Chapter 13
Flexible Molecules

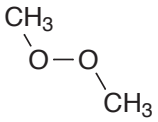
This chapter describes procedures available for calculations on flexible molecules.

Up to this point, we have not paid much attention to the different conformers that molecules might adopt. Many of the molecules dealt with so far are rigid or dominated by a single conformer and, where they are not, we have chosen to downplay the importance of conformation. In fact, the vast majority of molecules are not rigid and knowing the "right" conformer or conformers can be important to properly describing molecular spectra and properties and overall chemical behavior.

The five tutorials in this chapter illustrate a variety of approaches for dealing with flexible molecules. The first tutorial presents a molecule with only a single degree of (conformational) freedom. Here the focus is not only on identifying the "best" conformer, but also on rationalizing why it is the best. The second tutorial explores a molecule with two degrees of freedom. The focus here is on identifying all "reasonable" conformers and comparing the ordering of energies for these conformers with different theoretical models. The third tutorial involves a molecule with several degrees of conformational freedom and hundreds of conformers. Its goal is simply to find the lowest-energy conformer using practical methodology. The last two tutorials have more of a chemical focus. The first of these asks a question about the "price" that needs to be paid in order for a molecule to be properly oriented for an intramolecular reaction. The second asks a similar question, but this time with regard to a proper conformation for binding to a protein.

25 mins

Internal Rotation in Dimethylperoxide



Quantum chemical calculations may be called on to furnish data to parameterize empirical energy functions for use in molecular mechanics and/or molecular dynamics calculations. Perhaps most important are data relating to torsional motions where small changes torsional angles may lead to large changes in overall shape, as well as significant changes in molecular spectra and properties.

Any function chosen to represent the energy of rotation about a single bond needs to reflect the inherent periodicity, that is, it must repeat itself in 360°. The most common choice is a truncated Fourier series.

$$E^{torsion}(\omega) = k^{torsion1}(1 - \cos(\omega - \omega^{eq})) + k^{torsion2}(1 - \cos 2(\omega - \omega^{eq})) + k^{torsion3}(1 - \cos 3(\omega - \omega^{eq}))$$

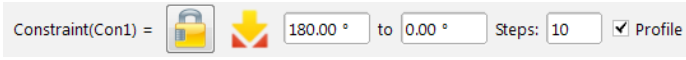
Here, ω^{eq} is the ideal dihedral angle and $k^{torsion1}$, $k^{torsion2}$ and $k^{torsion3}$ are parameters. The first (one-fold) term accounts for the difference in energy between *syn* and *anti* conformers, the second (two-fold) term for the difference in energy between planar and perpendicular conformers, and the third (three-fold) term for the difference in energy between eclipsed and staggered conformers.

In this tutorial, you will examine the energy for rotation about the oxygen-oxygen bond in dimethylperoxide, first using ωB97X-D/6-31G* density functional calculations to establish geometry and then using ωB97X-V/6-311+G (2df,2p)[6-311G*]calculations to get a more accurate account. Both ωB97X-D and ωB97X-V functionals account for long-range dispersive interactions, the former by an empirical correction and the latter more properly by explicit calculation. The 6-311+G(2df,2p) basis set is much more flexible than 6-31G*, but the calculation can be simplified using the so-called dual-basis set approximation where convergence is first reached with the smaller 6-311G* basis set. You will fit your data to truncated Fourier series.

1. Build dimethylperoxide. If the molecule is not already in an *anti* conformation, select **Measure Dihedral** from the **Geometry** menu (), *click* on COOC atoms in order, *click* inside the box to the right of **Dihedral...** and enter **180** (180°) using the number pad that appears. ***Do not minimize***.

2. Select **Constrain Dihedral** from the **Geometry** menu (). Select the COOC torsion (*click* on COOC atoms in order), and then *click* on at the bottom right of the screen. The icon will change to indicating that a dihedral constraint is to be applied.

3. *Check* the box to the left of **Profile** at the bottom right of the screen. This will result in two additional text boxes.



   Leave 180° in the original (leftmost) box alone, but change the value in the box to the right of **to** to 0°. *Click* inside the box and enter **0** using the number pad. **Steps** should be 10. If it is not, *click* inside the box and enter **10** using the number pad. What you have specified is that the dihedral angle will be constrained first to 180°, then to 160°*, etc. and finally to 0°. *Click* on .

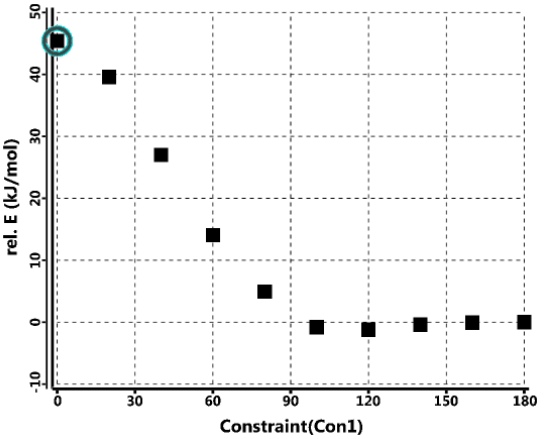
4. Select **Calculations...** from the **Setup** menu () and specify **Energy Profile** from the top menu to the right of **Calculate**, and **Density-Functional,** ω**B97X-D** and **6-31G*** from the three bottom menus. *Click* on **Submit** and accept the name ***dimethylperoxide***.

