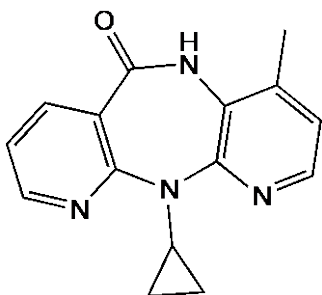


## MOLECULAR MODELLING EXERCISES

### EXERCISE 20.1 NEVIRAPINE

#### INTRODUCTION

Nevirapine (Fig. 1) is an antiviral agent that inhibits the viral enzyme reverse transcriptase. It is classed as a non-nucleoside reverse transcriptase inhibitor and binds to an allosteric binding site, unlike nucleoside inhibitors which bind to the active site. Further information on nevirapine and other non-nucleoside reverse transcriptase inhibitors can be found in section 20.7.3.2 in the textbook. Nevirapine has been identified as a chiral molecule although there is no asymmetric carbon centre present. In this exercise, we will examine the structure to see why the molecule is considered to be chiral.



**Figure 1** Nevirapine.

#### INSTRUCTIONS

It is suggested that you attempt to carry out the following instructions yourself before following the more detailed **Procedures** that follow. You may find the file entitled **Common Operations for ChemBio3D/Chem3D** a useful guide on how to carry out various operations. ChemDraw files for various structure can be found in the ChemDraw folder.

#### PART A

- \*Create the energy-minimised 3D-structure of nevirapine.
- \*Identify the number of rotatable bonds in the structure.
- \*Carry out a conformational analysis involving rotation round the bond between nitrogen and the cyclopropyl ring.
- \*Identify the most stable conformation generated.

#### PART B

- \*Choose the most stable conformation of nevirapine generated in part A
- \*Generate the opposite enantiomer of that conformation.
- \*Overlay the two structures to establish that they are different.
- \*Use molecular dynamics to assess the likelihood of pyramidal inversion for the nitrogen atom bearing the cyclopropyl group.

## PROCEDURES


There are various approaches that you can use to tackle these molecular modelling exercises. The following procedures illustrate how you might tackle this particular exercise, but they are not meant to be prescriptive. Note also that the results obtained may vary depending on the computer and the version of ChemBio3D or Chem3D used. For example, the specific conformations obtained from energy minimisation may differ, as may quantitative results such as steric energies.

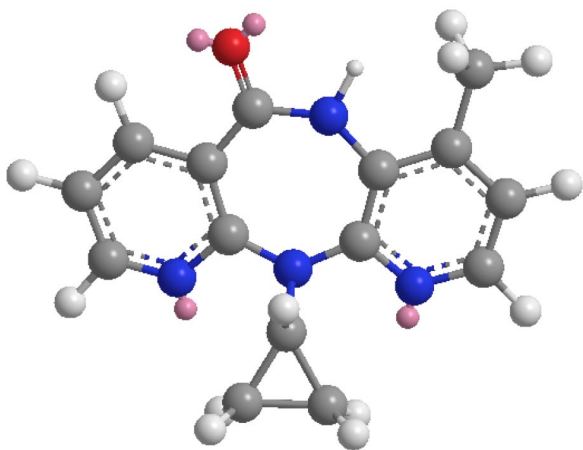
### PART A Structure and conformations of nevirapine.

#### 1. Create the energy-minimised 3D model of nevirapine.

\*Open **ChemBio3D** or **Chem3D**.

\*From the **File** menu, choose **Open**, then select the ChemDraw file for nevirapine. Click **Open**.

\*Energy minimise  the structure. The energy-minimised structure has a steric energy of 48.2 kcal/mol (Fig. 2).

