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COVER STORY

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COMBINATORIAL CHEMISTRY

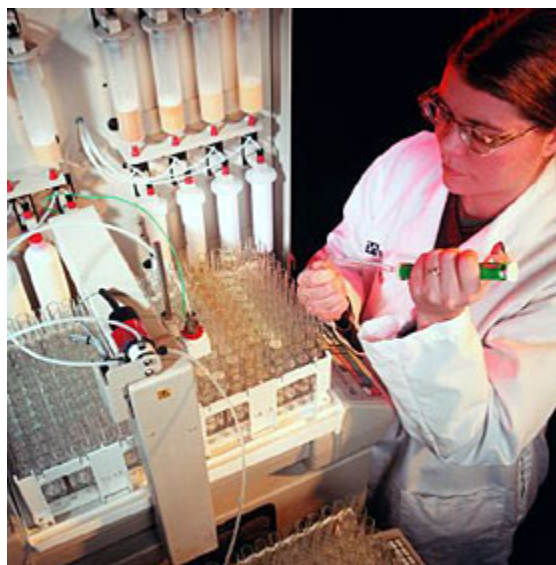
Advances in synthesis, purification, and analysis further refine the combinatorial approach, now a mainstream tool in drug discovery

STU BORMAN, C&EN WASHINGTON

Combinatorial Chemistry--a technology for synthesizing and characterizing collections of compounds and screening them for useful properties--was conceived about 20 years ago. Initially, the field focused primarily on the synthesis of peptide and oligonucleotide libraries.

In the 1990s, the focus of the field changed predominantly to the synthesis of small, druglike organic compounds. And many pharmaceutical companies and biotechnology firms now use it in their drug discovery efforts.

Questions arise from time to time about whether combinatorial chemistry has proved its mettle. Has it done any good? Have drugs been discovered with it that perhaps couldn't have been found any other way? And if combinatorial chemistry is such a great idea, why



MULTIPLICITY Combinatorial chemist performs parallel fractionation at Albany Molecular Research.

PHOTO COURTESY OF ALBANY MOLECULAR RESEARCH

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Up Cl

hasn't the concept been recognized with a Nobel Prize?

-- [Ch
Is](#)

Many compounds discovered combinatorially, including a couple mentioned in this article, have at least entered preclinical or clinical trials. That's some proof of the value of combinatorial chemistry. But the bottom line is that many researchers in academia, industry, and government already recognize it as an integral component of the drug discovery repertoire.



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Not that there's no point in scouring the rainforest for interesting natural products or designing drugs by "rational" means. Both are still done. It's just that the combinatorial approach is now used routinely to modify compounds from the rainforest or as a complement to rational design.

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Combinatorial chemistry has become established. It has changed drug discovery. It's a mainstream tool many drug companies would not want to do without. And as far as the Nobel Prize goes--it could happen.

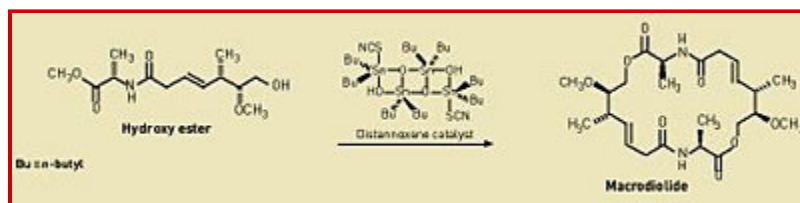
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Meanwhile, researchers continue to find ways to further enhance the capabilities of combinatorial chemistry, including these developments:

- A growing trend toward the synthesis of complex natural-product-like libraries, including the advent of carbohydrate-based libraries.
- An increased focus on "phase trafficking" techniques aimed at integrating synthesis with purification.
- Novel strategies for purification and analysis, such as the combinatorial use of supercritical fluid chromatography.
- And the application of combinatorial chemistry to new targets, such as nuclear receptors.

These and other developments were discussed at "Combinatorial Chemistry: Conventional Tools from Revolutionary Technology," an American Chemical Society ProSpectives conference held in Leesburg, Va., in September. The ACS ProSpectives program is a series of small conferences oriented toward industry scientists. The conference cochairs were Andrew P. Combs, head of a directed parallel synthesis group and a central nervous system medicinal chemistry project at Bristol-Myers Squibb, Wilmington, Del., and John C. (Jack) Hodges, senior director of cardiovascular chemistry at Pfizer Global R&D, Ann Arbor, Mich.

