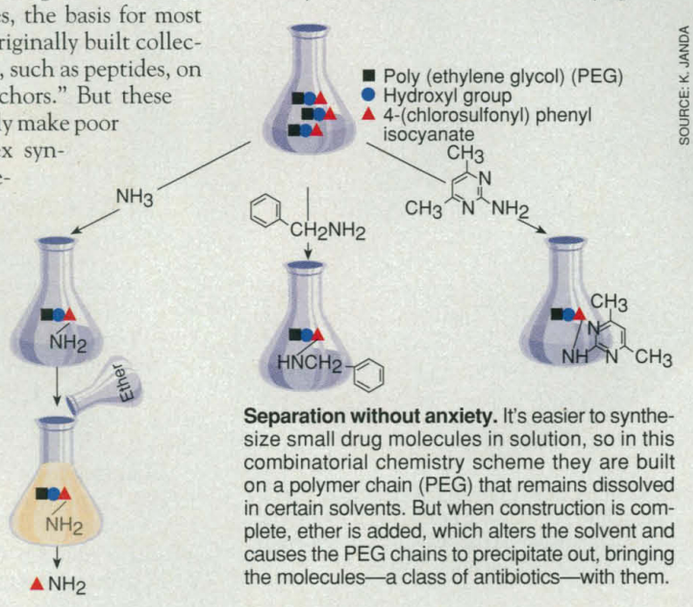
Combinatorial Chemistry Hits the Drug Market

Combinatorial chemistry is a hot field, and if interest from company job scouts is any gauge, it's only getting hotter. It burst on the scene in the early 1990s as a way to simultaneously create and then test thousands of related compounds for various kinds of activity, potentially fast-forwarding the process of drug discovery. And now "people on our project are getting calls from headhunters every day," says Steven Goldstein, who runs the combinatorial chemistry program at Pfizer in Groton, Connecticut.

The reason for the heated interest is that potential is becoming reality: Combinatorial chemists are finally learning how to handle small organic molecules, the basis for most drugs. The technique originally built collections of large molecules, such as peptides, on tiny polymer bead "anchors." But these large molecules generally make poor drugs, and the complex synthetic reactions that create small molecules are frequently incompatible with polymers. Now chemists have found reagents that happily associate with polymers. They've even gone a step further and optimized ways to do combinatorial chemistry in solution, eliminating the need to invent new reactions that accommodate solid supports. Because "essentially all of organic chemistry for the last 100 years has been done in solution," says Dale Boger, a chemist at the Scripps Research Institute in La Jolla, California, that's a big step forward. And still other researchers have figured out ways of combining solution- and solid-based methods.

Compounds discovered with the help of these new combinatorial techniques are beginning to move toward the clinic. Since last November, for instance, Eli Lilly, Pfizer, and Neurogen, a Branford, Connecticut–based drug discovery company, have each launched initial clinical trials of compounds designed to target central nervous system diseases, atherosclerosis, and obesity, respectively.

Lending support. Combinatorial schemes, of which there are many, all aim to assemble every possible permutation of a group of molecular building blocks. One of the most popular methods is the split-and-mix approach. Researchers start by attaching a single molecular building block—and a corresponding chemical tag—to a large pool of polymer beads or another type of solid support. These beads are split into, say, 10 batches, and a different building block–and-tag combo is then added to each of the separate batches. All the beads and their 10 molecular pairs are then mixed together and redivided into 10 batches, so that each batch now contains all 10 pairs. As another group of building blocks is added, the number of different combinations grows exponentially. Members of this "library" are then screened for activity against biological targets, such as the ability to prevent HIV from infecting a target cell (*Science*, 3 June 1994, p. 1399).



SOURCE: K. JANDA

Separation without anxiety. It's easier to synthesize small drug molecules in solution, so in this combinatorial chemistry scheme they are built on a polymer chain (PEG) that remains dissolved in certain solvents. But when construction is complete, ether is added, which alters the solvent and causes the PEG chains to precipitate out, bringing the molecules—a class of antibiotics—with them.

This technique's simplicity made it ideal for synthesizing peptides and oligonucleotides, whose amino acid and nucleic acid building blocks can be linked together using the same well-understood chemical reaction over and over again. But these compounds typically make poor drugs, as they "are quickly broken down by digestive enzymes," says Harvey Berger, CEO of Ariad Pharmaceuticals, a drug discovery company in Cambridge, Massachusetts. As well, Berger adds, the relatively large size of these molecules gives them "poor bioavailability," for they are typically too large to pass into the blood. So researchers have worked to adapt the technology to make small organic molecules, such as benzodiazepines (which include Valium and other tranquilizers), which at half the size of small peptides are less susceptible to these problems.

