Introduction to 1H-NMR Spectroscopy   Hydrogen NMR interpretation is more complex than 13C-NMR, but provides extra information that is unavailable from carbon NMR. In interpreting carbon NMR, we focused on how many unique carbon lines were present, and where they were located (chemical shifts). In hydrogen NMR, but two additional factors, "integration" and "splitting", are useful.

The four facets of 1H NMR spectroscopy:
1. Number of signal sets => the number of symmetry-unique hydrogens
2. Chemical shifts => chemical environment/hybridization/functional groups
3. Integration => how many hydrogen atoms cause a signal.
   - 3H   => CH_3 group (or 2H and 1H groups superimposed)
   - 2H   => CH_2 group (or two nonequivalent 1H groups superimposed)
   - 1H   => CH or OH group
4. Splitting   =>   information about how many H's are connected to adjacent carbons
   - N lines   => N-1 "neighbor" H's (when working from spectrum to structure)
   - N neighbors   => N+1 lines (when you know what a structure is, and you're trying to predict what it's spectrum should look like)

Summary of Steps in 1H NMR Interpretation: (Not all will be needed to get Answers Today)
1. **Count how many signal sets** you have. **This will tell you how many types of hydrogen-bearing carbons** you have.
   - Hydrogens attached to symmetry-equivalent carbons will give equivalent signals
2. **Check diagnostic "chemical shift" windows** of the lines **to provide yes-or-no answers regarding the presence or absence of key functional groups** in your molecule.
3. Check the integration of each signal set.
   - 3H   => CH_3 group 2H   => CH_2 group 1H   => CH or OH group
4. Check the splitting of each signal set.
   - For a signal set with N lines   =>   N-1 hydrogens will be attached to carbons directly connected to the carbon of the signal set

I. Number of Signal Sets
1. Nonequivalent H's have different chemical environments and give different signals
2. Symmetry-equivalent H's have the same chemical environment and give the same signal
   - The number of signal sets tells you how many different types of hydrogens are present
3. On an **achiral** molecule (alkenes excepted), hydrogens on a given carbon will be equivalent.
   - all three H's on a CH_3 group
   - both H's on a CH_2 group
4. The number of signal sets may sometimes differ from the number of carbons:
   a. Symmetry equivalent carbons and hydrogens
   b. Hydrogen-free Carbons: No attached H, no H signal!   (Carbonyl carbons rarely have H's…)
   c. OH Groups: OH as well as CH's give hydrogen signals
   d. CH_2 H's are NONEQUIVALENT in "Cis/Trans" Situations:
      - In **Alkenes**, or when there is a **chiral center** in the molecule.
5. Strategy Keys:
   a. If possible, determine how many signal sets you have in a spectrum. (Useful when working from spectrum to structure).
   b. For a particular structure, determine how many signal sets you should have. (Useful when matching unknown candidate structures with actual spectra, as in today's lab.)
   c. **End-Check: Check that the number of signal sets in your spectrum matches with the structure you believe you actually have! If not, structure needs correction!**
   d. **Beware of overlaps!**

II. "Chemical Shifts" of the Signal Sets

7's (6.5-8.4)   **Aromatic** sp^2 hybridized C-H's

3's (2.8-4.5)   **Oxygenated** sp^3 hybridized C-H's (halogenated and nitrogenated alkyl C-H's will also come in this window, although no candidates for today's lab). Oxygenated sp^3–carbons are routinely present for the following functional groups that contain oxygen single bonds:
- **alcohols**,
- **ethers**, or
- **esters**

2's (1.8-3.1)   **Allylic** sp^3 hybridized C-H's (sp^3 hybridized C-H's that has a double bond attached to the sp^3 hybridized C). Allylic signals routinely appear when one of the following double-bonded functional groups is present:
- **carbonyls**, (ketones, esters, aldehydes, acids, amides)
- **alkenes**, or
- **aromatics**

1's (0.7-2.0) sp^3 hybridized C-H's, with **no attached Functional Groups**
- **Note:** Many molecules with non-functional alkyl portions will give a lot of signal in this area.

