Perspective

Perspective Articles on the Utility and Application of Solution-Phase Combinatorial Chemistry

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J. Comb. Chem., **2000**, 2 (2), 89-103• DOI: 10.1021/cc990064+ • Publication Date (Web): 14 March 2000

JOURNAL OF combinatorial CHEMISTRY

Volume 2, Number 2 March/April 2000

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Received October 25, 1999

Combinatorial chemistry has become an important part of the discovery and optimization process for novel drugs, affinity ligands, and catalysts. The technology has been applied in both academic and industrial institutions to provide a number of unique approaches to satisfy the ever-growing need for new chemical entities with proven utility. While combinatorial chemistry has its origins in solid-phase synthesis, many have chosen the solution-phase route.

The following articles will discuss the benefits and challenges that are inherent in this decision to adopt solution-phase combinatorial chemistry strategies. These include a comparison of solution- vs solid-phase approaches (Boger, Coffen), the utility of solution-phase combinatorial chemistry in the arenas of lead optimization (Ellingboe, Gubernator) and asymmetric catalysis (Snapper), the integration of biocatalysis (Krstenansky and Michels), and the importance of process chemistry development (Baldino and Harris). The compilation provides a broad viewpoint on the current state of solution-phase combinatorial chemistry research.

Although solution-phase combinatorial chemistry has yet to reach its full potential, it has become a focal point in the future plans of academic research groups, large pharmaceutical companies, and in the biotechnology industry as described in the following articles. The potential of this technology is widely recognized, but only by addressing the limitations and bottlenecks described herein will it be able to deliver. The further integration of other disciplines such as high-throughput screening, computational chemistry, laboratory automation, and analytical chemistry both for characterization and purification may provide the needed leverage.

In closing, I would like to thank the Editor, Anthony W. Czarnik, for proposing the idea for this article and especially all of the authors for the quality and timeliness of their contributions.

Carmen M. Baldino and Michele R. Stabile-Harris.[1] Automated Solution-Phase Parallel Synthesis for Lead Discovery

Lead discovery in the pharmaceutical industry has traditionally been accomplished by screening companies' historical libraries. The development of ultra-high throughput screening technology and large numbers of genomic targets has prompted many companies to increase the number of test compounds in their screening repository. The urgency of this issue has caused companies to seek out external sources capable of providing a large influx of quality compounds.

The technology platform developed at ArQule uses automated solution-phase parallel synthesis to generate large numbers of novel compounds. One key advantage to the solution-phase approach is the ability to use a vast body of existing synthetic chemistry methodology which provides this technology platform with the same synthetic tools as those available to a medicinal chemist.

In order to design relevant drug-like compounds a number of factors have to be assessed such as drug-likeness (Lipinski rule violations), structural information about the biological

10.1021/cc990064+ CCC: $19.00
Published on Web 03/14/2000

target, and chemical diversity analysis of the proposed compounds relative to those already in the repository. These are but a few of the possible design elements that can be applied to library generation. However, regardless of the design criteria, one must have the requisite process chemistry and automated synthesis capabilities to produce the intended libraries.

This article illustrates our approach for automated process chemistry optimization for the production of large screening arrays. Over the course of the last four years, we have found that the development of an automated library protocol is most efficient when it is conducted using the same automated platforms as those employed in the final production. The early involvement of automation provides a substantial increase in the true performance of a library protocol throughout the production process. However, this level of efficiency comes at a price of increased cost in both process and analytical chemistry. Typically, our 200 000 compound Mapping Array Set® contains approximately 65 library protocols. The average purity is maintained above 85% for each of the arrays (UV, ELSD, and HPLC peak area), and the overall average of the Mapping Array Repository® as a whole is approaching 90%. Our automated chemistry development group conducts over 70 000 reactions annually in order to provide the necessary automated chemical protocols for construction of the Mapping Array Repository®.

Once the desired chemical template with the corresponding synthetic route is chosen, the chemical building blocks are assembled and the process development is initiated. The process involves the following steps: (1) synthetic route validation, (2) initial translation of the chemistry to automation, (3) optimization of reaction conditions, (4) chemical building block validation, (5) optimization of workup, (6) finalizing the analytical method, and (7) final analysis of the library protocol. A more detailed analysis of this process is provided in the following:

Step 1. Synthetic Route Validation

- Several final products are prepared and purified using the specified synthetic route.
- The pure compounds are fully characterized (^{1}H NMR, ^{13}C NMR, UV, IR, HPLC, MS, and X-ray crystallographic analysis when appropriate).
- The pure compounds are used as analytical standards for the final library QC analysis.
- The stability of the pure compounds to storage in DMSO and H_2O, or as dry samples exposed to the air, is assessed.

Step 2. Initial Translation of the Chemistry to Automation

- One plate of 80 compounds is produced using a fully automated process.
- Building blocks that vary in physical properties and reactivity are chosen.
- The purity and yield of each reaction step is assessed.
- The performance of the chemistry is evaluated using standard HPLC and MS methods.

Step 3. Optimization of Reaction Conditions

- Generally 2−12 plates of 80 compounds are required to fully understand the process chemistry of a 2−4 step library synthesis.
- Standard reaction parameters are optimized such as time, temperature, and solvent.
- The optimal number of molar equivalents of each reagent set required to drive the reaction to completion is determined.
- The optimal acid, base, or catalyst is determined.
- The requirement for agitation is assessed.
- Both the HPLC and MS methods are evaluated for final optimization.

Step 4. Chemical Building Block Validation

- The optimized synthetic protocol from step 3 is used to qualify the building blocks proposed for the library.
- The solubility of each reagent in the reaction solvent is determined.
- The reactivity of every building block in each reagent set is assessed against at least two building blocks in each of the other reagent sets.
- A detailed reactivity report is prepared for each reagent set for use in the final process optimization.

Step 5. Optimization of Workup

- Both solid-phase extraction and liquid−liquid extraction methods are tested.
- Quantification of the product after workup is addressed.
- Stability of the products to the workup conditions is also addressed.
- Reactions are scaled in order to deliver the desired quantity of product.

Step 6. Analytical Method Finalized

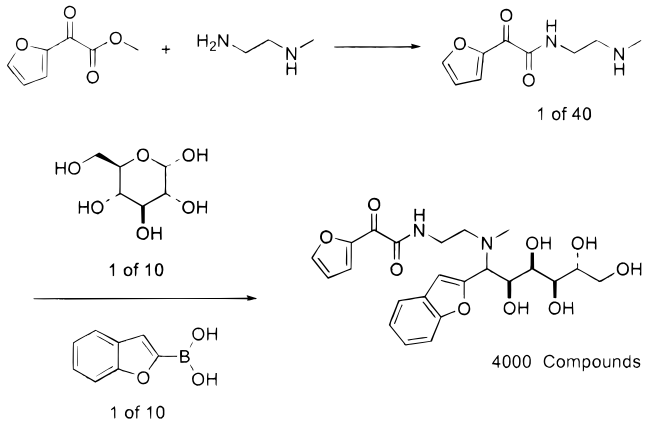
- Exact HPLC conditions are determined by analysis of the process chemistry QC data.
- Exact MS conditions are determined by analysis of the process chemistry QC data.

Step 7. Final Analysis of the Library Protocol

- A set of 2−4 plates of 80 compounds are produced using the optimized library protocol.
- 100% LCMS data is collected on the compounds.
- An average purity of >90% by HPLC with 100% MS confirmation is required to move into production.

The automated process chemistry optimization for ArQule's α-ketoamide-carbohydrate conjugate array provides a good example of the approach. The 4000 compound array combines two novel transformations and four unique building block sets to generate the desired compounds.

Synthetic Route Validation.[2−4]



Initial Translation of the Chemistry to Automation. α-Ketoamide formation required two plates of 80 reactions. The boronic acid carbohydrate coupling required an additional two plates of 80 reactions.

Optimization of Reaction Conditions. A key in the optimization of this chemistry was the identification of the reaction solvent (methanol). During the preparation of the α-ketoamides, a number of products tended to crystallize from methanol. In this case, 80 α-ketoamides were prepared and tested for solubility in methanol at 0.25 M. The compounds that passed the solubility test were then analyzed under the reaction conditions to verify reactivity. In the end, 40 α-ketoamides were used in the array production.

