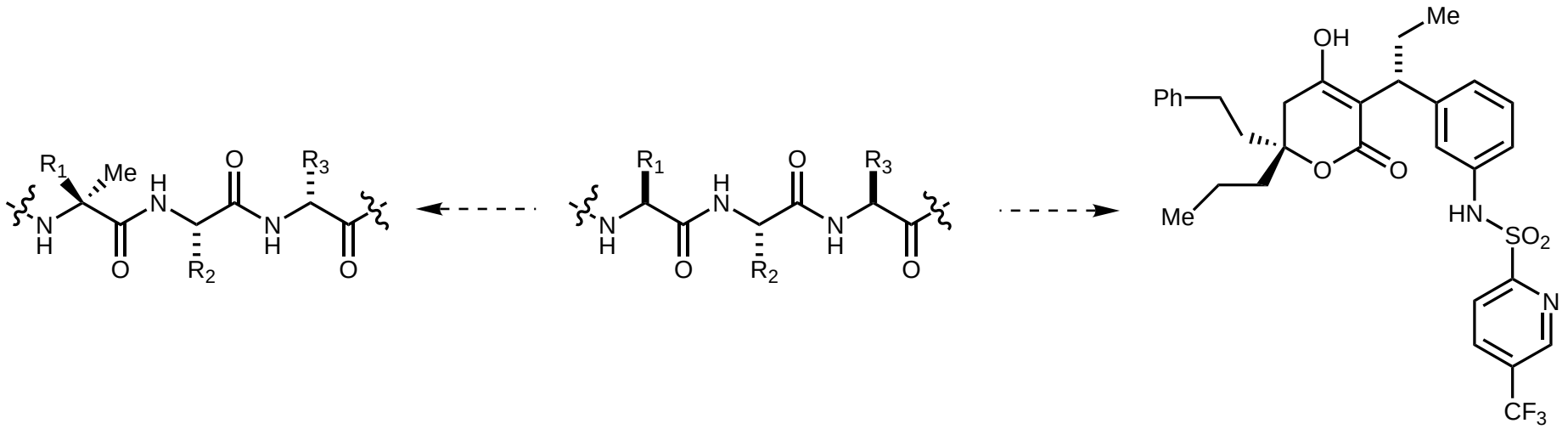
Recent Advances in the Design and Synthesis of Peptidomimetics



Overview

1. Background
2. Primary structure modifications
3. Secondary structure modifications
4. Applications to drug design and development

Gretchen Peterson

Evans Group Literature Seminar

04/13/01

Definitions

Peptidomimetic: A molecule bearing identifiable resemblance to a peptide that, as a ligand of a biological receptor, can imitate or inhibit the effect of a natural peptide

Isostere: Surrogate functionalities that are isosteric and/or isoelectronic with a peptide amide bond

Non-peptidomimetic: A molecule bearing *no* identifiable resemblance to a peptide or its isosteres, yet which imitates or inhibits the biological effect of a peptide

Agonist: A molecule (natural or synthetic) which *imitates* the function of a ligand of a biological receptor

Antagonist: A molecule (natural or synthetic) which *inhibits* the function of a ligand of a biological receptor

Peptide analog: A peptidomimetic where one or more sidechains have been modified

Psuedopeptides: A peptidomimetic where one or more peptide bonds have been replaced with an isostere

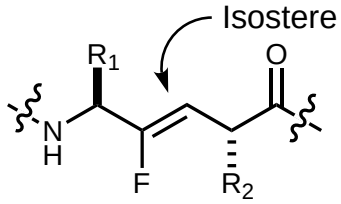
Depsipeptide: A pseudopeptide where the isosteric replacement is an ester bond

Retro-inverso peptide: A linear peptide whose amino acid sequence is reversed and the α-center chirality is inverted

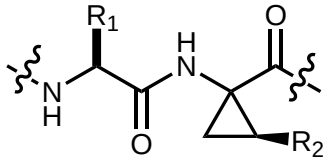
Exo-peptidase: A non-specific enzyme that degrades peptides by sequential hydrolysis of peptide bonds from either the amino (aminopeptidase) or carboxylic acid (carboxypeptidase) end of an amino acid

Endo-peptidase: A peptidase that cleaves an internal amide bond of a specific peptide

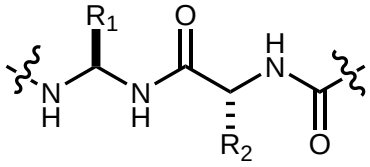
Pharmacophore: Structural region of a peptide responsible for interaction with its biological target



Pseudopeptide

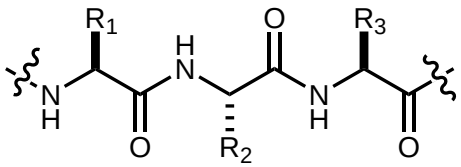


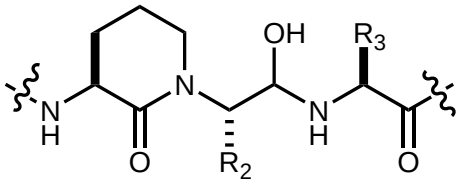
Peptide analog



Retro-inverso peptide

Why Peptidomimetics are Needed for Drug Potency





Disadvantages of Peptides as Drugs

1. Limited stability towards proteolysis by peptidases in the gastroeintesinal tract and in serum ($t_{1/2}$ on the order of minutes)

2. Poor transport properties from the intestines to the blood and across the blood-brain barrier due to high MW and lack of specific transport systems

3. Rapid excretion through the liver and/or kidneys

4. Inherent flexibility enables interaction with multiple receptors besides the target, and could result in undesired side-effects

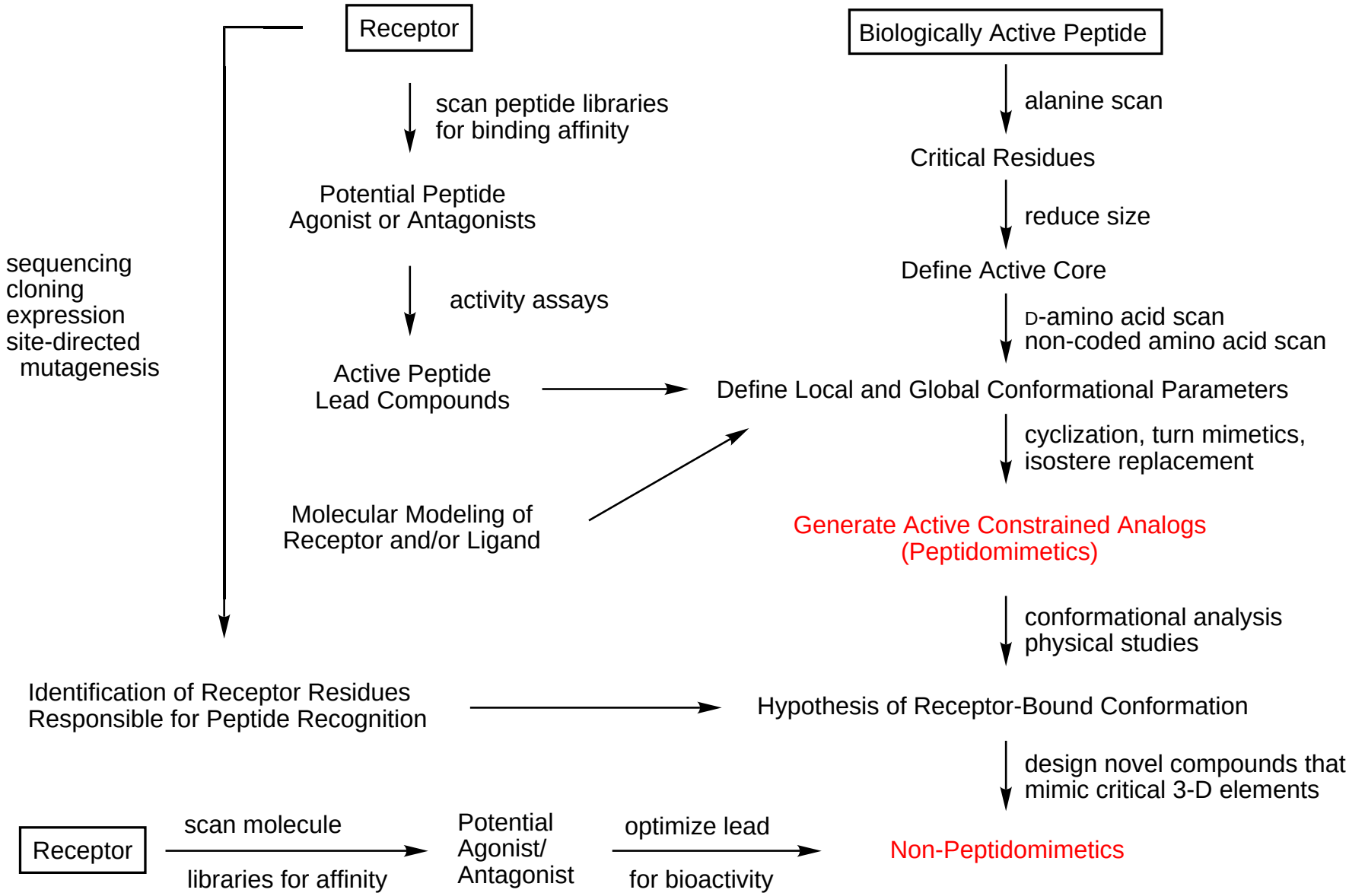
Advantages of Peptidomimetics as Drugs

1. Conformationally restrained structures can minimize binding to non-target receptors and enhance the activity at the desired receptor.

2. Addition of hydrophobic residues and/or replacement of amide bonds results in better transport properties through cellular membranes.

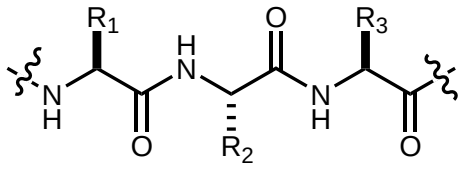
3. Isosteres, retro-inverso peptides, cyclic peptides and non-peptidomimetics all reduce the rate of degradation by peptidases and other enzymes.

Peptidomimetic Drug Design Principles



Adapted from *Adv. Drug. Res.* **1997**, *29*, 1-78

Determination of a Peptide's Active Conformational Parameters



1. What is the conformation of the biologically active core peptide?

2. How can the peptide be modified to rigidify that conformation?

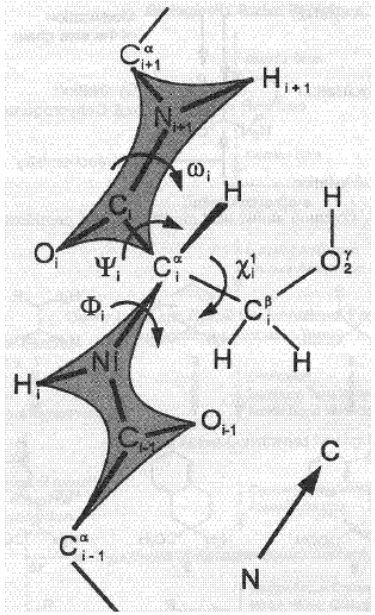
3. Do these modifications increase the peptide's bioavailability, receptor selectivity, and resistance to degradation?

Limitation of torsional angles
available to active site peptide residues

- Sequentially substitute D-amino acids and conformationally constrained amino acids for the natural residues in the target.

- Conformationally restricted amino acids must retain the crucial side-chain interactions with the receptor.

- Constrained amino acids can be categorized by the torsional angles that they restrict in the peptide.



Torsional Angles

ω: $N_{i+1} - C_i$

ψ: $C_i - C^{\alpha}_i$

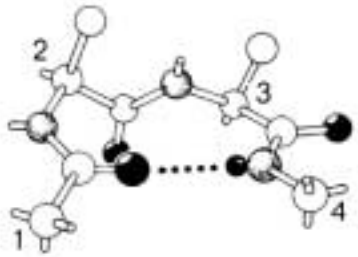
ϕ: $C^{\alpha}_i - N_i$

χ: $C^{\alpha}_i - C^{\beta}_i$

Peptide Secondary Structure Motifs

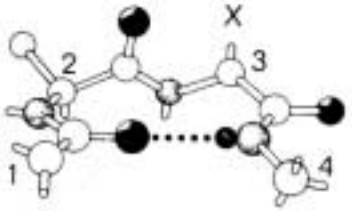
Torsional Angles
ω: N_{i+1} - C_i
ψ: C_i - C_i^{α}
ϕ: C_i^{α} - N_i
χ: C_i^{α} - C_i^{β}

α-helix (3.6_{10} helix)
ϕ = -57 °, ψ = -47 °

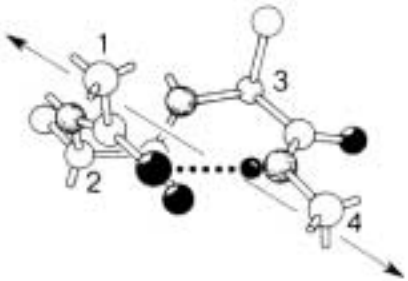


O N C

Type I



Type II

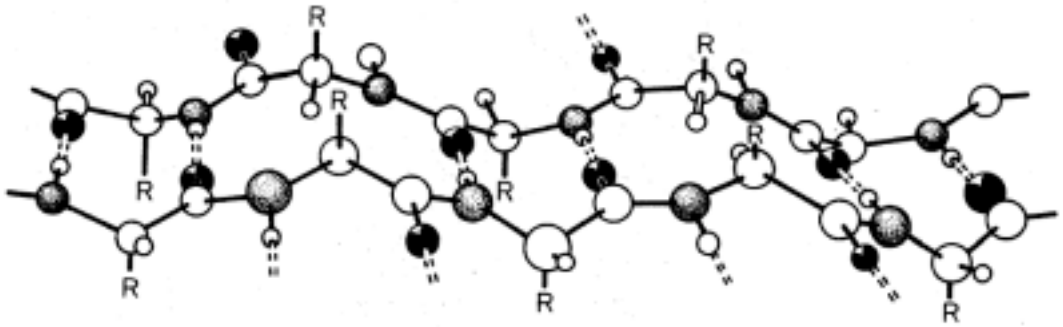


Type III
(3.0_{10} helix)

