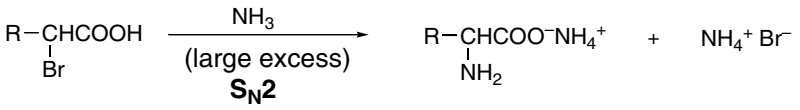
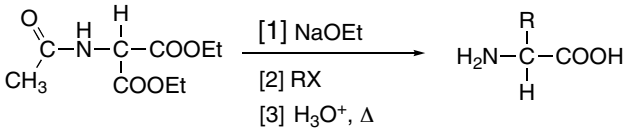
Chapter 28: Amino Acids and Proteins

♦ Synthesis of amino acids (28.2)

[1] From α-halo carboxylic acids by S_N2 reaction

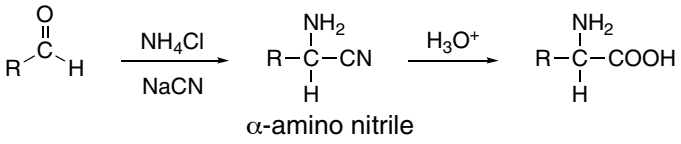


[2] By alkylation of diethyl acetamidomalonate



- Alkylation works best with unhindered alkyl halides—that is, CH_3X and RCH_2X.

[3] Strecker synthesis

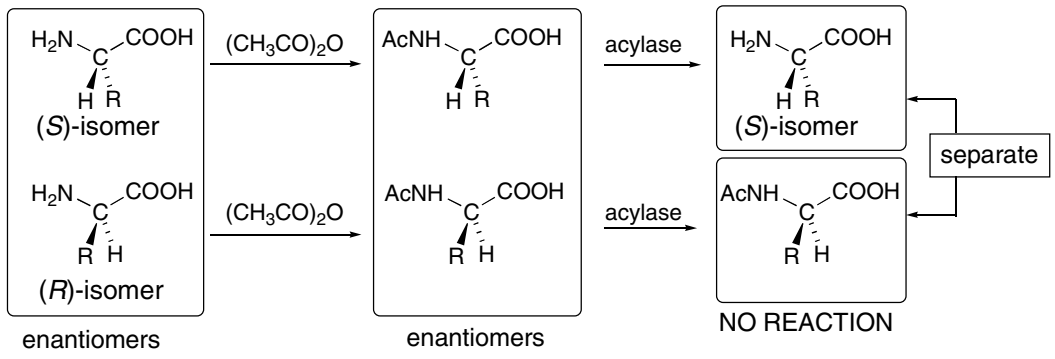


♦ Preparation of optically active amino acids

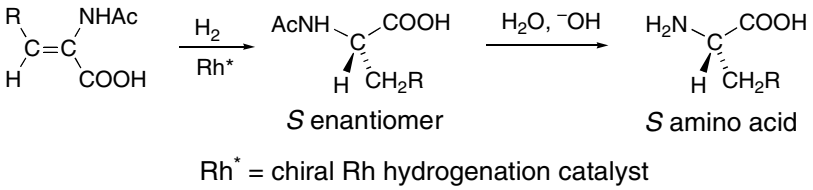
[1] Resolution of enantiomers by forming diastereomers (28.3A)

- Convert a racemic mixture of amino acids into a racemic mixture of *N*-acetyl amino acids [(*S*)- and (*R*)-$CH_3CONHCH(R)COOH$].
- React the enantiomers with a chiral amine to form a mixture of diastereomers.
- Separate the diastereomers.
- Regenerate the amino acids by protonation of the carboxylate salt and hydrolysis of the *N*-acetyl group.

[2] Kinetic resolution using enzymes (28.3B)



[3] By enantioselective hydrogenation (28.4)

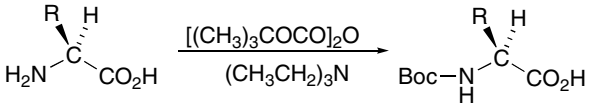


♦ Summary of methods used for peptide sequencing (28.6)

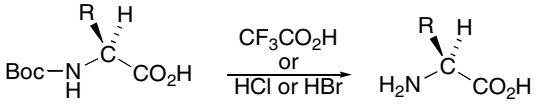
- Complete hydrolysis of all amide bonds in a peptide gives the identity and amount of the individual amino acids.
- Edman degradation identifies the N-terminal amino acid. Repeated Edman degradations can be used to sequence a peptide from the N-terminal end.
- Cleavage with carboxypeptidase identifies the C-terminal amino acid.
- Partial hydrolysis of a peptide forms smaller fragments that can be sequenced. Amino acid sequences common to smaller fragments can be used to determine the sequence of the complete peptide.
- Selective cleavage of a peptide occurs with trypsin and chymotrypsin to identify the location of specific amino acids (Table 28.2).

♦ Adding and removing protecting groups for amino acids (28.7)

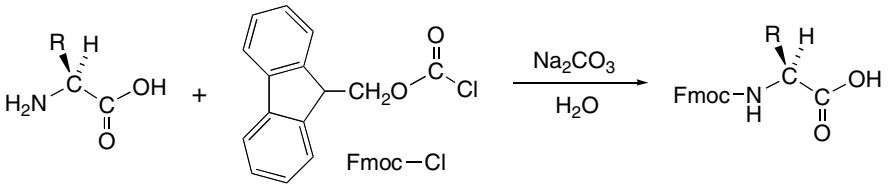
[1] Protection of an amino group as a Boc derivative



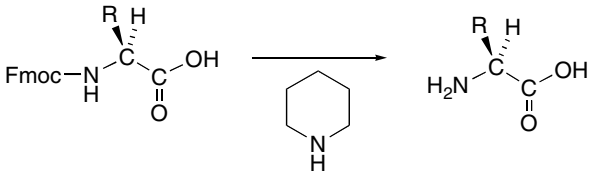
[2] Deprotection of a Boc-protected amino acid



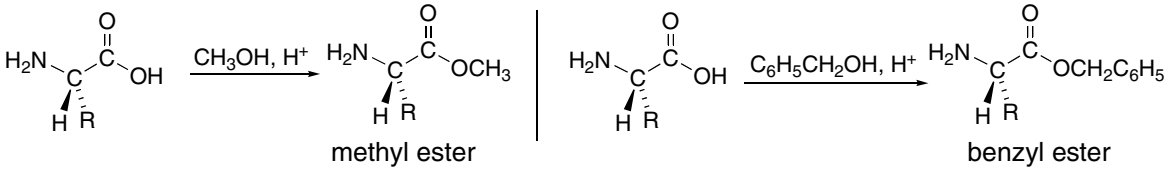
[3] Protection of an amino group as an Fmoc derivative



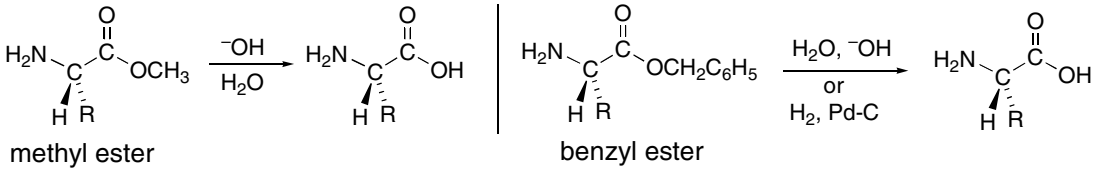
[4] Deprotection of an Fmoc-protected amino acid



[5] Protection of a carboxy group as an ester

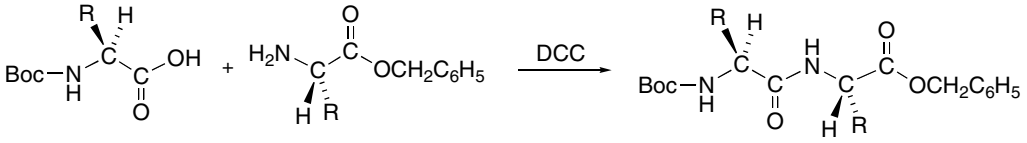


[6] Deprotection of an ester group



♦ Synthesis of dipeptides (28.7)

[1] Amide formation with DCC



[2] Four steps are needed to synthesize a dipeptide:

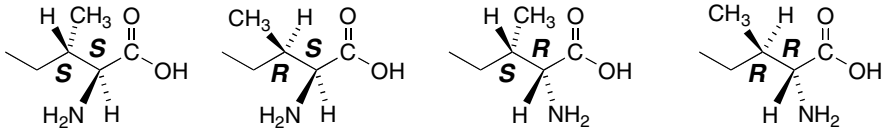
a. **Protect** the amino group of one amino acid using a Boc or Fmoc group.
b. **Protect** the carboxy group of the second amino acid using an ester.
c. Form the amide bond with **DCC.**
d. **Remove both protecting groups** in one or two reactions.

♦ Summary of the Merrifield method of peptide synthesis (28.8)

[1] Attach an Fmoc-protected amino acid to a polymer derived from polystyrene.
[2] Remove the Fmoc protecting group.
[3] Form the amide bond with a second Fmoc-protected amino acid using DCC.
[4] Repeat steps [2] and [3].
[5] Remove the protecting group and detach the peptide from the polymer.

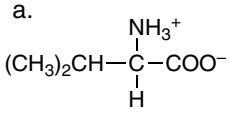
Chapter 28: Answers to Problems

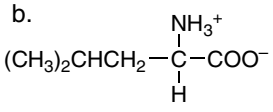
28.1

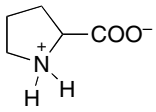


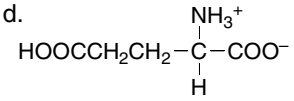
L-isoleucine

28.2

a. 

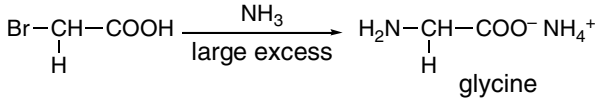
b. 

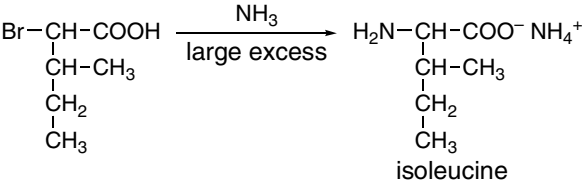
c. 

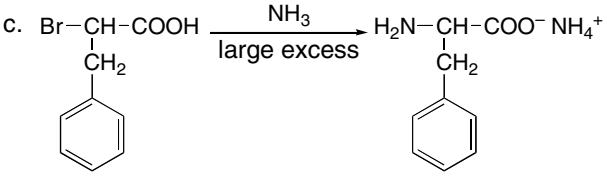
d. 

28.3 In an amino acid, the electron-withdrawing carboxy group destabilizes the ammonium ion ($-NH_3^+$), making it more readily donate a proton; that is, it makes it a stronger acid. Also, the electron-withdrawing carboxy group removes electron density from the amino group ($-NH_2$) of the conjugate base, making it a weaker base than a 1° amine, which has no electron-withdrawing group.

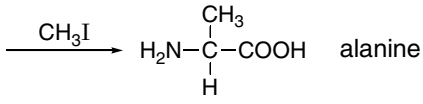
28.4 The most direct way to synthesize an α-amino acid is by **S_N2 reaction of an α-halo carboxylic acid with a large excess of NH_3**.

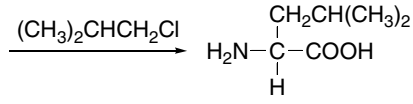
a. 

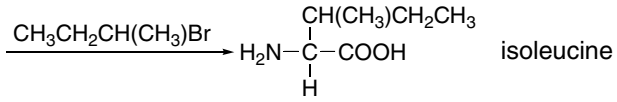
b. 

c. 

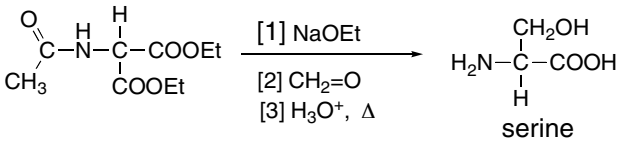
28.5

a. 

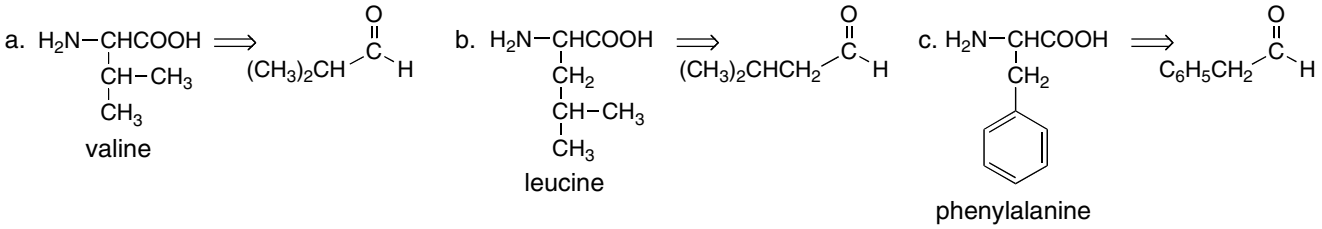
b. 

c. 

