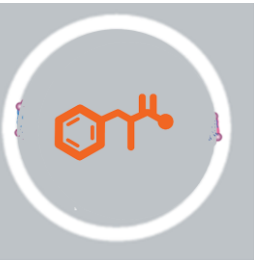


Modulo B3

Sintesi in fase solida di peptidi e proteine

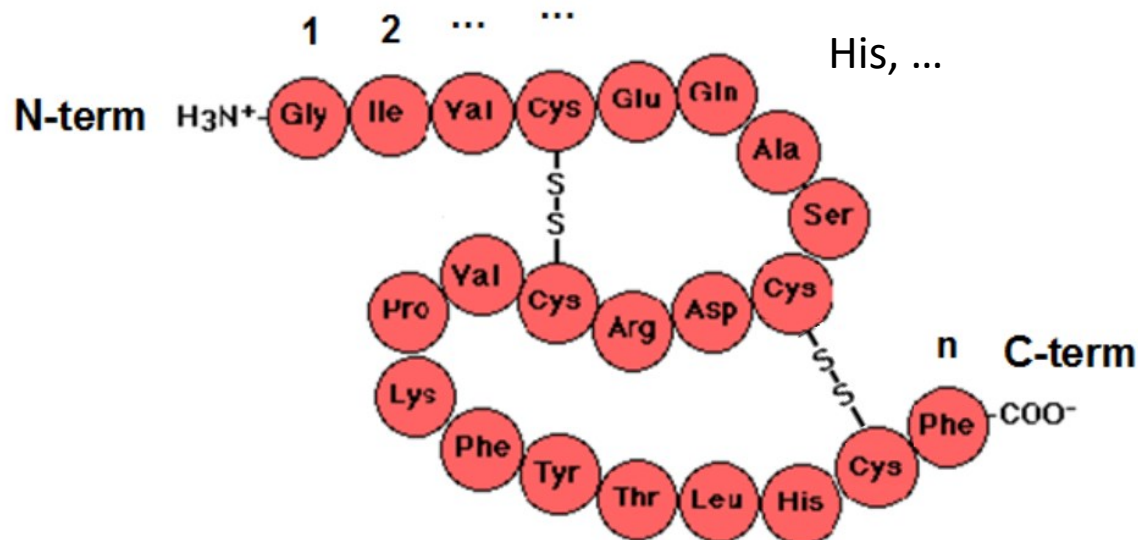
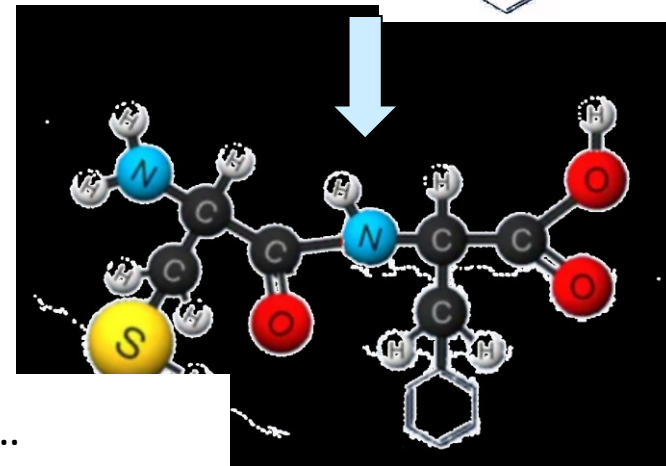
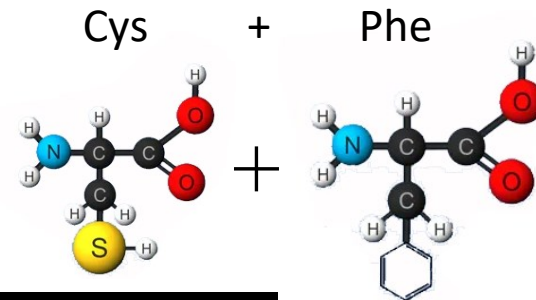


2023-24

SPPS – Sintesi in fase solida di peptidi e proteine

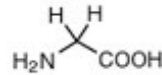
Protein production:

- Biochemical (extraction)
- Recombinant
- **Chemical synthesis**
- Semisynthesis

