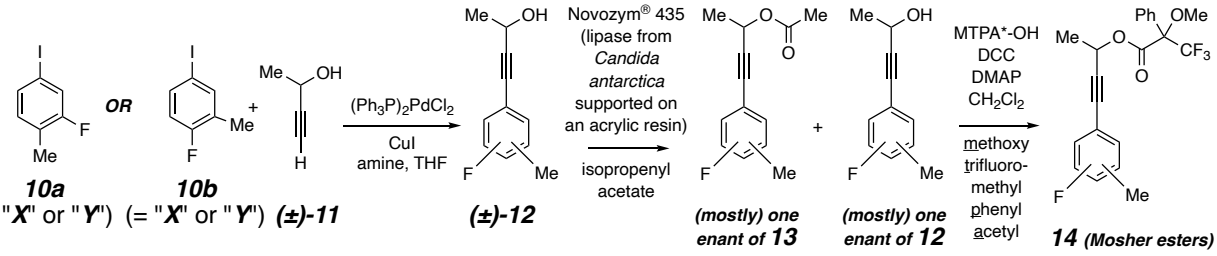
Experiment 4. Transition Metal- and Enzyme-Catalyzed Organic Reactions

Palladium Coupling, Lipase Kinetic Resolution, and Mosher Ester Analysis

Reaction Sequence



10a (= "***X***" or "***Y***") ***10b*** (= "***X***" or "***Y***") ***(±)-11*** ***(±)-12*** *(mostly) one enant of* ***13*** *(mostly) one enant of* ***12*** ***14*** *(Mosher esters)*

Palladium-catalyzed Arylation of Terminal Alkynes

Introduction:

The coupling of an aromatic halide and a terminal alkyne (R-C≡C-H) using Pd(0) catalysis has been extensively developed in recent decades and is one of the simplest methods available for this transformation. Variations of this process are known for alkenes, organozinc reagents, and organoboron species. *Twelve years ago (2010), on the day before this experiment was discussed here in the 2312 lecture,* Richard F. Heck (Delaware), Eiichi Negishi (Purdue), and Akira Suzuki (Hokaido) were announced as the winners of the 2010 Nobel Prize in chemistry; they were recognized *"for palladium-catalyzed cross couplings in organic synthesis."* The analogous coupling with a terminal alkyne is known as the Sonogashira (or Castro-Stephens) reaction. You will carry out this coupling using either 4-iodo-2-fluorotoluene (**10a)** or 5-iodo-2-fluorotoluene (**10b**) with a racemic sample of (±)-3-butyn-2-ol (**11**). This will generate a *racemic mixture* of the alkynol **12**. You will then use a lipase (an enzyme with esterase activity) that will preferentially catalyze the transesterification reaction of one enantiomer of **12** with isopropenyl acetate [CH_2=C(Me)OCOMe] to provide the acetate ester **13** (predominantly of one configuration) and the unreacted alcohol **12** (predominantly of the opposite configuration). You will separate these two compounds and then perform a *Mosher ester analysis* on the recovered alcohol to determine the absolute configuration of the less reactive enantiomer of **12**.

Experiments:

$Pd(PPh_3)_2Cl_2$ catalyzed coupling of an aryl iodide (10a or 10b) with alkyne 11[1]

Start with 2.0 mmol of one of the aryl iodides **10a** or **10b**. One of the TAs has provided on your bench a vial labeled as "**X**" or "**Y**", but not otherwise identified (that is, **X** could be either **10a** or its isomer **10b**, likewise for **Y**). This vial contains 275 microliters of the aryl iodide. Use ca. 2 mole percent (i.e., 0.02 equivalents) of the (precious/expensive) Pd catalyst and ca. 4 mol% of CuI. Use either diisopropylamine ($^{i}Pr_2NH$) or triethylamine (Et_3N) as the amine component of the solvent mixture; I recommend use of a cosolvent mixture of the amine and anhydrous tetrahydrofuran (THF) having a composition between 15 to 30 vol% of amine in THF. Once the catalysts and aryl idodide **10** are mixed, allow this solution/suspension to stir for 5-10 minutes and flush (or evacuate and refill) the headspace with nitrogen to replace most of the air (oxygen). Add 1.5 molar equivalents of alkyne **11**. If you observe formation of a solid precipitate within a few minutes at ambient temperature (this will be the ammonium iodide byproduct and serves as an indication that reaction is progressing reasonably rapidly), continue to stir the mixture at room temperature and monitor the reaction progress by TLC. If not, heat the reaction mixture in a warm water bath that has been pre-equilibrated to ca. 60 °C. Use of a tightly capped, screw-capped culture tube will minimize evaporative loss of solvent. Again, monitor the reaction progress periodically by TLC. The very non-polar aryl iodide **10** will be converted to the more polar alcohol **12**. Reflush the headspace with N_2 each time you take an aliquot and before replacing the screw cap. If the reaction progress appears to have stalled (i.e., stopped short of full consumption of the **10a** or **10b**), add a small amount of additional alkyne.

