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Diversity Oriented Synthesis

July 23, 2002

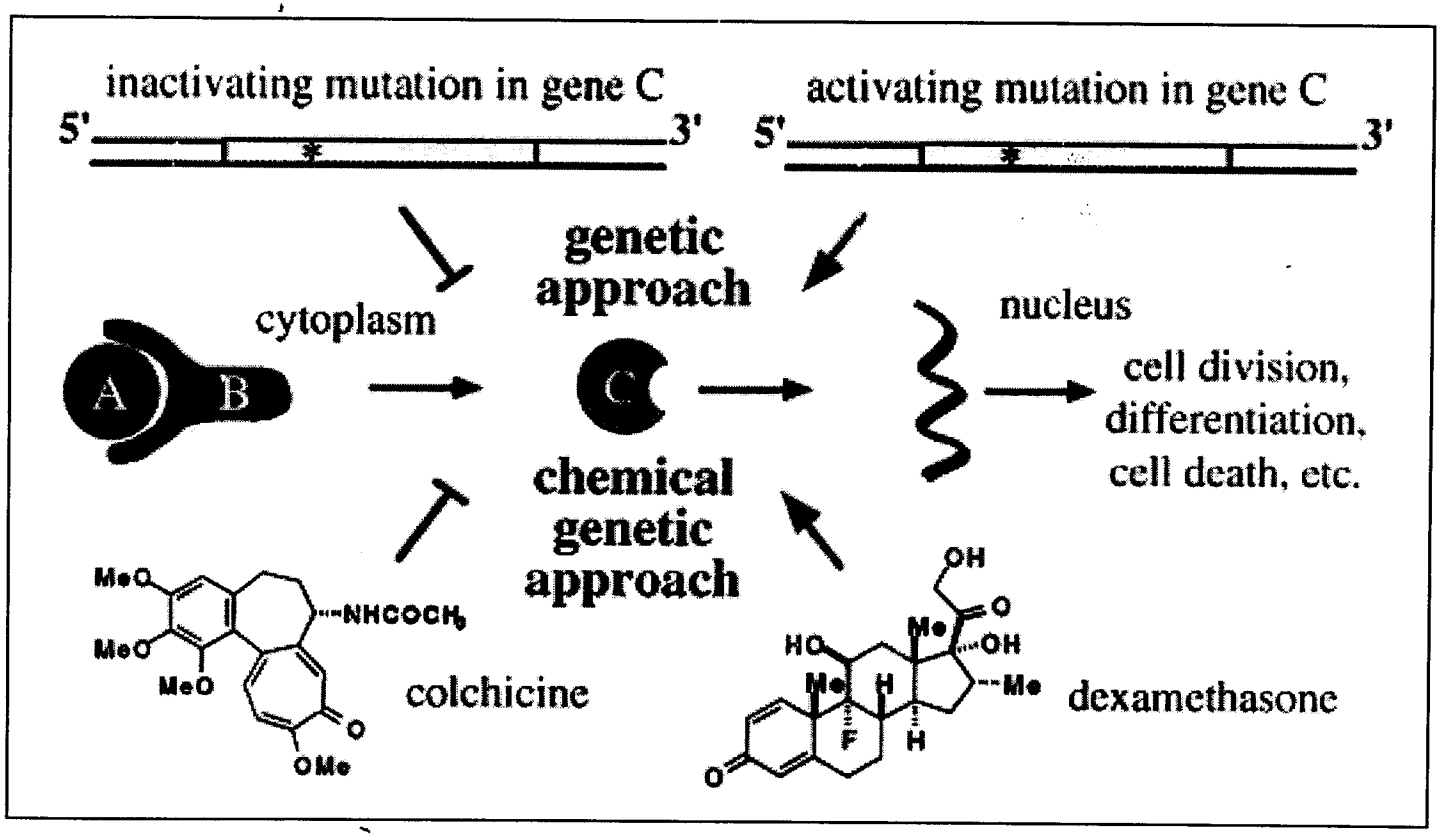
Target-Oriented Synthesis

A specific molecule is the target of a synthesis. Approach is effectively devised through retrosynthetic analysis.

Diversity-Oriented Synthesis

No target is specified. Design an approach to synthesizing a library of molecules to maximize DIVERSITY and COMPLEXITY with each subsequent reaction

How to Emulate Genetics with Chemistry



"When I initiated my independent studies….I knew nothing of biology, having never taken a course in modern biology"

Schreiber, S. L. *Bioorg. Med. Chem.* **1998**, *6*, 1127.

