BALAJI COLLEGE OF PHARMACY

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COMBINATORIAL CHEMISTRY

Definition

Combinatorial chemistry is a term used for the systematic and repetitive combination of a set of building blocks having different structures, yielding a large number of compounds, which are further screened for their biological activity. The collection of compounds obtained using combinatorial chemistry is termed as a library.

Combinatorial chemistry is also referred as matrix chemistry and is a subfield of chemistry which aims at synthesizing diverse and large number of molecules by using a small number of reagents in all possible combinations. In contrast, the chemical entities by traditional method were prepared one at a time

History

Bruce Merrifield had developed the idea for combinatorial chemistry in the year 1960 while working on synthesis of peptides. Bruce received the Nobel prize for his invention of solid-phase synthesis. In the year 1982, Arpad Furka devised the method of "portioning-mixing". This method was reinvented and published by two independent groups, Lam et al in Arizona and Houghten et al in California as "split-and-mix' method and "divide-couple-recombine" method respectively.

Mechanism Involved in Combinatorial Synthesis

Principle

A set of chemical building blocks are brought together to react in a particular chemical reaction sequence to form numerous and diverse compounds with all possible combinations of the building blocks.

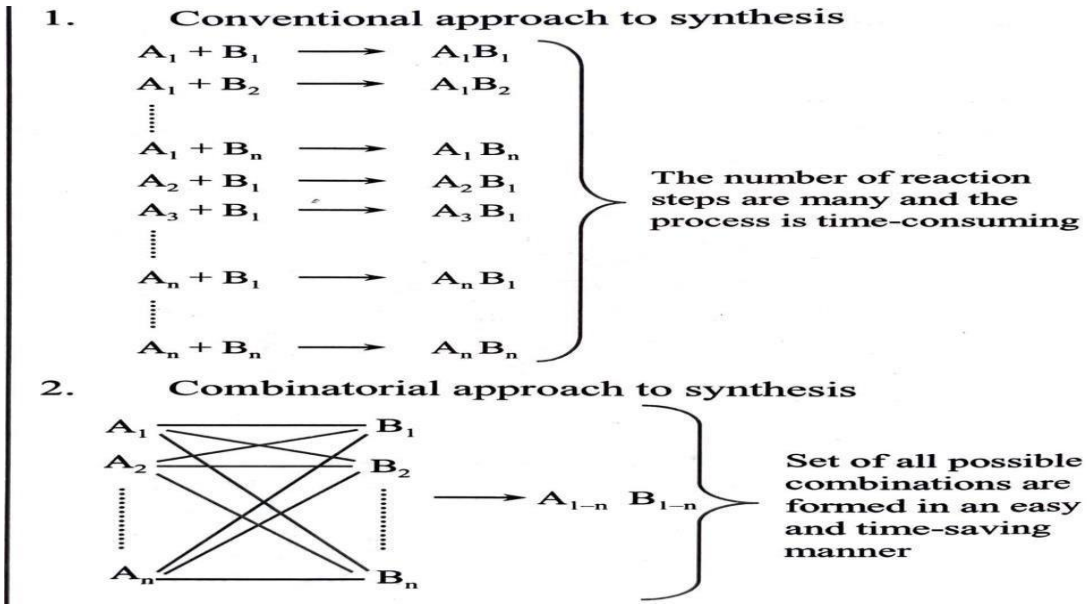
Mechanism

In the conventional or traditional methods of synthesis, a number of starting materials are made to react with each other but in a stepwise fashion. This approach makes the process time-consuming, expensive and laborious.

In case of combinatorial synthesis, the starting materials are made to react in all proportions simultaneously.

If two reactants are taken, then the first reactant is made to combine with all proportions of the second reactant and vice-versa.

Consider the synthesis of compounds with starting materials A and B with A_1 to An being the proportions of A and B_1 to Bn, being all the possible quantities of B.



Advantages

1.Large libraries of compounds can be created in a very short period of time.

2. The overall cost of producing a library of compounds by conventional synthesis is greater compared to the cost of combinatorial synthesis of the same library. Therefore, it is a cost-effective procedure.

