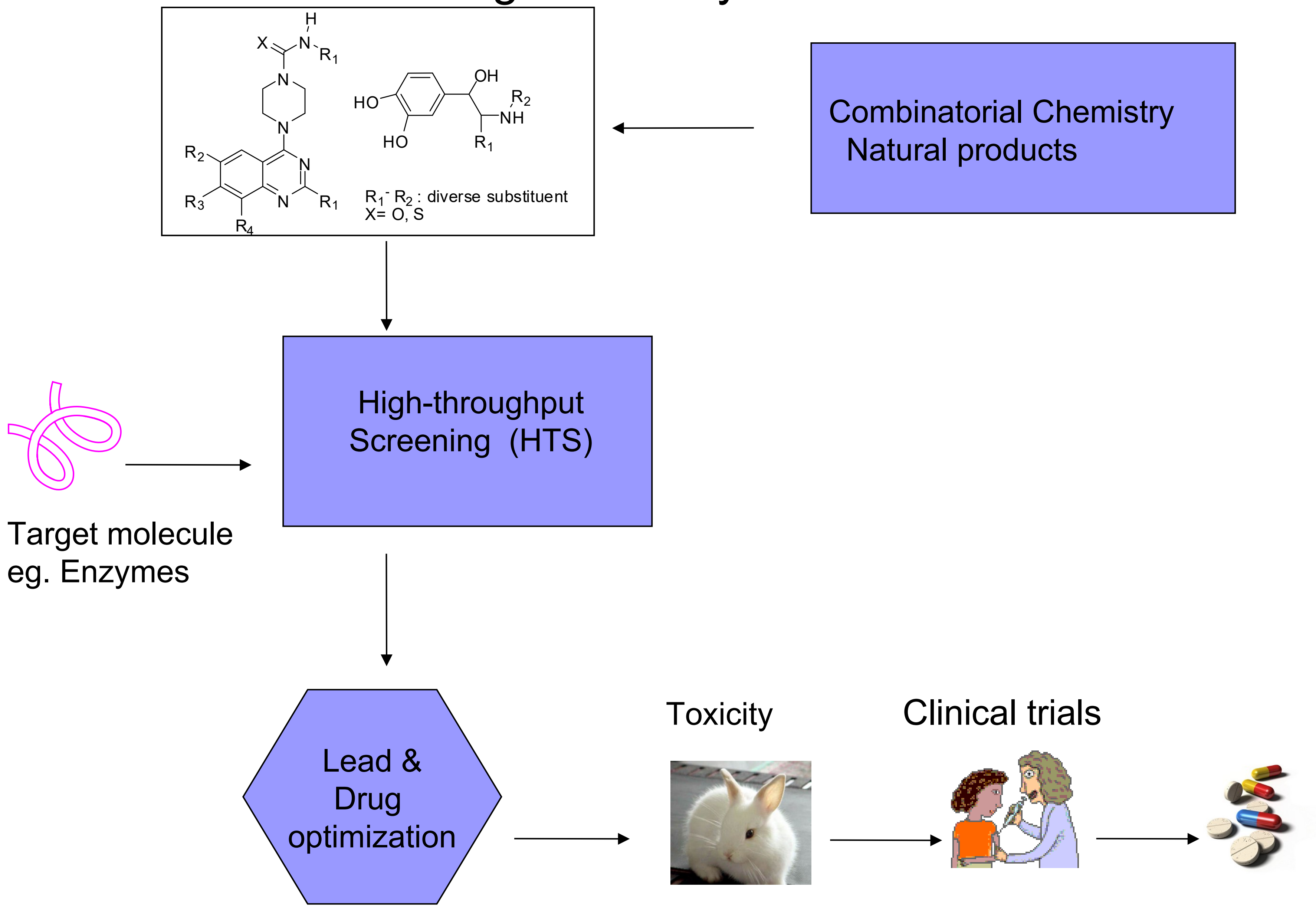
Combinatorial Chemistry As a Part of Drug Discovery Process

Drug Discovery Process



Combinatorial Chemistry

- Definition: the synthesis of chemical compounds as ensembles (libraries) and the screening of those libraries for compounds with desirable properties
- Potentially speedy route to new catalysts, materials and namely **drugs**
- Technique invented in the late 1980s and early 1990s to enable tasks to be applied to many molecules simultaneously

Combichem Techniques

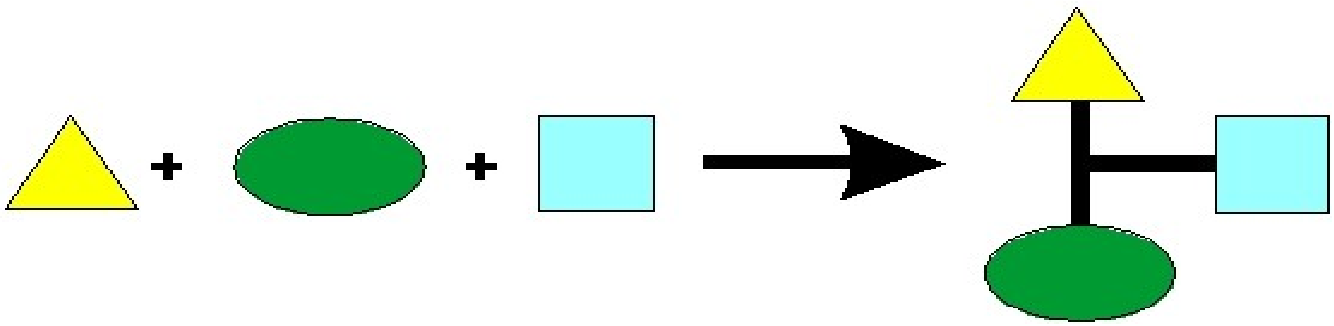
- Tools
  - Solid-phase synthesis – some reagents are anchored on resins
  - Sets of reagents (Monomers)
  - Linkers
  - Screening methods, preferably HTS (high-throughput screening)

Combichem Methods

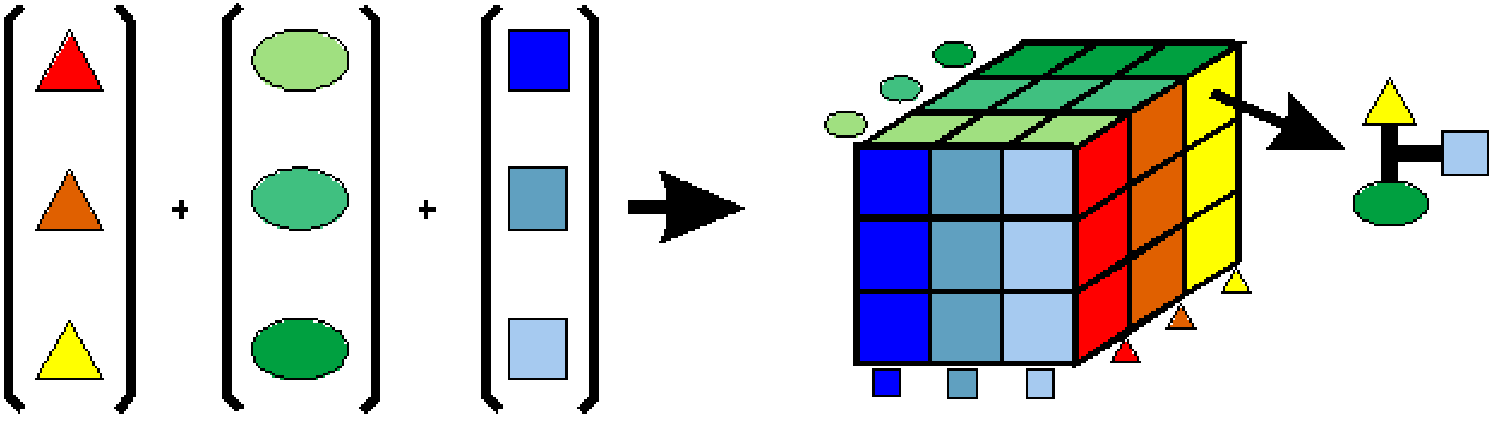
- Use of solid supports originally for peptide synthesis led to wider applications
- Products from one reaction are divided and reacted with other reagents in succession
  - Split-mix scheme: library size increases exponentially

Ilustration of a difference between classical and combinatorial synthetic approaches

