POLITECNICO DI MILANO

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry





- Parallel Synthesis / High-Throughput Screening.
- Combinatorial Chemistry.

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Combinatorial Chemistry and High Throughput Screening.

1. **Solid Phase Techniques**
   - 1.1. Advantages
   - 1.2. Requirements
   - 1.3. Examples of Solid Supports
   - **1.4.** Anchor or linker
     - 1.4.1. Merrifield resin for peptide synthesis (chloromethyl group)
     - 1.4.2. Wang resin
     - 1.4.3. Rink resin
     - 1.4.4. Dihydropyran resin
2. **Parallel Synthesis**
   - 2.1. Houghton's Tea Bag Procedure
   - 2.2. Automated parallel synthesis
   - **2.3.** Automated parallel synthesis of all 27 tripeptides from 3 amino acids
3. **Mixed Combinatorial Synthesis**
4. **Solution phase synthesis**

Breakthrough in Experimentation.

- Robotic sample preparation
- Miniaturization of reactors
- High-level automatization of sensors
- Pharmaceutical industry:
  - routine creation and testing of 1000 to 1000000 distinct compounds (libraries)
- Techniques are now also being applied in material development
- New companies:
  - Symyx (www.symyx.com)
  - Avantium (www.avantium.com)

High-throughput Screening.

- Typical cycle of experimentation:
  - thousands of reactions in few hours
  - few hours of statistical analyses
  - thousands of reactions in few hours
  - few hours of statistical analyses
  - ---
- New chemical may be developed in 3 weeks rather than 3 years:
  - Which statistical techniques are important?
  - How do the classical techniques of the previous lectures fit in?
  - Which new techniques are necessary?

Combinatorial Chemistry- a Historical Review.

1. **Solid-phase synthesis of peptides**
2. **Solid-phase synthesis of oligodeoxyribonucleotides**
3. **Traditional vs. combinatorial synthesis:**

   Mario Geysen: Multipin technique

   Richard Houghton: Tea bag technique

   Ronald Frank: SPOT technique

   John Ellman: First combinatorial synthesis of small heterocyclic compounds

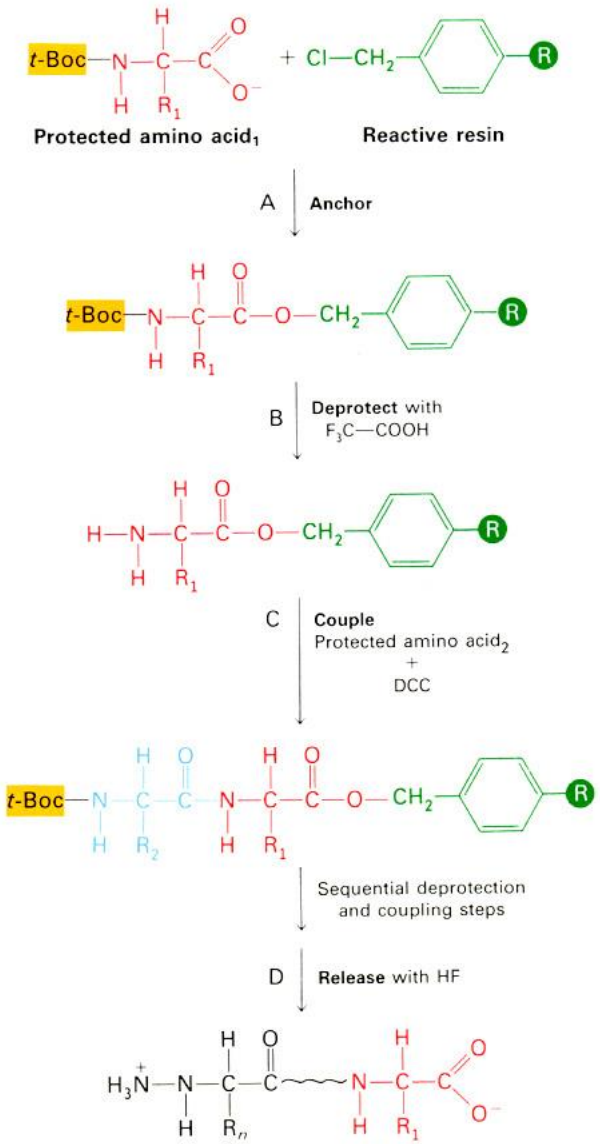
4. **Basic concepts of combinatorial chemistry**
5. **Combinatorial chemistry in drug development.**

1. Solid-phase Peptide Synthesis (SPPS) by Boc Method.

SPPS: developed by Merrifield, a Nobel laureate.*

Solid-phase synthesis vs. traditional solution synthesis:

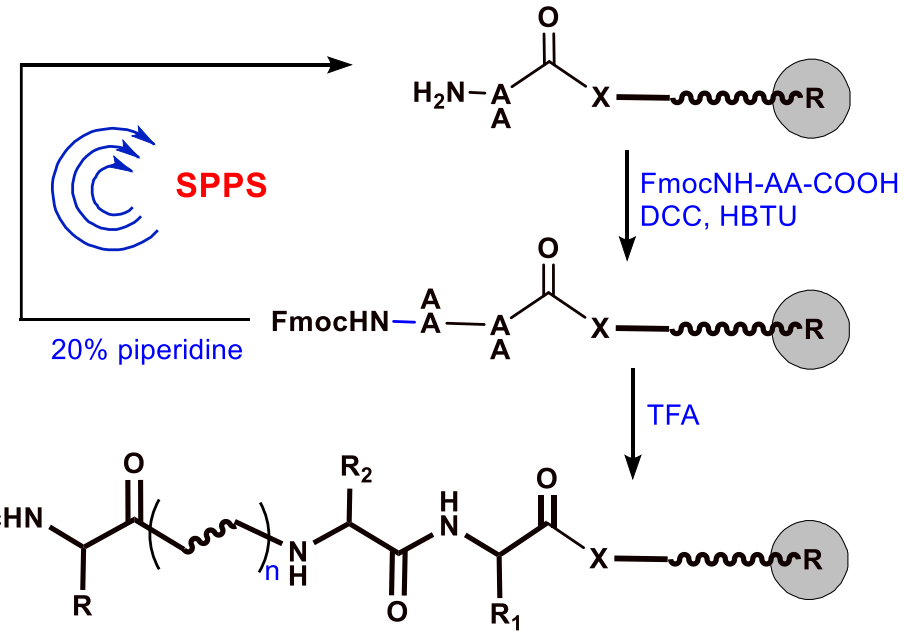
1. Easy product purification, especially suitable for multistep synthesis
2. Heterogeneous reaction, reactions are slower and less efficient than the solution reactions.
3. Recycling?



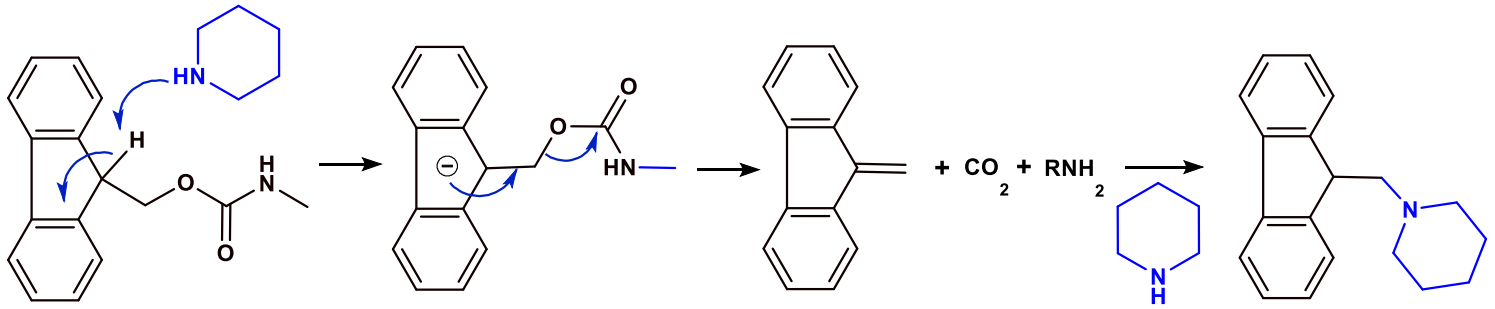
* R.B. Merrifield, Biochemistry 3 (1964) 1385.

Solid-phase Peptide Synthesis (SPPS) by Fmoc method.

Fmoc method



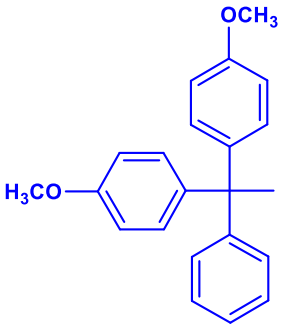
Fmoc deprotection



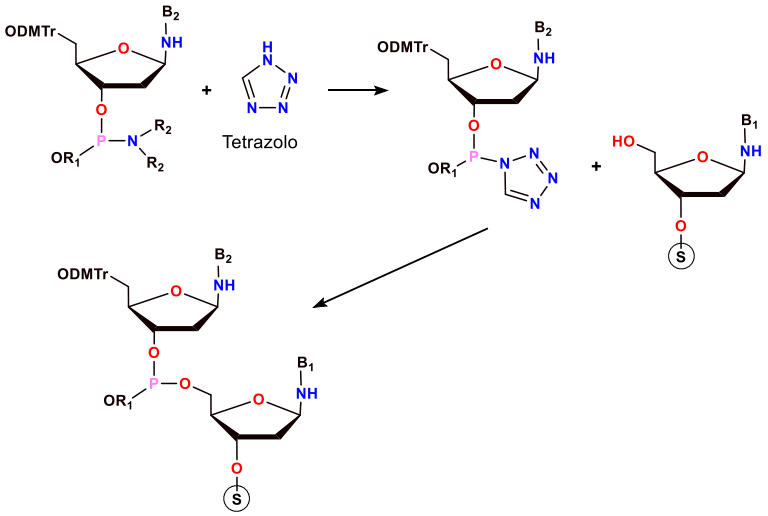
Chemical Synthesis of DNA Oligos.

Step 1: Terminal deprotection: a few min

CCl_3COOH(TCA) or $CHCl_2COOH$(DCA)



DMTr



R_1= β-cyanoethyl

B = base group
S = solid phase

Step 2: Coupling: 2-3min

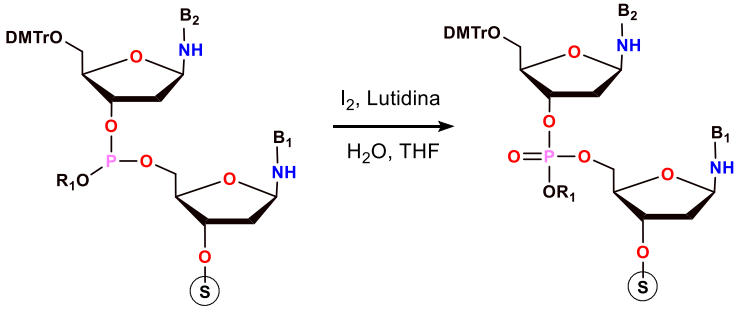
Step 3: Oxidation: I_2, a few min

Step 4: Capping: to block unreacted 5'-OH

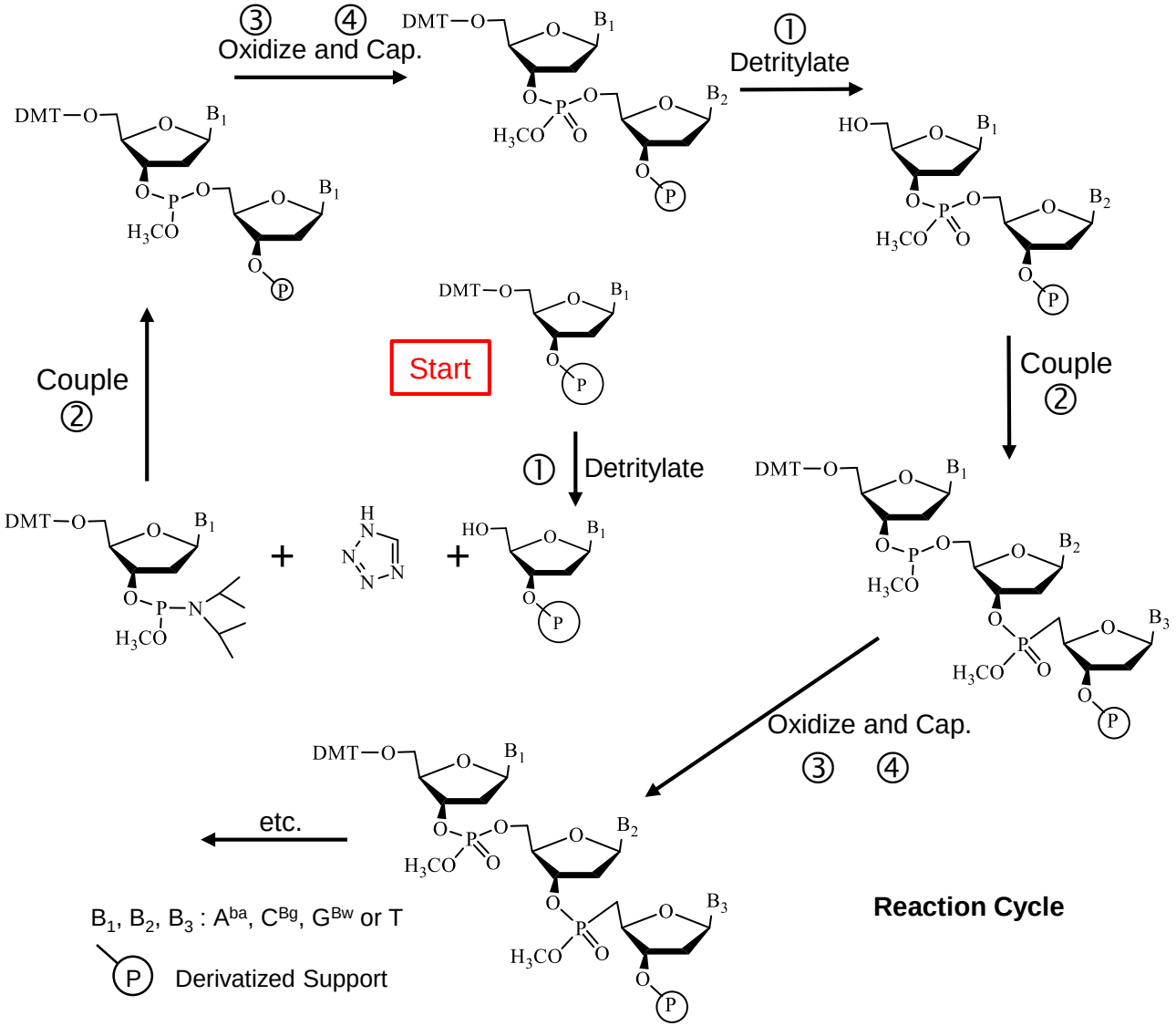
Ac_2O, lutidine, N-methylimidazole, THF

Deprotection: 30% NH_4OH, 5h at 50°C

Purification: HPLC, electrophoresis, ion-exchange cartridge, etc.



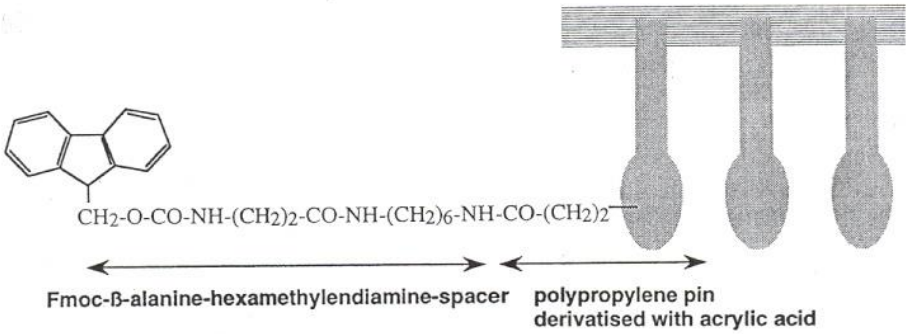
Synthetic Cycle.



Multipin Technique for Parallel Synthesis of Peptides.

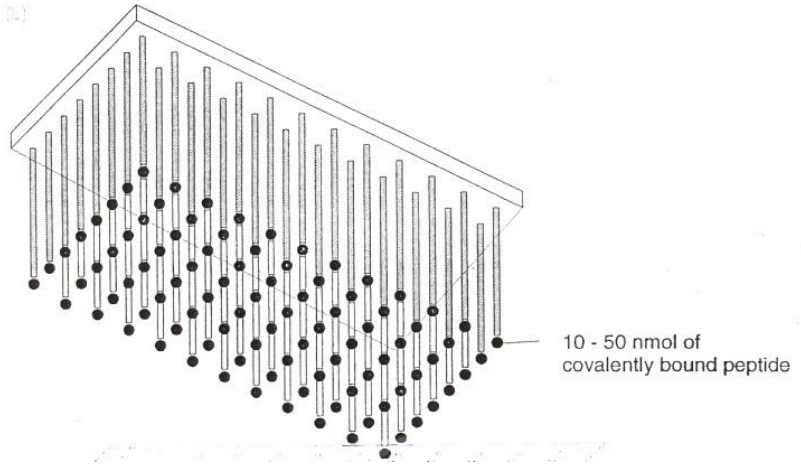
Geysen, HM; Rodda, HM; Mason, TJ. The delineation of peptides able to mimic assembled epitopes. In Synthetic Peptides as Antigens. Ciba Foundation Symposium 119; Porter, R., Wheelan, J., Eds.; Wiley: New York, 1986; pp131-149.

Washing and deprotection in a big pan.



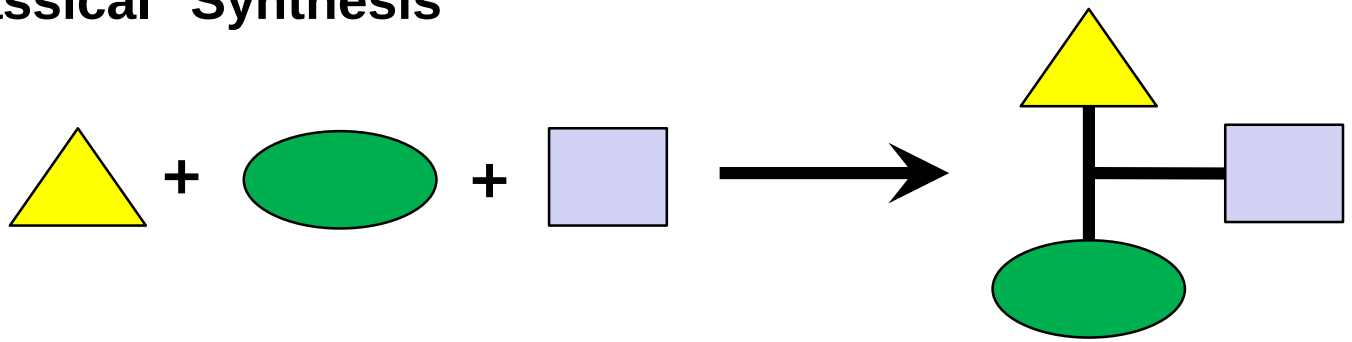
Coupling: use 96-well plate.

Protected AA building blocks in each well depends on the sequence of the target peptide on the pin it corresponds to.

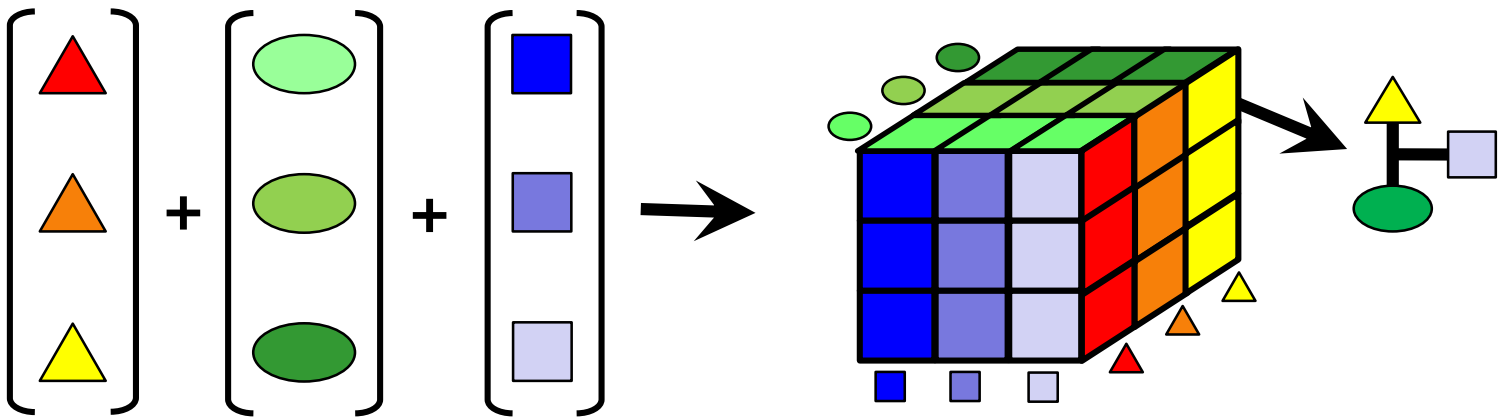


Classical Synthesis vs. Combinatorial Synthesis Approach.

