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 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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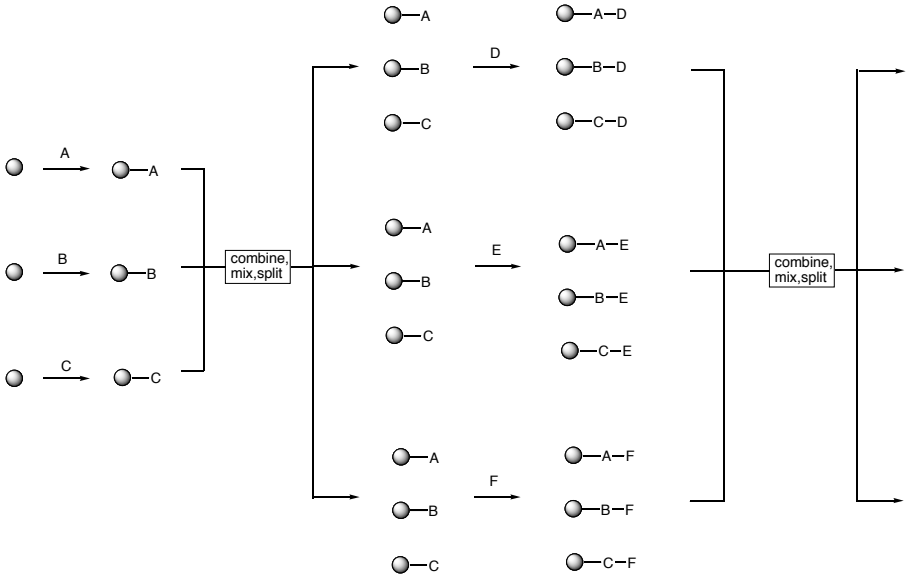
Split synthesis (mixture libraries)



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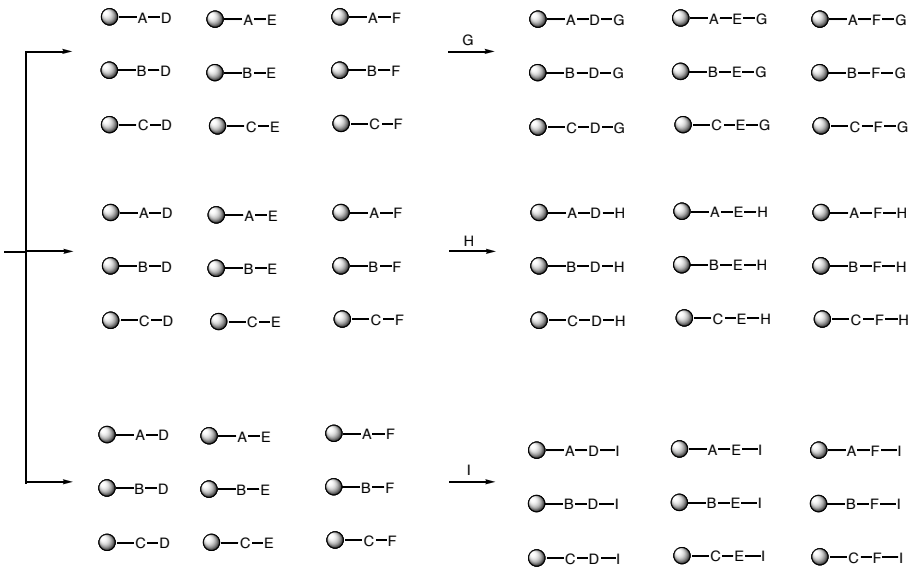
Split synthesis (con't)



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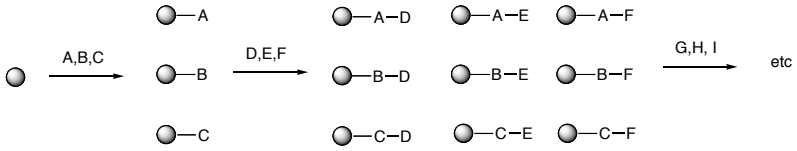
Why not?