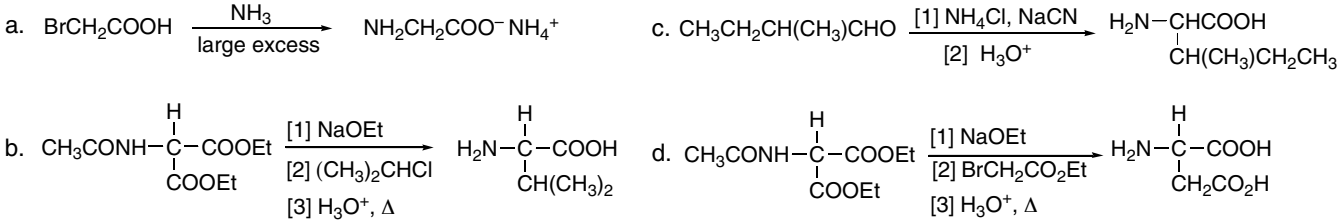
28.6



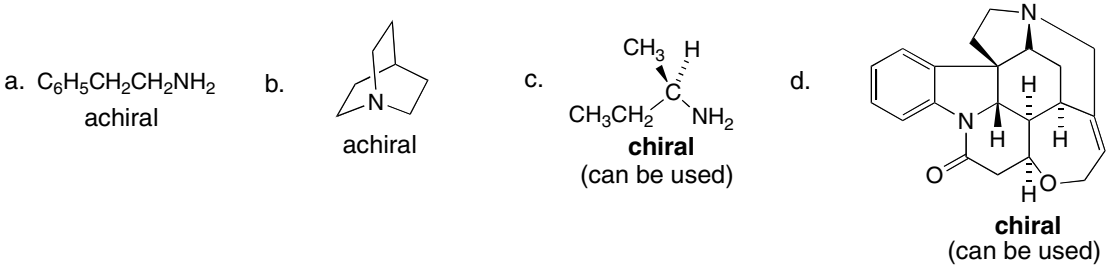
28.7



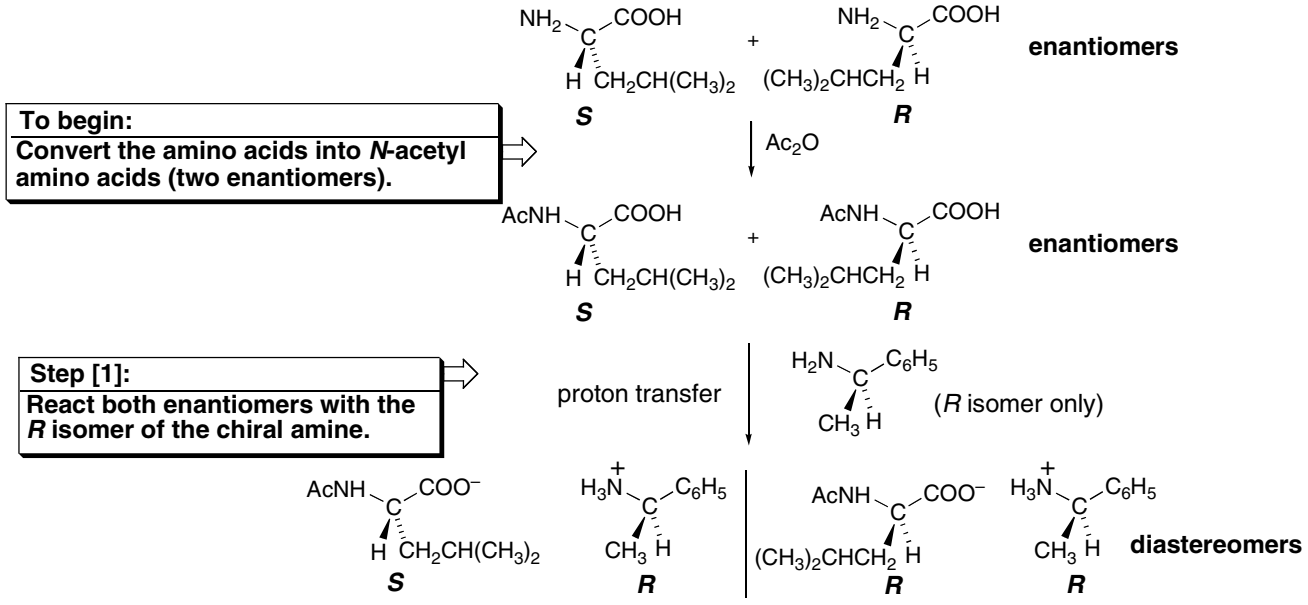
28.8



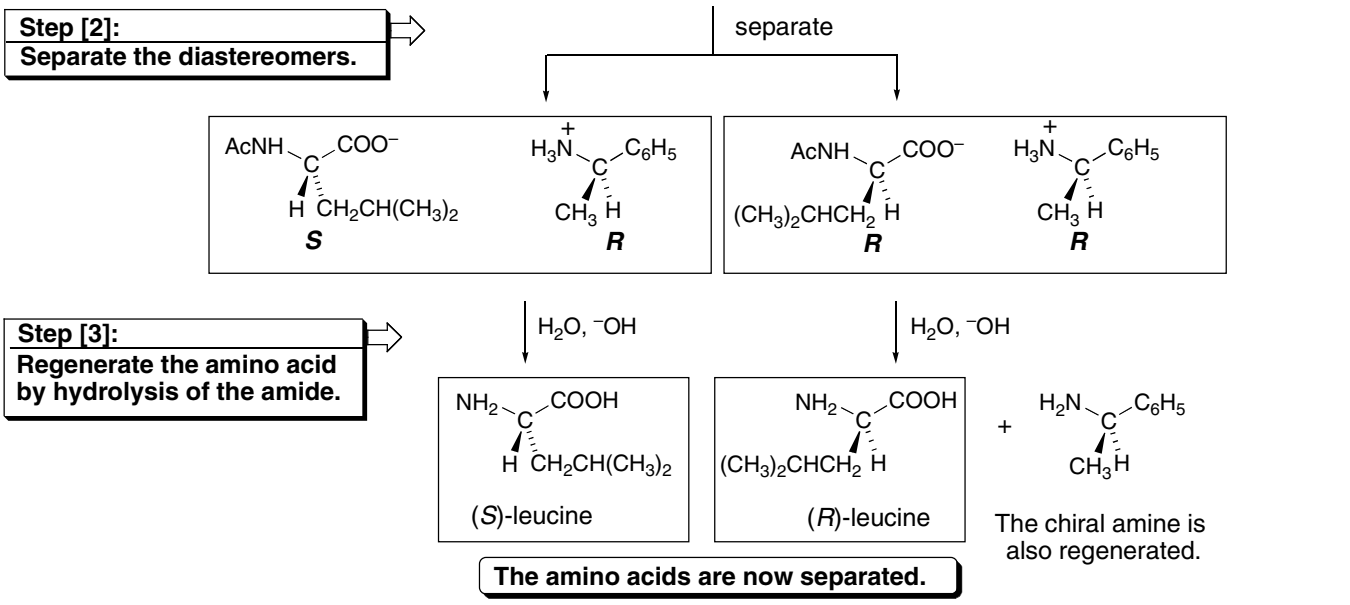
28.9 A chiral amine must be used to resolve a racemic mixture of amino acids.



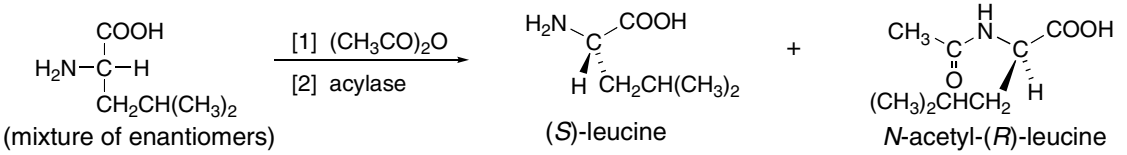
28.10



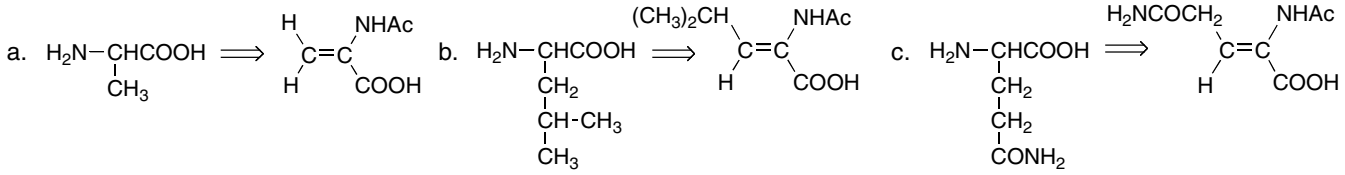
These salts have the *same* configuration around one stereogenic center, but the *opposite* configuration about the other stereogenic center.



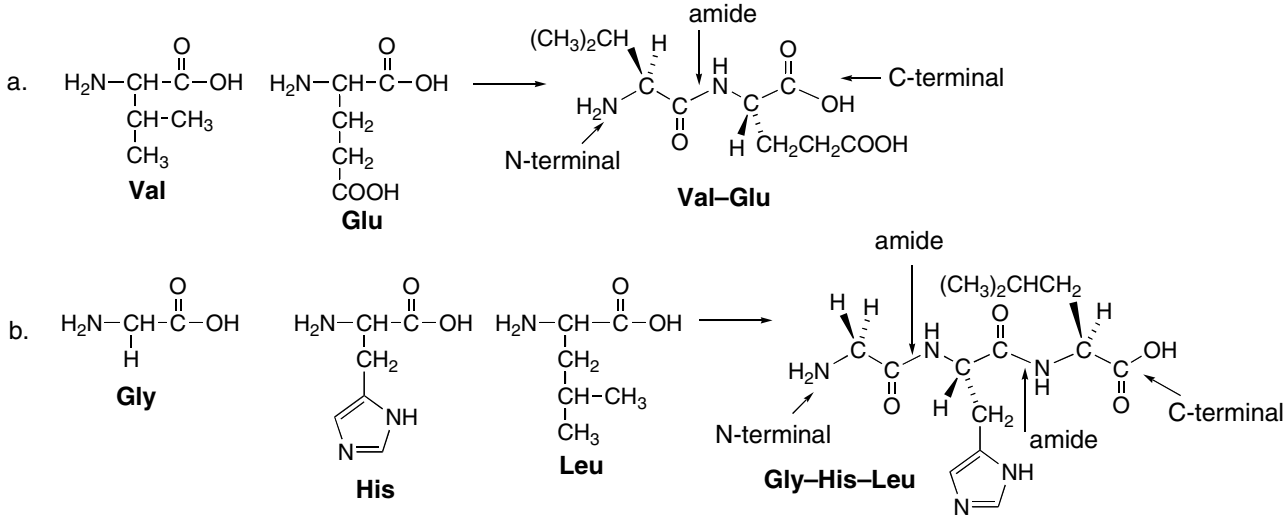
28.11



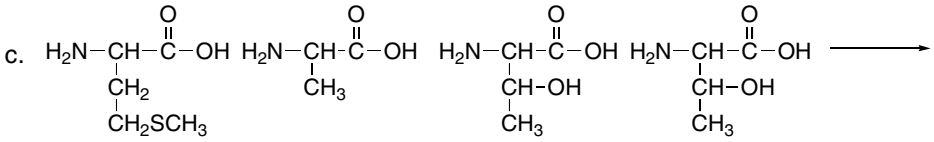
28.12



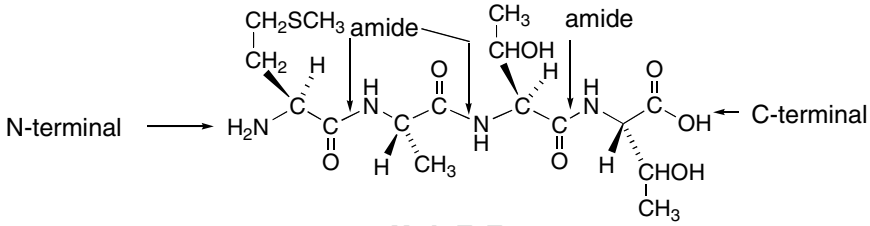
28.13 Draw the peptide by joining adjacent COOH and NH_2 groups in amide bonds.



c.



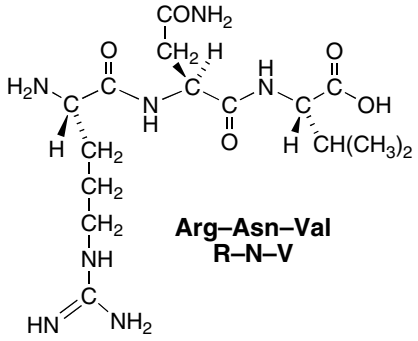
M **A** **T** **T**



M–A–T–T

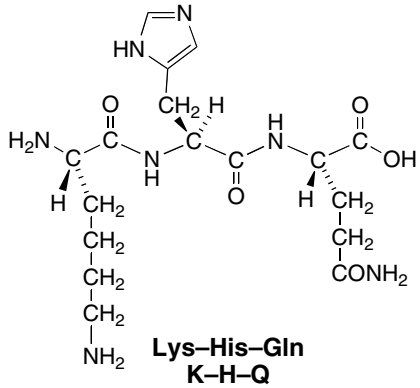
28.14

a.



Arg–Asn–Val
R–N–V

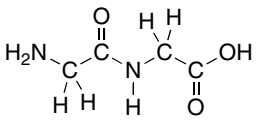
b.



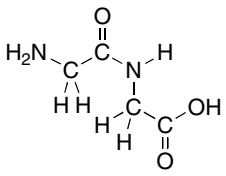
Lys–His–Gln
K–H–Q

28.15 There are six different tripeptides that can be formed from three amino acids (A, B, C): A–B–C, A–C–B, B–A–C, B–C–A, C–A–B, and C–B–A.

28.16 The *s*-trans conformation has the two C's oriented on *opposite* sides of the C–N bond. The *s*-cis conformation has the two C's oriented on the *same* side of the C–N bond.

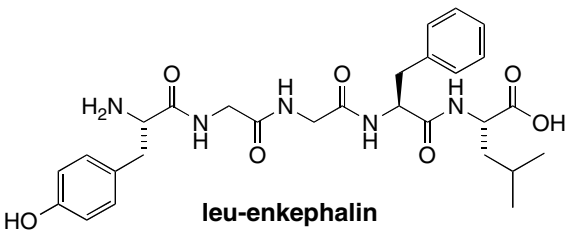


s-trans



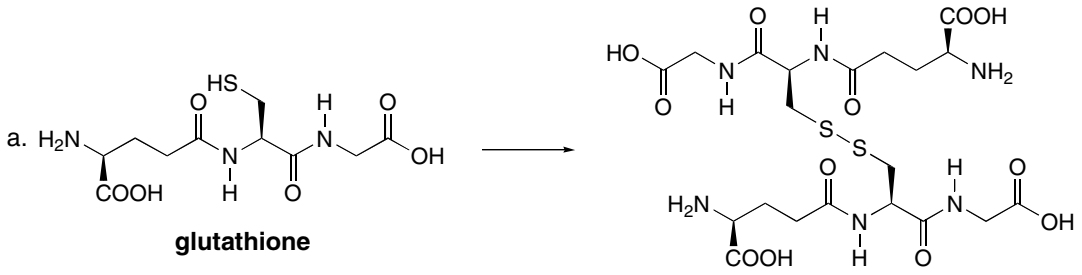
s-cis

28.17

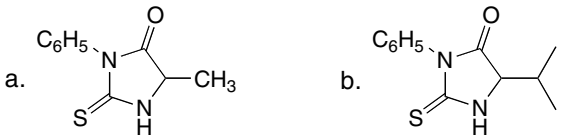


leu-enkephalin

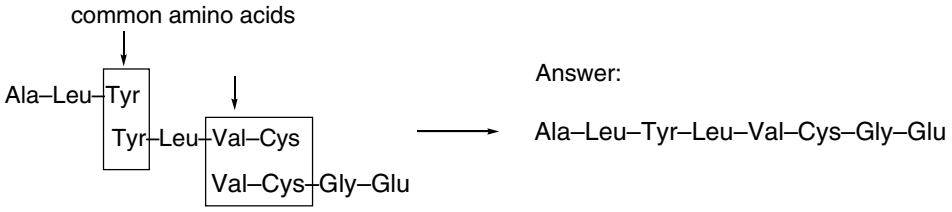
28.18



28.19



28.20 Determine the sequence of the octapeptide as in Sample Problem 28.2. Look for overlapping sequences in the fragments.



28.21 Trypsin cleaves peptides at amide bonds with a carbonyl group from Arg and Lys. Chymotrypsin cleaves at amide bonds with a carbonyl group from Phe, Tyr, and Trp.

a. [1] Gly–Ala–Phe–Leu–Lys + Ala
   [2] Phe–Tyr–Gly–Cys–Arg + Ser
   [3] Thr–Pro–Lys + Glu–His–Gly–Phe–Cys–Trp–Val–Val–Phe
b. [1] Gly–Ala–Phe + Leu–Lys–Ala
   [2] Phe + Tyr + Gly–Cys–Arg–Ser
   [3] Thr–Pro–Lys–Glu–His–Gly–Phe + Cys–Trp + Val–Val–Phe

28.22

Edman degradation gives N-terminal amino acid: → Leu–___–___–___–___–___–___–___

Carboxypeptidase identifies the C-terminal amino acid: → Leu–___–___–___–___–___–___–Glu