Chemical Building Block Validation.

building blocks	no. of reactions
carbohydrates	27
boronic acids	28
diamines	104
α-ketoamide	61
total	220

Optimization of Workup. The library was purified using Amberjet™, a strongly basic resin scavenger to remove excess boronic acid. Before use, the commercially available resin was washed with methanol and dried using low heat in a vacuum oven overnight. The washing procedure served two purposes: (1) to remove impurities from the resin and (2) to prepare a free-flowing resin for automated dispensing. The resin was added to the reaction vials and shaken for 30 min at room temperature. The reaction mixture with the resin was then filtered directly into fresh microtiter plates, the resin was rinsed twice with ethanol, and the filtrate was combined. After evaporation of the solvent, the final compounds were obtained with an average 80% mass recovery.

Final Analysis of the Library Protocol. The final test plate of 80 compounds (2 carbohydrates × 4 α-ketoamides × 10 boronic acids) was run, and an average purity of 91% was obtained. The final library production was 4000 compounds (40 α-ketoamides × 10 carbohydrates × 10 boronic acids) with an average purity of 85% by HPLC.

In conclusion, solution-phase automated parallel synthesis provides an efficient platform for the generation of large numbers of pure, novel, and drug-like molecules. We have initially focused on library protocol development as the key to providing high-quality chemistry products. The next areas of focus will be high-throughput purification and flow NMR. These technology enhancements will further increase the purity and the level of structural characterization of our compounds for lead discovery.

Dale L. Boger.[5] Solution-Phase Synthesis of Combinatorial Libraries: To Bead or Not To Bead?

Combinatorial chemistry has undergone rapid development and has provided a new paradigm for drug discovery. Our interest first started with our description of divergent synthesis[6] for exploring biological activity enlisting a common advanced synthetic intermediate and emerged as a serious endeavor upon encountering biological targets for which there were no natural product lead structures. Perhaps as a consequence of the original extension of combinatorial chemistry from peptide and oligonucleotide synthesis, the majority of approaches have relied on solid-phase synthesis techniques. A complement to adapting solution-phase chemistry to polymer-supported combinatorial synthesis is the development of protocols for solution-phase combinatorial synthesis. At a time when solid-phase techniques were first being exploited in the emerging field of combinatorial chemistry, we introduced a simple solution-phase protocol that is technically nondemanding, that takes advantatge of all the attributes of solution-phase synthesis, and that serve as part of the proprietary technology on which CombiChem, Inc. was founded (1992−94).[7−10] Given that both solution- and solid-phase sample manipulation are convenient and easily automated, the potential limitation to the solution-phase synthesis of chemical libraries is the isolation of the library members. Recognizing that if the advantages of the sample isolation characteristic of solid-phase synthesis could be incorporated into solution-phase synthesis, its nonlimiting scale, expanded repertoire of chemical reactions, direct production of soluble intermediates and final products for purification and assay, and the lack of requirements for linking, attachment/detachment, or capping strategies would make solution-phase combinatorial synthesis an especially attractive complement to solid-phase synthesis. The approach we introduced enlisted aqueous, acid, and base extractions for sample isolation, providing a simple and general approach to the solution-phase synthesis of chemical libraries on scales capable of providing 50−150 mg quantities of each member, Figure 1. In each step of the sequence, the reactants, unreacted starting materials, reagents, and their byproducts are removed by simple liquid/liquid or liquid/solid acid/base extractions, providing the desired intermediates and final compounds in high purities (>95%) irrespective of the reaction yields and without deliberate reaction optimization. Notably, schemes may be devised such that each intermediate, as well as the final product, may be not only isolated but also purified using this approach, and this distinguishes it from solid-phase techniques which typically facilitate isolation but preclude purification. In addition, our initial disclosure[7] also described the first use of solid-phase workup or quenching reagents (ion-exchange resins) to remove reactants and reaction byproducts in the synthesis of chemical libraries (solid/liquid extraction), although this is commonly

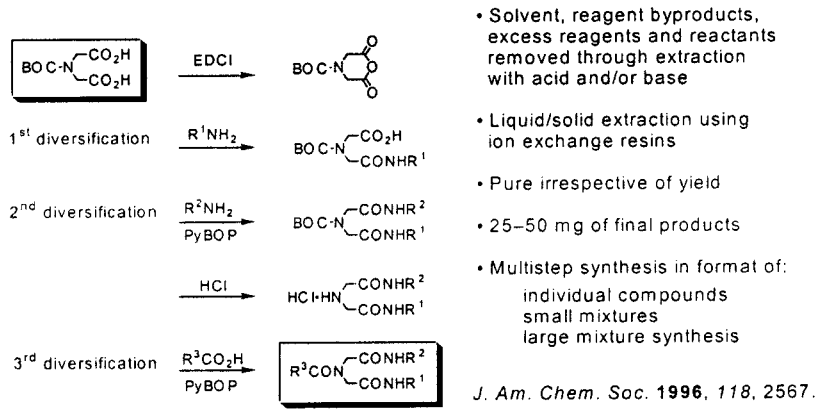


Figure 1. Multistep solution-phase synthesis of combinational libraries purification via liquid/liquid or liquid/solid extraction.

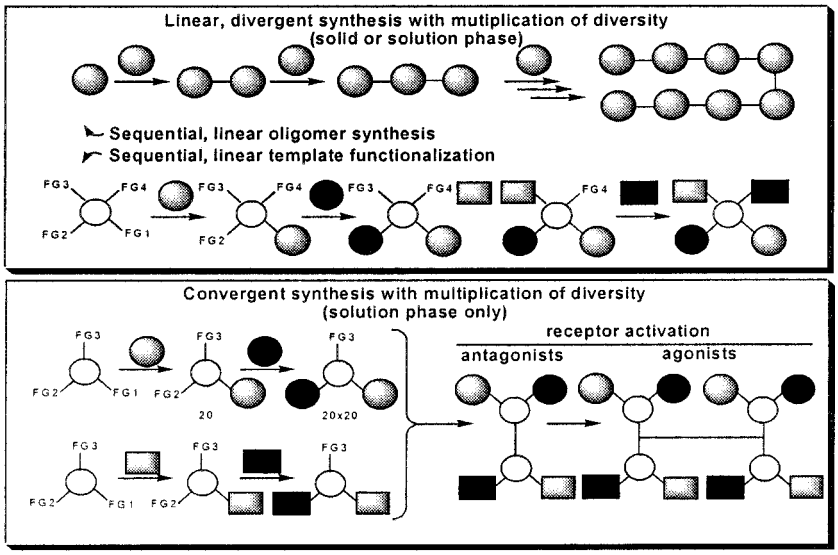


Figure 2.

overlooked or unrecognized. The protocol has been implemented in formats for the parallel synthesis of individual compounds[7−10] (1000 member libraries, individual compounds), modest sized libraries composed of small mixtures (1000−10 000 member libraries, 10−50 compounds/mixture),[11−13] or combinatorially assembled to provide large mixture compound libraries (25 000−1 000 000 member libraries, 10 000−28 000 compounds/mixture).[13,14] Notably, this latter mixture synthesis, which is easily conducted in solution, cannot be effectively conducted on the typical solid phases because of the need to use excess reagents to ensure complete reaction versus nonequivalent competing reaction rates which preclude formation of all intended compounds. Thus, the solution-phase protocol may be adopted in a format that is compatible with any screening objective or procedure, i.e., single compounds, small mixtures of 5−150 compounds, or large mixtures of compounds (>100−25 000).[15,16] In an opportunity that is not as easily accessible using solid-phase synthesis techniques, deconvolution of the large depository mixture libraries subjected to multiple assays can be conducted up front, in advance of the assay testing, by positional scanning or the complementary technique of deletion synthesis deconvolution[9] which we introduced. This has provided a powerful and rapid approach to lead identification. Thus, the solution-phase approach is convenient for both lead discovery or lead optimization and produces the library members on a scale (50−150 mg) that allows their repeated use in screening without resynthesis.[15,16] This ensures that the value of the libraries is not exhausted, but rather grows with time. Given the amounts of materials needed for each assay and the storage protocol we use (100 mM solution frozen in DMSO), they may be repeatedly dispensed for routine screening and are of the amounts that should ensure their continued availability throughout my scientific career. It is this feature along with its technically nondemanding implementation that we consider among its greatest attributes.

