Experiment 4: **Equilibrium Thermodynamics of a Keto-Enol Tautomerism Reaction**

<u>Reading:</u>

SGN: Experiment 21 (p.256-263), Experiment 43 (p.456-459).
Quanta: Nuclear magnetic resonance, Relaxation

All reactions tend towards equilibrium. Equilibrium occurs when the concentrations of the reactants and products remain constant over time. Synthetic chemists try design reactions that achieve equilibrium when the concentrations of the products are much greater than those of the reactants: these are reactions that have high yields, or "go to completion". However, there are many useful and important reactions that reach an equilibrium state containing significant amounts of both products and reactants. In this experiment, you will characterize the equilibrium thermodynamics of the keto-enol tautomerization reaction for 2,4-pentanedione (also named acetylacetone, or acac).

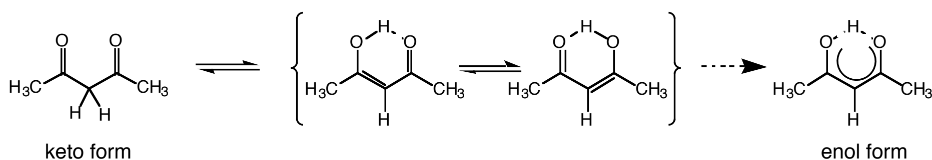

Figure 1: Keto-enol tautomerization reaction for 2,4-pentanedione. The two center enol structures are in fast exchange in this experiment, and can be treated as the single structure shown on the right.

The relative amounts of products and reactants at equilibrium is characterized by the equilibrium constant. For this reaction, K_{eq} is defined as:

$$K_{eq} = \frac{[enol]_{eq}}{[keto]_{eq}} \tag{1}$$

where the subscript *eq* indicates that all concentrations are measured at equilibrium. The overall tendency for this reaction to occur (proceed to the right) is governed by the change in the Gibbs free energy, which at any time during the reaction is given by:

$$\Delta G = \Delta G^0 + RT\ln Q \tag{2}$$

where ΔG^0 is the standard Gibbs free energy change, R is the gas constant, T is the temperature (in Kelvin), and Q is the reaction quotient. When the reaction reaches equilibrium, $\Delta G=0$ and $Q=K$, so that:

$$\Delta G^0 = -RT\ln K_{eq} \tag{3}$$

If the reactants are favored at equilibrium, $K_{eq}<1$, and $\Delta G^0>0$. Conversely, if the products are favored, $K_{eq}>1$, and $\Delta G^0<0$. Only if the equilibrium is perfectly balanced will $K_{eq}=1$, and $\Delta G^0=0$. Thus, the *standard* free energy change indicates the position of the equilibrium (amounts of products and reactants) while the *total* free energy change indicates which direction the reaction must proceed to get to equilibrium.

The change in Gibbs free energy is related to the changes in enthalpy (ΔH^0) and entropy (ΔS^0) that accompany the reaction:

$$\Delta G^0 = \Delta H^0 - T\Delta S^0 \qquad (4)$$

Fundamentally, it is the values of ΔH^0 and ΔS^0 that govern the equilibrium position for the reaction. This reflects the contributions from the two driving forces in nature: the tendency towards lowest energy, and the tendency towards greatest entropy. For example, reactions that go to completion (i.e., $K_{eq}>1$, and $\Delta G^0<0$) tend to be highly exothermic ($\Delta H^0<<0$) or highly endergonic ($\Delta S^0>>0$). Of course, this is not always the case, as there are many combinations of values of ΔH^0, ΔS^0 and T that will result in a negative value for ΔG^0. The reverse reasoning holds for reactions that tend not to proceed (i.e., $K_{eq}<1$, and $\Delta G^0>0$).

