

## CH 242 EXPERIMENT #5

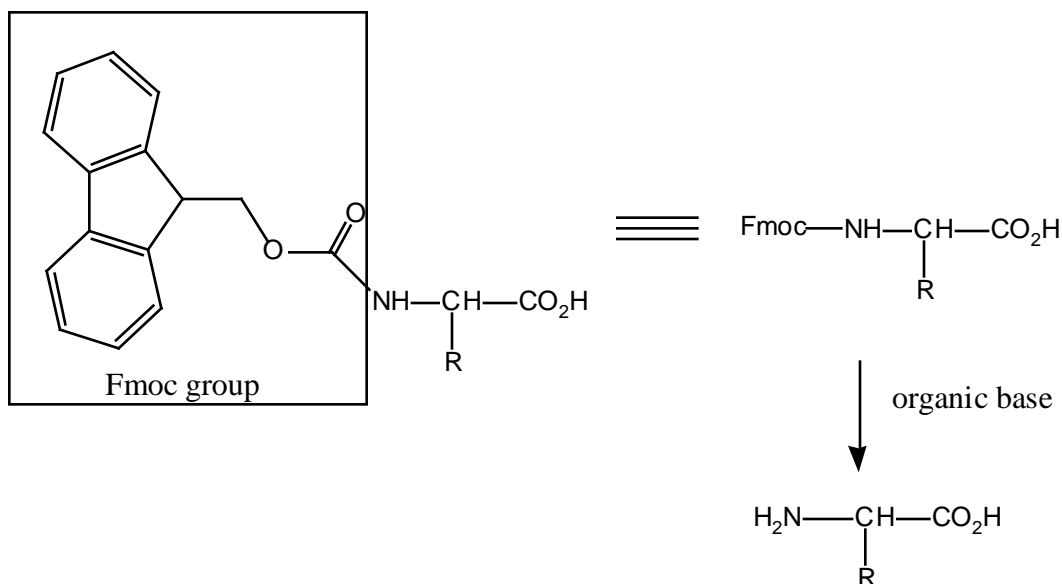
WEEK OF APRIL 1, 2002

### SOLID PHASE SYNTHESIS AND MASS SPECTROMETRIC ANALYSIS OF A FMOC-DIPEPTIDE

#### Background

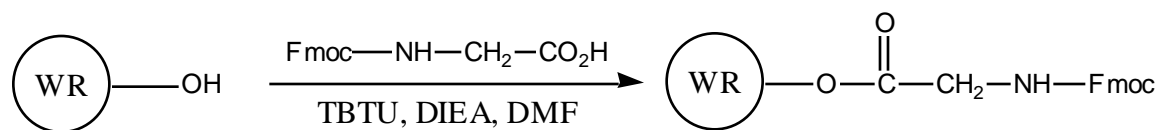
Solid phase synthesis refers to a process in which synthetic reactions are carried out on a solid support. Such an idea was developed by Bruce Merrifield to synthesize polypeptides (a string of amino acids held together by amide bonds) and earned him the Nobel Prize in 1984. Indeed, solid phase synthesis has revolutionized the area of synthetic organic chemistry and has been applied to the preparation of a wide variety of organic compounds including carbohydrates, peptides, and oligonucleotides. Solid phase synthesis typically offers many advantages over conventional synthesis in terms of efficiency as well as convenient work-up and purification procedures. In this experiment, you will prepare a dipeptide protected by a Fmoc group (see below) using a Wang resin as the solid support.

The fluorenylmethoxycarbonyl (Fmoc) group, shown in the box, is used to protect the amino end of amino acids. Amino acids protected in such a manner are known as Fmoc amino acids. The Fmoc group is usually quite stable in acid but may be readily removed by organic bases to release the amino group.



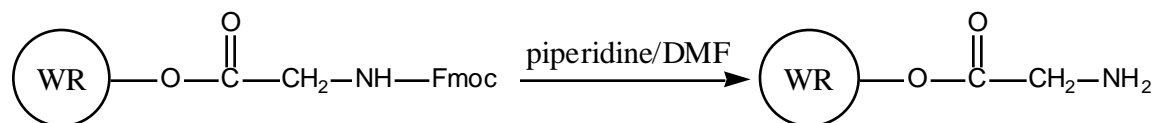
The Wang resin is an inert material bearing primary alcohol functional groups. These OH groups provide convenient "handles" to carry out the desired synthesis. For example, in this experiment we will form an ester linkage using the OH group on the resin and the carboxylic acid group of an Fmoc protected amino acid known as glycine. We will then remove the Fmoc protecting group to release the amino end that we will subsequently use to form an amide linkage with a second Fmoc protected amino acid. Finally, we will cleave the ester group with acid to release the Fmoc protected dipeptide from the resin. The analysis of this Fmoc dipeptide will be performed using mass spectrometry. Procedures for the individual steps are given below. Please consult your lab instructor for structures of key reagents and details regarding mechanisms.