5. When the calculations have completed (several minutes), they will go into a file named ***dimethylperoxide.Prof.M0001***. A prompt will ask you if you want to open this file. *Click* on **OK**. Align the conformers. Select **Align** from the **Geometry** menu (), select **Structure** from the **Align by** menu at the bottom right of the screen and, one after the other, *click* on both oxygens and on one of the carbons. Then *click* on the **Align by** button at the bottom right of the screen. Select **Spreadsheet** from the

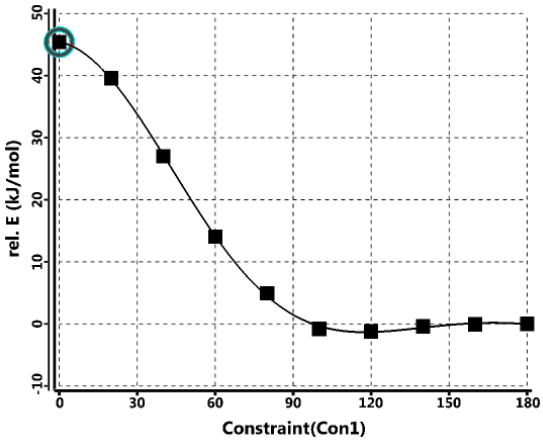
* The difference between constraint values is given by: (final-initial)/(steps-1).

Display menu ( ), and enter both the energies relative to the 180° conformer, and the COOC dihedral angles. First *click* on the label (**M0001**) for the top entry in the spreadsheet (the 180° conformer), then *click* on the header cell for the leftmost blank column, and finally, *click* on **Add...** at the bottom of the spreadsheet. Select **rel. E (kJ/mol)** from the quantities in the **Molecule List** tab, and *click* on the spreadsheet to release the dialog. To enter the dihedral angle constraints, select **Constrain Dihedral** from the **Geometry** menu ( ), *click* on the constraint marker attached to dimethylperoxide and *click* on at the bottom of the screen (to the right of the value of the dihedral angle). *Click* on .

6. Select **Plots** from the **Display** menu ( ). *Click* on at the top of the (empty) plot pane and select **Constraint(Con1)** from the **X Axis** menu and **rel. E(kJ/mol)** from the **Y Axes** list and then *click* **Create**.



To fit the points to a Fourier series, *click* on at the top of the plot pane, select **Fourier** to the right of **Curve** in the resulting dialog and *click* on **Done**.



7. To get a better account of the energy profile for rotation about the OO bond in dimethylperoxide, perform calculations with the ωB97X-V/6-311+G (2df,2p)[6-311G*] density functional model* (using the equilibrium geometries that you obtained from the ωB97X-D/6-31G* model). First, make a copy of ***dimethylperoxide.Prof.M0001***. Name it ***dimethylperoxide ωB97X-V***. Select **Calculations...** from the **Setup** menu ( ) and specify calculation of energy using the ωB97X-V/6-311+G (2df,2p)[6-311G*] density functional model. Make certain that **Global Calculations** (at the bottom of the dialog) is *checked* to signify that energy calculations are to be performed on all conformers. *Click* on **Submit**.

8. Energy calculations for all ten conformers will require several minutes to complete. When they are done, draw a new energy plot and compare it to the energy plot produced earlier.

9. Close all documents and dialogs.

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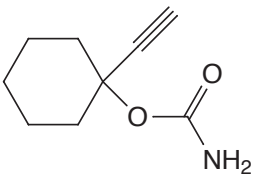
* This is different from the model provided in SSPD in that it employs the dual basis set approximation where 6-311G* is the supporting small basis set. Use of the dual basis set approximation leads to an order of magnitude reduction in cost with little noticeable difference in relative conformer energies.

30 mins

Ethinamate



Ethinamate is a prescription drug previously used for the treatment of insomnia. It involves two degrees of conformational freedom, rotation of the single bond to the carbonate group (two unique arrangements) and inversion of the six-member ring (two unique chair conformers).

1. Build or sketch ethinamate. *Click* on . Select **Calculations...** from the **Setup** menu ( ) and request a **Conformer Distribution** with the ωB97X-V/6-311+G (2df,2p)[6-311G*] density functional model using ωB97X-D/6-31G* geometries. This actually invokes a multistep recipe starting from a systematic conformer search using MMFF molecular mechanics and proceeding through several immediate steps to gradually reduce the number of conformers for the final energy calculation. *Click* on **Submit**, and accept the name ***ethinamate***.

2. The calculation will require several tens of minutes. When it completes, ***ethinamate*** will display the lowest-energy conformer but the total energy, dipole moment and Mulliken atomic charges* will refer to a Boltzmann weighted average over all accessible conformers. A ***Spartan*** document containing these conformers (***ethinamate_1***) has been returned. Open the latter document, enter the total energies into the spreadsheet. You will see that the total energy of the best conformer is in fact lower than that of the Boltzmann averaged value (found in ***ethinamate***).**

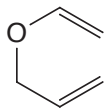
5. Close any open documents and dialogs.

* Natural charges are available only for the 6-311+G(2df,2p) basis sets. Electrostatic charges are too costly to calculate for molecules with large numbers of accessible conformers and are deliberately excluded.

** This may be surprising but is necessarily the case as the Boltzmann average while dominated by the lowest-energy conformer includes contributions from higher energy conformers.

