Organic synthesis toward small-molecule probes and drugs

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"Organic synthesis" is a compound-creating activity often focused on biologically active small molecules. This special issue of PNAS explores innovations and trends in the field that are enabling the synthesis of new types of small-molecule probes and drugs. This perspective article frames the research described in the special issue but also explores how these modern capabilities can both foster a new and more extensive view of basic research in the academy and promote the linkage of life-science research to the discovery of novel types of small-molecule therapeutics [Schreiber SL (2009) *Chem Bio Chem* 10:26–29]. This new view of basic research aims to bridge the chasm between basic scientific discoveries in life sciences and new drugs that treat the root cause of human disease—recently referred to as the "valley of death" for drug discovery. This perspective article describes new roles that modern organic chemistry will need to play in overcoming this challenge.

Organic Synthesis of Small-Molecule Probes and Drugs

Chemists and biologists have been exploring the functions of small molecules in living systems for over a century, beginning with the discovery of glucose, amino acids, vitamins, hormones, neurotransmitters, lipid mediators, and many others. Especially during the second half of the 20th century, small molecules have been used increasingly as probes ("tool compounds") of life processes. Notable early examples include the use of small-molecule neurotoxins and neurotransmitters to probe ion channels and neurotransmission (1, 2), phorbol esters to probe protein kinases and signal transduction (3, 4), and colchicine and cytochalasins to illuminate the molecular components of the cytoskeleton (5, 6). Understanding the types of targets and processes that can be modulated with small molecules helped define the principles that underlie the rational discovery of small-molecule therapeutics (7).

This outline of small-molecule science is being developed more fully in the 21st century (8). New approaches that ensure exquisite specificity of small-molecule/macromolecule interactions are enabling inferences to be drawn with greater confidence, including the "bump/hole strategy" (9) used with protein phosphatases and kinases (10, 11). In this approach, researchers introduce by mutation a "hole" in the drug target, typically by decreasing the size or increasing the flexibility of an amino acid side chain at a site contacting the small-molecule modulator. A complementary substituent is added to the small molecule (the "bump") that occupies the newly created hole in the protein encoded by the dominant drug-sensitive allele. This key substituent also prevents binding to the native drug target. Related to this is the increasingly common use of dominant drug-resistant alleles that encode fully functional protein targets of small-molecule modulators but that have side chains that prevent binding by the "native" small molecule and can, therefore, be used to determine the relevance of the target to the small molecule's cellular activities (12).

These methods, however, require the genetic engineering of model systems. The greatest advances have been in the methods that enable the discovery of small molecules targeting native proteins in cells and animals. Here, modern organic synthesis is playing an increasingly prominent role.

Organic synthesis is yielding probes of new types of targets and processes, including ones historically considered challenging, and these are informing drug discovery by testing new concepts in physiology with small molecules. Whereas processes including intracellular signal transduction, gene expression, and protein homeostasis were considered challenging just 20 y earlier, small-molecule probes of protein kinases, protein phosphatases, chromatin-modifying enzymes, and the proteasome demonstrated in the 1990s that these targets can be modulated with small molecules with outstanding selectivity and potency (13, 14). These same target classes are today central to many if not the majority of drug-discovery efforts. More recent studies showing that small molecules can be discovered that (*i*) modulate the cellular functions of extracellular growth factors (15) and intracellular transcription factors (16), proteins lacking enzymatic activities, and (*ii*) disrupt protein/protein interactions (17, 18), activate or inactivate autophagy (19), or cause cell types to adopt features characteristic of other cell types ("transdifferentiation") (20) suggest that these targets and processes may be the focus of future drug-discovery efforts (21).

When highly selective probes are combined with new methods to characterize cells comprehensively, including the genetic features of different cell lines, correlations can be derived that impact early drug discovery. For example, correlations between genetic variation and sensitivity toward highly selective small-molecule probes may facilitate the development of drugs tailored for the genetic features of disease associated with individual patients. Although this is perhaps most evident in diseases such as cancer where the penetrance of somatic mutations is often high (22, 23), new approaches have been described that reveal such correlations in heritable disease as well (24).

