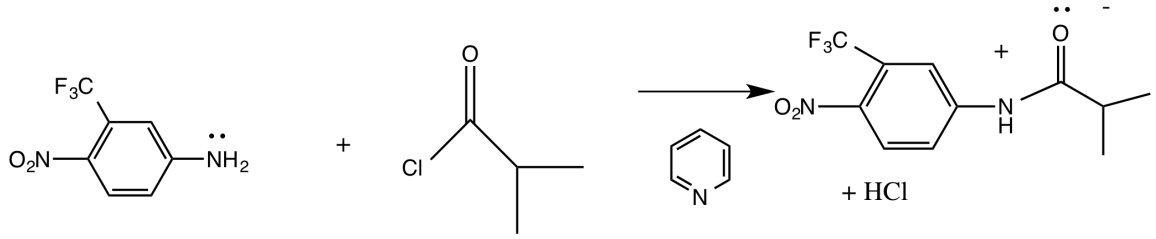
Synthesis of Flutamide[1]

Flutamide is an anti-androgen therapy that has long been used to treat prostate cancer. [2] It is still occasionally used today! In preparation for this lab and for your own edification and enjoyment, you should read up on the web about flutamide, its use in cancer treatment and its mechanism of action.

The synthesis of flutamide is based on the adaptation [1] of two literature synthesis [2,3] that are rooted in carboxylic acid derivative chemistry. The idea here is that if a carbonyl has a reasonable leaving group, a nucleophile can attack the carbonyl (delta plus site). When this occurs, the pi electrons that are initially transferred to the oxygen will flip back down and kick out the leaving group, resulting in a net substitution and the retention of the carbonyl group. These reactions are not reductions. If you calculate the oxidation state, you will note that the attacked carbon retains a +3 oxidation state . This concept is demonstrated below in general and for flutamide, specifically. This can be studied in your text book - you are encouraged to you use the text index. You can also read any of the references given below .

Overall reaction:

