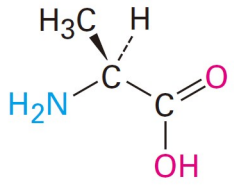
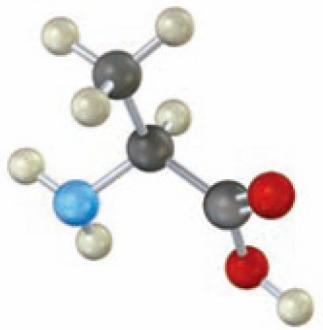


26

氨基酸，多肽和蛋白质

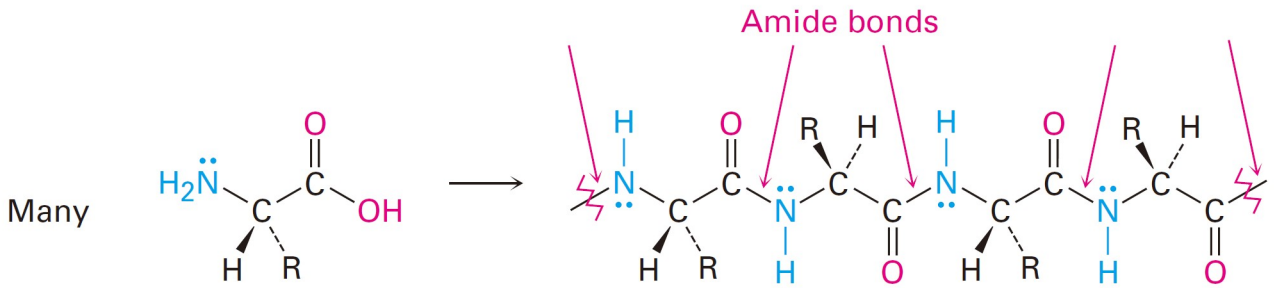
- 蛋白质是生命的载体和功能实现的物质基础。蛋白质水解以后的最小单元是氨基酸。一般少于 50 个氨基酸连成的链称为肽链。





Alanine, an amino acid

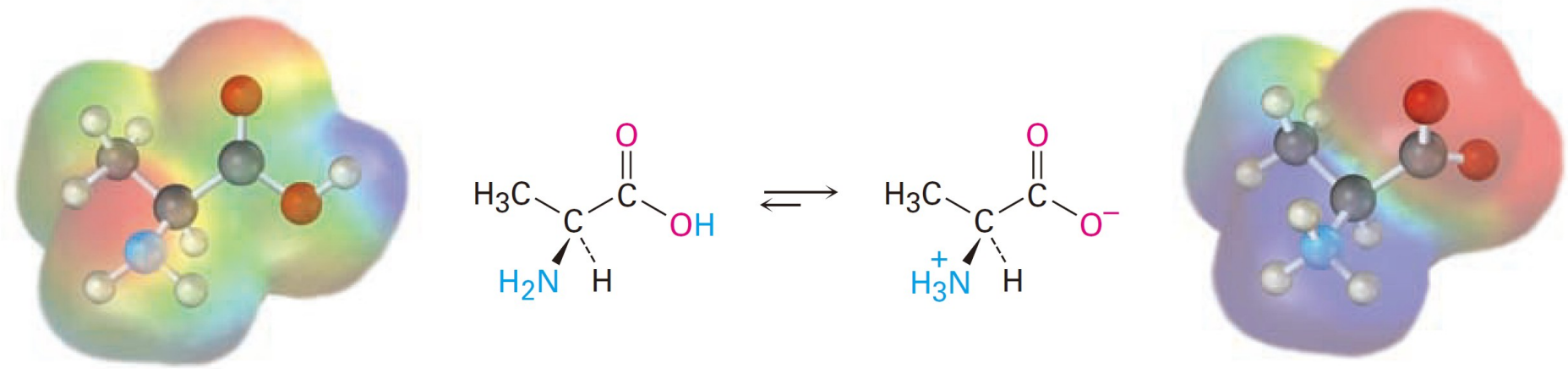
Their value as building blocks to make proteins stems from the fact that amino acids can join together into long chains by forming amide bonds between the $-NH_2$ of one amino acid and the $-CO_2H$ of another. For classification purposes, chains with fewer than 50 amino acids are often called **peptides**, while the term **protein** is generally used for larger chains.



26.1 氨基酸的结构

- 氨基酸，一般指 alpha 氨基酸，（氨）胺基处于羧基的 alpha 位。它在水溶液中一般以盐的形式存在。

We saw in **Sections 20.3 and 24.5** that a carboxyl group is deprotonated and exists as the carboxylate anion at a physiological pH of 7.3, while an amino group is protonated and exists as the ammonium cation. Thus, amino acids exist in aqueous solution primarily in the form of a dipolar ion, or **zwitterion** (from the German *zwitter,* meaning "hybrid").



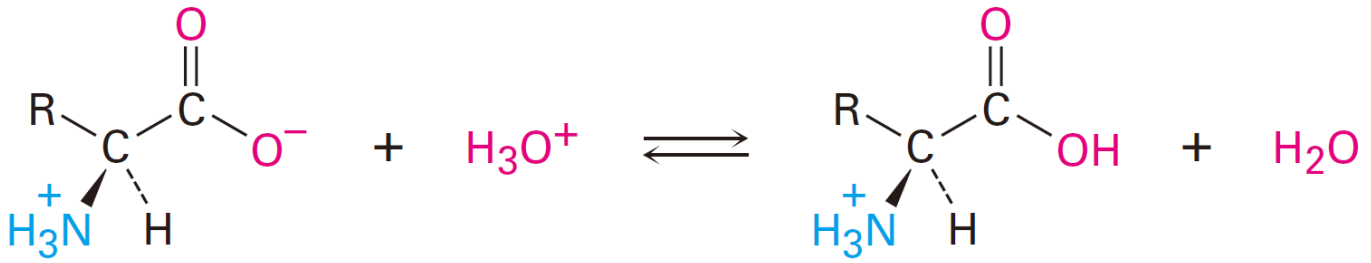
(uncharged) (zwitterion)

Alanine

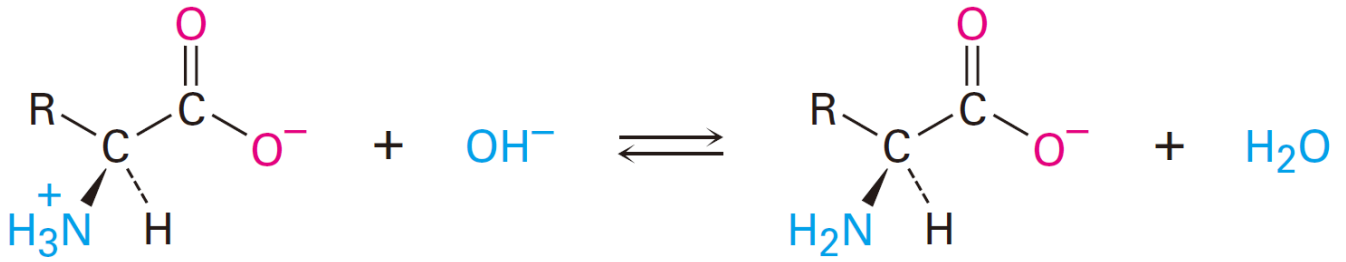
26.1 氨基酸的结构

- 氨基酸具有两性，既可以和酸反应也可以和碱反应。在酸性溶液中，它以氨离子存在；在碱性溶液中，它以羧酸阴离子存在。

In acid solution



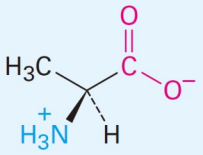
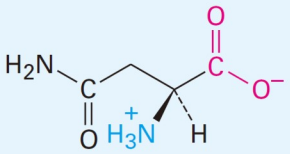
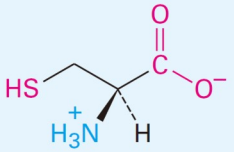
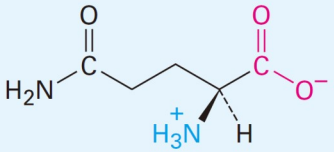
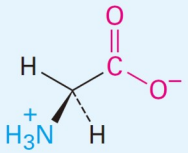
In base solution



26.1 氨基酸的结构

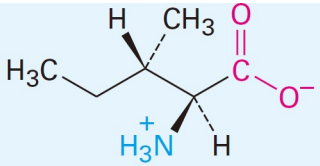
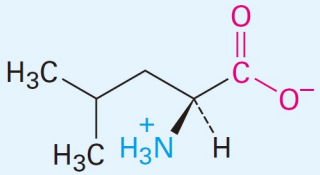
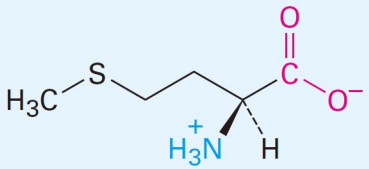
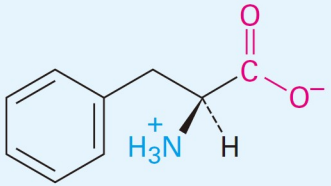
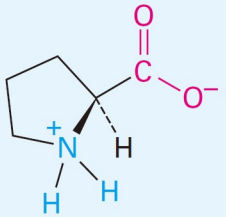
- 20 中常见的氨基酸

Table 26.1 The 20 Common Amino Acids in Proteins

Name	Abbreviations		MW	Stucture	pK_a α-CO_2H	pK_a α-NH_3^+	pK_a side chain	p*I*
Neutral Amino Acids								
Alanine 丙氨酸	Ala	A	89		2.34	9.69	—	6.01
Asparagine 天冬酰胺	Asn	N	132		2.02	8.80	—	5.41
Cysteine 半胱胺酸	Cys	C	121		1.96	10.28	8.18	5.07
Glutamine 谷氨酰胺	Gln	Q	146		2.17	9.13	—	5.65
Glycine 甘氨酸	Gly	G	75		2.34	9.60	—	5.97

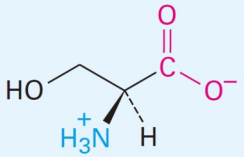
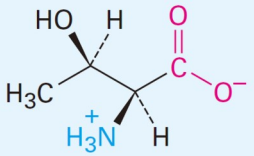
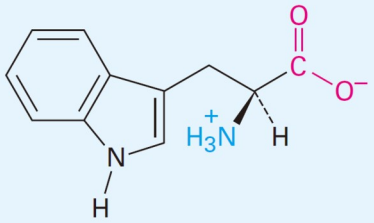
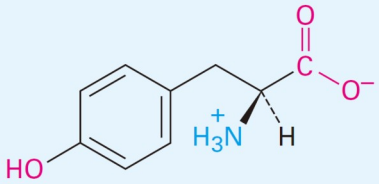
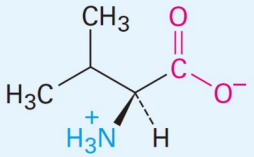
26.1 氨基酸的结构

- 20 中常见的氨基酸

Isoleucine 异亮氨酸	Ile	I	131		2.36	9.60	—	6.02
Leucine 亮氨酸	Leu	L	131		2.36	9.60	—	5.98
Methionine 蛋氨酸	Met	M	149		2.28	9.21	—	5.74
Phenylalanine 苯丙氨酸	Phe	F	165		1.83	9.13	—	5.48
Proline 脯氨酸	Pro	P	115		1.99	10.60	—	6.30

26.1 氨基酸的结构

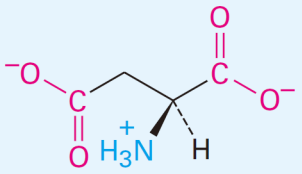
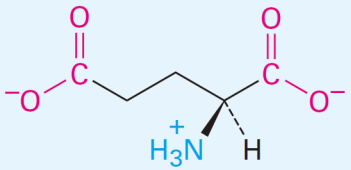
- 20 中常见的氨基酸

Name	Abbreviations		MW	Stucture	pK_a α-CO_2H	pK_a α-NH_3^+	pK_a side chain	p*I*
Neutral Amino Acids *(continued)*								
Serine 丝氨酸	Ser	S	105		2.21	9.15	—	5.68
Threonine 苏氨酸	Thr	T	119		2.09	9.10	—	5.60
Tryptophan 色氨酸	Trp	W	204		2.83	9.39	—	5.89
Tyrosine 酪氨酸	Tyr	Y	181		2.20	9.11	10.07	5.66
Valine 缬氨酸	Val	V	117		2.32	9.62	—	5.96