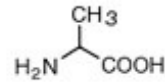


Caratteristiche degli amminoacidi

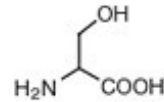
Small



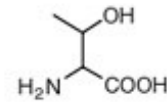
Glycine (Gly, G)
MW: 57.05



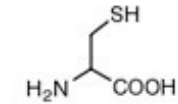
Alanine (Ala, A)
MW: 71.09



Serine (Ser, S)
MW: 87.08, pK_a ~ 16

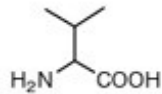


Threonine (Thr, T)
MW: 101.11, pK_a ~ 16

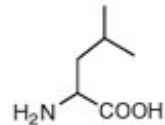


Cysteine (Cys, C)
MW: 103.15, pK_a = 8.35

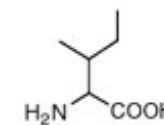
Hydrophobic



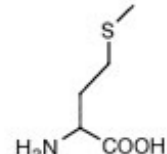
Valine (Val, V)
MW: 99.14



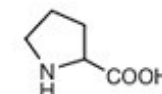
Leucine (Leu, L)
MW: 113.16



Isoleucine (Ile, I)
MW: 113.16

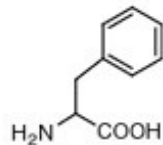


Methionine (Met, M)
MW: 131.19

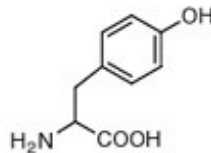


Proline (Pro, P)
MW: 97.12

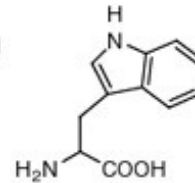
Aromatic



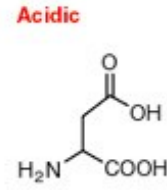
Phenylalanine (Phe, F)
MW: 147.18



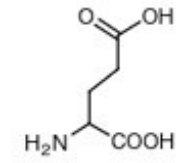
Tyrosine (Tyr, Y)
MW: 163.18



Tryptophan (Trp, W)
MW: 186.21

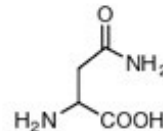


Aspartic Acid (Asp, D)
MW: 115.09, pK_a = 3.9

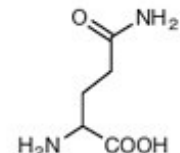


Glutamic Acid (Glu, E)
MW: 129.12, pK_a = 4.07

Amide

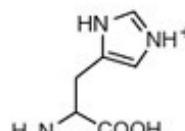


Asparagine (Asn, N)
MW: 114.11

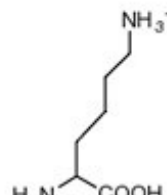


Glutamine (Gln, Q)
MW: 128.14

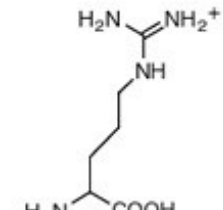
Basic



Histidine (His, H)
MW: 137.14, pK_a = 6.04



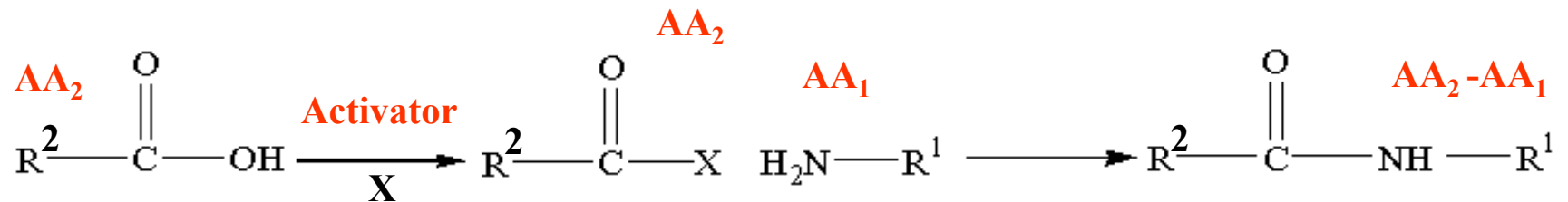
Lysine (Lys, K)
MW: 128.17, pK_a = 10.79



Arginine (Arg, R)
MW: 156.19, pK_a = 12.48

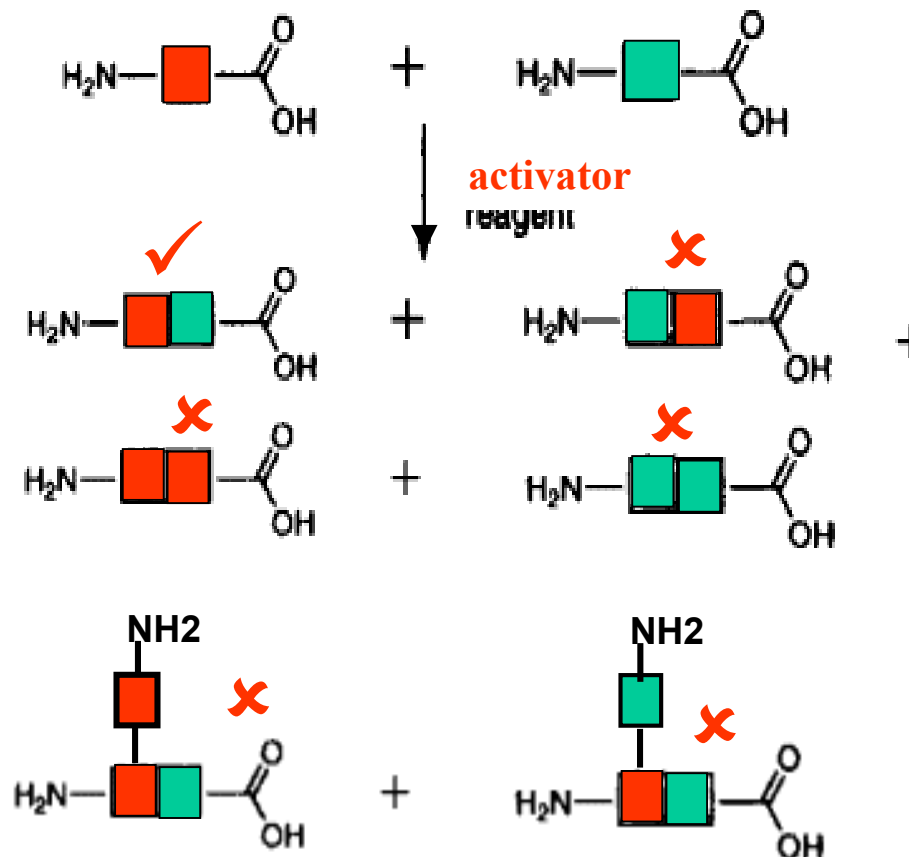
Amino acids have different reactive groups: α-ammino, α-carboxyl
side-chain ammino, amido, carboxyl, hydroxyl,
sulphydril, guanidino, imidazole, indole

