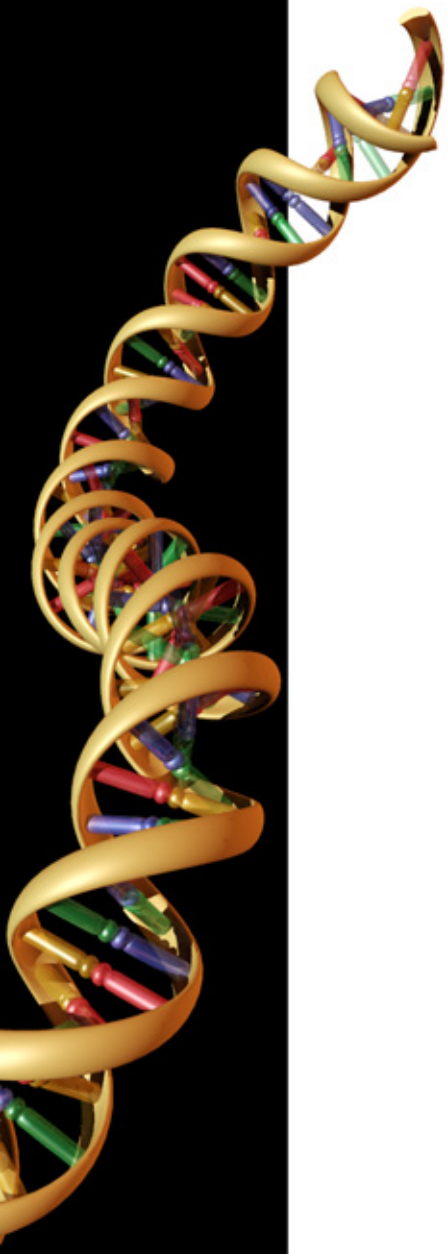
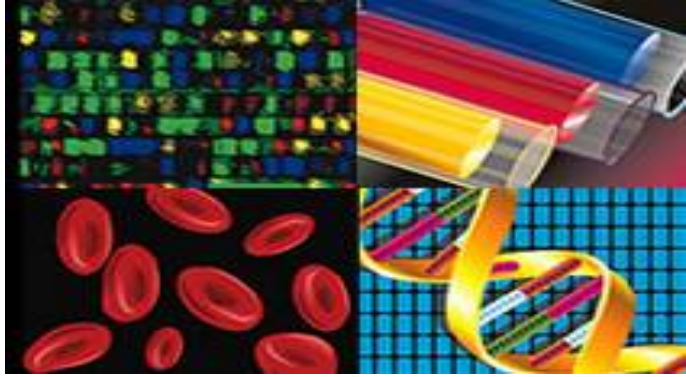


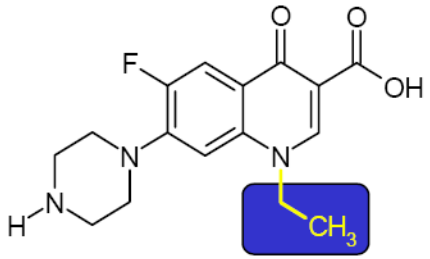


Chemical Library & Combinatorial Chemistry for Drug Discovery

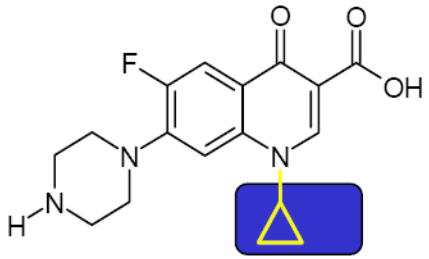

존재가능한
저분자 화합물의 수
10^{60-68}
Drug Space
6×10^{6}
Drug-like
$10^{20\sim40}$

The Game with the Large Numbers

Variation of a Lead Structure: Small Changes with Big Effects



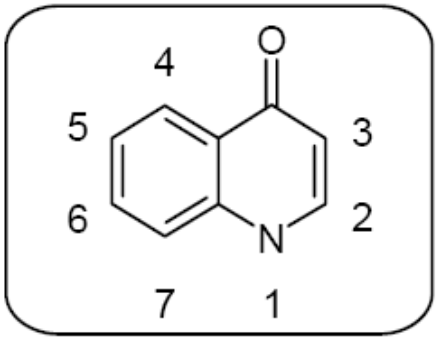
Norfloxacin



Ciprofloxacin

Sales 1997: 200 Mio 1.300 Mio USD

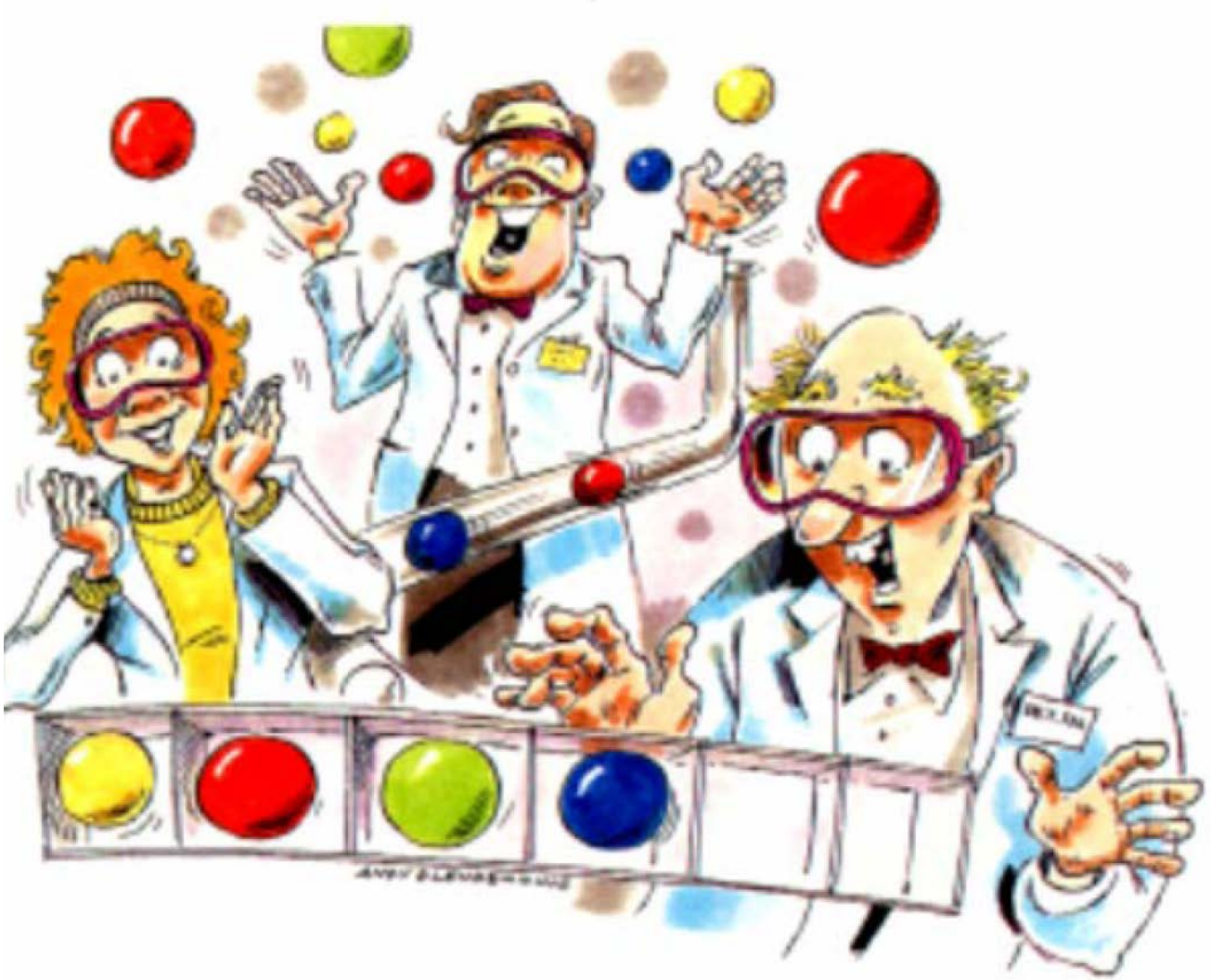
Challenge: how to find the best variation?



15 substituents per 7 positions

170.859.375 compounds

A Lottery for Medicinal Chemist



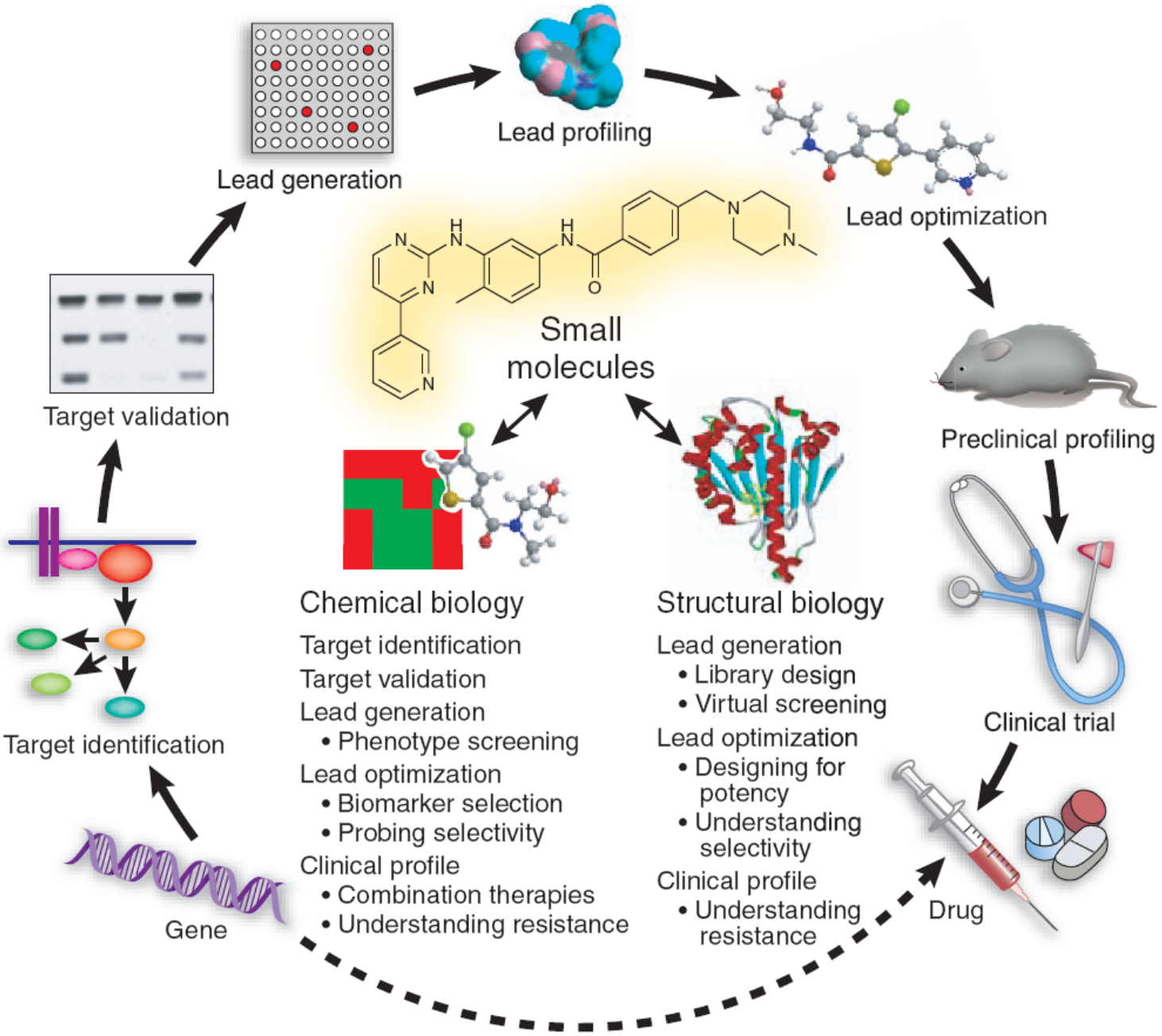
What is Chemical Libraries ?

A *collection of stored small molecules* usually used ultimately in high-throughput screening.

Each chemical has associated information stored in some kind of database with information such as the *chemical structure, purity, quantity, and physiochemical characteristics* of the compound.

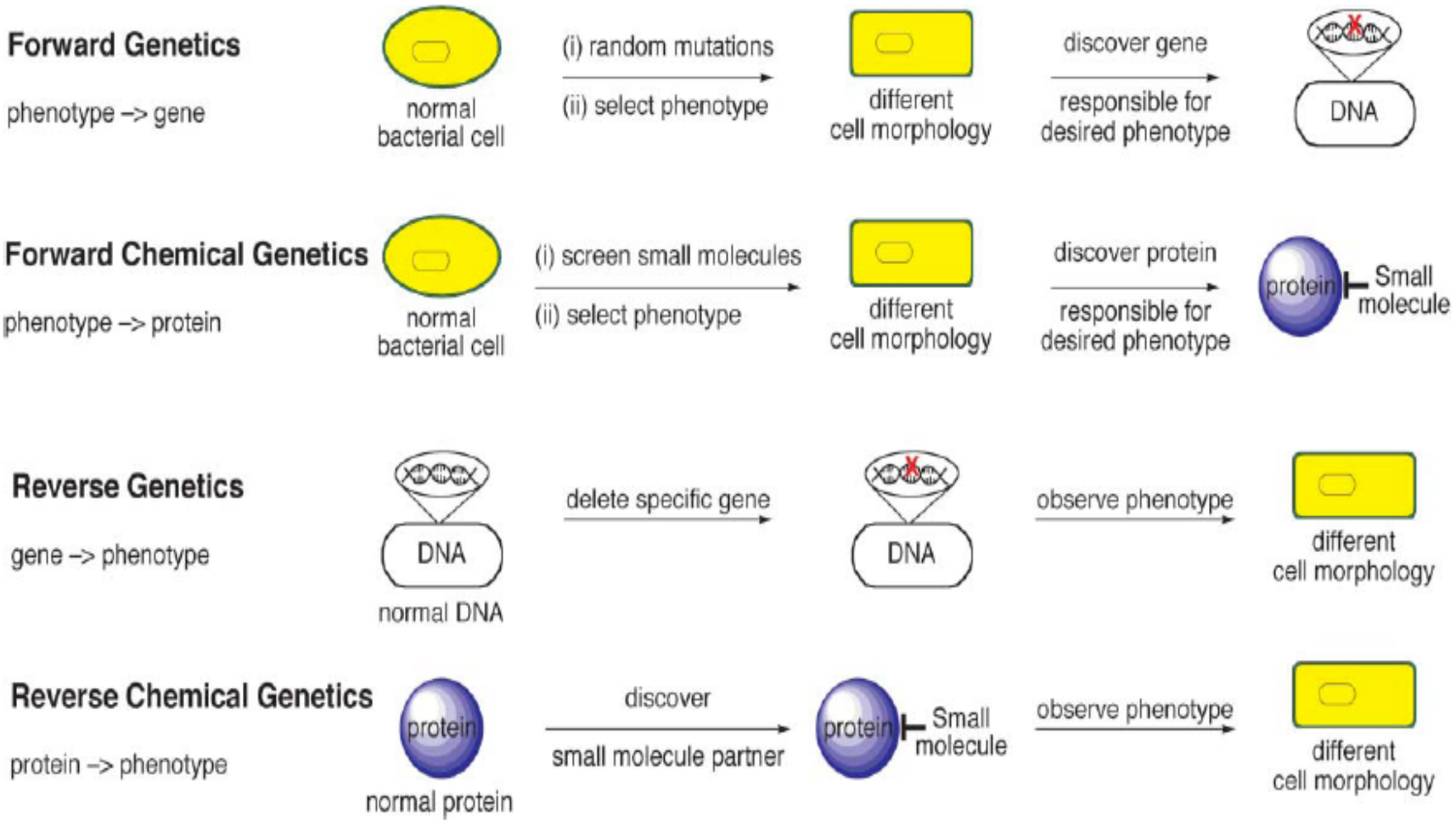
From Wikipedia

Chemical Libraries & Two main approaches in “Lead generation”



What is *Chemical Genetic* ?

The study of biological systems using small molecule intervention, instead of only genetic intervention



Forward Chemical Genetics

- Goal is *target identification*
- A wide variety of small molecules is screened in “black box assays”
- Those that cause a specific phenotype of interest are used to isolate and identify the protein target

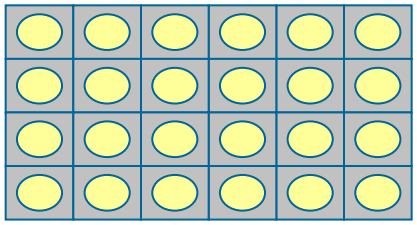
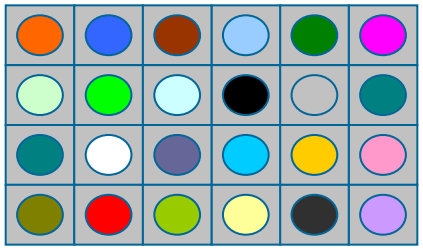


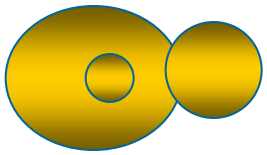
Plate with cells

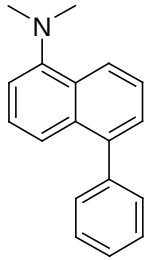
Add one compound per well



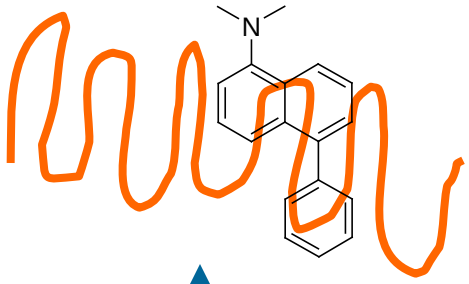
Select compound that produces phenotype of interest

Chemicals as ‘mutagens’



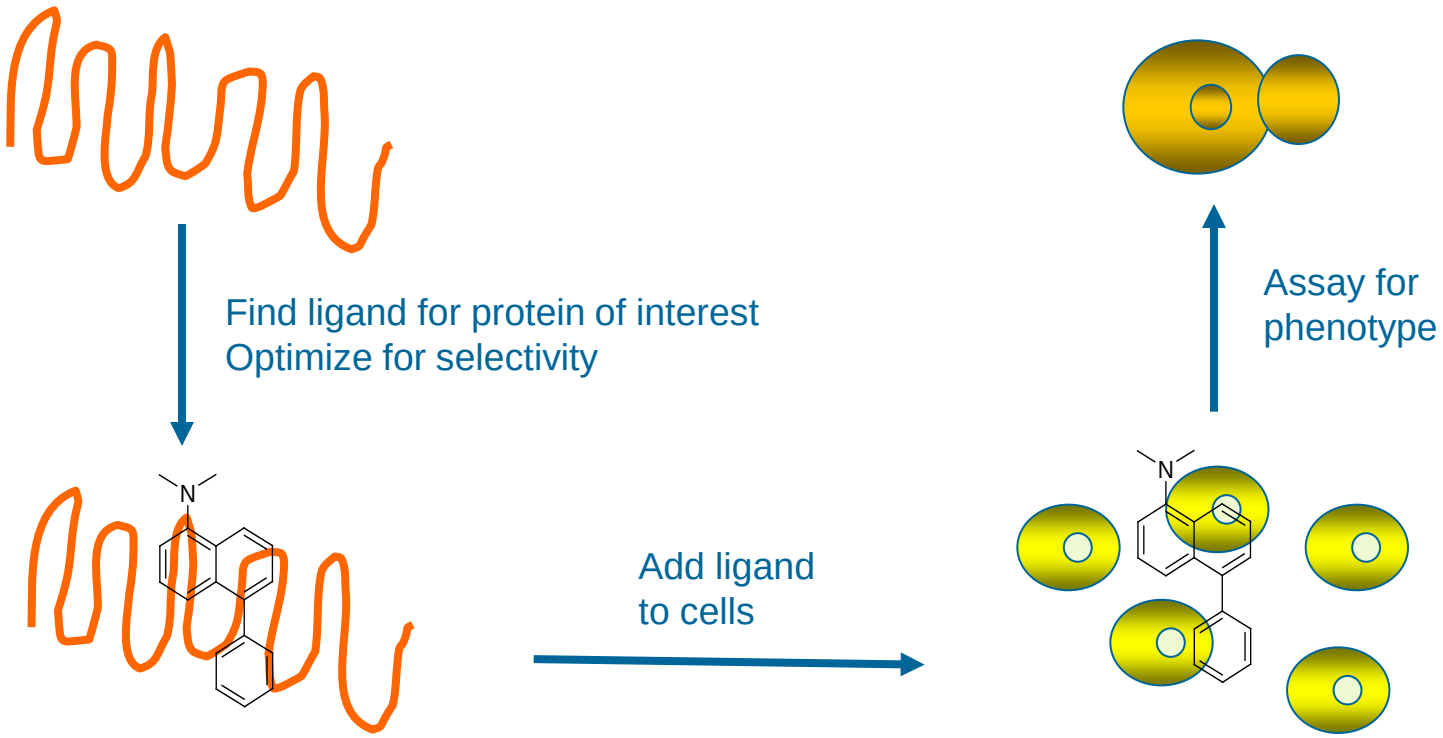


Identify protein Target (deconvolution)



Reverse Chemical Genetics
(Target Validation)

- Goal is *target function and validation*
- Use a known protein of unknown function
- Screen for compounds that bind to the protein
- Optimize for *selectivity* rather than potency



Requirements for Chemical Genetics

1st : A number of small molecules

- Combinatorial Chemistry based on TOS (Target-Oriented Synthesis), or DOS (Diversity-Oriented Synthesis)

2st: Proteins

- For forward chemical genetics, the identification of the small molecule-protein partner is a longstanding challenge

3st: Biological Assays

- To recognize and characterize the small molecule-protein interaction

What is Chemical Libraries ?

