Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

Prelude

- 2600 B.C. Mesopotamia: 1000 plant based drugs
- 1500 B.C The Ebers Papyrus: 700 plant based drugs
- 659 A.D The Tang Herbal: 850 plant based drugs

For thousands of years humans have used the natural flora and fauna to treat illness. In the last century this dependence forever changed. Advances in molecular biology beginning in the 1950's produced a deeper understanding of gene pathways and the molecular mechanisms that underlie disease. This insight coupled with the growing power of organic synthesis shifted attention from screening natural product extracts to the rational design of synthetic drugs. Further advances in the early 1980's allowing for the rapid identification, purification, and evaluation of validated disease targets gave rise to a new era of high-throughput screening. For the first time the ability to screen compounds was limited by the number of compounds available for screening. Combinatorial chemistry was created to fill this void. However, when combinatorial chemistry did not perform as expected, efforts to combine combinatorial chemistry and natural product synthesis ensued.

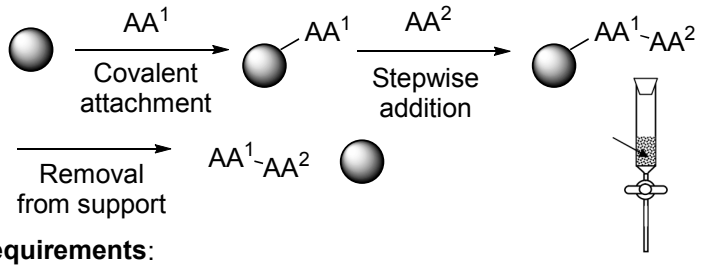
Combinatorial Chemistry: The generation of a large collection of compounds (library) by synthesizing all possible combinations of a set of smaller chemical structures (building blocks).

Solid Phase Combinatorial Chemistry

- Solid phase organic synthesis
- Multipin peptide synthesis
- Tea bag synthesis
- Split-and-mix synthesis
  - Iterative deconvolution
  - Chemical encoding
  - Radiofrequency encoding
- olomoucine
- sarcodictyin A

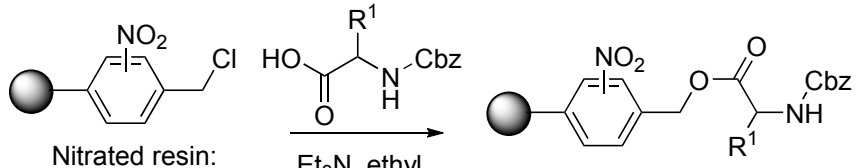
Origins

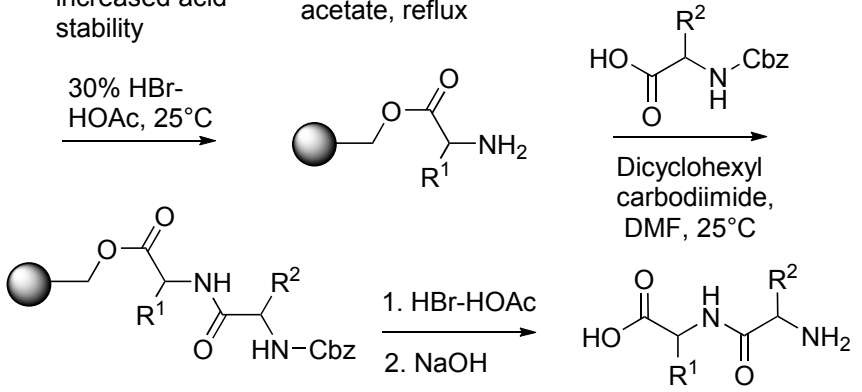
1963 Robert Merrifield invents solid phase peptide synthesis



Solid support requirements:
- Insoluble in all solvents
- Stable physical form allowing filtration
- Contain FG capable of covalent bond formation

Polymers investigated:
- Cellulose
- Polyvinyl alcohol
- Polymethacrylate
- Sulfonated polystyrene
- **Chloromethylpolystyrene divinyl benzene copolymer** (Merrifield resin)





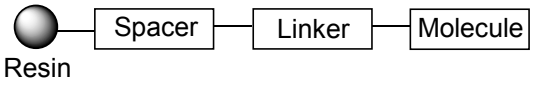
Nat. Prod. Rep., **2000**, 17, 215-234; *J. Am. Chem. Soc.* **1963** 85 (14), 2149-2154

Shenvi Lab Group Meeting
07/25/2016

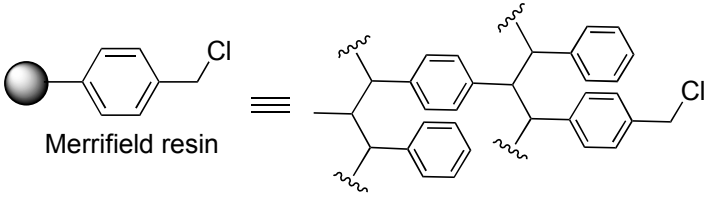
Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

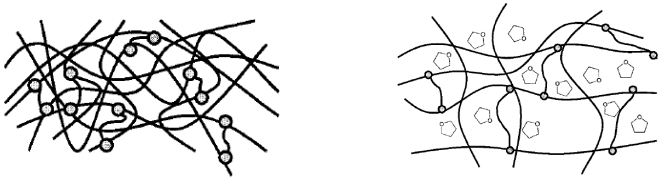
Solid Phase Organic Synthesis



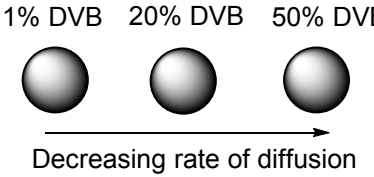
Resin: an inert matrix passive to chemistry
- TentaGel resin
- AgroGel resin
- Polystyrene divinyl benzene
  - gel-type structure
  - readily allows penetration of reagents and solvents inside of beads where chemistry takes place
  - most commonly used (1-2% DVB)
  - Cheap
  - Excellent chemical stability
  - Thermal stability dependent on DVB crosslinking (.5-5% ~130°C)

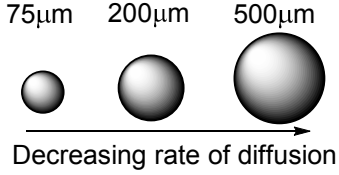


Swelling:
- Swelling of a polymer resin must occur before any reactions can occur
- Swelling of a cross linked polymer is equivalent to solvation of a linear polymer
- Solvent is taken up by cross-linked polymer resulting in an increase of volume
- Swollen resin becomes the solvent for reaction

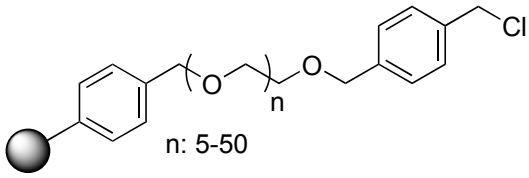


Diffusion:
- A molecule unable to reach its desired site of interaction is unable to react
- Barrier of interaction for molecules in solution is negligible (Brownian motion)
- Attachment of molecule to support diminishes Brownian motion
- Diffusion of reagents through beads influences reaction rate
  - Increasing degree of cross-linking decreases diffusion rates
  - Increasing bead diameter decreases diffusion rates