Strategies Used by Organic Chemists

Organic synthesis can contribute to the discovery of biologically active small molecules in several ways. By yielding structurally diverse small molecules having features well suited for binding macromolecules, it delivers starting points for probes or drugs. Structure/activity relationships resulting from the strategic synthesis of analogs are central to the identification of optimized variants of the starting compounds. Efficient syntheses of the optimized variants are essential for practical applications of probes and drugs. The research reports from authors in this special issue focus on advances in each of these three facets of organic synthesis. Strikingly, the contributions illustrate, overall, two primary strategies for discovering probes and drugs.

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The first strategy is inspired by naturally occurring small molecules named "natural products." For many years chemists have synthesized the natural products themselves with an eye toward advancing the capabilities of organic synthesis. This issue highlights instead short and modular syntheses of structural variants of specific natural products with an eye toward the discovery of small molecules having improved or novel properties as probes or drugs.

The second strategy is inspired by the structural complexity and diversity of the entire ensemble of natural products, rather than by specific ones. Here, chemists use modular syntheses of compounds having features such as intermediate ratios of atoms with sp^3 or sp^2 hybridization, multiple stereogenic elements, and rigidifying skeletal elements. These features enable the discovery of modulators of many disparate aspects of biology, including ones currently viewed as challenging. Chemists are learning to incorporate these features strategically to facilitate downstream optimization and manufacturing.

Even though these synthetic chemistry efforts are relatively recent, data are beginning to emerge that enable retrospective analyses of them. This special feature also illustrates computational science aimed at, among others, providing guiding principles for improving the rate of success in either of the two paths being explored.

Two recent and striking advances of these concepts in drug discovery are described next. These vignettes have been selected to illustrate the two approaches noted above.

Vignette: Organic Synthesis Exploring Structure/Activity Relationships of a Complex Natural Product and Yielding a New Breast Cancer Drug

The US Food and Drug Administration approved in late 2010 a new drug for treatment of metastatic breast cancers (25). Eribulin's structural complexity is unrivaled for a completely synthetic drug, and its discovery and large-scale manufacturing represent a landmark for the field of organic synthesis (Fig. 1). The inspiration for eribulin comes from an even more complex natural product, halichondrin B, which was isolated from a marine sponge. One of the authors of this special issue, Yoshito Kishi, pioneered the advance of eribulin through collaborations at Harvard University and the Eisai Research Institute (26). Exploiting their ability to synthesize halichondrin B and its structural variants, these chemists determined the influence of nearly every key structural feature found in halichondrin B. They discovered that a large component of the natural substance could be eliminated and that by adding back

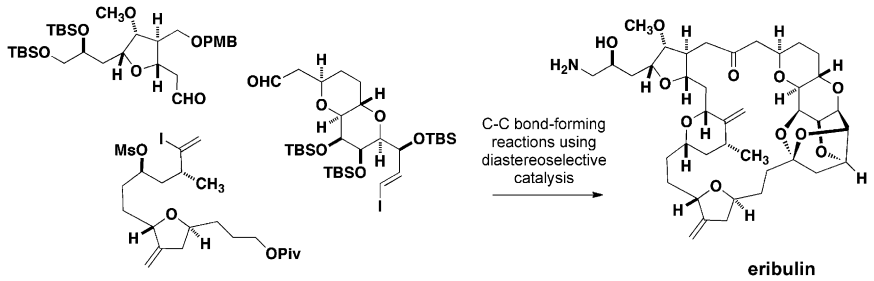

Fig. 1. Advances in diastereoselective catalysis facilitated the discovery of eribulin. Variations on Nozaki–Hiyama–Kishi reactions using chiral catalysts resulted in unprecedented control of stereochemistry during key convergent couplings involving the formation of carbon/carbon bonds. These coupling reactions were central to an efficient synthesis of eribulin, a novel drug recently approved for the treatment of advanced breast cancer.