Conceptually more important, it is adaptable to convergent as well as the typical divergent synthesis with introduction of diversity.[13−18] Thus, unlike the divergent synthesis of libraries which may be achieved by either solution- or solid-phase techniques, convergent syntheses are especially suited for solution-phase techniques and are precluded by conventional solid-phase techniques since the combining components typically would be on mutually exclusive solid phases, Figure 2.

Although early applications focused on the use of simple templates such as N-Boc-iminodiacetic acid anhydride[19] (Figure 1) which are sufficiently general as to be useful against most biological targets, recent efforts have also addressed both nonamide containing product libraries and complex natural products including distamycin,[20] triostin A, and HUN-7293, Figure 3.

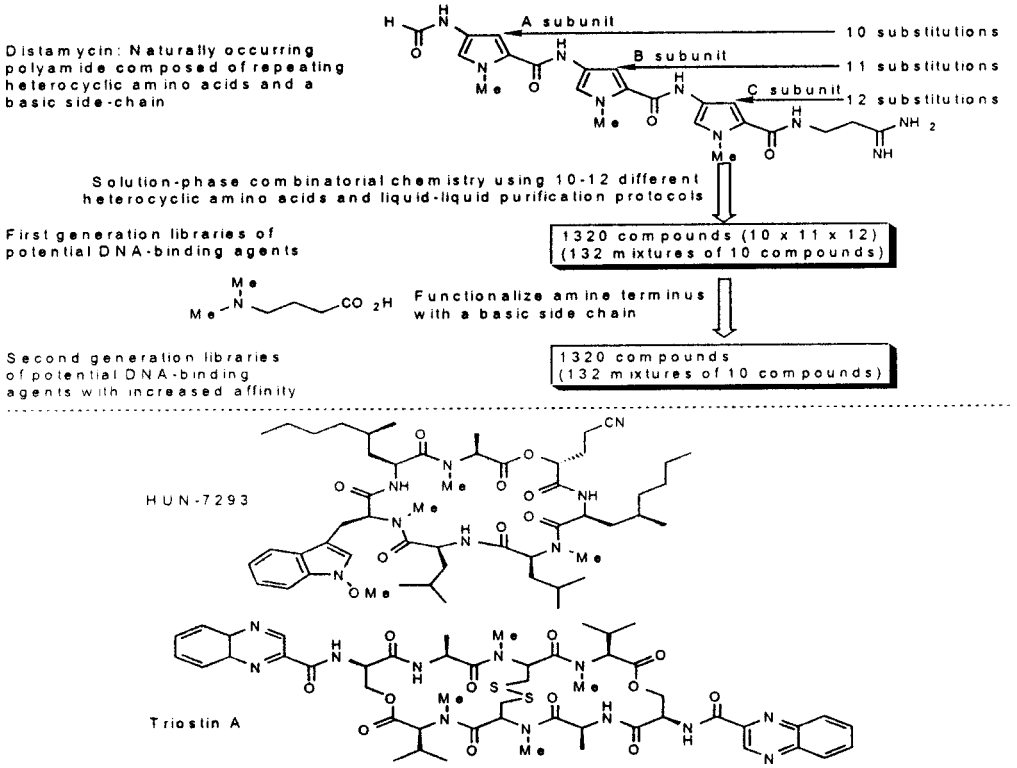


Figure 3. Multistep solution-phase synthesis of 2640 distamycin analogues.

As such, the approach whereby we use liquid/liquid or liquid/solid extractions to isolate and purify the intermediates and final products in the multistep synthesis of combinatorial libraries has provided a powerful approach to lead identification and optimization, even with a number of complex natural products. In addition, it served as one of the earliest prototypical examples of alternatives to solid-phase synthesis that have been utilized for the preparation of chemical libraries, it restablished the value of mixture synthesis, it stimulated interest in the development of various deconvolution schemes including those that may be conducted in advance of the screening, and useful extensions of this approach have now been disclosed by a number of groups.

David L. Coffen.[21] A Side by Side Comparison of the Features and Limitations of Solution- and Solid-Phase Methods

Combinatorial chemistry has its roots in solid-phase synthesis methods, and with equal certainty one can state that synthetic organic chemistry is rooted in solution-phase reactions. So neither method can be defined a priori as the *right* way to prepare compound libraries. In fact, the comparisons made herein present a strong case for the view that both methods bear features and limitations that are sufficiently complementary as to justify significant capability in both, if a discovery laboratory is to fully exploit the gain in function provided by combinatorial chemistry.

First the context in which these comparisons are made should be made clear. Both solution- and solid-phase combinatorial chemistries now come in many forms, depending on the type and scope of library required and the technology and instrumentation being employed. Here we are comparing methods for preparing relatively large libraries of at least 1000 individual compounds using highly automated parallel synthesis systems such as the Myriad system or ArQule's AMAP for solution phase and tagged, encapsulated aliquots of synthesis resin such as the Trega Teabag or IRORI AccuTag systems for solid phase.

The issues examined are listed below, together with +/− indications of how well each method addresses each issue. Comments and analysis are added to support the evaluation where appropriate. The issue list is deliberately ordered to convey a sense of clustered selection criteria.

Issue 1: Range of Accessible Reactions
Solution Phase: ++ Solid Phase: −

In principle, any chemistry and any reaction can be employed in solution-phase methods, including complex organometallic reagents, biocatalysis, etc. Few, if any, of the reactions and procedures documented in compendia such as *Organic Syntheses* and *Organic Reactions* could not be adapted to solution-phase combinatorial chemistry if suitable time and effort are applied. In contrast, a relatively small proportion of the synthetic repertoire has been adapted to solid-phase methods thus far.

Issue 2: Production of Congeneric Sets of Compounds in SAR Ordered Arrays
Solution Phase: ++ Solid Phase: −

The mechanical aspects of solution-phase parallel synthesis are such that this is almost an unavoidable outcome and makes this method particularly attractive for lead optimization. When solid-phase methods are applied most effectively, i.e., in a directed split and pool mode, the products may be presented in random order unless some pr-programmed presentation of the library has been introduced with appropriate software.

Issue 3: Use of In-Process Controls
Solution Phase: + Solid Phase: −

In-process control methods, particularly TLC and HPLC, are the cornerstone of quality outcomes in conventional synthesis. To some extent, at least, these techniques can be applied to solution-phase combinatorial chemistry, particularly so in the early stages of "convergent parallel synthesis" schemes.[22]

While solid-phase peptide synthesis is nicely monitored by the Kaiser (or ninhydrin) test for completeness of coupling, few, if any, comparable in-process methods have been developed for other kinds of solid-phase chemistry. The use of on-resin FTIR to monitor the introduction or consumption of carbonyl groups is probably the most useful technique developed thus far.

Issue 4: Effort Required To "Combinatorialize" a Synthetic Reaction or Scheme
Solution Phase: + Solid Phase: −

Adapting viable reactions to solution-phase combinatorial chemistry entails a set of problems which are routinely solved in a month or two. They are fairly standard (see Carmen Baldino's discussion of this topic earlier in this Perspective) and address things such as control of stoichiometry, capturing a broad range of reactivity within a single set of reaction conditions, and product quality assurance. However, adapting a standard reaction to solid-phase methods entails additional and frequently difficult problems associated with finding suitably robust and versatile linkers, points of attachment, and developing reliable cleavage methods. These problems can be particularly thorny when the goal is lead optimization of a lead structure that offers no convenient "handle" for attachment to a resin (see next issue).

Issue 5: "Navels"
Solution Phase: NA Solid Phase: −

Each compound in a library produced by solid-phase methods will generally have a structural feature, usually a functional group, associated with its covalent linkage to a resin bead. This feature, whimsically but appropriately referred to as a "navel", becomes an obligatory part of every compound in the library—they will all be amines, phenols, acids, etc. depending on the type of linker used. Such features are not always desirable, and considerable effort is being invested in the development of "tracerless" linkers—often silicon-based. Another approach utilizes intramolecular displacement cleavage strategies in which a (desirable) new ring is formed at the cleavage stage.

All of the above is irrelevant to solution-phase methods.