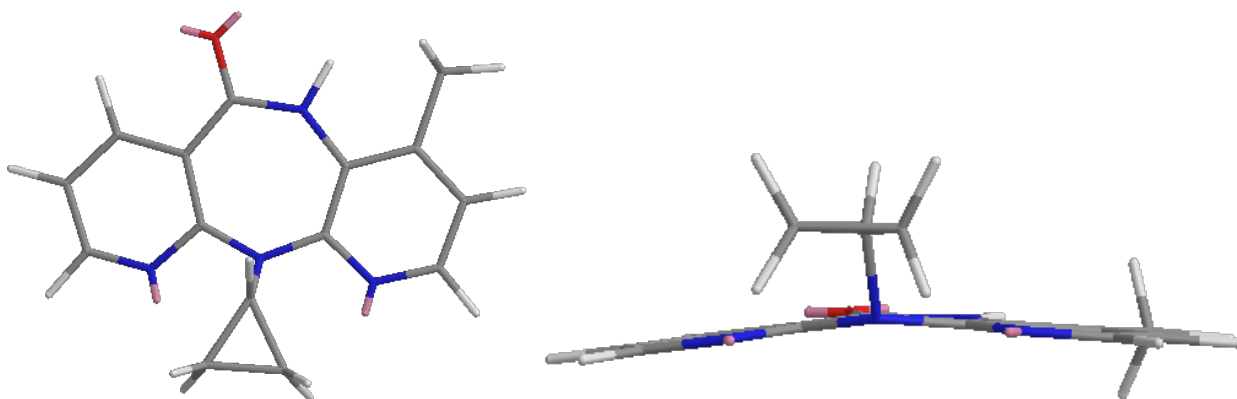


**Figure 2** Energy-minimised structure of nevirapine.

#### 2. Change the display mode to sticks.

\*From the **View** menu, choose **Model Display**, then **Display Mode**. Select **Sticks**.

The stick model demonstrates that the tricyclic ring system is slightly kinked to form a butterfly shape, with the *N*-cyclopropyl group pointing upwards (Fig. 3).

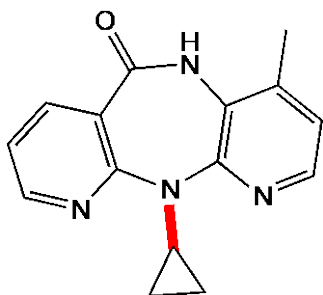


**Figure 3** Energy-minimised structure of nevirapine from two different perspectives.

#### 3. Identify any rotatable bonds that might be present.


- \*From the **Calculations** menu *choose* **Compute Properties**.
- \**Expand* **Molecular Topology** and *select* **Num Rotatable Bonds**.
- \**Click* **OK**.

The number of rotatable bonds can be found in the bottom window and is given as 1. Note that rotatable bonds in this context are defined as those that result in distinct differences in conformation. Rotatable bonds that only alter the relative positions of hydrogen atoms (e.g. C-CH<sub>3</sub>) are not included in the total. In this case, the only rotatable bond in terms of conformational analysis is the one between N and the cyclopropyl ring (Fig. 4).



**Figure 4** Rotatable bond in nevirapine in terms of conformational analysis.


#### 4. Generate a Dihedral Driver chart from rotation round the rotatable bond.

- \**Copy* and *paste* the energy-minimised structure into a new window.
- \**Choose* the **Select** tool .
- \**Click* on the rotatable bond between nitrogen and the cyclopropyl group.
- \*From the **File** menu, *choose* **Preferences** to open up a dialogue box.
- \**Click* the tab entitled **Dihedral Driver**.
- \*Use the slider bar in the new dialogue box to make the rotation angle 1°. Make sure that the tick box for minimization is unticked.
- \**Click* on **Apply** and **OK**.
- \*From the **Calculations** menu, *choose* **Dihedral Driver**, then **Single Angle Plot**. You will see rotation taking place round the rotatable bond. On completion, a chart should appear showing how the steric energy has altered as rotation takes place round the chosen bond (Fig. 4). If the chart is not visible, *click* on the tab for the **Dihedral Driver Chart**, which is just above the main window. If it is still not visible, you may have to enlarge the window containing the chart by dragging the margins. This is immediately above the main window.

#### 5. Identify stable conformations from the chart

The chart measures steric energy on the y-axis and a dihedral angle on the x-axis. The dihedral angle defines the conformation. On this run, the four atoms used to measure the dihedral angle are C(2)-N(1)-C(9)-C(10), where the numbers are the serial numbers defined by the program. The serial numbers in the structure can be identified by *hovering* the mouse over each atom in turn to produce a pop up window. Alternatively, the numbers can be displayed on the structure as follows (Fig. 4B).