3.The number of reactions required for the synthesis of library is less when compared to the reactions involved in individual synthesis of compounds present in the same library. Therefore, it is a time-effective method.

4. Since the library of several compounds is formed all the compounds can be assayed at once.

5.Yields highly diverse compounds which makes it ideal for new lead discovery.

Disadvantage

Large number of molecules are created which may not be bioactive. It can be said that combinatorial chemistry approach to synthesis is not a focussed method of synthesis.

Combinatorial Library

The set of products formed from a number of building blocks used in combinatorial synthesis is called a combinatorial library.

E.gs: Merck somatostatin mimics library, analog libraries etc

The size of a combinatorial library refers to the number of compounds present in it. It depends on the number of building blocks or reagents used in the reaction and also on the number of reaction steps. A combinatorial library usually consists of 10^2 to 10^5 compounds.

Types of Combinatorial Libraries

1. Based on Structure

On Structural basis, combinatorial libraries can be scaffold-based or backbone-based.

(a)Scaffold-Based Libraries

These libraries contain compounds with a common core-structure called scaffold. The scaffold may consist of several single building blocks for example amino acids and amino benzophenone.

(b)Backbone-Based Libraries

These libraries are synthesized using different building blocks.

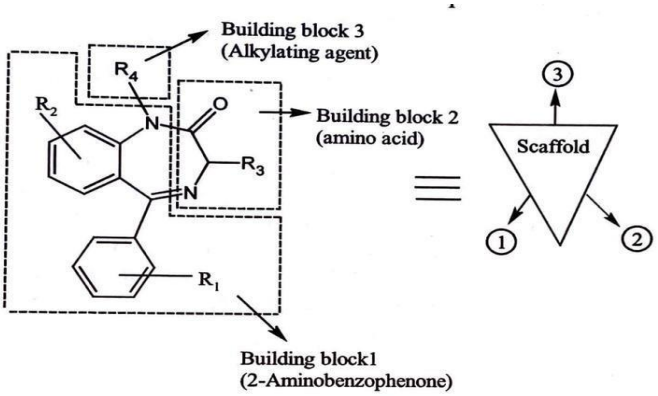


Figure: Scaffold-based Library Structure

b) Backbone-Based Libraries

These libraries are synthesized using different building blocks.

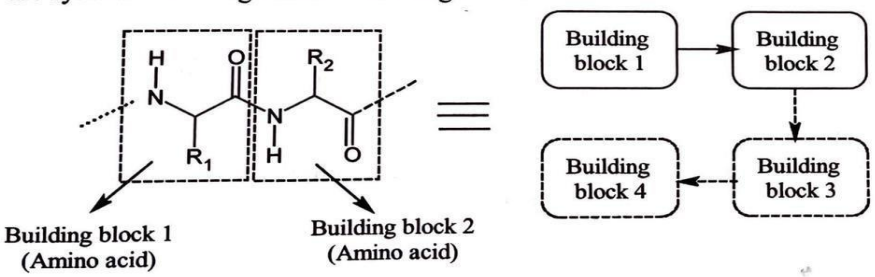


Figure: Backbone-based Library Structure

2.Based on the Approach Followed for Generation of Library

Based on the approach followed for synthesis, combinatorial libraries are classified as random libraries and focussed or targeted libraries.

Applications

The important applications of combinatorial chemistry have been discussed below.

1. Millions of structurally related compounds can be synthesized quickly by using combinatorial chemistry. It helps in finding the right combination of drug molecules.

2.Combinatorial chemistry provides fresh and promising leads for medicinal chemistry. Active lead compounds can be developed using combinatorial chemistry. These include

antineoplastic compounds, antiepileptic drugs, antimicrobials, benzodiazepine derivatives having various biological activities etc.

Example: Development of benzodiazepine derivatives which exhibited opioid receptor antagonist activity in addition to potent psychotic activities.

3. Traditionally, combinatorial synthesis was employed for compounds like peptides and oligonucleotides. But it is also being employed for synthesis of libraries containing compounds with a molecular weight of 500 Daltons or less.

