台灣大學開放式課程

有機化學乙

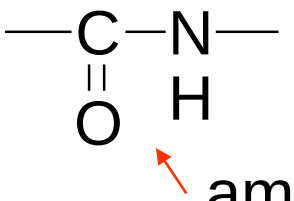
蔡蘊明　教授

Chapter 24 Amino acids and proteins

※ Introduction

✓Proteins

polyamides with α-amino acids as monomer
(~20 different α-amino acids)

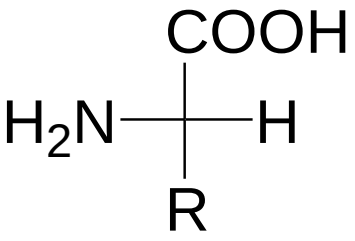


amide linkage

✓Primary structure

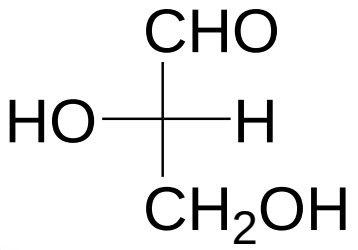
the sequence of α-amino acids
further folding → secondary and tertiary structures

✓ Most natural α-amino acids are L form



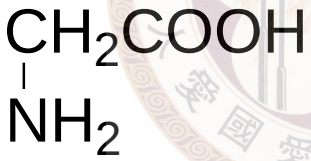
L-α-amino acid

cf.



L-glyceraldehyde

Exception:



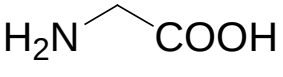
Glycine (甘胺酸)

✓20 α-Amino acids for protein synthesis

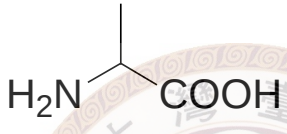
8 α-amino acids are essential ─ acquired from diet

Five sub-groups

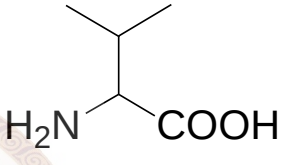
R: neutral



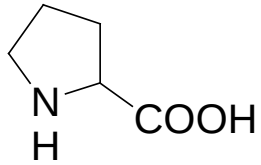
glycine



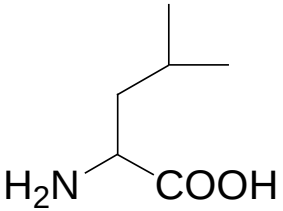
alanine



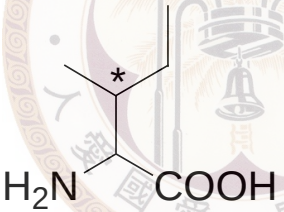
valine



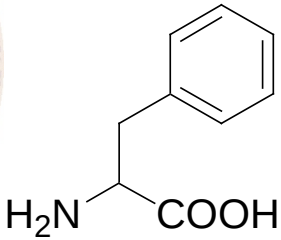
proline



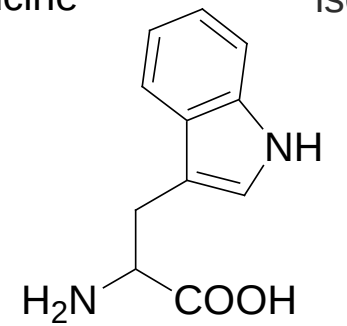
leucine



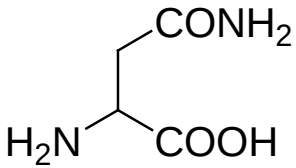
isoleucine



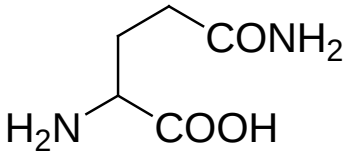
phenylalanine
(F)



tryptophan
(W)

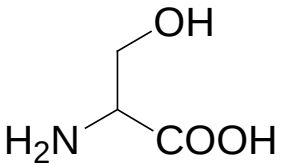


asparagine
(N)

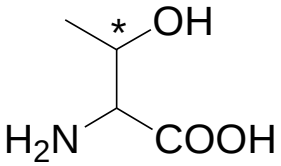


glutamine
(Q)

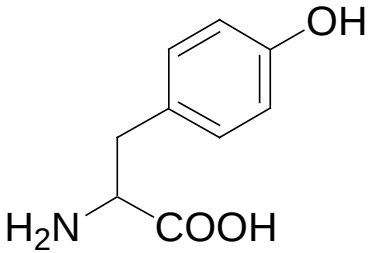
-OH



serine

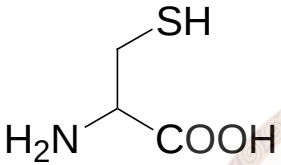


threonine

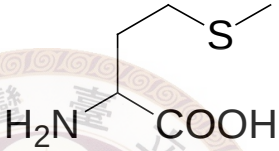


tyrosine
(Y)

-S

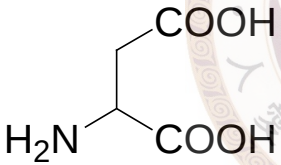


cysteine

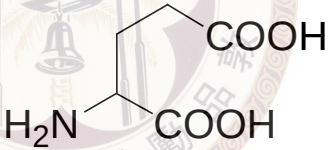


methionine

-COOH

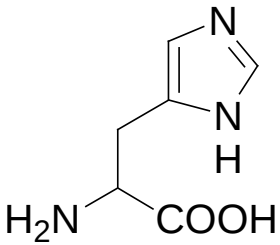


aspartic acid
(D)

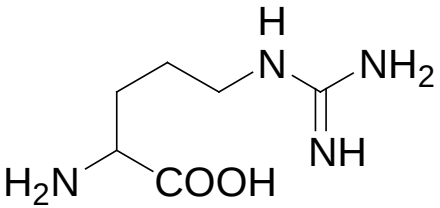


glutamic acid
(E)

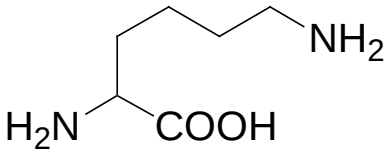
basic



histidine

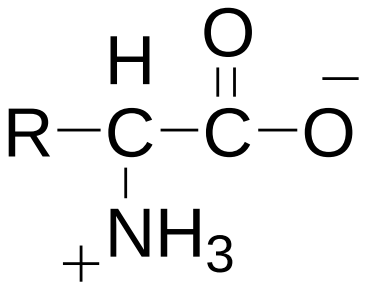


arginine
(R)

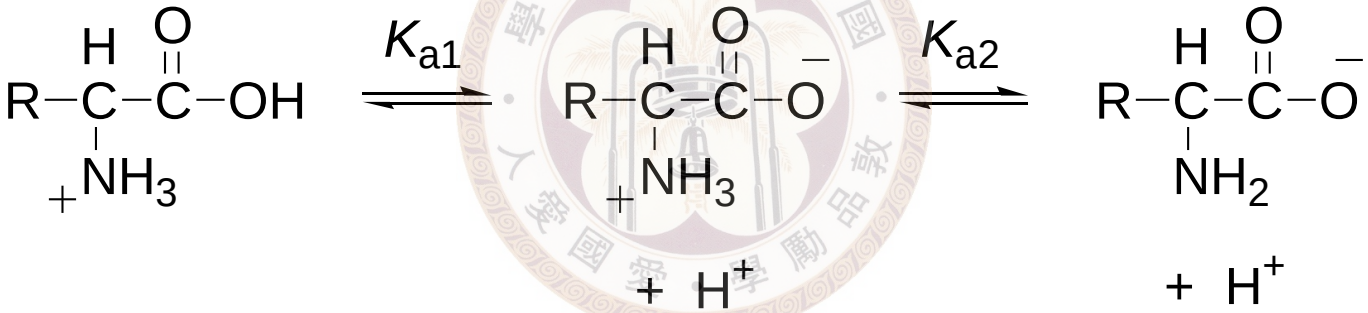


lysine
(K)

※ The dipolar ion structure



Amphoteric – both an acid and base



P (positive) **Z** (neutral) **N** (negative)

$$K_{a1} = \frac{[\mathbf{Z}][H^+]}{[\mathbf{P}]} \qquad K_{a2} = \frac{[\mathbf{N}][H^+]}{[\mathbf{Z}]}$$

$$K_{a2} = \frac{[\mathbf{N}][H^+]}{[\mathbf{Z}]}$$

When [**Z**] = [**N**]

