# Integrating combinatorial chemistry into the discovery pipeline

Often the most productive meetings are not the large conferences with hundreds of participants, but the small intimate ones. The *Integrating Combinatorial Chemistry into the Drug Discovery Pipeline* conference (14–15 September 1998, Arlington, VA, USA) was one of the latter. Attended by only 75–100 combinatorial chemists from both production and R&D, the meeting included presentations on the application of combinatorial chemistry to drug discovery and 'nuts and bolts' discussions on the day-to-day problems faced in a combinatorial chemistry laboratory.

## High-throughput purification and analysis

Combinatorial chemists have the ability to synthesize very large libraries very rapidly; they are also attempting highly challenging synthetic schemes in a parallel fashion. As more large libraries of complex molecules are prepared, there is a need for purification and analysis – this has been a difficult technical challenge. A pre-conference workshop addressed these challenges. Presentations from MDS-Panlabs and ArQule provided a look at the difficulties faced by their production chemists and how such difficulties are being solved. This was contrasted nicely by a presentation of the library generation effort at Novartis.

Cheryl Garr (MDS Panlabs, Bothell, WA, USA) presented a detailed overview of the library generation process at Panlabs, focusing on purification of large libraries. Panlabs routinely purifies 12,000 reaction products a month using two Biotage Parallax HPLC systems. Characterization of desired products is accomplished by flow-injection analysis/mass spectrometry (FIA/MS) and HPLC analysis. Overall purity averages 92% for a 10,000 component library.

A comparison of analytical characterization techniques was provided by James Kyranos (ArQule, Medford, MA, USA). The techniques presented [FIA/MS, HPLC/UV/ELSD (evaporative light-scattering detector), HPLC/MS and NMR] each have applications depending on the throughput required. Strengths and weaknesses of these techniques were highlighted, with comments on how to maximize the strengths of each by proper application. For example, FIA/MS is most readily used for synthesis confirmation, but it provides little information on purity. HPLC coupled to both a UV and an ELSD detector allows purity analysis, but at a lower throughput than FIA/MS. Thus, 'pseudo-HPLC/MS' was developed as a high-throughput procedure to provide an indication of purity and confirmation of synthesis – it is mainly used for confirmation of array synthesis and it involves a short HPLC gradient (30 s) coupled to MS peak determination. Similarly, a high-throughput preparative HPLC was shown, using MS as peak detection and a trigger for sample collection. Run times of 2–5 min are used for purification of 5–50 mg of material.

Frank Otto Gombert (Novartis Pharma, Basel, Switzerland) gave an overview of array production in Basel. Using a custom-designed pin apparatus and RF tagging enables pin sort-and-combine methodology for preparation of 1000-member libraries. Final purification of libraries by preparative HPLC/MS using 5 cm length columns and gradients of 5 min allows throughput of 160 compounds a day. Only the desired M+ is collected in the purification.

### *Glimpsing the future*

A glimpse into possible future combinatorial compound structure determination was given by Timothy Peck (Magnetic Resonace Microsensors Corp., Savoy, IL, USA). He described the use of microcoils for NMR coupled to HPLC or capillary electrophoresis systems to allow rapid NMR spectra determination of libraries.

During a panel discussion, it was generally agreed that HPLC, especially using short columns, will come to the forefront as a purification technique. This will represent a paradigm shift from traditional medicinal chemistry. There was also discussion concerning the need to purify all reactions and whether this was a shortcoming of chemistry development. It was concluded that purification would be necessary because of the very nature of combinatorial chemistry, especially with the more difficult chemistries where yields and purity are important.

### *Solid- or solution-phase?*

The question of which is the most efficient synthesis method for library generation was raised. Many delegates seemed to hold the view that solid-phase synthesis should be used for large library generation and solution phase for small-scale, directed lead optimization, but this was not the conclusion of the panel. Both Panlabs and ArQule favored solution-phase synthesis for large library generation, as no particular benefit in purity is provided by solid phase. Moreover, solid-phase chemistry is limiting in scale.

