Carbohydrate based resins for polymer assisted solution phase synthesis

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Abstract

Carbohydrate based beads can be derivatised to produce high loading resins which are comparable in both solvent compatibility and loading of required functionality to commercial polystyrene based resins, that are used in polymer assisted solution phase synthesis. The results from the application of a carbohydrate based resin to the acylation of amines and the bromination of alkenes and arenes are presented.

Acknowledgments

I would like to thank Millpore Ltd for providing the time and money to complete this work. I would also like to thank Simon and Les for their help in proof reading the experimental section. Also to Phil for helping with the biodegradation studies. A big thank you to Dr P. G. Steel who has had to put up with my bad english and grammer while reading this thesis.

I would like to dedicate this to Dr A. Chesney who sadly died recently, without whose help in both getting the project started and ideas to keep it going, this work would never have happened.

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1.0 Introduction

Whether it is solid phase synthesis, supported reagents or scavenger resins, combinatorial chemistry is based around solid supports. With the ever-increasing expansion of the field there are an ever-increasing number of new and novel supports being utilised. While most are based around synthetic polymers *e.g.* polystyrene, there are a number of supports based on naturally available polymers *e.g.* carbohydrates. These are used extensively in the biotechnology industry for the purification of proteins and therapeutic antibodies. The aim of this project was to apply these natural polymers to the field of combinatorial chemistry with particular focus on the area of solid supported sequestering reagents or scavenger resins.

1.1 Combinatorial Chemistry

In recent years, there has been an explosion of interest in the field of combinatorial chemistry.[1] This new technique has been exploited to allow large libraries of small molecules to be rapidly formed, which in turn has helped to accelerate the drug discovery process and has been extended to areas as diverse as new materials and catalysts. The field of combinatorial chemistry can be divided into two areas according to the nature of the reaction medium. These areas which are broadly related and yet conceptually different are;

1. Solid phase organic synthesis.
2. Solution phase synthesis.

1.2 Solid phase organic synthesis

The basis for solid phase organic synthesis (SPOS) was first described over 30 years ago by Merrifield, who prepared peptides on solid supports and this area continues to be a prime application of SPOS.[2] Since the initial preparation of peptides, the field grew steadily until it was realised that the methodology could be applied to the synthesis of a wide range of biologically active materials in a rapid, parallel fashion.[3] A particularly interesting early application of this methodology was in the synthesis of a library of 1,4-benzodiazapines **4**.[4] In this seminal work, a range of suitably substituted resins **1**, and a group of aromatic imines **2** were reacted to form a diverse library of supported imines **3**,

which were subsequently cyclised and simultaneously cleaved from the support in one step (Figure 1).

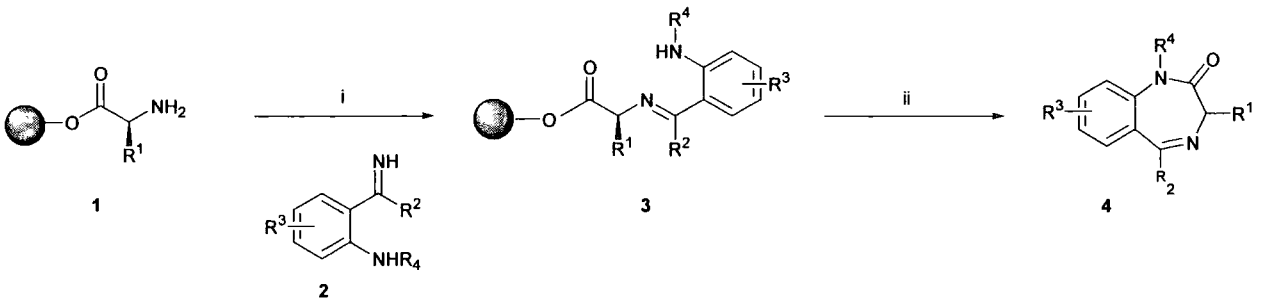


Figure 1 ***Reagents and conditions***; i) **2**, $ClCH_2CH_2Cl$, Δ; ii) TFA, Δ

The numerous advantages of this approach include;

1. The ability to utilise large excess of reagents to force reaction to completion.
2. Easy removal of excess reagents by simple filtration and wash sequences.
3. The potential for automation.
4. The production of libraries (in the thousands).

SPOS, however suffers from several disadvantages, the main being;

1. The linker often remains attached to the target molecule, which can be undesirable.
2. It often requires the use of special polymeric supports, with prerequisite surface functionalisation.
3. A limitation exists in the number of reactions that can be performed, due to potential interference by the polymer support.
4. There are difficulties in monitoring reactions in progress.

Chemists are currently targeting many of these problems, with particular emphasis being placed on the development of so called traceless linkers. Examples of these linkers are shown in Figure 2.

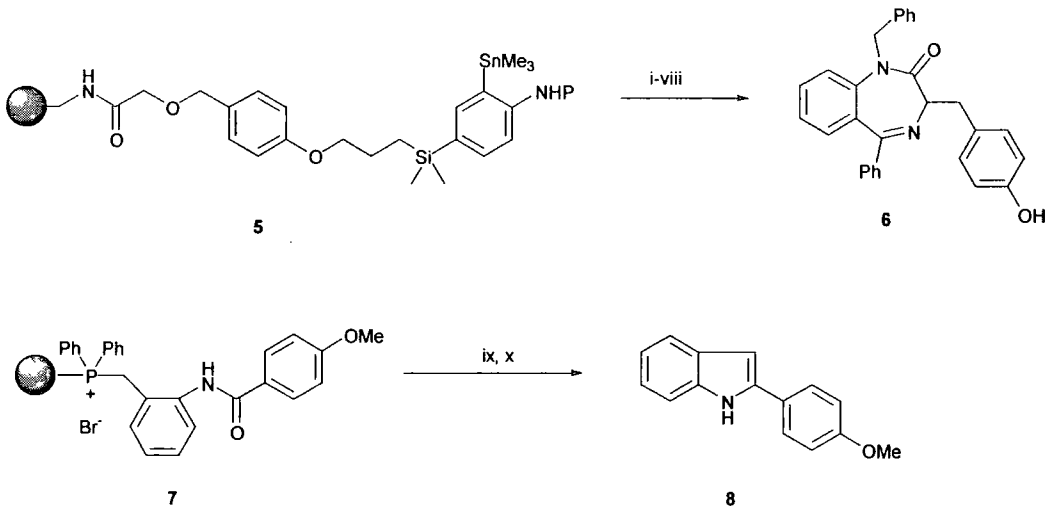


Figure2 ***Reagents and conditions***; i) Benzoyl chloride, $Pd_3dba_3.CHCl_3$; ii) 3% CF_3COOH; iii) Fmoc Tyrosine fluoride; iiii) 20% piperidine / DMF; v) 5% AcOH, 65°C; vi) Lithiated oxazolidinone, Benzyl halide, DMF; vii) $TFA/Me_2S/H_2O$ (85:15:5); viii) HF; ix) Toluene, DMF, Δ; x) KO^tBu, Δ, 45 min

Of those linkers shown in Figure 2, the material incorporating a silyl linker **5** has been employed in the synthesis of 1,4-benzodiazepines **6**,[5] but suffers from the problem that HF is required in the cleavage step. The phosphonium salt **7** can be readily prepared from triphosphine resin, and can be cleaved under mild conditions. For example the use of sodium methoxide[6] results in cleavage by hydrolysis whilst the use of potassium *tert*-butoxide results in cleavage by an intramolecular Wittig reaction, which has been used to prepare the indole derivative **8**.

The ease of this approach has led to a number of automated systems being developed, in order to allow for the faster and more efficient synthesis of libraries.[1c]

1.3 Solution phase combinatorial methods

Solution phase combinatorial chemistry has a number of advantages over SPOS, which are mainly due to the fact that standard solution phase reactions can be applied without the need to develop special polymers.[7] Simply because of this, reaction rates, kinetics and the behaviour of reagents are well understood, allowing the development chemist to plan reactions and anticipate any problems. It is also possible to follow reactions easily by standard analytical techniques *e.g.* HPLC, TLC, NMR and m/z. There is however, one main drawback with solution phase chemistry, purification of the product from excess reagents and by-products. Since standard purification techniques are time consuming and often therefore constitute the slowest part of any library synthesis, any method of avoiding

them will greatly enhance this area. Several novel approaches have therefore been developed specifically in order to overcome this obstacle. These include the use of equimolar quantities of reactants, high-throughput flash chromatography or preparative HPLC.[8] Arguably the most efficient and simple technique is Polymer Assisted Solution Phase (PASP) synthesis. This involves the use of a polymer to support reagents within a reaction and/or the purification of the reaction products by the application of scavenger resins.

1.3.1 Supported reagents

With the introduction of PASP a new generation of reagents has been introduced.[9] These solid supported reagents allow reactions to be carried out in the solution phase but without the associated problems of excess reagents having to be removed (Figure 3).

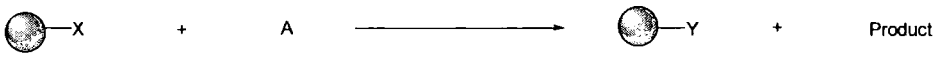


Figure 3

The main advantages of supported reagents are the ease of purification, the potential for automation and the fact that hazardous reagents are often rendered safer. However the disadvantages are cost and loading of the supported reagent. In addition, there are frequently problems producing the required reagent.

The number of reactions being reported involving supported reagents is growing all the time. In fact some important industrial processes have been carried out using supported reagents including the Mitsunobu reaction,[10] reductions,[11] halogenations,[12] coupling reactions,[13] catalysis,[14] and oxidation's.[15]

To demonstrate the synthetic use of these supported reagents or polymers for purification, the synthesis of natural products and pharmaceuticals has been reported by a number of groups. One example, by Ley *et al.*, [16] is shown below in Figure 4, in which Viagra was synthesised using no purification.

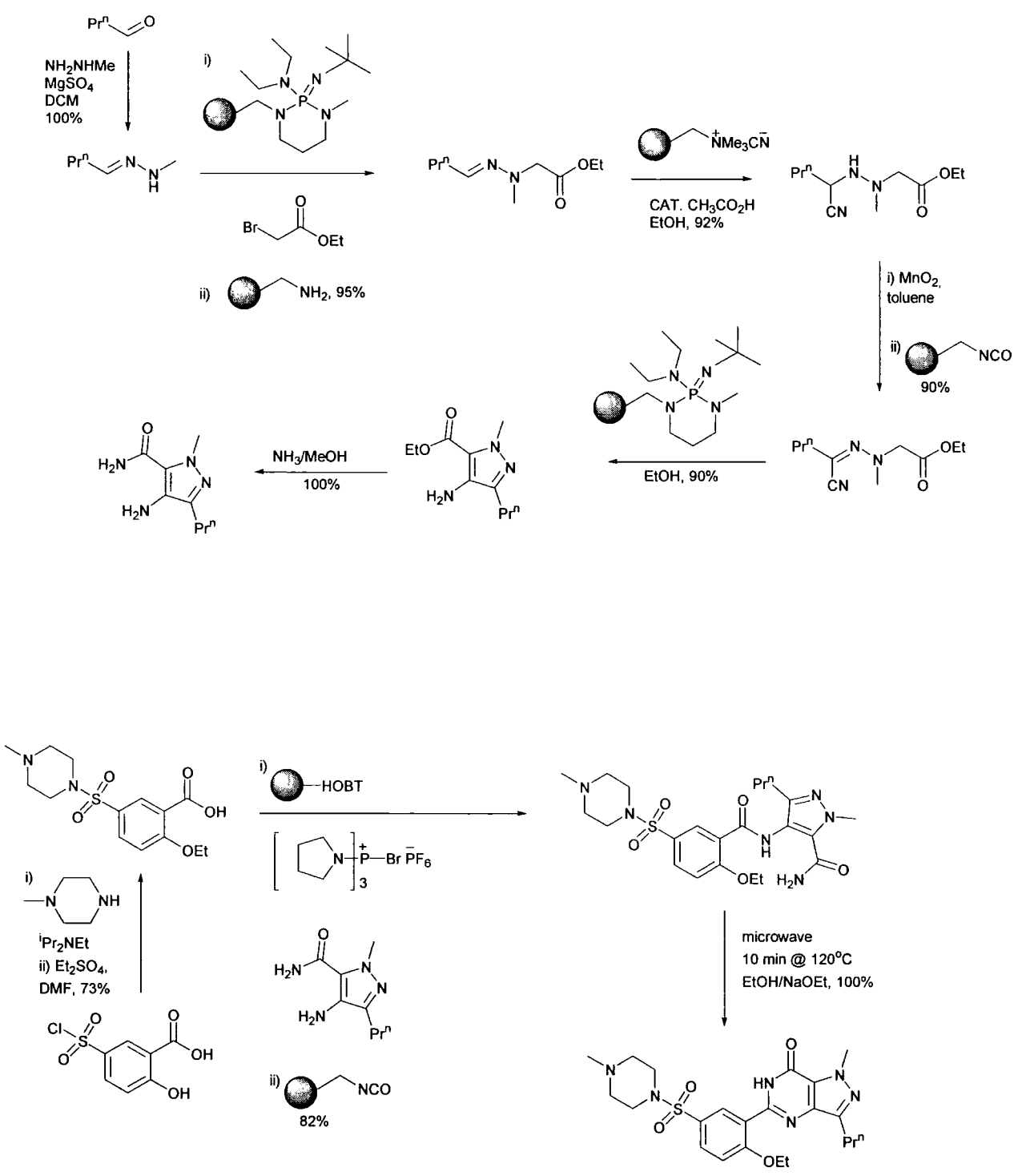
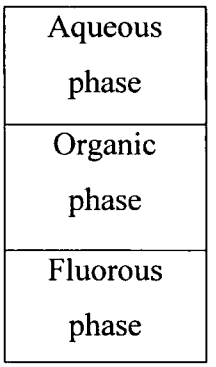

NH2NHMe
MgSO4
DCM
100%
i)
ii) NH2, 95%
CAT. CH3CO2H
EtOH, 92%
i) MnO2,
toluene
ii) NCO
90%
EtOH, 90%
NH3/MeOH
100%
i) HOBT
82%
i) iPr2NEt
ii) Et2SO4,
DMF, 73%
microwave
10 min @ 120°C
EtOH/NaOEt, 100%

Figure 4

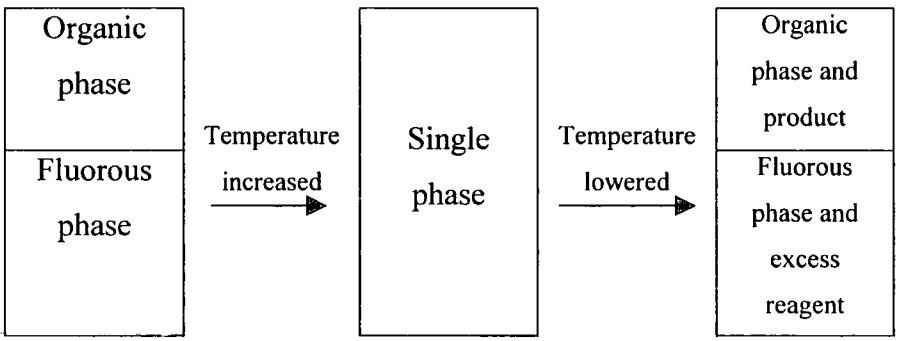
1.3.2 Fluorous phase

It has been known for decades that perfluorocarbons (PFC) can form immiscible layers with both organic and aqueous solvents.[17] Horváth introduced the term 'fluorous' phase as the analogue to the term 'aqueous' phase in 1994[18] to describe this third layer.



1.3.2.1 Phase extraction

With solution phase extraction being one of the simplest processes in the purification of organic compounds the ability of perfluorocarbons to provide a new phase, leads to a relatively new method of purification; fluorous phase extraction. Highly fluorinated compounds are relatively insoluble in traditional solvents, however they are soluble in the fluorous phase which makes it possible to remove either a fluorinated reactant or product by simply partitioning with a fluorinated solvent. An aspect of fluorous bi-phasic systems is that with certain organic solvents *e.g.* toluene, at elevated temperatures a single homogeneous layer is formed in which the reaction is carried out. When the reaction is completed and the temperature lowered the bi-phasic system reforms with, for example the fluorinated reactants in the fluorous layer and the product in the organic layer.



Adopting this system a number of fluorous reagents have been developed which include reducing agents[19] and metal catalysts incorporating fluorinated ligands [20] *e.g.* $[CF_3(CF_2)_5CH_2CH_2]_3SnH$ and $P(CH_2CH_2C_8F_{17})_3$.

1.3.2.2 Phase switching

Another strategy that can be adopted is phase switching.[21] Phase switching involves the transfer of excess reactants or side products to an alternative phase. An example of this is the removal of excess reagents with a scavenging resin; the reagent is switched from solution to the solid phase. This principle has been extended to incorporate the fluorous phase. However given that most products do not contain sufficient fluorine content to allow efficient partitioning, this approach requires the addition of a fluorine tag.

A reaction is carried out with two reactants A and an excess of B, which leads to the desired product and unreacted B. Excess B is reacted with a highly fluorinated reagent, the product of which is switched to the fluorous phase to leave only the product in the other layer. This approach, an example of chemical phase switching is illustrated below (Figure 5).