Issue 6: Larger Scale Resynthesis of Bioactive Library Members
Solution Phase: ++ Solid Phase: −

This is a nonissue for solution phase as only conventional scale-up problems have to be dealt with.

Scale-up of active compounds prepared by solid phase would ideally be done with a solution-phase version of the synthesis; but the transition can be complicated by the role of covalent linkers, "navels", relative rates of side reactions, etc.

Issue 7: Choice of Solvents
Solution Phase: + Solid Phase: −

Compatibility with automated fluid-handling devices is the only solvent restriction for solution-phase methods. Problems that may exist, such as loss of accuracy in dispensing small volumes of highly volatile solutions in ether or dichloromethane, can usually be overcome with less volatile alternatives such as dioxane or tetrachloroethylene.

Solvent choices are much more restrictive with solid-phase methods. While cross-linked polystyrene resin beads may be insoluble, polyethylene or polypropylene used in containers, pins, etc. will deform or dissolve in many solvents at elevated temperatures. Another restriction stems from the swelling/shrinking characteristics of cross-linked polystyrene beads. Dichloromethane, THF, DMF, toluene, and (marginally) DMSO are all suitable in this respect but ether, methanol, ethyl acetate, acetonitrile, and water are not. Tentagel resins are compatible with more solvents but introduce several more restrictions.

Issue 8: Operating Temperatures
Solution Phase: + Solid Phase: −

Solution-phase systems equipped to prevent condensation of water vapor (or icing up) at the low end, or equipped to condense/reflux solvent vapor at the high end, can easily operate in the −20 °C to +150 °C range. In contrast, +100 °C is generally at or beyond the upper limit for most solid-phase systems—room temperature to 80 °C being the "comfort zone". It should be noted, however, that very low-temperature operations involving, for example, reactive enolates generated at −78 °C are more easily handled in a directed split and pool solid-phase mode (fewer batches).

Issue 9: Heterogeneous Reagents
Solution Phase: + Solid Phase: −

Chemistry involving heterogeneous catalysts such as palladium on charcoal or reagents such as manganese dioxide demands only suitable agitation from solution-phase methods. However such reagents and catalysts are intrinsically incompatible with solid-phase synthesis methods.

Issue 10: Scavenger Resins
Solution Phase: + Solid Phase: NA

The use of functional group specific, reactive resins (e.g., resin-bound isocyanates for primary and secondary amines) is a significant advance in solution-phase methods. In appropriate cases it allows the use of excess reagents to drive reactions to completion, followed by facile removal of excess reagent from the product solution.

The technique has no relevance to solid-phase methods.

Issue 11: Cost of Reagents and Materials
Solution Phase: + Solid Phase: −

Solution-phase methods encourage parsimony in the use of reagents as any excess can become a product impurity. Conversely, solid-phase methods foster profligate use of reagents to drive reactions to completion. Added to these considerations is the fact that speciality resins, "hi-tech" linkers, and single-use resin containers can add substantial material costs to library production.

Issue 12: Abundance of Literature Precedents
Solution Phase: ++ Solid Phase: +

Given the existing corpus of synthetic organic chemistry, it is inconceivable that a competent combinatorial chemist could ever run out of interesting library ideas; such a notion suggests that medicinal chemistry could have an end-point. However, the literature favors those using solution-phase methods as most precedents can then be directly adapted to a combinatorial setting. The literature of solid-phase precedents from which solid-phase practitioners can draw is far more limited, but rapidly growing—a fact to which this journal attests.

Issue 13: Location-Based Sample Identification
Solution Phase: ++ Solid Phase: −

The mechanics of laying out reagent combinations in solution-phase parallel syntheses produce automatic linkage to a cornerstone of modern biology—the 96 well microtiter plate. The identity of compounds is spontaneously defined by coordinates of the space each one occupies in relation to all other members of the library. Things *are* where you put them! The downside of this system is that things must stay where you put them. A process step which may require a shift from parallel to serial processing, e.g., for extractive workup or chromatographic purification, entails risk of sample mix-up or complex mechanical and electronic control systems to preclude sample mix-up.

Solid-phase library syntheses end with cleavage of the products from the solid support, and at this stage, sample identification converges on the location-based system if the library is presented in microtiter plate format. The alternative is the use of individually labeled vials.

It should also be noted that the generation of SD files that link compound structures to samples in specific locations is more straightforward with solution-phase parallel synthesis and can be conveniently done as a first (or virtual) library production step.

Issue 14: Tagging-Based Sample Identification
Solution Phase: − Solid Phase: +

Tagging techniques exist for both micro- (e.g., cosynthesis of halogenated benzoates of fatty alcohols or differential isotopic labeling) and macro-sample identification. The latter may utilize simple labels, barcodes, 2D-barcodes, or radio frequency transponders. The use of passive tagging techniques allows free movement and transfer of in-process materials without loss of identity and, therefore, places no restriction whatsoever on the selection of process methods. For simple and obvious mechanical reasons, the use of tagging techniques is highly compatible with solid-phase methods but clumsy, at best, with solution methods.

Issue 15: Capital Investment Required
Solution Phase: − Solid Phase: ++

This issue is directed to systems capable of routinely producing big libraries numbering in the thousands or tens of thousands of compounds. For *highly automated* solution-phase parallel synthesis on this scale (≥00 000 compounds/year), an investment of several million dollars may be required to build and equip a suitable facility. The items required include properly ventilated space, robotic weighing and dissolution capability, programmable fluid handlers, reactor stations with provision for heating, cooling, and agitation, analytical systems both for in-process control and product QC, workup, evaporation, purification, plate replication, and operating software—just to list the essentials.

In sharp contrast, solid-phase methods for directed split and pool production of large libraries are conducted in a standard chemistry laboratory with minimal automation. Modest capital investments are required for tag-reading/sorting devices, apparatus for cleavage and delivery of compounds to plates, and centrifugal evaporation. The instrumentation requirements for product QC are, of course, the same.

Issue 16: Maintaining Inert Conditions
Solution Phase: − Solid Phase: +

Carrying out reactions which are sensitive to air, moisture, etc. in thousands of individual vessels imposes serious engineering requirements on the operating system.

By contrast, setting up 10−20 reaction flasks, each containing a few hundred porous resin containers, is a simple extension of conventional laboratory procedures for carrying out reactions of this type.

Issue 17: Mass-Action Reagent Excess
Solution Phase: − Solid Phase: ++

The mass-action effect of driving reactions to completion with excess reagent, a defining feature of solid-phase peptide synthesis, works equally well in solid-phase combinatorial chemistry. The effect on product quality is entirely positive since the excess reagents are simply washed away.

The use of excess reagents in solution phase can only be contemplated when facile removal of the excess is possible, e.g., because of volatility or when scavenger resins can be employed.

Issue 18: Library Transformations with No Change in Diversity
Solution Phase: − Solid Phase: +

On occasion a synthetic step is used in which all library members at some intermediate stage undergo a uniform transformation. An example of such a step would be palladium-catalyzed carbonylation of a set of aryl halides. In a solution mode, this would entail setting up *N* individual catalytic carbonylations, technically quite a difficult operation, *N* being the number of individual library members. However, in a solid-phase setting, the entire library (of resin aliquots in porous containers) is added to a single reaction vessel and all *N* are transformed in a *single* batch.

Issue 19: Protecting Groups
Solution Phase: − Solid Phase: +

Complex molecule synthesis relies heavily on protecting groups. However, their use in solution-phase library synthesis is restricted to situations where their removal gives volatile byproducts, e.g., Boc or Cbz groups.

The use of protecting groups in solid-phase methods is unrestricted because of the washout feature.

Certain chemistries that produce nonvolatile coproducts are also a problem in solution. For example, Mitsunobu reactions, which produce phosphine oxide and hydrazide coproducts, work very well in solid phase but require major adaptation of procedures for use in solution.

Issue 20: Multistep Syntheses

Solution Phase: − Solid Phase: +

Multistep synthesis conducted in normal solution-phase parallel synthesis leads to rapid deterioration of product quality because of incomplete reactions and accumulating byproducts. "Convergent parallel synthesis" affords some improvement if the early steps involve a limited number of intermediates which can be isolated and purified and the number of truly combinatorial steps is held to a minimum (≤ 3).

While solid-phase methods may lack in-process control, the fact that most byproducts, reagents, and other impurities are easily removed at each step makes solid-phase methods much more compatible with multistep syntheses.