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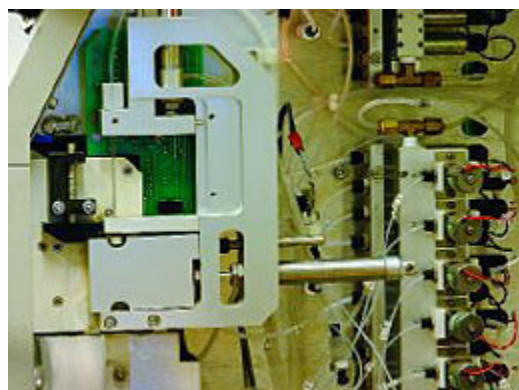


BIODIVERSITY Porco, coprincipal investigator James Panek, and coworkers at Boston University's new CMLD facility used a distannoxane transesterification catalyst to cyclodimerize in a single step an amino acid- and polyketide-containing hydroxy ester monomer to a 22-member macrodiolide--a compound that approaches the complexity of macrolide and macrodiolide antibiotics.

DIVERSITY-ORIENTED SYNTHESIS. Researchers today are showing "how one might consider combining natural product synthesis with combichem," Hodges said. "In the past, combichem has largely focused on simple tried-and-true synthetic sequences, whereas the natural product route tends to be infinitely more complicated and creative. Combichem is now growing into those more complex realms"--an approach called diversity-oriented synthesis.

"Diversity-oriented synthesis is taking on a new level," Combs added. "People are advancing it to the point where they're going after much more complex syntheses than in the initial years of combinatorial chemistry. They're tackling compounds with multiple stereocenters and very complex natural-product-like libraries, and that just wasn't happening three years ago."

Efforts to advance complex high-throughput synthesis got a boost this year from grants for two new [Centers of Excellence in Chemical Methodologies & Library Development \(CMLDs\)](#). At the ACS ProSpectives conference, the two principal investigators on those grants--chemistry professor [Peter Wipf](#) of the University of Pittsburgh and assistant professor of chemistry [John A. Porco Jr.](#) of [Boston University](#)--described current



AUTO CARBS Seeberger's automated oligosaccharide synthesizer (shown here in close-up) is a modified peptide synthesizer.

PHOTO BY FELICE FRANKEL, MIT

studies that exemplify the type of diversity-oriented synthesis projects that in the future will be carried out at their two centers.

Wipf and coworkers at Pitt have been synthesizing analogs of the complex natural product curacin A, an antimetabolic agent from blue-green algae, as potential leads for anticancer drugs. They have identified library compounds that exceed the potency and selectivity of the natural product.

And Porco and coworkers at BU recently used combinatorial synthesis to rapidly synthesize highly functionalized angular structures from chiral epoxyquinol dienes. They also developed a reaction sequence in which polyketide- and amino acid-containing hydroxy esters were cyclodimerized catalytically to form 22-member ring compounds that approach the complexity of macrolide and macrodiolide antibiotics.

"We are fundamentally interested in transformations where molecular complexity is generated rapidly," Porco said. "Future efforts will involve manipulation of variables such as ring size and stereochemistry to produce large numbers of complex macrocycles using cyclooligomerization reactions."

Natural-product-like libraries are also being constructed by postdoc Glenn C. Micalizio and coworkers in chemistry and chemical biology professor Stuart L. Schreiber's group at Harvard University. The researchers vary the structure of scaffolds--central structures on which a variety of functional groups are added or modified--to create diverse libraries. Combinatorial chemists have more typically varied the functional-group "decorations" on scaffolds, rather than the scaffolds themselves, to generate libraries.

Micalizio, grad student John P. Rearick, and coworkers recently created a complex natural-product-like library by using a branched network of boronic ester annulation reactions on a varied set of aldehyde scaffolds. The boronic ester annulations produced a range of boronic acid intermediates that were subsequently modified into functionalized enones, trisubstituted allenes, enediols, tetraols, polycyclic heterocycles, and 1,3-diols.

"There's been a gradual paradigm shift from the original idea of combichem, where you would take a single scaffold and make 10,000 or 20,000 compounds around it," Hodges said. "If you have lots of scaffolds with lots of functional groups on each one, diversity goes a lot further."

Associate professor [Peter H. Seeberger](#) and coworkers at Massachusetts Institute of Technology are bringing difficult-to-synthesize carbohydrates into the combinatorial fold. The researchers use automated solid-phase oligosaccharide synthesis to create large collections of complex carbohydrates from monosaccharide building blocks.



