TopSpin

Structure Analysis Tools

User Manual

Version 002

Innovation with Integrity

NMR

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Contents

Contents

1 Introduction

1.1 About this Manual

This manual is a reference to TopSpin multiplet analysis and chemical structure drawing. Once created, chemical structures can be displayed in multiplet mode and connected atoms can be connected to multiplets.

1.2 Conventions

1.2.1 Font and Format Conventions

Type of Information	Font	Examples
Shell Command, Commands, "All what you can enter"	Arial bold	Type or enter **fromjdx zg**
Button, Tab, Pane and Menu Names "All what you can click"	Arial bold, initial letters capitalized	Use the **Export To File** button. Click **OK**. Click **Processing**…
Windows, Dialog Windows, Pop-up Windows Names	Arial, initial letters capitalized	The Stacked Plot Edit dialog will be displayed.
Path, File, Dataset and Experiment Names **Data Path Variables** **Table Column Names** **Field Names (within Dialog Windows)**	Arial Italics	*$tshome/exp/stan/nmr/lists* *expno, procno,*
Parameters	Arial in Capital Letters	VCLIST

Program Code
Pulse and AU Program Names
Macros
Functions
Arguments
Variables
	Courier	`go=2`
		`au_zgte`
		`edmac`
		`CalcExpTime()`
		`XAU(prog, arg)`
		`disk2, user2`

AU Macro	Courier in Capital Letters	`REX` `PNO`

Table 1.1: Font and Format Conventions

1.2.2 File/Directory Conventions

<tshome> - the TopSpin home directory (default *C\:Bruker\topspin* under Windows and */opt/topspin* under LINUX)

<userhome> - the user home directory

Introduction

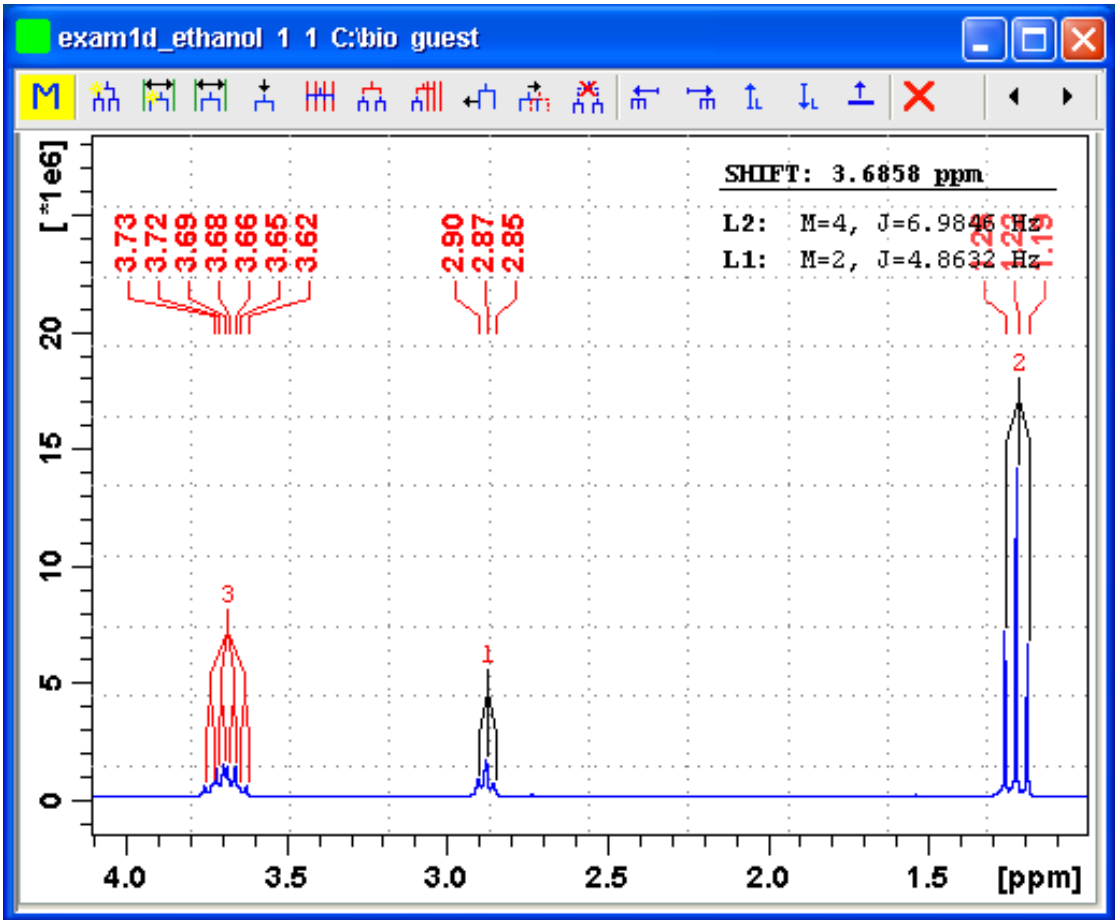
2 Multiplet Analysis

TopSpin offers a multiplet analysis package. This allows you to easily define multiplets and deduce chemical shifts, coupling constants, multiplicities and connections.

2.1 Automatic Multiplet Analysis

TopSpin 2.0 and newer allows fully automatic multiplet definition, which is the easiest and fastest way to perform multiplet analysis. Just take the following steps:

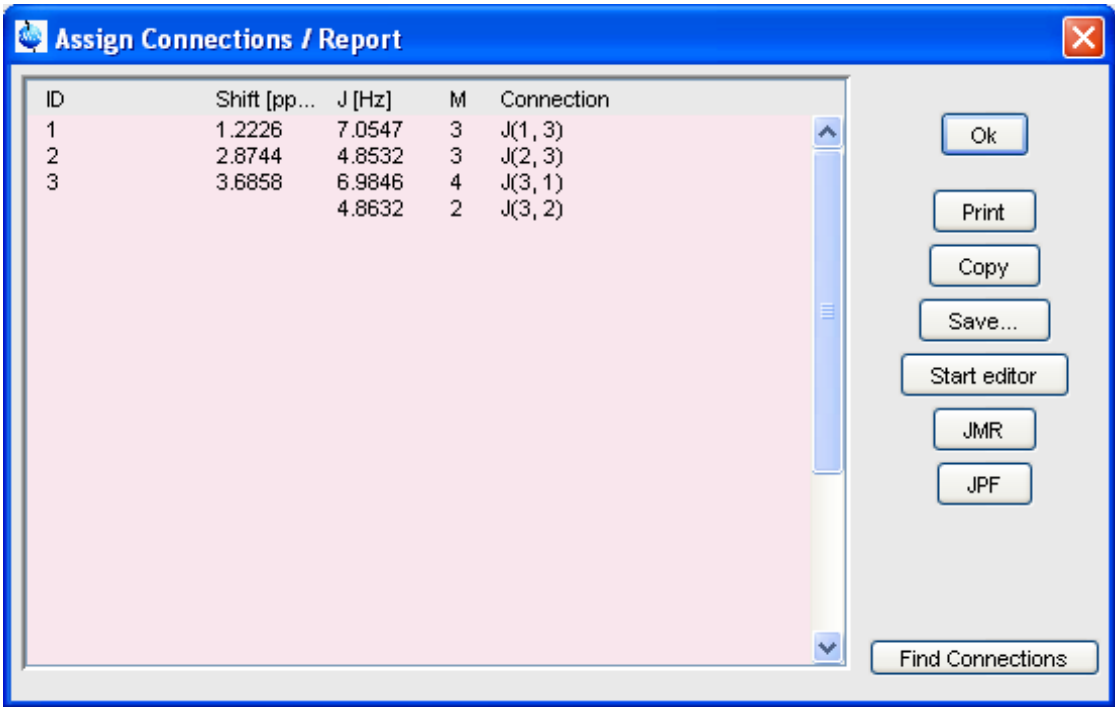
- Perform peak picking if this has not been done yet. To do this, enter the command **pp**, set the peak picking parameters in the appearing dialog and click **OK**.
- If necessary, zoom into the region that contains the multiplets of interest.
- Click **Analysis** => **Multiplet Analysis** => **Enter** or enter **mana** on the command line to switch to multiplet analysis mode. The data window tab bar will change to a toolbar:



The figure above shows the result of the multiplet analysis of an ethanol spectrum, with triplet 1 for the ethanol OH-group, triplet 2 for the CH3-group and multi-level multiplet 3 for the CH2-group.

- Click the  button at the left of the toolbar to perform automatic multiplet definition. The program searches for possible multiplets in the displayed region, according the options set for automatic multiplet creation (see *How to Set Multiplet Options [▶ 8]*). Only peaks in the peak list, as created in step 1, are used.

- Click the button to open the Report dialog. This shows a list of found multiplets, with the respective shifts, J-values, multiplicities and possible connections. Note that the actual connections have not been made yet!
- In the Report dialog, click the button **Find Connections** to define the connections. The column *Connection* will be updated to show the found connections.
- Print, edit or save the multiplet table using the respective buttons in the Report dialog.



To export the Multiplet Report, do one of the following:

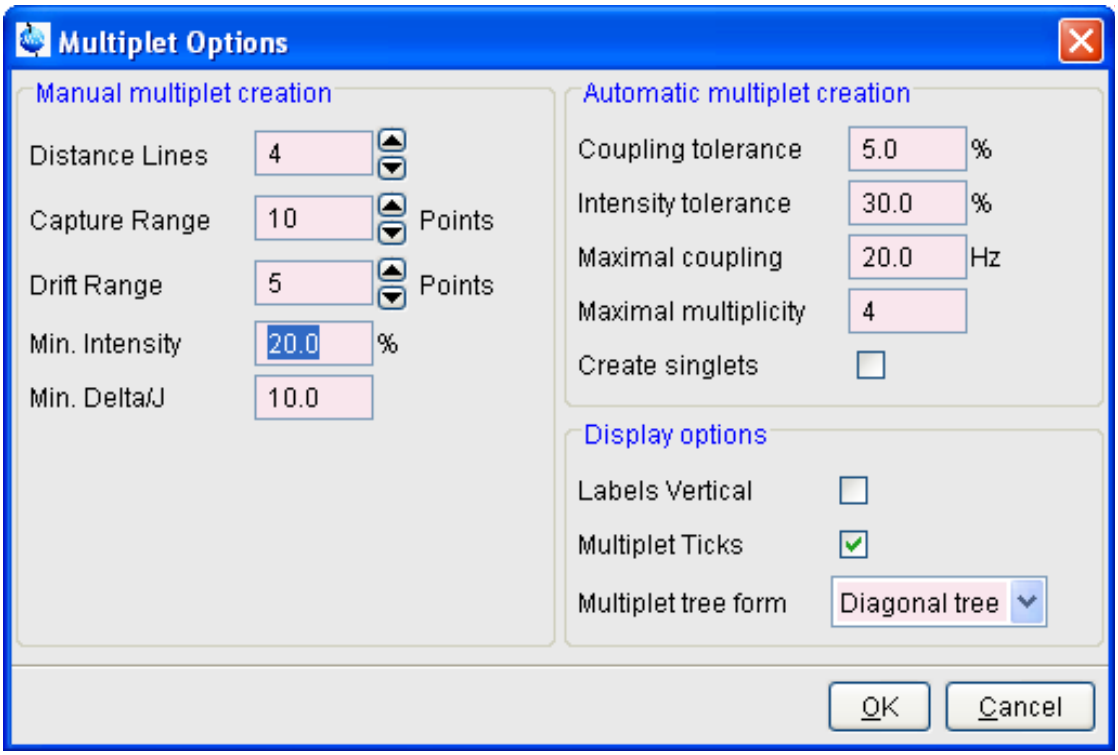
- Click **JMR**: this will export the report in JPR (Journal of Magnetic Resonance) format, e.g.:
  1H NMR (300 MHz, CDCl3)
  Shift ppm 1.22 (t, J=7.05 Hz, 3 H)
  Shift ppm 2.87 (t, J=4.85 Hz, 1 H)
  Shift ppm 3.69 (dq, J=4.86, 6.98 Hz, 2 H)
- Click **JFP**: this will export the report in JPF (Japanese Patent Format), e.g.:

No	(ppm)	Integ	Peak	J1(Hz)	J2(Hz)	J3(Hz)	Comment
1	1.22	50.1	T	7.05	0.0	0.0	
2	2.87	16.5	T	4.85	0.0	0.0	
3	3.69	33.4	Dq	4.86	6.98	0.0	

Table 2.1: Japanese Patent Format

2.2 How to Set Multiplet Options

Automatic multiplet definition is controlled by various parameters. To change these parameters, click the button on the toolbar, which will open the Multiplet Options dialog. Here you find parameters for automatic as well as manual multiplet definition. Manual methods are described in paragraph *Further Multiplet Definition Methods [▶ 10]*.



Options for automatic multiplet creation

These parameters are used by the modes "Automatically define multiplets" and "Automatically define multiplet by region".

- *Coupling tolerance*: the maximum difference in distance between peaks for them to have the same coupling constant.
- *Intensity tolerance*: the maximum difference in peak intensities for peaks to be part of the same level of a multiplet.
- *Maximal coupling*: the maximum of coupling constant to be searched.
- *Maximal Multiplicity*: the maximum of multiplicity (number of levels) of multiplet.
- *Create singlets*: defines singlets whether singlets will be created or not.

Options for manual multiplet creation

These parameters are used by the modes "Define Multiplets Manually", "Define Multiplets by Region" and "Free Grid Analysis".

- Distance lines (2-9): the default number of distance lines in the multiplet dialogs.
- Capture range (1 - 30): the search range for maximum intensity of peak position in manual mode.
- Drift range (1-30): the maximum difference in data points between line distances within one multiplet
- Min. Intensity: The minimum intensity of a peak compared to the reference peak to be accepted as a multiplet line.
- Min. Delta/J: the minimum ratio of the difference in chemical shift of the coupling groups and the coupling constant. Below this value, the coupling constant in the Report box is indicated with a question mark to suggest possible second order effect.
- Labels Vertical: displays multiplet labels 90° rotated.

Display Options

- Labels vertical: display multiplet labels (identifiers) vertical or horizontal.
- Multiplet ticks: display vertical tick on each peak in the multiplet.
- Multiplet tree form: shape of the multiplet tree (square of diagonal).

2.3 How to Connect Multiplets to Molecule Structure

Multiplets can be connected with molecule structures defined with **edstruc** (see --- FEHLENDER LINK ---). If the structure file has been saved to the dataset EXPNO, the structure will be displayed in the data window when you switch to multiplet mode.

Then there are three possible operations:

- Connect Selected Multiplet
- Disconnect Selected Multiplet
- Restore Atoms Selection

The corresponding commands are accessible from a popup menu that appears if you right-click the multiplet or the molecule.

2.3.1 Connect Selected Multiplet

To connect several atoms in the structure to a multiplet you have to:

- Select the required multiplet.
- Click on atoms that must be connected. The atoms will be selected. To select more than one atom use Ctrl or Shift key.
- Right-click on the molecule and choose **Connect Selected Multiplet**.

If you now select a multiplet, then the corresponding atoms will also be selected.

2.3.2 Disconnect Selected Multiplet

To disconnect the selected multiplet user should:

- Select the required multiplet.
- Right-click on the molecule and choose **Disconnect Selected Multiplet**.

2.3.3 Restore Atoms Selection

To restore the atoms selection for the currently selected multiplet:

- Right-click on the molecule and choose **Restore Atoms Selection**.

2.4 Further Multiplet Definition Methods

If the result of the automatic multiplet definition described above is not satisfactory, for example because there are too many overlapping peaks, TopSpin offers the following semi-automatic or manual definition methods:

- Automatically define multiplets by region
- Define multiplets by region
- Define multiplets manually
- Define multiplets by free grid
- Define multi-level multiplets by coupled grid

- Couple existing multiplets into a multi-level multiplet

These methods are described below for the ethanol CH3, OH and CH2 groups.