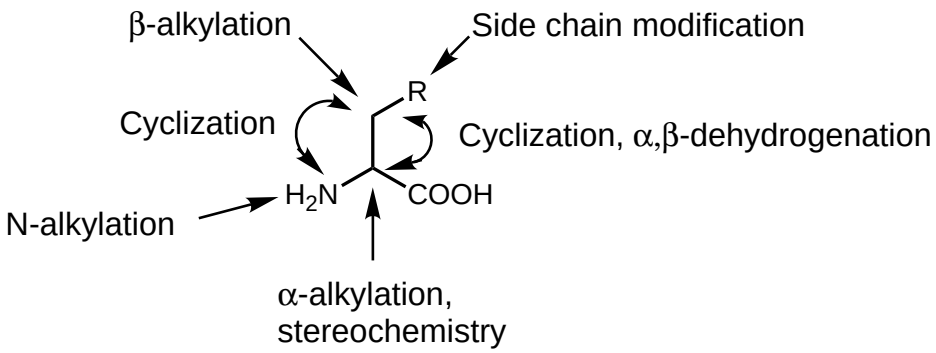
β-turns	ϕ_2	ψ_2	ϕ_3	ψ_3
I	-60	-30	-90	0
II	-60	+120	+80	0
III	-60	-30	-60	-30

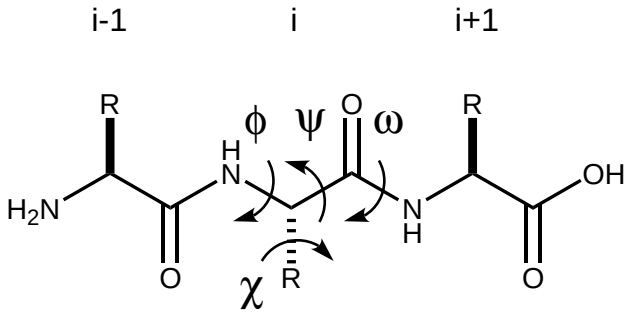
Adapted from *Adv. Drug. Res.* **1997**, *29*, 1-78

β-sheet
ϕ = -139 °, ψ = +135 °



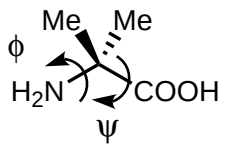
Conformational Constraints





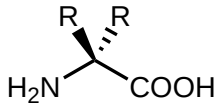
Modification	Conformational effect
1. Backbone *N*-alkylation	ϕ, ψ, χ are constrained, facilitates cis-trans amide bond isomerism
2. Backbone C_α-alkylation	ϕ, ψ are constrained to a helical or extended linear structure
3. D-Amino acid/proline substitution	Favors formation of β-turn structures
4. Peptide bond isosteres	ω can be fixed at 0 or 180° (olefins), or allowed greater freedom of rotation (i.e. -CH_2 S-)
5. Cyclic amino acids	ω can be biased to 0 or 180°, ϕ, ψ are biased towards formation of β-turns or γ-turns, χ can also be affected
6. Dehydroamino acids	Fix χ at 0 or 180°
7. β-alkylation	Constrain χ, may also affect backbone conformation

α-Alkylation of Amino Acids



α-Aminoisobutyric acid (Aib)

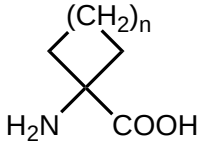
- Most widely studied α-alkylated amino acid
- Restricts ϕ, ψ to angles present in α or 3_{10} helices
- Review of its conformational effects in peptides: *Biochemistry* **1990**, *29*, 6747-6756.



R = Et (Deg), *i*-Pr, Ph

Dialkylglycine

- Preferred conformation is in an extended structure ϕ, ψ = 180°



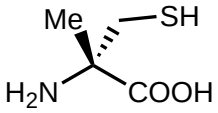
α-Aminocycloalkane carboxylic acid ($Ac^{n+3}c$)

- Preferred conformation is in a β-turn or 3_{10} helix
- Substitution of $Ac^{6}c$ into various positions of enkephalin, a peptide responsible for modulating pain response, resulted in a peptidomimetic with greater *in vivo* activity. Toniolo *Peptide Res.* **1989**, *2*, 275-281

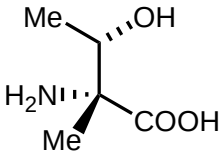
H-Tyr-Gly-Gly-Phe-Leu-OH

Enkephalin

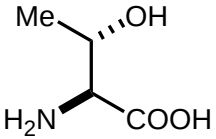
Synthesis of α-Methylamino Acids



α-Methylcysteine

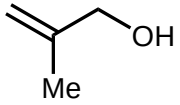


α-Methylthreonine



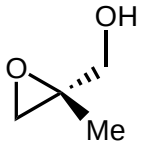
D-allo-threonines

- When incorporated in a peptidomimetic, these constrained amino acids have the potential for disulfide bond formation or other cyclizations to furthur rigidify the structure.



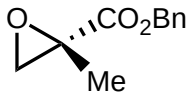
Sharpless Asymmetric Epoxidation

71% y.
> 95% ee



1. $RuCl_3•H_2O$, $NaIO_4$

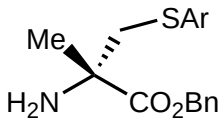
2. DCC, DMAP, BnOH
63% y. (2 steps)



1. NaN_3, 99% y.
2. PPh_3, 83% y.

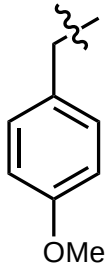
$BF_3•OEt_2$

RSH
78% y.



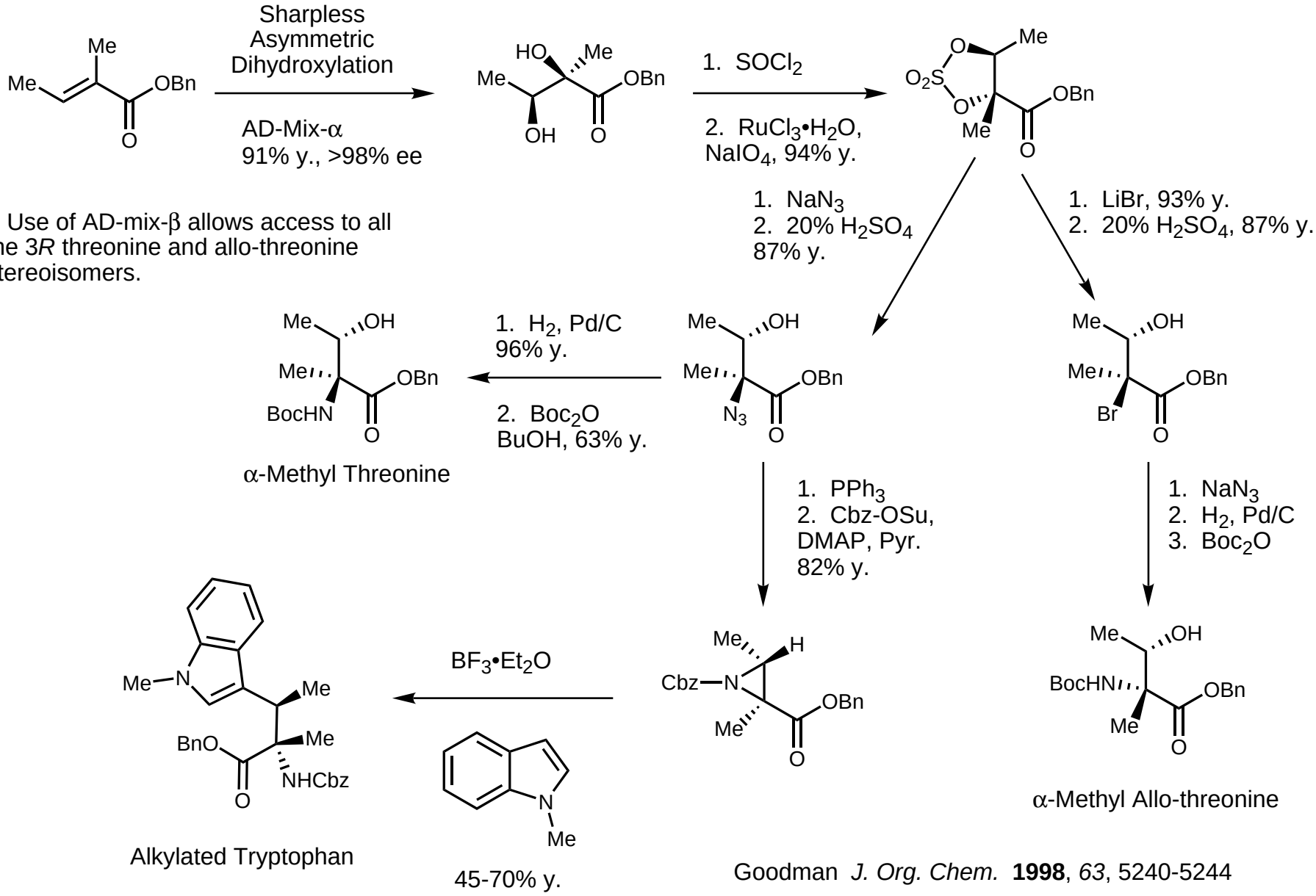
Protected
α-Methylcysteine

R =



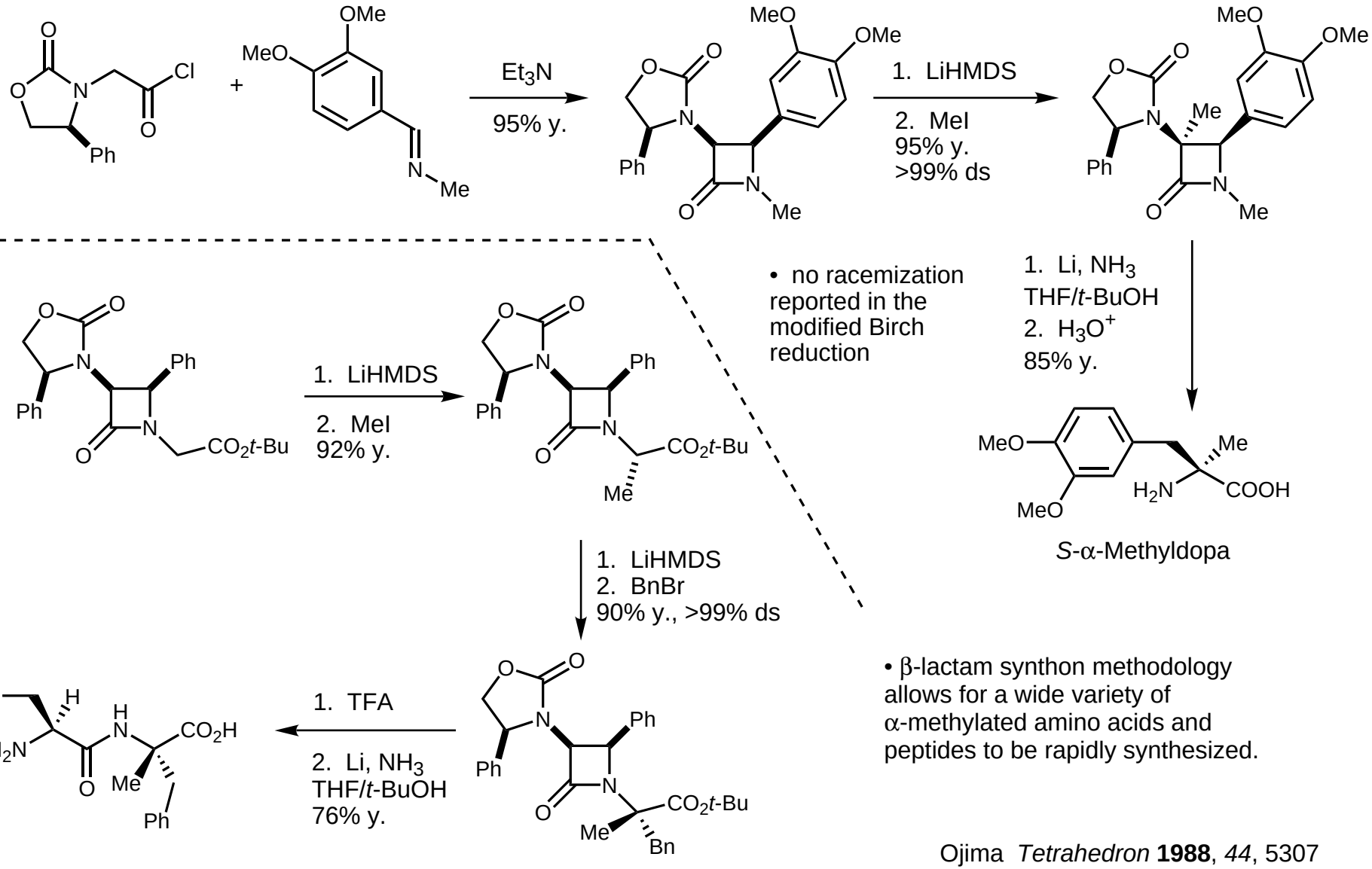
Goodman *Pure Appl. Chem.* **1996**, *68*, 1303-1308

Syntheses of α-Methylthreonines



Goodman *J. Org. Chem.* **1998**, *63*, 5240-5244

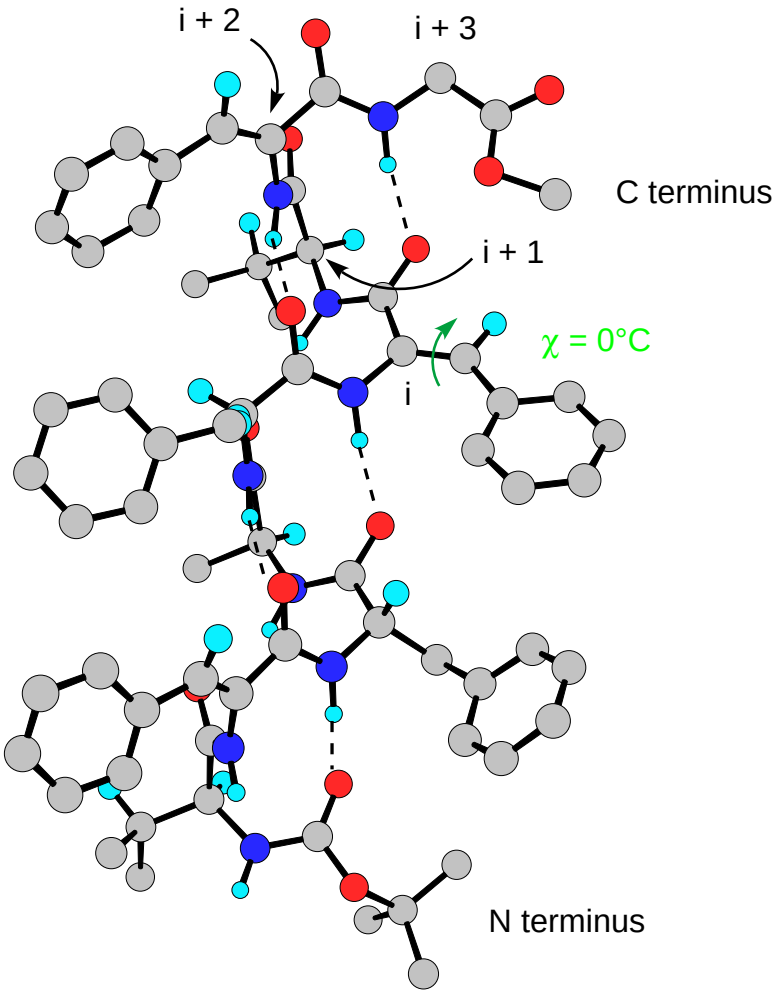
α-Methyl Amino Acids and Dipeptides From β-Lactams

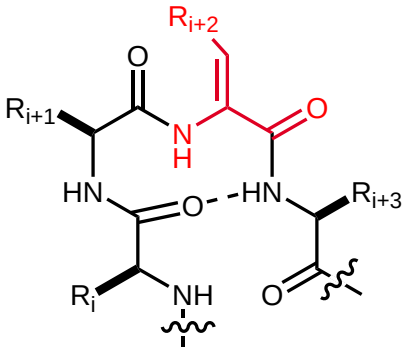


Ojima *Tetrahedron* **1988**, *44*, 5307

Staudinger reaction: Evans *Tetrahedron Lett.* **1985**, *26*, 3783

Dehydroamino Acids





- Favors the formation of β or γ-turns when placed in the (i+2) position of the putative turn sequence.