But synthesizing small druglike organic molecules on solid supports like beads has not been easy, says Daniel Kahne, a chemist at Princeton University. It takes a vast range of different reactions to build the chemical backbones, arms, and rings of small molecules. These reactions were originally designed to work in solution, and running them in a container filled with plastic beads can send them awry.

Recently researchers have begun chipping away at this problem. Earlier this year, for instance, Kahne, Columbia University chemist Clark Still, and their colleagues created the first combinatorial library of 1500 carbohydrate molecules, a class of small sugar compounds that act as receptors on the surface of many cells. To synthesize carbohydrates, researchers have traditionally used metal-containing catalysts that are hydrophilic: They prefer not to associate with hydrophobic molecules such as organic polymers. That has made it difficult to synthesize carbohydrates on polymer beads.

Kahne and his colleagues had discovered non–metal-containing catalysts that are less hydrophilic. And when the researchers used these reagents in conjunction with the solid support, they found that the reagents readily created the "glycosidic linkage" that stitches different sugar molecules together. Samuel Danishefsky, who also works in the area of carbohydrate synthesis and shares a joint appointment at Memorial Sloan Kettering Cancer Center and Columbia University in New York City, calls Kahne's effort "stunning," for even "making single carbohydrates is fairly difficult."

Kahne and his colleagues are not alone in their success. Other groups have also recently synthesized libraries of other small druglike molecules on solid supports, such as β-lactams, benzodiazepines, thiazolidines, and pyrrolidines. Peter Myers, chief operating officer of CombiChem, a small combinatorial chemistry company in San Diego, estimates that the number of reactions that have been optimized for the solid phase has jumped from just a handful just a few years ago to about 150, with more appearing every week.

The solution solution. These solid advances, however, do have limits. "The number of reactions you can put on the solid phase is still limited," says Myers. As well, he adds, in many cases it's still difficult to isolate more than a milligram of the desired target molecule, because the millions of beads or other solid supports take up most of the space in the reaction vessels.

The alternative is to run combinatorial schemes entirely in solution, doing away with beads and their ilk altogether, and thereby gaining the freedom to use a greater

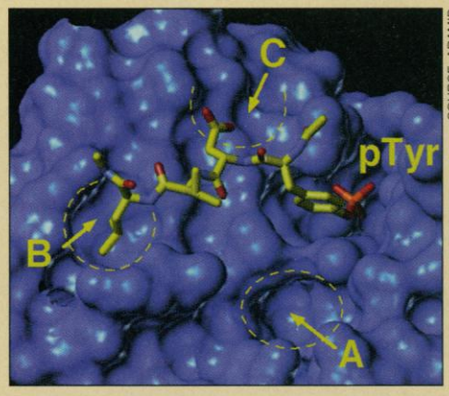
Aiming the Shotgun

Combinatorial chemistry is a shotgun approach. With it, chemists can rapidly turn out thousands or even millions of different compounds (see main text) in their search for an active drug or a probe molecule for research or diagnosis. But most of those compounds don't come close to binding to a target. So many researchers in business and academic research are mixing the power of combinatorial chemistry with the finesse of targeted, or rational, drug design to come up with focused libraries of compounds more likely to hit their targets.

"In the early days, combinatorial chemistry researchers said 'let's make millions of compounds by this technique' " and try them all, says Peter Myers, chief operating officer of CombiChem, a combinatorial chemistry company in San Diego. "That's still a valid approach. But it's relatively time-consuming, and screening millions of compounds [for biological activity] is relatively expensive and not easy," he adds.

Today, CombiChem and other combinatorial outfits are using techniques such as x-ray crystallography and computer-aided drug design to help them determine the structure and chemical behavior of proteins and other drug targets. They then use this information to figure out which types of small molecules are likely to have the proper shape and chemical attributes to bind to their targets. Chemists then build "biased" combinatorial libraries, using building blocks likely to create molecules with only those shapes and attributes. "It's clearly how the cleverer compounds are being made," says Steven Goldstein, who heads the combinatorial chemistry program at Pfizer's research laboratory in Groton, Connecticut.

Earlier this year, Stuart L. Schreiber and his colleagues at Harvard University reported the first study showing the potential payoff—and difficulties—of such focused libraries. The researchers were investigating the workings of a protein involved in intracellular communication known as the src protein. They knew that a particular region of the protein, the SH3 domain, had one unique protein-grabbing pocket, and suspected that it accounted for the specificity of src's interactions. By blocking the domain with a molecule of just the right shape, they hoped to glean clues to the region's function.



