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CHAPTER 1

COMBINATORIAL CHEMISTRY: ROLE IN LEAD DISCOVERY

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1.1 INTRODUCTION TO COMBINATORIAL CHEMISTRY

Accelerating the drug discovery process by finding new chemical moieties (NCEs) faster is a major objective in the field of medical research. Hunting for a drug among the vast numbers of chemotypes, is a daunting task as is evidenced by the small number of molecules that are approved as drugs even though significant resources are expended on this activity. The drug discovery process generally involves identification of a validated biological target (enzyme, receptor, DNA & RNA) and developing a biological assay to hunt for novel chemotypes as a starting point for drug design. After finding a novel chemotype with the required biological activity multiple rounds of optimization with respect to pharmacokinetics and drug metabolism lead to clinical candidates. Drug hunters have evolved various strategies in order to perform this task efficiently, among these combinatorial chemistry is a strategy that has been used to efficiently sample the large chemical space that is available to them. The starting point or the initial discovery of a novel chemotype with the required biological activity is often called a “lead compound” and this phase of drug discovery is often called the “lead discovery” phase. A suitable lead compound is a small molecule with measurable and reproducible activity in the primary assay(s). In case the target is unknown then random screening may be need to test many thousands or millions of compounds in order to discover a lead. Combinatorial chemistry is designed to support the lead discovery process. It is a technique by which a large number of structurally distinct molecules can be synthesized together at a time and submitted for focused or varied pharmacological assay. Combinatorial chemistry has enabled the identification of many leads in drug discovery programs.

1.2 PRINCIPLES OF COMBINATORIAL CHEMISTRY

“Combinatorial synthesis involves synthesis of diverse analogs under the same reaction conditions, in the same reaction vessel followed by identification of the biologically most active compound for further development using high-throughput screening” (Furka, 2007; Jung, 2008).

The main principle behind this combinatorial chemistry is the synthesis of products all at a time from all possible combinations of a given set of building blocks (starting material). The collection of these synthesized compounds is referred to as a combinatorial library. This title is normally used

when synthesis is done producing compound mixtures; however, if the step-wise synthesis of individual compounds is made then they are designated as ‘arrays’. The combinatorial libraries are generally structurally related by a central core structure, a common backbone, termed the scaffold. The total number of compounds within the combinatorial library is determined by the number of building blocks used per reaction, and the number of reaction steps, in which a new building block is incorporated. The number of all synthesized compounds (N) is given by the equation $N = b^n$, where b is an equal number of building blocks used in each reaction (1, 2, 3, ...) and n is the number of reaction steps, in which a new building block is introduced (Gallop, 1994; Houghten, 1991; Swartz, 2000).

1.3 COMBINATORIAL LIBRARIES

The creation of libraries composed of hundreds or thousands or millions of molecules by combining and mixing solid supports and various building blocks or by parallel addition of substrates at a time during the organic reaction is the main goal of combinatorial chemistry. A library of compounds is an ensemble of compounds that have originated from the same assembly strategy and building blocks. The members of a library exhibit a common core structure with different appended substituents. This assembly strategy defines the position and sequence of addition of the building blocks. The assembly strategy can be illustrated by the examples shown in Figure 1.1.

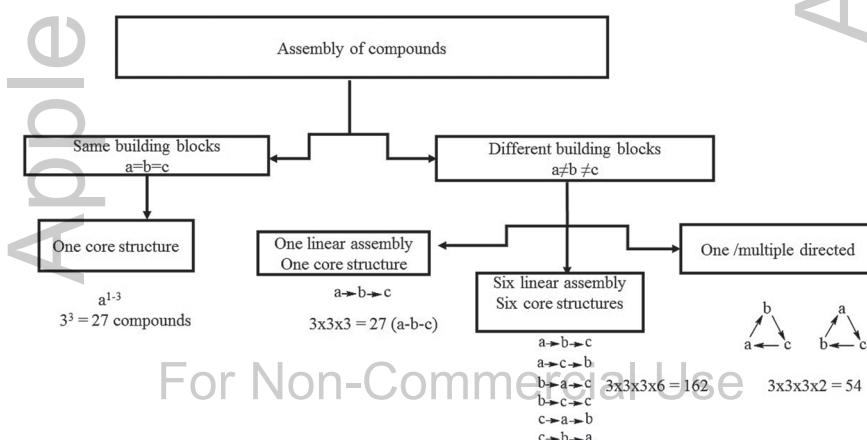


FIGURE 1.1 Strategy for preparation of assembly of compounds or libraries.

If a, b, and c are the three different building blocks assembled in a linear fashion, then the expected library would have either 27 products or in a nonlinear fashion the library would have 162 products with the basic core structure. If the building blocks are assembled in a cyclic fashion then the same building block assembly gives rise to 54 products in a library. In addition to this assembly strategy of reactive building blocks, the focus remains on the solid-phase chemistry that uses suitable resins and linkers on which the synthesis of the structurally diverse set of small-molecular weight compound libraries is achieved (Obrecht, 1998).

A library made by the process described above, suffers from low hit rates (low actives), partly because the library members possess poor structural diversity and have poor physicochemical properties, and partly because they are produced with the aim for quantity rather than quality. The key to synthesizing a diverse combinatorial library is a thorough knowledge about the relationship between the structure of a molecule and its physiological function.

An ideal combinatorial library should be relatively small and should contain chemically and functionally diverse compounds; each having a distinct biological activity, oral bioavailability and less toxicity (Welsch et al., 2010).

A good library, thus, has to take into account the requirements of both the products that will be made and the reagents that will be used to synthesize them. A general scheme for the design of a combinatorial library is demonstrated in Figures 1.2 and 1.3.

Following are the general considerations while designing a combinatorial library:

- a. overall similarity or diversity to a target;
- b. drug-like properties;
- c. predicted activity;
- d. deconvolution/decoding strategy;
- e. availability of reagents;
- f. cost of reagents;
- g. combinatorial constraints (Brown et al., 2000).

To achieve good success while synthesizing such a library, careful thought must be given in the following five steps:

- i) use of computational techniques to enumerate all possible compounds, creating a so called ‘virtual library’, keeping in mind factors such as reagent availability and synthetic feasibility;
- ii) choosing a subset out of these compounds which is representative of the virtual library;

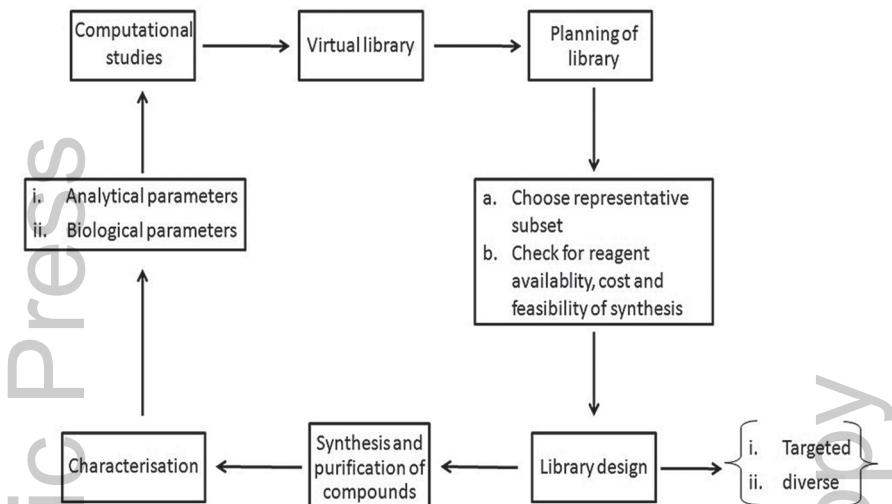


FIGURE 1.2 A schematic for the design, synthesis and characterization of a combinatorial library.

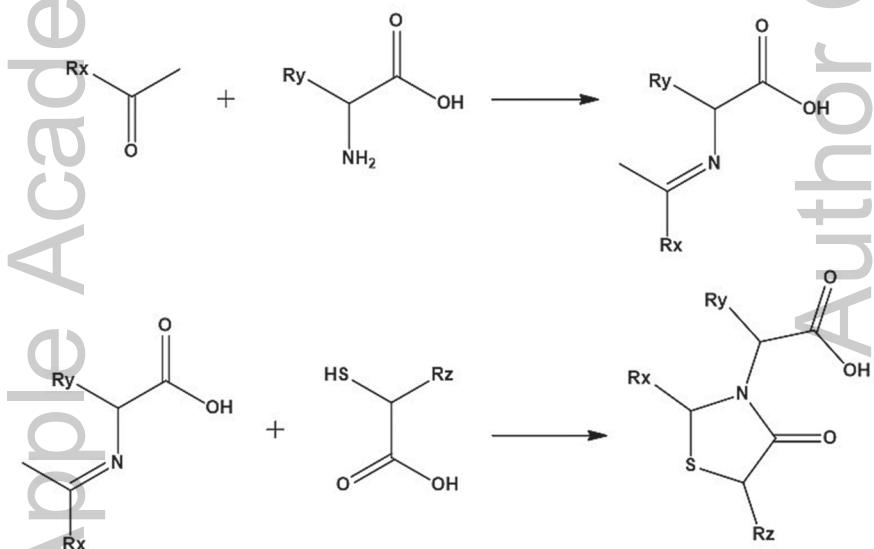


FIGURE 1.3 A general scheme to design a combinatorial library having three sites of variation.

- iii) library design;
- iv) purification followed by screening of the components in the library;
- v) determining the chemical structure of the active molecules (Lewis, 2002).

There are various methods for the preparation of a combinatorial library, the choice of the method being determined by the type of library desired. Some of these types will be discussed briefly.

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1.3.1 SCAFFOLD/BACKBONE BASED LIBRARY

The basic structural component that is the starting point for the production of a chemical library is termed the ‘scaffold’. It is a fixed part of the library on which functional groups may be attached or substituted, and it is common to all the compounds in the particular series. The scaffolds employed generally are ‘privileged structures’; molecules that are capable of acting as ligands for a wide array of receptors.

For a molecule to be successful as a drug, it has to be both selective and potent. These attributes are determined by the compound’s stereochemistry and rigidity. The stereochemistry depends on the number of chiral centers present in the molecule, which make the molecule selective through stereospecific binding on the target. Rigidity improves binding affinity as a consequence of the lower entropic loss on binding. Natural products, because of their functional role, are found with a large number of chiral centers and show greater rigidity compared to their synthetic counterparts, for these reasons, majority of scaffolds in use today are based on natural products.

Ideal properties of a scaffold:

- The scaffold should be easily synthesizable by simple reactions and inexpensive reagents.
- It should be readily available if the product is to be obtained from natural sources.
- It should be amenable to chemical modification and introduction of different functional groups.
- It should not have any highly reactive groups.
- It should be free from structural elements that could engender toxicity, such as alkylating agents or planar three ring aromatic systems.
- It should abide by Lipinski’s rule of five, that is,
 - i) the molecular weight should be less than 500.
 - ii) log P value should be less than 5.
 - iii) there should be less than 5 H-bond donors (calculated as the sum of O-H’s and N-H’s).

- iv) there should be less than 10 H-bond acceptors (calculated as the sum of O's and N's).

Scaffolds can be broadly classified into two types:

- (a) Functional scaffold: A compound having a fixed molecular function (e.g., antagonism at a particular receptor, inhibition of an enzyme) or a specific indication (e.g., antifungal or proapoptotic). A functional scaffold is used when there is a need to optimize properties of the lead (e.g., potency or bioavailability) while retaining the basic activity of the molecule. Their major use is for the generation of focused libraries which are target class-specific. However, their main drawback is their limited utility in the discovery of lead compounds for a wide range of targets, as they have been biased towards a target class-specific activity.
- (b) Structural scaffold: As the name suggests, structural scaffolds are molecules with specific structural features. The structural feature in question may be a particular ring system, chiral centers, functional groups and so on. These scaffolds may help in lead identification for a broad range of targets and are useful to increase the available chemical space. Since they do not have an activity bias, they are more universally applicable than libraries based on functional scaffolds (Brown et al., 2000; Eckard et al., 2010; Lewis2002; Welsch et al., 2010).

1.3.2 RANDOM/DIVERSE LIBRARIES

As mentioned previously, a diverse chemical library is a collection of compounds which aims to act at multiple targets. Since these compound collections are much larger as opposed to focused libraries, they have the obvious disadvantage of cost of reagents and screening. However, they may generate leads which may not be amenable by other methods because of the added chemical space they encompass. The logic is that greater the molecular diversity higher is the chance for a ligand to be recognized by a given receptor during screening.

1.3.3 VIRTUAL LIBRARIES

A virtual library contains all possible molecules that may be synthesized, taking into consideration the constraints of the chemistry being used and

reagents that are commercially available and compatible with the chemistry being employed. Care should be taken in the selection of a scaffold during the initial stages. Small, flexible scaffolds such as guanidine or lysine are preferred over hydantoins or benzodiazepines owing to the limitations in conformational space spanned by these ring structures. Commonly applied filters for scaffolds and substituents include hydrophobicity, molecular weight, hazardous or toxic features, price and biological relevance. Another commonly employed filter is ‘drug-likeness’ in that if they are to be found active against the target in question, then they should less likely show problems in their absorption, distribution, metabolism, excretion or toxicology, so as to shorten the drug discovery and development cycle (Fauchère et al., 1998).

1.3.4 TARGETED OR FOCUSED LIBRARIES

The common practice in drug discovery over the past few years is the creation of ‘diverse’ chemical libraries; compounds collections which aim to act at multiple targets or proteins (Harris et al., 2011) The logic behind such a design is that if more number of compounds are synthesized, greater will be the chemical space that is covered and hence greater the chances of generating a lead compound (Drewry and Young, 1999). But unfortunately, the synthesis and screening of such a large collection of compounds has proved to be a costly affair. As a result, the focus has now shifted towards building ‘targeted’ or focused’ libraries. These are libraries that are smaller, containing higher quality compounds that have been designed keeping in mind a specific target or protein. The obvious advantage of designing such a library is that they eliminate compounds which are unlikely to bind to the target; as a result, fewer number of compounds have to be screened to identify leads. Also, the hit rates observed with such a library are greater compared to diverse libraries, thus reducing the hit-to-lead timescale.

An understanding of the target is an essential prerequisite for designing a focused library. The design can be based on the structural information available about the target (e.g., kinase receptors, where an abundance of crystallographic data is available) or sequence or mutagenesis data (e.g., ion channels). An alternative approach can be based on known ligands for the target in question provided high quality structural information or prior knowledge of the binding site interactions is available. Targeted libraries are usually obtained as a subset of much larger collections using computational

techniques. These libraries commonly employ a single core entity with attachment points to which specific substituents, or side chains can be appended to arrive at the desired compounds. When selecting the scaffold and the substituents, synthetic feasibility must be given careful consideration. Generally, all possible combinations of compounds are considered and a subset is chosen for synthesis. Thus the process of selection of compounds out of all possibilities is very important (Harris et al., 2011). This selection process is governed by the chemical space of interest for the target in question, followed by finalization of the set of molecules which will represent that space. A filter may be the introduction of a constraint that the molecules must be ‘drug-like’, that is, they must have properties which give them a reasonably good chance of having good oral bioavailability and a decent pharmacokinetic profile (Drewry and Young, 1999).

Methods for generating targeted/focused libraries are:

(a) *In silico based design*

This involves use of available structural information such as is available in the Protein Databank to help dig out drug receptor interactions. In the absence of experimental structures, homology models can be built for many target proteins, since there is an abundance of available template proteins. The first step in *in silico* design involves choosing the right template or the scaffold. This is achieved by docking various core molecules into the binding site of the target. Once the scaffold is finalized, substituents are attached to each possible position on the template, for assessing substituents that provide the best fit for the target. These combinations of scaffold and substituents are then ‘scored’, and the best ones are chosen as the subset for further synthesis, based on electrostatic and steric complementarity with the target. The correct docking of the template along with the substituents is crucial for the success of this approach (Beavers and Chen, 2002).

(b) *X-ray crystal structure based design*

This method is used in the design of fragment based libraries when high resolution X-ray crystal structures of the target are available. This helps to generate small molecules of molecular weight lower than normal drug molecules. Fragments which bind well to the target are identified and subsequently linked to each other through a scaffold. The structure based chemical library that is generated is bioassayed later. However, a limitation of using X-ray crystallography to

design a library is the static nature of the crystal. Ligand-binding pockets are flexible in nature, and a protein-ligand structure solved by this method does not reflect this aspect, which might result in errors in the library design.