- Dehydroamino acid (*Z* isomer more synthetically accessible than *E*) rigidifies the conformation of the side chain. χ is fixed at 0°C (*Z*) or 180 °C (*E*).

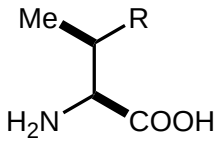
- Sequential placement of ΔPhe in a peptide gives repeated β-turns, which form a 3_{10} helix.

Boc-Val-ΔPhe-Phe-Ala-Phe-ΔPhe-Val-ΔPhe-Gly-OMe

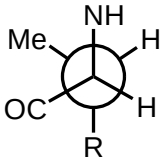
X-Ray Structure

Chuhan *J. Am. Chem. Soc.* **1992**, *114*, 9225-9226

β-Methylamino Acids



2*R*, 3*R*



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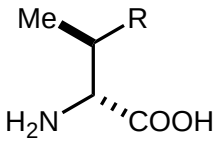
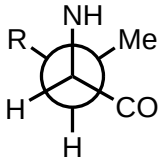
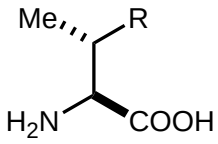


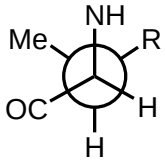
2*S*, 3*R*



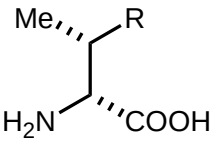
g^-



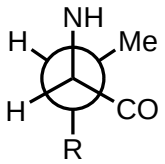
2*R*, 3*S*



g^+



2*S*, 3*S*



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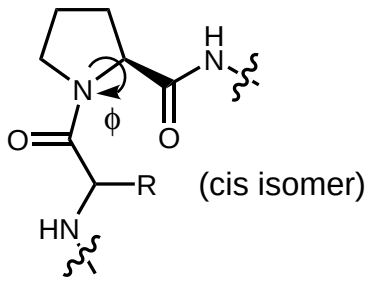
- Four configurations are accessible from varying the two stereocenters. Preferred conformations are shown.
- Systematic incorporation of β-MePhe into somatostatin peptidomimetics has resulted in a model for the ligand-receptor interaction, based on the activity changes induced by different configurations at the β center.

χ	descriptor
-60°	gauche$^-$ (g^-)
180°	trans (t)
+60°	gauche$^+$(g^+)

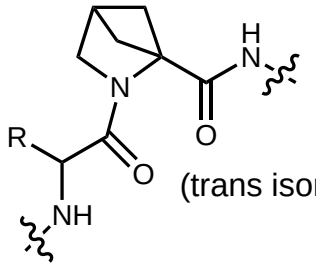
Goodman *J Am. Chem. Soc.* **1992**, *114*, 9390-9401

Conformational Restriction of Proline Analogs



(cis isomer)

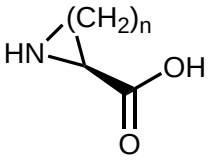
Proline (Pro)



(trans isomer)

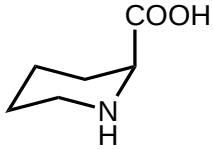
2,4-Methanoproline
(2,4-MePro)

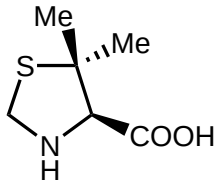
- ϕ is constrained to –65 ± 15°, preventing α-helix formation, and encourages formation of β-turns.
- Barrier to proline cis-trans isomerism is ~ 2 kcal/mol, whereas 2° amide barrier is 10 kcal/mol.
- 2,4-MePro prefers the trans isomer by 6-8 kcal/mol.



n=1 Azyline-2-carboxylic acid (Azy)
n=2 Azetine-2-carboxylic acid (Aze)
n=4 Pipecolic acid (Pip)

- Difference between the Azy, Aze, and Pro residues is largely steric bulk of the side-chain rather than the ϕ, ψ angles.
- Pipecolic acid prefers a chair conformation in which the carboxylic acid is axial.





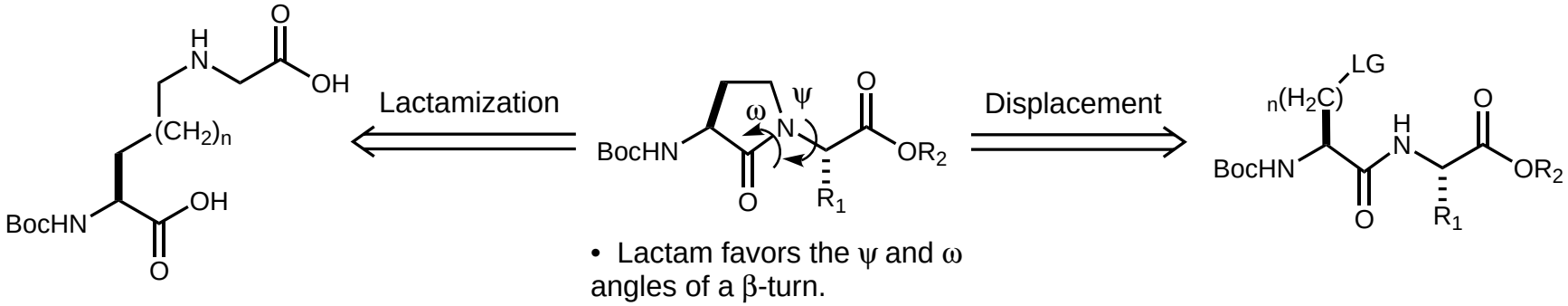
5,5-Dimethylthiazolidine-
4-carboxylic acid (Dtc)

- Allowed angles for ψ are in the γ-turn region
- Substitution of Dtc for Pro in angiotensin II, a key peptide in blood pressure regulation, resulted in a peptidomimetic with 39% greater agonist activity than the natural peptide.
J. Med. Chem. **1991**, *34*, 3036-3043

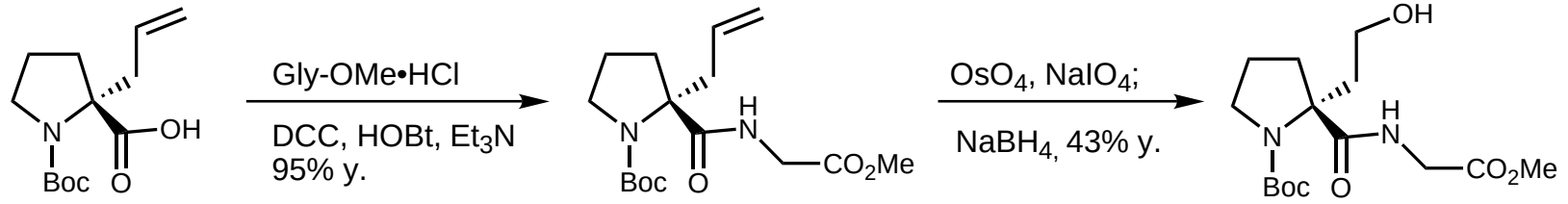
H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH

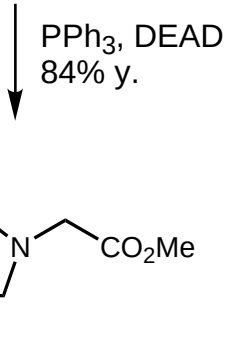
Angiotensin II

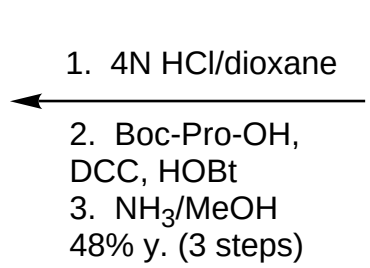
Cyclic Amino Acids Through Lactamization

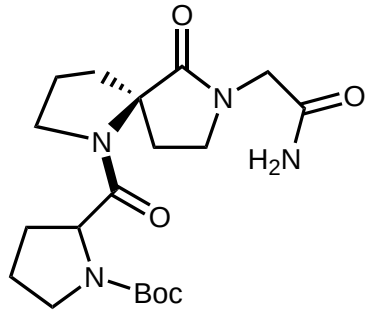


• Lactam favors the ψ and ω angles of a β-turn.





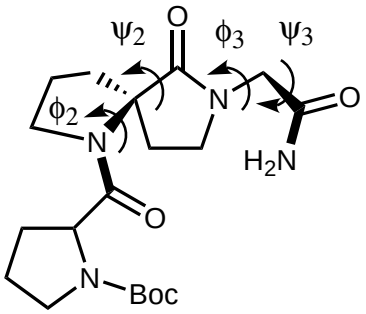


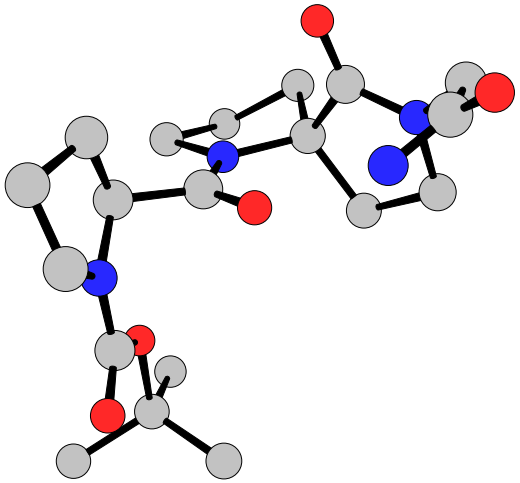


• Spirolactam combines the conformational restraints of both proline and the lactam to fix the tripeptide in a Type II β-turn.

Johnson *J. Org. Chem.* **1993**, *58*, 2334-2337

Use of Spirolactam to Form an Active Peptidomimetic





	ϕ_2	ψ_2	ϕ_3	ψ_3
X-ray structure	–50.9	128.7	91.1	–5.4
ideal Type II β-turn	–60	120	80	0

Johnson *J. Org. Chem.* **1993**, *58*, 2334-2337

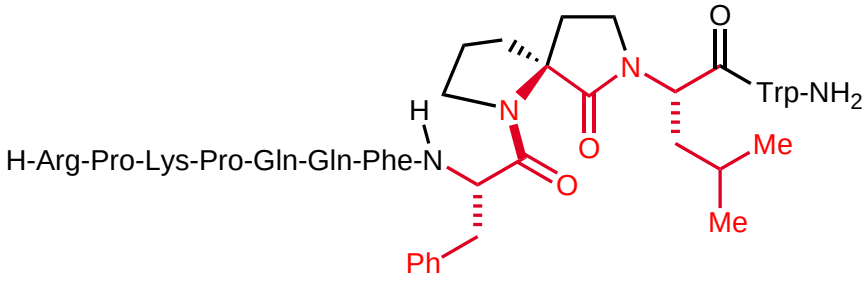
H-Arg-Pro-Lys-Pro-Gln-Gln-Phe-Phe-Gly-Leu-Met-NH_2

Substance P

- Substance P is a tachykinin, a nervous system modulator and transmitter, with theraputic potential in treating gastrointestinal inflamation, arthritis, Parkinson's and Alzheimer's disease.

- By substituting Gly, Pro and D-aa in structure-activity relationship (SAR) studies, a β-turn in the Phe-Gly-Leu portion of the peptide was believed to be crucial for receptor binding of Substance P.

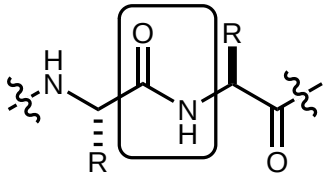
- Incorporation of the spiro-lactam into the optimized peptidomimetic GR71251 resulted in the most potent antagonist of the NK1 Substance P receptor known to date.



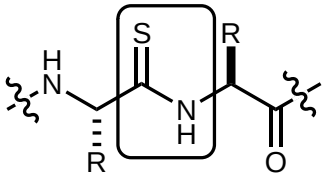
GR71251

Ward *J. Med. Chem.* **1990**, *33*, 1848-1851

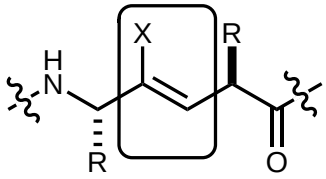
Common Amide Bond Isosteres



Peptide

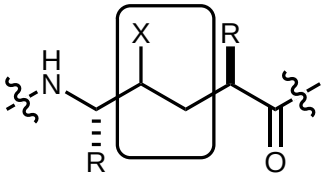


Thioamide isostere



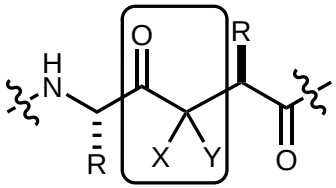
Trans-olefin isosteres

X = H, F



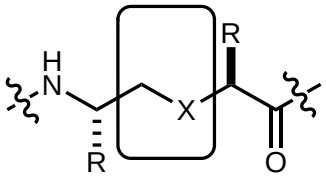
Ethylene isosteres

X = H, OH



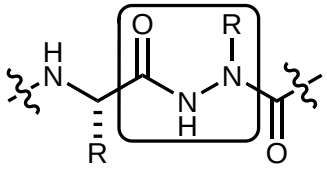
Ketomethylene isosteres

X = Y = H or F
X = H, Y = OH

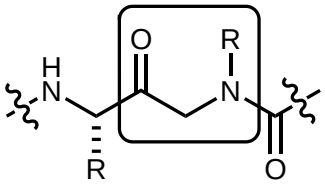


Methylene isosteres

X = S, S(O), O



Azapeptide isostere



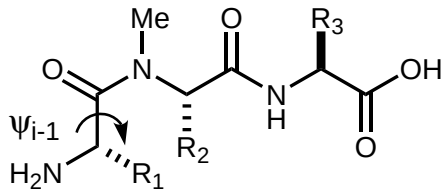
Peptoid isosteres

For a comprehensive review, see:

- Rieger, Evans Group Seminar, 1991
- Goodman *Burger's Medicinal Chemistry and Drug Discovery.* Ed. M. E. Wolff. New York, John Wiley & Sons, Inc., **1995**, 803-861.

