and did not rely upon a secondary reaction with a chromophore containing reagent. This is in contrast to well established screening methodologies such as umbelliferone-based screening which requires a secondary reaction to release the fluorescent product.
5.2.1 *Detection of dimedone - Proof of concept*

Dimedone was dissolved in a range of different DMSO/H₂O concentrations in a MTP which showed that the green colour formed was proportional to the DMSO concentration. The higher the DMSO concentration the richer the green colour. In order to prove that the colour and absorbance of copper II dimedone solution was linear with concentration, the absorbance of a range of concentrations of dimedone in DMSO/H₂O (3:1) containing copper II acetate was measured. The plot of concentration of dimedone against the absorbance at 417 nm is shown in figure 25. A linear regression fit showed a $R^2$ value of 0.991.

![Figure 25: Plot of dimedone concentration against absorbance in a DMSO solution containing copper II acetate](image)

5.2.2 *CVL-catalysed hydrolysis at varying enzyme concentrations*

We envisaged monitoring the progress of CVL-catalysed hydrolysis of dimedone-1,3-enol ester (R/S)-162 by observing green copper II dimedone formation at 417 nm. Complete dissolution of substrate to give a clear solution was required to ensure accurate absorbance readings. Complete substrate dissolution and optimum colour formation was obtained using a DMSO/H₂O (3:1) mixture. In order to establish that the absorbance at 417 nm was proportional to the concentration of CVL, and hence the rate of hydrolysis of dimedone-1,3-enol ester (R/S)-162, the standard curves shown in figure 26a and 26b were generated (scheme 102). The green colour of copper II dimedone chelate 191 in the MTP is shown in figure 26c.
Figure 26: (a) Plot of absorbance against time for varying CVL concentration; (b) plot of curve slope against the amount of CVL per MTP well and (c) MTP showing the green colour of copper II dimedone
Reagents and Conditions: \((R/S)-162\) (14 \(\mu\)mol/well), CVL (varying amounts), DMSO:H\(_2\)O:saturated Cu(O\(_2\)CMe)\(_2\) (aq) (75:15:10, 200 \(\mu\)L/well), 30 °C, 2 h.

**Scheme 102:** CVL-catalysed hydrolysis of enol ester \((R/S)-162\) with dimeredone detection

### 5.2.3 Determination of hydrolase activity in MTP

Eight lipases and two esterases, each identified as giving high conversion of \((R/S)-162\) from the HPLC screen (detailed in section 4.4.1), were then chosen to validate the new colorimetric screen. The kinetics of all ten hydrolysis reactions were easily monitored in a 96-well MTP, over a period of two hours, using a UV/Vis-plate reader. Figure 27 shows the time course of hydrolysis monitored by detection at 417 nm. Thus *Chromobacterium viscosum* lipase (CVL), *Rhizopus arrhizus* lipase (RAL), *Candida antarctica* lipase B (CAL-B), *Candida lipolytica* lipase (CLL) and *Mucor miehei* esterase (MME) were all identified as active biocatalysts, and in the same rank order of activity as determined by the HPLC conversions (section 4.4.1).

Interestingly, although esterases are notoriously less tolerant to organic solvents, MME retained activity despite the fact that the reaction was performed in high DMSO concentration.

Five hydrolases, namely *Pseudomonas cepacia* lipase (PCL), *Pseudomonas fluorescens* lipase (PFL), *Mucor javanicus* lipase (MJL), porcine pancreatic lipase (PPL) and *Candida lipolytica* esterase (CLE) showed unacceptable background UV at 417 nm and could not be analyzed using this technique. Background absorption in these cases may be due to additives present in commercially available hydrolase preparations.
We proposed that $E$ values for the hydrolase-catalysed hydrolysis of dimedone-1,3-enol ester (R/S)-162, could be calculated by monitoring the kinetics of (R)- and (S)-enantiomers in separate reaction wells. Separate enantiomers of enol ester 162 were synthesised from dimedone 24 and the corresponding (R)- and (S)-3-phenylbutyryl fluoride 161 (scheme 80, section 4.4.1). The kinetics of CVL-catalyzed hydrolysis of individual enantiomers of (S)-162 and (R)-162 showed the expected difference in rate (figure 28a) but appreciably less difference than expected based on $E = 88$ for CVL. We suspected that the lack in differentiation may be attributed to the elimination of competitive binding between the two enantiomers. Contrastingly, the corresponding CVL-hydrolysis of individual enantiomers in 10% acetonitrile/buffer showed an appreciable difference in rate (figure 28b).
The (S)-selectivity of CVL-catalysed hydrolysis was not altered in high DMSO concentration but the rate of hydrolysis was greatly reduced compared to the rate in 10 % acetonitrile-buffer. These results suggest that measurement of the difference in rate of hydrolysis for separate enantiomers may not be feasible for $E$ calculation.
5.3 Summary and further work

We have successfully shown how detection of copper-dimedone chelates can be used to monitor the kinetics of hydrolase catalysed hydrolysis of (R/S)-enol ester 162. Kazlauskas et al. recently demonstrated the utility of their ‘Quick B’ method to screen a library of esterase mutants based on Pseudomonas fluorescens esterase.

Further work includes utilising techniques established in this chapter for 'on-bead' screening of libraries of mutant enzymes based upon detection of resin bound CHD (scheme 103). The main advantage of 'on bead' screening for activity is that coloured beads can be hand picked and the enzyme that catalysed the reaction subsequently sequenced. The formation of on-bead metal-CHD chelates 192 and 193 are shown in scheme 103 however, the low loading of the CHD resin may mean that the colours formed are not bright enough for detection. A possible alternative is the formation of fluorescent dansyl hydrazone 194 which should allow for detection of lower CHD concentrations. The reaction between CHD and hydrazines of this type is not instantaneous so this technique may only provide end-point analysis of the enzymatic hydrolysis.

![Scheme 103: On-bead screening of mutant enzyme libraries using colorimetric or fluorimetric detection of metal-CHD chelates](image)

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Previous attempts at enzymatic hydrolysis of resin bound dimedone-1,3-enol esters have been low yielding so this reaction requires considerable optimisation including attempting the reaction at higher dilution, a technique which showed higher yields in studies by Rein Ulijn et al.\textsuperscript{151}
6. Experimental – Synthesis of CHD resin

6.1 Laboratory equipment

Solid phase reactions were carried out in Solvesso or chemglass sintered reaction flasks, reaction tubes and rotated on a blood rotator or shaken on a New Brunswick Scientific Gyrotory Water Bath Shaker (model G76). TLC was performed on precoated silica gel glass plates (Merck 60 F254, 0.25 mm). Plates were visualised using UV light or molybdenate (VII) dip. Column chromatography was carried out using pre-packed silica columns in a Biotage flash purification system. Chemicals were purchased from Aldrich, Acros or Novabiochem unless otherwise stated. Polystyrene resins were purchased from Argonaut Technologies and PEGA1900 resin was purchased from Polymer laboratories. All reagents and solvents were standard laboratory grade and were used as supplied unless otherwise stated. DMF refers to peptide synthesis grade.

IR absorption spectra were recorded on a Jasco-FT/IR-410 Spectrophotometer using standard techniques and νmax values are quoted in cm⁻¹. Resin IR spectra were recorded by swelling the beads in DCM between NaCl plates or crushed with a diamond anvil and viewed with a Perkin Elmer FT-IR Microscope and Spectrum 1000 spectrometer. UV spectra were collected on a Unicam UV/Vis Spectrometer and microtitre plate (MTP) readings were performed in a Molecular Devices Versamax tunable microplate reader using Softmax Pro software.

1H-NMR and 13C-NMR were recorded on a Varian Gemini 200 Bruker AC250 or Bruker AC400 spectrometer. The following abbreviations are used: δ, chemical shift; d, doublet; dd, doublet of doublets; dt, doublet of triplets; dq, doublet of quartets; i, ipso, J, coupling constant; m, multiplet; q, quartet; s, singlet; t, triplet. Chemical shifts are recorded in ppm using TMS as standard and coupling constants (J) are stated in hertz (Hz). Residual protic solvent, CHCl3 (δH 7.26, s) was used as the internal standard in 1H NMR spectra, and 13C NMR shifts were referenced using CDCl3 (δC 77.0, t) with broad band decoupling. Resins were analysed by Magic Angle Spinning (MAS) probe, swollen in CDCl3, in zirconium rotors on a Bruker AC600 Spectrometer. Chemical shifts for MAS-probe NMR were quoted to one
decimal place due to the formation of broad peaks and hence inaccuracy of the peak position.

Nominal mass spectra were recorded on a Micromass Platform-2 instrument under positive and negative ion electrospray conditions. Fast Atom Bombardment (FAB) high resolution mass spectra were recorded on a Kratos MS50TC instrument. LCMS was performed on a C18 Luna Phenomenex column using an acetonitrile (0.1 % TFA)/water (0.1 % TFA) gradient over 30 minutes. Preparative LC-MS was performed on a Micromass ZMD with Gilson 215 liquid handler, eluting with a water/acetonitrile gradient over 12 minutes.

Microwave reactions were carried out in a CEM Discover Microwave Synthesiser with Explorer Carousel. The reaction conditions were monitored using a pressure probe which was inserted through the septum of the reaction tube and black body irradiation measured to monitor the temperature of the sample. A special setting on this microwave, called ‘continuous cooling’, allows simultaneous microwave irradiation and cooling of samples in a stream of air thus enabling higher levels of irradiation throughout the time course of the reaction.

Analytical reverse phase HPLC was performed on a Waters 600 controller/pump utilizing a 996 photodiode array detector and equipped with a Phenomenex Sperclone C18 column on a eluting with acetonitrile in water at 1 ml min⁻¹ and analyzing at 210 nm. Normal phase HPLC was performed on a Waters 600 controller/pump utilizing a 486 tunable absorbance detector and equipped with a Chiracel-ODH column eluting with IPA in hexane at 0.5 ml min⁻¹ and analyzing at 210 nm. Preparative HPLC was performed on a Luna 5μ 250 by 10.0 mm column eluting with a water/acetonitrile gradient over 20 minutes.

CHN analysis was obtained for crystalline solids. A small sample was recrystallised three times then submitted for combustion analysis. CHN was not obtained for gummy solids or oils. Resin elemental analysis was performed by Medac Ltd.

Melting points were obtained on Gallenkamp melting point apparatus and are uncorrected. Optical rotations were performed on an AA1000 polarimeter from Optical Activity Ltd (measurements made at the sodium D-line). αd concentrations are given in g/100ml.
6.2 Determination of loading by Fmoc cleavage

The UV absorbance (A) at $\lambda_{\text{max}} = 301$ nm ($\varepsilon = 7800$ M$^{-1}$ cm$^{-1}$) of two pre-weighed amount of resin (wt in mg) in a specific volume of 10 % piperidine in DMF (v) was used to calculate the loading according to equation 9 deduced from the Beer Lambert equation. The absorbance was measured using quartz cuvettes, against a blank containing 10 % piperidine in DMF to give three concordant results. If the absorbance value was greater than one, the below equation does not apply so the solution was further diluted till it gave a suitable absorbance value. Standard dilution protocol: 1 mL of a 10 mL solution of 10 % piperidine in DMF (containing between 5 and 10 mg of resin) was diluted to 5 mL.

$$L_{\text{mmol/g}} = \frac{A \times v}{(\varepsilon \times 10^{-3} \times \text{wt})}$$  \hspace{1cm} \text{equation 9}$$

Graph 1 shows a plot of time against percentage of maximum loading for a standard solution of 10 % piperidine in DMF containing approximately ten milligrams of Fmoc-glycine coupled $N$-methyl aminomethyl Argopore and polystyrene resins. The Argopore resin shows maximum absorbance almost immediately but the polystyrene resin doesn't reach maximum absorbance till after half an hour. This is indicative of the time taken for dibenzofulvene to escape from the internal structure of the bead.

![Graph showing time against absorbance](image)

**Figure 29:** Plot of time against absorbance for a standard solution of 10 % piperidine/DMF, containing approximately 10 milligrams of resin
Therefore for an accurate Fmoc-UV analysis, polystyrene resin must be left for one hour to allow complete diffusion out of the bead. If the resins are left with 10% piperidine in DMF overnight no decrease in dibenzofulvene concentration was observed.

The method of determining loading by difference in weight was not found to be an accurate measure of resin loading, especially when using small quantities, and for this reason was not utilised.

6.3 Resin bead tests

6.3.1 Chloranil Test

Solution A: 2% acetaldehyde in DMF; Solution B: 2% p-chloranil in DMF.

1. Sample a few beads and wash with DMF,
2. Add 1-2 drops of both solutions A and B,
3. Stand at room temperature for 5 minutes,
4. Blue beads indicate the presence of primary or secondary amines.

6.3.2 Bromophenol Blue Test

Solution C: 2% bromophenol blue in DMAc.

1. Sample a few beads and wash with DMF,
2. Add 1-2 drops of solution C,
3. Stand at room temperature for 5 minutes,
4. Blue beads indicate the presence of primary or secondary amines.

6.3.3 TNBS Test

Solution D: 10% DIPEA in DMF; Solution E: 1% 2,4,6-TNBS in DMF

1. Sample a few beads and wash with DMF,
2. Add 1-2 drops of both solutions D and E,
3. Stand at room temperature for 5 minutes,
4. If necessary, wash beads with DMF to remove the red solution formed,
5. Red beads indicate the presence of primary amines.
6.3.4 Kaiser Test

Solution F: 5 g ninhydrin in 100 mL ethanol; Solution G: 80 g of liquefied phenol in 20 mL ethanol; Solution H: 2 mL of 0.001 M KCN (aq) in 98 mL pyridine

(1) Sample a few beads and wash with DMF,
(2) Add 1-2 drops of solutions F, G and H,
(3) Warm with a heat gun for 1 minute,
(4) If necessary, wash beads with DMF to remove the blue solution formed,
(5) Blue beads indicate the presence of primary amines.

6.4 Standard resin washing protocol

The resin was filtered under vacuum and washed with 2 × DMF, 2 × MeOH, 2 × DMF and 4 × DCM.

6.5 Birch reduction

6.5.1 1-Ethyl-3,5-dimethoxycyclohexa-2,5-dienecarboxylic acid 36

Ammonia (125 mL) was condensed into a 250 mL three-necked flask and 3,5-dimethoxybenzoic acid 35 (20 g, 109.8 mmol) and anhydrous THF (200 ml) added to form a clear solution. The mixture was cooled to −78 °C in a dry ice/acetone bath and lithium (1.92 g, 274.8 mmol) added portion-wise to form a highly coloured solution (red, green or blue). Bromoethane (8.62 mL, 120.8 mmol) was added dropwise to form a cloudy white solution. The solution was stirred at −78 °C for one hour then warmed slowly to room temperature over a further 19 hours whilst the ammonia evaporated into water. The solution was concentrated in vacuo to remove the THF and the residue dissolved in chilled DCM (150 mL) and water (80 mL). The mixture was cooled in an ice bath, 2M HCl (~100 mL) was added dropwise to pH 3 and the DCM layer separated. The aqueous layer was washed twice more with DCM (2 × 75 mL) and the combined organic layers washed with brine (100 mL), dried over anhydrous magnesium sulfate before being concentrated in vacuo to yield a pale
yellow crystalline solid (23.2 g, > 99 %). If necessary the crude mixture was recrystallised from dichloromethane/petroleum ether (1:1) to give a white crystalline solid. mpt; 132-136 °C (literature 141-144 °C); rf (10 % MeOH/DCM); 0.61; δH (CDCl3, 200 MHz); 0.81 (3H, t, 3J = 7.5 Hz, CH2CH3), 1.74 (2H, q, 3J = 7.5 Hz, CH3CH3), 2.76 (2H, s, H4), 3.60 (6H, s, 2 x OCH3), 4.65 (2H, s, H2/H6) ppm; δC (CDCl3, 63 MHz); 9.0 (CH2CH3), 31.6 (CH2CH3), 34.2 (C4), 50.8 (C1), 54.8 (2 x OCH3), 94.8 (C2/C6), 153.8 (C3/C5), 182.8 (C=O) ppm; νmax (nujol mull); ~3500 (br, OH), 3000-2700 (br, CH), 1698 (C=O), 1656 (C=C) cm⁻¹; m/z; found (FAB) found [M+H]+ 213.11271, C11H12O4 requires 213.11268; CHN; expected C 62.26, H 7.55, N 0.

6.5.2 1,4-Diethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid 37

![Chemical structure of 1,4-Diethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid 37](image)

Obtained as a by-product of the birch reduction of 3,5-dimethoxybenzoic acid 35 as a white crystalline solid. mpt; 124-128 °C; rf (10 % MeOH/DCM); 0.62; δH (CDCl3, 200 MHz); 0.67 (3H, t, 3J = 7.5 Hz, CH2CH3), 0.87 (3H, t, 3J = 7.5 Hz, CH2CH3), 1.74 (4H, m, 2 x CH2CH3), 2.91 (1H, t, 3J = 4.0 Hz, H4), 3.58 (6H, br s, 2 x OCH3), 4.77 (2H, s, H2/H6) ppm; δC (CDCl3, 63 MHz); 8.8 (CH2CH3), 8.9 (CH2CH3), 21.5 (CH2CH3), 34.7 (CH2CH3), 41.0 (C4), 49.5 (C1), 54.4 (2 x OCH3), 95.4 (C2/C6), 155.0 (C3/C5), 182.8 (C=O) ppm; νmax (nujol mull); ~3500 (br, OH), 3000-2700 (br, CH), 1778 (C=O), 1693 (C=O), 1639 (C=C) cm⁻¹; m/z; found (FAB) found [M+H]+ 241.14399, C13H17O4 requires 241.14398; CHN; expected C 65.00, H 8.33, N 0.

6.5.3 5-Ethyl-3-methoxy-1-oxo-cyclohex-2-ene-carboxylic acid 38

![Chemical structure of 5-Ethyl-3-methoxy-1-oxo-cyclohex-2-ene-carboxylic acid 38](image)
Obtained as a by-product of the birch reduction of 3,5-dimethoxybenzoic acid 35 as a white crystalline solid. **mpt**: 122-124 °C; **rf** (10 % MeOH/DCM); 0.39; δH (CDCl3, 400 MHz); 0.93 (3H, t, 3J = 7.5 Hz, CH2CH3), 1.75 (2H, q, 3J = 7.5 Hz, CH2CH3), 2.29 (1H, d, 2J = 16.5 Hz, H6), 2.43 (1H, d, 2J = 17.5 Hz, H6), 2.88 (1H, d, 2J = 16.0 Hz, H4), 2.89 (1H, d, 2J = 17.0 Hz, H4), 3.72 (3H, s, OCH3), 4.40 (1H, s, CH=CO), 5.80 (1H, s, OH) ppm; δC (CDCl3, 101 MHz); 9.0 (CH2CH3), 30.1 (CH2CH3), 36.9 (CH2), 44.4 (CH2), 48.0 (C5), 56.5 (OCH3), 102.3 (CH=CO), 177.4 (C3), 179.7 (C=O acid), 198.0 (C=O enol) ppm; νmax (nujol mull); 3000-2700 (br, CH), 1727 (C=O, acid) 1606 (C=C), 1583 (C=C) cm⁻¹; **m/z**: found (FAB) found [M+H]+ 199.09760 C10H15O4 requires 199.09703.

6.6 Synthesis on Argopore-MB resin – solution-phase analogues

6.6.1 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid methyl amide 39

![chemical structure](attachment:image)

Birch acid 36 (0.50 g, 2.36 mmol), TBTU (0.83 g, 2.60 mmol) and HOBt.H2O (0.16 g, 1.18 mmol) were dissolved in DMF (14 mL) and triethylamine (0.69 mL, 4.95 mmol) was added slowly. MeNH2.HCl (0.18 mg, 2.60 mmol) was added in one portion and the resulting cloudy mixture stirred overnight under argon to form a clear colourless solution. The mixture diluted with EtOAc (20 mL) and saturated aqueous NaHCO3 solution (20 mL). The EtOAc layer was separated and washed twice with water (2 × 20 mL) and brine (20 mL). The EtOAc layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a white crystalline solid (379 mg, 71 %). **mpt**: 137-140 °C; **rf** (10 % MeOH/DCM); 0.61; δH (CDCl3, 200 MHz); 0.69 (3H, t, 3J = 7.5 Hz, CH2CH3), 1.73 (2H, q, 3J = 7.5 Hz, CH2CH3), 2.69 (5H, m, H4 and NHCH3), 3.53 (6H, s, 2 x OCH3), 4.48 (2H, s, H2/H6), 5.89 (1H, br s, NH) ppm; δC (CDCl3, 63 MHz); 8.7 (CH2CH3), 26.4 (NCH3), 29.6 (CH2CH3), 31.2 (C4), 31.5 (CH2), 50.8 (C1), 54.4 (2 × OCH3), 95.6 (C2/C6), 153.7 (C3/C5), 176.7 (C=O amide) ppm; νmax (nujol mull); 3416 (NH), 3000-2800 (CH), 1694 (C=O secondary
amide), 1650 (C=C) cm$^{-1}$; m/z; found (FAB) [M+H]$^+$ 226.14430 C$_{12}$H$_{20}$NO$_3$ requires 226.14432.

6.6.2 1-Ethyl-3-hydroxy-5-oxo-cyclohex-3-ene carboxylic acid methyl amide 40

\[
\begin{align*}
&\text{Bis-enol ether 39 (0.2 g, 0.88 mmol) was dissolved in THF (2 mL) and 2M HCl (2 mL) added slowly. The mixture was stirred at room temperature for 3½ hours to give a clear colourless solution. The solution was concentrated in vacuo to remove the THF and the residue dissolved in chloroform (10 mL) and water (10 mL). The chloroform layer was separated and the aqueous layer washed once more with chloroform (10 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear yellow oil that decomposed rapidly (118 g, 68%). }
&\text{rf (10 % MeOH/DCM); 0.40; } \delta_H (\text{CDCl}_3, 400 \text{ MHz}) \text{ crude, enol and keto forms; 0.94 (3H, t, } ^3J = 7.5 \text{ Hz, CH}_2\text{CH}_3), 1.59 (1H, dq, } ^3J = 7.5 \text{ Hz, CH}_2\text{CH}_3), 1.80 (1H, dq, } ^3J = 7.5 \text{ Hz, CH}_2\text{CH}_3), 1.91 (1H, br s, NH), 2.12 (1H, d, } ^2J = 6.0 \text{ Hz, H2}), 2.14 (1H, d, } ^2J = 7.0 \text{ Hz, H2}), 2.31 (1H, d, } ^2J = 18.0 \text{ Hz, H6}), 2.38 (1H, d, } ^2J = 18.0 \text{ Hz, H6}), 2.59 (2H, d, } ^2J = 17.5 \text{ Hz, H4}), 2.73 (3H, s, NCH}_3), 5.30 (1H, s, CH=CO) \text{ ppm}; \delta_C (\text{CDCl}_3, 400 \text{ MHz}); 8.5 \text{ (CH}_2\text{CH}_3), 23.6 \text{ (NCH}_3), 25.3 \text{ (CH}_2\text{CH}_3), 45.9 \text{ (CH}_2), 42.5 \text{ (C5), 47.4 \text{ (CH}_2), 47.5 \text{ (CH}_2), 86.9 \text{ (C3), 174.1 \text{ (C=O amide), 205.2 \text{ (C=O keto) ppm; } \nu_{\text{max}} (\text{thin film); 3306 \text{ (NH), 2968 (CH), 2924 (CH), 2925-2800 (CH), 1716 (C=O keto), 1681 (C=O tertiary amide) cm}^{-1}; m/z \text{ found (FAB) [M+H]}^+ 198.11370 \text{ C}_{10}H_{16}NO_3 \text{ requires 198.11302.}}}
\end{align*}
\]
6.7 Solid-phase synthesis on Argopore-MB resin

6.7.1 Analysis of Argopore-MB-CHO 41

The resin was commercially available from Argonaut Technologies. The resin showed negative chloranil, bromophenol blue and TNBS tests. $v_{\text{max}}$ (diamond anvil): 3100-2800 (br, CH), 1679 (C=O aldehyde), 1601 (Ar C=C) cm$^{-1}$.

6.7.2 (2-Methoxy-4-hydroxypolystyrene-benzyl)-methyl-amine 42

Argopore-MB-CHO resin 41 (1 g, $L_\text{quote} = 0.83 \text{ mmol/g, 0.83 mmol}$), NaBH(OAc)$_3$ (880 mg, 4.15 mmol) and MeNH$_2\cdot$HCl (282 mg, 4.15 mmol) were shaken overnight in a 50 ml flask with DMF (10 mL) and AcOH (0.2 mL). The resin was washed with DMF and the coupling repeated overnight. The resin was washed according to the standard protocol but with two additional washes with 10 % DIPEA in DMF then dried overnight in the vacuum oven. The resin showed positive blue colouration with the chloranil and bromophenol blue tests. $v_{\text{max}}$ (diamond anvil): ~3300 (br, NH), 3100-2800 (br, CH), 1604 (Ar C=C) cm$^{-1}$.

6.7.3 Loading determination by Fmoc-UV analysis: [(2-Methoxy-4-hydroxypolystyrene-benzyl)-methyl-carbamoyl]-methyl-carbamic acid 9H-fluoren-9-ylmethyl ester 43

Methylamino resin 42 (0.1 g, $L_\text{max} = 0.82 \text{ mmol/g, 0.08 mmol}$) was swollen in DMF (0.5 mL). Meanwhile, Fmoc-Gly-OH (122 mg, 0.41 mmol) and TBTU (132 mg, 0.41 mmol) were dissolved in DMF (0.5 mL) then DIPEA (0.14 mL, 0.82 mmol) was
added and the total mixture stirred for 5 minutes. The contents of the flask were transferred to the isolute tube containing the resin and the mixture rotated overnight on the blood rotator. The resin was washed with DMF and the coupling repeated overnight. The resin was washed according to the protocol and dried overnight in the vacuum oven. The resin showed a negative chloranil test. \( L_{\text{uv}} = 0.67 \text{ mmol/g}; \nu_{\text{max}} (\text{DCM}); \sim3300 (\text{br, NH}), 3054 (\text{CH}), 2986 (\text{CH}), 1716 (\text{C=O carbamate}), 1651 (\text{C=O tertiary amide}), 1602 (\text{Ar C=C}) \text{ cm}^{-1} \).

6.7.4 Resin Cleavage: Methylcarbamoylmethyl-carbamic acid 9H-fluoren-9-ylmethyl ester 44

Resin bound amino acid 43 (51.6 mg, \( L_{\text{uv}} = 0.68 \text{ mmol/g}, 0.04 \text{ mmol} \)) was suspended in TFA (1.9 mL) and water (0.1 mL) and shaken for 2 hours. The resin was washed thoroughly with DCM and the combined washings dried over anhydrous magnesium sulfate before being concentrated \textit{in vacuo} to yield a gummy solid (7.8 mg, 72 %). \( \delta_{\text{H}} (\text{CDCl}_3, 200 \text{ MHz}); 2.75 (3\text{H}, \text{ d}, \beta J = 5.0 \text{ Hz, NHCH}_3), 3.78 (2\text{H}, \text{ m, CH}_2\text{NH}), 4.15 (1\text{H}, \text{ t, } \gamma J = 5.0 \text{ Hz, CH Fmoc}), 4.40 (2\text{H}, \text{ d, } \gamma J = 6.5 \text{ Hz, CH}_2\text{O}), 5.34 (1\text{H}, \text{ m, NHCH}_3), 7.25 (4\text{H}, \text{ m, ArH}), 7.51 (2\text{H}, \text{ d, } \gamma J = 7.0 \text{ Hz, ArH}), 7.70 (2\text{H}, \text{ d, } \gamma J = 7.0 \text{ Hz, ArH}) \text{ ppm}; m/z; \text{ found (ES}^+) [\text{M+Na}]^+ 333 \text{ C}^{13}\text{H}_{18}\text{N}_2\text{O}_3\text{Na requires 333; } \nu_{\text{max}} \text{ (resin, DCM);} 3200-2900 (\text{CH}), 1783 (\text{C=O}), 1601 (\text{Ar C=C}) \text{ cm}^{-1} \).

