Drugs of the Future 1998, 23(6): 643-654

High throughput purification methods in combinatorial solution phase synthesis

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Introduction

Combinatorial chemistry (1-4) represents an important opportunity in the identification of drug candidates for the vast majority of pharmaceutical companies. Solid phase synthesis (5-7) is the most popular method selected to achieve the synthesis of libraries despite the fact that solution (or liquid) phase has been the predominant reaction medium over the past century in synthetic organic chemistry. In the solid phase synthesis approach a substrate is attached covalently, via an intermediate molecule termed "linker", to an insoluble polymeric entity named solid support or resin (8). The reaction medium is heterogeneous and a large excess of reactants and/or reagents is normally used to ensure the completion of the reaction. Solid phase strategy allows an easy purification methodology by simple filtration and rinsing. Byproducts are washed off the polymeric support. A final cleavage step is needed to afford the final desired product. Despite the efficiency of the purification method, solid phase synthesis has some drawbacks related to the heterogeneity of the reaction medium: i) nonlinear kinetic behavior of reaction; ii) unequal distribution of the polymeric support in the reaction mixture; iii) problems of accessibility of the reagents onto the polymeric matrix; iv) poor swelling of some resins, in particular organic solvents; and v) difficulties in monitoring reactions. Overcoming these drawbacks of solid phase chemistry is often nontrivial; nevertheless, the recent literature shows exponentially growing interest in translating "traditional" solution organic reactions to solid phase as well as an important methodological effort to monitor reactions on solid phase.

Solution phase combinatorial synthesis (9-13) provides a homogeneous reaction medium and overcomes the drawbacks of a solid phase strategy. By default the time needed for chemistry assessment in the preparation of a library in solution phase is normally much less than for an equivalent solid phase synthesis approach. An easy purification method is required in solution phase combinatorial (or parallel) synthesis to facilitate automation. The throughput in solution phase automated synthesis is directly related to the facility of performing the purification process (workup, compound separation, *etc.*).

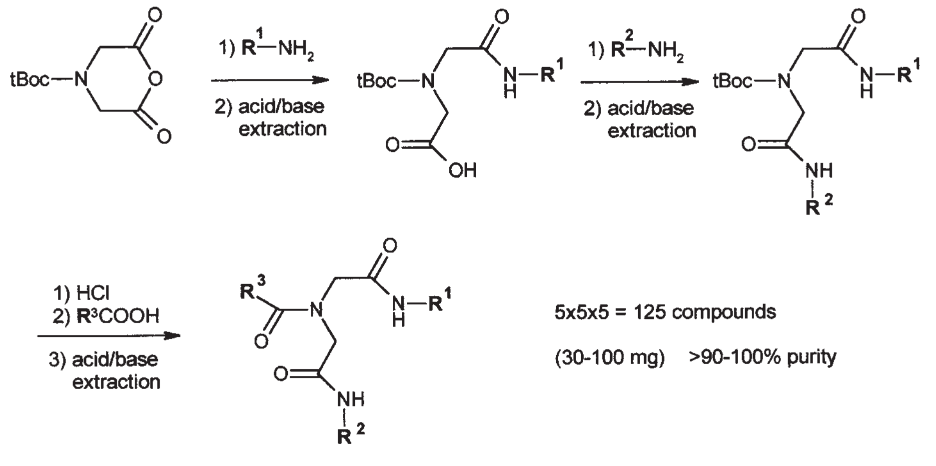
Herein we intend to provide an overview of the solution phase combinatorial synthesis that has appeared recently in the literature from the point of view of purification strategies. First, we will discuss the liquid-liquid extraction approach, describing the use of the traditional aqueous/organic phase separation as well as the recent use of the so-called fluorous phase. Next, solid phase extraction will be regarded as a replacement of liquid-liquid extraction strategy. In both approaches noncovalent interactions are involved. Functionalized solid supports, able to form covalent bonds with byproducts or final compounds, have been used with the aim of purification. This strategy is described in the section on covalent scavengers and resin capture approaches. Finally, macromolecules such as soluble polymers and dendrimers and their use as soluble supports in solution combinatorial synthesis will be discussed.

Some of the reported solution phase syntheses of libraries escape the boundaries of this review, for example, libraries which were created by reaction of equimolecular quantities of their components in excellent yield and good purity without the use of any purification methodology. This review also does not cover low throughput chromatographic methods such as flash chromatography or HPLC. We invite the interested reader to directly consult the primary literature for the solution synthesis of libraries as discretes (14-16) or in pools (17-22) which use these approaches.

Liquid-liquid extraction

Liquid-liquid extraction involves two nonmiscible liquids where a particular substance has a partition coefficient

Scheme 1



between the liquid phases. Aqueous/organic phase extraction is used extensively in traditional organic chemistry. Basic (amines) and acidic (carboxylic acids) substances should be separated by acid-base liquid-liquid extraction. This well-known purification principle has been used by Boger (23-27) as a key feature in the generation of small molecule libraries. Starting with an anhydride scaffold, and then sequentially adding amines and carboxylic acid derivatives as is shown in Scheme 1, Boger was able to synthesize a library of 125 amides in high purity (>90%) with a considerable quantity of final compound (30-100 mg). A similar approach has been used by Zhu (28) in the synthesis of a library of arylpiperazines. The approach is limited to acid-base substrates or reactants and their ability to form aqueous soluble salts.