Partial hydrolysis common amino acids

↓

Ala–Ser–Arg

Gly–Ala–Ser

↓

Gly–Ala–Ser–Arg → Leu–Gly–Ala–Ser–Arg–___–Glu

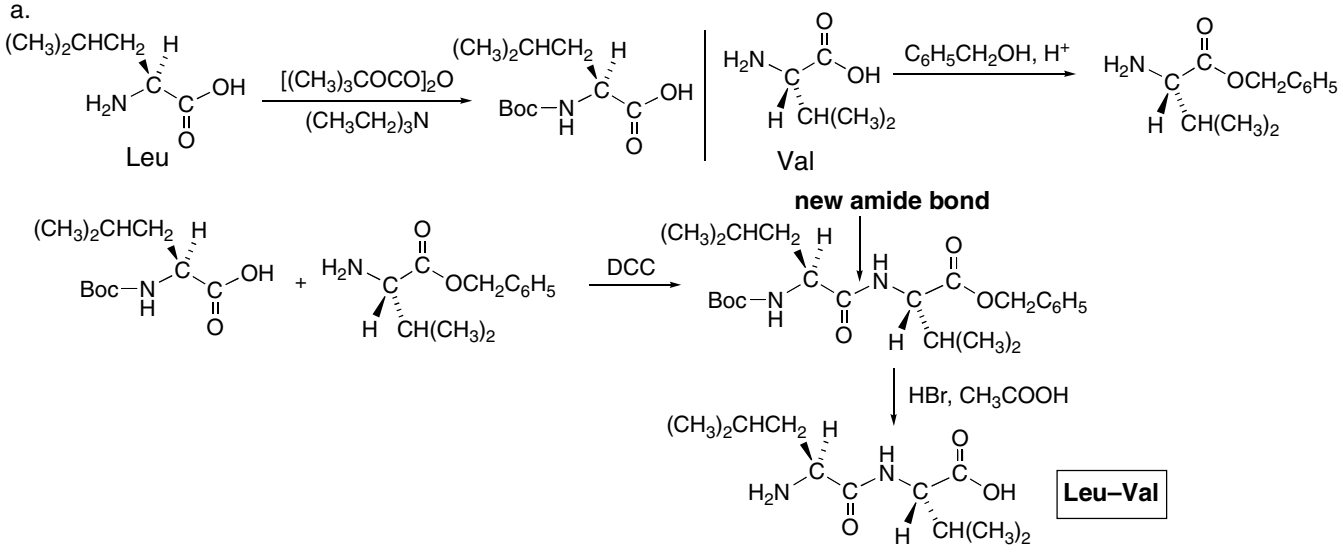
or

Leu–___–Gly–Ala–Ser–Arg–Glu

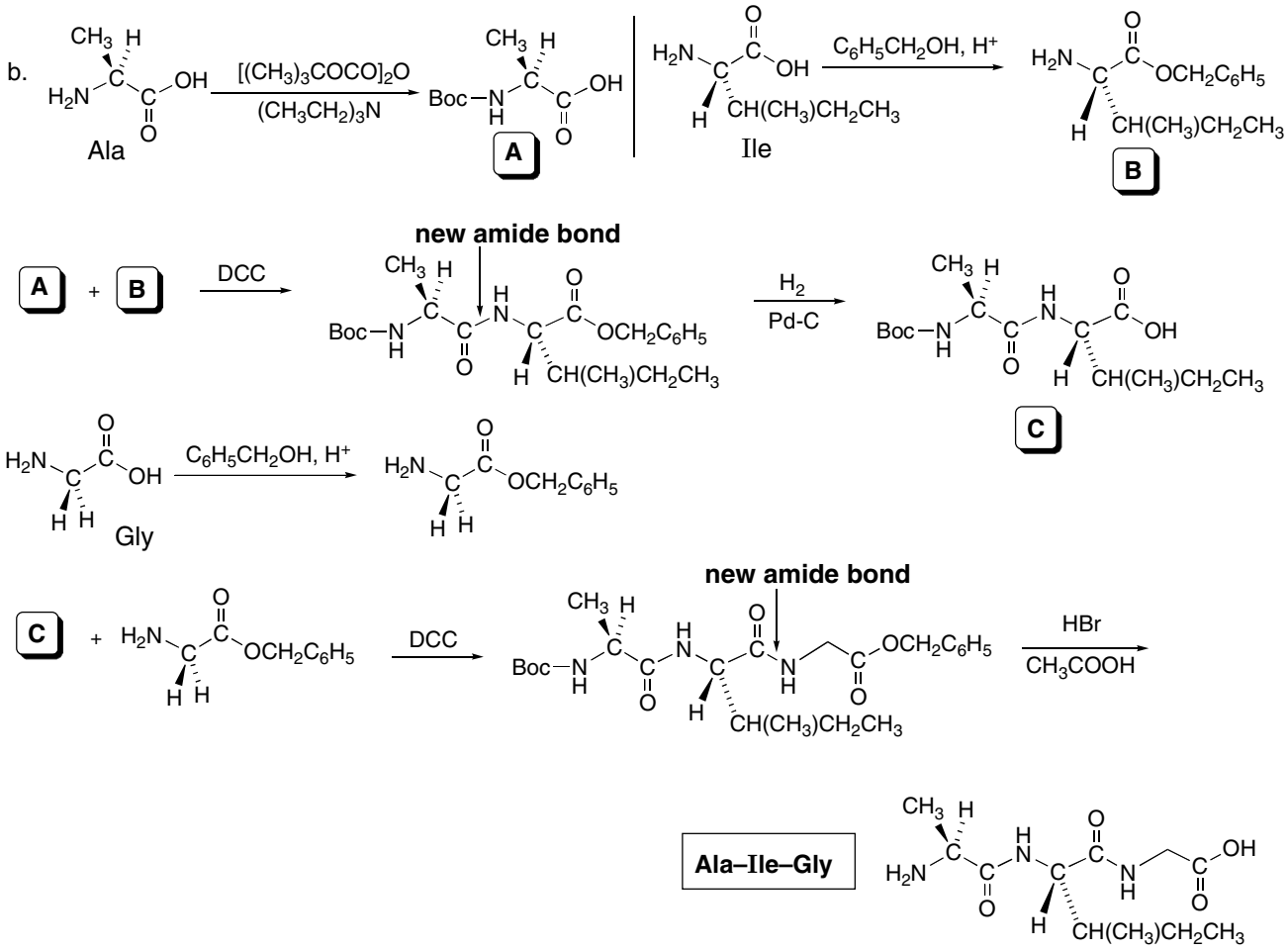
Cleavage by trypsin is after Arg and yields a dipeptide; therefore, this must be the peptide: → Leu–Gly–Ala–Ser–Arg–Phe–Glu

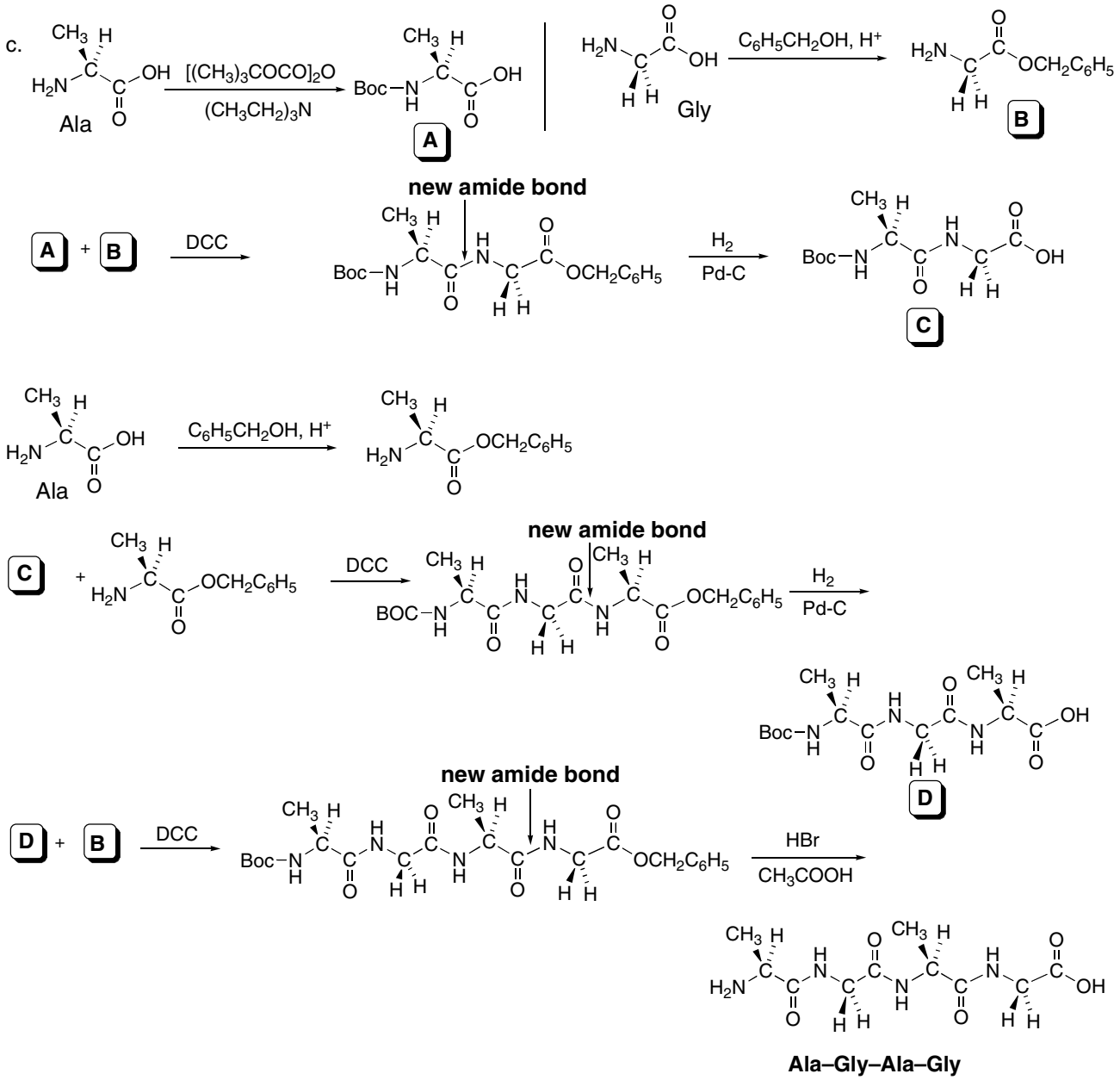
28.23

a.



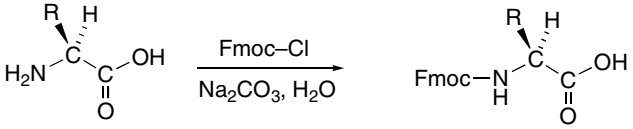
b.


Ala
[(CH3)3COCO]2O
(CH3CH2)3N
A
Ile
C6H5CH2OH, H+
B
new amide bond
DCC
H2
Pd-C
C
Gly
C6H5CH2OH, H+
HBr
CH3COOH
Ala–Ile–Gly

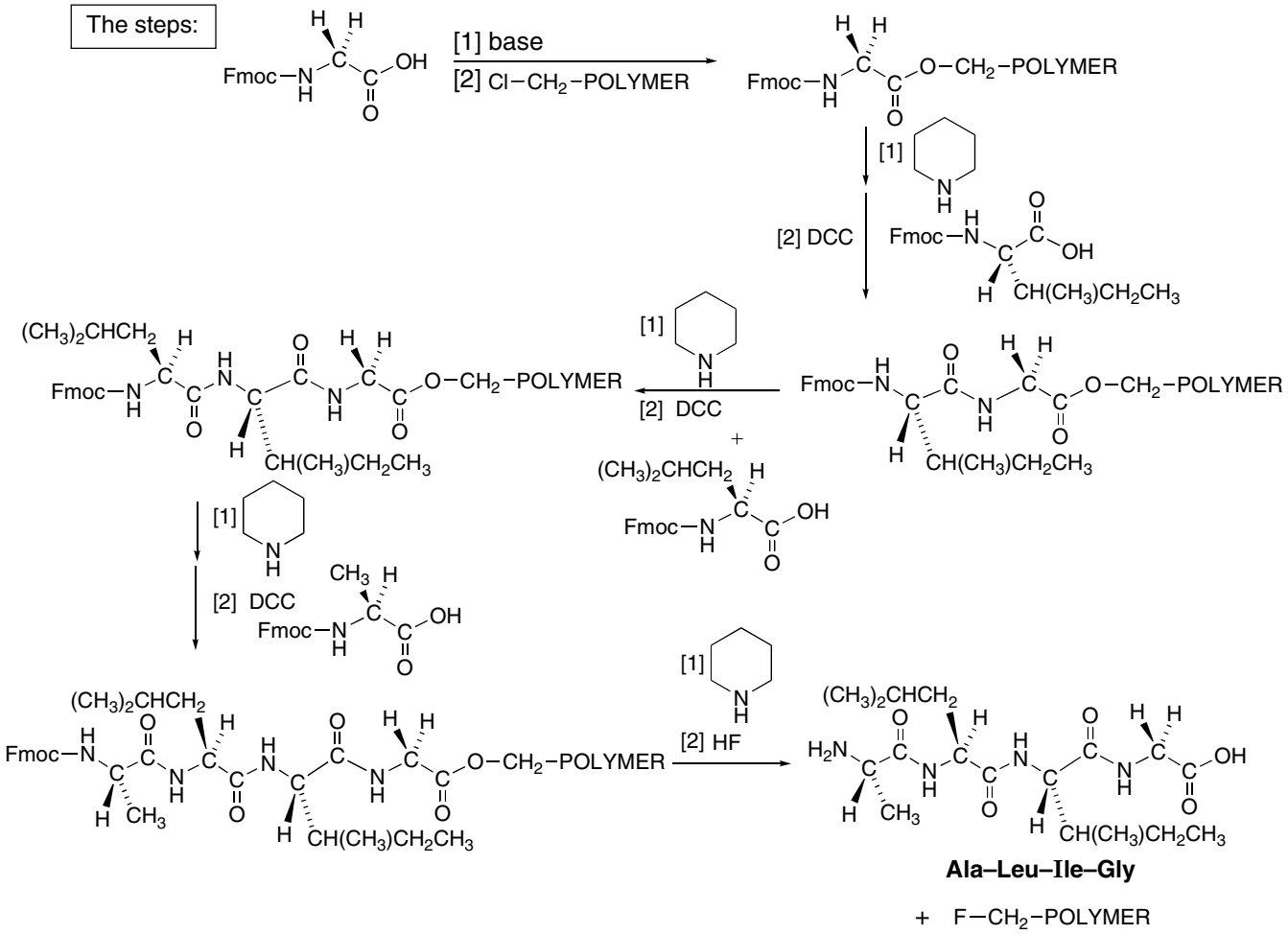

c.
Ala
[(CH3)3COCO]2O
(CH3CH2)3N
A
Gly
C6H5CH2OH, H+
B
new amide bond
DCC
H2
Pd-C
C
D
HBr
CH3COOH
Ala–Gly–Ala–Gly

28.24

All Fmoc-protected amino acids are made by the following general reaction:

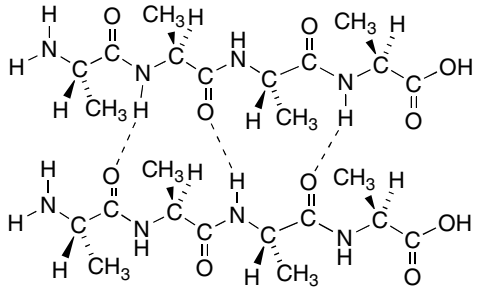


The steps:

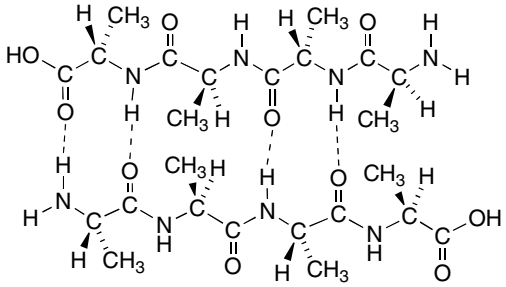


28.25 Antiparallel β-pleated sheets are more stable then parallel β-pleated sheets because of geometry. The N–H and C=O of one chain are directly aligned with the N–H and C=O of an adjacent chain in the antiparallel β-pleated sheet, whereas they are not in the parallel β-pleated sheet. This makes the latter set of hydrogen bonds weaker.

28.26 In a *parallel* β-pleated sheet, the strands run in the *same* direction from the N- to C-terminal amino acid. In an *antiparallel* β-pleated sheet, the strands run in the *opposite* direction.



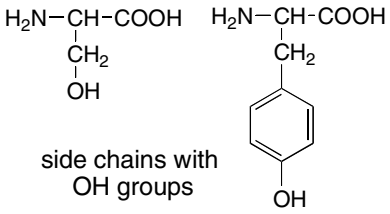
parallel



antiparallel

28.27

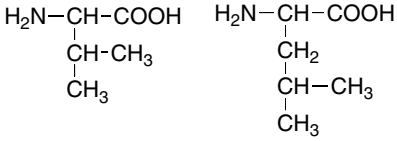
a. Ser and Tyr



side chains with OH groups

hydrogen bonding

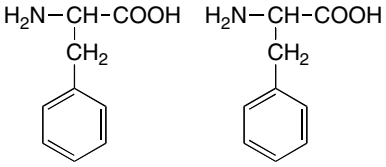
b. Val and Leu



side chains with only C–C and C–H bonds

van der Waals forces

c. 2 Phe residues

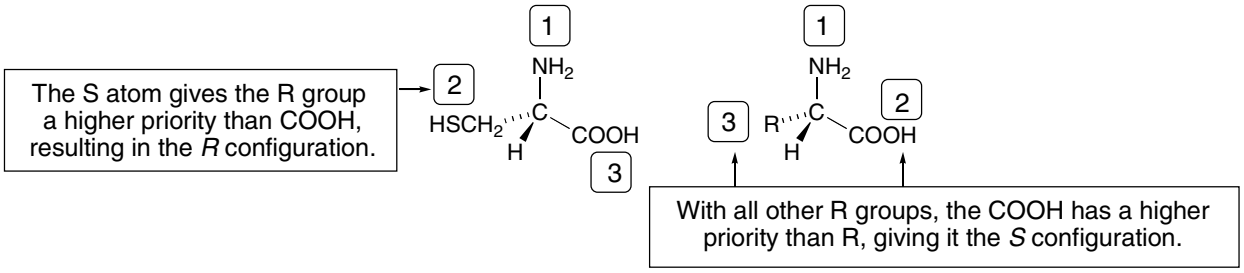


van der Waals forces

28.28 a. The R group for glycine is a hydrogen. The R groups must be small to allow the β-pleated sheets to stack on top of each other. With large R groups, steric hindrance prevents stacking.

