**Peptide Day 1**

**Experimental Procedure – Preparation of N-tert-Butoxycarbonyl L-Alanine**

1. Place 0.90 g of L-alanine in a 50-mL round-bottom flask containing a stirbar.
2. Add 5 mL *tert*-butanol and 5 mL 3 M NaOH.
3. Using the 5-mL syringe, transfer 2.5 mL of di-tert-butyl dicarbonate to the round-bottom flask and continue stirring for 45 min at room temperature.
4. Add 10 mL of water to the reaction mixture and transfer the solution to a separatory funnel. Rinse the round-bottom flask with 1–2 mL of water and transfer the rinse to the separatory funnel.
5. Add 25 mL of diethyl ether to the separatory funnel and shake the funnel gently, venting as necessary. Separate the layers and acidify the aqueous layer to pH 2 with 3 M HCl.
6. Extract the aqueous layer with two 10-mL portions of diethyl ether and then wash the combined organic layers with 10 mL of brine. Dry the organic layer over several spatula-tips full of anhydrous sodium sulfate and remove the solid by gravity filtration with a glass funnel.
7. Concentrate the solution to between 4-5 mL. If the solution is a little cloudy, add a spatula tip of sodium sulfate and decant into a new flask.
8. Add approximately 25 mL of hexanes to the ethereal solution, stir the solution with a glass stirring rod, and place the flask in an ice-water bath for 10 min. If no solid precipitates, scrape the bottom of the flask with a spatula until precipitate starts to form.
9. Weigh the recrystallized product, determine its melting point, and obtain an IR spectrum.

**Peptide Day 2**

**Experimental Procedure – Preparation of Methyl L-Phenylalaninate Hydrochloride**

1. Place 1.0 g of L-phenylalanine in a 25-mL round-bottom flask containing a one-stirbar, add 5 mL of MeOH, and begin stirring the mixture.
2. Add 0.5 mL of thionyl chloride dropwise using a syringe. Equip the round-bottom flask with a reflux condenser and heat the mixture, which should be homogeneous under gentle reflux for 45 min. (Note: Set the hotplate to 130 and do not start the 45 minutes until the solution has started to reflux).
3. Allow the reaction mixture to cool to room temperature in air for 5 minutes. The place round bottom flask into water for 10 minutes. Transfer the solution to a 100 mL beaker. Rinse the round-bottom flask with 1 mL methanol and transfer this rinse to the beaker.
4. Place the beaker in an ice-water bath and add 25 mL of diethyl ether. Leave in until precipitate forms (10-15 minutes).
5. Collect the white solid by vacuum filtration and rinse with 25 mL diethyl ether.
6. Weigh the recrystallized product, determine its melting point, and obtain an IR spectrum.

**Peptide Day 3**

**Experimental Procedure Preparation of Methyl N-*tert*-Butoxycarbonyl L-Alanyl-L-Phenylalaninate**

1. Place 0.63 g of methyl L-phenylalaninate hydrochloride in a 150-mL Erlenmeyer flask.
2. Add 10 mL of dimethylformamide and 0.3 mL of N-methylmorpholine to the flask, swirl the flask to mix the contents, and place the flask in an ice-water bath. Label the flask as Solution A.
3. Add 0.50 g of N-tert-butoxycarbonyl-L-alanine in a 100-mL round-bottom flask containing a one inche stirbar. Add 10 mL of dimethylformamide, and then using a 1-mL syringe transfer 0.3 mL of N-methylmorpholine to the flask. Place the flask in an ice-water bath and stir the solution for 5 min.
4. Using a 1-mL syringe add 0.4 mL of isobutyl chloroformate dropwise to the cooled solution in the round-bottom flask and continue stirring the mixture in the ice-water bath for 5–10 min.
5. Transfer Solution A into the round-bottom flask and continue to stir the reaction with cooling in the ice-water bath for 45 min.
6. Add 20 mL of water to the reaction mixture and transfer it to a separatory funnel. Rinse the round-bottom flask with a 30-mL portion of diethyl ether and transfer the rinse to the separatory funnel.
7. Shake the funnel gently, venting as necessary. Separate the layers and wash the organic layer with two 25-mL portions of 1 M HCl, a 25-mL portion of saturated sodium bicarbonate, and a 25-mL portion of brine.
8. Transfer the organic layer to a 125-mL Erlenmeyer flask and add several spatula-tips full of anhydrous sodium sulfate.
9. Decant the solution into a 150 mL beaker and air dry the sample until the ether is almost gone. Add 2-3 mL ether back into the beaker and swirl until the solid has dissolved.
10. Add approximately 15 mL of hexanes to the flask and place the flask in an ice-water bath for about 15 minutes. Use a spatula to scrape all the solid from the bottom of the flask.
11. Isolate the crystals by vacuum filtration and air-dry them.
12. Weigh the recrystallized product, determine its melting point, and obtain an IR spectrum.

**Peptide Day 4**

**Experimental Procedure – Preparation of Methyl L-Alanyl-L-phenylalaninate Trifluoroacetate**

1. Place 300 mg of methyl N-tert-butoxycarbonyl-L-alanyl-L-phenylalaninate in a 25 mL round-bottom flask containing a rice stir bar and add 6 mL of dichloromethane. Begin stirring the solution.
2. Using a syringe add 1.5 mL of trifluoroacetic acid to the flask and continue stirring the mixture for 30 min at room temperature.
3. Transfer the solution to a 100 mL beaker and remove the stir bar. Evaporate most of the solvent by air drying it and then add 4 mL of diethyl ether to the flask. Place the flask in an ice-water bath for 10-15 min to complete the precipitation.
4. Collect the white solid by vacuum filtration and air-dry it.
5. Weigh the recrystallized product, determine its melting point, and obtain an IR spectrum.