

Laurea Magistrale in Chimica

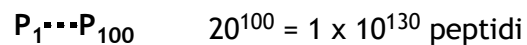
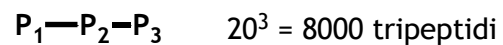
Laboratorio di Chimica Bioorganica

anno accademico 2020 – 2021

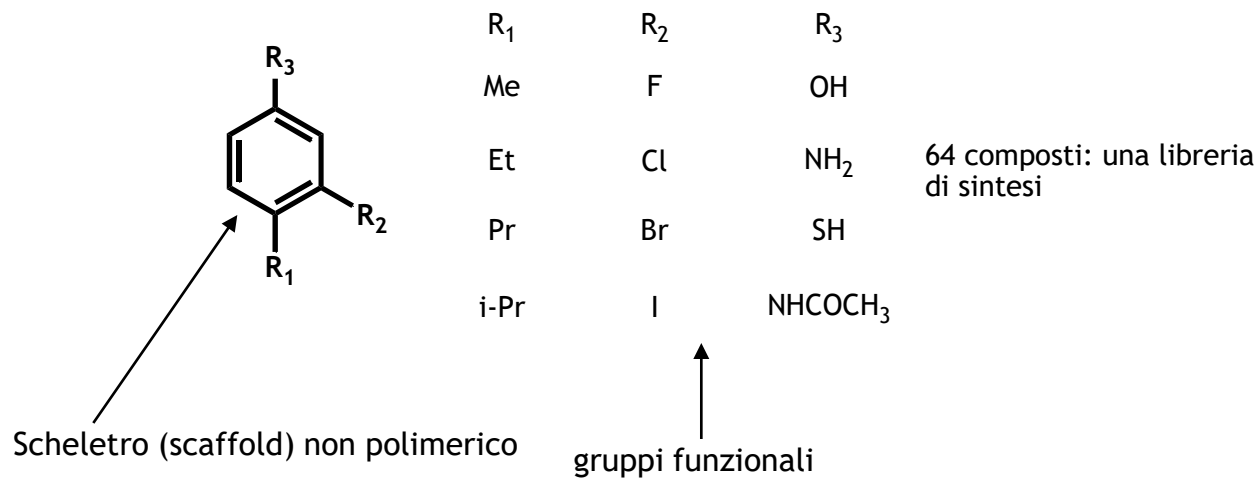
Prima esperienza

Due librerie ortogonali di accettori di Michael potenziali inibitori della calpaina 1 umana

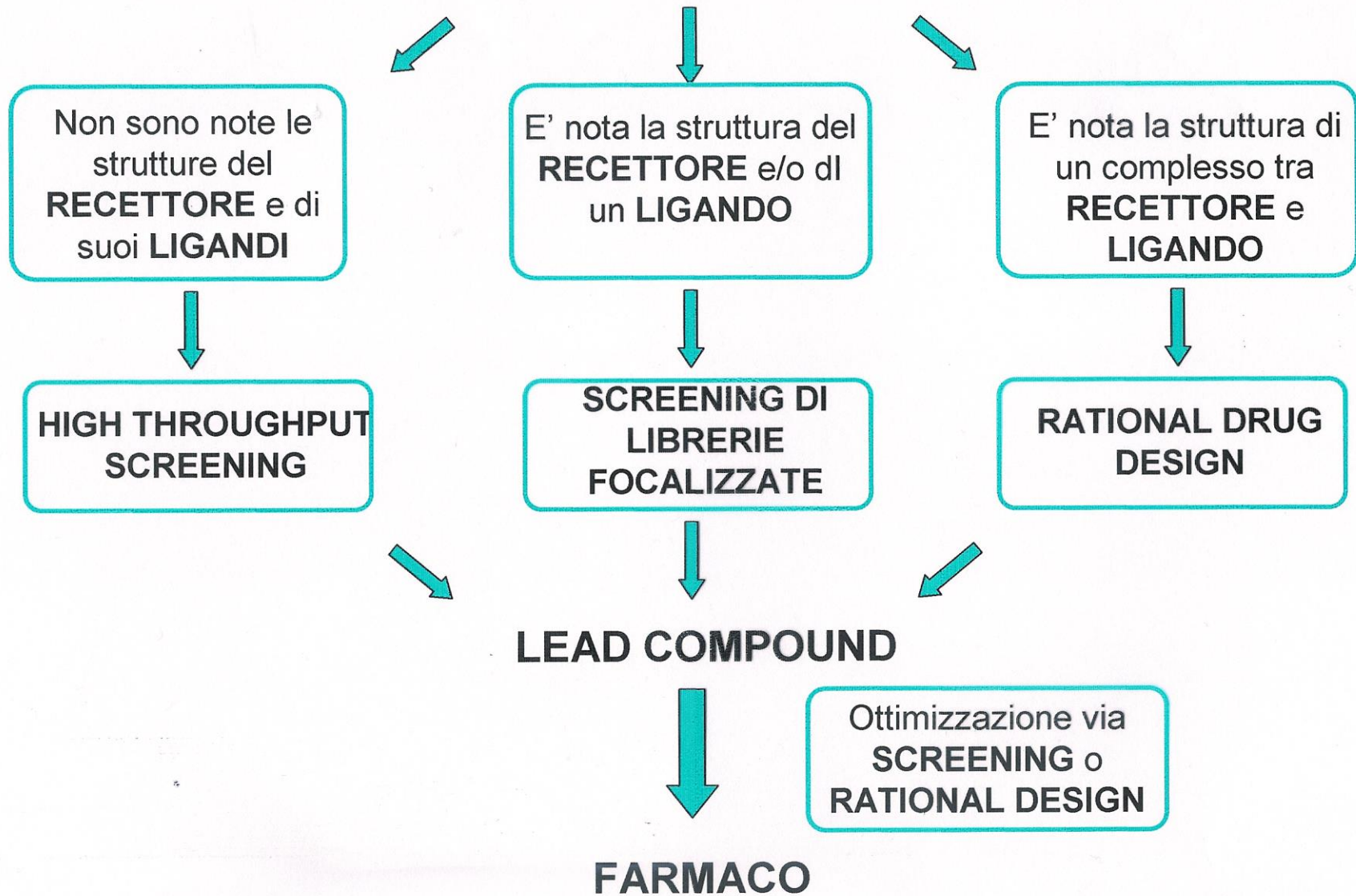
Introduzione alla chimica combinatoriale



