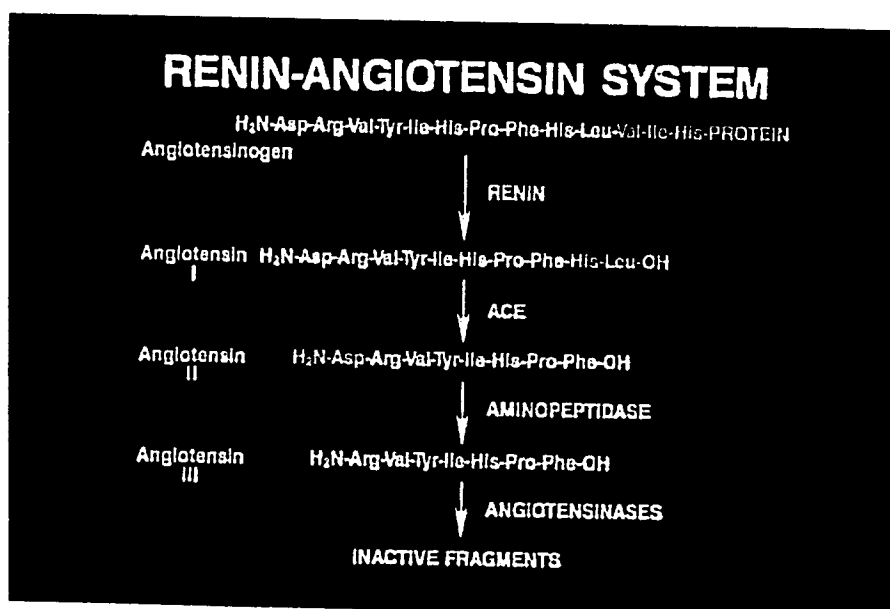


PROPERTIES OF A DRUG FOR CLINICAL USE

- HIGH POTENCY
- HIGH SPECIFICITY
- METABOLIC STABILITY
- LOW TOXICITY
- SIGNIFICANT ORAL ABSORPTION



METHODS OF OBTAINING THREE-DIMENSIONAL STRUCTURES

Experimental

X-ray crystallography
NMR

Theoretical

Modelling

ASPARTYL PROTEASES

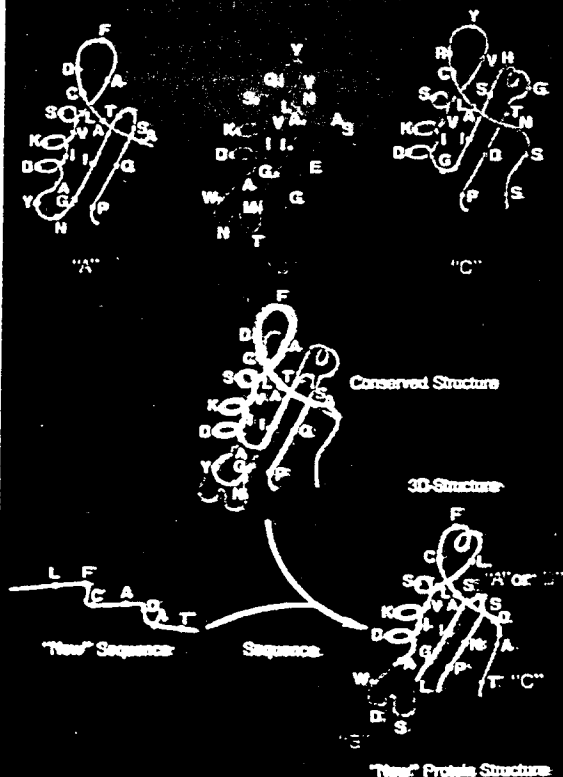
Enzyme	Three-Dimensional Structure	Amino Acid Sequence
Penicillopepsin	Yes	Yes