6.7.5 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid (2-methoxy-4-hydroxypolystyrene-benzyl)-methyl-amide 45

Birch acid 36 (178 mg, 0.84 mmol), TBTU (297 mg, 0.92 mmol) and HOBT.H_2O (56.7 mg, 0.42 mmol) were dissolved in DMF (2 mL) and triethylamine (128 \( \mu \text{L}, 0.92 \text{ mmol}) was added. The mixture was stirred at room temperature for 5 minutes. Meanwhile methylamino resin 41 (0.20 g, \( L_{\text{max}} = 0.82 \text{ mmol/g}, 0.17 \text{ mmol} \)) was
swollen in DMF (2 mL) and triethylamine (292 μL, 2.10 mmol) and DMAP (24.6 mg, 0.20 mmol) were added. The contents of the first flask were transferred to the isolute tube containing the resin and the resulting mixture shaken at room temperature overnight. The resin was washed with DMF and the coupling repeated overnight. The resin was washed according to the standard protocol and dried overnight in the vacuum oven. The resin showed a negative chloranil test. \( \nu_{\text{max}} \) (diamond anvil); 3100-2800 (br, CH), 1686 (C=O tertiary amide), 1654 (C=C), 1610 (Ar C=C) cm\(^{-1}\).

### 6.7.6 Resin Cleavage: 1-Ethyl-3-hydroxy-5-oxo-cyclohex-3-enecarboxylic acid methyl amide 40

![Diagram](image)

Resin bound tertiary amide 45 (350 mg, \( \text{L}_{\text{max}} = 0.72 \text{ mmol/g, 0.25 mmol} \)) was suspended in TFA (3.8 mL) and water (0.2 mL) and shaken for 2 hours. The resin was washed thoroughly with TFA/H\(_2\)O (95:5) and the combined concentrated in vacuo to yield a gummy solid (67 mg, > 99 %). Crude material (mixture of methoxycyclohexenone 46 and diketone 40) was purified by prep LC-MS to yield a colourless gummy solid (6.0 mg, 12.1 %). \( \text{rf} \) (10 % MeOH/DCM); 0.40; \( \delta_H \) (CDCl\(_3\), 400 MHz) enol and keto forms; 0.85 (3H, m, -CH\(_2\)CH\(_3\)), 1.42-1.78 (2H, m, CH\(_2\)CH\(_3\)), 2.07-2.99 (6H, m, 3 × CH\(_2\)), 2.70 (3H, s, NCH\(_3\)), 5.26 (1H, s, H\(_2\) enol), 5.70 (1H, br t, NH) ppm; \( \text{m/z} \); found (ES\(^+\)) [M+H]\(^+\) 198 \( \text{C}_{10}\text{H}_{16}\text{NO}_3 \) requires 198; \( \nu_{\text{max}} \) (resin, DCM); 3200-2900 (CH), 1783 (C=O TFA enol ester), 1601 (Ar C=C), cm\(^{-1}\).

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6.8 Synthesis on polystyrene resin – Solution-phase analogues

6.8.1 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid benzyl-
methyl-amide 47

Birch acid 36 (0.50 g, 2.36 mmol), TBTU (0.83 g, 2.60 mmol) and HOBT.H₂O (0.16 g, 1.18 mmol) were dissolved in DMF (14 mL) and triethylamine (0.26 mL, 2.60 mmol) was added slowly. N-Methylbenzylamine (0.34 mL, 2.60 mmol) was added dropwise and the resulting cloudy mixture stirred for 1½ hours under argon to form a yellow solution. The mixture diluted with EtOAc (20 mL) and aqueous 1M KH₂SO₄ solution (20 mL). The EtOAc layer was separated and washed once with saturated aqueous NaHCO₃ solution (20 mL), water (20 mL) and brine (20 mL). The EtOAc layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a crude pale yellow oil. The crude product was purified by column chromatography on silica eluting with 20 % ethyl acetate in petroleum ethers (40-60 °C) to give a clear colourless oil that crystallised out to form a sticky solid (0.61 g, 81 %). rf (10 % MeOH/DCM); 0.79; δH (CDCl₃, 200 MHz); 0.71 (3H, t, J = 7.5 Hz, CH₂CH₃), 1.84 (2H, q, ³J = 7.5 Hz, CH₂CH₃), 2.61 (1H, d, ²J = 21.0 Hz, H4), 2.83 (1H, d, ²J = 25.0 Hz, H4), 2.89 (3H, s, NCH₃), 3.39 (6H, br s, 2 x OCH₃), 4.47 (2H, s, CH₂Ph), 4.59 (2H, s, H₂/H₆), 7.24 (5H, m, ArH) ppm; δC (CDCl₃, 63 MHz); 8.2 (CH₂CH₃), 31.1 (CH₂CH₃), 33.3 (C4), 35.8 (NCH₃), 50.2 (C1), 52.9 (CH₂Ph), 54.1 (2 x OCH₃), 95.6 (C2/C6), 125.9 (ArC), 126.7 (ArC), 128.3 (ArC), 138.0 (i-ArC), 149.2 (C3/C5), 175.0 (C=O amide) ppm; νmax (nujol mull); 3200-2800 (CH), 1733 (C=O), 1689 (C=O amide), 1635 (C=C) cm⁻¹; m/z; found (FAB) [M+H]⁺ 316.19123 C₁₀H₁₈NO₃ requires 316.19127.
6.8.2 1-Ethyl-3-hydroxy-5-oxo-cyclohex-3-enecarboxylic acid benzylmethyl-amide 49

\[ \text{Bis-enol ether 47 (0.5 g, 1.59 mmol) was dissolved in THF (5 mL) and 2M HCl (5 mL) added slowly. The mixture was stirred at room temperature for 4 hours to give a clear colourless solution. The solution was concentrated in vacuo to remove the THF and the residue dissolved in chloroform (20 mL) and water (20 mL). The chloroform layer was separated and the aqueous layer washed once more with chloroform (20 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a hygroscopic white crystalline solid (0.50 g, > 99%).} \]

rf (10 % MeOH/DCM); 0.31; \( \delta_n \) (CDCl\(_3\), 200 MHz) keto form; 0.91 (3H, t, \( ^3J = 7.5 \) Hz, CH\(_2\)CH\(_3\)), 1.79 (2H, q, \( ^3J = 7.5 \) Hz, CH\(_2\)CH\(_3\)), 2.40 (2H, m, H\(_2\)), 2.94 (3H, s, NCH\(_3\)), 3.19 (4H, m, H\(_4\)/H\(_6\)), 4.52 (2H, s, CH\(_2\)Ph), 7.20 (5H, m, ArH) ppm; \( \delta_c \) (CDCl\(_3\), 63 MHz) enol and keto forms; 8.7 (CH\(_2\)CH\(_3\)), 8.8 (CH\(_2\)CH\(_3\)), 29.6 (CH\(_2\)CH\(_3\)), 30.5 (CH\(_2\)CH\(_3\)), 35.4 (NCH\(_3\)), 35.9 (NCH\(_3\)), 42.2 (CH\(_2\)), 48.0 (C5), 49.8 (CH\(_2\)), 53.3 (CH\(_2\)), 53.5 (CH\(_2\)), 54.9 (CH\(_2\)Ph), 103.5 (CH=CO), 127.4 (ArC), 127.6 (ArC), 128.6 (ArC), 136.4 (ArC), 172.5 (C3), 173.3 (C=O amide), 188.5 (C=O enol), 202.6 (C=O keto) ppm; \( \nu_{\text{max}} \) (nujol mull); 3500-3000 (br, OH), 3000-2500 (br, CH), 1712 (s, C=O keto), 1670-1630 (br, C=O amide), 1624 (C=C enol) cm\(^{-1}\); m/z; found (FAB) [M+H]\(^+\) 288.15993 C\(_{17}\)H\(_{22}\)NO\(_3\) requires 288.15997.

6.8.3 1-Ethyl-3-methoxy-5-oxo-cyclohex-3-enecarboxylic acid benzylmethyl-amide 48

\[ \text{Bis-enol ether 47 (50 mg, 0.16 mmol) was dissolved in DCM (0.5 mL) and aluminium chloride (105 mg, 0.79 mmol) added slowly. The mixture was stirred at} \]
room temperature for 4 hours to give dark orange solution. The solution was concentrated in vacuo to remove the THF and the residue dissolved in chloroform (20 mL) and water (20 mL). The chloroform layer was separated and the aqueous layer washed once more with chloroform (20 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a gummy orange solid (28.5 mg, 63 %). **rf** (10 % MeOH/DCM); 0.45; δ<sub>H</sub> (CDCl<sub>3</sub>, 400 MHz); 0.93 (3H, t, <sup>3</sup>J = 7.5 Hz, CH<sub>2</sub>CH<sub>3</sub>), 1.83 (2H, m, CH<sub>2</sub>CH<sub>3</sub>), 2.42 (1H, d, <sup>2</sup>J = 11.5 Hz, H<sub>6</sub>), 2.46 (1H, d, <sup>2</sup>J = 10.5 Hz, H<sub>6</sub>), 2.99 (3H, s, NCH<sub>3</sub>), 3.00 (1H, d, <sup>2</sup>J = 12.5 Hz, H<sub>4</sub>), 3.12 (1H, d, <sup>2</sup>J = 16.5 Hz, H<sub>4</sub>), 3.71 (3H, s, OCH<sub>3</sub>), 4.47 (1H, d, <sup>2</sup>J = 14.5 Hz, CH<sub>2</sub>Ph), 4.71 (1H, d, <sup>2</sup>J = 15.0 Hz, CH<sub>2</sub>Ph), 5.36 (1H, s, CH=CO), 7.17 (2H, m, ArH), 7.27 (3H, m, ArH) ppm; δ<sub>C</sub> (CDCl<sub>3</sub>, 63 MHz); 8.7 (CH<sub>2</sub>CH<sub>3</sub>), 29.8 (CH<sub>2</sub>CH<sub>3</sub>), 37.5 (NCH<sub>3</sub>), 38.8 (CH<sub>3</sub>), 46.4 (CH<sub>2</sub>), 49.5 (C<sub>5</sub>), 55.3 (CH<sub>2</sub>Ph), 55.9 (OCH<sub>3</sub>), 101.1 (CH=CO), 127.3 (ArC), 127.6 (ArC), 128.5 (ArC), 136.9 (i-ArC), 172.5 (C<sub>3</sub>), 177.4 (C=O amide), 196.7 (C=O enol) ppm; v<sub>max</sub> (nujol mull); 2917 (CH), 2848 (CH), 1653 (C=O enol), 1625 (C=C), 1613 (C=C) cm<sup>-1</sup>; m/z; found (FAB) [M+H]<sup>+</sup> 302.17560 C<sub>18</sub>H<sub>24</sub>NO<sub>3</sub> requires 302.17562.

6.9 Solid-phase synthesis on polystyrene resin

6.9.1 Analysis of formylpolystyrene 50

The resin was commercially available from Argonaut Technologies. The resin showed negative chloranil and bromophenol blue tests. δ<sub>H</sub> (MAS, 600 MHz, CDCl<sub>3</sub>); 1.5 (CH<sub>2</sub> PS), 1.8 (CH<sub>3</sub> PS), 6.6 (ArH PS), 7.6 (ArH PS), 10.0 (CHO) ppm; v<sub>max</sub> (diamond anvil); 3100-2800 (br, CH), 1702 (C=O aldehyde), 1604 (Ar C=C) cm<sup>-1</sup>.

6.9.2 N-Methylaminomethylpolystyrene 51
Formylpolystyrene resin 50 (20 g, \(L_{\text{quote}} = 1.41 \text{ mmol/g}, 28.2 \text{ mmol}\)), NaBH(OAc)\(_3\) (29.9 g, 141 mmol) and MeNH\(_2\).HCl (9.59 g, 141 mmol) were shaken for 18 hours in a conical flask with DMF (196 mL) and AcOH (4 mL). The resin was filtered through a sintered filter funnel and washed according to the standard protocol and dried overnight in the vacuum oven. The resin showed positive blue colouration with the chloranil test. 
\[\delta_H (\text{MAS}, 600 \text{ MHz}, \text{CDCl}_3); 1.5 (\text{CH}_2 \text{ PS}), 1.8 (\text{CH}_2 \text{ PS}), 2.4 (\text{NCH}_3), 3.6 (\text{CH}_2), 6.6 (\text{ArH PS}), 7.0 (\text{ArH PS}) \text{ ppm}; v_{\text{max}} (\text{diamond anvil}); ~3300 (\text{br}, \text{NH}), 3100-2800 (\text{br}, \text{CH}), 1601 (\text{Ar C=C}) \text{ cm}^{-1}.\]

6.9.3 Loading determination by Fmoc-UV analysis: [(Methylpolystyrene-methyl-carbamoyl)-methyl]-carbamic acid 9H-fluoren-9-ylmethyl ester 52

\[
\text{FmocHN}\text{O} \quad \text{N}\text{PS}
\]

N-Methylaminomethyl resin 51 (0.1 g, \(L_{\text{max}} = 1.38 \text{ mmol/g}, 0.14 \text{ mmol}\) was swollen in DMF (0.5 mL). Meanwhile, Fmoc-Gly-OH (204 mg, 0.69 mmol) and TBTU (221 mg, 0.96 mmol) were dissolved in DMF (0.5 mL) then DIPEA (240 \(\mu\text{L}, 1.38 \text{ mmol}) was added and the total mixture stirred for 5 minutes. The contents of the flask were transferred to the isolute tube containing the resin and the shaken at RT for 3 hours. The resin was washed according to the standard protocol. The coupling repeated overnight, the resin was washed according to the standard protocol again and dried overnight in the vacuum oven. The resin showed a negative chloranil test. \(L_{\text{av}} = 0.97 \text{ mmol/g}; \delta_H (\text{MAS}, 600 \text{ MHz}, \text{CDCl}_3); 1.4 (\text{CH}_2 \text{ PS}), 1.8 (\text{CH}_2 \text{ PS}), 2.7 (\text{NCH}_3), 4.0 (\text{CH}_2), 4.2 (\text{OCH Fmoc}), 4.4 (\text{OCH}_2), 6.6 (\text{ArH PS}), 7.0 (\text{ArH PS}), 7.4 (\text{ArH Fmoc}), 7.6 (\text{ArH Fmoc}), 7.7 (\text{ArH Fmoc}) \text{ ppm}; v_{\text{max}} (\text{diamond anvil}); 3413 (\text{br}, \text{NH}), 3060-2800 (\text{br}, \text{CH}), 1728 (\text{C}=\text{O carbamate}), 1659 (\text{C}=\text{O tertiary amide}), 1601 (\text{Ar C=C}) \text{ cm}^{-1}.\]

6.9.4 Capping Standard: \(N\)-Methylpolystyrene-\(N\)-methyl-acetamide 53
N-Methyl aminomethyl resin 51 (0.2 g, \( L_{\text{max}} = 1.32 \) mmol/g, 0.26 mmol) was shaken at room temperature in DMF (2 mL), with acetic anhydride (130 \( \mu \)L, 1.38 mmol), DIPEA (480 \( \mu \)L, 2.76 mmol) and DMAP (16.9 mg, 0.14 mmol) for 2 \( \frac{1}{2} \) hours. The resin was then filtered and washed according to the standard protocol then dried overnight in the vacuum oven. The resin showed a negative chloranil test.

\[ \delta H (\text{MAS}, 600 \text{ MHz, CDCl}_3); 1.4 \text{ (CH}_2 \text{ PS), 1.9 \text{ (CH}_2 \text{ PS), 2.1 \text{ (NCH}_3\text{), 2.8 \text{ (COCH}_3\text{), 4.5 \text{ (NCH}_2\text{), 6.6 \text{ (ArH PS), 7.0 \text{ (ArH PS) ppm; \nu}_{\text{max}} \text{ (diamond anvil); 3100-2800 (br, CH), 1656 (C=O tertiary amide), 1601 (Ar C=C) cm}^{-1}.} }\]

6.9.5 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid

methylenepolystyrene-methyl-amide 54

Birch acid 36 (26.2 mg, 123.4 mmol), was dissolved in DMF (94 mL). Meanwhile N-methylaminomethyl resin 51 (18.7 g, \( L_{\text{calc}} = 1.33 \) mmol/g, 24.9 mmol) was swollen in DCM (94 mL). The contents of the first flask were transferred to the conical flask containing the resin and DIC (19.3 mL, 123.4 mmol) was added dropwise. The resulting mixture was shaken at room temperature for 2 \( \frac{1}{2} \) hours and the resin filtered and washed with according to the standard protocol. The coupling repeated was repeated for 2 \( \frac{1}{2} \) hours, the resin washed according to the standard protocol again and dried overnight in the vacuum oven. The resin showed a negative chloranil test.

\[ \delta H (\text{MAS}, 600 \text{ MHz, CDCl}_3); 0.7 \text{ (CH}_2 \text{CH}_3\text{), 1.4 \text{ (CH}_2 \text{ PS), 1.8 \text{ (CH}_2 \text{ PS and CH}_2\text{CH}_3\text{), 2.7 \text{ (H}_4\text{), 2.8 \text{ (NCH}_3\text{), 3.2-3.6 \text{ (2 X OCH}_3\text{), 4.5 \text{ (H}_2\text{H}_6\text{ and NCH}_2\text{), 6.5 \text{ (ArH PS), 7.0 \text{ (ArH PS) ppm; \nu}_{\text{max}} \text{ (diamond anvil); 3100-2800 (br, CH), 1690 (C=O tertiary amide), 1636 (C=C), 1602 (Ar C=C) cm}^{-1}.} }\]
6.9.6 1-Ethyl-3-methoxy-5-oxo-cyclohex-3-enecarboxylic acid methylpolystyrene-methyl-amide 56

Bis-enol ether resin 54 (5 g, L_{max} = 1.31 mmol/g, 6.53 mmol) was shaken with TFA (4.5 mL), water (250 μL) and DMF (250 μL) for 2 ½ hours at room temperature. The resin was filtered and washed according to the standard protocol then dried overnight in the vacuum oven at 40 °C. The resin showed a negative chloranil test.

δH (MAS, 600 MHz, CDCl3); 0.9 (CH2CH3), 1.4 (CH2 PS), 1.8 (CH2 PS and CH2CH3), 2.4 (H6), 2.9 (NCH3), 3.1 (H4), 3.6 (OCH3), 4.5 (NCH2), 5.4 (CH=CO), 6.6 (ArH PS), 7.0 (ArH PS) ppm; ν (diamond anvil); 3200-2800 (br, CH), 1674 (C=O enol), 1631 (C=C), 1602 (Ar C=C) cm^{-1}.

6.10 Investigations into methoxycyclohexenone reactivity

6.10.1 3-Methoxy-5,5-dimethyl-cyclohex-2-enone 58

Dimedone 24 (10 g, 71.4 mmol) was dissolved in methanol (150 mL) then trimethylsilane (9.50 mL, 75.0 mmol) and DIPEA (26.1 mL, 150 mmol) added. The reaction mixture was stirred at room temperature under nitrogen overnight then concentrated in vacuo to give a clear oil. The oil was dissolved in ethyl acetate (100 mL) and washed with water, saturated aqueous NaHCO3, water again, then brine (all 100 mL). The ethyl acetate layer was concentrated in vacuo to yield a clear colourless oil (8.84 g, 80 %). \text{rf} (10 \% \text{MeOH/DCM}); 0.78; δH (CDCl3, 200 MHz); 1.51 (6H, s, 2 × CH3), 2.29 (2H, s, H6), 2.35 (2H, s, H4), 3.77 (3H, s, OCH3), 5.45 (1H, s, CH=CO) ppm; δC (CDCl3, 63 MHz); 28.1 (2 × CH3), 32.3 (C5), 42.5 (C4), 50.5 (C6), 55.5 (OCH3), 100.9 (CH=CO), 179.6 (C3), 199.4 (C=O enol) ppm; ν_{max} (thin film); 2959-2848 (br, CH), 1651 (C=O enol), 1606 (C=C) cm^{-1}; m/z; found (FAB) [M+H]^+ 155.10757 C9H15O2 requires 155.10720.
Oven dried magnesium turnings (0.16 mg, 6.49 mmol) were dry stirred under nitrogen for five minutes then two crystals of iodine added. Bromobenzene (0.51 mL, 4.87 mmol) in THF (2.5 mL) was added dropwise until the yellow colour of iodine disappeared and heat evolution had ceased. 3-Methoxy-5,5-dimethyl-cyclohex-2-enone 58 (0.5 g, 3.25 mmol) in THF (2.5 mL) was added and the resultant mixture stirred at room temperature for one hour. 2M HCl (10 mL) was added dropwise and the mixture stirred for a further 30 minutes at room temperature. The solution was quenched with saturated aqueous NaHCO₃ solution till pH 7 then the mixture extracted three times with ether (3 x 25 mL). The combined organic layers were washed with brine (25 mL), dried over anhydrous sodium sulfate and concentrated in vacuo to yield a yellow oil. The crude mixture was purified by column chromatography on silica eluting with 20 % ethyl acetate in petroleum ether (40–60 °C) to yield an orange oil. (325 mg, 50 %). \( \text{rf (30 \% EtOAc/pet ether); 0.45;} \)

\[ \delta_H (\text{CDCl}_3, 200 \text{ MHz}); 1.21 (6H, s, 2 \times \text{CH}_3), 2.42 (2H, s, H6), 2.73 (2H, s, H4), 6.50 (1H, s, \text{CH=CO}), 7.49 (3H, m, ArH), 7.61 (2H, m, ArH) \text{ ppm; } \delta_C (\text{CDCl}_3, 63 \text{ MHz}); 28.3 (2 \times \text{CH}_3), 33.6 (\text{C5}), 42.2 (\text{C6}), 50.8 (\text{C4}), 115.3 (\text{CH=CO}), 124.2 (\text{ArC}), 126.0 (\text{ArC}), 129.8 (\text{ArC}), 138.9 (\text{i-ArC}), 157.6 (\text{C3}), 200.2 (\text{C=O}) \text{ ppm; } \nu_{\text{max}} (\text{thin film}); 2958 (\text{CH}), 2869 (\text{CH}), 1660 (\text{C=O}), 1606 (\text{C=C}) \text{ cm}^{-1}; m/z; \text{found (FAB) } [\text{M+H}]^+ 201.12730 \text{ C}_{14}\text{H}_{17}\text{O requires 201.12794.} \]

Methyoxycyclohexenone 58 (1.61 g, 10.5 mmol) was stirred at 0 °C in an ice bath under nitrogen while oxalyl chloride (1.5 mL, 16.3 mmol) was added dropwise with care. When gas evolution had ceased the yellow solution was diluted with a drop of
DMF and DCM (2 mL) before being warmed to room temperature and stirred overnight. The product was purified by distillation to give a pale yellow oil (1.42 g, 86%). bpt; 65 °C at 0.4 mm Hg; rf (30% EtOAc/pet ethers 40-60 °C); 0.59; δH (CDCl3, 200 MHz); 1.16 (6H, s, 2 × CH3), 2.32 (2H, s, H6), 2.62 (2H, d, δJ = 1.0 Hz, H4), 6.28 (1H, t, δJ = 1.0 Hz, CH=CO) ppm; δC (CDCl3, 63 MHz); 27.1 (2 × CH3), 33.0 (C5), 46.9 (C6), 49.4 (C4), 126.3 (CH=CO), 155.6 (C3), 195.7 (C=O) ppm; νmax (thin film), 2962 (CH), 2872 (CH), 1681 (C=O), 1613 (C=C) cm⁻¹; m/z (FAB) found 159.05769 C8H12OCl requires 159.05767.

6.10.4 Loading determination by elemental analysis: 1-Ethyl-3-chloro-5-oxo-cyclohex-3-enecarboxylic acid methylpolystyrene-methyl-amide 61

Methoxycyclohexenone resin 56 (20 mg, 0.02 mmol) was swollen in DCM (1 mL) containing one drop of DMF. Oxalyl chloride (10 μL, 0.11 mmol) was added in one portion to give a red resin, which was rotated at room temperature overnight. The resin was filtered and washed with 3 × DMF, 3 × MeOH, 3 × DMF, 3 × DCM and 3 × Et2O before being dried in the vacuum over at 40 °C overnight. Lea = 1.33 mmol/g; δH (MAS, 600 MHz, CDCl3); 1.0 (CH2CH3), 1.5 (CH2 PS), 1.6 (CH2 PS), 1.8 (CH2CH3), 1.90 (CH2 PS), 2.7 (H4), 2.8 (NCH3), 4.6 (NCH2), 6.7 (ArH PS), 7.2 (ArH PS) ppm [the NMR showed no contaminant DCM which would affect the results of elemental analysis]; νmax (DCM); 3100-2800 (br, CH), 1690-1630 (C=O broad), 1631 (C=CH2), 1602 (Ar C=CH2) cm⁻¹.

6.11 Synthesis on macroporous Argopore resin

6.11.1 N-Methylaminomethyl macroporous polystyrene 63

Argonaut macroporous polystyrene resin 62 (1 g, Lquote = 1.05 mmol/g, 1.05 mmol) and MeNH2.HCl (0.36 g, 5.25 mmol) were dissolved in DMF (10 mL) and DIPEA (1.83 mL, 10.5 mmol) was added. The mixture was shaken for 18 hours in a round
bottomed flask with balloon attached then the resin was filtered through an isolute tube and washed according to the standard protocol. The coupling was repeated for a further 20 hours and the resin was washed according to the same protocol but then dried overnight in the vacuum oven. The beads gave a blue colouration with the chloranil test. \( \nu_{\text{max}} \) (DCM); 3100-2800 (br, CH), 1602 (Ar \text{C=C}) \text{ cm}^{-1}.

6.11.2 Loading determination by Fmoc-UV analysis: \( \left[(\text{Methylpolystyrene}-\text{methyl-carbamoyl})\text{-methyl-carbamic acid}\right] \text{9H-fluoren-9-ylmethyl ester 64}

\[
\text{FmocH}N\begin{array}{c}
\text{N}\\ \text{AP}
\end{array}
\]

The resin was synthesised according to the procedure in section 6.9.3. The resin showed a negative chloranil test. \( L_{\text{uv}} = 0.55 \text{ mmol/g} \); \( \nu_{\text{max}} \) (DCM); 3411 (NH), 3100-2800 (CH), 1722 (C=O carbamate), 1651 (C=O tertiary amide), 1601 (C=C) cm\(^{-1}\).

6.11.3 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid methylpolystyrene-methyl-amide 65

\[
\text{MeO} \quad \begin{array}{c}
\text{O}\\ \text{Af}
\end{array}
\]

The resin was synthesised according to the procedure in section 6.9.5. The resin showed a negative chloranil test. \( \nu_{\text{max}} \) (DCM); 3000-2800 (CH), 1691(C=O tertiary amide) 1631 (C=C), 1602 (C=C) cm\(^{-1}\).

