I. Introduction to Spectroscopy

Spectroscopy involves gaining information from the absorption, emission, or reflection of light from a sample. There are many other examples of spectroscopy in our experience, but three familiar real-life examples include:

1. X-rays. Dense bone absorbs x-ray radiation.
2. Grocery store scanners. A monochromatic laser is either absorbed (black bar) or reflected (white bar). The simple black-or-white lines with their yes-or-no absorption-or-reflection response essentially produces a binary code, from which products and prices can be determined.
3. Stop lights. A lens is adjusted at timed intervals to enable emission of green, red, or yellow light.

In organic chemistry, the most important type of spectroscopy is “NMR” ("Nuclear Magnetic Resonance" spectroscopy). NMR spectroscopy is routinely used for chemical analysis, whether to identify the structure of an unknown, to assess the purity of a product, or to determine ratios of isomers. This week we will use carbon-13 NMR; later we will use hydrogen NMR. Both of these will be used later in the year, especially during second semester lab. During second semester lecture, we will revisit NMR and spend time and a test on interpretation of NMRs. Magnetic Resonance Imagine (“MRI”) is an important hospital application of NMR. (The name was changed from NMR to MRI because some patients were afraid of the word “nuclear”!)

II. General Aspects of Spectroscopy Physics

The fundamental principles of chemical spectroscopy are illustrated below. Spectroscopy involves having quantized energy levels. You are familiar with the concept of quantized energy levels for electrons (1s, 2s, 2p, 3s, 3d etc.) and electron spins (spin up or spin down), but other things are also quantized (vibrational energies, rotational energies…).

Given that there is an exact energy gap between two quantized energy states, a photon of precise energy must be absorbed in order to excite a molecule from the ground state. When an excited state relaxes back to the ground state, that same photon is released. By measuring the exact frequencies of photons that are either absorbed or emitted, we can measure \(\Delta E\). The quantity of photons can tell us about how much material is absorbing or emitting.

The chemist must then be able to interpret what the frequencies of the photons mean in terms of chemical structure.

![General Picture of Energetics and Spectroscopy](image)

1. Quantized Energy Gaps
2. When a photon with exactly the right energy/frequency/wavelength is absorbed, a sample gets "excited" from its "ground state" to an "excited state"
3. When an exited state "relaxes" back to its ground state, the same \(\Delta E\) is involved, and a photon with the same energy/frequency/wavelength is released
III. NMR Physics

Certain nuclei (not all) have quantized “nuclear spins”. Being charged objects that spin, a result is that they are magnetic. (A circular flow of charge or electricity always produces a magnetic field, according to the “right hand rule” of electromagnetism.) Nuclei that have quantized spin states are referred to as “NMR active”. Just as electrons have quantized spin states (spin up or spin down), NMR-active nuclei also have quantized spin states, spin up or spin down.

Some NMR-active nuclei:  H-1, C-13, N-15, F-19, P-31, Si-29, Se-79, Sn-119
Some NMR-inactive nuclei:  C-12, N-14, O-16

The list of NMR inactive nuclei is somewhat unfortunate for organic chemistry! We are largely interested in the chemistry of carbon and the 2\textsuperscript{nd} row elements, but unfortunately the dominant isotopes for carbon, nitrogen, and oxygen are all NMR inactive! Fortunately at least carbon-13 is active. Although only 1\% of carbons are C-13, that’s still enough to give us useful information. Hydrogen is also NMR active, and can give us a lot of information (later…).

In the presence of an applied magnetic field, nuclear magnets can align with (spin down, $\alpha$) or against (spin up, $\beta$) the field. The energy gap between these spin states is quantized, and depends on the strength of the magnetic field. (As with a bar magnet, the stronger the field, the greater the preference for the magnet to line up correctly…). To “excite” a nucleus from the more stable $\alpha$ state to the less stable $\beta$ state, radiation with the correct photon frequency is required. When an excited nucleus relaxes back to the $\alpha$ state, a photon with that same frequency is emitted. Since magnetic field strength determines $\Delta E$, and $\Delta E$ determines $v$, the magnetic field thus determines the frequency of the radiation absorbed or emitted.

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When an external magnetic field is applied, will all nuclei have the same $\Delta E$ and the same photon frequency? No!

1. Different nuclei (H-1 versus C-13) have very different $\Delta E$. Thus an MRI can easily identify whether a particular nuclei is or is not present.

2. In different chemical environments, the same nucleus will have different $\Delta E$.

The second point is the key to 13C NMR. Although the external magnetic field (applied by the spectrometer) may be the same, different carbons in a molecule experience or “feel” different magnetic fields. This is due to the magnetic fields produced by local electrons and by other nuclei (because moving electrons function as “electron magnets” and moving nuclei function as “nuclear magnets”). The magnetic influence of local electrons and nuclei can reinforce or partially counteract the external field, so that every different carbon “feels” a different $H_{\text{actual}}$ ($H =$ magnetic field)

$$H_{\text{actual}} = H_{\text{applied}} + H_{\text{electrons}} + H_{\text{nuclei}}$$

$$H_{\text{actual}} \propto \Delta E \propto v$$
IV. The Actual Experiment  The actual steps in the experiment include:

1. Prepare the sample. For C-13, put 10 drops of sample into your NMR tube. Dilute to 1/3 full with CDCl3. (For H-1, put in 1 or 2 drops of sample.)
2. Insert the sample into the magnetic field. (We’ll use robotics for this.)
3. “Lock” the magnetic field. (So it doesn’t drift.)
4. “Shim” the applied magnetic field. (Make it as consistent as possible in the observation window, from top to bottom, front to back, left to right.)
5. Blast the sample with radiation to excite the nuclei. Rather than dialing through the different frequencies, a broad range of frequencies is applied so that all the carbon nuclei can get excited at the same time. After briefly blasting, the radiation is turned off.
6. Detect/listen to the signals (actually in the radio frequency!) as the excited nuclei relax and release photons. (Many different signals with different frequencies are released simultaneously, each with it’s own wavelength…)
7. Repeat the irradiate-then-detect sequence repeatedly to build up the weak signal.
8. “Fourier Transform” (mathematical operation) to deconvolute the complex signal pattern resulting from the many overlapping frequencies. The Fourier Transform enables the computer to identify all the individual photon frequencies that summed up to give the total signal. An imperfect analogy would be to have every possible radio station broadcasting at the same time; then the Fourier Transform would essentially be able to identify and pick out each station one at a time and make sense of it.

