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2. A Review on Combinatorial Chemistry

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Abstract:

The use of combinatorial chemistry techniques has been explored as an alternative to conventional approaches for the synthesis of compounds in the drug discovery process. This technique is the starting point for the development of synthesis concepts that were intended to cover and explore the chemical space without having to prepare every individual compound. Combinatorial Chemistry technologies were developed in response to the increased screening capacities that are available when drug discovery changed its screening paradigm from a pharmacology-based approach to target oriented lead finding. This article will illustrate technique used in combinatorial chemistry, some of the advances made in recent years and their application in the synthesis of different peptides, oligosaccharides and other molecules.

Keywords:

Combinatorial Chemistry, synthesis, solid phase, solution phase.

2.1 Introduction:

Combinatorial Chemistry is a technology for synthesizing and characterizing collections of compounds and screening them for useful properties was conceived about 20 years ago. Initially, the field focused primarily on the synthesis of peptide and oligonucleotide libraries in the 1990s, the focus of the field changed predominantly to the synthesis of small, drug like Organic compounds. And many pharmaceutical companies and biotechnology firms now use it in their drug discovery efforts. The drug discovery process became a highly parallel one, in which hundreds or even thousands of structures could be synthesized at one time. Sometime high throughput screening (HTS) has been performed for their in vitro assays, running assays in 96 well microtiter plates and by using laboratory robotics for pipetting and analysis.

The main purpose of computer assisted combinatorial chemistry is to generate thousands structurally diverse compounds as libraries, maximising their diversity, which are then considered in an experimental parallel automated synthesis and screening on the basis of their properties. The key issue is to integrate all important steps of CC/CT in a single, multidisciplinary approach.

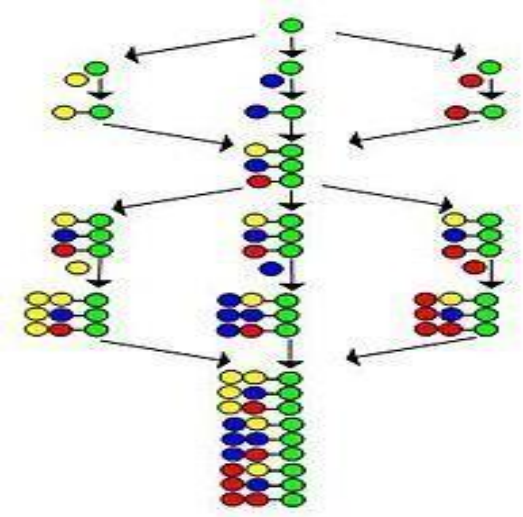
- The multipin procedures introduced by Geysen and his collegues – was used for the parallel synthesis of arrays of peptides. It is now considered as the prototype of the

powerful automatic machines now capable of preparing hundreds of different kinds of individual compounds in parallel.

- The " portioning - mixing method invented by Furka & his collegues – was developed to enable the users to prepare millions of new peptides in only a couple of days; also for synthesizing organic libraries.

Several others techniques developed for synthesis of large new compounds at a time were the biological method of preparation of peptide libraries, the light- directed spatially addressable parallel chemical synthesis. Researchers continue find ways to further enhance the capabilities of combinatorial chemistry, including these developments: A growing trend toward the synthesis of complex natural-product-like libraries, including the carbohydrate-based libraries, An increased focus on “phase trafficking” techniques are used for integrating synthesis with purification, Novel strategies for purification and analysis, such as the combinatorial use of supercritical fluid chromatography And the application of combinatorial chemistry to new targets, such as nuclear receptors. The goal of combinatorial chemistry able to synthesis, purify, chemically analyze, and biologically test all the structures in the library, using as few synthetic experiments as possible.

Combinatorial chemistry was first applied to the synthesis of peptides. In 1963 Merrifield introduced the efficient synthesis of peptides on a solid supports or resin. Combinatorial chemistry is of two types: first is solid phase combinatorial chemistry and second is solution phase combinatorial chemistry. Many countries have emphasized the urgent need to get acquainted with Combinatorial Technologies in order to enable local enterprises to remain competitive and economically viable in the coming decades and gain expertise on application practices of combinatorial technology. In view of global competition, CC/CT together with molecular modeling may be considered as powerful tools for the implementation and/or the increase of a country is capabilities in drug design, agro chemistry, new materials and new catalysts. The above considerations become even more significant if it is taken into account that many countries have abundant natural resources which are presently well below their proper exploitation. Combinatorial chemistry can enhance the potential of these resources.



Definition:

Combinatorial chemistry is one of the important new methodologies developed by researches in the pharmaceutical industry to reduce the time &costs associated with producing effective & Competitive new dugs. Modern combinational chemistry involves both the synthesis & screening of large sets of compounds called libraries. The libraries themselves can be always of individual compounds or mixtures. When two reactants, A &B combines, A is actually a mixtures of five components while B may be a composite of ten, so on complete of reaction, a mixture of 50 different compounds will be produced.

Combinatorial chemistry is one of the important new methodologies developed by academics & researchers in the pharmaceutical, agrochemical and biotechnology industries to reduce the time & costs associated with producing effective, marketable, & competitive new drugs. Simply put, scientists use combinatorial chemistry to create large population of molecules, or libraries that can be screened efficiently en masse. By producing larger diverse compound libraries, companies increase the probability that they will find novel compounds of significant therapeutic & commercial values. The field represents a convergence of chemistry & biology, made possible by fundamental advances in miniaturizations, robotics, and receptor development.

While combinatorial chemistry can be explained simply, its application can take a variety of forms, each requiring a complex interplay of classical organic synthesis techniques rational drug design strategies, robotics, scientific information management.

2.2 Synthetic Methods in Combinatorial Chemistry:

The various methods as used for synthesis of compounds in combinatorial chemistry. They all.

- Parallel synthesis leading to individual compounds.
- Combinatorial synthesis of mixtures.

Methods is which the no: of synthesized compounds remain constant in the consecutive series of coupling steps. These methods termed parallel procedures, are suitable for preparing series of individual compounds. They includes…….

