Future combinatorial strategies for chemistry and biology

Terry Hart, Novartis Institute for Medical Sciences, 5 Gower Place, London, UK WC1 6BN, tel: +44 207 928 9292, e-mail: terry.hart@pharma.novartis.com

The third *Combinatorial Approaches to Chemistry and Biology III* meeting (17–18 July 2001, Cambridge, UK) was attended by 180 delegates and by several companies exhibiting their latest product ranges of building blocks and equipment, mainly for use in combinatorial chemistry.

Protease inhibitors

The meeting opened with a plenary presentation from Jonathan Ellman (University of California, Berkeley, CA, USA) on 'Combinatorial approaches to inhibitor design and catalyst development'. A key feature of his talk was the new combinatorial method using fluorogenic positional scanning libraries to gain insights into protease subsite specificity[1]. He highlighted the pressing need to make selective, cell-permeable protease inhibitors to determine the function of the 1227 mammalian proteases that are predicted to account for 4% of the human genome.

Ellman reviewed his work on inhibitors of Cathepsin D (a typical aspartyl protease), which might be responsible for tau proteolysis and contribute to Alzheimer's disease[2]. The design of competitive, reversible inhibitors of cysteinyl proteases, such as cruzipain (thought to be essential for replication of the organism responsible for Chagas disease), with good pharmacokinetic profiles is much more scientifically challenging, and Ellman briefly described the development of libraries of such inhibitors. The essential ketone-binding motif, which reacts reversibly to the enzyme catalytic sulfhydryl centre, was attached to the resin via a readily cleavable hydrazone linkage. In the second part of his presentation, he described some recent work on library-enabling methodology using olefin metathesis, and carbon monoxide insertion using both ruthenium chemistry[3] and concomitant annulation chemistry[4].

Library design and synthesis

Bob Boyle (Millennium Pharmaceuticals, Cambridge, UK) discussed mixed approaches towards the synthesis of drug-like libraries using benzodiazepine-2,5-dione and dihydro-2*H*-isoquinolinone scaffolds. One thousand compound libraries of benzodiazepine-2,5-dione were prepared using the Ugi reaction in conjunction with a variety of scavenger resins. Modifying the procedure by attaching the isonitrile component to a resin via a cyclohexenyl skeleton improved the yields considerably, even with unreactive aromatic aldehydes. He also described an expedient method for oxadiazole formation using microwave technology and flash tubes. These were used both as the reaction container and for the subsequent purification to generate the desired products in isolated yields of 60%.

Neil Thomas (University of Nottingham, Nottingham, UK) described how a phage-display library of 16-residue proteins designed to mimic a β-hairpin motif can select for duplex DNA motifs and so find molecules that either have the potential to control gene expression or to combine with specific nucleotide sequences.

An extremely entertaining lecture entitled 'Never enough data' was given by Brian Warrington (GSK, Harlow, UK), who discussed the philosophical meaning of chemical diversity and how this impacted upon library design. Perhaps in agreement with many chemists in industry who now build libraries for hit generation, his conclusion was that, like many things in life, they should be done little but often.

It is also a fact of life that, the poorer the experimental design, the more difficult it is to analyze the data produced and the more worthless it becomes. Sean Rigby (University of Bath, Bath, UK) gave a detailed and somewhat abstract talk on the comparative merits of genetic algorithms, neural networks and simulated annealing in experimental design, especially with respect to the problems involved in designing new catalysts by combinatorial methods.

Solid-phase chemistry

Solid-supported reagents combined with scavenger resins were proposed by Steve Ley (University of Cambridge, Cambridge, UK) as a means of achieving high-yielding, complex, multi-step syntheses without the need for purifying intermediates. Microwave chemistry also featured highly in many of his syntheses and Sildenafil provided a powerful illustration of how combining these three methodologies could be used to synthesize a highly substituted drug[5]. Ley predicted that future areas for research would include new solid-supported reagents, possibly incorporated as stirrer bars, more robust beads and higher loading scavenger resins, possibly based upon dendridized silica gel.

1359-6446/01/$ – see front matter PII: S1359-6446(01)01972-9

Microwave chemistry featured again in an authoritative description by Chris Brain (Novartis Institute for Medical Sciences, London, UK) of the synthesis of heterocycles, such as oxazoles and oxadiazoles, using polymer-supported reagents. He also highlighted the need for workers in the field to start using specialized microwave-focused beam monocavity apparatus, not only for safety reasons, but also to obtain repeatable and publishable results[6–8].

Klaus Haaf (Aventis Crop Science, Parsippany, NJ, USA) described the development of a reliable solid-phase method using palladium-catalyzed [Pd(0)] chemistry for the solid-supported synthesis of phosphinates. This methodology neatly overcomes the difficulty of purifying the final diacid phosphinate products.

