Chapter 9
Spectra of Organic Molecules

The tutorials in this chapter illustrate applications involving the calculation of IR and proton and ^{13}C NMR spectra. The DP4 analysis is illustrated to identify which stereoisomer best fits an experimental ^{13}C NMR spectrum. Finally, the tutorials also illustrate the use of calculated IR spectra to identify an experimental unknown.

In addition to equilibrium geometries, conformations and conformational energy differences, reaction energies, as well as diverse molecular properties, calculations provide infrared and Raman spectra, NMR spectra and UV/visible spectra. IR and Raman spectra arise from the transitions between ground and excited vibrational states, NMR spectra from transitions between nuclear spin states and the UV/visible spectra from transitions between ground and excited electronic states.

Chemists use spectra, in particular, NMR spectra to provide essential clues needed to assign the structure of an unknown molecule. That is, features in the spectrum may be used to support a proposed structure assignment, whereas their absence suggests that the assignment is likely to be incorrect. On the other hand, a calculated spectrum starts with a known structure. A high degree of similarity with a measured spectrum may be taken as evidence that the calculated molecule is the same (or at least very similar to) that for which the spectrum was measured. Lack of similarity suggests that the two molecules are not the same.

The first two tutorials in this section deal with infrared structure and use the EDF2/6-31G* model. The first details the steps needed to calculate the infrared spectrum of methyl formate and to relate the spectrum to the underlying molecular structure, and the second illustrates use of the Spartan Infrared Database to identify a molecule based on its infrared spectrum. The remaining tutorials

deal with NMR spectroscopy and use the ωB97X-D/6-31G* model and (optionally) the SSPD database. The first of these details the steps involved in calculating and displaying a proton spectrum for 1-methylindole, and the second, the ^{13}C spectrum of caulophyline, both of which are rigid molecules. The third considers the ^{13}C NMR of *cis*-1,2-dimethylcyclohexane, a molecule that rapidly (relative to the time scale of the NMR experiment) moves between two equivalent conformers. Discussion of the NMR spectra of molecules with multiple degrees of conformational freedom and several accessible conformers is provided in a later chapter. The final tutorial examines the dependence of carbon chemical shifts on stereochemistry and illustrates the use of DP4 analysis to decide which best fits the experimental NMR spectrum.

10 mins

Infrared Spectrum of Methyl Formate

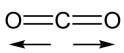
In the harmonic approximation, the frequency at which a diatomic molecule vibrates is proportional to the square root of the ratio of the force constant (the second derivative of the energy with respect to change in bond length) and the reduced mass (the product of the masses of the two atoms divided by their sum). Frequency increases with increasing force constant or stiffness of the bond and decreases with increasing masses of the atoms involved in the bond.

$$\text{frequency} \propto \sqrt{\frac{\text{force constant}}{\text{reduced mass}}}$$

In order to generalize this expression to a polyatomic molecule, it is necessary to use a coordinate system that leads to a diagonal matrix

of second energy derivatives (so-called ***normal coordinates***).* These differ from internal coordinates (depicting charges in specific bond lengths and angles), and for a polyatomic molecule will typically involve the motions of several (and likely all) atoms.

The intensity of absorption of infrared radiation by a diatomic molecule is proportional to the change in the dipole moment with change in bond length. Since the dipole moment for a homonuclear diatomic molecule does not change with distance, this means that molecules such as N_2 and O_2 are transparent in the infrared. The intensity of each of the individual lines in an infrared spectrum of a polyatomic molecule follows from the change in dipole moment along the associated normal coordinate. Some of the normal coordinates may not lead to a change in dipole moment, for example, the symmetric stretch in carbon dioxide where both CO bonds are simultaneously moving, and infrared absorptions will not be observed.



This tutorial illustrates the steps required to calculate and display an infrared spectrum and to compare it with an experimental spectrum. You will explore how the ease or difficulty of molecular motion (the value of the force constant) and changes in atomic masses affect frequency.

1. Build or sketch methyl formate. Select **Calculations...** from the

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* Consider the hypothetical case of a molecule with two coordinates, R_1 and R_2. Here the second derivatives form a matrix.

$$\begin{pmatrix} \frac{\partial^2 E}{\partial R_1^2} & \frac{\partial^2 E}{\partial R_1 \partial R_2} \\ \frac{\partial^2 E}{\partial R_1 \partial R_2} & \frac{\partial^2 E}{\partial R_2^2} \end{pmatrix}$$

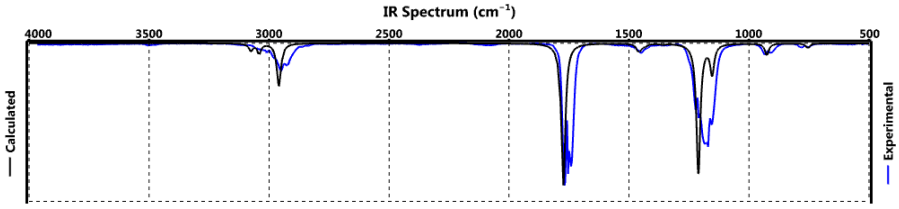
In order to associate the elements of this matrix with the individual coordinates, it is necessary to choose a coordinate system R' such that the off-diagonal second derivative is zero.

