Exercise 25-1 Select the amino acids in Table 25-1 that have more than one chiral center and draw projection formulas for all the possible stereoisomers of each which possess the \(\text{\(L\)}\) configuration at the \(\text{\(\alpha\)}\) carbon.

Exercise 25-2 Which of the amino acids in Table 25-1 are acidic amino acids and which basic amino acids? Which of the structures shown would have the most basic nitrogen? The least basic amino nitrogen? Give the reasons for your choices. (Review Section 23-7.)

Exercise 25-3 How would the general features of the plot of concentration of dipolar ion and charged species versus pH for glycine (Figure 25-1) change for 6-aminohexanoic acid, which has \(p(K_{a})\) values of 4.43 and 10.75? Give special attention to the position of the isoelectric point and the width of the pH range over which the dipolar ion is expected to be the most stable species present.

Exercise 25-4 Use Equations 25-1 and 25-2 to show that the isoelectric point of glycine is the average of the two \(p(K_{a})\) values for the acid dissociation of glycine.

Exercise 25-5

a. The equations for the acid-base equilibria of lysine in Section 25-2 show possible involvement of three forms of the monocation and three forms of the neutral acid. Arrange the three forms of each set in expected order of stability. Give your reasoning.

b. The conjugate acid of glutamic acid (Table 25-1) has three acid dissociation steps with \(p(K_{a})\) values of 2.19, 4.25, and 9.67. Write equations for the equilibria involved and assign \(p(K_{a})\) values to each. Do the same for arginine (Table 25-1) with \(p(K_{a})\) values of 2.17, 9.04, and 12.48. Calculate the isoelectric point for glutamic acid and for arginine.

Exercise 25-6 Indicate the approximate positions of \(\text{\(ce\{(C=O)\}\}}\) and \(\text{\(ce\{(N-H)\}\}}\) absorptions you would expect in the infrared spectra of (a) \(\text{\(ce\{(Cl)\}\}}\ \text{\(ce\{H\_3\}\}}\ \text{\(ce\{N\}\}}\ \text{\(ce\{CH\_2CO\_2H\}\}}\) and (b) \(\text{\(ce\{H\_2NCH\_2C\}\}}\ \text{\(ce\{O\_2\}\}}\ \text{\(ce\{Na\}\}}\).

Exercise 25-7 Sketch the NMR spectrum showing the splitting pattern and chemical shifts you would anticipate for alanine dissolved in an excess of \(\text{\(ce\{D\_2O\}\}}\). Do not neglect \(\text{\(ce\{H\-D\}\}}\) exchange (Section 9-10E and 9-10I).

Exercise 25-8 The reactions that lead to the blue color produce between ninhydrin and \(\text{\(\alpha\)}\)-amino acids are examples of reactions discussed previously in the context of carbonyl chemistry (see, for instance, Section 16-4C). Write mechanisms, based insofar as possible on analogy, for each of the steps involved in the ninhydrin test, using glycine as an example. Would you expect ammonia or methanamine to give the blue color? Explain.

Exercise 25-9 Explain why arginine elutes from an ion-exchange column using a buffer at pH 5-6, whereas glutamic acid elutes at pH 3.

Exercise 25-10 A cation-exchange resin can be prepared by radical-addition polymerization of phenylethene (styrene, Section 10-8) in the presence of about \(\text{\((2\,-\%(10\%)\)}\) 1,4-diethenylbenzene (1,4-divinylbenzene),
, followed by electrophilic sulfonation of the resulting polymer with \(\text{H}_2\text{SO}_4-\text{SO}_3\) (see Section 22-4G). Explain how these reactions lead to a *three-dimensional* insoluble polymer with linkages as shown below. Indicate the reaction mechanisms involved.

---

**Exercise 25-11** Consider a "hard" water comprised of dilute \(\text{MgCl}_2\). Ion exchange with resin\(\text{-S} \\overset{\ominus}{\text{O}_3}\overset{\oplus}{\text{Na}}\) replaces \(\text{Mg}^{2+}\) with \(\text{Na}^+\), and with resin\(\text{-SO}_3\text{H}\), \(\text{Na}^+\) is replaced by \(\text{H}^+\), thereby producing a dilute \(\text{HCl}\) solution. What kind of an ion-exchange resin would you need to remove the \(\text{Cl}^-\) from the \(\text{HCl}\) solution and produced "deionized" water? (Consider exchanging \(\text{Cl}^-\) for \(\text{OH}^-\)).