Note: Many of these operations are best done by a computer. (The Fourier Transform especially!) Each of these steps also involves a number of software commands. So that you can acquire data and focus on chemical interpretation of the data, rather than being totally distracted by learning a lot of software commands, many steps have been automated and programmed for you.

V. Interpreting C-13 NMR While the physics of what happens is interesting, for the most part you the chemist will be engaged in interpreting the data that comes out at the end. This is true for the use of many instruments in science and health care. You need to learn some basic operational skills so that you can use the instrument safely and accurately. But being able to interpret the data is really what you need to be able to do at the end.

We will run three types of C-13 NMR’s this week. The first, called “decoupled” C-13 NMR, will show a unique line for each type of carbon present. The second, called “DEPT” (Distortionless Enhancement by Polarization Transfer) will differentiate CH2 carbons (“evens”) from CH and CH3 carbons (“odds”), and will not include carbons that don’t carry any hydrogens. The third, called “coupled” C-13 NMR, will “split” the decouple carbon lines into doubles (for CH), triplets (for CH2), or quartets (for CH3). The three

Decoupled C-13 NMR

Summary of Decoupled C-13 NMR Interpretation:
1. **Count how many lines** you have. **This will tell you how many types of carbons** you have. (Symmetry equivalent carbons can at times cause the number of lines to be less than the number of carbons in your structure.)
2. **Check diagnostic frequency windows** (“chemical shift windows”) of the lines to provide yes-or-no answers regarding the presence or absence of key functional groups in your molecule.

1. **Number of Lines and Number of Symmetry-Unique Carbons**
   a. Each “unique” carbon gives a separate line.
      • This is due to having different electronic environments, and because spinning electrons create magnetic fields that counteract or reinforce the applied field.
   b. Symmetry duplicates give the same line.
      • If due to molecular symmetry two carbons have exactly the same chemical environment, naturally they will absorb and emit exactly the same photon frequency, and give exactly the same line in the spectrum.
2. “Chemical Shifts” of the Lines (This reflects the energies or photon frequencies/wavelengths associated with the lines.)

<table>
<thead>
<tr>
<th>Interval</th>
<th>Description</th>
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<tbody>
<tr>
<td>220-160</td>
<td>C=O carbonyl carbons, sp² hybridized</td>
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<tr>
<td>160-100</td>
<td>C alkene or aromatic carbons, sp² hybridized</td>
</tr>
<tr>
<td>100-50</td>
<td>C-O oxygen-bearing carbons, single bonds only, sp³ hybridized</td>
</tr>
<tr>
<td>50-0</td>
<td>C alkyl carbons, no oxygens attached, sp³ hybridized</td>
</tr>
</tbody>
</table>

a. Notice that sp² hybridized carbons come above 100, sp³ hybridized come below
b. Notice that oxygenated carbons come higher than non-oxygenated analogs. An sp³-hybridized carbon with an oxygen comes higher than without, just as an sp²-hybridized carbon comes higher with oxygen than without
c. **How do I process and use what I see from my Chemical Shifts?**
   - Check each of the four zones. Each one gives you a yes or no answer about the presence of absence of the featured group.
   - Check 220-160. Do I have any carbonyl carbons or not? Easy yes or no question.
   - Check 160-100. Do I have any alkene/aromatic carbons? Yes or no! If I do, then how many? If I have two, I probably have an alkene! If I have four to six, I probably have a benzene!
   - Check 100-50. Do I have an oxygenated sp³ carbon? Yes or no! Alcohols and esters will normally have one carbon in the 100-50 zone. Ethers will have two.
   - Check 50-0. I’ll almost always have some lines there! But how many should tell me how many types of non-oxygenated sp³ carbons I have.

### Using Chemical Shifts to Identify Functional Groups in Simple Molecules:
1. An alcohol should show one carbon in the 50-100 zone.
2. A ketone should show one carbon in the 160-220 zone.
3. An ester should show one carbon in each of the 50-100 and the 160-220 zones.
4. An aromatic should show at least four different carbons in the 100-160 zone.

3. **Signal Height/Size** Two tips:
   - a. Carbons without attached H’s are short. This is common for carbonyls and for substituted carbons in a benzene ring.
   - b. Symmetry duplication amplifies signal height. (If you have two copies of a carbon, the line will probably be taller than normal!)

4. **Subtracting the Solvent Lines: Don’t Count the 3-Line Triplet at 77**  
   Our samples are routinely diluted with CDCl₃, which has a carbon and thus gives a signal. Usually lots more solvent gets used than solute, so potentially the solvent lines could dominate. Fortunately deuterated carbons give a different look: CDCl₃ will give a 3-line “triplet” signal at 77. Ignore this signal! Don’t count it as three more unique carbons in your molecule! Don’t conclude that you have three oxygenated sp³ carbons!
5. **How do I know what’s a real line, from a carbon in my compound from an impurity that I should ignore?** No simple way! With experience or some advanced experiments you can often tell, but there is no automatic way to know. For today, if in doubt ask the instructor! The instructor will confirm which lines you should or shouldn’t consider in doing your analysis.

