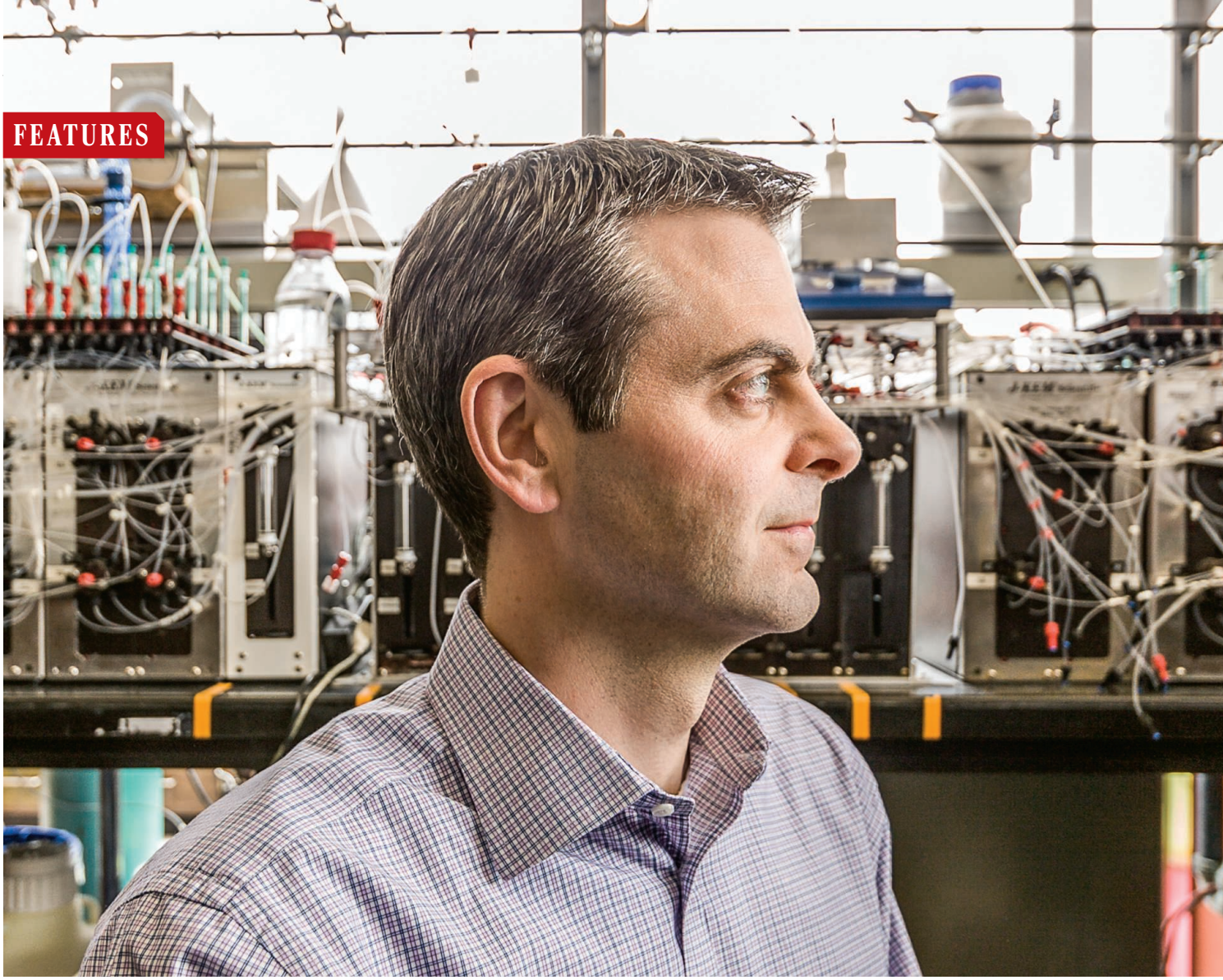

THE SYNTHESIS MACHINE

An automatic device that makes small organic molecules could revolutionize drug discovery

