Cathepsin K Inhibitor Development

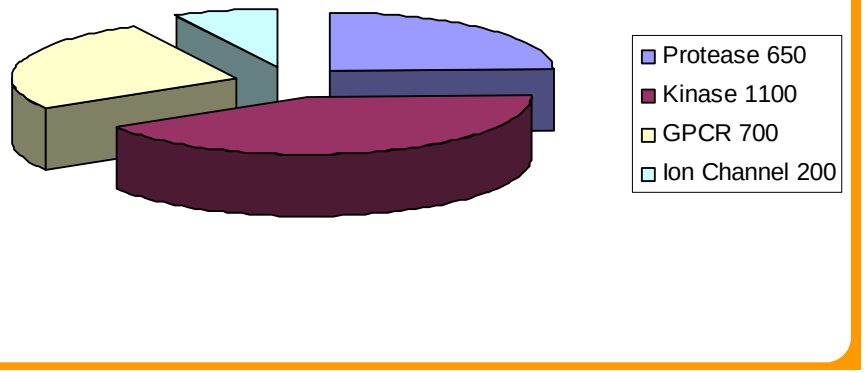
IASOC

September 16-21, 2006

Bertil Samuelsson

Medivir AB, Stockholm

The Drugable Human Genome



- **All marketed drugs addresses ~120 different targets**
- **The top 100 best-selling drugs are directed against only ~45 host proteins**
- **Very few new innovator targets are successfully developed each year**

Proteases:
- **Marketed drugs: ~40 from 7 classes**
- **In Clinical Trials: ~100 investigational drugs from 23 classes**

- The new "hot" target class

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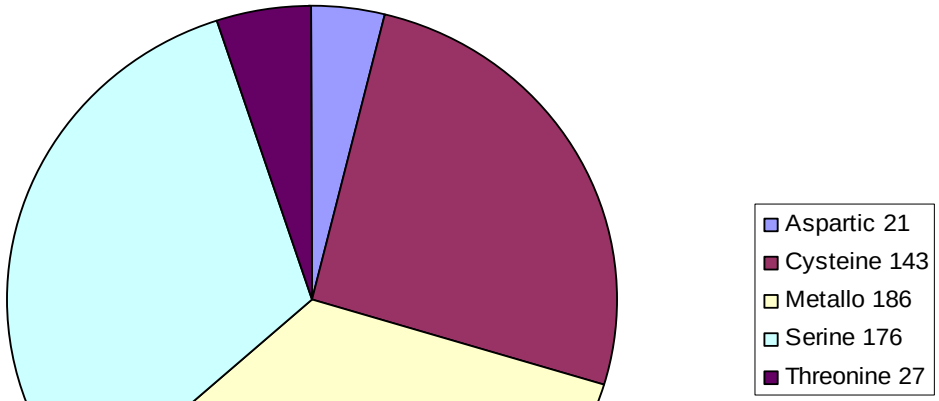
The Distribution of the Human Proteases

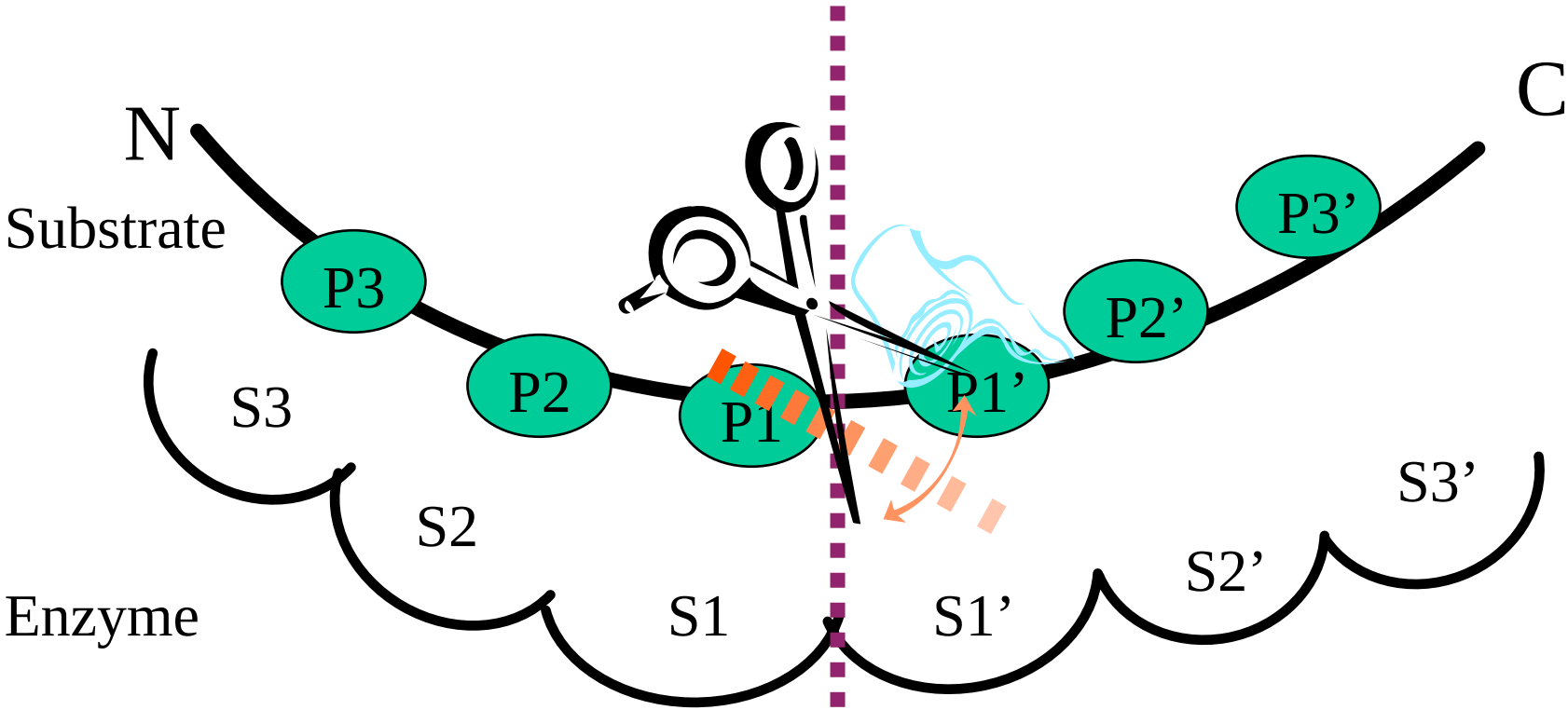


The serine, metallo and cysteine Classes are the largest

Puente *et al*, Nature Rev. Gen. **4**, 544-58, 2003

“Proteases at work”

Cleaves Bonds Between two Amino Acids – Activates or Inactivates Proteins



Schecter, I. and Berger, A., Biochem. Biophys. Res. Com., **1967**, 27, 157-62.

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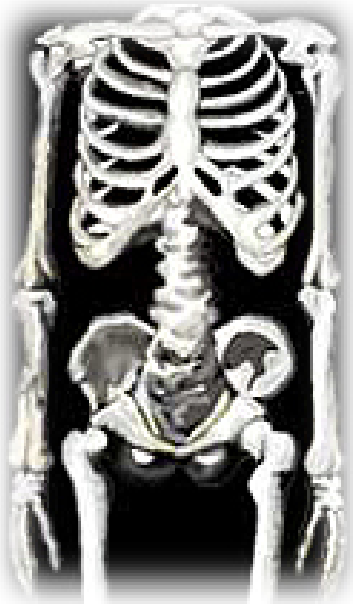
OSTEOPOROSIS

Cathepsin K Inhibitors –
Potential for Changing Treatment Paradigms



Many patients:

- Approx 100 million patients in major pharmaceutical markets have osteoporosis
- Incidence increasing
- Global market $11 billion by 2008

Bisphosponate issues:

- Irreversible inhibition of bone build-up
- Raising concern: Necrosis of the jaw

Cathepsin K:

- Selectively expressed in the osteoclasts
- Pivotal role in the degradation of bone matrix
- Attractive new mechanism of action
- Potential for bone forming activity and reduced fracture incidence
- Suitable for broad patient populations and both genders

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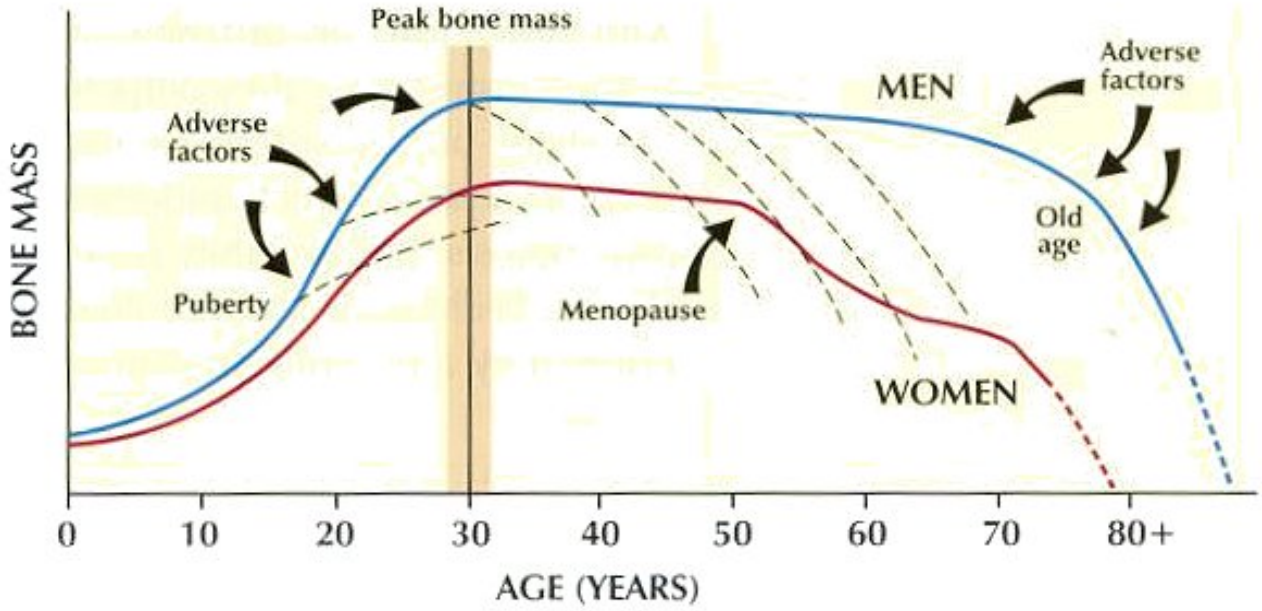
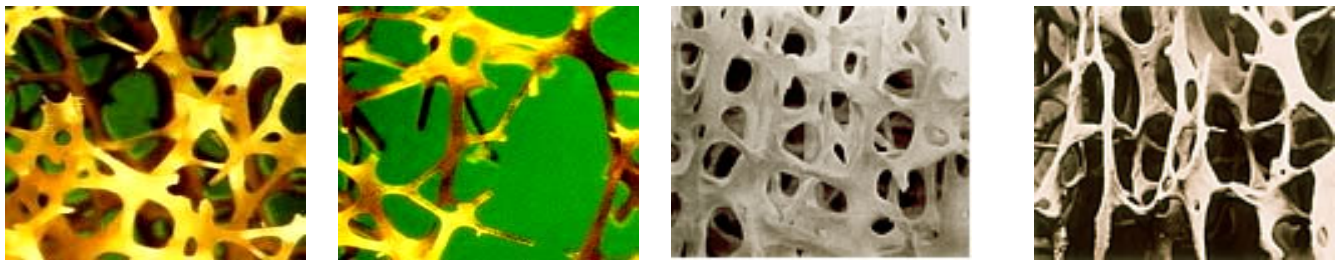
Osteoporosis





