

Combinatorial Chemistry: Generation of Molecular Diversity by Covalently Combining Members of a Set of Structurally Varied Chemical Building Blocks

I: Linear, Homo-Oligomeric Molecules

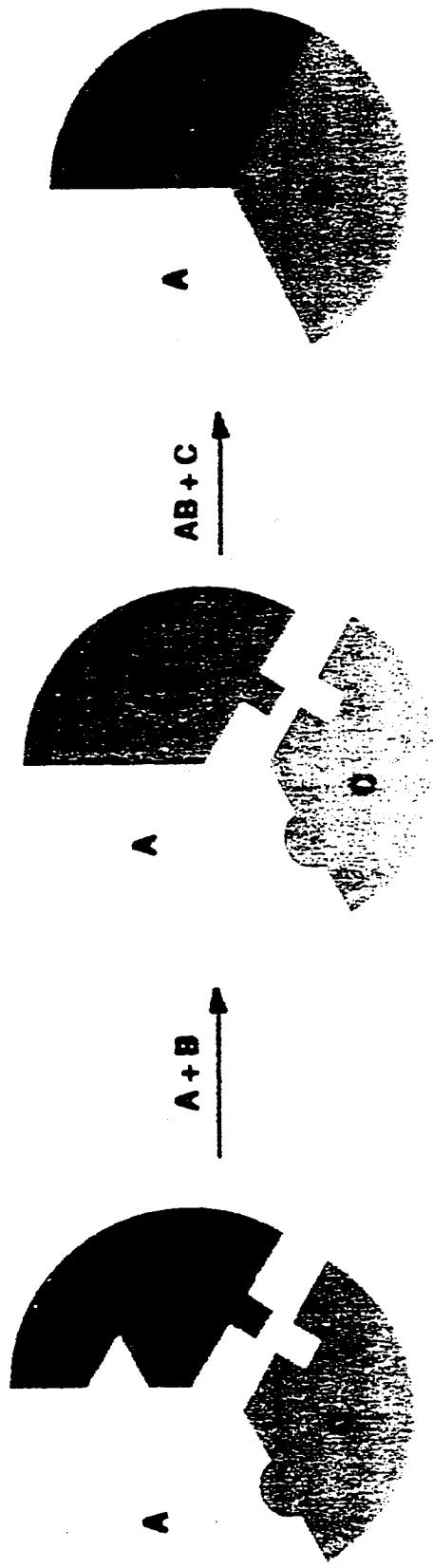
Iteration of a Single Coupling Chemistry using a Single Set of Building Blocks



Peptides, Oligonucleotides,
N-substituted glycines (peptoids), polycarbonates

II: Cyclic, Non-polymeric Scaffolds

Multiple Ligation Chemistries using Different Sets of Building Blocks



Benzodiazepines, Hydantoins, Diketopiperazines

Technology of Combinatorial Chemistry

**Encoded, Non-encoded, Spatial formats
Instrumentation issues
Information management**

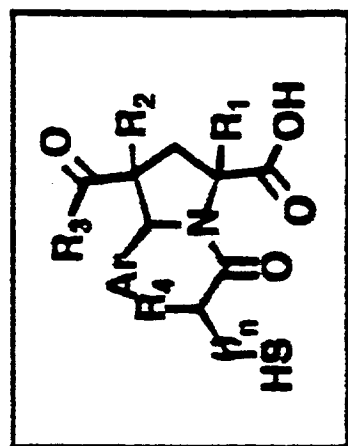
Chemistry of Combinatorial Chemistry

Strategy

Developing Solid Phase Organic Synthesis

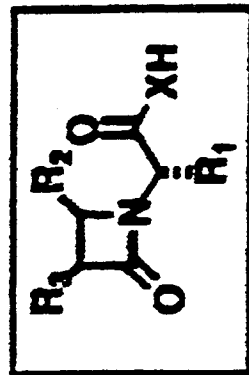
- Protease Inhibitor Libraries**
- Natural Product Libraries**
- Soluble diversity - supports & linkers**

Immobilized Imine: A Versatile Intermediate



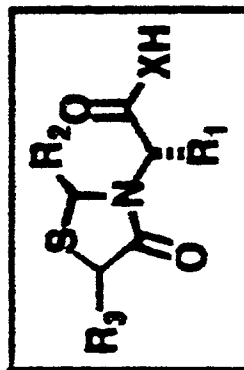
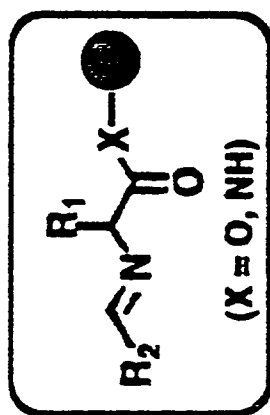
Prolines

M. Murphy et al.
J. Am. Chem. Soc.
1995, 117, 7029



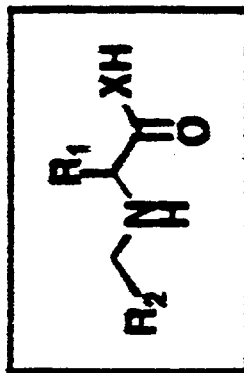
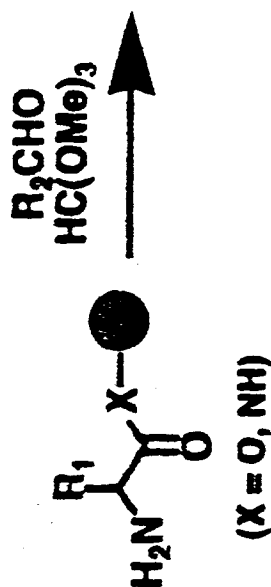
beta-lactams

B. Ruhland et al.
J. Am. Chem. Soc.
1996, 118, 253.



Thiazolidinones

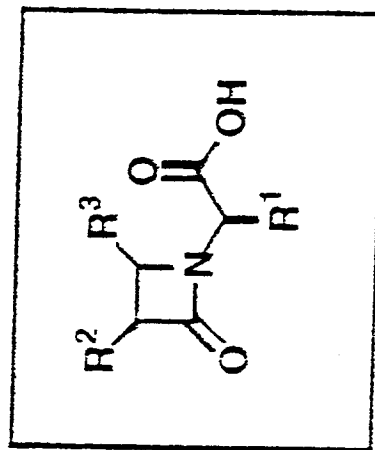
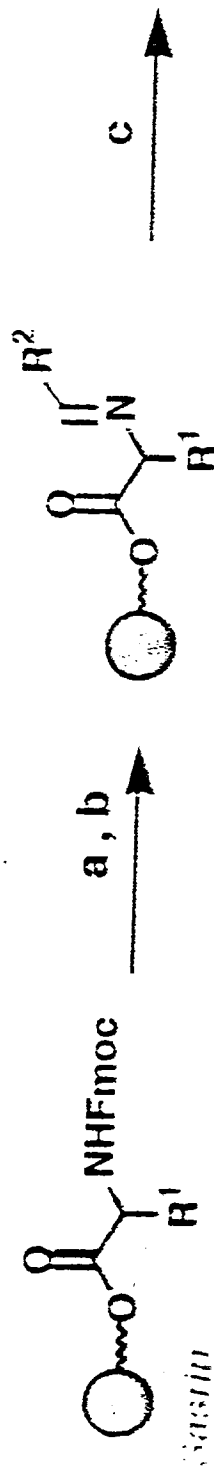
C. Holmes et al.
J. Org. Chem.
1995, 60, 7328.



Subetd amines

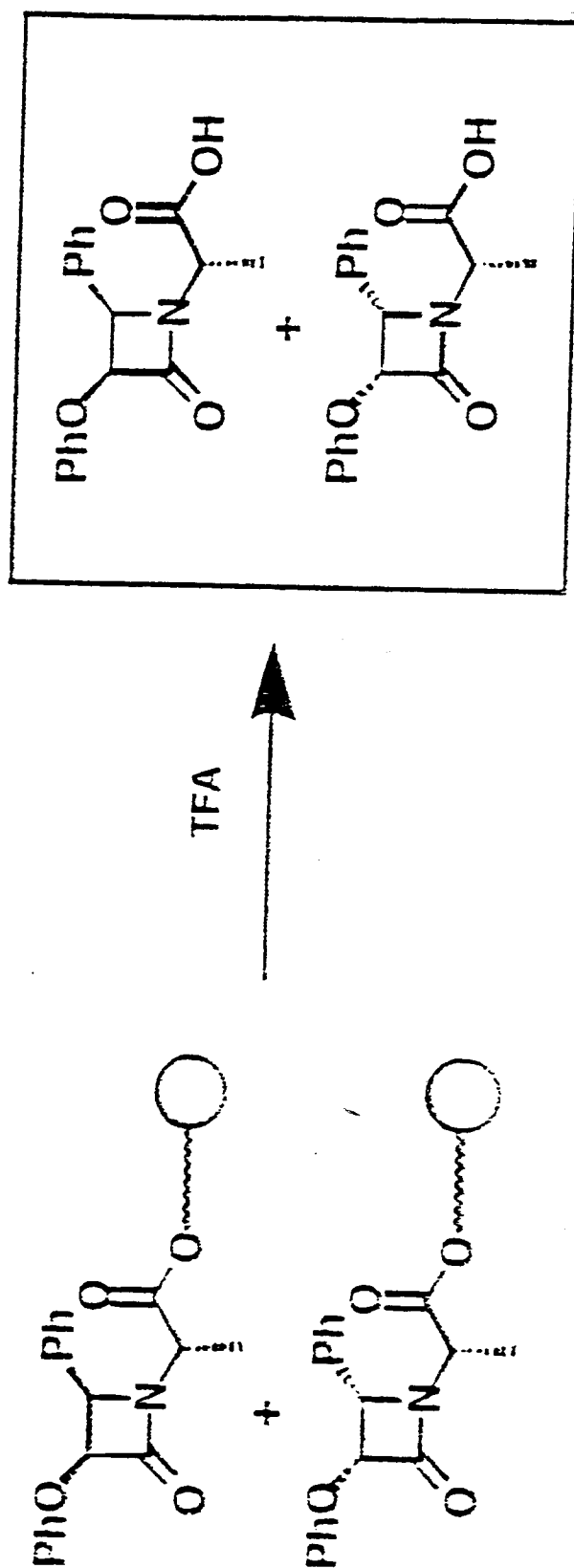
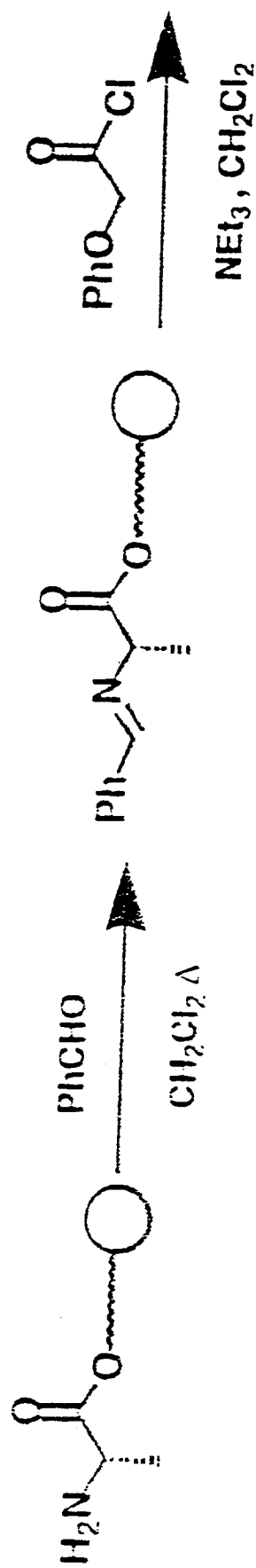
A. Szardenings et al.
J. Org. Chem.
1996, 61, in press.