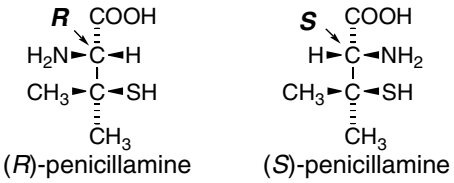
b. Silk fibers are water insoluble because most of the polar functional groups are in the interior of the stacked sheets. The β-pleated sheets are stacked one on top of another so few polar functional groups are available for hydrogen bonding to water.

28.29 All L-amino acids except cysteine have the ***S* configuration**. L-Cysteine has the *R* configuration because the R group contains a sulfur atom, which has higher priority.

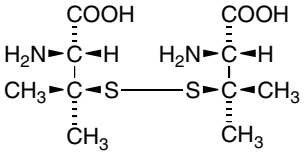


28.30

a.



b.

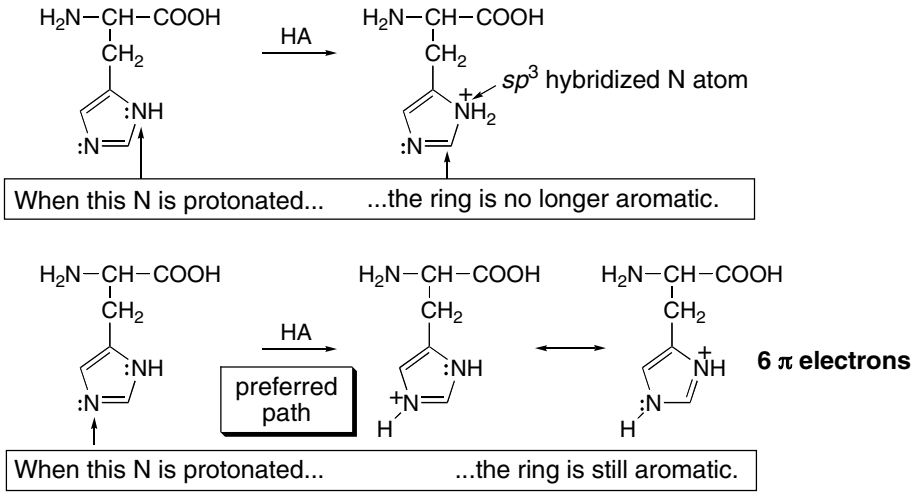


28.31 Amino acids are insoluble in diethyl ether because amino acids are highly polar; they exist as salts in their neutral form. Diethyl ether is weakly polar, so amino acids are not soluble in it. *N*-Acetyl amino acids are soluble because they are polar but not salts.

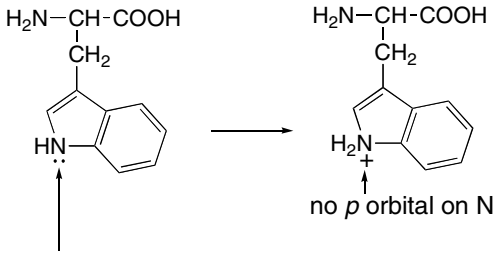
amino acid, a salt
H_2O soluble and ether insoluble

N-acetyl amino acid
ether soluble

28.32 The electron pair on the N atom not part of a double bond is delocalized on the five-membered ring, making it less basic.



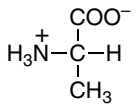
28.33

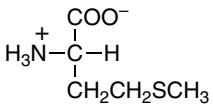


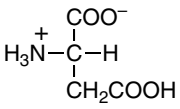
This electron pair is delocalized on the bicyclic ring system (giving it 10 π electrons), making it less available for donation, and thus less basic.

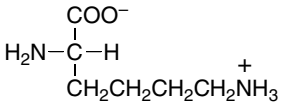
The ring structure on tryptophan is aromatic since each atom contains a *p* orbital. Protonation of the N atom would disrupt the aromaticity, making this a less favorable reaction.

28.34 At its isoelectric point, each amino acid is neutral.

a. 
alanine

b. 
methionine

c. 
aspartic acid

d. 
lysine

28.35

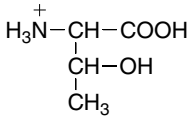
a. [1] glutamic acid: use the pK_a's 2.10 + 4.07
[2] lysine: use the pK_a's 8.95 + 10.53
[3] arginine: use the pK_a's 9.04 + 12.48

b. In general, the p*I* of an acidic amino acid is lower than that of a neutral amino acid.

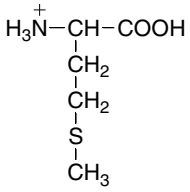
c. In general, the p*I* of a basic amino acid is higher than that of a neutral amino acid.

28.36

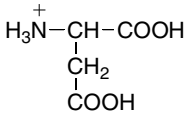
a. **threonine**
p*I* = 5.06
(+1) charge at pH = 1



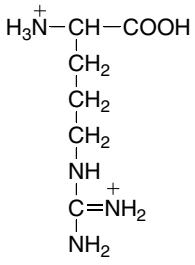
b. **methionine**
p*I* = 5.74
(+1) charge at pH = 1



c. **aspartic acid**
p*I* = 2.98
(+1) charge at pH = 1

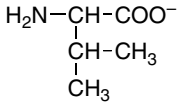


d. **arginine**
p*I* = 5.41
(+2) charge at pH = 1

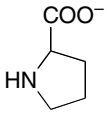


28.37

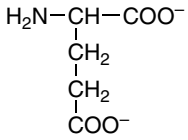
a. **valine**
pI = 6.00
(–1) charge
at pH = 11



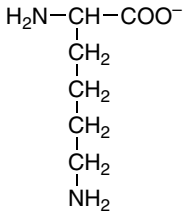
b. **proline**
pI = 6.30
(–1) charge
at pH = 11



c. **glutamic acid**
pI = 3.08
(–2) charge
at pH = 11

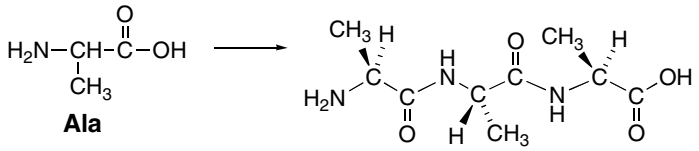


d. **lysine**
pI = 9.74
(–1) charge
at pH = 11



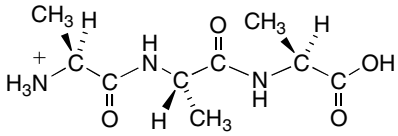
28.38 The terminal NH_2 and COOH groups are ionizable functional groups, so they can gain or lose protons in aqueous solution.

a.



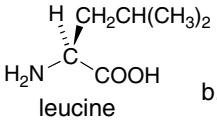
Ala **A–A–A**

b. At pH = 1

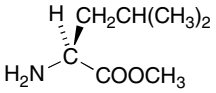


c. The pK_a of the COOH of the tripeptide is higher than the pK_a of the COOH group of alanine, making it less acidic. This occurs because the COOH group in the tripeptide is farther away from the $–NH_3^+$ group. The positively charged $–NH_3^+$ group stabilizes the negatively charged carboxylate anion of alanine more than the carboxylate anion of the tripeptide because it is so much closer in alanine. The opposite effect is observed with the ionization of the $–NH_3^+$ group. In alanine, the $–NH_3^+$ is closer to the $COO^–$ group, so it is more difficult to lose a proton, resulting in a higher pK_a. In the tripeptide, the $–NH_3^+$ is farther away from the $COO^–$, so it is less affected by its presence.

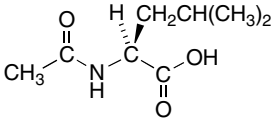
28.39


leucine

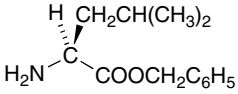
a. CH_3OH, H^+



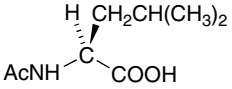
b. CH_3COCl, pyridine



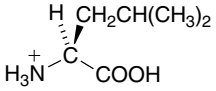
c. $C_6H_5CH_2OH$, H^+



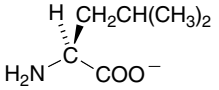
d. Ac_2O, pyridine



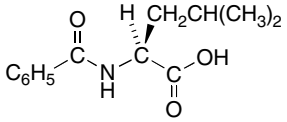
e. HCl (1 equiv)



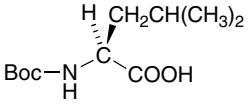
f. NaOH (1 equiv)



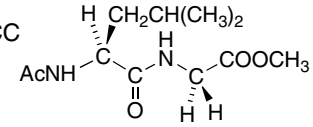
g. C_6H_5COCl, pyridine



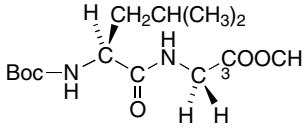
h. $[(CH_3)_3COCO]_2O$, $(CH_3CH_2)_3N$



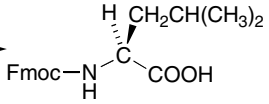
i. The product in (d), then $NH_2CH_2COOCH_3$ + DCC



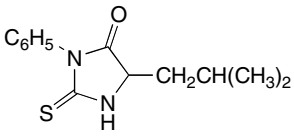
j. The product in (h), then $NH_2CH_2COOCH_3$ + DCC



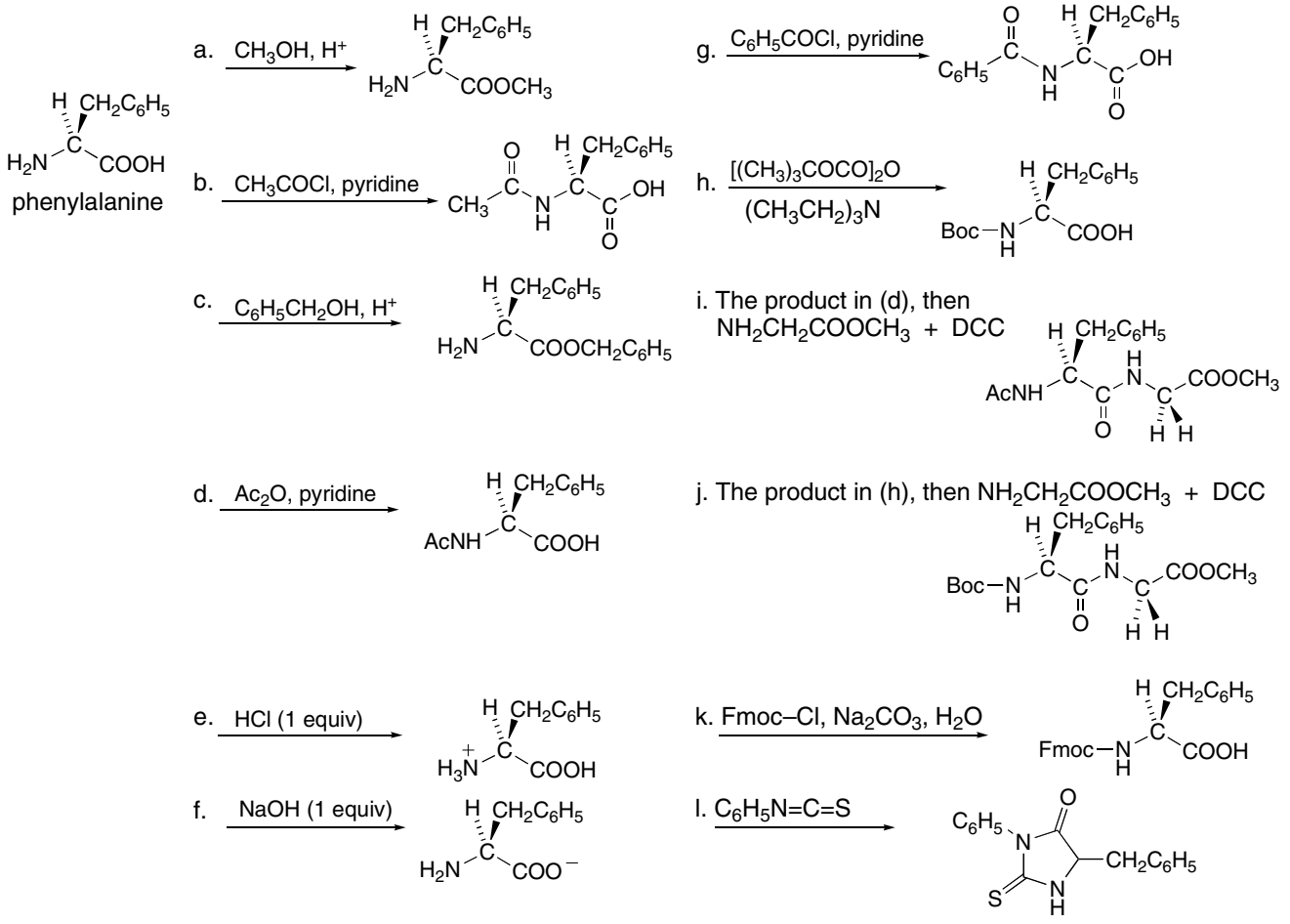
k. Fmoc–Cl, Na_2CO_3, H_2O



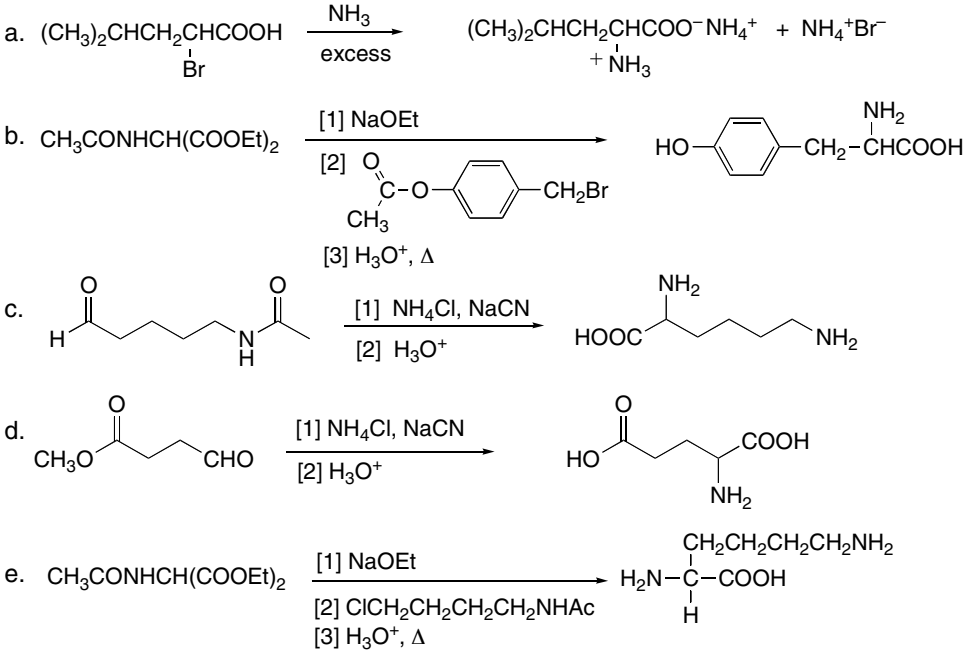
l. $C_6H_5N{=}C{=}S$



28.40

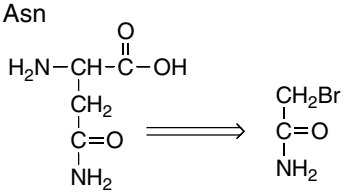

phenylalanine
a. CH3OH, H+
b. CH3COCl, pyridine
c. C6H5CH2OH, H+
d. Ac2O, pyridine
e. HCl (1 equiv)
f. NaOH (1 equiv)
g. C6H5COCl, pyridine
h. [(CH3)3COCO]2O
(CH3CH2)3N
i. The product in (d), then NH2CH2COOCH3 + DCC
j. The product in (h), then NH2CH2COOCH3 + DCC
k. Fmoc–Cl, Na2CO3, H2O
l. C6H5N=C=S

