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& COMBINATORIAL LIBRARIES

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Small Molecule Organic Chemical Diversity

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Techniques for Massively Parallel Synthesis of Small Organic Molecules

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Introduction

Discovery of new drugs is the goal of big pharmaceutical companies as well as small biotech firms. Combinatorial chemistry techniques, which help to speed up the process of finding new leads, in combination with computer-aided drug design methods will become the method of choice for generating new drug leads (for reviews see e.g. [1-3]; compilation of relevant literature is available on Internet [4]). However, until theoretical methods of drug design become more reliable, it will still be important to prepare and test large numbers of organic molecules to generate starting points for medicinal chemistry efforts.

Trega Biosciences (formerly Houghten Pharmaceuticals) has used the generation and screening of mixture based libraries followed by deconvolution into individual compounds or one step deconvolution from positional scanning libraries for a number of years [5]. Recently, we decided to complement these techniques by methods that allow rapid synthesis of individual organic molecules. However, to be able to cover significant diversity in a reasonable size library for initial screening of biological targets, it is necessary to prepare the new compounds at the rate of thousands of compounds per day rather than tens per day. Since there was no instrument with this synthesis capacity on the market [6], we decided to create the necessary system ourselves.

Results and Discussion

Tea Bag technology is an extremely powerful technique for the synthesis of hundreds of compounds. By compartmentalization of the solid support in polypropylene mesh pockets, all packets undergoing the same treatment can all be processed in one reaction vessel [7]. The process we have developed utilizes the tea bag method for the synthesis of hundreds of intermediates and combines it with the automated synthesis in microtiter plates for the synthesis of tens of thousands of compounds. The chemistry of the first steps of the synthesis is limited only by the compatibility of the material needed for the creation of the tea bags with the organic reaction conditions. Using polypropylene mesh Tea Bags, it is possible to work in a wide range of temperatures (from –78 °C to +100 °C) in a completely inert atmosphere, thus applying quite challenging chemistry for preparation of the intermediates. All tea bags, each containing 1-2g of solid support, are labeled and reacted with the same reagent in parallel fashion in vessels of volume up to

several litres. After all the steps of intermediate preparation have been performed, each bag is opened and a sample of the product is cleaved from the resin. The product is analyzed by LCMS and only bags containing intermediate of purity higher than 85% (based on UV trace of HPLC) qualify for the next step of the synthesis. In this step, the content of each bag is distributed into deep well polypropylene microtiter plates by multichannel pipetting of resin slurry and the rest of the synthesis is performed by dedicated robotic tools. If the bags were distributed into all 96 wells of the microtiter plate, the number of individual compounds prepared from N plates was 96 * N – that is the reason why this step is called bag explosion. It is, however, more advantageous from the diversity point of view, to distribute several bags per plate (e.g. one bag to only 48 or 32 wells), utilizing thus only 48 or 32 different building blocks in the last step of the synthesis (i.e. number comparable to number of building blocks used in the first steps).

There are several basic problems with automatic processing of multiplicity of reaction vessels. The first is the cost of the reactor. If the reaction vessel is complicated and expensive, it has to be recycled and cleaned for the next synthesis. The difficulties involved with this simple fact eliminates most of the vessel designs used in current automated systems. For the throughput of several thousand compounds a day, the reaction vessel has to be disposable. We have found the use of microtiter plates as the optimal solution. The second basic problem is the separation of solid and liquid phases after each reaction and washing step. Application of filtration material is always prone to filter clogging, especially when thousands of filtrations must be performed simultaneously. We have shown earlier that liquid phase can be recovered from the mixture by "surface suction", i.e. by slowly lowering flat end needles against the liquid surface while performing the strong suction through the needles [8] (Figure 1). In this

