Perspective

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Drug discovery is a competitive discipline that requires constant innovation and refinement as a combination of market, patient, and regulatory concerns require that company's balance their novel, clinically unvalidated molecular targets with validated targets (KO, ASO, siRNA) because the attrition rate for novel targets is substantial. The dramatic consolidations across the pharmaceutical industry in recent years clearly point to the complexities of modern drug discovery. With the high attrition rates (many Phase II and III efficacy failures) and limited human resources, drug discovery efforts must focus on a large and diverse collection of molecular targets, and judiciously employ enabling technologies and new paradigms to simultaneously develop multiple early stage programs to balance risk. Importantly, the goal at the outset of a nascent program is to rapidly provide target validation in vivo with a novel small molecule or deliver a "quick kill" for the program so that resources can be reassigned. Coupled with these concerns is the need to establish intellectual property to support broad generic patent claims early in the development process because chemical space is shrinking at an alarming rate and corporate screening collections are becoming ubiquitous.[1] Combinatorial chemistry emerged as a "white knight" with the potential to address all of these major issues facing the pharmaceutical industry.[1–5]

The 1990s witnessed a surge in combinatorial chemistry that infiltrated both academic and industrial laboratories. In the pharmaceutical industry, combinatorial chemistry, in the form of classical solid-phase organic synthesis and large compound libraries, promised to rapidly deliver new clinical candidates and drugs for company's struggling pipelines. By the early 2000s, it was clear to many companies that, after huge investments, combinatorial chemistry failed to deliver on its promises and most industrial combinatorial chemistry laboratories were disbanded.[1] Why? Drug discovery is not a simple numbers game. Synthesizing a library of 1000−10 000 molecules does not increase the odds of discovering a preclinical candidate. There are far too many caveats. The first of these caveats is library design. If the scaffold on which a 10 000-member library was prepared does not orient appended monomers in a biologically relevant way for a particular target, then the library will not afford active compounds. Second, to generate a 10 000 member library based on a single template requires a large number of monomers, and depending on the functional groups employed, the diversity in monomers may be slight, if any, providing a library of little diversity. As a result, all the compounds are topologically similar, and the SAR will appear flat or, if poorly designed for a particular biological

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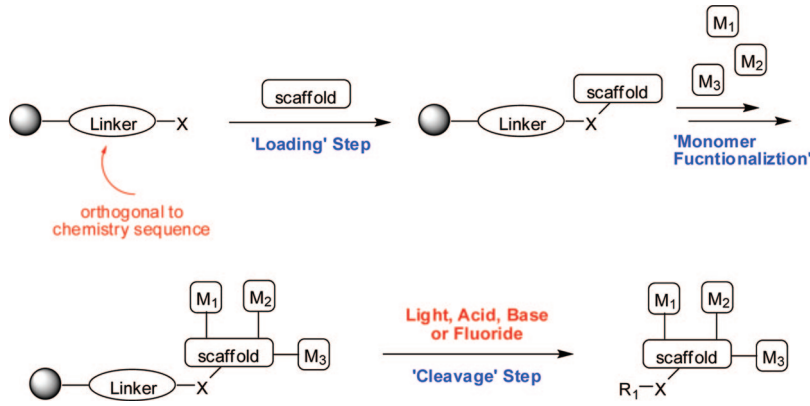

Figure 1. Overview of solid-phase organic synthesis.

target, inactive. Indeed, Sauer and Schwartz developed a computational tool that demonstrated that single-scaffold libraries, regardless of their size, are restricted to a limited number of molecular shapes, as opposed to smaller libraries designed around multiple scaffolds.[6] Because molecular shape is intrinsically linked to biological activity, the greater the structural diversity in a library, the better the odds of identifying ligands for a broad range of biological targets.[6]

Beyond structural diversity, the time required to design, synthesize, purify, and characterize a 1000−10 000-member library exponentially exceeded the time frame in which lead-optimization efforts operate; therefore, the project team had usually moved into new chemical space before the library was finally ready to be evaluated, rendering it irrelevant before screening occurred. Compound characterization with large libraries was always suspect because it typically relied solely on mass spectroscopy, suggesting the desired mass was "in the well" with little quantiation which resulted in many "false hits" and complex deconvulution exercises to identify the "active component" in a well. Finally, solid-phase chemistry and large-library synthesis required "experts" and was not embraced widely by classical medicinal chemists. It was felt that solid-phase synthesis was only useful for large-library synthesis because a one-step functionalization required a minimum of three synthetic steps (Figure 1): a loading step, a monomer incorporation step, and a cleavage step (light, acid, base, or fluoride). Moreover, each library member had an artificial "handle" at the cleavage site (unless a traceless linker was employed) usually an amine, acid, or hydroxyl moiety. Further contributing to the poor acceptance of combinatorial chemistry by medicinal chemists was the lack of direct translation of solution chemistry to the solid phase and the need to optimize the loading, diversity, and cleavage steps.[1]

While solid-phase chemistry has lost favor in small molecule drug discovery, it remains the preferred method for peptide synthesis, especially for peptide active pharmaceutical ingredients (APIs) and peptide-containing polymers.[7,8] As of 2007, solid-phase peptide synthesis is the method of choice for all phases of development for peptide pharmaceutical candidates, from discovery to commercial production.[7] Peptide APIs represent a significant class of pharmaceutical products with yearly sales in excess of $12 billion/year with a growth rate of ~4%, and the majority of these (>50%) are prepared via solid-phase peptide synthesis.[7] One such peptide, Leuprolide, has achieved blockbuster status with worldwide sales in excess of $ 2 billion/year. Without question, solid-phase synthesis maintains a pivotal role in modern drug discovery and development.[8]

Historically, the scope, mission, and technology platforms of lead-optimization groups varied considerably across the drug discovery industry leading to highly variable success rates.[2–5] Some organizations had clearly defined "hand-offs" criteria that fragmented lead optimization into a hit-to-lead phase and a chemical lead-optimization phase. Hit-to-lead focused on optimizing screening hits, usually by library synthesis (solution or solid phase), for target potency with minimal concern for selectivity, ancillary pharmacology, and pharmacokinetics (PK). Hits meeting certain potency criteria and displaying robust structure–activity-relationships (SAR) would then be "handed-off" to a second group for the lead-optimization phase, wherein more classical medicinal chemistry (single compound synthesis and intense DMPK profiling) would occur.[2–5]

After several years of combinatorial chemistry back-lash, the science behind combinatorial chemistry is now at the forefront of modern drug discovery. As a result, lead optimization in drug discovery has changed significantly in the past five years, and no longer needs to be fragmented into separate hit-to-lead and lead-optimization phases; however, centralized groups with expertise in parallel and library synthesis do add value.[2–5] Major advances have been made in high-throughput screening (HTS) technologies, which have enabled detection of novel modes of target modulation. Once dominated by radioligand binding assays and limited to detection of classical agonists and antagonists by HTS, kinetic imaging plate readers, such as FDSS and FLIPR, allow for the identification of allosteric ligands which provide positive and negative modulation of both known and novel targets, offering new chemotypes and improved selectivity and safety profiles.[9,10] Chemical lead optimization, from evaluation of screening hits to preclinical candidate identification, is now a seamless process drawing upon new technologies for accelerated synthesis, purification, and screening. Directed, iterative compound libraries are now employed throughout the lead-optimization continuum with single compound synthesis restricted to an "as needed" basis for complex, multistep chemistry. With the incorporation of DMPK (drug metabolism and pharmacokinetics) inputs at the initiation of a lead-optimization program, molecules are

