Solid-phase synthesis of combinatorial libraries

Michal Lebl

Address
Spyder Instruments Inc and Illumina Inc
9390 Towne Center Drive, Suite 200
San Diego
CA 92121
USA
Email: michal@5z.com

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Developments in the solid-phase synthesis of combinatorial libraries during 1998 are reviewed with emphasis on the rapid synthesis of libraries, new techniques for analysis of reaction progress and synthetic results, application of solid-phases as reagents, and synthesis of particular molecular entities. An alternative to solid-phase synthesis - fluorous-phase synthesis - is mentioned as an emerging technique that may compete with solid-phase synthesis in the future.

Keywords Library, mega-arrays, solid-phase

Introduction

It is extremely difficult to select and review only 30 papers published in a single year (1998) in an area of research that is expanding as rapidly as the solid-phase synthesis of combinatorial libraries. In the last year, we registered 384 papers and patents which qualified in both categories of solid-phase organic synthesis (excluding synthesis of peptides and oligonucleotides) and library creation. The inclusion of peptide synthesis increased this number further to 425. The creation and use of libraries was a topic of 1038 communications. In every issue of *Tetrahedron Letters*, there are at least two papers dealing with solid-phase synthesis. The reader of this review may be surprised by the relatively low incidence of articles from *Tetrahedron Letters* that have been cited, however, it was felt that due to the presence of this journal in almost all scientific libraries, indicating alternative literature sources may prove more useful. (All articles and patents are compiled in a database available on the Internet at *www.5z.com/divinfo*; the site is maintained and updated by the author of this review.) The selection of the material in this article was therefore influenced by the personal choice of the reviewer and particular emphasis was placed on publications describing novel approaches, emerging trends and simple solutions to problems encountered in solid-phase synthesis. There are no 'background' references quoted in this article, with the exception of the special issue of *Chemical Reviews* [1•], which may qualify as a general introduction to the area of combinatorial chemistry, and a rather unusual review of the history of combinatorial chemistry (including some very personal comments) written by the scientists who were opening this field in the 1980s and 1990s [2•]. There have been a number of books published concerning solid-phase and combinatorial libraries (for details see the above-mentioned Internet link), which give very comprehensive information. A really outstanding book is *The Combinatorial Index* by Barry Bunin [3••], which is an indispensable tool that should not be absent from any serious chemical laboratory that deals with combinatorial chemistry.

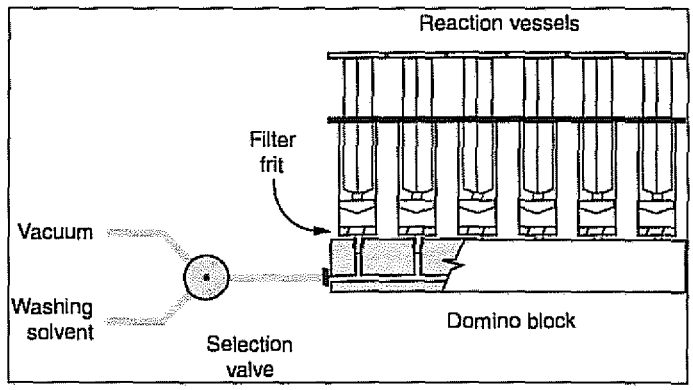
The following areas were considered of special interest for those working in disciplines applying solid-phase library approaches: (i) rapid creation of mega-arrays of organic molecules; (ii) developments in solid supports and linkers; (iii) solid-phases used as reagents; (iv) solid-phase synthesis of particular molecular types; (v) analytical characterization of prepared compounds and analytical tools for following organic reactions on solid support; (vi) new techniques utilizing the principle of solid-phase synthesis, but not using solid support; (vii) new applications of solid-phase libraries for studying intermolecular interactions; and (viii) interesting molecules found in combinatorial libraries.

Rapid creation of organic compounds mega-arrays

Since the keyword 'library' became so popular recently, it is being abused by some authors of scientific communications to 'dress up' their contributions. For example, an experiment in which four compounds were synthesized in parallel can be seen to be called 'a library synthesis'. Even though there is no magic number to determine if a compound collection should be called 'a library', four compounds would qualify rather as 'a small bookshelf'. A more accurate statement would be.... "four compounds were synthesized in parallel". The parallel synthesis of several compounds is nothing new and productive chemists were practicing it long before the word library was introduced to organic and inorganic chemistry, and long before the techniques of 'tea bag' and 'pin' synthesis, invented by Houghten and Geysen, were established as standard methodologies for multiple parallel syntheses. Some of these techniques have been repackaged to attract laboratories starting out in the solid-phase arena, eg, the radiofrequency (Rf) tag labeled MicroKan™ approach of IRORI. The principle was a solid support encapsulated in polypropylene mesh packets labeled with a human or machine-readable code (alphanumeric or bar-code label), which was improved by the inclusion of Rf tags used for tracking laboratory animals. The clear relationship to the tea bag technique is obvious from the fact that IRORI sells these MicroKans under license to a tea bag patent. This Rf tag technique is actively used in numerous locations and various chemistries have been applied to it, eg, the synthesis of a library of 432 tyrphostins, reported by IRORI's chemists [4•].

Several papers concerned with simple techniques have been published, including one in which the labeling of synthetic compartments was simplified for manual operation by inclusion of colorized plastic or glass beads and using color-coded caps [5]. This technique was exemplified on an uncomplicated synthesis of 96 pseudopeptides. Another technique involves the use of continuously divideable solid supports (membranes, threads) for the synthesis of non-statistical libraries; the technique was improved by Frank, who pre-labeled cellulose filter paper by laser printer [6]. By using scissors to cut out the pieces after each synthetic step and manually re-sorting the cut pieces, a library of 400 defined mixtures was obtained. It is speculated that this technology is applicable to the synthesis of up to 10,000-member libraries, with a last segment size of 2 x 2 mm.