Sintesi peptidica



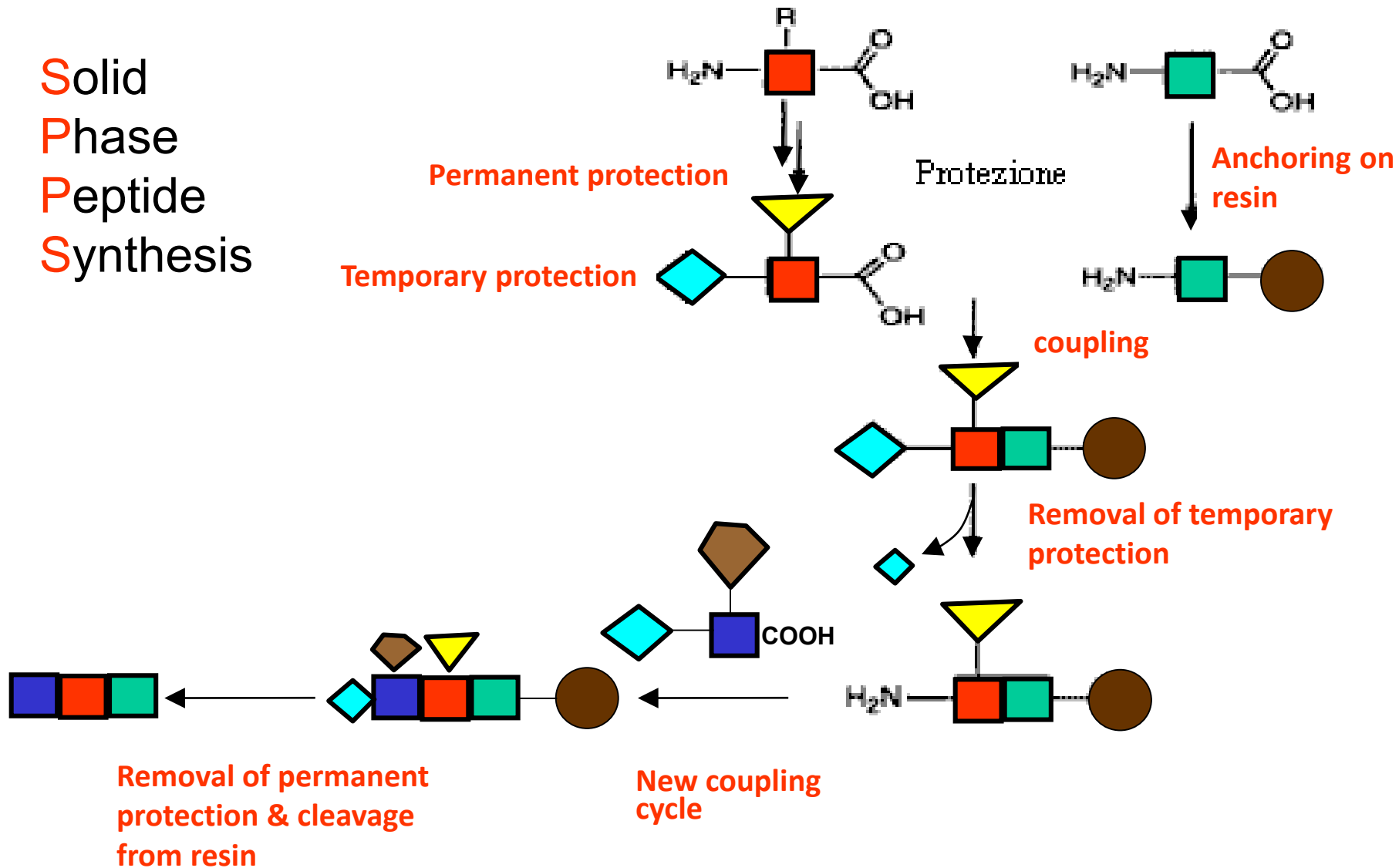
Solution synthesis without protection

Problem with
side products

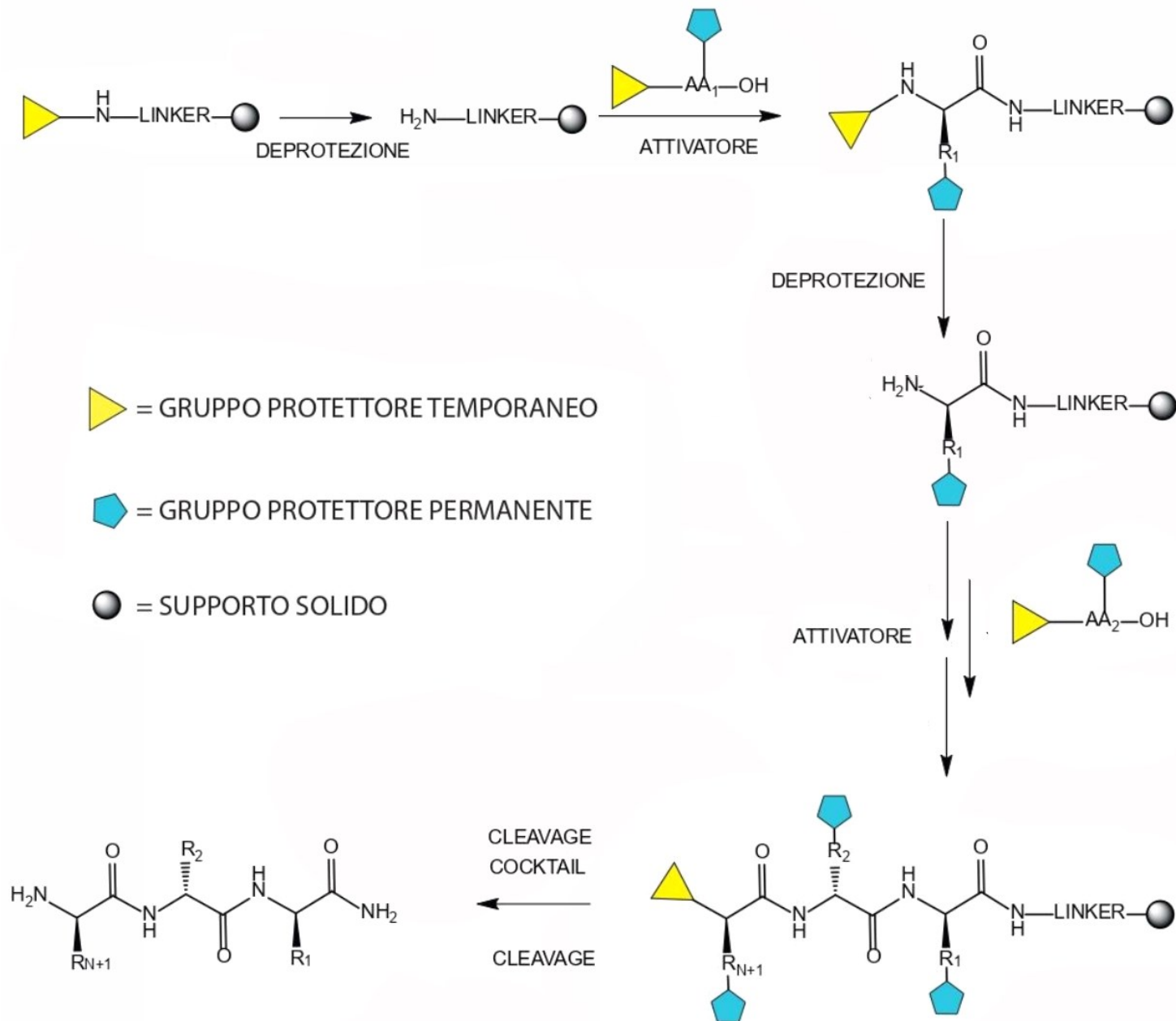


SPPS – Solid Phase Peptide Synthesis

Solid Phase Peptide Synthesis



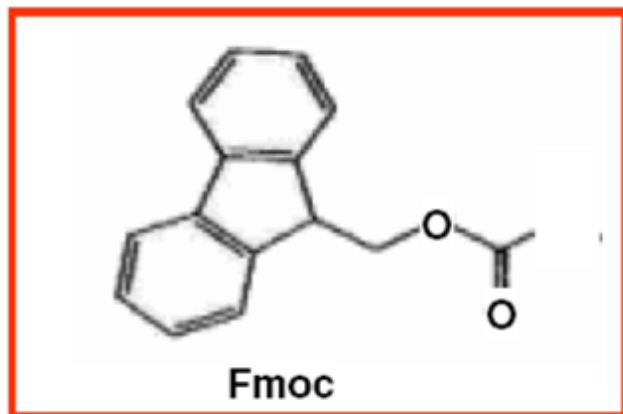
SPPS – Solid Phase Peptide Synthesis (cont.)



Protezione – temporanea e permanente