1. Portioning mixing (PM) synthesis
2. Parallel Synthesis methods

2.2.1 The Portioning – Mixing Synthesis:

The portioning mixing method is based as Merrifield's solid phase procedure:

In solid phase combinatorial chemistry, reagents or products are attached to solid supports such as polystyrene beads—is the most traditional form of phase trafficking. In solid-phase organic synthesis, it’s easy to purify products by filtration, it’s possible to do mix-and-split

synthesis (a technique used to make very large libraries), excess reagents can be used to drive reactions to completion, and syntheses can be automated easily. Solid phase chemistry has some advantages over the solution-phase. First, in solid-phase synthesis, large excesses of reagents can be used to drive reactions to completion; these excess reagents can then be removed at the end of the reactions by filtration and washing. Second, because of easy separation of reagents and products, solid phase chemistry can be automated more easily than solution chemistry. Separation of compounds bound to the solid support from those in solution is accomplished by simple filtration.

Solid support used in Solid phase synthesis:

Most solid state combinatorial chemistry is conducted by using polymer beads ranging from 10 to 750 μm in diameter. The solid support must have the following characteristics for an efficient solid-phase synthesis:

a. Physical stability and of the right dimensions to allow for liquid handling and filtration;
b. Chemical inertness to all reagents involved in the synthesis;
c. An ability to swell while under reaction conditions to allow permeation of solvents and reagents to the reactive sites within the resin;
d. Derivatization with functional groups to allow for the covalent attachment of an appropriate linker or first monomeric unit.

The compounds to be synthesized are not attached directly to the polymer molecules. They are usually attached by using a linker moiety that enables attachment in a way that can be easily reversed without destroying the molecule that is being synthesized and allow some room for rotational freedom of the molecules attach to the polymer.

Types of solid that are used:

a. **Polystyrene resins** in this Polystyrene is cross linked with divinyl benzene (about 1% crosslinking). polystyrene resin are suitable for nonpolar solvents.
b. **Tenta Gel resins** Polystyrene in which some of the phenyl groups have polyethylene glycol (PEG) groups attached in the para position. The free OH groups of the PEG allow the attachment of compounds to be synthesized. PEG Containing resins are suitable for use in polar solvents.
c. **Polyacrylamide resins** like super blue these resin swell better in polar solvent, since the contain amide bonds, more closely resemble biological materials.
d. **Glass and ceramic beads** these type of solid supports are used when high temperature and high pressure reaction are carried out.

Linkers used in solid phase synthesis:

To support the attachment of a synthetic target, the polymer is usually modified by equipping it with a linker. Linker must be stable under the reaction conditions, but they must be susceptible to a cleavage. Some specialized linker have been developed to meet particular reaction or product conditions this type of linker is known as traceless linkers, it can be cleaved from the resin with no residual functionality left.

This type of linkers allows the attachment of aryl and alkyl products that do not have OH or NH functionality example of these linker include silyl group (-Si(CH3)2) that is sensitive to acid and can be cleaved to give un substituted phenyl or alkyl product.

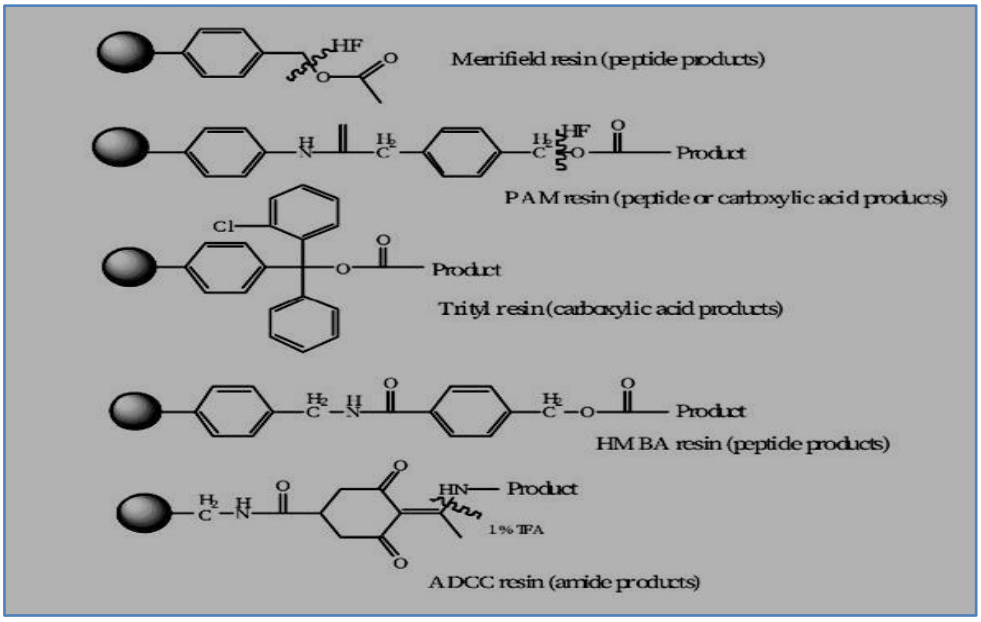
A new class of linkers was developed known as safety-catch linkers which is inert to synthetic condition and chemically transformed to allow final liberation of the product from the resin.

Now a ultraviolet light sensitive protecting groups are used, like affymax group is used in the synthesis of carboxylic acid and carboxamide products. Some groups have used linkers that can only be cleaved by enzymes.

A novel linker possessing selenocyanate and masked carboxylic acid was developed for the solid-phase synthesis of dehydropeptides. This linker was used to demonstrate the synthesis of the model compound of RGD-conjugated dehydropeptide.

Oxabicyclo norbornenes constitute a convenient and readily cleaved linker for solid-phase organic synthesis. A simple and inexpensive furfuryl- substituted resin has been shown to capture and release maleimide dienophiles under conditions compatible with intermediate synthetic steps.

A new linker based on a chroman system is developed for the side-chain anchoring of Arg and other guanidine-containing molecules. The system is compatible with the Fmoc/tBu solid-phase strategy, because the release of the final product is achieved by treatment with TFA in the presence of Scavengers.

