Drugs of the Future 2003, 28(7): 659-678

CCC: 0377-8282/2003

Review Article

From the combinatorial chemistry boom to polymer-supported parallel chemistry: established technologies for drug discovery

Montserrat Cano and Shankar Balasubramanian*
University Chemical Laboratory, University of Cambridge,
Lensfield Road, Cambridge CB2 1EW, UK.
Correspondence and present address: Medicinal Chemistry
Department, R&D Center, Ferrer Group, Juan de Sada 32,
08028 Barcelona, Spain

CONTENTS

Abstract

The application of combinatorial chemistry has been changing since it was first described almost two decades ago. This review highlights relevant innovative ideas using the combinatorial chemistry approach, focusing on the present and future scope of this technology and its general acceptance among the scientific community. Recent developments include solid-supported reagents, catalysts, scavengers and purification; resin technology (polymer-supported chemistry on monolithic disks); reporter resins for solid-phase organic chemistry; dynamic combinatorial libraries; natural product-based libraries and chemical genetics; and microreactors.

Introduction

The term combinatorial chemistry can be broadly defined as the preparation of numerous organic compounds (*i.e.*, libraries) through rapid simultaneous, parallel or automated synthesis (1). The driving force to increase synthetic output has arisen from the capability of screening compounds for a particular biological activity in a high-throughput fashion. To accelerate synthetic organic chemistry processes, the solid-phase format, based on the Merrifield method of peptide synthesis (2), has been adopted. Early library synthesis was based on the parallel preparation of up to 10,000 discrete compounds (3-5). In this approach, a monomer is first immobilized on a resin bead and after several transformations on the solid support, a more elaborated small molecule is obtained (Fig. 1). The main advantages include the use of excess solution phase reagents to drive reaction equilibria to completion, the ease of purification by filtration and the potential for automation. The attachment of the starting material and the cleavage of the final product are additional steps necessary for solid-phase synthesis. Initial efforts in the emerging field were also focused on the development of new and more versatile linker molecules which permit mild cleavage reaction conditions and also withstand a wider range of chemistries (6). Furthermore, a systematic adaptation of a wide variety of synthetic organic processes to the solid phase has been reported in the literature (7-9). An important consideration when embarking on solid-phase organic synthesis is the difficulty in analyzing compounds attached to the support. This has been addressed by developments in analytical technology such as magic angle spinning (MAS) NMR or FT-infrared spectroscopy to characterize compounds on individual beads.

The concept of Furka’s split-and-mix procedure has had a major impact on combinatorial chemistry (10), permitting the synthesis of large compound mixtures in the format of one compound on each bead (11) to yield large numbers of spatially segregated small molecules. As illustrated in Figure 2, a quantity of resin is split into equal-sized portions that are placed into separate reaction vessels. The resin from all of the vessels is then recombined, mixed thoroughly and reapportioned into the requisite number of reaction vessels to perform the second synthesis step that adds diversity. By repeating the split,

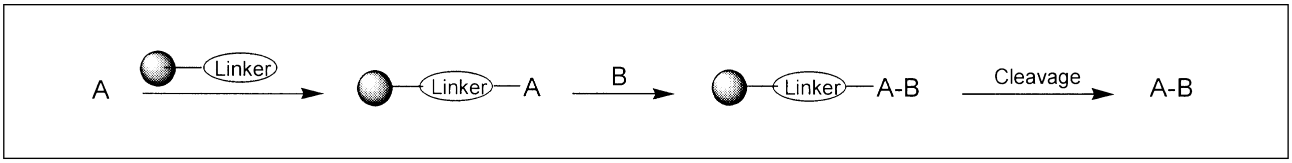


Fig. 1. Solid-phase synthesis.

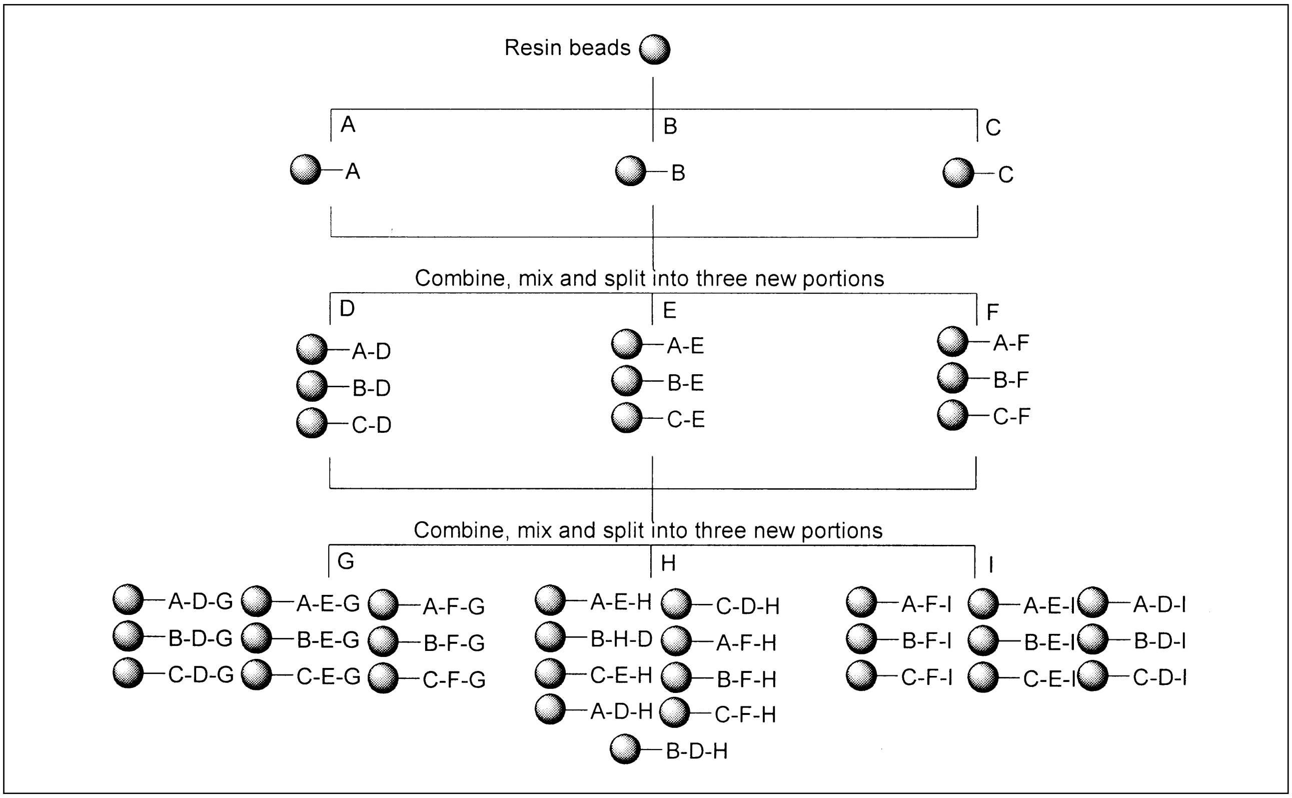
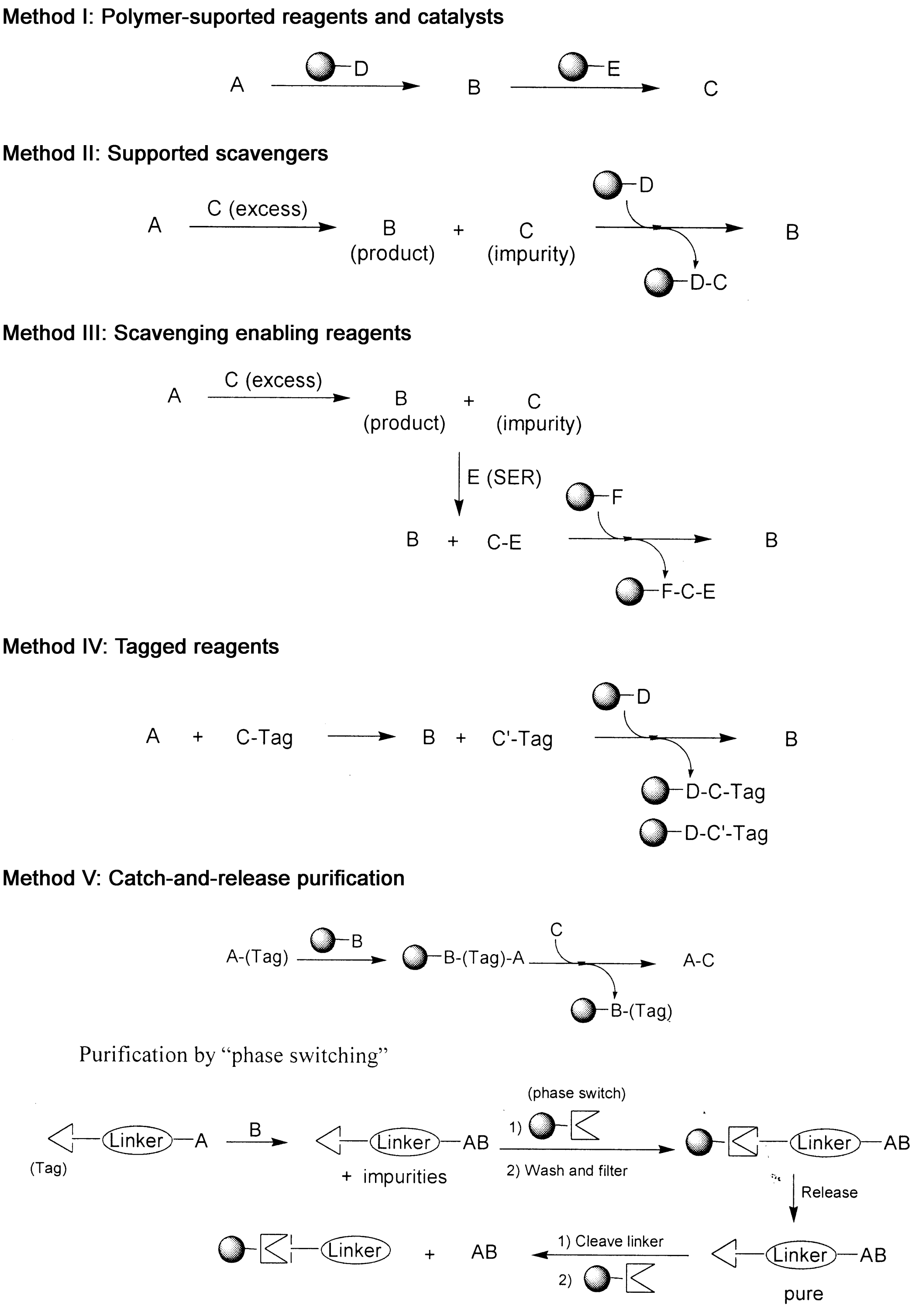


Fig. 2. Split-and-mix synthesis.

react and mix operations, compounds incorporating all possible combinations of the sets of different building blocks are generated. Because each bead reacts with only one type of building block at each cycle, only one type of product is displayed on each bead. The technique has the advantage that it drastically reduces the number of reactions required to produce a large number of compounds as compared to parallel synthesis. To take full advantage of this approach, a compatible high-throughput assay is essential to screen the resultant combinatorial library. The identity of the active compound from a screened mixture must be deduced by either using a suitable analytical technique on the product released from a single bead tested positive or or suitable encoding and deconvolution procedures. The encoding method traces the reaction history of the compound by incorporating an identifiable tag onto the bead at each step in the synthesis (12). A potential problem with screening a combinatorial mixture is that any activity may result from the sum of several moderately active compounds, therefore leading to a weakly active structure.

Owing to some of the uncertainty and the issues of reproducibility with split-and-mix libraries and screening of mixtures, the pharmaceutical industry has largely adopted the strategy of producing individual compounds in 5-50 mg quantities by parallel synthesis. To this end, organic synthesis on solid support still offers the advantages of high-throughput and automation. However, development and optimization of reaction conditions on solid phase can be time-consuming depending on the chemistry and its compatibility with high-throughput analytical methods. To solve the same problem, the approach of polymer-assisted solution-phase chemistry (PASP) has been further developed and adopted. This methodology is based on immobilizing the reagent on solid phase while leaving the substrate and product in solution. Unreacted starting materials or side products are removed from the solution via reaction with a functionalized "quench" resin

Scheme 1: Methods used in PASP synthesis



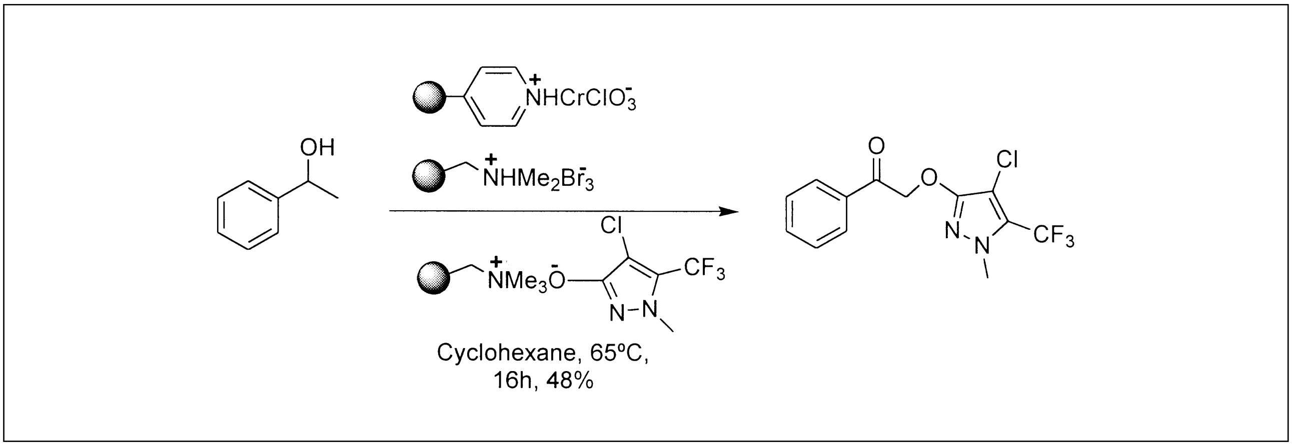


Fig. 3. One-pot transformation using solid-supported reagents that is not possible in solution.

to leave a relatively pure product in solution. Placing the desired product in solution greatly facilitates the monitoring of reactions.

