Experiment 27

Advanced Spectroscopy

Reading: Handbook for Organic Chemistry Lab, section on NMR Spectroscopy (Chapter 20). Organic Chemistry by Marc Loudon, 6th ed., pp. 656-662 (Section 13.9).

This experiment builds on the NMR theory you learned in the NMR Spectroscopy lab, and on the practical aspects of running spectra that you learned in the Practical NMR lab. If you have not done these experiments, you should speak to the lab director immediately to arrange to make them up in another section. There is no quiz or prelab for this experiment.

Proton NMR is the most widely-used tool in organic chemistry, but often, other methods are necessary. Sometimes it is necessary to change the solvent conditions before the proton NMR is useful (for example, by using a shift reagent or a D_2O shake). Sometime you need to look at a different nucleus entirely (carbon NMR). Sometimes you need to find the molecular weight of your compound before you can start to figure out its formula and structure (mass spectroscopy). All of these methods will be covered during today's lab.

The required parts of this experiment are marked with "Exercise." For these parts, you should write up the results into a Word document. This report should be written individually, and is due in one week. Some of the exercises require you to submit spectra. This should be done in a group of 4-5 people; you can divide up this work between yourselves however you like. Once you receive the spectra, share them with each other so you can each work them up individually. As a reminder, instructions are posted at www.orgchemboulder.com/Labs/Software/ for downloading spectra from the NMR server, so even if you don't manage to get the spectra from your group members, you can still go and download a copy for yourself. Once you have the spectra, work them up as you learned to do in the Practical NMR lab last semester, and submit these spectra as part of your individually-written report.

Shift Reagents

Often, multiple different protons in a compound will have such similar chemical environments that their chemical shifts overlap. This often makes it difficult to analyze the structures of compounds with sparse functional groups, for instance long-alkyl-chain alcohols or steroids.

This problem can be overcome by adding a small amount of a shift reagent to the NMR sample. The most commonly-used shift reagent is tris(7,7,-dimethyl-1,1,2,2,2,3,3-heptafluoroocta-7,7-dimethyl-4,6-dionato) europium(III), commonly referred to as Eu(fod)$_3$ or just Eufod (Figure 27-1).
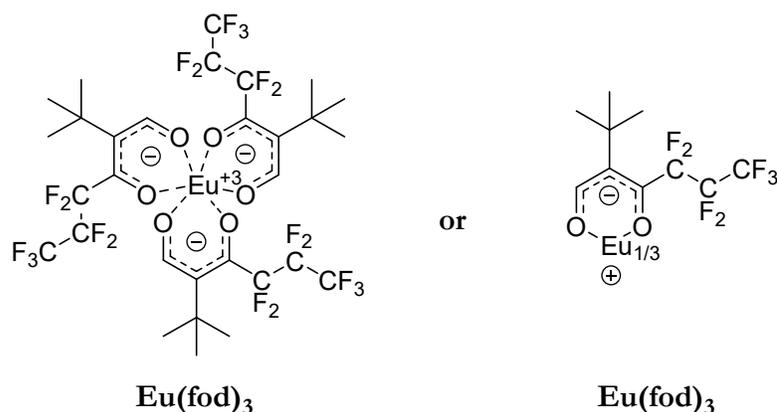

Eu(fod)$_3$ or   **Eu(fod)$_3$**

Figure 27-1: The structure of Eu(fod)$_3$.

Experiment 27: Advanced Spectroscopy

When a compound is placed in an NMR tube with Eu(fod)₃, it complexes onto the metal. Complexation means that two available electrons on the compound – for instance, the lone pair of an alcohol – contribute both of the electrons needed to form a bond to the transition metal. This is also known as a dative bond or a coordination bond.) Once complexed, the molecule experiences a change in the local magnetic field, because the europium nucleus is paramagnetic. This means that there are two cone-shaped regions where the chemical shift of the compound is increased, depending on how close it is to the europium nucleus (Figure 27-2).
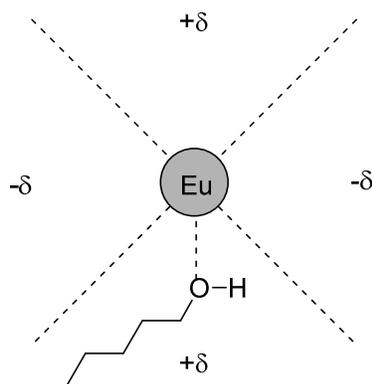

Figure 27-2: The europium nucleus is surrounded by zones where the chemical shift is increased or decreased.