<table>
<thead>
<tr>
<th>Practical Use of Decoupled C13 NMR:</th>
</tr>
</thead>
<tbody>
<tr>
<td>• How many lines/carbons do I have?</td>
</tr>
<tr>
<td>• How many are carbonyls (160-220), how many are aromatic (100-160), how many are oxygenated (50-100), and how many are non-oxygenated (0-50)?</td>
</tr>
<tr>
<td>• Do the chemical shifts suggest ketone, ester, aromatic, or alcohol?</td>
</tr>
</tbody>
</table>

**DEPT NMR: Distinguishing C and CH2 carbons from CH and CH3 carbons.**

The DEPT experiment is done in conjunction with a decoupled C-13 NMR. While more elaborate and even more informative versions of DEPT are available, at the cost of extra time, this week we will use a basic version (called DEPT 135).

<table>
<thead>
<tr>
<th>Use the following DEPT clues:</th>
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<tbody>
<tr>
<td>• <strong>CH2 carbon lines will usually point down</strong></td>
</tr>
<tr>
<td>• CH and CH3 carbon lines will usually point up</td>
</tr>
<tr>
<td>• Carbons with no directly attached hydrogens will not appear.</td>
</tr>
</tbody>
</table>

**Practical:** Look at your decoupled C13 spectrum beside your DEPT spectrum.

- Which carbons are “quaternary” carbons? (the ones with no hydrogens vanish from your DEPT spectrum.
  - These are usually carbonyls or substituted aromatic carbons.
- Which carbons are CH2 carbons?
  - Are any of these oxygenated? (in the 50-100 zone)?
- How many CH/CH3 carbons do you have, and in which zones?

**Coupled C-13 NMR**

Whereas “decoupled” C-13 NMR spectra show nice sharp singlet lines for each type of carbon, “coupled” C-13 spectra show the following:

- CH3: four lines (“quartet”)
- CH2: three lines (“ triplet”)
- CH: two lines (“ doublet”)
- C (no hydrogens): one line (“ singlet”)

The information is complementary to DEPT information in that both help to differentiate among different carbons.

**Coupled C-13 is not used real often in the real world for at last two reasons:**

- Signal to noise: The signal to noise is a lot worse than in decoupled C-13 or DEPT NMR, and the cost of improving the signal-to-noise is taking more time.
- Overlap: With coupled C-13 NMR, there are a lot more lines, and overlapping of lines becomes normal and confusing for non-simple molecules.

**Practical:** Use the coupled carbon can differentiate CH3 from CH carbons
General: Each pair should obtain spectra and identify at least two of the unknowns. Each individual must have run one of the two samples. (If you don’t have or want a partner, just prepare and run two yourself.)

Queued Samples: Samples can be queued up and run in automated sequence on lab day. Once the sequence is running, (assuming no problems), each sample will require five minutes combined. (This includes loading from the autosampler; running the decoupled carbon; running the DEPT; and running the coupled carbon.) All 24 students could have their samples run within 2 hours.

Individual Samples and Signing up for NMR Time: A paper signup sheet will be in the NMR lab. If you don’t want to wait and run in the lab-day sequence queue, you could sign up for a time later on your own. A typical student should be able to finish within 15 minutes. I’m often gone after 6 pm, so running during daytime hours when I can help you if you get stuck is probably smart.

Prepare the sample
1. Put about 10 drops of sample into an NMR tube.
   - Perhaps an easier load is to use one of the long pipets; if you fill it just up to where the skinny tube widens, that should be fine. The loading is not critical; anything in the 5-15 drops range should be fine.
2. Then add CDCl₃ solvent until the NMR tube is about 1/3 full. Using one of the short pipets, this is somewhat less than a “full squeeze”. Again, the volumes are not critical.
3. Put a cap on the sample. Prepare the sample during lab time, even if you aren’t going to run it for a few days.

Load and Run the Samples, and Print the Results: See the NMR Usage Instructions.
   - For this week, you will run the sequence of experiment called C-DEPT

Printing note:
   - The instrument can auto-print the three spectra, but you will also want to print copies for your partner.
   - A copy machine could be used to do this, or
   - The prints could be done from the NMR, see the NMR Usage Instructions

Unknown Candidates

Simple Ketones

Esters

Alcohols

Aromatic Carboxyls
C13-NMR Interpretation

1. **Count how many lines** you have. This will tell you how many types of carbons you have. (Symmetry equivalent carbons can at times cause the number of lines to be less than the number of carbons in your structure.)
   a. Each “unique” carbon gives a separate line.
   b. Symmetry duplicates give the same line.
   c. If there are more carbons in your formula than there are lines in your spectrum, it means you have symmetry.

2. **Check diagnostic frequency windows** (“chemical shift windows”) of the lines to provide yes-or-no answers regarding the presence or absence of key functional groups in your molecule.
   - 220-160 C=O carbonyl carbons, sp² hybridized
   - 160-100 C alkene or aromatic carbons, sp² hybridized
   - 100-50 C-O oxygen-bearing carbons, single bonds only, sp³ hybridized
   - 50-0 C alkyl carbons, no oxygens attached, sp³ hybridized

3. **Use DEPT and/or Couple C13 NMR to Differentiate C, CH, CH₂, and CH₃ carbons.**

