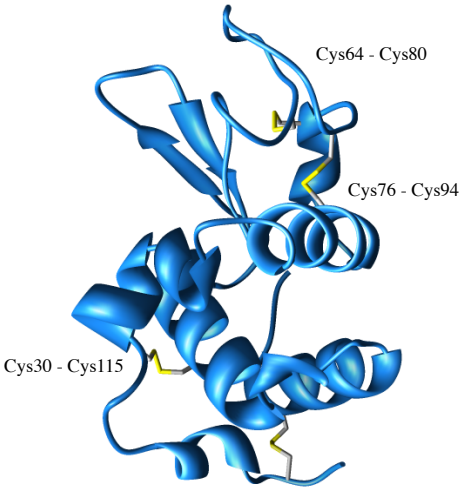
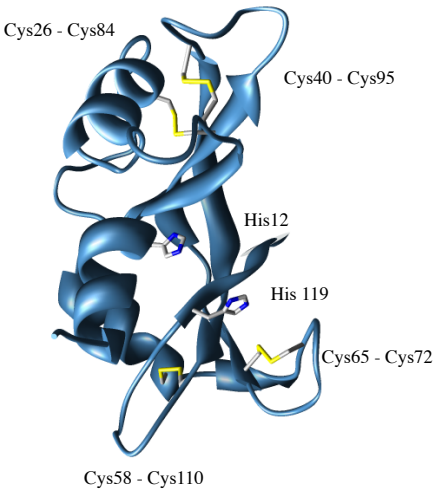
Disulfide bonds

lysozyme
pdb code: 1AZF



ribonuclease
pdb code: 1ALF



113

Combinatorial Chemistry: molecular diversity

"Synthesis and Applications of Small Molecule Libraries."
Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555-600.
"Design, Synthesis, and Evaluation of Small-Molecule Libraries."
Ellman, J. A. *Acc. Chem. Res.* 1996, 29, 132 -143.
Combinatorial Chemistry, Nicholas K. Terrett, Oxford University Press, London, 1998

pharmaceutical industry- drug discovery

Lead Cmpd —optimization (synthesis)→ **Drug** —10 years, > $ 1 Billion→ **$ $ $**

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

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

Number of marketable drugs per compounds that undergo preliminary biological testing: $\frac{1}{10{,}000}$ ← Rational drug design ← Combinatorial chemistry

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Peptides: poor bioavailability, poor transport, easily metabolized: poor drug candidates.

However, they are the natural substrates for many enzymes and receptors (drug targets) and have well-defined conformations: excellent lead compounds

Enzymes: converts a substrate to a distinct product

Receptor: binds a ligand (no reaction), causing a chain of physio-chemical events leading to a pharmacological response.

Agonist: substance that interacts (binds) with a receptor and elicits an observable response

Antagonist: substances inhibits the affect of an agonist, but has no biological activity of its own

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Example of molecular diversity:

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

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

20^4 = 160,000 different tetra-peptides !

now include the 19 D-amino acids (20 L + 19 D = 39)

39^4 = 2.3 million different tetra-peptides !!

now include 20 unnatural amino acids

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

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

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

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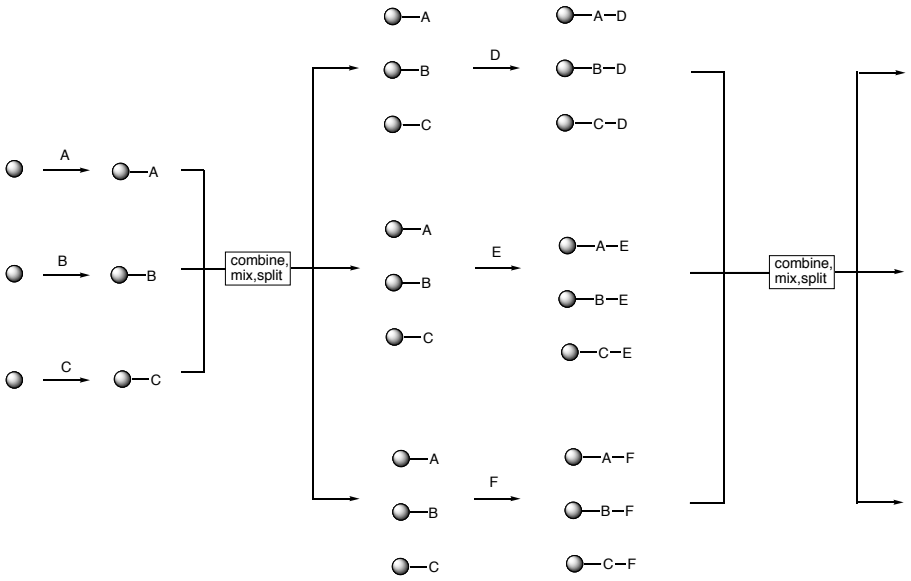
Split synthesis (mixture libraries)