Example: Synthesis of 1, 4-benzodiazepine library.

4. Human viral diseases like HIV-1, RSV and herpes simplex viruses 1 and 2 infections can be prevented by using antibody libraries synthesized through combinatorial chemistry.

5. Combinatorial synthesis has been applied in the development of enzyme inhibitors like carbamates and tetrahydro acridines (acetylcholinesterase enzyme inhibitors).

6. Combinatorial chemistry has been developed as a tool for lead optimization.

Example: Optimization of leukotriene D_4 antagonist was achieved using combinatorial chemistry.

7. Qualitative and quantitative characterization of drug database can be achieved through combinatorial libraries.

8. Combinatorial chemistry is used in the development of thinner insulators for the electronic gadgets.

9.Artificial nose is a device which is being developed to identify small traces of known molecules in a compound.

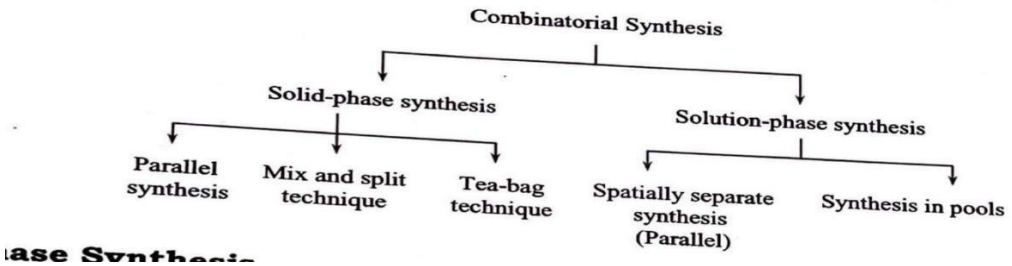
It is also used in the detection of landmines and toxic substances. It is constructed using optical fibres which have chemicals attached at one of their ends. These chemicals bind with traces of other chemicals and produce a colour change. These large libraries of chemicals are synthesized using combinatorial chemistry.

10. The inorganic molecules which emit phosphorescence when excited by light or electrons are called phosphors. These are used in lighting and computer monitors. Large libraries of phosphors of varied colours can be created using combinatorial synthesis.

Example: 'Christmas library' synthesized by symyx technologies, is a phosphor library consisting of 25,000 different phosphors using the combinatorial synthesis approach.

Techniques in Combinatorial Synthesis

A chemical library may be formed by either of the two modes of combinatorial synthesis; solid-phase synthesis or solution-phase synthesis.



- **Solid-Phase Synthesis**

Principle

Solid-phase synthesis utilizes a solid support for reaction between reagents. The first building block is linked to the support and made to react with the second building block. Finally, the product is formed on the solid support.

Requirements

The prerequisites for solid-phase synthesis include,Only one active centre of the molecule should be available at a time for the reaction to proceed.

Solid Supports

A solid support which can be a cross-linked insoluble polymeric material generally a resin. It should be inert to the ongoing synthesis.

Table: Examples of Solid Supports

	Examples	Comments
1.	Polystyrene resin	Prepared by the addition of 1% divinyl benzene to the polymerization mixture. It has good swelling ability and mechanical stability
2.	Tentagel	Co-polymer of polystyrene-polyoxaethylene graft
3.	Pepsyn	Polyamide resin especially used for peptide synthesis
4.	PEGA resin	Co-polymer of polyacrylic amide-ethylene glycol

Note: These resins are amphiphilic in nature and are used for the synthesis of polar compounds.

- **Protecting Groups**

Protection groups are useful for masking or protecting functional groups of the molecules which do not take part in the reaction. These are used to mask the groups not involved in the synthesis.