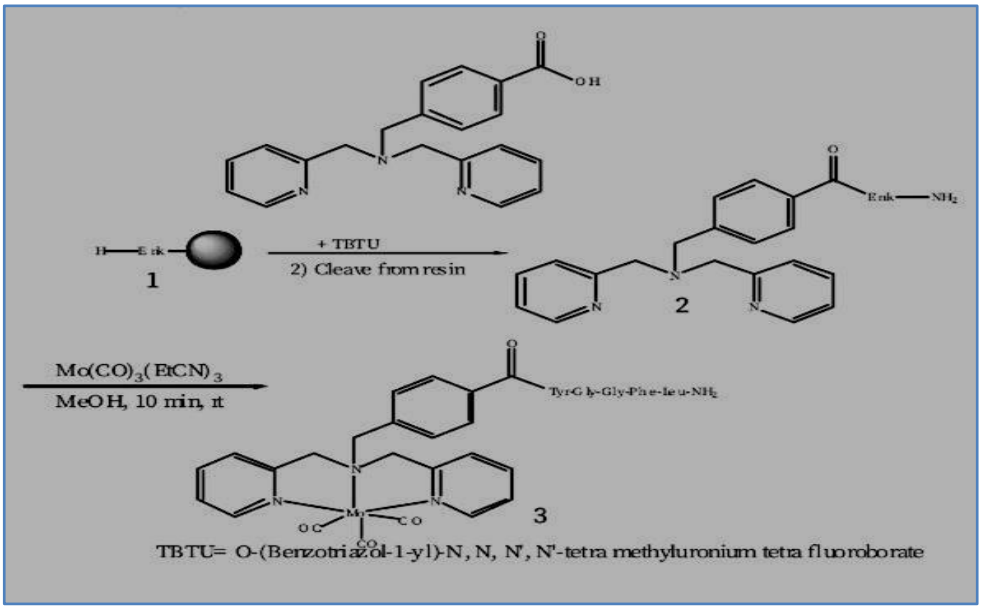


2.2.2 Application of solid phase synthesis:

A. Solid-Phase Synthesis of Peptide –Metal-Complex Conjugates:

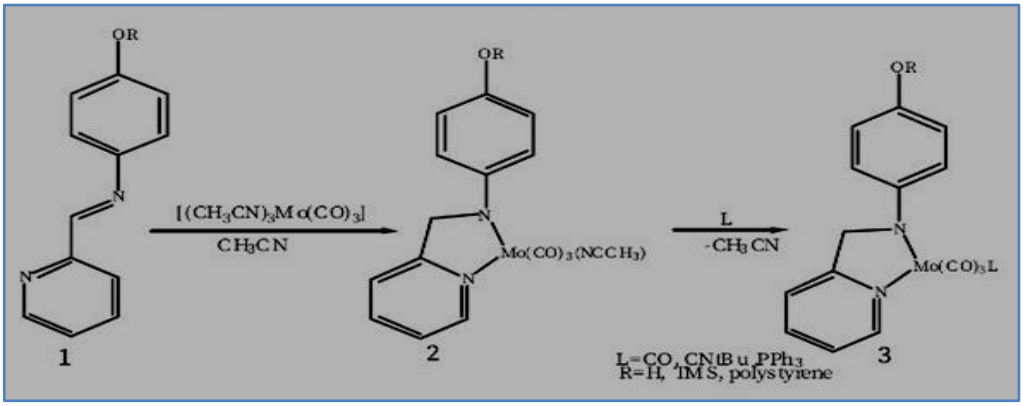
Solid-phase synthesis of inorganic complexes was established by Heinze, Metzler-Nolte, Reedijk and others. Coordination and organometallic chemistry on solid-phase were typically studied in the context of catalyst performance. Recently, solid-phase synthesis using insoluble resins as solid support was used to synthesize metal complexes based on peptide backbone ligands. These coordination compounds find applications in biochemistry as well as in medicinal chemistry. Resin-bound chelates were prepared in such a manner that upon the addition of suitable metal salts the target metal complexes were selectively released from the resin and used.

a. Synthesis of Bis(2-picolyl)amine (bpa) molybdenum conjugate: In case when the attachment of a metal complex to the peptide on the solid support is not desirable, e.g. with radioactive metal isotopes, an innocent anchoring group can be attached to the peptide during solid-phase synthesis. The ligand– peptide conjugate is then cleaved from the resin, purified and the metal label is only added in solution immediately prior to use of the bioconjugate.



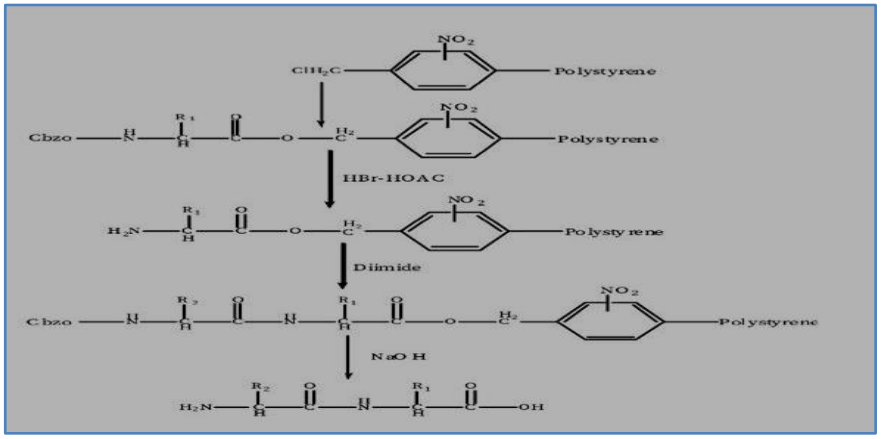
b. Bidentate Schiff base metal conjugates: A solid-phase synthesis approach for molybdenum carbonyl complexes was developed by Heinze. neither peptide coupling nor metallated amino acids are used, because it illustrates that complex organometallic transformations are possible on solid support. A specific resin and linker system allows coordination under solid-phase reaction conditions and the cleavage of the metal complex from the solid support. Bidentate Schiff base **1** was used as the ligand. The phenolic hydroxyl group allows the attachment to the solid support. A silyl ether based linker was

chosen due to its stability under basic and acidic conditions and the possibility to cleave with fluoride ions, which are expected to be unreactive towards most metal complexes. In solution high temperature and rather harsh oxidative reaction conditions are necessary to synthesize the desired tricarbonyl compounds. Such harsh conditions have to be avoided in solid-phase chemistry with polystyrene resins as the molybdenum precursors can react with the aromatic residues of the support. Heinze and co- workers used [(CH3CN)3Mo(CO)3] as a Mo(CO)3 source and under mild reaction conditions the intensely blue coloured complexes **2** and **3** formed rapidly and having excellent yields. However, acetonitrile, a rather poor solvent for resin swelling, had to be used in a mixture with toluene. The cleavage was performed with tetra-*n*-butylammonium fluoride in dichloromethane and resulted in deeply coloured solutions of the deprotonated complexes.