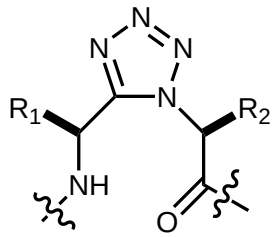
Methods for Constraining Peptide Cis-Amides

N-Methyl amides



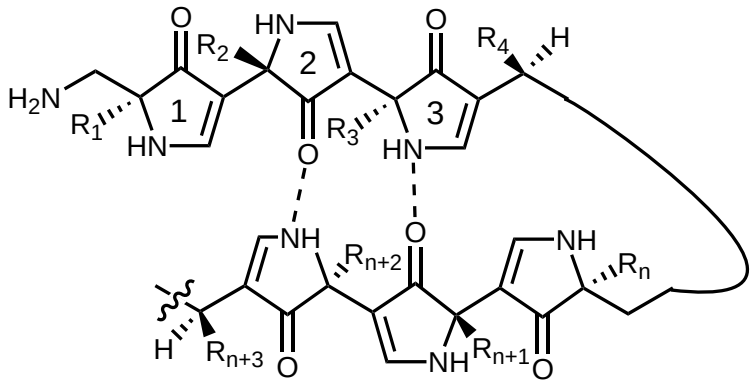
- The cis-amide is only 0.6 kcal/mol higher in energy than the trans isomer.
- The increase in flexibility is tempered by the steric restrictions imposed by the Me group. ψ_{i-1} is restricted to $60<\psi<180$ in both the cis- and trans-amide.

Tetrazoles



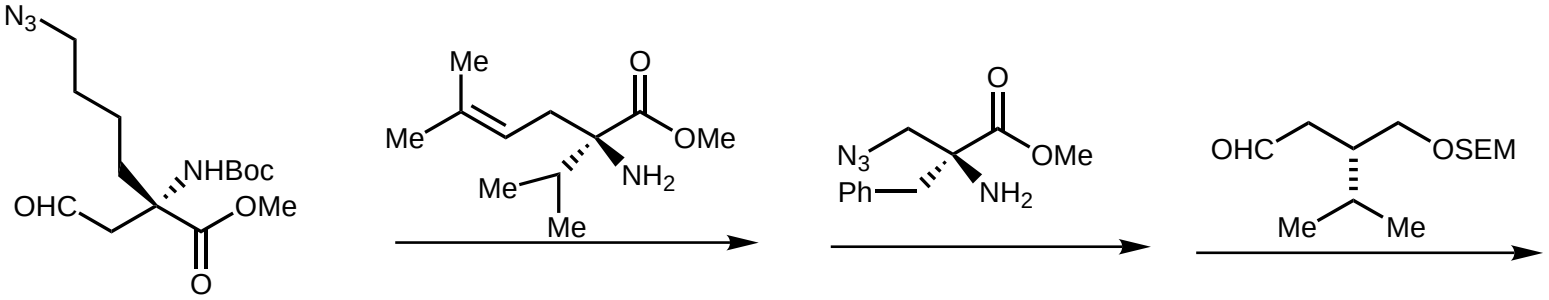
- The tetrazole locks the amide in a cis configuration, and is more easily synthesized than a cis-olefin isostere.
- Similar or enhanced activity of a tetrazole peptidomimetic indicates a cis-amide bond is favorable for receptor binding.
- Absence of activity can be due to the increased steric bulk of the tetrazole, not necessarily that a cis-amide bond is not the bioactive conformation.

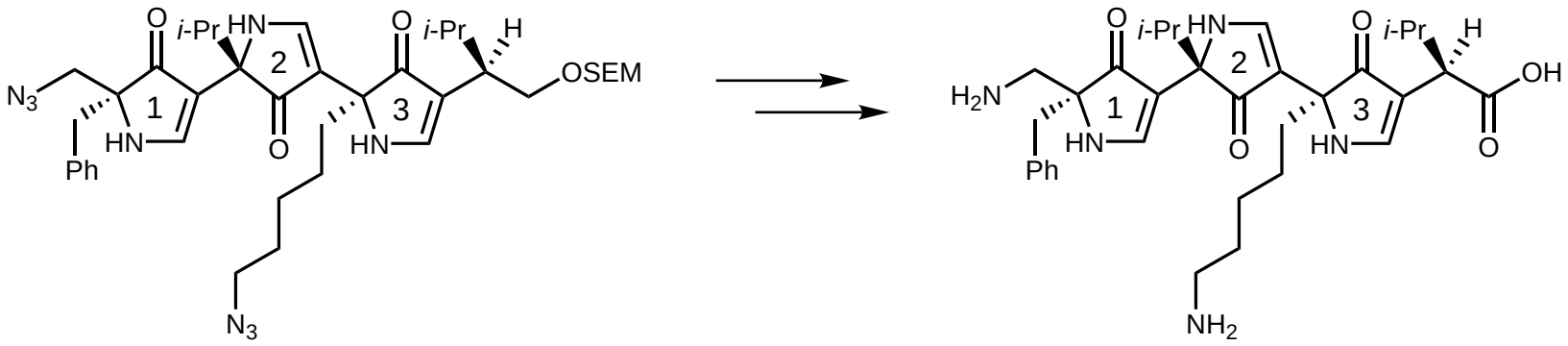
Pyrrolinone Peptidomimetic Scaffold



- The pyrrolinone backbone fixes ϕ, ψ, ω to the angles near that of an anti-parallel β-pleated sheet (ideal is $\phi = -139°$, $\psi = 135$)

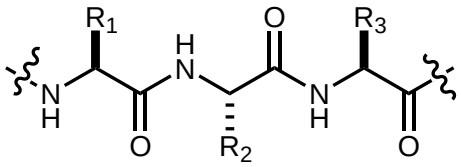
- Oligomer is amenable to iterative synthesis for a wide variety of amino acids.



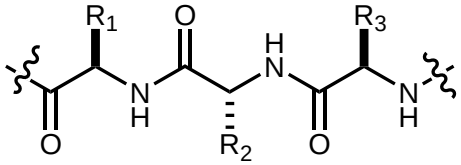


Smith *Org. Lett.* **2000**, *2*, 2037-2040

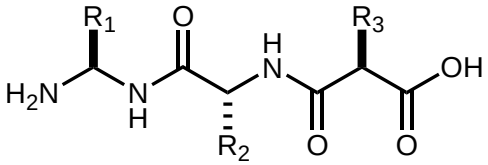
Retro-Inverso Peptidomimetics



Normal peptide



Retro-inverso peptide



End group modified
retro-inverso peptide

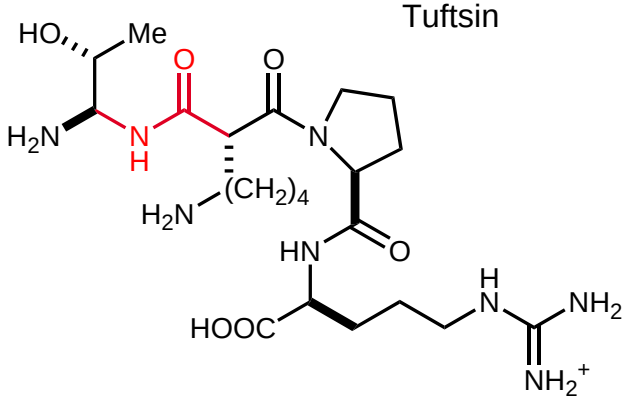
- The reversal of the amide bond direction minimizes peptidase degradation, thereby increasing the mimetics' *in vivo* half-life dramatically.
- The inversion of each sterocenter from L to D preserves the 3-D orientation of the side chain residues of the original peptide.
- Reversal of the carboxyl and amino termini reverses the charge structure of the peptide and may be the cause of their low biological activity.
- End group modifications increased the complementarity of the peptidomimetic with the native peptides and has resulted in several potent peptidomimetics. A retro-inverso section can also be embedded in a larger normal peptide.

- Tuftsin is a immune system stimulator which is completely degraded *in vivo* in 8 min.
- The retro-inverso peptidomimetic shows less than 2% hydrolysis after 50 min and retention of bioactivity.

Verdini *J. Med. Chem.* **1991**, *34*, 3372-3379

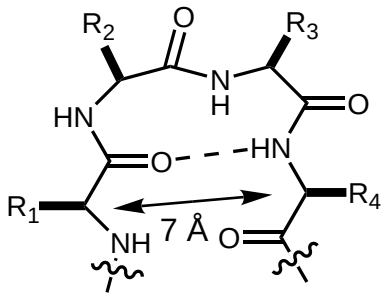
H-Thr-Lys-Pro-Arg-OH

Tuftsin



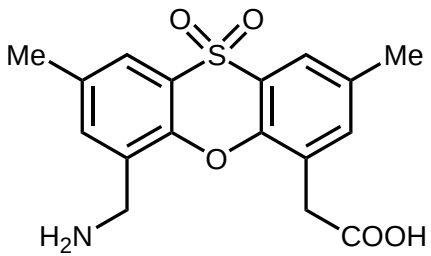
Pseudopeptide

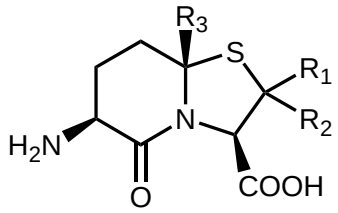
β-Turn Non-peptidomimetics

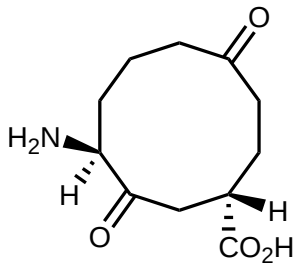


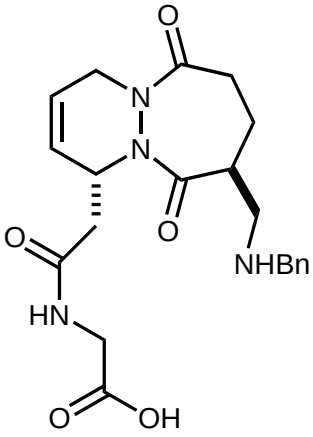
β-turn

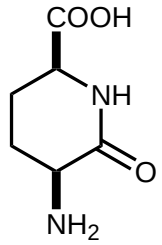
Representative β-turn mimetics









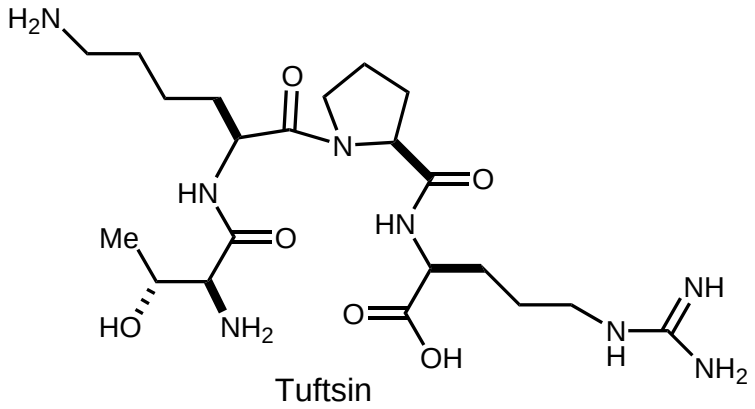


- β-turns are the most frequently mimicked protein secondary structures.

- A β-turn is defined as a tetrapeptide sequence where the distance between α-C_i and α-C_{i+3} is ≤ 7Å. The turn can be stabilized by chelation of a cation, such as Ca^{2+}, or intramolecular hydrogen bond(s).

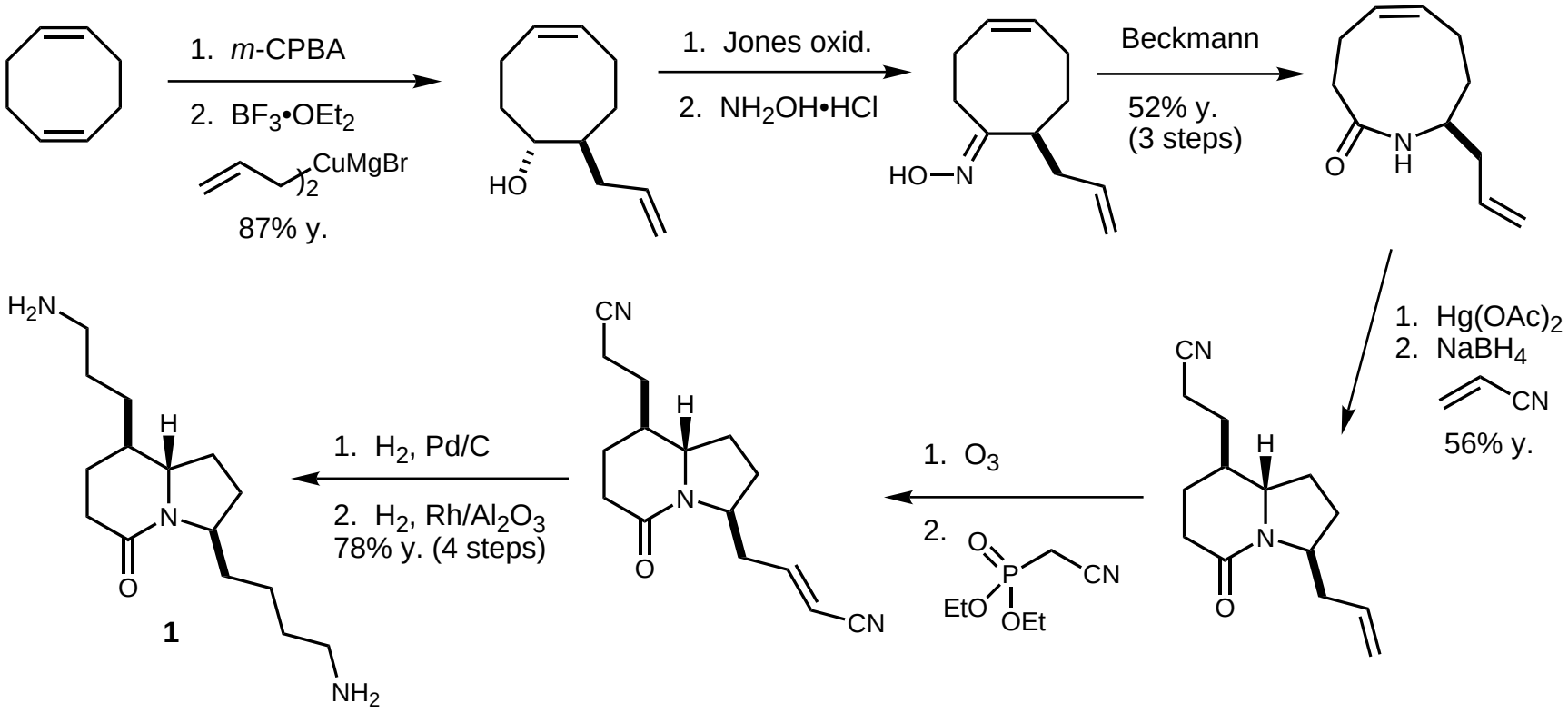
- Ideal mimic will have a rigid scaffold that orients the sidechain residues in the same direction as the natural peptide, while conferring better solubility and/or resistance to enzymatic degradation.