"Classical" Synthesis



"Combinatorial Chemistry Approach"



Example of Molecular Diversity and Synthesis.

Tetra-peptide: H_2N-A-B-C-D-CO_2H

- consider only the 20 natural amino acids (L-series)

  20^4 = 160,000 different tetra-peptides !
- now include the 19 D-amino acids (20 L + 19 D = 39)

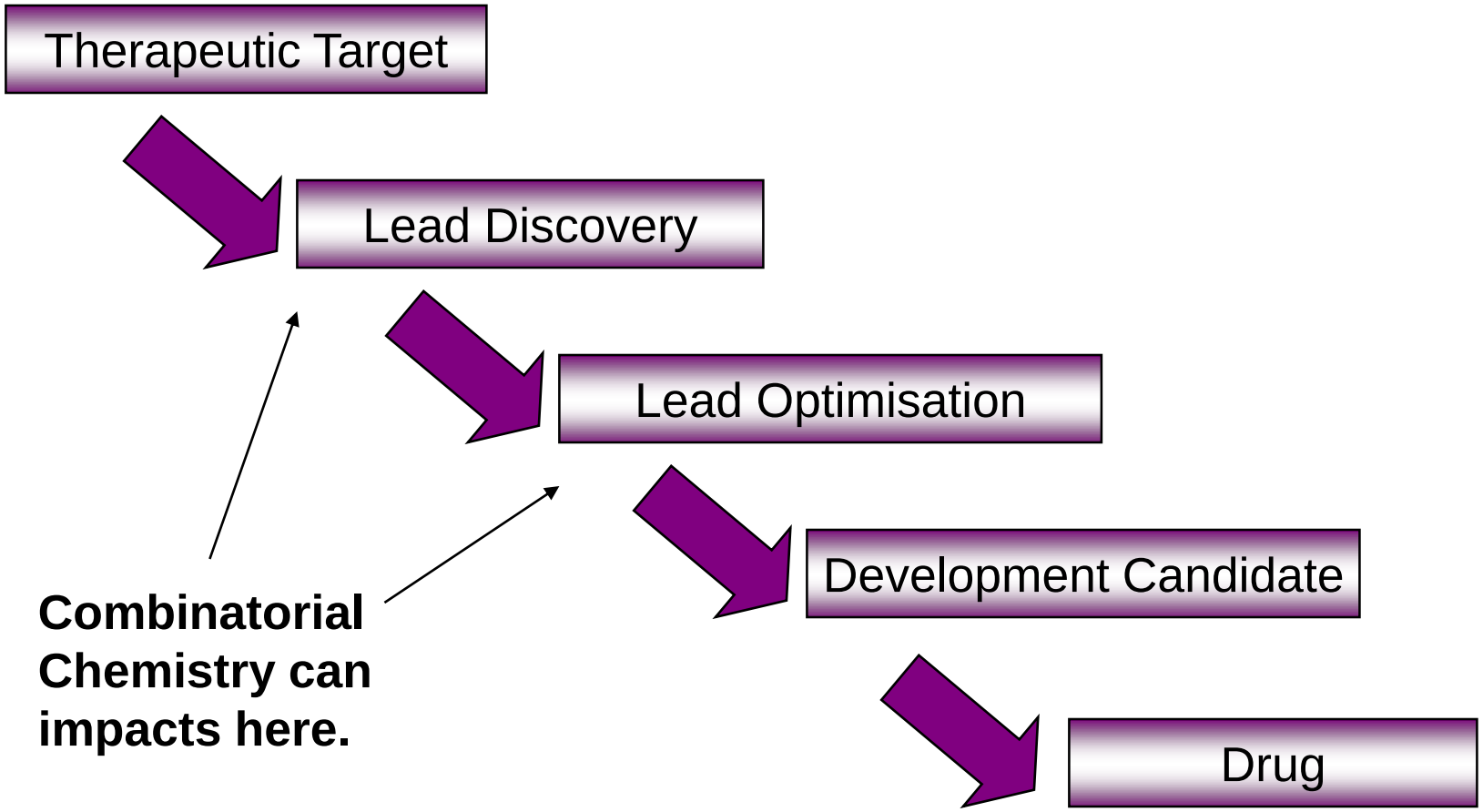
  39^4 = 2.3 million different tetra-peptides !!
- now include 20 unnatural amino acids

  59^4 = 12 million different tetra-peptides !!!!

Combinatorial chemistry: method by which a family (library) of related compounds (structurally & synthetically) can be prepared and evaluated (screened).

For multi-step synthesis, one must use solid-phase synthetic approach in order to expedite purification of intermediates

Combinatorial Chemistry within Drug Design.



Pharmaceutical Industry - Drug Discovery.

Lead Compound —optimization (synthesis)→ **Drug** —10 years, 400 – 800 million $→ **$ $ $**

Lead identification: literature (open & patent)
nature (natural products)

Careful optimization of a lead structure via chemical synthesis
““methyl-ethyl-butyl-futile game””

Number of marketable drugs per compounds that undergo preliminary biological testing.

$$\frac{1}{10{,}000}$$

← Rational drug design
← Combinatorial chemistry

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Solid Phase Organic Synthesis - Catalysis on Polymeric Supports.

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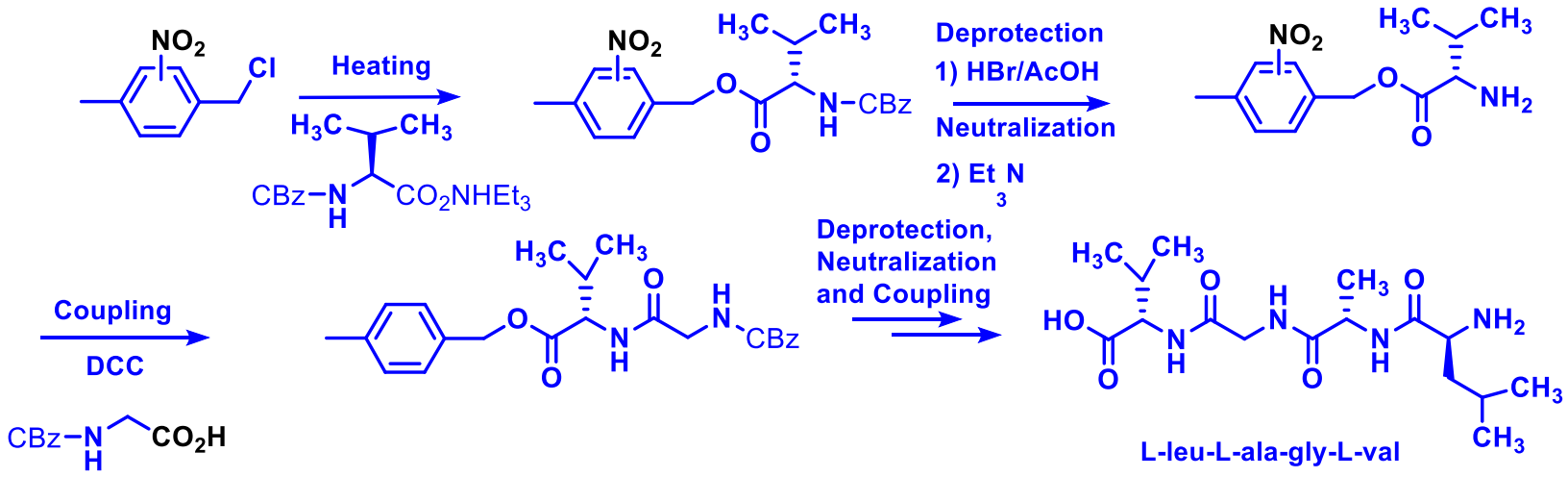
Solid Phase Organic Synthesis (SPOS).

The synthesis in solid phase is a methodology allowing to realize chemical transformations with the aid of an insoluble polymer. Therefore it is also known as polymer assisted synthesis and when applied to organic chemistry it takes the acronym of “SPOS” = Solid-Phase Organic Synthesis.



The approach was introduced by R. B. Merrifield, Nobel price for Chemistry in 1984.

R. B. Merrifield, J. Am. Chem. Soc. 1963, *85,* 2149

Solid Phase Techniques.

- Reactants are bound to a polymeric surface and modified whilst still attached. Final product is released at the end of the synthesis. Automation is possible.

Advantages

- Specific reactants can be bound to specific beads
- Beads can be mixed and reacted in the same reaction vessel
- Products formed are distinctive for each bead and physically distinct
- Excess reagents can be used to drive reactions and are easily removed
- Reaction intermediates adhere to bead and should not be isolated/purified
- Individual beads can be separated to isolate individual products
- Polymeric support can be regenerated and re-used after cleaving the product

Requirements

- A resin bead or a functionalized surface to act as a solid support
- An anchor or linker
- A bond linking the substrate to the linker. The bond must be sufficiently stable
- A means of cleaving the product from the linker at the end • Protecting groups for functional groups not involved **in the synthesis.**

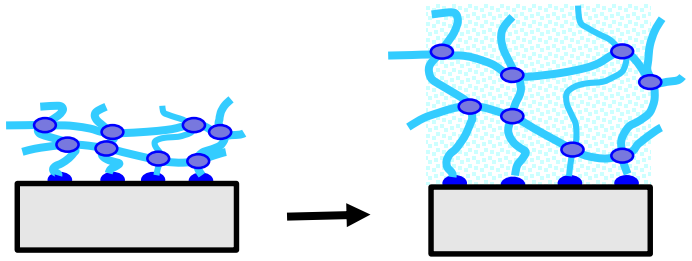
Solid Phase Techniques (2).

Examples of Solid Supports:

- Partially cross-linked polystyrene beads hydrophobic in nature causes problems in peptide synthesis due to peptide folding
- Sheppard's polyamide resin - more polar
- Tentagel resin - similar environment to ether or THF
- Beads, pins and functionalized glass surfaces.

Beads must be able to swell in the solvent used, and remain stable.

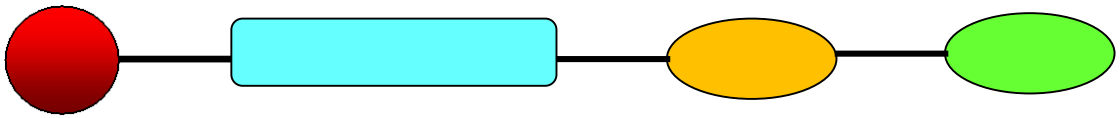
Most reactions occur in the bead interior Resin bead.



Swelling by solvent

Structure of a Polymer for SPOS.

The material we can distinguish four well differentiated components:



Solid Support (Core) Spacer Linker Scaffold

Core is usually a 1% crosslinked insoluble polymer. Typical are copolymers of polystyrene and divinylbenzene in case with polyethyelenglycol.

Spacer: if present, is usually a molecular fragment (usually a polyethylene glycol) connecting the solid support to linker via a covalent bond. Give more solution-like reactivity with lower resin loading

Linker: if present, provides an orthogonal method for releasing the scaffold

Scaffold is the part that we are interested in doing chemistry on and releasing at the end of the synthesis.

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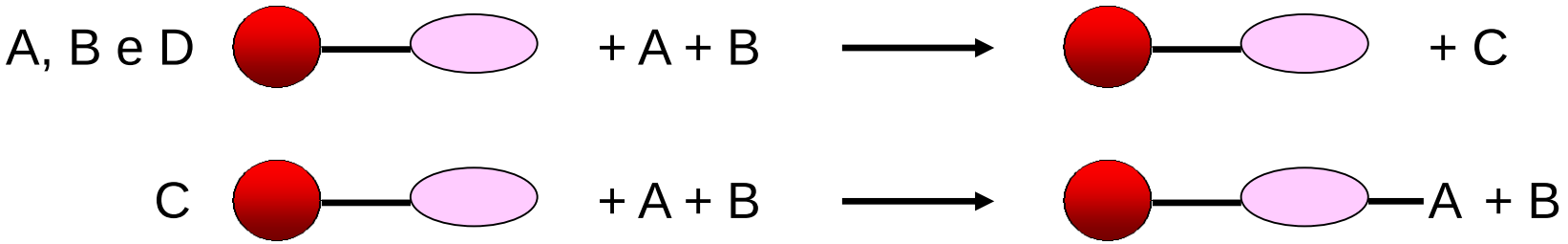
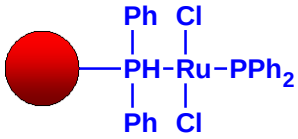
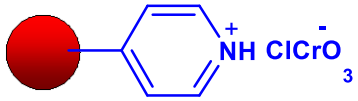
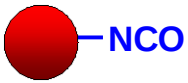
Scaffolds and Reactive Terminal Functional Groups.

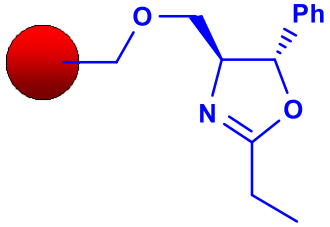
Reactive Polymers for Solid Phase Organic Synthesis:











A) Catalyst B) Oxidant C) “Blocking” D) Chiral Auxiliary

Benefits of SPOS.

There are a series of basic reasons which make SPOS a advantageous methodology in comparison with the classical liquid phase synthesis:

1. Simplified reaction procedures: purification of reaction product can be made through a simple filtration and washing of the resin.
2. Thermodynamic and kinetic influence on the reaction course: It allows to use big excess of reactive in solution (polymeric protecting groups) or on resin (polymeric reactive), with high reaction yield.
3. Resin regeneration: Resin or polymeric reactive can be regenerated to be used in a new reaction.
4. High dilution principle: Controlling the equivalent capacity of resin (< 0.8 mmol/g), unwanted coupling reactions can be eliminated and the intramolecular cyclization reactions can be favored.
5. Automation is possible : Automation is typically used in the Chemical Combinatorial technique and in the Solution Phase Array technique.

Disadvantages of SPOS.

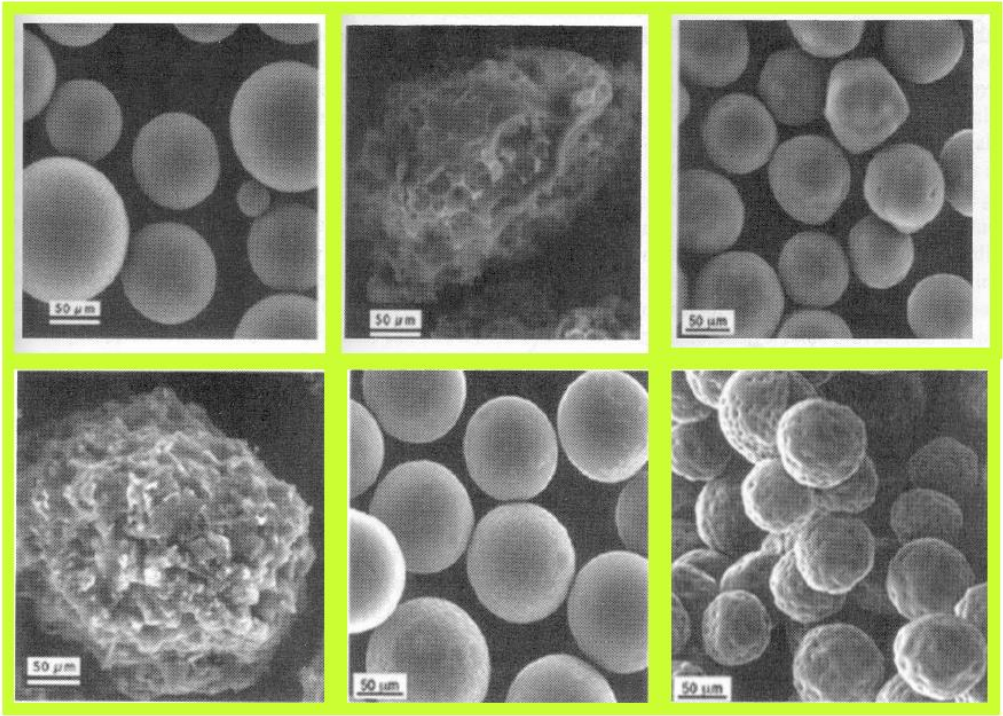
The main disadvantages of the polymer assisted chemistry are:

1. Development of a synthetic method: Since this is a solid-liquid reaction (kinetics controlled by reactive diffusion into polymer pores) and it occurs on solid support (which must be stable), the reaction conditions in solution cannot be directly applied, therefore must be optimized (with selection of polymeric protecting groups).
2. Limitations of solid supports and “linkers”: “linkers” and solid supports, although developed commercially, are limited in number.
3. Monitoring of reactions: Using polymeric protecting groups the real time monitoring of reactions are limited because the forming product remains linked to the polymer.
4. Additional synthetic stages: Working by addition-elimination reactions, more stages must be added to the current solution syntheses.
5. Scale: Generally the method is applied to synthetize product samples lower than 1 g, but in some cases it has been applied during the production.

Types of Solid Support.

Polymeric supports are used to immobilize substrates, reagents and catalysts.

Polymeric support is insoluble in most cases, although sometimes used soluble polymers. The polymers used in the synthesis of organic molecules belong to four classes:



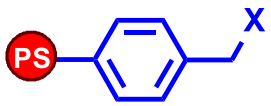
1) Crosslinked organic polymers: are insoluble in organic solvents, and can be micro-porous or macro-porous (i.e. Polystyrene).
2) Linear organic polymers: soluble in organic solvents and insoluble in other where can be precipitated (i.e. Polyethylenglycol).
3) Dendrimers: whose solubility depends on polarity and shape
4) Inorganic supports : porous glass, silica, alumina, clay, graphite.

Types of Supports.

Supports consisting of crosslinked organic polymers are the common:

- Most applications use two types of supports:
  - poly(styrene-co-divinylbenzene) PS-DVB
  - poly(ethylenglycol-co-styrene-co-divinylbenzene) PEG-PS-DVB.

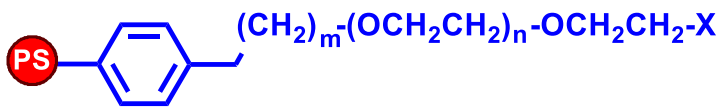
PS-DVB



X = Cl (Merrifield), OH, NH_2

Size:	**100-200 mesh (75-15 μm)** **200-400 mesh (38-75 μm)**
Equivalent capacity:	**0,5-4 mmol/g**
Chemical compatibility:	**DMF, DCM, THF, Toluene.** **Termally stable** **Incomplatible with protic solvents**

PEG-PS-DVB



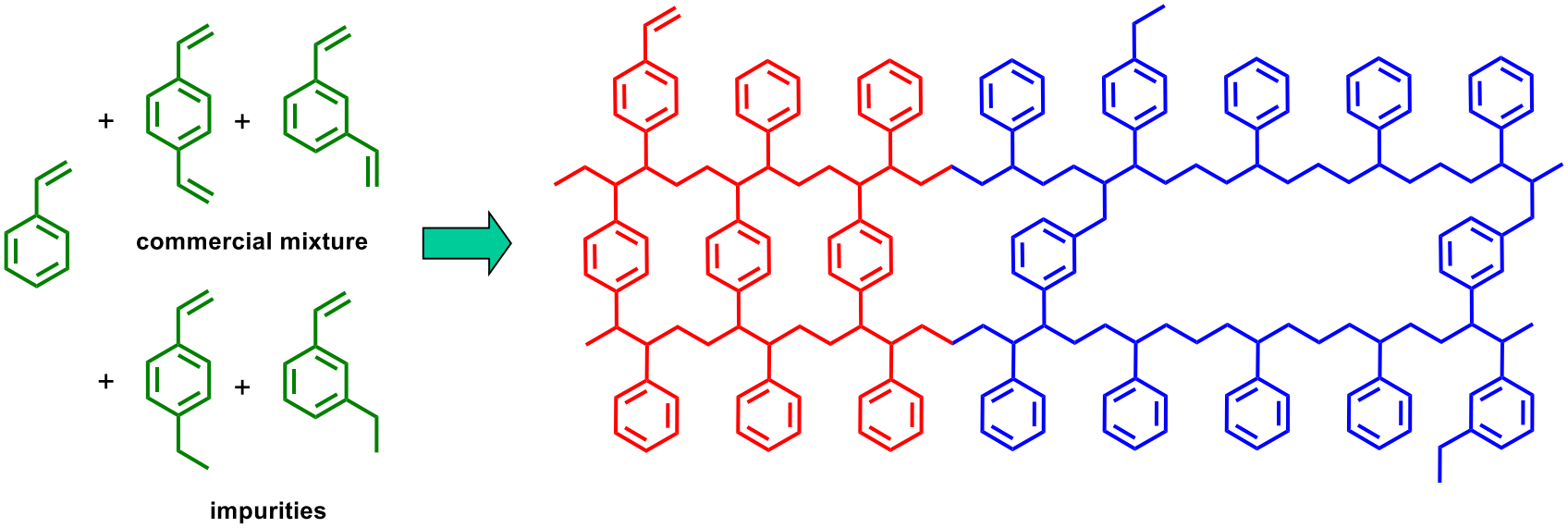
X = Br, OH, NH_2, SH, COOH

	100-200 mesh (75-15 μm)
	0,2-0,5 mmol/g
	Protic and non protic solvents

Cross-linked Polystyrene.