6.12 Linker synthesis

6.12.1 4-\(\left[(\text{9H-fluoren}-9\text{-ylmethoxycarbonyl})\text{-methyl-amino}\right]\text{-butyric acid 68}

\[
\text{FmocHN}\begin{array}{c}
\text{O}\\ \text{OH}
\end{array}
\]

4-(Methylamino)butyric acid hydrochloride 67 (938 mg, 6.40 mmol) was dissolved in 10% aqueous \( \text{Na}_2\text{CO}_3 \) solution (25 mL) and cooled to 0 °C in an ice bath.
Fluorenyl methylchloroformate (1.82 g, 7.04 mmol) in 1,4-dioxane (10 mL) was added slowly to the stirred solution and the pH was maintained at pH 8-9. The solution was stirred at room temperature for 3 hours. The reaction mixture was then added to water (400 mL) and extracted with ether (2 × 200 mL). The aqueous was acidified with conc. HCl (aq) to pH 3 and extracted with EtOAc (400 ml). The combined organic layers were dried over anhydrous magnesium sulfate and concentrated in vacuo to give a white crystalline solid (1.65 g, 76 %). r.f (60 % EtOAc/pet ethers 40-60 °C) 0.10; δH (CDCl₃, 250 MHz); 1.57 (1H, t, 3J = 7.0 Hz, CH₂), 1.84 (1H, t, 3J = 6.5 Hz, CH₂), 2.07 (1H, t, 3J = 7.5 Hz, CH₂), 2.32 (1H, t, 3J = 7.0 Hz, CH₂), 2.85 (3H, d, 3J = 8.0 Hz, NCH₃), 3.05 (1H, t, 3J = 7.0 Hz, CH₂), 3.32 (1H, t, 3J = 7.0 Hz, CH₂), 4.11 (1H, d, 3J = 7.0 Hz, CH Fmoc), 4.21 (1H, d, 3J = 5.0 Hz, CH₂O), 4.42 (1H, d, 3J = 7.0 Hz, CH₂O), 7.30 (4H, m, ArH), 7.56 (2H, d, 3J = 7.0 Hz, ArH), 7.75 (2H, d, 3J = 7.5 Hz, ArH) ppm; δC (CDCl₃, 63 MHz); 22.5 (CH₂), 30.8 (CH₂), 34.2 (NCH₃), 47.2 (CH₂), 47.9 (CH₂), 67.0 (CH₂), 119.8 (ArC), 124.7 (ArC), 126.9 (ArC), 127.5 (ArC), 141.2 (i-ArC), 143.9 (i-ArC), 156.1 (C=O carbamate), 178.2 (C=O acid) ppm; ν max (thin film), 3000-2800 (CH), 1701 (C=O) cm⁻¹; m/z; found (FAB) [M+H]+ 340.15440, C₂₀H₂₂N₂O₄ requires 340.15488.

6.12.2 (3-Methylpolystyrene-carbapamoyl-propyl)-methyl-carbamic acid 9H-fluoren-9-ylmethyl ester 69

4-[(9H-Fluorene-9-ylmethoxy carbonyl)-methyl-amino]-butyric acid 68 (4 g, 11.8 mmol), EDC.HCl (2.26 g, 11.8 mmol), HOBT.H₂O (1.59 g, 11.8 mmol), DIPEA (4.1 mL, 23.6 mmol) and DMF (17 mL) was added to a 100 mL round bottomed flask containing N-methylaminomethylpolystyrene 51 (1.71 g, L max = 1.33 mmol/g, 2.27 mmol). The mixture was rotated at room temperature for 2 ½ hours then the resin was filtered and washed according to the standard protocol. The coupling was then repeated for a further 2 ½ hours and the resin washed again according to the standard protocol and dried in the vacuum oven overnight. The resin showed a negative chloranil test. L max = 0.64 mmol/g; δH (MAS, CDCl₃, 400 MHz); 1.7 (CH₂), 1.9 (CH₂ and CH₂ PS), 2.1 (CH₂), 2.3 (CH₂), 2.8 (NCH₃), 2.9 (NCH₃), 3.1 (CH₂), 3.35 (CH₂), 168
4.20 (CH), 4.42 (CH₂), 6.50 (ArH PS), 7.04 (ArH PS), 7.25 (ArH), 7.31 (ArH), 7.55 (ArH), 7.68 (ArH) ppm; νmax (DCM): 3100-2800 (CH), 1696 (C=O, carbamate), 1641 (C=O, amide), 1641 (C=O, amide), 1601 (Ar C=C) cm⁻¹.

6.12.3 N-Methylaminomethylpolystyrene-4-methylamino-butyramide 70

Fmoc-protected amino resin 69 (2.23 g, Lcalc = 0.64 mmol/g, 1.42 mmol) was rotated in 20% piperidine in DMF (20 mL) for 5 mins. The resin was washed with DMF and the procedure repeated. The resin was then washed according to the standard protocol and dried in the vacuum oven overnight. The resin showed a dark blue colouration with the chloranil and bromophenol blue tests. δH (MAS, CDCl₃, 400 MHz); 1.8 (CH₂ and CH₂ PS), 2.1 (CH₂), 2.3 (2 × NCH₃), 2.7 (CH₂), 6.5 (ArH PS), 7.0 (ArH PS) ppm; νmax (DCM): 3100-2800 (CH), 1638 (C=O, amide), 1601 (Ar C=C).

6.12.4 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid [3-(methylpolystyrene-methyl-carbonyl)-propyl]-methyl-amide 71

Birch acid 36 (2.3 g, 10.4 mmol) and DIC (1.7 mL, 10.8 mmol) were dissolved in DMF (9 mL). The solution was added to a pre-swollen mixture of amino resin 70 (1.76 g, Lcalc = 0.75 mmol/g, 1.32 mmol) in DCM (9 mL). The mixture was rotated at RT for 2½ hours. The resin was filtered and washed according to the standard protocol. The coupling was repeated for a further 2½ hours and the resin washed according to the standard protocol again before being dried in the vacuum oven overnight. The resin gave a negative chloranil test. δH (MAS, CDCl₃, 400 MHz); 0.7 (CH₂CH₃), 1.4 (CH₂), 1.8 (CH₂CH₃ and CH₂ PS), 2.3 (CH₂), 2.8 (CH₂), 2.9 (NCH₃), 3.0 (NCH₃), 3.3 (CH₂), 3.5 (OCH₃), 3.7 (CH₂), 4.5 (H₂/H₆), 6.5 (ArH PS), 7.05 (ArH PS) ppm; νmax (DCM): 1691 (C=O tertiary amide), 1601 (Ar C=C) cm⁻¹.
6.13 Alternative enol ether protecting groups – silyl enol ethers

6.13.1 3,5-Dihydroxy-benzoic acid methyl ester 74

\[
\begin{align*}
\text{HO} & \quad \text{4} \quad \text{3} \quad \text{OH} \\
\text{6} \quad \text{5} & \quad \text{O} \\
& \quad \text{1} \\
& \quad \text{CO}_2\text{Me}
\end{align*}
\]

3, 5-Dihydroxybenzoic acid 73 (5 g, 32.5 mmol) was dissolved in anhydrous methanol (50 mL) then concentrated sulfuric acid (10 mL) was added dropwise. The mixture was refluxed at 80 °C for 4 ½ hours then concentrated *in vacuo* to remove the methanol. The residue was dissolved in ethyl acetate (100 mL) and washed with saturated aqueous NaHCO₃, water and brine (all 50 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to give a white crystalline solid (6.14 g, >99%). *m.p.; 168-170 °C (literature 170 °C);* *r.f. (10 % MeOH/DCM); 0.32; *δ*₇ (CD₃OD, 200 MHz); 3.91 (3H, s, CO₂CH₃), 6.54 (1H, t, *³J* = 2.5 Hz, H4), 6.99 (2H, d, *³J* = 2.5 Hz, H2/H6), 9.70 (2H, s, 2 × OH) ppm; *δ*₂ (CDCl₃, 63 MHz); 51.1 (CO₂CH₃), 105.4 (ArC), 106.8 (ArC), 107.4 (ArC), 131.6 (i-ArC), 158.3 (C=O) ppm; *ν* max (nujol mull); 3237 (OH), 3100-2854 (CH), 1694 (C=O ester), 1635 (C=C), 1602 (Ar C=C) cm⁻¹; *m/z; found (ES⁺) [M-H]⁺ 167 C₈H₇O₄ requires 167.

6.13.2 General procedure: Synthesis of silanyloxy benzoic acid methyl esters 75 and 76

\[
\begin{align*}
R_3O & \quad \text{OR}_3 \\
\text{CO}_2\text{Me} & \quad \text{75 R = TBDMS} \\
\text{76 R = TIPS}
\end{align*}
\]

3,5-Dihydroxy-benzoic acid methyl ester 74 (1 g, 5.95 mmol) was dissolved in DMF (17.5 mL) and trialkysilyl chloride (TBDMS-Cl or TIPS-Cl) (12.2 mmol) with imidazole (2.03 g, 29.8 mmol) added portionwise. The mixture was stirred at room temperature for 4 ½ hours then diluted with water (100 mL) and ethyl acetate (100 mL). The ethyl acetate layer was separated and washed with brine (50 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield the products as crude oils.

170
3,5-Bis-(tert-butyl-dimethyl-silanyloxy)-benzoic acid methyl ester 75 was used without further purification as the crude oil (> 99%). \( \text{rf} \) (2 % EtOAc/hexane); 0.23; \( \delta_H \) (CDCl\(_3\), 200 MHz); 0.12 (12H, s, 2 × Si(CH\(_3\))\(_3\)), 0.97 (18H, s, 2 × SiC(CH\(_3\))\(_3\)), 3.87 (3H, s, CO\(_2\)CH\(_3\)), 6.50 (1H, t, \( ^3J = 2.5 \) Hz, H4), 7.12 (2H, d, \( ^3J = 2.5 \) Hz, H2/H6) ppm; \( \delta_C \) (CDCl\(_3\), 63 MHz); -4.6 (4 × Si(CH\(_3\))\(_2\)), 18.1 (2 × SiC(CH\(_3\))\(_3\)), 25.5 (2 × SiC(CH\(_3\))\(_3\)), 52.0 (CO\(_2\)CH\(_3\)), 114.4 (C4), 116.7 (C2/C6), 131.7 (C1), 156.4 (C3/C5), 166.7 (C=O) ppm; \( v_{\text{max}} \) (thin film); 2955 (CH), 2931 (CH), 2886 (CH), 2859 (CH), 1729 (C=O), 1590 (Ar C=C) cm\(^{-1}\); m/z; found (ES\(^+\)) [M+H]\(^+\) 397 C\(_{20}\)H\(_{37}\)O\(_4\)Si\(_2\) requires 397.

3,5-Bis-triisopropylsilanyloxy-benzoic acid methyl ester 76 was purified by column chromatography on silica eluting with 5 % ethyl acetate in hexane to give a clear colourless oil (2.53 g, 89 %). \( \text{rf} \) (2 % EtOAc/hexane); 0.33; \( \delta_H \) (CDCl\(_3\), 200 MHz); 1.17 (36H, d, \( ^3J = 5.0 \) Hz, 2 × Si[CH(CH\(_3\))\(_2\)]\(_3\)), 1.30 (6H, m, 2 × Si[CH(CH\(_3\))\(_2\)]\(_3\)), 3.96 (3H, s, CO\(_2\)CH\(_3\)), 6.68 (1H, t, \( ^3J = 2.5 \) Hz, H4), 7.22 (2H, d, \( ^3J = 2.5 \) Hz, H2/H6) ppm; \( \delta_C \) (CDCl\(_3\), 63 MHz); 13.0 (2 × Si[CH(CH\(_3\))\(_2\)]\(_3\)), 18.3 (2 × Si[CH(CH\(_3\))\(_2\)]\(_3\)), 52.6 (CO\(_2\)CH\(_3\)), 114.6 (C4), 116.4 (C2/C6), 132.1 (C1), 157.3 (C3/C5), 167.4 (C=O) ppm; \( v_{\text{max}} \) (thin film); 3100-2800 (CH), 1729 (C=O), 1589 (Ar C=C) cm\(^{-1}\); m/z; found (ES\(^+\)) [M+H]\(^+\) 481 C\(_{26}\)H\(_{49}\)O\(_4\)Si\(_2\) requires 481.

6.13.3 3,5-Bis-(tert-butyl-dimethyl-silanyloxy)-1-ethyl-cyclohexa-2,5-dienecarboxylic acid methyl ester 77

Ammonia (19 mL) was condensed into a 100 mL three necked flask attached to a condenser. 3,5-Bis-(tert-butyl-dimethyl-silanyloxy)-benzoic acid methyl ester 75 (0.5 g, 1.26 mmol) dissolved in THF (6 mL) was added using a glass pipette and the contents of the flask cooled to −78 °C. s-Butanol (115 μL, 1.26 mmol) and THF (4 mL) were added followed by lithium metal (22.1 mg, 3.15 mmol) to form a dark blue solution. The coloured solution was stirred at −78 °C for 20 minutes then bromoethane (94.5 μL, 1.32 mmol) added dropwise to form an orange solution. The
mixture was stirred at -78 °C for a further hour then the condenser removed and the ammonia allowed to evaporate into water overnight. The brown solution formed was diluted with water (50 mL) and extracted three times with ethyl acetate (3 × 50 mL). The combined ethyl acetate layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield an orange oil. The crude oil was purified by chromatography on silica eluting with 2 % ethyl acetate in hexane to yield a clear colourless oil (0.23 g, 43 %). 

### 6.13.4 3,5-Bis-(tri-isopropyl-silanyloxy)-1-ethyl-cyclohexa-2,5-dienecarboxylic acid methyl ester 78

![molb](attachment:0.png)

Ammonia (70 mL) was condensed into a 250 mL three necked flask attached to a condenser. 3,5-Bis-(triisopropyl-silanyloxy)-benzoic acid methyl ester 76 (1 g, 2.08 mmol) dissolved in THF (30 mL) was added using a glass pipette and the contents of the flask cooled to -78 °C. s-Butanol (191 µL, 2.08 mmol) was added followed by lithium metal (46.8 mg, 5.20 mmol) to form a dark blue solution. The coloured solution was stirred at 0 °C for 20 minutes then bromoethane (156 µL, 2.18 mmol) added dropwise to form a white cloudy solution. The ammonia allowed to evaporate into water over three hours and the THF removed by concentrating in vacuo. The white crystalline solid formed was diluted with ethyl acetate (50 mL) and washed two times with water (2 × 50 mL) and once with brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a yellow oil. The crude oil
was purified by chromatography on silica eluting with 2% ethyl acetate in hexane to yield a clear colourless oil (179 mg, 17%). *rf* (10% EtOAc/pet ethers 40-60 °C) 0.54; δH (CDCl3, 200 MHz); 0.87 (3H, t, 3J = 7.5 Hz, CH2CH3), 1.16 (36H, d, 3J = 5.5 Hz, 2 × Si[CH(CH3)2]3), 1.23 (6H, septet, 3J = 5.0 Hz, 2 × Si(CHMe2)3), 1.73 (2H, q, 3J = 7.5 Hz, CH2CH3), 2.81 (2H, s, H4), 3.69 (3H, s, CO2CH3), 4.90 (2H, s, H2/H6) ppm; δC (CDCl3, 93 MHz); 9.3 (CH2CH3), 13.0 (2 × Si[CH(CH3)2]3), 18.4 (2 × Si[CH(CH3)2]3), 33.5 (CH2CH3), 35.5 (C4), 51.3 (C1), 52.1 (CO2CH3), 104.2 (C2/C6), 149.0 (C3/C5), 176.7 (C=O) ppm; vmax (thin film) 3000 - 2800 (CH), 1733 (C=O), 1696 (C=O), 1658 (C=C), 1588 (C=C) cm⁻¹; m/z; found (FAB) [M+H]+ 511.36212, C28H55O4Si2 requires 511.36389.

### 6.14 Alternative protecting groups - β-diketone protection

#### 6.14.1 1-Ethyl-3-hydroxy-5-oxo-cyclohex-3-enecarboxylic acid 80

Birch acid 36 (11.5 g, 54.2 mmol) was dissolved in THF (100 mL) and 2M HCl (100 mL) was added dropwise. The mixture was stirred at room temperature for three hours then concentrated *in vacuo* to remove the THF. The resultant mixture was diluted with ethyl acetate (100 mL) and the ethyl acetate layer was separated. The aqueous layer was washed with brine (100 mL) before being dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield an orange solid. The crude material was triturated (with slight warming) in 50% ethyl acetate in hexane to give white crystals (7.58 g, 75%). **mp**: 163-165 °C; *rf* (20% MeOH/DCM); 0.18; δH (DMSO, 200 MHz) 0.88 (3H, t, 3J = 7.0 Hz, CH2CH3), 1.67 (2H, q, 3J = 7.5 Hz, CH2CH3), 2.34 (2H, m, H6), 2.68 (2H, m, H2), 5.21 (1H, s, CH=CO) ppm; δC (d4-MeOD, 93 MHz) 8.0 (CH2CH3), 31.7 (CH2CH3), 40.3 (C2/C6), ~50 (observed by d4-MeOD peak, C1), 103.3 (CH=CO), 177.1 (C3), ~190.0 (C=O) ppm; vmax (nujol mull) 3000 - 2800 (CH), 1708 (C=O acid), 1607 (C=O), 1511 (C=C) cm⁻¹; m/z; found (FAB) 185.08147 C9H13O4 requires 185.08138.
6.14.2 General procedure: Synthesis 1-Ethyl-3-alkoxy-5-oxo-cyclohex-3-enecarboxylic acids 81 to 83

1-Ethyl-3-hydroxy-5-oxo-cyclohex3-enecarboxylic acid 80 (0.5 g, 2.72 mmol) and p-toluenesulfonic acid monohydrate (52 mg, 0.27 mmol) were dissolved in the corresponding alcohol (30 mL). The mixture was heated at reflux for five hours then concentrated in vacuo to remove the alcohol. The resultant mixture was diluted with ethyl acetate (100 mL) and the ethyl acetate layer washed twice with water (2 × 100 mL) and once with brine (100 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to yield the product 1-Ethyl-3-iso-propyloxy-5-oxo-cyclohex-3-enecarboxylic acid 81 was not purified and was obtained as a clear pale yellow oil that crystallised on standing to give white crystals (539 mg, 88 %). mpt; 78-80 °C; rf (20 % MeOH/DCM); 0.55; δH (CDCl₃, 200 MHz); 0.98 (3H, t, 3J = 7.5 Hz, CH₂CH₃), 1.35 (6H, d, 3J = 6.5 Hz, CH(CH₃)₂), 1.80 (2H, q, 3J = 7.5 Hz, CH₂CH₃), 2.33 (2H, m, H₆), 2.95 (2H, m, H₂), 4.50 (1H, septet, 3J = 6.0 Hz, CH(CH₃)₂), 5.43 (1H, s, CH=CO) 9.31 (1H, br s, CO₂H) ppm; δC (CDCl₃, 93 MHz); 8.7 (CH₂CH₃), 21.5 (CH(CH₃)₂), 31.5 (CH₂CH₃), 37.3 (C6), 44.0 (C2), 47.7 (C1), 71.8 (CH(CH₃)₂), 102.6 (CH=CO), 175.3 (C3), 180.0 (C=O acid) 198.0 (C=O enol) ppm; ν max (thin film) 2978 (CH), 2937 (CH), 1726 (C=O acid), 1589 (C=O) cm⁻¹; m/z; found (FAB) [M+H]+ 227.12789, C₁₂H₁₉O₄ requires 227.12833.

1-Ethyl-3-sec-butyloxy-5-oxo-cyclohex-3-enecarboxylic acid 82 was not purified and was obtained as a clear colourless oil that crystallised on standing to give white crystals (775 mg, > 99 %). mpt; 103-105 °C; rf (20 % MeOH/DCM); 0.53; δH (CDCl₃, 200 MHz); 0.74 (3H, t, 3J = 7.5 Hz, CH₂CH₃), 0.84 (3H, t, 3J = 7.0 Hz, CHMeCH₂CH₃), 1.18 (3H, d, 3J = 6.0 Hz, CH(CH₃)CH₂CH₃), 1.50 (2H, m, CHMeCH₂CH₃), 1.67 (2H, q, 3J = 7.5 Hz, CH₂CH₃), 2.32 (2H, m, H₆), 2.79 (2H, m, H₄), 4.14 (1H, m, CHMeCH₂CH₃), 5.31 (1H, s, CH=CO) 9.72 (1H, br s, CO₂H) ppm; δC (CDCl₃, 93 MHz); 9.1 (CH₃), 9.9 (CH₃), 19.0 (CH₂CH₃), 28.9 (CH₂CH₃),
31.7 (CH₂CH₃), 37.5 (C₆), 44.3 (C₄), 48.0 (C₁), 82.3 (CHCH₃), 102.8 (CH=CO), 176.3 (C₃), 180.1 (C=O acid) 198.7 (C=O enol) ppm; \( \nu_{\max} \) (thin film) 2969 (CH), 2935 (CH), 2880 (CH), 1717 (C=O acid), 1606 (C=O), 1578 (C=C) cm⁻¹; m/z; found (FAB) [M+H]⁺ 241.14401, C₁₃H₂₀O₄ requires 241.14398.

1-Ethyl-3-allyloxy-5-oxo-cyclohex-3-enecarboxylic acid 83 was purified by column chromatography on silica eluting with 10% methanol in dichloromethane to yield a clear colourless oil (465 mg, 76%). \( \text{rf} \) (20% MeOH/DCM); 0.58; \( \delta_H \) (CDCl₃, 200 MHz); 0.85 (3H, t, \( ^3J = 7.5 \) Hz, CH₂CH₃), 1.68 (2H, q, \( ^3J = 7.5 \) Hz, CH₂CH₃), 2.22 (1H, d, \( ^2J = 16.5 \) Hz, H6), 2.37 (1H, d, \( ^2J = 17.0 \) Hz, H6), 2.78 (1H, d, \( ^2J = 12.0 \) Hz, H2), 2.87 (1H, d, \( ^2J = 12.5 \) Hz, H2), 4.32 (2H, d, \( ^3J = 5.5 \) Hz, CH₂CH=CH₂), 5.26 (3H, m, CH=CO and CH=CH₂), 5.89 (1H, m, CH=CH₂), 8.98 (1H, br s, CO₂H) ppm; \( \delta_C \) (CDCl₃, 93 MHz); 9.1 (CH₂CH₃), 31.8 (CH₂CH₃), 31.1 (C₆), 44.4 (C₂), 48.0 (C₁), 70.0 (CH₂CH=CH₂), 103.1 (CH=CO), 119.5 (CH=CH₂), 131.5 (CH=CH₂), 176.3 (C₃), 180.1 (C=O acid) 197.0 (C=O enol) ppm; \( \nu_{\max} \) (thin film) 3083 (CH), 2969 (CH), 2938 (CH), 2883 (CH), 1725 (C=O acid), 1597 (C=C) cm⁻¹; m/z; found (FAB) [M+H]⁺ 225.11300 C₁₂H₇O₄ requires 225.11268.

6.14.3 1-Ethyl-3-alkoxy-5-oxo-cyclohex-3-enecarboxylic acid methylpolystyrene-methyl-amide resins 84 to 86

Acids 81 to 83 (0.54 mmol), were dissolved in DMF (0.5 mL) and DIC (84 μL, 0.54 mmol) was added dropwise. Meanwhile N-methylaminomethyl resin 51 (100 mg, \( L_{\max} = 1.33 \) mmol/g, 0.13 mmol) was swollen in DCM (0.5 mL) the the contents of the first flask were transferred to the conical flask containing the resin. Each resulting mixture was shaken at room temperature for 2 ½ hours and the resins filtered and washed with according to the standard protocol. The coupling repeated was repeated for 2 ½ hours, the resins washed according to the same protocol and dried overnight in the vacuum oven.

1-Ethyl-3-iso-propoxy-5-oxo-cyclohex-3-enecarboxylic acid methyl polystyrene-methyl-amide resin 81 gave a negative chloranil test. \( \delta_H \) (MAS,
CDCl₃, 400 MHz); 0.9 (CH₂CH₃), 1.3 (2 × CH(CH₃)₂), 1.4 (CH₂ PS), 1.8 (CH₂CH₃), 2.4 (CH₂), 2.8 (NCH₃), 2.9 (CH₂), 4.4 (CH₂NMe), 4.6 (CHMe₂), 5.3 (CH=CO), 6.1 (ArH PS), 7.1 (ArH PS) ppm; νₓ max (DCM); 3000-2800 (CH), 1644 (C=C enol), 1602 (Ar C=C) cm⁻¹.

1-Ethyl-3-sec-butyloxy-5-oxo-cyclohex-3-enecarboxylic acid methylpolystyrene-methyl-amide resin 82 gave a negative chloranil test. δH (MAS, CDCl₃, 400 MHz); 0.9(2 × CH₂CH₃), 1.3 (CHCH₃), 1.4 (CH₂ PS), 1.7(CH₂ PS), 1.9 (2 × CH₂CH₃), 2.5 (CH₂), 2.8 (NCH₃ and CH₂), 4.2 (CHCH₃), 4.6 (NMeCH₂), 5.4 (CH=CO), 6.5 (ArH PS), 7.0 (ArH PS) ppm; νₓ max (DCM); 3100-2800 (CH), 1645 (C=C), 1634 (C=C), 1601 (C=O) cm⁻¹.

1-Ethyl-3-allyloxy-5-oxo-cyclohex-3-enecarboxylic acid methylpolystyrene-methyl-amide resin 83 gave a negative chloranil test. δH (MAS, CDCl₃, 400 MHz); 0.9 (CH₂CH₃), 1.4 (CH₂ PS), 1.7 (CH₂ PS), 2.1 (CH₂CH₃), 2.4 (CH₂), 2.9 (NCH₃ and CH₂), 4.4 (CH₂CH=CH₂), 4.6 (NMeCH₂), 5.3 (CH=CO), 5.4 (CH₂CH=CH₂), 5.9 (CH₂CH=CH₂), 6.5 (ArH PS), 7.0 (ArH PS) ppm; νₓ max (DCM); 3000-2800 (CH), 1626 (C=C enol), 1602 (Ar C=C) cm⁻¹.

6.15 C4-Acylation and alkylation

6.15.1 2-Hydroxy-6,6-dimethyl-4-oxo-cyclohex-2-enecarboxylic acid ethyl ester 89

Anhydrous ethanol (5 mL) was added to an oven dried three necked flask and sodium (0.2 g, 8.74 mmol) added portionwise. Once the sodium had dissolved diethylmalonate (1.33 mL, 8.74 mmol) was added via a dropping funnel. Ethanol (1 mL) was used to rinse out the funnel then 4-methylpent-3-en-2-one 88 (1 mL, 8.74 mmol) added to form a yellow solution. The funnel was rinsed again with ethanol (1 mL) and the mixture heated under reflux for 45 minutes to form a cloudy solution. The mixture was diluted with water (50 mL) and ethyl acetate (50 mL) and 2M HCl (10 mL) added to pH 2 to give a clear colourless solution. The ethyl acetate layer
was separated and the aqueous layer washed twice more with ethyl acetate (2 × 50 mL). The combined ethyl acetate layers were washed with water (50 mL) then brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil. The crude oil was purified by column chromatography on silica eluting with 5 % MeOH in DCM to yield the product (1.39 g, 75 %). The absence of enolic proton NMR peaks can be explained by enol-keto tautomerism in deuterated methanol. rf (10 % MeOH/DCM); 0.41; δH (CD3OD, 250 MHz); 1.40 (6H, s, 2 × CH₃), 1.54 (3H, t, 3J = 7.0 Hz, OCH₂CH₃), 2.37-2.50 (1H, m, H5), 2.85-2.92 (1H, m, H5), 3.41 (1H, s, CHCO₂Et), 4.43 (2H, q, 3J = 7.0 Hz, OCH₂CH₃) ppm; δC (CD3OD, 63 MHz); 12.5 (2 × CH₃), 24.1 (CH₂), 26.8 (CH₂), 34.0 (C6), 42.1 (OCH₂CH₃), 60.3 (CHCO₂Et), 169.1 (C=O ester) ppm; νmax (thin film) 3435 (OH), 2969 (CH), 1729 (C=O ester), 1605 (C=C) cm⁻¹; m/z; found (ES⁺) [M+H]+ 213 C₁₁H₁₇O₄ requires 213.