[2+2] Cycloaddition Synthesis of β -Lactams



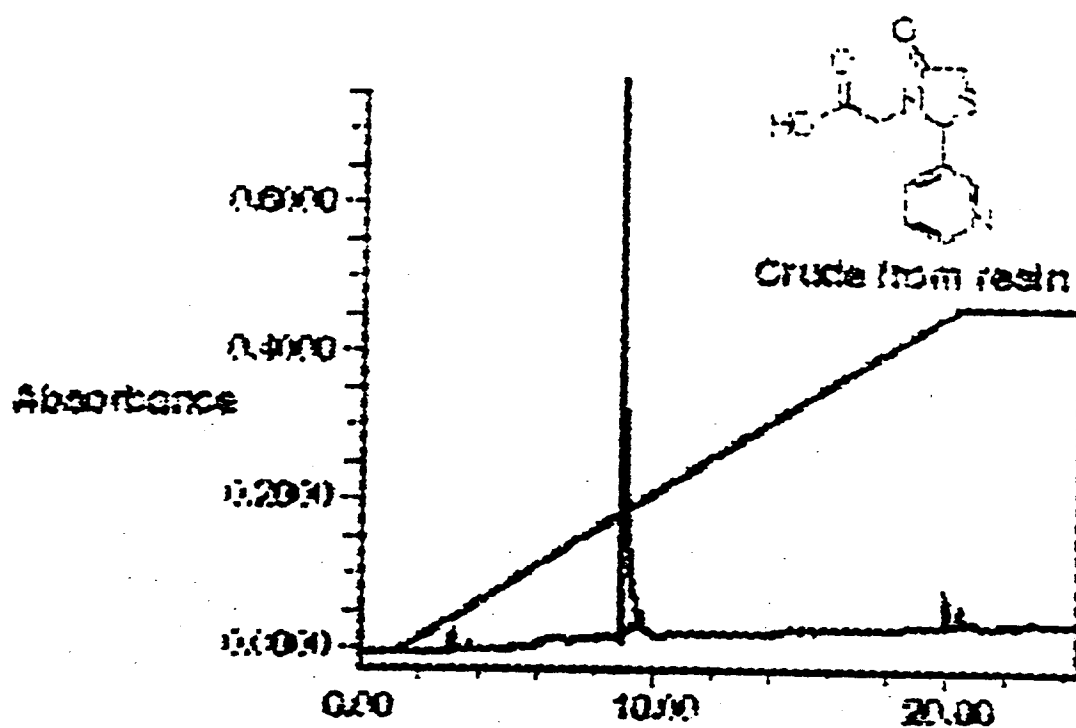
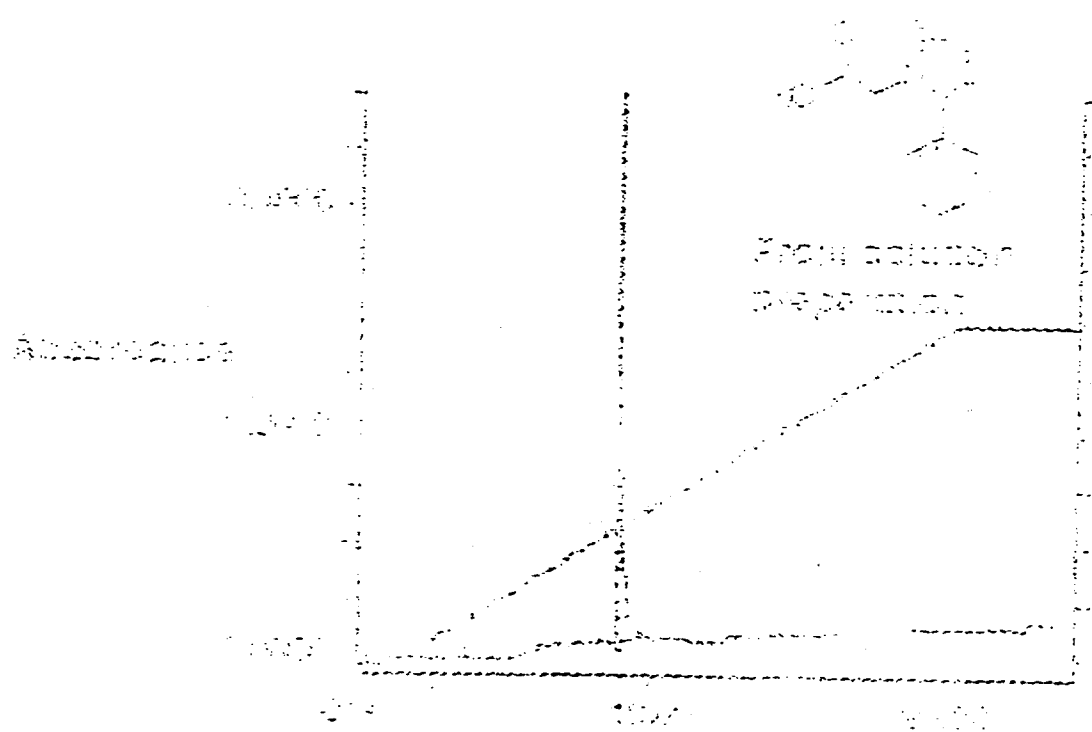
- (a) 30% piperidine in NMP, 1hr ;
- (b) 1M R^2CHO in 1/1 $\text{CH}(\text{OMe})_3$ / DCM, 3hrs;
- (c) 1M Ketene, 1.4 M Et_3N in DCM, 0°C to 23°C 15hrs;
- (d) 3% TFA in DCM.

Synthesis of β -Lactams



Two diastereomers

HPLC Analysis of Thiazolidinone Synthesis - Solution vs Solid Phase



Analytical Combinatorial Chemistry

Cleave and Characterize NMR on Beads

Gel phase NMR - E. Glralt, et al Tetrahedron, 40, 4141 (1984)

Fast C¹³ NMR - G. Look, et al, J. Org. Chem., 59, 7588 (1994)

Varian Nanoprobe - ¹H NMR - W. Fitch, et al J. Org. Chem. 59, 7955 (1994)