"CLASSICAL" SYNTHESIS

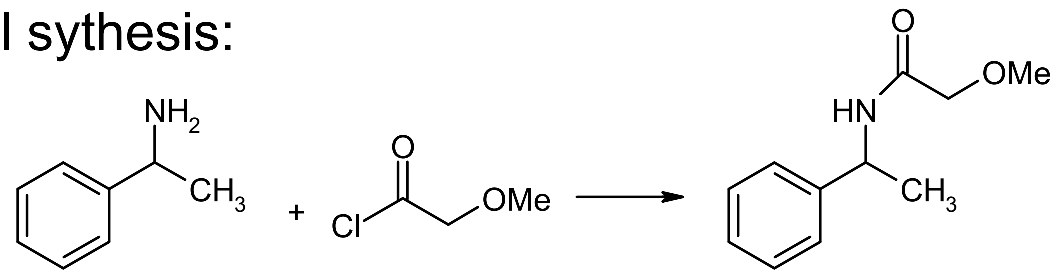


"COMBINATORIAL CHEMISTRY APPROACH"
combinatorial synthesis approach



Classical and combinatorial synthesis

Classical sythesis:



1 Reactant * 1 Reactant = 1 Product

Combinatorial synthesis:

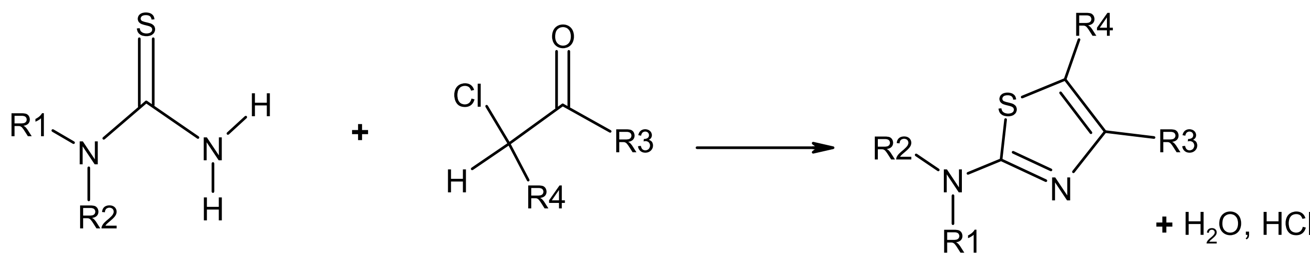
Eg.: 50 Reactants * 20 Reactants = 1000 Products

In more stages eg.: 50 * 20 * 20 = 20 000 Products

Synthetic methods for reaching of combinatorial libraries

The goal is to enable to get many compounds by the same chemical reactions

Parallel Synthesis (*diversity orientiented*)

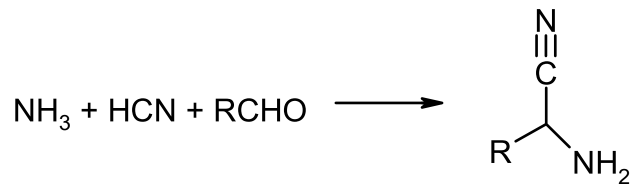


Problem: Many reaction vessels are necessary mainly if a synthesis has many stages.

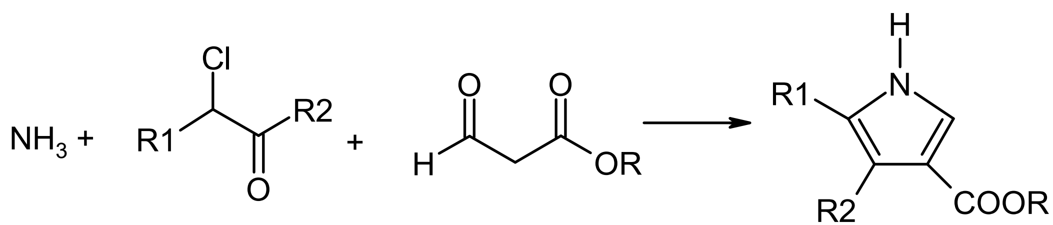
Solution proposal: multi-component reactions

Historic Milestones

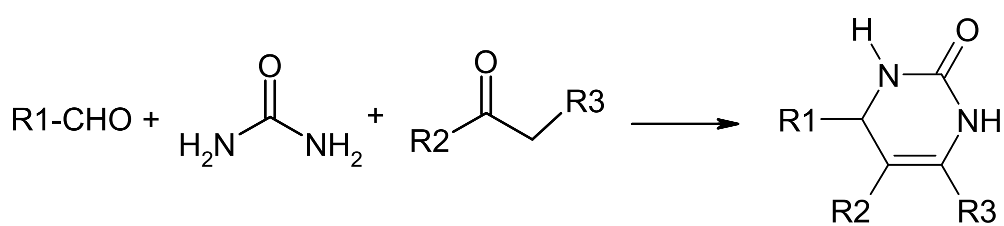
A.Strecker (1850)



A.R. Hantzsch (1890)

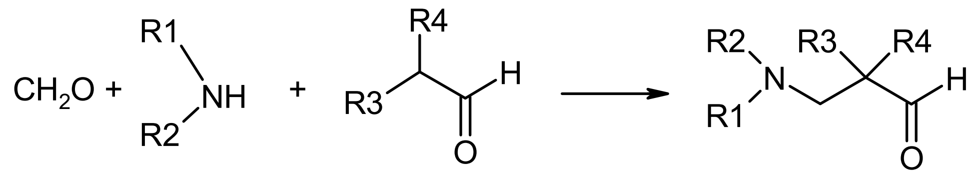


P. Biginelli (1893)

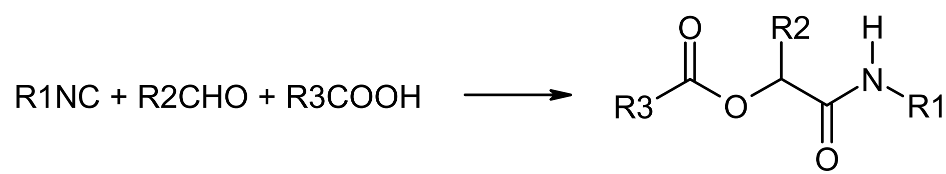


Historic Milestones

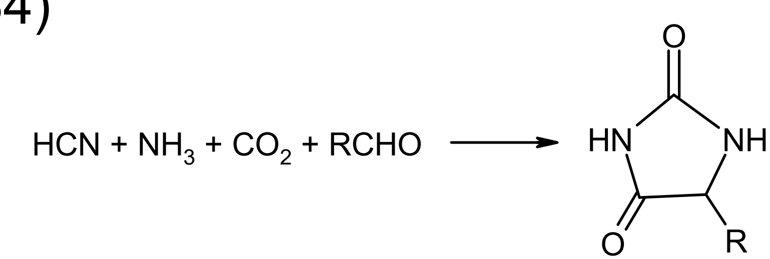
C. Mannich (1912)



M. Passerini (1921)

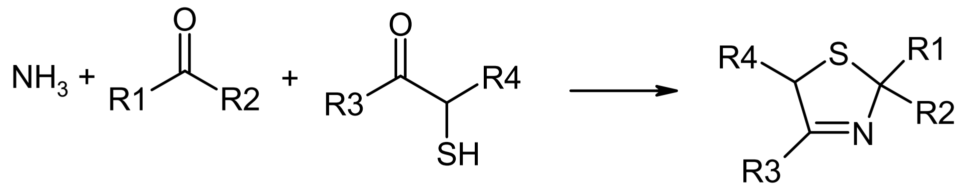


H.T. Bucherer (1934)

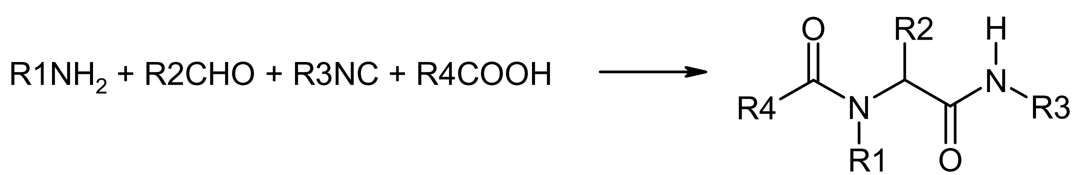


Historic Milestones (III)