This last development might at first be seen as a reversion to the classical chemistry approach, but it does embrace a significant change in chemical synthesis methodology. The boom of the combinatorial chemistry concept has led to the establishment of polymer-aided parallel chemistry as an essential technology that will undoubtedly lead to an acceleration in the process of drug discovery. The choice of using either solid-phase organic chemistry (SPOC) or PASP chemistry will depend on the compound synthesis in question and the purpose of the study. The combination of traditional medicinal chemistry thinking and the polymer-aided technology as a whole (SPOC or PASP) will form a major part of the new era for organic synthesis.

The aim of this review is to highlight some of the innovative approaches recently developed that have enhanced the field and will provide a robust platform for the future. These include solid-supported reagents, catalysts, scavengers and purification; resin technology (polymer supported chemistry on monolithic disks); reporter resins for SPOC; dynamic combinatorial libraries; natural product-based libraries and chemical genetics; and microreactors.

Polymer-assisted solution-phase chemistry

Polymer-assisted solution-phase (PASP) synthesis uses suitable modified polymers, either during the course of the reaction and/or during the purification process, to facilitate the generation of solution-phase chemical libraries (13). In this process substrates and products remain in solution while the reagents required to mediate the chemical transformation are on solid supports. PASP synthesis is based on the use of several methods outlined in Scheme 1.

The polymer-bound reagents (14-20) or catalysts (21-23) transform a substrate to a new chemical product and the excess or spent reagent may be removed by filtration (Method I, Scheme 1). They can provide the benefits of reduced toxicity or odor as compared with solution analogues. Interestingly, mixtures of reagents that are incompatible in solution may be used in combination when supported on a solid phase due to site isolation. An impressive example of a multistep synthesis involving a combination of three different polymeric reagents in one is shown in Figure 3, to afford depicted pyrazole in 48% overall yield (24).

Methods II-V shown in Scheme 1 are designed to facilitate the purification step. The removal of excess unreacted starting materials or by-products from a reaction mixture is typically achieved by employing polymer-supported reagents with complementary reactivities. Four classes of polymer-assisted purification have been reported as follows.

1) Solid-supported scavengers (Method II) are functionalized resins that covalently react with surplus reactants or by-products, which can then be removed by filtration. For example (14, 15), amino resins have been used to scavenger acid chlorides, acid anhydrides, aldehydes, ketones, imines, isocyanates, isothiocyanates and sulfonyl halides.

2) Sequestration-enabling reagents (SERs, Method III) transform by-products into chemically tagged species capable of polymer-supported removal. An example is the use of electrophilic tetrafluorophthalic anhydride (25) to react with an excess of amine forming an addition product which is effectively removed by a polymer-bound amine (Fig. 4).

3) Chemically tagged reagents (Method IV) are bifunctional molecules with the desired reactive site and a functional group tag which facilitates capture by scavenger resins. For example, a tertiary amine-tagged carbodiimide can be used for a Pfitzner-Moffat oxidation protocol and then be scavenged by ion exchange resin (Fig. 5) (26).

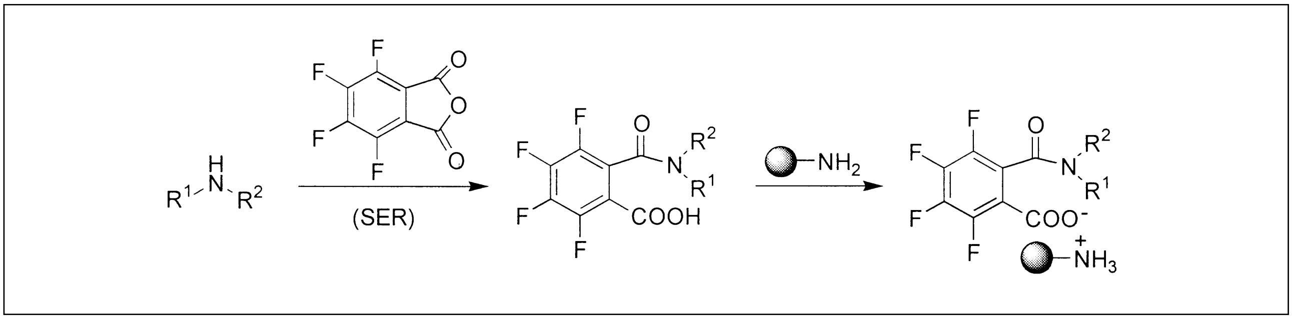


Fig. 4. Tetrafluorophthalic anhydride, a sequestration enabling reagent for amines.

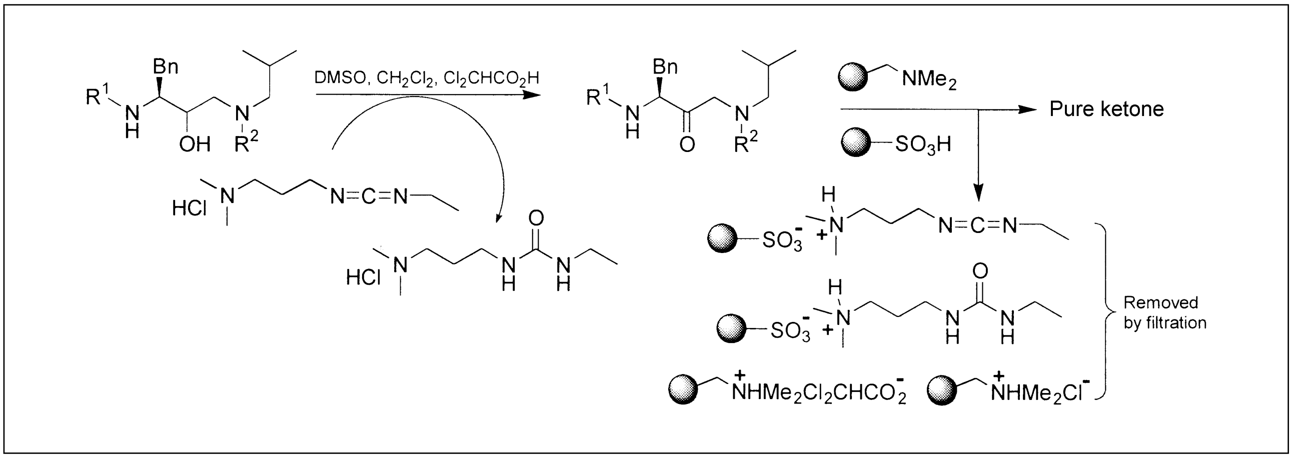


Fig. 5. Tagged carbodiimide in Pfitzner-Moffat oxidations.

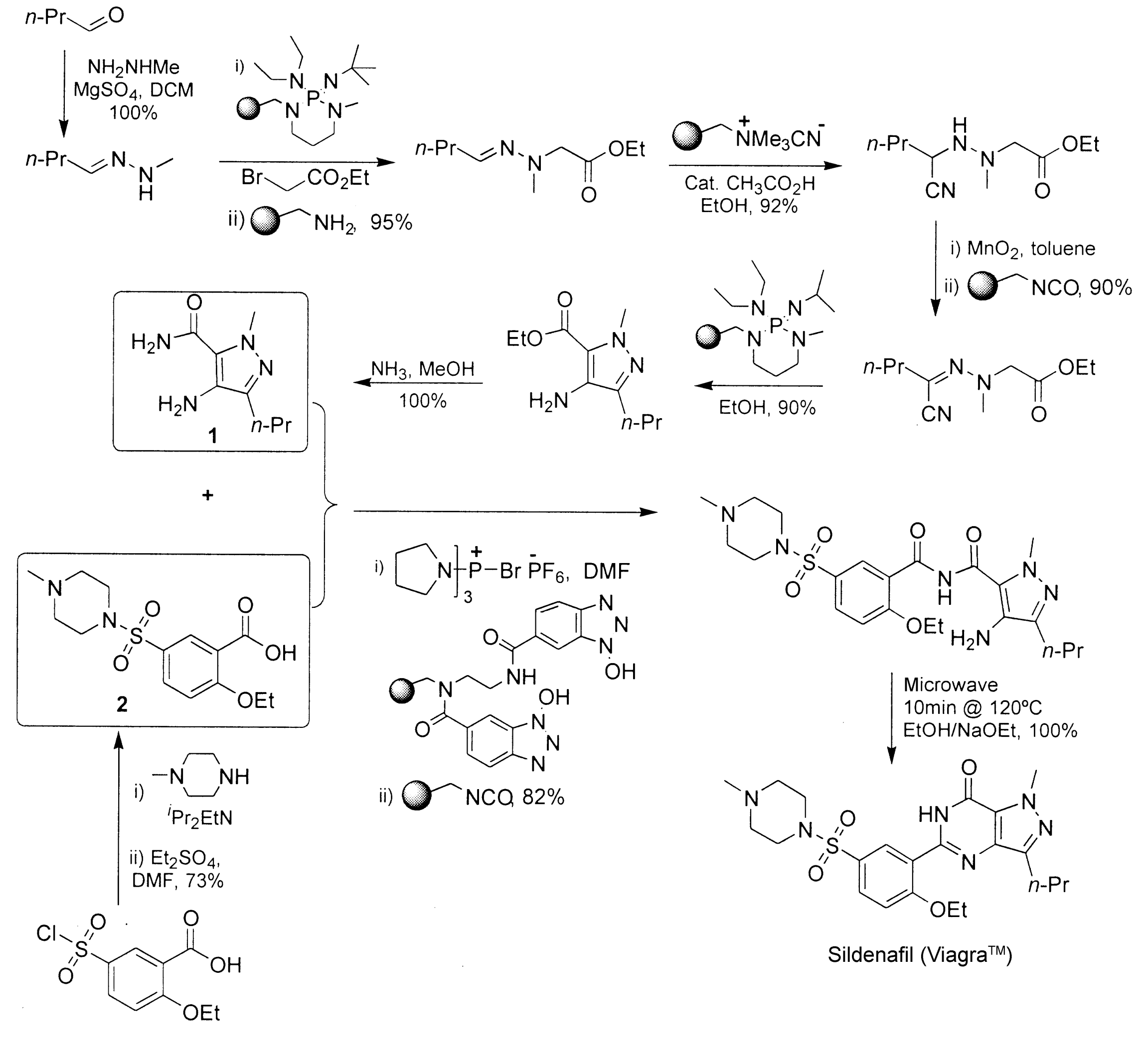
4) Purification by catch-and-release (27) (method V) is based on the capture of a desired product by either selective covalent derivatization with a functionalized resin or precipitation and subsequent filtration followed by release of the desired product in a pure form. An example of this is the capture of a vinyl boronate derivative from solution via a Suzuki coupling reaction with a polymer-bound aryl iodide (28).

The resin capture approach can use a tagged reactant leading to a tagged product to give a chemically more general approach of separation. In this way, crown-ether-tagged peptides have been readily separated from the reaction mixture by affinity chromatography using aminomethylated (in TFA form) polystyrene columns (29). A quinoline tag has also been used, which precipitates when protonated by mineral acid (30), and a 2-pyridyldimethylsilyl tag for acidic extraction of synthetic intermediates (31). A similar strategy is described using anthracene-tagged ester substrates in conjunction with an *N*-benzylmaleimide resin and a reversible Diels-Alder cycloaddition as a chemoselective removal of the tagged molecules (Fig. 6) (32). A more sophisticated strategy uses a covalent derivatization of the reactant with an organic metal-chelating tag (bipyridine ester derivative) to perform a selective and reversible capture of the synthetic intermediate after every chemical step in a sequence by complexion to a resin-bound copper(II) (33).

The combined power of functionalized polymers, having all the described advantages, has been applied to several automated parallel synthesis and multistep sequences (14, 15). An impressive example, shown in Scheme 2, is the synthesis of sildenafil (34), a potent and selective inhibitor of the enzyme phosphodiesterase type five (PDE-5), in 7 steps without using conventional purification techniques. A key feature of this work is that a convergent synthetic strategy was used. The heterocyclic amine **1** was assembled in 6 steps from readily available *n*-butanal. Coupling of the carboxylic acid **2** and amine **1** proceeded smoothly in the presence of a polymer-supported hydroxybenzotriazole and bromo-trispyrrolidinophosphonium hexafluorophosphate (pyBrOP). This process allows simultaneous activation of the acid **2** and separation from ester impurities by simple filtration. The last cyclodehydration of the resulting amide was performed using sodium ethoxide under microwave conditions to afford gram quantities of the target product in excellent overall yield.

