Table 2. Platform technologies for discovering anti-infectives

Company	Technology
GPC	Pathogen DNA database
GTC	Pathogen DNA database, proteomics and gene chips
MedImmune	Vaccine for uropathic *Escherichia coli*
Microcide	Targeted genomics
Peptide Therapeutics	Bacterial protease inhibitors
RiboGene	Translation factors

View, CA, USA) discussed his company's development of protegrin IB367, a synthetic protegrin analogue containing 17 amino acids with a broad spectrum of activity against gram-positive and gram-negative bacteria and yeasts. The protegrins are limited to topical use because of systemic toxicity and are not absorbed by topical or oral application. Protegrin IB367 is being developed both as a topical treatment for oral mucositis in cancer patients and as an aerosol formulation for the treatment of respiratory infections in cystic fibrosis patients. Phase I oral mucositis trials have shown the drug to be safe and to decrease counts of oral bacteria. This drug, however, requires six times-a-day dosing. A double-blind, placebo-controlled Phase II trial is currently in progress and results should be available sometime this year. Meanwhile, an aerosol formulation has already been shown to be safe in Phase I studies in cystic fibrosis patients.

Jon de la Harpe (AMBI Inc., Tarrytown, NY, USA) spoke on peptide antimicrobials for multidrug-resistant bacteria. NISIN, a 35 amino acid-bacterial peptide used as a food preservative, has now been formulated for the treatment of colitis caused by *Clostridium difficile*. Another bacterial protein, lysostaphin, has been shown to be effective in a rabbit model of *S. aureus* endocarditis. However, the emergence of resistant strains, could limit the use of lysostaphin to adjunctive therapy.

One approach to the problem of bacterial resistance was outlined by Patrick Scannon (XOMA, Berkeley, CA, USA). The company's lead product, Neuprex™ (BPI_{21}), is a 193 amino acid-protein fragment derived from a bactericidal glycoprotein from human neutrophils. This drug is active against gram-negative bacilli and augments the antibacterial activity of several antibiotics, including vancomycin, against gram-positive species. Clinical studies are under way to evaluate Neuprex for adjunctive therapy in gram-positive infections with a Phase III study examining its effects against the gram-negative bacteria, pediatric meningococcemia.

In closing the conference, Mair Powell of the Medicines Control Agency (London, UK) reviewed regulatory changes in Europe as they relate to the development of antibiotics for emerging resistant pathogens. New regulations include the listing of resistance rates in drug labeling, with the requirement that companies update their resistance data every five years.

The consensus at the conference was that the industry understands the challenge posed by emerging superpathogens and has developed some promising drugs. Although this is certainly no time for complacency, it is clear that intensive research and development, combined with the latest technologies, can succeed in finding ways to defeat a host of highly resourceful and constantly evolving microbial adversaries.

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Combinatorial chemistry and HTS – feeding a voracious process

Referring to the spectacular speed with which combinatorial chemistry has become accepted as routine, Richard Houghten, President of the Torrey Pines Institute for Molecular Studies (La Jolla, CA, USA) and one of the pioneers in the field, is reported as saying, 'Whereas ten years ago a good medicinal chemist might make 50 to 100 compounds a year, that same chemist is probably expected to make in the thousands or tens of thousands today.' (*Chemical & Engineering News*, 6 April 1998). This serves to demonstrate the power of combinatorial chemistry and associated high-throughput screening (HTS) techniques. These technologies offer the potential to significantly accelerate the drug discovery process and have been embraced by the pharmaceutical industry and the biotechnology companies alike, where there is constant pressure to reduce the time and costs involved in getting a new drug onto the market.

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This article reports on a novel development that is designed to relieve the reagent supply bottlenecks that can develop when the number of syntheses being performed increases vastly.