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)

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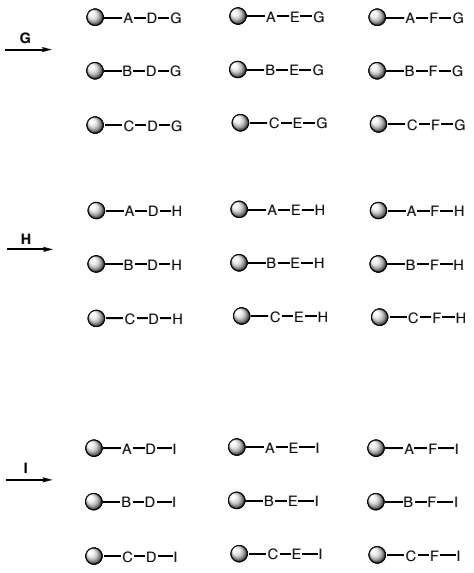
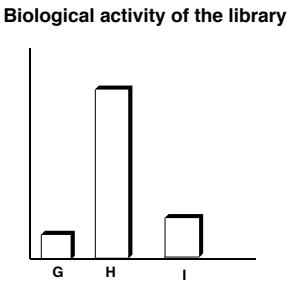
Deconvolution of the library via biological activity and sub-library re-synthesis





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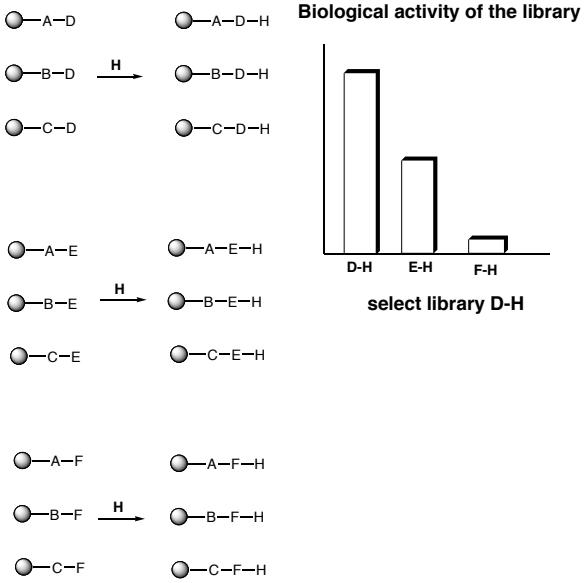

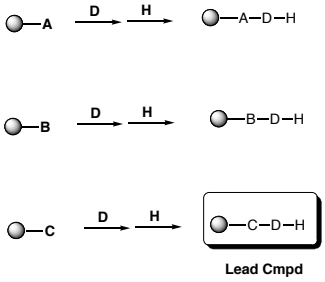

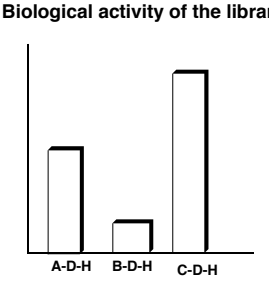

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Problems:

- 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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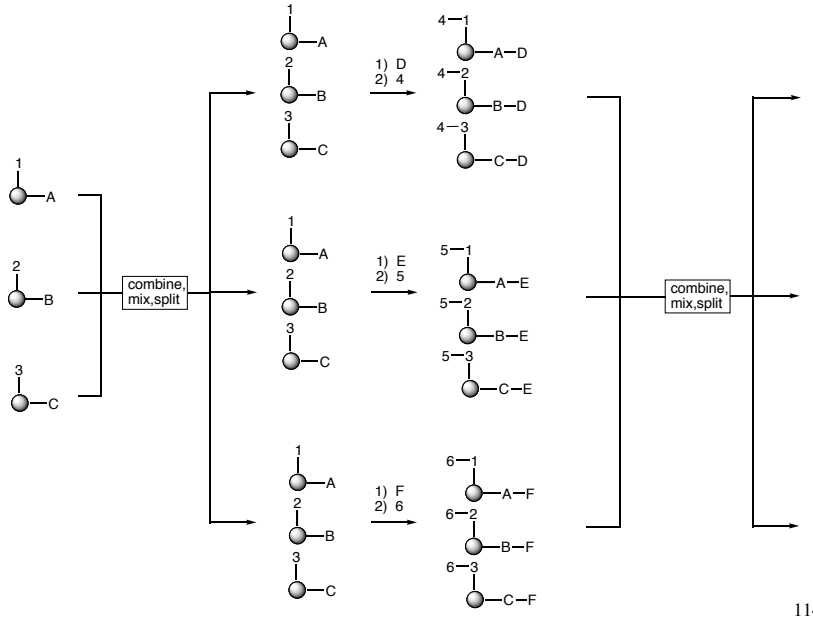
Encoded Libraries