An important source in Drug Discovery

From Wikipedia

Research Trends in Chemical Libraries…

<국외 연구동향>

Big Pharma : GSK, Merck, Pfizer, etc…

- 수백만종 이상의 라이브러리 확보

National Institute of Health

- **The Molecular Libraries Program** 실시

: NIH Chemical Genomic Center (NCGC)
Molecular Libraries Screening Center Network
(MLSCN) 구축

- **Molecular Libraries and Imaging roadmap** 구축

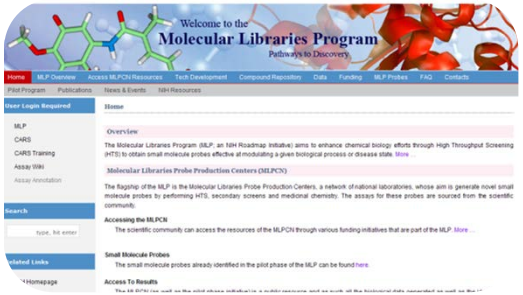
: Molecular Libraries Probe Production Center Network
Molecular Imaging and Contrast Agent Database
Imaging Probe Development Center

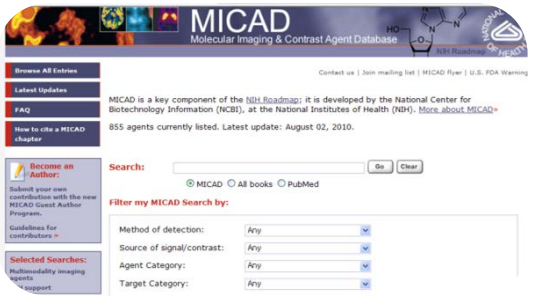
- **PubChem Database** 개발

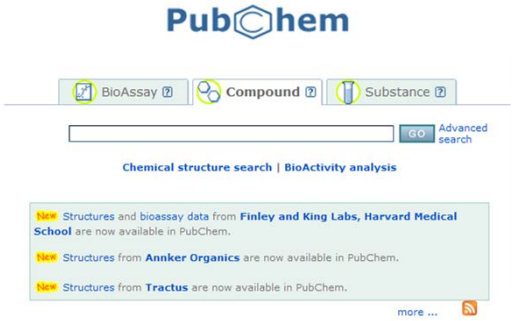
: NCGC, MLSCN, MPLCN, MICAD, IPDC등의 정보 저장

Havard University : ChemBank

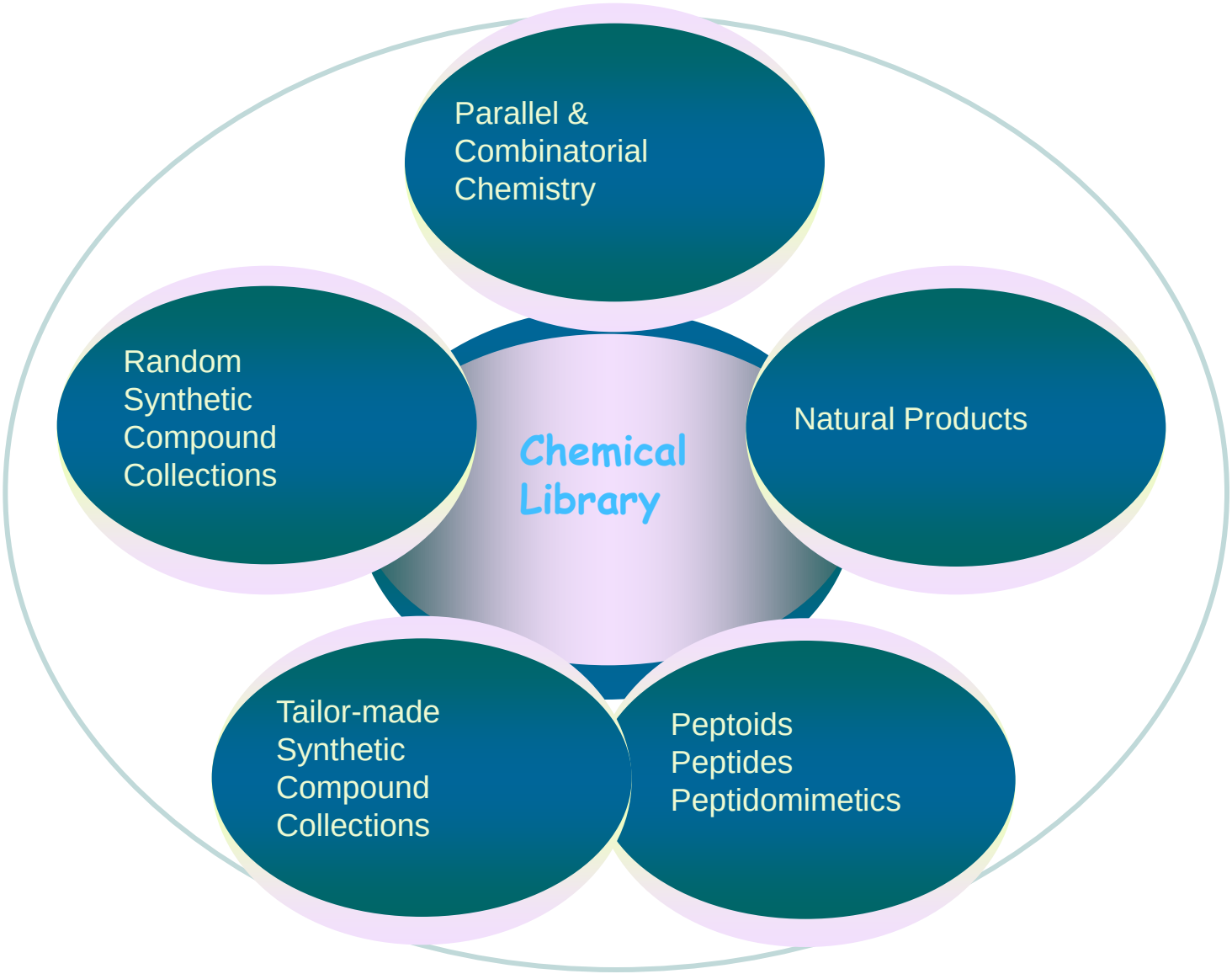
: Structure DB, Biological Assay Data, Data Download
PubChem 통해 정보제공







Sources for Chemical Library



Types of Library -01

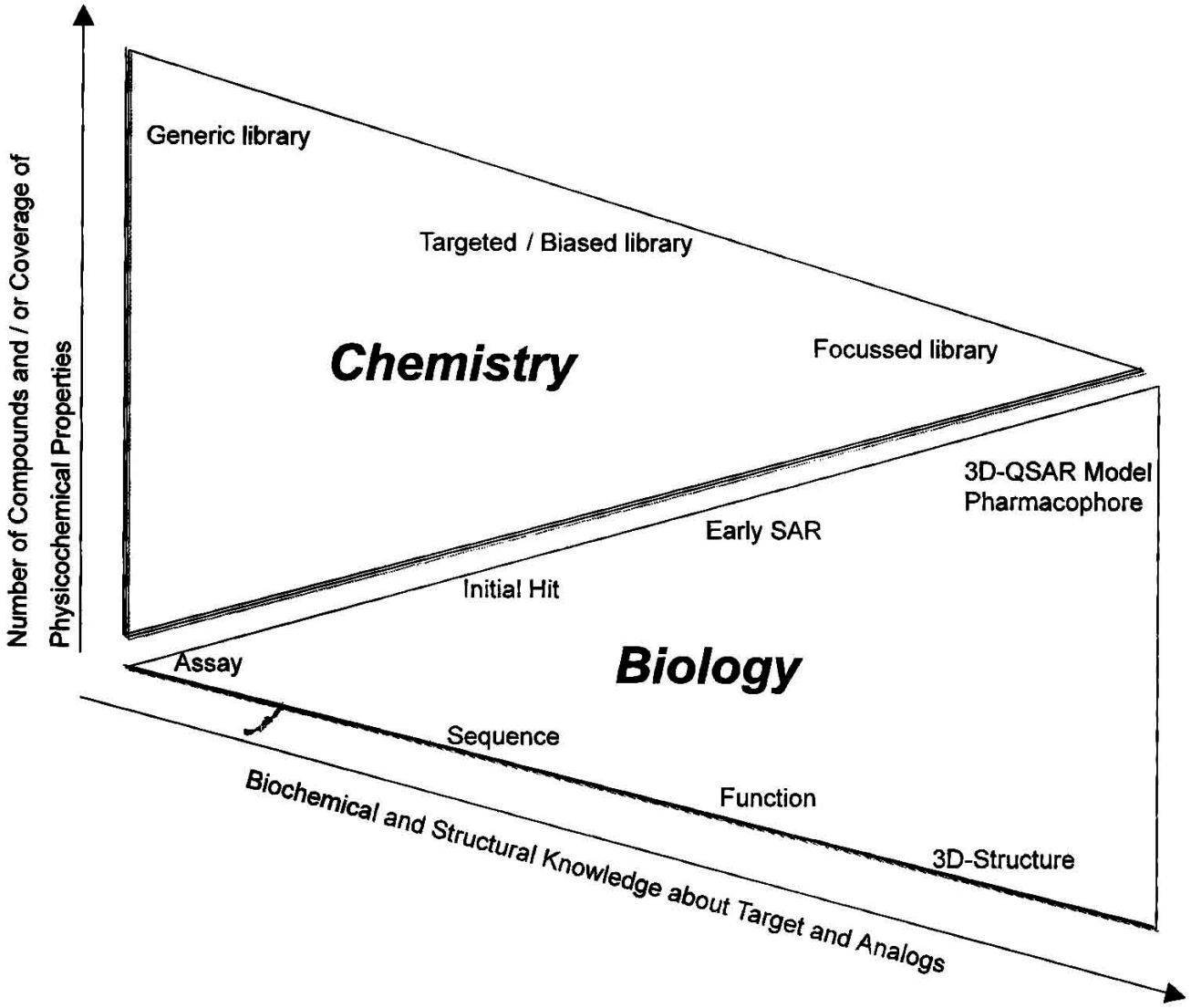


Figure 13.1. Library design depending on knowledge about biological targets and analogues.

Types of Library -02

Random Libraries

- multiple libraries
- many targets
- highly diverse
- mixtures
- > 5.000 compounds
- solid phase synthesis
- non-purified compounds
- on bead screening, if possible

Focused or targeted Libraries

- Template- scaffold library
- one target
- high structural similarity
- single compounds
- << 5,000 compounds
- synthesis in solution, solid phase
- pure compounds
- screening in solution

Types of Library Synthesis

- **Combinatorial Synthesis**
  The Game with the Large Numbers
- **Diversity Oriented Synthesis**
- **Biology Oriented Synthesis**

Combinatorial Synthesis

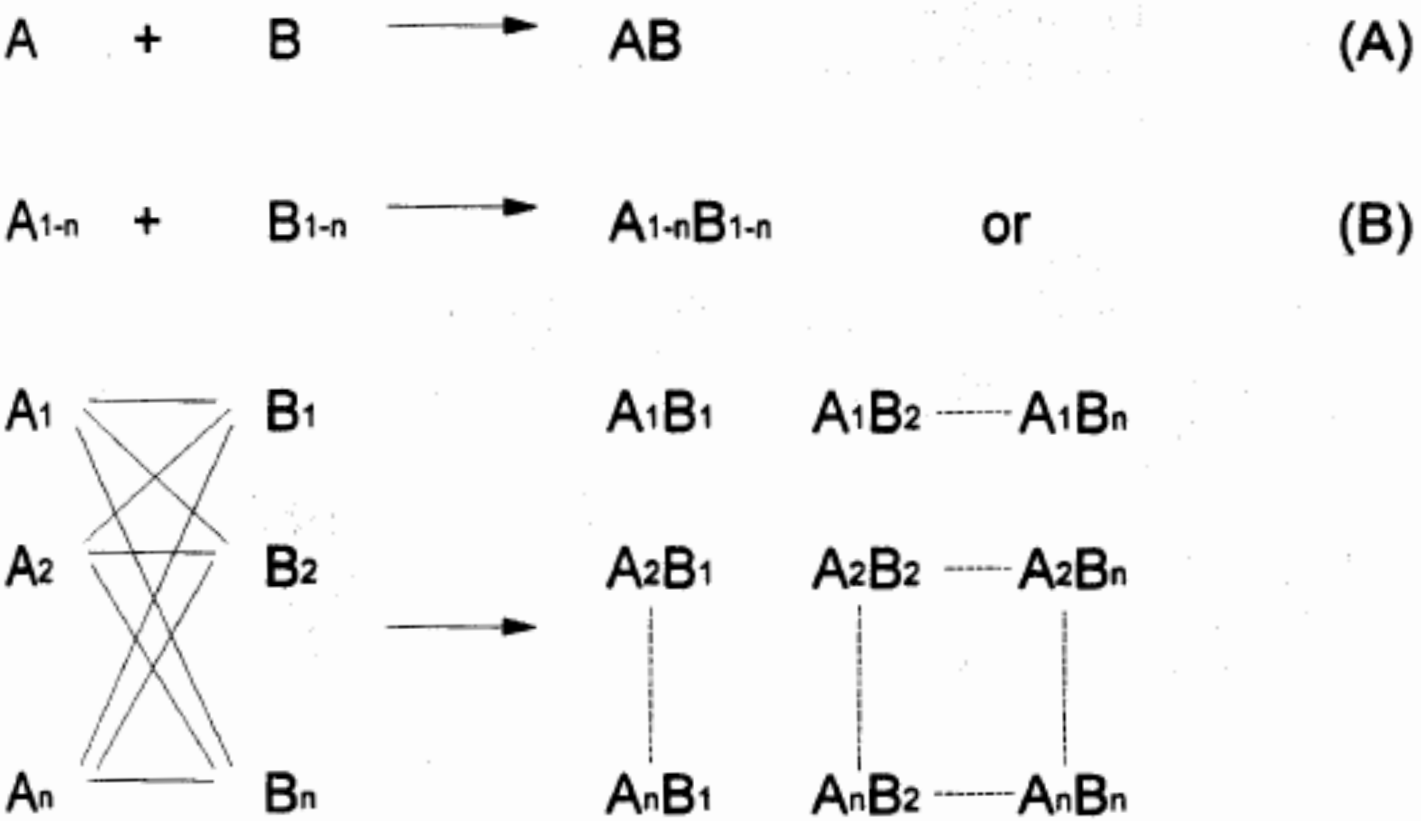


Figure 1.2. (A) In general, in a conventional synthesis one starting material A reacts with one reagent B resulting in one product AB. (B) In a combinatorial synthesis different building blocks of type A (A1–An) are treated simultaneously with different building blocks of type B (B1–Bn) according to combinatorial principles, i.e. each starting material A reacts separately with all reagents B resulting in a combinatorial library A1–nB1–n.

Principles of Combinatorial Chemistry

- **The basic principle of combinatorial chemistry is *to prepare a large number of different compounds at the same time***
- **Instead of synthesizing compounds in a conventional one-at-a-time manner.**

- **The characteristic of combinatorial synthesis is that different compounds are *generated simultaneously under identical reaction conditions in a systematic manner,* so that ideally the products of all possible combinations of a given set of starting materials (termed building blocks) will be obtained at once**
- **The collection of these finally synthesized compounds is referred to as *a combinatorial library.***

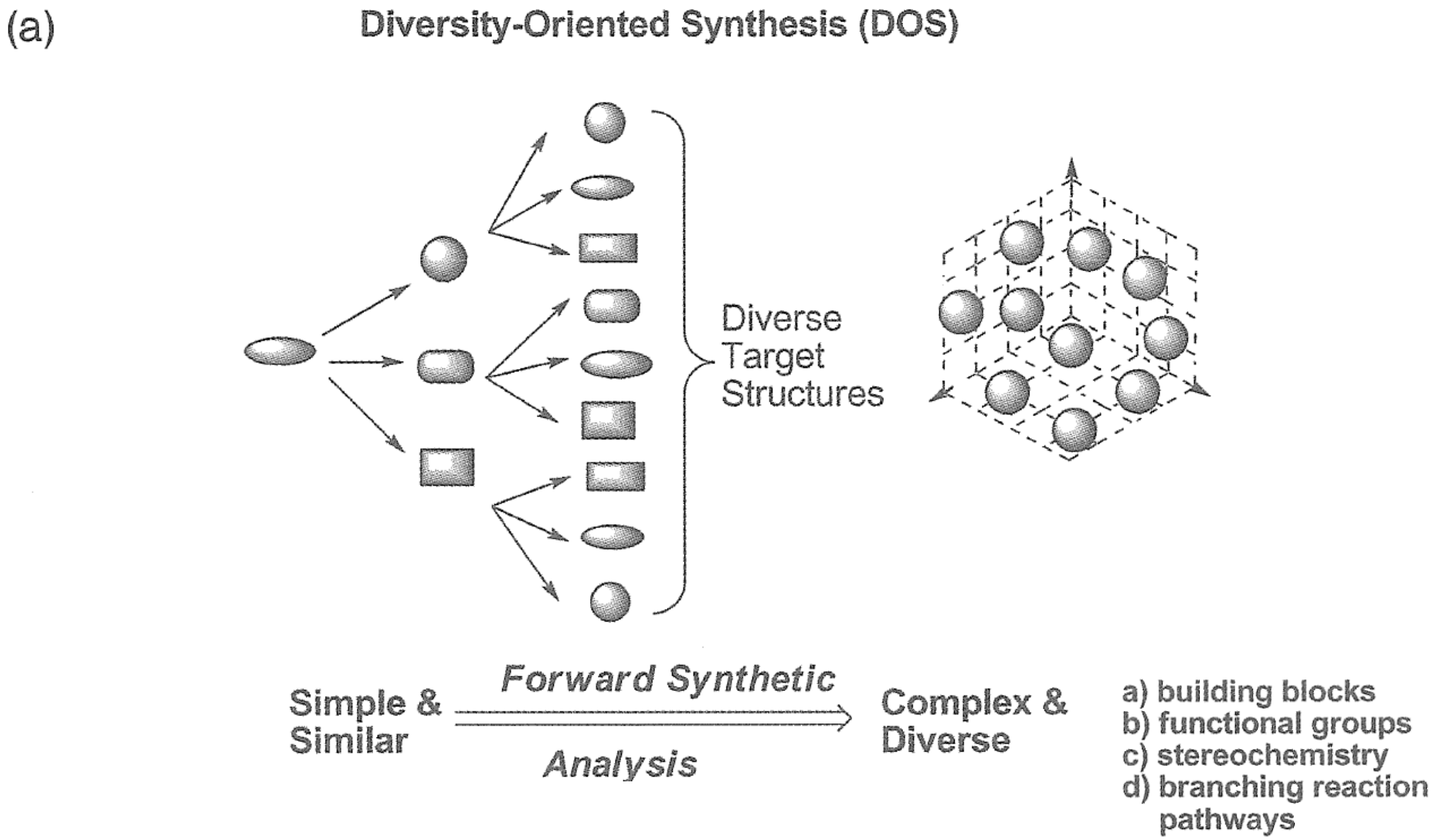
Types of Library Synthesis

- ***Diversity Oriented Synthesis***

Aims of Diversity Oriented Synthesis

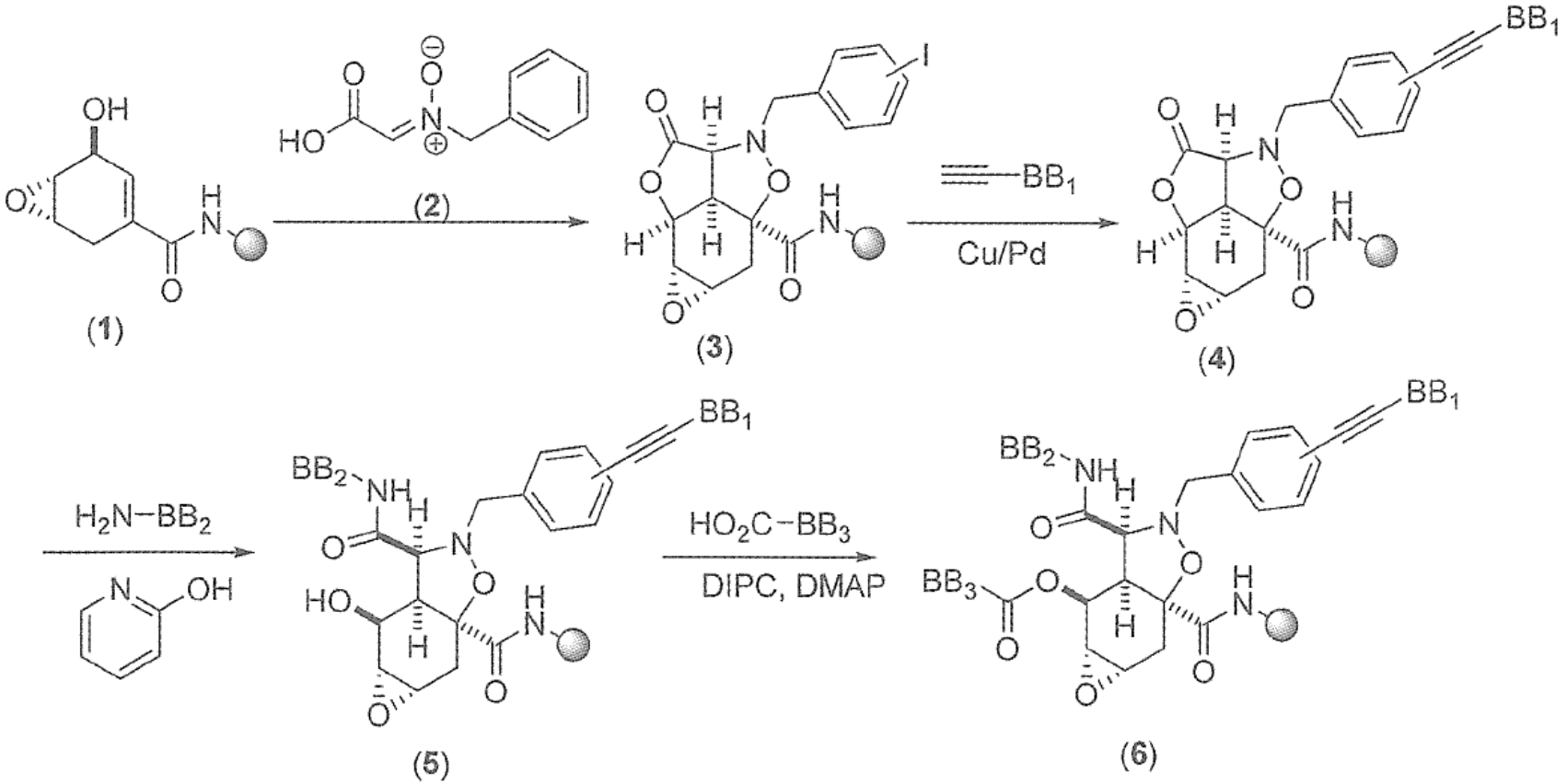
Diversity oriented synthesis aims at building structurally complex, diverse architectures in a high-throughput manner [5]. This strategy emerged because of the growing need to access diverse and often natural product-like skeletons that could be further utilized in library generation. Unlike combinatorial synthetic approaches that were often focused on the generation of aromatic and heterocyclic compound libraries, the establishment of three-dimensional structural complexity by exploring stereo- and enantio-selective reactions on a solid phase is one of the main characteristics of DOS (Box 3.1). The libraries generated by DOS are meant to provide small-molecule chemical probes for the study of cellular processes and are not biased by a given biological target.