novel structural elements, each introduced by chemical synthesis, they acquired biological properties necessary for use in humans. Most strikingly, the daunting challenge of synthesizing such a complex drug with the efficiency required for patient use worldwide was enabled by the development of new and powerful chemical transformations, including carbon/carbon bond-forming reactions of large synthetic fragments. The reactions are enabled by transition metal catalysts that deliver outstanding control of diastereoselectivity. This new capability of organic synthesis enables systematic variation of stereochemistry at a large number of the stereogenic atoms in eribulin, thereby illuminating structure/activity relationships (Fig. 1).

Vignette: Organic Synthesis Exploring Novel Structural Features of High-Performance Hits in Screens and Yielding a Promising Starting Point for a New Mechanism-of-Action Malaria Drug

An interdisciplinary effort involving synthetic organic chemists and infectious disease biologists resulted in the discovery of a small molecule that kills malarial parasites by a novel mechanism-of-action (27). This study provides a striking example of organic synthesis yielding modest-sized collections of highly novel small molecules for challenging small-molecule screens. Only several months earlier, a malaria drug discovery effort was reported taking a similar approach but using >2 million candidate compounds having conventional structural features—most notably enriched in heterocyclic rings and atoms having sp^2 hybridization—that yielded active compounds, but none that have been advanced as drug candidates (28). In contrast, Rottmann et al. discovered an advanced drug candidate having antiparasitic activity via a new mechanism-of-action from merely 10,000 diverse compounds synthesized by collaborating chemists to have structural features found in naturally occurring small molecules. These include the features that correlate with highly selective binding by small molecules recently reported by another contributing author of this special issue, Paul Clemons. The Clemons study illustrated that selective compounds (ones that bind only 1 of >100, sequence-unrelated proteins) have an increased proportion of atoms with sp^3 hybridization, an intermediate degree of stereochemical complexity, and rigidifying skeletal elements (29).

The compound discovered by Rottmann et al. resulted from a short, modular diversity synthesis strategy ("build/couple/pair") that entails syntheses (or purchase) of small building blocks, including chiral ones, coupling them intermolecularly and pairing remaining functional groups intramolecularly to yield rigidifying rings (Fig. 2) (30). This strategy provides stereochemically and skeletally diverse compounds in a small number of steps. It is especially useful in optimization efforts (medicinal chemistry in drug discovery efforts) because it enables nearly every atom to be modified without having to develop new synthetic pathways. Each aspect of this synthetic strategy was used brilliantly in this promising advance in malaria drug discovery.

Special Issue

This special issue of PNAS includes contributions from 20 leading scientists in organic synthesis and related disciplines. The contributions highlight short and modular syntheses of structural variants of natural products having novel properties as probes and drugs. They also describe modular syntheses of compounds both poised for optimization and having stereochemical complexity and skeletal diversity. They describe numerous illustrations of the identification of small molecules having novel biological activities. This special feature also highlights research at the interface of organic synthesis and computational science. These