(c) *Library design based on NMR*

The technique, abbreviated as ‘SAR by NMR’, uses a high-field NMR spectrometer and large quantities of a pure, labeled protein. Isotopes such as ^{15}N , ^{19}F or ^{13}C are used to monitor protein-ligand interactions, provided there is adequate structural information on the binding site. Very weak binders (having activity in the millimolar range) can be detected by this method (Orry et al., 2006).

Assessment of the virtual library/subsequent synthesis: Virtual libraries are invariably too large to be synthesized in their entirety, and are frequently redundant in terms of the chemical space they cover. A process termed as ‘library subsetting’ is employed to obtain a representative subset of this virtual library, and this subset is termed as a ‘design library’. Library subsetting involves assessment of the virtual library in order to quantify its diversity (Pandeya and Thakkar, 2005). The objective of these investigations is to obtain maximum diversity while keeping the library as small as practically possible. Structural descriptors are used as a means of assessing the molecular diversity, which provide information about hydrophobic, electronic, steric parameters etc., which can separate active moieties from inactive ones. The information gleaned from such a study depends to a large extent upon the choice of descriptors used. Such a library should display ‘neighborhood behavior’, that is, minor changes in diversity should produce minor changes in biological activity, which can be reflected in a plot of the changes in the biological activity vs. the difference in descriptor values. This process generates a set of compounds that is maximally diverse, out of which any number can be synthesized depending on the desired size of the design library (Fauchère et al., 1998).

The subset of molecules chosen for synthesis should be representative of the chemical space covered by the entire library. An important factor that governs the decision to synthesize a set of compounds is the diversity it adds to the existing compound collection. The collection may be diverse with respect to the chemical space it covers, but the information it contains should not overlap with that present in the preexisting collection.

While classifying a library as a diverse one, care should be taken to mention whether the diversity refers to the substituents attached to the scaffold or

the product molecule as a whole. Generally, it is easier to analyze diversity in substituents. For example, a diversity analysis carried out in a scaffold with three points of diversity, using ten different substituents would require screening of a total of 30 compounds. However, analysis of the fully enumerated product set would involve 1,000 compounds. Analysis of diversity in substituents, although convenient may not be a reliable method as the fragments may interact with each other. For this reason diversity calculations are generally performed on the final products (Drewry and Young, 1999).

1.4 CHARACTERIZATION OF LIBRARIES OBTAINED FROM COMBINATORIAL SYNTHESIS

In the last few years, combinatorial technology has rapidly evolved from the production of peptide libraries to synthesis of small organic compounds. Synthesis as well as screening has become largely an automated process. Because of this, the bottleneck has shifted from production of compound libraries to analytical characterization. Analytical characterization involves use of various available analytical techniques to comprehensively dig out information about the designed library. Ideally, analytical characterization should not only provide the medicinal chemist with information about the final quality of the synthesized library, but also help him during the synthesis of the said library with vital parameters such as optimization of reaction conditions, selection of building blocks, etc. Thus the key to a successful characterization of a compound library is carrying out chemical and analytical work in parallel with compound library development.

During analysis of any compound obtained as the product of a chemical reaction, a synthetic chemist seeks to address three key issues: identity (have we been successful in synthesizing what we wanted?), quality (is our compound a pure one?, have we also generated some undesirable side products?), and quantity (how much have we made?, what is the yield?). Traditionally, analysis involves structure elucidation using both Nuclear Magnetic Resonance (NMR) and high resolution Mass Spectrometry (MS) with additional confirmation provided by IR spectroscopy. Purity estimation is carried out by chromatographic techniques, either HPLC or TLC with UV/VIS detection.

By definition, a combinatorial library is a collection of compounds that is highly diverse, synthesized in small amounts, and which may be a mixture

of several components. This obviously means that the analytical chemist will have to work with a set of compounds that have varying properties, develop unbiased methods which apply to the entire collection, provide results in a short span of time, cost effective, all these while working with a small quantity of the material. It goes without saying that this will put a strain on the analytical method to be used. The technique involved should be fast, robust, informative, sensitive, and should be conveniently combined with separation techniques (for ease of handling mixtures of compounds). Furthermore, analytical demands are different at various stages of library development. In the early stages, information content is crucial when it becomes important to elucidate the structure of the desired compound and any other side product. During evaluation of building blocks and optimization of reaction conditions, the priority is to combine the analytical method with a separation technique. At the final stages, when thousands of compounds are to be analyzed, the primary requirement is high throughput. We shall first consider the strategies to be used in different stages of library development, followed by an application of the methods to various types of libraries.

1.4.1 ANALYTICAL METHODS USED FOR FINAL CHARACTERIZATION OF COMPOUND LIBRARIES

After library development has passed the stage of optimization and development, the focus is more on high-throughput rather than detailed analysis. Thousands of samples have to be analyzed for characterization of the production library. At this stage, analysis is carried out to provide confirmation that the intended compound has been successfully synthesized. Other goals are quantification of the product and purity assessment.

- a. Confirmation of structure using mass spectrometry: It is the method of choice for high throughput structure confirmation as the technique is based on the measurement of a very basic parameter of a compound – the mass to charge ratio. The technique does not depend on the presence of any functional groups or chromophores in a molecule. It is a highly sensitive method, femto moles of a sample can be measured easily. It is also fast, with analysis times of only a few seconds. The ionization techniques commonly employed are electrospray ionization (ESI), chemical ionization (CI) and matrix assisted laser desorption/ionization (MALDI).

- b. NMR: Although NMR is a highly informative technique for compound characterization, NMR has limited application in this field because of the following drawbacks. It is slow, insensitive, consumes expensive deuterated solvents and requires homogenous samples.
- c. Infrared (IR) Spectroscopy: IR spectroscopy is mostly used for functional group identification. The applications include determining the extent to which the reactant gets converted to the product, and observing the time taken for completion of the reaction, by observing the changes in the intensity of bands which correspond to specific functional groups.
- d. Purity assessment: The technique of choice is high performance liquid chromatography (HPLC). HPLC is ideal for high throughput screening, since it is easy to automate, robust, has high resolving power and is not biased towards certain features of a molecule, such as charge.
- e. Quantification: Quantification along with the requirement of high throughput is very challenging. Neither UV nor MS detectors are capable of carrying out quantitative analysis without a reference standard. The key to this is to use a proper reference standard and arrive at conclusions that are based on a combination of results from various analytical techniques.

1.5 GENERAL METHODS OF COMBINATORIAL SYNTHESIS

1.5.1 BIOLOGICAL LIBRARY APPROACH

A biological library is made up of a pool of microorganisms, which express various polypeptides on their surfaces. Each microorganism used in the library display shows one kind of peptide sequence, and represents a clone. Every clone which is part of the library can be replicated multiple times, and the resulting progeny will express the same polypeptide on its surface. A specific sequence of DNA coding for the polypeptide is inserted into the microorganism for library construction (either into a plasmid or into its genome). Library construction usually starts with synthesis of degenerated oligonucleotides. The nucleotides are single stranded; they are made double stranded and ligated into appropriate vectors. This is followed by

insertion into host cells where they undergo replication and are displayed (Figure 1.4) (Miertus and Fassina, 2014).

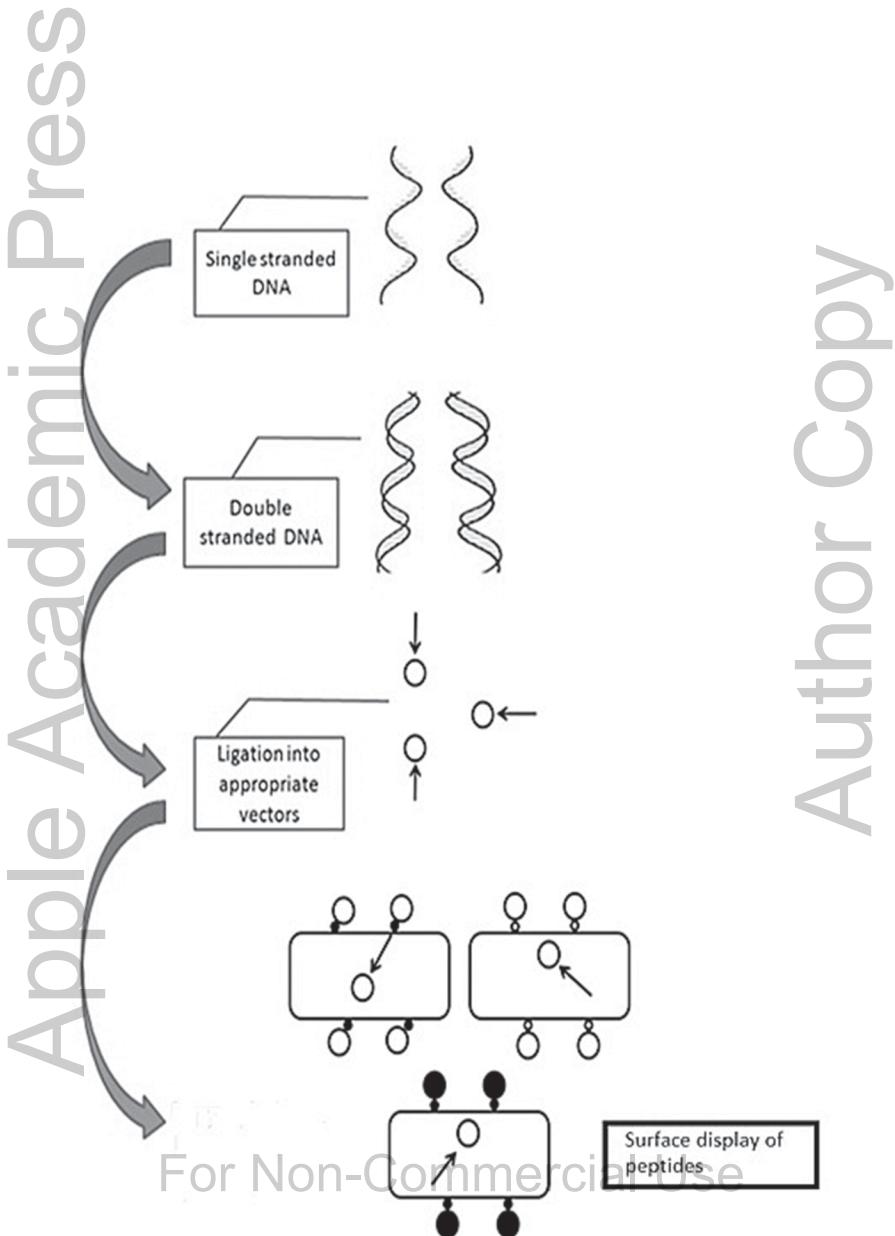


FIGURE 1.4 Construction of a biological display library.

1.5.1.1 Plasmid Display

The major applications of plasmid display lie in the field of protein engineering. Phage display and polysome have been used, but in most cases, their use is limited to the display of peptide libraries.

Plasmid display is much more efficient in displaying target proteins because:

1. As compared to the limited space on the surface, larger number of proteins can be produced in the cytoplasm
2. Following gene expression, subsequent translocation into the cell surface is not required
3. The DNA structure employed in this display has high stability and so different conditions (salt concentration, temperature, pH) can be used for screening.

The methodology employed is as follows: plasmid display involves directly linking proteins to plasmid encoding target genes in the cytoplasm. It is the process of synthesizing a protein *in vivo*, followed by linking it to the plasmid in the host cell's cytoplasm as shown in Figure 1.5. The process involves fusing the target proteins to an anchor, which is mainly the DNA binding proteins, which then binds to the appropriate DNA sequence in the plasmid. Thus the target protein is linked to the plasmid via the DNA binding protein, forming a plasmid protein complex. This complex is purified using an affinity resin and eluted. The plasmid is repurified and transformed into competent *E. coli* cells on an agar plate. The transformed cells can be used for the next round of screening. Formation and maintenance of a strong protein

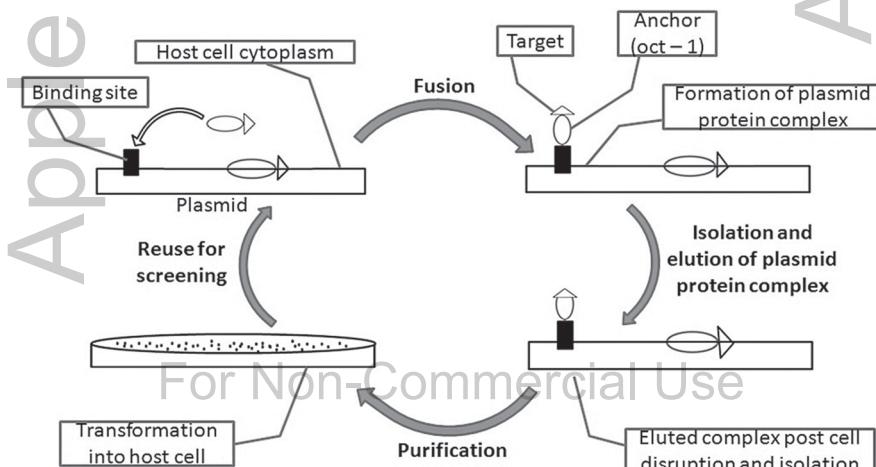


FIGURE 1.5 Plasmid display.

plasmid complex during screening are the most important issues that need to be addressed. Thus it becomes important to choose the anchor very carefully.

1.5.1.2 Polysome Approach

Although libraries displayed on phages or plasmids are a rich source of ligands for numerous targets, both display systems depend on *in vivo* gene expression, and the diversity and size of the library is eventually determined by the biological constraints and transformation capacity of the host microorganism. Polysome libraries are *in vitro* display systems, which, as the name suggests, displays the library on polysomes. They have the advantage of not being dependent on bacterial transformation, and they can also generate libraries which are much larger and much more diverse compared to other cell based systems. It is very useful for peptide expression. A discussion on the construction of a peptide library using polysome display follows (Figure 1.6).

1.5.1.2.1 Library Construction

The method begins with the construction of a DNA library which is made up of random peptide coding sequences. This library is incubated in a DNA dependent, *E. coli* coupled *in vitro* translation/transcription system. After terminating protein synthesis with an appropriate reagent such as

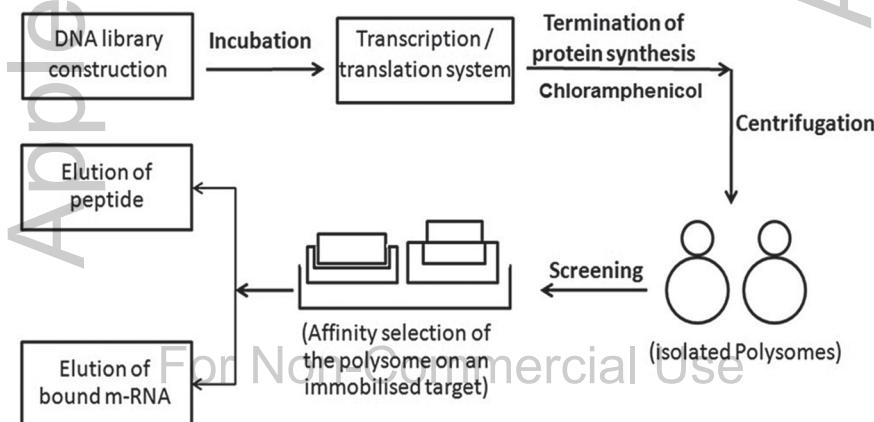


FIGURE 1.6 Polysome display of peptide libraries.

chloramphenicol, the polysomes are isolated by high speed centrifugation. The polysomes consist of peptides, which are linked to their corresponding encoding mRNAs. These are screened using affinity selection of the peptides on an immobilized target. The bound mRNA is dissociated using EDTA and by polymerase chain reaction, can be used to produce a template for new rounds of in vitro selection and synthesis. Identification of the enriched peptides is carried out by cloning the template and sequencing a portion of it. Their binding specificities may be determined using appropriate assays.

1.5.1.3 Phage Display Libraries

Phage display is a convenient method to generate and screen combinatorial libraries for various purposes. It is mostly used for the design of peptide libraries. Using concepts of molecular biology, it is possible to design phage libraries which display 10^{10} different peptides, and subsequently screen them for peptide ligands to metals, proteins, substrates of proteases or cell surfaces within a few weeks.

