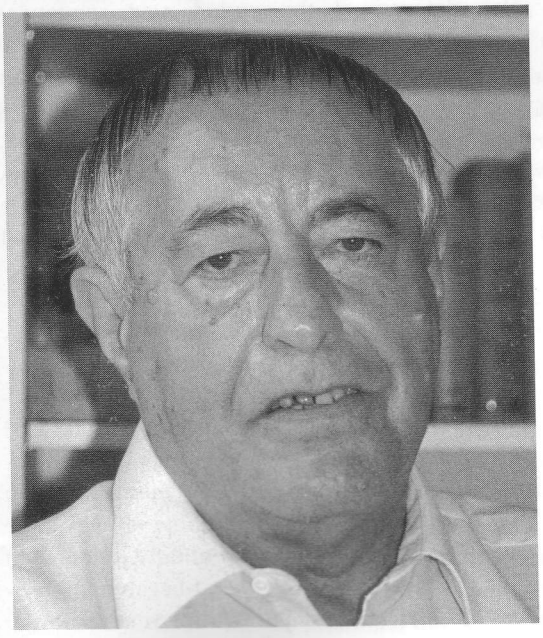
Árpád Furka, Pioneer of Combinatorial Chemistry, Is Still Full of Ideas

ISTVÁN HARGITTAI

Árpád Furka (b. 1931 in Romania) is Professor of Organic Chemistry at Eötvös University in Budapest. He received a high school teacher's diploma in chemistry and physics from the University of Szeged in 1955, his Diploma in Chemistry and his Dr. rer. nat. degree from the same university in 1959, and his Cand. Sci. (equivalent to a Ph.D.) and D.Sc. degrees from the Hungarian Academy of Sciences in 1961 and 1971, respectively. He has been at Eötvös University since 1961, with a postdoctoral stint at the University of Alberta in Edmonton, Canada, in 1964–65 and an extended sabbatical at the Advanced ChemTech company in Louisville, Kentucky, in 1995–99. He is best known for having developed the "portioning–mixing method for the synthesis of combinatorial libraries." Having originally formulated his ideas in 1982, he first communicated them in 1988, and that date may be considered the beginning of combinatorial chemistry. In 1996, he received the Leonardo da Vinci Award of Excellence from the Moet Hennessy–Louis Vuitton Foundation, and in 1999, the Academy Award from the Hungarian Academy of Sciences. Professor Furka published an article in our magazine about his research [The Chemical Intelligencer 1999, 5(1), 22–27] but I thought his personal background and his latest ideas were also of interest, and I recorded a conversation with him in September 1999 in Budapest. Below are some excerpts from his narrative.



Árpád Furka in September 1999. (Photo by I. Hargittai.)

I was born in 1931 of Hungarian parents, in Kristyor, Romania. This village is in Transylvania in a region of gold mines, and my father worked for one of the mining companies. He was originally from this region but my mother was from the Great Hungarian Plain. My parents had six children. Our mother tongue was Hungarian but I went to a Romanian school. In 1942, my mother decided to return to Hungary and she took the children (except one of my married sisters) with her to Kunágota, where she had come from originally. My father stayed behind because he didn't want to forfeit his pension. We were poor, my mother was ill, and there was hardship. I started working when I was still a child to contribute to the family in any way I could, and I didn't continue my studies after I completed the mandatory general school, when I was 14. There was unemployment and I had all kinds of odd jobs. For example, I helped in harvesting and the payment was in-kind; the wheat I earned fed us during the winter.

I always regretted that I couldn't study and eagerly read every book that came my way. Then, in 1950, I was among those who were offered the possibility of attending an accelerated course and completing my secondary education in one year instead of the customary four years. It was tough. We lived in the school and could leave only on weekends. We got a good edu-

©2000 SPRINGER-VERLAG NEW YORK, INC.

The Furkas in 1959 in Szeged (courtesy of Professor Furka).

cation but everything was stripped to the bare essentials. Our certificate was called something like a special maturation, and this term has had a certain connotation in Hungary. To some, it signifies a deficient education; to others, tough perseverance. What I missed was the cultural environment that others, more fortunate than I, spent their childhood in. In any case, I regained some of the lost years in my education.

