Developing combinatorial libraries provides access to high-quality diverse chemical compound collections that give scientists powerful tools to discover lead molecular structures with a wide range of physiological properties. The birth of combinatorial chemistry can be traced to the first sequential peptide synthesis on solid phase in the 1960s. The merits of using solid phase synthesis include the ability to use excess reagents to push reactions to completion and the easy purification of the resin bound product through filtration and washing. While solid phase peptide synthesis is highly developed and robust, the same cannot be said for every reaction on solid phase. The use of solid phase synthesis for non-peptide library production has a number of drawbacks associated with it. The additional steps required for attachment and removal of the linker as well as the inability to easily monitor reaction progress can make the process cumbersome. But most significantly the adaptation of standard solution reactions to solid phase methods entails additional and frequently difficult problems such as linker compatibility, points of substrate attachment and achieving efficient and complete reaction conversions. Multi-step synthesis of non-peptide libraries can be extremely difficult as the chemistry being used is often not as highly optimised as peptide synthesis and results in a poor quality of the final library. This has lead to the resurgence of solution phase chemistry as the preferred method of non-peptide library generation in industry but often exploiting variants of the solid phase principles. The advantages of using solution phase synthesis include the vast literature of reactions to draw from and the absence of extra steps associated with solid phase synthesis.

One major drawback of solution phase organic synthesis lies in the purification and isolation of the individual products particularly in library generation when multi-step synthesis is required. A number of approaches involving the use of solid supported reagents and scavenger resins have emerged to aid the purification bottleneck. These techniques mirror the advantages of solid phase synthesis, as the excess reagents used or reaction byproducts can be separated from the product by simple filtration. Solid supported reagents and scavenger resins can provide an easy means of work-up for a solution phase reaction thereby eliminating the need for further purification techniques. A key advantage of using solid phase synthesis is that an excess of reagents can be used to drive reactions to completion. If this approach is adopted for solution phase, the problem of how to remove the excess reagent must be solved.
For example, if a substrate B is reacted with an excess of A, then the product AB will be contaminated with the remaining excess of A. Removal of this excess reagent upon reaction completion can be achieved by employing a suitable scavenger resin. The scavenger resin is added and reacts with the excess A, resulting in A being covalently bonded to the resin. Filtration separates the solution of product AB from scavenger resin sequestered A (Scheme 1).

Probably the most important advantage in using a functionalised polymer as a scavenger to remove excess reagents or byproducts is the simplification of product work-up, separation and isolation. The need for complex separation, extraction and chromatographic techniques is replaced by simple filtration. With linear polymers, techniques such as precipitation, sedimentation and ultrafiltration can be used. In addition, resins provide the opportunity for automation and thus high-throughput synthesis.

The most important and obvious disadvantage is the additional cost in using a supported scavenger. This might be well offset by the potential advantages, particularly if the scavenger can be regenerated. In addition there is always a possibility that the desired product itself may undergo a side reaction with the scavenger. A report in the early 1980s lists dozens of polymeric reagents that have found applications as catalysts, protecting groups, substrate carriers, analysis (sensors), ion exchange, detection of reaction intermediates, chromatography, enzyme immobilisation and others. Polymeric phosphine reagents, sulfonium salts, halogenating agents, redox, acylating and alkylating reagents, and polymer bound nucleophiles can all be used, in principle, as scavengers as well as for organic synthesis. In two parts, this article will focus on scavenger strategies in combinatorial chemistry and organic synthesis over the past 25 years, including:

Part 1
- Polymeric scavengers
- Reactive filtration

Part 2
- Ion Exchange scavengers for product purification
- Sequestration enabling techniques
- Alternative resins
- Polyaromatic scavenger reagents
- Fluorous quenching scavenger protocols
- Microwave-assisted scavenging protocols

**Polymeric scavengers**

In 1997, the concept of complementary molecular reactivity and molecular recognition was introduced as a method of purification for parallel solution phase combinatorial library generation. This technique is now more commonly known as scavenging and was proposed to remove excess reagents or byproducts in combinatorial library generation. Polymeric scavengers are typically electrophilic or nucleophilic in nature and are designed and used to sequester specific reagents. They are also known as ‘quenching agents’ or ‘sequestering agents’.