Development of β-Turn Mimetics



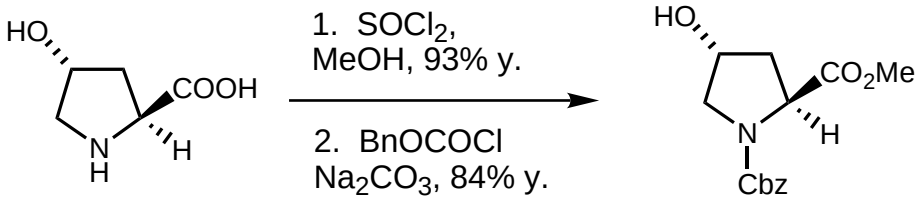
Tuftsin

- A folded structure was thought to be present in the receptor-bound conformation of the immunoregulator tuftsin.
- **1** was the first β-turn mimetic ever synthesized.
- **1** exhibited some bioactivity in a dose dependent manner, but extensive studies were not undertaken.

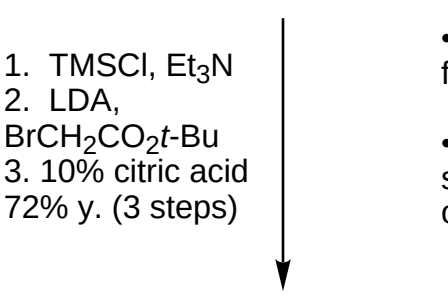


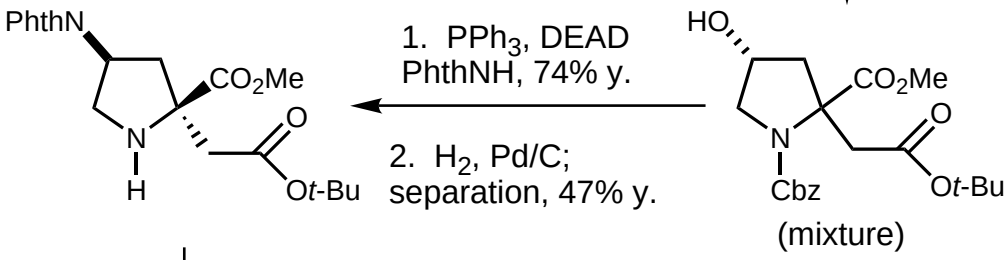
Kahn *Tetrahedron Lett.* **1986**, *27*, 4841-4844

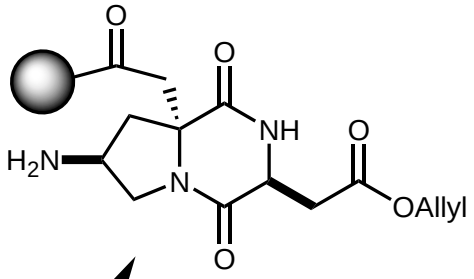
β-Turn Mimetics for Solid Phase Synthesis

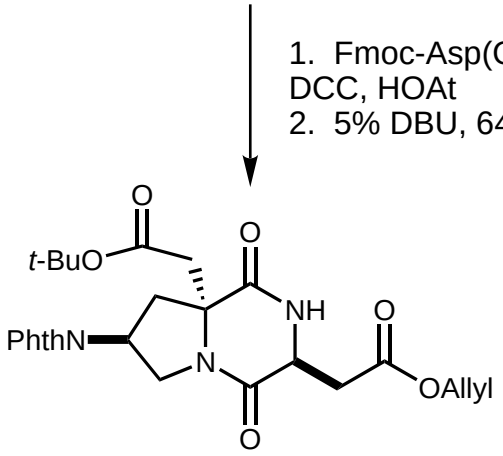


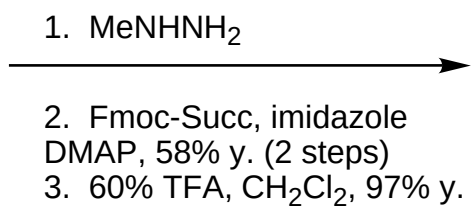
- The peptide sequence -Asn-Pro-Asn-Ala- has been identified as a key target to mimic for developing an effective malaria vaccine. It is present on the surface of *Plasmodium falciparum* parasite which is recognized by the human immune system.
- Solution phase NMR studies indicate a preference for a Type I β-turn in the -Asn-Pro-Asn-Ala- region.
- Solid phase amino acid synthesis facilitates the screening of a number of turn mimetics to find the optimal geometry/stability.

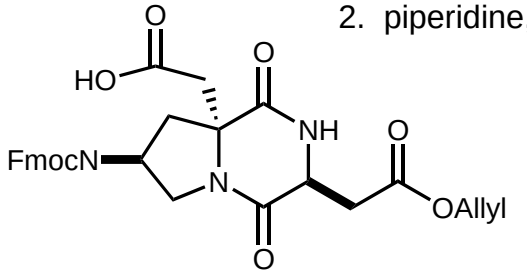


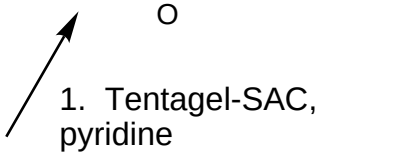






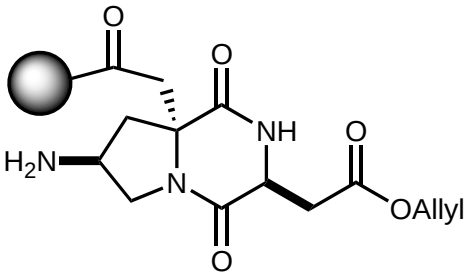






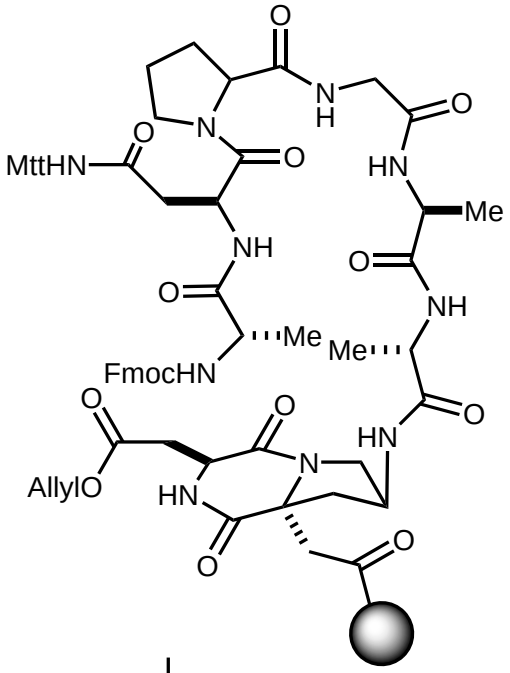
Robinson *Synlett.* **1999**, 429-441

Elaboration of Turn Mimetic



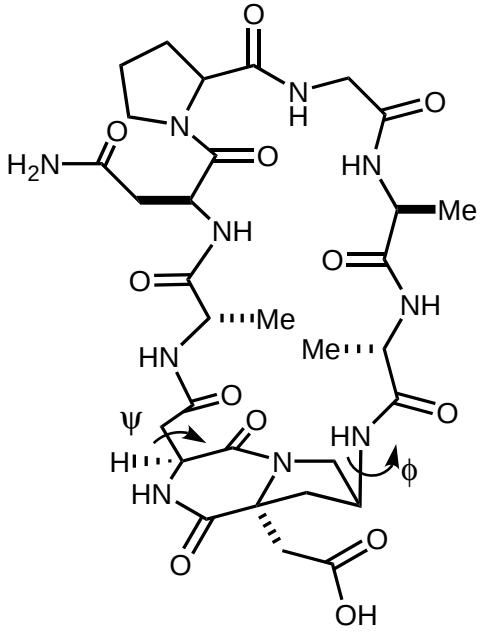
1. peptide coupling: HBTU, HOBt, Fmoc-aa, DIEA, DMF

2. Fmoc removal: piperidine, DMF
3. repeat sequence



1. $Pd(PPh_3)_4$, NMM, AcOH, DMF
2. piperidine, DMF
3. BOP, DIEA, NMP
4. TFA

(Mtt = methyltrityl)

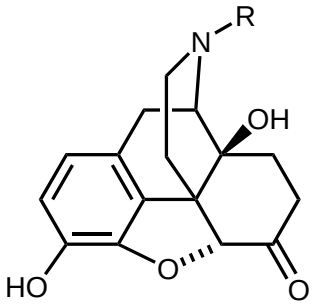


- Attachment of this peptidomimetic to a carrier protein and injection into mice resulted in production of anti-sera to malaria sporozoites.

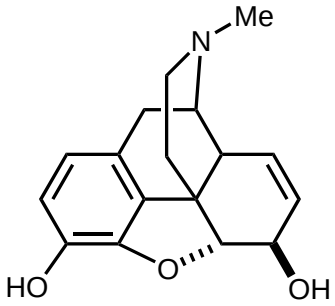
- NMR studies of the macrocycle indicate the illustrated ϕ and ψ were in close agreement with a Type I β-turn.

Robinson *Synlett.* **1999**, 429-441

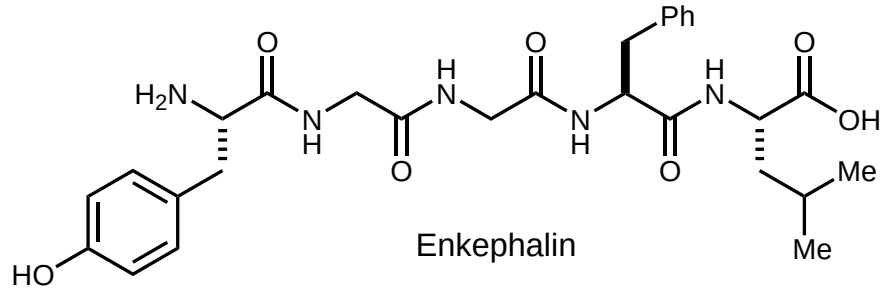
Morphine: a Non-peptidomimetic For Enkephalin



R = Me: Oxymorphone, μ agonist
R = Allyl: Naloxone, μ antagonist



Morphine

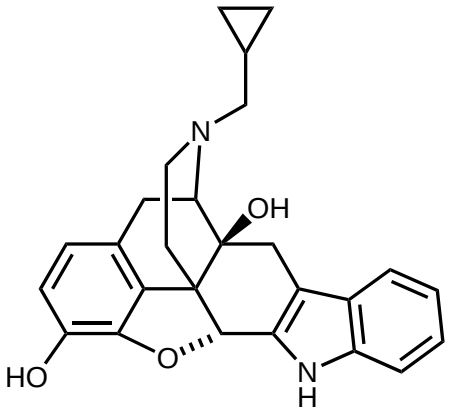


Enkephalin

Message: Binds to the receptor

Spacer

Address: Sequence bound by carrier proteins to direct the peptide to the correct receptor after synthesis



Naltrindole

δ antagonist

- Morphine, one of the oldest known analgesics, binds to the opiate receptors. Three receptors have been identified: κ, μ, and δ. Enkephalin is specific for δ, while dynorphin binds to the κ receptor. An endongenous μ peptide ligand has not been identified yet.

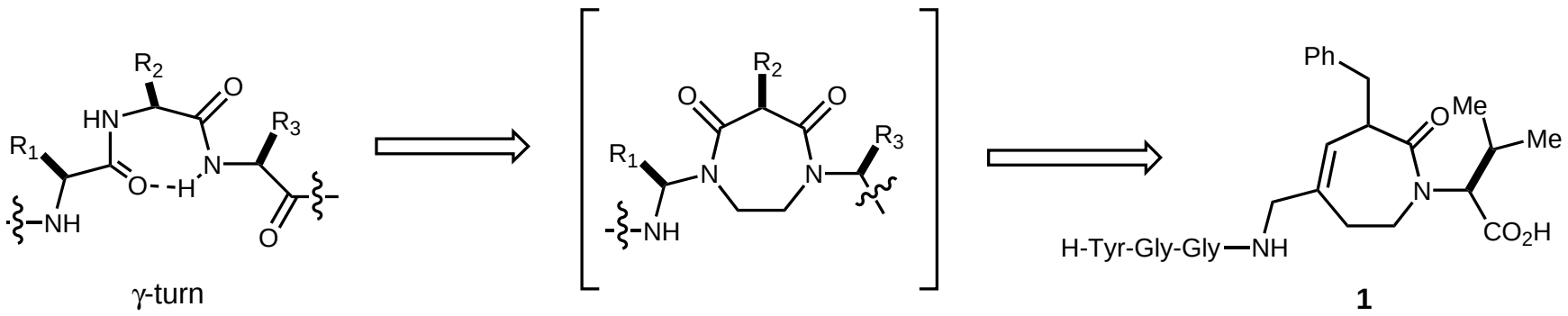
- Exact nature of the binding interaction between either natural or synthetic ligand and a receptor has yet to be elucidated.