Rhinopuspapsin	Yes	Yes
Endothelapsin	Yes	Yes
Renin (Human)	No	Yes
Renin (Mouse Submaxillary)	Not Yet	Yes

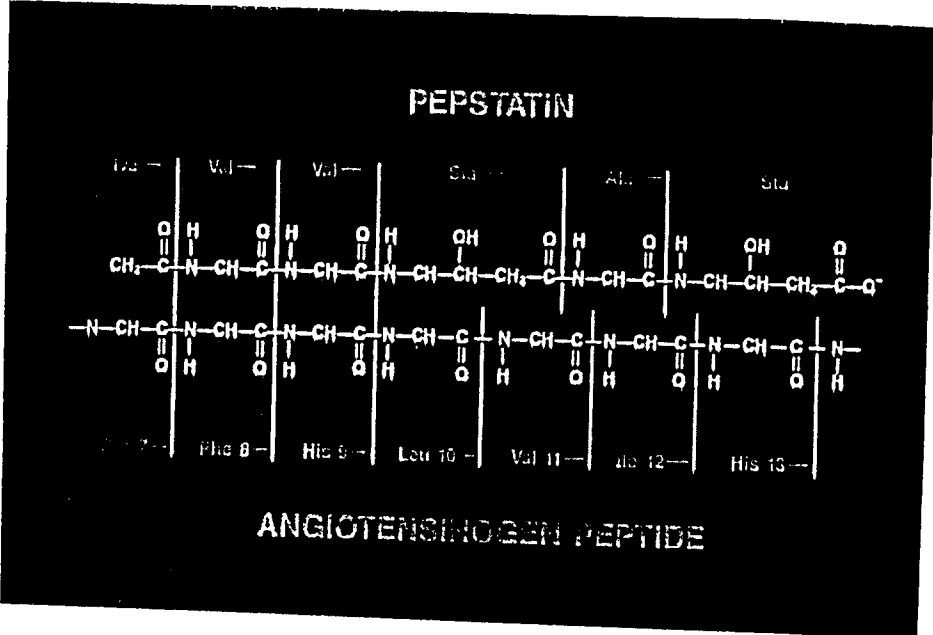
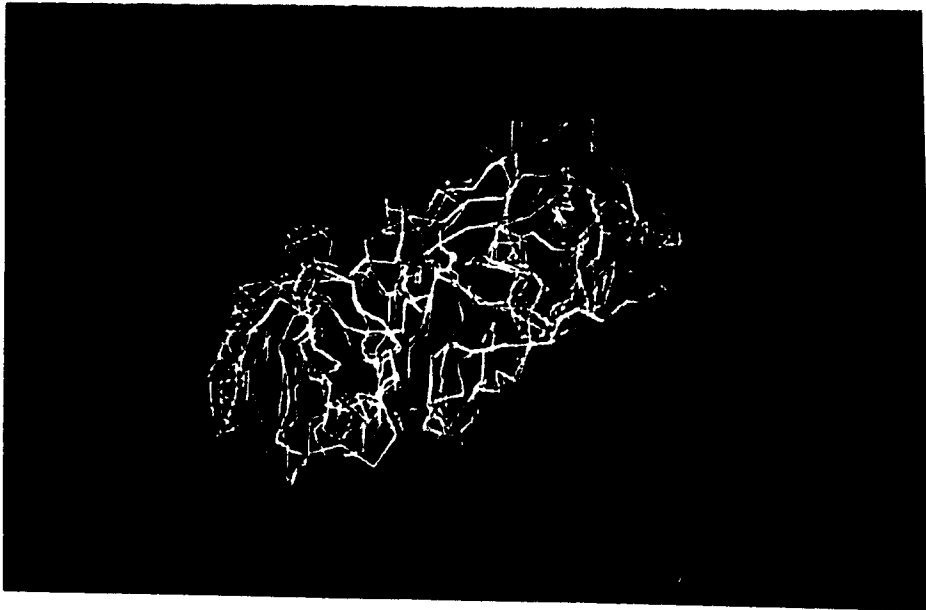
SEQUENCE HOMOLOGY

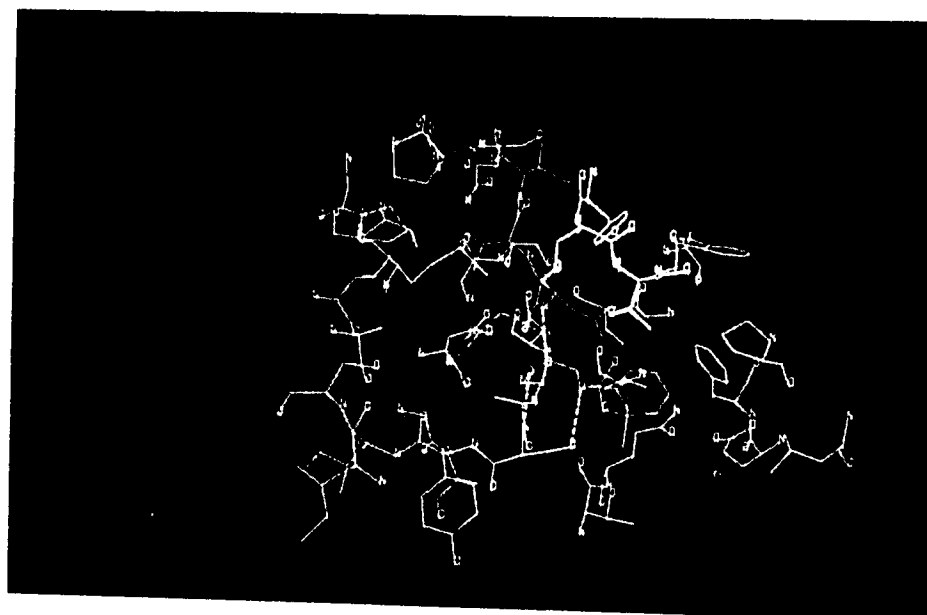
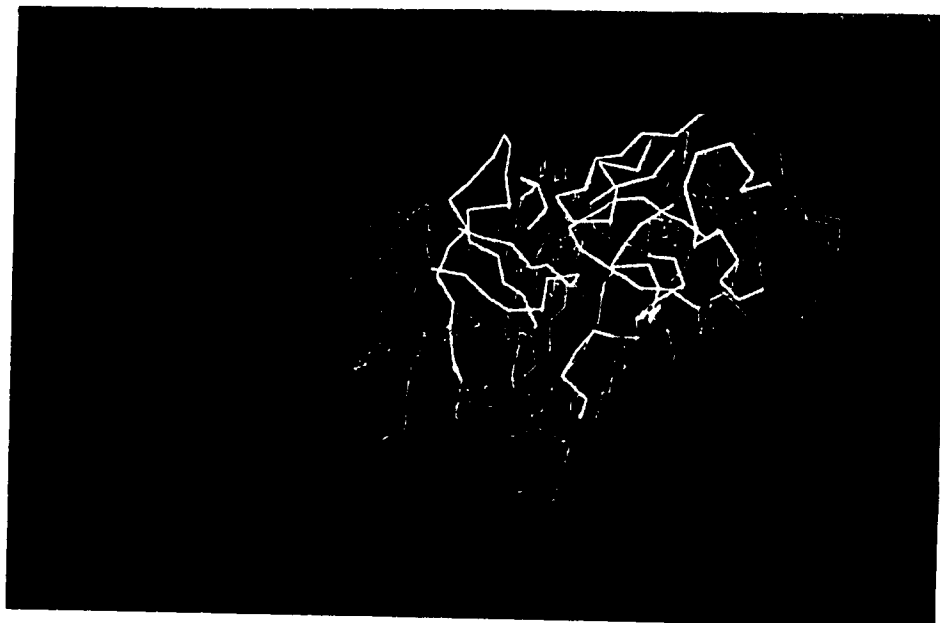
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A :  ATCDFALSVKIDAYN--GIT--SQP
B :  SNQ-VVLSVKIDAWNTMGIN--AEG
C :  SSNACRYVLSVKID----GISHGTDP