COCHAIRS Combs (left) and Hodges.

BRISTOL-MYERS SQUIBB PFIZER PHOTO PHOTO

Seeberger's automated carbohydrate synthesizer delivers solvents and reagents to a reaction chamber where sugar building blocks are combined with support-bound carbohydrates in a programmed manner. After several cycles, oligosaccharide products are born.

Seeberger described a strategy for using the synthesizer to make libraries of heparin-like glycosaminoglycans. Glycosaminoglycans are involved in growth factor and receptor interactions, blood coagulation, and plaque formation. New glycosaminoglycan agents created combinatorially might therefore prove useful as medications for cancer, viral infections, heart disease, and neurodegenerative diseases, among other conditions.

Up to now, Seeberger and coworkers have validated their synthetic approach by constructing only a small number of glycosaminoglycan variants in solution phase. But their first automated runs are under way, and the researchers are simultaneously pursuing a second research goal--the solid-phase synthesis of heparin chips (heparin combinatorial arrays).

"Of all the talks I saw at the conference, I would say that Seeberger's wowed me the most," Hodges said. "To have simplified and automated carbohydrate chemistry as well as he has is absolutely remarkable." The work could eventually lead to a "machine that would make different carbohydrates in different wells" of a combinatorial array, he said.

"Seeberger has done what nobody had been able to accomplish to date," Combs said. "By automating the synthesis of oligosaccharides, he took a job that once required years of a chemist's time and reduced it to days on a solid-

phase synthesizer. That's quite an accomplishment. It's the carbohydrate equivalent of the solid-phase synthesis of peptides"--the technique developed in the 1960s by biochemistry professor and Nobel Prize winner R. Bruce Merrifield of Rockefeller University.

Seeberger's and Micalizio's work "shows how far diversity has come since the early days of synthesizing libraries," Combs said. This progression reflects the growing influence of major academic synthetic organic chemistry groups on the art of combinatorial synthesis, he noted.

Studies by professor of medicinal chemistry [Ron Grigg](#) and coworkers at the University of Leeds, in England, also exemplify this trend. "From the perspective of a real hard-core synthetic organic person, Ron has been able to show how different types of cascading cyclization reactions can be brought to bear on diversity-oriented synthesis," Combs said. A cascade is a series of reactions that occur spontaneously.

"Ron uses cascading cyclizations to make some very complex molecules in very few steps," Hodges noted. "In a parallel synthesis, the more steps there are, the harder it is to get all your reactions to work. He has this amazingly fun set of multicomponent and multiring-forming reactions that get a lot done very quickly." An example is a novel palladium-indium diastereoselective cascade allylation reaction developed recently by Grigg's group, in which imines were converted to a diverse set of N-tosyl and N-aryl homoallyl amine products [*Chem. Commun.*, **2002**, 1372].

Microwave-assisted synthesis can also improve combinatorial productivity. Using a laboratory microwave oven, "reactions that took days can be done in minutes, usually with higher yields and potentially easier work-ups," said senior principal research scientist Christopher Sarko of Boehringer Ingelheim Pharmaceuticals, Ridgefield, Conn. "The days of the old oil bath"--a traditional lab apparatus for heating reactions--"are behind us."

Sarko and coworkers conducted an experiment to determine the time that can be saved by using microwave oven heating in combinatorial synthesis. One scientist used a microwave system to develop a combinatorial library, and another used conventional thermal heating. The two scientists were not permitted to communicate on methods and results. The goal of the experiment was the generation of three diverse library compounds with a liquid chromatography-mass spectrometric (LC-MS) purity exceeding 85%.

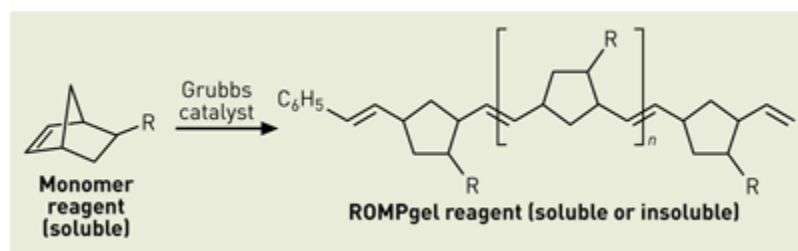
It took 37 days to develop the library thermally and two days to develop it with the microwave oven--about an 18-fold productivity increase. Sarko and coworkers calculated the return-on-investment time (break-even point) for adopting microwave synthesis to be 5.8 months. According to Sarko, major vendors of laboratory microwave heating equipment include Milestone (Soriso, Italy), [Personal Chemistry](#) (Uppsala, Sweden; [C&EN, Feb. 11, page 17](#)), and CEM Corp. (Matthews, N.C.).

