

Tightening the nuts and bolts

From its beginnings as a mere laboratory curiosity, combinatorial chemistry has today emerged as a powerful tool for the creation of libraries consisting of chemically diverse compounds. The screening of combinatorially derived libraries can provide biologically active compounds for use as both probes in fundamental research and lead structures in drug development. Traditionally, such lead compounds have either been isolated from natural sources or through organic synthesis of logically designed molecules. However, random natural-product screens rely on serendipity and the rational design of biologically active compounds has proved to be a more arduous task than anticipated. Combinatorial techniques based on organic synthesis are a powerful alternative because large numbers of chemically diverse compounds can be generated in a controllable manner. A recent international conference and workshop* focused on recent progress in the field of combinatorial technology. In this report, we highlight some of the very exciting work presented at this meeting.

The field of combinatorial chemistry initially focused on peptide chemistry. This was due, in part, to the wide variety of biological activities associated with peptides. In addition, the well-developed peptide-protective-group and coupling chemistries, in particular solid-phase protocols, were relatively easily modified for combinatorial purposes. For example, routine solid-phase synthesis of a tetrapeptide library using 20 amino acids by the split-pool protocol yields $20^4=160\ 000$ distinct compounds in a matter of days. However, peptides are rarely membrane permeable and are rapidly degraded by the proteolytic machinery of living cells. A large effort has therefore been directed towards making the vast number of chemical transformations currently employed in organic synthesis more accessible to the combinatorial chemist.

Novel solution- and solid-phase chemistries

In solid-phase approaches, the chemical nature of the linker attaching the molecule to the solid support is crucial. After attachment of the first building block, the linking moiety must be stable to the subsequent synthetic transformations required for library construction. In addition, the final release of the library molecules must be facile and should preferably not leave any undesirable functionality on the target molecules. D. C. Rees (Organon Laboratories, Motherwell, UK) described a linker designed for the synthesis of tertiary amines, which is a pharmacologically interesting class of compounds. The synthetic sequence starts with an acrylate resin and, in three steps, results in a quaternary amine attached to the solid support. The tertiary amine is subsequently released by elimination with weak base, thus regenerating the acrylate resin. The advantage of this strategy is that nonquaternized compounds will remain attached to the solid support, resulting in high purity for the final product even if the overall yield is low. D. S. Brown (Zeneca, Macclesfield, UK) discussed the synthesis of an acid-labile Merrifield α -methoxyphenyl (MAMP) resin and its application in the automated parallel synthesis of libraries. This linker resin appears to be versatile and suitable for a wide variety of chemistries, such as peptide and peptoid synthesis, carbanion chemistry, palladium-catalysed couplings, and the synthesis of heterocyclic compounds.

Compound libraries are predominately prepared either by parallel synthesis or by split-pool combinatorial techniques. Parallel synthesis provides arrays of discrete, spatially segregated compounds. Split-pool synthesis results in combinatorially derived bead pools, in which each bead carries a single compound. Both approaches require optimization and uniform reaction conditions for each chemical transformation in order to make the library synthesis successful and manageable. This challenging task was illustrated by M. Plunkett (Arris Pharmaceuticals, San Francisco, CA, USA) and J. P. Mayer (Amgen, Boulder, CO, USA).

M. Plunkett discussed the solid-phase syntheses of peptide and peptidomimetic libraries. One of these libraries employed 4-ketoproline as a scaffold to which building blocks such as amines, carboxylic acids, anhydrides, isocyanates and sulfonyl chlorides can be attached at different positions. J. P. Mayer shared his results obtained in the development of solid-phase methodology for the synthesis of nitrogen-containing heterocycles such as quinazolinones and benzimidazoles. As both presentations illustrated, seemingly simple and reliable transformations must be carefully optimized in order to obtain uniform conditions that are useable for library synthesis. This optimization is generally carried out by the synthesis of a set of model compounds to evaluate various polymeric supports, linkers, building blocks, reagents, solvents, etc. After each synthetic step, intermediates are isolated and characterized with, for example, high-performance liquid chromatography (HPLC), nuclear magnetic resonance or mass spectrometry (MS). Moreover, to ensure that the overall optimization process is successful, alternative synthetic pathways should already have been considered at the library-planning stage.

Automation

The repetitive nature of combinatorial chemistry and the handling of large numbers of chemicals, reaction vessels, etc. suggest that automation would reduce repetitive manual work and improve throughput in synthesis as well as screening. Using the knowledge gained from peptide- and oligonucleotide-synthesizer development, a considerable number of automated organic synthesizers are now commercially available from different companies. J. M. Cassells (The Technology Partnership, Royston, Herts., UK) described the Myriad system for automated synthesis. This system includes a commercially available 24-reaction-vessel synthesizer suitable for lead-compound optimization and process development as well as a large core system currently under testing. The core system is designed for high-throughput synthesis of large libraries and carries out operations such as reagent handling, incubation and product transfer. Advanced systems like this can be further developed to

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include selection, weighing and preparation of reagents and building blocks as well as on-line analysis.

