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RESCUING COMBICHEM

Diversity-oriented synthesis aims to pick up where traditional combinatorial chemistry left off

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Combinatorial chemistry, the rapid synthesis and screening of collections (libraries) of varied compounds to identify agents with desired functional properties, has been subject to a lot of criticism lately. The complaint is that combichem hasn't lived up to its hype as a speedy and prolific source of new biologically active agents and drugs.

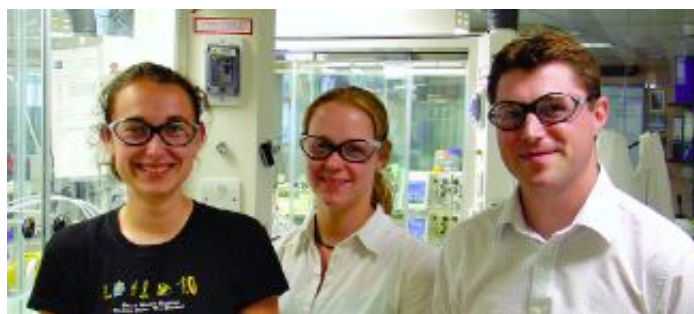
Perhaps the field's low point was "Drug Industry's Big Push Into Technology Falls Short," an article in the Feb. 24 *Wall Street Journal* that, despite a few qualifying statements, essentially put combichem through the wringer and hung it out to dry. That article followed C&EN's own reappraisals (Oct. 13, 2003, [page 77](#); Oct. 27, 2003, [page 45](#)). The field has been so discredited lately that even the term "combinatorial chemistry" has begun to lose its cachet and luster, with some researchers beating the bushes for a better label.

At the same time that combichem's fortunes have been declining, a potential solution to some of its problems has been ascending. Combichem's potential savior is diversity-oriented synthesis (DOS)—the synthesis of relatively small libraries of organic molecules that are structurally more complex, have a greater variety of core structures, and possess richer stereochemical variation than those produced by traditional combichem.

"The term 'combinatorial chemistry' goes back to the mix-and-split era of chemistry, when pieces were added together just for the sake of creating new combinations, and that doesn't reflect where the field is today," comments James B. Summers, divisional vice president for advanced technology at Abbott Laboratories. "Many industry groups are dropping that term from their lexicon. 'Diversity-oriented synthesis' and 'high-throughput chemistry' are the types of phrases that are now being bandied about a little bit more."

DOS had its beginnings in the late 1990s. Since then, a number of academic research groups either have tried DOS or have become actively involved in investigating and developing it. The pharmaceutical industry has begun to show significant interest in it as well.

Whether DOS will solve some of the problems of combinatorial chemistry remains to be seen. Many scientists believe that various aspects of combichem deserve to be saved, and DOS currently seems to provide the best hope for the field's revival. Combichem may indeed have been burned by its critics, but DOS now seems to be rising phoenix-like from its ashes.





DOS TEAM Spring (right) and graduate students (from left) Gemma Thomas and Emma Wyatt in the DOS lab at the University of Cambridge. The group has DOS collaborations with industry. "In fact, Gemma is supported by GlaxoSmithKline and Emma by AstraZeneca," Spring says.

UNIVERSITY OF CAMBRIDGE PHOTO

WHAT DISTINGUISHES DOS from traditional combichem? For one thing, DOS libraries typically consist of tens to hundreds of compounds, versus the tens of thousands to millions of compounds produced in many traditional combinatorial syntheses. Also, most DOS compounds have cyclic architectures and resemble natural products—which are often designed by nature to be biologically active from the start—whereas compounds generated by traditional combichem weren't necessarily that complex.

"Natural products are usually shaped in a very distinctive fashion," University of Pittsburgh chemistry professor [Peter Wipf](#) says. "That's what we're trying to accomplish in DOS. The question we ask ourselves is, 'How can we make molecules that are every bit as interesting as natural products in as few steps as possible?'"

DOS syntheses are thus designed to be simple. "While DOS-generated compounds tend to be very complex, they are obtained by short, efficient synthetic routes," says Program Director John M. Schwab of the National Institute of General Medical Sciences (NIGMS). "It's critical that compounds that are going to be developed into clinical candidates or into marketed drugs be readily accessible."