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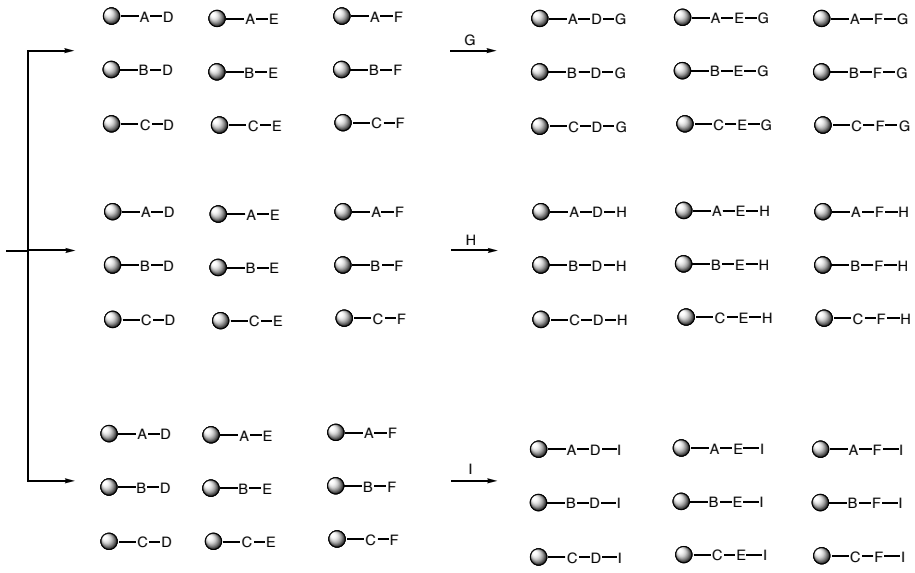
Split synthesis (con't)



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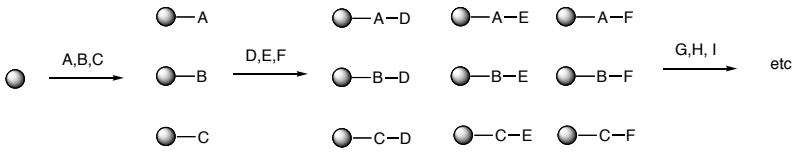
Why not?

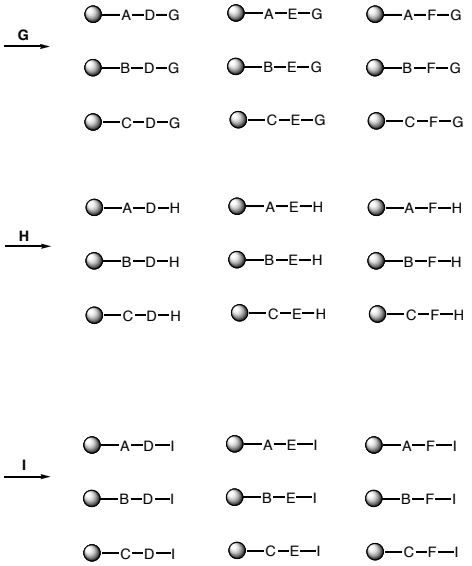


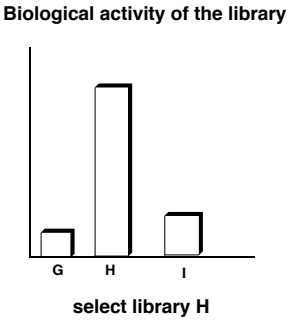
Reactivity of the coupling reaction may be different and this could bias the library

Split synthesis approach, all compounds are equally represented (each coupling is individually controlled)