An obvious advantage of combinatorial chemistry is the rapid generation of information such as the 512 compounds formed in a three-step synthesis with 8 building blocks ($8 \times 8 \times 8 = 512$). An equally obvious disadvantage is that problems are multiplied in the same manner – one mistake in one of the steps yields 64 failures ($1 \times 8 \times 8 = 64$). J. C. Phelan (Arris Pharmaceuticals, San Francisco, CA, USA) pointed out that people are inherently error prone and that automation greatly reduces the number of human errors. He described several approaches to minimize events that result in low-quality libraries and screening data that are difficult to interpret. For example, efficient monitoring during automated library synthesis creates a record of mistakes that do occur. Library utility is thus preserved in spite of mistakes such as a swap of two building blocks.

Parallel library synthesis provides discrete compounds with the identity of a certain library member known during the complete synthetic and screening process. In split-pool synthesis, libraries are generally screened as mixtures, with the identity of an active compound determined by an encoding technique such as chemical tags. Radio-frequency (RF)-encoded synthesis was recently introduced as a technique that combines the advantages of parallel and split-pool synthesis. R. Brown (IRORI, La Jolla, CA, USA) described the use of RF-encoded reactors in library synthesis. Each reactor contains an RF tag with a unique identity and a small amount of resin (35 mg); the reactors are permeable to various reagents and can be used in combination with ordinary chemistry glassware. Each RF tag encodes a specific sequence of chemical steps and the reactors are treated in a split-pool fashion. After each step, a scanning station is used to sort the reactors according to their RF tag so that the chemical history of each reaction vessel can be followed by computer. Thus, each reactor represents only one compound (cf. parallel synthesis) even though the synthesis is carried out according to the split-pool protocol.

Intelligent libraries

The information density of combinatorial approaches requires efficient ways to record, manipulate and interpret large amounts of data. S. Rose (BioFocus, Settingbourne, UK) described computational approaches for the selection of library monomers and the evaluation of screening data. Two- and three-dimensional fingerprints and calculated physicochemical properties can be used to divide a large number of monomers, such as amines, into clusters. Subsequently, small numbers of amines can be selected from these clusters with the aim of maximizing the library diversity. Thus, this intelligent-library approach can expand library diversity without increasing the actual size of the library. Bioassay-screening data can also provide clusters of active compounds in a similar fashion. This information can, in turn, be used in the design and synthesis of second-generation libraries.

Integration with other technologies

It is clear today that combinatorial technologies can be used not only for drug discovery but also for medicinal chemistry, that is, the process of developing a biologically active compound into a functional drug. M. A. Poss (Bristol-Myers Squibb, Princeton, NJ) described efforts to integrate automated synthesis with medicinal chemistry. These efforts were illustrated by a solid-phase protocol developed for the synthesis of angiotensin-II-receptor antagonists. Derivatives of a known antagonist were synthesized in order to obtain new structure-activity data. His results show that reliable combinatorial protocols, preferably in an automated parallel mode, have the potential to accelerate the medicinal-chemistry process in drug development.

M. Elofsson and C. M. Crews focused on integrating combinatorial chemistry and genomics. The human genome has yet to be fully sequenced but it is already clear that a vast number of novel protein families will be discovered. Protein-family members are often closely related in sequence, but their specific function can vary dramatically. Current techniques for determining protein function are inadequate to manage the torrent of information from the genome-

sequencing projects. Novel cell-based assays are currently being developed for screening combinatorial libraries, with the aim of identifying cell-permeating ligands for any given protein. The ultimate goal is to develop an assay that is capable of screening combinatorial compound libraries against cDNA-encoded protein libraries in a multiplex fashion, thus providing a catalogue of ligand-protein pairs. Through derivatization, protein-binding ligands can be used in further studies to determine the function of target proteins.

Characterization and purification

The postconference workshop focused on analytical characterization and purification of compounds. A library synthesized with split-pool techniques is generally not characterized before screening. Instead, the identity and chemical structure of active compounds are usually determined by encoding technologies, the result then being confirmed by resynthesis and rescreening. In parallel synthesis or the synthesis of mixtures of moderate size, there is an option to characterize and purify compounds before biological evaluation. D. B. Kassel (CombiChem, San Diego, CA, USA) described rapid compound characterization and purification HPLC with ultraviolet (UV) and MS detection. For each product of a parallel synthetic strategy, the molecular weight is known; by monitoring the HPLC eluent with MS, the target compound can be identified and isolated in a preparative mode. This approach reduces the number of fractions collected, and the identity of the target compound is verified during the analysis-purification phase. Furthermore, throughput can be greatly increased by reducing the column length and increasing the flow rate: samples of 50 mg can be purified within 10 min using a 50-mm-long column and a flow rate of 50 ml min^{-1} .