Vectors: Most phage display systems involve a bacteriophage that infects *E. coli*. The most popular bacteriophage is M13. Their genomes are small and can therefore be easily used for the construction of large libraries. They do not cause cell lysis unlike other bacteriophages. The M13 bacteriophage particle consists of one DNA molecule and five coat proteins. As displayed in Figure 1.7, these coat proteins are protein products of 5 genes, namely genes 3, 6, 7, 8, and 9, and are abbreviated as p3, p6, p7, p8 and p9, respectively.

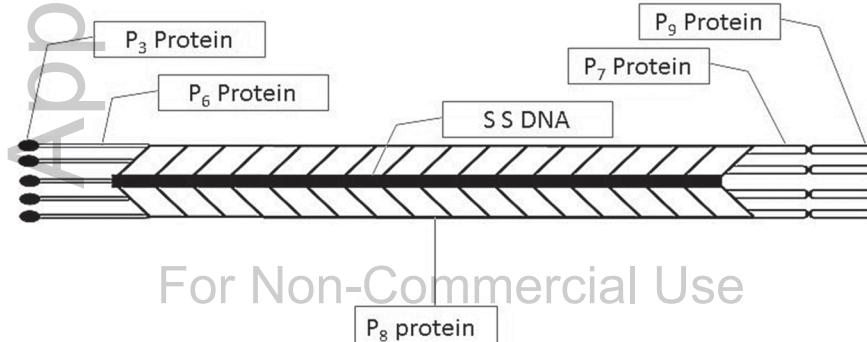


FIGURE 1.7 The M13 bacteriophage particle.

The coat proteins package the DNA into a stable molecule that is infectious for *E. coli* (Huang et al., 2011).

Design/Infection: The phage particle is first attached to the *F. pilus* of a male *E. coli* cell. This is mediated by the pIII coat protein. Following entry of the bacteriophage into the cell, its coat is removed and DNA replication is initiated using the host. The DNA is packaged into a new phage particle, which is extruded via the cell wall into the medium (Miertus and Fassina, 2014).

Advantages of phage display combinatorial peptide libraries:

- i) Phage display is perfect for the construction of peptide libraries.
- ii) The generated libraries are highly stable, and can remain so for an indefinite period of time if stored under proper conditions.
- iii) Phage libraries can be conveniently renewed by infection of bacteria and harvesting the particles from cultures.
- iv) Ligands of interest can be identified easily, in a short span of time and with modest effort.
- v) Using DNA sequencing, the identity of selected peptides can be deduced very simply and at a low cost.
- vi) Large libraries can be represented in a very small volume. This makes screening of the libraries a very simple process (Huang et al., 2011).

1.5.2 CHEMICAL APPROACH LIBRARY SYNTHESIS USING PARALLEL SOLID PHASE METHOD

As the name implies, parallel synthesis involves synthesis of a series of compounds carried out simultaneously. Each reaction is carried out in a different vessel and all the operations are carried out in parallel. At the end of the reaction, the products are individually cleaved from the support and collected in designated vessels. The advantage of parallel synthesis is that the number of operations carried out is the same; the only change is that it requires serial transport of the solvents and reagents into each reaction vessel. The collection of compounds prepared by this method is called compound libraries.

1. *Geyzen's Multipin method:* The Multipin method was developed by Geyzen, and was originally designed for synthesis of peptides. The apparatus involved a series of microtiter plates as reaction vessels, and cover plates with polyethylene pins that fitted into the vessels. The tip of the polyethylene rods (also referred to as ‘pins’) were

coated with polyacrylic acid. The wells contained a solution of the coupling reagents and the amino acids used to build the peptides. The coated tips of the pins were immersed into the wells, and kept in solution until the reactions were complete. The peptide sequence depends on the order of amino acids added to the well. The assay can be carried out while the peptides are still attached to the rods. (Furka, 2007). Screening is carried out using enzyme linked immunosorbent assay (ELISA), where the binding affinity of the peptides to antibodies is determined. (Pandeya and Thakkar, 2005). Recent applications of the multipin method include studies on T-cell proliferation, Substance P-receptor binding and SAR studies on ligands for endothelin receptors (Zhao and Lam, 1997).

2. *Houghton's tea-bag approach:* Developed by Houghten, the tea bag method enables preparation of over 200 fully characterizable peptides at a time, on a much larger scale as compared to the Multipin method. The technique involves placing solid phase resins in solvent permeable polypropylene bags, which are called 'tea-bags' (Rinnová and Lebl, 1996). Amino acids are then coupled to the solid phase resins by immersing the bag in a solution of individual activated monomers (Pandeya and Thakkar, 2005). These are then grouped, according to sequence of amino acids that will be attached in a particular synthetic step. Washing and deprotection are carried out by mixing the tea bags in a vessel, followed by their separation and characterization (Rinnová and Lebl, 1996). The advantages here are smaller number of operations and smaller number of reaction vessels (Furka, 2007). Applications include epitope mapping and studying antigen antibody interactions (Rinnová and Lebl, 1996).
3. *SPOT technique of Frank:* The SPOT technique is similar to the Multipin method, but more economical and easier to perform. The only difference is that cellulose is used as the solid support in place of polyethylene pins. In the presence of certain activating reagents, the solution of protected amino acids are 'spotted' on to the functionalized cellulose paper (Zhao and Lam, 1997). These spots can be considered as reaction vessels where the reactions take place, and as many as 2000 peptides can be synthesized at a time (Furka, 2007) (Figure 1.8). By treating the cellulose membrane with appropriate reagents, the entire synthetic procedure can be carried out simultaneously. The peptides that are formed can be either analyzed directly

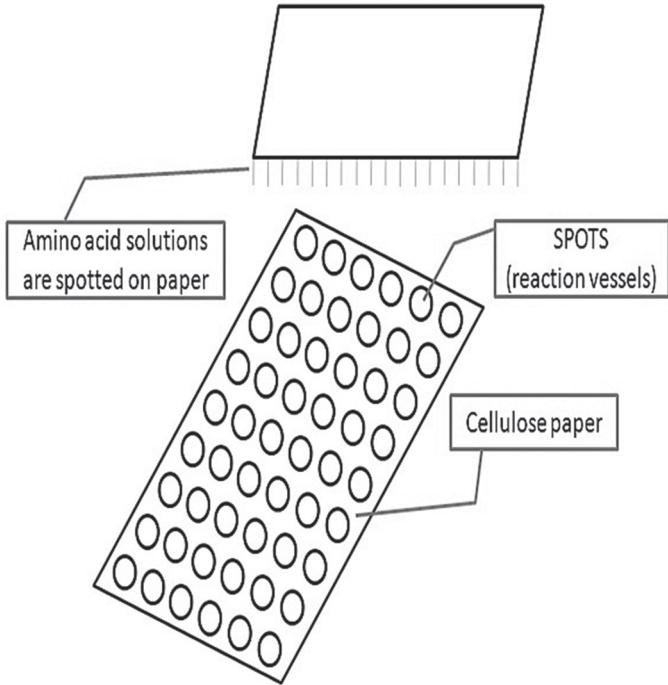


FIGURE 1.8 SPOT synthesis.

using ELISA based studies or cleaved and applied in solution (Zhao and Lam, 1997).

4. *Light directed peptide synthesis:* This technique is based on the principle of spatially addressable synthesis, in which the identity of a compound depends on its location on a substrate. Here, the addition of a reagent is carried out on predetermined sites on the solid support. The technique is a combination of photolithography and solid phase peptide synthesis. It is demonstrated in Figure 1.9. The substrate is attached to a photolabile protected covalent linker, the protecting group usually being a nitroveratryloxycarbonyl (NVOC) group. With use of light, the photolabile linker is removed. Removal of these groups, also called deprotection, causes activation of selected areas. The activation is followed by amino acid coupling which occurs selectively in the activated areas. The amino acid solution is removed, and a different region on the substrate is activated, followed by coupling. The product is determined by the deactivation pattern, and the sequence of reactants

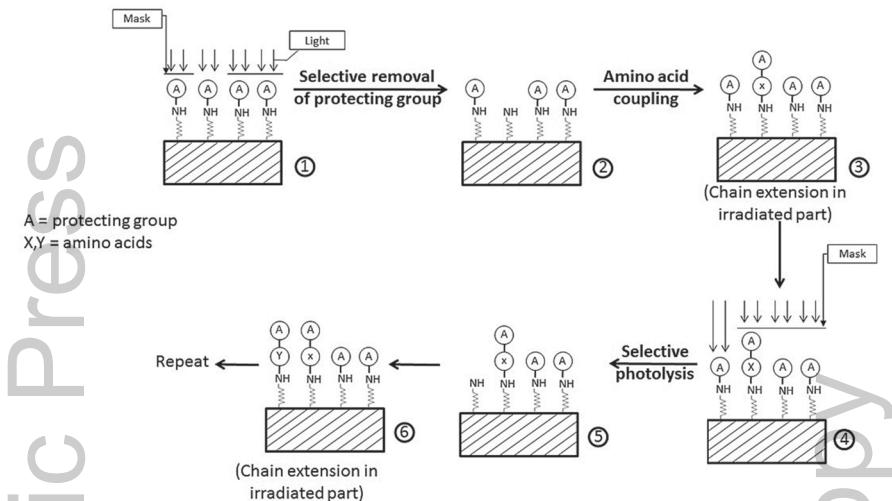


FIGURE 1.9 Spatially addressable light directed peptide synthesis.

used. Since photolithography is used, the total number of compounds that can be made depends on the number of sites that can be addressed with proper resolution. Direct assessment of the compound is possible since the position is precisely known (Pandeya and Thakkar, 2005).

1.6 APPLICABILITY OF COMBINATORIAL TECHNOLOGY

Over the years scientists have explored the applications of combinatorial chemistry in peptide, oligonucleotide as well as small molecule synthesis. Solid phase synthesis demands highly optimized repetitive reactions that are suitable for automation. Early work on small molecule combinatorial chemistry was reported by Leznoff and co-workers (Wong et al., 1974) and Frechet and co-workers (Fréchet and Seymour, 1978). It was realized that different solid supports are required for nonpeptide and peptide synthesis. Furthermore, unless each reaction step in a solid-phase synthesis is optimized, poor quality products are often the result. Strong interest in the synthesis of small molecules was finally kindled by Ellman and Bunin in 1992, when they illustrated the synthesis of benzodiazepine analogs prepared by rapid parallel synthesis, yielding compounds of pharmaceutical interest (Bunin and Ellman, 1992). After the success story of benzodiazepines synthesis, combinatorial synthesis became a well-established tool in

the pharmaceutical industry for generation of large sets of small molecules for lead finding and optimization (Dolle, 2002; Golebiowski et al., 2001). Combinatorial chemistry is mainly divided into solution phase and solid phase synthesis.

1.6.1 SOLUTION PHASE SYNTHESIS

Organic synthesis has traditionally been done in solution phase in which starting materials and reagents are dissolved in a solvent to generate a homogeneous mixture for the reaction to proceed. Solution phase organic reactions are “worked up” after every step in a multisynthesis through a series of extractions whereby the mixture is partitioned between an organic phase and an aqueous phase of known pH. Reaction progress as well as product identity and purity may be checked by well-established chromatographic and spectroscopic methods and the time needed for chemistry development in the preparation of libraries in solution phase is much less than for solid-phase approaches. In contrast to the large number of advantages of solution-phase parallel synthesis, there is one major disadvantage, namely the “purification problem”. Solution and solid-phase synthesis are both convenient and easily automated, the limitation to solution-phase parallel synthesis is the isolation of the desired compounds. Thus, the throughput ratio of libraries in automated solution-phase synthesis is directly proportional to the work-up procedures and to the purification process. Therefore, easy and efficient purification methodologies are required for high speed solution-phase synthesis (Golebiowski et al., 2001). The following methods are used in the solution phase synthesis of combinatorial chemistry.

1. Solution phase combinatorial synthesis using monomethyl polyethylene glycol (OMe-PEG) solid support. It is amphiphilic in nature and soluble in water as well as in many organic solvents (e.g., methylene chloride, ethanol, toluene, acetone, and chloroform) except in diethyl ether. The synthesis is started by reacting the acid group of a building block to the hydroxy group of OMe-PEG (Figure 1.10). The product is precipitated by adding diethyl ether and the excess reagent and other impurities are removed by washing. The solid product is redissolved in fresh solvent and the second stage of synthesis is carried out using a similar reaction and washing procedure. At the end of the synthesis the product may be cleaved from

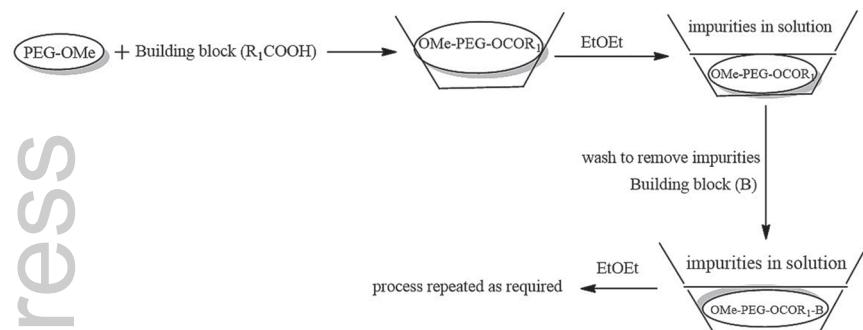


FIGURE 1.10 Solution phase libraries using monomethyl PEG.

the OMe-PEG, purified and assayed. In some cases the product is assayed when it is still attached to the OMe-PEG. This approach may be carried out using either the parallel synthesis or split and mix methods, the latter being carried out while the products of a stage are in solution (Han and Janda, 1996).

Dendrimers have been used as soluble supports in solution phase combinatorial chemistry in a similar fashion to OMe-PEG. Chemically dendrimers are branched oligomers (small polymers) with regular structures (Figure 1.11). This method helps the user to attain a high yield of synthesized compounds as multiple copies of each molecule are synthesized per dendrimer. The final product is released from the dendrimer and purified, and its structure is determined using the history of the process and/or standard analytical techniques. An important advantage of this technique is that it usually gives high yields (Kim et al., 1996).

- Polyfluorinated organic compounds are frequently used as soluble support in solution phase combinatorial synthesis even though it is insoluble in water and organic solvents but soluble in liquid

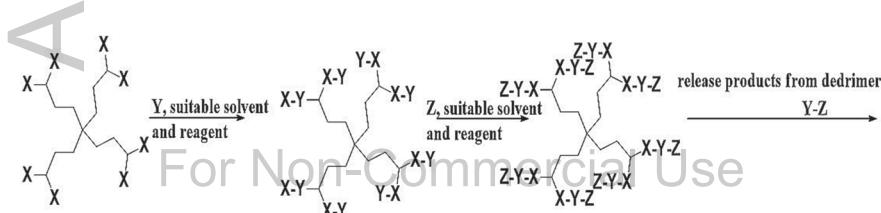


FIGURE 1.11 Representation of product formation using dendrimers.

