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REVIEW ON COMBINATORIAL CHEMISTRY

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ABSTRACT

In the new era of medicinal chemistry the target in focused on the preparation of chemical libraries for the generation of new lead for drug discovery. A traditional chemist can synthesize 100-200 compounds per year. Combinatorial chemistry is one of the important new methodologies developed by researchers in the pharmaceutical industry to reduce the time and costs associate with producing effective and competitive new drugs. It is used to synthesize large number of chemical compounds such as proteins & peptides by combining sets of building blocks. This review focus on various current newly available solid supports, Linkers and other accessories employed in combinatorial organic synthesis.

KEYWORDS: Combinatorial Chemistry, Solid supports, Linkers.

INTRODUCTION

Solid phase synthesis is methodology where organic synthesis involves one of the reactant attached to an insoluble material referred as solid supports. Such method applicable to synthesis of peptides and Oligonucleotide. Combinatorial approaches were originally based on premise that the probability of finding a molecule in a random screening process is proportional to the number of molecules subjected to the screening process. In its earliest expression, the primary objective of combinatorial chemistry focused on the simultaneous generation of large numbers of molecules and on the simultaneous screening of their activity. Combinatorial chemistry has emerged because of genomics and new efficient biological screening strategies and is widely cited as a paradigm shift in the way that new small molecule lead structures will be identified. The first combinatorial chemistry experiments were applied to the study of epitopes. The short sequences of amino acids responsible for antibody recognition and binding to proteins. The development of new processes for the

generation of collection of structurally related compounds (libraries) with the introduction of combinatorial approaches has revitalized random screening as a paradigm for drug discovery and has raised enormous excitement about the possibility of finding new and valuable drugs in short times and at reasonable costs (Figure 1).

However the advent of this new field in drug discovery did not obscure the importance of "classical" medicinal chemistry approaches, such as, computer-aided rational drug design and QSAR for example, but catalyzed instead their evaluation to complement and to be integrated with combinatorial technologies.[1]

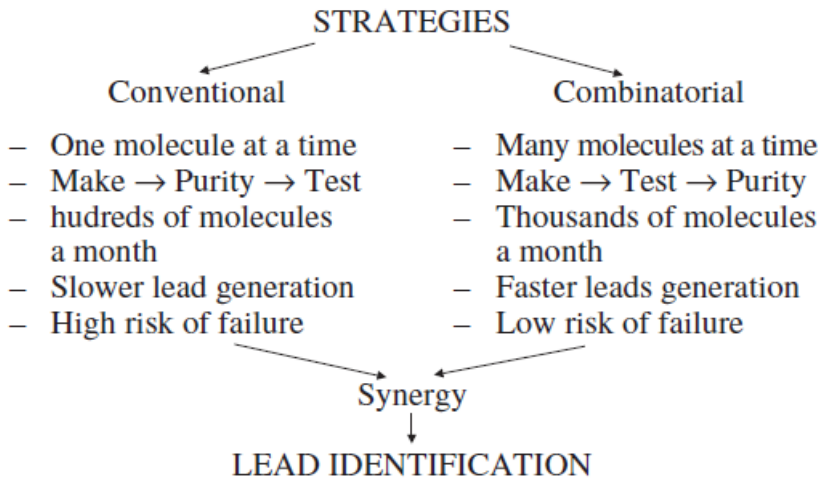


Fig.1:Principal characteristics of conventional v/s combinatorial strategy of drug discovery.

Steps involved in combinatorial synthesis:[2]

Solid Phase Peptide Synthesis (SPPS) can be defined as a process in which a peptide anchored by its C-terminus to an insoluble polymer is assembled by the successive addition of the protected amino acids constituting its sequence.

Each amino acid addition is referred to as a cycle consisting of:

a) Cleavage of the Nα-protecting group

b) Washing steps

c) Coupling of a protected amino acid

d) Washing steps

As the growing chain is bound to an insoluble support the excess of reagents and soluble by-products can be removed by simple filtration. Washing steps with appropriate solvents ensure the complete removal of cleavage agents after the deprotection step as well as the elimination of excesses of reagents and by-products resulting from the coupling step.

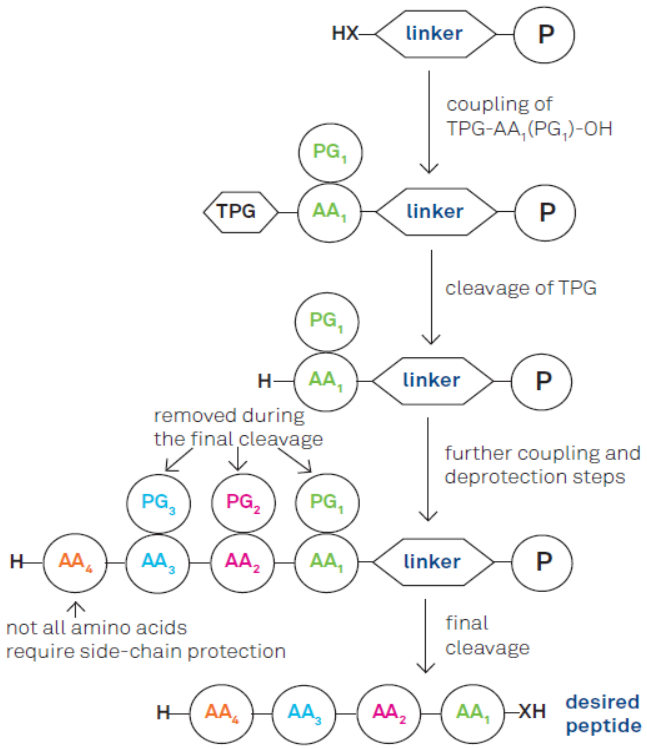


Fig .2: General scheme of SPPS.

