Introduction
In this second half of the semester project, you will synthesize a small molecule library using solid-phase coupling strategies. In the past, we had a very specific target molecule that we wanted to synthesize. However, this year you will have the CHOICE in how you assemble the various components and in the process you will create a unique target structure. The various components are as follows:
(a) linker (you can choose the length from our inventory)
(b) amino acids (select from our inventory)
(c) substituted 2-nitrocinnamic acid (that you synthesized in the first sub-project)
(d) isothiocyanates (select from our inventory)

You do not need to have all of these components, but you will need to have at least the substituted 2-nitrocinnamic acid as it serves as the library scaffold. The only other restrictions are that (a) you must split your library at self-selected points in the synthesis such that in the end you create a multi-product library of 6-10 compounds and (b) your library possesses a reasonable amount of structural diversity (achieved by building block selection). An example scheme follows for a 6 compound library:
You must propose a strategy to Dr. Timm by March 4, 2005. If you are so inclined, you may also propose to add a building block and to consider incorporating it into the structure in place of one of the others listed above.

This part of the project begins the week of March 28, 2005.

**Resin washing strategy (to be done after EACH AND EVERY reaction):**
The solvent is drained from the vessel and the resin is washed thoroughly using the following protocol:
1. wash 2 times with reaction solvent (usually DMF) with 1 minute of rotation in between washings.
2. wash 2 times with DCM with 1 minute of rotation in between washings.
3. wash 1 times with THF with 1 minute of rotation in between the washings.
4. wash 1 time with iPrOH with a 1 minute rotation in between.
5. wash 1 time with MeOH with a 1 minute rotation in between.
6. wash 1 time with DCM and the solvent is drained from the vessel.

Complete coupling is determined by Ninhydrin analysis. The resin is stored in the refrigerator in a labeled beaker until the next lab period.

Estimated time for washing = 1 hour

**Representative Coupling Procedures**

(a) **Linker attachment:**
600 mg (*this quantity can be adjusted*) of Wang resin (1.1 mmol/g) is measured out into a medium or large solid phase reaction vessel. The resin is swelled with 5 mL of DMF and is shaken at room temperature for 30 minutes. To the resulting suspension, is added 10 equiv. of CDI (1,1-carbonyldiimidazole). The suspension is shaken for 2 h at room temperature. The solvent is drained from the reaction vessel under vaccum (*using the filtration apparatus*) and the resin is washed thoroughly with DMF. The beads are resuspended in 4 mL of DMF. 20 equiv. of your selected 1,X -diaminoalkane is dissolved in 2 mL of DMF and added to the reaction vessel. The contents are shaken overnight (*usually 8-12 h of reaction time*) at room temperature. Upon completion the solvent is drained and washed according to the procedure above. [Alternatively, the solvent can be drained, rinsed quickly with DMF and washed more thoroughly at a later time.]

Estimated time for this reaction step = first part (3 hours), second part (8-12 hours, most of which is reaction time – maximum reaction time is 4 hours), washing (1 hour)

NOTE: If coupling is INCOMPLETE after 4 hours, drain the solution, wash, and repeat the coupling a 2nd time.

(b) **Splitting (accomplished before each step in which multiple synthons are introduced):**
The resin is suspended in 5 mL of DMF and then split into X number of equivalent fractions (using now the small reaction vessels). The solvent is drained and the vessels can be stored or carried on immediately to a reaction.

Estimated time for this reaction step = splitting (0.5 hour)

(c) **Coupling of fmoc-protected amino acids:**
DMF (1.5 mL) is added to each vessel along with 4 eq. (based upon the mmol of resin in each tube -- now 200 mg per tube -- how many mmol is that?) of your selected fmoc- protected amino acid(s). Add 4 eq. HOBt to the vessel followed by 4 eq. of DIC. The mixture is shaken for 30 min. at room temperature to swell the resin and commence reaction. After 30 min, 2.5 eq. of Hunig's base (diisopropyl ethylamine) is added to each vessel and the entire mixture is left on the rotator at room temperature. After 1 h, the beads are checked via Ninhydrin analysis to determine completeness of reaction. Ninhydrin analysis is repeated each hour until the reaction is complete. Upon completion the solvent is drained and washed according to the procedure above.
[Alternatively, the solvent can be drained, rinsed quickly with DMF and washed more thoroughly at a later time.]

Estimated time for this reaction step = coupling + reaction (2.5-4.5 hours, most of which is reaction time), washing (1 hour)

(d) Removal of an fmoc protecting group:
The fmoc group is removed (deprotection) by diluting each remaining reaction vessel with 2 mL of 20% piperidine in DMF. The reaction vessel is left on the rotator for 45 minutes. The solvent is drained and the resin is washed thoroughly with DMF. Ninhydrin analysis is performed to confirm fmoc deprotection. Upon completion the solvent is drained and washed according to the procedure above. [Alternatively, the solvent can be drained, rinsed quickly with DMF and washed more thoroughly at a later time.]