0-5 (anywhere!)   **Alcohol** O-H hydrogens
 (normally 1.5-3.5 range)

How do I process and use what I see from my Chemical Shifts?

1. **Recognize OH's**. Because an OH can come anywhere, it can easily cause mistaken conclusions. An OH in the 2's, for example, can falsely make you think that you have an allylic C-H when you really don't. Thus it is really helpful to recognize OH's when they appear so that they don't confuse you. **Three recognition factors for OH signals:**
    1. They always **integrate for 1H**, never for 2H or 3H

    2. They usually **appear as singlets**. The only way to have a 1H singlet is for it to be an OH.

    3. If you have an alcohol OH signal, of course you will also have some C-H signals in the 3.0-4.5 area. (For the hydrogens on the hydroxy-bearing carbon.)

2. **Check each of the zones. Each one gives you a yes or no answer about the presence of absence of the featured group**.
    - Do I have something in the 7's? (Other than a solvent singlet…)? If yes   => aromatic

    - Do I have something in the 3's? If yes   => alcohol, ether, or ester (or OH)

    - Do I have something in the 2's? If yes   => ketone, aromatic, or alkene (or OH)

    - Do I have something in the 1's? If yes   => some nonfunctional alkyl carbons (or OH)

3. End-Check: Check that the functional groups indicated by your chemical shift information match with the structure you believe you actually have! If not, structure needs correction!

Miscellaneous Chemical Shifts Notes
1. <u>Approximate 1's, 2's, 3's, and 7's and spillover</u>: The regions are somewhat approximate, and have some spillover. But it's still useful to basically talks about the "1's", "2's", "3's", etc. to discuss the major windows. Even though something might actually come at 4.2, it's still useful to refer to that as appearing in the "3's" group and make conclusions accordingly. I'll still refer to something as coming in the "1's" group even if it comes at 0.8.
2. <u>Hybridization</u>: sp^2 hybridized C-H's come above 5, sp^3 hybridized C-H's come below
3. Oxygenated C-H's come higher than non-oxygenated analogs.
4. When two functional groups are impacting, chemical shifts change. (If a CH2 group is doubly allyic, it won't show in the 2's. If a CH2 is doubly oxygenated, it won't show in the 3's.)
   - For this introductory lab, you won't need to worry about this.
5. OH's are wildcards because they can come anywhere, and can cause confusion.

III. Integration   Unlike in carbon NMR, the sizes of H-NMR signal sets are very useful and informative.
1. The signal area ("integral") is proportional to the number of hydrogens causing the signal.
   CH_3 => CH_2 =>   CH or OH   =>

2. The key is not the signal height, but rather the signal **area**.
   - The signal **area** is measured by "integration lines". Differentiate integration marks, and what they mean, from signal lines themselves.
3. Relative areas correlate ratios of H's. **Convert to simple whole-number ratios** (and round off freely).

1:1 => CH2 to CH2, or CH3 to CH3	1.5:1 => 3H:2H (CH3 to CH2)
2:1 => 2H:1H (CH2 to OH)	5:2 => 5H:2H (C6H5 to CH2, common with aromatics)
3:1 => 3H:1H (CH3 to OH)	6:1 => Common with isopropyls, CH(CH3)2

4. <u>Clean sets involving equivalent H's give clean, symmetric signal sets</u>:
   a. 1H => CH or OH
   b. 2H => CH_2
   c. 3H   => CH_3
   d. 6H => 2 equivalent CH_3 groups
5. <u>Unsymmetrical messy sets involving overlapping signal sets.</u> (these will routinely not look nice and symmetric…)
   a. 3H => OH overlapping a CH_2
   b. 4H   => two overlapping but not exactly equivalent CH_2 groups; or a CH_3 overlapping an OH or CH
   c. 5H => common in the 7's, for 5 overlapping arene H's; also common in the 1's, when a CH_3 and CH_2 overlap

How do I process and use what I see from my Integrations?
1. **Distinguish "Clean" Signal Sets from Overlapping Signal Sets**
   o Clean ones look symmetric, overlapping sets do not