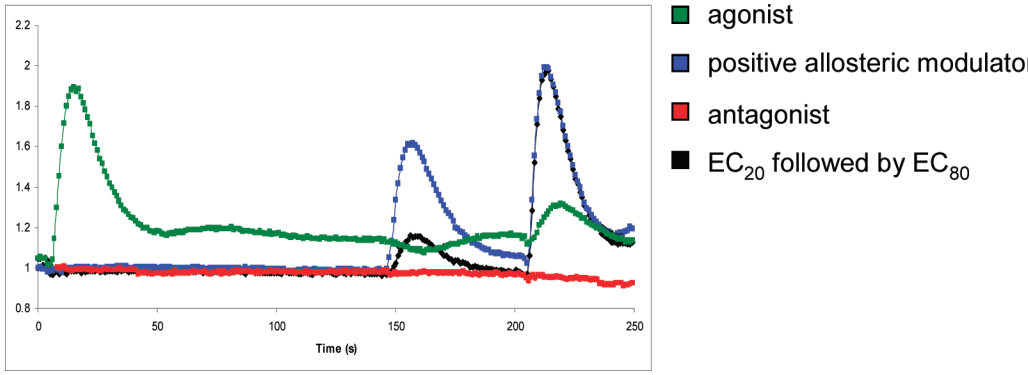

Figure 2. Triplicate screen to identify allosteric modes of target modulation: (black) vehicle along with an EC_{20} and EC_{80} of agonist; (green) wave-form profile of an agonist; (red) wave-form profile of an antagonist (flow-up necessary to distinguish orthosteric versus allosteric antagonist); and (blue) wave-form profile of a potentiator, also kown as a positive allosteric modulator. A single screen generates entire spectrum of hits with dramatically different pharmacological effects on a molecular target.

not solely optimized for target potency and selectivity, but also with respect to reducing protein binding, improving pharmacokinetics, and diminishing CYP inhibition.[1–14] Moreover, "closed loop" work-flows are in place so that chemical synthesis and primary screening data operate on a one week turn around for hundreds of compounds/week, with DMPK data cycling every other week to improve compound design and provide expedited timelines for the development of proof of concept compounds to rapidly provide "go/no go" decision points for novel molecular targets and deliver preclinical candidates with limited human resources. To avoid the negative stigma surrounding combinatorial chemistry in both industrial and academic laboratories, this new paradigm for lead optimization is coined "technology-enabled synthesis" or TES; however, a more accurate moniker would be "technology-enhanced medicinal chemistry".[15–24]

Advances in High-Throughput Screening Technologies

The science of combinatorial chemistry has also impacted and influenced high-throughput screening by advocating a philosophy that values the ability to automate complex biological assays, allow screening of difficult-to-screen targets and to detect novel mechanisms of target modulation. The historical HTS paradigms valued the use of automation to only increase throughput; however, the focus now is to faithfully execute complex tasks with high precision and answer many questions in parallel, a strength of combinatorial chemistry. Modern HTS facilities employ automated screening systems composed of state-of-the-art liquid handling, plate readers, incubators, and other instruments to support a wide-variety of cell-free and cell-based assays ranging from enzyme assays on purified proteins to phenotypic screens on model organisms, such as *Caenorhabditis elegans* and zebrafish embryos.[9,10,25–28] Advances in analysis software allows for information-rich assay forms, primarily in cell-based or organism-based environments, with read modes based on either parallel acquisition of kinetic data using instruments like the Hamamatsu FDSS and FLIPR kinetic imaging plate readers[28] or on object-based screening using high spatial resolution devices such as automated microscopes or the BlueShift Isocyte.[29] Both of these read modes yield complex, information rich data sets. While the analysis and storage of such data can be quite challenging, major advances have been made in the development of analysis software and storage of large, complex data sets. The ultimate success of a drug discovery campaign is directly linked to the ability to acquire, synthesize, store, present compounds, and collect/analyze data from the biological systems for which proof of concept compounds and preclinical candidates hope to be discovered.[9,10,25–29]

Triplicate Screen to Identify Classical and Allosteric Modes of Target Modulation. Assay miniaturization follows in the path blazed by combinatorial chemistry science. Miniaturization of assays employing kinetic imaging plate readers allow for the development of robust high-throughput calcium mobilization-based assays that detect the activation/inhibition of molecular targets through both classical and allosteric modes of target modulation. For instance, one can measure receptor-induced intracellular release of calcium by using an imaging-based plate reader that makes simultaneous measurements of calcium levels in each well of a 384-well plate. In a novel triplicate screening paradigm (Figure 2), either vehicle or a test compound was added to cells expressing a receptor of interest, such as a GPCR, that has been loaded with fluorescent dye, Fluo-4. After a brief incubation period, a submaximally effective (EC_{20}) concentration of the orthosteric agonist was added, followed by a nearly maximal (EC_{80}) concentration added 1 min later. In this manner, modern HTS scientists are able to screen for and identify, classical agonists/antagonists, allosteric potentiators, and antagonists simultaneously, maximizing the efficiency of each screen and delivering a diverse collection of hits for the chemists to optimize. This paradigm affords the medicinal chemists with options, both in terms of modulatory mechanism for their therapeutic target and in terms of chemotype, for the lead optimization campaign in a manner previously unavailable and predicated on the founding principles of combinatorial chemistry.[30]

Of course, technology has not only advanced for the screening of GPCRs but also for kinases and ion channels. Kinase screening now employs both low and high concentrations of ATP to identify both ATP-competitive and allosteric inhibitors. Numerous technology platforms have recently appeared for ion channels targets, such as highly automated

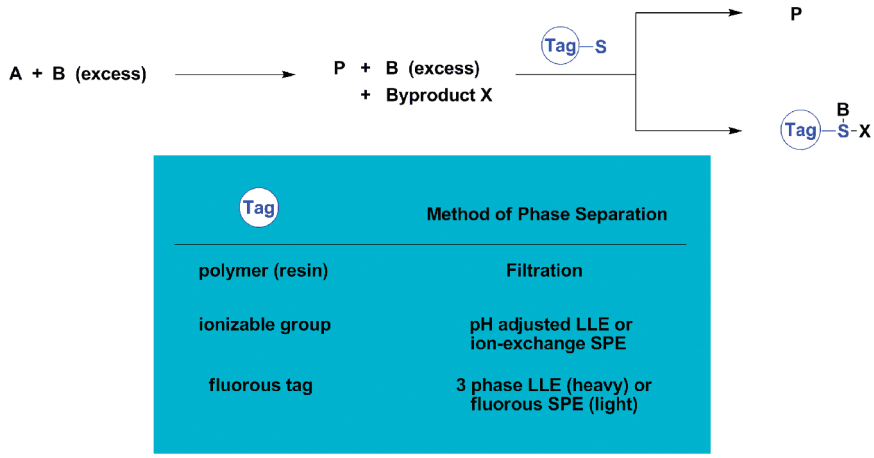

Figure 3. Solution-phase parallel synthesis and phase switching.

ion-works and Q-patch, which avoid the need for burdensome and slow single patch-clamp experiments, which allow for libraries of analogues to be screened each week against both open and closed states of the ion channels.[25–30] In short, the science of combinatorial chemistry has fundamentally altered HTS paradigms.