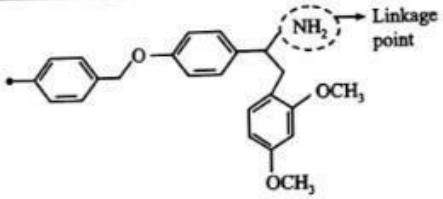
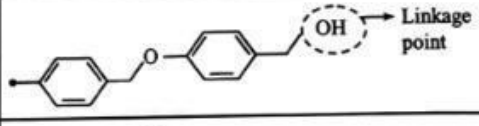
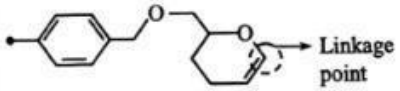
E.gs: Fluro methoxy carbonyl benzyl ester (FMOC), tertiary butyloxy carbonyl (TBOC).

- **Linkers**

Linkers are the bridges (covalent bonds) that join the reactant and the resin and are useful for maintaining the space between the bulky resin and the smaller reactant molecule to avoid the problem of steric hindrance during the process of synthesis. Its presence also makes the removal of the final product from the resin quite easy.

The linkers are selected based on the functional group present on the substrate and the functional group desired in the product.

Table: Resins with Linker Groups

	Linker Resins	Suitable for	Structures
1.	The rink resin	Attachment of carboxylic acid and the release of carboxyamide	
2.	The wang resin	Attachment and release of carboxylic acids	
3.	The dihydropyran derivative resin	Attachment of alcohol	

Mechanism

Mechanism

Building block 1(A) is first linked to a solid support with its active group exposed. The second building block (B) is added to this support-A complex. B binds at the exposed active centre of A. The chain is elongated in a similar way. The product formed remains attached to the support and can be cleaved using suitable technique. The excess reagents and by products can be removed from the product by simply washing the support with an appropriate solvent.

Example

Bruce Merrifield had used the solid-phase synthesis for generating a chain of peptides.

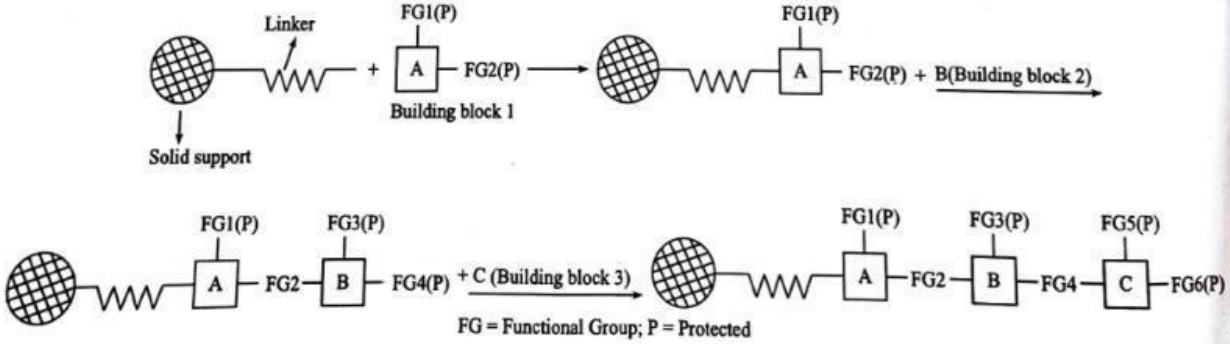


Figure: Solid-phase Synthesis

Advantages

1. Removal of excess reagent or by-products very easy. These can be removed simply by washing with appropriate solvent.

2. The solid polymeric support may be reused by regeneration of the resin if possible.

Techniques Involved in Solid-Phase Synthesis

1. Parallel Synthesis

In parallel synthesis, the products are formed in separate vessels but simultaneously. As several reactions are allowed to occur simultaneously, therefore multiple compounds are obtained at the same time. The compounds are obtained in pure form and as individual entities but not as mixtures. During the synthesis, the building blocks are fixed to beads (if using grid well in a plastic plate) or crowns (if using pins or grids of plastic rods). The products formed on the resin are removed by using the methods specific to the linker present on the resin. The structures of the products can be determined by studying the history of synthesis using the grid references of the wells. These structures can be reconfirmed by employing instrumental methods like NMR, mass spectroscopy.