Issue 21: Use of Bifunctional Reagents

Solution Phase: − Solid Phase: +

Attempts to use bifunctional reagents such as diamines, diacids, diols, bis-epoxides, etc. in a monofunctional mode with solution-phase methods rarely give anything better than a statistical mixture of unreacted, monoreacted, and bis-reacted products (an exception is the high-fidelity mono-acylation of symmetrical diamines with α-ketoesters[23]).

The circumstances of soli-phase reactions lead to entirely different results. Only when two functional groups are widely separated (by >10 linear chain atoms) will both react, producing a new cross-link in the polymer. In the general case, one group reacts and the second remains poised for the next dissolved reagent to be added. For instance, the reaction of bis-isocyanates with aminomethyl-polystyrene readily affords an isocyanate scavenger resin.[24]

Issue 22: Access to Split and Pool Amplification

Solution Phase: − Solid Phase: ++

In time, the split and pool synthesis protocol may be appreciated as one of the most powerful mechanical levers ever devised for synthesis. However, its early application to the production of vast mixture libraries contributed to the hyperbolic (and largely unmet) expectations which formed around combinatorial chemistry in its neonascent years. There may be some very specific applications of split and pool in solution-phase methods but this is generally regarded as a formula for making a programmed mess.

The development of chemically passive tagging systems for solid-phase synthesis vastly enhanced the utility of the split and pool protocol. The enormous gain-in-function offered by split and pool can now be used to produce huge libraries of "pure", single compounds in multi-milligram quantities with minimal synthetic effort. For example, the production of a 160 000 compound library can be achieved in just 80 discrete synthetic operations if the library design embraces 4 sets of building blocks with 20 members in each set.

Conclusion. In terms of 22 routine operational issues viewed in terms of the respective features and limitations of solution- and solid-phase methods, the two show remarkable complementarity. The conclusions to be drawn are (A) that an optimally equipped and staffed combinatorial chemistry laboratory should afford ready access to both technologies and (B) that the sole arbiter of which one to use in a given instance should be Chemistry.

John W. Ellingboe.[25] Solution-Phase Synthesis in Lead Optimization

The use of solution-phase synthesis methods in drug discovery programs is increasing at a steady rate throughout the pharmaceutical industry. While this activity was started in core combinatorial chemistry groups who continue to be the primary practitioners, it is gradually being adopted by medicinal chemists across all groups. Of the various methods used in combinatorial chemistry, including solid-phase synthesis, solution-phase synthesis, fluorous-phase synthesis, and combinations of the above, solution-phase synthesis is still the most general approach to array production. Lead generation and lead optimization are the two basic uses of combinatorial chemistry in drug discovery, and the greatest near term impact is being realized in the latter area. The role of solution-phase synthesis in lead optimization, as well as issues and opportunities associated with solution-phase synthesis will be discussed in this article.

Solution-phase parallel synthesis provides an efficient and thorough route to optimizing an early lead in a drug discovery program. Much attention is focused on shortened optimization times that can be realized through parallel synthesis. This is certainly an important consequence, but of equal or perhaps greater importance is the more thorough examination of SAR that can be achieved through parallel synthesis. When compounds are made and tested in a serial fashion, it is likely that not all possible combinations of several sets of substituents will be prepared. Thus, the optimal combination within a series of compounds may be missed. However, if all possible combinations of several sets of substituents are made, the optimal compound within a series will be identified. This may have important consequences later in development where a compound with better potency or selectivity will fare better. Thus, even if significant time savings are not realized during the discovery phase, there may be longer term increases in efficiency. This consequence will become more concrete as more high-throughput methods are developed for assessing drug-like qualities such as solubility, absorption, metabolism, and toxicity. It will then be possible to use solution-phase parallel synthesis to optimize not only in vitro characteristics such as potency but also characteristics that are important in drug development.

As the range of assays for testing compounds expands to include those for drug-like qualities, purity requirements for individual compounds will become more stringent. To generate a meaningful set of data for each compound which can be used to rank or prioritize hits from an assay, it will be necessary that the purities be similar and high. Automated preparative HPLC purification has become straightforward,

and we use it routinely to purify all solution-phase parallel synthesis products. Routine purification has also had the effect of decreasing up front chemistry optimization time. Rather than spending months optimizing a reaction to give 80+% purity, one can spend days purifying compounds after synthesis and more quickly get the target compounds tested. It is still important that reactions in general proceed in good yield, but a 50% crude product purity can be accepted if purification is straightforward.

Additional methods that contribute to the purity of compounds involve combinations of solution- and solid-phase chemistry. The use of scavenging reagents attached to a solid support to trap excess reagents, unreacted starting materials, and byproducts can significantly improve the purities of products. Another strategy combining solid and solution phases is the temporary sequestration of a reactive intermediate on a solid support during a reaction such that it is only made available for the desired reaction pathway and not for side reactions. We have found this strategy very useful in areas such as reductive alkylation. This process is an example of a transient phase switching. Changing phases at each step of a synthesis is also practical in some cases. Since there are some reactions that are well optimized in the solid phase or that proceed better in solid phase, it may make sense to use these for part of a synthesis while using solution-phase methods for other steps in a synthesis. We have used this type of phase switching to produce several arrays. Thus, the division between solid- and solution-phase synthesis seems to be blurring somewhat. For a given array target, the choice of using a solution- or solid-phase approach may have to be evaluated for each step.

In addition to the expanding scope of methodologies available for parallel synthesis, there are still a number of opportunities in which solution-phase synthesis has been underutilized. A relatively unexplored area of solution-phase parallel synthesis is the use of natural products as scaffolds. Since programs with the goal of optimizing natural products tend to rely on derivitization of existing functional groups, such as alkylating hydroxy groups or acylating amino groups, they offer a good opportunity for the use of solution-phase parallel synthesis. With a polyfunctional natural product, derivitization reactions can be nonselective and can give low yields. However, with the automated preparative HPLC technology that is now available, the solution-phase parallel synthesis of natural product derivatives should become more routine.

Klaus Gubernator.[26] The State of Solution-Phase Combinatorial Chemistry Research

Solution-phase chemistry is the synthesis method of choice for evolutionary chemistry. Rapid succession of synthesis, purification, and testing characterizes evolutionary chemistry.[27] Large combinatorial virtual libraries of compounds that could in principle be synthesized are searched using predictive models. Only modest numbers of individually selected members of these libraries are synthesized per evolution cycle. The advantages of solution phase chemistry are (1) rapid development of new synthetic schemes, (2) easy miniaturization and automation, (3) broad choices of synthetic methods and conditions. The main disadvantage is production of varying yields and typically a variety of byproducts, but this can be compensated by purifying every compound through an automated LC-MS system.

Virtual libraries are built from synthetic knowledge and lists of available reagents. The scope of the reaction defines the composition of the virtual library of compounds that could in principle be synthesized.

Most of the reactions used are one-pot solution-phase chemistries, some are multicomponent,[28,29] and others are multistep reactions. Initial reaction research consists of identifying reaction sequences that employ forward compat-

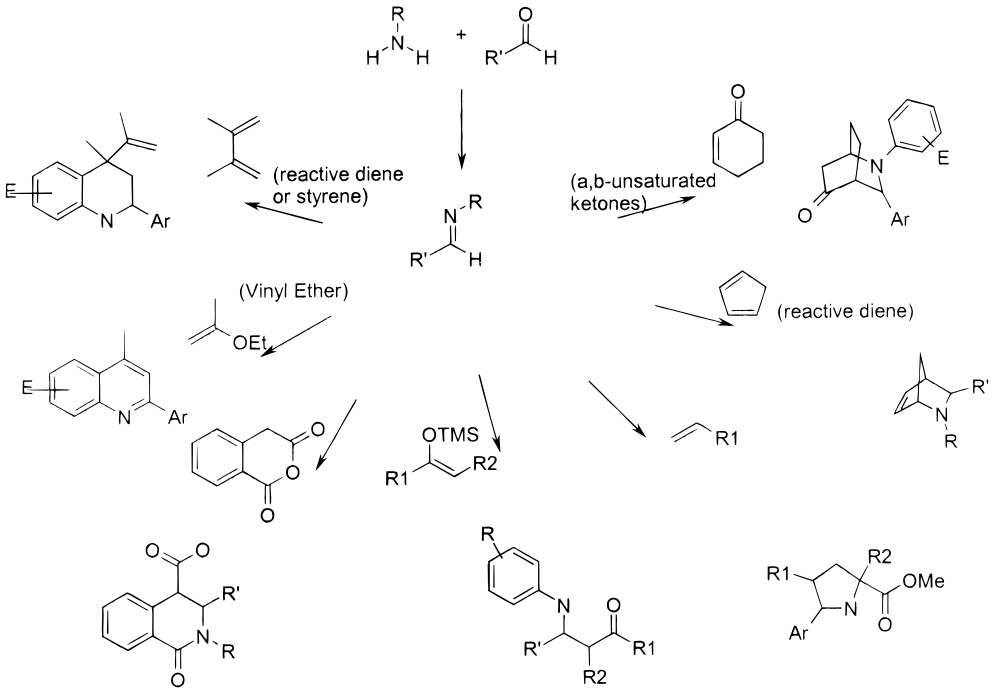


Figure 4. Imine-based reactions.