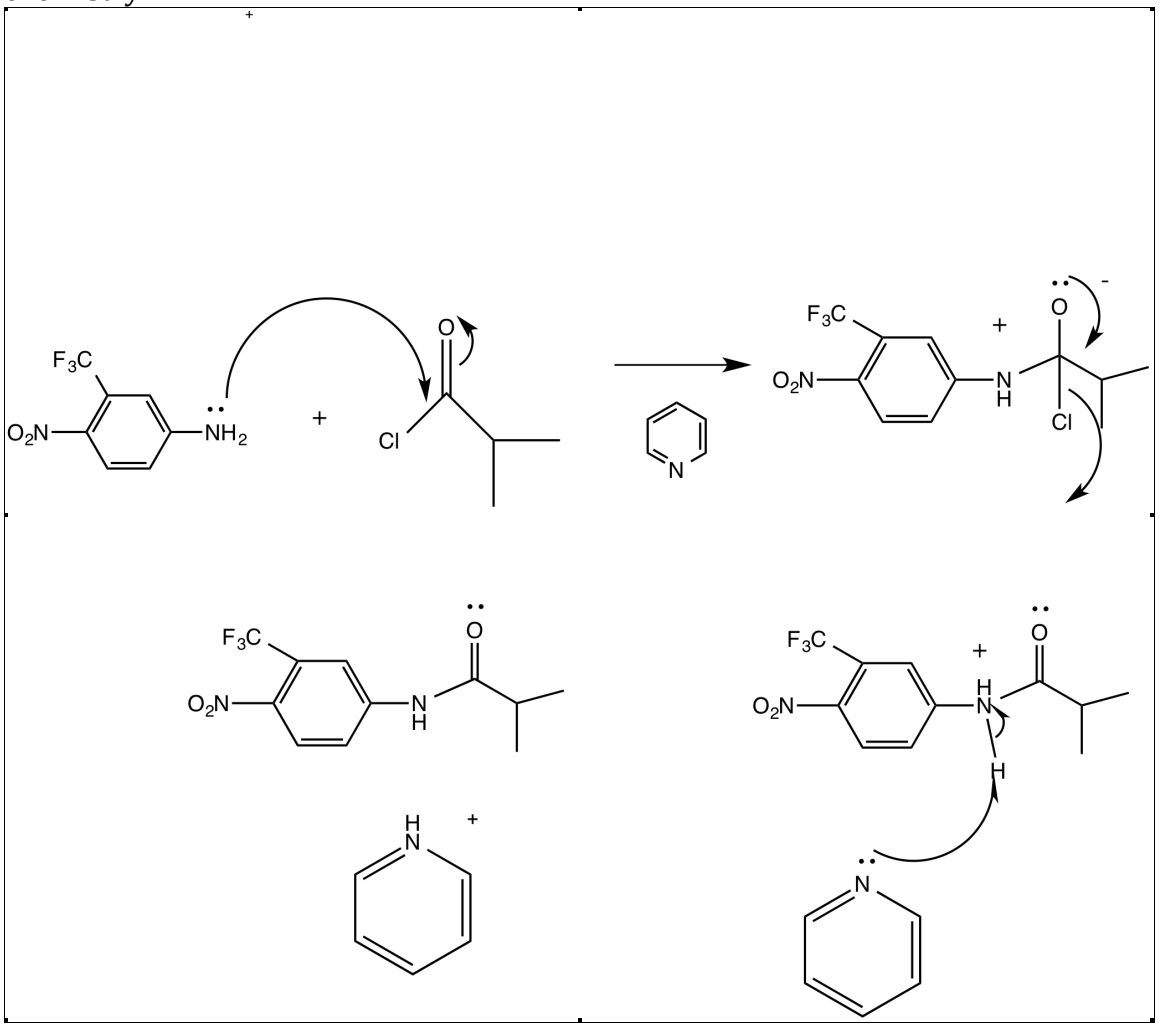


Note: this is a net substitution reaction.

Generalized reaction of carboxylic acid derivative with "good leaving group" and nucleophile:

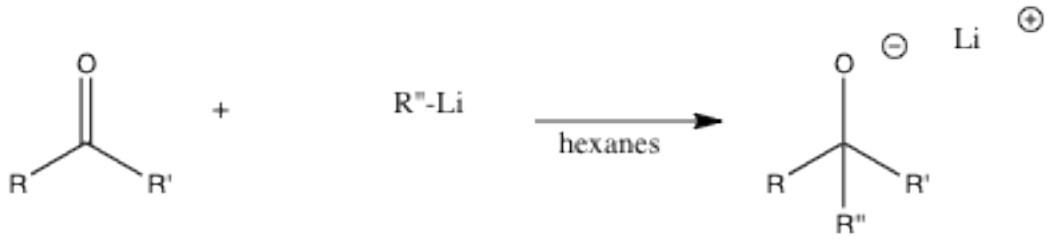
The "flip"

Mechanism for Flutamide synthesis an example of carboxylic acid derivative chemistry.



You might want to note that this chemistry is distinct from Grignard and organolithium chemistry or hydride-type chemistry in that in these reactions, there is no leaving group and the pi electrons are transferred to the oxygen. In Grignard/hydride reactions, the electrons do not flip down, but rather "stick" on the oxygen, after which they are protonated, resulting in an alcohol. This is shown in general, below. Again, if you need to you can refer to you text.

The “stick”



We have demonstrated two major pathways – the “stick” and the “flip” - these are not official terms. which is conjugate addition.

Thin Layer Chromatography – A Brief Description and General Instructions

1. Obtain a Thin Layer Chromatography (TLC) sheet from the desiccator. This sheet will be about the size of a playing card, but much thinner. One side is a very thin coating of silica gel (a very polar stationary phase) that is in a binder. The silica gel is impregnated with a fluorescent compound that will glow green or orange depending on the compound impregnated. When exposed to UV light, the plates will look sort of like those glow sticks and necklaces kids have in the summer. One side is white and sort of rough, the back side is plastic or aluminum. You will do your work on the rough side.

2. You should draw a line with a pencil very lightly about 1 cm up from the lower edge of the TLC plate. This is the origin. See the diagram below.

3. Preparation of standards. Take a spatula tip of each of the starting materials in a vial and add about 1 mL of dichloromethane. This is sort of an estimate of the solid and solvent and estimating is good and OK. You should also make a small solution of your reaction mixture/crude product. If it is liquid, you should take a drop or two and dissolve it in about 1 mL of dichloromethane. Again, the solutions are just approximate. The goal is to study the components of the reaction vs. the starting materials. New spots observed after elution and visualization indicate product, whereas spots seen at the same location as the starting material standards indicate incomplete reaction.