$$\begin{pmatrix} \frac{\partial^2 E}{\partial R'_1{}^2} & 0 \\ 0 & \frac{\partial^2 E}{\partial R_2'^2} \end{pmatrix}$$

Setup menu (), and request an equilibrium geometry using the EDF2/6-31G* density functional model. *Check* **IR** to the right of **Compute** and *click* on **Submit**. Accept the name ***methyl formate***.

> The IR spectrum of methyl formate is in SSPD and you could have just retrieved it instead of having to calculate it.

2. After the calculation has completed (several minutes), select **Spectra** from the **Display** menu (). *Click* on the in the toolbar at the top of the spectra pane and select from the palette of icons. The experimental IR spectrum of methyl formate is available in the freely available NIST database. *Click* on the again and select from the icon palette. Calculated (in red) and experimental (in blue) infrared spectra are now superimposed.



3. *Click* on either the up or down triangles that define a cursor and slide the mouse while holding down the left button across the spectrum. Position it over the intense line in the (calculated) spectrum near ≈1774 cm^{-1}. Note that the molecular model (on screen above the spectra pane) vibrates. The motion corresponds to stretching of the CO double bond. Position it over the intense line near ≈1211 cm^{-1}. This motion corresponds to a combination of stretching motions of the two CO single bonds.

4. Select **Save As** from the **File** menu () to make a copy of methyl formate; name it ***methyl formate d3***. *Click* on to enter **View** mode. Select **Properties** from the **Display** menu () and *click* on one of the three hydrogen atoms on the methyl group to bring up the **Atom Properties** dialog. Change **Mass Number** from **1** to **2 Deuterium**. Repeat for the other two

methyl group hydrogen atoms. Resubmit the calculation* (it will require only a few seconds) by selecting **Submit** from the **Setup** menu.

5. Compare the frequencies of the undeuterated and deuterated forms of methyl formate, and identify which change the most and which change the least. To do this open the original ***methyl formate*** document and display the calculated IR spectra. To examine the calculated frequencies, *click* on the **Tables** icon (). A table of frequencies and intensities is presented. Examine the frequencies associated with CH vibrations on the methyl group. Repeat the procedure examining frequencies associated with CD vibrations on the methyl group. You can move between the documents by *clicking* on the appropriate tab at the bottom of the screen.

6. Close all documents and any open dialogs.

2 mins

Searching *Spartan*'s Infrared Spectral Database

Pattern matching ("fingerprinting") a measured infrared spectrum to a collection of experimental spectra contained in a database is common practice and is appropriate for identifying molecules that have previously been characterized. However, NMR has replaced infrared spectroscopy as the method of choice for structure assignment of "new" (previously uncharacterized) molecules. Pattern matching to databases of calculated infrared spectra is not routine. One reason is that the results of calculations (a set of vibrational frequencies and intensities) do not look like experimental infrared spectra, at least, spectra obtained at normal temperatures. However, a fit of the calculated data to a Lorentzian function in which peak width at half height is treated as a parameter makes the two spectra visually quite similar, not at all surprising as this loosely corresponds to temperature. In fact an infrared spectrum measured at low temperature comprises

* Because force constants (second derivatives) do not depend on mass, the calculation is very simple and will require only a few seconds.

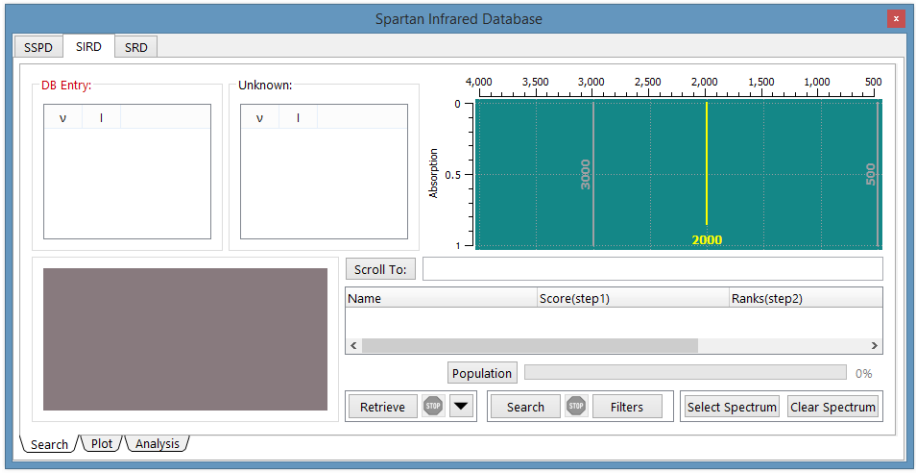
a series of sharp lines (rather than bands).