A convenient workup for this reaction mixture consists of removing ca. 90% of the reaction solvent on a rotary evaporator, adding several mL of EtOAc to the residue, and filtering the resulting suspension/slurry through a small plug of silica gel (ca. 5 cm height in a dispo pipet). This should remove most of the ammonium salts, CuI, and palladium species. [Note: Use of low load levels (≤2-4 mol%) of the Pd and Cu catalysts has advantages in purifying the product of this reaction.] Concentrate the filtrate from the dispo pipet/SiO_2 filtration and purify that residue by flash chromatography (not MPLC, please), using the proper elution solvent that you identify by tlc analysis. Characterize the purified product so obtained by the usual battery of spectroscopic methods. For another example of a publication that contains a typical experimental procedure, see reference 2. Please place all silica gel solid waste used in both the small column clean-up and the flash column in a separate waste container labeled for "palladium waste." This can/should be the same container in which we earlier put the Pd/C on Celite® catalyst following the hydrogenation of pulegone.

Structure of "X" and "Y".

You will be able to determine the structure of your starting fluoroiodotoluene (i.e., whether it was **10a** or **10b**) from a critical analysis of certain ^1H NMR spectra. In particular, the coupling constants will be quite informative. See Question #2 for a problem that you should solve first; this will give you experience in doing this kind of analysis, where coupling to the fluorine atom is also present.

For **X**, the ^1H NMR spectrum in C_6D_6 (deuterobenzene) is interpretable.
For **Y**, the ^1H NMR spectrum in DMSO-d_6 (deuterated dimethyl sulfoxide) is interpretable.
These two .mnova files have been uploaded to the website, so you do not need to record these spectra.

Please add a paragraph at the end of your report where you describe the logic you have used (i.e., your coupling constants analysis) to reach your assignment of the structure of your **X** or **Y**.

Enzyme-catalyzed transesterification of (±)-12 with *iso*-propenyl acetate

I have looked up exemplary procedures using Reaxys for carrying out this transesterification reaction. Many variants exist. One at this citation (ref. 3) uses vinyl acetate (instead of isopropenyl acetate) as the solvent. Others use a cosolvent of various hydrocarbon solvents (toluene, hexanes, etc.) with the alkenyl acetate. We will use a commercially available lipase called Novozym® 435[4] (lipase acrylic resin from *Candida antarctica*, recombinant, expressed in *Aspergillus niger*), which comes in powdered form (the reagent is actually the enzyme immobilized/deposited on an inert acrylic resin). It's activity is specified to be >5,000 Units/g.

Use only ~25 mg of the Novozym® 435 catalyst in your reaction (it is expensive), regardless of how much **(±)-12** you use. Use the nutator to agitate your reaction suspension; *do not magnetically stir the mixture because that can denature the enzyme, destroying its effectiveness as a catalyst*. GCMS is the best method for monitoring the progress of this reaction (note that the acetate has about twice the sensitivity or detector response as the alcohol and, recall, you are looking for ca. 50% consumption of the alcohol). Filter or decant the reaction solution away from the Novozym® powder, once you have deemed the reaction to be ca. 50% complete. Separate the acetate ester product **13** from the unreacted alcohol **12** by flash chromatography or MPLC, whichever you like and find up to the task. Characterize your sample of pure acetate ester product **13** in the usual fashion. Verify (^{1}H NMR and GCMS properties) that your recovered alcohol **12** is the same compound, although now enriched in one enantiomer, as that with which you started. There is no need to take its IR spectrum.

Mosher ester analysis to determine the absolute configuration of the unreacted 12

First, read reference #5. You can find a link to this paper at my group's "Older Publications" website (http://hoye.chem.umn.edu/content/older_publications); search for "Mosher ester" and look for the 2007 publication.

Plan to prepare either the *R*- or the *S*-Mosher ester using *N,N*'-dicyclohexyl carbodiimide (DCC) as the coupling agent. I will announce teams of (2-3) partners where all have started with the aryl iodide **10** having the same substitution pattern (i.e., "**X**" or "**Y**"). Decide which one of you will make the *R*- and which of you the *S*-Mosher ester. You will be sharing your NMR data with each other. Use your collective data for the *R*- and the *S*-Mosher esters and deduce the absolute configuration of the recovered alcohol **12**. See "Box 1" in reference 5 for a model of how to report the data from your Mosher ester analysis.

