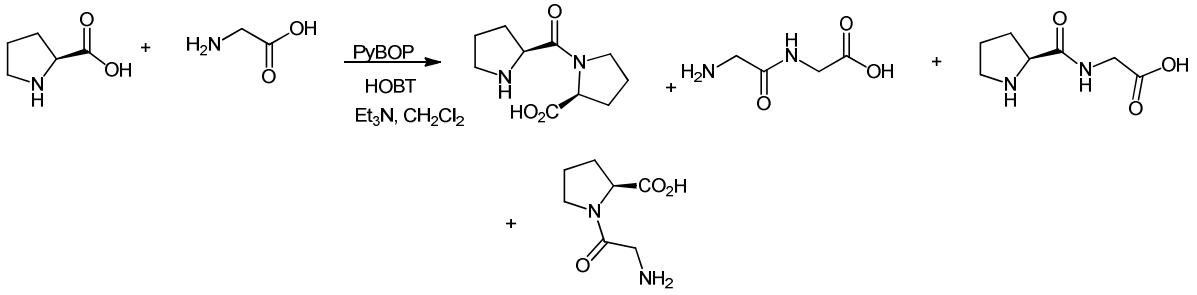
Lecture 18

November 22, 2011

2. Protecting groups: Let's move on now to a discussion of protecting groups.

Protecting Groups:

Consider the following reaction:

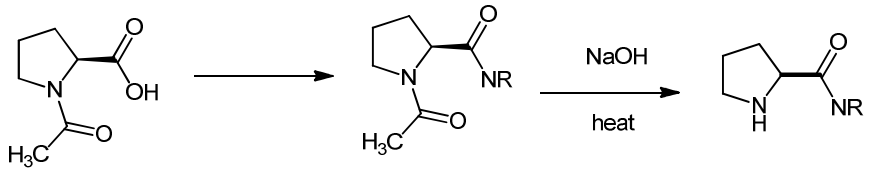


If I have two peptides and no protecting groups, I end up with a mess of four amino acids that can be a real pain to separate.

You want to react one amine group and one carboxylic acid group, and protect the functional groups that you don't want to react.

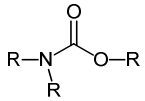
In choosing a protecting group, you need to consider the reaction conditions necessary to deprotect it after the synthesis is over – when you want your free peptide – and whether those reaction conditions are going to harm your peptide in any way.

For example, in the following reaction the acetyl group (COMe) acts as a protecting group for the nitrogen in the five membered ring. Once the free carboxylic acid forms a new amide bond. you can remove the acetyl group. Unfortunately that requires harsh conditions (strong base, and heat) that racemizes the chiral center. BIG PROBLEM.

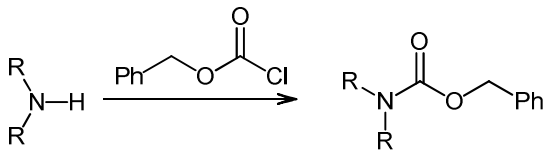


The most common protecting groups are carbamates. We will talk about three of them today.

General structure of a carbamate:

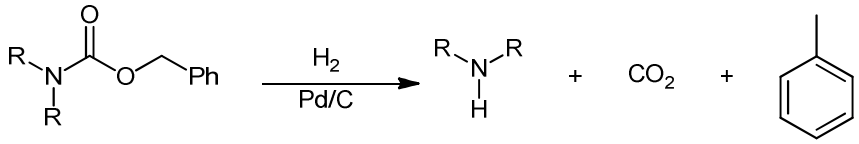


1. Cbz protecting group – carbobenzoxy – you can protect the amine by reacting it with the Cbz chloride



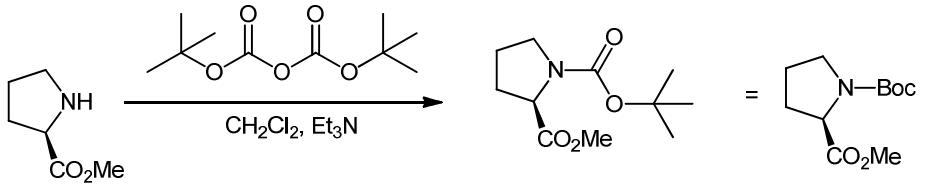
R_2N-Cbz

After the rest of the molecule (represented by the R group) is done reacting, you can take off the Cbz group by hydrogenation –

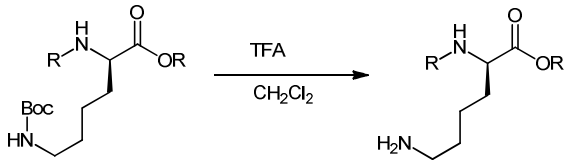


This is a useful protecting group because the side products (carbon dioxide, toluene) are so easily separable.

