Principles of Combinatorial Chemistry

Christopher Rose

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1. Basic ideas & concepts

Basic idea of combinatorial chemistry:

- Preparation of a large number of different compounds at the same time
- High throughput- screening provides the most promising substances

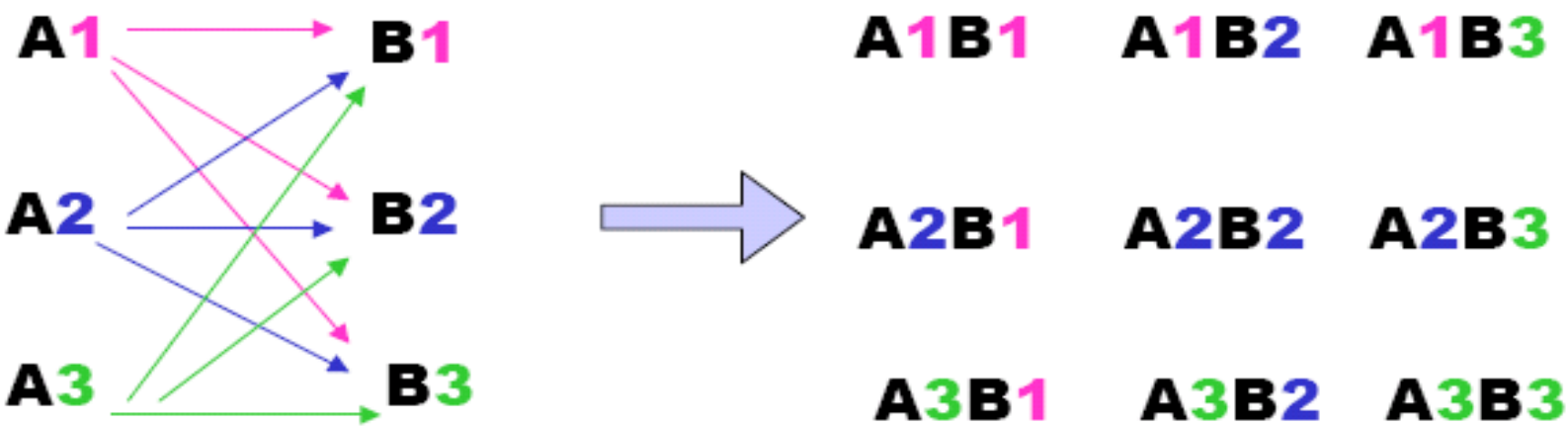
Conventional Reaction: $\mathbf{A + B \longrightarrow A\text{-}B}$

Combinatorial Chemistry: $\mathbf{A(1\text{-}n) + B(1\text{-}n) \longrightarrow A(1\text{-}n) - B(1\text{-}n)}$

⇨ Combinatorial Chemistry as a valuable tool in drug discovery and material science

Combinatorial Libraries:

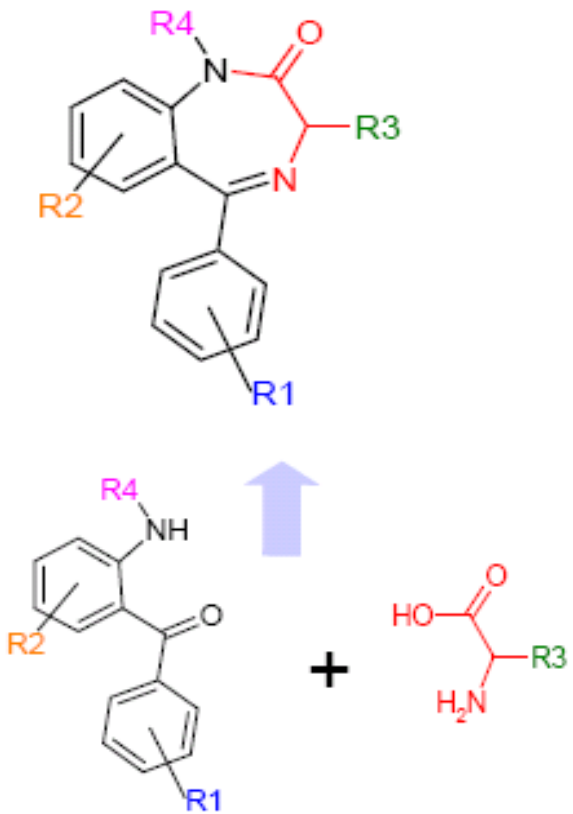
- Def.: Collection of finally synthesized compounds
- Size: depends on the number of building blocks used per reaction and the number of reaction steps, in which a new building block is introduced
- Typical: 10^2 up to 10^5 compounds