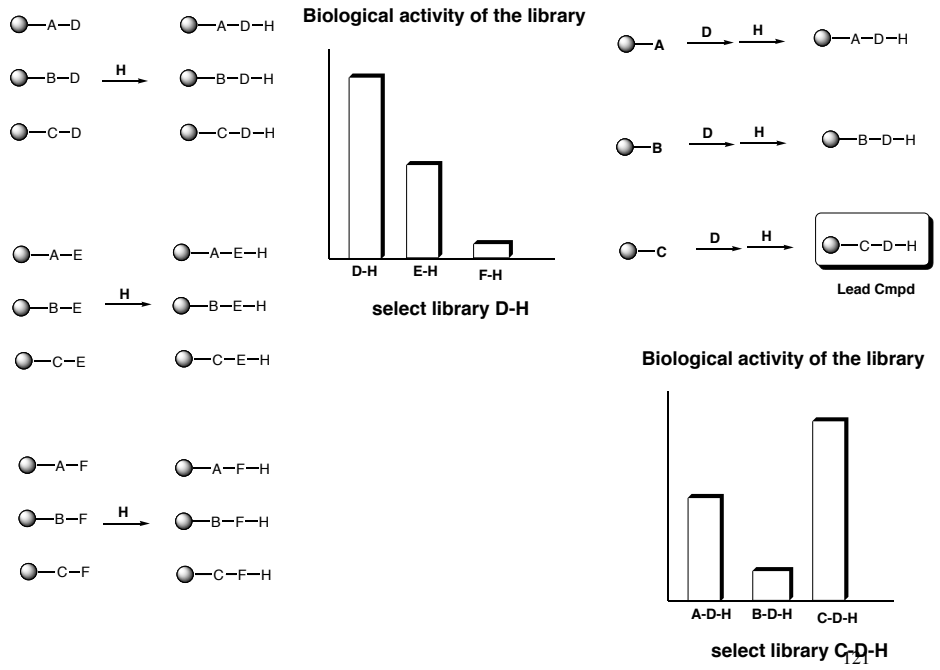
119

Deconvolution of the library
via biological activity and sub-library re-synthesis





120



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Problems:

- deconvolution of the library can be labor intensive
- can be fooled by low concentrations of highly active compounds
- activity is dependent upon the compound and its concentration
- activity observed is the the combined activity of the entire library

Advantage:

- can synthesize a very large number of compounds very quickly and relatively easily depending on the chemistry.

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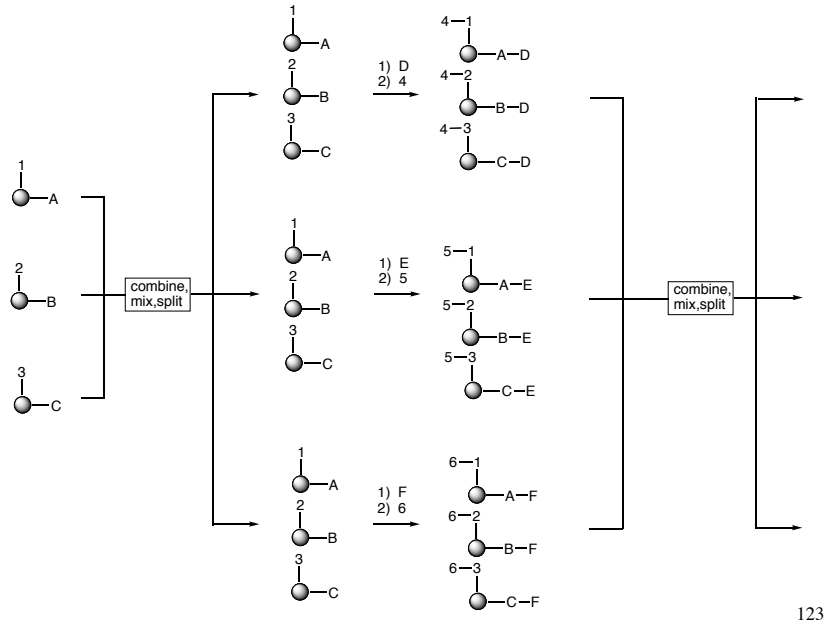
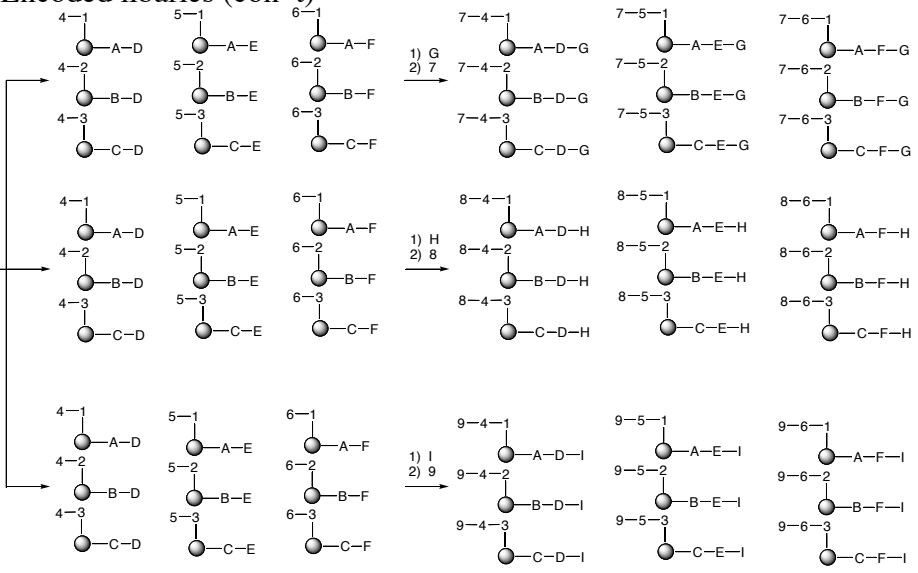
Encoded Libraries