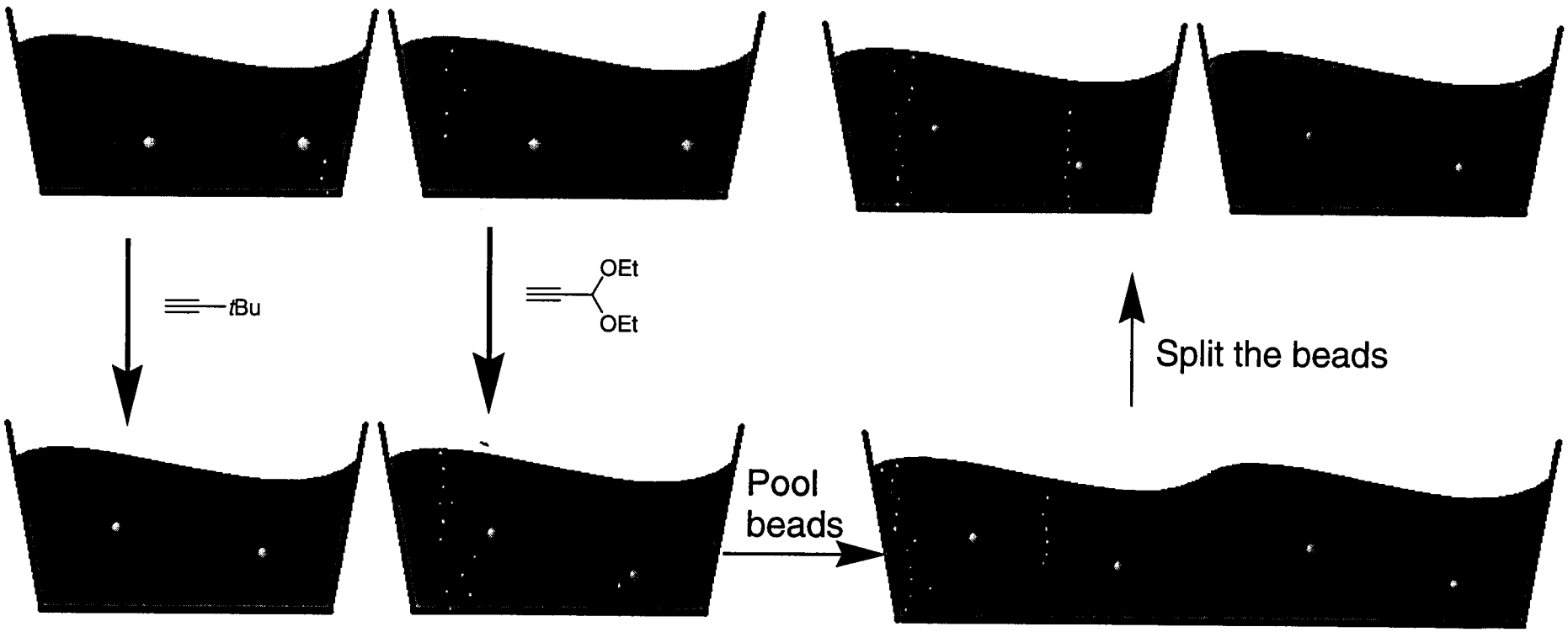
Chemical Genetics vs Classical Genetics

	Pros	Cons
Classical Genetics	Can activate or inactivate ("knock out") ANY protein by gene mutation i.e. Genetic approach is general Deletion of a gene is specific	Genetic approach to understanding protein function is very time-consuming
Chemical Genetics	Some protein-binding natural products can approach the specificity of a gene "knock out" in altering protein function Small-molecule (in)activation of protein provides *instantaneous* alteration of function in a cell Molecules can potentially be used to alter/control certain protein functions. i.e. synthetic organic chemistry bridging another connection between biology and medicine	Most natural products lack the inherent specificity of a gene "knockout" Highly specific protein-ligands (small molecules) still come primarily from nature, and only exist for a miniscule amount of proteins

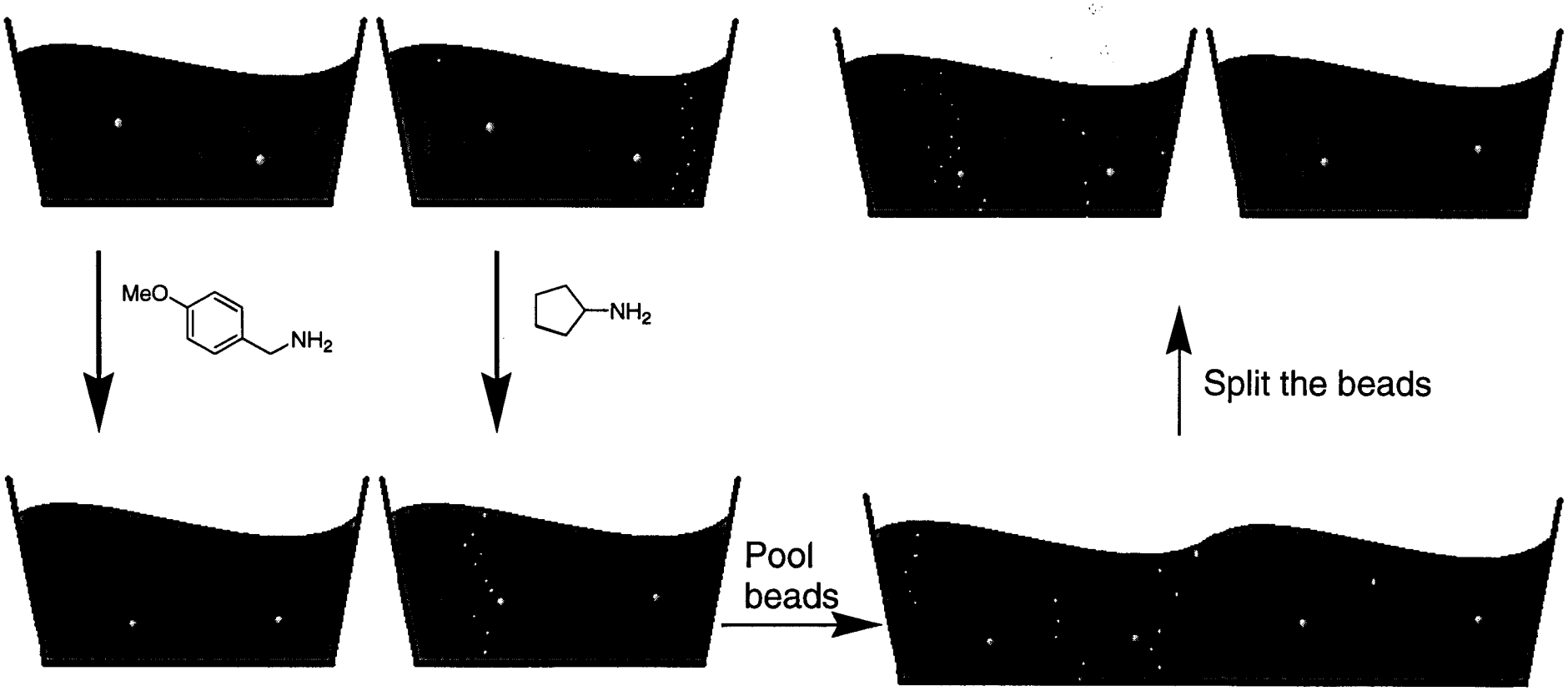
HOW DO YOU MAKE CHEMICAL GENETICS MORE PROMISING?

Split-Pool Synthesis or the "One-Bead / One Compound" Approach

Synthesis beads are split and placed
into separate reaction tubes
(typically, 10^6 synthesis beads are used)