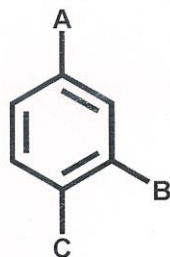
Una libreria di origine biologica, ad es. fagica: $10^6 - 10^8$ membri



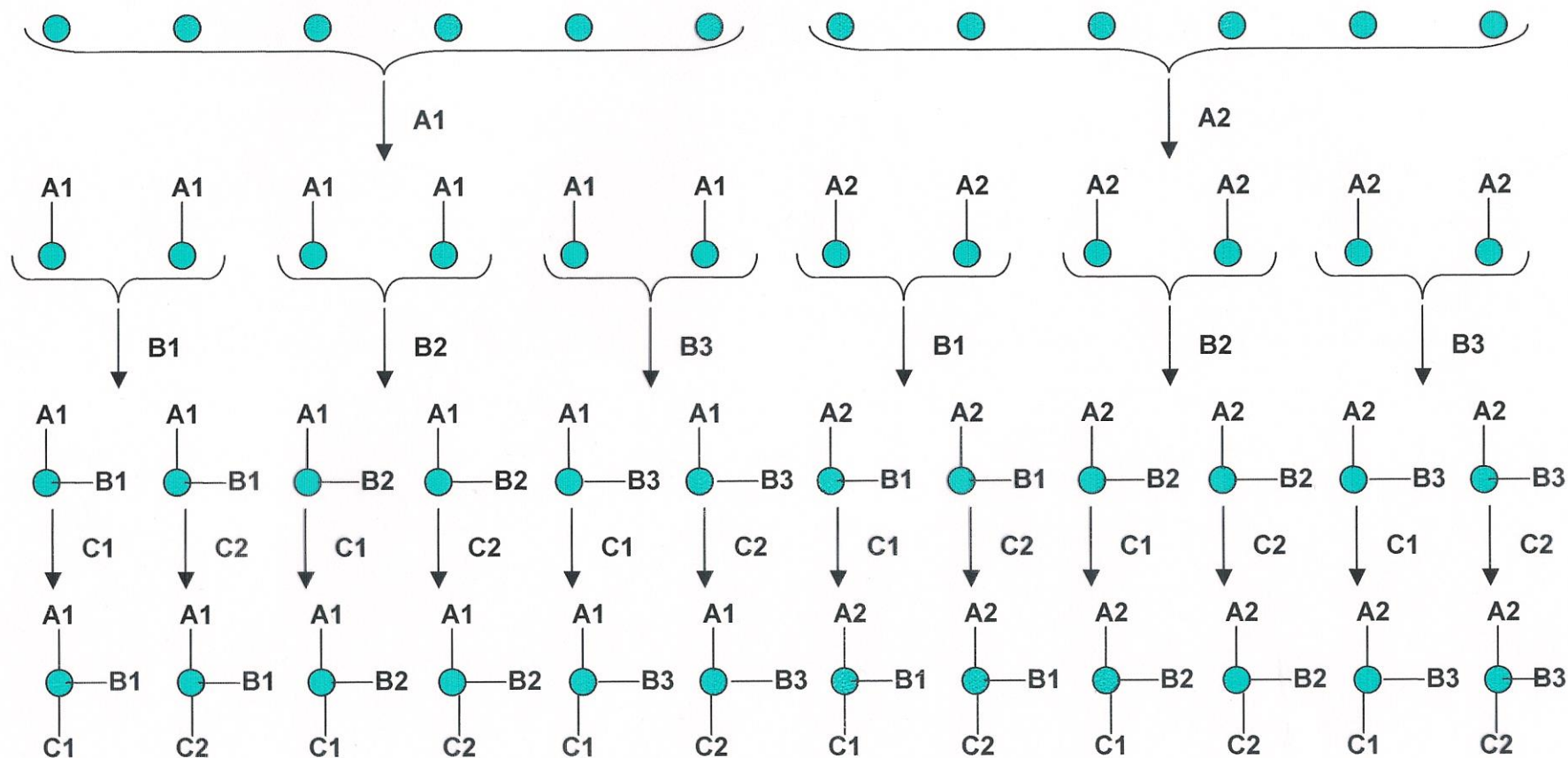
OBIETTIVO



SINTESI PARALLELA DI:

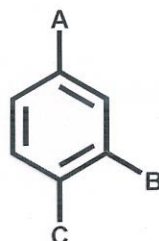


A1, A2 - B1, B2, B3 - C1, C2

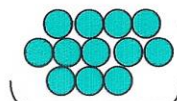


2+6+12=20 reazioni per 12 composti

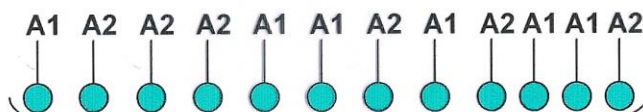
SINTESI IN MISCELA DI:



A1, A2 - B1, B2, B3 - C1, C2



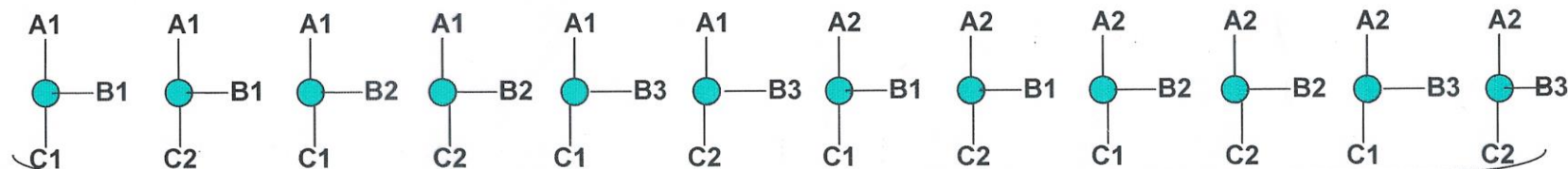
50% A1 + 50% A2



33% B1 + 33% B2 + 33% B3

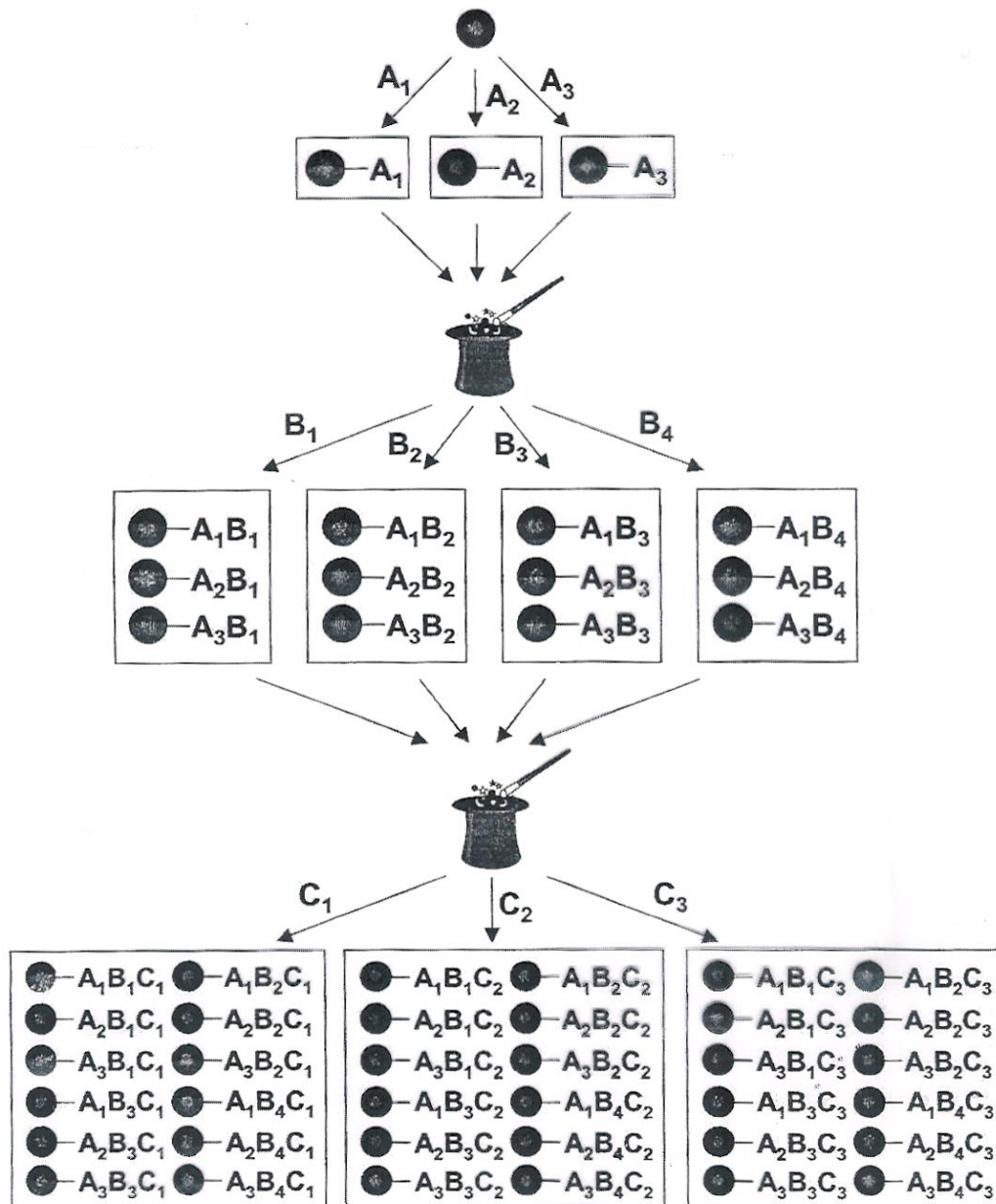


50% C1 + 50% C2



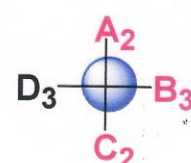
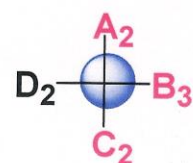
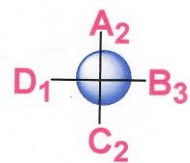
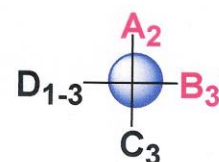
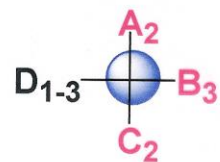
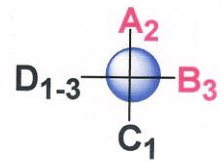
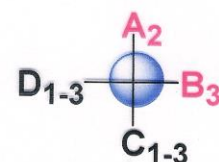
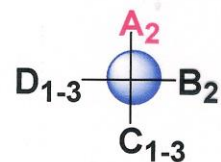
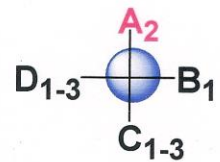
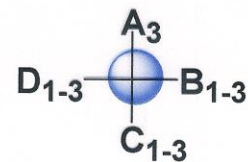
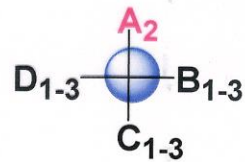
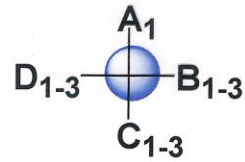
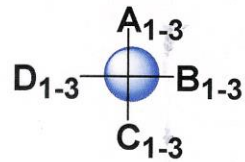
Tre reazioni per una libreria di 12 composti

