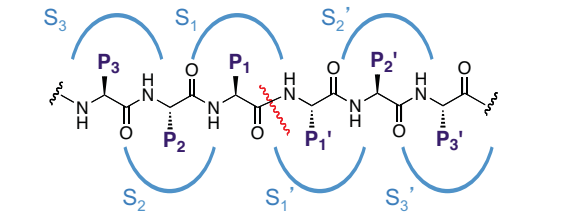
The Discovery of Telaprevir: A Direct-Acting Antiviral Inhibitor of the Hepatitis C NS3•4A Protease

IASOC 2012
24 September 2012 | John Maxwell, PhD
Vertex Pharmaceuticals, Inc.

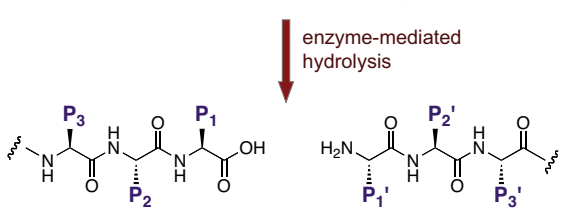
Protease Introduction

- Proteases (or proteinases or peptidases) are hydrolytic enzymes that catalyze the selective hydrolysis of peptide amide bonds
- Perform key role in generating and degrading bioactive proteins and peptides
- Widespread: Proteases represent ~2% of human genome
- Four major classes of protease:
  - Aspartic Protease
  - Metalloprotease
  - Cysteine Protease
  - Serine Protease
- Each class possesses mechanistic and structural characteristics
- Therapeutic targets in a wide variety of diseases

Proteases: hydrolytic enzymes that cleave peptide bonds

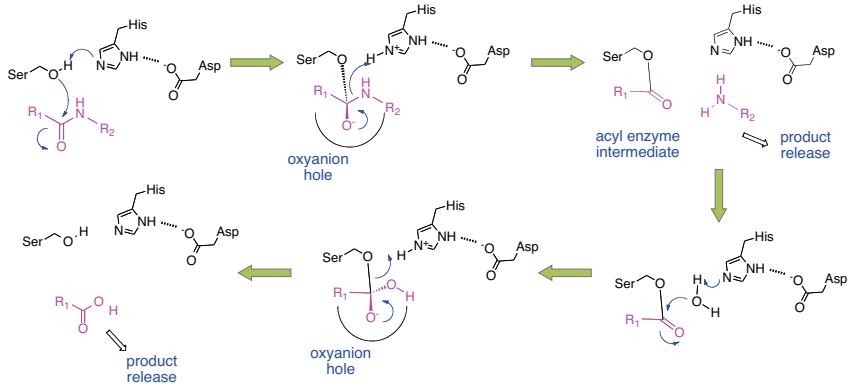


P - peptide position
S - enzyme subsite



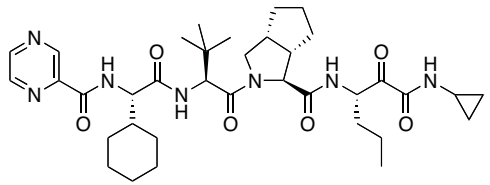
Examples
- pro-peptide hydrolysis
- active peptide degradation

Mechanism of Hydrolysis: Serine Protease



- characterized by Asp-His-Ser catalytic triad
- covalent acyl-enzyme mechanism
- inhibited by covalent and non-covalent means

Telaprevir: Direct-Acting Antiviral NS3•4A Protease Inhibitor for the Treatment of HCV Infection



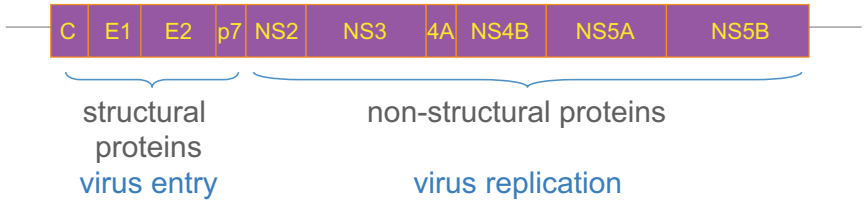
A reversible covalent serine protease inhibitor for the treatment of hepatitis C

- Hepatitis C background
- Discovery of telaprevir (medicinal chemistry)

Hepatitis C

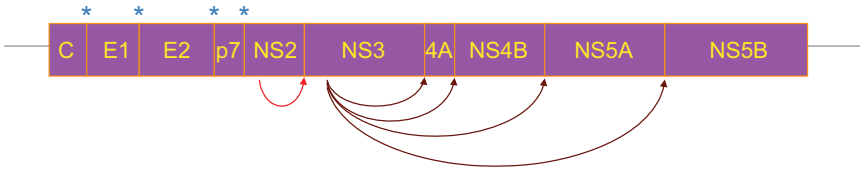
- ~ 200 million infected globally
- 2-3 million new infections per year
- Leading cause of chronic hepatitis, liver cirrhosis, liver cancer

Hepatitis C Virus: Structural Biology



- HCV is a member of the flaviviridae family
- RNA virus; 6 genotypes, multiple subtypes
- several, clinically validated targets
  - NS3/4A
  - NS5A
  - NS5B
- NS3 is a serine protease
- responsible for cleavage or precursor protein into active components