**Scheme 1:** Scavenger resins to assist in the removal of excess reagents

**Scheme 2:** An early example of polymer scavenging of the allergen isoalantolactone

**Scheme 3:** Multi-step synthesis using polymeric scavengers
In 1980, Frechet et al exploited the use of an insoluble polymer bound nucleophilic primary amine to selectively bind and remove α-methylene-γ butyrolactone allergens from complex mixtures. Contact dermatitis results from an allergic reaction with sesquiterpene lactones, but these potentially harmful compounds can be effectively removed by scavenging. A dilute solution of the allergen eg isoalantolactone and scavenger polymer was stirred at room temperature for 24 hours to effectively remove typically >95% of the allergen (Scheme 2).

Solid supported nucleophiles and electrophiles have been used to simplify the work-up and purification of parallel non-peptide small molecule libraries. The authors prepared ureas, thioureas, amides, sulfonamides and carbamates utilising a nucleophilic scavenging agent to remove the excess isocyanate, acyl chloride or sulfonyl chlorides following simple filtration (Table 1, entry 1).

This work also demonstrated the use of an electrophilic polymer supported isocyanate to sequester an excess of secondary amine following alkylation with alkyl halides and epoxides (Table 1, entries 2,3).

In the same report, multi-step sequences were carried out providing urea products in high purity and yield following the sequential use of polymer supported scavenger reagents (Scheme 3).

**Reactive filtration**

Most commercial scavenging resins are available in bead form and are easily prepared, widely used and have good mechanical stability. However, drawbacks include size variations in the resin and slow
diffusion through the pores containing the reactive sites as well as handling challenges such as electrostatic charge that make automation difficult. Tripp et al developed a reactive filtration technique involving grafted macroporous polymer monolithic disks as an alternative to beads that can be used in the purification of combinatorial libraries. Polyehtylene encased porous poly(chloromethylstyrene-co-divinylbenzene) disks were prepared by polymerisation in a cylindrical glass mold and cut to a disk format (Figure 1).

Following attachment of a free radical azo initiator, the polymerisation of 2-vinyl-4,4-dimethylazlactone was initiated from the surface (Scheme 4).

The use of these disks as scavenging filters to remove various amines from solutions in flow-through operations was demonstrated by effective removal of amines in a very short period of time from their solutions in a variety of solvents including alcohols and water (Scheme 5, Table 2).

There are two important requirements for monoliths in flow through systems (i) high permeability to allow high flow through rates and (ii) a high loading capacity. However, high permeability is usually obtained with large pore size which results in diminished loading capacity. This was overcome by grafting a functional polymer on to the surface of the monoliths with relatively large pore size which allowed for good permeability without compromising loading. Reactive filtration flow-through procedures remove the excess of undesired reagents from solutions in a short amount of time and complement solution phase combinatorial library generation.

An alternative to grafting is to polymerise the continuous phase of an internal phase emulsion. This technique produces a material called PolyHIPE which has a more open permeable structure and a high surface area (Figure 2). Monolithic polymer supports and scavengers were prepared via nucleophilic displacement of chloride in poly(4-vinylbenzyl chloride-co-divinylbenzene) PolyHIPE materials. Reactions of monolithic PolyHIPE with tris(2-aminoethyl)amine, 4-aminobutanol, tris(hydroxymethyl)aminomethane, morpholine and hexamethylenetetramine led to functionalised polymers with amino and hydroxyl functionalities with high degrees of conversion. 4-Chlorobenzoyl chloride was efficiently and rapidly scavenged from solution by the tris(2-aminoethyl)amine derivative of monolithic poly(4-vinylbenzyl chloride-co-divinylbenzene) PolyHIPE at ambient temperature.