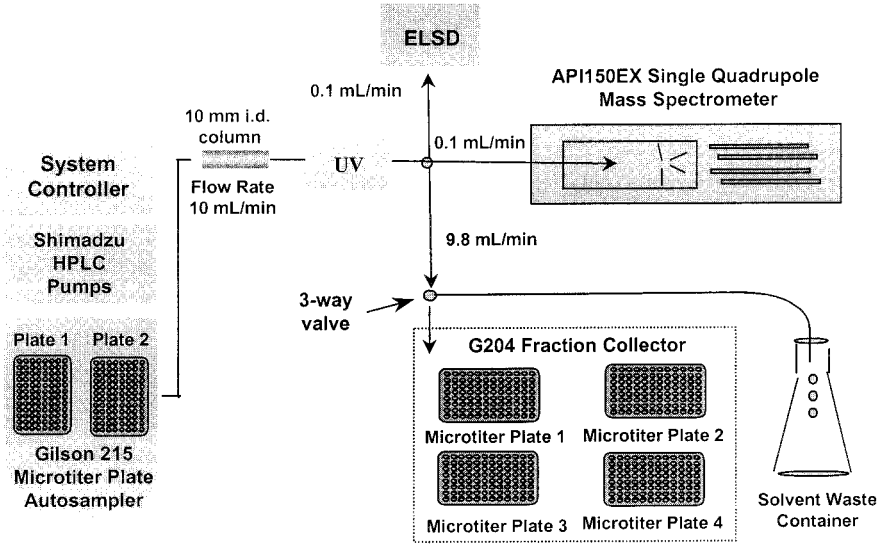


Figure 5. HPLC/MS/ELSD purification−quantification platform.

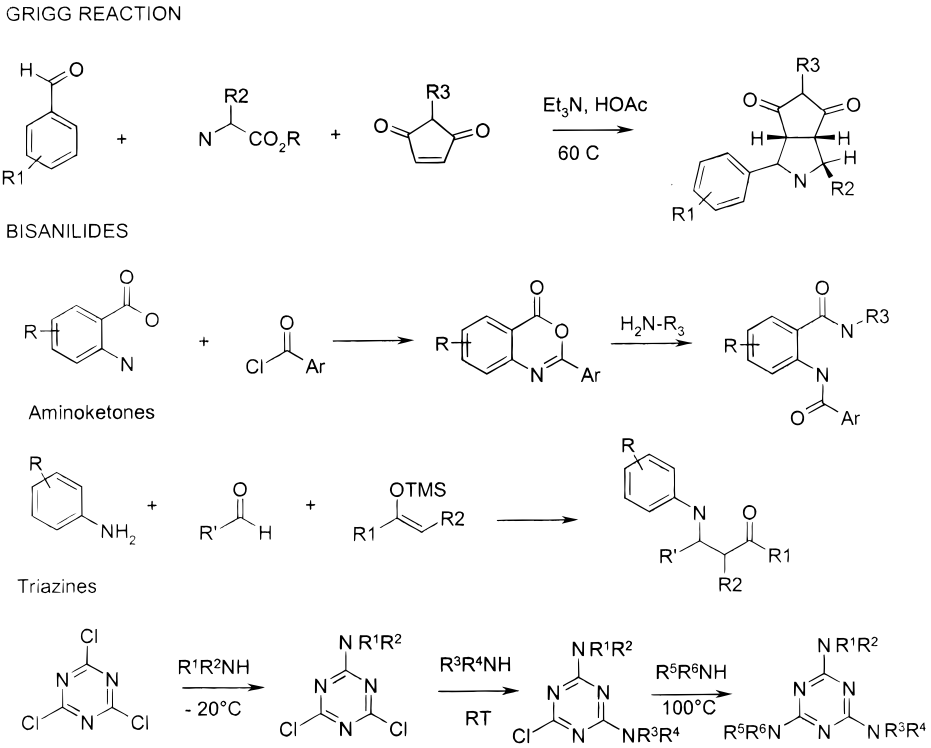


Figure 6. Some reactions used in the thrombin benchmark experiment.

ible reagents and require no separation other than evaporation between the steps.

The richest source, so far, of viable reactions is imine-based chemistry. Both aldehydes and amines are commercially available in large numbers and are of very diverse structures. These two components alone may be used to generate libraries of millions of compounds. Reacted with a third component, even of limited diversity, very large virtual libraries can be defined. Many of these reactions occur under mild conditions in convenient solvents (Figure 4).[30]

We have recently developed a large variety of combinatorial reactions on a robotic platform. A disposable 96 well microtiter-shape reactor allowing heating, cooling, shaking, and maintaining inert atmosphere is being used on a standard Tecan liquid handler. The 400 mL reaction volume is typically used to synthesize at the 10 mM scale. Specialized software integrates with the design process and the purification platform. All process related design, synthesis, purification, and screening data are captured in a central database. See Figure 5.

After dilution with an appropriate solvent, the crude reaction mixture is then injected into the automated LC/MS system. Only products with the desired molecular ion are collected into microtiter plates. At the same time, the isolated product is quantified using an evaporative light scattering (ELSD) detector. This occurs in a single operation. The system is set up to automatically purify and quantify multiple synthesis plates per day per instrument. Typical isolated yields range from 30 to 80%. Since most primary biological

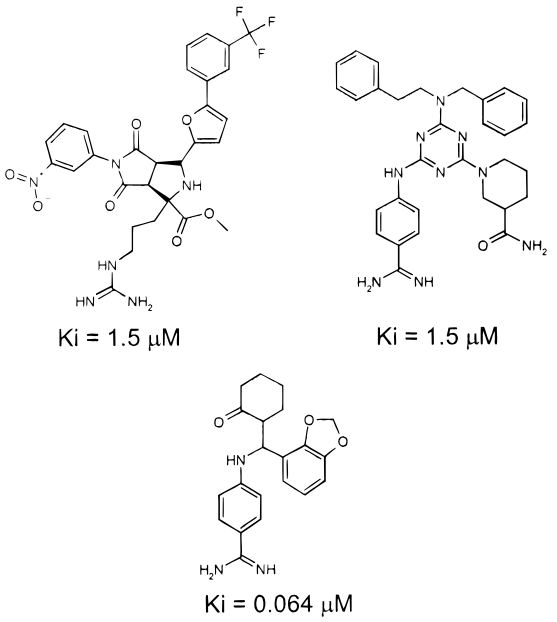


Figure 7. Examples of synthetic actives (human thrombin K_i).

assays require only very small quantities, even 10% yield can be useful for the discovery process.

In a recent benchmarking experiment, human thrombin inhibitors have been synthesized. The molecules were selected from a 250 000 molecule virtual library defined by five reactions. Examples[5−10] of these are depicted in Figure 6.

A predictive model has been constructed from 60 active molecules identified in the literature and about 6000 inactives identified by screening a diverse compound collection (Figure 7). The model is based on multiple 3D conformations of all of these compounds, and it reflects the consensus 3D requirements that discriminate actives from inactives. This model is then used to select promising candidate molecules from the virtual library which are then considered for synthesis. The average time for completing selection, synthesis, purification, and screening was 5−6 days. For a total of 891 compounds, synthesis was initiated, and 634 compounds were isolated in sufficient quantity (1 mmol) and purity (>85%) for screening. In the thrombin fluorogenic assay, 150 compounds were active (<25 mM K_i), and some compounds were in the 50 nM range.