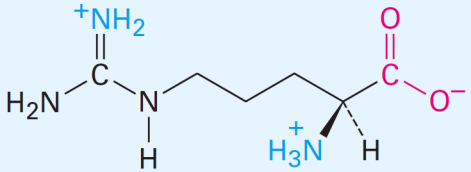
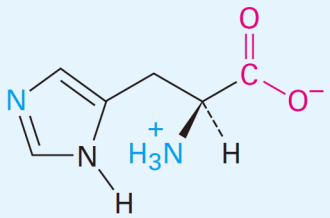
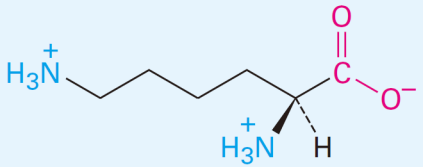
26.1 氨基酸的结构

- 20 中常见的氨基酸

Acidic Amino Acids

Aspartic acid 天冬氨酸	Asp	D	133		1.88	9.60	3.65	2.77
Glutamic acid 谷氨酸	Glu	E	147		2.19	9.67	4.25	3.22

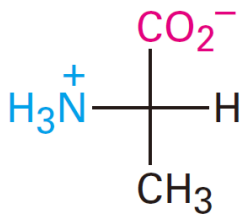
Basic Amino Acids

Arginine 精氨酸	Arg	R	174		2.17	9.04	12.48	10.76
Histidine 组氨酸	His	H	155		1.82	9.17	6.00	7.59
Lysine 赖氨酸	Lys	K	146		2.18	8.95	10.53	9.74

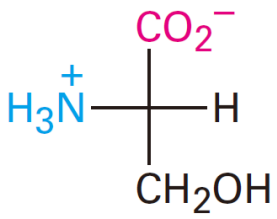
26.1 氨基酸的结构

- 天然的氨基酸都是 L 构型的。回忆 D 构型糖，羟基向右； L 构型氨基酸，氨基向左。

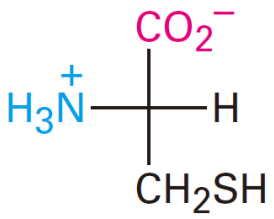
(Section 25.3), the naturally occurring α-amino acids are often referred to as L amino acids.



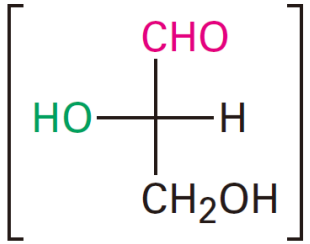
L-Alanine
(*S*)-Alanine



L-Serine
(*S*)-Serine



L-Cysteine
(*R*)-Cysteine



L-Glyceraldehyde

26.1 氨基酸的结构

- 练习

Problem 26.1

How many of the α-amino acids shown in Table 26.1 contain aromatic rings? How many contain sulfur? How many contain alcohols? How many contain hydrocarbon side chains?

Problem 26.2

Eighteen of the nineteen L amino acids have the *S* configuration at the α carbon. Cysteine is the only L amino acid that has an *R* configuration. Explain.

Problem 26.3

The amino acid threonine, (2*S*,3*R*)-2-amino-3-hydroxybutanoic acid, has two chirality centers.

(a) Draw a Fischer projection of threonine.

(b) Draw a Fischer projection of a threonine diastereomer, and label its chirality centers as *R* or *S*.

26.2 氨基酸的等电点

- 一定 pH 条件下，某种氨基酸接受或给出质子的程度相等，分子所带的净电荷为零，此时溶液的 pH 值就称为该氨基酸的等电点。

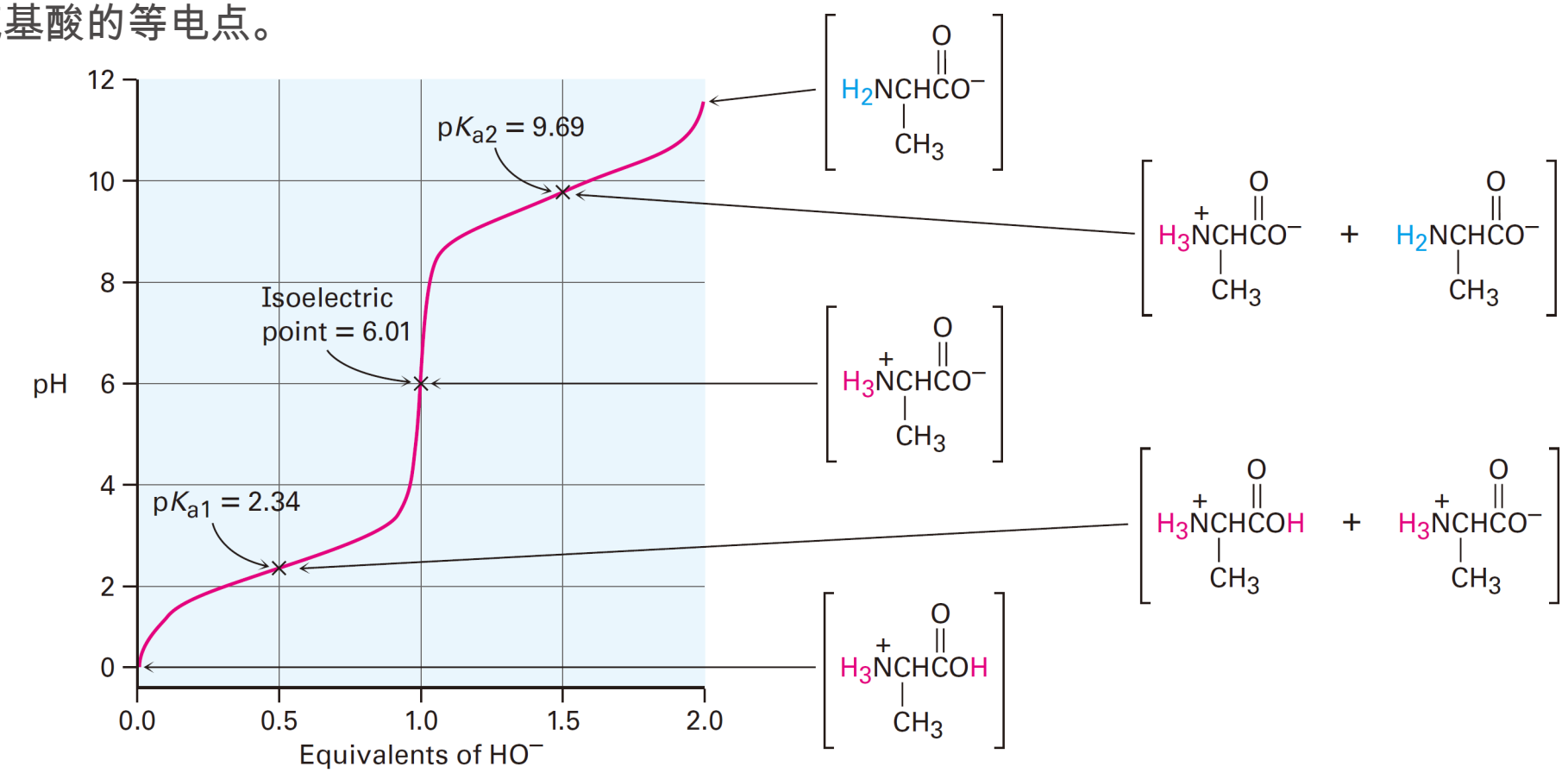
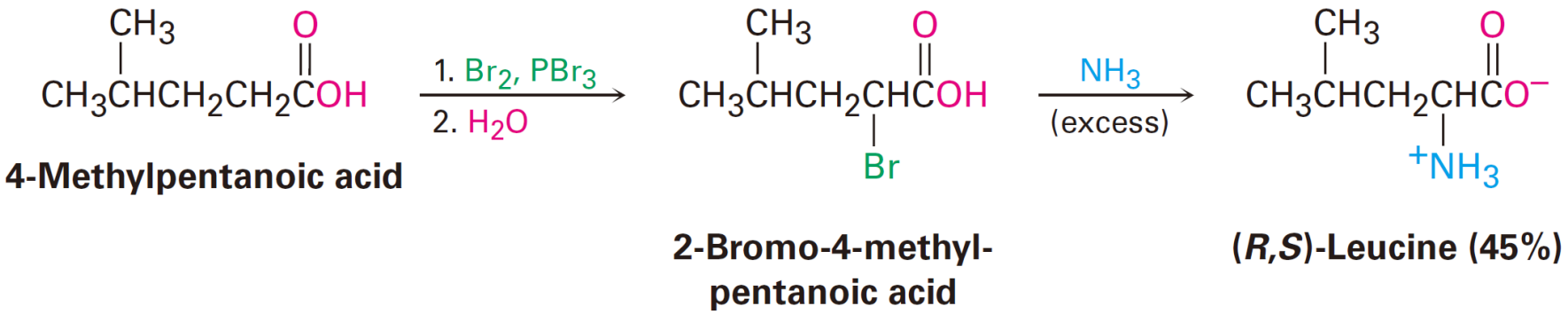


Figure 26.1 A titration curve for alanine, plotted using the Henderson–Hasselbalch equation. Each of the two legs is plotted separately. At pH < 1, alanine is entirely protonated; at pH = 2.34, alanine is a 50 : 50 mix of protonated and neutral forms; at pH = 6.01, alanine is entirely neutral; at pH = 9.69, alanine is a 50 : 50 mix of neutral and deprotonated forms; at pH > 11.5, alanine is entirely deprotonated.

26.3 氨基酸的合成

- S_N2 亲核取代反应



4-Methylpentanoic acid

2-Bromo-4-methyl-pentanoic acid

(*R,S*)-Leucine (45%)

Problem 26.5

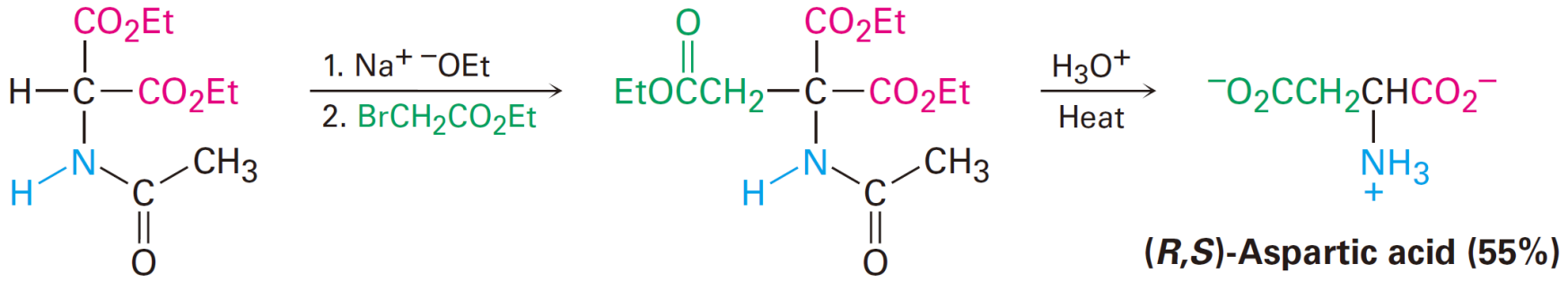
Show how you could prepare the following α-amino acids from the appropriate carboxylic acids:

(a) Phenylalanine **(b)** Valine

26.3 氨基酸的合成

- 胺基丙二酸酯合成法

The Amidomalonate Synthesis



Diethyl acetamidomalonate

(*R,S*)-Aspartic acid (55%)

Problem 26.6

What alkyl halides would you use to prepare the following α-amino acids by the amidomalonate method?