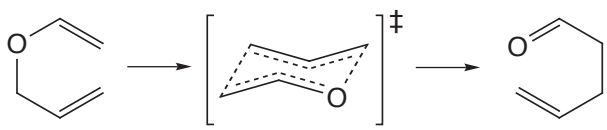
5 mins

Allyl Vinyl Ether



The **Equilibrium Conformer** and **Conformer Distribution** selections under **Calculations** in the **Setup** menu directly access the MMFF molecular mechanics model and either the ωB97X-V or B97M-V density functional models with the 6-311+G(2df,2p) [6-311G*] basis sets. They represent the extremes in terms of cost computation and are intended to be used in two situations with entirely different expectations. There are numerous models and combinations of models in between and the purpose of this tutorial is to explore one of them. Here we seek a semi-quantitative account of the energy required to go from the "best" conformer of a molecule to a conformer poised for chemical reaction. This is different than the expectation of the following tutorial which is to accurately account for the NMR spectrum of a flexible molecule.

Allyl vinyl ether undergoes Claisen rearrangement, the mechanism presumes a chair arrangement of the reactant.



Is this the lowest-energy conformer (global minimum) or is additional energy required to properly orient the molecule for reaction? To find out, you need to locate all the conformers of allyl vinyl ether, identify the best chair structure and evaluate its energy relative to that of the actual global minimum. You will first carry out a conformational search using the MMFF molecular mechanics model, and then refine relative conformer energies using single-point ωB97X-D/6-31G* density functional calculations based on 3-21G Hartree-Fock equilibrium geometries. The results are not expected to be as good as those that might be obtained from better geometry and especially energy calculations, but they should be sufficient for the objective at hand.

1. Either build  or sketch  allyl vinyl ether.

2. Select **Calculations...** from the **Setup** menu () and specify **Conformer Distribution** from the top menu to the right of **Calculate** and **Molecular Mechanics** and **MMFF** from the two bottom menus. *Click* on **Submit** and accept the name ***allyl vinyl ether***.

3. When completed, it will give rise to a series of low-energy conformers* placed in a new file ***allyl vinyl ether.Conf.M0001***.** A prompt will ask you if you want to open this file. *Click* on **OK**.*** Select **Spreadsheet** from the **Display** menu (). Size the spreadsheet such that all rows (corresponding to different conformers) are visible at one time. *Click* on **Add...** at the bottom of the spreadsheet. *Click* on the **E (kJ/mol)** button from the **Molecule** tab and *click* on the spreadsheet to remove the **Add** dialog. Energies for each of the different conformers will be added to the spreadsheet. Examine the lowest-energy conformer (the top entry). Is it in a chair conformation suitable for Claisen rearrangement? If not, identify the lowest-energy conformer that is suitable. You can keep two or more conformers on screen at the same time by *checking* the boxes immediately to the left of the molecule labels (the leftmost column) in the spreadsheet. To get a clearer idea of structural similarities and differences, align the conformers. Select **Align** from the **Geometry** menu () and select **Structure** from the **Align by** menu at the bottom right of the screen. A message will appear at the bottom left of the screen.

Click on oxygen and on the two carbons of the vinyl group bonded to oxygen to designate them as alignment centers. Each will be marked by a red circle. If you make a mistake, *click* on the circle and it will disappear. When you are done, *click* on the **Align by** button at the bottom right of the screen. *Click* on  .

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* By default, only conformers within 40 kJ/mol of the global minimum will be kept. This can be changed (see **Conformational Search** in **Appendix D**).

** **M0001** is the default label of the molecule you built. You can change it by altering the **Labels** field in the **Molecule Properties** dialog (**Properties** under the **Display** menu; **Chapter 22**).

*** To avoid confusion, it is a good idea to close the original file ***allyl vinyl ether***.

4. To obtain a better estimate of the energy required to twist allyl vinyl ether into a conformer suitable for Claisen rearrangement, perform ωB97X-D/6-31G* energy calculations using 3-21G geometries. Select **Save As** from the **File** menu () to make a copy of ***allyl vinyl ether.M0001***. Name it ***allyl vinyl ether density functional***. Using the copy, delete all conformers except the global minimum and the lowest-energy conformer that appears to be most closely poised for Claisen rearrangement. Select each conformer to be discarded, and then select **Delete Molecule** from the **File** menu (). Alternatively, *click* on the cell in the spreadsheet containing the label for the molecule to be deleted, and then *click* on **Delete** at the bottom of the spreadsheet. When you are done, the spreadsheet should contain only two entries.

5. Select **Calculations...** from the **Setup** menu () and specify an **Energy** calculation and **ωB97X-D Density Functional** method with the **6-31G*** basis set. Also, specify **Hartree-Fock** and **3-21G** from the **Start from** menu. Make certain that **Global Calculations** at the bottom of the dialog is checked to specify that the dialog settings apply to both conformers. *Click* on **Submit**.

6. When completed, examine the conformer energies. Select **Spreadsheet** from the **Display** menu. *Click* on the **Add** button at the bottom of the spreadsheet and *click* on the **rel.E (kJ/mol)** button from the **Molecule List** tab. *Click* on the spreadsheet to release the **Add** dialog. What is the energy needed to go from the global minimum to a conformer poised to undergo Claisen rearrangement?