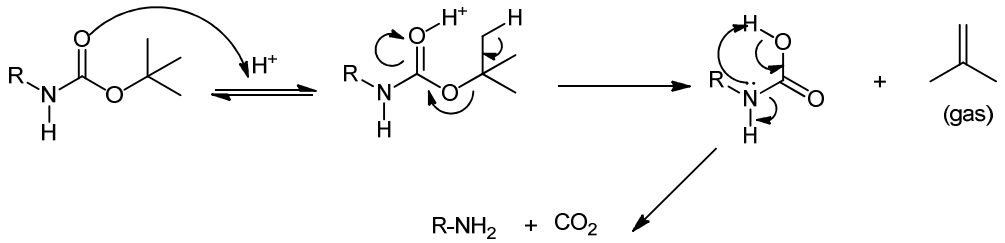
2. Boc protecting group – you protect the amine by reacting it with Boc-anhydride:



And when it's done reacting, the Boc group is removed with trifluoroacetic acid (TFA) (relatively strong acid):

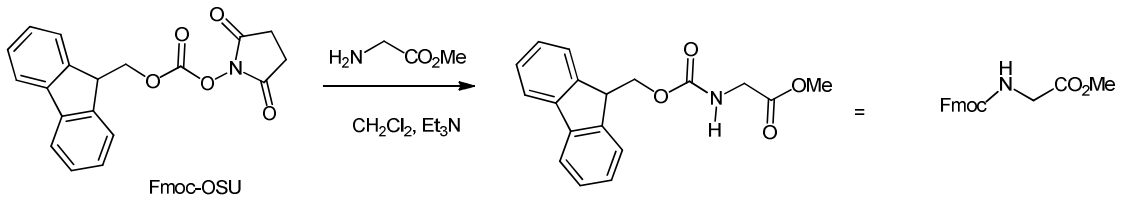


Mechanism of deprotection:

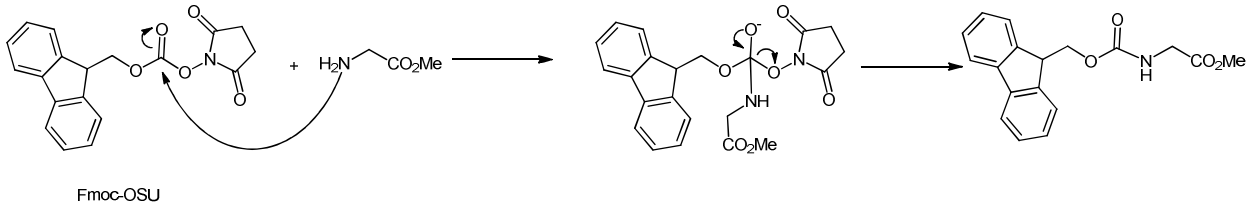


3. F-moc protecting group – abbreviation for 9-fluoromethoxycarbonyl

The free amine is protected by reacting with Fmoc-chloride or Fmoc-OSU:

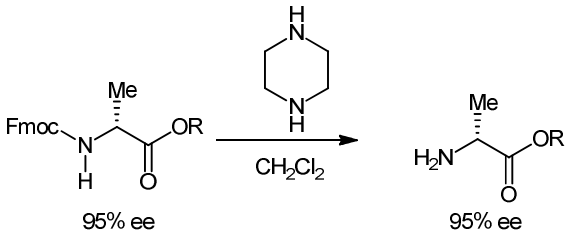


The mechanism for F-moc protection is pretty straightforward:

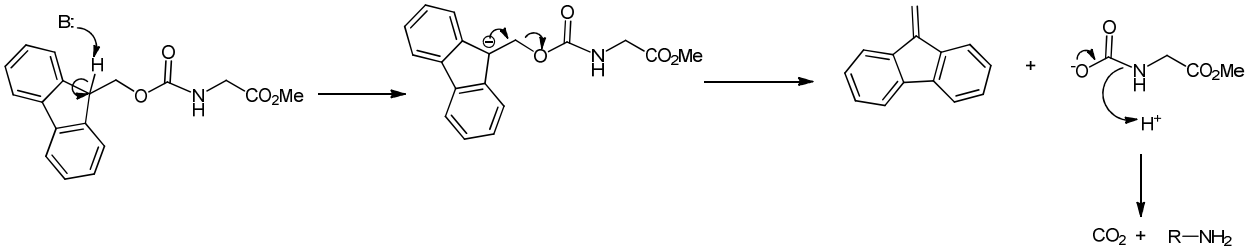


Nucleophilic attack of the amino group, followed by displacement of the succinimide leaving group, to generate a protected amino acid.