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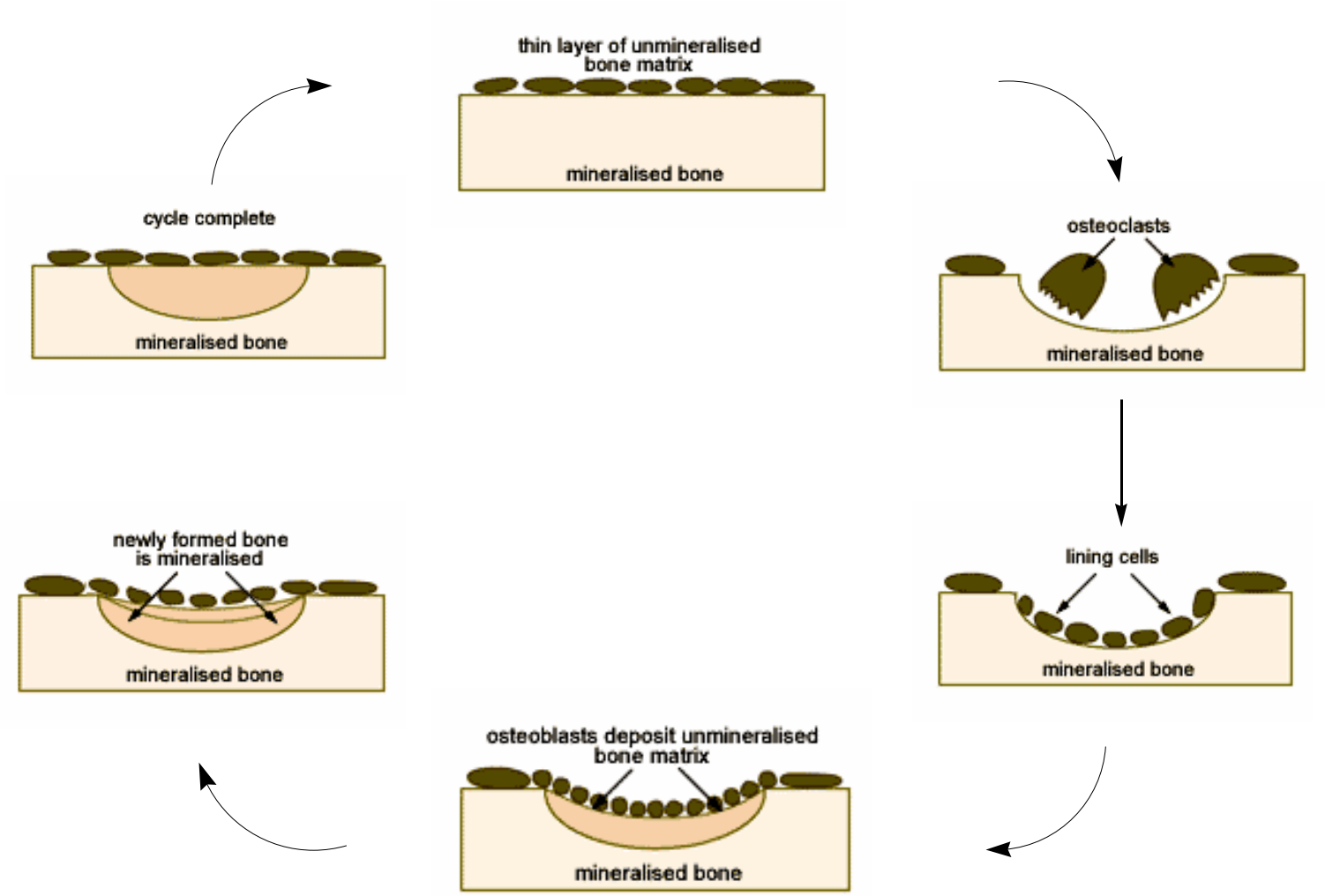
Bone Metabolism

- **Bone is a form of connective tissue**
  - type I collagen fibrils
  - hydroxyapatite crystals
- **Unceasing process of remodelling**
  - osteoblasts
  - osteoclasts
    - large multinucleated cells
    - originate from hemopoietic stem cells
    - precursor cells released into the bloodstream
    - collect at sites of bone resorption

Bone Remodelling Process



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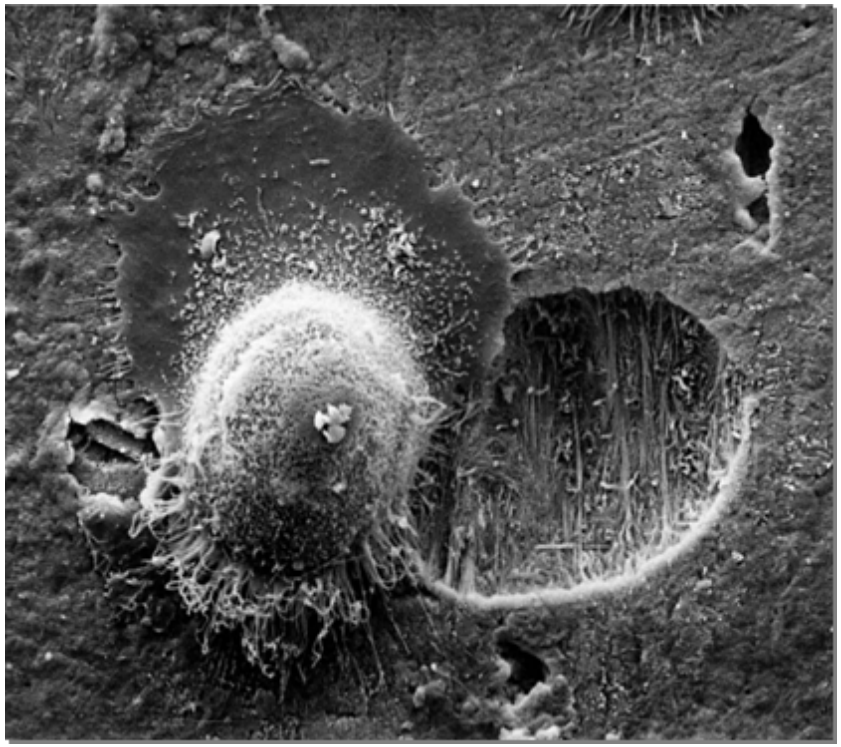
The Molecular Target: Cathepsin K

The osteoclast



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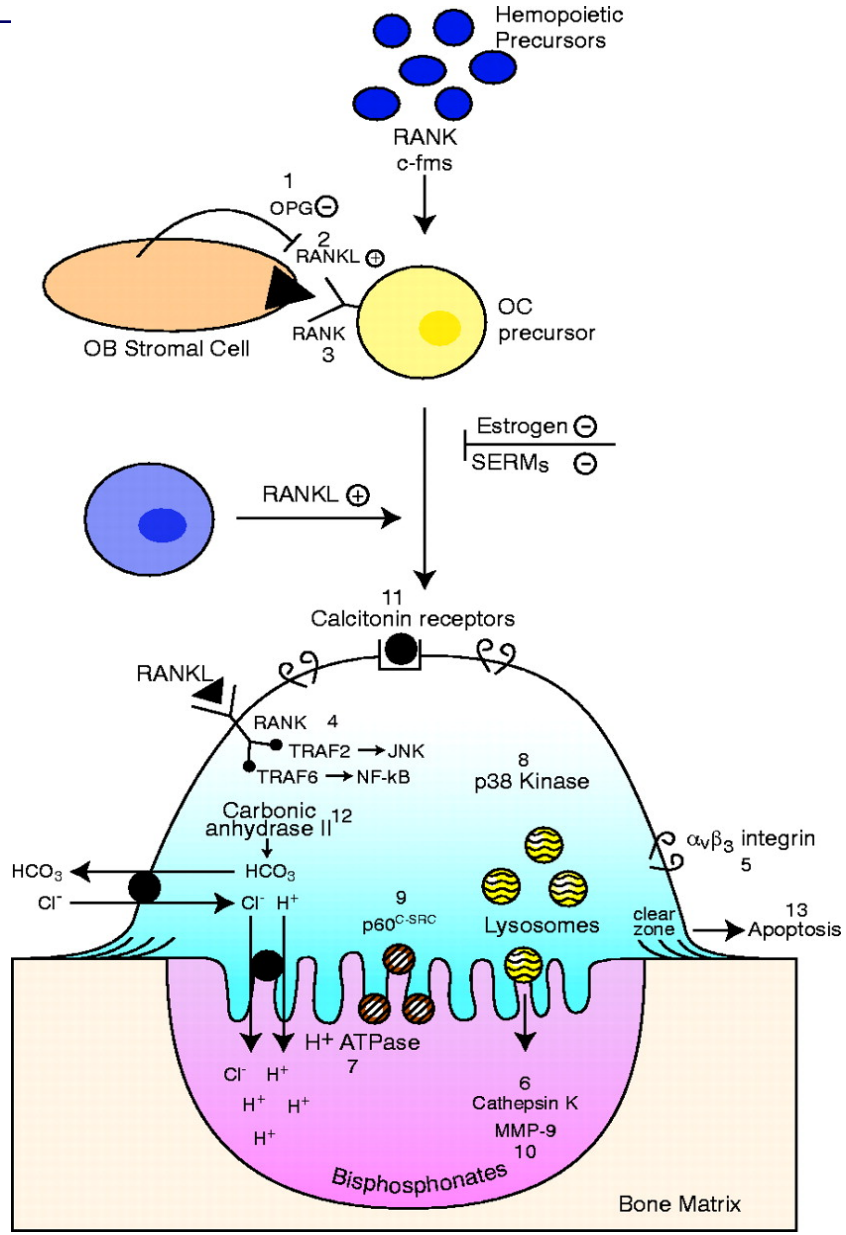

Cathepsin K

- Cysteinyl proteinase
- Papain superfamily
- 73 % Homology to cathepsin S
- 76 % Homology to cathepsin L
- Highly expressed during bone resorption
- Only known mammalian protease to solubilise collagen by cleavage of type I & II collagen
  - Within triple helix
  - In telopeptide region

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Role of Cathepsin K in Bone Remodelling

- Cathepsin K genetic deficiency leads to pycnodysostosis
- Antisense inhibition of cathepsin K mRNA
  - Blocks protein translation
  - Leads to dose dependent inhibition of osteoclast pit formation
- Cathepsin K knockout mice have been shown to develop osteopetrosis
- Animal studies have shown that inhibition of cathepsin K leads to a reduction in bone resorption in cynomolgus monkeys in a dose dependent manner

- Cathepsin K is a critical enzyme in bone resorption
- Cathepsin K inhibitors are developed to provide therapy for bone disorders such as osteoporosis

C1 Family – Selectivity is Important

Cathepsin	Tissue Expression	mouse phenotype
B	Ubiquitous	Reduced apoptosis
L	Ubiquitous	Defective CD4 selection, hair loss, increased mortality
V	Thymic	-
K	Osteoclasts	Pycnodysostosis
S	Antigen-presenting cells	Defective antigen presentation
H	Ubiquitous	-
W	CD8+ T cells	-
F	Macrophages, Ubiquitous	-
C	Myeloid cells, Ubiquitous	Hyperkeratosis, periodontitis
O	Ubiquitous	-
Z	Ubiquitous	-

?