By **Robert F. Service**, *in Urbana, Illinois*

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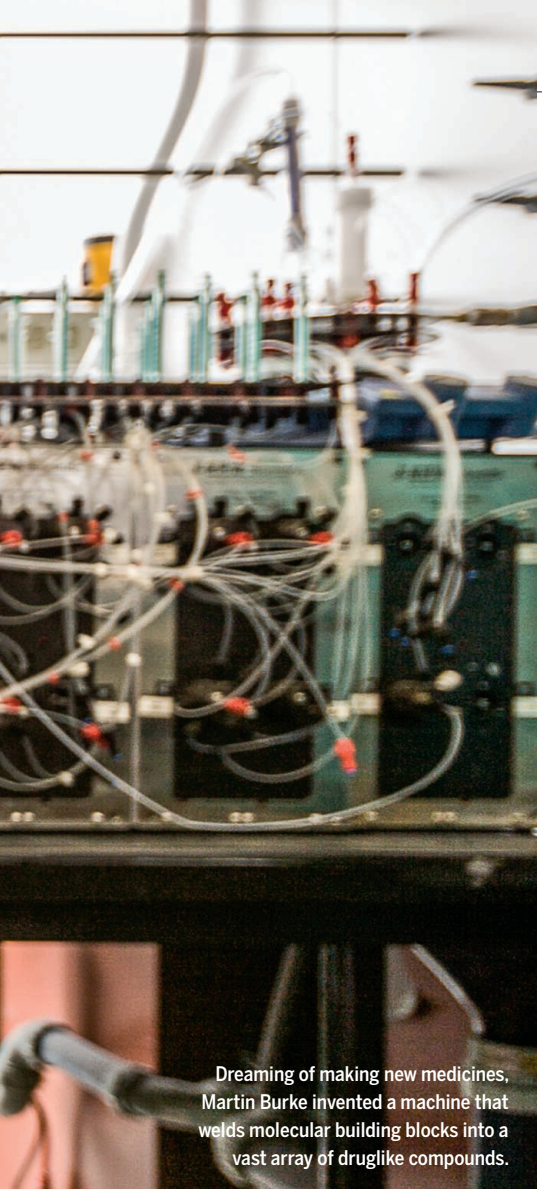


Dreaming of making new medicines, Martin Burke invented a machine that welds molecular building blocks into a vast array of druglike compounds.

Organic chemistry is an ordeal—just ask most science majors. There are the bewildering names of molecules, the elements, bonds, reactions, and reagents. There are the recipes, lab work, and late nights hovering over flasks. There are the separations, purifications, and analysis. Even for experts, making molecules is slow, painstaking work.

"We think we can change that," says Martin Burke, a chemist at the University of Illinois, Urbana-Champaign. And to drive the point home, he offers to transform a chemistry neophyte—me—into a synthetic chemist.

Burke steers me into Room 456 of the Roger Adams Laboratory building and toward a black lab bench holding a contraption about the size of one of those industrial espresso machines you see at Starbucks. Atop it sits two aluminum blocks, drilled through with 2.4-centimeter-wide holes, for holding nine vials. A tangle of thin tubes connects all the different pieces. But its basic principle is simple: It's a chemistry version of a highway cloverleaf, intended to steer ingredients from one place to another. Burke and his students call it simply "The Machine."

Michael Schmidt, a second-year graduate student in Burke's lab, gives me a recipe for crocacin C, an antifungal compound first synthesized in 2000 by three Australian chemists. Plastic vial number 1, which contains a pinch of a white crystalline powder, goes in slot number 1. Vial number 2, with a different white crystalline powder, goes in slot number 2, and so on. Schmidt has me connect a few spaghetti-width tubes to feed in water, organic solvent, air, and nitrogen gas. Then I press "run" on the Dell laptop below the lab bench, and my work is done.

But The Machine is just getting started. With a soft whir, it proceeds through dozens of steps: preparing, reacting, purifying, washing, preparing, reacting, purifying, washing, and on and on. Two days later, Burke sends word by e-mail that my first total synthesis is complete. The result: 8.6 milligrams of an off-white, powdery crocacin C.