123

Encoded libaries (con't)



CODE: 1= A 2= B 3= C 4= D 5= E 6= F 7= G 8= H 9= I

Cleave the marker and analyze the code. Each unique marker codes for a unique peptide sequence

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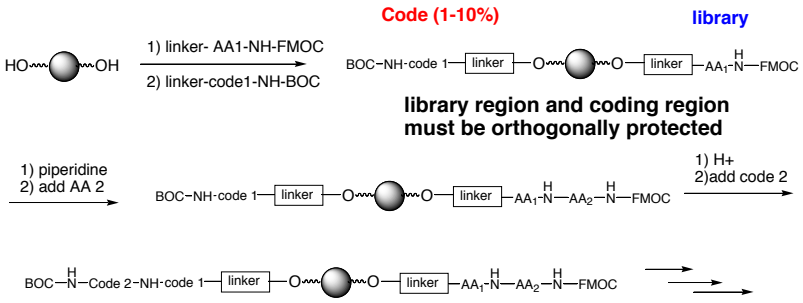
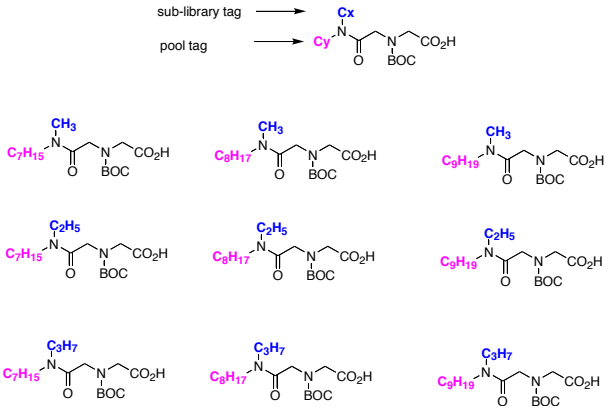
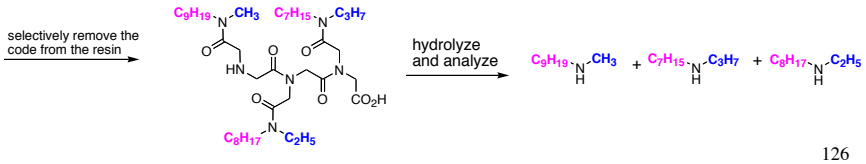
Synthesis of an encoded library