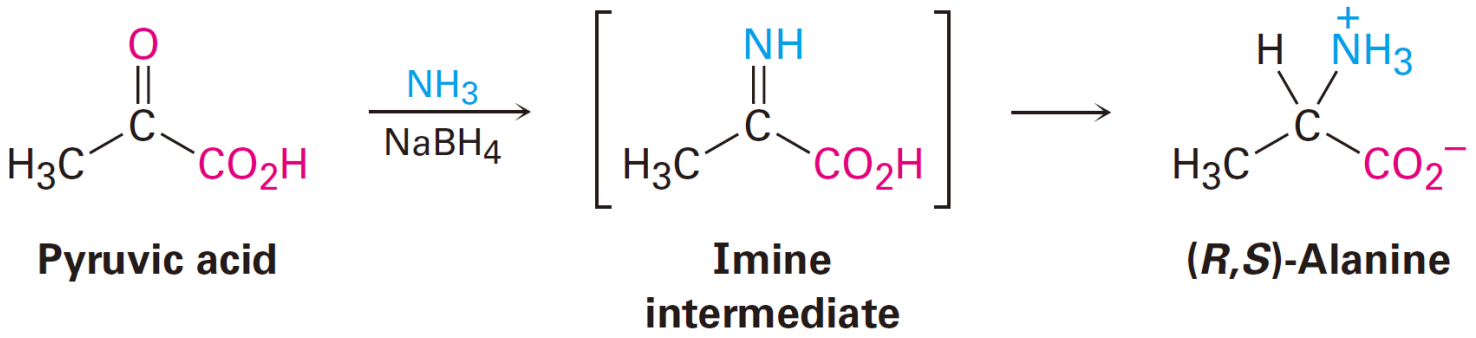
(a) Leucine **(b)** Histidine **(c)** Tryptophan **(d)** Methionine

26.3 氨基酸的合成

- alpha 酮酸的还原胺化

Reductive Amination of α-Keto Acids

Yet another method for the synthesis of α-amino acids is by reductive amination of an α-keto acid with ammonia and a reducing agent. Alanine, for instance, is prepared by treatment of pyruvic acid with ammonia in the presence of $NaBH_4$. As described in **Section 24.6**, the reaction proceeds through formation of an intermediate imine that is then reduced.

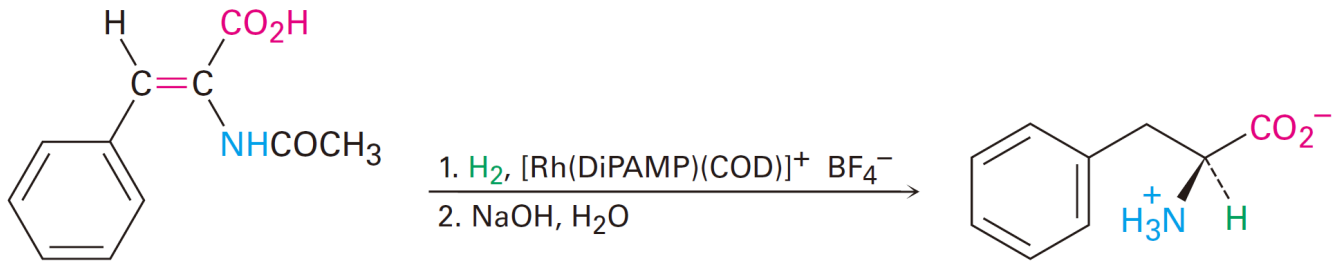


Pyruvic acid **Imine intermediate** **(*R*,*S*)-Alanine**

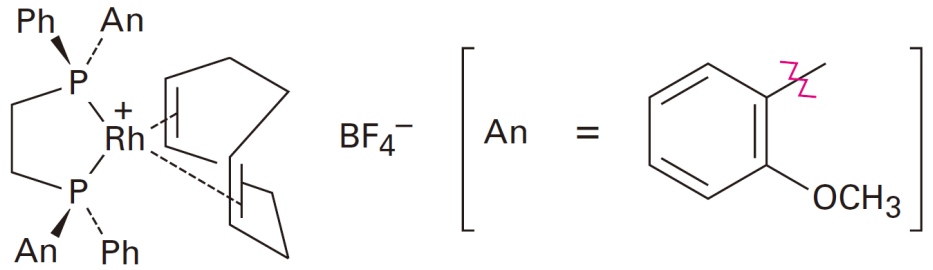
26.3 氨基酸的合成

- 不对称合成（对映选择性合成）。光活性的膦配体与中心金属 Rh 配位，造成一个不对称的反应环境。当底物接近金属中心时，只有其中一个面可以被进攻，因此得到单一的对映异构体。回想：三价膦的孤对电子不能自由翻转，所以有手性。胺的孤对电子可以翻转，因此胺没有手性问题。

不对称氢化，获得2001年诺贝尔化学奖。



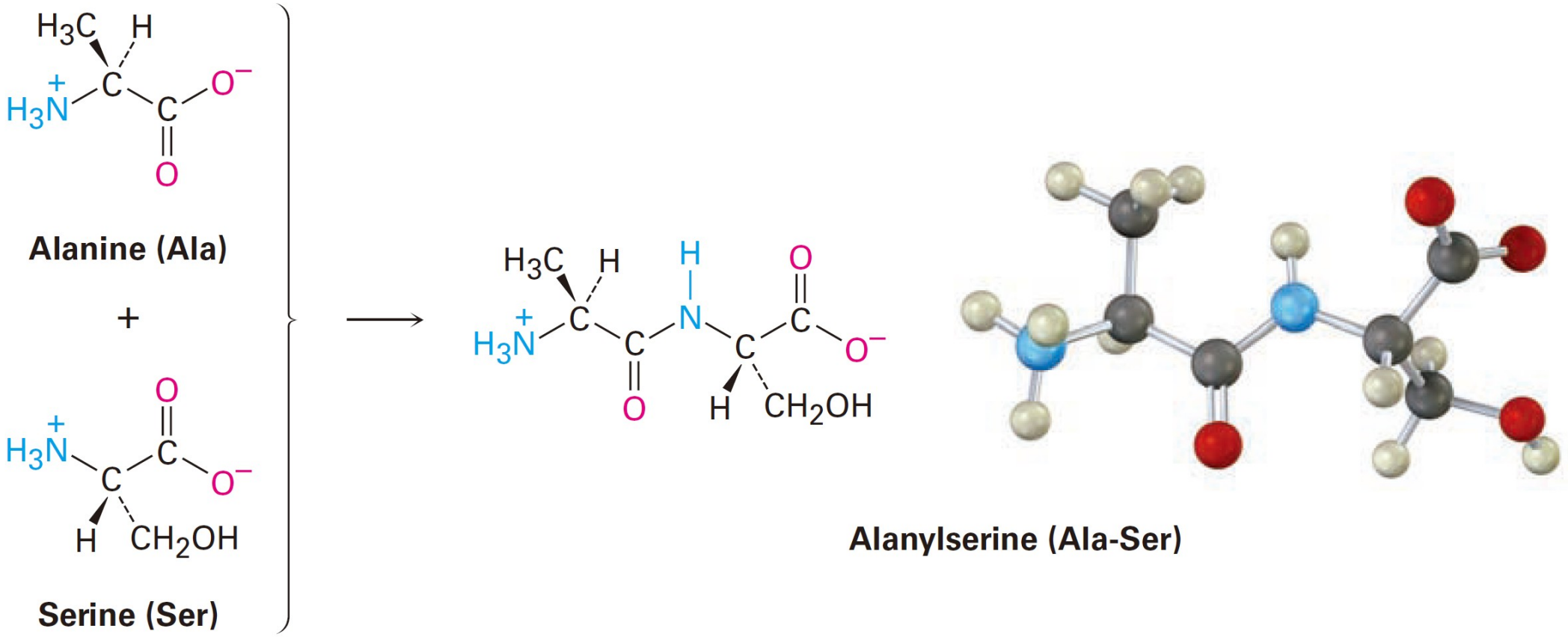
A (*Z*) enamido acid **(*S*)-Phenylalanine**