<table>
<thead>
<tr>
<th>Type of C</th>
<th>Name</th>
<th>DEPT-135</th>
<th>Coupled C13</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH₃</td>
<td>Methyl</td>
<td>Up</td>
<td>Quartert (q)</td>
</tr>
<tr>
<td>CH₂</td>
<td>Methylene</td>
<td>Down</td>
<td>Triplet (t)</td>
</tr>
<tr>
<td>CH</td>
<td>Methane</td>
<td>Up</td>
<td>Doublet (d)</td>
</tr>
<tr>
<td>C (no attached hydrogens)</td>
<td>Quaternary</td>
<td>Absent</td>
<td>Singlet (s)</td>
</tr>
</tbody>
</table>

4. **Aromatics, Symmetry, and C-13 Signals.** Most aromatics have symmetry, and both the number of aromatic lines and the splitting of the aromatic lines can be indicative of the substitution pattern on a benzene. Mono- and para-disubstituted benzenes have symmetry.
   - 4 lines s, d, d, d Monosubstituted benzene. (Has symmetry)
   - 4 lines s, s, d, d Para-disubstituted benzene. (Has symmetry)
   - 6 lines s, s, d, d, d, d Or tho- or meta-disubstituted benzene. (Has no symmetry)

5. **Signal Height/Size**
   a. Carbons without any attached H’s are short. This is common for carbonyls (aldehydes are the only carbonyl carbons that have hydrogens attached) and for substituted carbons in a benzene ring.
   b. Symmetry duplication multiplies signal height (if you have two copies of a carbon, the line will probably be taller than normal!)
Using Automation

1. Sample Preparation
   - Carbon NMR: 10 drops plus CDCl3
   - Proton NMR: 1 drop, dilute with CDCl3 solvent to 1/3 tube depth
   - 2D NMR: 10 drops plus CDCl3.

2. Add sample to a Spinner/Turbine
   - Adjust depth by placing the turbine into the golden depth finder, and gently slide the sample till the tube just barely reaches the bottom

3. Load sample/turbine into autosampler
   - Press the round white Access Request Button on the panel below the sample trays/doors
   - Wait until the “status” light turns to a solid yellow, and the message panel reads “door unlocked”
   - Gently open the doors, and swap your sample/turbine(s) into the autosampler.
     o Remember which site(s) you placed it into!
   - Note: DO NOT JUST GRAB OPEN THE AUTOSAMPLER DOOR WHILE IT IS LOCKED. YOU CAN DAMAGE THE ALIGNMENT BY FORCING IT OPEN WHEN IT IS LOCKED. IF THE STATUS LIGHT IS GREEN, YOU MAY NOT OPEN THE DOORS!
   - Note: Samples can be added in this way to the autosampler even while the instrument is running somebody else’s sample.

4. Login from scratch
   - User name: nmr
   - Double click on the VNMRJ Icon (upper left).
   - Hit OK (nmr does not currently require a password, although that may change...)

5. Login from within VNMRJ: click Unlock (note: if VNMRJ is left open, as it should be for all of this week, then there will be a screensaver that goes black after a period of non-use. To get back in you will need to “unlock” the screen.

6. Select/Prepare for the experiment, for individual samples that are not part of a big sequence: push the following buttons or address the following things from the menu
   1) New Study (push button on lower left)
   2) Experiment Selection: C-DEPT (on the left. The “UserStudies” folder at the lower left-hand corner of the Experiment Selector folders must be opened for this to be available.)
   3) Node/site identification
      - Click on the button showing where in the autosampler your sample is.
   4) Sample Name (fill in your name or names)
   5) Solvent selection: check that CDCl3 is listed
   6) Comment box: Enter your names, first name and last name
   7) Lock?: switch to “No (a lock=n)”
   8) Shim: unclick box, do not shim. (shimming takes extra time and isn't needed for fast C13)

8. Submit the experiment(s) by clicking the bright green Submit button on the lower left side.
9. **What’s happening automatically during the run:** The experiment(s) will now run on its own. We’ve turned some of these steps off, but the normal procedure is:
   a. Sample insertion
   b. Lock (omitted this week)
   c. Tune (omitted this week)
   d. Shim (omitted this week)
   e. Spinner turns on
   f. Experiment acquisition
   g. Fourier transformation
   h. Data processing
   i. Printing

10. **Copying Instructions: Autosampler Rapid Sequencing/Queueing**
    a. To copy the same instructions to a different sample, click on a different node to copy the instructions, then:
       1. change the Sample Name
       2. change the comment name
       3. click submit.
    b. To copy the experiment and apply it to multiple samples at once:
       1. Click on the first of the multiple samples.
       2. Control click on the last of the multiple samples.
       3. All of these samples will get almost identical names, so in the comment box, designate who belongs with which node, for example:
          44 Craig Jasperse and Tammy Jasperse
          45 Aaron Rodgers and Greg Jennings
          46 Peter, James, and John
       4. click submit.

11. **Printing extra copies from the Queue**
    1. Under **Study Queue** on the left, change the View to **Spectrometer** (you may need to click the “done” button first)
    2. A key will show up next to each completed node
    3. Click on the appropriate key to open the experiments that were run on your sample.
    4. Double-click on an experiment to load it into the viewscreen.
    5. Below the viewscreen, click **Process** (3rd from left)
    6. Click **Plot** (2nd from bottom)
    7. Click **Automatic Plot Page** (top left)
    8. Repeat steps 4-7 to plot the DEPT and coupled carbon spectra

12. **Opening and Printing Using the Data Folders**
    1. Open VNMRJ
    2. Click on the Folder icon, upper left, the icon is right below the “Edit” menu.
    3. If you’re lucky, it will go directly to the home/nmr/vnmrsys/data/chem355 folder. If you’ve named your sample uniquely, you can double-click on the folder.
    4. Double click on CARBON_01.fid to get the decoupled carbon spectrum to show in the view screen.
    5. Print as described in steps 4-7 above.
    6. Click on the Folder icon again, and now double click on DEPT_01.fid, and repeat the print process.
    7. Click on the Folder icon again, and now double click on CARBON_02.fid to get the coupled carbon spectrum, and repeat the print process.
    8. To get the Folder icon to go back to the main chem355 folder, click on the Folder icon again, then click ONCE only on the a little icon that shows an arrow up
C-13 NMR Lab Handin

Name

For each of the two unknowns that you will analyze, hand in the following:

1. For each of the 15 unknown candidates shown on the next page, predict how many carbons you’d get for each box. Hand in this page.