SPLIT AND COMBINE LIBRARY SYNTHESIS

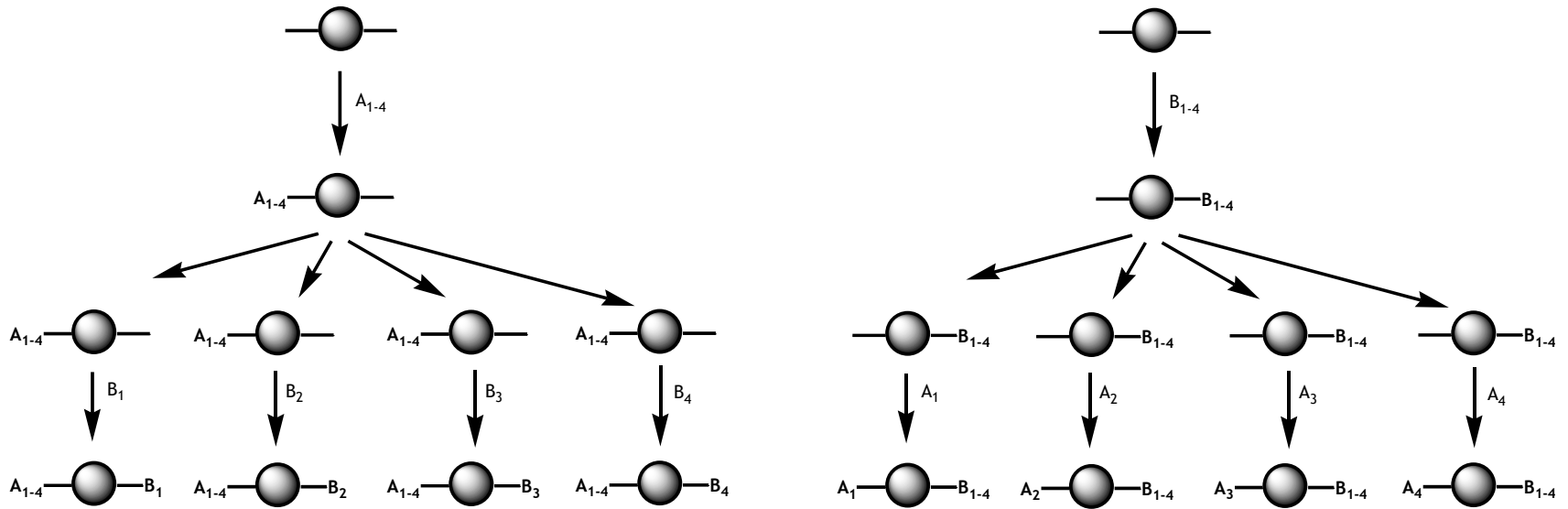


Sintesi in miscela di una libreria composta di una gerarchia di sottolibrerie

DECONVOLUZIONE ITERATIVA

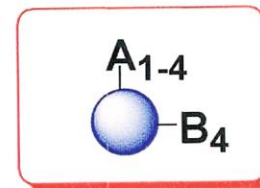
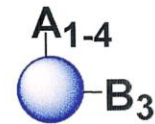
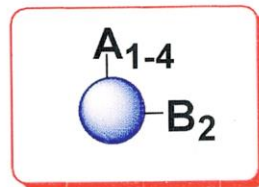
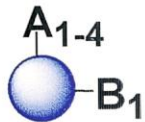
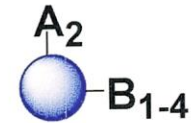
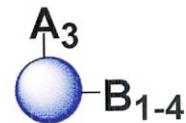
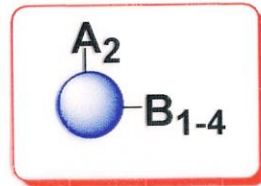
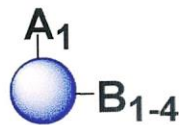
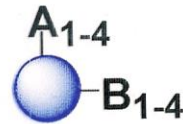


Sintesi in miscela di librerie ortogonali



100 A, 100 B: 10000 composti, 10000 reazioni per la sintesi parallela pura. 202 reazioni in quattro passaggi per la sintesi di librerie ortogonali.

