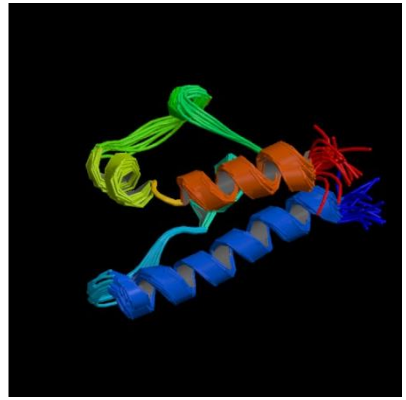
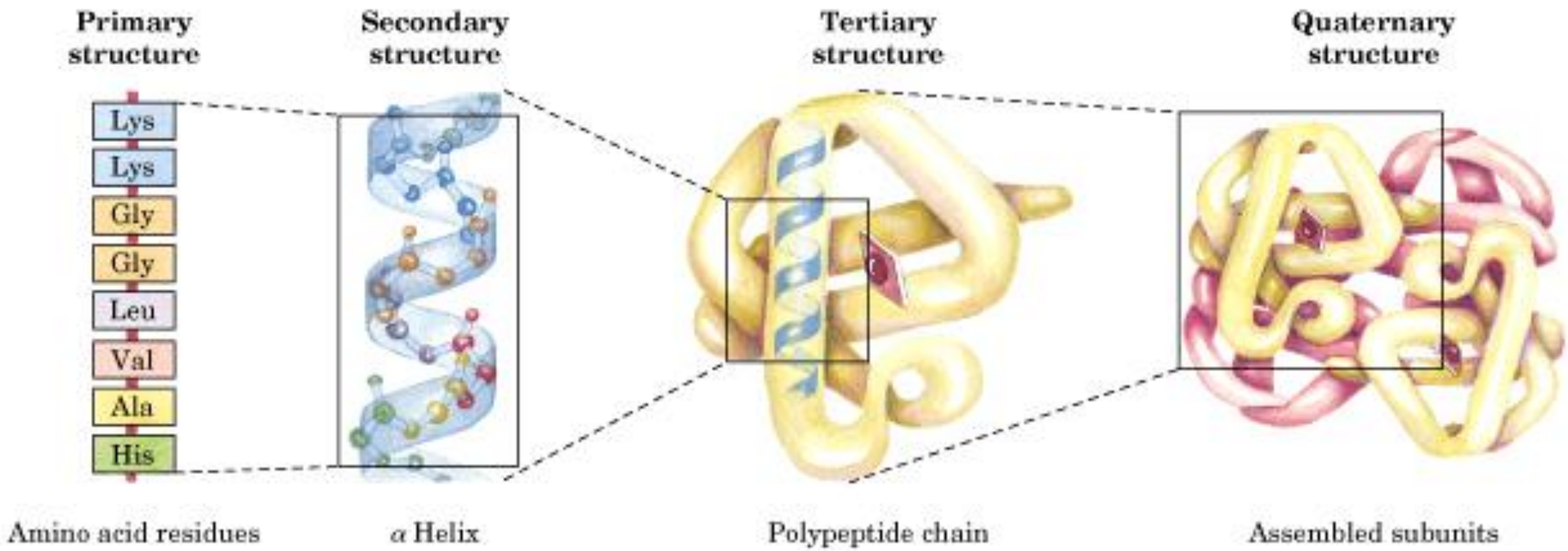
Tema 5:



Frederick Sanger
Premio Nobel en Química
en 1958 y en 1980

Proteínas
Estructura primaria
Secuenciamiento y síntesis de péptidos



Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain
Assembled subunits

1 Hidrólisis química total y análisis cuali-cuantitativo de aminoácidos

2 Determinación de grupos terminales

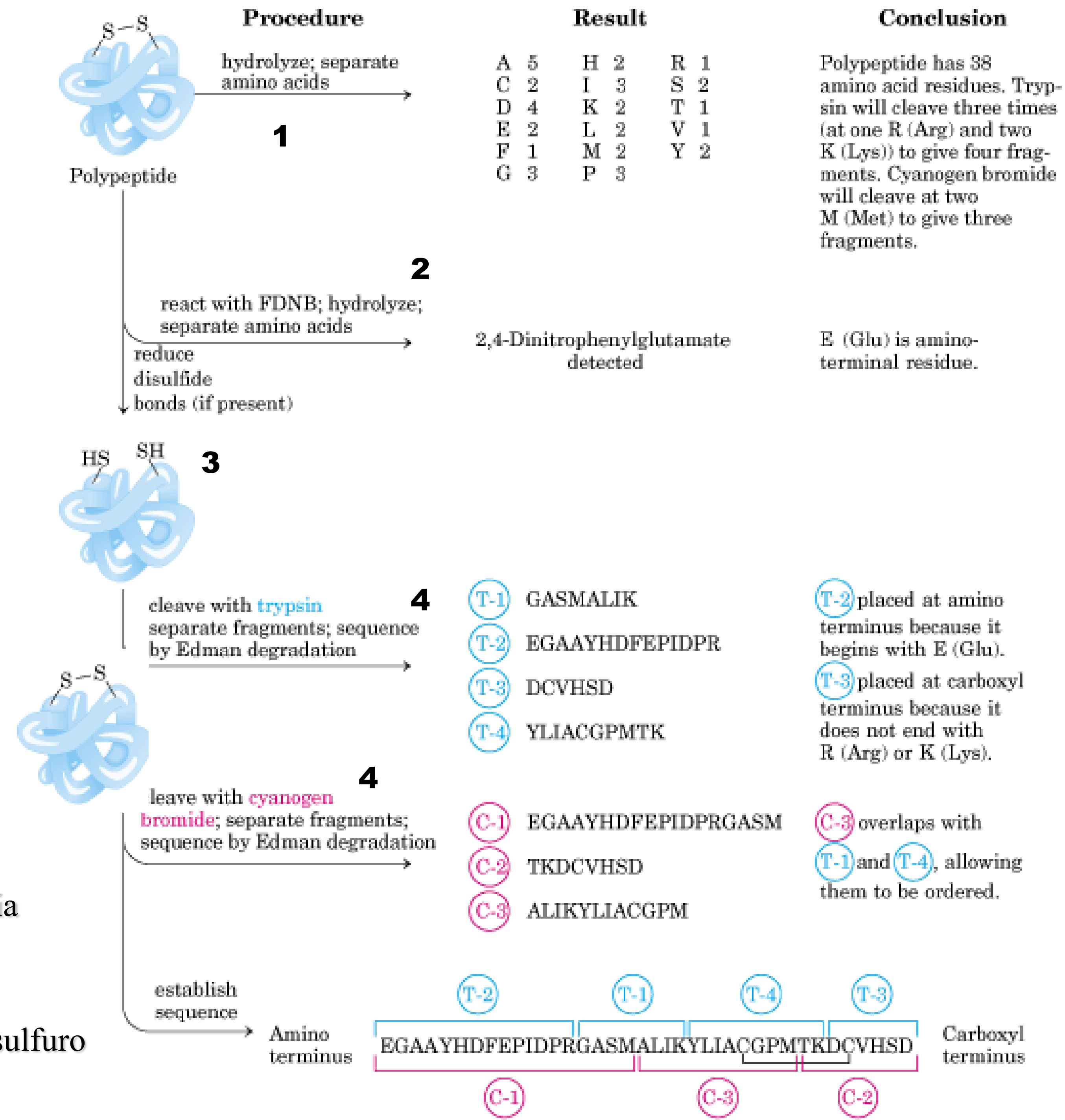
3 Reducción u oxidación de puentes disulfuro

4 Hidrólisis parcial

Secuenciación de péptidos

Establecimiento de secuencia

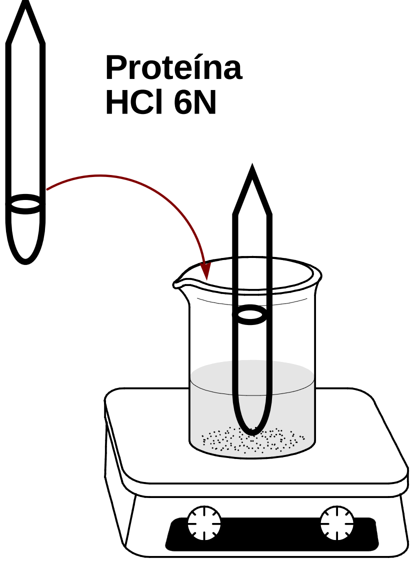
Localización de puentes disulfuro



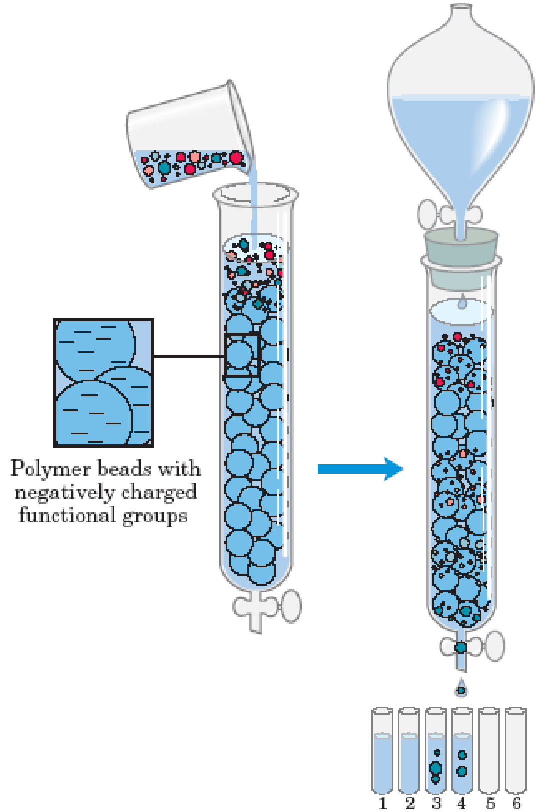
1

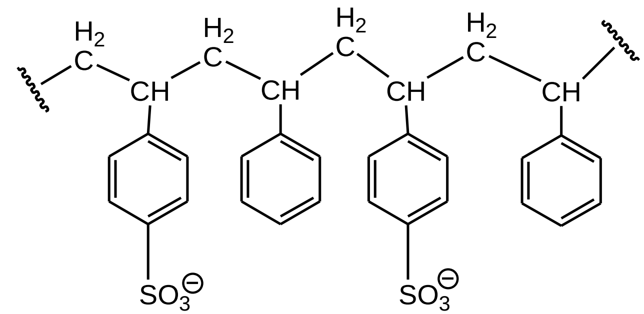
Hidrólisis química total y análisis cuali-cuantitativo de aminoácidos

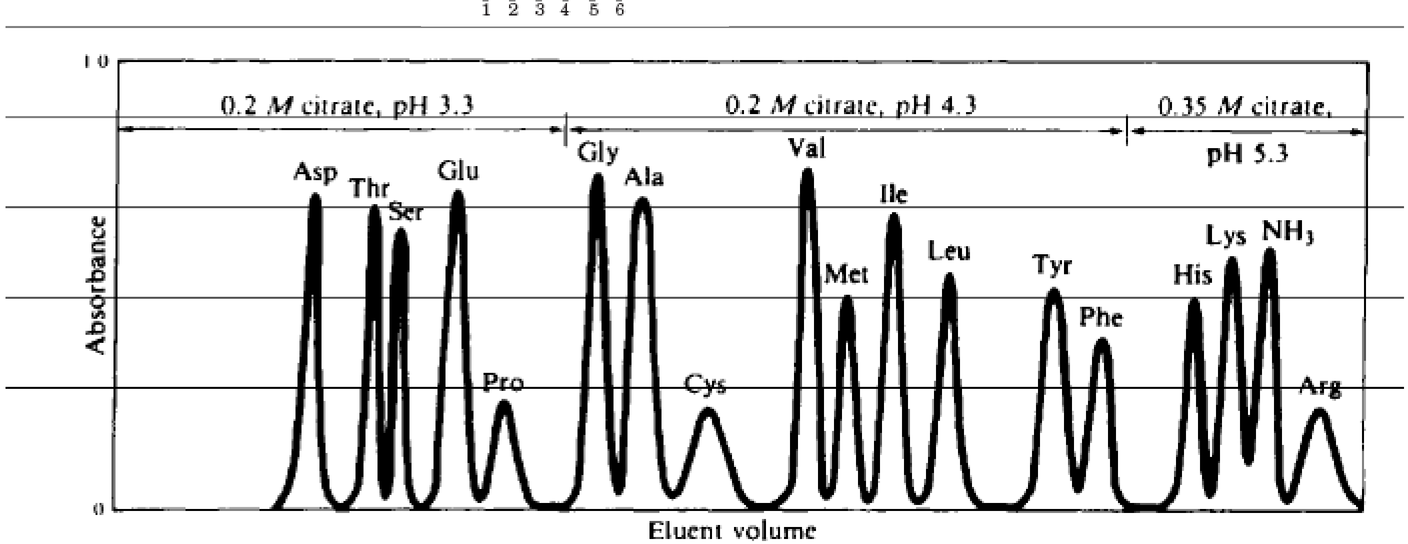
Separación resina de intercambio catiónico