→ $K_{a2} = [H^+]$

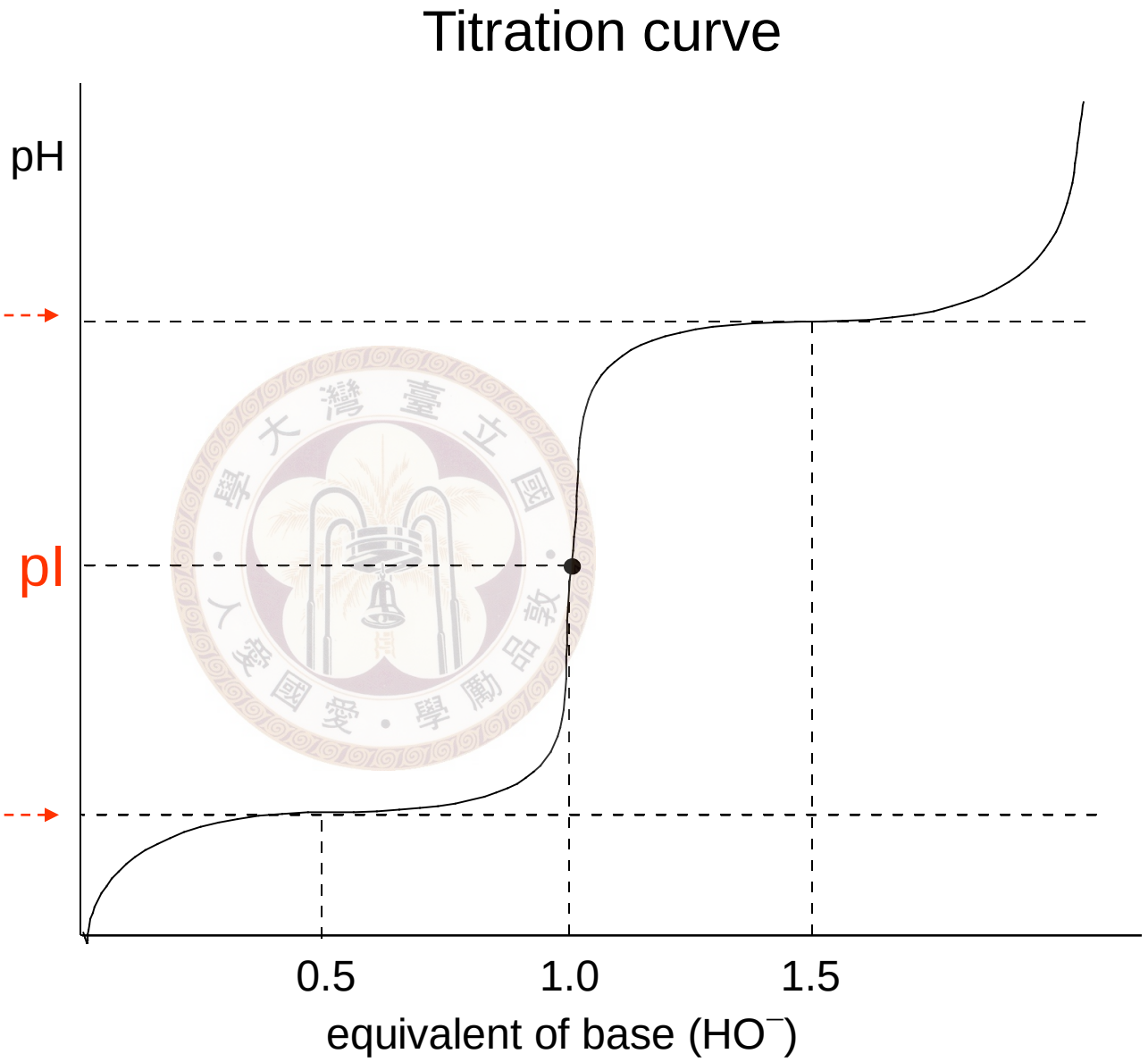
→ $pK_{a2} = pH$

$$K_{a1} = \frac{[\mathbf{Z}][H^+]}{[\mathbf{P}]}$$

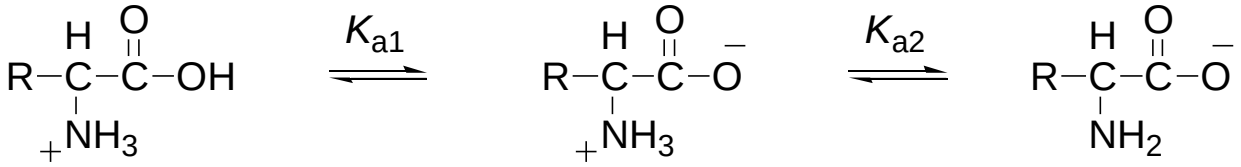
When [**Z**] = [**P**]

→ $K_{a1} = [H^+]$

→ $pK_{a1} = pH$



★ Isoelectric point



$$K_{a1} = \frac{[\mathbf{Z}][H^+]}{[\mathbf{P}]} \qquad K_{a2} = \frac{[\mathbf{N}][H^+]}{[\mathbf{Z}]}$$

$$pK_{a1} = -\log[H^+] - \log\frac{[\mathbf{Z}]}{[\mathbf{P}]} \qquad pK_{a2} = -\log[H^+] - \log\frac{[\mathbf{N}]}{[\mathbf{Z}]}$$

$$pK_{a1} + pK_{a2} = 2pH - \log\frac{[\mathbf{N}]}{[\mathbf{P}]}$$

When $[\mathbf{N}] = [\mathbf{P}] \rightarrow pK_{a1} + pK_{a2} = 2pH$

The net charge of amino acid is neutral

⟹ The isoelectric point

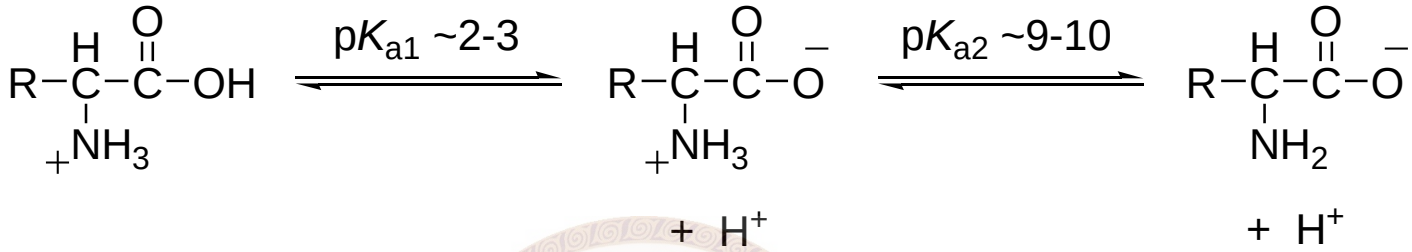
(the dipolar ion has the highest concentration)

⟹ At this point $pH = \frac{pK_{a1} + pK_{a2}}{2} = pI$

∗ different amino acid has different pI

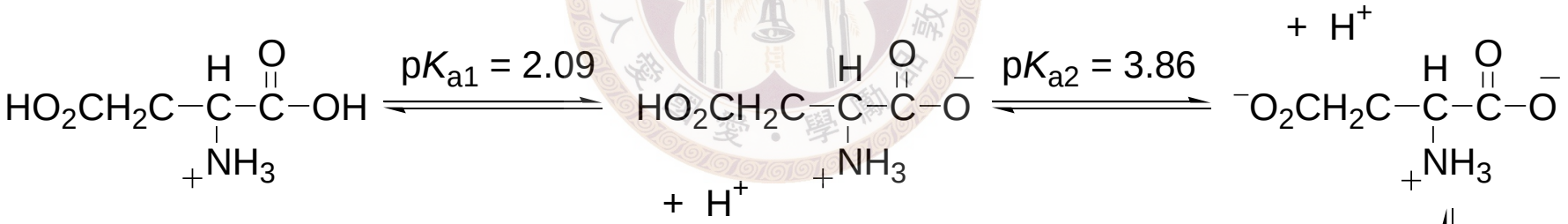
✓In general:

When R is neutral

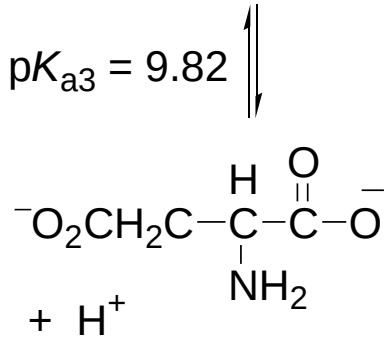


pI ~6

When R is acidic

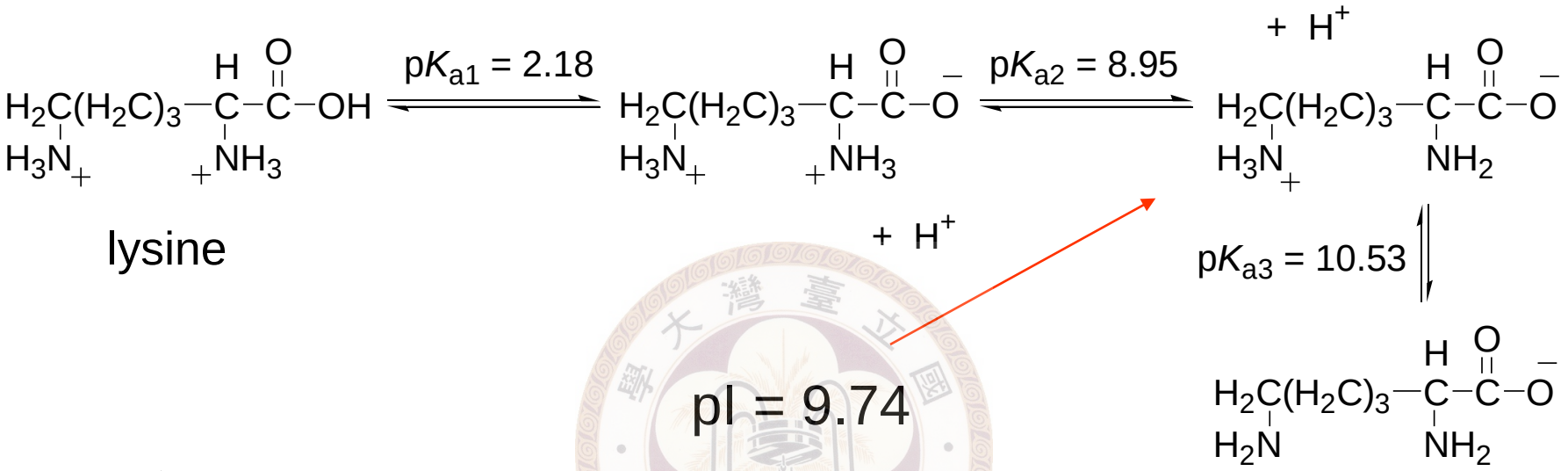


aspartic acid

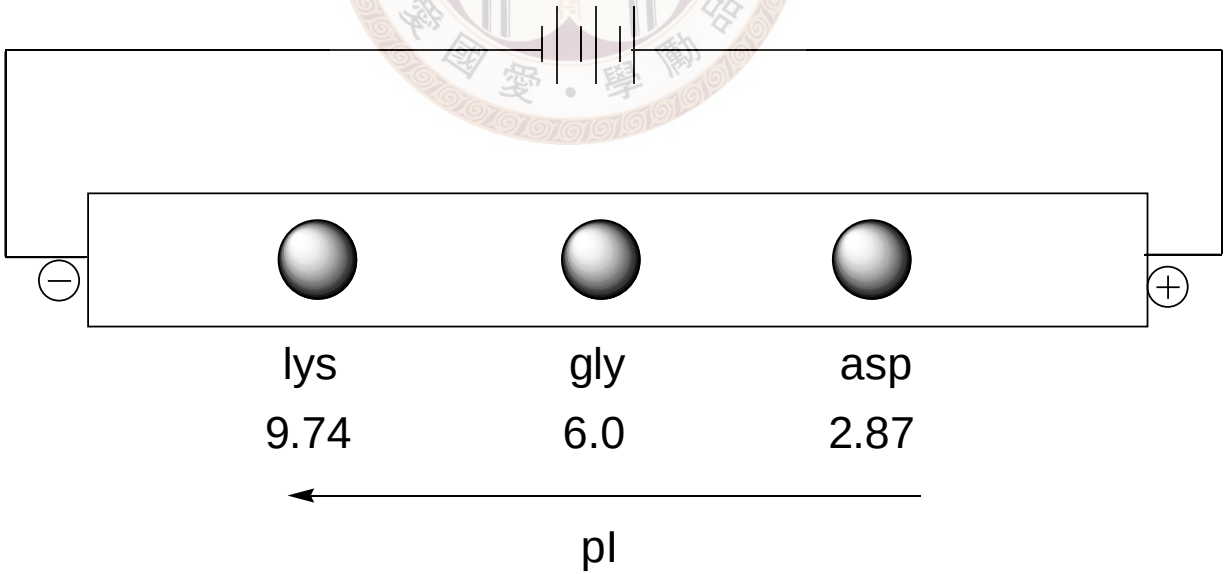


pI = 2.87

When R is basic

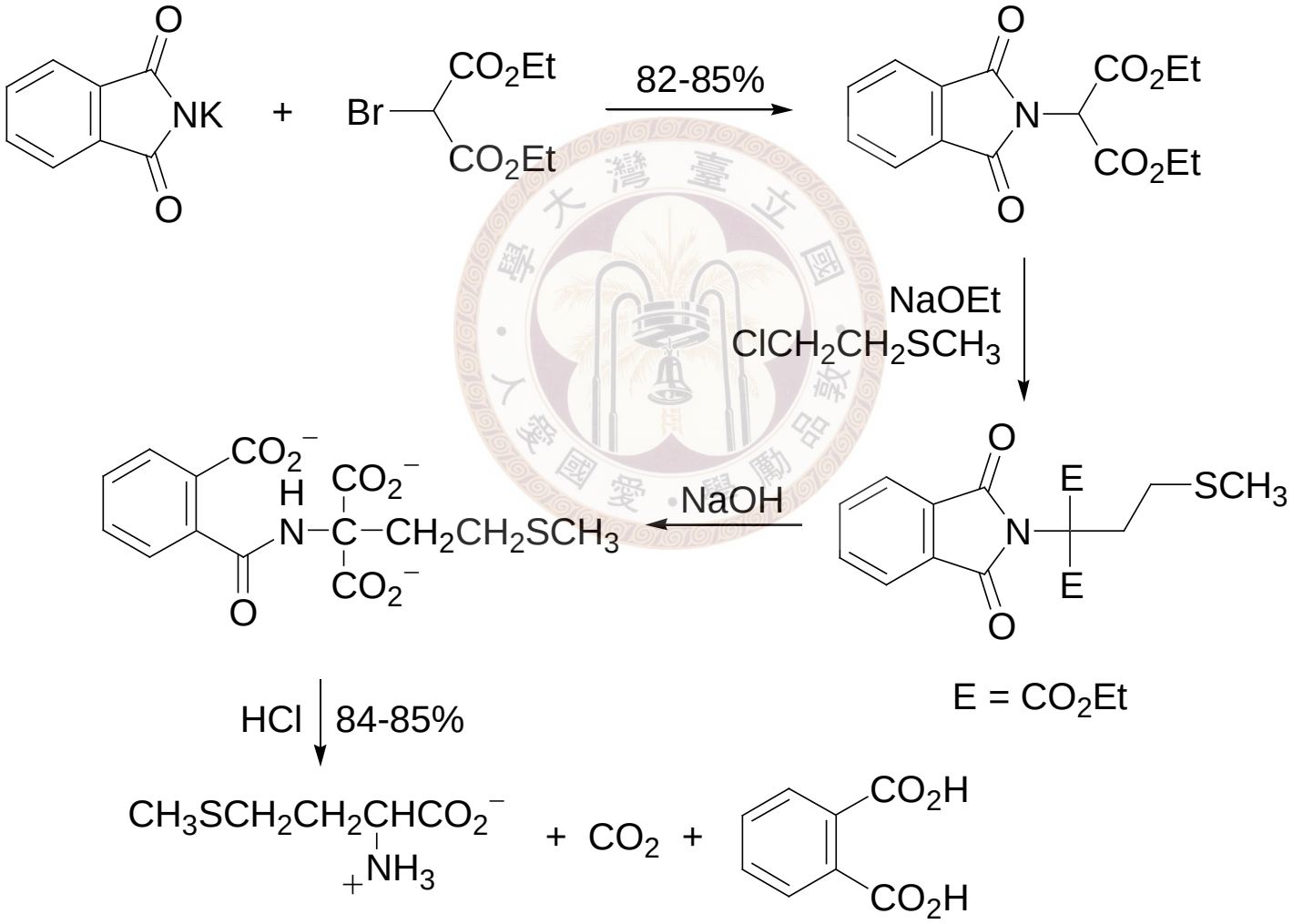


✓ Electrophoresis

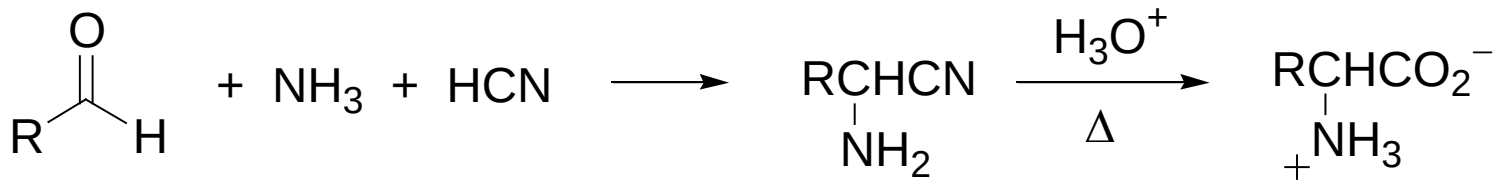


※ Lab synthesis

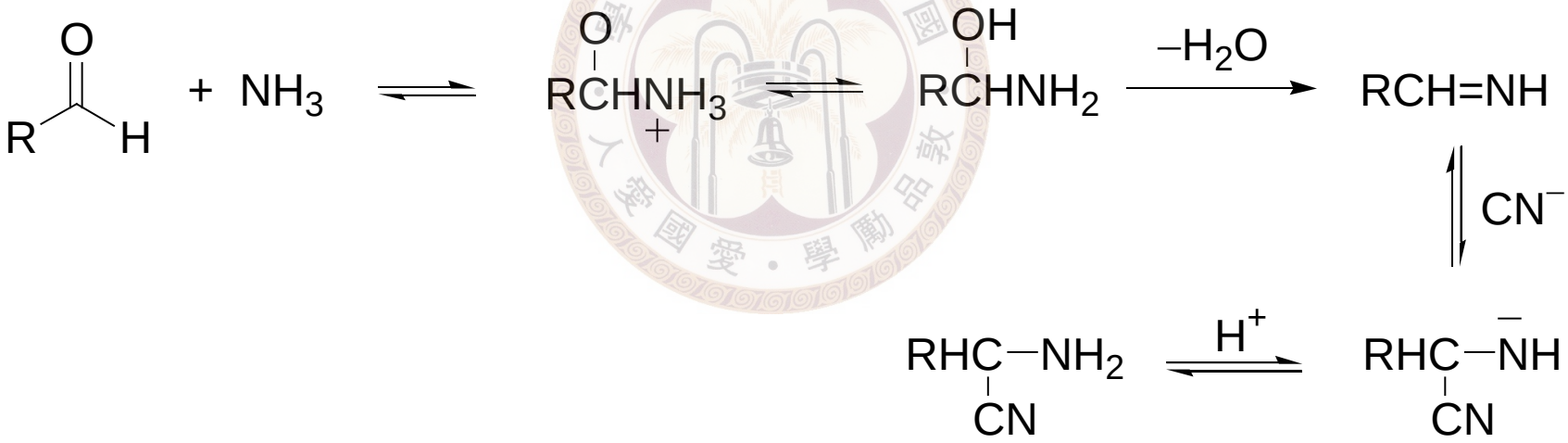
◎ From potassium phthalimide



◎ Strecker synthesis

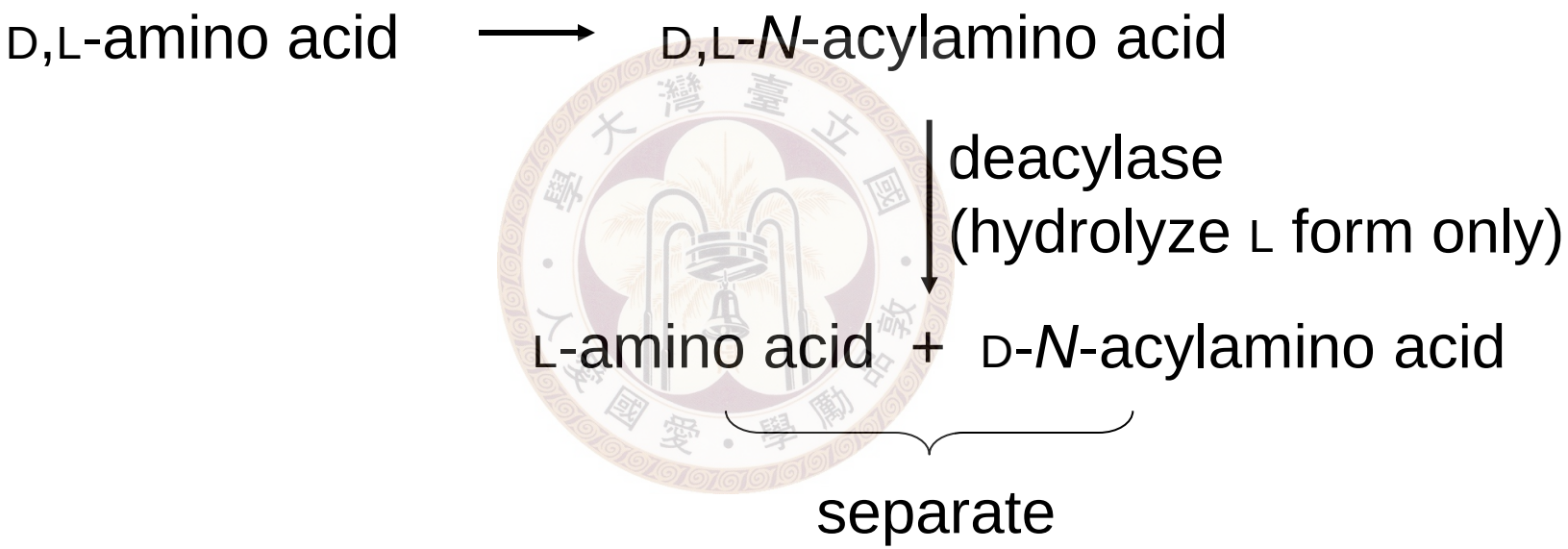


Mechanism:



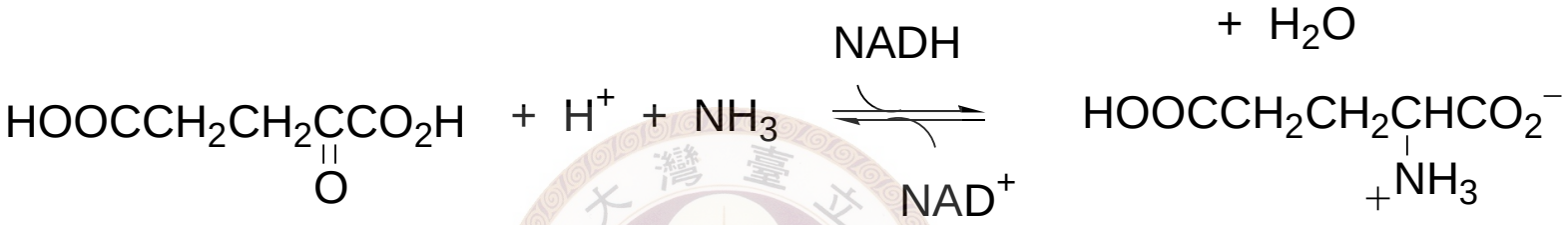
※ Resolution of D,L-amino acids

✓ An enzymatic method



※ Biosynthesis of amino acids

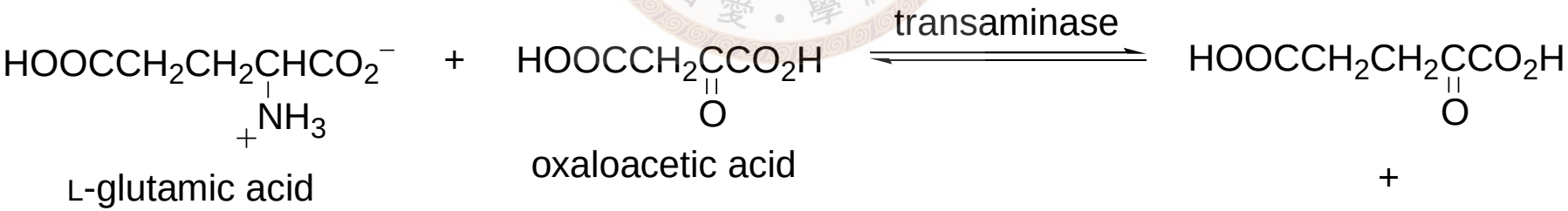
✓ Reductive amination



α-ketoglutaric acid from carbohydrate

L-glutamic acid

✓ Transamination



L-glutamic acid

oxaloacetic acid

L-aspartic acid