F. Asinger (1958)



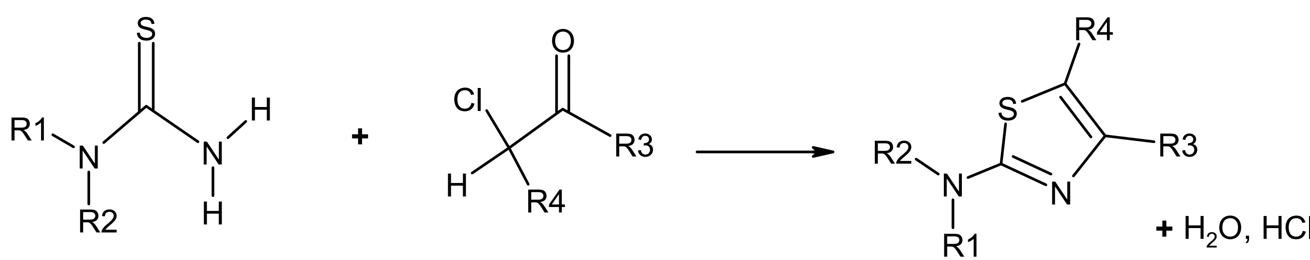
I. Ugi (1959)



Synthetic methods for reaching of combinatorial libraries (II)

The goal is to enable to get many compounds by the same chemical reactions

Parallel Synthesis (*diversity orientiented*)

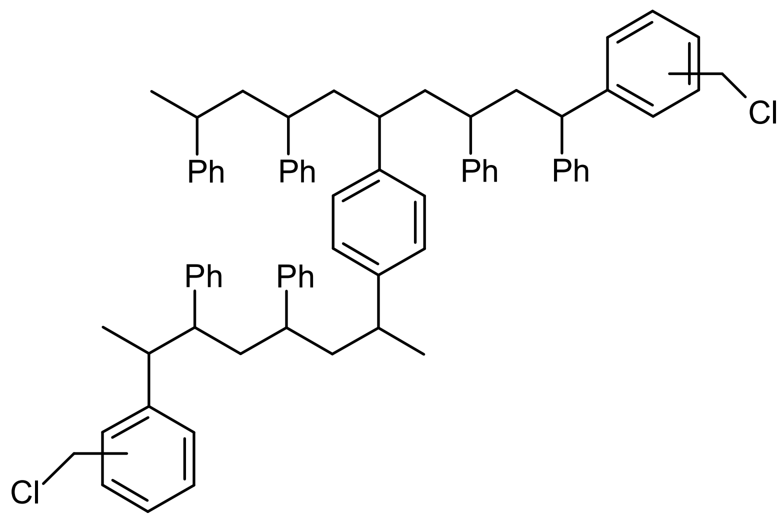


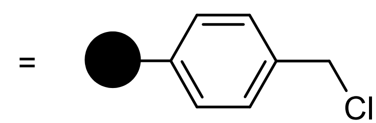
One of the biggest problems is the isolation of a product from the reaction mixture mainly in non-crystalline compounds and small amounts.

Proposal of a solution: Temporary fixing to a solid holder

Solid phase synthesis

Anchoring on a polymer resin with suitable functional groups
small polystyrene balls = *beads*





Crosslinked co-polymer of polystyrene with 1-1.5% divinylbenzene

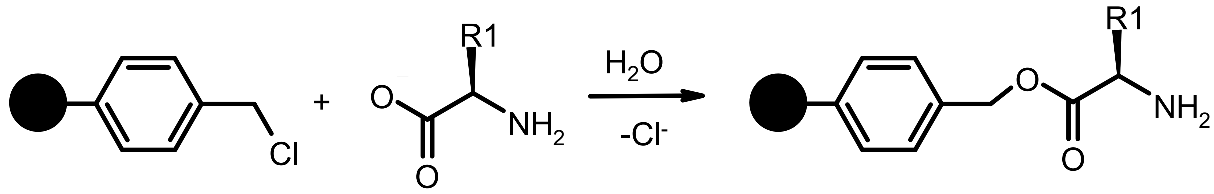
Solid phase synthesis

Originally developed for synthesis of long chain polypeptides.

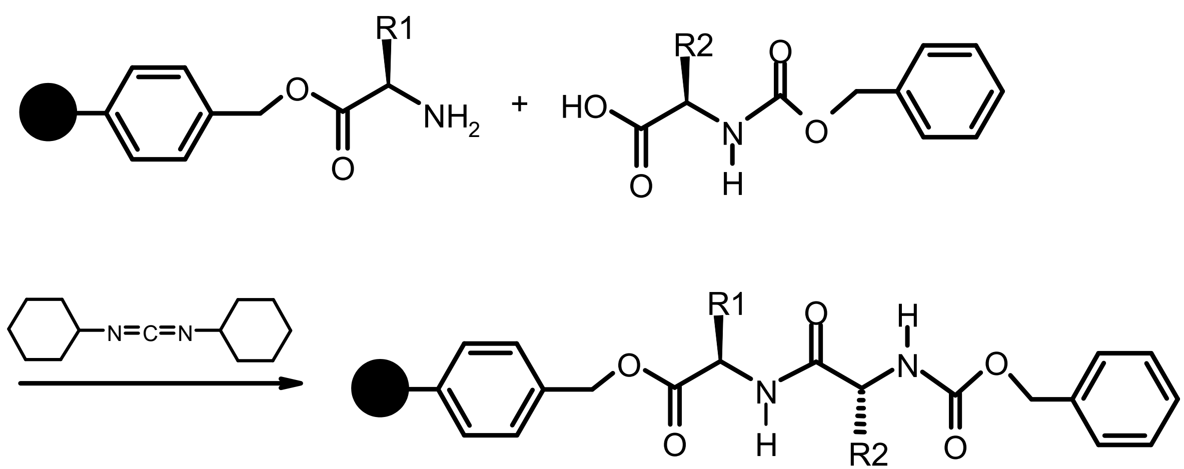
The peptide remains anchored on polystyrene beads.

An example of Merrifield:

1. C-terminal amino acid

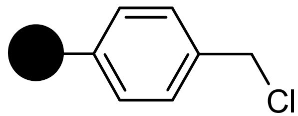


2. N-Boc protected amino acid (with benzyloxycarbonyl)

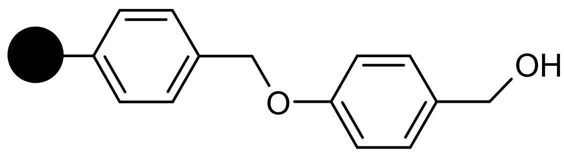


Lit. R. B. Merrifield *J.Am.Chem.Soc.* **85** (1963) 2149.