2. Attach the six NMR’s that you should have printed. These should be:
   a. The decoupled C13 NMR for your sample
   b. The DEPT spectrum for your sample
   c. The coupled C13 NMR for your sample.
   d. The decoupled C13 NMR for your partner’s sample
   e. The DEPT spectrum for your partner’s sample
   f. The coupled C13 NMR for your partner’s sample.

3. Directly on each the two decoupled NMR’s, write down which unknown it was. (“Unknown A”, “Unknown B”…).

4. Then draw the chemical structure for each of the two unknown, again directly on the decoupled NMRs.

5. Write a letter by each of the carbons in your structures (see examples below)

6. Next to each carbon line in the decoupled NMR, assign the letter of the carbon that best fits.
   • Note: in some cases there will be ambiguity. So for example, you might have two lines in the same zone and write “C or D” by each of them, for example.

   **Example of drawing molecules and then Lettering each of the carbons**

   ![Molecules and Lettering Example]

   **Unknown Candidates**

   **Simple Ketones**
   ![Simple Ketones]

   **Esters**
   ![Esters]

   **Alcohols**
   ![Alcohols]

   **Aromatic Carbonyls**
   ![Aromatic Carbonyls]
A. For each Structure, fill in the number of carbons under each category column.

<table>
<thead>
<tr>
<th>Structure</th>
<th>C’s</th>
<th>C=O</th>
<th>Aryl</th>
<th>C-O</th>
<th>0-50</th>
<th>C</th>
<th>CH2</th>
<th>CH/CH3</th>
<th>CH</th>
<th>CH3</th>
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<td>1.</td>
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I. Background

In 1912 Victor Grignard received the Nobel prize in chemistry for his work on the reaction that bears his name, a carbon-carbon bond-forming reaction by which almost any alcohol may be formed from appropriate alkyl halides and carbonyl compounds. The Grignard reagent RMgBr is easily formed by redox reaction of an alkyl halide with magnesium metal in anhydrous diethyl ether solvent.

\[ \text{R-Br} + \text{Mg} \rightarrow \text{RMgBr} \quad \text{RMgBr} = \text{R}^- + \text{Mg}^{2+} + \text{Br}^- \]

The Grignard reagent can be viewed as an ionic species consisting of carbanion \( \text{R}^- \), with a Mg\(^{2+} \) counterion and an additional Br\(^- \) counterion. The carbanion \( \text{R}^- \) is very reactive, and functions both as an extremely strong base and an extremely strong nucleophile.

Some of its reactions are shown below:

- **It reacts as a strong base with water or alcohols.**
  - Conversion from less stable R\(^- \) to more stable HO\(^- \) or RO\(^- \) is favorable.

- **It reacts as a strong nucleophile with carbonyl groups aldehydes, ketones, and esters.**
  - Conversion from less stable R\(^- \) to more stable RO\(^- \) is favorable, followed by protonation to give alcohols ROH.
II. Overview of Our Experiment
Our experiment is shown below. During week one we will generate the Grignard reagent (step one) and react it with the ester (step two). During the second week we will neutralize the alkoxide (step three), isolate the alcohol, purify the alcohol by recrystallization, and do product analysis.

\[
\begin{align*}
2 \text{PhBr} + 2 \text{Mg} & \xrightarrow{\text{anhydrous ether}} 2 \text{MgBr} + \text{PhH} \\
\text{Bromobenzene} & \quad \text{mw 157 g/mol} \\
\text{d: 1.49 g/mL} & \quad \text{24.3 g/mol}
\end{align*}
\]

\[
\begin{align*}
1 \text{PhCO}_2\text{CH}_3 & \xrightarrow{1 \text{equiv. MgBr}_2} \text{PhCH}_3 \text{Br} + 1 \text{equiv. Mg} \\
\text{Methyl Benzoate} & \quad \text{mw = 136 g/mol} \\
\text{d: 1.094 g/mL} &
\end{align*}
\]

\[
\begin{align*}
\text{PhH} + \text{PhH} & \xrightarrow{1 \text{equiv. Mg}} \text{PhH} + \text{PhH} \\
\text{H}^+ & \xrightarrow{\text{H}^+} \text{PhH} + \text{PhH} \\
\text{Triphenylmethanol} & \quad \text{mw=260.3 g/mol} \\
\text{melting range: 158-160} &
\end{align*}
\]
The overall mechanism is illustrated above. The carbanion is generated by electron transfer from magnesium metal. The reactive carbanion then attacks electrophilic carbonyl to give an anionic intermediate (step one). This unstable intermediate rapidly eliminates a methoxide anion (step two). The resulting ketone is attacked again (step three). The resulting anion waits patiently until next laboratory period, at which time acid will be added to protonate the anion (step four).

**Byproducts and Potential Problems** There are two main byproducts and three problems.

1. **The first side product is biphenyl, Ph-Ph**, which is formed in competition with the Grignard reagent PhMgBr. Following initial electron transfer, the phenyl radical Ph• can either accept another electron leading to the desired carbanion, or combine with another phenyl radical to make biphenyl.