The most used polymeric support in solid phase organic synthesis is poly(styrene-co-divinylbenzene).

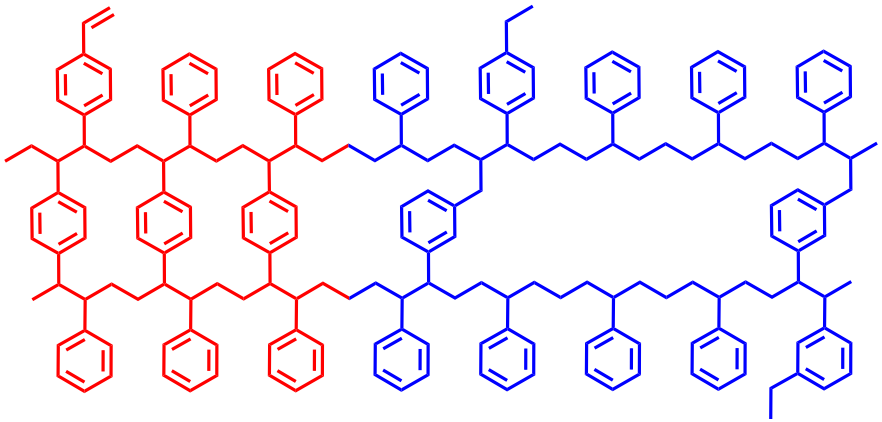
The main problem presented by this polymer for its use in synthesis is its inhomogeneity.



Origin of Inhomogeneity.

1. Different reactivity of *m*-DVB and *p*-DVB:
   - As concerns the first vinyl group, the *meta* isomer reacts with styrene faster than *para* isomer.
   - *p*-DVB reacts preferentially with itself (dense microstructure).
   - As concerns the second vinyl group, *m*-DVB reacts at a rate similar to the one of styrene, therefore is it more regularly introduced.
2. Polymer modification after its synthesis:
   - Washing the polymer with good solvents it dry with collapsed pores .
   - Washing the polymer with bad solvents it dry with wider pores.

Dense regions rich in *para* monomer.

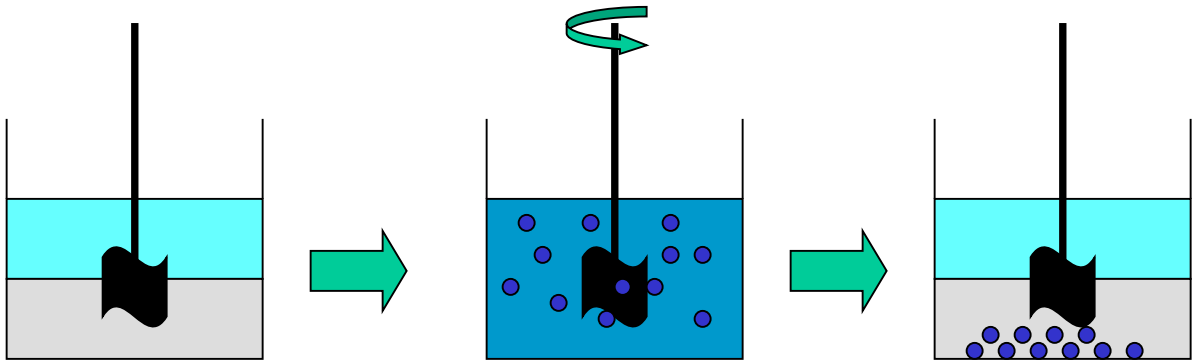


Homogeneous regions but spaced by *meta* monomer.

Synthesis of Cross-linked Polystyrene.

The preferred technique for the formation of resins for synthesis is the suspension polymerization. It differs from other techniques:

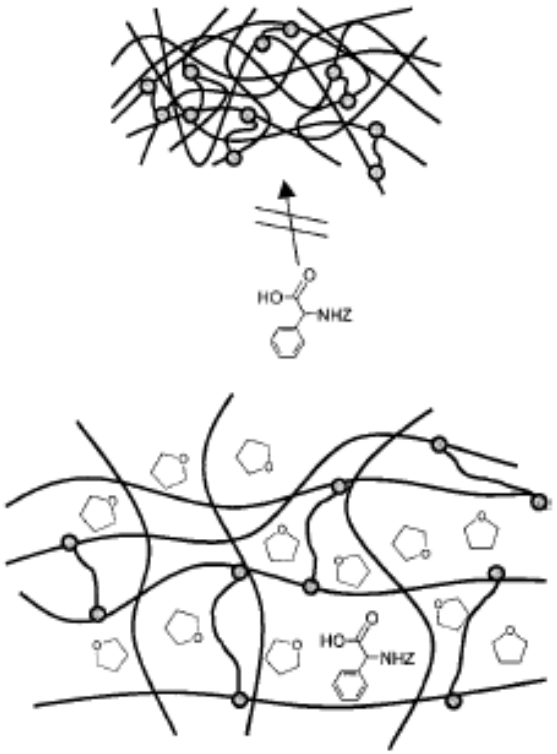
- Is a biphasic system: monomer phase (consisting of monomer and organic solvent –"porogen"-) and aqueous phase.
- Use a stabilizer as additive: a mixture of inorganic salts and polar organic species (polyvinylalcohol), to lower the surface tension of droplets and inhibits its aggregation.
- Produces nearly perfect spheres. by formation of monomer droplets in aqueous phase (mechanical stirring).



Properties of Cross-linked Polystyrene.

The "Swelling" of a crosslinked polymer is equivalent to solvation of a linear polymer:

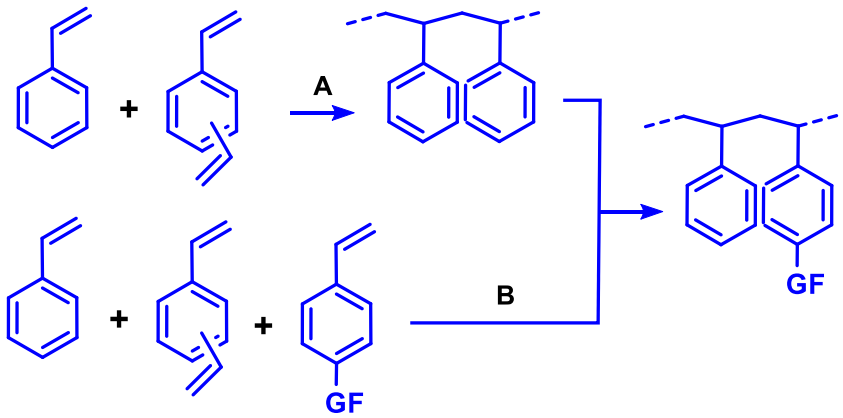
- The solvent penetrates into the crosslinked polymer and occupies the spaces between the polymer chains, producing an increase in volume.
- Crosslinking of the polymer creates as a weave that prevents the movement of the chains of polymer necessary to form a solution.
- The swelled polymer is the real solvent of solid phase reaction, but it present an high viscosity.

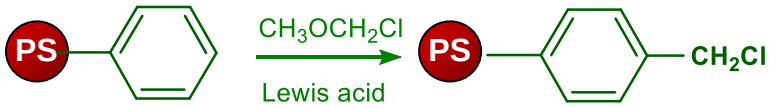


- There is no direct relationship between the amount and kinetics of imbibition reaction.
- The crosslinking degree has a big impact on imbibition capacity trough dissipated configurational entropy. An higher crosslinking make more rigid the polymer, the dissipated entropy is lower and imbibition is difficult.

Derivatized Polystyrene Resins.

The use of derivatized polystyrene resins is because the alkyl framework is chemically inert whereas benzene rings are reactive and can be easily modified (by aromatic electrophilic substitution (i.e. chloromethylation) or nucleophilic substitution (litiations)). Moreover, several cheap functionalized styrene monomers are available.





Lewis acid: $SnCl_4$, $ZnCl_2$, $BF_3{\cdot}OEt_2$

costant behavior needs 19%Cl

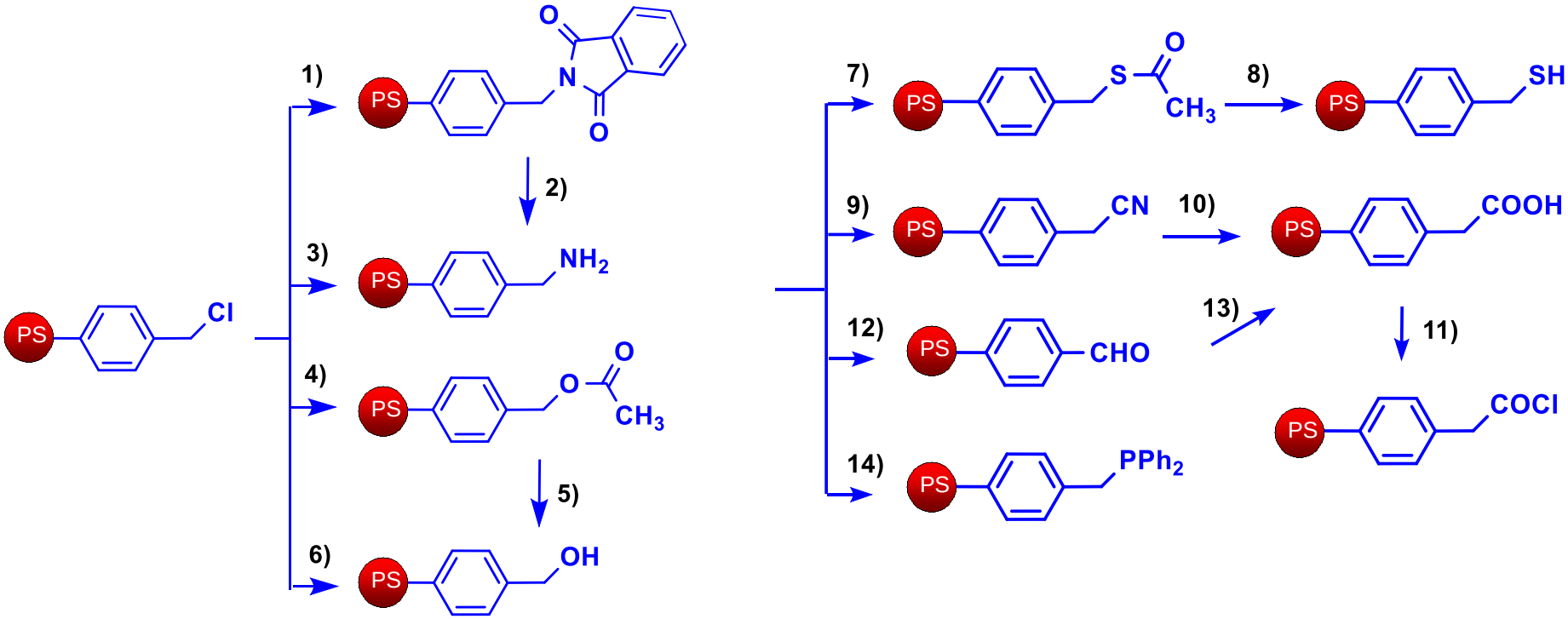
para-/*orto*-: 95:5

Method A: It has the advantage of reacting only rings and positions of equal accessibility. The disadvantage is that reactions are slow and difficult to monitor with problems of yields and byproducts.

Method B: Ensures the exact position of functionalization and a high degree of substitution, but requires the synthesis of appropriate monomers.

Derivatized Polystyrene Resins.

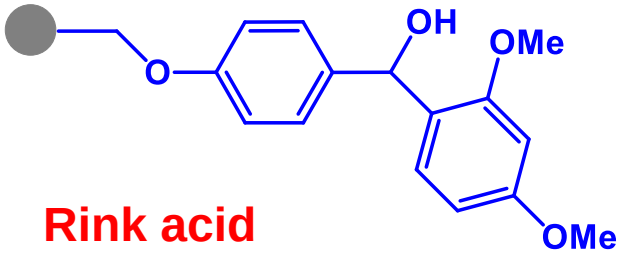
By functionalization of chloromethylpolystyrene:



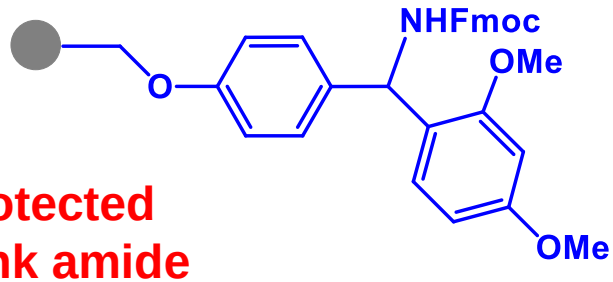
1) Potassium phthalimide, DMF; 2) NH_2NH_2, EtOH; 3) NH_3, DCM; 4) KOAc, DMA, 85 ºC, 24h; 5) HAL, ether, 4h; 6) KOH, 1-pentanol, reflux, 24h; 7) KSAc, DMF; 8) $LiBH_4$, ether; 9) NaCN, DMF, H_2O, 120 ºC, 20h; 10) H_2SO_4, AcOH, H_2O, 120 ºC, 10h; 11) $SOCl_2$, toluene, 110 ºC, 24h; 12) DMSO, $NaHCO_3$, 155 ºC, 6h; 13) m-CPBA, DME, 55 ºC, 19h; 14) $ClPPh_2$, Li, THF.

SPOS Synthesis – Linkers.

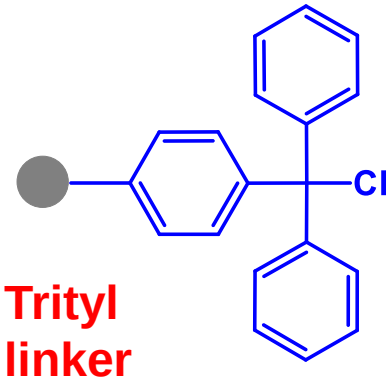
Other linkers



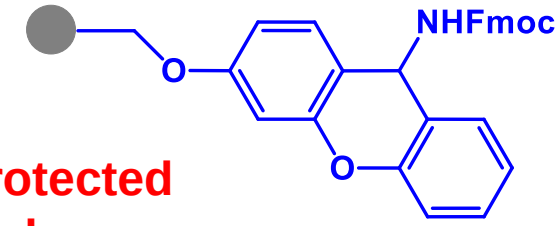
Rink acid linker



Protected Rink amide linker



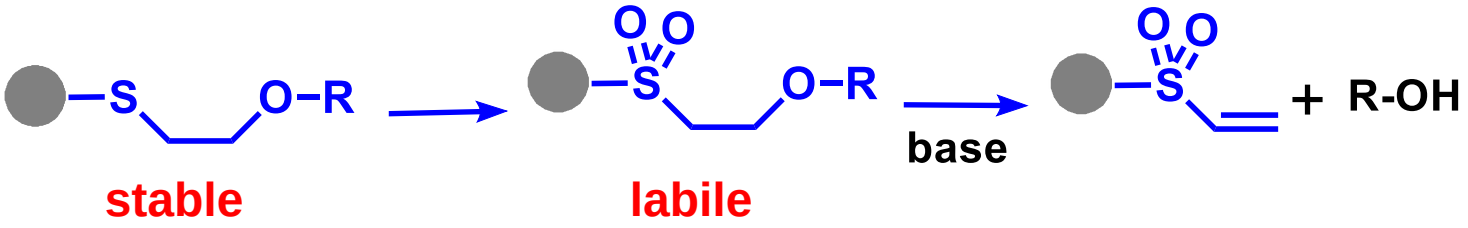
Trityl linker

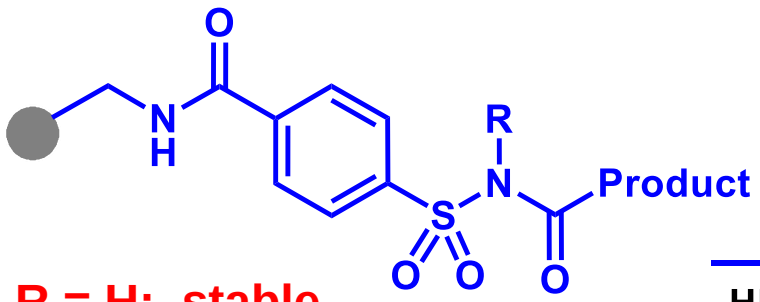


Protected Sieber linker

SPOS Synthesis – Linkers.

Safety catch linker:





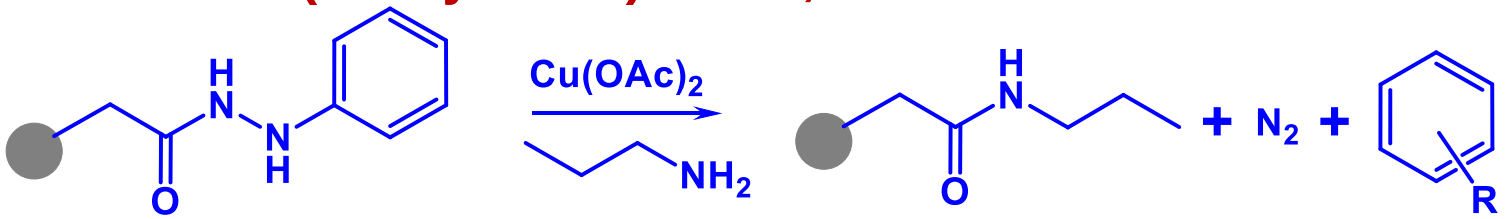
$^-$OH → product-COOH

HNR_1R_2 → product-$CONR_1R_2$

R = H: stable

+ CH_2N_2 → R = CH_3: labile

Traceless (safety catch) linker;



Solid Phase Synthesis: Protecting Groups.

Few protecting groups used in solid phase synthesis.

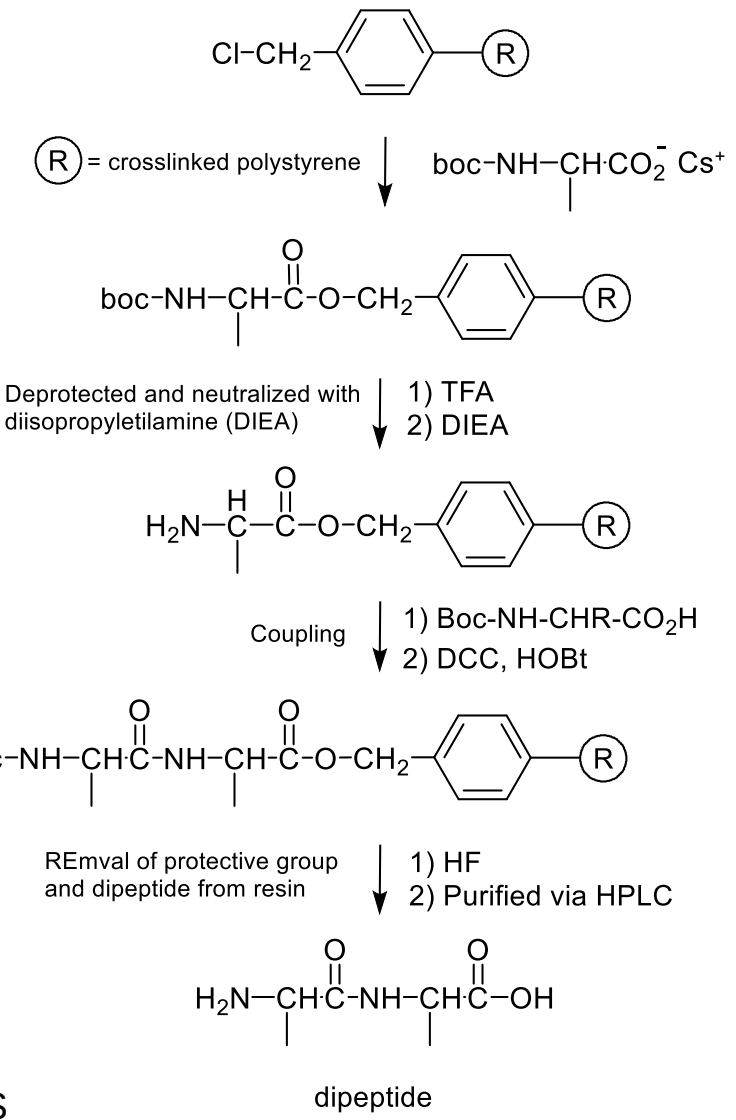
- For amines.
  - Boc (t-butoxycarbonyl)
  - Fmoc (9-fluorenylmetoxycarbonyl)
  - Tmsec (2-[trimethylsilyl]ethoxycarbonyl)

- For carboxylic acids.
  - Tert-Butyl ester (t-butyl ester)
  - Fm ester (9-fluorenylmethyl ester)
  - Tmse ester (2-[trimethylsilyl]ethyl ester)

Solid Phase Peptide Synthesis – the Steps Involved.

