Drug Discovery on the Assembly Line

In the search for new drugs, diversity is strength. Combinatorial chemistry generates lots of molecular diversity, all at once—and keeps track of what it creates

Henry Ford didn't invent the car. But he is acclaimed as an industrial genius just the same because he developed the assembly line and in so doing changed the way everything is manufactured. Likewise, a growing group of chemists isn't the first to synthesize new drug candidates. But they have created a way of turning out candidate compounds quickly and affordably, and their work is changing the way R&D is done in the drug industry. "We have a new technology that will not only cut years off the time that it takes to develop new drugs and save millions of dollars in R&D costs, but one that is changing the whole notion of drug discovery and refinement," says Adele M. Haley, chief drug industry analyst for the Stover-Haley-Noyes Life Sciences Advisory Group.

The new technology sweeping the industry is called combinatorial chemistry, a way of churning out hundreds—or hundreds of millions—of compounds with known structures. "By simultaneously creating compounds with shapes, charges, and electrostatic characteristics covering a tremendous range of possibilities, combinatorial chemistry dramatically improves the odds of finding at least one new substance that will bind to some target [in the body] with extraordinary affinity," says Thomas Ingolia, director of natural products and biotechnology research at Eli Lilly. That, he adds, "is really what drug discovery is all about."

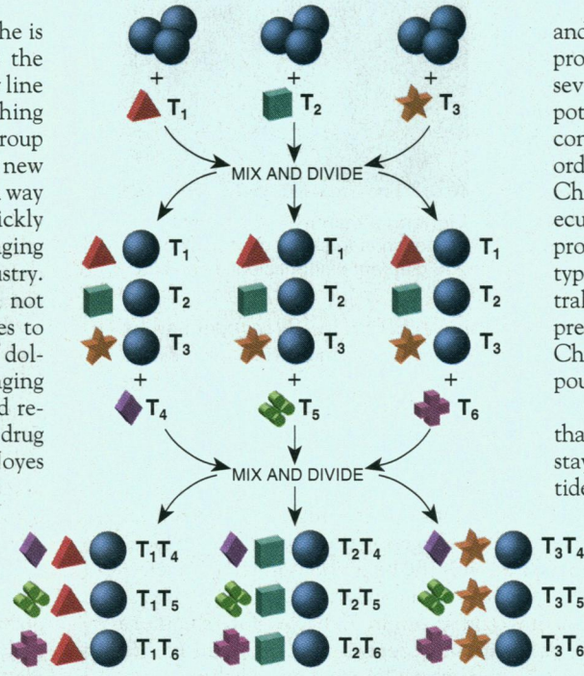
Classical synthetic chemistry was limited in the array of candidates it could muster because chemists could only make the molecular shapes and characteristics they could imagine. And while the world's forests, oceans, and compost piles are a treasure-trove of unexpected molecular entities, sorting this trove requires collecting thousands of natural specimens and assaying them for biological activity. Then comes the even larger task of isolating and purifying the active ingredient, determining its structure, and most difficult of all, synthesizing it. Combinatorial chemistry consolidates these laborious tasks into one quick succession of steps.

The basic strategy is to assemble every possible combination of a given set of chemical building blocks while simultaneously recording which ones have been used and in what order, then assay the resulting molecules all at once—and refer to the record to determine the identity of any that look promising. Since the strategy was introduced by researchers at Affymax Pharmaceuticals in 1991, it has triggered a combinatorial explosion of its own, blossoming into a multitude of variants based on different molecular building blocks and strategies for keeping track of the product molecules. It has also sparked a proliferation of start-up drug discovery companies exploiting variations of the combinatorial chemistry theme.

These start-ups and the established pharmaceutical companies that are building their own in-house combinatorial teams are aiming to streamline a process that takes an average of 12 years and costs $359 million, according to the U.S. Office of Technology Assessment. With combinatorial chemistry, says Larry Gold, co-inventor of one combinatorial technology and founder of NeXagen in Boulder, Colorado, "it is now possible to come up with several solid drug candidates in a matter of months instead of years."