NEW:  TADACFL-LSVKIDAWDSLGIS--SNP
    
```

MOLECULAR MODELING







PROTECTION FROM PEPTIDASE ENZYMES ACTION

- Change the character of the peptide bond
- Change the amine acid recognized by the cleaving agent
- Protect the peptide bond

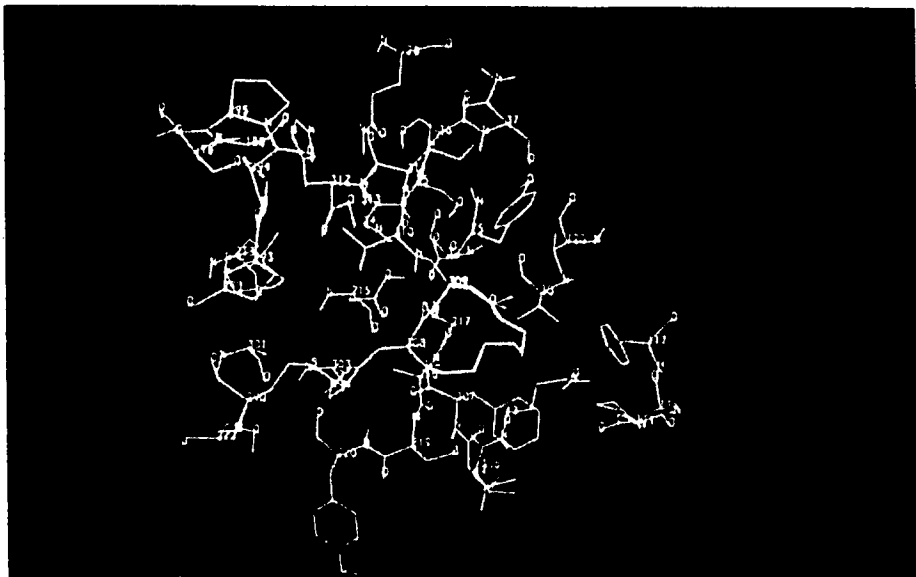
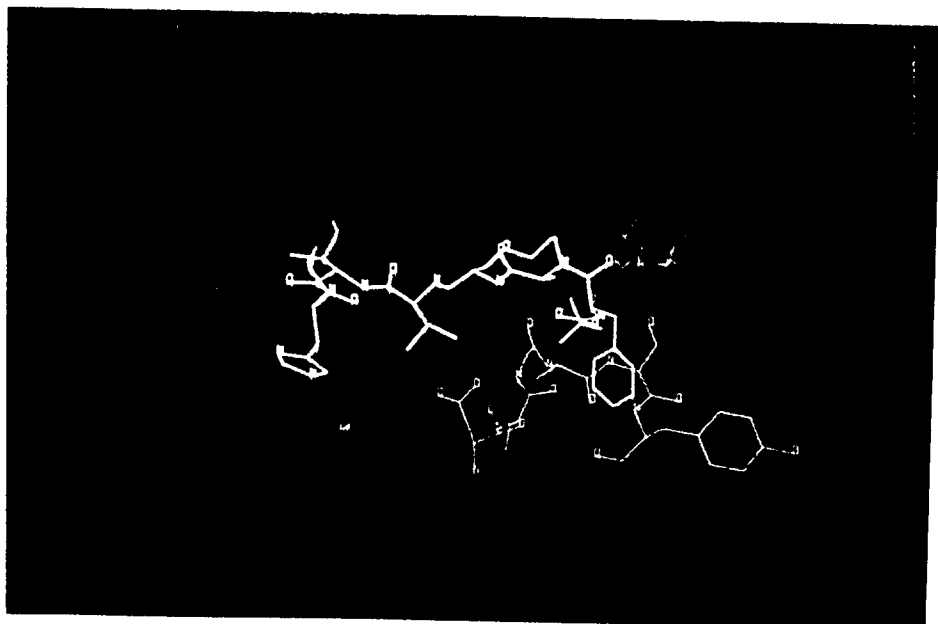


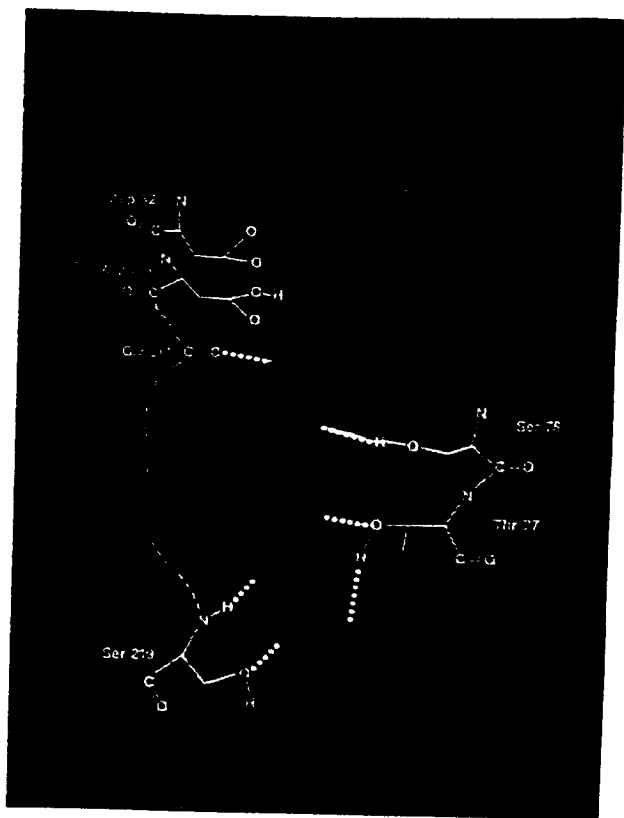
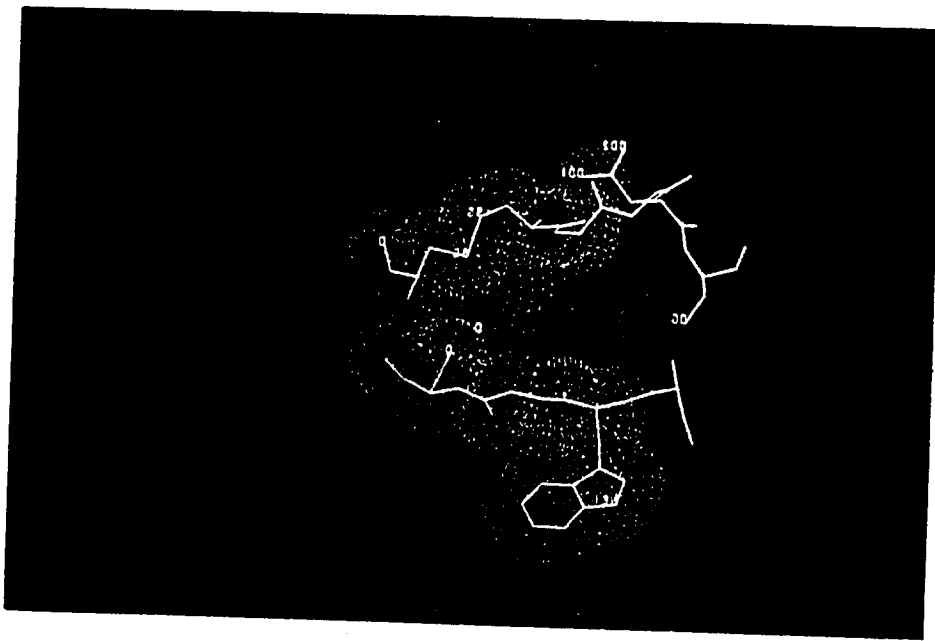
TABLE OF POTENCY, SPECIFICITY
AND METABOLIC STABILITY