Stereochemical diversity is a key property of DOS libraries. "A concept that's stronger in DOS than it ever was in combichem is the use of relative and absolute stereochemistry to gain additional selectivity, preorganization, and greater structural diversity overall," Wipf says. "DOS uses effectively this very well known concept of stereochemistry to shape scaffolds differently as unique three-dimensional surfaces."

The presence of cyclic motifs in most DOS compounds is another important contributor to their unique properties. Linear molecules tend to be quite flexible. "They may do a lot of things, but none of them very well," Wipf says. "A cyclic molecule, through its preorganization and its presentation in three-dimensional space, is much more selective and much more distinctive in its interactions. It goes to certain targets, but it doesn't bind at all to others. Generally that's what you want."

Overall, conventional combichem libraries often consisted of flat, aromatic heterocyclic compounds with no stereogenic centers, notes [Prabhat Arya](#), senior research officer in the chemical biology program at the National Research Council of Canada, Ottawa. Researchers haven't been all that successful in identifying interesting lead compounds from such libraries. The natural-product-like compounds produced in DOS, with their highly complex three-dimensional architectures and stereochemically diverse structures, have a much better shot at interacting with desired molecular targets and exhibiting interesting biological activity, he says. "Five to six years ago, molecules like that were missing from the combinatorial chemistry scene," but since then DOS has increasingly been bringing them forward, Arya says.



Schreiber
HARVARD
UNIVERSITY
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Aubé
UNIVERSITY OF
KANSAS PHOTO



Tan
MSKCC PHOTO



Waldmann
MAX PLANCK
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SOME SCIENTISTS maintain that DOS is not really all that new or different from conventional combichem. "It is incorrect to say that combinatorial methods have failed," says [Richard A. Houghten](#), founder and president of Torrey Pines Institute for Molecular Studies, San Diego. "In fact, virtually every pharmaceutical company in the world has existing programs using one or more methods that came directly, or were derived from, combinatorial approaches." What is currently occurring with DOS, Houghten says, "is the consolidation and/or evolution of the more useful of the many and varied methods making up combichem into more focused approaches in which the more practical of the older methods are melded and given more appropriate and descriptive names."

And DOS isn't the only game in town when it comes to developing druglike compounds, Wipf points out. The related approaches of click chemistry and dynamic combinatorial chemistry also focus on chemical shape, he says.

In click chemistry, developed by chemistry professor [K. Barry Sharpless](#) and coworkers at Scripps Research Institute, a biological receptor, such as an enzyme, is permitted to select the best fitting partial ligands (ligands that don't occupy the entire binding site) from a range of modules that are capable of reacting with one another. After the modules bind to the site, their reactivity toward each other induces them to combine, forming a compound that blocks and thus inhibits the entire catalytic site ([C&EN, Feb. 16, page 63](#)).

In dynamic combinatorial chemistry, a target receptor is exposed to a library of potential ligands, each of which is formed by reversible combinations of small building-block compounds in an equilibrium solution. Selective binding of one of the ligands to the target lowers the ligand's concentration in solution, causing the equilibrium to shift so that more of the strongly binding ligand forms. The system thus enhances the amount of strongly binding species and discriminates against poorly binding ones, resulting in selection. The upshot is that the target receptor selects its own ligand, just as with click chemistry.

"In DOS, we're using our own creativity and sometimes developing new chemistry to generate defined shapes," Wipf says. "Click chemistry is also shape-oriented, and dynamic combichem selects for a certain shape in a dynamic equilibrium system. These two concepts are evolving parallel to DOS. Like DOS, they are trying to take combichem to another level and away from the random way of making libraries." At this point, DOS is the most highly developed and most widely practiced of the three shape-optimizing approaches.

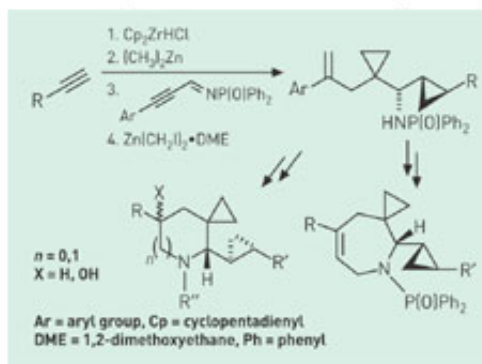
[Stuart L. Schreiber](#), professor of chemistry and chemical biology and a Howard Hughes Medical Institute investigator at Harvard University, has been instrumental in the conception and development of DOS. His group first reported on the DOS concept in 1998. However, DOS-related ideas had been around before.