2. **The second side product is benzene (Ph-H), resulting from protonation of the carbanion.** The carbanion is supremely basic, so if there is any water in the solvent or in the glassware, or if moist air is allowed to enter the reaction mixture, some of the carbanion will be protonated. **Great care is thus required to ensure “dry”, water-free conditions.**

3. **The third problem is getting the magnesium to actually do the electron transfers!** Pure magnesium is an active metal, so active that any magnesium that has been exposed to air is inevitably coated with a film of magnesium oxide on its surface. This oxide film blocks the bromobenzene from actually contacting active magnesium, and thus prevents the requisite electron transfer. **For a Grignard reaction to work, it is necessary that fresh active magnesium be exposed.** Otherwise no electron transfer from magnesium to bromobenzene can take place, no carbanion can be formed, and no reaction proceeds. We will use two techniques, iodine activation and physical crushing, to activate our magnesium.

4. **The fourth problem is unreacted starting material.** (Could be the Ph-Br, the Mg, and/or the ester).
III. Procedure: Week One

Note: All equipment and reagents must be dry!

Phase 1: Preparing the Grignard Reagent

1. Dig out the following pieces of glassware:
   a. 250-mL round-bottomed flask
   b. “Claisen” two-branched connecting adapter (piece #9 in your kit)
   c. reflux condenser (piece #12 in your kit)
   d. separatory funnel with stopper
   e. drying tube packed with calcium chloride
   f. stick the drying tube into the rubber end of the thermometer adapter

2. Clamp the 250-mL round-bottomed flask to a vertical rod. If possible, use a clamp with grips that are either pure metal or else have non-flammable white coating rather than gray rubber coating. (Rubber clamps will melt and stink when subjected to Bunsen-burner flame!)

3. Light your Bunsen burner and pass the flame over the flask until there is no more steam visible on the surface of the glass.

4. As soon as the steam is gone from the flask, add the Claisen adapter to the flask and flame dry it as well

5. As soon as the steam is gone from both the flask and the adapter, add the reflux condenser to the flask, and flame dry as best you can.

6. While everything is still hot, attach the drying tube into the top of the reflux condenser, add the separatory funnel with it’s stopper on into the other arm of the Claisen adapter.
   - At this point, the interior should be entirely closed from wet air getting in. The separatory funnel blocks out one side, and any air coming in through the column must pass through the drying tube.

7. Weigh out about 2 grams of magnesium metal. (Record weight)

8. When the glassware is cool enough to handle, add tubing to the condenser so that you can run a slow stream of tap water through the condenser. Reassemble the array as quickly as possible.

9. When the glassware is cool enough to handle, lift out the condenser and pour in the magnesium, then replace the condenser as soon as possible.

10. Pour 40 mL of ether into the separatory funnel and put stopper back on.

11. Measure out 9.0 mL of bromobenzene in a graduated cylinder. Record the volume as accurately as possible.

12. Ask the instructor to add one small chip of iodine into the separatory funnel. (The iodine can be added directly to the round-bottomed flask, or to the separatory funnel before the ether is added).

13. Drain the bromobenzene/ether/iodine solution into the round-bottomed flask.
   - **The iodine serves two functions.**
     a. The first is as an **indicator**. The color will disappear when the magnesium is activated and is able to do redox chemistry with bromobenzene.
     b. The second is as an **activator**. Iodine is sometimes able to chemically “clean” the surface of the magnesium so that fresh, active magnesium is exposed so that it can do redox chemistry with bromobenzene. However, it doesn’t often work!

   - **Make a mental picture of how much magnesium you have to begin with, so you can remember later on for comparison.**

14. If the redox chemistry of the Grignard reaction initiates, the iodine color will go away, the solution will begin to get hot, there will be some bubbling, and things may become slightly cloudy.

15. If there is no indication of reaction after two minutes, beg the instructor to come over to crush some magnesium. Note: If yours starts without need for crushing, specifically note this in your writeup.

16. The instructor will use a glass rod to try to crush some of the pieces of magnesium firmly against the bottom of the flask. This will expose fresh, active magnesium that should be able to initiate the redox chemistry and the formation of the Grignard reagent. Trying to crush very very hard magnesium pieces inside a glass flask is dangerous, though; it’s easily possible to punch a hole in the glass. So if somebody is going to poke a hole in your flask, let it be the instructor so he can take the blame rather than doing it yourself!
17. The reaction should be so exothermic that it will be self-boiling for some time. If the rate of boiling subsides, apply a heating mantle (connected to the Variac, not directly to the wall outlet) and apply heat to maintain a good rate of boiling. A power setting of 20-25 is good; do not exceed 25.

18. Maintain boiling for one hour.
   - **Note:** notice how the reflux condenser works. The bottom flask can be boiling hot (which facilitates maximum reaction rate), but the condenser enables you to liquify and recycle all of the boiling solvent.
   - **Keep good procedural and observational notes of everything that you see and do!**

Phase 2: Things to do during the Grignard Hour…
Once the reaction is clearly going, prepare for Phase 3, in which you will add the methyl benzoate ester electrophile to the carbanion that you are making. And do the calculations that you will eventually need to include in your report.

1. Calculate what **volume** (in mL) it will take to add 5.0 grams of liquid methyl benzoate (density = 1.094 g/mL).
2. Calculate the number of **moles** used for magnesium, bromobenzene, and methyl benzoate.
3. Calculate the **overall theoretical yield** (in grams) for your final product of next week, triphenylmethanol (mw = 260 g/mol).
   - To do this, you must **first identify** which of the three reactants (Mg, PhBr, or PhCO₂CH₃) is the **limiting reactant**
   - To do this, you must factor in the overall stoichiometry, which is not all 1:1:1:1. (Given your calculated moles of Mg, how many moles of Ph₃COH could you make? Given your calculated moles of PhBr, how many moles of Ph₃COH could you make? Given your calculated moles of PhCO₂CH₃, how many moles of Ph₃COH could you make?)
   - In calculating theoretical yield for a multistep reaction, theoretically every step will be perfect. (We know otherwise, but we’re talking theoretical yield here…) Thus you don’t need to calculate or measure quantities for any intermediates. **Your limiting reactant and theoretical yield should consider only original reactants and final product, all things which are easily quantified.**
4. After the Grignard solution has reacted for one hour, check to see how much magnesium is left. Any qualitative estimate of about how much is left? (None? 10%? 50%?)
   - What implications might this have on your possible yield? Is it necessary for all of your magnesium to have reacted completely in order to get 100% yield? Or could you get 100% yield even if some of your magnesium remains unreacted?