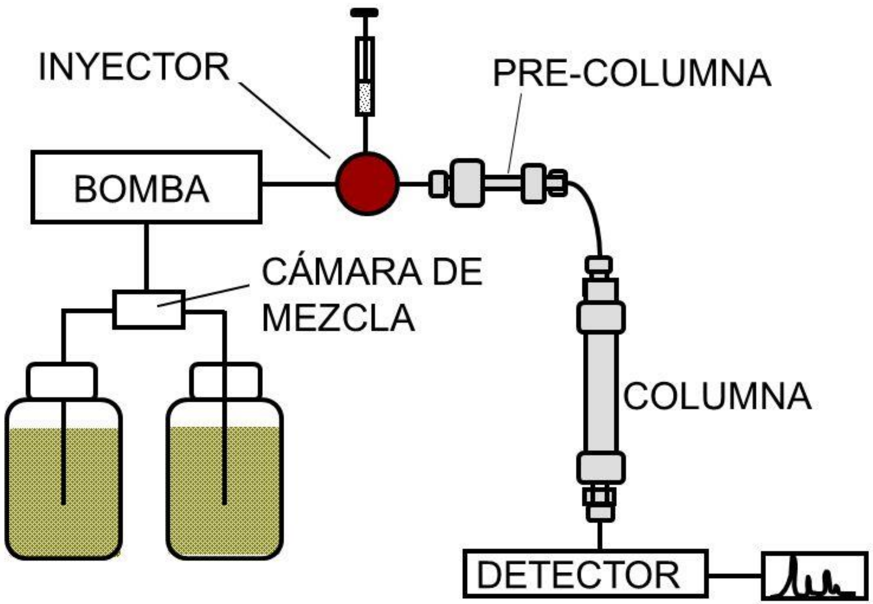
105-110ºC
24h

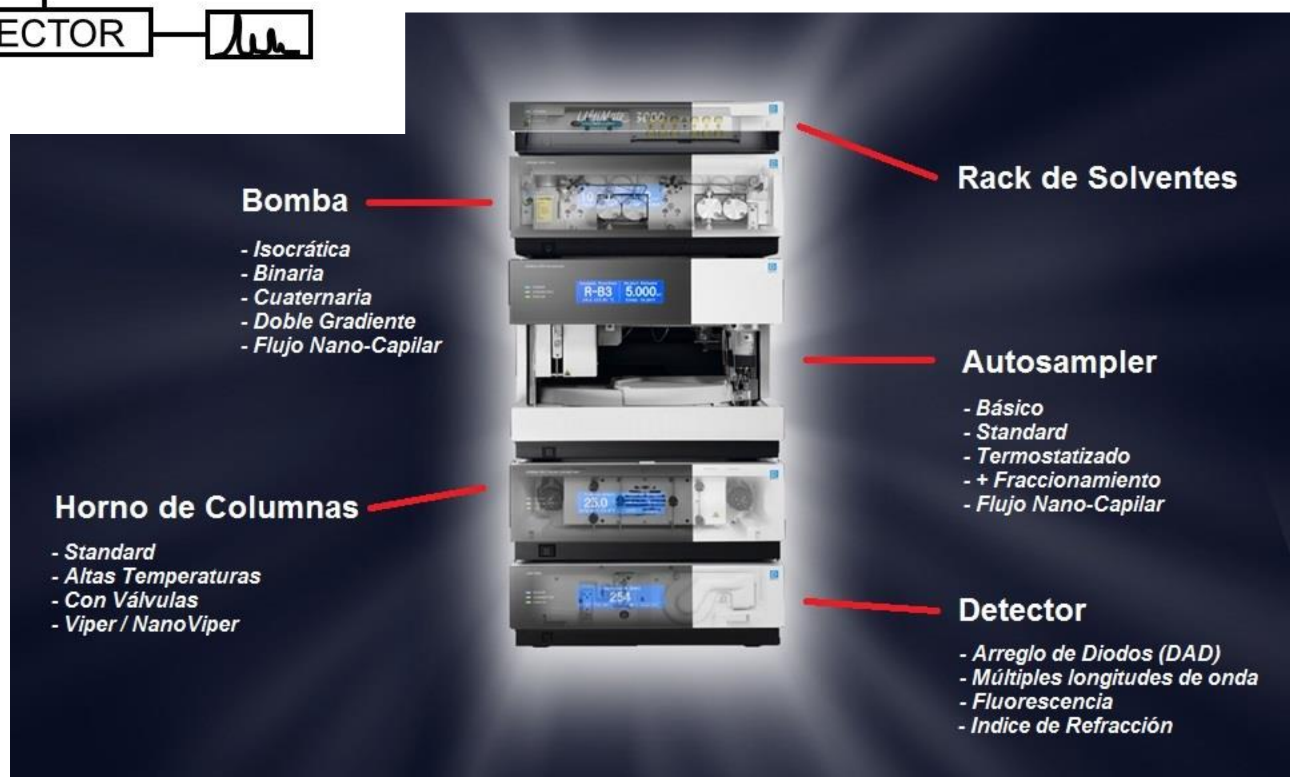


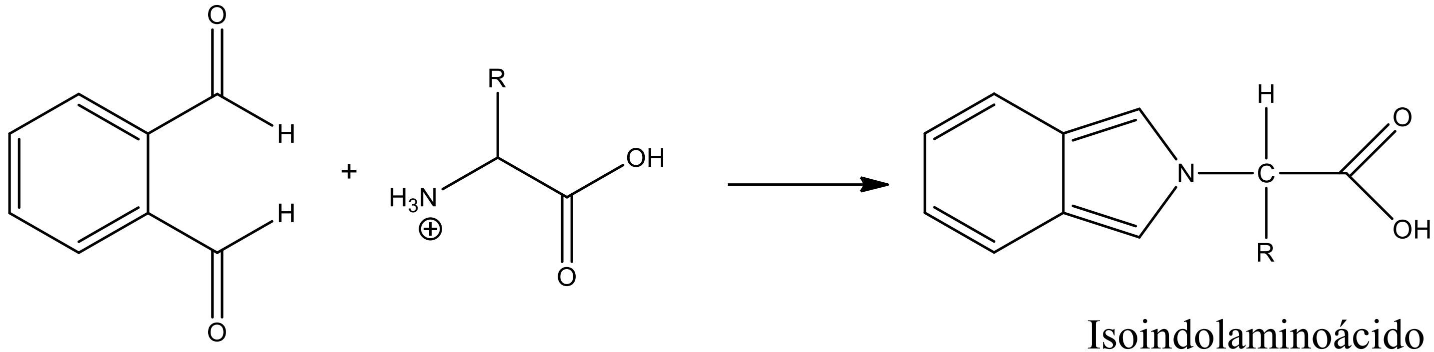




Esquema del equipo de HPLC







Isoindolaminoácido (fluorescente)

o-ftaldehído

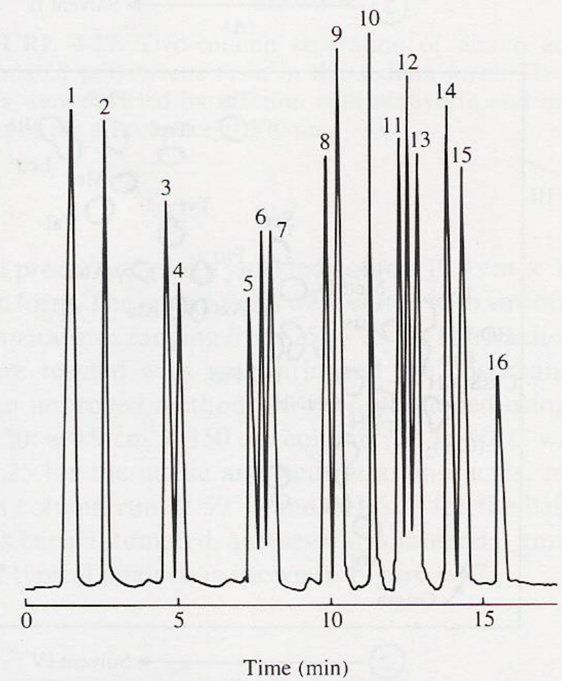
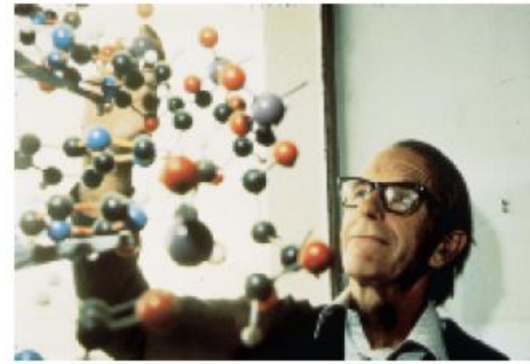


FIGURE 4-29. HPLC analysis of *o*-phthalaldehyde amino acids on a reversed-phase Microsorb Short-one C18 column (Rainin, Woburn, MA) using a 1.7 mL/min gradient elution of solvent A (0.1 M sodium acetate adjusted to pH 6.2 with acetic acid) and solvent B (methanol) with fluorescence detection. Peaks: 1 = Asp, 2 = Glu, 3 = Ser, 4 = His, 5 = Gly, 6 = Thr, 7 = Arg, 8 = Ala, 9 = Tyr, 10 = Abu, 11 = Met, 12 = Val, 13 = Phe, 14 = Ile, 15 = Leu, 16 = Lys.