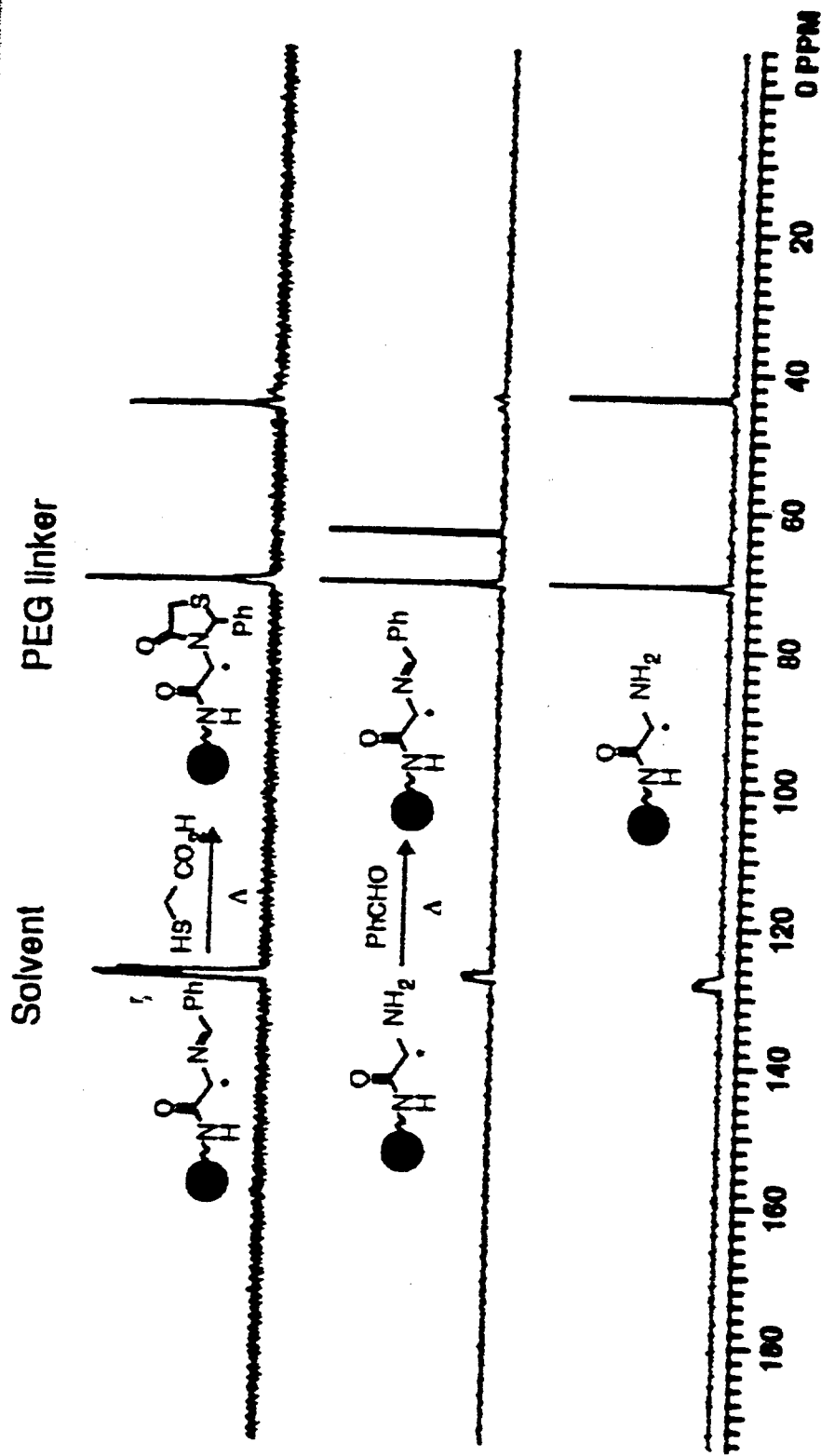
Mass Spectroscopy

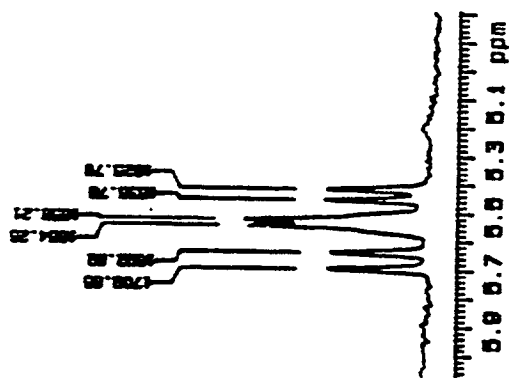
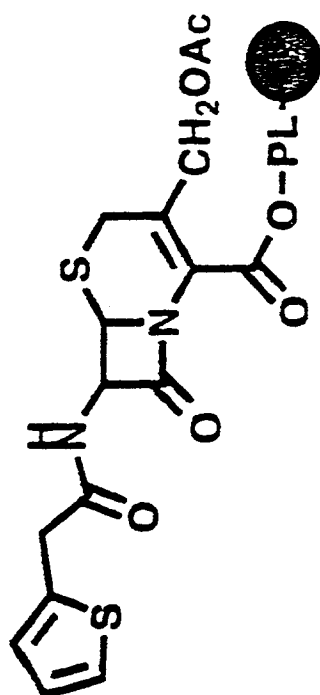
Time of Flight - S. Benkovic, et al , Science 264, 399 (1994)

Infrared Spectra

β -lactams B. Ruland, G. Detre, Affymax unpublished

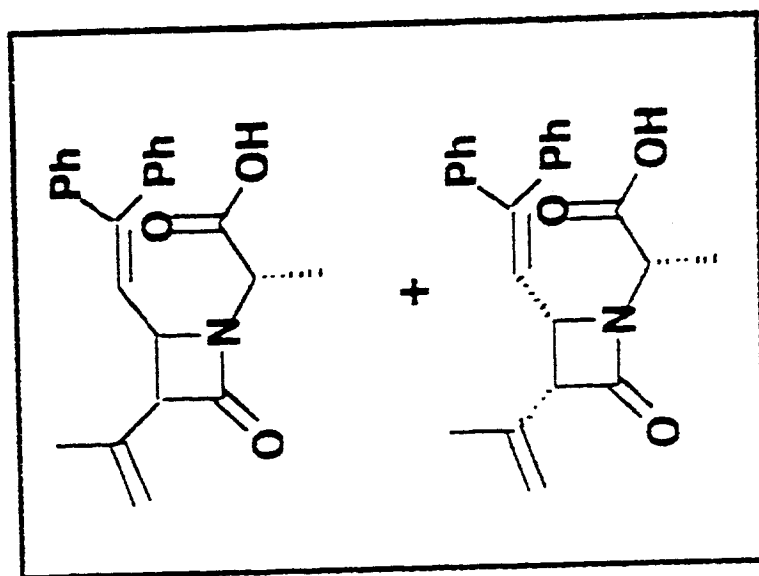
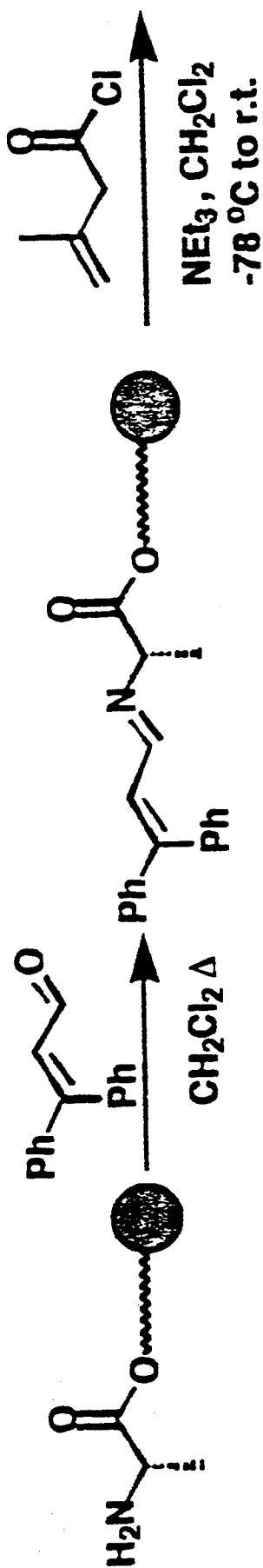
Fast ^{13}C n.m.r. Analysis of Solid-phase Thiazolidinone Synthesis





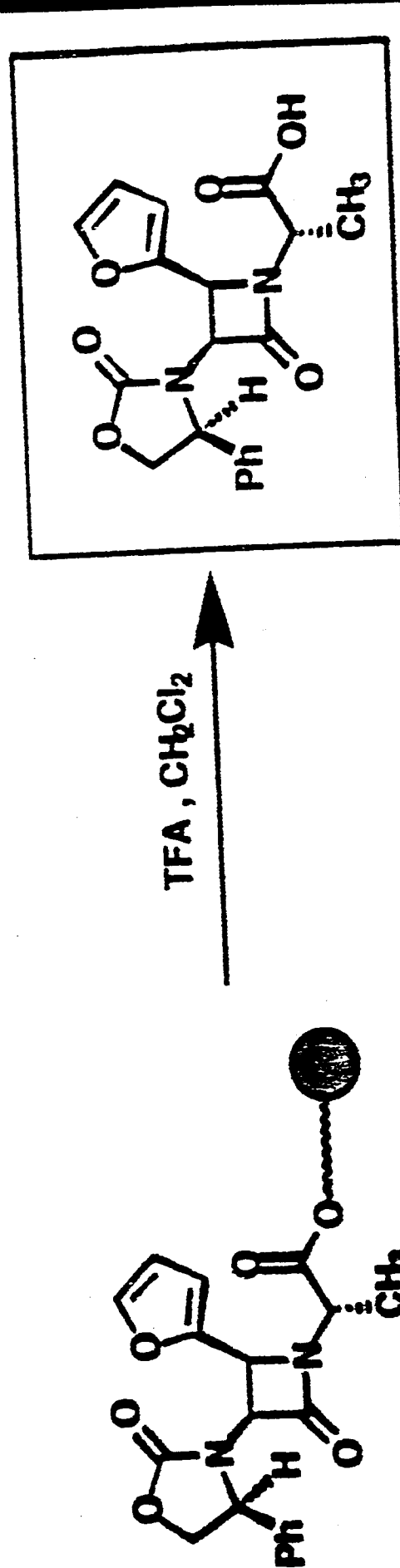
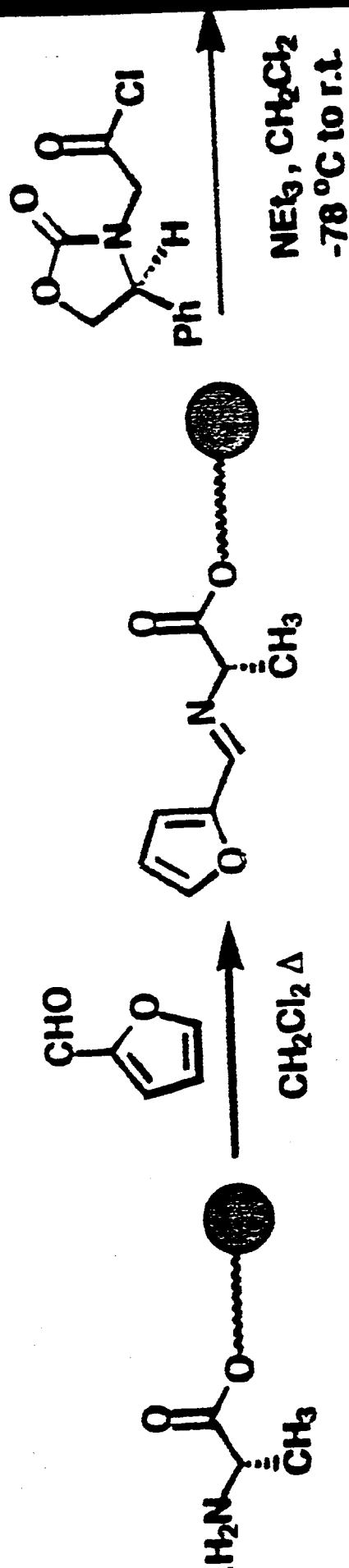
ppm