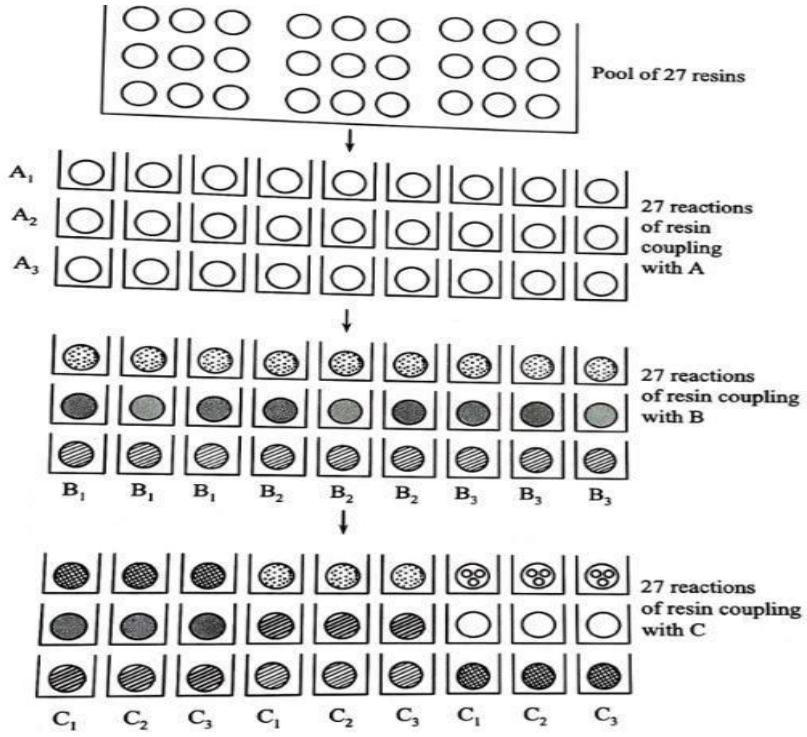


Figure: Parallel Synthesis

Advantages

1. Single product is obtained in a single vessel therefore; it is considered to be pure.

2. The structure of the compound can be determined from its location.

3. As the process follows 'one vessel-one compound' principle, screening for biological activity becomes easier.

Disadvantages

1. Only medium sized libraries can be generated.

2. Several vessels are required.

3.Large number of reactions have to be performed.

Once the active mixture has been identified, the next step involves the determination of active compounds which can be identified using deconvolution or encoding methods.