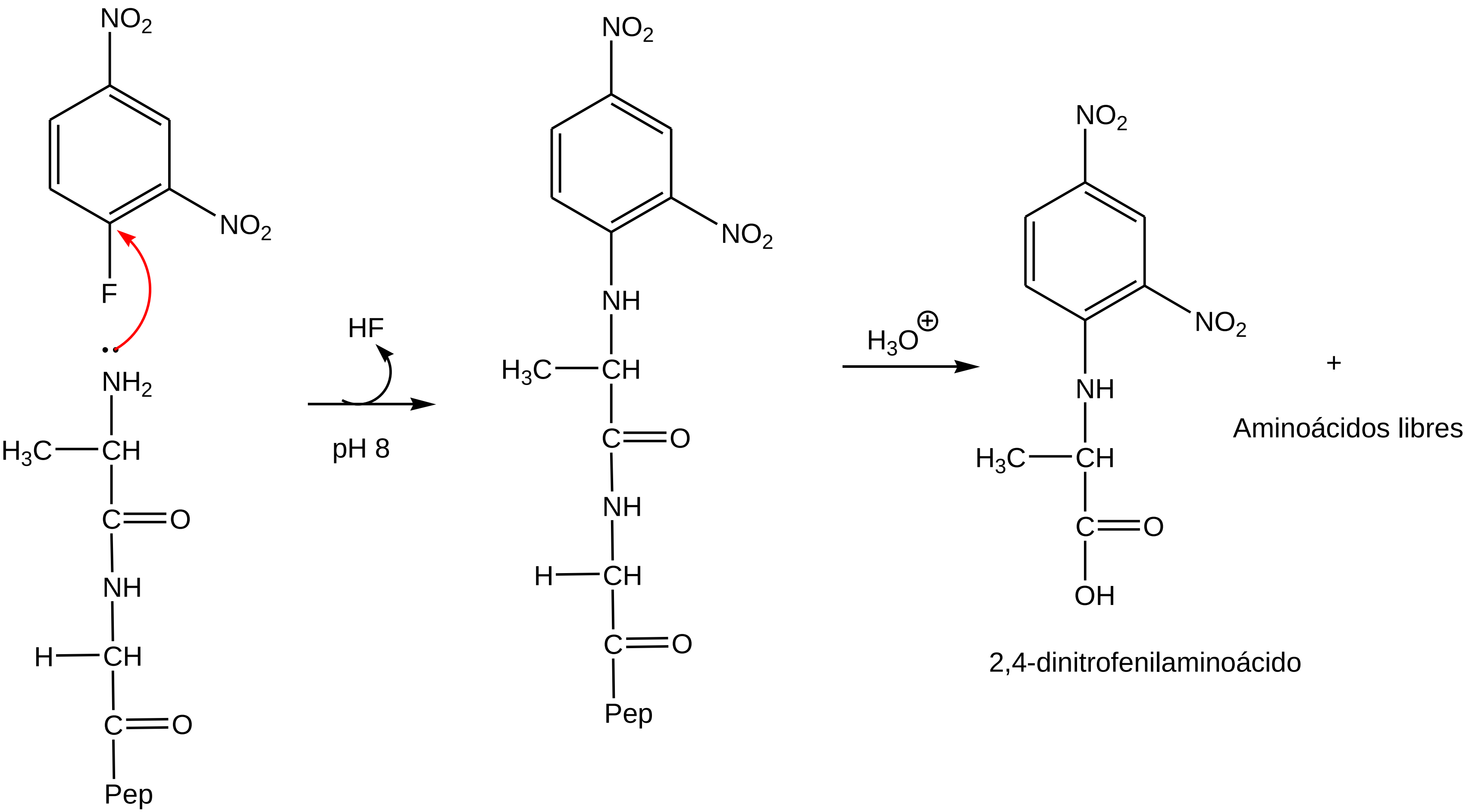
2

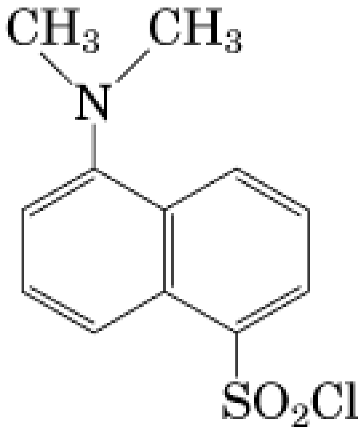
Determinación de grupos terminales

Reacción de Sanger

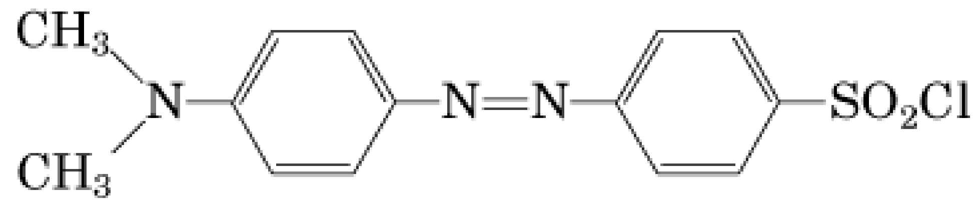


Frederick Sanger

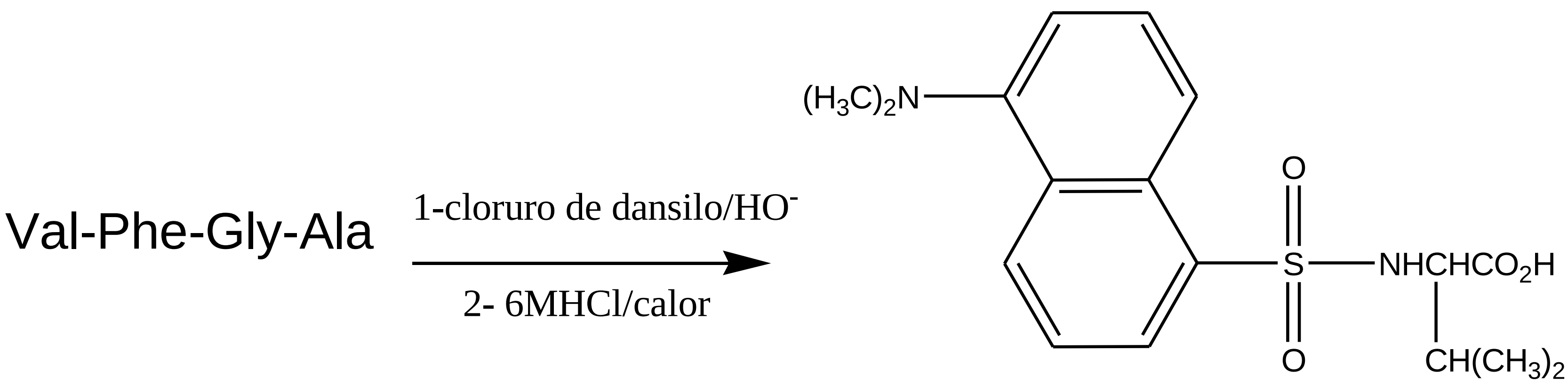




Dansyl chloride



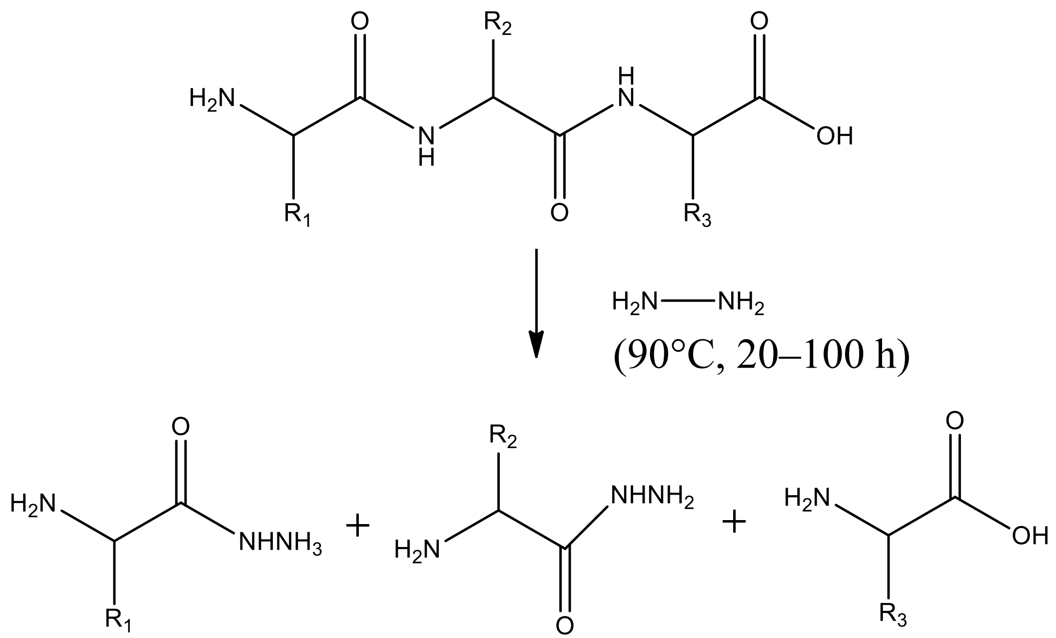
Dabsyl chloride



Determinación de carboxilo terminal

-Hidrazinólisis (NH_2-NH_2)

Un péptido que es tratado con hidrazina (90°C, 20–100 h) genera **amino-acil hidrazidos** de todos los aminoácidos excepto el residuo C-terminal que se puede identificar cromatográficamente comparado con los estándares del aminoácido.

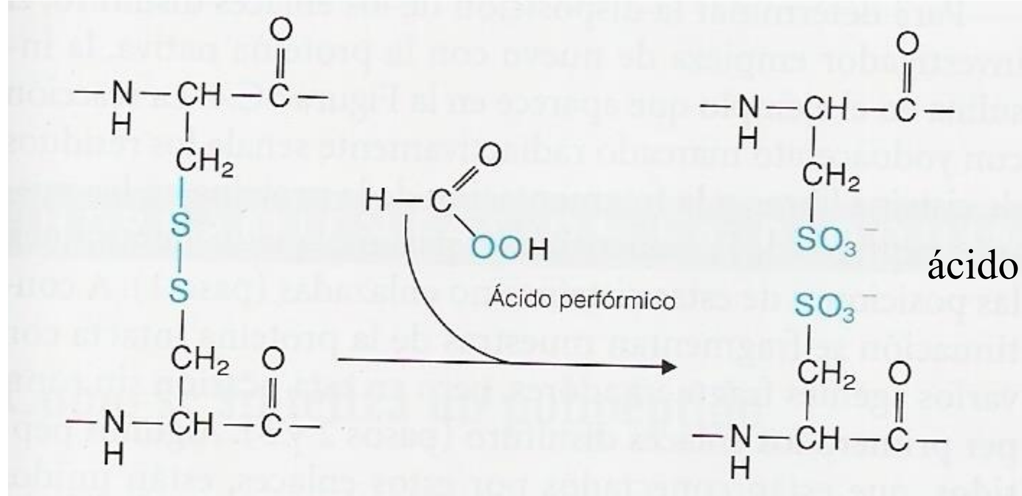


-Carboxipeptidasa A

Enzima que hidroliza selectivamente el último enlace peptídico

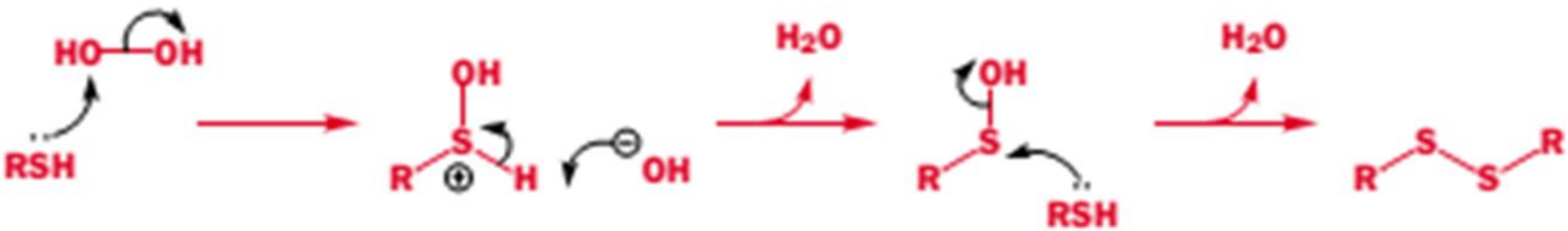
3
Reducción u oxidación de puentes disulfuro

Oxidación

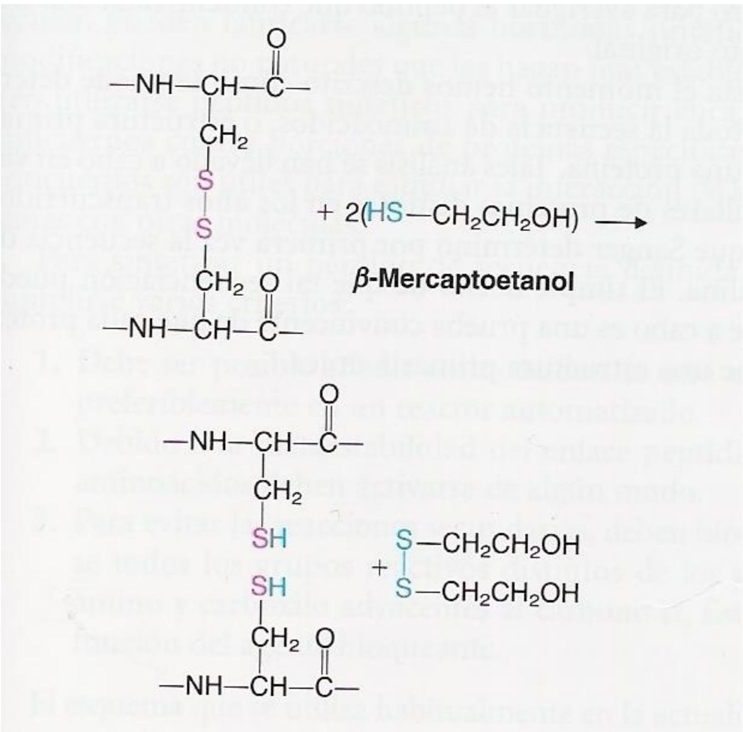


ácido cisteico

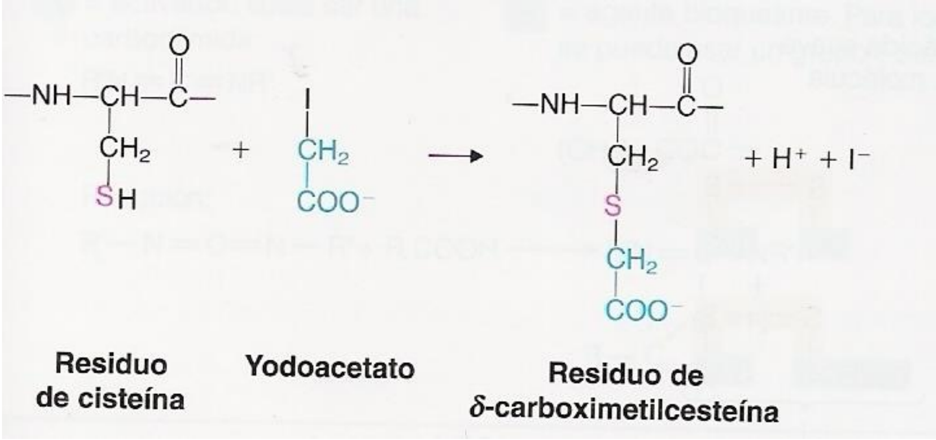
En presencia de peróxidos..