Practical calculations assume that the frequency of an infrared absorption is proportional to the square root of the second derivative of the energy at a minimum in the potential surface for a particular vibrational coordinate, that is, to the curvature of the surface. The so-called quadratic approximation leads to a surface that is too steep, resulting in calculated frequencies that are larger than measured frequencies. For the most part, the error is systematic, with calculated frequencies being between 3% and 15% larger than measured frequencies, depending on the theoretical model. Hartree-Fock models show an error near the top of the range, while density functional and MP2 models show an error near the bottom of the range. Semi-empirical models do not show a consistent error pattern. Such a systematic error can be compensated for, at least in part, by incorporating a single linear scaling parameter into the fitting function.

Spartan fits calculated infrared spectra to the measured infrared spectra using a Lorentzian function that incorporates two parameters, a non-linear parameter accounting for peak width and a linear scaling parameter.

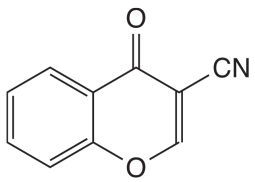
This tutorial illustrates the way in which searches of the Spartan Infrared Database (SIRD) are setup and carried out and the results examined. SIRD is an additional access route to the molecules contained in the Spartan Spectra and Properties Database (SSPD) and derives from EDF2/6-31G* calculations. You may choose from a small selection of measured infrared spectra (or supply your own spectra as a .dx file).

1. You need to have a ***Spartan*** document open in order to access SIRD. Select **New Build** from the **File** menu (), and then select **Databases** from the **Search** menu (). *Click* on the **SIRD** tab to bring up the **Spartan Infrared Database** dialog.

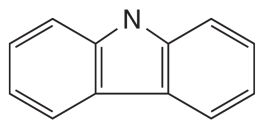


Click on **Select Spectrum** at the bottom right of the dialog. Navigate to the ***spectra of organic molecules*** subdirectory under the ***Tutorials*** directory.*

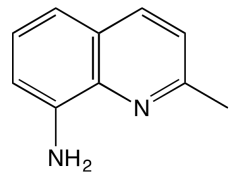
Select one of the following files and *click* on **Open**.



chromone-3-carbonitrile



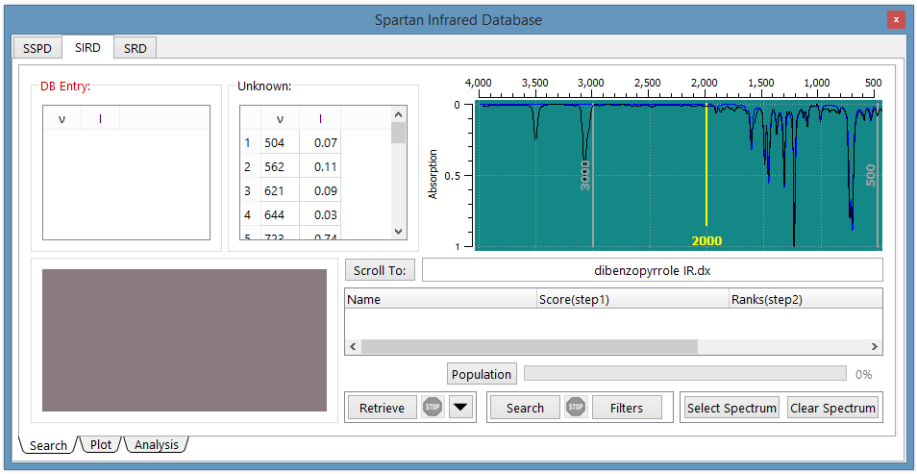
dibenzopyrrole



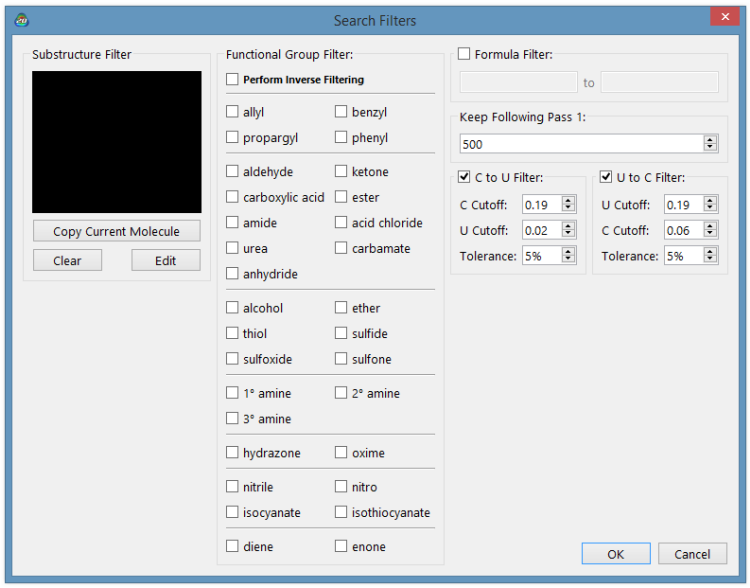
8-amino-2-methylquinoline

The experimental infrared spectrum of the selected molecule will appear in the window at the top right of the dialog with the name underneath. Immediately to the left is a scroll box containing the most intense lines in the spectrum (obtained from a fit of the experimental spectrum to a Lorentzian function).

