

Organic Spectroscopic Methods

Chemistry 136 – Spring 2016

Chem 136 is meant to develop your problem solving skills. We will review some theory to understand experimental methods, in particular for NMR, but we will go in depth into solving spectroscopy problems. NMR spectroscopy will be covered in detail using 1D and 2D techniques. Additional and useful spectroscopic techniques such as IR, Mass Spectrometry, and X-ray crystallography will also be covered.

Instructor: Prof. Yves Rubin (y Rubin@ucla@gmail.com)

Course website: <http://rubingroup.org/home/yves-rubin/teaching/chem136/>

Lecture: Thursdays, 12:00 - 1:40 PM, Young Hall, Room 3069

Office Hours: Monday, 2:00 – 2:50 PM in Molecular Sciences 4515 (you can also reach me in my office during most afternoons)

To reach me for quick or last minute questions, you can email me at the address above.

Textbook Support: “*Introduction to Spectroscopy*” (<http://bit.ly/1ncDZNZ>)
by Pavia, Lampman, Kriz, Vyvyan, 5th Edition, CENGAGE Learning

Other useful sources:

- *High Resolution NMR Techniques in Organic Chemistry* by T.D.W. Claridge, Pergamon, 2nd Edition, 2008
- *Structure Elucidation by NMR in Organic Chemistry* by Breitmaier, Wiley, 3rd Edition, 2002
- *Spectroscopic Methods in Organic Chemistry* by Hesse, Meier, Zeeh, Thieme, 2nd Edition, 2008
- *Introduction to Spectroscopy* by Pavia, Lampman, Kriz, Vyvyan 4th Edition, Harcourt College Publishers, 2008

Problem Sets: Problem sets will be handed out weekly. You are expected to show in detail how you have analyzed spectra, so that we can follow your logical deductions when grading

Problem sets are due *before* the start of the class one week following handing them out

Problem Set 1:	2.1, 2.3, 2.4, 2.5, 2.8, 2.9, 2.10 (IR)	Due April 7
Problem Set 2:	5.17, 5.19, 5.20, 5.21, 5.22, 5.23 (simple ¹ H NMR)	Due April 14
Problem Set 3:	7.3, 7.4, 7.9, 7.10, 7.13, 7.14 (¹ H NMR)	Due April 21
Problem Set 4:	7.16, 7.17, 7.18, 7.22, 7.22, 7.26 (¹ H NMR)	Due April 28
Problem Set 5:	6.3, 6.4, 6.5, 6.9, 6.10, 6.11, 6.15 (¹³ C NMR)	Due May 12
Problem Set 6:	8.2, 8.3, 8.8, 8.9, 8.10, 8.11, 8.15, 8.19 (¹ H NMR)	Due May 19
Problem Set 7:	11.2, 11.9, 11.10, 11.14, 11.31 (combined problems)	Due May 26
Problem Set 8:	9.2, 9.3, 9.4, 9.7, 9.8 (2D NMR)	Due June 3 (TA)

Course Syllabus

Week	Lecture Date	Topic	Material
1	March 31	<i>Introduction + Infrared Spectroscopy Theory & Example Problems</i>	Pavia Chapter 2
2	April 7	<i>The NMR Phenomenon – Introduction to NMR Theory</i>	Pavia Chapter 5
3	April 14	<i>Chemical Shifts and Coupling Constants, 1D NMR</i>	Pavia Chapters 5, 7
4	April 21	<i>Chemical Shifts and Coupling Constants, Advanced Techniques</i>	Pavia Chapters 7, 8
5	April 28	<i>Chemical Shifts and Coupling Constants, Advanced Techniques</i>	Pavia Chapters 7, 8
6	May 5	<i>X-ray Crystallography (Prof. Duilio Cascio, <u>Young Hall 3069</u>)</i>	Handouts
7	May 12	<i>Date to be confirmed: MIDTERM EXAM (in class and open-book)</i>	
8	May 19	<i>Introduction to 2D Homonuclear NMR</i>	Pavia Chapter 9
9	May 26	<i>2D Homo- and Heteronuclear NMR</i>	Pavia Chapter 9
10	June 2	<i>Problems in Class</i>	Handouts
		FINAL EXAM Monday, June 6, 2016, 6:30pm-9:30pm	