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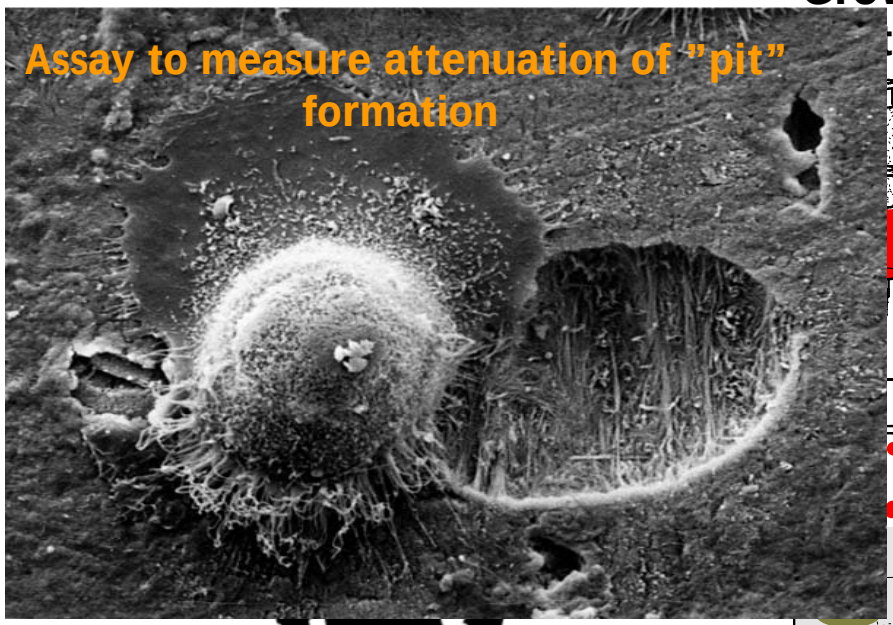
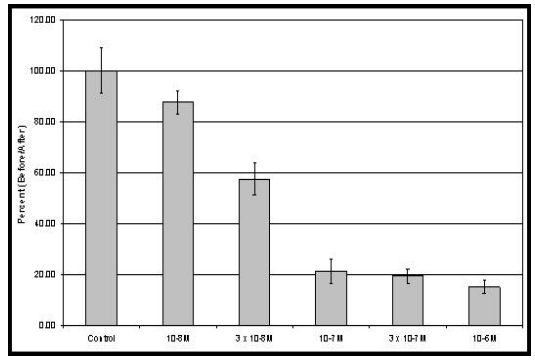
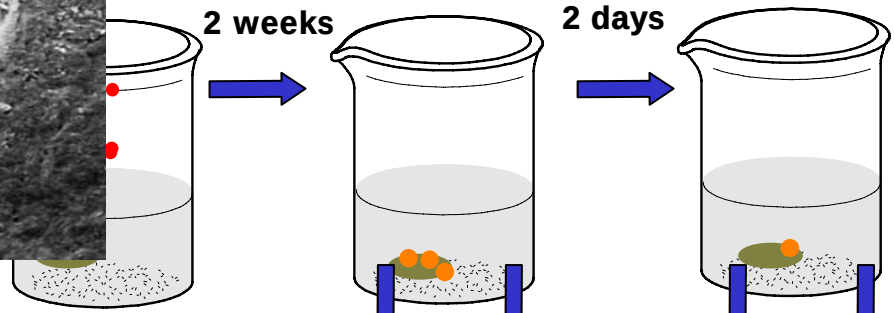
Development of a Human Osteoclast Resorption Assay - Key Cell-based test



Growth
tors

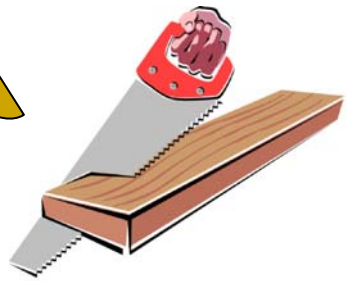
Inhibitor





CrossLaps Assay

CrossLaps Assay



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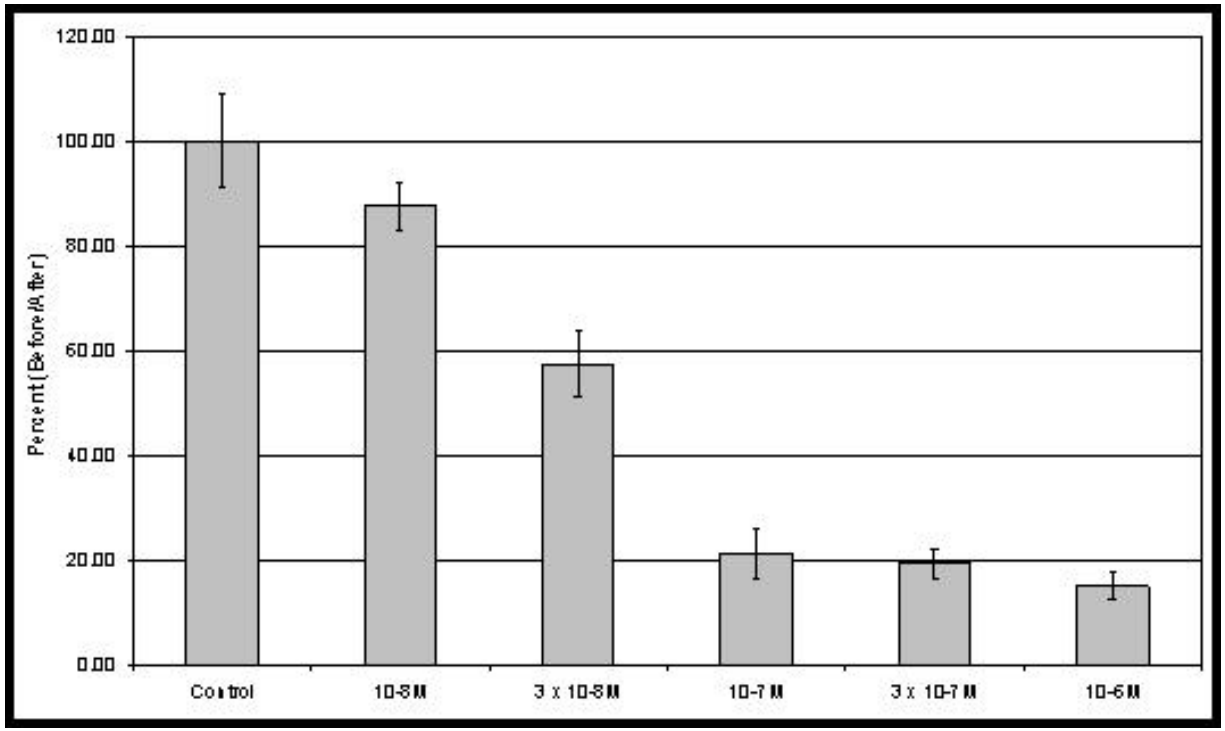
Human Osteoclast Assay



- In-house inhibitors exhibit excellent potency in the human osteoclast resorption assay,coupled with high potency in the isolated enzyme assay and selectivity against related enzymes

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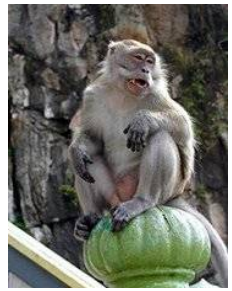
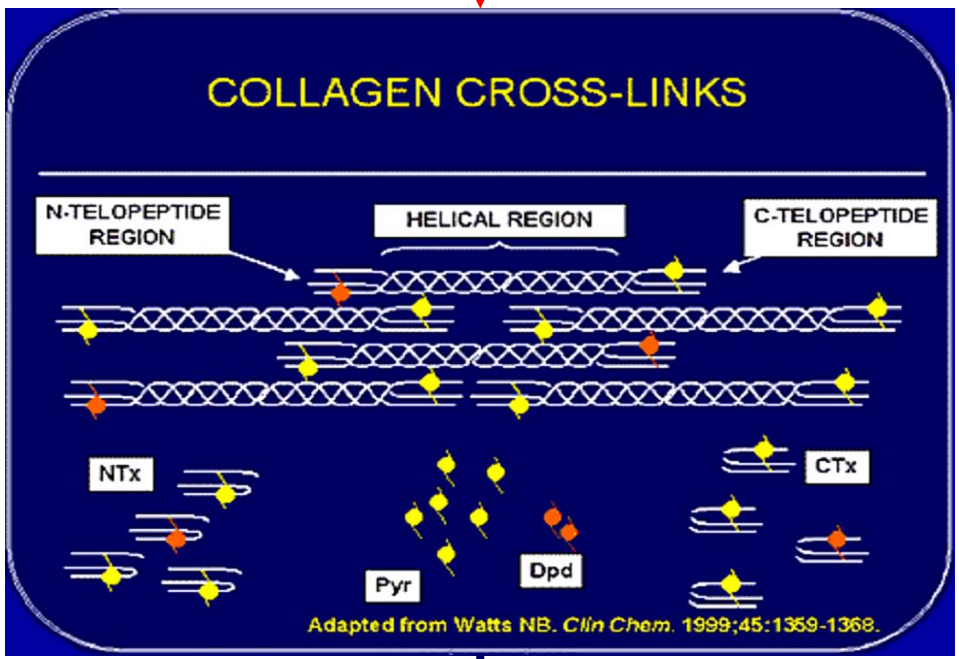
In vivo **efficacy can be Demonstrated in Cynomolgus:**
CTx is a bone resorption biomarker