"The nice thing about microwave heating is that you can do synthesis optimization really fast," Hodges commented. "You can try, say, 20 different conditions for the same reaction in a matter of hours, rather than weeks. And then once you get the right synthesis method, you can use the same instrument and go kind of one at a time to build a library. Nobody yet has a parallel-synthesis microwave device that would let you run, say, 96 microwave-irradiated reactions all at once. That may come someday. But in the meanwhile, microwave ovens are so fast that you can run serial reactions and still get a lot done. It's a new and coming technology in combinatorial chemistry."

Another emerging concept is the use of microreaction devices for miniaturizing combinatorial synthesis and screening. By reducing the space in which reactions take place, microreaction devices can make it faster and easier to optimize synthetic yield and selectivity, evaluate new reaction pathways, and carry out screening procedures, according to Managing Director Wolfgang Ehrfeld of Ehrfeld Mikrotechnik, Wendelsheim, Germany.

Characteristic dimensions of microreaction technology range from the submillimeter to submicrometer range, compared with centimeters or more in conventional reactions. "There's been lots of talk of microreaction technology," Combs said, "but I think Ehrfeld gave a convincing talk that this is actually going to be emerging soon."

Researchers are showing "how one might consider combining natural product synthesis with combi-chem."



COMBINATORIAL ROMP Ring-opening metathesis polymerization of a derivatized norbornene (shown) or other soluble monomer is used to create a ROMPgel reagent, which can be soluble or insoluble. The monomer can be polymerized after it's been used in a combinatorial reaction and then filtered from solution--a process called impurity annihilation--or the monomer can be polymerized first and then used as a supported reagent in parallel synthesis.

COURTESY OF ANTHONY BARRETT

PHASE TRAFFICKING. The most familiar way to synthesize most organic compounds is with classical solution-phase reactions, in which all the reagents, intermediates, and products remain in solution. "The beauty of pure solution-phase synthesis is the large lexicon of reactions one can perform," explained Daniel L. Flynn, president of Deciphera Pharmaceuticals, Natick, Mass. However, purifying products formed by solution synthesis can be difficult. "In solution-phase synthesis, typically an organic chemist will spend 10% of the time running a reaction and 90% of the time purifying it," Flynn said.

That's why chemists are increasingly exploring phase trafficking, an alternative to conventional solution-phase synthesis in which synthesis and purification are carried out more or less simultaneously. In phase trafficking, reagents, by-products, or products are directed into a separate phase so the products can be isolated easily from the reaction mixture. Phase-trafficking methods "can be mixed and matched to enable high-throughput synthesis of a wide array of compounds," Flynn noted.

Solid-phase organic synthesis--an approach in which reagents or products are attached to solid supports such as polystyrene beads--is the most traditional form of phase trafficking. With solid-phase organic synthesis, it's easy to purify products by filtration, it's possible to do mix-and-split synthesis (a technique used to make very large libraries), excess reagents can be used to drive reactions to completion, and syntheses can be automated easily. However, relative to solution-phase synthesis, solid-phase synthesis often requires more development time (to adapt familiar solution-phase reactions to the solid-phase milieu) and additional linkage and cleavage steps are needed (to attach compounds to supports and later

detach them). In addition, solid-phase reactions tend to be slower and product yields are more limited.

A new generation of phase-trafficking techniques was introduced in the 1990s, when Hodges and Flynn developed polymer-supported scavenging techniques that can be used combinatorially. With these techniques, you carry out a reaction in solution and afterward use polymer-supported affinity agents to scavenge reagents and by-products out of solution. Filtering the solution to remove the scavenger leaves the product in solution phase. In many cases, this method eliminates the need to purify products chromatographically.

Another phase-trafficking technique currently being developed by several research groups is the use of ring-opening metathesis polymerization (ROMP) to create ROMPgels--soluble or insoluble oligomeric or polymeric reagents and scavengers that can be used in combinatorial synthesis. ROMPgels are made by using Grubbs catalysts to carry out ROMP reactions on strained alkenes such as norbornene and 7-oxanorbornene. Functional groups on the alkene monomers are the active agents used to mediate combinatorial reactions or cleanups.