Split-Pool Synthesis or the "One-Bead / One Compound" Approach

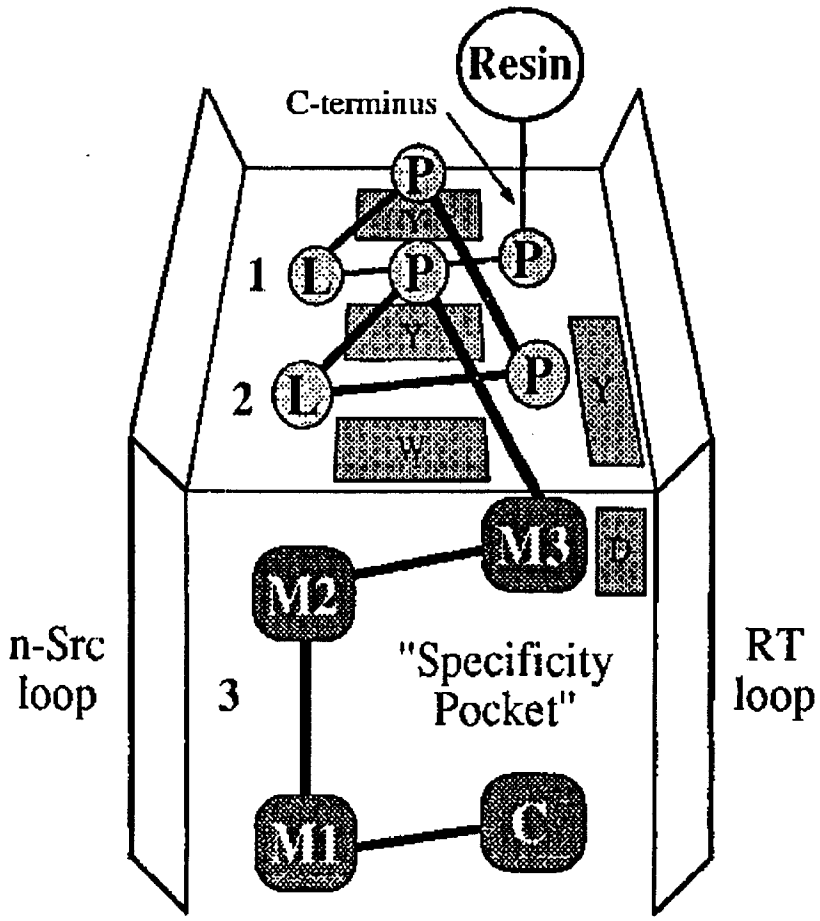


Features of Split-Pool Synthesis

- Final solution of beads is separated in an arrayer and prepped for subsequent assays
- Ideally, all possible combinations of building blocks are coupled
- Allows for rapid construction and collection of numerous diverse compounds that can be separated by the beads they are attached to

Approach has been to build libraries of compounds related to a known protein binder, i.e. still target oriented.

Use of Split-Pool Synthesis in Target Oriented Synthesis - the SH3 Binding Domain



Src Homology 3 (SH3) domains are protein modules that mediate inter- and intramolecular protein interactions in molecules ranging from components of the cytoskeleton to transducers of cellular signaling

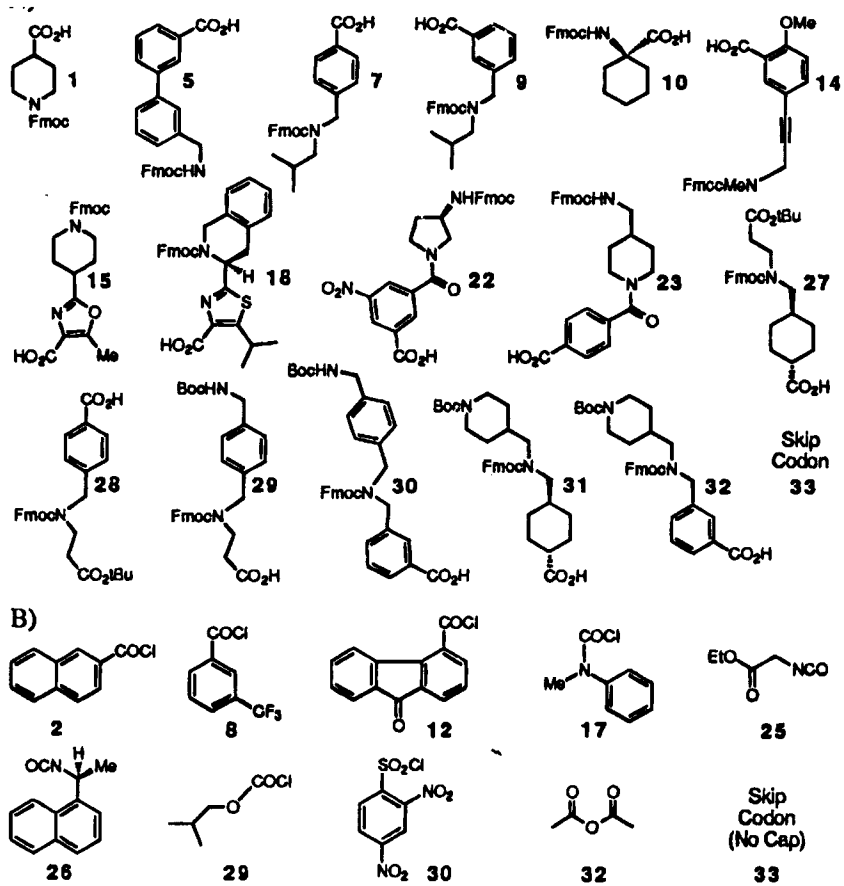
Can use a common low-affinity biasing sequence (PLPPLP) to position elements attached to it in the third pocket. (Third pocket binding is primary determinant of ligand specificity).

Split-pool approach with 3 monomers (M1, M2, M3) and a capping reagent (C).

Non-peptide sequences can be found that effectively binds specificity pocket

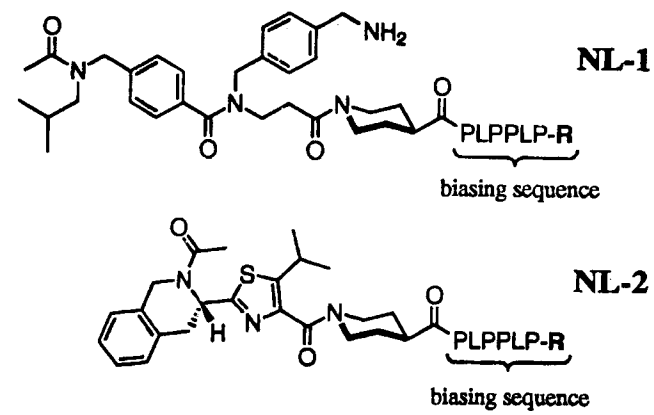
Phase 1, Generation and Screening of library

Monomers used (A) and Capping reagents (B)



library of ~1.1 million compounds created, 15 beads found specific for SH3 domains.

Most contained 1 in M1 position, and either 18 or 29 in M2 position. No other trends observed