Grading:	Lab Reports (50 pts for 1 st , 75 pts each for 2 nd to 5 th)	350 points
	8 Problem Sets (25 pts each for IR, NMR, adv. NMR, MS)	200 points
	Midterm Exam	100 points
	<u>Final Exam</u>	200 points
	Total	850 points

Laboratory Guidelines

The laboratory component of this course emphasizes the purification and determination of unknown chemical structures using physical (m.p., b.p. color, etc) and spectroscopic methods (IR, NMR, MS). There is no specific “recipe” or directions provided to carry out these experiments, except for Experiment 1. Guidelines and techniques will be given in class, but you will need to put yourself in a “detective” frame of mind and devise purification and analytical methods for each compound. The TAs will be there to provide guidance, but they have been asked *not* to give you specific recipes. The whole purpose of this course is to teach you how to think creatively to solve problems, which seems quite unsettling at first, but it is a great way to learn.

Except for Lab Report 1, all lab reports will consist of a completed “Unknown Report Sheet”, **which must include original spectra** acquired by the NMR TA, as well as a clear analysis of the data by yourself (see example report online).

Teaching Assistants:	Section 1A (Tue/Thu) Lab 6110
	Nathan Dupper ndupper@chem.ucla.edu
	Craig Lee cjlee@chem.ucla.edu
	Section 1C (Tue/Thu) Lab 6086
	Yolanda Li yolandali@chem.ucla.edu
	Alec Parenzan parenzan@chem.ucla.edu
	Section 1B (Wed/Fri) Lab 6110
	Lingchao Cai cailc@chem.ucla.edu
	Alec Parenzan parenzan@chem.ucla.edu

Schedule of Unknowns and Lab Reports:

Experiment	Type	Distribution/Start Date	Report Due Date ¹
1	Qualitative Analytical Tests (1 g)	March 31/April 1	Thursday, April 14 ²
2	Mixture of 2 unknowns (solid, 500 mg)	April 7/8	Thursday, April 28
3	Natural Product Extraction	April 21/22	Thursday, May 12
4	TBD	May 5/6	Thursday, May 26 ³
5	Artificial Flavor Synthesis	May 19/20	Monday, June 6 ³

Notes:

¹ All lab reports are due in class *at the beginning of lecture*, **10 pts will be taken off if late!**

² This is not a full lab report, use the “Organic Qualitative Analysis” handout to prepare this report, which is available online in Word or PDF format.

³ In addition to standard IR and NMR characterization, you will be expected to request two sets of 2D experiments (COSY, NOESY, HMQC, or HMBC) on only one of your samples for either experiments 4 or 5.

NMR Data Processing Background

Being able to plot your own NMR experiments is an invaluable experience, and also a lot of fun! Each one of you will be learning how to process NMR data from your own samples that will be run at night by the NMR TA using a high field NMR spectrometer (400 or 500 MHz instruments). You will be processing these acquired data with specialized software on a PC (Fourier transform and plotting). While some aspects of the NMR processing are quite easy, they may seem dauntingly complex at first because processing requires a set of commands that you need to get familiar with. Thus, you will be taught how to process the NMR FID data using software installed on a PC located in the labs. Instruction on processing of FID data, will be done during regular lab hours. You will be asked to come in groups of 4–5 students to learn the different aspects of the NMR signal processing.

Instruction and Experiment Schedule:

Week 1: *no instruction* (carry out your Qualitative Analytical Tests for Experiment 1)

Week 2 or 3: data processing instructions in class

Weeks 3–10: process ^1H and ^{13}C NMR data *acquired for you by the NMR TAs* on your own samples for Experiments 2 – 5 (you will first need to separate and purify your unknowns, then prepare NMR samples of each in a deuterated solvent, e.g. CDCl_3 , and label the NMR tubes with your initials and experiment number, TAs will designate an area in the lab where to store these NMR tubes for acquisition after hours)

Weeks 8–10: same as weeks 3–10, but with additionally *two sets* of required 2D NMR experiment on a *single unknown* besides the usual 1D ^1H and ^{13}C NMR experiments. You can request a *total of two* out of the following 2D experiments: COSY or NOESY, HSQC INEPT135 which gives multiplicity information) or HMBC (long range ^1H - ^{13}C correlation).