NeXagen scientists, for example, last year took less than 4 months to generate a library of short, chemically stabilized chains of nucleotides, screen them for the ability to bind and block basic fibroblast growth factor (a promoter of cell proliferation), and move several of the compounds into animal tests as potential treatments for cancer and other conditions. And in what may be a speed record, it took only a few weeks for chemists at Chiron to develop several small organic molecules that bind specifically to a family of G-protein-linked receptors, the most common type of neurotransmitter receptor in the central nervous system. Says Walter Moos, vice president for chemical therapeutics at Chiron, "Older methods of making one compound at a time just can't compete."

Combinatorial chemists are quick to admit that the strategy is no panacea. So far, its mainstays have been peptides and oligonucleotides—polymers consisting of repeated units. Such mix-and-match molecules were the natural choice for working out the basic technology, but they are fragile and difficult to administer compared to most existing drugs. Over the past year or so, however, several groups have succeeded in applying combinatorial strategies to the simple rings and chains that are the building blocks of small organic molecules, which constitute the vast majority of drugs available today.



Add and react. In one combinatorial chemistry scheme, molecular building blocks *(shapes)* and their matching chemical tags (*T*) are linked one-by-one to plastic beads. After each successive addition, the beads are mixed and then divided into new pools, leading to a combinatorial explosion.

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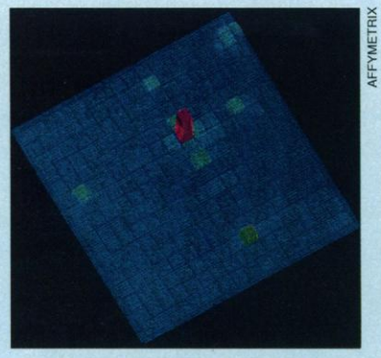
The key feature setting apart the various combinatorial techniques is how they keep track of the vast molecular libraries they generate. Affymax's Encoded Synthetic Libraries (ESL) approach, perfected last year, relies on easy-to-read chemical labels, written as strings of nucleotides. In ESL, each potential drug candidate is grown on a 10-micron plastic bead, side-by-side with its tag. A three-nucleotide codon identifies each molecular building block.

The numbers game

The process begins when the first building block and its codon are added to a single large pool of beads and attach themselves to its surface. Then the pool is split into as many batches as there are building blocks—20, if the subunits are amino acids—and a different building block and matching codon is added to each one, creating 20 different two-unit combinations. Now the subsets are recombined, redivided into 20 new pools, and again reacted with one of the amino acids and then its codon, resulting in 400 different three-unit combinations. Repeating this process eight times with all 20 amino acids

Putting Genes on a Chip

Now that combinatorial chemistry is showing its mettle by spinning out candidate disease treatments (see main text), researchers at Affymetrix hope it will prove equally adept at diagnosis. By merging silicon-patterning techniques borrowed from microelectronics with combinatorial chemistry, this small biotech start-up in Palo Alto is producing intricate checkerboards of different nucleotide sequences anchored to a silicon chip. These chips, they say, can serve as detectors of aberrant genetic sequences, which would bind to specific sites on the checkerboard.



Lighting a match. A fluorescent probe binds to sites on a matrix of 256 different eight-nucleotide chains.

"Basically, we're writing down genetic sequence information in a way that is easily accessible using standard hybridization techniques," says Stephen Fodor, who with colleagues at parent company Affymax developed the technology, very-large-scale immobilized polymer synthesis (VLSIPS), in 1991. Fodor and his colleagues originally envisioned VLSIPS as a way to generate and screen drug candidates, but other techniques have since surpassed it for drug development. Where Affymetrix hopes VLSIPS will shine, however, is in the testing of genetic and viral diseases. They also think that it might speed gene sequencing—a hope shared by the Human Genome Project, which has funded the company to explore the technology.