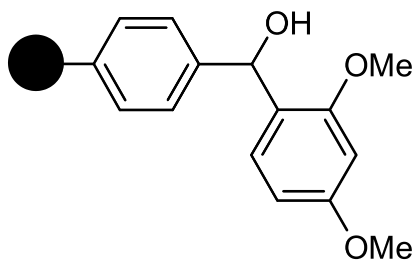
Linkers for synthesis on solid phase



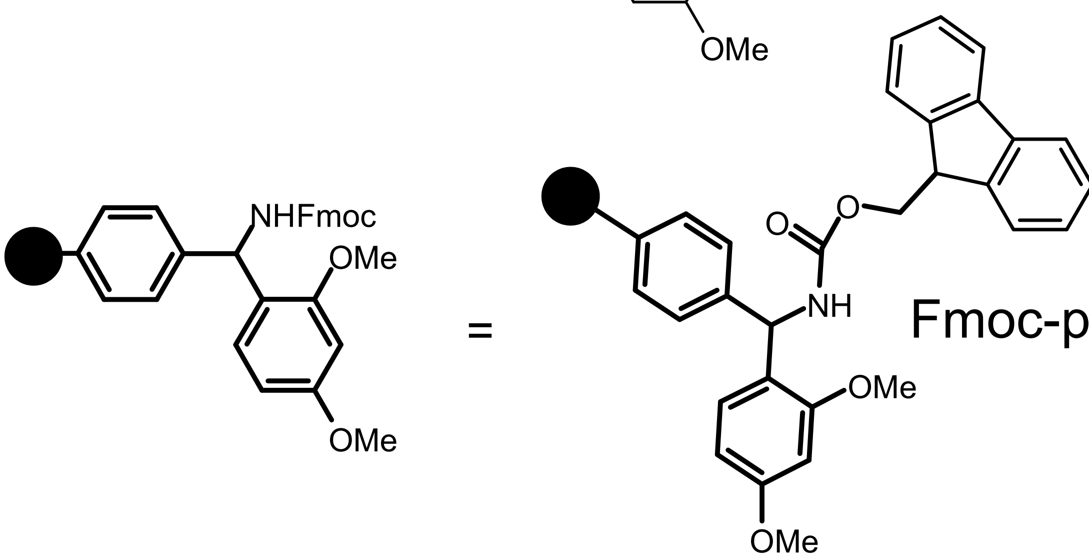
Merrifield resin



Wang resin

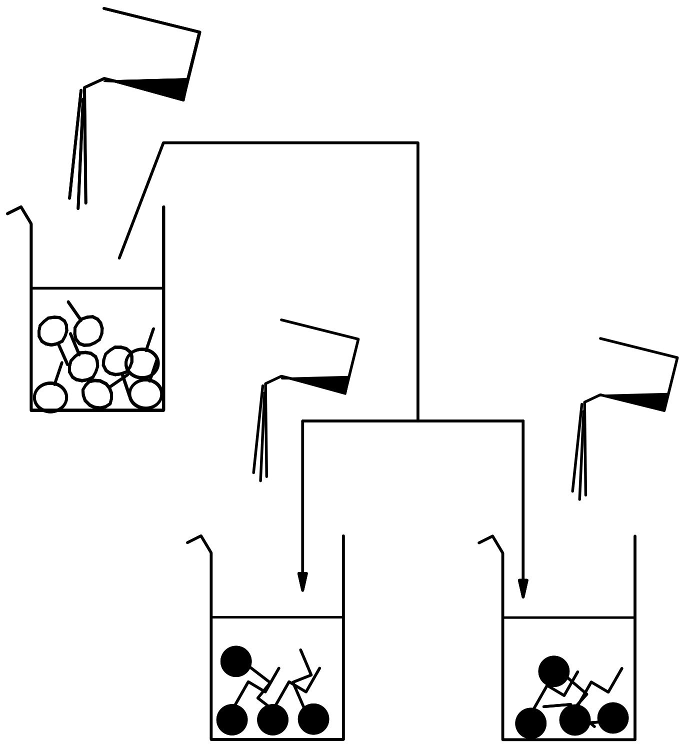


Rink acid linker



Fmoc-protected Rink amine linker

„Split-and-pool strategy“



If we start from one scaffold we can modify the substitition pattern in every new *pool* by *splitting*

Possible technical solution: magnetic beads

Synthesis of Peptide Libraries

The division of the product enables an efficient parallel synthesis for example for the construction of orthogonal libraries:

Systematic vary of the amino acid at the Xth position of a protein. Required for the epitop-mapping on antibodies

Ac-DVXXXX-NH2 20^4 peptides

↓

Ac-DVPXXX-NH2 20^3 peptides

↓

↓

Ac-DVPDYA-NH2

A successful restriction of the most active amino acid sequence (*split-and-mix*)

Synthetic methods for generation of combinatorial libraries

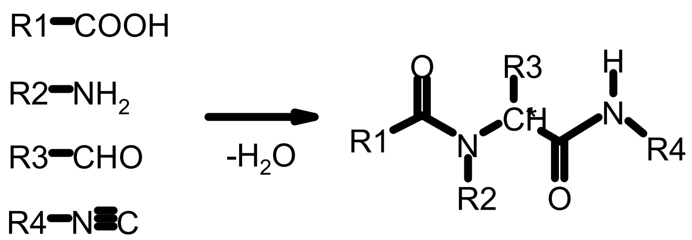
The reliable synthetic steps are required for the parallel synthesis for the construction of compound libraries, which are possible to perform also by means of synthetic robots, e.g. reactions such as

- reductive amination
- acylation
- Hantzsch synthesis of 2-aminothiazols
- Suzuki coupling (building of a C-C bond)
- Ugi condensation (dipetides)

Synthesis of peptides / Anchored on a solid phase

Ugi 4-components condensation

The reaction of an isonitrile with a carboxyl group and an aldehyde leads to a α-acyloxy-amide (Passerini reaction). If an amine is then added the appropriate bisamide is formed:



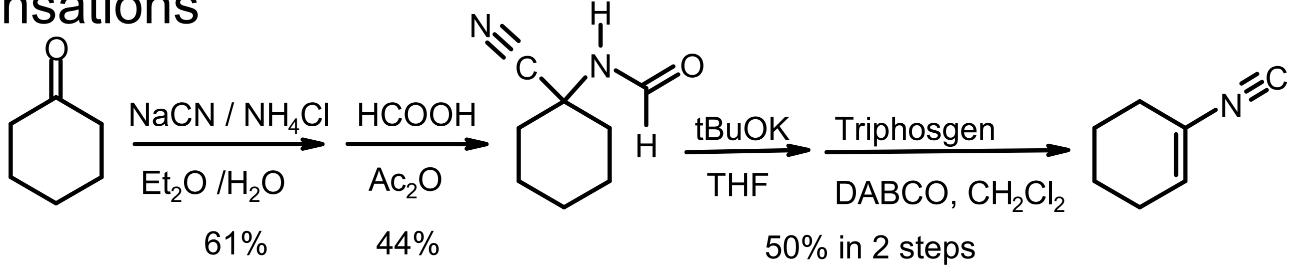
Disadvantage: only few isonitrils are commercilally available; their smell is very unpleasant

Solutions:

- Usage of an universal isonitrile which serves as a precursor for further products
- Formation of an isonitrile *in situ*

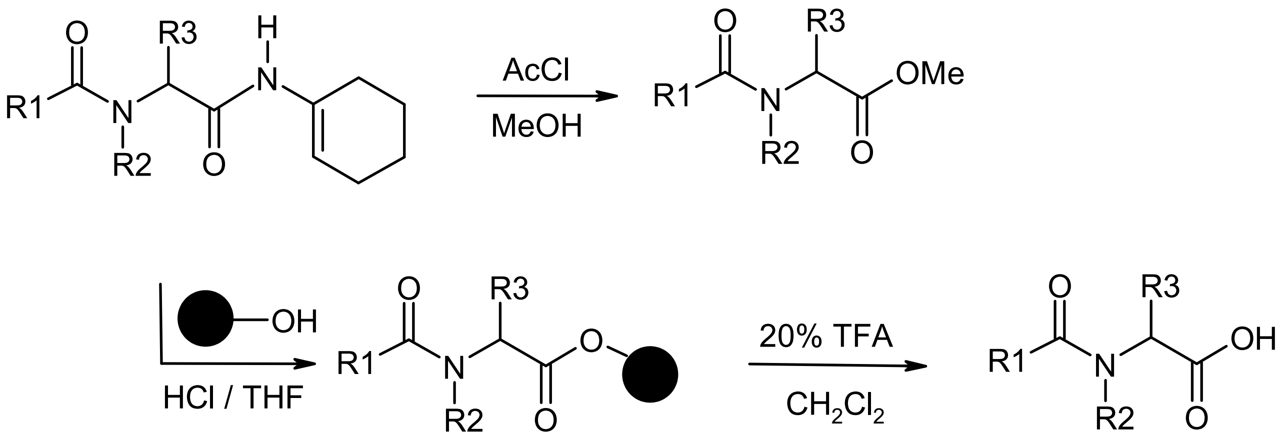
Synthesis of peptides / Anchored on a solid phase

1-isocyanocyclohexene as a versatile convertible isonitrile for Ugi-condensations



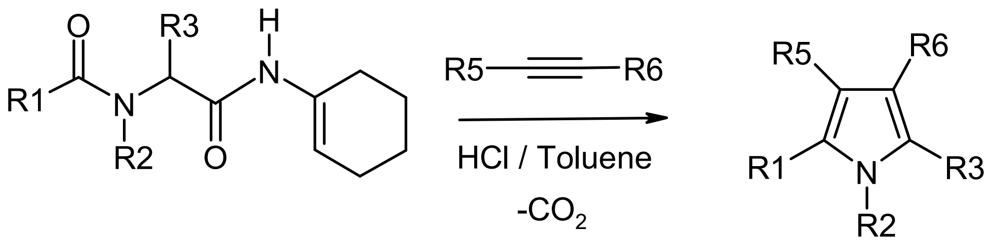
Enables a series of of products and also capture to a resin :

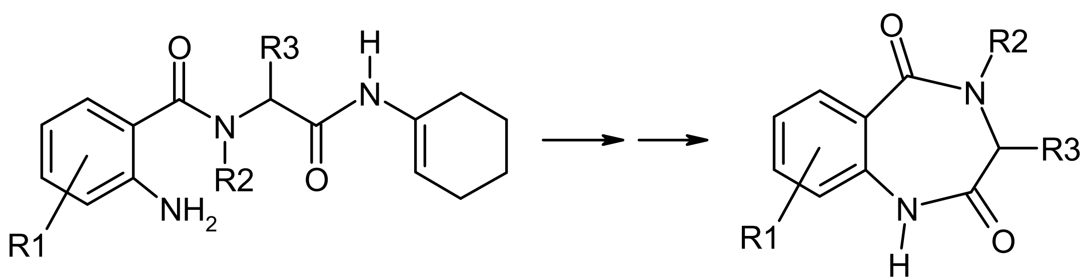
Keating & Armstrong *J.Am.Chem.Soc.* **118** (1996) 2574