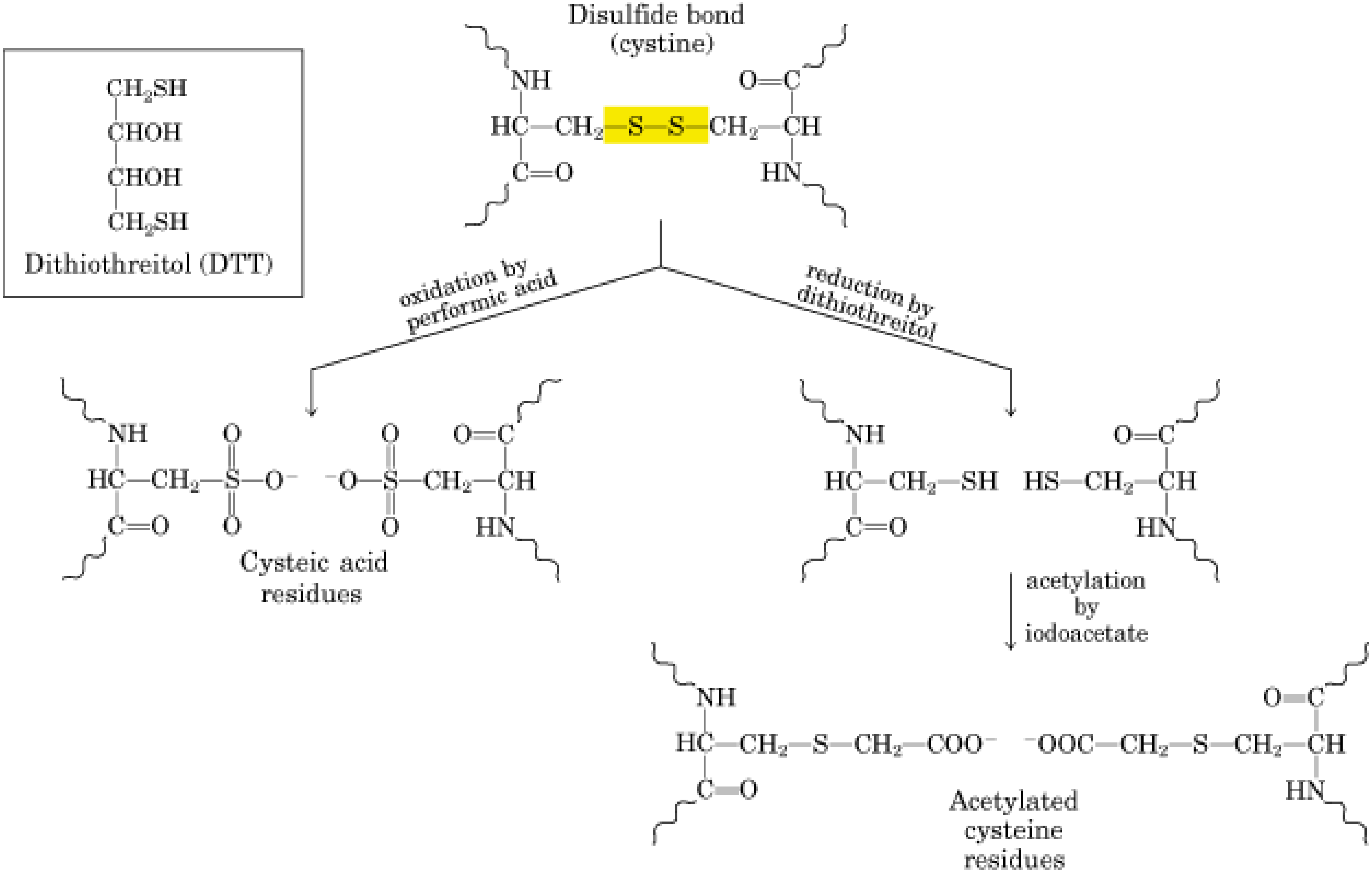


Reducción



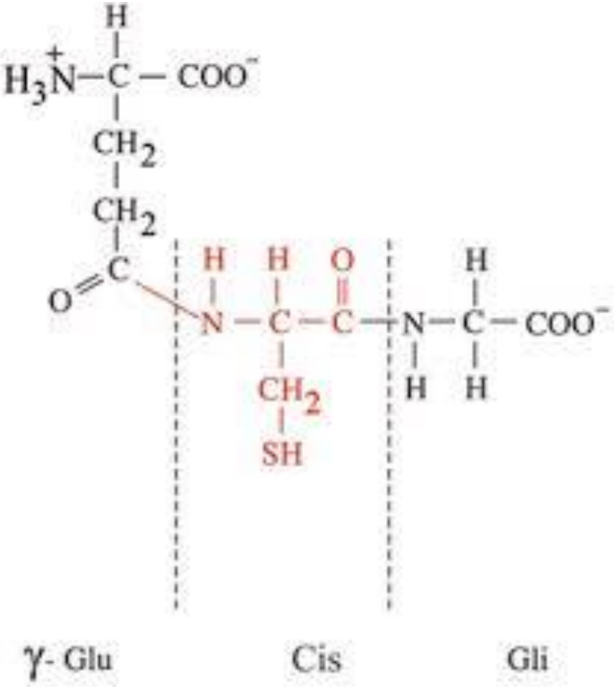
Bloqueo de sulfhidrilos libres





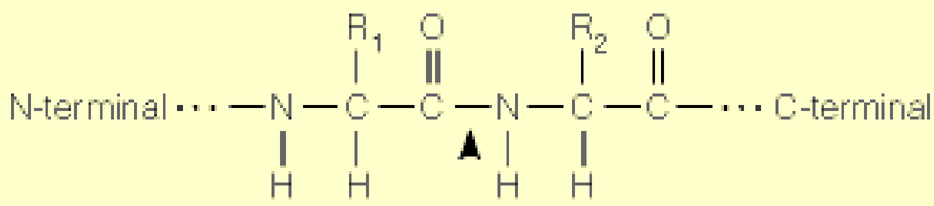
Disulfide bond
(cystine)
Dithiothreitol (DTT)
oxidation by
performic acid
reduction by
dithiothreitol
Cysteic acid
residues
acetylation
by
iodoacetate
Acetylated
cysteine
residues

Glutatión: un antioxidante natural



4
Hidrólisis parcial

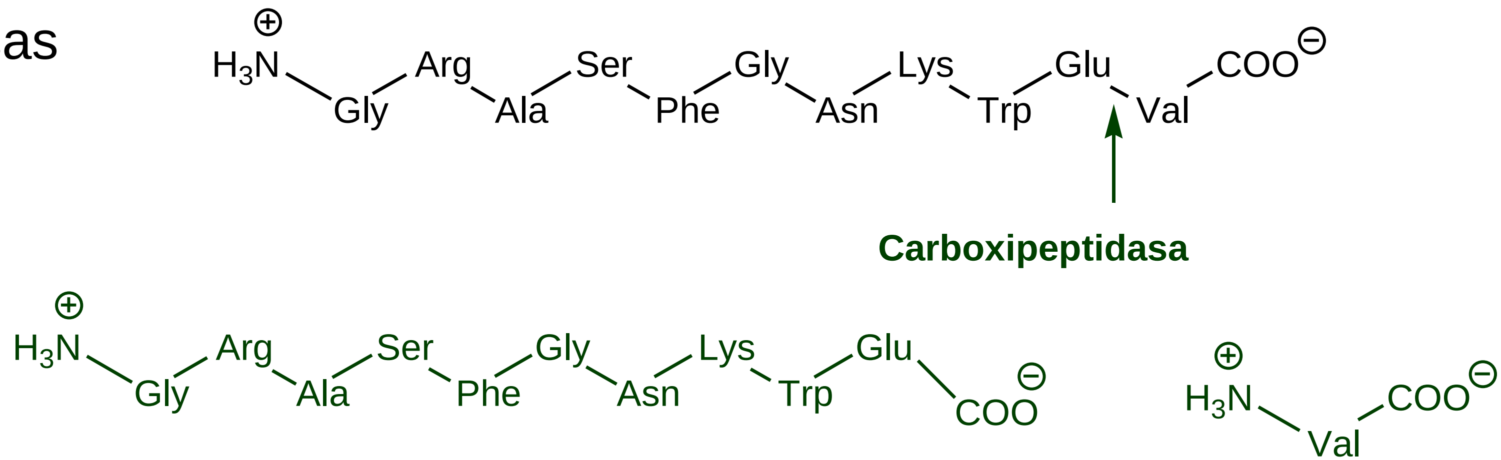
Hidrólisis enzimática



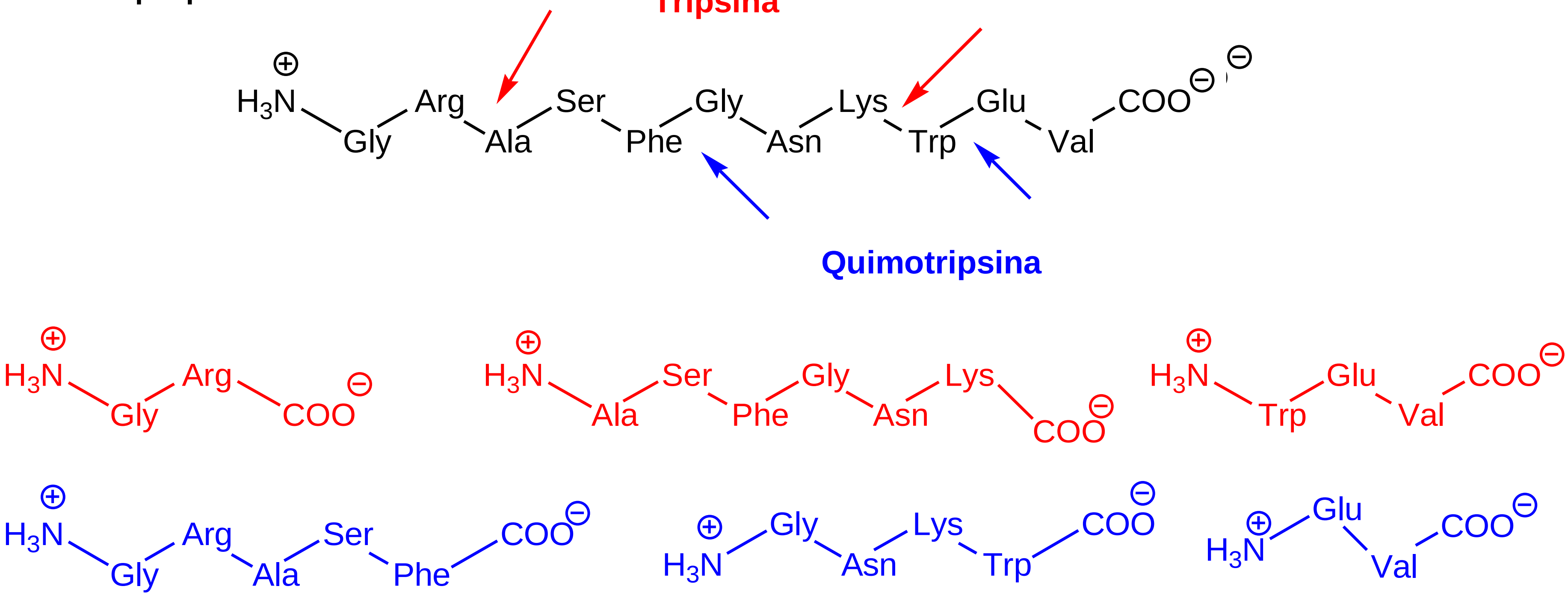
Enzyme	Preferred Site[a]	Source
Trypsin	R_1 = Lys, Arg	From digestive systems of animals, many other sources
Chymotrypsin	R_1 = Tyr, Trp, Phe, Leu	Same as trypsin
Thrombin	R_1 = Arg	From blood; involved in coagulation
V-8 protease	R_1 = Asp, Glu	From *Staphylococcus aureus*
Prolyl endopeptidase	R_1 = Pro	Lamb kidney, other tissues
Subtilisin	Very little specificity	From various bacilli
Carboxypeptidase A	R_2 = C-terminal amino acid	From digestive systems of animals
Thermolysin	R_2 = Leu, Val, Ile, Met	From *Bacillus thermoproteolyticus*

[a]The residues indicated are those next to which cleavage is most likely. Note that in some cases preference is determined by the residue on the N-terminal side of the cleaved bond (R_1) and sometimes by the residue to the C-terminal side (R_2). Generally, proteases do not cleave where proline is on the other side of the bond. Even prolyl endopeptidase will not cleave if R_2 = Pro.