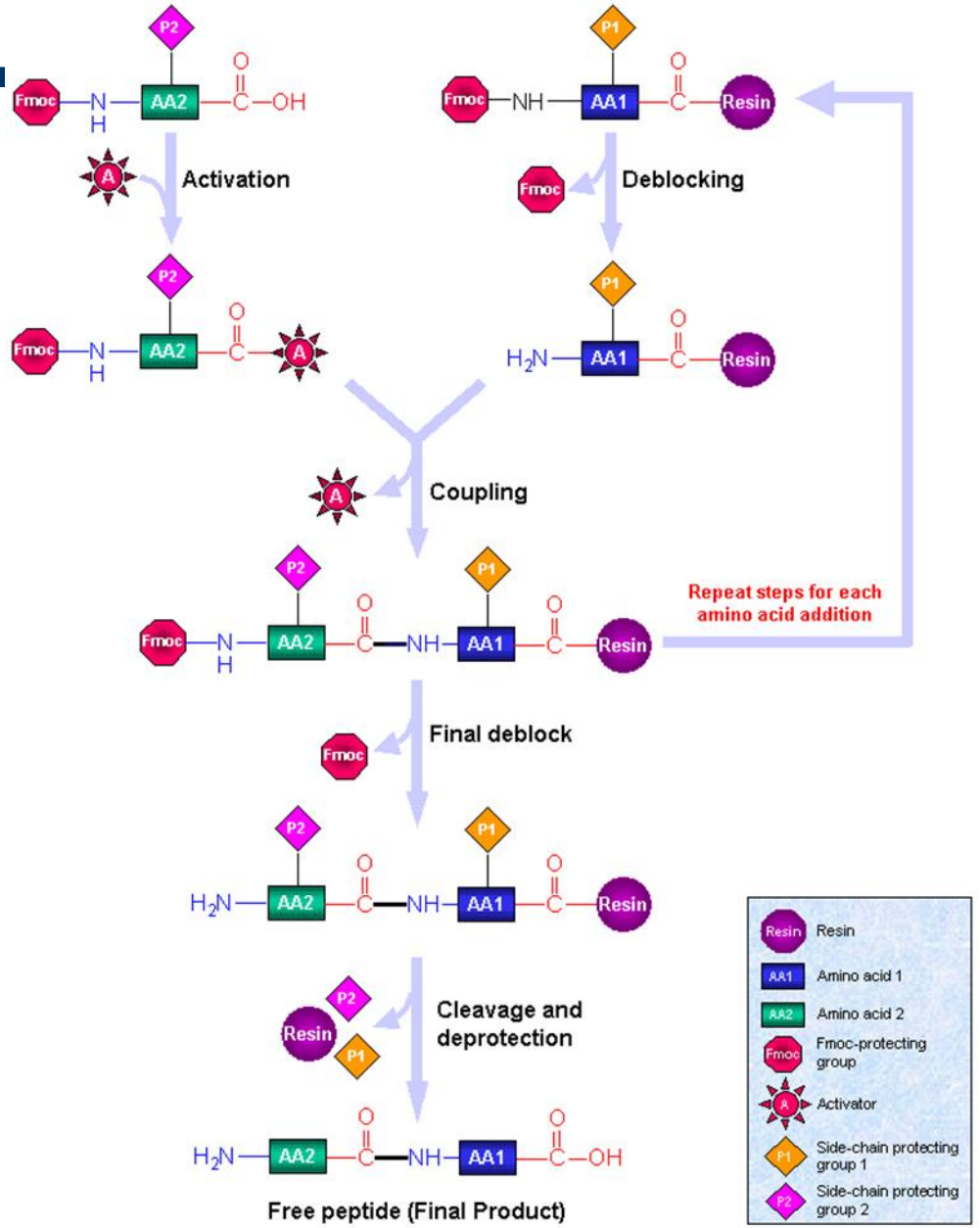
1. N-protected amino acid (Boc or Fmoc) is attached to bead by the C-terminus
2. Deprotect N-terminus, rinse.
3. Couple: Add solution of activated next amino acid. Let react, rinse.
4. Repeat deprotection and coupling for each subsequent monomer
5. Cleave peptide from resin.

Note: synthesize C to N



Scheme based on Merrifield's methodology for Boc-based SPPS

Solid Phase Peptide Synthesis.



Solid Phase Organic Chemistry (Pro/Con).

Products are insoluble

- Easier to manipulate physically;
- Easier to clean up, can wash exhaustively;
- Can use excess reagents to drive reactions to completion:
- No bimolecular reactions (infinite dilution);
- Can’t use Solid Phase Reagents (SPR);
- Modified kinetics (generally slower, greater rate distribution, all sites not equal);
- Requires new analytical methods;
- Requires linking chemistry (limits reaction conditions, constrains product structure).

Solution Phase Organic Chemistry (Array)

More compounds means less time per compound.

This requires:

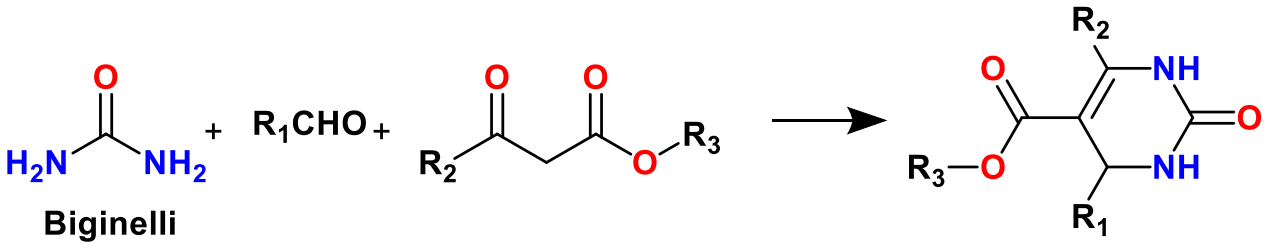
- Good generalized procedures
- Short synthetic sequences
- High yield reactions
- Stoichiometric addition of reactants
- Requires development of parallel workup and purification methods.

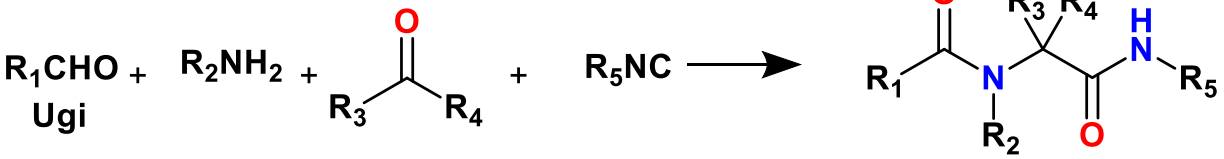
Products are soluble;

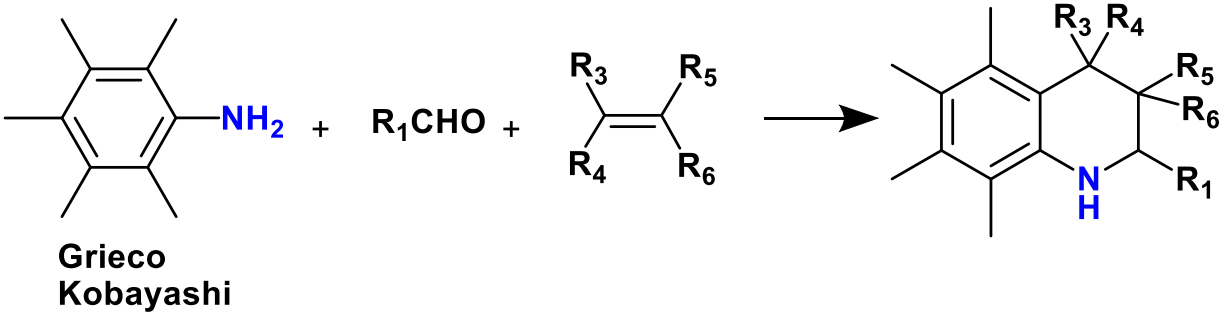
- Byproducts and excess reagents are also soluble and accumulated with each step
- Direct analysis is much easier (tlc, nmr, ms, hplc, lc/ms)
- Kinetics are uniform and familiar
- Use of solid phase reagents (SPR's) is possible
- No linkers required, less excluded chemistry.

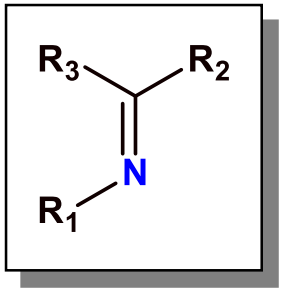
Solution Phase Organic Chemistry (Array).

Multiple Component Condensation Reactions:



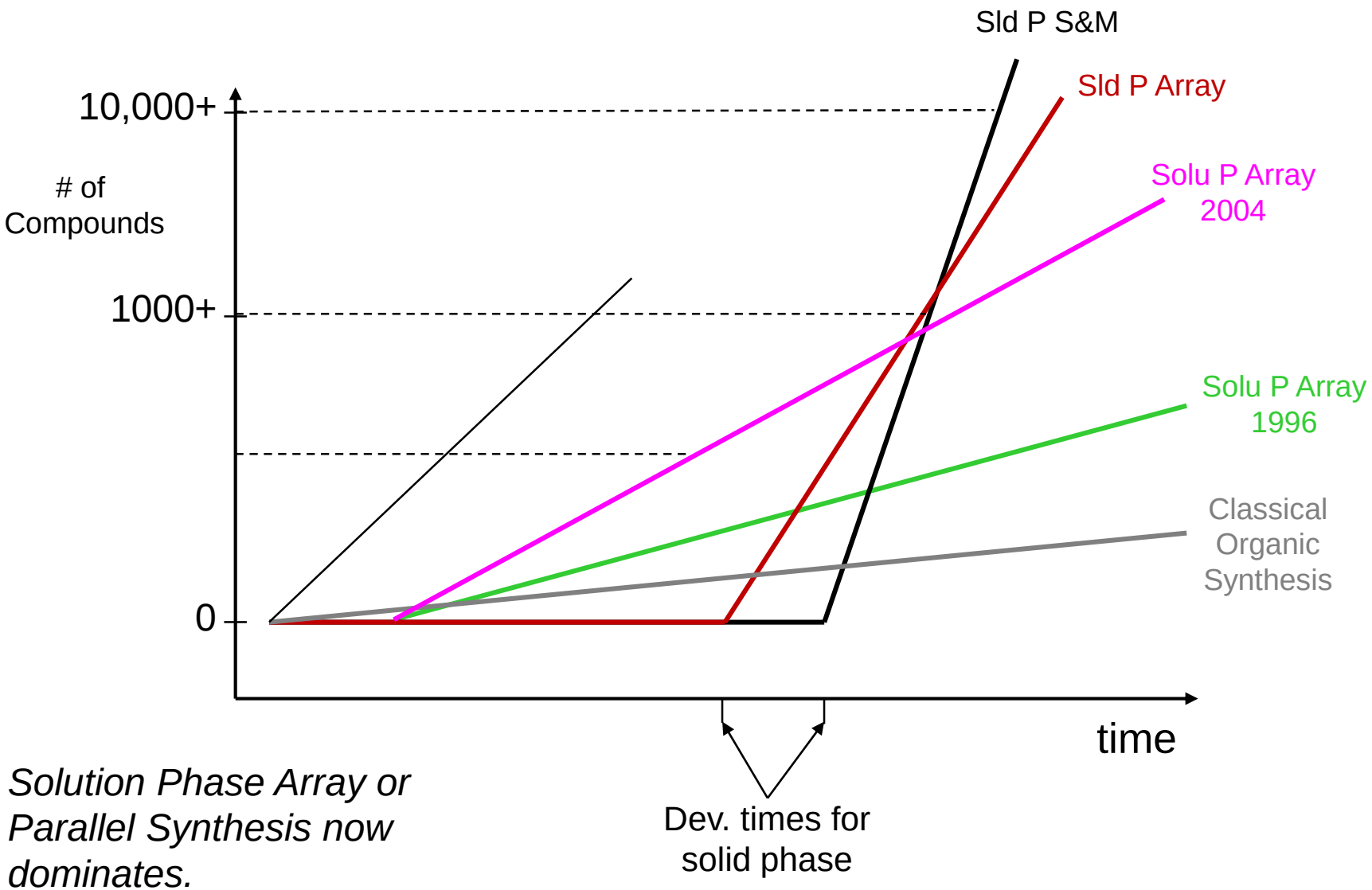






Armstrong, R.W., Combs, A.P., Tempest, P.A., Brown, S.D., & Keating, T.A. *Acc.. Chem. Res.*, 29, 123-131 (1996).

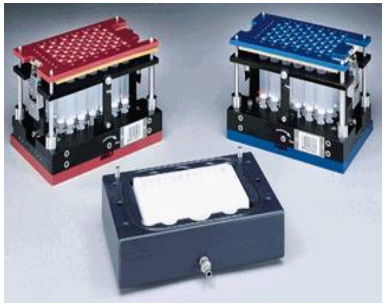
Trends over the Last Decade.



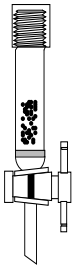
Solution Phase Array or Parallel Synthesis now dominates.

Commercial Apparatus for Solid Phase.

Little Stuff



FlexChem
www.robsci.com
www.scigene.com



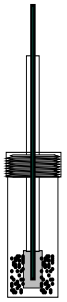
MiniBlock
www.bohdan.com
www.Autochem.com

Polyfiltronics/Whatman
www.whatman.com

Charybdis Technologies
www.spike.cc

Big Stuff

Argonaut
- Quest 210
- Nautilus 2400
- Trident

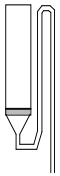


Bohdan Ram

Tecan Combitec

Advanced Chemtech
496

Myriad Core



Purification Methods.

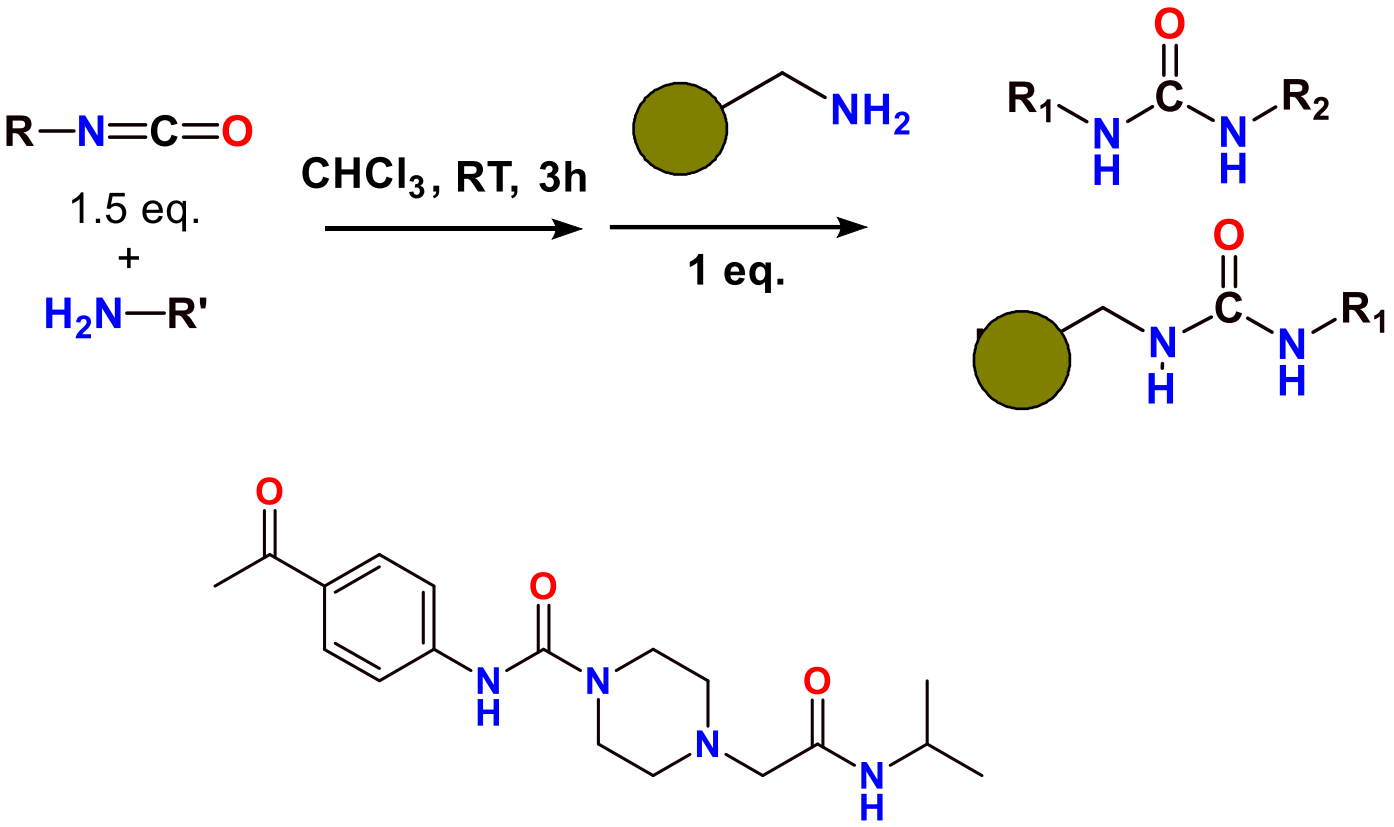
Solid Phase:

- **Wash exhaustively**
- **Product dependent cleavage**

Solution Phase - Parallel Purification:

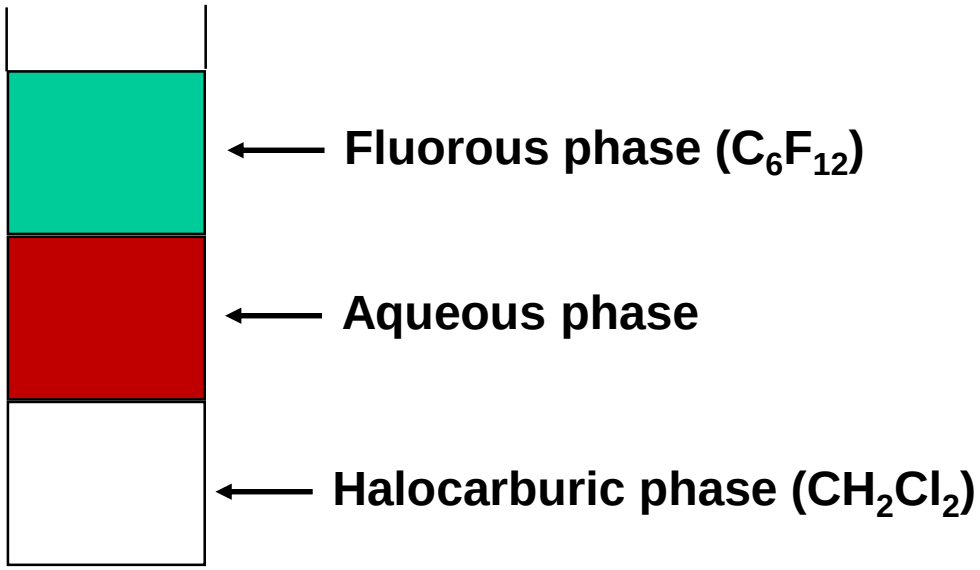
- **Extraction**
  - **liquid-liquid, acid/base**
  - **SPE, scavenging resins**
  - **Fluorous Synthesis**
- **Chromatography**
- **MIP (molecular imprinted polymers)**

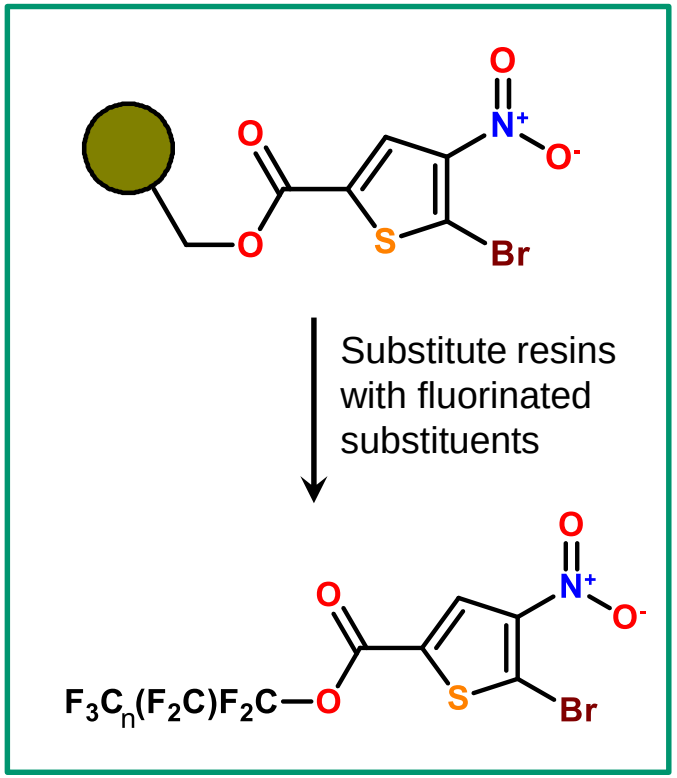
Scavenging Resins.



S. W. Kaldor, J. E. Fritz, J. Tang, E. R. McKinney, *Biorganic & Med. Chem. Lett.*, 6, 3041-3044 (1996).

Fluorous Synthesis.