1-isocyanocyclohexene as a versatile convertible isonitrile for Ugi-condensations

Possibilities of further reactions of Ugi Products



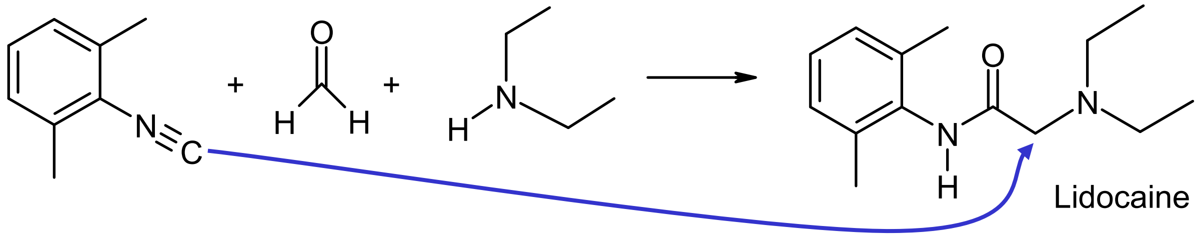


1,4-Benzodiazepine-2,5-dione

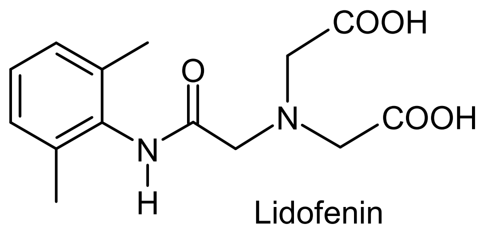
Keating & Armstrong *J.Am.Chem.Soc.* **118** (1996) 2574

3 components Ugi condensation

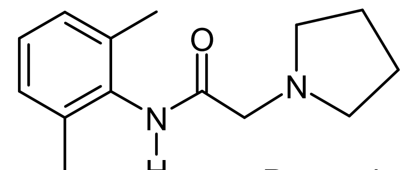
Paralell synthesis of local anesthetics of anilide type
(Morphochem, Munich)