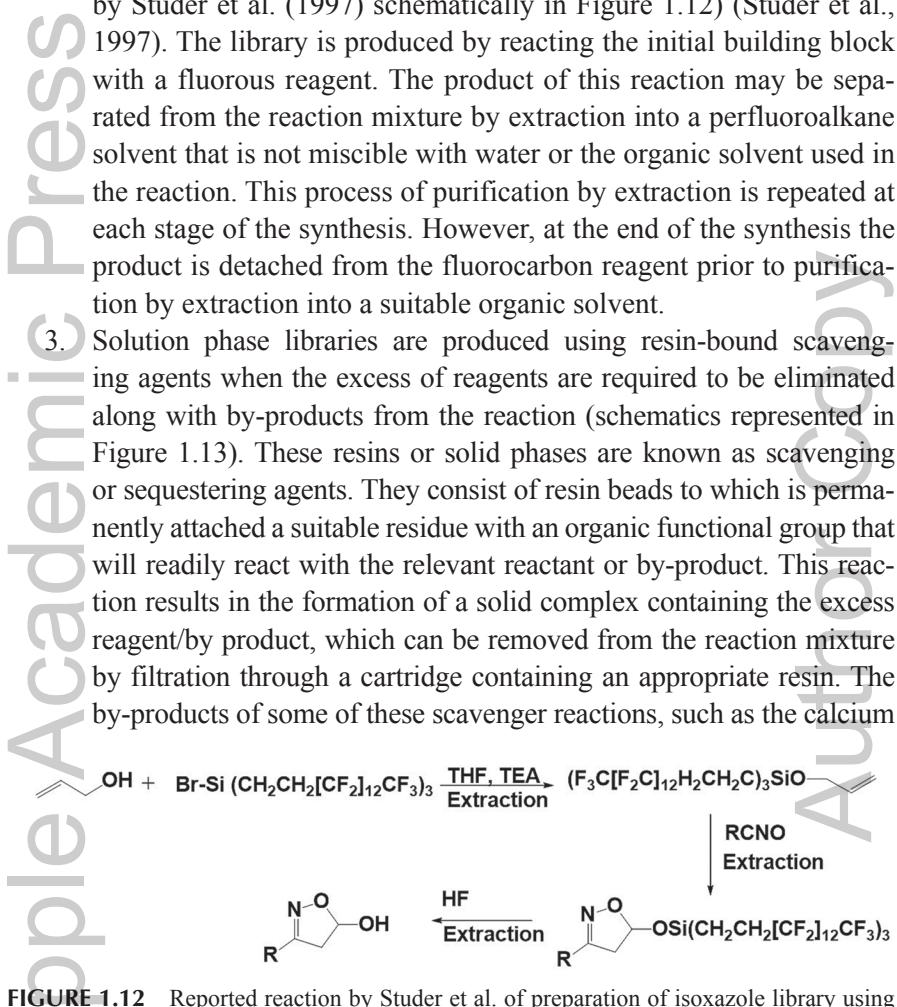


FIGURE 1.12 Reported reaction by Studer et al. of preparation of isoxazole library using fluorous resin.

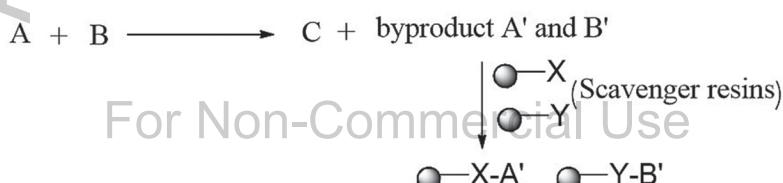


FIGURE 1.13 Schematics of resin bound scavengers to capture products.

sulphonate resin used to remove excess of tetrabutyl ammonium fluoride (TBAF) from a number of desilylation reactions, are also solid and may be removed by filtration. Resin-bound scavengers for the removal of by-products of both the reaction and the reagents operate in the same way as those used for the removal of excess reagents, except they are normally used during the reaction. For example, carboxylic acids have been sequestered by the use of the anionic Amberlite-68 resin and 4-nitrophenol has been removed by ion exchange with a quaternary ammonium hydroxide resin. The use of these sequestering agents during the reaction usually helps to drive the reaction to completion.

4. Resin capture of products: In this technique the resin has a functional group that can sequester the product. However, there is also the possibility of breaking the bond linking the product to the resin to form the original functional group of the product. At the end of the reaction the product is captured on the resin and the excess reagents, and reagent by-products are washed away with suitable solvents. The product is released from the resin, dissolved in a suitable solvent and the resin removed by filtration. For example, Blackburn et al. synthesized a library of 3-aminoimidazo[1,2-*a*]pyridines and pyrazines by this technique. They used a cation exchange resin to capture the products (Jung, 2008).

1.6.1.1 Solid Phase Synthesis

Solid-phase synthesis was invented by Merrifield in 1963; he used polystyrene resin beads to aid the synthesis of peptides. This strategy was expanded and further investigations on solid-phase synthesis towards organic compounds was carried by many other groups including Leznoff, Camps, Frechet, Rapaport in the 1970s (Früchtel and Jung, 1996; Hermkens et al., 1996; Thompson and Ellman, 1996). The ideal properties of a solid support are that it should be insoluble in the solvents used in the solid phase synthesis and should not react with the reagents used in the synthesis. The organic reactions take place mostly at the surface and sometimes inside the solid support. These supports are mostly used in the form of small resin beads that swell in the solvents used in the synthesis. The reactions in other kinds of supports take place only at the surface. These supports are polymers or glass beads, rods, sheets etc. and with the exception of their surface layer do not swell in the solvents. Solid

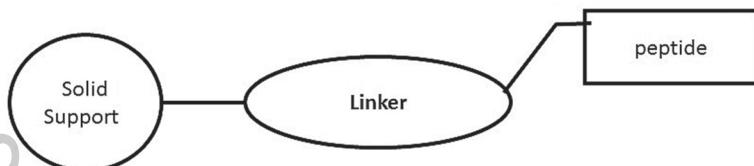


FIGURE 1.14A Attachment of Linkers, protective groups in peptide synthesis.

supports are usually made up of a core and a linker. The starting molecule of the synthesis is attached to the support *via* the linker (Figure 1.14A). The core provides insolubility to the support, determines the swelling properties, while the linker directs the functional group for attachment of the starting molecule. Reactions in solid-phase chemistry are selective and efficient and consequently the synthesis must be planned carefully. Otherwise, the purification of the final products can be a challenge. The scale of solid-phase synthesis is limited and generally restricted by the amount of the solid support and its loading capacity; the preparation of multimilligram quantities can be cumbersome and expensive for large combinatorial libraries (Jung, 2008). Following is a brief on the different types of supports and linkers used in solid phase synthesis.

1. *Polymeric Supports:* Cross-linked polystyrene resins are the most commonly used supports for solid phase synthesis. These resins are synthesized from styrene and divinylbenzene by suspension polymerization. The ratio of divinylbenzene to styrene determines the density of cross-links. Higher crosslink density increases the mechanical stability of the beads. Lowering the crosslink density, on the other hand, increases swelling and increases the accessibility of the functional groups buried inside the beads. The bead size of the resin is an important factor for the combinatorial synthesis. Smaller the resin, faster the reactions, but use of very small beads may cause problems during filtration. Normally 1–2% divinylbenzene is used with styrene. Cross-linked polystyrene is very hydrophobic so it swells only in an apolar solvent like THF, toluene, DCM, dioxane, CAN, DMF and methanol but not in water (Merrifield, 1963; Merrifield et al., 1966).

Polyethylene glycol grafted polystyrene support (PEG) has a 1–2% polystyrene core with ethylene glycol chains covalently attached. The PEG chains render hydrophilic character to the resin and it swells in water and methanol but poorly in ethanol and ether (Jung, 2008).

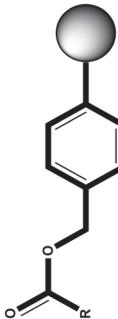
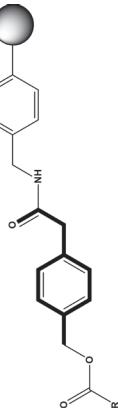
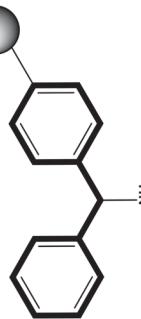
Glass beads with controlled pore size can also be considered as supports. These supports are mechanically stable, they can be functionalized by attaching linkers but they do not swell in solvents. Sometimes macroscopic objects can be used as solid supports are known as SynPhase crowns or SynPhase lanterns. In this technique polymer chains are grafted into the surface of an appropriate monomer and then the terminal chains are connected with the linker group, for example, styrene with polyolefin when grafted by radiation acts as a solid support (Geyzen et al., 1984; Morales and Bunin, 2003).

Mimotopes are devices with molded polypropylene surface on which is grafted either a hydrophilic copolymer of methacrylic acid/dimethyl acrylamide or the relatively hydrophobic polystyrene. The polymer is then suitably derivatized to allow the incorporation of a linker system. Another solid support lantern is made up of uniformly spaced flat rings. They are of two types D-series and I-series. D-series have larger surface area or volume ratio and have a higher loading capacity of 35 $\mu\text{mol}/\text{unit}$. Lanterns are mainly used for the synthesis of libraries of small molecules (Vaino and Janda, 2000; Wu et al., 2003).

2. *Linker:* A linker is abifunctional molecule, that binds irreversibly to the solid support (resin) and exhibits a reversible binding site for the coupling of desired molecules so that further chemical reactions may be carried out. The linker remains unchanged during the synthesis and on cleavage of the product remains attached to the solid support. The solid support attached to the linker is reusable. Cleavage of the bond between the linker and the synthesized library depends on the strength of its attachment to the resin. The synthesized library can be cleaved from the linker at the end of the synthesis either with acid, base or nucleophilic reagents, hydrogenolysis, enzymatic, palladium-catalyzed, photochemical, or by oxidative and reductive cleavage methods.

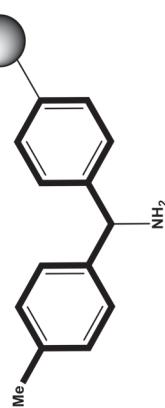
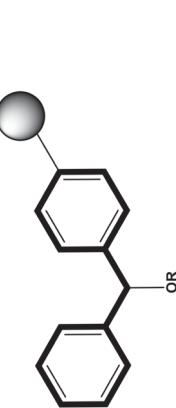
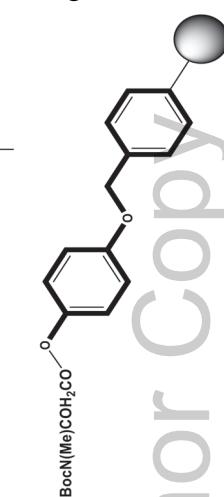
A large number of linkers have been prepared and discussed in a number of reviews. Linkers allowing the cleavage of a certain functional group have been named as mono-functional linkers. However, if an attachment is made cleavable to generate more than one functional group, it is named as multifunctional linkers. Linkers that are copolymerized into resin beads can be either of the integral or nonintegral type. Those which are not part of the polymer core can

TABLE 1.1 List of Linkers, Anchoring Groups and Cleavage Reagents and Deprotecting Reagents

Type	Linker	Anchoring group	Cleavage reagent	Ref.
Linkers cleaved using electrophilic reagents				
Strong Acid	Free carboxylic acid	Alcohols	HF or Trifluoromethane sulfonic acid (TFMSA)	[40– 42]
Cleavable Linker	of benzyl oxy carbonyl (Cbz) or Boc N-protected amino acids			
	R=p-Nitrophenol/N-Oxasuccinimide, tertiary-amine		50% TFA in CH_2Cl_2	[43]
Phenylacetamido-methyl (PAM) linker	Peptide ester			
Strong Acid	Marshall benzhydryl amine linker (MBHA)		TFMSA and HBF_4^- / thioanisole in TFA	[44– 47]
Cleavable Linker	Carboxamide			

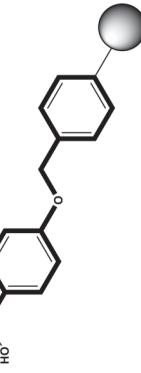
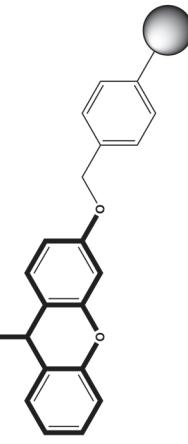
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Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Benzhydrol				HBr in TFA, or HF/ansole to cleave the MBHA	
OMPPA (4-(3-Hydroxy-4-methoxybenzyl)phenylacetic acid linker)				1–2% TFA in CH2Cl2	[48]
Wang Linker <i>p</i> -Alkoxybenzyl alcohol linker				On cleavage with HF it undergoes intramolecular cyclization	
Mild acid cleavable linker				1–10% TFA in CH2Cl2	[49, 50]

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TABLE 1.1 (Continued)
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Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Mild acid cleavable linker	SASRIN linker (superacid sensitive resin)	Acids		1% TFA  	[51, 52]

$X = Cl/OH/NHR/NHFmoc$

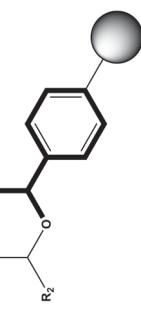
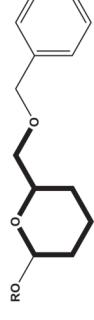
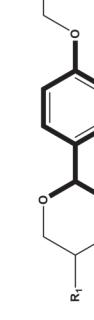
i) TFA from 10% to 95% in CH_2Cl_2
ii) Higher concentrations of TFA are required for peptide amide cleavage (generally 95% in CH_2Cl_2).

Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Sieber linker		Carboxamide or peptide-amides		% TFA in CH ₂ Cl ₂ after 1 min	[54]
Indole linker		Chloroformates, isocyanates, sulfonyl chlorides, or acids		TFA in CH ₂ Cl ₂ (2–50%)	[55]
Mild acid cleavable linker	Trityl linkers			anhydrous 2% TFA or dry HBr	[56– 58]

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R= H / 2'-Cl/4'-Me/4'-OMe

Type	Linker	Anchoring group	Cleavage reagent	Ref.
Ketal linkers	 a carbonyl group onto a diol-based linker or diols onto a carbonyl linker	 	10% TFA in CH_2Cl_2 with a trace amount of water or methanol	[59, 60]

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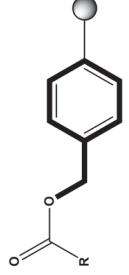
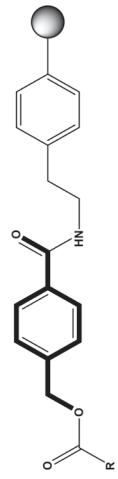
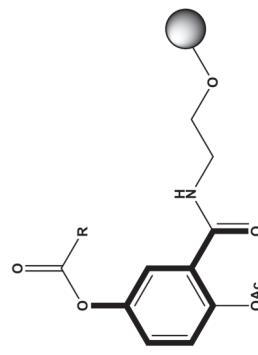
Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Semicarbazone linker	Ketone (trifluoromethyl ketone)			Aq. HCl and acetic acid	[62]
Mild acid cleavable linker	Imine, enol, ether and enamine linkers		Ketones or C-terminal peptide	3% TFA in CH2Cl2	[63]
t-Alkoxycarbonyl based linker			Immobilize amine to release 1° and 2° amines or N-terminal peptide	TFA	[64]
Aryltriazene linker using diazonium salt			1° and 2° amines	10% TFA	[64]

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TABLE 1.1 (Continued)
Linkers cleaving using nucleophilic reagents

Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Oxygen nucleophile	Saponification	Acid or alcohol		Hydrazine, ammonia, ethanolamine, methylamine	[65, 66]
Enzyme cleavable	4-acyloxybenzyl linker	Acid		Lipase or peptidase; Exception protease gives only 1–2% yield	[67, 68]
Nucleophilic transesterification	4-Hydroxymethylbenzoic acid (HMBAA)	Ester		MeOH/ MeONa/ THF, MeOH/ Et3N, K2CO3, CH2N2	[69]

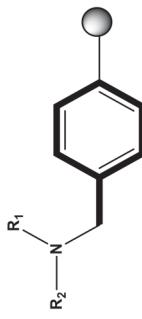
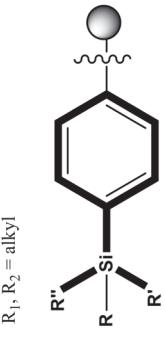
Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Nitrogen nucleophile	Sulfonate-based polystyrene linker		^{1°} Amines, imidazoles, thiolates	[70, 71]	
	Diketopiperazine cyclo release		C-terminal proline and glycine anchored peptides	0.1 M Acetic acid DCM	[72]
	Hydantoin Pyrazolones		Peptide linkage	Et3N, MeOH	[73]
	Nitrogen nucleophile			ArHNHNH2	[74]

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Type	Linker	Anchoring group	Cleavage reagent	Ref.
Safety Catch Linkers sensitive to nucleophiles upon activation	Sulfide or sulfone safety catch	2-Aminopyrimi- dines	<i>m</i> CPBA, R ₁ R ₂ NH ₂	[76, 77]
Kenner Safety catch linker	1° Amines		1° Amines	[78]
Boc benzamide activation	Alcohol		LiOH, 5% H ₂ O ₂	[79]
Wieland Safety-Catch Linker	Amine		BnNH ₂	[80]

Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Carbon Nucleophile	Thioester linker			Grignard reagent	[81]
Halogen nucleophile	Wang linker	Secondary amines or N-benzyl tertiary amines		α -chloroethylchloroformate	[82]
				TBAF, AcOH, and THF	[83]

$R = Cl / OR /$
 $R' = Me / Ph / Pr / nBu$
 $R'' = Me / Ph / iPr / nBu$

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Type	Linker	Anchoring group	Cleavage reagent	Ref.	
Photocleavable linker	o-Nitrobenzyl-Based Linkers, Nitrobenzhydryl linker (NBH) Nitrobenzhydrylamine linker (NBHA)	Boc-peptide	Sonication and a mixture of CH_2Cl_2 /trifluoroethanol	[84]	
					[85]

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Type	Linker	Anchoring group	Carrier	Cleavage reagent	Ref.
Multidetachable linkers	Boc-aminoacyl-4-[4-(oxymethyl)phenylacetoxymethyl]-3-nitrobenzamidomethyl resin	Different families of compounds		Acidolysis or hydrogenolysis for benzyl ester and photolysis for other ester	[86]

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be considered as nonintegral (or grafted) in nature. Although the core structure of the linker may remain unchanged, the group placed between the linker and the support can modify the cleavage conditions and also alter the degree of linker cleavage. These groups are called linker attachments (Jung et al., 2007). Table 1.1 shows the classes of frequently used linkers, anchoring groups and cleavable reagents.