Scavenging experiments used resin with 5.3mmol of NH/NH₂ groups per gram to sequester 4-chlorobenzyl chloride from solution; a

![Scheme 4: Preparation of macroporous disks](image)

![Scheme 5: Grafted monolithic disks as scavengers for amines](image)

<table>
<thead>
<tr>
<th>AMINE</th>
<th>SOLVENT</th>
<th>AMOUNT SCAVENGED %</th>
</tr>
</thead>
<tbody>
<tr>
<td>benzylamine</td>
<td>tetrahydrofuran</td>
<td>74.7</td>
</tr>
<tr>
<td>phenethylamine</td>
<td>tetrahydrofuran</td>
<td>76.9</td>
</tr>
<tr>
<td>butylamine</td>
<td>dichloromethane</td>
<td>78.0</td>
</tr>
<tr>
<td>diethylamine</td>
<td>dichloromethane</td>
<td>90.1</td>
</tr>
<tr>
<td>3,5-dimethylaniline</td>
<td>tetrahydrofuran</td>
<td>47.6</td>
</tr>
</tbody>
</table>

*Conditions. Reaction mixture: 0.2mmol of t-butyliocyanate, 0.3mmol of amine, 1.5mL of solvent; porous disc: 5x3mm diameter; grafted with 20% 2-vinyl-4,4-dimethylazlactone and 2% DVB; flow rate 3mL/h, residence time 8min.*
Combinatorial Chemistry

3.35-fold molar excess of amino groups was used (Scheme 6).

After one-hour reaction time, the acid chloride was no longer detectable in the mixture. A similar experiment was performed under flow through conditions. A solution of 4-chlorobenzyl chloride in dichloromethane was passed through a column containing a mon(2-aminoethyl)amine derivative of vinyl benzyl chloride/divinylbenzene (VBC/DVB) PolyHIPE. At a flow rate of approximately 20mL/h and ambient temperature, 86.8% of the acid chloride was scavenged after the first pass-through of the solution, increasing to 98.5% after the second-pass through of the same solution. For comparison, the scavenging ability of a commercial trisamine resin (Argonaut PS-Trisamine, loading 3.1mmol/g NH/NH2 by elemental analysis) was found to be slower: 82% of acid chloride was scavenged after two minutes, 90% after 10 minutes and 99% after 30 minutes. These preliminary results indicate that highly permeable monolithic PolyHIPE supports are advantageous in solution phase organic synthesis. The monolithic format simplifies reagent transfer and other manipulations, furthermore flow-through procedures allow more rapid transformations.

Ion exchange scavengers for product purification

Solid phase extraction is a purification technique that temporarily sequesters the product or byproduct to the solid phase. Normal phase, reverse phase and ion exchange chromatography are all variants of solid phase extraction. Ion exchange resins provide a useful method of purification for the separation of ionic compounds.
Cationic and anionic resin washes using ion exchange resin are readily automated with conventional liquid handling robotics for high throughput sample purification. This was exploited for preparation and purification in the parallel synthesis of a library of amide analogues (Scheme 7)\(^1\). More than 225 analogues were prepared by this automated procedure in an average yield of 75% and an average HPLC purity of 90%.

A related report describes the use of ion exchange resins as scavengers for parallel solution phase amide libraries. The authors investigated the use of nine different basic ion exchange resins in the purification of amides derived from acid chlorides and found that Amberlite IRA-68, a weakly basic resin, provided products of highest purity (>99%)\(^2\). Following the reaction, the excess acid chloride was quenched with water and the basic ion exchange resin absorbed the resulting carboxylic acid from solution leaving behind the pure amide product (Scheme 8).

A solid phase extraction method using Dowex 1x8-400 format anion exchange resin was used for the capture of carboxylic acids in a 96-well parallel array\(^3\). For example, a reaction between compounds A-X and Y-B-CO\(_2\)H in the presence of suitable coupling reagents resulted in a mixture containing the product A-B-CO\(_2\)H as well as reagent and X-Y byproducts (Scheme 9).