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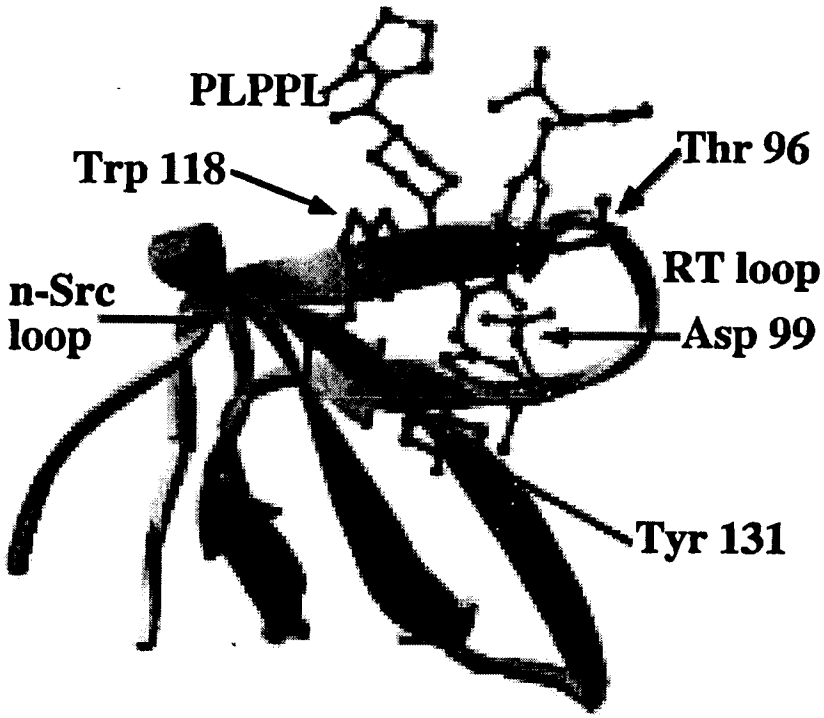
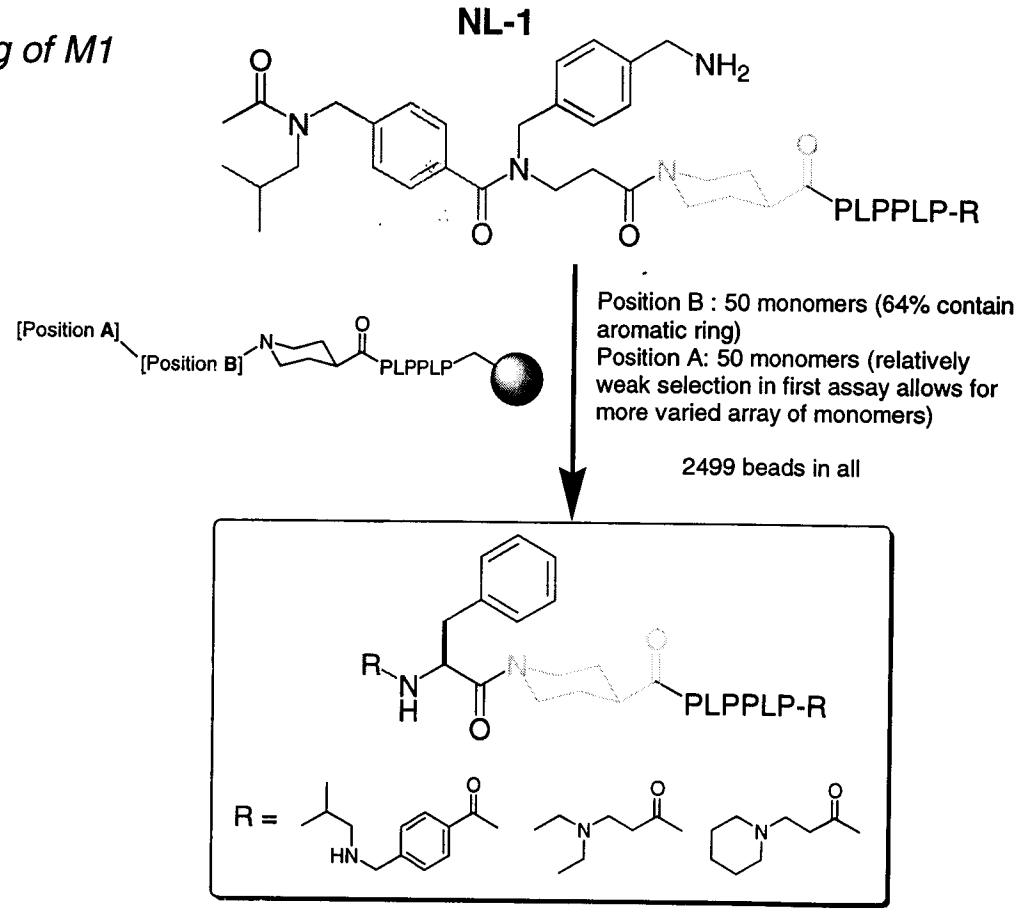
Phase 2, Generation and Screening of library

(1) Hydrophobic interaction Thr 96 – aromatic ring of M1
(2) Benozyl carbonyl – Asp-99
(3) Aromatic-aromatic interaction, Tyr 131 – M2





- These are examples of "biased" libraries. i.e. based on knowledge of the binding site
- They show its still hard to predict anything!
- Development of cell-permeable ligand will help elucidate protein function in vivo

Understanding a New Concept – Diversity Oriented Synthesis

WHAT IF THIS WAS CONDUCTED WITH NO SPECIFIC TARGET ? (PROTEIN OR ITS SMALL-MOLECULE BINDER)

A

target-oriented synthesis ⇨ **retrosynthetic analysis**

begin with: complex target or collection of targets (e.g., "focused library")
end with: simple building blocks/starting materials

diversity-oriented synthesis ⟶ **forward synthetic analysis**

begin with: simple building block(s)
end with: large collection of structurally complex and diverse compounds

B

forward synthetic analysis

structural complexity
↓ use of:

(a) pairs of complexity-generating reactions where product (rx 1) = substrate (rx 2)
(b) conformational analysis[7]