### Step 1: Attaching Fmoc-glycine to the Wang resin through an ester linkage.



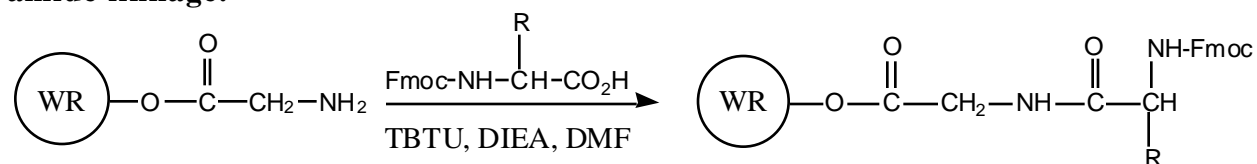
Pour the suspension of resin in dimethylformamide (DMF) into a fritted column after making sure that the stopcock is closed. In a clean test tube dissolve 0.5 mmol of Fmoc-glycine and 0.5 mmol of *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) in 2 mL of DMF. Add 1 mL (containing 1 mmol) of the diisopropylethylamine (IPEA) solution in DMF. Wait for five minutes. In the meantime open the stopcock of the column and drain the DMF into a 125 mL Erlenmeyer flask. Close the stopcock and add the contents of the test tube into the column. Let stand with occasional shaking for forty five minutes.

### Step 2: Removing Fmoc to release the amino group.



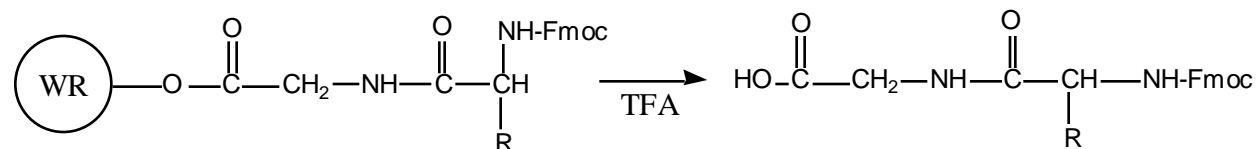
Open the stopcock and drain the solution from the column into the same Erlenmeyer flask that you used before. Add 5 mL of DMF to the column and drain. Repeat with another 5 mL of DMF. Then close the stopcock and add to the column 5 mL of a solution containing 20% piperidine in DMF. Let stand for 15 minutes with occasional shaking.

### Step 3: Attaching another Fmoc-amino acid to the resin-bound glycine through an amide linkage.



In a clean test tube dissolve 0.5 mmol of Fmoc-amino acid and 0.5 mmol of *O*-benzotriazol-1-yl-*N,N,N',N'*-tetramethyluronium tetrafluoroborate (TBTU) in 2 mL of DMF. Add 1 mL (containing 1 mmol) of the IPEA solution in DMF. Wait for five minutes. In the meantime open the stopcock of the column and drain the DMF into the same Erlenmeyer flask that you used before. Add 5 mL of DMF to the column and drain. Repeat with another 5 mL of DMF. Close the stopcock and add the contents of the test tube into the column. Let stand with occasional shaking for forty five minutes.

### Step 4: Cleaving the Fmoc-dipeptide from the resin.

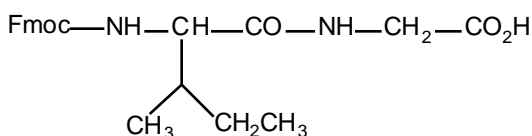
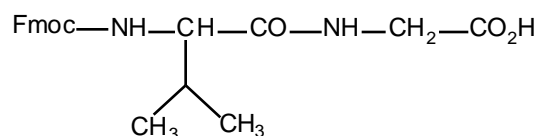
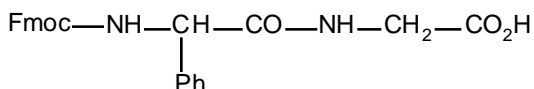


Drain the DMF solution in the column into the same Erlenmeyer flask that you used before. Add 5 mL of dichloromethane to the column and drain. Repeat with another 5 mL of dichloromethane. Finally, close the stopcock, and add 2 mL of dichloromethane to the column. Mix thoroughly

using a Pasteur pipet and transfer a portion of the resin into a 2 mL plastic centrifuge tube. Carefully remove as much of the dichloromethane as possible from the centrifuge tube with a pipet so that only the resin remains at the bottom. To the resin add 20  $\mu\text{L}$  of trifluoroacetic acid (TFA). [*Caution! Trifluoroacetic acid is potentially dangerous. Be sure to seek your lab instructors help in dispensing this acid.*] Wait for 15 minutes and add to the tube 1.5 mL of a 50:50 solution of methanol and water. Analyze the contents of the tube using mass spectrometry.

### PRELAB

- (a) Read section 25.3c in your text for an overview of peptide synthesis.  
 (b) Calculate the molar masses for the following Fmoc-dipeptides.



### LABORATORY REPORT

Your lab report should discuss all aspects of your experimental observations, results and conclusions. Finally, answer the questions below.

#### QUESTIONS

- (a) What is the role of TBTU in steps 1 and 3? Provide a mechanism for ONE of those two steps.  
 (b) Provide a reasonable mechanism for the removal of the Fmoc group by piperidine.  
 (c) Using procedures learned in this experiment, outline a protocol to prepare the following tripeptide.