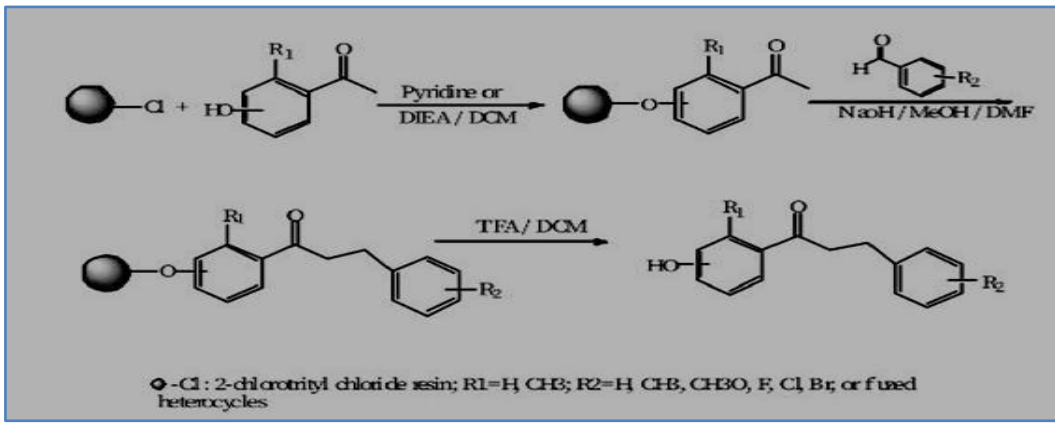
B. Synthesis of a Tetra-peptide:

A new approach to the chemical synthesis of polypeptides was investigated. It involved the stepwise addition of protected amino acids to a growing peptide chain which was bound by a covalent bond to a solid resin particle. This provided a procedure whereby reagents and by-products were removed by filtration, and the recrystallization of intermediates was eliminated. The advantages of the new method were speed and simplicity of operation. The feasibility of the idea was demonstrated by the synthesis of the model tetrapeptide L-leucyl-L-alanylglycyl-L-valine. To provide a point of attachment for the peptide the polystyrene resin was partially chloromethylated. The product was then nitrated or brominated. The resulting substituted chloromethyl polystyrene was treated with the triethylammonium salt of the first protected amino acid in the proposed peptide chain to give a substitute dbenzyl ester linkage. This was the stable covalent bond which held the growing peptide chain in the solid phase on the supporting resin. Protecting group which was used throughout the syntheses to be reported was the carbobenzoxy group. It was selected because it could be removed readily and completely by hydrogen bromide in glacial acetic acid stituted carbobenzoxy-L-valyl polymer even in 10% HBracetic acid there was also considerable loss of ester. After nitration the rate of removal of carbobenzoxy was decreased, but the loss of ester was reduced to a very small level. With 30% HBr the carbobenzoxy group was removed in **2** to **4** hr., while the ester cleavage remained low level for at least **6** hr.



C. Solid Phase Synthesis of Chalcones by Claisen-Schmidt Condensations:

In order to accelerate the development of relatively inexpensive antimalarials that are effective against chloroquine-resistant strains of Plasmodium falciparum, a methodology for the solid phase synthesis of chalcone (l, 3-diphenyl- 2-propen-lone) analogues reasonably having high yields and purity. In a manual peptide synthesis vessel a mixture 3- or 4-hydroxyacetophenone and 2- chlorotritylchloride resin (l00 mg, l.l-l.6 mmol/g) in anhydrous dichloromethane (3 mL) was shaken for l h at room temperature. Resin was washed with DMF (3x), MeOH (2x) and DCM (3x) and dried in vaccum. The resin-attached aldehydes (l eq.) or methyl ketones (l eq.) were condensed with either substituted methyl ketones (l0 eq.) or substituted aldehydes (l0 eq.) with NaOH (0.1 eq.) in l0% MeOH-DMF (3 mL total) at room Temperature for 24 h. Resins were washed in the same sequence as the first step described above. The product was cleaved with TFA/DCM at room temperature for 20 min. Determination of product purity is done by HPLC.

