Integrated Drug Discovery Technologies

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Combinatorial Chemistry: The History and the Basics

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I. DEFINITION

What is combinatorial chemistry? There have been several opinions, some formulated very sharply, but most expressing what combinatorial chemistry is not. At the end of the conference "Combinatorial Chemistry 2000" in London, we organized a discussion session because we wanted to answer this question and make sure that our understanding of the term reflects the fact that at least some operations in the synthesis of the group of chemical compounds is performed in combinatorial fashion. Unfortunately, when it came to the public vote, the scientists in the audience voted for a much broader definition of combinatorial chemistry. The majority expressed the opinion that combinatorial chemistry is defined by the design process, i.e., that compounds designed by the combination of building blocks (and synthesized by whatever means) are the subject of combinatorial chemistry. In the literature, the term combinatorial chemistry is used very often; however, the definition is found rarely. Seneci [1] says that "combinatorial chemistry refers to the synthetic chemical process that generates a set or sets (combinatorial libraries) of compounds in simultaneous rather than a sequential manner." *Journal of Combinatorial Chemistry* defines combinatorial chemistry as "a field in which new chemical substances—ranging from pure compounds to complex mixtures—are synthesized and screened in a search for useful properties."

But do we really need to define combinatorial chemistry before discussing the history of this branch of science? Must we have consensus about the term combinatorial chemistry before we start the new journal with the term in its title? (There are already several journals with the term combinatorial chemistry on their

cover.) Apparently not, and the precise definition is probably not as important as the fact that the novel techniques are being widely accepted and applied as needed for a variety of projects, starting from finding new drug candidates and ending in discovery of new inorganic materials.

II. HISTORY

Maybe the best introduction to combinatorial chemistry is through its brief history. In 1959, the young chemist Bruce Merrifield, had the idea that it would be extremely beneficial to modify the sometimes unpredictable behavior of growing peptide chain intermediates by attaching the chain to the polymeric matrix, the properties of which would be very uniform from step to step [2–5]. His invention of solid-phase synthesis, for which he was awarded the Nobel Prize [4], changed the field of peptide synthesis dramatically. Synthesis of oligonucleotides followed immediately [6]; however, solid-phase synthesis of organic molecules was pursued basically only in the laboratory of Professor Leznoff [7,8]. Even though solid-phase synthesis was more or less accepted in the chemical community, it took another 20 years before the new ways of thinking about generation of a multitude of compounds for biological screening brought combinatorial chemistry to life. Pressure from biologists motivated the development of combinatorial chemistry. Chemists could not keep up with the demand for the new chemical entities. Big pharmaceutical companies started to screen their entire collections of chemical compounds against new targets, and the rate at which these collections grew seemed unsatisfactory. Ronald Frank in Germany [9], Richard Houghten in California [10], and Mario Geysen in Australia [11] devised ways to make hundreds of peptides or oligonucleotides simultaneously by segmenting the synthetic substrate–solid support. Frank used cellulose paper as the support for the synthesis of oligonucleotides. Cutting the circles of the paper and reshuffling the labeled circles for each cycle of the coupling was a very simple way to generate hundreds of oligos. Houghten enclosed classical polystyrene beaded resin in polypropylene mesh bags, later called "tea-bags" or "T-bags," and used them for parallel synthesis of hundreds of peptides. The principle was the same: combine the bags intended for coupling the same amino acid and resort the bags after each cycle of coupling. Geysen used functionalized polypropylene pins arranged in the fixed grid. Each pin was then immersed in a solution of activated amino acid pipetted into the individual wells of microtiter plate. Pins were not resorted after each step, but the common steps of the synthesis (washing, deprotection) were done by introduction of the pins into the bath containing appropriate solvent. These techniques cleared the way for the arrival of real combinatorial techniques applied to general organic chemistry and not only to the specific arena of peptides and oligonucleotides.