D. P. Curran, M. Hoshino, *J. Org. Chem.,* **1996**, *61***,** 6480-6481.
D. P. Curran and Z. Luo, Fluorous Synthesis with Fewer Fluorines (Light Fluorous Synthesis): Separation of Tagged from Untagged Products by Solid-Phase Extraction with Fluorous Reverse Phase Silica Gel, *J. Am. Chem. Soc*., **1999**, *121*, 9069. http://fluorous.com

POLITECNICO DI MILANO

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry





Parallel Synthesis.

Prof. Attilio Citterio

Dipartimento CMIC “Giulio Natta”

https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/

Automated Parallel Synthesis.

Aims:

- To use a standard synthetic route to produce a range of analogues, with a different analogue in each reaction vessel, tube or well
- The identity of each structure is known
- Useful for producing a range of analogues for SAR or drug optimization.

Automated parallel synthesis:

- Automated synthesizers are available with 42, 96 or 144 reaction vessels or wells
- Use beads or pins for solid phase support
- Reactions and work ups are carried out automatically
- Same synthetic route used for each vessel, but different reagents
- Different product obtained per vessel.

Combinatorial Synthesis in Solution.

Synthesis in Solution.

+ reaction conditions are usually more easily adapted to a large variety of substituents
+ no attachment / cleavage steps
+ no limitations in synthesis-scale
- strict limitations in reagent excesses
- extensive and time consuming, chromatographic purifications are often necessary
- parallelisation and automation usually requires more initial effort

Synthesis in solution is especially suited for short reaction-sequences:
"One-pot" syntheses, multi-component reactions

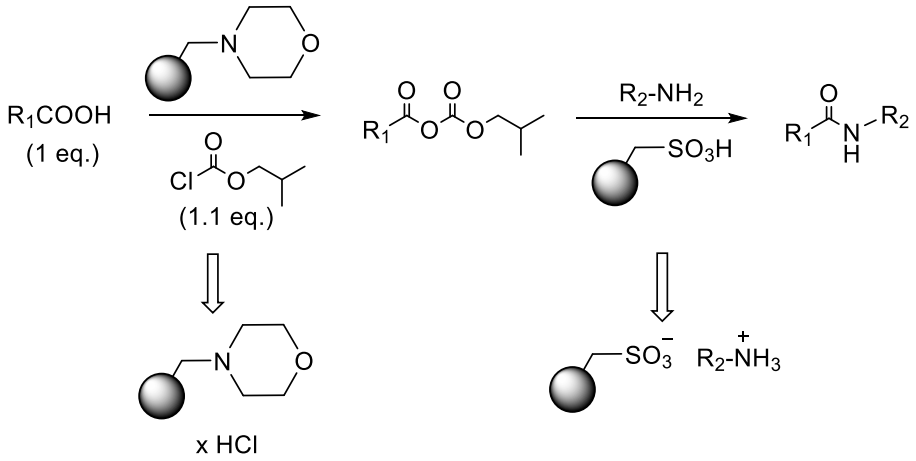
Solid Phase Synthesis.

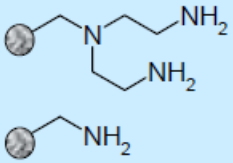
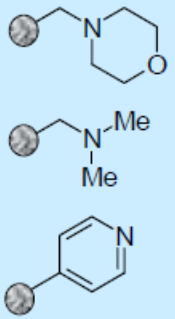
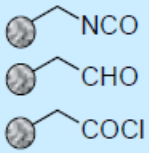
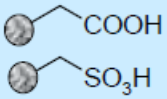
+ purification procedures achieved by simple filtration which can be easily automated
+ excess of reagents can be used to drive reactions to completion
+ synthesis of mixtures (split and combine)
+ easy to automate
- reaction conditions have to be established for each case
- difficult on-bead characterization of the products
- chemistry on polymers is relatively expensive: polymers, solvents
- resins and linkers limit the number of possible reactions (orthogonality).

Parallel Synthesis in Solution.

Work-up Procedures: ***Liquid-solid phase extractions using polymer-bound scavengers***

- Auxiliary bases and acids are covalently linked to insoluble supports
- Advantage: Excess of reagents and by-products can be removed by simple filtration.

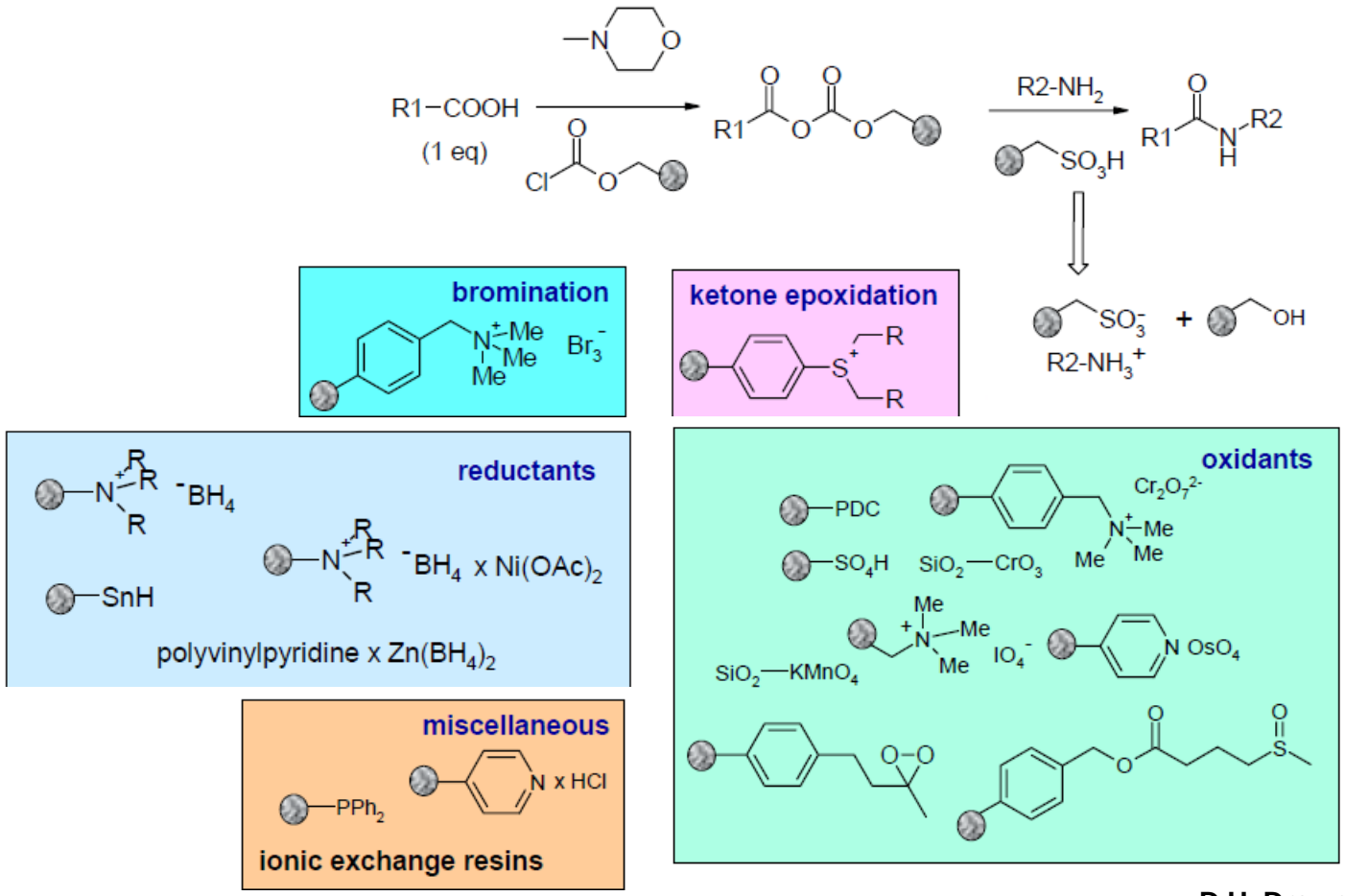


nucleophilic	basic	electrophilic	acidic
			

Parallel Synthesis in Solution.

Special Methods: polymer-bound reagents or catalysts.

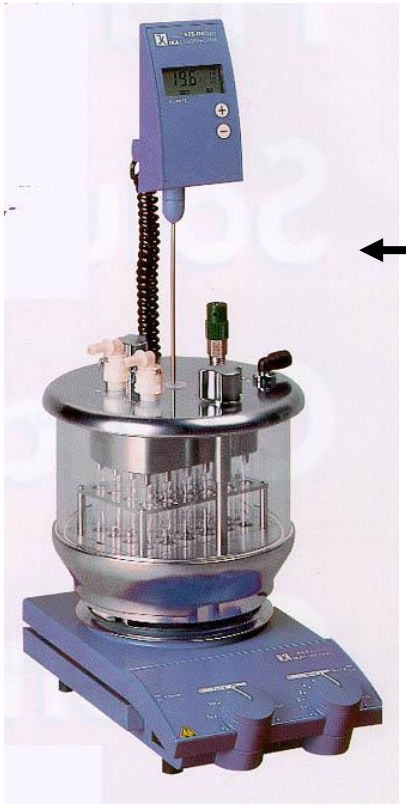
"Inverse" solid phase synthesis:



D.H. Drewry, D.M. Coe, S. Poon
Med. Res. Rev. 19, 97-148 (1999)

Equipments for CC.

Equipment **varies considerably** from traditional ‘organic synthesis’.



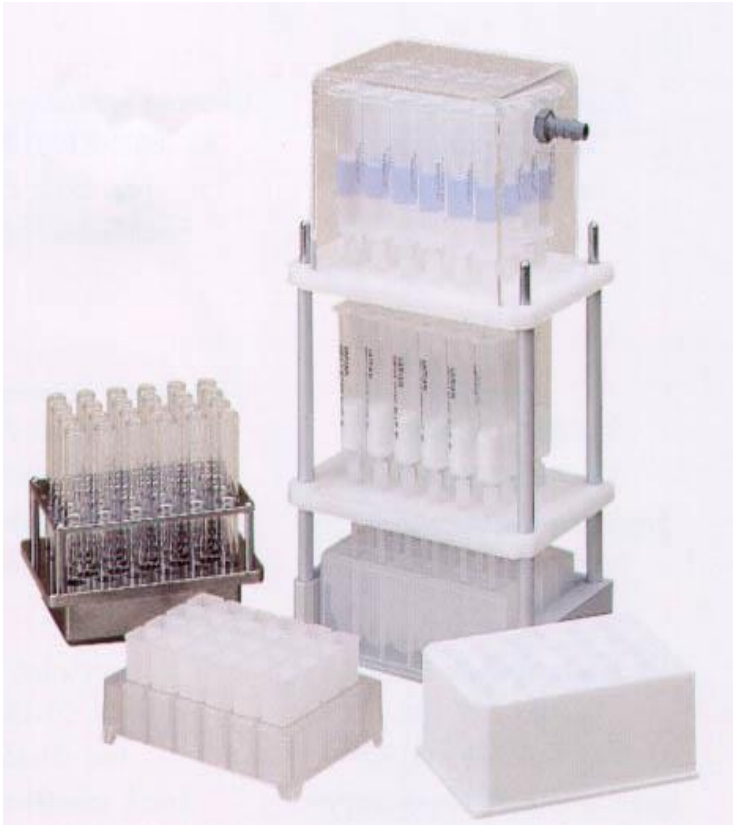
A 24 position ‘personal synthesizer’ for parallel solution and solid supported reagent based chemistry 0.3 - 3 ml per reaction tube. The equipment has the same ‘footprint’ as a standard 24 well microtitre plate allowing for rapid transfer *via* robotics to **bioassay** 24 well plates.

A 6 place reaction station that allows 6 × 100ml reactions in parallel with stirring, heating, *in vacuo* or at pressure (with or without inert atmosphere). Ideal for parallel synthesis of **building blocks** for the parallel synthesizers shown before.

Purifications in Parallel Synthesis.

A Stacker parallel purification system.

24 well titre plate footprint allowing filtration, phase separation and Solid Phase Extraction (SPE) techniques. Works in conjunction with the personal parallel synthesizer shown earlier.



Parallel Synthesis.

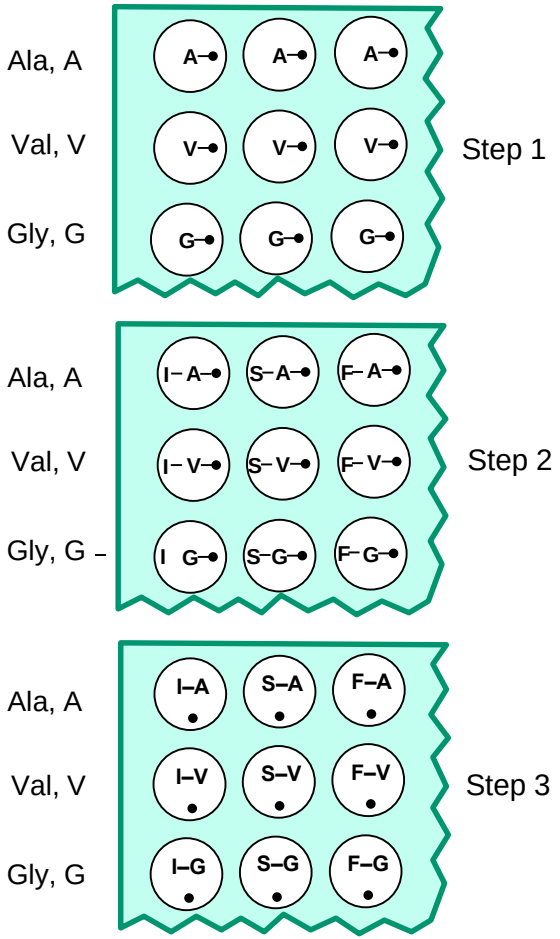
Simultaneous synthesis of multiple products in different reaction vessels with aid of automation. (Increased productivity 100-fold or more).

Each well has one compound, and can be identified by its position on the grid.

Example: Parallel synthesis of a 96-member library of dipeptides in a microtiter plate with 8 rows and 12 columns. (partially shown)

- Step 1: each row starts with a different amino acid attached to a bead
- Step 2: each column adds a different second amino acid. (results in 96 different dipeptides)
- Step 3: Remove dipeptide from the bead
- Step 4: Test each for biological activity.

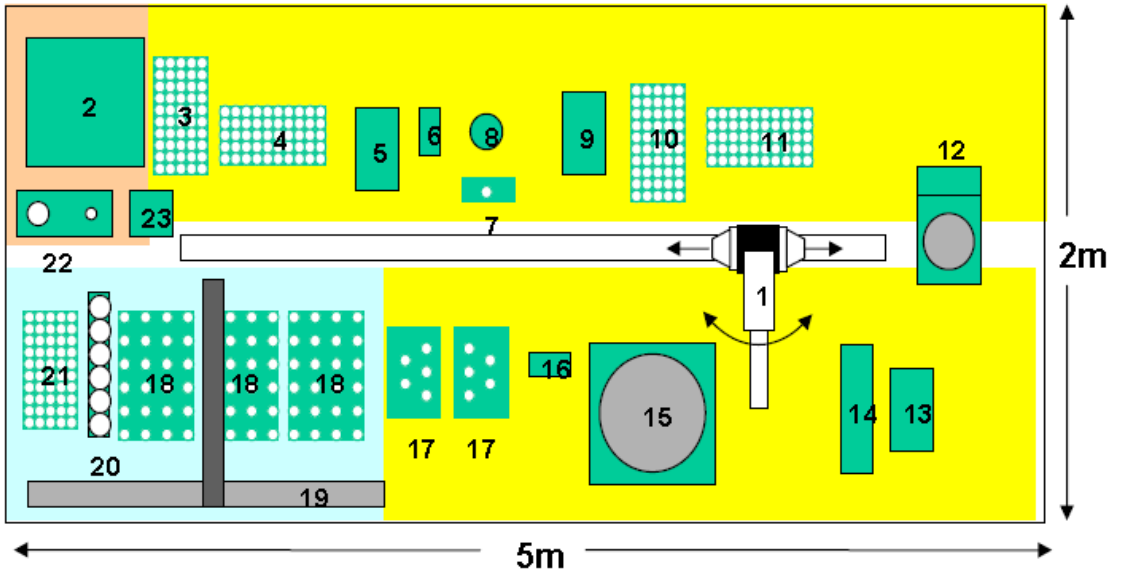
Note: pin and well grid technique also.



Synthesizers for Automated Synthesis in Solution.

1. ISRA-system

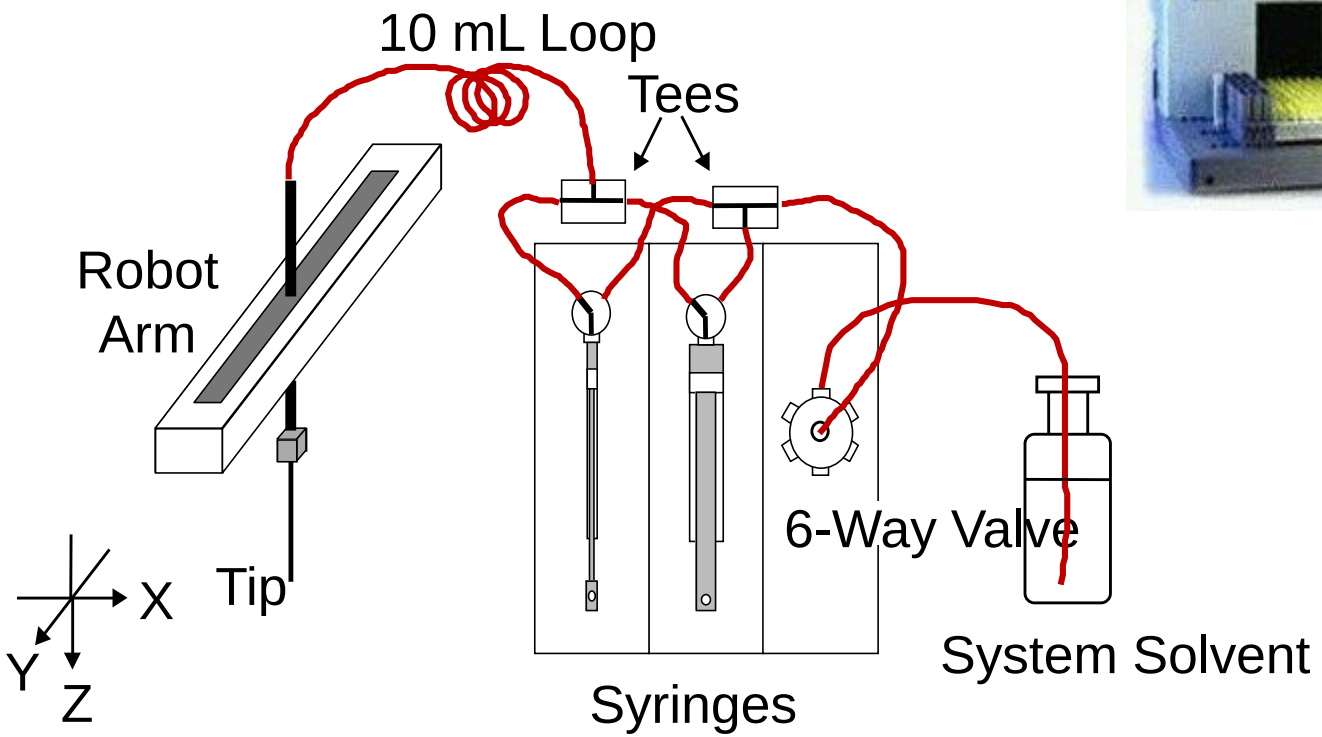
- Requirements: All procedures of a synthesis-laboratory have to be automated:
- weigh in, synthesis, work-up, HPLC-analysis, lab journal
- Automation of this complete process is extreme difficult. No commercial solutions.
- Advantage: fully-automatic, parallel synthesis laboratory, operating 24 h a day.