Administer compound →



← Take blood sample



Monitor decrease in bone resorption

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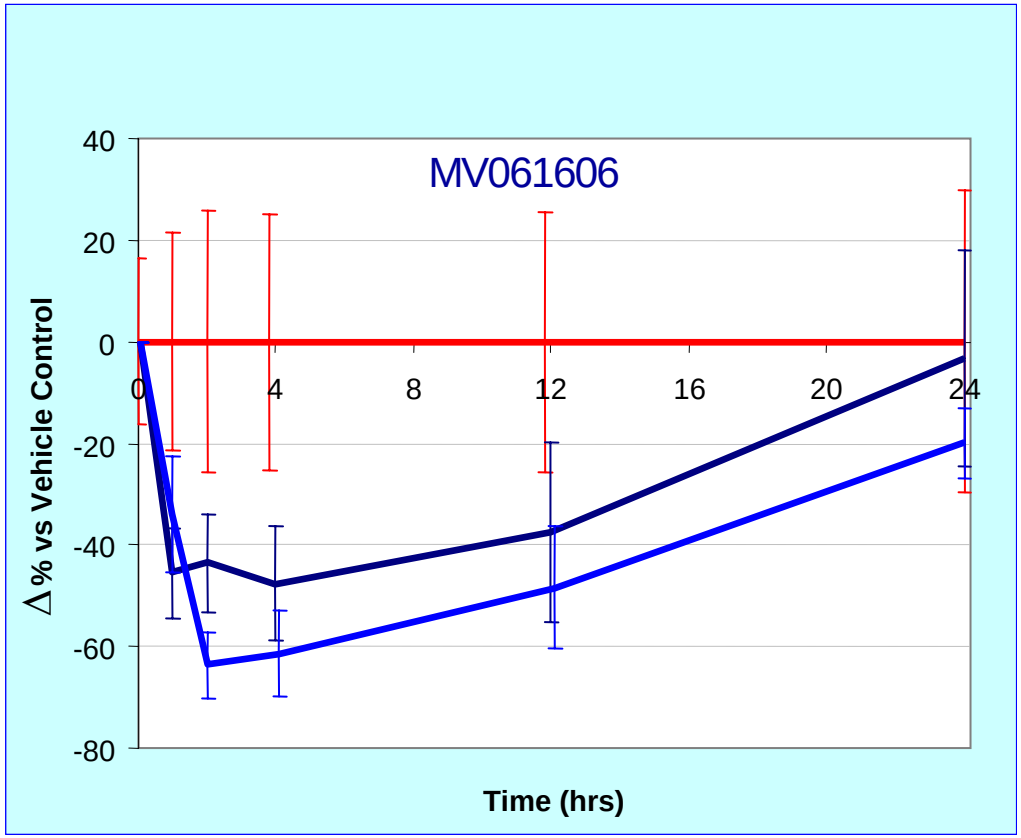
CTx Measurement in intact Monkeys

- Oral dosing in cynomolgus (5 mg/kg and 16 mg/kg)



In vivo efficacy demonstrated in monkeys based on biomarkers

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Evolution of the Cathepsin K Inhibitor Program at Medivir

- Novel leads provided by screening Medivir's compound libraries and RAPID™
- RAPiD & FOCUS
  - RAPiD generic library
    - >120,000 FRET compounds
    - Abz-B-C-D-E-Tyr(NO_2)-Asp-NH_2
    - Natural/non-natural amino acids, dipeptide mimetics
  - Substrate and inhibitor recognition sequence information obtained
  - Selectivity against other related enzymes
  - Initial SAR
- To date > 2500 inhibitors synthesised in-house providing advanced SAR
  - Fully reversible binding
- X-ray crystal structure information and SBDD have driven synthesis
- Early focus of *in vitro* and *in vivo* DMPK has ensured drug-like properties of the inhibitors developed

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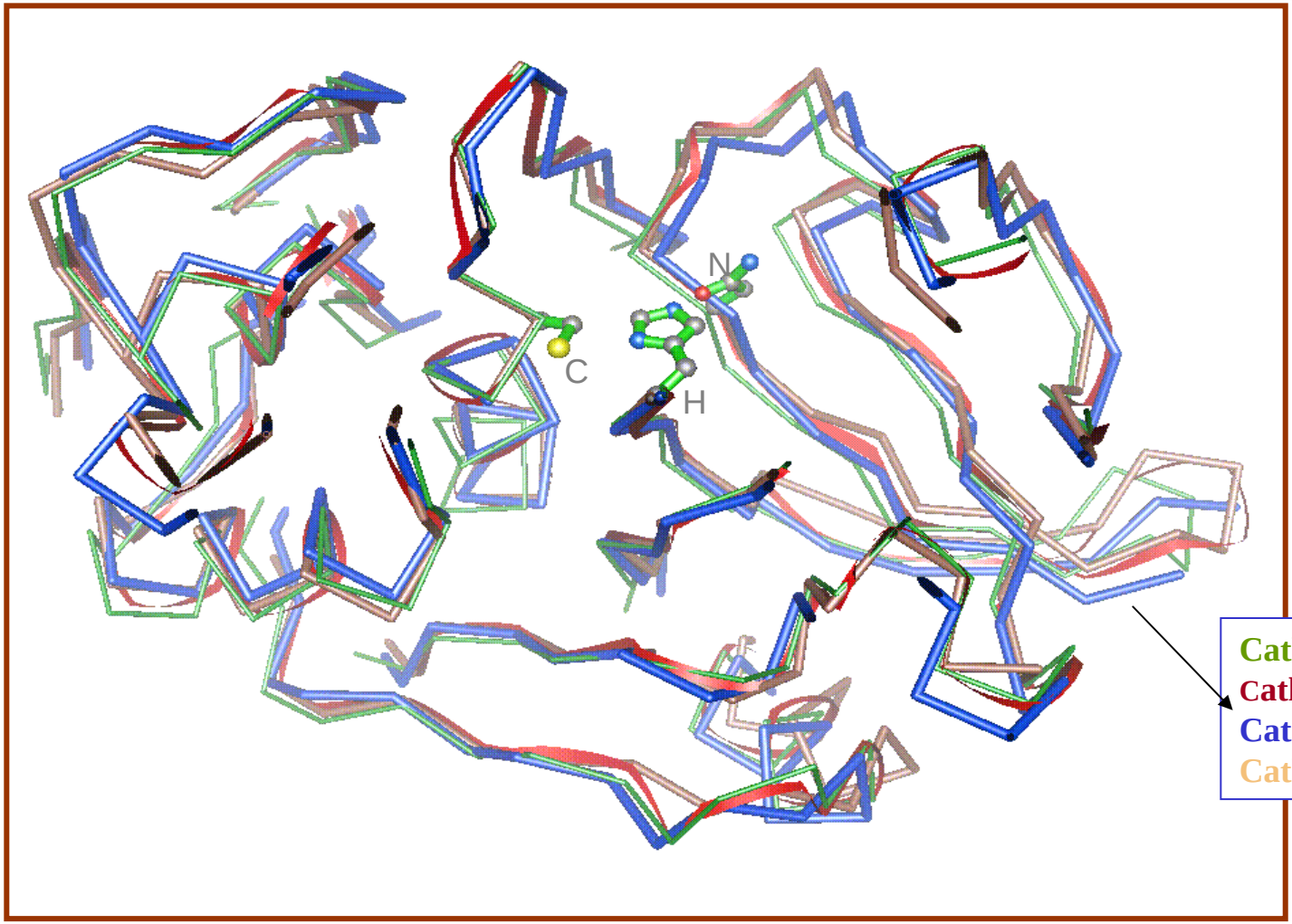
Comparison of catalytic domains



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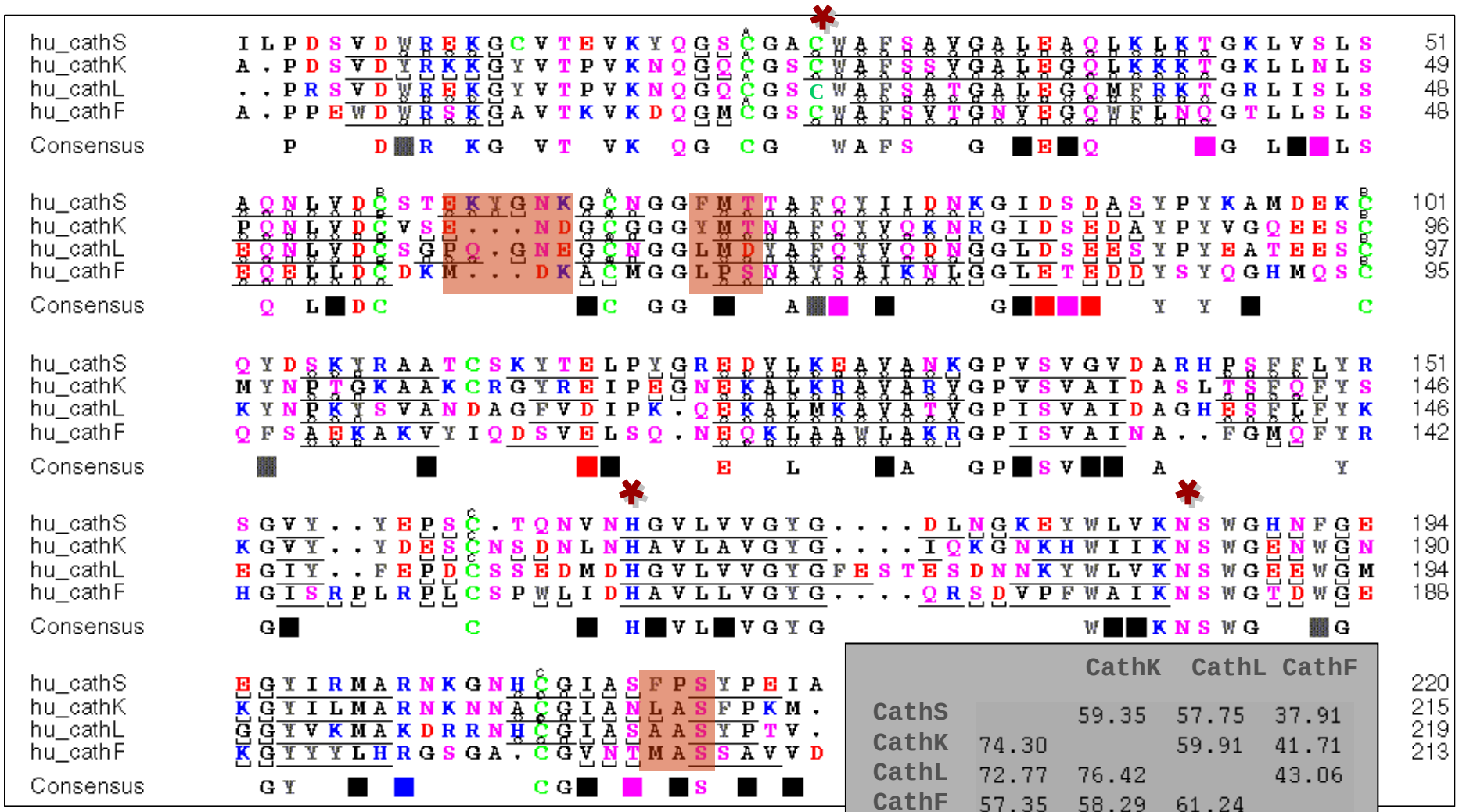
Comparison of the primary sequences Cathepsins S,K,L and F



	CathK	CathL	CathF
CathS	59.35	57.75	37.91
CathK	74.30	59.91	41.71
CathL	72.77	76.42	43.06
CathF	57.35	58.29	61.24