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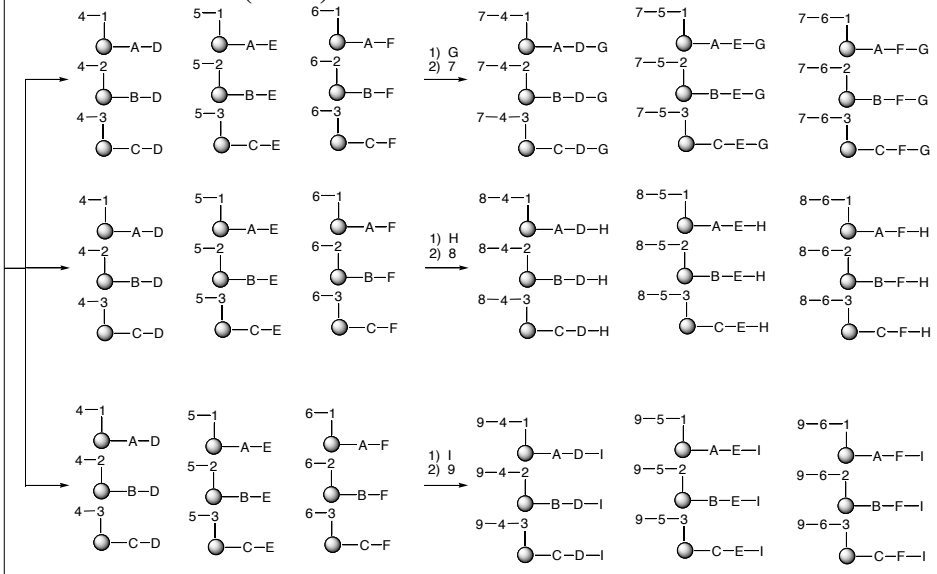
Encoded libaries (con't)



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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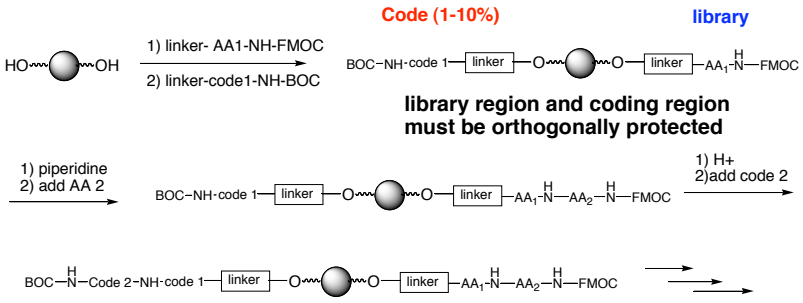
Synthesis of an encoded library



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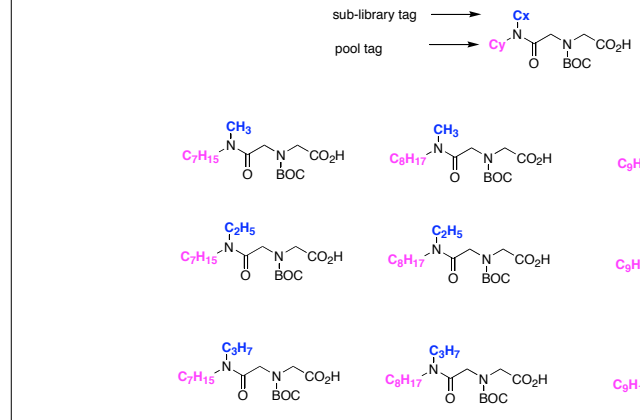
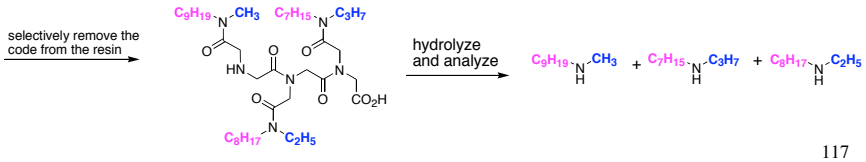


Reading the code



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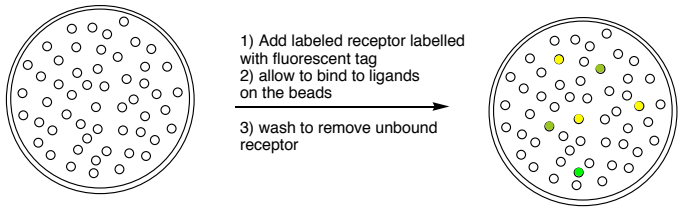
"On-Bead" Assay for Receptor Binding using a Fluorescently Labeled Receptor



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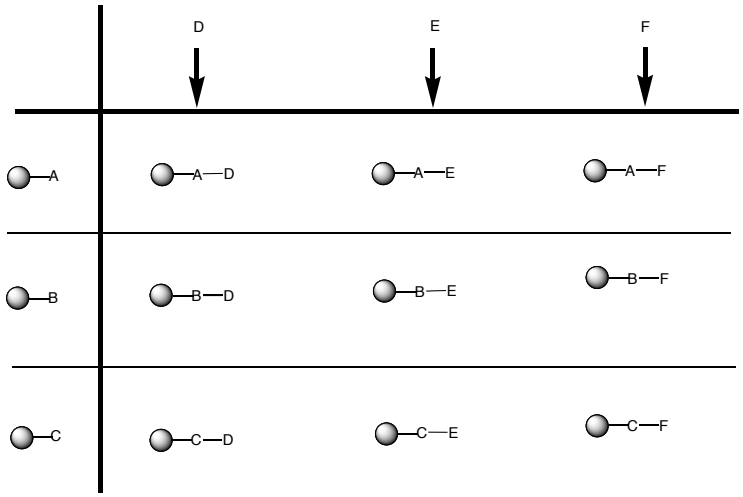
Spatially Addressable, Parallel Synthesis