This experiment demonstrated that a single optimization cycle yields active compounds in four of five synthetic classes. Most remarkably, none of the five classes were represented in the training set of known active and inactive molecules used to construct the predictive model.

It is therefore concluded that a rich choice of solution-phase reactions utilized in a rapid iterative process is an efficient discovery approach.

John L. Krstenansky and Peter C. Michels.[37] Solution-Phase Combinatorial Biocatalysis

The potential impact and applications of combinatorial biocatalysis can perhaps best be appreciated/understood by examining the natural function of enzyme catalysts and the evolutionary processes that created them. Nature has long practiced solution-phase divergent synthesis to create the unparalleled complexity of natural products. The broad array of chemistries required for the production of organic biomolecules all must occur under mild and uniform conditions within the living cell. Some of these reactions (such as aromatic or aliphatic hydroxylation, demethylation, decarboxylation, etc.) are difficult to reproduce using purely chemical means under any conditions. Since most natural products are polyfunctional and chiral, a high degree of catalytic selectivity is important. Thus, by necessity, enzymes have evolved to catalyze reactions with high catalytic efficiency, high selectivity, and with few byproducts on the full range of structures observed in Nature.

These characteristics allow biocatalysis to complement and augment the many strengths of traditional organic synthesis. In particular, enzyme reagents allow efficient catalysis for several reaction chemistries quite difficult to achieve by chemical synthesis. Also, the high selectivity and mild conditions for the broad range of biocatalytic reactions make them particularly suitable for modifying complex, polyfunctional, or labile molecules in a single step at high yield. Many of these characteristics of biocatalysis also allow extensive reaction schemes to be executed/automated using simple equipment.

Historically, biocatalysis has been used to identify cost-effective alternatives to chemical processes for the production of chemicals on a large scale. Relatively little attention has been paid to establishing *broad* biocatalytic synthetic capabilities in a single lab for chemical synthesis in a research format. The approaches differ in the following way: For efficient processes one needs to find a single, inexpensive and stable biocatalyst system that efficiently and cleanly transforms a given precursor molecule to the desired product. For research purposes, one needs many biocatalytic systems with broad and complementary specificities that need not be as highly efficient in their transformation to be useful.

EnzyMed has chosen to make such a broad, research-directed, biocatalysis platform and the smooth integration of this platform with traditional organic synthesis, its core technology. A major obstacle to these goals is the poor solubility of many compounds of interest in aqueous systems (the natural environment of biocatalytic systems) and, correspondingly, the substantial loss of activity of enzymes in organic solvents. To address these challenges, we have spent considerable effort to examine methods for using a variety of biocatalysts in organic solvents. In building our synthetic platform, we explore the range of conditions (solvents, temperatures, catalyst preparation, and immobilization techniques, etc.) and variety of substrates that each biocatalyst can accept and then use the resulting information to define a particular 'toolbox.' These 'toolboxes' have been developed over time until enough had been assembled to represent a useful synthetic platform that can transform virtually any molecule presented to it, similar to the biocatalytic abilities observed in Nature. Each of these 'toolboxes' (now numbering >500) has been miniaturized and transferred to a common screening platform that allows for rapid, parallel performance and analysis of hundreds of reactions to identify catalysts for each substrate and the resultant products from each reaction. Useful, effective

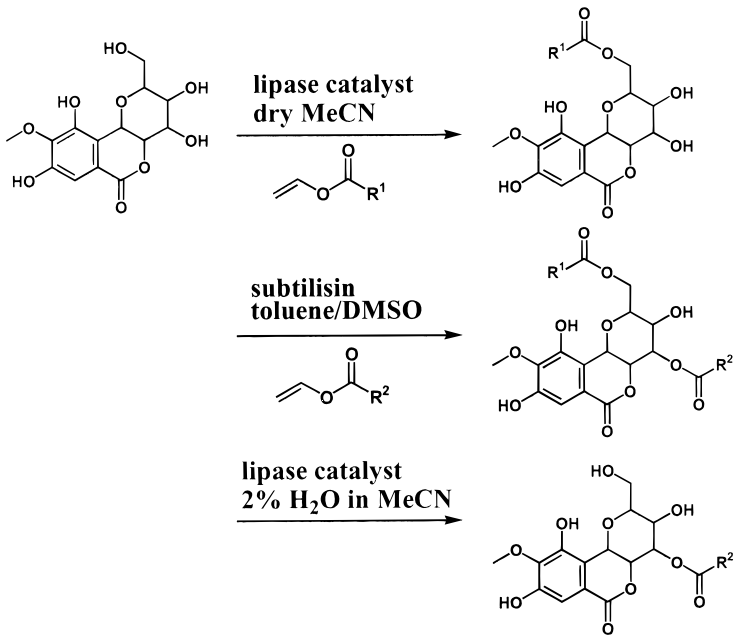


Figure 8.

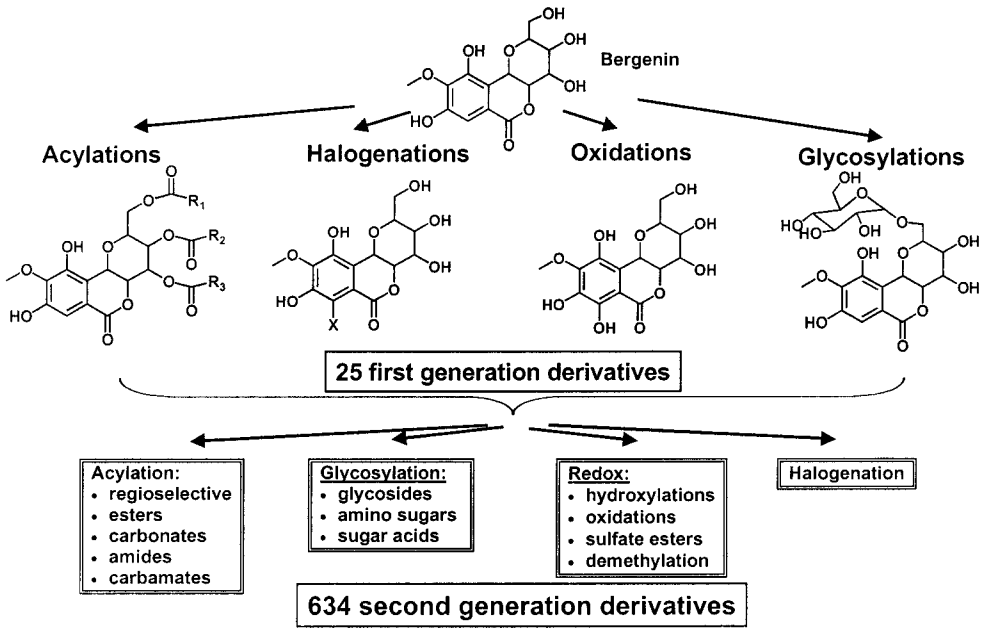


Figure 9.

catalysts can then be applied serially and in a combinatorial fashion to produce libraries of derivatives from each starting compound.

As an example, bergenin is a polyhydroxylated natural product. After rapid screening of the 'toolboxes' it was found that *Candida antarctica* lipase selectively acylated the 11-hydroxyl group in anhydrous acetonitrile while subtilisin in dry toluene/DMSO acylated both the 4- and 11-hydroxyl groups. By combining these reactions individually or serially in both the forward and reverse directions (acylation/hydrolysis) we could selectively make any combination of selectively 4- and 11-, mono- and diacylated derivatives of bergenin (Figure 8). Additionally, these reactions could be applied in combination with other biocatalytic modifications, such as halogenations oxidations and glycosylations, to produce a library of derivatives of the original starting compound (Figure 9). Thus, combinatorial syntheses are achieved in this derivatization approach by serially combining biocatalytic and chemical steps rather than, as in most combinatorial chemistry, combining reagents in a single reaction scheme to create a library of compounds. This alternative approach allows for the efficient creation of diversity from existing lead molecules or from existing libraries of molecules.