Hepatitis C Virus: Structural Biology



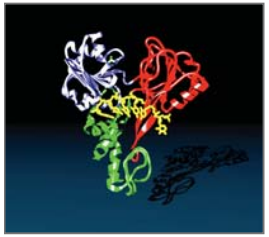
* host-mediated proteolysis
→ NS2 autoproteolysis
→ NS3 proteolysis

HCV Protease Inhibitor Discovery: Original Strategy

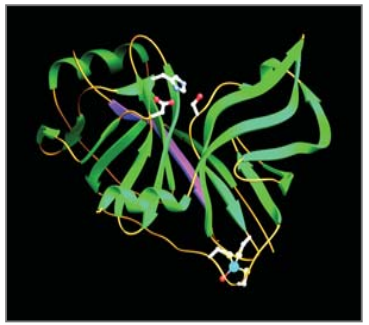
- ***Apply structure-based drug design***
- ***Apply mechanism-based inhibition***
- ***Target the liver***
  - HCV replication occurs in the liver
  - High liver concentration/replicon IC_{50} ratio

    $[C_{avg\ liver}] > 10x\ IC_{50}$

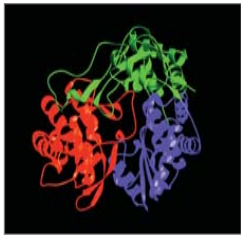
Hepatitis C: Viral Enzyme Structures



NS3 Helicase + RNA

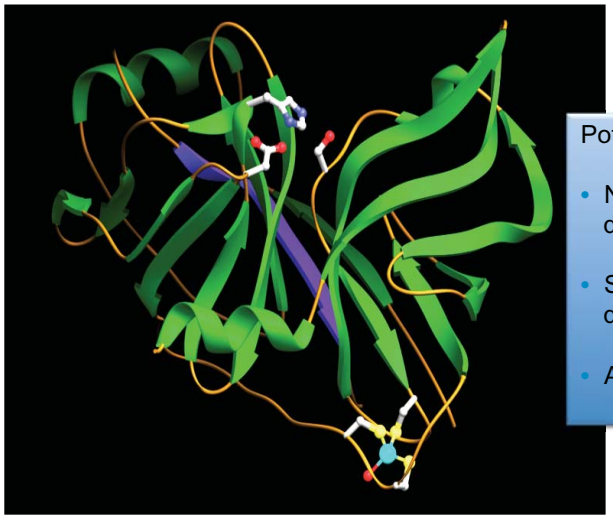


NS3•4A Protease



NS5B Polymerase

NS3•4A Protease Crystal Structure

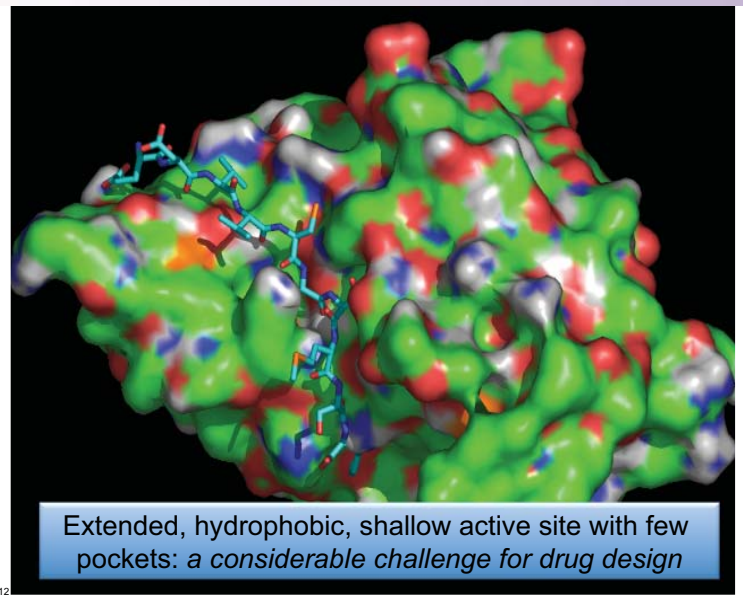


Potential for Inhibition

- NS4A cofactor displacement
- Structural zinc atom displacement
- Active Site Blockage

First X-ray crystal structure of the HCV NS3•4A protease:
Kim et al., Cell **1996**, *87*, 343-355.

The NS3•4A Protease Active Site

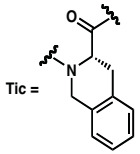


Extended, hydrophobic, shallow active site with few pockets: *a considerable challenge for drug design*

Truncation Studies: Challenges Highlighted

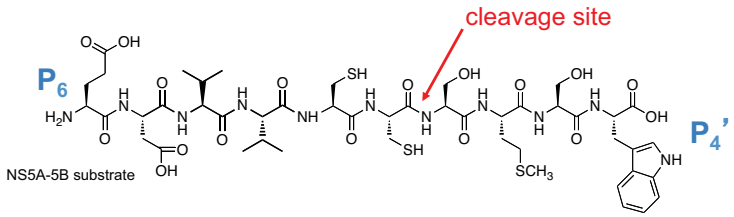
Peptide Sequence	Ki (μM)
P6……..P1 P1'……..P4'	
H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	0.34
H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-OH	27
H-Glu-Asp-Val-Val-Leu-Cys-Tic-Nle-OH	17
H-Glu-Asp-Val-Val-Leu-Cys-Tic-OH	14
H-Asp-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	4.4
H-Val-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	79
H-Val-Leu-Cys-Tic-Nle-Ser-Tyr-OH	500
H-Leu-Cys-Tic-Nle-Ser-Tyr-OH	2000