The contributions to ΔG^0 from enthalpy and entropy can be experimentally determined by measuring the equilibrium constant as a function of temperature. Combining equations (3) and (4), we have:

$$\ln K_{eq} = -\frac{\Delta H^0}{RT} + \frac{\Delta S^0}{R} \qquad (5)$$

From equation (5), we can see that a plot of $\ln K_{eq}$ versus $1/T$ will yield values for ΔH^0 and ΔS^0.

In this experiment you will use NMR spectroscopy to measure the equilibrium constant for the keto-enol tautomerization of 2,4-pentanedione as a function of temperature in two different solvents. From this data, you will determine the standard enthalpy and entropy changes for the reaction in each solvent, which will allow you to discover the roles of entropy and enthalpy (energy) in the tautomerization reaction. You will also be able to gain some insight on the role of solvent in stabilizing or destabilizing the two forms of 2,4-pentanedione.

An NMR spectrum of 2.4-pentandione in $CDCl_3$ is shown in Figure 2. The spectrum represents a mixture of keto and enol forms due to the equilibrium reaction in Figure 1. The spectrum is expanded to show the peaks of interest at ~5.3 ppm, 3.4 ppm, ~2.0 ppm, and ~1.8 ppm.

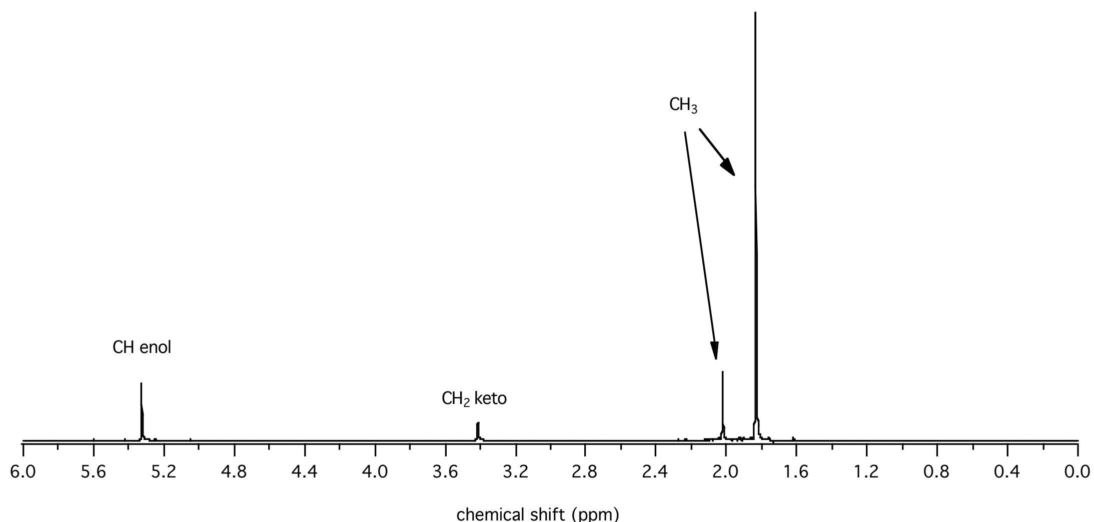

Figure 2: 300 MHz NMR spectrum of 2,4-pentanedione in $CDCl_3$ (expanded view). The methyl protons from the keto and enol forms have distinct chemical shifts.

In an NMR spectrum, the integrated area under a peak is proportional to the concentration of that type of proton in solution. This means that the area is proportional to the concentration of the

compound in solution scaled by the number of protons per compound that contribute to the peak. There are two sets of protons that can be compared to obtain the equilibrium constant for reaction (1): *(a)* the protons on carbon 3, or *(b)* the methyl protons. In case *(a)*, the enol CH peak arises from one proton per molecule, while the keto CH_2 peak arises from two protons per molecule. This means that one of the integrated areas should be divided by two in order to obtain relative concentrations. Let's suppose we had a mixture that was 1:1 keto:enol. In this case, we would expect the keto peak to be twice as large (there are twice as many protons contributing to that signal). But the concentration in this example is 1:1, so we should divide the keto CH_2 area by two to obtain the correct concentration ratio. Thus, using the peaks arising from the protons on carbon 3:

$$K_{eq} = \frac{[enol]_{eq}}{[keto]_{eq}} = \frac{(CH \ \ peak\ area)}{(CH_2 \ \ peak\ area / 2)} \tag{6a}$$