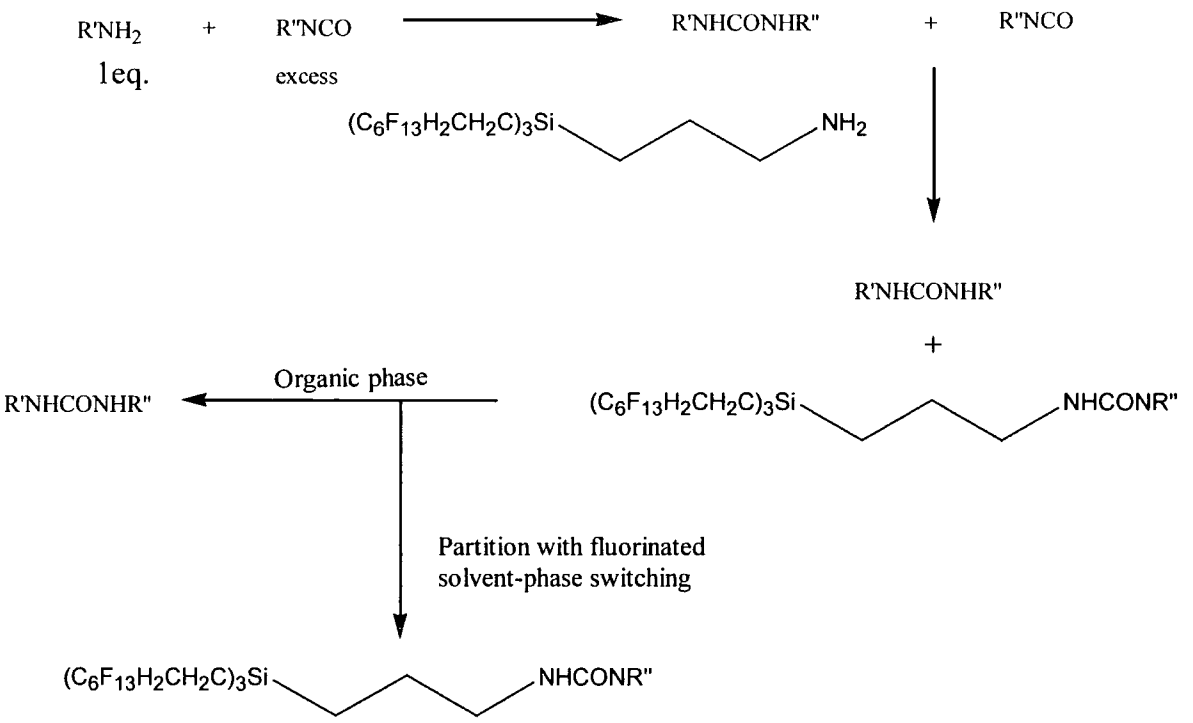


Figure 5

The fluorinated amine is not only switching phase it is also removing the excess isocyanate and is therefore acting as a scavenger.

1.4 Scavenger resins

The main advantage of using a scavenger resin, is the ease of purification of the mixture and the simplicity of its use. During solution phase synthesis a large excess of one reagent is often added, in order to force the reaction to completion. This however results in the problem of product purification due to contamination by the excess reagent. Scavenger resins work by removing this unwanted reagent by reacting it with active surface groups on the resin. In order to facilitate this, the scavenger resin must possess a complimentary functionality with which to react with any unreacted reagent. This then allows rapid removal of the excess reacted reagent by filtration, leaving only the desired product in solution as shown schematically in Figure 6.

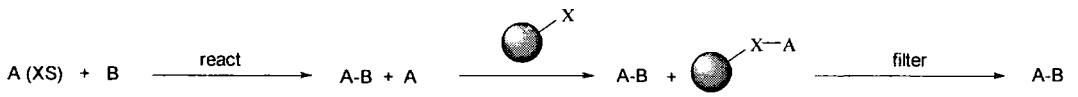


Figure 6

This complementary molecular reactivity lends itself to the use of multiple scavengers to remove more than one by-product or reagent at the same time. Even if the functionalities on the resins are mutually incompatible the rate of reaction in solution will far exceed the rate of reaction between the resins.[22]

The base matrix to be employed as a scavenger resin requires certain characteristics:

1. It must be stable to the reaction conditions being employed.
2. There should be no contamination by leakage of ligand.
3. It should be cheap, easy to handle and non-toxic.
4. It should possess a wide compatibility with different solvents including water.

As most scavengers are based on polystyrene cross-linked with divinyl benzene, they meet all of the above requirements except cost and compatibility with water. Some selected examples of functional groups attached to polystyrene and employed as scavenger resins are contained in Table 1.

Table 1. Some common functional groups employed on scavenger resins.

Functional Group	Reactant	Reference
$-NH_2$	electrophiles, acid chlorides, isocyanates	23
-NCO	amines, thiols, alkoxides,	23, 24
-SH	halides, mesylates, tosylates	25
$-SO_2Cl$	catch and release of alcohols	26
$-SO_3H$	general acid, quenching reagent, amine scavenger	22, 27
$-NR_2$	protons	23, 28

As can be seen from Table 1, a relatively small number of functional groups allows a wide range of reactions to be performed utilising this technology. In addition to those functional groups listed above, a much larger range of specific materials have been prepared, and these have been recently reviewed in the literature.[29] An excellent example of the application of both supported reagents[30] and scavenger resins to achieve the synthesis of a small library (Figure 8) is shown in Figure 7. The trisubstituted ureas **9–13** were prepared in excellent yields and high purities, with simple filtration steps as the key purification strategy.[24]

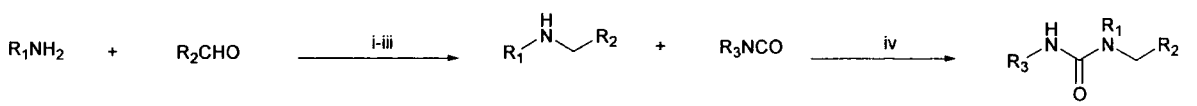


Figure7 *Reagents and conditions*; i) MeOH, r.t, 1 h; ii) Amberlite IRA-400 BH_4 resin, r.t.; iii) polystyrene carboxaldehyde, CH_2Cl_2, overnight, r.t; iv) $CHCl_3$, 1 h, aminomethylated polystrene, 1 h, filter.

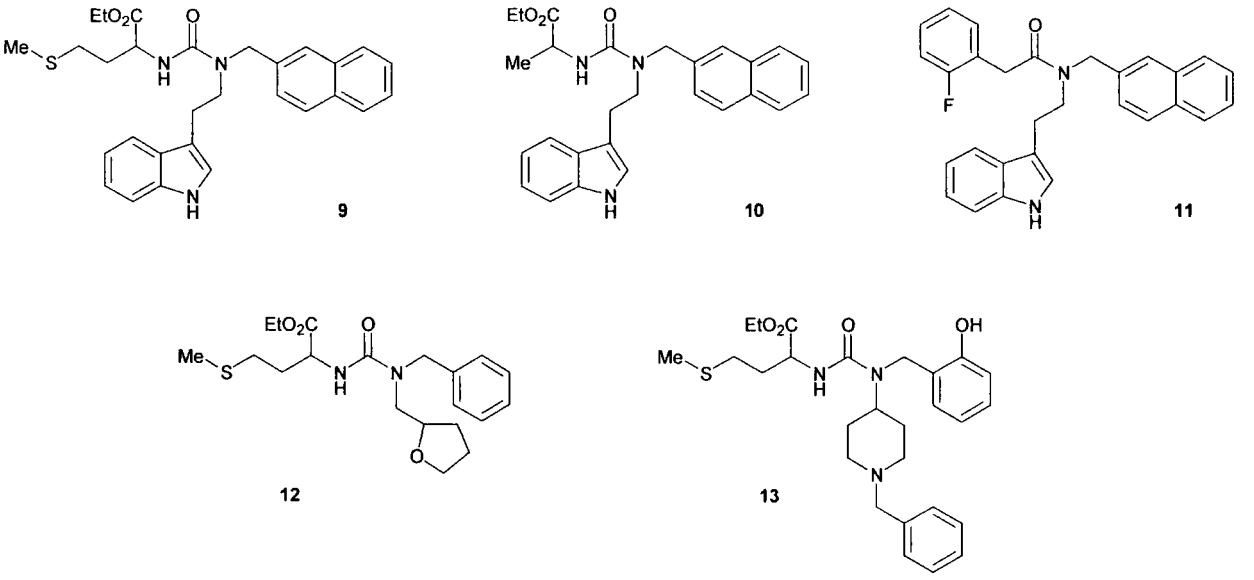


Figure 8

1.5 Conclusion

Thus, the application of scavenger resins appears to be a growth area, with many advances in both the nature and complexity of the resins being employed as part of a multi-faceted approach to solution phase high throughput synthesis. However new materials are still required.

2.0 Results and discussion

2.1 Introduction

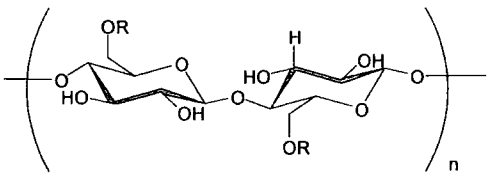
As combinatorial chemistry grows so does the need for new and novel supports. The aim of this project was to explore the possibility of using natural carbohydrate polymers as the solid support in the area of PASP, with particular emphasis on scavenger resins. To our knowledge this has not been previously explored. The results of this are discussed in the following chapters.

2.2 The Application of Derivatised Cellulose Resins in Combinatorial Chemistry

Cellulose derivatives in the form of insoluble beads may potentially be prepared by two fundamental and yet complimentary methods;

1 The derivative can be made in a soluble form, the cellulose converted to a bead and then fixed to provide a stable bead.
2 The bead cellulose can be prepared with the requisite porosity and physical characteristics, and then it is subsequently derivatised in a suitable manner.

A wide variety of physical properties can be rapidly introduced into cellulose materials by simple chemical modification of the basic polymer unit.[31] Polymers with a number of interesting characteristics such as variable water solubility have been prepared by such methods, and have found wide application in the food, agrochemical and biochemical / biotechnological fields.[32] Of particular interest to this project, is the potential for cellulose to undergo facile alkaline etherification to afford cellulose ethers.[33] Such ethers are polymeric substances characterised by the partial substitution of the hydroxyl groups within the cellulose framework. The number of ether groups generated per monomer unit is defined as the degree of substitution (DS), which can reach a maximum of three, when all of the available glucopyranose hydroxyls have been reacted. There are many examples of such ethers in the literature, with some of the earliest examples of simple ethers being methylcellulose **14**,[34] carboxymethyl cellulose **15**[35] and benzyl cellulose **16**[36] (Figure 9).



14, R = CH_3
15, R = $CH_2CO_2CH_3$
16, R = CH_2Ph

Figure 9 Examples of cellulose ethers with a degree of substituion of 1

Activated cellulose has found widespread use in the synthesis of peptides and oligonucleotides. One method involves the use of a chemically activated membrane. The hydroxyl groups of the membrane undergo esterification with an N-protected amino acid. The protecting group is then removed to give an even distribution of reactive amino groups. A solution of a second amino acid is then applied to the membrane surface generating a dipeptide. However, this solution is only added in small discrete spots normally between 3-12mm in diameter. Any remaining amino groups are blocked by acylation and the N-terminus of the dipeptide anchor is released for analysis and further reaction. This method is generally referred to as SPOT synthesis.[37] Another method involves the use of cellulose discs.[38] The disc is first reacted with an activating group, which then allows an amino acid to be coupled on the surface. The discs are then packed into a column and all subsequent addition, de-protection and cleavage steps are carried out under continuous flow conditions. Other methods involve the activation of a disc or string.[39] These are then divided equally and then reacted with a different amino acid. This procedure is repeated as many times is necessary to build the required number of peptides of the desired length.

2.3 The Preparation of Derivatised Beaded Cellulose

Of the two approaches, by far the most commonly used is the latter, and given the ready availability of Perloza MT-100 and related spherical cellulose beads, we elected to follow this route in the work described in this thesis.

Perloza resins have previously been successfully derivatised by a number of strategies, and relatively large peptides have been synthesised on the surface,[40] or immobilised on it *via* a chemical spacer.[41] The development of novel chemistries on these materials was not pursued, instead the resultant materials were employed as chromatography adsorbents for the purification of biomolecules from crude feedstocks.[42] Amongst the most useful

reactions previously described were those employing either a bromination,[43] or free radical addition[44] to allyl moieties pendant on the matrix surface. The activation of the allyl matrix **18**, *via* these protocols to give respectively a bromohydrin resin **17**, and an amine functionalised resin **19**, by the addition of cysteamine, are shown in Figure 10.

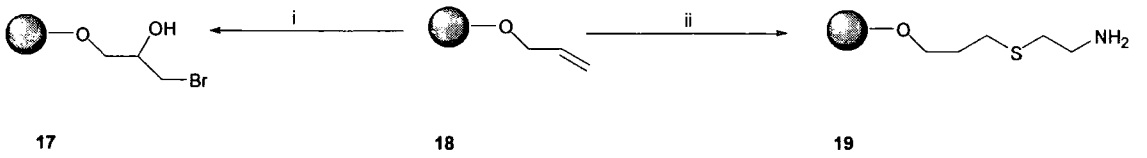


Figure 10 *Reagents and conditions*; i) NBS, H_2O or BrOH; ii) $H_2NCH_2CH_2SH$, UV.

The first scavenger resin we elected to prepare was that containing a *tris*-(2-aminoethyl)-amine moiety, which could be employed to scavenge excess electrophiles from solution. The route to the final trisamine resin **22** is shown in Figure 11, and began with the previously documented addition of allyl bromide to the Perloza resin to form matrix **18**.[27] Subsequent hydrobromination of this material using bromine water, afforded resin **17**, which was reacted with *tris*(2-aminoethyl)amine in methanol to afford the target resin, with the last step presumably proceeding *via* the intermediate epoxy substituted resin **21**.

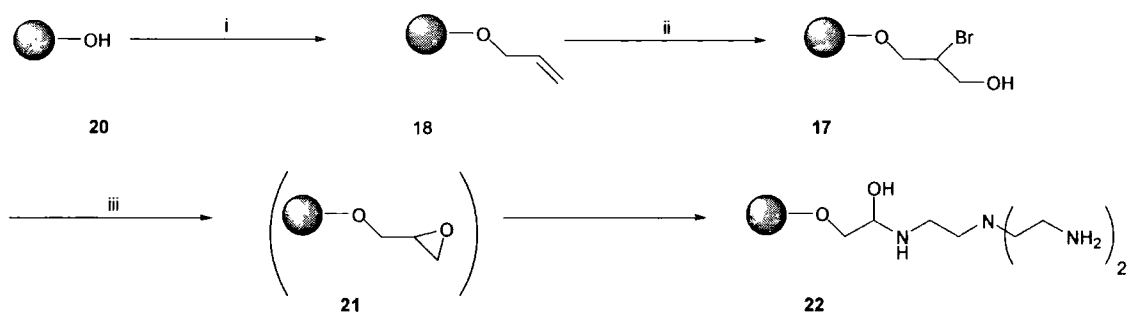


Figure 11 *Reagents and conditions*; i) NaOH, $BrCH_2CHCH_2$; ii) HOBr; iii) $(NH_2CH_2CH_2)_3N$, MeOH.

In this scenario, the *tris*-(2-aminoethyl)amine acted as the base to remove the proton and the resultant bromide. Attempts to improve the conversion of brominated allyl groups into amine termini by the addition of a non-reactive second base such as triethylamine did not prove successful, and the use of slight excess of the desired amine proved most efficient.

2.4 Investigation of the swelling properties of resin 22

Initially it was decided to examine the swelling properties of the resin in a variety of solvents, in order to determine the scope and limitations of cellulose beads in solvents with different properties. The swelling of the matrix was determined by washing 10 ml of the resin settled from an aqueous slurry with 1,4-dioxane to remove any water and then to wash the settled resin with the solvent of choice. The solvated resin was then resuspended in approximately 10 ml of the desired solvent, thoroughly slurried and allowed to settle in a graduated column, from which the final volume could be read. The results of these experiments are contained in Table 2. It should be noted that 10 ml of resin **22** in its aqueous form has a dry mass of approximately 1 g, as determined by residual mass measurements after oven drying at elevated temperatures for 16 h. Previous attempts to re-swell desiccated Perloza resins have shown that the pore structure is destroyed during the desiccation procedure, and that re-swelling is inefficient.[39] Our attempts to dry the resin using freeze drying as a mild method of water removal confirmed the previous findings, since even under these conditions re-swelling the resin after desiccation proved impossible.

Table 2. The swelling properties of resin **22** in various solvents.

Solvent	**Volume/ml**	**Solvent**	**Volume/ml**
water	10.0	tetrahydrofuran	9.0
1,4-dioxane	9.0	methanol	9.0
dichloromethane	8.0	ethyl acetate	9.0
toluene	8.0	acetonitrile	9.0
dimethylformamide	9.0	diethyl ether	10.0

As can be seen from Table 2, the resin has a relatively narrow range of swelling volumes, with the minimum being observed for dichloromethane and toluene. The fact that diethyl ether gives such a high swelling volume relative to water is interesting, and the reason for this apparently anomalous behaviour has yet to be determined.

This narrow range of swelling volumes is a great asset when employing this material as a scavenger resin, as differential swelling in mixed solvent systems can be avoided. A second benefit is that an almost constant volume of solvent is present within the pores of

the matrix, which remain relatively unaffected in various solvents, *i.e.* they do not collapse or over-expand, and are thus equally accessible in all the environments tested.