* For Windows, this directory is found in ***Program Files/Wavefunction/Spartan20***. For security reasons, the program file directory is protected. Copy the folder to your desktop or to another location available to the user prior to opening it in ***Spartan***. For Linux, this is found in the directory where ***Spartan*** was installed. For Macintosh, this is located at the top level on the ***Spartan20*** disc image.



2. *Click* on **Filters** at the bottom right of the dialog to bring up the **Search Filters** dialog.



This provides for substructure and formula filters as well as functional group filters. You can, if you wish, *check* an appropriate entry. For example, if you have selected chromone-3-carbonitrile as the "unknown", *checking* ***nitrile*** from among the **Functional Group Filters** would limit the search to molecules with nitrile functionality. *Click* on **OK** to exit the dialog.

3. *Click* on **Search** at the bottom of the (**Spartan Infrared Database**) dialog. The search may require a minute or two. When

it has completed, scan the list of hits at the bottom of the dialog for the name of your query. It will be at or near the top of the list.* *Click* on the name. The calculated infrared spectrum (red) will be superimposed onto the fit of the experimental spectrum (blue) in the window at the top right of the dialog.

4. Close all documents and any open dialogs.

10 mins

Proton NMR Spectrum of 1-Methylindole

Proton NMR spectroscopy was the first tool available to chemists that allowed definitive assignment of the molecular structures of complex organic molecules. By the 1970's, it had largely replaced infrared spectroscopy and to a large extent chemical proofs of structure. ^{13}C NMR is now more dominant, but proton NMR remains an essential tool in the chemist's arsenal.

An NMR spectrum follows from the fact that nuclei possess spins that can either align parallel or antiparallel to an applied magnetic field, giving rise to different nuclear spin states. The relative energy of these states (ΔE) depends on the nucleus and on the strength of the applied magnetic field, by way of a simple relationship:

$$\Delta E = \gamma \hbar B_0$$

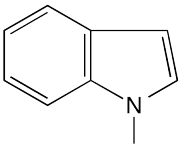
γ is the gyromagnetic ratio (a constant for a given type of nucleus), $\hbar$ is Planck's constant divided by 2π and B_0 is the strength of the magnetic field ***at the nucleus***. While the two nuclear spin states are normally in equilibrium, this equilibrium can be upset by applying a second magnetic field. The absorption of energy as a function of field strength (a ***resonance***) between the states can then be detected.

The key to the utility of the magnetic resonance experiment is that the energy at which a nucleus resonates depends on its location in the molecule, and is measureably different for each (chemically) distinct

* This will not always be the case. Errors inherent to the calculations as well as the absence of detail may result in better matches to "incorrect" but closely related molecules. Of course, a query may also not be in the database, so it is possible to find only related molecules.

nucleus. The reason is that the applied magnetic field is weakened by electrons around the nucleus. Nuclei that are well shielded by the electron cloud will feel a lesser magnetic field than those that are poorly shielded, and will show a smaller energy splitting. The difference, given relative to a standard, is termed a ***chemical shift***. By convention, both proton and ^{13}C chemical shifts (treated later in this chapter) are reported relative to tetramethylsilane (TMS) as a standard.

While each *unique* proton in a molecule gives rise to a single line (resonance) in the spectrum, the spins on nearby protons add or subtract to the external magnetic field. This leads to a splitting of lines, the splitting pattern depends on the number of neighboring protons and their geometry. In practice, only two-bond (H—C—H) and three-bond (H—C—C—H) interactions or coupling are important. Discounting splitting, the intensity of the lines is approximately proportional to the number of equivalent protons that contribute.* For example, the proton NMR spectrum of 1-methylindole would be expected to show seven lines, six with unit intensity corresponding to the protons on the indole ring and one line with three times the intensity corresponding to the three equivalent methyl group protons.



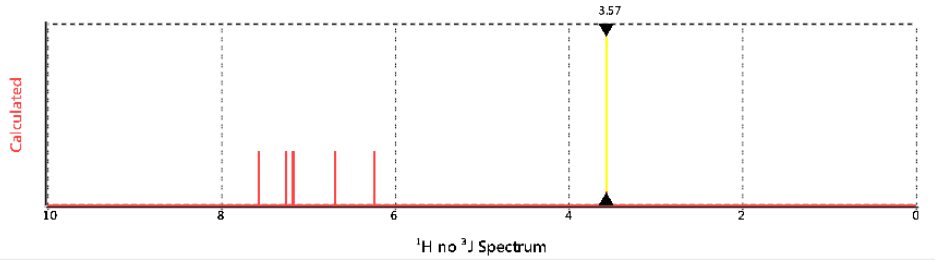
In this tutorial, you will use the ωB97X-D/6-31G* model to calculate the proton NMR spectrum of 1-methylindole and compare it with the experimental proton spectrum in the absence of three-bond HH coupling.