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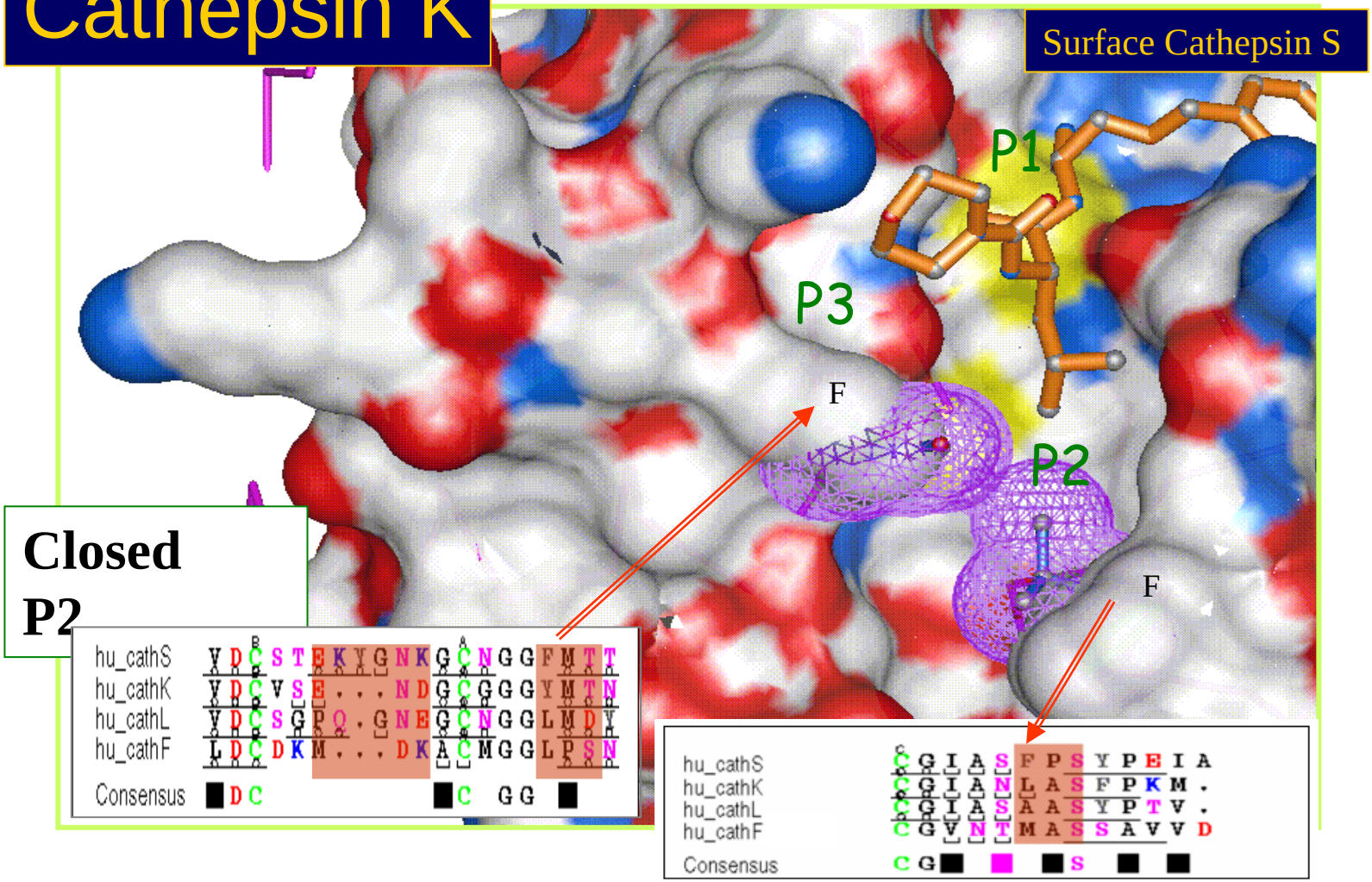
Cathepsins : basis for selectivity

Cathepsin K



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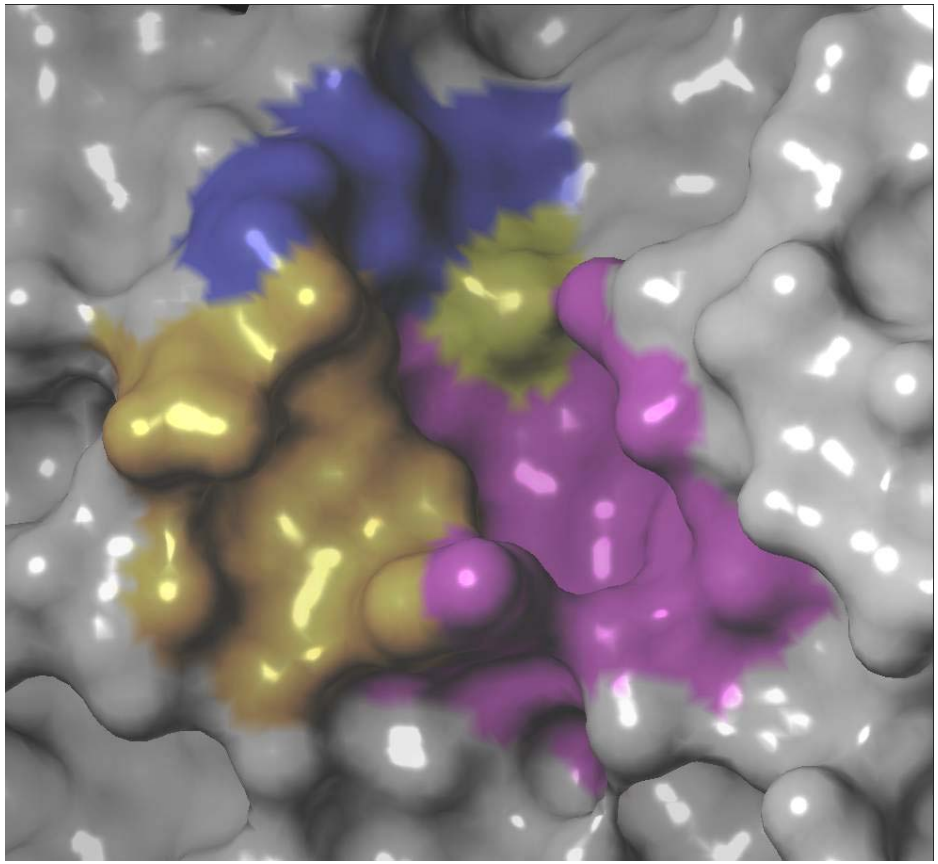
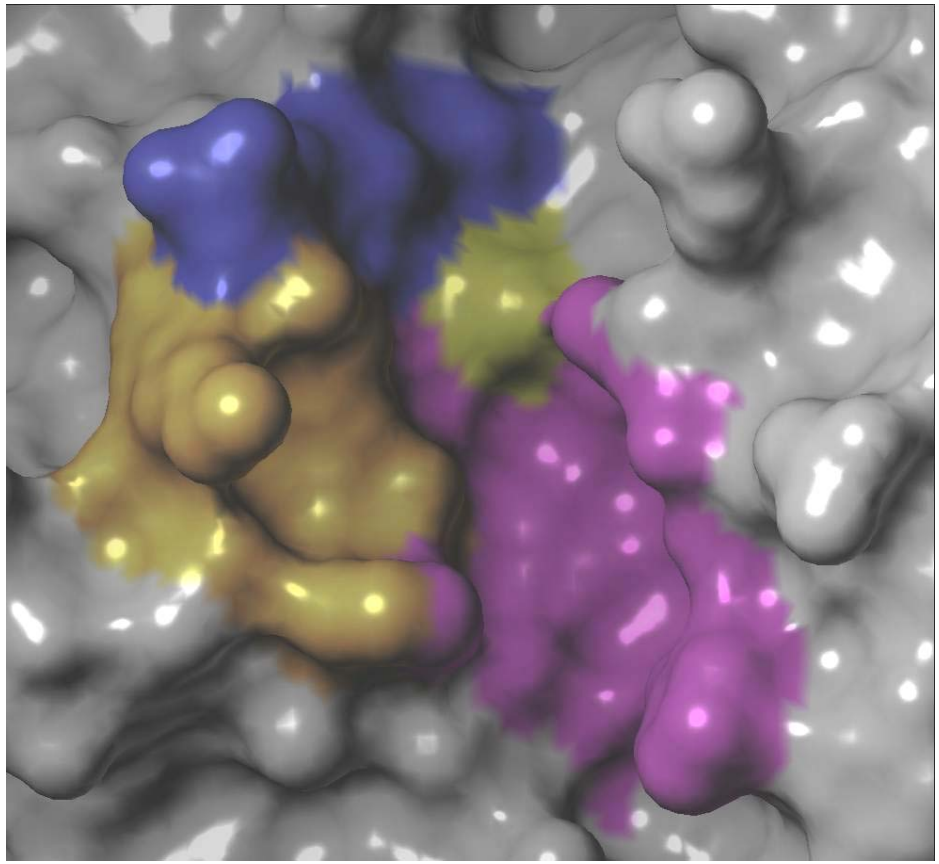
Visualization of Cathepsin K and S Active Sites





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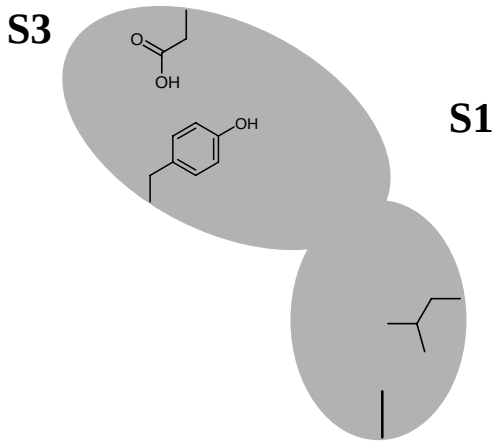
Cathepsin K Enzyme

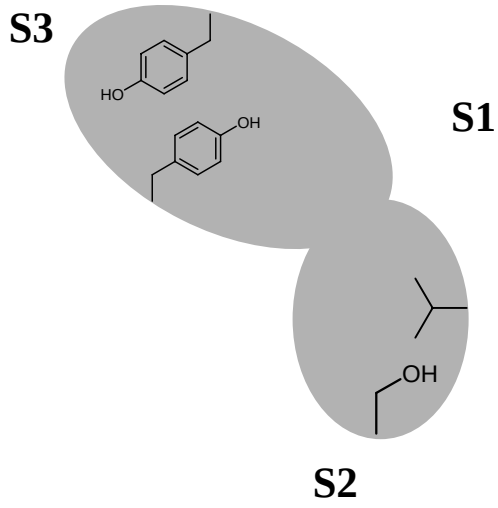
	S3	
Amino Acid Residue	61	67
Human	Asp	Tyr
Rat	Tyr	Tyr

	S2	
Amino Acid Residue	134	160
Human	Ala	Leu
Rat	Ser	Val

Human



Rat



- **Species differences need to factored in**

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Cathepsin K selectivity was factored in from the start