3. *Protecting Groups:* Generally in combinatorial chemistry protecting groups are used when a chemical reaction is to be carried out selectively at one reactive site in a multifunctional compound, and other reactive sites must be temporarily blocked. The protecting group must react selectively in good yield to give a protected substrate. The protective group must be selectively removed in good yield by readily available, preferably nontoxic reagents that do not attack the regenerated functional group. The protective group should form a derivative (without the generation of new stereogenic centers) that can easily be separated from side products associated with its formation or cleavage. Protecting groups are subcategorized as “Orthogonal protection” if two functional groups are protected using two different protecting reagents and later in the synthesis the multiply protected groups are removed one at a time (Figure 1.14B). Applications of protection and deprotection steps in organic synthesis will help to control the chemistry. The protecting groups used for different functional groups and the deprotecting reagents are tabulated in the Table 1.2.

A, B, and C :
Protecting groups
L: Linker
P: protecting group
for amine
X= O or NH yields
peptide acids or
amides

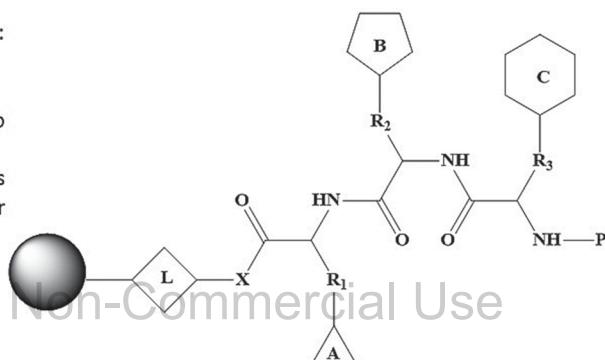


FIGURE 1.14B Orthogonal Protecting groups.

Protecting groups	Protection reaction	Cleavage reagent	Reference
Amino functional group ($R-NH_2$)	Amino-protection in peptide synthesis	HBr/AcOH, HBr/TFA	[87]
Benzylcarbonyl (Z) group		TFA	[88, 89]
<i>t</i> -Butyloxycarbonyl (Boc) (Boc_2O)		Basic condition 20% piperidine in DMF	[90-93]

TABLE 1.2 (Continued)

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Protecting groups

Protecting groups	Protection reaction	Cleavage reagent	Reference
Tetrachlorophthaloyl (TCP)		15% hydrazine DMF	[94]
Allyloxycarbonyl (Alloc) group		Pd(PPh3)4 and morpholine	[95-97]
2-(4-biphenylyl)isopropoxycarbonyl (Bpoc)		t-Butyl group	[98-101]
Trityl (Trt),		t-Butyl group	[100]

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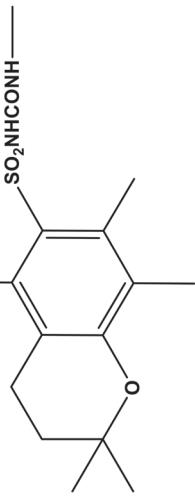
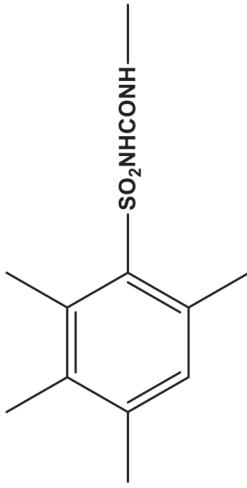
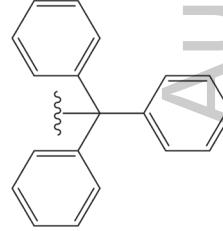
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Protecting groups	Cleavage reaction	Reference
α , α -Dimethyl-3,5-dimethoxybenzyl oxy carbonyl (Ddz)	t-Butyl group [100]	
2-(4-Nitrophenylsulfonyl) ethoxycarbonyl (Nsc)	20% piperidine and DMF, Dioxane in DMF(1:1)	
Guanidino group	Saponification, HBr/ AcOH, HF, catalytic hydrogenation but not by TFA	
Benzyl ester	TFA	
t-Butyl ester	[106, 107]	

Protecting groups	Protection reaction	Cleavage reagent	Reference
Allyl(Al)		Pd(Ph_3P) ₄ (0.1eq) and scavengers (PhSiH_3 10eq), DCM	[108, 109, 110]
Phenacyl(pac)		Sodium thiophenoxy or Zn in AcOH	[111, 112]
		TBAF	[113]
		Photolysis	[114]
		Photolysis	[115, 116]

Protecting groups	Cleavage reagent	Reference
Alcoholic and phenolic hydroxyl group	Protection reaction	
Benzyl ether	HF, HBr/AcOH, catalytic hydrogenolysis	[117]
Triyl ether	CF3CO2H/t-BuOH	[118]
Silyl ethers	HCl or TFA	[119]
	(Me can be replaced with Et/ iPr)	
Tetrahydropyranyl ether	Pyridinium p-toluenesulfonate (PPTS) in EtOH Or TsOH in MeOH	[120, 121]
Benzoate	K2CO3/KOH	[122]
t-Butyl carbonate(Boe)	TFA	[88, 89]
Trichloroethylcarbonate	Zn, AcOH	[123]

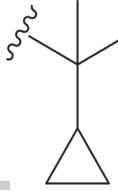
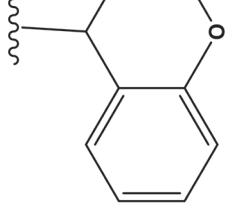
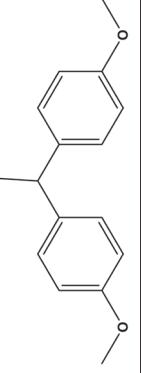
Protecting groups	Protection reaction	Cleavage reagent	Reference
Allyl carbonate		Pd ₂ (dba) ₃ , dppe, Et ₃ NH in THF	[124]
Perfluoroalkylsulfonate		KOH or palladium-Pd(OAc) ₂ mediated reductive cleavage	[125]
Carbonyl-protecting Groups Acetal/ketal methanol in dry HCl or MeOH (MeO) ₃ CH ₃		TFA, CHCl ₃ , H ₂ O in 1,3-dithiane and a dioxolane acetal or TsOH, acetone.	[126, 127]
S,S'-dialkylacetal (RSH in HCl, or RSSi(CH ₃) ₃ , ZnI ₂ , Et ₂ O.)		m-CPBA; Et ₃ N Ac ₂ O, H ₂ OorHg(ClO ₄) ₂ , MeOH, CHCl ₃	[128, 129, 130]
Guainidino group	O ₂ N-NHCNH-	Resists HBr/ AcOH but cleaved by liq. HF	[131]
Nitroguanidine	"	"	

Protecting groups	Protection reaction	Cleavage reagent	Reference
2,2,5,7,8-Pentamethylchroman-6-sulphonyl (Pmc) group		TFA [132]	
4-Methoxy-2,3,6-trimethylbenzenesulphonyl (Mts)		TFA (less sensitive and takes few hours to cleave)	[133]
Amide groups (in side chains of asparagine and glutamine)		TFE-EDT-H2O(EDT-ethanedithiol)	[134, 135]
Tritylation			

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TABLE 1.2 (Continued)
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Protecting groups	Protection reaction	Cleavage reagent	Reference
Cyclopropylmethylcarbinyl (Cpd)		TFA-thioanisole-EDT-anisole (90:5:3:2)	[136, 137]
9-Xanthenyl (Xan)		TFA scavengers	[138, 139, 140]
4,4'-Dimethoxybenzhydryl (Mbh)		TMSBr (trimethylsilyl)boronide thioanisole EDT-m-cresol in TFA	[141, 142]

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1.7 SPLIT AND MIX SYNTHESIS

Arpad Furka and co-workers invented the mix and split method in 1988. This method is used to generate large number of libraries as it produces one type of compound on each bead. Mix and split synthesis is executed in following three simple steps: first the solid support is divided into equal portions, each portion is coupled individually with one building block as shown in Figure 1.15. The final product is then mixed, homogenized and divided into equal portions for the next step of reaction with different building blocks.

Mix and split technique forms all products in any reaction vessel and the products are evenly distributed among the reaction vessels for the next reaction step. This method was mainly designed for peptide libraries. However, it has been used widely for organic libraries. As organic molecules are prepared by multistep synthesis, one can easily implement the split and mix synthesis for preparation of organic libraries. However, if every step needs to be screened for activity, then chemical tags are attached to the solid supports during the synthetic steps and they must be different from those that are applied for coupling of the building blocks (the two reactions need to be orthogonal). This process is called encoding or chemical tagging of the resin. The chemical tag must be cleavable at the end of the synthesis from the beads separately from

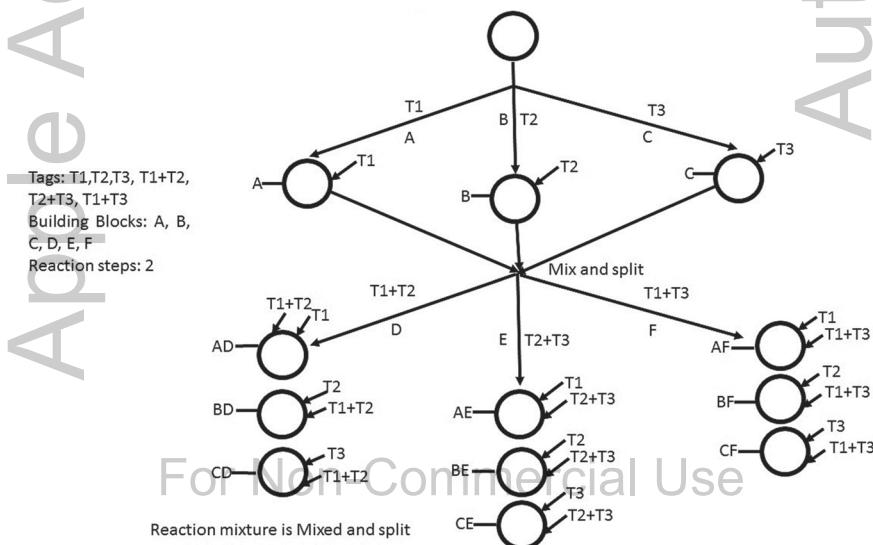


FIGURE 1.15 Mix and Split Technique.

the products. It must reveal information of the route of the beads by a simple analysis using spectroscopic methods rather than determining the structure of the compound. Chemical tagging can be done using peptide or oligonucleotide sequences as chemical tags (Brenner and Lerner, 1992; Needels et al., 1993; Nielsen et al., 1993; Nikolaiev et al., 1992) or by binary encoding method where the coding units are halobenzenes carrying a hydrocarbon chain with varying length attached to the solid support with a cleavable spacer (Kerr et al., 1993). Generally, separate linker functionality is used for the tagging sequence to prevent interference with the target chemistry. If the compound libraries are prepared in small concentrations in μmol to nmol yield, the solid support is then enclosed with electronic transponders into permeable capsules at the time of the reaction. Electronic transponders are used to encode the reagents and to encode the order of their addition. Radiofrequency signals can then be used to decode upon completion of the reaction. A capsule/microreactor contains 0.3 mL polypropylene with mesh sides capable of holding (in addition to the electronic chip) 30 mg of resin. About 15–30 μmol of compound can be prepared in each capsule. However, this technique is generally limited in many laboratories as it requires computers and readers, and because of the limited space present in a microreactor to carry out the reactions (Moran et al., 1995; Nicolaou et al., 1995). Another system of optical coding encompasses laser optical synthesis chips (LOSCs). The supports are $1 \times 1 \text{ cm}^2$ polystyrene grafted square plates. The medium carrying the code is a $3 \times 3 \text{ mm}^2$ ceramic plate in the center of the support. The code is imprinted into the ceramic support by a CO_2 laser in the form of a two-dimensional bar code. Before each synthetic step, the pooled chips are scanned and sorted according to the principles of the combinatorial synthesis as directed by a computer. This modified technique was demonstrated in the synthesis of a 27-membered oligonucleotide library (Xiao et al., 1997). The visual tagging technique came into picture recently to overcome the drawbacks observed in radiofrequency tagging. It is a color-coding strategy. This method helps to distinguish 96 different microreactors when 12 different colored caps and 8 different colored beads are placed among the resin beads. An equal portion of resin is divided into 8 reaction vessels for attachment of 8 different building blocks. Upon completion of the reaction the resin in the vessel is separated into 12 equal portions and placed in a microreactor with a few colored beads (color beads used for this purpose are same for each pool but different for each reactor). One capsule from each pool of 12 is removed and capped with the same colored cap. A second capsule from each pool is removed and

capped with a different colored cap and so on (step B). After color tagging the capsules are combined and mixed and divided into 12 reactors as per their cap color (Guiles et al., 1998).

1.8 SCREENING

Combinatorial libraries generated from genomics, proteins, and peptides libraries are screened by HTS technology. This method can screen large compound libraries at a rate of few thousand compounds per day or per week. The main goal of the technique is to accelerate the drug discovery process. It is of vital importance, because parallel and combinatorial chemical synthesis generates a vast number of novel compounds. HTS implements fluorescence resonance energy transfer (FRET) and homogeneous time resolved fluorescence (HTRF) techniques for identification of novel hits. Initially combinatorial libraries are screened for primary assay. This assay is less quantitative than biological assays. If a compound from an examined library gives a positive result or “HIT” then a precise secondary screening is conducted and the IC_{50} is calculated. Secondary screening is performed by adopting biological and biochemical tests (Figure 1.16) (Evans et al., 1988; Gallop, 1994; Gordon et al., 1994; Szymański et al., 2011). Two main strategies are practiced for screening the combinatorial libraries:

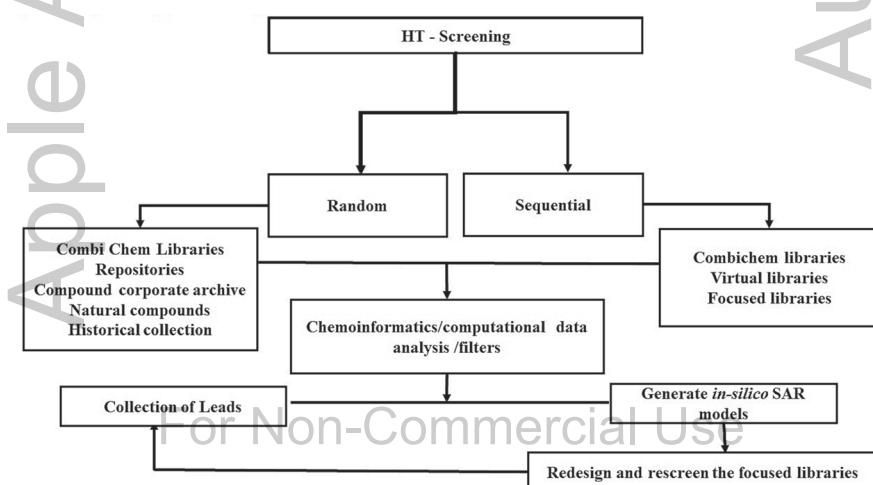


FIGURE 1.16 High throughput screening protocol.