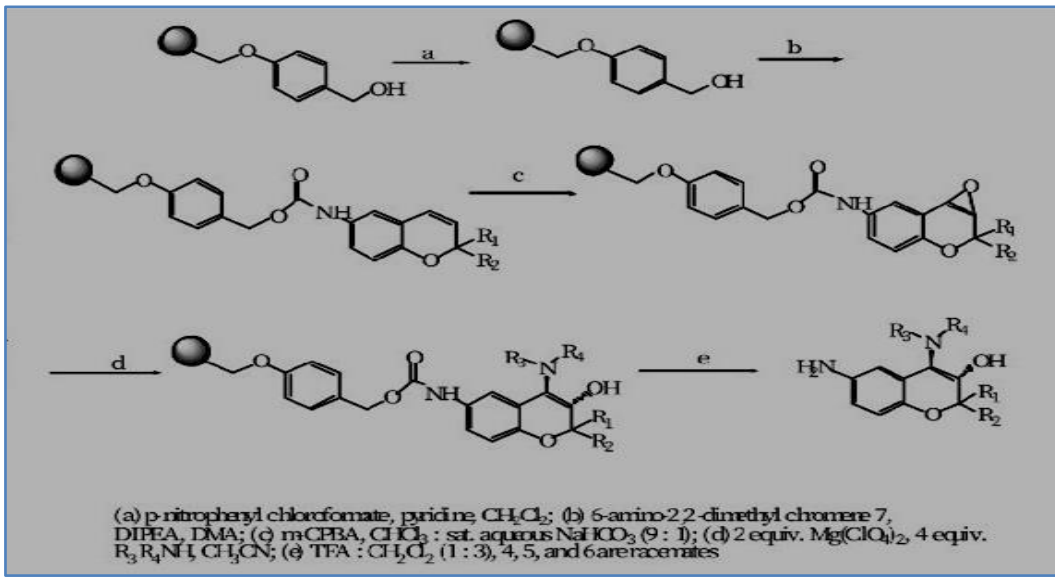


D. Synthesis of Benzopyran Derivatives Using Two-Phase Solvents system:

Solid-phase organic synthesis has emerged as a powerful technique in generating combinatorial libraries of small organic molecules which is useful for drug discovery. Heterocyclic compounds provide scaffolds on which pharmacophores can be arranged to

yield potent and selective drugs. A variety of heterocycles have been synthesized on solid support. A successful application of the epoxides **4** to generate the 3-hydroxy-4-amino substituted benzopyran library. We selected the Wang resin **1** as a polymer support, the hydroxy group of the Wang resin is useful in the introduction of 6-aminochromenes **6** through the carbamate linker which also serves as an efficient protection group for the amino group against the subsequent oxidation and alkylation reactions.

The benzopyran derivatives **6** were finally liberated from the resin by trifluoroacetic acid (TFA). In the first step, the 4-nitrophenyl carbonate resin **2** was prepared by adding pyridine in CH2Cl2 to the Wang resin 1 in the presence of p- nitrophenyl chloroformate in CH2Cl2. The reaction of carbonate resin **2** with 6- amino-2,2-dimethyl chromene and Ndiisopropylethylamine (DIPEA) in N, N dimethylacetamide (DMA) afforded the carbamate resin 3 and the progress of the reaction was verified by the complete disappearance of the carbonate peak at 1760 cm-1 in the IR. It was found that the two-phase solvent system comprised of chloroform and saturated aqueous NaHCO3 was quite satisfactory. Under this condition the desired epoxide resin was obtained in good yield. We assumed that the success of this reaction was due to the basic aqueous solution's ability to remove excess m-chlorobenzoic acid quite effectively. The hydroxyl compounds 6 can also be used for further combination with acylating agents to preparing diverse chemical libraries for biological evaluation.



(a) p-nitrophenyl chloroformate, pyridine, CH_2Cl_2; (b) 6-amino-2,2-dimethyl chromene 7, DIPEA, DMA; (c) m-CPBA, $CHCl_3$: sat. aqueous $NaHCO_3$ (9 : 1); (d) 2 equiv. $Mg(ClO_4)_2$, 4 equiv. R_3R_4NH, CH_3CN; (e) TFA : CH_2Cl_2 (1 : 3), 4, 5, and 6 are racemates

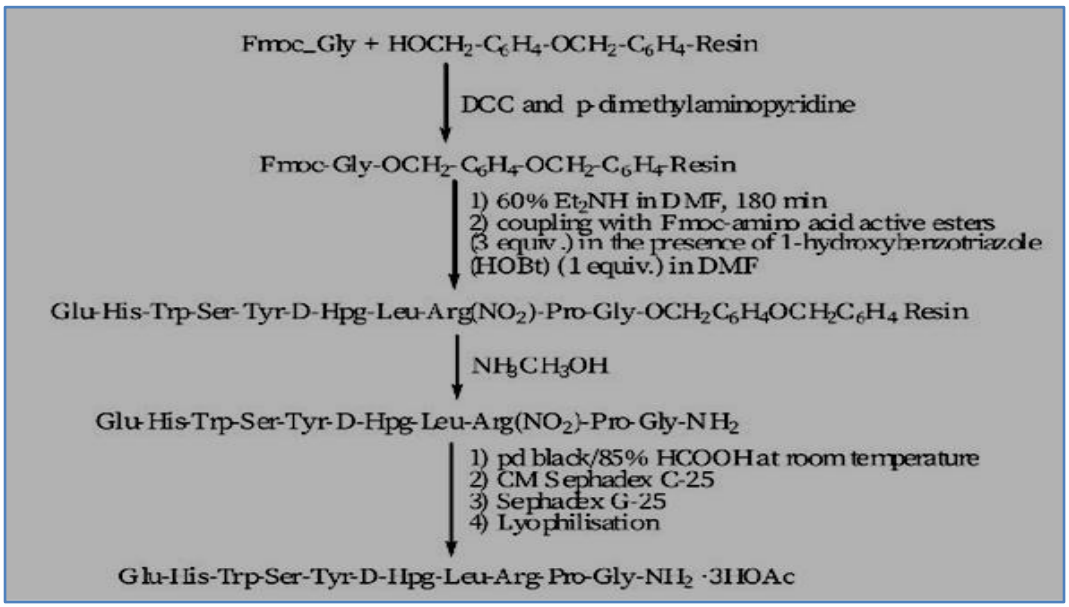
E. Synthesis of luteinizing hormone releasing hormone analogues using 9- fluorenyl-methyloxycarbonyl amino acid:

Synthesis of the hypothalamic hormone, luteinizing hormone releasing hormone (LHRH) and its agonists and antagonists by using acid labile protecting groups like Boc, Z, etc., for a-amino or side-chain protection generally involves final treatment with anhydrous liquid hydrogen fluoride leading to contamination of the final product with closely related impurities there by necessitating extensive purificaiton. In solid phase synthesis of peptides, use of base labile 9 fluorenylmethyloxycarbonyl (Fmoc) group for Na-protection

would allow milder conditions to be employed during the synthesis in addition to the requirement of minimal side-chain protection and this strategy was followed for the synthesis of LHRH analogues. The purity of the final peptides was demonstrated by paper chromatography on Whatman No. 1 chromatography paper strips by ascending method by using the following solvent systems:

A.*n*-BuOH-HOAc-H2O (4:5:5, upper phase, v/v)

B. *n*-BuOH-HOAc-H2O-pyridine (30:6:24:20, v/v)

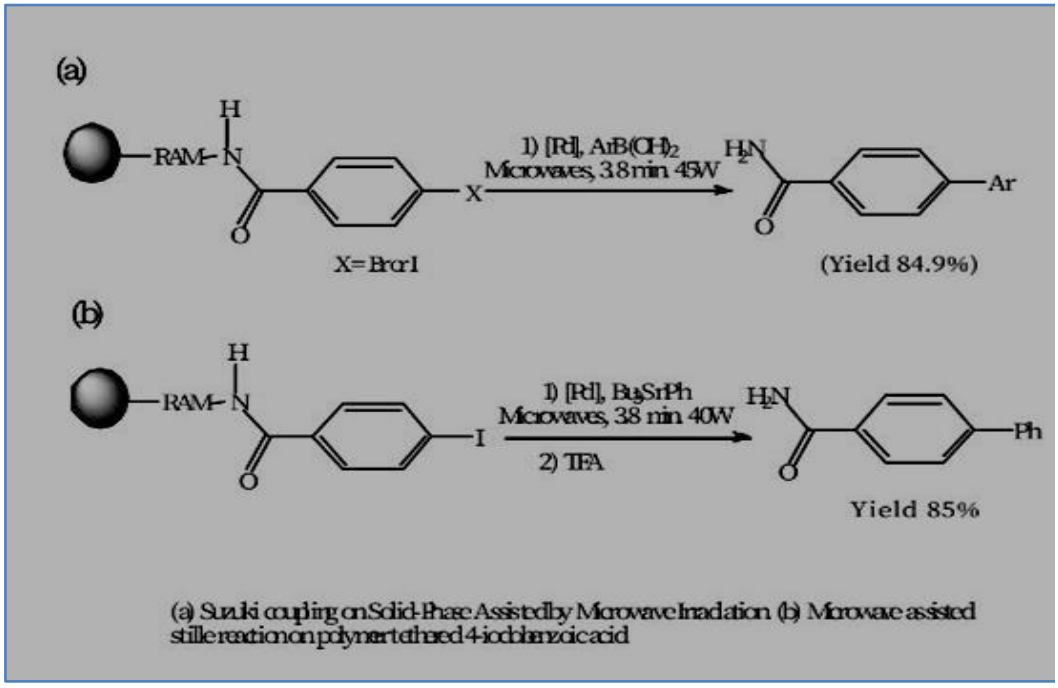


F. Microwave–assisted combinatorial synthesis:

The combination of microwave power to solid phase synthesis is quite logical. Rate accelerations and high loadings for several solid-phase protocols have been reported with reaction time being reduced in some cases from hours to a few minutes.

The combination of solid phase synthesis and microwave heating is receiving attention and this combination has enormous potential for better results Larhardetal. have demonstrated that highly useful Suzuki and Stille reactions could be conducted under flash-heating conditions using a single mode cavity and would afford better yields.

They reported microwave-assisted palladium-catalyzed coupling of aryl and heteroaryl boronic acids with iodo- and bromo-substituted benzoic acids, anchored to Tenta Gels RAM, provided high isolated yields of coupled products after a reaction time of 3.8 min. Suzuki and Stille reactions worked readily on a polymeric support consisting of a benzoic acid linked to Rinkamide on polyethylene glycol (PEG) grafted polystyrene (TentaGel). The polymer was found to be stable under these harsh conditions.

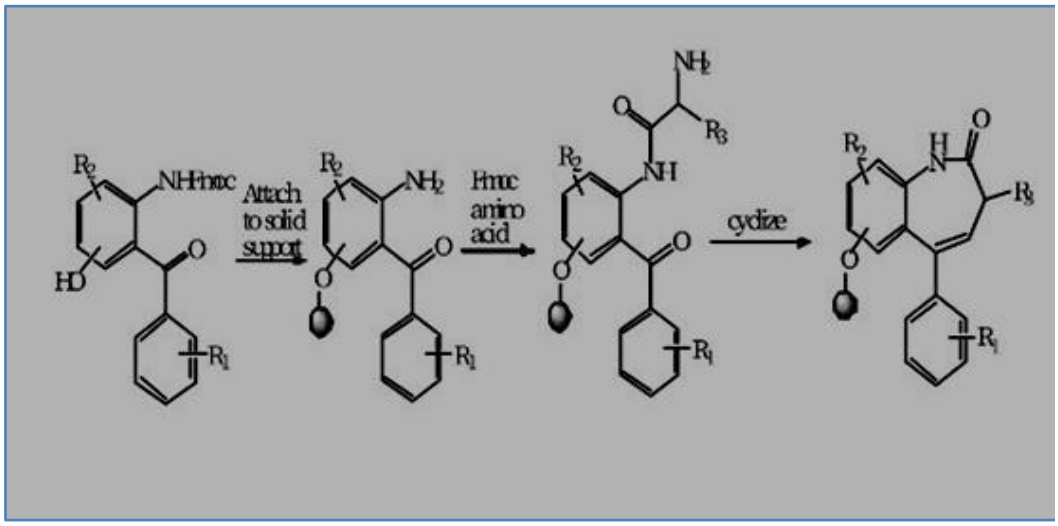


(a) Suzuki coupling on Solid-Phase Assisted by Microwave Inadation (b) Microwave assisted stille reaction on polymer tethered 4-iodobenzoic acid

G. Synthesis of 1, 4 benzodiazepines:

The choice of benzodiazepines was inspired because of the medicinal importance of these materials and their resemblance to peptides.

Here the library was constructed by a combination of three reactants. In the synthesis 1, 4 benzodiazepines Fmoc is used as a common protecting group and detachment of solid support is done by tetrafluoroacetic acid.