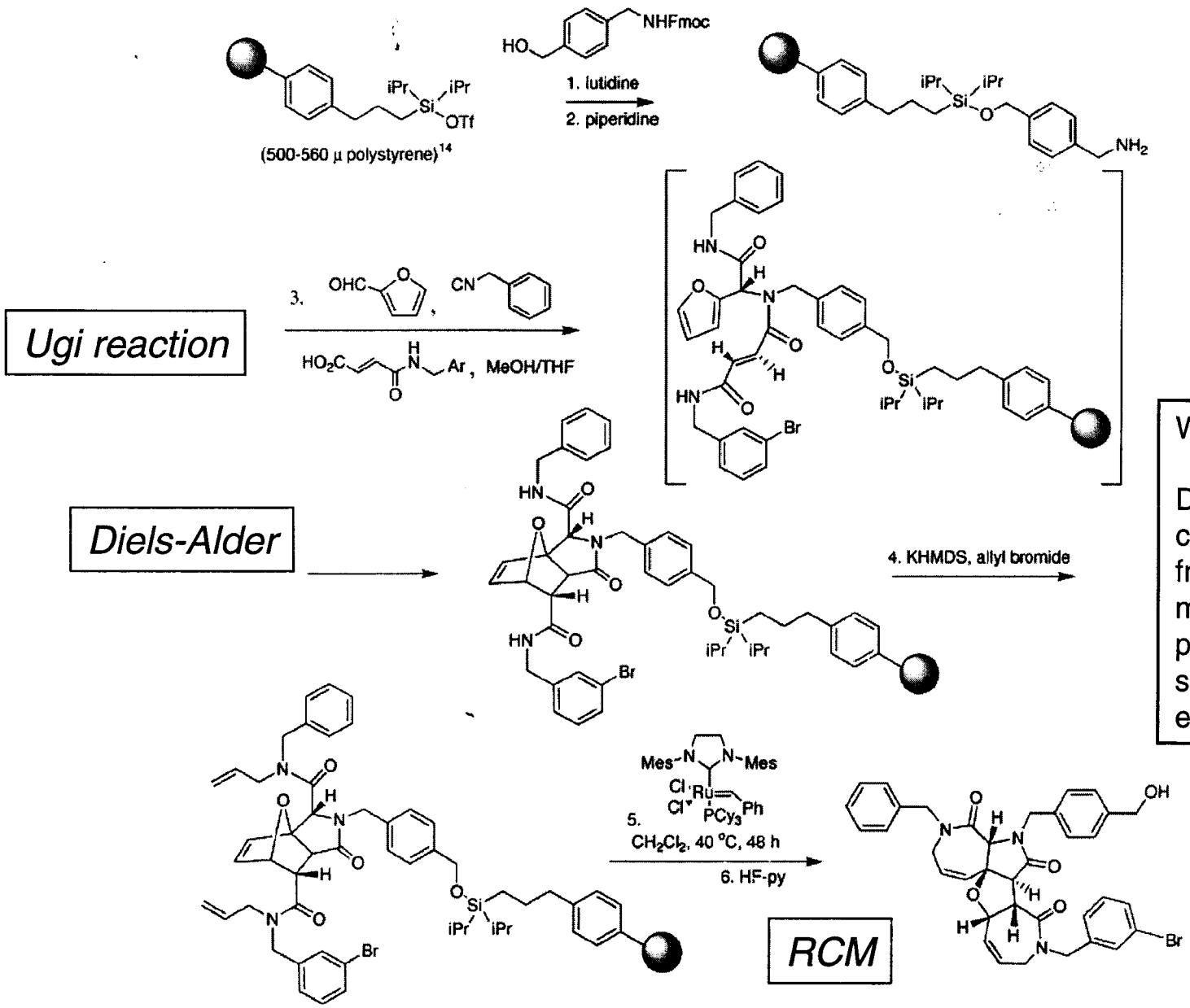
structural diversity
use of: ↓

(a) building blocks[4,5]
(b) stereochemistry[8]
(c) branching reaction pathways[8]

→ integrated analysis ←

Schreiber, S. L. *Science*, **2000**, *287*, 1964. Lee, D.; Sello, J. K.; Schreiber, S. L. *Org. Lett*, **2000**, *2*, 709.

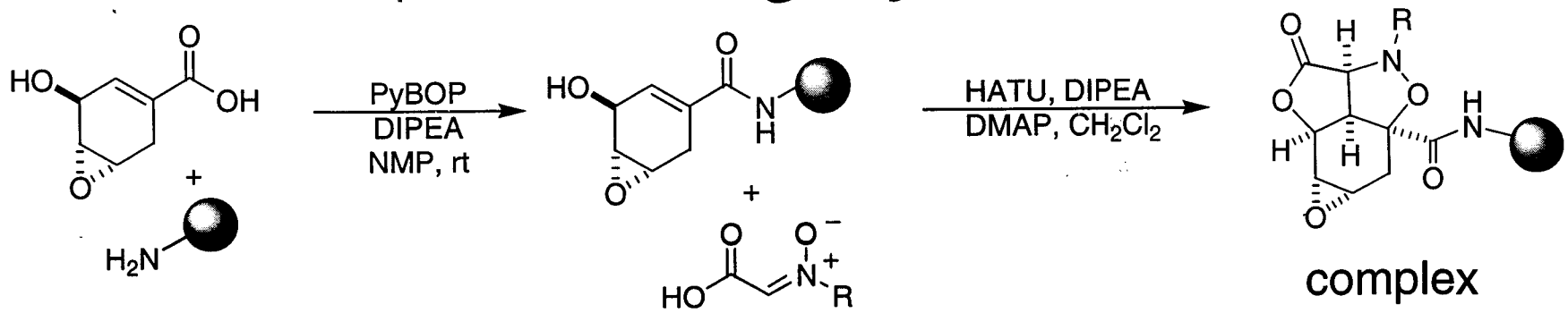
Complexity-Generating Reactions



Why focus on rings?

Decreasing degrees of conformational freedom in small molecules can facilitate protein binding and specificity by reducing entropic cost of binding

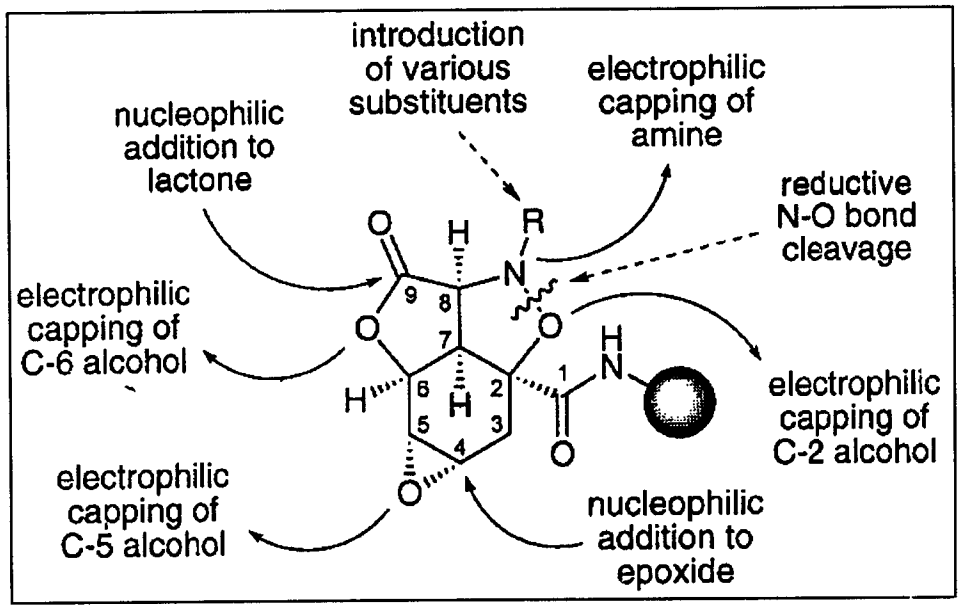
Complexity- and Diversity-Generating Synthesis