Tic =



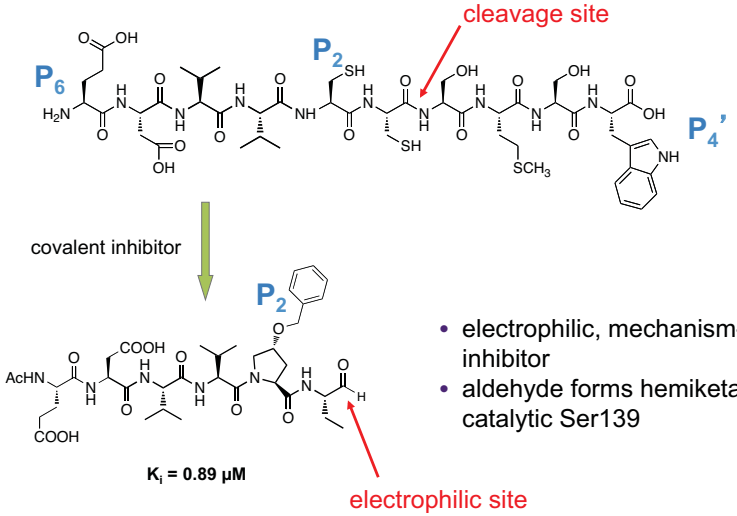
- Significant protease tolerability and pocket flexibility deduced from model studies
- Charged residues at P5 and P6 important

Starting Point: The Natural Substrate



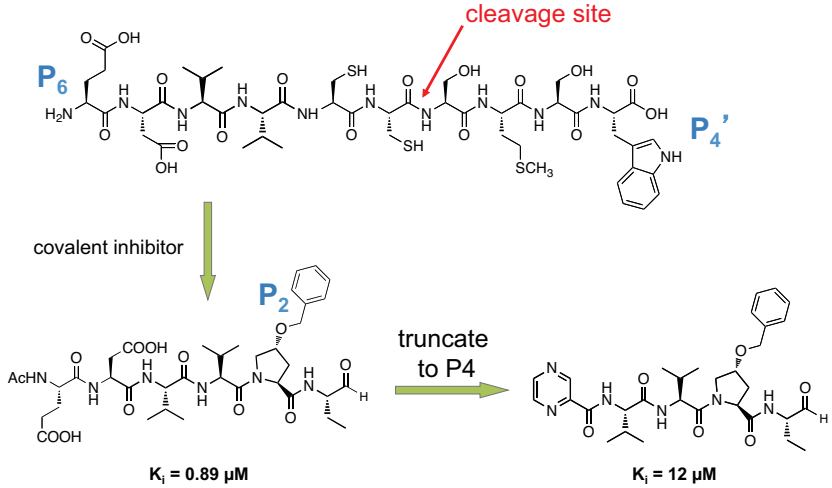
- Minimally active substrate sequence spans 10 amino acid residues
- Cys recognized at P_1
- Long path from here to drug!

From the Natural Substrate to P1-P4 Aldehyde Inhibitor

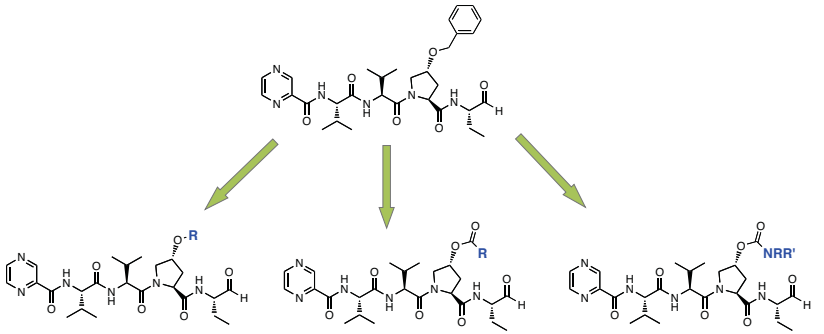


- electrophilic, mechanism-based inhibitor
- aldehyde forms hemiketal with catalytic Ser139

From the Natural Substrate to P1-P4 Aldehyde Inhibitor

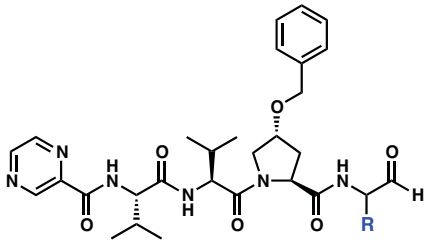


Early Optimization Focuses on P_2



R	K_i (μM)	R	K_i (μM)	R, R'	K_i (μM)
CH_2Ph	12 (L)	Ph	5.8 (D, L)	Ph, H (L)	22.3
CH_2-1-Naphthyl	2.9 (D, L)	1-Naphthyl	1.9 (D, L)	1-Naphthyl, H (L)	12.5
CH_2-2-Naphthyl	1.7 (D, L)	2-Naphthyl	0.4 (D, L)	 (D, L)	0.89