6.15.2 2-Hydroxy-6,6-dimethyl-4-oxo-cyclohex-2-enecarboxylic acid 90

Ester 89 (0.41 g, 1.93 mmol) was dissolved in 1,4-dioxane (12 mL) and lithium hydroxide monohydrate (179 mg, 4.26 mmol) in water (4 mL) added dropwise. Water (0.5 mL) was added and the yellow solution heated to 80 °C for 6 hours. The mixture was dissolved in ethyl acetate (100 mL) and 2M HCl (50 mL). The ethyl acetate layer was separated and the aqueous layer washed twice more with ethyl acetate (2 × 25 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear yellow oil (281 mg, 79 %). The absence of enolic proton NMR peaks can be explained by enol-keto tautomerism in deuterated methanol. rf (10 % MeOH/DCM); 0.11; δH (CD3OD, 200 MHz); 1.35 (6H, s, 2 × CH₃), 2.23 (1H, s, H5), 2.45 (1H, s, H5), 2.92 (1H, s, CHCO₂H) ppm; δC (CD3OD, 63 MHz); 24.9 (2 × CH₃), 33.8 (C6), 50.1 (CHCO₂H), 50.9 (CH₂), 170.2 (C=O acid), 208.8 (C=O enol) ppm; νmax (thin film) 3429 (OH), 2967 (CH), 1710 (C=O acid), 1636 (C=C) cm⁻¹; m/z; found (ES⁺) [M+NH₃⁺] 201 C₉H₁₅NO₄ requires 201.
6.15.3 4-Methylpolystyrene-3-hydroxy-5,5-dimethyl-cyclohex-2-enone

Diisopropylamine (2.10 mL, 15.0 mmol) was added to chilled anhydrous THF (10 mL) in a three necked flask. "BuLi (9.4 mL, 1.6M in hexanes, 15.0 mmol) was added drowise and the solution stirred at 0 °C for 30 mins in a dry ice/acetone bath. After this time the solution was cooled to -78 °C and dimesdone (1g, 7.14 mmol) and DMPU (4.32 mL, 35.7 mmol) in THF (5 mL) added over 5 minutes to give a cloudy cream solution. After stirring under nitrogen for one hour at -78 °C, Merrifield resin I (0.92 g, L\text{\textit{quote}} = 3.89 mmol/g, 3.57 mmol) and sodium iodide (0.54 g, 3.57 mmol) were added in one portion keeping the mixture under nitrogen. THF (1 mL) was added to wash the resin down the sides of the flask and the mixture allowed to reach room temperature before being shaken overnight under an argon balloon. The resin was filtered and washed with 2 × MeOH, 2 × MeOH/2M HCl (1:1), 2 × DMF, 2 × DMF/2M HCl (1:1), 2 × DMF/H\text{\textit{2O}} (1:1) then washed according to the standard protocol then dried in the vacuum overnight. δ\text{\textit{H}} (MAS, CDCl₃, 600 MHz); 1.2 (2 × CH₃), 1.4 (CH₂ PS), 2.8 (CH₂), 6.7 (ArH PS), 7.1 (ArH PS) ppm; ν\text{\textit{max}} (DCM); 3054 (CH), 2986 (CH), 1715 (C=O keto), 1691 (C=O enol), 1603 (Ar C=C) cm⁻¹.

6.15.4 [2-(3-Benzyl-2-hydroxy-4,4-dimethyl-6-oxo-cyclohex-1-enyl)-2-oxoethyl]-carbamic acid 9H-fluoren-9-ylmethyl ester

Fmoc-Gly-OH (374 mg, 1.26 mmol) was dissolved in DMF (0.5 mL) with DMAP (15 mg, 1.26 mmol) and DIC (197 μL, 1.26 mmol) added. Diketone resin 91 (0.1 g, 0.26 mmol) was swollen in DCM (0.5 mL) and solutions mixed. The reaction mixture was then rotated at RT overnight then the resin filtered and washed according to the standard protocol. The coupling reaction was repeated overnight...
and the resin washed according to the same protocol then dried in the vacuum oven overnight. $L_{uv} = 0.23 \text{ mmol/g}; \delta_H (\text{MAS, } CDCl_3, 600 \text{ MHz}); 1.0 (2 \times \text{CH}_3), 1.4(\text{CH}_2 \text{ PS}), 2.5 (\text{CH}_2), 4.0-4.8 (\text{CH Fmoc, NHCH}_2 \text{, OCH}_2 \text{ Fmoc}), 6.7 (\text{ArH PS}), 7.1(\text{ArH PS}), 7.7 (\text{ArH Fmoc}), 7.9 (\text{ArH Fmoc}) \text{ ppm}; v_{\text{max}} (\text{DCM}); 3054 (\text{CH}), 3027 (\text{CH}), 2985 (\text{CH}), 2959 (\text{CH}), 2850 (\text{CH}), 1771 (\text{C=O carbamate}), 1716 (\text{C=O keto}), 1689 (\text{C=O enol}), 1651 (\text{C=C}), 1604 (\text{Ar C=C}) \text{ cm}^{-1}.

### 6.16 Synthesis of high loading resins

#### 6.16.1 Fmoc-Lys(Boc)-N-methylamidomethylpolystyrene resin 95

![Fmoc-Lys(Boc)-N-methylamidomethylpolystyrene resin 95](image)

$N$-Methylaminomethylpolystyrene resin 51 ($50 \text{ mg, } L_{\text{calc}} = 1.323 \text{ mmol, } 0.07 \text{ mmol}$) was swollen in DCM ($0.25 \text{ mL}$). Fmoc-Lys(Boc)-OH ($162 \text{ mg, } 0.35 \text{ mmol}$) was dissolved in DMF ($0.25 \text{ mL}$) and DIC ($54 \mu\text{L, } 0.35 \text{ mmol}$) was added dropwise. This solution was added to the isolute tube containing the resin and the mixture rotated at room temperature for $2 \frac{1}{2} \text{ hours}$. The resin was filtered and washed according to the standard protocol and dried overnight in the vacuum oven. The resin gave a negative chloranil test. $L_{uv} = 0.59 \text{ mmol/g}; v_{\text{max}} (\text{DCM}); 3421 (\text{NH}), 1712 (\text{C=O carbamate}), 1644 (\text{C=O tertiary amide}), 1602 (\text{Ar C=C}) \text{ cm}^{-1}.$

#### 6.16.2 Attempted synthesis of high loading $N$-methylaminomethyl polystyrene resin 97

Merrifield resin 1 ($50 \text{ mg, } L_{\text{quote}} = 3.89 \text{ mmol g}^{-1}, 0.19 \text{ mmol}$) was swollen in DMF ($0.5 \text{ mL}$) containing MeNH$_2$HCl ($66 \text{ mg, } 0.97 \text{ mmol}$) and DIPEA ($330 \mu\text{L, } 1.90 \text{ mmol}$). The mixture was rotated at room temperature for 24 hours then the resin filtered and washed according to the standard protocol and dried overnight in the vacuum oven to give a crunchy textured resin. The resin beads gave a dark blue colouration with $p$-chloranil so were taken on to the next stage without analysis.
6.16.3 Loading determination by Fmoc-UV analysis: [(Methylpolystyrene-methyl-carbamoyl)-methyl]-carbamic acid 9H-fluoren-9-ylmethyl ester 99

\[
\text{FmocHN} \quad \text{N} \quad \text{PS} \\
\text{O}
\]

The resin was synthesised according to the procedure in section 6.9.3. The resin showed a negative chloranil test. \( L_{\text{uv}} = 0.30 \text{ mmol/g}; \nu_{\text{max}} (\text{DCM}); 3436 (\text{NH}), 3054 (\text{CH}), 2984 (\text{CH}), 2926 (\text{CH}), 2850 (\text{CH}), 2787 (\text{CH}), 1730 (\text{C}=\text{O carbamate}), 1675 (\text{C}=\text{O tertiary amide}), 1603 (\text{Ar C} = \text{C}) \text{ cm}^{-1}.

6.16.4 Analysis of commercially available trisamine resin 100

\[
\text{H}_2\text{N} \quad \text{N} \quad \text{PS} \\
\text{NH}_2 \quad \text{R} \quad \text{D}
\]

The resin was commercially available from Argonaut Technologies. The resin showed positive chloranil, Kaiser and TNBS tests. \( \delta_{\text{H}} (\text{MAS, CDCl}_3, 600 \text{ MHz}); 1.30 (\text{CH}_2 \text{PS}), 2.55 (\text{CH}_2\text{CH}_2 \text{PS}), 3.30 (\text{NH}), 4.37 (\text{NCH}_2\text{Ar}), 6.57 (\text{ArH PS}), 7.07 (\text{ArH PS}) \text{ ppm}; \nu_{\text{max}} (\text{DCM}); 3200 (\text{broad NH}), 3060 (\text{CH broad}), 1681 (\text{C}=\text{C}) \text{ cm}^{-1}.

6.16.5 Loading determination by Fmoc-UV analysis: Fmoc-Gly-tris-(2-aminoethyl)aminomethylpolystyrene resin 101

\[
\text{FmocHN} \quad \text{N} \quad \text{D} \\
\text{O}
\]

The resin was synthesised according to the procedure in section 6.9.3. The resin showed a negative chloranil and Kaiser tests. \( L_{\text{uv}} = 1.42 \text{ mmol/g}; \nu_{\text{max}} (\text{DCM}); 3409 (\text{NH}), 3316 (\text{NH}), 3100-2800 (\text{CH}), 1734 (\text{C}=\text{O carbamate}), 1650 (\text{C}=\text{O tertiary amide}), 1599 (\text{Ar C}=\text{C}) \text{ cm}^{-1}.
6.16.6 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid tris-(2-aminoethyl)aminomethylpolystyrene-amide 102

Birch acid 36 (12.9 mg, 60.8 mmol), was dissolved in DMF (45 mL) and DIC (9.51 mL, 60.8 mmol) was added dropwise. This solution was added to tris-(2-aminoethyl)aminomethyl polystyrene resin 100 (9 g, L_{calc} > 5 mmol/g, > 45 mmol) pre-swollen in DCM (45 mL). The resulting mixture was shaken at room temperature for 2 ½ hours and the resin filtered and washed according to the standard protocol. The coupling repeated was repeated for 2 ½ hours, the resin washed according to the same protocol and dried overnight in the vacuum oven. The resin gave a negative chloranil test. δH (MAS, CDCl3, 600 MHz); 0.76 (CH2CH3), 1.10 (CH2CH2), 1.40 (CH2), 1.78 (CH2 PS and CH2CH3), 2.58 (H4), 2.79 (NCH3), 3.21 (NH), 3.54 (2 x OCH3), 4.32 (NCH2), 4.54 (H2/H6), 6.56 (ArH PS), 7.07 (ArH PS) ppm; νmax (DCM); 3414 (NH), 3100-2800 (br, CH), 1691 (C=O tertiary amide), 1653 (C=O secondary amide), 1621 (C=C), 1602 (Ar C=C) cm⁻¹.

6.16.7 1-Ethyl-3-methoxy/hydroxy-5-oxo-cyclohex-3-enecarboxylic acid tris-(2-aminoethyl)aminomethylpolystyrene-amide 103

Bis-enol ether resin 103 (5 g, L_{max} > 5 mmol/g, > 25 mmol) was shaken with TFA (45 mL), water (2.5 mL) and DMF (2.5 mL) for 2 ½ hours at room temperature. The resin was filtered and washed according to the standard protocol then dried overnight in the vacuum oven. δH (MAS, CDCl3, 600 MHz); 0.89 (CH2CH3), 1.10 (CH2CH2), 1.40 (CH2 PS), 1.72 (CH2CH3), 2.39 (H6), 3.04 (H4), 3.26 (NH), 3.63 (residual OMe), 5.30 (CH=CO), 6.56 (ArH PS), 7.05 (ArH PS) ppm; δC (MAS, CDCl3, 600 MHz); 9.0 (CH2CH3), 33.0 (CH2CH3), 36.5 (NMe), 41.0 (CH2), 48.0 (CH2), 40.5 (C5), 54.0 (CH2), 57.0 (residual OMe), 101.5 (CH=CO), 128.0 (broad ArC PS),
145.5 (i-\text{ArC PS}), 162.0 (C=O enol ether), 175.5 (C=O amide) ppm; $\nu_{\text{max}}$ (DCM); 3330 (NH), 3200-2800 (br, CH), 1666 (C=O), 1603 (Ar C=C) cm$^{-1}$.

6.16.8 Loading determination by elemental analysis: -1-Ethyl-3-chloro-5-oxo-cyclohex-3-enecarboxylic acid tris-(2-aminoethyl)aminomethyl polystyrene-amide 104

The resin was synthesised according to the procedure in section 6.10.4. $L_{\text{ea}} = 2.75$ mmol/g; $\delta_{\text{H}}$ (MAS, 600 MHz, CDCl$_3$); 0.9 (CH$_2$CH$_3$), 1.4 (CH$_2$CH$_3$ and CH$_2$ PS), 1.9 (CH$_2$), 4.2 (NHCH$_2$Ar), 6.6 (ArH PS), 7.1 (ArH PS) ppm; $\nu_{\text{max}}$ (DCM); 3418 (NH), 3055 (CH), 2976 (CH), 1690 - 1600 (C=O and C=C) cm$^{-1}$.

6.17 Synthesis of CHD-polystyrene resin

6.17.1 1-Ethyl-3-hydroxy-5-oxo-cyclohex-3-enecarboxylic acid methylaminomethylpolystyrene-amide (CHD) resin 57

3-Methoxy cyclohexen-1-one resin 56 (1 g) was stirred with TFA (1.67 mL), water (1.67 mL) and DMF (1.67 mL) in the microwave synthesiser at 110 $^\circ$C (50 W) for 10 minutes. The resin was filtered and washed with 2 x DMF, 2 x MeOH then shaken in $^\text{BuNH}_2$ for five minutes to remove any TFA enol ester 111 ($\nu_{\text{max}} = 1781$ cm$^{-1}$). The resin was further washed according to the standard protocol then dried overnight in the vacuum oven. The resin gave a positive brown colouration with 1M FeCl$_3$.H$_2$O aqueous solution indicating 1,3-diketone formation. The loading of CHD resin was determined to be 0.69 mmol/g by elemental analysis of 3,5-benzoyl enol ester resin x (see section x). $\delta_{\text{H}}$ (MAS, 600 MHz, CDCl$_3$); 0.8 (CH$_2$CH$_3$), 1.4 (CH$_2$ PS), 1.8 (CH$_2$ PS and CH$_2$CH$_3$), 1.9 (CH$_2$), 2.8 (NCH$_3$), 2.9 (CH$_2$), 4.5 (broad
NCH$_2$), 5.0 (CH=CO), 6.6 (ArH PS), 7.0 (ArH PS) ppm; $\nu_{\text{max}}$ (DCM); 3200-2800 (br, CH), 1715 (small, C=O keto), 1674 (C=O enol), 1631 (C=C enol), 1602 (Ar C=C) cm$^{-1}$.

6.17.2 *Trifluoracetic acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester 112*

Dimedone 24 (0.5 g, 3.57 mmol) was dissolved in TFA (5 mL) and stirred at room temperature for 5 minutes. The resultant yellow solution was concentrated in vacuo to yield an unstable yellow oil (888 mg, > 99 %). rt, decomposed on silica; $\delta_H$ (CDCl$_3$, 200 MHz); 1.20 (6H, s, 2 x CH$_3$), 2.48 (4H, s, H4/H6), 5.91 (1H, s, CH=CO) ppm; $\delta_H$ (CD$_3$OD, 200 MHz); 1.24 (6H, s, 2 x CH$_3$), 2.42 (4H, s, H4/H6), 5.25 (1H, s, CH=CO) ppm; $\delta_C$ (CD$_3$OD, 93 MHz); 26.4 (2 x CH$_3$), 31.5 (C5), 49.4 (CF$_3$), 41.6(C6), ~48.0 (C4, obscured by MeOD peak), 99.5 (CH=CO), 178.7 (C=O enol ester), 200.9 (C=O enol) ppm; $\nu_{\text{max}}$ (thin film); 2962 (CH), 2937 (CH), 2879 (CH), 1783 (C=O ester), 1666 (C=O enol) cm$^{-1}$; m/z; found (ES$^+$) 141 [dimedone+H]$^+$ requires 141, decomposition under ES$^+$ and FAB conditions.
7. Experimental – Applications of CHD resin

7.1 Mini block screening

CHD resin 57 (150 mg) was shaken overnight with each of the substrates:

a) benzaldehyde  k) benzoyl chloride
b) 2-methylvaleraldehyde  l) trimethylacetylechloride (pivoyl chloride)
c) 2,4-dimethyl-3-pentanone  m) o-toluic acid
d) 2-methoxyacetophenone  n) 4-methylvaleric acid
e) benzylamine  o) bromobenzene
f) cyclohexylamine  p) triphenylmethylichloride
g) piperidine  q) diethyl malonate
h) 2-methylpiperidine  r) diethylphenyl malonate
i) aniline  s) 2,4-dinitrophenylhydrazine
j) o-toluidine  t) cyclopentenone

Standard 55.5 mM solutions of each compound in dichloromethane were prepared using the Bohdan Neptune and 1 mL of each substrates dispensed to the resin in Bohdan Mini Blocks using the robotic arm. The resin was shaken overnight using a mini block shaker then the resin filtered and washed three times with DCM. The resins were analysed by MAS-probe NMR and FT-IR.

7.1.1 Benzoic acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester 115

Dimedone 24 (0.5 g, 3.57 mmol) was dissolved in DMF (18 mL) and benzoyl chloride (456 µL, 3.93 mmol) was added dropwise. The mixture was stirred at room temperature overnight for four hours. The mixture was diluted with ethyl acetate (100 mL) and 2M HCl (100 mL). The ethyl acetate layers was separated and washed with saturated aqueous sodium bicarbonate, water and brine (all 100 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to give a clear colourless oil (625 mg, 72 %). \( \text{rf} (20 \% \text{EtOAc/pet ethers 40-60 °C}); 0.39; \delta_H (\text{CDCl}_3, 200 \text{MHz}); \)
1.08 (6H, s, 2 × CH$_3$), 2.26 (2H, s, H4), 2.49 (2H, s, H6), 5.99 (1H, s, CH=CO), 7.49 (3H, m, ArH), 8.03 (2H, m, ArH) ppm; δ$_C$ (CDCl$_3$, 93 MHz); 28.7 (2 × CH$_3$), 33.1 (C5), 42.7 (C4), 51.3 (C6), 117.3 (CH=CO), 129.0 (ArC), 130.7 (ArC), 134.3 (ArC), 168.3 (C1), 169.1 (C=O enol ester), 200.0 (C=O enol) ppm; $\nu$$_{max}$ (thin film); 3064 (CH), 2961 (CH), 2871 (CH), 1739 (C=O enol ester), 1673 (C=O enol) 1645 (C=C), 1601 (Ar C=C) cm$^{-1}$; m/z; found (FAB) [M+H]$^+$ 245.11787 C$_{15}$H$_{17}$O$_3$ requires 245.11777.

### 7.2 Elucidation of acylation mechanism

#### 7.2.1 6-Deutero-3-methoxy-5,5-dimethyl-cyclohex-2-enone D6-58

Anhydrous THF (25 mL) was added to a round bottomed flask and cooled to 0 °C under nitrogen in a dry ice/acetone bath. Diisopropylamine (2.81 mL, 21.4 mmol) was added in one portion followed by $^9$BuLi (13.4 mL, 1.6 M in hexanes, 21.4 mmol). The resultant yellow solution was stirred for 30 minutes at 0 °C then cooled to −78 °C. Methoxycyclohexenone 58 (2 g, 13.0 mmol) in DMPU (9 mL) and THF (16 mL) was added dropwise and the mixture stirred at −78 °C for 1 hour. Deuterium oxide (5 mL, 0.28 mmol) was added in one portion and the solution allowed to warm up to room temperature overnight. The mixture was concentrated in vacuo to remove the THF then diluted with ethyl acetate (100 mL) and deuterium oxide (50 mL). The ethyl acetate layer was separated and then washed with 1 M HCl (50 mL), saturated aqueous NaHCO$_3$ solution (50 mL) and brine (50 mL). The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to yield a clear pale yellow oil (1.21 g, 60 %). $rf$ (10 % MeOH/DCM); 0.78; δ$_H$ (CDCl$_3$, 200 MHz); 1.51 (6H, s, 2 × CH$_3$), 2.29 (1H, s, CHD), 2.35 (2H, s, H4), 3.77 (3H, s, OCH$_3$), 5.45 (1H, s, CH=CO) ppm; δ$_C$ (CDCl$_3$, 63 MHz); 28.1 (2 × CH$_3$), 32.3 (C1), 42.5 (C4, CH$_2$ on dept), 50.5 (C6, CH on dept), 55.5 (OCH$_3$), 100.9 (CH=CO), 179.6 (C3), 199.4 (C=O) ppm; $\nu$$_{max}$ (thin film); 3453 (NH), 3100-2800 (br, CH), 1651 (C=O enol), 1602 (C=C) cm$^{-1}$; m/z; found (ES$^+$) [M+H]$^+$ 156 C$_7$H$_{14}$DO$_2$ requires 156.
7.2.2 4-Deutero- and 6-deutero-benzoic acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester D4-115 and D6-115

6-Deutero-3-methoxy-5,5-dimethyl-cyclohex-2-enone D6-58 (0.5 g, 3.23 mmol) was dissolved in dichloromethane (16 mL) and benzoyl chloride (935 μL, 8.05 mmol) was added dropwise. The mixture was stirred at room temperature for two days under nitrogen. The mixture was diluted with water (50 mL) and stirred at room temperature for 5 minutes to destroy the acid chloride. The dichloromethane layer was separated and washed with saturated aqueous NaHCO₃ solution (50 mL), 10 % aqueous KHSO₄ solution (50 mL) and brine. The organic layer was dried over anhydrous sodium sulfate and concentrated in vacuo to give a clear colourless oil which was purified by column chromatography on silica eluting with 20 % ethyl acetate in petroleum ether (40-60 °C) to yield a clear colourless oil (160 mg, 20 %). rf (20 % EtOAc/pet ethers 40-60 °C); 0.39; δH (CDCl₃, 200 MHz); 1.14 (6H, s, 2 × CH₃), 2.32 (1.5H, s, H4), 2.55 (1.5H, s, H6), 6.04 (1H, s, CH=CO), 7.50 (2H, m, ArH), 7.62 (1H, m, ArH), 8.07 (2H, m, ArH) ppm. The sample showed a mixture of compounds and hence deuterium scrambling. No further analysis was obtained.

7.2.3 Monitoring the kinetics of acylation

CHD resin 57 (0.1 g, 0.69 mmol) was reacted with benzoyl chloride (41 μL, 0.35 mmol) and acetic acid (20 μL, 0.35 mmol) in DCM (1 mL). The resin samples were either rotated at room temperature or stirred in the microwave synthesiser at 110 °C (300 W) for a set amount of time. The resin was filtered, washed according to the standard protocol then analysed by FT-IR to monitor the C=O enol ester peak intensity relative to the C=C peak intensity.

7.2.4 Monitoring the kinetics of release

Resin bound enol ester 114b (0.1 g, 0.69 mmol) was reacted with benzylamine (varying concentrations) in methanol (1 mL) and stirred in the microwave synthesiser
at 125 °C (200 W) for a set amount of time using continuous cooling. The resin was filtered, washed according to the standard protocol and analysed by FT-IR to monitor the C=O enol ester peak intensity relative to the C=C peak intensity.

7.3 Library synthesis

7.3.1 Microwave synthesis of CHD 1,3-enol esters resins 114b to 114f

Resin bound enol esters 114b to 114f were prepared by the reaction of CHD resin 57 (1 g, 0.69 mmol) with acid chloride (3.45 mmol) and acetic acid (197 µL, 3.45 mmol) in DCM (4 mL) and stirred in the microwave synthesiser at 110 °C (300 W) for 10 minutes. The resin was filtered and washed according to the standard protocol plus an additional wash with 10 % DIPEA in DMF. The coupling was repeated in the microwave for a further 10 minutes and the resin washed using the same protocol and dried overnight in the vacuum oven.

Resin bound benzoyl enol ester 114b: δ_H (MAS, 600 MHz, CDCl₃): 0.9 (CH₂CH₃), 1.4 (CH₂PS), 1.8 (CH₂PS and CH₂CH₃), 1.9 (CH₂), 2.8 (NCH₃), 2.9 (CH₂), 4.8 (NCH₂), 6.1 (CH=CO), 6.6 (ArH PS), 7.0 (ArH PS), 8.1 (ArH) ppm; ν_max (DCM); 3200-2800 (br, CH), 1738 (C=O enol ester), 1673 (C=O enol), 1632 (C=C enol), 1602 (Ar C=C) cm⁻¹.

Resin bound 4-methoxybenzoyl enol ester 114c: δ_H (MAS, 600 MHz, CDCl₃): 0.9 (CH₂CH₃), 1.4 (CH₂PS), 1.8 (CH₂PS and CH₂CH₃), 2.0 (CH₂), 2.8 (NCH₃), 2.9 (CH₂), 4.8 (NCH₂), 6.0 (CH=CO), 6.6 (ArH PS), 7.0 (ArH PS), 8.0 (ArH) ppm; ν_max (DCM); 3200-2800 (br, CH), 1731 (C=O enol ester), 1674 (C=O enol), 1631 (C=C enol), 1603 (Ar C=C) cm⁻¹.

Resin bound 3,5-dichlorobenzoyl enol ester 114d: L_EA = 0.65 mmol g⁻¹; δ_H (MAS, 600 MHz, CDCl₃): 0.9 (CH₂CH₃), 1.4 (CH₂PS), 1.9 (CH₂PS), 2.0 (CH₂CH₃), 1.9 (CH₂), 2.8 (NCH₃), 2.9 (CH₂), 4.8 (NCH₂), 6.1 (CH=CO), 6.6 (ArH PS), 7.0 (ArH
PS), 7.5 (ArH), 8.0 (ArH) ppm; $\nu_{\text{max}}$ (DCM); 3200-2800 (br, CH), 1739 (C=O enol ester), 1673 (C=O enol), 1626 (C=C enol), 1601 (Ar C=C) cm$^{-1}$.

**Resin bound butyryl enol ester 114e**: $\delta_H$ (MAS, 600 MHz, CDCl$_3$); 0.9 (CH$_3$CH$_2$CH$_2$), 1.0 (CH$_2$CH$_3$), 1.4 (CH$_2$), 1.7 (CH$_3$CH$_2$CH$_2$), 1.8 (CH$_2$ PS and CH$_2$CH$_3$), 1.9 (CH$_2$), 2.3 (CH$_3$CH$_2$CH$_2$), 2.8 (NCH$_3$), 2.9 (CH$_2$), 4.8 (NCH$_2$), 6.1 (CH=CO), 6.6 (ArH PS), 7.0 (ArH PS) ppm; $\nu_{\text{max}}$ (DCM); 3200-2800 (br, CH), 1731 (C=O enol ester), 1674 (C=O enol), 1632 (C=C enol), 1601 (Ar C=C) cm$^{-1}$.

**Resin bound isobutyryl enol ester 114f**: $\delta_H$ (MAS, 600 MHz, CDCl$_3$); 0.9 (CH$_2$CH$_3$), 1.2 (Me$_2$CH), 1.4 (CH$_2$ PS), 1.8 (CH$_2$ PS and CH$_2$CH$_3$), 1.9 (CH$_2$), 2.6 (CH(CHOH)$_2$), 2.8 (NCH$_3$), 2.9 (CH$_2$), 4.8 (NCH$_2$), 6.2 (CH=CO), 6.6 (ArH PS), 7.0 (ArH PS) ppm; $\nu_{\text{max}}$ (DCM); 3200-2800 (br, CH), 1739 (C=O enol ester), 1674 (C=O enol), 1632 (C=C enol), 1602 (Ar C=C) cm$^{-1}$.

7.3.2 **Microwave release of amides 127a to 127p**

Resin bound enol esters 114b to 114f (0.1 g, 0.02 mmol wrt capture and release) were reacted with amine (0.4 mmol) in MeOH (1 mL) and stirred in the microwave synthesiser at 125 °C (200 W) for 30 minutes on constant irradiation. Dowex-50WX (0.25 g, 1.2 mmol) was added to the reaction mixture and the suspension stirred at room temperature for 2 hours. The resin was filtered and washed with $2 \times$ MeOH (2 × 1 mL), $2 \times$ DCM (2 × 1 mL) and $1 \times$ MeOH (1 mL). The filtrate was concentrated *in vacuo* and the yield calculated from the weight of product. The product was analysed by LC-MS at 220 nm using an acetonitrile (0.1 % TFA)/water (0.1 % TFA) gradient over 30 minutes on a C18 Luna Phenomenex column. The results of analysis are shown in table 7.