2. **For the Clean sets, the integration tells you what kind of group you have**
   a. 1H => CH or OH   (methine or hydroxyl group)
   b. 2H => CH_2 (methylene group)
   c. 3H   => CH_3 (methyl group)
   d. 6H => 2 equivalent CH_3 groups

3. **End-Check: Check that the "groups" your integration shows match with the structure you believe you actually have! If not, your structure needs to be corrected!**

IV. Splitting Hydrogen signals are routinely split into multiple lines. The number of lines in a signal set tell us nothing about "the signal" C-H's themselves that cause the signal (whether it's a CH_3 or CH_2 group, or whether it's allylic or oxygenated…). But the splitting tells us something else that is really useful: what kind of CH groups are attached to the group of interest! It provides great information about "neighbor groups" and helps explain how the components of an organic molecule are sequenced.

Rules of "Splitting"

❑ **N-1 Rule:**   **N lines   => N-1 neighbor H's** (H's directly attached to carbons attached to the signal C-H group causing the signal)
  - The N-1 Rule is useful when <u>working from spectrum to actual structure</u>
❑ **N+1 Rule:**   **N neighbor H's   => N+1 lines**
  - The N+1 Rule is useful when <u>working from structure to actual spectrum</u>

1. OH hydrogens don't participate in splitting ~75% of the time. About 25% of time they do.
2. C-H hydrogens participate in splitting (always)
3. For today's labs and for simple molecules, the N-1/N+1 rules are good. The rules actually work only if the neighbor H's are equivalent. The rule can break down when some of the neighbor H's differ significantly from each other
4. Splitting from H's further distant than neighbor carbons sometimes occurs, but usually the amount of splitting is too small to worry about
5. Physics Origin: hydrogens are quantized little magnets. Neighbor hydrogen magnets can align so as to either reinforce (spin up) or counteract (spin down) the external magnetic field

```
N+1 Rule (Given structure, how many lines a spectrum should give)
```

Neighbors	2	3+2	2	0	Neighbors	0	-	2	2+3	2
Lines	3	6	3	1	Lines	1	-	3	6	3

(Notice: OH doesn't split...)

```
N-1 Rule (Given spectrum, how many neighbors a structure should have)
```

Lines	1 (s)inglet	Lines	2 (d)oublet
Neighbors	0	Neighbors	1

Lines	3 (t)riplet	Lines	4 (q)uartet
Neighbors	2	Neighbors	3 etc.

6. Splitting nicknames:
  - 1 line => singlet (s)   2 lines => doublet (d)   3 lines => triplet (t)
  - 4 lines => quartet (q) 5 lines => pentet (p)   >5 lines => multiplet (m)

How do I process and use what I see from my Splitting?
1. Use **integration** for a given signal to determine if your signal set is a CH_3, CH_2, or CH group
2. Then use the number of lines in the signal set and the N-1 Rule to see how many hydrogens must be present on <u>neighboring</u> carbons that are attached to your signal set
3. **End-Check: Check that the structure you believe you actually have would give the splitting you are actually seeing in your spectrum. If not, your structure needs to be corrected!**

V. Standard Summary Report and/or Prediction Formats   There is a standard summary report format for H-NMR's which addresses chemical shift, integration, splitting, and the source hydrogens.

Ex: $CH_3OCH_2CH_2CH_2C(O)CH_3$ (I'll number the carbons from left to right...)

Summary Report Format

Shift	Int	#lines(split)	Source
3.79	2H	3 (t)	(CH2-2)
3.48	3H	1 (s)	(CH3-1)
2.34	2H	3 (t)	(CH2-4)
2.16	3H	1 (s)	(CH2-6)
1.55	2H	5 (p)	(CH2-3)

Prediction Format

Source	Shift	Int	#lines(split)
(CH3-1)	3's	3H	1 (s)
(CH2-2)	3's	2H	3 (t)
(CH2-3)	1's	2H	5 (p)
(CH2-4)	2's	2H	3 (t)
(CH2-6)	2's	3H	1 (s)

VI. Miscellaneous

1. **Subtracting the Solvent Lines: Don't Count the Singlet at 7.26** $CDCl_3$ is routinely used as solvent, but is contaminated by trace $CHCl_3$ which gives a signal at 7.26.