Figure 1. Scheme of the principle of washing in the Domino Block synthesizer.



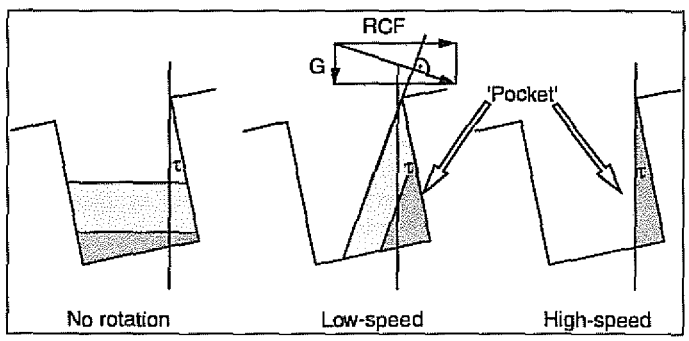
The original tea bag technique was used for the preparation of hundreds of solid support-bound intermediates, which were 'exploded' (distributed into individual wells) to deep-well microtiter plates. Tens of thousands of wells were then processed simultaneously, products cleaved from the resin in gaseous hydrogen fluoride, and sizeable libraries (10,000 to 60,000 members) prepared [7]. This technology became the basis for the preparation of large libraries, which are distributed under the trademark Chem.Folio™ by Trega Biosciences Inc (*http://www.trega.com/*).

The generation of tea bags requires a small investment in the tools necessary for their preparation, whilst the use of plastic syringes, described by Krchnák in 1989, does not require any special skills or equipment. Polypropylene syringes are filled with pre-cut plastic frit (syringes suitable for synthesis are available commercially) and all of the reagents are introduced through the needle. This simple disposable reactor was tested in numerous organic syntheses and can be arranged in a Multiblock, in which 42 syringes are processed simultaneously [8], or in Domino Blocks [9], where syringes with a 'frozen piston' are arranged for the washing step in four blocks of 12 or 24 syringes (Figure 1). A vacuum is applied to the syringes and by switching the valve, solvent is introduced. This simple arrangement allows for the simultaneous processing of up to 96 syringes, which would be difficult using a manual regimen.

A new approach to processing solid support in wells of a microtiter plate using a Spyder Instruments Inc synthesizer (*http://www.5z.com/spyder/technology3.html*), allowed the synthesis of up to 768 individual compounds in one batch [10]. In this synthesizer, the plates are attached to the perimeter of a centrifuge rotor and tilted towards the center of rotation. By spinning the rotor, the liquid overflows the wall of the well, while the solid support stays in the pocket created by the tilt of the plate (Figure 2). Cross-contamination does not occur since the liquid leaving one well never enters another well, but instead passes in to the inter-well space of the microtiter plate.

Another synthesizer, constructed by ProtoGene Laboratories, was designed for 96-well multititer plates and is equipped with filters at the bottom of each plate [11]. In this synthesizer, the plate is transported in the x-direction under an array of nozzles (eight banks of eight nozzles movable in the y-direction, and six banks of 48 fixed nozzles), so that each well can receive the appropriate reagent or solvent from any reservoir. Each line is equipped with a solenoid valve and liquids are driven by nitrogen pressure. The entire system is kept under slight overpressure of an inert gas and can be temperature-controlled in the range 5 to 80 °C. The images of the synthesizer are available on the Internet (*http://www.protogene.com/images/projects/large_combi2.jpg*).

Figure 2. A tilted centrifugation technique applied to a solid-phase synthesizer.



The situation of sedimented beads in the well during centrifugation of the tilted plate. During the high speed centrifugation, everything, with the exception of the pocket, is removed from the well.
(*Reproduced by permission of the Association of Laboratory Automation and Lebl M.* ***A new approach to automated solid-phase synthesis based on centrifugation of tilted plates.*** *The Journal of the Association for Laboratory Automation (1998) 3(8):59-61*).

The development of new instruments will continue, but simple techniques for array preparations will find their way into the laboratories much faster, since it is easier to adopt techniques for which no significant investment is needed, than to find funds to purchase expensive instruments.

Developments in solid supports and linkers

Solid-phase synthesis has been applied to a wide variety of organic and inorganic reactions, sometimes without detailed knowledge of the properties of the solid support, and with the only prerequisite that the reaction works. More recently, the focus has shifted to the influence solid supports have on reaction kinetics, intra- and intermolecular interactions, solvation of substrate and carrier, and selective adsorption of reaction components. A number of issues relevant to this were discussed in a comprehensive article by Bing Yan [12], and solid support and linker chemistries are the topic of a review by Blackburn in *Biopolymers - Peptide Science* [13].

Although useful solid supports are commercially available, the magical 'universal support' does not exist yet. Even solid supports which have proved successful for the syntheses of various molecular types, eg, benzhydrylamine resins, could be significantly improved by optimizing methods for their preparation [14].

Attachment to the resin can be of utmost importance; the product should be easily cleaved at the end of the synthesis, but stable throughout the transformations during the synthesis, and moreover, the attachment should not leave its 'mark' on the final compound. The concept of traceless linkers, where the site of linker attachment to the cleaved compound is not readily apparent, is very popular, even though the 'tracelessness' of a linker can be questionable. A variety of traceless linkers exist, including novel traceless linkers based on selenium chemistry. Here the aryl-selenium-alkyl bond is cleaved homolytically and selenium is retained on the aryl residue as tributylstannyl phenyl selenide [15].