7. Close all open documents and dialogs.

2 hours

^{13}C Chemical Shifts Depend on Conformation

At normal temperatures, the time for nuclear spin relaxation is very long relative to time required for equilibration among conformers. This means that for each of the carbon chemical shifts in the NMR spectrum of a flexible molecule, $^{13}\mathbf{C}$, will be a weighted average of the shifts NMR of the individual conformers, $^{13}C_i$.

$$^{13}\mathbf{C} = \Sigma_i \, \omega_i \, ^{13}C_i$$

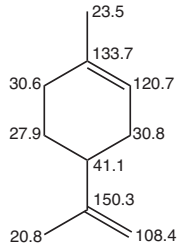
The weight, ω_i, is given by the Boltzmann equation, and depends on its energy, ε_i (relative to that of the lowest-energy conformer, ε_0) and on temperature, T. Summation is over all conformers (including the lowest-energy conformer), g_i is the number of times that conformer i appears in the overall distribution and k is the Boltzmann constant.

$$\omega_i = g_i \exp - [\varepsilon_i - \varepsilon_0/kT]/\Sigma_j \{\exp - [\varepsilon_j - \varepsilon_0/kT]\}$$

In practice, conformers that are 10 kJ/mol or more above the lowest-energy conformer make a negligible contribution to the total (at room temperature). However, in order to actually know which conformers are important and to construct a proper Boltzmann average, it is necessary to examine all of them.

In this tutorial, you will use the protocol first introduced with ***Spartan'18*** to calculate the ^{13}C NMR spectrum of limonene. This comprises two parts, the first a multi-step protocol starting from MMFF molecular mechanics and ending with a series of large basis set density functional calculations to obtain "accurate" Boltzmann weights, and the second a set of small basis set density functional calculations to obtain ^{13}C chemical shifts. The two parts are then combined to produce a Boltzmann averaged ^{13}C spectrum.

1. Build or sketch limonene.



2. Enter experimental ^{13}C chemical shifts (shown above) either on the sketch or 3D model. If you choose to add to the sketch, select **Edit Sketch** ( ) from the **Build** menu and then *click* on ($^{1}H/^{13}C$) from the row of icons above the sketch pad. *Click* in turn on each carbon, *click* inside the box that appears to its right, use the number pad that comes up to type in the chemical shift and *click* on **Enter**. If you choose instead to add to the 3D model, select **Expt. Chem. Shifts** [$^{1}H/^{13}C$] from the **Expt. Data** menu. As with the sketch, *click* in turn on each carbon, *click* inside the box that appears to its right, use the number pad to type in the chemical shift and *click* on **Enter**.

3. Select **Calculations...** from the **Setup** menu, **NMR Spectrum** (**Density Functional**)*, **ωB97X-D** and (**6-31G***)* from the top three menus to the right of **Calculate** and **ωB97X-V/ 6-311+G(2df,2p)[6-311G*] Weighted Average** and **ωB97X-D/ 6-31G*** from the bottom two menus. *Click* on **Submit**.

4. The calculation may require an hour or more.** When completed, bring up the **Properties** dialog (**Display** menu) and *click* on the **NMR** tab. Statistics related to the fit between calculated and experimental ^{13}C shifts are provided on the left at the center of the dialog that results, specifically **RMS**, **Max Absolute** and **Mean Absolute** errors. If the **Boltzmann Average** tab is checked, these correspond to a Boltzmann weighted average over conformers. If the **Best Fit** tab is checked, they instead correspond to the conformer which best fits measured values. You will see that

* Pre-selected menu items cannot be altered.

** If you don't want to wait, a pre-calculated file is available. For Windows, this directory is found in ***Program Files/Wavefunction/Spartan20***. It must be copied 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 of the ***Spartan'20*** disc image.

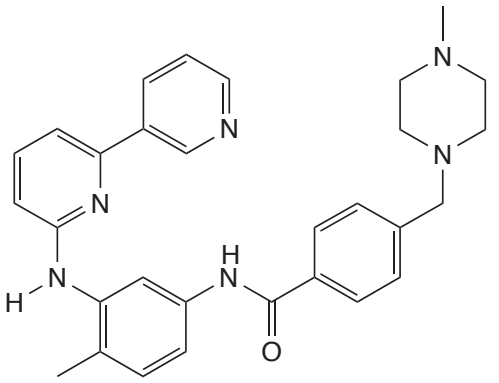
both measures provide good concurrence between calculated and measured ^{13}C chemical shifts.

Spartan provides two different ways to assess the fit between calculated and measured ^{13}C chemical shifts. The first and clearly the more rigorous measure involves constructing a proper Boltzmann-weighted average. This requires accurate conformer energies and associated Boltzmann weights which in turn functionals that take proper account of long-range interactions such as the ωB97X-V functional and large basis sets such as 6-311+G(2df,2p). While such an approach can be applied to medium-size organic molecules with moderate conformational flexibility, it can involve many hours of computer time. The second approach only requires that a set of "good" conformers be identified, and from among these selects the conformer ^{13}C chemical shifts for which best fit measured values. Lacking the need for accurate Boltzmann weights, it can rely on simpler functionals such as ωB97X-D where long range interactions are dealt with empirically and smaller basis sets such as 6-31G*, and as a result, is an order of magnitude faster.

5. The structure that has been returned corresponds to that with the lowest ωB97X-V/6-311+G(2df,2p)[6-311G*] energy (the leading contributor in the Boltzmann average). A secondary file, **limonene_Conf**, has been created (but not opened). This contains NMR chemical shift calculations for all conformers.
6. Close any open documents and dialogs.

2 mins

Gleevec. Protein Bound vs. Free Conformer



gleevec

Gleevec (Glivec, Imatinib) is a protein kinase inhibitor used in anticancer therapy. It specifically targets a protein kinase coded for in the rogue gene ***bcr-abl***. Several crystal structures for gleevec docked in protein kinases are available in the PDB. In this tutorial, you will examine one of them* to establish whether or not the conformation of gleevec in the protein is identical (or similar) to that of the free (gas-phase) molecule, and if not, what energy penalty is to be paid to adopt the protein-bound conformation. The next tutorial will return to this same structure and extract a pharmacophore (gleevec's footprint).