9 different compounds

Distinction: Scaffold- based vs. Backbone- based libraries

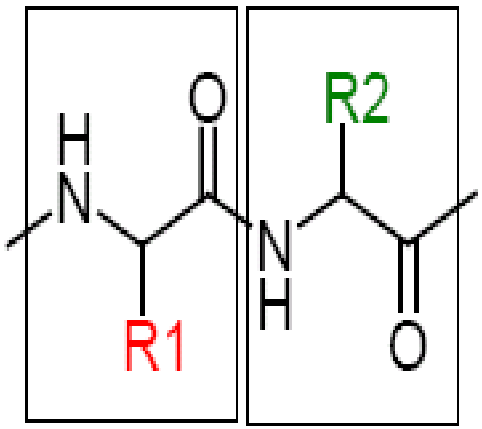
1. Scaffold- based libraries:



Definition: Core- structure, which all compounds of the library have in common

- Scaffold can consist of several single building blocks (here: Aminoacid and Aminobenzophenone)

2. Backbone- based libraries:



Building block A Building Block B

Further Examples:

Nucleic Acids, Carbohydrates

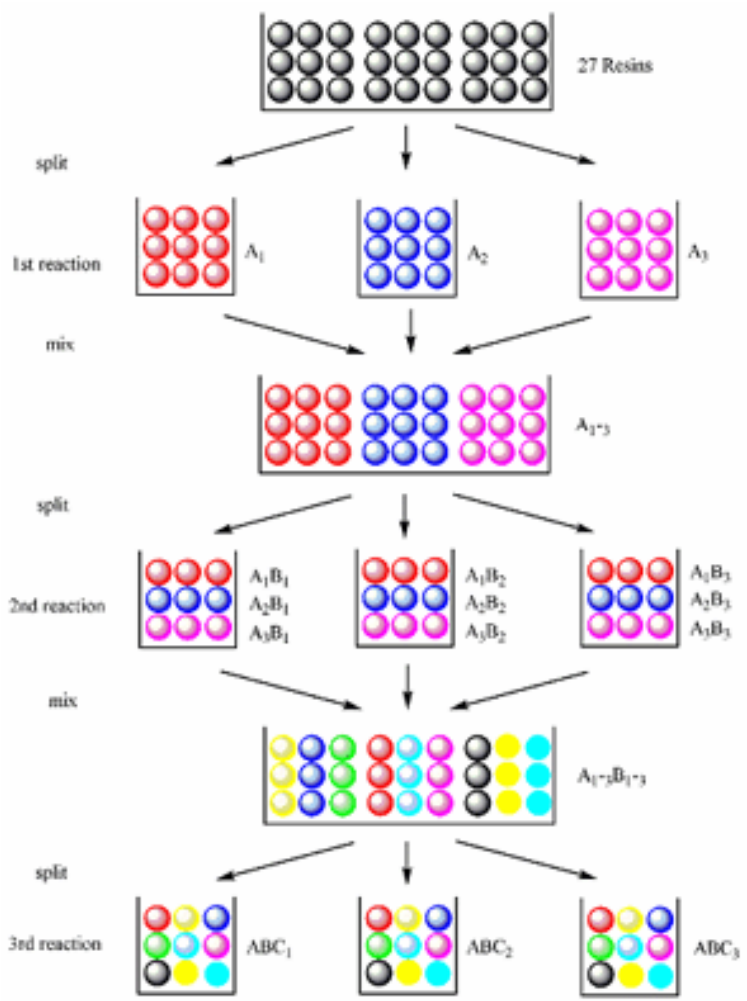
- Main function: Lead identification & lead optimization e.g. in the drug discovery process