Very close to the 'ideal' are linkers which are based on the principle of a 'safety catch', where the linker is inert to the conditions of the synthesis, and only after very specific activation can it be cleaved using relatively mild conditions [13]. In this case, the use of enzymes may become very convenient for the activation of the linker, eg, cleavage of an acetyl group by lipase RB001-05, generating a phenolate which fragments to quinone methide and releases the desired compound (Figure 3) [16].

Solid-phase used as a reagent

A solid-phase can be useful for quenching reactions and eliminating an excess of reagents in solution-phase synthesis. A polymer-supported quench (PSQ) methodology has been applied to the one-pot, three-component synthesis of multi-substituted 4-thiazolidinones [17]. In this synthesis, aldehyde, amine and mercaptoacetic acid were mixed and the unreacted components were removed by quenching with polymer-bound thiols and amines. In special cases, two solid supports can be considered, eg, in the synthesis of trisubstituted amines the release of product from the resin by Hofmann elimination was achieved by addition of the ion exchange resin Amberlite IRA-95. The Amberlite resin acts as a scavenger generating free amine from the salt in a catalytic cycle [18].

Polymeric reagent can be used to 'fish out' product from a solution synthesis by selective attachment, purification by washing and release from the polymer. This principle was recently tested, by Janda and Hori, on the selective purification of β-amino alcohols involving attachment to boranes immobilized on polyethyleneglycols. Intermittently formed 1,3,2-oxazaborolidines were isolated and amino alcohols were regenerated by treatment with acid [19].

A solid support can actually become a part of the synthesized molecule, or it can 'vanish' in the last step of the synthesis. This principle is illustrated in Figure 4, where the polymer prepared by ring opening metathesis polymerization (ROMP) forms a substrate on which the transformations (attachment) of R groups can be performed. After completion of the synthetic processes, the polymer can be disassembled into monomeric units, potentially with the introduction of additional functionalities. Barrett and coworkers demonstrated the principle of a vanishing support on the transformation of substituted oxanorbornene into 3-aza-8-oxabicyclo[3.2.1]octanes, with three potential points of diversity [20••]. The polymer was disassembled by ozonolysis with dimethylsulfide work-up and the generated dialdehydes were subjected to reductive amination without any purification. Products were obtained in yields comparable to standard solution methods (~30%).

Figure 3. An enzyme activated linker.

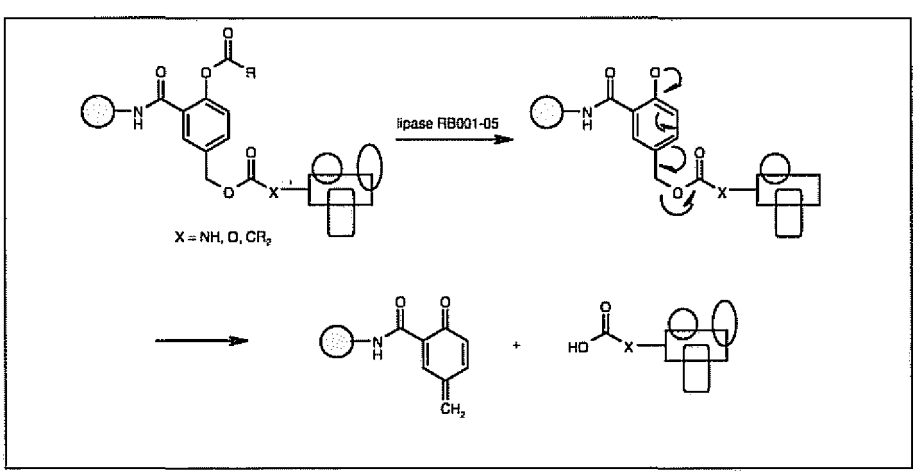
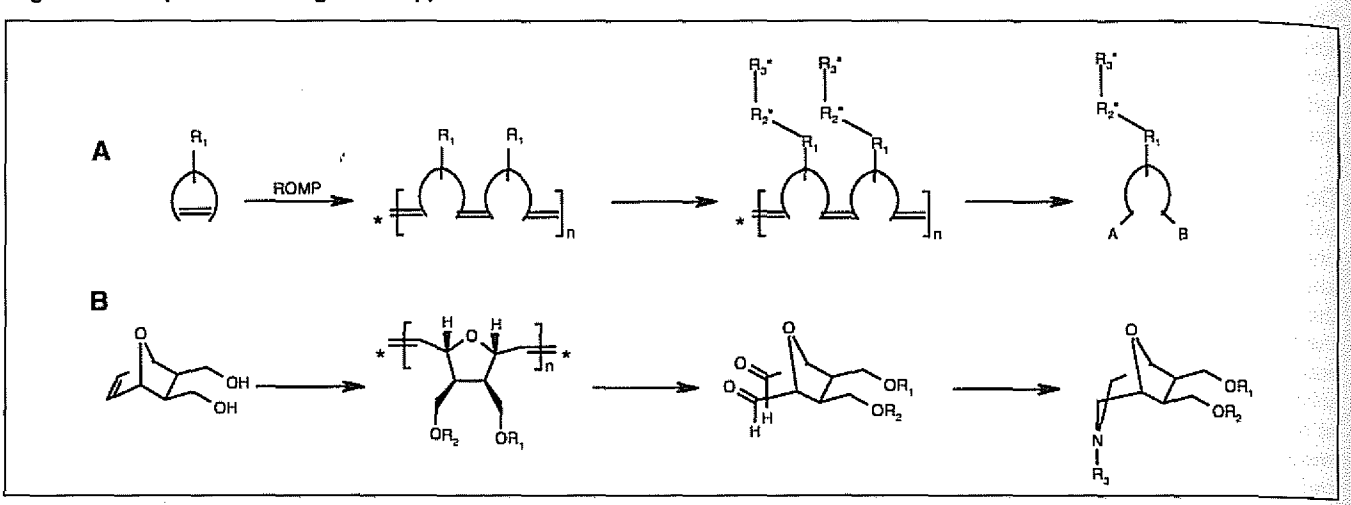


Figure 4. Principle of vanishing solid support.