7.3.3 **Resin recycling**

Resin formed after one round of benzamide 127a capture and release was subjected to a second round of synthesis according to section 7.3.1 and 7.3.2.
7.4 CHD as an electrophilic and nucleophilic scavenger resin

7.4.1 2-[(2′-(Benzy1-methyl-carbamoyl)-5′-ethyl-3′-hydroxy-1′-oxo-cyclohexyl]phenyl-methyl]-5-ethyl-3-hydroxy-1-oxo-cyclohex-2-ene-carboxylic acid benzyl-methyl-amide 130

Diketone 49 (150 mg, 0.52 mmol) was dissolved in anhydrous DCM (2.5 mL) and benzaldehyde (58 µL, 0.57 mmol) added dropwise. The mixture was stirred at room temperature under argon over four days. The mixture was diluted with DCM (10 mL) and water (10 mL). The DCM layer was separated and the aqueous layer washed twice more with DCM (2 x 10 mL). The combined organic layers were dried over anhydrous sodium sulfate and concentrated in vacuo to yield pale yellow crystals. The crude product was purified by preparative HPLC to yield a white crystalline solid (75 mg, 43 %). mpt; 174-176 °C; r f (40 % EtOAc/pet ethers 40-60 °C) 0.33; δH (CDCl3, 400 MHz); 0.96 (6H, t, J = 7.5 Hz, 2 × CH2CH3), 1.83 (4H, q, J = 7.5 Hz, 2 × CH2CH3), 2.32 (2H, d, J = 17.5 Hz, CH2), 2.37 (2H, d, J = 20.0 Hz, CH2), 3.03 (6H, s, 2 × NCH3), 3.26 (2H, d, J = 17.0 Hz, CH2), 3.32 (2H, d, J = 17.0 Hz, -CH2), 3.62 (1H, s, CHPh), 4.42 (2H, d, J = 14.5 Hz, CH2Ph), 4.93 (2H, d, J = 15.0 Hz, CH2Ph), 5.43 (1H, s, CH), 7.18 (15H, m, ArH) ppm; δC (CDCl3, 101 MHz); 9.1 (CH2CH3), 31.2 (CH2CH3), 33.2 (NCH3), 36.1 (CHPh), 43.5 (CH2), 44.0 (CH2), 48.7 (C1), 53.9 (CH2), 116.7 (CH=CO keto small), 125.5 (ArC), 127.4 (ArC), 127.7 (ArC), 128.0 (ArC), 128.3 (ArC), 128.5 (ArC), 129.1 (ArC), 138.3 (C3), 173.2 (C=O) ppm; νmax (thin film); 3000-2800 (br, CH), 1667 (C=O), 1626 (C=C), 1597 (Ar C=C) cm⁻¹; m/z; found (FAB) [M+H]+ 663.34288 C41H42N2O6 requires 663.34341.
7.4.2 3-Benzylamino-5-ethyl-1-oxo-cyclohex-2-enecarboxylic acid benzylmethyl-amide 131

![Chemical Structure]

Diketone 49 (50 mg, 0.17 mmol) was dissolved in DCM (0.9 mL) and benzylamine (21 μL, 0.19 mmol) was added dropwise. The mixture was stirred at room temperature overnight to give a yellow solution which was concentrated in vacuo to yield a gummy yellow solid. The crude product was purified by preparative LC-MS to yield a yellow oil (30.7 mg, 47 %). \( t_{R} \) (10 % MeOH/DCM) 0.53; \( \delta_{H} \) (CDCl\(_3\), 400 MHz); 0.91 (3H, t, \(^3J = 7.5\) Hz, CH\(_2\)CH\(_3\)), 1.73 (1H, sextet, \(^3J = 7.5\) Hz, CH\(_2\)CH\(_3\)), 1.89 (1H, sextet, \(^3J = 7.5\) Hz, CH\(_2\)CH\(_3\)), 2.39 (1H, d, \(^2J = 15.0\) Hz, H6), 2.57 (1H, d, \(^2J = 17.0\) Hz, H4), 2.89 (3H, s, NCH\(_3\)), 3.01 (1H, d, \(^2J = 17.0\) Hz, H4), 3.14 (1H, d, \(^2J = 15.0\) Hz, H6), 4.31 (2H, s, NHCH\(_2\)), 4.44 (1H, d, \(^2J = 15.0\) Hz, CH\(_2\)Ph), 4.61 (1H, d, \(^2J = 15.0\) Hz, CH\(_3\)Ph), 5.39 (1H, s, CH=CO), 6.52 (1H, s, NH), 7.27 (10H, m, ArH) ppm; \( \delta_{C} \) (CDCl\(_3\), 101 MHz); 9.2 (CH\(_2\)CH\(_3\)), 30.1 (CH\(_2\)CH\(_3\)), 30.3 (CH\(_2\)CH\(_3\)), 36.4 (NCH\(_3\)), 40.6 (CH\(_2\)), 44.5 (CH\(_2\)), 47.9 (CH\(_2\)), 50.4 (C5), 54.0 (CH\(_2\)), 95.7 (CH=CO), 127.9 (ArC), 128.1 (ArC), 128.4 (ArC), 129.1 (ArC), 129.3 (ArC), 136.3 (i-ArC), 137.1 (i-ArC), 168.2 (C3), 1173.0 (C=O amide), 194.0 (C=O enol) ppm; \( \nu_{max} \) (thin film); 3258 (NH), 3100-2800 (br, CH), 1676 (C=O), 1624 (C=C), 1547 (Ar C=C) cm\(^{-1}\); \( m/z \); found (ES\(^+\)) [M+H]\(^+\) 377.22206 C\(_{24}\)H\(_{29}\)N\(_2\)O\(_2\) requires 377.22290.

7.5 CHD as an allyl cation scavenger resin

7.5.1 Benzyl-carbamic acid allyl ester 133

![Chemical Structure]
Benzylamine 132 (1.02 mL, 9.33 mmol) was dissolved in pyridine (10 mL) and anhydrous DCM (10 mL) in a round bottomed flask under argon at room temperature. Allyl chloroformate (1.09 mL, 10.36 mmol) was added dropwise over two minutes and the mixture stirred at room temperature for a further two hours. After this time the mixture was diluted with water (100 mL) and DCM (100 mL). The DCM layer was separated and the aqueous layer washed with DCM (2 \times 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo and azeotroped with toluene to yield a pale yellow oil (1.14 g, 64\%). \textit{rf} (10 \text{\%} \text{MeOH/DCM}); 0.47; \delta_H (CDCl_3, 200 MHz); 4.44 (2H, d, $^2J = 6.0$ Hz, CH$_2$Ph), 4.67 (2H, d, $^3J = 6.0$ Hz, CH$_2$CH=CH$_2$), 5.20 (1H, br s, NH), 5.29 (1H, dd, $^3J = 1.5$ Hz, $^2J = 9.0$ Hz, CH$_2$CH=CH$_2$ cis), 5.37 (1H, dd, $^3J = 1.5$ Hz, $^2J = 17.0$ Hz, CH$_2$CH=CH$_2$ trans), 6.00 (1H, m, CH$_2$CH=CH$_2$), 7.39 (5H, m, ArH) ppm; \delta_C (CDCl$_3$, 63 MHz); 44.7 (CH$_2$Ph), 65.3 (CH$_2$CH=CH$_2$), 117.4 (CH$_2$CH=CH$_2$), 127.1 (ArC), 128.3 (ArC), 132.5 (CH$_2$CH=CH$_2$), 138.2 (i-ArC), 156.0 (C=O) ppm; $v_{\text{max}}$ (thin film); 3346 (NH), 3000-2800 (CH), 1697 (C=O carbamate) cm$^{-1}$; \textit{m/z}; found (ES$^+$) [M+Na]$^+$ 214 C$_{11}$H$_{13}$NO$_2$Na requires 214.

7.5.2 \textit{Analysis of 3-benzylamino-5,5-dimethyl-cyclohex-2-enone 134}

Obtained as a by-product in the attempted Pd-catalysed Alloc deprotection of benzyl-carbamic acid allyl ester 133, as a yellow solid (0.52 g, 55\%). \delta_H (CDCl$_3$, 200 MHz); 1.01 (6H, s, 2 $\times$ CH$_3$), 2.12 (2H, s, H6), 2.14 (2H, s, H4), 4.12 (2H, d, $^2J = 5.0$ Hz, CH$_2$Ph), 4.69 (1H, br s, NH), 7.25 (5H, m, ArH) ppm; \textit{m/z}; found (ES$^+$) [M+H]$^+$ 230 C$_{13}$H$_{20}$NO requires 230.

7.5.3 2-Allyloxy carbamylamino-propionic acid ethyl ester 136

Obtained as a by-product in the attempted Pd-catalysed Alloc deprotection of benzyl-carbamic acid allyl ester 133, as a yellow solid (0.52 g, 55\%). \delta_H (CDCl$_3$, 200 MHz); 1.01 (6H, s, 2 $\times$ CH$_3$), 2.12 (2H, s, H6), 2.14 (2H, s, H4), 4.12 (2H, d, $^2J = 5.0$ Hz, CH$_2$Ph), 4.69 (1H, br s, NH), 7.25 (5H, m, ArH) ppm; \textit{m/z}; found (ES$^+$) [M+H]$^+$ 230 C$_{13}$H$_{20}$NO requires 230.
D/L-Alanine ethyl ester hydrochloride 135 (2g, 13.0 mmol) was dissolved in anhydrous DCM (20 mL) and pyridine (20 mL). The mixture was cooled in an ice bath and stirred under nitrogen whilst allyl chloroformate (1.52 mL, 14.3 mmol) was added dropwise. The clear colourless solution was stirred at room temperature overnight. The mixture was then dissolved in water (50 mL) and DCM (50 mL) and the DCM layer separated. The aqueous layer was washed with DCM (2 × 50 mL) and the combined DCM layers washed with water (50 mL), brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a pale yellow oil (2.11 g, 81 %).  

**rf** (10 % MeOH/DCM); 0.69; **δ**\(_{H}\) (CDCl\(_3\), 250 MHz); 1.36 (3H, t, \(^3J=7.0\) Hz, CH\(_2\)CH\(_3\)), 1.49 (3H, d, \(^3J=7.0\) Hz, CHCH\(_3\)), 4.28 (2H, q, \(^2J=7.0\) Hz, -\(\text{CH}_2\text{CH}_3\)), 4.47 (1H, m, CHCH\(_3\)), 4.65 (2H, d, \(^3J=5.5\) Hz, CH\(_2\)CH=CH\(_2\)), 5.29 (1H, dd, \(^3J=1.5\) Hz, \(^2J=10.5\) Hz, CH\(_2\)CH=CH\(_2\) cis), 5.39 (1H, dd, \(^3J=1.5\) Hz, \(^2J=17.0\) Hz, CH\(_2\)CH=CH\(_2\) trans), 5.50 (1H, br s, NH), 6.00 (1H, m, CH\(_2\)CH=CH\(_2\)) ppm; **δ**\(_{C}\) (CDCl\(_3\), 63 MHz); 13.9 (CH\(_2\)CH\(_3\)), 18.5 (CHCH\(_3\)), 49.4 (CHCH\(_3\)), 61.3 (CH\(_2\)CH\(_3\)), 65.5 (CH\(_2\)CH=CH\(_2\)), 117.5 (CH\(_2\)CH=CH\(_2\)), 132.5 (CH\(_2\)CH=CH\(_2\)), 155.3 (C=O carbamate), 172.9 (C=O ester) ppm; \(ν_{\text{max}}\) (thin film); 3348 (NH), 2985 (CH), 2941 (CH), 1724 (C=O carbamate and ester) cm\(^{-1}\); m/z; found (FAB) [M+H]\(^{+}\) 202.10789 C\(_9\)H\(_{16}\)N\(_2\)O\(_4\) requires 202.10793.

### 7.5.4 Analysis of 2-(5,5-dimethyl-3-oxo-cyclohex-1-enylamino)-propionic acid ethyl ester 137

![Chemical Structural Formula](image)

Obtained as a by-product in the attempted Pd-catalysed Alloc deprotection of 2-allyloxy carbonylamino-propionic acid ethyl ester 136, as a yellow oil (24.3 mg, 8 %). **δ**\(_{H}\) (CDCl\(_3\), 200 MHz); 1.00 (6H, s, 2 × CH\(_3\)), 1.23 (3H, t, \(^3J=7.0\) Hz, CH\(_2\)CH\(_3\)), 1.38 (3H, d, \(^3J=7.5\) Hz, CHCH\(_3\)), 2.12 (2H, s, H\(_4\)), 2.16 (2H, s, H\(_6\)), 4.03 (1H, q, \(^3J=7.0\) Hz, CHCH\(_3\)), 4.16 (2H, q, \(^3J=7.0\) Hz, CH\(_2\)CH\(_3\)), 5.01 (1H, s, CH=CO) ppm; m/z; found (ES\(^{+}\)) [M+H]\(^{+}\) 240. C\(_{13}\)H\(_{22}\)NO\(_3\) requires 240.
7.5.5 Carbonic acid allyl ester benzyl ester 139

Benzyl alcohol 138 (4.78 mL, 46.2 mmol) was dissolved in anhydrous THF (23 mL) and pyridine (5.98 mL, 74.0 mmol). The mixture was cooled in an ice bath and stirred under nitrogen whilst allyl chloroformate (8.58 mL, 74.0 mmol) was added dropwise. The cloudy white solution was allowed to warm to room temperature and stirred overnight. The mixture was then dissolved in water (50 mL) and ethyl acetate (50 mL) and the organic layer separated. The aqueous layer was washed with ethyl acetate (2 × 50 mL) and the combined organic layers washed with water (50 mL), brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil (9.41 g, > 99 %). \( rf (20 \% \text{MeOH/DCM}) \); 0.50; \( \delta_H \) (CDCl₃, 200 MHz); 4.72 (2H, d, \( \delta_J = 6.0 \text{ Hz, CH}_2\text{CH=CH}_2 \)), 5.25 (2H, s, CH₂-Ph), 5.35 (1H, d, \( \delta_J = 9.0 \text{ Hz, CH}_2\text{CH=CH}_2\text{ cis} \)), 5.42 (1H, d, \( \delta_J = 22.0 \text{ Hz, CH}_2\text{CH=CH}_2\text{ trans} \)), 5.99 (1H, ddd, \( \delta_J = 7.0, 10.0, 23.0 \text{ Hz, CH}_2\text{CH=CH}_2 \)), 7.44 (5H, m, ArH) ppm; \( \delta_C \) (CDCl₃, 63 MHz); 68.4 (CH₂CH=CH₂), 69.5 (CH₂-Ph), 118.8 (CH₂CH=CH₂), 128.2 (ArC), 128.4 (ArC), 131.4 (CH₂CH=CH₂), 135.1 (i-ArC), 154.8 (C=O carbonate) ppm; \( \nu_{\max} \) (thin film); 3089 (CH), 3067 (CH), 3034 (CH), 2986 (CH), 2954 (CH), 2893 (CH), 1747 (C=O carbonate) 1650 (C=C) cm⁻¹; \( m/z \); found (FAB) [M+H]⁺ 193.908692 C₁₁H₁₃O₃ requires 193.08647.

7.5.6 Deprotection of carbonic acid allyl ester benzyl ester 139: Allyl cation scavenging with dimedone

Dimedone 24 (1.09 g, 7.81 mmol) was added to an oven dried 50 mL round bottomed flask under argon then anhydrous THF (6 mL) added. Carbonic acid allyl ester benzyl ester 139 (0.3 g, 1.56 mmol) dissolved in THF (2 mL) was added in one portion. Tetrakis(triphenylphosphine) palladium (0) (90 mg, 78 \( \mu \text{mol} \)) was added in one portion whilst keeping the solution under argon and the mixture stirred at room
temperature for 30 minutes. The mixture was filtered through two plugs of celite and concentrated in vacuo to yield a brown solid. The crude material was purified by column chromatography on silica eluting with 20 % ethyl acetate in petroleum ethers (40–60 °C) increasing concentration to 30 % ethyl acetate to yield benzyl alcohol 138 as a pale yellow oil (160 mg, 95 %). rf (20 % EtOAc/pet ethers 40–60 °C); 0.22; δH (CDCl3, 200 MHz); 2.66 (1H, br s, OH), 4.71 (2H, s, CH2Ph), 7.43 (5H, m, ArH) ppm.

7.5.7 Analysis of 2,2-diallyl-5,5-dimethyl-cyclohexane-1,3-dione 140

Di-allylated dimedone analogue 140 was obtained from deprotection of carbonic acid allyl ester benzyl ester 139 as brown crystals (68.8 mg, 40 %) after column chromatography on silica eluting with 20 % ethyl acetate in petroleum ethers (40–60 °C) increasing concentration to 30 % ethyl acetate. rf (20 % EtOAc/pet ethers 40–60 °C); 0.42; δH (CDCl3, 200 MHz); 0.97 (6H, s, 2 × CH3), 2.47 (2H, s, CH2), 2.51 (2H, s, CH2), 2.54 (4H, s, 2 × CH2CH=CH2), 5.06 (2H, dd, 3J = 2.0 Hz, 2J = 10.0 Hz, 2 × CH2CH=CH2 cis), 5.13 (2H, dd, 3J = 2.0 Hz, 2J = 10.0 Hz, 2 × CH2CH=CH2 trans), 5.58 (2H, m, 2 × CH2CH=CH2 cis), ppm; δc (CDCl3, 63 MHz); 28.6 (2 × CH3), 30.6 (C5), 38.7 (2 × CH2), 51.9 (CH2CH=CH2), 119.3 (CH2CH=CH2), 132.2 (CH2CH=CH2), 208.6 (C=O keto) ppm; νmax (thin film); 3079 (CH), 3013 (CH), 2956 (CH), 2871 (CH), 1726 (C=O keto) 1696 (C=C), 1639 (C=C) cm−1; m/z; found (ES+) [M+H]+ 221 C14H21O2 requires 221.
7.5.8 Deprotection of carbonic acid allyl ester benzyl ester 139: Allyl cation scavenging with CHD resin 114

CHD resin 103 (1 g, \( L_{\text{calc}} = 2.78 \text{ mmol/g, 2.78 mmol} \)) was swollen in THF (8 mL) and carbonic acid allyl ester benzyl ester 139 (214 mg, 1.11 mmol) in THF (2 mL) added dropwise. The mixture was stirred at room temperature under argon while tetrakis(triphenylphosphine) palladium (0) (1.28 mg, 1.11 \( \mu \text{mol} \)) added in one portion. The mixture was stirred at room temperature overnight then the resin filtered and washed with 2 \( \times \) MeOH and 4 \( \times \) DCM and the filtrate concentrated \textit{in vacuo} to yield a clear colourless oil requiring no further purification. (104 mg, 87 %). \( \text{rf} \) (20 \% MeOH/DCM); 0.22; \( \delta_{\text{H}} \) (CDCl\(_3\), 200 MHz) 2.02 (1H, br s, OH), 4.84 (2H, s, CH\(_2\)), 7.45 (5H, m, ArH) ppm; \( \delta_{\text{C}} \) (CDCl\(_3\), 63 MHz); 65.2 (CH\(_2\)Ph), 126.9 (ArC), 127.5 (ArC), 128.4 (ArC), 140.7 (i-ArC) ppm; \( \nu_{\text{max}} \) (thin film); 3330 (OH), 3087 (CH), 3063 (CH), 3031 (CH), 2918 (CH), 2873 (CH), 1654 cm\(^{-1}\); \( m/\text{f} \); found (ES\(^+\)) [M-OH]\(^+\) 91 C\(_7\)H\(_7\) requires 91, \( \delta_{\text{H}} \) (Resin 143, MAS, 600 MHz, CDCl\(_3\)); 0.8 (CH\(_2\)CH\(_3\)), 1.5 (CH\(_2\) PS), 1.8 (CH\(_2\) PS and CH\(_2\)CH\(_3\)), 2.4 (CH\(_2\)), 2.8 (CH\(_2\)CH=CH\(_2\)), 3.6 (residual OMe), 5.1 (CH=CH\(_2\)), 5.8 (CH=CH\(_2\)), 6.6 (ArH PS), 7.1 (ArH PS) ppm.

7.6 CHD as a BAL – solution phase analogues

7.6.1 Acetic acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester 144
Dimedone 24 (1 g, 7.14 mmol) was dissolved in anhydrous DCM (30 mL) and pyridine (0.87 mL, 10.7 mmol) added. Acetyl chloride (0.76 mL, 10.7 mmol) was added dropwise to form a pale yellow solution which was stirred at room temperature for one hour. The mixture was dissolved in DCM (20 mL) and water (50 mL). The DCM layer was separated and washed with 1.5M aqueous potassium hydrogen sulfate solution (50 mL), saturated aqueous sodium bicarbonate solution (50 mL) and brine (50 mL) before being dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil (1.37 g, > 99%). 

\[ \text{rf (10 \% MeOH/DCM); } 0.77; \delta_{\text{H}} (\text{CDCl}_3, 200 \text{ MHz}); 1.14 (6\text{H, s, } 2 \times \text{CH}_3), 2.25 (3\text{H, s, COCH}_3), 2.30 (2\text{H, s, H4}), 2.45 (2\text{H, s, H6}), 5.94 (1\text{H, s, CH=CO}) \text{ ppm}; \delta_{\text{C}} (\text{CDCl}_3, 63 \text{ MHz}); 21.1 (\text{COCH}_3), 27.9 (2 \times \text{CH}_3), 32.9 (\text{C5}), 42.0 (\text{C4}), 50.7 (\text{C6}), 116.3 (\text{CH=CO}), 167.3 (\text{C1}), 167.9 (\text{C=O enol ester}), 199.3 (\text{C=O enol}) \text{ ppm; } \nu_{\max} (\text{thin film}); 2961 (\text{CH}), 2873 (\text{CH}), 1771 (\text{C=O enol ester}) 1674 (\text{C=O enol}), 1644 (\text{C=C}) \text{ cm}^{-1}; m/z; \text{found (FAB) [M+H]}^+ 183.10241 \text{ C}_{10}\text{H}_{15}\text{O}_3 \text{ requires 183.10212.}

7.6.2 2-Acetyl-3-hydroxy-5,5-dimethyl-cyclohex-2-enone 145

By acetone cyanohydrin rearrangement:

Acetic acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester 144 (1.37 g, 7.53 mmol) was dissolved in anhydrous acetonitrile (40 mL) and triethylamine (4.23 mL, 30.1 mmol) added. Acetone cyanohydrin (69 \mu L, 0.75 mmol) was added dropwise to form a yellow solution, which was stirred at room temperature overnight. The mixture was dissolved in petroleum ethers (40–60 °C) (50 mL) and chilled 1M HCl (50 mL). The organic layer was separated and washed with chilled water (25 mL) and brine (50 mL) before being dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil (0.43 g, 31 %).

By in situ DMAP rearrangement:

Dimedone 24 (1 g, 7.14 mmol) was dissolved in anhydrous DCM (36 mL) then pyridine (865 \mu L, 10.7 mmol) and DMAP (1.13 g, 10.7 mmol) added. Acetic
anhydride (1.01 mL, 10.7 mmol) was added dropwise to form a clear colourless solution, which was stirred at room temperature for 3 days. The mixture was dissolved in DCM (20 mL) and 1.5 M aqueous KHSO₄ solution (50 mL). The organic layer was separated and washed with water (50 mL) and brine (50 mL) before being dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear yellow oil (1.13 g, 87%). \( \text{rf} (20 \% \text{EtOAc/pet ethers } 40-60 \, ^\circ \text{C}; 0.30; \delta_H (\text{CDCl}_3, 250 \, \text{MHz}); 1.01 (6H, s, 2 \times \text{CH}_3), 2.29 (2H, s, \text{H}6), 2.40 (2H, s, \text{H}4), 2.56 (3H, s, \text{COCH}_3), 18.06 (1H, s, \text{OH enol}) \, \text{ppm}; \delta_C (\text{CDCl}_3, 63 \, \text{MHz}); 28.0 (2 \times \text{CH}_3), 28.4 (\text{COCH}_3), 30.5 (\text{C}5), 46.7 (\text{C}6), 52.3 (\text{C}4), 112.2 (\text{C}3), 197.8 (\text{C}=\text{O}), 200.3 (\text{C}=\text{O}) \, \text{ppm}; \nu_{\text{max}} (\text{thin film}); 2959 (\text{CH}), 2872 (\text{CH}), 1668 (\text{C}=\text{O} \, \text{enol}), 1560 (\text{C}=\text{C}) \, \text{cm}^{-1}; \, \text{m/z}; \) found (ES⁺) [M+H]⁺ 183 C₁₀H₁₅O₃ requires 183.

7.6.3 [2-(2-Hydroxy-4,4-dimethyl-6-oxo-cyclohex-1-enyl)-2-oxo-ethyl]-carbamic acid 9H-fluoren-9-ylmethyl ester 146

\[
\text{N} \quad \text{Fmoc} \\
\text{O} \quad \text{O} \\
\text{H} \\
\text{C} \quad \text{C} \\
\text{H} \quad \text{H} \\
\text{OH}
\]

Dimedone 24 (200 mg, 1.43 mmol) and DMAP (192 mg, 1.57 mmol) were dissolved in anhydrous DMF (7 mL) and stirred at room temperature for 30 minutes. Meanwhile Fmoc-Gly-OH (467 mg, 1.57 mmol) and EDC.HCl (302 mg, 1.57 mmol) were dissolved in anhydrous DMF (7 mL) and stirred at room temperature for the remaining 20 minutes. The contents of the second flask were transferred to the first and the total mixture stirred at room temperature for 1½ hours under nitrogen. The mixture was diluted with ethyl acetate (25 mL) and washed three times with water (3 \( \times 25 \, \text{mL} \)) and brine (25 mL) before being dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a foamy white solid. The crude product was purified by column chromatography on silica eluting with 40 \% ethyl acetate in hexane to give a white crystalline solid (241 mg, 40 \%). \( \text{mp} \); 68-72 \( ^\circ \text{C} \); \( \text{rf} (50 \% \text{EtOAc/hexane}); 0.30; \delta_H (\text{CDCl}_3, 200 \, \text{MHz}); 1.09 (6H, s, 2 \times \text{CH}_3), 2.36 (2H, s, \text{H}5), 2.56 (2H, s, \text{H}3), 4.26 (1H, t, 3 \text{J} = 6.0 \, \text{Hz}, \text{CH Fmoc}), 4.41 (2H, d, 3 \text{J} = 7.0 \, \text{Hz}, \text{NHCH}_2), 4.67 (2H, d, 3 \text{J} = 6.0 \, \text{Hz}, \text{OCH}_2), 5.47 (1H, br t, 2 \text{J} = 7.0 \, \text{Hz}, \text{NH}), 7.35
(4H, m, ArH), 7.62 (2H, d, \(3J = 7.0\) Hz, ArH), 7.76 (2H, d, \(3J = 7.0\) Hz, ArH), 16.94 (1H, br s, OH enolic) ppm; \(\delta_c\) (CDCl\(_3\), 250 MHz) 28.0 (2 × CH\(_3\)), 29.6 (C5), 30.9 (C4), 45.2 (C3), 47.0 (CH Fmoc), 50.6 (CH\(_2\)), 66.9 (CH\(_2\)), 111.1 (CH=CO), 119.8 (ArC), 125.0 (ArC), 126.9 (ArC), 127.5 (ArC), 141.1 (i-ArC), 143.8 (C2), 156.3 (C=O carbamate), 194.9 (C=O enol) 200.5 (C=O enol), 220.0 (C=O keto) ppm; \(\nu_{\text{max}}\) (nujol mull); 3439 (NH or OH), 3000-2500 (br, CH), 1725 (C=O carbamate), 1665 (C=O enol), 1609 (Ar C=C) cm\(^{-1}\); m/z; found (FAB) [M+H]\(^+\) 420.18117 C\(_{25}\)H\(_{26}\)N\(_0\)\(_5\) requires 420.18110

7.7 CHD as a BAL – C-acyl derivatives

7.7.1 4-Acetyl-1-ethyl-3-hydroxy-5-oxo-cyclohex-3-enecarboxylic acid methylpolystyrene-methyl-amide resin 147

CHD resin 57 (100 mg, \(L_{\text{calc}} = 0.69\) mmol/g, 0.07 mmol) was swollen in DMF (0.5 mL) and acetonitrile (0.5 mL). DIPEA (79 \(\mu\)L, 0.57 mmol) and acetyl chloride (40 \(\mu\)L, 0.57 mmol) were added to the resin mixture to form a yellow solution, followed by acetone cyanohydrin (52 \(\mu\)L, 0.57 mmol). The mixture was rotated at room temperature for two days then filtered, washed according to the standard protocol and dried in the vacuum over overnight. \(\delta_H\) (MAS, 600 MHz, CDCl\(_3\)); 1.0 (CH\(_2\)CH\(_3\)), 1.5 (CH\(_2\) PS), 1.6 (CH\(_2\) PS and CH\(_2\)CH\(_3\)), 2.1 (CH\(_2\)), 2.2 (residual enol ester COCH\(_3\)), 2.7 (COCH\(_3\)), 6.6 (ArH PS), 7.0 (ArH PS), 18.4 (OH enolic) ppm; \(\nu_{\text{max}}\) (DCM); 3200-2800 (br, CH), 1738 (small, residual C=O enol ester), 1666 (C=O enol), 1622 (C=C enol), 1602 (Ar C=C) cm\(^{-1}\).
7.7.2 \{2-[4-(Methylpolystyrene-methyl-carbamoyl)-4-ethyl-2-hydroxy-6-oxo-cyclohex-1-enyl]-2-oxo-ethyl\}-carbamic acid 9H-fluoren-9-ylmethyl ester resin 148a

CHD resin 57 (500 mg, \(L_{\text{max}} = 0.69 \text{ mmol/g}, 0.35 \text{ mmol}) was swollen in DCM (2.5 mL) and DMF (2.5 mL) containing Fmoc-Gly-OH (512 mg, 1.73 mmol), DMAP (211 mg, 1.73 mmol) and DIC (270 \(\mu\)L, 1.73 mmol) and rotated at room temperature overnight. The resin was filtered, washed according to the standard protocol and the coupling repeated overnight. The resin was washed according to the standard protocol and dried in the vacuum oven overnight. \(L_{\text{uv}} = 0.50 \text{ mmol/g; } \delta_\text{H} (\text{MAS, 600 MHz, CDCl}_3); 1.0 (\text{CH}_2\text{CH}_3), 1.5 (\text{CH}_2 \text{PS}), 1.6 (\text{CH}_2 \text{PS and CH}_2\text{CH}_3), 2.1 (\text{CH}_2), 5.1 (\text{CH} = \text{CO}), 6.6 (\text{ArH PS}), 7.1 (\text{ArH PS}), 7.7 (\text{ArH Fmoc}), 7.8 (\text{ArH Fmoc}) \text{ ppm; } \nu_{\text{max}}(\text{DCM}); 3200-2800 \text{ (br, CH)}, 1721 (\text{C=O carbamate}), 1614 (\text{C=C}) \text{ cm}^{-1}.