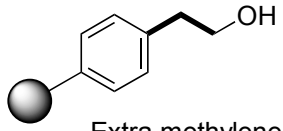




Spacers: act to distance chemistry from the solid support
- Most commonly chains of polyethylene glycol or extended methylene chains
- Tailors swelling properties (more "solution-like")
- Generally improves solvent compatibility
- Can alter linker properties



Hydroxy-methyl polystyrene



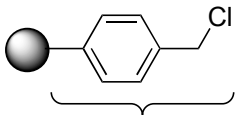
Extra methylene unit increases acid stability

Linkers: Covalently connects molecules to solid support
- Provides means for chemical cleavage
- Must be compatible with planned chemistries
- Two types:
  - Integral: solid support forms all or most of the linker
  - Nonintegral: linker attached to the resin core

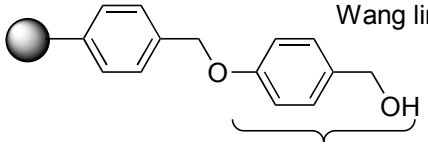
Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

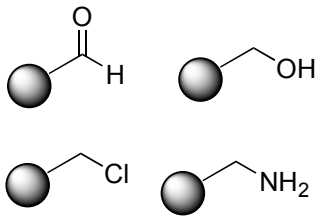
Benjamin Huffman

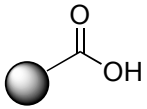


Integral linker



Nonintegral linker

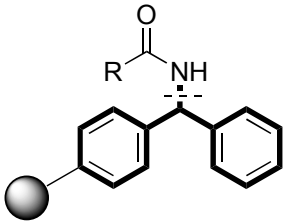




- Functional groups attached to polystyrene resins

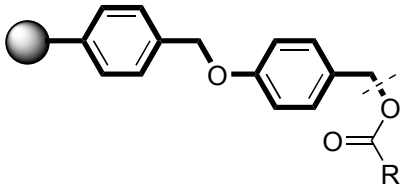
- >200 linkers made
- 8 general classes

Strong acid cleavable linkers:



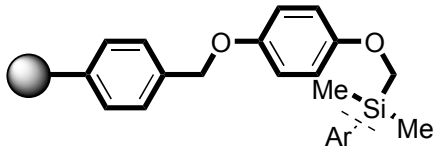
- Benzhydrylamine (BHA) linker
- Stable to many reactions
- Can be difficult to remove
- Selective cleavage of C-N bond
- Clevage conditions: HF/anisole (93:7)

Mild acid cleavable linkers:



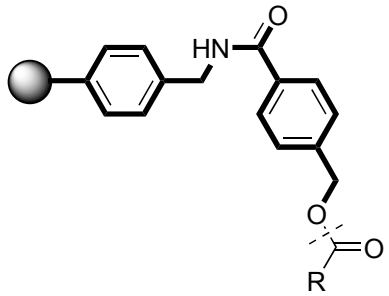
- Wang linker
- Acid sensitivity dependent on stability of carbocation formed
- Cleavage conditions: 50% TFA/DCM

Silicon linkers



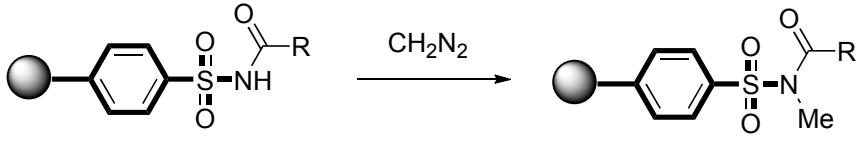
- Dimethylphenoxymethylsilyl linker
- Si orthogonal to many organic transformation
- Cleavage conditions: fluoride or mild acid

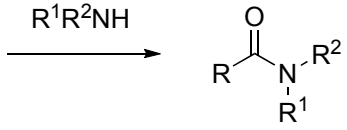
Nucleophilically cleaved linkers



- 4-hydroxymethylbenzoic acid (HMBA) linker
- Electron withdrawing groups enhance reactivity
- Cleavage conditions: 1M NaOH

Safety-catch linkers





- Acylsulfonamide linker
- Resistant to acidic conditions
- Insensitive to nucleophiles
- Alkylation activates amide bond
- nucleophilic cleavage with amine

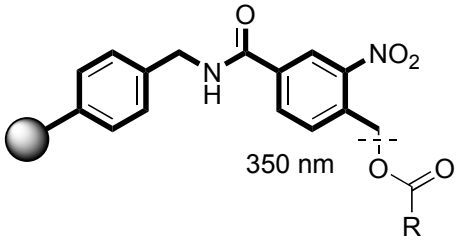
Chem. Rev. **2000**, 100, 2091-2157

Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

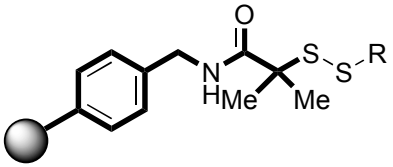
Benjamin Huffman

Photocleavable linkers



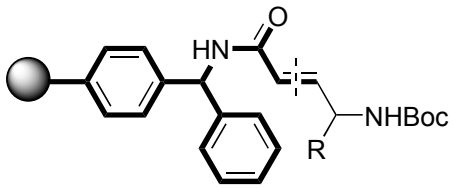
- *o*-nitrobenzyl linker
- Photolysis
  - Mild
  - Orthogonal
- Widely used in carbohydrate, nucleotide, and peptide synthesis
- Application toward nonoligomeric synthesis is limited (SM absorb light)

Reductively cleaved linkers

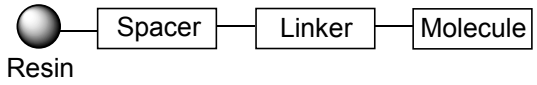


- Disulfide based linker
- Four main approaches:
  - Catalytic hydrogenation
  - Disulfide reduction
  - Deselenization
  - Hydride addition
- Cleavage conditions: TCEP, dioxane/water

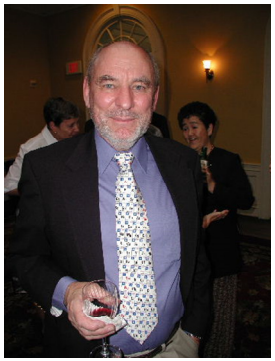
Oxidatively cleaved linkers



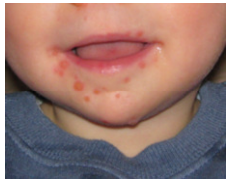
- α,β unsaturated based linker
- Two main approaches:
  - Ozonolysis of an alkene
  - Oxidation of sulfur- or selenium-based linker
- Cleavage conditions: ozone, thiourea