4. The standards and the reaction mixture should be spotted on the TLC plate as indicated on the diagram below. The idea is to use a different capillary pipet (these will be available in the lab) for each compound. You dip the capillary into the solution being spotted and it will enter the capillary via capillary action (no pun intended) and then it is a good idea to blot it a bit on a paper towel so you don’t get a big blob of liquid on the end of the capillary. Then, lightly spot the solution on its mark on the TLC plate. The idea is to

spot it and get the smallest spot possible. Then reapply it several times going for a small concentrated spot. You need to let the dichloromethane evaporate between each application. You will do this for the standards and the reaction as diagramed. It may take you a couple plates to get the hang of it. It is important for all members of your group to get the hang of this technique.

5. Realize it is really important to spot about a cm up from the bottom of the plate, to keep the spots about a cm apart and to spot in from the edge of the plate about a cm as diagramed.

6. **Preparing the Eluting chamber.** We will use a makeshift eluting chamber that will be either a 250 mL or 400 mL beaker with about a 0.5-1.0 cm of dichloromethane (if you are dissatisfied with your TLC results, meaning the Rf values are too close). You need to use a piece of filter paper as diagramed below to saturate the beaker's atmosphere with the eluting solvent. Use a watch glass to cover the chamber.

7. **Eluting the TLC plate.** Dip your TLC plate in the solvent. It is important that the solvent does not immediately touch your spots. If it does, you need to adjust the level of the eluting solvent or spot a new plate. The former is preferable. The plate should be dipped, but also resting against the wall of the beaker. The back side toward the beaker, but on the opposite side of the beaker from the filter paper. The solvent rises up the plate by capillary action. It will take about ten minutes. In this process, there is a competition for the compounds by the silica gel on the plate (very polar – the stationary phase) and the solvent system (varying degrees of polarity, but less polar than the stationary phase – this is the mobile phase). The compounds are in equilibrium between the stationary phase (the silica) and the mobile phase (the solvent). If the compound is extremely polar it will spend most of its time interacting with the silica gel and it won't move much. If it is of very low polarity (provided it is soluble in the eluting solvent), it will spend most its time in the solvent and will move way up the plate. Compounds move to varying degrees depending on their polarity. Compounds that are the same, will elute to the same point on the plate, so your standard lanes will help you identify compounds in the reaction mixture.

8. When the solvent is about a cm. from the top of the plate, you should remove the plate and lightly mark the level of the solvent. This is called the solvent front and its level is significant. Let the solvent completely evaporate off in the hood. The spots are visualized (most of the compounds we are working with are white and are not readily visible) with a UV lamp. Be careful not to expose your hands or your neighbors to the UV lamp. The background will fluoresce as previously described and your compounds should show up as blue or black spots in the background. Lightly circle them with a pencil.

Procedure:

This reaction will be done in groups of two or three and written up as a group.

1. Obtain a clean, dry, two dram vial with a pressure releasing cap. Also obtain a micro stir bar (please keep close guard on this stir bar – return it to your instructor or TA at the end of lab). Weigh out 0.10 gram of 4-nitro-3-trifluoromethylaniline and add this to the 2 dram vial. Be extremely careful and only use the spatula provided. Do not waste this very expensive material. Add the stir bar and cap the vial with the pressure releasing cap.

2. Using only the syringes provided, add 2 mL of pyridine through the septum cap to the same vial. Ask your TA or instructor about how to remove the pyridine from the bottles with a syringe. After adding the solvent (pyridine) you should observe a yellow color. If you are curious use your text index to find the section in your text where the origin of color in most organic molecules is described.

3. Place the solution in an ice bath for five or ten minutes.

4. Using the Eppindorf pipet provided, add 100 microliters of isobutyryl chloride . Watch out, this reagent is a lachrymator (Why? Consider the reaction of the reagent with the water that is found in your eyes). It must be measured in the hood. You will syringe this material drop wise into your reaction. The reaction occasionally turns red at this point, but this color is rarely observed and not necessary for success.

5. The vial should be placed in the pie reactor which will be set at 98 degrees celcius. Check to be sure it is stirring.

6. While waiting run a TLC of the starting materials for the reaction. Small vials of diluted standards can be made by taking a spatula (a pinch very little) of the amine and dissolving it in a vial with about ten to twenty drops of dichloromethane. A drop of isobutyryl chloride can be dissolved in another vial with ten to twenty drops of dichloromethane. Conserve materials and share the standards. The TLC plates will be available in a dessicator and you should review running TLC from last semester. Elute the TLC plate in dichloromethane. Visualize the plate with the UV lamp. This will serve as a great reminder of last semesters skills.