7.8 CHD as a BAL – direct C-acylation via enamine formation

7.8.1 1-Ethyl-3-isobutylamino-5-oxo-cyclohex-3-ene carboxylic acid methylpolystyrene-methyl-amide resin 149

CHD resin 57 (100 mg, \(L_{\text{calc}} = 0.69 \text{ mmol/g}, 0.07 \text{ mmol}) was swollen in toluene (1 mL) containing isobutylamine (56 \(\mu\)L, 0.57 mmol) and the mixture heated in the microwave at 145 °C (300 W) for 10 minutes. The resin was filtered and washed according to the standard protocol and dried in the vacuum oven overnight. \(\delta_\text{H} (\text{MAS, 600 MHz, CDCl}_3); 0.9 (\text{CH(CH}_3)_2), 1.0 (\text{CH}_2\text{CH}_3), 1.5 (\text{CH}_2 \text{PS}), 1.6 (\text{CH}_2 \text{PS and CH}_2\text{CH}_3), 1.9 (\text{CH}_2\text{CH(CH}_3)_2), 2.1 (\text{CH}_2), 2.9 (\text{CH(CH}_3)_2), 5.1
(CH=CO), 6.6 (ArH PS), 7.1 (ArH PS) ppm; \( \nu_{\text{max}} \) (DCM); 3426 (NH), 3319 (NH), 3200-2800 (br, CH), 1601 (Ar C=C) cm\(^{-1} \).

7.8.2 \{2-[4-(Methylpolystyrene-methyl-carbamoyl)-4-ethyl-2-hydroxy-6-oxo-cyclohex-1-enyl]-2-oxo-ethyl\}-carbamic acid 9H-fluoren-9-ylmethyl ester resin 148b

Enamine resin 149 (500 mg, \( L_{\text{max}} = 0.66 \) mmol/g, 0.33 mmol) was swollen in DMF (5 mL) containing Fmoc-Gly-OH (787 mg, 2.65 mmol) and EDC.HCl (509 mg, 2.65 mmol) and rotated at room temperature overnight. The resin was filtered, washed according to the standard protocol and the coupling repeated overnight. The resin was swollen in DMF/2M HCl (1:1) and rotated at room temperature for 4 hours. The resin was washed according to the standard protocol and dried in the vacuum over overnight. \( L_{\text{av}} = 0.22 \) mmol/g; \( \delta_{\text{H}} \) (MAS, 600 MHz, CDCl\(_3 \)); 0.9 (residual CH(CH\(_3\))\(_2 \)), 1.0 (CH\(_2\)CH\(_3 \)), 1.5 (CH\(_2\) PS), 1.6 (CH\(_2\) PS and CH\(_2\)CH\(_3 \)), 1.9 (residual CH\(_2\)CH(CH\(_3\))\(_2 \)), 2.1 (CH\(_2 \)), 2.9 (residual CH(CH\(_3\))\(_2 \)), 5.1 (CH=CO), 6.6 (ArH PS), 7.1 (ArH PS), 7.7 (ArH Fmoc), 7.8 (ArH Fmoc) ppm; \( \nu_{\text{max}} \) (DCM); 3426 (residual NH), 3319 (residual NH), 3200-2800 (br, CH), 1721 (C=O carbamate), 1614 (C=C) cm\(^{-1} \).
7.9  CHD as a BAL– amino acid coupling

7.9.1  2-[1-((4,4-Dimethyl-2,6-dioxo-cyclohexylidene)-ethylamino]-propionic acid ethyl ester 150

2-Acetyl-3-hydroxy-5,5-dimethyl-cyclohex-2-enone 145 (0.5 g, 2.75 mmol) and D/L-alanine ethyl ester 135 (467 mg, 3.02 mmol) were dissolved in anhydrous DCM (14 mL) and the mixture stirred at room temperature under argon whilst DIPEA (847 µL, 6.04 mmol) was added dropwise to give a very pale yellow solution. The mixture was stirred at room temperature under argon for 24 hours then diluted with DCM (50 mL) and washed with 2M HCl (50 mL), water (50 mL) and brine (50 mL). The DCM layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil which crystallised on standing to give white crystals (0.78 g, > 99 %). mpt; 55-56 °C; rf (5 % MeOH/DCM); 0.67; δH (CDCl3, 200 MHz); 1.03 (6H, s, 2 × CH3), 1.29 (3H, t, 3J = 7.0 Hz, CH2CH3), 1.58 (2H, d, 3J = 7.5 Hz, CHCH3), 2.38 (4H, s, H3/H5), 2.52 (3H, s, C=CCH3), 4.25 (2H, q, 3J = 7.0 Hz, CH2CH3), 4.43 (1H, m, 3J = 7.5 Hz, CHCH3), 13.74 (1H, d, 3J = 6.0 Hz, NH) ppm; δC (CDCl3, 63 MHz); 13.9 (CH2CH3), 18.1 (CHCH3), 18.6 (C=CCH3), 28.1 (2 × CH3), 29.8 (C4), 51.6 (CH2CH3), 52.7 (C3/C5), 61.9 (CH2CH3), 108.0 (C=CMe), 170.5 (C=CMe), 172.5 (C=O ester), 197.7 (C=O) ppm; νmax (thin film); 2957 (CH), 2870 (CH), 1739 (C=O ester), 1639 (C=C), 1579 (C=C) cm⁻¹; m/z; found (FAB) [M+H]+ 282.17043 C15H24NO4 requires 282.17053.

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2-Acetyl-3-hydroxy-5,5-dimethyl-cyclohex-2-enone 145 (74 mg, 0.41 mmol) and L-Fmoc-Dap-OH (200 mg, 0.61 mmol) were dissolved in DMF (20 mL) and the mixture stirred at room temperature under argon for 24 hours then diluted with ethyl acetate (50 mL) and washed with 2M HCl (50 mL), water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil which crystallised on azeotroping with toluene to give fluffy white crystals (224 mg, > 99 %). mpt: 98-99 °C; rf (10 % MeOH/DCM); 0.31; δ_H (CDCl_3, 200 MHz); 0.96 (6H, s, 2 × CH_3), 2.35 (4H, s, H3/H5), 2.54 (3H, s, C=CCH_3), 3.93 (2H, br m, NHCH_2CH), 4.14 (1H, t, J = 7.0 Hz, CH Fmoc), 4.33 (2H, m, OCH_2), 4.46 (2H, m, J = 5.0 Hz, NHCH_2CH), 5.87 (1H, d, J = 5.0 Hz, NH Fmoc), 6.10 (1H, br s, CO_2H), 7.23 (4H, m, ArH Fmoc), 7.52 (2H, d, J = 7. Hz, ArH Fmoc), 7.69 (2H, d, J = 7.5 Hz, ArH Fmoc), 13.28 (1H, s, NHCH_2CH) ppm; δ_C (CDCl_3, 63 MHz); 18.0 (C=rCCH_3), 28.1 (2 × CH_3), 30.1 (C4), 44.5 (C5), 46.9 (NHCH_2CH), 52.0 (C3), 53.7 (CH Fmoc), 67.2 (OCH_2 Fmoc), 108.3 (C=CMe), 119.9 (ArC), 124.9 (ArC), 127.0 (ArC), 127.7 (ArC), 128.1 (ArC), 128.9 (ArC), 141.2 (i-ArC), 143.3 (i-ArC), 155.7 (C=O carbamate), 170.4 (C=CMe), 175.5 (C=O acid), 190.0 (C=O) ppm; ν_{max} (thin film); 2956 (CH), 2928 (CH), 2891 (CH), 2869 (CH), 1721 (C=O acid/carbamate), 1620 (C=C), 1567 (C=C) cm^{-1}; m/z; found (ES^+) [M+H]^+ 491 C_{24}H_{31}N_2O_6 requires 491; [α]_D^{22} +86.1 (c 1.01, CHCl_3).
7.9.3 3,6,6-Trimethyl-1,5,6,7-tetrahydro-indazol-4-one 152

2-Acetyl-3-hydroxy-5,5-dimethyl-cyclohex-2-enone 145 (2.5 g, 13.7 mmol) was dissolved in THF (69 mL) and hydrazine hydrate (4.71 mL, 151 mmol) was added dropwise. The mixture was stirred at room temperature for 1 1/2 hours under nitrogen. The mixture was concentrated in vacuo to remove the THF the water (50 mL) and ethyl acetate (50 mL). The ethyl acetate layer was separated and washed with water (50 mL) and brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a pale yellow oil that crystallised out to form cream crystals (1.21 g, 50%). Upon standing a white crystalline solid precipitated from the aqueous washing which was removed by filtration and dried in the vacuum oven at 40 °C overnight to give a white crystalline solid (0.93, 38%).

mpt; 97-98 °C; rf (10 % MeOH/DCM); 0.41; δH (CDCl3, 200 MHz); 1.04 (6H, s, 2 × CH3), 2.30 (2H, s, H5), 2.50 (3H, s, CH3C=N), 2.64 (2H, s, H7) ppm; δC (CDCl3, 63 MHz); 11.9 (CH3C=N), 28.3 (2 × CH3), 35.4 (C6), 36.2 (C5), 52.9 (C7), 114.5 (C3a), 144.8 (C7a), 194.5 (C=O) ppm; νmax (thin film); 3202 (NH), 2960 (CH), 2896 (CH), 2873 (CH), 1658 (C=O enamine), 1579 (C=C enamine), 1504 (C=N) cm⁻¹; m/z; found (ES⁺) [M+H]⁺ 179 C10H15N2O requires 179.

7.9.4 2-[1-/4-(Methylpolystyrene-methyl-carbamoyl)-4-ethyl-2,6-dioxocyclohexylidene]-ethylamino]-propionic acid ethyl ester resin 153

2-Acetyl resin 147 (50 mg, Lcalc = 0.24 mmol/g, 12 µmol) was swollen in DCM (0.5 mL) containing D/L-alanine ethyl ester 135 (41 mg, 0.27 mmol) and DIPEA (76 µL,
0.54 mmol). The resin was rotated at room temperature overnight then filtered and washed according to the standard protocol and dried in the vacuum oven. $\delta_H$ (MAS, 600 MHz, CDCl$_3$); 1.0 (CH$_2$CH$_3$), 1.4 (CO$_2$CH$_2$CH$_3$), 1.6 (CH$_2$ PS and CH$_2$CH$_3$), 2.0 (residual enol ester COCH$_3$), 4.3 (CO$_2$CH$_2$CH$_3$), 6.7 (ArH PS), 7.2 (ArH PS), 18.4 (OH enolic) ppm; $\nu_{\text{max}}$ (DCM); 3100-2800 (br, CH), 1739 (C=O ester), 1633 (C=C enol) cm$^{-1}$.

7.9.5 Resin Cleavage: 2-Acetylamino-propionic acid ethyl ester 156

Alanine ethyl ester coupled resin 153 (500 mg, $L_{\text{max}} = 0.23$ mmol/g, 0.12 mmol) was mixed with acetic acid (5 mL) containing ammonium molybdate (240 mg, 1.23 mmol) and hydrogen peroxide (0.38 mL, 3.92 mmol). The mixture was heated in the microwave at 110 °C (50 W) for 10 minutes after which time the resin was filtered and washed with 2 × H$_2$O, 1 × MeOH and 4 × DCM. The DCM layer was separated and the aqueous layer washed with DCM (2 × 50 mL). The combined organic layers were washed with brine (50 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil (30 mg, 39%). The product was apparent by NMR but not very clean. $m/z$ found (ES$^+$) [M+Na]$^+$ 182 C$_7$H$_{13}$NO$_3$Na requires 182.
7.9.6 3-\{1\-f4\-(Methypolystyrene-methyl-carbamoyl)-4-ethyl-2,6-dioxo-cyclohexylidene\}-ethylamino\}-2-(9H-fluoren-9-ylmethoxycarbonylamino)propionic acid resin 157

2-Acetyl resin 147 (100 mg, $L_{calc} = 0.24$ mmol/g, 24 μmol) was swollen in DMF (2 mL) containing L-Fmoc-Dap-OH (71 mg, 216 μmol). The DMF mixture was sonicated to ensure complete dissolution of amino acid. The resin was rotated at room temperature overnight then filtered and washed according to the standard protocol and dried in the vacuum oven. The resin showed negative chloranil and Kaiser tests. $L_{uv} = 0.22$ mmol/g; δH (MAS, 600 MHz, CDCl3); 0.9 (CH$_2$CH$_3$), 1.4 (CH$_2$ PS), 1.9 (CH$_2$ PS and CH$_2$CH$_3$), 2.1 (residual enol ester COCH$_3$), 2.4 (2 × CH$_2$), 2.85 (C=CCH$_3$), 4.1-4.7 (CH Fmoc, OCH$_2$, NHCH$_2$CH), 5.7 (NH Fmoc), 6.6 (ArH PS), 7.1 (ArH PS and ArH Fmoc) ppm; νmax (DCM); 3100-2800 (br, CH), 1733 (C=O carbamate/acid), 1633 (C=C enol), 1602 (Ar C=C) cm$^{-1}$.

7.9.7 2-Amino-3-\{1\-f4\-(methypolystyrene-methyl-carbamoyl)-4-ethyl-2,6-dioxo-cyclohexylidene\}-ethylamino\}-propionic acid resin 158

Fmoc-Dap-OH coupled resin 157 (68 mg, $L_{uv} = 0.22$ mmol/g, 15 μmol) was swollen in DMF (0.8 mL) containing piperidine (200 μL). The mixture was rotated at room temperature for two hours and the resin filtered, washed according to the standard
protocol and dried in the vacuum oven overnight. The resin showed dark blue colouration with both the chloranil and Kaiser tests and red colouration with the TNBS test. δH (MAS, 600 MHz, CDCl3); 0.9 (CH₂CH₃), 1.4 (CH₂ PS and CH₂CH₃), 2.0 (residual enol ester COCH₃), 6.6 (ArH PS), 7.0 (ArH PS) ppm; νmax (DCM); 3100-2800 (br, CH), 1736 (C=O acid), 1633 (C=C enol), 1601 (Ar C=C) cm⁻¹.

7.9.8 3-[1-[4-(Methylpolystyrene-methyl-carbamoyl)-4-ethyl-2,6-dioxo-cyclohexylidene]-ethylamino]-2-(2-tert-butoxycarbonylamino-propionylamino)-propionic acid resin 159

Amino resin 158 (50 mg, Lmax = 0.23 mmol/g, 12 μmol) was swollen in DMF (1 mL) containing L-Boc-Ala-OH (11 mg, 58 μmol), TBTU (19 mg, 58 μmol) and DIPEA (16 μL, 115 μmol). The resin was rotated at room temperature overnight then filtered and washed according to the standard protocol and dried in the vacuum oven. The resin showed negative chloranil and Kaiser tests. δH (MAS, 600 MHz, CDCl3); 0.9 (CH₂CH₃), 1.5 (C(CH₃)₂ Boc), 1.6 (CH₂ PS), 1.9 (CH₂ PS and CH₂CH₃), 2.1 (residual enol ester COCH₃), 6.6 (ArH PS), 7.1 (ArH PS) ppm; νmax (DCM); 3100-2800 (br, CH), 1737 (C=O acid), 1715 (C=O carbamate), 1666 (C=O enol), 1632 (C=C), 1601 (Ar C=C) cm⁻¹.
8. Experimental - Dimedone-1,3-enol esters in hydrolase catalysed reactions

8.1 Synthesis of dimedone-1,3-enol esters

8.1.1 (±)-3-Phenylbutyryl fluoride (R/S)-161

(±)-3-phenylbutyric acid (R/S)-160 (1.07 g, 6.49 mmol) was dissolved in anhydrous DCM (33 mL) and pyridine (0.52 mL, 6.49 mmol) added dropwise. The mixture was stirred at -20 °C under nitrogen in a dry ice/acetone bath and cyanuric fluoride (3.50 mL, 41.5 mmol) added dropwise to form a white precipitate. The mixture was allowed to warm to room temperature over 1 hour then ice (50 mL) and DCM (10 mL) added. The DCM layer was separated and the aqueous layer washed twice with DCM (2 x 50 mL). The combined organic layers were washed with ice (20 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil (1.01 g, 93%).

\[ \text{rf (0.1% AcOH in 10% MeOH/DCM): 0.75; } \delta_H (\text{CDCl}_3, 200 \text{ MHz}): 1.49 (3H, d, \^J = 8.0 \text{ Hz, CHCH}_3), 2.88 (2H, m, CH}_2\text{CHCH}_3), 3.40 (1H, m, CHCH}_3), 7.39 (5H, m, ArH) \text{ ppm; } \delta_C (\text{CDCl}_3, 63 \text{ MHz}): 20.6 (\text{CHCH}_3), 34.8 (\text{CH}_2\text{CHCH}_3), 40.2 (\text{CHCH}_3), 125.6 (\text{ArC}), 126.0 (\text{ArC}), 127.8 (\text{ArC}), 143.3 (i-ArC), 164.6 (C=O) \text{ ppm; } \nu_{\text{max}} (\text{thin film}): 3087 (\text{CH}), 3064 (\text{CH}), 3031 (\text{CH}), 2970 (\text{CH}), 2932 (\text{CH}), 2878 (\text{CH}), 1842 (C=O acid fluoride) \text{ cm}^{-1}; m/z \text{ found (ES)} [2M+H]^+ 333 C_{20}H_{23}O_2F_2 \text{ requires 333.}

(R)-(−)-3-Phenylbutyryl fluoride (R)-161: (R)-(−)-3-phenylbutyric acid (R)-160 (0.2 g, 1.22 mmol) was treated as above to yield a clear colourless oil (215 mg, > 99%).

\[ \delta_H, \delta_C, \nu_{\text{max}} \text{ and } m/z \text{ data as for (±)-3-phenylbutyryl fluoride (R/S)-161; } [\alpha]_D^{22} -12.1 \text{ (c 1.07, CHCl}_3). \]

(S)-(−)-3-Phenylbutyryl fluoride (S)-161: (S)-(−)-3-phenylbutyric acid (S)-160 (0.5 g, 3.05 mmol) was treated as above to yield a clear colourless oil (400 mg, 79%).

\[ \delta_H, \delta_C, \nu_{\text{max}} \text{ and } m/z \text{ data as for (±)-3-phenylbutyryl fluoride (R/S)-161; } [\alpha]_D^{22} +12.7 \text{ (c 1.02, CHCl}_3). \]
8.1.2 (±)-3-Phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R/S)-162

(±)-3-Phenylbutyryl fluoride (R/S)-161 (0.5 g, 3.01 mmol) was dissolved in anhydrous DCM (20 mL) and dimedone 24 was added. DIPEA (1.05 mL, 6.02 mmol) was added dropwise to form an orange solution that turned dark red then blue over 5 minutes. The mixture was stirred at room temperature under nitrogen for 2 hours. The mixture was diluted with 1M HCl (15 mL) and the DCM layer separated and washed with saturated aqueous NaHCO₃ (2 × 15 mL), brine (15 mL), dried over anhydrous magnesium sulfate and concentrated *in vacuo* to yield a bright blue oil. The crude material was purified by column chromatography on silica eluting with 5 % MeOH in DCM to yield a pale yellow oil (771 mg, 90 %).

**rf** (10 % MeOH/DCM); 0.75; δₜ (CDCl₃, 200 MHz); 1.04 (6H, s, 2 × CH₃), 1.36 (3H, d, 3J = 7.0 Hz, CHCH₃), 2.18 (2H, d, 3J = 3.5 Hz, H₄), 2.21 (2H, d, 3J = 5.0 Hz, H₆), 2.74 (2H, d, 3J = 7.0 Hz, CH₂CHMe), 3.31 (1H, m, CHMe), 5.74 (1H, t, 3J = 1 Hz, CH=CO), 7.26 (5H, m, ArH ppm; δₜ (CDCl₃, 63 MHz); 22.1 (CHCH₃), 28.2 (2 × CH₃), 33.3 (C₅), 36.8 (CHCH₃), 42.1 (CH₂), 43.1 (CH₂), 50.9 (CH₂), 116.7 (CH=CO), 126.9 (ArC), 128.8 (ArC), 144.8 (i-ArC), 168.3 (C₁), 169.1 (C=O ester), 199.6 (C=O enol ppm; vₘₙₐₓ (thin film); 3062 (CH), 3029 (CH), 2962 (CH), 2872 (CH), 1763 (C=O enol ester), 1672 (C=O enol) 1602 (Ar C=C) cm⁻¹; m/z; found (FAB) [M+H]+ 287.16435 C₁₈H₂₃O₃ requires 287.16472; HPLC; (Reverse phase, Phenomenex Sphereclone C18 column, 50 % MeCN in H₂O, 0.1 % TFA modifier) 18.2 mins; (Normal phase, Chiracel-ODH column, 10 % IPA in hexane, 0.1 % TFA modifier) peaks resolved to baseline at 20.41 (R) and 23.46 (S) minutes.

(R)-(−)-3-Phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R)-161: Dimedone 1 (84 mg, 0.6 mmol) was treated with (R)-(−)-3-Phenylbutyryl fluoride (R)-161 (210 µL, 0.6 mmol) as above to yield a clear colourless oil (127 mg, 74 %). δₜ, δₜ, vₘₐₓ and m/z data as for (±)-3-phenylbutyryl acid 5,5-dimethyl-3-oxo-
cyclohex-1-enyl ester (±)-3-Phenylbutyryl fluoride (R/S)-162; [α]_D^22 -44.0 (c 1.00, CHCl₃); HPLC; (Normal phase, Chiracel-ODH column, 90 % IPA in hexane, 0.1 % TFA modifier) 20.25 mins.

(S)-(−)-3-Phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (S)-162:
Dimedone 1 (84 mg, 0.6 mmol) was treated with (S)-(−)-3-Phenylbutyryl fluoride (S)-161 (210 μL, 0.6 mmol) as above to yield a clear colourless oil (148 mg, 86%). δ_H, δ_C, ν_max and m/z data as for (±)-3-phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R/S)-162; [α]_D^22 +39.6 (c 0.984, CHCl₃); HPLC; (Normal phase, Chiracel-ODH column, 90 % IPA in hexane, 0.1 % TFA modifier) 23.51 mins.

8.2 Enzymatic screen – general procedure

3-Phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R/S)-162 (85.8 μg, 3 μmol) was dissolved in 10 % MeCN/0.1 M KPₒ buffer (pH = 7.0) and added to the enzymes (1 mg) under study in individual eppendorf tubes and to one empty tube as a control. The tubes were rotated at room temperature overnight then 0.1 % TFA in MeCN (0.5 mL) was added to each tube to inactivate and precipitate the enzyme. The mixtures were filtered and analysed by HPLC on a reverse phase Sphereclone C18 column eluting with 50 % MeCN in H₂O (0.1 % TFA modifier) over 20 minutes. The reaction was repeated to a conversion less that 50 % in order to calculate an E value. The sample was dissolved in 10 % IPA in hexane for analysis by normal phase HPLC on a Chiracel-ODH column eluting with 10 % IPA in hexane (0.1 % TFA modifier). HPLC; (Normal phase, Chiracel-ODH, 10 % IPA in hexane, 0.1 % TFA modifier) (±)-3-Phenylbutyric acid (R/S)-160: peaks resolved to baseline at 9.38 (R) and 11.89 (S) minutes, Dimedone 24: peak at 8.31 minutes. The results of the screen are detailed in table 8.
8.3 Reagents for hydrolase catalysed transesterification

8.3.1 (±)-Methyl 3-phenylbutanoate (R/S)-163

(±)-3-Phenylbutyric acid (R/S)-160 (1 g, 6.09 mmol) was dissolved in methanol (9 mL) and concentrated sulfuric acid (2 mL) added. The mixture was heated at reflux for 2 ½ hours. The mixture was concentrated in vacuo to remove the methanol then dissolved in ethyl acetate (100 mL) and washed with saturated aqueous sodium NaHCO₃ solution (50 mL, to pH 9), water (50 mL) and brine (50 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a clear colourless oil (1.09 g, > 99 %). \( \text{rf} \) (10 % MeOH/DCM); 0.81; \( \delta_{\text{H}} \) (CDCl₃, 200 MHz); 1.40 (3H, d, \( ^3J = 7.0 \) Hz, CHCH₃), 2.68 (2H, dd, \( ^3J = 7.5, 5.4 \) Hz, CH₂CHCH₃), 3.38 (1H, m, CHCH₃), 3.71 (3H, s, OCH₃), 7.78 (5H, m, ArH) ppm; \( \delta_{\text{C}} \) (CDCl₃, 63 MHz); 21.6 (CHCH₃), 36.3 (CHCH₃), 42.5 (CH₂CHCH₃), 51.3 (COCH₃), 126.2 (ArC), 126.5 (ArC), 128.3 (ArC), 145.5 (i-ArC), 172.7 (C=O) ppm; \( \nu_{\text{max}} \) (thin film); 3062 (CH), 3029 (CH), 2965 (CH), 1738 (C=O ester), 1604 (Ar C=C) cm\(^{-1}\); m/z; found (ES\(^{+}\)) [M+Na\(^{+}\)] 201, C₁₁H₁₄O₂Na requires 201; \text{HPLC}; (Normal phase, Chiracel-ODH, 10 % IPA in hexane, 0.1 % TFA modifier) peaks resolved to baseline at 9.42 (R) and 14.64 (S) minutes.