RAPiD combinatorial library and

FOCUS software provide SAR and selectivity data from protease pre-screens

Cathepsin S

Cathepsin K

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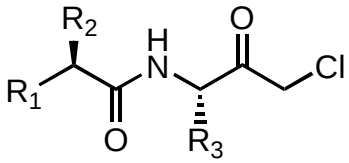
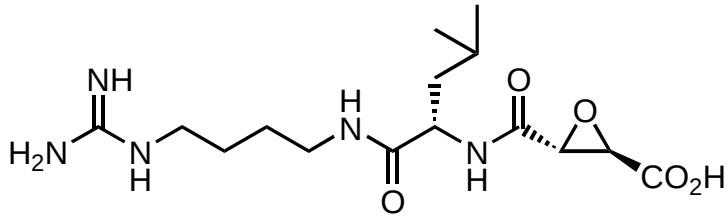
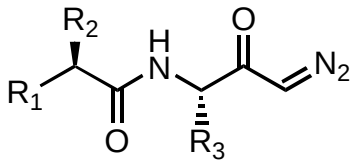
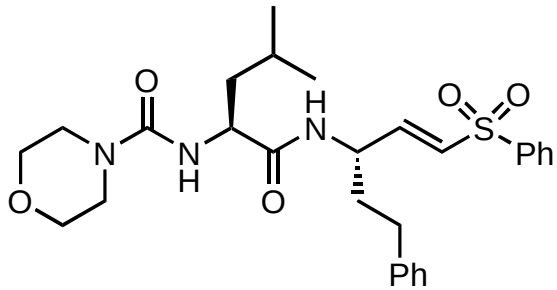
Exemplar Classical Cysteine Protease Inhibitors

Irreversible

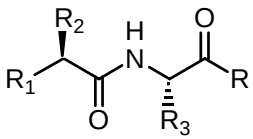


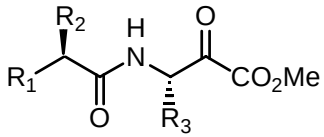


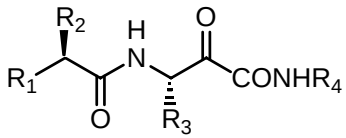


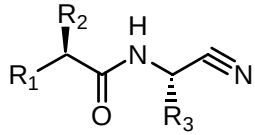


Reversible









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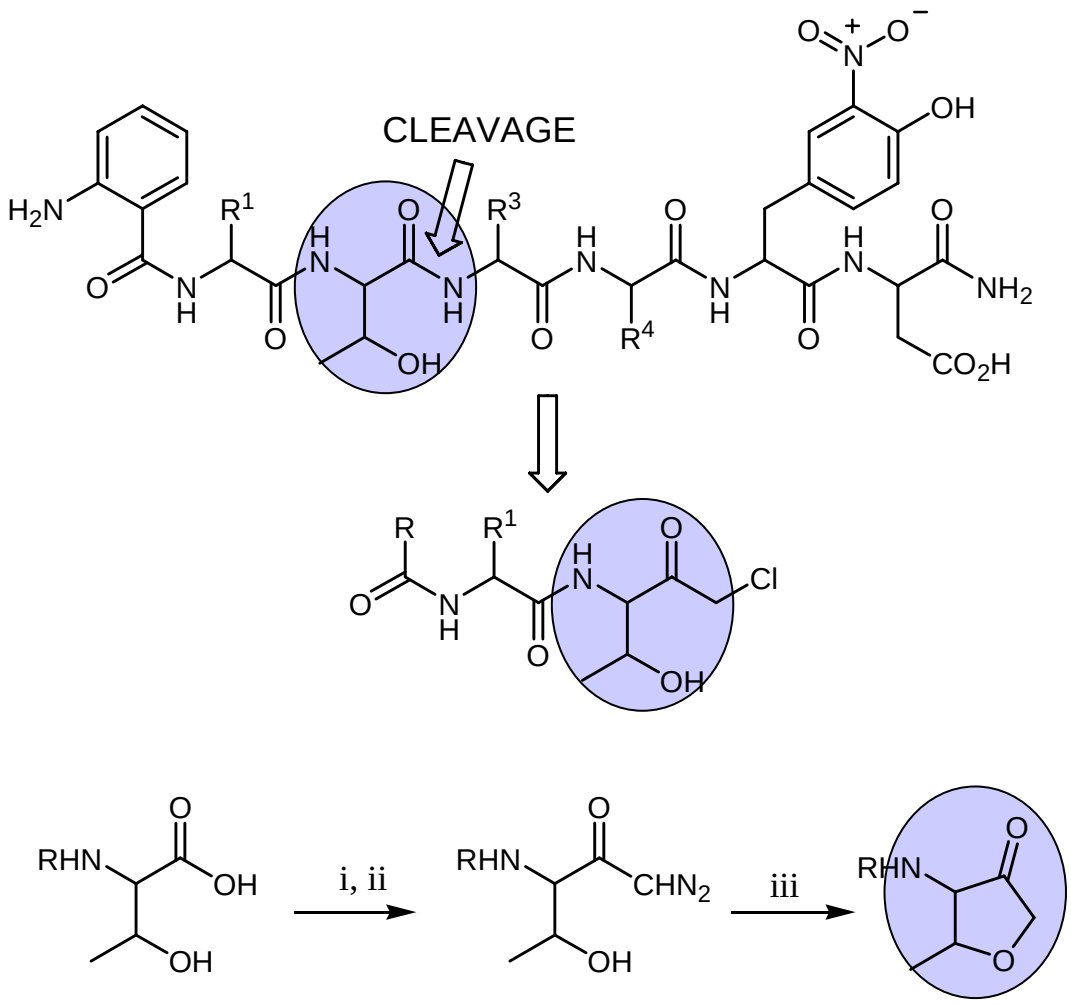
Cathepsin K Inhibitor Design



i. ^{i}BuOCOCl, NMM; ii. CH_2N_2; iii. LiCl in AcOH

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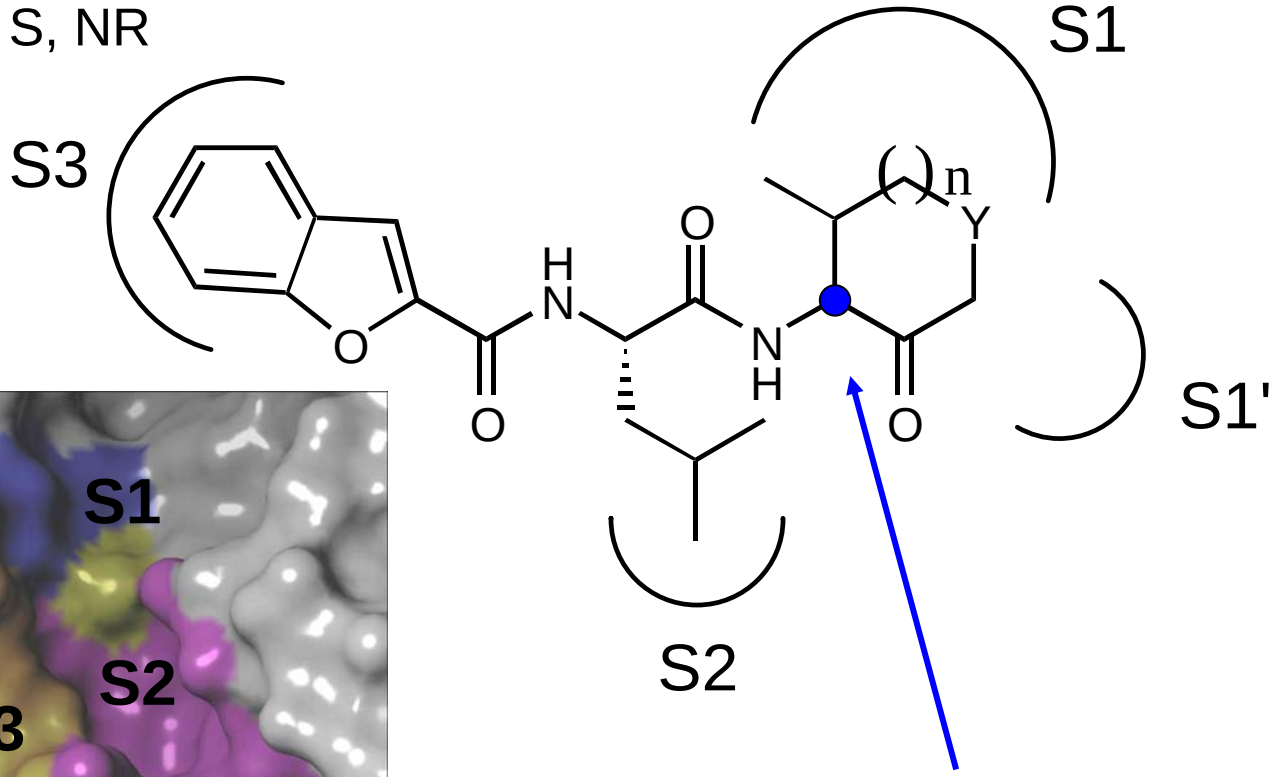
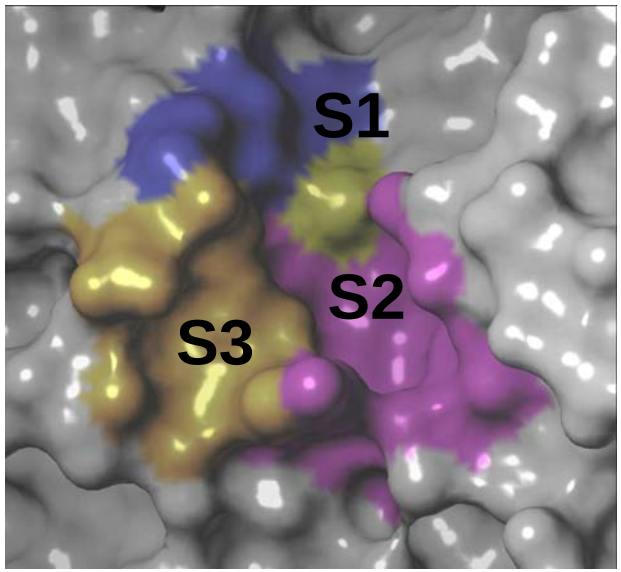
SAR Modifications

n = 0, 1, 2 or 3

Y = O, S, NR





Potential development issue: chirally labile position

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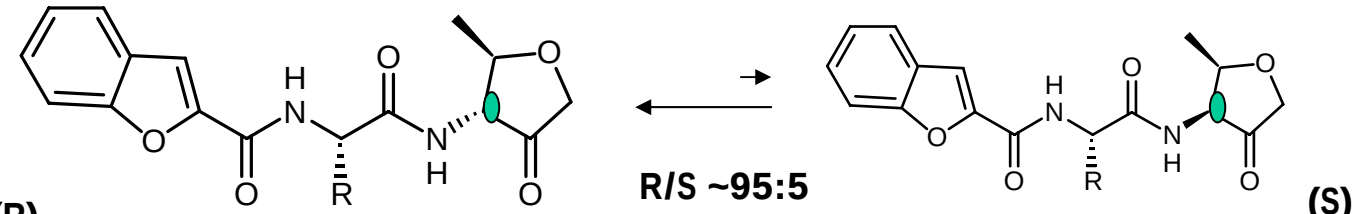
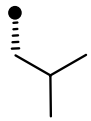
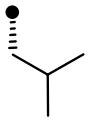
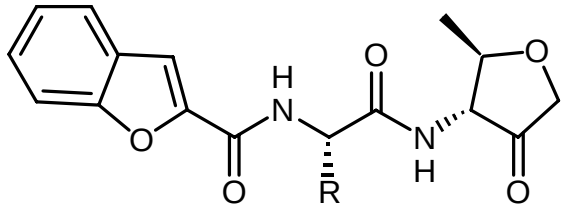
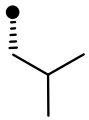
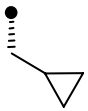
The (S)-Isomer is the More Active