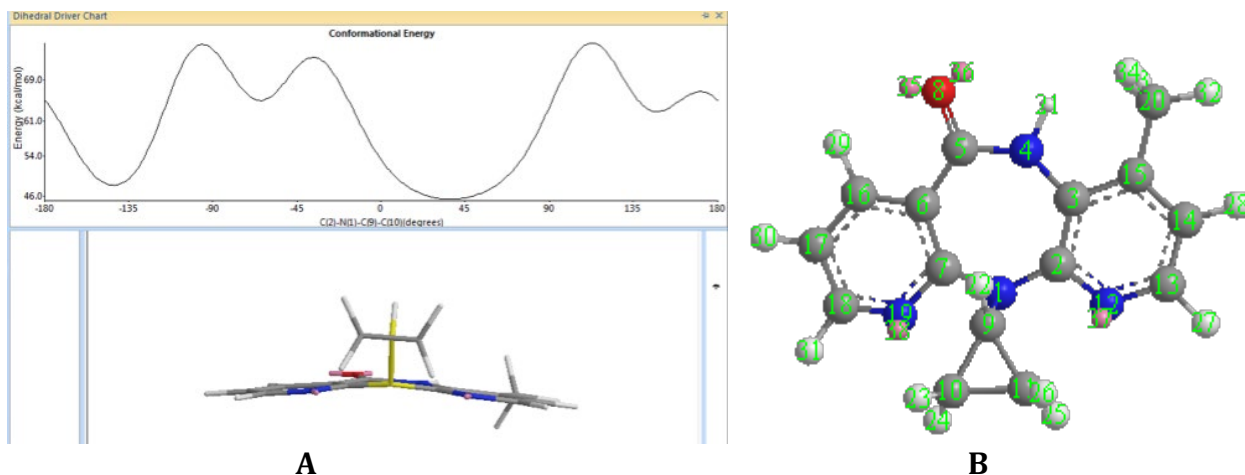
- \*From the **View** menu, *choose* **Model Display**, then **Show Serial Numbers**.
- \*To hide the serial numbers again, go back into the **View** menu, *choose* **Model Display**, then *click* on **Show Serial Numbers**.

\*With the shift key depressed, use the **Select** tool  to *click* on the four atoms that define the dihedral angle C(2)-N(1)-C(9)-C(10).

- \*From the **Structure** menu, *choose* **Measurements**, then **Display Dihedral Angle**.

The angle is -144°. This is the dihedral angle for the energy-minimised conformation used to generate the Dihedral Driver Chart. From the dihedral chart, it is clear that this is the second most stable conformation with a steric energy of 48.2 kcal/mol.

\*To identify a specific conformation, *click* on the plot line. The molecule in the main window will be converted to the relevant conformation, and a pop window will show the energy and the dihedral angle measurement for that conformation.



**Figure 4** A) Dihedral Angle Chart for nevirapine. B) Identification of serial numbers

\*Identify the most stable conformation. This is found at a dihedral angle of  $34^\circ$  with a steric energy of 45.55 kcal/mol.

\**Click* on this part of the plot to display the conformation, then carry out an energy minimization. The resulting structure has a steric energy of 41.78 kcal/mol (Fig. 5). A comparison with figure 3 shows a significant alteration in the position of the cyclopropyl ring. It is now orthogonal to the tricyclic ring system. In figure 3 the cyclopropyl ring was roughly coplanar with the tricyclic ring system.

\**Copy and paste* the conformation shown in figure 5 into a new window for Part B of the exercise.



**Figure 5** Most stable conformation identified by the Dihedral Driver Chart.

## PART B The enantiomers of nevirapine.

### 1. Identify any stereochemistry in the structure

In this part, we will study the most stable conformation of nevirapine with steric energy 41.78 kcal/mol (Fig. 5).

\*Go to the **Structure** Menu and *choose* **Detect Stereochemistry**. No stereochemistry or chiral carbon centre is detected. However, the structure can exist as two enantiomers.