• Concept of Diversity Oriented Synthesis

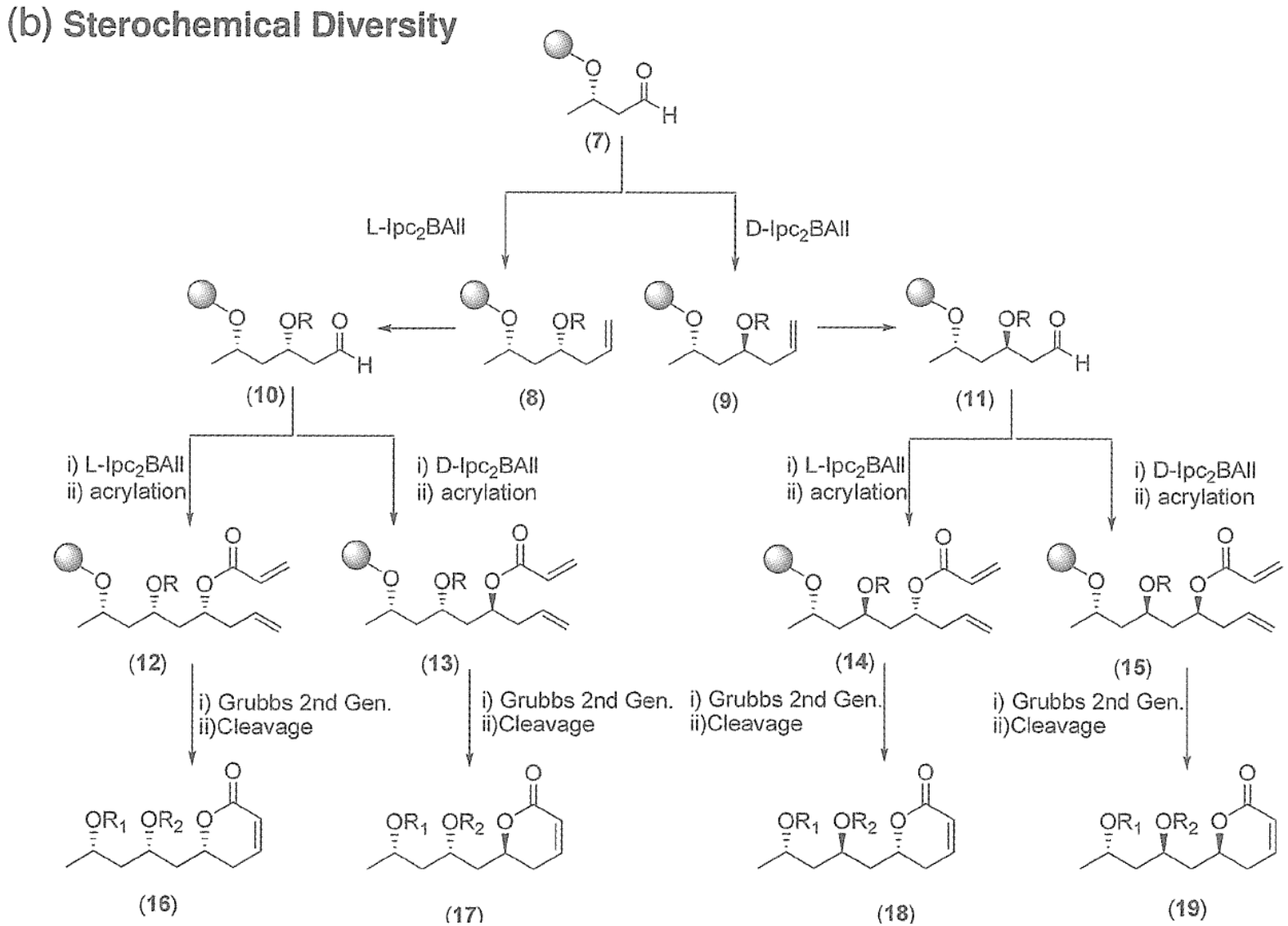


- **Diversity Oriented Synthesis**

(a) **Appending or Building Block Diversity**

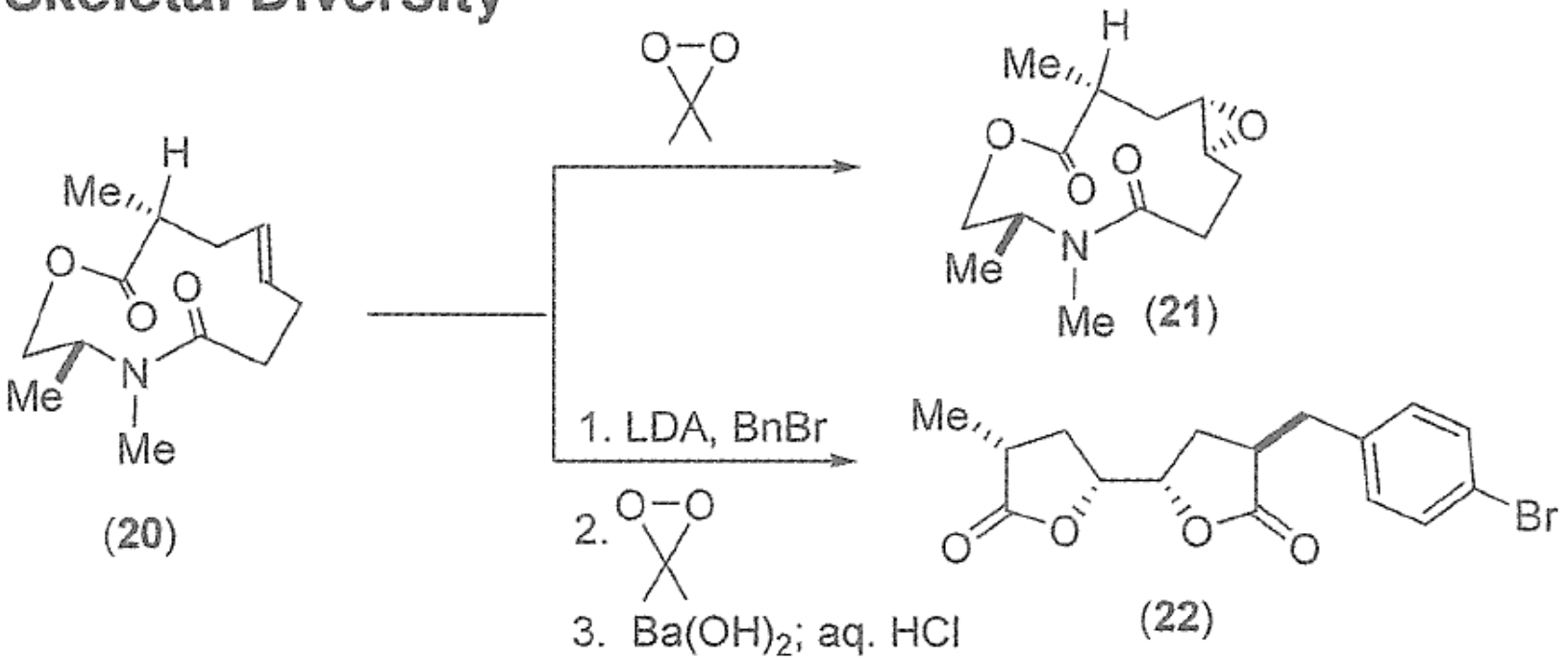


- **Diversity Oriented Synthesis**

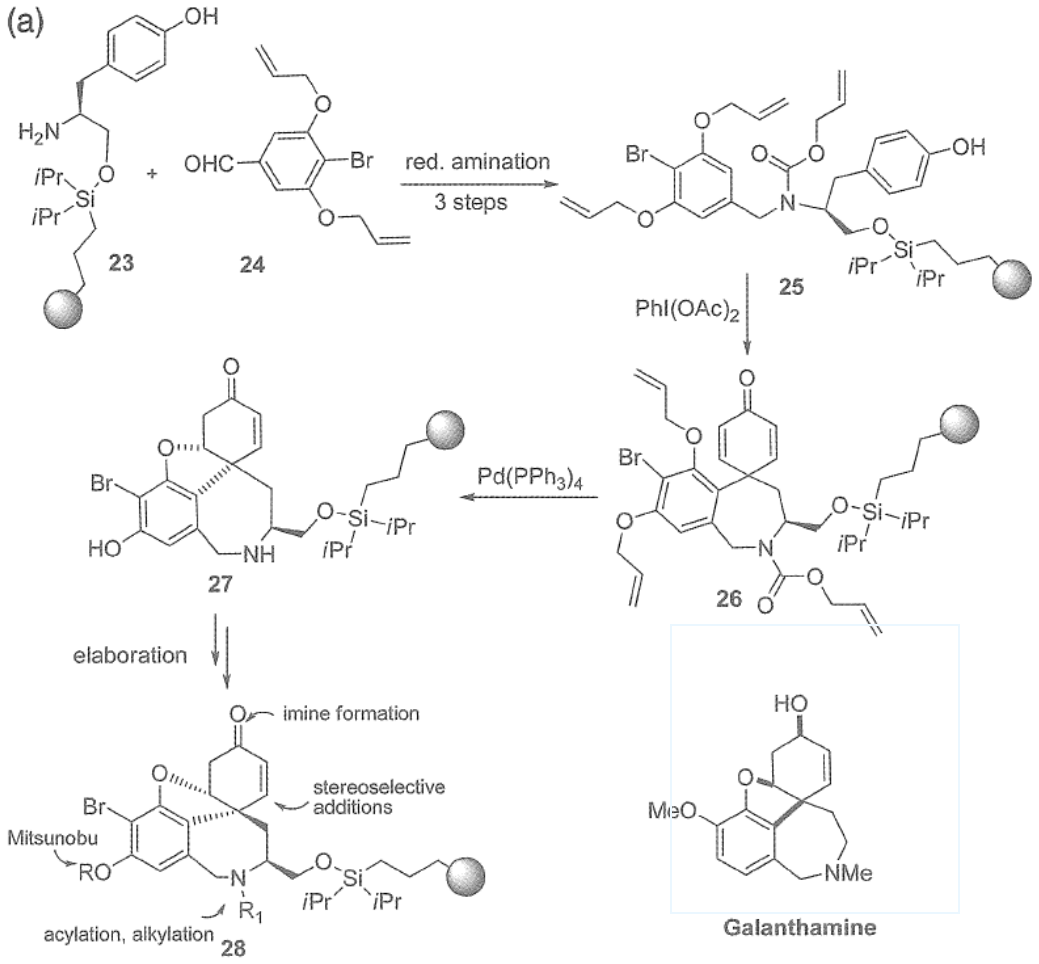


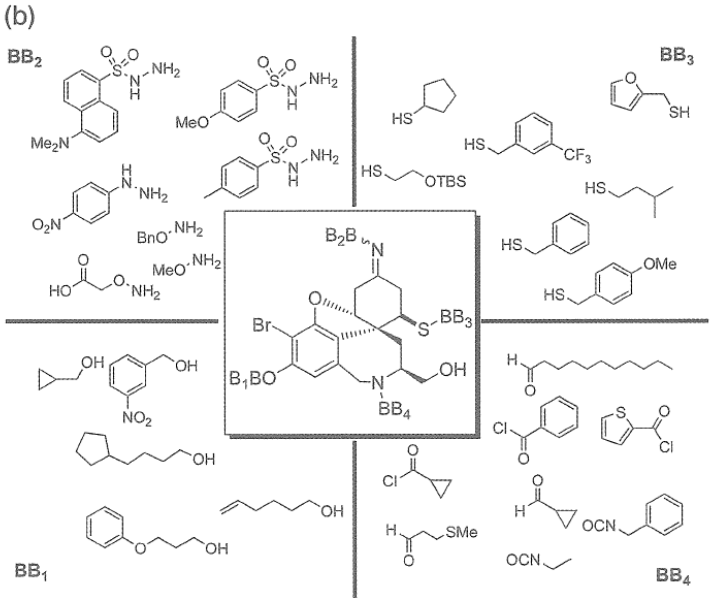
- **Diversity Oriented Synthesis**

(c) Skeletal Diversity



- **DOS Based Library Synthesis and Evaluation**





- **DOS Based Library Synthesis and Evaluation**

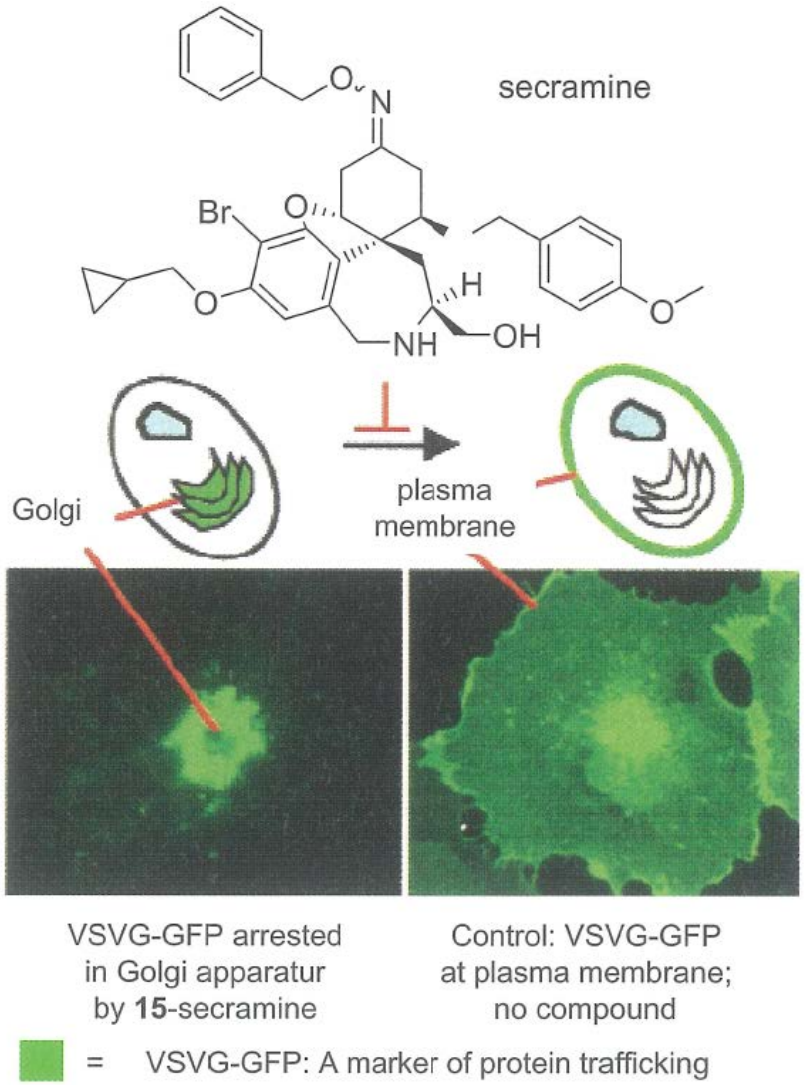


Figure 3.3 Discovery of Secramine, a galanthamine-like molecule that perturbs protein trafficking. (Reprinted

Considerations in DOS-Library Design

1. **The reaction evaluation (a new synthetic route & innovative scaffold).**

- A new scaffold must be synthetically accessible thru a novel, elegant route
- It must be evaluated that building blocks are compatible with particular synthetic scheme.

2. **The library uniqueness evaluation**

- Every new compound is unique
- There is *a higher probability* of finding a given biological activity in clusters of *similar molecules where activity has been already detected* than dissimilar ones.

3. **The library diversity evaluation**

- To design a generic library for lead finding, the consideration of two very similar compounds does not enhance the ability to find different types of biological activity.
- This concept is the key motivating factor for the design of optimally diverse compound libraries (*J. Biomol. Screen*. 1996, *23*, 3-25.)
- The library itself must exhibit a wide coverage of the physico-chemical property space.
- Consider diversity, representativity, complementarity.

4. **The reactant/product diversity evaluation**

- Use diversity-based selection on reactants
- The most diverse library will be obtained by a pure diversity selection on the full product matrix.
- Sometimes it is not very practical, as the combinatorial scheme is violated.

Types of Library Synthesis

- **Biology Oriented Synthesis (BIOS)**

Aims of Biology Oriented Synthesis

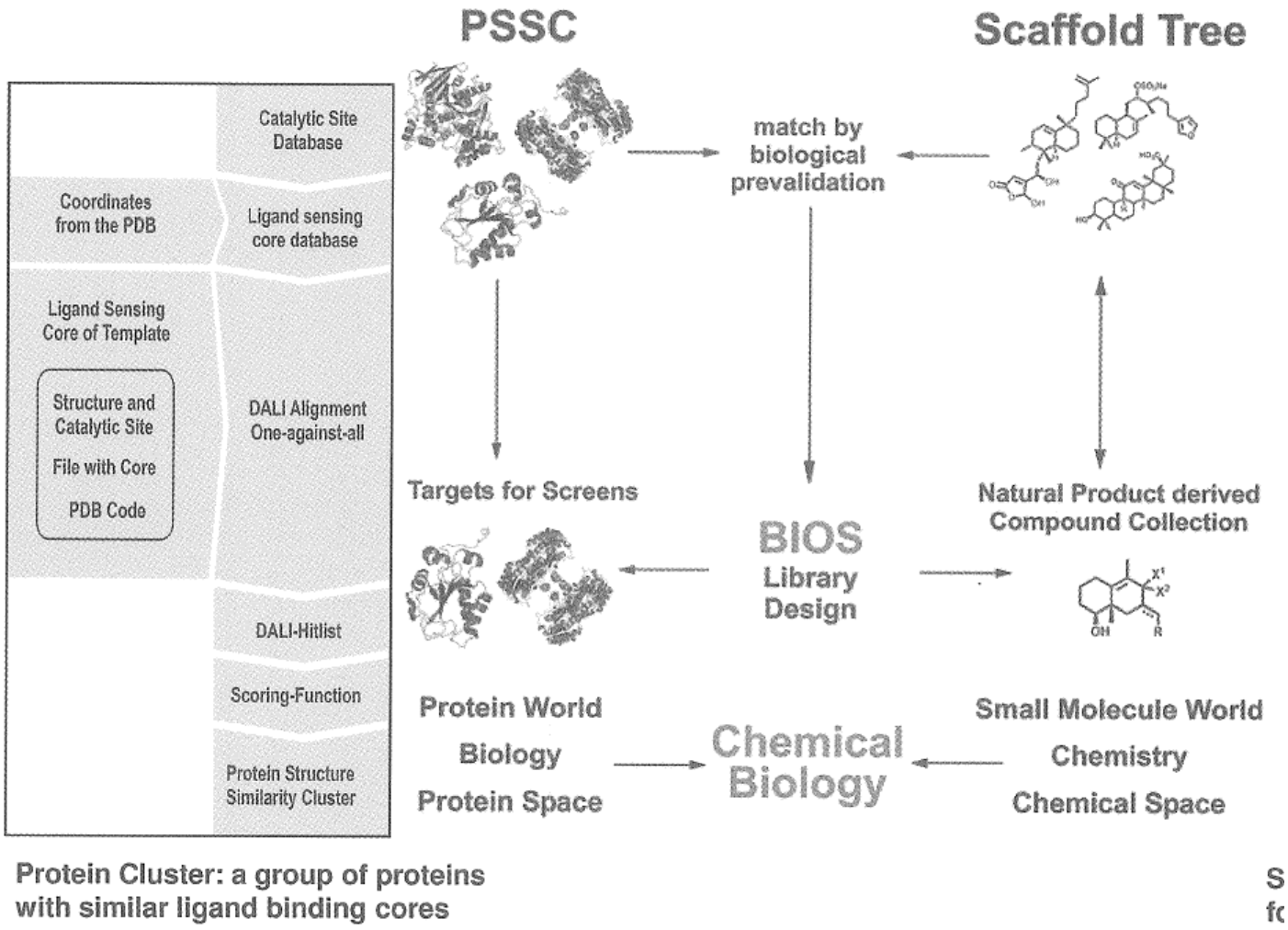
BIOS takes the structures of natural products, and often other small molecules with proven biological relevance, as the basis for design and hypothesis generation. The frameworks of inherently biologically relevant molecules are characterized by an extra element of quality and relevance of the libraries to be synthesized. The concept of BIOS on the one hand originated from the insight that, during the evolution of proteins, only a small fraction of all possible amino acid combinations that could have been probed by biosynthesis [13] was actually explored by nature. The three-dimensional folds of proteins (which typically are the targets for natural products and other bioactive molecules) have been shown to be even more conserved during evolution than their underlying sequence since similar three-dimensional structures can be formed by different amino acid sequences [14]. On the other hand, this structural conservatism in the protein world is paralleled by a relatively small number of basic natural products classes. These complementary elements of conservatism in bioactive small molecules (natural products and other bioactive molecules) and their targets, that is proteins, were merged [15] into a new concept for library synthesis, termed BIOS (Box 3.3).

The Similarity Principle

- 'Similarity Principle' Formulated by Johnson & Maggiora in 1990. (Maggiora, G. M.; Johnson, M.A. *Concepts and Applications of Molecular Similarity*, Wiley, New York, 1990, pp99-117.)
- Structurally similar molecules are assumed to have similar physico-chemical & biological properties.
- This principle leads to the design & evaluation of compound libraries spanning a wide range of chemical & biological properties and to the prediction of target properties for new molecules using known values for similar compounds.
- The use of very similar molecules for primary screening does not enhance the probability to find different types of biological activities, while using dissimilar molecules should enhance the probability for finding interesting leads on different targets.
- Similarity radius: compounds within this radius of another molecule were shown to have comparable biological properties.
- Taking a large variety of compounds within an initial virtual library, an optimal procedure would select only dissimilar compounds outside this similarity radius, leading to a more diverse subset, which increases the probability of finding lead.

- **Concept of Biology Oriented Synthesis (BIOS)**

Key hypothesis = Similar proteins bind Similar ligands

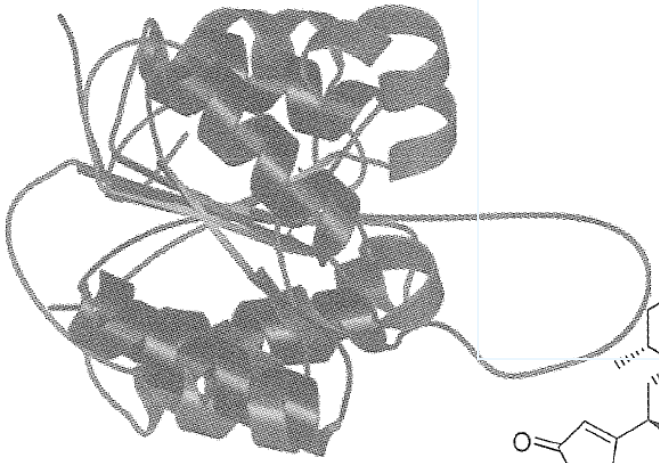


PSSC = protein structure similarity

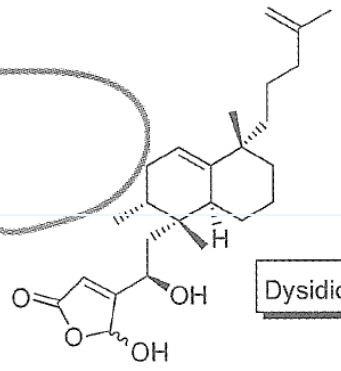
- # BIOS Based Library Synthesis and Evaluation

a. The target of Dysidiolide = Cdc25A
b. Protein structure similarity clustering with Cdc25A
c. Identifying 11-beta-HSD and AChE

Cdc25A and 11-β-Hydroxysteroid-Dehydrogenase



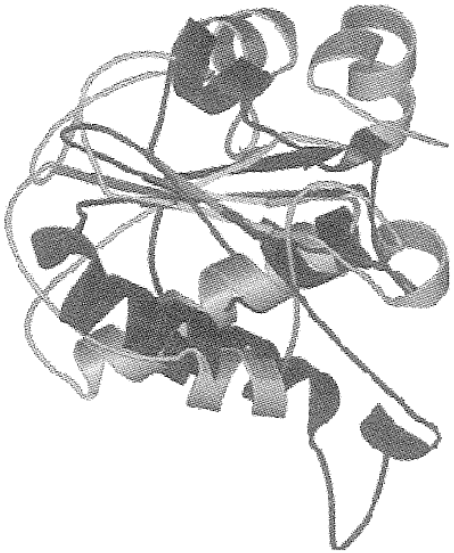
Cdc25A and 11-β-HSD: 80 Residues
RMSD 4.13 Å
Sequence identity 5.0%



Dysidiolide

Cdc25A:9.4 μM

Acetylcholine Esterase and Cdc25A phopshatase



AchE and Cdc25A proteins:49 Residues
RMSD 2.74 Å,
Sequence identity 8.2%

- **BIOS Based Library Synthesis and Evaluation**

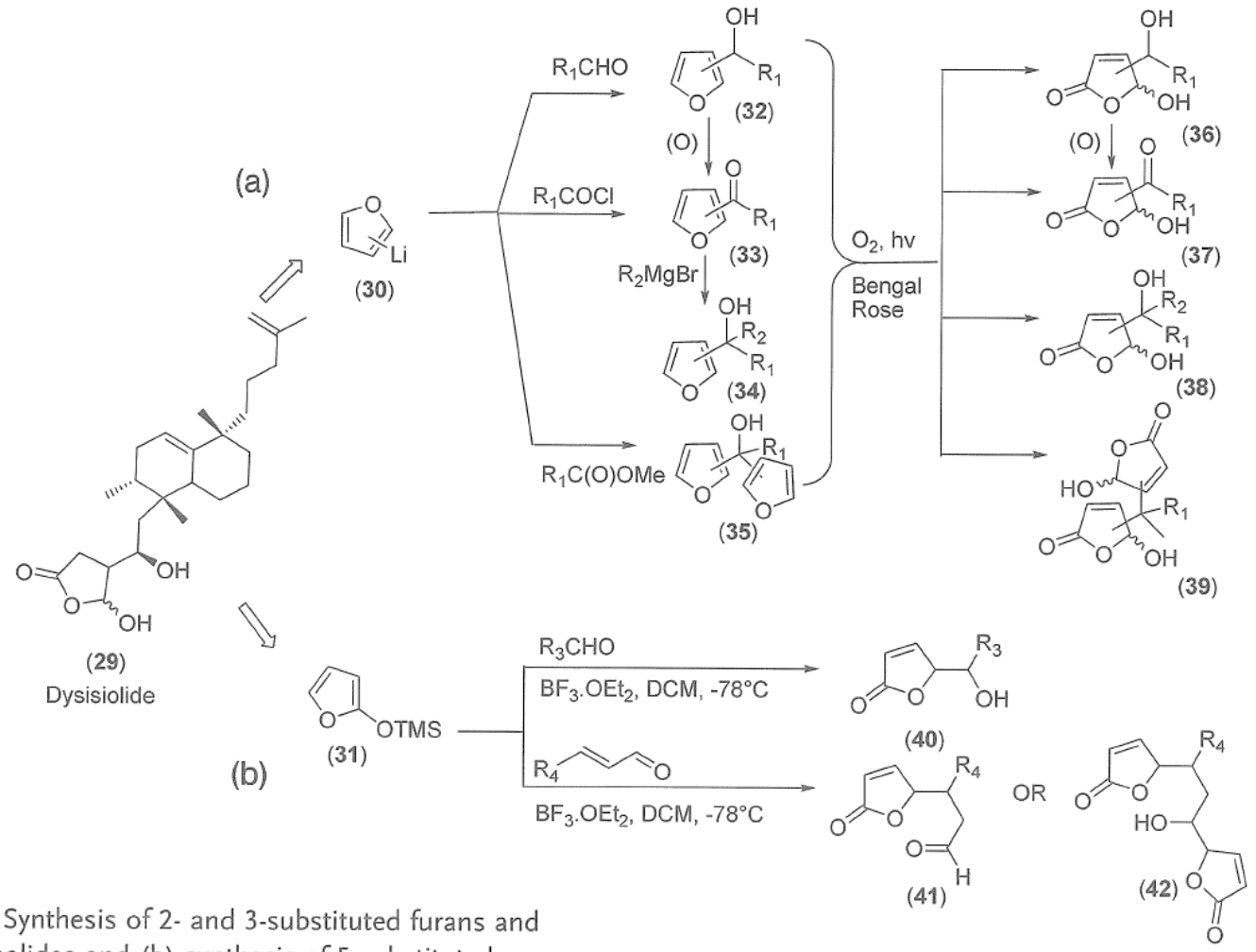


Figure 3.4 (a) Synthesis of 2- and 3-substituted furans and γ-hydroxybutenolides and (b) synthesis of 5-substituted butenolides and bisbutenolides.

- **BIOS Based Library Synthesis and Evaluation**

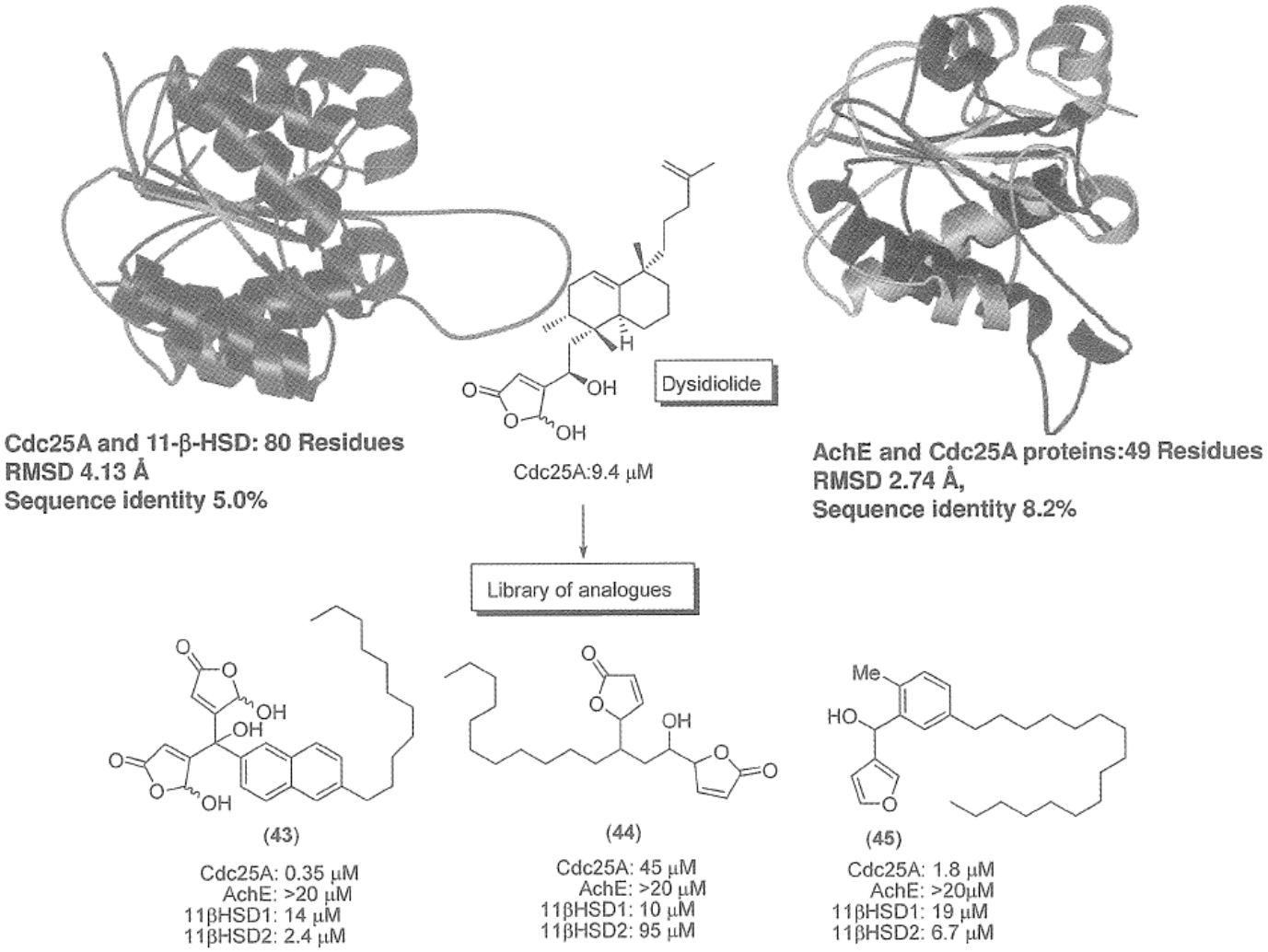


Figure 3.5 Application of BIOS for targeting protein cluster with common ligands.