SPS of β -Lactams



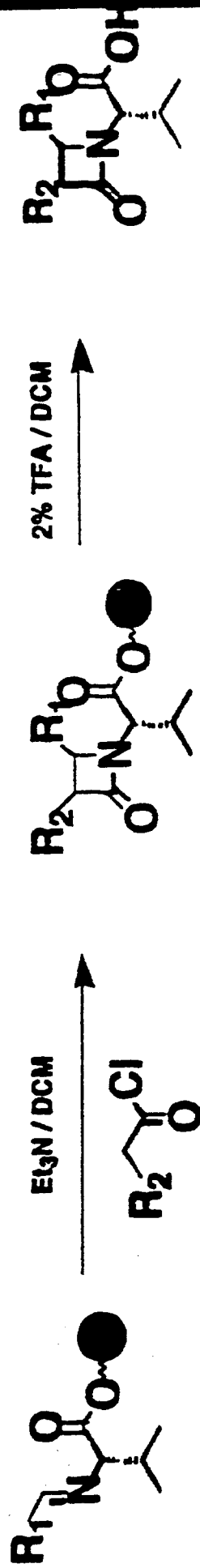
Two diastereomers

SPS of β -Lactams



Chiral ketone precursor yields one cis lactam diastereomer

Rehearsal for β -Lactam Library



R_1	R_2	Yield (%)	Yield (%)	Yield (%)
		100%	90%	100%
		100%	90%	100%
		51%	78%	77%

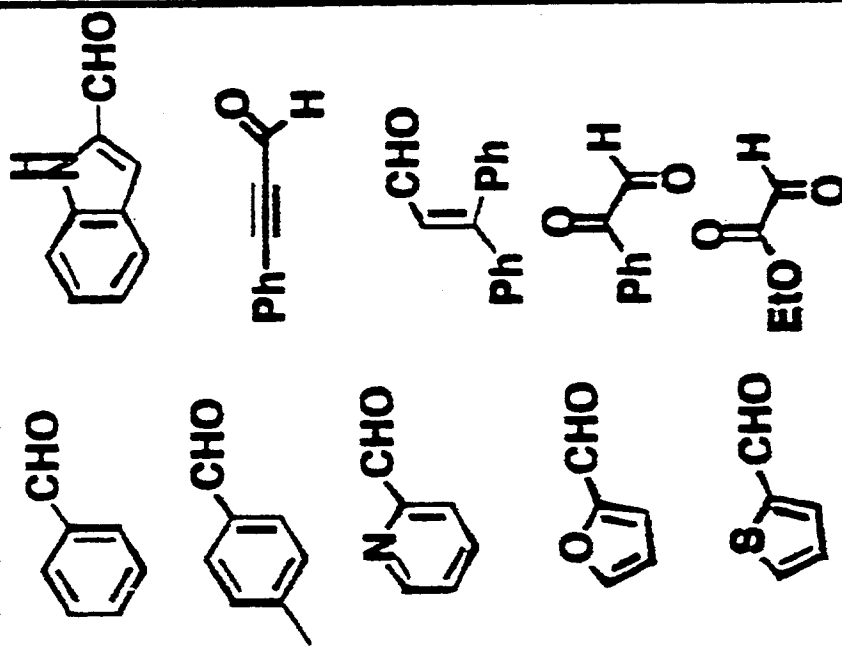
Building Blocks for a β -Lactam Library

3 Building Block Families - Representatives

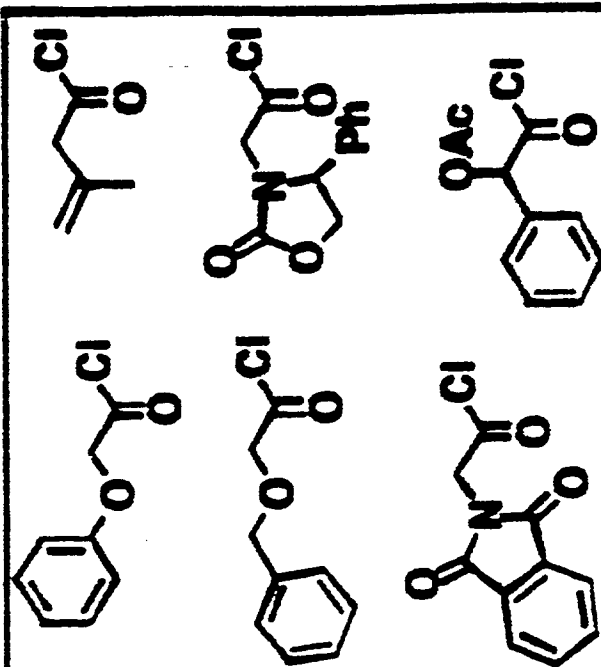
Amines (16)

Gly	Asp
Abu	Glu
Ala	Lys
Val	Asn
Leu	Gln
Ile	Hls
Ser	Phe
Thr	Tyr

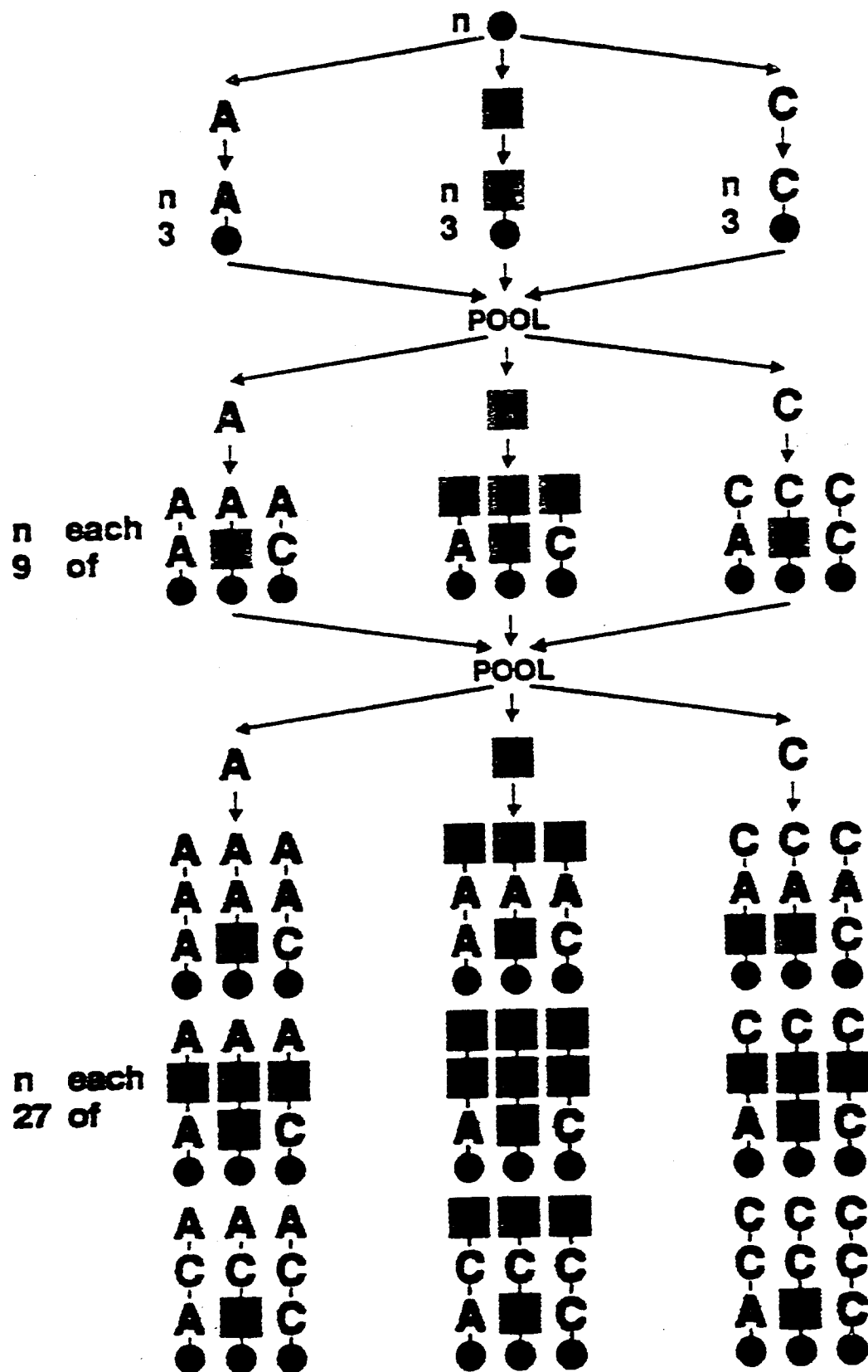
Aldehydes (10)



Ketene Precursors (6)