Solution Phase Parallel Synthesis for Lead Optimization

The chemical technologies and platforms for chemical lead optimization have undergone a major paradigm shift in the past 10 years. In the 1990s, hit-to-lead efforts were driven by combinatorial chemistry and characterized by large (1000–10 000-member) solid-phase libraries which required months to synthesize and characterize.[1,31] Often, by the time the library was ready for screening, the SAR of the program or the lead series have moved on, and the value of the library was minimal.[1,31,32] Or in the worst case, the library was screened, but the upon resynthesis of actives, the active species could not readily be elucidated requiring complex deconvolution steps and concerns over synergistic efficacy. As a result, most pharmaceutical companies disbanded their combinatorial chemistry groups and lead optimization relied primarily on single compound synthesis or small collections (less than 12) of compounds. Inspired to make the lead-optimization process more efficient, while retaining the classical medicinal chemistry feel, the concept of solution-phase parallel synthesis began to gain favor, and technologies rapidly began to accrue to enable this new approach.[33–40] In the last five years, major advances were made in the availability of polymer-supported reagents and scavengers and the advent of precision controlled single-mode microwave synthesizers for organic synthesis, along with the development of robust mass-directed preparative HPLC purification platforms have revolutionized and accelerated lead optimization by taking advantage of the science of combinatorial chemistry.[24,33–41]

Solution-Phase Parallel Synthesis (SPPS). SPPS employs the principles of excess from combinatorial chemistry to drive reactions to completion, but in contrast to classical solid phase chemistry, the product remains in solution where reaction progress can be monitored by traditional methods (TLC, LCMS). Key to the success of SPPS was the development of both resin-bound reagents and "scavenging reagents". Scavenging (quenching) reagents are highly effective tools for the rapid purification and isolation of the desired product(s) from a solution phase reaction by forming either covalent or ionic bonds with excess reactants or reaction byproduct. In general terms, scavenging can be considered a "phase-switching" technique, wherein a chemoselective reaction is employed to switch the phase of one product relative to another by virtue of a "tag" attached to the scavenging reagent.[33–41] There are three major classes of scavenging reagents categorized by the nature of the phase tag: solid-phase polymers, ionizable functional groups, and fluoroalkyl chains.[33–43] In a typical scenario, an excess of reactant **B** is combined with **A** to provide product **P**, along with **B** and other reaction byproducts **X**, in a homogeneous solution phase reaction. Then, **B** and **X** are chemoselectively removed from solution in a subsequent scavenging step with a scavenging reagent **1** linked to a phase tag. After separation of the resulting phases, the product, **P**, is obtained in high purity by simple evaporation of the solvent (Figure 3). However, in today's high-throughput medicinal chemistry laboratories, all compounds are purified by HPLC to ensure robust, reliable SAR is obtained and that analytically pure samples are available for DMPK assays. The only instances where scavenged-only libraries are employed is hit explosion from an HTS screen, where SAR is not driving program decisions, but instead, prioritizing which series to initiate hit-to-lead programs.

The most commonly used tags are solid-phase polymers, and hence, a wealth of literature centers on the applications of polymer-supported scavenger reagents to transfer a captive species from the organic liquid phase to the solid-phase for removal by filtration. Indeed, this approach has gained widespread acceptance because of the commercial availability of a diverse array of electrophilic and nucleophilic polymer-supported scavenging reagents, along with an abundance of polymer-supported reagents. Moreover, because of site isolation (>99% of functional groups within core of resin bead), "cocktails" of polymer-supported reagents and scavengers can be used simultaneously.[33–43]

Another commonly used tagging strategy involves linking a scavenger to an ionizable functional group, such as a COOH ($pK_a < 5$) or an NR2 ($pK_a > 10$). In this instance, the captured species can be selectively phase transferred by either of two methods. Classically, pH-adjusted liquid/liquid extraction was employed to transfer the desired product into the organic liquid phase with the tag transferring into the liquid phase. More recently, solid-phase extraction (SPE) on an ion-exchange cartridge emerged as a parallel synthesis friendly approach that transfers the desired product into the SPE cartridge eluent.[36] SPE is a very attractive method for purification because a crude reaction mixture is simply applied to a disposable silica plug, grafted with either a sulfonic acid (SCX = strong cation exchange) or a tertiary amine (SAX = strong anion exchange), and neutral molecules are eluted off with methanol, while ionizable functional groups are retained on the SPE cartridge. Unfortunately, this strategy impacts the diversity of a library by limiting the presence of ionizable groups to either neutral or orthogonally charged library members.[37]

Fluorous chemistry, pioneered by Curran and co-workers, represent a third tagging strategy. Relying on the affinity that fluoroalkyl chains have for each other and the phobia that they exhibit toward both organic molecules/solvents and aqueous solvents, researchers began examining fluorous tags as a means of phase switching.[43] Initially, efforts centered on "heavy" fluorous tags (60% or more fluorine content by molecular weight, i.e., eighteen or more difluoromethylene, CF_2, groups) that used liquid/liquid phase separation to isolate fluorous-tagged molecules from untagged organics. Typically, a three-phase liquid/liquid extraction, requiring an organic layer, aqueous layer, and a fluorous layer (a perfluorohexane such as FC-72) delivers pure material. More recently, fluorous solid-phase extraction (FSPE) employing fluorous silica gel (reverse-phase silica gel with a fluorocarbon bonded phase) has been developed to effectively separate both "heavy" fluorous-tagged molecules, as well as "light" fluorous-tagged molecules (4−10 CF_2 groups), from untagged organics. The FSPE columns, referred to as Fluoro*Flash*columns, retain the fluorous-tagged material when eluted with a fluorophobic solvent, such as 80/20 MeOH/H_2O, allowing the untagged organic molecule to rapidly elute from the column. Homogeneous reaction kinetics, generality with respect to charged and neutral functional groups, and a variety of efficient phase-separation options have spurred a dramatic increase in the development of fluorous scavenging reagents and protocols.[43]

Microwave-Assisted Organic Synthesis (MAOS). Microwave-assisted organic synthesis (MAOS), fueled by the development of precision controlled, single-mode microwave reactors with robotic autosamplers for serial synthesis, has had a profound impact on organic and parallel synthesis. Reaction times are reduced by orders of magnitude; a diminution in side product formation is typically observed, and MAOS reactions are readily scalable. Moreover, MAOS reactions tend to be general in scope and lend themselves to the synthesis of libraries to rapidly develop SAR. These advantages, easily appreciated when considering established routes with successful reactions, are even more valuable when working out robust conditions for novel reactions and allowing one to approach reaction development from a combinatorial chemistry perspective. Exploratory reactions can be conducted in minutes to hours instead of days and speculative, higher-risk ideas can be pursued with minimal time investment, yet with complete testing of a hypothesis. Indeed, MAOS allows any chemistry to be pursued in parallel (via 60-position autosamplers) and allows chemistries historically avoided for library synthesis (mulitcomponent reactions, organometallics, transition-metal -catalyzed couplings, etc.) to be completed successfully in minutes.[24,44,45] Other MAOS instruments allow for parallel microwave heating employing rotor systems[46] or special materials that allow multiple MAOS reactions to occur simultaneously,[47] which work by heating reaction vessels first instead of the reaction materials themselves as in the serial autosamplers.