For example, associate professor Robert W. Armstrong and a coworker at the University of California, Los Angeles, came up with a concept for skeletal or scaffold diversity a year earlier [[7](#)].

Am. Chem. Soc., **119**, 7607 (1997)]. And Houghten and coworkers proposed diversifying existing libraries with additional functional groups in their "libraries from libraries" approach [*Proc. Natl. Acad. Sci. U.S.A.*, **91**, 11138 (1994)].

Nevertheless, since the late 1990s, the DOS approach has been systematized and refined by 'Schreibers group more than any other, as well as by a number of academic and commercial spin-offs from Schreiber's group. "The real melding of all ideas on trying to maximize structural diversity and complexity and trying to synthesize compounds as efficiently as possible has been through Stuart," says [David R. Spring](#), a fellow in the department of chemistry at the University of Cambridge and a former Schreiber postdoc. "Without a doubt, he's the major name in the field."

The Schreiber group's first paper on the DOS concept [*J. Am. Chem. Soc.*, **120**, 8565 (1998)] reported a 2 million-compound library derived from shikimic acid. "That first effort showed that we could make a library of complex molecules with stereochemical features," says the paper's lead author, [Derek S. Tan](#), now an assistant professor at Memorial Sloan-Kettering Cancer Center, New York City. "It relied heavily upon mixing and matching of different building blocks. That's how we got very rapidly to large numbers."



MULTICOMPONENT SUCCESS Wipf holds a rack from an automated parallel flash separation station at Pittsburgh's CMLD. In one recent study, Wipf and coworkers developed a new one-pot multicomponent condensation (first reaction) and used it to produce an intermediate that was converted (second reaction) into 5-, 6-, and 7-membered heterocycles: pyrrolidines (bottom left, $n = 0$), piperidines ($n = 1$), and azepines (bottom right), respectively.

UNIVERSITY OF PITTSBURGH PHOTO

DOS HAS EVOLVED considerably since that work. "The scaffolds were all very similar within that library," whereas now "people have moved to having multiple scaffolds or backbones in the same DOS library to increase the structural diversity," Tan says. "The other caveat is that the shikimic acid library contained only about 15 ng of each compound. Despite efforts to develop miniaturized screening formats, [such small amounts] proved to be impossible to work with. So we've moved back to larger synthetic formats that yield milligram quantities of each compound. That allows you to purify every compound in the library, if desired, and it gives you plenty of each stock solution to do multiple screens and conduct follow-up experiments. The other direction people are going is to make significantly smaller libraries. In my lab, we're targeting libraries on the order of 1,000 compounds. I think it's safe to say no one is going to make million-compound DOS libraries again."

A major achievement of Schreiber's group was the development of an overall strategy for achieving skeletal diversity [*Science*, **302**, 613 (2003)]. The approach uses split-pool synthesis to preencode skeletal diversity combinatorially in synthetic starting materials. Libraries of small molecules that are highly varied structurally are then produced very efficiently from the preencoded substrates. Schreiber and coworkers specialize in identifying compounds that can be

used in chemical genetics, the use of small organic molecules as probes of protein function and biological processes.

Schreiber is also director of Harvard's Center for Chemical Methodologies & Library Development (CMLD), one of four centers with strong DOS programs that have been established recently with funding from NIGMS. The other three CMLDs are at the University of Pittsburgh, Boston University, and the University of Kansas.

CMLDs are multi-investigator research centers set up to develop methods for design, synthesis, analysis, and handling of DOS libraries. According to NIGMS, "Each center hosts a library synthesis core facility that serves two purposes: to validate newly developed methodologies for application to DOS and to apply newly developed chemical methodologies and strategies to the generation of chemical diversity libraries for high-throughput biological screening."

The specific mission of Harvard's CMLD "is to bring high-throughput technology and automation science to bear on DOS, both in terms of reaction and catalyst discovery and in the implementation of reagents and catalysts in effective DOS pathways," Schreiber says. The center operates as a kind of sociological experiment, he notes. The idea is "to bring chemists and biologists together, planning experiments from the get-go that are critically interdependent," Schreiber says. "An example is the design of assays that enable chemists to assess the merits of their DOS pathways by experimentally measuring the impact of stereochemical and skeletal diversity on the variance of biological outcomes. These experiments are performed in parallel with the development of DOS pathways, so that feedback is provided in real time rather than after the fact."