---

**Exercise 25-12**

- a. Draw the structure of the azlactone derived from \(\text{L-phenylalanine and ethanoic anhydride.}\)
- b. Which of the hydrogens in this azlactone would you expect to be the most acidic? Explain.
- c. Why do chiral azlactones derived from amino acids such as \(\text{L-phenylalanine racemize easily on heating in ethanoic acid in the presence of ethanoate ion?}\)

---

**Exercise 25-13** Explain why glycine itself, as the dipolar ion, reacts with nitrous acid to eliminate nitrogen, whereas the ethyl ester of glycine forms ethyl diazoethanoate.

---

**Exercise 25-14** Show how the following amino acids may be prepared from the indicated method and starting materials:

- a. glutamic acid from 2-oxopentanedioic acid (\(\alpha\)-ketoglutaric acid) by the Strecker method
- b. leucine from 2-methyl-1-propanol by the phthalimidomalonic ester synthesis
- c. aspartic acid from ethyl chloroethanoate by the \(\text{N}\)-formylaminomalonic ester synthesis

---

**Exercise 25-15** Suggest a synthetic route to proline from hexanedioic acid (adipic acid) that involves the transformations \(\text{CO}_2\text{H}\rightarrow\text{NH}_2\), and \(\text{CH}_2\text{CO}_2\text{H}\) to \(\text{CHBrCO}_2\text{H}\). Specify the reagents required to accomplish each step.

---

**Exercise 25-16** The structure of the hormonal peptide oxytocin is abbreviated to

\[
\text{CYS- TYR- ILE- GLN- ASN- CYS- PRO- LEU- GLYNH}_2
\]
Exercise 25-17 What problems might be encountered in using the 2,4-dinitrofluorobenzene method for determination of end groups on Gly-Lys-Ala? Explain.

Exercise 25-18 The tripeptide, eisenine, has only one free carboxyl group, does not react with 2,4-dinitrofluorobenzene, and on complete hydrolysis yields 2 moles of \(\text{(L-)}\)-glutamic acid, 1 mole of \(\text{(L-)}\)-alanine, and 1 mole of ammonia. Alanine is indicated to be the \(\text{(C-)}\)-terminal amino acid. Write a structure for eisenine that is in accord with the above facts.

Exercise 25-19* Eledoisin is a peptide isolated from the salivary glands of *eledone*, a Mediterranean eight-armed cephalopod. The peptide is a powerful hypotensive agent. Deduce a possible structure from the following information:

2. No free amino \(\text{(N-)}\)-terminal group or free carboxyl \(\text{(C-)}\)-terminal group can be detected.
3. Chymotrypsin hydrolysis forms two peptides, L and M. Their compositions are

   \[
   L = \text{Ala, Asp, Glu, Lys, Phe, Pro, Ser (unsequenced)}
   \]
   \[
   M = \text{Ile-Gly-Leu-Met(\text{NH}_2)} \text{ (sequenced)}
   \]

   (At this point you should be able to deduce the sequence of five amino acids at the \(\text{(C-)}\)-terminus of eledoisin.)

4. Trypsin hydrolysis gives two peptides, P and Q, with the indicated compositions:

   \[
   P = \text{Gly, Lys, Pro, Ser}
   \]
   \[
   Q = \text{Ala, Asp, Gly, Ile, Leu, Met, Phe}
   \]

   (At this point, you can deduce two possible sequences for Q.)

5. Trypsin hydrolysis of L gives a peptide of composition Ala, Asp, Phe which, with 2,4-dinitrofluorobenzene, gives the 2,4-dinitrophenyl derivative of aspartic acid.
6. Partial acid hydrolysis of eledoisin gives several dipeptides, among them Ser-Lys and Pro-Ser.

Exercise 25-20 A hexapeptide was subjected to the transformations diagrammed below. (The commas between the amino acids indicate the sequence is unknown or unspecified.) Deduce the structure of the hexapeptide.

Exercise 25-21 How could an optically pure \(\text{(N-)}\)-acylamino acid racemize and lead to racemic \(\text{(N-)}\)-acylpeptides as the result of a peptide coupling reaction wherein the carboxyl group of the amino acid was converted to an anhydride group? (Review Section 25-5A.)