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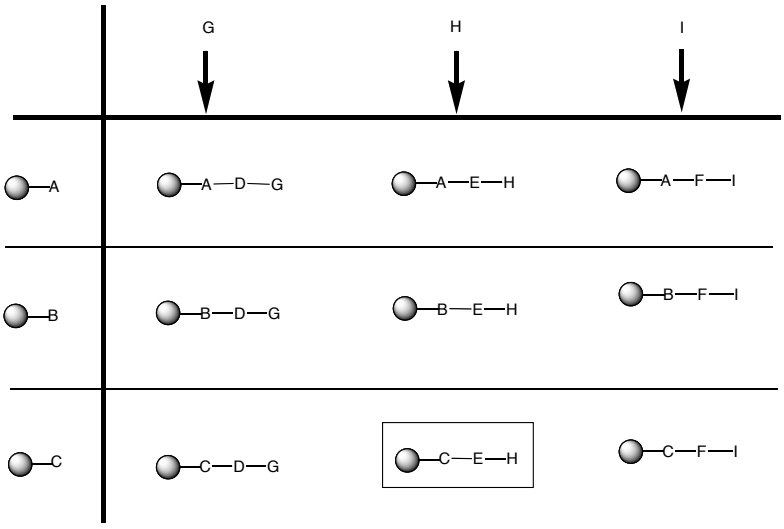
Spatially Addressable, Parallel Synthesis (con't)



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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

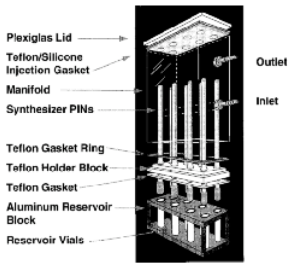


Figure 1. Schematic representation of an eight-PIN synthesizer. The apparatus consists of an array of gas dispersion tubes (PINS), a reservoir block with multiple reaction wells, a holder block, a manifold, and gaskets. Clamps are not depicted.

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

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)

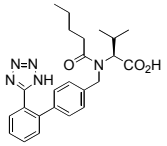
Zn^{2+} protease — angiotensin converting enzyme (ACE)

Asp–Arg–Val–Tyr–Ile–His–Pro–Phe

angiotensin II

(vasoconstriction → high blood pressure)

ACE inhibitors



Diovan

Captopril

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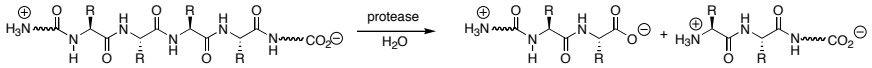
Proteases: catalyzes the hydrolysis of peptide bonds



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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Carboxypepidase:
General base mechanism

Nucleophilic mechansim (acyl enzyme complex)

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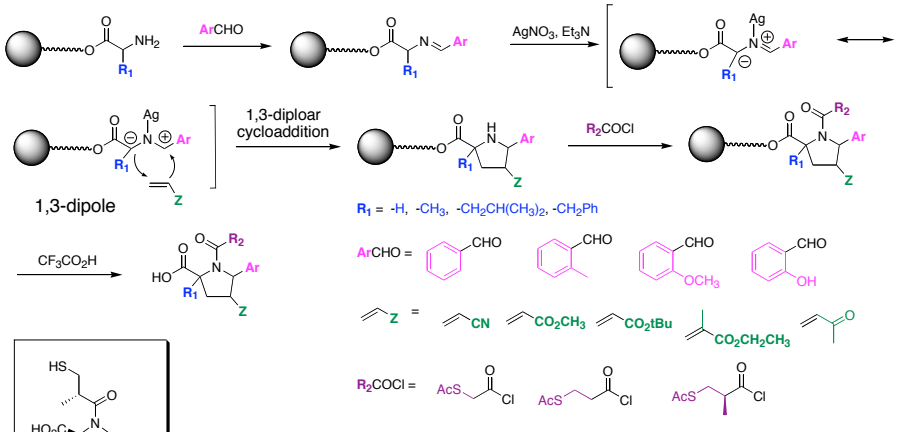
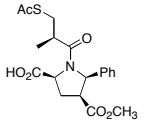
ACE inhibitor Library



