Peptides and proteins are very important in biology. As a result, synthesis of these molecules has become very important, allowing for the laboratory study of model compounds that can give us insight into how proteins work, as well as pharmaceutically important compounds.

Structurally, amide or peptide bonds are very stable and resistant to carboxyl substitution. That stability makes optimal structures from which to construct proteins. Despite being composed of very long chains of linked amino acids, proteins actually have some limits on their conformational flexibility (their "floppiness"). That allows proteins to more reliably hold a particular shape.

Both the stability and the structural rigidity of peptides arises from the nature of the peptide bond. The pi donation that hinders nucleophiles from substituting at the carbonyl is pronounced enough that it can be considered to form an additional bond. Thus, peptides behave as though they contain C=N bonds rather than C-N bonds. X-ray structure determinations show that the peptide nitrogens in proteins are trigonal planar, not pyramidal. In addition, many peptides exhibit cis-trans isomerism. For every peptide bond, two different isomers can occur, depending on whether a substituent attached to nitrogen is on the same side of the C=N bond as the carbonyl oxygen or the opposite side.

The great stability of these structures does not mean they are easy to make. Part of the difficulty stems from the fact that amino acids are difunctional. In order to form long chain structures, amino acids must be able to react twice: once with an amine, to grow in one direction, and once with a carboxylic acid to grow in the other direction. In other words, an amino acid contains both a nucleophile and an electrophile.

Suppose we were to try to make the dipeptide, ala-phe. This peptide contains an alanine connected to a phenylalanine through a peptide bond. The peptide bond is formed between the carboxylic acid of alanine and the amine of phenylalanine. Assuming the amino acids do react together to form the peptide, combining these two reactants would likely produce a mixture of four dipeptides:

\[
\text{ala-phe} \quad \text{ala-ala} \quad \text{phe-phe} \quad \text{phe-ala}
\]

In other words, peptide formation from amino acids is non-selective.

**Problem CX9.1.**

Draw structures for the four peptides formed by combining phe and ala.

**Problem CX9.2.**

What tripeptides would be produced by mixing ala, gly and val?
Problem CX9.3.

Simply combining these peptides might not result in any peptide formation at all. Why not?

In laboratory syntheses, a number of techniques have been used to make peptide synthesis selective. Most frequently, protecting groups are used. A protecting group "masks" one of the two functional groups on an amino acid, but leaves the other one open. If one amino acid has its amine protected, it can only react via its carboxylic acid. If the other amino acid has its carboxylic acid protected, it can only react via its amino group. Only one combination will result.

The key to protecting groups is that the reaction used to mask one of the functional groups must be reversible. You must be able to take the protecting group back off when it is no longer needed.

Carboxylic acids are normally protected as esters. Esters can be removed via acid- or base-catalyzed hydrolysis (as can amides, but esters are more reactive, being farther up the ski hill). Amines are normally protected as amides. However, we need to be able to remove specific amides here: the ones that mask the amines, not the ones that we have formed to link two amino acids together. As a result, in peptide synthesis, amines are usually protected as carbamates. Carbamates can be cleaved more easily than amides.

Problem CX9.4.

Propose a reason for the relative reactivity of carbamates and amides.

An additional complication in peptide synthesis is that amines and carboxylic acids do not really exist together. Instead, a proton is transferred from the carboxylic acid to the amine, forming a salt. The carboxylate is no longer very electrophilic, due to its negative charge. Because of its positive charge, the ammonium ion is no longer very nucleophilic.

To get around this problem, a number of coupling agents have been developed. A coupling agent can temporarily convert the carboxylate anion into a more reactive electrophile. To do so, it exploits the nucleophilicity of the carboxylate anion. After donating to the coupling agent, the carbonyl compound becomes more electrophilic.

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