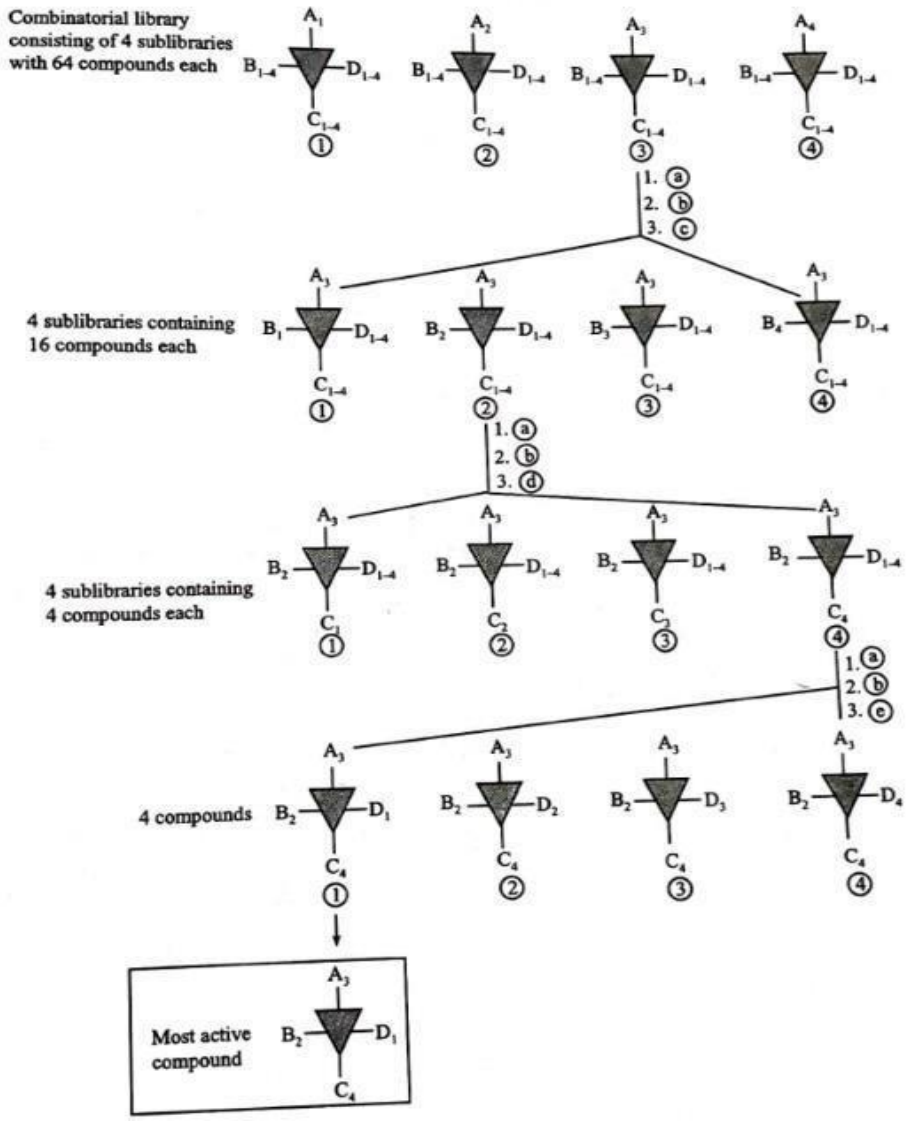
1. **Deconvolution**

Deconvolution is the process of identifying and isolating the most active compound from a mixture of compounds.

It can be performed either by iterative deconvolution or deconvolution by positional scanning.

a. **Iterative Deconvolution**

It involves the preparation of sublibraries by selecting of a defined building block. The sublibraries are screened for their bioactivity and the group with highest bioactivity is selected and further set of sublibraries are synthesized from it.The cycle is repeated till a single compound possessing high bioactivity is obtained.



In the figure,

ⓐ = Screening for bioactivity

ⓑ = Identification of group with high bioactivity

ⓒ = Resynthesis of pool ③ which shows high bioactivity

ⓓ = Resynthesis of pool ② which shows high bioactivity

ⓔ = Resynthesis of pool ④ which shows high bioactivity.

Disadvantages

1.To maintain the solubility of compounds in the pool, the compounds are taken in low concentrations. Therefore, it is difficult to detect activity of moderately active compounds.

2. It is suitable for screening only small number of compounds.

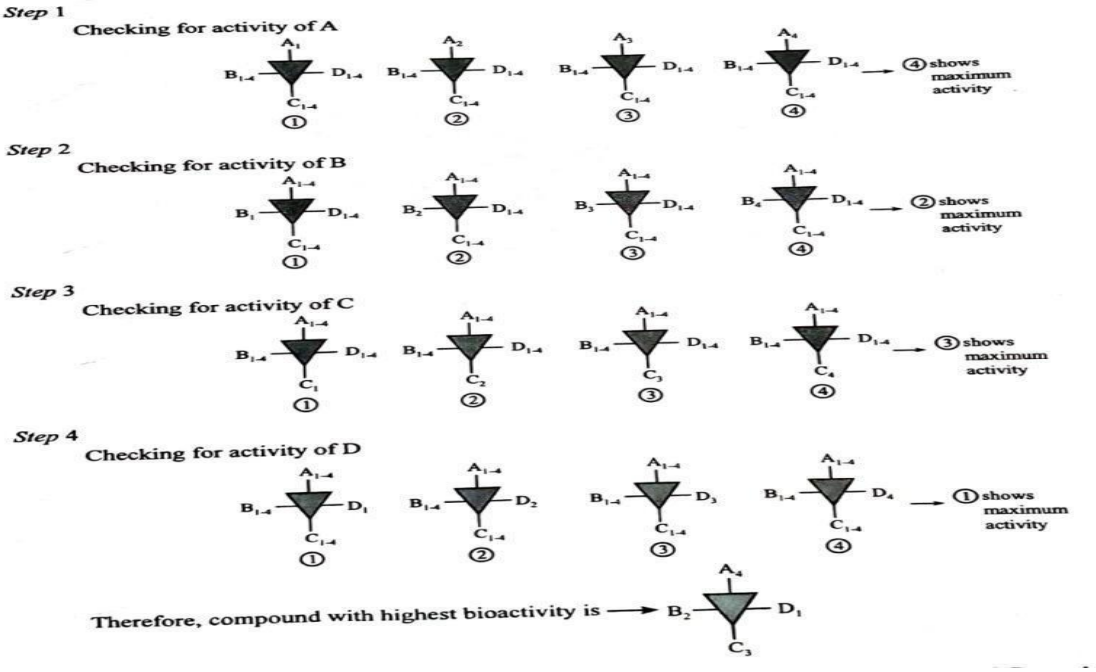
3.Biological activity produced may be synergistic, therefore a pool of moderately active compounds may be mistaken to contain highly potent compounds.