Crocacin C is just one of a host of organic molecules that Burke's machine can make. On page 1221 of this issue, Burke and his colleagues report that they've used their automated synthesizer to produce a wide variety of molecules—stringlike linear compounds, rings, and bowls—all with bonds twisting this way and that. Like an automated DNA synthesizer, the new machine works by snapping together premade building blocks. And because thousands of such building blocks are already sold commercially, the machine could speed the production of potentially billions of different organic compounds that can be tested as new drugs, agricultural compounds, and materials.

"This is an amazing technology," says Cathleen Crudden, an organic chemist at Queen's University in Kingston, Canada. "It's a huge advance in small-molecule synthesis." Peter Seeberger of the Max Planck Institute of Colloids and Interfaces in Potsdam, Germany, agrees. "It's an important milestone, and there will be more to come," says Seeberger, who pioneered his own automated synthesizer for a different kind of molecule, the oligosaccharide sugar chains that cap many proteins and lipids. Improved automation, he and others say, will give biologists and many other researchers unprecedented access to compounds they need, while freeing organic chemists to pursue projects more challenging than churning out molecules they already know how to make. "The better we make this tool and the more we make it available, the more it will enhance science," Seeberger says.

ORGANIC CHEMISTRY has largely resisted automation since the first organic molecules were synthesized in the 1820s. The primary exception has been biopolymers, molecules made with a small number of building blocks that are all linked with the same type of chemical bond. That single bond acts like a train hitch capable of linking boxcars together in any desired order. Today, dedicated synthesizers churn out three such biopolymers: DNA fragments called oligonucleotides, protein fragments called peptides, and oligosaccharides.

This automation has produced powerful results. According to the business research firm MarketsandMarkets, by 2019 the oligonucleotide synthesis business will be worth $1.7 billion. Synthetic peptide-based drugs, meanwhile, already account for more than $14 billion in sales annually. "If you could do similar things with small molecules, it would have a huge impact," says Richard Whitby, an organic chemist at the University of Southampton in the United Kingdom. That's because these molecules not only represent the backbone of the pharmaceutical industry, but are also used in countless other products such as dyes, agricultural chemicals, light emitters, and biological probes.

That hasn't been possible, because small molecules assume a near-infinite variety of shapes. One recent report, for example, calculated that small molecules containing just carbon, oxygen, and nitrogen could be assembled in 10^{60} different ways, more than the number of atoms in the universe. "You need a lot of reactions to put together complex molecules," Crudden says.

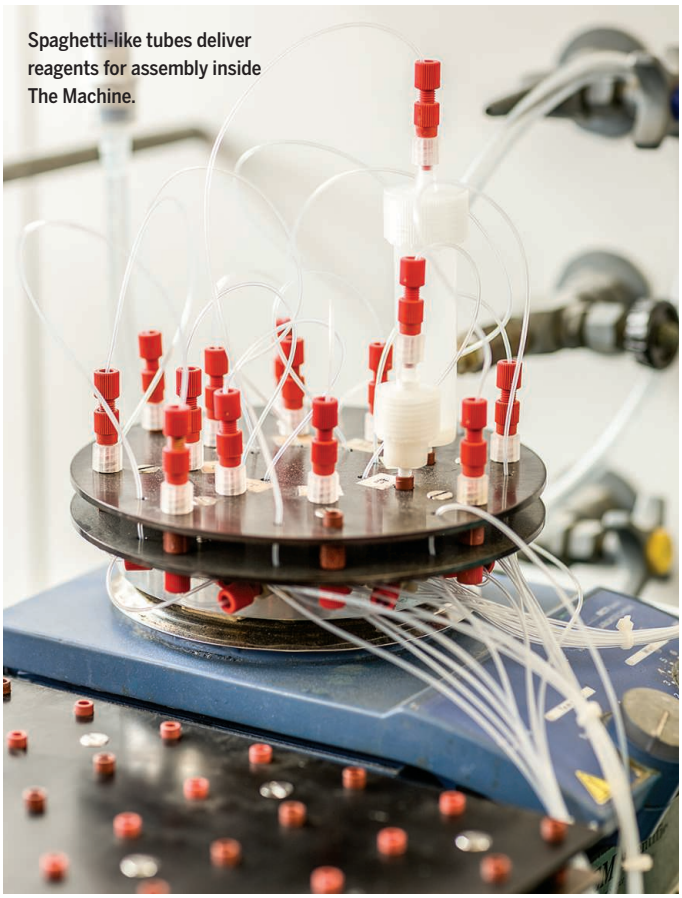
Making organic molecules has always been less like linking boxcars than like building furniture. Woodworkers can use many kinds of wood, linked with a variety of joints: mortise and tenon, dovetail, lap joints, and so on. And like craftsmen joining pieces of wood, organic chemists must ensure that each bond is oriented in the proper direction. Attach a carbon atom facing away from the core of the molecule instead of inward, and the biological result could be as useless as a chair with one leg jutting skyward. As a result, except for automating several common industrial reactions, organic chemists tend to rely on complex recipes to forge bonds one at a time, slowly building their molecules in dozens or hundreds of steps. Most synthetic chemists consider that methodical approach the only way to ensure they make exactly what they want. "That has been the dogma for 180 years," Burke says.