Deconvoluzione di librerie ortogonali

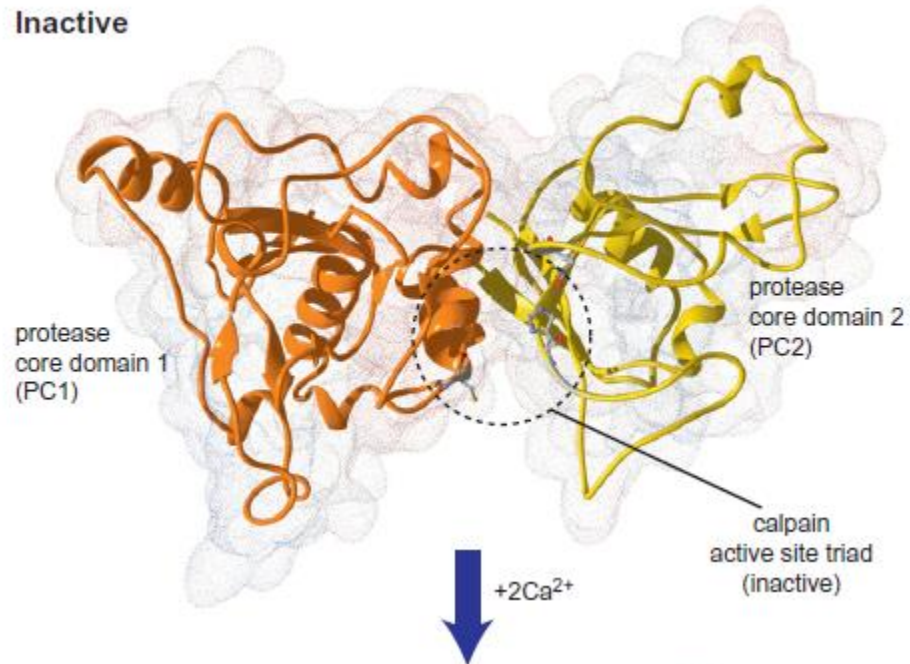


Attivi: A_2B_2 e A_2B_4



La calpaina 1

Inactive



Active

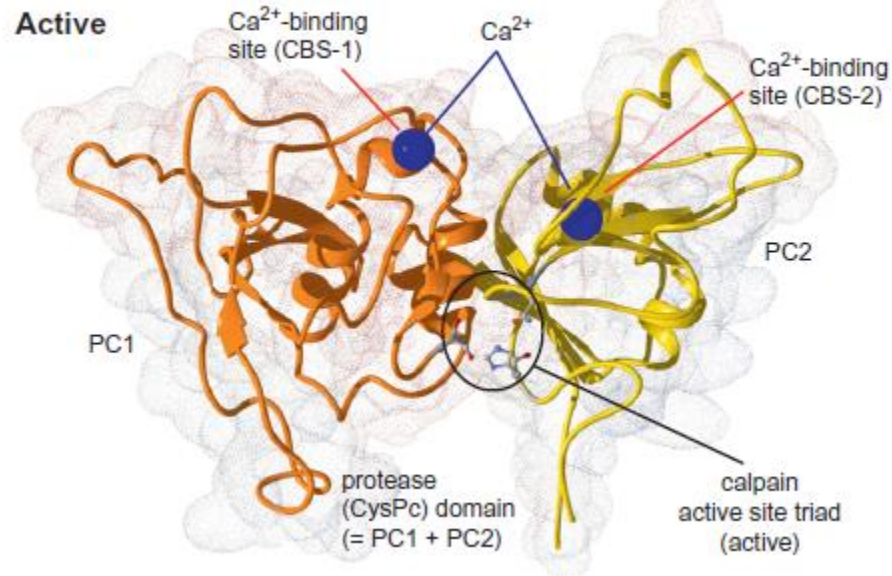
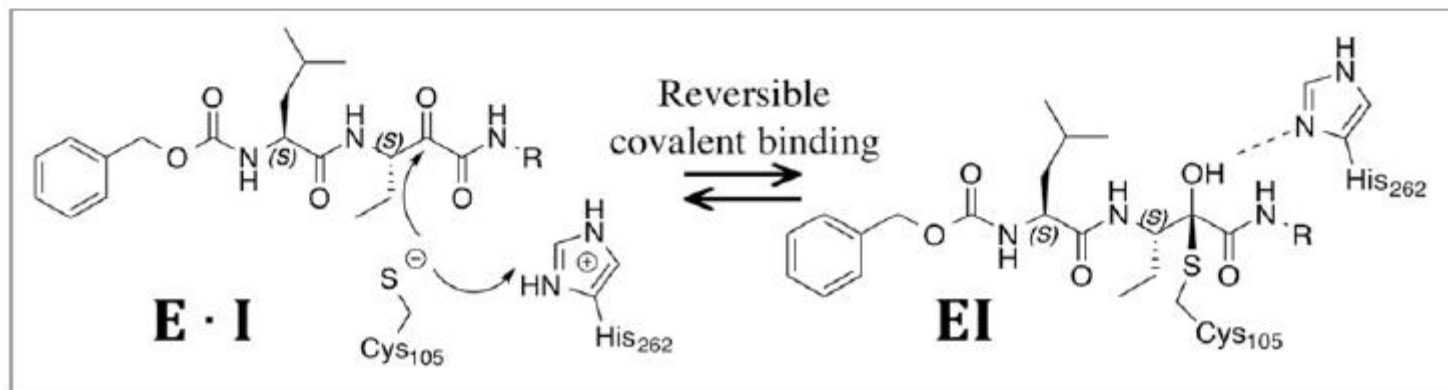
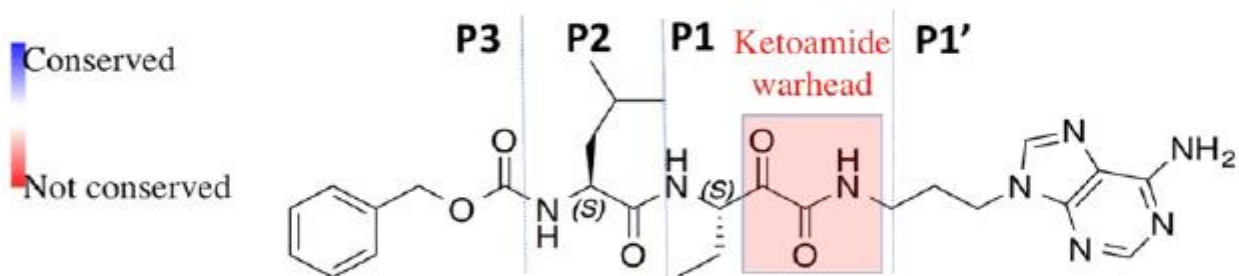
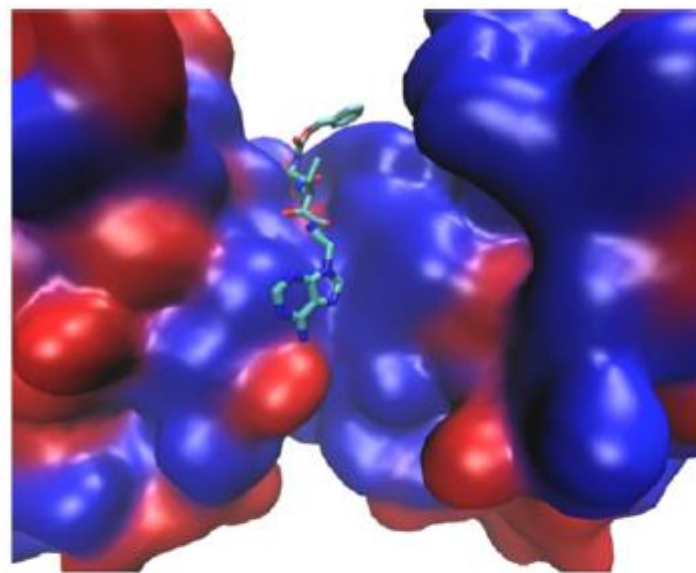
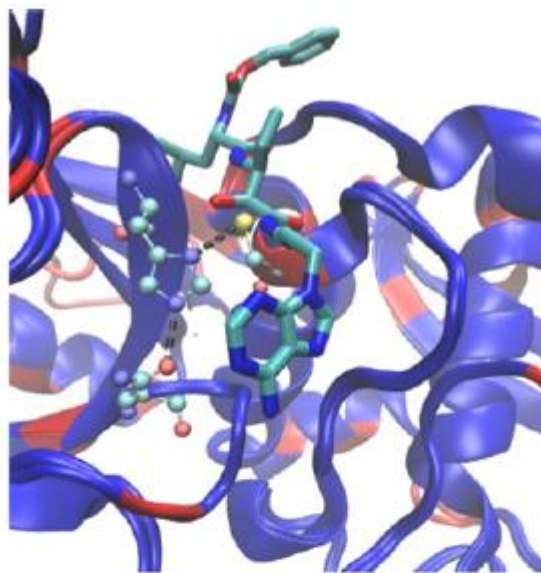
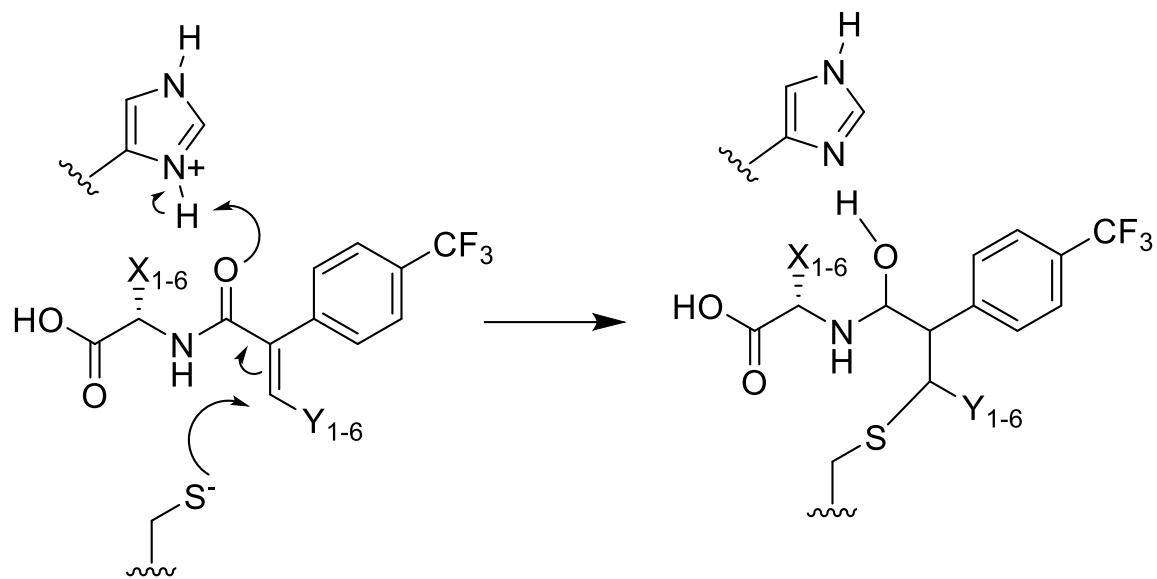
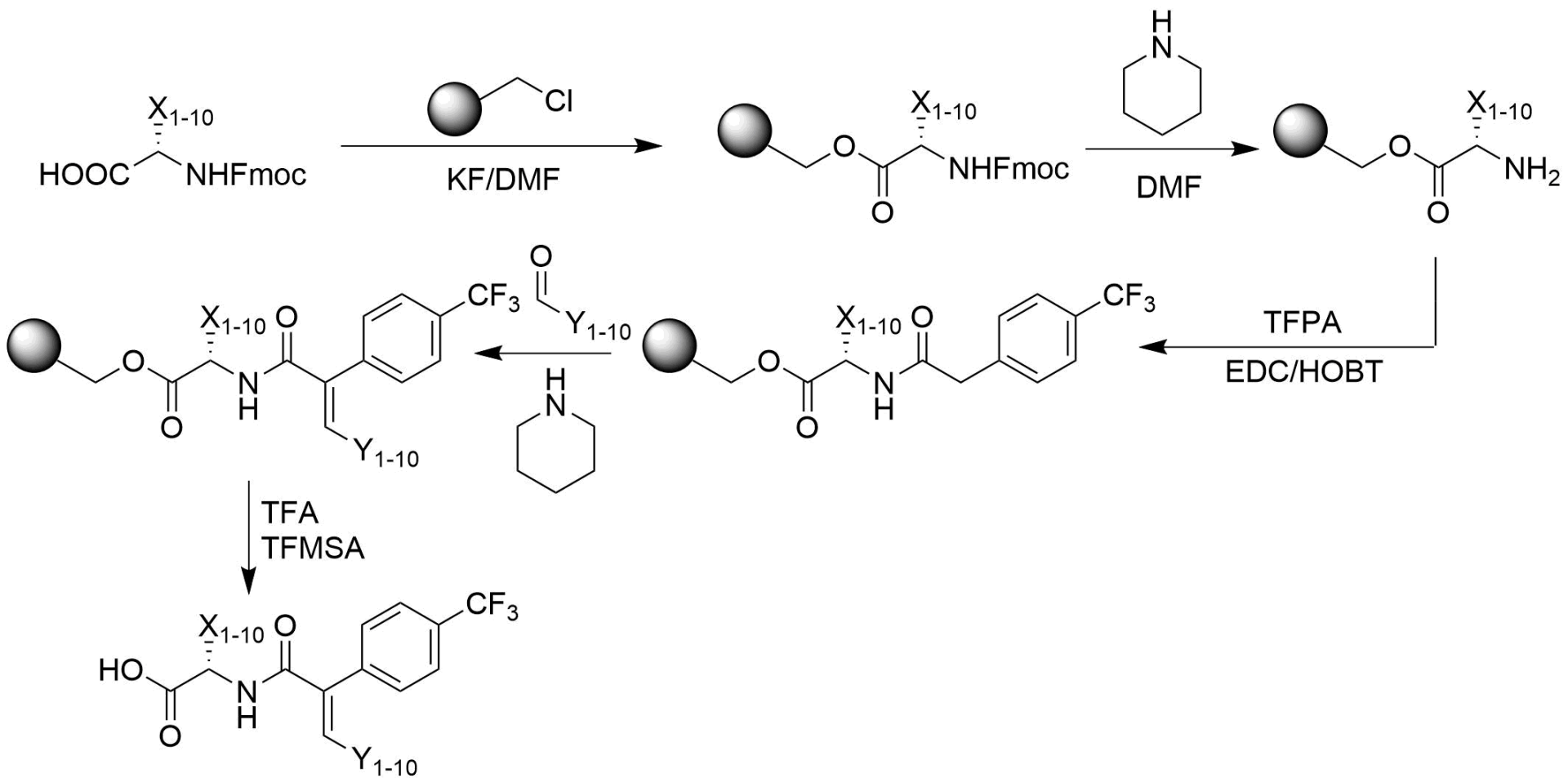