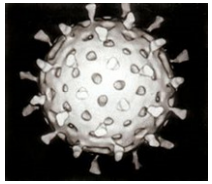


Multipin Peptide Synthesis



- **1984** Mario Geysen invents Multipin peptide synthesis (**parallel synthesis**)
- Research objective: to identify the immunogenic epitope of the coat protein for the virus that causes Hand, Foot, and Mouth Disease (HFMD)
- Elucidating immunogenic epitope could aid vaccine development
- Methods to rapidly synthesize and evaluate peptides did not exist

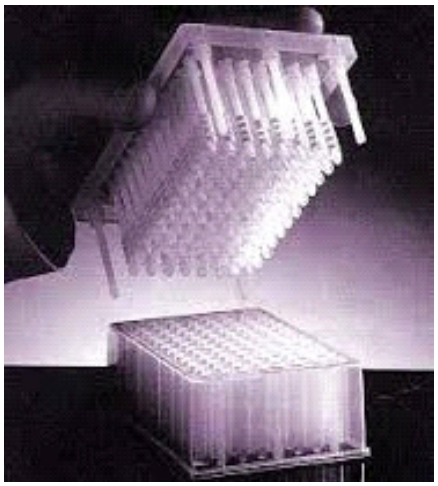


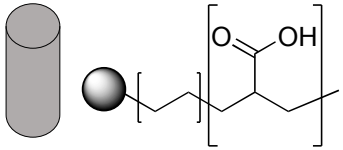


NH_2-T-T-S-A-G-E-S-A-(204 AA's)-L-COOH

hexapeptide 3
hexapeptide 2
hexapeptide 1

208 member hexapeptide library





- Polyethylene rods 6% acrylic acid
- 4 mm diameter x 40 mm length

- Reactions carried out in teflon tray

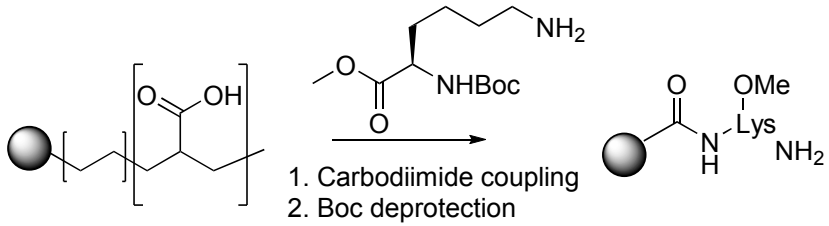
- Geysen apparatus: format and spacing of a microtiter plate

Proc. Natl Acad Sci. USA **1984**, 81, 3998-4002

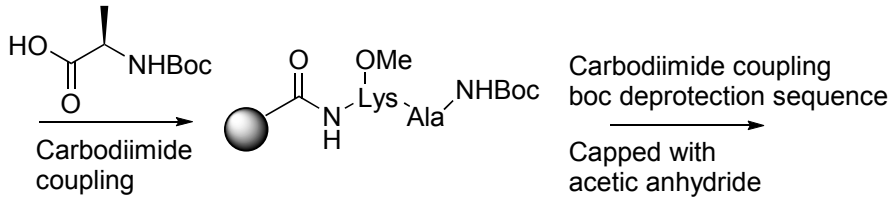
Shenvi Lab Group Meeting
07/25/2016

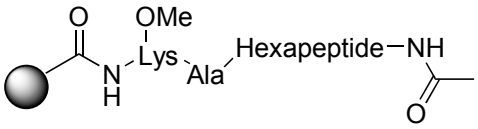
Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

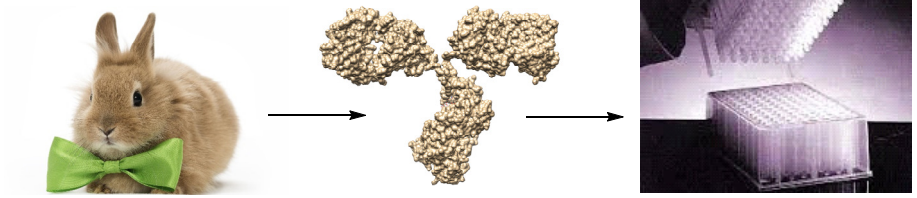
Benjamin Huffman



poly(ethylene-co-acrylic acid)

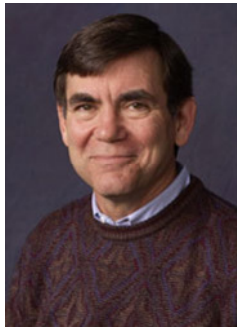




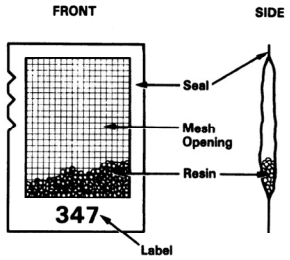


- Antisera isolated from infected rabbits and placed in wells
- Binding against immobilized peptides evaluated using a horseradish peroxidase assay
- Immunogenic epitope: XXLQXLA (X is any AA)
- First parallel synthesis of peptide libraries
- Maximum yields 300 nmol/pin
- Purity an issue

Tea Bag Synthesis



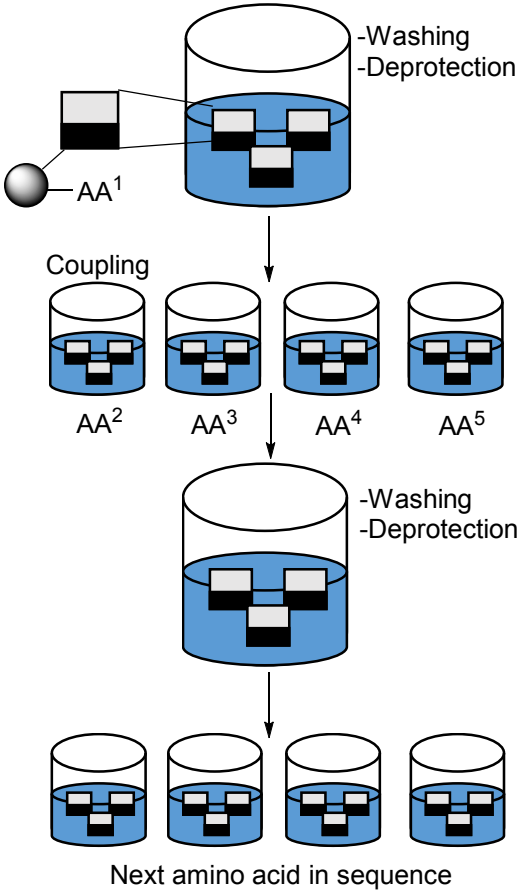
- **1985** Richard Houghten invents Tea bag synthesis
- Research objective: To provide a method allowing for the rapid synthesis of a large number of peptides