Aqueous workup is one of the primary purification steps in the parallel synthesis of arrays in solution phase (13). The separation of the organic/aqueous phases may be performed with a commercially available hydrophobic membrane in a propylene cartridge in order to separate a dichloromethane or chloroform phase from the aqueous phase. Adsorbent packing devices have also been developed to adsorb the aqueous phase. A novel separation method has been developed at Glaxo Wellcome (13) consisting of cooling the organic/aqueous phases to -20 °C in the presence of an array of pins. After the freezing process, the aqueous phase is removed as ice attached to the pins. This procedure has been termed the “lollipop” method.

The specific physical behavior of the perfluorocarbon or “fluorous” phase (29, 30) has been exploited by Curran with combinatorial aims (31, 32). Generally, the fluorous phase consists of perfluoro hydrocarbons (*e.g.*, perfluoro hexanes) which are not soluble either in water or in most organic solvents. A relatively large quantity of perfluorinated moieties (fluorous tag) is required to dissolve organic molecules in fluorous solvents (Fig. 1). This concept has been used previously by Horvath and Rabai (29) in the preparation and use of fluorous catalysts which can be easily recovered by simple liquid-liquid extraction between an aqueous/organic and fluorous phase (containing the fluorous catalyst).

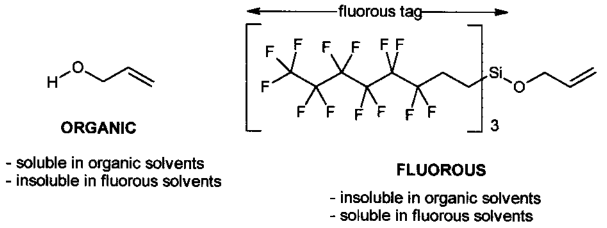
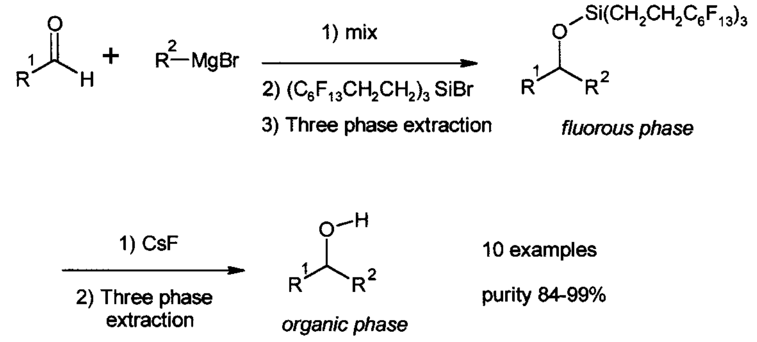


Fig. 1.

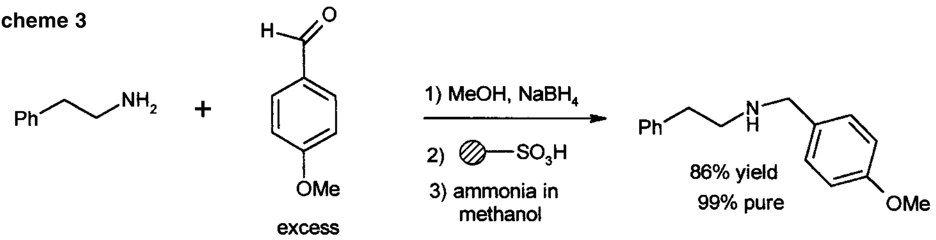
A key aspect in the fluorous phase approach was to find a suitable solvent able to dissolve all the reagents, substrates, reactants and/or catalysts with different natures (organic, fluorous) in order to obtain homogeneous reaction conditions. Diethyl ether, carbon tetrachloride and tetrahydrofuran are the most soluble organic solvents in fluorous solvents. Benzotrifluoride (BTF) is a mixed solvent that shows powerful dissolving properties for organic and fluorous entities (31, 33, 34).

So far, two classes of fluorous tags have been used by Curran: perfluorinated tin and silyl derivatives (Fig. 1). The purification strategy is common for the use of fluorous catalysts (30), fluorous reagents (34-37), fluorous substrates (38, 39), fluorous reactants (40-42) or fluorous “phase switch” (32) approaches. A good partition coefficient between fluorous/organic and/or fluorous/aqueous phases is required in order to separate fluorous molecules from the rest of the components. In the case of the “phase switch” approach (32) (taking one of several examples described by Curran), the bromo perfluorosilane reacts exclusively with the alkoxy bromo magnesium intermediate generated during the addition of Grignard

Scheme 2



Scheme 3



reagents to several aldehydes (Scheme 2). After purification by liquid-liquid extraction between fluorous, organic and aqueous phases (three-phase extraction) the fluorous fraction was treated with cesium fluoride and the alcohol derivatives were recovered in the organic phase with good purities.