In 1951, I was sent to the University of Szeged to become a high school teacher of chemistry and physics. I didn't mind that I wasn't even asked about my preference because I always liked sciences. My dream, though, had been to become an astronomer. At the university, my speedy high school education successfully withstood the test, and not only did I keep up with my peers, often I could even help them in their studies.

In 1955, I got my teacher's certificate and went to teach high school physics in Makó, in southeastern Hungary. After one year, however, I was called back to the University of Szeged, where I was charged with teaching in the Department of Chemical Technology. Gábor Fodor, who was Professor of Organic Chemistry, was directing our research. My first project was acyl migration, and I enjoyed it a great deal. The upheaval in 1956 interrupted my university work, which had barely started some weeks before. I used the forced break of several months to study English. I was then happy to resume my research when the conditions permitted it. Professor Fodor soon left Szeged for Budapest and later he immigrated first to Canada and then to the United States. He is now Professor Emeritus at West Virginia University.

In 1961, I was offered a position at Eötvös University and was transferred to Budapest. Again, this was not my own doing. After I had already been in the Department of Organic Chemistry for quite a while, I learned that its head, Professor Victor Bruckner, had wanted to fill the opening with someone else when I was virtually forced down his throat. This let me understand why I never became one of his favorite co-workers. I never felt fully at home in Professor Bruckner's department although there was no open hostility and nobody hindered my work in any way. My alienation was as much my own doing as their lack of trust in me. A small episode will give you a sense of the atmosphere that I contributed as much as anybody else to creating. Professor Bruckner used to have a weekly afternoon tea for his senior co-workers. He must have been somewhat reluctant to invite me, but nevertheless he sent word to me that I would be welcome at these afternoon teas. However, I sent word back that I didn't like tea. That must have sounded awfully rude, and it was, I see that now. But at that time I didn't drink tea and I took the invitation literally.

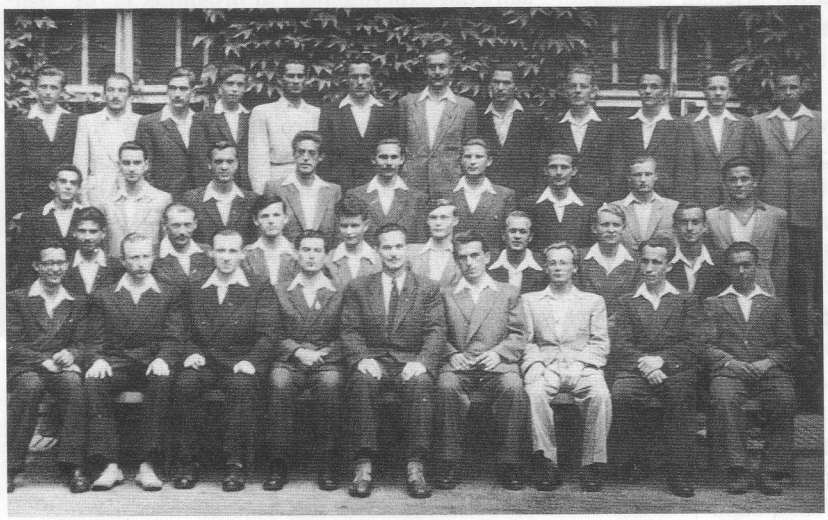
Nevertheless, I enjoyed working in the new field of peptide chemistry. Just the other day, I found a notebook of mine from those days in which I had recorded some of my ideas. I used to take my ideas to Professor Bruckner but he never showed any interest in them. One of these ideas, in about 1962, was a solid-state synthesis of peptides.

In 1964, I got a postdoctoral fellowship from the National Research Council of Canada and spent one year at the University of Alberta. That year with Professor L. B. Smillie was very fruitful. Smillie had learned a technique at Cambridge for the determination of the position of disulfide bridges in proteins, using a combination of oxidation and diagonal paper electrophoresis. He also determined the amino acid sequences around the five disulfides of chymotrypsinogen B. Based on his preliminary work, I almost completed the sequence determination of this protein of 245 amino acids during my one-year stay. Smillie's reputation got a boost from this work, and, shortly afterward, he established a peptide research institute with himself as director. Last time we met was in 1993 at a symposium.