Beyond the speed advantage two additional merits of MAOS and modern reactors should be highlighted: precision and reaction scope. As has been noted in this text and elsewhere, the benefits of MAOS have been studied in multimode "kitchen microwaves" for decades; what prevented acceptance in the wider community was irreproducibility because of a lack of pressure and temperature control. In addition, kitchen microwaves use multimode resonators, which lead to a heterogeneous field and local "hot" spots, but despite this disadvantage, early work demonstrated the utility of MAOS. Modern systems (Figure 4) provide a homogeneous field, precise control of temperature and pressure, and little resemblance to kitchen microwaves. Importantly, the mechanism of heating in a microwave reactor is quite different from classical thermal convection heating with a heating mantle or oil bath. MAOS relies on dipolar oscillations and ionic conduction, that is, molecular friction, to generate heat and afford uniform heating of the sample. In contrast, conventional thermal heating relies on heat transfer from the walls of a reaction vessel and affords nonuniform heating of the sample (Figure 4). This uniform heating and rapid time to set temperature delivers reproducible results with fewer side products, and as a result, higher chemical yields.[24]

MAOS technology has also significantly impacted library design and synthesis and can be considered a "diversity engine".[17] For example, when presented with a small heterocycle as a hit from an HTS, MAOS technology allows one to not only rapidly synthesize and evaluate substitutions on the parent heteocyclic scaffold but also to synthesize and evaluate multiple heterocyclic templates with diverse substituents in parallel (Figure 5).[44,48] Therefore, a single library will contain multiple heterocyclic cores, with varying degrees of basicity and topology, while at the same time broadening the generic scope for composition of matter patents.[44,48] This is but a single example of how MAOS has influenced and impacted modern parallel synthesis and is highly reminiscent of library strategies employed in early combinatorial chemistry.

Library Purification: The Evolution of Mass-Directed Preparative HPLC. Despite the purity obtainable by SPPS scavenging (typically >90%) and the high purities obtained

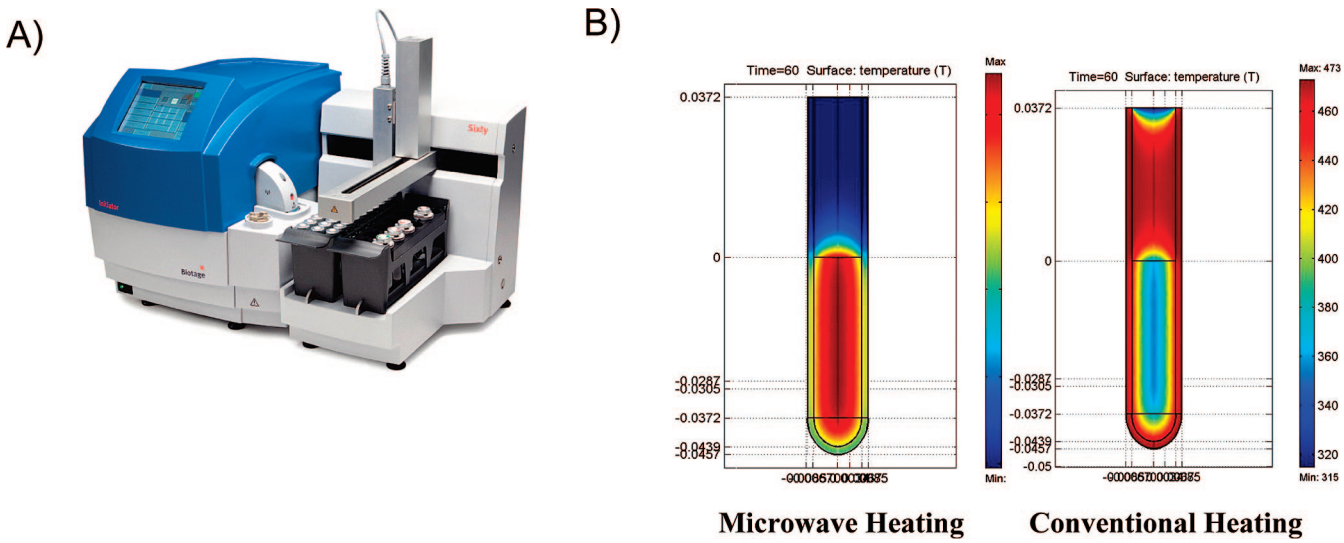

Figure 4. Microwave assisted organic synthesis: (A) a single-mode microwave reactor for organic synthesis and (B) comparison of surface temperature between microwave and conventional heating.

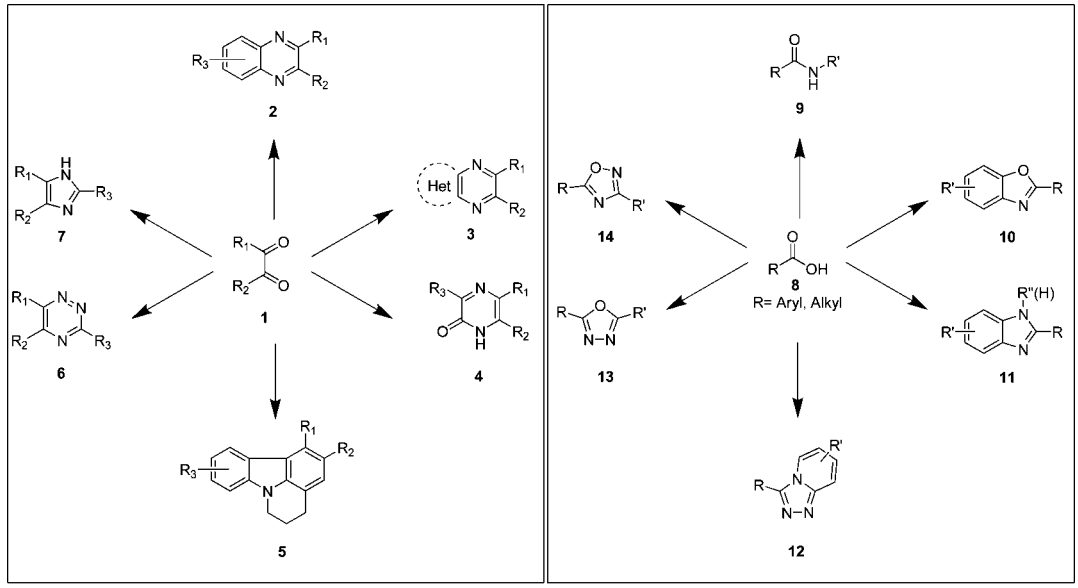

Figure 5. MAOS as a diversity engine to rapidly access diverse heterocyclic scaffolds from common intermediates with abbreviated reaction times and high chemical yields.