Solid phase extraction

Solid phase extraction (SPE) involves the partition of a mixture of components between a solid (sorbent) and a liquid (43). The interactions responsible for the sequestration into the solid support are noncovalent (ionic, Van der Waals, hydrophobic). Those interactions can be modulated by the physical properties of the liquid phase (eluent). Conceptually, there are no major differences between liquid-liquid extraction and solid phase extraction (called solid-liquid extraction as well), but SPE overcomes the formation of emulsions, is amenable to very dilute solutions and can be easily automated. The sorbents commonly used (commercially available as cartridges) are silica gel, reverse phase silica gel and ion exchange resins. Ion exchange resins have been the most commonly used for combinatorial applications. Recently, Curran (44) has proposed the use of fluorous reverse phase silica gel as an efficient sorbent in SPE of fluorous compounds.

Ion exchange resins are able to differentiate charged species from neutral molecules. Molecules which may undergo a proton transfer could be complexed by ionic forces to ionic exchange resins. This ionic labelling process is reversible depending on the ionic strength of the medium (pH).

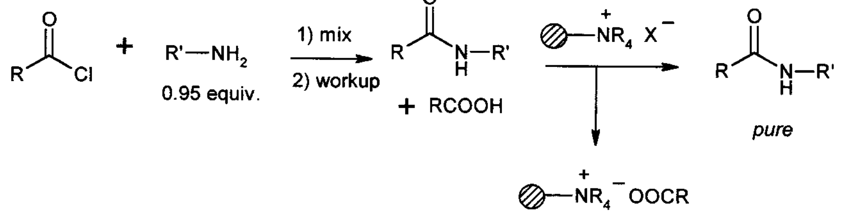
Substrates, byproducts and intermediates having the ability to form ions have been purified by ion exchange solid phase extraction protocols in the solution phase synthesis of libraries.

Siegel (45) described the use of strong cation exchange resins to purify a library of amines generated by reductive amination (Scheme 3). An excess of aldehyde was used to ensure the completion of the reaction and the crude reaction was passed through phenyl sulfonic acid polystyrene resin, allowing the selective extraction of library components as ammonium charged species. Elution with a methanolic solution of ammonia recovered the desired amines with excellent purities.

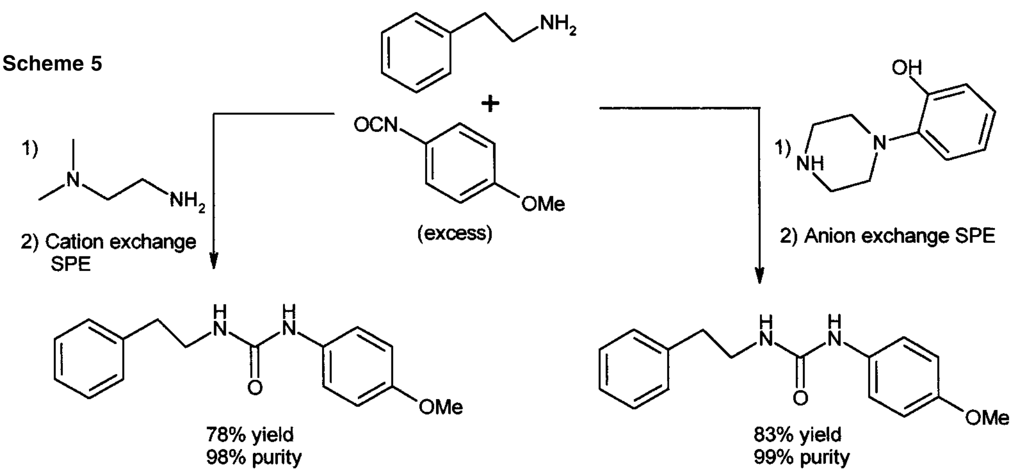
Byproducts (*e.g.*, unreacted substrates) can be retained by the use of ion exchange resins. Suto (46) was able to retain unreacted acid residues by the use of a basic ion exchange support (Scheme 4).

Solution scavengers have been used by Siegel (45) to transform a neutral compound into an ionizable species that can be captured by an ion exchange solid support. The excess of isocyanate in the generation of a library of urea derivatives was removed by reaction with either dimethylaminoethyl amine or a phenolic piperazine derivative (solution phase scavengers). The byproducts were removed by ionic interactions with a cationic and anionic exchange resin, respectively (Scheme 5).