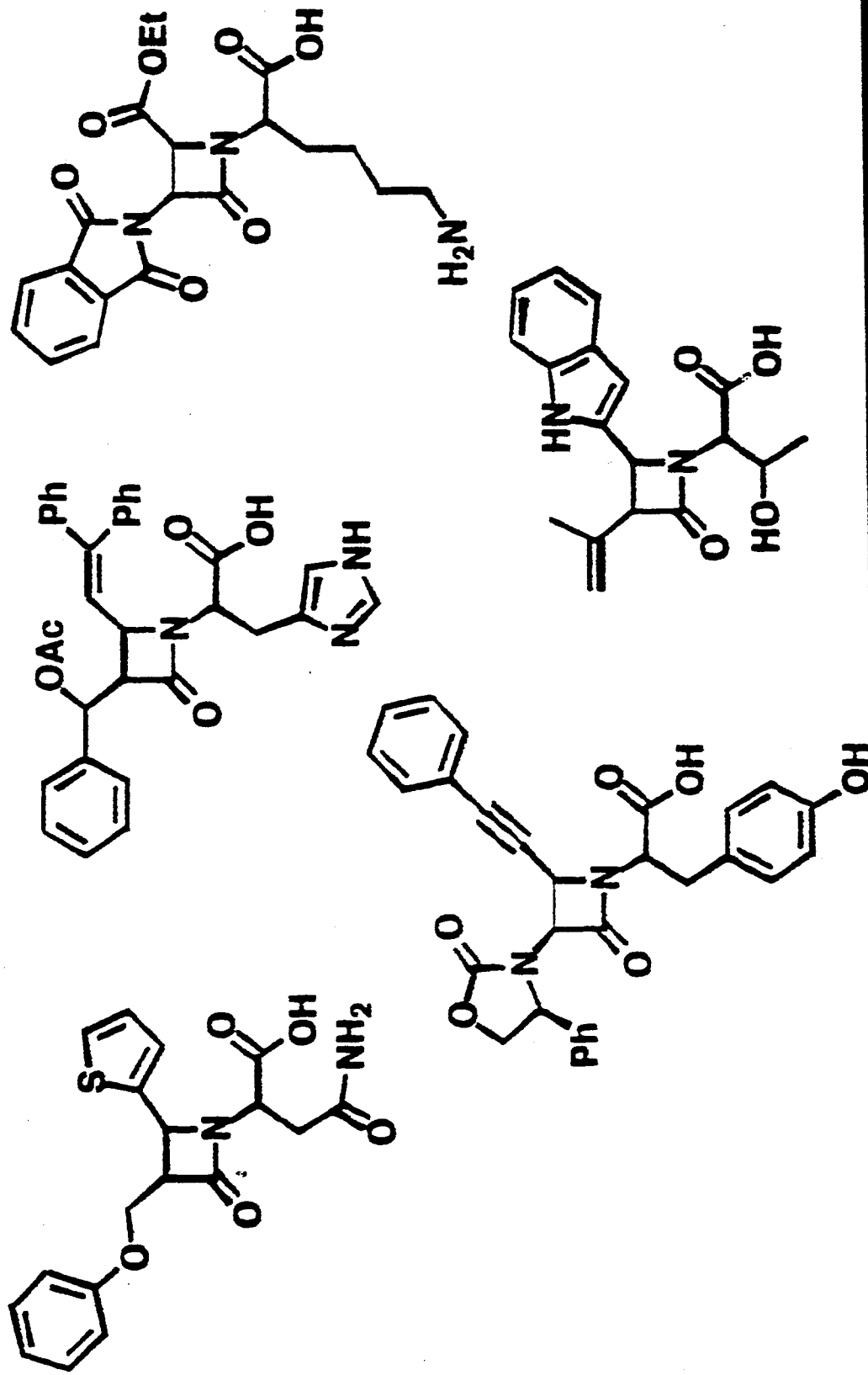
Combinatorial Library Synthesis by Resin "Split/Pool" Method



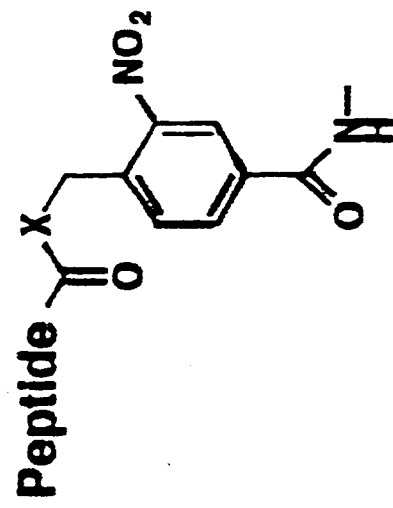
(After Furka et al, Int. J. Peptide Protein Res. 37, 487 (1991))