In practical terms this means that as you add more and more Eu(fod)₃ to your NMR sample, all the protons shift further and further to the left, but the protons closest to the complexing group tend to shift further. You can use this to spread out the peaks of your spectrum and get a clear view of the splitting on each individual peak. In terms of how much shift reagent to use, a typical starting point is 0.2 molar equivalents of Eu(fod)₃.

The easiest way to compare two spectra is to stack them in MestreNova. First, go to Stack → Stack Options, and check the box next to "Auto scale the intensities when stacking". Then select one of the spectra you wish to stack in the left pane, and select the other spectrum by holding down the shift key and clicking it. Once both spectra are selected, go to Stack → Stack Spectra. They will show up in the same window, allowing for an easy peak-by-peak comparison. An example is shown below in Figure 27-3.
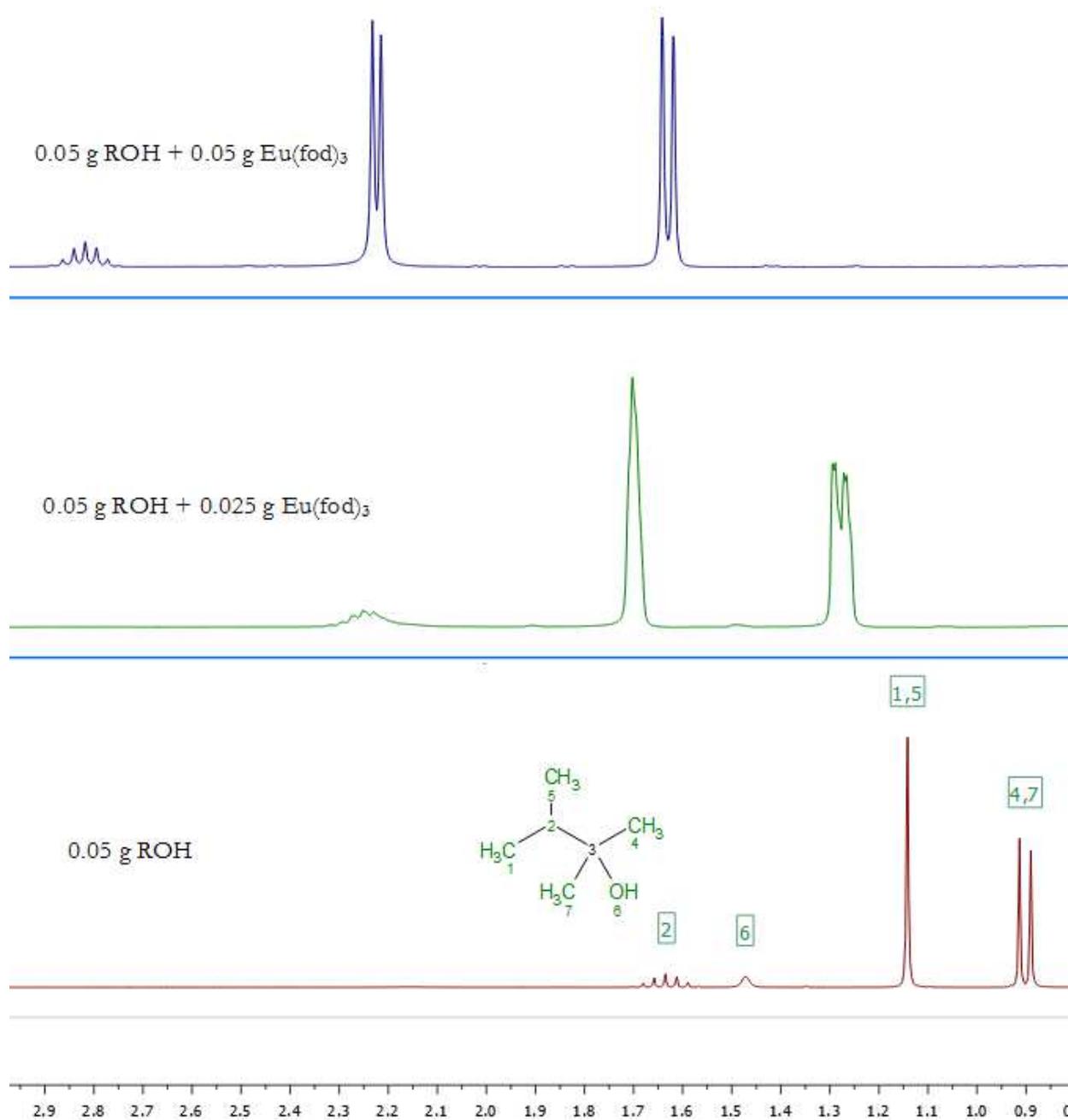
Figure 27-3: An example of stacked NMR spectra showing the addition of increasing amounts of Eu(fod)$_3$, from bottom to top. The ppm scale at the bottom is the same for all spectra.