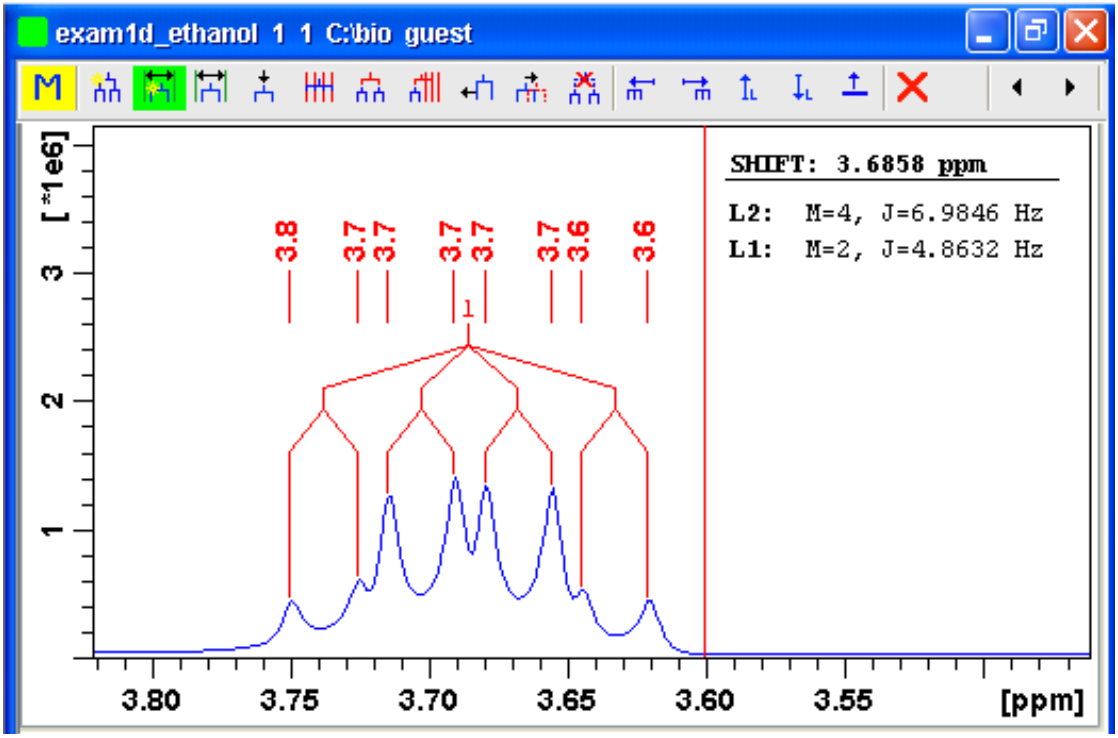
Automatically Define Multiplets by Region

This method is the same as "Automatically Define Multiplets", except that only a user-defined region is analysed. The program searches for possible multiplets in the defined region, according the options set for automatic multiplet creation (see par. 2.2). Only peaks in the peak list are used.

To use this method, take the following steps:

1. Click the  button (it turns green).
2. Click-hold the left mouse button on one side of a multiplet region, move the mouse and release it at the other side of the region. The multiplet will be displayed.
3. Repeat step 2. for all multiplets to be defined.
4. Click the  button to leave the automatic region mode.

In the following figure, the multi-level multiplet of the ethanol CH2-group shown.



See also

How to Set Multiplet Options [▶ 8]

2.4.1 Define Multiplets by Region

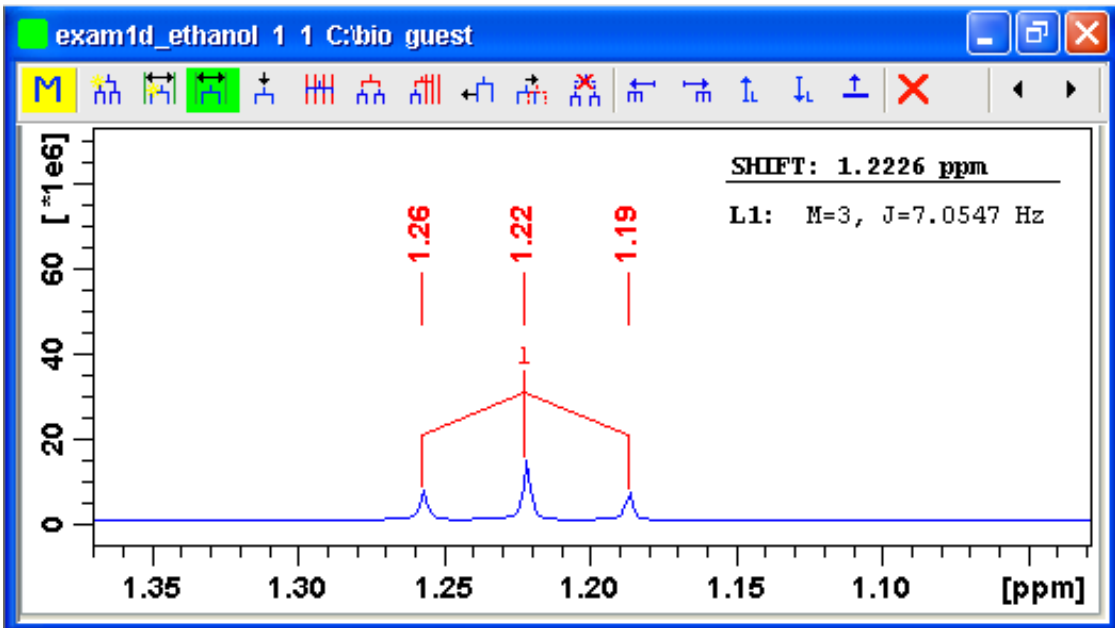
As in the previous method, a multiplet is created in a user-defined region. However, no multiplet search is done, the peaks in the region are simply defined as a multiplet. Only peaks in the peak list that have the minimum intensity as set in the multiplet options (see par 2.2) are used.

To use this method, take the following steps:

1. Click the  button (it turns green).

2. Click-hold the left mouse button on one side of the multiplet region, move the mouse and release it at the other side of the region. The multiplet will be displayed.
3. Click the button to leave the region mode.

The following figure shows the defined multiplet of the ethanol CH3-group.



See also

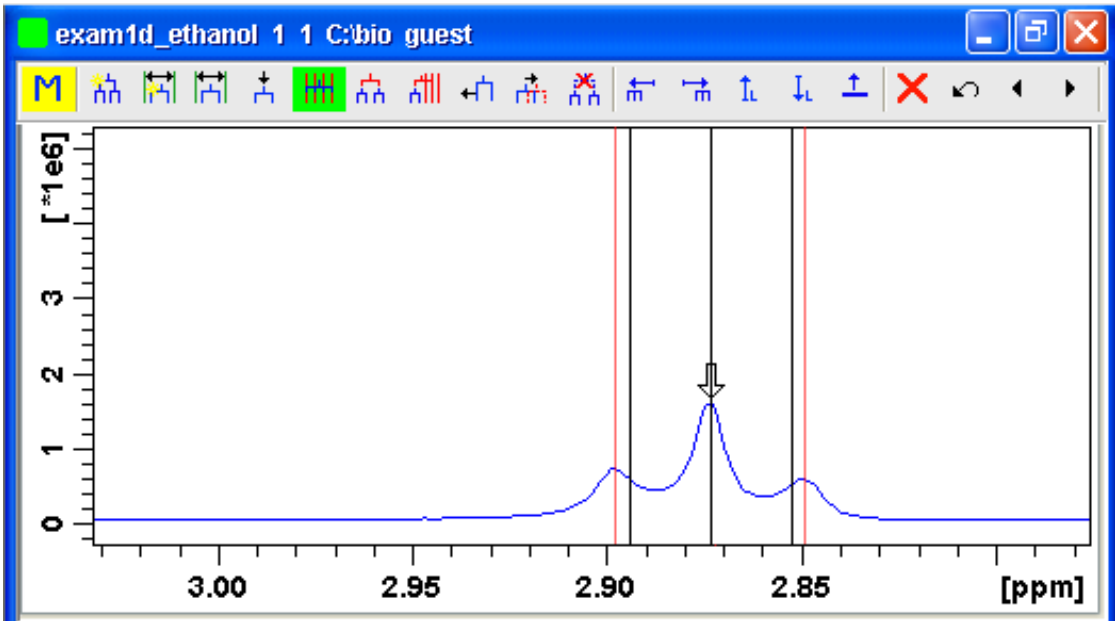
How to Set Multiplet Options [▶ 8]

2.4.2 Free Grid Analysis

With this method, you can define a multiplet by assigning one peak manually and all other peaks by free-grid analysis. The grid consists of a predefined number of distance lines. A multiplet is defined by searching for the largest peak within the capture range each distance line. Peaks are taken from the peak list created by peak picking, or, if no peak picking was done, found by maximum intensity search.

To use this method, take the following steps:

1. Zoom in on the region around the desired multiplet.
2. Click the button (it turns green). A cursor line will appear along with a second (faint) line. Note that the faint line jumps to the largest peak within the capture range of the cursor line.
3. Right-click in the data window and set the number of distance lines to the number of peaks in the multiplet.
4. Move the cursor line towards the central peak of the multiplet until the faint line coincides with it and click the left mouse button. The peak will be marked. For a multiplet with an even number of peaks, just take one of the central peaks.
5. Move the mouse to the left or to the right until the distance lines coincide with the remaining multiplet peaks and click the left mouse button. The multiplet will be displayed.
6. Click the button to leave this mode.



In the figure above, the triplet of the ethanol OH-group is being defined. The central peak is defined and the black grid lines are within the capture range of the other peaks as shown by the faint red lines on the peak maxima.

2.4.3 Define Multiplet Manually

With this method, you can define a multiplet by manually assigning individual peaks. No prior peak picking is required. If, however, peaks have been picked, they are used.

To use this method, take the following steps:

1. Zoom in on the region around the desired multiplet.
2. Click the button (it turns green) and move the cursor into the data window. A cursor line will appear along with a second (faint) line.
3. Move the cursor line towards the left peak of the multiplet until the faint line coincides with it (note that the faint line automatically jumps to the largest peak within the capture range). Click the left mouse button. The peak will be marked. Note that clicking a marked peak again will unmark it.
4. Repeat step 3 for the remaining peaks of the multiplet.
5. Right-click in the data window and select **Define Multiplet** in the popup menu. The multiplet will be displayed.
6. Click the button to leave this mode.

2.4.4 Couple Existing Multiplets into a Multi-Level Multiplet

With this method, you can define a multi-level multiplet by coupling already defined multiplets.To do that:

1. Click the button (it turns green).
2. Select the multiplets to be coupled by left-clicking them.
3. Right-click in the data window and select **Define Multiplet** in the popup menu.The multi-level multiplet will be displayed.
4. Click the button to leave this mode.

Note that only multiplets of the same level can be coupled.

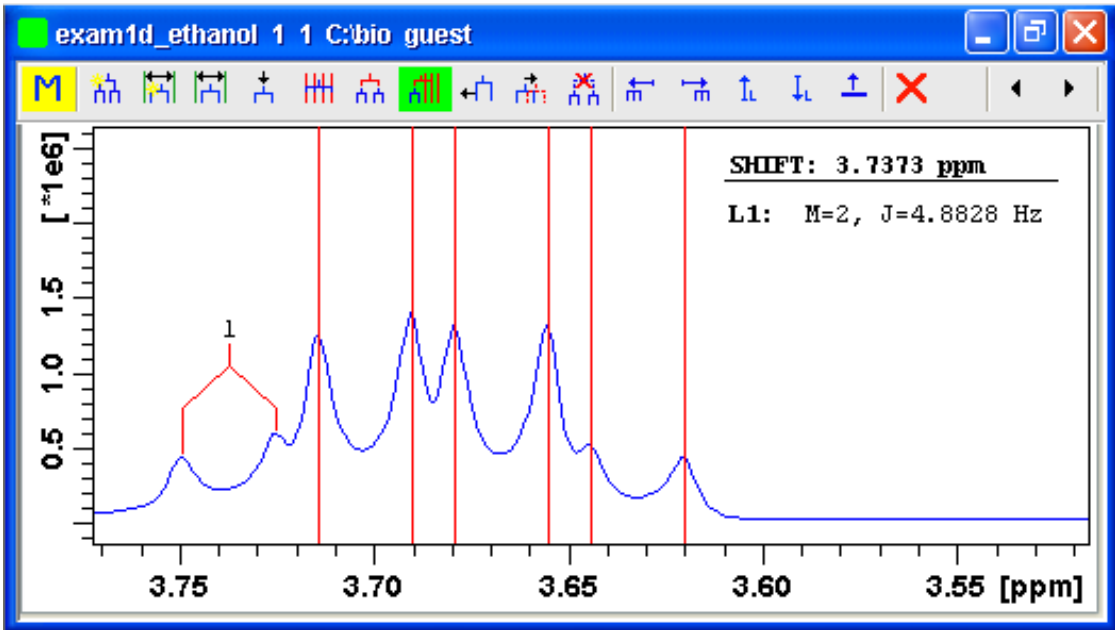
2.4.5 Define Multi-level Multiplet by Coupled Grid

With this method, you can define a multi-level multiplet by defining one level with one of the methods above and using coupled grid analysis to define the next level.

To use this method, take the following steps:

1. Zoom in on the region around the desired multi-level multiplet.
2. Define the leftmost or rightmost multiplet using one of the methods described above, e.g. "Define Multiplets by Region".
3. Click the  button (button turns green).
4. Right-click in the data window and set the multiplicity to the number of these multiplets in the multi-level multiplet.
5. Move the mouse to the left or to the right until the grid lines coincide with the multiplet peaks and click the left mouse button. The multi-level multiplet is defined now.
6. Click the  button to leave this mode.

In the figure below, the multi-level multiplet of the ethanol CH2-group is being defined. The left-most doublet has already been defined with automatic definition by region. The (red) lines of the coupled grid are positioned on the remaining six peaks of the multiplet.



2.5 How to Decouple Multi-Level Multiplets

You can remove one of the couplings of a multi-level multiplet, i.e. decouple it, as follows:

1. Click the desired multi-level multiplet to select it.
2. Click the button  .

Decoupling a multi-level multiplet splits it into a set of multiplets. It removes the highest level of the multiplet and leaves the submultiplets unconnected.

2.6 How to Select Multiplets/Levels

2.6.1 Selecting a Multiplet

You can select a particular multiplet simply by clicking it in the data field. The currently selected multiplet is displayed in the color of the second spectrum (default red). Alternatively, you can select a multiplet from the toolbar as follows:

 Select the previous multiplet.

 Select the next multiplet.

2.6.2 Selecting a Level in a Multi-Level Multiplet

Multi-level multiplets can be defined for a group which couples with multiple other groups, for example the ethanol CH2-group. Each level can be selected and designated.

 Select the next level (up) of a multi-level multiplet.

 Select the previous level (down) of a multi-level multiplet.

2.7 How to Designate Multiplets

A level of a multi-level multiplet can be designated to connect with another multiplet. To do that:

Right-click in the multiplet and choose **Designate Multiplet**

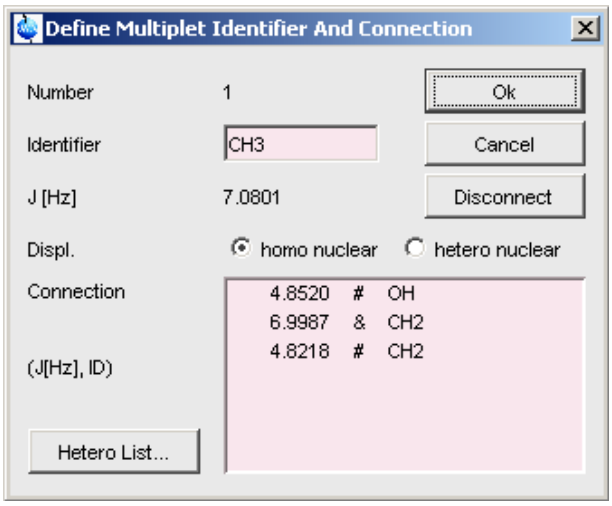
The designated level is displayed in the color of the fourth spectrum (default purple). Note that the *selected* level can be different from the *designated* level.