1. Build or sketch 1-methylindole. Select **Calculations...** from the **Setup** menu (). Specify calculation of equilibrium geometry using the ωB97X-D/6-31G* density functional model. *Check* **NMR** to the right of **Compute** and *click* on **Submit**. Accept the name ***1-methylindole***. The calculation will require several

* More generally, the relative intensity of the sum of the lines corresponding to a proton that has been split is approximately proportional to the number of chemically equivalent protons.

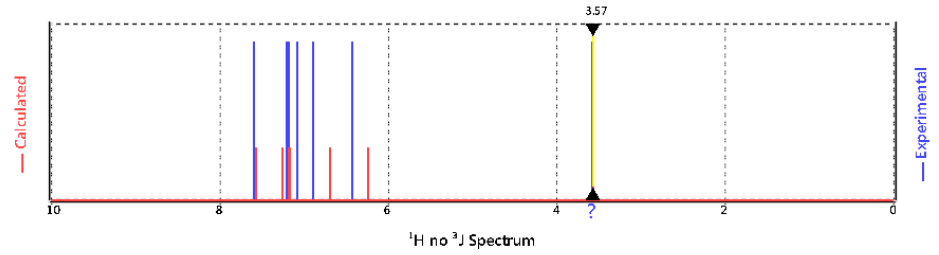
minutes.*

2. When the calculation has completed (or after you have retrieved results from SSPD), select **Spectra** from the **Display** menu ( ). *Click* on in the bar at the top of the spectra pane and select Calculated (proton NMR spectrum in which there is no HH coupling).



Move the mouse while holding down the left button over the spectrum. When you intersect a line, a numerical value for the chemical shift appears at the top of the spectrum and the proton is highlighted in the structure model.

3. *Click* again on and this time select Experimental (experimental proton NMR spectrum with HH coupling constants set to 0). The experimental spectrum will be retrieved from the freely available NMRShiftDB database** and displayed with the calculated spectrum. You will need to be online.



The comparison gives you an idea of the level of agreement that can be expected between calculated and experimental proton spectra.

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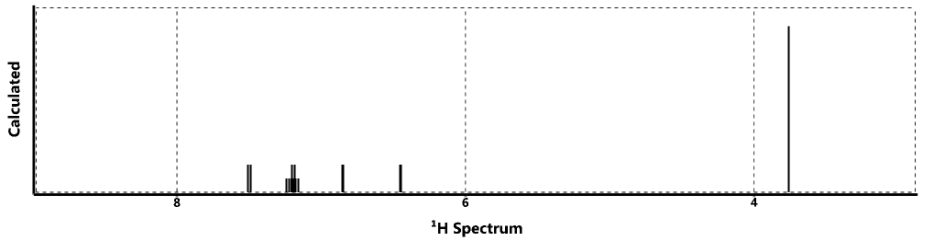
* 1-methylindole is in the Spartan Spectra and Properties Database (SSPD). You can, if you like, avoid doing any calculations and simply retrieve it. In this case, *click* on the name at the bottom of the screen, make sure the ωB97X-D model is selected and *click* on **Replace** in the dialog that results.

** The NMRShiftDB is primarily a collection of ^{13}C spectra, although some proton spectra including that for 1-methylindole are available.

4. *Click* again on ✚ and select ☐ ^{1}H Calculated (calculated proton NMR spectrum). The calculated proton spectrum that now appears accounts for three-bond HH coupling.



You can focus in on details by a combination of zooming the spectrum (scroll wheel) and shifting the displayed range (move the mouse while holding down the right button). You will see that lines due to protons at C_2, C_3, C_4 and C_7 are doublets, while those due to protons at C_5 and C_6 are quartets (doublet of doublets).

5. Close ***1-methylindole*** and any open dialogs.

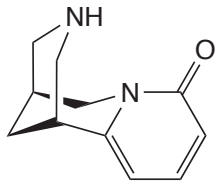
20 mins

^{13}C NMR Spectrum of Cytisine

There are several reasons why NMR spectroscopy, in particular, ^{13}C NMR, is one of the most important routine analytical techniques available for characterizing organic molecules. The analysis is straightforward, can be carried out quickly requiring relatively small samples and is non-destructive. The resulting spectrum is quite simple, comprising but a single line for each and every unique carbon.* However, assigning ^{13}C spectra is by no means trivial, even for seemingly simple molecules. The problem is that the positions of the lines in the spectrum (the chemical shifts) are very sensitive to the environment in which the carbons find themselves. Experiments exist to separate ^{13}C resonances based on the number of attached hydrogens, however, where two or more carbons in a molecule reside in "similar environments", it may be very difficult to distinguish them. This is particularly problematic for quaternary carbons.