1. Retrieve a protein structure from the PDB. Select **Access PDB Online...** from the **File** menu ( ). Type ***1opj*** into the box to the right of PDB ID and *click* on **Open**.

   If you are not online, you may skip this step as the structure is available as ***1opj*** in the ***medicinal chemistry*** sub-directory (***tutorials*** directory).**

   The protein will be represented by a ribbon model. Red sections correspond to parts of the chain that are α helices, while blue

* B. Nager *et al*, Cell, **112**, 859 (2003): PDB identification 1opj.

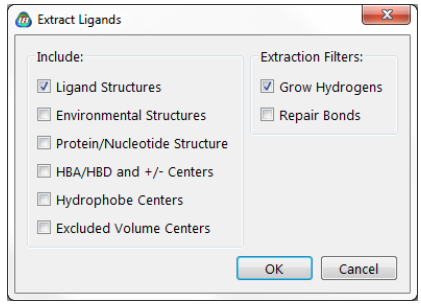
** For Windows, this directory is found in ***Program Files/Wavefunction/Spartan20***. It must be copied to another location available to the user prior to opening it in ***Spartan***. For Linux, this is found in the directory where ***Spartan*** was installed. For Macintosh, this is located at the top level of the ***Spartan'20*** disc image.

regions correspond to parts that are β sheets (the remaining regions are green). A Ramachandran plot (the ϕ and ψ torsional angles connecting the individual amino acids) allows you to see the clustering of α helices and β sheets. To display, select **Ramachandran Plot** from the **Model** menu ( ). The colors of the dots in the resulting plot map one-to-one with the colors of the ribbon model. When you are done, *click* on to remove the plot.

> The colors on the ribbon display may be modified, by selecting **Configure...** from the **Model** menu ( ) and *clicking* on the **Ribbons** tab. Coloring **By Residue** will give each amino acid its own unique color.

The protein you have brought in from PDB incorporates two sets of bound molecules, depicted in the model as two sets of translucent spheres. Gleevec is the larger of the two incorporated molecules and is designated by its ***PDB HET*** code ***STI***.

2. Select **Extract Ligands** from the **File** menu ( ). Select (*click* on) one of the gleevec molecules inside the protein structure and *click* on the **Extract Ligands** button at the bottom right of the screen. This leads to the **Extract Ligands** dialog.



Check **Ligand Structures** under **Include** and **Grow Hydrogens** under **Extraction Filters** inside the dialog (if other options are selected, remove them) and *click* on **OK**.

3. A ball-and-spoke model for gleevec will appear on screen. (You no longer need the protein; select and close it.) Put a copy of gleevec on the clipboard. With the molecule selected, choose **Copy** from the **Edit** menu or right *click* on the background and select **Copy** from the menu that appears.

4. Select **Build New Molecule** (not **New Build**) from the **File**

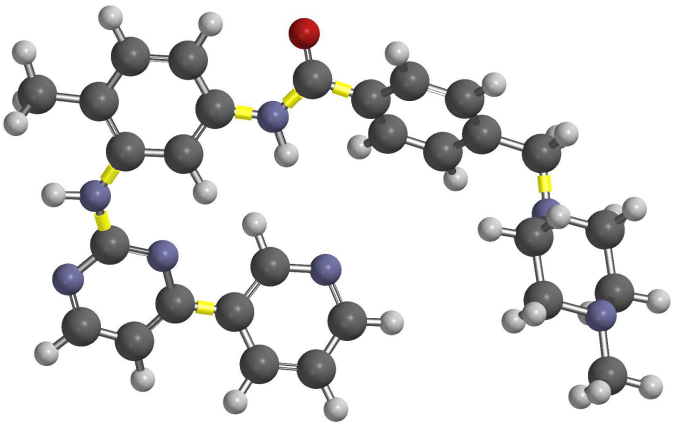
menu ( ). Select **Clipboard** from the organic model kit and *click* anywhere on screen. *Click* on . You now have two copies of gleevec in a single document. You can step between them using the step buttons at the bottom left of the screen.

5. Select the first copy and then select **Calculations...** from the **Setup** menu ( ). **Equilibrium Geometry** from the top left menu to the right of **Calculate** and **Molecular Mechanics** and **MMFF** from the two bottom menus. The conformation will not be altered, but bond lengths and angles will be optimized.

> Structure optimization is necessary in order to establish the energy difference between protein-bound and free conformations of gleevec. The resolution of protein X-ray crystallography is not adequate to establish bond lengths and angles to chemical accuracy.

Remove the checkmark from **Global Calculations** at the bottom of the dialog to signify that your choice (geometry optimization) only applies to this copy of gleevec. *Click* on **OK** (not on **Submit**).

6. You will use the second copy of gleevec as a starting point for a conformational search. First, remove some degrees of conformational freedom in order to shorten the computer time for the conformational search. With the second copy, select **Set Torsions** from the **Geometry** menu ( ). Your model will be augmented with yellow cylinders and circles to designate flexible bonds and rings, respectively. Remove the markers (circles) on the piperazine ring by *double clicking* on each in turn. The conformation of the piperazine ring will not change during the search. The model should now appear as below.



7. Select **Calculations...** from the **Setup** menu ( ). Select **Equilibrium Conformer** from the top left menu to the right of **Calculate** and (as before) **Molecular Mechanics** and **MMFF** from the two bottom menus. *Click* on **Submit**, and provide the name ***gleevec protein bound vs. free conformer***.