2.8 How to Define Multiplet Identifiers

To define multiplet identifiers:

1. Left-click the multiplet.
2. Right-click the multiplet and select **Define Multiplet Identifier** in the popup menu.
3. Specify the multiplet identifier in the appearing dialog and click **OK**

Alternatively, multiplet identifiers can be defined by clicking the  button and double-clicking the respective multiplet lines in the appearing Report dialog. This will open the Identifier dialog:



Just fill out the field **Identifier** and click **OK**.

Note that the dialog also offers a button to **Disconnect** the current multiplet and a field *Connection* that shows all possible connections using the following flags:

& : the current connection

: a non-existing connection

! : a different existing connection

2.9 How to Connect/Disconnect Multiplets

Once the multiplets of a spectrum are defined, you can define the connections between them.

To make all connections:

1. Click the button to open the Report dialog.
2. The multiplets appear in the order in which they have been defined. Newly defined multiplets appear with numbers as ID's and no multiplet connections (x,0) defined.
3. Click the button **Find Connections** to make the multiplet connections:
   - Set the maximum difference between related couplings or accept the default.
   - Set the lower limit for couplings or accept the default.
   - Check the box **Change already defined Connections** if applicable.
   - Click **OK** to define the connections.

Note that the Report dialog also offer buttons for Printing the multiplet information, editing it, copying it to the clipboard and saving it to a text file.

To disconnect all multiplets:

1. Click one of the two multiplets that you want to connect. If this is a multi-level multiplet, also select the proper level using the or button.
2. Right-click and select **Disconnect Multiplets** from popup menu.

To define individual connections:

1. Select the first multiplet. In case of a multi-level multiplet, click the or button to select the required level.
2. Right-click and select **Designate Multiplet** from popup menu.

3. Select the second multiplet. In case of a multi-level multiplet, click the  or  button to select the required level.
4. Right-click and choose the **Connect Multiplets** from popup menu.

Alternatively, you can open disconnect a multiplet from the Identifier dialog.

2.10 How to Shift a Multiplet or Multiplet Line

To horizontally shift a multiplet:

 Click this button (turns green), left-click-hold the desired multiplet and move the mouse to the left or to the right. Release the left button when the multiplet is at the desired position. Click this button again to leave this mode (buttons turns gray).

To horizontally shift an individual line:

 Click this button (turns green) and move the vertical cursor line into the capture area of the desired peak. The peak will be marked by a faint line. Then left-click-hold and move the mouse to the left or to the right. Release the left button when the line is at the desired position.

To vertically shift a multiplet:

 Click this button (turns green) and move the mouse to put the horizontal line cursor above or below the multiplet tree. Then left-click to shift the multiplet to that position.

2.11 How to Remove Multiplet Definitions

 Remove the currently selected multiplet. Clicking this button several times allows you to remove all multiplets.

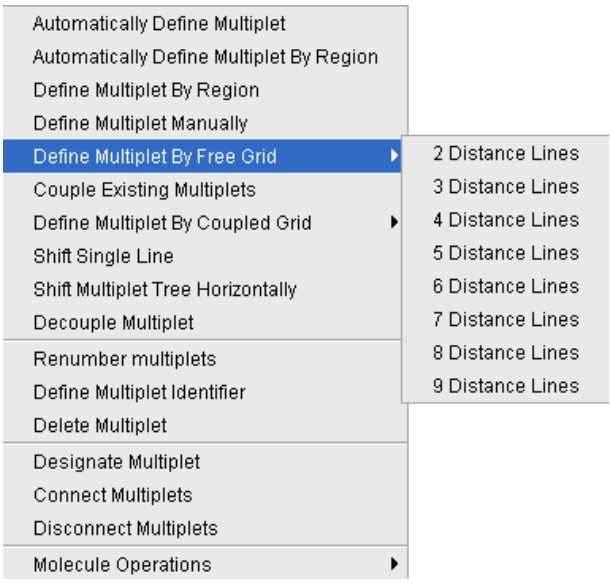
2.12 Miscellaneous Multiplet Functions

 Show Daisy multiplets.

 Undo the last multiplet action.

 Redo the last multiplet action.

Toolbar functions are also available from a popup menu which appears when you right-click in the data window:



2.12.1 How to Save and Close Multiplet Analysis

When you have finished multiplet analysis you can save your work and exit from multiplet mode as follows:

Save multiplet analysis.

Save multiplet analysis and quit.

Quit multiple analysis mode.

The multiplet analysis result is saved in the files *multiplet.txt* and *mol2ppm.txt* in the *procno* data directory. The saved multiplets are automatically shown when you re-enter multiplets analysis mode.

2.13 More information on Multiplet Analysis

An example of manual multiplet analysis of a 2,3-Dibromopropionic acid in CDCl3 sample can be found under:

Help => **Manuals** => [**Acquisition User Guides**] **1D and 2D Step-by-Step - Advanced**

3 Solids Lineshape Analysis

3.1 Introduction

TopSpin offers Solids Line Shape Analysis allows you to simulate and fit calculated spectra to various experimental 1D solid NMR spectra. The following fitting models are available:

- Gauss/Lorentz
- Chemical Shift Anisotropy
- Quadrupolar Central Peak of the +/- 1/2 Transition of a Quadrupolar Nucleus
- All Quadrupolar Transitions of a Quadrupolar Nucleus
- The Combination of the Chemical Shift Anisotropy and Quadrupolar Interaction

You can simulate powder spectra of static or rotating samples at single or double axis conditions. Both rotation angles can be set. The inner and outer rotating speeds are freely adjustable. For rotating samples, a maximum of ten rotation side bands and five DOR bands can be set. You can simulate one 1D spectrum with a maximum of 25 observable nuclei, i.e. 25 sites of a nucleus. Ten other nuclei can be defined as dipolar coupling partners of the observed nucleus (TopSpin 2.0 allows only one observed nucleus (site) if you define heteronuclear dipolar couplings).

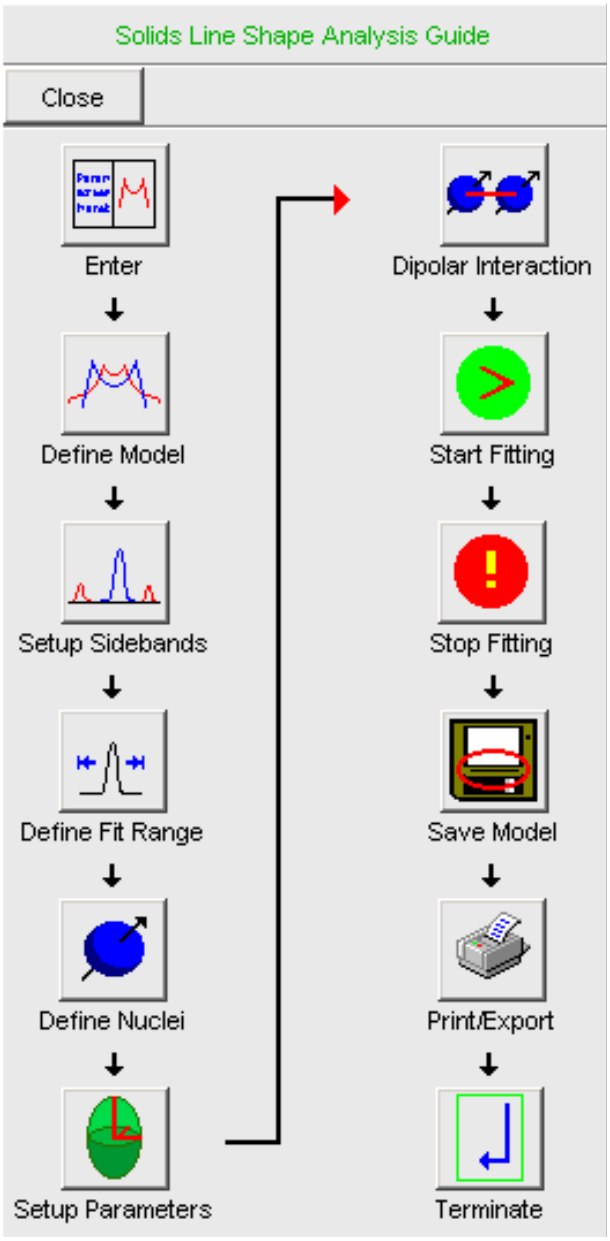
3.2 Switch to Line Shape Analysis Mode

Before starting Solids Line Shape Analysis, the 1D spectrum must be properly phase corrected and baseline corrected.

To switch to Solids Line Shape Analysis mode:

- Click **Analysis** => **Solids Lineshape Analysis** [**solaguide**]

This opens the workflow as shown in the following figure:



Clicking **Enter** here will split the data window in two. Experienced users can enter this mode directly and skip the workflow with the command **sola**.

The right part of this window is the data window showing the 1D experimental and calculated spectrum. The left part is the parameter window with five panels, where the second one, the **Main** panel is selected by default.

3.3 The Simulation Procedure

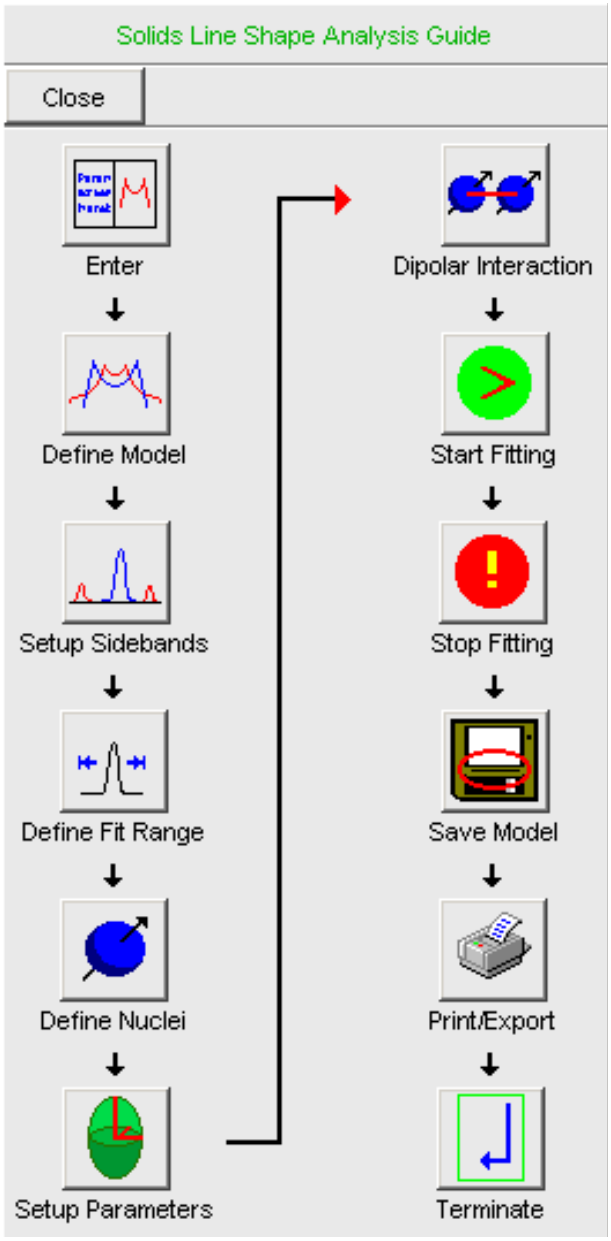
The simulation procedure consists of the following steps:

3.3.1 Set Optional Parameters

In the **Main** panel, you find some parameters which you can normally leave unchanged. See the section *Simulation Details* below for more information. If a simulation on the current dataset has already been done and stored, you can read this by clicking **Open**. If not, you will start from scratch and setup the simulation as described below.

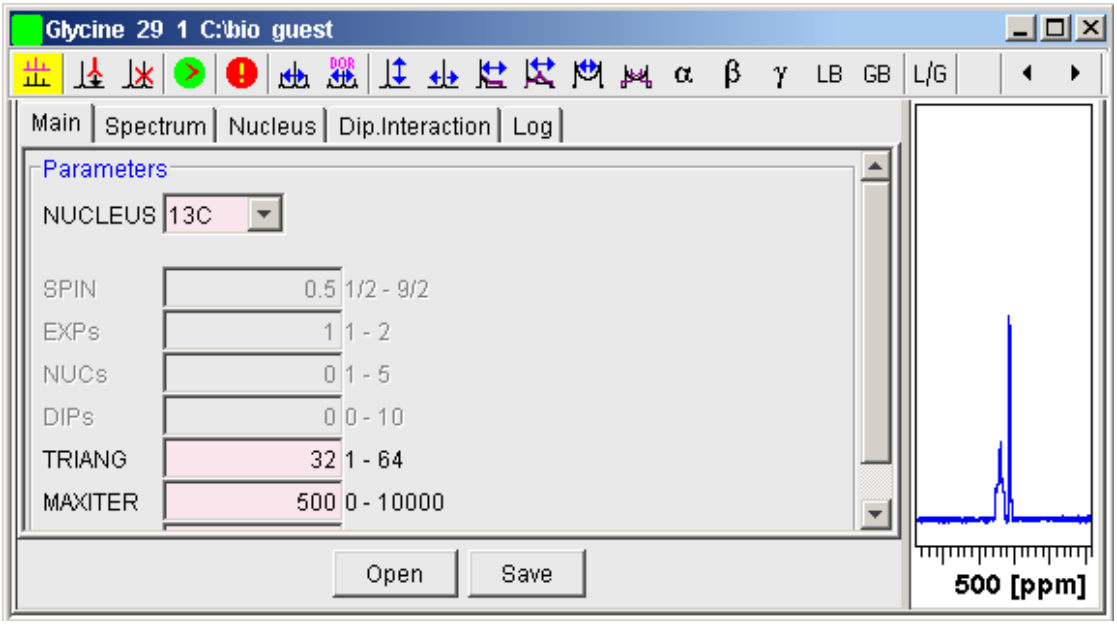
Define the Model

Click the **Spectrum** panel and select the **Model** according to your experiment. You can refine the experimental conditions by checking one of the following boxes:



- **All** for all quadrupolar transitions.
- **DOR** for performed double rotation experiments.
- **Sync** for rotor synchronized experiments.

When a parameter is greyed, this means it cannot be changed for the selected model or it is related to one of the other (checked) parameters.



3.3.2 Define Rotation Parameters

From the **Spectrum** panel, set the values for:

- MASR - rotation speed of the single axis MAS (VAS) experiments or DOR outer axis speed.
- DORR - inner rotation speed in DOR experiment.

and set their checkmark if they must be optimized during the simulation.

Set the value for:

- SBands: the number of side bands on one side of the central transition.

3.3.3 Define the Spectral Region

Typically, non-overlapping experimental peaks are fitted in separated simulations. Before each simulation, the region to which it is applied must be defined.

- Zoom in on the region to be simulated.
- Right-click in the data window choosing **Define Fitting Region Using Display Region** or click **DefReg** in the panel window.

This will set the parameters F1P and F2P in the Spectrum panel. Alternatively, you can enter the values in the respective parameter fields.