Plan to derivatize ca. 10 mg of your sample of recovered alcohol **12**. This will be the smallest scale reaction that you will perform all semester. Part of the reason for this stems from the fact that the Mosher acid derivatizing agent is rather expensive [ca. $180 per gram (!) from Aldrich]. But it is also good for you to gain experience working on this type of mini-scale reaction.

Plan to perform the reaction in your smallest size culture tube or in a 1 dram, screw-cap vial. Make sure you have a teflon-lined cap that fits your vessel snuggly so that the solvent does not evaporate. Combine the alcohol **12**, DCC (2.0 equivalents of the DCM stock solution in the hood), and the proper *R*- or *S*-enantiomer of the Mosher acid (2.0 equivalents) in the tube. You should also use 15 mol% (0.15 molar equivalents) of 4-dimethylaminopyridine (DMAP) as a base catalyst; on this scale, that translates to 1.0 mg of DMAP. To accurately deliver that small of an amount of the DMAP, you will use the concept of a stock solution. Weigh out ca. 10 mg of

DMAP into a test tube. Add enough methylene chloride (DCM), the eventual reaction solvent, to arrive at a concentration of 2 mg of DMAP per 1 mL of DCM. For example, if you happen to weigh 11 mg of DMAP, add 5.5 mL of DCM. Make sure this solution is mixed to homogeneity. Finally, deliver 0.5 mL of this stock solution to the reaction vessel. That aliquot will contain 1.0 mg of DMAP. It is particularly important to keep reaction mixtures relatively concentrated (i.e., using small solvent volumes) when performing small-scale experiments. Cap the tube and *gently* agitate the contents to achieve homogeneity. After that it is not necessary to stir the reaction mixture any longer. Monitor the reaction progress by tlc. It may (?) be done in an hour or two but there is no harm in leaving the mixture incubate for a day or longer. Once you judge the reaction to be complete, add 3 mL of Et_2O and 2 mL of 10% aq. HCl to the culture tube or vial. Recap and shake to effect a mini-separation. Withdraw the top ether layer. Dry it with a small amount of $MgSO_4$ in a small Erlenmeyer flask or a test tube. Filter (cotton plug in a disposable pipet) and concentrate the filtrate. Record a ^{1}H NMR spectrum of this crude product mixture.

Purify your Mosher ester by a mini-flash column (pipet column) or MPLC using the smaller diameter column (the attenuation should be set to, probably, 8x-16x on the RI detector because you need that extra sensitivity to ensure the detector will "see" the relatively small amount of materials coming through the column; ask a TA for help in adjusting the attenuation). Collect GC/MS, IR, and ^{1}H NMR data for your pure ester. We will *not be evaluating your yield* of purified product for this transformation, because it is challenging, at least for new investigators, to get efficient mass recovery when working with such small amounts of material. Provide the spectral (MS, IR, NMR) data and write up the procedure in the usual way. In addition, provide a table of the comparative NMR chemical shifts for the *R*- and *S*-Mosher esters, similar to the one shown on page 2455 of ref #5. Finally, give a short discussion (3-4 sentences) of how you have used those data to deduce the configuration of the more reactive enantiomer of alcohol **12**.

Lab Report Questions (Please answer in your own words):

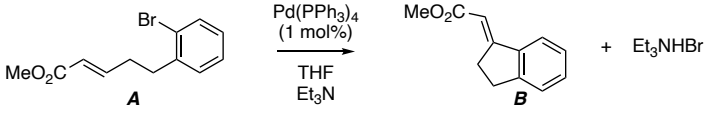
1 (5 pts). How many unique positional (or constitutional) isomers exist for benzene derivatives having two bromine two are chlorine substituents (i.e., derivatives having the formula $C_6H_2Br_2Cl_2$)? Draw them.

2 (12 pts). A TA once collected a proton (**Bromofluorotoluene1HNMR.fid**) and a carbon (**Bromofluorotoluene13CNMR.fid**) NMR spectrum of an isomer of a bromofluorotoluene. These spectra, recorded at 200 and 50 MHz, respectively, are available for download from the course website. The *substitution pattern* of the compound that gave rise to these spectral data is *different from* that of either of the starting iodo analogs **10a** or **10b** that you will use for the first reaction above. However, by interpreting the spectra in each of these .fid files, you will learn valuable skills of analysis that you will use in unraveling the spectral data of each of your products in this experiment. Print a copy of these ^{1}H and ^{13}C NMR spectra and interpret each spectrum directly on that printout. Deduce and label each resonance in the proton NMR spectrum with the value of the coupling constants it contains. *Turn this in with your report as your answer to this question*. It turns out that ^{19}F is an NMR-active nucleus with spin ±1/2 (the same as ^{1}H or ^{13}C). Its natural abundance is >99%. It couples to nearby protons and carbons and you will need to take this into account in your interpretations. Refer to the "Table of Coupling Constants and Chemical Shift Data" on the following page for guidance. *Working through this problem early* in the stage of your doing Experiment 4 will *significantly help* in your interpretation of the NMR spectra you collect for your three products **12**, **13**, and **14**.