2.5 Application of resin 22 to scavenge electrophiles from solution

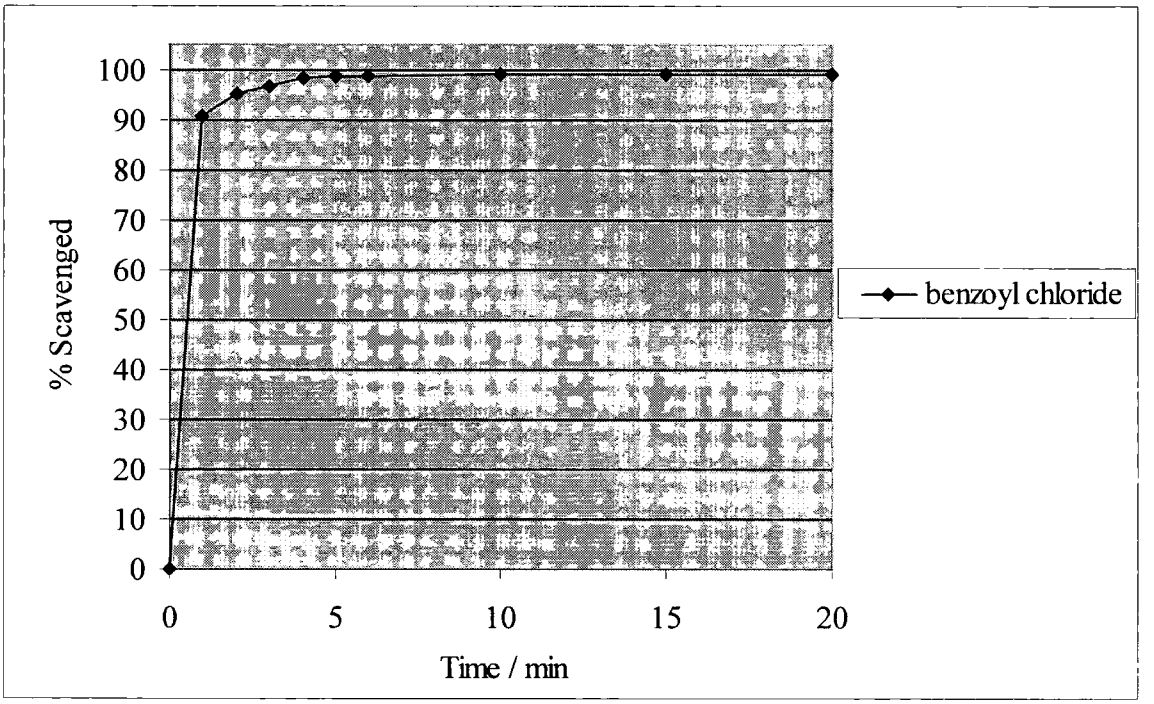
Having determined that the resin would swell appreciably, but in a controlled fashion in a number of solvents, we elected to determine whether the material would indeed remove an electrophile from an organic solution, and if so, how rapidly this could be achieved. We therefore solubilised a known amount of benzoyl chloride in dry dichloromethane and measured the absorbance of the resulting solution at the λ_{max} of 295nm. To this solution was added approximately 2.5 'NH_2 equivalents' of resin **22** and the mixture stirred. At set time intervals, aliquots of the solution were removed, filtered to remove any resin and the absorbance measured to determine the amount of benzoyl chloride which had been removed. The results of this study are contained in Table 3.

Table 3 The rate of removal of benzoyl chloride from solution by resin **22**.

Time/min	Abs 295 nm	% Scavenged
0	2.33	0
1	0.22	90.7
2	0.118	94.9
3	0.080	96.6
4	0.040	98.2
5	0.035	98.5
6	0.030	98.7
10	0.025	98.9
15	0.020	99.2
20	0.020	99.2

The results of this experiment are represented graphically in Figure 12, which clearly shows that the initial scavenging is extremely rapid, with over 90% of the benzoyl chloride being removed in the first minute. The reaction then gradually slows, with 99% scavenging being reached after approximately 6-10 minutes, after this point changes in the optical density were too small to demonstrate that no benzoyl chloride was present at all, *i.e.* 100% scavenging.

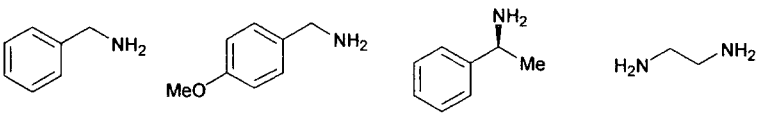
Figure 12 The rate of removal of benzoyl chloride from solution by resin **22**.



Having determined that the resin was capable of removing electrophiles from solution rapidly and efficiently, and that the swelling properties of the material meant that it was compatible with organic solvents, we elected to look at the formation of amides and ureas using excess electrophiles.

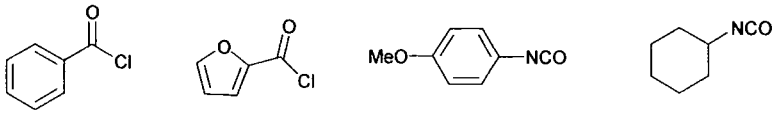
We chose to use the set of amines **23** – **26** and electrophiles **27** – **30** shown in Figure 13, to generate a defined library of amides and ureas **31** – **44**. In each case, 1.5 equivalents of the electrophile were employed, in order to drive the reactions to completion prior to scavenging.

Amines



23 24 25 26

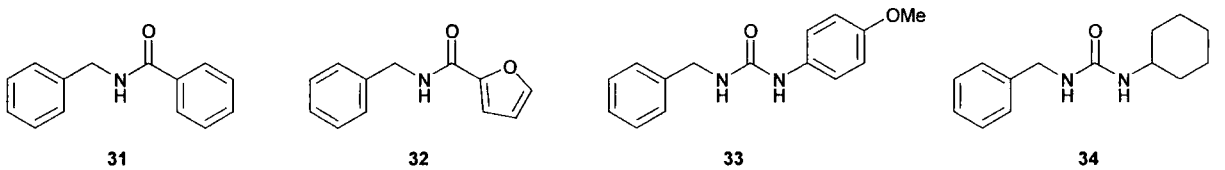
Electrophiles



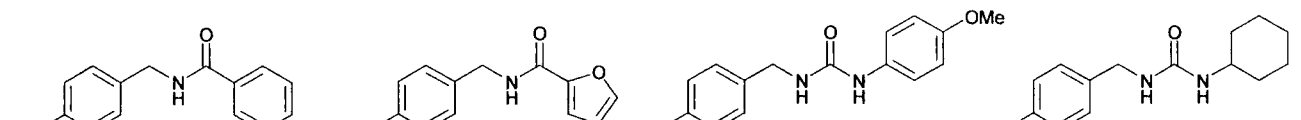
27 28 29 30

Combined to afford

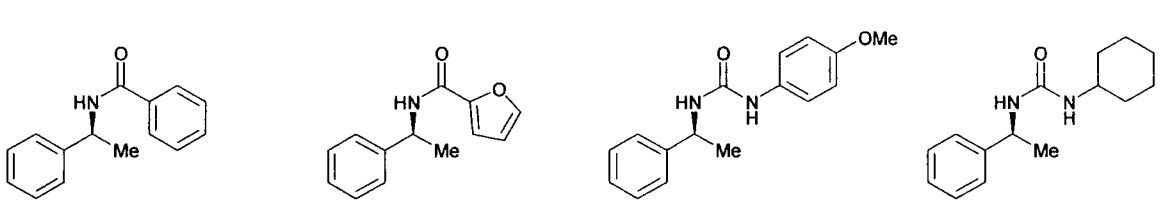
Reaction Products



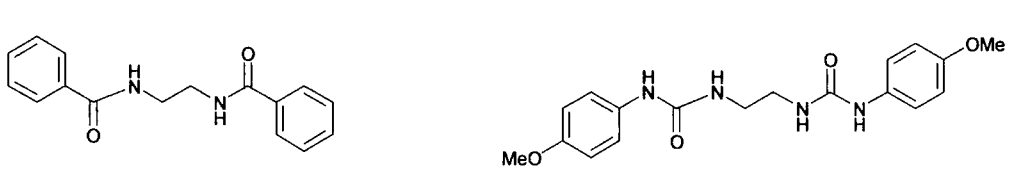
31 32 33 34



35 36 37 38



39 40 41 42



43 44

Figure 13

The yields obtained from this set of reactions were determined on the crude, non-purified materials, and the purity of the individual library components was determined by high performance liquid chromatography. The yield and purities for the compounds are listed in Table 4.

Table 4. The yield and purities of the amide and urea library.

Compound	% Yield	% Purity
31	99	>95
32	74	>95
33	97	>95
34	84	>95
35	95	>95
36	96	>95
37	93	>95
38	91	>95
39	93	>95
40	89	>95
41	95	>95
42	97	>80
43	nd	nd
44	nd	nd

As can be seen from Table 4, the formation of mono-amides or mono-ureas proceeded in excellent overall yields with the slight exception of **32**, and with high purities being obtained. The one exception to the high purities was the cyclohexyl urea **42**, which was relatively insoluble and gave a low HPLC resolution and purity. The formation of *bis*-amide **43** and *bis*-urea **44** was more problematic, due to the extreme insolubility of these materials. Attempts to prepare these compounds using more polar solvents than the dichloromethane usually employed did not alleviate matters, and the proton spectra of the materials obtained from the reactions were inconclusive as to the structure or purity of the materials.

Having established that a suitably derivatised cellulose bead could perform as a scavenger resin in organic solution, we elected to look at resin functions for which there was no literature precedent.

2.5.1 Application of resin 18 to scavenge excess bromine

Our attention was immediately drawn to the previously prepared allyl substituted resin **18**, and we reasoned that since it could be easily activated with bromine water, it should act as a scavenger for electrophilic bromine. In order to test this theory, we elected to brominate double bonds, to form the corresponding dibromo species. Under standard reaction conditions, the excess bromine normally employed to drive such reactions to completion would either be removed by aqueous base treatment, or by distillation, and it was hoped that simply employing resin **18** would allow the use of excess halogen without recourse to either of these strategies. The reactions chosen to test this theory are shown in Figure 14, with the yields and purities of the isolated materials are contained in Table 5.

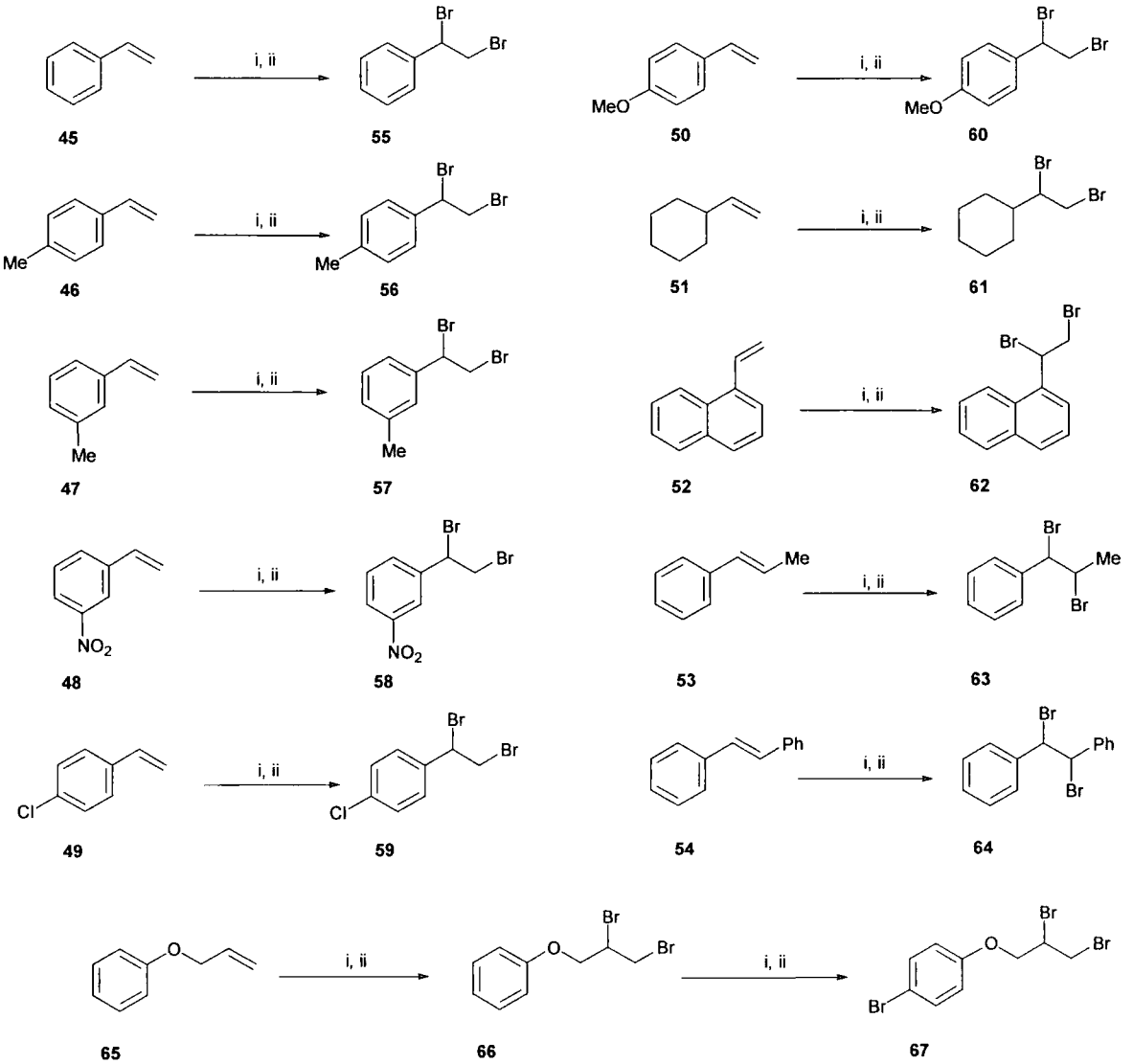


Figure 14 *Reagents and conditions*; i) Br_2 , CH_2Cl_2, RT, 30 min; ii) resin **18**

Table 5 Results of the bromination of alkenes with excess bromine.

Alkene	Product	% Yield	% Purity GC	% Purity HPLC
45	**55**	95	>95	>95
46	**56**	98	43	>85
47	**57**	98	>90	>95
48	**58**	100	>75	>95
49	**59**	94	>95	>95
50	**60**	95	65	>95
51	**61**	90	>95	>95
52	**62**	92	37	>90
53	**63**	80	70	>95
54	**64**	94	>80	>90
65	**67**	95	90	>90

The bromination reactions of the selected alkenes occurred in universally high yields, and with the purities of the product as determined by HPLC all greater than 95%, with the sole exception of **56** which was obtained with a purity of 85%. The purities of the materials were initially determined by GC, however the loss of HBr to form the bromoalkene was observed in GC-mass spectra of the materials and presumably this reaction was responsible for the lower purities observed with this technique

An interesting point was noted during the attempted preparation of dibromo ether **66** from allyl phenyl ether **65**. As the reaction proceeded, more bromine than the 1.5 equivalents calculated had to be added in order to retain a red-brown colour within the solution, this was also accompanied by the production of acidic white fumes as the reaction progressed. Upon workup and analysis, the target compound **66** was not isolated, instead both proton NMR and mass spectral evidence from the crude product pointed to the addition of one bromine atom to the aromatic ring, to afford **67**.

With the evidence of bromination on the aromatic ring to give **67** it seemed logical to explore this reaction further. To further investigate this it was decided to look at the bromination of phenols. The results of which can be seen in Figure 15 and the yields and purities are contained in Table 6.

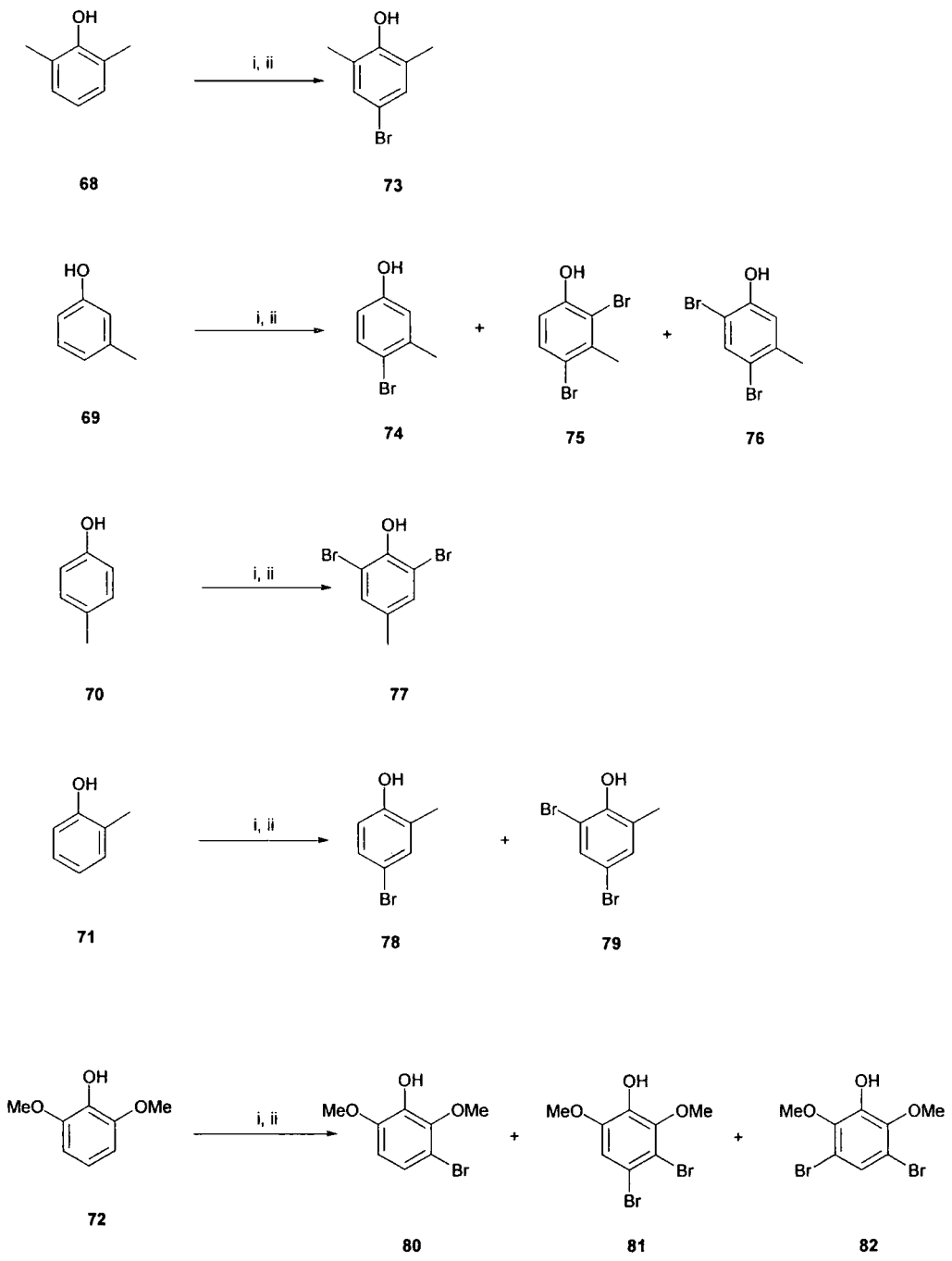


Figure 15 *Reagents and conditions*; i) Br_2 , CH_2Cl_2, RT, 30 min; ii) resin **18**

Table 6. Yields and purities for the bromination of phenols.