There are now a significant number of commercially available functionalized polymers to enable the broad

Scheme 2: Polymer-supported synthesis of sildenafil



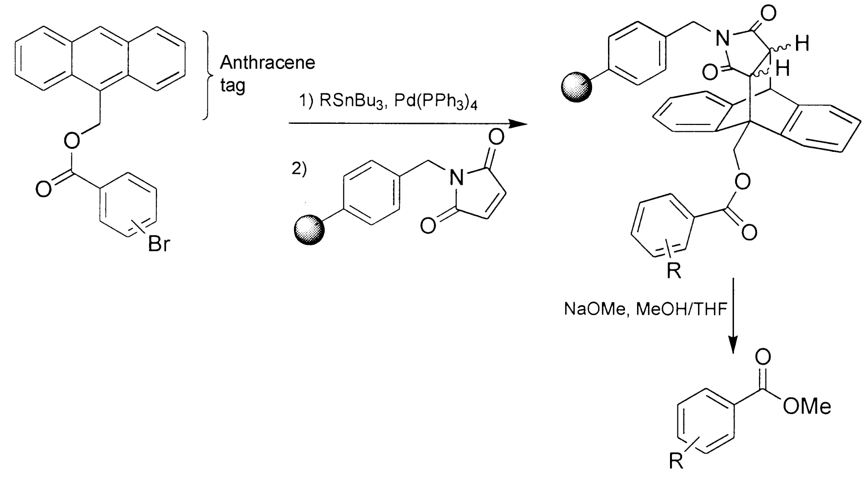


Fig. 6. Anthracene-tagged ester substrates removed by Diels-Alder reaction with solid-supported maleimide.

application of PASP synthesis in compound library generation.

Advances in resin handling

The vast majority of polymers used in combinatorial synthesis to date are prepared as cross-linked beads of sizes generally between 80-500 μm by suspension polymerization. A number of reviews have appeared on resin types (35-38) and their physical properties (39-42).

Interest in further accelerating the process of combinatorial synthesis has led to automation with different ways of handling beads. In the area of multiple parallel synthesis, major advances have been made by the introduction of tea bags (43), crowns (44, 45) and IRORI Kans (46, 47), all of which are based on the handling of discrete packets of resin beads. However, these methodologies still involve preparatory steps that include filling, sealing and labeling of the "containers" and recovery of tagging devices which increases the complexity of handling.

Other new formats of polymer supports which have recently emerged are polymer discs and plugs. These materials present a non-swelling highly cross-linked macroporous structure that have pores and are well suited for flow-through applications. This macroscopic structure itself can be taken as a discrete packet of resin beads with a tailored loading higher than individual beads, thus simplifying handling.

Monolithic polymer discs were first reported by Hird *et al.* (48) for a Suzuki type reaction. Subsequently, Tripp *et al.* applied this technology for the immobilization of scavengers for amines (49, 50) or acylating agents (51). These investigators increased polymer substitution without reducing permeability by grafting reactive chains onto the surface of the pores of the monoliths. These types of supports are macroporous poly(chloromethylstyrene-co-di-vinylbenzene)monoliths prepared by direct polymerization of the mixture of chloromethylstyrene, divinylbenzene, initiator and porogen. The mixture is placed within shrinkable polyethylene (PE) tubing that acts as a mold inside a sealed glass tube (Fig. 7a). After polymerization, the glass tube is removed and the monolith sliced into disks of required thickness. The PE tubing enables flow only in the axial direction. The grafting of reactive monomer at the pore surface within the disk is undertaken by first derivatizing the chloromethyl groups with an azo initiator (Fig. 7b). Finally the pores are filled with a solution of monomer with the required functionality and heated to achieve polymerization initiated by radicals anchored at the pore surface.

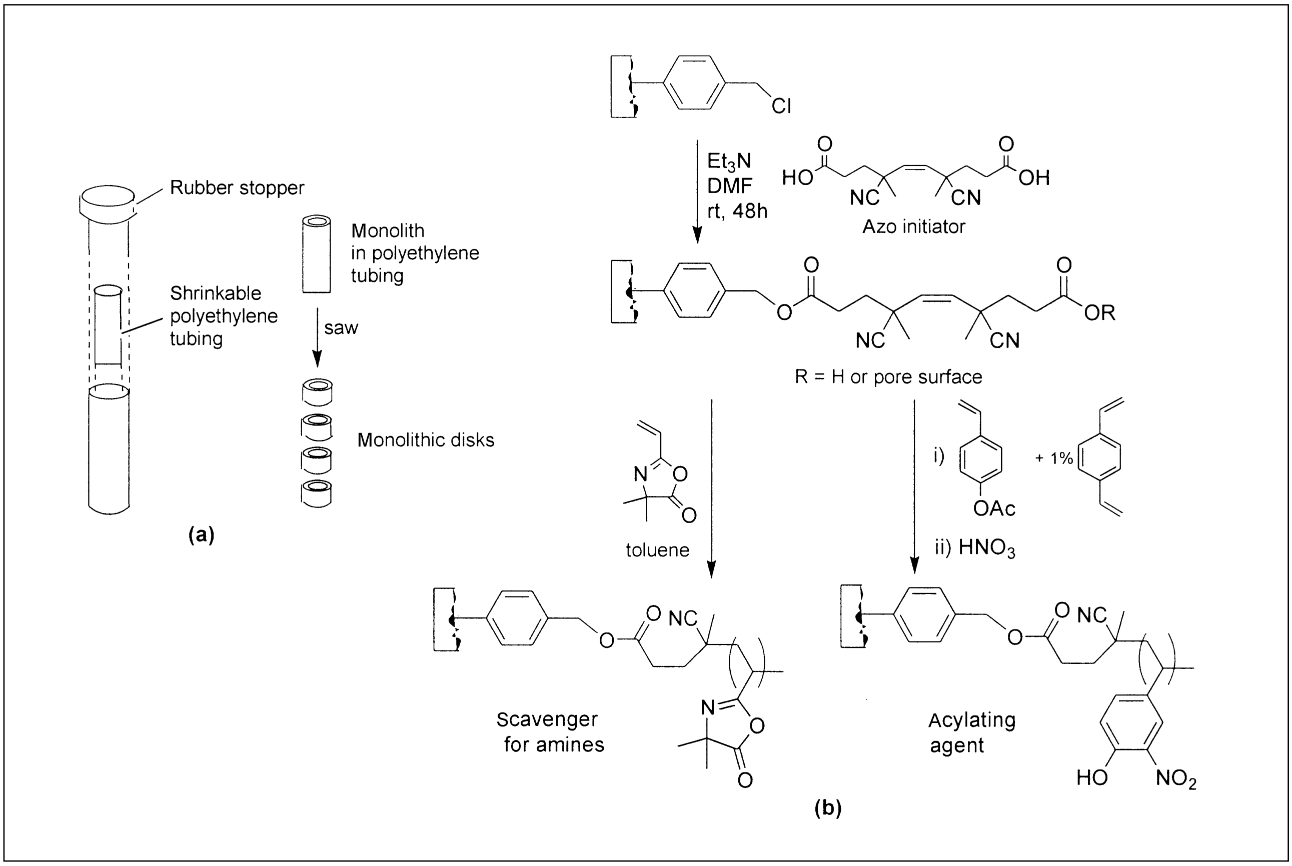


Fig. 7. (a) Preparation of macroporous disks. (b) Preparation of monolith grafted with functionalized monomer.

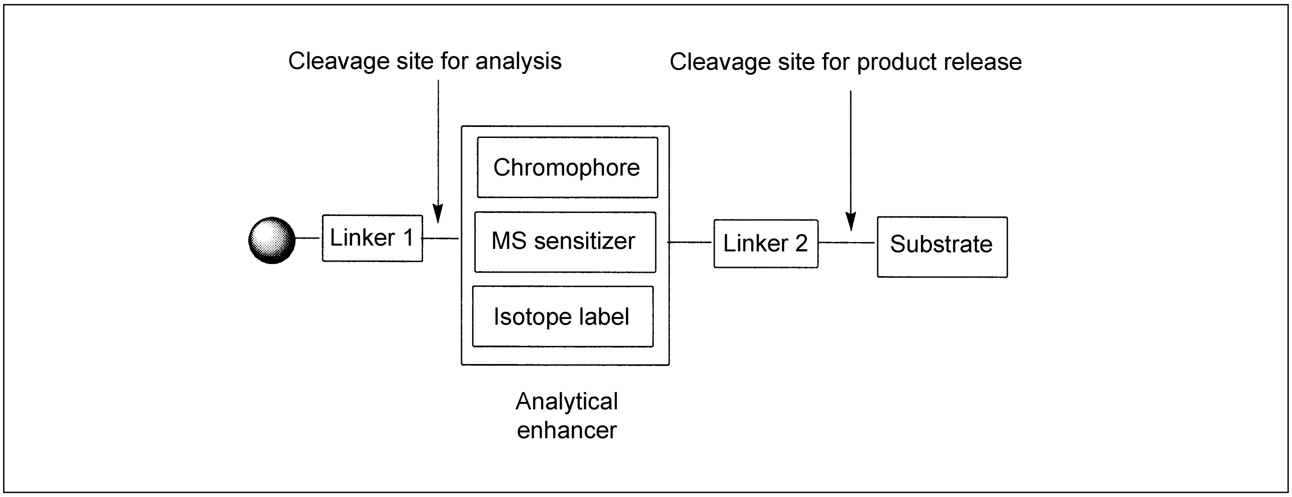


Fig. 8. Analytical construct containing a UV-chromophore.

Plugs are based on resin sintering within an inert polymer matrix (52). This method is applicable to various resin types (polystyrene [PS], polystyrene-polyethyleneglycol graft polymer [PS-PEG]) either pre- or postloaded with virtually any linker. The preparation is based on blending a 1:1 proportion of ultra-high molecular-weight polyethylene and the desired synthesis resin to a homogeneous mix. This is then loaded into polytetrafluoroethylene (PTFE)-lined molds and passed through a sintering oven under a nitrogen atmosphere. By increasing the temperature of the mixture, the polyethylene matrix softens to obtain plugs of resin with the mold shape. These plugs are porous macroscopic frameworks where the resin is embedded acting as minireactors. Each plug has a defined loading, a high permeability to solvents and an even void volume allowing swelling of synthesis beads to occur. The structural integrity of the plug is stable to a wide range of organic solvents, acids and bases. The performance of chemistry carried out on different resin types in a plug and their comparison with identical loose resin showed virtually identical results. The potential of this new format was demonstrated when an encoded library was prepared by fitting each resin plug with commercially available radiofrequency transponders through a hole in the center of the plug. The noninvasive retrieval of information from any plug was unambiguous and instantaneous, and was applied to synthesize a 100 compound library. Further development of this approach will use these resin plugs as scavengers, supported reagents and catalysts in a similar way as the monolithic polymer discs.

Analytical constructs on resin for rapid analysis of solid-phase chemistry

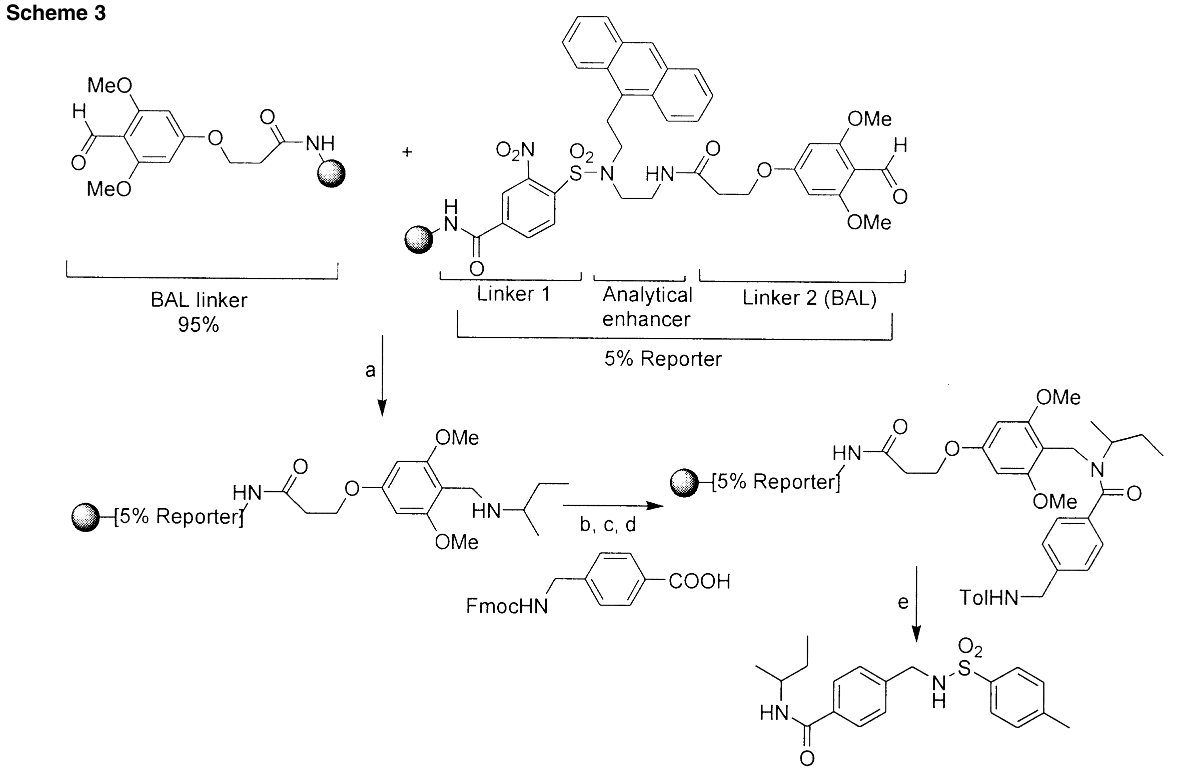
Chemistry validation on solid phase is a time-consuming process due to the lack of a fast, simple and sensitive method for rapid analysis. Analytical tools available to study resin-bound products have greatly improved. Examples include single-bead MAS NMR spectroscopy (53), gel-phase [^{13}C]-NMR spectroscopy, single-bead FT-IR spectroscopy (54), heteronuclear NMR methods including [^{31}P] (55), [^{19}F] (56), [^{15}N] (57) and [^{13}C]-(58-60)-enriched linker or substrates. However, these methods are still limited by low throughput and sensitivity.