At the University of Kansas' CMLD, research by associate professor of chemistry [Paul R. Hanson](#), President Daniel L. Flynn of Deciphera Pharmaceuticals, Lawrence, Kan., and coworkers on phase trafficking—the use of norbornenyl tags and ring-opening metathesis polymerization to shuttle reagents and products in and out of solution—is probably the most mature project and the one that's had the most notable success to date," chemistry professor and center Director [Jeffrey Aubé](#) says. Other themes at the center include the use of organometallic chemistry to create novel libraries; the synthesis of small druglike heterocycles, such as quinolones and enamines; the synthesis of peptidomimetic and carbohydrate-mimic libraries; and multicomponent reactions.

Multicomponent reactions are also being developed at the University of Pittsburgh's CMLD. In one study, a new one-pot multicomponent condensation produced an intermediate that was subsequently converted into 5-, 6-, and 7-membered heterocycles that are "important building blocks of many known drugs," says Wipf, director of Pittsburgh's CMLD. "The study showed that you can make druglike molecules very quickly as end products of a diversified multicomponent condensation."

MEANWHILE, at Boston University's CMLD, notable achievements include the development of a number of novel DOS libraries and the deployment of a DOS protocols database as a shared resource. Work reported last year showed how cyclodimerization could be used to rapidly assemble complex polyketide-like macrodiolides from simple but enantioenriched hydroxy ester starting materials. "It was a very nice illustration of how underutilized reaction methods can be used to produce stereochemically diverse complex templates," chemistry professor and CMLD Codirector [James S. Panek](#) says. The database, Synthesis Protocols, lists solution- and solid-phase library synthesis procedures and is publicly accessible. "That's directly related to the goals of the NIGMS-funded centers, which are to make the methodologies readily available," associate professor of chemistry and CMLD Codirector [John A. Porco Jr.](#) says.

Outside the CMLDs, a number of other groups are working to develop and use DOS methods that until recently have been unavailable. One such group is that of [Herbert Waldmann](#), a professor at the Max Planck Institute of Molecular Physiology, Dortmund, Germany. "Our goal is to develop the chemistry required to make biologically relevant compound libraries," he says. "It means we will have to [transport] the entire armamentarium of organic synthesis to library

synthesis, and that is not easy—not at all. Once one has this set of tools, one can go for complicated libraries if they are required."

Waldmann and coworkers are developing a DOS-based approach called natural-product-guided compound library development, which could dramatically shorten the process of finding ligands for proteins. The researchers identify a natural product that binds to a specific protein, synthesize a DOS library of analogs with structures similar to the ligand, and search structure databases for protein binding sites that are structurally similar to that of the original protein. The library will then often contain ligands for the related proteins as well.

For example, Waldmann and coworkers made a library of analogs of a phosphatase ligand and found that the library also contained ligands for acetylcholinesterase and dehydrogenase, both of which have catalytic sites structurally similar to that of the phosphatase. "We obtained hit rates in the 2 to 3% range"—much higher than a typical non-DOS library would yield, Waldmann says.

Meanwhile, in Canada, Arya's group is developing stereocontrolled DOS methods leading to the library generation of natural-product-like complex polycyclic derivatives, such as indoline- and tetrahydroquinoline-based scaffolds. Arya notes that "indoline and tetrahydroquinoline derivatives are considered highly privileged druglike scaffolds in medicinal chemistry"—where "privileged" refers to a tendency to bind many different target structures.

Chemistry professor [K. C. Nicolaou](#) of Scripps Research Institute and the University of California, San Diego, and coworkers have taken substructural features from natural products and used those as starting points for DOS library design. For example, they prepared 10,000 benzopyran-based compounds and found promising bioactive agents in the library.

Other DOS efforts have been or are being carried out by groups such as those of Tan at Memorial Sloan-Kettering; assistant professor of chemistry [Young-Tae Chang](#) at New York University; assistant professor Luis E. Martinez Jr. at the University of Texas, El Paso; assistant professor of chemistry [Karl A. Scheidt](#) at Northwestern University; Harvard professor of chemistry and chemical biology [Matthew D. Shair](#); research fellow [Jared Shaw](#) at Harvard Medical School; and professor of chemistry [Douglass F. Taber](#) at the University of Delaware, Newark. For example, Shair and coworkers identified a secretory pathway inhibitor called secramine in a galanthamine-based DOS library.