\*Save this structure as Enantiomer I.

### 2. Generate the opposite enantiomer of nevirapine.

\**Copy* and *paste* enantiomer I of nevirapine into a new window.

\*From the **Structure** menu, *choose* **Reflect Model**, then **Invert Through Origin**. The resulting structure is the mirror image or enantiomer of enantiomer I.

\**Save* this structure as Enantiomer II (Fig. 6).

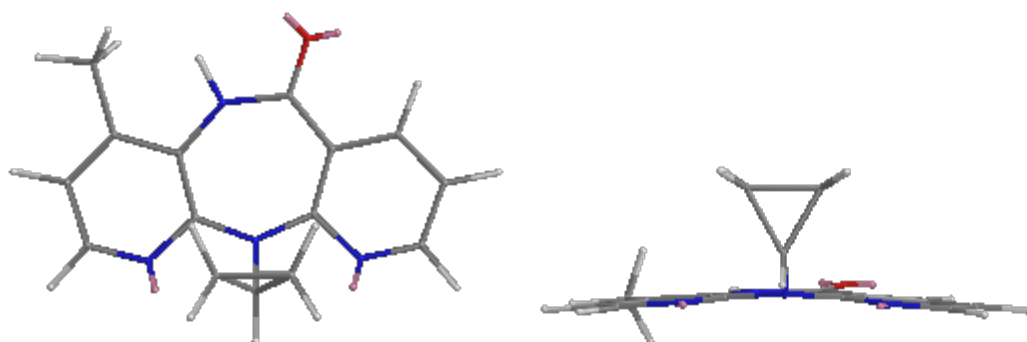


Figure 6 Opposite enantiomer of nevirapine.

### 3. Align the two enantiomers in a new window.

\*From the **File** menu *choose* **New** to open a new window.

\**Copy* and *paste* enantiomers I and II into the new window.

\**Align* the structures such that the cyclopropyl rings are similarly orientated. You can move one structure without moving the other, by *selecting* the structure then *moving* it with the shift key depressed.

### 4. Recolour one of the enantiomers

\**Choose* the **Select** tool .

\*With the Shift key depressed, *click* on all the carbon atoms in one enantiomer.

\**Hover* the mouse cursor over any of the chosen atoms, then *right click* the mouse to produce a menu.


\*From the menu, *select* **Color**, then **Choose color**.

\**Select* your chosen colour from the colour palette, then *click* on **OK**.

\*Repeat the process to colour the other elements in the structure.

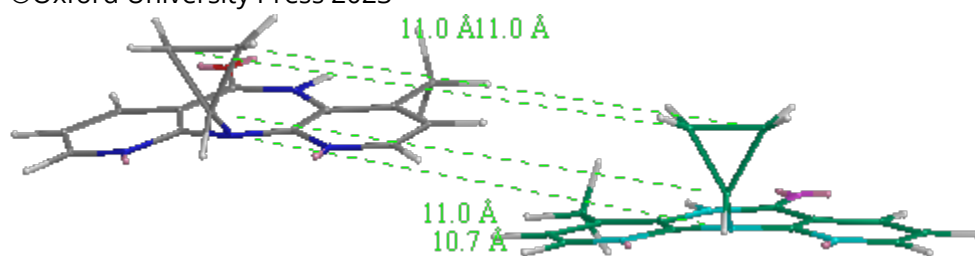
### 5. Pair up atoms ready for the overlay.

Pair up the carbon atoms of the cyclopropyl ring, as well as the nitrogen atoms to which the cyclopropyl rings are attached.

\*With the Shift key depressed, use the **Select** tool  to *select* an atom in one structure and a corresponding atom in the other structure.

\*From the **Structure** menu, *choose* **Measurements**, then *choose* **Display Distance Measurement**.

\*Repeat this process for other pairs of atoms (Fig. 7).