Scheme 4



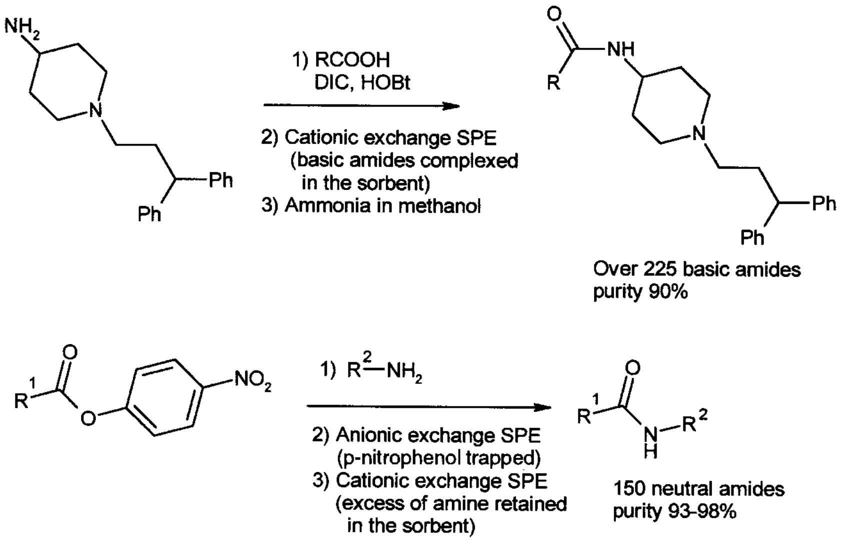
Scheme 5



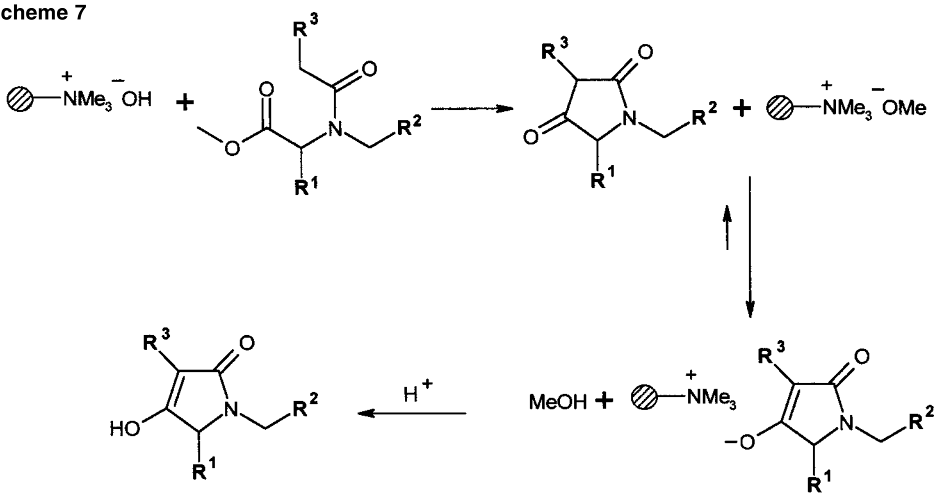
Lawrence (47) similarly described the synthesis of a library of over 225 basic amides with a >90% purity and a neutral amide library of 150 compounds with a >93% purity (Scheme 6). Automation was performed using a commercially available liquid handler and a SPE workstation. Both basic and neutral amide libraries were prepared on 25-300 mg scale.

Ganesan (48) employed an ion exchange resin (Amberlyst A-26, HO- form) as reagent and purification agent (Scheme 7) in the Dieckmann condensation. The

Scheme 6



Scheme 7



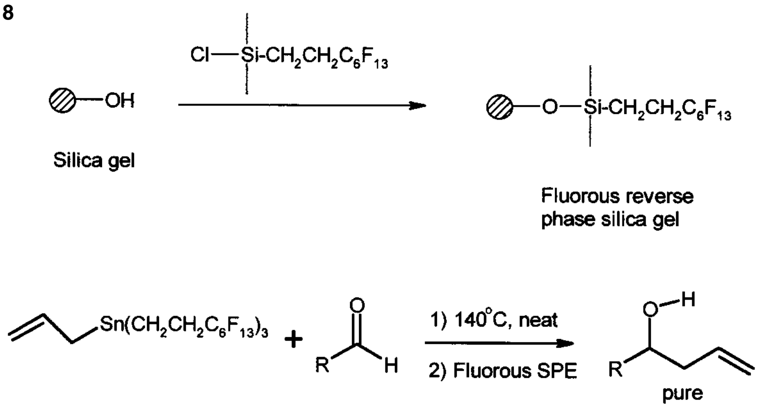
pyrrolidinedione (tetramic acid) binds to the resin and the rest of the components are washed away. Preliminary results showed that pure final compounds were released by acid treatment.

Silica gel modified with dimethyl{2-(perfluorohexyl) ethyl}silyl chloride was used as solid support in the solid phase extraction of fluorous tin derivatives (Scheme 8). Fluorous byproducts were trapped by the fluorous reverse phase silica gel sorbent after the addition of the allyl tin reagent to several aldehydes was performed. Curran (44) partitioned the crude reaction mixture between the fluorous reverse phase silica gel and acetonitrile (Scheme 8). The fluorous solid phase retained all the compounds bearing perfluorinated moieties. Allyl alcohols were obtained in good purity after evaporation of the acetonitrile phase. The fluorous derivatives could be extracted from the solid support with an apolar solvent such as hexane. No major differences were found between liquid-liquid extraction with fluorous solvents and solid phase extraction using the fluorous reverse phase solid phase extraction.