- Polypropylene mesh packet
- 74 μm mesh opening
- Dimensions: 15 x 20 mm

Advantages:
- Purity average: 84%
- Obtained up to 500 μmol of each peptide

Disadvantages:
- Still time intensive: 260 peptides 3380 couplings 4 weeks



Proc. Natl Acad Sci. USA **1985**, 82, 5131-5135

Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

Split and Mix Synthesis

- **1982** Arpad Furka conceptualizes split and mix synthesis
- **1988** first publication
- Enables the synthesis of peptide mixtures containing millions of components
- While working on determining the sequence of chymotrypsinogen-B, Furka thought about how many sequences were possible

- 245 amino acids and 20 possible amino acids
- $20^{245} = 5.65 \times 10^{318}$ combinations
- Estimated mass of the universe: ~ 10^{53} Kg
- Mass of protein mixture: ~ 10^{295} Kg

Number of possible peptide sequences:
- Dipeptides: 400
- Tripeptides: 8,000
- Tetrapeptides: 160,000
- Pentapeptides: 3,200,000
- Hexapeptides: 64,000,000

- With an elongation rate of 1 amino acid per day it would take 43.8 thousand years to synthesize all possible pentapeptides

- Synthesis could be optimized by reducing the number of necessary coupling steps

Discrete libraries → Mixture libraries

- Divide solid support into even portions equal to number of building blocks

- Couple a different building block to each portion

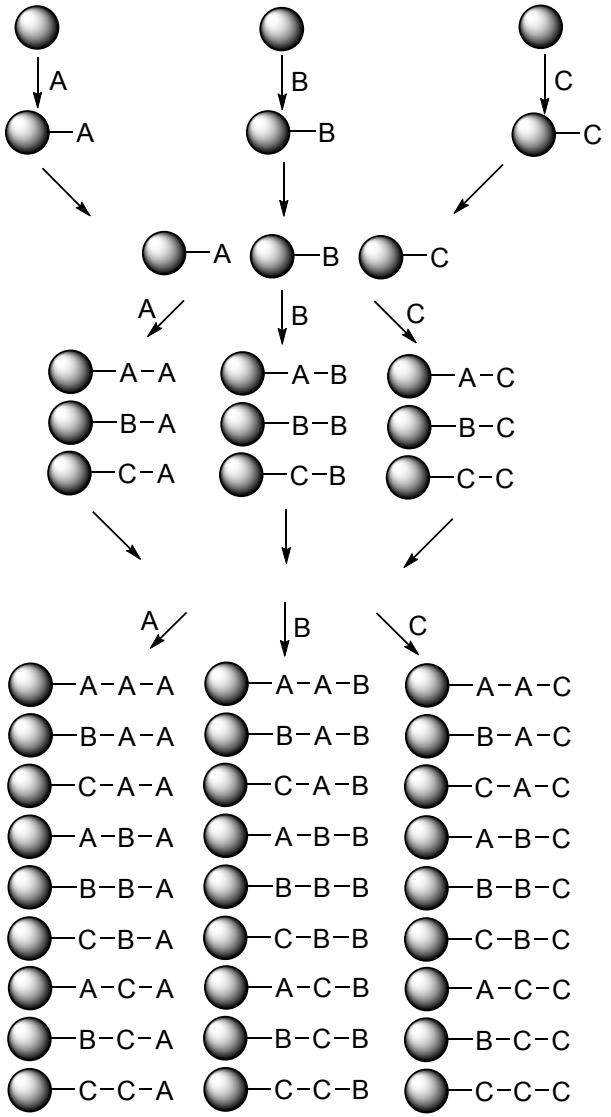
- Mix portions together

Split and mix:
- 27 membered library
- contained in 3 reaction vessels
- 9 reactions

Tea bag and Multipin:
- 27 membered library
- contained in 27 reaction vessels
- 81 reactions

Using 3 building blocks:
cycle 1: 3
cycle 2: 9
cycle 3: 27
cycle 4: 81

Using 20 building blocks:
cycle 1: 20
cycle 2: 400
cycle 3: 8,000
cycle 4: 160,000



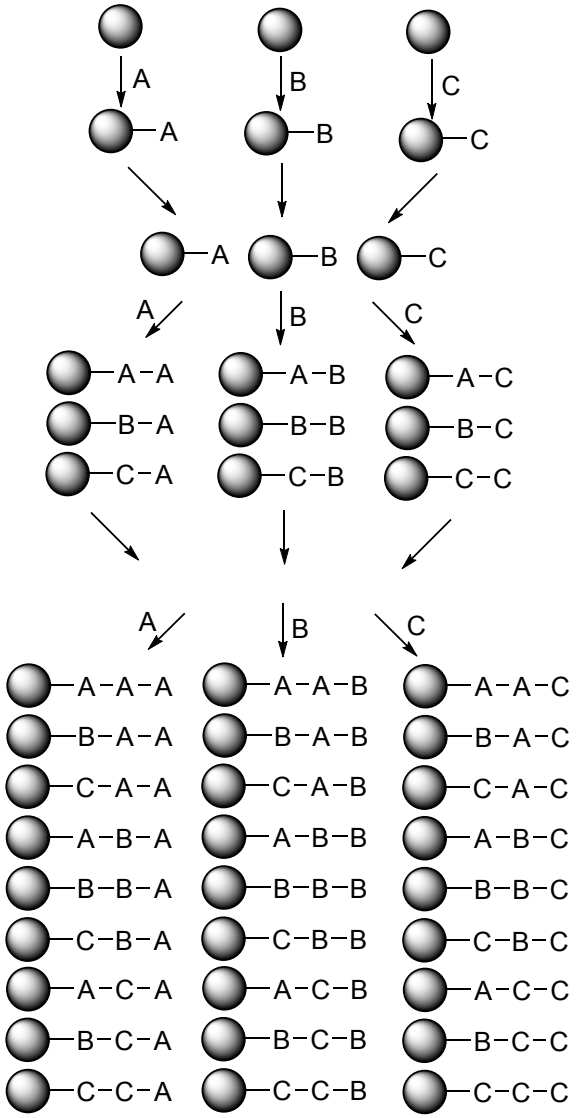
Abstr. 10th Intl. Symp. Med. Chem., **1988**, 288; *Int. J. Peptide Prot. Res.* **1991**, 37, 487 -493

Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

Iterative Deconvolution



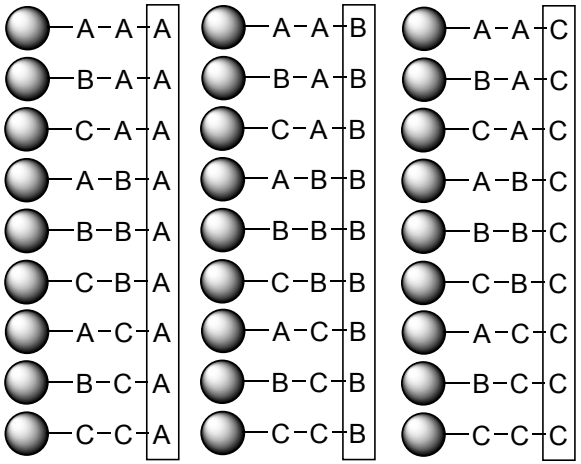
Identification techniques:

Screening:
- Iterative deconvolution

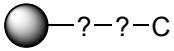
Encoding:
- Chemical encoding
- Radiofrequency encoding

- Sample set aside before mixing

- Sample set aside before mixing

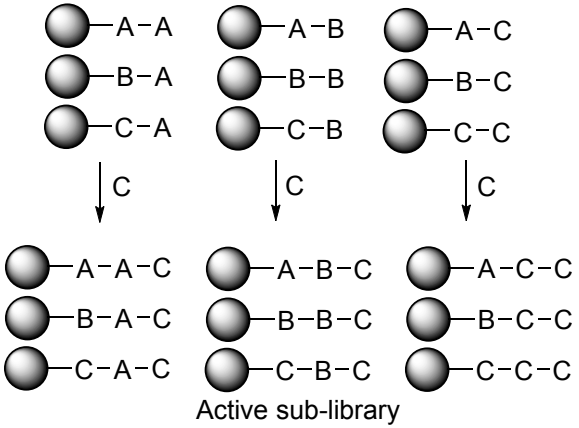


-Screen entire library

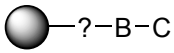


Active component

Active sub-library



- Take sample set aside from cycle 2
- Synthesize sub-library in separate reaction vessels
- Screen sub-library



Active component

Active sub-library

- Process repeated until sub-library small enough for individual synthesis

Nucleic Acids Res. **1993**, 21, 1853 -1856; *J. Med. Chem.* **1995**, 38, 344 -352.

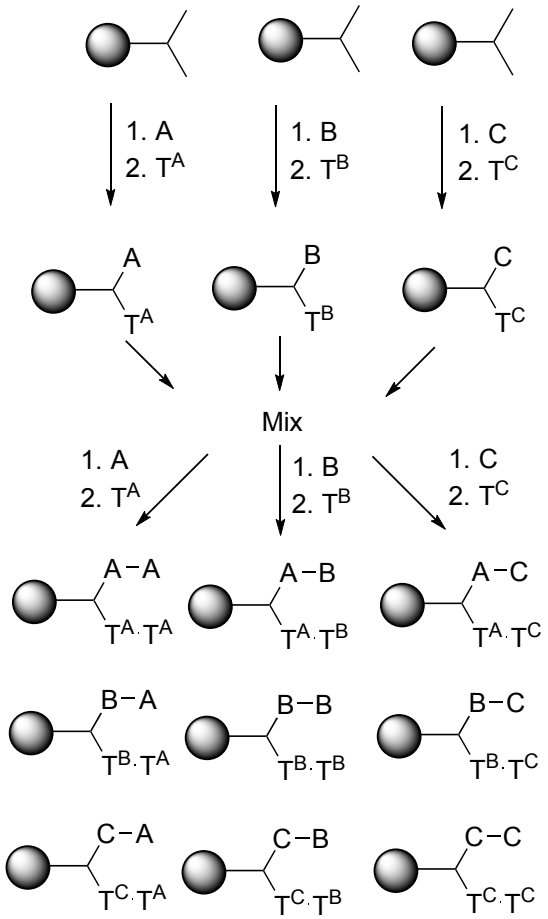
Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

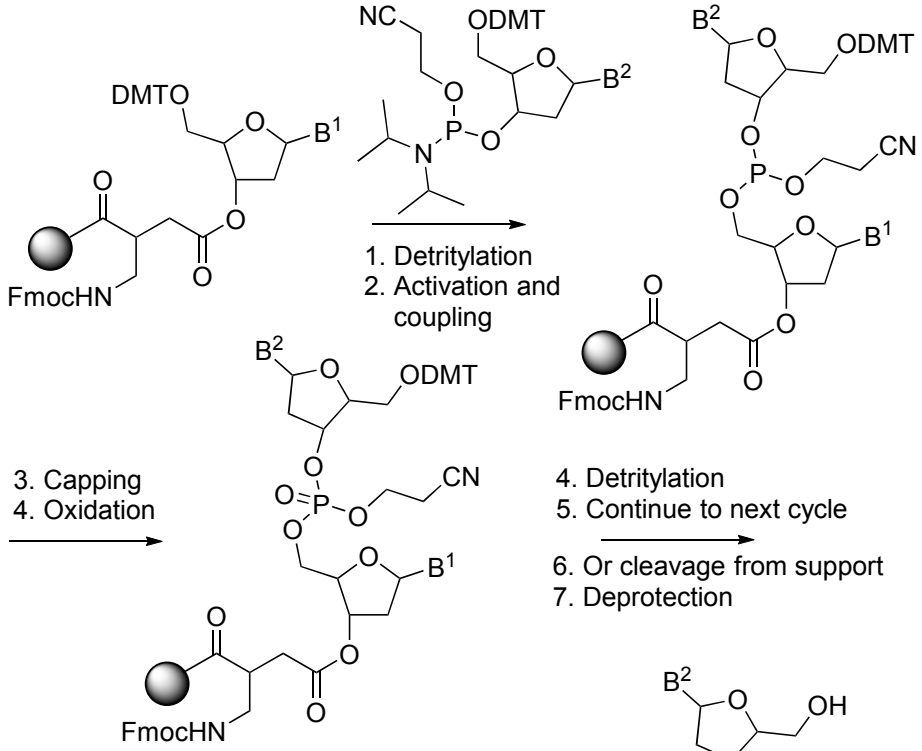
Chemical Encoding

General Strategy:



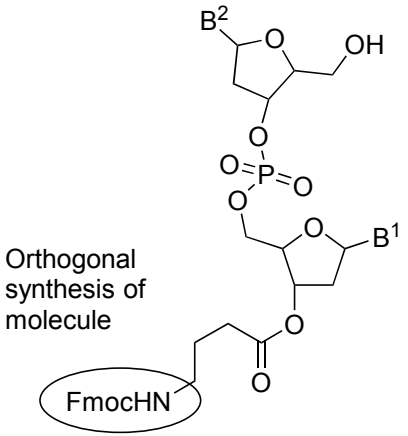
- Alternate stepwise synthesis of molecule and tag
- Linker must be capable of housing both molecule and tag
- Chemical tag must:
  - Be amendable to high sensitivity detection and decoding
  - Be stable to the reagents used in synthesis
- Most commonly used chemical tags:
  - Peptides
  - Oligonucleotides
- Oligonucleotides can be amplified by PCR
- Identity elucidated through sequencing

- Phosphoramidite method



Common reagents

1. Detritylation: Trichloroacetic acid
2. Activation: cat. tetrazole, MeCN
3. Capping: acetic anhydride, N-methyl imidazole, pyridine, THF
4. Oxidation: Iodine, pyridine, water
7. Deprotection: Conc. NH_4OH