2. **Subtracting the Reference Line: Don't Count the Line at 0**   A reference chemical [$(CH_3)_4Si$] is included to define where "zero" is.

3. **Subtracting the Water Line:**   Often a little moisture will be in the solution. This will often appear somewhere around 1.6, but it wanders depending on hydrogen-bonding factors.

4. **Subtracting the Acetone Line?**   Acetone shows a singlet at around 2.15. If acetone has been used to rinse but hasn't dried yet, this will appear. But it normally integrates incorrectly.

5. **How do I know what's a real signal versus a signal arising from an impurity that I should ignore?**   For today, if in doubt ask the instructor! The instructor will confirm which lines you should or shouldn't consider in doing your analysis. However, one useful recognition tip is that if something integrates badly, it's likely a contaminant.   Integrals are supposed to be nice whole-number ratios (1:1, 2:1, 3:2, etc.)

6. **Beware of Overlapping.** Overlapping is most routine in the benzene area (7's), and also in the alkyl area (1's), but happens elsewhere as well. OH signals often overlap other signals. For this week, if in doubt ask.

Summary of 1H-NMR Interpretation

## I. Number of Signal Sets

II. "Chemical Shifts" of the Signal Sets

7's (6.5-8.4)	**Aromatic** sp^2 hybridized C-H's

3's (2.8-4.5)   **Oxygenated** sp^3 hybridized C-H's. Oxygenated sp^3–carbons are routinely present for the following functional groups that contain oxygen single bonds:
- **alcohols**,
- **ethers**, or
- **esters**

2's (1.8-3.1)   **Allylic** sp^3 hybridized C-H's (sp^3 hybridized C-H's that has a double bond attached to the sp^3 hybridized C). Allylic signals routinely appear when one of the following double-bonded functional groups is present:
- **carbonyls**, (ketones, esters, aldehydes, acids, amides)
- **alkenes**, or
- **aromatics**

1's (0.7-2.0) sp^3 hybridized C-H's, with **no attached Functional Groups**
- **Note:** Many molecules with non-functional alkyl portions will give a lot of signal in this area.

0-5 (anywhere!)   **Alcohol** O-H hydrogens
 (normally 1.5-3.5 range)

III. Integration   These **must be simple whole-number ratios** (2:1, 3:1, 3:2, etc..)

IV. Splitting
- ❑ **N-1 Rule: N lines   =>   N-1 neighbor H's** (H's directly attached to carbons attached to the C-H group causing the signal)
  - The N-1 Rule is useful when working from spectrum to actual structure
- ❑ **N+1 Rule: N neighbor H's   =>   N+1 lines**
  - The N+1 Rule is useful when working from structure to actual spectrum

Note: OH hydrogens don't participate in splitting (normally)

Hydrogen NMR, The Experiment. What you need to do, an Overview.