Recently, rapid and unambiguous analysis of reactions on resin has been demonstrated using a “dual-linker analytical construct” on resin (Fig. 8). This method relies on the use of a linear combination of two chemically orthogonal linkers (linker 1 and 2) separated by an analytical enhancer component. Cleavage at linker 1 affords the substrate attached to the analytical unit which facilitates LC-MS characterization of the associated product. At the completion of the synthetic sequence, orthogonal cleavage at linker 2 cleanly releases the substrate of interest in a convenient manner. The modular nature of the linkers, analytical enhancer and solid support allow the system to be tailored for different chemistries.

A recent development of this concept utilizes an analytical fragment composed of an amine group (generated at the point of cleavage) which enhances detection by electroscopy mass spectroscopy (*i.e.*, a MS sensitizer), an isotope label which gives rise to a characteristic split-peak pattern in the mass spectrum for ease of peak analysis (a MS splitter) and a distinct chromophore to enable quantification by UV-HPLC. The chromophores used to date are dansyl (61) and anthracene (62) derivatives with intense absorption maxima at a remote wavelength (339 and 386 nm, respectively) free of absorbances from other species, thus enabling detection of material released from a single resin bead.

In this way, rapid analysis of an ongoing synthetic sequence on solid phase is achieved by cleavage at linker 2 of small resin aliquots and subsequent analysis of the resultant solution by LC/MS. These analytical construct

Scheme 3



Analytical fragments, resulting from cleavage of linker 1 with mercaptoethanol, were analyzed by LC/MS and HPLC. HPLC areas were measured at 386 nm, detecting one major peak. Product molecular ions (MH+) were measured using an LC-MS instrument (Thermoquest LCQ in ESI+ mode) and were in agreement with the expected product for each step. Reagents and conditions: (a) (i) *sec*-$BuNH_2$ (5 equiv), AcOH (5 equiv), NMP, 1-5 h, (ii) Bu_4NBH_4 (5 equiv), AcOH, NMP, 2 h; (b) Fmoc protected benzoic acid (10 equiv), PyBOP (10 equiv), DIPEA (20 equiv), CH_2Cl_2:DMF (1:4), 30 min; (c) piperidine:DMF (1:4), 30 min; (d) *p*-toluenesulfonylchloride (2 equiv), Et_3N (4 equiv), MeCN, 2 h; (e) TFA:CH_2Cl_2 (1:1), 1 h.

systems have been used for the direct monitoring of organic reactions on solid supports (62-66), for rapid reaction scanning of solid-phase chemistry (67, 68) and kinetics (69) and for the analysis of split-and-mix libraries (70).

The most recent modification of the analytical constructs has generated a “reporter resin”. This relies on the mixing of a small proportion (5%) of analytical construct-linked resin with unmodified resin (95%) prior to a solid-phase synthesis, to track the progress of reactions occurring on the whole. This concept was demonstrated with the synthesis of the model library shown in Scheme 3 (71). The chemical sequence was successfully monitored at each step by analytical cleavage and confirmed with MAS NMR spectroscopic analysis. The use of a small quantity of the reporter resin facilitates the development of solid-phase synthesis routes and enables a level of quality control of library products prior to isolation.

These analytical construct resins are proven tools for the analysis of solid-phase organic reactions that directly address a significant limitation in the development of resin chemistry.

Dynamic combinatorial libraries

Dynamic combinatorial chemistry (DCC) (72-77) has recently emerged as an elegant and potentially powerful strategy for the discovery of receptor-ligand systems. In a dynamic combinatorial library (DCL) each library member is assembled from building blocks using reversible chemistry (dynamic chemistry). As a consequence, all products can interconvert to give a thermodynamically controlled product distribution selected from all possible combinations. Addition of a target molecule to this mixture can shift the product distribution in favor of molecules that most strongly bind to the target at the expense of the other molecules (amplification by molecular recognition). If at this point the reversible chemistry of the mixture is switched off, the major library members can be isolated and characterized. This approach constitutes a chemical process under a selection pressure, resulting in a one-pot molecular evolution. In principle, the dynamic combinatorial approach integrates template-directed preparation of the desirable compound and screening in one process, without the need for individual synthesis of a large

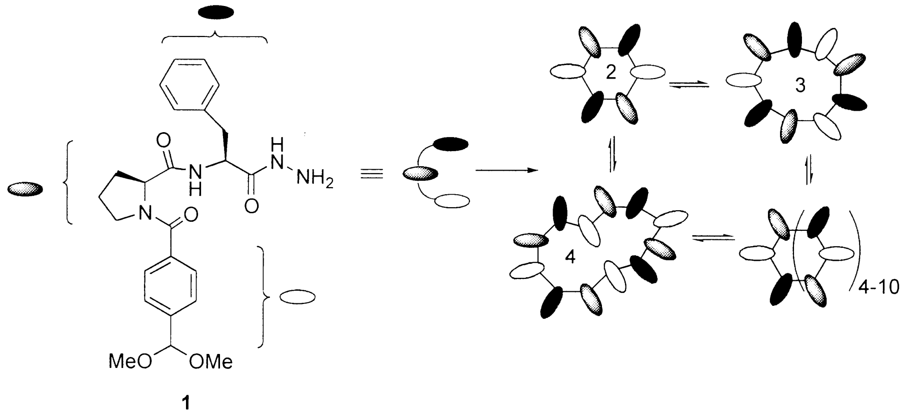


Fig. 9. Acid-catalyzed hydrazone chemistry to generate a DCL.

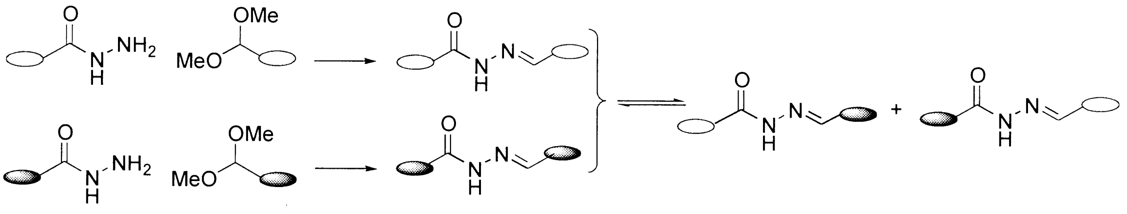


Fig. 10. Reversible cyclization of building block **1**.

number of compounds and the identification of active compounds by deconvolution or parallel screening procedures.

To date, a few examples have demonstrated the feasibility of this concept (78, 79). Most of the examples have concentrated on the development of chemistries that allow controlled reversible bond formation as a proof of concept. DCLs have been generated noncovalently through metal ligand exchange (80-84) and hydrogen bonding networks (85-90), and covalently deploying a range of reversible reactions such as transesterification (91-93), disulfide exchange (94-97), allylpalladium chemistry (98) and transimination (99-102) or hydrazone chemistry (103).

An example of a new receptor identified and isolated from a covalent DCL (104) using hydrazone chemistry is shown in Figure 9. By mixing hydrazides and protected aldehydes under acidic conditions, both hydrazone formation and efficient exchange of the generated hydrazones occur (Fig. 10). The resulting equilibrium mixture can be frozen by increasing the pH of the reaction mixture.

Compound **1** (Fig. 9) bearing three molecular recognition groups, was selected as a building block to prepare a library of macrocycles with potential to take part in hydrogen bonding, pi interactions and Lewis acid/base interactions. Acid catalyzed cyclization of building block **1** generated a DCL of macrocycles from dimer to undecamer as characterized by ESI-MS and HPLC. This DCL was screened for its affinity for cations. When the dynamic library was exposed to quaternary ammonium iodides, KI, RbI or CsI, no significant changes in the product distribution occurred. However, upon addition of NaI a significant shift toward the cyclic trimer was observed which was even more pronounced when LiI was introduced into the reaction mixture (up to 98% of trimer formation). The selected trimer was isolated by successive filtration through a basic resin (neutralization) and silica gel. The interaction between Li^+ and the amplified trimer was studied in detail, using [^{1}H]-NMR, [^{13}C]-NMR, [^{7}Li]-NMR and FT-IR, spectroscopies to show that this new receptor is rather flexible and changes its conformation upon binding. Such flexible receptors, although widespread in nature, are extremely difficult to create by design. This illustrates the potential of the dynamic combinatorial approach for the discovery of novel conformationally flexible host (or guest) molecules.

Natural product-based libraries and chemical genetics

Planning a library synthesis in a drug discovery program often involves the preparation of small molecules to be screened for their ability to bind a preselected protein target. The synthesis of natural product libraries can be planned by retrosynthetic analysis of a starting hit structure or natural product-based template. This approach is supported by the fact that half the drugs on the market are direct descendants of natural products (105, 106), although it might be argued that historically drug discovery has been biased by a significant component of natural product screening. In recent years, several solid-phase approaches to natural product-based libraries have been developed (107-111). The earliest efforts were focused on the immobilization of complex natural product skeletons (derived from either semisynthesis or total synthesis) onto solid support to be further combinatorial diversified at several positions. More recently, a few groups have managed the total synthesis of natural products on solid support enabling the use of multicomponent building block pools and the introduction of variations at all sites of the natural product including the basic structure itself.

The strategies exploit the concept of library construction based on privileged structures, a term first proposed by Evans *et al.* (112) to describe select structural types from a natural source that bind to multiple, unrelated classes of protein receptors as high-affinity ligands. These privileged structures are typically rigid, polycyclic heteroatomic systems capable of orienting functional groups in a well-defined three-dimensional space. Furthermore, this template needs to accommodate the installation of a maximum degree diversity via solid-phase split-and-pool synthesis (113-115). Libraries have been inspired from a specific nonpeptidic natural product, a hybrid structure or simplified mimetic of a natural product (107-111), in addition to biopolymers (peptides, nucleic acids, oligosaccharides) (116). However, natural products still cover a diversity space not yet available from synthetic libraries. Theoretical studies from Lee and Schneider *et al.* (117) compare natural product databases with different classes of drug molecules, revealing natural product-based scaffolds not present in trade drugs which could generate potential drug-like libraries.