1 robot
2 HPLC
3 starting rack
4 filter rack
5 filtration station
6 capping station
7 lid transfer port
8 lid magazine
9 drying station
10 drying columns
11 coolable rack
12 balance
13 phase separation
14 phase detection
15 centrifuge
16 hand over station
17 evaporator
18 reaction blocks
19 pipetting station
20 reagent rack
21 reactant rack
22 HPLC-transfer
23 vortexer station

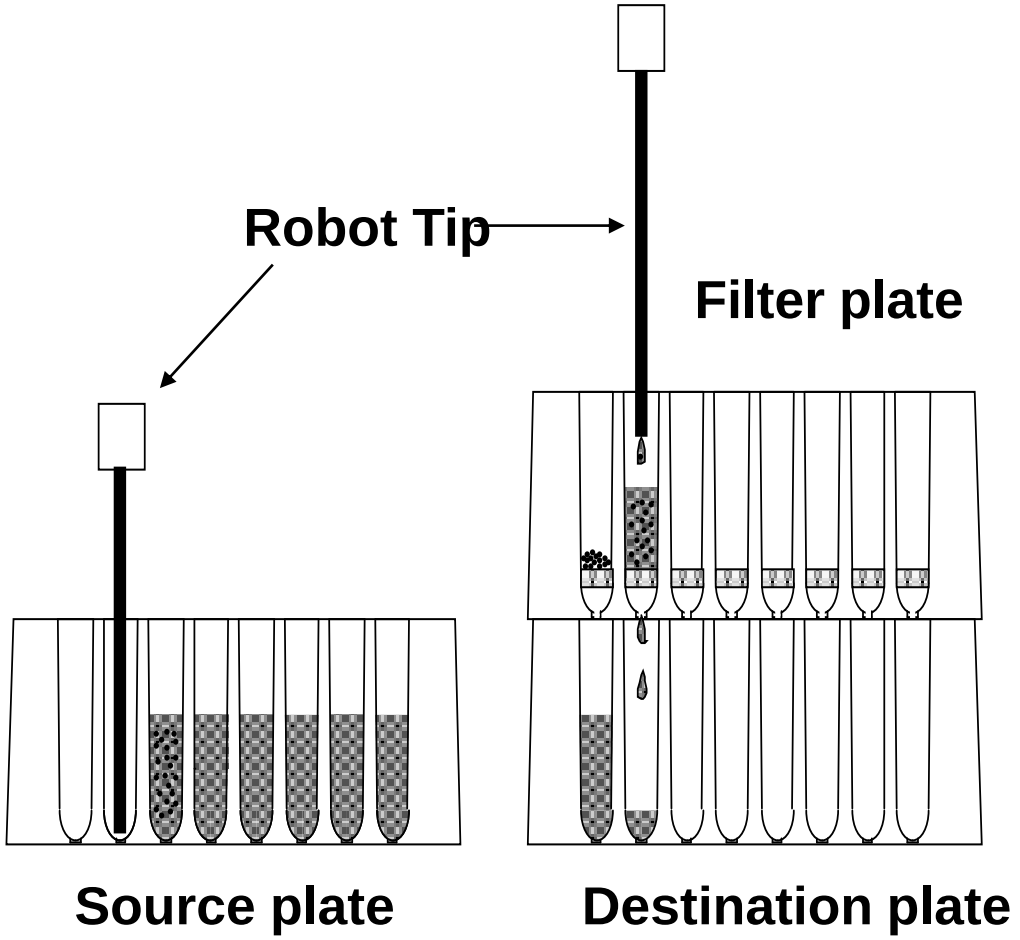
synthesis | Work-up | Analysis

Liquid Handling Robots.



Filtration.

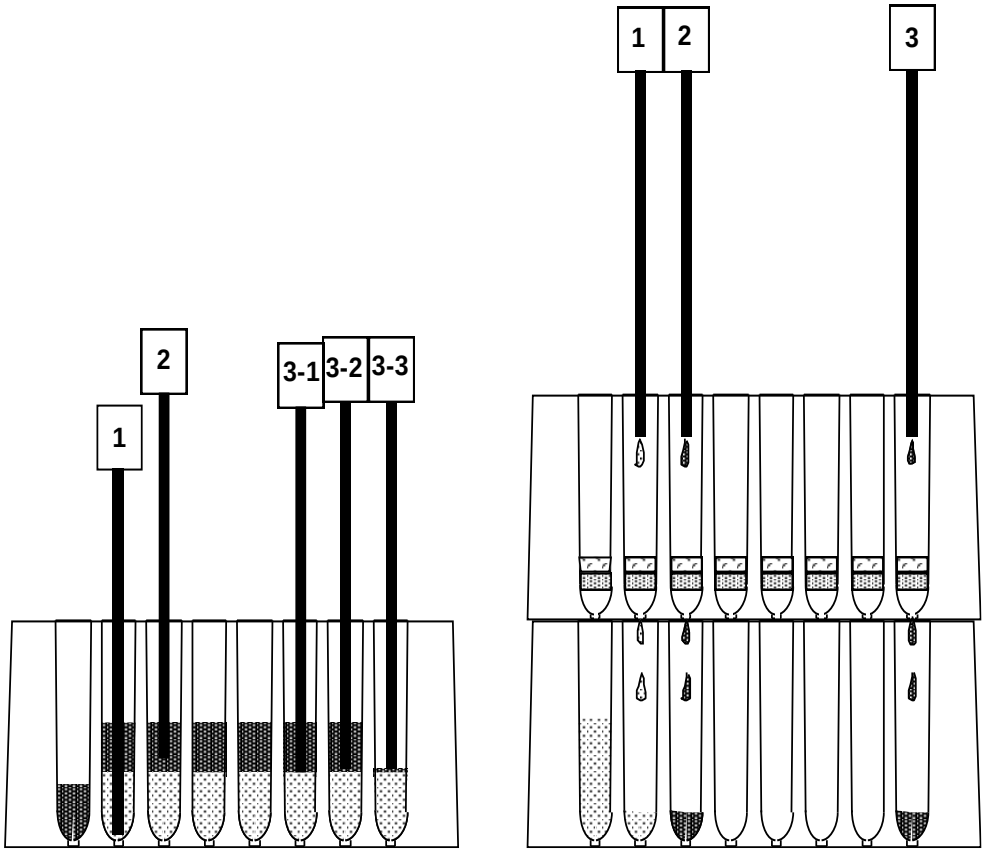
- Salt Removal.
- Covalent and Ionic Scavenging Resin Removal.



Extractions.

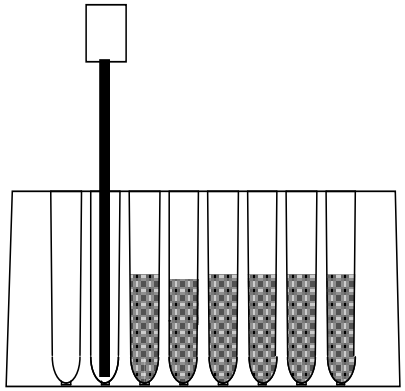
Liquid-Liquid

1. Positional Heavy Solvent Extraction
2. Positional Light Solvent Extraction
3. Liquid Detection Light Solvent Extraction.

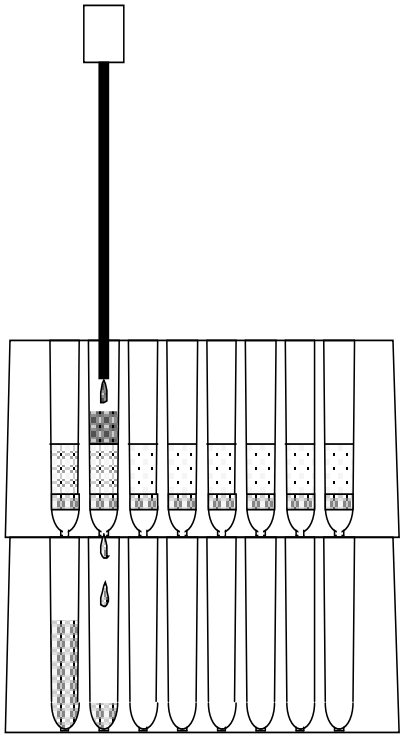


Chromatography and SPE.

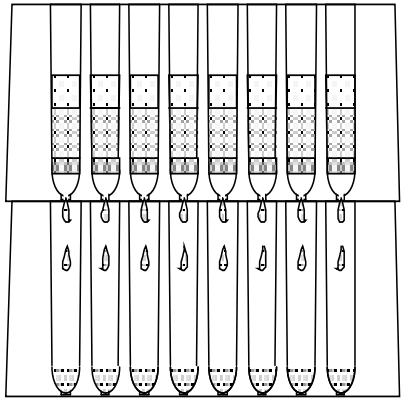
- ✓ Silica Gel
- ✓ Fluorous Silica Gel
- ✓ C_{18}
- ✓ Ion Exchange



1. Dissolve Samples in a suitable solvent

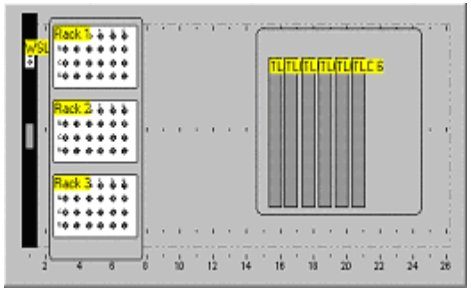


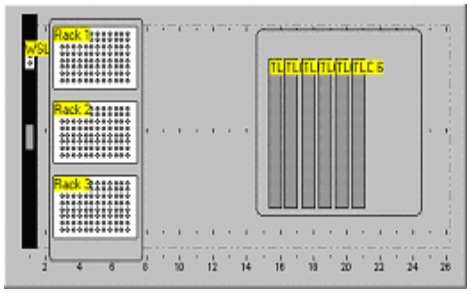
2. Transfer to little chromatography columns

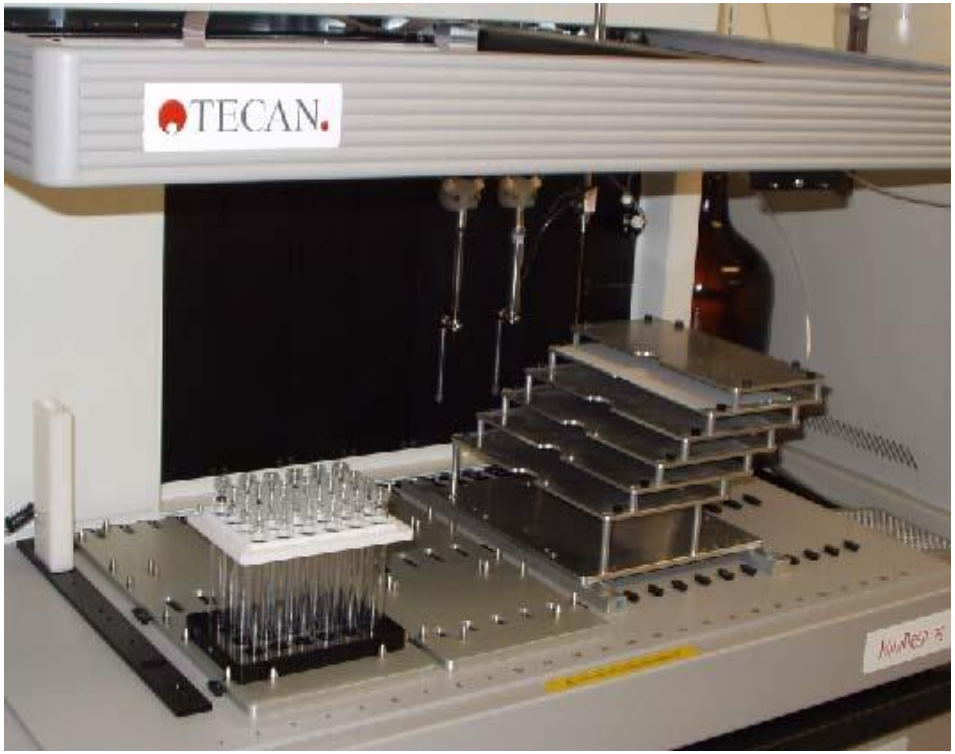


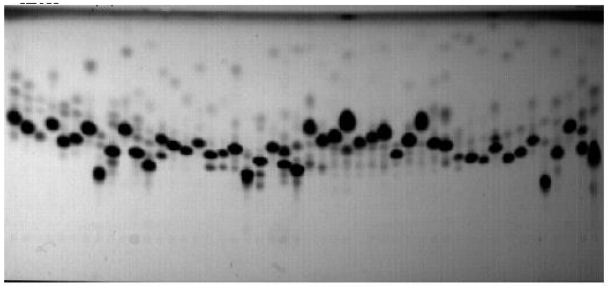
3. Elute clean products and/or collect fractions

Robotic TLC Plate Spotting.



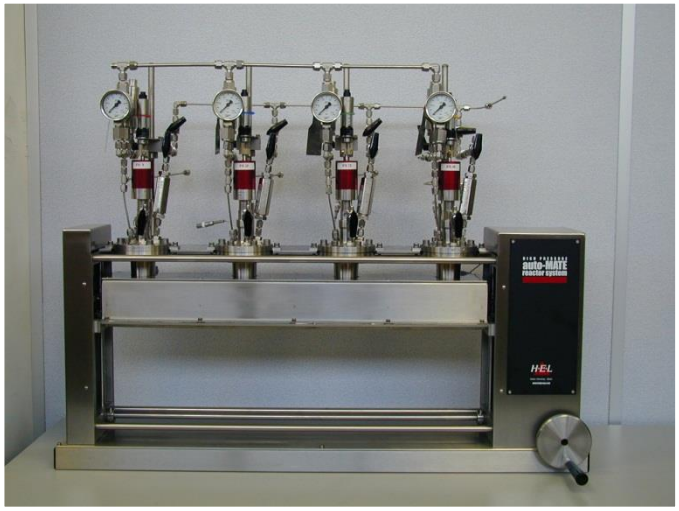






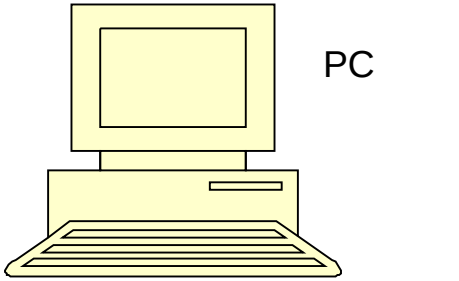
Detected spots

Reaction Optimization and Combinatorial Chemistry.

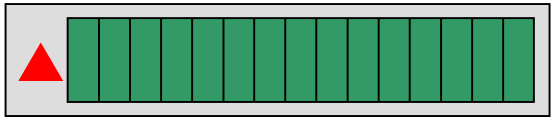


- "Design, Synthesis, and Evaluation of Small-Molecule Libraries." Ellman, J. A. *Acc. Chem. Res.* 1996, 29, 132 -143.
- Combinatorial Chemistry, Nicholas K. Terrett, Oxford University Press, London, 1998
- Combinatorial methods for development of sensing materials, Springer, 2009. ISBN 978-0-387-73712-6
- QSAR and Combinatorial Science, 24, Number 1 (Feb. 2005)

Better Processes – Faster.

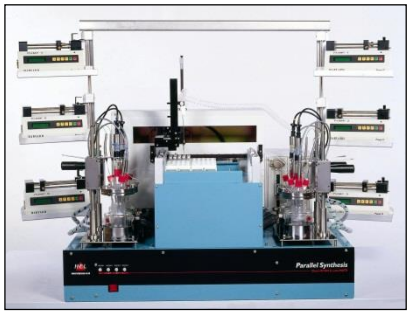


PC



ELECTRONIC
INTERFACE

PARALLEL
SYNTHESIS



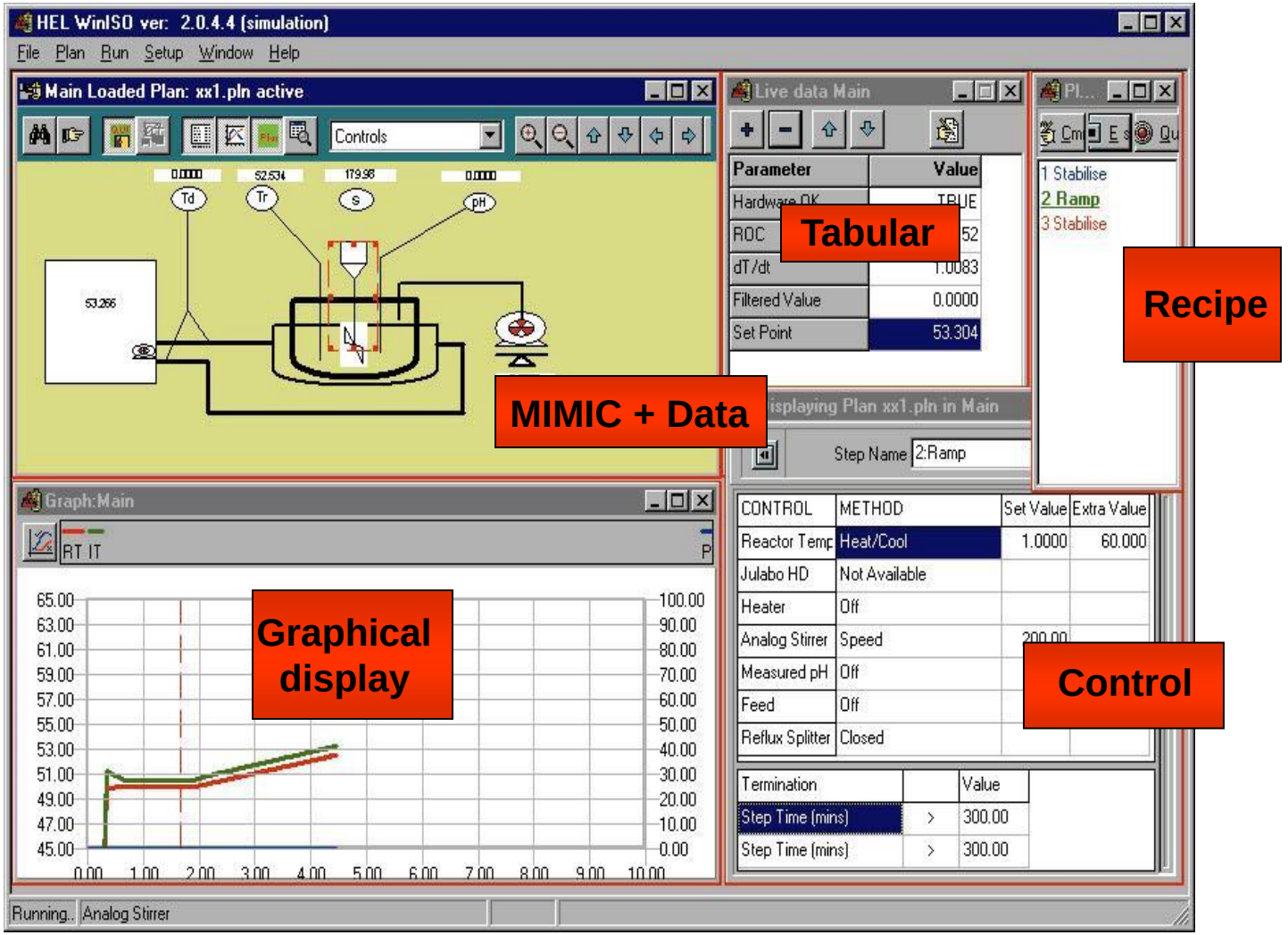
BENCH & PILOT
SCALE REACTORS

ADIABATIC
REACTORS

Better Processes – Faster.

- Most common systems.
- Automated liquid feeds, *T*, *P* control, stirring and distillation.
- User interface: Completely flexible recipe editor.

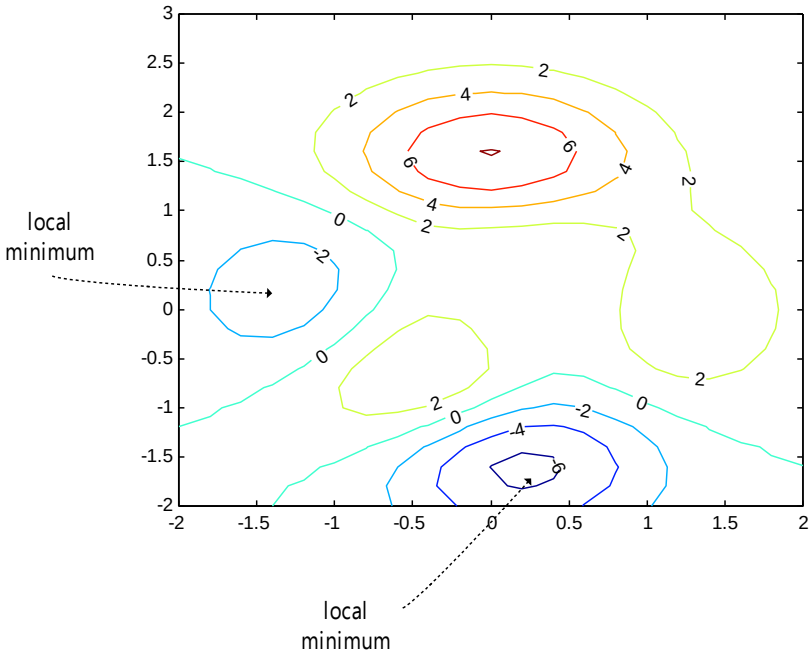
Real Time Control.

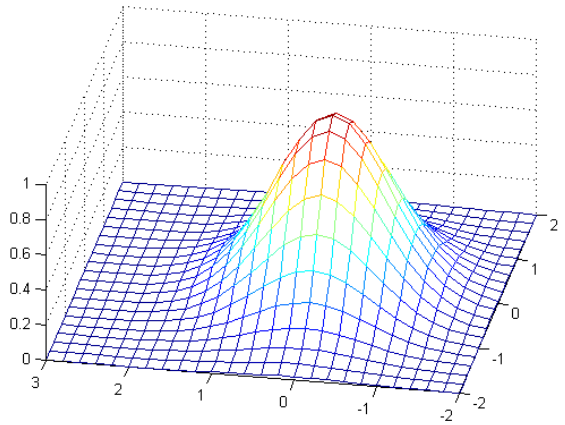


Pilot Scale Unit.

- 0.05- 20 litre high pressure reactor.
- Continuous or Semi-batch with several different feeds including:
  - volatile liquids
  - pyrophorics
  - flammable gases
- Main challenges
  - mechanical design
  - explosion proof
- Standard User Interface.
- Tubular reactor systems are highly customizable and can be made to various lengths and diameters and engineered for various pressures and temperatures.