Currently, UV-absorbance measurement is the most-general method used for monitoring HPLC eluents. However, many compounds do not contain chromophores, which is a requirement for UV detection, and so other detection systems have been investigated for use with combinatorial approaches. G. D. Dollinger

(Chiron Corp., Emeryville, CA, USA) described the Chemiluminescent Nitrogen Detector as a valuable complement to the UV detector. Although not of universal utility, this nitrogen detector has a potential in pharmaceutical development, because most drugs and biologically interesting molecules contain nitrogen atoms. The detector allows accurate quantification even with limited amounts of impure samples. The analytical system described combines UV and nitrogen detection with MS. A single analytical run thus provides information on sample purity, concentration and identity.

Liquid chromatography is one of the most powerful techniques for sample purification, but it is expensive and throughput is limited, even with highly automated systems. Liquid-liquid extraction, on the other hand, is a fast and simple purification technique. S. Hadida (University of Pittsburgh, PA, USA, currently at CombiChem) gave a talk about the 'fluorous' phase, which has unique properties in comparison with water and organic solvents. For example, a reaction carried out with a fluorous reagent such as the commercially available $(C_6F_{13}CH_2CH_2)_3SnH$ can be purified by extraction with water, an

organic solvent like dichloromethane and perfluorinated hexanes. Inorganic salts will dissolve in the aqueous phase, the product can be isolated from the dichloromethane phase and the fluorous reagent ends up in the fluorous phase; the target compound is obtained in high purity in the organic phase. This liquid-liquid-extraction protocol has been further developed into a solid-phase-extraction method using fluorous silica gel.

Summary

The conference made it clear that combinatorial chemistry now has become an established subdivision of organic chemistry. The pharmaceutical industry has recognized the potential of combinatorial libraries in the search for lead compounds. In addition, it is now obvious that combinatorial methods have great potential in medicinal chemistry. As a consequence, large efforts are being made to adopt the large number of transformations employed in organic chemistry for combinatorial purposes. Several presentations also indicated that parallel synthesis of large numbers of discrete compounds is emerging as a complement to split-pool synthesis of compound mixtures. Furthermore, the nature of

combinatorial chemistry has spurred the development of fully automated systems capable of carrying out a majority of the operations required in parallel or split-pool library synthesis. The advances made in high compound throughput have, in turn, driven the development of new strategies for compound characterization and purification. The post-conference workshop focused on several of these techniques for the analysis and purification of libraries. Automated systems for rapid characterization and purification are now available, and it is obvious that the recent developments have resulted in systems that can cope with the number of compounds prepared in high-throughput synthesis. In summary, the conference and workshop created an interactive forum to discuss current issues in depth. Furthermore, the speakers were generous in sharing their results and experiences in detail, which resulted in a creative and inspiring atmosphere.

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Dielectric estimation of microbial biomass using the Aber Instruments Biomass Monitor

The recent article by Olsson and Nielsen, *On-line and in situ monitoring of biomass in submerged cultivations*¹, contained a number of inaccuracies regarding both the dielectric method for measuring cellular biomass on-line and *in situ*, and its exploitation in the Aber Instruments Biomass Monitor that we would like to correct.

The glossary states that the 'dielectrical [sic] permittivity consists of the capacitance (the ability to store electrical charges) and conductance (the ability to conduct electrical charges)

of the subject'. It does not. The dielectric permittivity is the capacitance normalized to take into account the geometry of the electrodes. Permittivity does not have a conductivity term, and conductivity is the conductance normalized to take into account the geometry of the electrodes. To understand the basis of the method, it is useful to recognize the relevant units, which are for capacitance Farads (usually pF), for conductance Siemens (usually mS) and for conductivity $S\ m^{-1}$. Permittivity is dimensionless.

Many reviews and books describe this, including those aimed at biologists (e.g. Refs 2-4). This incorrect definition of the dielectric permittivity inevitably means that most of the basis of the dielectric method is simply misrepresented. In addition, Olsson and Nielsen refer readers to Matanguihan *et al.*⁵, which is a poor choice for a discussion of biological dielectrics as its abstract, which is what most people will read, also conflates capacitance and permittivity.

Many groups⁶⁻¹¹ have published work on the use of the Aber Instruments Biomass Monitor, which exploits the dielectric/capacitance method via the β -dielectric dispersion for on-line and real-time measurement of biomass. It is not true that 'the effect of the medium conductance has to be calibrated before the cell concentration can be determined'. The effect of conductivity is entirely well understood (and

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