Members of β -Lactam Library

Representative Examples - 5 of ~ 960

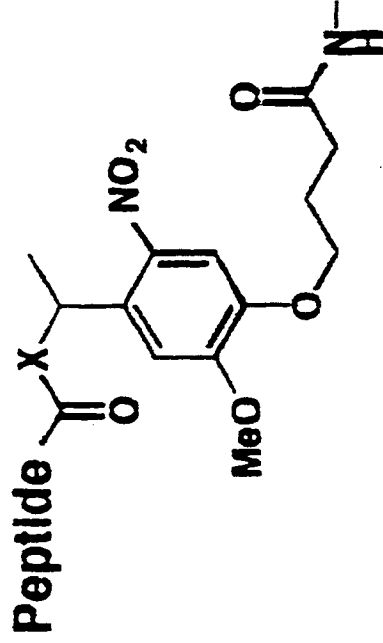


Photocleavable Linker for Solid Phase



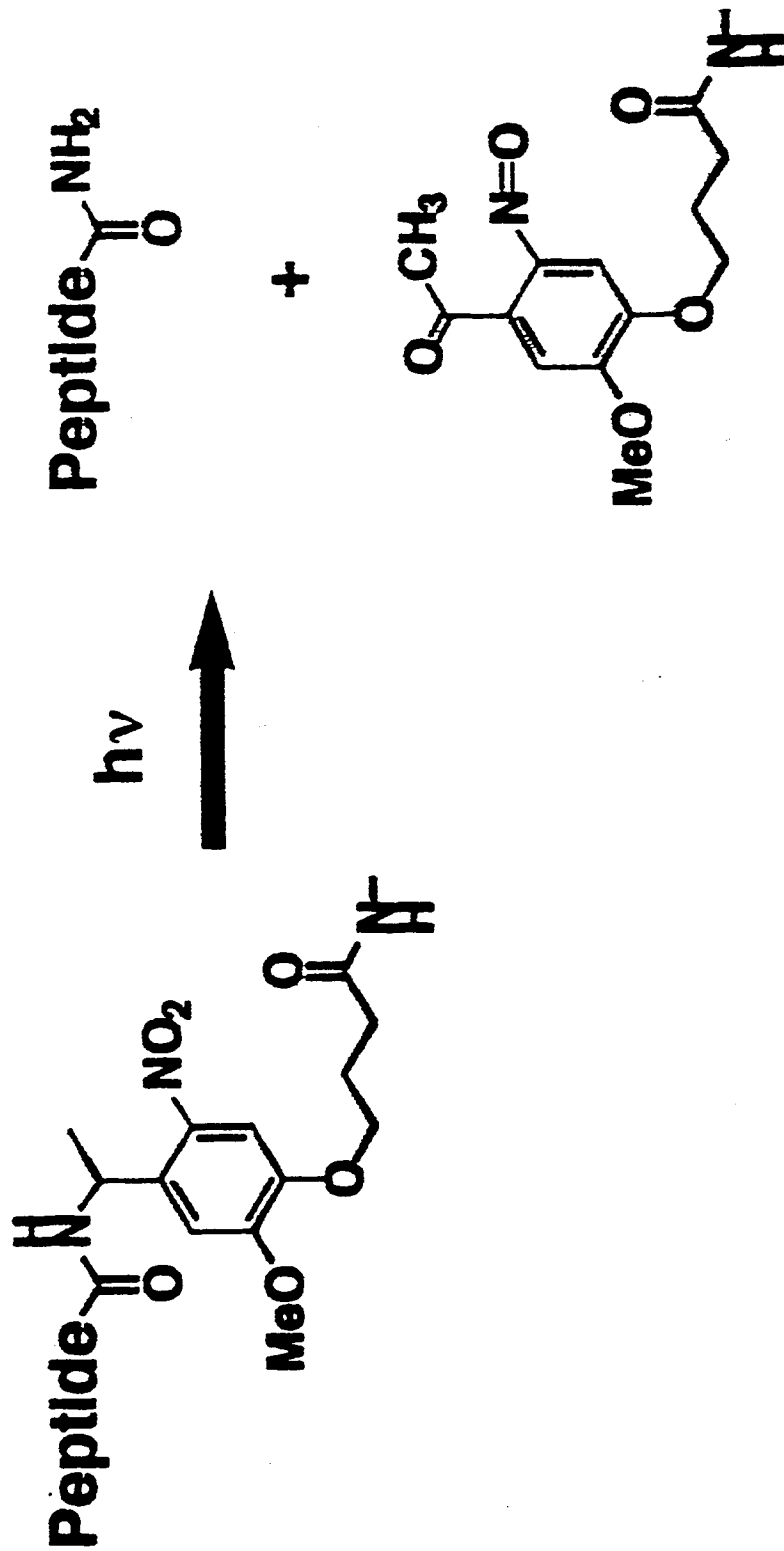
$h\nu$

$X = O \rightarrow$ Peptide acid
 $X = NH \rightarrow$ Peptide amide



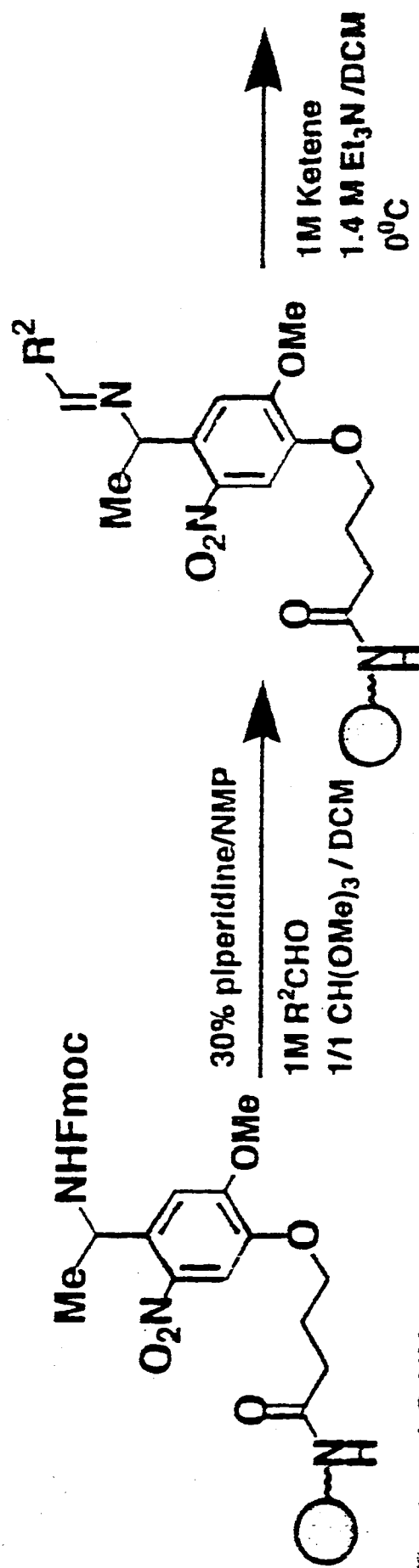
D. Rich, J. Amer. Chem. Soc., 97, 1575 (1975)
 G. Barany, Int. J. Peptide Protein Res., 36, 31 (1990)

Products of Photocleavage

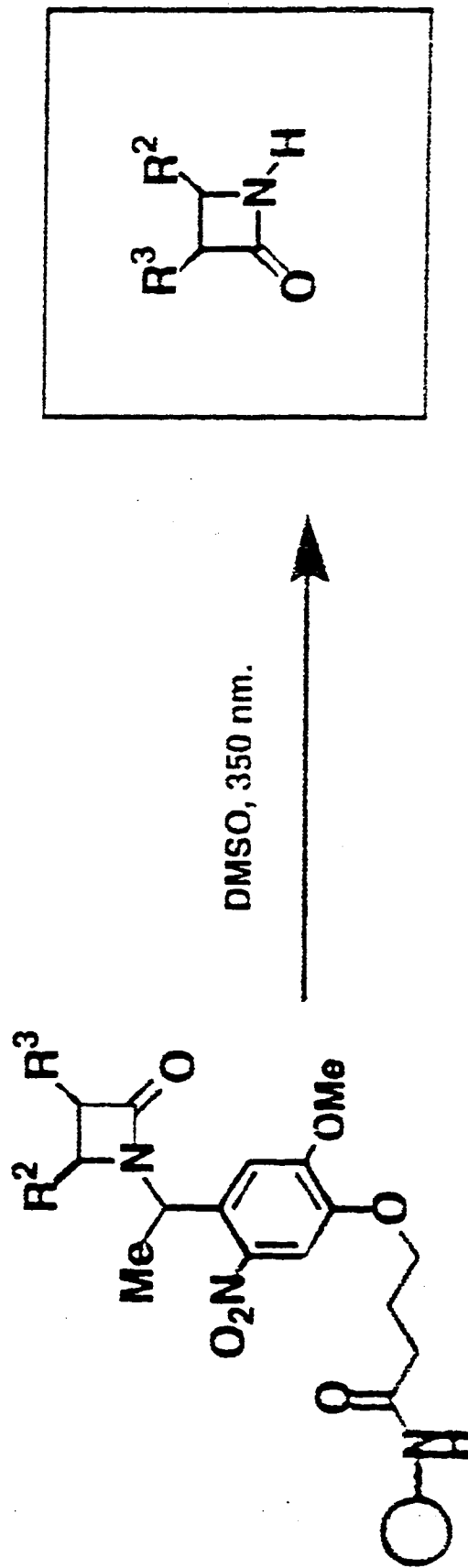


Ref. C.Holmes, L.C. Dong, Unpublished Observations

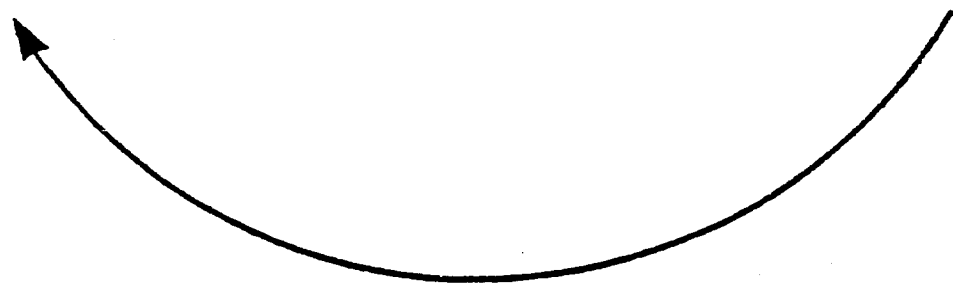
SP Synthesis of N-Unsubstituted 2-Azetidinones



Tentagel S NH₂

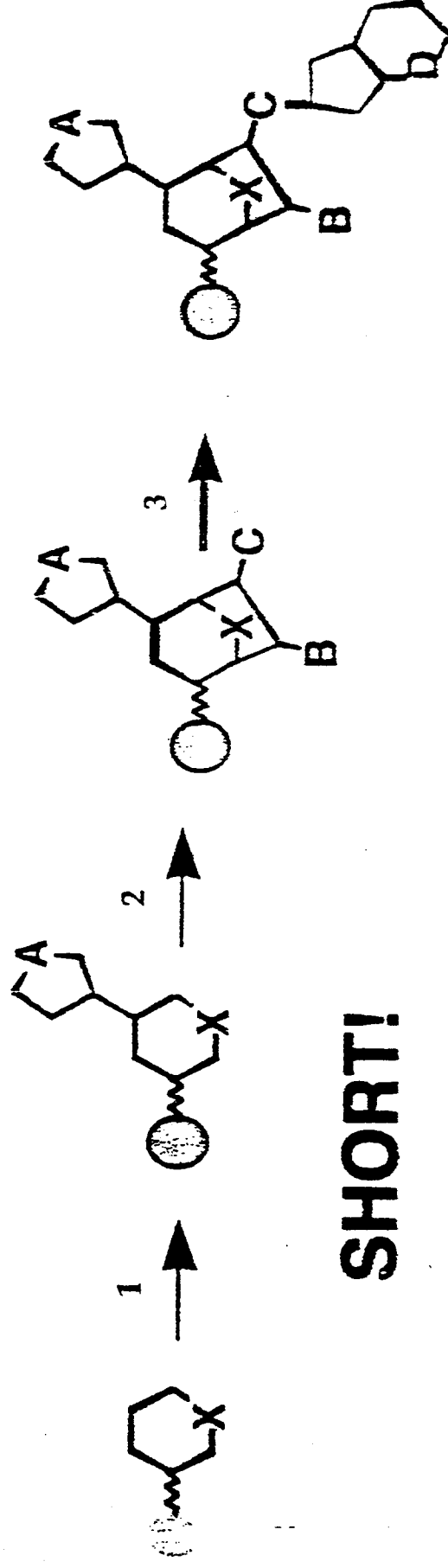


The Combinatorial Chemistry Process

1. Library Design
 2. Building Block Selection
 3. Chemical Rehearsal - Quality Control
 4. Library Synthesis
 5. Biological Screening
 6. Compound Identification
 7. Activity Confirmation
 8. Interpretation
- 

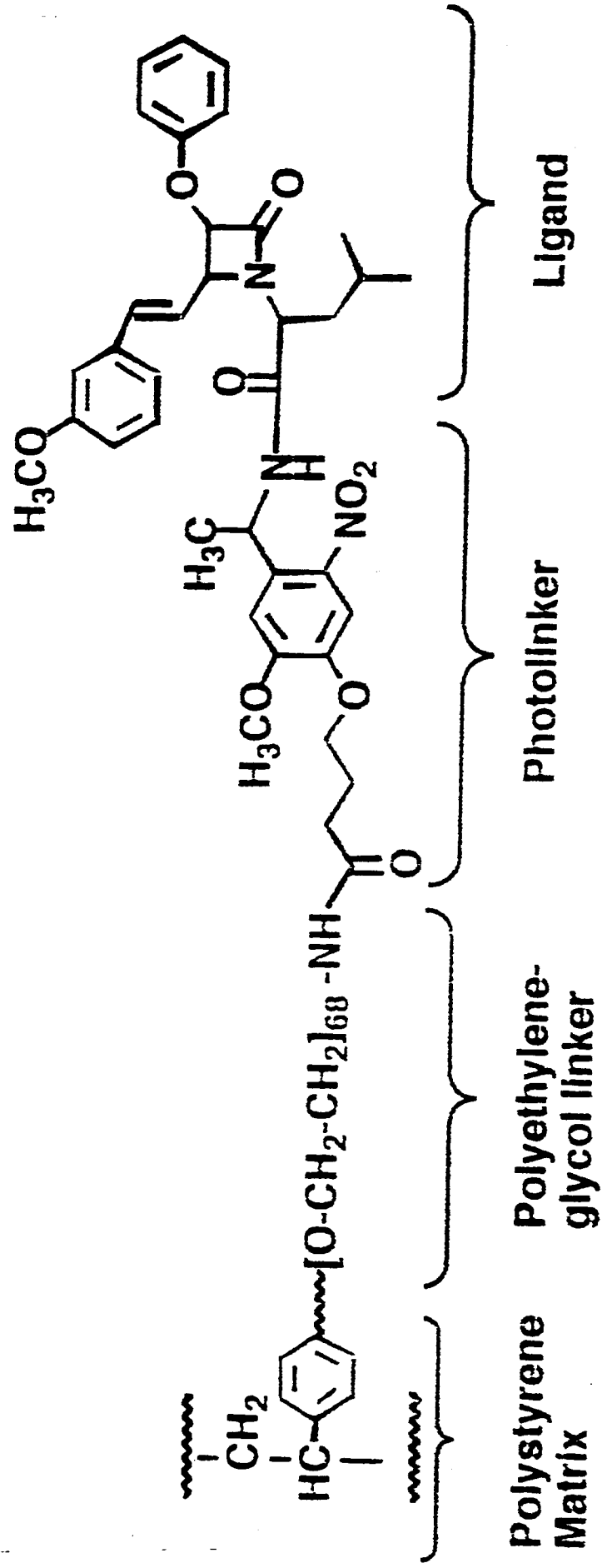
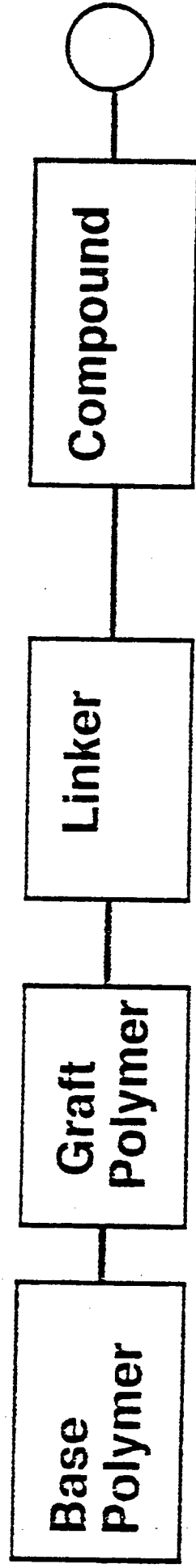
What Needs to Happen in Combichem?