Upon my return to Budapest, I couldn't continue this kind of work, as I lacked the necessary instrumentation, in particular, an amino acid analyzer. The head of the department made all decisions about what should be purchased. So I turned my attention to working out new methodologies, such as the C-terminal peptide isolation and how to enhance the solubility of enzymatic hydrolysates of proteins. Eventually, I defended my D.Sc. degree and, in 1972, I became full professor. Later on, I cooperated for years with the

Chinoin Pharmaceutical Company in Budapest. We isolated natural peptides, determined their sequences, and then synthesized them and their analogues. At that time, I began dreaming about the possibility of preparing full series of small peptides, not only selected sequences. Although this seemed impossible using the available techniques, I kept thinking about it. Finally, and quite unexpectedly, I found the solution.

My initial thoughts about what is known today as combinatorial chemistry were also recorded in my notes. I described a technique for synthesizing mixtures of very large numbers of peptides simultaneously, and it included a methodology for selecting the active peptides among them. Thus, this was a description of combinatorial chemistry. We performed the synthesis part but, lacking partners for the rest of the work, the selection methodology, known today as the iteration method, was not tested in practice. I was looking for partners, but neither the biologists I approached nor Chinoin was interested in my proposal. However, Ms. Éva Somfai, who was in charge of patents at Chinoin, suggested to me that I have my notes notarized, which I did [1], hence the date of 1982 on my notes. Ms. Somfai didn't think my proposal was suitable for a patent since it was about a research methodology. Later, however, others filed similar patents. At that time, I couldn't even think about filing a patent by myself as our salaries were meager. Since I couldn't do anything without a sponsor, and there wasn't any, only my notarized notes have remained from that time.



The special maturation class (chemistry–biology orientation) in Eger, 1951 (courtesy of Professor Furka).

Two colleagues of mine, Dr. Ferenc Sebestyén and Dr. József Gulyás, worked on the experimental methodology, but electrophoresis did not clearly show the products. Mamo Asgedom, Dr. Sebestyén's doctoral student from Ethiopia, proved very successful in this project. Originally, we identified the peptides with a two-dimensional high-voltage paper electrophoresis technique. This technique was based on an observation made by R. E. Offord in the early sixties. Offord worked in Cambridge, where many peptides had been isolated and purified by paper electrophoresis. He had collected all the data and found that the charge and molecular mass of the peptides determined their mobility. He had set up a formula [2], and I incorporated this formula into software to identify the peptides on the two-dimensional electrophoretic maps. This software gave us a computer-predicted peptide map, which could be compared with the experimentally established one. A built-in normalization procedure made the match between the prediction and the experiment perfect.

As for publishing my methodology, I presented it for the first time in poster sessions at two international symposia in 1988, one in Prague [3] and the other in Budapest [4]. Following the two meetings, I submitted our first article in February 1990, and it appeared, after an initial rejection and a long and painful delay, in 1991 [5]. Subsequently, it proved very important that I had presented my methodology to international audiences in 1988 and that the contents of my posters had appeared in print. They have been much cited, and today there can be no doubt as to the priority of my discovery, and I don't think that there is any doubt either.

While I can rightly maintain that I initiated what is called today combinatorial chemistry, I would like to mention prior work by H. M. Geysen [6], whose multipin technique preceded my methodology. This technique produced single substances by parallel reactions, and thus it was not truly combinatorial. Yet another technique also described by Geysen involved applying mixtures of amino acids in couplings that gave combinatorial mixtures [7]. Due to the differences in the reactivity of the amino acids, however, the peptides were formed in unequal molar quantities, and even the composition of the mixtures was uncertain. For this reason, the technique did not find wide application.

Our two posters didn't have much impact initially. It takes more than two posters from Budapest to catch people's attention. Also, they didn't show how one could select the useful products from among all those present in the mixtures. It was a painful experience when, in my absence, Sebestyén presented our methodology to the Commission of Peptide Chemistry of the Hungarian Academy of Sciences and our peers rudely rejected it. This was between our 1988 posters and the appearance of our paper in 1991. Since then, the situation has changed, and in 1999 I received the Award of the Hungarian

Academy of Sciences.