Captopril
(ACE Inhibitor)

240 cmpds, each are multiple stereoisomers



K_i ~ 160 pM (3x better than Captopril)

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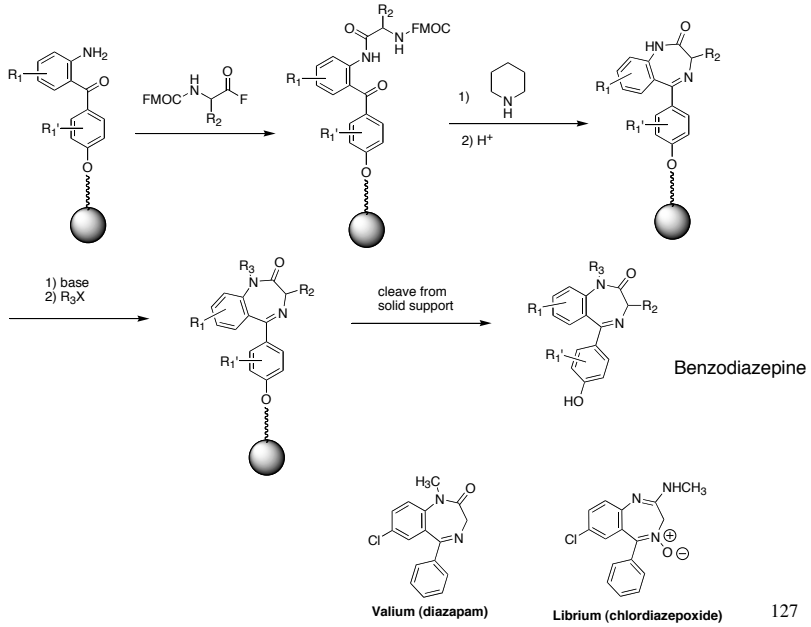
Parallel Synthesis of a Benzodiazepine Library



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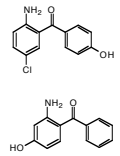
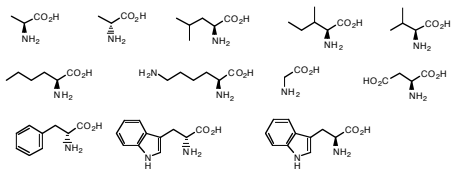
Parallel Synthesis of a Benzodiazepine Library

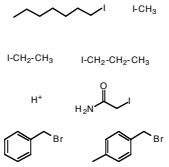
2-aminobenzophenones



α-amino acids



alkylating agents



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

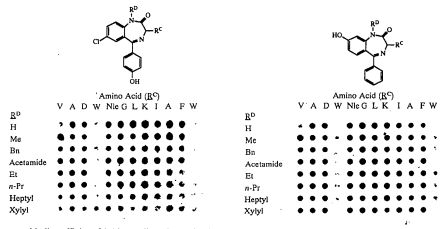


FIG. 3. Receptor binding affinity of 1,4-benzodiazepine derivatives. Receptor binding affinity of the 1,4-benzodiazepine derivatives at 7.5 μM is monitored by the percent displacement of ^{125}I-labeled CCK-8 from the CCK A receptors as observed by the relative signal intensity upon PhosphorImager exposure. 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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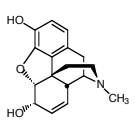
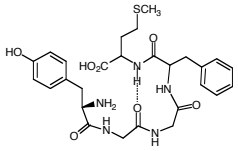
Peptide Mimetics or Peptidomimetics



Morphine

Tyr-Gly-Gly-Phe-Met (met-enkephalin)



Desirable pharmacological properties

1. metabolic stability
2. good bioavailability
3. high affinity ($K_i \sim 10^{-9}$) and specificity for a given receptor or enzyme
4. minimal side effects

Natural ligands for receptors or enzymes are often peptides:
peptides are good lead structures but poor drug candidates

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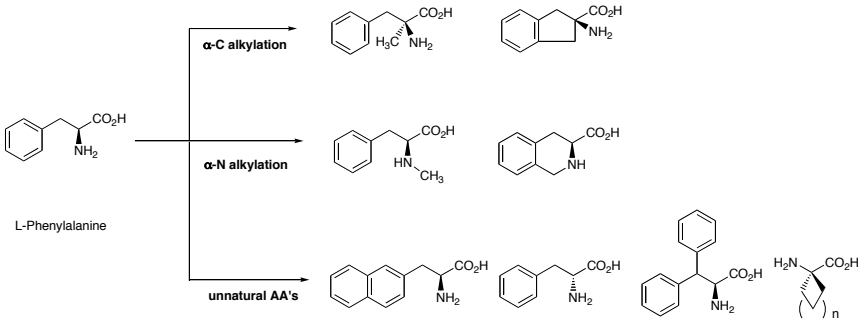
Peptidomimetics Strategies