X = O, NH, AA = Amino Acid, PG = Protecting Group, P = Polymer Support, TPG = Temporary Protecting Group

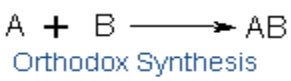
Table 1: 50 Years of history - A choice of key Dates.[2]

Year	Authors	Development
1963	Merrifield	Development of SPPS, insoluble carrier: Crosslinked polystyrene; Nα-protecting group: Boc
1967	Sakakibara	HF-cleavage
1970	Pietta & Marshall	Introduction of BHA-resin for the synthesis of peptide amide MBHA-resin: Matsueda & Stewart 1981
1970	Carpino & Han	Fmoc, a base labile Nα-protecting group
1973	Wang	Development of p-alkoxybenzyl alcohol resin (Wang resin, cleavage: TFA; Nα-protection: Bpoc

1976	Burgus & Rivier	Application of preparative reversed phase HPLC for the purifi cation of peptides prepared by Boc SPPS
1977	Barany et al.	The concept of "orthogonal" protection schemes
1978	Meienhofer et al.	Fmoc/tButyl strategy. Carrier: p-alkoxybenzyl alcohol resin; Nα-protection: Fmoc; side-chain protection: TFA- labile, e.g. Boc, tBu; final cleavage: TFA
1985	Houghten and others	Simultaneous parallel peptide synthesis, synthesis of peptide libraries (T-bags, pins, etc.)
1985	Rapp and others	Polystyrene-polyethylene glycol grafts e.g. TentaGel
1987	Rink and others	Introduction of various TFA-labile linkers for the Fmoc/tBu SPPS of peptide amides
1987	Sieber	"Xanthenyl linker" for the Fmoc/tBu SPPS of fully protected peptide amides, cleavage: 1% TFA/DCM
1987	Mergler et al.	Development of 2-methoxy-4-alkoxybenzyl alcohol resin SASRIN (Super Acid Sensitive ResIN) for the Fmoc/tBu SPPS of fully protected peptide fragments, cleavage: 1% TFA/DCM
1988	Barlos et al.	2-Chlorotritylchloride resin for the Fmoc/tBu SPPS of fully protected peptide fragments, cleavage: AcOH/TFE/ DCM (1:1:3) or HFIP/DCM (1:4)
1993	Hobbs de Witt, Ellman and others	Combinatorial Chemistry; Solid Phase Organic Synthesis (for rapid synthesis of libraries of small molecules)
1995	Mutter et al.	Pseudoproline dipeptides
2002	Gogoll and others	Microwave-accelerated SPPS
2003	White and others	Fmoc SPPS of long peptides (100 AA)

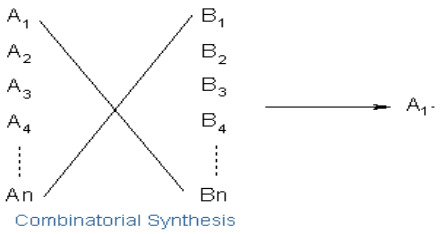
Principle of combinatorial chemistry

Combinatorial chemistry is a technique by which large numbers of structurally distinct molecules may be synthesised in a time and submitted for pharmacological assay. The key of combinatorial chemistry is that a large range of analogues is synthesised using the same reaction conditions, the same reaction vessels. In this way, the chemist can synthesise many hundreds or thousands of compounds in one time instead of preparing only a few by simple methodology. In the past, chemists have traditionally made one compound at a time. For example compound A would have been reacted with compound B to give product AB, which would have been isolated after reaction work up and purification through crystallisation, distillation, or chromatography.



Orthodox Synthesis

In contrast to this approach, combinatorial chemistry offers the potential to make every combination of compound A_1 to An with compound B_1 to Bn.



The range of combinatorial techniques is highly diverse, and these products could be made individually in a parallel or in mixtures, using either solution or solid phase techniques. Whatever the technique used the common denominator is that productivity has been amplified beyond the levels that have been routine for the last hundred years.

Libraries[1]

Collection of structurally related compound (peptides, oligonucleotides, oligosaccharides, organic molecules) obtainable by chemical or biological means simultaneously as a mixture and screened for activity as a mixture of compounds, without any isolation protocol step. Identification of active compounds derives from the synthesis/production protocolused to generate the library. Great acceleration of leads identification since millions of different compounds can be screened simultaneously.

