reliable method of delivering proteins into cells could therefore lead to the development of highly specific drugs. Dowdy's group has already demonstrated several possible therapeutic applications *in vitro*. They fused caspase-3, an apoptosis promoter, to the PTD and replaced the endogenous caspase cleavage sites with sites specifically cleaved by the HIV protease. When this was added to media containing Jurkat T cells, some of which had been previously infected by the HIV virus, only the infected cells were killed [Vocero-Akbani, A.M. *et al.* (1999) *Nat. Med.* 5, 29–33]. It should therefore be possible to generate similar protein chimeras that would be effective against other pathogens that are dependent on specific proteases. A similar technique has been used to selectively kill cancer cells. Transducing a PTD-linked tumour suppressor protein into cultured tumour cells induced cell-cycle arrest followed by apoptosis. Although cell-cycle arrest was also induced in normal cells transduced by the same tumour suppressor protein, these cells did not die.

Many questions must be answered before protein drugs based on this technology can reach the clinic. It is possible that the PTD might be immunogenic, although Dowdy believes that any such problem could be overcome. 'We are now working with synthetic PTDs. Each of these sequences is more efficient than the HIV PTD. It is very unlikely that all possible synthetic transduction domains will induce an immune response.' Although it could be very difficult to target a particular cell type using this technique, it is likely to enable the application of protein therapy to human disease.

Clare Sansom

Combinatorial approaches to chemistry and biology

The second Royal Society of Chemistry–BMCS Conference on *Combinatorial Approaches to Chemistry and Biology* took place at Churchill College (Cambridge, UK) on 30 June–1 July 1999. This international meeting of 250 participants was heavily oversubscribed, mainly because generous industrial support had enabled the organizers to assemble 17 multidisciplinary speakers, each experts in their respective combinatorial fields. A broad range of sciences that now involve combinatorial approaches were represented. Several recurrent themes also emerged that highlighted recent developments in the field, including knowledge-based library design, polymer-supported reagents (PSRs) and catalysts, and new methods for carbon–carbon bond formation.

Polymer supporting reagents

The impact of PSRs (e.g. chemical reagents such as oxidants, reducing or dehydrating agents and organopalladium catalysts, which are attached to a polymer and are not free in solution) in synthetic organic chemistry is already very notable, and it is clear that this is just the beginning of their application in solution-phase parallel synthesis. Graham Showell (Cambridge Combinatoria, Cambridge, UK) reviewed the status of PSRs in combinatorial chemistry using, for example, the impressive solution synthesis of epibatidine devised by Steven Ley's group (Cambridge University, UK). Epibatidine is an unusual pyridine derivative that was only discovered in 1992 on the skin of poisonous frogs and is of great interest because it is >200-fold more active than morphine as an analgesic [Habermann, J., Ley, S.V. and Scott, J.S. (1999) *J. Chem. Soc., Perkin Trans. 1* 10, 1253–1256]. David Drewry (GlaxoWellcome, Research Triangle Park, NC, USA) then described the PSR work of GlaxoWellcome's combinatorial technology team. Subsequently, Michael South (Searle Discovery Research, St Louis, MO, USA) presented the elegant work of Monsanto, who are developing multistage reactions that incorporate the versatility of PSR in many of its different guises (e.g. as reactants, reagent scavengers and as by-product traps). These three lectures emphasized the commonly accepted advantages of PSR in the synthesis of smaller libraries (<2000 components) such as the ease of chemistry validation, analysis, purification and scale-up. One of the major future objectives will be to increase the versatility and loading of these reagents, while decreasing their cost, so that they can be used more effectively in the multistage synthesis of drug-like molecules.

New technologies

Chris Abell (Cambridge University, Cambridge, UK) described the use of Atomic Force Microscopy to determine ligand-binding from libraries. Meanwhile, James McCann (Cambridge

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Sensors, Cambridge, UK) outlined the current impact of microfluidics on genomic analysis and biosensors, and the inevitable impact it will have on high-throughput screening and automated, continuous flow-chemical synthesis, a theme later mentioned by Brian Warrington (SmithKline Beecham, Harlow, UK) during his 'state-of-the-science' closing lecture. In particular, McCann described how miniaturized chemical reactors can be easily printed onto plastic chips, and emphasized that the two important advantages of this proprietary approach are the low set-up costs and rapid circuit-design turn-around.