1. Amino acid modification
2. Dipeptide analogues (isosteres and TS analogues)
3. Peptide backbone modifications
4. Secondary structure mimics

Amino Acid Modifications: changes (restricts) conformational degrees of freedom of the peptide



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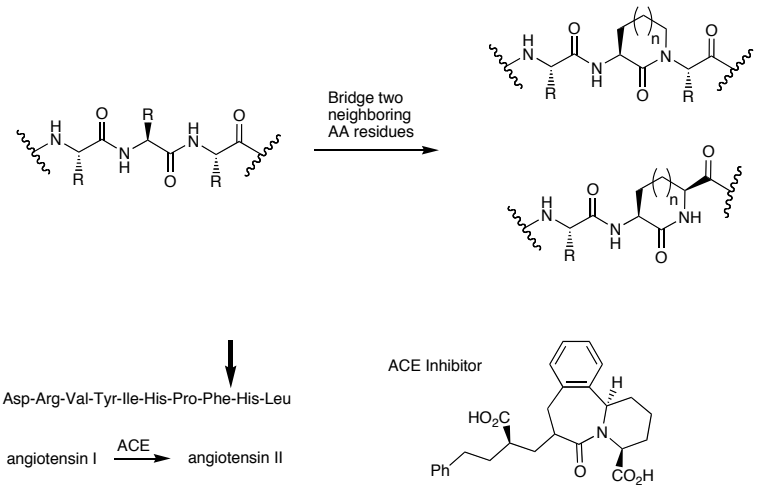
Dipeptide analogues



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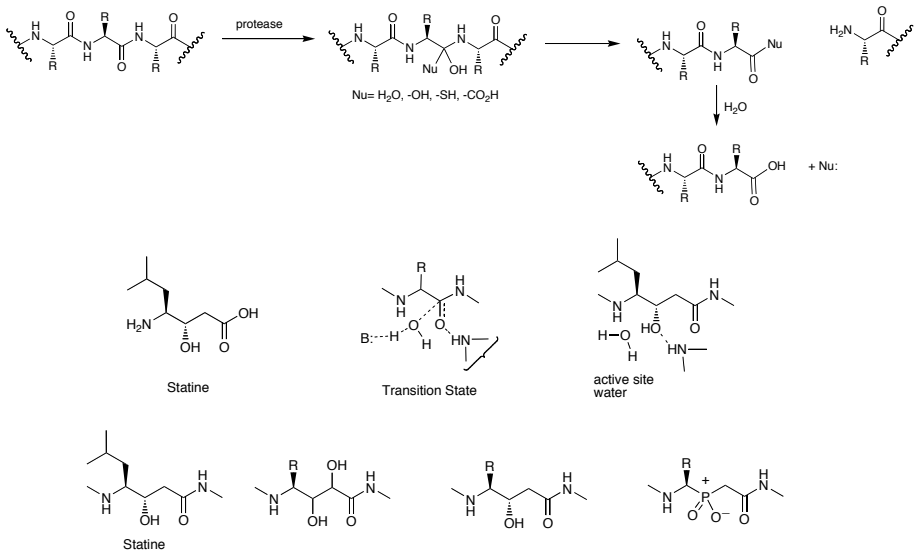
Transition state analogues for a protease:



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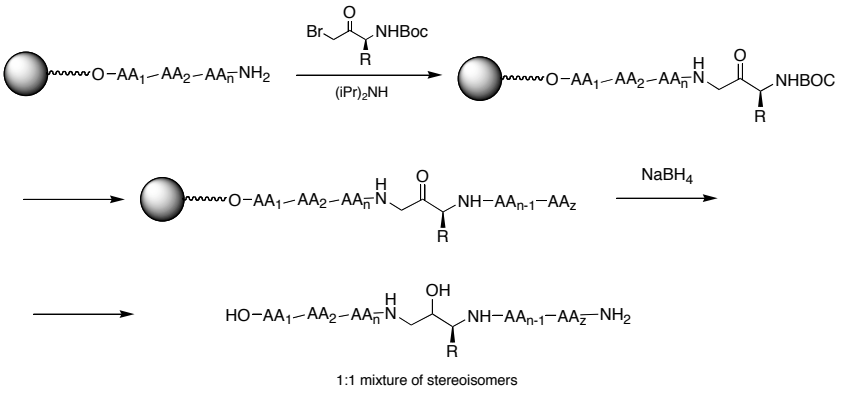
TS analoges via combinatorial synthesis