"I can make compounds on a ROMPgel that you can't make on polystyrene supports," said organic chemistry professor [Anthony G. M. Barrett](#) of Imperial College of Science, London. And the activity and reaction rate of ROMPgels are high because they are flow-through materials, whereas conventional solid supports only carry active groups on their surfaces [*Chem. Rev.*, **102**, 3301 (2002)].

"Typically with polystyrene reagents, one would use three or four equivalents, and the reactions are slow," Barrett said. "With ROMPgels, we would typically use about 1.2 equivalents, and the reactions are fast."

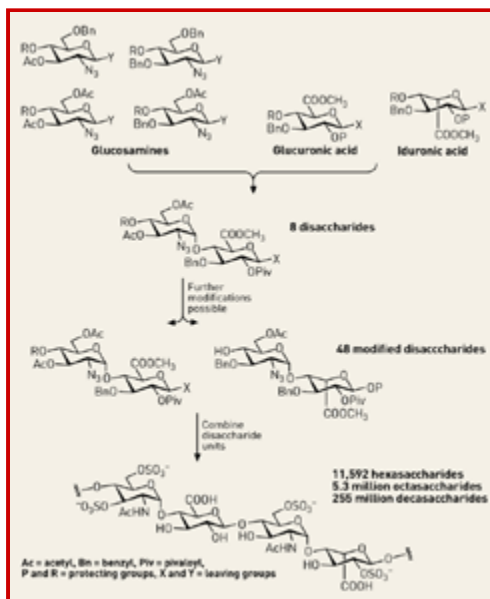
One way to carry out a combinatorial reaction with a ROMPgel is to synthesize a functionalized ROMP monomer, use it as a soluble reagent to carry out a transformation, and then polymerize it. Excess reagent, whether modified in the reaction or not, thus forms a polymer that falls out of solution, whereas the desired synthetic product stays in solution. This technique--which Barrett calls "impurity annihilation"--permits solution-phase parallel synthesis to be carried out with minimal need for additional purification.

ROMPgels can also be used as bifunctional reagents and as sequestration-enabling reagents (SERs). A bifunctional reagent is a soluble monomeric reagent with both chemical

reactivity and phase-trafficking components. And an SER reacts with an impurity that's difficult to remove and converts it to a sequesterable species.

It's also possible to make relatively short (20–60 unit) ROMP reagents, which are soluble in many traditional organic solvents but can be readily precipitated from solution using methanol or ether. They therefore "retain favorable reaction kinetics associated with homogeneous solution-phase synthesis, yet require only precipitation and filtering from a pertinent solvent as the sole purification protocol," said associate professor of chemistry [Paul R. Hanson](#) of the University of Kansas.

FULL SIZE - CLICK IMAGE



COMBI CARBS Seeberger and coworkers are developing an automated parallel synthesis of heparin-like glycosaminoglycans. Each of four glucosamines is added to a glucuronic or iduronic acid, forming eight disaccharides. The disaccharides are modified and combined, and the resulting oligosaccharides are deprotected and sulfated.

ADAPTED FROM SEEBERGER ET AL., CHEMISTRY-A EUROPEAN JOURNAL (IN PRESS)

In fact, "there's a whole myriad of applications" of ROMPgel technology, said Flynn, who recently reviewed its various permutations [*Curr. Opin. Drug Discovery Dev.*, **5**, 571 (2002)]. "It's a liberal system and a very forgiving one," he said. "One simply makes the choice--When do you want to introduce the reagent or scavenger, and when do you want to get rid of it?"

Soluble ROMP reagents are related to a liquid-phase combinatorial synthesis strategy developed in 1995 by chemistry professor [Kim D. Janda](#) and coworkers at Scripps Research Institute. In that technique, synthesis is carried out on a soluble polyethylene glycol (PEG) support, and the polymer is precipitated out of solution at the end of the reaction by changing the solvent.

"Although PEG is a very powerful and effective soluble support, its limitations, including low loading [low density of active groups], have driven efforts toward the development of new designer polymer systems," Hanson said. ROMP reagents are higher loading than PEG reagents because each monomer unit is derivatized with a functional group, whereas each PEG polymer is only derivatized at its end, Hanson noted.

