Microtechnologies and miniaturization

Modern microtechnologies and miniaturization approaches are enabling new advancements in automated and miniaturized combinatorial synthesis and high-throughput screening. The excellent IBC conference entitled *Microtechnologies and Miniaturisation: Tools, Techniques and Novel Applications for the Pharmaceutical Industry*, held in Berlin in December 1997, provided an overview on the latest developments in the field of theoretical and practical applications for miniaturization in chemistry and biology. The conference speakers focused on three major areas:

- Screening, detection and assay miniaturization.
- Genomic applications and technologies.
- Synthesis.

High-density microtitreplate format

Dr Kevin Oldenbourg (Du Pont Merck, Wilmington, DE, USA) presented the state of the art in the miniaturization of microtitre-plate-based assays. A specially designed 9,600-well microtitreplate with a cavity volume of 0.2 µl can be used, for example, in immunoassays with soluble enzymes. It was shown that this format offers savings in reagents, enzymes and time. The 9,600-well plate requires new methods for liquid handling and detection; these were developed in collaboration with Imaging Research (St Catherine's, Canada) and Bio Dot (Irvine, CA, USA).

Microstructuring of polymers and glass

Dr Wolfgang Ehrfeld (Institute for Microtechniques, Mainz, Germany) described recent developments of the LIGA technique. LIGA, a combination of deep lithography, microelectroforming and moulding processes, enables the microstructuring of polymers. Such materials have wide applications including microfabricated microfilters, micromembranes, self-filling micromembrane pumps, microextraction modules, tips for scanning-near-field microscopy and 3D microreactors

Miniaturized analysis

Dr Michael Knapp (Caliper, Palo Alto, CA, USA) presented Caliper's technology combining micromachining with active microfluidics for miniaturized, integrated biochemical and cellular analysis. In substrates made of glass or plastic, interconnected channel systems are created with dimensions of approximately 50 µm wide by 10 µm deep. Electrokinetic actuation of fluid and material movement within the custom-designed channel network is achieved by introducing electric fields at each channel terminus. The nature and magnitude of the electric field is determined by custom electronics and computer control. A gating fluidic logic is created by simultaneous determination of fluid or material flow coming from the different parts of the chip, allowing sub-nanolitre control with great precision accomplished without moving parts. Molecular partition can be achieved in the channel structures, using strategies borrowed from capillary electrophoresis. In this way, it is possible to emulate all components of bench-scale experiments including biochemical conversions, separations and detection. Ultra-high throughput versions of these elements allow for massive information generation using very small amounts of material. Data quality is so high that it is possible to envisage creating databases of biochemical data that are conventionally difficult to perform and reproduce.

Piezoelectronic dispensing and DNA chips

As an example of miniaturisation in genomic applications and technologies scientists from the Max Planck Institute for Molecular Genetics (Berlin, Germany) presented the world-wide first 2D piezoelectronic dispensing module for biotechnological applications. At present, the module consists of 16 individually addressable dispensers and allows the rapid aspiration and dispensing of 100 pl volumes into all microtitreplate formats with a 4.5 mm well pitch. This piezoelectronic technology is mainly used to produce DNA arrays with a density of more than 2,500 clones cm^{-2}. Specific results on oligonucleotide-fingerprinting assays and expression-analysis experiments on microdispensed DNA arrays were shown. Another application of the multihead piezo-inkjet dispenser is to equip targets for MALDI-MS with 1,500 digested proteins for fast peptide mapping. The data shown demonstrated that the spectra obtained from 100 pl microdispensed spots are identical to conventional MALDI target loading procedures obtained by manual loading of liquids in the µl range.

Dr Ulrich Certa (Hoffmann-La Roche, Basel, Switzerland) illustrated the use of Affymetrix light-directed oligonucleotide synthesis on silicon chip technology for the quantification of mRNAs on different bacterial transcripts. In addition to this technique, in which 1,500 genes can be monitored in parallel, results obtained with plasmid chip arrays (PCA) were shown. This fast technique allows differential library screening and the analysis of subtractive hybridization experiments without any PCR steps.

Enhancing PCR

Dr Michael Steinwand (Perkin Elmer Applied Biosystems, Bodenseewerk Perkin-Elmer, Überlingen, Germany) discussed the possibility of making PCR faster and more robust, at the same time increasing throughput by using higher reaction density and lowering costs by reducing reagent consumption. The practical work described in the presentation included the adaptation of the TaqMan™ fluorescent-detection chemistry to miniaturized formats and a peltier-driven miniaturized thermocycler integrated within a CCD-based detection system [as described by Taylor *et al.*

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(1997) *Nucleic Acids Res.* 25, 3164–3168].