BURKE HAS THE TALL and trim build of a long-distance runner and something of a marathoner's persistence. In November 1998, as an M.D.-Ph.D. student at Harvard University, he met a 22-year-old patient with cystic fibrosis, a disease caused by the lack of a protein that normally forms an ion channel in cell membranes. Without the channel, the salt balance in patients' lung tissue is disrupted, making sufferers prone to infections. The patient, a bright and inquisitive young woman, asked Burke ever more detailed questions about the disease, and he described the exact genetic mutation at fault. Finally, she asked: If doctors knew so much about her disease, why couldn't they fix it? "That conversation changed my life," Burke says.

Burke knew that modern medicines are tailored to block overactive proteins, not to create missing ones. But he also knew that, in some cases, small drug molecules may be able to substitute for them. He had learned about one such molecule in one of his early organic chemistry classes at Harvard. Amphotericin B (Amph B) is an antifungal compound made by bacteria. The molecule links up with multiple copies of itself, along with cell membrane molecules called sterols, to create an ion channel. Researchers had long believed—though not proven—that Amph B kills fungi by punching holes in their cells. Whatever the mechanism, Amph B was a lifesaving drug for patients with dangerous fungal infections. Unfortunately, the drug is also highly toxic, causing such severe side effects that patients often refer to it as "amphiterrible."

As Burke was finishing his M.D.-Ph.D. in 2005, he hoped to confirm Amph B's cell-killing mechanism and then tweak the compound to make it less toxic. But his long-range vision was even bolder: He wanted to harness the molecule's channel-making properties to help cystic fibrosis patients. If it worked, it could serve as a sort of molecular prosthetic device for ensuring the proper salt balance in lung tissues—perhaps not as good as the real thing, but good enough to improve patients' lives.

The initial hope was short-lived. "We quickly realized that the bottleneck was synthesis," Burke says. Amph B is a complicated molecule containing more than 38 carbon atoms, and the only known synthesis, published in 1987, involved more than 100 steps. Making enough of the molecule—along with near-variants of it—to figure out the biology of pore formation would take years, if not decades.



Spaghetti-like tubes deliver reagents for assembly inside The Machine.

"This is an amazing technology. It's a huge advance in small-molecule synthesis."

Cathleen Crudden, Queen's University

Burke envied his close friend and fellow Harvard Ph.D. student, Rahul Kohli, who was studying the biological activity of large ring-shaped peptides. At the end of each week, Kohli and Burke got together at The Cellar, a Cambridge, Massachusetts, bar, to catch up over a beer. "I was blown away at how fast his research was moving," Burke says. Kohli's advantage boiled down to the automated synthesizer that churned out a library of peptides for him to study. "While we were drinking, some machine was making more [peptides] for him. I became insanely jealous, and wondered 'How can we do the same thing for small molecules?' "

Burke realized that many of the amino acid building blocks Kohli's synthesizer was assembling are structurally complex. They come in a wide variety of shapes and sizes, some with single- or double-ringed sidecars, others without. Yet a machine readily strings them together, using a single kind of bond. "The complexity is all in the building blocks, and you buy those in a bottle," Burke says.

