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Combinatorial Chemistry

Combinatorial chemistry is a term created about 1990 to describe the rapid generation of multitudes of chemical structures with the main focus on discovering new drugs. In combinatorial chemistry the chemist should perform at least one step of the synthesis in combinatorial fashion. In the classical chemical synthesis, one synthetic vessel (flask, reactor) is used to perform chemical reaction designed to create one chemical entity. Combinatorial techniques utilize the fact that several operations of the synthesis can be performed simultaneously.

Historically, the first papers bringing the world's attention to combinatorial chemistry were published in 1991, but none of these papers used the term combinatorial chemistry. Interestingly, they were not the first papers describing the techniques for preparation of compound mixtures for biological evaluation. Previously, H. Mario Geysen's lab had prepared mixtures of peptides for identification of antibody ligands in 1986. Other laboratories heavily engaged in synthesizing multitudes or mixtures of peptides were Richard A. Houghten's laboratory in San Diego and Árpád Furka's laboratory in Budapest. The recollections of the authors of these historical papers were published in the journal dedicated to combinatorial chemistry, *Journal of Combinatorial Chemistry*.

Since the goal of combinatorial chemistry is the discovery of new compounds with interesting properties (biological as new pharmaceuticals or physical as new materials), the chemists want not only to make as many compounds as possible as quickly as possible, but to make these compounds as different from each other as possible in order to cover what is referred to as chemical space. This space stretches over all theoretically possible structures and conformations of all compounds within a given range of size. When the structures of a set of compounds made by combinatorial chemistry are evenly distributed over the respective chemical space, one of the compounds has a better statistical chance of being identical or at least similar to the "optimal" structure (conformation) for a desired property (e.g., biological activity), as compared to a set of compounds that cover only a fraction of the chemical space.

A large set of related synthetic compounds is typically called a library (or combinatorial library). Libraries range in complexity from a few dozen up to millions of compounds. The central feature of combinatorial libraries is that all compounds making up the library represent combinations of two or more "building blocks" which are connected by chemical reactions.

Combinatorial libraries can be classified based on their composition or synthetic history (see Figure 7). Peptide-like (oligomer) libraries are composed of repeated units of similar building blocks connected by repetition of the same (or similar) chemical reaction. Glucose-like (scaffolded) libraries are based on the multifunctional scaffold, the functional groups of which are selectively employed in attachment of various building blocks. Benzodiazepine-like (condensed) libraries are created by connecting building blocks capable of forming unique structures depending on the order of performed reactions, where original building blocks may not be readily identifiable within the resulting library structure (various strategies and building block types can be used for forming the same resulting structures). Libraries can be structurally homogeneous or heterogeneous; that is, the compounds can have identical or variable "scaffolds" or "backbone." All combinatorial libraries can also be complete (containing all theoretically possible combinations of used building blocks), or incomplete (containing only a fraction of all possible compounds). A complete combinatorial library is composed of all possible permutations of the building blocks at their respective positions. If the scaffold of a library has three attachment points (prospective diversity positions), and ten different building blocks are used for each diversity positions, then the complete combinatorial library is composed of $10^3 = 1000$ compounds.

Synthesizing a combinatorial library can be rather straightforward, as the same protocol is typically used for all compounds, so that the synthesis method has to be worked out only