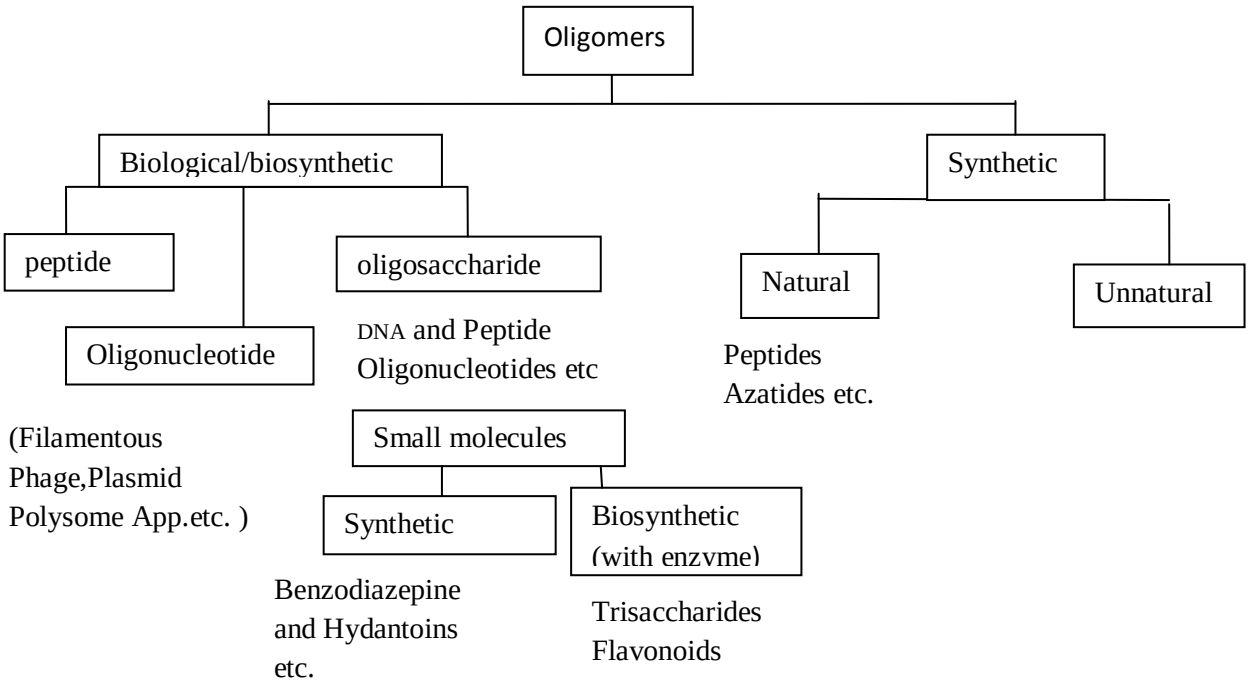


Fig.3: The basic sources of molecular diversity generated by combinatorial approach.

Dynamic combinatorial chemistry

Under thermodynamic control, combinatorial chemistry is known as dynamic combinatorial chemistry. In a dynamic combinatorial library, all constituents are in equilibrium. The library members are interconverted into one another via reversible process in that covalent or non-covalent interactions are involved.[5] From the available building blocks, dynamic combinatorial chemistry consists of using the target as a template which is build with best complement(s). In the field of supramolecular chemistry, where DCC is rooted by where molecular diversity generated by the use of self-assembling systems through the reversible association of a few components. The concentrations of the different species in a library will be dependent upon the intrinsic stability of the various library members. In particular, stabilization of a particular library member through noncovalent interactions with an added template will alter the positions of the equilibria governing the system (Figure 4). Such a shift in equilibrium position will ideally lead to an increased production – an "over expression" or "amplification" of the stabilized library member at the expense of the other species in the mixture. In this way, a library may be probed for species with affinity for a given target molecule use of self-assembling systems through the reversible association of a few components.[4]

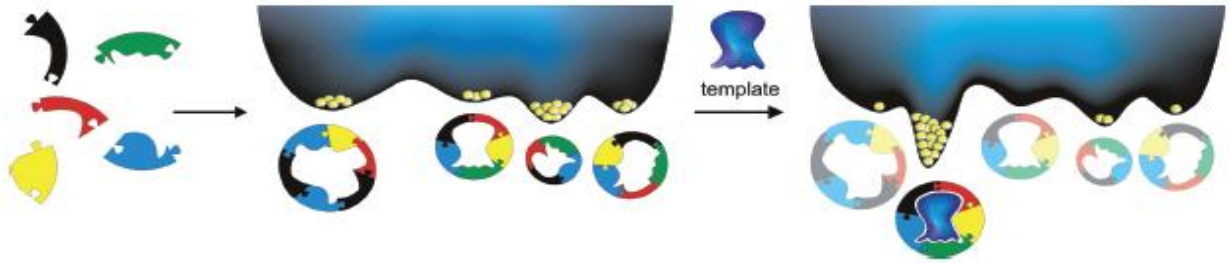


Fig.4: A DCL is formed from the different combinations of several building block. Ideally, addition to the library of a template will alter the library distribution to amplify the receptor that forms the most stable complex with the template.

Method involved in combinatorial synthesis:[3]

There are two approach by which the combinatorial libraries can be generated:

1) Biological library approach

a) Filamentous phage approach

b) Plasmid approach

c) Polysome approach

2) Spatially addressable parallel solid phase library approach

a) Multi-pin methodology

b) Tea bag methodology

c) SPOTS membrane method

d) Light directed peptide synthesis on resin support

1)Biological approach to generate molecular diversity

The use of biological system for the generation of peptide diversity mimics the evolution creation of protein diversity.Artifical evolution can be greatly enhanced by the introduction of diversity in to the system at a much higher rate than that occurs naturally. The source of the diversity in the combinatorial chemical synthesis is the structure of oligonucleotides.Oligonucleotide synthesis is a well characterized chemistry that allows tight control of the composition of mixture created.The degenerated sequence produced are then cloned and expressed as peptides.

Peptide displayed on phage particles

Emil Fischer synthesized the first dipeptide, called glycylglycin and coined the term "peptide". Peptide synthesis has become one of the most important methodologies in bioorganic chemistry. The automation of solid-phase synthesis has permitted a great variety of researchers to utilize synthetic peptides. This method involves displaying the peptide on the much less complex surface of the bacteriophage particles. [6] In 1988, Parmely and Smith proposed the use of filamentous phage to display random oligopeptides on the amino terminal of the viral PIII coat protein. This was accomplished by the insertion of a stretch of random deoxynucleotide into PIII gene of filamentous phage with the help of ECoRI restriction endonuclease.

2)Spatially addressable parallel library approach

The desire to develop and explore SAR around peptide lead compound has placed tremendous demands on the productivity of peptide chemistry.

a) Multiple pin methodology

In this method,the synthesis, of peptides takes place on polyethylene pins (4×40 mm) functionalized with acrylic acid arranged in 96 well formats. The well contain activated amino acid monomers.Peptide synthesis is carried out at the end of a spacer (e.g.NB-Fmoc-β-

alanyl-1,6-diaminohexane).Screening is done by means of enzyme linked immunosorbent assay (ELSIA) to determine the binding capability of covalently bound peptide to antibodies.

Fig.5: Multiple pin methodology

b) Tea bag

Houghten first developed this method of multiple peptide synthesis. The peptide synthesis occurs on resin that is sealed inside polypropylene bags.Amino acids are coupled to th resin by placing the bag in solution of the appropriate individual actiated solution of the appropriate individual activated monomers.All common steps such as resin washing and amino group deprotection are performed simultaneously.At the end of synthesis,each bag contains a single peptide.