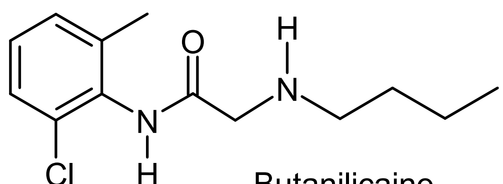
Lidocaine



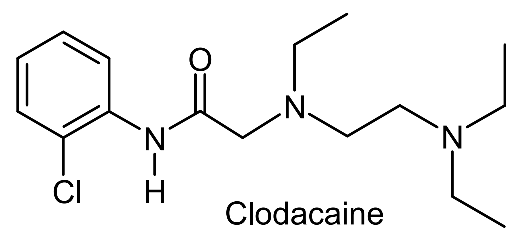
Lidofenin



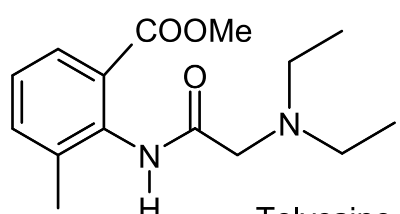
Pyrrocaine



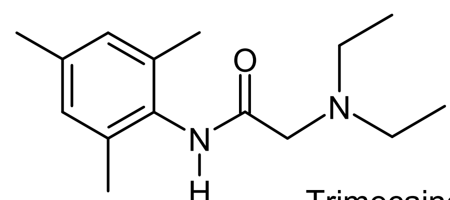
Butanilicaine



Clodacaine

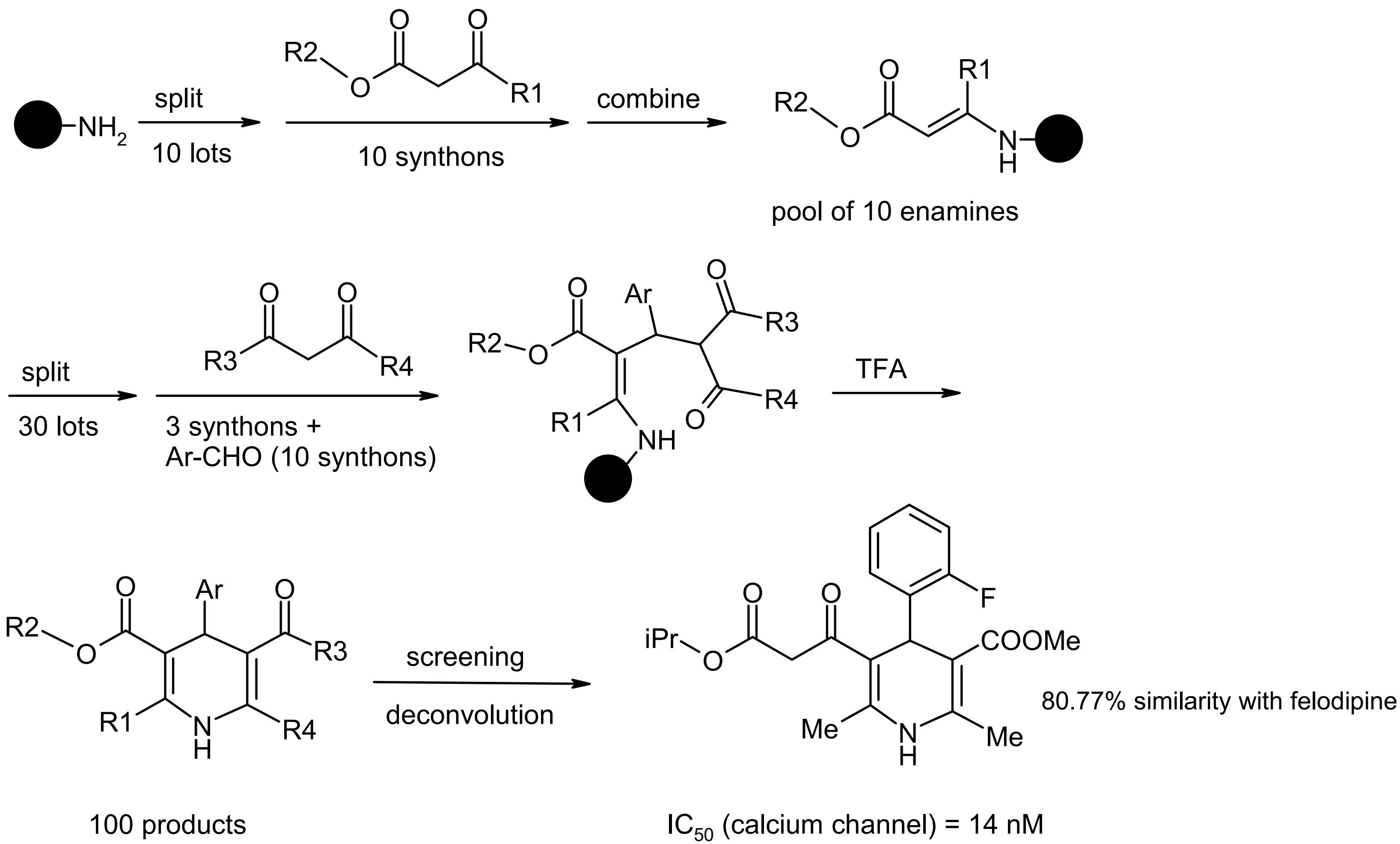


Tolycaine



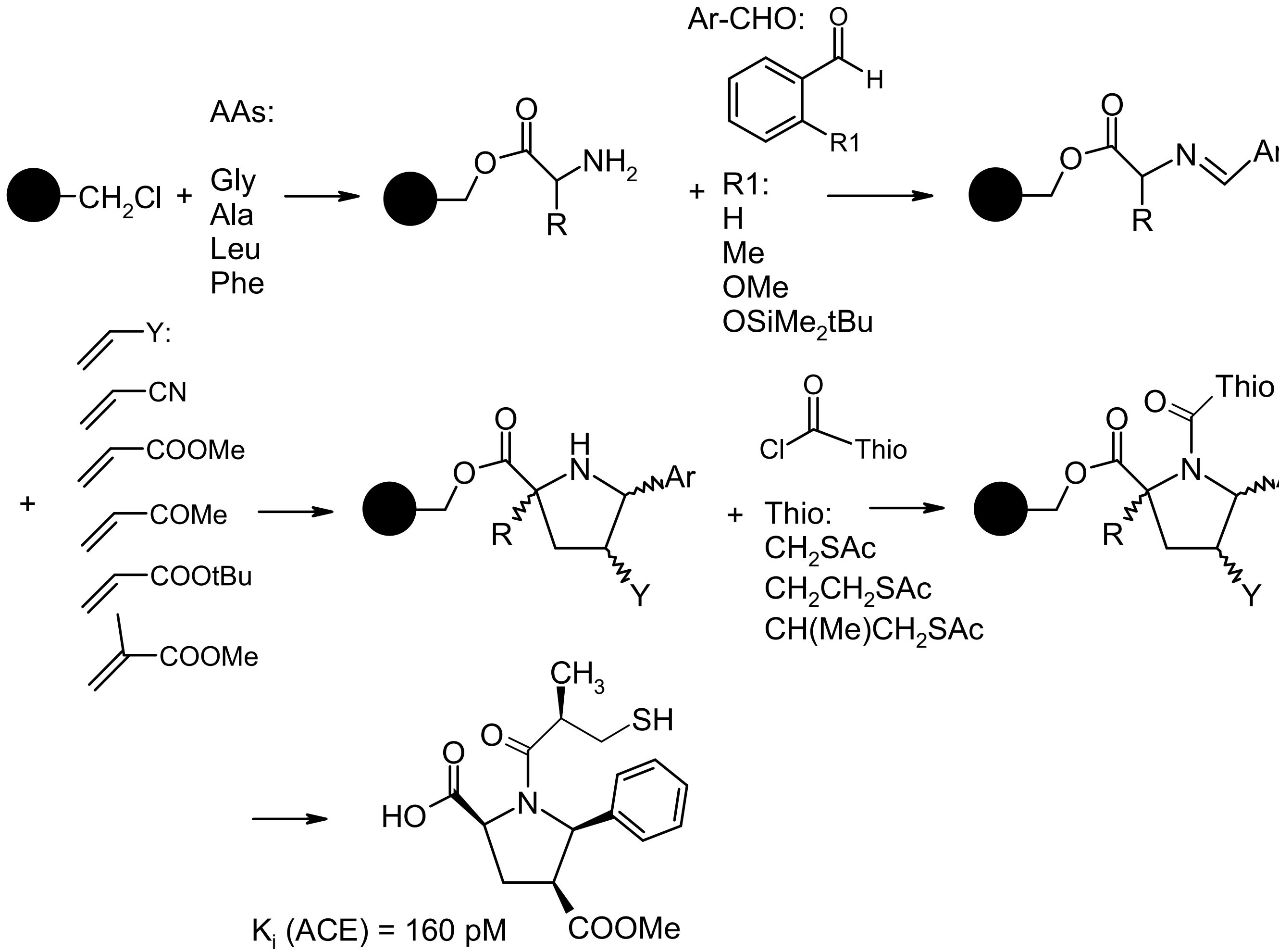
Trimecaine

Combinatorial dihydropyridine library



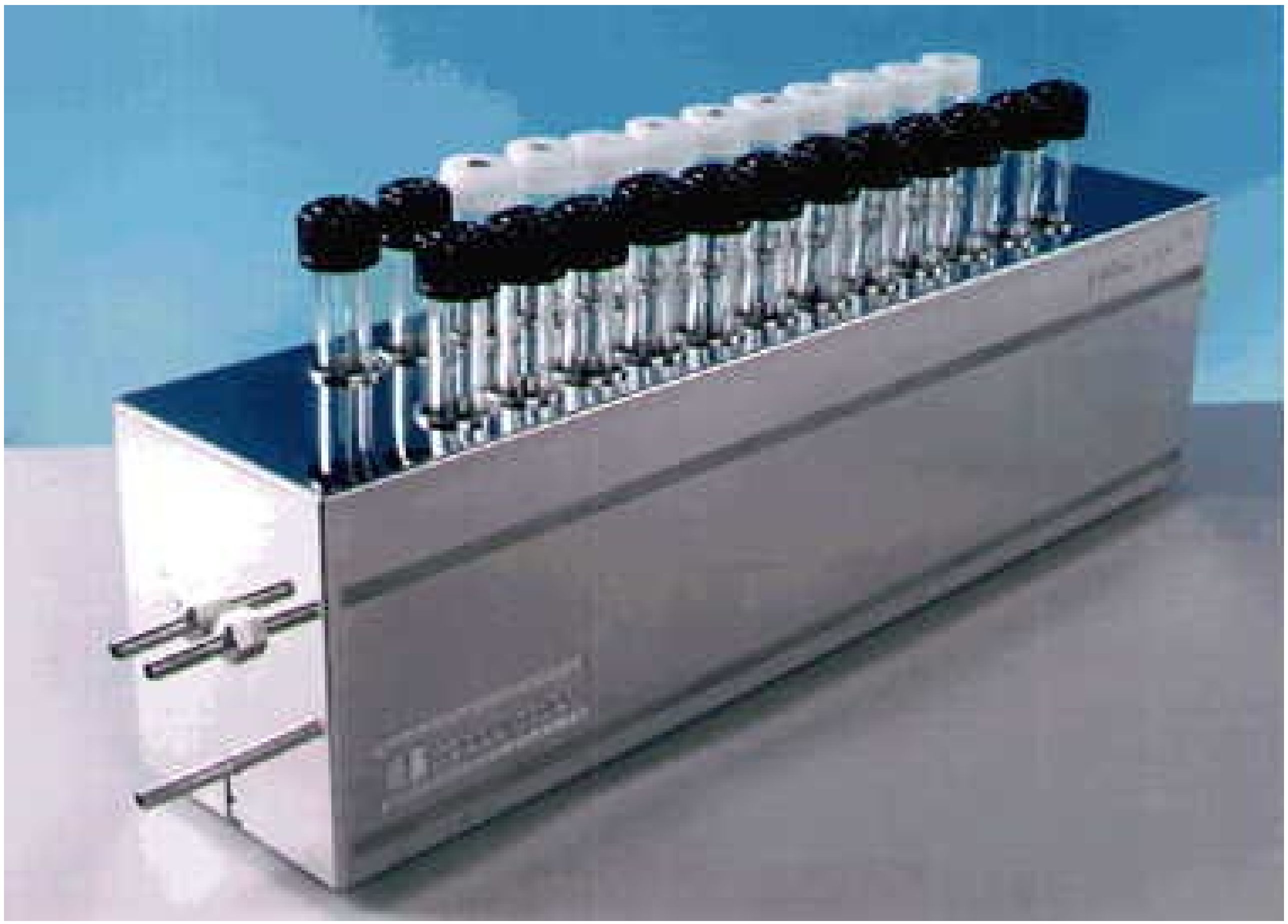
Lit: K.C. Nicolaou et al. in *Handbook of Combinatorial Chemistry*, VCH Wiley (2000) pp. 659-660

Angiotensin converting enzyme (ACE)-Inhibitors Library



Lit: M.M. Murphy et al. *J.Am.Chem.Soc*, **117** (1995) 7029

Multireactor vessels



Problems with Early Combichem Libraries

- Many compounds had undesirable properties:
  - Size (molecular weight)
  - Solubility
  - Inappropriate functional groups