A-NUMBER	COMPOUND	$IC_{50}(\mu M)$		$t_{1/2}$ (min) CHYMOTRYPSIN CLEAVAGE
		HUMAN RENIN	MOUSE RENIN	
56853	X-His-Y	0.3	6.5	—
56834	X-Ala-Y	1.4	0.5	10-15
57327	X-Ala-Y	2.7	1.0	—
57285	X-Ala ^{"10"} -Y	>100	>100	—
60427	X-Ala ^{"12"} -Y	69	0.4	—
60788	X-Ala ^{"14"} -Y	2.7	3.8	not cleaved

X = Boc-Phe

Y = R-Val-His-His-OMe





No.	Structure								IC ₅₀ (nM)	
	P ₃	P ₂	P ₁	R	P ₁ '	P ₂ '	P ₃ '	OMe		
1	Boc	Phe	His	Leu	R	Val	Ile	His	OMe	300
2	Boc	—	His	Leu	R	Val	Ile	His	OMe	No inhibition
3	Boc	—	—	Leu	R	Val	Ile	His	OMe	No inhibition
4	Boc	Phe	His	Leu	R	Val	Ile	—	OMe	2300
5	Boc	Phe	His	Leu	R	Val	NHCH ₂ Ph	—	—	370
6	Doc	Phe	His	Cal*	R	Val	NHCH ₂ Ph	—	—	34

* Cal = Cyclohexylalanine

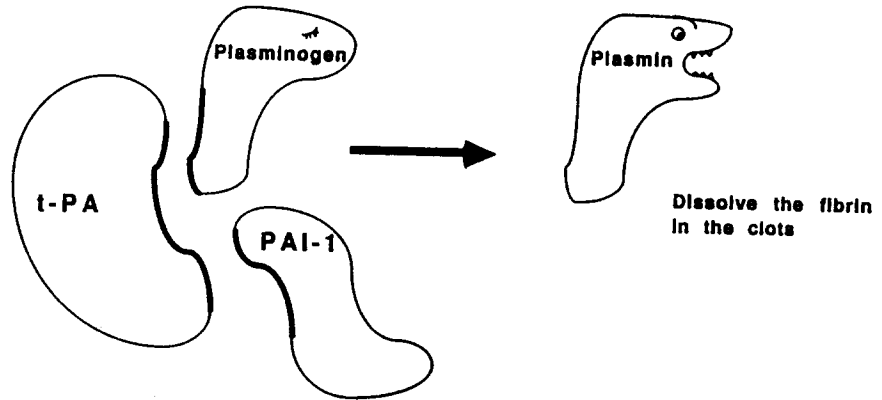
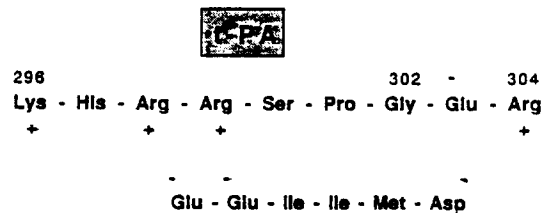


TABLE 1 Sequence alignment of human t-PA with trypsin

Trypsin	16		37		39	41		60
t-PA	IVGGYTCCGAN	TVPYQVSLNS	GYH	FCGGSLINSQ	WVVSAAHCYK		