28.41

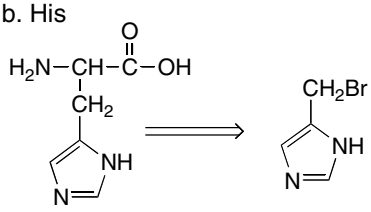

a. (CH3)2CHCH2CHCOOH
Br
NH3
excess
(CH3)2CHCH2CHCOO⁻NH4⁺ + NH4⁺Br⁻
⁺NH3
b. CH3CONHCH(COOEt)2
[1] NaOEt
[2]
CH2Br
[3] H3O+, Δ
c.
[1] NH4Cl, NaCN
[2] H3O+
d.
[1] NH4Cl, NaCN
[2] H3O+
e. CH3CONHCH(COOEt)2
[1] NaOEt
[2] ClCH2CH2CH2CH2NHAc
[3] H3O+, Δ

28.42

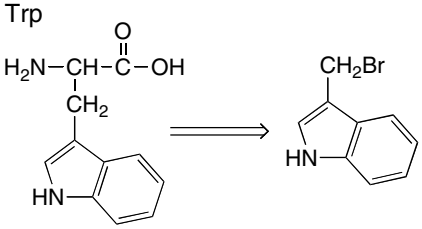
a. Asn



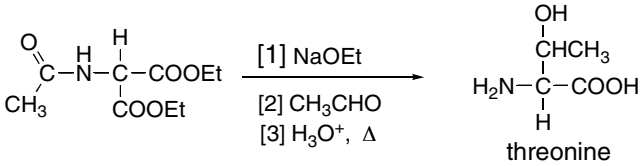
b. His



c. Trp

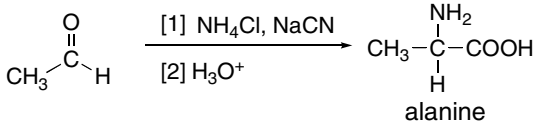


28.43

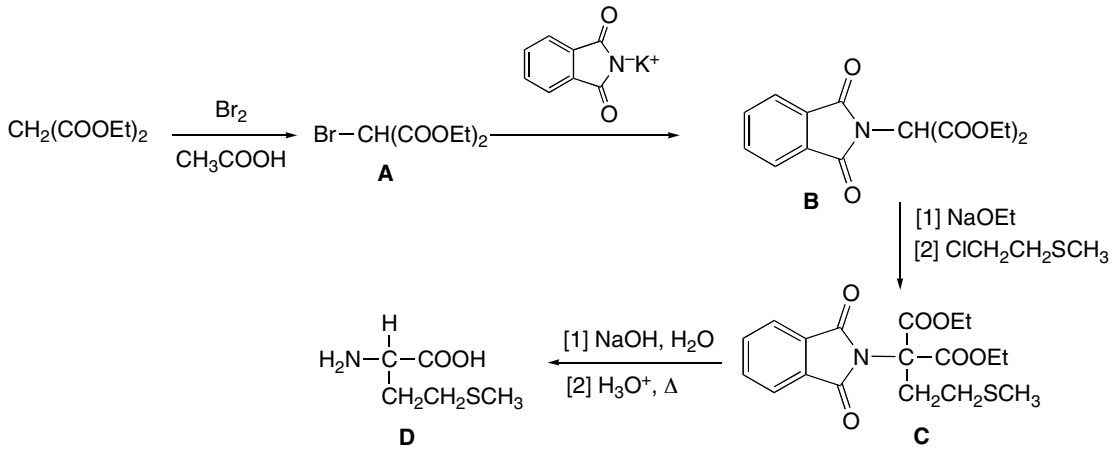


28.44

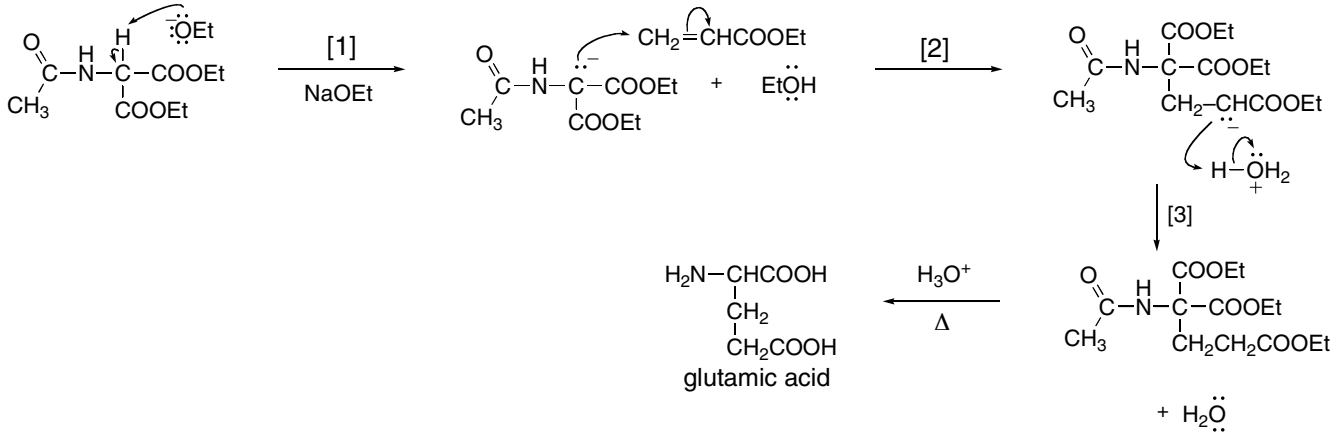
a. $CH_3CHO \xrightarrow[CH_3COOH]{Br_2} BrCH_2CHO \xrightarrow[H_2SO_4,\ H_2O]{CrO_3} BrCH_2COOH \xrightarrow[\text{excess}]{NH_3} {}^{+}H_3NCH_2COO^{-}$ glycine

b. 

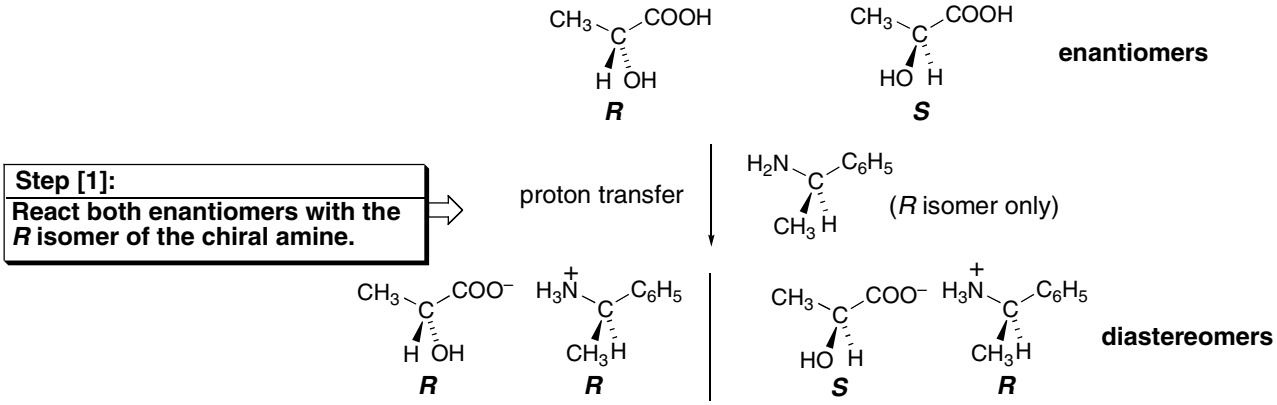
28.45



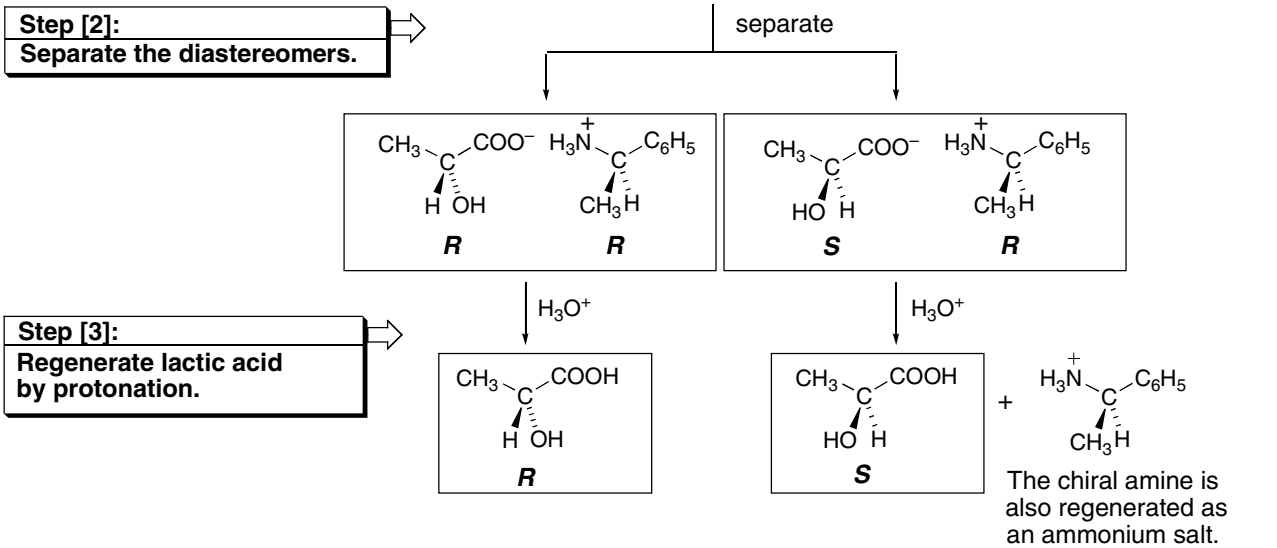
28.46



28.47

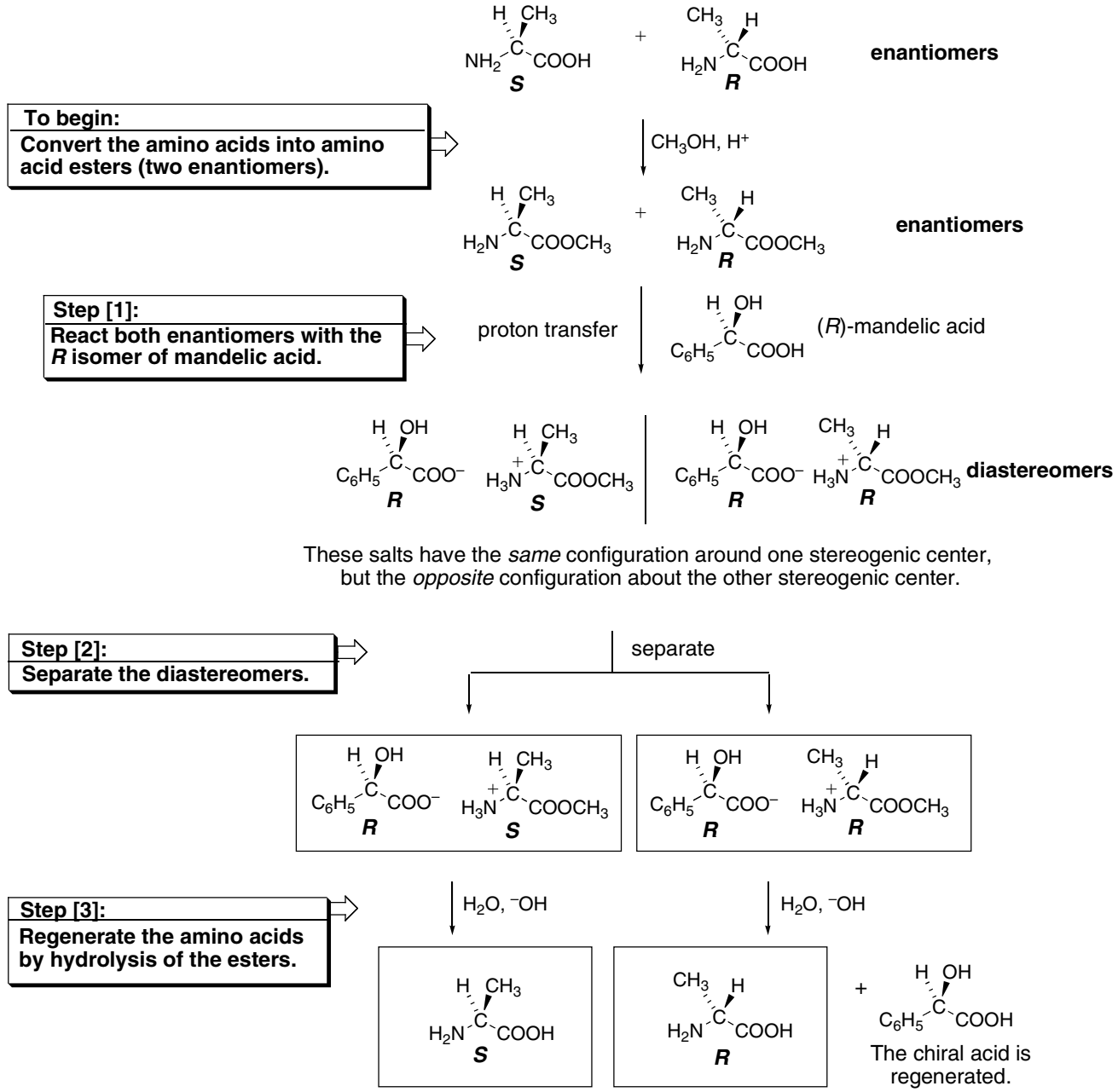


These salts have the *same* configuration around one stereogenic center, but the *opposite* configuration about the other stereogenic center.

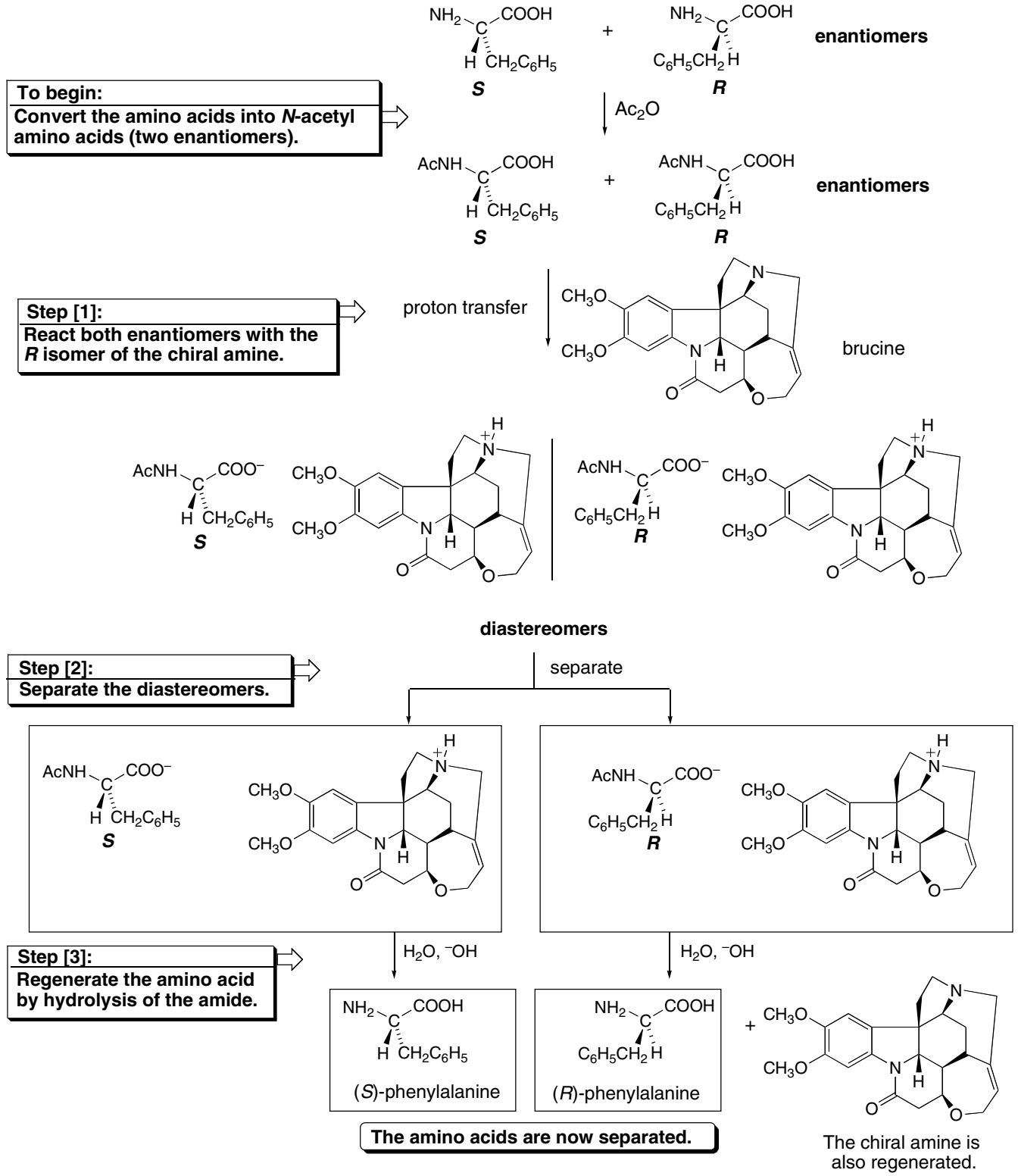


The chiral amine is also regenerated as an ammonium salt.