The order of how well each functional group complexes to europium goes in the general order shown in Figure 27-4.
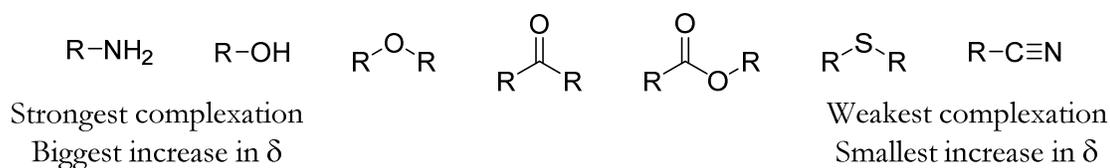

Figure 27-4: Different functional groups are shifted by different amounts in the presence of a shift reagent.

Experiment 27: Advanced Spectroscopy

To use a shift reagent, you need a solvent that is free of other, competing complexing groups. For this reason, deuterated water and deuterated acetone are not good choices. Commercially available $CDCl_3$ contains a small amount of ethanol-d6 as a stabilizer, and may also contain a small amount of water if it has been left open to atmosphere too long. To remove these impurities, you can filter the $CDCl_3$ through a pipette filled with silica gel, although this will not be necessary for today's experiment. Since the shift reagent itself also complexes easily to water, it is quite hygroscopic and will absorb moisture form the air. The bottle should be left capped as much as possible to slow down this process. Your sample itself must also be free of water.

Initially you should take an NMR spectrum of your pure compound by itself, so you know where each peak is located. Then you can run more spectra with slightly more shift reagent added each time, until the spectrum is spread-out enough to give you a clear view of each peak. If you were doing this in a research lab, you might keep using the same tube, weighing out a little more shift reagent each time and then immediately collecting the next spectrum. In the interests of time for this course, you will just use three separate tubes.

Exercise 1: As a group, choose one of the unknown alcohol samples - make sure you copy down the molecular formula on the label. Your group should collect three spectra, using the quantities given in the table below, each dissolved in 0.5 mL $CDCl_3$. To weigh out your alcohol, stand it up in a beaker on the scale, tare the scale, and then pipet the alcohol into the NMR tube a drop at a time.

	Alcohol	Eu(fod)₃
1	0.05 g	0 g
2	0.05 g	0.025 g
3	0.05 g	0.05 g

Stack all three spectra together in MestreNova. What is the structure of your unknown alcohol?

D₂O Shakes

Most of the time, hydrogen-bonding protons in your molecule are a lot more variable in width, position and splitting than non-hydrogen-bonding protons. For example, the table below shows the chemical shift of methanol (CH_3OH) in different solvents. Note that the CH_3 group only moves around over a range of 0.24 ppm, but the OH group moves around over a range of 2.91 ppm, over ten times as much.

Solvent	CH₃ peak of methanol	OH peak of methanol
CDCl₃ (chloroform-d)	3.40	1.10
CD₃CN (acetonitrile-d3)	3.28	2.16
CD₃COCD₃ (acetone- d6)	3.31	3.12
CD₃SOCD₃ (DMSO-d6)	3.16	4.01

Other factors also affect the locations of hydrogen-bonding protons, such as temperature, concentration, and whether there are any other compounds available to hydrogen-bond with. Depending on conditions, the proton may or may not couple to adjacent protons on the molecule. Overall, hydrogen-bonding protons are somewhat problematic to predict or interpret.

You can pin down exactly which protons are hydrogen-bonding by using a very easy technique known as a "D_2O Shake". First, run a spectrum of your compound as you normally would. Then, add a drop or two of D_2O to the tube, shake to mix, and run the sample again. This causes all the hydrogen-bonding protons in your compound to "disappear" by forcing them to exchange for deuterium. D_2O shakes exploit the fact that hydrogen-bonding protons exchange readily with each other (Figure 27-5).

$$\text{OH} \quad + \quad D_2O \quad \underrightarrow{\text{shake}} \quad \text{OD} \quad + \quad \text{HOD}$$

Figure 27-5: The proton-deuterium exchange that occurs during a D_2O shake.

After the shake there will be a new peak for HOD, but this will probably be in a different location – perhaps further away from the product peaks you wish to examine. Performing a D_2O shake also means that the hydrogen-bonding proton will no longer split any other protons on your molecule.