Discovery of the new type of ligand

Synthetic Methodology for Library Construction

- **Solid-Phase Organic Synthesis**

  The compound library have been synthesized

  on solid phase such as resin bead, pins, or chips

- **Solution-Phase Organic Synthesis**

  The compound library have been synthesized

  in solvent in the reaction flask

Solid phase synthesis techniques

1. Overview
   - Introduction
   - Resins & Linkers
   - Protecting groups
   - Building blocks

2. Practical Synthesis
   - Peptide
   - Oligonucleotide
   - Small molecule

Introduction

What is a solid phase organic synthesis?

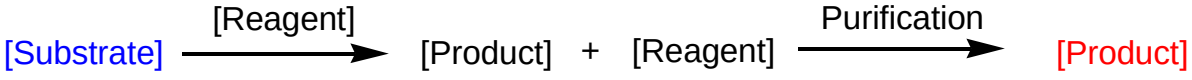
Organic reactions carried out on substrates
that are covalently attached to a polymeric support

Advantages of solid phase versus solution phase synthesis?

- Synthetic intermediates don't have to be isolated
- The excess reagents are just washed away each step
- It is often quicker and easier than solution phase
- The process can easily be automated using robots

Solid phase synthesis

vs Solution phase synthesis

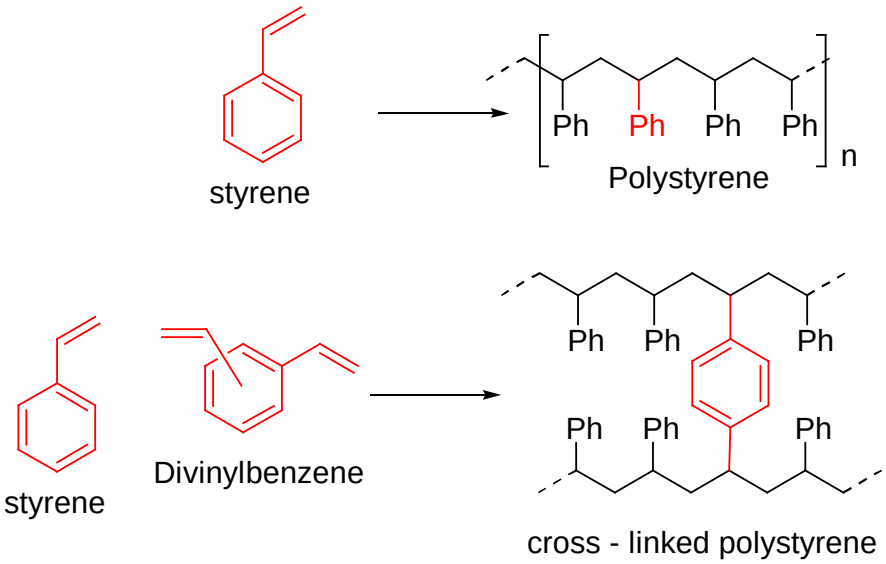


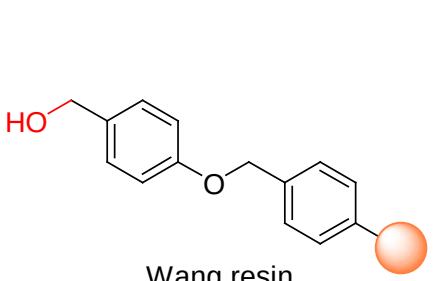
Resin

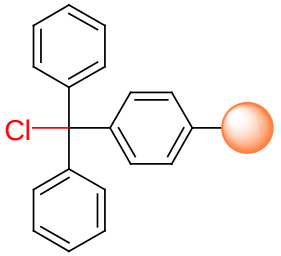
What is a resin?

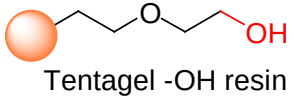
- The polymeric backbone that synthesis is performed on
- Different resins have different swelling properties

1. Polystyrene (PS): (cheap!): Swells in non-polar solvents
2. Polyethylene glycol (PEG): Swells in polar and non-polar solvents
3. Many others exist with different swelling properties



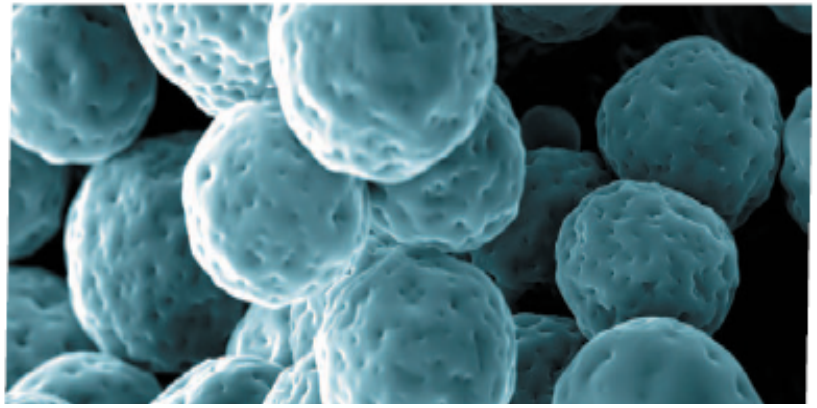




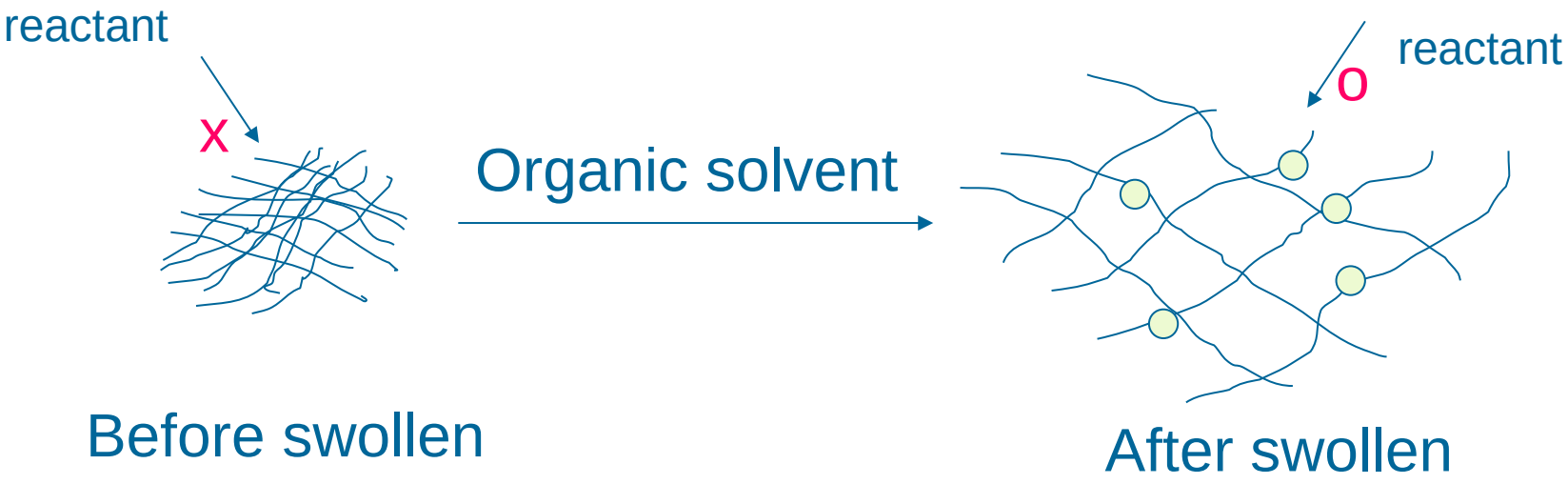


Swelling?

SEM (Scanning Electron Micrograph) of CLEAR resin particles: The structure is nearly 100% cross-linked. The polyethylene glycol backbone makes the resin fully accessible to a wide range of solvents and reagents including aqueous solutions. Unlike liquid phase PEG resins, CLEAR particles are easy to filter and resistant to all but the most harsh acid or base solutions.

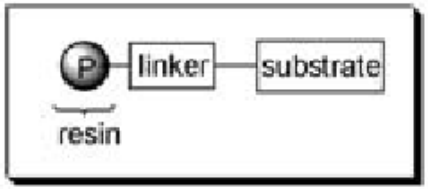


Trega Biosciences Inc. TL, 39, 8951 (1998)



Linker

- What is a linker?
  - An intermediate organic structure that the resin and substrate are covalently attached to:



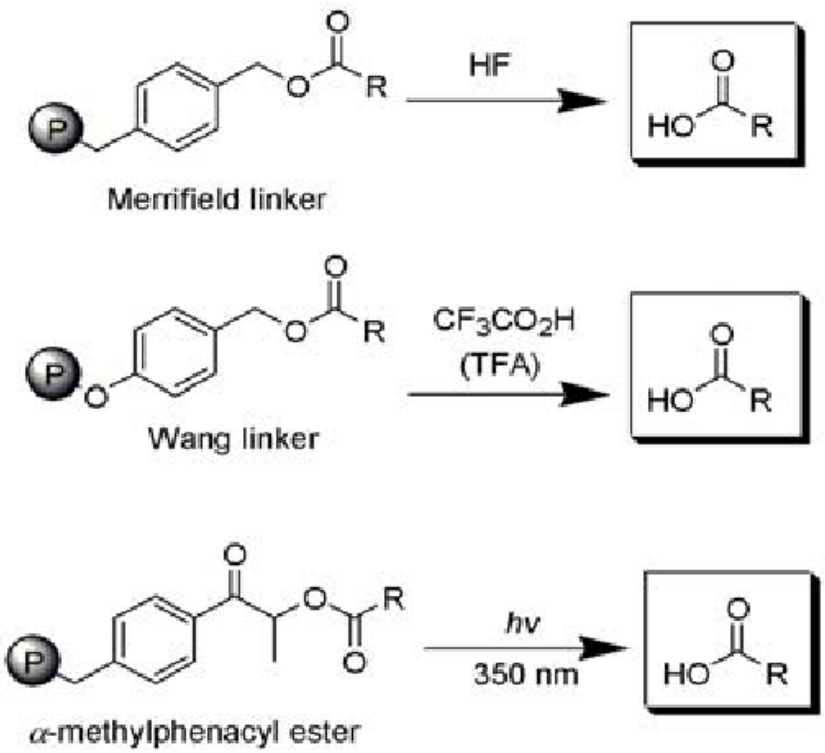
  - Different linkers can be cleaved by different reaction conditions! (e.g. acid, photolysis, nucleophilic attack, etc.)
  - Different linkers can be used to unmask different functional groups on the substrate upon cleavage!

For ideal linker

- Efficient attachment to the resin
- Efficient loading to the desired compounds
- Clean and fast release of product
- Stable through all the synthetic steps

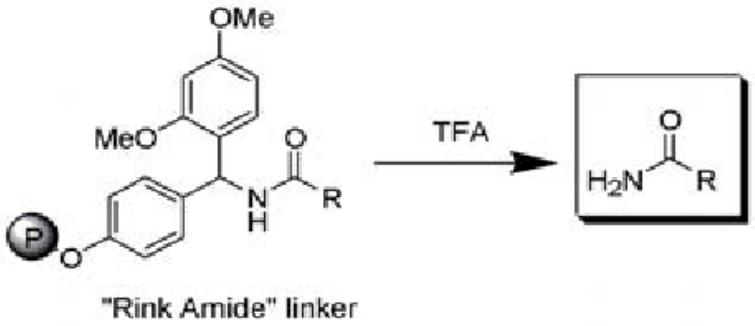
Solid phase synthesis: resins and linkers

- Examples of linkers commonly used in solid phase peptide synthesis:

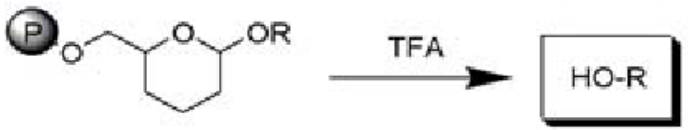


Solid phase synthesis: resins and linkers

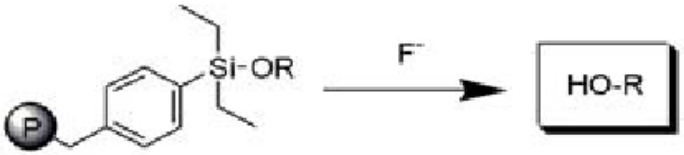
- A couple more linkers:



"Rink Amide" linker



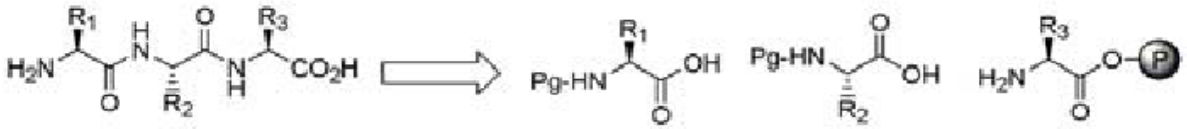
THP linker



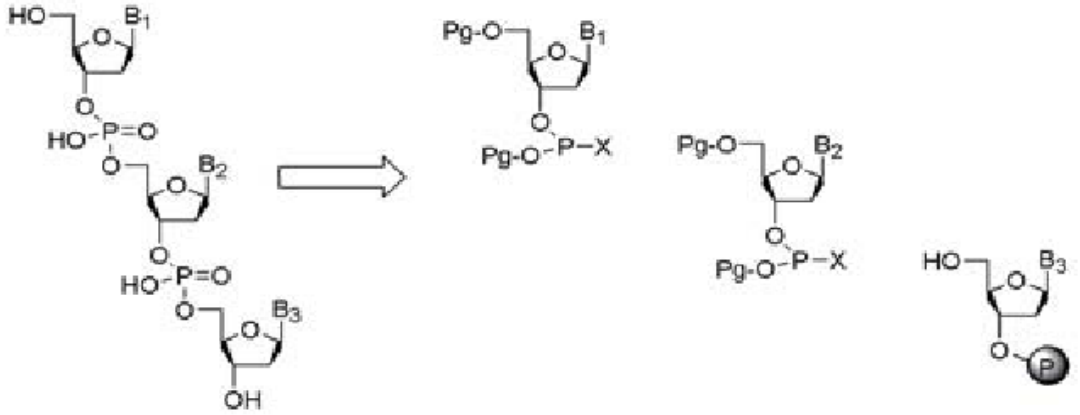
Silyl linker

Solid phase synthesis: building blocks

- Solid phase allows rapid attachment of building blocks to make new molecules.
- What classes of molecules can be synthesized?
- Three examples that we'll address:
  - Peptides (from amino acid building blocks):



  - DNA oligomers (from nucleotide building blocks):



  - Almost anything else (small molecules, etc.)

Practical Synthesis

- Peptide
- DNA
- Small molecule

Solid phase synthesis: protecting groups

- What is a protecting group?
  - A species conjugated to a functional group that blocks the reactivity of that group
  - Good protecting groups are easily attached and removed using mild reaction conditions
  - There are many hundreds of protecting groups, but only a small subset are practical for solid phase synthesis

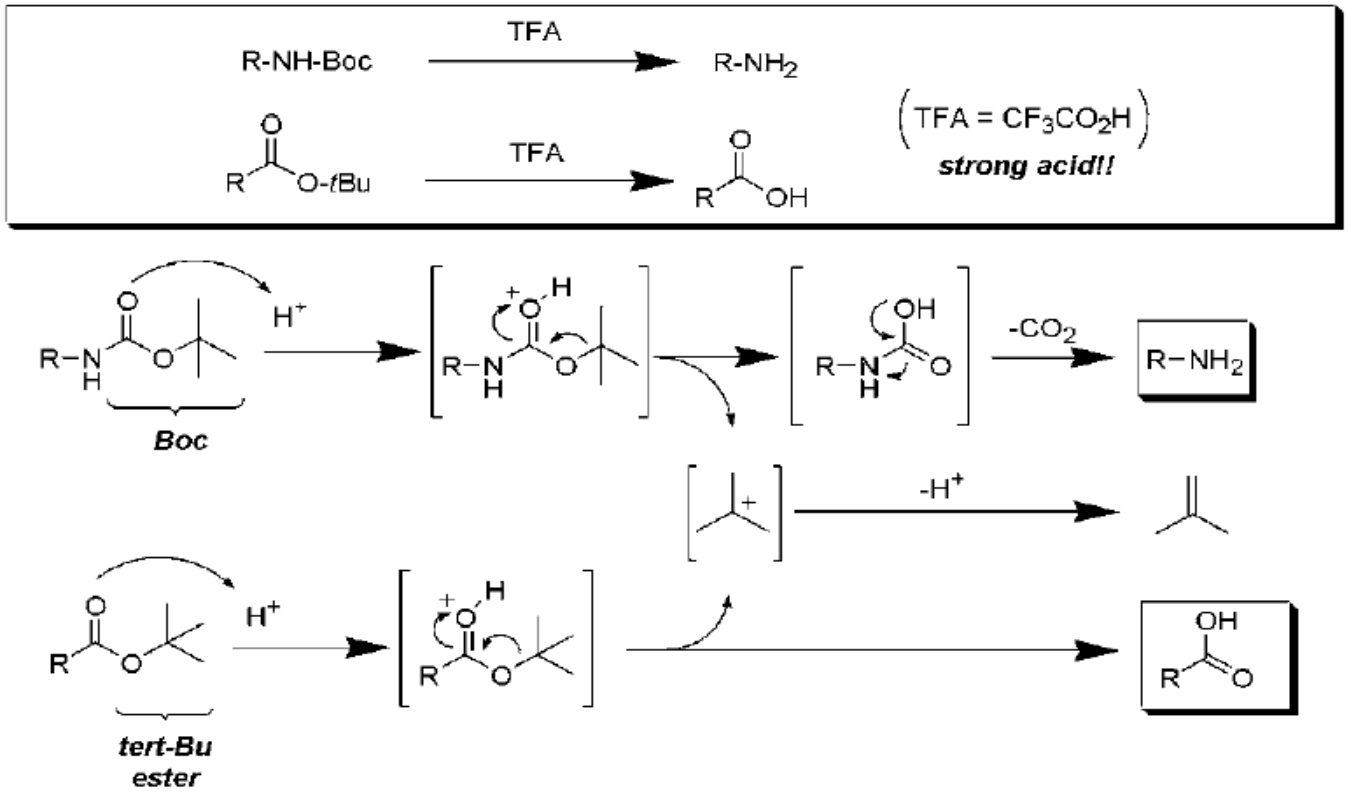
- A few protecting groups used in solid phase synthesis:

	For amines	For carboxylic acids
Removed w/acid:	Boc	*tert*-Bu ester
Removed w/base:	Fmoc	Fm ester
Removed w/fluoride:	Tmsec	Tmse ester
Removed w/Pd^0	Alloc	Allyl ester
Removed w/thiols:	Nosyl	n/a

- All of these protecting groups are "orthogonal"
  - (Boc can be removed in the presence of Fmoc, etc.)

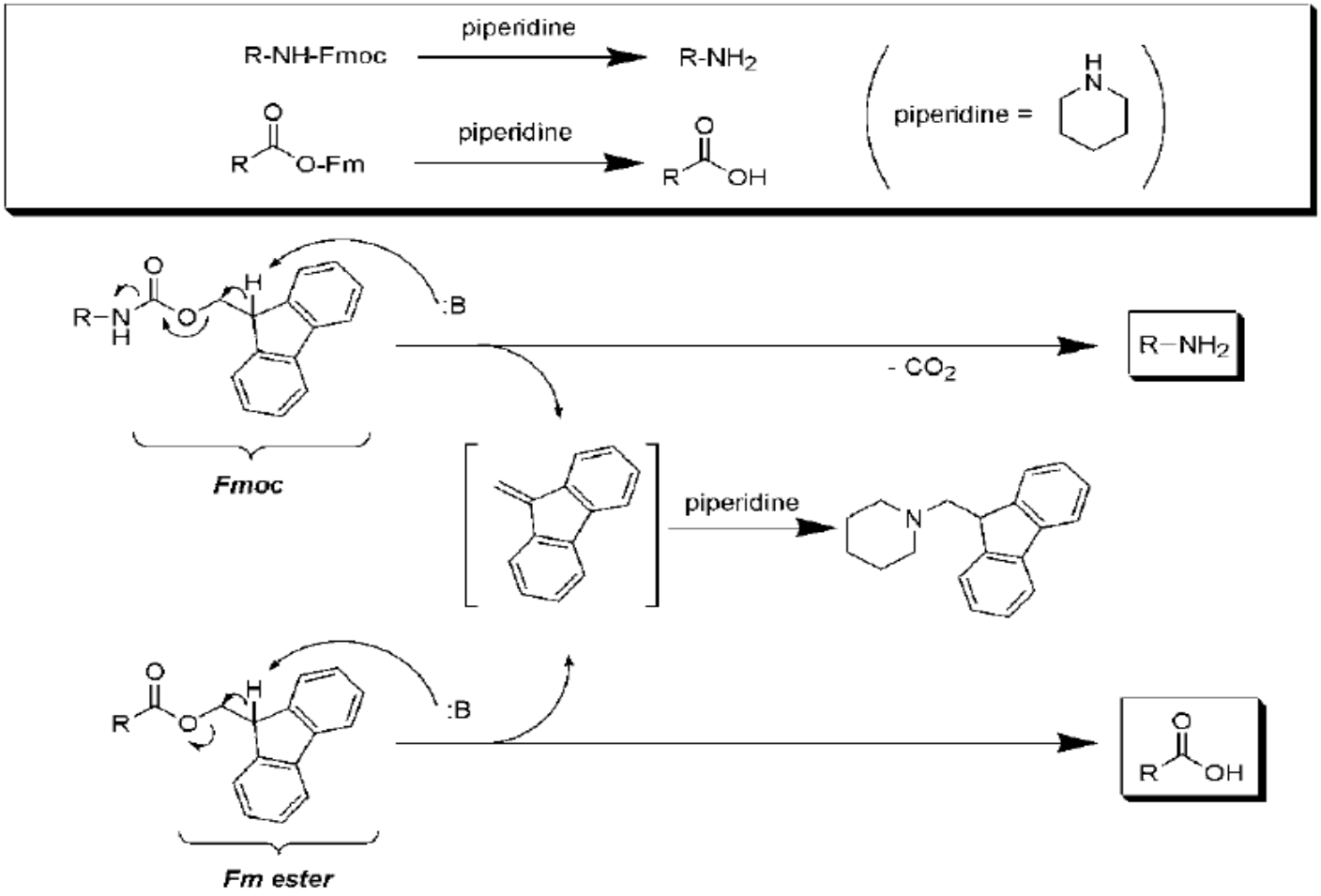
Solid phase synthesis: protecting groups

- Acid labile protecting groups: Boc and *tert*-Bu ester



Solid phase synthesis: protecting groups

- Base labile protecting groups: Fmoc & Fm ester



Solid phase synthesis: to make peptides

- Now let's make peptides – there are two general strategies:
  - Boc peptide synthesis strategy (uses Merrifield linker)
  - Fmoc peptide synthesis strategy (uses Wang linker)