In case *(b)*, the enol CH_3 peak arises from six protons per molecule, and the keto CH_3 peak arises from six protons per molecule. Thus, using the methyl peaks:

$$K_{eq} = \frac{[enol]_{eq}}{[keto]_{eq}} = \frac{(CH_3^{enol} \ peak\ area)}{(CH_3^{keto} \ peak\ area)} \tag{6b}$$

In theory, both ways of calculating the equilibrium constant should yield the same results.

The full NMR spectrum of 2,4-propanedione in $CDCl_3$ is shown in Figure 3:

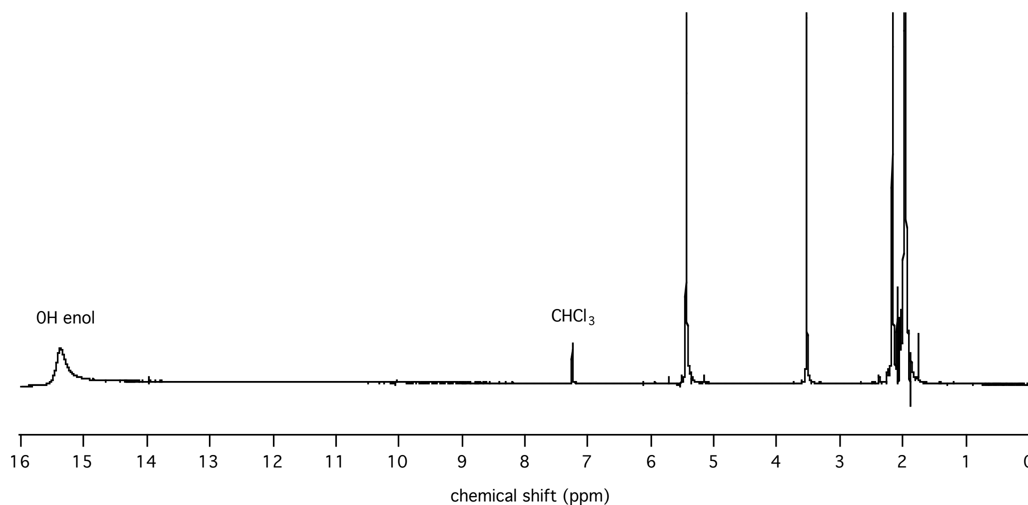

Figure 3: Full 300 MHz NMR spectrum of 2,4-pentanedione in $CDCl_3$. The vertical scale is greatly expanded from that in Figure 2.

The residual $CHCl_3$ signal can be seen in Figure 3 at 7.25 ppm, as can the quite broad OH signal from the enol form. The signal is broad due to the fast exchange between the two enol forms. There is a small amount of contamination of the sample that is apparent at such a large scale expansion.

The second solvent that you will use in this experiment is DMSO-d6, which is very polar. Note that the two methyl peaks may not be distinct in DMSO-d6, and that the residual DMSO peak appears as a quintet at 2.50 ppm.

⇨ *Do the following in your notebook before you come to lab:*

1. Using the relative sizes of the CH and CH_2 peaks in Figure 2, determine which CH_3 peak arises from the enol form, and which is from the keto form.
2. Look up the proton chemical shifts, boiling points, freezing points, densities, dielectric constants and dipole moments of $CHCl_3$ and DMSO; and the freezing point, boiling point, molecular weight, and density of 2,4-pentanedione. Enter all of these values in a table in your notebook, being sure to include complete references to the source of your information.
3. Calculate in your notebook the volume of 2,4-pentanedione that is required to make solutions that are 0.20 mole fraction in 2,4-pentanedione, using a solvent volume 0.50 mL. Calculate also the molarities of these solutions.