Parallel synthesis

An interesting case study in parallel synthesis was given by Brian Maloney (Evotec, Hamburg, Germany), who outlined the successful working collaboration between a large multinational (DuPont Agriculture, Wilmington, DE, USA) and a service company (Evotec) to provide libraries for screening. The problems of target confidentiality and feedback of biological data to the service company were not perceived to be a problem by either company and, apparently, contributed to the success of this collaboration. Large compound-libraries based upon Suzuki reactions[9] and nucleophilic substitutions of the 4,6-dichloro-5-nitropyrimidine scaffold were presented.

In a presentation entitled 'A unified approach to parallel synthesis and purification', Ben Moshiri (Mettler-Toledo-Boham, Royston, UK) described the versatility of the MiniBlock™ synthesizer, which had been developed in conjunction with Bristol-Myers Squibb (New York, NY, USA). Moshiri focused on its ability to perform a variety of solution- and solid-phase chemistries, and solid-phase and solid–liquid extraction purification in parallel.

Mark Gardener (Pfizer, Sandwich, UK) discussed the discovery of leads through an integrated approach to parallel synthesis. During their first four years, the Pfizer hit-generation group used mix-and-split chemistry to generate libraries for screening but, because of the screening chaos these mixtures caused, they now, like most of the pharmaceutical industry, tend to make and screen single compounds. He exemplified the effectiveness of this new approach with the design of inhibitors of an unspecified kinase. A 625-component library of drug-like, substituted pyrimidines was prepared using 'rule-of-five' selection criteria and polar surface-area considerations. After bioassay, three weakly active molecules (~10 μM) were detected. Resynthesis of a similar library, but based around the new lead motif, produced drug-like compounds with a tenfold increase in activity.

Claire Newton (Millennium Pharmaceuticals, Cambridge, UK) described parallel-processing methods, using a Tecan™ robot for identifying the best process for the Suzuki coupling of an indene boronic acid with an aryl bromide[10]. Rapid evaluation of a variety of conditions using different palladium complexes resulted in 25-times less catalyst being required. In a similar way, an alternative zirconium catalyst for the polymerization of ethylene was quickly identified.

Other highlights

David Embiata-Smith (GSK, Stevenage, UK) gave a detailed description of a process-chemistry case history in which high-throughput experimentation (HTE), together with multivariate data analysis, was used to rapidly optimize a variety of multicomponent reactions, including Wittig and Sonogashira reactions. The way that reactions moved quickly from the bench onto the kiloscale was extremely impressive. There followed an overview of the lead-finding process at GSK by David Hunter (GSK, Harlow, UK). In their 'Compound Factory', they have successfully integrated automated-synthesizers, work-up stations, evaporators and analytical and purification systems. He emphasized the importance of screening single compounds and the need to design fewer libraries, but which contain thousands of molecules, specifically targeted towards particular biologically important motifs, such as G-protein-coupled receptors (GPCRs), ion channels and nuclear receptors. The aim is to provide chemists in the lead-optimization phase with high-quality lead molecules as well as some associated SAR data. The challenge for library designers now, is to decide which drug-like compounds of those that can be made need to be synthesized.

A robust, user-friendly system called AutoChem, was described by Ruben Tomassi (Novartis, Summit, NJ, USA), which can automate solution-phase organic reactions and purify mixtures by reverse-phase HPLC – ideal for chemists who want to explore SARs rapidly. The system, which is based on equipment developed by Gilson (Middleton, WI, USA), costs ~US$77,000 to build, is capable of temperature control (from –25 to +100 °C) and can use both resin-bound reagents and on-line solid-phase extraction (SPE). Its efficiency was exemplified by the synthesis of a wide range of reactions, including reductive aminations, acylations and sulfonylations, and various palladium-mediated reactions such as Suzuki couplings, Mitsunobu reactions and deallylations. In general, AutoChem can routinely prepare and purify 48 compounds per day, ranging from 7 to 40 mg of product.

A charismatic and challenging presentation on the latest advances in microreactor chemistries was given by Steve Haswell (University of Hull, Hull, UK), who illustrated the rapid advances in this technology. He described microreactors made of borosilicate glass, with a network of channels in the 50–300 μm range, and electric fields used to control the flow of reagents through the system. It was evident that a wide range of

chemistries can now be used for microreactors[11,12]. The realistic possibility that this technology can be used in a continuous-flow process for plant-scale synthesis probably came as a shock to most of the audience, who, like myself had previously only considered this useful for miniature reactions. Apparently, on the picolitre scale, a flow of reagents of 2 ml min^{-1} at 0.1 M concentration will produce 1–2 tons of material per year!