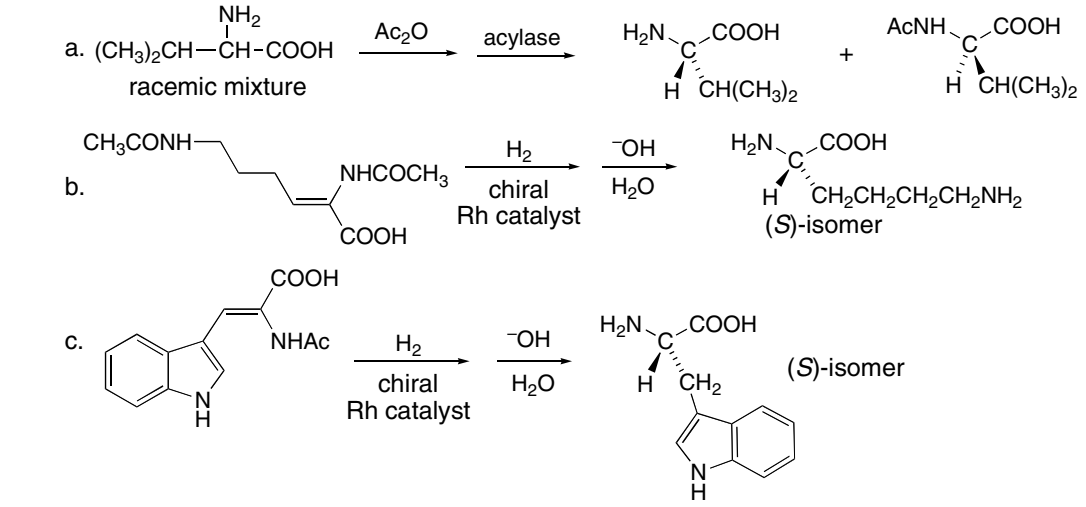
28.48


enantiomers
To begin:
Convert the amino acids into amino acid esters (two enantiomers).
CH3OH, H+
enantiomers
Step [1]:
React both enantiomers with the R isomer of mandelic acid.
proton transfer
(R)-mandelic acid
diastereomers
These salts have the same configuration around one stereogenic center, but the opposite configuration about the other stereogenic center.
Step [2]:
Separate the diastereomers.
separate
Step [3]:
Regenerate the amino acids by hydrolysis of the esters.
H2O, −OH
H2O, −OH
The chiral acid is regenerated.

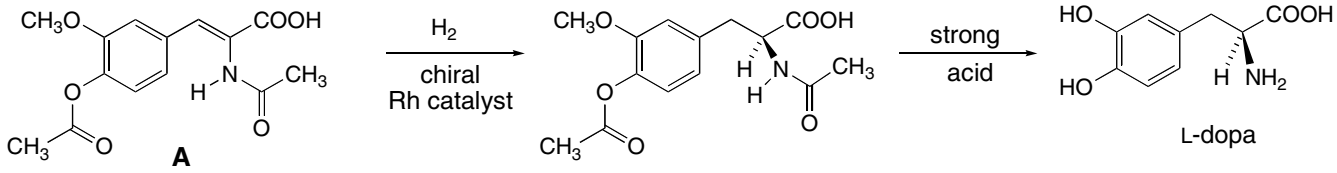
28.49


To begin:
Convert the amino acids into N-acetyl amino acids (two enantiomers).
enantiomers
Ac2O
enantiomers
Step [1]:
React both enantiomers with the R isomer of the chiral amine.
proton transfer
brucine
diastereomers
Step [2]:
Separate the diastereomers.
separate
Step [3]:
Regenerate the amino acid by hydrolysis of the amide.
H2O, −OH
H2O, −OH
(S)-phenylalanine
(R)-phenylalanine
The amino acids are now separated.
The chiral amine is also regenerated.

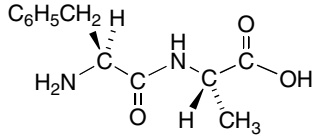
28.50



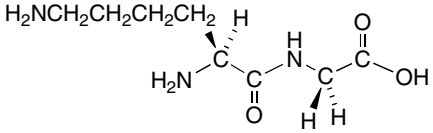
28.51



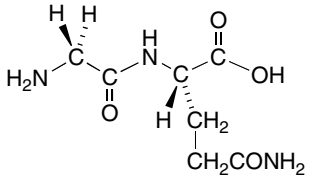
28.52

a. 

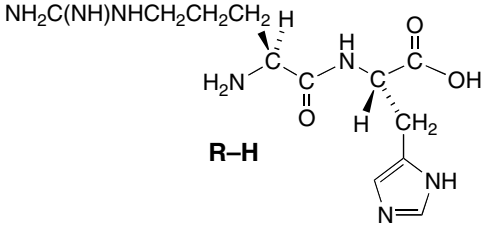
Phe–Ala

c. 

Lys–Gly

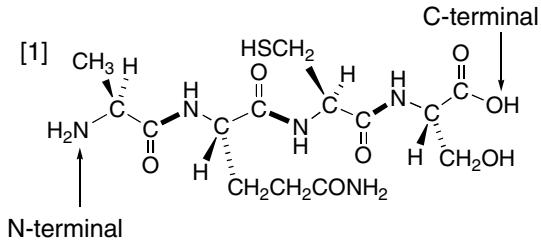
b. 

Gly–Gln

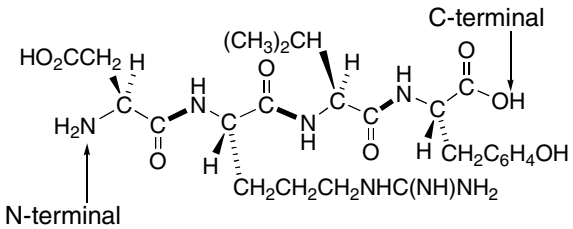
d. 

R–H

28.53 Amide bonds are bold lines (not wedges).

[1] 

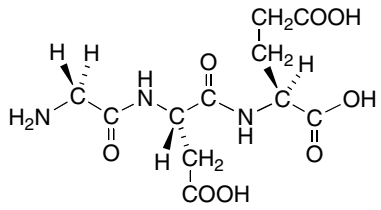
Ala–Gln–Cys–Ser
A–Q–C–S

[2] 

Asp–Arg–Val–Tyr
D–R–V–Y

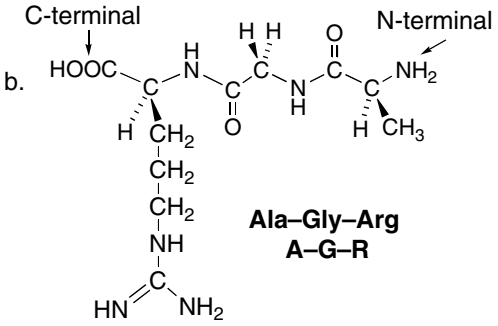
28.54 Name a peptide from the N-terminal to the C-terminal end.

a.



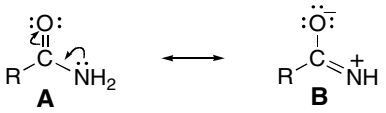
Gly–Asp–Glu
G–D–E

b.



Ala–Gly–Arg
A–G–R

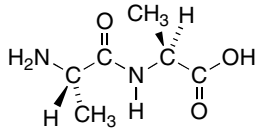
28.55 A peptide C–N bond is stronger than an ester C–O bond because the C–N bond has more double bond character due to resonance. Since N is more basic than O, an amide C–N bond is more stabilized by delocalization of the lone pair on N.



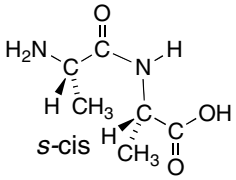
A **B**

Structure **B** contributes greatly to the resonance hybrid and this shortens and strengthens the C–N bond.

28.56 Use the principles from Answer 28.16.



s-trans



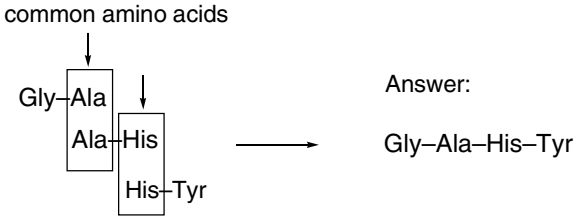
s-cis

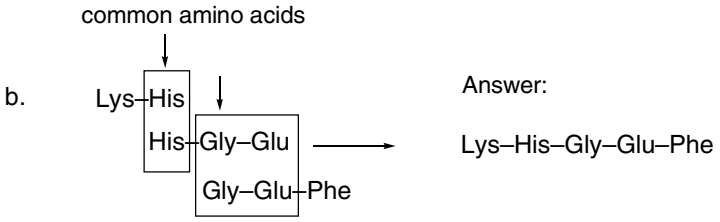
28.57

a. A–P–F + L–K–W + S–G–R–G
b. A–P–F–L–K + W–S–G–R + G
c. A–P–F–L–K–W–S–G–R + G
d. A + P–F–L–K–W–S–G–R–G

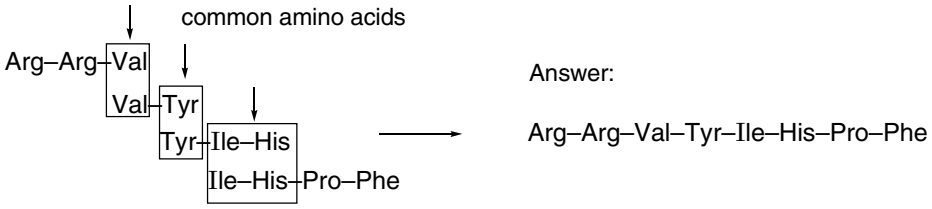
28.58

a.

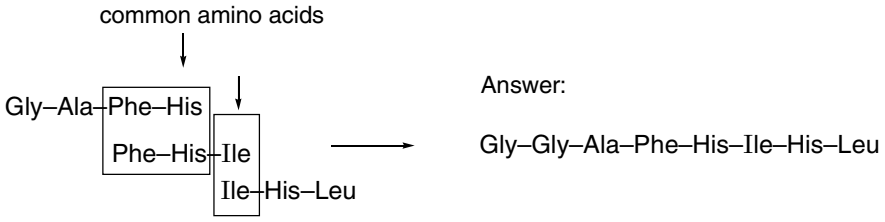




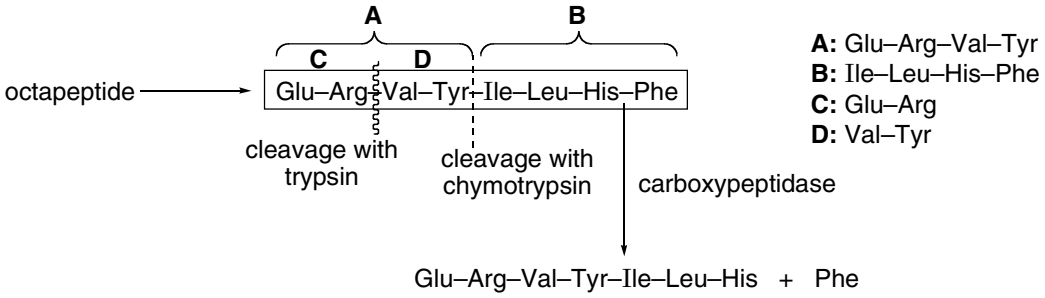
28.59



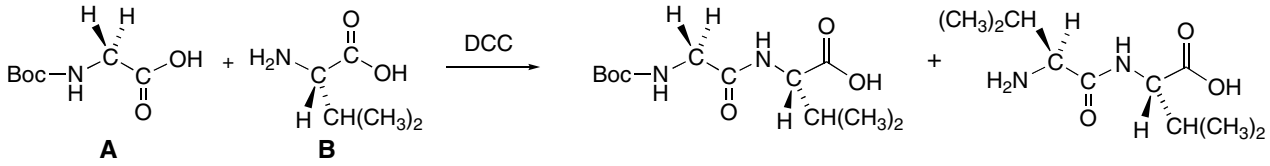
28.60 Gly is the N-terminal amino acid (from Edman degradation), and Leu is the C-terminal amino acid (from treatment with carboxypeptidase). Partial hydrolysis gives the rest of the sequence.



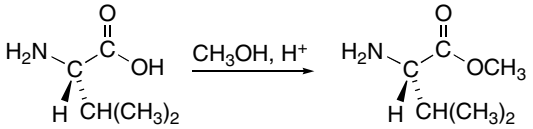
28.61 Edman degradation data give the N-terminal amino acid for the octapeptide and all smaller peptides.

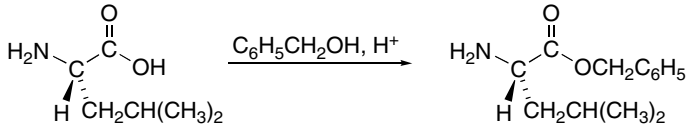


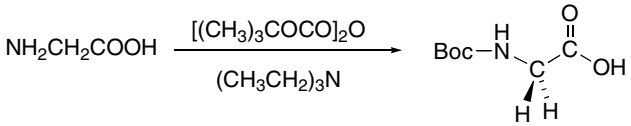
28.62 **A** and **B** can react to form an amide, or two molecules of **B** can form an amide.

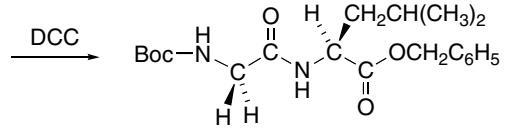


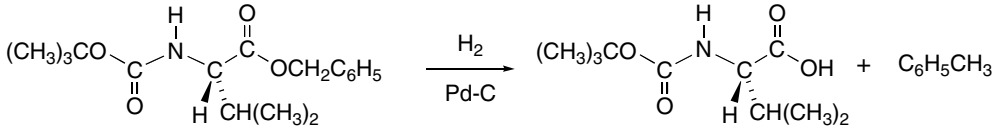
28.63

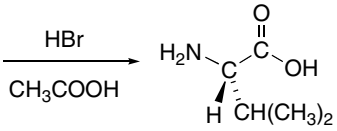
a. 

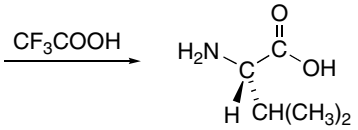
b. 