The latest issues encountered in the development and analysis of hits arising from larger split-mix libraries (>2000) were addressed by Stephen McKeown (GlaxoWellcome, Stevenage, UK) and Robin Carr (GlaxoWellcome). They are currently developing several novel combined encoding and HPLC–MS methodologies to identify screening hits and highlighted the need for higher loading and improved quality of linkers and resins. To overcome these problems, Wolfgang Brill (Novartis) reviewed the latest developments using Crowns (Chiron, Mimotopes, Australia) and high-loading beads for parallel synthesis of single compounds, and demonstrated how their manipulations could be automated to provide useful lead-finding libraries.

Novel catalysts

Shu Kobayashi (University of Tokyo, Tokyo, Japan) stressed that the major advantage of combinatorial chemistry for the organic chemist in drug discovery synthesis was increased efficiency [Kobayashi, S. *et al.* (1999) *Chem. Soc. Rev.* 28, 1–16]. In his opinion, because of conflicting scientific and commercial demands, there was currently little synergy between the chemistry required for natural product synthesis and for compound library synthesis. As an example, Kobayashi identified the need to develop new, robust carbon–carbon bond-forming chemistries suitable for library synthesis, and then developed the results of this concept using polymer-supported thiosilylenolate esters.

Of particular note was the reaction with substituted hydrazones in a three-component coupling procedure using the novel catalyst, microencapsulated (PS-Mc) scandium triflate, which produced a high yield of the corresponding cyclic pyrazolones. The great attraction of the PS-Mc technique is that the catalyst can be easily removed and recycled without loss of activity. The use of catalytic quantities of PS-Mc osmium tetroxide produced repeatedly high yields of diols even after using the catalyst five times.

The general utility of hydrazide linkers was again highlighted by Stephan Brase (Institute for Organic Chemistry, Aachen, Germany) with his work on the development of traceless linkers in a salt-free Heck reaction [Brase, S. *et al.* (1999) *Angew. Chem., Int. Ed. Engl.* 38, 1071–1073; Brase, S. *et al.* (1998) *Angew. Chem., Int. Ed.* 110, 3614–3616]. The yields were variable, but the purity of the products was high (>95%) and, most interestingly, amino acids could be connected to the hydrazide linker by the N-terminus and then further manipulated without racemization at the α-carbon.

Amir Hoveyda (Boston College, MA, USA) presented work on designing new catalytic reactions with reference to an asymmetric Strecker synthesis [Krueger, C.A. *et al.* (1999) *J. Am. Chem. Soc.* 121, 4284–4285]. The catalyst, a chiral Schiffs base, appears to generate high enantiomeric excess values (>95%) through the formation of a reactive complex with the low levels of hydrogen cyanide, which was conveniently generated *in situ* from the slow degradation of trimethylsilyl cyanide. Using a similar compound mining approach, Tim Powers (Simyx, Santa Clara, CA, USA) described the design and use of polymer-supported palladium and nickel libraries to identify better homogenous Ziegler–Natta-type catalysts, which are widely used by the industry to polymerize ethylene and propylene.

Knowledge-based libraries

A contemporary combinatorial theme in combinatorial chemistry is the design of smaller, knowledge-based libraries with a high information content. The aim is to use *a priori* knowledge to design libraries that will quickly reveal the structure–activity relationships of, for example, compounds that bind to a biological protein or even act as efficient catalysts for a new reaction.

Following on from his published combinatorial work on the synthesis of transition-state inhibitors of aspartyl proteases, Jonathan Ellman (UC Berkeley, CA, USA) outlined the use of knowledge-based library design in the design mechanism of base inhibitors of cysteinyl proteases. This philosophy was generally supported by David Hollinshead (AstraZeneca, Alderley Park, Macclesfield, UK) who reviewed the impact of combinatorial chemistry in the lead optimization phase of a variety of medicinal chemistry programmes at AstraZeneca. It is noteworthy that one of their current main objectives is to synthesize single compounds, each weighing 100 mg, in libraries of 50–100 compounds. After the early 1990's fevered rush to generate vast compound libraries that often delivered little useful information, this minimalist approach to lead optimization now appears to be the current industry trend. Perhaps this reflects a cost-effective balance between knowledge-based library design, the efficiency of current synthetic technology and information turn-around time.