Portoghese *J. Med. Chem.* **1990**, *33*, 1714-1720

Determination of Active Secondary Structure

H-Tyr-Gly-Gly-Phe-Leu-OH

Enkephalin



γ-turn

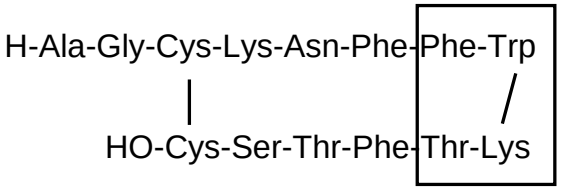
1

- **1** was designed to probe whether enkepalin's bioactive conformation contained a γ-turn. This turn mimetic concept had successfully been used in developing inhibitors of thrombin, a protease involved in blood coagulation.
- No binding activity was observed at any of the opiate receptors.
- Does enkephalin then not contain a γ-turn?
- Negative results are inconclusive, not proof of the absence of the element in question.

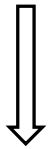
Factors affecting binding: ring conformation, dipole, steric fit, hydrogen bonding, hydrophobicity

Huffman *Tetrahedron* **1993**, *49*, 3479-3488

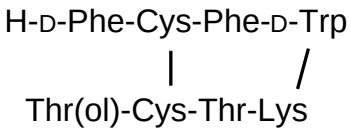
Somatostatin: Early Investigations



Somatostatin



Suspected β-turn
in boxed region



Octreotide

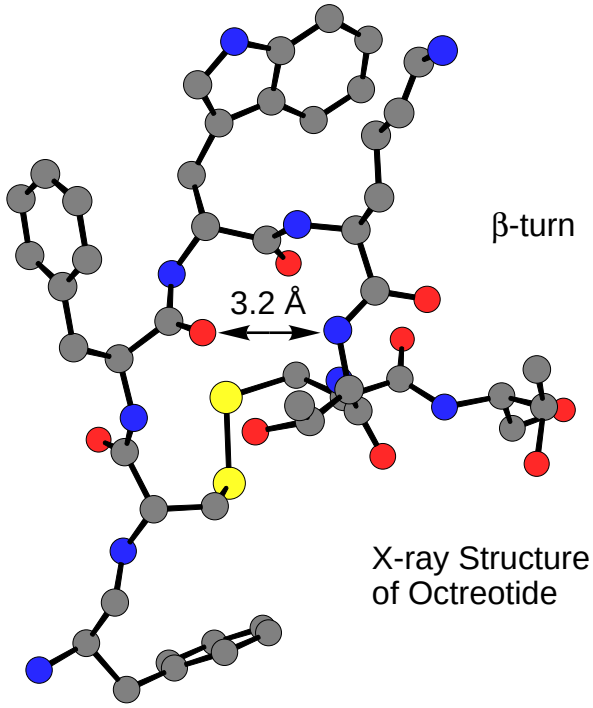
- Octreotide has similar biological activity to somatostatin and is a drug of first choice for the treatment of several carcinoid syndromes.

Thr(ol) = L-threoninol

- Somatostatin is a cyclic peptide formed by a disulfide bond between two Cys residues, and found in the brain and digestive organs.

- Biological activity includes the inhibition of growth hormone, regulation of lymphocyte production in the brain, regulation of glucagon/insulin pathway (in pancreas), and digestive tract motility and blood supply.

- Main theraputic use is in the treatment of ulcers and bleeding in the gastrointestinal tract, but somatostatin has a prohibitively short half life *in vivo*.

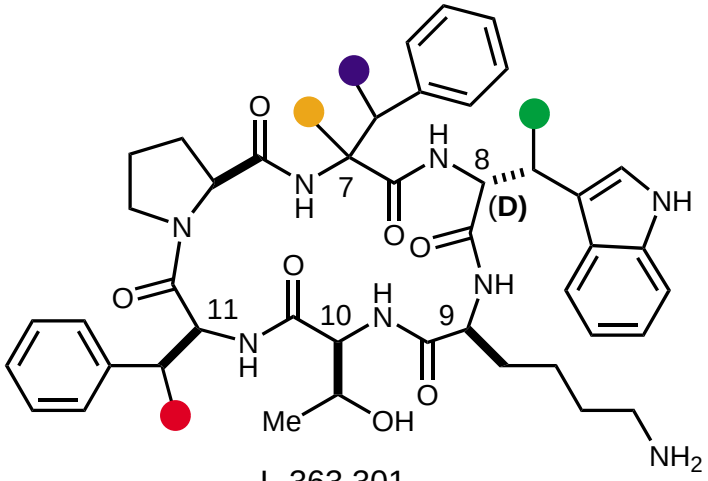


X-ray Structure
of Octreotide

Synthesis: Pless *Life Sci.* **1982**, *31*, 1133-1140

X-ray: Sheldrick *Acta. Cryst. D* **1995**, 48-59

Somatostain Active Site Elucidation



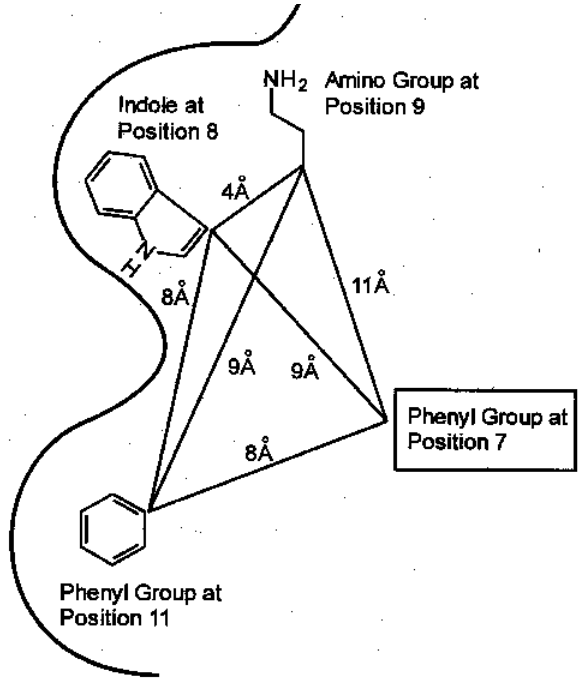
L-363,301
IC_{50} = 1 nM

- L-363,301, first synthesized in 1982, was selectively methylated at the 7, 8, and 11 positions in the putative β-turn region.
- NMR and SAR studies of the derivatives led to a model for the somatostatin pharmacophore in which residues 8, 9 and 11 were important for activity.

Modification	IC_{50} (nM)
(2*S*, 3*R*)-β-Me-7	1
(2*S*, 3*S*)-β-Me-7	1
(*S*)-α-Me-7	>1000
(*R*)-α-Me-7	>1000
(2*R*, 3*S*)-β-Me-8	<1
(2*R*,3*R*)-β-Me-8	>1000
(2*S*, 3*R*)-β-Me-8	10
(2*S*, 3*S*)-β-Me-8	>1000
(2*R*, 3*S*)-β-Me-11	50
(2*R*,3*R*)-β-Me-11	>1000
(2*S*, 3*R*)-β-Me-11	>1000
(2*S*, 3*S*)-β-Me-11	>1000

2D-NMR and

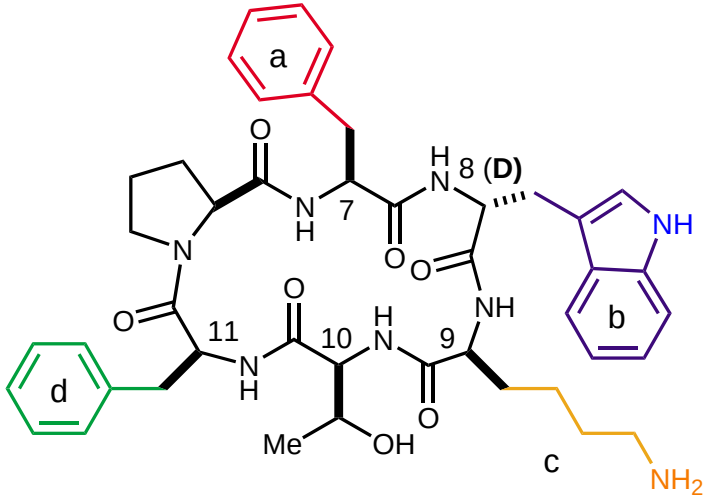
Analysis of Conformer Populations



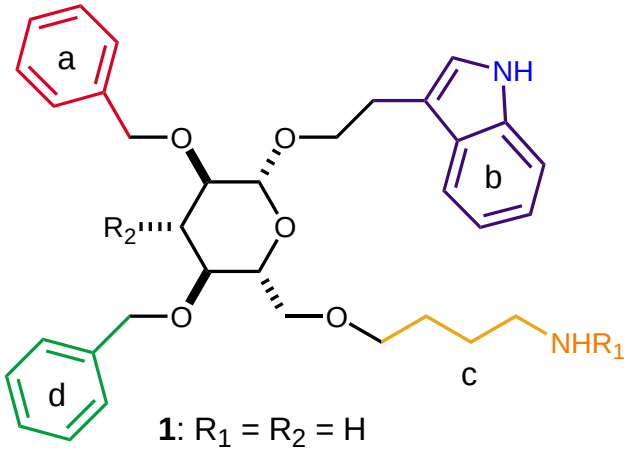
Pharmacophore Model

Goodman *J. Am. Chem. Soc.* **1992**, *114*, 9390-9401

Glucose Scaffold for Non-peptidomimetic of Somatostatin



L-363,301



1: $R_1 = R_2 = H$
2: $R_1 = CH_3CO$, $R_2 = OCH_2Ph$

G-Protein Coupled Receptor	**Biological Activity (IC_{50}, μM)**		
	L-363,301	**1**	**2**
somatostatin	0.001	15	1.3
	low conc.: agonist high conc.: antagonist		agonist (all conc.)
β_2-adrenergic		3 antagonist	——
NK1		0.18 agonist	0.06 antagonist

Distance (Å)

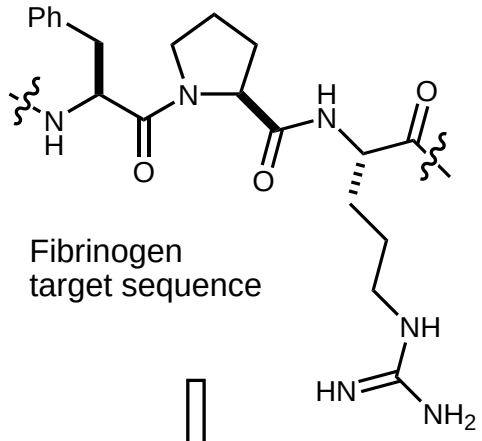
	a-b	a-c	a-d	b-c	b-d	c-d
L-363,301	7.1	11.3	9.2	7.3	9.2	14.1
2	5.6	10.6	8.0	6.6	10.3	13.5

- Computer modeling, combined with previous biological studies of somatostain peptidomimetics, led to the design of a glucose-based non-peptidomimetic.

- Glucose shows promise in development of non-peptidomimetics for several G-protein coupled receptors, but selectivity must be fine-tuned for the desired activity.

Hirshmann *J. Am. Chem. Soc.* **1993**, *115*, 12550-12558

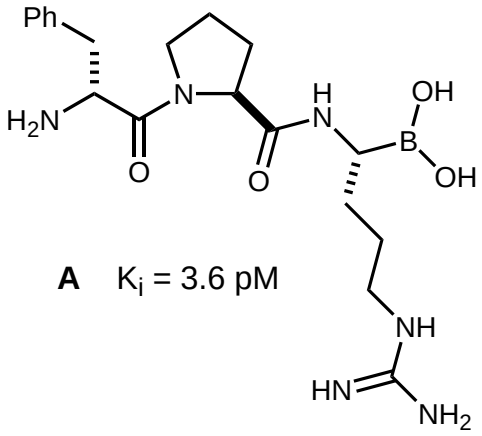
Peptidomimetic Thrombin Inhibitors



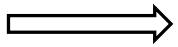
Fibrinogen
target sequence

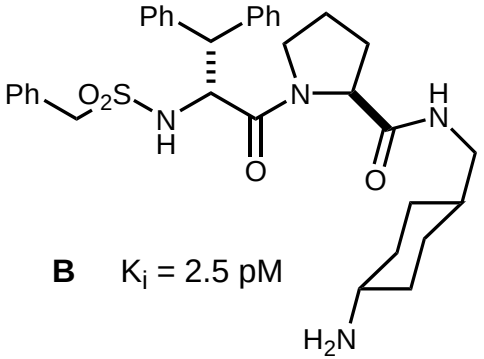
- Thrombin is a serine protease that promotes blood coagulation by cleavage of fibrinogen to the active protein fibrin. Contraction and dilation of blood vessels are also affected. Antagonists could be theraputic in treating arteriosclerosis and thrombosis.

- Early antagonist design focused on non-hydrolyzable transition state mimetics of the -Phe-Pro-Arg- sequence adjacent to the substrate cleavage site.