(R)-(−)-Methyl 3-phenylbutanoate (R)-163: (R)-(−)-3-Phenylbutyric acid (R)-160 (0.1 g, 0.61 mmol) was treated as above to yield a clear colourless oil (95 mg, 87 %). \( \delta_{\text{H}}, \delta_{\text{C}}, \nu_{\text{max}} \) and m/z data as for (±)-methyl 3-phenylbutanoate (R/S)-163; \text{HPLC}; (Normal phase, Chiracel-ODH, 10 % IPA in hexane, 0.1 % TFA modifier) peak at 9.44 minutes; \([\alpha]_D^{22} -29.4 \) (c 1.02, CHCl₃) [literature \([\alpha]_D^{24} -25.3 \) (c 1.07, CHCl₃)].

8.3.2 (±)-Vinyl 3-phenylbutanoate (R/S)-164
(±)-3-Phenylbutyric acid (R/S)-160 (5 g, 30.5 mmol) and mercury (II) acetate (97.2 mg, 0.3 mmol) were dissolved in vinyl acetate (16.8 mL, 182.7 mmol) and stirred at room temperature under argon for 30 minutes. Concentrated sulfuric acid (10 µL) was added and the mixture heated under reflux for 4 hours to form a yellow solution. The solution was diluted with ethyl acetate (100 mL) and washed with saturated aqueous sodium bicarbonate solution (100 mL) and brine (100 mL) before being dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a yellow oil. The crude material was purified by column chromatography on silica eluting with 20 % ethyl acetate in petroleum ethers (40–60 °C) to yield a clear colourless oil (3.68 g, 64 %). 

\[ \text{rf (20 % EtOAc/pet ethers 40-60 °C): 0.57; } \delta_{\text{H}} \text{(CDCl}_3, 200 \text{ MHz): 1.33 (3H, d, } J = 7.0 \text{ Hz, CHCH}_3), 2.66 (2H, dd, } J = 7.5, 7.0 \text{ Hz, CH}_2\text{CHCH}_3), 3.28 (1H, m, CHCH}_3), 4.53 (1H, dd, } J = 6.5 \text{ Hz, }^3J = 1.5 \text{ Hz, CH=CH}_2 \text{cis}), 4.84 (1H, dd, } J = 14.0, \quad ^3J = 1.5 \text{ Hz, CH=CH}_2 \text{trans}), 7.25 (6H, m, ArH and CH=CH}_2 \text{ppm); } \delta_{\text{C}} \text{(CDCl}_3, 63 \text{ MHz): 14.0 (CHCl}_3), 21.6 (\text{CHCH}_3), 36.0 (\text{CH}_2\text{CHCH}_3), 97.5 (\text{CH=CH}_2), 126.4 (\text{ArC}), 126.5 (\text{ArC}), 128.4 (\text{ArC}), 140.9 (\text{CH=CH}_2), 145.1 (i-\text{ArC}), 169.2 (C=O) \text{ ppm; } \nu_{\max \text{(thin film): } 3088 (\text{CH}), 3030 (\text{CH}), 2969 (\text{CH}), 1752 (\text{C=O}), 1646 (\text{C=C}), 1604 (\text{Ar C=C}) \text{ cm}^{-1}; } m/z; \text{ found (FAB) [M+H]^+ 191, C}_{12}\text{H}_{15}\text{O}_2 \text{ requires 191; HPLC; (Normal phase, Chiracel-ODH, 1 % IPA in hexane, 0.1 % TFA modifier) peaks not fully resolved at 10.44 (R) and 10.87 (S) minutes.}

(R)-(−)-Vinyl 3-phenylbutanoate (R)-164: (R)-(−)-3-Phenylbutyric acid (R)-160 (0.5 g, 3.05 mmol) and mercury II acetate (9.7 mg, 30.5 µmol) were dissolved in vinyl acetate (1.68 mL, 18.3 mmol) and stirred at room temperature under argon for 30 minutes. Concentrated sulfuric acid (10 µL) was added and the mixture heated in the microwave at 145 °C (200 W) for 5 minutes to form a brown solution. The solution was concentrated in vacuo and purified directly by column chromatography on silica eluting with 20 % ethyl acetate in petroleum ethers (40–60 °C) to yield a clear colourless oil (0.33 g, 57 %). \( \delta_{\text{H}}, \delta_{\text{C}}, \nu_{\max \text{ and m/z data as for (±)-vinyl 3-phenylbutanoate (R/S)-164; HPLC; (Normal phase, Chiracel-ODH, 1 % IPA in hexane, 0.1 % TFA modifier) peak at 10.45 minutes; } [\alpha]_D^{22} -21.6 \text{ (c 1.02, CHCl}_3) \text{ [literature } [\alpha]_D^{22} -19.8 \text{ (c 1.01, CHCl}_3)].

(S)-(−)-Vinyl 3-phenylbutanoate (S)-164: (S)-(−)-3-Phenylbutyric acid (S)-160 (0.5 g, 3.05 mmol) was treated as above in the microwave to yield a clear colourless oil
(0.28 g, 48 %). δH, δC, νmax and m/z data as for (±)-vinyl 3-phenylbutanoate (R/S)-164; HPLC; (Normal phase. Chiracel-ODH, 1 % IPA in hexane, 0.1 % TFA modifier) peak at 10.86 minutes; [α]D 22 +21.0 (c 1.00, CHCl3) [literature [α]D 22 +20.7 (c 1.08, CHCl3)].

8.4 Monitoring the kinetics of hydrolysis – general procedure

Fourteen samples of ester (3 μmol) were each dissolved in 10 % MeCN/0.1M KP buffer (pH = 7.0) (1 mL) containing CVL (0.1 mg) in individual eppendorf tubes (with one tube containing no CVL as control) and rotated at room temperature for t = 5, 10, 15, 20, 30, 45, 60, 90, 150, 270, 480, 960 and 1440 minutes. After the correct time the samples were added to ethyl acetate containing 0.1 % TFA (1 mL). The ethyl acetate layer was separated and filtered through a plug of anhydrous magnesium sulfate before being concentrated in vacuo. The product was dissolved in 1 % IPA in hexane (1 mL) for analysis by normal phase HPLC. HPLC; Normal phase, Chiracel-ODH, 1 % IPA in hexane, 0.1 % TFA modifier) (±)-3-Phenylbutyric acid (R/S)-160: peaks resolved to baseline at 16.88 (R) and 28.09 (S) minutes.

8.5 Synthesis of CHD on PEGA1900 resin

8.5.1 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid methylPEGA1900-methyl-amide resin 166

Birch acid 36 (106 mg, 0.50 mmol) was dissolved in DMF (2.5 mL) and DIC (78 μL, 0.50 mmol) was added dropwise. Meanwhile commercially available aminomethyl-PEGA1900 resin 165 (5 g, L-quote = 0.2 mmol/g, 0.10 mmol) was swollen in DCM (2.5 mL). The contents of the first flask were transferred to the isolute tube containing the resin. The resulting mixture was shaken at room temperature for 2 ½ hours and
the resin filtered and washed according to the standard protocol. The coupling repeated was repeated for 2 ½ hours, the resin washed according to the same protocol and the resin stored in the freezer under methanol. The resin gave negative chloranil and Kaiser tests. \( v_{\text{max}} \) (DCM); 3418 (NH), 3201 (NH), 3054 (CH), 2985 (CH), 2871 (CH), 1674 (C=O), 1652 (C=C), 1634 (C=C), 1615 (Ar C=C) cm\(^{-1}\).

8.5.2 1-Ethyl-3-hydroxy-5-oxo-cyclohex-3-ene carboxylic acid methylaminomethylPEG\(_{1900}\)-amide (CHD) resin 167

![Bis-enol ether resin 166](image)

Bis-enol ether resin 166 (1 g, \( L_{\text{max}} = 0.2 \) mmol/g, 0.2 mmol) was rotated for 2 ½ hours at room temperature in TFA (4.5 mL), water (1.67 mL) and DMF (1.67 mL). The resin was filtered and washed with 2 x DMF, 2 x MeOH then shaken in \( \text{\(^{3}\)BuNH}_2 \) for five minutes to remove any TFA enol ester (\( v_{\text{max}} = 1781 \) cm\(^{-1}\)). The resin was further washed according to the standard protocol and stored in the freezer under methanol. The resin gave a positive brown colouration with 1M FeCl\(_3\).H\(_2\)O aqueous solution indicating 1,3-diketone formation. \( \delta_H \) (MAS, 600 MHz, CDCl\(_3\)); 0.75 (CH\(_2\)CH\(_3\)), 0.85 (CH\(_2\)CH\(_3\)), 1.05 (CH\(_2\) backbone), 1.55 (CH\(_2\)CH\(_3\)), 1.60 (CH\(_2\)CH\(_3\)), 2.30 (H2), 2.35 (CH\(_2\)), 2.60 (CH\(_2\)), 2.65 (CH\(_2\)), 3.30-4.60 (PEG backbone peak) ppm; \( v_{\text{max}} \) (DCM); 3347 (NH), 3200 (NH), 3055 (CH), 2985 (CH), 2878 (CH), 1669 (C=O, enol), 1621 (C=C) cm\(^{-1}\).

8.6 Synthesis of racemic 1,4-enol ester \((R/S)-169\) and subsequent release

8.6.1 \((\pm)-3\)-Phenylbutyryl chloride \((R/S)-168\)

\((\pm)-3\)-Phenylbutyric acid \((R/S)-160\) (5 g, 30.5 mmol) was dissolved in anhydrous DCM (30.5 mL) and containing DMF (3 drops). The mixture was cooled in an ice
bath and oxalyl chloride (2.79 mL, 31.9 mmol) was added dropwise with stirring under argon. The mixture was stirred for a further 3 hours in the ice bath to give a pale yellow solution. The mixture was concentrated in vacuo to give an orange solution which was distilled under vacuum to give a clear colourless oil (4.40 g, 79%).

**bpt;** 60 °C (0.5 mm Hg) *rf* (0.1 % AcOH in 10 % MeOH/DCM); 0.49; δ_H (CDCl₃, 200 MHz); 1.44 (3H, d, ²J = 7.0 Hz, CHCH₃), 3.22 (2H, m, CH₂CHCH₃), 3.40 (1H, m, CHCH₃), 7.39 (5H, m, ArH) ppm; δ_C (CDCl₃, 63 MHz); 20.3 (CHCH₃), 35.8 (CH₂CHCH₃), 54.2 (CHCH₃), 125.7 (ArC), 127.8 (ArC), 128.0 (ArC), 143.0 (i-ArC), 164.6 (C=O) ppm; ν_max (thin film); 3086 (CH), 3063 (CH), 3031 (CH), 2969 (CH), 2931 (CH), 2876 (CH), 1799 (C=O acid chloride) cm⁻¹; *m/z*; decomposed under ES⁺ and FAB conditions.

### 8.6.2 3-Phenyl-butyric acid 5-methylPEGₐ₁₉₀₀-carbamoyl-5-ethyl-3-oxo-cyclohex-1-enyl ester resin (R/S)-169

![Chemical Structure](image)

CHD resin 167 (5 g, 0.1 mmol) was swollen in DCM (50 mL) containing (±)-3-phenylbutyryl chloride (R/S)-168 (347 mg, 1.90 mmol). The mixture was shaken overnight then filtered and washed according to the standard protocol and stored in the freezer under methanol. δ_H (MAS, 600 MHz, CDCl₃); 0.80 (CH₂CH₃), 0.85 (CH₂CH₃), 1.05 (CH₂ backbone), 1.21 (CHCH₃), 1.55 (CH₂CH₃), 3.31-4.64 (PEGA backbone peak), 7.28 (ArH), 7.26 (ArH) ppm; ν_max (DCM); 3337 (NH), 3197 (NH), 3054 (CH), 2985 (CH), 2928 (CH), 2866 (CH), 1673 (C=O, enol) cm⁻¹.
8.6.3 Resin cleavage: Standard release of \((\pm)-3\text{-phenylbutyric acid (R/S)}\)-164

Resin bound \((\pm)-3\text{-phenylbutyryl enol ester (R/S)}\)-169 (200 mg MeOH swollen) was weighed into an isolute tube and washed prior to reaction with \(5 \times \text{H}_2\text{O}, 5 \times \text{H}_2\text{O}/\text{MeCN (1:1)}\) and \(5 \times \text{H}_2\text{O}\). The resin was swollen in 0.1M aqueous NaOH (1 mL) and rotated for 1 hour at room temperature. The resin was filtered and washed with \(5 \times \text{H}_2\text{O}, 5 \times \text{H}_2\text{O}/\text{MeCN (1:1)}, 5 \times \text{H}_2\text{O}\) and \(5 \times \text{EtOAc}\) and the filtrate collected. The EtOAc layer was separated and filtered through a plug of anhydrous magnesium sulfate. The resin was further washed with \(5 \times \text{DCM}\) and again the DCM layer collected and filtered through a plug of anhydrous magnesium sulfate. The combined organic layers were concentrated in vacuo and the resultant product dissolved in 1 % IPA in hexane (1 mL) for analysis by normal phase HPLC on a Chiracel-ODH column eluting with 99 % hexane, 1 % IPA (0.1 % TFA modifier). The peak area observed gave a calculated resin loading of 19 \(\mu\text{mol} \cdot \text{g}^{-1}\) for resin swollen in methanol and 130 \(\mu\text{mol} \cdot \text{g}^{-1}\) for dry resin. The peak area was used as a standard for the release of acid from the resin in subsequent kinetic resolution experiments.

8.6.4 Resin cleavage: Attempted CVL-catalysed release of \((\pm)-3\text{-phenylbutyric acid (R/S)}\)-168
Resin bound (±)-3-phenylbutyryl enol ester (R/S)-169 (200 mg MeOH swollen, $L_{\text{calc}} = 0.19 \text{ mmol/g, 38 } \mu\text{mol}$) was weighed into an isolute tube and washed prior to reaction with $5 \times H_2O$, $5 \times H_2O/MeCN (1:1)$ and $5 \times H_2O$. The resin was swollen in 10 % MeCN/0.1 M KP$_1$ buffer (pH = 7.0) containing CVL (1 mg) and rotated for one hour at room temperature. The resin was filtered and washed with $1 \times 2M \text{ HCl}$, $5 \times H_2O$, $5 \times H_2O/MeCN (1:1)$, $5 \times H_2O$ and $5 \times \text{EtOAc}$ and the filtrate collected. The EtOAc layer was separated and filtered through a plug of anhydrous magnesium sulfate. The resin was further washed with $5 \times \text{DCM}$ and again the DCM layer collected and filtered through a plug of anhydrous magnesium sulfate. The combined organic layers were concentrated in vacuo and the resultant product dissolved in 1 % IPA in hexane (1 mL) for analysis by normal phase HPLC on a Chiracel-ODH column eluting with 99 % hexane, 1 % IPA (0.1 % TFA modifier). (S)-(+)3-Phenylbutyric acid (R/S)-160 (2 %) with e.e. = 51 % was observed.

8.7 Kinetic resolution of 3-phenylbutyric acid (R/S)-169

8.7.1 General procedure

CHD resin 167 (200 mg MeOH swollen, $L_{\text{calc}} = 19 \text{ mmol/g, 3.8 } \mu\text{mmol}$) was weighed into an isolute tube and washed prior to reaction with $5 \times H_2O$, $5 \times H_2O/MeCN (1:1)$ and $5 \times H_2O$. Acylating agent (R/S)-163 or (R/S)-164 (38 $\mu$mol) was added directly to the resin followed by the hydrolase (1 mg) in 0.1M KP$_1$ buffer (pH = 7.0) (1 mL). The resin was rotated at room temperature for 16 hours then washed with $5 \times H_2O$, $5 \times \text{DMF/H}_2O (1:1)$, $5 \times \text{DMF}, 5 \times H_2O, 5 \times \text{DMF/H}_2O (1:1)$, $5 \times H_2O, 5 \times H_2O/MeCN (1:1)$ and $5 \times H_2O$ before further rotation in 0.1M aqueous NaOH (1 mL) for 1 hour at room temperature. The resin was filtered and washed with $1 \times 2M \text{ HCl}$, $5 \times H_2O$, $5 \times H_2O/MeCN (1:1)$, $5 \times H_2O$ and $5 \times \text{EtOAc}$ and the filtrate collected. The EtOAc layer was separated and filtered through a plug of
anhydrous magnesium sulfate. The resin was further washed with $5 \times \text{DCM}$ and again the DCM layer collected and filtered through a plug of anhydrous magnesium sulfate. The combined organic layers were concentrated \textit{in vacuo} and the resultant product dissolved in 1% IPA in hexane (1 mL) for analysis by normal phase HPLC on a Chiralcel-ODH column eluting with 99% hexane, 1% IPA (0.1% TFA modifier). The results of kinetics resolution are shown in table 9.

8.7.2 \textit{Multiple acylation}

CHD resin 167 (200 mg MeOH swollen, $L_{\text{calc}} = 19 \text{ mmol/g, 3.8 } \mu\text{mol}$) was weighed into an isolute tube and washed prior to reaction with $5 \times \text{H}_2\text{O}$, $5 \times \text{H}_2\text{O/MeCN (1:1)}$ and $5 \times \text{H}_2\text{O}$. Vinyl ester (R/S)-164 (7.2 mg, 38 $\mu$mol) was added directly to the resin followed by CVL (1 mg) in 0.1M KP$_1$ buffer (pH = 7.0) (1 mL). The resin was rotated at room temperature for 16 hours then washed with $5 \times \text{H}_2\text{O}$, $5 \times \text{DMF/H}_2\text{O (1:1)}$, $5 \times \text{DMF}$, $5 \times \text{H}_2\text{O}$, $5 \times \text{DMF/H}_2\text{O (1:1)}$, $5 \times \text{H}_2\text{O}$, $5 \times \text{H}_2\text{O/MeCN (1:1)}$ and $5 \times \text{H}_2\text{O}$. The acylation step was repeated either once or two times more then the resin samples treated as for section 8.7.1. The results of multiple acylation are shown in table 9.

8.7.3 \textit{Control with non lipase}

Two samples of CHD resin 167 (200 mg MeOH swollen, $L_{\text{calc}} = 19 \text{ mmol/g, 3.8 } \mu\text{mol}$) were weighed into an isolute tube and washed prior to reaction with $5 \times \text{H}_2\text{O}$, $5 \times \text{H}_2\text{O/MeCN (1:1)}$ and $5 \times \text{H}_2\text{O}$. Vinyl ester (R/S)-164 (7.2 mg, 38 $\mu$mol) or (±)-3-phenylbutyric acid (R/S)-160 (6.2 mg, 38 mmol) was added directly to each resin sample in 0.1M KP$_1$ buffer (pH = 7.0) (1 mL). No lipase was added. The resins were then treated as for section 8.7.1. Negligible levels of 3-phenylbutyric acid 160 were observed in each case indicating no background acylation.

8.7.4 \textit{Pre-equilibration of lipase}

CHD resin 167 (200 mg MeOH swollen, $L_{\text{calc}} = 19 \text{ mmol/g, 3.8 } \mu\text{mol}$) was weighed into an isolute tube and washed prior to reaction with $5 \times \text{H}_2\text{O}$, $5 \times \text{H}_2\text{O/MeCN (1:1)}$ and $5 \times \text{H}_2\text{O}$. CVL (1 mg) in 0.1M KP$_1$ buffer (pH = 7.0) (1 mL) was added to the resin and the mixture rotated at room temperature for one hour prior
to addition of vinyl ester (R/S)-164 (7.2 mg, 38 \mu mol). The resin was then treated as for section 8.7.1 to yield (R)-(\-)3-phenylbutyric acid (R/S)-160 (53 \%) with e.e. = 90 \%.

8.7.5 Pre-reaction of methyl ester (R/S)-163 with CVL

CHD resin 167 (200 mg MeOH swollen, \( L_{\text{calc}} = 19 \text{ mmol/g, 3.8 \mu mmol} \)) was weighed into an isolute tube and washed prior to reaction with \( 5 \times H_2O, \ 5 \times H_2O/MeCN \ (1:1) \) and \( 5 \times H_2O \). Methyl ester (R/S)-163 (6.8 mg, 38 mmol) was suspended in 0.1 M KP\(_b\) buffer (pH = 7.0) (1 mL) containing CVL (1 mg) and allowed to react for 1 hour at room temperature before addition to the resin. The resin was then treated as for section 8.7.1 to yield (R)-(\-)3-phenylbutyric acid (R/S)-160 (30 \%) with e.e. > 99 \%.

8.7.6 Resin recycling

CHD resin 167 (200 mg MeOH swollen, \( L_{\text{calc}} = 19 \text{ mmol/g, 3.8 \mu mmol} \)) was treated as for section 8.7.1. The resin was washed with \( 5 \times H_2O, \ 5 \times H_2O/MeCN \ (1:1) \) and \( 5 \times H_2O \) and then subjected to a second reaction, again as in section 8.7.1 to yield (R)-(\-)3-phenylbutyric acid (R/S)-160 (18 \%) with e.e. = 87 \%.

8.7.7 Solid-phase timecourse of transesterification

Four samples of CHD resin 167 (200 mg MeOH swollen, \( L_{\text{calc}} = 19 \text{ mmol/g, 3.8 \mu mmol} \)) were weighed into isolute tubes and washed prior to reaction with \( 5 \times H_2O, \ 5 \times H_2O/MeCN \ (1:1) \) and \( 5 \times H_2O \). Vinyl ester (R/S)-164 (7.2 mg, 38 \mu mol) was added directly to the resin samples followed by CVL (1 mg) in 0.1 M KP\(_b\) buffer (pH = 7.0) (1 mL). The resin was rotated at room temperature for 1, 2, 4 and 8 hours respectively then the samples treated as for section 8.7.1.

8.8 Primary amide synthesis and release from resin

8.8.1 (\pm)-3-Phenyl-butyramide (R/S)-175
3-Phenybutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R/S)-162 (200 mg, 0.69 mmol) was dissolved in anhydrous methanol (3.5 mL) and stirred under nitrogen in an ice bath. Once cooled ammonia in methanol (7N, 0.5 mL, 3.50 mmol) was added dropwise. The mixture was allowed to warm to room temperature and stirred for three hours. The mixture was diluted with ethyl acetate (10 mL) and 1.5M aqueous KHSO₄ solution (20 mL). The ethyl acetate layer was separated and washed with saturated aqueous NaHCO₃ solution, water and brine (all 20 mL). The organic layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a white crystalline solid (86 mg, 75 %). mpt; 99-100 °C; rf (10 % MeOH/DCM); 0.33; δH (CDCl₃, 200 MHz); 1.25 (3H, d, ²J = 7.0 Hz, CHCH₃), 2.37 (1H, dd, ²J = 15.0, ³J = 7.0 Hz, CH₂CHCH₃), 2.50 (1H, dd, ²J = 15.0, ³J = 7.0 Hz, CH₂CHCH₃), 3.20 (1H, m, CHCH₃), 5.31 (1H, br s, NH₂), 5.64 (1H, br s, NH₂), 7.20 (5H, m, ArH) ppm; δC (CDCl₃, 63 MHz); 21.1 (CHCH₃), 36.1 (CHCH₃), 44.2 (CH₂CHCH₃), 125.8 (ArC), 126.1 (ArC), 127.8 (ArC), 127.9 (ArC), 145.1 (i-ArC), 173.7 (C=O) ppm; v max (thin film); 3357 (NH), 3187 (NH), 3028 (CH), 2858 (CH), 2925 (CH), 1658 (C=O amide), 1625 (C=C) cm⁻¹; m/z; found (ES⁺) [M+H]⁺ 164, C₁₀H₁₄N₀ requires 164, [M+Na]⁺ 186, C₁₀H₁₃NONa requires 186; HPLC (Normal phase, Chiracel-ODH, 5 % IPA in hexane, 0.1 % TFA modifier) peaks resolved to baseline at 44.89 (R) and 48.54 (S) minutes.