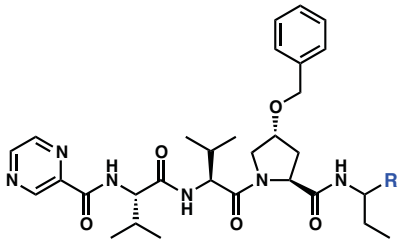
SAR at P_1: Small, Lipophilic Moieties Are Preferred



- S_1: specificity pocket
- Provides selectivity over the clotting cascade enzymes
- Consensus sequence for all substrates includes a cysteine residue at P_1, not compatible with an electrophilic warhead

R	K_i (μM)
	12
	3.1
	3.8
	>50
	>50

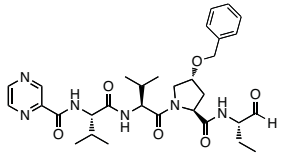
Can the Aldehyde Be Replaced?



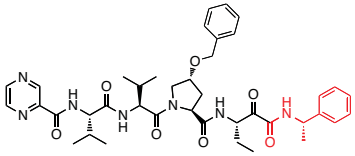
R	K_i (μM)
CHO	12
COOH	> 50
$COCF_3$	> 50
$COCH_2Cl$	> 50
$COCF_2CO_2C_2H_5$	> 50
$COCF_2CF_3$	18

Most conventional electrophiles do not work well

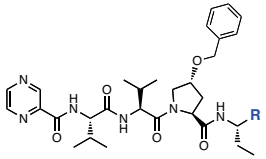
α-Ketoamides Showed Promise

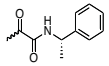
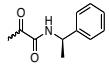


K_i = 12 μM

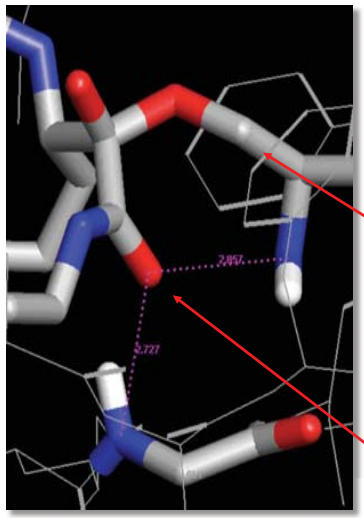


K_i = 0.92 μM



R	K_i (μM)
	0.9 (L)
	17 (L)
	32 (L)

Ketoamide Bidentate Binding Motif

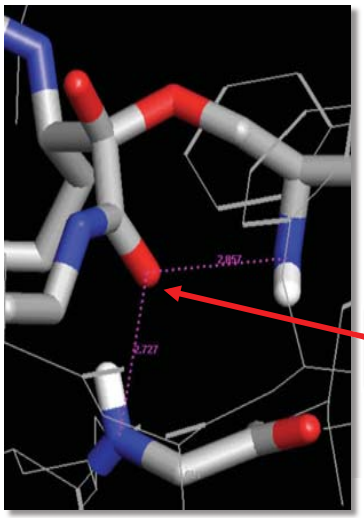


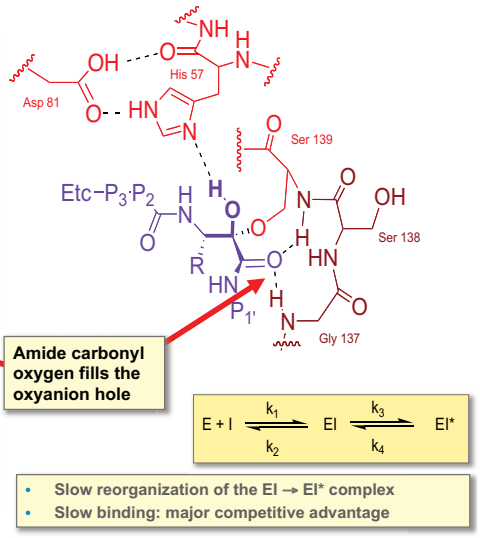
- Reversible covalent attachment of serine to keto-carbonyl carbon
- Amide carbonyl bound in oxyanion hole

Catalytic Serine

Amide Carbonyl

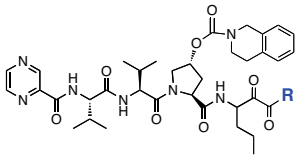
Ketoamide Bidentate Binding Motif





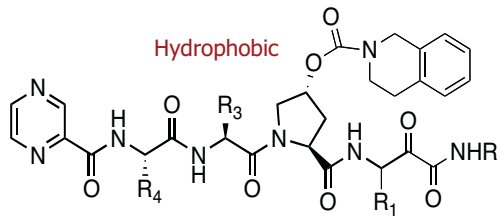
- Slow reorganization of the EI → EI* complex
- Slow binding: major competitive advantage

Ketoamides: Early Potential for Enzyme and Cell Activity



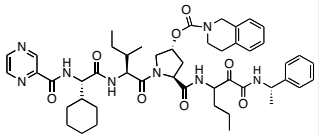
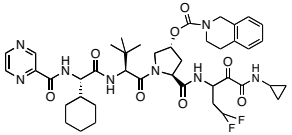
R	K_i (μM)	Replicon IC_{50} (μM)
	0.22	0.31
Ala	0.13	>10
Phe	0.026	>10
NH-CH_2Ph	0.22	3.2
NH-Cyclopropyl	0.42	2.3