Phase 3: Reacting the Grignard Reagent with the Methyl Benzoate
1. After the hour is up, let the reaction cool down (an ice-water bath might help).
2. Add **15 mL of ether** to your separatory funnel. (Stopcock closed).
3. Add **5.0 grams of methyl benzoate** to your separatory funnel by syringe. (Remember, you calculated this volume in Phase 2….)
4. Remove the cold bath (if you have one on), then drain the ester/ether solution into the round-bottomed flask, slowly so that the reaction doesn’t overheat to much. Try to shake the solution around as much as possible (hard to do when it’s clamped!) If things start to boil hard, reapply the cold bath.
   - Record your observations!
5. If everything is added without excessive boiling, try to shake everything up, and give it five minutes or so to continue reacting.
6. If the reaction is still hot, cool it with the ice bath.
7. Remove all the glassware from the top of the round-bottomed flask, and stuff in a rubber stopper.
   - **Note:** it is essential that the solution isn’t hot when you do this. If it is, then when it cools it will create a vacuum and suck the stopper in…)
   - **Note:** it is essential that the vigorous exothermic reaction is done before you stopper the flask. Otherwise if stirring or further reaction generates enough heat, it will cause the ether to boil and blow the stopper off!
8. Stash the round-bottomed flask with the chemicals and the stopper into a secure spot in your drawer, and wait till next lab to finish!
IV. Procedure: Week Two

1. Record your observations for what your mixture looks like at this point.

2. Remove the stopper, and add about 40 grams of ice and 50mL of 2M sulfuric acid
   • The acid will react exothermically with both the anion and unreacted magnesium. The ice is there
     simply to absorb the heat.

3. Swirl well to promote hydrolysis and break the solid clumps. Use a spatula to break up the chunks.

4. In the process, three things should happen:
   • The anion should be protonated, giving the neutral organic alcohol product. This should partition
     into the organic ether layer.
   • Magnesium salts should be ionic, so they should partition into the aqueous layer.
   • Unreacted leftover magnesium metal will react with the acid to give molecular hydrogen. That’s
     what causes the bubbling. (1 Mg + 2 H⁺ → Mg²⁺ + H₂ gas)

5. Pour the mixture into your separatory funnel. (The magnesium doesn’t need to be totally dissolved…)

6. Pour an additional 10 mL of sulfuric acid and 10 mL of ether into your flask, swirl to try to dissolve up
   anything left on the walls, and pour into the separatory funnel. (These need not be measured, just pour
   some in approximately.)

7. Drain off the bottom aqueous layer into a beaker.

8. Add another 20 mL of sulfuric acid into the separatory funnel, shake it up, and drain off the aqueous
   layer again. Pour the combined aqueous layers into the aqueous waste bottle in the hood.

9. Drain the organic layer from the separatory funnel into an Erlenmeyer flask.

10. Add about 5 grams of sodium sulfate to “dry” the ether layer. Add additional scoops if the sodium
    sulfate is all clumped up (indicating that there may be too much water for the sodium sulfate to handle).

11. Plug your long-stem funnel with a little glass wool

12. Pour the ether solution through the glass-wool plugged funnel into a different Erlenmeyer flask. The
    wool should be sufficient to filter off the solid sodium sulfate, and only allow the solution to get into
    the flask. Rinse your original flask and the sodium sulfate with an additional portion of ether.
    • At this point, your solution should be free of water and of magnesium salts. Other than the ether
      solvent itself, you should have nothing but the desired product and organic contaminants.

13. Prepare a sample for GC-MS analysis. Take out one pipet, and add it to your 25-mL beaker. Add a
    solvent (ether or acetone or whatever organic solvent is available in the hood) to dilute to 10 mL. Take
    one pipet of this diluted mixture into your 10-mL Erlenmeyer, and dilute this to almost 10 mL. Take
    one pipet of this diluted solution and place it into a GC-MS vial. Label it. You can save it and run it
    later (you’ll need to run another one later anyways.)

14. Make a TLC plate with five pencil marks for five tracks ready:
    a. Authentic biphenyl
    b. Authentic methyl benzoate
    c. Crude mixture
    d. Purified mixture
    e. Post-crystallization solvent

15. Take a capillary droplet from your mixture, and put it on the “crude mixture” spot. Take droplets from
    the authentic biphenyl and methyl benzoate bottles in the hood and apply them as well. Save the plate
    until you’ve finished purifying the product, at which point you’ll be able to apply your last spot.

16. Add 25 mL of “ligroin” solvent (all hydrocarbons, mostly hexanes, but not pure) to your ether solution.
    The product is more soluble in ether than in hydrocarbons, so you are essentially adding some “bad
    solvent” to facilitate a mixed solvent recrystallization.
17. Add a boiling stick to your organic solution

18. Now heat your solution on a hot plate. A power setting around 5 or 6 might be a good starting guess?

19. Boil the solution down to 20-25 mL or so. (Crystals may start to form before this, depending on your yield. But if you stop boiling as soon as the first crystals form, you’ll still have too much solvent and will get a low yield.) Add another 20 mL ligroin and again boil down to around 20-25 mL.