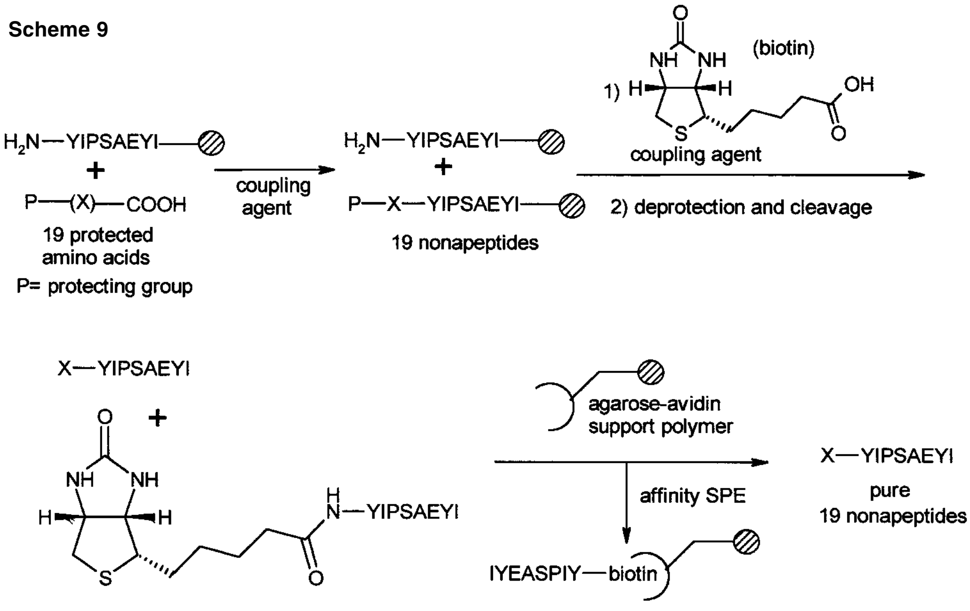
As mentioned above, a substrate linked to a solid support or soluble polymer is purified by filtration and rinsing. Usually, once the cleavage is performed, the product is not purified further. However, recent examples have appeared in the literature where after cleavage from the polymer resin the final compound, in solution, was purified by SPE (49, 50).

An interesting approach was described by Quesnel (49) in the purification of a peptide library based on capping and posterior affinity chromatography using the avidin-biotin system (Scheme 9). The solid support, which has been previously linked to the octapeptide YIPSAEYI, was coupled with 19 amino acids (all the natural ones except cysteine). The unreacted octapeptide was capped with biotin, then deprotection of the amino acids and cleavage from the resin was performed. The mixture was incubated in a commercially available avidin-agarose

Scheme 8



Scheme 9



solid support. In the sorbent the *N*-biotinylated octapeptide was effectively retained. In solution the mixture of the 19 nonapeptides remained free of the octapeptide which may interfere in the biological test. Treatment with guanidine-HCl (pH 1.6) induced the reversible denaturation of the avidin. The unmobilized avidin in agarose beads was reusable.

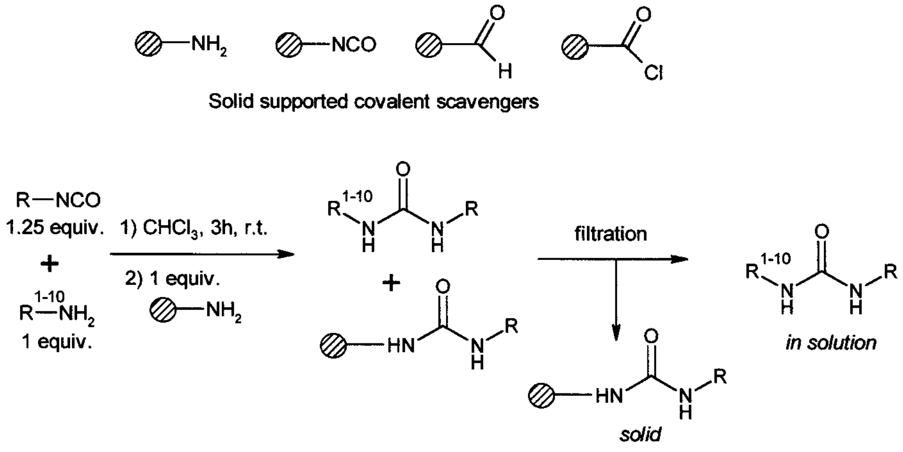
Covalent scavengers and resin capture approaches

Kaldor and Siegel (51, 52) have used functionalized solid supported resins (Scheme 10) with electrophilic or nucleophilic character to trap unreacted starting materials or known impurities. The quenching proceeds via a covalent bond between the functionalized resin and the byproducts. Therefore, only the desired product remains in solution and is obtained by filtration and rinsing. The use of excess reactants and/or reagents in order to drive the reaction to completion is thus possible.