SOURCE: ARAMID

Designer combinations. To create a molecule that binds to this signaling protein *(blue)*, researchers picked molecular building blocks that fit into binding pockets (A, B, C, and pTyr), put them together in many ways using combinatorial methods, and came up with compounds that fit just right.

After reviewing computer models of the structure of the SH3 binding site, the researchers came up with 33 small-molecule building blocks that they believed had the shape and chemical attributes needed to bind to src's unique pocket. They then used conventional combinatorial chemistry synthesis techniques to create over 1 million compounds from these components. But none of the compounds bound well to the SH3 domain.

Because x-ray studies had already shown that larger molecules—peptides—only bind to src in a specific orientation, Schreiber and his colleagues suspected that their compounds just weren't lined up the right way to fit into the pocket. So the researchers added a short sequence of amino acids—which other work had shown to bind loosely to neighboring pockets in the SH3 domain—to their mix of target compounds. Their hope was that the amino acids would bind to one of these other pockets and hold the rest of the target compound in the proper orientation to fit into the selective binding pocket. The scheme worked. In the 10 January issue of the *Journal of the American Chemical Society*, the researchers reported finding several tight binders, one which bound more than 50 times tighter to the src protein than to other members of this protein family, suggesting the pocket–small molecule interaction was responsible. It is, says Roger Tung, who runs the combinatorial chemistry program at Vertex (a biotech company based in Cambridge, Massachusetts), "an excellent example of the application of computational chemistry to combinatorial synthesis."

Nor is it the only example: Researchers at a small Cambridge, Massachusetts–based biotech firm called Ariad Pharmaceuticals are using the same synthesis of combinatorial and computational techniques to find small molecules that bind to other cell-signaling proteins, such as ZAP, a protein involved in triggering the body's immune response. Ariad researchers hope this effort will lead to new immunosuppressant drugs. And other biotechs and pharmaceutical giants say they're combining the techniques to look for drugs to fight everything from arthritis to asthma.

–R.F.S.

variety of reagents and reactions. Researchers typically start with an array of spatially separated reaction containers, such as a 96-well plate, and place a different combination of molecular building blocks in each well, keeping track of the reactants. Instead of producing a different compound on different beads, here the result is a unique compound in each well.

But solutions have problems. Because the compound isn't conveniently attached to the surface of a bead, researchers have trouble isolating it from the mixture of reagents, reaction byproducts, and solvents in each one of thousands of wells. Conventional schemes for performing such separations tend to be slow. But earlier this year, in the 28 February issue of the *Journal of the American Chemical Society*, Boger and his colleagues reported a way to speed things up.

They found that in organic solvents, they could separate acidic and basic reagents from the product molecules by adding a water solution of additional acids or bases. The water-bound acids and bases either donate or swipe a proton from reagents in the solvent mixture, giving the reagents a net charge. These charges allow the reagent molecules to dissolve in water, as water molecules themselves are polar, meaning they can effectively separate charges in different regions; then the reagent-laden water and the organic solvent separate, like oil and water. Boger discards the water, then evaporates the remaining organic solvents, leaving just the purified small organic molecules.

"It's an interesting solution technique," says Rob Armstrong, a chemist at the University of California, Los Angeles, who also runs the chemistry program at Amgen, a biotech company in Thousand Oaks, California. But he adds that it's limited to reactions that use acids and bases as reagents. Other researchers, however, are already beginning to extend the strategy. University of Pittsburgh chemist Dennis Curran, for instance, has developed a similar technique to extract either reagents or the small molecules themselves into other liquids, known as perflurocarbons.

The best of both worlds. Another strategy combines the advantages of solution-based synthesis with the ease of extraction that comes from attaching the molecules to a solid support. One of the most provocative of these hybrid combinatorial schemes binds small-molecule building blocks to a polymer chain that has the ability to dissolve in certain solvents with the small molecules still attached. That allows scientists to carry out small-molecule synthesis in solution, and then change the solvent causing the polymer to be insoluble, so it—and its attached compounds—can be easily fished out.

The chain is known as polyethyleneglycol, or PEG. Kim Janda and his colleagues at the Scripps Research Institute reported last year in the 3 July issue of the *Proceedings of the National Academy of Sciences* that they used it to anchor arylsulfonamides, a class of known antibiotics, that they built up combinatorial step by combinatorial step in a solution of methylene chloride. When these reactions were complete, the researchers then added ether to their mixture, which caused the PEG molecules and their small molecular attachments to precipitate out of solution. They could then simply be filtered out. Using a related strategy, Armstrong and his colleagues use an all solution-based synthesis to construct libraries of small molecules. They then add insoluble polymers that contain linker groups specifically designed to home in on the target molecules. Once this attachment is made, the researchers simply filter out the polymers, and the small molecules come along for the ride.