Another promising phase-trafficking approach is fluororous mixture synthesis, a technique developed by chemistry professor [Dennis P. Curran](#) and coworkers at the University of Pittsburgh. Fluororous mixture synthesis makes it possible to run reactions on a number of fluororous-tagged starting materials and then easily separate and identify the reaction products.

First, fluororous tags of different chain lengths are added to a series of starting materials. The tagged substrates are mixed and taken through a multistep reaction sequence. Fluororous chromatography is then used to separate the tagged product mixtures, based on the fluorine content of the different tags. Finally, compounds in the separate solutions are "detagged" to release the final products.

Previously, only small libraries were accessible by this route. But the technique has now been adapted to moderate-size libraries of 100 to 1,000 compounds by Curran; Wei Zhang of Fluororous Technologies, Pittsburgh; and coworkers.

They recently used fluororous mixture synthesis to prepare a 560-member library of analogs of the natural product mappicine [*J. Am. Chem. Soc.*, **124**, 10443 (2002)]. The procedure required 90 reactions, compared with 630 for a corresponding parallel synthesis. And the separation phase of the procedure required only 80 chromatographic steps, whereas 560 would have been needed with parallel synthesis.

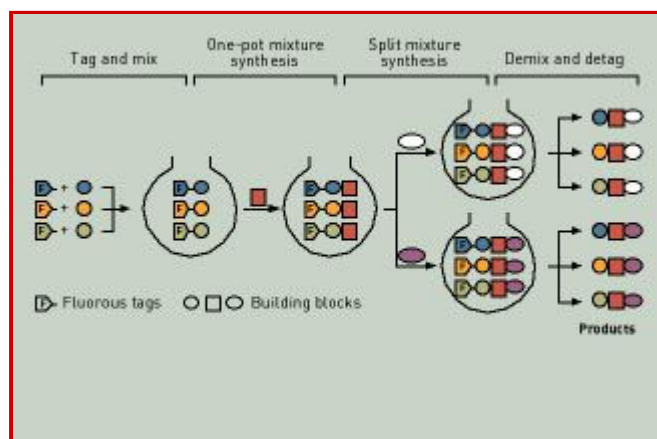
A new medium for solid-phase organic synthesis was also discussed at the conference. Combinatorial solid-phase organic synthesis requires the use of multiple aliquots of loose resin beads, which can be difficult to handle and keep track of. A number of technologies have been designed to make it easier to manipulate and track solid-support beads, including the following:

- Tea bag synthesis, developed by [Richard A. Houghten](#), president of Torrey Pines Institute for Molecular Studies, San Diego, in which small mesh bags are used to hold aliquots of resin.

- Directed sorting technology, marketed by the Irori unit of Discovery Partners International, which uses "kan" microreactors containing resin and miniature encoding devices.
- SynPhase Lanterns, offered commercially by Mimotopes, in Clayton, Australia, in which reactive sites are grafted on solid polymer surfaces.

Now, Polymer Laboratories, Amherst, Mass., has introduced StratoSpheres, rigid foamlike plugs in which resin beads are immobilized within an inert porous polyethylene matrix. The plugs "entrap a predetermined quantity of resin in such a way that it is free to react but is otherwise constrained inside the porous matrix," explained Polymer Laboratories Vice President Aubrey Mendonca. "They generally have significantly higher levels of functional group loading compared with equivalent-sized grafted or container devices." A future generation of StratoSpheres will have a broader range of shapes and dimensions and the capacity for encoding, he noted.

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MIX IT UP In fluorine mixture synthesis, different fluorous tags (blue, orange, and green) are linked to starting materials. Tagged compound mixtures are put through a reaction sequence, the products are separated, and the tags are removed to yield relatively pure products.

COURTESY OF FLUOROUS TECHNOLOGIES

PURIFICATION AND ANALYSIS. Combinatorial chemists already have in hand effective techniques for purifying many types of compound collections and for quantitating their purity, but some gaps still need to be filled. Speakers at the ACS ProSpectives conference addressed some of these needs and discussed ways in which combinatorial purification and analysis continue to advance.

By screening compounds for activity as mixtures, researchers can cover a lot of ground quickly. In fact, screening of mixtures is the traditional method used to evaluate compounds extracted from natural sources, such as microbes and plants. However, when compounds are synthesized and screened as mixtures, "usually, not only are you screening the things you intended to make, but you're screening a lot of debris that's in there with it," Hodges said. "That really muddies the waters as to what's active at the end of the day. That's one of the things that gives screening of mixtures a bad name."