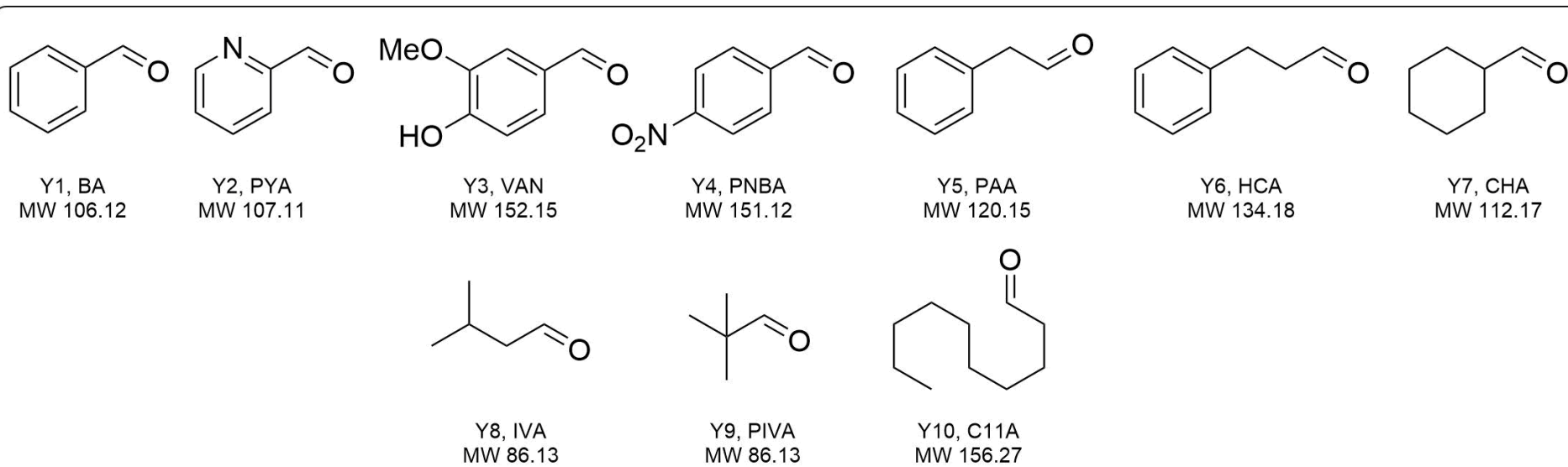
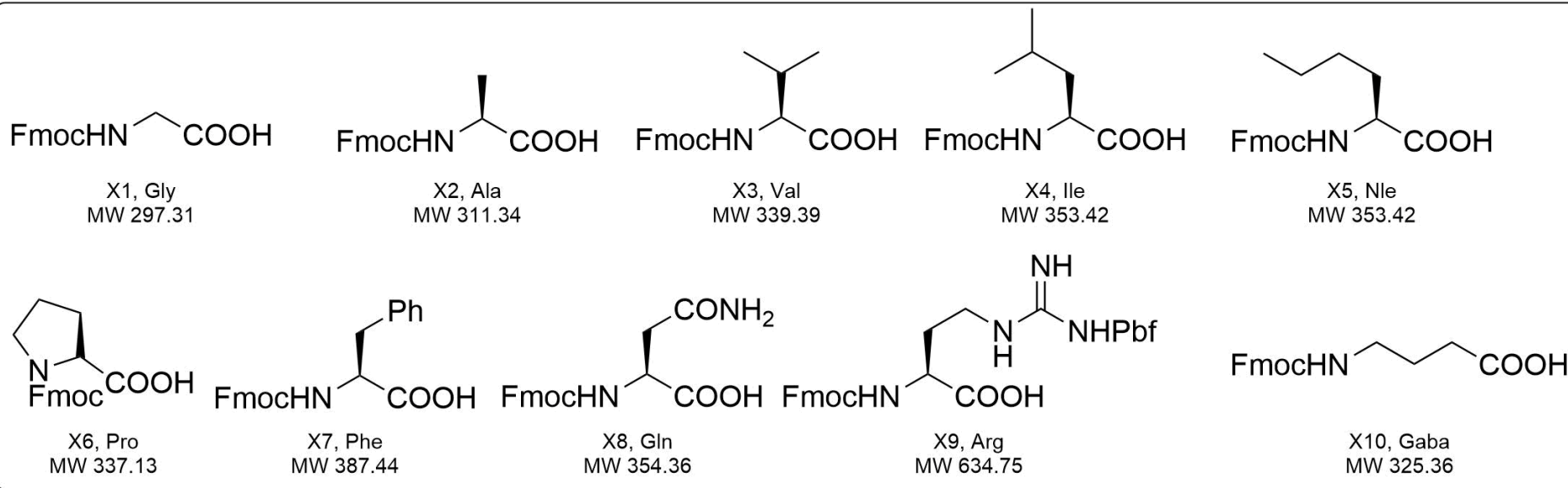
FIGURE 454.3 Schematic three-dimensional structures of inactive and active calpain CysPc domains. Schematic three-dimensional ribbon structures superimposed on the surface-type structures of the inactive (Ca^{2+} -free) and active (Ca^{2+} -bound) forms of human and rat m-calpain using PDB data, 1KFX and 3DF0, respectively [45,47]. The active protease (CysPc) domain is formed by fusion of the PC1 and PC2 core domains after each of these domains binds a single Ca^{2+} . The active site is circled in black. Blue balls represent Ca^{2+} .