General Guidelines for Experiment 2

The main portion of this lab course is based on the separation and purification of a mixture of two unknown compounds, on two solids or a mixture of solid/liquid. There is no specific “recipe” for separation and purification, because several methods can be used and each can be as effective. However in general, whether this is done in this lab course or in an organic research group, the following methods and guidelines are useful to follow:

1) Observe:

- a. What colors can you see in the mixture of compounds, are there separate particles/crystals? (are both colorless, or mixture of color and no color?) Color gives you an indication of a conjugated π -system, and virtually 80% of compounds you will encounter are based on aromatic rings.
- b. Dissolve a minute amount of this mixture in a common organic solvent like CH_2Cl_2 or ethyl acetate. How soluble is it? Low solubility is usually related to high-polarity molecules, or large flat aromatic systems that have strong π - π interactions between individual molecules.
- c. To address the question of high-polarity, also test water solubility. Some highly polar molecules like diols or triols (e.g. glycerin), or amino acids, can be water soluble. Remember that this is a mixture of two compounds, so it is unlikely both will have the same properties.
- d. The more likely scenario for point c above is that the highly polar molecule will be a carboxylic acid and it will not be water-soluble. However, it can be made water-soluble by conversion to its salt with dilute NaOH or KOH. This can be easily exploited to do an extraction-based separation, where the mixture of the two

compounds is dissolved into diethyl ether and extracted with 1M Na₂CO₃ solution. The formed salt goes into the aqueous phase (bottom in this instance), and the nearly pure neutral second unknown remains in the organic phase. Talk to your TAs for further information.

2) Prep yourself for some TLC:

- a. The most widely used method to purify compounds and mixtures of compounds is column chromatography, based on the selective physical interaction of compounds in a solvent (eluent) with the surface of a solid support, usually silicagel. To determine the best solvent polarities that you will use for the preparative chromatography, you will need first to do TLC (thin layer chromatography), which is by far the most useful method to monitor separation of compounds (and the progress of a reaction in fact).
- b. For your first TLC, try the following approach:
 - i. Dissolve a small amount (2-5 mg) of your compound in a low-boiling solvent (it evaporates quicker upon loading), preferably CH₂Cl₂ or diethyl ether.
 - ii. Spot your compound at the baseline of your TLC plate (see handout on class website) at **two** different concentrations (one should be faint blue under UV light, the other strongly blue, unless both compounds have no conjugated double bonds). This will ensure that you can see other impurities in the higher concentration lane if they are present. For the most part, you should see only two spots once the TLC has been eluted.
 - iii. **Elution solvents:** Start first with a non-polar solvent such as CH₂Cl₂ or hexanes. Since most compounds will have at least some polar substituents (OH, OMe, COOR, NO₂, etc), they are likely to elute very slowly (or not at all) with these two solvents. First elute the TLC and mark the front of the solvent once it reaches within 5-10 mm of the top. After evaluating your eluted TLC plate under UV light or with a stain reagent (vanillin, KMnO₄, or phosphomolybdate), mark the location of the spots, and calculate their retention factor (R_f , see TLC handout).
 - iv. **More about elution solvents:** More than likely, you will have to elute several TLC plates to determine the best *starting* solvent mixture for the next step, preparative chromatography. You should never start preparative chromatography with a solvent system that gives an elution R_f factor higher than 0.1–0.15 for the upper most spot, otherwise this compound will elute much too quickly with the solvent frontline, and the compounds are unlikely to get separated at all. To quickly choose from different solvents, spread your choices to two other solvent polarities on the solvent polarity chart, for example if you first chose hexanes, then use CH₂Cl₂ and ethyl acetate (or perhaps EtOAc and acetone if you first chose CH₂Cl₂ and your compound was not eluting). Run those concurrently to get faster results, since each TLC elution can take up to 10–15 minutes. Once you have a good idea of the polarity of your compound, start using mixtures of solvent, e.g. hexanes/CH₂Cl₂ in 9:1 ratio. Often, even a small proportion of the more polar solvent can have a dramatic result on elution R_f factors for your unknowns.