If product M.W. is in the range 450-750
Assume each Building Block is approx. M.W. 150
Then 3-5 BB's must be interconnected per molecule
Upper limit of only 2-4 reactions are necessary



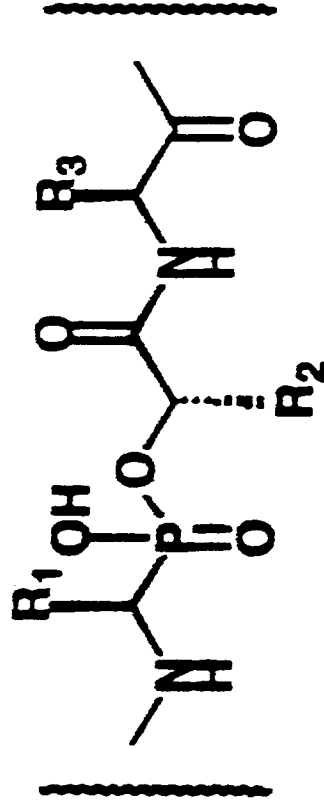
SHORT!

What is the Structure of Compounds on Bead ?

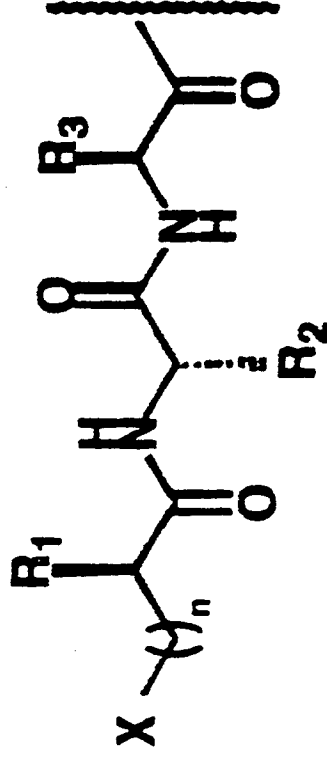


Protease Inhibitor Libraries

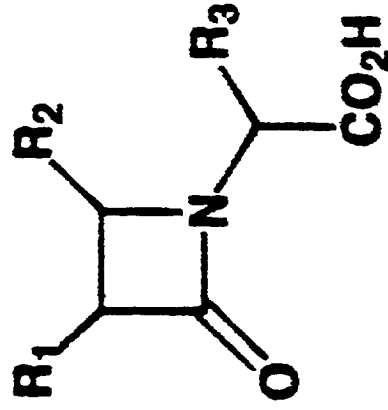
**Phosphonic Acids
(Aspartyl and Metallo)**



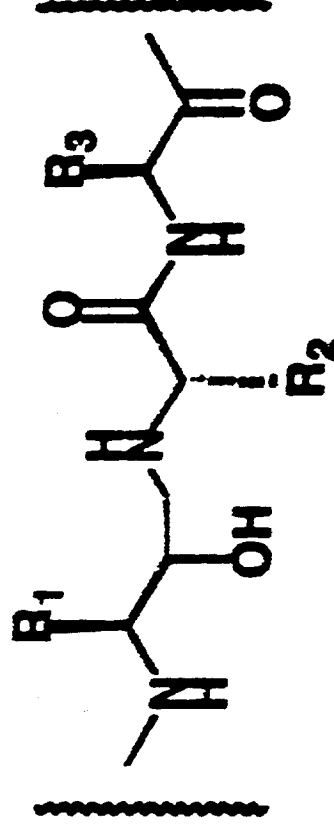
**Mercaptans
Hydroxamates(metallo)**



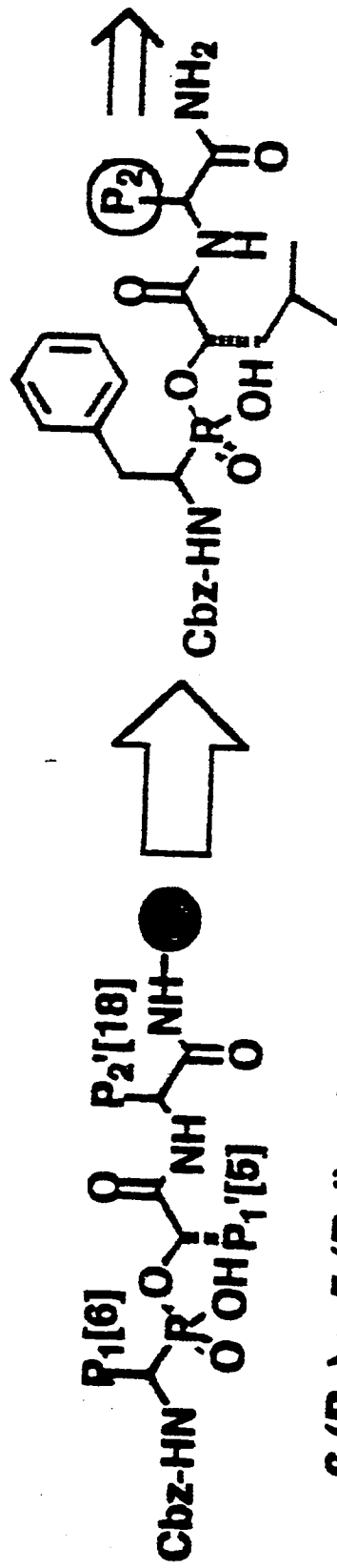
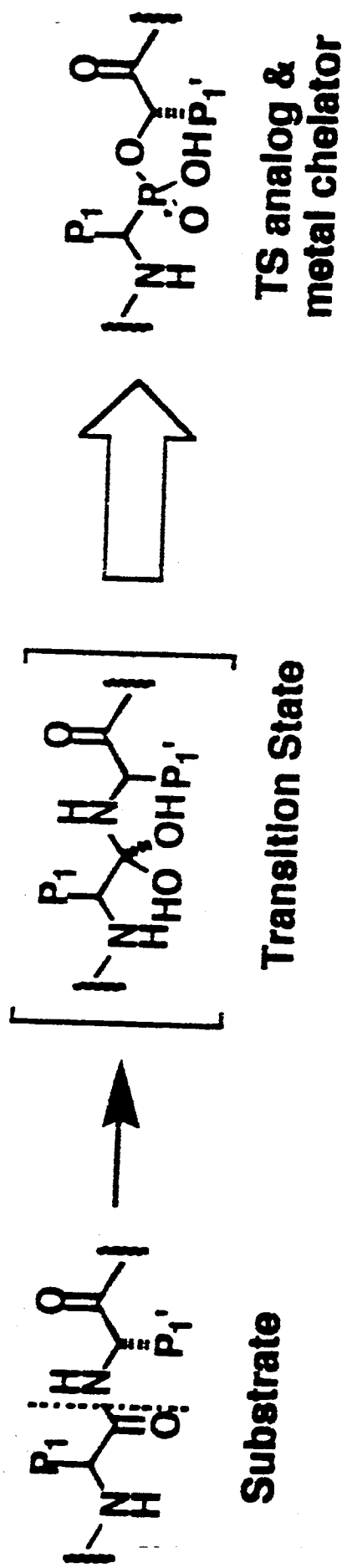
β -Lactams (serine)