by MAOS (also typically >90%), modern lead-optimization programs require >95% purity of all compounds that contribute to the development of SAR and that advance into DMPK assays. This is in sharp contrast to classical combinatorial chemistry where library purity was allowed to dip to ~70% in most laboratories, which required resynthesis to establish robust SAR. In the 1990s, many laboratories employed UV-directed preparative HPLC and, often, multichannel units to increase throughput. While this approach worked, purification of a single sample might lead to >30 fractions per sample, which then required analysis by analytical LCMS to identify which fractions contained the desired product.[36,49] In 2000, several vendors launched preparative LCMS units that offered mass-directed fractionation. Now, purification of a single crude sample afforded only one or two pure fractions, a significant advance. Further modifications for library purification included DMSO slugs to bracket sample injections or "at-column dilution" to provide robust chromatography and prevent in-line sample precipitation before the column. These modified systems were capable of purifying, in a single pass, 60−80 compounds per day with purity levels exceeding 98%; however, the systems required an expert chromatographer to develop custom gradients for each sample in a library.[41,49] Recently, several vendors launched analytical-to-preparative (A2Prep) LCMS software packages that addressed the need for a dedicated, expert chromatographer to operate each prep LCMS instrument. With analytical-to-preparative software, a file containing the compound ID and exact mass for each sample in a library to be purified is uploaded into the preparative LCMS system, which then electronically accesses the analytical LCMS data and extracts the retention time of the mass of interest from the crude sample chromatograms. The preparative LCMS system analytical-to-preparative software then calculates a customized gradient for each sample in a library and therefore reduces the need for an experienced chromatographer to achieve excellent first pass

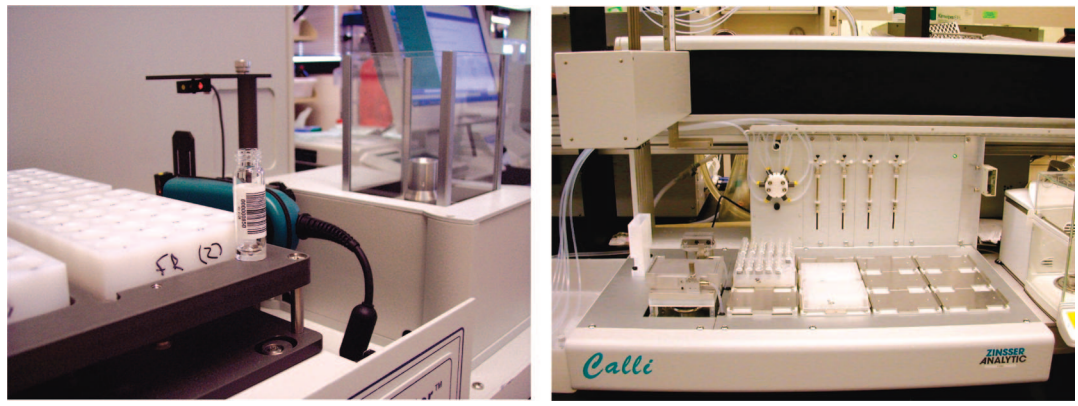

Figure 6. Automated sample handling. Custom bar-coded vials, automated weighing stations, and dilution robots enable hundreds of compounds to be processed and delivered to biologists each day.

purification results. This feature also allows the instrument to run unattended overnight and further increases operational efficiency.

Modern Postpurification Sample Handling and Compound Characterization. Modern parallel synthesis, that is, high-throughput medicinal chemistry, laboratories have borrowed a page from the industrial revolution (i.e., the automotive industry) and developed highly efficient assembly lines for postpurification sample handling and compound characterization. Automated weighing systems with bar-code readers scan and record weights on unique bar-coded vials into which pure compounds from the preparative LCMS systems are transferred for concentration in a sample evaporator (Figure 6). After the drying step, the bar-coded vials with pure, solid sample are transferred to a liquid handling robot. This instrument scans each bar code, weighs the vial, and determines the net weight of the pure product. This data file is merged with a registration file containing the molecular weight of the compound, and the system software then calculates the volume of DMSO required to dilute the samples to a preset concentration for screening. The system then dilutes the samples, transfers the DMSO stock solution to a 96-well plate, and generates an electronic plate map file for submission to the primary screen, in vitro drug metabolism assays, pharmacokinetic cassettes, and for flow-cell NMR (vide infra). With this highly automated workflow, a single scientist can oversee the postpurification sample handling of thousands of samples per week.[1–5,41]

Lack of complete and in some cases any quantitative compound characterization has been a major shortcoming of combinatorial chemistry and early high-throughput medicinal chemistry laboratories, which led to poor adoption by traditional medicinal chemists. Technology has once again advanced so that every member of a compound library can be fully characterized to the same standard as a single compound prepared by a traditional medicinal chemist. After purification by preparative LCMS, a final analytical LCMS is generated for each sample at two wavelengths (214 and 254 nM), and also evaporative light scattering detection (ELSD) is performed. The LCMS vials are then delivered to a QTOF mass spectrometer system with a 100-position autosampler and UPLC frontend for accurate mass measurement (high-resolution mass spectroscopy) determinations. ^{1}H NMR spectra are obtained for each sample. Initially, NMR tubes were prepared for each sample, and chemists took advantage of NMRs with autosamplers. More recently, flow-cell NMR and solvent suppression software allows for quality NMR spectra to be obtained from DMSO stock solutions in 96-well plate.[50] Importantly, this ensures high purity samples, prepared in directed libraries, to drive lead-optimization programs and generate quality SAR at the same level as compounds prepared by singleton synthesis.

Accelerating Drug Metabolism and Pharmacokinetics

Streamlining and reducing drug discovery timelines requires that chemical lead-optimization campaigns involve more than just target potency optimization. New technology, inspired by combinatorial chemistry science, allows early lead optimization campaigns to address and consider multiple parameters and inputs for each round of iterative library synthesis. These inputs allow for the rapid development of potent compounds with drug-like profiles, as opposed to just compounds with optimized potency. These data also provide quick kills (go/ no go) to individual leads or series and allow the lead-optimization effort to redirect resources to more productive compounds/chemical series.[1–5,12–24]

A great deal of time, effort, and attention has been applied to the miniaturization and DMSO compatibility of in vitro drug metabolism assays with tremendous success. Today, a 48-member library in a 96-well plate of DMSO stock solution can be rapidly evaluated in cytochrome P450 inhibition assays (CYP3A4, 2D6, 2C9), protein binding assays (rat, dog, human), logP, hERG binding, and other standard assays to assess drugability.[12–15,51,52] Previously, medicinal chemists were forced to select a limited number of compounds to evaluate in these assays, and often only the most potent analogs would be chosen for screening. Unfortunately, the most potent compounds were not necessarily the ones with the most promise as preclinical candidates. Being able to acquire these data for an entire library provides opportunities to pursue leads within a series with the most balanced potency and DMPK profiles. This is of critical importance in lead optimization to ensure that drug-like leads are being pursued and further refined to avoid falling into a potency well from which drugability is difficult to obtain. Similarly, cassette dosing of compounds in liver microsomes and hepatocytes enables evaluation of an entire library in short