3) Column chromatography:

- a. Packing silicagel, loading a sample, and eluting solvent through the preparative column will be explained by your TAs in the lab. There is an art in packing the silicagel support well, so that the entire column is homogeneous and gives great separation. I prefer the wet loading method, which tends to give the best results for prepping the column. After loading the column with the silicagel slurry and letting solvent come down to near the top of the silicagel column, don't forget to gently tap the column with a rubber hose to achieve tight packing of the silicagel particles. Otherwise, solvent channels will form during chromatography and poor separation may result. Once elution starts, you will need to collect fractions of the solvent in test tubes, but only once you detect a trace of compound in the eluent after placing a spot on a fresh TLC plate and visualizing a blue sport under UV light. Otherwise, you will collect loads of nearly pure solvent in test tubes for no good purpose.

4) Column chromatography – Final Steps:

- a. While you are collecting fractions of eluting solvent from your column, begin to spot a 2-inch wide TLC plate with each fraction (e.g. 10–15 lanes, corresponding to that many fractions), making sure you spot repeatedly the same fraction to get enough material deposited on your TLC (otherwise it will be hard to detect the presence of a mixture if one compound is present in excess to the other, which is the whole point of making sure you collect only fractions that have a unique compound). This way you can save yourself a lot of time by “multitasking” (or dual-tasking here). Keep in mind that performing one column chromatography operation well usually takes at least 1 hour, and easily two or even longer. You cannot start a preparative column at 5 pm, so you need to budget your time and prepare yourself well in advance. To compound the issue, leaving a half-finished column overnight and coming to finish it the next day gives usually poor results. The compounds diffuse slowly over the time and bands of different compounds become not well-separated.
- b. After collecting only the fractions that have pure compound, evaporate the solvent on the Rotavap to dryness, and dissolve the crystalline residue in a very small amount of low-boiling solvent. Then you can transfer this solution to a smaller flask, evaporate it again, and then recrystallize your compound for the ultimate purification step. **Crystallization** is THE best way to gain high purity in a compound. It is done in the pharmaceutical industry on tons of material, and since their purity has to be extreme, crystallization is often done more than once. For your purpose, once should be enough.
- c. Once you have pure crystals of your compounds, record their melting point. High purity will give you a narrow melting range, which will help you confirm the structure of your unknown once you know more about its features from spectroscopy.

5) Spectroscopic characterization:

- a. First record an IR spectrum, it is easy and this gives you loads of information on the nature of your compound.
- b. Prepare samples of your two unknowns in two pairs of NMR tubes:
 - i. For ^1H NMR, dilute solutions are better; place 5-10 mg of compound in a small round-bottomed flask or test tube and dissolve it into ~ 0.5 mL of *deuterated* solvent (CDCl_3 , $\text{DMSO-}d_6$, $\text{acetone-}d_6$, etc). Make sure ALL the compound is dissolved, then filter the solution through a cotton pad placed into a pipette that is inserted into the NMR tube. Solid particles give bad spectra!
 - ii. For ^{13}C NMR, you need to “freaking” load up your sample, since ^{13}C sensitivity is so low; place 20-100 mg of compound in a small round-bottomed flask or test tube and dissolve it into ~ 0.5 mL of *deuterated* solvent (CDCl_3 , $\text{DMSO-}d_6$, $\text{acetone-}d_6$, etc). Repeat the following steps from point i. above.

6) Preparation for Laboratory Report:

- a. Science is based on observation and a precise recording of the observed data. Thus, you will need to write concisely but accurately what you observe during your separations and spectroscopic evaluations.
- b. An example of a good report is provided on the CHEM 136 website. I will explain in class how to evaluate difficult coupling patterns in proton NMR spectra.
- c. It is key that you attach a plot of each of your spectra to the report, and assign specific features such as peaks, integrals (^1H NMR), or coupling patterns (^1H NMR) on those spectra. Otherwise, we have no way to understand how you came to your conclusion of the identity of your compound. You will also need to formulate a brief narrative of how you came to your conclusion. Use online sources (including chemical literature) to reference data on your compound.