H-NMR Unknown Candidates

K1   K2   K3   K4   K5   K6

E1   E2   E3   E4   E5

B1   B2   B3   B4   B5

A1   A2   A3   A4   A5
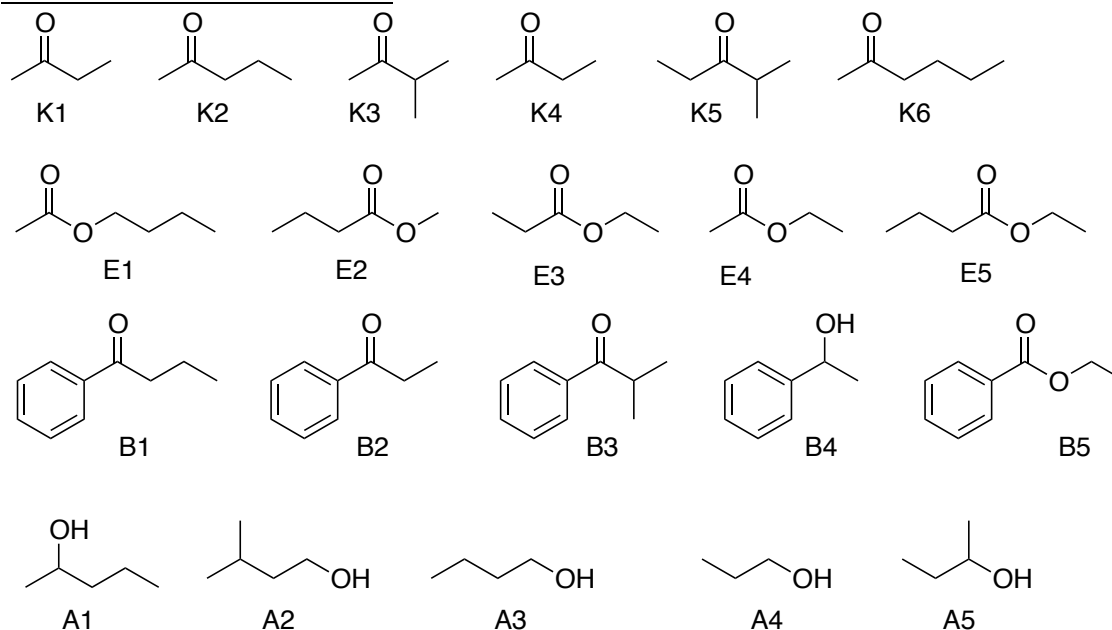
Lab and Lab Report Requirements
1. **Prepare and run one sample**.
   - Using a syringe, add 0.1 mL of unknown sample. Add this first.
   - Add 0.8 mL of CDCl3 solvent.
   - Cap and shake to ensure mixing.
   - The experiment run will be "Proton8"

2. **Identify and interpret at least two NMR spectra**.
   - You can get the "other" NMR by opening somebody else's data from the queue or from the data folder.
   - It may be your partner's data, but it doesn't need to be.
   - If you do match with a partner, it might be more fun for consulting.
   - There is a handin form later.

3. Get at least one full print for each of the two unknowns.
   - Horizontal expansions are not required but may be very helpful to be able to see and interpret the splitting
   - Manual integrations are not required but may be very helpful to recognize the integration values

4. Predict what some other structures would give. There is a handin form for this later.

5. Due date:

User's Guide to NMR: General
- For help, see Dr. Jasperse, Hagen 407J, phone 477-2230   Draft 10/28/15

1. **Add sample to a Spinner/Turbine**

2. **Adjust depth** by placing the turbine into the golden depth finder

3. **Load sample/turbine into autosampler.**
   - Press the round white **Access Request Button** on the panel below the sample trays/doors
   - **Wait until "status" light turns to a solid yellow, and the message panel reads "door unlocked"**

4. **Opening Program on Computer: Usually already open**, and usually to correct "operator"
   - If not open: Operator: Should be your class or research group   Password: none.
   - To switch operator, click **Logout** from submit mode and select the correct operator

5. **"Submit" vs "Spectrometer" modes:** New Study/Submit Queue to submit; Spectrometer to print/view
   - Click "**New Study**" button (lower left) to jump from Spectrometer to Submit mode
   - Click "**Cancel**" button (lower left) to exit Submit queue and go to Spectrometer:

6. **Experiment Selection** (from within Submit mode). Usually preselected for organic labs.
   - If not already in New Sample/submit queue mode, push **New Study** button on lower left
   - **Proton8** is the normal H-NMR experiment, under the "UserStudies" folder
   - For some classes/operators, **Proton8** has been set to open by default, since most NMR's are regular H-NMR's
   - Add experiments as needed from the Experiment Selector.
   - To **edit or delete**: **right click** on experiment and select "Open Experiment" or "Delete Experiment"

7. **3 Step Submission** (assuming the experiment already specified, and still/already in Submit mode).
   a. Fill **Sample Name** (for both computer filing and printout recognition)
   b. **Click Sample Spot**: Click on the button showing your sample site. (Remember/record! ☺)
   c. **Submit**: clicking the red **Submit** button on the lower left side.
   - **Note**: Can repeat this 3-step sequence for new samples/new students, if running same experiment