8. When the calculations have completed (perhaps minutes), select **Spreadsheet** from the **Display** menu ( ). *Click* inside leftmost the cell for the second molecule (the one on which a conformational search was performed) then *click* inside the header cell for a blank data column. *Click* on **Add** at the bottom of the dialog and select **Rel. E** from the scroll box in the dialog that appears and **kJ/mol** from the **Energy** menu. The relative energy of the protein bound conformer is provided in the spreadsheet. Is it close to zero meaning that the protein-bound conformer will be present in significant amount in a sample of free gleevec?

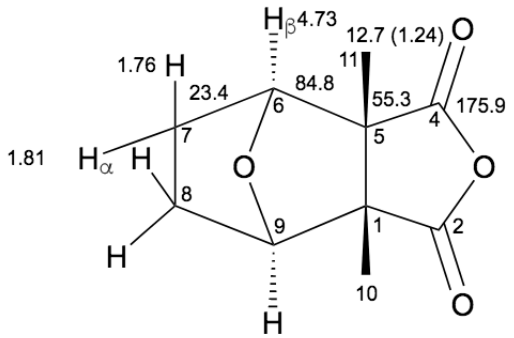
9. Close ***gleevec protein bound vs. free conformer*** and any open dialogs.

15 mins

HMBC Spectrum of Cantharidin

Cantharidin provides a very simple example for the way in which an HMBC spectrum is set up and displayed and the results compared with assigned experimental CH couplings. The molecule is rigid eliminating concerns about conformation and its structure has been confirmed by X-ray crystallography.

1. Build or sketch cantharidin. Enter both experimental proton and ^{13}C chemical shifts as shown below (the 1.24 in parenthesis following the ^{13}C shift of 12.7 for the C_{10} and C_{11} are the associated proton shifts).*



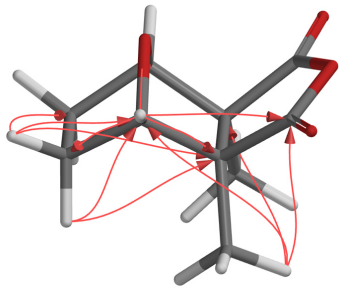
2. In 3D mode, select **Expt. Chem. Shifts** from the **Expt Data** menu or *click* on each chemically unique carbon and hydrogen, *click* on **Edit** to the right of **Expt. Chem. Shifts** at the bottom right of the screen, type in the chemical shift in the number pad and *click* on **Enter**.

3. Observed 2 and 3-bond CH couplings are as follows (see numbering above):

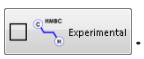
H_6	C_4, C_5, C_7, C_9
$H_{6\alpha}$	C_5, C_6, C_7
$H_{7\beta}$	C_5, C_6, C_7
H_{10}	C_2, C_4, C_9

* Experimental data from: D. Sicker, K-P. Zeller, H-U Siehl and S. Berger, **Natural Products**, **Isolation**, **Structure Elucidation and History**, Wiley -VCH, Wenheim, Germany, 2018.

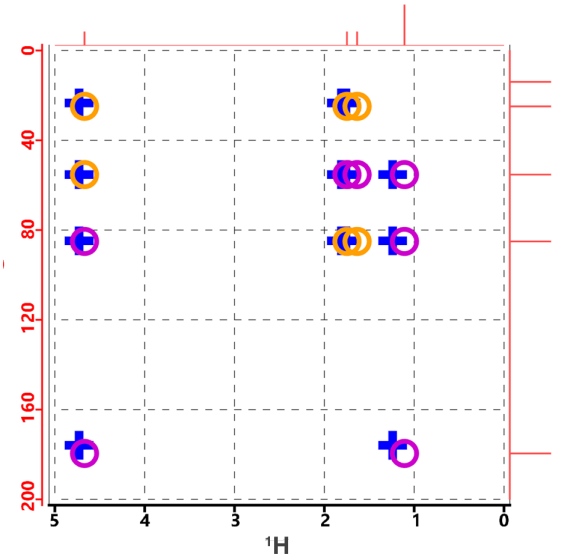
In 3D mode, select **CH Couplings** from the **Expt. Data** menu or *click* on [J^{CH}]. *Clicking* on one of the four hydrogens noted on the previous page, for example H_6 will result in all carbons where 2 or 3-bond CH coupling might be observed being highlighted. *Click* on one of these, C_4, C_5, C_7 or C_9, in this example, will draw an arrow from hydrogen to carbon. *Click* again on the same hydrogen to specify the couplings for the other carbons. Repeat for the remaining hydrogens. Your 3D model (tube representation) should look as follows.



4. Cantharidin is rigid, so the multi-step procedure involving different possible conformers is not necessary. Select **Calculations** from the **Setup** menu and specify calculation of equilibrium geometry using the wB97X-D/6-31G* model. *Check* **NMR** to the right of **Compute** and select **Calculated (Fermi Contact)** from the menu to the right of **Coupling Constants**. You have requested both chemical shifts and coupling constants to be calculated, the latter using the so-called Fermi contact approximation. This is nearly as accurate and significantly faster than the full coupling constant calculation. *Click* on **Submit**.

5. When the job completes (a few minutes), select **Spectra** from the **Display** menu (or *click* on ), *click* on  at the top left of the spectra display that occupies the lower half of the screen and select  . *Click* again on  and select  . Calculated and experimental HMBC spectra will be superimposed and appear at the center of the spectra display area. *Click* on  to bring them onto the main screen and then *click* on  at the top right of the spectra display area to dismiss it.