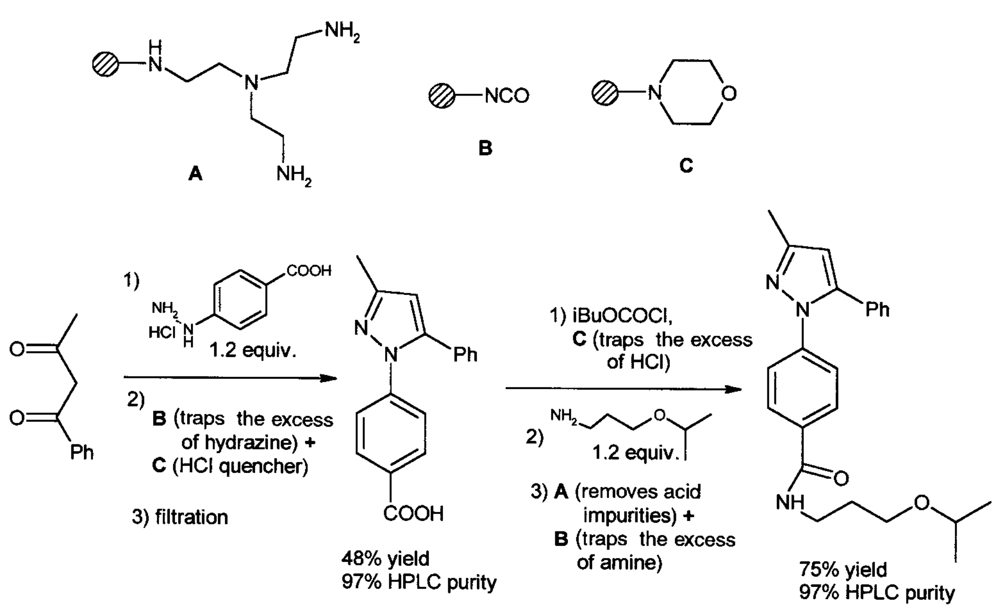
Kaldor (53) used a nucleophilic solid support scavenger (Scheme 10) in the synthesis of a library of 4000 ureas (400 pools of 10-compound mixtures). The solid supported amino nucleophile quenched the excess of isocyanates, leaving the desired ureas in good purity.

Hodges (54) described a similar approach using a polymer-supported quenching methodology in solution phase synthesis. Primary amines, isocyanates and

Scheme 10



Scheme 11



tertiary amines supported on a polymer were used in the synthesis of pyrazole derivatives, as shown in Scheme 11.

Armstrong introduced the "resin capture" concept (55). In a solution phase synthesis, once the product is formed it reacts selectively with a functionalized solid support. Impurities and unreacted substrate(s) remain in solution and are washed away by simple filtration and rinsing.

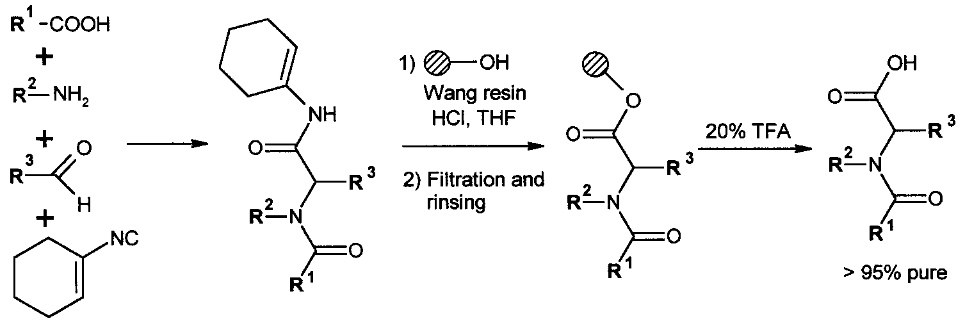
Multicomponent reactions such as Ugi condensations (Scheme 12) were used as a proof of the resin capture purification approach (56). The reactivity of the vinyl amide moiety allowed the specific reaction with a supported alcohol (Wang resin) in the presence of acid. After filtration and cleavage, the final carboxylic acids were obtained in >95% purity.

Other examples of resin capture have been described by Armstrong using Suzuki coupling reactions during the synthesis of tetrasubstituted ethylenes (55, 57).

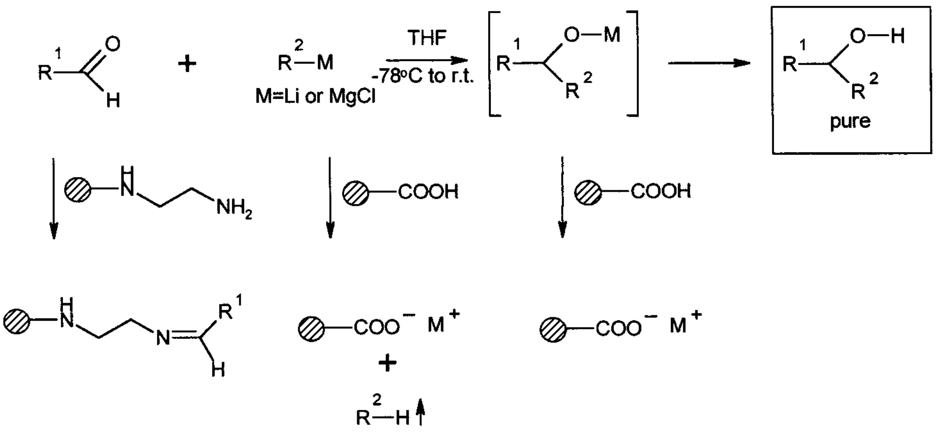
Quenching of products by functionalized resins have been described in the past for analytical purposes (58-60).