※ Determination of protein sequence

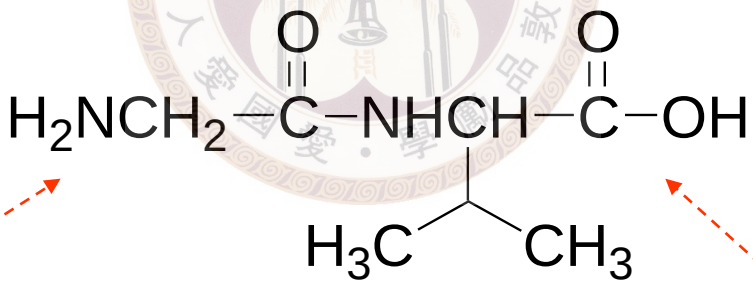
✓ Nomenclature

Proteins and polypeptides

polyamides with MW < 10000 → polypeptides

polyamides with MW > 10000 → proteins

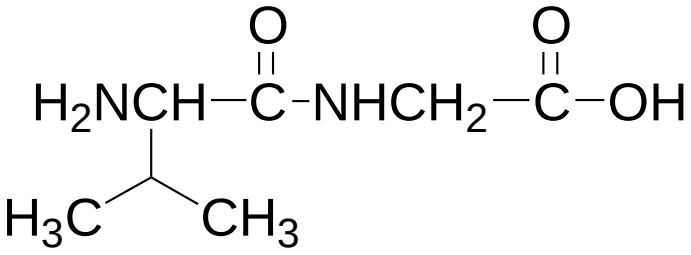
dipeptide



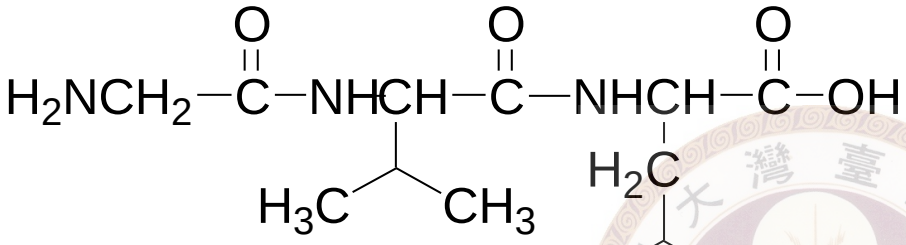
amino end (N-terminal)

glycylvaline (Gly•Val)

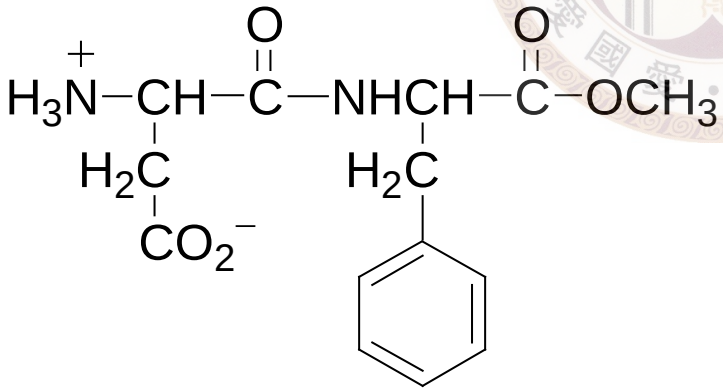
carboxy end (C-terminal)



valylglycine
(Val•Gly)



glycylvalylphenylalanine
(Gly•Val•Phe)



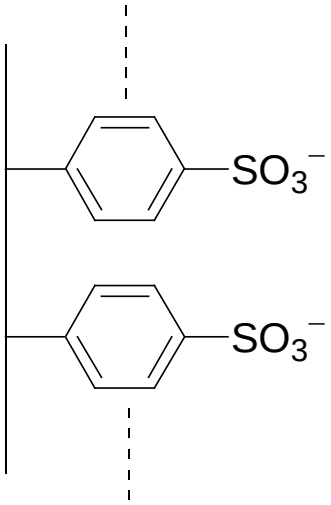
aspartylphenylalanine methyl ester
(aspartam; NeutraSweet) – 一種代糖

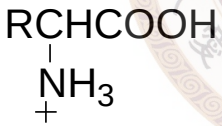
✓ Detection

protein $\xrightarrow[24\ h]{6\ M\ HCl}$ individual amino acids

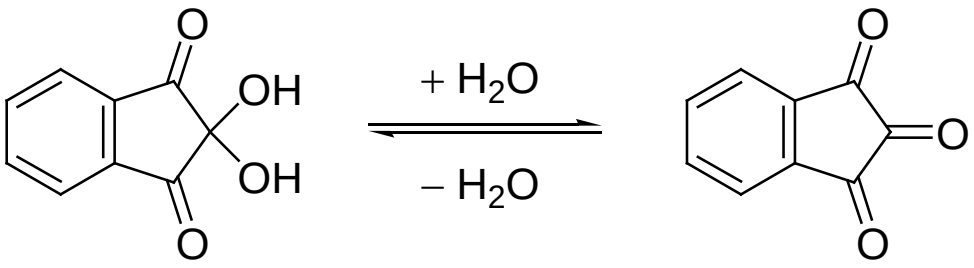
↓

amino acid analyzer
(using cation-exchange resin)