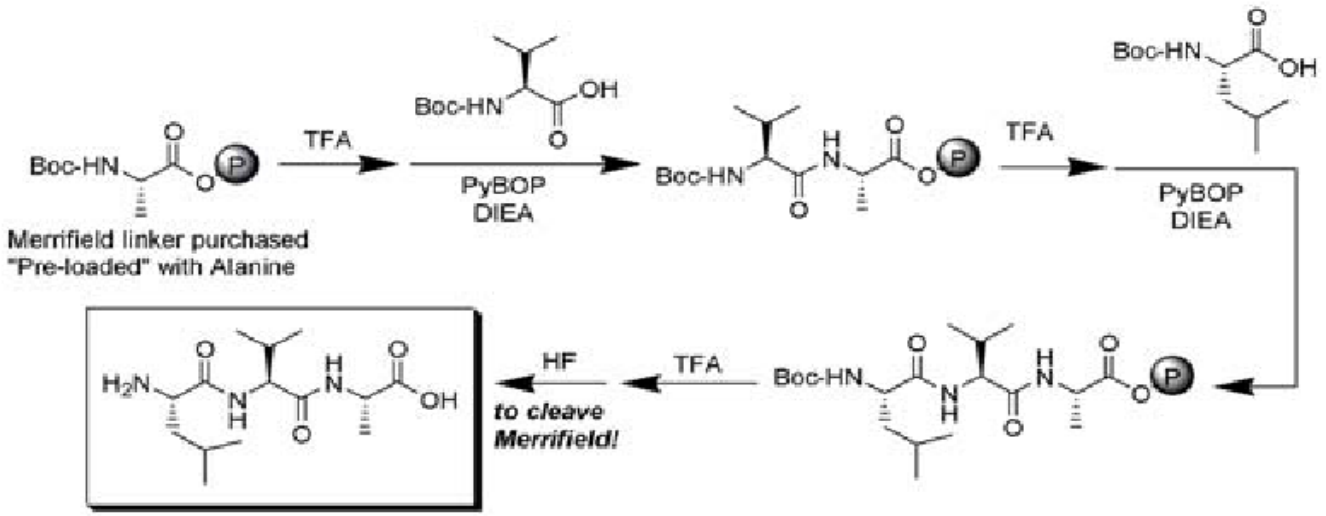
- The two strategies use different protecting groups for the growing peptide chains (Boc or Fmoc)

- Furthermore, the resins are cleaved by different reagents:
  - Merrifield linker cleaved by HF (*nasty / toxic / eats glass !!!*)
  - Wang linker cleaved by TFA (*not that bad*)

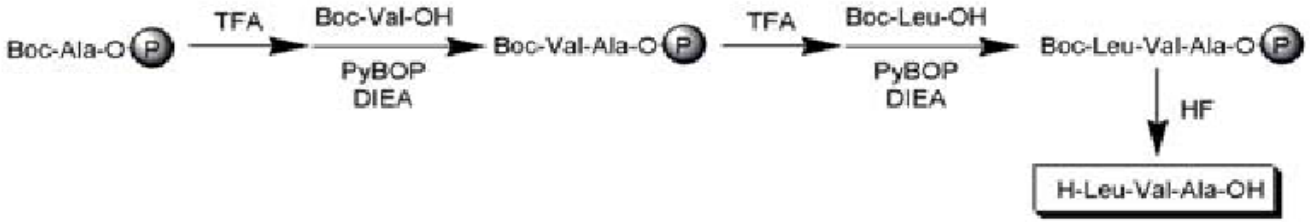
- Due to the nastiness of HF, the Boc-Merrifield strategy has mostly been replaced by the Fmoc-Wang strategy

Solid phase synthesis: to make peptides

- First is the "Boc strategy" of SPSS: (making H-Leu-Val-Ala-OH):

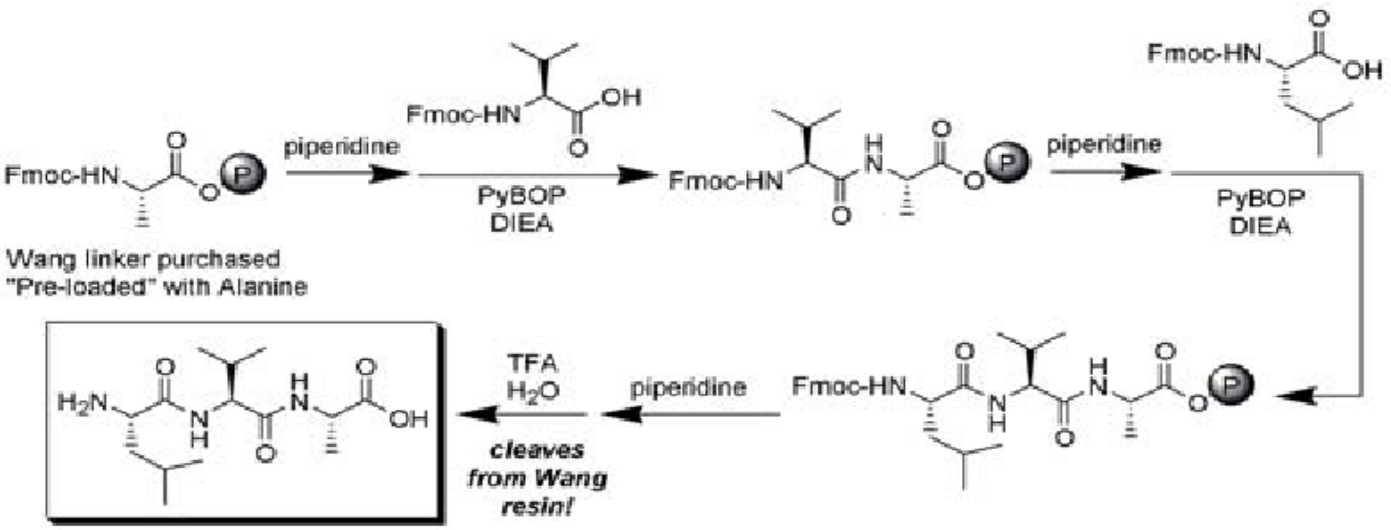


- The sequence can be abbreviated like this:

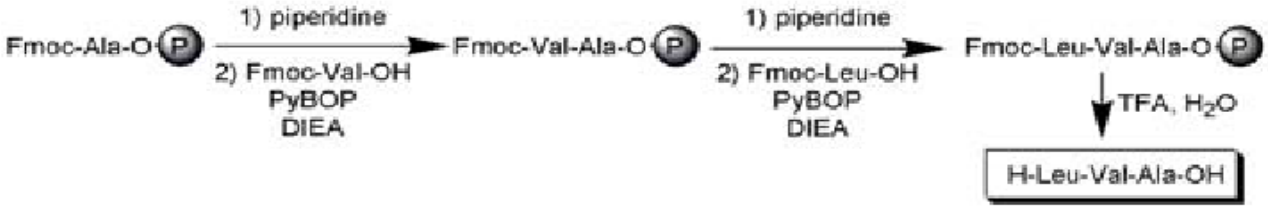


Solid phase synthesis: to make peptides

- And here is the "Fmoc strategy" of SPSS: (H-Leu-Val-Ala-OH):

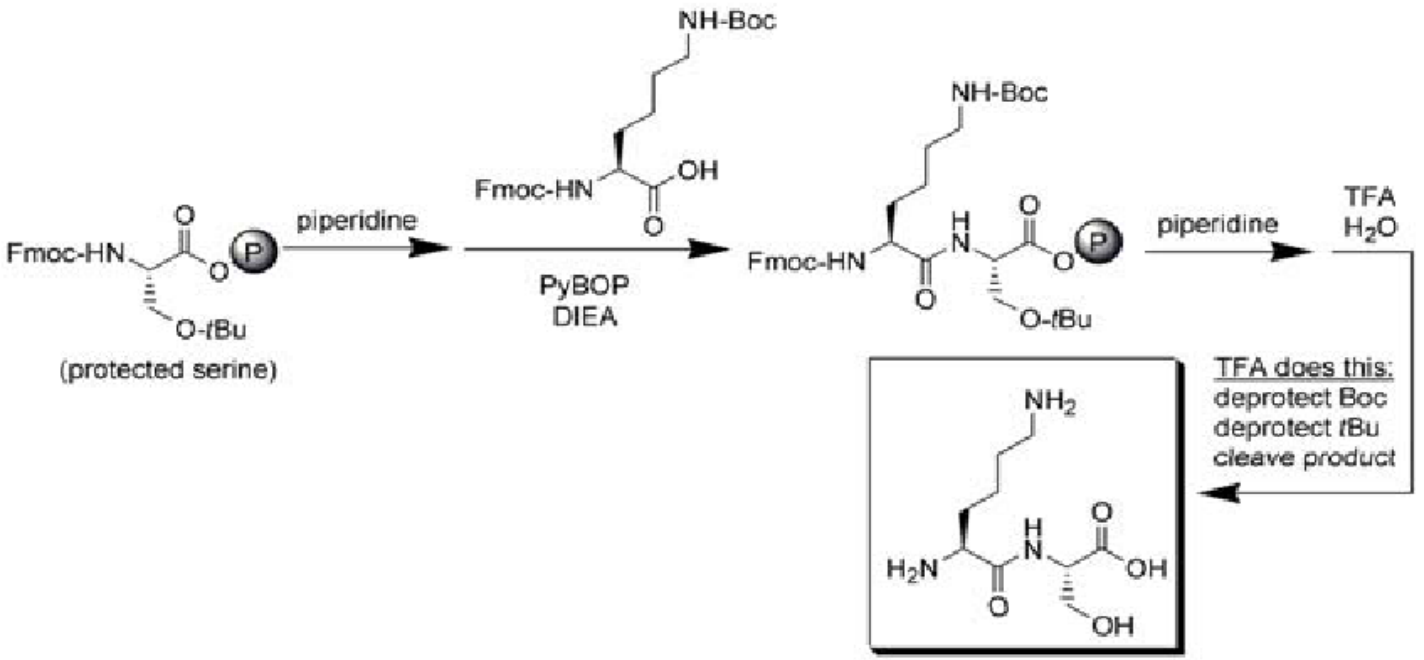


- The sequence can be abbreviated like this:



Solid phase synthesis: to make peptides

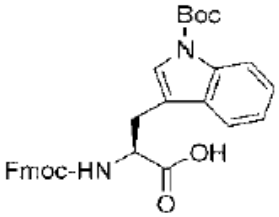
- How does one deal with reactive side chains of amino acids?
  - They are masked with "semi-permanent" PGs <u>until cleavage</u>:
  - Example: H-Lys-Ser-OH:



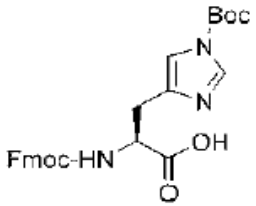
Solid phase synthesis: to make peptides

- There are Fmoc-building blocks for all 20 natural amino acids
- The PGs below are removed by acid during cleavage from resin

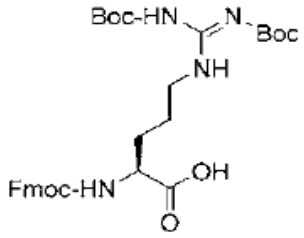
Fmoc-Ala-OH	Fmoc-Leu-OH
Fmoc-Arg(Boc) $_2$-OH	Fmoc-Lys(Boc)-OH
Fmoc-Asn(Trt)-OH	Fmoc-Met-OH
Fmoc-Asp(O-*t*Bu)-OH	Fmoc-Phe-OH
Fmoc-Cys(Trt)-OH	Fmoc-Pro-OH
Fmoc-Glu(O-*t*Bu)-OH	Fmoc-Ser(O-*t*Bu)-OH
Fmoc-Gln(Trt)-OH	Fmoc-Thr(O-*t*Bu)-OH
Fmoc-Gly-OH	Fmoc-Trp(Boc)-OH
Fmoc-His(Boc)-OH	Fmoc-Tyr(O-*t*Bu)-OH
Fmoc-Ile-OH	Fmoc-Val-OH



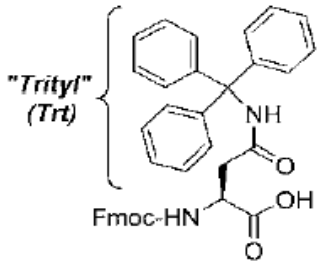
Fmoc-Trp(Boc)-OH



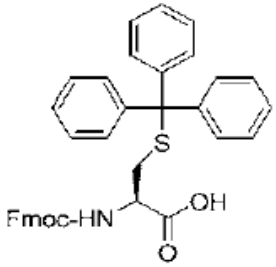
Fmoc-His(Boc)-OH



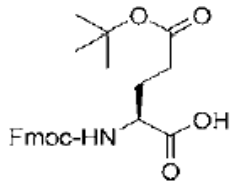
Fmoc-Arg(Boc)$_2$-OH



Fmoc-Asn(Trt)-OH



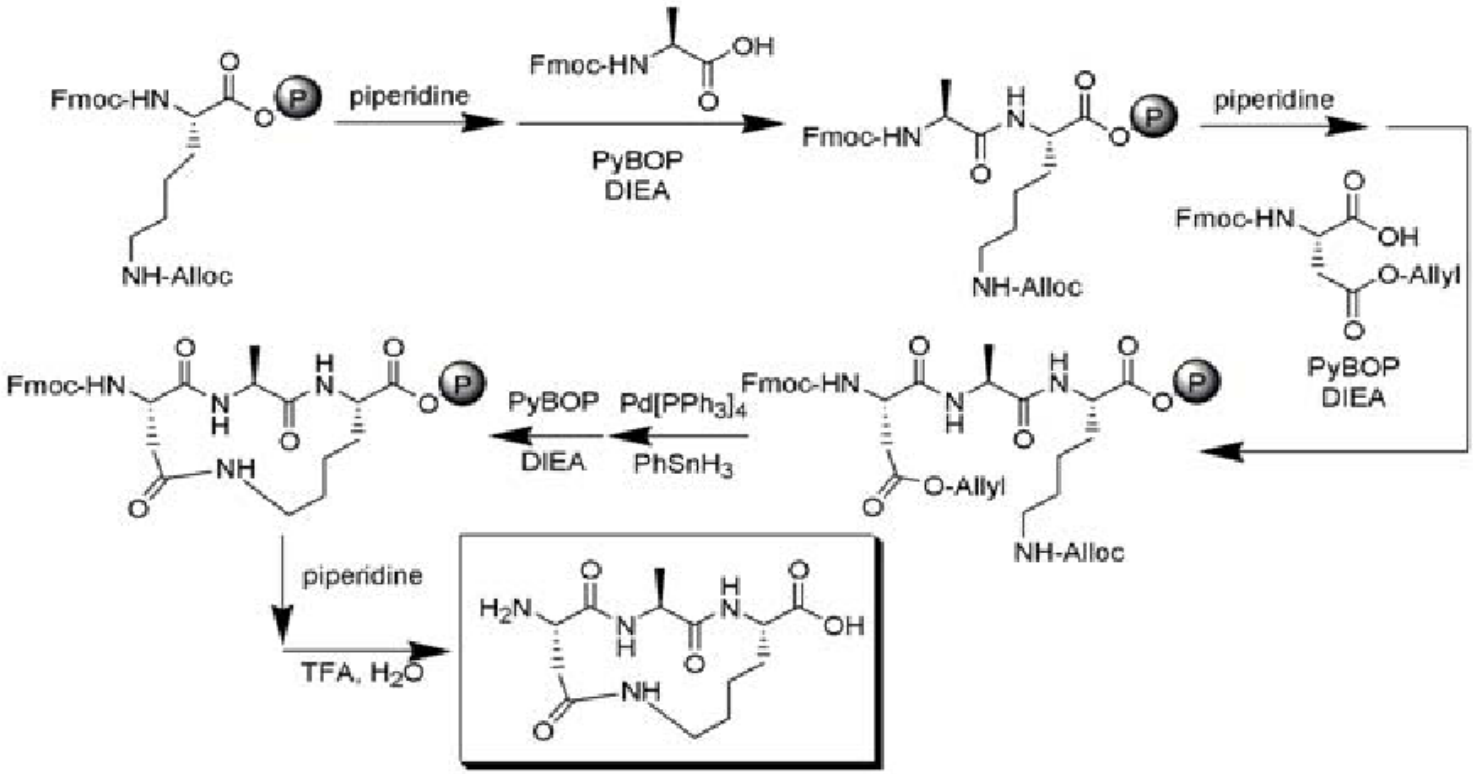
Fmoc-Cys(Trt)-OH



Fmoc-Glu(O-*t*Bu)-OH

Solid phase synthesis: to make peptides

- A cool application of peptide synthesis: peptide cyclization
- This sort of approach can be used to synthesize many biologically relevant cyclic peptides



Solid phase synthesis: to make peptides

- Brief tidbit: Peptidomimetics

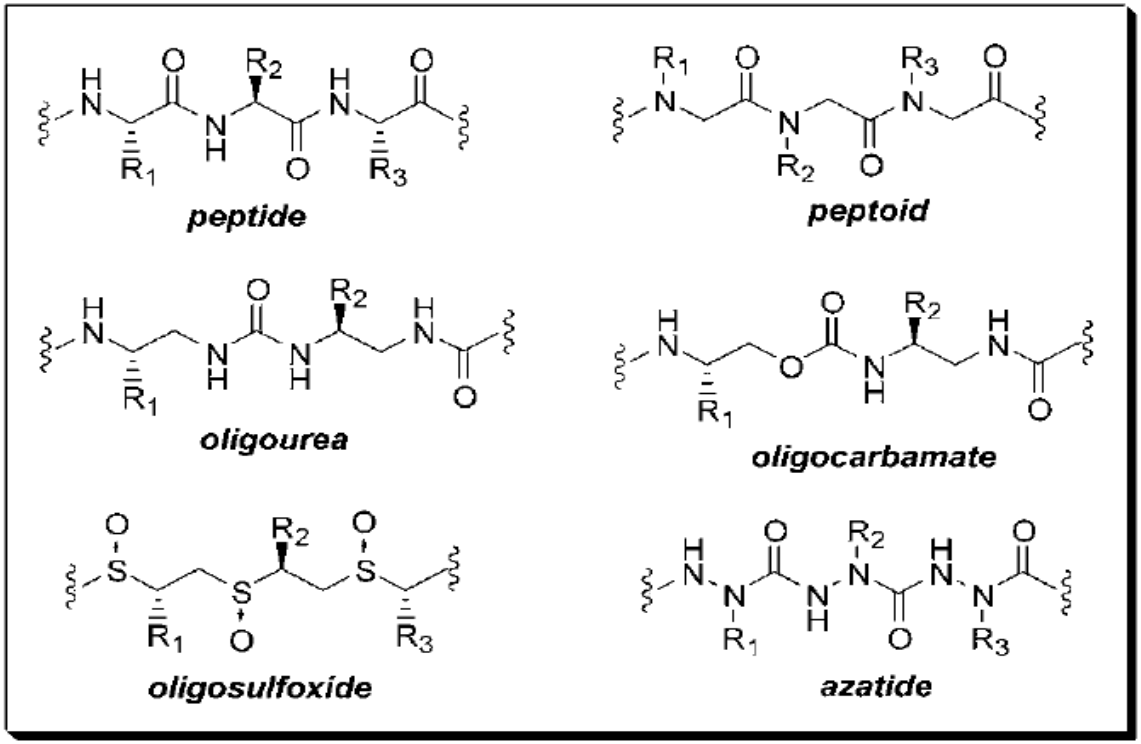
- Peptides are generally bad drugs, why? Poor bioavailability
  - Easily proteolyzed
  - High molecular weight
  - Often too polar

- These pharmacokinetic issues can be solved using peptidomimetics:
  - Peptidomimetics have modified structural features to limit the undesirable characteristics of peptides.
  - This is a very large topic that we'll only briefly mention here.

- One class of peptidomimetics: pseudopeptides
  - Modification of the "polyamide" peptide backbone

Solid phase synthesis: to make peptides

- Brief tidbit: Peptidomimetics: Pseudopeptides
  - All of these can be constructed by solid phase using appropriate building blocks



Solid phase synthesis: to make DNA

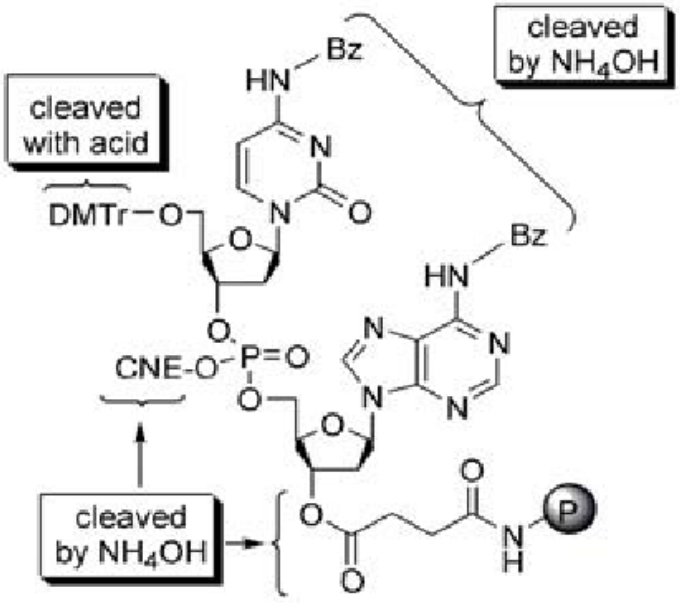
- The concept is very similar to peptide synthesis:
  - Linear sequence of reactions connecting nucleotide building blocks

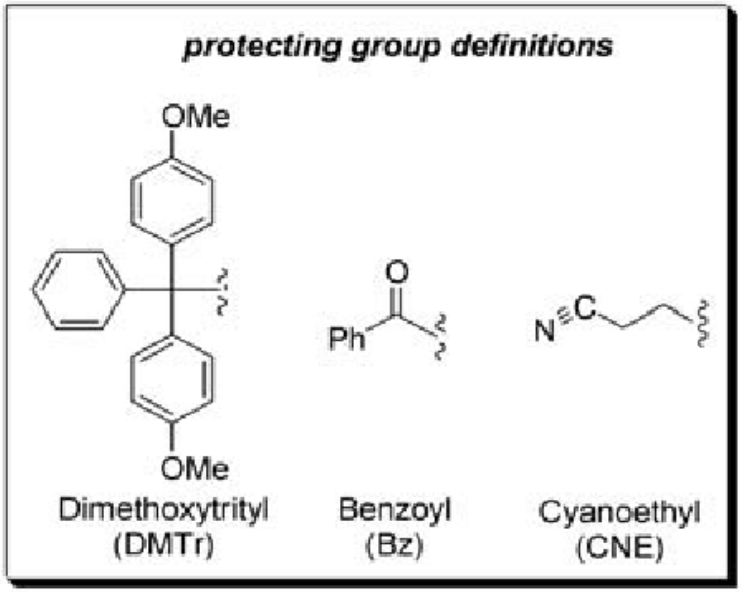
- Why synthesize DNA?
  - Primer synthesis (for PCR)
  - Synthesis of radiolabeled DNA fragments / probes

    and for many other molecular biology reasons that I'm ignorant about!
  - Useful "tag system" in combinatorial chemistry (later lecture)

Solid phase synthesis: to make DNA

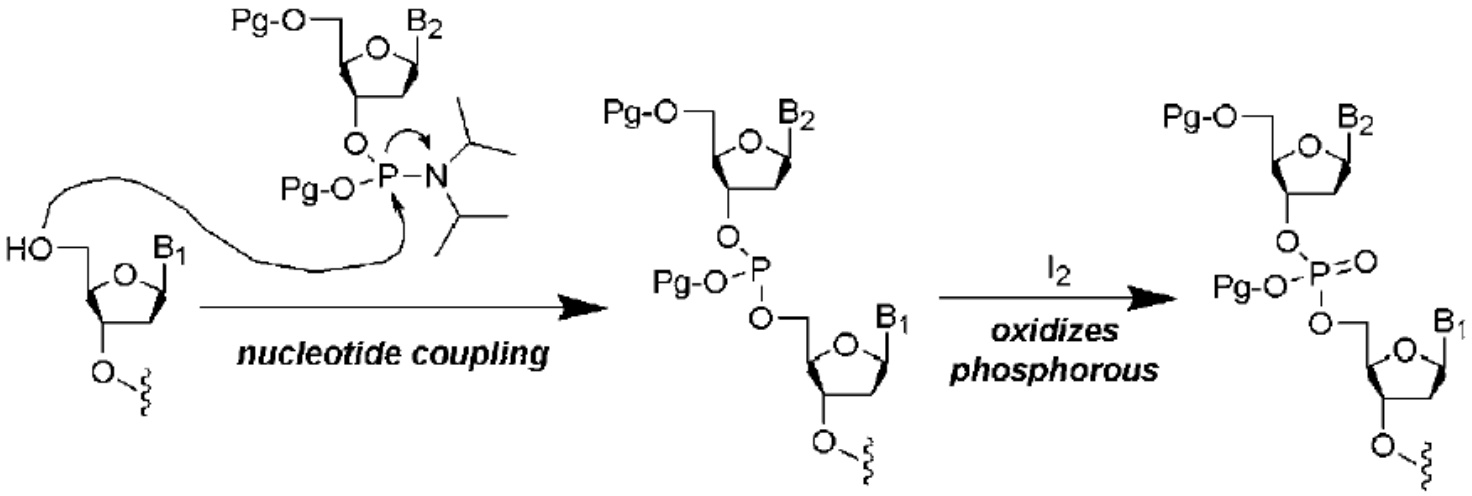
- The strategies for the linker and protecting groups are different
  - The linker and "semi-permanent" PGs cleave w/ aqueous base
  - The growing sugar backbone PG cleaves w/ acid