VLSIPS starts with a silicon chip coated with a nucleotide linked to a light-sensitive chemical "block" group that prevents further reactions. Light shining through a mask illuminates specific grid squares on the coated chip, triggering the removal of the block group in those spots. The chip is then incubated with the next photoprotected nucleotide, which binds to the exposed spots. Another round of exposure and reaction binds a third nucleotide to a different set of grid sites, and so on. In 32 cycles, for example, a carefully planned sequence of masking patterns and reactions can create all 65,536 possible chains of eight nucleotides, each one in a known location on the chip.

The chip can then probe a solution of fluorescent-labeled DNA fragments for the presence of specific sequences. Complete sequence matches show up as squares of bright fluorescence, while single-base mismatches yield dimly lit squares. The matches could betray the presence of genetic defects, such as the cystic fibrosis gene, or pathogen strains, says Fodor. Together, the matches and the single-base mismatches could also reveal the complete nucleotide sequence in a set of DNA fragments, he adds.

That's still a ways off, but in the meantime Fodor and his colleagues are pushing ahead with combinatorial diagnostics; they plan to begin shipping diagnostic chips to researchers and clinicians by the end of the year. Says Adele M. Haley, a drug industry analyst, "Affymetrix's technology is going to be a big winner. It will be fully automated and should be capable of running upwards of 100,000 tests simultaneously. That should offer significant savings for big clinical laboratories." And in today's world of medical cost-cutting, any savings usually translates into success.

–J.A.

creates a library of the 25.6 billion possible nonapeptides, each identified by a unique string of 24 nucleotides.

To assay the activity of such molecular libraries, Affymax chemists mix the beads with a fluorescent-tagged version of the receptor, antibody, or other molecule that is the target of the desired drug. Beads bearing molecules that bind to the labeled target fluoresce, and a commercially available fluorescence-activated cell sorter can pick them out from among the inactive beads—a rapid alternative to the painstaking purification and isolation required to extract a promising compound from a natural specimen. Then it's simply a matter of reading each bead's DNA tag, using the polymerase chain reaction (PCR) and standard sequencing technology, to identify its molecular cargo.

Rather than creating labels, scientists at Chiron, Parke-Davis, Sphinx Pharmaceuticals, and other companies rely on spatial location to track their compounds. At Chiron, for example, compounds are grown on an array of polyethylene pins manipulated by a robot arm. In a carefully calculated sequence, the pins are dipped into sets of minute test tubes containing reactive building blocks. A different subset of pins is exposed to each successive building block, with the result that each pin ends up carrying a different combination of building blocks. The pins can then be exposed to a fluorescent-tagged version of the drug target, and the identity of a compound that has a strong affinity for the target can be determined from the pin's position.

A third approach forgoes any labeling scheme—something that's possible if the building blocks are nucleotides, whose sequence can readily be deciphered. NeXagen, for example, begins with a primer sequence, then uses a battery of oligonucleotide synthesizers to put it through 25 to 30 cycles of nucleotide addition. After each cycle, the growing oligonucleotides from each synthesizer are mixed, then split into new batches. The result is trillions of different oligonucleotides, which can be mixed with target molecules. PCR and automated sequencing can then reveal the identity of the molecular needles-in-a-haystack that bind to the target.

This field is so young and is breeding its own technical combinations so rapidly that no two companies use exactly the same technique. (Indeed, most firms have applied for patents to cover their variants of combinatorial chemistry.) But there doesn't seem to be much arguing about whose approach is best. "I'm sure each company takes pride in its method, but no one has a monopoly on good combinatorial methods," says James Bristol, a vice president at Parke-Davis. "What's important is that this technique speeds up the early phases of the drug discovery process and may reduce the overall cost of discovering a new chemical entity,"

And the strategy not only betters the odds of finding an active compound; because it creates such a large pool of contenders, the candidate compounds are often more specific than those found in nature or synthesized by traditional means. Barry Polisky and his colleagues at NeXagen, for example, recently created a pool of 10^{14}—that's a hundred trillion—different RNA molecules containing a 38-nucleotide random sequence.