(R)-(−)-3-Phenyl-butyramide (R/S)-175: (±)-3-Phenylbutyric acid (R/S)-160 (0.5 g, 3.05 mmol) and triethylamine (446 µL, 3.20 mmol) were dissolved in chloroform (15 mL) and isobutylchloroformate (415 mL, 3.20 mmol) was added dropwise whilst cooling in a salt/ice bath. The mixture was stirred for 15 minutes then ammonia gas bubbled through the mixture for 10 minutes. The mixture was allowed to warm to room temperature over one hour then the mixture worked up as above to yield a white crystalline solid (0.61 g, > 99 %). δH, δC, v max and m/z data as for (±)-3-phenyl-butyramide (R/S)-175; [α]D²² −28.0 (c 1.00, CHCl₃) [literature [α]D²⁴ −30.9 (c 1.01, CHCl₃)].
8.8.2 Resin cleavage: Standard release of (±)-3-phenyl-butyramide (R/S)-169

(+)-3-Phenylbutyryl enol ester (R/S)-169 (200 mg MeOH swollen) was weighed into an isolute tube and washed prior to reaction with 5 × H2O, 5 × H2O/MeCN (1:1) and 5 × H2O. The resin was swollen in ammonia in methanol (7N, 1 mL) and rotated for 1 hour at room temperature. The resin was filtered and washed with 1 × 2M HCl, 5 × H2O, 5 × H2O/MeCN (1:1), 5 × H2O and 5 × EtOAc and the filtrate collected. The EtOAc layer was separated and filtered through a plug of anhydrous magnesium sulfate. The resin was further washed with 5 × DCM and again the DCM layer collected and filtered through a plug of anhydrous magnesium sulfate. The combined organic layers were concentrated in vacuo and the resultant product dissolved in 5 % IPA in hexane (1 mL) for analysis by normal phase HPLC on a Chiracel-ODH column eluting with 95 % hexane, 5 % IPA (0.1 % TFA modifier). The resin was washed with 5 × H2O, 5 × H2O/MeCN (1:1) and 5 × H2O and subjected to hydrolysis in 1M NaOH (aq) (1 mL) and treated as for section 8.7.1 to check for complete enol ester cleavage. The peak area for ammonia release corresponded to 74 % cleavage of enol ester. The peak area was used as a standard for the release of primary amide from the resin in the subsequent experiment.
8.8.3 Transesterification and release of (±)-3-phenyl-butyramide (R/S)-175

(CHD resin 167 (200 mg MeOH swollen, L_{calc} = 19 mmol/g, 3.8 μmmol) was weighed into an isolute tube and washed prior to reaction with 5 × H_{2}O, 5 × H_{2}O/MeCN (1:1) and 5 × H_{2}O. Vinyl ester (R/S)-164 (7.2 mg, 38 μmol) was added directly to the resin followed by CVL (1 mg) in 0.1 M KP buffer (pH = 7.0) (1 mL). The resin was rotated at room temperature for 16 hours then washed with 5 × H_{2}O, 5 × DMF/H_{2}O (1:1), 5 × DMF, 5 × H_{2}O, 5 × DMF/H_{2}O (1:1), 5 × H_{2}O, 5 × H_{2}O/MeCN (1:1) and 5 × H_{2}O before further rotation in ammonia in methanol (7N, 1 mL) for 1 hour at room temperature. The resin was filtered and washed with 1 × 2M HCl, 5 × H_{2}O, 5 × H_{2}O/MeCN (1:1), 5 × H_{2}O and 5 × EtOAc and the filtrate collected. The EtOAc layer was separated and filtered through a plug of anhydrous magnesium sulfate. The resin was further washed with 5 × DCM and again the DCM layer collected and filtered through a plug of anhydrous magnesium sulfate. The combined organic layers were concentrated in vacuo and the resultant product dissolved in 5 % IPA in hexane (1 mL) for analysis by normal phase HPLC on a Chiracel-ODH column eluting with 95 % hexane, 5 % IPA (0.1 % TFA modifier). (R)-(−)-3-phenyl-butyramide (R/S)-175 (32 %) was obtained with e.e. > 99 %.
8.9 Synthesis and subsequent hydrolysis of C5 modified analogues

8.9.1 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid benzylamide 176

Birch acid 36 (1 g, 4.72 mmol), TBTU (1.67 g, 5.19 mmol) and HOBr.H₂O (0.38 g, 2.36 mmol) were dissolved in DMF (24 mL) and triethylamine (0.72 mL, 5.19 mmol) was added slowly. Benzylamine (0.57 mL, 5.19 mmol) was added dropwise and the resulting pale yellow mixture stirred for 1½ hours under argon to form a yellow solution. The mixture diluted with EtOAc (50 mL) and aqueous 1M KHSO₄ solution (50 mL). The EtOAc layer was separated and washed once with saturated aqueous NaHCO₃ solution (50 mL), water (50 mL) and brine (50 mL). The EtOAc layer was dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a crude pale yellow oil. The crude product was purified by column chromatography on silica eluting with 20 % ethyl acetate in petroleum ether to give a clear colourless oil that crystallised out to form white crystals (1.11 g, 78 %). mp; 86-87 °C; rf (10 % MeOH/DCM); 0.71; δH (CDCl₃, 200 MHz); 0.82 (3H, t, 3J= 7.5 Hz, CH₂CH₃), 1.88 (2H, q, 3J= 7.5 Hz, CH₂CH₃), 2.71 (2H, dd, 2J= 22.0 Hz, H4), 3.61 (6H, s, 2 x OCH₃), 4.44 (2H, s, CH₂Ph), 4.61 (2H, s, H2/H6), 6.31 (1H, s, NH), 7.26 (5H, m, ArH) ppm; δC (CDCl₃, 63 MHz); 8.7 (CH₂CH₃), 31.1 (CH₂CH₃), 33.3 (C4), 43.3 (CH₂Ph), 51.0 (C1), 54.4 (2 x OCH₃), 95.5 (C2/C6), 127.1 (ArC), 127.5 (ArC), 128.2 (ArC), 128.5 (ArC), 138.6 (i-ArC), 153.9 (C3/C5), 176.0 (C=O) ppm; νmax (thin film); 3343 (NH), 3200-2800 (br, CH), 1733 (C=O), 1692 (C=O), 1653 (C=C), 1606 (Ar CC) cm⁻¹; m/z; found (FAB) [M+H]⁺ 302.17605 C₁₈H₂₄NO₃ requires 302.17562.
8.9.2 1-Ethyl-3-hydroxy-5-oxo-cyclohex-3-enecarboxylic acid benzylamide 177

Bis-enol ether 176 (0.36 g, 1.20 mmol) was dissolved in THF (2.9 mL) and 12 M HCl (2.9 mL) added slowly. The mixture was stirred at room temperature for 2 hours to give a clear colourless solution. The solution was concentrated in vacuo to remove the THF and the residue dissolved in chloroform (20 mL) and water (20 mL). The chloroform layer was separated and the aqueous layer washed once more with chloroform (20 mL). The combined organic extracts were washed with brine (10 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a hygroscopic white crystalline solid (0.31 g, 95\%). mpt; 79-80 °C; rf (10 % MeOH/DCM); 0.26; δH (CD3OD, 200 MHz); 0.78 (3H, t, 3J = 7.5 Hz, CH2CH3), 1.65 (2H, q, 3J = 8.0 Hz, CH2CH3), 2.09 (2H, s, CH2C=O keto), 2.35 (2H, d, 3J = 17.0 Hz, 2 × CH2), 2.82 (2H, d, 3J = 17.0 Hz, 2 × CH2), 4.29 (2H, d, 3J = 6.0 Hz, CH2Ph), 7.18 (5H, m, ArH), 8.24 (1H, br s, NH) ppm; δC (CD3OD, 63 MHz); 6.9 (CH2CH3), 30.8 (CH2CH3), 39.7 (CH2), 40.9 (CH2), 41.0 (CH2), 42.3 (CH2), 48.0 (C5), 126.1 (ArC), 126.4 (ArC), 127.5 (ArC), 138.4 (i-ArC), 174.5 (C=O amide), 205.0 (C=O keto) ppm; νmax (nujol mull); 3314 (NH), 3000-2500 (br, CH), 1636 (C=C), 1603 (Ar C=C) cm⁻¹; m/z; found (FAB) [M+H]+ 274.14495 C16H20NO3 requires 274.14432.

8.9.3 (S)-(−)-3-Phenyl-butyric acid 5(R/S)-benzylcarbamoyl-5-ethyl-3-oxo-cyclohex-1-enyl ester (5R/S, 3S)-178
(S)-(+-)-3-Phenylbutyryl fluoride (R/S)-161 (0.22 g, 1.33 mmol) was dissolved in anhydrous DCM (6 mL) and diketone 177 (0.33 g, 1.21 mmol) was added. DIPEA (0.42 mL, 2.42 mmol) was added dropwise to form an orange solution that turned dark red then blue over 5 minutes. The mixture was stirred at room temperature under nitrogen for 1 hour. The mixture was diluted with 1M HCl (15 mL) and the DCM layer separated and washed with saturated aqueous NaHCO₃ (2 ×15 mL), brine (15 mL), dried over anhydrous magnesium sulfate and concentrated in vacuo to yield a bright blue oil. The crude material was purified by column chromatography on silica eluting with 50 % ethyl acetate in petroleum ethers (40–60 °C) to yield a pale yellow oil (0.31 mg, 61 %). rt (10 % MeOH/DCM); 0.82; δH (CDCl₃, 200 MHz); 0.75 (3H, t, 3J = 7.5 Hz, CH₂CH₃), 1.28 (3H, d, 3J = 9.5 Hz, CHCH₃), 1.59 (2H, m, CH₂CH₃), 2.27 (2H, m, CH₂), 2.68 (4H, m, CH₂ and CH₂CHMe), 4.04 (1H, sextet, 3J = 8.0 Hz, CHMe), 4.33 (2H, d, 3J = 5.5 Hz, CH₂Ph), 5.67 (1H, t, 3J = 2.5 Hz, NH), 6.33 (1H, br q, CH=CO), 7.19 (10H, m, ArH) ppm; δC (CDCl₃, 63 MHz); 8.4 (CH₂CH₃), 21.8 (CHCH₃), 35.8 (CH₂), 36.6 (CHCH₃), 42.7 (CH₂), 43.8 (CH₂), 45.3 (CH₂), 47.9 (C₅), 117.2 (CH=CO), 126.7 (ArC), 126.7 (ArC), 127.3 (ArC), 127.6 (ArC), 128.5 (ArC), 137.9 (i-ArC), 144.4 (i-ArC), 167.8 (C1), 169.3 (C=O ester), 172.8 (C=O amide), 196.8 (C=O enol) ppm; νmax (thin film); 3398 (NH), 3398 (CH), 3030 (CH), 2968 (CH), 1736 (C=O enol ester), 1647 (C=O enol) cm⁻¹; m/z; found (FAB) [M+H]+ 420.21764 C₂₆H₃₀N₄ requires 420.21748; HPLC; (Normal phase, Chiracel-ODH column, 10 % IPA in hexane, 0.1 % TFA modifier) peaks resolved to baseline at 56.15 (R) and 70.53 (S) minutes; d.e. = 7 %; [α]b° 22 +29.0 (c 1.00, CHCl₃).
8.9.4 Monitoring the kinetics of hydrolysis of (S)-(+)\text{-}3-\text{Phenyl\text{-}butyric acid 5(R/S)-benzylcarbamoyl\text{-}5-ethyl\text{-}3\text{-}oxo\text{-}cyclohex\text{-}1\text{-}enyl ester (5R/S, 3S)-178}}

(S)-(+)\text{-}3-\text{Phenyl\text{-}butyric acid 5-benzylcarbamoyl\text{-}5-ethyl\text{-}3\text{-}oxo\text{-}cyclohex\text{-}1\text{-}enyl ester (5R/S, 3S)-178}} was treated as for general procedure 8.4. No appreciable difference in rate was observed for each diastereoisomer.

8.10 Screening for hydrolase activity by detection of copper-dimedone chelates

8.10.1 Detection of dimedone – Proof of concept

Dimedone 24 (varying amounts) was dissolved in DMSO (150 \mu L/well), saturated aqueous copper II acetate solution (20 \mu L/well) and un-buffered water (30 \mu L/well) in a MTP. The absorbance of each solution was measured on a plate reader at 417 nm and plotted against the concentration of dimedone to give figure 25.

8.10.2 CVL catalysed hydrolysis at varying enzyme concentrations

(\pm)-3-\text{Phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R/S)-162}} (4 mg/well, 14 \mu mol) was dissolved in DMSO (150 \mu L/well) and saturated aqueous copper II acetate solution (20 \mu L/well) in a MTP. CVL samples (0.1, 0.3, 0.5, 0.7 and 1 mg) were dissolved in un-buffered water (30 \mu L/well) and added directly to the required reaction well. The UV-absorbance at 417 nm was monitored as described above against a background sample containing no enzyme, over a two hour time period at 30 °C. The results of this experiment are shown in figure 26.
8.10.3 Determination of enzymatic activity in MTP

(±)-3-Phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R/S)-162 (4 mg/well, 14 µmol) was dissolved in DMSO (150 µL/well) and saturated aqueous copper II acetate solution (20 µL/well). The enzyme (1 mg/well) were dissolved in un-buffered water (30 µL/well) and added directly to the required reaction well. The UV-absorbance at 417 nm was monitored as described above against a background sample containing no enzyme, over a two hour time period at 30 °C. The results of this screen are shown in figure 27.

8.10.4 Monitoring the kinetics of hydrolysis of (R)-(−)- and (S)-(+)3-phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R)-162 and (S)-162

The kinetics of hydrolysis were monitored by HPLC according to the general procedure detailed in section 8.4. For the UV-timecourse, (R)-(−) and (S)-(+)3-phenylbutyryl acid 5,5-dimethyl-3-oxo-cyclohex-1-enyl ester (R)-162 and (S)-162 (4 mg/well, 14 µmol) were dissolved in DMSO (150 µL/well) and saturated aqueous copper II acetate solution (20 µL/well). CVL (0.1 mg) was dissolved in un-buffered water (30 µL/well) and added directly to the required reaction well. The UV-absorbance at 417 nm was monitored as described above against a background sample containing no enzyme, over a two hour time period at 30 °C. The results of this experiment are shown in figure 28.
9. Bibliography

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Solid-Supported Cyclohexane-1,3-dione (CHD): A "Capture and Release" Reagent for the Synthesis of Amides and Novel Scavenger Resin

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Received December 19, 2002

ABSTRACT

A three-step synthesis of cyclohexane-1,3-dione (CHD) resin 6 on polystyrene resin is described. Resin 6 was used to prepare an amide library of high purity by microwave-assisted serial "capture and release" and can be recycled for this purpose. High-loading CHD resin 10 was also shown to scavenge allyl cations in solution.

Polymer-supported reagents and scavenger resins† have been widely used to aid the production of a large number of target libraries by combinatorial chemistry.‡ This technique affords ease of purification and isolation of compounds and is widely applicable to the pharmaceutical industry in order to meet the high demand for novel compounds as drug candidates. During the past five years, many new resins have become available, including those able to scavenge amines, acid chlorides, alcohols, and aldehydes.

Dimedone 1 is well-known to be able to react with amines§ and aldehydes¶ in solution as well as scavenge allyl cations in the palladium-catalyzed deprotection of allyl carbamates and carbonates.

We envisaged that polymer-supported dimedone would be useful as a scavenger resin for a wide variety of functionalities, including cations, nucleophiles and electrophiles. Previous examples of solid-supported dimedone analogues include the work of Bycroft§ based on a Dde protecting group with dimedone linked to the solid support via the C2 ring position. We envisaged attaching dimedone to the solid phase via the gem-dimethyl C5 position, therefore leaving the 1,3-diketone moiety free.

Herein we report the synthesis of the novel functionalized resin 6 from cheap and readily available starting materials and demonstrate its utility as a multifunctional solid-phase reagent.

The synthesis of cyclohexan-1,3-dione (CHD) resin 6 via bis-enol ether 4 is shown in Scheme 1. 1-Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid 3 was prepared by the Birch reduction and alkylation of methylaminomethyl polystyrene resin 2 is commercially available but can be prepared by the reductive amination of formylpolystyrene using DIC in 1:1 DCM/DMF to give resin bound bis-enol ether 4.

Attempts to convert bis-enol ether 4 to CHD 6 using a variety of acidic conditions proved to be unsuccessful, resulting in incomplete hydrolysis and the formation of resin-bound 3-methoxy cyclohexen-1-one 5. We postulate that the resulting in incomplete hydrolysis and the formation of resin-bound 3-methoxy cyclohexenone intermediate acts as a thermodynamic sink for this reaction, thus preventing complete enol ether hydrolysis. The corresponding solution-phase reaction proceeds to the diketone in less than 1 h. This demonstrates how the kinetics of solid-phase reactions can differ markedly from those in solution.

Enol ester 7a was reacted with benzylamine in methanol at room-temperature overnight to yield benzamide 8a in high purity (95% by LC-MS), thus demonstrating the use of CHD resin as a capture and release reagent for the synthesis of amides.

"Resin capture—release" methodology can be used to aid impurity removal and facilitate product purification. Functionalized polymers developed for the synthesis of amides include polymer-supported dimethylamino pyridine (PS-DMAP) and polymer-supported dimethylaminopyridine hydrochloride (PS-DMAP.HCl). These reagents have been shown to be effective in the synthesis of amides, particularly in solution-phase reactions.

Examples of the use of polymer-supported reagents in conjunction with microwave heating have been reported. A complete hydrolysis of 3-methoxy cyclohexen-1-one resin 5 was achieved by microwave heating of a 1:1:1 TFA/H2O/THF suspension at 110 °C, 50 W for 10 min in a microwave synthesizer (CEM Explorer), followed by washing with butylamine to give CHD 6. Higher TFA concentrations resulted in acidic cleavage of the linker, whereas direct hydrolysis of bis-enol ether resin 4 resulted in incomplete hydrolysis. Resins 4–6 were fully characterized by FT-IR and MAS-probe 1H NMR.

Parallel robotic screening of a range of potential reactive functional groups (e.g., aldehydes, amines, acid chlorides, hydrazines) indicated that CHD resin 6 reacted with benzoyl chloride to give enol ester 7a (Scheme 2). The addition of acetic acid was essential for preventing base-catalyzed migration of the acyl group from O—C.

Reagents and conditions: (a) PhCOCl, MeCO₂H, DCM, rt, overnight; (b) PhCH₂NH₂, MeOH, rt, overnight.

Reagents and conditions: (a) DIC, 1:1 DCM/DMF, 2.5 h, rt, double coupling; (b) 90:5:5 TFA/H₂O/DMF, rt, 2.5 h; (c) 1:1:1 TFA/H₂O/DMF, α, 50 W, 110 °C, 10 min; (d) BuNH₂ wash.

\[ \text{Scheme 1} \]

\[ \text{Scheme 2} \]

\[ \text{8a} \]
Table 1. Capture and Release of Amides

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\(^a\) Yields by weight. \(^b\) Purity by LC-MS at 220 nm. \(^c\) Resin recycling.

DMAP,\(^{16}\) 1-hydroxy-benzotriazole (PS-HOBT),\(^{17}\) and tetrafluorophenol (PS-TPF).\(^{18}\)
To gain further insight into the kinetics of microwave-assisted synthesis of amides using CHD resin 6, we decided to monitor the reaction using FT-IR. Comparing carbonyl and carbon—carbon double-bond peak intensities in FT-IR allowed the level of acylation to be determined. High levels of acylation were achieved using microwave-assisted heating compared to lower acylation levels at room temperature (Figure 1). To ensure maximum acylation, CHD resin 6 was subjected to a double-microwave acylation twice for 10 minutes. The loading with respect to capture and release was deemed to be 0.2 mmol/g by elemental analysis.

The release of amide into solution was also accelerated by microwave heating. In similar kinetic studies of the release reaction, residual enol ester was observed on the resin unless excess amine was present. Excess amine was scavenged from the reaction mixture using Dowex-50WX sulfonic acid resin. Higher release levels were observed when the resin mixture was subjected to continuous microwave irradiation while being continuously cooled, compared to runs without cooling.\(^{19}\)

CHD resin 6 was used to prepare a library of amides\(^{20}\) shown in Table 1 in varying purity and yields. Aromatic enol esters generally gave higher yields and purities of their corresponding amides than aliphatic enol esters. Aniline gave lower yields overall, presumably due to reduced amine nucleophilicity. Upon second use of CHD resin 6, amide 8a was obtained in 63% yield (by weight) and 100% purity (by LCMS at 220 nm), thus demonstrating the ability to recycle CHD resin 6 (Table 1, entry 1).

\(^{19}\) CEM Discover microwave allows continuous cooling of samples, thus enabling higher levels of irradiation over the time-course of the reaction. The temperature of the reaction mixture was monitored using a temperature and pressure probe inserted through the seal of the reaction tube.
\(^{20}\) Synthesis of resin bound enol-carbonates was achieved, but release of carbamates occurred in poor yield and purity.

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By attachment of carboxylic acid 3 to commercially available trisamine resin 9, high-loading CHD resin 10 was prepared using the same procedure as before (Scheme 3).22 The loading of resin 10 was determined by elemental analysis to be 2.78 mmol/g. The scavenging ability of CHD resin 10 was demonstrated in the palladium-catalyzed O-arene deprotection of alloc benzyl alcohol 11 (Scheme 3). Insufficient scavenging was observed with lower-loading resin 6. Evidence for C-allylation of the resin was given by the presence of C-allyl signals at 5.1 and 5.6 ppm in the MAS-probe 1H NMR. Benzyl alcohol 12 was obtained in 87% yield with minimal formation of allyl benzyl ether byproduct, thus eliminating the need for column chromatography.

(22) Dendritic linker of resin 10 was unstable to microwave irradiation and thus was not hydrolyzed to completion. The resin was used as a mixture of methoxy cyclohexenone and CHD functionalities.

Acknowledgment. This investigation was generously supported by funds provided by Organon Laboratories, Ltd., and the Engineering and Physical Sciences Research Council (EPSRC No. 00316713). The authors thank CEM for provision of a Discover microwave synthesizer, Dr. Ian Sadler for his NMR assistance, and Dr. Dan Fletcher at Organon Laboratories for MAS-probe analysis of resins.

Supporting Information Available: Detailed experimental procedures and characterization of resins 2 and 4–7(a–e). This material is available free of charge via the Internet at http://pubs.acs.org.

OL027503P
Lipases are industrially important enzymes that have found widespread use in the resolution of chiral alcohols, amines and carboxylic acids.\(^1\) In view of their diverse substrate specificity they have also been used as reagents for the solution-phase parallel synthesis of compounds based upon pharmacophoric scaffolds.\(^2\) However, in comparison with proteases, little is known about the activity of lipases toward resin-bound substrates,\(^3\) an area of contemporary interest with applications in solid-phase synthesis\(^4\) including enzyme-cleavable linkers.\(^5\) Herein we report the first example of a lipase-catalyzed acylation reaction on a resin-bound substrate, the results of which may have applications in parallel solid-phase synthesis and screening methodologies.

We recently reported the synthesis of resin-bound cyclohexane-1,3-dione (CHD) and demonstrated its use as a capture and release reagent for the synthesis of amides.\(^6\) Furthermore, we have shown that esters of cyclic-1,3-diketones are good substrates for lipases and esterases\(^7\) and thus sought to combine these two ideas to investigate lipase resolutions on resin-bound substrates. Screening of a range of lipases and esterases against enol ester (R/S)-1 (Scheme 1) identified Chromobacterium viscosum lipase (CVL) as possessing good activity and high enantioselectivity ([\(\varepsilon\)] = 88),\(^8\) and hence this enzyme was selected for initial studies. Previous work has shown that enzyme-catalyzed reactions on solid-phase are greatly enhanced by the use of PEGA\(_{1900}\) resin (commercially available from Polymer Laboratories, Ltd.) which swells in aqueous solvents.\(^9\) The resin also has an open-pore structure allowing large enzyme molecules access to the resin active sites.\(^10\)

PEGA\(_{1900}\) supported CHD resin 6 was prepared as shown in Scheme 2. Ethyl-3,5-dimethoxy-cyclohexa-2,5-dienecarboxylic acid 3, obtained by the Birch reduction and alkylation of 3,5-dimethoxybenzoic acid,\(^11\) was coupled (\(\times 2\)) directly to PEGA\(_{1900}\) resin 4 using DIC in DCM/DMF (1:1) to give resin bound bis-enol ether 5. Acidic hydrolysis of 5, using a TFA/H\(_2\)O/DMF (90:5:5) mixture, yielded CHD resin 6. The loading of CHD resin 6 was determined as 19 \(\mu\)mol/g (swollen) and 130 \(\mu\)mol/g (dry), by coupling with (R/S)-3-phenylbutyryl chloride followed by standard sodium hydroxide release from the resin bound racemate.

PEGA\(_{1900}\) supported CHD resin 6 was subjected to CVL-catalyzed acylation using (R/S)-methyl 3-phenylbutanoate 7 and (R/S)-vinyl 3-phenylbutanoate 8 (Scheme 2) to yield resin-bound 1,3-enol ester 9. As is typical for solid-phase reactions, a large excess (approximately 10 equiv) of acylating agent was used to ensure high conversions. The reaction was performed in aqueous potassium phosphate buffer at neutral pH in the absence of organic solvent. Under these conditions, the concentration of hydrophobic acylating agent at the reaction site, within the PEGA\(_{1900}\) microenvironment, is high.\(^12\) After overnight reaction, the resin was extensively washed, followed by acyl group release from the resin (aqueous NaOH) to give 3-phenylbutyric acid 2 which was analyzed by HPLC (Chiracel-ODH column) to determine the yield and enantiomeric excess (ee) of the reaction (Table 1). In each case the predominant enantiomer observed was the (R)-acid 2 rather than the expected (S)-acid despite the known preference for CVL to catalyze hydrolysis of the (S)-enantiomer in solution (Scheme 1). Similar results were obtained with Pseudomonas cepacia lipase (PCL) and

Appendix 2

### Table 1. Lipase-Catalyzed Resolution by "Capture and Release"

<table>
<thead>
<tr>
<th>entry</th>
<th>ester</th>
<th>lipase</th>
<th>product</th>
<th>yield</th>
<th>ee</th>
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<td>(R/S)-7</td>
<td>CVL</td>
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<tr>
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</tr>
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<td>&gt;99</td>
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</table>

*Calculated by comparison of HPLC peak areas with a control [NaOH hydrolysis of chemically synthesized racemic enol ester]. \(^d\) ee = 96%. \(^e\) ee = 92%. \(^d\) Double acylation. \(^e\) Triple acylation.

## Scheme 1. CVL-Catalyzed Hydrolysis of Dimedone 1,3-Enol Esters

\[
\text{(R/S)-1} \quad \text{(S)-2 ee = 95%} \quad \text{(R)-1 ee = 72%}
\]

*Reagents and conditions: CVL, 10% MeCN/0.1 M KP\(_1\) buffer (pH = 7.0), rt, 1.5 h (49%).

## Scheme 2. Synthesis of CHD-PEGA\(_{1900}\) Resin 6 and Lipase-Catalyzed Resolution Involving a "Capture and Release" Strategy

\[
\begin{align*}
\text{(R or S)-2} & \quad \text{Lipase} = 10 \mu\text{mol/g (swollen)} \\
& \quad 130 \mu\text{mol/g (dry)}
\end{align*}
\]

*Reagents and conditions: (i) DIC, DCM/DMF (1:1), rt, 2.5 h \(\times 2\); (ii) TFA/H\(_2\)O/DMF (90:5:5), rt, 2.5 h; (iii) lipase, 0.1 M KP\(_1\) buffer (pH = 7.0), rt, 16 h; (iv) 0.1 M NaOH, rt 1 h.

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\(^1\) University of Edinburgh.

\(^2\) Organon Laboratories Ltd.
porcine pancreatic lipase (PPL), both of which are also known to be (S)-selective. Use of the methyl ester 7 (Table 1, entry 1) resulted in a reasonable yield of (R)-acid 2 with modest ee, whereas the more reactive vinyl ester 8 (Table 1, entry 2) gave a lower yield but excellent ee. Subjecting the resin to a second acylation reaction, after washing, resulted in an increased yield (78%) (Table 1, entry 7) which could be further enhanced (86%) with a triple acylation (Table 1, entry 8).

To probe this unexpected enantiospecificity, the acylation reactions were repeated using enantiotomerically enriched acyl donors. When (R)-vinyl ester 8 (ee = 96%) was used, the (R)-acid 2 was obtained in high yield and ee (Table 1, entry 3). However, switching to the enantiotomerically enriched (S)-vinyl ester 8 (ee = 92%) also gave the (R)-acid 2 in low yield but high ee (Table 1, entry 4). In this case the lipase selectively catalyses acylation of the minor contaminant (R)-enantiomer, in the presence of excess (S)-enantiomer.

The reaction in the absence of lipase showed negligible levels of formation of 3-phenylbutyric acid 2 by HPLC, eliminating the possibility of background reaction between CHD resin 6 and vinyl ester 8. Similarly, the corresponding solution-phase reactions, in the absence of lipase, between dimedone and either vinyl ester 8 or 3-phenylbutyric acid 2 showed no evidence of conversion.

The enantiospecificity of the reaction can be rationalized by considering the competing enzymatic hydrolysis of the (R)- and (S)-enantiomers of 8 in a lipase-catalyzed parallel kinetic resolution analogous to that reported by Rakels et al. (Figure 1).

(S)-Vinyl ester 8 undergoes rapid formation of an acyl-enzyme intermediate followed by hydrolysis to form (S)-acid 2. Solution-phase hydrolysis of vinyl ester 8 revealed that the (S)-enantiomer is completely hydrolyzed in less than 15 min under these conditions. The corresponding hydrolysis of the (R)-enantiomer is much slower than that of the (S)-enantiomer allowing the enzyme intermediate to undergo transesterification to form the resin bound (R)-enol ester 9. An alternative scenario, which we cannot exclude at present, involves initial acylation by both (R)- and (S)-esters (k_{SR} > k_{SR}) followed by rapid hydrolysis of the (S)-enantiomer (k_{SR} > k_{SR}), thus releasing (S)-acid into solution with accumulation of the (R)-enantiomer on the resin.

The difference in ee for transesterification with methyl versus vinyl ester (Table 1, entries 1 and 2) is consistent with the faster rate of hydrolysis of vinyl versus methyl ester. Previous unpublished studies in our laboratories have shown that diffusion of enzymes into PEGA_{1900} resin occurs over a period of ca. 1 h. When CVL was allowed to equilibrate with the resin for 1 h before addition of vinyl ester 8, the ee of the liberated (R)-acid 2 decreased to 90%. Under these conditions the (S)-vinyl ester 8 presumably undergoes a small degree of transesterification onto the CHD resin, prior to hydrolysis. Longer equilibration times did not lead to further reductions in ee.

In conclusion, we have demonstrated for the first time that the lipase-catalyzed kinetic resolution of racemic esters can be carried on solid-phase using an acylation/deacylation capture and release strategy. The reactions exhibit enantiospecificity which is consistent with the operation of a parallel kinetic resolution process. Such resin-mediated reactions should be amenable to automation, particularly using parallel synthetic approaches, and in view of the broad substrate specificity of available lipases they can be exploited for the preparation of combinatorial libraries suitable for screening against biological targets of interest.

Acknowledgment. This investigation was generously supported by funds provided by Organon Laboratories, Ltd., the Engineering and Physical Sciences Research Council (EPSRC No. 00316713), and the Wellcome Trust. We thank Dr. Ian Sadler for his help with the MAS-probe NMR spectra of resin samples.

Supporting Information Available: Experimental preparations for ester (R/S)-1, resins 5, 6, esters 7, 8, and standard procedures for lipase-catalyzed hydrolyses and transesterifications (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

References


(14) Attempts to provide evidence for this scenario, by carrying out the CVL-catalysed hydrolysis of CHD resin loaded with (R/S)-9, lead to very low levels of cleavage of carboxylic acid from the resin.

JA037922X

Figure 1. Explanation of enantiospecificity of reaction.