Experimental Procedure:

Apparatus: 300 MHz Bruker NMR Spectrometer; 2,4-pentanedione, $CDCl_3$, DMSO-d6, NMR tubes, microsyringes. Note that the NMR tubes and syringes are quite expensive, so care should be taken in preparing samples!

Caution: The magnet in any NMR spectrometer produces a significant magnetic field that extends beyond the immediate region of the spectrometer. Modern magnets have stray fields that are powerful enough to erase credit cards and destroy watches. For this reason, you should always ***place your watch, wallet, and any other metal jewelry, etc. on the table near the door before you approach the spectrometer console!***

Since some time will need to be spent getting comfortable with the instrument, each group will acquire only two spectra and the data will be shared by the class. Each group will be responsible for acquiring data at two temperatures in a given solvent (see Table 1). You will have approximately 40 minutes during lab time to acquire your data. This means that you must come to lab earlier than your scheduled time to prepare your sample.

Table 1: Temperatures for the study of the keto-enol tautomerization of 2,4-pentanediol.

Time	*Group #*	$CDCl_3$ (*Day 1*)	*Group #*	DMSO-d6 (*Day 2*)
2:15	*(Loh)*	25°	*(Loh)*	50°
3:00	1	29°, 33°	4	55°, 60°
3:40	2	37°, 41°	5	65°, 70°
4:20	3	45°, 50°	6	75°, 80°

There are two keys to obtaining good data in any NMR experiment:

(1) achieving thermal equilibrium in your sample before acquiring data, and
(2) good shimming of the magnetic field (that is, making the magnetic field as homogeneous as possible around your sample).

This experiment is particularly sensitive to both of these factors. To begin, insert your sample in the spectrometer and set the temperature controller to the correct temperature. It will take approximately 5-10 minutes for your sample to reach thermal equilibrium once the thermostat has stabilized. To help reduce waiting time, the previous person will have set the temperature for your

first spectrum before you come in. Be sure that you do the same for the next person when you complete your acquisition.

Setting the Lock Signal: While you are waiting for the sample to equilibrate, you can set up the spectrometer parameters. Begin by turning the spinner on (press the **spin** button). In routine NMR work, the sample is spun (typically at ~20 Hz) to average out some of the field inhomogeneities in the x-y plane. Next, you need to optimize the deuterium lock signal. To do this, first press the **field** button and adjust the knob until the sweep pattern is symmetrical about the center of the screen. This sets the frequency of the deuterium signal coming from the deuterated solvent in your sample. Now press the **lock phase** button, and turn the knob until the left and right peaks in the sweep signal are of equal heights. This corrects the phase of the lock signal (you will see phasing again for the proton signal when you work up your data). Finally, press the **lock** button. As the spectrometer locks on the deuterium signal that you just set, the signal will climb up the screen and flatten out. You will have achieved a stable lock signal when the lock light stops flashing.

Shimming: Now comes the most important, most time-consuming, and most "black art" part of NMR spectroscopy: shimming the field. The term "shim" comes from construction, where thin pieces of wood are used to level or even out the major pieces of interest. In NMR, shimming is performed by running a variable low current through different sets of small metal coils to produce small magnetic fields with different orientations. These are used to adjust the field around your sample in order to make the field as homogeneous as possible. A well-shimmed spectrum will have very narrow, Lorentzian (rather bell-shaped) peaks without jagged tops or lopsided edges (see Figure 4).