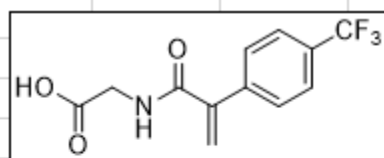
<i>Disease with a primary cause other than calpains</i>				
Atherosclerosis	Calpain-1 and -2 (human, mouse)	Aggravating or causative	Calpain inhibitors	25
Brain ischaemia	Calpain-1 and -2 (human, mouse)	Aggravating or causative	Calpain inhibitors	24,44
Cardiovascular disorders	Calpain-1 and -2 (human, mouse)	Aggravating or causative	Calpain inhibitors	23,39,114,116,117,119
Cataracts	Calpain-1 and -2 (human, mouse)	Aggravating or causative	Calpain inhibitors	92,134,136–139
Neurodegenerative disorders (e.g. Parkinson disease, amyotrophic lateral sclerosis, spinocerebellar ataxia type 3 and lissencephaly)	Calpain-1 and -2 (human, mouse)	Aggravating or causative	Calpain inhibitors	14–17,19
Retinitis pigmentosa	Calpain-1 and -2 (human, mouse)	Aggravating or causative	Calpain inhibitors	141,142
Cancers	Calpain-1, -2, CAPN3 and CAPN9 (human, mouse)	Preventive, aggravating or causative	Calpain inhibitors and/or gene therapy	29,85,86,145,147–159
Alzheimer disease	Calpain-1 and -2 (human, mouse)	May be aggravating or causative	Potentially calpain inhibitors	20,110
Muscular dystrophies	Calpain-1 and -2 (human, mouse)	May be aggravating or causative	Potentially calpain inhibitors	130
<i>Disease model</i>				
Cardiac scar	Calpain-1 and -2 (human, mouse)	Preventive	Potentially gene therapy	120
Dilated cardiomyopathy	Calpain-1 and -2 (human, mouse)	Preventive	Potentially gene therapy	121
<i>Infectious disease</i>				
Fungal infection (opportunistic infection)	PalB/Rim13 (fungus)	Aggravating or causative	Calpain inhibitors	181,182
Malaria	Calpain-1 and -2, Pf-calpain (human*, mouse*, parasite)	Aggravating or causative	Calpain inhibitors	41,178
Periodontitis	Tpr (bacteria)	Aggravating or causative	Calpain inhibitors	12
Trypanosomiasis and leishmaniasis (e.g. African sleeping sickness)	Calpain-1 and -2, ClpGM6 (human*, mouse*, parasite)	Aggravating or causative	Calpain inhibitors	180
Schistosomiasis	Smp-157500 (parasite)	Aggravating or causative	Vaccine (rSm-p80)	10,225
<i>Calpainopathies</i>				
Autosomal dominant neovascular inflammatory vitreoretinopathy	CAPN5 (human, mouse)	Aggravating or causative	Calpain inhibitors	34,167
Eosinophilic oesophagitis	CAPN14 (human, mouse)	Preventive	Potentially gene therapy	35,36
Gastric ulcer	CAPN8, CAPN9 (human, mouse)	Preventive	Potentially gene therapy	33
Limb-girdle muscular dystrophy type 2A	CAPN3 (human, mouse)	Preventive	Potentially gene therapy	32
Spastic paraplegia 76	CAPN1 (human, mouse)	Preventive	Potentially gene therapy	48
Type 2 diabetes	CAPN10 (human, mouse)	Not definable	Unclear	251,252