20. Remove from heat, and let cool slowly to grow your crystals, first to room temperature and then to 0ºC.

21. Filter your crystals with Buchner funnel and aspirator.

22. Rinse with cold solvent. (What to use?)

23. Take a droplet from the solvent and put it on the tlc plate in the “post-crystallization solvent” spot

24. Take about 0.2 grams of your crystals (needn’t be bone dry) and dissolve in 3 mL of ether. Then take a capillary and put a droplet of this purified material onto your tlc plate in the “purified” spot.

25. Prepare a sample for GC-MS analysis. Take out one pipet of the solution you made in the previous step, and add it to your 25-mL beaker. Add a solvent (ether or acetone or whatever organic solvent is available in the hood) to dilute to 10 mL. Take one pipet of this diluted mixture into your 10-mL Erlenmyery, and dilute this to almost 10 mL. Take one pipet of this diluted solution and place it into a GC-MS vial. Label it.

26. You can run this GC-MS and the “crude” GC-MS. You will be able to evaluate the purity of your sample, and compare it to the purity you had prior to crystallization. You should also be able to use the library to perhaps evaluate what contaminants were present prior to purification and what contaminants perhaps remained after crystallization.

27. Run the tlc in designated solvent (10% ethyl acetate/hexane?), and analyze by UV and the “dip” solution.
   • Mark down the results, with the following questions in mind:
     o Is biphenyl present in the crude mix? In the purified material?
     o Is methyl benzoate present in the crude mix? In the purified material?
     o Any other side products in the crude?
     o Did recrystallization purify the material at all?
     o Did crystallization get all of the product out of the solvent?

28. Take a melting range on your final product. (Should melt above 150º, so heat accordingly)

29. Get your final mass.
Lab Report Requirements and Format: The following layout is standard for a “synthesis reaction” report. Provide the parts and information in the sequence specified.

1. Title = Reaction Summary
   For an organic reaction, there is no point in having a Worded Title: The chemical reaction is the best title summary of what you did!

2. Listing of all Chemicals Used
   • This should include all chemicals used, including solvents.
   • For each chemical, you should include the actual quantity used and measured. For example, with the methyl benzoate you measured a volume by syringe, rather than by weighing on a balance. So you should list the volume you actually used rather than just the weight.
   • If a person was later to repeat your experiment, they should be able to look at this list and know all the chemicals they’d need to have on hand and in what quantities, in order to complete the experiment.
   • In some cases, there may be considerable roundoff (you needn’t keep precise record of the quantity of solvent that was used, for example, or of sodium sulfate drying agent…)
   • For reactants that might possibly be limiting reactants and might possibly factor into calculation of the theoretical yield, however, you must include more than just the quantity of chemical used. You should also include a conversion from what you measured into the number of moles used.

3. Calculation of Theoretical Yield
   • Specify which chemical is the limiting reactant
   • Given moles of limiting reactant, calculate theoretical moles of product
   • Given moles of product, calculate theoretical grams of product.
   • Note: Why do this so early in report?
     o First, because it fits in near your mole calculations above.
     o Second, if calculated in advance, as with most research, you know which chemical is limiting and thus must be measured most carefully, but you also know which are in excess and thus need not be measured with equal precision.
     o Third, it’s nice to know approximately how much material is expected, so you can recognize whether your actual results are reasonable or way off.

   • For this particular experiment, the “procedure” section will be by far the biggest portion of your report.
   • This should be a concise but detailed description of things, including:
     o What you actually did (even if not recommended or not from recipe)
     o All observations should be included. These include all observed changes, such as:
       • Changes in color
       • Changes in solubility (formation of precipitate or cloudiness…)
       • Formation of bubbles
       • Changes in temperature (like, reaction became hot…)
     o Time and temperature details:
       • Whenever you heat something or cool something, the procedure should specify
       • Specify times. Whether you boiled for 5 minutes or 5 hours matters!
   • Writing details: As a record of what actually happened, the report must be written in past tense, not command tense. (Teachers in other classes may have different requirements). But you are not obligated to avoid references to “I” or “we” in this class.

5. Product Analysis
   • Any NMR, mp, bp, TLC information, and GC-MS. For this report, mp, gc-ms, and TLC information must be included.
   • Final yield and percent yield information. (include detailed TLC picture and interpretation…)

6. Discussion/Summary. Need not be long, but any conclusions or excuses would go here…”

7. Answers to any assigned Questions
Assigned Questions, Grignard Lab

1. Draw a detailed, step-by-step mechanism for the reaction you actually did: (on attached sheet?)

2. Triphenylmethanol can also be prepared by the reaction of PhMgBr with diethylcarbonate (CH₃CH₂O)₂C=O, followed by H⁺ workup. Draw a detailed, step-by-step mechanism for the following reaction: (on attached sheet?)

3. If you hadn’t bothered to flame-dry your glassware or used a drying tube, what byproduct would have formed?

4. If the methyl benzoate you used had been wet (contained water), what byproduct would have formed? (Note: the answer for this problem may or may not be the same as for previous problem.)

5. Your yield was considerably less than 100%. Discuss where you think things might have come up short. You may wish to differentiate reaction things (reasons or evidence that you didn’t have complete chemical conversion) versus isolation things (reasons or evidence that you didn’t isolate all of the product that was actually made chemically). (It’s possible that your TLC may support or disprove some possible explanations.)

6. Given the quantities of chemicals used in this recipe, one could conceivably have gotten a 100% chemical yield without having completely reacted all of the magnesium, or without having completely reacted all of the bromobenzene. But it would not have been possible to get 100% chemical yield if the methyl benzoate didn’t react completely. Explain.