Marc L. Snapper.[38] Journal of Combinatorial Chemistry: Perspective Article

My colleague Amir Hoveyda and I have had a longstanding interest in developing better ways of synthesizing optically pure molecules. In this regard, the introduction of

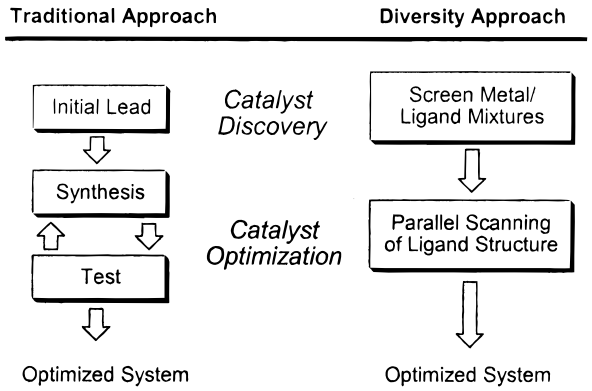


Figure 10. Strategies for reaction development.

new catalytic asymmetric transformations is a vital area of research. Discovering better ways of making necessary molecules faster is particularly important given the globally competitive nature of the chemical and pharmaceutical industry.

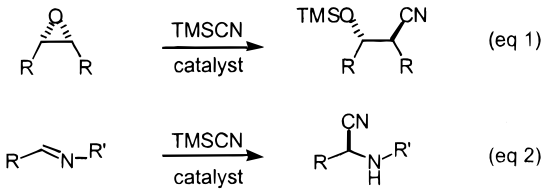
Fortunately, the value of research in asymmetric catalysis has been established clearly through several seminal contributions. Typically, the practitioners of asymmetric reaction development start with some preliminary information indicating a possible pathway of success (Figure 10, traditional approach). Guided by this lead information and their resulting mechanistic hypotheses, preliminary compounds are prepared and tested for the desired reactivity. The results are then fed back into the hypothesis, allowing improved designs to be incorporated into their reaction systems. This cycle of synthesis and testing continues until a solution that meets their needs and objectives is obtained.

Notwithstanding the demonstrated power of this traditional strategy, we were curious to see whether diversity techniques used so successfully within the context of biological and pharmaceutical challenges could also be applied to the development of new catalytic enantioselective reactions. The potential for this strategy was clear: reaction development through a diversity approach would not be limited by uncertain mechanistic hypotheses,[39] the opportunity for serendipitous discovery should be magnified, and importantly, the time required to reach suitable solutions could be considerably reduced.

Given that these diversity strategies were a radically different and unproven formula for reaction discovery, and knowing that the likelihood of discovering a new catalyst for a known reaction should be fairly similar to finding a catalyst for an unknown transformation, we decided that the true test of this diversity approach would be best evaluated in the development of an unknown asymmetric transformation. The reactions we examined are illustrated in Scheme 1.[40]

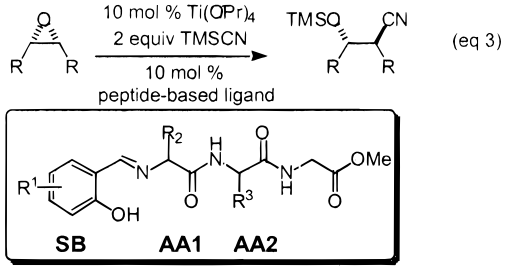
The first significant boost to our program came in 1995 with a report from the Ellman laboratories.[41] The Ellman team used a diversity strategy in the optimization of ligands for an asymmetric addition of diethylzinc to aldehydes. The Ellman paper was then followed in 1996 by reports from the Gilbertson[42] and Burgess/Sulikowski[43] laboratories also highlighting the use of diversity strategies for reaction

Scheme 1. Asymmetric Reactions Developed through a Diversity Approach



development. Clearly we were not alone in recognizing the potential of this methodology.

However, unlike these early contributions, we sought to develop a new asymmetric catalytic process. In 1997, we reported on the desymmetrization of *meso*-epoxides with TMSCN, a reaction that had not been previously carried out in an enantioselective fashion (eq 3).[44] We found that Ti(O*i*-Pr)$_4$ modified by chiral peptide-based ligands catalyzes the asymmetric addition of cyanide into *meso*-epoxides. The development of this transformation was similar to most reaction development protocols except for how suitable catalysts were identified. We reasoned that di- and tripeptide-based ligands were particularly well suited for a diversity-based catalyst optimization strategy. These ligands can be prepared from readily available chiral building blocks in a combinatorial fashion on solid support using established procedures.



Indeed, we identified reactive and selective catalysts for this new transformation through a combinatorial selection process, but the variety of subunits available for our modular ligand system offered significantly greater diversity than we could readily evaluate. For instance, if there are 20 commercially available hydroxyl aldehydes (SB) and 20 amino acids, these individual subunits could be combined to make 20^3, or 8000, different ligands. Due to our inability to examine rapidly this many ligands in an asymmetric transformation, protocols that sample the diversity space were required.

We found that an iterative approach to ligand optimization yielded attractive results. In this method each of the three subunits in the modular ligand are optimized independently and successively (Figure 11). For example, one amino acid of the ligand system (AA1) is varied while the other two subunits are held constant. *tert*-Leucine was found optimal for the first position, and this structural element was then retained in successive generations. The second position (AA2) was altered, and *O*-*tert*-butyl-threonine was identified as the amino acid that offered the highest selectivity when *tert*-leucine was in the AA1 position. Finally, 3-fluorosalicylaldehyde was selected as the best hydroxyl aldehyde

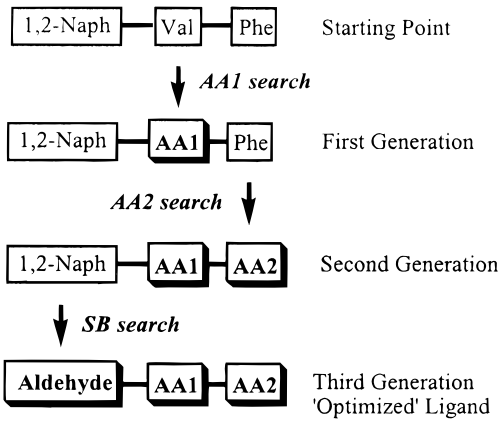
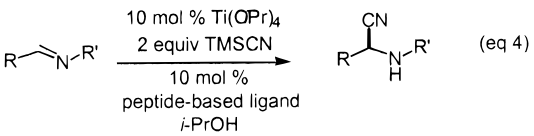


Figure 11. Iterative optimization of modular ligand.

Schiff base (SB) when *tert*-leucine was held constant in AA1 and *O*-*tert*-butyl-threonine was in AA2. This iterative screening of <1% of the 8000 possible ligands (20 + 20 + 20 ligands) led to a catalyst that afforded approximately a 95:5 ratio of enantiomers (er) for the opening of cyclohexene oxide with TMSCN (compared with a 63:37 er for the initially selected random ligand).

Is this the most selective ligand of the 8000 possibilities? Are the contributions to the reaction selectivity from the individual subunits additive? Are there cooperativity effects between the subunits that are missed by looking at only a subset of ligand structures? We will not know the answers to these and other questions until all the ligands from this diversity set are prepared and evaluated in the transformation. Nevertheless, reasonable results are obtained rapidly without the need of costly equipment, an aspect of the reaction development strategy that makes it accessible to anyone.

Can this optimization strategy be used to discover other catalytic asymmetric processes? Recently, we have applied the same ligand optimization strategy toward the development of an asymmetric Strecker reaction.[45] Equation 4 illustrates the transformation. Again, we find that new peptidyl ligands, which effect the desired reaction with high selectivity and yield, are identified through this diversity approach.



What does the future hold for combinatorial catalysis? Similar reports on the use of diversity strategies in reaction development are appearing at an increasing pace.[46] Its place as a useful tool for developing new or improved reactions has been established, and as this new methodology is explored further, the boundaries of its utility will become better defined.

Moreover, the prospects for using diversity strategies to answer other chemical questions are bright and exciting. Almost any area where the parameters influencing a system are not fully understood can benefit from a diversity-based strategy. Molecular recognition, material design, process optimization, and total synthesis are just a few endeavors that either have or should explore further the applicability of combinatorial methodologies.

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CC990064+