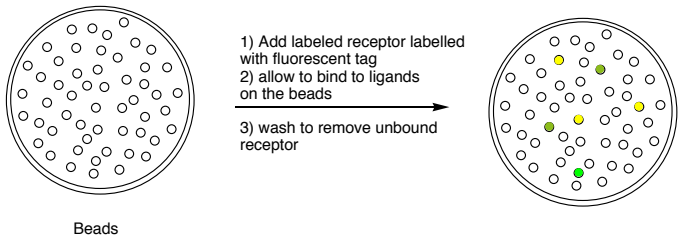




Reading the code



"On-Bead" Assay for Receptor Binding using a Fluorescently Labeled Receptor



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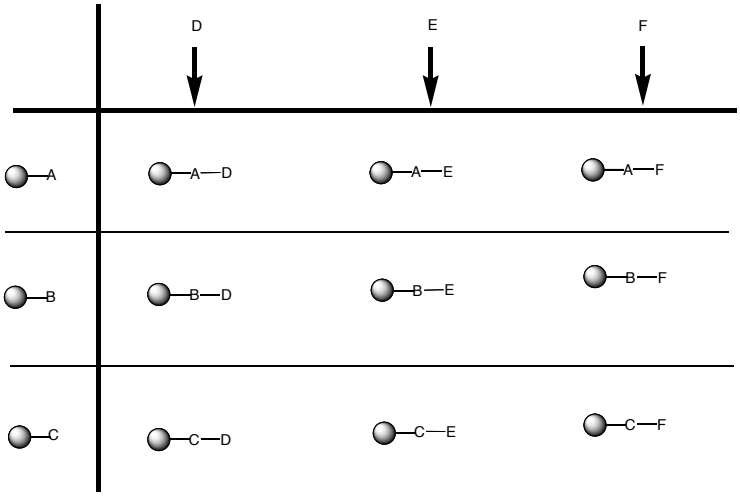
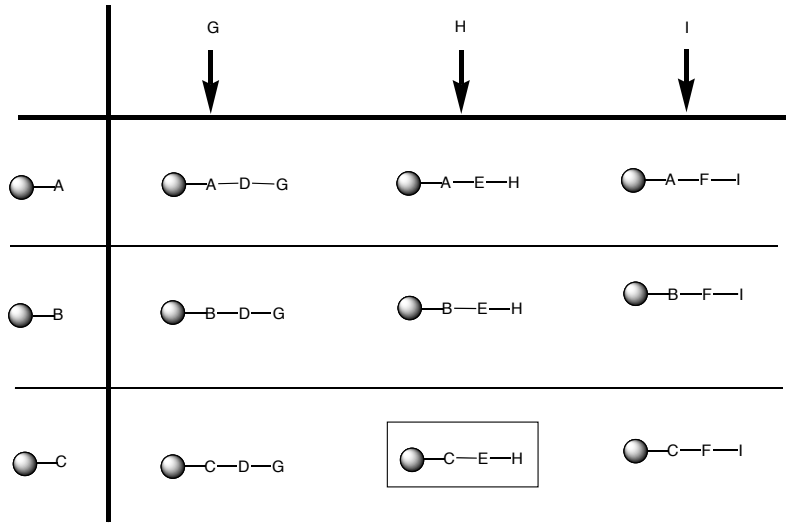
Spatially Addressable, Parallel Synthesis



128

Spatially Addressable, Parallel Synthesis (con't)



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Spatially addressable, parallel libraries

Advantage:

- synthesizing pure compounds, no need to deconvolute the library

Disadvantage

- libraries tend to be much smaller

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Diversomer apparatus

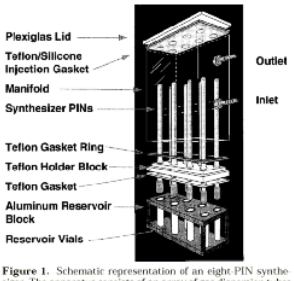

Figure 1. Schematic representation of an eight PIN synthesizer. The apparatus consists of an array of gas dispersion tubes (PINS), a reservoir block with multiple reaction wells, a holder block, a manifold, and gaskets. Clamps are not depicted.

Figure 2. A now-dated photograph of the 40-array apparatus interfaced to the x,y,z robot used for liquid delivery (September 1993).

Acc. Chem. Res. **1996**, *29*, 114-122

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ACE Inhibitors

Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu–Val–Ile–His–Asn
angiotensinogen

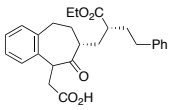
aspartyl protease | renin

Asp–Arg–Val–Tyr–Ile–His–Pro–Phe–His–Leu
angiotensin I
(little biological activity)

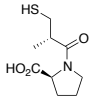
Zn^{2+} protease | angiotensin converting enzyme (ACE)

Asp–Arg–Val–Tyr–Ile–His–Pro–Phe
angiotensin II
(vasoconstriction → high blood pressure)