[Rh(*R*,*R*-DiPAMP)(COD)]$^+$ BF_4^-

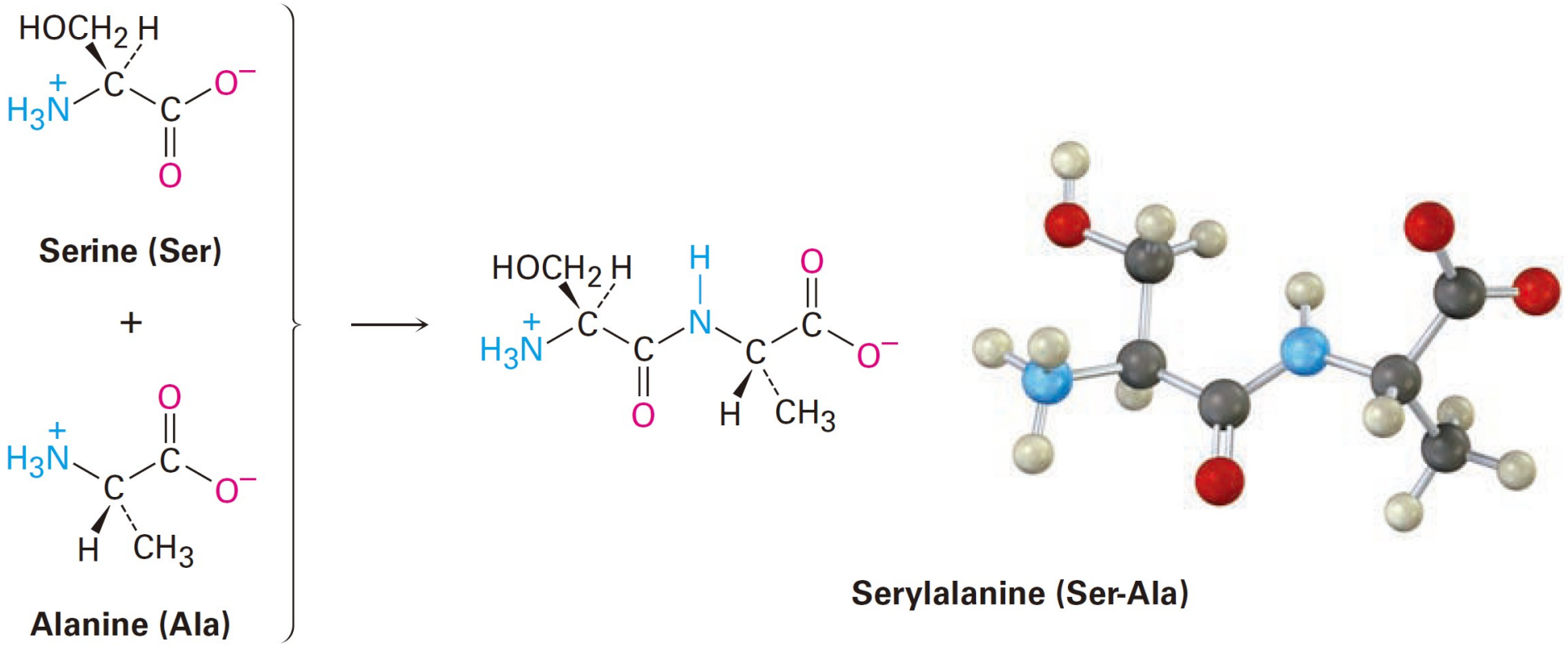
26.4 肽和蛋白质

- 两个氨基酸，首尾通过羧基和氨基形成酰胺键，得到肽。



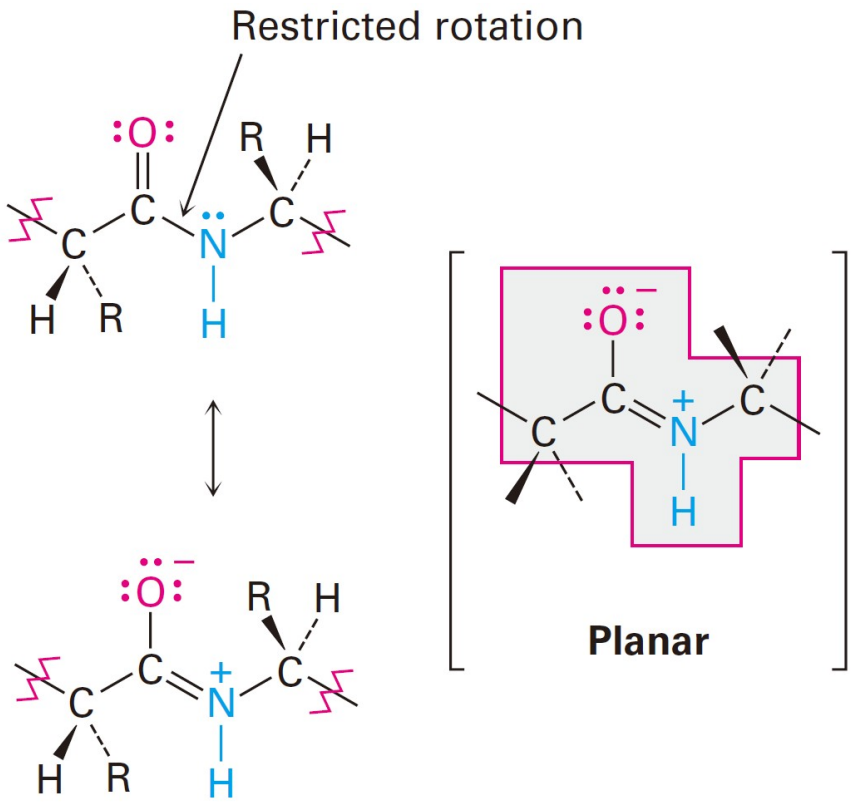
26.4 肽和蛋白质

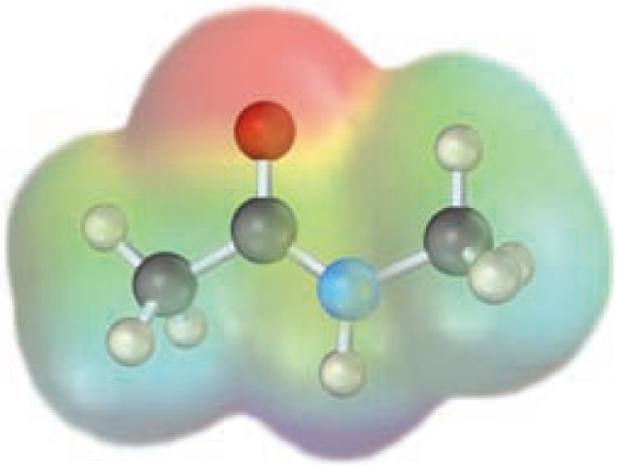
- 这种连接具有次序性。比较下面的反应和前一页的反应，有什么不同？



26.4 肽和蛋白质

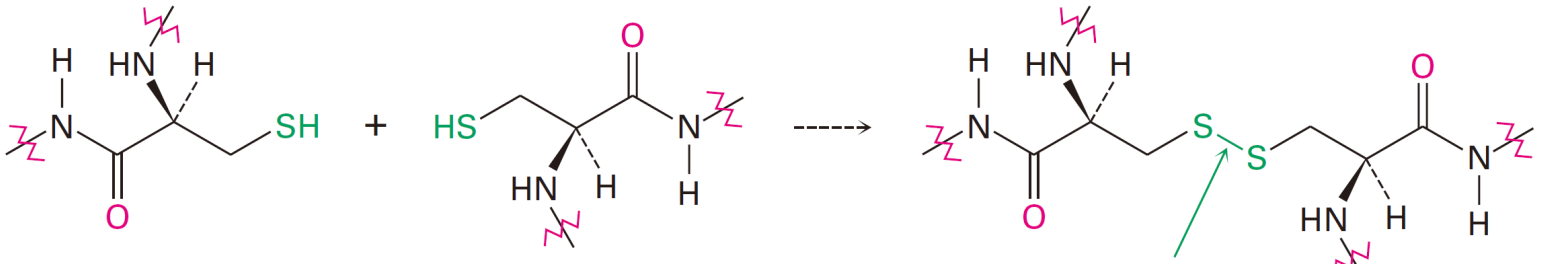
- 酰胺键的共振。粉色框内的原子处于同一平面。





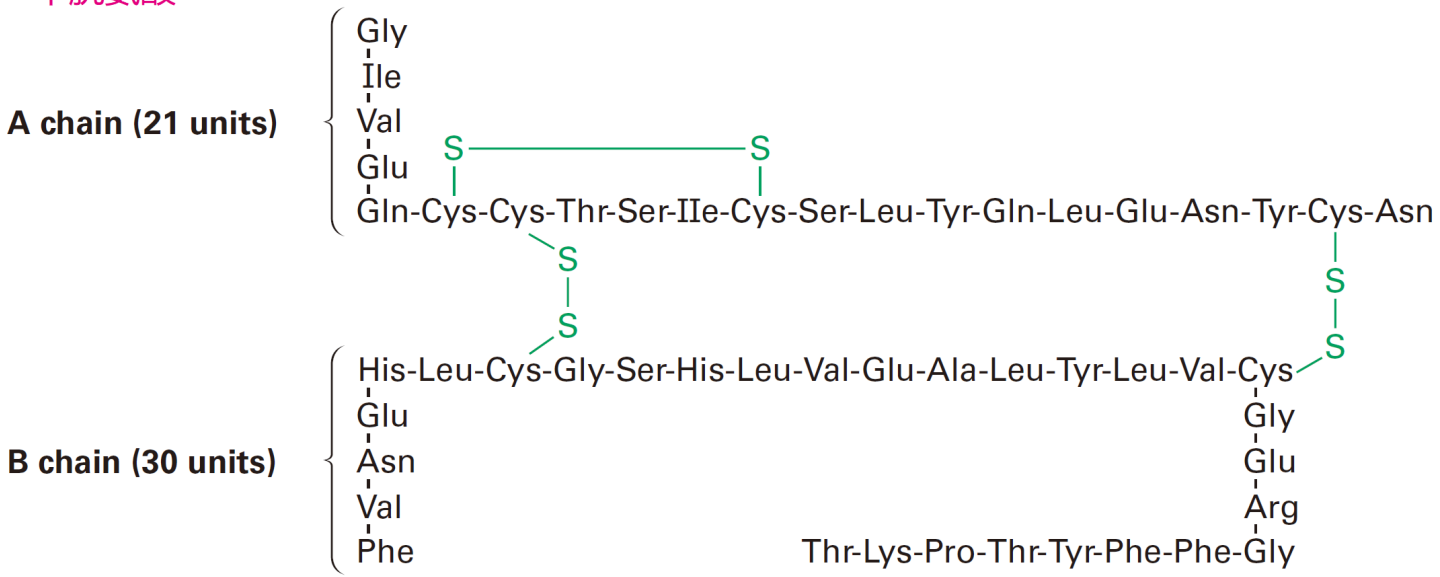
26.4 肽和蛋白质

- 氨基酸通过酰胺键的连接形成肽链。肽链间的连接除了酰胺键以外，还有其他形式，比如巯基相连形成 S–S（二硫）键



半胱氨酸

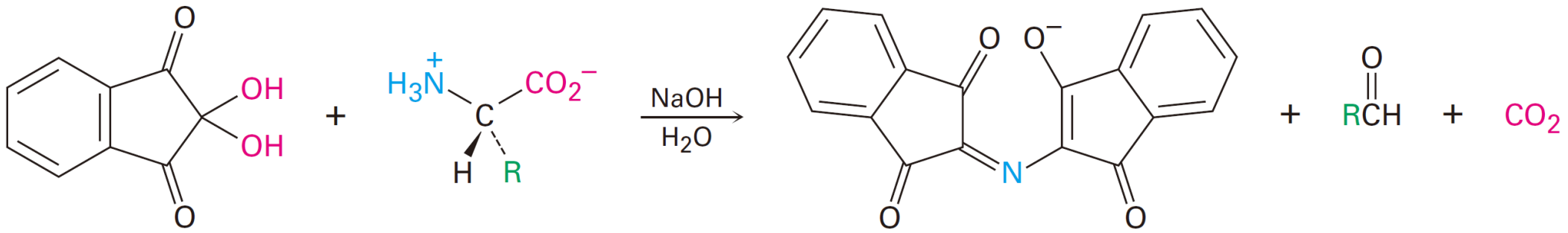
二硫键



Insulin 胰岛素

26.5 肽链的氨基酸分析

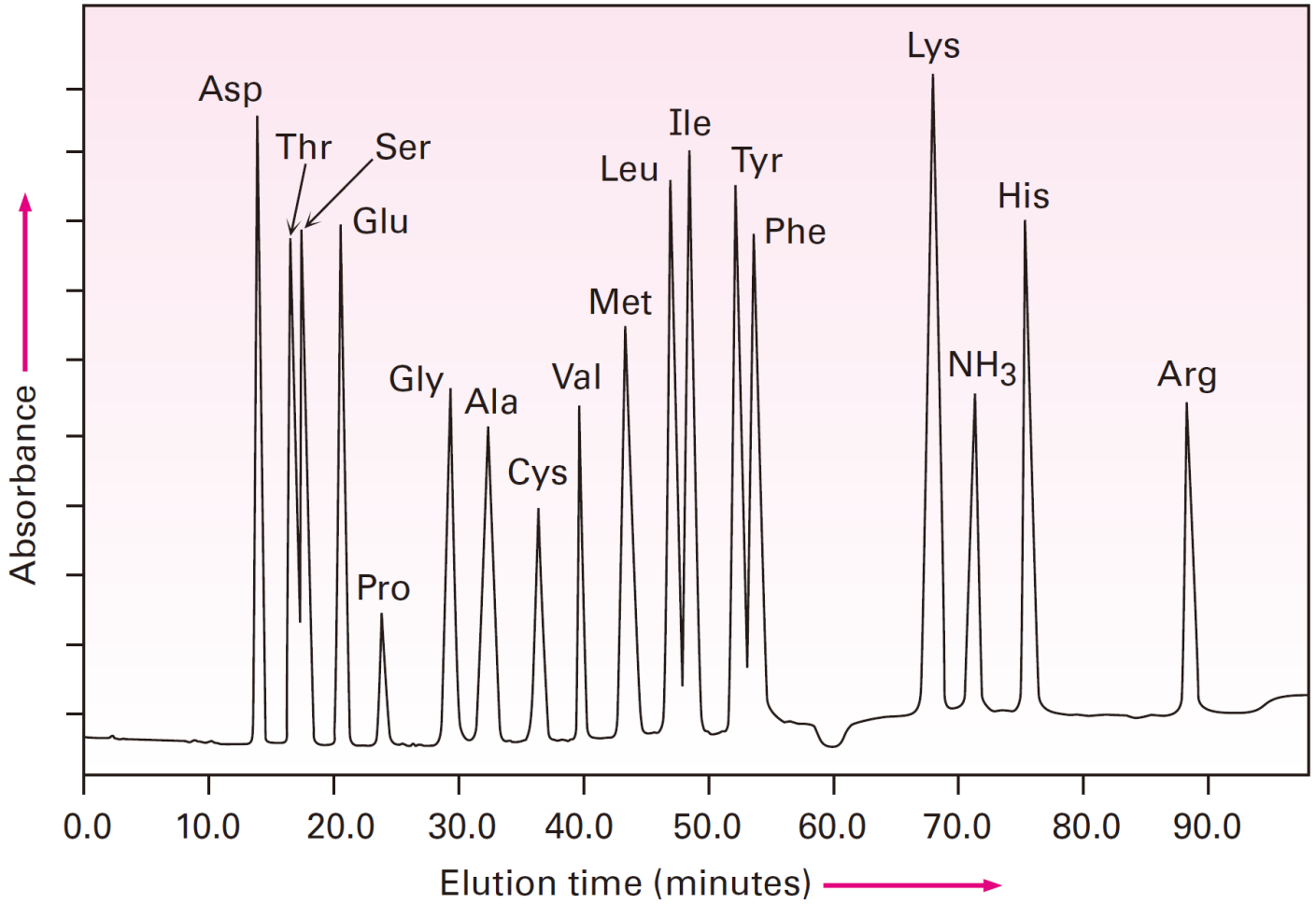
- 分析蛋白质或肽链的结构，我们首先要回答三个问题：有哪些氨基酸，它们各自有多少个，以及连接顺序怎样。下面这个反应可以快速检测氨基酸类型的化合物，但不能告诉是什么氨基酸。