For biologists and biochemists, working with mixtures was absolutely natural—well, it was natural also for natural products chemists—however, organic chemists were (and still are) horrified when mixtures were mentioned. Therefore, development of specific binders selected from the astronomically complex mixtures of RNA by selective binding and amplification of selected molecules by polymerase chain reaction (PCR) was accepted enthusiastically, and papers describing it were published in *Science* [12] and *Nature* [13,14]. (Larry Gold and his colleagues were adventurous enough to build a company around this technology—NeXstar—now merged with Gilead, in Colorado.) Relatively fast acceptance was given to the techniques generating specific peptides on the surface of the phage, panning for the binding sequences and amplification of the phage [15,16], described by Smith. Again, the approach was basically biological. However, earlier attempts to publish papers describing the use of synthetic peptide mixtures for determination of epitopes in *Nature* were unsuccessful; the world was not ready for chemical mixtures. Geysen's seminal paper was eventually published in *Molecular Immunology* [17] and did not find a large audience. In this paper the mixture of amino acids was used for the coupling at the defined positions, thus generating large mixtures of peptides. Mixtures showing significant binding were "deconvoluted" in several steps to define the relevant binding peptide sequence at the end.

The pioneer in development of the methods for creating the equal mixtures (of peptides) was Arpad Furka in Hungary. His method of "portioning-mixing" was invented in 1982 (*http://szerves.chem.elte.hu/Furka/index.html*) and presented as posters in 1988 and 1989 [18,19]. The method was not noticed until 1991, when it was reinvented and published in *Nature* by two independent groups, Lam et al. in Arizona ("split-and-mix" method) [20] and Houghten et al. in California ("divide–couple–recombine" method) [21]. Technology of deconvolution of mixtures was the basis of formation of Houghten Pharmaceuticals, Inc., later renamed Trega Biosciences, Inc. (Leon, Germany). Finding the active molecule requires synthesis of the second (and third, and fourth etc.) generation mixtures of lower complexity based on the activity evaluation of the most active mixture from the first round of screening. An alternative method is positional scanning in which mixtures of the same complexity with defined building blocks in all positions of the sequence are screened and the importance of individual blocks is ascertained. The combinations of all "important" residues are then assembled in the possible "candidate sequences," which are then tested individually [22]. The use of mixture-based libraries was reviewed recently [23].

Portioning-mixing (split-and-mix, divide–couple–recombine) is a simple but powerful method that not only allows generation of equimolar mixtures of compounds but is also the basis of one-bead-one-compound technology for the screening of individual compounds (as recognized by Lam [20,24,25]). In this modifica-

tion, the synthetic compounds are not cleaved from the resinous bead, and binding is evaluated by assay performed directly on the bead. The structure of a compound residing on positively reacting bead is then established by direct methods or by reading "the code" associated with that particular bead. The one-bead-one-compound technique can be modified for the release of the compound to solution [26], or to semisolid media [27], to allow for the use of assays not compatible with solid-phase limitation. Again, this technology jump-started the first combinatorial chemistry company, Selectide Corporation, in Tucson, Arizona (now part of Aventis).

III. SMALL ORGANIC MOLECULES

Libraries of peptides and oligonucleotides were relatively easy to handle both in the mixture and in the individual one-bead-one-compound format. Determination of structure of peptide and/or oligonucleotide is made relatively easy by sequencing requiring picomolar or even lower amounts of material. At the same time synthetic methodologies for their synthesis are well developed. However, a good candidate for new successful drug is being sought between "small organic molecules." Libraries containing nonoligomeric organic compounds were obviously the next step in the development of combinatorial chemistry. Jonathan Ellman recognized this need and developed a method for solid-phase parallel synthesis of benzodiazepines [28]. His publication, together with published results from Parke-Davis [29] and Chiron [30,31], started a flood of communications about application of solid-phase synthesis to preparation of enormous numbers of different categories of organic compounds, with the major focus on heterocyclic molecules. (Numerous compilations of solid-phase syntheses were published; see, for example, [32–35], and a dynamic database of all relevant publications is available on the Internet (*http://www.5z.com/divinfo*).).

Transformation of one-bead-one-compound libraries to the arena of small organic molecules requires methods allowing simple and unequivocal determination of the structure from the individual bead containing picomolar amounts of analyzable material. This problem was addressed by inclusion of "tagging" into the synthetic scheme [36–39]. The structure of the relevant molecule is determined by reading the "tag." The most elegant method for tagging was developed by Clark Still [37]. Again, as a rule in this field, the result was formation of a new company, Pharmacopeia. In this method, the tagging of the organic molecule is achieved by a relatively small set of halogenated ethers attached to the bead as a defined mixture in each step of the synthesis, forming digital code (each molecule of the tagging substance is either present—digit 1—or absent—digit 0), evaluated after detachment from the bead by gas chromatography.

It did not take long before the combinatorial techniques were applied to material science [40–44]. These libraries are produced usually in a spatially

addressable form and were used to find new supraconductive, photoluminescent, or magnetoresistive materials.