Phenol	Yield (%)	Purity (%)
68	97	87
69	52	90[a]
70	78	78
71	92	94[a]
72	98	98[a]

a In these cases a mixture of products was obtained and the purity refers to that determined by HPLC for the combined yield of brominated phenol products.

The resulting number of multiple products is due to the position of the substituted group on the aromatic ring. In the reaction involving 2,6-dimethylphenol (**68**) the methyl groups block both ortho positions, leaving only the para position to react. In reaction **70** it is the opposite. The methyl at position 4 allows only the ortho positions to react resulting in one product. Reactions **69** and **71**, which have both, an ortho and para position available gave multiple products due to the reaction giving mixtures of mono and di-substitution. The meta position in 2,6-dimethoxyphenol (**72**) is capable of reacting due to the stronger electron donating effect of the 2 methoxy groups overriding the hydroxy. This has, in effect, made the meta position (to the hydroxy) into an ortho/para position relative to the methoxy groups.

2.6 New Matrix Evaluation Studies

Having established that Perloza MT-100 was a useful base matrix for the introduction of chemical groups capable of scavenging excess reagents in selected reactions, we elected to look at other base matrices in order to determine if improved materials could be prepared. We selected the resins GCL-2000 (regenerated cellulose) and agarose (seaweed) based upon the following criteria;

1. They are readily available commercially in large quantities.
2. They are known to be capable of suitable chemical derivatisation utilising standard techniques.

3. They are prepared from natural and therefore renewable sources.

GCL-2000 is a regenerated cellulose acetate matrix, which has been cross-linked with epichlorohydrin. This not only improves the mechanical strength of the matrix, but also locks the pore size to 2 x 10^6 Da, which was determined by size exclusion chromatography. This value is almost twice that quoted for Perloza MT-100 1 x 10^6 Da, and it was hoped that the increased pore dimensions would increase access for reagents, and products, due to improved diffusion rates.

The agarose material chosen possessed a pore size comparable to that of the Perloza MT-100, but since it is formed from a different carbohydrate source (seaweed), the actual pore structure is different to that of a cellulose bead.

Figure 16. Structure of agarose.

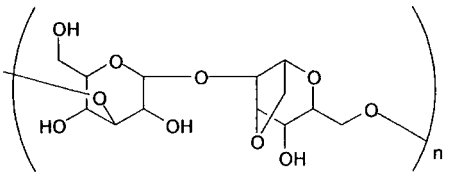
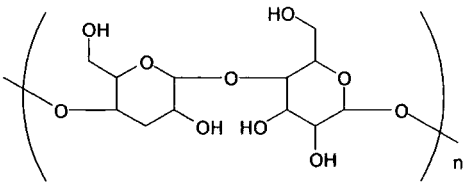


Figure 17.Structure of cellulose.



Cellulose is a linear polymer of β-D-glucopyranose with 1,4-glycosidic links whereas agarose has a primary structure consisting of alternating residues of D-galactose and 3-anhydrogalactose. The secondary and tertiary structures of agarose can be thought of as single fibres spun into a yarn of multiple fibres.

The methods for manufacturing both the agarose and cellulose beads are based around similar principles. In both processes, the base carbohydrate is dissolved in water either by chemical derivatisation or by the application of heat to form a melt. The former process is usually employed for cellulose, when a triacetate is normally prepared, whereas for agarose

a melt process is generally preferred. In the second step of the preparation, mixing the carbohydrate solution with an immiscible solvent such as toluene or xylene forms an emulsion. Finally, bead formation from the emulsion is achieved by removing the derivatisation *e.g.* cleavage of a triacetate (regenerated cellulose acetate) and by decreasing the temperature of the emulsion (agarose). As the temperature is reduced, insoluble beads are formed, which precipitate from the reaction mixture. In general, the beads are then removed by filtration, washed to remove residual solvent and sieved to achieve the desired particle size distribution. The bulk physical properties of the resin beads such as hardness, and particle size distribution are controlled by a number of factors. These include the initial percentage of carbohydrate added, the degree and type of derivatisation and the rate of temperature decrease in the precipitation stage.

In order to determine whether these other matrices (agarose and GCL-2000) were suitable for the preparation of scavenger resins, they were activated in a manner identical to that employed previously with the Perloza MT-100, in order to form both allyl and trisamine resins. As before loading values were determined by either bromine titration (for allyl groups) or CHN (amines). From Table 7 it can be seen that the ligand density measured on these matrices was comparable to the levels previously obtained using the Perloza MT-100 resin.

Table 7. A comparison of the loadings of allyl and amine groups on the different matrices.

Resin	Allyl level (mmol/g)	amine level (mmol/g)
Perloza MT-100	2.0	2.2
GCL 2000	1.7	2.6
Agarose	2.3	2.9

Once it had been established that the two target resins could support a sufficiently high ligand density for our purposes, we chose to study the swelling volume in various solvents, since this is one of the most important physical properties of any resin which is to be employed in scavenging reactions. The swelling volumes for each material were determined by washing a 10 ml aqueous sample of the resin with 1,4-dioxane (3 x 10 ml) and then with the solvent of choice (3 x 10 ml). The resin was then re-suspended in a calibrated column with the appropriate solvent (10 ml) and left to settle for 24 h. The final volume was then read from the calibration marks. In order to determine how these

carbohydrate resins compared to the polystyrene based resins, which are commonly employed at present, we included a polystyrene based trisamine resin sold by Argonaut. In the case of this resin, 1 g of the material was suspended in 20 ml of the solvent of choice, after first washing with 1,4-dioxane.

Table 8. A comparison of solvent swelling volumes (ml/dry g) for trisamine resins.

Solvent	Perloza	GCL-2000	Agarose	PS-Trisamine
Dichloromethane	8.0	12.0	10.5	5.0
Acetonitrile	9.0	11.0	10.5	3.0
1,4-dioxane	9.0	12.0	11.0	5.5
Methanol	9.0	12.0	11.0	4.0
Diethyl ether	10.0	10.5	10.5	1.0
Toluene	8.0	10.5	10.5	4.0
DMF	9.0	14.5	15.0	5.0
Tetrahydrofuran	9.0	11.0	11.0	5.0
Ethyl acetate	9.0	11.0	11.5	5.0
Water	10.0	12.0	11.0	1.0

From the results shown in Table 8 it is apparent that both the GCL-2000 and the agarose resins have very similar swelling properties despite originating from different carbohydrate sources and having been prepared by different procedures.

Indeed, even though the Perloza and the GCL-2000 are both cellulose-based resins, there is a significant difference in their swelling properties. With the GCL-2000 and the agarose resin, the fact that there is little change in volume (except for DMF) when going from an aqueous solvent system to an organic solvent is probably due to the cross-linking step used to increase their mechanical and chemical stability which also locks the pores.
In the agarose and the GCL-2000 resins, the pore size has been fixed, and some hydroxyl groups of the carbohydrate backbone have been utilised in order to achieve this. However, these hydroxyl groups are still present in the Perloza resin, and may somehow inhibit the DMF from acting as a porogen and opening up the primary structure of the resin to cause swelling. Given that the Perloza swells evenly in a broad range of solvents, this rather puzzling lack of swelling in a polar aprotic solvent seems anomalous. In order to fully test the theory that free hydroxyl groups are in some way involved, it would be necessary to test the swelling properties of all three resins in other polar aprotic solvents such as

hexamethylphosphoramide (HMPA) or N-methyl pyrrolidone. However, this study was not undertaken due to time constraints, but may throw light on this apparent anomaly in the future.

The more open and rigid pore structure of the carbohydrate resins compared with standard crosslinked polystyrene, allows higher diffusion rates of reactants thereby increasing the ability of the resin to remove any excess reagent. Also the carbohydrate resins have a wider compatibility with solvents compared to the polystyrene based resin, which is important if a solvent change is required, or if the reaction is to be carried out in a mixture of solvents. However, possibly the most important application of these materials is due to their pore accessibility and overall compatibility with aqueous and near-aqueous systems. With the ever-increasing drive to more environmentally friendly reactions, it is becoming more desirable to use water as the solvent. As this becomes more commonplace there will be a greater need for water compatible reagents.[45] To address this problem a new generation of resins have been developed which have hydrophilic groups (usually polyethylene glycol) grafted onto a polystyrene base.

In order to fully determine the utility of the agarose and GCL-2000, it was decided to compare them in two standard reactions, for which it had been demonstrated that the Perloza resins were very efficient.
To this end, the allyl functionalised versions of both resins were utilised to scavenge excess bromine from the bromination of styrene in dichloromethane (Figure 18.).

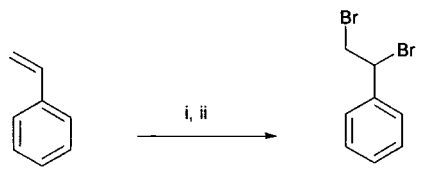


Figure 18 *Reagents and conditions* i) Br_2, DCM; ii) Resin **18**

The results are shown alongside the Perloza resin results in Table 9 for comparison.

Table 9. The scavenging of bromine in CH_2Cl_2 with allyl resins.

Resin	Yield % [1]	Purity % [2]
Perloza MT-100	95	>95
GCL-2000	85	>95
Agarose	95	>95

[1] Isolated yield after removal of the solvent and drying *in vacuo*.

[2] Determined by HPLC of the isolated material

In a second test reaction, the Trisamine resin was used to scavenge excess benzoyl chloride from the reaction with benzylamine to form the benzamide.

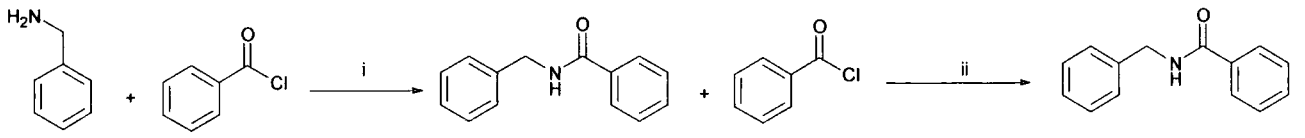


Figure 19 *reagents and conditions* i) DCM, pyridine; ii) Resin **22**

The results are tabulated in Table 10

Table 10. The scavenging of benzoyl chloride from organic solution with trisamine resins.

Resin	Yield % [1]	Purity % [2]
Perloza MT-100	95	95
GCL-2000	83	95
Agarose	89	95

[1] Isolated yield after removal of solvent and drying in vacuo.

[2] Determined by GC or HPLC of the isolated material

From Tables 9 and 10 it can be seen that both yield and purity of the products are comparable. The results indicate that even though all three resins are from different carbohydrate sources and made by different procedures there is very little difference in their efficiency at removing excess reagents. Surprisingly, given the fact that the GCL-2000 has twice the pore size of the agarose and Perloza there was not a significant difference in its ability to function as a scavenger. If anything, it produced the lowest yields and this is possibly due to the product being retained in the pores reflecting its

original use in size exclusion chromatography. Given the fact that the quoted pore size is an exclusion limit, reflecting a maximum value, it is not related to the overall pore size distribution. Consequently this polymer may well contain a large number of smaller pores which could retain the small molecules used in this study more effectively. This could be overcome by prolonged washing but this wasn't explored at this time.

One possible advantage the GCL-2000 could have is the rate at which it can remove excess reagents. Due to the increased pore size excess reagents may be able to enter the pores faster and therefore be removed quicker, however due to lack of time these kinetic parameters were not investigated.

2.7 Nucleophilic scavenger

Having successfully established that the trisamine resin **22** could readily remove excess electrophiles from solution, we decided to study a scavenger resin designed to remove excess nucleophiles from solution. Having examined the literature, it became apparent that there are a number of potential targets for a nucleophilic scavenger. These include resins substituted with aldehyde,[46] acid chloride[47] and anhydride moieties.[48] We elected to pursue the preparation of an aldehyde since it was known from the literature that carbohydrates could be readily oxidatively cleaved to prepare aldehyde substituents. A second reason for the choice of the aldehyde group is that they are relatively stable in the aqueous conditions we wished to work in, in sharp contrast to anhydrides and acid chlorides, which are inherently unstable in aqueous solution. Various methods of obtaining the aldehyde group on the resin were considered, the initial approach focusing on the periodate cleavage of diols. This approach was indeed attempted, but it was discovered that under the conditions required to achieve a sufficiently high level of activation to be of use as a scavenger resin, degradation of the resin backbone occurred. Indeed, in the reactions attempted with high concentrations of acidic periodate (pH 4.0), as shown in Figure 20, high levels of aldehyde groups were produced, but the resin beads degraded, and leaching of the material was observed.

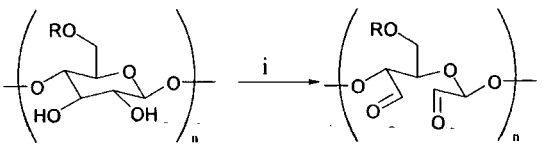


Figure 20 *Reagents and conditions*;i) $NaIO_4$, pH4, acetate buffer.

Having established that the periodate route was unsuitable for our requirements, an alternative method utilising ozone to oxidise an immobilised alkene to the corresponding aldehyde group was attempted.[49] Ozone was chosen for three principal reasons; firstly because the reaction is clean, giving only the desired aldehyde on the resin, secondly it is easy to perform and finally, the work up is a simple filtration and water wash. In order to avoid the possibility of β-elimination, as shown in Figure 21, of the newly formed aldehyde under the basic conditions employed in this reaction, a chain length of at least 4 carbons is required.

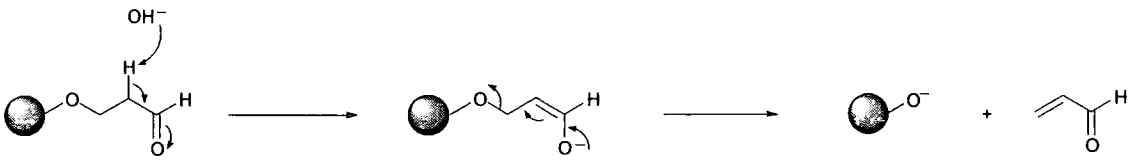
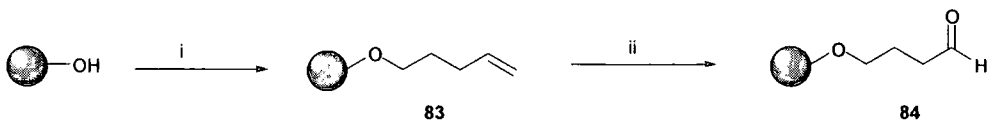


Figure 21

To achieve this, 5-bromo-1-pentene was attached to the resin *via* an ether linkage under basic conditions. However, since utilising already-established procedures employing strong aqueous sodium hydroxide often resulted in the elimination of bromine before the carbohydrate hydroxyls could react, an alternative route had to be developed. It has been reported[50] that it is possible to alkylate the carbohydrate-based molecule cyclodextrin utilising sodium hydroxide in dimethyl sulphoxide, however in our hands using resin beads, this reaction gave only low levels of activation even after seven days. The problem of activation was finally overcome by employing the non-nucleophilic base sodium hydride in anhydrous dimethyl formamide. Utilising sodium hydride alone resulted in low levels of activation in the region of 0.78mmol/g, so improvements to this procedure were considered. Iodides are known to undergo a more facile S_N2 process and consequently the reaction was repeated with the addition of KI with the idea of generating the iodide *in situ*. Gratifyingly, the addition of potassium iodide to this reaction increased the level of alkene immobilised to 3.3mmol as determined by bromine titration to give resin **83**.

Having achieved an acceptable level of activation, the next step was to treat the resin with ozone to afford the desired aldehyde. This procedure was readily accomplished by stirring the alkene resin **83** in dichloromethane at –78°C, whilst bubbling ozone into the rapidly stirred solution (Figure 22).



83 **84**

Figure 22 *Reagents and conditions;* i) NaH, DMF, KI, 5-bromo-1-pentene; ii) Ozone, DCM, -78°C

Once a deep blue colour was observed in the suspension, after 1.5 h, the ozone addition was stopped, oxygen passed through until the blue colour had disappeared and then dimethylsulphide added. The resin was then washed with dichloromethane, tetrahydrofuran (THF), THF/water 50:50, THF/water 75:25 and finally with water to give resin **84**. The aldehyde level was determined using the established Purpald®[51] assay which had been calibrated for the resin. The assay consistently gave a loading of 1mmol/g, which indicates a conversion level of approximately 30% of the total groups available. This is probably due to the reaction being stopped too early, when the alkene groups situated on the outside of the pore structure have reacted, but those located deeper within the pores have not. To achieve complete conversion of all the alkene groups to aldehydes the reaction must be allowed to continue for an extended period of time to allow the ozone to diffuse into the pore structure to reach the internal groups and react with them. This was however was not explored due to time constraints.

2.8 Synthesis of amino alcohols

The preparation of amino alcohols via the ring opening of epoxides with amines is a useful reaction often leading to compounds with interesting biological properties. Given the ease of formation of bromohydrins from alkenes using resin **18** this extension to generate epoxides, and subsequently amino alcohols seemed logical. In particular the possibility of performing such reactions using only supported reagents and scavenger resins was considered (Figure 23).