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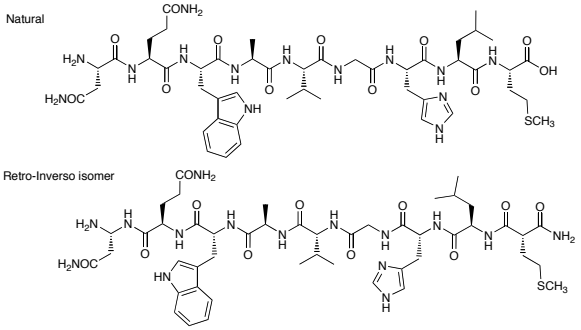
Peptide Backbone Modifications: Increase biological half-life

Retro-Inverso Isomers: Change from L- to D-amino acids and inverse N to C sequence



Interesting idea, but the retro-inverso isomers usually showed much weaker binding. However, they were significantly more resistant to enzymatic digestion.

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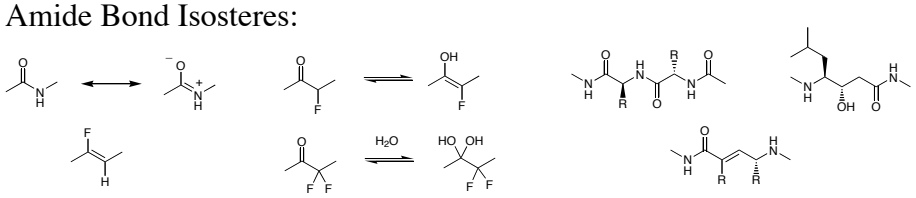
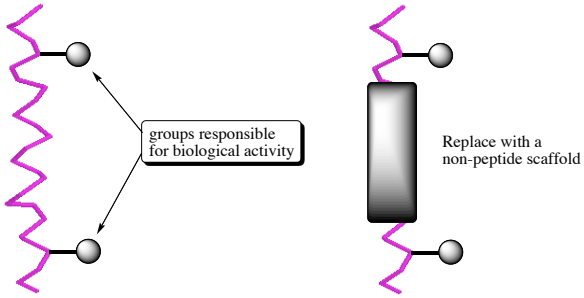
Amide Bond Isosteres:



Secondary Structure Mimics:

main 2° structures: α-helix, β-sheet, β-turns, γ-turns

α-helix mimic



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Vasoactive Intestinal Peptide (VIP): 28 AA helical peptide. Bronchodilator-asthma treatment "α-helical Peptidomimetic for Vasoactive Intestinal Peptide (VIP)" G. L. Olson et al. *J. Med. Chem.* **1993**, *36 (21)*, 3039-3049.

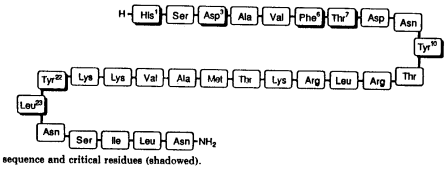
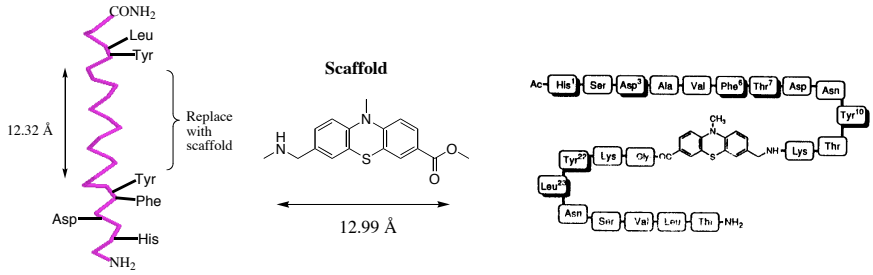


Figure 8. VIP sequence and critical residues (shadowed).



"Ala-scan" determine which residues are needed for binding

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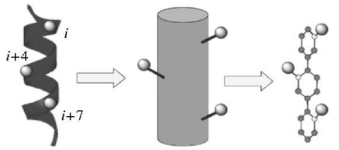
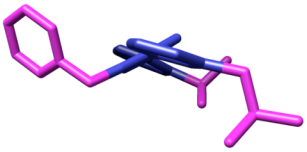
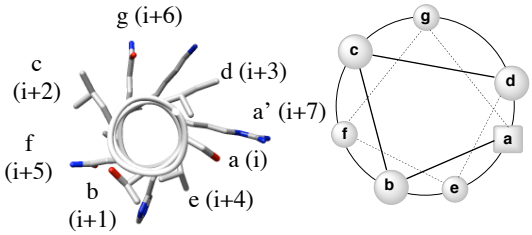
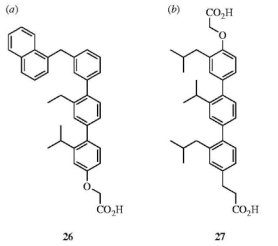
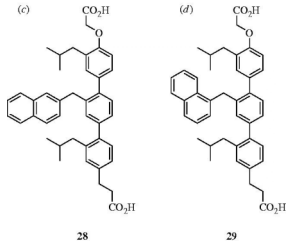
Ter-phenyl as an α-helix mimic