Another important consideration that was raised concerned the vast quantities of data and information produced during research and what to do with it. It was agreed that all data should be captured, even if only a small portion is currently being used, as saving data for future use was deemed imperative.

## Combinatorial and medicinal chemistry synergy

As combinatorial chemistry is becoming entwined in drug discovery, we are

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seeing advances in several areas of the field. Combinatorial chemistry has, up to now, been seen solely as a method of preparing large screening libraries, but increasingly, parallel synthesis is being used to generate SAR quickly in the development of a drug candidate. It is being used as an extension of traditional medicinal chemistry and as such is becoming an integral tool in medicinal chemistry.

The development of a potent (low nanomolar) and selective thrombin inhibitor by Merck is a prime example of this strategy. Joseph Vacca (Merck & Co., West Point, PA, USA) presented how rapid synthesis techniques of combinatorial chemistry were applied to traditional medicinal chemistry to optimize efficiently the potency of this thrombin inhibitor. Taking a potent hydrophobic lead, and using rapid screening of libraries prepared on solid phase, the SAR of the lead was determined. Then using modelling and crystallography, changes were made to decrease the lipophilicity, while maintaining potency. It was particularly interesting to see that much of the rapid synthesis was used to optimize the oral bioavailability of the inhibitor.

### *ICE inhibitors*

Both Scott Harbeson (Vertex Pharmaceuticals, Cambridge, MA, USA) and Joseph Warmus (Parke-Davis Pharmaceuticals, Ann Arbor, MI, USA) provided examples of the use of combinatorial chemistry to design interleukin-converting enzyme (ICE) inhibitors. Harbeson described how directed arrays, based on ideas from computer-aided ligand design, can drive a project rapidly towards a clinical candidate. Initial library design around a screen hit against ICE centered on filtering a virtual library to obtain a set of potent, synthetically accessible drug-like molecules. Targeted libraries were prepared, assayed and analysed. Computer-aided design, based on the biological results, was used to design further targeted libraries. Reiteration of this cycle allowed rapid optimization of the lead compound.

A different class of ICE inhibitor was investigated using directed-array synthesis by Warmus. He showed how reactions could be rapidly optimized for automation using batch method optimization. Multiple reaction conditions set up in parallel and analyzed by HPLC are used to determine the ideal set of conditions.

## New chemistry and techniques

A key factor in the growth of combinatorial chemistry has been the development of new technologies and automation. Two prime examples of new methods to handle the parallel processing of reactions were presented at this meeting. Michal Lebl (Trega Biosciences, San Diego, CA, USA) described a unique method involving simultaneous washing of resin in a 96-well format by centrifugation. Plates are centrifuged at an angle of 9° to remove solvent, yet trap resin in the plates. It allows the efficient washing of four plates at a time.

Joseph Guiles (Hoechst Marion Roussel, Bridgewater, NJ, USA) showed how color-coding can simplify mix-and-sort synthesis in Irori Microkans. By using inexpensive colored glass beads and colored caps that fit the Microkans, a reaction can be tracked without an RF tag inserted. This allows both a cost savings and the ability to load more resin into the cans. Another use of the caps is for reaction monitoring when using RF tags. Inclusion of a few duplicate reactions with colored caps allows easy identification of Microkans for analysis during a reaction step.

### *Purification methods*

John Parlow (Monsanto, St Louis, MO, USA) provided an overview of polymer-supported reaction purification, termed polymer-assisted solution phase (PASP) by the group at Monsanto. He presented many of the techniques and reagents available for removal of starting materials, reagents and by-products in a solution-phase combinatorial synthesis. Polymer-supported quench of unreacted starting materials, products and reaction by-products, can be achieved by either simple quenching resins or by sequestering techniques. Also, 'tagging' of reagents can allow simple removal by polymer-bound sequestering or quenching reagents. Using these techniques in combination allows efficient purification of solution-phase libraries.