3.3.4 Define Nuclei Parameters

For each observable nucleus (site), a set of parameters (see below) must be set. To do that:

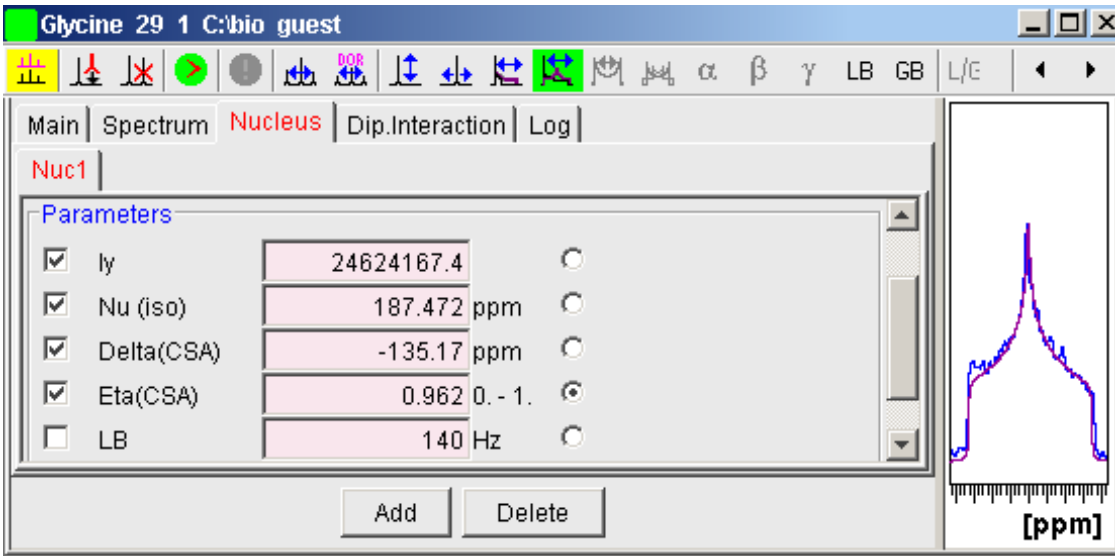
- Click the **Nucleus** panel or the  button.
- Click **Add** if the nucleus is not shown yet.
- Adjust the nuclei parameters until the calculated spectrum approximately fits the experimental spectrum.You can do that as follows:

Enter the values in the parameter field.

or

Click the radio button to the right of the parameter, click-hold the corresponding toolbar button (colored green) and move the mouse horizontally.

Note that the calculated spectrum in the data field is automatically updated as you adjust a parameter:



- Check the parameters which must be optimized during the simulation.

3.3.5 Define Dipolar Coupling Nuclei (if they exist)

Dipolar coupling nuclei can be defined if only one observe nucleus is defined. To set dipolar coupling parameters:

- Click the **Dip. Interaction** panel.
- Click **Add** if the nucleus is not shown yet.
- Set the nuclei parameters as follows:

Enter the values in the parameter field.

or

Click the radio button to the right of the parameter, click-hold the corresponding toolbar button (colored green) and move the mouse horizontally.

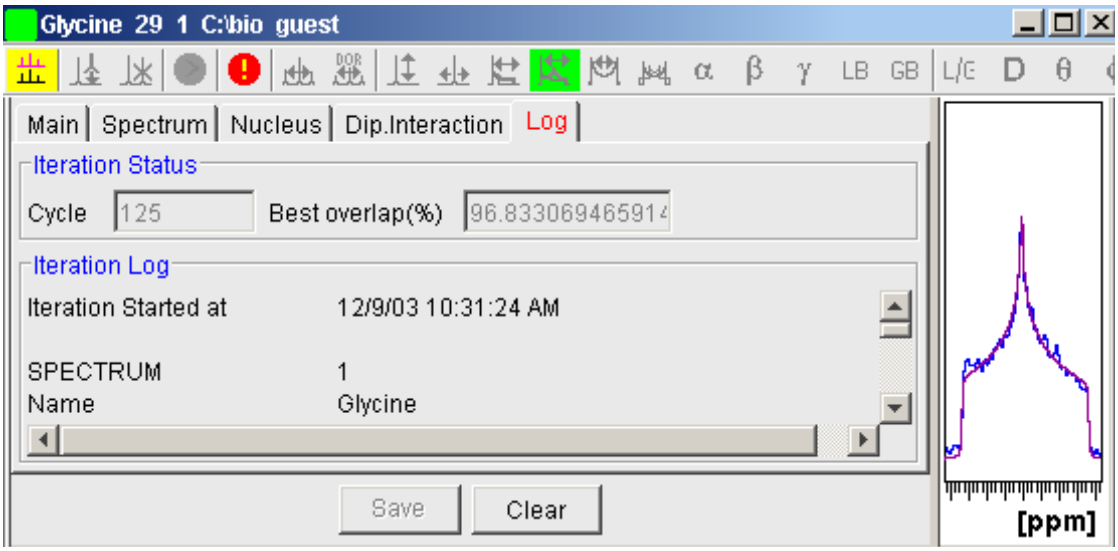
- Check the parameters to be optimized during the simulation.

3.3.6 Start the Simulation

Now you can start the iterative simulation. To do that:

- Click the  toolbar button.

The simulated spectrum is displayed in the data window and continuously updated:



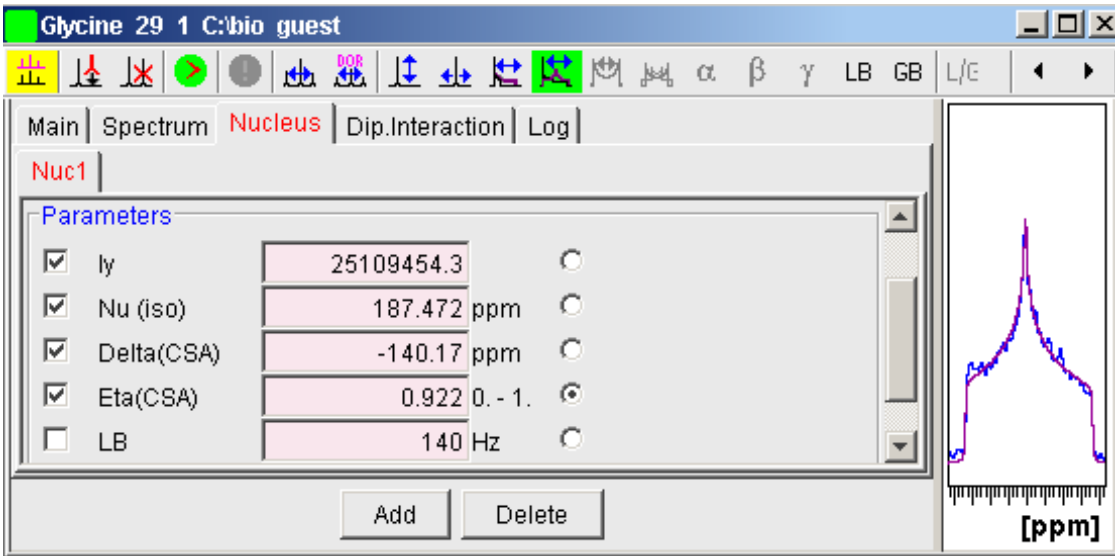
The parameter window will switch to the **Log** panel showing:

- *Iteration Status*, including the iteration *Cycle* and the B *est Overlap* percentage so far.
- *Iteration Log* with the starting parameters and the results of the fit. Parameters which are marked with an asterisk have been optimized during the simulation. They are automatically updated in the respective panels.

To save the Log panel information, click the **Save** button.

To clear the Log panel, click the **Clear** button.

During the simulation process, you can freely switch to other panels to view the parameter being optimized. After the simulation has finished, the **Nucleus** panel will show the optimized values:



Abort the Simulation

To abort a running simulation:

- Click the  toolbar button.

After a few seconds the iteration stops and the best spectrum will be shown in the data window.

3.3.7 Save the Simulation

After the simulation process is finished for all spectral regions of interest:

- Switch to the *Main* panel.
- Click **Save**

to save all parameters.

3.3.8 Exit Solids Lineshape Analysis

To leave the solids analysis mode:

- Click the  toolbar button.

3.4 Simulation Details

Basic parameters

The Main panel shows you a list of basic parameters:

- NUCLEUS: the observe nucleus. By default, this parameter is set to the value of the acquisition parameter NUC1.
- SPIN: spin of the observe nucleus. It is automatically set according to the selected NUCLEUS.
- EXPs: number of experimental spectra.
- NUCs: number of nuclei (sites of the observe nucleus).
- DIPs: number of dipolar coupling nuclei.
- TRIANG: number of triangles involved in powder spectrum simulation with several random oriented crystallites. The default value of 32 generally results in a good quality spectrum.
- MAXITER: maximum number of iterations.
- SSIZE: initial step size for the iterated parameters. The value represents the fraction of the initial parameter value. It ranges from 0.0 to 1.0 with a default of 0.1.

The values of NUCs and DIPs will automatically be updated when you add or delete nuclei from the **Nucleus** and **Dip.Interaction** panels.

Spectrum parameters

Spc1

TopSpin 2.0 supports only one experimental spectrum to be fitted.

Models

Available fitting models are: *Gauss/Lorentz*, *CSA, QUAD central*, *QUAD all*and *QUAD & CSA.*

Experimental Spectrum

Shows the datapath variables of the experimental spectrum.

Parameters

The following parameters are available.

- MASR - rotation speed of the single axis MAS (VAS) experiments or DOR outer axis speed.
- DORR - inner rotation speed in DOR experiment.

- Angle and AngleInt - the outer and inner rotation angles.
- SBands and DORBands - number of calculated side bands.
- F1 and F2 - the left and right edge of the experimental spectrum.
- F1P and F2P - the limits of the region to be fitted. These must be within the F1-F2 range. To define F1P and F2P interactively, expand the spectrum and right-click in the data window choosing **Define Fitting Region Using Display Region** or click **DefReg** in the panel window.

The displayed parameters SI, O1P, SF, SW, SWH, LB, GB and SR are used by the fitting calculations. They are defined by the corresponding processing parameters (command **edp**).

3.4.1 Nucleus Parameters

The section *Model* shows the spectrum model type, which was selected in the **Spectrum** panel. The section *Parameters* contains the nucleus dependent model parameters. The available parameters and the corresponding toolbar buttons are:

 Iy - Signal intensity.

 Nu(iso) - isotrope chemical shift given in ppm.

 Delta(CSA) - Chemical shift anisotropy parameter in ppm (can be positive, negative or zero).

 Eta(CSA) - Asymmetry parameter (0 ≤ Eta ≤1).

 CQ(Quad) - Quadrupolar coupling constant in kHz.

 Eta(Quad) - Asymmetry parameter of the quadrupolar coupling tensor. (0 ≤ Eta ≤ 1)

 Alpha Euler angle of the ’CSA & Quad’ tensor.

 Beta Euler angle of the ’CSA & Quad’ tensor.

 Gamma Euler angle of the ’CSA & Quad’ tensor.

 Line broadening parameter (half width if GB=0).

 Gauss broadening parameter. If GB>0 then LB must be negative.

 Gauss component of the Gauss/Lorentz ratio. 0.0 ≤ GL ≤ 1.0Lorentz curve: GL=0, Gauss curve: GL=1. Used only by the *Gauss/Lorentz* model

 Dipolar coupling.

 Theta (dipolar).

 Phi (dipolar).

3.4.2 Dipolar Neucleus Parameters

TopSpin 2.0 supports a maximum of ten dipolar coupling partners. Note, however, that you can only simulate one observable nucleus (site) at a time if you define dipolar coupling nuclei.

- NUCLEUS - Coupling nucleus partner.
- Spin - Nucleus dependent Spin (Read only).

- D(dip) - Dipolar coupling constant.
- Angle2 - Euler angle of dipolar coupling vector.
- Angle3 - Euler angle of dipolar coupling vector.

Note that Dipolar couplings are invariant to the first Euler angle (rotation around the Z-axis), so this angle value cannot be set.

3.4.3 The Simplex Algorithm

The Simplex iteration minimizes the least square difference of the experimental spectrum and the superimposed simulated spectra between F1P and F2P. The 'Best overlap %' value is determined as described below.

Calculate the area between the curves of the experimental and the calculated spectra.

A(dif) = Sum(Abs(Yexp(i)-Ycalc(i)))

Calculate the area of the experimental spectrum.

A(exp) = Sum(Abs(Yexp(i)))

Compare these to area values.

Overlap(%) = 100*(1-A(dif)/A(exp))

Overlap(%) = 100, if A(dif)=0. This is the theoretical maximum of the signal overlap.

Overlap(%) = 0, if the calculated spectrum is similar to the experimental one, with same area, but they do not overlap.

Overlap(%) > 0, but <100. Partial overlap.

Overlap(%) > 70-90, good agreement.

Overlap < 0. No agreement. Change the initial parameters and start a new simulation.

4 DNMR- Analysis

4.1 Introduction

4.1.1 DNMR General

DNMR (Dynamic NMR) Lineshape Analysis is a program to simulate temperature dependent NMR spectra, interactively set up and iteratively refine the model parameters to get the best fit of the measured and simulated 1D NMR spectra.

The mathematical calculation is based on the steady-state solution of the time-dependent Schrödinger equation written in the Liouville-von Neumann equation form, which contains the averaged density matrix of the spin system [1-5]. The Hamiltonian operator contains the interaction of the spin system with B_1 field, the isotrope chemical shifts of nuclei up to seven, the scalar couplings and chemical exchanges among the nuclei. The relaxation is treated phenomenologically. All these Hamiltonian members and the density matrix are written using super-operator formalism.

The spectrum simulator can calculate the superposition of DNMR spectra of more than one spin system. These systems can be completely independent from each other or they can have common physical parameters, like natural half widths (LB), molar fractions or reaction speed values of the chemical exchange processes as super parameters. Another level of the simplification of the spin systems is the use of spin groups characterized by pseudo-spins. For example two magnetically equivalent methyl groups can be treated as a single nucleus with 6/2 pseudo-spin.

4.1.2 Examples

The use of this DNMR module is demonstrated on two samples. The first sample is dimethyl acetamide (Me2NCOMe) in DMSO-d6, where the dimethyl amino group's rotation is hindered around the amide bond. Over the room temperature N-methyl proton signals broaden, overlap and, finally, coalesce. This system will be described by a mutual exchange model.

The second sample is N,N-diisopropyl carbamic acid trimethyl silyl ester (iPr2SIC) in toluene-d8. Over the room temperature the diisopropyl amino group's rotation is hindered around the amide bond. In this case the isopropyl methine septets show strong temperature dependence in the proton NMR spectrum. This system will be described by a mutual exchange model of scalar coupled nuclei. The diisopropyl amine contamination of the sample is described with an other spin system.

The same iPr2SIC sample will be used to demonstrate the lineshape analysis of a non-mutually exchanging system as well. Below the room temperature the rotation of isopropyl groups is hindered and two non-equally populated rotamers appear. Altogether four inequivalent methine positions result in three methine septets with different intensities.

4.2 Example 1: Me2NCOMe

4.2.1 Introduction

The Me2NCOMe dataset contains temperature dependent 1H NMR spectra of dimethyl acetamide in DMSO-d6 solvent. This compound exhibits hindered rotation around the amide bond. Above room temperature the N-methyl signals coalesce.