Further Optimization at P_1, P_3 and P_4 Leads to Cell-Potent HCV Protease Inhibitors

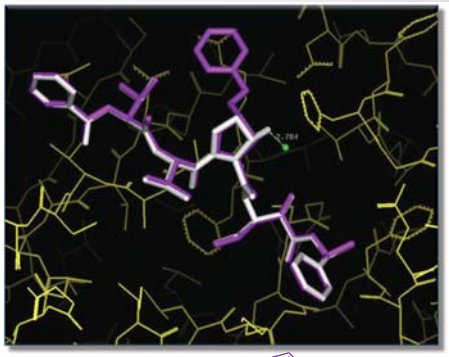


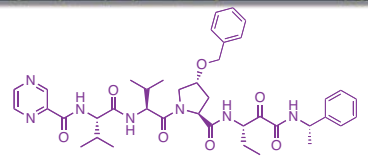
THIQ P_2 Displays Poor Liver Exposure in Mice

- Liver & plasma concentrations over 8h determined in mice following a single 50 mg/kg dose

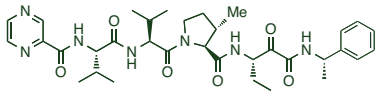
Structure	MW, cLogP	Replicon IC_{50} (μM)	C_{avg} Liver (μM)	C_{avg} Plasma (μM)
	879, 8.4	0.027	0.02	0.14
	837, 5.5	0.049	0.01	BDL

Smaller Proline-Based P_2 Affords Similar Enzyme Affinity





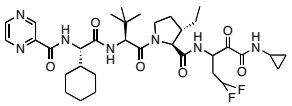
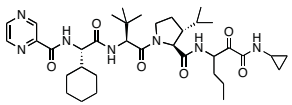
K_i = 0.92 μM



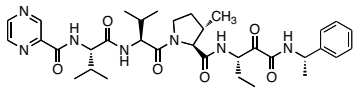
K_i = 1.4 μM

Smaller Proline-Based P_2 Trends Toward Better Exposure

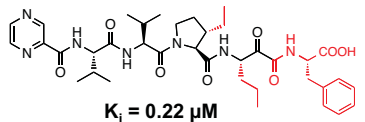
- Liver and plasma concentrations over 8h determined in mice following a single 50 mg/kg dose

Structure	MW, cLogP	Replicon IC_{50} (μM)	C_{avg} Liver (μM)	C_{avg} Plasma (μM)
	690, 4.1	0.37	0.38	0.67
	682, 5.9	0.91	**14.6**	**2.27**

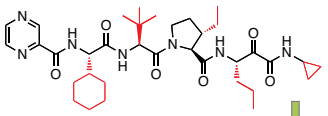
Optimization to Bicyclic P_2 Leads to Telaprevir