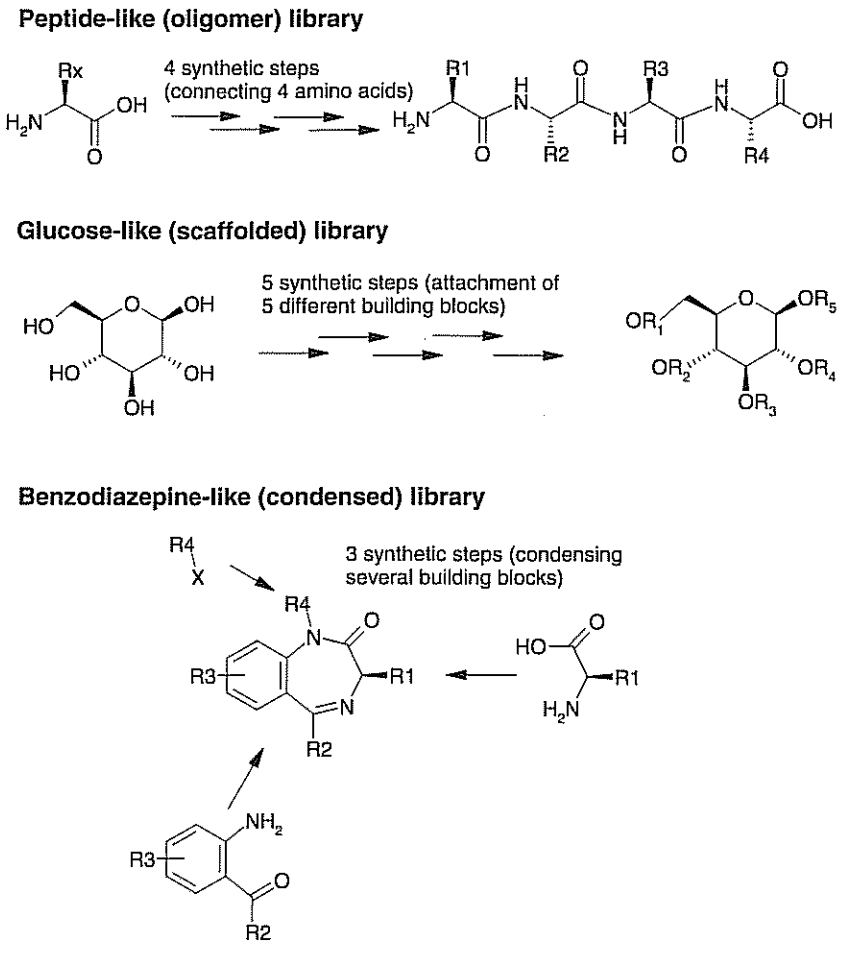

Figure 7. Different library types—peptide-like (oligomer) libraries, glucose-like (scaffolded) libraries, and benzodiazepine-like (condensed) libraries. Oligomeric libraries are built by connecting similar building blocks by repetition of one (or several) reactions—peptides and oligonucleotides are typical examples. Scaffolded libraries are constructed by modification of individual functional groups on the template (scaffold) molecule. In condensed libraries it may be difficult to trace the character of building blocks used for their construction.

once. This, however, is not always as easy as it may sound (with issues covering the choice of synthetic strategy, in solution or on solid support, chemistry of attachment of the first building block, protection and deprotection strategies, release from solid support, etc.), as the optimal reaction conditions can vary greatly among the different building blocks used for a particular step.

An important prerequisite for combinatorial chemistry is the availability of methods for parallel synthesis. Prototypical of combinatorial chemistry techniques is Houghten's "tea bag" method. In this technique the solid support (functionalized polystyrene resin) is sealed in packets made of polypropylene mesh (Figure 8), which is permeable for solvents and reagent solutions. Up to several hundreds of such resin packets can be processed simultaneously in common reaction vessels. After each step the packets are resorted for the next synthesis step. Resorting is either based on readable alphanumeric labels, or it can be simplified by enclosing a radio-frequency tag in the tea bag. In this way up to a thousand compounds can be synthesized using a reasonable number of reaction vessels. For example in the peptide synthesis, only 20 reactors with individual amino acids are required for the synthesis of basically unlimited number of (natural) peptides of any length in parallel.

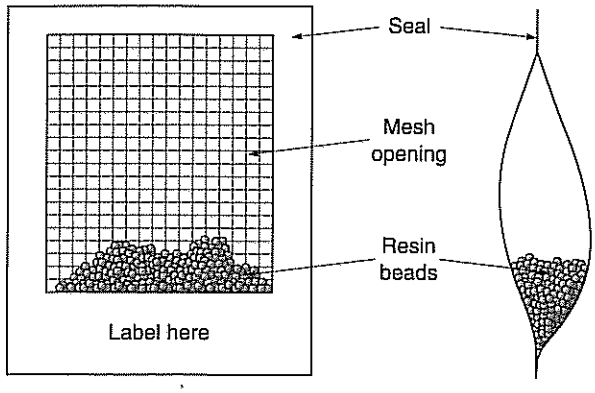

Figure 8. Schematic drawing of the "tea-bag."