A flexible approach

While the origins of combinatorial chemistry can be traced back to solid-phase peptide synthesis in the 1960s, today's applications only began to take shape in the late 1980s and became widespread approximately four years ago. Inevitably, the rapid and general acceptance of combinatorial chemistry, together with the continual drive forward in developing the technologies, has placed novel demands on the chemists and the suppliers of both instrumentation and reagents. This is an area where progress cannot be made without the close co-operation of both parties. Hence, in this relatively young and fast-moving field, a flexible approach and a degree of consensus on method- and product-development and reagent supply is necessary.

From the perspective of a reagent supplier, this has a particular resonance. The proliferation of instrumentation for automated synthesis, HTS and analysis has ensured that chemists have the capability to synthesize and screen extensive compound libraries and to take them through the process of lead optimization. However, traditional methods of supplying chemicals and other reagents are not adequate to provide for this massive process.

According to Mark Bradley of the Department of Chemistry at Southampton University (Southampton, UK), who is currently bidding to develop Europe's first Combinatorial Centre of Excellence, there is a growing need for reagent suppliers to become a 'one-stop shop', offering easy access to all the materials required for combinatorial chemistry and drug discovery. It can be envisaged that this should include the building blocks for synthesis, resins, solid-phase materials and disposables.

More specifically, the pharmaceutical industry has an immediate practical requirement for a wide array of chemicals to supply combinatorial synthesis programmes and parallel purification processes. Because of the sheer scale of the operations, the preparation of reagents for synthesis or screening programmes has become an onerous and costly task and is often the rate-limiting step. The UK Automated Synthesis Forum, an informal grouping of pharmaceutical and agrochemical companies, has been active in pursuing common issues of reagent supply.

Compiling the starting materials required for the generation of several thousands of compounds using automated and combinatorial chemistry procedures is one of the most tedious and labour-intensive steps in the initial stages of drug discovery.

The Filling Station (Sigma-Aldrich, Poole, UK) is a new concept in reagent supply for drug discovery applications and was developed in cooperation with the major pharmaceutical companies. This service delivers a customized package in which the user selects the required chemicals, specifies the quantities and packaging (which is designed to fit the automated instrumentation in use) and dictates the delivery date, at which time all reagents will be supplied together. Reagents can be supplied in many different formats, including 96-well plates, vials and robotic reaction vessels.

This service delivers the necessary building blocks in the required formats for synthesis. Focused libraries of monomers are selected from a comprehensive database to meet the customer's exact specifications of physical characteristics, molecular shape, size, specific active groups, functional properties and other parameters. Similarly, the Filling Station can deliver compounds for HTS. As the company has a database of more than 150,000 compounds they can supply most combinations of reagents.

The following examples illustrate two ways in which the service is being used in drug development applications.

Automated organic synthesis

With the advent of combinatorial chemistry, new approaches are required for reagent supply. This means pre-determined formulation of reagents, the supply of multiple bottles and delivery in specific bottle sizes. Nick Hird (formerly at SmithKline Beecham, now at Takeda Chemical Industries Ltd, Osaka, Japan) has stated that in his view, pharmaceutical companies would prefer the adoption of industry standards, but that this is currently impractical given the plethora of instrumentation available. Suppliers, though, must deliver products that support high-throughput synthesis. SmithKline Beecham (Harlow, UK) is using the Filling Station as a means of outsourcing the collection of materials for chemistry applications. The external provision of reagents improves efficiency, removing the need to dedicate internal personnel to sourcing, weighing and mixing, and allowing better deployment of valuable resources.

The service was used as part of a project undertaken by Hird and Bill MacLachlan (SmithKline Beecham) to evaluate a new automated synthesizer, the Myriad Core System from Mettler Toledo Myriad Ltd (Royston, UK). One project involved the process of retrieval and precise weighing of 28 reagents each day, which previously would have taken two hours for one person. Instead, following an in-house assessment of needs, an order for compounds was placed through the Filling Station, and the critically important delivery date was agreed and met. Delivery is a key factor in the whole outsourcing process, as all reagent sets must be delivered together and on time, to fit in with the planning and implementation of complex synthesis programmes. MacLachlan concluded that the greatest benefit of this equipment was that all

reagents were supplied directly in appropriate vials ready for automation. The provision of material accurately pre-weighed meant that the only requirement was to add solvent, after which the vials could then be placed directly on the robot.