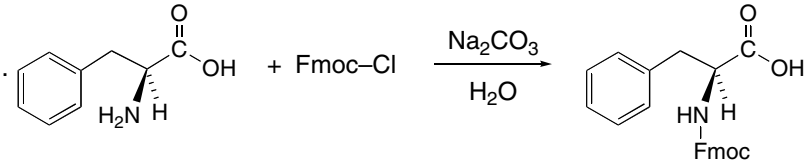
c. 

d. product in (b) + product in (c) 

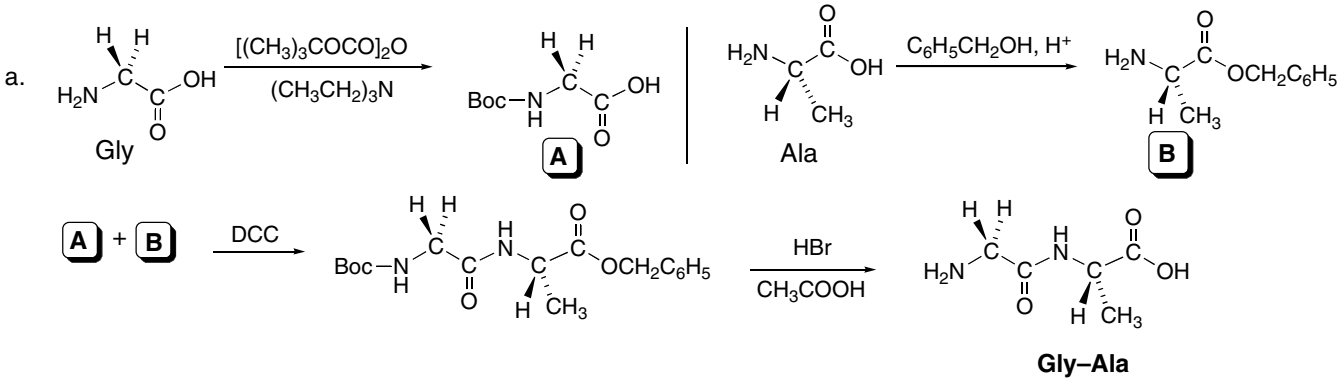
e. 

f. starting material in (e) 

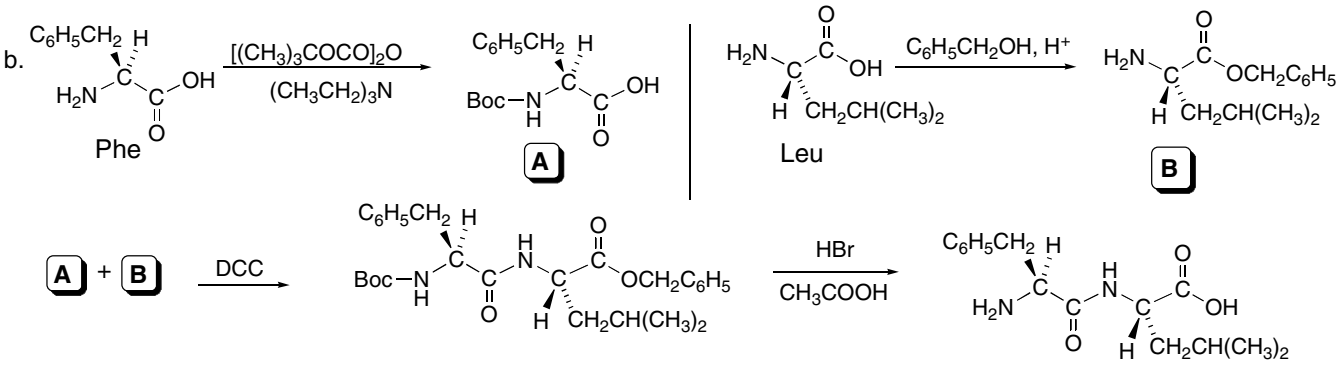
g. product in (e) 

h. 

28.64

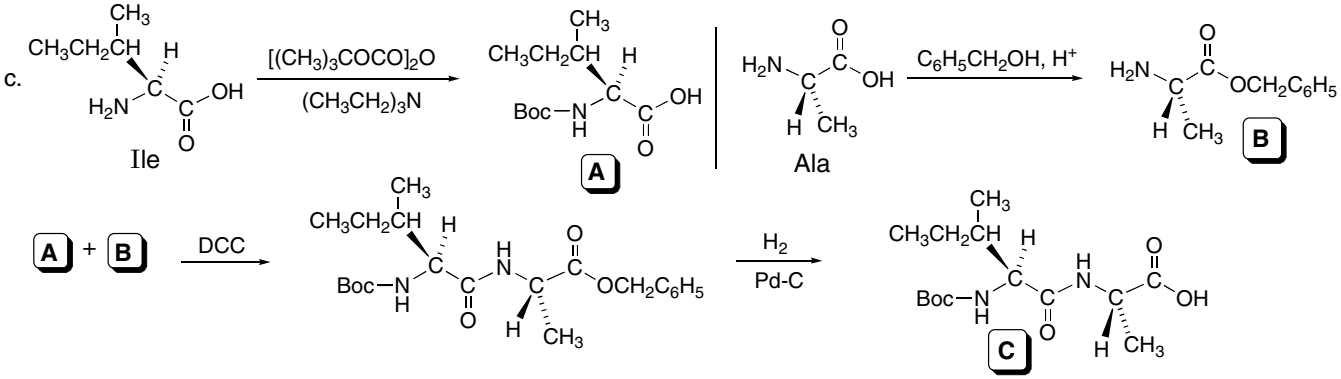
a. 

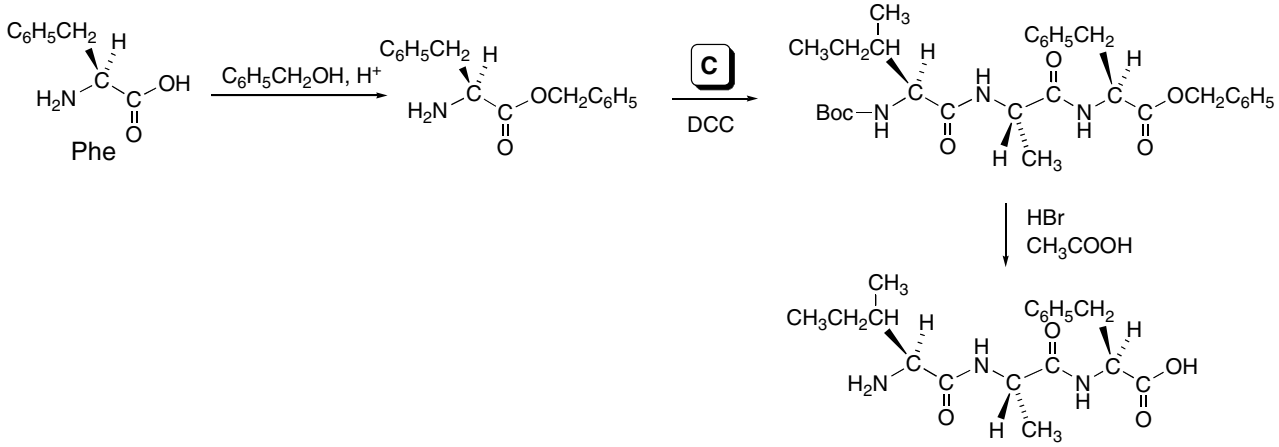
b.



Phe–Leu

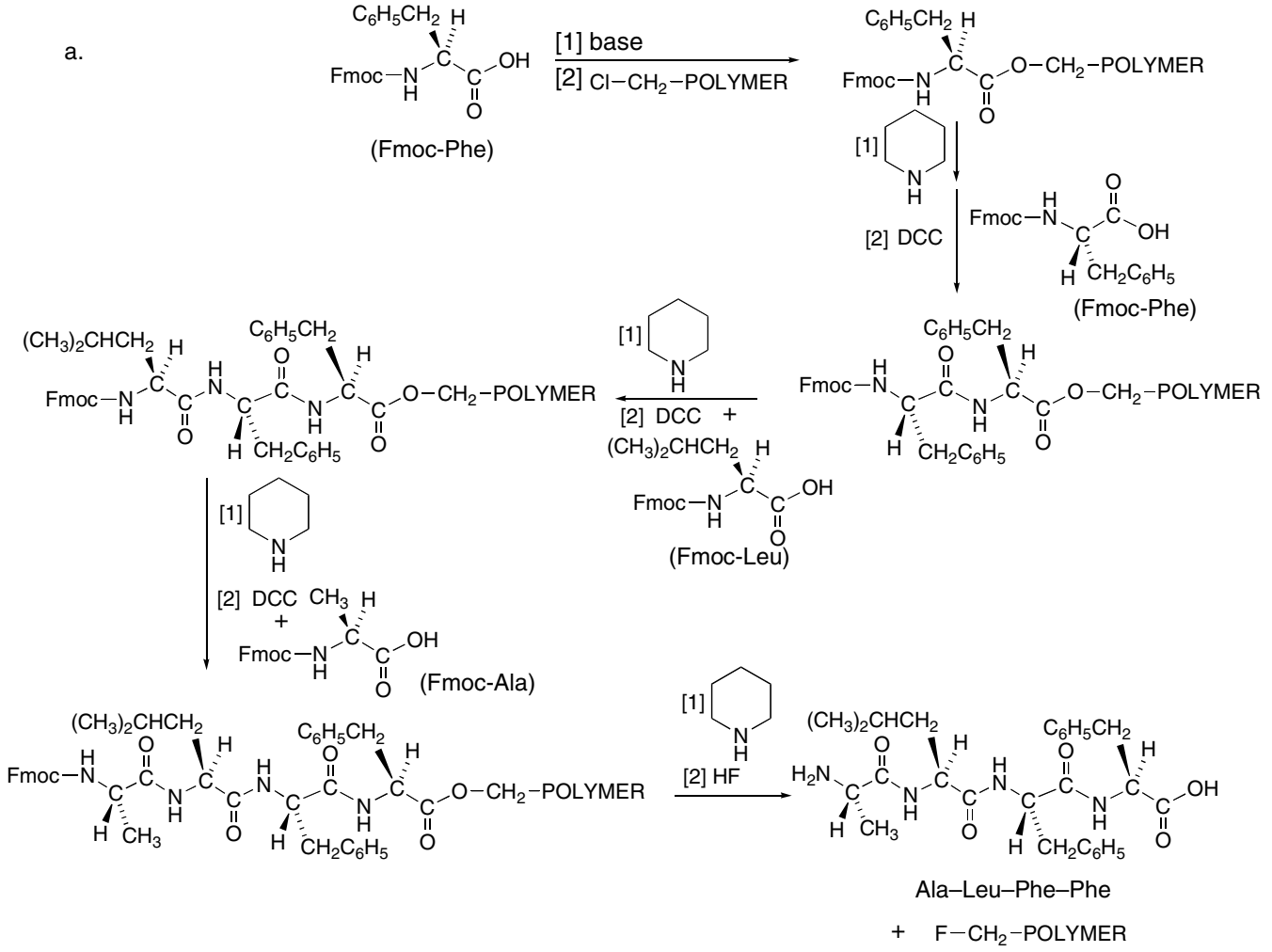
c.



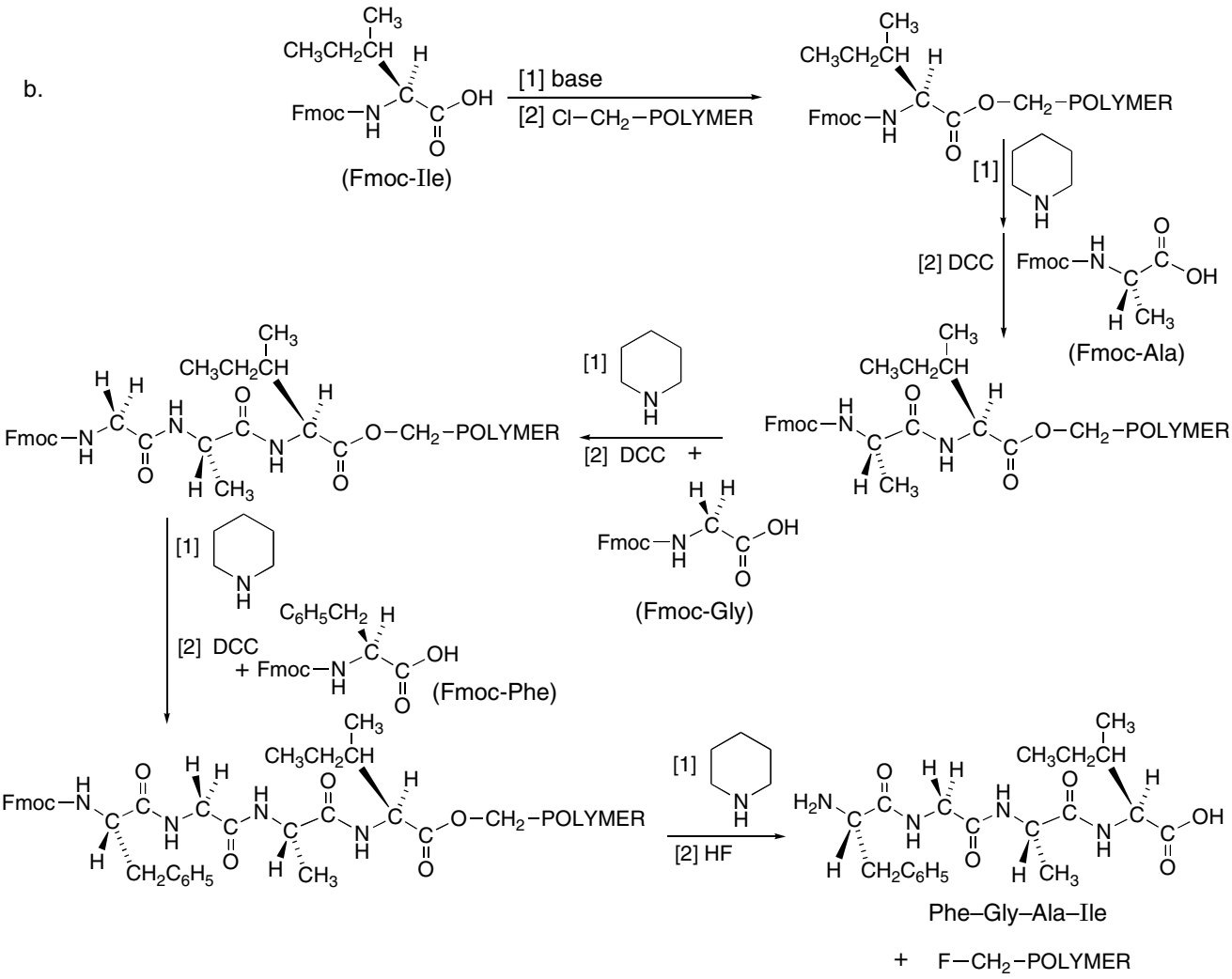


Ile–Ala–Phe

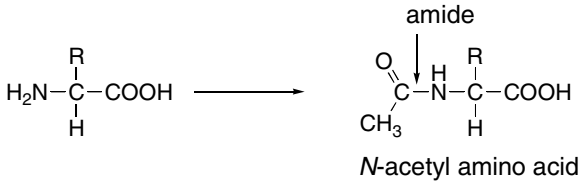
28.65 Make all the Fmoc derivatives as described in Problem 28.24.



b.

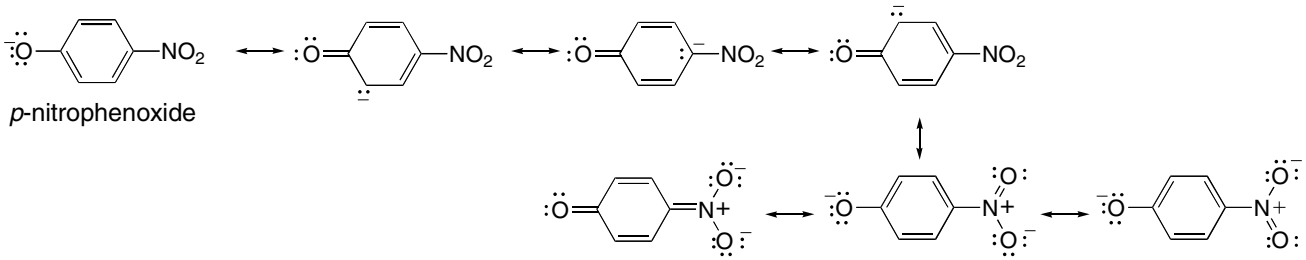


28.66 An acetyl group on the NH_2 forms an amide. Although this amide does block an amino group from reaction, this amide is no different in reactivity than any of the peptide amide bonds. To remove the acetyl group after the peptide bond is formed would require harsh reaction conditions that would also cleave the amide bonds of the peptide.



28.67

a. A *p*-nitrophenyl ester activates the carboxy group of the first amino acid to amide formation by converting the OH group into a good leaving group, the *p*-nitrophenoxide group, which is highly resonance stabilized. In this case the electron-withdrawing NO_2 group further stabilizes the leaving group.