- Different approaches to generate new libraries:

  - „Random libraries“
  - „Focused Libraries“

2. Synthetic methods & techniques

Split- Pool- Synthesis



- Splitting of the resin, coupling with building block A_1- A_3
- Pooling, washing, deprotection
- Splitting, coupling with B_1- B_3
- Pooling, washing, deprotection
- Splitting, coupling with C_1- C_3

- After a Split- Pool- synthesis: just one single compound is bound to each resin bead

  ⇨ „one- bead- one- compound“ library

- Split- Pool- Procedure requires a solid support

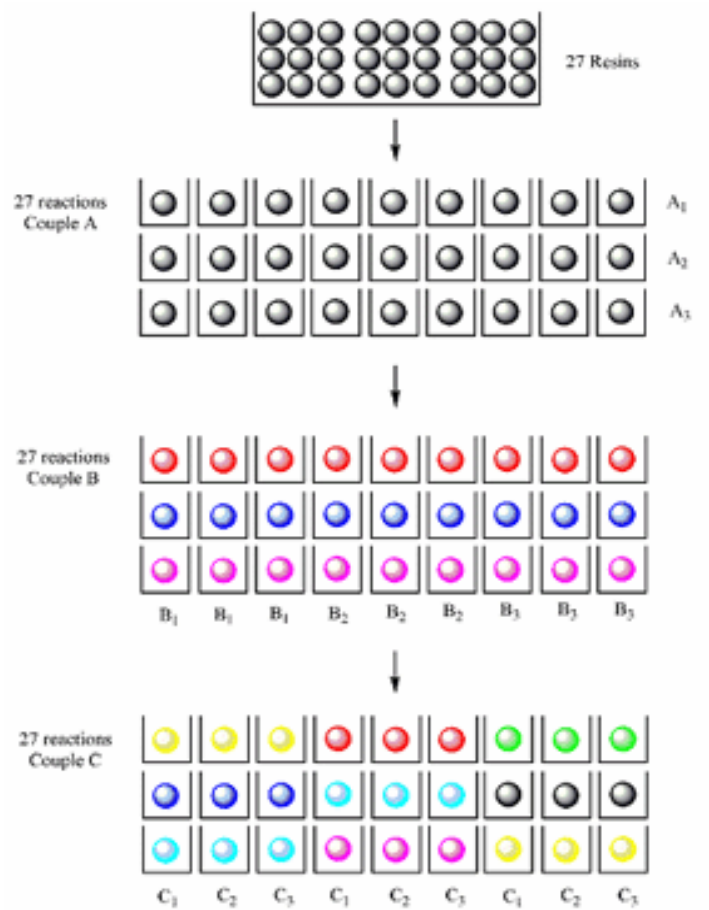
Advantages:

- Only few reaction vessels required
- Method of choice for large libraries (up to 10^5 compounds)

Disadvantages:

- Threefold amount of resin beads necessary
- Only little amounts of the synthesized compounds available

Parallel Synthesis



- Coupling with building block A_1- A_3 (1/3 of the resin beads for each building block), then washing, deprotection
- Coupling with building block B_1- B_3 (1/3 of the resin beads for each building block), then washing, deprotection
- Coupling with building block C_1- C_3 (1/3 of the resin beads for each building block), then washing, deprotection

- Concept: Compounds are synthesized in parallel using spatially separated compartments
- „One vessel - one compound“- philosophy
- Solid supported - as well as solution chemistry is possible

Advantages:

- Each compound is substantially „pure“ in its location
- Defined location provides the structure of a certain compound
- Easier biological evaluation

Disadvantage:

- Applicable only for medium libraries (several thousand compounds)

Techniques:

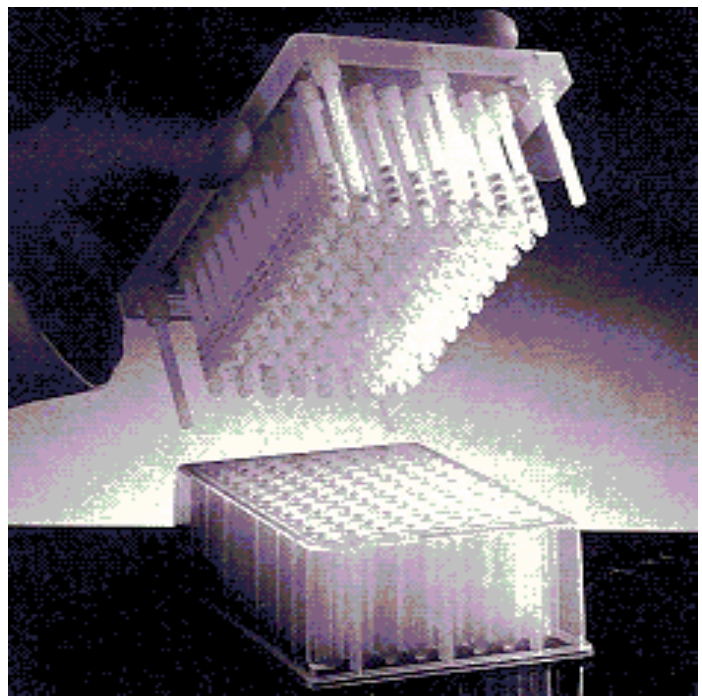
- **Conventional Technique: Using resin beads**

Reaction vessels: 96 well Microtiterplate (MTP) or an array of chromatography tubes etc.

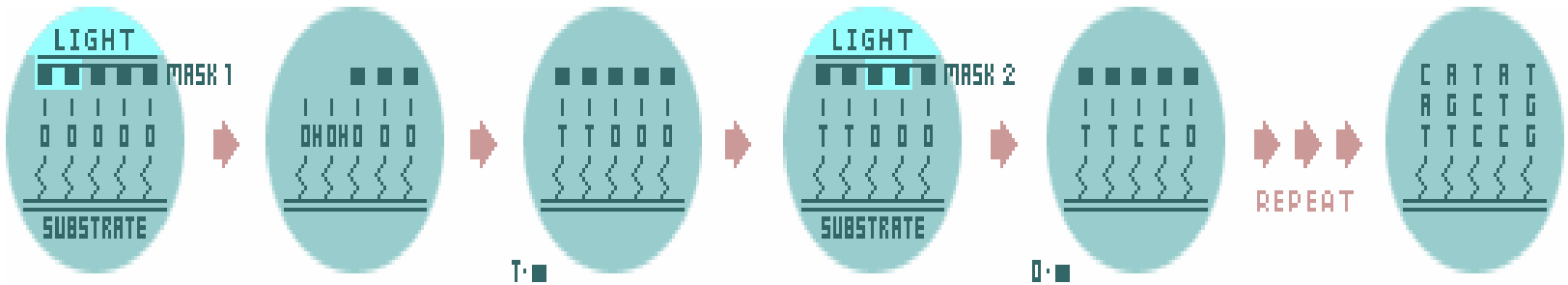
- **Multipin-Technique:**

Array of polimeric pins serves as solid support.

Polymer is functionalised with a variety of linkers



- **Light- directed synthesis on silica wafers (based on semiconductor photolithography):**



Key Points:

- Each set of building blocks contains a photolabile protecting group
- Only the building blocks which have been exposed to light can be coupled with another building block