The NeXagen chemists then screened these molecules for the ability to discriminate between theophylline, a common anti-asthma medication, and caffeine, which differs from theophylline by only a single methyl group. One of the oligonucleotides had an affinity for theophylline 10,000 times greater than its affinity for caffeine—discrimination 100 times better than that of the monoclonal antibody now used to monitor blood theophylline levels (*Science*, 11 March, p. 1425). That feat is of more than academic interest; a more selective theophylline assay would increase the safety of using the drug to control asthma.

A farewell to elegance?

Such feats haven't won over the entire community of organic chemists. Stalwarts mourn the loss of the elegant chemistry required to assemble a complex molecule piece by piece.

As a result, says Thomas N. Salzmann, vice president for chemistry at Merck, "not everyone here is a convert" to combinatorial chemistry—although he adds that the resistance is "not for any good scientific reasons."

Yet even the most ardent advocates of combinatorial chemistry say they will never put traditional organic chemists out of business. "We still need their creativity to develop new chemistry, new reactions that we can use in our brute-force methods," says Jack Baldwin, president of Pharmacopia, a year-old drug discovery company. What's more, combinatorial chemistry has had to cede some tasks to traditional synthetic chemistry because of a practical drawback.

The drawback is that the kinds of molecules best suited to the combinatorial approach—peptides and nucleic acids—can't be taken by mouth; they are either too large to be absorbed in the gastrointestinal tract, or they get digested along the way. To cope with that limitation, officials at some companies, such as NeXagen and Protein Engineering, are targeting diseases severe enough that injections will be acceptable, such as cancer and AIDS. Or they are adopting other goals: using combinatorial libraries for diagnostic purposes (see box) or as guides for traditional chemical synthesis.

"We can use these high-affinity binders not as drugs themselves, but as models from which small organic molecules can be crafted with the appropriate spatial and biophysical characteristics," explains Edward Cannon, Protein Engineering's chief executive officer. By studying the shapes created by combinatorial chemistry, for example, his company has identified the structural features that are the key to inhibiting elastase—an enzyme that plays a role in inflammatory diseases—and several enzymes involved in clotting.

These limits may only be temporary. As combinatorial chemists move beyond nucleic acids and peptides to small organic molecules, they may be able to compete head-to-head with traditional drug discovery techniques. Creating small molecules in combinatorial fashion, says Affymax's Gordon, "is definitely a challenge, but we and many others are finding that small molecule synthesis is doable." Michael Pavia of Sphinx Pharmaceuticals explains that "it's not that it's harder to do with small organics; it's just that we need to develop the ability to do traditional organic chemistry on solid supports."

At Parke-Davis, for example, Sheila Hobbs DeWitt, using the company's DIVERSOMERS technology, has created an array of ringed heterocycles (compounds containing oxygen, nitrogen, or sulfur). Among them are benzodiazepines—the family that includes the drugs Valium and Xanax—and hydantoins, which include Dilantin. Still, Gordon adds, it will probably be a few years before combinatorial synthesis of small molecules rivals the power it has demonstrated with peptides and nucleic acids.

When it does, the main problem combinatorial chemists may face is their technique's very efficiency. Most assay systems are not designed to handle billions of compounds. Then there is the glut of information that comes from testing such a large number of compounds with known structures. "In theory, combinatorial chemistry provides the perfect data set for sophisticated structure-activity relationship analysis," says Merck's Salzmann. In reality, says Chiron's Moos, "we can now generate so much data so fast that we're in danger of becoming overwhelmed." In some respects, combinatorial chemistry may be too much of a good thing.