A powerful, yet simple method for manual or semiautomated solid-phase synthesis of mixtures of up to millions of compounds is the "one-bead–one-compound" approach (Figure 9). It has also been referred to as "split-and-mix" or "divide–couple–recombine" approach, and is based on coupling each building block to separate portions of the solid-phase resin, followed by combining and mixing all resin portions, before dividing the resin again for the next synthesis step. By repeating this procedure three more times, and using 20 different building blocks for each synthesis step, a library of 160,000 (20^4) compounds can be readily prepared. This process yields libraries containing an individual, unique compound on each resin bead. After assembling the library on the resin, it can be either cleaved for bioassays in solution, or left on the resin for solid-phase assays. The bio-assays are typically performed on single beads, so that the screening format of one-bead–one-compound libraries is that of single compounds, rather than compound mixtures. The one-bead–one-compound library principle is based on the statistical distribution of the particles in the process. However, this statistical nature of the process can be eliminated by the use of continuously divideable solid supports, such as membranes or threads. In this case, all members of the library are guaranteed to be prepared, and none of them is prepared in more than one copy.

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Further Reading

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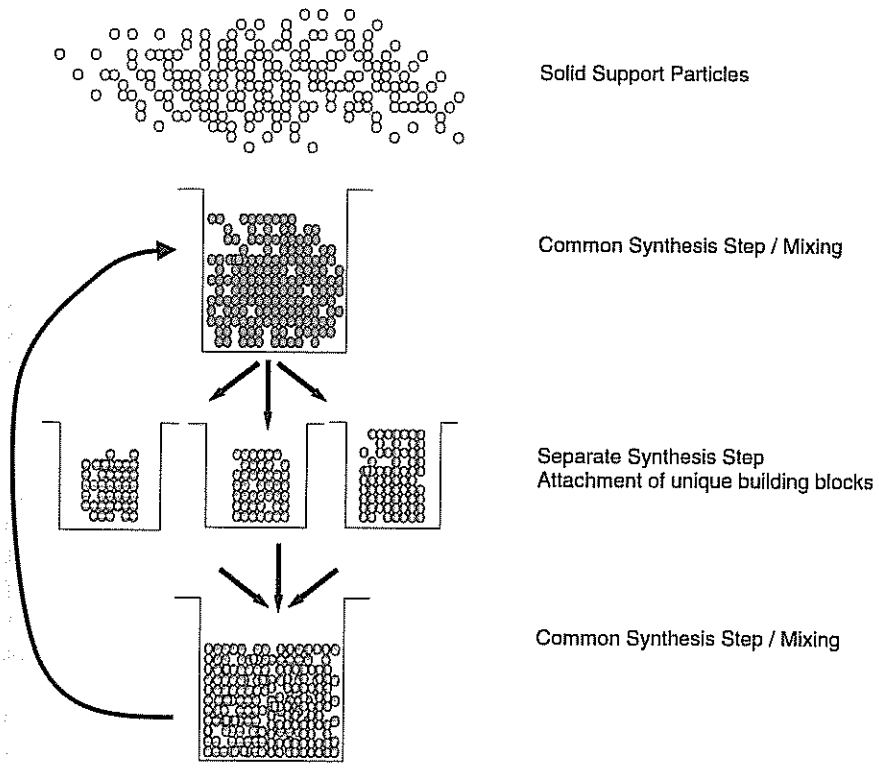

Figure 9. Principle of one-bead–one-compound library synthesis. Resin particles are exposed to only one reagent at a time and therefore each particle can contain only one structure. The process of separating the solid support into aliquots and mixing them is repeated as many times as there are steps of the library building using the unique building blocks. A multistep synthesis of a library, in which three of the steps use the various building blocks (10 different building blocks are used in each step), would generate $10 \times 10 \times 10 = 1000$ different bead populations. If 1 gram of 130-micrometer polystyrene beads were used for the synthesis (1,000,000 beads), there will be in average 1,000 beads carrying the same compound. If only 1,000 beads are used for the synthesis (very unlikely), the chance of having any particular structure represented in the library would be only about 70 percent.

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Useful Websites

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