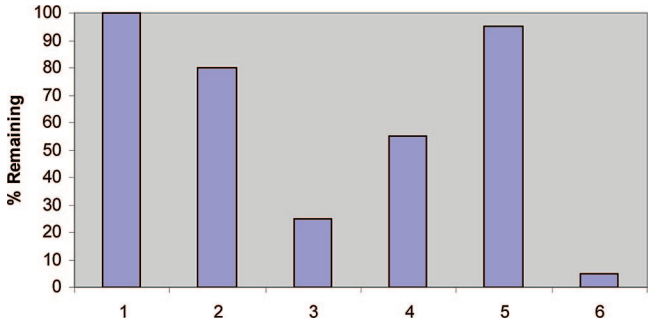

Figure 7. Liver microsome cassette experiment. Simulataneous evaluation of a positive control (or first clinical candidate, compound **1** in the graph above) and five test compounds (compounds **2**–**6**) from a library in a single-well experiment in fortified rat liver microsomes after a 2 h incubation period (dog, nonhuman primate, and human microsomes afford similar results). From this experiment, compound **5** is about as stable as the first clinical candidate and therefore is an interesting compound. In contrast, compound **6** is unstable in microsomes and need not be considered further. This rapid, qualitative assessment of stability allows entire libraries to be evaluated.

order.[12–15,51,52] This is especially valuable in the lead optimization of a back-up clinical candidate program, wherein the clinical candidate is the positive control and new compounds (typically 5−6 per cassette) are viewed qualitatively as more or less stable than the first clinical candidate (Figure 7). Once an in vitro/in vivo correlation can be established for a given series, these rapid cassette experiments can drive a lead-optimization program and require only intermittent in vivo experiments.[12–15,51,52]

Resource and practical constraints prohibit acquiring single rat and dog (PK) pharmacokinetics (i.v. and p.o.) for every member of a library; however, the compound with the best PK may not be the most potent analog in a library, and knowledge of this data is crucial for lead optimization. In fact, chemical lead optimization programs have been guided solely by optimization of PK. With the success of in vitro cassette paradigms for microsomal and hepatocyte stability, the concept was recently extended to in vivo PK in rats and dogs (Figure 8) so that an entire library could be evaluated in vivo employing a limited number of animals.[12,15,53] However, there are some caveats. Combinatorial oral dosing to determine oral bioavailability (*%F*) in cassette format generally proved to be not very reproducible nor in agreement with single PK experiments. In contrast, i.v. cassette dosing, in both rats and dogs, proved highly reproducible and within the error of a single PK experiment and is a valuable tool to determine qualitative rates of clearance between five to six new compounds and an internal control of known clearance.[12,15,53] PK cassettes employ an overall low dose of test compounds to minimize potential drug−drug interactions. These rapid cassette experiments prioritize which compounds from a library should then be studied in single i.v./p.o. single animal PK studies. As shown in Figure 8, compound **2** has qualitatively lower intrinsic plasma clearance rate in a rat PK cassette experiment than an internal control compound with known bid (twice daily predicted dosing) PK (Cl = 12

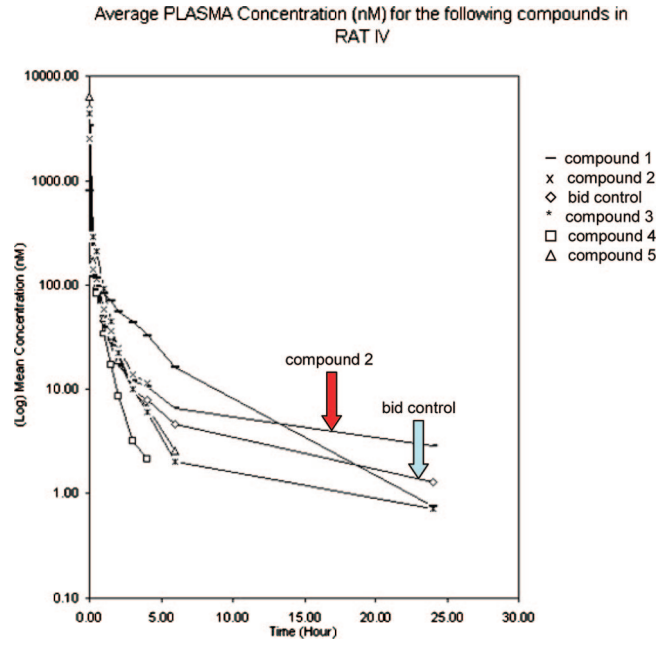

Figure 8. Pharmacokinetic evaluation of libraries: cassette dosing in rats to qualitatively evaluate clearance rates relative to a bid control compound. In this case, a compound with known bid PK is employed as a positivie control and five new compounds are evaluated relative to the control compounds intrinsic clearance. Compound 2 in the cassette is qualtitatively equivalent or better than the bid control, so it will be followed up in a single i.v./p.o. PK experiment. The other four test compounds have higher clearance than the control and do not need to be evaluated further.

mL/min/kg), whereas the other four compounds in the cassette have higher clearance and need not be studied further.

Expedited, Closed Loop Work Flow for Lead Optimization

Combining all of the above-mentioned technologies and paradigms for synthesis, screening, and DMPK evaluation affords an aggressive, expedited process for chemical lead optimization derived again from the founding principles of combinatorial chemistry.[1,16–25] This protocol allows 1–2 synthetic chemists to support a chemical lead-optimization effort with accelerated timelines delivering proof of concept compounds within 6 months and clinical candidates within 12 months of the initiation of a lead-optimization campaign.

Independently, the technologies and strategies described herein provide improvements for chemical lead optimization; however, when they become closely aligned with screening and DMPK resources in a closed loop paradigm, the impact on drug discovery is exponential (Figure 9). Starting from an HTS hit, considerable attention is first devoted to library design, without question the most important component of a successful lead-optimization effort. Library design changes over the course of a lead-optimization campaign. The initial design strategy is to explode SAR around a screening hit and to be as diverse as possible with respect to monomer input and analog synthesis to rapidly identify productive changes for further optimization. In addition, this component of lead optimization is often conducted in parallel, wherein a single chemist will simultaneously synthesize diversity

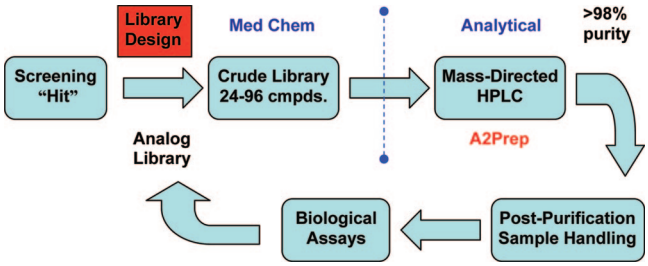

Figure 9. Expedited closed-loop lead-optimization paradigm.