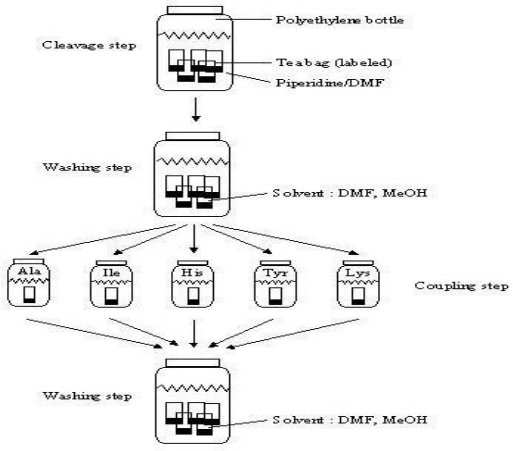


Fig.6: Tea bag method

c) SPOTS membrane method

Frank (1992) has followed Geysens strategy except that a cellulose membrane or paper was used instead of the polyethylene pins as the solid support for peptides synthesis.

d) Light directed spatially addresssable parallel chemical synthesis

In this method identity of a compound is given by its location on a synthesis.Here the combinatorial process is carried out by controlling the addition of a chemical reagent to specific location on a solid support.

This technique combines two technology: i) Solid phase peptides synthesis chemistry ii)Photolithography

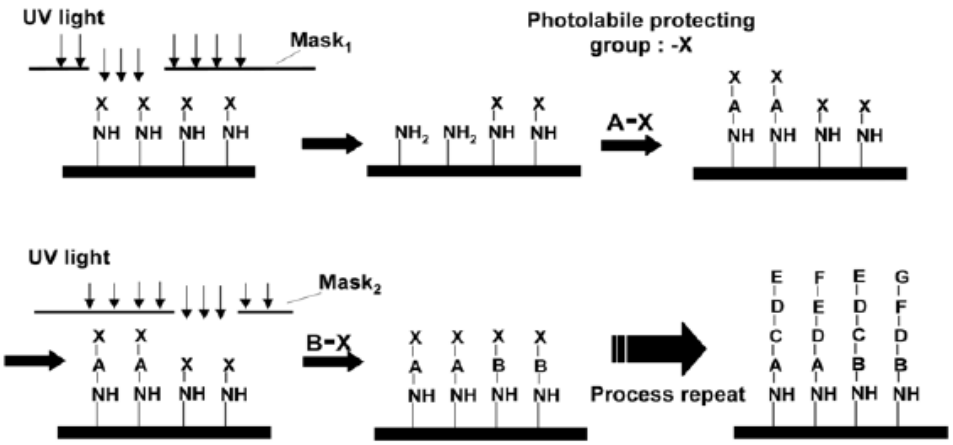


Fig.7: Light-directed,spatially addressable parallel chemical synthesis (Fodor etal.,1991).[7]

Combinatorial chemistry can be applied to i)Solution phase synthesis ii) Solid phase synthesis

i) Solution phase synthesis

The solution phase synthesis involves conducting chemical reaction simultaneously, preferably in a well-ordered sets of (arrays) of reaction vessels in solution for example, the preparation of small array of amides which consists of placing different acid chlorides and amines in each of matrix reaction vessel (along with tertiary amine to neutralize liberated hydrochloric acid),incubating and performing liquid-liquid extraction .Evaporation of amines give crude amides,which can be tested directly in biological assay.

Examples (N.K. Terrett 2014), A library of imidazo[1,2-a]pyridines was synthesised by the reaction of readily available aldehydes and 2-aminopyridines in a mixture of nitroalkane and DMF.This transformation presumably occurs by a sequential aza Henry reaction/cyclisation /denitration.[10]

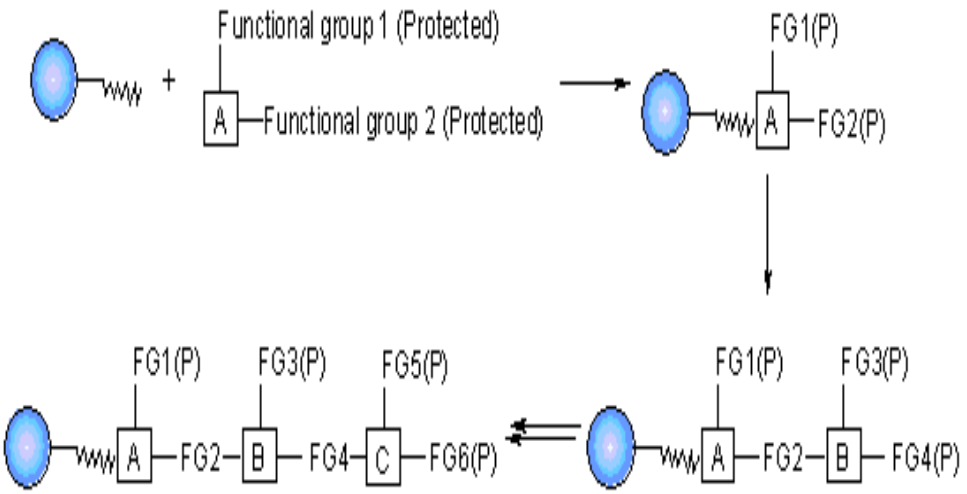
Disadvantages

In this method number of reagents are taken together in solution,it can result in several side reactions and may lead to polymerization giving a tarry mass.Therefore to avoid this, the new approach is developed in which all-chemical structure combinations are prepared separately,in parallel on a giving building block using an automated robotic apparatus.Hundreds and thousands of vials are used to perform the reactions and laboratory robots are programmed to deliver specific reagents to each vial.

ii) Solid phase synthesis

In this method,the reaction is carried out on a solid support such as resin beads,a range of different starting materials can be bound to separate resin beads, which are mixed together, such that all the starting material can be treated with another reagent in a single experiments. Since the products are bound to solid support, excess reagents or by-products can be easily removed by washing with appropriate solvent. Large excess of reagent solvints can be used to drive the reaction to completion.Intermediates in a reaction sequences are bound to the bead and need not be purified.Individual beads can be separated at the end of the experiment to get individual products; the polymeric support can be regeneratedamd reused if appropriate cleavage conditions and suitable anchor/linker groups are chosen. There are certain advantages of the solid phase synthesis over the solution phase synthesis,which includes synthesis on a polymeric support greatly, simplifies the problem of product isolation from reaction mixture, moreover we can take the advantage of the support-tethered diversity in the design of convenient receptor binding assay for library evaluation.