The combination of these two technologies allows PCR reactions to be observed and optimized in real time within various microstructured glass, silicon or plastic reaction vessels. Each material shows a different influence on the performance of the PCR process in terms of thermal behaviour or surface effects. Finally, a procedure was proposed for parallel sequence-specific detection by PCR. A chip containing 48 microreaction chambers connected by a microstructured manifold is preloaded with the reagents and then filled in one shot with the sample. Positive wells (containing the sequence-specific primer) emit fluorescence and, hence, can be discriminated from wells without any signal.

Miniaturized analytical thermocyler

Allen Northrup of Cepheid (Santa Clara, CA, USA) presented a battery-driven, miniaturized thermocycler: the Miniature Analytical Thermal Cycler Instrument (MATCI). Northrup demonstrated the analytical potential of this instrument with quantitative detection of *Salmonella* and other bacteria, including an extraordinary detection accuracy across ten different strains, within 30 min.

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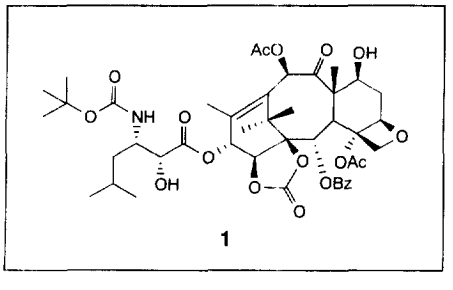
Drug discovery at IKCOC-7

The 7th International Kyoto Conference on New Aspects of Organic Chemistry (IKCOC-7) was held on 10–14 November 1997 and was attended by over 900 delegates. This brief report highlights work relevant to drug discovery that was presented at the meeting. Professor Iwao Ojima (Stony Brook State University of New York, NY, USA) presented his work on taxoid antitumor agents. A problem with taxol and taxotere therapy is multidrug resistance (MDR) caused by overproduction of P-glycoprotein. The Ojima group has identified a new taxoid (**1**, SBT101131) that is two orders of magnitude more active than taxol and taxotere in an apoptosis assay against MDR cells. This compound has been selected for development.

Ojima and coworkers have identified a common pharmacophore for taxol (**2**), the epothilones (**3**) and discodermolide (**4**). These three compounds appear to bind competitively to the same β-tubulin site. According to this pharmacophore, the thiazole group of the epothilones overlays with the benzamide of taxol, while the 16-membered ring of the epothilone overlays with the south and southwestern parts of taxol, which is in a conformation where the phenyl rings of the benzoyl group and the β-amino acid side-chain are relatively close together. This analysis may lead to the design of novel taxol and epothilone derivatives.



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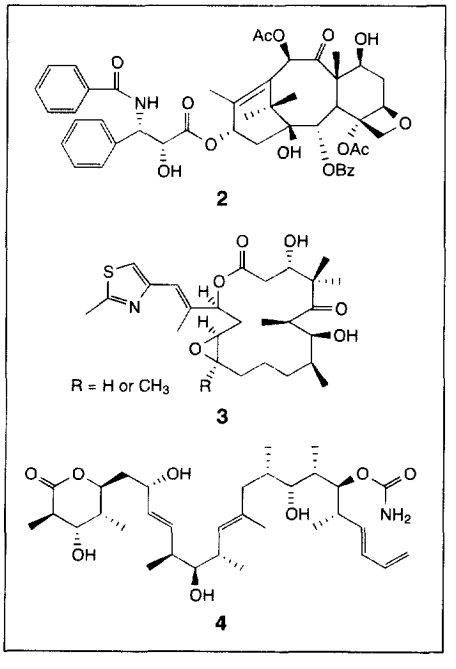

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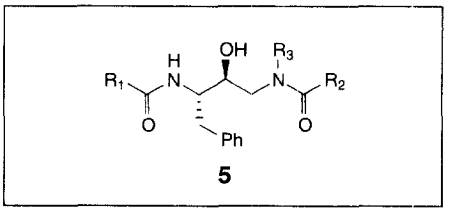

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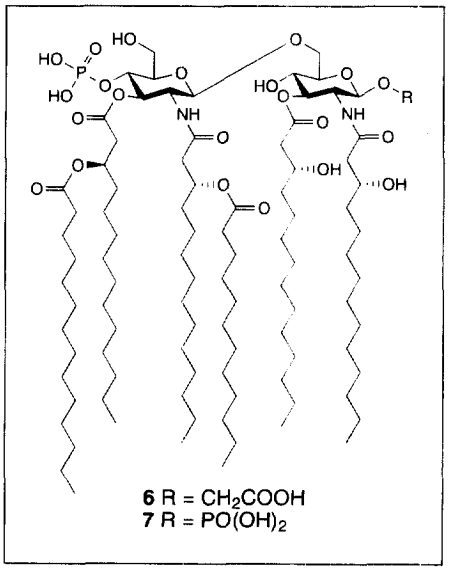

Combinatorial chemistry

As part of the conference, there was a mini-symposium on combinatorial

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