4.It is a laborious, expensive and time-consuming process.

b. Deconvolution by Positional Scanning

Deconvolution by positional scanning involves the preparation of separate sublibraries which contain a single defined building block at one position and a mixture of building blocks at other positions. Therefore, the number of sublibraries formed will be equal to the number of variable positions in the substitution pattern. This process follows a hybrid of parallel and mix and split methods of synthesis, followed by screening for bioactivity determination.

Synthesis of 16 sublibraries each containing 64 compounds.



Disadvantages

1.If there is no clearly defined substituent for a particular position, then all the combinations of substituents have to be synthesized which becomes tedious.

2. Compared to iterative deconvolution, there are lesser chances of identifying potent compounds using this method.

2.Encoding Methods

Encoding methods include chemical tagging method and mechanical tagging method.

(i) Chemical Tagging Method

A chemical molecule is attached to the linker on the resin. Each time a reaction occurs (reagent binds to resin), a code compound is attached to the tag. The product can be identified by assaying the chemical tag with appropriate method.

Oligonucleotides, peptides, haloaromatic tags, secondary amine tags etc., attached to the resin through amide linkages.

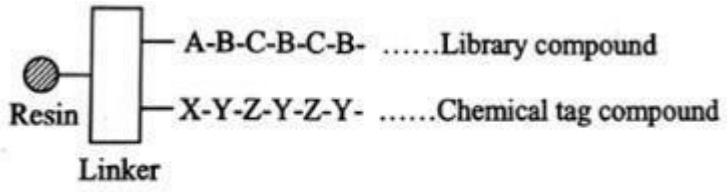


Figure: Chemical Tagging Method

(ii)Mechanical Tagging Method

In this method, resin beads are enclosed within small, porous packages. These packages are labelled with visual labels, electronic tags, radiofrequency tags, etc. When radio-frequency tags are attached to the resin, the tag is first read and then the bead is sent to a specific reaction vessel as per the information on the tag. This type of mechanism is known as direct sorting.

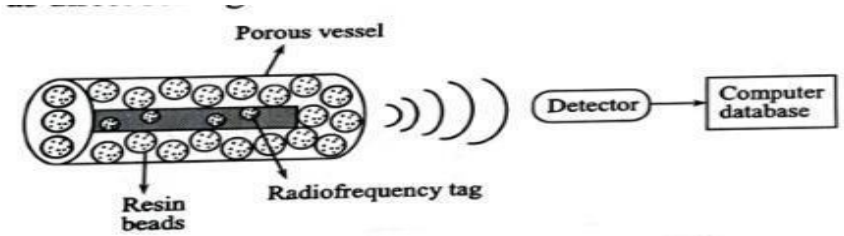


Figure: Schematic Representation of Radiofrequency Tagging

Advantages of Mix and Split Technique

1. Mix and split technique is a time and cost-effective technique.

2. Large libraries can be synthesized using very few reactions.

3.Less number of reaction vessels are required.