The use of solid support for organic synthesis relies on three interconnected requirements:



1) A cross linked, insoluble polymeric material that is inert to the condition of synthesis.
2) Some means of linking the substrate to this solid phase that permits selective cleavage of some or all of the product from the solid support during synthesis for analysis of the extent of reaction(s), and ultimately to give the final product of interest.
3) A chemical protection strategy to allow selective protection and deprotection of reactive groups.

Types of solid support

1) Polymeric solid support
2) A linker
3) Protecting groups

1) Polymeric solid support

In solid phase support synthesis, the solid support is generally based on a polystyrene resin. The most commonly used resin supports for SPS include spherical beads of lightly cross linked gel type polystyrene (1–2% divinylbenzene) and poly (styrene-oxyethylene) graft copolymers which are functionalised to allow attachment of linkers and substrate molecules. Each of these materials has advantages and disadvantages depending on the particular application.

Example N.K. Terrett (2014) the synthesis of 3'-[N-(fluorenylmethoxycarbonyl)-amino]-5'carboxymethyl derivative of all four natural ribonucleosides from cheap chiral pool compounds. Using standard solid phase Fmoc-chemistry, the synthesis of fully amide-linked RNA analogues of small oligonucleotides containing all four nucleoside amino acids has been described.[9]

2) Linkers

The group that joins the substrate to the resin bead is an essential part of solid phase synthesis. The linker is a specialised protecting group, in that much of the time, the linker will tie up a functional group, only for it to reappear at the end of the synthesis. The linker must not be affected by the chemistry used to modify or extend the attached compound. And finally the cleavage step should proceed readily and in a good yield. The best linker must allow attachment and cleavage in quantitative yield.

Strategies of linker[8]

The first strategy is to link through functionality already present in the desired target molecule,such as in peptide and oligonucletide synthesis.The second approach is a cyclative cleavage whereby the linking functional group is somehow incorporated into the final molecule. The third approach is the introduction of an auxiliary functional group such as phenol,amide etc for linker attachment.

Example:O-Nitrobenzyl photolabile linker

Christopher P.Holmes (1993) The use of photolabile molecule as a linker for the cleavage of peptides from solid support are widely used. o- Nitrobenzyl derived from 4-(bromomethyl)-3-nitrobenzoic acid has been the most widely employed photolabile support for generation of both peptide acids and amides. Photolabile amide protecting groups for C-termini of peptides which rely on the same basic o-nitrobenzyl chemistry have also been reported.

Types of linker [5]

a) Cyclative cleavage

b) Tracelss linkers

c) Cleavage diversification

d) Recyclable/reusable resin

e) Asymmetric induction

f) Partial/sequential release linkers

g) Biocompatible linkers

a) Cyclative cleavage

Induction of cleavage by cyclisation during the final step of a synthesis has the major advantage that reaction products which are not capable of cyclisation, for instance, due to an complete transformation earlier in the reaction sequence remain attached to the solid phase.

Example: An insoluble cross-linked poly-(4- hydroxy-3-nitrostyrene) resin was employed and was treated with protected peptides to give an insoluble polymer containing an activated ester group as the linker. Deprotection of the amine end of the peptide incurred intramolecular cyclisation to afford cyclic peptides in high yield and purity.

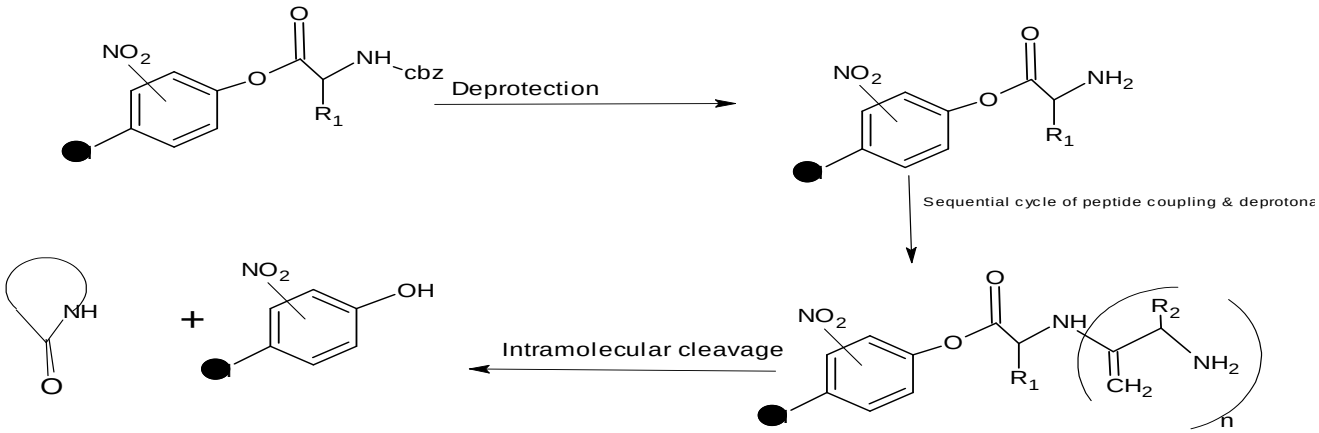


Fig.8: Cyclative cleavage in solid phase synthesis of a cyclic peptide.

a) Traceless Linkers

In traceless cleavage a functional group is excised leaving behind no trace or' memory' of the solid phase synthesis. Such libraries may be highly desirable should residual functionality have the potential to exert a negative effect on the biological or chemical activity of the target molecule.