**Figure 7** Alignment and pairings for the enantiomers.

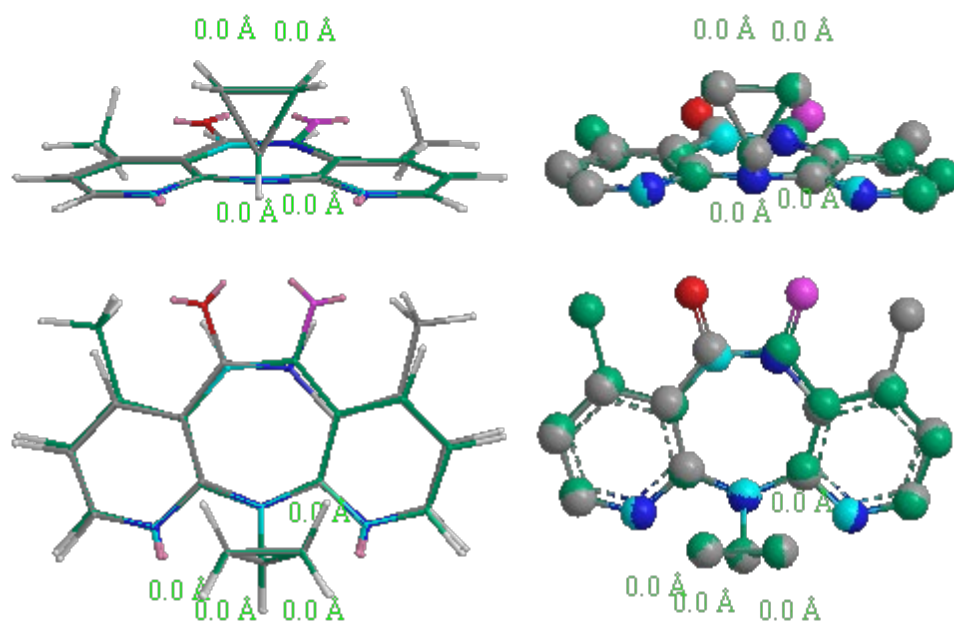
### 6. Overlay the structures.

\* From the **Structure** menu, choose **Overlay**, then *click* on **Minimise**. A dialogue box will appear.

\*Retain the default values and *click* on **Start**.

\*If the overlay is not perfect, select one of the structures and move it manually such that the paired atoms are closer, then repeat the overlay minimisation. It should be possible to get an overlay where there the paired atoms are perfectly matched up (Fig. 8).

The overlays demonstrate that the methyl substituents for each structure are in different positions. This is also the case for the carbonyl groups. The two structures can be viewed as enantiomers with the chiral centre being the nitrogen atom bearing the cyclopropyl ring. However, an amine nitrogen atom is rarely counted as a chiral centre since pyramidal inversion normally occurs. For nervirapine to be chiral, pyramidal inversion of this nitrogen atom must be hindered.



**Figure 8** Overlay of the two enantiomers from different perspectives and in different display modes.

### 7. Assess the likelihood of pyramidal inversion using molecular dynamics.

The chirality of the molecule is related to the rigid butterfly shape of the molecule. If this is totally rigid then the nitrogen is essentially a chiral centre. However, if the nitrogen could invert along with inversion of the butterfly shape, then the enantiomers could interconvert.

Check how feasible this might be by carrying out a molecular dynamics operation on either of the enantiomers.

\*From the **Calculations** menu, *choose* **MM2**, then **Molecular Dynamics**.

\*A table will come up showing default entries that include a Target Temperature of 300K.

\*Keep the default values and *click* **Run**. You will see the molecule vibrating and adopting various conformations. You could also try a higher temperature such as 450K. You should find that the nitrogen atom and the tricyclic ring system are resistant to inversion. Therefore, the enantiomers are unlikely to interconvert at room temperature.

### **8. Repeat the molecular dynamics run on the original energy-minimised conformation**

\**Copy* and *paste* the original energy minimised structure obtained in part A into a new window.

\*Energy minimise the structure and the steric energy should still be 48.2 kcal/mol.

\*Repeat the molecular dynamics procedure at 300 K

You should find that the less stable conformation shown in figure 2 flips relatively readily at 300 K. In doing so, it creates the opposite enantiomer with the cyclopropyl substituent in its most stable orientation. As a result, the structure does not invert back again.