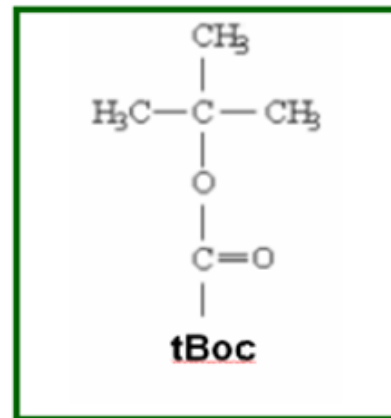
Protezione temporanea sul gruppo α -amminico

Fluorenilmetossicarbonile



labile in base
(20% piperidina)

*tert*Butossicarbonile

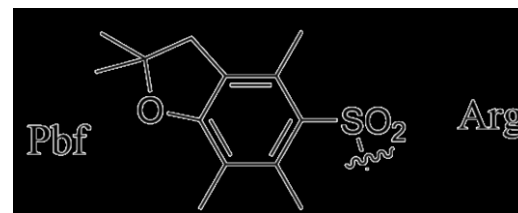
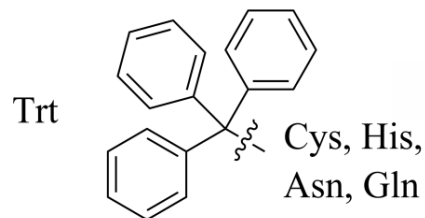
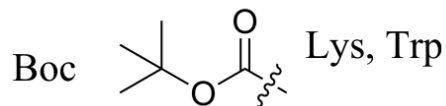
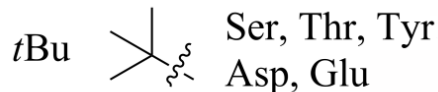


labile in acido
(TFA)