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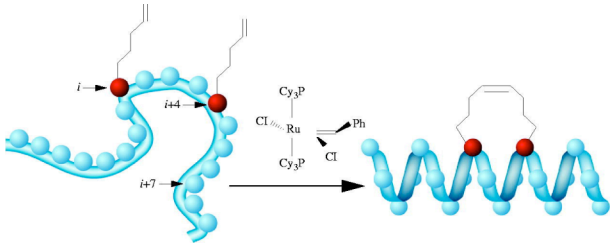
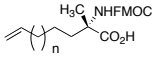
"stapled" α-helices





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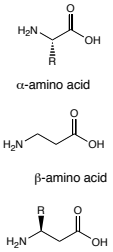
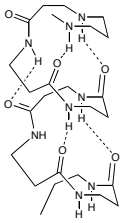
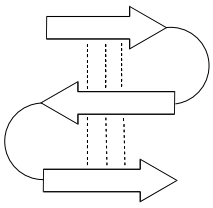
β-amino acids

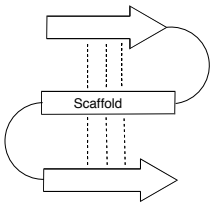


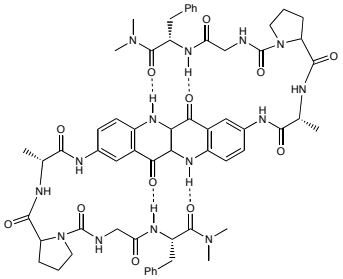


oligomeric β-amino acid
will fold into a helical structure
with 3 AA's per turn

β-Sheet mimics







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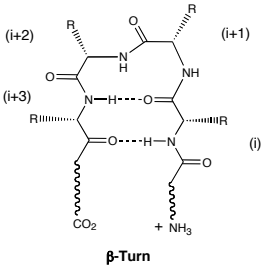
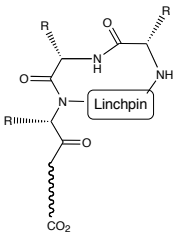
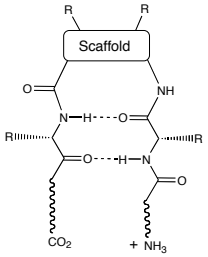
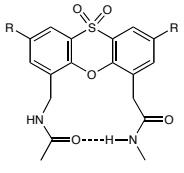
β-Turn Mimics:

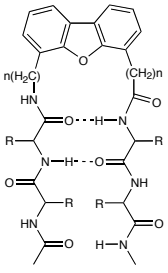


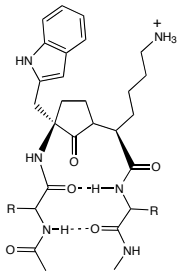
β-Turn











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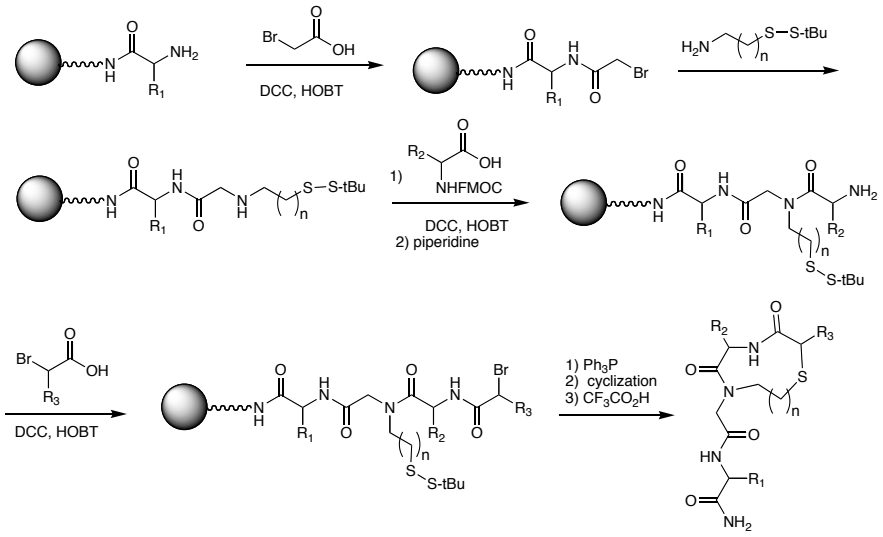
Combinatorial synthesis of linchpin β-turn mimic



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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