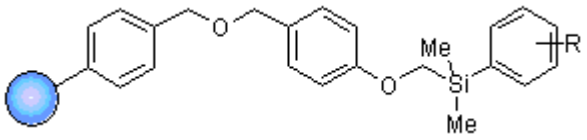


Fig.9:Traceless silyl linker

The first and most widely explored of this traceless linker is the silyl linker. Silicon attached to a phenyl group can undergo a protodesilylation reaction cleaving the silicon – aryl bond when treated with acid.

b) Cleavage diversification

An alternative linkage strategy is to facilitate a range of cleavage approaches thus allowing for further product diversification in the final step. Although this strategy does not yield a C-H bond, it does not leave any memory of the nature of the linkage and instead, a range of different functionalities are introduced by the reagents used for cleavage.

Example:

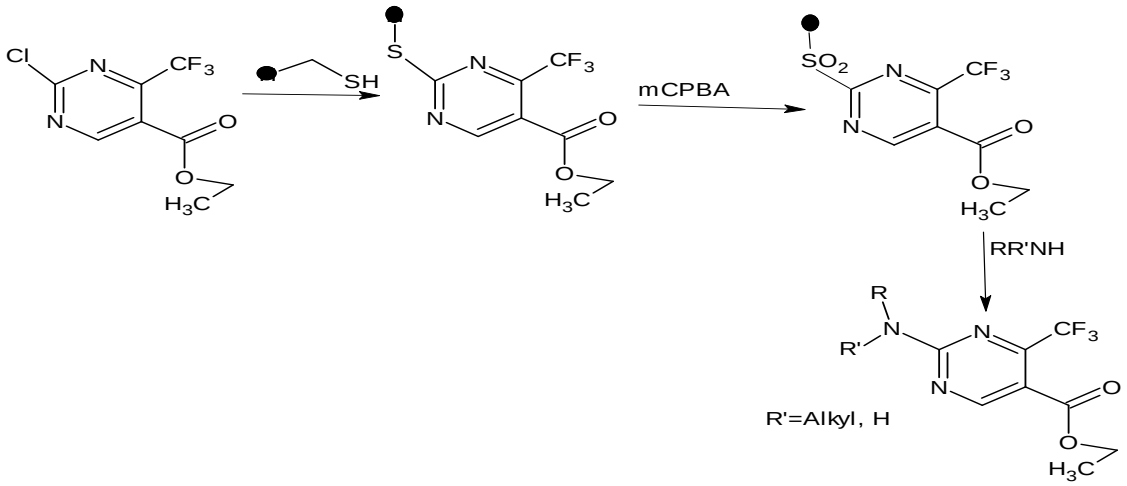


Fig.10: A sulfur-based linker providing diversification upon cleavage.

c) Recyclable/reusable resin

A linker is the ability to recycle that is, the linker structure can be regenerated upon substrate release, thus allowing for another synthetic cycle to begin on the same resin.

Example: Recyclable benzyl and aryl sulfone analogues that are operate by the same mechanism as the REM resin being derivatised by a Michael addition and activated by quaternisation.

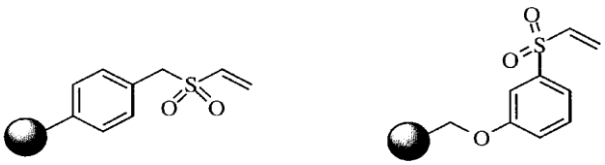


Fig.11: Benzyl and aryl sulfone analogues of the REM resin.

d) Asymmetric Induction

They ideally are recyclable are those which induce stereoselectivity and therefore incorporate chiral auxiliaries.

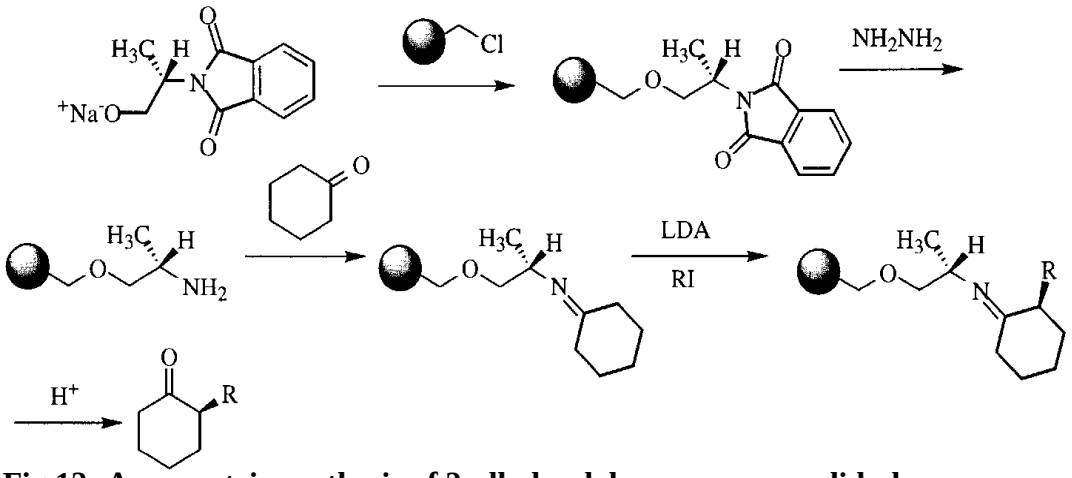


Fig.12: Asymmetric synthesis of 2-alkylcyclohexanones on solid phase.

e) Partial/Sequential release linkers

Linker system is the ability to allow for controlled partial cleavage rather than releasing all of the compound bound to resin particles at once.

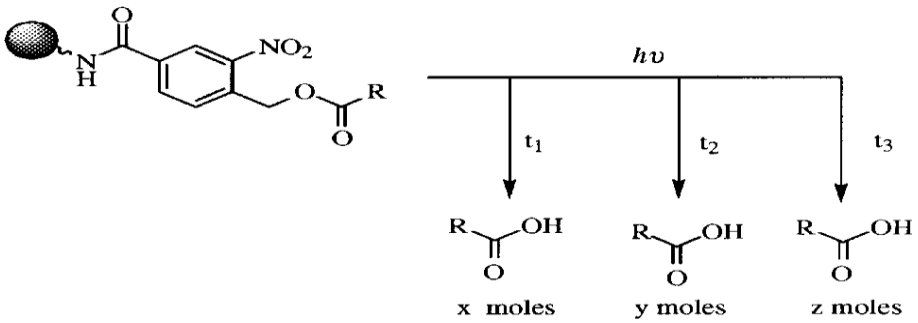


Fig.13: Partial release by controlled irradiation using photolabile linker.

f) Biocompatible linkers

A o-nitrobenzyl photolabile linker has the advantage that it is biocompatible and can be cleaved within aqueous buffer and then screened. This feature clearly offers the potential to interface solid phase organic synthesis with biological screening.