DOS HAS ALSO begun to be widely utilized, at least on a trial basis, in industry. "Industry is certainly moving very strongly in that direction," Wipf says. "People in industry feel a little bit tainted by their previous experience of combichem, so they don't really know what label they should use. But I think DOS, much more than the traditional form of combichem, is what most of them are doing right now."

Summers agrees that DOS "is being widely initiated across industry programs." Abbott has a high-throughput organic synthesis lab that creates libraries for lead optimization studies and also carries out DOS syntheses of targeted libraries, which are screened for activity against specific diseases.

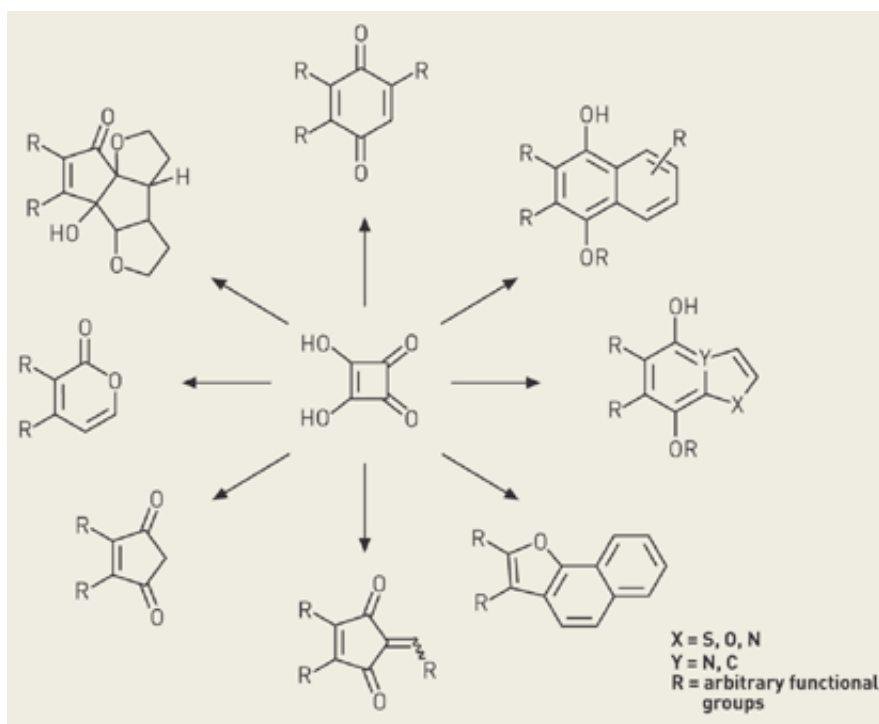
A few small companies are also focused on DOS. Infinity Pharmaceuticals, Cambridge, Mass., "was a spin-off of early work done in [Schreiber's] lab," Vice President for Chemical Technology Michael A. Foley says. "We felt confident that DOS could actually be practiced on an industrial scale. As we had hoped, our compound collection has shown great promise." The company has identified inhibitors of protein-protein interactions with levels of activity in the low-double-digit nanomolar range, and it's investigating some of these as potential therapeutics.

"The libraries have higher information content because of DOS, and we have been able to harness that information to move more programs forward more efficiently than we otherwise could have done," Foley says. "We have moved compounds all the way through animal studies at this point and found them to behave very well."

Other companies focused on DOS include VivoQuest, Valley Cottage, N.Y., which is screening natural-product-based DOS libraries against a range of targets and has identified preclinical candidates in its hepatitis C virus and oncology programs. And a new Merck spin-off named Edelris will market DOS libraries as sources of lead compounds for medicinal chemistry applications. Edelris is being formed by Jean-Yves Ortholand, head of combichem at Merck Sant", Ly, Lyon, France, and colleagues. You probably heard the name here first—a Google search of "Edelris" last week yielded zero hits.

DOS is also the focus of a number of academic-industrial collaborations. For instance, Spring and coworkers at Cambridge have consulted or collaborated on DOS projects with Novartis, AstraZeneca, GlaxoSmithKline, Pfizer, Eli Lilly, Syngenta, and DanioLabs. For instance, a DOS compound from Spring's DanioLabs collaboration has shown interesting activity in a zebra fish model of inflammatory bowel disease. Mark Ladlow, head of the GlaxoSmithKline Cambridge Technology Centre, who collaborates with Spring, says DOS can potentially be used to generate "enriched compound sets, from which the likelihood of identifying a lead structure with the desired biological properties is significantly increased."