Could that same strategy work for small molecules? After getting his degree, Burke decided to find out. He put together a proposal for a research program, as he searched for an academic job. The day he interviewed at Illinois, Burke says, "they gave me an offer and I accepted on the spot." He's been working to fulfill his vision ever since.

THE FIRST TASK was to identify the best reaction to link building blocks together. The answer seemed clear. In the 1970s, a Japanese chemist named Akira Suzuki from Hokkaido University discovered a way to use palladium metal as a catalyst to link carbon atoms on two separate molecules, while leaving everything else about those molecular pieces untouched. Suzuki's trick was to adorn a carbon atom on one of the two molecules with a so-called boronic acid: a boron atom attached to two alcohols $(OH)_2$. To a carbon on the other molecule he attached an element such as iodine or bromine, known in chemistry parlance as halogens. When the palladium brings the two couplers together, it links the carbon tied to the boronic acid with the carbon joined to the halogen and throws away the boronic acid and halogen. Today, says Max Planck's Seeberger, this so-called Suzuki coupling is one of the most powerful and widely useful reactions in all of organic chemistry.

Burke had his connector. By synthesizing building blocks with a boronic acid group on one side and a halogen on the other, he could snap them together like a ball and hitch. But he needed one more critical piece: a way to control the reaction, so the catalyst would not go on linking building blocks in random combinations ad infinitum. To build a molecule step by step, Burke says, "we needed a switch."

In 2007, he and his colleagues found it: a molecule called MIDA that wraps itself around the boron and shuts down its reactivity. Now, Burke's team could start with one building block that did not contain a halogen but had an exposed boronic acid, then add a second building block that had both a halogen and a boronic acid capped

with MIDA. Because the capped boronic acid couldn't react, the setup forced the halogen on the second building block to react with the exposed boronic acid on the first. Presto, the two were linked. Burke's team could then remove MIDA from the just-forged tandem, add another building block with a halogen and capped boronic acid, and repeat the process (see diagram, below).

The strategy worked. In 2012, Burke's team reported in the *Proceedings of the National Academy of Sciences* that they had used their technique to make a derivative of Amph B, which could not make ion channels but still killed fungal cells. The result proved that the conventional wisdom was wrong: Amph B kills fungal cells not by poking holes in them but by binding the sterols that cells need for their membrane proteins to work properly, among other functions. Without the sterols, the cells can't survive. Beyond Amph B, in 2008 Burke and colleagues reported in the *Journal of the American Chemical Society* (*JACS*) that they had used their technique to make a wide variety of druglike compounds called polyenes. Later, they calculated that just 12 MIDA boronate building blocks would enable them to synthesize more than 75% of the 2839 polyenes known to be made by natural organisms. They were off and running.

THE TROUBLE WAS, THEY WERE STILL doing all of their assembly work by hand. To automate it, they needed to solve new problems. The biggest was finding a way to purify whatever tandem molecules they made—separating finished molecules from partially completed ones, unused building blocks, and leftover reagents.

Automated peptide, DNA, and oligosaccharide synthesizers can do that because the building blocks for each class of these biopolymers have a chemical handle in common. Using that handle, researchers tether the molecules they are building to a solid anchor such as a plastic bead. Between synthetic steps, the machines just hang on to the beads and wash the excess reagents away. But no such common handle exists for small molecules. Then, in 2008, by chance, Burke's team discovered that MIDA boronates stick to sandlike silica particles when two organic solvents—methanol and ether—are both present but then drop off when a different solvent, known as THF, is added. The technique gave the researchers a way to catch and release their compounds at will. Now, to purify compounds, they could simply run them through a silica-containing vial—no beads involved. This simple solution was "the key discovery" that enabled them to automate their chemistry, says Southampton's Whitby.