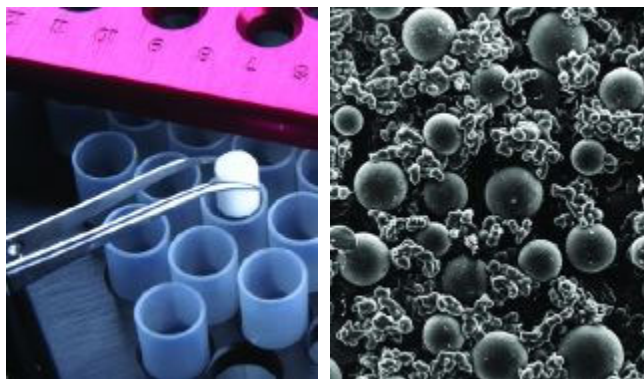
At the conference, associate professor [Michael G. Organ](#) of York University, Toronto, proposed one solution to this problem--the use of frontal affinity chromatography-mass spectrometry (FAC-MS) to screen mixtures.

FAC separates a mixture of compounds in a mobile phase by the compounds' affinity for an immobilized enzyme or other target, and electrospray MS monitors ions generated from each separated component. Together, they make it possible to separate and identify compounds in the mixture that bind strongly to a target. "Thus, the speed advantage of assaying mixtures can be realized while still treating compounds individually," Organ noted.

"A big trade-off with mixture screening is that you usually have to deconvolute--go back and remake each active compound individually," Combs said. "With FAC-MS, you don't."

Researchers at ChemRx, South San Francisco, a unit of Discovery Partners International, have developed an Accelerated Retention Window (ARW) method that rapidly cleans up large combinatorial libraries to 90% purity or greater. ARW calculates the LC solvent gradient composition that would be needed to elute a compound by preparative LC at a predetermined accelerated retention time. Using this setting makes it possible to collect the compound efficiently as a clean individual fraction.

"The ARW process has been used in the purification of more than 180,000 druglike compounds in the last 24 months," said ChemRx Vice President and General Manager Nathan Collins. "Compounds were produced and purified to meet client specifications in 2- to 50-mg quantities at greater than 90% purities."



RESIN PLUG In StratoSphere plugs (left), synthesis beads are immobilized in a flow-through polyethylene matrix (shown here at 50X magnification).

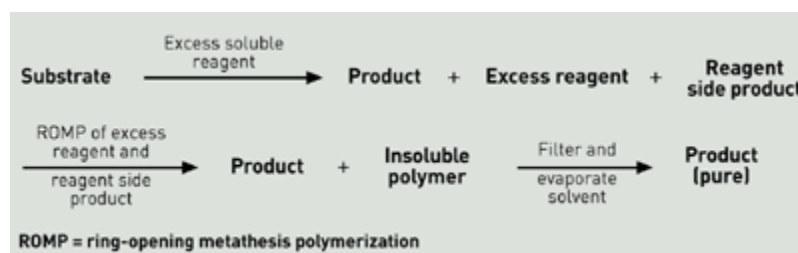
COURTESY OF POLYMER LABORATORIES

Another group has developed a set of techniques that make it easier to use LC-MS to purify parallel-synthesis libraries. Effective LC-MS purification conditions can be hard to work out, and generic LC-MS system settings are frequently problematic. This is particularly evident in labs where synthetic chemists carry out their own separations without assistance from chromatographic specialists.

Senior research scientist Karl F. Blom and coworkers at Bristol-Myers Squibb have now developed an LC sample-loading technique called "at-column dilution" that aids preparative LC of parallel libraries and a method-optimization protocol that facilitates LC-MS analysis of the library compounds [*J. Comb. Chem.*, **4**, 295 (2002)]. The at-column dilution technique was adapted in part from an approach reported earlier by Thomas E. Wheat of Waters Corp., Milford, Mass. The two techniques establish specific LC conditions and MS fraction collection parameters for each sample in an automated way, using customized software. Together, they improve chromatographic resolution by about a factor of three, compared to generic or universal prep LC methods, and increase the reliability and effectiveness of the MS analyses.

An emerging technology for purification of combinatorial libraries is supercritical fluid chromatography (SFC). "The lore that SFC can only analyze a small percentage of the structural types HPLC can handle has proven to be totally invalid for druglike molecules," said Larry Truesdale, director of combinatorial chemistry technologies for Pfizer, San Diego. "SFC is generally superior to HPLC due to its milder chromatographic environment and the greater number of theoretical plates available." The low cost of SFC solvents (such as CO₂) and waste disposal is also advantageous.