Conclusion

Two clear messages emerged from this meeting. Firstly, the definition of the term 'combinatorial' has now widened

to include a diverse range of chemical and biochemical protocols, most of which seek to maximize both the rate of empirical information obtained and its associated value. Secondly, combinatorial methodology is now an accepted procedure that, through careful design, has now been successfully used in a variety of applications. Furthermore, through the development of new reagents and techniques, combinatorial methodology will play an increasingly important role in most scientific research programmes.

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Plastics in cancer therapy

Reducing the side effect profile and increasing the efficacy of anti-cancer therapies might be possible through attachment of anti-tumour agents to bulky polymer molecules, according to Ruth Duncan (School of Pharmacy, University of London, UK) speaking at the Royal Society of Chemistry's annual conference in Edinburgh (UK) on 10 September 1999. The idea behind this approach is to improve tumour targeting and reduce accumulation of the agents in healthy tissue.

Polymers and plastics have historically been reserved for medical appliances such as replacement hip joints and heart valves rather than used for pharmacology. However, as Duncan explained, water-soluble polymer–drug conjugates have an interesting property in that, after intravenous injection, they cannot cross the cell membranes of healthy tissues. This is because the tight endothelial gap junctions of normal blood vessels inhibit their entry. However, the newly formed blood vessels that supply tumour cells are much 'looser' in architecture so that bulky macromolecules, such as polymers, can cross the membranes into the tumour tissue. These 'leaky' blood vessels therefore provide a gateway for selective delivery.

Current approaches

The administration of chemotherapeutic agents without selective targeting wastes much of the drug and can cause cytotoxic damage to healthy tissues leading to the well-known symptoms of chemotherapy, such as nausea, diarrhoea and hair loss. Duncan also highlighted that secondary (metastatic) tumours that have spread from an original site of disease are notoriously difficult to locate and target using conventional agents. She believes the way to overcome these problems is to use an appropriate targeting mechanism.

Much effort has focused on a 'magic bullet' for tumour targeting, in particular, ones based on antibody technology and liposomes. However, these early approaches have had their own problems such as immunogenicity, poor stability or being difficult to synthesize. 'The design of tailor-made polymer conjugates allows the sophisticated use of chemistry to provide a synthetic approach that can overcome many of the early difficulties as well as being an inexpensive and industrially practical approach', says Duncan.

Polymer–drug conjugates

The idea of using polymeric macromolecules for carrying a drug directly to a tumour was first proposed in the mid-1970s by the polymer chemist Helmut Ringsdorf (University of Mainz, Germany) with whom Duncan collaborated during her PhD [Ringsdorf, H. (1975) *J. Polym. Sci., Polym. Symp.* 51, 135–153]. Duncan and her colleagues at the Centre for Polymer Therapeutics (London, UK) are now investigating several polymer–drug conjugates using anticancer agents such as doxorubicin and cisplatin [Duncan, R. *et al.* (1999) *Eur. J. Cancer* 35, 994–1002]. Preliminary clinical trials have so far produced promising results [Cassidy, J. *et al.* (1999) *Clin. Cancer Res.* 5, 83–94].

Water-soluble synthetic polymer conjugates based on *N*-(2-hydroxypropyl)-methacrylamide (HPMA) copolymers developed in collaboration with the Institute of Macromolecular Chemistry (Prague, Czech Republic) were the first to enter clinical trials. One such agent, PK1 (Fig. 1), is an HPMA–copolymer conjugate of doxorubicin with a peptidyl spacer cleavable by intracellular lysosomal cysteine protease enzymes, which are found at high levels in certain tumour types associated with poor prognosis such as colorectal, lung, breast and refractory cancers. After intravenous injection, PK1 selectively accumulates in tumour tissue because of the increased vascular permeability of this tissue. After internalization, the drug is cleaved from its polymer carrier inside the lysosomal compartment and is distributed within the tumour cells destroying them. Duncan highlighted that careful design of the spacer linking the cytotoxic agent to the polymer is vitally important as to minimize the exposure of healthy tissue to the drug, the linker must only be cleaved within the tumour.

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