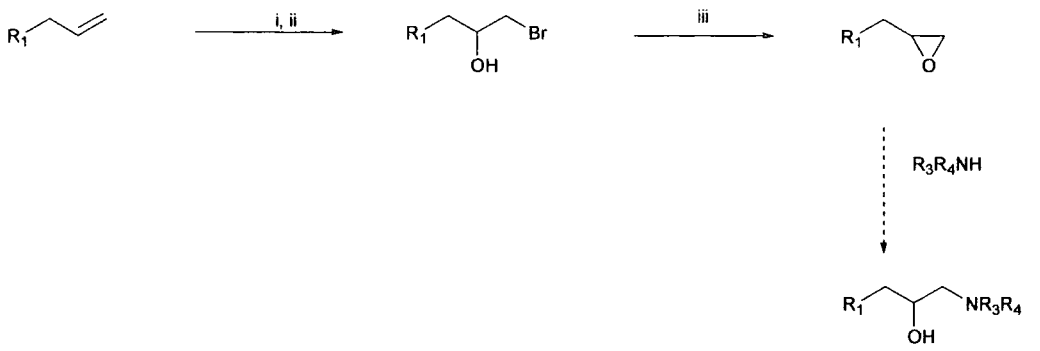
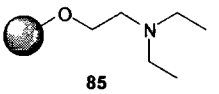


Figure 23 Reagents and conditions; i) BrOH, 4 h; ii) resin 18; iii) Resin **85**

2.8.1 Epoxide formation

As described in previous sections, bromohydrins could be efficiently produced using bromine water. The next step required the cyclisation of the bromohydrin to produce the epoxide. The normal procedure for such reactions is to use K_2CO_3 and anhydrous MeOH, however it was decided to look at the possibility of utilising a supported base.

Although resin **22** is basic it is also nucleophilic and could competitively react with the bromohydrin and the resultant epoxide. Indeed resin **22** is actually made by reacting a bromohydrin with trisamine, therefore a non-nucleophilic base was required. These are commonly tertiary amine bases (Figure 24) and a readily available example of this is an agarose supported triethylamine equivalent (**85**) commonly used in ion exchange chromatography.



85

Figure 24

This resin was initially washed with NaOH to remove any absorbed CO_2 and then washed with water to remove excess NaOH. This was then added to the already purified bromohydrin (from styrene and bromine water) in water. The reaction was left to mix for 24 h then extracted with DCM. Upon analysis of the products by NMR and comparing with an authentic styrene oxide sample, it appeared that the reaction had worked and the

epoxide had been formed. With this encouraging initial reaction a number of different alkenes were converted to the bromohydrin and then treated to form the epoxide. However upon analysis of the final product it was obvious that the reaction had not worked leaving only the bromohydrin. Further investigation indicated that the initial success was possibly due to residual NaOH being retained in the internal pores of the polymer. Due to time restraints the use of other supported non-nucleophilic bases was not investigated.

2.9 Bi- phasic reaction

With the resin being compatible with both water and organic solvents an attempt was made to see if a reaction could be carried out in a bi-phasic system and purified using the resin without the added steps of solvent exchange. To demonstrate this vinylanisole was dissolved in dichloromethane and brominated using bromine water. The bromine transferred from the aqueous phase into the organic layer after 10 min of mixing, this was left for a further 30 min after which time there was no colour change in the organic layer. The allyl resin, still in water, was then added and stirred vigorously for 30 min. During this time the organic layer began to de-colourise indicating that the resin was removing excess bromine from the reaction. After the work up of the reaction the product was found to be the di-bromide not the bromohydrin normally associated with bromine water reactions. This was explained by the migration of the bromine into the organic layer and essentially performing a normal bromination reaction with the vinylanisole. The increased reaction time is due to the fact that the resin is sitting at the interface between the aqueous layer and the organic layer. This results in a greatly reduced surface area from which to remove the excess bromine.

2.10 Biodegradation studies on the base matrix

The potential for biodegrading the base cellulose resin was investigated since it would demonstrate yet another unique advantage of this material over the non-degradable, cross-linked polystyrene resins currently employed in solid phase synthesis. In nature, cellulose units are readily broken down by a variety of microorganisms, which often employ the enzyme cellulase to convert subunits **86** into glucose **87**.[52] Due to this, there exists the

potential to metabolise the sugars produced, in order to provide energy for other biological processes within the organism. In our scenario, this last step was to be attempted by the conversion of any glucose fragments produced into ethanol **88** by a fermentation step, utilising brewers yeast in conjunction with the cellulase (Figure 25).

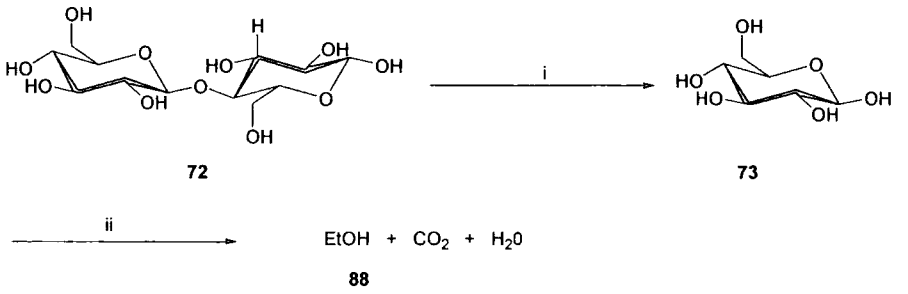


Figure 25 *Reagents and conditions;* i) Cellulase, pH 5.2, 35°C 48h; ii) Brewers yeast, 48h

The anaerobic fermentation of hemicellulose sugars to produce ethanol **88** utilising a strain of *bacillus stearothermophilus* has recently been patented,[53] and other reports demonstrate not only the relative ease of this conversion, but also serve to demonstrate its potential economic and environmental benefits.[54] Although this thermophilic fermentation will not proceed when non-digested cellulose is employed as the feedstock, it was hoped that the use of cellulase would at least demonstrate the biodegradation possibilities of these resins in a laboratory context, without utilising a bacterial broth, or prior acid digestion of the beads.[55]

It was therefore decided to examine the ability of the enzyme cellulase to degrade the resin. It was also decided that brewers' yeast should be added to selected samples in order to determine whether the reaction outlined in Figure 25 could be driven across to ethanol by fermentation of the glucose formed in the initial step. In each reaction, cellulase (100 mg) and/or yeast (100 mg) were added to approximately 1 ml (aqueous volume) of Perloza MT-100 resin in sodium acetate buffer (pH 5.2). The suspensions formed were then incubated at a pre-set temperature for forty-eight hours prior to analysis. Two 'blank' control reactions were also carried out; the first contained no cellulase or yeast, and the second containing only yeast. On completion of the incubation, four parameters were measured in order to ascertain the extent of any reaction. These were;

1. The final pH of the mixture.
2. The final settled volume of resin.

3. The amount of glucose present as determined by a glucose hexokinase (HK) assay kit.[56]
4. The final mass of the recovered resin after washing and drying at 100°C.

The determination of glucose using the HK assay kit is dependent upon the sequence of enzymatic reactions outlined in Figure 26. In these reactions, glucose **87** is initially converted to glucose-6-phosphate **90** by hexokinase, with the phosphate group being transferred from adenosine triphosphate (ATP) **89**, generating adenosine diphosphate (ADP) **91** as a by-product. The phosphate **90** is converted to 6-phosphoglutonate **93**, with concomitant interconversion of nicotinamide adenine dinucleotide (NAD) **92,** present in the mixture, into the hydrogenated form NADH **94**. The actual amount of glucose after a pre-set time can then be quantitatively determined by measurement of the absorbance of the solution at 340 nm, with reference to internal controls.

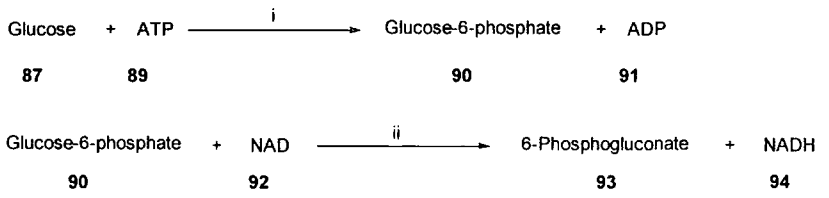


Figure 26 *Reagents and conditions;* i Hexokinase; ii) Glucose-6-Phosphate Dehydrogenase

The amount of glucose present can easily be determined by equation 1, where ΔA equals the total test blank absorbance, TV is the total assay volume, F is the dilution factor required to obtain a measurable absorbance, and SV is the sample volume.

$$\text{mg Glucose} = \frac{(\Delta A)\,(TV)\,(\text{molecular weight of glucose})\,(F)}{(E)\,(d)\,(SV)\,(\text{conversion factor } \mu g \text{ to mg})}$$

$$= \frac{(\Delta A)\,(TV)\,(180.2)\,(F)}{(6.22)\,(1)\,(SV)\,(1000)}$$

$$= \frac{(\Delta A)\,(TV)\,(0.029)\,(F)}{(SV)}$$

Equation 1. The determination of glucose utilising glucose assay kit.

The results of this group of incubation reactions are outlined in Table 11.

Table 11. Results of biodegradation studies on Perloza MT-100.

Sample[a]	Cellulase	Yeast	Final pH	Final vol/ml	Glucose/mg[b]	mass/mg[c]
1	no	no	5.23	1.0	0	105
2	yes	no	5.23	1.0	36	73
3[d]	yes	yes	5.30	1.0	46	nd
4[e]	yes	yes	5.27	0.95	44	nd
5	no	yes	5.30	1.1	0	nd
6	yes	no	5.21	1.0	64	61
7	yes	no	5.21	1.0	82	47

nd = not determined

[a] samples run at 35 °C, except sample 6 (45 °C) and sample 7 (55 °C). [b] determined by glucose assay with internal blanks. [c] final dry mass of residual resin after solvent washing and heating to 100 °C to remove water. [d] cellulase and yeast added simultaneously. [e] cellulase added initially, and yeast after 24 h.

As can be seen in Table 11, the final pH of the reaction mixtures, and the volume of the resin obtained did not alter drastically in any of the reactions. These two factors although easily determined were therefore not employed as measures of reaction efficiency.

The final mass of the recovered resins did vary markedly from reaction to reaction, except where yeast was employed. In these cases, no final mass was recorded due to the presence of yeast cells, which could not be removed from the mixture to allow accurate measurements to be made. In all cases, the glucose assay kit proved to be capable of measuring the sugars produced and provided a second measurable factor.

When no cellulase was present (entries 1 and 5), no glucose was produced, and the final mass of the resin was identical to that measured for the initial resin (approximately 105-110 mg dry). Addition of cellulase alone (entries 2, 6 and 7) led to the increasing formation of glucose as measured by assay, and a simultaneous drop in the final mass of resin. Interestingly, the trend towards more glucose and lower resin mass was in line with an increase in reaction temperature, a fact which was not predicted based upon standard non-thermophilic enzymatic behaviour. In any case, enzyme inhibition by heat does not appear to be a problem within the admittedly limited confines of this experiment. Simply heating the resin alone in acetate buffer does not lead to the production of glucose or to a

decrease in resin mass, indicating as expected that the enzyme cellulase is required for degradation to occur. The mass balance in entries 2, 6 and 7 is greater than 100% since only 105-110 mg (1 ml) of resin were added to each tube. This is presumably due to inaccuracies in the measurements, and not due to contamination of the reactions by cellulase by-products, since mixing cellulase and acetate buffer with no resin gave a negative result in the subsequent glucose assay.

The addition of yeast to the cellulase reactions either simultaneously as in entry 3, or after 24 h incubation as in entry 4, led to a slight increase in the amount of glucose observed. These reactions serve to illustrate two points; firstly that the presence of yeast at any time does not inhibit the action of cellulase, and secondly that it actually appears to increase the amount of glucose present. This second fact is interesting given that the yeast was added to effectively decrease the amount of glucose present by converting it to ethanol (Figure 25). The higher level of glucose could be due to a fermentation process producing ethanol, and thus reducing initial inhibition of the cellulase. Such end-product inhibition could potentially be caused by a rapid initial build up of glucose, thereby reducing enzymatic activity. Consequently, a reduction in the initial glucose concentration would favour the enzymatic degradation process.

Overall, it can be seen that the resin can be biodegraded by the action of an enzyme, and that the degree of degradation can be easily quantified either by measurement of the final resin mass, or by enzymatic assay of the glucose produced.

2.11 Summary

The activation of beaded cellulose with functional groups capable of scavenging electrophiles from organic solutions has been achieved. Of the two materials so far prepared, the trisamine gel **22** performs efficiently in removing both acid chlorides and isocyanates from solution, whilst the novel allyl resin **18** scavenges bromine. The two gels therefore allowed the rapid synthesis of a number of target compounds, which were isolated in high yields and in excellent purities.

The resin itself has also been shown to be biodegradable by the action of the enzyme cellulase, and this demonstrates a significant advantage of these materials over the traditional resins employed to produce scavenger matrices, which are prepared from cross linked polystyrene. The fact that the cellulose is a renewable resource also makes the

application of these gels more environmentally benign. Another advantage compared to the traditional polystyrene resins is the compatibility with aqueous and bi-phasic systems.

3.0 Experimental Section

3.1 General Details

Proton NMR spectra were recorded on a Bruker 250 spectrometer at 250 MHz with tetramethylsilane as the internal standard. Mass spectra were recorded on a Varian VG7070E spectrometer operating in either electron impact (EI) mode at 70 eV or in chemical ionisation mode (CI) with ammonia as the impingent gas. Infra-red spectra were recorded on a Perkin-Elmer 1615 FTIR operated from a Grams Analyst 1600 package or on a Perkin-Elmer Paragon 1000 using a 'Golden Gate' press. Elemental analyses were performed on a Carlo-Erba Strumentazione instrument. Melting points were obtained using an Electrothermal 9200 instrument, and are uncorrected. High Performance Liquid Chromatography (HPLC) was performed on a Varian system with detection at 254 nm. The stationary phase employed was a 20 cm Hypersil reverse phase column, with MeOH : H_2O 85 : 15 as the mobile phase. Capillary Gas Chromatography was performed on a Perkin-Elmer 8410 using an RH5 column. Perloza MT-100, particle size 100-250 μm was obtained from Lovochemie (Prague) and was washed with water to remove preservatives prior to derivatisation. All other reagents were of commercial quality, and solvents were dried, where stated, by standard procedures. The rollermixer employed to mix reaction tubes was a Stuart Scientific SRT 2. The glucose (HK) assay kit was purchased from Sigma-Aldrich. UV measurements were performed on a Camspec m330 Uv/Vis spectrophotometer.

3.2 Preparation of allyl resin 18

To a suspension of suction-dried Perloza MT-100 (100 g), in H_2O (50 ml) were added allyl bromide (28 g, 20 ml, 0.23 mol) and 10M NaOH (10 ml). Water was added, to give a final volume of 200 ml, and the resultant suspension was stirred overnight at rt. After 18h the resin was filtered, washed with H_2O (3 x 100 ml), acetone (3 x 100 ml) and H_2O (3 x 100 ml). Oven dried samples of the allyl matrix were titrated using a standard solution of bromine in water (1% v/v) which showed the average loading of allyl groups to be 1.9 mmol/g (maximum is 16.9 mmol/g).

3.3 Preparation of trisamine resin 22

Allyl-activated resin **18** (100 g, 0.19 mol) was hydro brominated by the addition of excess bromine-water (1% v/v). After 1 h completion of the reaction was indicated by the persistence of colour in the supernatant, the resin was washed with H_2O (3 x 100 ml), 1,4-dioxane (3 x 100 ml) and MeOH (3 x 100 ml). The volume of the resin was adjusted to 140 ml with methanol, whereupon *tris*-(2-aminoethyl)amine (29.3 g, 30 ml, 0.20 mol) was added. The suspension was stirred at rt for 16h, after which time the resin was washed with MeOH (3 x 100 ml) and H_2O (3 x 100 ml) and suction dried. Elemental analysis on an oven dried sample found; C, 42.35; H, 6.37; N, 3.04. Nitrogen content 2.2 mmol/g (maximum 16.9 mmol/g).

3.4 Determination of the swelling properties of trisamine resin 22

A slurry of trisamine resin **22** and H_2O (10 ml, 1 : 1 v/v) was allowed to settle in a graduated column, with the level being adjusted by the addition or removal of slurry, until a resin volume of 10 ml was achieved. The settled bed was then washed with H_2O (10 ml), 1,4-dioxane (2 x 10 ml) and the solvent of choice (3 x 10 ml). Once the final wash was completed, an extra 10 ml of the solvent of choice was added, and the column ends sealed. The column was shaken vigorously to ensure the formation of an even slurry, and allowed to settle over 16 h. On completion of settling, the volume of the resin was recorded.

3.5 Determination of the reaction rate of trisamine resin 22 with benzoyl chloride

Benzoyl chloride (25 μl, 0.2 mmol) was added to anhydrous CH_2Cl_2 (20 ml), and a uv/vis absorption spectrum taken, which gave an absorption maximum at 295 nm. The optical density of the solution at the λ_{max} was recorded. Trisamine resin (5 ml aqueous volume, 0.55 mmol NH_2 groups) which had been washed with 1,4-dioxane (2 x 10 ml) and CH_2Cl_2 (2 x 10 ml) was added to the benzoyl chloride solution, and the resultant suspension mixed thoroughly. A 1 ml aliquot of the supernatant solution was removed at 1 min intervals, filtered through a 45 μm disc filter, and the absorbance measured at 295 nm.

3.6 Preparation of pent-1-ene resin 83

Agarose resin (20 ml) was washed with 1,4-dioxane (3 x 20 ml), THF (3 x 20 ml) and DMF (3 x 20 ml). The resin was then re-suspended in DMF (60 ml) to which was added sodium hydride (3.26 g, 82 mmol, 1.2 eq). The suspension was stirred vigorously for 5 min after which time 5-bromopent-1-ene (10 ml, 68 mmol, 1 eq) and potassium iodide (22.6 g, 136 mmol, 2 eq) were added. The suspension was then left stirring for 60 h at 25 °C. The resin was then washed with DMF (5 x 20 ml), MeOH (5 x 20 ml) and H_2O (5 x 20 ml).