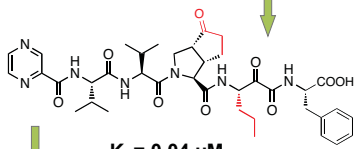
K_i = 1.4 μM



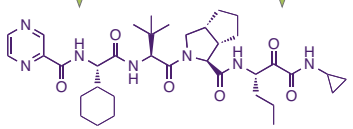
K_i = 0.22 μM



K_i = 0.15 μM



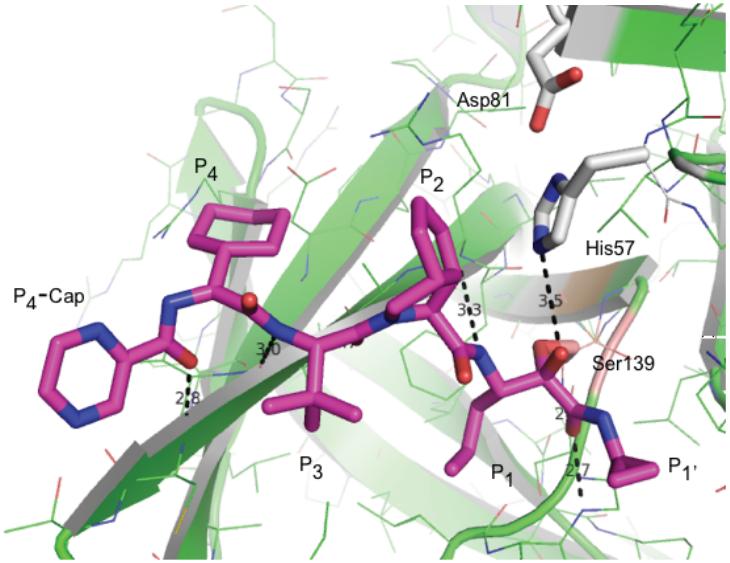
K_i = 0.04 μM



Telaprevir

K_i = 0.044 μM

Telaprevir – HCV Protease: Key Binding Interactions



Telaprevir: Pharmacokinetics in Preclinical Species

IV and PO PK

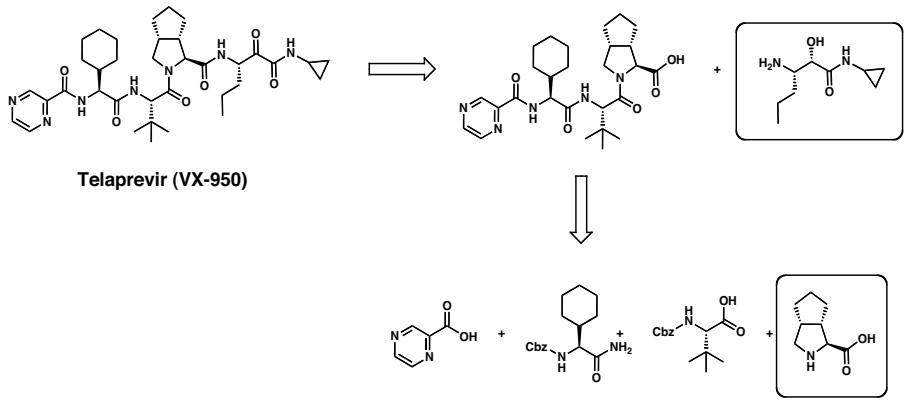
	Vss (L/kg)	$T_{1/2}$ (h)	Cl (mL/min/kg)	%F
Rat	5.81	1.7	54	25.0
Dog	1.84	0.9	42	41

Liver exposure

		C_{max} (μM)	C_{min} (μM)	AUC (μg*h/mL)	C_{max}/IC_{50}	C_{min}/IC_{50}
Rat	Plasma	0.49	0.04	2.23	0.79	0.11
	Liver	19.9	3.30	78.5	**27.7**	**9.30**
Dog	Plasma	1.35	0.13	3.62	1.69	0.36
	Liver	3.29	0.46	8.31	**3.90**	**1.30**

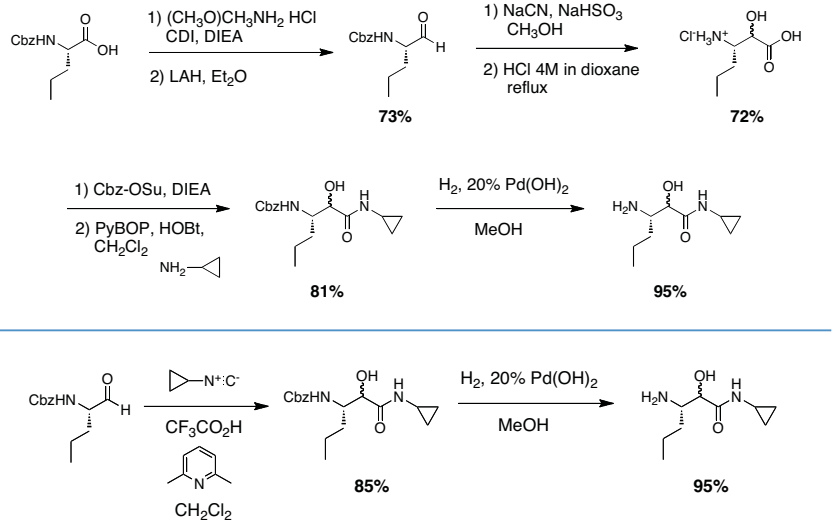
Single 30 mg/kg dose in propylene glycol

Telaprevir: Synthetic Building Blocks

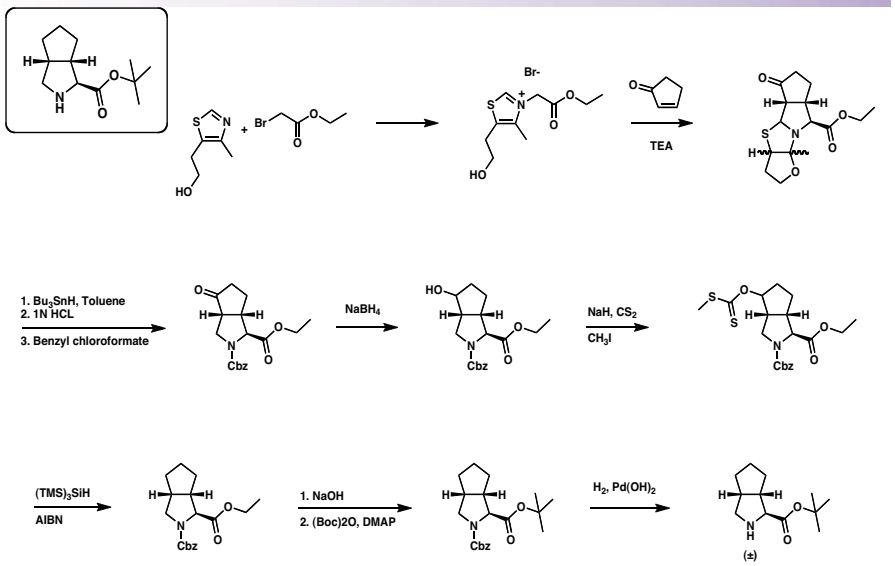


Telaprevir (VX-950)