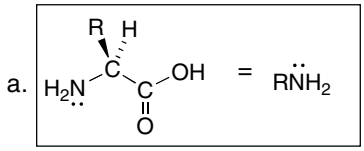


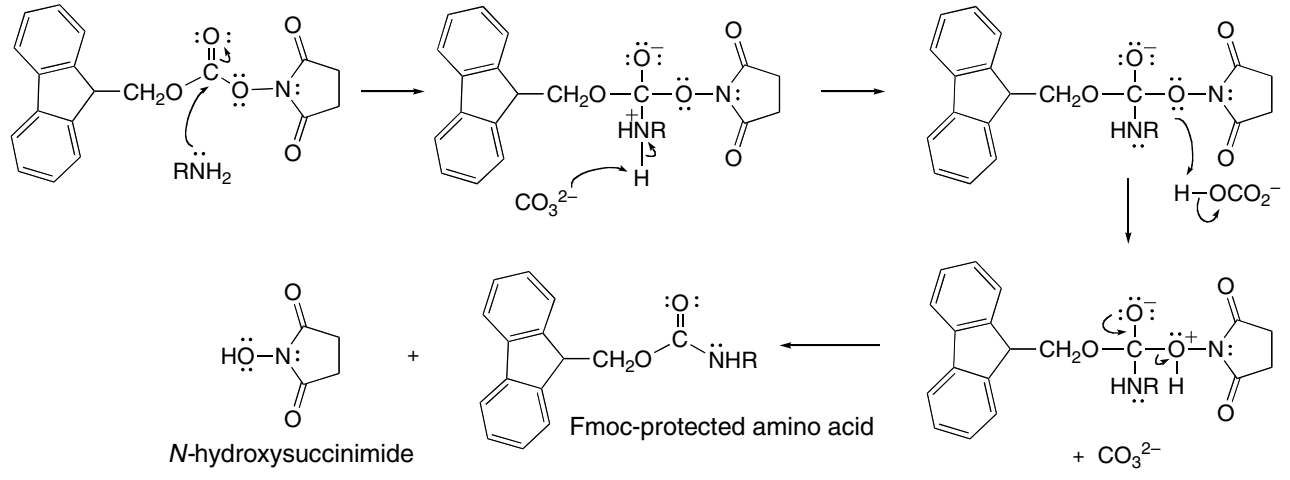
p-nitrophenoxide

The negative charge is delocalized on the O atom of the NO_2 group.

b. The *p*-methoxyphenyl ester contains an electron-donating OCH_3 group, making $CH_3OC_6H_4O^-$ a poorer leaving group than $NO_2C_6H_4O^-$, so this ester does not activate the amino acid to amide formation as much.

28.68

a. 

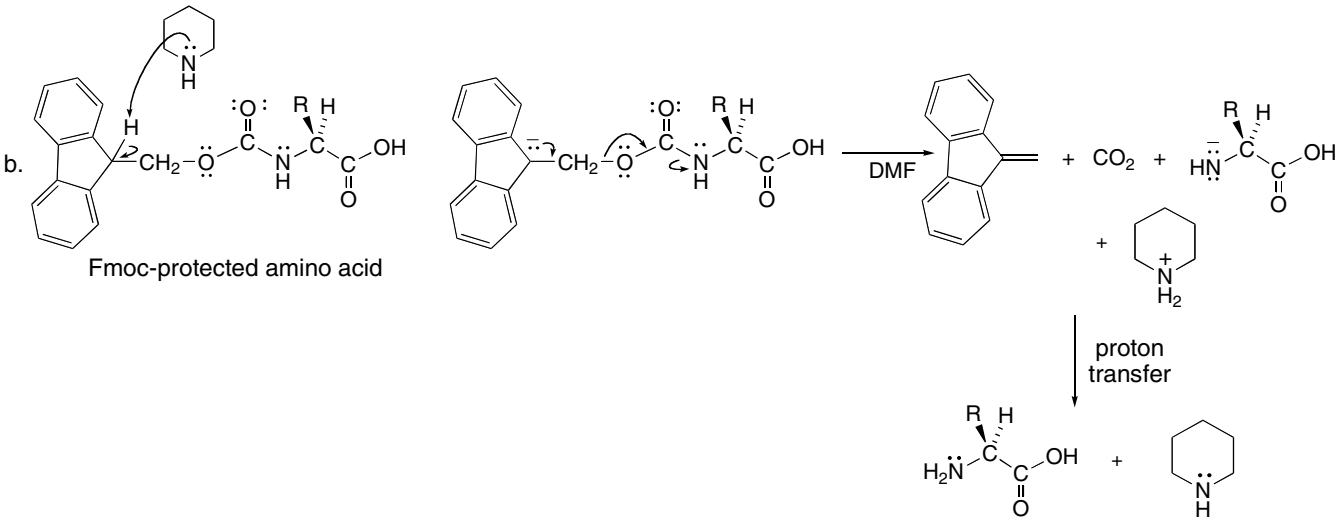


N-hydroxysuccinimide

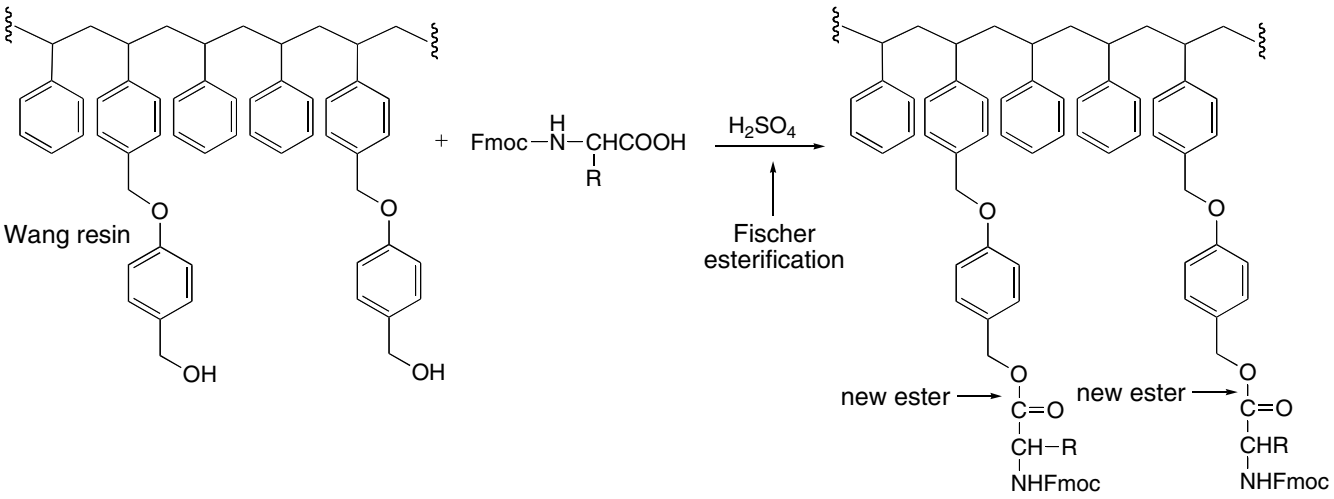
Fmoc-protected amino acid

+ CO_3^{2-}

b.

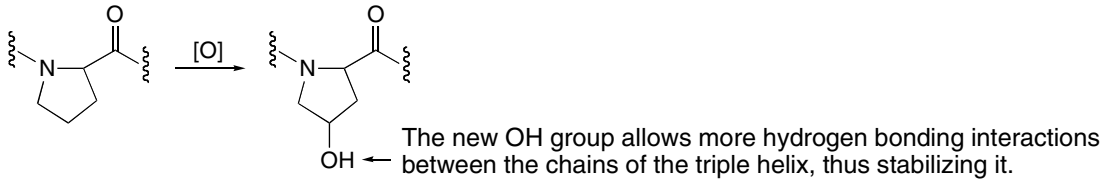


28.69 Reaction of the OH groups of the Wang resin with the COOH group of the Fmoc-protected amino acids would form esters by Fischer esterification. After the peptide has been synthesized, the esters can be hydrolyzed with aqueous acid or base, but the conditions cannot be too harsh to break the amide bond or cause epimerization.

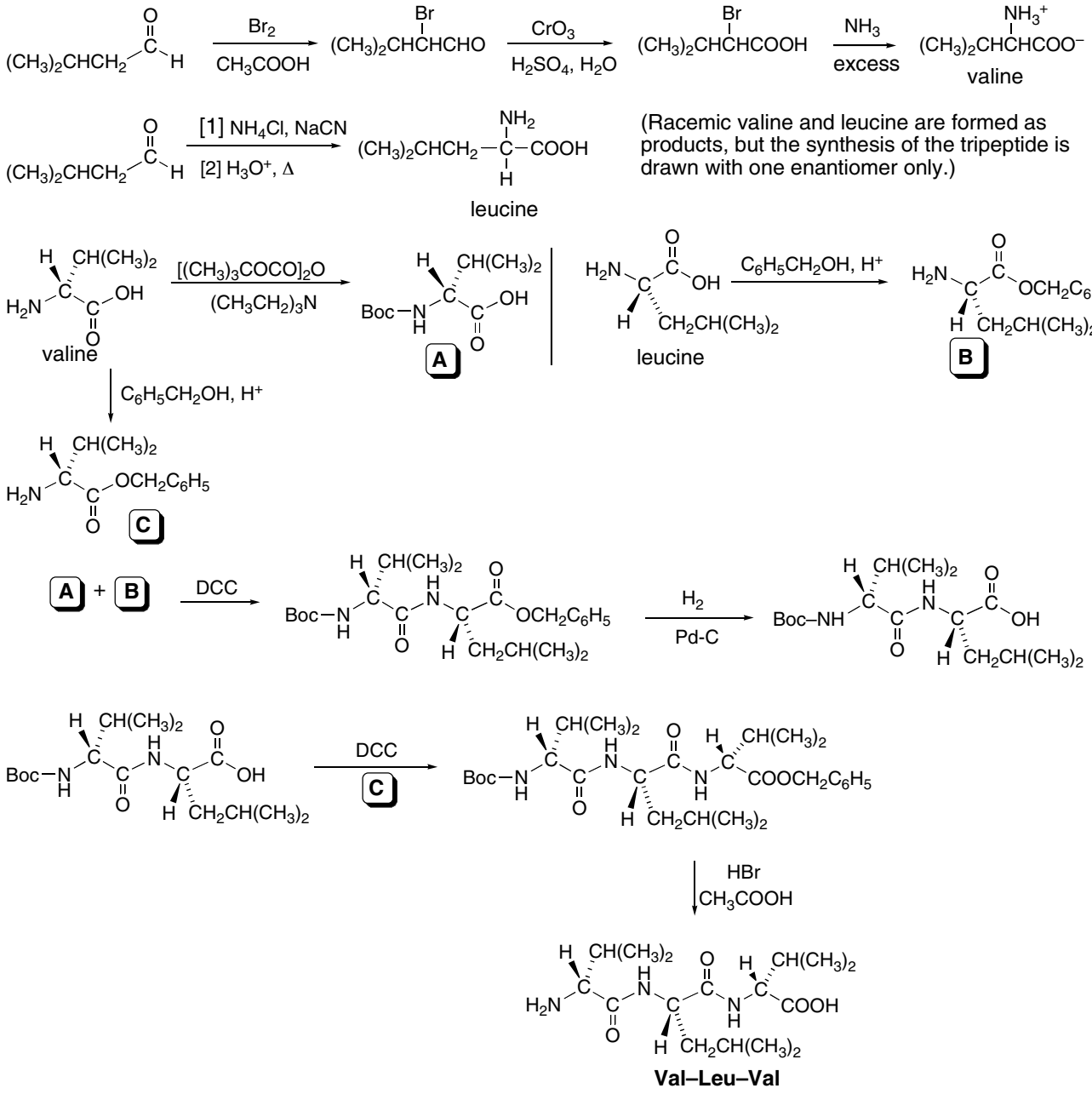


28.70 Amino acids commonly found in the interior of a globular protein have nonpolar or weakly polar side chains: isoleucine and phenylalanine. Amino acids commonly found on the surface have COOH, NH_2, and other groups that can hydrogen bond to water: aspartic acid, lysine, arginine, and glutamic acid.

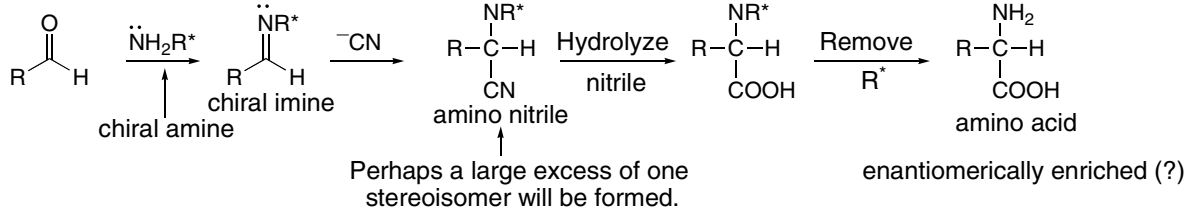
28.71 The proline residues on collagen are hydroxylated to increase hydrogen bonding interactions.



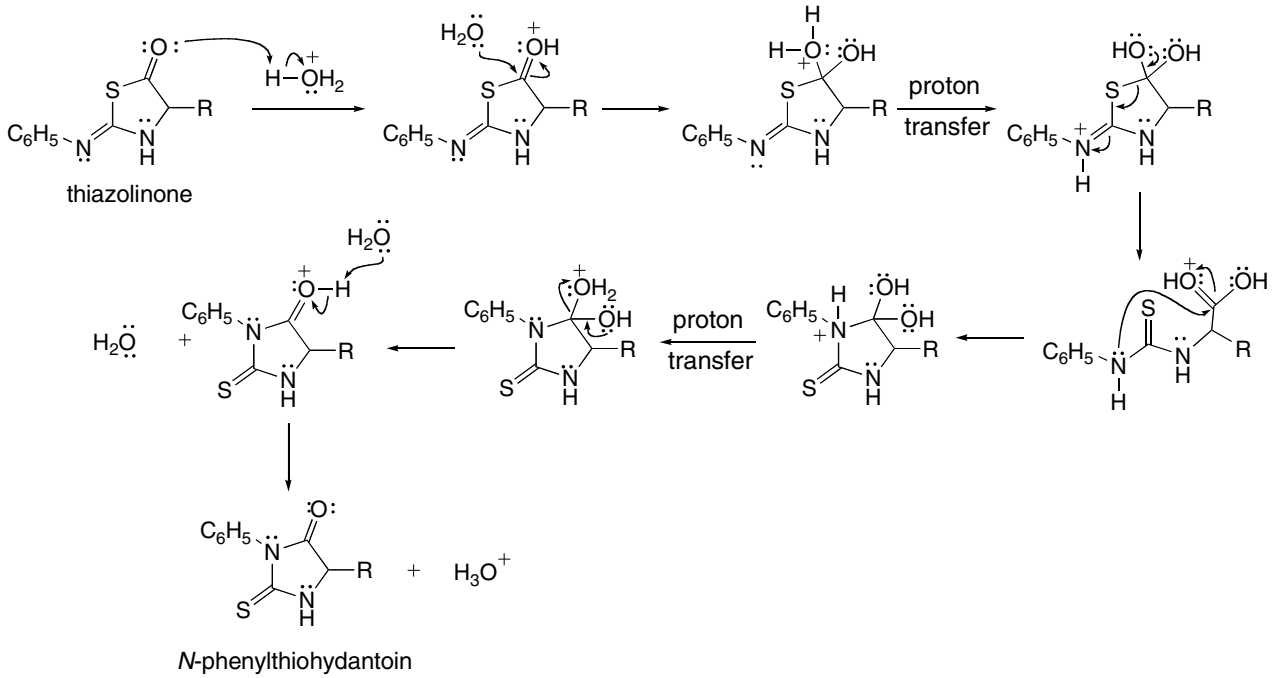
28.72



28.73 Perhaps using a chiral amine R*NH_2 (or related chiral nitrogen-containing compound) to make a chiral imine, will now favor formation of one of the amino nitriles in the Strecker synthesis. Hydrolysis of the CN group and removal of R* would then form the amino acid.



28.74


thiazolinone
proton transfer
proton transfer
N-phenylthiohydantoin