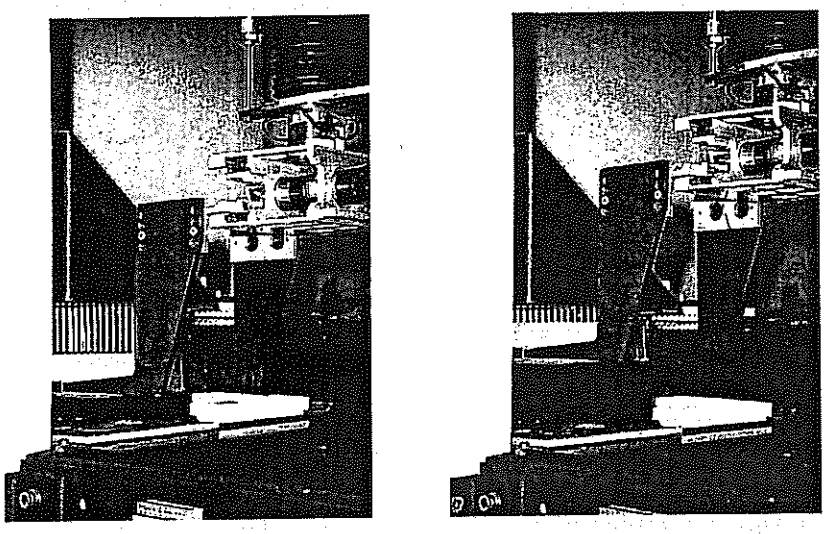

Figure 1. *Microtiter plate being lifted by the robotic gripper against the flat top needle array. Starting position (left), end of move (right).*

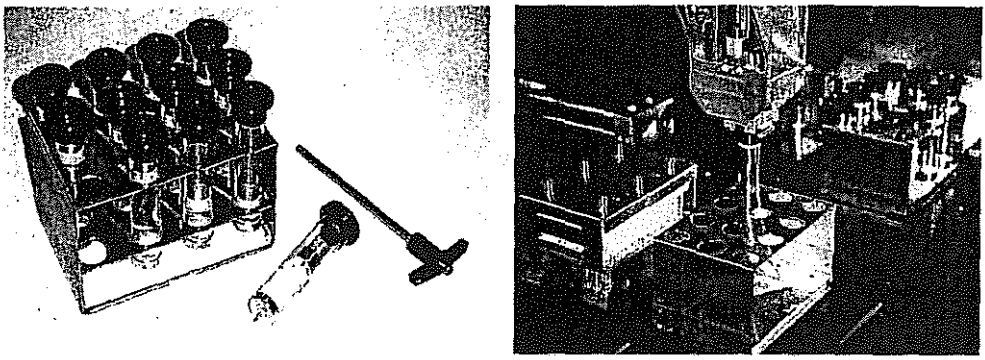

Figure 2. *Syringes for storage of building block and reagent solutions (left). Syringe actuating tool picking the syringe for delivery (right).*

way, only the surface layer of the liquid is influenced and the bulk of the solvent is not disturbed. Therefore, the needle tips can get very close to the sedimented solid phase particles without removing any solid phase beads. Elimination of liquid is not as efficient as by filtration, but "surface suction" can be performed simultaneously on hundreds of wells without the danger of filter occlusion. We have shown with a sample synthesis that the washing performed by surface suction is as effective as filtration, it just requires one or two more repetitions. The washing is performed by a Yaskawa robotic platform capable of processing 60 microtiter plates in one batch without manual interference. The plates, after washing, are transferred onto a Quadra 96 – 96 channel pipettor. Then building block solutions and other reagents were added to the plates by pipetting from master plates (microtiter plates filled with the appropriate solution in each well). Master plates were created by a Packard Canberra Multiprobe 104 - 4 channel pipettor - by pipetting from the stock solutions of building blocks stored in 50ml Falcon tubes. To avoid exposure of the building blocks solutions to air oxygen and humidity, we have closed the pipettor in an inert atmosphere enclosure filled with dry nitrogen. Alternatively, we have solved this third major problem of the synthesis - storage and distribution of reagents under inert atmosphere - by storing the solutions in syringes (Figure 2). In this way, the solution is stored in a container that is always full, the only connection to the atmosphere being the end of the needle. During storage the syringes reside in racks supported by a foam polypropylene pad. For the solution distribution, the syringe is attached to the gripper, the gripper "soft-lends" on the piston in the syringe and delivers the given amount of the liquid into the appropriate well. After complete depletion of the syringe content, the plunger is attached to the piston and the syringe is manually recharged (possibly in the inert atmosphere enclosure) with new solution.