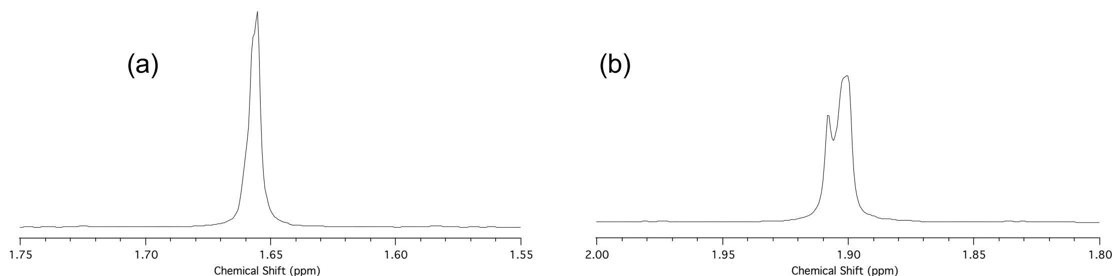

Figure 4: Examples of spectra obtained with (a) a pretty good shim, and (b) a poor shim. The region around the peak has been expanded to observe the true line shape.

The shim settings should be fairly close to ideal already, so all you need to do is adjust the **Z** and $\mathbf{Z^2}$ shims until you get a maximum lock signal. A good shim file has been saved in case you really screw up the shims and can't get them back. To load this file, type: **RSH C313F04S.001** (for $CDCl_3$ samples) or **RSH C313F04S.002** (for DMSO-d6 samples). To check the quality of your shim, you will need to take a spectrum.

Acquiring a Spectrum: To acquire a spectrum, you will need to set the following parameters:

- load acquisition parameters into the NMR computer
- set the pulse width, or tip angle (we will use a 30° pulse - see below)
- set the receiver gain (the degree of amplification of the digital signal)
- set the number of scans to be collected and later averaged
- set the relaxation delay between scans

In a standard NMR spectrum, the bulk magnetization vector is tipped from its equilibrium alignment along the z-axis (vertical in NMR instruments, horizontal in MRI instruments) to a new orientation by the application of a small of magnetic field pulse. The detector sits in the *xy*-plane, so the maximum signal will be obtained if the vector is tipped by 90° (so that it lies in the *xy*-plane). The longer the pulse (or the greater the pulse power), the greater the tip angle. Since our samples are very concentrated, they are likely to produce a very strong signal, which can overload the detector electronics. Therefore, we will use only a 30° pulse. According to the latest calibration on our instrument for ^{1}H nuclei, a pulse lasting 8.75 μs produced a 90° tip angle.

Modern NMR spectrometers are Fourier transform instruments - this means that the entire spectrum of data is obtained at once (in the time domain), then mathematically transformed to produce a spectrum in the frequency domain. One of the advantages of an FT instrument is that noise can be reduced by adding several spectra prior to Fourier transforming the data - the random noise cancels (in part), while the signal is enhanced. Since our samples are very concentrated, we should expect a very high signal-to-noise ratio, so only a few scans are necessary. For the same reason, we will start with a very low receiver gain. The relaxation delay is the time the instrument waits between scans in order to allow the magnetization vector to completely return to equilibrium.

To acquire a spectrum, type in the following commands:

- **RJ H010602.001** (press ENTER): loads the acquisition parameters into the NMR computer
- **PJ** (press ENTER twice): loads the processing parameters into the NMR computer
- **RG** (press ENTER) **1** : (sets the receiver gain)
- **PW** (press ENTER) **2.9**: (sets the pulse width = 8.75 μs/3, or tip angle = 90°/3=30°)
- **NS** (press ENTER) **8** : (sets the number of scans to be averaged)
- **RD** (press ENTER) **2** : (sets the time (in seconds) to wait between scans)
- **GS** (press ENTER) : (initiates a test scan)

If everything is set up correctly, you should see a FID (Fourier induction decay: the raw NMR signal) appear on the screen. The FID represents the decay in the magnetization in the *xy*-plane versus time as the magnetization vector relaxes back to equilibrium along the *z*-axis. (see Figure 5). The oscillations in the decay represent the sum of all of the proton Larmor frequencies in the sample. The initial signal amplitude should be approximately 2 blocks high on the NMR computer screen. If the amplitude is too low, increase the size of **RG** (the receiver gain). Once the amplitude is set, press the CONTROL and K buttons to kill the test scan procedure. Enter **ZG** to begin the actual data acquisition.