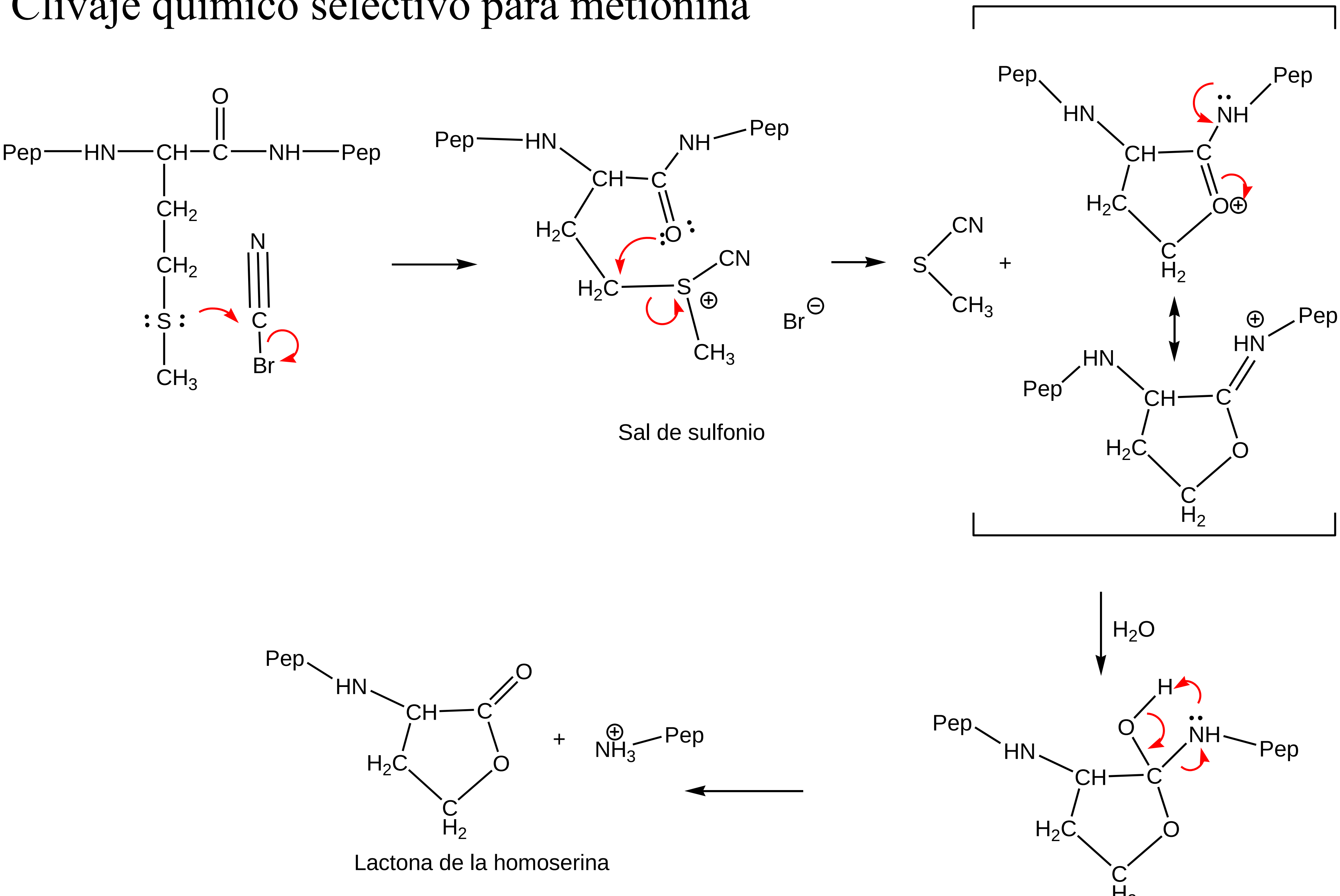
Exopeptidasas



Endopeptidasas

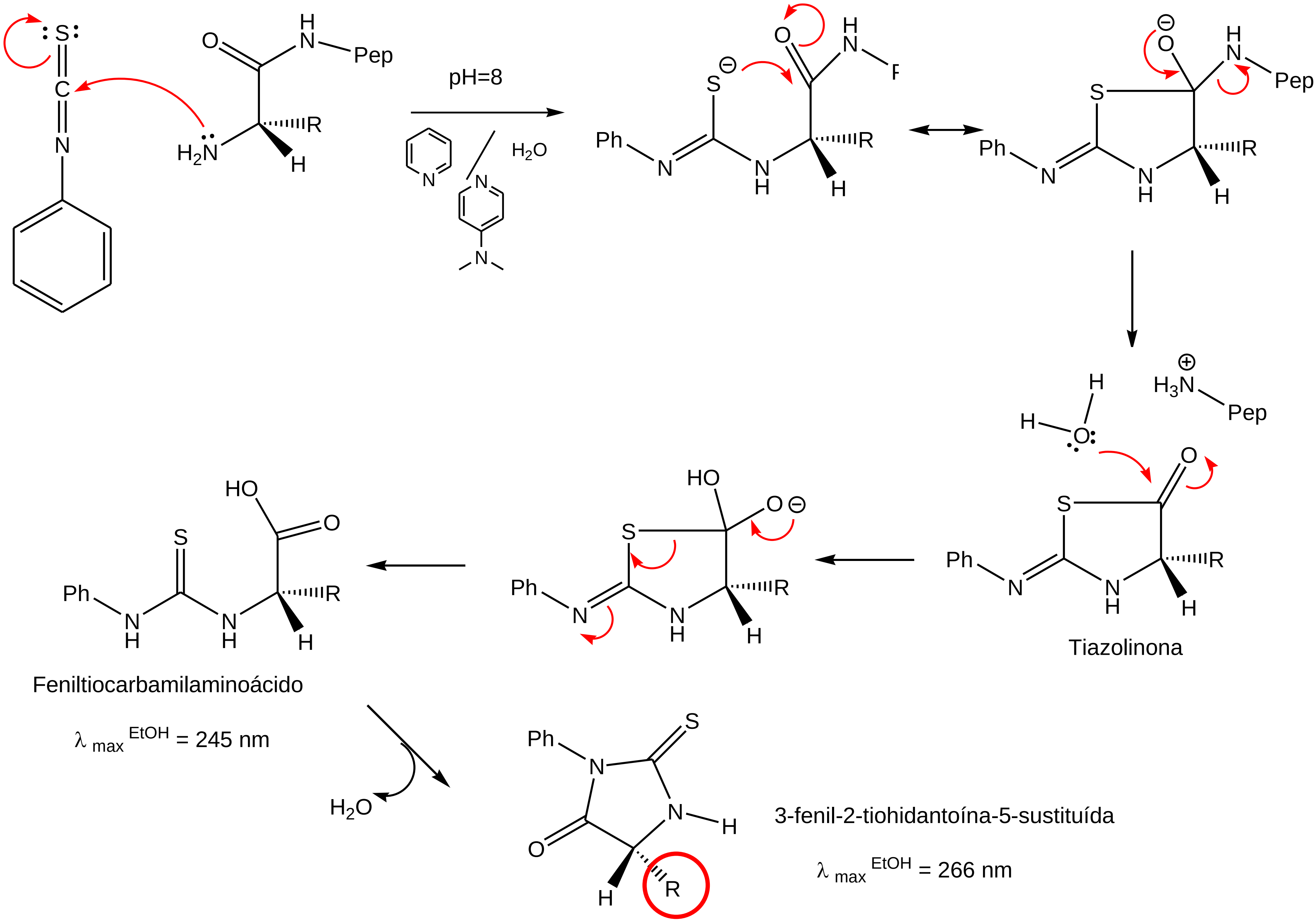


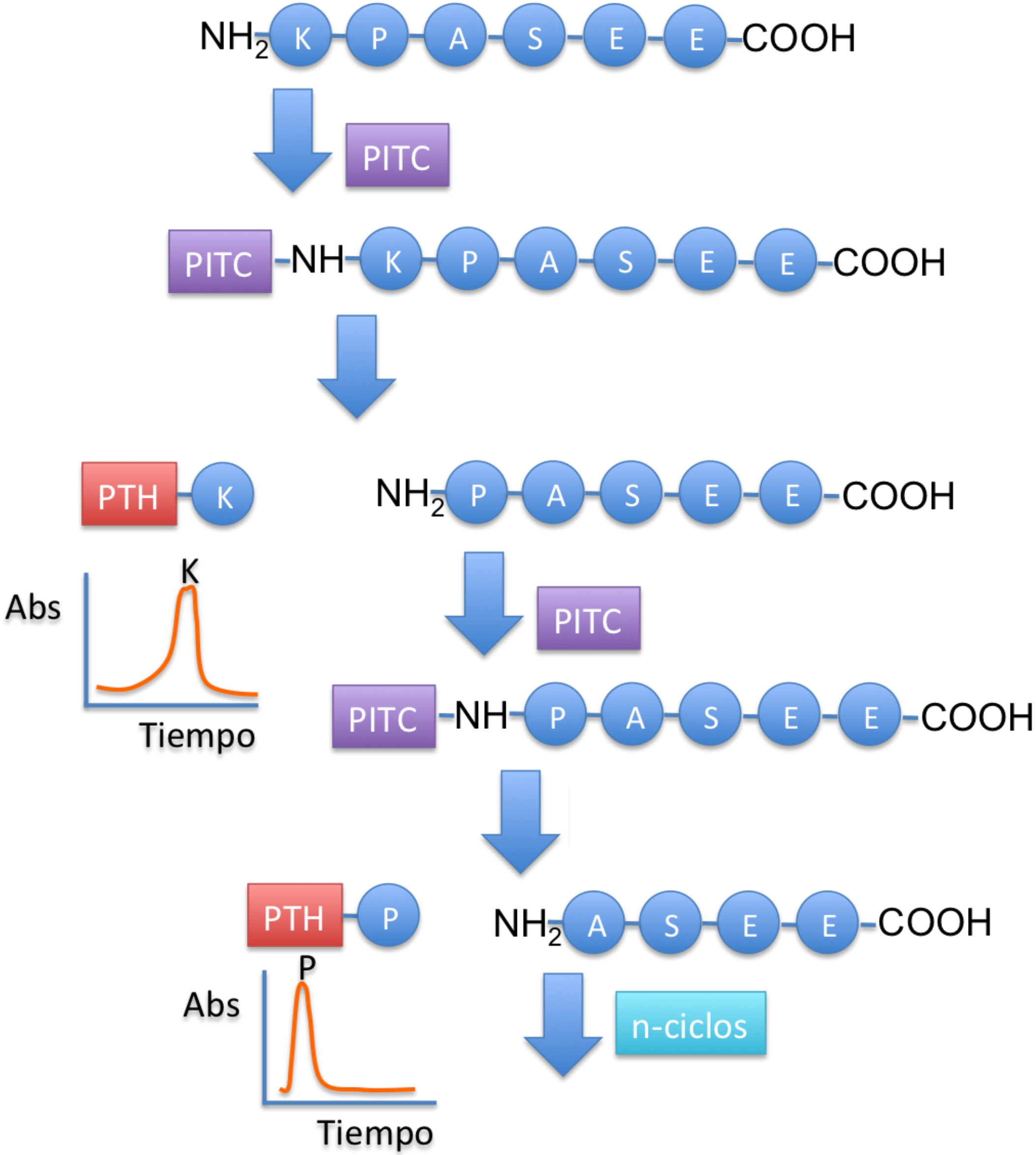
Clivaje químico selectivo para metionina



5
Secuenciación de péptidos

Degradación de Edman





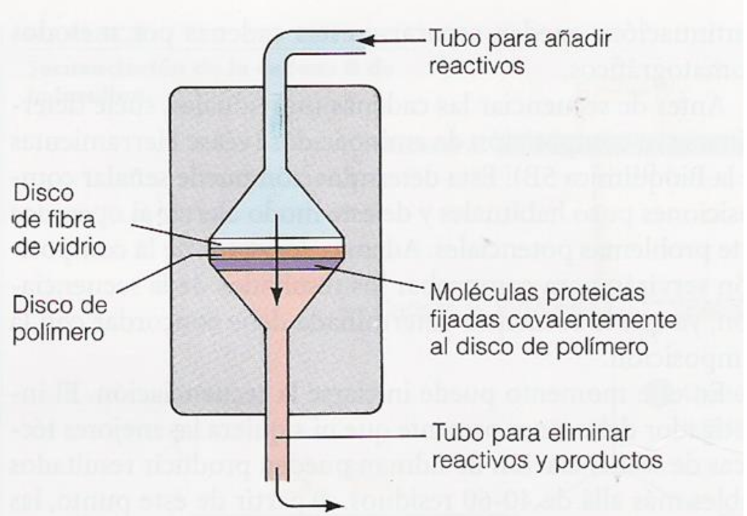


FIGURA **5C.3**

Recipiente de reacción en un secuenciador automático de fase sólida.

6
Localización de puentes disulfuro

Sobre la proteína con enlaces SS intactos

Digestión con enzimas/rvos qcos

Separación de los fragmentos resultantes

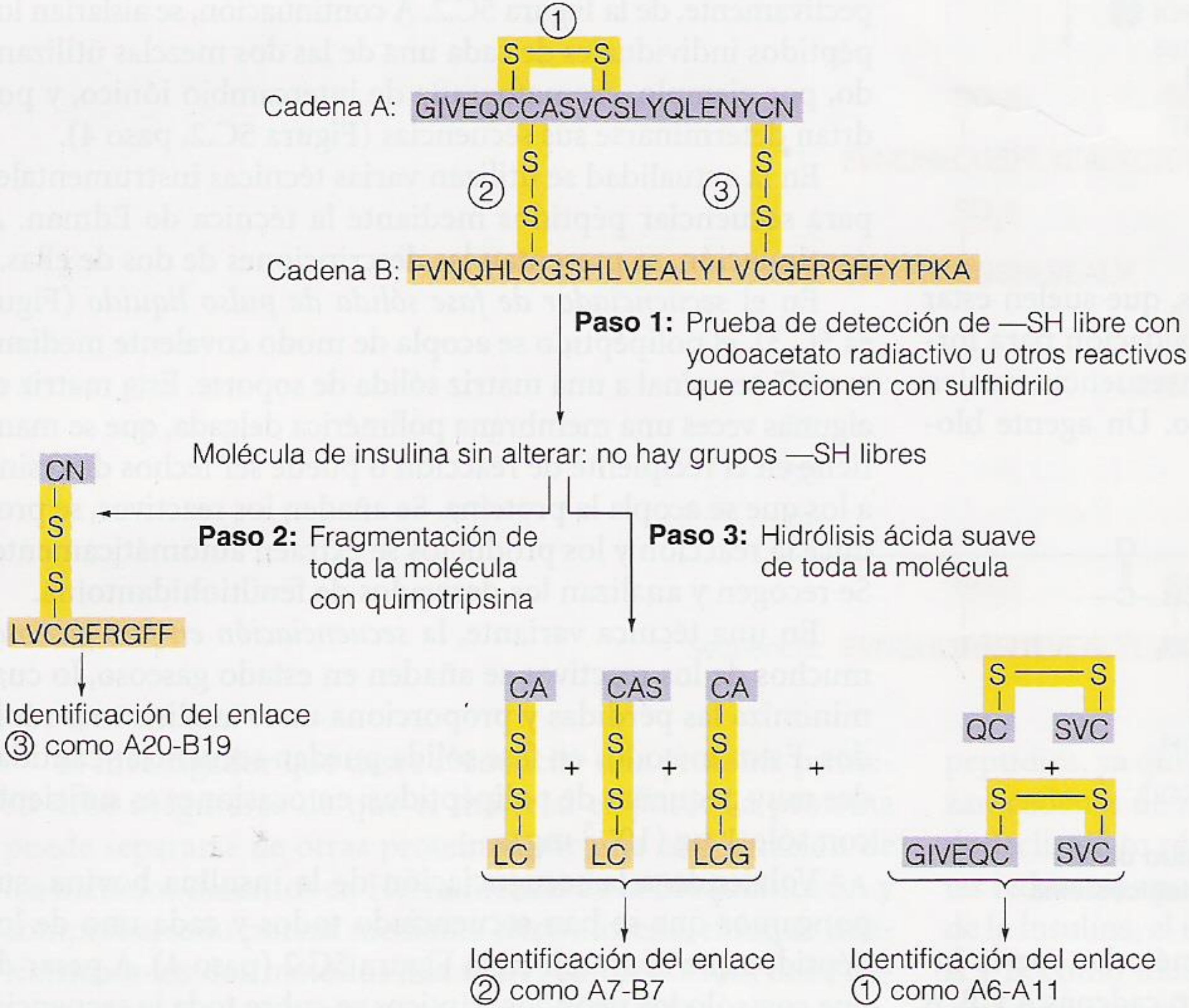
A cada fragmento

- Alquilación
- Hidrólisis

Sólo los –SH libres aparecen alquilados

- Reducción
- Alquilación
- Hidrólisis

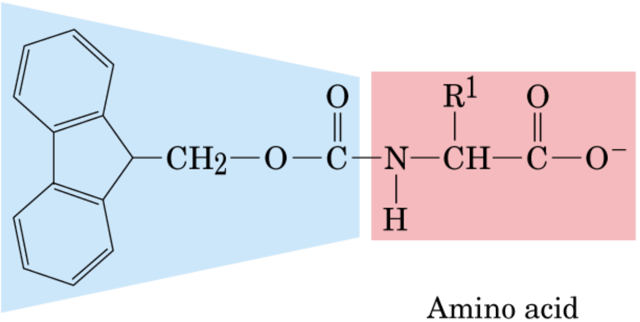
Aparecen alquilados los –SH libres y los SS