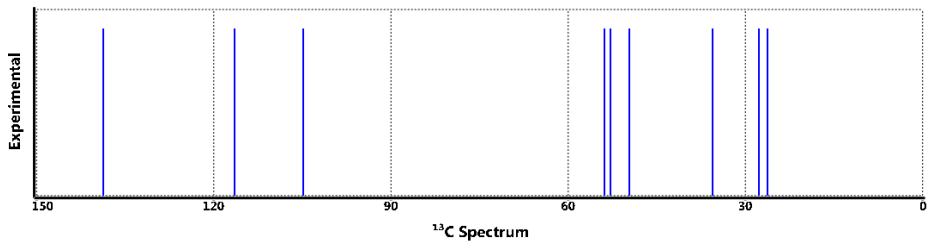
* CH coupling does split carbon resonances but is almost always eliminated by what is termed proton decoupling.

This tutorial uses the alkaloid cytisine to illustrate the use of calculated ^{13}C spectra to assist in assigning the measured spectrum of the molecule.



1. Either build or sketch cytisine. Select **Calculations...** from the **Setup** menu ( ) and specify calculation of equilibrium geometry with the ωB97X-D/6-31G* density functional model. *Check* **NMR** to the right of **Compute** and leave the NMR setting at **Current Model**. *Click* on **Submit** and accept the name ***cytisine***. The job will require several minutes to complete*.

2. When the calculation is done (or after you have retrieved the molecule from SSPD), select **Spectra** from the **Display** menu ( ). *Click* on ✚ in the bar at the top of the spectra pane and select ^{13}C Experimental (experimental ^{13}C spectrum). The experimental ^{13}C spectrum is drawn.



3. Use the calculated spectrum to associate the individual lines in the experimental ^{13}C spectrum with specific carbons in the structure of cytisine. *Click* on ✚ and this time select ^{13}C Calculated (calculated ^{13}C spectrum). The calculated spectrum (in red) will be superimposed on top of the experimental spectrum (in blue). This both gives an impression of the performance of the quantum

* Cytisine is available in the Spartan Spectra and Properties Database (SSPD). If you decide to use this instead of doing the calculations, *click* on the name at the bottom of the screen, make sure that ωB97X-D is selected from the menu in the dialog that results and *click* on **Replace**.

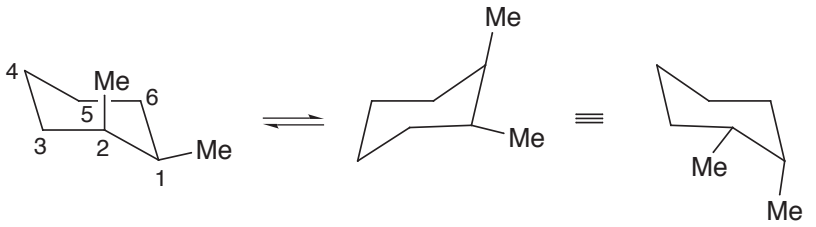
chemical calculations,* and also allows you to assign the lines in the experimental spectrum to specific carbons. *Click* on one of the triangles that designate the cursor and move the mouse while holding down the left button over the spectrum. A "hit" on a line will display the value of the chemical shift and will highlight the carbons responsible for this line in the structure model.

4. Close ***cytisine*** and any open dialogs.

10 mins

^{13}C NMR Spectrum of *cis*-1,2-Dimethylcyclohexane

At normal temperatures, the NMR spectrum of a conformationally-flexible molecule represents a (Boltzmann-weighted) average of the NMR spectra of all accessible conformers. Only when the temperature is lowered will the spectrum reveal its components. *cis*-1,2-dimethylcyclohexane is a special case where the (two) conformers are actually the same.



The room temperature ^{13}C NMR spectrum comprises only four lines at 34.9, 31.9, 24.2 and 16.4 ppm relative to TMS, corresponding to an equal weighting of C_1 and C_2, C_3 and C_6, and C_4 and C_5 and the two methyl carbons, respectively. When the sample is cooled the spectrum reveal eight distinct lines.

1. Either build or sketch *cis*-1,2-dimethylcyclohexane. Select **Calculations...** from the **Setup** menu ( ) and specify calculation of equilibrium geometry with the ωB97X-D/6-31G* density functional model. *Check* **NMR** to the right of **Compute** and leave the NMR setting at **Current Model**. *Click* on **Submit** and accept the name ***cis-1,2-dimethylcyclohexane***.

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* ^{13}C chemical shifts from ωB97X-D/6-31G* calculations have been empirically corrected.

The calculation will take less than 10 minutes.