"We estimate the cost of running SFC is about half the cost of HPLC," Truesdale said. "This difference is very important when one is doing hundreds of thousands of separations a year." SFC instrumentation is expensive, but Truesdale noted that costs could plummet as the technology matures. "SFC is obviously a direction that a lot of pharmaceutical companies are going to want to take," Combs commented.



IMPURITY ANNIHILATION In this technique, polymerization and subsequent removal of excess reagent and side-product make it possible to perform solution-phase parallel synthesis with minimal additional purification. COURTESY OF ANTHONY BARRETT

APPLICATIONS. One combinatorial application discussed at the ACS ProSpectives conference was the discovery of an inhibitor for NS3-4A protease, an enzyme produced by the infectious hepatitis C virus. Scott Harbeson and fellow medicinal chemists at Vertex Pharmaceuticals, Cambridge, Mass., used solid-phase mix-and-split synthesis to prepare a combinatorial library of peptidyl aldehydes for rapid mapping of binding subsites on the protease. "We then prepared small, focused libraries of discrete compounds to elaborate the structure-activity relationships for subsites of particular interest," Harbeson noted. This led to the identification of VX-950, an orally dosable candidate for treatment of hepatitis C viral infection, which is now in preclinical development.

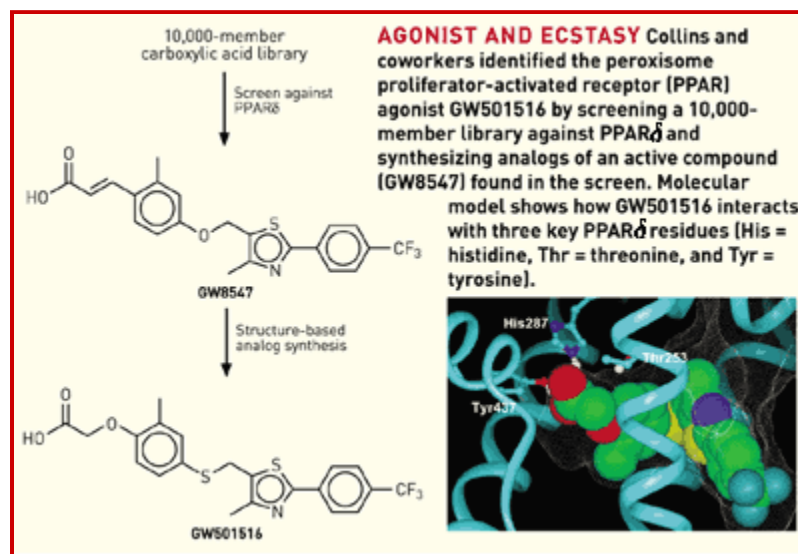
Recent combinatorial studies at GlaxoSmithKline, Research Triangle Park, N.C., have focused on peroxisome proliferator-activated receptors (PPARs) and other ligand-activated transcription factors in the nuclear receptor protein superfamily. Jon L. Collins and coworkers at GlaxoSmithKline made a 10,000-member carboxylic acid library of potential PPAR-targeted agents. The library was screened against PPAR δ , and an active pool of compounds was deconvoluted to find an active agent, called GW8547. Optimization of that compound using structure-based analog synthesis led to the identification of GW501516 as a potent, selective PPAR δ agonist. The compound is now a clinical candidate for treatment of cardiovascular problems associated with an "orphan disease" called metabolic syndrome X [*Proc.*

Natl. Acad. Sci. USA, **98**, 5306 (2001)].

"The utilization of target-biased library design and solid-phase parallel array synthesis were critical in accelerating the discovery of GW501516 as a potent, selective PPAR δ agonist," Collins said. The work "exemplifies the potential utility of combinatorial chemistry in accelerating drug discovery."

According to Combs: "Collins gave one of the most beautiful presentations on how combinatorial chemistry can be used in the pharmaceutical environment to understand the function of nuclear receptors. It was a really nice demonstration of making libraries, finding hits, taking the hits and optimizing them, and actually going into animals and discovering what each one of these receptors is responsible for--what its function is. It really showed combinatorial chemistry is not just a technology we're developing, but one that's actually being used, and very effectively."

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COURTESY OF GLAXOSMITHKLINE

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