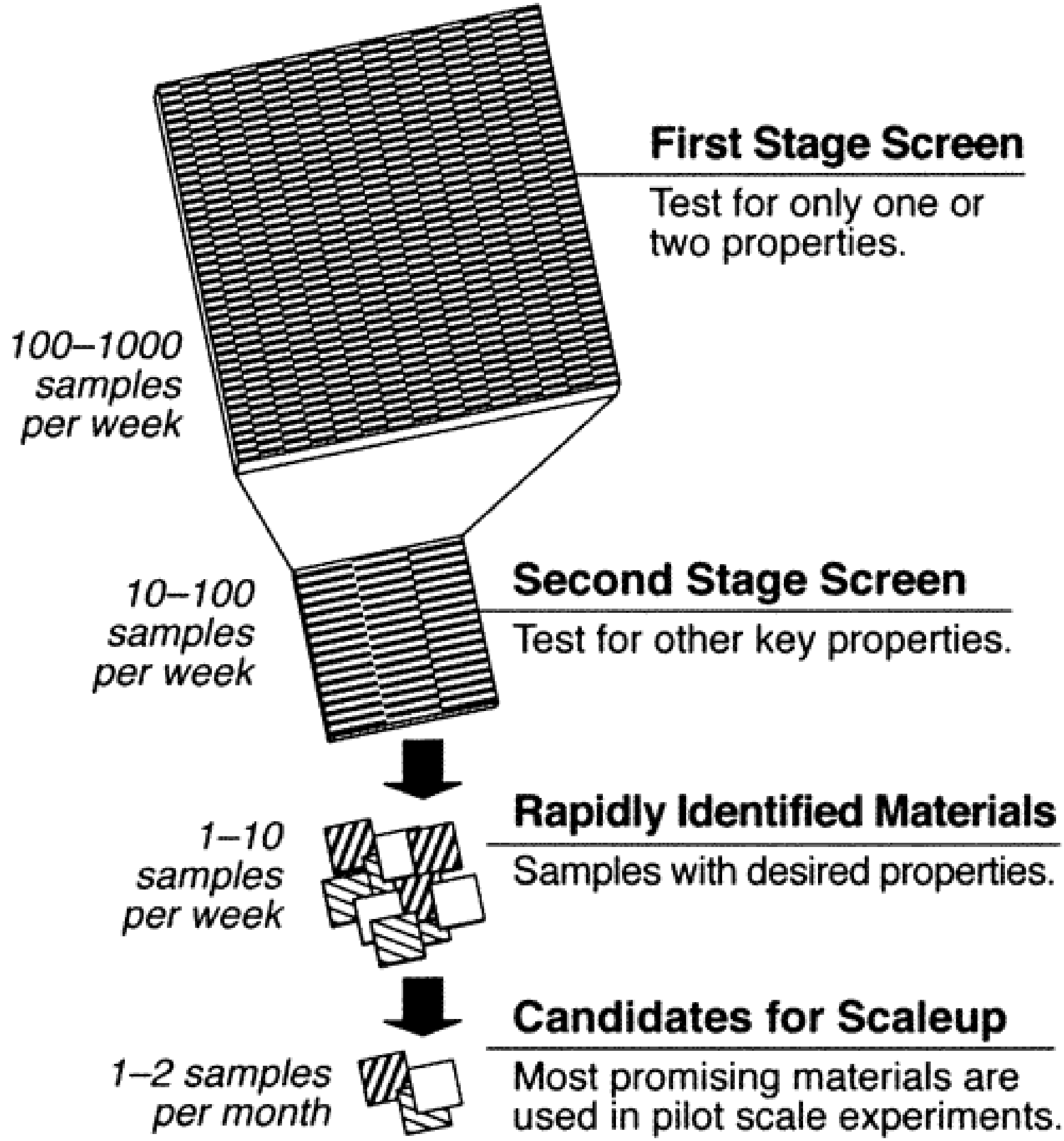
Criticism of the Technique

- Early libraries were often based on a single skeleton (scaffold - basic structure)
- Limited number of skeletons accessible
- Individual library members were structurally similar
- Compounds tended to be achiral or racemic
- Initial emphasis on creating mixtures of very large numbers of compounds is now out of favor

DIVERSE AND FOCUSED LIBRARIES

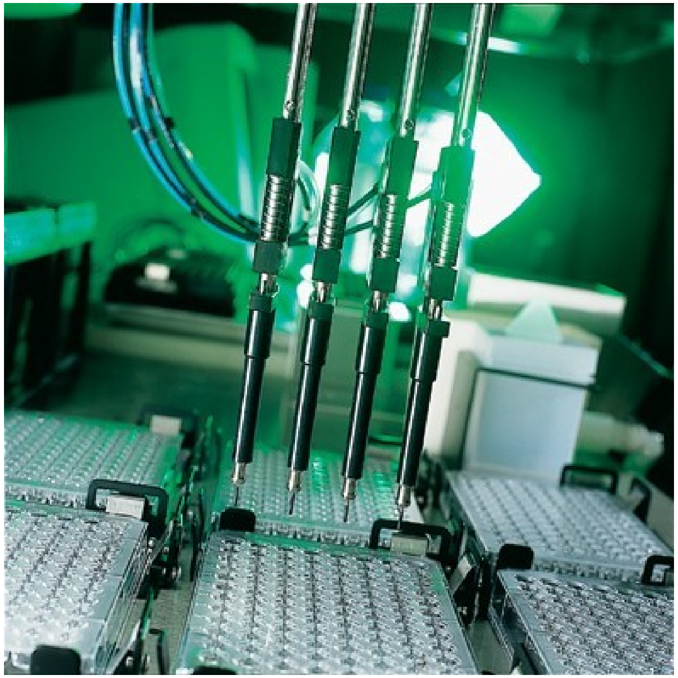
- Many early disappointments led to:
  - Design of smaller, more focused libraries with much information about the target
    - May concentrate on a family of targets (e.g., proteases or kinases)
  - Use of more diverse libraries when little is known about the target
    - "Primary screening" libraries
    - Give broad coverage of chemistry space
  - Selection of compounds with "drug-like" physicochemical properties

Multistage screening



High-throughput Screening (HTS)

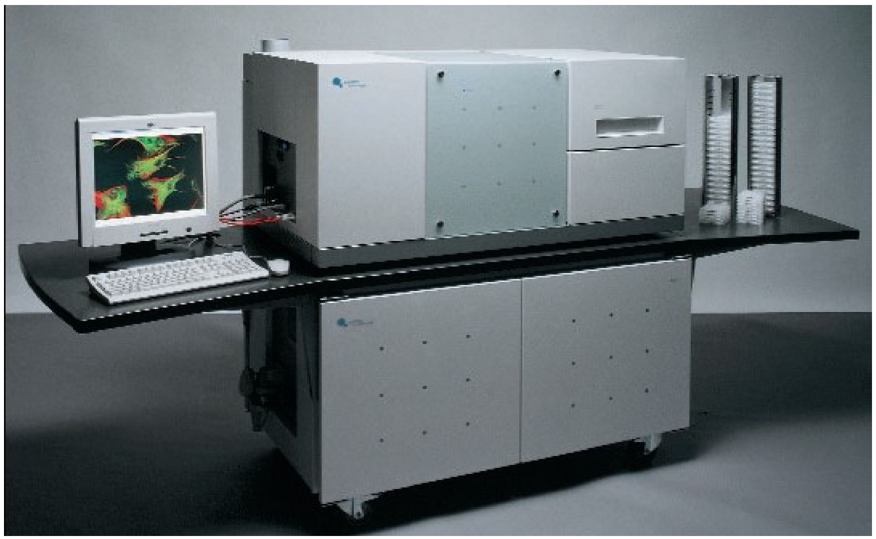
- A process of assaying a large number of compounds against biological targets.
- Up to 100,000 compounds can be analyzed in a day.
- Robots can usually prepare and analyze many plates simultaneously.



http://www.metprog.org.uk/images/manufacturing_icon.jpg

High-Throughput Screening at the University of Cincinnati

Opera Imaging Reader



>50,000 multi-color data points/24 hours

Applications

Whole cell fluorescence assays
  Cell viability, cell differentiation, cell proliferation, cytotoxicity, apoptosis, transporter phenomena

Cell signaling assays
  Calcium flux, second messengers, ion channels, membrane potential

Gene expression assays
  Expression of house-keeping and reporter genes, gene activity and protein regulation, RNAi

Membrane receptor assays
  Ligand binding, receptor activation and desensitization, translocation and endocytosis, recruitment of signaling molecules

Translocation assays
  Target molecule redistribution

Morphological assays
  Neurite outgrowth, cell differentiation, cell adhesion and spreading

Compound Repository

Haystack Neat Compound Storage

- Capacity = 200,000 bottles
- Current = 207,000 bottles
- Freezer storage when appropriate