2. When the calculation is done, select **Spectra** from the **Display** menu (), *click* on in the bar at the top of the spectra pane and select 

(calculated ^{13}C spectrum). There should be only four lines, the positions of which should correlate fairly well with what is observed experimentally at room temperature (within 1.5 - 2 ppm). ***Spartan*** has recognized that there are two equivalent structures and has calculated the average.

3. Close ***cis-1,2-dimethylcyclohexane*** and any open dialogs.

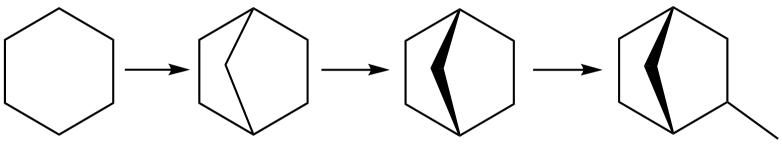
10 minutes

Stereochemical Assignments from ^{13}C Spectra

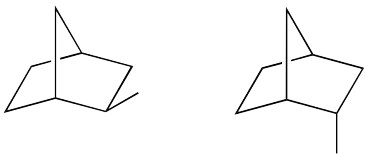
NMR spectroscopy, in particular ^{13}C spectroscopy, is without doubt the method of choice to establish the three-dimensional structure of organic molecules. Only X-ray diffraction provides more definitive results, although the requirement of a crystalline sample often limits its application. It is now practical to routinely calculate the NMR spectra of moderate size (MW on the order of 500 amu) organic molecules. Accurate calculations provide a "virtual NMR spectrometer" offering organic chemists an entirely new paradigm for structure determination, that is direct comparison of a measured spectrum with calculated spectra for one or more chemically reasonable candidates.

In this tutorial, you will first obtain ^{13}C chemical shifts for *endo* and *exo* stereoisomers of 2-methylnorbornane using the ωB97X-D density functional model, then enter the experimental shifts for one of the two isomers and use the DP4 metric to decide which provides the better match. Note that isomers **MUST** be labeled identically in order to make meaningful comparisons.

1. Sketch 2-methylnorbornane. Start with a six-member ring, draw a one-carbon bridge between two carbons, add "up" stereochemical markers. Finally, add a carbon to the 2 position.



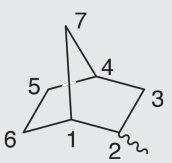
You need to add a stereochemical marker to distinguish between *endo* and *exo* stereoisomers, but before you do so, copy the sketch to the clipboard. *Right click* on screen and select **Copy** from the menu. Add the marker to give the *endo* stereoisomer. Select **Sketch New Molecule** (not **New Sketch**) from the **File** menu, *right click* on screen and select **Paste** from the menu. Add the marker to give the *exo* stereoisomer. *Click* on to give 3D structures for both isomers.*



2. Bring up the spreadsheet, *left click* on the **Label** cell to select all molecules, then *right-click* and choose **Rename Selected Using SSPD**. Specify calculation of equilibrium geometry using the ωB97X-D/6-31G* density functional model and *check* the **NMR** box. Make sure **Global Calculations** is *checked* and submit the job. The job (two molecules) will require a few minutes to complete.

3. When the calculations are completed, select the first molecule, bring up the **Properties** dialog and one after the other *click* on the individual carbons. *Click* on the **Edit** drop down menu to the right of **Expt Chem Shift:** and use the number pad to enter the experimental shift values given in the table below:

Experimental ^{13}C NMR Data



C_1	42.2	C_5	30.6
C_2	34.6	C_6	22.4
C_3	40.7	C_7	38.9
C_4	38.2	CH_3	17.4

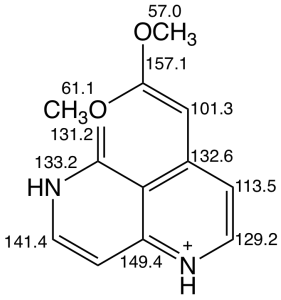
* Building the structures in this way assures that the atom labels will be consistent between the two stereoisomers. This is a requirement to obtain proper DP4 results.

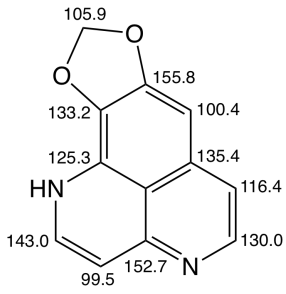
4. *Click* on  to revert the **Atom Properties** dialog to the **Molecular Properties** dialog. *Click* on the **NMR** tab (in the **Properties** dialog). *Click* on  to the left of **DP4** in the dialog. Bring up the spreadsheet to see the DP4 score for the two stereoisomers. The scale is from 0 to 100 with the larger numbers corresponding to better fits. (You can also examine DP4 scores from the **Properties** dialog if you flip between the stereoisomers.)

5. Close the document and any open dialogs.

Aaptamine: Protonated or Not?