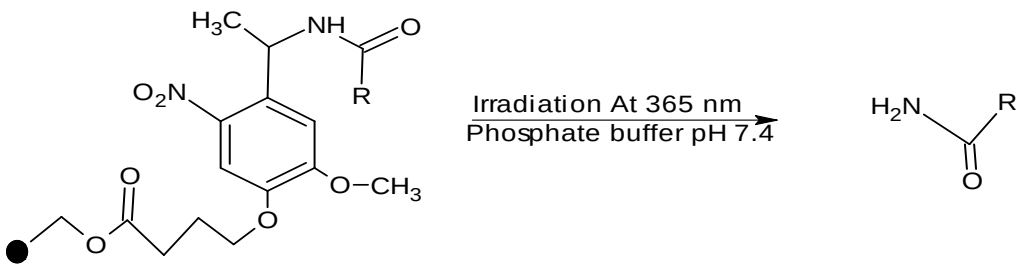


Fig.14: Photolabile linker for direct release of compound into the biological screen.

3) Protecting group/ reagent

Protecting groups are important for blocking and regenerating certain functional groups in a reaction sequence.

Example: Fmoc (Fluoromethoxy carbonyl benzyl ester) and Boc (Tertiarybutyloxycarbonyl). N.K. Terrett(2015), synthesized peptide hydrazides using a new hydrazone resin, obtained via acylation of aminomethyl polystyrene by the Fmoc-hydrazone of pyruvic acid, shown that the hydrazone linker is completely stable during standard Fmoc solid phase peptide synthesis, it can tolerate 5% TFA in dichloromethane thus permitting selective removal of acid-labile protecting groups.

N.K. Terrett (2015), synthesized the Polymer supported guanidinylation of a peptide chain on a polymeric support under microwave conditions, using S-alkylisothioureas, pyrazole carboxamidine, and guanidine as guanidinylating reagents, the best results were obtained with N, N”-di-benzyloxycarbonyl- S-methylisothiourea and N, N”-di-(2-chlorobenzyloxycarbonyl)- S-methylisothiourea with reagents containing Boc groups was accompanied by side reactions.

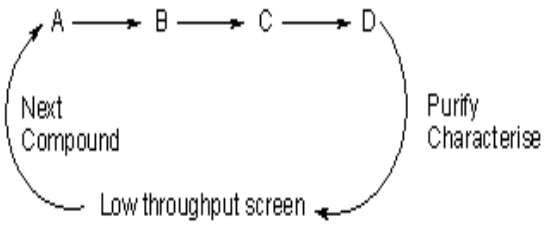
Two distinct mechanisms for elaborating molecular diversity may be defined by:

1) Parallel synthesis and 2) Split and mix synthesis

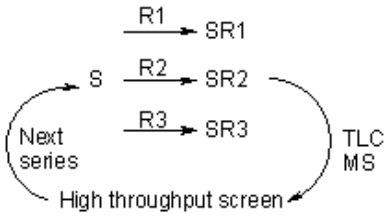
1) Parallel solution phase synthesis

Parallel synthesis of peptide libraries is based on a simple theory of combinatorial chemistry. Geysen et al. reported the first approach of peptide library synthesis by multi-pin technology (Geysen et al., 1984). Peptide libraries are synthesized in individual reaction chambers, and therefore, each product is pure, separated, well determined. However, this method generates only a small set of peptides library. Consequently, an automated synthesis system is required for the synthesis of large pool of peptide library.

Orthodox Analogue Synthesis and Screening



Parallel analogue Synthesis and Screening



Orthodox synthesis usually involves a multistep sequence, e.g. from A through to the final product D, which is purified and fully characterized before screening. The next analogue is then designed, guided by the biological activity of the previous compound, prepared, and then screened. This process is repeated to optimise both activity and selectivity.

In contrast parallel analogue synthesis involves reaction of a substrate S with multiple reactants, R_1, R_2, R_3 … R_n, to produce a compound library of n individual products SR_1, SR_2, SR_3 … SR_n. The library is screened, usually without purification, and with only minimal characterisation of the individual compounds, using a rapid throughput screening technique.

Advantage

a) No deconvolution is required.

b) No risk of synergistic effects leading to false positive results during screening.

2) Split and mix synthesis

The portioning-mixing (PM) procedure is one of the earliest combinatorial synthetic methods. It was introduced 10 years ago, in 1988. The procedure is illustrated with three different chemical units. These units have two reactive areas so that they can be coupled one to another forming for example a chain. The molecules are usually "grown" out from a solid support, typically a polymer bead that is used to " carry" the result of the reactions through the system.This makes it easy to separate the product from the reactants (not linked to the bead).

At each stage the reactions are carried out in parallel. After the first stage we have three different types of bead, each with only one of the different units on them. The starting material is split in 'n' portions, reached with n building blocks, and recombined in one flask for the second step, this procedure is repeated. This method is particularly employed for solid phase synthesis.