	R =	Cath K (K_i μM)	Cath L (K_i μM)
(S)		0.004	0.52
(R)		0.020	0.75

P2 Modifications



R =	Cath K (K_i μM)	Cath L (K_i μM)
	0.3	> 100
	0.02	0.75
	2.12	9.41
	0.82	4.24

Small branched P1 groups preferred

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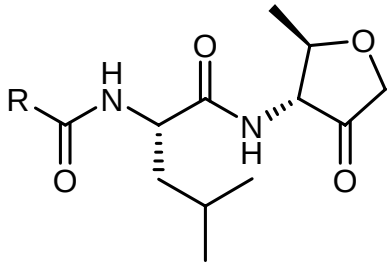
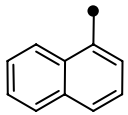
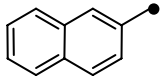
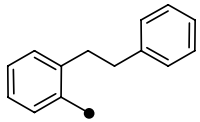
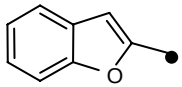
P3 Modifications



R =	Cath K (K_i μM)	Cath L (K_i μM)
	9.5	0.35
	0.13	0.67
	7.6	0.76
	0.02	0.75

The benzofuran P2 capping group provides good potency and selectivity

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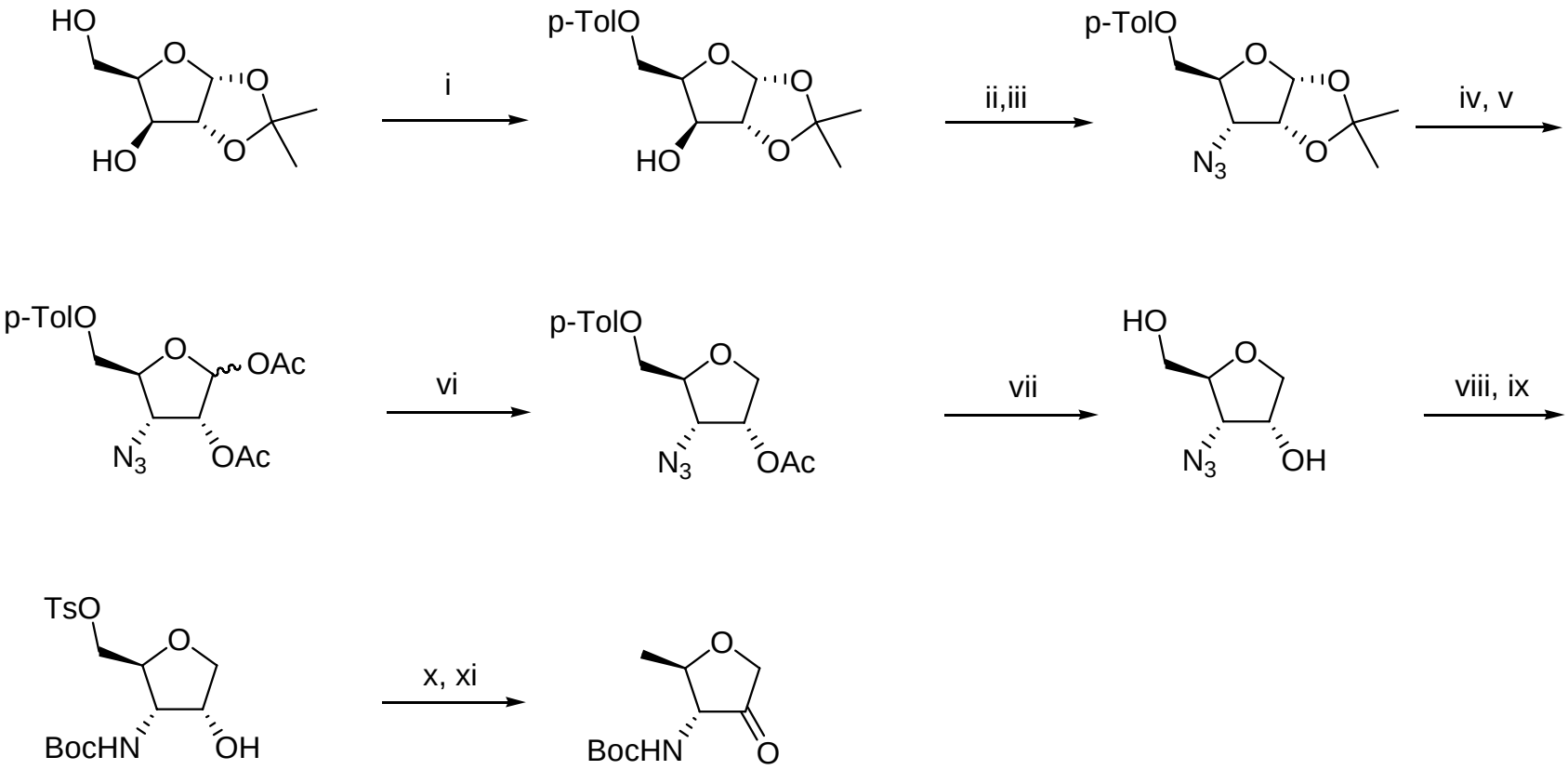
Synthesis of the P1 Furanone Template



i. *p*-TolCl, pyridine, 72 % yield; ii. Tf_2O, pyridine, ; iii. NaN_3, 30 % yield over two steps; iv. 75% HCO_2H; v. Ac_2O, pyridine, 74 % yield over two steps; vi. TMSOTf, Et_3SiH, 50 % yield; vii. K_2CO_3, MeOH, 80 % yield; viii. H_2/Pd/C, Boc_2O, ix. TsCl, pyridine, 95 % over two steps; x. $LiAlH_4$; xi. Dess Martin Periodinane, 70 % yield over two steps

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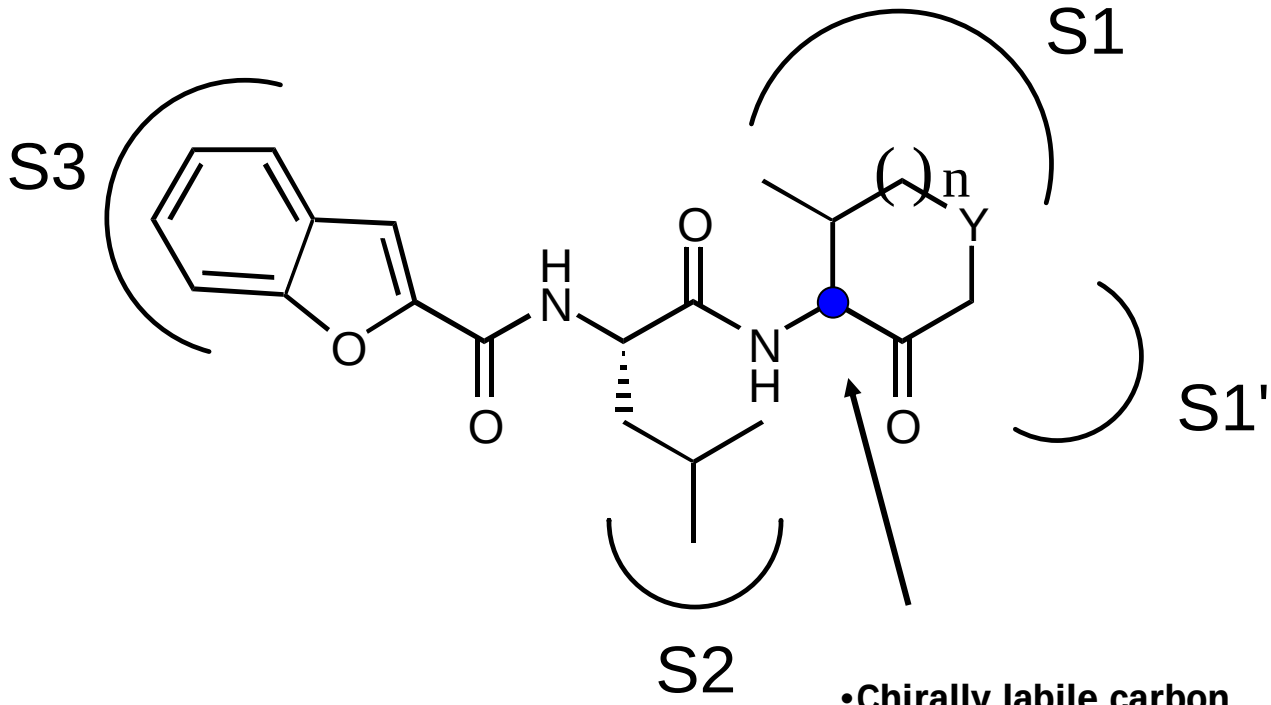
Design of New P1 groups



n = 0, 1, 2 or 3

Y = O, S, NR

- **Chirally labile carbon**

- **Chirally stable P1 templates desirable**
- **Should retain potency/DMPK properties**

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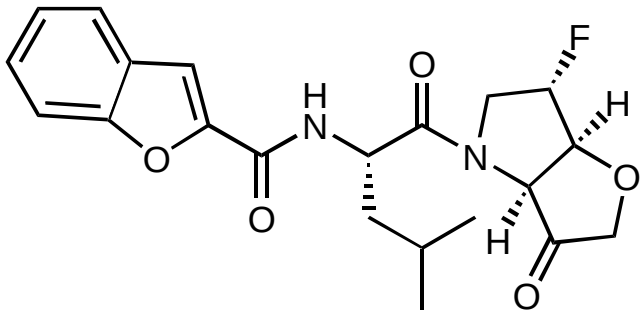
Example of Non-epimerizable P1 Groups



- A P1 fused 5:5 ring system designed
- Maintains the preferred (*S*)-chirality
- Chirally stable