Another method of purification involving the use of fluorous-phase chemistry was described by Dennis Curran (University of Pittsburgh, PA, USA). The immiscible nature of fluorinated solvents with both organic and aqueous systems enables a novel method of purification. Design of fluorous reagents, such ax fluorous tributyl tin hydride, will allow efficient removal of the reagents from the organic phase by washing with fluorous solvents. Fluorous silica gel has also been prepared for a simple solid-phase extraction system based on fluorous reagents.

## Information explosion

The vast increase in new compound production has lead to an explosion in the amount of data produced. However, the ability to capture and use these data has been a major problem for combinatorial and medicinal chemists, and it is only now that it is being addressed.

Two examples of integrated data-management solutions were presented. Kimberley Heuer described the 'in-house' system developed by Searle (Skokie, IL, USA). The system contains a database of available reagents, which allows selection and array design to be integrated. It also allows an automation procedure to be set up for easy transfer to a robotics laboratory. A unique feature of the system is its ability to

schedule biological assays to which the final compounds are sent. A logic system automatically selects compounds that meet a specified set of criteria, and such compounds can then be sent for secondary or tertiary screens. All the data from the assays are automatically captured and are available to the chemist.

David Chapman (Afferent Systems, San Francisco, CA, USA) presented a commercial approach to data handling. With the Afferent system, enumeration of libraries uses a reaction sequence rather than the R-group core that many other systems use. This enables some chemistry to be enumerated, such as a Diels–Alder reaction, which cannot otherwise be enumerated by R-group cores. It also allows electronic capture of synthetic protocols, which thus acts as a 'corporate memory' for future chemists. Modules that provide a single user interface for multiple synthetic instruments are also available, and new modules that will allow an integrated analytical data and biological data retrieval system are in development.

### Final impressions

The meeting provided an excellent opportunity to discuss with fellow combinatorial chemists current technologies and common difficulties found in combinatorial chemistry labs. The mix of chemists from many production companies and directed R&D groups allowed cross-fertilization of techniques and chemistry. The small size allowed free discussion throughout the event. Surely many of the participants left the meeting with ideas of how to improve the workflow in their own labs.

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# Book review

**Clinical Trials of Genetic Therapy with Antisense DNA and DNA Vectors**

Edited by Eric Wickstrom, Marcel Dekker, 1998. $185 (xvii + 427 pages, hardback) ISBN 0-8247-0085-6

In the September issue of *Drug Discovery Today*, we inadvertently gave the wrong publisher of this book reviewed by David E. Szymkowski, who, in his opening paragraph, summarizes the book in the following way:

'Coming soon after the first NDA for an antisense compound, *Clinical Trials of Genetic Therapy with Antisense DNA and DNA Vectors* is a timely and comprehensive review of the potential applications of oligonucleotide- and vector-based gene therapy to treat disease. Wickstrom has assembled contributions from 20 research groups, incorporating late-stage preclinical R&D with results from the first clinical trials of genetic therapies. Development and clinical trial data for these compounds are often not easily accessible in the scientific literature, and this book provides both a readable introduction for newcomers and a useful update for those familiar with the field.'

For the full review of this book see *Drug Discovery Today* (1998) 3, 403.

## In short…

An investigational new drug application to inaugurate Phase I clinical trials of a nitric oxide (NO) neutralizing compound is to be filed by **Medinox** (San Diego, CA, USA) in early 1999.

The company claims to have developed a unique class of compound that has the ability to absorb, and thus remove, excess NO while conserving the quantities necessary for normal physiological functions. This is an alternative to blocking the NO synthase enzyme pathway, which can lead to deleterious effects.

The NO molecule acts as a signaling agent in the nervous system. An excess production of NO by neurons can contribute to fatal hypotension, arthritis, neurodegenerative diseases and brain-cell death after cerebrovascular accidents such as stroke. The company claims that if the good safety and efficacy profile of their compounds is reaffirmed through the clinical trials, a novel therapeutic prototype for the treatment of haemorrhagic shock and stroke may develop that could be routinely administered during transport of a patient to hospital. Currently, they have three compounds in preclinical development with the potential for treating a broad spectrum of disease states.