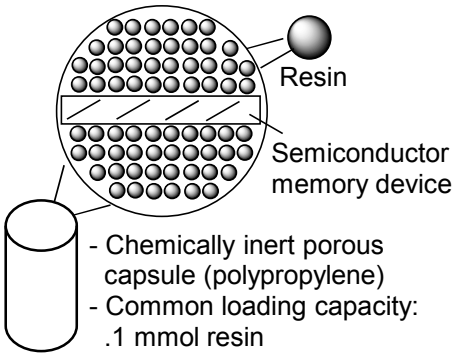
Proc. Natl. Acad. Sci. USA **1992**, 89, 5381 -5383; *J. Am. Chem. Soc.* **1993**, 115, 9812 -9813

Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

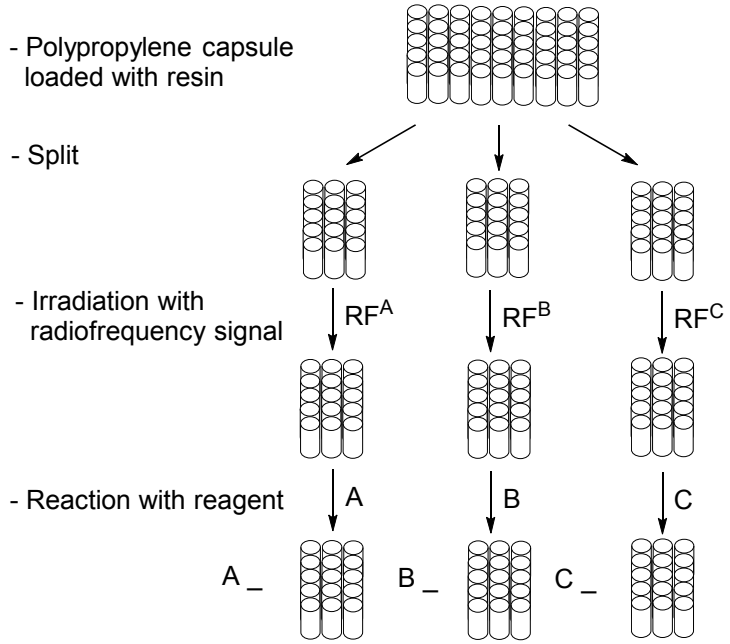
Benjamin Huffman

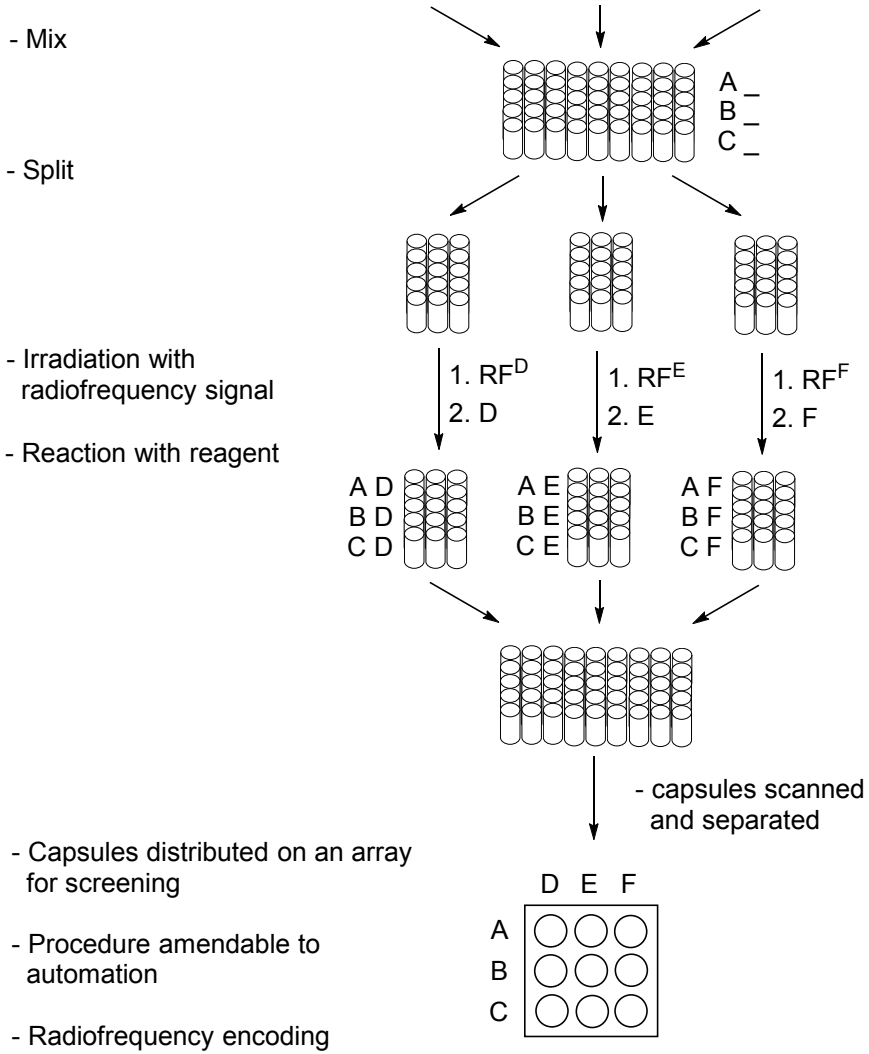
Radiofrequency Encoding



- Chemically inert porous capsule (polypropylene)
- Common loading capacity: .1 mmol resin

- Chemical encoding may be incompatible with synthetic methods used to generate the library
- Radiofrequency encoding allows noninvasive transmission and retrieval of synthetic history
- Semiconductor memory device capable of receiving, storing, and emitting radiofrequency signals





- Capsules distributed on an array for screening
- Procedure amendable to automation
- Radiofrequency encoding strategy used in several natural product analog syntheses

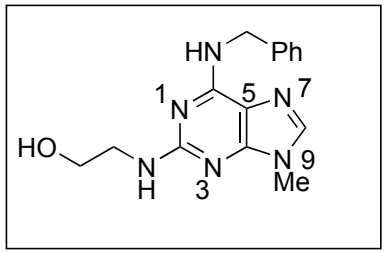
Angew. Chem. Int. Ed. Engl. **1995**, 34, 2289-2291; *J. Am. Chem. Soc.* **1995**,117, 10787-10788

Shenvi Lab Group Meeting
07/25/2016

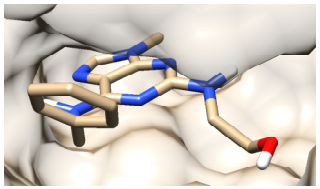
Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