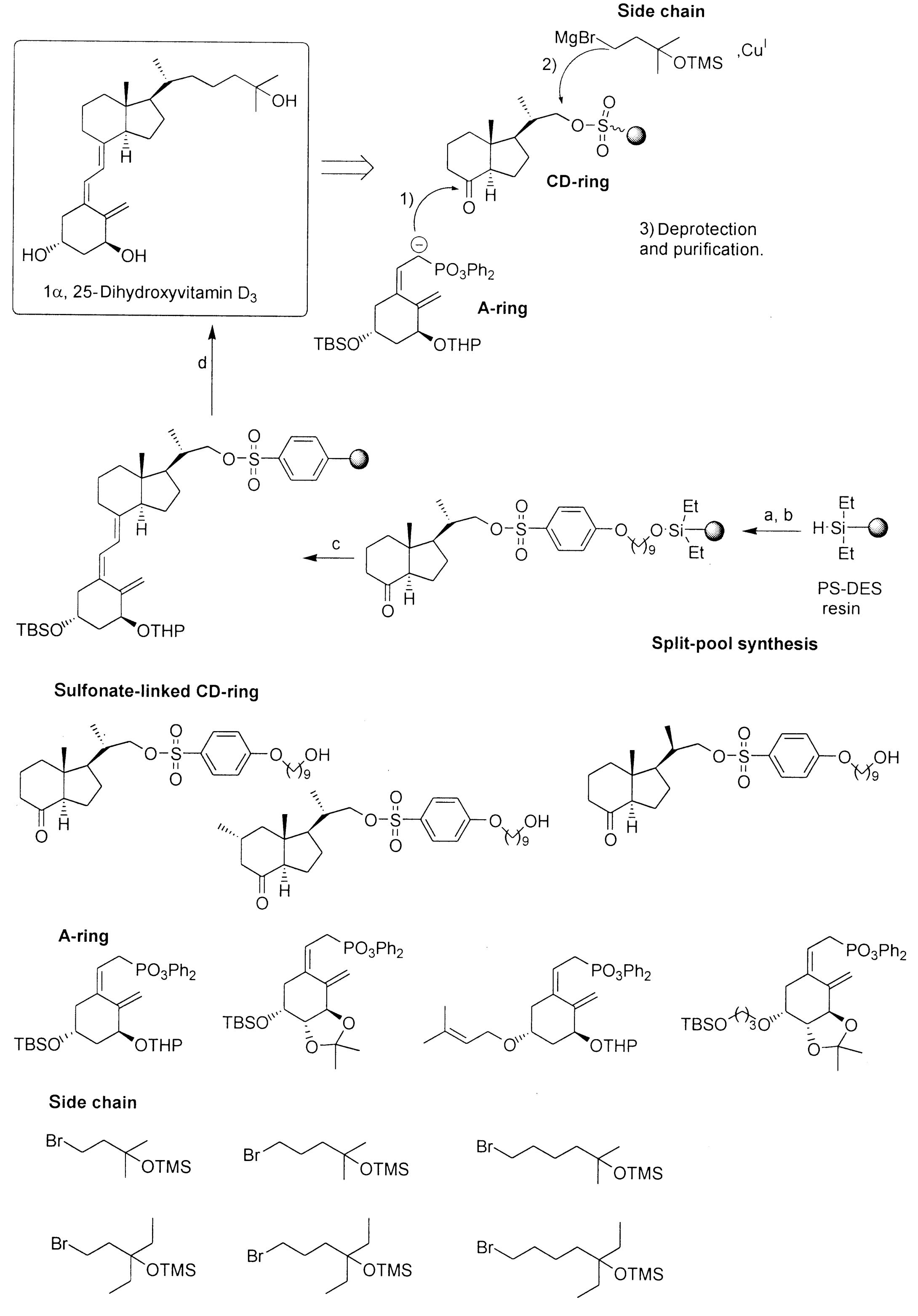
A recent and worthy example is the vitamin D_3 library synthesized by Hijikuro *et al.* (118). 1α,24-Dihydroxyvitamin D_3 (Scheme 4), the biologically active metabolite of vitamin D_3, is recognized as a steroid hormone (119, 120). The natural hormone exhibits a variety of physiological activities, such as regulation of calcium and phosphorus metabolism, cell differentiation and proliferation, and the immune system (121). Recently, to separate these activities and to enhance specificity, a number of analogues have been synthesized as single compounds using solution-phase chemistry (118). Doi *et al.* reported the first solid-phase synthesis of the 11-hydroxyvitamin D_3 system by combining three components; the A-ring, the CD-rings and the side chain (Scheme 4) (122). An improved synthesis amenable for library preparation (118) was carried out by a split-pool methodology using radiofrequency encoded (REC) chemistry (123). The sequence began with the loading of the sulfonate-linked CD-rings onto chlorinated PS-DES resin. Horner-Wadsworth-Emmons reaction of solid supported CD-rings with lithiated A-rings yielded the corresponding triene. Sequential one-pot coupling of several side chains with the resulting immobilized sulfonate and concomitant cleavage from the polymer support by Cu^I-catalyzed Grignard reaction gave the library members as crude single compounds. The workup and purification of these products was performed in parallel using a manual parallel synthesizer. The biological evaluation of the resulting 72-member library of vitamin D_3 analogues will establish structure-activity relationships for this molecular scaffold.

Research in genomics and proteomics coupled with the use of microarray technologies is expected to provide massive quantities of data relating to novel genes and proteins. While such advances offer many opportunities, a significant challenge in the "postgenomics" and proteomics era is to identify highly specific small-molecule ligands for any protein of interest to elucidate novel protein function and the development of new drugs. Diversity-oriented synthesis has been adopted as an alternative approach to the previous target-oriented synthesis to address this broad scientific goal (124). Collections of vast numbers of complex and diverse compounds are screened for detectable biological effects in a cell or whole organism. Once a particular biological effect is observed, the function of a novel protein or gene can be inferred. This concept is similar to genetic methods, whereby mutations in genes are introduced and the resulting biological effects are examined. It has therefore been called the chemical genetic approach (Fig. 11) (125-127). If a specific small-molecule agonist and antagonist is found for every protein, it should be possible to exert complex control of the biology using small molecules alone. To address this goal, chemists are preparing libraries composed of complex, natural product-like compounds. This approach has been facilitated by the ability to produce huge collections of compounds by combinatorial methods and the use of modern high-throughput screening techniques.

Initial attempts have used highly functionalized polycyclic derivatives as a basis to introduce diversity at several positions in the template. For example, Tan *et al.* have developed a synthesis of a shikimic acid derivative by tandem acylation and 1,3-dipolar cycloaddition chemistry (Fig. 12) (128). The highly functionalized scaffold was reported to have been further derivatized to give a library of some 2 million polycyclic compounds.

Recently, a boronic ester annulation strategy has been reported to lead to several different skeletal arrays (129). This approach is based on a ring closing metathesis of allylic or propargylic unsaturated boronic esters to afford a diverse family of heterocycles (*e.g.*, structures **A** and **B**, Fig. 13). Further modification of these double bond-containing products through, for example, a dihydroxylation (sequence I, Fig. 13) or a Diels-Alder reaction (sequence II, Fig. 13) yields structurally and stereochemically diverse compounds by the same approach.

Scheme 4



Reagents and conditions: (a) 1,3-dichloro-5,5-dimethylhydantoin (3 equiv), CH_2Cl_2, rt, 1 h; (b) sulfonate-linked CD-ring (3 equiv), imidaxole (4 equiv), CH_2Cl_2, rt, 6 h, 95% yield. (c) A-ring (8 equiv), *n*-BuLi (7.5 equiv), THF, –40 to –10 °C, 3 h. (d) Side chain (15 equiv), $CuBr\text{-}Me_2S$ (1 equiv), THF, rt, 3 h; CSA, MeOH, H_2O, 30 °C, 6 h, 47% from CD-ring solid supported resin.

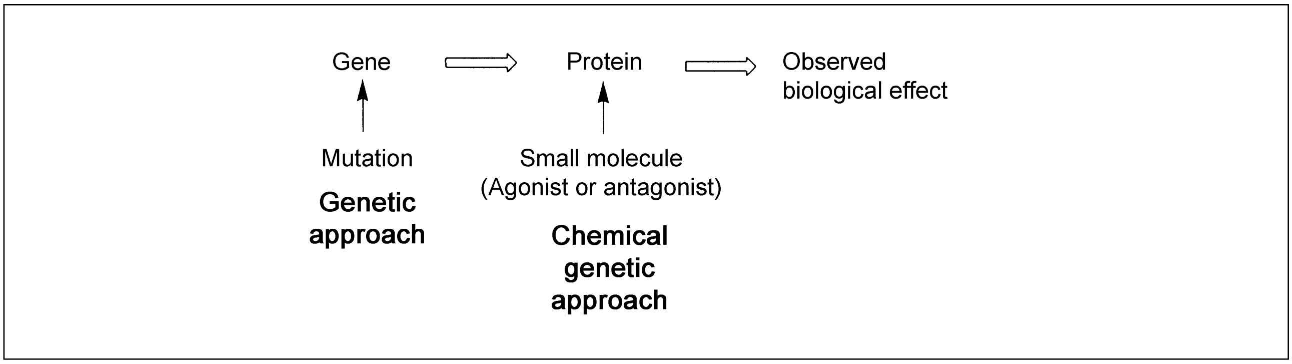


Fig. 11. Genetic and chemical genetic approaches.

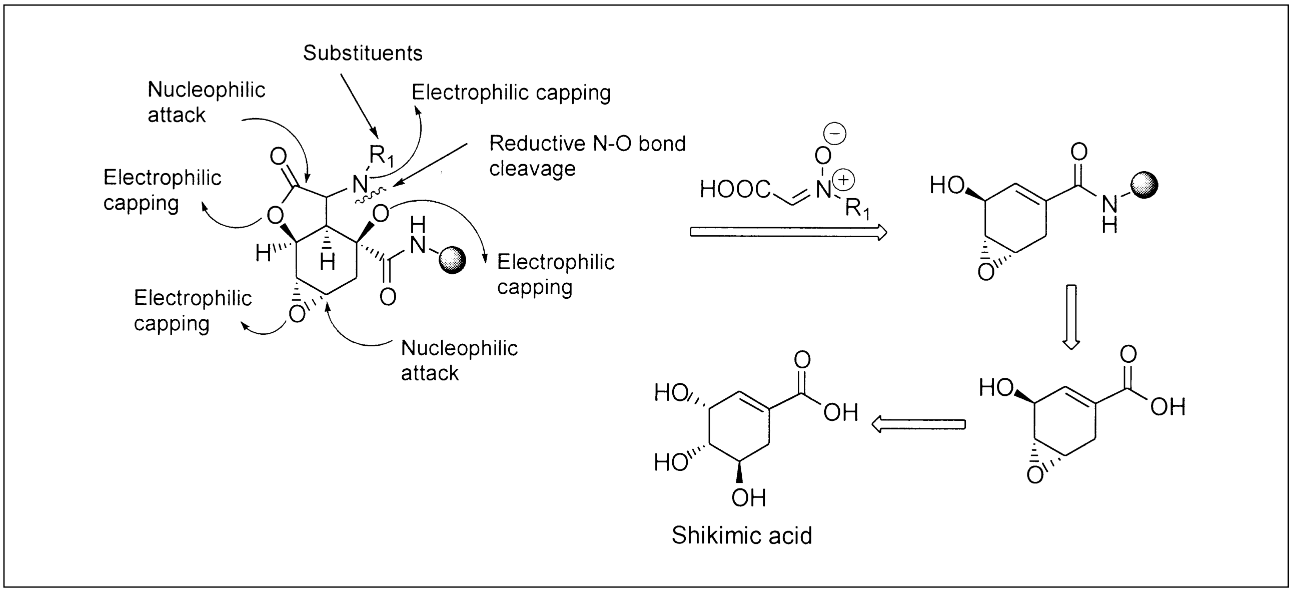


Fig. 12. Retrosynthetic sequences to generate highly functionalized polycyclic derivatives.

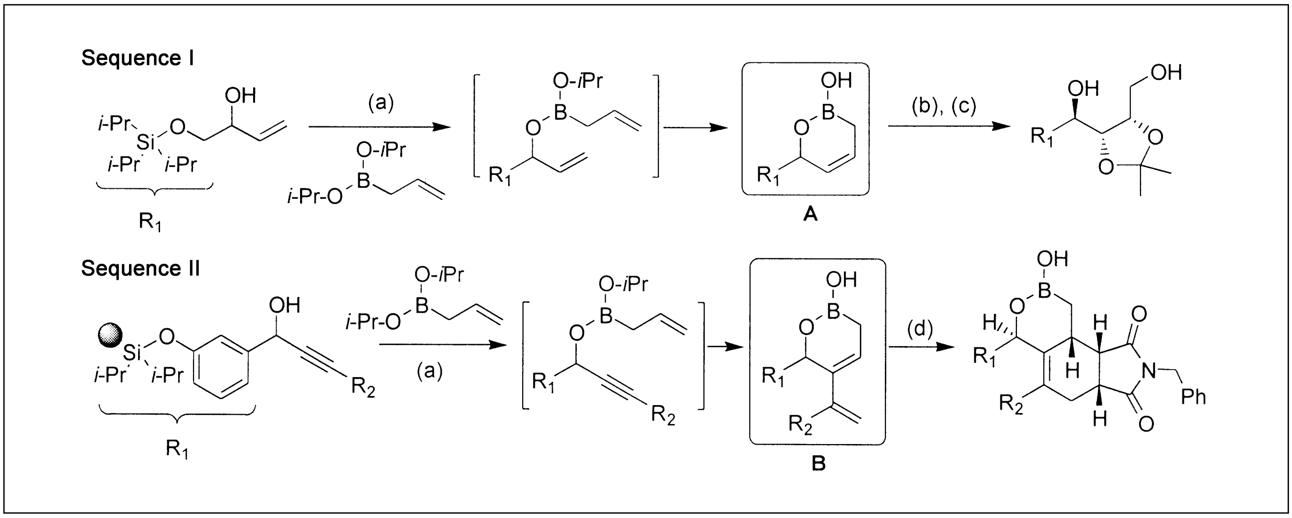


Fig. 13. Reagents: (a) Grubbs' catalyst (20 mol%), DCM, reflux, then H_2O. (b) OsO_4, NMO, acetone, pH 7 buffer: then 2,2-dimethoxypropane, DCM, PPTS. (c) H_2O_2, NaOH, THF. (d) *N*-benzylmaleimide, PhMe, 70 °C. PST = pyridinium *p*-toluene sulfonate.