(A) ROMP - ring opening metathesis polymerization; and (B) an example of the transformation of a 7-oxanorbornene derivative into trisubstituted 3-aza-8-oxabicylco[3.2.1]octane.

The principle of the resin activation/capture (REACAP) technology was developed by scientists at Sibia Neurosciences Inc [21]. In this technique, the reactive intermediate is formed on the resin and can be transformed into a stable, covalently attached molecule. The unreacted reactive intermediate is quenched and removed from the resin, leaving only desired product on the resin. An example of this principle is given in Figure 5, where the acylpyridinium complex formed on the resin reacts with the Grignard reagent forming dihydropyridone. The unreacted acylpyridinium complex collapses back to starting components during the work-up, thus simplifying the purification procedure.

Solid-phase synthesis of particular molecular types

Solid-phase synthesis methodology is being actively reviewed, and besides Bunin's classic [3••] there are two important reviews: Hermkens' coverage of solid-phase synthesis literature [22], and Brown's yearly review of developments in solid-phase organic synthesis [23]. The following examples illustrate the fact that essentially any type of molecule can be prepared using solid-phase synthetic techniques. The question that remains, however, is whether it makes sense to prepare a particular compound by solid-phase synthesis.

Oligosaccharides

The solid-phase synthesis of oligosaccharides is covered in a review by Seeberger and Danishefsky [24]. Papers published in this field reflect an increased interest in oligosaccharides as the source of molecular diversity [25,26]. Solid-phase synthesis methods appear sufficiently mature to be applied to the preparation of dodecasaccharides [27], and the synthesis of saccharide libraries is exemplified by a paper describing a two-directional approach for their preparation [28]. An interesting approach to solid-phase synthesis of oligosaccharides employs enzymatic coupling on Sepharose™ as the support [29].

β-strand mimetics

A recent paper from Kahn's laboratory describing the synthesis of libraries of β-strand mimetics is an example of simple chemistries involving Diels-Alder cycloaddition for building libraries of bicyclic peptide mimetics in which inhibitors of thrombin, trypsin, tryptase and kallikrein were found [30].

Figure 5. Example of RECAP technology.

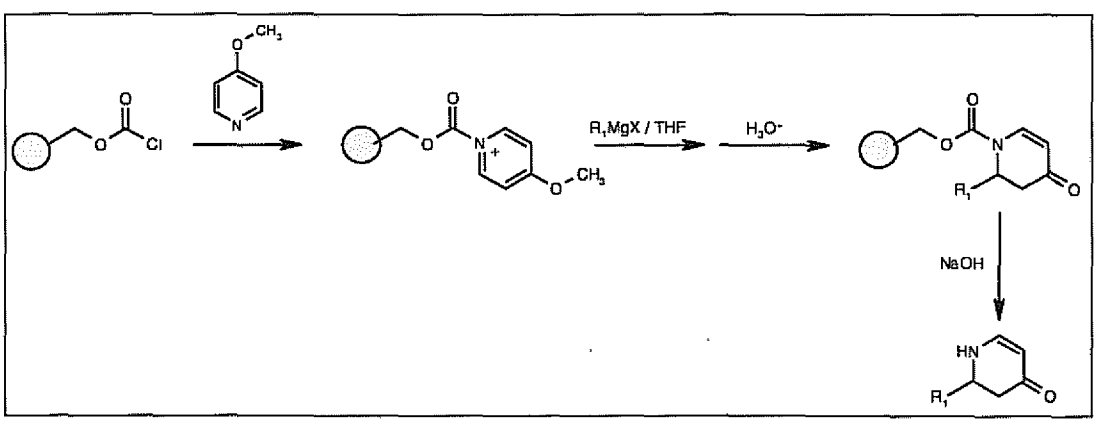
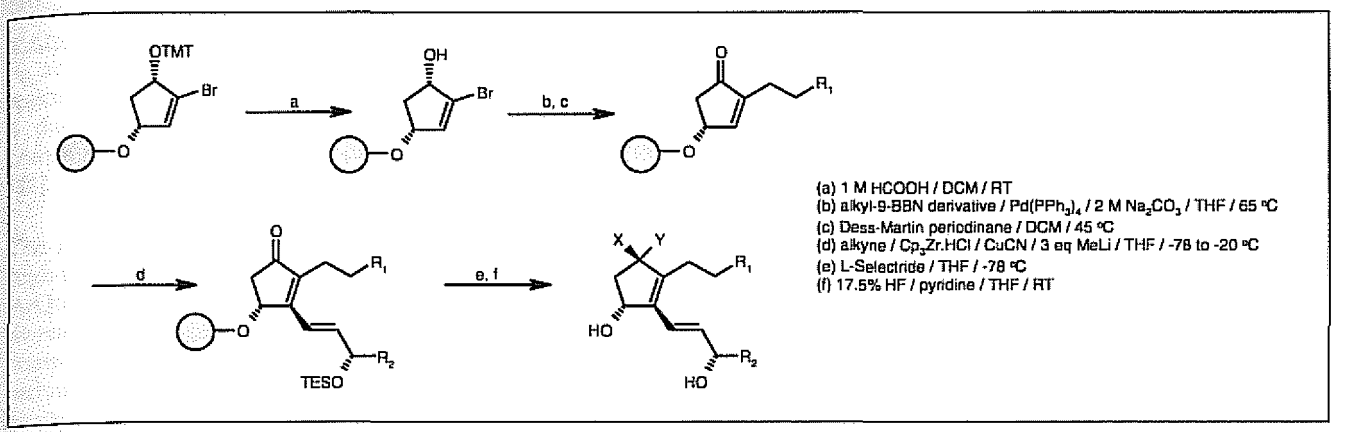


Figure 6. Synthesis of prostaglandins on solid support.