Factorial Design in Optimization by Design of Experiments.





Design of experiments:

- Full factorial design with replication

vs.

- One-Factor ANOVA:
- Separates total variation observed in a set of measurements into:
  - Variation within one system
    - Due to random measurement errors
  - Variation between systems
    - Due to real differences + random error
- Is variation(2) statistically > variation(1)?
- One-factor experimental design.

POLITECNICO DI MILANO

School of Industrial and Information Engineering

Course 096125 (095857)

Introduction to Green and Sustainable Chemistry





Combinatorial Synthetic Chemistry

Prof. Attilio Citterio

Dipartimento CMIC “Giulio Natta”

https://iscamapweb.chem.polimi.it/citterio/it/education/course-topics/

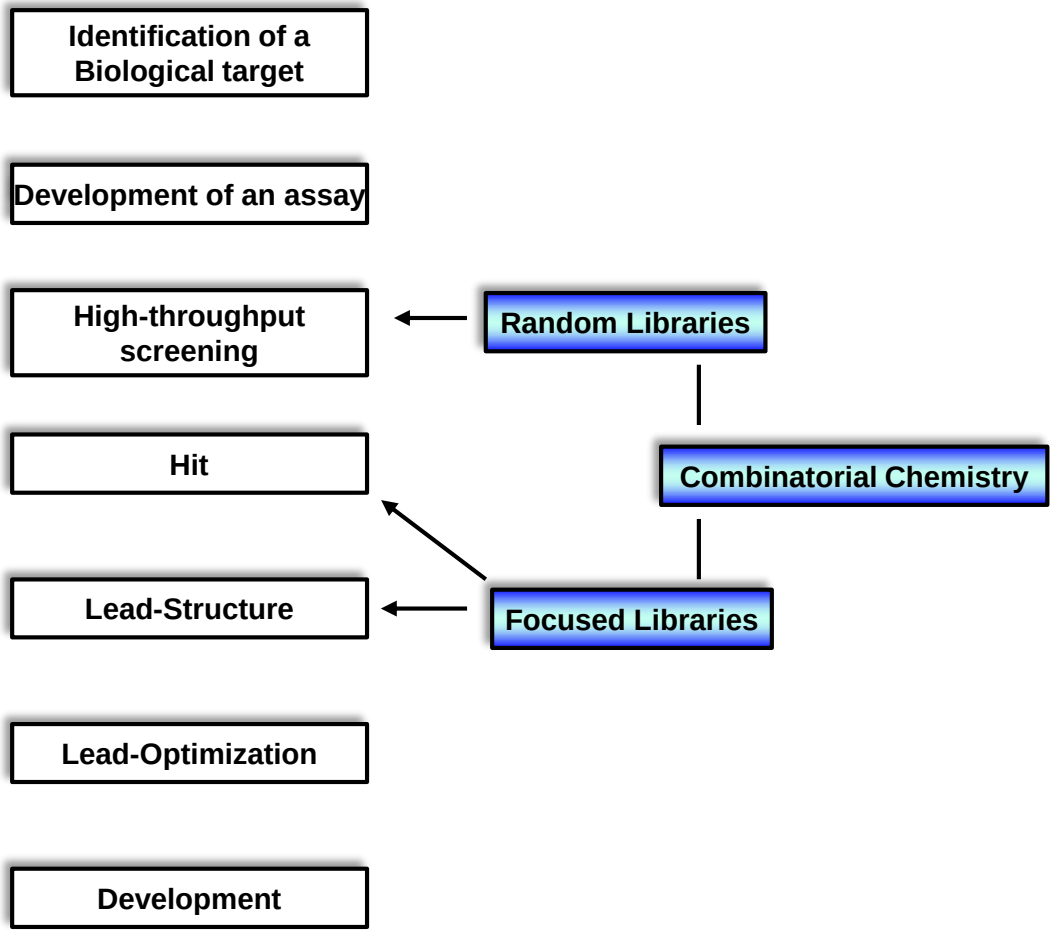
Exploring Molecular Diversity: Combinatorial Chemistry.

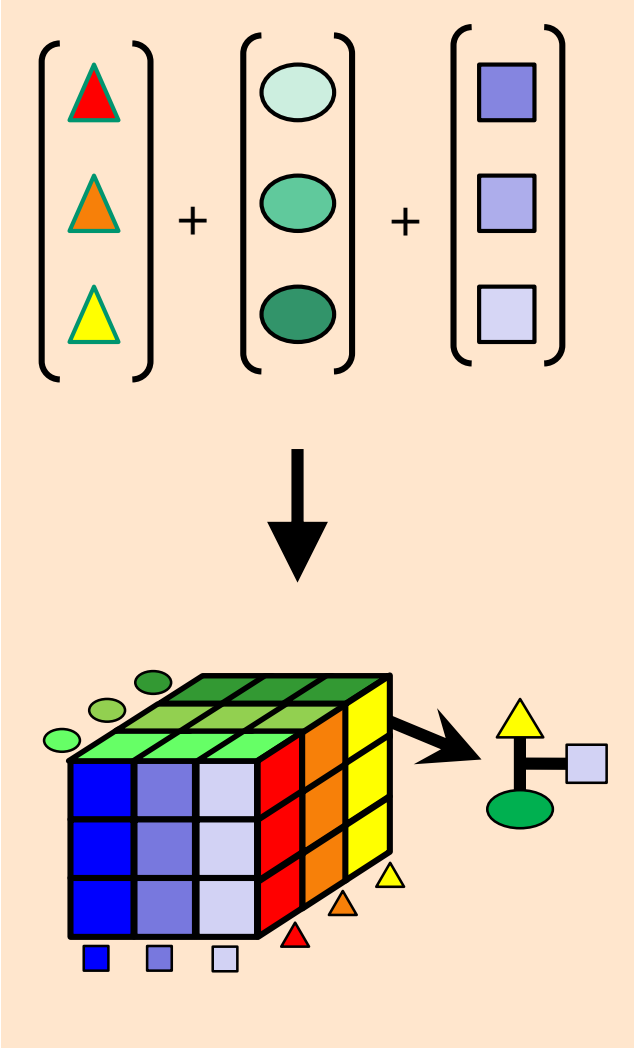
Combinatorial Chemistry concerns the design and preparation of product “libraries” having similar structures, based on a “lead” molecule, to verify biological properties and their optimization in the development of new drugs for the pharma industry.

Combinatorial Chemistry generates a multitude of chemically related (”congeneric”) compounds, so-called ”combinatorial libraries“ or single products (“parallel synthesis”) or their mixtures (“combinatorial synthesis”), through a combination of a group or groups of reactants in only few synthetic stages consistent in dissolution or adsorption in solid phase, which allow for a fast structural elucidation (“deconvolution and tagging”), a faster recognizing of structure-activity relationships (“SAR”) and the possibility to automation.

Technique invented in the late 1980s and early 1990s.

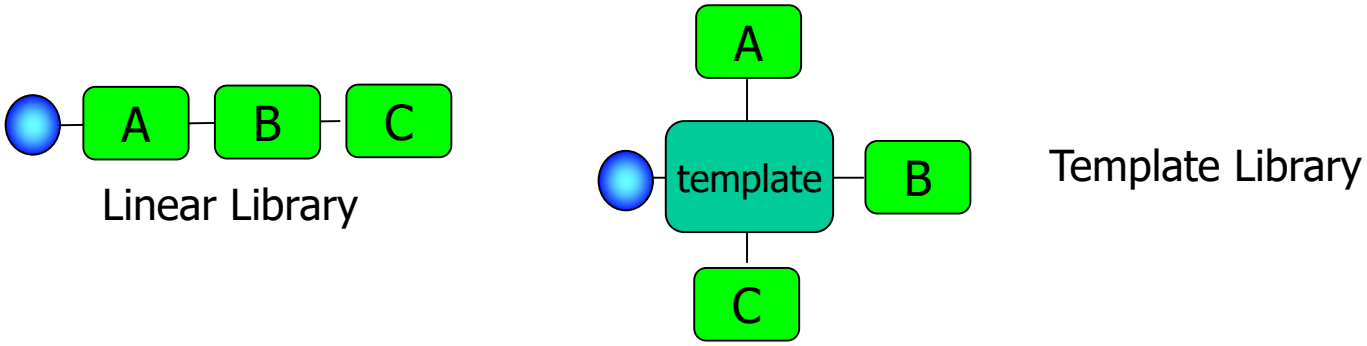
Combinatorial Synthesis Approach.





Combinatorial Synthesis.

- Multiple reactions in one reaction vessel, quickly generating a very large set of diverse products known as a combinatorial library. Usually the reaction is the same, but reactants are different. Number of compounds possible: $b \cdot x$ (x = # steps).
- Design of combinatorial synthesis: sequential (a) or template (b):

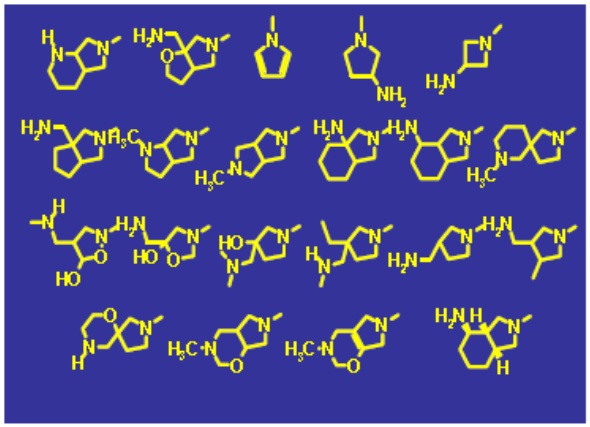


- Synthetic generation of molecular diversity is conceptually and experimentally rooted in solid phase peptide synthesis: Extension of this to organic molecules has only occurred after 1990.

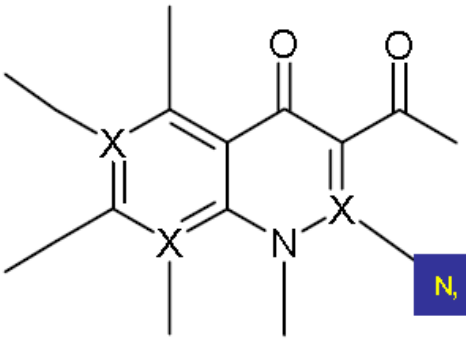
Combinatorial Synthesis.

Sources of Chemical Diversity:

Unlimited!



20

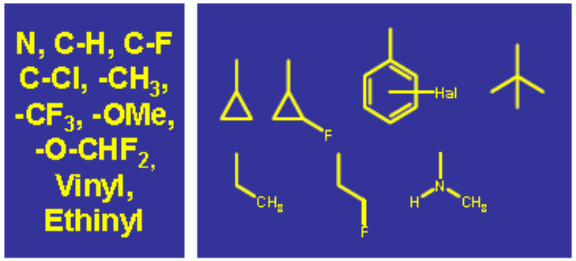


>3

-OH
or
prodrugs
e.g.. Ester

N, CH,

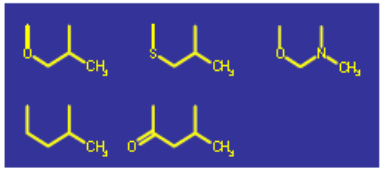
2



7

N, C-H, C-F
C-Cl, -CH_3,
-CF_3, -OMe,
-O-CHF_2,
Vinyl,
Ethinyl

10



5

8

-NH_2, CF_3
F, H,
CH_3, OH,
Vinyl,Ethinyl

No variation
allowed

6

H, F, Cl
-NH_2, -CN,
-SMe

20 x 10 x 7 x 3 x 8 x 6
= 201,600

Mixed Combinatorial Synthesis.

Aims:

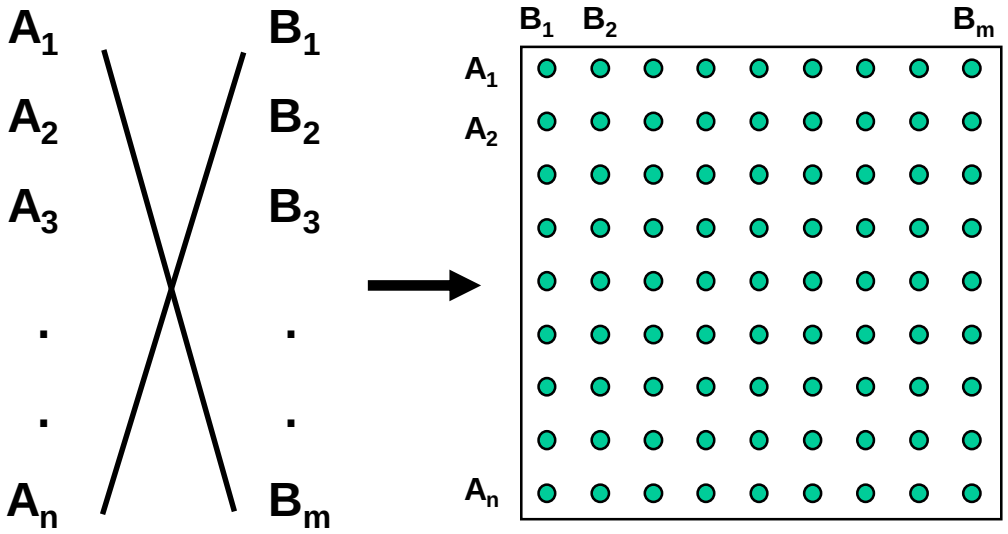
- To use a standard synthetic route to produce a large variety of different analogues where each reaction vessel or tube contains a mixture of products;
- The identities of the structures in each vessel are not known with certainty;
- Useful for finding a lead compound;
- Capable of synthesizing large numbers of compounds quickly;
- Each mixture is tested for activity as the mixture;
- Inactive mixtures are stored in combinatorial libraries;
- Active mixtures are studied further to identify active component.

Combinatorial Chemistry - How does it differ from traditional synthesis?

Traditional Synthesis

- 1 chemist =
  50 compounds/year

Combinatorial Synthesis

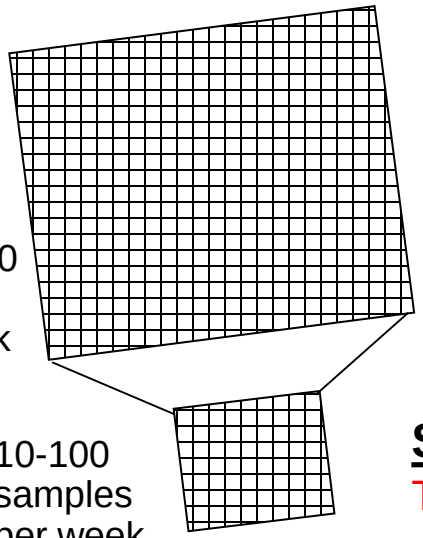


n × m products

- 10^3-10^4 compounds/experiment

Multistage Screening.

High Throughput Screening Using Combinatorial Methods.



First Stage Screen
Test for only one or two properties

Second Stage Screen
Test for other key properties

Conventional Screening Using Established Methods.

1-10
samples
per week

Rapidly Identified Materials
Samples with desired properties

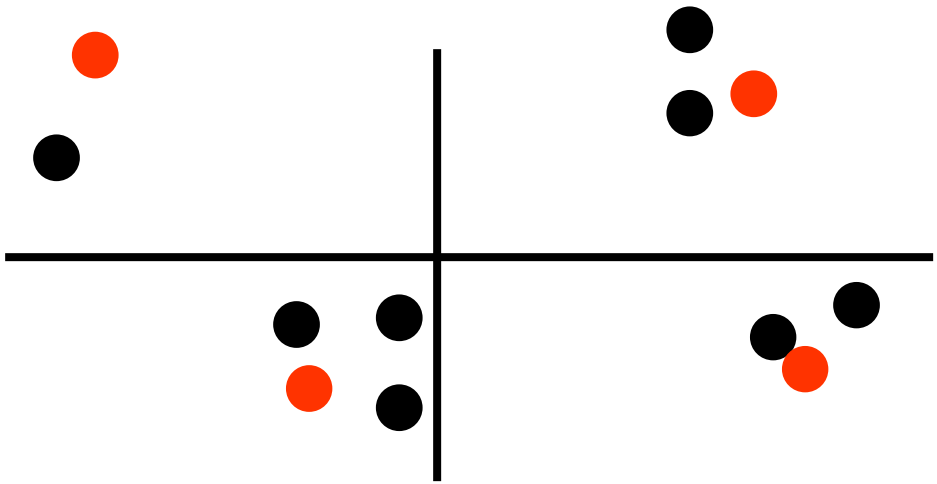
1-2
samples
per month

Candidates for Scale up
Most promising materials are Used in pilot scale experiments

Experimental Strategies: Combinatorial Organic Synthesis.

- Structural descriptors are calculated for each compound
- Similarity coefficients are calculated between compound pairs
- Compounds are selected using multivariate methods (based on clustering, dissimilarities, etc.).

Possible because target is single compound!

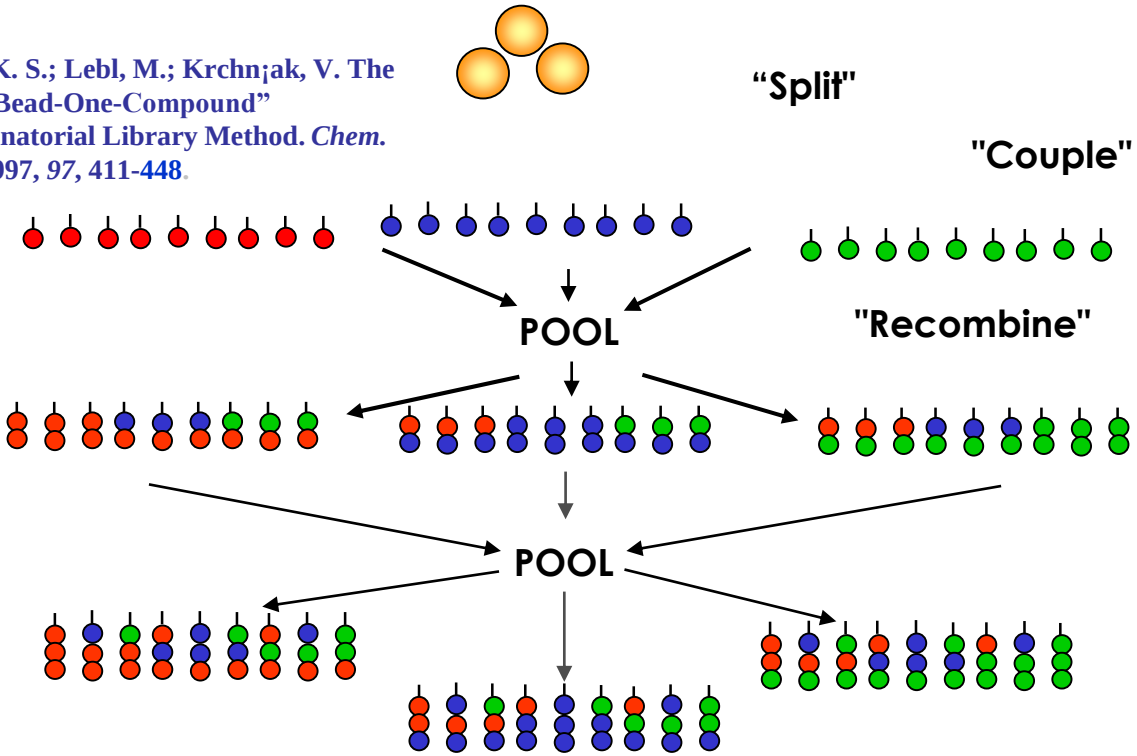


Mixed Combinatorial Synthesis.

The Mix and Split Method Combinatorial procedure involves five separate syntheses using a mix and split strategy.

Example - Synthesis of all possible tripeptides using 3 amino acids (Standard methods would involve 25 separate syntheses).

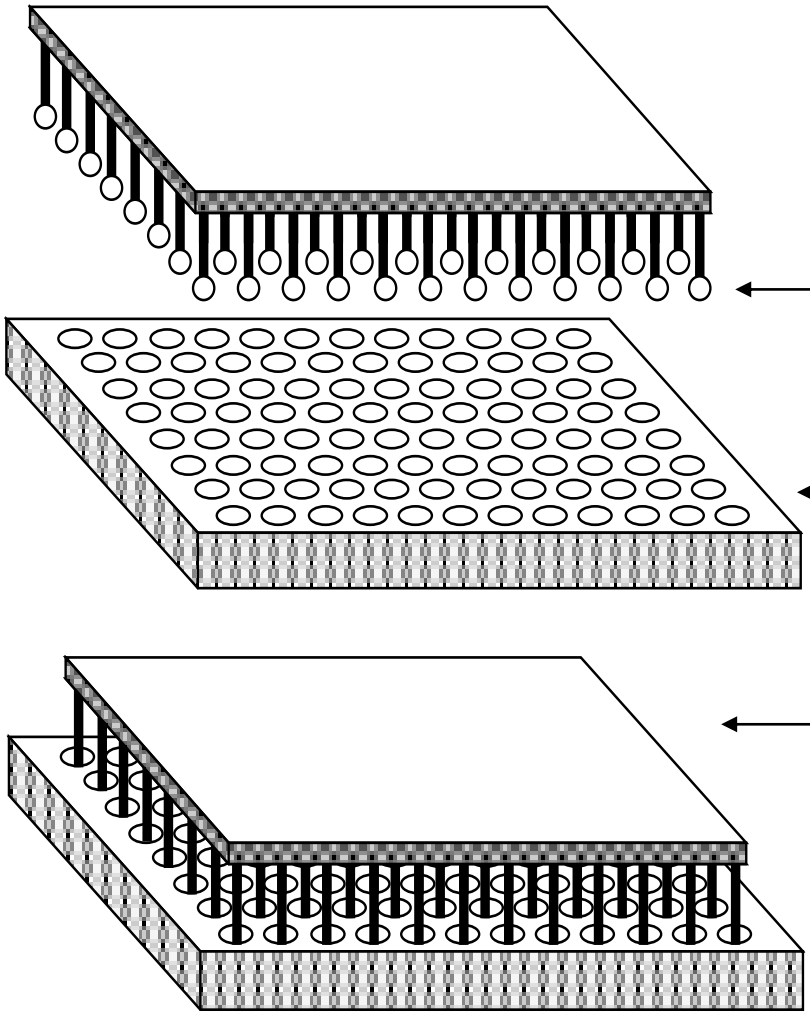
Lam, K. S.; Lebl, M.; Krchn¡ak, V. The "One-Bead-One-Compound" Combinatorial Library Method. *Chem. Rev.* 1997, *97*, 411-448.