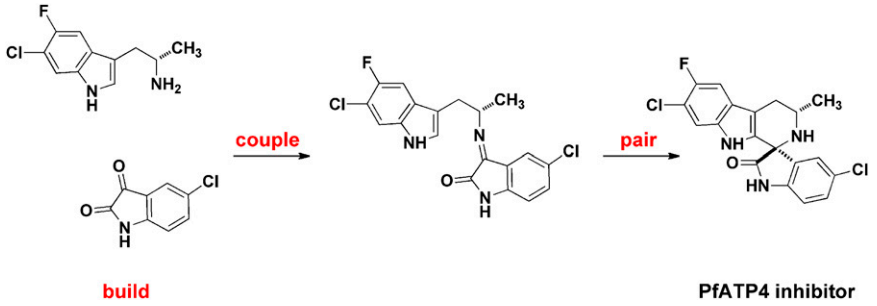

Fig. 2. Build/couple/pair strategy of diversity synthesis yielding, following optimization studies of an initial "hit" in a live/dead malarial parasite screen, a promising malaria drug candidate having a novel mechanism of action. This strategy in organic synthesis can yield candidate probes or drugs having structural features that correlate with highly selective binding, including to proteins lacking enzymatic activity. It can also yield compounds well suited for optimization ("follow-up chemistry" for probes and "medicinal chemistry" for drugs). In this example, chemists synthesized or purchased building blocks (only two of many shown) having "orthogonal" functionality that permitted intermolecular coupling followed by intramolecular pairing of indole and imine functionality. Novel spirocyclic products of the indicated Pictet–Spengler reaction yielded a starting point for a drug discovery effort in malaria. The ease of optimization of the starting point facilitated the discovery of an extraordinary compound that eliminates the malaria parasite in an animal model by a novel mechanism of action (27).

efforts are providing guiding principles for improving the rate of success in either of the two paths being explored. Although many of the contributed papers stress the foundational role of three additional areas of organic synthesis, (*i*) synthetic methodology, (*ii*) total synthesis, and (*iii*) medicinal chemistry, the papers do not focus on these areas per se.

Beyond the Special Issue: Organic Synthesis in the Future

The advances in organic synthesis outlined in this special issue are facilitating the discovery of powerful probes with increasing frequency—including ones that modulate historically challenging targets and processes. However, increasingly sophisticated small-molecule probes, for example, ones that function with high specificity in animals, may also have the potential to help redefine the scope of "basic research" in the academy with important consequences for drug discovery in the future. Here, I offer a proposal for achieving this goal (Fig. 3).

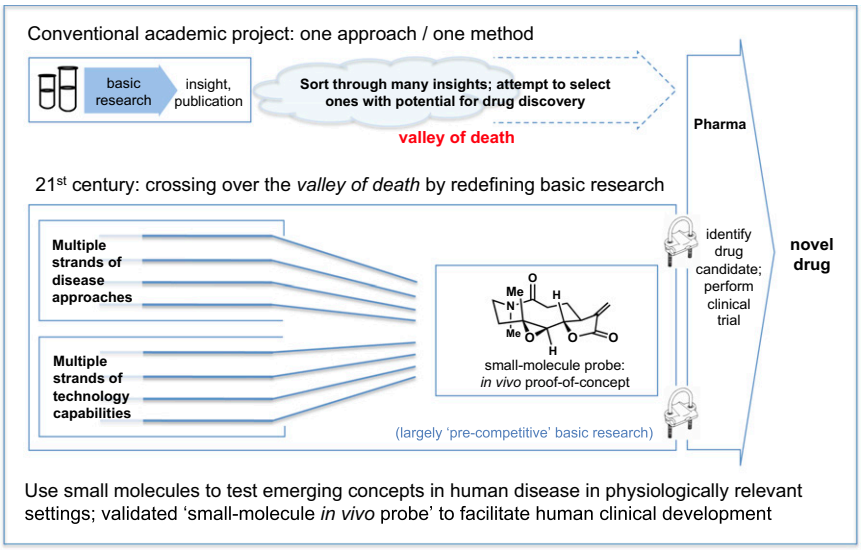

Fig. 3. Proposal for bridging the valley of death. (*Upper*) Current academic research yields concepts potentially related to human health, but their relevance to human clinical outcomes is difficult to assess—thereby contributing to the valley of death (31). (*Lower*) Building on and expanding recent models of academic research [for example, the National Institutes of Health-sponsored Molecular Libraries Probe Centers Network (MLPCN) and Centers of Excellence in Chemical Methodology and Library Design (CMLD)] provide a solution, where basic research spans new concepts in human disease that are tested using small molecules in physiologically relevant settings. Here, it is anticipated that small-molecule probes (or drugs) with mechanisms of action that are rigorously established are used in disease models to test hypotheses. Positive outcomes provide much greater confidence in the merits of a full drug discovery and development program by, for example, the pharmaceutical industry. In some instances, for example in rare and neglected diseases or ones requiring new approaches to clinical research, academic efforts might also entail the development of the clinical candidate.