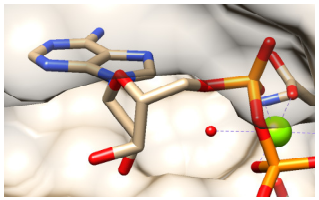
Olomoucine



CDK2: Olomoucine complex PDB: 1W0X

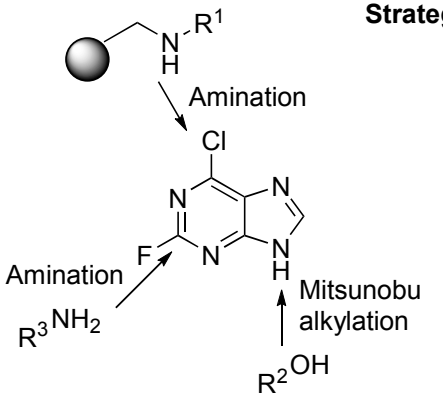


CDK2:ATP complex PDB: 1HCK



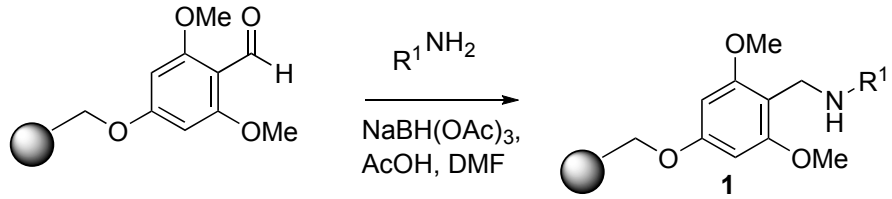
- Isolated by Meijer in 1994
- Competitive inhibitor of CDK2/cyclin A (7μM)
- Purine core rotated 160°
- Shultz develops library synthesis in 1996
- Generates diversity at C2, C6, and N9

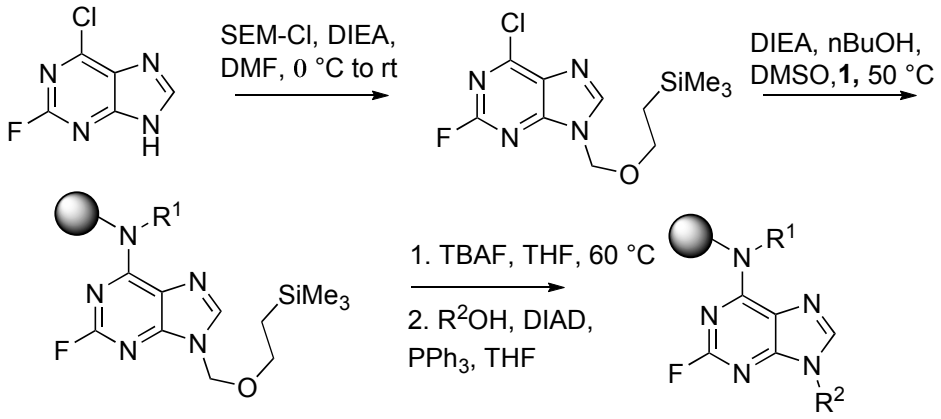
Strategy

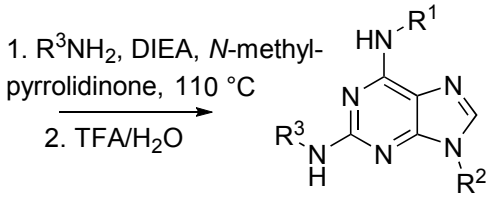


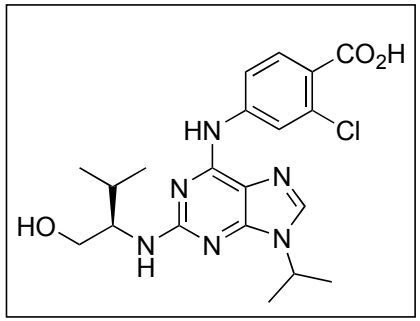
- Resin: polystyrene DVB
- Linker: (BAL)-derivatized 4-methylbenzhydrylamine
- Library size not disclosed
  - "Several hundred"
- Parallel synthesis in 96-well Geysen apparatus
- Screened against CDK2/cyclin A complex

Library Generation



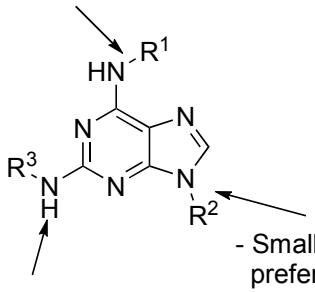






- Purvalanol B: 1000 fold increase in potency over olomoucine

- 3- and 4- substituted benzylamine and analine improve potency



- Small alkyl chain preferred

- Chiral amino alcohols improve potency

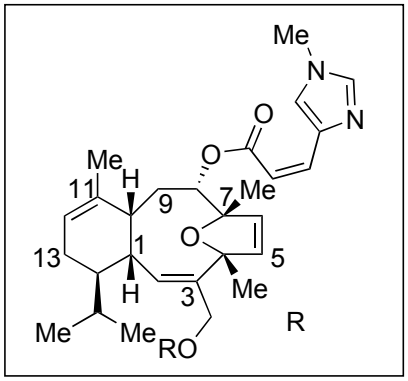
J. Am. Chem. Soc. **1996**, 118, 7430-7431; *Science.* **1998**, 281, 533-538

Shenvi Lab Group Meeting
07/25/2016

Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

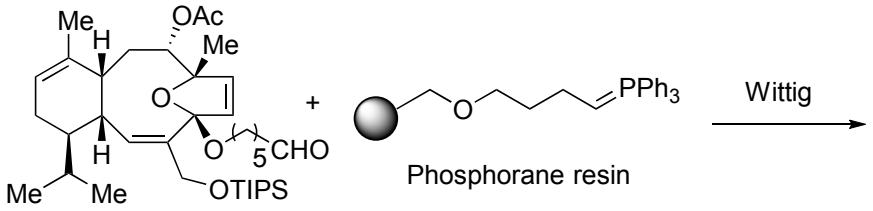
Sarcodictyin A



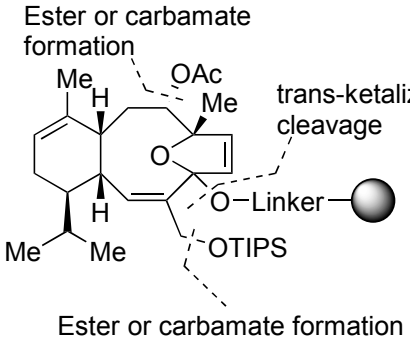
- Isolated from Mediterranean stoloniferan coral in 1987
- Potent antitumor activity
  - Perturbs tubulin-microtubulin dynamics resulting in cell death
- Nicolaou reports hemisynthetic approach to analogs
- Three points of diversification: C3, C4, and C8
- Split and mix approach using radiofrequency encoding

