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Library synthesis using solution phase capping of solid phase derived intermediates

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Abstract

An efficient protocol for the preparation of combinatorial libraries is described. The methodology first deploys solid phase chemistry to synthesize a 96-well array of intermediates linked to resin via a latent reactive functionality such as a benzyl ether, *tert*-amine or thioether. Cleavage from the resin is accompanied by transformation of the linker to a reactive functionality, namely a benzyl halide, *sec*-amine or thiol. Aliquots of this array of reactive intermediates are each capped with a different solution phase reactant to deliver the final library in 96-well format, ready for high throughput screening. © 2000 Elsevier Science Ltd. All rights reserved.

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The recent renaissance of small molecule solid phase synthesis has been driven by the demand for large, diverse compound collections for use in high throughput screening.[1] The many organic reactions now documented on a solid support,[2] together with advances in automated synthetic chemistry, have led to the widespread acceptance of solid phase chemistry for the generation of libraries. However, despite its usefulness, solid phase chemistry is not without its drawbacks. The library produced often contains residual polar functionality, typically an acid or amide, where the compounds were linked to the resin. This has led to the development of cyclisation–cleavage,[3] traceless linkers[4] and activation–displacement protocols.[5] Solid phase libraries also require lengthy and extensive development efforts to devise general reaction conditions for diverse reactants. These difficulties are compounded by the limited choice of analytical methods, poor reaction kinetics and additional variables such as resin type and loading. The chief virtue of solid phase chemistry lies in its generation of relatively pure materials free from excess reagents and byproducts. This is in contrast to solution phase library synthesis, which suffers from the accumulation of reaction byproducts during multi-step schemes, prompting the development of supported reagent[6] and resin scavenge protocols.[7]

Our approach to the challenge of library synthesis, outlined in Scheme 1, sought to capitalize on the strengths of both solid and solution phase chemistries by coupling them together in series. The method-

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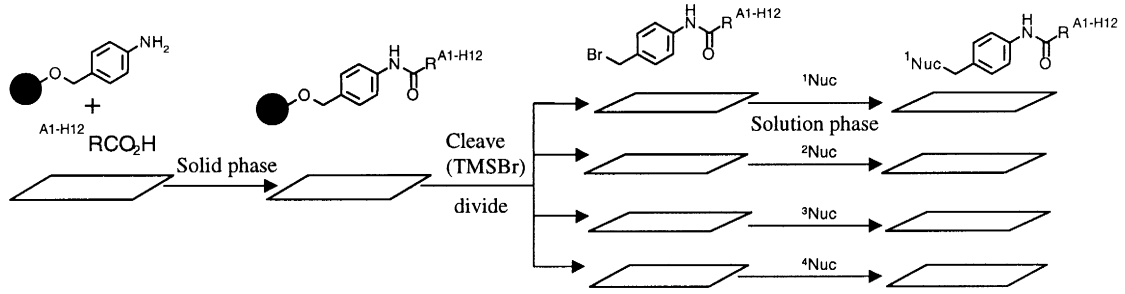
ology first deploys solid phase chemistry to synthesize a 96-well array of resin-bound intermediates linked to the support by a latent reactive functionality. Cleavage from the resin is accompanied by transformation of the linker to a reactive functionality. Aliquots of this array of reactive intermediates are each capped with a different solution phase reactant to deliver the final library in a 96-well format ready for high throughput screening.

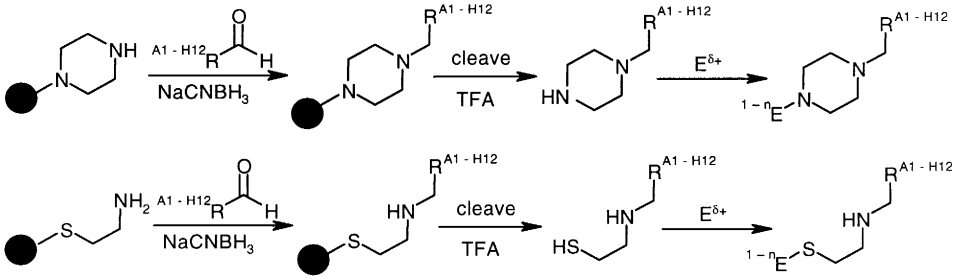


Scheme 1.