Legame alla resina e protezione permanente sulle catene laterali

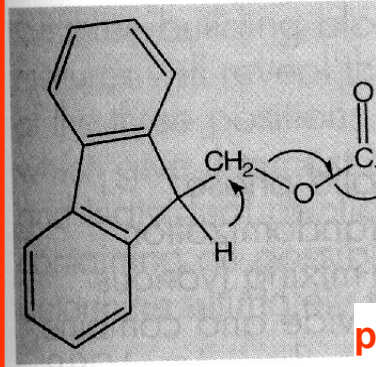
- Labile in acido (TFA)
- Ortogonale

- Labile in acido molto forte (HF)
- Parzialmente ortogonale



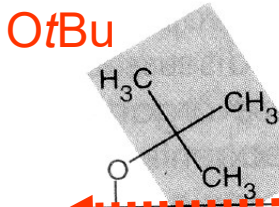
SPPS summary

Fmoc

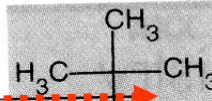


piperidine

tert-butile
OtBu



OtBu



TFA

TFA

Fmoc/*tert*-butile chemistry

resina

linker

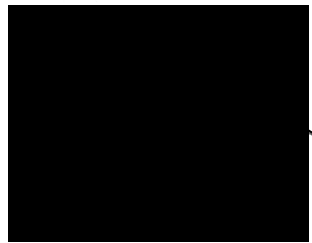
orthogonal protection (acid/base deprotection)

tBoc/benzyl chemistry

nonorthogonal
protection

oBz

tBoc



TFA

HF

resina

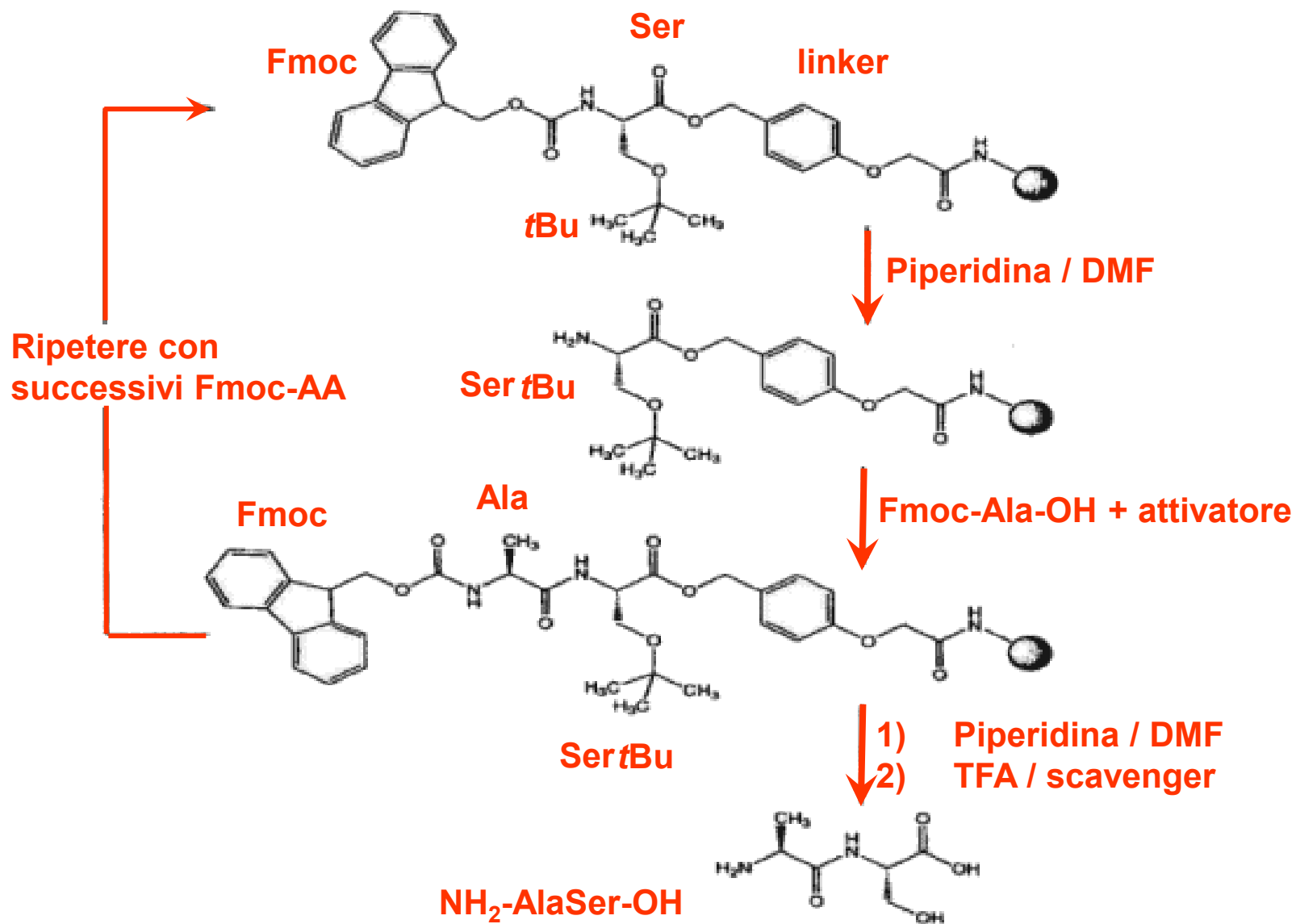
• Fmoc / *tert*-butile:

- Fmoc base-labile (20% piperidine)
- Side-chains/resin link acid-labile (TFA)

• tBoc / benzyl:

- tBoc acid-labile (TFA)
- side-chains/anchor strong acid labile (hydrofluoric acid, HF)