Recently, I've worked out a new approach to improve the applicability of my original methodology for drug research. This improvement is aimed at producing larger quantities of the desired products and at differentiating between the products. The original approach yielded very small quantities on each bead of the solid support, and you had to determine the structure of the substance on each bead in order to identify it. Professor Nicolaou of the Scripps Institute in San Diego has suggested a new approach in which the beads of the solid support are enclosed in a permeable capsule together with an electronic chip. This chip carries information about the identity of the product formed in the capsule. After each synthetic step, the capsules are sorted and regrouped. Those capsules that will be exposed to the same reagent in the next synthetic step are grouped together. This approach preserves the productivity of my original technique but, at the same time, offers the advantages of the parallel synthesis: larger quantities and known products.

My latest methodology, however, would eliminate the need for the chip or any other labeling of the solid-support units. Instead of labeling the support units, we arrange them into spatially ordered groups. The spatial arrangement is preserved during the chemical reactions and, before the next reaction step, the units are rearranged according to a predetermined pattern. With the use of appropriate software, a computer can track the synthetic history of each support unit and can predict the spatial position of each product. In model experiments, we prepared 125 tripeptides using Chiron crowns as solid-support units. We formed spatially ordered groups by stringing the crowns on polyethylene fish line. We used a simple, manually operated device to rearrange the crowns before the second and third coupling steps. After sorting, of course, the crowns were stringed again. Many different patterns can be used for regrouping, and each of them needs different software to predict the final position of the products. For our manual device, the "semiparallel" pattern gave the optimal sorting speed, and the prediction of the positions of the tripeptide sequences on the final three strings proved to be perfect. My present goal is to build an automatic machine, which would enhance the speed of sorting by a factor of 10 to 100. Alas, our grant application has been turned down by NIH because the technical description of our proposed automatic device was considered to be incomplete.

I have recently returned to Budapest after an extended sabbatical in the United States and am continuing my work at Eötvös University. I am 68 and I know my abilities. I'm good at generating one new idea after another, but I'm poor at making contacts with people and securing the necessary funding for my experiments. The lack of proper conditions for creative activities makes my situation almost impossible. But I don't want to ascribe all my difficulties to my surroundings. My own abilities have severe limitations and I just have to live with them.

If I had to single out the most significant of my current ideas that I would like to put into practice, that would be an improvement in the testing of the products of the combinatorial syntheses. My suggested approach wouldn't just test them for a few selected applications, but for all possible targets. The current testing techniques may leave products of great drug potential untapped because of the limitations in their testing. My new ideas would make a significant thrust in the direction of more complete testing. Once we would start the experiments I'm dreaming of, the continuation would be self-propelling. Today my dream may sound just as unrealistic as combinatorial chemistry seemed not such a long time ago.

If I could have three wishes, they would be good health, seeing more of the world, and getting my research funded. I could make annual support of $50,000 go very, very far.

During the past decade or so, a truly new field of chemistry has emerged. According to many, it has also led to a revolution in drug research. All the big pharmaceutical companies have started combinatorial chemistry groups and have invested millions of dollars in the field. The development of new drugs, however, takes time and the results will emerge gradually. There's also secrecy surrounding this kind of work. Beyond its application in drug research, this methodology is beginning to be employed in other fields. Yet the biggest benefit of this new field may not even be the new substances it produces but its impact on our way of thinking.

REFERENCES

1. See *http://www.win.net/kunagota.*
2. Offord, R. E. *Nature* **1966,** *211,* 591
3. Furka, Á.; Sebestyén, F.; Asgedom, M.; Dibó, G. In *Highlights of Modern Biochemistry,* Proceedings of the 14th International Congress of Biochemistry; VSP: Utrecht, The Netherlands, 1988; Vol. 5, p 47.
4. Furka, Á.; Sebestyén, F.; Asgedom, M.; Dibó, G. Abstracts, 10th International Symposium of Medicinal Chemistry, Budapest, Hungary, 1988; p 288, Abstract P-168.
5. Furka, Á.; Sebestyén, F.; Asgedom, M.; Dibó, G. *Int. J. Peptide Protein Res.* **1991,** *37,* 487.
6. Geysen, H. M.; Meloen, R. H.; Barteling, S. J. *Proc. Natl. Acad. Sci. U.S.A.* **1984,** *81,* 3998.
7. Geysen, H. M.; Rodda, S. J.; Mason, T. J. *Mol. Immunol.* **1986,** *23,* 709.