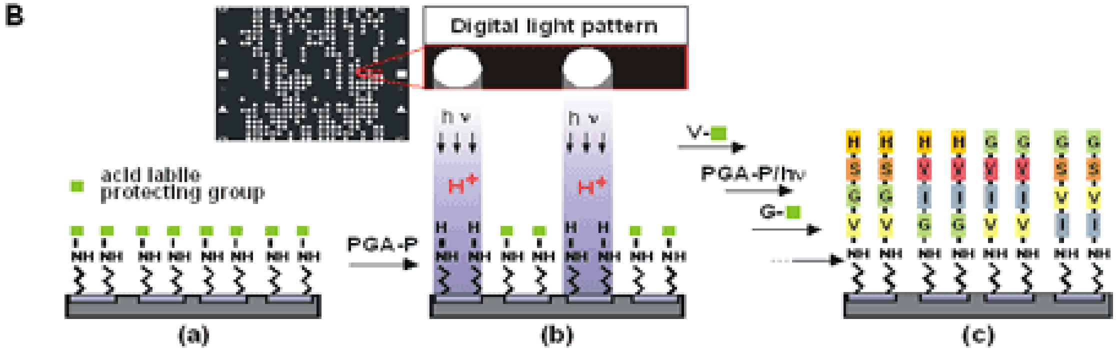


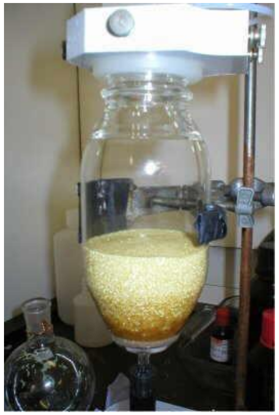
①
S — S
Cadena A: GIVEQCCASVCSLYQLENYCN
S
S
②
③
S
S
Cadena B: FVNQHLCGSHLVEALYLVCGERGFFYTPKA
Paso 1: Prueba de detección de —SH libre con yodoacetato radiactivo u otros reactivos que reaccionen con sulfhidrilo
Molécula de insulina sin alterar: no hay grupos —SH libres
CN
S
S
LVCGERGFF
Paso 2: Fragmentación de toda la molécula con quimotripsina
Paso 3: Hidrólisis ácida suave de toda la molécula
Identificación del enlace ③ como A20-B19
CA
CAS
CA
S
S
LC
LC
LCG
+
+
+
S — S
QC
SVC
+
S — S
GIVEQC
SVC
Identificación del enlace ② como A7-B7
Identificación del enlace ① como A6-A11

Amino acid sequence (protein)	Gln–Tyr–Pro–Thr–Ile–Trp
DNA sequence (gene)	CAGTATCCTACGATTTGG

Síntesis de péptidos

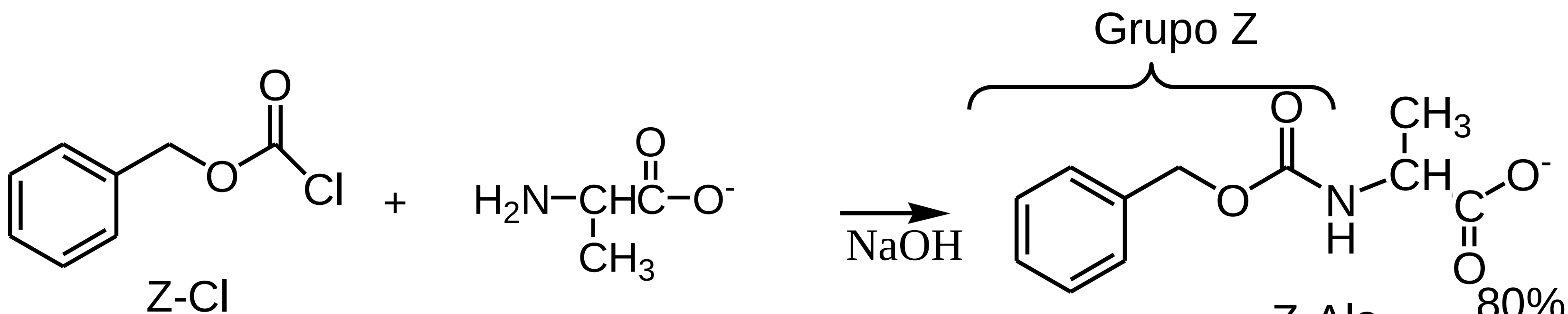






PROTECCIÓN DE GRUPO AMINO

Grupo Z

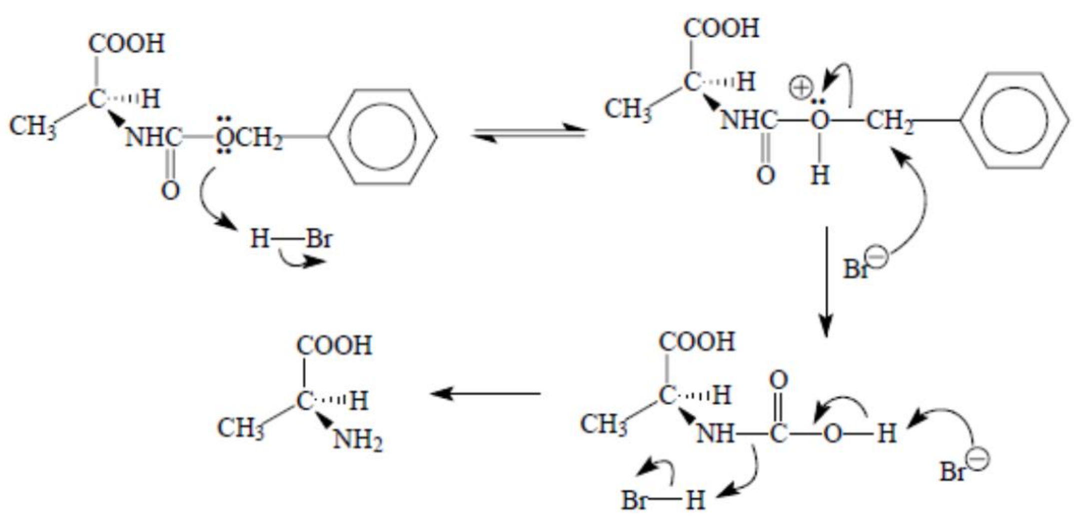


Z-Cl

Coroformiato de benzilo

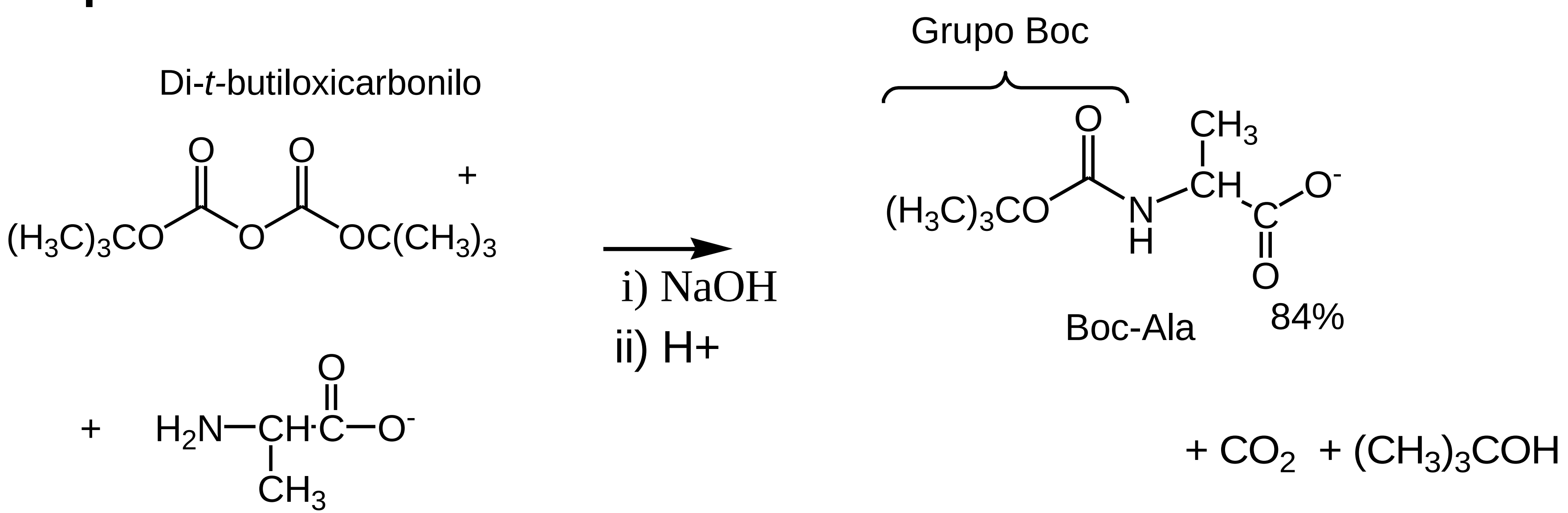
Z-Ala

Desprotección

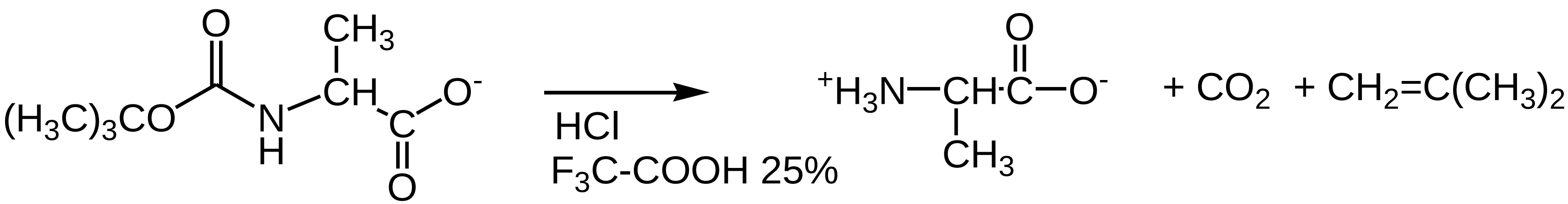


PROTECCIÓN DE GRUPO AMINO

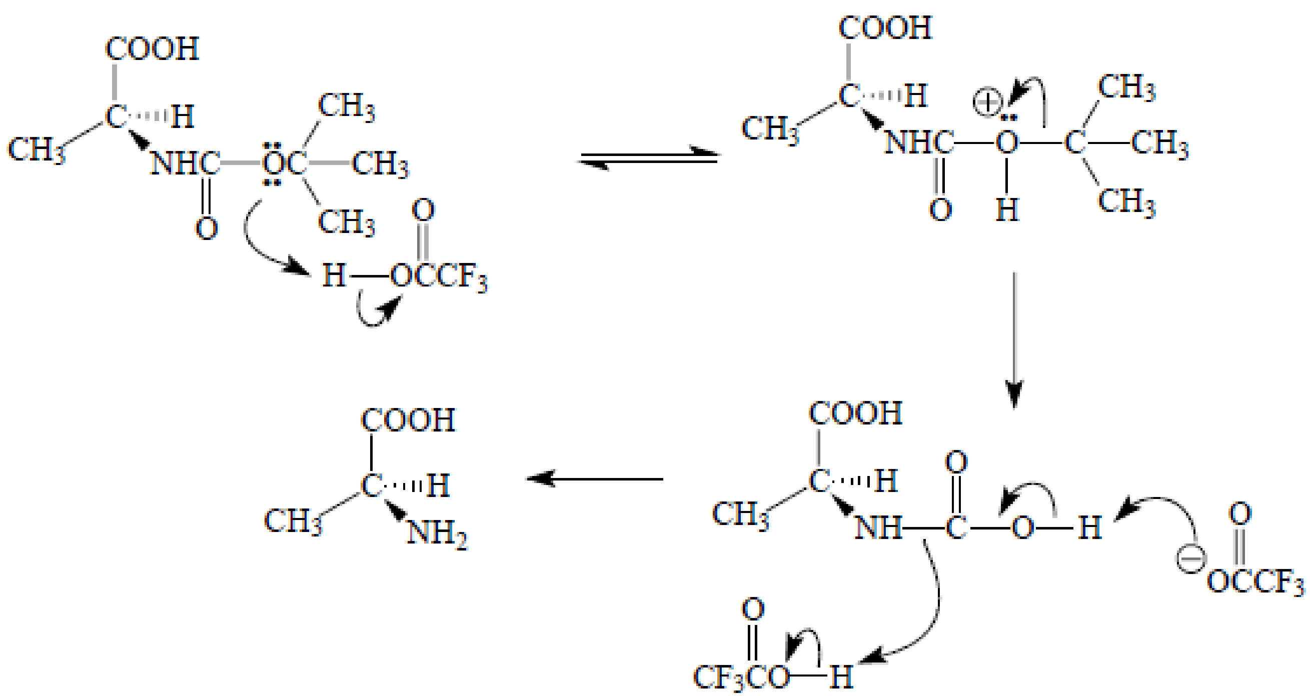
Grupo BOC



DESPROTECCIÓN DE Boc



Boc-Ala



SÍNTESIS EN FASE SÓLIDA

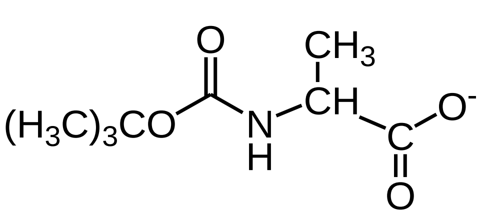
La síntesis en fase sólida es una metodología que permite realizar transformaciones químicas con la ayuda de un polímero insoluble. También se conoce como síntesis asistida por polímeros. Ya que las transformaciones sobre los compuestos orgánicos son, con mucho, las más habituales, también se denomina síntesis orgánica en fase sólida (SPOS *Solid-Phase Organic Synthesis*).

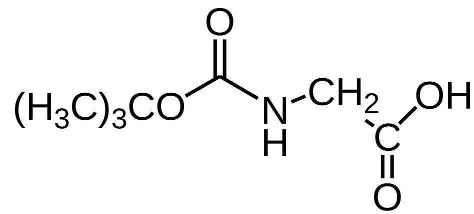
El primero químico en desarrollar esta metodología fue **Robert Bruce Merrifield**, (*J. Am. Chem. Soc.* **1963**, *85*, 2149), por lo que obtuvo el Premio Nobel de Química en 1984 "por el desarrollo de una metodología para la síntesis química sobre un soporte sólido".