libraries around four to six hits to expediently identify the best leads for further optimization. After this initial diversity-oriented explosion, library design must become more focused to impact drug discovery goals: *random libraries do not accelerate programs*. It is important to approach directed library design from a medicinal chemistry perspective and assemble the library as a collection of single compounds designed to address a particular issue. For example, the design of a 24-member library should involve careful thought regarding what would the first four single compounds to synthesize be to test a hypothesis, increase potency, improve PK, etc. Then, for each of the first four analogs synthesized, consider what the next four analogs should be if the first changes were productive or nonproductive. This exercise in library design generates quality data that drives a lead-optimization program toward proof of concept compounds and clinical candidates very quickly.[1,16–25]

Another key feature of the closed loop approach to lead optimization involves division of labor and the transfer of samples from medicinal chemists to the analytical chemists. In this paradigm, the medicinal chemists design and synthesize the compound libraries (24−96 compounds) and obtain analytical LCMS reports for each member of the library. At this point, the medicinal chemists transfers the crude samples to the analytical chemists who purify the libraries by mass-directed preparative HPLC to >98% using analytical-to-preparative software, perform all postpurification sample handling and coordinate submission of samples, in a 96-well plate format, to the biologists and DMPK personnel for screening (vide supra).[16–25,54] If resources allow, this division of labor affords opportunities for the medicinal chemists to focus on library design, develop and optimize new chemistries, and pursue multiple lead series in parallel.[1,16–25,54]

The success of this paradigm hinges on rapid screening and dissemination of data to the medicinal chemists so that the next iteration of library synthesis can be initiated. To facilitate this, the delivery of compounds is coordinated with the biologists and assays are run the same day that the compound libraries are delivered. Biological data is then returned within 24 h of receipt of the libraries. This allows lead optimization to operate on a one week turn around between the initiation of chemical synthesis and the generation of primary assay data. Secondary or selectivity data typically trail primary data by 1–2 days. As these data trigger the need for DMPK information, DMPK data typically follows one week after the initial assay data is obtained. Overall, this expedited process parallels traditional singleton medicinal chemistry work flows but generates data on hundreds of compounds in the time it used to take to evaluate just a few compounds. Moreover, this protocol allows 1–2 synthetic chemists to support a chemical lead-optimization effort with accelerated timelines delivering proof of concept compounds within 6 months and clinical candidates within 12 months of the initiation of a lead-optimization campaign. It is important to note that this lead-optimization paradigm requires collaboration, close and frequent communication with biology and DMPK colleagues, sophisticated databases to store the volumes of data generated, and a major investment in technology.[1,16–25]

Conclusion

The science that established combinatorial chemistry as important new discipline in the 1980s and 1990s, has re-emerged and infiltrated every subdiscipline within modern drug discovery and has profound impact. The application of combinatorial chemistry science has revolutionized high-throughput screening paradigms, chemical lead optimization, library purification and postpurification sample handling, as well as in vitro and in vivo drug metabolism and pharmacokinetic assays. Although no longer in the spotlight and heralded as the savior of the drug industry, combinatorial chemistry is alive and well: actually, combinatorial chemistry science is more prevalent and widespread than ever before.

References and Notes

(1) (a) Glaser, V. *Gen. Engin. Biotech. News* **2005**, *25*, 31–37. (b) Weller, H. N.; Nirschl, D. S.; Petrillo, E. W.; Poss, M. A.; Andres, C. J.; Cavallaro, C. L.; Echols, M. M.; Grant-Young, K. A.; Houston, J. G.; Miller, A. V.; Swann, R. T. *J. Comb. Chem.* **2006**, *8*, 664–669. (c) Patel, D. V.; Gordon, E. M. *Drug Discovery Today* **1996**, *1*, 134–144. (d) Lee, A.; Brietenbucher, J. G. *Curr. Opin. Drug Discovery Dev.* **2003**, *6*, 494–508.

(2) Gillespie, P.; Goodnow, R. A., Jr. *Annu. Rep. Med. Chem.* **2004**, *39*, 293–304.

(3) Keseru, G. M.; Makara, G. M. *Drug Discovery Today* **2006**, *11*, 741–748.

(4) Bleicher, K. H.; Boehm, H-J.; Mueller, K.; Alanine, A. I. *Nat. Rev. Drug Discovery* **2003**, *2*, 369–378.

(5) Meador, V.; Jordan, W.; Zimmermann, J. *Curr. Opin. Drug Discovery Dev.* **2002**, *5*, 72–78.

(6) (a) Sauer, W. H. B.; Schwarz, M. K. *Chim. Int. J. Chem.* **2003**, *57*, 276–283. (b) Sauer, W. H. B.; Schwarz, M. K. *J. Chem. Inf. Comput. Sci.* **2003**, *43*, 987–1003.

(7) Verlander, M. *Int. J. Pep. Res. Ther.* **2007**, *13*, 75–82.

(8) Breitenkamp, R. B.; Ou, Z.; Breitenkamp, K.; Muthukumar, M.; Emrick, T. *Macromolecules* **2007**, *40*, 7617–7624.

(9) Wood, M.; Smart, D. In *Receptors: Structure and Function*, 2nd ed.; Stanford, C., Horton, R., Eds.; Oxford University Press: New York, 2001; pp 175−191.

(10) Wood, M. D.; Jerman, J.; Smart, D. *Recent Res. Dev. Neurochem.* **2000**, *3* (Pt. 1), 135–142.

(11) Huang, R.; Qian, M.; Chen, S.; Lodenquai, P.; Zeng, H.; Wu, J.-T. *Int. J. Mass Spectrom.* **2004**, *238*, 131–137.

(12) Halladay, J. S.; Wong, S.; Jaffer, S. M.; Sinhababu, A. K.; Khojasteh-Bakht, S. C. *Drug Metabol. Lett.* **2007**, *1*, 67–72.

(13) Bu, H-Z.; Magis, L.; Knuth, K.; Teitelbaum, P. *Rapid Commun. Mass Spectrom.* **2001**, *15*, 741–748.

(14) Cai, Z.; Sinhababu, A. K.; Harrelson, S. *Rapid Commun. Mass Spectrom.* **2000**, *14*, 1637–1643.