Experiment No. 320 demonstrates the slow rotation at 320 K. Two sharp N-Methyl groups are visible at 2.95 and 2.79 ppm:

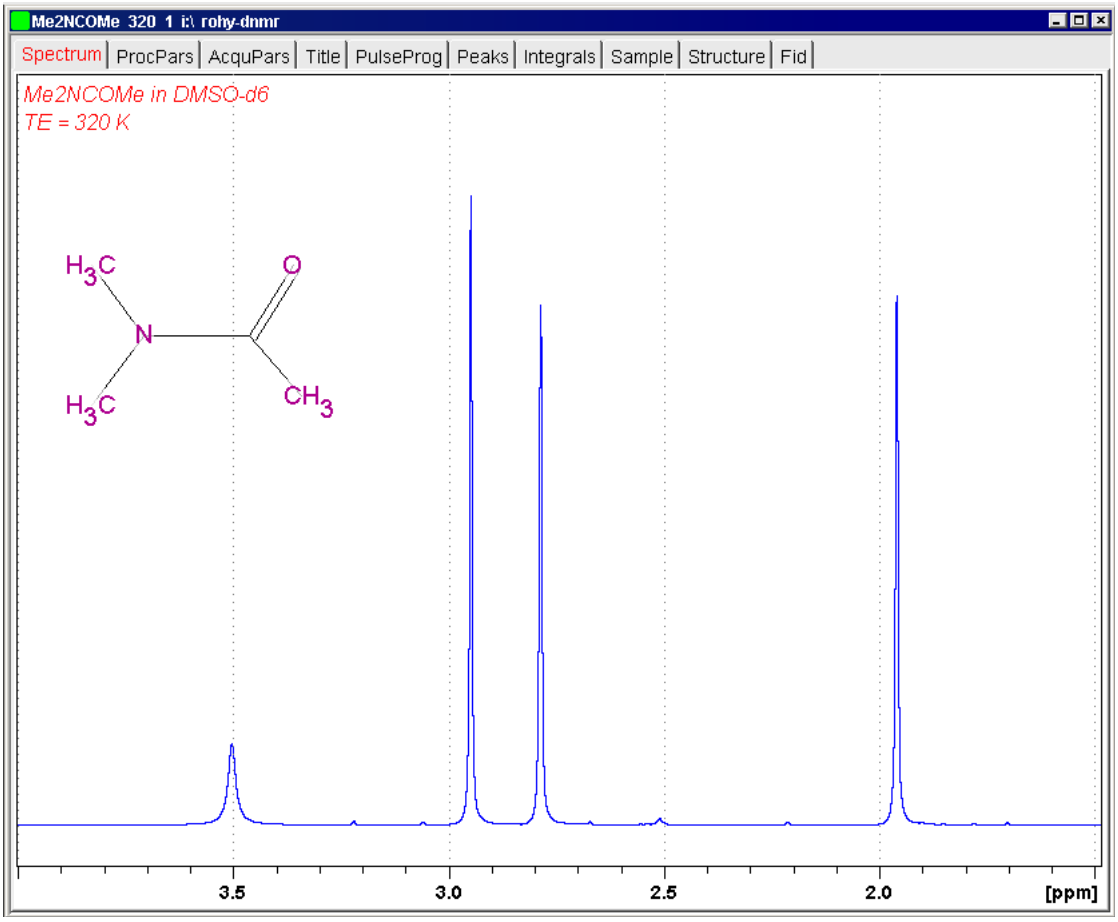


Figure 4.1: Dimethyl acetamide 1H NMR spectrum at 320 K

The signal at 1.96 ppm is also sharp and belongs to the NCOMe Methyl group. The water content of the sample results in a smaller and broad signal at 3.5 ppm. This signal moves in up-field direction during the heating of the sample. This is a known behaviour of H-bonded protons.

In Experiment No. 350 (at 350 K) the rotation is still slow but observable. N-Methyl group signals are broad but separated, since the temperature is below the coalescence point:

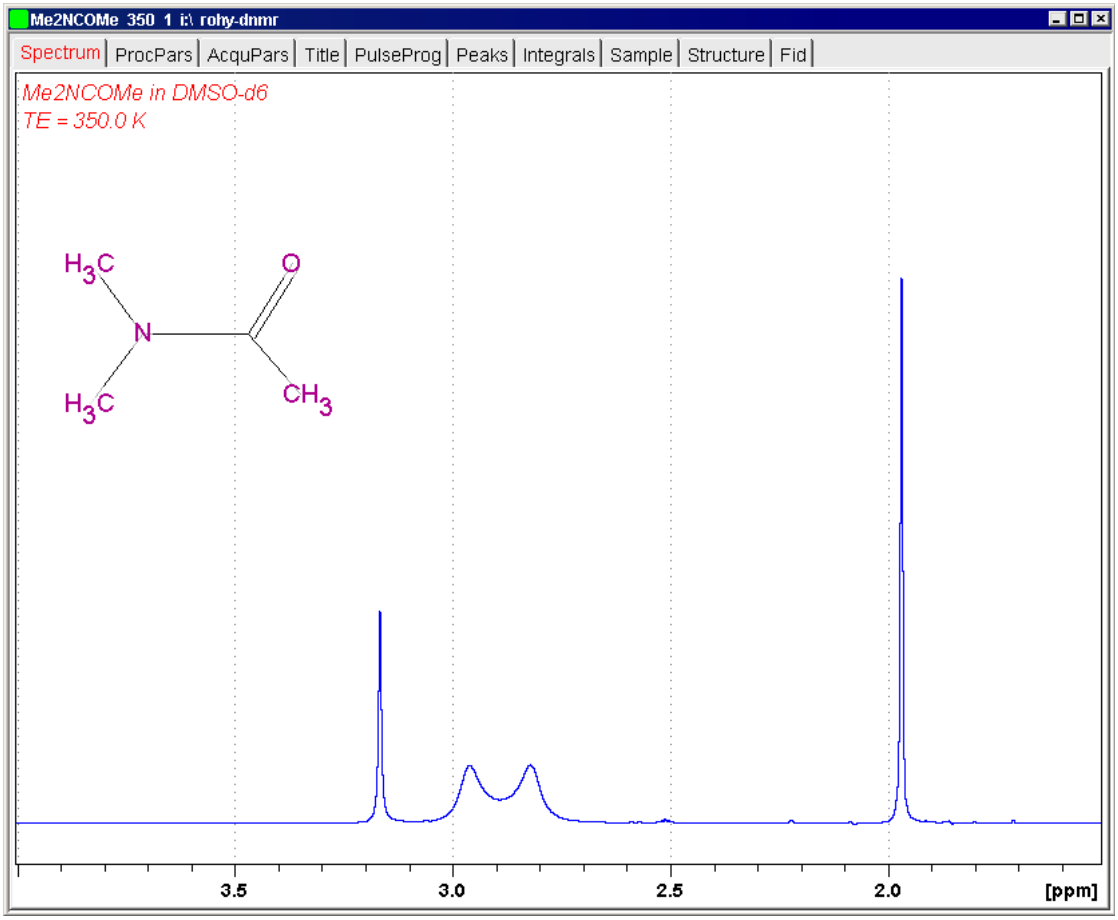


Figure 4.2: Dimethyl acetamide 1H NMR spectrum at 350 K

Spectrum No. 370 shows only one broad N-Methyl signal. This indicates that the rotation speed is higher than in spectrum No. 350:

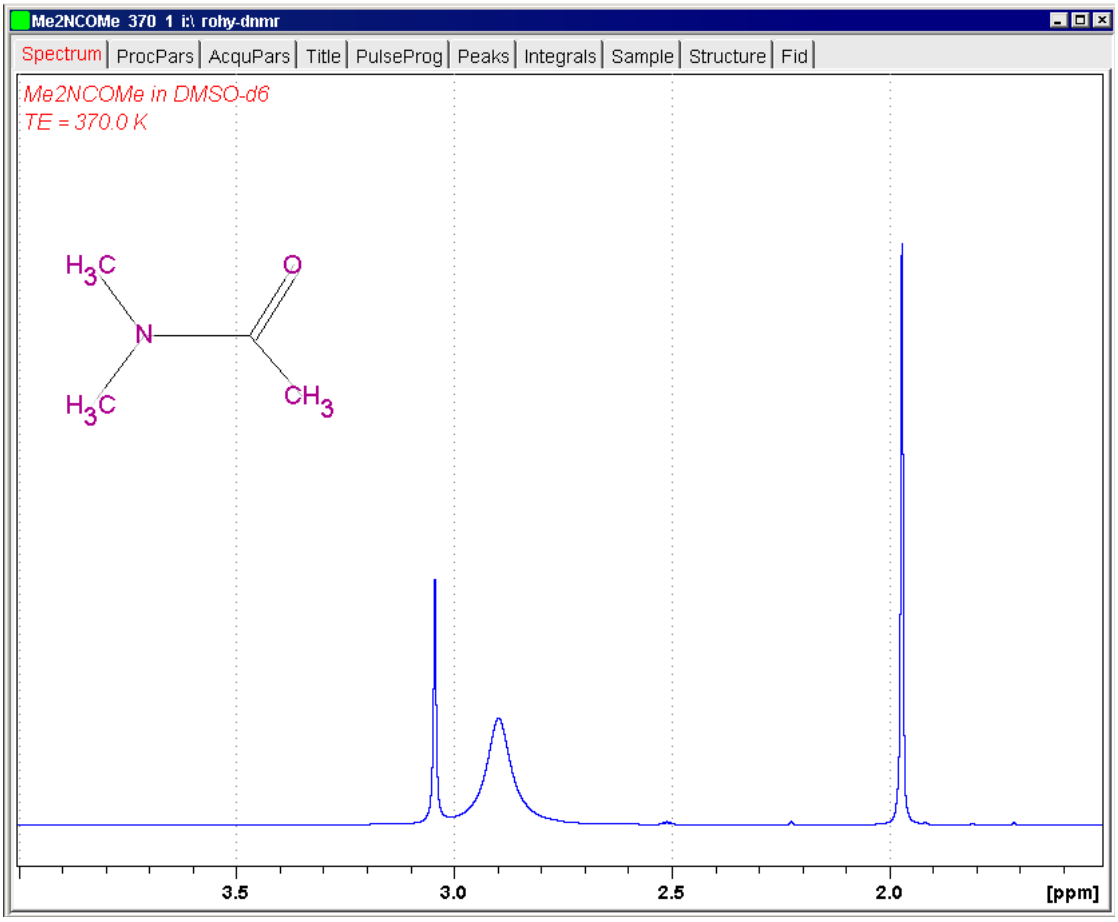


Figure 4.3: Dimethyl acetamide 1H NMR spectrum at 370 K

Finally, spectrum No. 420 belongs to measurement at 420 K, where the intra-molecular exchange is very fast and only one sharp N-Me signal is observed at 2.92 ppm:

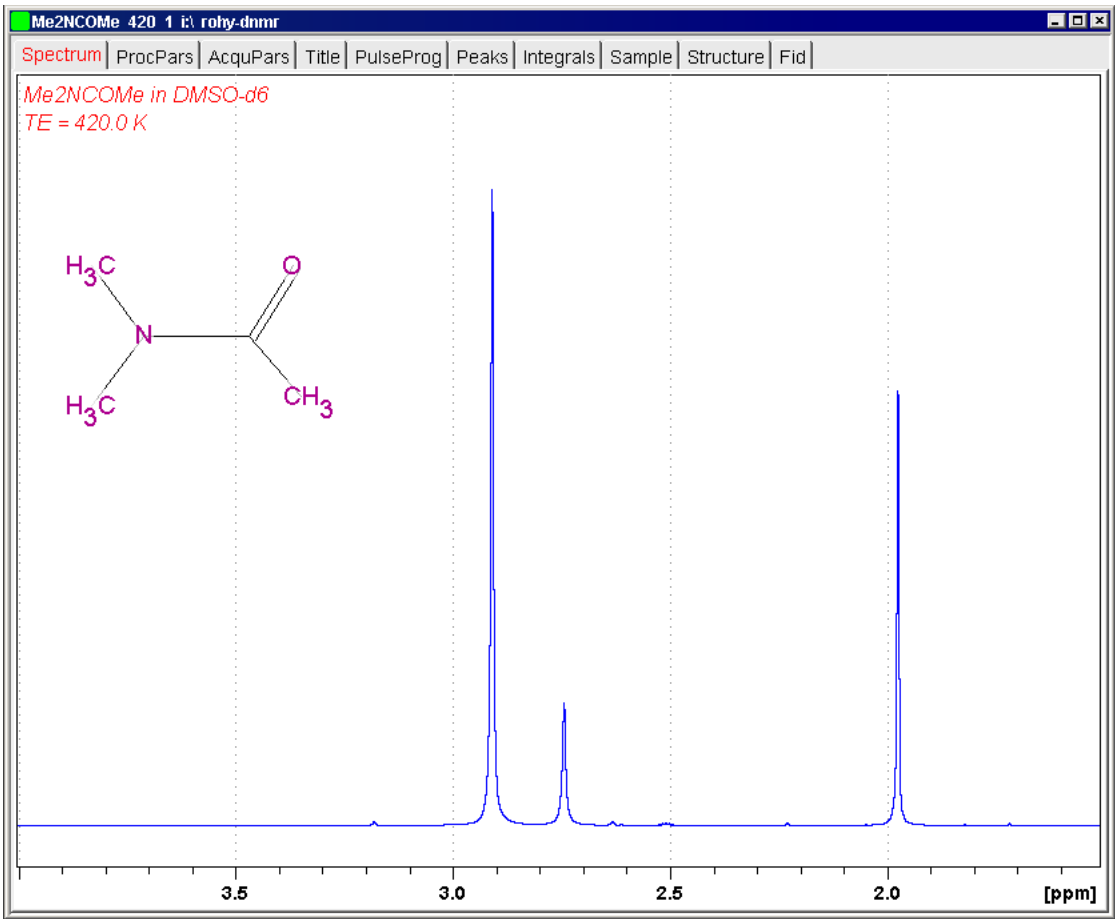


Figure 4.4: Dimethyl acetamide 1H NMR spectrum at 420 K

4.2.2 DNMR Lineshape Analysis

Open spectrum No. 350 of Me2NCOMe. Choose **DNMR Lineshape Analysis** menu item of **Analysis** pull-down menu:

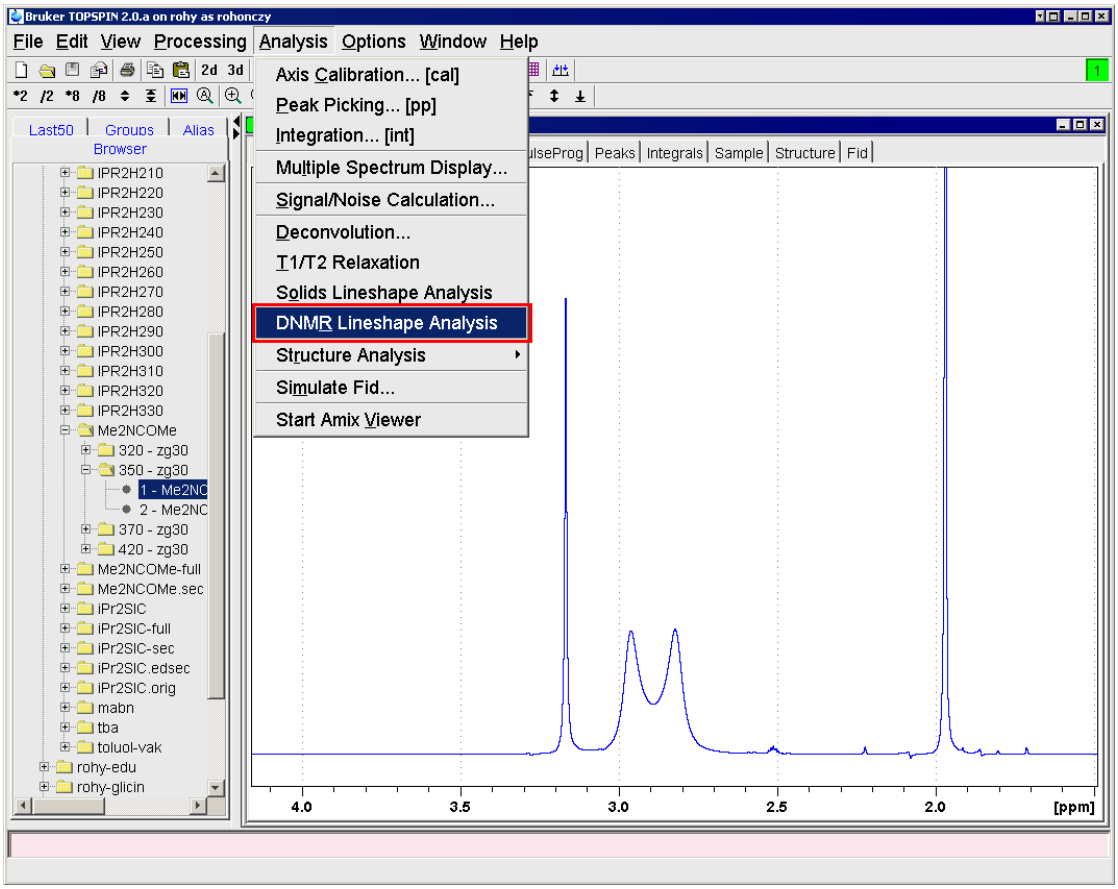


Figure 4.5: Open DNMR Lineshape Analysis

The spectrum panel splits into two windows. The left window shows the parameter panel of the spectrum analysis while the right one the experimental and simulated spectra:

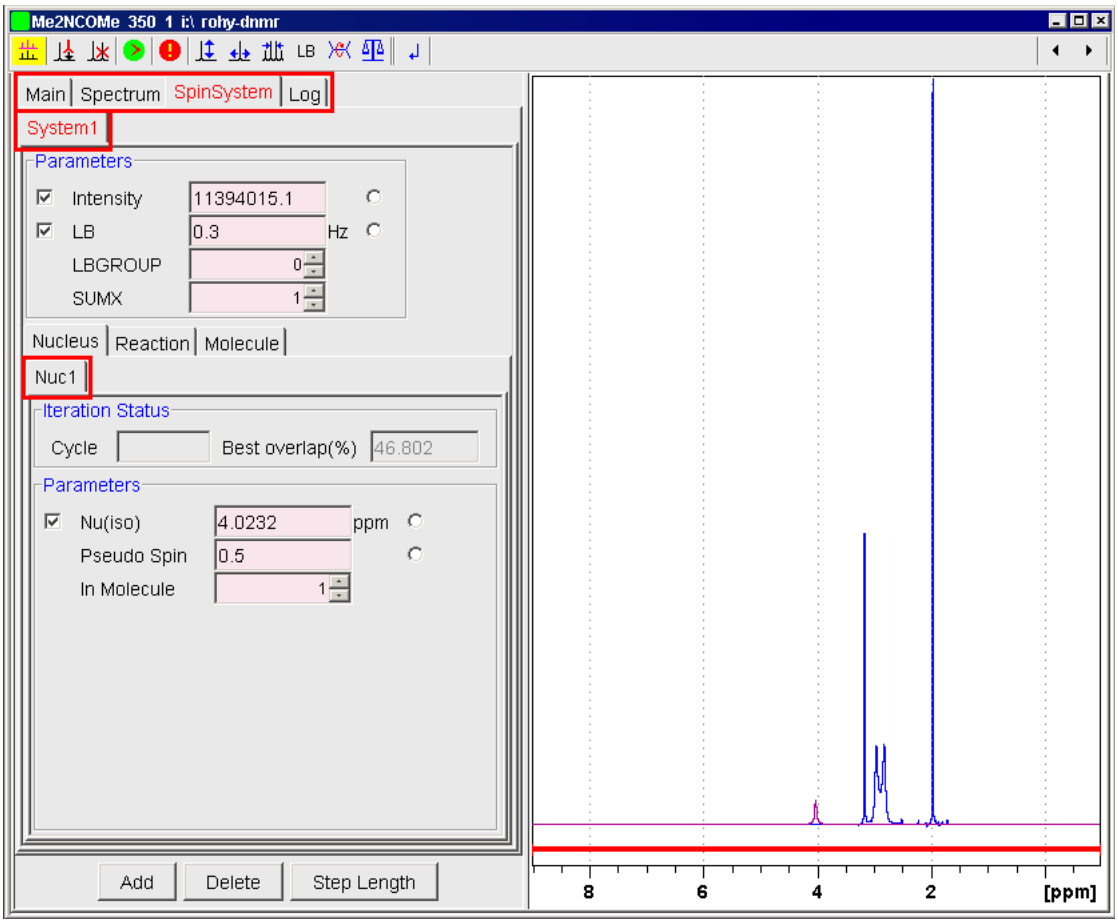


Figure 4.6: The DNMR Lineshape Analysis window

The parameter window contains four tabbed parameter panels. These are the **Main**, **Spectrum**, **SpinSystem** and **Log** panels. Now the **SpinSystem** panel is visible. The **System1** spin system is automatically generated and it contains one nucleus, called **Nuc1**.

Spin systems can contain up to six nuclei, which are in interactions in quantum-mechanical sense. It is useful to apply several small spin systems instead of few bigger ones. **System#** panels contain the common parameters of a spin system, like Intensity, LB (line broadening) etc. and three internal tabbed panels: **Nucleus**, **Reaction** and **Molecule**.

4.2.3 Building up the Spin System

Click on **Spectrum** tab to enter the Spectrum panel and set F1P = 4.0 and F2P = 1.6 ppm to define the fit range. The red horizontal bar on the spectrum display shows this restricted spectrum range. Only this range will be used in the lineshape fitting calculation:

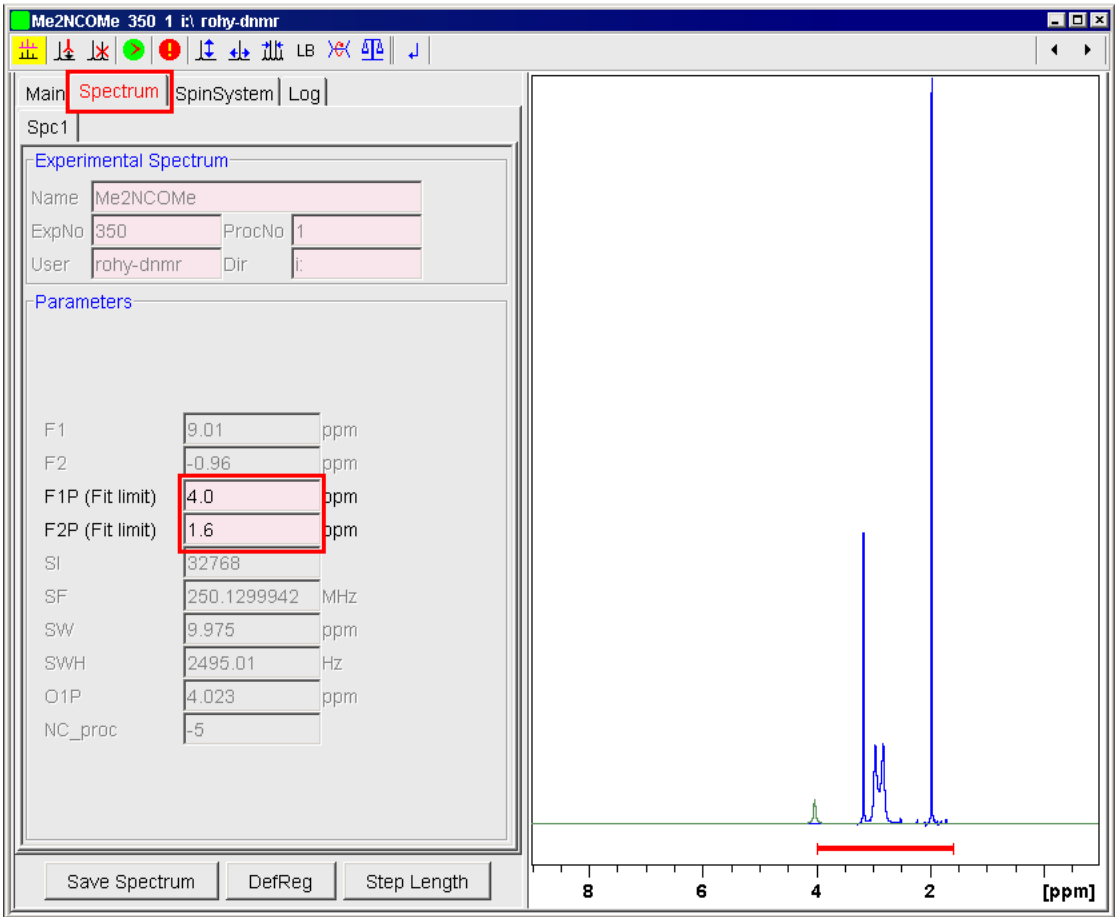


Figure 4.7: The Spectrum panel is used to set the fit range.

Click on **SpinSystem** tab to return to the spin system panel.

Define the first spin system which contains all the three methyl groups of the dimethyl acetamide: since there is no visible scalar coupling between the groups, it is possible to describe them as singulets with 0.5 pseudo spin. Drag the (horizontal movement) icon horizontally and place the calculated curve to 2.96 ppm. Click on the **Add** button underneath. Nuc2 is added to the Nucleus panel. Move the second curve to 2.82 ppm. Add a third nucleus and move it to 1.97 ppm in a similar way. Set their common intensity and LB parameters numerically or you can drag the small ■ (Intensity) and ■ icons in the upper toolbar as well. Set LB = ca. 1.1 value to fit to the experimental line broadening of the NCO-Me group at ca. 2 ppm.

Click on the small check boxes at the beginning of the parameter rows to mark the Intensity and LB parameters. Only these marked parameters will be refined during the iterative refinement procedure:

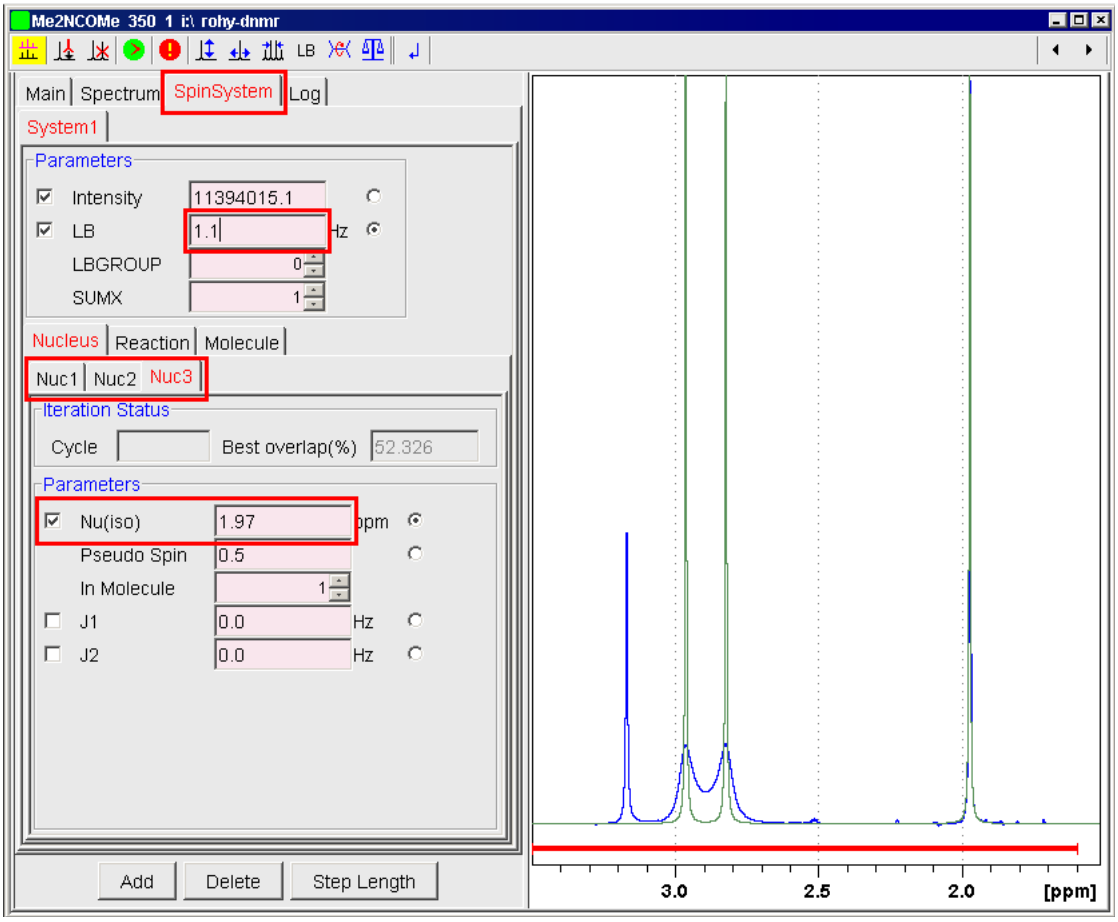


Figure 4.8: Define the Nu and LB parameters of the three methyl groups

Select a parameter panel (now **Nuc1** or **System1**) and click on **Step Length** button. The background color of the variable parameters change to yellow. This is the parameter difference set-up mode. Now you can give the starting step length of the Simplex type parameter refinement calculation:

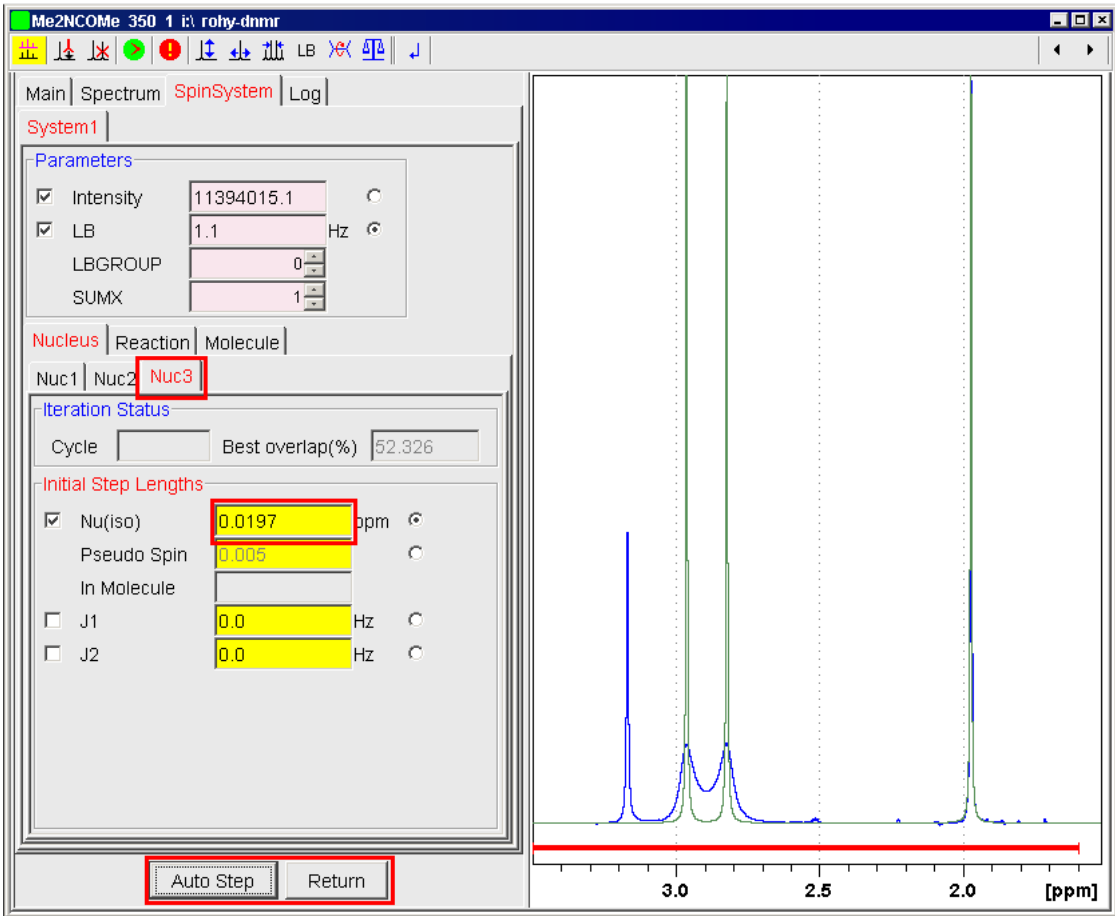


Figure 4.9: Define the step length of the Nu parameter.