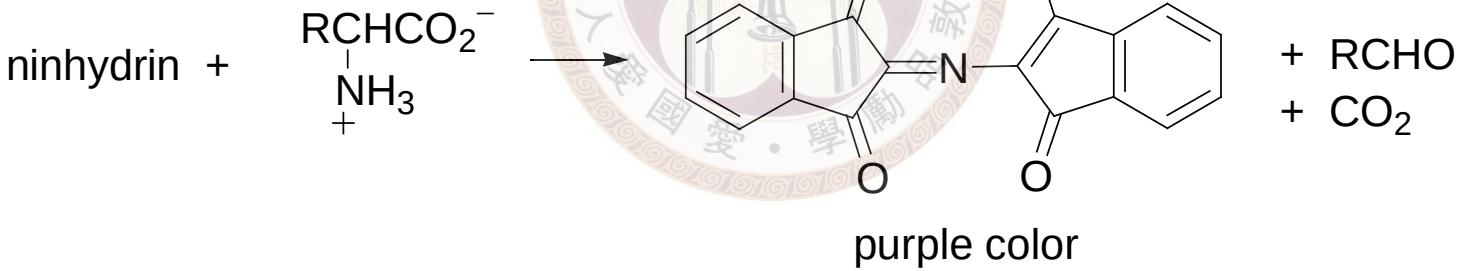




Different amino acids are absorbed differently
→ separation

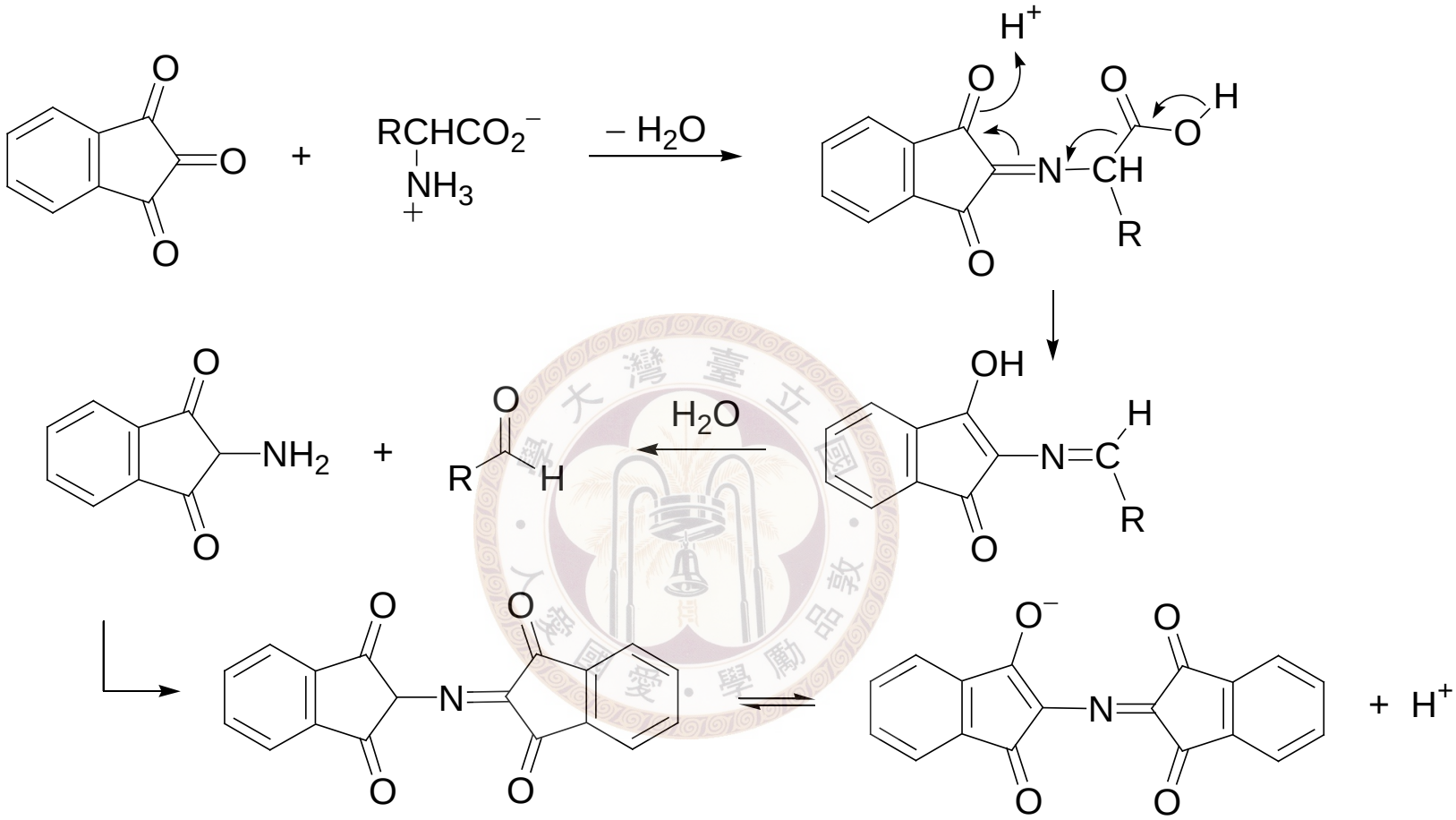


ninhydrin indane-1,2,3-trione



purple color

Mechanism:



※ Sequence determination

Covalent structure of protein – primary structure

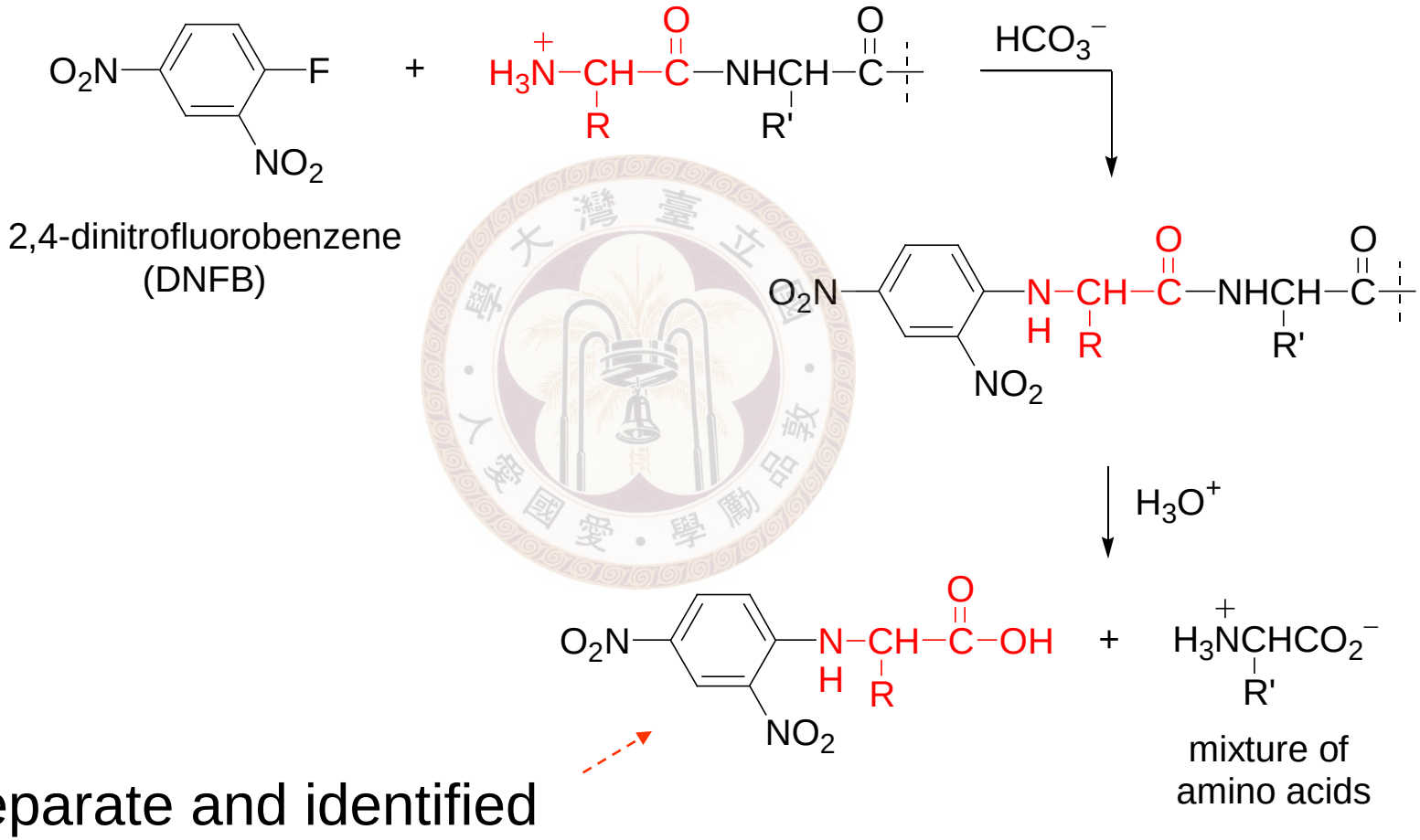
⟹ The amino acid sequence

✓Composition information
MW information
} exact number of amino acids can be determined