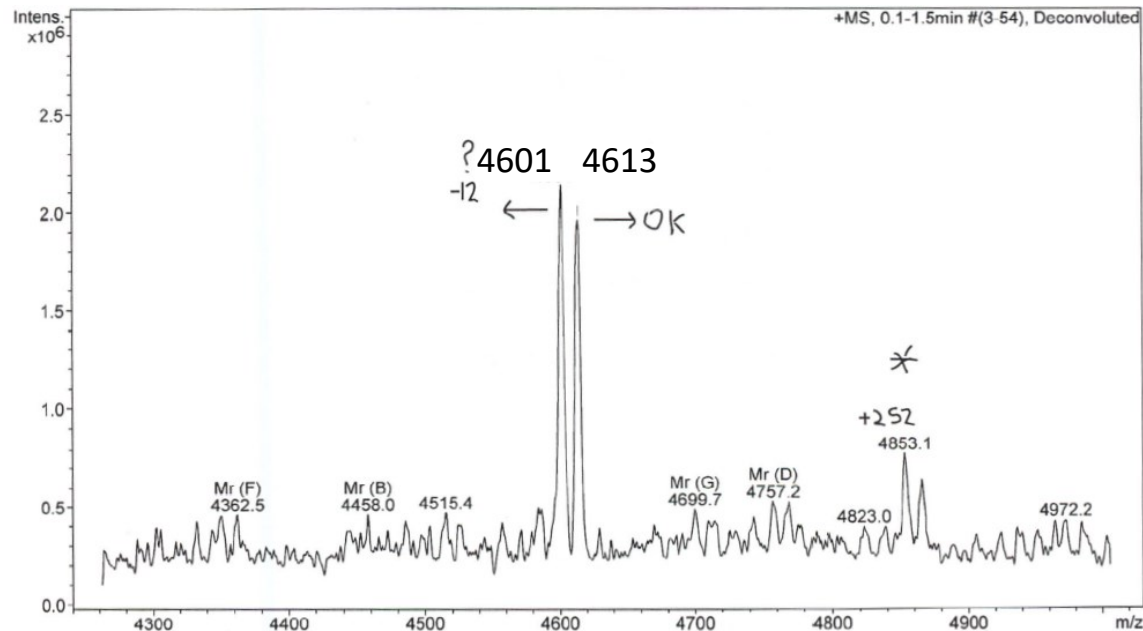
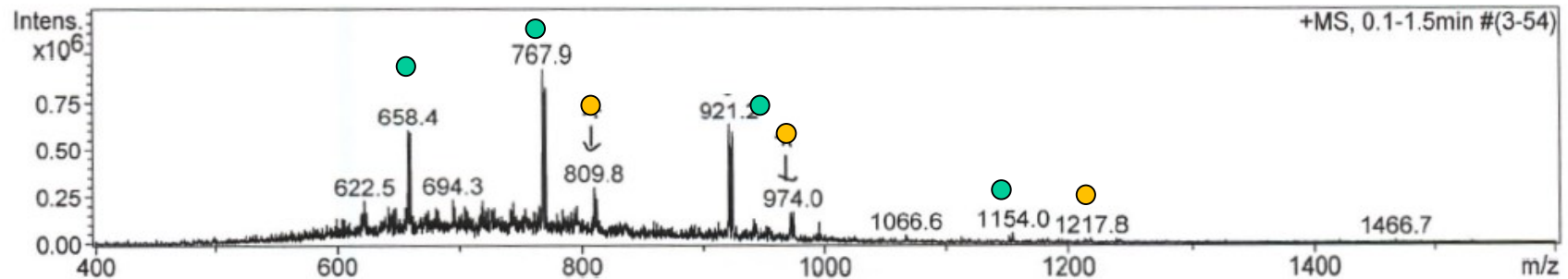
SPPS Fmoc – cicli di sintesi