Polyketides

Boron-mediated aldol reactions with aldehydes were described as a convenient way for the stereocontrolled synthesis of 1,3,5-triols [31]. 4-Penten-1-ol was attached to the resin through a silyl linker and the double bond was ozonolyzed. The generated aldehyde was reacted with preformed chiral boron enolate with the induction of two steric centers. The aldol product was reduced *in situ* with $LiBH_4$ and the third chiral center was created. The final product was obtained in 71% yield with > 96% diastereoselectivity. This approach is amenable to the synthesis of polyketide libraries.

Prostaglandins

Prostaglandins are delicate structures requiring highly sophisticated techniques for their synthesis. Ellman's group has taken on the challenge to prepare prostaglandins on solid-phase as an approach to the rapid preparation of arrays of this class of molecule [32••]. Synthesis of the 1-series prostaglandins is illustrated in Figure 6. The selectively protected diol was attached through a dibutylsilyl linker, which was selected for its mild cleavage conditions, ie, diluted HF/pyridine. The final cleavage step required optimization of hydroxyl protection, and the trimethoxytrityl group was found to be optimal (cleaved by 1 M formic acid in dichloromethane in 5 min). The first diversity site was introduced by Suzuki cross-coupling with alkylboranes, which could be generated *in situ* by hydroboration of terminal alkenes. The secondary alcohol was oxidized by Dess-Martin periodinane and the second diversity site was introduced by the addition of vinyl cuprates, prepared *in situ* from terminal alkynes by hydrozirconation followed by transmetalation. Products were obtained in 49 to 60% yield with > 95% diastereomeric purity.

Heterocycles

An elegant approach to heterocyclic molecules containing the 1-acyl-3-oxopiperazine skeleton was published by Patek's group [33••]. This strategy employs tandem *N*-acyliminium ion cyclization - a nucleophilic addition ring-forming process in which the linker to the resin serves as both the protecting group and activator for the process (see Figure 7). Bromoacetaldehyde is attached to the resin by transacetalization and the bromine is displaced by the amine. This process can be monitored by a convenient and relatively underutilized ion-selective electrode technique [34]. In the next step, the amino acid is coupled and acylated with an acyl containing a strategically sited nucleophilic group (or group that can potentially form a nucleophile). This nucleophilic group can be an amino group of α-amino acid, a thiol group of cysteine, an amino group of *o*-substituted benzoic acid, or a C-3 atom of the indole ring of tryptophan. Cleavage of the linker in formic acid leads to the formation of an *N*-acyliminium ion, which is intramolecularly quenched by the nucleophile, thus forming a multicyclic heterocyclic system.

Multicomponent condensations

Advantages of solid-phase synthesis are utilized in multicomponent condensation reactions. For example, the synthesis of tetrahydroquinolines via condensation of substituted anilines, electron-rich olefins and aldehydes. In principle, any component of the reaction can be attached to the solid support, and in this article, the authors have examined the attachment of aldehydes and olefins [35].