ACE Inhibitors



Benzazepril



Captopril

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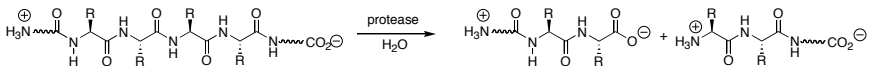
Proteases: catalyzes the hydrolysis of peptide bonds



1. Serine protease
2. Cysteine protease
3. Aspartyl protease
4. Zinc (metallo) protease

ACE: zinc protease (no x-ray or NMR structure),
compared to carboxypeptidase or thermolysin
important catalytic groups: Glu-270, His-196, His-69
(catalytic triad)

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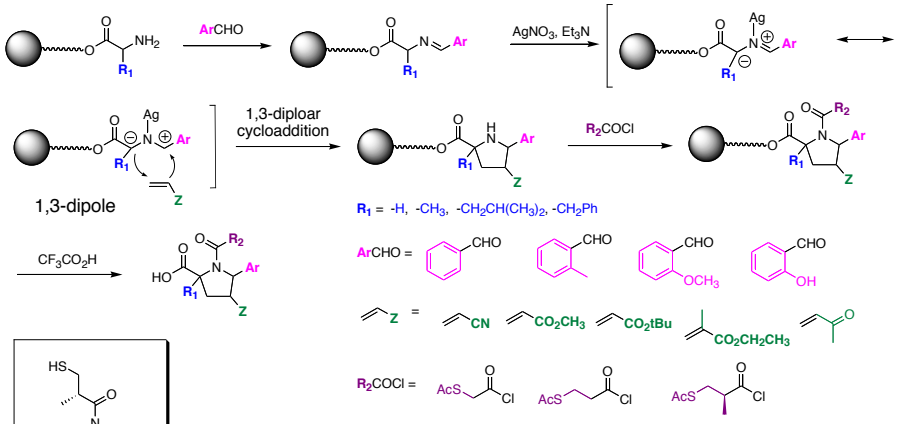
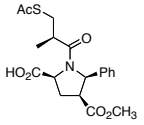
ACE inhibitor Library



Captopril
(ACE Inhibitor)

240 cmpds, each are multiple stereoisomers



K_i ~ 160 pM (3x better than Captopril)

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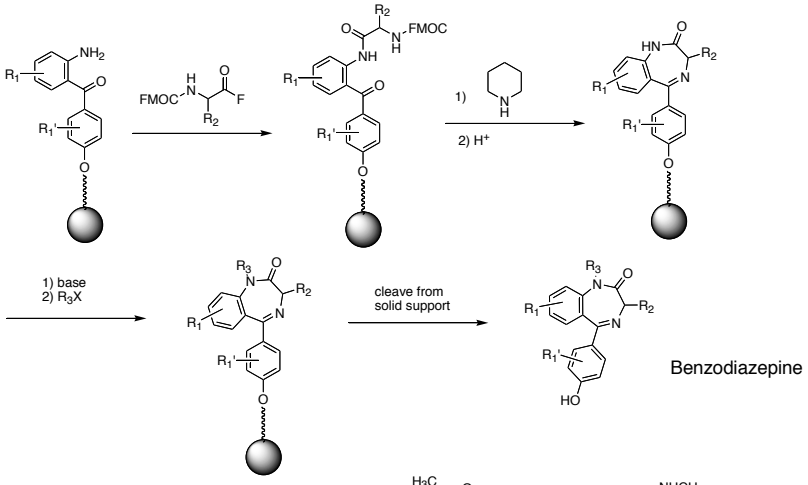
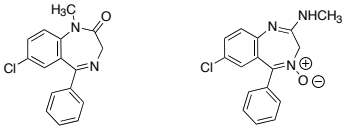
Parallel Synthesis of a Benzodiazepine Library





Valium (diazapam)

Librium (chlordiazepoxide)

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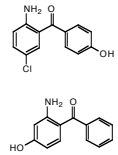
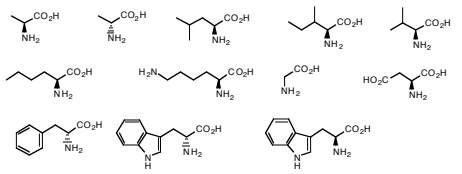
Parallel Synthesis of a Benzodiazepine Library