simple

complex

R = diversity element



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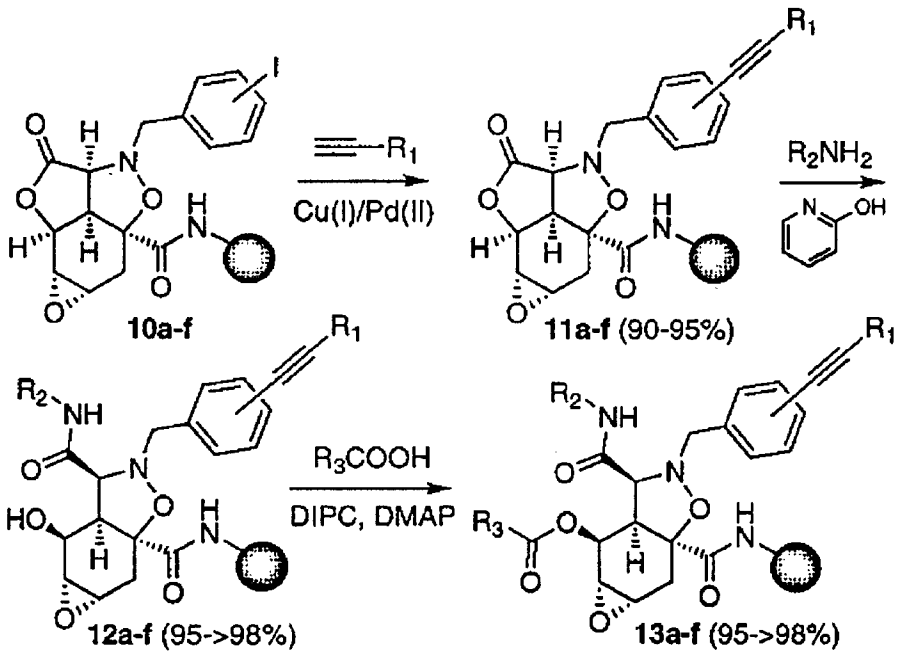
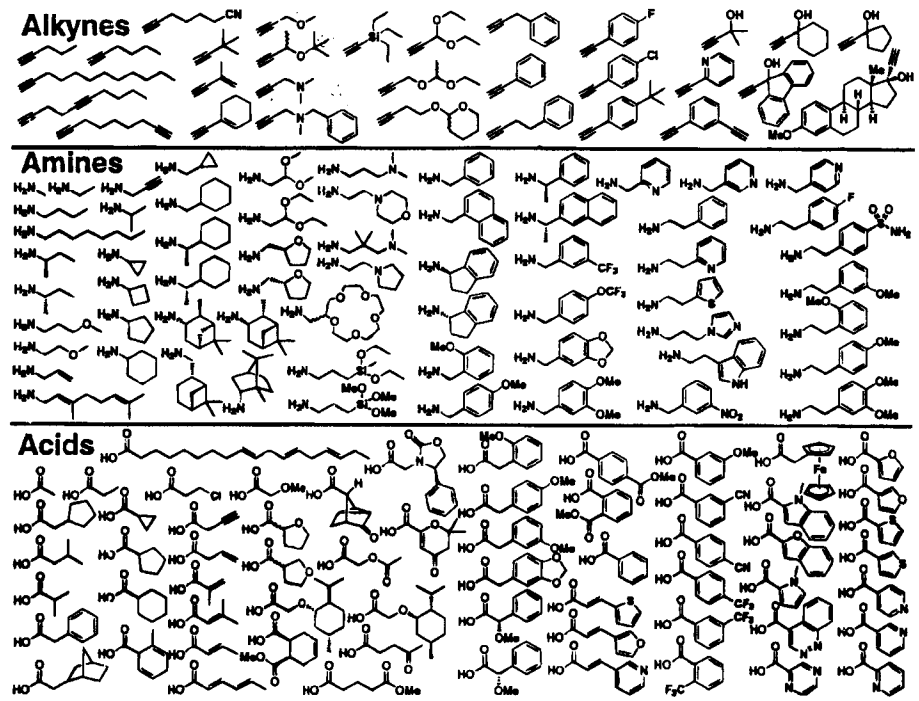
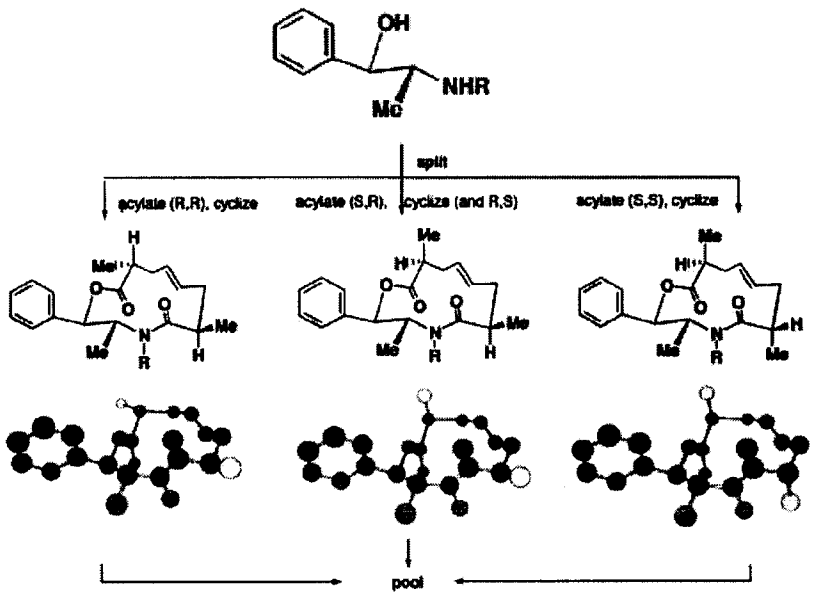
Complexity- and Diversity-Generating Synthesis





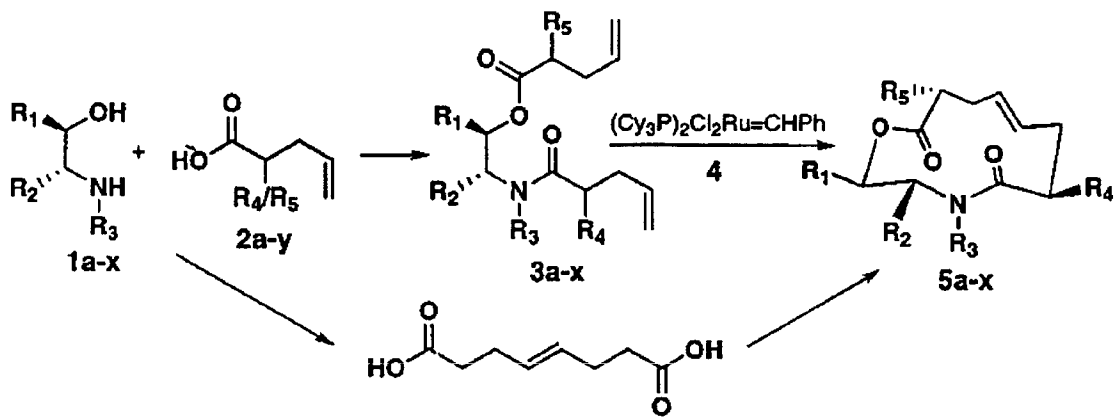
30 alkynes, 62 primary amines, and 62 carboxylic acids were used with skip codon at each step
Grand total = 2.18 million compounds (entire process took three weeks)