   - **Comment box**: (can add comments for the paper printout). (Control C to cut and Control K to paste)
     Other submission options of possible use for advanced labs, research, or offsite Concordia users:
     Solvent; **Autoplot** (offsite Concordia users should turn this off); **Email**; **Email Address** (offsite Concordia users should set this correctly!☺); **Lock**: (with non-deuterated solvent run unlocked), **Shim** (with non-deuterated solvent run 1H PFG); **Tune**

8. **Opening Completed Samples for Printing and Processing. ("Spectrometer Mode" required)**
   - Must be in "Spectrometer" mode, not "Submit" mode.
   - If in submit mode, "Submit" button will display (lower left). Click "**Cancel**" to exit Submit mode.
   - In "Spectrometer" mode, must have "Zones" map displayed (96 sample nodes show). Click on little circle icon (🔵) to the upper left of the spectra-display panel, if zones map not already open.
   f. Right click on sample number
   g. Click "Show Study"
   h. Click on file folder name located on the left
   i. Then **double click** on spectrum you want to view to load it into the spectra-display viewscreen.
   j. Process > **Auto Plot** or **Print**. See next page for more detailed printing and processing instructions.
   - Re-click the little circle icon (🔵) to get back to zone map in order to open other files
   - To return to "Submission" mode in order to run more samples, click "New study"

9. **Logout:** Click **"Logout"** button underneath spectrum-display from Submit Mode.

10. <u>Plotting (when wanting non-automatic plots)</u>
- Must be in the process mode. (Highlight "Process" beneath the spectrum display)
a. Click "**Auto Plot"** or "**Print**" button, way on lower right corner of page.
b. Re-click if you want to print additional copies for the other students
  - Note to offline Concordia users: this "plot" command will print to MSUM NMR-room printer. ☺
  - For advanced labs or research groups, additional plot preferences are available in the process mode by clicking "**Plot** (Beneath spectrum display, 2nd from bottom underneath "Start")

11. <u>Horizontal Expansions</u>
- With spectrum displayed on screen, use a panel of display icons on the far right.
a. Click on the magnifying glass icon (6th icon down, 🔍)
b. Move your cursor to the left end of the zone you want to expand, then **<u>hold down left mouse button</u>** and slide it to the other end of the zone you want to expand.
  - To return to the full display, you can either click on the 3rd icon (✖) or the 5th icon (🔍).
  - If the lines aren't tall enough, type "vsadj" (vertical scale adjust) on the command line.

12. <u>Manual Integration: Defining Integrals Yourself (see #13 to also give nice integral numbers)</u>
- With spectrum displayed, must be in the process mode ("Process" beneath the spectrum display)
a. Choose "**Integration**" (Beneath spectrum display towards left, 2nd underneath "Start")
b. Hit "**Clear Integrals**" button (slightly further to the right and lower down from previous button)
c. Hit "**Interactive Resets**" button (immediately above the "clear integrals" button) and define
  1. Move cursor beyond the left end of the signal you want to integrate.
  2. Left-mouse click-and-release
  3. Move the cursor to the right of the signal, and again click-and-release. Everything between the two "clicks" will be integrated.
  4. Repeat this for each area you want to integrate.
d. Click very top cursor icon (▮▮▮) to the right of the display screen to regain normal cursor function

13. <u>Setting Nice Integral Numbers (</u>While already in integration mode following steps a-d above**<u>)</u>**
a. Click cursor on one of your integral regions
b. Click "Normalize Area to" "**<u>Single Peak</u>**" below "Set Integral Area" panel underneath the display
c. Set "integral area" to some nice whole number (1, 2, or 3, depending on your molecule)
d. Click the "set integral value" button
  - If it says "cursor is outside of integral region", then reset the cursor on an integral of choice, and re-click the "set integral value" button again.
  - Click "**Auto Plot"** (lower right) in order to print.