Addition of the anion exchange resin extracted the carboxylic acid from the mixture to give the resin bound A-B-CO\(_2\)\(^-\) (II). The non-ionic impurities were easily removed by filtration and solvent washing of the resin. The purified product A-B-CO\(_2\)H was then obtained after treatment of the resin (II) with a volatile solvent acid such as HCO\(_2\)H. A 12-membered carboxylic acid test library was prepared utilising the Stille coupling and purified using the DOWEX 1x8-400 formate resin to give products in an average (1H NMR determined) yield of 49% and an average HPLC purity of 95% (Figure 3).

Incompatible functional groups such as acids and bases can co-exist in the same reaction vessel when used in resin format. This is believed to be possible because of the relative isolation of the functional sites within the bead. The authors demonstrated this with the parallel Moffat oxidation of hydroxyethylamines to ketones using an amine encoded diimide (Scheme 10)\(^4\). A simple filtration provided the ketone products with no detection of starting material or reagent byproducts by spectroscopic analysis.

Polystyrene DVB supported derivatives of tris(2-aminoethyl)amine and methyl isocyanate can be
used to quench excess reactants from crude reaction products of the solution phase parallel synthesis of ureas, thioureas, sulfonamides, amides and pyrazoles (Scheme 11)\(^\text{15}\).

The products were isolated by a single filtration followed by solvent evaporation. The mechanical simplicity of this methodology allows the rapid parallel purification of crude reaction products, single compounds or mixtures, obtained via solution phase combinatorial chemistry. Since these resins are expensive, they are particularly useful for small-scale work where the labour, solvent and silica gel savings are considerable.

A PSQ methodology was used for the parallel preparation and purification of dihydropyridones and a subsequent ‘libraries from libraries’ approach gave aminopiperidines\(^\text{16}\). The dihydropyridone scaffold was assembled via a solution phase acid catalysed hetero Diels-Alder reaction. The procedure reported by Affymax\(^\text{17}\) for imine library synthesis was used. An equimolar mixture of aldehyde and primary amine was stirred for one hour in triethylorthoformate and the solvent was evaporated. After evaporation, the resulting imines were used directly in the Lewis catalysed hetero Diels-Alder reaction with Danishefsky’s diene. The polymer supported polyamine removes the byproduct diene and any unreacted imine giving crude product yields of 50-95\% and purities of 80-95\% (Scheme 12).

Nicolaou et al also reported a ‘libraries from libraries’ approach\(^\text{18}\) for the creation of diverse natural product like libraries of benzopyrans\(^\text{19}\). An initial primary benzopyran library was generated using a solid phase split and pool synthesis and a directed sorting technique (Scheme 13). This primary library was organised into a 96-well format after cleavage resulting in one compound per well. A suitable epoxidising agent (volatile DMDO) was then added to each well to give the corresponding epoxides which were subsequently reacted with various nucleophiles including alcohols and amines to generate a second library of ring opened epoxides. Any unreacted excess nucleophiles were then scavenged, for example the excess amine was removed with a polymer bound isocyanate while excess alcohols were readily evaporated. A third generation library was possible via derivatisation of the ring-opened epoxides upon reaction with electrophiles. This included acetylation of the resulting secondary alcohol, with scavenging of the excess acetylating agent with polymer bound tris(2-aminoethyl)amine. Another derivative involved the resulting secondary amine (formed from reaction of the epoxide with a primary
amine) and isocyanate, the excess of which was scavenged with a polymer bound tris(2-aminoethyl)amine. The scavenger resins were removed by filtration and the products obtained after concentration.

Taddei et al developed a soluble supported scavenger for sequestering alcohols, thiols, triphenylphosphine and triphenylphosphine oxide (Scheme 14).