	IKGGLFADIA	SHPWQAAIFA	KRRRSP	EAR	LCCGILISSC	WILSAAHCFO		
	276		296		304			325
Trypsin	61							105
t-PA	S.....GIQV	RLGEDNINVV	EG.NECFISA	SKSIVHPSYN	SNTLNNDIHL			
	ERFPPHLLTV	ILGR.TYRVV	PGEEEQKFEV	EKYIVHKEFD	DDTYDNDIAL			
	326							374
Trypsin	106							150
t-PA	IKLKSA....	ASLNSRVAS	ISLPT..SCA	SAGTQCLISG	WGNTKSSGTS			
	LQLKSDSSRC	AQESSV.VRT	VCLPPADLQL	PDWTECELSG	YGRHEALSPF			
	375							423
Trypsin	151							190
t-PA	YPDVLKCLKA	PILSDSSCKS	A..YFGQITS	NMFCAGYLE	GG.....KDS			
	YSERLKEAHV	RLYPSSRCTS	QHLHRTVTD	NMLCAGDTRS	GGPQANLHDA			
	424							473
Trypsin	191		214					240
t-PA	CQGDSSGGPVV	CS...GKIQ	GIVSWGSGCA	QNIKPGVYTK	VCHYVSNIKQ			
	CQGDSSGGPLV	CLHDGRMTLV	GIISWGLGCG	QNDVPGVYTK	VTHYLDNIKD			
	474							523
Trypsin	245							
t-PA	TIASH							
	HMRP							
	527							



A del 296 - 302

B Arg → Ser 304

C Arg → Glu 304

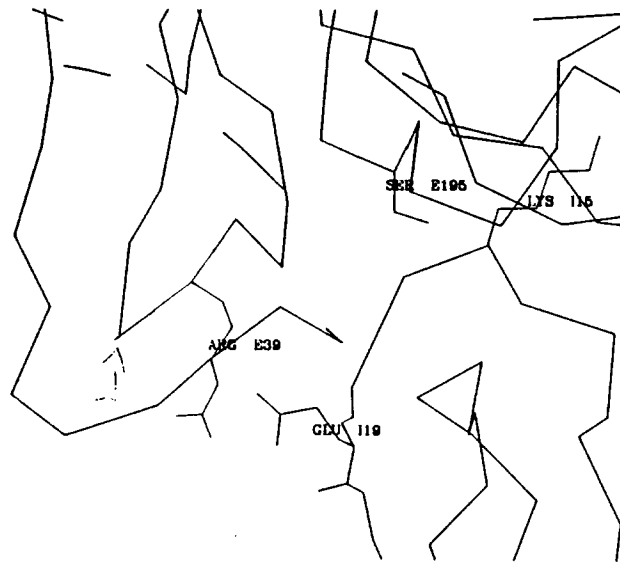