NMR spectra for aaptamine* and closely-related natural product methylenedioxyaaptamine** have been reported. The ^{13}C chemical shifts noted below are nearly identical despite the fact that aaptamine is shown as protonated, whereas its methylenedioxy analogue is not.***





Are one of the assignments for aaptamine or methylenedioxy-aaptamine incorrect insofar as the state of protonation? Could aaptamine not be protonated in water (the solvent the NMR was taken in) or could methylenedioxyaaptamine be protonated in methanol (the solvent its NMR was taken)? To find out, perform NMR calculations on both molecules in both protonated and non protonated forms.

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* H. Nakamura, J. Kobayashi, and Y. Ohizumi, Tetrahedron. Lett, **23**, 5535 (1982).

** T. Hamada, Y. Matsumoto, C.-S. Pham, T. Kamada, S. Onitsuka, H. Okasura, T. Iwagawa, N. Arima, F. Tani and C.S. Vairappan, **Nat.** Prod. Commun., **14**, 1 (2019).

*** We have reversed the experimental assignment for C_6 and C_7 in aaptamine and for C_8 and C_9 for methylenedioxyaaptamine.

1. Build or sketch aaptamine. Select **Expt. Chem. Shifts** from the **Expt Data** menu, *click* on each chemically unique carbon, *click* on **Edit** to the right of **Expt. Chem. Shifts** at the bottom right of the screen, type in the chemical shift in the number pad and *click* on **Enter**. When you are done, right *click* on the background and select **Copy** from the menu that appears to make a copy of both the structure and its chemical shifts. You may or may not need this copy.

2. Select **Calculations** from the **Setup** menu. Select **NMR Spectrum**, (**Density Functional**), ω**B97X-D** and (**6-31G***) from the top four menus to the right of **Calculate** and ω**B97X-D/** ω**B97X-D/6-31G* Energy** and **HF/3-21G** from the bottom two menus. Make certain that **Total Charge** is set to **Cation**. *Click* on **Submit** at the bottom of the dialog.

3. The job will require several tens of minutes as it involves a sequence of calculations involving the possible conformers. When it has completed, bring up the **Properties** dialog (**Display** menu) and *click* on the **NMR** tab. Check on the **Best Fit** (not **Boltzmann Average**) tab at the center of the dialog. The **RMS**, **Max Absolute** and **Mean Absolute** statistics are those for the conformer that best fits the experimental data. RMS is probably the best of these measures and a value of <2 ppm is generally considered a good fit. Do you agree with the author's assignment of aaptamine as protonated under the conditions of the NMR experiment?

4. If you do agree, skip to step 5. If not, select **New Build** from the **File** menu, right *click* on screen and select **Paste** from the menu that appears. The copy that you made of aaptamine appears on screen. Select **Delete** from the **Build** menu () and *click* on the hydrogen attached to nitrogen at position 4 (see diagram on the previous page). You also need to delete the free valence as otherwise it will turn into hydrogen. Repeat the calculation being certain that **Total Charge** is now set to **Neutral**. Does the deprotonated form of aaptamine provide an acceptable fit?

5. Build or sketch methylenedioxyaaptamine as the deprotonated

form (as depicted in the paper). Enter the ^{13}C chemical shifts in the same way as before. Again, make a copy by right *clicking* on the background and selecting **Copy** from the menu.

6. This molecule is rigid so you can replace the multi-step procedure needed for aaptamine by a simple (and less costly) structure optimization. Inside the **Calculations** dialog (**Setup** menu) select **Energy**, **Ground** and **Gas** from the three top menus to the right of **Calculate** and **Density Functional**, **ωB97X-D** and **6-31G*** from the three bottom menus. Select **Equilibrium Geometry** from the menu to the right of **Start From:** and **Hartree-Fock** and **3-21G** from the two menus that then appear. *Click* inside the **NMR** box to the right of **Compute** near the bottom of the dialog. You have requested an NMR calculation using the ωB97X-D/6-31G* density functional model but with a geometry obtained from the HF/3-21G model. After you make certain that **Total Charge** is set to **Neutral**, *click* on **Submit**.

7. This job will require only a few minutes. When it has completed, bring up the **Properties** dialog (**Display** menu) and *click* on the NMR tab. Based on the RMS statistic, are the calculations for the non-protonated form of methylenedioxyaaptamine in accord with the experimental NMR? If they are, you are done. If not, go to the next step.

8. Select **New Build** from the **File** menu. Right *click* on the background and select **Paste** from the menu. Inside the organic model kit, select **-H** from the list of atomic hybrids and *double click* on screen nearby to the (non-protonated) nitrogen (N_4). *Click* on  at the bottom of the model kit, *click* on the free valence on the hydrogen and *double click* on the nitrogen. *Click* on  at the bottom of the model kit. Setup the **Calculations** dialog as before (step 6) to **Cation**. Submit and after the job completes, bring up the NMR properties dialog. Is the calculated spectrum now in good accord with the experiemental data?

9. Close all open **Spartan** documents and dialogs.