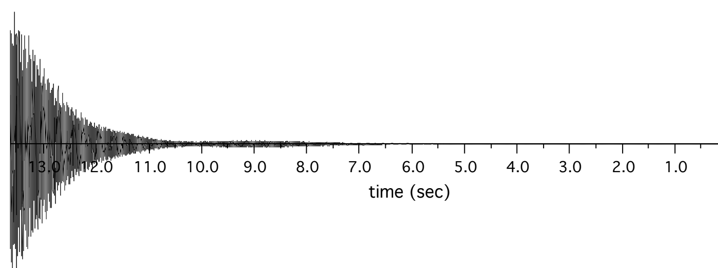

Figure 5: Sample FID of a reasonably well-shimmed sample. Notice the smooth exponential decay of the signal envelope. FID's that look bumpy or deformed are usually the result of poor shimming.

Data Processing and Visualization: To view your spectrum and check your shim, you need to export the data from the NMR computer to the PC. At the spectrometer console, save your spectrum by typing: **WR C313XXXY.00Z**, where XXX is the temperature in Kelvin, Y is your group number, and Z is the spectrum index (i.e.: 1 for the first attempt, etc.). Press the ENTER key: the screen will flash and you should get the prompt back if the file has been saved.

Move to the PC computer and open the ASPLINK window. Enter **6** (options), and set the data directory to **C:\NMRLINK\CHM313\FALL04**. Enter **1** (import), and type the name of your file (C313XXXY.00Z). When the import is complete, you can minimize the ASPLINK window. To view and process the spectrum, open the NUTS window. Open your file using the **FILE…OPEN** menu option. The program will ask you if you want to use autodetect import; choose **YES**. You should see your FID when the file has been loaded. To process the data, type **FT** (Fourier transform), then **PH** (phase). Click the right mouse button and drag the mouse back and forth until the baseline is flat (phased), then release the button and hit ENTER. Double click the left mouse button on a region near one of the peaks and highlight that peak. Click with the right button to zoom in on the peak - check to be sure the peak is a smooth, narrow Lorentzian shape (see Figure 4). If there are jagged edges or lumps you will need to reshim and reacquire a spectrum. Once you have a good spectrum, save a copy of the original FID as **GOODXXX.YQQ** (where QQ are your initials) in the C:\NMRLINK\CHM313\FALL04\ folder.

Calculations:

Save a copy of each of the good spectra on a floppy diskette and transfer these files into your Student Files folder on one of the Macs or a PC that has the NUTS software package. The Mac version of NUTS is called MacNutsPPC. Open the first spectrum file, and transform and phase the spectrum. Expand the vertical scale and find the residual solvent peak. Place your cursor on the center of the peak and type **S** while holding down the mouse button. A dialog box will appear: enter the chemical shift of the solvent peak. Set the display range to be 6 ppm - 0 ppm using the zoom control.

To integrate the peak areas, type **ID** (integral subroutine) then type **B** (baseline correct the integrals). You should see an integral line that is parallel to the baseline where there are no peaks, but which jumps vertically at each peak. If this is not observed, hit return to exit the integration routine and type **BC** to correct the spectrum baseline. Re-enter integration mode. Cut the integrals close to each peak using the left mouse button (a NUTS manual is available in the lab if you get stuck; there is also a help menu in the NUTS program). If you make a mistake, typing **C** will clear all broken integrals. Also cut one integral where there are no peaks peaks – this integral should have an intensity of zero when properly phased. Next place your cursor on the pentandione methyl protons peak and type **V** while holding down the mouse button. Enter a relative value of **100** in the dialog box that appears - this scales all of the integrals to a nice number. Type **T** to show the integral values on the screen. Phase the spectrum by typing **PH** and sliding the mouse to the left or right while holding down the right mouse button. Once the spectrum is well-phased, record the integral values in a table in your notebook. Also print a copy of this spectrum, and label it with the file name, solvent, and temperature. Tape this spectrum into your notebook.