6. Expand the spectra on the main screen by first *clicking* on either the left vertical axis (**^{13}C**) or the bottom horizontal axis (**^{1}H**) and then using the scroll wheel. Alternatively, on touch screen devices, pinch your fingers together after selecting an axis. The scale of the ^{13}C axis (0 - 200 ppm) is OK, but change the proton axis to range from 0 - 5 ppm. *Click* on the axis to select and enter **5** to replace the default value of 10 ppm and *press* **Enter**. The HMBC plot should appear as follows.



The crosses represent experimental couplings and the open circles calculated couplings. Orange circles designate 3 bond couplings and magenta circles designate 4-bond couplings. You can see details (proton and ^{13}C chemical shifts in ppm and, in the case of calculated values, coupling constants in H_3) by *clicking* inside the circles or at the center of the crosses.

7. Close all open files and dialogs.

5 hours

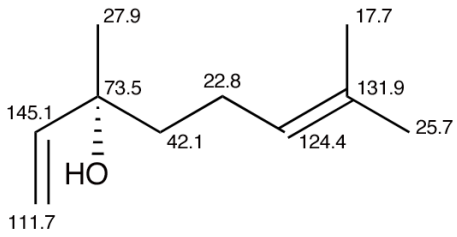
Linalool: Calculating the NMR Spectrum of a Flexible Molecule

Spartan provides two different pathways, each supported by an automated multi-step protocol, for calculating proton and ^{13}C spectra as well as 2D COSY and HMBC spectra of flexible molecules. The more rigorous and more costly in terms of required computation involves construction of a proper Boltzmann-weighted average, in practice, calculation of accurate Boltzmann weights for all accessible conformers followed by calculation of chemical shifts and (optionally) coupling constants for each of the conformers. Boltzmann weight calculation is the limiting step as it requires density functional models that account explicitly for long-range van der Waals or dispersive interactions coupled with large basis sets. Calculations of moderate size (MW in the range of 500 amu) with 100 or more conformers can easily consume many hours on present generation personal computers.

The second less costly and less rigorous approach replaces the need for accurate Boltzmann weights with the less demanding task of identifying the low-energy conformers and then choosing from among these conformers the one that best fits the experimental NMR data. Of course, this requires that the experimental data exist! Simpler density functional models with much smaller basis sets are suitable.

In this tutorial, you will calculate ^{13}C chemical shifts for the natural product linalool using the simpler procedure and compare results both to experimental values as well as those obtained from Boltzmann averaging.

1. Build or sketch linalool. Enter the ^{13}C shifts (see figure below). 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 a chemical shift using the number pad and *click* on **Enter**. When you are done, *click* on  .



2. Open the **Calculations** dialog (**Calculations** from the **Setup** menu) and select **NMR Spectrum** (**Density Functional**), **ωB97X-D** and (**6-31G***) from the four top menus to the right of **Calculate** and **ωB97X-D/6-31G*** and **3-21G** from the two bottom menus. This specifies calculation of energies and chemical shifts from the ωB97X-D/6-31G* density functional model using Hartree-Fock 3-21G equilibrium geometries. *Click* on **Submit**.

3. The job will take several hours. When completed, bring up the NMR properties dialog (**Properties** from the **Display** menu and *click* on **NMR**). *Click* on the **Best Fit** tab at the center left of the dialog to see how well the calculated shifts fit the experimental data. RMS is perhaps the best measure where values less than or close to 2 ppm indicating a good match.

4. The calculation you performed selects the conformer that best fits the experimental NMR data rather than constructing a proper Boltzmann-weighted average. You can do the latter if you wish (it will require significantly more computer time) by changing the entries in the two bottom menus to the right of **Calculate** in the **Calculations** dialog to **ωB97X-V/6-311+G(2df,2p) [6-311G*] Weighted Average** and **ωB97X-D/6-31G***. When this job completes, you need to select the **Boltzmann Average** tab in the **NMR** properties dialog instead of the **Best Fit** tab. The errors should be identical or similar to:

   RMS 1.88
   Max Absolute 3.36
   Mean Absolute 1.60

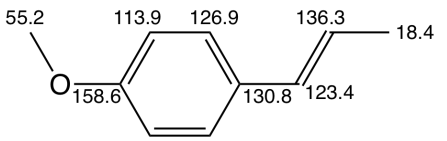
   Both procedures provide a good description in this case.

5. Close any ***Spartan*** documents and open dialogs.

1 hour

Anethole: Boltzmann Averaging ^{13}C Chemical Shifts

The natural product anethole provides a very simple illustration of obtaining ^{13}C chemical shifts as a Boltzmann-weighted average over different conformers.



The molecule is small and there are only two conformers, so the task can be completed using only a few minutes of computer time.

1. Build or sketch anethole. Enter ^{13}C experimental chemical shifts (provided in the drawing above). Select **Expt. Chem. Shift** from the **Expt. Data** menu and in turn *click* on each unique carbon*, *click* on **Edit** to the right of **Expt. Chem. Shift** at the bottom right of the screen, use the number pad to enter the value of the shift and *click* on **Enter**.

2. Inside the **Calculations** dialog (**Calculations** from the **Setup** menu), select **NMR Spectrum**, (**Density Functional**), ω**B97X-D** and **(6-31G*)** from the four top menus to the right of **Calculate** and ω**B97X-V/6-311+G(2df,2p)[6-311G*] Weighted Average** and ω**B97X-D/6-31G*** from the two bottom menus. You have requested that Boltzmann weights be obtained in a multi-step procedure for the (two) conformers of anethole using the ωB97X-V/6-311+G(2df,2p)[6-311G*]//ωB97X-D/6-31G* density functional model, and following this, chemical shifts for the conformers be calculated with the empirically corrected ωB97X-D/6-31G* density functional model, allowing that the Boltzmann average to be obtained. *Click* on **Submit** at the bottom of the dialog.