Role of Organic Synthesis in Redefining Basic Research and Bridging the "Valley of Death"

Academic research has provided many insights into biology that have medical potential. However, academic research often does not provide enough information ("validation") to help the pharmaceutical industry prioritize which insights merit the enormous investment required to develop drugs. The United States Cures Acceleration Network (CAN) Act explicitly calls out this chasm between basic scientific discoveries and new treatments—labeling it the valley of death (31). CAN legislation was created to provide funding necessary to bridge the valley of death. However, effective strategies are needed for CAN and other scientific support mechanisms to achieve this important goal.

Advances in organic synthesis and the increased focus on the *science of drug discovery* in the academy in general provide a useful foundation for bridging this gap in knowledge. Modern, interdisciplinary projects are enabling basic research to progress to a more advanced stage where *emerging concepts in human disease can be tested with small-molecule probes or drugs in physiologically relevant conditions*.

The ability to manipulate DNA or RNA in cells and animals provides extraordinary tools to infer the functions of genes. However, these inferences often lead to uncertainties in terms of the effects of small-molecule drugs targeting not DNA or RNA, but proteins encoded by genes. This is all too familiar to drug developers, who invest enormous time, effort, and resources only to determine in many instances that the hypotheses lack medical relevance. The frequent failure of drug candidates in phase 2 and phase 3 clinical trials due to unexpected lack of efficacy or unanticipated mechanism-based toxicity is the painful consequence. Testing emerging concepts with highly selective probes (or drugs in some cases) in defined models of human disease would enable pharmaceutical companies to prioritize drug-development investments with much greater confidence. The impact of investments in basic research would be enhanced considerably.

To redefine basic research and to impact therapeutic discovery as described above, actually crossing over the valley of death by using small-molecule compounds to test hypotheses (Fig. 3), future research undertakings would be structured in ways not

readily funded currently. These undertakings would tackle audacious challenges of relevance to human disease, incorporate modern methods of understanding targets and mechanisms of action of tool compounds, and deliver advanced probes or even "proof-of-concept drugs" that function in disease models. These models of disease include human primary cells in three-diminsional cocultures or organ cultures and genetically defined animals engineered to mimic the precise basis of human disease.

Modern organic synthesis in the academy can contribute to this goal by providing the following enhanced capabilities and materials:

i) Improved methods and strategies in organic synthesis will be needed, likely guided by advances in computational science, to accelerate the discovery of small molecules having novel biological activities. *Novel small molecules* available only via modern organic synthesis should be *made widely and freely available*, especially for inclusion in small-molecule screening collections.

ii) A disciplined approach to yielding probes and drugs that *target the root cause of disease*, rather than an opportunistic one that exploits what is currently feasible, will advance the science of drug discovery. In some cases, this will require that organic synthesis be directed toward extremely challenging drug targets and processes, ones viewed today as difficult to modulate with small-molecule drugs but revealed by human biology to be essential for safe and effective therapies.

iii) Organic synthesis projects should be selected carefully with a critical analysis of the potential *impact on human health* as a key factor. These projects provide a forum for advancing an understanding of the core principles of organic chemistry as effectively as projects less connected to human health. However, they offer the potential for extending our definition of basic research and for actually bridging the valley of death.

Organic synthesis is a venerable field that is redefining itself, hopefully in a way that will accelerate the discovery of future therapeutics. The continued growth, redirection, and evolution of the field is essential for scientists to realize the promise of human biology in the 21st century.

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