3.7 Preparation of butanal resin 84

Pentene resin (10 cm^3, 260 μmol/ml) was washed with 1,4-dioxane (3 x 10 ml) and CH_2Cl_2 (3 x 10 ml). Ozone was then passed through a suspension of the resin in CH_2Cl_2 (30 ml) for 1.5 h at –78 °C until the solution went blue. The ozone generator was then turned off and oxygen passed through the stirred suspension until the blue colour disappeared, at this point dimethylsulfide (250 μl, 5 eq) was added and the suspension was allowed to warm to rt over1 h. The resin was filtered, then washed with CH_2Cl_2 (3 x 10 ml), THF (3 x 10 ml), THF/H_2O 75 : 25 (3 x 10 ml), THF/ H_2O 25 : 75 (3 x 10 ml) and H_2O (3 x 10 ml).

3.8 Purpald® assay

A stock solution (2.77 mM) was prepared by dissolving propionaldehyde (100 μl) in a volumetric (500 ml) and adjusting the volume up to the mark with water. From the stock solution a standard curve was prepared (see Table 12 for quantities).

Table 12 Purpald® Standard Curve.

Volume of propionaldehyde (ml)	Volume of water (ml)	Propionaldehyde (μmol)
0	4.0	0
0.4	3.6	1.11
0.75	3.25	2.08
1.25	2.75	3.46
2.0	2.0	5.54
4.0	0	11.08

The Purpald® (4-amino-3-hydrazine-5-mercapto-1,2,4-triazole) solution was prepared by dissolving Purpald® (0.5 g) in 1M sodium hydroxide (50 ml) immediately before use. To 4 ml of H_2O a known amount (ml or mg) of sample was added. To the sample and standard curve 1 ml of the fresh Purpald® solution was added and left to mix for 30 min. The absorbance of the solution was measured at 525 nm using a Camspec m330 uv/vis spectrophometer.

3.9 General Procedures for the preparation of amides and ureas using trisamine resin 22

Procedure A

To a mixture of the amine (0.4 mmol, 1 eq.) and pyridine (95 mg, 97 µl, 1.2 mmol, 3 eq.) in anhydrous CH_2Cl_2 (2 ml) in a glass screw-top vial at 0 °C, was added the electrophile (0.6 mmol, 1.5 eq.). The vial was sealed, the solution was allowed to warm to 20 °C, and was then mixed on a roller mixer. After 2 h, trisamine resin **22** (5 ml aqueous volume, 0.55 mmol NH_2 groups) which had been washed with 1,4 dioxane (3 x 5 ml) and CH_2Cl_2 (2 x 5 ml) was re-suspended in 5 ml of CH_2Cl_2 and transferred to the reaction vessel. The mixture was agitated on a roller mixer. After 1 h, the resin was filtered and washed with CH_2Cl_2 (4 x 10 ml). The combined filtrates were evaporated under reduced pressure, and the resulting residue was dried using a freeze dryer for 2 h to afford the desired products.

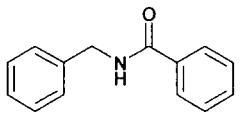
Procedure B

As procedure A except the amine was suspended in 10 ml of anhydrous CH_2Cl_2, and resin **22** was added without the aid of additional solvent.

Procedure C

As procedure A, but with MeCN as the solvent, in place of CH_2Cl_2 in all stages.

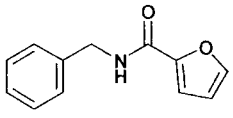
3.10 Results for amides and urea synthesis



31

***N*-Benzylbenzamide 31**

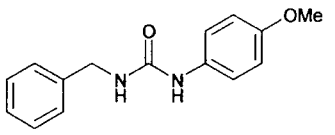
Using procedure A, benzylamine (43 mg, 44 µl, 0.4 mmol, 1 eq.) and benzoyl chloride (84 mg, 70 µl, 0.6 mmol, 1.5 eq.), gave **31** (84 mg, 99%) as a white solid, m.p. 104-105 °C (literature[57] m.p. 106-107 °C); δ_H (250 MHz; $CDCl_3$) 7.79-7.71 (2 H, m, Ph), 7.55 - 7.20 (8 H, m, Ph), 6.40 (1 H, br s, NH) and 4.50 (2 H, d, J = 5.5, CH_2Ph); *m/z* (EI) 211 (M^+, 73%), 105 (95) and 77 (100); HPLC purity >95%.



32

N-Benzyl-2-furancarboxamide 32

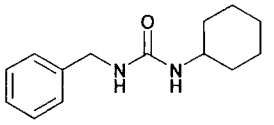
Using procedure A, benzylamine (43 mg, 44 µl, 0.4 mmol, 1 eq.) and 2-furfuroyl chloride (78 mg, 59 µl, 0.6 mmol, 1.5 eq.), gave **32** (75 mg, 74%) as a white solid, m.p. 108-110 °C (literature m.p. 110-112 °C [58]); δ_H (250 MHz; $CDCl_3$) 7.38 (1 H, dd, J = 3.0 and 1.0, 5-H furyl), 7.30-7.23 (5 H, m, Ph), 7.05 (1 H, dd, J = 2.5 and 1.0, 3-H furyl), 6.70 (1 H, br s, NH), 6.40 (1 H, dd, J = 2.5 and 3.0, 4-H furyl) and 4.50 (2 H, d, J = 5.5, CH_2Ph), *m/z* (EI) 201 (M^+, 73%) and 95 (100); HPLC purity > 95%.



33

1-Benzyl-3-(4′-methoxyphenyl)urea. 33

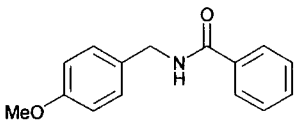
Using procedure C, benzylamine (43 mg, 44 μl, 0.4 mmol, 1 eq.) and 4-methoxybenzyl isocyanate (89 mg, 79 μl, 0.6 mmol, 1.5 eq.) gave **33** (93 mg, 97%) as an off white solid m.p. 160-163 °C (literature[59] m.p. 166.5-167.5°C); δ_H (250 MHz; $CDCl_3$) 7.35-7.25 (5 H, m, Ph), 7.17 (2 H, d, J = 8.5, 2′, 6′-H Ar), 6.82 (2 H, d, J = 8.5, 3′, 5′-H Ar), 6.05 (1 H, br s, 2 x NH), 4.40 (2 H, s, CH_2Ph) and 3.78 (3 H, s, MeO); HPLC purity >95%.



34

1-benzyl-3-cyclohexyl urea 34

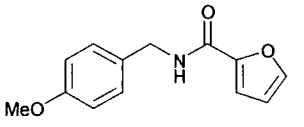
Using procedure B, benzylamine (43 mg, 44 μl, 0.4 mmol, 1 eq.) and cyclohexyl isocyanate (75 mg, 77 μl, 0.6 mmol, 1.5 eq.) gave the product **34** (78 mg, 84%), as a pale yellow solid m.p. 162-163 °C (literature[60] m.p. 162-164°C); δ_H (250 MHz; $CDCl_3$) 7.38-7.23 (5 H, m, Ph), 4.70 (1 H, br s, NH), 4.37 (2 H, d, J = 5.5, CH_2Ph), 3.50 (1 H, br s, NH), 1.99 - 1.81 (2 H, m, cyclohexyl), 1.72-1.59 (3 H, m, cyclohexyl), 1.38-1.01 (6 H, m, cyclohexyl); *m/z* (EI) 232 (M^+, 52%) and 91 (100); HPLC purity > 95%



35

***N*-(4-Methoxybenzyl)benzamide 35**

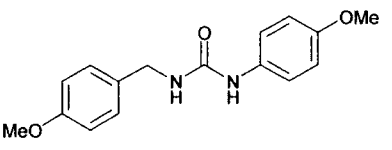
Using procedure B, with the addition of dried MeCN (2 ml), 4-methoxybenzylamine (55 mg, 52 μl, 0.4 mmol, 1 eq.) and benzoyl chloride (84 mg, 70 μl, 0.6 mmol, 1.5 eq.) as the electrophile gave the product **35** as a white solid (91 mg, 95%) m.p. 93-95 °C (literature[61] m.p. 97.5-99°C); δ_H (250 MHz; $CDCl_3$) 7.72-7.67 (2 H, m, Ph), 7.43-7.34 (3 H, m, Ph), 7.20 (2 H, d, J = 8.5, 2, 6-H Ar), 6.81 (2 H, d, J = 8.0, 3, 5-H Ar), 6.36 (1 H, br s, NH), 4.51 (2 H, d, J = 5.5, CH_2Ph) and 3.70 (3 H, s, MeO); *m/z* (EI), 241 (M^+, 83%) and 105 (100); HPLC purity >95%.



36

Furan-2-carboxylic acid-(4′-methoxybenzyl)amide, 36

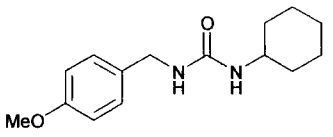
Using procedure A, 4-methoxybenzylamine (55 mg, 52 μl, 0.4 mmol, 1 eq.) and 2-furfuroyl chloride (78 mg, 59 μl, 0.6 mmol, 1.5 eq.) gave the product **36** as a white solid (88 mg, 96%) m.p. 83-85 °C; δ_H (250 MHz; $CDCl_3$) 7.35 (1 H, dd, J = 2.0 and 1.0, 5-H furyl), 7.22 (2 H, d, J = 8.5, 2′, 6′-H Ar), 7.05 (1 H, dd, J = 2.5 and 1.0, 3-H furyl), 6.80 (2 H, d, J = 8.5, 3′, 5′-H Ar), 6.58 (1 H, br s, NH), 6.40 (1 H, dd, J = 2.5 and 2.0, 4-H furyl), 4.43 (2 H, d, J = 5.5, CH_2Ar) and 3.70 (3 H, s, MeO); *m/z* (EI), 231 (M^+, 62%) and 95 (100); HPLC purity >95%.



37

1-(4-methoxybenzyl)-3-(4′-methoxyphenyl)urea 37

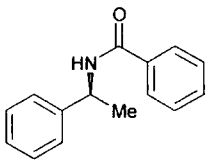
Using procedure B, with the addition of dried MeCN (2 ml), 4-methoxybenzylamine (55 mg, 52 µl, 0.4 mmol, 1 eq.) and 4-methoxyphenyl isocyanate (90 mg, 78 µl, 0.6 mmol, 1.5 eq.) gave the product **37** as a pale yellow solid (106 mg, 93%) m.p. 192-193 °C; δ_H (250 MHz; DMSO-D_6) 8.26 (1 H, br s, NH); 7.28 (2 H, d, J = 9.0, 2′, 6′-H Ar), 7.20 (2 H, d, J = 8.5, 2, 6-H Ar), 6.88 (2 H, d, J = 8.5, 3′, 5′-H Ar), 6.80 (2 H, d, J = 9.0, 3, 5-H Ar), 6.40 (1 H, br t, J = 6.0, NH), 4.20 (2 H, d, J = 5.5, CH_2Ar), 3.72 (3 H, s, CH_3O) and 3.68 (3 H, s, MeO); *m/z* (EI), 286 (M^+, 31%) and 123 (100).



38

1-cyclohexyl-3-(4-methoxybenzyl)urea 38

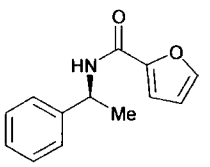
Using procedure A, 4-methoxybenzylamine (55 mg, 52 µl, 0.4 mmol, 1 eq.) and cyclohexylisocyanate (75 mg, 77 µl, 0.6 mmol, 1.5 eq.) gave the product **38** as an off white powder (95 mg, 91%) m.p. 156-157 °C; δ_H (250 MHz; $CDCl_3$) 7.23 (2 H, d, J = 8.5, 2, 6-H Ar), 6.85 (2 H, d, J = 8.5, 3, 5-H Ar), 4.58 (1 H, br s, NH), 4.23 (2 H, d, J = 5.5, CH_2Ph), 3.78 (3 H, s, MeO), 3.50 (1 H, m, cyclohexyl), 2.05-1.81 (2 H, m, cyclohexyl), 1.78-1.52 (4 H, m, cyclohexyl) and 1.40-1.02 (4 H, m, cyclohexyl); *m/z* (EI), 262 (M^+, 78%) and 121 (100); HPLC purity >95%.



39

_N_-(S)-1-phenylethyl benzamide 39

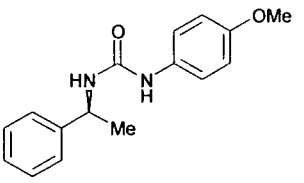
Using procedure A, (*S*)-α-methylbenzylamine (48 mg, 51 μl, 0.4 mmol, 1 eq.) and benzoyl chloride (84 mg, 70 μl, 0.6 mmol, 1.5 eq.) gave the product **39** as an off white powder (84 mg, 93%) m.p. 115-120 °C (literature[62] m.p. 122-124 °C); δ_H (250 MHz; $CDCl_3$) 7.78 (2 H, d, *J* = 8.5, Ph), 7.51- 7.24 (8 H, m, Ph), 6.40 (1 H, br s, NH), 5.34 (1 H, q, *J* = 7.0, C*H*CH_3) and 1.60 (3 H, d, *J* = 7.0, CHC*H*$_3$); *m/z* (EI), 225 (M^+, 41%) and 105 (100); HPLC purity >95%.



40

Furan –2-carboxylic acid – _N_-(*S*) 1-phenylethyl amide 40

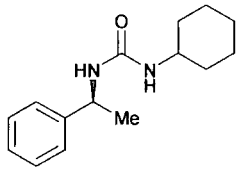
Using procedure A, (*S*)-α-methylybenzylamine (48 mg, 51 μl, 0.4 mmol, 1 eq.) and 2-furfuroyl chloride (78 mg, 59 μl, 0.6 mmol, 1.5 eq.) gave the product **40** as a white solid (77 mg, 89%); m.p. 88-90 °C (literature[63] m.p. 95-96 °C); δ_H (250 MHz; $CDCl_3$) 7.38-7.11 (6 H, m, Ph + furyl), 7.00 (1 H, dd, *J* = 2.5 and 1.0, 3-H furyl), 6.60 (1 H, br s, NH), 6.40 (1 H, dd, *J* = 2.5 and 1.0, 4-H furyl), 5.22 (1 H, q, *J* = 7.0, C*H*CH_3) and 1.50 (3H, d, *J* = 7.0, CHC*H*$_3$); *m/z* (EI), 215 (M^+, 22%) and 95 (100); HPLC purity >95%.



41

1-(4-methoxyphenyl)-3-((S)-1′-phenylethyl) urea 41

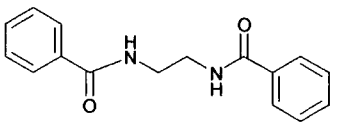
Using procedure A, (*S*)-α-methylbenzylamine (48 mg, 51 μl, 0.4 mmol, 1 eq.) and 4-methoxyphenyl isocyanate (90 mg, 78 μl, 0.6 mmol, 1.5 eq.) gave the product **41** as a white solid (103 mg, 95%). m.p. 177-178°C (literature[64] m.p. 185-186 °C); δ_H (250 MHz; $CDCl_3$) 7.38-7.23 (5 H, m, Ph), 7.18 (2 H, d, *J* = 8.0, 2, 6-H Ar), 6.86 (2 H, d, *J* = 8.5, 3, 5-H Ar), 6.08 (1 H, br s, NH), 4.97 (1 H, q, *J* = 7.0, C*H*CH_3), 4.83 (1 H, br s, NH), 3.80 (3 H, s, MeO) and 1.45 (3 H, d, *J* = 7.0, CHC*H*$_3$); *m/z* (EI), 270 (M^+, 22%) and 123 (100); HPLC purity >95%.



42

1-Cyclohexyl-3-((S)-1′-phenylethyl) urea 42

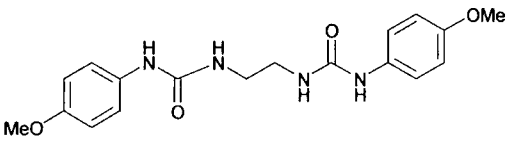
Using procedure B, (*S*)-α-methylbenzylamine (48 mg, 51 μl, 0.4 mmol, 1 eq.) and cyclohexylisocyanate (75 mg, 77 μl, 0.6 mmol, 1.5 eq.) gave the product **42** as a white solid (92 mg, 97%). m.p. 136-138 °C (literature[65] m.p. 178-180 °C); δ_H (250 MHz; $CDCl_3$) 7.37-7.20 (5 H, m, Ph), 4.74 (1 H, q, *J* = 7.0, C*H*CH_3), 4.20 (1 H, br s, NH), 3.50 (1 H, m, cyclohexyl), 1.89-0.82 (10 H, m, cyclohexyl) and 1.44 (3 H, d, *J* = 6.5, CH C*H*$_3$); *m/z* (EI), 246 (M^+, 49%) and 105 (100); HPLC purity >80%



43

Attempted preparation of 1,2-diaminoethyl benzamide 43

Using procedure C, 1,2-diaminoethane (35 mg, 40 µl, 1 eq.) and benzoyl chloride (141 mg, 116 µl, 1.0 mmol, 2.5 eq.) with additional anhydrous MeCN (5 ml), to afford a light brown solid m.p. 177-178 °C (literature[66] m.p. 259-260 °C). The proton NMR was inconclusive as to the structure of the product.



44

***N, N'*-Diaminoethane-*N, N'*-4 methoxy benzyl urea. 44**

Using procedure B, 1,2-diaminoethane (35 mg, 40 µl) and 4-methoxybenzylisocyanate (149 mg, 130 µl, 1.0 mmol, 2.5 equiv.) solubilised in anhydrous MeCN (5 ml), gave the product as a light brown solid m.p. >220 °C. The proton NMR was inconclusive as to the structure of the product.