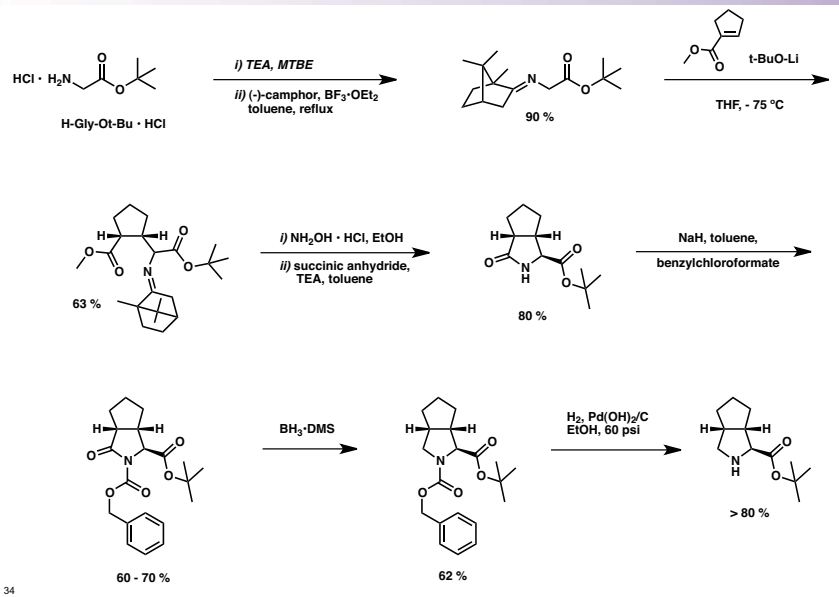
α-Hydroxyamide Synthesis



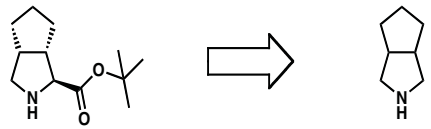
Bicycloproline P2 synthesis – 1st MedChem Route



P2 Synthesis via Camphor Auxiliary

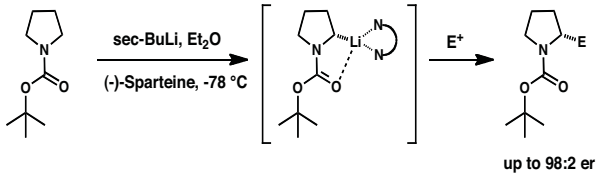


Synthesis of Telaprevir



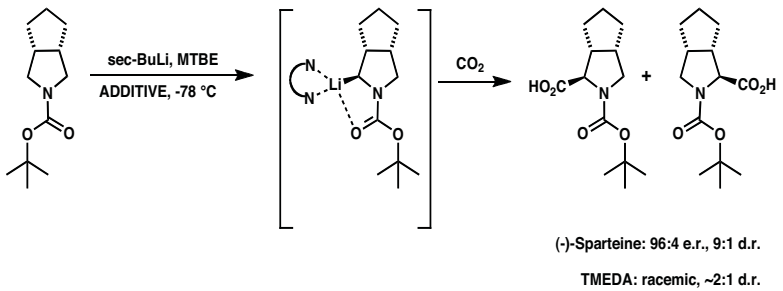
- Achiral starting material
- Requires an enantioselective synthetic step
- Or requires resolution

Enantioselective alpha-lithiation of pyrroldine



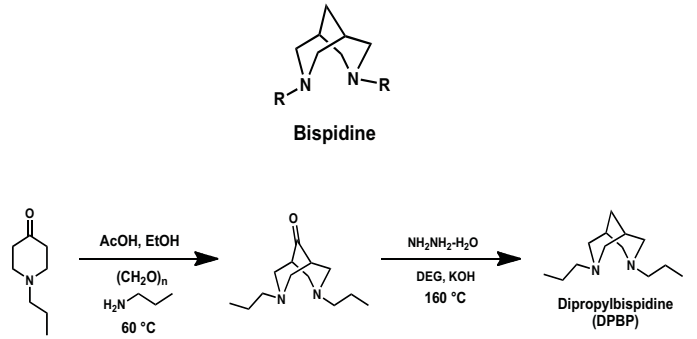
- E^+ = TMS, CO_2H, Ph_2COH, CH_3, etc.
- Kerrick, S. T.; Beak, P. *J. Am. Chem. Soc.* **1991**, *113*, 9708.
- Beak, P. et al *J. Am. Chem. Soc.* **1994**, *116*, 3231.

(-)-sparteine as ligand



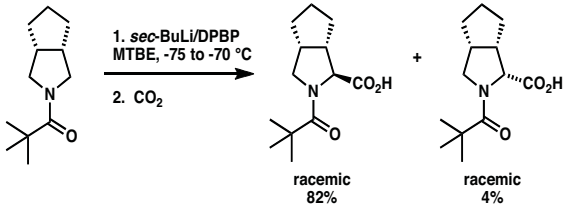
- Good enantioselectivity
- Good diastereoselectivity
- Wrong enantiomer

Development of an “achiral sparteine”



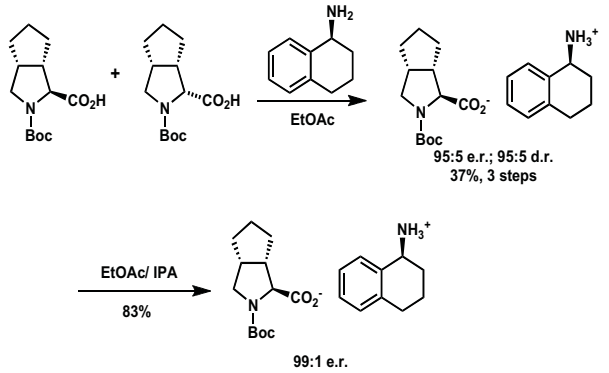
- DPBP: isolated by distillation
- 57% overall yield