Estimated time for this reaction step = reaction (1 hour), washing (1 hour)

(e) Coupling of nitrocinnamic acids:
To each of the small vessels is added 1 mL of DMF and the mixture is shaken for 20 min. at room temperature to swell the resin. Subsequently, 2 equiv. DIC and 2 equiv. HOBT are added to each reaction vessel and the contents are shaken for an additional 20 minutes. During this 2nd reaction time, vials (varying number depending upon splitting) are prepared with 2 equiv. of the nitrocinnamic acid, 2.2 equiv. triethylamine, and 1 mL of DMF. The contents of each vial are added to the appropriate reaction vessel and the entire mixture is left on the rotator at room temperature. After 1 h, the beads are checked via Ninhydrin analysis to determine completeness of reaction. Ninhydrin analysis is repeated each hour until the reaction is complete. Upon completion the solvent is drained and washed according to the procedure above. [Alternatively, the solvent can be drained, rinsed quickly with DMF and washed more thoroughly at a later time.]

Estimated time for this reaction step = coupling + reaction (2.5-4.5 hours, most of which is reaction time – maximum reaction time is 4 hours), washing (1 hour)

NOTE: If coupling is INCOMPLETE after 4 hours, drain the solution, wash, and repeat the coupling a 2nd time.

(f) Sn reduction of aromatic NO₂ to NH₂ to allow further coupling:
The contents of EACH REACTION VESSEL are suspended in 1 mL of DMF and the contents are shaken at room temperature for 20 minutes. To each vessel is added 20 equiv. of SnCl₂ and the reaction was allowed to proceed for 48 h at room temperature on the rotator. Upon completion the solvent is drained and washed according to the procedure above. [Alternatively, the solvent can be drained, rinsed quickly with DMF and washed more thoroughly at a later time.]

Estimated time for this reaction step = coupling + reaction (48 hours, most of which is reaction time), washing (1 hour)

(g) Isothiocyanate coupling:
The contents of the vessels are drained and the resin is washed thoroughly as before. The resin is left to dry under vacuum for 2-3 hours. Once dry, the reaction vessels can be stored in the refrigerator in a labeled beaker or carried on to the next part.

The resin in each of the reaction vessels is suspended in 1.5 mL of dry DMF followed by the addition of 20 equiv. of phenylisothiocyanate. The reaction is left to proceed overnight on the rotator at room temperature. Upon completion the solvent is drained and washed according to the procedure above. [Alternatively, the solvent can be drained, rinsed quickly with DMF and washed more thoroughly at a later time.]
Estimated time for this reaction step = drying (2-3 hours) coupling + reaction (8-12 hours, most of which is reaction time), washing (1 hour)

(h) TFA cleavage of compounds from the resin for analysis (done throughout synthesis to verify achievement of synthetic intermediates and at the end of the synthesis to release final products): A SMALL sample of resin from EACH VESSEL is set aside in a small reaction vessel for TFA cleavage and LCMS analysis. EACH sample is suspended in 1.5 mL of a cleaving solution containing 40% CH\textsubscript{2}Cl\textsubscript{2}, 50% TFA, and 10% triethylsilane. The mixture is shaken for 1.5 h at room temperature, at which time the liquid is drained into a vial, washed with the DCM, and the solvent is concentrated on the rotary evaporator or under stream of nitrogen gas.

Estimated time for this reaction step = reaction (1.5 hours, most of which is reaction time), rotary evaporation (20 minutes)

(i) Ninhydrin test for primary amines
1. Remove a small portion of resin beads (10-15) and transfer into a small (GSMS) vial.
2. Add 2-3 drops of Reagents A, B, and C, respectively.
3. Heat the vial on the hot plate for 5 minutes.
4. Evaluate color as follows:
   a. Colorless of faint blue: complete coupling (no free primary amine present)
   b. Dark blue solution/colorless beads: nearly complete coupling (some free primary amine remains)
   c. Light blue solution/dark blue beads: incomplete coupling (many free primary amine remains)
   d. Dark blue solution/dark blue beads: no coupling (all free primary amines present)

NOTES:
(1) Coupling is not always complete – as indicated by Ninhydrin analysis. In the event that coupling is incomplete, a second coupling using the same quantities and reaction conditions is recommended.
(2) You are expected to acquire complete characterization of all synthetic intermediates as well as products which includes the following information:
   a. Mass/% yield (final compounds ONLY)
   b. HPLC (for purity)
   c. APCI-MS
   d. \textsuperscript{1}H-NMR
   e. \textsuperscript{13}C-NMR
   f. Ninhydrin analysis

Useful References:
(a) Available through Dr. Timm:
   - *Advanced ChemTech Handbook of Combinatorial, Organic, and Peptide Chemistry* (useful for general procedures for coupling and cleavage as well as listing of key references and various reactions that can be employed in solid-phase organic synthesis)

(b) Available through the Wilmot Library:
   - *Combinatorial Chemistry* by Nicholas K. Terrett
   - *Combinatorial Chemistry: Synthesis and Application* edited by Stephen R. Wilson, Anthony W. Czarnik
   - *Stimulating Concepts in Chemistry* edited by Fritz Vögtle, J. Fraser Stoddart, Masakatsu Shibusaki
   - *Journal of Combinatorial Chemistry* (available through ACS web editions)