You can use **Auto Step** button to suggest step lengths, which are a few percents of the parameter values. But, if the parameter value is 0.0, the suggested step length will be 0.0 as well. In this case you should give a different number to be able to vary this parameter. Finally, click on **Return** button to return to parameter mode. The background color will be gray again.

4.2.4 Building up the Exchange Model

Click on **Reaction** panel to define the chemical exchange reaction. In this compound a mutual exchange results in the broadening of the two N-Methyl group signals. Set the Exchanges values to 2. Nucleus #1 exchanges with nucleus #2 during the amide bond rotation (1 → 2 and 2 → 1). Set Exchange "#1 From" = 1, "To" = 2, "#2 From" = 2 and "To" = 1. Set "k" = ca. 30 Hz. You can give this value numerically or by dragging the toolbar icon. Do not forget to mark the parameter and set its difference value as well:

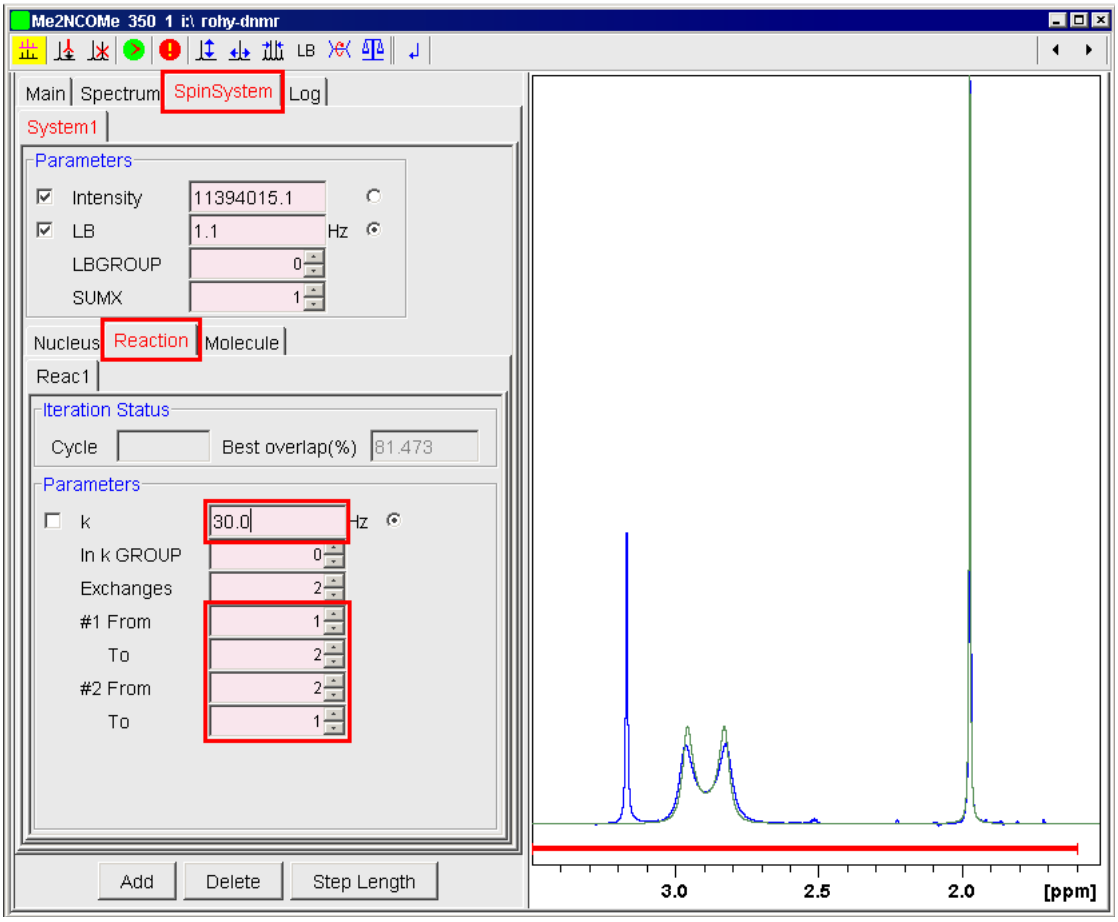


Figure 4.10: Define the exchanges and the speed parameter.

4.2.5 Define Molecules

This system contains only one molecule. After the rotation the molecule remains the same. Its molar fraction x =1.0 and its stoichiometric coefficient is equal to -1 (reactants of the reactions have negative integer coefficients while products have positive ones). These are the default parameters of the **Molecule** panel. So it is not necessary to change any value on the **Molecule** panel in this case:

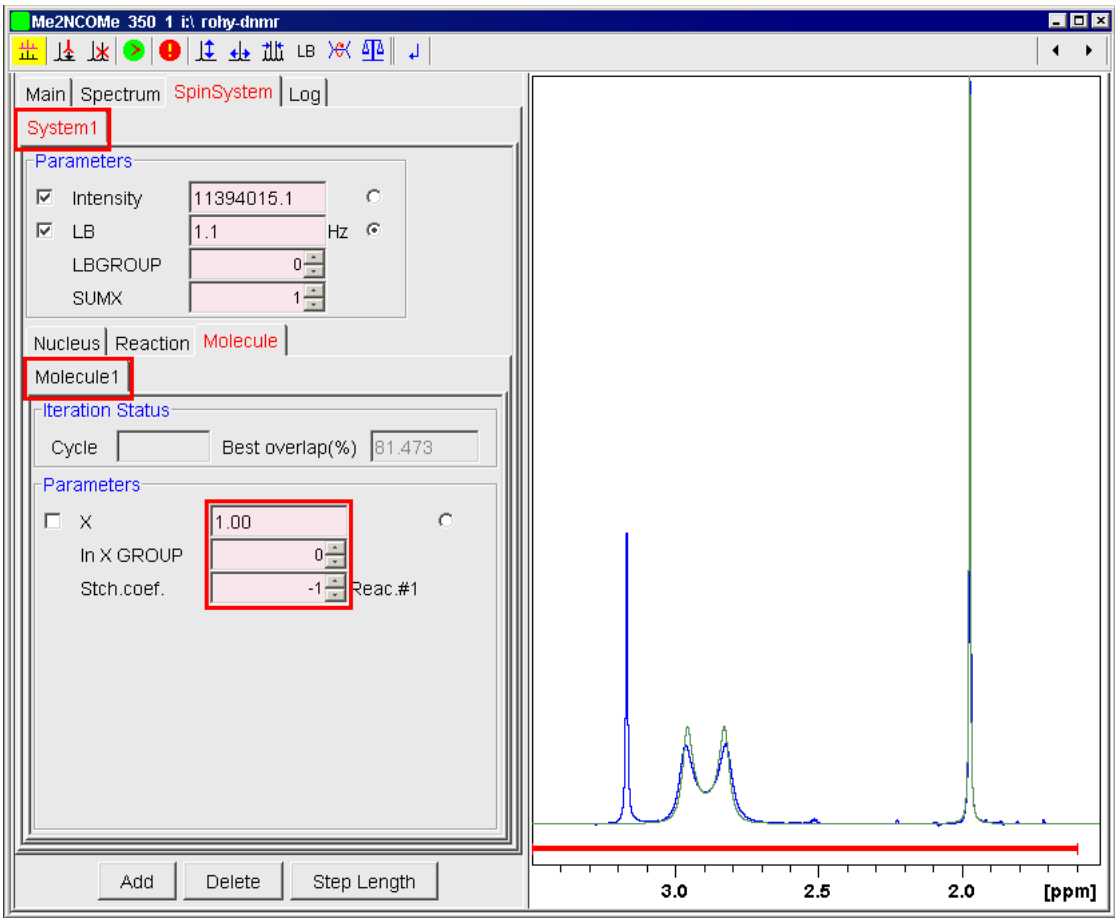


Figure 4.11: The Molecule Panel

The Molecule panel contains the molar fraction and stoichiometric coefficients of a molecule.

4.2.6 Add a Second Spin System

Now add a second spin system which describes the water signal. Click on **System1** tab to select it. Then click on the icon in the toolbar or on **Add** button underneath. A second spin system called **System2** tab appears with **Nuc1**. Move this signal to 3.17 ppm and modify their parameters (LB and Intensity) to get best overlap with the experimental signal. Do not forget to set their difference values and mark them as variable parameters:

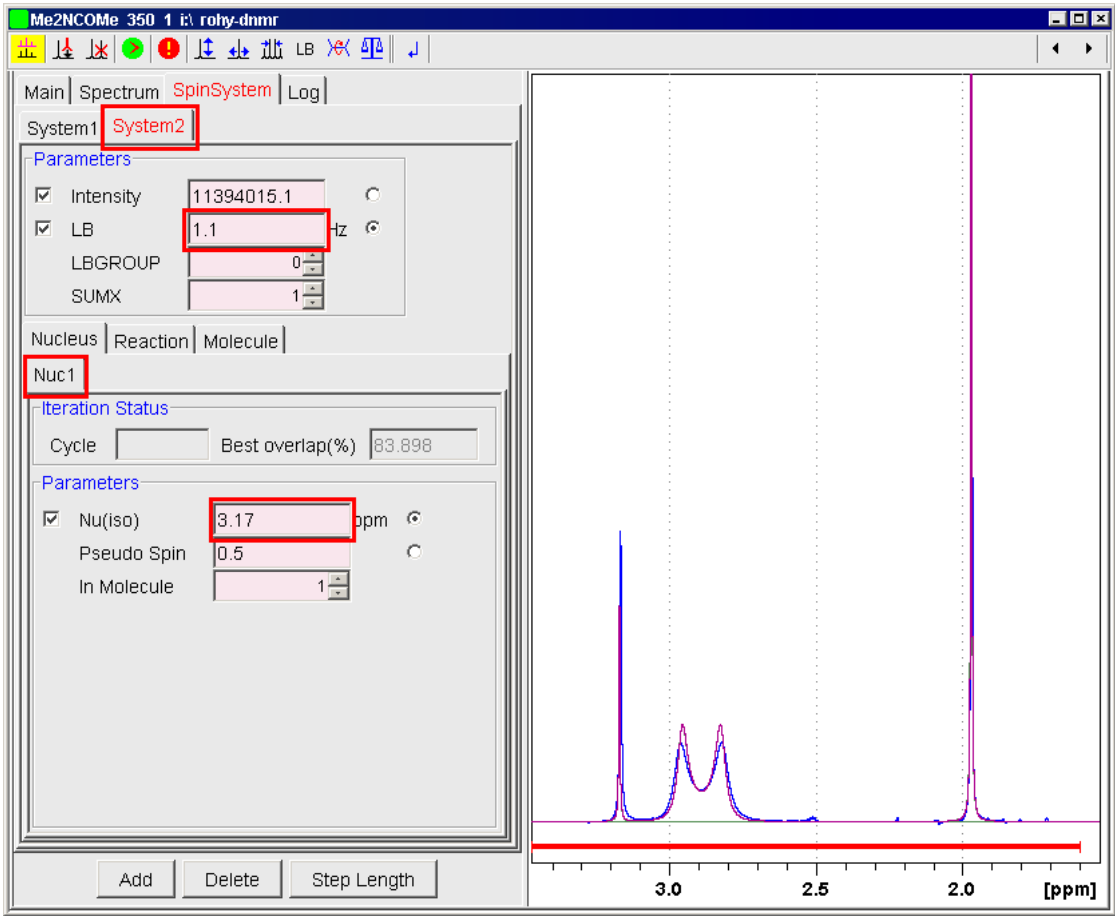


Figure 4.12: Define the second spin system to simulate the water signal

4.2.7 Save the Model

Before the refinement, save the parameter set. Click on **Main** tab to enter Main panel. Click on **Save** button underneath of the panel. A "Save model" dialog window pops up. Give a model name, "model-1" for example:

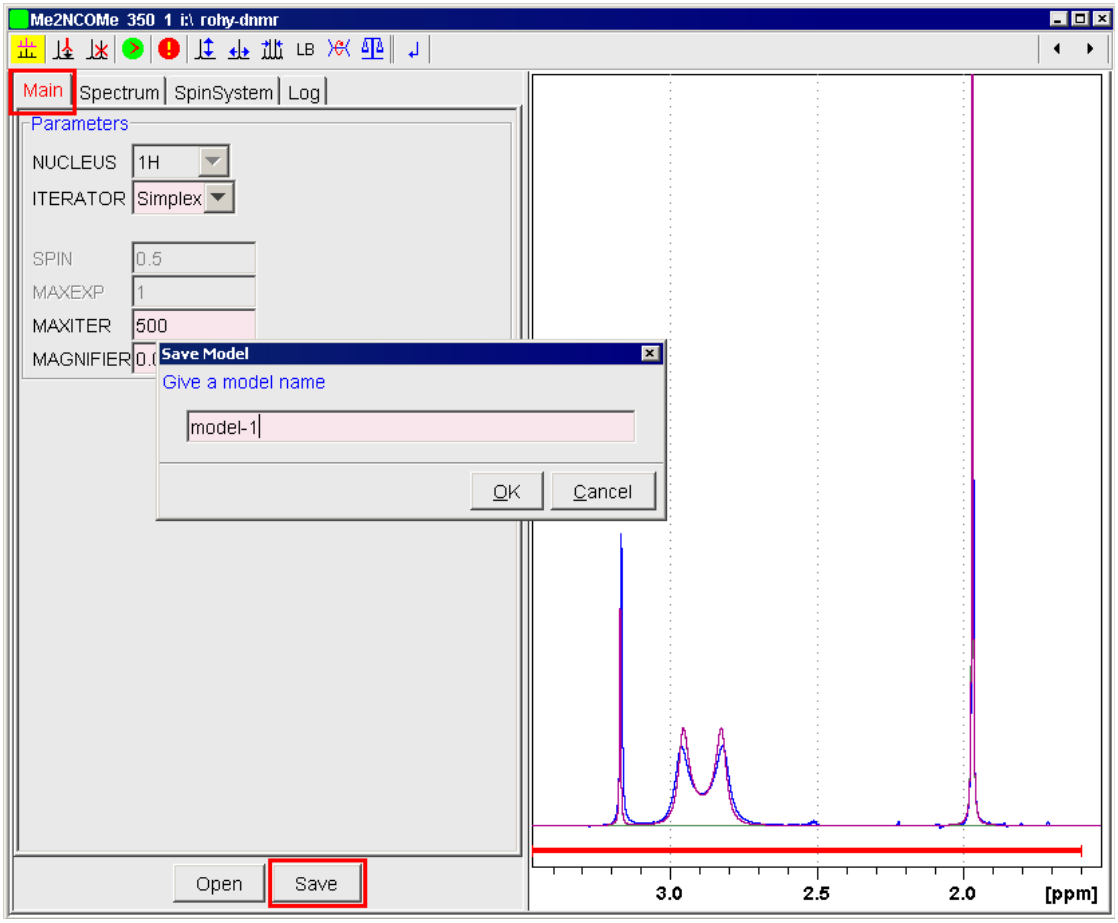


Figure 4.13: Save the model.

Now the model is ready to refine its variable parameters.