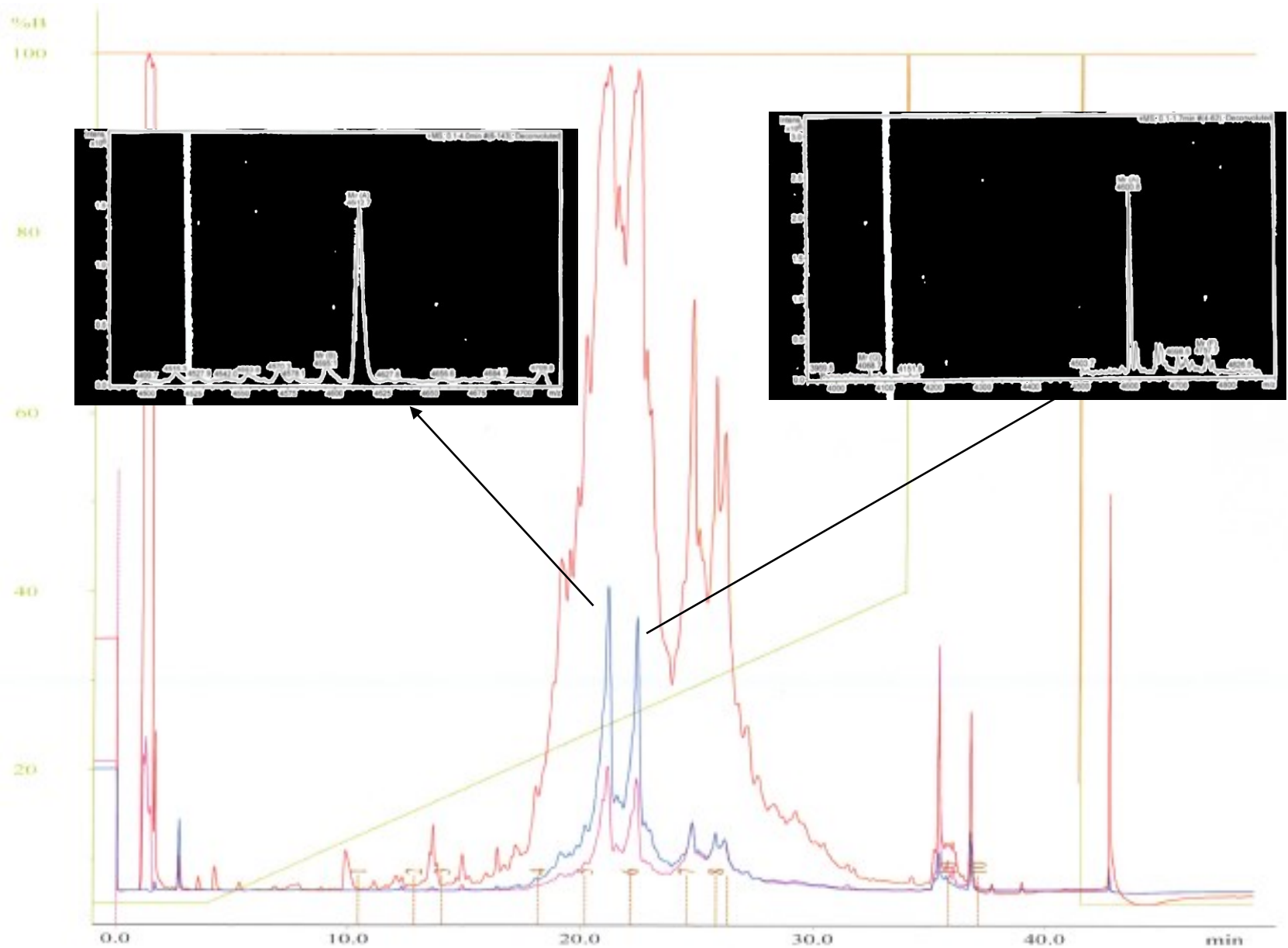
SPPS – esempio di sintesi

Sintesi di un peptide antimicrobico di mitile

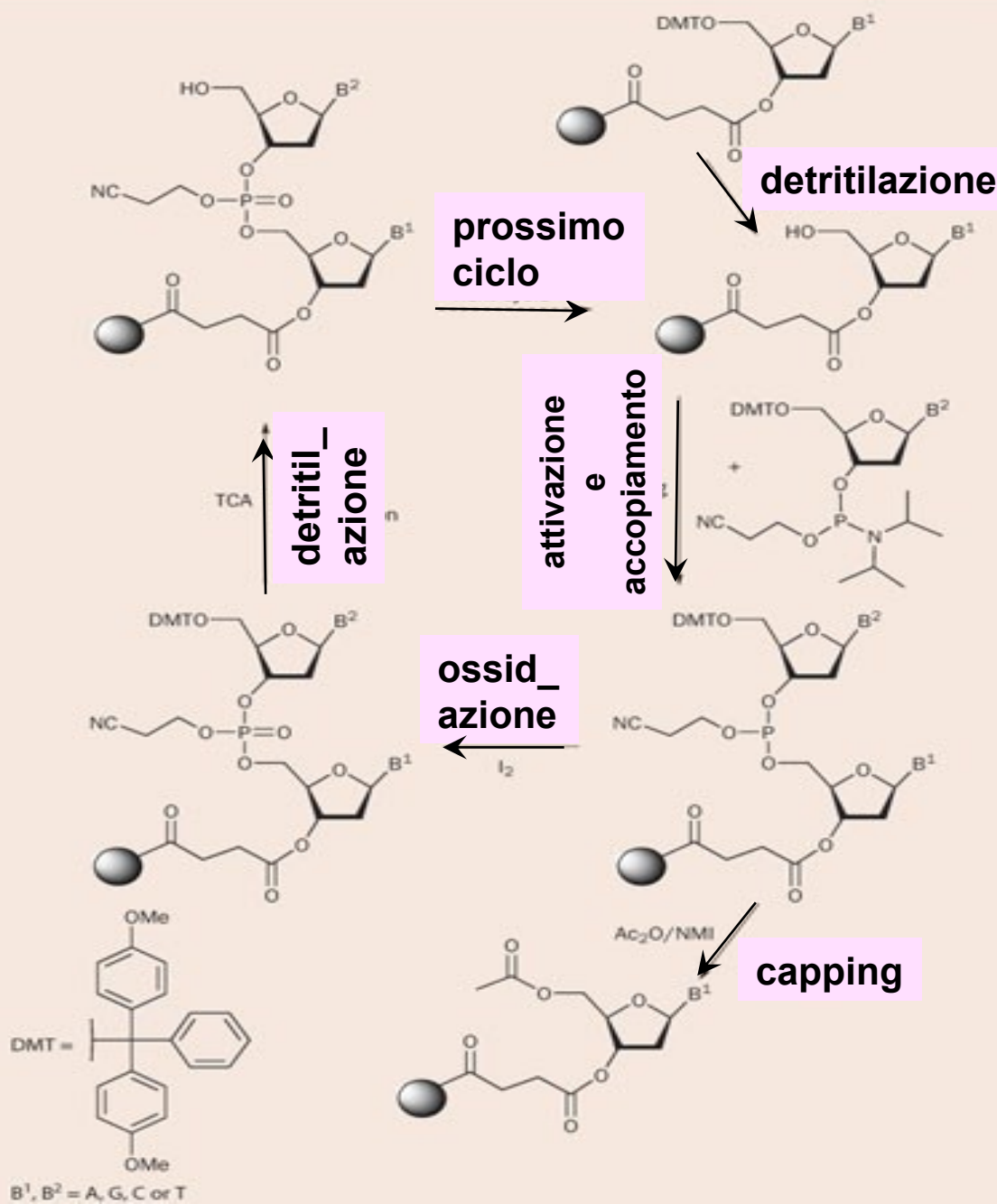
YSWPRMPRIPLPRYPYPRYPG MW = 4613 Da



SPPS – esempio di sintesi (cont.)