Diversity-Increasing Reactions through Stereochemistry



Many RCM reactions for medium-sized ring synthesis are optimized for protecting groups, stereochemistry, and functionalities present. This is not practical for split-pool based synthesis

Selecting the proper large (12 membered) rings with low torsional strain allow one to by-pass many of of these limitations



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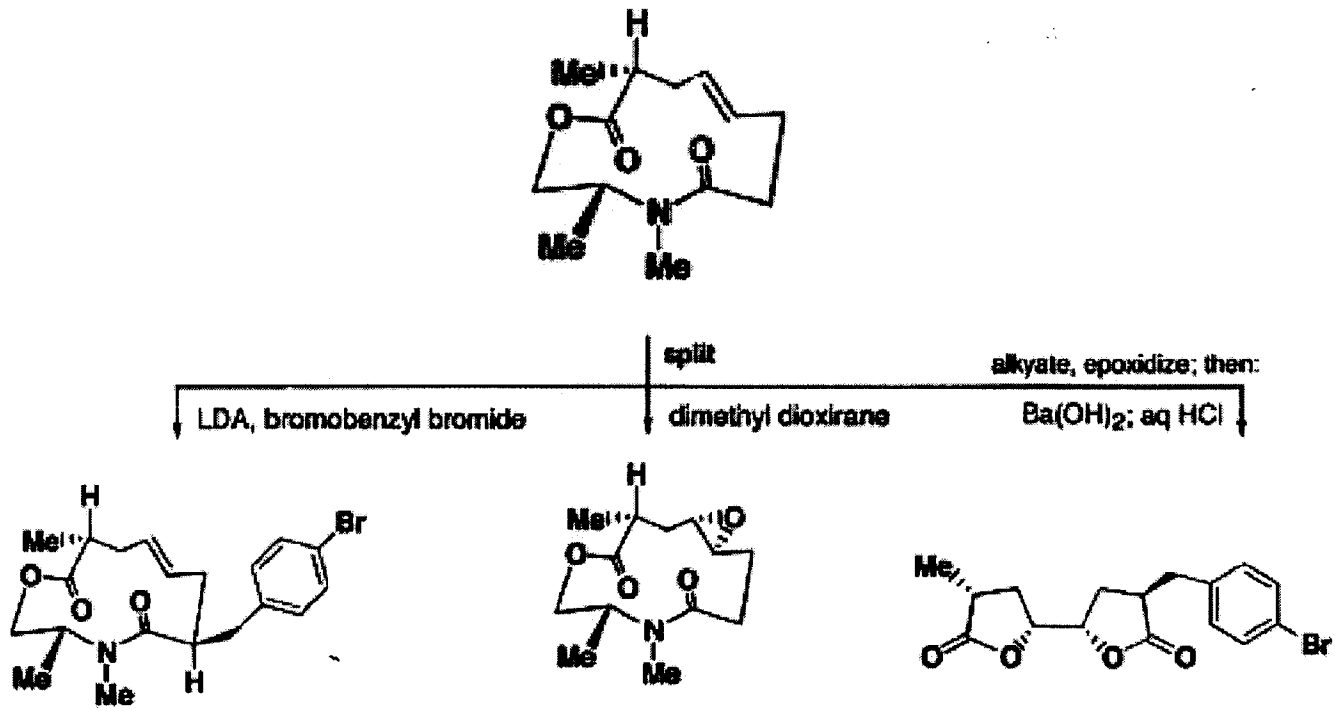
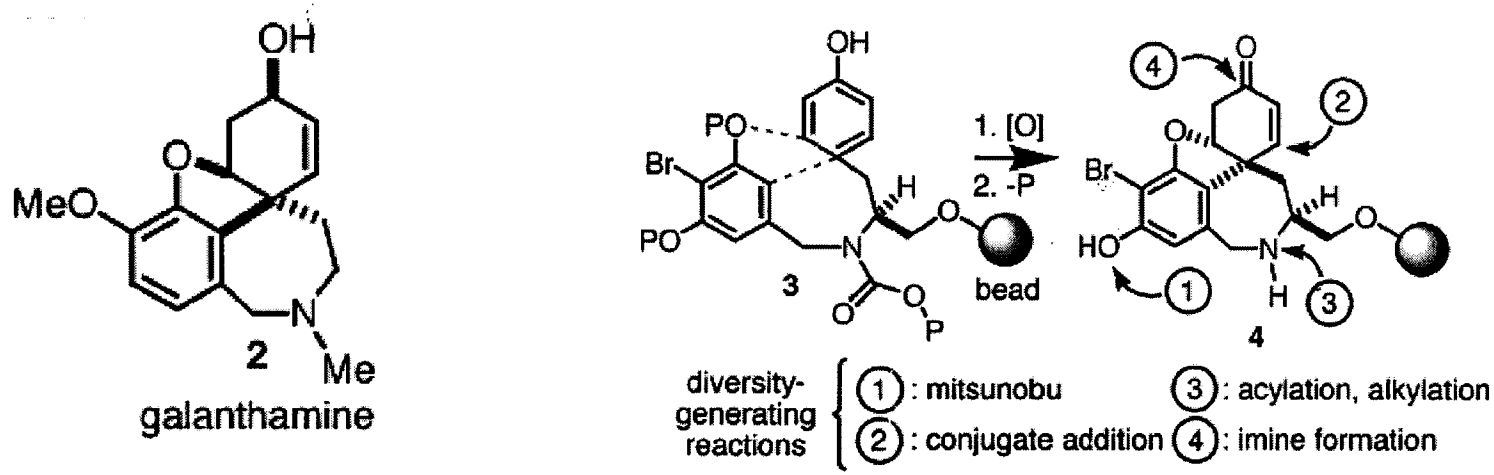
Diversity-Increasing Reactions through Branching