Ninhydrin + **α-Amino acid** → **(purple color)** + RCHO + CO_2

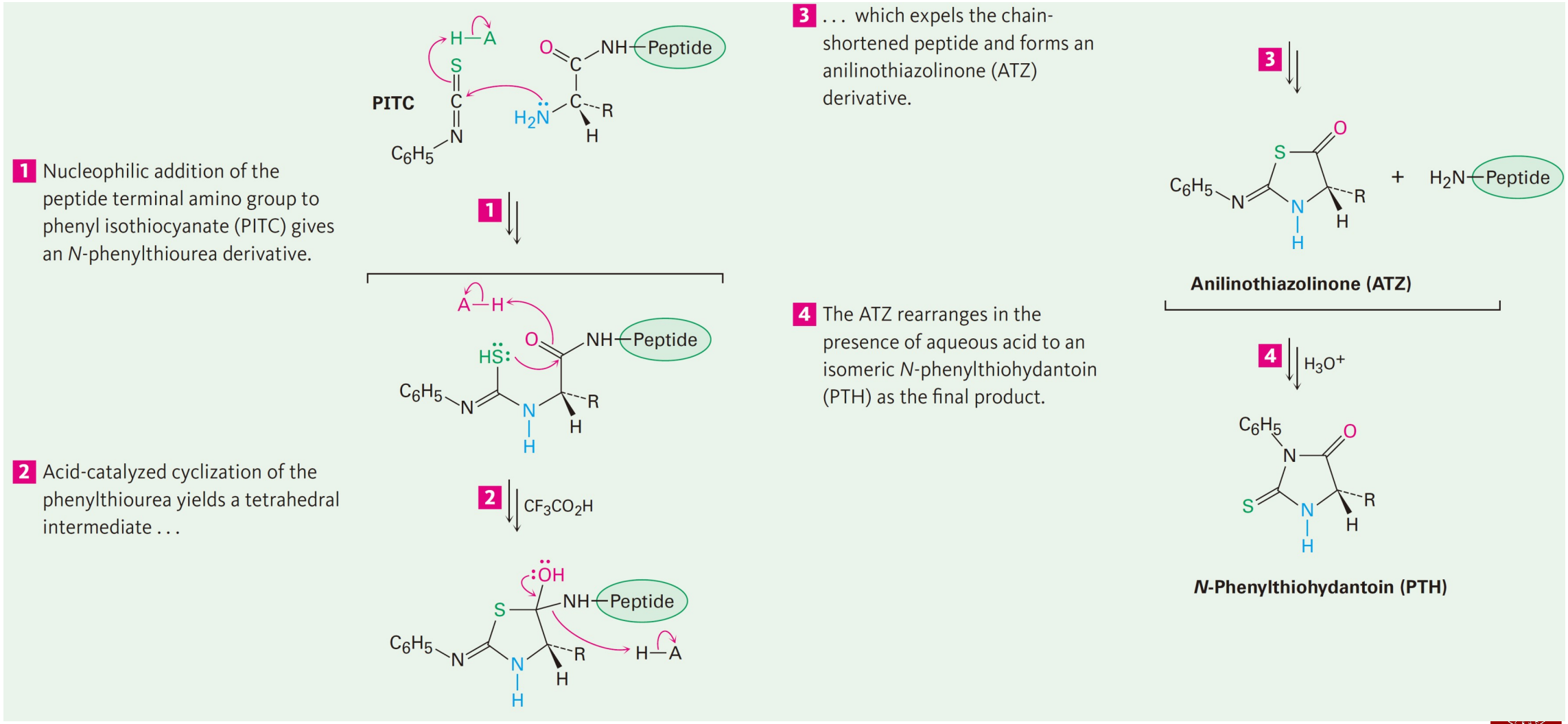
26.5 肽链的氨基酸分析

- 1972 年诺贝尔化学奖授予 William Stein 和 Stanford Moore ，以表彰他们在氨基酸序列分析做出的贡献。这里，用很强的反应条件让蛋白质或者肽链彻底水解，得到氨基酸。然后通过色谱分析，得到它们的种类和数量。



26.6 肽链的 Edman 降解

- N 末端氨基酸残基被异硫氰酸苯酯修饰，然后从多肽链上切下修饰的残基，再经层析鉴定，余下的多肽链（少了一个残基）被回收再进行下一轮降解循环。



26.6 肽链的 Edman 降解

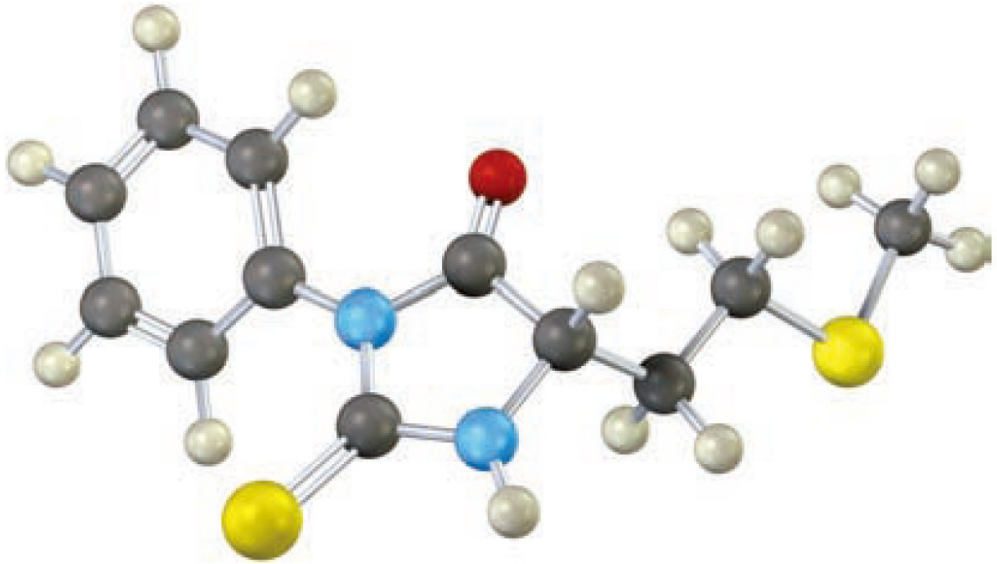
- 练习

Problem 26.12

The octapeptide angiotensin II has the sequence Asp-Arg-Val-Tyr-Ile-His-Pro-Phe. What fragments would result if angiotensin II were cleaved with trypsin? With chymotrypsin?

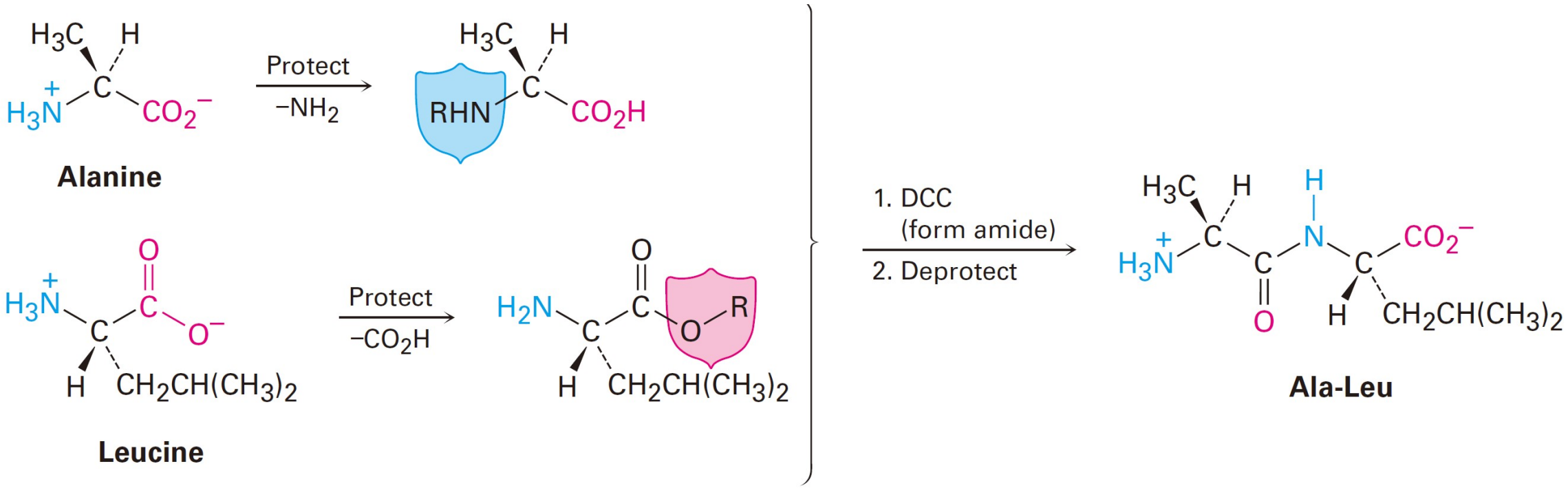
Problem 26.13

What is the N-terminal residue on a peptide that gives the following PTH derivative on Edman degradation?