Burke and his students pushed on to make their machine. It took a couple of years of designing and redesigning the apparatus, working with engineers in the university's machine shop to create the parts, and writing the computer code needed to follow each recipe step by step. Along the way, they added other steps to link building blocks in different orientations and turn some of their linear chain-like compounds into more rigid ringed molecules, both key advances in making a broader array of natural products.

~260 thousand
Known small-molecule natural products

70–75
Percentage that could be made with 5000 MIDA boronate building blocks

~200
MIDA boronate building blocks now commercially available

FOR NOW, IT'S UNCLEAR how many small organic molecules Burke's synthesis machine can make. With about 5000 building blocks, he estimates, it could make 70% to 75% of the nearly 260,000 small-molecule natural products known to exist. "If we can do that, we can shift the rate-limiting step from synthesis to understanding function," Burke says. "I think organic chemistry is hungry for this."

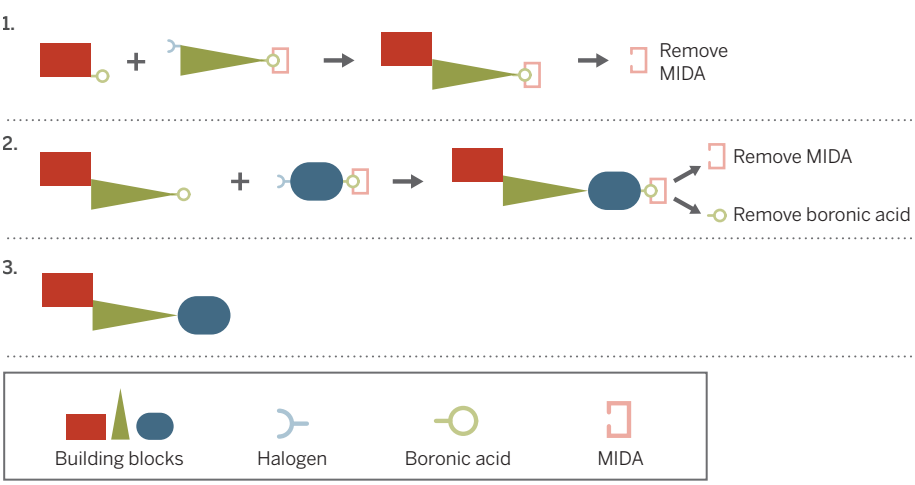
Still, many organic chemists remain resistant to using automation, Seeberger says. "Some people feel threatened." And so far, only about 200 building blocks with both the crucial halogen and MIDA-capped boronic acid linkers are commercially available. Thousands more, however, sport just the boronic acid or the halogen and can be used by the machine as the first or last building block. Nevertheless, for the technology to reach its potential, other chemists will have to embrace it and produce many more full-fledged building blocks.

Burke is not waiting. He is using the synthesizer to return to his favorite compound, Amph B. He says his team has already made a derivative that kills fungi but leaves human cells alone. Fungi and human cells rely on different sterols to make their cell membranes, and in a 2013 paper in *JACS*, Burke's team reported making an Amph B derivative that binds the fungal sterol, called ergosterol, but not cholesterol, the human version. That selectivity has already yielded a less toxic version of the drug, at least in cell culture. Last month, the team passed much of that work on to a new biotech startup that Burke co-founded, Revolution Medicines, which has also acquired the intellectual property for commercializing Burke's synthesis machine.

Burke says he has also begun to use his new synthesis machine to return to his dream of making molecular prosthetics to help cystic fibrosis patients and others. He says his team is already working to make new Amph B derivatives capable of forming the ion channels that cystic fibrosis patients lack. The end of that story remains to be written. But the potential of a new approach to automating organic chemistry is just starting to unfold. ■

A huge chemical menu, from standardized ingredients

To make a small molecule, The Machine links a carbon attached to a boronic acid (○) to a carbon attached to a halogen (⊃). A MIDA group (⌐) stops the reaction. Then the MIDA group is removed and another component added.



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