Merrifield, B. (1986). Solid Phase Synthesis. Science *232*, 341 - 347
(This is a transcript of Merrifield's Nobel Award address.)

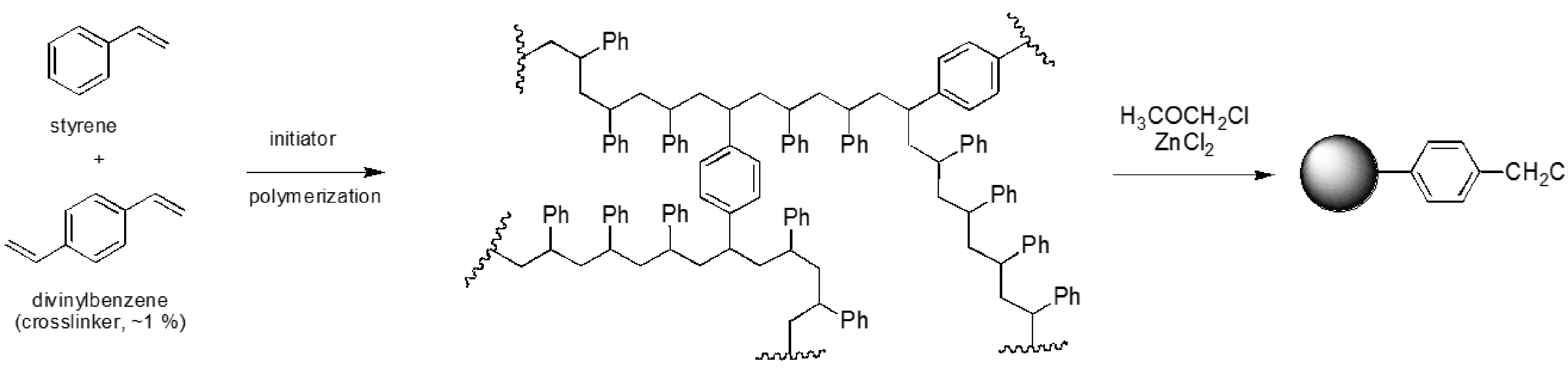
MÉTODO DE MERRIFIELD

Objetivo: síntesis del dipéptido alanilglicina
Materiales: Boc-Ala y Boc -Gli



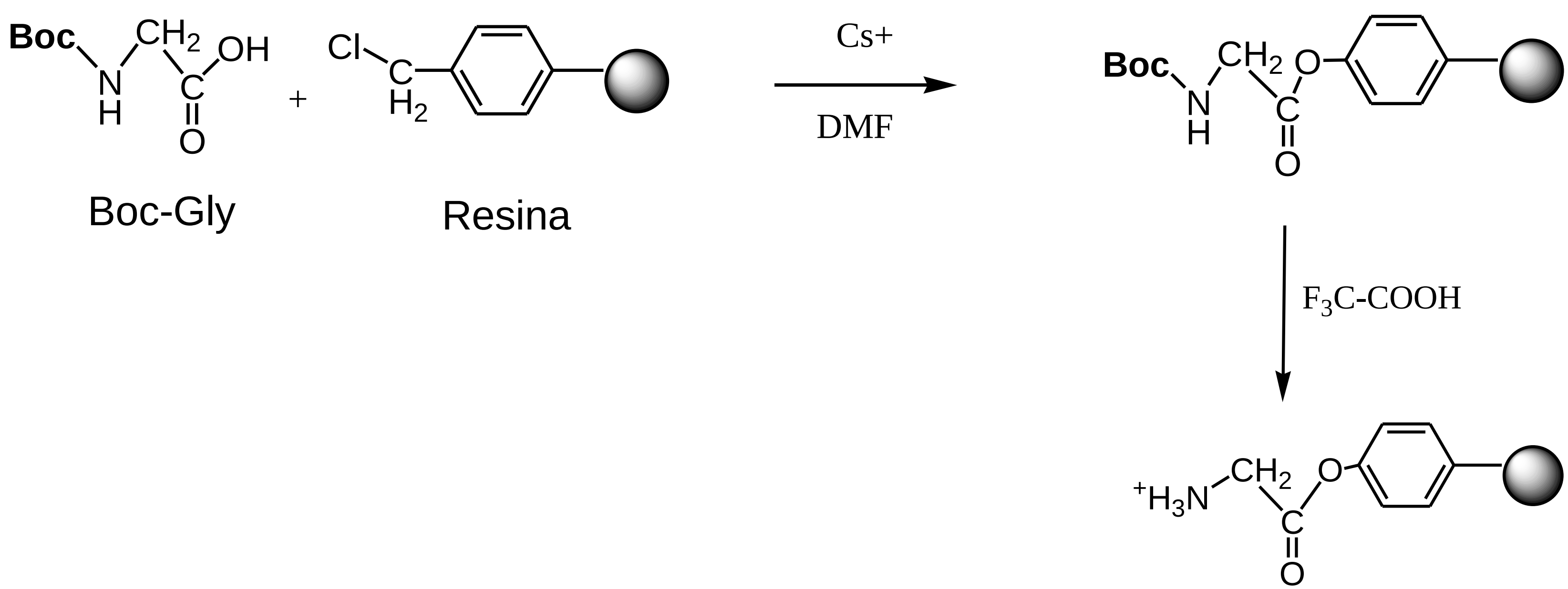


Resina de Merrifield, poliestireno

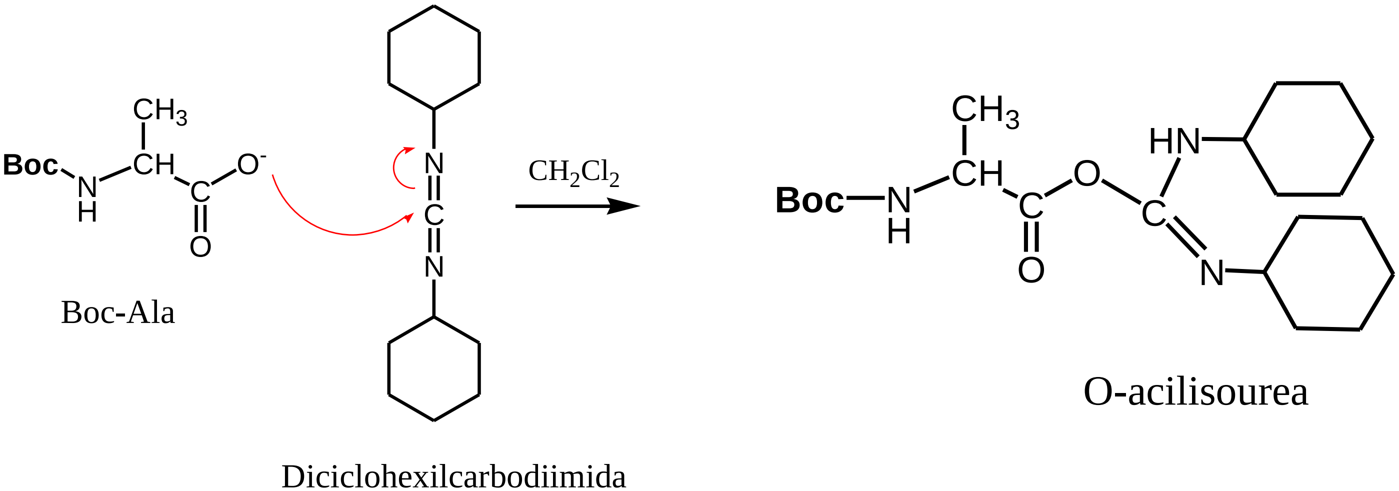


MÉTODO DE MERRIFIELD

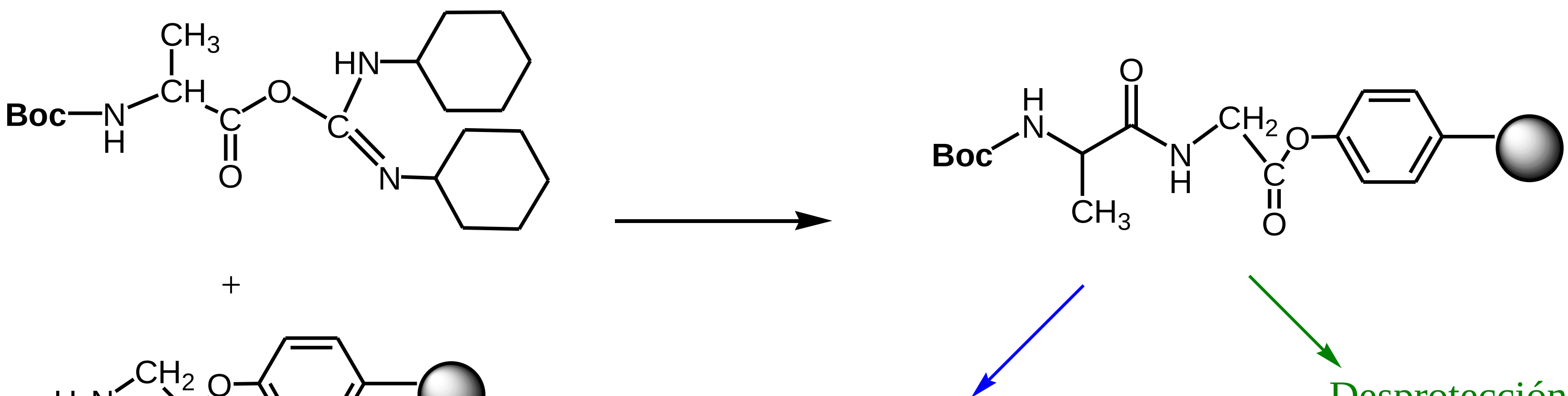
1-Unión a la resina del aa carboxilo terminal



2-Activación de grupo carbonilo

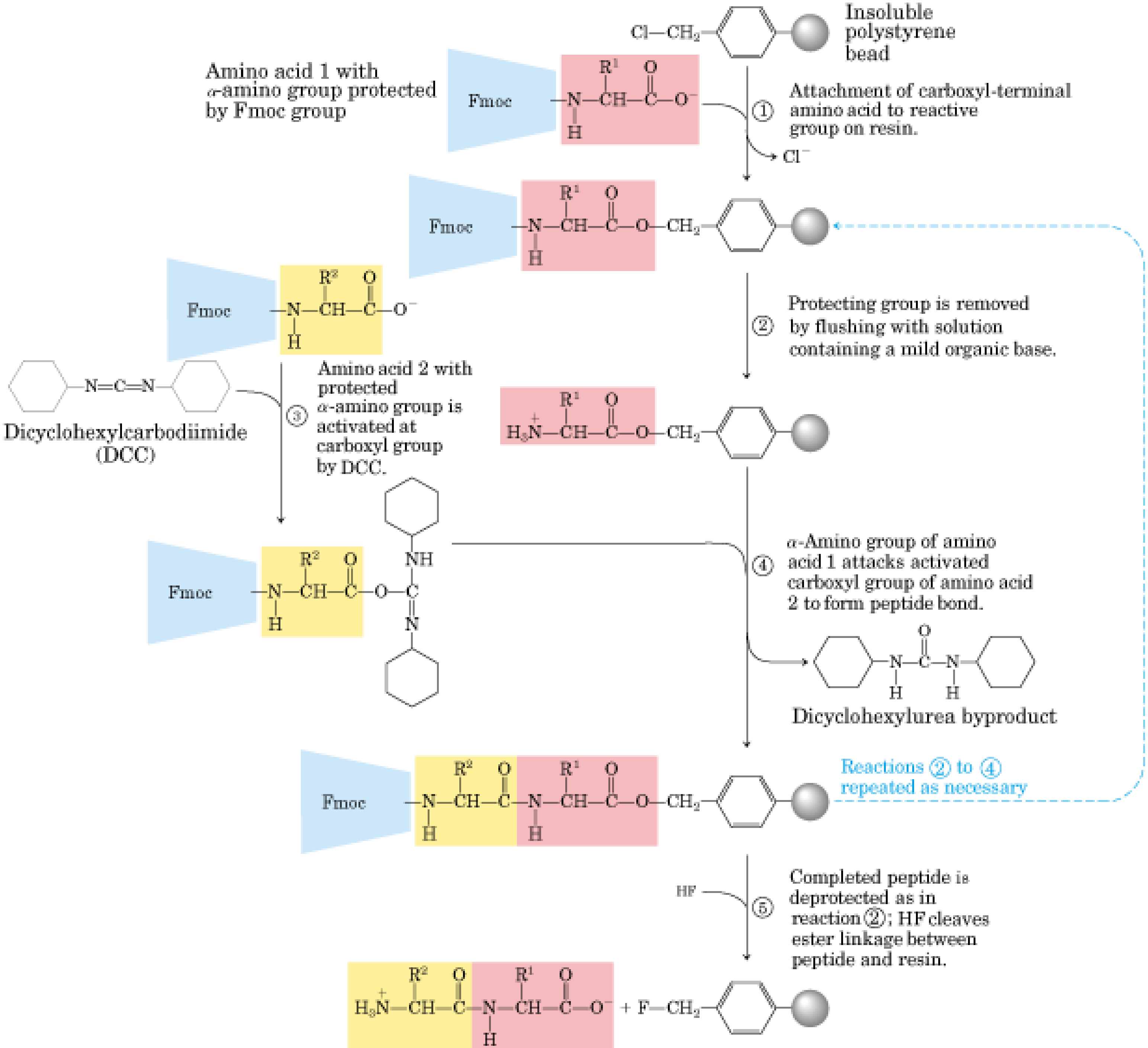


3-Unión peptídica



Desprotección y elongación de cadena
hacia extremo amino terminal
(Repetir pasos 1 y 2)

Desprotección final
i) F3C-COOH 25%
ii) HF



Insoluble polystyrene bead
Amino acid 1 with α-amino group protected by Fmoc group
Attachment of carboxyl-terminal amino acid to reactive group on resin.
Protecting group is removed by flushing with solution containing a mild organic base.
Dicyclohexylcarbodiimide (DCC)
Amino acid 2 with protected α-amino group is activated at carboxyl group by DCC.
α-Amino group of amino acid 1 attacks activated carboxyl group of amino acid 2 to form peptide bond.
Dicyclohexylurea byproduct
Reactions ② to ④ repeated as necessary
Completed peptide is deprotected as in reaction ②; HF cleaves ester linkage between peptide and resin.