✓ Sequence determination
} terminal residue analysis
} partial hydrolysis

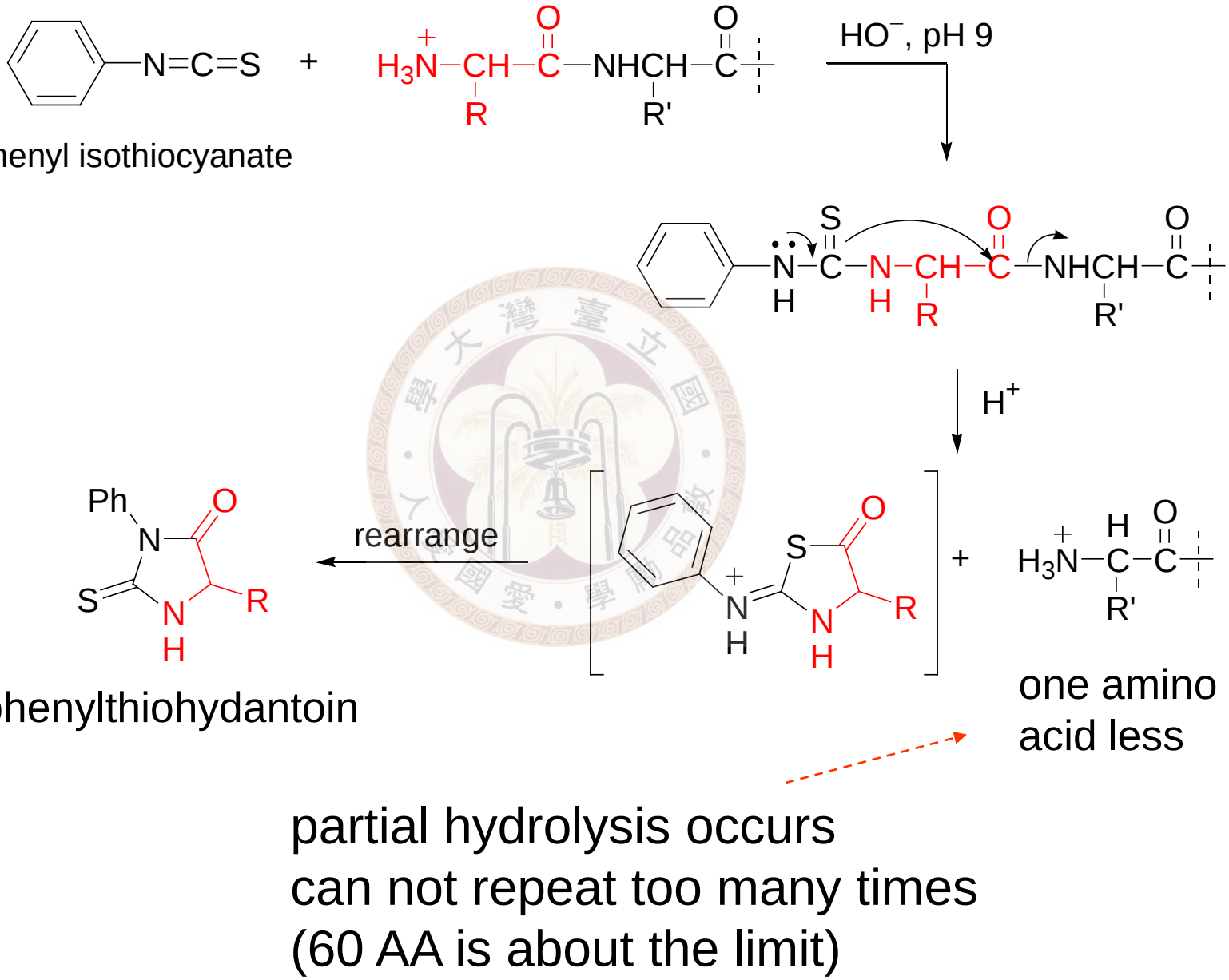
◎ Terminal residue analysis

✓ Sanger method



Separate and identified

✓ Edman method



partial hydrolysis occurs
can not repeat too many times
(60 AA is about the limit)

✓Carboxypeptidase method

Hydrolyze C-terminal one by one

Follow the progress of growing amino acids

Becomes more complicate as the time goes

◎ Partial hydrolysis

Use dilute acids or enzyme

→ cut protein into smaller fragments

→ identify each fragment

Enzyme cuts at specific site

例 trypsin cleaves carboxyl side of arg, lys

with extra amino group

chymotrypsin cleaves carboxyl side of phe, tyr, trp

with aryl side chain

Examples:

A pentapeptide

Val_2, Leu, His, Phe

Sanger method: N-terminal → Val

Carboxypeptidase: C-teminal → Leu

⟹ Val-(Val, His, Phe)-Leu

Partial hydrolysis

⟹ Val•His + His•Val + Val•Phe + Phe•Leu

Ans:

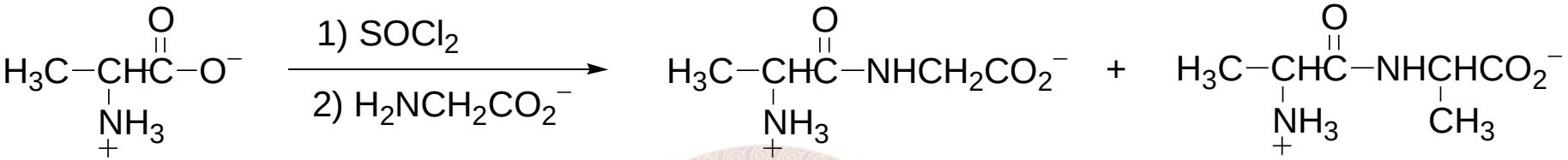
Val•His

His•Val ⟹ Val•His•Val•Phe•Leu

Val•Phe

Phe•Leu

※ Protein and polypeptide synthesis



Ala

Ala♦Gly

Ala♦Ala

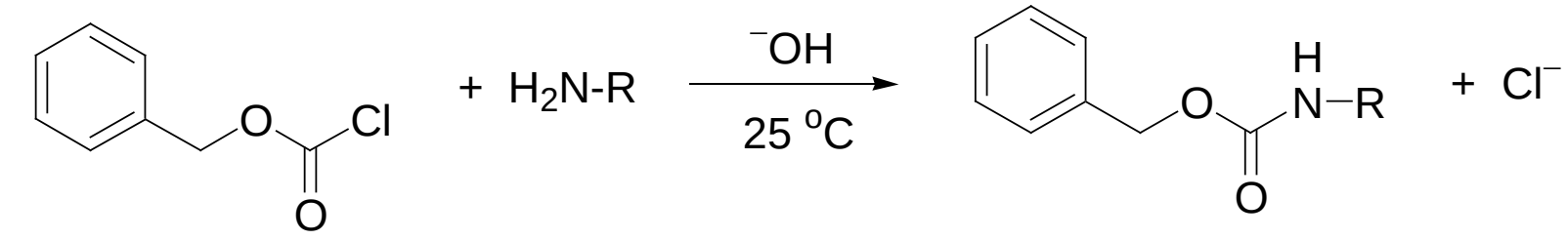
+ Ala♦Ala♦Ala + Ala♦Ala♦Gly + ...

A mixture is obtained

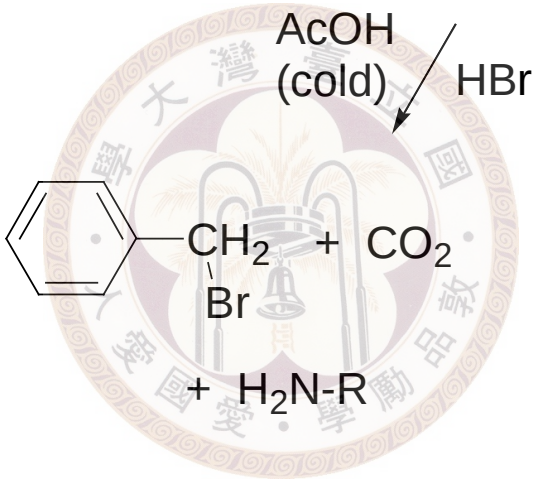
Solution:

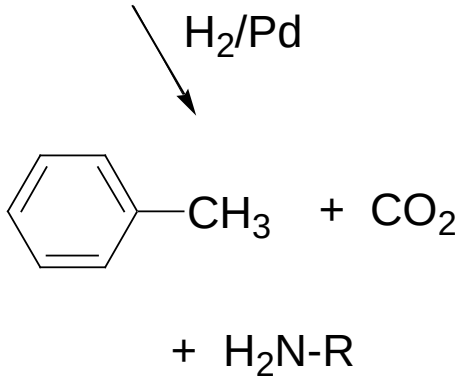
protection

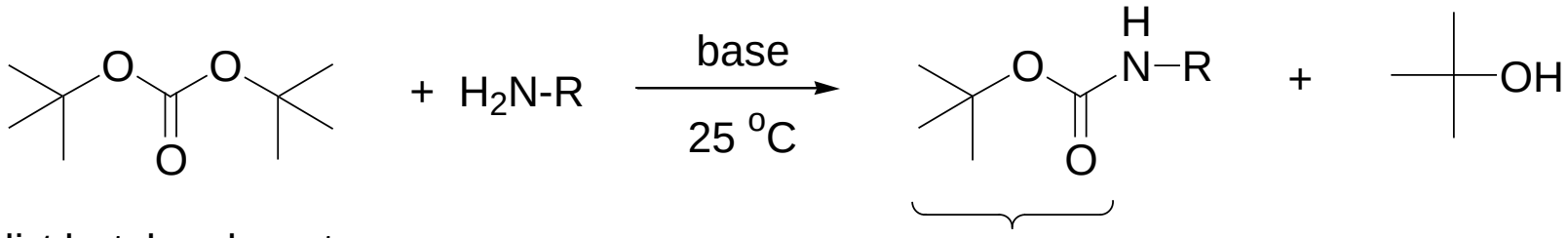
◎ Protection



benzyl chloroformate

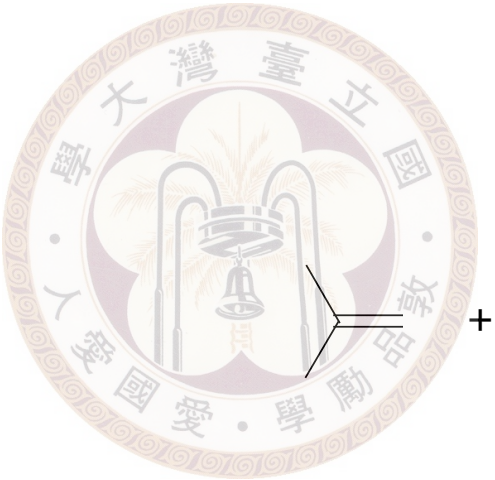


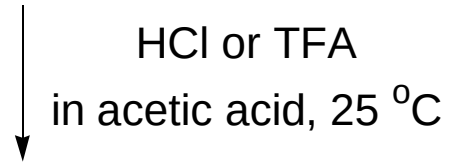


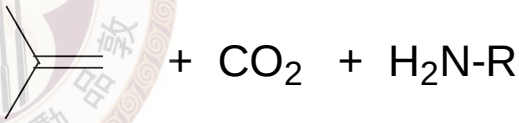


di-*t*-butyl carbonate

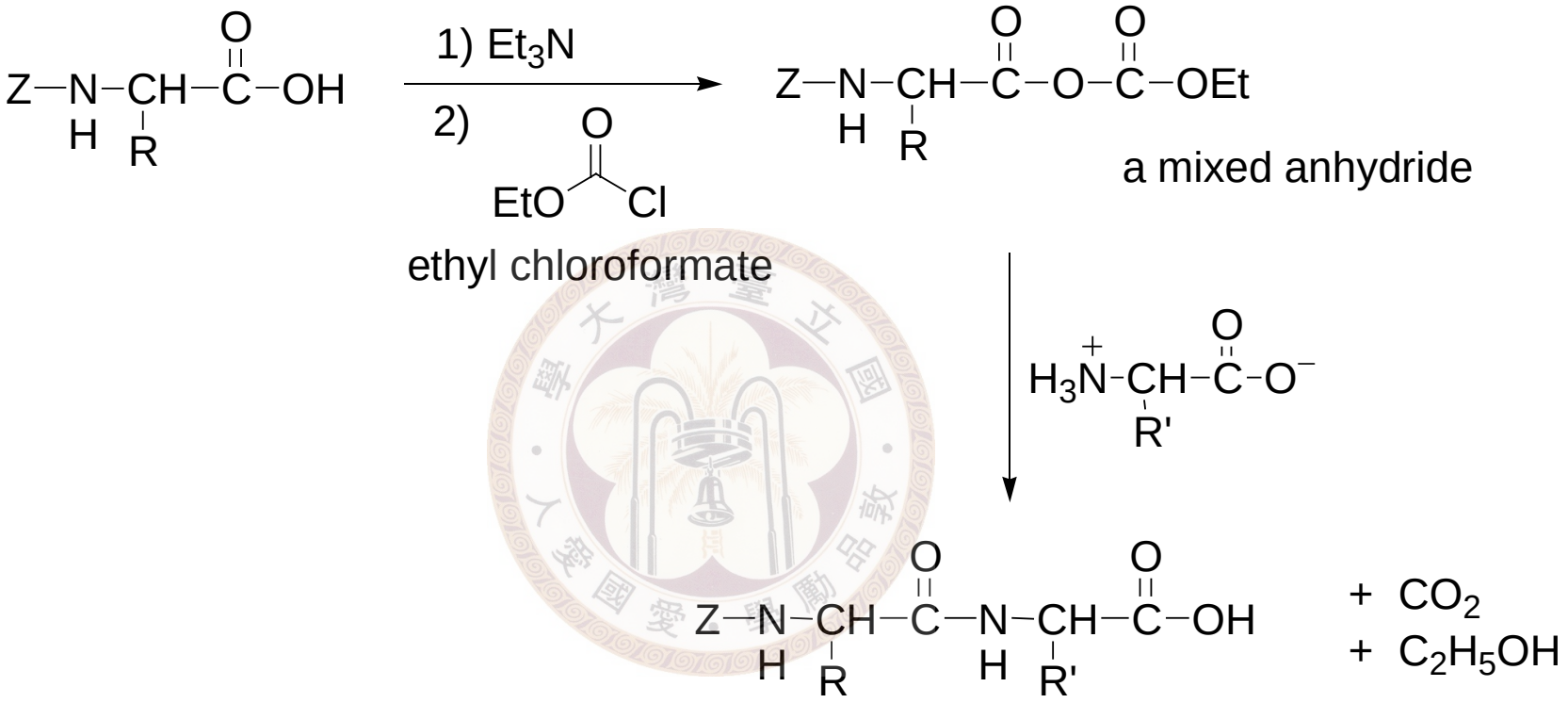
t-butyloxycarbonyl
(BOC group)