However, the degree to which DOS will be picked up by industry shouldn't be overemphasized. "DOS is a change in the way we do things, but that doesn't mean it will become the only way things are going to be done in the future," Aubé says. "Is DOS going to make an impact and have an influence? I think it definitely will. But does that mean nobody is going to be doing structure-based drug design in the future? No. Obviously, DOS and structure-based drug design are complementary technologies."



DIVERSIFICATION In a 1997 paper, Armstrong and a coworker described an early DOS-like concept by showing varied compound shapes that could be derived from squaric acid.

AS TO WHETHER DOS will ultimately prove successful, the jury's still out. One criticism is that DOS libraries are often synthesized speculatively, without regard to specific biological targets, and that such undirected efforts can turn out to be wasteful and inefficient.

Certainly, further elaboration and development of the DOS approach will be needed. For example, DOS compounds "include a lot of stereochemical information, and pharma usually tries to avoid stereochemical complexity—even so much as a single stereocenter," Schwab says.

"This issue will need to be resolved."

In addition, "There are those who argue that while structural complexity may lead to high affinity, it may also lead to a potential for promiscuity—that is, nonselectivity," Schwab notes. The argument has been made, he says, "that each time you build in a new appendage for increasing affinity, you are also providing a hook that can interact with proteins other than the intended target."

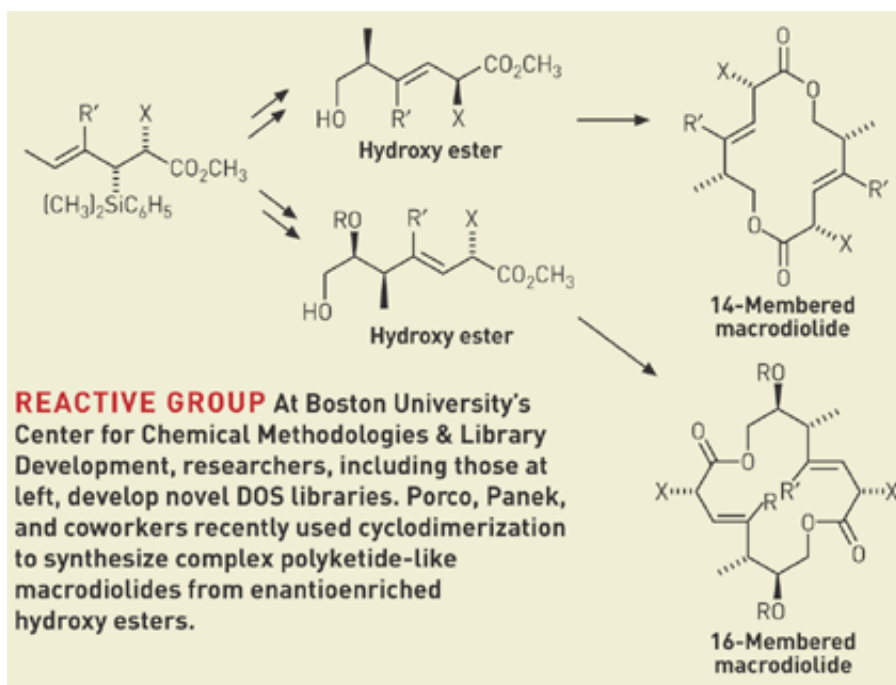


Boston University Photo

Furthermore, "DOS still requires a boost in technology," Wipf says. "If you look at the instrumentation and automation that's available, it's not sufficient to make DOS work as effectively as we would like. We're still waiting for technologies—like the integrated lab-on-a-chip—that would give us an order of magnitude in speed beyond what's been accomplished so far."

Nevertheless, Waldmann says, "What one clearly can see in the past two or three years is that the first generation of combichem—be it for basic biology or for drug development—is now being translated into a second generation. In this second generation, there's more emphasis on quality than on quantity."

Can DOS fix combichem? "If by creating enough diversity one can address a larger fraction of the space that is available to nature, then DOS will be a valid way to go," Waldmann says.



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