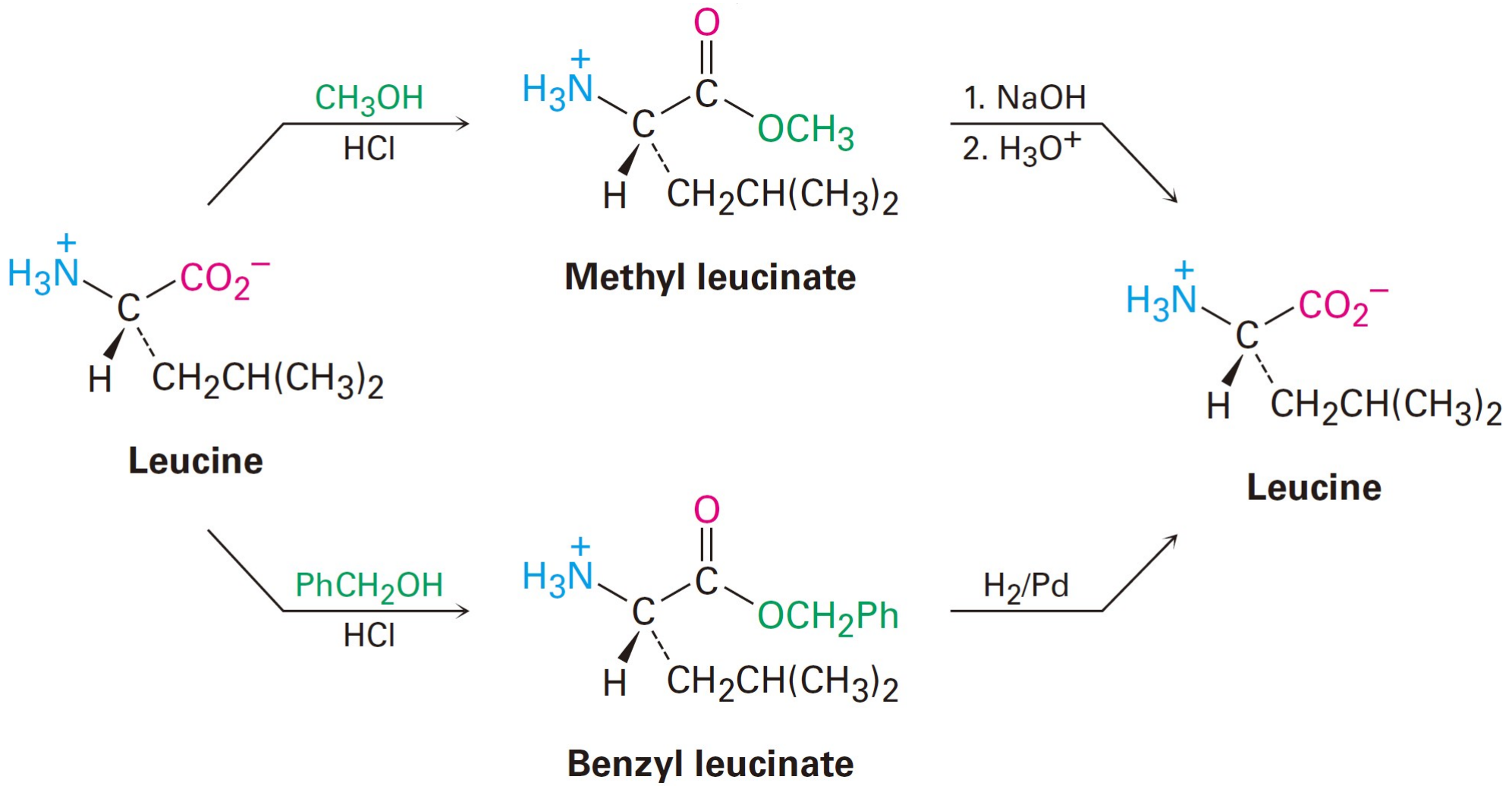
26.7 肽的合成

- 两种氨基酸直接形成肽键会得到两种不同次序的肽链。因此，我们需要保护一个氨基酸的氨基和另一个氨基酸的羧基，然后让其反应得到单一次序的肽链。



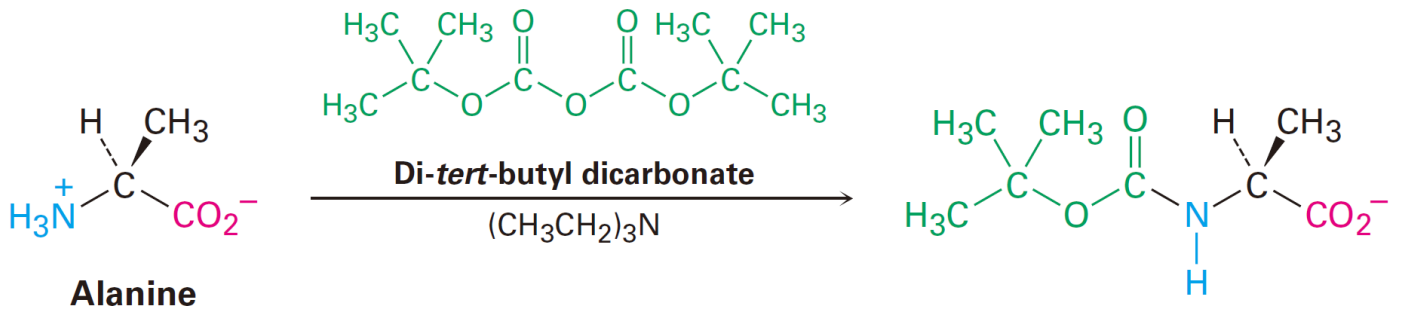
26.7 肽的合成

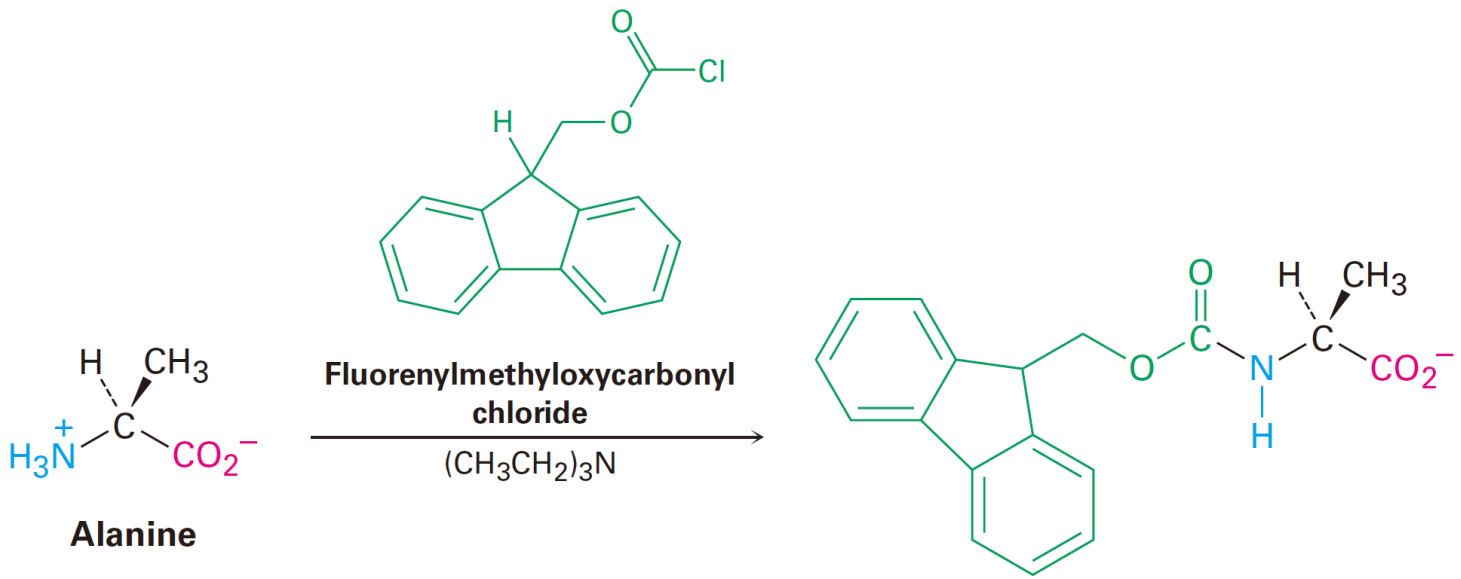
- 对 C 端的保护（对羧酸的保护）及去保护



26.7 肽的合成

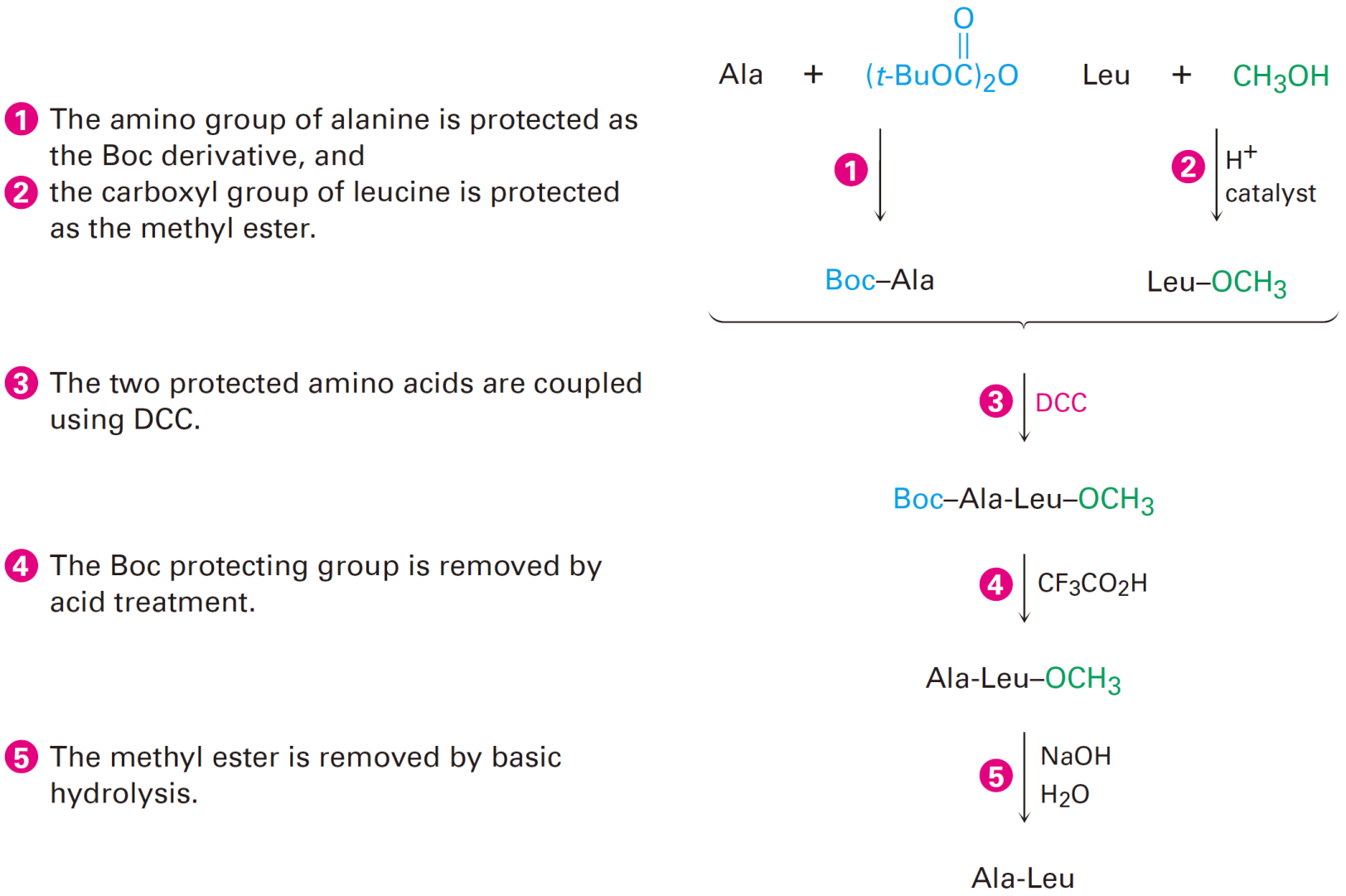
- 对 N 端的保护（对氨基的保护）及去保护





26.7 肽的合成

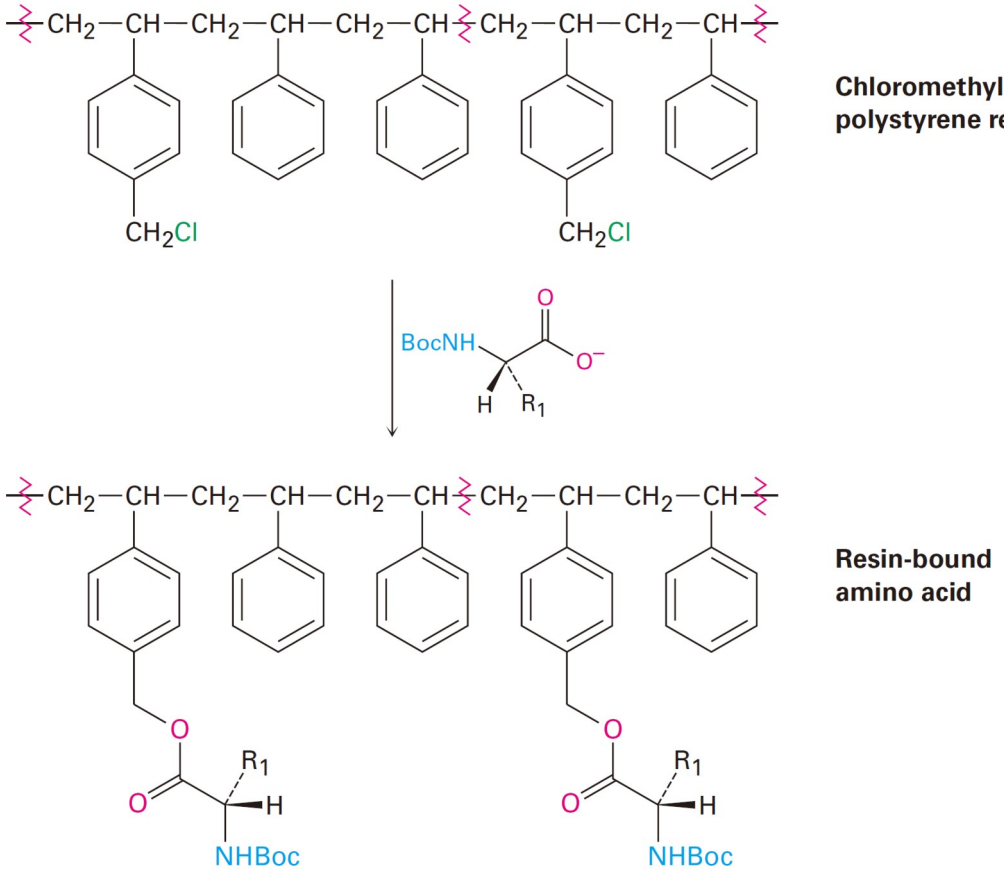
- 一个完整的保护—连接—去保护过程。



1. The amino group of alanine is protected as the Boc derivative, and
2. the carboxyl group of leucine is protected as the methyl ester.
3. The two protected amino acids are coupled using DCC.
4. The Boc protecting group is removed by acid treatment.
5. The methyl ester is removed by basic hydrolysis.

26.8 Merrifield 固相合成方法

- 固相合成是指在固体表面上进行的化学合成。核心在于多步反应的中间产物一直连接在高分子载体之上，反应中的小分子试剂与低分子副产物均能用过滤除去，分离提纯十分方便。该方法是由美国化学家梅里菲尔德于 1963 年报道，于 1984 年获得诺贝尔化学奖。这种方法不但广泛运用于多肽合成，还能用在寡糖、寡核苷酸的合成上，极大的促进了合成化学的发展。

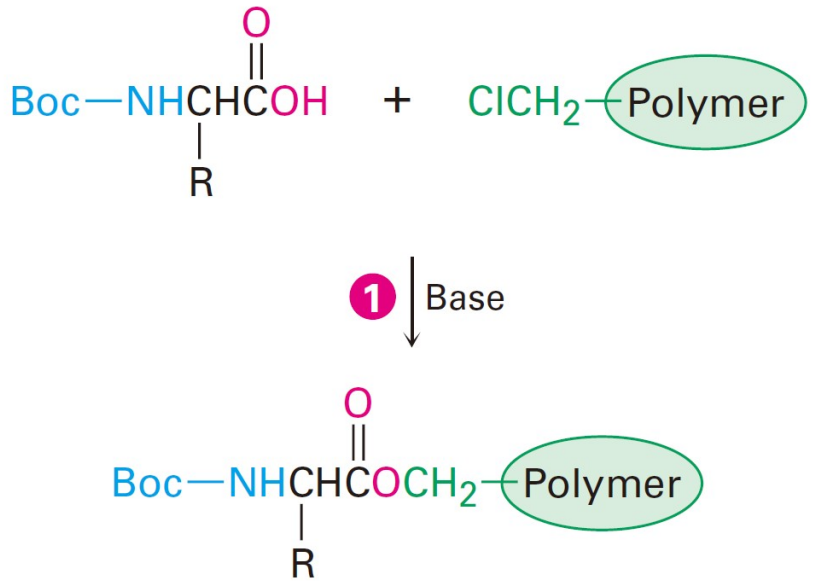


Chloromethylated polystyrene resin

Resin-bound amino acid

26.8 Merrifield 固相合成方法

- 具体步骤： 1. 将第一个氨基酸首先连接到高分子载体上；



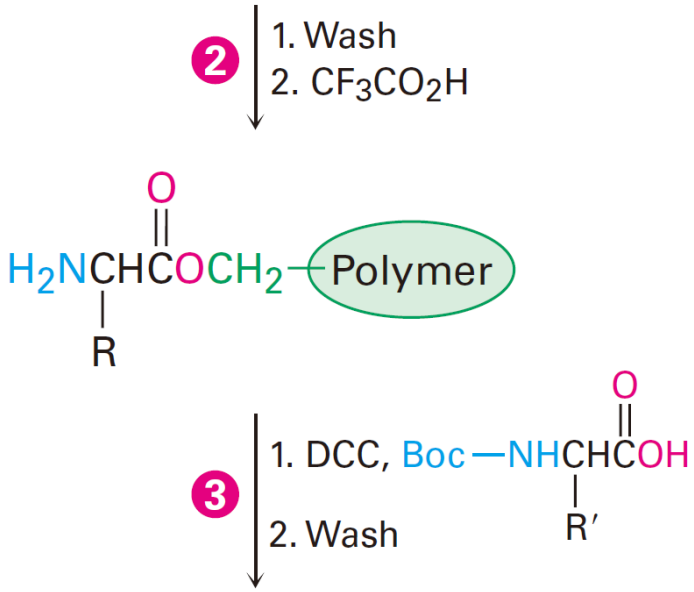
1 A Boc-protected amino acid is covalently linked to the polystyrene polymer by formation of an ester bond (S_N2 reaction).