(15) Lindsley, C. W.; Wisnoski, D. D.; Leister, W. H.; O'Brien, J. A.; Lemiare, W.; Williams, D. L., Jr.; Burno, M.; Sur, C.; Kinney, G. G.; Pettibone, D. J.; Miller, P. R.; Smith, S.; Duggan, M. E.; Hartman, G. D.; Conn, P. J.; Huff, J. R. *J. Med. Chem.* **2004**, *47*, 5825.
(16) Lindsley, C. W.; Zhao, Z.; Leister, W. H.; Robinson, R. G.; Barnett, S. F.; Defeo-Jones, D.; Jones, R. E.; Hartman, G. D.; Huff, J. R.; Huber, H. E.; Duggan, M. E. *Bioorg. Med. Chem. Lett.* **2005**, *15*, 761–765.
(17) Wolkenberg, S. W.; Lindsley, C. W. *Discovery Dev.* **2004**, *4*, 1–5.
(18) Zhao, Z.; O'Brien, J. A.; Lemiare, W.; Williams, D. L., Jr.; Jacobson, M. A.; Sur, C.; Pettibone, D. J.; Tiller, P. R.; Smith, S.; Hartman, G. D.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5968–5972.
(19) Nanda, K. K.; Nolt, M. B.; Cato, M. J.; Kane, S. A.; Kiss, L.; Spencer, R. H.; Wang, J.; Lynch, J. L.; Regan, C. P.; Stump, G. L.; Li, B.; White, R.; Yeh, S.; Bogusky, M. J.; Bilodeau, M. T.; Dinsmore, C. J.; Lindsley, C. W.; Hartman, G. D.; Wolkenberg, S. E.; Trotter, B. W. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5897–5901.
(20) Wolkenberg, S. E.; Zhao, Z.; Kapitskaya, M.; Webber, A. L.; Pertukhin, K.; Tang, Y. S.; Dean, D. C.; Hartman, G. D.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2006**, *16*, 5001–5004.
(21) Lindsley, C. W.; Zhao, Z.; Leister, W. H.; O'Brien, J. A.; Lemiare, W.; Williams, D. L., Jr.; Chen, T-B.; Chang, R. S. L.; Burno, M.; Jacobson, M. A.; Sur, C.; Kinney, G. G.; Pettibone, D. J.; Tiller, P. R.; Smith, S.; Tsou, N. N.; Duggan, M. E.; Conn, P. J.; Hartman, G. D. *ChemMedChem* **2006**, *1*, 807–811.
(22) Zhao, Z.; Wisnoski, D. D.; O'Brien, J. A.; Lemiare, W.; Williams, D. L., Jr.; Jacobson, M. A.; Wittman, M.; Ha, S.; Schaffhauser, H.; Sur, C.; Pettibone, D. J.; Duggan, M. E.; Conn, P. J.; Hartman, G. D.; Lindsley, C. W. *Bioorg. Med. Chem. Lett.* **2007**, *17*, 1386–1391.
(23) Lindsley, C. W.; Wolkenberg, S. E.; Shipe, W. *Drug Discovery Today: Technol.* **2005**, *2*, 155–161.
(24) Lindsley, C. W.; Weaver, D.; Conn, P. J.; Marnett, L. *ACS Chem. Biol.* **2007**, *2*, 17–20.
(25) Ueki, T. *Nippon Yakurigaku Zasshi* **2007**, *129*, 276–280.
(26) Macarron, R. *Drug Discovery Today* **2006**, *11*, 277–279.
(27) DeSimone, R. W. *Drug Discovery Today* **2003**, *8*, 156.
(28) For information on Hamamatsu FDSS, see: www.hamamtsu.com.
(29) For information on BlueShift Isocyte, see: www.blueshiftbiotech.com.
(30) Rodriguez, A.; Williams, R.; Jones, C.; Niswender, C.; Meng, X.; Lindsley, C. W.; Conn, P. J. *Nat. Chem. Biol.* , .
(31) Dorwald, F. Z. *Organic Synthesis on Solid Phase*; Wiley-VCH, Weinheim, Germany, 2000.
(32) Ellingboe, J. W. *Curr. Opin. Drug Discovery Dev.* **1999**, *2*, 350–357.
(33) Kuroda, N.; Hird, N.; Cork, D. G. *J. Comb. Chem.* **2006**, *8*, 505–512.
(34) Altorfer, M.; Ermert, P.; Faessler, J.; Farooq, S.; Hillesheim, E.; Jeanguenat, A.; Klumpp, K.; Maienfisch, P.; Martin, J. A.; Merrett, J. H.; Parkes, K. E. B.; Obrecht, J-P.; Pitterna, T.; Obrecht, D. *Chimia* **2003**, *57*, 262–269.
(35) Booth, R. J.; Hodges, J. C. *Acc. Chem. Res.* **1999**, *32*, 18–26.
(36) Kaldor, S. W.; Siegel, M. G. *Curr. Opin. Chem. Bio.* **1997**, *1*, 101–106.
(37) Siegel, M. G.; Hahn, P. J.; Dressman, B. A.; Fritz, J. E.; Grunwell, J. R.; Kaldor, S. W. *Tetrahedron Lett.* **1997**, *38*, 3357–3360.
(38) Ley, S. V.; Baxendale, I. R.; Longbottom, D. A.; Meyers, R. M. *Drug Discovery Dev.* **2007**, *2*, 51–89.
(39) Zhang, M.; Flynn, D. L.; Hanson, P. R. *J. Org. Chem.* **2007**, *72*, 3194–3198.
(40) Parlow, J. J. *Curr. Opin. Drug Discovery Dev.* **2005**, *8*, 757–775.
(41) Leister, W. H.; Strauss, K. A.; Wisnoski, D. D.; Zhao, Z.; Lindsley, C. W. *J. Comb. Chem.* **2003**, *5*, 322–329.
(42) Kyranos, J. N.; Lee, H.; Goetzinger, W. K.; Li, L. Y. T. *J. Comb. Chem.* **2004**, *6*, 796–804.
(43) Gladysz, J. A., Curran, D. P., Horvath, I. T., Eds. *Handbook of Fluorous Chemistry*; Wiley-VCH: New York, 2004.
(44) Shipe, W. D.; Yang, F.; Zhao, Z.; Wolkenberg, S. E.; Nolt, M. B.; Lindsley, C. W. *Heterocycles* **2006**, *70*, 665–689.
(45) Kappe, C. O.; Dallinger, D. *Nat. Rev. Drug Discovery* **2006**, *5*, 51–63.
(46) Pisani, L.; Prokopcova, H.; Kremsner, J. M.; Kappe, C. O. *J. Comb. Chem.* **2007**, *9*, 415–421.
(47) Kremsner, J. M.; Kappe, C. O. *J. Org. Chem.* **2006**, *71*, 4651–4658.
(48) Wang, Y.; Sarris, K.; Sauer, D. R.; Djuric, S. W. *Tetrahedron Lett.* **2007**, *48*, 2237–2240.
(49) Blom, K. F. *J. Comb. Chem.* **2002**, *4*, 295–301.
(50) Curran, S. A.; Williams, D. E. *Appl. Spectrosc.* **1987**, *41*, 1450–1454.
(51) Kariv, I.; Rourick, R. A.; Kassel, D. B.; Chung, T. D. Y. *Comb. Chem. High Throughput Screening* **2002**, *5*, 459–472.
(52) Kenakin, T. *Nat. Rev. Drug Discovery* **2003**, *2*, 429–438.
(53) Huang, R.; Qian, M.; Chen, S.; Lodenquai, P.; Zeng, H.; Wu, J-T. *Int. J. Mass Spectrom.* **2004**, *238*, 131–137.
(54) Warrington, B. H. *Lab. Autom. Chem. Ind.* **2002**, 157–209.

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