The easiest way to compare two spectra is to superimpose them in MestreNova. Since the majority of the peaks have not changed locations, it is more useful to show the two spectra superimposed directly on the same baseline, instead of stacked together on the same page. Select multiple spectra as you did before, then go to Stack → Superimpose Spectra. They will show up right on top of each other, allowing for an easy peak-by-peak comparison. An example is shown below in Figure 27-6, for the compound menthol. The pure compound's spectrum is shown in dark red, and the post-D_2O shake version is in lighter blue. This makes it easier to notice that the OH peak at 1.46 ppm has disappeared after the shake, although all other peaks remain.
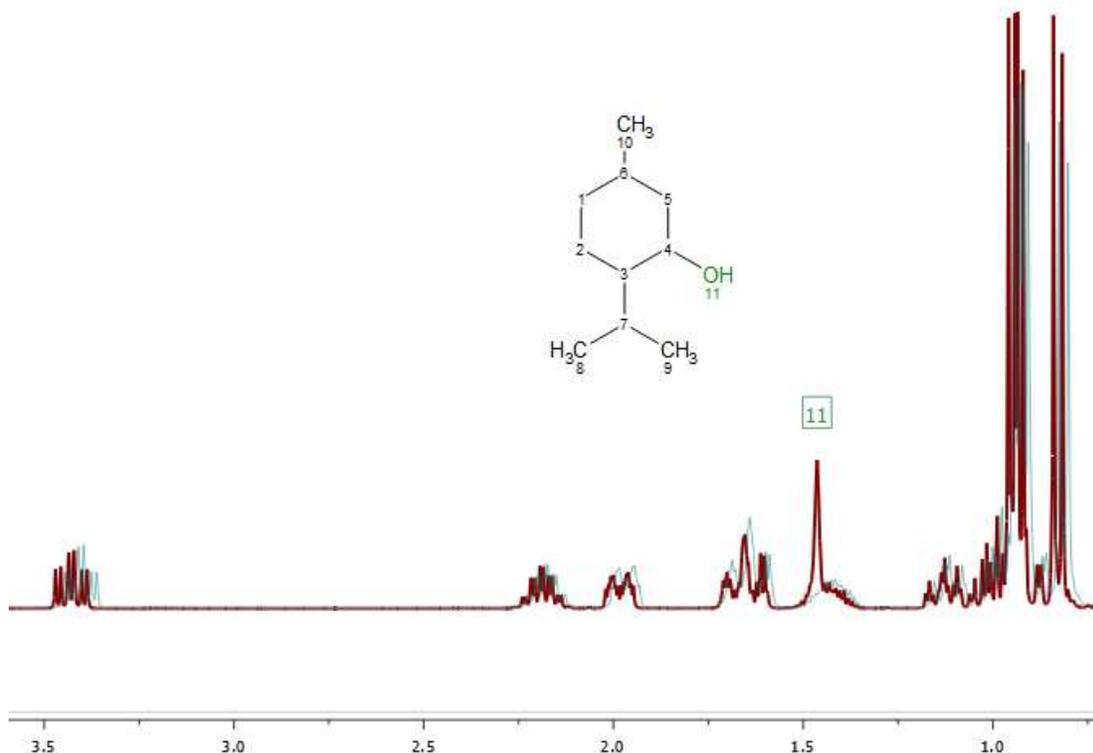

Figure 27-6: The spectrum of menthol, before and after a D_2O shake. The peak corresponding to the OH group, labeled as 11, disappears after the shake.

Exercise 2: One person in your group should submit an NMR sample of eugenol (available in your lab) without D₂O, and one of you should submit a sample of eugenol with a couple of drops of D₂O added. Both samples should still use CDCl₃ as the solvent. Superimpose the spectra in MestreNova. Which peak is caused by the hydroxyl group in eugenol?

Carbon NMR

For this part of the lab, you should read section 13.9 in your textbook.

In practical terms, running a ^{13}C NMR is almost identical to running a ^{1}H NMR, except that you need to specify that you are collecting a carbon spectrum. You can collect multiple spectra of the same sample, so you will only need to prepare a single NMR tube.

Using the standard parameters for a carbon spectrum, the instrument will take approximately half an hour to collect your spectrum. This is because it takes many more scans to collect a strong enough signal. Opening the spectrum and working it up in MestreNova is the same as it was for proton NMRs. In addition, many of the same resources can be used to obtain literature spectra and predictions.

Exercise 3: Work through problems 13.30 – 13.32 in your textbook.

Exercise 4: With your group, choose one of the unknown painkillers - the choices are acetaminophen, aspirin, caffeine, and ibuprofen. Submit an NMR sample of your unknown by choosing "HandC" from the dropdown menu – this will collect first a proton, then a carbon spectrum. Determine the identity of the unknown by comparing it to literature spectra in SDBS or elsewhere.