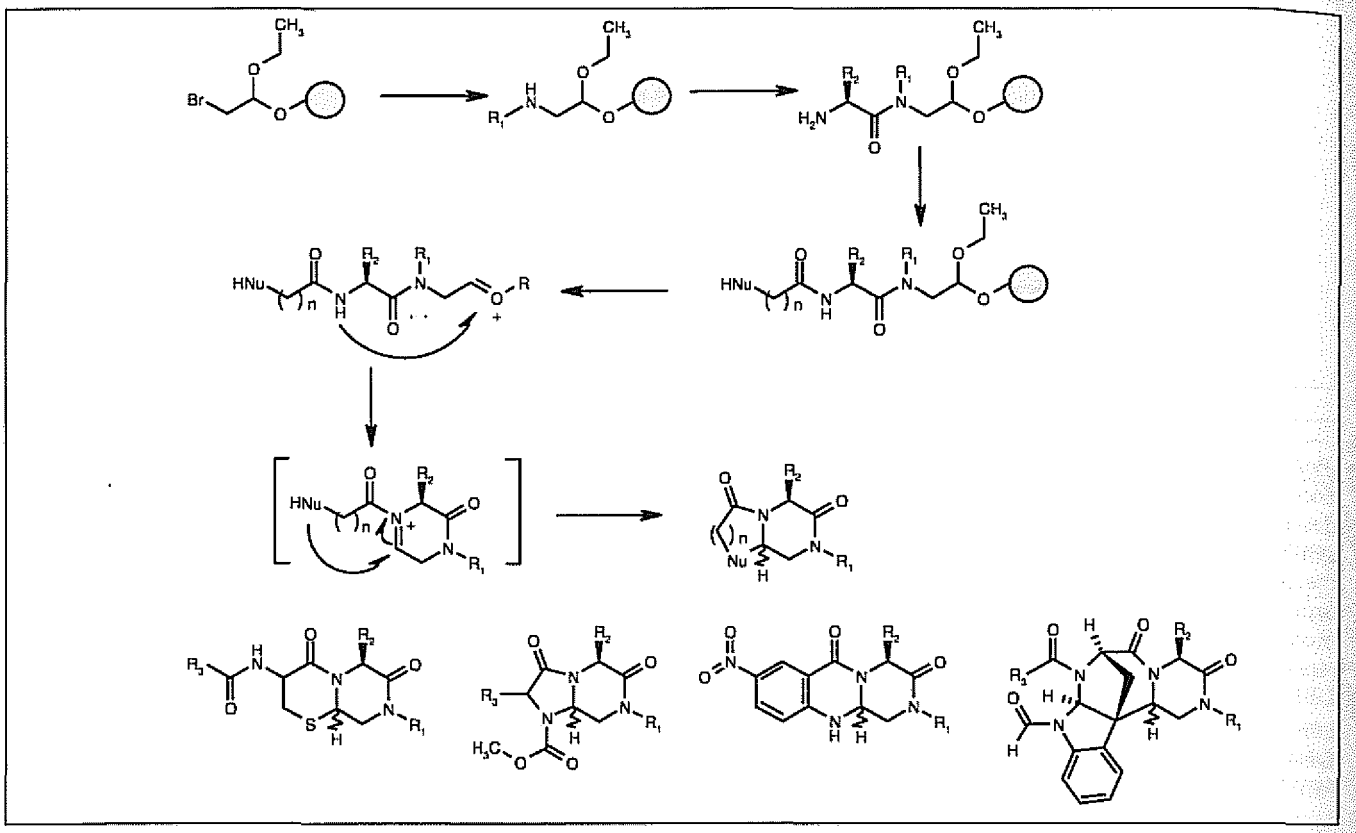
Polyguanidiniums

The synthesis of oligomeric guanidiniums was optimized and up to tetra-guanidinium oligomers were prepared. The process utilizes carbodiimide-mediated protected thiourea coupling to mono-protected diamines [36].

Analytical characterization and analytical tools used in solid-phase synthesis

The need for analytical support for the successful development of solid-phase organic reactions is evident. Analysis of the intermediates in solid-phase synthesis usually requires taking a sample, cleaving the product from the support and applying classical analytical techniques, eg, HPLC, MS or NMR. This approach is impossible in the case of split-and-mix synthetic strategies, where every bead contains a different product, and it is slow and inconvenient in all other cases. (There are the added problems that the intermediate is, unlike the product, sensitive to cleavage conditions, and the result of analysis can be completely irrelevant to the quality of the final product.)

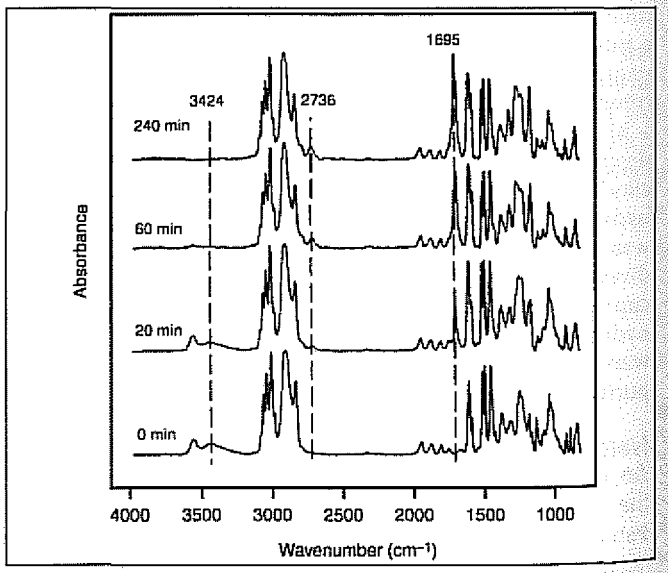
Figure 7. The synthesis of a 3-oxopiperazine skeleton by tandem *N*-acyliminium ion cyclization - a nucleophilic addition ring-forming process.



Non-invasive techniques for analytical evaluations have been developed. One advantage of mass spectroscopy (MS) is its extreme sensitivity, and although the molecules analyzed are destroyed, consumption is negligible and the method can be characterized as non-destructive if the compounds do not have to be detached from the solid support prior to the analysis. Secondary ion MS has been applied to monitor the progress of solid-phase synthesis on plastic pins (crowns) [37]. In this case, the solid support was subjected to primary ion bombardment and the emitted characteristic ions indicated both the solid support and the synthesized compound. Analyzed crowns could be used for further synthesis.

The evaluation of functional group transformations on solid-phase does not require full structural analysis of the synthetic product. Fourier transform infrared spectroscopy (FTIR) is a convenient method of following a reaction by identifying functional groups of intermediates. An experiment can be performed at the level of an individual bead, and the reaction can be followed in real time (see Figure 8). The results of a transformation can be quantitated based on the intensities of particular bands. The FTIR technique developed by Novartis (where this technique is used as an equivalent of TLC) was summarized by Bing Yan [38•]. Raman spectroscopy at the level of individual beads has been used to tag beads by including Raman- and/or IR-active groups [39]. One problem with this technique is with the compatibility of tags with the chemistry to be performed on the solid-phase. However, 'FTIR tagging' has been employed for simultaneous mapping of several hundred polystyrene beads and for the selective detection of an individual component of a small library of isoxazolidines, based on scanning the beads at singular wavelengths typical for individual functional groups [40••].

Figure 8. Reaction progress monitored at the level of an individual bead by FTIR spectroscopy.