Features:

a. **Efficiency:** It can be seen that starting with a single substance, the no: of compounds is tripled after each coupling step. First 3 x 1 = 3 resin bound dipeptides, then 3 x 9 = 27 resin bound tripeptides & finally 3 x 27 = 81 tetrapeptides are formed. If a 20 different amino acids are used in the synthesis, the no. of peptides in each coupling step is increased by a factor of 20. The total no: of the synthesized peptides can be expressed by a simple formula 20', when n is the no. of amino acids.
b. **Formation of all possible sequence combinations:** Consecutive execution of the three simple operations (portioning, coupling, mixing) ensures with mathematical accuracy – the formation of all possible sequence combinations of amino acid building blocks used in the synthesis. This combinatorial principle embodied in PM synthesis captured the imagination of many researchers & had a profound effect on the development of the field.
c. **Formation of compounds in one-to-one molarities:** It is very important to prepare libraries in which the constituents are present in equal molar quantities. Otherwise, a low activity component, if present in a large amount, may show a stronger effect than a highly active component present lower quantity. The PM method was designed to comply with requirement of 1:1 molar ratio. Before each round of couplings, the resin is thoroughly mixed, then divided into homogenous equal portions. This ensures that the previously formed peptides are present in equimolar quantities in each portion.
d. The PM synthesis determination of the structure of the various organic compds is not as simple as sequencing peptide, the bead are usually encoded. The building blocks of the encoding tags are attached to the beads in parallel with the organic building blocks of the library. Two types of encoding are:
e. **Encoding with sequence:** When encoding by sequences, the encoding tags are peptides or oligo nucleotides. Their sequences encode both identity of the organic reagents coupled to the bead & the order of coupling. The white, black, gray & white square encode the organic reagents represented by white, black & gray circles & their white – black. Gray – white coupling order.
f. **Binary coding:** In binary encoding system the coding units are halobenzenes carrying a varying length hydrocarbon chain attached to the bead through a cleavable space. It is simply their presence which codes for the organic building blocker & their position.
g. Reducing the No. of the varied amino acids:
h. Reducing the No. of varied positions:

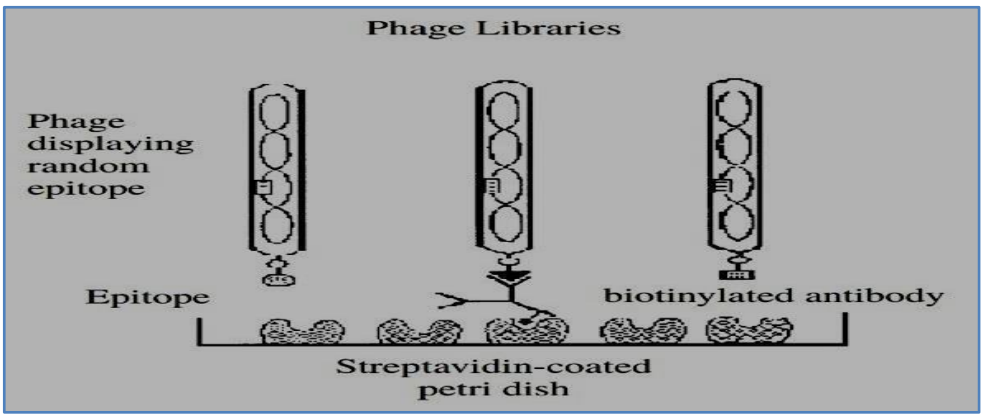
The Mixed Reagen Method of The Synthesis of Combinatorial Liberties.

Combinatorial peptide libraries can be prepared by using mixture of amino acids in the acylation step of the solid phase synthesis. Although the method is even more efficient than PM procedure, it has serious disadvantages. The one to one molar ratio of the formed compound cannot be assured due to the differences in the coupling rates of amino acids. There differences can in part be compensated by proper adjustment of the concentration of amino acids in the coupling mixtures. The method has been applied mainly in preparation of peptides libraries, but non peptide libraries have also been sythesized. Because mixtures of reactants are used in couplings, at the end of the synethsis of all library components are present on every bead. This means that only mixtures can be prepared by their method.

2.3 The Biologycal Method:

Biological methods for library preparation are mainly limited to peptide or oligonucleotide libraries. For peptide libraries, methods are based on the construction of a pool of clones each one expressing a different peptide on its surface. The peptides are fused to proteins normally expressed on the surface of the microorganism used. Phage display libraries are the most commonly used. Screening is accomplished by incubation of the target molecule, adsorbed to a solid support, with the phage population. Active phages will bind the target even after extensive washing steps. Target-bound phages are isolated and propagated by infection of *E. coli* and subjected to an additional round of adsorption to the immobilized target.

This procedure increases both the number of active phages and the stringency of selection, since harsher condition may be employed in the washing steps to reduce the number of non-specifically bound phages. As for the case of synthetic libraries, iterative cycles of adsorption, washing, elution and propagation in *E. coli* are performed to enrich the phage population in the active or in few active sequences. Active phages may then be subjected to DNA sequencing in order to decode the active peptide sequence. The use of biological display libraries for the isolation of peptide ligands is an interesting alternative to chemical libraries. Since 1985. when this technique was first published, many fields of research have benefited from its use. Web resources relevant to Combinatorial Chemistry and Combinatorial Technologies. There is a sort of information explosion accompanying the development of Combinatorial Chemistry and Combinatorial Technologies and an almost exponential growth of publications and patents in the field. At the same time, several web sites have been established providing updated information.



2.4 Solution Phase Combinatorial Chemistry:

Most ordinary synthetic chemistry takes place in solution phase. The use of solution phase techniques has been explored as an alternative to solid-phase chemistry approaches for the preparation of arrays of compounds in the drug discovery process. Solution-phase work is free from some of the constraints of solid-phase approaches but has disadvantages with respect to purification.