26.8 Merrifield 固相合成方法

- 2. 然后去除 N 端的保护基；
- 3. 连接下一个氨基酸；

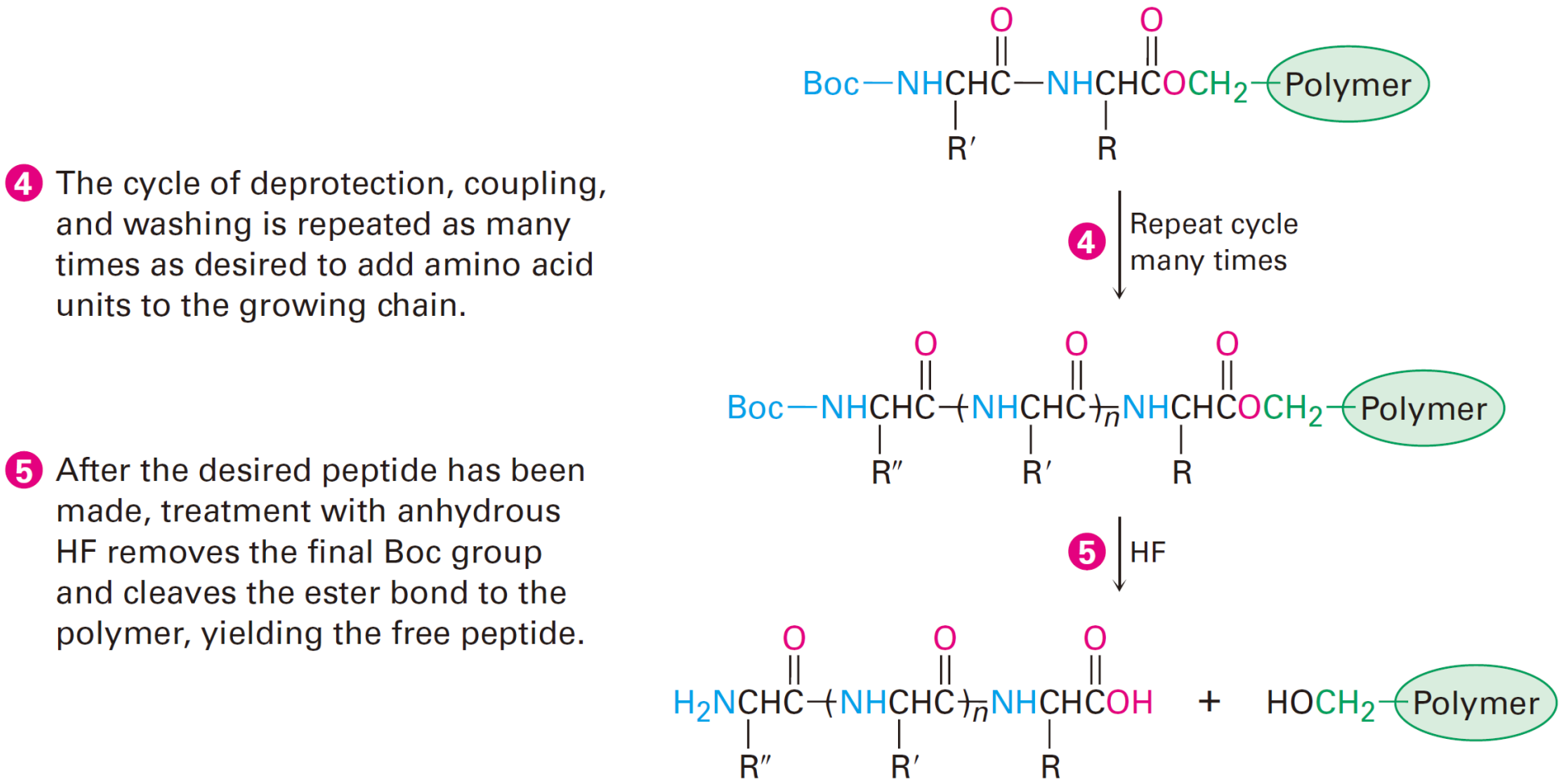
❷ The polymer-bonded amino acid is washed free of excess reagent and then treated with trifluoroacetic acid to remove the Boc group.

❸ A second Boc-protected amino acid is coupled to the first by reaction with DCC. Excess reagents are removed by washing them from the insoluble polymer.



26.8 Merrifield 固相合成方法

- 4. 重复以上步骤；
- 5. 最后将得到的氨基酸序列，也即多肽，从高分子上切下来，完成合成。

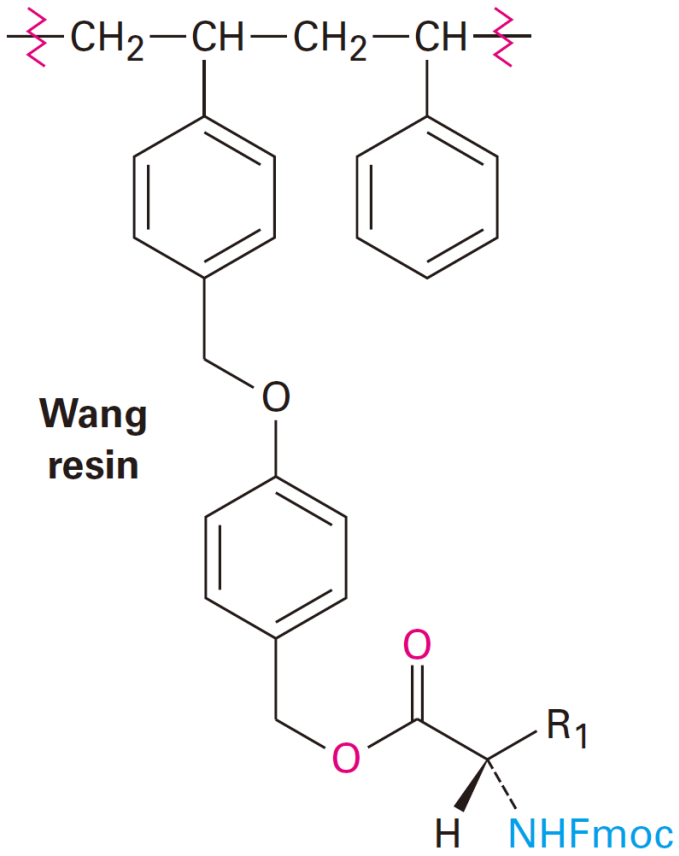


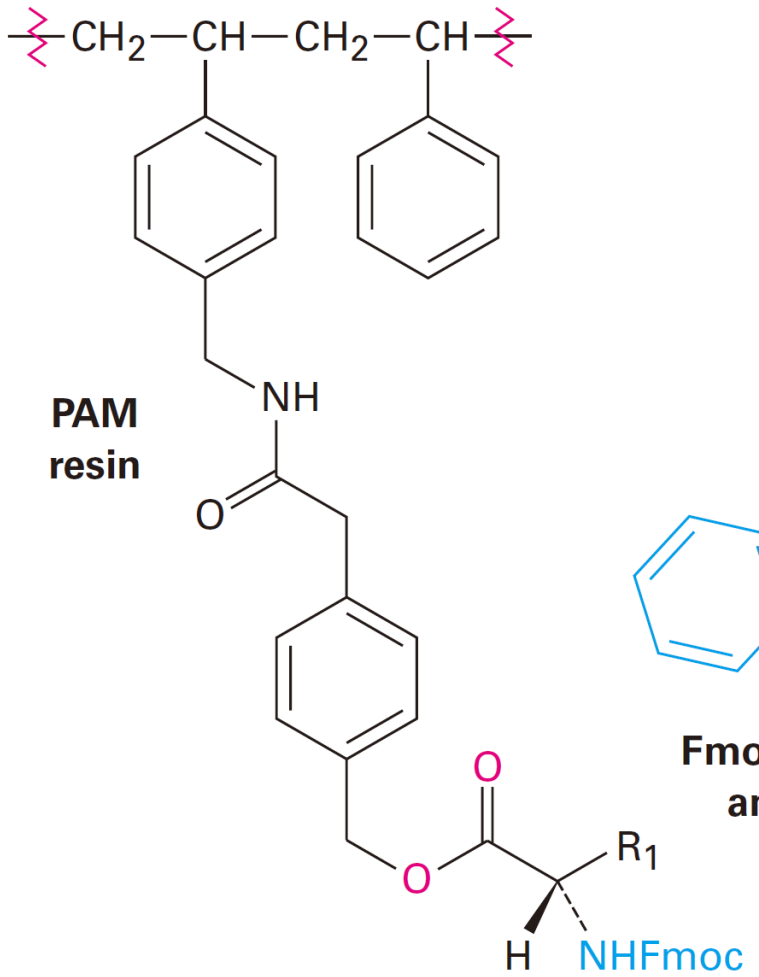
4 The cycle of deprotection, coupling, and washing is repeated as many times as desired to add amino acid units to the growing chain.

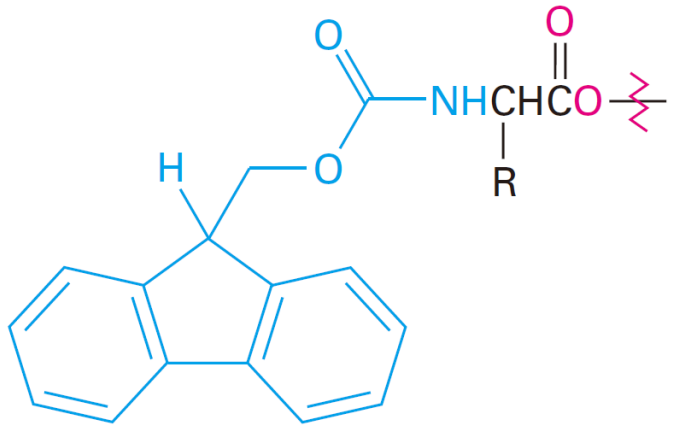
5 After the desired peptide has been made, treatment with anhydrous HF removes the final Boc group and cleaves the ester bond to the polymer, yielding the free peptide.

26.8 Merrifield 固相合成方法

- 一些不同的高分子树脂载体







Fmoc-protected amino acid

26.8 Merrifield 固相合成方法

The Nobel Prize in Chemistry 1984



Photo from the Nobel Foundation archive.

Robert Bruce Merrifield

Prize share: 1/1

The Nobel Prize in Chemistry 1984 was awarded to Robert Bruce Merrifield "for his development of methodology for chemical synthesis on a solid matrix."



Merrifield 教授来访北大
邢其毅教授接待（1994）

26.9 蛋白质的结构

- 蛋白质的一、二、三、四级结构

  - The **primary structure** of a protein is simply the amino acid sequence.
  - The **secondary structure** of a protein describes how *segments* of the peptide backbone orient into a regular pattern.
  - The **tertiary structure** describes how the *entire* protein molecule coils into an overall three-dimensional shape.
  - The **quaternary structure** describes how different protein molecules come together to yield large aggregate structures.