Tony Czarnik (Sensors for Medicine and Science, Germantown, MD, USA) gave an overview, in his inimitable style, of the advantages of radiofrequency tags for communicating information in a non-destructive way, and how they can be used in conjunction with implanted microfluorimeters as sensors to monitor fluctuating glucose levels in type 1 diabetic patients.

A rather complex talk was given by Hicham Fenniri (Purdue University, West Lafayette, IN, USA) on dual-recursive deconvolution (DRED), a spectroscopic method for screening large, resin-supported compounds. The concept is based on the observation that polymeric resin beads can be 'barcoded' by varying the amount and type of monomer present before polymerization. The subsequent decoding of the bead, which would reveal the identity of the attached compound, can then be performed using near-IR and Raman spectroscopy and standard laser-based barcode readers[13–16].

Conclusion

It is evident that a combination of powerful, cutting-edge technologies, such as microwave technology with polymer-supported reagents and palladium catalysts, will rapidly change laboratory chemistry. In the case of microreactors, this might well have an effect on process- and plant-scale chemistry. In terms of library design, the current trend within the industry appears to be the need to make smaller, targeted libraries (1000s) of drug-like molecules as singles for hit generation, and even smaller, iterative libraries for lead explosion (100s).

It is now approximately 10 years since the concept of combinatorial chemistry or parallel processing first entered the pharmaceutical arena. Judging from the number of library hits that are now in the optimization phase, and allowing for the time lag in publishing commercially sensitive preclinical research, we can realistically expect to hear the first, full case-histories of 'from library hit to clinic' to enter the public domain in the near future.

References

1 Harris, J.L. *et al.* (2000) Rapid and general profiling of protease specificity by using combinatorial fluorogenic substrate libraries. *Proc. Natl. Acad. Sci. U. S. A.* 97, 7754–7759

2 Bi, X. *et al.* (2000) Novel Cathepsin D inhibitors block the formation of hyperphosphorylated tau fragments in hippocampus *J. Neurochem.* 74, 1469–1477

3 Szewczyk, J.W. *et al.* (2001) A Mass spectrometric labeling strategy for high-throughput reaction evaluation and optimization: Exploring C-H activation *Angew. Chem., Int. Ed. Engl.* 40, 216–219

4 Tan, K.L. (2001) Annulation of alkenyl-substituted heterocycles via rhodium-catalyzed intramolecular C-H activated coupling reactions. *J. Am. Chem. Soc.* 123, 2685–2686

5 Baxendale, I.R. and Ley, S.V. (2000) Polymer-supported reagents for multi-step organic synthesis: application to the synthesis of sildenafil. *Bioorg. Med. Chem Lett.* 10, 1983–1986

6 Larhed, M. and Hallberg, A. (2001) Microwave-assisted high-speed chemistry: a new technology in drug discovery. *Drug Discov. Today* 6, 406–416

7 Edwards, P. (2001) More microwave reactors required. *Drug Discov. Today* 6, 614

8 Brain, C.T. (2001) Are microwave instruments just expensive hot-plate stirrers? *Drug Discov. Today* 6, 663–664

9 Havelkova, M. (1999) The Suzuki–Miyaura cross coupling reactions of 6-halopurines with boronic acids leading to 6-aryl and 6-alkenylpurines. *Syn. Lett.* 7, 1145–1147

10 van Beek, J.A.M. *et al.* (1997) Indenyl compounds and catalyst components for the polymerization of olefine. US5,646,322

11 Haswell, S.J. *et al.* (2001) The application of micro reactors to synthetic chemistry. *J. Chem. Soc. Ser. Chem. Commun.* 391–398

12 Watts, P. *et al.* (2001) The synthesis of peptides using micro reactors. *J. Chem. Soc. Ser. Chem. Commun.* 991–998

13 Fenniri, H. *et al.* Barcoded resins: A new concept for polymer-supported combinatorial library self-deconvolution. *J. Am. Chem. Soc.* (in press)

14 Ghose, A.K. *et al.* (1999) A knowledge-based approach in designing combinatorial or medicinal chemistry libraries for drug discovery. 1. A qualitative and quantitative characterization of known drug databases. *J. Combi. Chem.* 1, 55–68

15 Fenniri, H. *et al.* (2001) Towards the DRED of resin-supported combinatorial libraries: A non-invasive methodology based on bead self-encoding and multispectral imaging. *Angew. Chem., Int. Ed. Engl.* 39, 4483–4485

16 Lawrence, R.N. (2001) Recursive deconvolution for real-time lead identification. *Drug Discov. Today* 6 (Suppl.) S2–S3