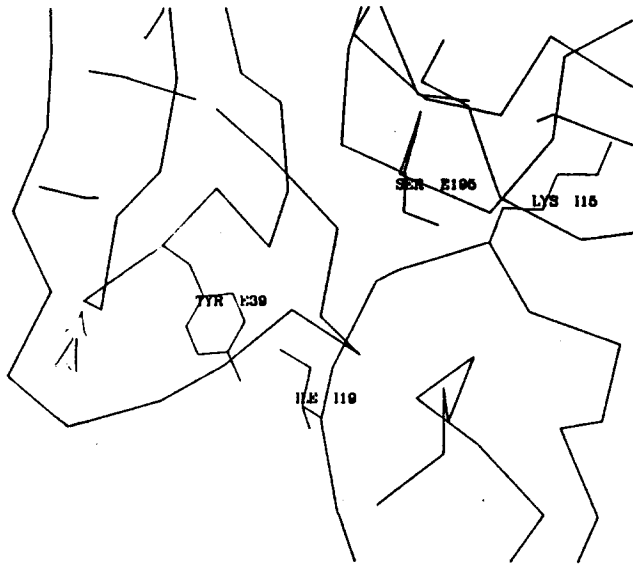
Mutations

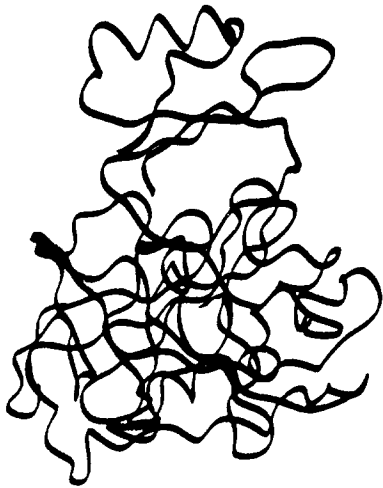
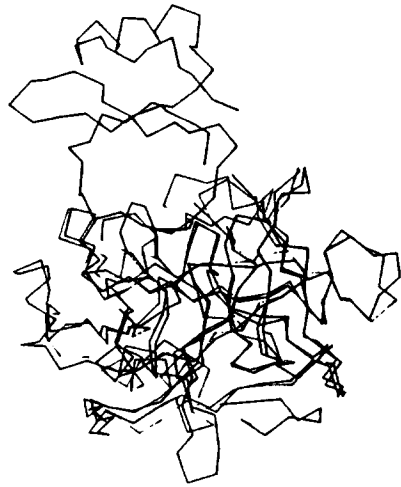
A Retains 95% of activity

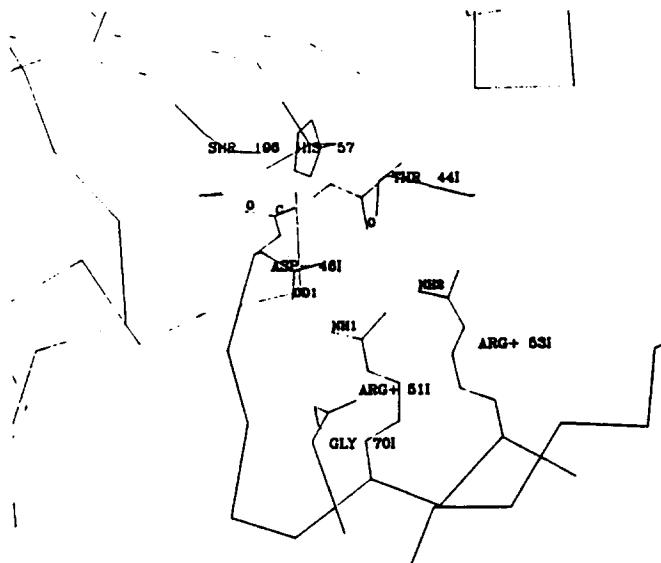
B = 4 times more PAI-1

C = 25 times more PAI-1

Results





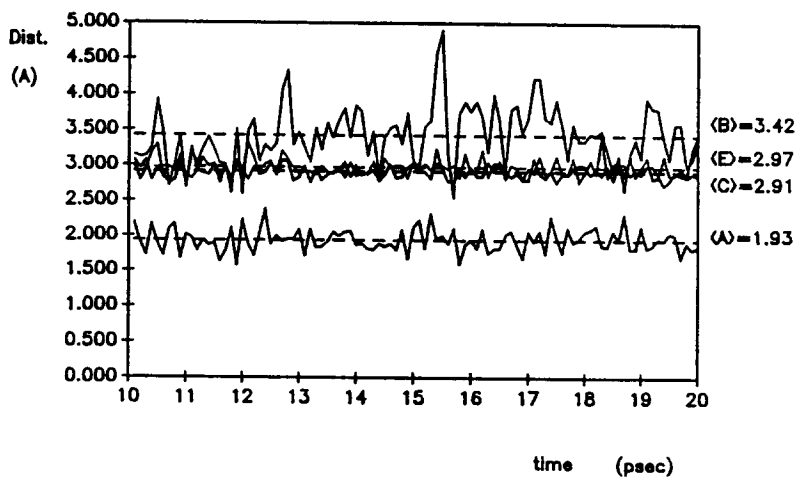


A = O(451)---HN(193)

B = O(451)---HN(195)

C = C(451)---OG(195)

E = OG(195)---NE2(57)



D2 = HN(216)---O(431)

D5 = HN(193)---O(451)

D6 = O(41)---HN(471)