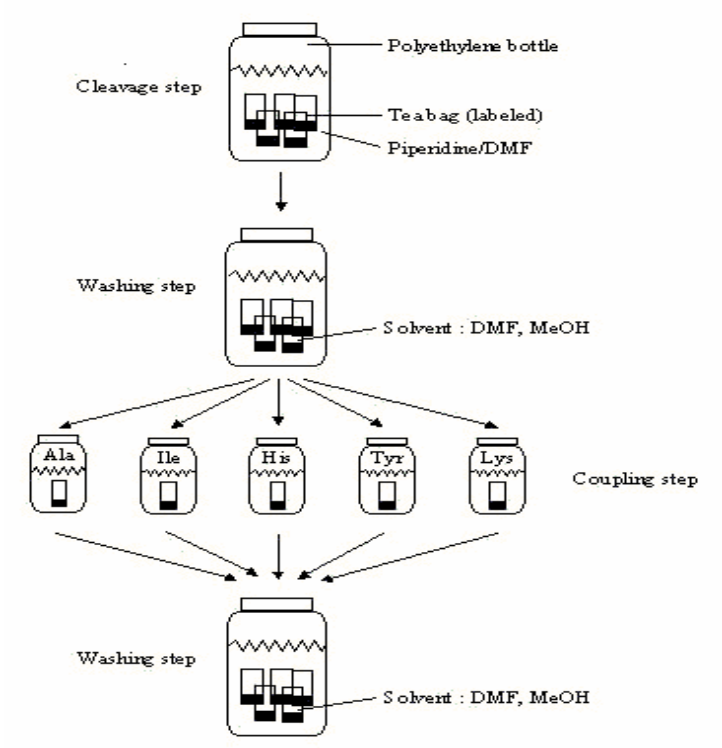
⇒ Pattern of masks and the sequence of protecting groups define the final structure of the compounds synthesized

⇒ Each member of the library is synthesized at a specific location on a functionalised silica wafer

- Libraries: 50000 compounds located in a 50μm square site

➢Compromise between parallel- and split- pool- synthesis: Tea- Bag- Method (Houghten et al., 1984)

- „Tea Bag“: 15 x 22 mm mesh packets filled with resin beads
- One single bead is (formally) replaced by a „macrobead“ (= teabag), that contains many small ones
- Split- Pool- Protocol occurs batchwise



Advantages:

•Greater Quantity of each compound is available at once (structural characterisation)

•Labelling of the tea bags: Easier identification of each compound

Characterisation of combinatorial libraries

- Analytical characterisation
- Biological evaluation

Analytical characterisation:

Evaluating the success of a combinatorial synthesis by determining the yield and the purity of the compounds

Separate single compounds:

- Purification by conventional techniques (e.g. chromatography), determination of the yield by weighing the substances, confirmation of purity by elemental analysis or NMR-spectroscopy

Compound mixtures:

- Highly sensitive methods are required

  ⇒ Mass spectroscopy coupled with HPLC or capillary electrophoresis (CE)

Biological evaluation:

Determining the most (bio-)active substance of a mixture

→ Bioassays (ELISA, FIA,...)

Methods:

1. On bead - screening:

- Compounds are still covalently attached to the solid support
- The solid-bound library is treated with a labelled biological target (receptor)
- Selection of the labelled beads (highly automated methods!) followed by structural characterisation
- Requirement: Solid support/ Linkers have to be water-soluble

Advantages:

- Useful for huge libraries (>10^5 compounds)
- Time - saving compared to other methods
- Re-use of the already assayed library

Disadvantage:

- Solid - bound compounds often show different bioactivity compared to free substances

2. Deconvolution

- Preparation of sets of sublibraries (Each of them contains compounds, where the identity of one single building block is known; the building blocks at the remaining positions contain all possible variations)
- Screening of the sublibraries provides the mixture with the highest bioactivity.
- Different proceedings: Iterative deconvolution/ deconvolution by positional scanning

Problems:

- Preparation of the sublibraries may be time-consuming
- The sublibrary with the highest bioactivity determined does not necessarily contain the most bioactive compound (synergistic effect of multiple compounds)

Sources:

- G. Jung: Combinatorial Chemistry, Synthesis, Analysis, Screening, p. 1- 34, Wiley VCH, Weinheim 1999 (Chemistry Library: 86/VK5500J95)
- Pictures: http://www.nvu.edu/classes/ytchang/book/c007.html