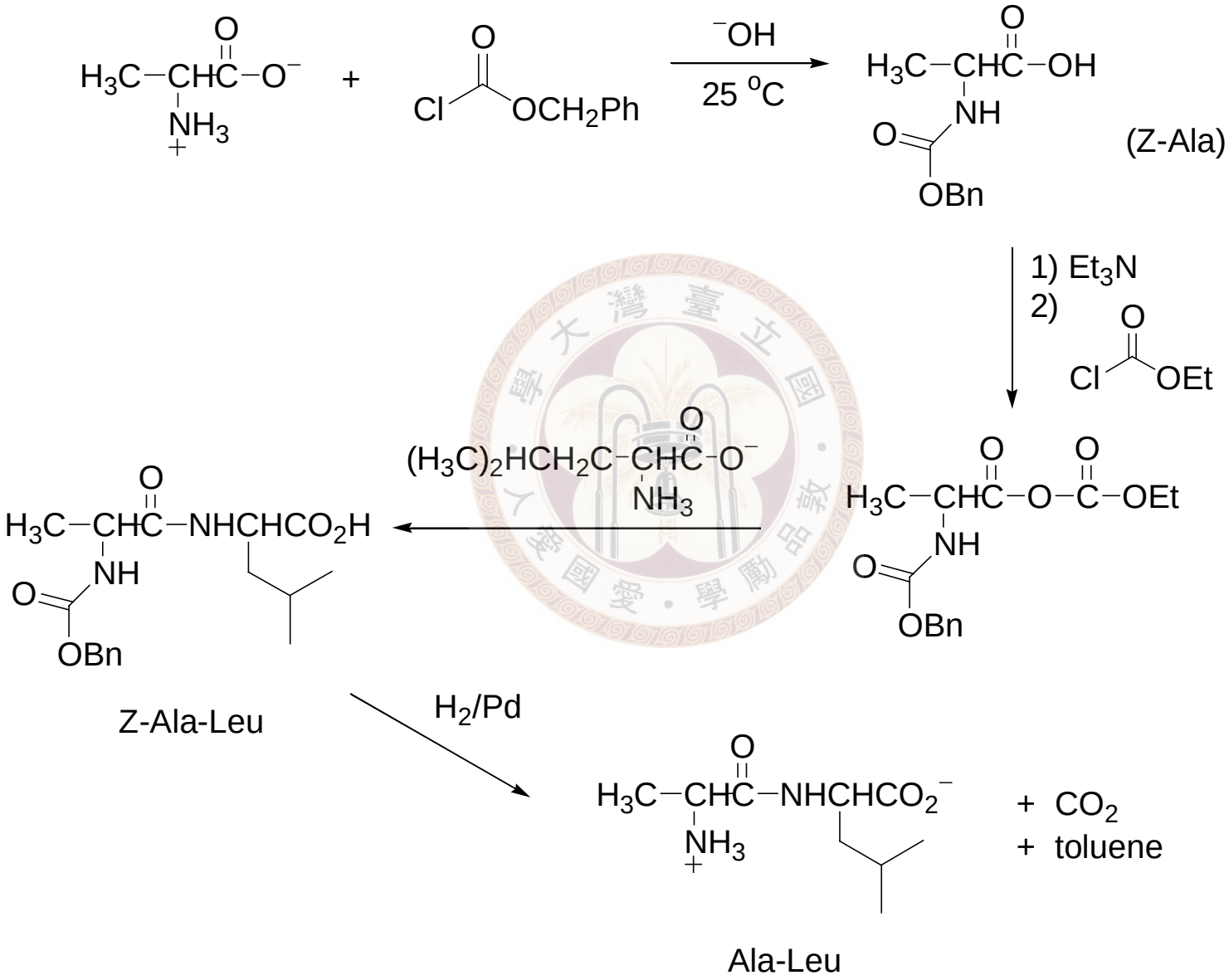




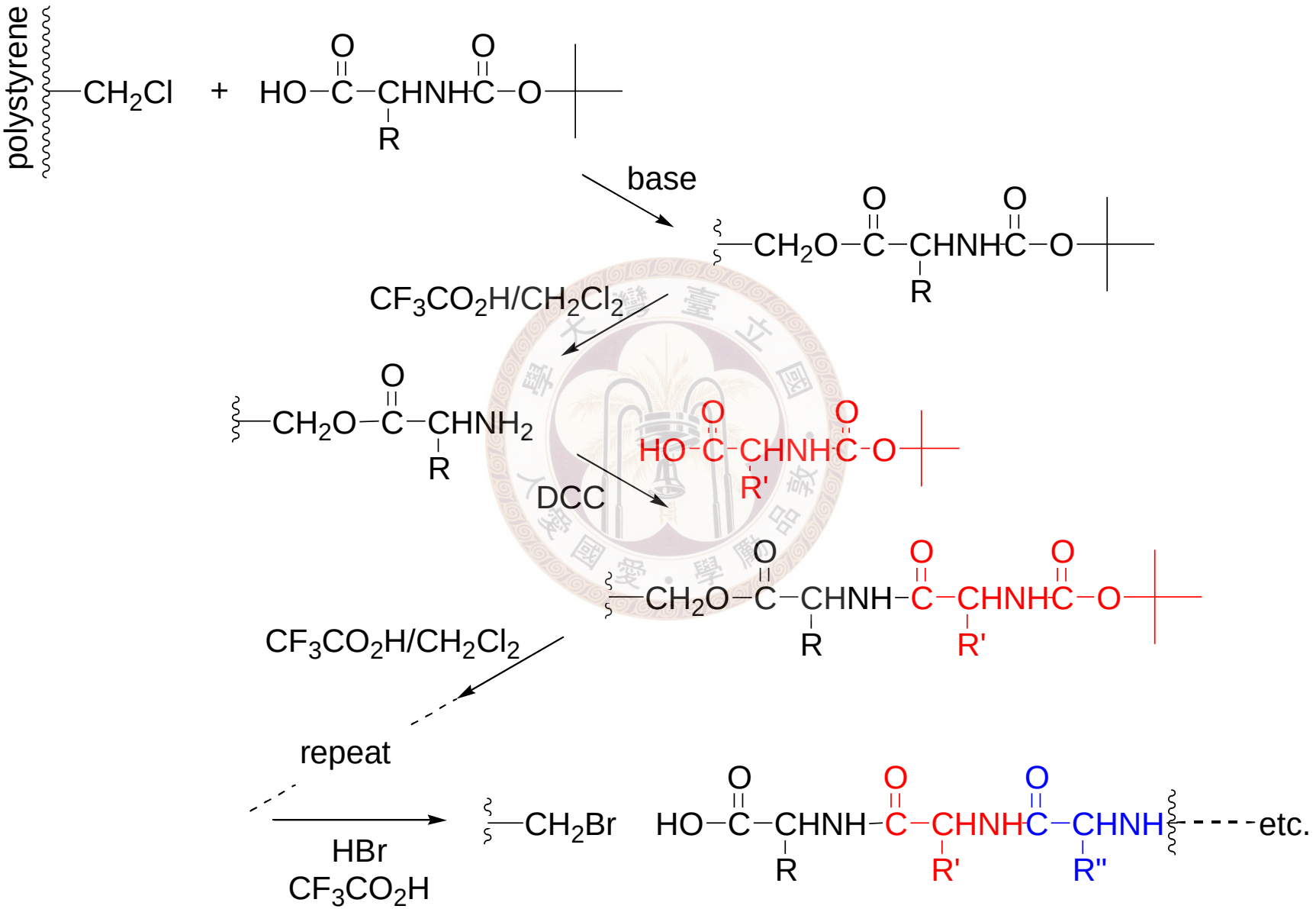
◎ Activation of carboxyl group



◎ Synthesis


H3C–CHC–O⁻
O
NH3+
+
Cl
OCH2Ph
⁻OH
25 °C
H3C–CHC–OH
NH
OBn
(Z-Ala)
1) Et3N
2)
Cl
OEt
(H3C)2HCH2C–CHC–O⁻
NH3
H3C–CHC–O–C–OEt
H3C–CHC–NHCHCO2H
Z-Ala-Leu
H2/Pd
H3C–CHC–NHCHCO2⁻
+ CO2
+ toluene
Ala-Leu

★ Merrifield method – polymer-bonded synthesis



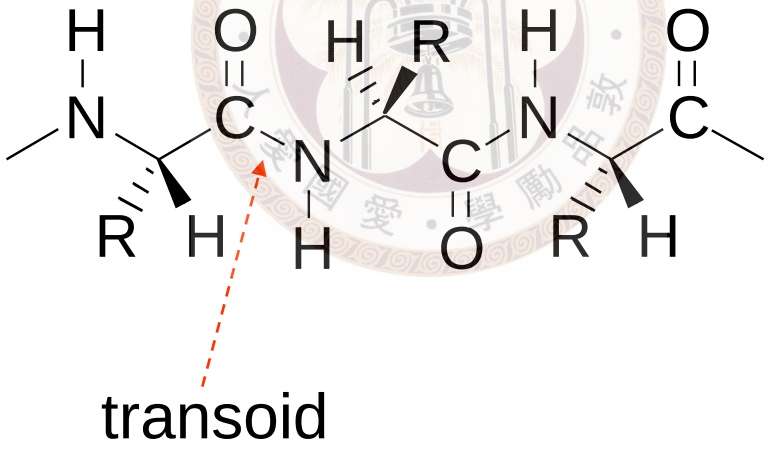
Successful for the synthesis of ribonuclease

- an 124 amino acid residues protein
- overall yield: 17%
- average yield for each step: 99% (in six weeks)

※ Protein structure

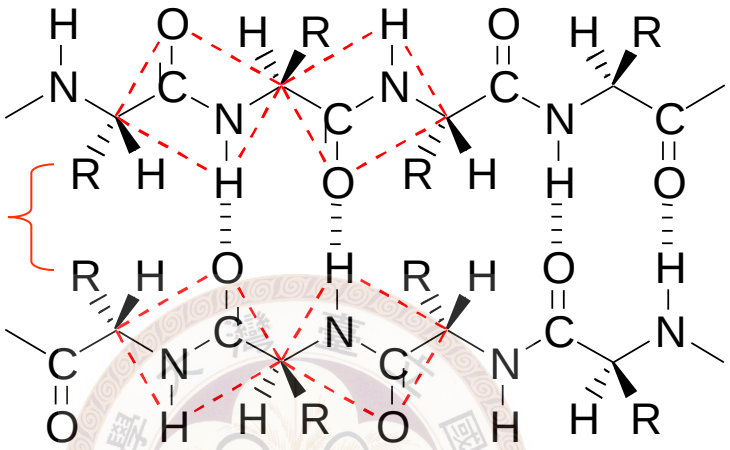
✓ Primary structure – the sequence

✓ Secondary structure: α-helix and β-sheet

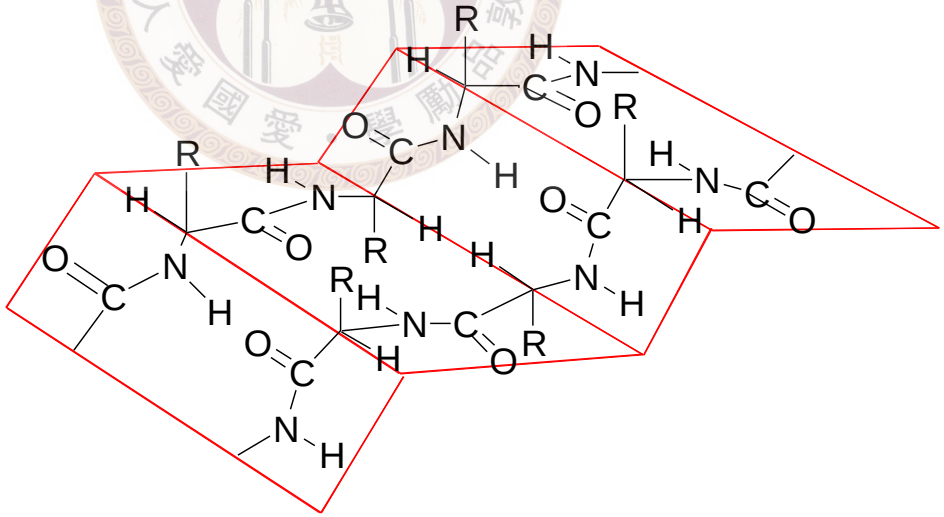


β structure

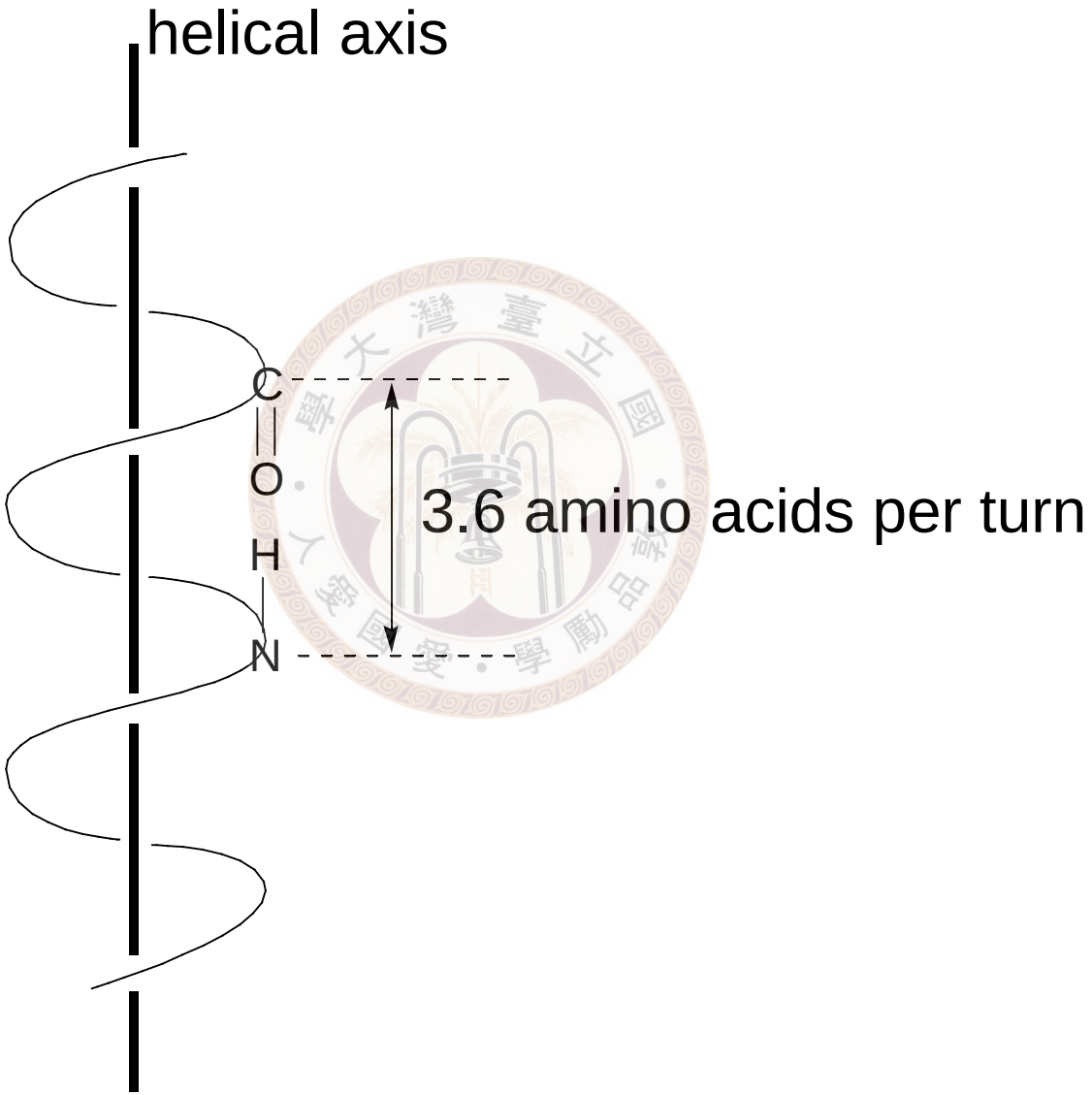
bad interaction
if planar



exists as
β-pleated sheet
to avoid steric
interaction



α structure: helical



✓Tertiary structure

overall three dimensional structure

determined by stability

- Hydrophilic part sticking outside
- Lipophilic part sticking inside
- Disulfide bonding
- Salt bridges