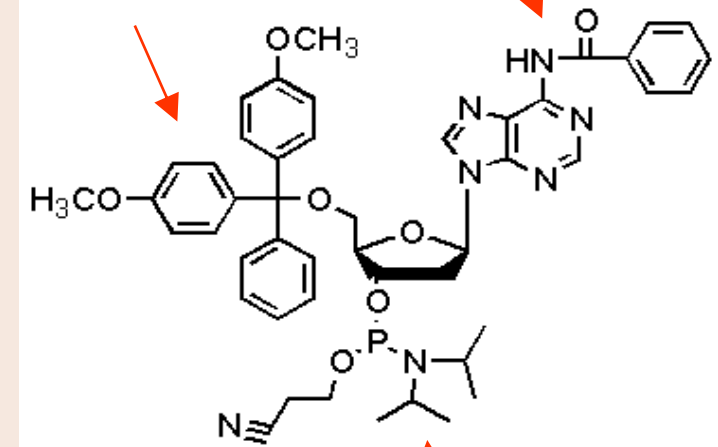
Sintesi in fase solida di DNA



Residuo nucleotidico protetto

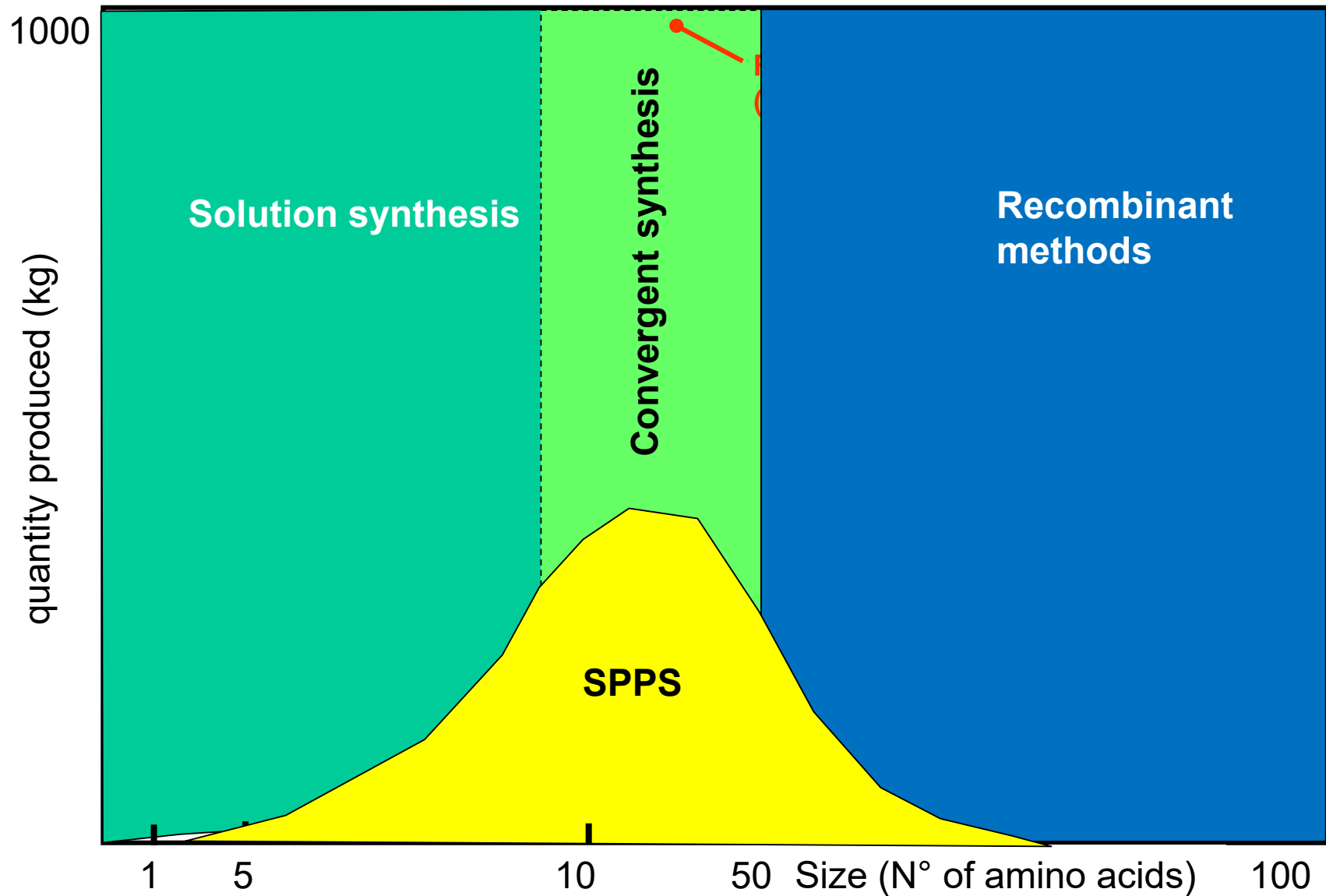
dimetossitritile DMT

Bz benzoi



N-6-benzoyl-deoxyadenosine phosphoramidite

Poly peptide synthesis – Choice of method



Synthetic peptide applications

- **USO TERAPEUTICO** - AIDS, controllo nascite, antitumorali, controllo della crescita, ipertensione, immunostimolazione e soppressione, infertilità, osteoporosi, diabete
(fuzeon, insulina, interferone/interleuchine, calcitonina)
- **USO VETERINARIO** - fattori di crescita, ormoni, vaccini
- **PRODOTTI ALIMENTARI** - dolcificanti, esaltatori antiossidanti
(aspartame)
- **AGRICOLTURA** - erbicidi, insetticidi, metabolismo vegetale, antibiotici
- **PRODOTTI INDUSTRIALI** - fibre, sostanze adesive

Miliardi di \$ per anno