–Joseph Alper

ATMOSPHERIC CHEMISTRY

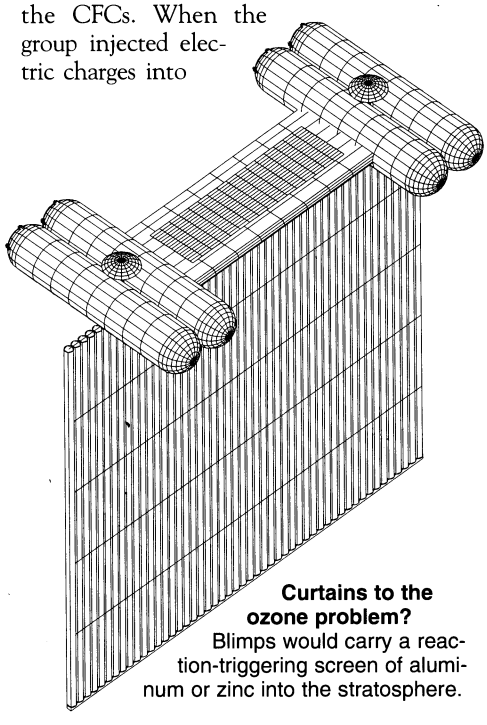
A High-Flying Fix for Ozone Loss

Alfred Wong knows he's in danger of overreaching when he talks about extending his lab-scale chemical reaction to the scale of the globe. But the University of California, Los Angeles, plasma physicist thinks the chemical transformation he has seen in a chamber of gases has implications too important for the global atmosphere to keep silent, in spite of a blast of criticism from atmospheric scientists. Wong's bold claim: Twenty or so panels of zinc or aluminum, each at least the size of a football field, carried aloft by balloons, could sustain a global version of the laboratory process—and provide a quick fix for stratospheric ozone loss.

That implication is buried in a paper in the 9 May *Physical Review Letters*, where Wong and his colleagues describe a scheme for inactivating the chlorine atoms, unleashed by manmade chlorofluorocarbons (CFCs), that destroy ozone in the stratosphere. The paper mainly describes laboratory results, but Wong's grander claim was the subject of a UCLA press release—and ensuing coverage by television networks and print media, including the Associated Press and *Newsweek*. "It was very well received," noted a spokesman in the UCLA press office.

By the press perhaps, but not by atmospheric scientists, who cite daunting technical problems. "It is a very clever idea, but it gets messy," says Earle Williams, who studies atmospheric electrical phenomena at the Massachusetts Institute of Technology (MIT).

Atmospheric chemists have little quarrel with the findings Wong's group described in the paper. In a coffin-sized chamber, the group monitored a simplified stratospheric brew including CFCs and low levels of ozone molecules, which were constantly created by ultraviolet light but were depleted by the CFCs. When the group injected electric charges into the chamber, ozone levels increased sharply. The researchers concluded that, by converting reactive chlorine atoms to virtually inert chloride ions, the charges had stymied the ozone-destroying process.



ALFRED WONG

Curtains to the ozone problem? Blimps would carry a reaction-triggering screen of aluminum or zinc into the stratosphere.

In their paper, Wong and his colleagues limited themselves to raising the possibility that the process could be scaled up, without saying how. But in an interview, Wong spelled out his scheme. Into the Arctic and Antarctic stratospheres, where the worst ozone depletion takes place, he would fly 10 to 20 balloon-borne platforms carrying sheets, wires, or curtains of a metal whose electrons can easily be dislodged by ultraviolet light. As in the laboratory, Wong believes some of the charges would find their way to the reactive chlorine atoms, converting them into inactive chloride ions that could then be collected by other, positively charged surfaces. Wong says he has been working with an engineering firm to draw up plans for the platforms, which would cost $25 million each. The total of $500 million for the full fleet would be a bargain—if it restored the ozone layer.

Some prominent atmospheric scientists don't dismiss the idea of global remediation. "If you have a serious problem [like ozone depletion]...you have to think about remediation," notes F. Sherwood Rowland of the University of California, Irvine, who with Mario Molina, now of MIT, first described the ozone-destroying chemistry of CFCs in 1973. Ralph Cicerone, a colleague of Row-