Solar (Solution Archive) – DMSO solutions

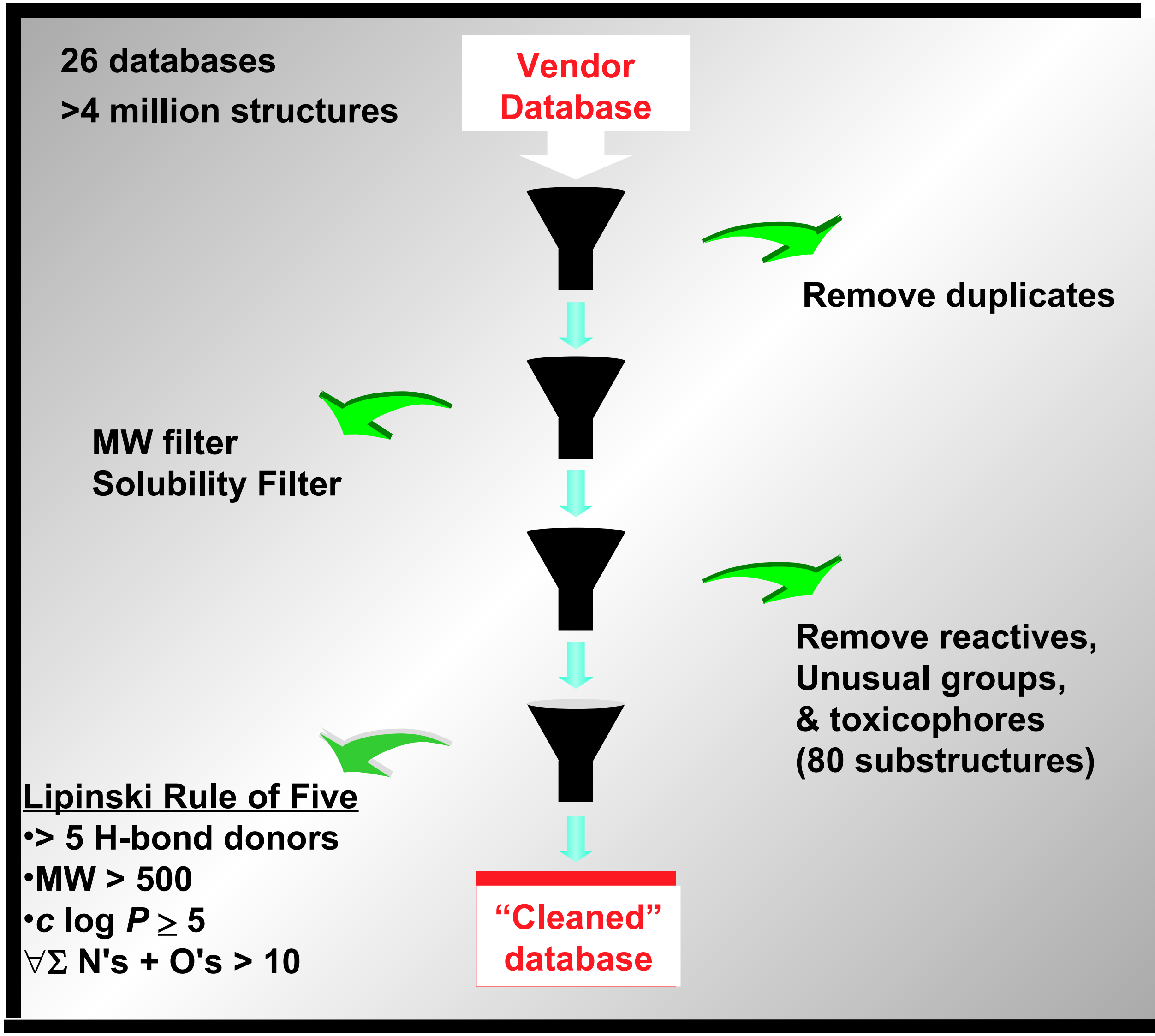
- Capacity = 1.8 million tubes, 10,000 deep well
- (96) plates, 13,600 shallow well (384) plates
- Current = 338,000 compounds in 383,400
- tubes, 1862 deep wells and 2332 shallow wells

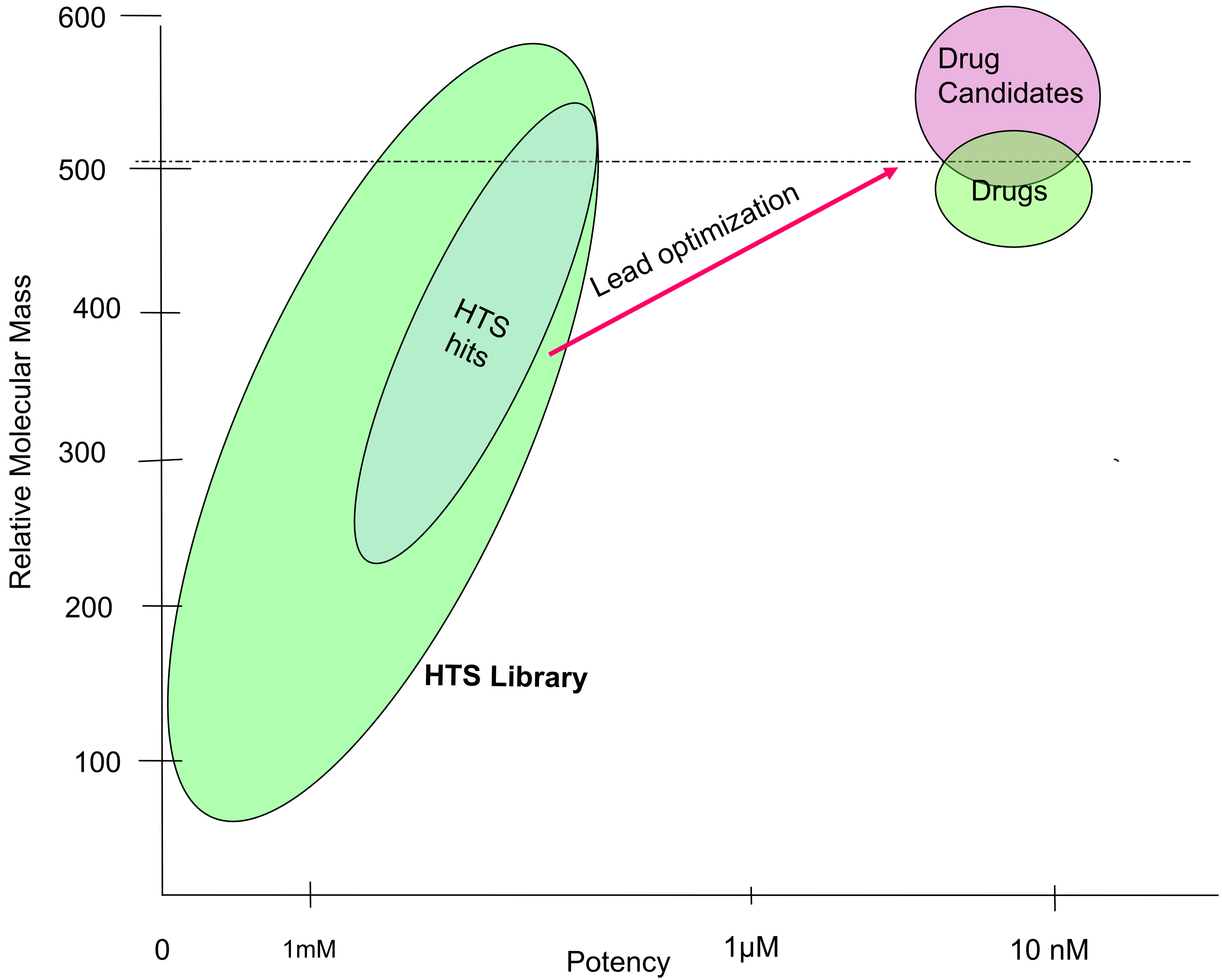
Compound handling and dissolution instruments

Housed at P&G's Mason Business Center (~3500 sq. ft.)

GRI Compound Library

- It's NOT just a numbers game – compound selection can greatly enhance screening efficiency
- Originally from P&G Pharma and represents a $22M investment
- Selected based on drug-like properties and to maximize structural diversity within a 6-dimensional "drug-like" space
- Both external (commercial suppliers) and internal discovery and combinatorial chemistry programs used as sources
- ~250,000 compounds
- Software to rapidly expand around structural leads identified




600
500
400
300
200
100
Relative Molecular Mass
0
1mM
Potency
1µM
10 nM
HTS Library
HTS hits
Lead optimization
Drug Candidates
Drugs

Traditional Discovery v. Combi-Chem

- Expensive, slow
- Sequential learning
- Goal: Reduce cost
- Emphasizes scientific knowledge
- Sequential search
- Requires years of training
- Art, hand-crafting
- Values experience
- Bottle-neck is analoguing
- Flexible across compounds
- Near 100% purity

- Cheap (?), fast
- Trial & error
- Goal: Reduce time
- Emphasizes process knowledge & skill
- Parallel search
- Threatens stakeholders
- Brute force
- Complements traditional
- Bottle-neck is data processing
- Applies to some compound families
- Partial purity