Fmoc is deprotected by treating it with a secondary amine base like piperidine.



The mechanism of F-moc deprotection is shown below:

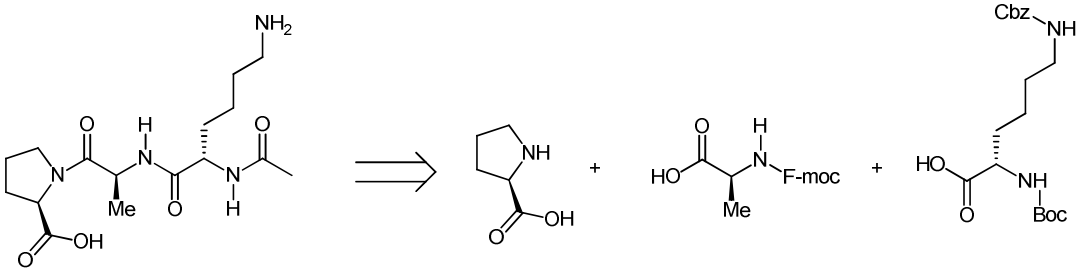


The base attacks the fluorenyl proton – this proton is slightly acidic because when you form the anion, that negative charge can be stabilized by the entire aromatic system. The anion then kicks off the carbamate, which decomposes to lose carbon dioxide and form the desired free amine product.

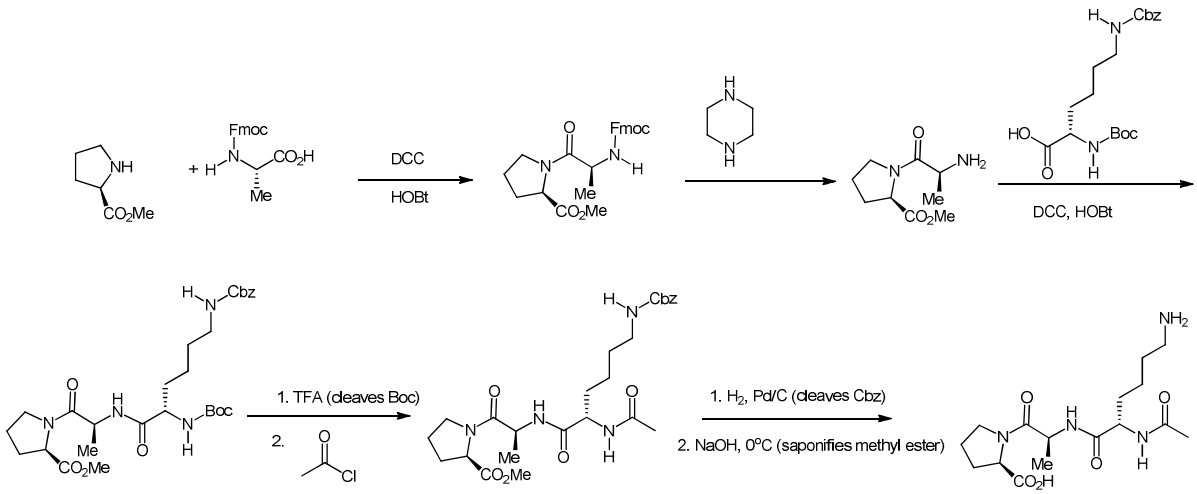
So that concludes the protecting groups for amines. The carboxylic acid also needs to be protected (if it is not the part of the amino acid that you want to react). This is typically done with a tert-butyl ester (or another mild ester).

Let's take a sample peptide and think about how to synthesize it both retrosynthetically and in the forward direction.

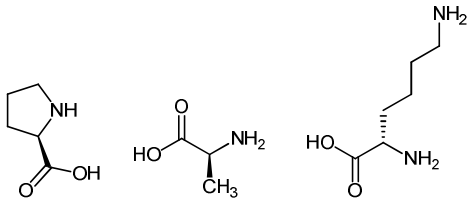
Retrosynthesis:



Forward direction:



Retrosynthetically, you should think to yourself that you need to break an amide bond – between the nitrogen and the carbonyl group – to get back to free, unprotected amino acids as starting materials. In this particular case, you also have an acetyl group (COMe) on the nitrogen, which does not come from an amino acid. Our starting amino acids are proline, alanine, and lysine. The structures of each of these three amino acids are shown below:

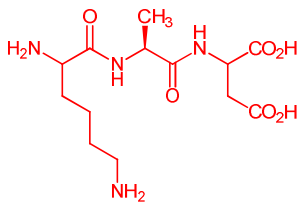


proline alanine lysine

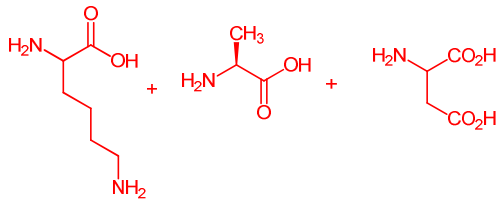
In the forward direction, you should remember to protect whatever functionalities are not reacting at that particular point in time. For example, the CO2H on the starting proline is protected as a methyl group. The nitrogen on the alanine is protected with Fmoc. Once the first coupling step is done, Fmoc is deprotected so that it can react with the next amino acid, lysine. Lysine also has a side chain that needs to be protected – the free NH2 group. In this case you should make sure that the lysine you introduce has different protecting groups on the NH2 of the side chain and the NH2 of the primary amino acid chain. This will allow you to deprotect one and leave the other intact until you are done with the whole reaction sequence. After lysine is attached, you can deprotect the Boc and react it with acetyl chloride to generate the final amide bond. TFA is used to deprotect Boc and NaOH is used to hydrolyze the ester (protecting group on the C terminus of the peptide).

Side note: all peptides have an N terminus (where the free amino end group is) and a C terminus (where the carboxylic acid group is). Peptides are usually grown in the C to N direction.

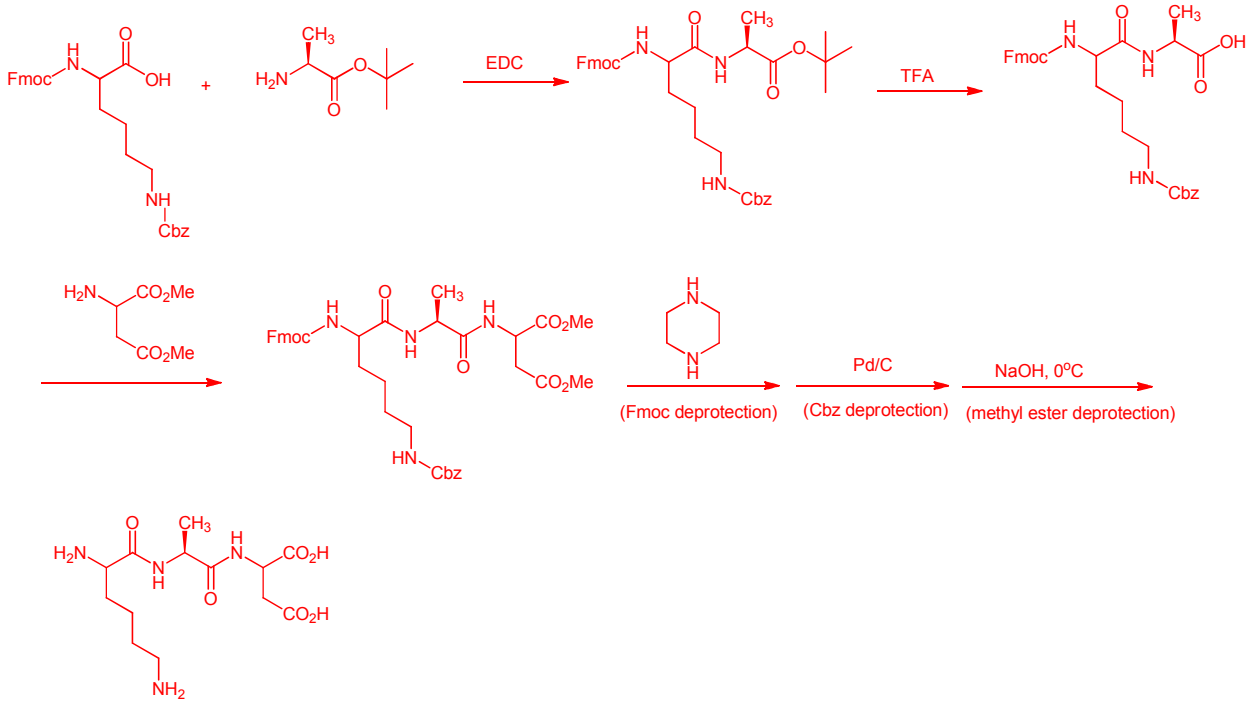
Here's one more practice problem: Design a synthesis of the peptide shown below, including all protecting groups and reagents:



The first step is to figure out what amino acids this is made from, by disconnecting at the amide bond:



And now we can do the forward direction:



We are going to continue our peptide discussion next time. I will post a problem set on peptide chemistry, as well as an opportunity for you to earn a little bit of extra credit in this class.