1.8.1 RANDOM SCREENING

It is also known as primary library or mass random screening. The compound libraries obtained from repositories, corporate compound archives, and historical collections or from natural products are screened for primary assay. The assay is used as a filter to identify promising hits. A major goal of this method of screening is to increase the chance of finding biologically active compounds from an unbiased and diverse library with a new and unexpected structural scaffold as a lead candidate. For identifying a hit from the diverse compounds using random screening method, there is no requirement for pharmacophore knowledge or information on enzyme–ligand interactions.

1.8.2 SYSTEMATIC SCREENING

This method works in association with chemoinformatic technology. The main feature of this approach is that only a small set of the library is assayed and results are statistically analyzed. From this a model for structure activity relationship is prepared and the next set of libraries are planned to improve the activity. This step is repeated several times to identify the most promising compounds for lead optimization. Chemoinformatics methods play an important role while designing libraries of combinatorial synthesis. The methods applied include pharmacophore based virtual screening, diversity selection, recursive partitioning, *in silico* prediction methods for drug likeness or lead likeness.

1.9 STRATEGIES FOR SYNTHESIS OF SMALL MOLECULE LIBRARIES

Small molecules libraries are prepared using various strategies of which the more popular ones are listed here: (Mannhold et al., 2006)

1. *Unbiased/Random Libraries:* These libraries are generated when insufficient knowledge is available on the molecular target and it mainly follows a method that is driven by synthesis. They are envisioned to generate a diverse collection of chemical structures, for the identification of “hits” for any number of targets. Normally, the screening hit results are further investigated, with the goal of generating validated new “lead” structures. These libraries are commonly

referred to as “lead identification”, “lead discovery” or “lead finding” libraries. Typically, an unbiased library will consist of structures having a common chemical core or scaffold (or template) and in some cases the compound scaffold is described by a “privileged structure” (DeSimone et al., 2004; Guo and Hobbs, 2003; Horton et al., 2003; Horton et al., 2002; Nicolaou et al., 2000). The unbiased libraries are further divided into two categories: compound libraries and natural product libraries. Compound libraries consist of molecules which are prepared in house or purchased from external vendors. Natural libraries consists of molecules isolated from various sources (Jia, 2003; Ortholand and Ganesan, 2004).

2. *Directed Libraries:* Also known as focused libraries. Many compounds in this type of library are defined by the pharmacophoric feature of the target. This library may be designed on the basis of privileged structures. Privileged structures are the dataset of active molecules available against multiple targets (Bondensgaard et al., 2004; Müller, 2003).

1.10 DECONVOLUTION/IDENTIFYING THE HIT FROM THE SCREENING

Deconvolution is used to identify a HIT/bioactive/potent molecule from the multicomponent combinatorial libraries. It is mainly divided into three types, iteration, positional scanning, and omission.

1. *Iteration Method:* In this method the bioactive molecule is identified by iterative synthesis. It can be demonstrated by the example of tripeptide synthesis. The original library is initially released from the solid support for biological assays. Normally along with the generated library the product in the last step of coupling is separated and given for screening. If any molecule in the library turns out to be bioactive then the terminal amino acid in the library is identified from the secondary library. To identify the second position amino acid the second last step in the tripeptide before mixing and coupling are separated and then coupled with the bioactive terminal amino acid previously screened for bioactivity. The bioactive hit is known as the last two sequences of bioactive amino acids. To identify the first amino acid in the hit one needs to separate the first step coupled

amino acids products and further combine then with the next two bioactive sequences of amino acids which is then screened. The most bioactive molecule reveals the sequence of the bioactive tripeptide (Geyzen et al., 1984; Houghten, 1991).

2. *Positional Scanning:* In case of the iterative method chemists are required to prepare the sublibraries to identify the hit molecule. This is accomplished by prepreparing the set of libraries that identifies the active hit in the positional scanning method. This concept was first explained in a patent filed by Furka et al. (Furka, 1995) in May, 1992 and subsequently put into practice by Pinilla et al. (Houghten et al., 1994). In case of positional scanning a sublibrary is prepared at every step of the combichem reaction along with the original library. Then the biological testing is carried out to identify the N-terminal amino acids at the every step of sublibrary. In principle, along with the original library, sublibraries are also prepared and screened. If the result is positive then all components of the set are required to be tested. From the result, the amino acid sequence of the bioactive peptide can be deduced. Furka explained the model of 20 building blocks of a pentapeptide. Along with the synthesis of the main library few sublibraries aliquots were prepared. While synthesizing the combichem libraries at every step after coupling the amino acids, one fifth of the total resin is removed before the mixing and recombining step and that library is called step 1 sublibrary. Similarly in steps 2, 3, 4 and 5 one fifth of the resin is separated before the coupling and mixing step and labeled as sublibrary 2, 3, 4 and 5, respectively. All of these sublibraries are released from the solid support and screened along with the original library to identify the hit.

3. *Omission Libraries:* Omission libraries can be prepared using full libraries by omitting one amino acid in all coupling positions. One must remember that the same amino acid is omitted irrespective of its number or position occupied in the sequences in all coupling positions (Câmpian et al., 1998). If the prepared bioactive peptide contains the omitted amino acid that means the omitted amino acid is responsible for the bioactivity on the other hand if the prepared peptide gives negative results in the presence of the omitted amino acid it means that the omitted amino acid is not responsible for the biological activity. This technique is simpler and less laborious than iterative screening and positional scanning methods.

1.11 ROLE IN DRUG DISCOVERY

Combinatorial chemistry can contribute to drug discovery by helping to identify biologically active compounds in conjunction with high-throughput screening thus accelerating the discovery of lead compounds with a desired pharmacological profile. Combinatorial chemistry has the power to generate highly diverse compound collections for random screening. It is one of the most efficient tool to identify lead molecules in a short span of time with minimum cost. It is heartening to note that some compounds are undergoing clinical trials that were discovered by combinatorial chemistry (Figure 1.17). The impact of combinatorial chemistry on lead identification is dictated by the size and composition of the library.

As of date we have witnessed two generations of methods in combinatorial chemistry. In the first-generation the main focus was on peptide and oligonucleotide synthesis and in the second generation researchers directed their interest on small-molecule chemistry. Many pharma companies have been an integral part of the development of technologies in the second-generation combinatorial chemistry venture. One among many is Pfizer; which from the beginning used methods in combinatorial chemistry that were developed in-house. Another pharma company, Sepracor has spawned a combinatorial chemistry subsidiary of Dainippon Sumitomo Pharma America Holdings Inc., Eli Lilly got a foot-hold in this technology by acquiring Sphinx and

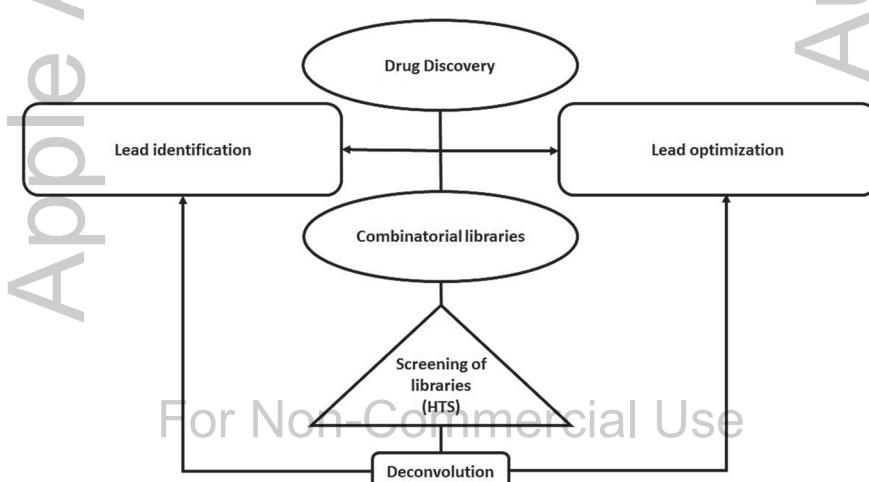
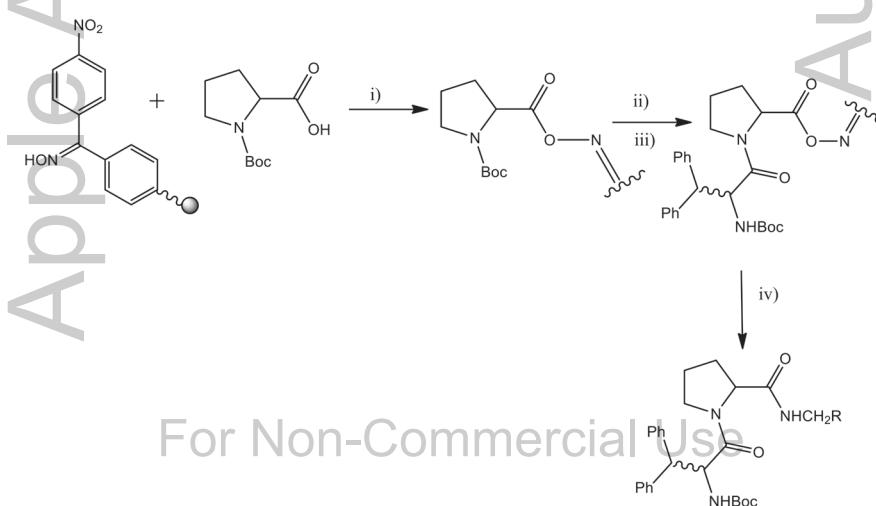


FIGURE 1.17 Drug discovery using combinatorial approach.

Glaxo took over Affymax to kick-start their combinatorial process. Presently the current trend in drug discovery is to combine combinatorial chemistry with structure-based drug design, molecular biology and biological target identification. Arris Pharmaceutical, Ariad Pharmaceuticals, Chiron, 3-Dimensional Pharmaceuticals, and Neurogen are some pharma companies that have taken this approach. Combinatorial chemistry requires huge investment in terms of and infrastructure and other facilities.

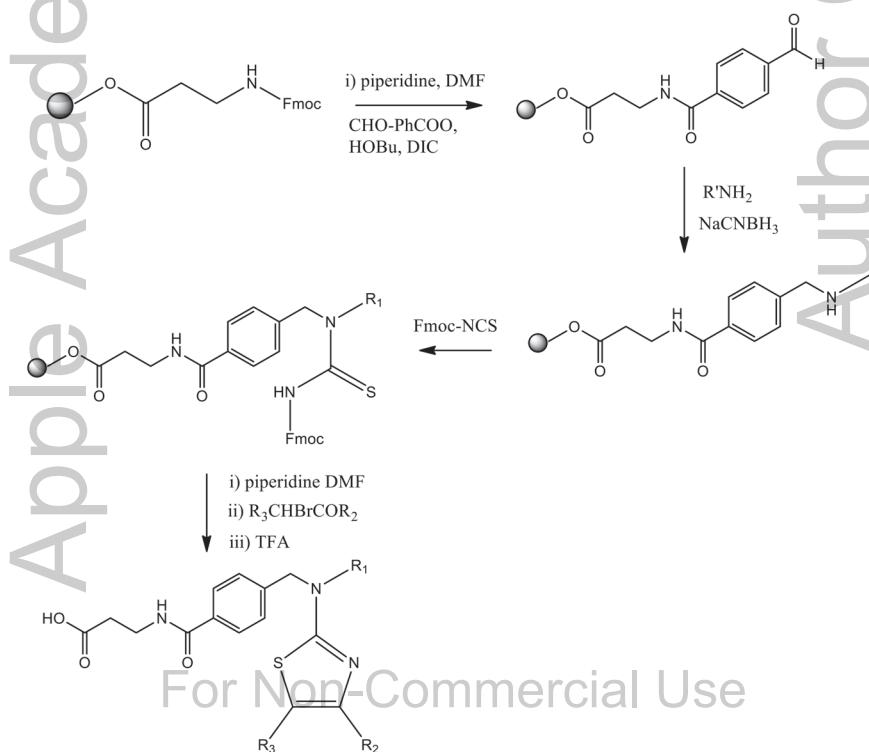
Pfizer has a compound in early clinical trials that researchers there wouldn't have discovered without combinatorial chemistry. Gunther Jung (Jung, 2008) has tabled a the list of industries that are progressing in the field of combinatorial chemistry synthesis, analysis and screening and has also reviewed molecules that are in clinical trials which are the products of combinatorial chemistry (Burgess and Lim, 1997). Several reviews have covered the companies (at present more than 180) that are using combinatorial chemistry in early drug discovery phase and have also listed a large number of leads are now in preclinical stage of development. In spite of the vast efforts by several companies, it is disheartening to note that there is no drug till date on the market that has been exclusively discovered by the application of combinatorial chemistry (Thayer, 1996). The following paragraphs gives a glimpse of some efforts by pharma companies to use combinatorial chemistry in the drug discovery process. The nineteenth century saw many scientists aggressively applying combinatorial chemistry tools to



SCHEME 1 Synthesis of dipeptides by Merck Lab using the Kaiser resin.

obtain leads. In 1999, Merck lab used a target based approach (the serine proteinase enzyme thrombin) and combinatorial tools to improve the bioavailability of a lead molecule L-371912 with K_i of 5 nM and prepared a library of 200 compounds. From the primary library they successfully developed L-372460 with K_i of 1.5 nM with improved bioavailability (Lumma et al., 1998). The group used a Kaiser oxime resin which was coupled to Boc protected proline using dicyclohexylcarbodiimide (DCC) (Scheme 1). In the next step deprotection was carried out with trifluoroacetic acid followed by HOBr/EDC coupling to Boc-D-diphenylalanine to get dipeptide attached to the resin. The peptide was cleaved from the resin with a collection of amines RCH_2NH_2 and triethylamine at room temperature. The released amides were deprotected with TFA to give the desired inhibitors.

(i) DCC, CH_2Cl_2 , (ii) CF_3COOH , CH_2Cl_2 , (iii) Boc (D)diPhe, DCC, CH_2Cl_2 , and (iv) RCH_2NH_2 , 3–6 days, 25°C, TFA.



Scheme 2 Synthesis of thiazole library.

Roland Dolle has successfully reviewed the literature covering the period from 1999 to 2010 and delivered to readers a vast dataset of chemical libraries that have been designed against various targets. We quote here a significant example in this section:

hGluR: Glucagon maintains glucose homeostasis during the fasting state by promoting hepatic gluconeogenesis and glycogenolysis. Antagonizing the glucagon receptor is expected to result in reduced hepatic glucose overproduction, leading to overall glycemic control and a possible treatment for type 2 diabetes. Over 800 thiazole analogs were prepared using Fmoc- β -Ala-Wang resin which was deprotected and coupled to 4-formylbenzoic acid which on reductive amination with primary amines followed by treatment with isocyanate gave the thiourea. Condensation of the Fmoc protected thiourea with α -bromoketones lead to the thiazole library (Scheme 2) (Madsen et al., 2009).

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KEYWORDS

- chemistry
- combinatorial
- drug discovery
- library
- synthesis
- technology

REFERENCES

- Akaji, K., et al. (1990). Tetrafluoroboric acid as a useful deprotecting reagent in Fmoc-based solid-phase peptide syntheses (Fmoc = fluoren-9-ylmethoxycarbonyl). *J. Chem. Soc., Chem. Commun.*, 4, 288–290.