蛋白质的一级结构是氨基酸的序列。

蛋白质的二级结构描述了肽主链的片段如何定向成规则模式。alpha螺旋和beta折叠

三级结构描述了整个蛋白质分子如何卷成一个整体的三维形状。

四级结构描述了不同的蛋白质分子如何聚集在一起以产生大的聚集结构。

26.9 蛋白质的结构

- alpha 螺旋

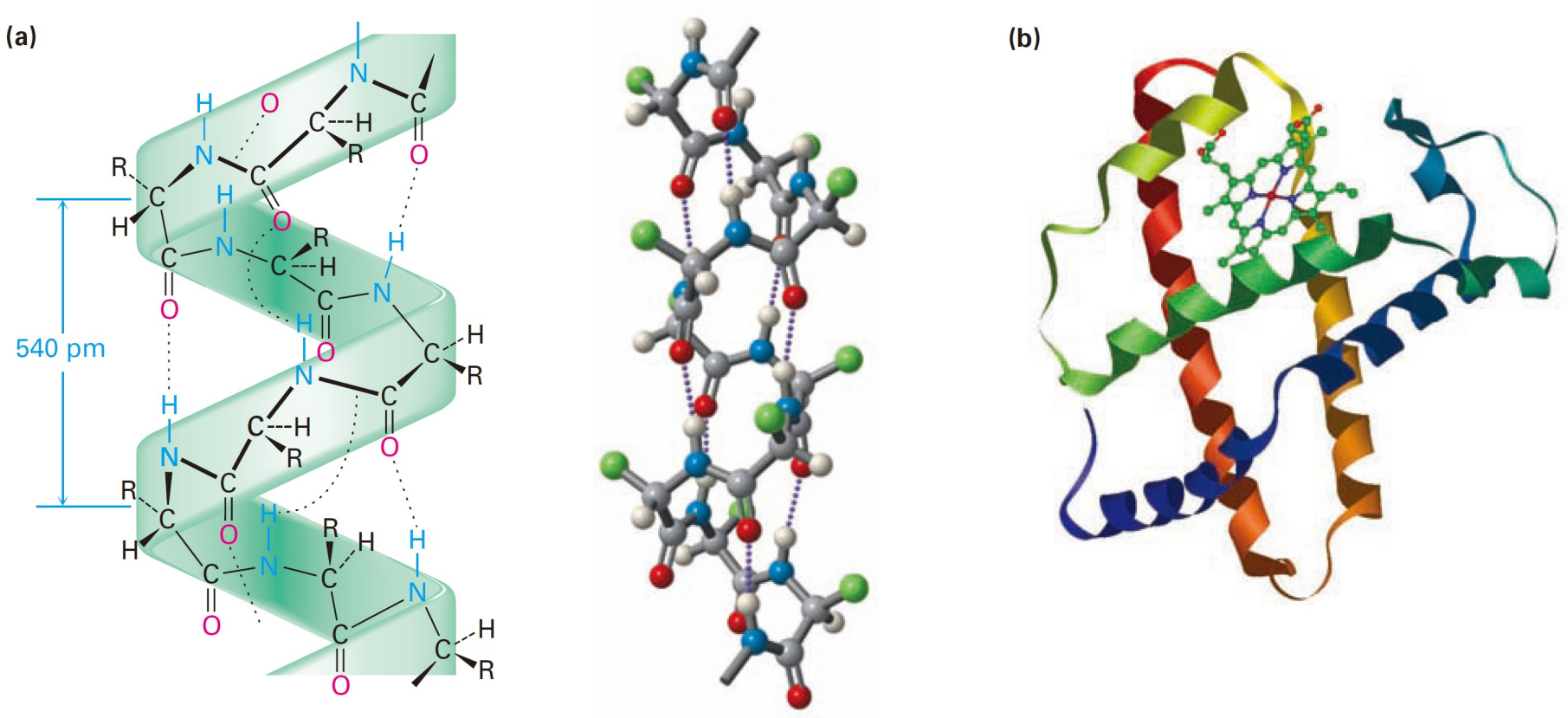


Figure 26.5 **(a)** The α-helical secondary structure of proteins is stabilized by hydrogen bonds between the N—H group of one residue and the C=O group four residues away. **(b)** The structure of myoglobin, a globular protein with extensive helical regions that are shown as coiled ribbons in this representation.

26.9 蛋白质的结构

- beta 折叠

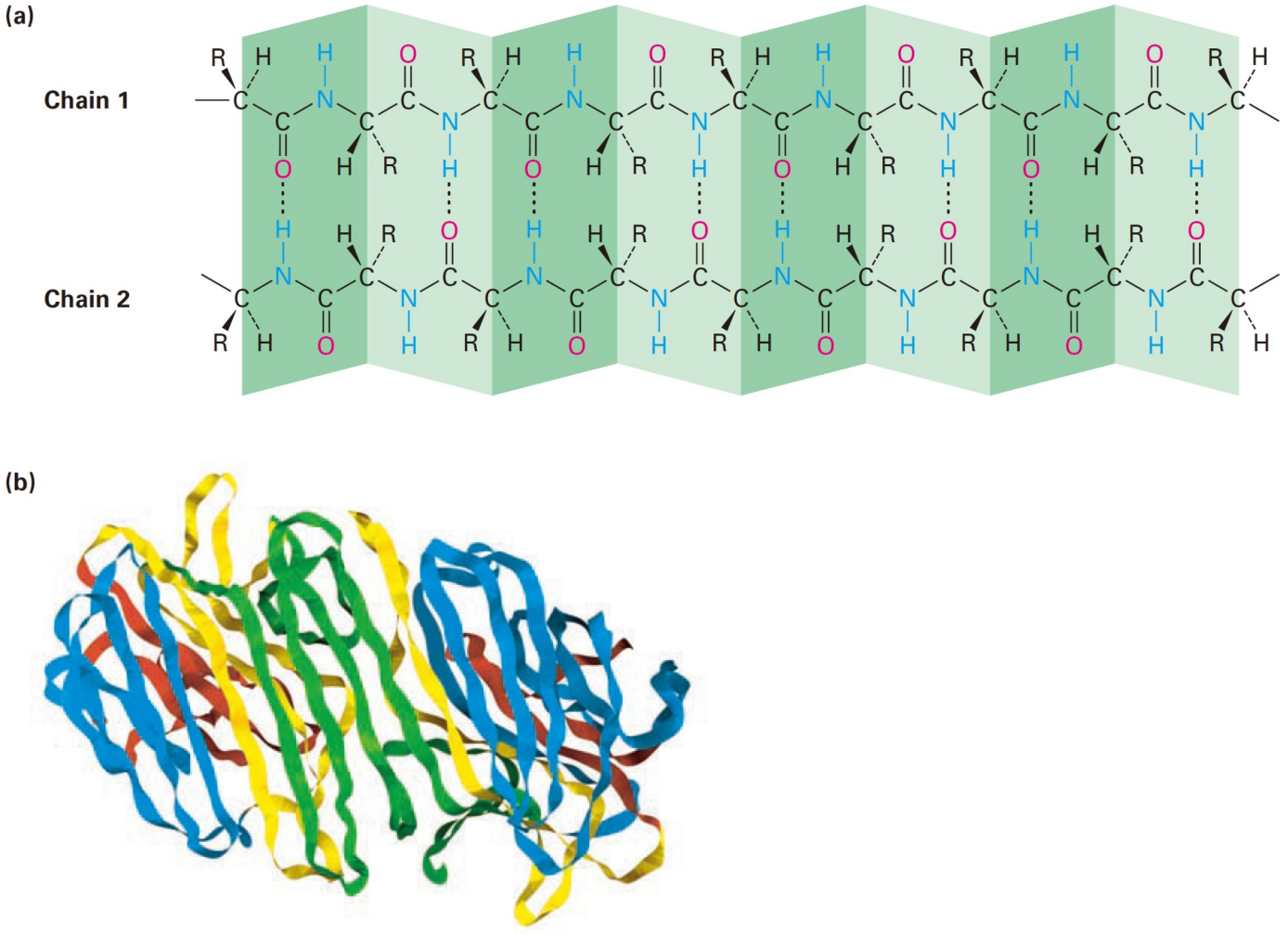
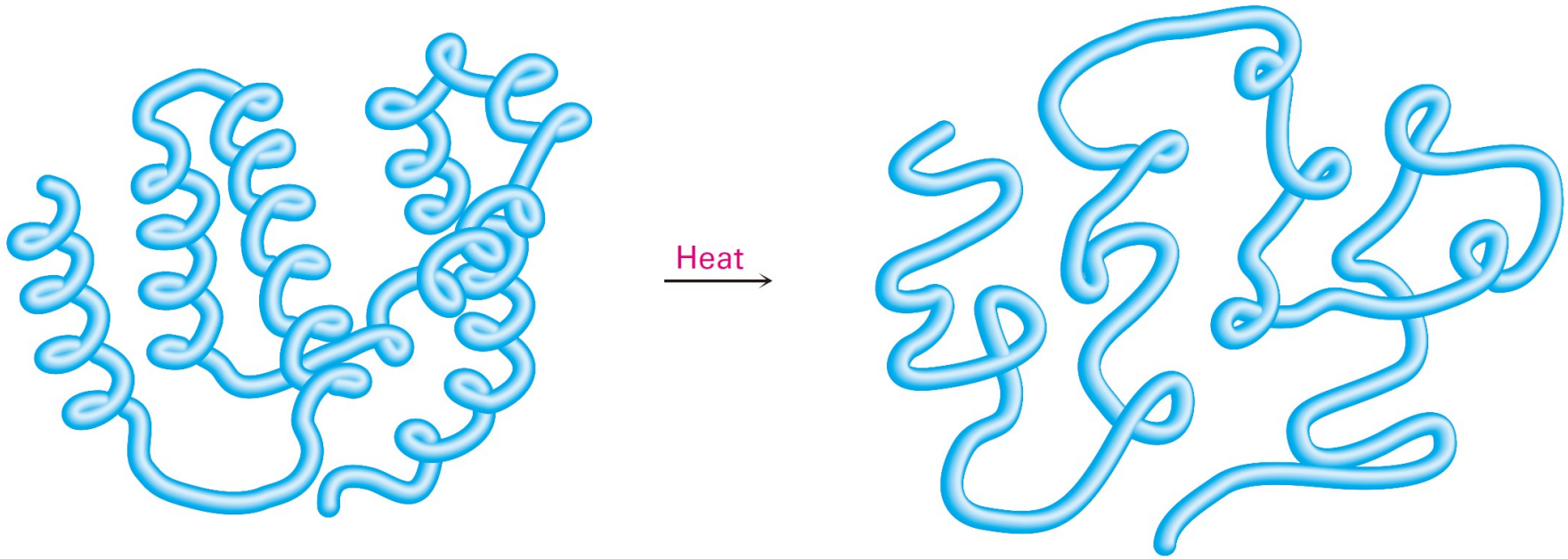


Figure 26.6 **(a)** The β-pleated sheet secondary structure of proteins is stabilized by hydrogen bonds between parallel or antiparallel chains. **(b)** The structure of concanavalin A, a protein with extensive regions of antiparallel β sheets, shown as flat ribbons.

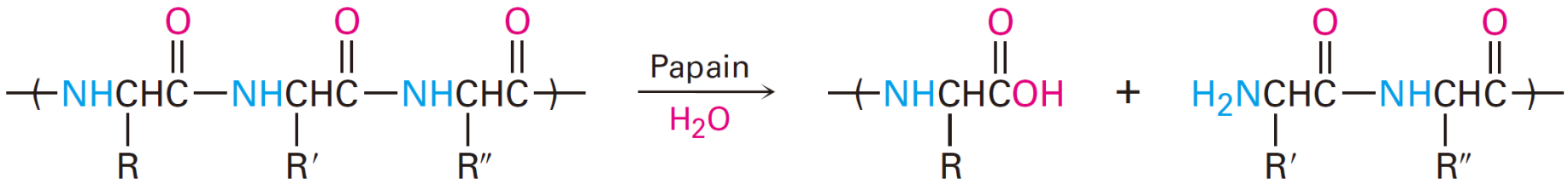
26.9 蛋白质的结构

- 蛋白质的变性：变性伴随着物理和生物特性的变化。



26.10 酶

- 酶是一种物质——通常是一种大蛋白质——作为生物反应的催化剂。



Enzymes function through a pathway that involves initial formation of an enzyme–substrate complex E · S, followed by a multistep chemical conversion of the enzyme-bound substrate into enzyme-bound product E · P and final release of product from the complex.

$$E + S \rightleftharpoons E \cdot S \rightleftharpoons E \cdot P \rightleftharpoons E + P$$

26.10 酶

- 改变反应路径，使反应更容易进行。