We have solved automatic closing of the multititer plates by attaching a flexibly mounted array of teflon balls on a spring-loaded, silicon rubber supported plate (Figure 3). Incubation during shaking was achieved with an Ameritech orbital shaker/oven which we have modified for processing 64 microtiter plates at a time. After the incubation and washing steps, the resin in the microtiter plates is dried and the organic molecules are detached from the resin. This is the fourth major problem of multiple

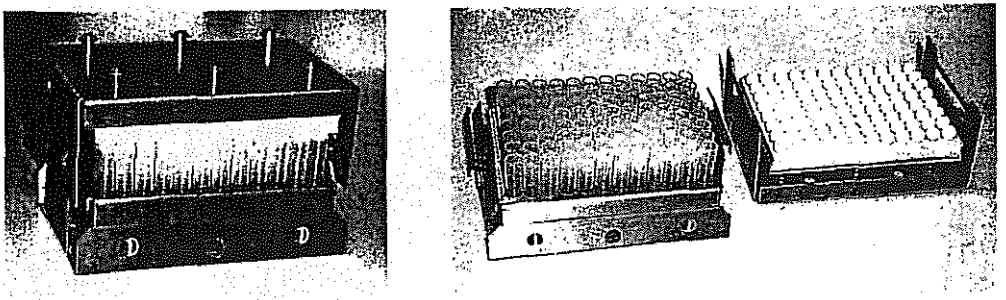

Figure 3. *"Robot friendly" closing of microtiterplates. Closed plate (left), open plate and flexibly mounted ball array (right).*

synthesis - parallel cleavage of thousands of molecules. We have applied a very convenient, but not very popular, reagent, which can be used in a gas form - hydrogen fluoride. Plates with dry resin were placed in a polypropylene chamber, the chamber was flushed with dry nitrogen and then filled with gas HF. After two hours at room temperature, the chambers were flushed with nitrogen and the plates dried in a vacuum. Then the plates were placed onto the Packard Canberra Multiprobe 208 and the organic compounds extracted by repeated addition and removal of acetic acid. Extracts were placed into microtiter plates (80 wells per plate), frozen and lyophilized. The content of each well was analyzed by flow injection mass spectroscopy and a fraction (~10%) of the production was analyzed by HPLC.

We have used this system for the synthesis of several libraries in the form of individual compounds per well and we have reached the capacity of 5000 compounds per day. We believe that the combination of the tea-bag technique for the synthesis of intermediates with automated stations for the processing of hundreds of microtititer plates into which the intermediates were "exploded" is the most efficient way to prepare realistic size libraries of carefully selected individual compounds.

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References

1. Abelson, J.N. (Ed.), *Methods in Enzymology. Combinatorial Chemistry.* Academic Press, San Diego, 1996.
2. Wilson, S.R. and Czarnik, A.W. (Eds.), *Combinatorial Chemistry. Synthesis and Application.* John Wiley & Sons,Inc., New York, 1997.
3. Krchnák, V. and Lebl, M., *Molecular Diversity* 1 (1996) 193.
4. Lebl, M., Leblova, Z., Compilation of papers in molecular diversity field. http://www.5z.com.
5. Ostresh, J.M., Blondelle, S.E., Dörner, B. and Houghten, R.A., In Abelson, J.N. (Ed.), *Methods in Enzymology. Combinatorial chemistry.* Academic Press, San Diego, 1996, p.220.
6. Cargill, J. F. and Lebl, M., *Current Opinion Chem. Biol.* 1 (1997) 67.
7. Houghten, R. A., *Proc. Natl. Acad. Sci. U.S.A.* 82 (1985) 5131.
8. Krchnák, V., Weichsel, A. S., Lebl, M., and Felder, S., *Bioorg. Med. Chem. Lett.* 7 (1997) 1013.