- Albericio, F. (2000). Orthogonal protecting groups for $\text{N}\alpha$ -amino and C-terminal carboxyl functions in solid-phase peptide synthesis. *Peptide Science*, 55(2), 123–139.
- Albericio, F., et al. (1984). Solid phase synthesis and HPLC purification of the protected 1–12 sequence of apamin for rapid synthesis of apamin analogs differing in the C-terminal region. *Tetrahedron*, 40(21), 4313–4326.
- Alcaro, M. C., et al. (2004). On-resin head-to-tail cyclization of cyclotetrapeptides: optimization of crucial parameters. *Journal of Peptide Science*, 10(4), 218–228.
- Anderson, G. W., & Callahan, F. M. (1960). t-Butyl Esters of Amino Acids and Peptides and their Use in Peptide Synthesis. *Journal of the American Chemical Society*, 82(13), 3359–3363.
- Anderson, G. W., & McGregor, A. C. (1957). t-Butyloxycarbonylamino acids and their use in peptide synthesis. *Journal of the American Chemical Society*, 79(23), 6180–6183.
- Atherton, E., et al. (1979). The polyamide method of solid phase peptide and oligonucleotide synthesis. *Bioorganic Chemistry*, 8(3), 351–370.
- Atherton, E., Sheppard, R. C., & Wade, J. D. (1983). Side chain protected N α -fluorenylmethoxycarbonylamino-acids for solid phase peptide synthesis. N α -Fluorenylmethoxycarbonyl-NG-4-methoxy-2,3,6-trimethylbenzenesulphonyl-L-arginine. *Journal of the Chemical Society, Chemical Communications*, 19, 1060–1062.
- Baxter, E. W., et al. (1998). Arylsulfonate esters in solid phase organic synthesis. II. Compatibility with commonly used reaction conditions. *Tetrahedron Letters*, 39(9), 979–982.
- Beavers, M. P., & Chen, X. (2002). Structure-based combinatorial library design: methodologies and applications. *Journal of Molecular Graphics and Modelling*, 20(6), 463–468.
- Bergmann, M., & Zervas, L. (1932). Über ein allgemeines Verfahren der Peptid-Synthese. *Berichte der deutschen chemischen Gesellschaft (A and B Series)*, 65(7), 1192–1201.
- Bodanszky, M. (1984). Alkyl esters of amino acids. *International Journal of Peptide and Protein Research*, 23(1), 111–111.
- Böhm, G., et al. (1998). A novel linker for the attachment of alcohols to solid supports. *Tetrahedron Letters*, 39(22), 3819–3822.
- Bondensgaard, K., et al. (2004). Recognition of privileged structures by G-protein coupled receptors. *Journal of Tetrahedron Letters*, 47(4), 888–899.
- Bourgault, S., Letourneau, M., & Fournier, A. (2007). Development of photolabile caged analogs of endothelin-1. *Peptides*, 28(5), 1074–1082.
- Bräse, S., et al. (1999). Triazenes as robust and simple linkers for amines in solid-phase organic synthesis. *Tetrahedron Letters*, 40(11), 2105–2108.
- Brenner, S., & Lerner, R. A. (1992). Encoded combinatorial chemistry. *Proceedings of the National Academy of Sciences*, 89(12), 5381–5383.
- Brown, R. D., Hassan, M., & Waldman, M. (2000). Combinatorial Library Design for Diversity, Cost Efficiency, and Drug-Like Character. *Journal of Molecular Graphics and Modelling*, 18(4), 427–437.
- Bunin, B. A., & Ellman, J. A. (1992). A general and expedient method for the solid-phase synthesis of 1, 4-benzodiazepine derivatives. *Journal of the American Chemical Society*, 114(27), 10997–10998.
- Burgess, K., & Lim, D. (1997). Resin type can have important effects on solid phase asymmetricalkylation reactions. *Chem. Commun.*, 8, 785–786.
- Butwell, F. G., Haws, E. J., & Epton, R. (1988). Advances in ultra-high load polymer-supported peptide synthesis with phenolic supports: 1. A selectively-labile c-terminal spacer group for use with a base-mediated n-terminal deprotection strategy and fmoc

- amino acids. in Makromolekulare Chemie. Macromolecular Symposia. Wiley Online Library.
- Câmpian, E., et al. (1998). Deconvolution by omission libraries. *Bioorganic & Medicinal Chemistry Letters*, 8(17), 2357–2362.
- Carey, R. I., et al. (1997). Preparation and properties of N α -Boc-amino acid pentafluorophenyl esters. *The Journal of Peptide Research*, 49(6), 570–581.
- Carpino, L. A. (1957). Oxidative Reactions of Hydrazines. IV. Elimination of Nitrogen from 1, 1-Disubstituted-2-arenesulfonhydrazides 1–4. *Journal of the American Chemical Society*, 79(16), 4427–4431.
- Carpino, L. A., & Han, G. Y. (1970). 9-Fluorenylmethoxycarbonyl function, a new base-sensitive amino-protecting group. *Journal of the American Chemical Society*, 92(19), 5748–5749.
- Carpino, L. A., & Han, G. Y. (1972). 9-Fluorenylmethoxycarbonyl amino-protecting group. *The Journal of Organic Chemistry*, 37(22), 3404–3409.
- Carpino, L. A., Schroff, H. N., Chao, H.-G., Mansour, E. M. E., & Albericio, F. (1995). Peptides 1994, *Proceedings of the 23rd European Peptide Symposium*, 155–156.
- Carpino, L. A., & Chao, H. G. (1995). *H. Chem. Abstr.*, 124, 146865.
- Chucholowski, A., et al. (1996). Novel Solution-and Solid-Phase Strategies for the Parallel and Combinatorial Synthesis of Small-Molecular-Weight Compound Libraries. *CHI-MIA International Journal for Chemistry*, 50(11), 525–530.
- Colvin, E., Raphael, R., & Roberts, J. (1971). The total synthesis of (\pm)-trichodermin. *Journal of the Chemical Society D: Chemical Communications*, 15, 858–859.
- Conti, P., et al. (1997). A new cleavage strategy for the solid-phase synthesis of secondary amines. *Tetrahedron Letters*, 38(16), 2915–2918.
- Corey, E., et al. (1981). *Tetrahedron Letters*, 22(36), 3455–3458.
- Corey, E., Niwa, H., & Knolle, J. (1978). Total synthesis of (S)-12-hydroxy-5, 8, 14-cis,-10-transeicosatetraenoic acid (Samuelsson's HETE). *Journal of the American Chemical Society*, 100(6), 1942–1943.
- Cros, E., et al. (2004). N-Tetrachlorophthaloyl (TCP) Protection for Solid-Phase Peptide Synthesis. *European Journal of Organic Chemistry*, 17, 3633–3642.
- DeSimone, R., et al. (2004). Privileged structures: applications in drug discovery. *Combinatorial Chemistry & High Throughput Screening*, 7(5), 473–493.
- Dolle, R. E. (2002). Comprehensive survey of combinatorial library synthesis: 2001. *Journal of Combinatorial Chemistry*, 4(5), 369–418.
- Dressman, B. A., Spangle, L. A., & Kaldor, S. W. (1996). Solid phase synthesis of hydantoins using a carbamate linker and a novel cyclization/cleavage step. *Tetrahedron Letters*, 37(7), 937–940.
- Drewry, D. H., & Young, S. S. (1999). Approaches to the design of combinatorial libraries. *Chemometrics and Intelligent Laboratory Systems*, 48(1), 1–20.
- Eckard, P., et al. (2010). Five Natural Product-Based, Chemically and Functionally Diverse Libraries. *Combinatorial Synthesis of Natural Product-Based Libraries*, 99.
- Ellison, R. A. L., E. R., & Chiu, C.-W., (1975). *Tetrahedron Lett*, 499.
- Estep, K. G., et al. (1998). Indole resin: A versatile new support for the solid-phase synthesis of organic molecules. *The Journal of Organic Chemistry*, 63(16), 5300–5301.
- Evans, B., et al. (1988). Methods for drug discovery, development of potent, selective, orally effective cholecystokinin antagonists. *Journal of Medicinal Chemistry*, 31(12), 2235–2246.

- Evans, D. A., et al. (1977). Thiosilanes, a promising class of reagents for selective carbonyl protection. *Journal of the American Chemical Society*, 99(15), 5009–5017.
- Fauchère, J.-L., et al. (1998). Combinatorial chemistry for the generation of molecular diversity and the discovery of bioactive leads. *Chemometrics and Intelligent Laboratory Systems*, 43(1), 43–68.
- Fréchet, J. M., & Haque, K. E. (1975). Use of polymers as protecting groups in organic synthesis. II. Protection of primary alcohol functional groups. *Tetrahedron Letters*, 16(35), 3055–3056.
- Fréchet, J. M., & Nuyens, L. J. (1976). Use of polymers as protecting groups in organic synthesis. III. Selective functionalization of polyhydroxy alcohols. *Canadian Journal of Chemistry*, 54(6), 926–934.
- Fréchet, J. M., & Seymour, E. (1978). Use of Polymers as Protecting Groups in Organic Synthesis. VII. Preparation of Monobenzoates of Acyclic Triols. *Israel Journal of Chemistry*, 17(4), 253–256.
- Friede, M., et al. (1991). Incomplete TFA deprotection of N-terminal trityl-asparagine residue in fmoc solid-phase peptide chemistry. *Peptide Research*, 5(3), 145–147.
- Früchtel, J. S., & Jung, G. (1996). Organic chemistry on solid supports. *Angewandte Chemie International Edition in English*, 35(1), 17–42.
- Funakoshi, S., et al. (1988). Combination of a new amide-precursor reagent and trimethylsilyl bromide deprotection for the Fmoc-based solid phase synthesis of human pancreatestatin and one of its fragments (Fmoc= fluoren-9-ylmethoxycarbonyl). *Journal of the Chemical Society, Chemical Communications*, 24, 1588–1590.
- Furka, A. (1995). History of combinatorial chemistry. *Drug Development Research*, 36(1), 1–12.
- Furka, Á. (2007). *Combinatorial Chemistry Principles and Techniques*.
- Fyles, T. M., & Leznoff, C. C. (1976). The use of polymer supports in organic synthesis. V. The preparation of monoacetates of symmetrical diols. *Canadian Journal of Chemistry*, 54(6), 935–942.
- Gallop, M. A., et al. (1994). Applications of Combinatorial Technologies to Drug Discovery. 1. Background and Peptide Combinatorial Libraries. *Journal of Medicinal Chemistry*, 37(9), 1233–1251.
- Gayo, L. M., & Suto, M. J. (1997). Traceless linker: Oxidative activation and displacement of a sulfur-based linker. *Tetrahedron Letters*, 38(2), 211–214.
- Genet, J. P., Blant, E., Savignac, M., Lemeune, S., Lemaire-Audoire, S., & Bernard, J. M. (1993). *Synlett J.* 680–682.
- Geyzen, H. M., Meloen, R. H., & Barteling, S. J. (1984). Use of peptide synthesis to probe viral antigens for epitopes to a resolution of a single amino acid. *Proceedings of the National Academy of Sciences*, 81(13), 3998–4002.
- Givens, R. S., et al. (2000). *Journal of the American Chemical Society*, 122(12), 2687–2697.
- Golebiowski, A., Klopfenstein, S. R., & Portlock, D. E. (2001). Lead compounds discovered from libraries. *Current Opinion in Chemical Biology*, 5(3), 273–284.
- Gordon, E. M., et al. (1994). Applications of combinatorial technologies to drug discovery. 2. Combinatorial organic synthesis, library screening strategies, and future directions. *Journal of Medicinal Chemistry*, 37(10), 1385–1401.
- Guiles, J. W., Lanter, C. L., & Rivero, R. A. (1998). A visual tagging process for mix and sort combinatorial chemistry. *Angewandte Chemie International Edition*, 37(7), 926–928.

- Guo, T., & Hobbs, D. W. (2003). Privileged structure-based combinatorial libraries targeting G protein-coupled receptors. *Assay and Drug Development Technologies*, 1(4), 579–592.
- Han, H., & Janda, K. D. (1996). Azatides: Solution and liquid phase syntheses of a new peptidomimetic. *Journal of the American Chemical Society*, 118(11), 2539–2544.
- Han, Y. S., Sole, N. A., Tejbrant, J., & Barany, G. (1996). *Pept. Res*, 9, 166.
- Hanessian, S., & Xie, F. (1998). Polymer-bound p-alkoxybenzyl trichloracetimidates: Reagents for the protection of alcohols as benzyl ethers on solid-phase. *Tetrahedron Letters*, 39(8), 733–736.
- Harris, C. J., et al. (2011). The design and application of target-focused compound libraries. *Combinatorial Chemistry & High Throughput Screening*, 14(6), 521.
- Heathcock, C. H., & Ratcliffe, R. (1971). Stereoselective total synthesis of the guaiazulenic sesquiterpenoids. alpha.-bulnesene and bulnesol. *Journal of the American Chemical Society*, 93(7), 1746–1757.
- Hendrickson, J. B., & Kandall, C. (1970). The phenacyl protecting group for acids and phenols. *Tetrahedron Letters*, 11(5), 343–344.
- Hermkens, P. H., Ottenheijm, H. C., & Rees, D. (1996). Solid-phase organic reactions: a review of the recent literature. *Tetrahedron*, 52(13), 4527–4554.
- Horton, D. A., Bourne, G. T., & Smythe, M. L. (2002). Exploring privileged structures: the combinatorial synthesis of cyclic peptides. *Journal of Computer-Aided Molecular Design*, 16(5–6), 415–431.
- Horton, D. A., Bourne, G. T., & Smythe, M. L. (2003). The combinatorial synthesis of bicyclic privileged structures or privileged substructures. *Chemical Reviews*, 103(3), 893–930.
- Houghten, R. A., et al. (1991). *Generation and Use of Synthetic Peptide Combinatorial Libraries for Basic Research and Drug Discovery*.
- Houghten, R., et al. (1994). Optimal peptide length determination using synthetic peptide combinatorial libraries. in *Peptides-American Symposium*. Ecom Science Publishers.
- Huang, R., Pershad, K., Kokoszka, M., & Kay, B. K. (2011). *Phage-Displayed Combinatorial Peptides. Amino Acids, Peptides and Proteins in Organic Chemistry: Protection Reactions, Medicinal Chemistry, Combinatorial Synthesis*, Volume 4, 451–471.
- Hulme, C., et al. (1998). Novel safety-catch linker and its application with a Ugi/De-BOC/Cyclization (UDC) strategy to access carboxylic acids, 1,4-benzodiazepines, diketopiperazines, ketopiperazines and dihydroquinoxalinones. *Tetrahedron Letters*, 39(40), 7227–7230.
- Imoto, M., et al. (1988). Synthetic approach to bacterial lipopolysaccharide, preparation of trisaccharide part structures containing KDO and 1-dephospho lipid A. *Tetrahedron Letters*, 29(18), 2227–2230.
- Jia, Q. (2003). *Stud. Nat. Prod. Chem.*, 29, 643–718.
- Jung, G. (2008). *Combinatorial Chemistry: Synthesis, Analysis, Screening*. John Wiley & Sons.
- Jung, G. (2008). *Combinatorial Peptide and Nonpeptide Libraries: A Handbook*. John Wiley & Sons.
- Jung, N., Wiehn, M., & Bräse, S. (2007). Multifunctional linkers for combinatorial solid phase synthesis, in *Combinatorial Chemistry on Solid Supports*. Springer. 1–88.
- Kenner, G., McDermott, J., & Sheppard, R. (1971). The safety catch principle in solid phase peptide synthesis. *Journal of the Chemical Society D: Chemical Communications*, 12, 636–637.

- Kerr, J. M., Banville, S. C., & Zuckermann, R. N. (1993). Encoded combinatorial peptide libraries containing nonnatural amino acids. *Journal of the American Chemical Society*, 115(6), 2529–2531.
- Kim, R. M., et al. (1996). Dendrimer-supported combinatorial chemistry. *Proceedings of the National Academy of Sciences*, 93(19), 10012–10017.
- Kishi, Y., Fukuyama, T., & Nakatsuka, S. (1973). New method for the synthesis of epidithiodiketopiperazines. *Journal of the American Chemical Society*, 95(19), 6490–6492.
- Koh, J. S., & Ellman, J. A. (1996). Palladium-Mediated Three-Component Coupling Strategy for the Solid-Phase Synthesis of Tropane Derivatives. *The Journal of Organic Chemistry*, 61(14), 4494–4495.
- König, W., & Geiger, R. (1970). Eine neue Amid-Schutzgruppe. *Chemische Berichte*, 103(7), 2041–2051.
- Kowalski, J., & Lipton, M. A. (1996). Solid phase synthesis of a diketopiperazine catalyst containing the unnatural amino acid (S)-norarginine. *Tetrahedron Letters*, 37(33), 5839–5840.
- Lenard, J., & Robinson, A. B. (1967). Use of Hydrogen Fluoride in Merrifield Solid-Phase Peptide Synthesis. *Journal of the American Chemical Society*, 89(1), 181–182.
- Lewis, R. A. (2002). The design of small-and medium-sized focused combinatorial libraries, in *Molecular Diversity in Drug Design*. Springer. 221–248.
- Lloyd-Williams, P., et al. (1991). Solid-phase synthesis of peptides using allylic anchoring groups. An investigation of their palladium-catalyzed cleavage. *Tetrahedron Letters*, 32(33), 4207–4210.
- Loedder, M., et al. (1998). Misacylated transfer RNAs having a chemically removable protecting group. *The Journal of Organic Chemistry*, 63(3), 794–803.
- Loffet, A., & Zhang, H. (1993). Allyl-based groups for side-chain protection of amino-acids. *International Journal of Peptide and Protein Research*, 42(4), 346–351.
- Lumma, W. C., et al. (1998). Design of novel, potent, noncovalent inhibitors of thrombin with nonbasic P-1 substructures: rapid structure-activity studies by solid-phase synthesis. *Journal of Medicinal Chemistry*, 41(7), 1011–1013.
- Lytle, M. H. H. (1992). Peptides Chemistry and Biology. *Proceedings of the 12th American Peptide Symposium*, 583–584.
- MacCoss, M., & Cameron, D. J. (1978). Facile deprotection of nucleoside derivatives by using trifluoroacetic acid. *Carbohydrate Research*, 60(1), 206–209.
- Madsen, P., et al. (2009). Human glucagon receptor antagonists with thiazole cores. A novel series with superior pharmacokinetic properties. *Journal of Medicinal Chemistry*, 52(9), 2989–3000.
- Mannhold, R., et al. (2006). *High-Throughput Screening in Drug Discovery*. Vol. 35. John Wiley & Sons.
- McArthur, C. R., et al. (1982). Polymer supported enantioselective reactions. II. α -Methylation of cyclohexanone. *Canadian Journal of Chemistry*, 60(14), 1836–1841.
- Mergler, M., et al. (1988). Peptide synthesis by a combination of solid-phase and solution methods I: A new very acid-labile anchor group for the solid phase synthesis of fully protected fragments. *Tetrahedron Letters*, 29(32), 4005–4008.
- Mergler, M., et al. (1988). Peptide synthesis by a combination of solid-phase and solution methods II synthesis of fully protected peptide fragments on 2-methoxy-4-alkoxy-benzyl alcohol resin. *Tetrahedron Letters*, 29(32), 4009–4012.
- Mergler, M., et al. (1999). Solid phase synthesis of fully protected peptide alcohols. *Tetrahedron Letters*, 40(25), 4663–4664.