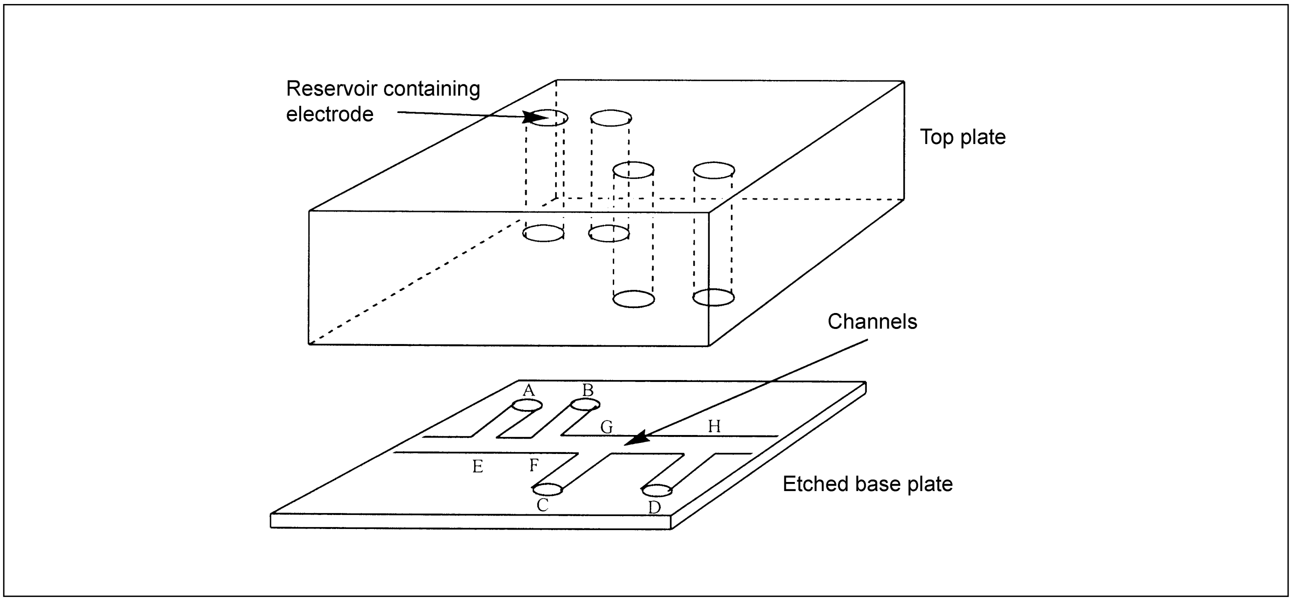


Fig. 14. A basic microreactor. For a bimolecular reaction of the type X + Y → Z, reactant X is introduced into reservoir A and suitable voltage is applied across electrodes within the reservoirs A and D to fill the A-D channel with X. Reactant Y is introduced into reservoir B and a voltage across BC is used to fill the B-C channel. The reaction of Y within the flowing stream of X will take place in the E-H channel, which can also be controlled by the application of voltages across A-D.

From microreactors to the "lab-on-a-chip" concept

During the past 10 years, the development of microflow injection analysis (μFIA) and micrototal analytical systems (μTAS), which exploit electroosmotic flow (EOF) and electrophoretic separation, has achieved a faster and cheaper way of performing chemical analysis (130). The demand for high-throughput parallel processing for combinatorial applications has led to the use of similar technology to construct microreactors for organic chemical synthesis.

Apparatus and principles

A microreactor involves a 2-dimensional network of micron sized channels in a solid substrate surface using wet etch, laser ablation, embossing, micromachining or microlithography techniques (131-133). A top cover is then bonded to form a capillary, as shown in Figure 14. Holes drilled through the top cover allow connection to the channels forming the reagent reservoirs and also positioning of electrodes. This form of fabrication typically generates channels with a rectangular cross-section of depth 10-200 μm, width 50-200 μm and length in the cm range. The flow-through of the microreactor manifold is generated by the application of suitable voltages across the electrodes within the desired reservoirs. This leads to an EOF which has no moving parts and minimal back pressure. EOF is used to move reagents and solvents around the system of channels. In addition, due to the high electric field (200 V/cm of channel) associated with the EOF, variations in the electrophoretic mobility of individual species enables separation to be achieved. The combination of both types of mobility can be used to model and control the spatial and temporal position of components in a microreactor system as a function of applied voltages, allowing the processes to be readily automated and computer-controlled (130). By varying the potentials across each channel section, it is possible to rapidly optimize the relative flows of different reagents, or to inject plugs of one reagent into a stream of another, or introduce a number of reagents in a specified sequence for multistep reactions. Microreactors have been successfully coupled to mass spectrometers (134), NIR (134), Raman (135) and gas chromatography spectrometers for online analysis (136).

Some direct advantages of microreactors include rapid heat transfer owing to the decrease in linear dimensions. Micromixers can reduce reagent mixing times to milli- or nanoseconds. The miniaturization reduces exposure to toxic or hazardous materials, and the enclosed nature of the microreactors means greater containment of chemicals. Other properties include localized control of concentration gradients, separation of reaction products and the possibility of eliminating unwanted side reactions (137). Although the small size of the microreactors would seem to preclude industrial scale synthesis, it has been shown (138) that with only 1,000 microreactors operating continuously, 1 kg of material could be produced in a day. The main attraction of this approach is the ability to maintain the high level of control and selectivity of chemical reactions.

Applications of microreactors in synthesis

The microreactor offers the opportunity to quickly perform a large number (many hundreds) of reactions in parallel to either explore and optimize reaction parameters or library generation. Microreactors have so far been successfully employed for gas- and liquid-phase chemistry. The introduction of supported reagents in microreactors has allowed the testing and optimization of catalytic processes. For example, the gas-phase partial hydrogenation of cyclododeca-1,5,9-triene, cycloocta-1,5-diene and benzene has been carried out using a microreactor impregnated with an organic solution of palladium(II) or Ru/Zn derivative (138). A solution-phase example developed a catalytic synthesis of 4-cyanobiphenyl using a modified Suzuki coupling reaction (Fig. 15) (139). The heterogeneous catalyst (1.8% palladium on silica) was incorporated as microporous silica frits within the reactor manifold and reagent and products were flushed through using EOF. The aryl halide and boronic acid were alternately pulsed over a catalyst bed of palladium on silica leading to a great improvement in yield from less than 5% to 68% by simulating the catalytic cycle. A superacid catalyst (sulphated zirconia) has also been immobilized on to the surface of a microreactor top plate whereby the pumping of hexan-1-ol produced dehydration to hex-1-ene (140).

Hazardous chemical processes such as nitration of toluene (141) or elemental fluorination of organic substrates (142) have been carried out using stainless steel or nickel microreactors, respectively. In these examples, conversion efficiencies are improved or comparable to results from conventional reactors, while many of the associated safety risks are minimized.

The preparation of a series of nitrostilbene ester compounds using Wittig chemistry has also been carried out in microreactor device (Fig. 16). First, stoichiometry and reaction diversity were investigated obtaining an increase in reaction efficiency over the conventional batch method (143). Then, the same system was used to introduce

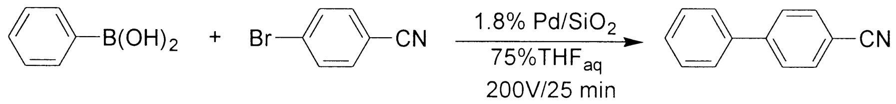


Fig. 15. Suzuki reaction carried out in a microreactor.

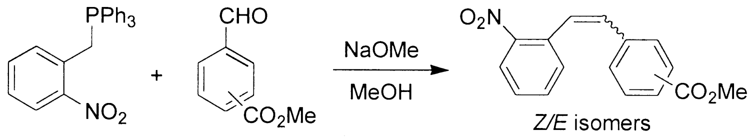


Fig. 16. Wittig reaction carried out in a microreactor.

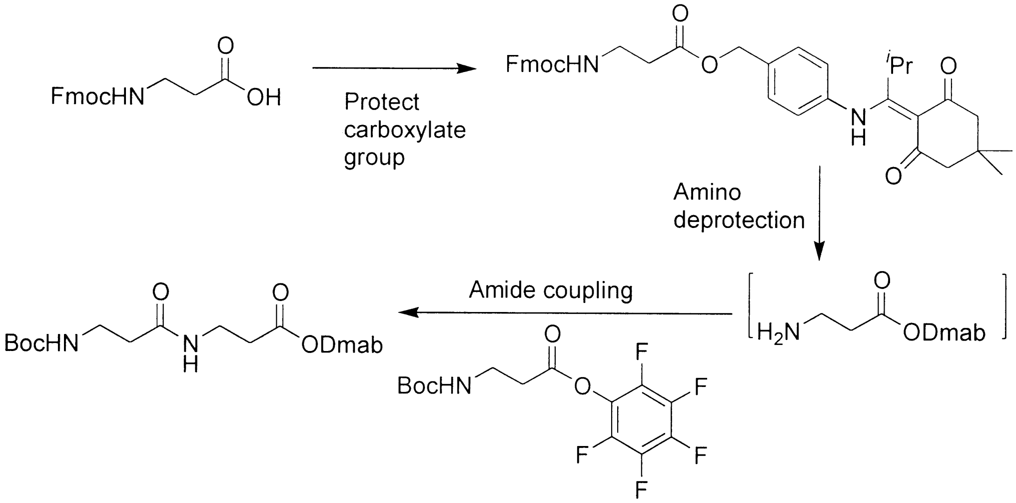


Fig. 17. Multistep peptide synthesis performed in a microreactor.

stereoselective control of the chemical reaction by applying electrical fields which generate controlled localized concentration gradients of the reagent streams leading to a variation in the *Z/E* ratio (144).

A major benefit of this methodology is the given capability of serially performing multistep reactions, culminating in target- or diversity-based synthesis. For example, dipeptides have been prepared using microreactors in quantitative yield in a period of just 20 min, compared with batch reactions where only moderate yields (40-50%) were obtained in a 24-h period (Fig. 17) (145).

Microreactors are a paradigm shift which have taken synthetic chemistry some way toward realizing the "lab-on-a-chip" concept. By analogy with conventional silicon chips, such a device could lead to massive parallel chemical processing in the future, well beyond the limits of current technologies for parallel high-throughput synthetic chemistry.

References

1. Thayer, A.M. *Combinatorial chemistry becoming core technology at drug discovery companies.* Chem Eng News 1996, 12: 57-64.

2. Merrifield, R.B. *Solid phase synthesis. I. The synthesis of a tetrapeptide.* J Am Chem Soc 1963, 85: 2149-54.

3. Gallop, M.A., Barrett, R.W., Dower, W.J., Fodor, S.P.A., Gordon, W.M. *Application of combinatorial technologies to drug discovery. I. Background and peptide combinatorial libraries.* J Med Chem 1994, 37: 1233-51.

4. Pinilla, C., Appel, J., Blondelle, S. et al. *A review of the utility of soluble peptide combinatorial libraries.* Biopolymers 1996, 37: 221-40.

5. Geysen, H.M., R.H., Barteling, S. *Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid.* Proc Natl Acad Sci USA 1984, 81: 3998-4002.

6. Guiller, F., Orain, D., Bradley, M. *Linkers and cleavage strategies in solid-phase organic synthesis and combinatorial chemistry.* Chem Rev 2000, 100: 2091-158.

7. Brown, R.C.D. *Recent developments in solid-phase organic synthesis.* J Chem Soc Perkin Trans I 1988, 3293-320.

8. Sammelson, R.E., Kurth, M.J. *Carbon-carbon bond-forming solid-phase reactions. Part II.* Chem Rev 2001, 101: 137-202.

9. Franzén, R.G. *Utilization of Grignard reagents in solid-phase synthesis: A review of the literature.* Tetrahedron 2000, 56: 685-91.

10. Furka, A., Sebesteyn, F., Asgedom, M., Dibo, G. *General method for rapid synthesis of multicomponent peptide mixtures.* Int J Pept Protein Res 1991, 42: 487-93.

11. Lam, K.S., Lebl, M., Krchnák, V. *The "one-bead-one-compound" combinatorial library method.* Chem Rev 1997, 97: 411-48.

12. Baldwin, J.J., Dolle, R. *Reconvolution tools for solid-phase synthesis.* In: A Practical Guide to Combinatorial Chemistry. Czarnik, A.W., DeWitt, S.H. (Eds.). American Chemical Society: Washington DC 1997, 155-74.

13. Parlow, J.J., Devraj, R.V., South, M.S. *Solution-phase chemical library synthesis using polymer assisted purification techniques.* Curr Opin Chem Biol 1999, 3: 320-36.

14. Kirschning, A., Monenschein, H., Wittenberg, R. *Functionalized polymers - emerging versatile tools for solution-phase chemistry and automated parallel synthesis.* Angew Chem Int Ed 2001, 40: 650-79.

15. Ley, S.V., Baxendale, I.R., Bream, R.N. et al. *Multi-step organic synthesis using solid-supported reagents and scavengers: A new paradigm in chemical library generation.* J Chem Soc Perkin Trans I 2000, 3815-4196.

16. Drewry, D.H., Coew, D.M., Poon, S. *Solid-supported reagents in organic synthesis.* Med Res Rev 1999, 19: 97-148.

17. Shuttleworth, S.J., Allin, S.M., Sharma, P.K. *Functionalized polymers: Recent developments and new applications in synthetic organic chemistry.* Synthesis 1997, 1217-39.

18. Kaldor, S.W., Siegel, M.G. *Combinatorial chemistry using polymer supported reagents.* Curr Opin Chem Biol 1997, 1: 101-6.

19. Akelah, A., Sherrington, D.C. *Application of functionalized polymers in organic synthesis.* Chem Rev 1981, 81: 557-87.

20. Fréchet, J.M.J. *Synthesis and applications of organic polymers as supports and protecting groups.* Tetrahedron 1981, 37: 663-83.

21. De Miguel, Y.R. *Supported catalysts and their applications in synthetic organic chemistry.* J Chem Soc Perkin Trans I 2000, 4213-21.

22. Jandeleit, B., Schaefer, D.J., Powers, T.S. et al. *Combinatorial material science and catalysis.* Angew Chem Int Ed Engl 1999, 38: 2495-532.

23. Lindner, E., Schneller, T., Auer, T. et al. *Chemistry in interphases - a new approach to organometallic syntheses and catalysis.* Angew Chem Int Ed Engl 1999, 38: 2155-74.

24. Parlow, J.J. *Simultaneous multi-step synthesis using polymeric reagents.* Tetrahedron Lett 1995, 36: 1395-6.

25. Parlow, J.J., Flynn, D.L. *Solution-phase parallel synthesis of a benzoxazinone library using complementary molecular reactivity and molecular recognition (CMR/R) purification technology.* Tetrahedron 1998, 54: 4013-31.