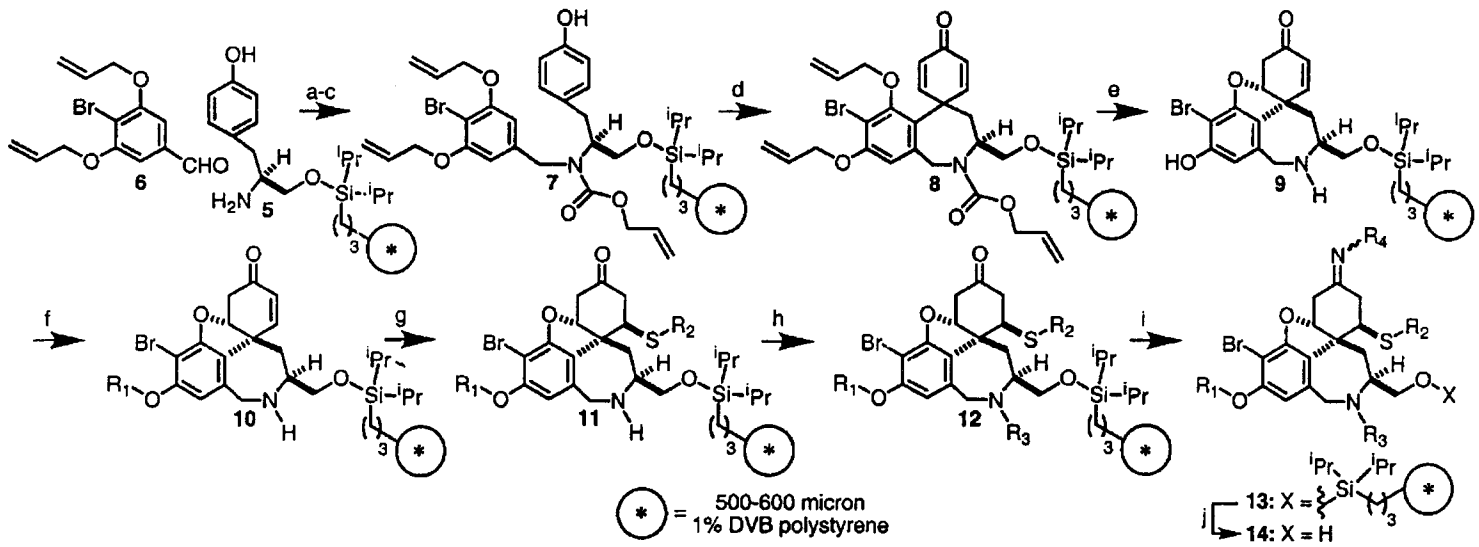
(employing same substrate, different reactions)



Most difficult to effectively apply to split-pool synthesis but allows for excellent diversity

Biomimetic Diversity-Oriented Synthesis





[a] Reagents: (a) 6, $CH(OCH_3)_3-CH_2Cl_2$, wash then $NaBH_3CN$, AcOH, MeOH−THF, 23 °C. (b) allylchloroformate, $^iPr_2EtN$, CH_2Cl_2, 23 °C. (c) piperidine, THF, 23 °C. (d) $PhI(OAc)_2$, $(CF_3)_2CHOH-CH_2Cl_2$, 23 °C. (e) $Pd(PPh_3)_4$, morpholine−THF, 23 °C. (f) R_1OH, PPh_3, DIAD, THF, 0 °C (2×). (g) R_2SH, 2,6-lutidine, ^nBuLi, THF 0 → 40 °C. (h) R_3CHO, AcOH, MeOH−THF, then $NaBH_3CN$ in MeOH, 23 °C or R_3COCl, 2,6-lutidine, CH_2Cl_2, 23 °C or R_3NCO, CH_2Cl_2, 23 °C, (i) R_4NH_2, AcOH, MeOH−CH_2Cl_2, 23 °C. (j) HF−pyridine, THF, 23 °C then TMSOMe.

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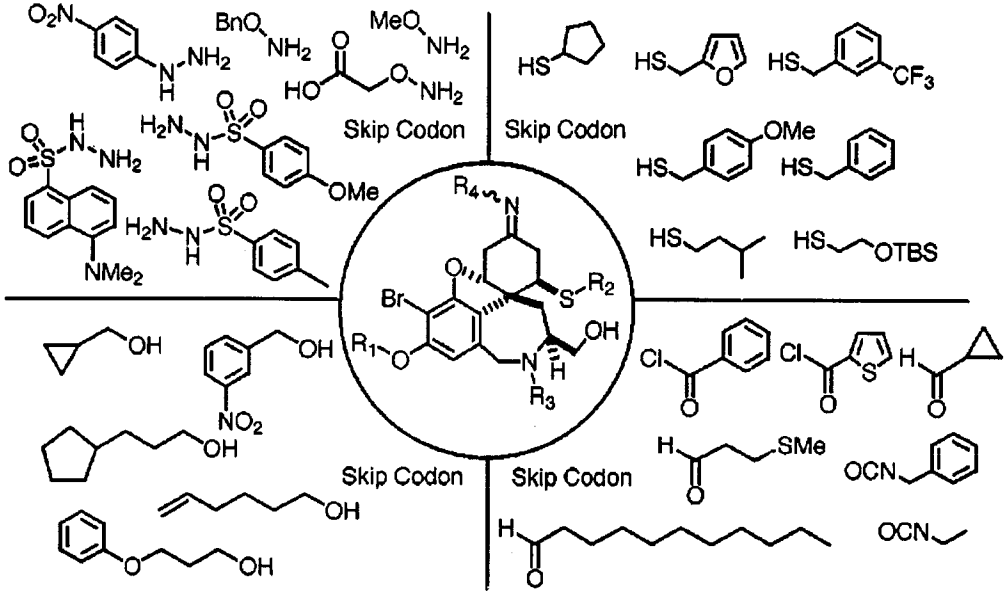
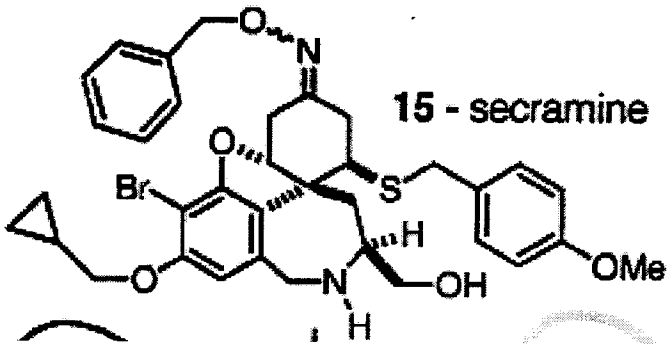
Biomimetic Diversity-Oriented Synthesis





- Library of 2527 molecules made
- Screened for ability to block secretory pathway
  (protein shuttling from entoplasmic reticulum to Golgi apparatus)
- 15 found to be a potent inhibitor
- Galanthamine is a potent acetylcholinesterase inhibitor, but has no affect on secretory pathway!

Conclusions

- "An ambitious goal of diversity-oriented synthesis is to design a synthetic pathway leading to a collection of compounds with a large number of different scaffolds, in the limit where each compound has a unique scaffold:
- "diversity-oriented synthesis, will likely play a role in drug discovery in the future"
- "Our ability to plan currently lacks guidance from our growing knowledge of small molecule-binding sites on biological macromolecules"
- "Not one of the steps has been optimized, but there appears to be no theoretical impediments, no insurmountable activation barriers associated with any individual step"
- "Will the full power of stereoselective methods in synthesis be brought to bear in split-pool or massively parallel synthesis of millions and billions natural product-like compounds?....It is true that even the first goal has not been demonstrated, but I cannot imagine that in a young chemists lifetime, it will not be accomplished....the impact on life sciences will be significant."

S. L. Schreiber

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