14. <u>Other Processing Options for Advanced Users/Research Groups/2D-NMR</u>
1. Peak Picking	4. Insets	6. Absolute Concentration Integration
2. Vsadj	5. Arraying spectra	7. 2D NMR processing, including varying the signal intensity
3. wp=2p sp=2p plot		

15. <u>Opening Spectra From the Data Folders</u>
- Click on the Folder icon and find your class or research professor's folder
- Double-click on the folder with your name.
- Double click on the experiment file
- To get the Folder icon to go back up a step, click on the Folder icon again, then click ONCE only on the little icon that shows an arrow up

16. <u>Getting the last sample out and replacing with a Lock Sample (if auto-eject isn't turned on)</u>
a. In "Spectrometer" mode, display "zones" map (▣)
b. Right click on sample 48 => select "Sample in Magnet" (3rd choice from the bottom) => OK.

17. <u>Logout:</u> Click **<u>"Logout"</u>** button underneath spectrum-display

Hydrogen NMR Lab Handin   **Name**

A. For each of the following chemicals, completely fill out the "Prediction Format" tables to predict what you would expect.

Standard Prediction Format: Ex: $CH_3OCH_2CH_2CH_2C(O)CH_3$ (Carbons numbered left to right…)

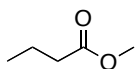| Source	Shift	Int	#lines(split)
(CH3-1)	3's	3H	1 (s)
(CH2-2)	3's	2H	3 (t)
(CH2-3)	1's	2H	5 (p)
(CH2-4)	2's	2H	3 (t)
(CH2-6)	2's	3H	1 (s)

Notes:
- Write numbers or letters next to each carbon in each structure to be used for identification purposes
- For the number of lines, you can include a number, you are not required to use the s, d, t, q descriptors
- For the chemical shifts for OH hydrogens, just write in "???"
- For OH hydrogens, assume they are 1line singlets and that they do not split CH hydrogens
- For the chemical shifts for others, enter either "1's", "2's", "3's", or "7's"
- Aromatic hydrogens tend to overlap. For aromatic hydrogens, just combine them all. For example, write "aryl" for source; "7's" for chemical shift; "5H" for integration, and leave # lines empty.
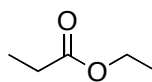
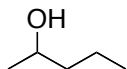Structure   Predicted NMR

Source	Shift	Integ.	# lines

Structure   Predicted NMR
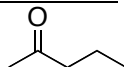
Source	Shift	Integ.	# lines

Source	Shift	Integ.	# lines

Source	Shift	Integ.	# lines

OH

Source	Shift	Integ.	# lines
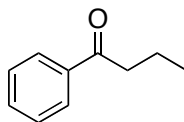
Source	Shift	Integ.	# lines

B. Draw the structures and use the Standard Summary Report format to interpret the features of the two unknows, the NMR that you ran and the other NMR that you interpreted.

Example **Standard Summary Report:** $CH_3OCH_2CH_2CH_2C(O)CH_3$ (Numbered from left to right…)

Shift	Int	#lines(split)	Source
3.79	2H	3 (t)	(CH2-2)
3.48	3H	1 (s)	(CH3-1)
2.34	2H	3 (t)	(CH2-4)
2.16	3H	1 (s)	(CH2-6)
1.55	2H	5 (p)	(CH2-3)

1. Which unknown did you run yourself? (H1, H2, …) _____

2. Draw its structure and make up numbers or letters next to each carbon.

Structure   Actual NMR

Chemical Shift (to the nearest 0.1 ppm)	Integ.	# lines	Source

3. If you know, which was the other NMR that you analyzed? (H1, H2, …) _____
    • Note: you don't need to know or report this if you don't actually know, which is well possible…

4. Draw the chemical structure and make up numbers or letters next to each carbon.

Structure   Predicted NMR

Chemical Shift (to th nearest 0.1 ppm)	Integ.	# lines	Source

C. Attach your NMR printouts. At minimum, include the unexpanded NMR printouts for both of the two samples. If you printed out horizontal expansions or printouts with manual integrations, you can include those too.