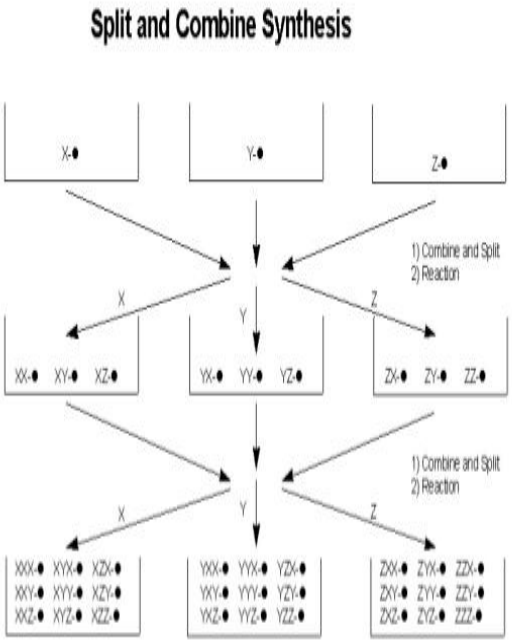


Fig.15: Split and mix synthesis

Advantages

a)Large libraries are readily available.

Limitations

1.Complex mixtures are formed.

2.Deconvolution or tagging is required.

3.Syergistic effect may be observed during screening, leading to false positive.

Criteria for selection of building block

The selection depends on the availability of large number of diverse, fairly complex, easily acecessible starting material. Member of building blocks should reflect broad array of physiological properties, e.g. functionality, charge, conformation etc.

An essential element of the combinatorial discovery process is that one must be able to extract the information made available by library. This can be achieved by library screening methods. There are two types of screening.

1) Random screening

In this, the task is to identify a lead compound in the absence of any structural information about active molecules.

2) Directed screening

In this, the objective is to evaluate closely related structure analogues of lead molecule, to establish SAR and optimize biological activity.

The importance of differentiating between random screenings versus directed screening is particularly related to the design of assay for library evaluation.

In broad terms, the assay procedures are grouped into three categories:

1) Isolation of ligand by affinity purification.

2) Binding of receptor to immobilized ligand.

3) Testing the activity of libraries of soluble compounds.

1) Isolation of ligand by affinity purification

Two important methods have been employed to achieve purification of peptide/nucleic acid complexes. The first involves incubation of a receptor in solution with the mixture of compounds. After allowing sufficient time for binding, the receptor is captured by immoboilizing it on anti-receptor antibody. The second approach is for pre-immobilization of the receptor on beads, or a chromatography support, followed by capture of the complex. In both cases, a solid support facilitates the separation and washing of receptor bound complex. To enhance the probability of isolating peptide ligands with biological function, the receptor should be active when immobilized. Immobilization of receptor protein on beads can be accomplished by covalent attachment. Biotinylation and immobilization on streptavidin or capture with high affinity non-blocking antibodies. But the first two processes may result in inactive protein and also some drug discovery targets may not be readily available as soluble receptor.

2) Building of receptor to immobilized ligand

The various libraries of compounds bound to solid support (pins, beads, etc.) can be screened by detecting direct binding of a labeled receptor to an immobilized ligand; the identity of the ligand is determined directly by mass spectroscopy. There are numbers of important issues related to this assay, first the ability of a receptor to interact with a tethered ligand may be influenced by the site or nature of its covalent bonding with support and the chemical nature of the linkage between the ligand and support may affect receptor ligand interaction.

3) Testing the activity of libraries of soluble compounds

This is a classical method of screening which is desired for biological activity is to test soluble compounds one at a time in a competition-binding assay, enzyme inhibition assay or in a cell-based bioassay.

This approach has been applied to library screening by releasing small compounds synthesized on solid support (e.g. pins, beads). The compounds on individual beads are released locally onto a lawn of confluent mammalian cells and cause activation of cells in the area surrounding the bead. The bead is responsible for cell activation is isolated and detected by various methods. But, the activity may contain some high affinity compound and some low affinity compound, which will cause difficulty in separation.

4) Encoding

The combinatorial libraries are collection of unique molecules, which are the source of molecular diversity. By the process of screening the number of this library that has “desirable properties” are sorted out. It is now very important to learn the identity of “winning” library member. The process of identification of active compound in a mixture is known as Encoding.

a) Positional encoding (iterative resynthesis and rescreening)

In this method the resynthesis and rescreening is carried out to know the identity of the active compound. After checking back we isolate the active compound.

Limitation

1) In large pools compound with modest activity cannot be detected.
2) Activity in any given pool is generally a result of the sum of more than one active compounds, the most active pool may therefore not contain the most potent compound.

3) Iterative resynthesis and biological testing can be time consuming and laborious.

b) Chemical encoding

This method is used for peptide libraries. The vast numbers of RNA sequence in a small volume of solution are generated. The solution was passed through an affinity column to which the ligands are bound. The RNA sequences that bound tightly to immobilize ligand eluted more slowly than others. The small amounts of sequence can be determined by using existing analytical techniques. By establishing the RNA sequence of 'Winner' its chemical identity becomes known.

c) Electronic encoding

This technique uses a micro electronic device called a Radio frequency (RF) memory tag. The tag measuring 13×3mm is encased in heavy walled glass and contains the following: A silicon chip, onto which laser-etched a unique binary code ,a rectifying circuit ,with which absorbed RF energy is converted to D.C. electrical energy, a transmitter/receiver circuit, an antenna, through which energy is received and rf signals are both received and sent.

Limitations of combinatorial chemistry

A major problem in the combinatorial organic synthesis is the difficulty of confirming the degree to which the expected chemistry has proceeded on the entire population of the substrate molecule. In the characterization of combinatorial products the presence of "by-products" combine with the difficulty of detecting these compounds, will cause problems, if one mistakenly concludes that screening "hit" is expected product.

CONCLUSION

This method has been considered as a most important advancement in medicinal chemistry and widely exploited by pharmaceutical industries in drug discovery whether the aim is a broad discovery search for optimization of a lead combinatorial chemistry, is a process for integration of synthesis and screening. In this competitive market, pharmaceutical industries must require efficient research work to stand in market and combinatorial chemistry offers higher productivity at lower expenses. Thus technology has definitely decreased the cost associated with the drug research and increased the chances of finding new lead molecule with a short time, promising drug leads have already been developed using combinatorial libraries method and several are currently in preclinical study further, in conjunction with the advent of combinatorial chemistry and molecular modeling technology, combinatorial

chemistry can now be applied to various new drug target's developed from our recent understanding of the molecular basis of diseases.

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