3 (2 pts). Why is ^{13}C NMR spectroscopy considerably less sensitive than ^{1}H NMR spectroscopy? Why do you not observe coupling between two adjacent (i.e., directly bonded) carbon atoms?

4 (6 pts). Explain the origin of each of the following peaks present in the mass spectrum of 1,2-dibromobenzene: *m/z* 234, 235, 236, 237, 238, and 239 amu (their relative intensities are ca. 52, 4, 100, 7, 48 and 4, respectively).

5 (5 pts). Provide a mechanism for the Heck reaction that converts **A** into **B** under the following conditions (and according to the balanced equation).



References

1 Mukai, C.; Hirose, T.; Teramoto, S.; Kitagaki, S. *Tetrahedron* **2005**, *61*, 10983-10994.

2 Kondo, Y.; Shiga, F.; Murata, N.; Sakamoto, T.; Yamanaka, H. *Tetrahedron* **1994**, *50*, 11803-11812.

3 Stereoselective modification of *N*-(α-hydroxyacyl)-glycinesters via palladium-catalyzed allylic alkylation. Horn, A.; Kazmaier, U. *Org. Lett.* **2019**, 4595–4599.

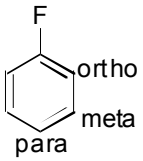
4 Novozym 435: the "perfect" lipase immobilized biocatalyst? Ortiz, C. et al. Catal. Sci. Technol. **2019**, *9*, 2380–2420.

5 Mosher ester analysis for the determination of absolute configuration of stereogenic (chiral) carbinol carbons. Hoye, T. R.; Jeffrey, C. S.; Shao, F. *Nature Protocols* **2007**, *2*, 2451–2458. (link)

Tables of Coupling Constants (*J*) and Chemical Shifts (δ) Useful for Assigning the ^{1}H NMR Spectra in Experiment 4

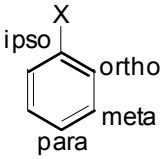
Fluorine/Carbon coupling constants (Hz)[1]

Carbon	coupling constants (Hz)
ipso	245.1
ortho	21.0
meta	7.8
para	3.2



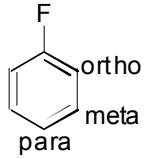
Effect of aromatic ring substituents on carbon chemical shifts.
(128.5 ppm + value found below)[3]

X	ipso	ortho	meta	para
F	33.6	-13.0	1.6	-4.4
Br	-5.4	3.3	2.2	-1.0
I	-31.2	8.9	1.6	-1.1
Me	9.2	0.7	-0.1	-3.0
alkyne	-6.2	3.6	0.4	-0.3



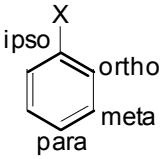
Fluorine/Proton coupling constants (Hz)[5]

Proton	coupling constants (Hz)
ortho	6.2-10.3
meta	3.7-8.3
para	0-2.5



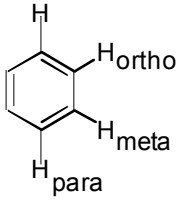
Effect of aromatic ring substituents on proton chemical shifts.
(7.26 ppm + value below)[4]

Substituent, X	ortho	meta	para
F	-0.29	+0.02	-0.23
Br	+0.17	-0.11	-0.06
I	+0.38	-0.23	-0.01
Me	-0.20	-0.12	-0.22
alkyne	+0.16	-0.03	-0.02



Proton/Proton coupling constants (Hz)[2]

Proton	coupling constants (Hz)
ortho	6.5-8.5
meta	1.0-3.0
para	0-1.0



Sources:

1-4.) Pretsch, E; Bühlmann, P.; Affolter, C. *Structure Determination of Organic Compounds: Tables of Spectral Data,* Sponger, 2000.
1.) pp. 113
2.) pp. 180
3.) pp. 97-99
4.) pp. 182-183
5.) Jackman, L; Sternhell, S. Applications of Nuclear Magnetic Resonance Spectroscopy in Organic Chemistry, 2nd Ed, Pergamon Press, pp. 349, 1969.