DPBP performs well as ligand for lithiation

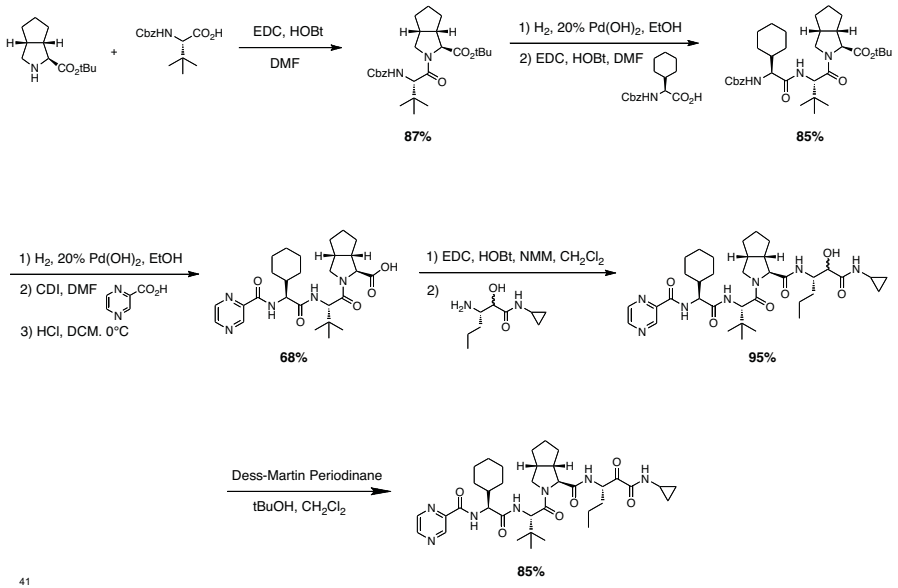


- DPBP recovered in 95% yield
- Recycled 5 times before re-purification
- Enantiomeric resolution
- Diastereomeric enrichment

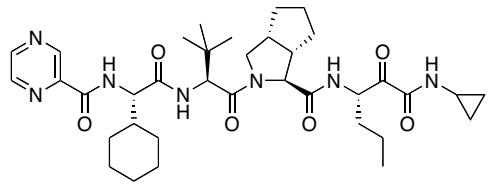
Final product resolved with chiral 1º amine



Synthesis of Telaprevir



Telaprevir: Direct-Acting Antiviral NS3•4A Protease Inhibitor for the Treatment of HCV Infection



Key Attributes

- Inhibits HCV protease (K_i* = 7nM, IC_{50} = 350nM)
- Advantageous enzymatic mechanism of action ($t_{1/2}$ ~1h)
- Exhibits potent and sustained anti-HCV activity *in vitro*
- Reduces liver damage in an animal model of HCV
- Orally bioavailable, good drug load, liver and plasma levels

Research Team Acknowledgements

Chemistry
- Robert Perni
- Shawn Britt
- Minzhang Chen
- Shu-Hui Chen (Lilly)
- Kevin Cottrell
- John Court
- Lawrence Courtney
- David Deininger
- Yong Dong
- Luc Farmer
- Raymond Forslund
- Jeremy Green
- Scott Harbeson
- Andy Jones
- Valdas Jurkauskis
- Rhonda Levin
- Young-Choon Moon
- John Munroe (Lilly)
- Ethan O' Malley
- Janos Pitlik
- Wayne Schairer
- Jerry Tanoury
- Mark Tebbe (Lilly)
- Roger Tung

Enzymology/ Biochemistry:
- Cynthia Gates
- Yu-Ping Luong
- James Landro
- Scott Raybuck
- John Thomson
- David Livingstone
- Edward Fox
- Stephen Chambers
- Maureen Dwyer
- May Wang (Lilly)

Virology / Cell Biology:
- John Alford
- Randal Byrn
- Joe Colacino (Lilly)
- Dan Frantz
- John Glass (Lilly)
- Tom Hoock
- Ann Kwong
- Chao Lin
- Kai Lin
- Carlos Lopez (Lilly)
- Sue Ma
- William Markland

PK, Formulations:
- Pravin Chaturvedi
- Caroline Decker
- Kirk Dinehart
- Gurudatt Chandorkar
- Elaine Kolaczkowski
- John Hickey
- Rick Panicucci
- Angie Heiser
- Stephanie Sweetana (Lilly)
- Nathan Yumibe (Lilly)

X-ray and Modeling:
- Joseph Kim
- Yunyi Wei
- Govinda Rao

Toxicology/ Clin. Pharm./Clinical:
- Edward Yau
- Enne Ette
- Chuck Braun (Lilly)
- John Pottage

Management
- Steve Lyons
- John Thomson
- Vicki Sato
- Gail Cassell (Lilly)

Project Planning
- Krista Evans
- Kyle Kelly
- Randy Miller
- Mary McGill-Maxwell
- Other contributors from both Vertex and Eli Lilly