This approach was initially used to prepare libraries based upon bifunctional benzyl alcohols. For example, 4-hydroxymethylaniline was attached to methoxytrityl resin as the benzyl ether and acylated with 96 carboxylic acids using standard solid phase methods in fritted, 2 ml, 96-well, polypropylene reaction boxes as depicted in Scheme 1.[8] The solid phase benzyl ether intermediates were cleaved and converted into benzyl bromides in a single step by treatment with TMSBr.[9] The reactions were then evaporated and the benzyl bromides re-dissolved in DMSO. Freshly prepared aliquots of these stock solutions were transferred to 96-well, 1 ml tubes and each array reacted with a different nucleophile as shown in Scheme 1. Our choice of simple substitution reactions in the second step was guided by the knowledge that they proceed rapidly at room temperature with few byproducts in polar, aprotic solvents (DMSO, DMF) which are suitable media for delivering compounds into biological screens. This novel cleavage–activation of solid supported benzyl ethers to benzyl bromides is especially advantageous since the resulting electrophiles are reacted with nucleophilic partners. These are available commercially in much larger numbers and diversity (thiols, amines, NH-heterocycles, phenols and carboxylates) than are electrophiles.

The scope of the protocol can be extended by using a number of standard linkers followed by cleavage and capping with various electrophilic reagents $E^{\delta+}$ shown in Scheme 2. Attachment of a template to the trityl resin via a *tert*-amine linkage, followed by elaboration using a variety of solid phase reactions and subsequent cleavage as a secondary amine, opens up a wide range of solution phase chemistries such as acylation with acyl halides and reductive alkylation. Likewise, functionalised thiols were also readily linked to methoxytrityl resin, elaborated and then cleaved with dilute TFA solutions. The resulting thiols react smoothly with a wide range of alkyl halides.

The final products were generated as 0.25 ml 10 mM solutions in DMSO. An array of intermediates derived from solid phase reactions using resin loadings of the order of 0.5 mmol/g and 100 mg of resin per well can be capped with 20 different solution phase reagents. This level of product multiplication has allowed 10 000's of individual compounds to be synthesised by a single chemist. Since each solid and solution phase reactant gives rise to multiple products in the final library, every reactant used was carefully selected beforehand to ensure it reacted cleanly under the conditions employed. LC/MS analysis of representative library samples have shown that the vast majority of library compounds are produced in over 50% purity. Furthermore, all the templates and reactants used were chosen such that the product



Scheme 2.

molecular weights and physical properties would lie within the drug-like range[10] for orally absorbed compounds.

Commercially available disposable polypropylene 'plates' with well volumes of one or two millilitres were used throughout the synthetic sequence. This allows for the use of 96 at a time pipetting robotics,[11] which minimizes the time spent in the liquid transfer which becomes very significant where large libraries are prepared with single compounds in each library tube. The process also facilitates the easy tracking and indexing of the library. No reformatting is necessary for screening. All that is required is transfer and dilution of product arrays into test plates, again using parallel pipetting robots with disposable pipettes. The library compounds can be visualized as a three dimensional array with substructural features from the initial solid phase step in the xy plane and those derived from the solution phase reaction in the z co-ordinate (see Scheme 1). Clusters of hits from screening often occur vertically through the library where the principal pharmacophore was formed in the initial step, or they occur as clusters in a single plate where the pharmacophore is produced in the second synthetic step. In either case some SAR may often be obtained from the initial screening results. The disadvantage of this approach is that substructures from each step recur throughout the library. However, this is mitigated by the great diversity of templates, solid phase reactions and capping reagents which may be employed.

The application of this two step protocol for library generation offers several advantages over other approaches. Since the functionality derived from solid phase linkage is capped with a variety of reagents in the solution phase step, the problem of residual polar functionality is eliminated. The accumulation of reaction byproducts is minimized since there is only a single, efficient solution phase step, and the effort expended in optimizing the initial solid phase reaction is fully exploited through the use of each solid phase product many times as the substrate for subsequent solution phase reactions. This protocol can be summarized as '*solid phase reaction, solution phase multiplication*' and can be regarded as an application and extension of the safety catch[12] or chameleon linker[13] approach.

In conclusion, a powerful methodology for the rapid high throughput synthesis of discrete compounds for biological screening has been developed. These libraries have produced many interesting hits in a variety of biological screens and these will be reported in due course.

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8. We used Microlute™ 2 ml fritted boxes from Porvair Sciences UK.
9. TMSBr cleavage procedure: A 20% solution of TMSBr in 1,2-dichloroethane (2 ml) was filtered through 100 mg of resin contained in each well of a Porvair reaction box and the filtrate collected in a standard 2 ml 96-well plate directly underneath. The collection box was heat sealed with a polyethylene film and left to stand at room temperature for seven days. The TMSBr solution was removed by evaporation in a vacuum dessicator after punching holes in the polyethylene film. The benzyl bromide intermediates were dried under vacuum for three days before being dissolved in DMSO prior to solution phase capping.
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