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Permutational Organic Synthesis in Addressable Microreactors (POSAM™): An Efficient, Inexpensive and Versatile Solid-Phase Protocol for the Preparation of Libraries of Compounds on the 0.01 to 1.0 millimole (or Larger) Scale.

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Abstract. Apparatus for the solid-phase synthesis of libraries of 10 milligramme quantities of compounds consisting of machined frit glass microreactors and precision engineered jacketed microreactor vessel tubes, is described. The apparatus provides an inert environment for reactions requiring some of the most basic and acidic conditions used in conventional organic synthesis over the temperature range -80 to 200 °C and is suitable for the synthesis of libraries of upto 600 members.

The quest to produce libraries of structurally diverse bioactive molecules both quickly and cheaply has led to intense activity in the area of solid-phase organic synthesis (SPOS) since 1992.[1,2] Solid-phase peptide and oligonucleotide synthesis had been perfected and automated prior to 1992 but, the more diverse and demanding conditions required for the conventional organic synthesis of non-polymeric materials in the solid-phase posed many new challenges. Some of these have now been met and the repertoire of new reactions that can be achieved on solid supports is impressive and has been reviewed comprehensively.[3-5] Underpinning these advances have been the advents of new resin materials,[6] resin functional groups,[6] linkers,[7] reagents,[8] resin cleavage protocols,[9] and in combinatorial chemistry, new deconvolution methods.[10,11]

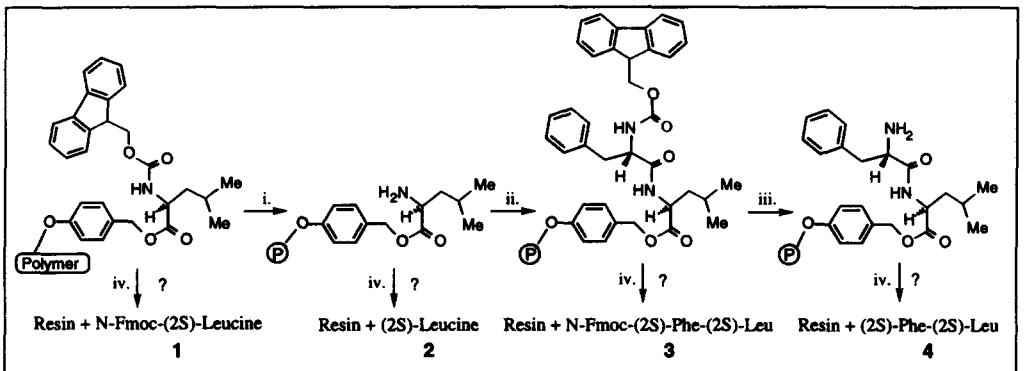
In considering the limitations of existing SPOS and deconvolution protocols it was noted that single resin bead libraries provide very small amounts of each unique material (~10-500 pmol), and are only useful when suitably sensitive biological screens are available. Such libraries are difficult to deconvolute and check the purity of and therefore, require resynthesis for proper evaluation. It was also noted that in order to cover a much wider range of conventional organic chemistry, any new protocols should be able to support reactions performed under extremely harsh conditions, including those required for unstabilised carbanion chemistry and those requiring the use of hot organic solvents.

In accord with these considerations we set ourselves three boundary conditions: a) to synthesise single compounds, so that the putative composition of any given library member would be known; b) to work on a scale that would allow the full characterisation of any given library member, and; c) to use inert materials (only PTFE and glass) for all of the components that would be exposed to solvents/reagents. These conditions focussed our thoughts on four operational requirements: a) to contain, and; b) to label upto a thousand

individual compound library members in individual chambers such that; c) selected library members could be reacted in parallel with prechosen reagents and then; d) be reorganised for subsequent chemical treatments.

In order to contain the individual chemical compounds comprising the library, closable frit glass microreactor chambers of various shape, size and frit porousity were constructed. Polyproplene microreactors have been used by other groups, however, these would be less compatable for the diverse range of chemistry we wish to employ.[11] The available volume inside the microreactors varied from approximately 200-900 mm^3. The various microreactors were charged with preloaded N-Fmoc-(2S)-leucine-Wang resin ester[12] (preswollen in DMF, 25 mg dry weight) and were closed.

For each type of microreactor a series of three chemical reactions was performed. First the microreactor was treated with a solution of 20% piperidine in DMF, to remove the Fmoc group, and was then washed in solvent. Second, the washed microreactor was treated with a solution of PyBOP-activated N-Fmoc-(2S)-phenylalanine, in order to form a resin-bound N-Fmoc protected dipeptide, and was then washed, and; third the microreactor was again treated with 20% piperidine in DMF and was then washed with DMF and finally methanol, to "shrink" the resin. The contents of each microreactor were then removed and the products were cleaved from the resin using 95:2.5:2.5 TFA:water:triethylsilane. The TFA solution was concentrated under reduced pressure and the residue was dissolved in deuteriated water for analysis by ^1H- and ^{13}C-NMR spectroscopy. Control reference standards showed that each of the four possible products, N-Fmoc-leucine **1**, leucine **2**, N-Fmoc-phenylalanylleucine **3**, and phenylalanylleucine **4** could be distinguished in mixtures on the basis of NMR spectra and that the quantities of individual components could be assessed. Thus, it was possible to determine which steps were causing problems simply by examining the product distribution in the residue of the hydrosylate, Scheme 1.