Fluorenil-9-metoxicarbonil (Fmoc)

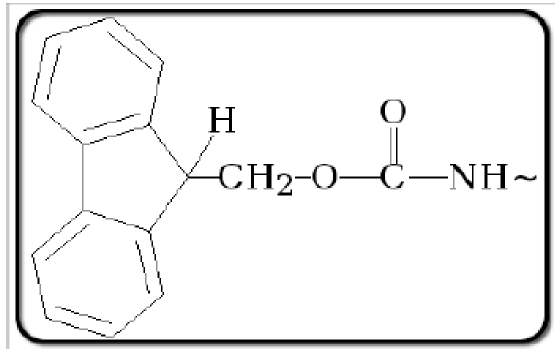
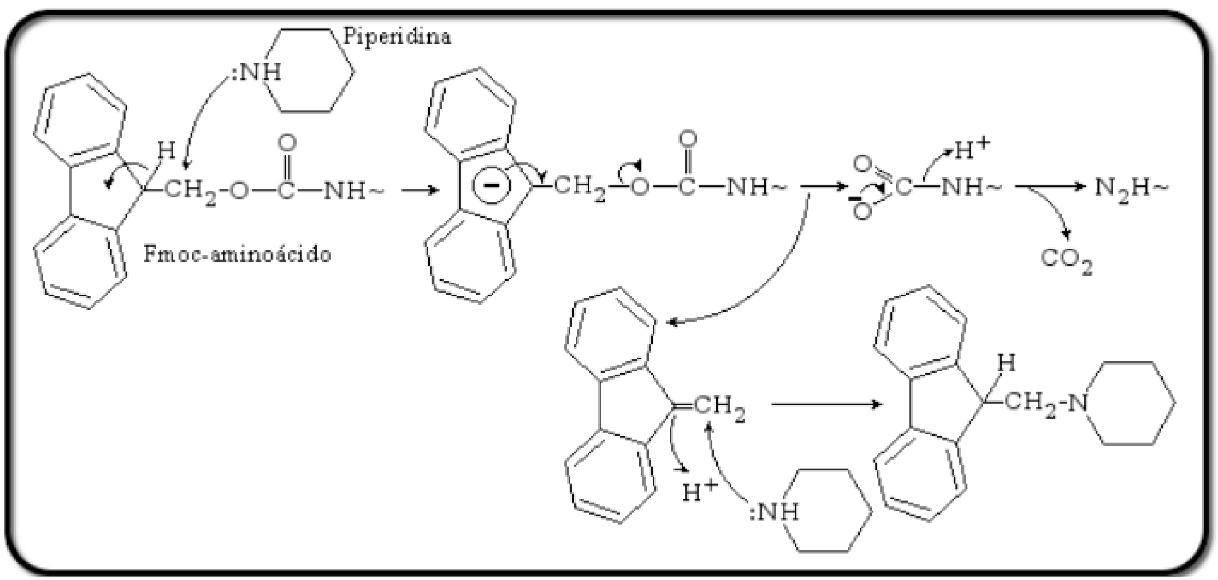
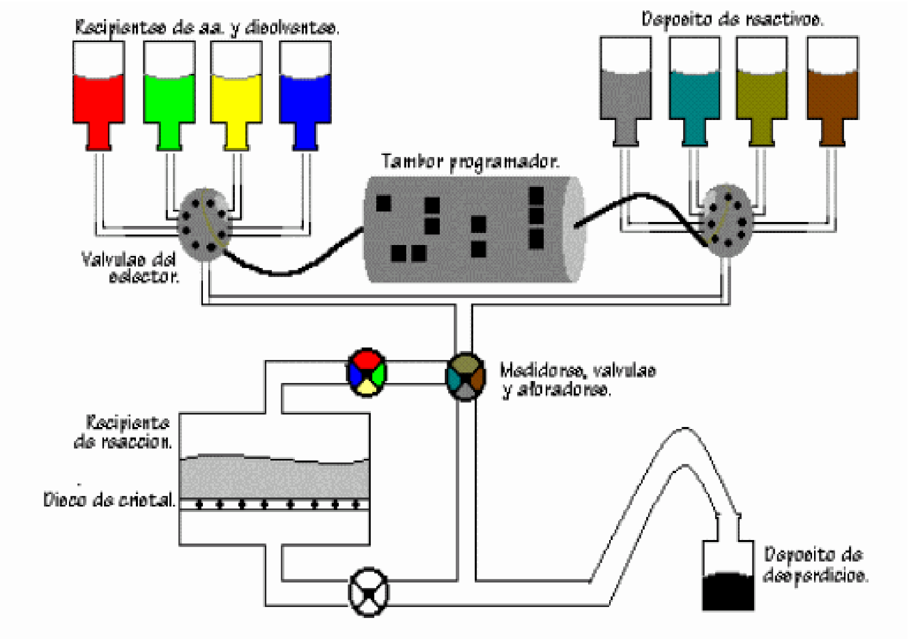


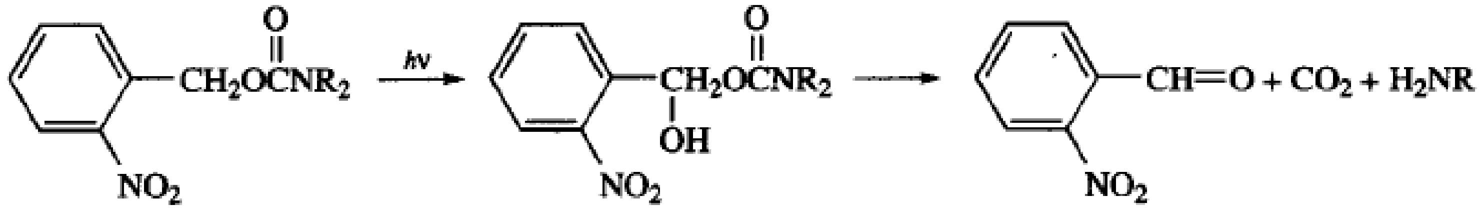
Figura 8. *Grupo protector Fmoc*

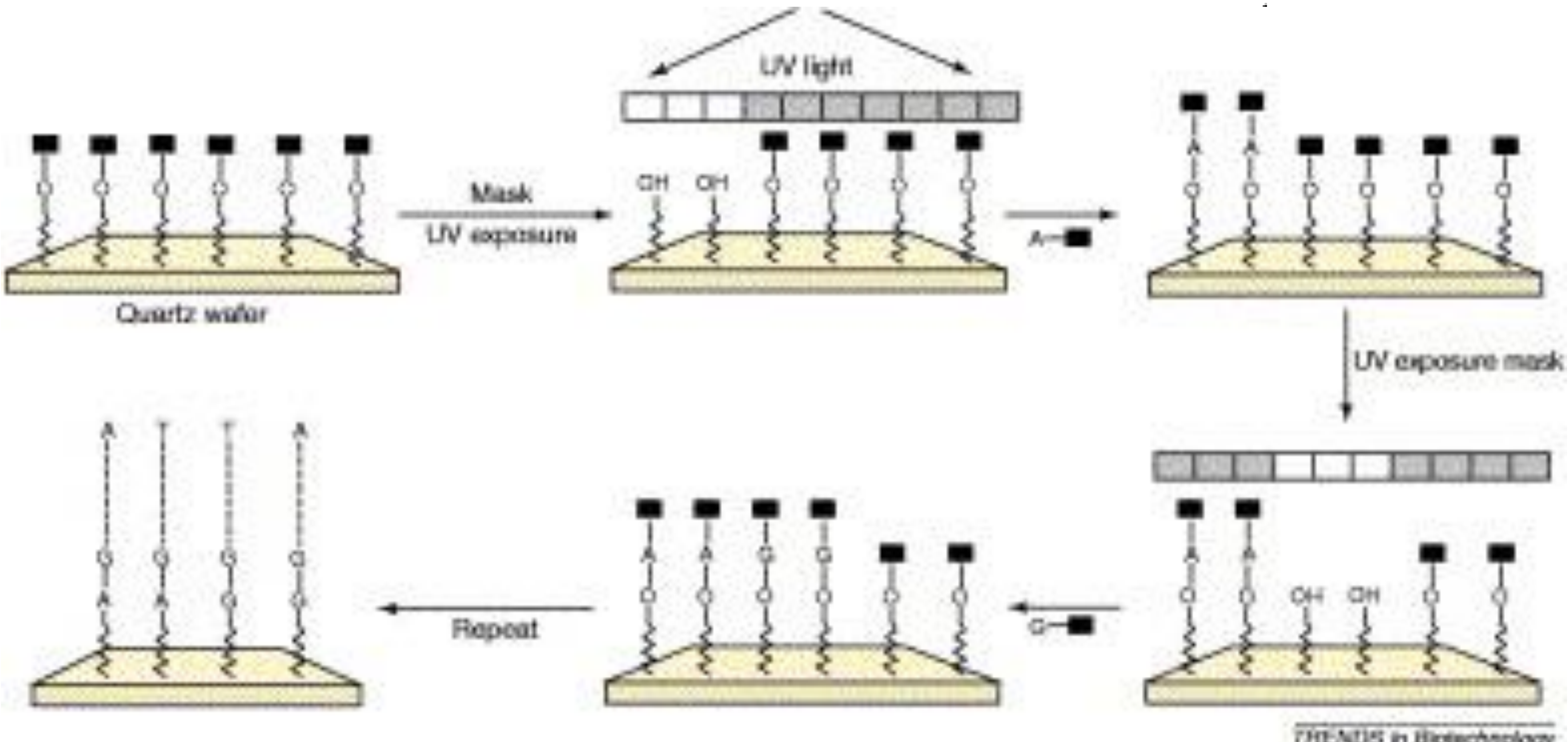
Desprotección



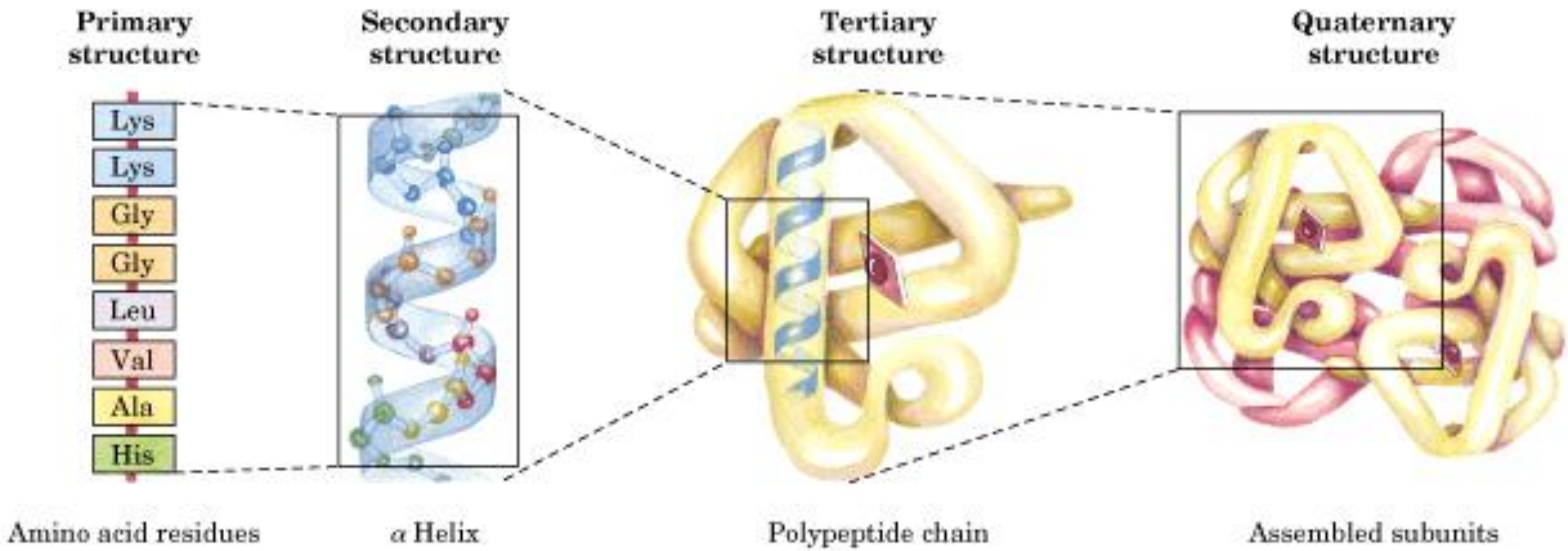

Recipientes de aa. y disolventes.
Deposito de reactivos.
Tambor programador.
Valvulas del selector.
Medidores, valvulas y aforadores.
Recipiente de reaccion.
Disco de cristal.
Deposito de desperdicios.

Síntesis combinatoria





Syro II is a fully automatic, computer controlled peptide synthesizer based on a two arms pipetting robot.


Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Lys
Lys
Gly
Gly
Leu
Val
Ala
His
Amino acid residues
α Helix
Polypeptide chain
Assembled subunits

BIBLIOGRAFÍA

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-BIOCHEMISTRY. Lubert STRYER Ed. Freemon
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