Disadvantages of Mix and Split Technique

1. Large quantity of resin beads are required.

2. The compounds synthesized are very little in amount.

3. Biological screening of compounds is difficult as a pool of moderately active compounds may be mistaken for a pool containing a few highly potent molecules due to synergistic effect.

3. Tea-Bag Method

Tea-bag method involves both parallel and mix and split methods of synthesis and was introduced by Houghton et al in the year 1986. This method involves the use of 15 x 20 mm mesh bags made of polypropylene which are properly sealed and labelled. The mesh-size of the tea-bag is such that it does not allow the resin beads to escape, but allows the entry of solvents and soluble reagents.

Individual tea-bags are immersed in separate vessels containing appropriate reagents. They can be pooled together, if the synthesis requires common reaction steps and also for resin deprotection, washing and neutralization steps. Thus, a single compound gets formed in a single tea bag and multiple compounds are formed as the number of tea-bags used are increase. Therefore, this process is said to be a mix of parallel mix and split synthesis and combines their advantages as well.

Solution-Phase Synthesis

Introduction

Solution phase synthesis of single compound libraries utilizes parallel synthesis technique whereas libraries of mixtures of compounds can be synthesized by separately reacting each of the members of a set of similar compounds with the same mixture of all the members of the second set of compounds.

The reactions involved in the synthesis are carried out in solution without any solid support. It may involve the use of soluble polymers instead.

Consider the example of reaction between 40 acid chlorides and 40 amines, leading to the formation of amides.

RCOCI + RNH → RCONHR+ HC

Acid chloride Amine Amide

In the first step of synthesis, each acid chloride (A) is made to react with an equimolar mixture of all 40 amines (B,1- 40).

In the second step, each amine (B) is made to react with an equimolar mixture of all acid chlorides (A,1- 40). The compounds formed were screened for biological activity.

Identifying the most biologically active acid chloride from the set will help in defining the acyl part (RCO) of the most biologically active amide. Similarly, identifying the most biologically active amine from the mixture will help in identifying the most active amide containing the amine residue.

In parallel synthesis, separate vessels are used for the formation of individual compounds whereas for synthesis in pools, lesser number of vessels are used as the compounds are formed as mixtures pooled together.

Techniques Involved in Solution-Phase Synthesis

1. Spatially Separate Synthesis/Parallel Synthesis

In this method, the compounds are synthesized in a spatially separate manner or in a parallel array. One example which can be considered for spatially separate synthesis in solution-phase is the Ugi reaction. The advantages and disadvantages of spatially separate synthesis is same as parallel synthesis.

Ugi Reaction

An isocyanide, an aldehyde, an amine and a carboxylic acid react in the presence of methanol, to give a major and a minor product. Several such reactions may be performed using 'n' moles of the reactants and the bioactive molecules can be selected from the range of products obtained by employing genetic algorithms.

2. Synthesis in Pools

Solution-phase synthesis in pools (i.e., the compounds in the library are pooled together) has been demonstrated through various experiments.

Examples

1. Smith was responsible for synthesizing a library of 1600 amides or esters.

2. Pirrung reported the 'indexed' combinatorial approach of synthesizing a library consisting of 54 carbamates, formed as a result of the reaction between 9 alcohols and 6 isocyanates.

3. Rebek synthesized a library by reacting a molecule of acid chloride, with an equimolar mixture of a variety of protected amines.

Disadvantage

Solution-phase synthesis offers difficulty in removing the impurities in each step of the synthesis.

Table: Differences between Solid-phase and Solution-phase Synthesis

	Solid-phase synthesis	Solution-phase synthesis
1.	The amount of products formed is small	Large amounts of products can be synthesized
2.	Product can be purified easily using techniques like washing, filtration, etc	Purification of the product is difficult
3.	It involves extra reaction steps of linkage and cleavage	Linkage and cleavage steps are not involved in this process
4.	Only limited chemical reactions can be performed	Wide range of chemical reactions can be performed
5.	The process has been automated successfully	Automation of solution-phase synthesis is not highly developed
6.	Reagents can be used in large volumes to drive the reaction to completion	Reagents cannot be used in large volumes