*(Reproduced by permission of the ACS and Yan B. **Monitoring the progress and the yield of solid-phase organic reactions directly on resin supports.** Accounts of Chemical Research (1998) **31**:621-630).*

The NMR analysis of resin-bound molecules is usually characterized by broad lines (with the exception of TentaGel resins). Significant improvement in the quality of spectra can be achieved by the application of 2D-spin-echo correlated spectroscopy [41]. NMR has been shown to be applicable as a detector for HPLC, and its use was exemplified by identifying individual isomers of dimethoxybenzoylglycine [42].

The production of a large number of compounds requires high-throughput analytical evaluation of their quality. ArQule Inc and CombiChem Inc have independently developed strategies for the analysis of tens of thousands of compounds in a month. The use of a combination of various techniques is needed for reliable evaluation of compound purity depending on ionizability, chromophore content, and volatility of the product and impurities. The combination of flow injection MS and HPLC, with both UV and evaporative light scattering (ELSD) detection is necessary for the quantitative estimation of compound purity [43]. CombiChem Inc developed the PrepLCMS system, which is capable of analytical evaluation and preparative purification of synthesized compounds [44••]. This system utilizes a real time mass spectrometric signal to trigger fraction collection of only relevant (containing appropriate molecular ion) fractions from the preparative run. A chemiluminescent nitrogen detector was evaluated as a universal detector for nitrogen containing molecules and proved to be a widely applicable, sensitive (down to picomolar levels) alternative to UV and ELSD detectors [45•].

MS is the method of choice for decoding encoded one-bead-one-compound libraries. An elegant example of an encoded ladder synthesis, using partial blocking of a growing chain of glycopeptide with chemically similar building blocks, was published by Carlsberg Laboratory chemists [46]. A library of 300,000 glycopeptides was screened for binding to C-type lectin and specific ligands were decoded after photolytic release of the ladder from individual beads.

Investment in analytical equipment is necessary to speed up progress in this area. The development of analytical instrumentation has been extremely rapid and the cost of instruments is beginning to become more reasonable. Additionally, the formation of central laboratories serving several users and laboratories utilizing completely automatic instruments, operating 24 hours a day, 7 days a week, is not uncommon.

New techniques utilizing solid-phase synthesis, but not using solid support

Solid-phase synthesis is an efficient and convenient technique for the synthesis of organic molecules because it replaces the sometimes unpredictable behavior of synthetic intermediates with the predictable behavior of solid supports. However, reactions are performed in a heterogeneous phase and only soluble reagents can be used with reactants attached to the solid support. A technique, which would simulate the convenience of solid-phase synthesis and at the same time allow synthesis in solution would be welcome, and fluorous synthesis may hold a promise in this area [47•].

Fluorous-phase synthesis

In fluorous-phase synthesis, one of the reagents is attached to a high fluorine content block ('fluorous tail'), which assures that it always has a tendency to stay in a fluorocarbon-based solvent layer. Because some fluorocarbon-based solvents are immiscible (or only partially miscible) with both organic solvents and water, and this phase is, in most cases, the one with highest density, its properties can be used to mimic the solid-phase principle of synthesis. The fluorous-phase synthesis technology is at an early stage of development, and the general process for its application to combinatorial synthesis is only speculative. Fluorous-phase combinatorial synthesis should proceed according to the following steps. In the first step, the reaction vessels are charged with a fluorous phase, eg, benzotrifluoride, and the first component of the synthesis with an attached fluorous tail (a block containing a high proportion of fluorine atoms) is delivered to all compartments. Subsequently, the reaction is performed by addition of the building blocks and reagent and after an appropriate incubation the reaction mixture is worked up. The work-up includes formation of multiple liquid phases and the removal of upper phases. (This can be accomplished, for example, by centrifugation of a tilted plate using the instrument mentioned earlier in this review (Figure 2).) After the desired number of building block addition steps and washings, the final compound is present in the reaction vessel attached to the fluorous tail. The final compounds can be utilized either directly attached to the fluorous tail, or can be cleaved from the fluorous tail and purified by extraction. Examples of fluorous-phase synthetic protocols can be found in the literature [47•,48-52].

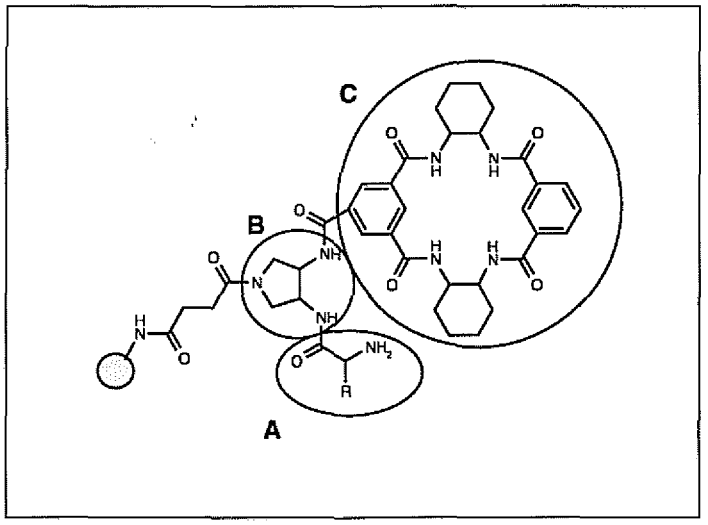
New applications of solid-phase libraries for studying intermolecular interactions

A new field has opened up for the application of solid-phase libraries in optical sensor arrays. Ultra-small solid particles (0.5 to 5 microns in diameter) can be immobilized in the etched end of a fiber-optic bundle, creating a miniaturized microtiter plate with wells of femtoliter volume. Since the beads are held firmly in place, the identity of each bead can be ascertained by the application of different encoding schemes and the array can be used as a sensor, or it can be used as a template for the identification of biomolecule binders. A density of $> 4.4 \times 10^9$ wells/cm^2 can be achieved, allowing for the generation of an unimaginable amount of data in a single experiment [53••].