Exercise 25-22 Suppose there is \(1\%\) formation in each step of the wrong isomer of the acylating component in an otherwise quantitative 100-step peptide synthesis. What is the yield of the desired polypeptide isomer?
Exercise 25-23* The following are acyl derivatives known to react with amines to give \(\text{N}\)-acylated amines:

\[
\begin{align*}
(1) & \quad R-C-CO-OC_2H_5 \\
(2) & \quad R-C-CO-N_N \quad \text{NO}_2 \\
(3) & \quad R-C-Cl \\
(4) & \quad R-C-CO_2H_5 \\
(5) & \quad R-C-OC(CH_3)_3 \\
(6) & \quad R-C-N_N
\end{align*}
\]

Arrange these reagents in expected order of reactivity with the free amino group of a carboxyl-protected peptide, \(\text{H}_2\text{N}\)-peptide\(\text{-X}\), where \(\text{H}_2\text{N}\)-peptide\(\text{-X}\) is the carboxyl-protecting group. Give your reasoning and indicate what disadvantages each reagent may have as to side reactions, undesirable byproducts, and so on. It may be useful to review Sections 18-7A and 24-3.

Exercise 25-24 Consider two routes to the synthesis of a tetrapeptide:

\[
\begin{align*}
(1) & \quad \text{Asn} \xrightarrow{\text{Phe}} \text{Asn-Phe} \xrightarrow{\text{His}} \text{Asn-Phe-His} \xrightarrow{\text{Pro}} \text{Asn-Phe-His-Pro} \\
(2) & \quad \text{Asn} \xrightarrow{\text{Phe}} \text{Asn-Phe} \xrightarrow{\text{His-Pro}} \text{Asn-Phe-His-Pro}
\end{align*}
\]

If each coupling step proceeds in \(\text{80\%}\) yield, which of the two routes would give the highest overall yield?

Exercise 25-25 Indicate the steps that would be necessary to attach each of the amino acids listed to the \(\text{N}\)-terminus of a peptide chain. Assume that any side-chain functions in the peptide are suitably protected, but do not assume that the amino acids will couple with the peptide without suitable protection of their functional groups.

a. lysine \\
b. aspartic acid \\
c. cystine \\
d. serine

Exercise 25-26 Show how each of the following substances may be synthesized starting with the individual amino acids. Indicate the reagents needed in each step.

a. glutamylglycine (Glu-Gly) \\
b. Tyr-Ala-Val

Exercise 25-27* A resin known as Sephadex that is useful in gel filtration is prepared from a polysaccharide that is cross-linked into a three-dimensional matrix with "epichlorohydrin",

\[
\begin{align*}
\text{CH}_2\text{CH} & \text{CH}_2\text{Cl}
\end{align*}
\]

. The degree of cross-linking determines the pore size of the gel. Write equations, specifying the conditions as closely as possible, for reactions whereby a glucose unit of one polysaccharide chain could be linked to the glucose of another chain through an epichlorohydrin molecule.

Exercise 25-28 Hemoglobin, the protein responsible for carrying oxygen from the lungs to the body tissues, contains \(0.355\%\) iron. Hydrolysis of \(100 \text{ g}\) of hemoglobin gives \(1.48 \text{ g}\) of tryptophan. Calculate the
minimum molecular weight of hemoglobin that is consistent with these results.

**Exercise 25-29** Devise a way to use a stereospecific hydrolytic enzyme for resolution of \((D)\),\((L)\)-alanine.

**Exercise 25-30** The proteolytic enzyme, papain, differs from chymotrypsin in having cysteine, or a labile derivative thereof, as part of its active site. The enzyme is deactivated by substances that form complexes with, or react with, \(-\ce{SH}\) groups and the activity is restored by reactions expected to regenerate an \(-\ce{SH}\) group. Work out a schematic mechanism for cleavage of a peptide chain with papain that involves acylation of the critical \(-\ce{SH}\) group of papain.

(One of the most interesting features of papain is that more than 100 of its total of 185 amino-acid residues may be removed with the aid of an aminopeptidase to give a fragment with considerable enzymatic activity.)

**Exercise 25-31** Why would the intermediate addition product of thiamine to pyruvic acid be expected to decarboxylate readily? Support your answer by analogy; see Section 18-4.

**Exercise 25-32** Write equations for a base-induced decomposition of the modified thiamine coenzyme, \((11)\), to ethanal and thiamine pyrophosphate.

**Exercise 25-33**

a. The lactim-lactam equilibrium of 2-hydroxypyrimidine lies on the side of the lactam, yet the benzenol-cyclohexadienone equilibrium lies far on the side of benzenol (Section 26-1). Explain what factors make for the large difference in the positions of these two equilibria. (Bond energies and review of Section 24-1A may help in showing you the differences between these systems.)

b. Use bond energies to decide whether the following equilibrium with 2-aminopyrimidine is likely to be more, or less, favorably to the right than the corresponding equilibrium \((19a) \rightleftharpoons (19b)\). Give your reasoning.

\[
\begin{align*}
\text{N} & \text{H} \\
\text{N} & \text{N} \\
\text{N} & \text{N} \\
\text{H} & \text{H}
\end{align*}
\]

\[
\begin{align*}
\text{N} & \text{H} \\
\text{N} & \text{N} \\
\text{N} & \text{N} \\
\text{H} & \text{H}
\end{align*}
\]

c. Show how the resonance method could be used to predict whether cytosine or 2-hydroxypyrimidine would have the greater tendency to be more stable in the lactam rather than the lactim form.