IV. SYNTHETIC TECHNIQUES

Although the pressure to produce more compounds was visibly coming from pharmaceutical companies, most of the new techniques were developed at academic institutions. Big companies still did not embrace the new techniques possibly due to the fact that they are quite simple and inexpensive to implement. Pharmaceutical companies do not want simple solutions; they would rather invest in enormous automation projects. In the end the managers are judged by the budget they were able to invest, and a big room full of robotic synthesizers definitely looks impressive. Another major factor is the "visibility" of the compound produced. Production of 100 nmoles of the compound (about 50μg of an average organic compound), which can make 100 ml of 1 μM solution (enough for 1000 biological assays), is unacceptable—simply because it is not "visible." Companies usually require 5–50 mg of the compound (more than enough for 1 million assays) just to "have it on the shelf." And techniques providing 100 nmoles are definitely cheaper and require less automation than techniques needed to make milligram quantities of the compound.

A very elegant technique for synthesizing numerous organic compounds in parallel was introduced by Irori in San Diego. This company was based on the idea that it is possible to label individual polymeric beads with the readable radiofrequency tag, which will be built during the split-and-mix synthesis of any type of molecule. Even though this very ambitious goal has not yet been achieved, the technique of "Microkans"—small containers made from polymeric mesh material containing inside beads used for solid phase synthesis together with radiofrequency tag [45,46]—is used in numerous laboratories [47]. The most recent incarnation of this technique (based on the original principle of "tea-bag" synthesis of Houghten [10]), is the labeling of small disks containing 2–10 mg of synthetic substrate, called "NanoKans," by a two-dimensional bar code on a small ceramic chip [48].

On the other hand, thousands of compounds can be synthesized relatively inexpensively in polypropylene microtiter plates using either "surface suction" [49] or "tilted centrifugation" [50]. However, nothing can be more economical and versatile for synthesis of up to couple of hundred compounds than disposable polypropylene syringes equipped with polypropylene frits, as introduced by Krchnak [51]. A syringe is charged with the solid support of choice, and all steps of the synthesis are performed by aspirating appropriate reagents using needles and (if needed) septum-closed bottles. The operation of syringes can be simplified by the use of domino blocks [52].

V. PHILOSOPHY AND CRITERIA

The different approaches to the synthesis of libraries illustrate the different philosophies of laboratories and companies. The same difference in thinking can be found in the value given to the purity of prepared compounds. Different companies apply different criteria. However, in the end you will always hear: "We do not accept anything worse than 80 (75, 85, 70)% purity." Well, what purity is being talked about? High-performance liquid chromatography with ultraviolet detector? All compounds would have to have the same absorbtion coefficient. Or evaporative light-scattering (ELS) detector? Slightly better. Or mass spectroscopic (MS) purity? There is nothing like MS purity! Maybe nuclear magnetic resonance (NMR), but who can evaluate several hundreds or thousands of NMR spectra each day? Anyway, what does this number tell you? Only a rough approximation of how many potentially good leads you will miss by not looking at the samples at all. The only really important information that the chemist should provide to the biologist is whether he or she can guarantee the preparation of the same sample tomorrow or a year from now. Does he or she have the stable, well-rehearsed protocol and reliable source of starting materials? If yes, every biologist should be happy to screen his or her compounds. If the biological activity is found in the impure sample, the likelihood that the active component of the mixture can be found after isolation of all components is pretty high. By the way, the probability that the activity is higher than observed in the mixture is also high. And, as a free bonus, the active species might not be the one that was targeted but rather the side product of unexpected (and hopefully novel) structure. This would make the patent people happy. For a long time I did not meet a combinatorial chemist who did not have a story about active compound being a side product.

We could go on discussing combinatorial chemistry, but because this text is intended to be an introduction to the history of the fields, we will stop here and refer readers to the published literature. The histories and personal recollections of the pioneers in this field were compiled in the inaugural issue of *Journal of Combinatorial Chemistry* [53], and a similar format was used for a history of solid-supported synthesis [54,55]. In addition to books on the subject of combinatorial chemistry and solid-phase synthesis [56–74], we recommend attendance at biannual symposia on solid-phase synthesis and combinatorial techniques [75], organized by Roger Epton. Reading of recent review articles [32–35,76–88] is also helpful. We also direct readers to the Internet site compiling all papers published in this exciting and rapidly growing field, which can be found at *http://www.5z.com/divinfo.*

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