Catalytic reactions can be conveniently followed by IR-thermographic detection. In this technique, a library of potential catalysts synthesized on polymeric beads is introduced into a solution of the substrate. The beads containing the catalyst are heated and can be visualized through the IR-thermographic camera. This technique was applied for the selection of catalysts for a simple acylation reaction [54••].

Still and coworkers are studying the interactions of small molecules with simple receptors. In a recent study, they observed amplification of a receptor for the solid-phase-bound tripeptide D-Pro-L-Val-D-Val. A two-armed receptor was discovered and modified by the introduction of a disulfide-based linker which can undergo a trans-sulfidation reaction. The equilibrium between unsymmetrical disulfide

Figure 9. Library of chiral selectors composed from three modules.



A 15 D- or L-amino acids
B RR- and SS-stereoisomers
C RRRR- and SSSS-stereoisomers

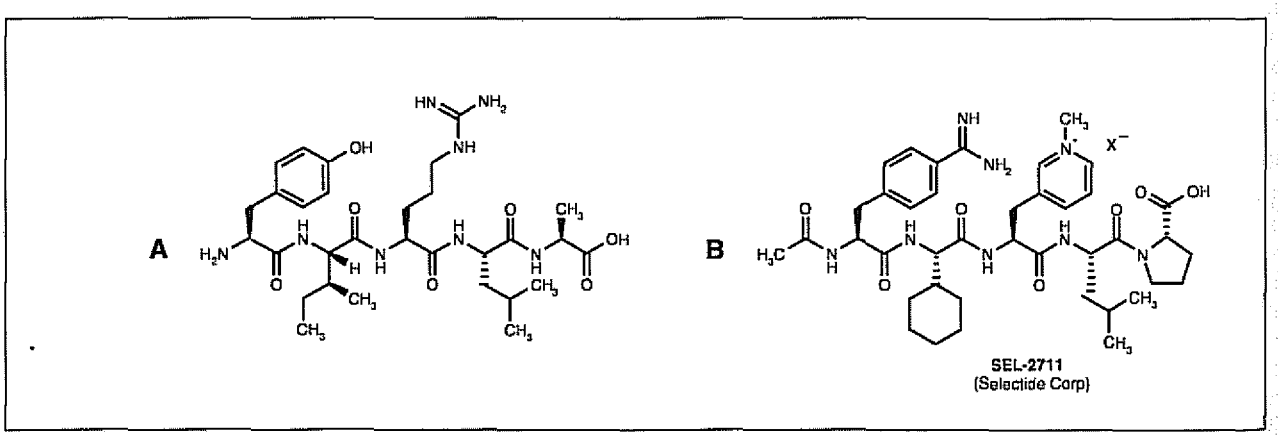
and symmetrical 'receptor' was shifted by 100-times in the presence of peptidic ligand. This paper shows that ligates for small molecules can be 'constructed' by assembling fragments on a solid-phase-bound template [55]. Another paper from the same laboratory describes the synthesis of libraries of chiral selectors composed from three modules (see Figure 9). Even though the library was relatively small, very powerful chiral selectors were found [56•].

Interesting molecules found in combinatorial libraries

The question, "If combinatorial libraries work so well, where are the new leads coming from them?", is less frequently asked as results are gradually published. Scientists working in industry knew that interesting molecules were being synthesized using these techniques, but most of the really interesting results were highly confidential and could only be published after patent application. An exhaustive review of leads found (and published) in combinatorial libraries has been recently published [57].

An inhibitor of factor Xa, discovered by Selectide Corp, is an example of an interesting molecule arising from a combinatorial library. This molecule resulted from the screening of a simple octapeptide library [58•] of 1.7×10^{10} members prepared in 1993. The original hit was modified by replacing the amino acids with an unnatural replacement (Figure 10), leading to a compound with high specificity (K_i = 3 nM for factor Xa and 40 μM for thrombin), stability (no loss of activity upon incubation with plasma proteins and proteinases), and reasonably bioavailability. SEL-2711 is now being clinically evaluated for its hemostatic properties. The early results were published after 5 years, in 1998 [58•].

Figure 10. Inhibitors of factor Xa.



A The molecule originally identified from the library.
B The optimized compound SEL-2711.

The one-bead/one-compound method was used by two groups to discover new ligands for integrin GPIIb/IIIa. In the first paper, the design of libraries of linear and cyclic oligocarbamates in which the structural features believed to be important for integrin interaction, ie, carboxylic and guanidino functionality were represented are described [59•]. Not surprisingly, ligands of a comparable affinity to the natural ones were found. In the second method, a library of D-peptides led to a ligand completely lacking the classical functionalities, Arg and Asp [60•]. The sequence, D-Pro-D-Tyr-D-Leu, appears to be a novel motif exploiting hydrophobic areas of the binding site. The results originally obtained in 1992 were not published until 1999 for commercial reasons.

Conclusion

The only conclusion that can be drawn at this point, is that the area of solid-phase synthesis of combinatorial libraries is growing extremely rapidly as are applications and techniques to evaluate it. The only limitation to an even wider application is the limitation of the scientist's own imagination. This review touches on some of the key points in solid-phase synthesis published during 1998, but because of the shear breadth of this field some material has been omitted.

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