**Exercise 25-34** Write equations for the mechanistic steps involved in hydrolysis of adenine deoxyribonucleoside to deoxyribose and adenine. Would you expect the reaction to occur more readily in acidic, basic, or neutral solution? (Review Section 16-4C).

**Exercise 25-35** The following steps have been used in the synthesis of 1-\((D)\)-glucosylcytosine:
Write the structures of the various substances given, and as detailed a mechanism as you can for the reaction of the bromo compound with 2,4-diethoxypyrimidine. Would you expect the reaction of 1-bromoglucose tetraethanoate with 2,4-diethoxypyrimidine to yield significant amounts of 6-ethoxy-1-(tetraethanoyl-D-glucosyl)-2-pyrimidone? Give your reasoning.

**Exercise 25-36** The enzyme, *acetoacetate decarboxylase*, converts 3-oxobutanoic (acetoacetic) acid to 2-propanone and carbon dioxide. Investigation of the nature of the catalytically active site has been carried on by F. Westheimer and his coworkers with the following results. First, 3-oxobutanoic acid labeled with \(^{18}\)O at the ketone group and decarboxylated in ordinary water in the presence of the enzyme gives 2-propanone containing no \(^{18}\)O. Second, the enzyme-substrate complex combines with hydrogen cyanide and decarboxylation stops. However, if a solution of the hydrogen cyanide-deactivated enzyme-substrate complex is dialyzed (i.e., placed in a cellophane bag immersed in flowing water to permit separation of low-molecular-weight water-soluble materials from the enzyme by diffusion through the cellophane; peptides and proteins having molecular weights greater than 6000 to 10,000 do not diffuse through cellophane), then the enzyme is recovered fully active. Third, the mild reducing agent sodium borohydride, which reacts with \(\text{C-N}\) but not \(\text{C=C}\) or amide carbonyl groups, reduces the enzyme-substrate complex made from \(^{14}\)C-labeled 3-oxobutanoic acid to give a product that is not enzymatically active and that retains essentially all the \(^{14}\)C on dialysis without regenerating the enzyme. Sodium borohydride treatment of the enzyme-substrate-hydrogen cyanide complex followed by dialysis regenerates fully active enzyme. Borohydride reduction of the enzyme-substrate complex, prepared from 3-oxobutanoic acid labeled at the 2- and 4-positions with \(^{14}\)C, followed by complete hydrolysis, gives 1 mole of \((\text{N}-2\text{-propyl-}\text{C})\text{-amino-2-aminohexanoic acid.}\)

Write a stepwise mechanism for the enzyme-induced decarboxylation, clearly indicating the nature of the bonding between the substrate and enzyme. Show how your mechanism can accommodate hydrogen cyanide inhibition and the results of the borohydride reactions. Utilize the results of the discussion of the ease of decarboxylation of various acids in **Section 18-4** to deduce possible structural requirements for the active site so that decarboxylation of the enzyme-substrate complex can occur more readily than the uncatalyzed decarboxylation.

**Exercise 25-37** Figure 25-29 shows an unusually well-resolved \(^{13}\)C NMR spectrum of the enzyme lysozyme (Table 25-3 and Figure 25-15) taken with proton decoupling. The closely spaced peaks on the left side of the spectrum are of the carbonyl groups. The peaks in the center are of unsaturated and aromatic carbons, while those on the right are of the aliphatic amino acid carbons. The five sharp resonances marked at about \(110 \text{ ppm}\) with * arise from tryptophan carbons marked with * in (22): 

![Tryptophan structure](image)

a. How many tryptophan residues does the \(^{13}\)C spectrum indicate to be present in lysozyme?
b. Lysozyme contains \(\text{S-S}\) bonds, and when these \(\text{S-S}\) bonds are cleaved by reduction, the resonances marked * in Figure 25-29 have much smaller chemical-shift differences. Explain why this might be so.

Figure 25-29: Carbon-13 NMR spectrum at \(45.3 \, \text{MHz}\) of lysozyme, \(0.015 \, \text{M}\) in water solution, taken with proton decoupling (Section 9-10L).

Contributors