For presentation purposes, you will create a stacked plot in Excel for each solvent set (see *Using Excel, Part IV*). In order to import the NMR data into Excel, you need to export the data in the processed spectrum in NUTS as a text file. *Do this before you open the next spectrum!* To export the spectrum as a text file, in NUTS choose **File … Export File … PPM, Intensity ASC data pairs**.

Give the exported file a name that makes sense. In Excel, choose **File … Open** and navigate to the text file that you just created. A dialog box will pop up asking you how you want Excel to read the data. Choose **Delimited** and hit the NEXT button, then click the **Tab** and **Comma** boxes. Keep hitting the NEXT button until your data appears in the spreadsheet - it should be in two columns: **PPM** and **Intensity**. Delete all data outside of the 0-6 ppm range.

Process the remaining spectra in the same way. Copy all of the **PPM, Intensity** data from a single solvent into one spreadsheet, and follow the instructions in *Using Excel, Part IV* to make the stacked plot. You should end up with 2 stacked plots, one for all the $CDCl_3$ spectra and one for all the DMSO-d6 spectra. Remove the legend from the plots. Print each one on a full page with a complete Figure # and title, and write in the temperature for each spectrum on the plot by hand.

For the $CDCl_3$ spectra, use equations (6a) and (6b) to calculate the equilibrium constant at each temperature. (Each group will probably have slightly different values at this point since the integrals will be cut slightly differently.) Enter your values for each set (*a* or *b*) of K_{eq} and T (in Kelvin) in an LLS spreadsheet, then fit and plot the data according to equation (5). Calculate values for ΔH^0 and ΔS^0 with 95% confidence limits in units of kJ/mol and kJ/mol·K, respectively. You should end up with two sets of values for K_{eq}, ΔH^0 and ΔS^0, which should be fairly similar to each other.

Repeat the analysis for the DMSO-d6 spectra, then calculate ΔH^0 and ΔS as for the $CDCl_3$ spectra.

Wrapping it Up:

Your notebook should have one table of all of the meaningful equilibrium data (i.e.: integrated areas, K_{eq}, T, etc.), and another table of final results for $\Delta H°$ and $\Delta S°$, with units where appropriate and error limits. Look up and record in your notebook as many relevant literature values as possible: *J. Chem. Ed.*, vol. 79 (2002), 707-714.

Discuss (don't just answer the question as posed - support your ideas with experimental evidence) the following physical aspects of this experiment:

- Describe the physical interpretations of the signs on $\Delta H°$ and $\Delta S°$ for the reaction in $CDCl_3$ (use the signs on the "true", or literature, values in this discussion). Can you justify these observations based on your understanding of organic chemistry?
- Compare the equilibrium constants for the reaction in $CDCl_3$ and DMSO-d6 at the same temperature. How does changing the solvent polarity affect the equilibrium? What raw data do you have to support this observation? How do enthalpy and entropy affect the equilibrium position of the reaction, and what is the role of temperature in the equilibrium position? How can you explain the role of temperature in terms of the physical interpretations of $\Delta H°$ and $\Delta S°$?
- Compare the values of $\Delta H°$ and $\Delta S°$ for the reaction in $CDCl_3$ and in DMSO-d6 (use the signs on the "true", or literature, values in this discussion). How does changing the solvent polarity affect reactant vs. product stability and reactant vs. product entropy? Can you justify these observations based on your understanding of organic chemistry and bond behavior?
- Assess the accuracy and precision of your data by comparing your results to the literature values, and comparing your results for the $CDCl_3$ solutions obtained using data sets *(a)* and *(b)*.