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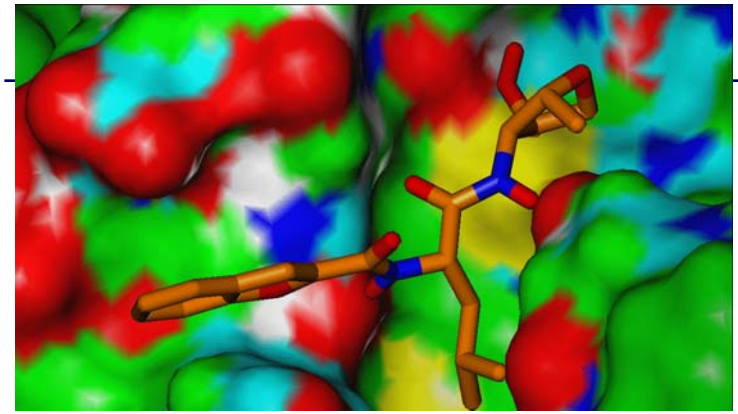
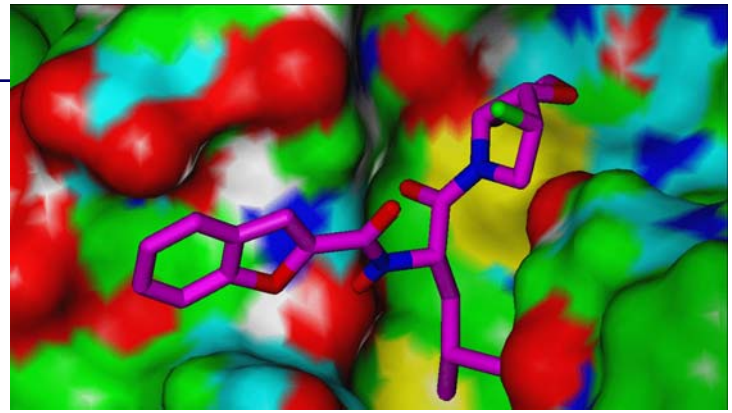
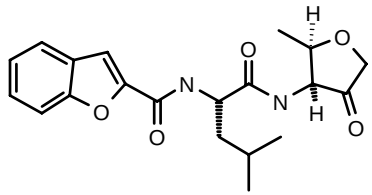
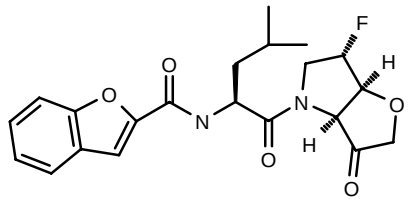
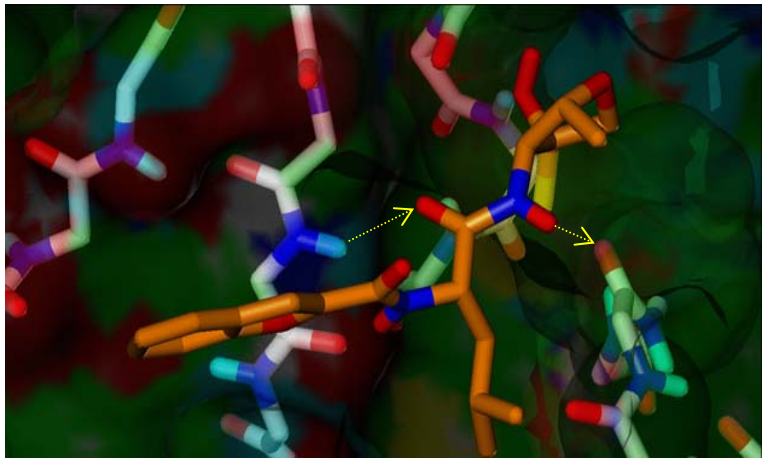
Will it Bind? - Docking of Methyl Furanone & Fluorobicycle Ligands to Cathepsin K

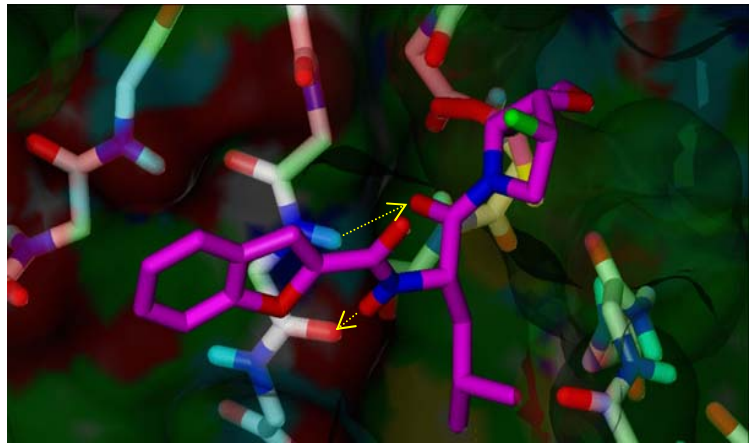












- The P1-P2 amide N-H hydrogen bond to the enzyme is lost while the overall placement and direction of the amide remains similar

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Overlay of Bound Conformation of Methyl-Furanone and Fluorobicycle P1s

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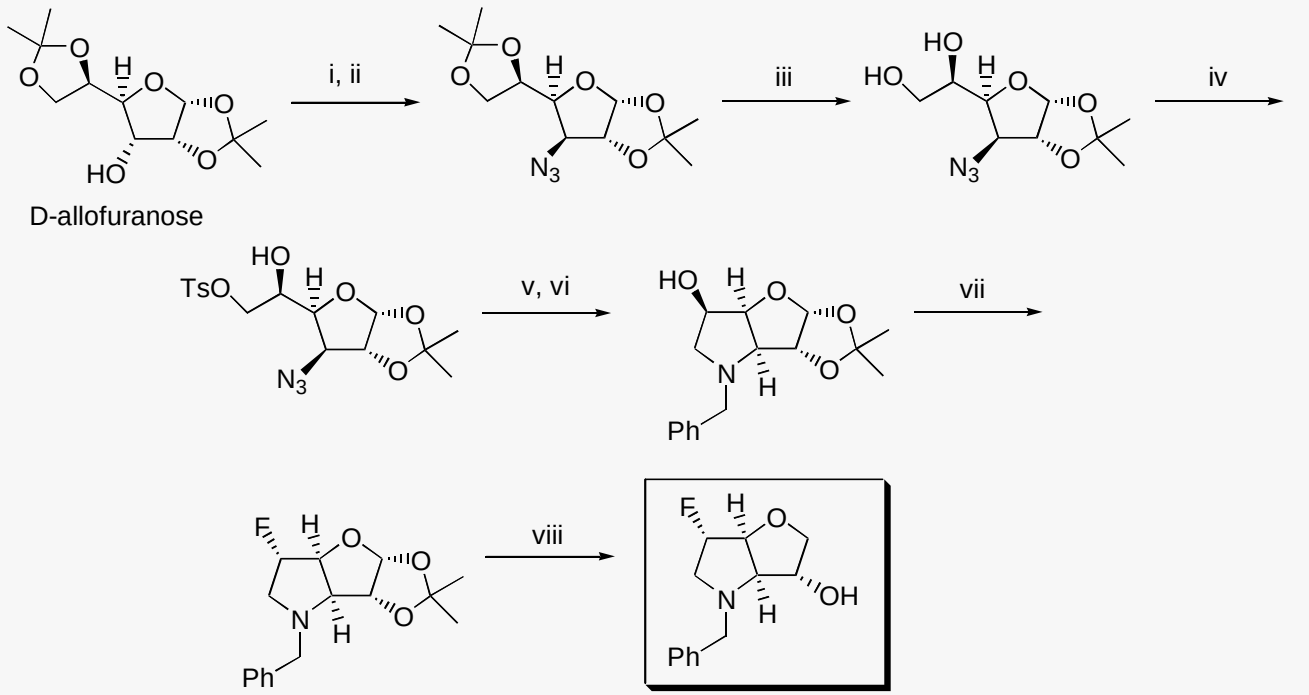
Synthesis of Novel P1 Bicyclic Template



i. Tf_2O, pyridine; ii. NaN_3, 60 % yield over two steps; iii. 70 % AcOH, Δ, 80 % yield: iv. *p*-TsCl, pyridine, 72 % yield; v. 10 % Pd on C, H_2; vi. PhCHO, $NaCNBH_3$, 74 % yield over two steps; vii. Deoxofluor, pyridine, 65 % yield; viii. a. TFA, H_2O; b. Ac_2O, pyridine: c. HBr, AcOH; d. $LiAlH_4$, 62 % yield over four steps

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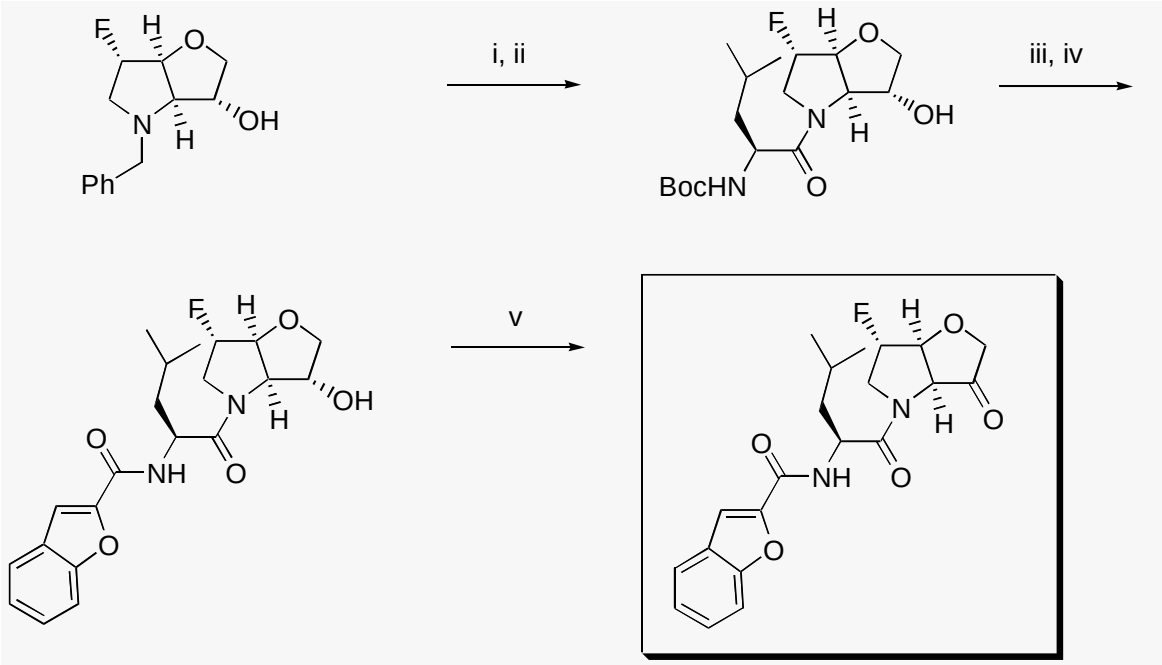
Synthesis of a Cathepsin K Inhibitor



i. 10 % Pd on C, H_2; ii. Boc-LeuOH, WSC, HOBt, NMM, 85 % yield over two steps; iii. TFA, H_2O; iv. 2-Benzofurancarboxylic acid, WSC, HOBt, NMM, 80 % yield over two steps; v. Dess-Martin periodinane, 65 % yield

- High potency displayed: K_i value of 2.6 nM
- > 100 fold selectivity over related Cathepsins
- Good overall DMPK properties
- Very promising inhibitor class

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Clinical Pipeline of Cathepsin K Inhibitors

	Merck	P&G/Sanofi-Aventis*	GSK	Novartis	Eli Lilly
Launched	**Fosamax** Bisphoshonate	**Actonel** Bisphoshonate	**Boniva** Bisphoshonate	**Zometa** Bisphoshonate	**Evista** SERM
				Miacalcin Calcitonin	**Forteo** PTH
Pipeline	**L-001037536** **Cathepsin K, P I**		**Relacatib** **Cathepsin K, P I**	**Aclasta** Bisphosphonate, P III	
	c-3578 α-v / β-3 antagonist, P I		**NPS-423562** PTH, P I	**AAE-581** **Cathepsin K, P II**	
			NPS-2143 PTH, P I	**AFG -495** **Cathepsin K, suspended**	

* Sanofi-Aventis: ongoing preclinical cat K program

- **Several pharmaceutical companies with a major osteoporosis franchise are now developing cathepsin K inhibitors**

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