core MW 273.21

		X1	X2	X3	X4	X5	X6	X7	X8	X9	X10	
	MW aldeide ↓	75.07	89.1	117.15	131.18	131.18	114.89	165.2	132.12	174.2	104.12	← MW aa
Y1	106.12	418.36	432.39	460.44	474.47	474.47	458.18	508.49	475.41	517.49	447.41	
Y2	107.11	419.35	433.38	461.43	475.46	475.46	459.17	509.48	476.4	518.48	448.4	
Y3	152.12	464.36	478.39	506.44	520.47	520.47	504.18	554.49	521.41	563.49	493.41	
Y4	151.12	463.36	477.39	505.44	519.47	519.47	503.18	553.49	520.41	562.49	492.41	
Y5	120.15	432.39	446.42	474.47	488.5	488.5	472.21	522.52	489.44	531.52	461.44	
Y6	134.18	446.42	460.45	488.5	502.53	502.53	486.24	536.55	503.47	545.55	475.47	
Y7	112.17	424.41	438.44	466.49	480.52	480.52	464.23	514.54	481.46	523.54	453.46	
Y8	86.13	398.37	412.4	440.45	454.48	454.48	438.19	488.5	455.42	497.5	427.42	
Y9	86.13	398.37	412.4	440.45	454.48	454.48	438.19	488.5	455.42	497.5	427.42	
Y10	156.27	468.51	482.54	510.59	524.62	524.62	508.33	558.64	525.56	567.64	497.56	

[illegible]

Laboratorio di chimica bioorganica 2020/21			La quantità finale di prodotto richiesta nelle librerie è tale da poter preparare 10 µL di madre 10 mM, servono dunque 100 nmoli.		
Reagenti a Disposizione			Scala di 30 µmol	Pesata in mg o vol in µL	Lavoriamo su una scala di 30 µmoli, per avere quantità pesabili di reagenti
Merrifield Resin HL capacità di carico:	1.2 mmol/g			25	1. Procedura per il carico - Yajma et al., Tetrahedron 1988, 44, 805-819. Basandosi sulla capacità di carico della resina, che è di 1.2 mmol/g, ed utilizzando 1.5 eq di amminoacido, si pesano le quantità in grammi indicate a fianco di ciascun amminoacido. La quantità di resina necessaria è 25 mg. Gli amminoacidi vengono sciolti/sospesi in 250 µL di DMF. Si aggiunge la resina e un agitatore magnetico. Si aggiunge 5 mg di potassio fluoruro. Si mantiene la miscela a 50°C per 24 ore. Si centrifuga e si lava il pellet di resina con DFM, poi con acqua e DMF 1:1, metanolo e acqua 1:1, e infine metanolo. La resina viene seccata.
	PM	densità			
Fmoc-Gly-OH	297.31			13	
Fmoc-Ala-OH	311.34			14	
Fmoc-Val-OH	339.39			15	
Fmoc-Leu-OH	353.42			16	
Fmoc-Nle-OH	353.42			16	2. Procedura per la rimozione della protezione Fmoc La resina viene sospesa in 450 µL di una miscela di piperidina e DMF 20:80 v/v La miscela viene agitata per 2 minuti, centrifugata, risospesa nella miscela piperidina/DMF, agitata per 5 min, filtrata e lavata con DMF
Fmoc-Pro-OH	337.13			15	
Fmoc-Phe-OH	387.44			17	
Fmoc-Gln-OH	354.36			16	
Fmoc-Arg(Pbf)OH	634.75			29	3. Procedura per il coupling La resina viene sospesa in 450 µL di DMF. 31 mg (5 eq) di TFPA vengono sciolti in 50-100 µL di DMF. 31 mg (5.5 eq) di HOBt vengono sciolti nel minimo volume di DMF e aggiunti alla resina. Infine vengono aggiunti 32mg di EDC e la sospensione viene lasciata in agitazione per 4 ore. La resina viene quindi lavata con DMF e metanolo.
Fmoc-Gaba-OH	325.36			15	
BA	106.12	1.04		2	15
PYA	107.11	1.126		1	14
VAN	s	152.15	1	2	23
PNBA	s	151.12	1	2	23
PAA	deliq.	120.15	1.03	2	17
HCA		134.18	1.019	2	20
CHA		112.17	0.926	2	18
IVA		86.13	0.803	2	16
PIVA		86.13	0.793	2	16
C11A		156.27	0.825	3	28
KF		58.1		5	
TFPA		204.15			
EDC		191.7			
HOBT		135.12			
TFMSA					
TFA					
DMF					
DCM					
MeOH					
Et2O					
Pipe					