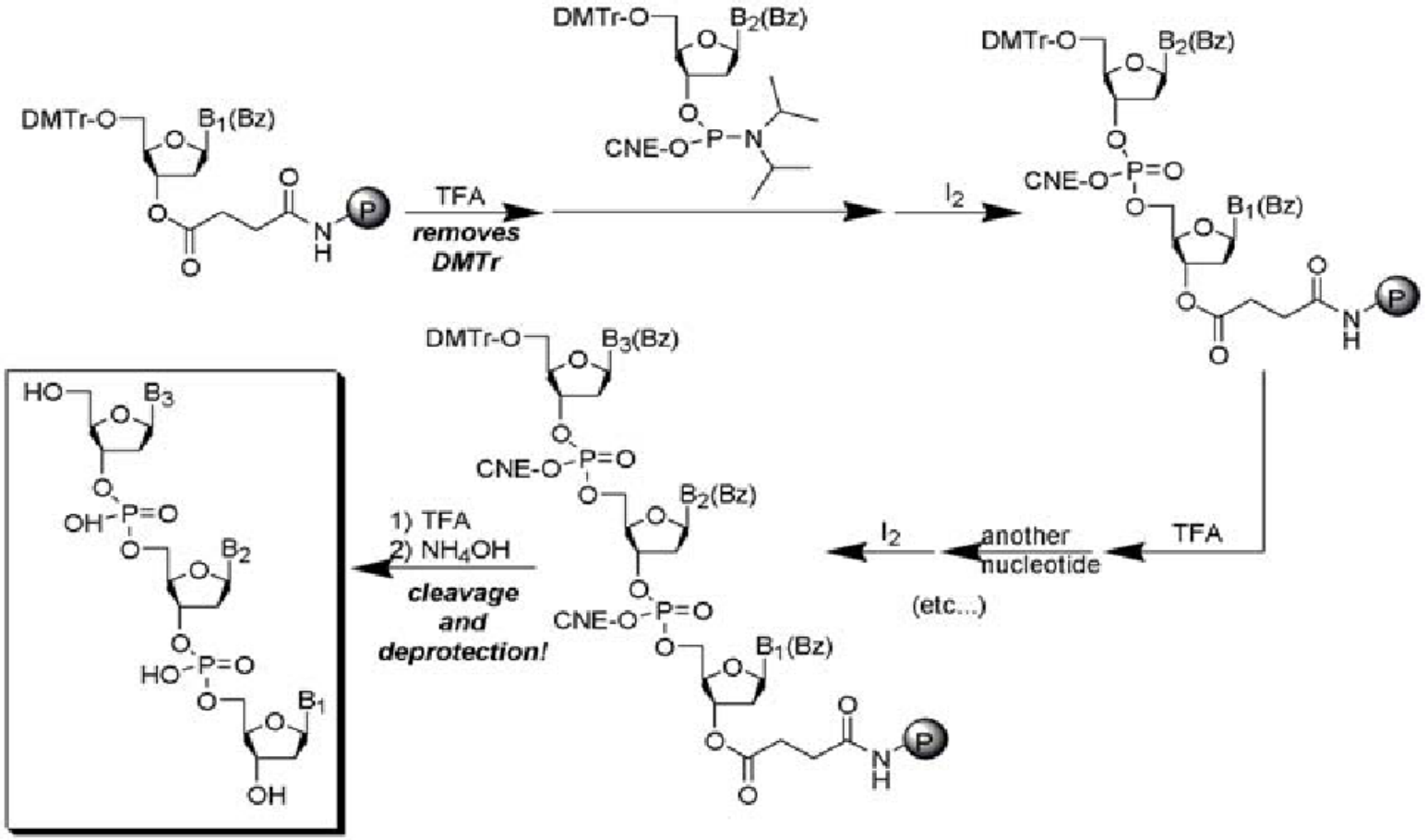
Solid phase synthesis: to make DNA

- DNA synthesis is done using the "phosphoramidite" strategy.
- This a two step process:
  - Coupling of the nucleotide fragments
  - Oxidation of the coupled phosphorous atom to the phosphate oxidation state



Solid phase synthesis: to make DNA

- One brief example: synthesis of a short oligonucleotide

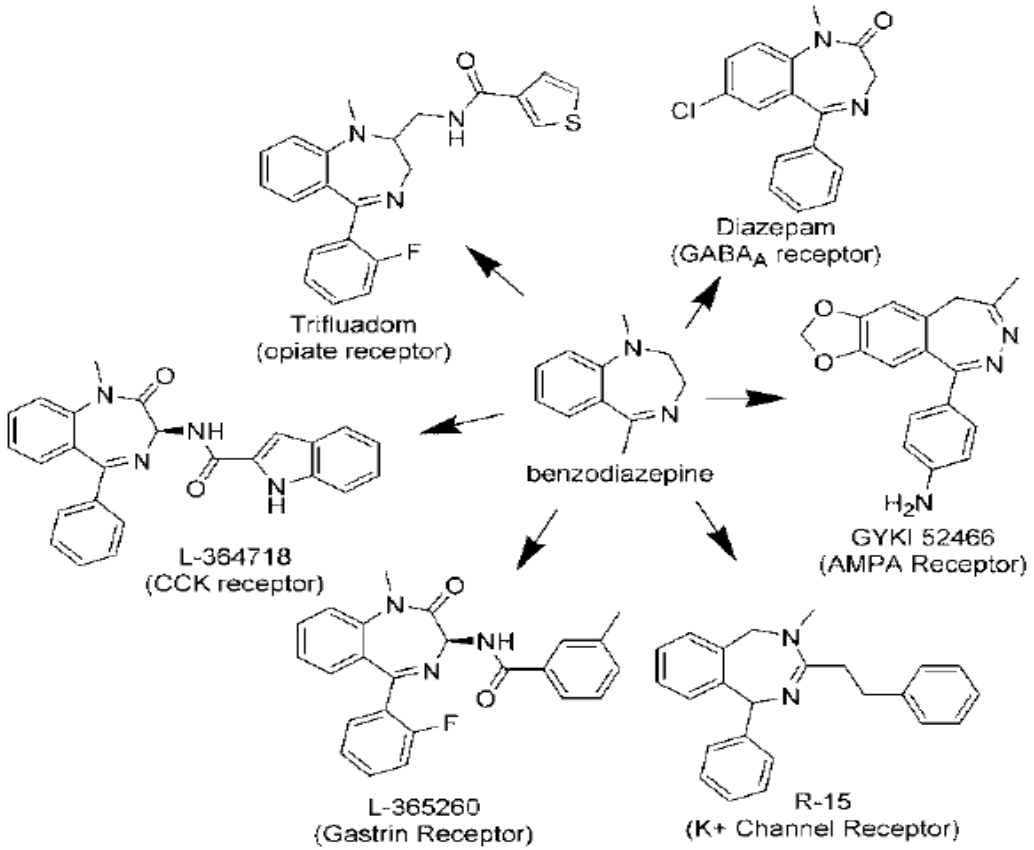


Solid phase synthesis: "small molecules"

- What do we mean by "small molecules?"
  - By this we typically mean "non-biopolymers" (biopolymers being peptides, DNA, etc.)
- Why are small molecules interesting?
  - They typically have drug-like properties and are designed to be bioavailable
  - Most modern drugs are considered small molecules
- This will be covered only very briefly here
  - The chemistry requires a much larger vocabulary of synthetic reactions! (Way more than you're expected to know now)
- We'll just present two examples of the small molecule "scaffolds" concept

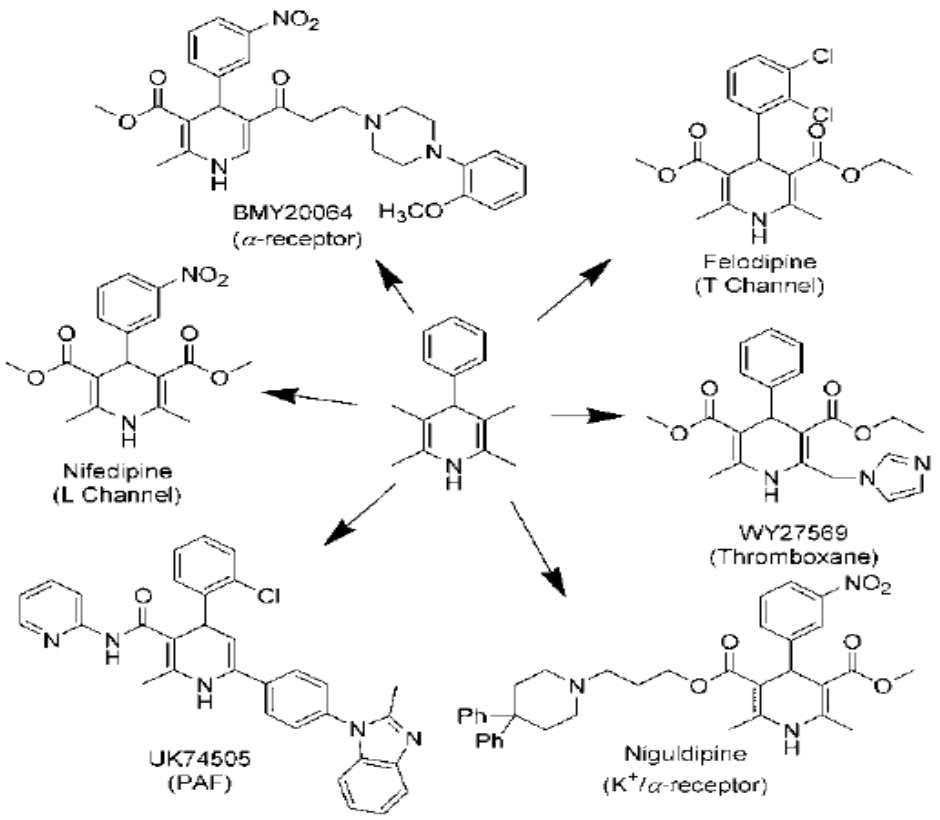
Solid phase synthesis: "small molecules"

- Example of small molecule scaffold: benzodiazepines

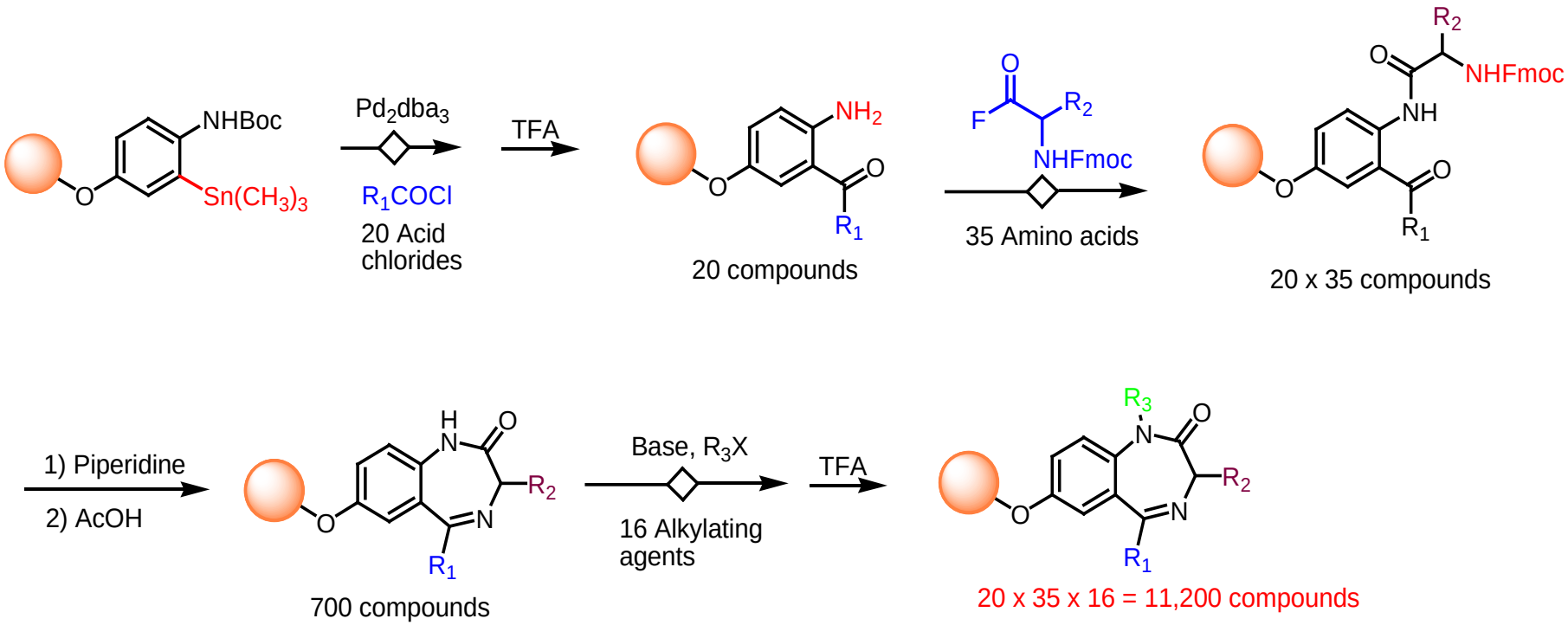


Solid phase synthesis: "small molecules"

- Example of small molecule scaffold: 1,4-dihydropyridines



Synthesis of 1,4-benzodiazepines



Solution Phase Synthesis

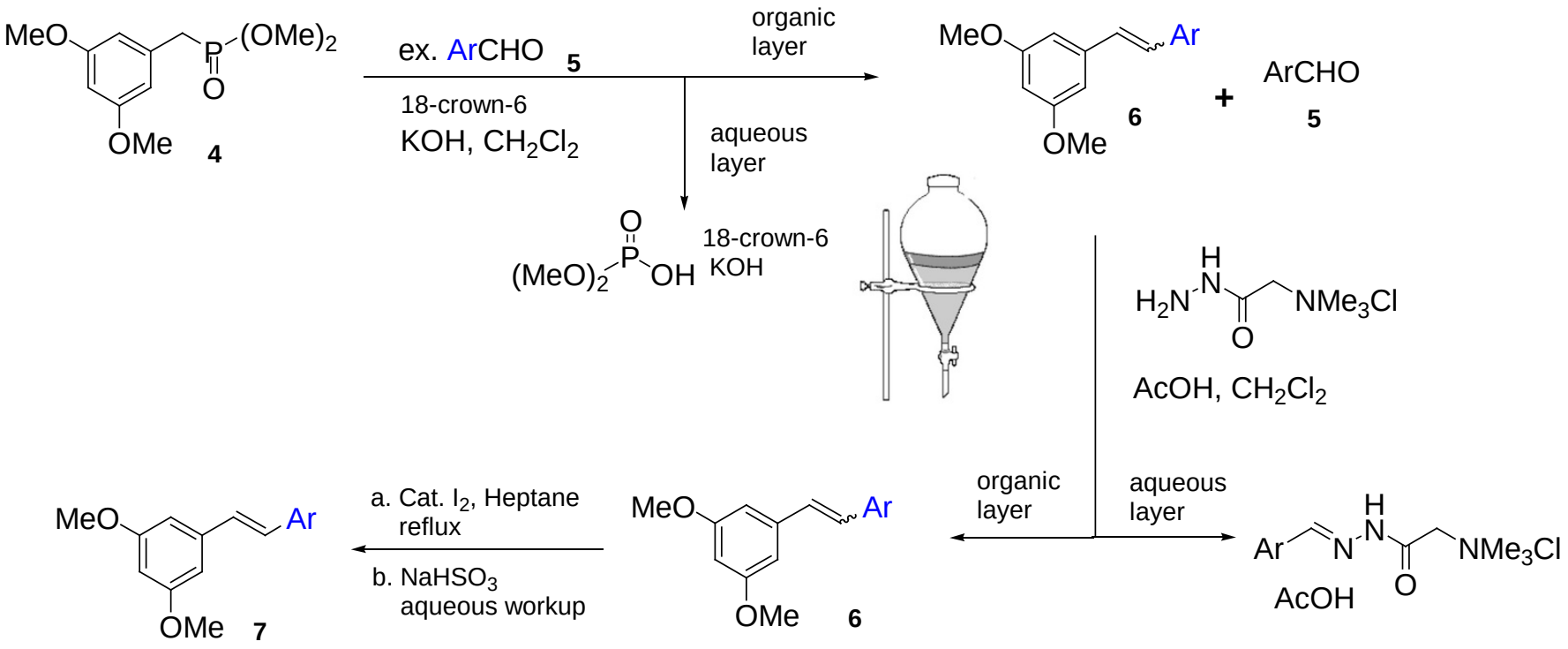
Three major techniques for solution phase synthesis:

- Liquid/Liquid Extraction
- Solution phase synthesis using scavenger resins
- Fluorous phase synthesis

The goal in both cases:

- **High throughput synthesis** making large amounts of compounds quickly

Solution Phase Synthesis of Stilbene Libraries



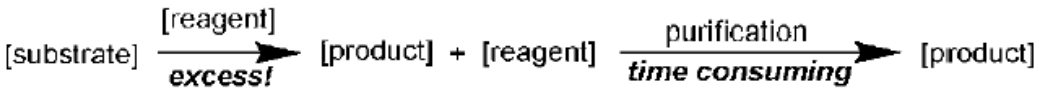
Sanghee Kim, Hyojin Ko, Jae Eun Park, Sungkyu Jung, Sang Kwang Lee, Young-Jin Chun *J. Med. Chem.* **2002**, *45*, 160-164.

Solution Phase Synthesis: Scavenger Resins

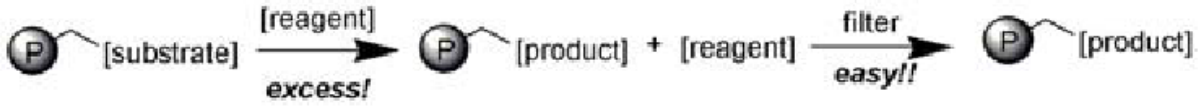
- What is it?
  - Do normal organic synthesis in solution phase
  - Use "scavenger resins" to consume excess reagents
  - The resin is then simply filtered away
  - This helps avoid time consuming purification

Solution Phase Synthesis: Scavenger Resins

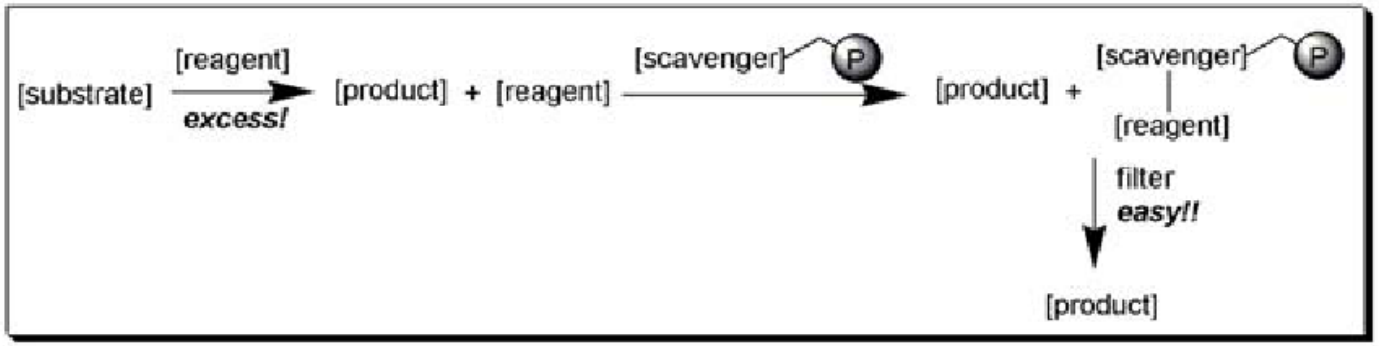
- Traditional solution phase synthesis:



- Solid phase synthesis (last lecture):



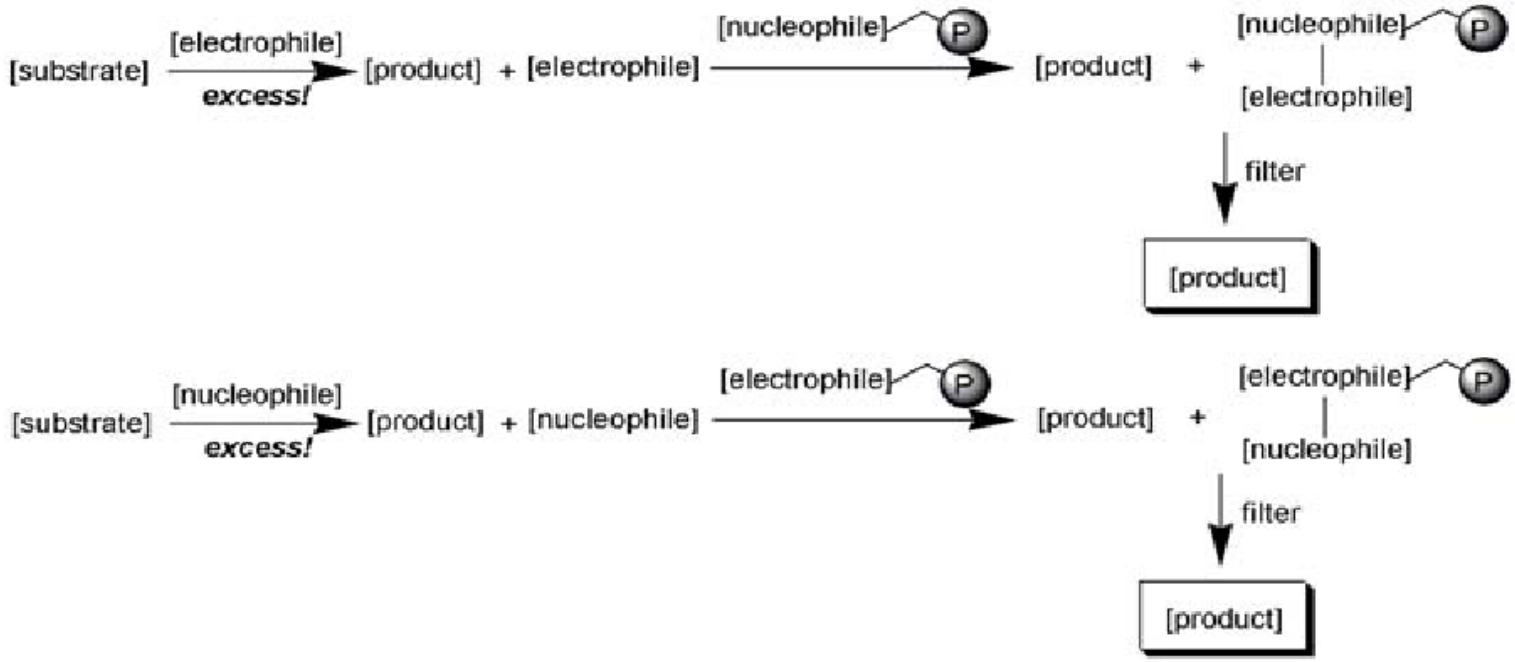
- Solution phase synthesis with scavenger resins (this lecture):



Solution Phase Synthesis: Scavenger Resins

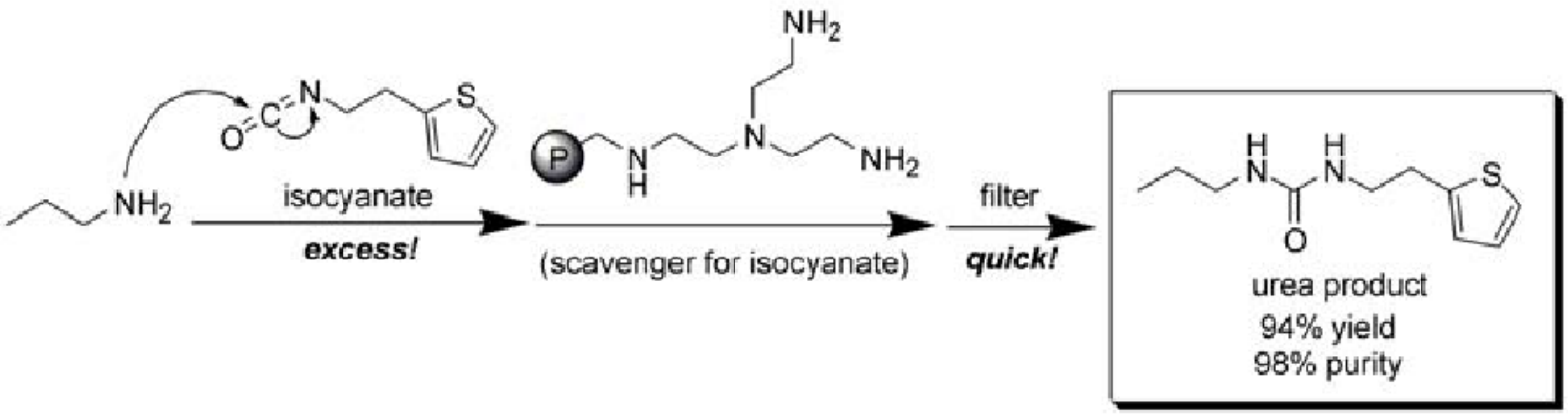
- We'll consider two strategies in using scavenger resins

- The issue is whether the reagent is electrophilic or nucleophilic
  - If the reagent is electrophilic, use a nucleophilic scavenger!
  - If the reagent is nucleophilic, use an electrophilic scavenger!