A K_i = 3.6 pM

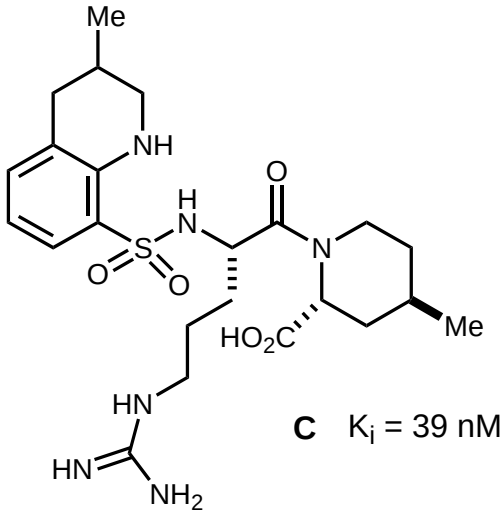




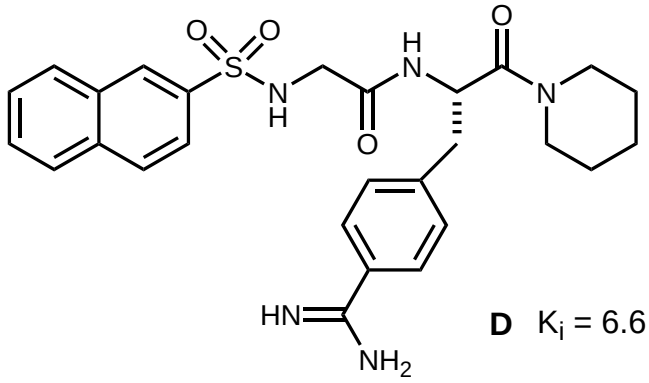
B K_i = 2.5 pM

Development of Thrombin Peptidomimetics

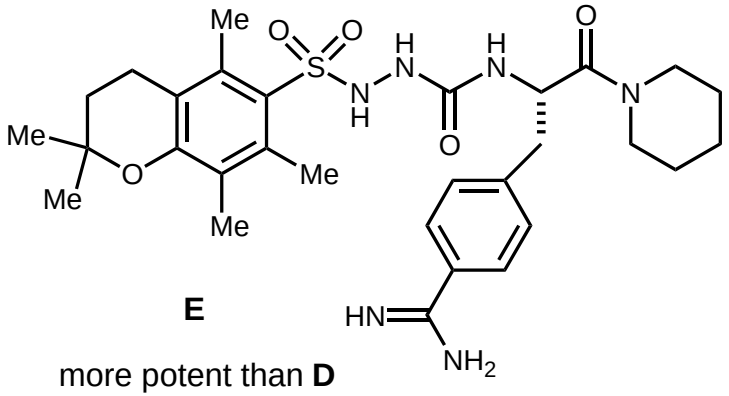
B ⇒



C K_i = 39 nM ⇒



D K_i = 6.6 nM ⇓



E

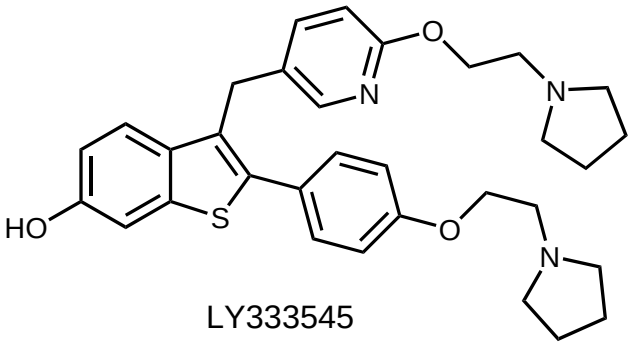
more potent than **D**

- Can you assume **D** is binding in the same pocket as the fibrinogen substrate does?

- X-ray structural studies indicate **C** and **D** bind to a different site in the enzyme through their hydrophobic residues,which causes collapse of the active site pocket. Enzymatic activity is thereby inhibited.

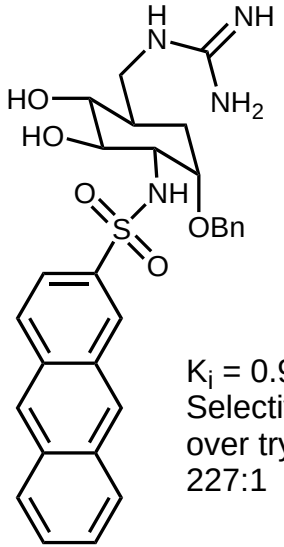
Review summarizing the progression:
Gante *Angew. Chem. Int. Ed. Eng.* **1994**, *33*, 1699-1720

Non-peptidomimetic Thrombin Inhibitors



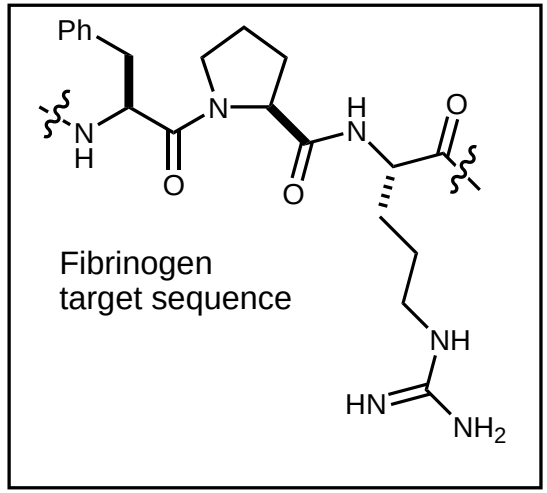
LY333545

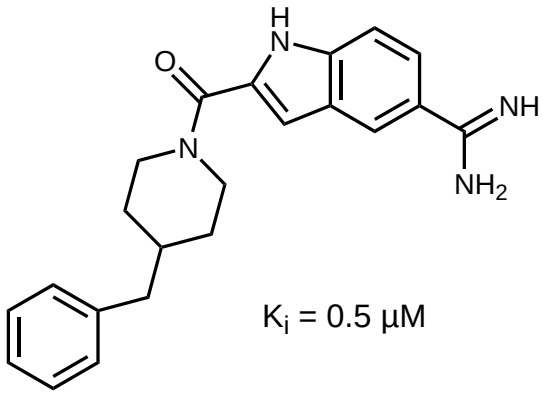
• developed from a library screening lead
Sall *Book of Abstracts, 214th ACS National Meeting* **1997**, A58



K_i = 0.9 µM
Selectivity over trypsin: 227:1

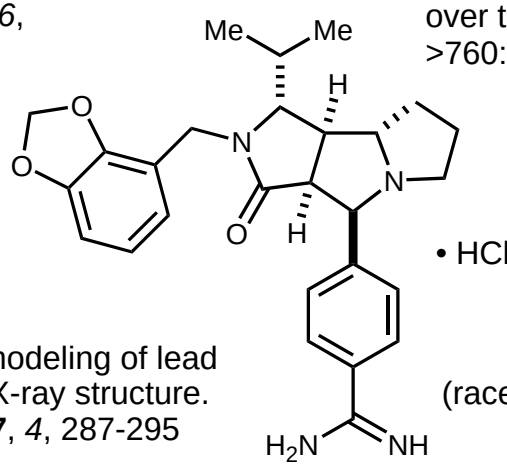
• developed by using a bioactive template (glucose) and altering the periphery to give desired activity. *Angew. Chem. Int. Ed. Eng.* **1997**, *36*, 751-752.





K_i = 0.5 µM

• developed by computer modeling studies of the indole nucleus docked in the thrombin X-ray structure.
Wery *Protein Sci.* **1997**, *6*, 1412-1417



K_i = 13 nM
Selectivity over trypsin: >760:1

• HCl

(racemic)

• developed by computer modeling of lead molecules docked into the X-ray structure.
Diederich *Chem. Biol.* **1997**, *4*, 287-295

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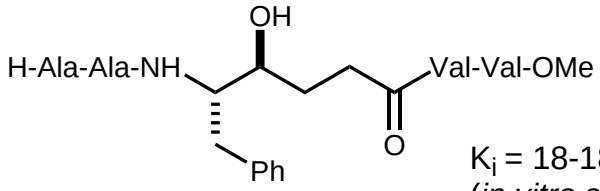
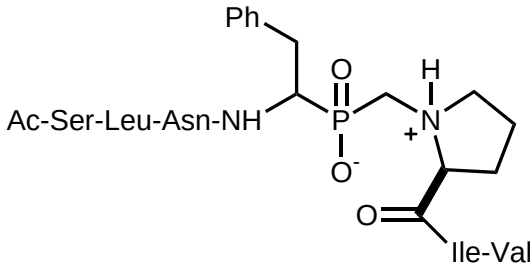
Peptidomimetic HIV-1 Protease Inhibitors



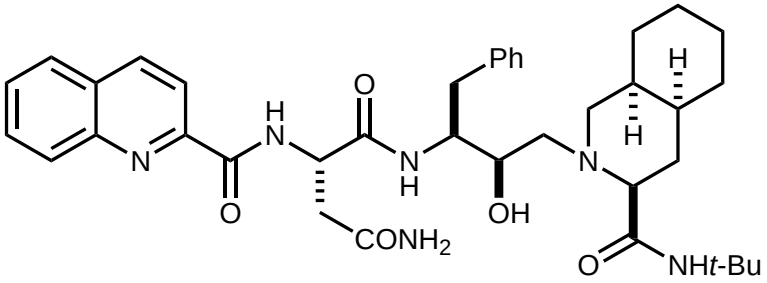
K_i = 18-180 nM
(*in vitro* against protease)

Dreyer *Proc. Natl. Acad. Sci.* **1989**, *86*, 9752-9756



K_i = 8.2 nM
(*in vitro* against protease)

Janda *J. Am. Chem. Soc.* **1992**, *114*, 7604-7606



Saquinavir (Ro 31-8959)

Roberts *Science* **1990**, *248*, 358-361

- HIV-1 protease is an aspartyl protease, meaning that the active site of the enzyme contains the triad -Asp-Thr(Ser)-Gly-. The protease is involved in synthesis of the virion's structural proteins. Inhibition of the protease results in non-infectious virions.

- The first approach to inhibition was to form transition-state analogues, where the scissile amide bond was replaced with a non-hydroyzable peptide isostere.

- Most isosteric replacements centered around the Phe-Pro cleavage site in the protease substrate.

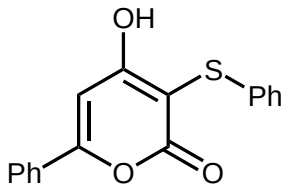
- Saquinivir has an IC_{50} = 2 nM in HIV infected cells.

- Excellent selectivity for HIV protease over human proteases was observed. Less than 50% inhibition of apartyl proteases was seen at 10 µM.

- Oral bioavailabitlity is good (plasma levels stay above required inhibitory concentration for several hours.)

- This drug has been through clincal trials and is currently an established drug for the treatment of AIDS.

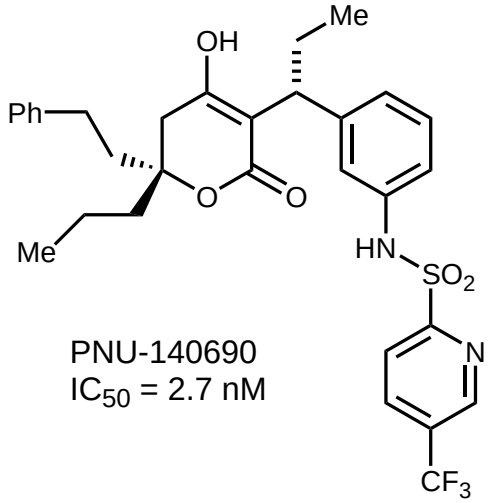
Non-Peptidomimetic HIV-1 Protease Inhibitors



Lead Structure

IC_{50} = 3 µM

Optimization



PNU-140690

IC_{50} = 2.7 nM

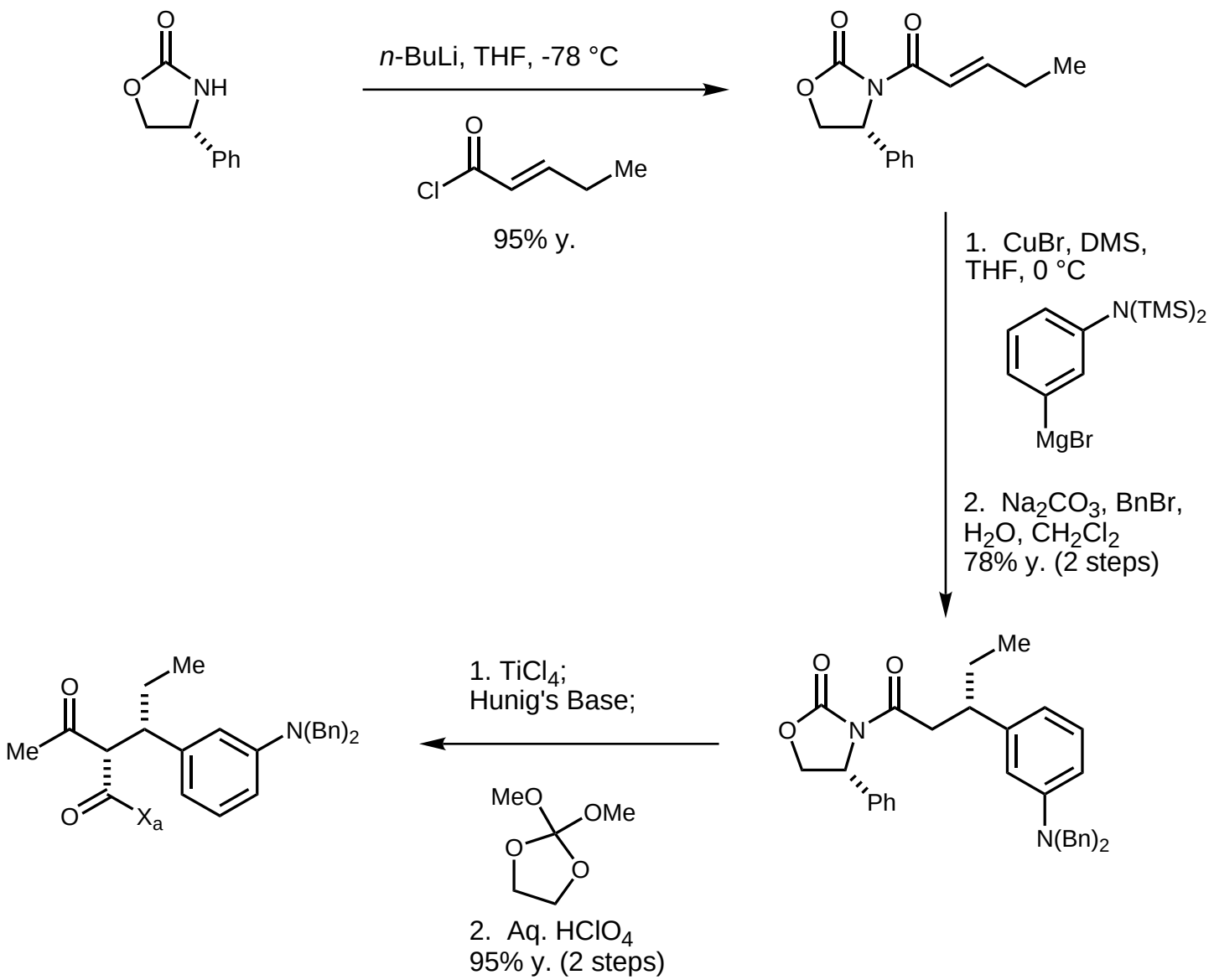
- Lead found in a high volume library screen at Pharmacia & Upjohn in 1994. The pyrone inhibits HIV-1 protease but has no anti-viral activity.

- Optimization of the core structure to the potent inhibitor PNU-140690 involved computer modeling studies (docking the molecule into the X-ray crystal structure of HIV-1 protease), SAR studies, and pharmacokinetics.