27 tripeptides

Number of ligands = N^n where N=number of building blocks & n = number of cycles; When N=100 and n=6: 1,000,000,000,000 possible structures

Geysen Pin Method (for parallel synthesis of peptides).



Resins attached to pins in an 8×12 array format

Reagents or wash solvents in a 96 deep-well plate

Drop it in to run reactions or wash resins

Kits available
www.mimotopes.com

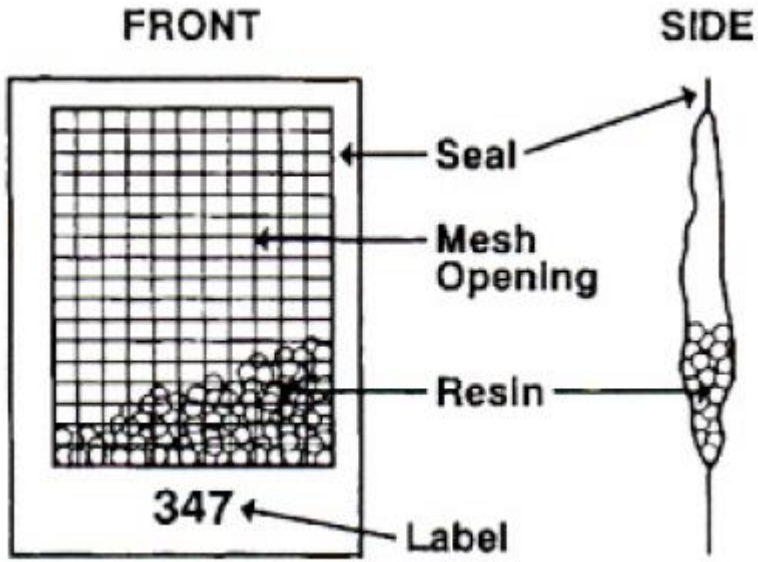
Tea bag technique for parallel synthesis of peptides.

Richard A. Houghten, PNAS USA 1985, 82, 5131-5135

Washing and deprotection:
done together;

Coupling:
Sort the bags in to groups according to the AA to be coupled;

Each group is coupled separately.

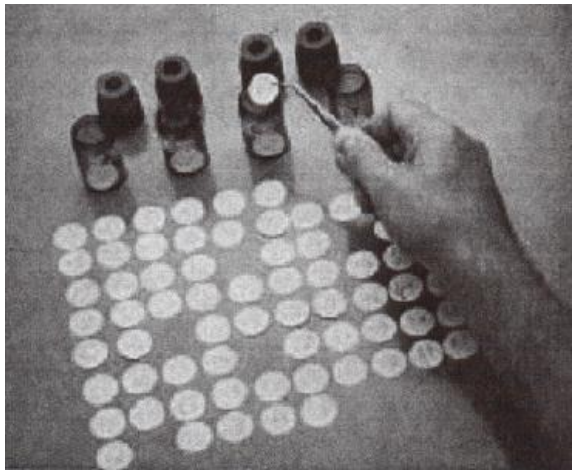


SPOT technique for parallel synthesis of oligonucleotides.

Ronald Frank et al. *Nucl. Acids Res*. 1983, 11, 4365-4377

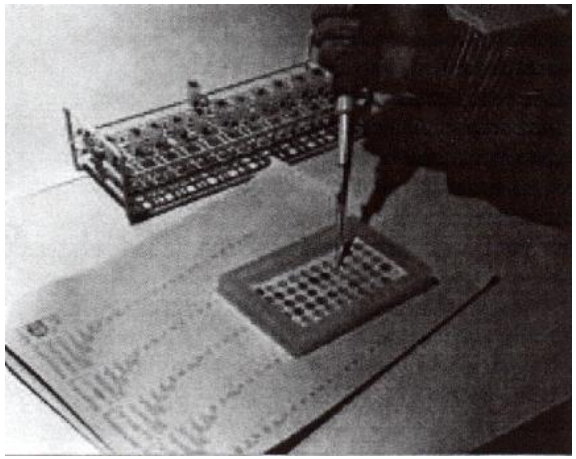
Filter disc method: one oligo per disc
Washing, deprotection, and oxidation:
done together.

Coupling:
Put the discs into four different vials
containing dA, dC, dT, dG building blocks
according to the oligo sequence of each disc.



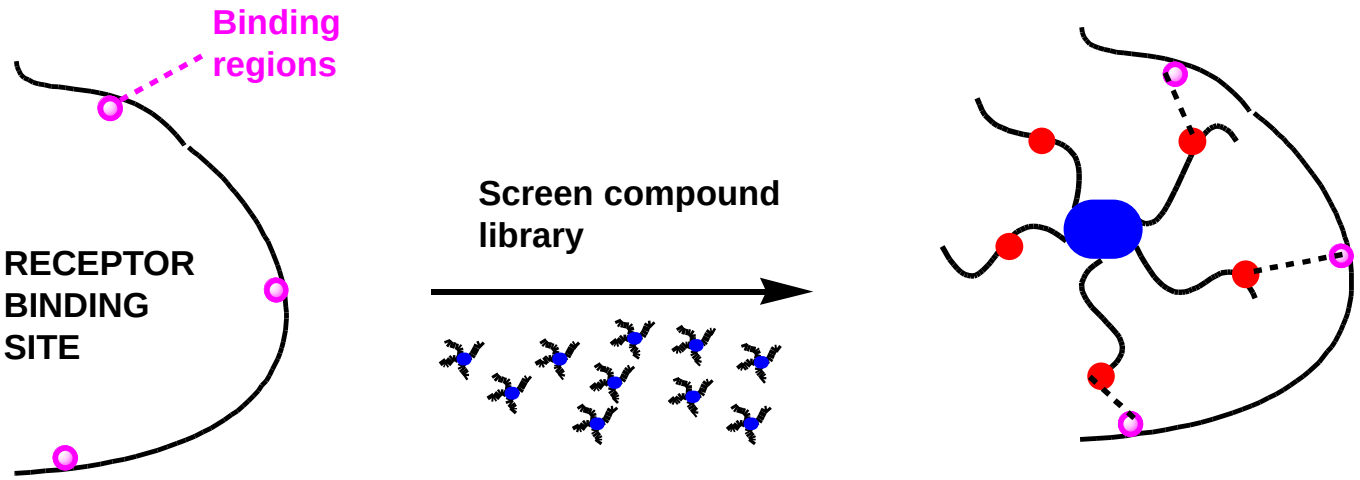
SPOT method: one oligo per disc
Washing, deprotection, and oxidation:
done together.

Coupling:
Pipette coupling solution
containing dA, dC, dT,
dG building blocks onto the discs
according to the sequence.



Planning a Combinatorial Syntheses: Scaffolds.

- ‘Spider’ scaffolds preferable for exploring conformational space;
- Allows variation of functional groups around whole molecule to increase chances of finding suitable binding interactions.

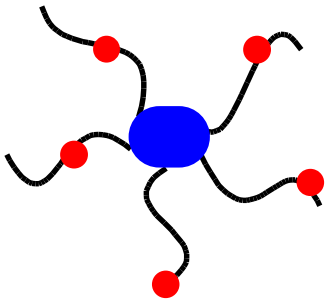


Molecular weight of scaffold should be low to allow variation of functionality, without getting products with a MW > 500.

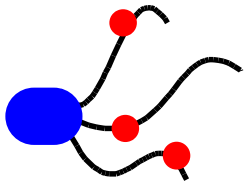
Planning a Combinatorial Syntheses: Scaffolds (2).

Tadpole scaffolds

- variation restricted to a specific region round the molecule;
- less chance of favorable interactions with a binding site.



'Spider' Scaffold with 'dispersed' substituents

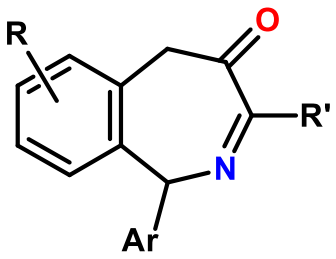


'Tadpole' scaffold with 'restricted' substituents

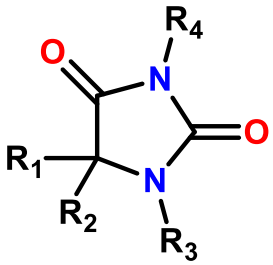
Privileged scaffolds

- scaffolds which are common in medicinal chemistry and which are associated with a diverse range of activities;
- benzodiazepines, hydantoins, benzenesulphonamide, etc.

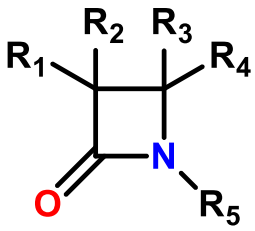
Planning a Combinatorial Syntheses: Examples of Scaffold Libraries.



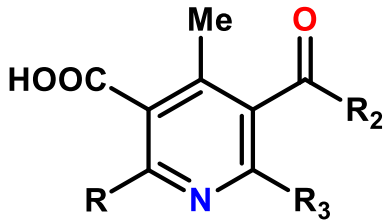
Benzodiazepines



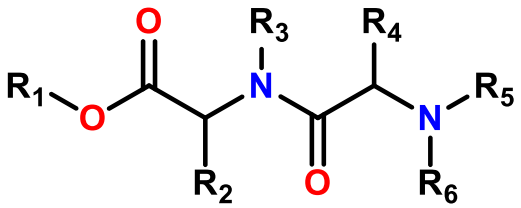
Hydantoins



β-Lactams



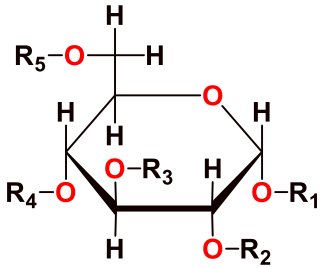
Pyridines



Dipeptides

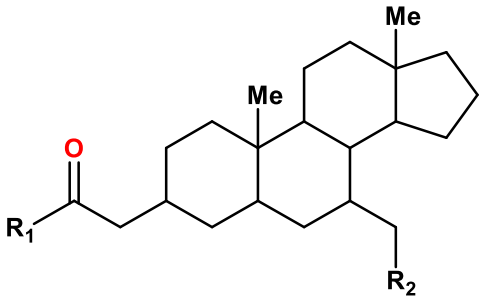
- Good scaffolds
- Spider like
- Low molecular weight
- Variety of synthetic routes available

Planning a Combinatorial Syntheses: Scaffolds - poor examples.



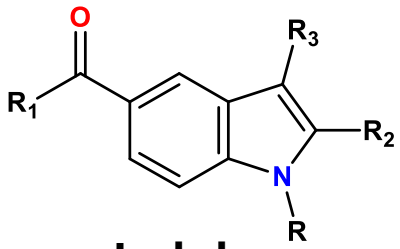
Glucose

Spider like and small molecular weight - good points.
But multiple OH groups.
Difficult to vary R_1-R_5 independently.



Steroid

MW relatively high.
Restricts N° of functional groups to keep MW < 500.
Relatively few positions where substituents easily added.

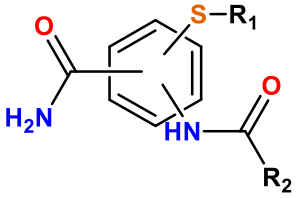
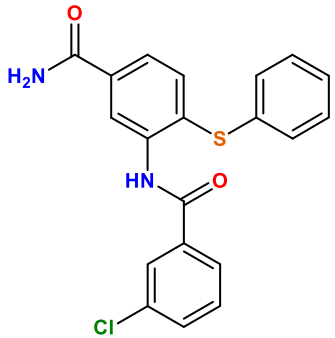
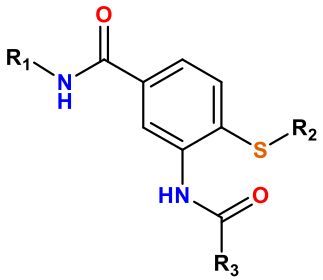
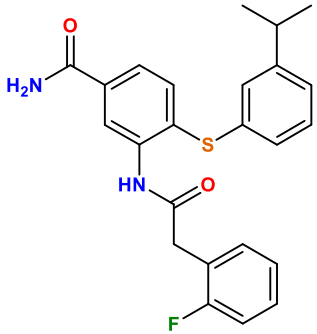


Indole

Tadpole like scaffold.
Restricted region of variability.

Combinatorial Chemistry.

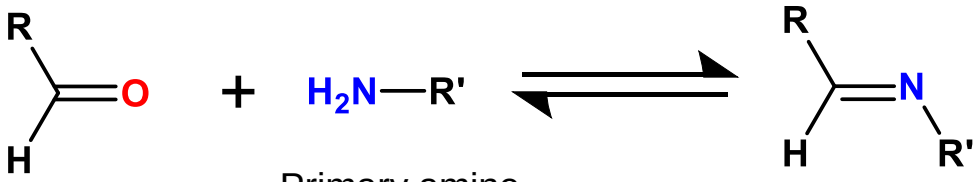
Hit finding → **Lead finding**

Random Library	HTS: First Hit	Targeted Library	Lead Structure
			
5000 compounds	EC_{50} 10 μM	500 compounds	EC_{50} 20 nM

Final Optimization Program

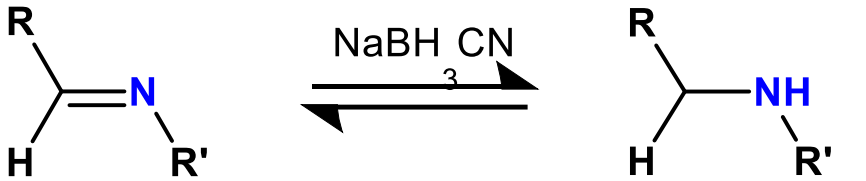
Dynamic Combinatorial Chemistry: Example - Ligands for Carbonic Anhydrase.

- Reaction - reversible formation of imines:



Aldehyde — Primary amine — Imine

- Reaction carried out in presence of carbonic anhydrase
- Three aldehydes and four amines present as building blocks
- Sodium cyanoborohydride added to ‘freeze’ the mixture.

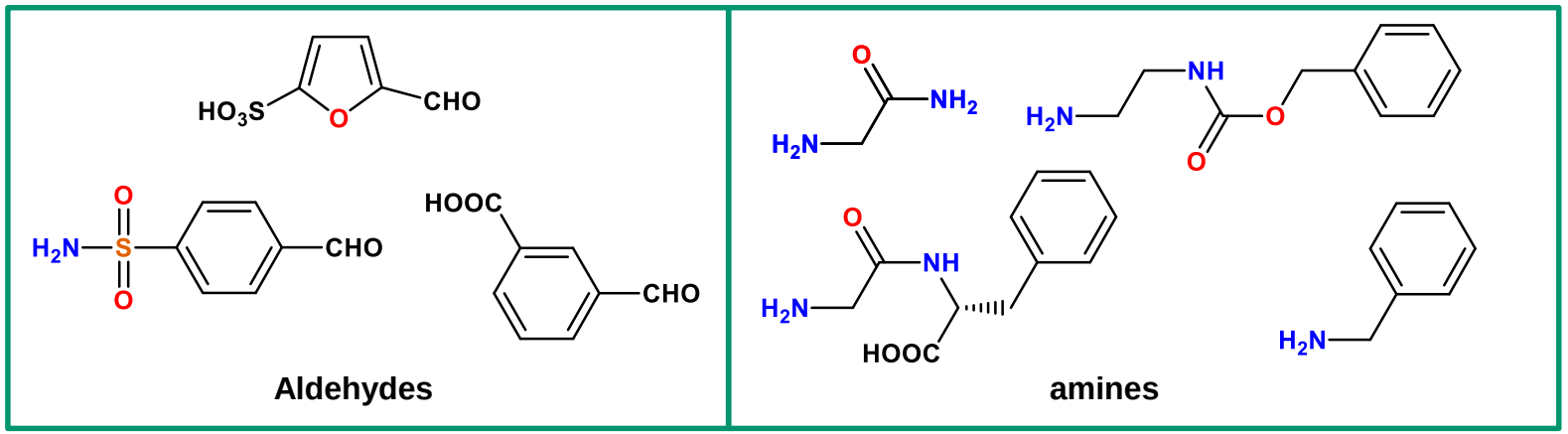


Imine — Secondary amine

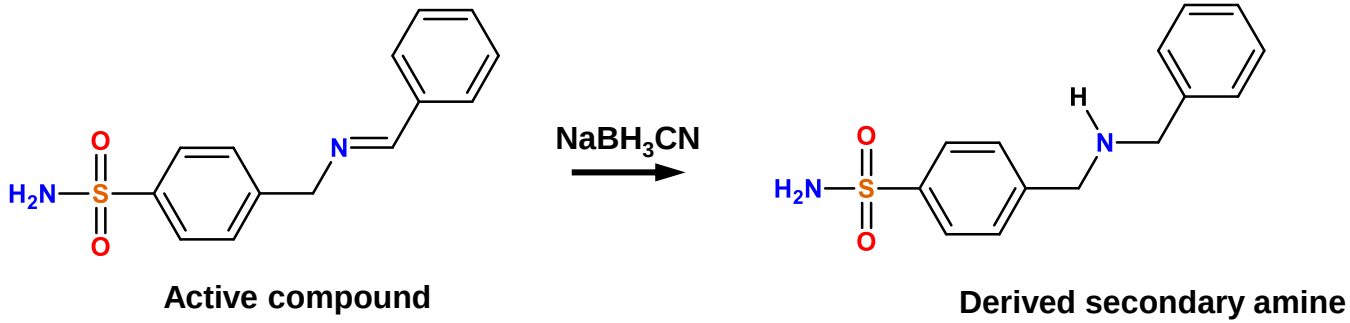
- Products quantified and identified
- Experiment repeated in absence of target to identify amplified product(s)
- Amplified product is not necessarily present in greatest amounts.

Dynamic Combinatorial Chemistry: Example - Ligands for Carbonic Anhydrase.

Building blocks



Amplified product



High Throughput Screening.

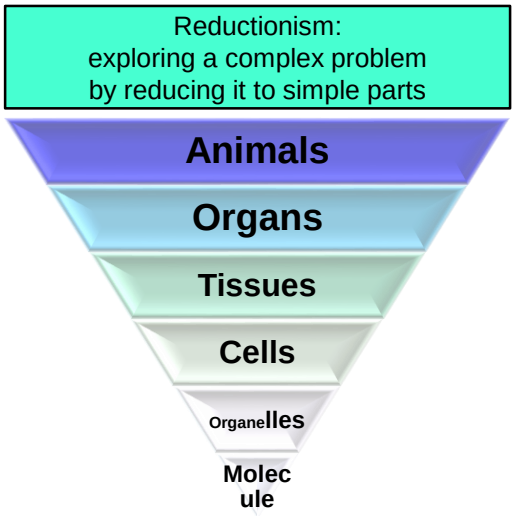
Molecular screening systems

- Measuring parameter: Binding

  receptor ⟷ ligand
  antibody ⟷ antigen
  DNA ⟷ DNA binding protein

- Measuring parameter: Enzymatic function

  Substrate turnover
  Modification of a target molecule



Subcellular screening systems

Binding or enzyme assays using subcellular components such as membrane preparations or cellular fractions

Cellular screening systems

- Measuring parameters: Cellular Events

  Secretion
  Triggering of intracellular ion fluxes
  Generation of signaling molecules
  Stimulation of gene expression

→ Significantly more complex

Combinatorial Chemistry Bibliography.

1. Comprehensive survey of combinatorial library synthesis: 1998, 1999, 2000, And 2001, 2002, 2004, 2005, 2006, J. Comb. Chem.
2. Combinatorial Peptide and Nonpeptide Libraries, A Handbook, Ed. Gunther Jung, 1996, VCH, Weinheim, New York, Basel
3. Combinatorial chemistry : synthesis and application / edited by Stephen R. Wilson, Anthony W. Czarnik. New York ; Chichester : Wiley, 1997.
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