Solution Phase Synthesis: Scavenger Resins

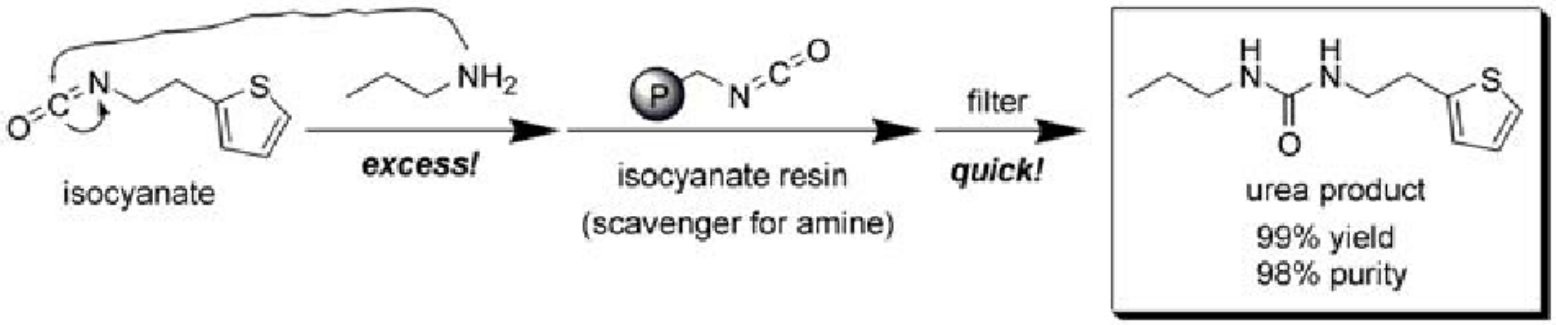
- Electrophiles scavenged by a nucleophilic resin:
  - Example 1: urea synthesis



Solution Phase Synthesis: Scavenger Resins

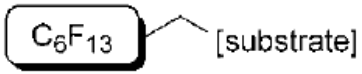
- Or alternatively…

- Nucleophiles scavenged by an electrophilic resin:
  - Example 1: urea synthesis

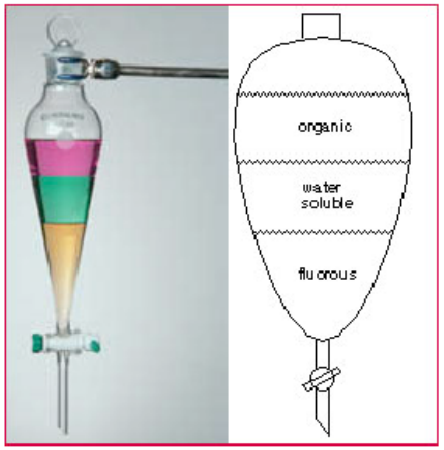


Solution Phase Synthesis: Fluorous technology

- What do we mean by "fluorous"?
  - A molecule with long tag containing many fluorine atoms – e.g.:



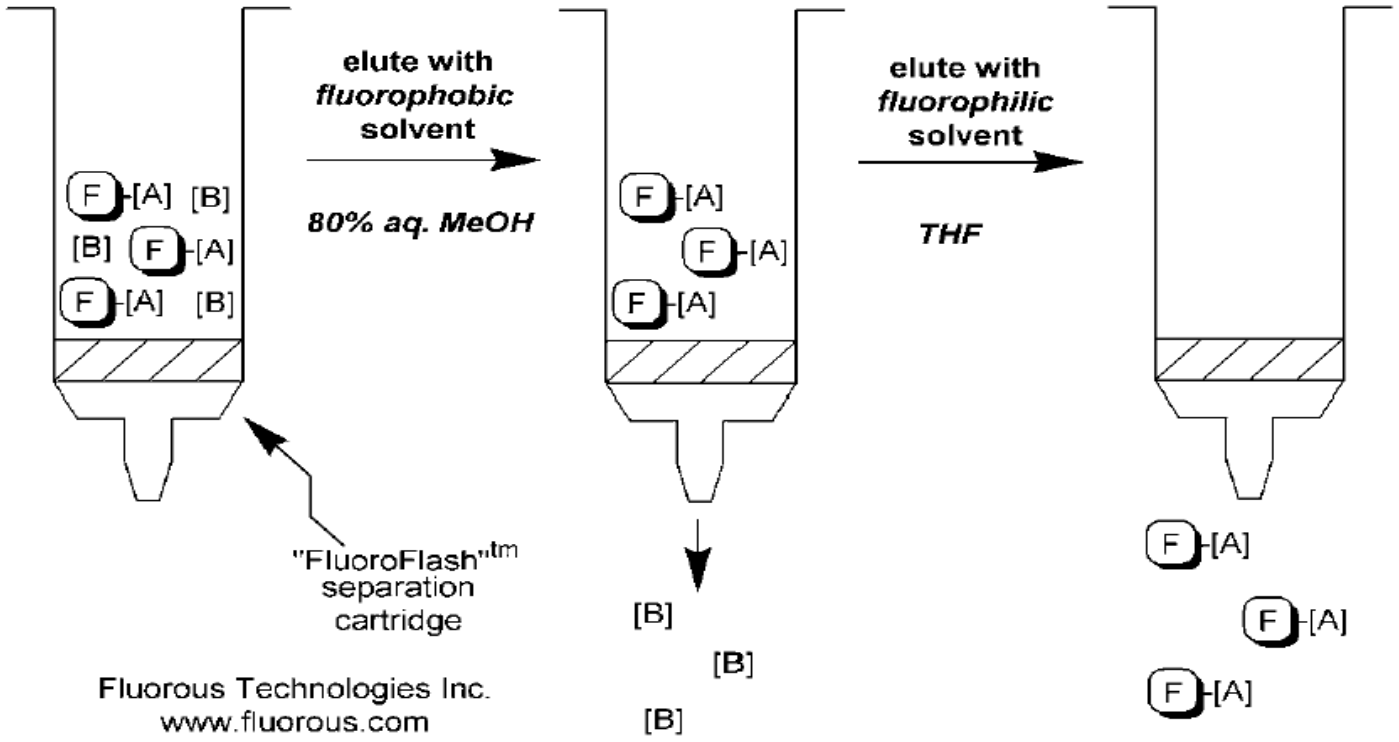
- What is special about fluorous molecules?
  - They have very weird chemical properties!
  - e.g. They can be extracted using "fluorous solvents"
  - Anything "non-fluorous" doesn't get extracted!



Solution Phase Synthesis: Fluorous technology

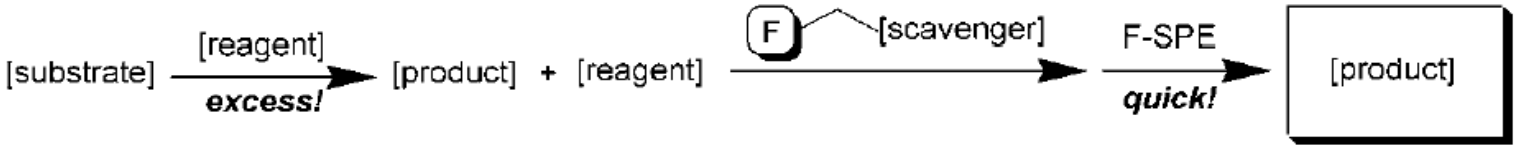
- A newer/quicker separation strategy

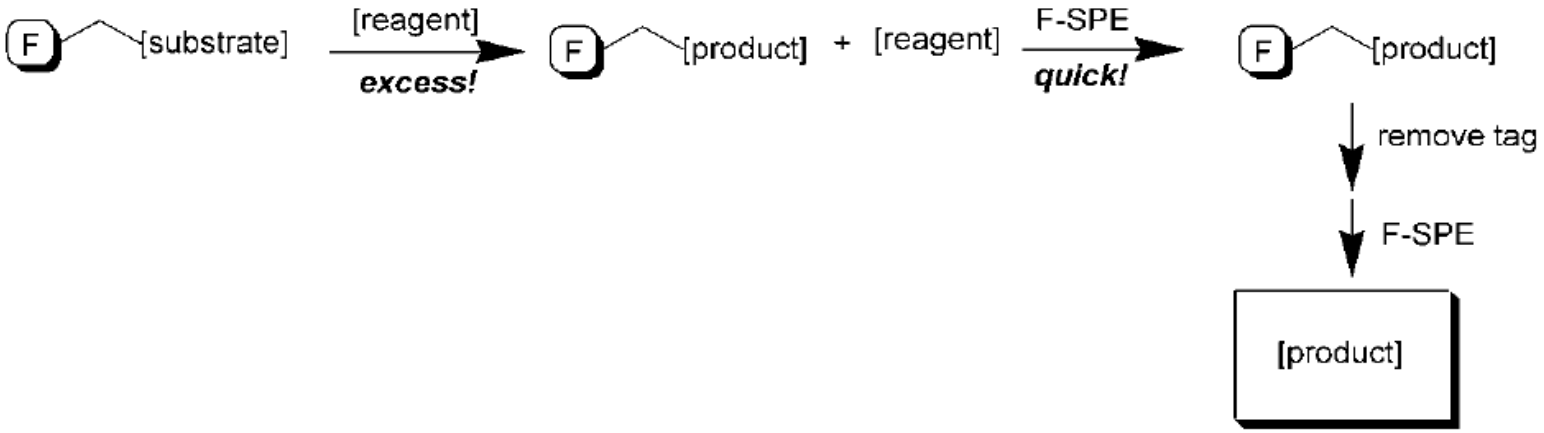
  "Fluorous solid phase extraction" (**F-SPE**)



Solution Phase Synthesis: Fluorous technology

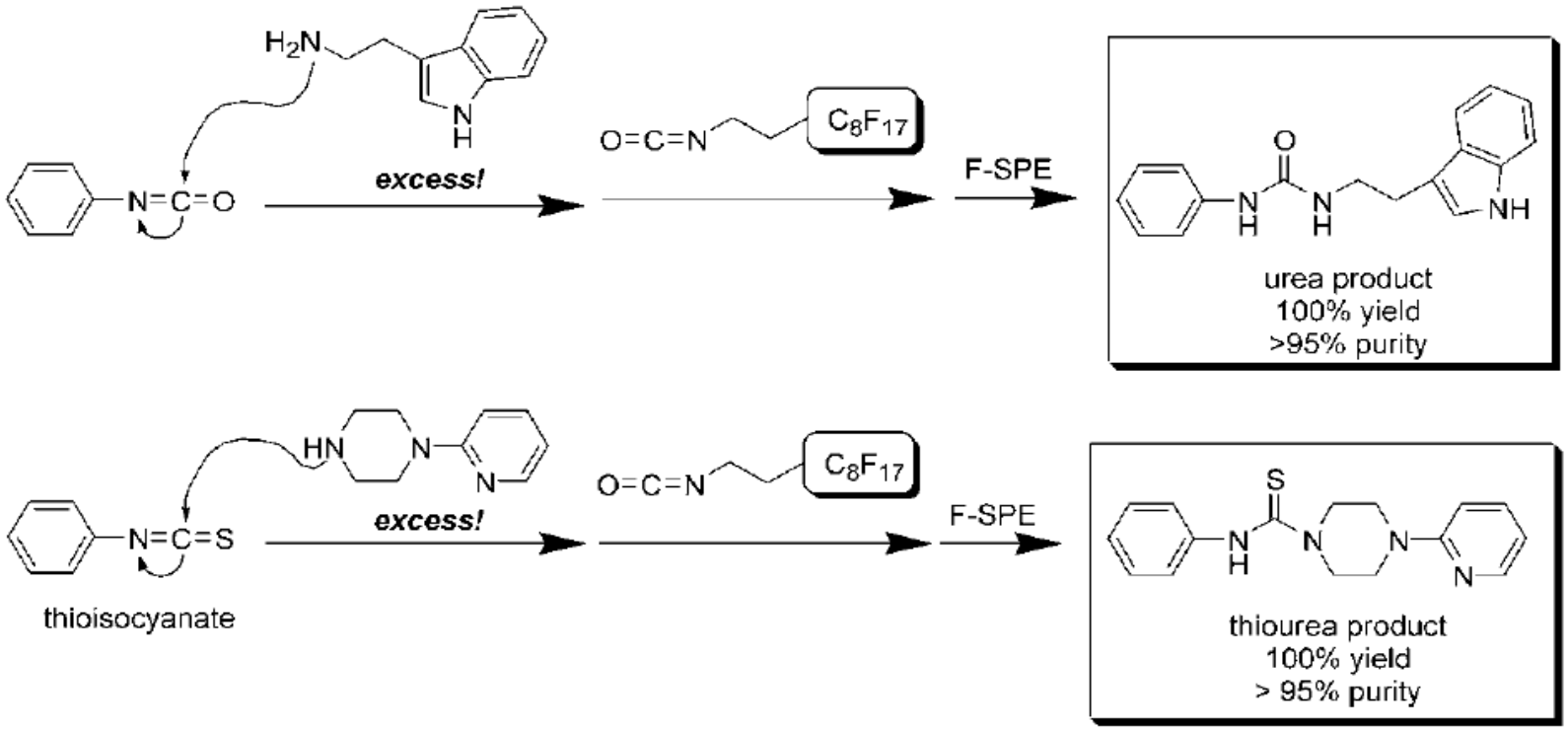
- Two general fluorous strategies we'll quickly look at:





Solution Phase Synthesis: Fluorous technology

- Nucleophiles scavenged by an electrophilic fluorous scavenger
  - Examples: urea and thiourea synthesis



Solid or Solution Phase Combinatorial Synthesis?

Solid Phase

+ Easy purification

+ Easy automation

+ Split and mix synthesis

+ Pseudo-dilution effects

- Adapt chemistry to solid phase and develop linking/cleaving strategies

- Reaction monitoring difficult

- Limited scale

- Expensive; polymers, excess reagents

Useful for the synthesis of larger series of compounds

Solution Phase

+ Chemistry not limited by support or linker

+ Monitor by traditional techniques

+ Purification possible after each step

+ Unlimited amounts(scales) available

+ Avoids extra steps for linking, etc

+ Mixture or parallel synthesis

- Parallelization and automation requires more initial effort

- Time consumimg purifications

- Removal of excess reagents and reactants limits scope

Efficient for small libraries

Synthetic Strategies towards Combinatorial Libraries

1. Parallel Synthesis towards Combinatorial Libraries
2. Tea Bag method
3. Multipin method
4. Split-mix (Split-pool) Synthesis towards Combinatorial Libraries
5. Reagent Mixture Synthesis towards Combinatorial Libraries

Parallel and Combinatorial Synthesis

- We'll address these two synthetic problems:
  - [a] tiny library: dipeptides consisting of 2 amino acids:
    - H-AA^1-AA^2-OH
    - The two amino acids we'll use are **Ala** and **Gly**
    - There will be **2*2 = 4** compounds in this library!
  - [b] larger library: tripeptides consisting of 3 amino acids:
    - H-AA^1-AA^2-AA^3-OH
    - The three amino acids we'll use are **Ala**, **Gly**, and **Trp**
    - There will be **3*3*3 = 27** compounds in this library!

H-Ala-Ala-OH	H-Gly-Ala-OH
H-Ala-Gly-OH	H-Gly-Gly-OH

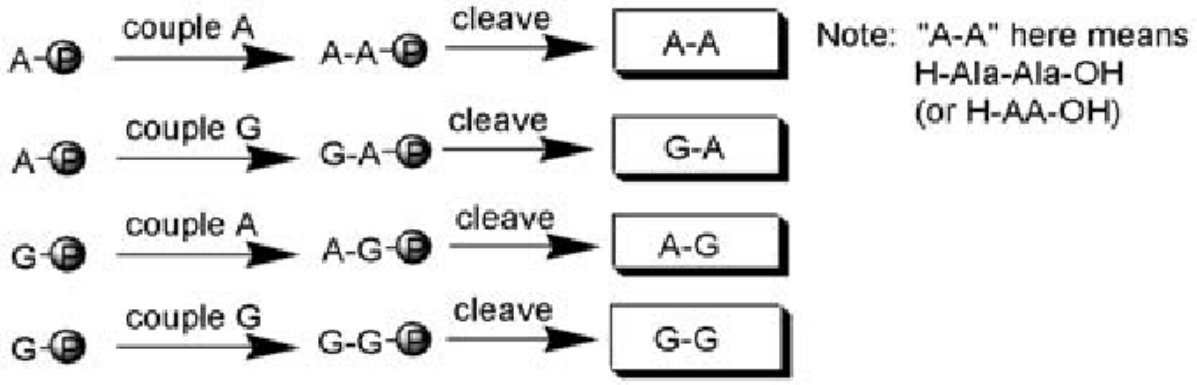
tiny library [a]

H-Ala-Ala-Ala-OH	H-Ala-Gly-Ala-OH	H-Ala-Trp-Ala-OH
H-Ala-Ala-Gly-OH	H-Ala-Gly-Gly-OH	H-Ala-Trp-Gly-OH
H-Ala-Ala-Trp-OH	H-Ala-Gly-Trp-OH	H-Ala-Trp Trp-OH
H-Gly-Ala-Ala-OH	H-Gly-Gly-Ala-OH	H-Gly-Trp-Ala-OH
H-Gly-Ala-Gly-OH	H-Gly-Gly-Gly-OH	H-Gly-Trp-Gly-OH
H-Gly-Ala-Trp-OH	H-Gly-Gly-Trp-OH	H-Gly-Trp Trp-OH
H-Trp-Ala-Ala-OH	H-Trp-Gly-Ala-OH	H-Trp-Trp-Ala-OH
H-Trp-Ala-Gly-OH	H-Trp-Gly-Gly-OH	H-Trp-Trp-Gly-OH
H-Trp-Ala-Trp-OH	H-Trp-Gly-Trp-OH	H-Trp-Trp Trp-OH

larger library [b]

Parallel and Combinatorial Synthesis

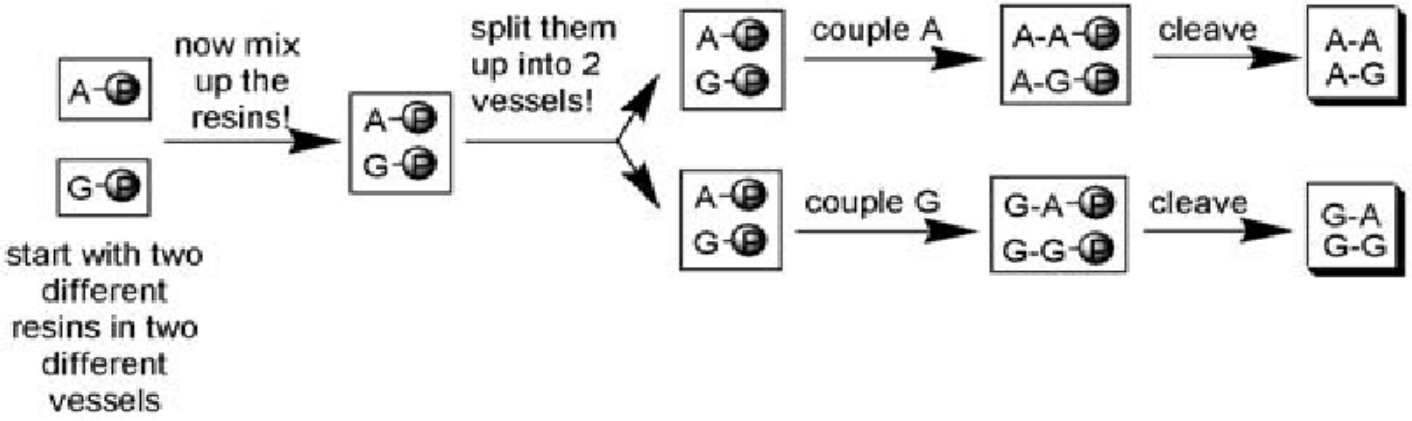
- Parallel synthesis of the tiny library [a]



- Four individual dipeptides isolated
- 4+4=8 reactions that were carried out

Parallel and Combinatorial Synthesis

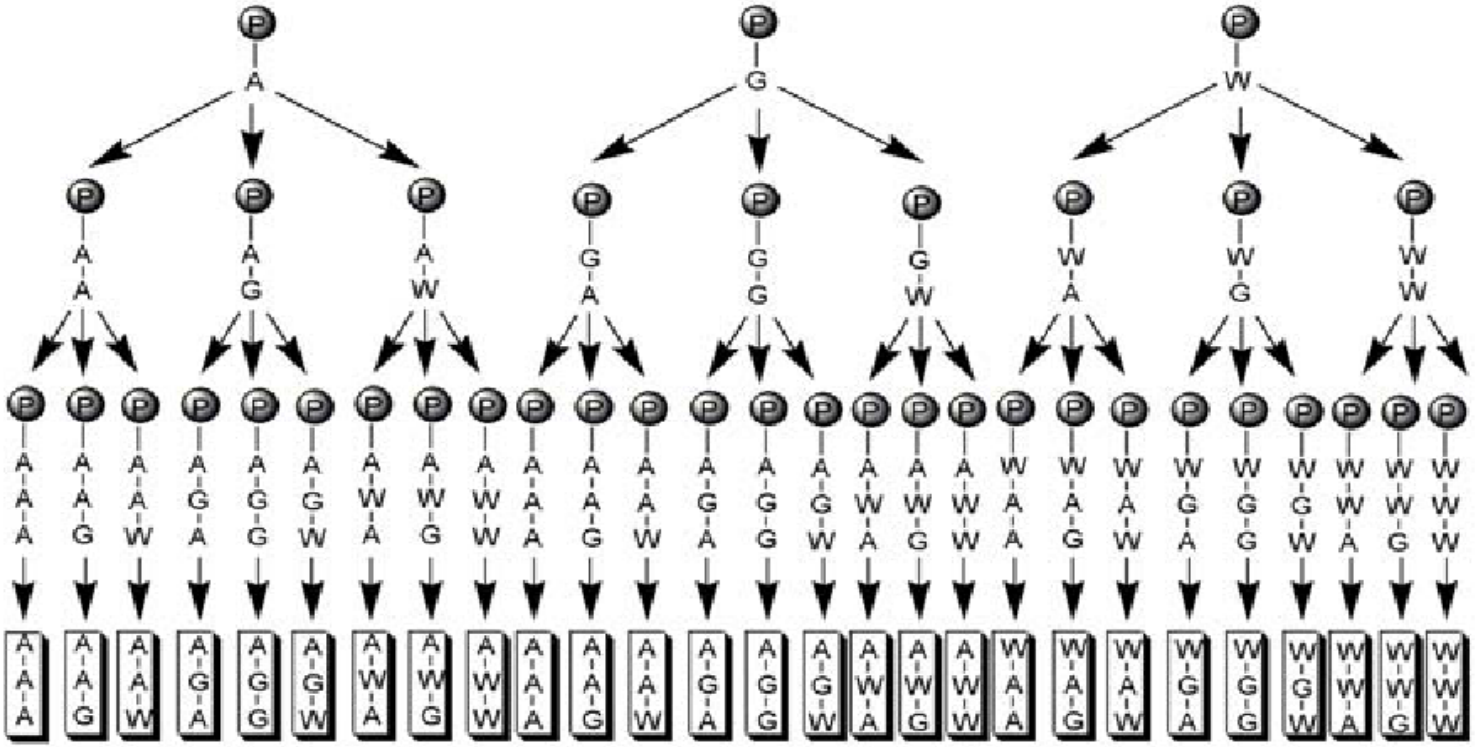
- Combinatorial synthesis of the tiny library [a]:
  - This is also called the "mix and split" method



- Two groups of two dipeptides each are isolated
- 2+2=4 reactions that were carried out
- Hmm… we're making mixtures of compounds, is that ok? (yes)

Parallel and Combinatorial Synthesis

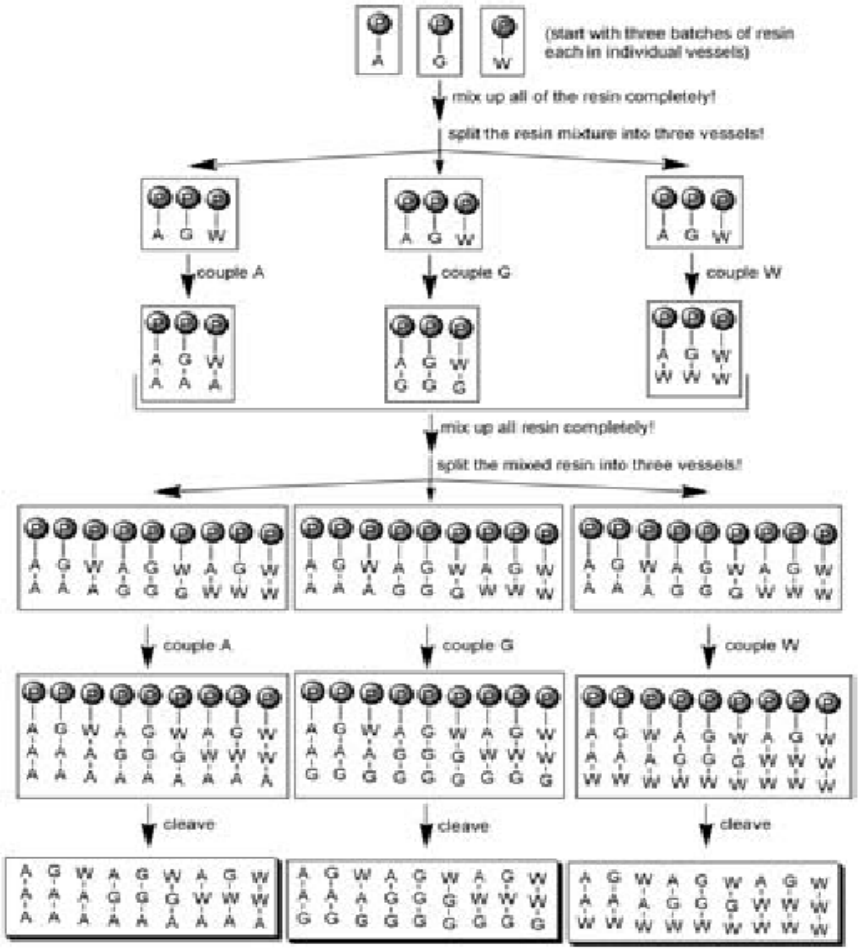
- Parallel synthesis of the larger library [b]



- 27 individual tripeptides isolated
- (3*3) + (3*9) + (3*9) = 63 reactions carried out

Parallel and Combinatorial Synthesis

- Combinatorial synthesis of the larger library [b]



Note:

3+3+3 = only 9 rxns done!