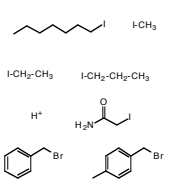
2-aminobenzophenones



α-amino acids



alkylating agents



2 (2-aminobenzophenone) x 12 (amino acids) x 8 (alkyl halides) = 192 compounds

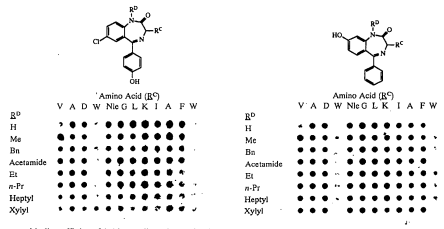


FIG. 3. Receptor binding affinity of 1,4-benzodiazepine derivatives. Receptor binding affinity of the 1,4-benzodiazepine derivatives at 7.5 μM is monitored by the percent displacement of ^{125}I-labeled CCK-8 from the CCK A receptors as observed by the relative signal intensity upon PhosphorImager exposure. Group R^C corresponds to the side chain of the amino acid that is incorporated into the benzodiazepine structure. The absolute configuration for most compounds is *S*, except for the derivatives in the last three columns (incorporating amino acids A, F, and W), where the absolute configuration is *R*. Nle, norleucine; Bn, benzyl; *n*-Pr, *n*-propyl. In the receptor binding assay, the two benzodiazepine derivatives prepared from valine with R^C = H were replaced with compound **9** (Table 1).

Cholecystikinin (CCK)
CCK-8: Asp-Tyr(SO3H)-Met-Gly-Trp-Met-Asp-Phe-NH2

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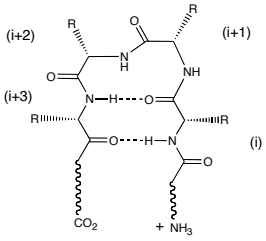
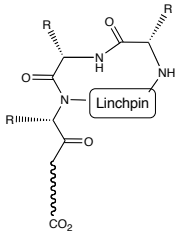
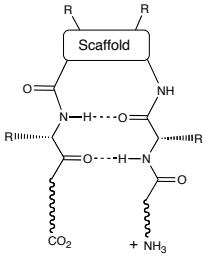
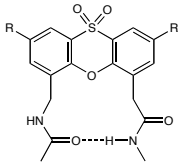
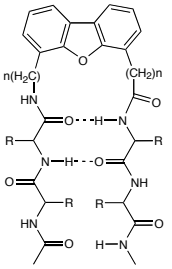
β-Turn Mimics:

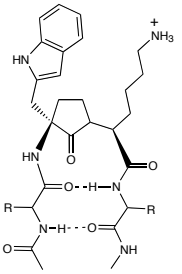












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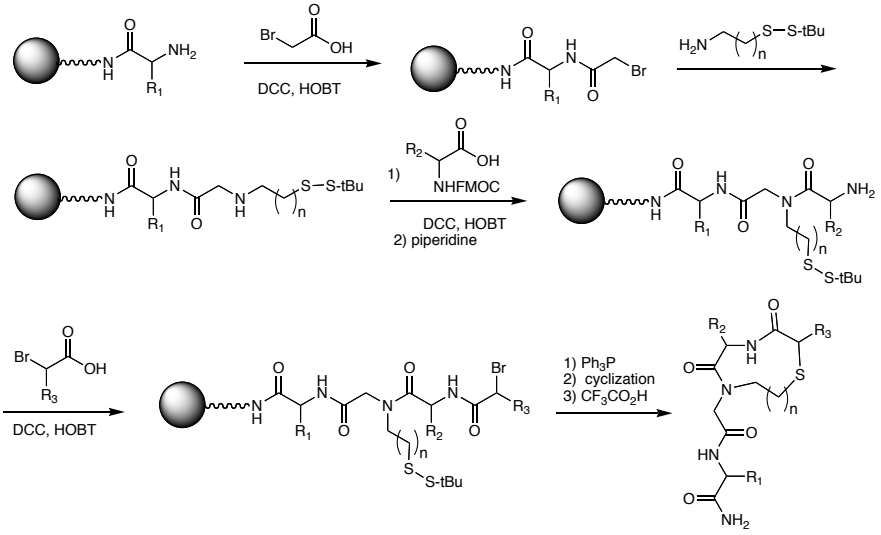
Combinatorial synthesis of linchpin β-turn mimic



Evaluated for Somatostatin receptor binding

n= 1,2 x R_2= 34 AA x R_3= 10 = 1292 β-turn mimics

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