3.11 General Procedures for the preparation of bromine derivatives using allyl resin 18

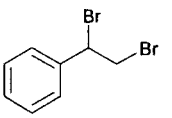
Procedure A

To a mixture of the alkene (1 mmol, 1 eq.) in anhydrous CH_2Cl_2 (2 ml) was added bromine in CH_2Cl_2 (1% v/v) over 10 min with stirring until a yellow colour persisted. An excess of allyl resin **18** (5 ml, 1 mmol) which had been washed with 1,4-dioxane (3 x 5 ml) and CH_2Cl_2 (3 x 10 ml) was added and allowed to mix until the solution decolourised. The resin was filtered and washed with CH_2Cl_2 (3 x 5 ml). The washes were combined with the initial filtrate and concentrated under reduced pressure to afford the product.

Procedure B

The reaction was performed as per reaction A, using THF as the solvent in place of CH_2Cl_2 in all stages. Bromine water (1% v/v) was used in place of bromine in CH_2Cl_2.

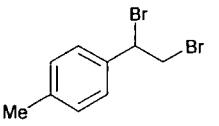
3.12 Results for bromination reactions



55

(1,2-Dibromoethyl)benzene 55

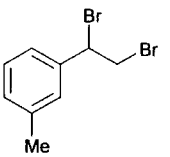
Using procedure A, styrene (104 mg, 50 µl, 1mmol) gave the product **55** (251 mg, 95%) as a white solid m.p. 70-72 °C (literature[67] m.p. 74-75 °C); δ_H (250MHz; $CDCl_3$) 7.40-7.28 (5 H, m, Ph) 5.17 (1 H, dd, J = 10, 5.5 CHBr) and 4.15-3.98 (2 H, m, CH_2Br); *m/z* (EI) 266 (M^+, 2.5%), 264 (M^+, 5.0), 262 (M^+, 2.5), 185 (100) and 183 (95); HPLC purity >95%



56

1-(1, 2-Dibromoethyl)-4-methylbenzene 56

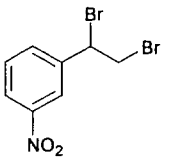
Using procedure A, 4-methyl styrene (118 mg, 131 µl, 1mmol) gave the product **56** (271 mg, 98%) as an off white solid, m.p. 35-37 °C (literature[68] m.p. 44-44.5 °C); δ_H (250 MHz; $CDCl_3$) 7.32 (2 H, d, J = 8.0, 2,6-H Ar), 7.16 (2 H, d, J = 8.0, 3,5-H Ar), 5.07 (1 H, dd, J = 5.0, 10.0, CHBr), 4.03-3.92 (2 H, m, CH_2Br) and 2.30 (3 H, s, CH_3); *m/z* (EI) 276 (M^+, 1.8%), 278 (M^+, 3.6), 280 (M^+, 1.7) and 118 (100); $\nu_{max}cm^{-1}$ 2916, 1610, 1513, 1430, 1378, 1134, 910, 818, 718 and 666; HPLC purity >85%



57

1-(1, 2-Dibromoethyl)-3-methylbenzene 57

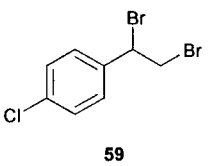
Using procedure A, 3-methyl styrene (118 mg, 132 µl, 1mmol) gave the product **57** (273 mg, 98%) as an off white solid, m.p. 46-47 °C (literature[69] m.p. 45 °C); δ_H (250 MHz; $CDCl_3$), 7.31-7.17 (4 H, m, Ar), 5.15 (1 H, dd, J = 6.0 and 10.0, CHBr), 4.12-4.00 (2 H, m, CH_2Br) and 2.38 (3 H, s, CH_3); *m/z* (EI) 280 (M^+, 1.4%), 278 (M^+, 3.0), 276 (M^+, 1.6) and 118 (100); ν_{max} cm^{-1} 1605, 1522, 1428, 1225, 1157, 1130, 789 and 700; G.C purity >90%; HPLC purity >95%.



58

1-(1, 2-Dibromoethyl)-3-nitrobenzene 58

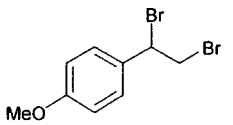
Using procedure A, 3-nitro styrene (149 mg, 139 µl, 1mmol) gave the product **58** (314 mg, 98%) as an off white solid m.p. 76-78 °C; δ_H (250 MHz; $CDCl_3$) 8.27 (1 H, d, J = 2.0, 2-H Ar), 8.21 (1 H, d, J = 8.0, 4-H Ar), 7.72 (1 H, d, J = 1.5, 6-H Ar), 7.58 (1 H, t, J = 8.0, 5-H Ar), 5.20 (1 H, dd, J = 5.0, 11.0, CHBr) and 4.18-3.97 (2 H, m, CH_2Br); *m/z* (EI) 311 (M^+, 0.14%), 309 (M^+, 0.25), 307 (M^+, 0.12) and 103 (100); ν_{max} cm^{-1} 1525, 1348, 1229, 1132, 813, 786, 691 and 671; G.C purity >75%; HPLC purity >95%



59

1-Chloro-4-(1,2-dibromoethyl)-benzene 59

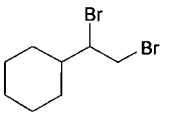
Using procedure A, 4-chlorostyrene (138 mg, 120 µl, 1mmol) gave the product **59** (281 mg, 94%) as a white solid m.p. 42-44 °C (literature[70] m.p. 42.6-43.8 °C); δ_H (250 MHz; $CDCl_3$) 7.38 (4 H, m, Ar), 5.20 (1 H, dd, J = 11.0, 5.5, CHBr) and 4.10-3.97 (2 H, m, CH_2Br); *m/z* (EI) 302 (M^+, 0.5%), 300 (M^+, 2.0), 298 (M^+, 3), 296 (M^+, 1), 221 (23), 219 (100) and 217 (76). HPLC purity >95%



60

1-(1, 2-dibromoethyl)-4-methoxybenzene 60

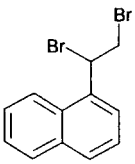
Using procedure A, 4-vinylanisole (134 mg, 132 µl, 1mmol) gave the product **60** (277 mg, 98%) as an off white solid m.p. 74-76 °C (literature[71] m.p. 76-80 °C); δ_H (250 MHz; $CDCl_3$) 7.32 (2 H, dd, J = 6.5 and 4.5, 2, 6-H Ar), 6.89 (2 H, dd, J = 6.5 and 4.5, 3, 5-H Ar), 5.18 (1 H, dd, J = 10.5 and 5.5, CHBr), 4.11 (2H, m, CH_2Br) and 3.80 (3 H, s, CH_3O); $\nu_{max}cm^{-1}$ 1606, 1511, 1455, 1250, 1025 832 and 732; G.C purity >65%; HPLC purity >95%



61

1, 2-Dibromoethyl-cyclohexane 61

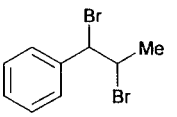
Using procedure A, vinyl cyclohexane (110 mg, 136 µl, 1mmol) gave the product **61** (243 mg, 90%) as a light brown oil. δ_H (250 MHz; $CDCl_3$) 4.18-4.07 (1 H, m, CHBr), 3.82-3.63 (2 H, m, CH_2Br), 1.90-1.51 (6 H, m, cyclohexyl) and 1.38-1.01 (5 H, m, cyclohexyl); *m/z* (EI) 272 (M^+, 0.05%), 270 (M^+, 0.1), 268 (M^+, 0.05) and 109 (100); G.C purity >95%. HPLC purity >95%



62

1-(1, 2-Dibromoethyl)-naphthalene 62

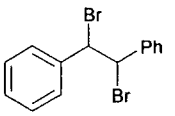
Using procedure A, 2-vinyl naphthalene (154 mg, 1mmol) gave the product **62** (288 mg, 92%) as an off white solid m.p. 75-76 °C; δ_H (250 MHz; $CDCl_3$) 7.95-7.82 (4 H, m, Ar), 7.58-7.47 (3 H, m, Ar), 5.37 (1 H, dd, J = 9.0 and 7.0, CHBr) and 4.18 (2 H, d, J = 7.0, CH_2Br); *m/z* (EI) 316 (M^+, 0.55%), 314 (M^+, 1.1), 312 (M^+, 0.55) and 154 (100); ν_{max} cm^{-1} 1610, 1489, 1120, 822, 745 and 692; HPLC purity >90%.



63

1,2-Dibromopropyl benzene 63

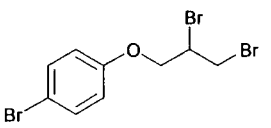
Using procedure A, *trans* β-methyl styrene (118 mg, 130 µl, 1mmol) gave the product **63** as a pale yellow solid (222 mg, 80%) m.p. 59-61 °C (literature[72] m.p. 65 °C); δ_H (250 MHz; $CDCl_3$) 7.46-7.31 (5 H, m, Ph), 5.07 (1 H, d, J = 10.0, CHBr), 4.67-4.58 (1 H, m, CHBr) and 2.03 (3 H, d, J = 6.5, CH_3); *m/z* (EI) 280 (M^+, 1%), 278 (2), 276 (1), 199 (63), 197 (64) and 117 (100); ν_{max} cm^{-1} 1454, 1378, 1147, 999, 765 and 691; G.C purity >70%; HPLC purity >95%.



64

***trans*-1,2-Dibromo-(1, 2-diphenyl)-ethane 64**

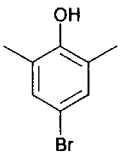
Using procedure A, *trans* stilbene (180 mg, 1mmol) gave the product **64** as a pale yellow solid (338 mg, 94%) m.p. 158-162 °C (literature[73] m.p. 239-240.3 °C); δ_H (250 MHz; $CDCl_3$) 7.54-7.38 (6 H, m, Ph), 7.19 (4 H, s, Ph) and 5.48 (2 H, s, 2 x CH); *m/z* (EI) 342 (M^+, 1%), 340 (2), 338 (1) and 258 (100); ν_{max} cm^{-1} 1467, 1360, 1164, 981, 752 and 679; G.C purity >80%; HPLC purity >90%.



67

1-bromo-4-(2, 3-dibromopropoxy benzene) 67

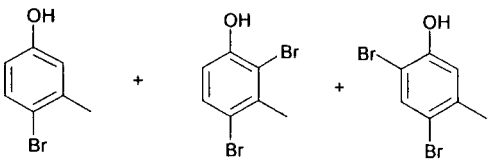
Using procedure A, allyl phenyl ether (134 mg, 137 µl, 1mmol) gave the product **67** (355 mg, 95%) as a pale brown oil; δ_H (250 MHz; $CDCl_3$) 7.40 (2 H, d, J = 9.0, 2,6-H Ar), 6.81 (2 H, d, J = 9.0, 3, 5-H Ar), 4.32-4.10 (3 H, m, CH_2Br, OCH_2), and 3.90 (2 H, d, J=7.0, CHBr); *m/z* (EI) 296 (M^+, 16.0%), 294 (31.8), 292 (18.0) and 174 (100); $\nu_{max}cm^{-1}$ 1589, 1579, 1488, 1455, 1242 and 821; G.C purity >90%; HPLC purity >90%.



73

4-bromo-2,6-dimethylphenol 73

Using procedure A, 2,6-dimethylphenol (122 mg, 1mmol) gave the product **73** (193 mg, 97%) as a pale brown solid m.p. 76–77 °C (literature[74] m.p. 79-80 °C); δ_H (250 MHz; $CDCl_3$) 7.05 (2 H, s, Ar), 4.58 (1 H, br s, OH) and 2.10 (6 H, s, CH_3); *m/z* (EI) 202 (M^+, 70%), 201 (10), 200 (75) and 121 (100); HPLC purity ~87%.



74 75 76

The bromination of 3-methylphenol 76

Using procedure A, 3-methylphenol (108mg, 104μl) gave a mixture (138mg 52%) of 4-bromo-3-methylphenol **74**, 2,4-dibromo-3-methylphenol **75** and 4,6-dibromo-3-methylphenol **76** as a pale yellow oil (2 : 31 : 67); HPLC purity >90%.

4-bromo-3-methylphenol 74

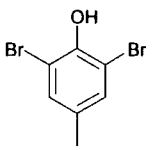
δ_H (250 MHz; $CDCl_3$) 7.20 (1 H, s, Ar), 6.70 (1 H, d, J = 4.0, Ar), 6.55 (1 H, d, J = 4.0, Ar) 5.80 (1 H, br s, OH) and 2.36 (3 H, s, CH_3); *m/z* (EI) 188 (M^+, 28%), 186 (30), 107 (68), 106 (11), 105 (10), 79 (30), 78 (42) and 77 (100).

2,4-dibromo-3-methylphenol 75

δ_H (250 MHz; $CDCl_3$) 7.40 (1 H, d, J = 5.0, Ar), 6.78 (1 H, d, J = 5.0, Ar), 5.63 (1 H, br s, OH) and 2.56 (3 H, s, CH_3); *m/z* (EI) 268 (M^+, 48%), 267 (7), 266 (100), 265 (4), 264 (52), 187 (44), 186 (10) and 185 (47).

4,6-dibromo-3-methylphenol 76

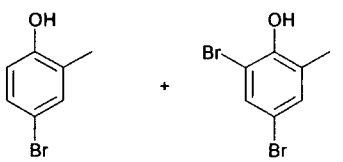
δ_H (250 MHz; $CDCl_3$) 7.60 (1 H, s, Ar), 6.90 (1 H, s, Ar), 5.44 (1 H, br s, OH) and 2.30 (3 H, s, CH_3); *m/z* (EI) 268 (M^+, 54%), 267 (7), 266 (90), 265 (25), 264 (44), 187 (54), 186 (13), 185 (61) and 77 (100).



77

2-bromo-4-methylphenol 77

Using procedure A, 4-methylphenol (108 mg, 104 µl) gave the product 77 (145 mg, 78%) as a colourless oil; δ_H (250 MHz; $CDCl_3$) 7.12 (1 H, s, Ar), 6.92 (1 H, d, J = 5.0, Ar), 6.80 (1 H, d, J = 5.0, Ar), 5.21 (1 H, br s, OH) and 2.20 (3 H, s, CH_3); *m/z* (EI) 188 (M^+, 31%), 187 (16), 186 (34), 185 (14) and 107 (100); HPLC purity >78%.



78 79

The bromination of 2-methylphenol 78 and 79

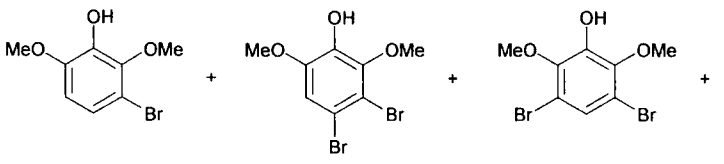
Using procedure A, 2-methylphenol (108 mg, 104 µl, 1mmol) gave a mixture (172 mg 92%) of 4-bromo-2-methylphenol **78** and 4,6-dibromo-2-methylphenol **79** as a colourless oil. (72 : 28); HPLC purity ~94%.

4-bromo-2-methylphenol 78

δ_H (250 MHz; $CDCl_3$) 7.17 (1 H, d, J = 5.0, Ar), 7.12 (1 H, s, Ar), 6.62 (1 H, d, J = 5.0, Ar), 5.58 (1 H, br s, OH) and 2.20 (3 H, s, CH_3); *m/z* (EI) 188 (M^+, 62%), 186 (66) and 107 (100).

4,6-dibromo-2-methylphenol 79

δ_H (250 MHz; $CDCl_3$) 7.40 (1 H, s, Ar), 7.22 (1 H, s, Ar), 5.7 (1 H, br s, OH) and 2.25 (3 H, s, CH_3); *m/z* (EI) 268 (14%), 167 (5 %), 266 (26 %), 265 (9 %), 264 (14 %), 187 (34 %), 185 (37 %), 78 (81 %) and 77 (100 %).



80 **81** **82**

The Bromination of 2,6-dimethoxyphenol

Using procedure A, 2,6-dimethoxyphenol (154 mg, 1mmol) to give a mixture (284 mg, 98%) of 3-bromo-2,6-dimethoxyphenol **80**, 3,4-dibromo-2,6-dimethoxyphenol **81** 3,5-dibromo-2,6-dimethoxyphenol **82** as a pale yellow oil, (40:40:20).

3-bromo-2,6-dimethoxyphenol 80

δ_H (250 MHz; $CDCl_3$) 7.02 (1 H, d, J = 5.5, Ar), 6.58 (1 H, d, J = 5.5, Ar), 5.80 (1 H, br s, OH) and 3.80 (6 H, s, OCH_3); *m/z* (EI) 234 (M^+, 98%), 233 (20), 232 (100), 231 (20), 219 (37), 218 (9), 217 (40), 191 (11) and 189 (13).

3,4-dibromo-2,6-dimethoxyphenol 81

δ_H (250 MHz; $CDCl_3$) 6.90 (1 H, s, Ar), 5.64 (1 H, br s, OH) and 3.86 (6 H, s, OCH_3); *m/z* (EI) 314 (M^+, 34%), 312 (67), 310 (35), 299 (8), 297 (17), 295 (14), 254 (25), 253 (16), 252 (16), 251 (23), 190 (30), 189 (11), 188 (35), 187 (16) and 77 (100).

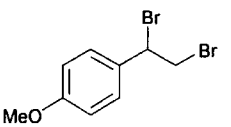
3,5-dibromo-2,6-dimethoxyphenol 82

δ_H (250 MHz; $CDCl_3$) 7.28 (1 H, s, Ar), 5.60 (1 H, br s, OH) and 3.88 (6 H, s, OCH_3); *m/z* (EI) 314 (M^+, 49%), 312 (100), 310 (52), 299 (21), 297 (42), 295 (22), 254 (25), 253 (15), 252 (15), 251 (20), 190 (10), 189 (10), 188 (18) 187 (12).