Solid phase extraction involves noncovalent interactions between the sorbent and the liquid phase. Covalent scavenger and resin capture approaches use a covalent bond to force the targeted compound in solution to switch phase (from liquid to solid phase). Both approaches have been used simultaneously by Flynn (61) who proposed the "complementary molecular reactivity and recognition" (CMR/R) purification approach. With the aim of avoiding a linkage between the substrate and a solid support, Flynn was able to quench the excess of reactant, reagent,

Scheme 12



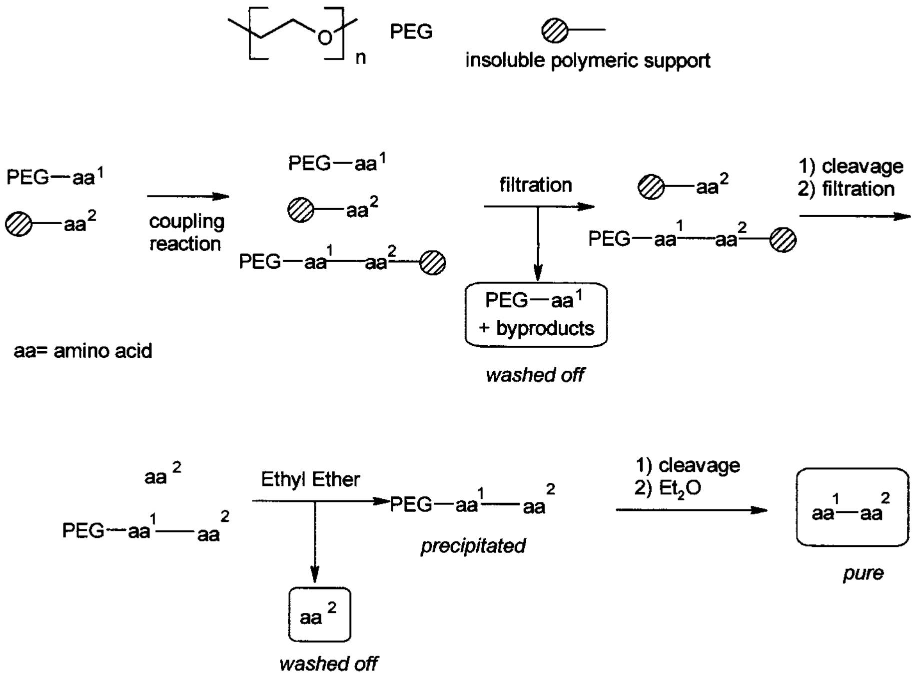
Scheme 13



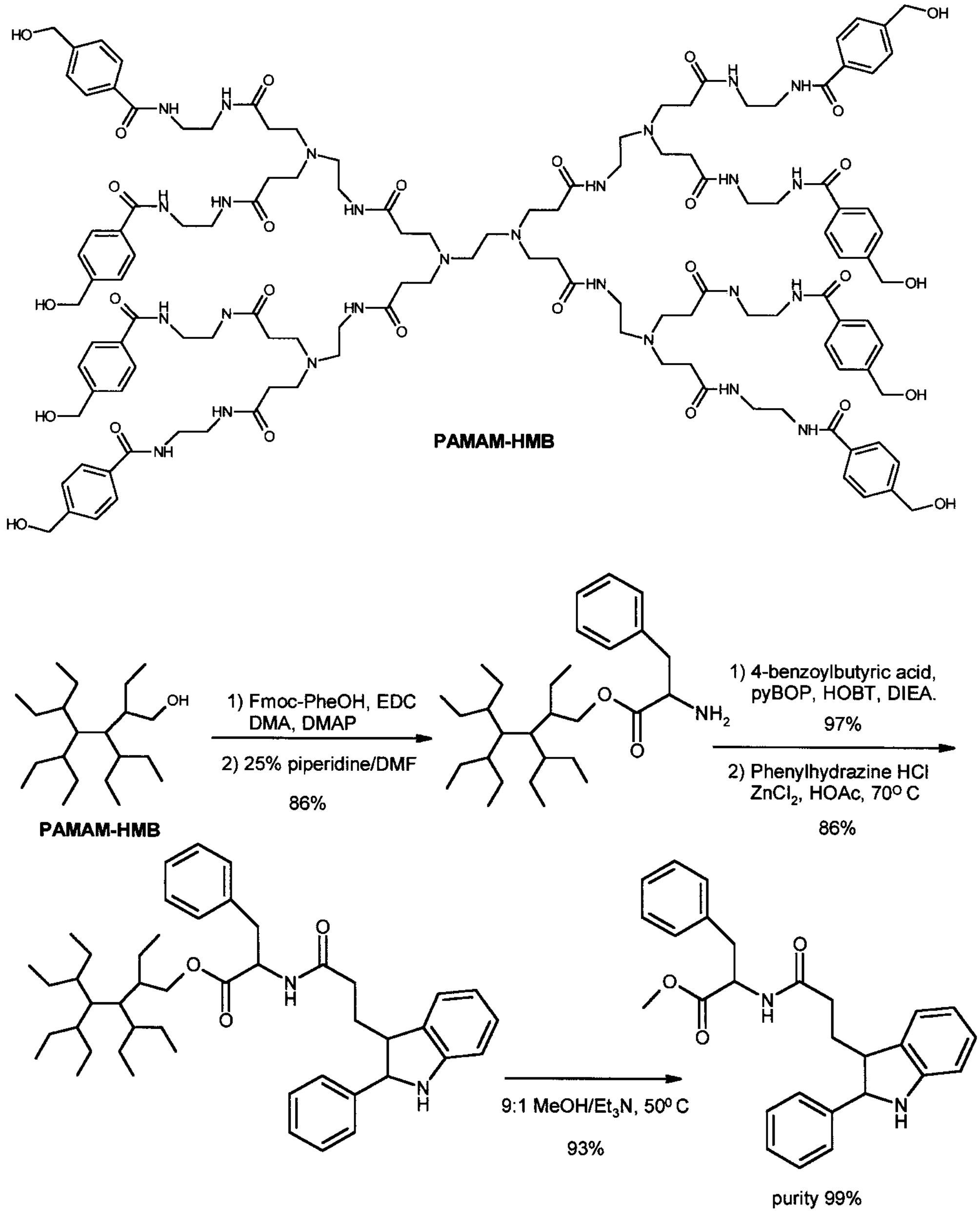
catalyst or even intermediates by specific resin supports (61). He exploited the intrinsic properties of each molecule present in the reaction and artificially tagged some components of the reaction to obtain a complementary reactivity. By quenching all the undesired byproducts of the reaction mixture, the desired product was obtained in solution after a simple filtration. The approach is illustrated in Scheme 13. The addition of alkyl metal derivatives to several aldehydes afforded a library of secondary alcohols. It is noteworthy to mention the possibility of using several resins simultaneously with functional groups that are incompatible in solution. The recognition of the components in the reaction mixture can be of covalent nature or not. The crude reaction was treated simultaneously with a mixture of resins containing a primary amine functionality and a carboxylic acid functionality. A liquid workup was not required to obtain pure compounds (>95% purity, 5 examples).