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* ***Spartan*** detects identical atoms (in this example the two carbons ortho and the two carbons meta) to the methoxy group. You only need to enter experimental data for unique atoms.

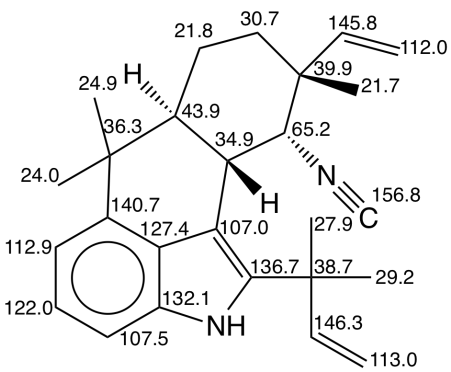
3. When completed (probably 10-20 minutes), bring up the NMR properties dialog (**Properties** under the **Display** menu and *click* on the **NMR** tab. Make sure that **Boltzmann Average** tab (not **Best Fit**) tab is selected. The reported error statistics indicate an excellent fit of calculated to experimental ^{13}C shifts.

4. Close any open ***Spartan*** documents and dialogs.

several hours

Ambiguine H. DP4 Analysis to Assign Stereoisomer

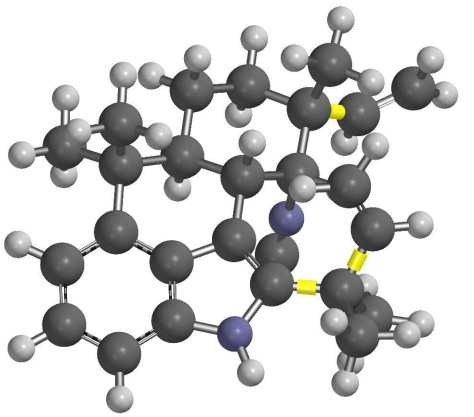
The structure assigned to the natural product ambiguine H is shown below. It contains four stereocenters, two of which designated R dictate the disposition of substituents attached to the incorporated trans-decalin bicyclic.



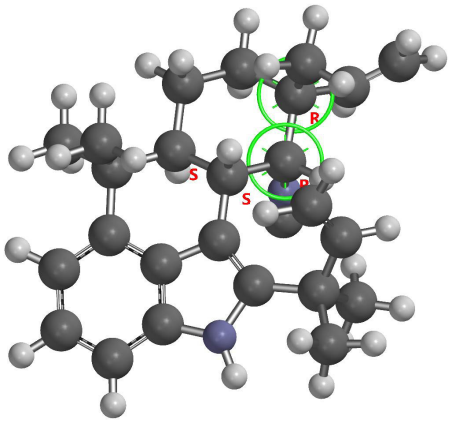
In this tutorial, you will use the DP4 metric to confirm or refute the experimentally assigned stereochemistry about these two centers. This involves chemical shift calculations on the four distinct structures each of which is conformationally flexible.

1. Build or sketch ambiguine H. To enter the experimental ^{13}C chemical shifts, select **Expt. Chem. Shift** from the **Expt. Data** menu. *Click* on each carbon in turn, *click* on **Edit** to the right of **Expt. Chem. Shift** at the bottom right of the screen, use the number pad to type in a value and *click* on **Enter**.

2. Rotatable bonds should be properly marked, but it is a good idea to check. Select **Set Torsions** from the **Geometry** menu. The three bonds shown in the figure below should be marked (encircled with yellow cylinders). If any are not, *double click* on the bond to select. If on the other hand, any other bonds are selected, *double click* to deselect.



3. Make a list of the four stereoisomers. Select **Generate Isomers** from the **Geometry** menu. In turn, *click* on each of the two stereocenters bearing substituents. They will be encircled in green (see figure below). *Click* on **Generate** list to the right of **Isomers** at the bottom right of the screen. Select **New Document** from the dialog that appears and *click* on **OK**. The four isomers (including the RR stereoisomer that you started with) will be placed in a new ***Spartan*** document. *Click* on **Save** (**File** menu) and name it ***ambiguine H isomers***. It's a good time to close the original document.



4. Bring up the **Calculations** dialog (**Calculations** from the **Setup** menu) and select **NMR Spectrum**, (**Density Functional**), ωB97X-D and **6-31G*** from the four top menus to the right of **Calculate** and ωB97X-D/6-31G* and **HF/3-21G** from the two bottom menus. What you have requested is done "under the hood" in two steps. First, NMR spectra for all conformers for each of the four stereoisomers are calculated, allowing the conformer for each isomer that best fits the experimental ^{13}C chemical shifts based on its DP4 score to be identified. Second DP4 scores for this set of "best" are evaluated. Make certain that **Global Calculations** at the bottom of the dialog is checked before you *click* on **Submit**.

5. The job will take several hours to complete. It involves calculations on as many as 108 molecules (up to 27 conformers on each of the 4 stereoisomers). When it is done, bring up the NMR properties dialog (**Properties** under the **Display** menu and *click* on the **NMR** tab). Step through the four isomers and identify the one with the highest DP4 score. Is this the one that has been assigned based on its NMR spectrum? Are any other isomers competitive?

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