**Hydroxyethyl Amines
(Aspartyl and Metallo)**



Protease Inhibitor Libraries: Peptidyl Phosphonates

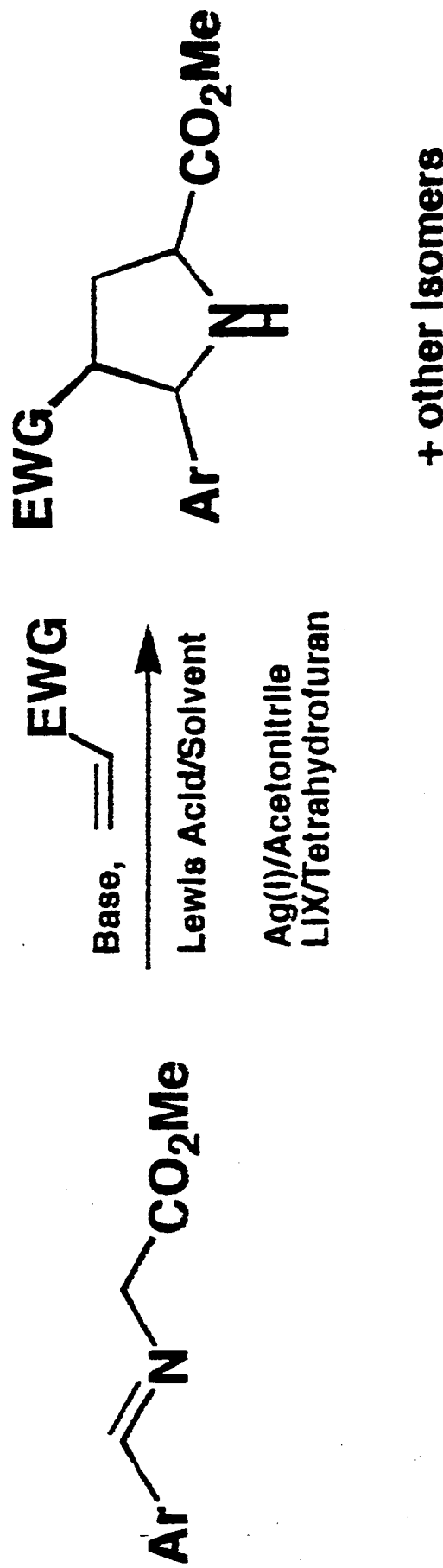


$6 (P_1) \times 5 (P_1') \times 18 (P_2')$
 $= 540$ member library

P ₂ '	K _i
Arg	64 nM
His	57 nM
Gln	127 nM
Ala	49 nM

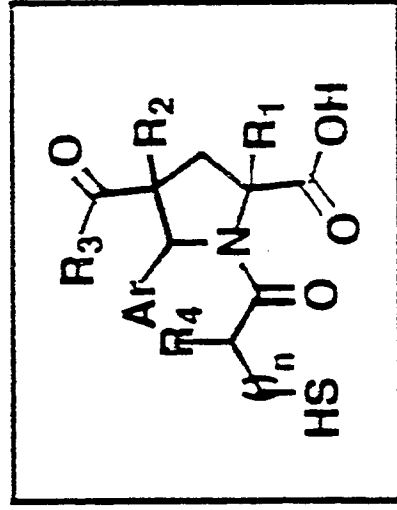
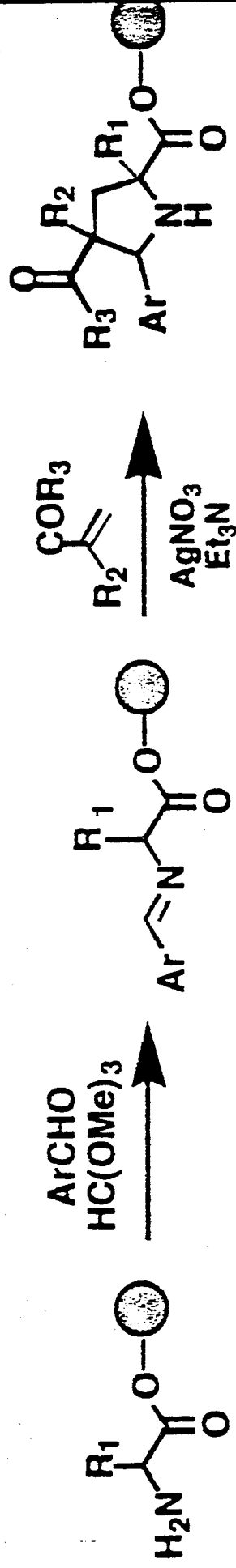
Combinatorial Approach Employing [3+2] Cycloaddition

Lewis Acid Catalyzed Cycloadditions

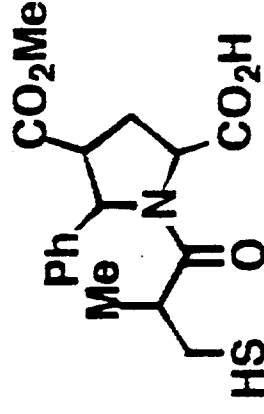


R. Grigg *Tetrahedron* 1989, 45, 4649
O. Tsuge *J. Org. Chem.* 1988, 53, 1384.

Mercaptoacyl Proline Library



4 (R_1) x 4 (Ar) x 5 (R_2/R_3) x 4 (R_4)
= 240 member library



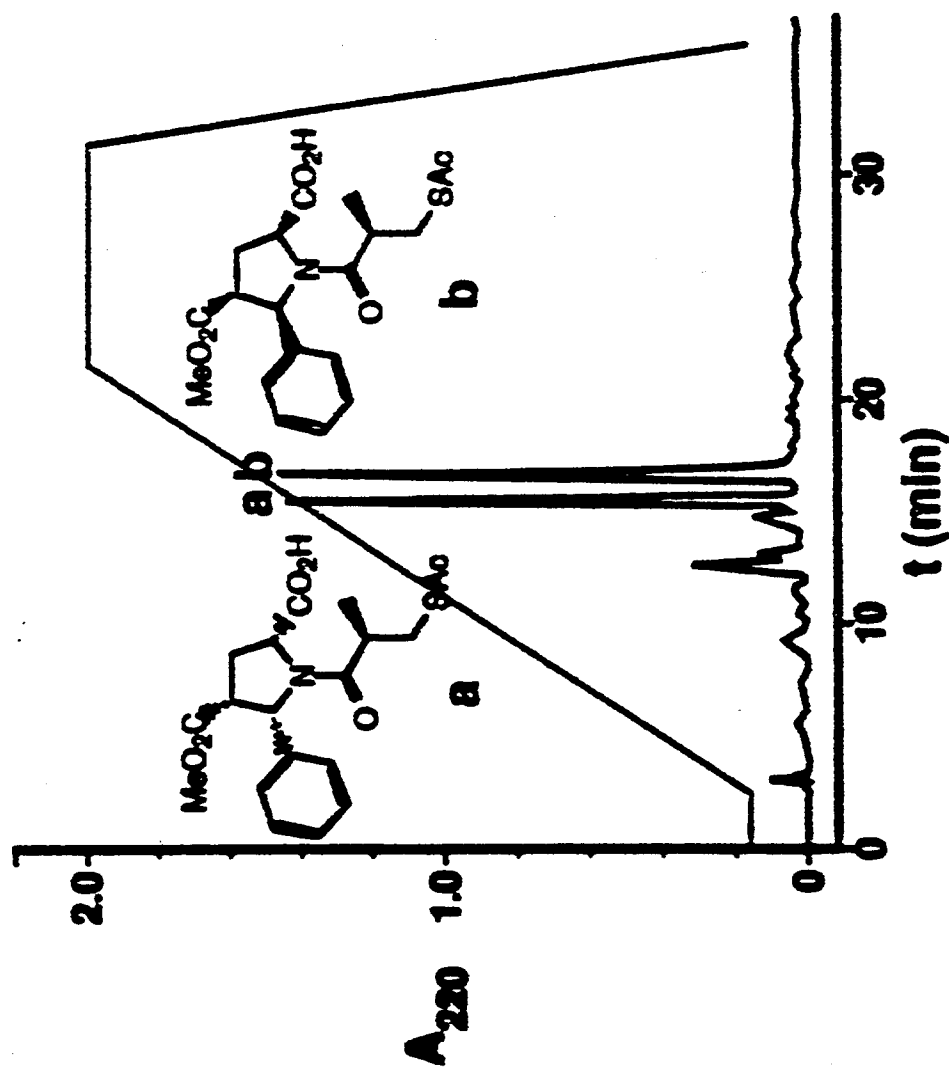
$K_I = 160 \text{ nM (ACE)}$

Mercaptoacyl Proline Library

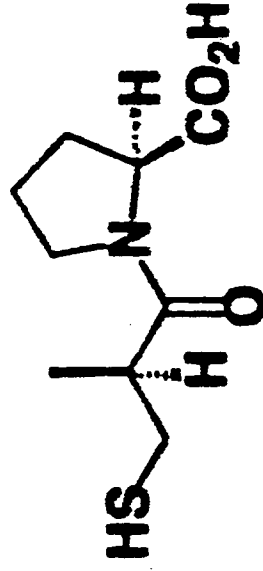
Library Procedure

- **Split / Pool synthesis - library of ~ 500 members**
- **4 level deconvolution - specifies 4 BB's**
- **Preparative SP Synthesis with 4 specified BB's**
- **Separation of 2 diastereoisomers**
- **Measurement of ACE inhibitory activity**

HPLC Analysis of ACE-inhibitory Mercapto Isobutyryl Prolines from Preparative Solid-phase Synthesis

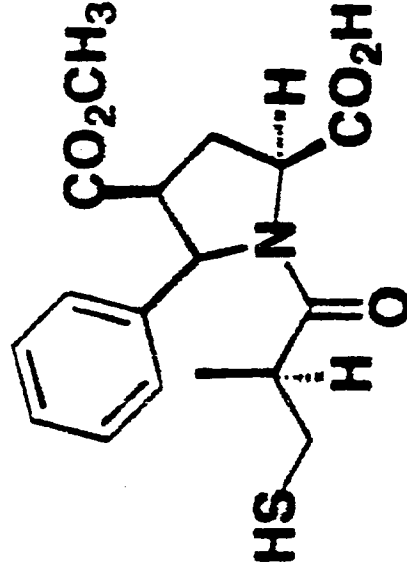


Identification of a Highly Potent ACE Inhibitor from a Combinatorial Proline Library



Captopril

Ki = 450 pM



Ki = 160 pM

M.M. Murphy, J.R. Schullek, E.M. Gordon, and M.A. Gallop.
J. Amer. Chem. Soc., 117, 7029-30 (1995).