26. Flynn, D.L., Crich, J.Z., Devraj, R.V. et al. *Chemical library purification strategies based on principles of complementary molecular reactivity and molecular recognition.* J Am Chem Soc 1997, 119: 4874-81.

27. Brown, S.D., Armstrong, R.W. *Post-condensation modifications of Ugi four-component condensation products: 1-Isocyanoclohexene as a convertible isocyanide. Mechanism of conversion, synthesis of diverse structures, and demonstration of resin capture.* J Am Chem Soc 1996, 118: 2574-83.

28. Brown, S.D., Armstrong, R.W. *Synthesis of tetrasubstituted ethylenes on solid support via resin capture.* J Am Chem Soc 1996, 118: 6331-32.

29. Zhang, S., Fukase, S., Kusumoto, S. *New methodology for high throughput solution phase synthesis: Affinity purification by using crown ether and ammonium ion interaction.* Tetrahedron Lett 1999, 40: 7479-83.

30. Perrier, H., Labelle, M. *Liquid-phase synthesis with solid-phase work-up: Application to multistep and combinatorial syntheses.* J Org Chem 1999, 64: 2110-13.

31. Yoshida, J.-I., Itami, K., Mitsudo, K. et al. *2-Pyridylsilyl group as a multifunctional "phase-tag" for solution phase synthesis.* Tetrahedron Lett 1999, 40: 3404-6.

32. Wang, X., Parlow, J.J., Porco, J.A. *Parallel synthesis and purification using anthracene-tagged substrates.* Org Lett 2000, 2: 3509-512.

33. Ley, S.V., Massi, A., Rodríguez, F. et al. *A new phase-switch method for application in organic synthesis programs employing immobilization through meta-chelated tagging.* Angew Chem Int Ed 2001, 40: 1053-5.

34. Baxendale, I.R., Ley, S.V. *Polymer-supported reagents for multi-step organic chemistry: Application to the synthesis of sildenafil.* Biorg Med Chem Let 2000, 10: 1983-6.

35. Sherrington, D.C. *Preparation, structure and morphology of polymer supports.* J Chem Soc Commun 1998, 2275-86.

36. Hodge, P. *Polymer-supported organic reactions. What takes place in the beads?* Chem Soc Rev 1997, 26: 417.

37. Svec, F., Fréchet, J.M.J. *Subnanometer-diameter wires isolated in a polymer matrix by fast polymerization.* Science 1996, 276: 782-4.

38. Rana, S., White, P., Bradley, M. *Influence of resin cross-linking on solid-phase chemistry.* J Comb Chem 2001, 3: 9-15.

39. Fréchet, J.M.J., Darling, G.D., Itsuno, S., Lu, P.Z., Demeftahi, M.V., Rolls, W.A. **title?** Pure Appl Chem 1988, 60: 353-64.

40. Meldal, M. *Properties of solid supports.* In: Methods in Enzymology: Solid-Phase Peptide Synthesis. Fields, G. (Ed.). Academic Press: London 1997, 83-109.

41. Vaino, A.R., janda, K.D. *Solid-phase organic synthesis: A critical understanding of the resin.* J Comb Chem 2000, 2: 579-96.

42. Vaino, A.R., Goodin, D.B., Janda, K.D. *Investigating resins for solid phase organic synthesis: The relationship between swelling and microenvironment as proved by EPR and fluorescence spectroscopy.* J Comb Chem 2000, 2: 330-6.

43. Houghten, R.A. *General method for the rapid solid-phase synthesis of large numbers of peptides: Specificity of antigen antibody interaction at the level of individual amino acids.* Proc Natl Acad Sci USA 1985, 82: 5131-5.

44. Bray, A.M., Chiefari, D.S., Valerio, R.M., Maeji, N.J. *Rapid optimization of organic reactions on solid phase using the multipin approach. Synthesis of 4-aminoproline analogs by reductive amination.* Tetrahedron Lett 1995, 36: 5081-4.

45. Virgilio, A.A., Bray, A.A., Zhang, W. et al. *Synthesis and evaluation of a library of peptidomimetics based upon the β-turn.* Tetrahedron 1997, 53: 6635-44.

46. Nicolaou, K.C., Winssinger, N., Vourloumis, D., Ohshima, T., Kim, S., Pfefferkorn, J., Xu, J.-Y., Li, T. *Solid and solution phase synthesis and biological evaluation of combinatorial sarcodictyin libraries.* J Am Chem Soc 1998, 120: 10814-26.

47. Nicolaou, K.C., Xiao, X.-Y., Parandoosh, Z., Senyei, A., Nova, M.P. *Radiofrequency encoded combinatorial chemistry.* Angew Chem Int Ed Engl 1995, 34: 2289-91.

48. Hird, N., Hughes, I., Hunter, D., Morrison, G.J.T., Sherrington, D.C., Stevenson, L. *Polymer discs: An alternative support format for solid phase synthesis.* Tetrahedron 1999, 55: 9575-84.

49. Tripp, J.A., Stein, J.A., Svec, F., Fréchet, J.M.J. *"Reactive filtration": Use of functionalized porous polymer monoliths as scavengers in solution-phase synthesis.* Org Lett 2000, 2: 195-8.

50. Tripp, J.A., Svec, F., Fréchet, J.M.J. *Grafted macroporous polymer monolithic disks: A new format of scavengers for solution-phase combinatorial chemistry.* J Comb Chem 2001, 3: 216-23.

51. Tripp, J.A., Svec, F., Fréchet, J.M.J. *Solid-phase acylating reagents in new format: Macroporous polymer disks.* J Comb Chem 2001, 3: 216-23.

52. Atrash, B., Bradley, M., Kobylecki, R., Cowell, D., Reader, J. *Revolutionizing resin handling for combinatorial synthesis.* Angew Chem Int Ed Engl 2001, 40: 938-41.

53. Gotfredsen, C.H., Grötli, M., Willert, M., Meldal, M., Duus, J.O. *Single-bead structure elucidation. Requirements for analysis of combinatorial solid-phase libraries by Nanoprobe MAS-NMR spectroscopy.* J Chem Soc Perkin Trans I 2000, 1167-71.

54. Chan, T.Y., Chen, R., Sofia, M.J., Smith, B.C., Glennon, D. *High throughput on-bead monitoring of solid phase reactions by diffuse reflectance infrared Fourier transform spectroscopy (DRIFTS).* Tetrahedron Lett 1997, 38: 821-4.

55. Johnson, C.R., Zhang, B. *Solid phase synthesis of alkenes using the Horner-Wadsworth-Emmons reaction and monitoring by gel phase ^{31}P-NMR.* Tetrahedron Lett 1995, 36: 9253.

56. Svensson, A., Fex, T., Kihlberg, J. *Preparation of fluorinated linkers: Use of F-19 NMR spectroscopy to establish conditions for solid-phase synthesis of pilicide libraries.* J Comb Chem 2000, 2: 736-48.

57. Swayze, E.E. *The solid phase synthesis of trisubstituted 1,4-diazabicyclo[4.3.0]nonan-2-one scaffolds: On-bead monitoring of heterocycle forming reactions using N-15 NMR.* Tetrahedron Lett 1997, 38: 8643-6.

58. Giralt, E., Rizo, J., Pedroso, E. *Application of gel-phase ^{13}C-NMR to monitor solid phase peptide synthesis.* Tetrahedron 1984, 40: 4141.

59. Lorge, F., Wagner, A., Mioskowski, C. *Improved procedure for routine C-13 NMR analysis of Merrifield resin bound molecules.* J Comb Chem 1999, 1: 25-7.

60. Jamieson, C., Congreve, M.S., Hewitt, P.R., Scicinski, J.J., Ley, S.V. *Development and application of a carbonyl-^{13}C-enriched backbone amide linker for solid phase reaction monitoring.* J Comb Chem 2001, 3: 397-9.

61. Conti, N., Dal Cin, M., Gehanne, S., Piao, A., Seneci, P., Zaramella, A. *Dansylated analytical constructs: LC/MS quantitation of compounds from solid-phase synthesis.* Combinatorial Chemistry 2000 (July 11-14, London) 2000.

62. Williams, G.M., Carr, R.A.E., Congreve, M.S. et al. *Analysis of solid-phase reactions: Product identification and quantification by use of UV-chromophore-containing dual-linker analytical constructs.* Angew Chem Int Ed Engl 2000, 39: 3293-6.

63. Carrasco, M.R., Fitzgerald, M.C., Oda, Y., Kent, S.B.H. *Direct monitoring of organic reactions on polymeric supports.* Tetrahedron Lett 1997, 38: 6331-4.

64. McKeown, S.C., Watson, S.P., Carr, R.A.E., Marshall, P. *A photolabile carbamate based dual linker analytical construct for facile monitoring of solid phase chemistry: "TLC" for solid phase?* Tetrahedron Lett 1999, 40: 2407-10.

65. Lorthioir, O., McKeown, S.C., Parr, N.J., Washington, M., Watson, S. *Practical synthesis of a new analytical construct: Thiopyrimidine safety-catch linker for facile monitoring of solid-phase chemistry.* Tetrahedron Lett 2000, 41: 8609-13.

66. Klein, S.I., Molino, B.F., Czekaj, M. et al. *Design of a new class of orally active fibrinogen receptor antagonists.* J Med Chem 1998, 41: 2492-502.

67. Murray, P.J., Kay, C., Scicinski, J.J., McKeown, S.C., Watson, S.P., Carr, R.A.E. *Rapid reaction scanning of solid phase chemistry using resins incorporating analytical constructs.* Tetrahedron Lett 1999, 40: 5609-12.

68. Cano, M., Ladlow, M., Balasubramanian, S. *Studies on the chemical stability and functional group compatibility of the benzoin photolabile safety-catch linker using an analytical construct.* J Comb Chem 2022, 4: 44-8.

69. Scicinski, J.J., Congreve, M.S., Jamieson, C. et al. *Solid-phase development of a L-hydroxybenzotriazole linker for heterocycle synthesis using and analytical constructs.* J Comb Chem 2001, 3: 387-96.

70. Lorthioir, O., McKeown, S.C., Parr, N.J. et al. *Single bead characterization using analytical constructs: Application to quality control of libraries.* Anal Chem 2001, 73: 963-70.

71. Congreve, M.S., Ladlow, M., Marshall, P. et al. *Reporter resins for solid-phase chemistry.* Org Lett 2001, 3: 507-10.

72. Brady, P.A., Sanders, J.K.M. *Selection approaches to catalytic systems.* Chem Soc Rev 1997, 26: 327-36.

73. Ganesan, A. *Strategies of dynamic integration of combinatorial synthesis and screening.* Angew Chem Int Ed Engl 1998, 37: 2828-31.

74. Lehn, J.M. *Dynamic combinatorial chemistry and virtual combinatorial libraries.* Chem Eur J 1999, 5: 2455-63.

75. Allusive, A.V., Lehn, J.M. *Dynamic combinatorial chemistry: Evolutionary formation and screening of molecular libraries.* Curr Top Microbiol Immunol 1999, 243: 159-72.

76. Sanders, J.K.M. *Adventures in molecular recognition. The ins and outs of templating.* Pure Appl Chem 2000, 72: 2265-74.

77. Lehn, J.M., Eliseev, A.V. *Chemistry-dynamic combinatorial chemistry.* Science 2001, 291: 2331-2.

78. Cousins, G.R.L., Furlan, R.L.E., Ng, Y.F., Redman, J.E., Sanders, J.K.M. *Identification and isolation of a receptor for N-methyl alkylammonium salts: Molecular amplification in a pseudo-peptide dynamic combinatorial library.* Angew Chem Int Ed Engl 2001, 40: 1757-60.

79. Calama, M.C., Timmerman, P., Reinhouds, D.N. *Guest-templated selection and amplification of a receptor by non-covalent combinatorial synthesis.* Angew Chem Int Ed 2000, 39: 755.

80. Huc, I., Krische, M.J., Funeriu, D.P., Lehn, J.M. *Dynamic combinatorial chemistry: Substrate H-bonding directed assembly of receptors based on bipyridine-metal complexes.* Eur J Inorg Chem 1999, 1415-20.

81. Albrecht, M. *From molecular diversity to template-directed self-assembly. New trends in metallo-supramolecular chemistry.* J Incl Phenom Macrocycl Chem 2000, 36: 127-51.

82. Albrecht, M., Blau, O., Fröhlich, R. *An expansible metallo-cryptand as a component of a supramolecular combinatorial library formed from di(8-hydroxyquinoline) ligands and gallium(III) or zinc(II) ions.* Chem Eur J 1999, 5: 48-56.

83. Baxter, P.N.W., Lehn, J.M., Rissanen, K. *Generation of an equilibrating collection of circular inorganic copper(I) architectures and solid-state stabilization of the dicopper helicate component.* Chem Commun 1997, 1323-4.

84. Klekota, B., Hammond, M.H., Miller, B.L. *Generation of novel DNS-binding compounds by selection and amplification from self-assembled combinatorial libraries.* Tetrahedron Lett 1997, 38: 8639-42.

85. Arterburn, J.B., Rao, K.V., Perry, M.C. *Solid-supported hydrazine substrate for labeling estradiol ligands with rhenium.* Angew Chem Int Ed Engl 2000, 39: 771-4.

86. Hof, F., Nuckolls, C., Rebek, J. *Diversity and selection in self-assembled tetrameric capsules.* J Am Chem Soc 2000, 122: 4251-2.