Trichlorotriazine was reacted with MeO-PEG-OH (Mw 5000) to give a PEG-dichlorotriazine (PEG-DCT) that was used as a soluble electrophilic scavenger20. PEG-DCT was added at the end of reactions to completely remove nucleophilic reactants or byproducts. The reaction was considerably faster than with Wang resin-based systems (45min vs 12h). After precipitation of the polymer with diethyl ether, the desired product was isolated by filtration and evaporation. A disadvantage is that the PEG loading is low (0.2mmol/g), but the trichlorotriazine component is quite cheap.

Marsh et al developed novel high loading scavenger resins functionalised with triazine dendrimers (Figure 4)21. These resins gave comparable efficiency in acid chloride scavenging to commercial resins at significantly lower concentration due to the increase in the number of active functional groups.

These methods are suitable for medicinal chemistry applications. In 2002, Maltais et al reported the parallel solution phase synthesis of a new family of type 3 17ß-hydroxysteroid dehydrogenase inhibitors that were purified via polystyrene-based scavenger resins (methylisocyanate, piperidinomethyl, aminomethyl) (Scheme 15)21.

Sequestration enabling techniques

In some cases, it is difficult to remove reagents from solution phase reactions since a suitable scavenger does not exist or the reagent does not have an appropriate functionality. Further functionalisation can be used to convert the reagent into a form that can be scavenged with an ion exchange resin.

Parlow et al demonstrated the use of tetrafluorophthalic anhydride as a sequestration enabling reagent (SER) (Scheme 16)22. This allows for the derivatisation of moderately reactive amines and subsequent sequestration by basic scavenger resins. For example, aniline was reacted with the electrophiles benzyl chloroformate, benzoyl chloride, benzenesulfonyl chloride and phenylisocyanate. Tetrafluorophthalic anhydride was added to the reaction mixture after 24 hours. The polyamine scavenger resin was then added to sequester the
carboxy tagged aniline derivative, the excess SER, excess electrophiles and byproduct HCl. Following filtration and evaporation the products were obtained in high purity. A drawback with tetrafluorophthalic anhydride is its slow rate of conversion with electron-deficient anilines.

The use of ‘catch-and-release’ strategies for the preparation of heterocycles is accompanied by a cyclisation-cleavage step. Parts of the linker can become part of the product which is released into solution. A gel-type polystyrene-sulfonyle-hydrazide resin which was originally developed for carbonyl scavenging can also serve as a linker for carbonyl compounds in the solid phase synthesis version of the Hurd-Mori reaction for 1,2,3-thiadiazole synthesis (Scheme 17) [23].

A recent review describing rapid purification methods for combinatorial libraries also provides a useful list of scavenger resins and sequestration enabling reagents [24]. In the forthcoming Part 2 of this article, alternatives to traditional scavenging methods will be introduced. These include new resins, the use of polymeric and fluorous scavenger reagents and applications of microwave technology for accelerating scavenging reactions.

Acknowledgement

Support for our research programme in chemical methodologies and library synthesis by the NIH, in particular the NIGMS CMLD programme (P50-GM067082) is gratefully acknowledged.

Peter Wipf is Professor of Chemistry and Professor of Pharmaceutical Sciences at the University of Pittsburgh, USA. He also serves as the Director of the Combinatorial Chemistry Centre and the Director of the Centre for Chemical Methodologies and Library Development at Pittsburgh. His research interests are in the areas of natural product total synthesis, organometallic and heterocyclic methodologies, and combinatorial chemistry.

Claire Coleman obtained her PhD in 2003 with Dr Donal O’Shea at University College, Dublin, Ireland and is now a postdoctoral researcher with Professor Wipf at the University of Pittsburgh. She also serves as the Assistant Director of the Centre for Chemical Methodologies and Library Development at Pittsburgh. Her interests include natural product synthesis and microwave assisted combinatorial library generation.
Combinatorial Chemistry

Scheme 16: The use of tetrafluorophthalic anhydride as an SER

Scheme 17: 1,2,3-Thiadiazoles prepared via ‘Resin Capture’ of ketones

References