7. After thirty minutes in the reactor, remove your vial and let it cool. Remove a drop of the reaction and add it to ten to twenty drops of dichloromethane.

Run a TLC of this reaction mixture vs. the standards prepared earlier. Do you see the emergence of a new product? Is the new product colored in visible light? How is it different from the starting material amine.

8. If there is significant amount of the amine left (very obvious what color is it in visible light?) put the cap back on tightly (it should already be on) and ptu it back in the reactor for another thirty minutes. Test it by TLC again following the same procedure. At some point the reaction stagnates in regard to progress.

9. Prepare a small beaker with thirty grams of ice (this can be quite approximate) and pour the reaction mixture into the ice. What is happening in this step? Can you write the chemistry? Be curious, not afraid. Every reaction has many questions to be asked and further research that can be done to answer them. Always ask questions. Rinse the vial in the hood with a small amount of water and add this to the beaker.

10. Allow the reaction to stir until all the ice melts. Your product should precipitate out. If it does not or if it looks difficult to isolate via micro vacuum filtration, the product will be isolated by extraction.

11. If you have a solid isolate it by micro vacuum filtration. Be sure to build a trap. Stick the micro filter paper down with a small amount of water and when the solid is isolated, rinse it with a small amount of water.

12. If your product is not precipitating or is difficult to isolate, it can be extracted. Transfer your liquids to a small extraction funnel and extract the mixture three successive times with ten mL of dichloromethane. The combined dichloromethane extracts can be dried over a small amount of sodium sulfate and then evaporated to dryness. It is fine to just leave the capped solution in your locker.

Depending on time, the rest of the lab can be done next week. NMRs can only be run next week.

13. The solid is re-crystallized using micro techniques from toluene. Review recrystallization by viewing the available video. Think about the recrystallization of your Diels-Alder product. Micro technique means that you should use your smallest Erlenmeyer and add 1 mL increments of hot toluene to dissolve the compound.

14. If you compound was extracted it will probably be evaporated by this time. It should be a solid and can be recrystsallized as in 13 above.

15. To earn your way onto the NMR and running spectra in general, run a TLC plate of your isolated crystalline material in dichloromethane.

16. When you demonstrate you have a pure sample by TLC – you may have to recrystallize again – then, you can run your spectra.

17. Run a thin film IR, proton NMR, GCMS and possibly a carbon NMR.

The laboratory write up for this lab will be limited to the following.

Brief Introduction: The purpose of the lab, any modifications of the procedure and a literature reference for the procedure.

The main reaction

The reaction mechanism. Though it is given, work through it, think about it, just don't copy it.

The mass of product obtained:

The percent yield: show calculation

The expected melting point: Give reference to any literature comparison.

The melting point range. Yes it should be a range.

The IR and Interpretation. Give reference for any literature comparison.

The proton NMR and interpretation: Give reference for any literature comparison.

The GCMS trace and the resulting molecular weight of the product from the GCMS.

The carbon-13 NMR interpretation of any carbon-13 run in the section.

A brief discussion of yield, purity and identity. This should be a one or two pages. NO MORE. The most likely issues with this lab are – incomplete reaction – how will this effect yield, purity and identity. How is this problem manifested in your results, including spectra? Please refer to the specific Nerz-Notes that will be posted this week.

This brief report will be turned into your TA as per the on-line schedule. One report per group. All materials should be placed in your TA's folder.

The learning in these labs should come from the direct correlation with lecture, lab lectures should be studied and the background should be studied, the biology

should be read and understood. You should gain much more about spectral interpretation. Note how references are presented as citations. You will do them this way in your final paper. It is expected that you will review procedures you have not done in a while, even if that means reviewing past videos.

See you in class and office hours.

References

1. Ryan G. Stabile and Andrew P. Dicks *J. Chem. Educ.*, **2003**, *80*, 1439.

2. http://en.wikipedia.org/wiki/Flutamide#Use_in_prostate_cancer,

3. Baker, J. W.; Bachman, G. L.; Schumacher, I.; Roman, D. P.; Tharp, A. L. *J. Med. Chem*. **1967**, *10*, 93–95.

4. Neri, R. O.; Topliss, J. G. Substituted Anilides As Anti-An- drogens. U.S. Patent 4144270, March 13, 1979.