87. Cardullo, F., Calama, M.C., Snellink-Rüel, B.H.M. et al. *Covalent capture of dynamic hydrogen-bonded assemblies.* Chem Commun 2000, 367-8.

88. Calama, M.C., Hulst, R., Fokkens, R., Nibbering, N.M.M., Timmerman, P., Reinhoudt, D.N. *Libraries of non-covalent hydrogen-bonded assemblies, combinatorial synthesis of supramolecular systems.* Chem Commun 1998, 1021-2.

89. Berl, V., Huc, I., Lehn, J.M., DeCian, A., Fischer, J. *Induced fit selection of a barbiturate receptor from a dynamic structural and conformational/configuration library.* Eur J Org Chem 1999, 11: 3089-94.

90. Huc, I., Krische, M.J., Funeriu, D.P., Lehn, J.M. *Dynamic combinatorial chemistry: Substrate hydrogen-bonding directed assembly of receptors based on bipyridine-metal complexes.* Eur J Inorg Chem 1999, 9: 1415-20.

91. Rowan, S.J., Reynolds, D.J., Sanders, J.K.M. *Effects of shape oil thermodynamic cyclisations of cinchona alkaloids.* J Org Chem 1999, 64: 5804-14.

92. Brady, P.A., Sanders, J.K.M. *Thermodynamically controlled cyclisation and interconversion of oligocholates: Metal ion templates of "living" macrolactonisation.* J Chem Soc Perkin Trans I 1997, 3237-53.

93. Lukeman, P.S., Sanders, J.K.M. *Macrolactone-based dynamic combinatorial libraries of cholate monomers bearing recognition functionality.* Tetrahedron Lett 2000, 41: 10171-4.

94. Ramström, O., Lehn, J.M. *In situ generation and screening of a dynamic combinatorial carbohydrate library against concanavalin A.* Chembiochem 2000, 1: 41-8.

95. Hioki, H., Still, W.C. *Chemical evolution: A model system that selects and amplifies a receptor for the tripeptide* D*-Pro-*L*-Val-*D*-Val.* J Org Chem 1998, 63: 904-5.

96. Royo, M., Contreas, M.A., Giralt, E., Albericio, F., Pons, M. *An easy entry to a new high-symmetry, large molecular recognition studies and de novo protein design. Solvent modulation of spontaneous formation of a cyclic monomer, dimer or trimer from a bis-cysteine peptide.* J Am Chem Soc 1998, 120: 6639-50.

97. Otto, S., Furlan, R.L.E., Sanders, J.K.M. *Dynamic combinatorial libraries of macrocyclic disulfides in water.* J Am Chem Soc 2000, 122: 12063-64.

98. Kaiser, G., Sanders, J.K.M. *Synthesis under reversible conditions of cyclic porphyrin dimers using palladium-catalysed allyl transesterification.* Chem Commun 2000, 1763-4.

99. Nazarpack-Kandlousy, N., Zweigenbaum, J., Henion, J., Eliseev, A.V. *Synthesis and characterization of a mixture-base library of grime ethers based on a common aromatic scaffold.* J Comb Chem 1999, 1: 199-206.

100. Polyakov, V.A., Nelen, M.I., Nazarpack-Kandlousy, N., Ryabov, A.D., Eliseev, A.V. *Imine exchange in O-aryl and O-alkyl oximes as a base reaction for aqueous "dynamic" combinatorial libraries. A kinetic and thermodynamic study.* J Phys Org Chem 1999, 12: 357-63.

101. Huc, I., Lehn, J.M. *Virtual combinatorial libraries: Dynamic generation of molecular and supramolecular diversity by self-assembly.* Proc Natl Acad Sci USA 1997, 94: 2106-10; erratum in Proc Natl Acad Sci USA 1997, 94: 8272.

102. Goodwin, J.T., Lynn, D.G. *Template-directed synthesis: Use of a reversible reaction.* J Am Chem Soc 1992, 114: 9197-8.

103. Cousins, G.R.L., Poulsen, S.-A., Sanders, J.K.M. *Dynamic combinatorial libraries of pseudo-peptide hydrazone macrocycles.* Chem Commun 1999, 1575-6.

104. Furlan, R.L.E., Ng, Y-F., Otto, S., Sanders, J.K.M. *A new cyclic pseudopeptide receptor for Li^+ from a dynamic combinatorial library.* J Am Chem Soc 2001, 123: 8876-7.

105. Newman, D.J., Cragg, G.M., Snader, K.M. *The influence of natural products upon drug discovery.* Nat Prod Rep 2000, 17: 215-34.

106. Grifo, F., Newman, D., Fairfield, A.S., Bhattacharya, B., Grupenhoff, J.T. *The origins of prescription drugs.* In: Biodiversity and Human Health. Grifo, F., Rosenthal, J. (Eds.). Island Press: Washington 1997, 131-144.

107. Dolle, R.E. *Comprehensive survey of combinatorial library synthesis: 1999.* J Comb Chem 2000, 2: 383-433.

108. Watson, C. *Polymer-supported synthesis of non-oligomeric natural products.* Angew Chem Int Ed Engl 1999, 38: 1903-8.

109. Wessjohann, L.A. *Synthesis of natural-product-based compound libraries.* Curr Opin Chem Biol 2000, 4: 303-9.

110. Arya, P., Baek, M.-G. *Natural product-like chiral derivatives by solid-phase synthesis.* Curr Opin Chem Biol 2001, 5: 292-302.

111. Hall, D.G., Manku, S., Wang, F. *Solution- and solid-phase strategies for the design, synthesis and screening of libraries based on natural product templates: A comprehensive survey.* J Comb Chem 2001, 3: 125-50.

112. Evans, B.E., Rittlee, K.E., Bock, M.G. et al. *Methods for drug discovery: Development of potent, selective, orally effective cholecystokinin antagonists.* J Med Chem 1988, 31: 2235-46.

113. Nicolaou, K.C., Pfefferkorn, J.A., Roecker, A.J., Cao, G.-Q., Barluenga, S., Mitchell, H.J. *Natural product-like combinatorial libraries based on privileged structures. 1. General principles and solid-phase synthesis of benzopyrans.* J Am Chem Soc 2000, 122: 9939-53.

114. Nicolaou, K.C., Pfefferkorn, J.A., Mitchell, H.J. et al. *Natural product-like combinatorial libraries based on privileged structures. 2. Construction of a 10,000-membered benzopyran library by directed split-and-pool chemistry using nanokans and optical encoding.* J Am Chem Soc 2000, 122: 9954-67.

115. Nicolaou, K.C., Pfefferkorn, J.A., Barluenga, S., Mitchell, H.J., Roecker, A.J., Cao, G.-Q. *Natural product-like combinatorial libraries based on privileged structures. 3. The "libraries from libraries" principal for diversity enhancement of benzopyran libraries.* J Am Chem Soc 2000, 122: 9967-76.

116. St.-Hilaire, P.M., Meldal, M. *Glycopeptide and oligosaccharide libraries.* Angew Chem Int Ed 2000, 39: 1162-79.

117. Lee, M.-L., Schneider, G. *Scaffold architecture and pharmacophoric properties of natural products and trade drugs: Application in the design of natural product-based combinatorial libraries.* J Comb Chem 2001, 3: 284-9.

118. Hijikuro, I., Doi, T., Takahashi, T. *Parallel synthesis of a vitamin D_3 library in the solid phase.* J Am Chem Soc 2001, 123: 3716-22.

119. Norman, A.W., Bouillon, R., Thomasset, M. (Eds.). Vitamin D: Gene Regulation. Structure Function Analysis and Clinical Application. Walter de Gruyter and Co.: Berlin 1991.

120. Yamamoto, K., Masuno, H., Choi, M. et al. *Three-dimensional modeling of and ligand docking to vitamin D receptor ligand binding domain.* Proc Natl Acad Sci USA 2000, 97: 1467-72.

121. Bouillon, R., Okamura, W.H., Norman, A.W. *Structure-function relationships in the vitamin D endocrine system.* Endocr Rev 1995, 16: 200-57.

122. Doi, T., Hijikuro, I., Takahashi, T. *An efficient solid-phase synthesis of the vitamin D3 system.* J Am Chem Soc 1999, 121: 6749-50.

123. Nicolaou, K.C., Winssinger, N., Vourloumis, D. et al. *Solid and solution phase synthesis and biological evaluation of combinatorial sarcodyctiyn libraries.* J Am Chem Soc 1998, 120: 10814-26.

124. Schreiber, S.L. *Target-oriented and diversity-oriented organic synthesis in drug discovery.* Science 2000, 287: 1954-69.

125. Schreiber, S.L. *Chemical genetics resulting from a passion for synthetic organic chemistry.* Biorg Med Chem 1998, 6: 1127-51.

126. Blackwell, H.E., Perez, L., Stavenger, R.A. et al. *A one-bead, one-stock solution approach to chemical genetics: Part 1.* Chem Biol 2001, 8: 1167-81.

127. Clemons, P.A., Koehler, A.N., Wagner, B.K. et al. *A one-bead, one-stock solution approach to chemical genetics: Part 2.* Chem Biol 2001, 8: 1183-95.

128. Tan, D.S., Foley, M.A., Stockwell, B.R., Shair, M.D., Schreiber, S.L. *Synthesis and preliminary evaluation of a library of polycyclic small molecules for use in chemical genetic assays.* J Am Chem Soc 1999, 121: 9073-87.

129. Micalizio, G.C., Schreiber, S.L. *A boronic ester annulation strategy for diversity-oriented organic synthesis.* Angew Chem Int Ed 2002, 41: 152-4.

130. Fletcher, P.D.I., Haswell, S.J., Paunov, V.N. *Theoretical consideration of chemical reactions in micro-reactors operating under electro-osmotic and electrophoretic control.* Analyst 1999, 124: 1273-82.

131. Haswell, S.J. *Development and operating characteristics of microflow injection analysis systems based on electro-osmotic flow: A review.* Analyst 1997, 122: 1R.

132. Kricka, L.J., Wilding, P. *Micromechanics and nanotechnology*. In: Handbook of Clinical Automation, Robotics and Optimization. Kost, G.J. (Ed.). John Wiley & Sons: New York 1996, 45.

133. Duffy, D.C., McDonald, J.C., Schueller, O.J.A., Whitesides, G.M. *Rapid prototyping of microfluidic systems in poly(dimethylsiloxane) macromolecular folding*. Anal Chem 1998, 70: 4974-84.

134. Mitchell, M.C., Spikmans, V., Bessoth, F., Manz, A., De Mello, A. *Towards organic synthesis in microfluidic devices: Multicomponent reactions for the construction of compound libraries.* In: Micro Total Analysis Systems 2000. van den Berg, A., Olthius, W., Bergveld, P. (Eds.). Kluwer Academic Press: Dordrecht 2000, 463-5.

135. Reshni, K.A., Morris, M.K., Johnson, B.N., Burns, M.A. *On-line detection of electrophoretic separations on a microchip by Raman spectroscopy.* In: Micro Total Analysis Systems 1998. van den Berg, A., Olthius, W., Begveld, P. (Eds.). Kluwer Academic Press: Dordrecht 1998, 109-12.

136. Doku, G.N., Haswell, S.J., McDreedy, T., Middleton, R.J. *Preliminary studies into the direct interfacing of a micro reactor to a gas chromatographic instrument.* Analyst 2001, 126: 133-5.

137. Haswell, S.J., Middleton, R.J., O'Sullivan, B., Skelton, V., Watts, P., Strying, P. *The application of micro reactors to synthetic chemistry.* Chem Commun 2001, 391-8.

138. Dietzsch, E., Hönicke, D., Fichtner, M., Schubert, K., Weissmeier, G. *The formation of cycloalkenes in the partial gas phase hydrogenation of c,t,t-1,5,9-cyclododecatriene-c,t-1,5-cyclooctadiene and benzene in microchannel receptors*. In: Microreaction Technology: 4th Int Conf IMRET 4. Topical Conference Proc AIChE Spring Natl Meet (March 5-9, Atlanta) 2000, S89-99.

139. Greenway, G.M., Haswell, S.J., Morgan, S.O., Skelton, V., Styring, P. *The use of a novel microreactor for high throughput continuous flow organic synthesis.* Sens Actuatirs B 2000, 63: 153-8.

140. Wilson, N.G., McCreedy, R. *On chip catalysis using a lithographically fabricated glass micro reactor the dehydration of alcohols using sulfated zirconia.* Chem Commun 2000, 733.

141. Burns, J.R., Ramshaw, C. *A microreactor for the nitration of benzene and toluene.* In: Microreaction Technology: 4th Int Conf IMRET 4. Topical Conference Proc AIChE Spring Natl Meet (March 5-9, Atlanta) 2000, S133.

142. Chambers, R.D., Spink, R.C.H. *Micro reactors for elemental fluorine*. Chem Commun 1999, 883-4.

143. Skelton, V., Greenway, G.M., Haswell, S.J. et al. *The preparation of a series of nitrostilbene ester compounds using micro reactor technology.* Analyst 2001, 126: 7-10.

144. Skelton, V., Greenway, G.M., Haswell, S.J. et al. *The generation of concentration gradients using electroosmotic flow in micro reactors allowing stereoselective chemical synthesis.* Analyst 2001, 126: 11-3.

145. Watts, P., Wiles, C., Haswell, S.J., Pombo-Villar, E., Styring, P. *The synthesis of peptides using micro reactors.* Chem Commun 2001, 990-1.