Scheme 1 *Reagents and conditions*: i. 20% piperidine in DMF; ii. N-Fmoc-(2S)-Phe pretreated with PyBOP, NMM and DMF; iii. 20% piperidine in DMF; iv. 90:2.5:2.5 TFA:water:Et_3SiH

For example, if N-Fmoc-leucine **1** was detected in the cleavage hydrosylate (step **iv**) after exposing the loaded resin to each of the reagents in steps **i-iii**, then it would be apparent that the piperidine in step **i** was not reaching, or reacting with, the resin efficiently. Detection of leucine **2** would indicate that either step **i** had worked and that the peptide coupling step **ii** had not worked or that step **i** had not worked and that step **iii** had worked. Detection of N-Fmoc-phenylalanylleucine **3** would indicate that steps **i** and **ii** had worked but that step **iii** had not and the detection of phenylalanylleucine **4** would indicate that all of the steps had worked.

Each type of microreactor was subjected to each of the steps **i-iii** under a wide range of conditions. The quantities of reagent were varied, the number and duration of the washing steps was varied and the method of exposing the microreactor to the various reagent solutions was tested. On one hand the microreactors were immersed in the reagent solutions to assess the passive reaction of the functionalised resin with the reagent. Under these conditions reactions were very slow and did not proceed to completion and significant quantities of N-Fmoc-leucine **1** were detected. On the other hand all methods of active agitation including stirring, ultrasonication and forced solution flow through the microreactor led to the formation of products **2** and, in smaller quantities, **4**. This encouraging result indicated that either step **i**, the first Fmoc deprotection step was only partially sucessful, or, that step **ii** was being undermined by the presence of excess piperidine left over from step **i** in the solvent gel surrounding the resin beads inside the microreactor. The problem was rectified by including extra washing steps and the quantitative conversion (> 97%) of Fmoc-leucine loaded Wang resin ester to the required dipeptide **4** was achieved using cylinderical microreactors (Figure 1) and recirculating (pump) or reciprocating (two syringe) forced flow methods with low solvent and solution volumes.

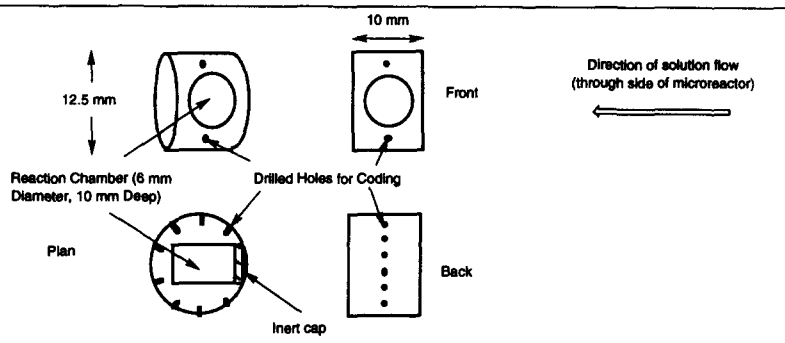


Figure 1. POSAM™ Microreactor Design

Microreactor body is constructed of high density scintered (frit) glass of pore size < 40 μm. Inert cap is constructed of silicon rubber (push fit) or PTFE (threaded grub screw). Coding holes accept coloured PTFE pegs and the entire microreactor, when fitted with the PTFE screw cap, is resistant to 2.5M butyl lithium in hexane for > 72 h. at 30°C.

In order to expose more than one resin-bound compound to a given reagent solution and thereby perform concurrent parallel syntheses, the optimum arrangements for stacking the microreactors in columns was explored. Jacketed vessel tubes were constructed from lengths of 13 mm internal diameter glass tubing and were fitted with a sinter filter at one end, the bottom. Initially nine microreactors were stacked within the vessel tube (separated by inert washers) and the top of the tube was closed with a screw on ground-glass joint fitted with a scinter filter such that a PTFE coated spring was compressed between the uppermost microreactor and the bottom of the scinter filter in the vessel tube cap, Figure 2. By using this arrangement, under optimised conditions[13] quantitative conversion (> 95%) of Fmoc-leucine loaded Wang resin ester to the required dipeptide **4** was achieved in each microreactor. There was no variation in the efficiency of the conversion of the starting resin to the product **4** with the position of the microreactor within the vessel tube and the use of longer vessel tubes containing larger numbers of microreactors gave the same result. Thus the method is suitable for efficient parallel synthesis on a useful scale.

The utility of the protocol in preparing a tripeptide library and the versatility of the apparatus in supporting Grignard chemistry and also acid-catalysed condensation reactions in hot toluene has been

established and will be reported on shortly.[13]

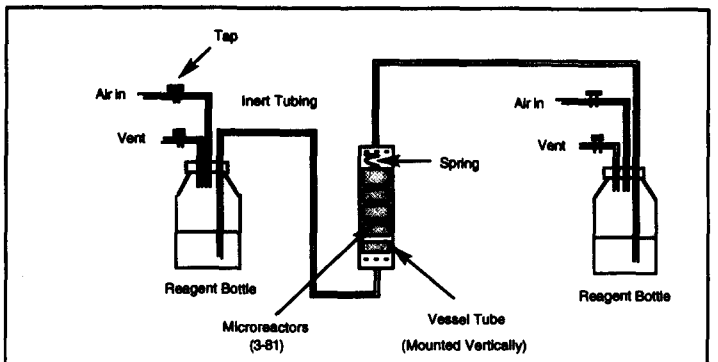


Figure 2. POSAM™- Microreactor Vessel Tube

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Abbreviations PTFE, polytetrafluoroethylene; NMM, N-methyl morpholine; PyBOP, Benzotriazole-1-yl-oxy-tris-pyrrolidino-phosphonium hexafluorophosphate; TES, triethylsilane; TFA, trifluoroacetic acid; Wang, Wang resin.

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