Stephen Kaldor, who heads combinatorial chemistry research at Eli Lilly in Indianapolis, calls such hybrid schemes "very powerful," for they "combine the best of both worlds" of solution and solid-phase combinatorial chemistry. But at this point, many researchers believe that it's too early to tell whether hybrids or one of the solution-only techniques will end up edging out the competition in the race to become the combinatorial standard. "We now have a lot of different options people can use," says Kahne. And the options, like chemical building blocks themselves, can be combined in many different ways.

–Robert F. Service

RADIOASTRONOMY

Upgrade to Improve Arecibo's Vision

Poking through the early morning mists on 16 May, a faceted aluminum shell the size of a six-story building rose slowly to its place at the center of a curved, 90-meter arm sitting 137 meters above a huge dish. "It was a perfect operation," says Cornell University astronomer Paul Goldsmith about the telescope that residents of nearby Arecibo, Puerto Rico, call El Radar.

The shell is part of a $26 million upgrade that will sharpen the eyesight of the combination radio telescope and radar facility operated by Cornell's National Astronomy and Ionosphere Center (NAIC), which Goldsmith directs. Funded by the National Science Foundation and NASA, the improvements to the 33-year-old facility are expected to allow researchers to search for superfast radio pulsars, understand in greater detail the nature of the whirling gases in galaxies of the early universe, and learn more about the chemistry of the cloudy birthplaces of stars within the Milky Way. "The future looks pretty bright for us," says Alexander Wolszczan, a pulsar observer at Pennsylvania State University. The upgrade will also extend Arecibo's lead as "the world's most powerful radar," says Don Campbell, NAIC's associate director, allowing it to map nearby astronomical objects in ever-finer detail.

The shell, or dome, that was moved into place this month lets radio waves stream through a 13-meter "pupil hole" on its underside to the 305-meter-diameter dish nestled in the Puerto Rican hills. It will contain two strangely curved reflectors along with powerful new transmitting and receiving equipment—the equivalent of an optics package—that will greatly extend the observatory's high-frequency vision and boost its sensitivity across the spectrum.

The package will largely replace a more cumbersome and limited system based on "line feeds"—dangling lengths of leaky wave guides. Most parabolic antennas can be rotated to focus radiation to a single point, but Arecibo is too large to move. Instead, its dish was given a spherical shape that focuses a given beam of radiation into a line parallel to its original direction. The line feeds are tapered waveguides up to 29 meters long with "zillions of slots" to let radiation in and out, says Michael Davis, project scientist for the upgrade. They hang from a moveable feed arm and reshuffle the waves to bring them in phase to a receiver at the top of the feed. But each feed works only over a narrow band of frequencies, and they perform poorly at wavelengths shorter than about 10 centimeters—just where many astrophysical studies start to get interesting.

Moveable feast. The new dome houses reflectors that will give astronomers a better look into space.

The new setup, says Davis, "does it with mirrors"—a pair of reflectors that will bring the radiation to a point. Because the reflectors don't rely on reshuffling the phases, they can work over a continuous range of wavelengths. Incoming radiation will stream upward from the dish and bounce off the reflectors in succession, focusing at a chosen radio receiver within the dome. The observatory's high-frequency vision will then be limited only by the millimeter-size irregularities in the dish, limiting reception to wavelengths of 2 or 3 centimeters, corresponding to frequencies of 10 billion to 15 billion hertz. "Instead of having a narrow, blinkered view of the universe," says Goldsmith, "we'll be able to look at any frequency we want."

The new reflectors can also handle radiation from a larger area of the dish, providing up to a sevenfold increase in the observatory's sensitivity for radioastronomical studies. Radar studies of asteroids, comets, and planetary surfaces will also benefit from a new, megawatt transmitter housed in the dome, helping to boost the overall radar sensitivity by a factor of 20. This increase, for example, will allow radar astronomers to map asteroids millions of kilometers away to a resolution of 15 meters. The extra high-frequency reception is also just what astronomers need to study phenomena ranging from very fast pulsars to the birth of stars and planets in the largest molecules of dense, dark, cold clouds.

The upgrade is expected to be completed later this summer after the installation of a second reflector now sitting in an assembly plant in Sterling, Virginia. But astronomers can hardly wait to add to Arecibo's already illustrious track record, which includes the discovery of millisecond pulsars and planets around a pulsar, as well as the first accurate determination of Mercury's rotation rate. Says Cornell's Martha Haynes: "I asked [Goldsmith] at lunch today, 'So now that the dome is lifted, when are the proposals due?' We're ready."

–James Glanz