3.13 Bi-phasic reaction

To a solution of 4-vinylanisole (134 mg, 132 µl, 1mmol) in CH_2Cl_2 (10 ml) was added an equal volume of bromine water such that where was an excess of available bromine water (10 ml, 1.5mmol, 1.5 eq). The reaction was left in the dark for 2 h, after which time all the bromine had transferred to the organic layer. Aqueous allyl resin 18 (10 ml, 2mmol, 4 eq) was added and left for 1 h with occasional shaking over which time the excess bromine was removed. The suspension was filtered, the organic layer separated from the aqueous and placed under reduced pressure to remove the solvent

3.14 Result of bi-phasic reaction



95

1-(1,2-dibromoethy-1-yl)-4-methoxybenzene

Using procedure 3.13 4-vinylanisole (134 mg, 132 µl) gave the product **95** (257 mg, 85%) as an off white solid m.p 72-75°C(literature[75] m.p. 76-80 °C); δ_H (250 MHz; $CDCl_3$), 7.37 (2 H, dd, J = 6.5 and 4.5, 2, 6-H Ar), 6.91 (2 H, dd, J = 6.5 and 4.5, 3, 5-H Ar), 5.16 (1 H, dd, J = 10.5 and 5.5, CHBr), 4.08 (2H, m, CH_2Br) and 3.82 (3 H, s, CH_3O *m/z* (EI) 290(M^+, 46.12%), 292 (M^+, 90.52), 294(M^+, 90.52) and 89 (100).

3.15 Biodegradation studies on Perloza MT-100

Samples of Perloza MT-100 (1 ml aqueous) were settled in graduated columns. The materials were each washed successively with water (2 x 5 ml) and aqueous sodium acetate (50mM, pH 5.0, 3 x 5 ml). The resins were then transferred into screw-top vials, and sodium acetate buffer (10 ml) was added to each. Cellulase (100 mg) and/or brewers yeast (100 mg) were then added as detailed in Table 11 (page 36). The vials were sealed, and the mixtures were incubated at 35-55 °C (Table 11) for 48 h. On completion of the reaction, the pH of the solution was recorded, and the mixture poured into a graduated column, from which the volume of gel was measured. The resin was washed with acetate buffer (10 ml) and the elutions pooled for glucose analysis. The resin was then washed with water (3 x 5 ml), 1,4-dioxane (3 x 5 ml) and diethyl ether (3 x 5 ml) and dried in an oven at 100 °C for 2 h, at which point the dry mass was recorded.

3.16 Analysis of cellulase reactions by glucose assay.

To a sample of glucose hexokinase (HK) assay kit reagent (1 ml) in a sample tube, was added a sample (10 µl) of the filtrate from the cellulase reaction. The tube was sealed, and mixed at 25 °C for 15 minutes. After this time, the optical density was recorded at 340nm, with water used as the blank sample. Two blank samples were also prepared, the first containing water (10 µl) and assay reagent (1 ml) and the second made up from water (1 ml) and glucose standard solution (10 µl) obtained from the assay kit. The optical densities of these solutions were recorded, and combined to give the total blank. The amount of glucose present in each sample was determined by use of the equation on page 35.

3.17 Bromohydrin collapse.

Styrene (115 μl, 1 mmol) was dissolved in acetone (5 ml) and then treated with bromine water (10 ml, 1.5 eq) until a permanent yellow/orange colour persisted after 1 h. Allyl resin was added and the suspension shaken until the colour disappeared. The resin was filtered, washed with water (2 x 5 ml) and the filtrates combined. To this solution diethylaminoethyl resin (20 ml, 4 mmol, 4 eq) was added and left stirring for 4 h. The resin was filtered, washed with water (2 x 20 ml) and the filtrates combined. The solution was then extracted with dichloromethane (3 x 70 ml), the extracts combined and dried with magnesium sulphate. The combined filtrates were then evaporated under reduced pressure.

4.0 References

1 For general reviews of combinatorial chemistry see ; a) L. A. Thompson and J. A. Ellman, *Chem. Rev.*, 1996, **96**, 555. b) E. M. Gordon, M. A. Gallop and D. V. Patel, *Acc. Chem. Res.*, 1996, **29**, 144. c) S. H. DeWitt and A. W. Czarnik, *Acc. Chem Res.*, 1996, **29**, 114.

2 R. B. Merrifield and J. A. Ellman., *J. Am. Chem. Soc.*, 1992, 114, 10997.

3 H. M. Geyson., R. H. and S. J. Barteling, *Proc. Natl. Sci. U.S.A.* 1984, **81**, 3998.

4 B. A. Bunin and J. A. Ellman, *J. Am. Chem. Soc.*, 1992, **114**, 10997

5 M. J. Plunkett and J. A. Ellman, *J. Org. Chem* 1995, **60**, 6006.

6 I. Hughes, *Tetrahedron. Lett.*, 1996, **37**, 7595

7 For a general overview of solution phase methods see; A. T. Merritt., *Combinatorial Chemistry and High Throughput Screening*, 1998, **1**, 57.

8 For an overview of purification methods in high throughput synthesis see; R. Ferrito and P. Seneci, *Drugs. Fut.*, 1998, **23**, 643.

9 For a recent review of supported reagents see; A. Kirsschning, H. Monenschein and R. Wittenberg, *Angew. Chem. Int. Ed.*, 2001, **40**, 650.

10 A. R. Tunouri, D. Dutta and G. I. Georg, *Tetrahedron Lett.*, 1998, **39**, 8751.

11 (a)E. J. Enholm and J. P. Schulte, *Org.Letts.*, 1999, **1**, 1275. (b) A. Kirschning, *J. Prakt. Chem.* 2000, **342**, 508. (c) P. Boussaguet, B. Delmond, G. Dumartin and M. Pereyre, *Tetrahedon Lett.*, 2000, **41**, 3377.

12 (a) C. Yaroslavsky and E. Katchalski, *Tetrahedron Lett.*, 1972, 5173.(b) K. M. Brummand and K. D. Gesenberg, *Tetrahedron Lett.*, 1999, **40**, 2231.

13 B.O. Buckman, M. M. Morrissey and R. Mohan, *Tetrahedron Lett.*, 1998, **39**, 1487.

14 (a) D. J. Bayston, J. L. Fraser, M. R. Ashton, A. D. Baxter, M. E. C. Polywka and E. Moses, *J. Org. Chem.*, 1998, **63**, 3137; (b) R. T Halle, B. Colasson, E. Schulz, M. Spagnol and M. Lemaire, *Tetrahedron Lett.*, 2000, **41**, 643; (c) S. B. Garber, J. S. Kingsbury, B. L. Gray and A. H. Hoveyda, *J. Am. Chem. Soc.*, 2000, **122**, 8168.

15 Y-L. Zhong and T. K. M. Shing, *J. Org. Chem.*, 1997 **62**, 2622.

16 I. R. Baxendale and S. V. Ley, *Bioorg. Med. Chem. Lett.*, 2000, **10** (17), 1983.

17 R.L. Scott, *J. Am. Chem. Soc.*, 1948, **70**, 4090.

18 I.T. Horváth and J. S. Rabái, *Science*, 1994, **266**, 72.

19 D. P. Curran, S. Hadida, S. Y. Kim and Z. Luo, *J. Am. Chem. Soc.*, 1999, **121**, 6607.

20 J. J. J. Juliette, D. Rutherford, I. T. Horvath and J. A. Gladysz, *J. Am. Chem. Soc.*, 1999, **121**, 2696.

21 B. Linclau, A. K. Sing and D. P. Curran, *J. Org. Chem.*, 1999, **64**, 2835.

22 D. L. Flynn, J. Z. Crich, R. V. Devraj, S. L. Hockerman, J. J. Parlow, M. S. South and S. S. Woodward, *J. Am. Chem. Soc.*, 1997, **119**, 4874.

23 R. J. Booth and J. C. Hodges., *J. Am. Chem. Soc.*, 1997, **119**, 4882.

24 (a) S. W. Kaldor, M. G. Siegel, J. E. Fritz, B. A. Dressman and P. J. Hahn, *Tetrahedron. Lett.*, 1996, **37**, 7193. (b) S. W. Kaldor, J. E. Fritz, J. Tang and E. R. McKenney, *Bioorg. Med. Chem. Lett.*, 1996, **6**, 3041. (C) M. W. Creswell, G. L. Bolton, J.C. Hodges and M. Meppea, *Tetrahedron*, 1998, **54**, 3983.

25 S. E. Ault-Justus, J. C. Hodges and M. W. Wilson, *Biotechnol. Bioeng.*, 1998, **61**, 17

26 (a) J. K. Rueter, S. O. Nortey, E. W. Baxter, G. C. Leo and A. B. Reitz, *Tetrahedron. Lett.*, 1998, **39**, 975. (b) E. W. Baxter, J. K. Rueter, S. O. Nortey and A. B. Reitz, *Tetrahedron Lett.*, 1998, **39**, 979.(c) T. Takahashi, S. Ebata and T. Doi., *Tetrahedron Lett.*, 1998, **39**, 1369.

27 a) L. M. Gayo-Fung and M. J. Suto, *Tetrahedron Lett.*, 1997, **38**, 513. b) M. G. Siegel, P. J. Hahn, B. A. Dressman, J. E. Fritz, J. R. Grunwell and S. W. Kaldor, *Tetrahedron Lett.*, 1997, **38**, 3357. c) M. J. Suto, L. M. Gayo-fung, M. S. S. Palanki and R. Sullivan, *Tetrahedron.*, 1998, **54**, 4141

28 a) B. V. Yang, D. O'Roarke and J. Li, *Synlett.*, 1993, 195. b) P. Conti, D. Dermot, J. Cals, H. C. J. Ottenheim and D. Leysen, *Tetrahedron Lett.*, 1997, **38**, 2915

29 a) D. L. Flynn, R. V. Devraj and J. J. Parlow, *Curr. Opin. Drug. Disc. Develop.*, 1998, **1**, 41. b) J. J. Parlow, R. V. Devraj and M. S. South., *Curr. Opin. Chem. Biol.*, 1999, **3**, 320.

30 For examples of supported reagents in organic synthesis see; A. Chesney *Green Chemistry*, 1999, **1**, 202.

31 For books detailing the chemical modification of cellulose materials see; a) *Cellulose and its' Derivatives: Chemical and Biochemical Applications*, ed. J. F. Kennedy, Ellis Horwood (Chichester), 1989. b) *Cellulose; Structural and Functional Aspects*, eds. J. F. Kennedy, G. O. Phillips and P. A. Williams, Ellis Horwood (Chichester), 1989. c) D. Klemm, B. Philipp, T. Heinze, U. Heinze and W. Wagenknecht, *Comprehensive Cellulose Chemistry*, vol **2**, Wiley-VCH (Chichester), 1998.

32 P. Gemeiner, M. J. Benes, and J. Stamberg, *Chem. Papers*, 1989, **43**, 805.

33 For a detailed review of cellulose ether formation, see; A. B. Savage, A. E. Young

and A. T. Maasberg in *Cellulose and Cellulose Derivatives*, ed. E. Ott, Interscience (London), 1954.

34 J. K. Chowdray, *Biochem Z*, 1924, **148**, 76.

35 E. Jansen German Patent 332,203 (1921).

36 M. Gomberg and C. C. Butler, *J. Am. Chem. Soc.*, 1920, **42**, 2060.

37 R. Frank, *Tetrahedron*, 1992, **42**, 9217.

38 R. Frank and R. Döring, *Tetrahedon*, 1988, **44**, 6031.

39 a) F. Dittrich, W. Tegge and R. Frank, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 2351, b) M. Stanková, S. Wade, K. S. Lam and M. Lebl, *Peptide Res.* 1994, **7**, 292.

40 a) D. R. Englebretsen and D. R. K. Harding, *Peptide, Res.*, 1993, **6**, 320. b) D. R. Englebretsen and D. R. K. Harding, *Int. J. Peptide Res.*, 1994, **43**, 546. c) D. R. Englebretsen and D. R. K. Harding, *Peptide, Res.*, 1994, **7**, 136. d) D. R. Englebretsen and D. R. K. Harding, *Peptide, Res.*, 1993, **7**, 322.

41 D. R. Englebretsen, M. J. Scanlon and M. L. West, *Biomed. Peptides, Proteins & Nucleic Acids*, 1996, **2**, 47.

42 a) S. C. Burton, N. W. Haggarty and D. R. K. Harding, *Biotechnol. Bioeng.*, 1997, **56**, 45. b) S. C. Burton and D. R. K. Harding, *J. Chromatogr. A.*, 1998, **778**, 30.

43 S. C. Burton and D. R. K. Harding, *J. Chromatogr. A.*, 1997, **775**, 29.

44 S. C. Burton and D. R. K. Harding, *J. Chromatogr. A.*, 1997, **775**, 273.

45 T-P. Loh and L-L. Wei, *Tetrahedron Lett.*, 1998, **39**, 323.

46 M. W. Creswell, G. L. Bolton, J. C. Hodges and M. Meppen, *Tetrahedron*, 1998, **54**, 3983.

47 B. Raju, J. M. Kassir and T. P. Kogan, *Bioorg. Med. Chem. Lett.*, 1998, **8**, 3043.

48 (a) T. Arnauld, A. G. M. Barrett, S. M. Cramp, R. S. Roberts and F. J. Zécri, *Org. Lett.*, 2000, **2**, 2663. (b) G. M. Coppola, *Tetrahedron Lett.*, 1998, **39**, 8233.

49 C. Sylvain, A. Wagner and C. Mioskowski, *Tetrahedron Lett.*, 1997, **38**, 1043.

50 P. S. Bates, D. Parker and A. F. Patti, *J. Chem. Soc. Perkin Trans. 2*, 1994, 657.

51 H. B. Hopps, *Aldrichimica Acta,* 2000, **33**, 28.

52 a) R. G. H. Siu, *The Microbial Decomposition of Cellulose*, Reinhold (NY), 1951. b) *The Biosynthesis and Biodegradation of Cellulose*, eds. C. H. Haigler and P. J. Weimer, Dekker (NY), 1991.

53 Patents granted to B. Hartley and B. S. Hartley, Elsworth Biotechnology Ltd; WO 8809379 A (1988), EP 370023 B (1990) and US 5182199 A (1993).

54 V. Richert and A. Zimmer, *Genetic. Eng. News.*, 1998, Oct1, 21.

55 For a discussion on recent advances in the degradation of non-soluble cellulose materials by enzymes, see; B. Saake, S. Horner and J. Puls, in *Cellulose Derivatives; Modification, Characterisation and Nanostructures*, eds T. H. Heinze and W. G. Glasser, ACS Symposium Series 688, American Chemical Society (Washington), 1998, Chp 15.

56 For the application of the hexokinase assay in glucose determination, see; a) R. J. L. Bondar and D. C. Mead, *Clin. Chem.*, 1974, **20**, 586. b) A. Kunsst, B. Draeger and J. Ziegenhorn, in *Methods of Enzymatic Analysis*, ed. H. U. Bergmeyer, Academic Press (NY), 1984, vol **2**, p. 163.

57 D. Dube and A. A. Scholte, *Tetrahedron Lett.*,1999, **40** (12), 2295.

58 Shephard, *J. Chem. Soc.*, 1971, **928.**

59 M. Tanno, s. Sveyoshi and S. Kamiya, *Chem. Pharm. Bull.*, 1990, **38** (1), 49.

60 T. Patonay, E. Patonay-Peli, L. Zolnai and F. Mogyorodi, *Synth. Commum.*, 1996, **26**(22) 4253.

61 W. R. Vaughan and R. D. Carlson, *J. Am. Chem. Soc.*, 1962, **84**, 769.

62 T. Yokomatsu, A. Arakawa and S. Shibuya, *J. Org. Chem.*, 1994, **52**(12), 3506.

63 Mndshojan, *Dokl. Akad. Nauk Arm. SSR.*, 1953, **17**, 119.

64 J. H. Ryoo, H. Kuramochi and H. Omokawa, *Biosci. Biotechnol. Biochem.*, 1998, **62**(11), 2189.

65 J. Barlvenga, C. Jimenez, C. Najera and M. Yus, *J. Chem. Soc. Perkin. Trans. 1.*, 1983, 3, 591.

66 D. R Marshall, P. Reynolds-Warnhoff, E. W. Warnhoff and J. R Robinson, *Can. J. Chem.*, 1971, **49** (6), 885.

67 D. H. R. Barton, M. Mhichael, D. Philip, K. G. Marathe, G. A. Poulton and P. J. West, *J. Chem. Soc., Perkin. Trans. 1.*, 1973, **15**, 1574

68 C Schwartzman, *J. Am. Chem. Soc.*, 1956, **78**, 322.

69 C Schwartzman, *J. Am. Chem. Soc.*, 1956, **78**, 322.

70 I. Wender, H. Greenfield, S. Metlin and M. Orehin *J. Am. Chem. Soc.*, 1952, **74**, 4079.

71 J. Weinstock, D. L. Ladd, J. W. Wilson, C. K. Brush, N. C. F. Nelson, G. Gallagher Jr, M. E. McCarthy, J. Silvestri, H. M. Sarau and K. E. Flaim, *J. Med. Chem.*, 1986, **29**(11), 2315.

72 T. Adachi and K. Otsuki, *Chem. Pharm. Bull.*, 1976, **24**, 2803.

73 I. J. Borowitz, D. Weiss and R. K. Crouch, *J. Org. Chem.*, 1971, **36** (16), 2377.

74 J. H. Espenson, Z. Zhu and T. H. Zauche, *J. Org. Chem.*, 1999, **64** (4), 1191.

75 J. Weinstock, D. L. Ladd, J. W. Wilson, C. K. Brush, N. C. F. Nelson, *J. Med. Chem.*, 1986, **29**(11), 2315.