Other examples of the CMR/R concept have been described by Parlow (62, 63).

Scheme 14



Scheme 15



Recrystallization/precipitation of soluble polymer supports

Recently, organic synthesis on soluble polymer supports was extensively reviewed by Gravert and Janda (64, 65). We want only to briefly highlight some aspects of this approach.

Some polymeric entities are soluble in organic solvents, thus allowing a homogenous reaction medium. These soluble polymers can be precipitated or recrystallized in specific solvents. Among the polymeric entities that have been used so far, polyethylene glycol (PEG) is the most popular. This homopolymer is soluble in water and in a wide range of organic solvents such as dichloromethane, dimethylformamide and pyridine (66). PEG is insoluble in hexane, diethyl ether and *tert*-butyl methyl ether. PEG has the tendency to form helical crystal structures in cool methanol or ethanol. These macromolecular properties allow the use of an excess of reagents/reactants and once the reaction is complete precipitation is induced by diluting with ethyl ether. Then, simple filtration affords the functionalized PEG derivative in pure form.

One of the major drawbacks in solid phase synthesis, as well as in the soluble polymer support approach, is that unreacted substrates become byproducts once the cleavage of the polymeric substance is performed. PEG supported substrates in solution, though, are able to react with insoluble polymeric resin building blocks. Orthogonal purification methodology has been accomplished (64, 67), as shown in Scheme 14. In this solid-liquid phase method it is not necessary to optimize the reaction conditions to ensure completion of reaction in order to obtain pure compounds, thus reducing the time necessary to assess the chemistry for the synthesis of the library.

PEG attached compounds have been tested in some biological screens where they displayed full biological activity (68, 69) in comparison with the related free compounds. PEG intermediates can be analyzed in a nondestructive manner with traditional techniques (NMR, IR, *etc.*). Nevertheless, a PEG strategy shows some limits in the use of aqueous workups due to the solubility of the polymer support in water. PEG is also insoluble in ethyl ether and tetrahydrofuran at low temperatures; hence, reactions could not be performed in these solvents under homogeneous conditions.

Size exclusion chromatography of dendritic supports

Dendrimers are branched oligomers with well-defined architecture. Dendrimers possess macromolecular properties allowing a size-selective purification methodology such as size exclusion chromatography or ultrafiltration. Dendrimers can be attached to a substrate under homogeneous reaction conditions. Synthesis on dendritic supports can be monitored by traditional methodologies such as NMR, IR and mass spectroscopy. The specific purification method allows the use of an excess of reactants and/or reagents as for solid phase synthesis.

Kim (70) prepared a dendritic support from the commercially available starburst polyamidoamine PAMAM by coupling with 4-hydroxymethyl benzoic acid (HMB). He synthesized a mini-library of (3x3x3) indole subunits in order to validate the approach (Scheme 15). The purification of the dendritic intermediates was accomplished by size exclusion chromatography on Sephadex LH-20. The sequence of reactions was monitored by NMR spectroscopy. The size exclusion chromatography elution conditions of the different dendritic species were very similar; in fact, this was one of the key purification features to obtain pure compound with a unique purification protocol. Columns could be used dozens of times.

Conclusions and outlook

Simple purification methods have been developed in solution phase combinatorial synthesis offering a competitive alternative to the solid phase approach. Phase separation is the basis of automated purification. This phase differentiation in solid phase synthesis is guaranteed by the covalent attachment of the substrate to the insoluble resin. As we have documented, this phase differentiation in a solution phase strategy can be accomplished by different techniques with the possibility of being automated and providing a high throughput synthesis of small molecules. Most of the approaches mentioned above are in the preliminary phase of development and we foresee their rapid growth in the near future.

Acknowledgements

The authors would like to thank Dr. Colin Leslie for helpful discussions.

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