3 groups of 9 tripeptides each isolated

Parallel and Combinatorial Synthesis

- Summary: # of reactions carried out using both methods:

	parallel	combinatorial
library [a]:	**8**	**4**
library [b]:	**63**	**9**

- What if we were making tripeptides with all 20 L-amino acids?

20*20+(20*20*20)*2	3*20
= 16400(!)	**= 60**

- Or tripeptides with all 40 L- and D- amino acids?

40*40+(40*40*40)*2	3*40
= 129600(!)	= **120**

Parallel and Combinatorial Synthesis

- Summary: # of reactions vessels with both methods:

	parallel	combinatorial
library [a]:	**4**	**2**
library [b]:	**27**	**3**

- What if we were making tripeptides with all 20 L-amino acids?

20*20*20	**20**
= **8000(!)**	

- Or tripeptides with all 40 L- and D- amino acids?

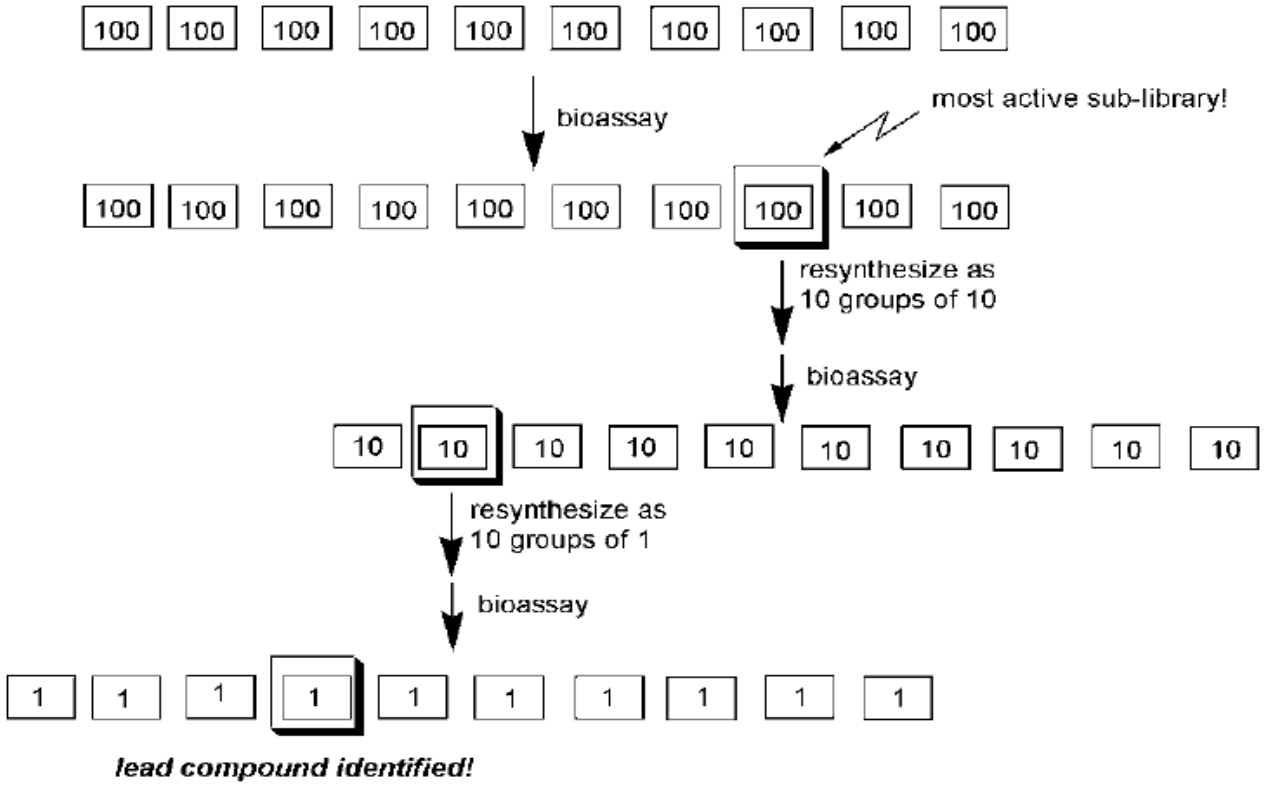
40*40*40	**40**
= **64000(!)**	

Parallel and Combinatorial Synthesis

- So now we have large compound libraries – how do we screen them for biological activity?
  - Parallel synthesis
    - Each individual compound can be assayed directly!
      (with whatever biological target: receptor, enzyme, DNA, etc.)
  - Combinatorial synthesis
    - Each compound is exists in a sub-library
    - But we want to find specific lead compounds!
    - Thus each sub-library of compounds is screened as a whole!
    - When a "hit" is found, we then "deconvolve" the sub-library
    - Deconvolution eventually leads to the single active compound!
    - We'll discuss one example of a deconvolution method now

Parallel and Combinatorial Synthesis

- Combinatorial synthesis: deconvolution
  - "Iterative deconvolution"
    Assume a library of 1000 compounds (10 pools of 100):



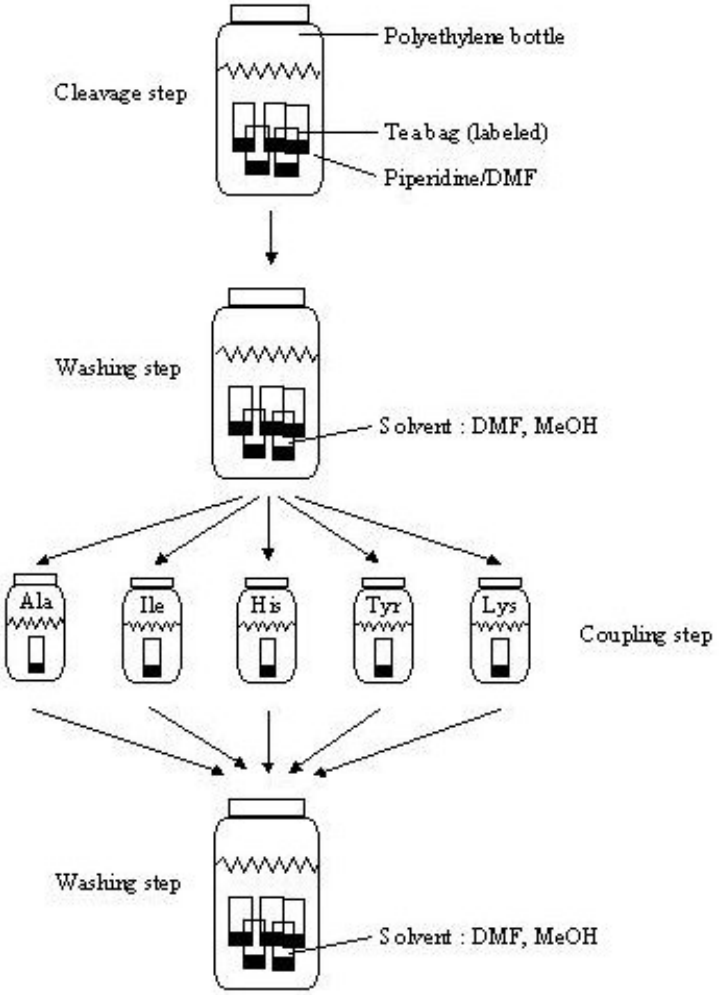
Parallel and Combinatorial Synthesis

- Combinatorial synthesis: deconvolution
  - "Iterative deconvolution"
    - This was a very early method in combinatorial synthesis
    - It's a pain because sub-libraries must be resynthesized! (very time consuming)
    - Thus mostly replaced by other deconvolution methods
      - For example:
        - "barcoding" the structure of the each compound (with chemical tags, etc.)
        - … or beads embedded with microchips that transmit radio-frequency signals describing the chemical structure of each compound

Tea-Bag Method

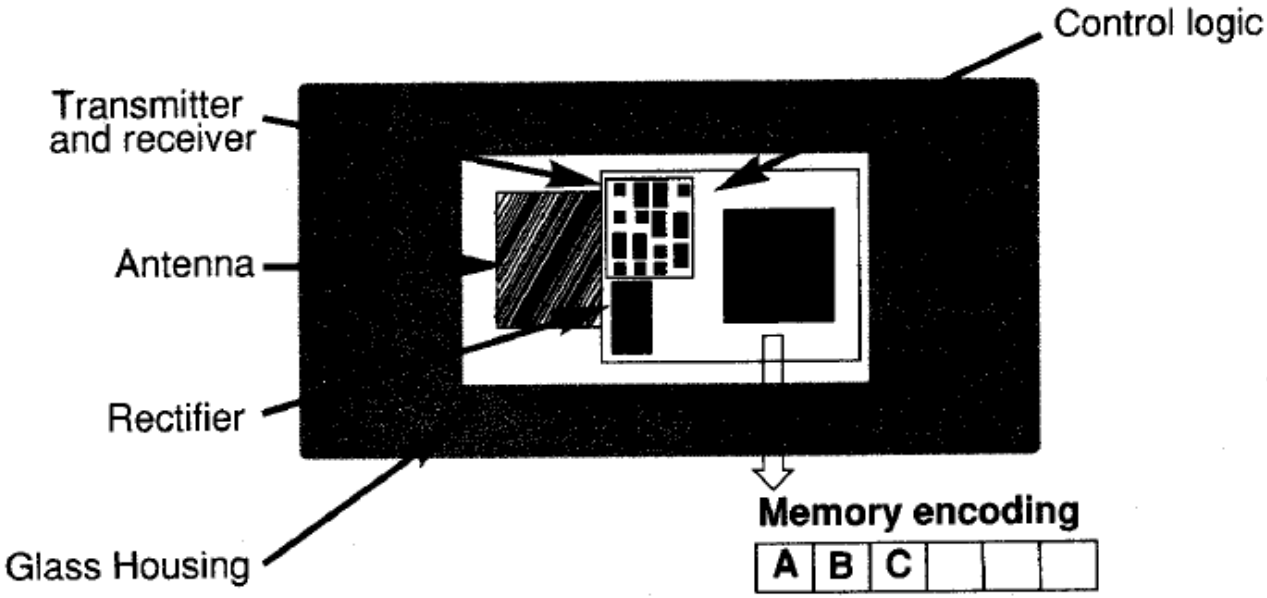
- Polyethylene bag with fine holes, similar to real tea bag, are filled with resins and each bag is put in the different reaction vessels to carry out reaction.

- the bag takes the role of filter and preventing resin mixing between reactions, and by labeling each bag, the synthesized compound structure can be identified.



Electronic Encoding

- Radiofrequency memory chips allow libraries to be tagged in a machine-readable form
- The chips (8 x 1 mm) can be incorporated into various reaction platforms (e.g. beads, tubes, bags, pins or cans)

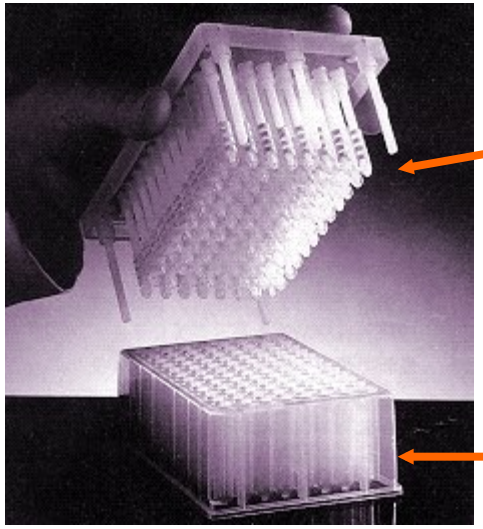


Nova, M.; Nicoloau, K. C. *et al. Angew. Chem., Int. Ed. Eng.* **1995**, *34*, 2289.

Armstrong, R. W. *et al. J. Am. Chem Soc.* **1995,** *117*, 10787.

Multipin Method – Parallel ?

- Synthesize on polyacrylate grafted polyethylene rods
- Utilize conventional solid phase synthesis methods
- Preparation of up to 10,000 spatially separate compounds by this method



Growing peptide on a pin
(Individual pins with crowns,
1 to 7 mmol loading capacity)

reagents, reactants in 96-well plate

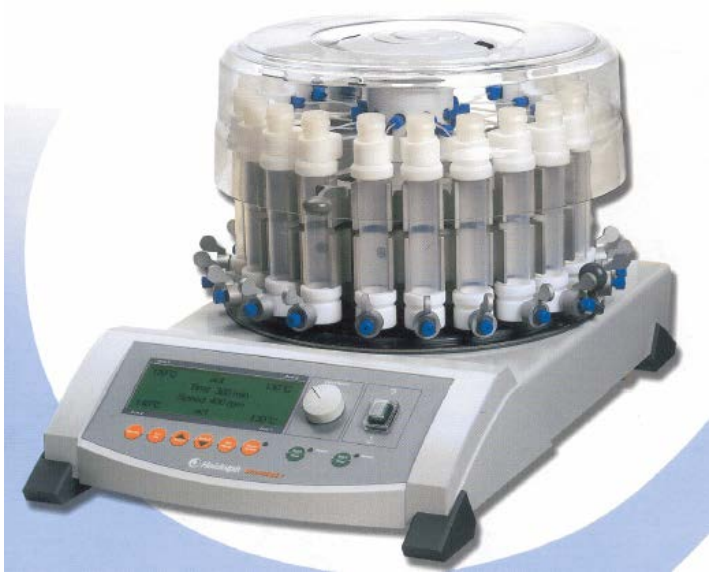
Comparison of Combinatorial Synthetic Strategies

Technique	Single compound /mixture	Speed of synthesis	SAR retrieval	Utility
split and mix	mixture (one compound / bead)	moderate	slow	lead identification lead optimization
encoded split and mix	mixture (one compound / bead)	moderate	moderate	lead identification lead optimization
parallel synthesis	single	slow	fast	lead optimization
mixture synthesis (deconvolution)	mixture	fast	slow (fast)	lead identification

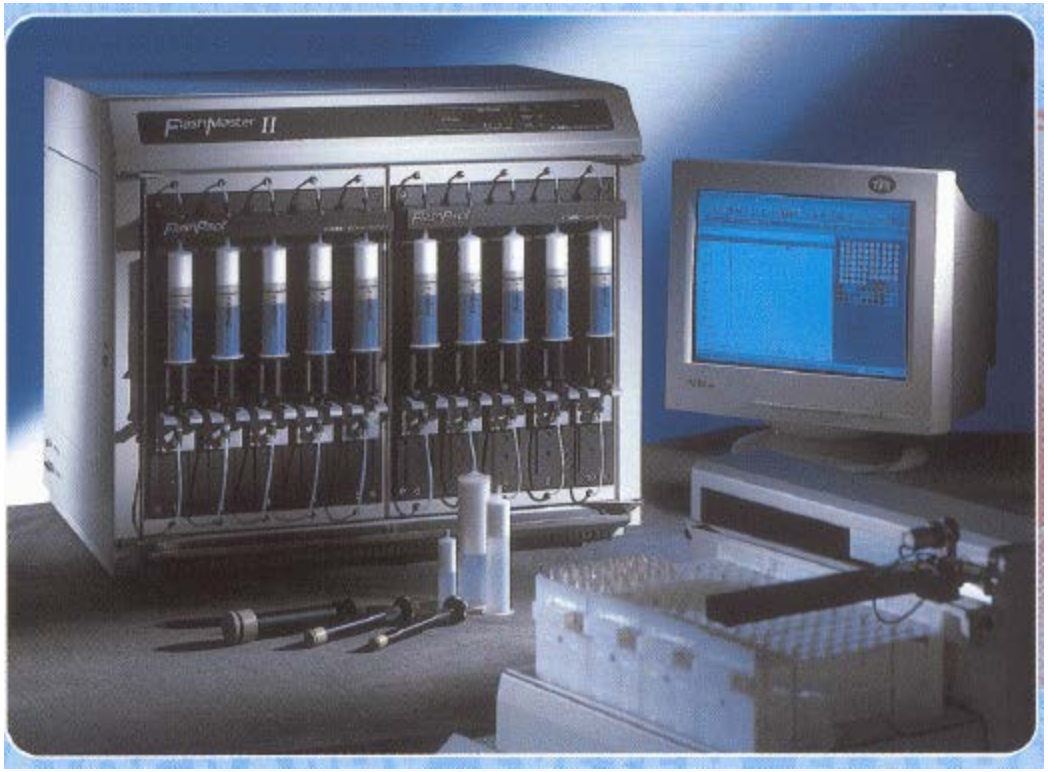
Guiles, J. W. *et. al. Angew. Chem. Int. Ed. Eng* **1998**, *37*, 926

Parallel Synthesizer

Quest 210



Automated Flash Chromatography System



FlashMaster

Solvent Evaporation System

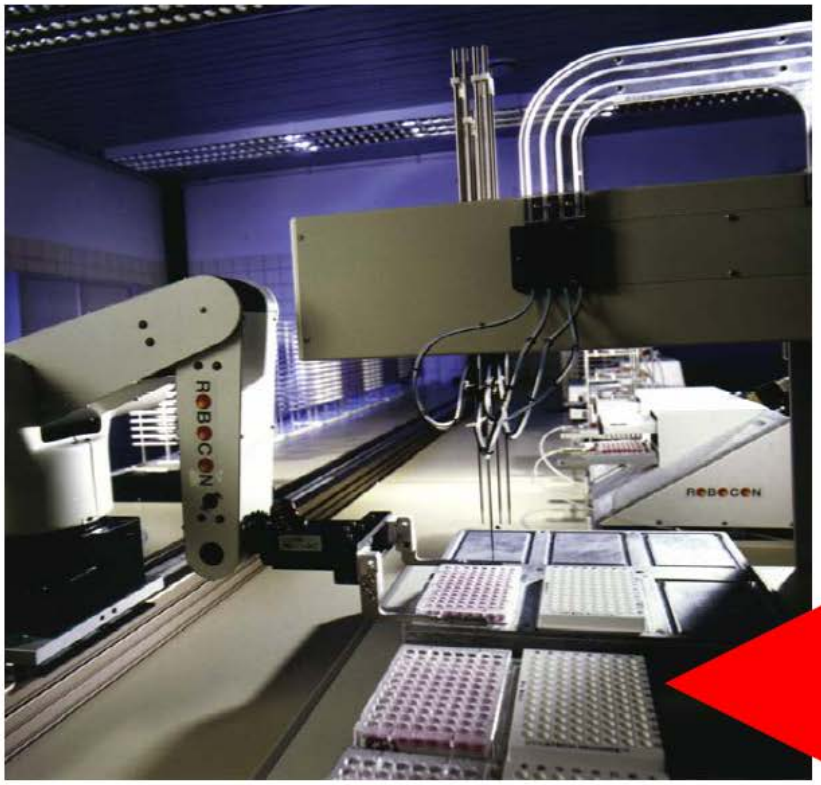
Large LED displays and simple keys make the DD-4X easy to program for a wide variety of applications. The membrane keypad is sealed for long-life in harsh environments.

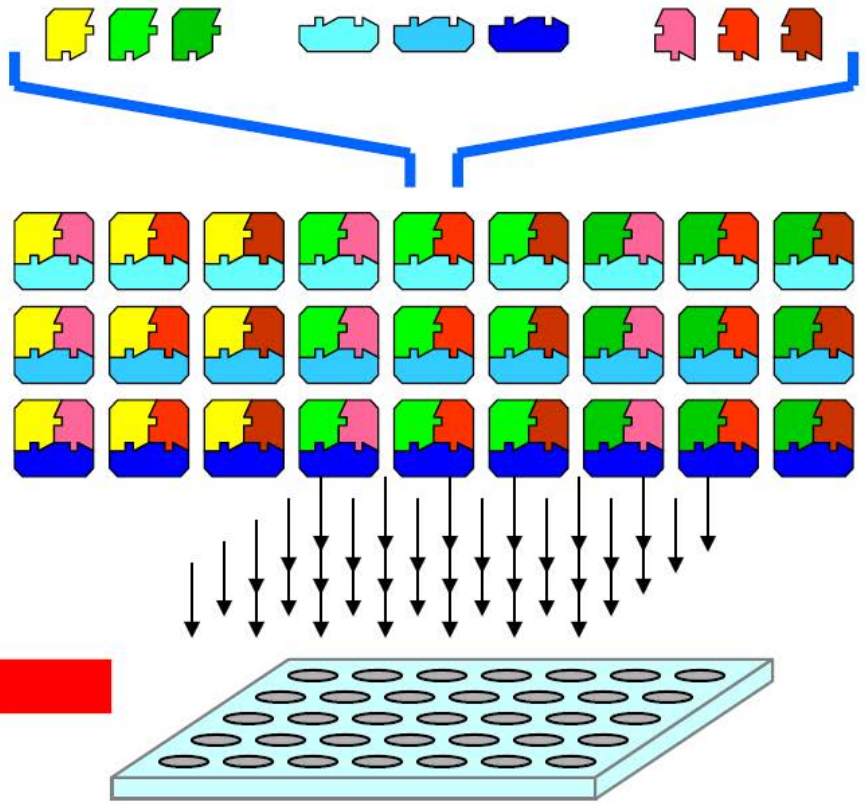
Fast interchangeable rotors for a wide range of applications.

Genevac

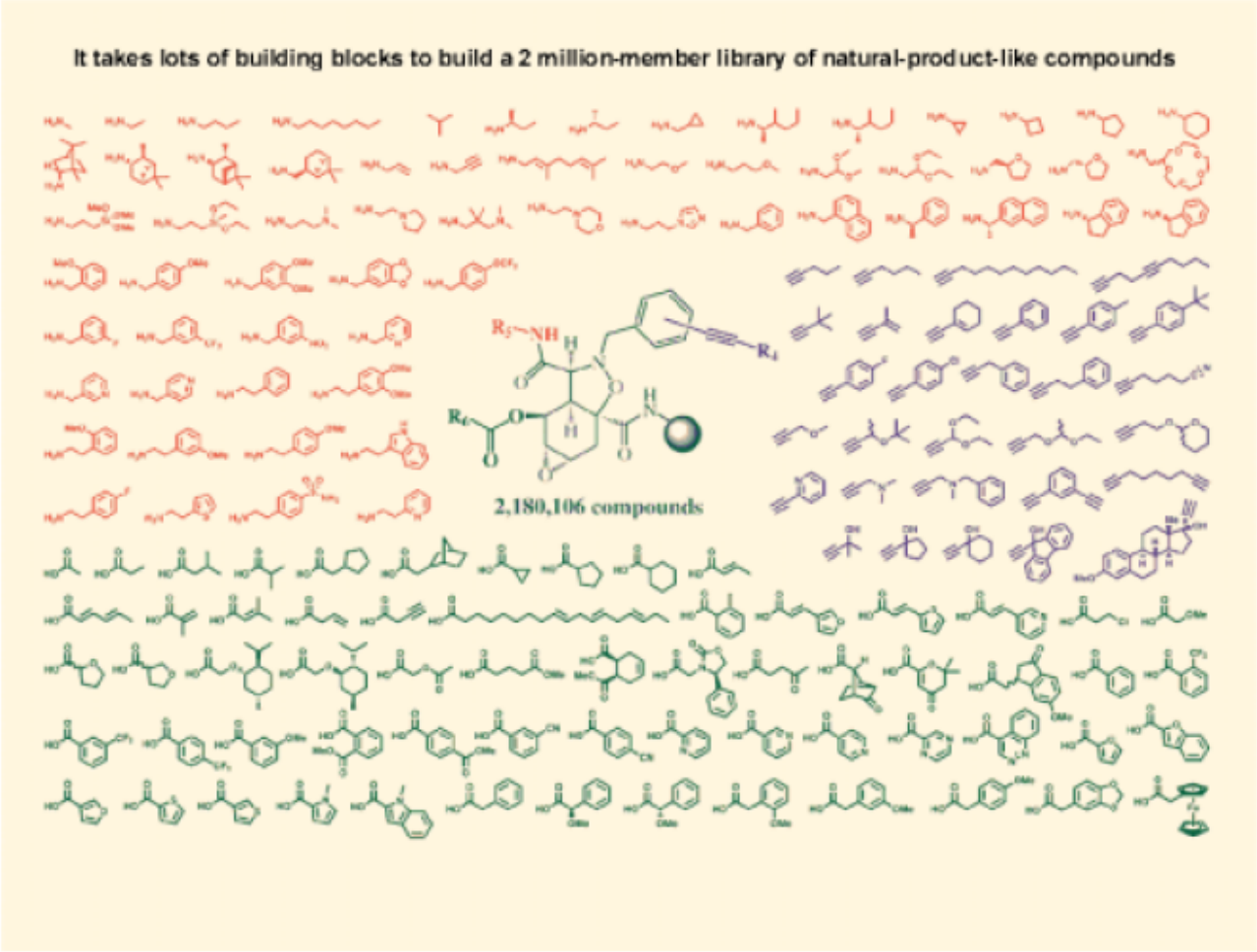
Chemical Library in Drug Discovery

biological testing → chemical synthesis





2 Million-member Library of Natural-Product-like Compounds by Schreiber



Derek S. Tan, Michael A. Foley, Brent R. Stockwell, Matthew D. Shair, and Stuart L. Schreiber*
Journal of the American Chemical Society; **1999**; *121*(39); 9073-9087.