R = Me: sarcodictyin A
R = Et: sarcodictyin B

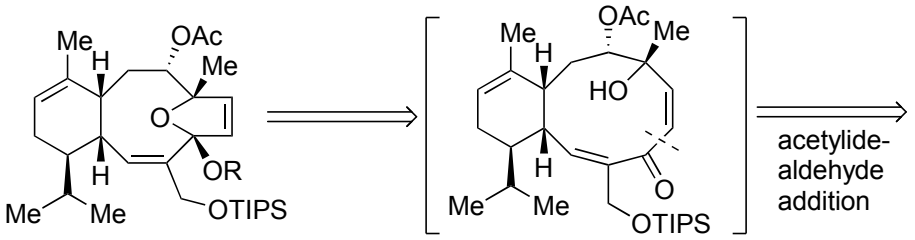
Strategy



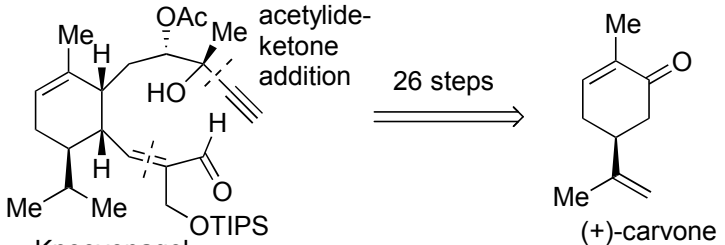
Key intermediate



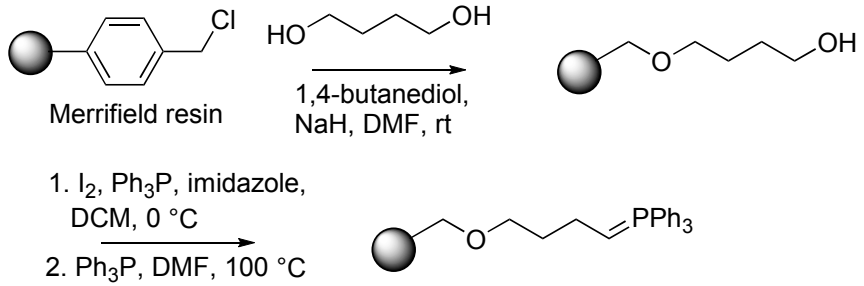
Key Intermediate Retrosynthesis



Key intermediate



Resin Preparation



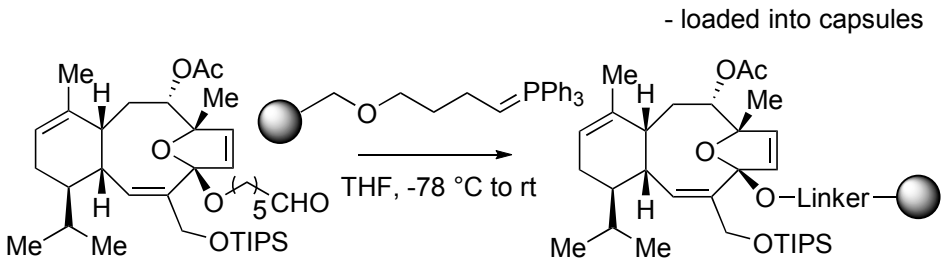
J. Am. Chem. Soc. **1998**, 120, 10814-10826; *J. Am. Chem. Soc.* **1998**, 120, 8661-8673

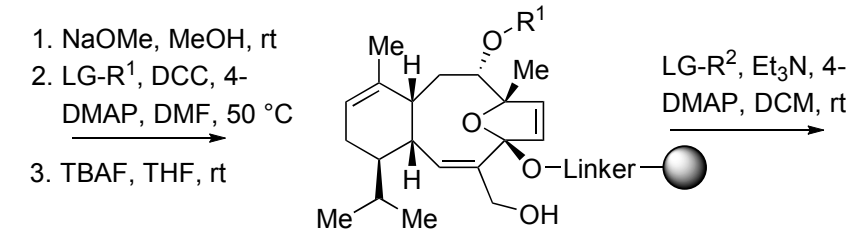
Shenvi Lab Group Meeting
07/25/2016

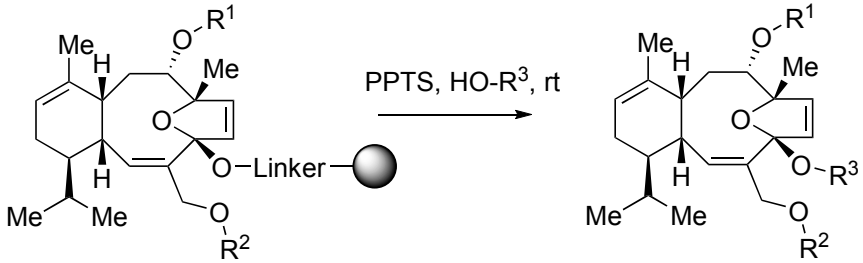
Solid Phase Combinatorial Chemistry: Methods and Application to Natural Products

Benjamin Huffman

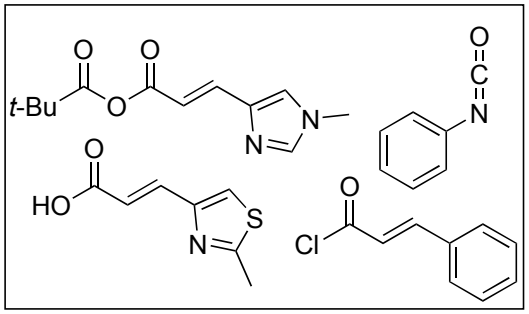
Library Generation



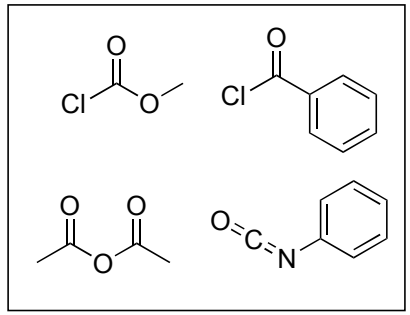




LG−R1

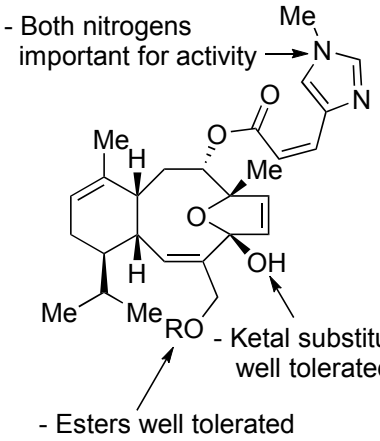


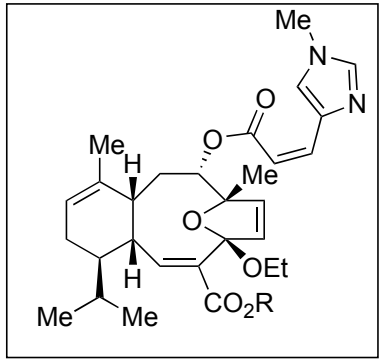
LG−R2



HO−R3

MeOH, EtOH, ^{n}PrOH, CF_3CH_2OH





SAR-85
- Improved potency:
  - 2-fold increase 1A9 cells
  - 10-fold increase 1A9PTX10
  - 2-fold increase 1A9PTX22

- 66 membered library
- Several analogs with improved potency

J. Am. Chem. Soc. **1998**, 120, 10814-10826; *J. Am. Chem. Soc.* **1998**, 120, 8661-8673