Outsourced dispensary

Richard Shute (AstraZeneca R&D, Macclesfield, UK) highlighted the importance of correct materials being available at the right time, by the fact that if one reagent is not there when synthesizing one compound a day, there is unlikely to be a major disaster. However, a missing reagent when synthesizing thousands of compounds might stop the entire production line.

Shute has also been instrumental in communicating the needs of pharmaceutical companies to reagent suppliers. Historically, chemists have enjoyed the financial benefits of buying chemicals in bulk and have been willing to weigh out materials. However, the arrival of effective robotics and the advent of high-throughput procedures have created sudden bottlenecks that must be overcome. Many organizations are therefore looking to reagent suppliers to meet the specific needs of pharmaceutical, biotechnology and other drug discovery companies. In sharp contrast with the early days of large-scale automation, users have now gained experience and are in a strong position to communicate their exact requirements to suppliers. The onus is now on suppliers to move with or ahead of the technology.

Shute's group is working on the generation of large general screening libraries to support lead discovery, creating a major resource for several years to come. The use of the service is proving useful at the reagent scoping stage before the full production line. This determines which reagents will work with the chemistry that has been developed and before the library design is completed.

In addition to the primary advantage of being able to specify exact reagent and formatting requirements, Shute cited a number of collateral benefits derived from the use of the service. One benefit is that, because companies no longer have to buy reagents in bulk, if any reagents do not work, they have avoided investment in large quantities that might remain unused and pose disposal problems. Thus the immediate costs of outsourcing the 'dispensary' are recouped further down the line. Similarly, many of their reagents are highly reactive and bulk buying leads to storage problems, wastage and doubts about the long-term activity of materials. In addition, because they are not investing in large quantities, a certain amount of oversampling is possible.

Ordering, reagent supply, packaging, custom labelling and delivery are designed to work smoothly. However, for the future, Shute would like to be able to access comprehensive informatics and databases via the Internet.

High-throughput screening

As well as its obvious advantages in combinatorial processes and lead optimization, the service also has applications in HTS for the identification of lead compounds, where there must be absolute confidence in the purity and structural conformation of the materials ordered. Furthermore, to avoid unnecessary screening, users can specify their exact requirements and not rely on an 'off-the-shelf' package from the supplier. Materials can also slot straight into the HTS programme and can be supplied in multiwell format, leaving designated wells blank. Another advantage of this system is that, because materials are procured from bulk supplies, subsequent purchase of the material with matching specifications will be possible for further investigation once promising leads have been identified.

Conclusion

The Filling Station concept appears to meet the expressed needs of pharmaceutical and other research companies for outsourcing compounds for combinatorial chemistry and HTS in drug discovery. Initial experiences have been positive and reinforce the importance of a close partnership between researchers and reagent suppliers in this rapidly moving field to ensure that the potential benefits of new technologies are fully realized. For the future, it is hoped that the ability to provide the correct quantity of reagents, at the correct time and in the correct place will significantly reduce the bottlenecks for combinatorial chemistry and ensure a timely supply of reagents for HTS. It is also hoped that the range of novel and useful building blocks will expand, informatics will improve and that a truly flexible approach to fulfilling the scientists needs will be achieved.

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In short...

An exclusive agreement has been signed between **Shield Diagnostics** (Dundee, UK) and **Gemini Holdings plc** (Cambridge, UK) to develop a DNA-diagnostic tool to test for the COL1A1 gene variation, which is an early and strong indication of a predisposition to osteoporosis or brittle bone disease. This test could then also be used to predict other indications such as the risk of bone loss in patients prescribed long-term steroid therapy.