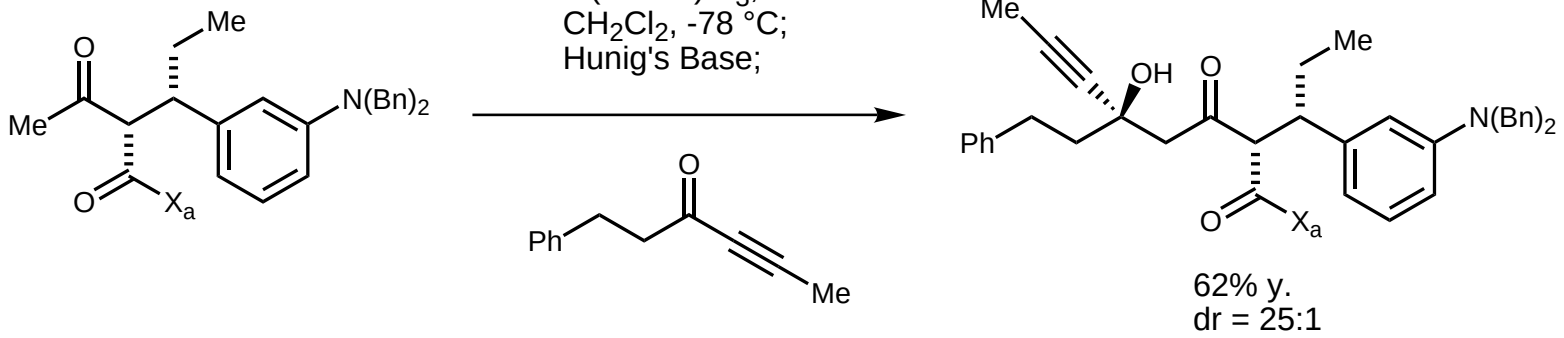
- PNU-140690 is effective against some strains of HIV-1 that have developed resistance to saquinivir, indinavir and other peptide-based protease inhibitors.

Hagen *J. Med. Chem.* **1997**, *40*, 3707-3711

Asymmetric Synthesis of PNU-140690

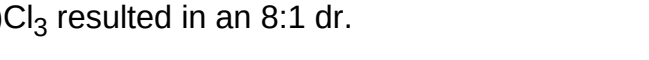


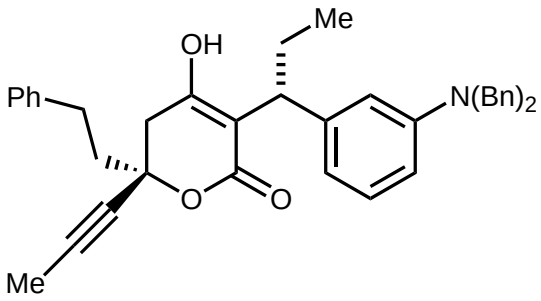
Asymmetric Synthesis of PNU-140690

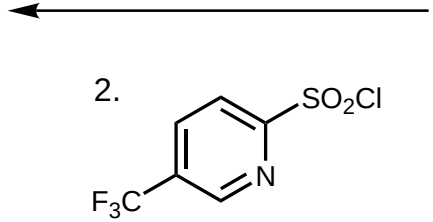


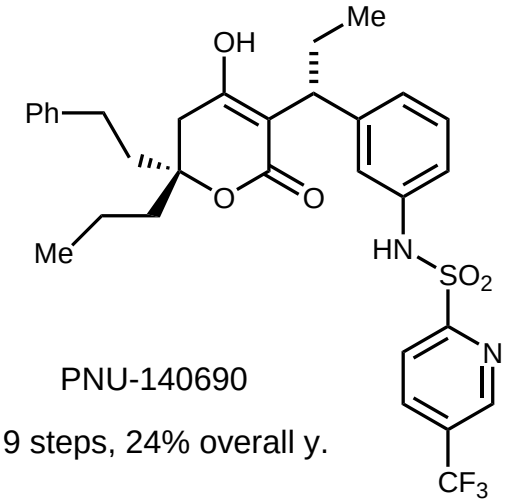
62% y.
dr = 25:1

- Use of Ti(O*i*-Pr)Cl_3 resulted in an 8:1 dr.







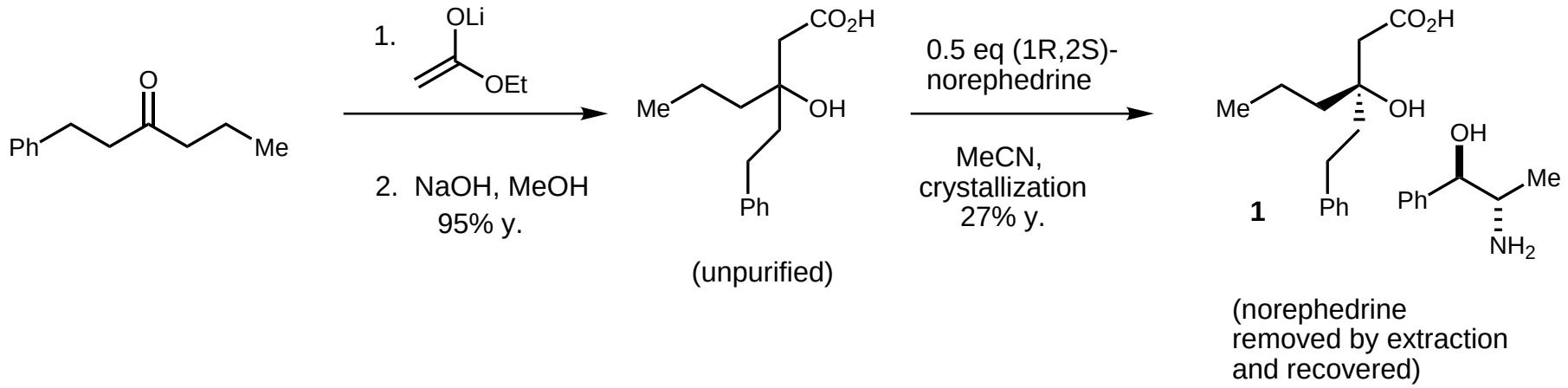


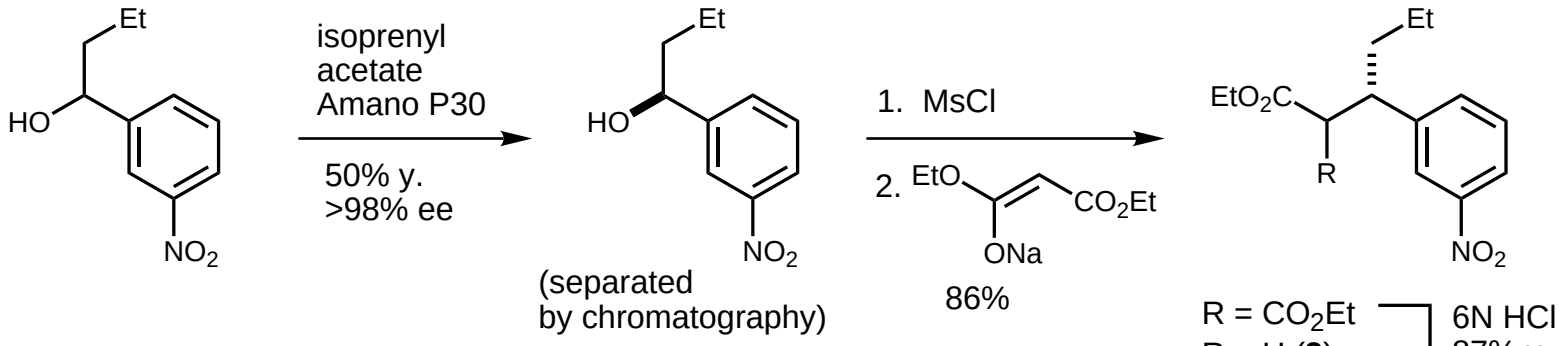
PNU-140690

9 steps, 24% overall y.

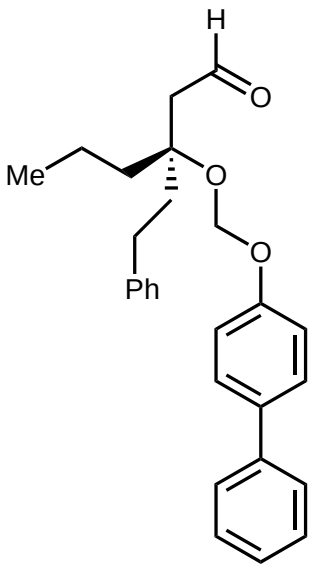
Gammill *J. Am. Chem. Soc.* **1997**, *119*, 3627-3628

Tapinavir (PNU-140690) Process Route



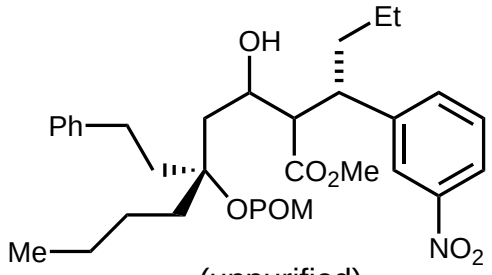


Tapranavir (PNU-140690) Process Route



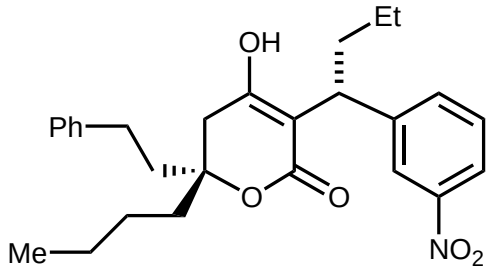
2, NaHMDS, THF, -80 °C

90% y.



(unpurified)

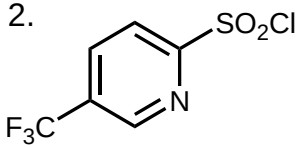
1. PCC, 99% y.
2. H_2SO_4, 84% y.
3. NaOH, MeOH, 75% y.



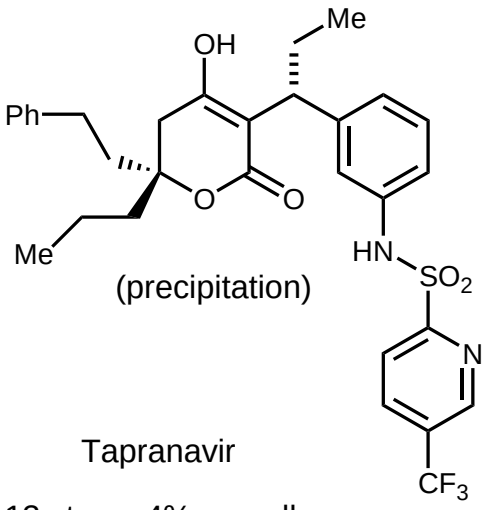
(recrystallized)

1. H_2, Pd/C, quant.

2.



pyr., CH_2Cl_2
78% y.



(precipitation)

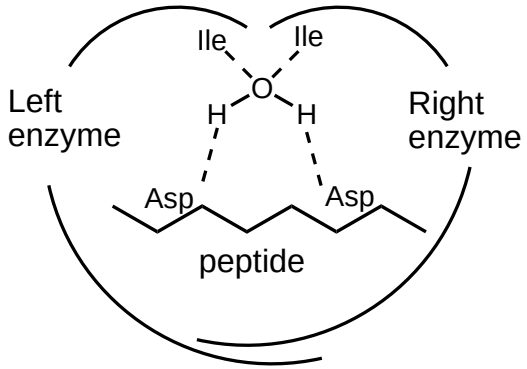
Tapranavir

13 steps, 4% overall y.

Gage *J. Org. Chem.* **1998**, *63*, 7356

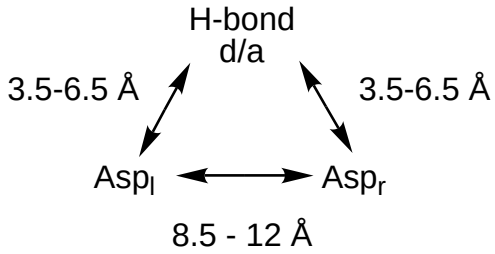
Rational Design of HIV Protease Inhibitor

Analysis of HIV protease
dimer-ligand X-ray structures

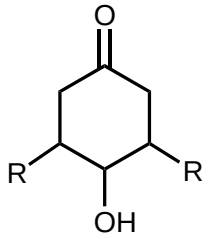


Results in

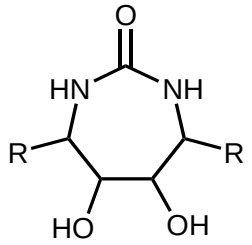
Pharmacophore model



3D-database
search



Lead structure
optimization
for increased
inhibitor binding



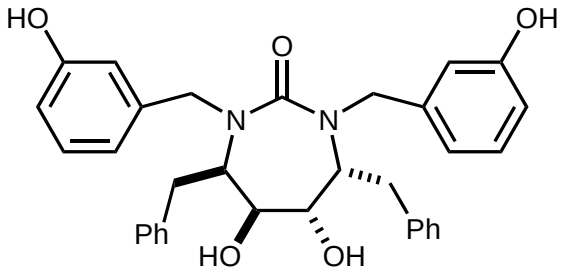
Dock analogs
of structure

into HIV protease
dimer X-ray structure

1. Set of likely inhibitors

2. Prediction of how stereocenters will affect binding, and thereby activity.

Biological
assays



IC_{50} = 36 nM

Lam *Science* **1994**, *263*, 380-384

Summary

1. Compared to native peptides, peptidomimetics and non-peptidomimetics offer significant advantages as drug candidates. The benefits include increased bioavailability, better transport through cellular membranes, decreased rate of excretion, and decreased hydrolysis by peptidases.

2. A tremendous variety of constrained amino acids have been developed to rigidify a particular amino acid or peptide sequence. Control over steric and electronic factors can be exercised through peptide backbone modificatons as well.

3. Failure of a mimetic to give the expected bioactivity is INCONCLUSIVE for determining if that conformational element exists in the receptor-bound conformation of the native peptide. For instance, if a β-turn mimetic shows no activity, the differences in side chain orientation, electronic factors, preferential binding to another target (*in vivo* systems), etc. may be the cause.

4. Biologically active mimetics support the existence of the desired element being present in the receptor-bound conformation of the peptide. However, the binding site of an antagonist may be different than active site, where the native peptide is bound.

5. Mimetics are usually designed based on analogy to the native peptide structure, then optimized to give the best possible pharmacokinetic properties.

6. The forefront of the field is the rational design of non-peptidomimetics using computer modeling, database searching, combinatorial chemistry and screening approaches, and analysis of X-ray structure data.

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