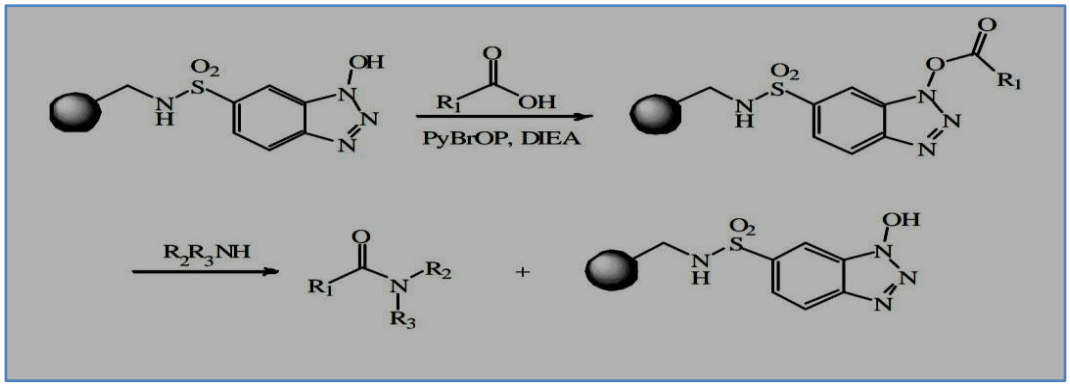
In solution phase synthesis we use soluble polymer as support for the product. PEG is a common vehicle which is used in solution phase synthesis it can be liquid or solid at room temperature and show varying degrees of solubility in aqueous and organic solvent. By converting one OH group of PEG to methyl ether (MeO-PEG- OH) it is possible to attached a carboxylic acid to the free OH and use in solution phase combinatorial synthesis. Another common support which is used in solution phase synthesis is liquid Teflon consisting mainly of long chain of (-CF2-) groups attached to a silicon atom. When these phases are used as a soluble support for synthesis the resulting product can be easily separated from any organic solvent.

A. Synthesis of Polymer by Solution Phase Combinatorial Chemistry: Tartar and co workers reported the synthesis of polymer supported

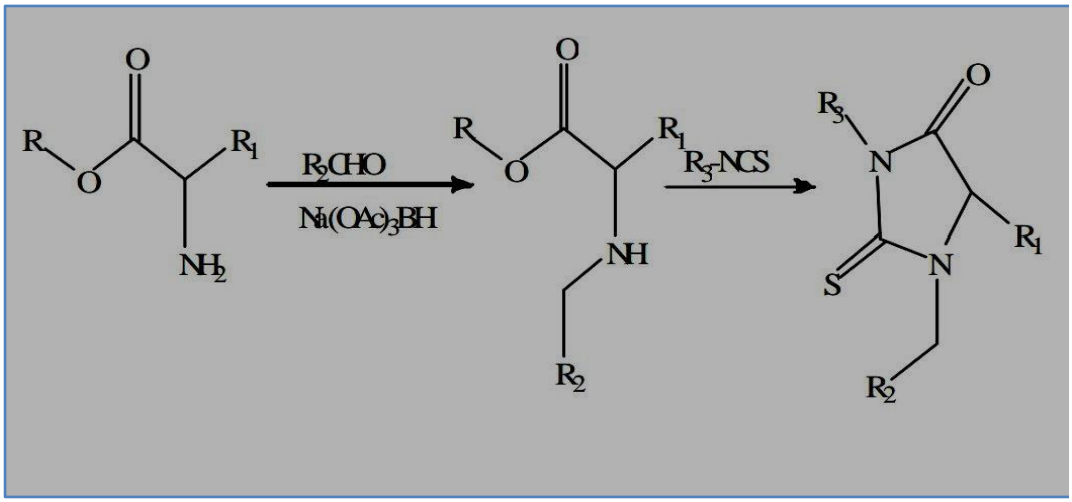
1-hydroxybenzotriazole. Reaction of the reagent with a carboxylic acid in the presence of an activating agent afforded the polymer bound activated ester which was reacted with amines to liberate the amide in solution.

Supported electrophilic, nucleophilic or ionic reagents used to remove impurities from solution have been termed scavenger reagents; polymer supported quenching reagents (PSQ) or complementary molecular reactivity/ molecular recognition polymer (CMR/R polymer). Use of such reagents provides a versatile counterpart to the approach. Booth and Hodges utilized a high loading amine resin derived from chloromethylpolystyrene and tris (2- aminoethyl) amine in the preparation of ureas, thioureas, sulphonamides and amides.

B. Synthesis of Thiohydantoins:



Sim and Ganesan developed a one-pot three component synthesis of thiohydantoins using the reductive amination of amino esters with aromatic aldehydes and sodium triacetoxyborohydride followed by the reaction with an isocyanate in the presence of triethylamine. The thiohydantions were isolated by an aqueous work-up protocol which incorporated the addition of glycine to convert unreacted reagents into water soluble materials. The methodology was used in the preparation of an array of 600 discrete compounds.



C. Solution Phase Synthesis of Biologically Important Oligosaccharides:

We departed from the traditional goal of oligosaccharide total synthesis striving for maximum convergency and followed a linear synthesis approach based on monosaccharide building blocks.

Using this method similar to that practiced for peptides and oligonucleotides we assembled several complex structures.

a. **Synthesis of High Mannose Structures of HIV gp120:** We completed the synthesis of a series of highly branched mannosides found on gp120 of HIV. Two different trisaccharides, a hexa-, and a nonasaccharide were prepared in conjugatable form. These structures were used to investigate the interaction of cyanovirin-N, a highly potent topical anti-HIV agent, with gp120. In collaboration with Barry O'Keefe (NCI) and Angela Gronenborn (NIH) isothermal calorimetry and high-field NMR were used to establish the minimal binding sequence and to map the binding site on the protein.
b. **Synthesis of Oligosaccharide Antigens Involved in Cancer and Bacterial Infections:** Cell surface carbohydrates act as biological markers for various tumors and are involved in bacterial and parasitic infections. Specific carbohydrate structures are found on particular cell populations and may be used to induce a specific immune response. These complex structures require reliable methodologies for their assembly. The Lewis antigens, a class of glycosphingolipids, are essential for cellular adhesion and recognition. In addition to their role in normal cellular adhesion processes such as the inflammatory response they have been implicated in many types of cancer and bacterial infections. We developed new synthetic routes for the modular assembly of the Lewis antigens as demonstrated on the example of H-type II that lend themselves to automation. Other tumor-associated antigens including Gb3 have also been prepared.

The oligosaccharides obtained from these syntheses are currently being attached to surfaces to enable rapid screening of carbohydrate-protein interactions.

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