- Merrifield, R. B. (1963). Solid phase peptide synthesis. I. The synthesis of a tetrapeptide. *Journal of the American Chemical Society*, 85(14), 2149–2154.
- Merrifield, R. B., Stewart, J. M., & Jernberg, N. (1966). Instrument for automated synthesis of peptides. *Analytical Chemistry*, 38(13), 1905–1914.
- Miertus, S., & Fassina, G. (2014). Combinatorial Chemistry and Technologies: Methods and Applications. CRC Press.
- Mitchell, A. R., Erickson, B. W., Ryabtsev, M. N., Hodges, R. S., & Merrifield, R. B. (1976). tert-Butoxycarbonylaminocetyl-4-(oxymethyl) phenylacetamidomethyl-resin, a more acid-resistant support for solid-phase peptide synthesis. *Journal of the American Chemical Society*, 98(23), 7357–7362.
- Miyashita, M. Y., & Grieco, P. A. (1977). *A. J. Org. Chem.*, 44, p. (1438).
- Mojsov, S., & Merrifield, R. (1981). Solid-phase synthesis of crystalline glucagon. *Biochemistry*, 20(10), 2950–2956.
- Montero, A., et al. (2007). Synthesis of a 24-Membered Cyclic Peptide-Biphenyl Hybrid. *European Journal of Organic Chemistry*, 8, 1301–1308.
- Morales, G. A., & Bunin, B. A. (2003). *Combinatorial Chemistry*. Academic Press.
- Moran, E. J., et al. (1995). Radio frequency tag encoded combinatorial library method for the discovery of tripeptide-substituted cinnamic acid inhibitors of the protein tyrosine phosphatase PTP/B. *Journal of the American Chemical Society*, 117(43), 10787–10788.
- Mullen, D. G., & Barany, G. (1988). A new fluoridolyzable anchoring linkage for orthogonal solid-phase peptide synthesis: design, preparation, and application of the N-(3 or 4)-[[4-(hydroxymethyl) phenoxy]-tert-butylphenylsilyl] phenyl pentanedioic acid monoamide (Pbs) handle. *The Journal of Organic Chemistry*, 53(22), 5240–5248.
- Müller, G. (2003). Medicinal chemistry of target family directed master keys. *Drug Discovery Today*, 8(15), 681–691.
- Needels, M. C., et al. (1993). Generation and screening of an oligonucleotide-encoded synthetic peptide library. *Proceedings of the National Academy of Sciences*, 90(22), 10700–10704.
- Nefzi, A., et al. (1997). Solid phase synthesis of heterocyclic compounds from linear peptides: cyclic ureas and thioureas. *Tetrahedron Letters*, 38(6), 931–934.
- Neises, B., & Steglich, W. (1978). Simple method for the esterification of carboxylic acids. *Angewandte Chemie International Edition in English*, 17(7), 522–524.
- Nicolaou, K., et al. (1995). *Angewandte Chemie International Edition in English*, 34(20), 2289–2291.
- Nicolaou, K., et al. (2000). Natural product-like combinatorial libraries based on privileged structures. 2. Construction of a 10,000-membered benzopyran library by directed split-and-pool chemistry using NanoKans and optical encoding. *Journal of the American Chemical Society*, 122(41), 9954–9967.
- Nielsen, J., Brenner, S., & Janda, K. D. (1993). Synthetic methods for the implementation of encoded combinatorial chemistry. *Journal of the American Chemical Society*, 115(21), 9812–9813.
- Nikolaiev, V., et al. (1992). Peptide-encoding for structure determination of nonsequenceable polymers within libraries synthesized and tested on solid-phase supports. *Peptide Research*, 6(3), 161–170.
- Nugiel, D. A., Cornelius, L. A., & Corbett, J. W. (1997). Facile preparation of 2, 6-disubstituted purines using solid-phase chemistry. *The Journal of Organic Chemistry*, 62(1), 201–203.

- Obrecht, D., & Villalgordo, J. M. (Eds.). (1998). *Solid-supported combinatorial and parallel synthesis of small-molecular-weight compound libraries* (Vol. 17). Philadelphia: Elsevier Mansion Ltd.
- Orlowski, R. C., Walter, R., & Winkler, D. (1976). Study of benzhydrylamine-type polymers. Synthesis and use of p-methoxybenzhydrylamine resin in the solid-phase preparation of peptides. *The Journal of Organic Chemistry*, 41(23), 3701–3705.
- Orry, A. J., Abagyan, R. A., & Cavasotto, C. N. (2006). Structure-based development of target-specific compound libraries. *Drug Discovery Today*, 11(5), 261–266.
- Ortholand, J.-Y., & Ganesan, A. (2004). Natural products and combinatorial chemistry: back to the future. *Current Opinion in Chemical Biology*, 8(3), 271–280.
- Pandeya, S., & Thakkar, D. (2005). Combinatorial chemistry: A novel method in drug discovery and its application. *Indian Journal of Chemistry*, 44, 335–348.
- Pietta, P., & Marshall, G. R. (1970). Amide protection and amide supports in solid-phase peptide synthesis. *Journal of the Chemical Society D: Chemical Communications*, 11, 650–651.
- Pon, R. T., & Yu, S. (1997). Hydroquinone-O, O'-diacetic acid as a more labile replacement for succinic acid linkers in solid-phase oligonucleotide synthesis. *Tetrahedron Letters*, 38(19), 3327–3330.
- Poupart, M.-A., et al. (1999). Solid-phase synthesis of peptidyl trifluoromethyl ketones. *The Journal of Organic Chemistry*, 64(4), 1356–1361.
- Quesnel, A., & Briand, J. P. (1998). Incomplete trifluoroacetic acid deprotection of asparagine-trityl-protecting group in the vicinity of a reduced peptide bond. *The Journal of Peptide Research*, 52(2), 107–111.
- Rabanal, F., et al. (1990). Study on the stability of 9-fluorenylmethoxycarbonyl in catalytic hydrogenation conditions. In *anales de quimica. Real soc espan quimica facultad de fisica quimica ciudad univ, 3 madrid, spain*.
- Ramage, R., et al. (1999). Comparative studies of NSC and FMOC as $\text{N}\alpha$ -protecting groups for SPPS. *Journal of Peptide Science*, 5(4), 195–200.
- Ramage, R., Green, J., & Blake, A. J. (1991). An acid labile arginine derivative for peptide synthesis: N-G-2,2,5,7,8-pentamethylchroman-6-sulphonyl-L-arginine. *Tetrahedron*, 47(32), 6353–6370.
- Rinnová, M., & Lebl, M. (1996). Molecular diversity and libraries of structures: Synthesis and screening. *Collection of Czechoslovak Chemical Communications*, 61(2), 171–231.
- Roeske, R. (1963). Preparation of t-Butyl Esters of Free Amino Acids. *The Journal of Organic Chemistry*, 28(5), 1251–1253.
- Rosenthal, K., Erlandsson, M., & Undén, A. (1999). 4-(3-Hydroxy-4-methylpentyl) phenyl-acetic acid as a new linker for the solid phase synthesis of peptides with Boc chemistry. *Tetrahedron Letters*, 40(2), 377–380.
- Rueter, J. K., et al. (1998). Arylsulfonate esters in solid phase organic synthesis. I. Cleavage with amines, thiolate, and imidazole. *Tetrahedron Letters*, 39(9), 975–978.
- Sabirov, A. N., et al. (1997). FMOC-and NSC-Groups as a Base Labile N (α)-Amino Protection: A Comparative Study in the Automated SPPS. *Protein and Peptide Letters*, 4, 307–312.
- Samukov, V. V., Sabirov, A. N., & Pozdnyakov, P. I. (1994). 2-(4-Nitrophenyl) sulfonylethoxy-carbonyl (NSc) group as a base-labile α -amino protection for solid phase peptide synthesis. *Tetrahedron Letters*, 35(42), 7821–7824.

- Sauerbrei, B., Jungmann, V., & Waldmann, H. (1998). An Enzyme-Labile Linker Group for Organic Syntheses on Solid Supports. *Angewandte Chemie International Edition*, 37(8), 1143–1146.
- Shimonishi, Y., Sakakibara, S., & Akabori, S. (1962). Studies on the Synthesis of Peptides Containing Glutamine as the C-Terminal. I. Protection of Amide-Nitrogen With Xanthyl Group During Peptide Synthesis. *Bulletin of the Chemical Society of Japan*, 35(12), 1966–1970.
- Sieber, P. (1987). A new acid-labile anchor group for the solid-phase synthesis of C-terminal peptide amides by the Fmoc method. *Tetrahedron Letters*, 28(19), 2107–2110.
- Sieber, P. A., R. H. Eisler, K., Kamber, B., Riniker, B., Rink, H. (1977). 543–545.
- Sieber, P., & Riniker, B. (1991). Protection of carboxamide functions by the trityl residue. Application to peptide synthesis. *Tetrahedron Letters*, 32(6), 739–742.
- Simkins, R., & Williams, G. (1952). The nitration of guanidine in sulfuric acid. Part I. The reversible conversion of guanidine nitrate into nitroguanidine. *J. Chem. Soc.*, 3086–3094.
- Sola, R., Méry, J., & Pascal, R. (1996). Fmoc-based solid-phase peptide synthesis using Dpr (Phoc) linker. Synthesis of a C-terminal proline peptide. *Tetrahedron Letters*, 37(51), 9195–9198.
- Stelakatos, G., Paganou, A., & Zervas, L. (1966). New methods in peptide synthesis. Part III. Protection of carboxyl group. *Journal of the Chemical Society C: Organic*, 1191–1199.
- Story, S. C., & Aldrich, J. V. (1992). Preparation of protected peptide amides using the Fmoc chemical protocol. *International Journal of Peptide and Protein Research*, 39(1), 87–92.
- Studer, A., et al. (1997). Fluorous synthesis: A fluorous-phase strategy for improving separation efficiency in organic synthesis. *Science*, 275(5301), 823–826.
- Swartz, M. E. (Ed.) (2000). *Analytical Techniques in Combinatorial Chemistry*. CRC Press.
- Szardening, A. K., et al. (1999). Identification of highly selective inhibitors of collagenase-1 from combinatorial libraries of diketopiperazines. *Journal of Medicinal Chemistry*, 42(8), 1348–1357.
- Szymański, P., Markowicz, M., & Mikiciuk-Olasik, E. (2011). *International Journal of Molecular Sciences*, 13(1), 427–452.
- Tam, J. P. (1985). A gradative deprotection strategy for the solid-phase synthesis of peptide amide using p-(acyloxy) benzhydrylamine resin and the SN2 deprotection method. *The Journal of Organic Chemistry*, 50(25), 5291–5298.
- Tam, J. P., Heath, W. F., & Merrifield, R. (1983). An SN2 deprotection of synthetic peptides with a low concentration of hydrofluoric acid in dimethyl sulfide: evidence and application in peptide synthesis. *Journal of the American Chemical Society*, 105(21), 6442–6455.
- Tam, J. P., Heath, W. F., & Merrifield, R. (1986). Mechanisms for the removal of benzyl protecting groups in synthetic peptides by trifluoromethanesulfonic acid-trifluoroacetic acid-dimethyl sulfide. *Journal of the American Chemical Society*, 108(17), 5242–5251.
- Thayer, A. M. (1996). Combinatorial chemistry becoming core technology at drug discovery companies. *Chemical & Engineering News*, 74(7), 57–57.
- Thompson, L. A., & Ellman, J. A. (1994). Straightforward and general method for coupling alcohols to solid supports. *Tetrahedron Letters*, 35(50), 9333–9336.
- Thompson, L. A., & Ellman, J. A. (1996). Synthesis and applications of small molecule libraries. *Chemical Reviews*, 96(1), 555–600.

- Tietze, L. F., Steinmetz, A., & Balkenhol, F. (1997). Solid-phase synthesis of polymer-bound β -ketoesters and their application in the synthesis of structurally diverse pyrazolones. *Bioorganic & Medicinal Chemistry Letters*, 7(10), 1303–1306.
- Vaino, A. R., & Janda, K. D. (2000). Solid-phase organic synthesis: a critical understanding of the resin. *Journal of Combinatorial Chemistry*, 2(6), 579–596.
- Vlattas, I., et al. (1997). The use of thioesters in solid phase organic synthesis. *Tetrahedron Letters*, 38(42), 7321–7324.
- Wang, S.-S. (1973). p-Alkoxybenzyl alcohol resin and p-alkoxybenzyloxycarbonylhydrazide resin for solid phase synthesis of protected peptide fragments. *Journal of the American Chemical Society*, 95(4), 1328–1333.
- Wang, S., et al. (1974). Solid phase synthesis of bovine pituitary growth hormone-(123–131) nonapeptide. *International journal of peptide and protein research*, 6(2), 103–109.
- Welsch, M. E., Snyder, S. A., & Stockwell, B. R. (2010). Privileged Scaffolds for Library Design and Drug Discovery. *Current Opinion in Chemical Biology*, 14(3), 347–361.
- Wong, J. Y., Manning, C., & Leznoff, C. C. (1974). Solid Phase Synthesis and Photochemistry of 4, 4'-Stil-benedicarbaldehyde. *Angewandte Chemie International Edition in English*, 13(10), 666–667.
- Woodward, R., et al. (1966). The total synthesis of cephalosporin C1. *Journal of the American Chemical Society*, 88(4), 852–853.
- Wu, Z., et al. (2003). Synthesis of tetrahydro-1, 4-benzodiazepine-2-ones on hydrophilic polyamide synphase lanterns. *Journal of Combinatorial Chemistry*, 5(2), 166–171.
- Xiao, X. Y., et al. (1997). Combinatorial chemistry with laser optical encoding. *Angewandte Chemie International Edition in English* 36(7), 780–782.
- Zehavi, U., & Patchornik, A. (1973). Oligosaccharide synthesis on a light-sensitive solid support. I. Polymer and synthesis of isomaltose (6-O-. alpha.-D-glucopyranosyl-D-glucose). *Journal of the American Chemical Society*, 95(17), 5673–5677.
- Zhang, K., et al. (2013). Destruction of perfluorooctane sulfonate (PFOS) and perfluoroctanoic acid (PFOA) by ball milling. *Environmental Science & Technology*, 47(12), 6471–6477.
- Zhao, Z.-G., & Lam, K. S. (1997). Synthetic peptide libraries, in *Annual Reports in Combinatorial Chemistry and Molecular Diversity*. Springer. 192–209.
- Zinner, H. (1950). Notiz über Mercaptale der d-Ribose. *Chemische Berichte*, 83(3), 275–277.

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