4.2.8 Iterative Parameter Refinement

Click on "Start" icon in the toolbar. "Log" window appears and you can see the starting parameters. In "Cycle" and "Best overlap(%)" boxes you can see the actual parameters of the iteration:

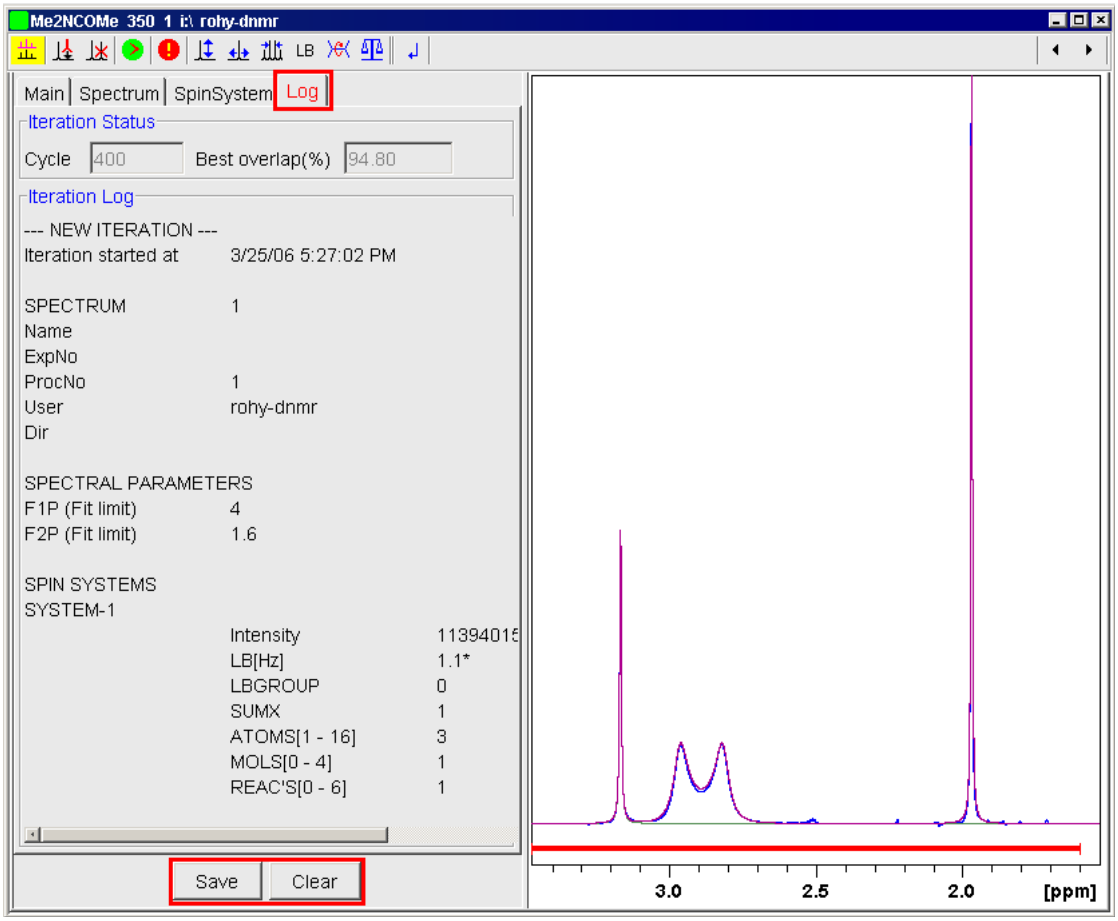


Figure 4.14: The Log panel with the Save and Clear buttons.

During the iteration you can switch to **SpinSystem** panel and select **Nucleus**, **Reaction** and **Molecule** panels where you can see the best fit values of the variable parameters. You can stop the iteration by pressing icon in the toolbar.

It is possible to save the iteration log as a *fitlog.txt* file in the processing directory by pressing **Save** button on **Log** panel.

Finally select **Spectrum** panel and click on **Save Spectrum** button. Give an empty procno to save the simulated spectrum:

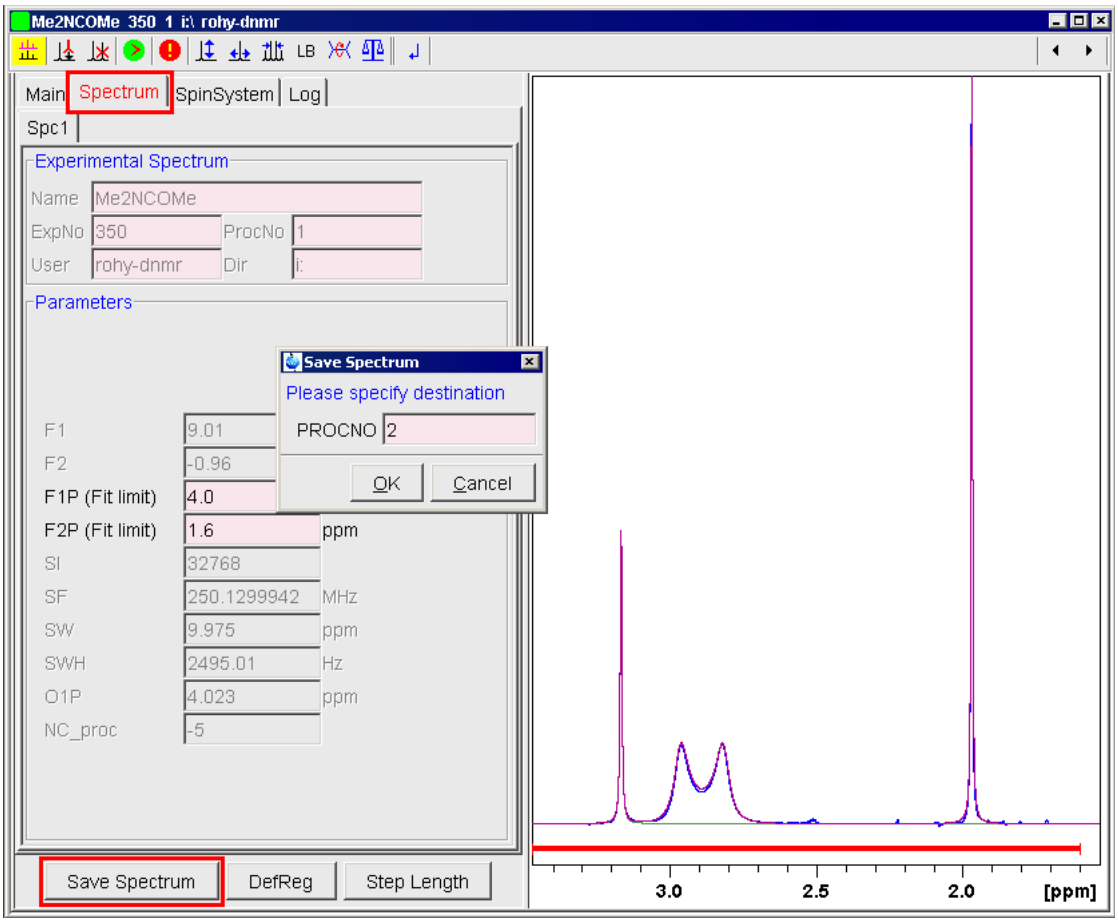


Figure 4.15: Save the simulated spectrum with an unused procno.

4.2.9 Exit the DNMR Module

You can click on ↲ exit icon on the toolbar to exit the DNMR module.

4.2.10 Use the Model in an Other Experiment

Now you can find fitparfix-model-1 and fitparfine-model-1 files in the original processing directory. You can copy them into the processing directories of expno 320, 370 and 420, respectively. If you open one of these experiments and enter to the DNMR module, you can read the model parameter set previously saved. Go to **Main** panel and click on **Open** button. Select the required model and click on **OK**:

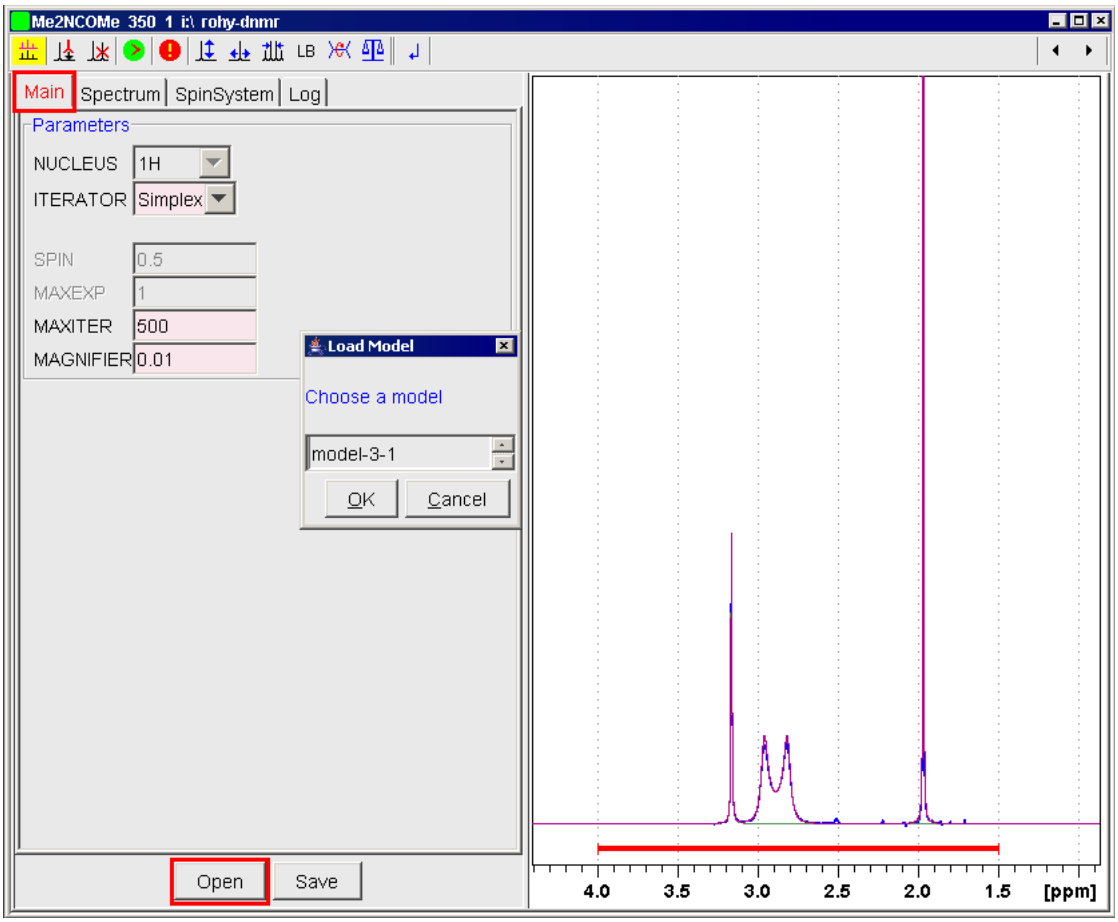


Figure 4.16: Open a model.

After that you can modify the fit parameters (at least the reaction speed parameter) and start the parameter refinement at this temperature.

4.3 Example 2: iPr2SIC – Mutually Exchanging Spin System

4.3.1 Introduction

The iPr2SIC compound is diisopropyl carbamic acid trimethyl silyl esther (formula: (iPr)2NC(O)OSiMe3) which was solved in toluene-d8 and its 1H NMR spectrum was measured. Methine septets of iPr-groups show very strong and complex temperature dependence [6].

In the higher temperature range (280 K -360 K) simultaneous rotations around the two N-C(iPr) bonds are very fast, while the speed of rotation around the N-C(O) amide bond is much slower. At 280 K the two chemically inequivalent methine septets of the fast rotating isopropyl groups (at 3.97 ppm and 3.50 ppm) are separated with 1:1 intensity ratio in respect to the hindered rotation around the amide bond. The higher the temperature is the less the hindrance. This results in a strong broadening of the isopropyl methine groups at 310 K. Above the coalescence point, at 330 K, the methine signal (at 3.73 ppm) is sharper again and shows septet multiplicity. Finally, at 360 K, the septet is very sharp at 3.73 ppm due to the fast rotation and the averaged environment of the methine groups:

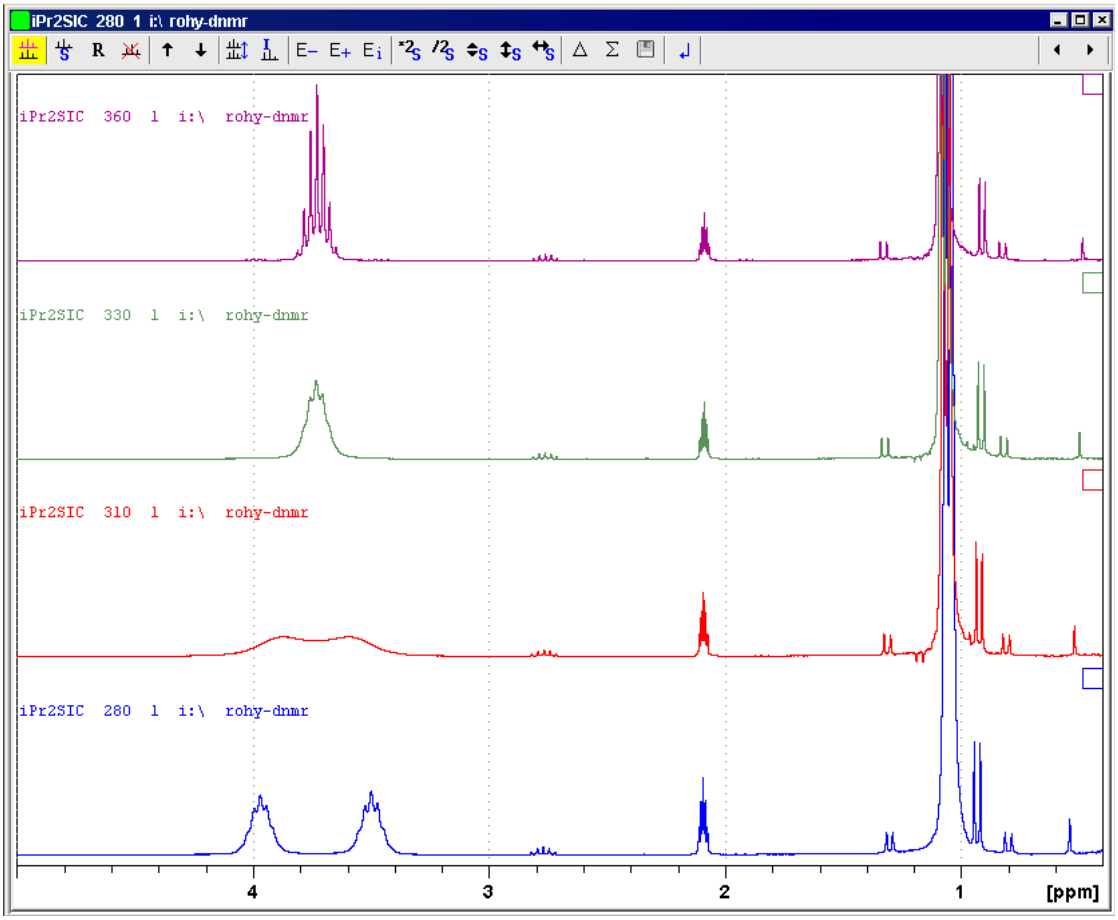


Figure 4.17: Temperature dependent 1H NMR spectra of iPr2SIC in toluene-d8 in the temperature range of 280 - 360 K.

4.3.2 Build up a Scalar Coupled Spin System

Open the spectrum No. 280 of iPr2SIC. Choose the **DNMR Lineshape Analysis** menu item of **Analysis** pull-down menu.

Select **Spectrum** tab and set F1P = 5.0 ppm and F2P = 2.2 ppm as fit range limits:

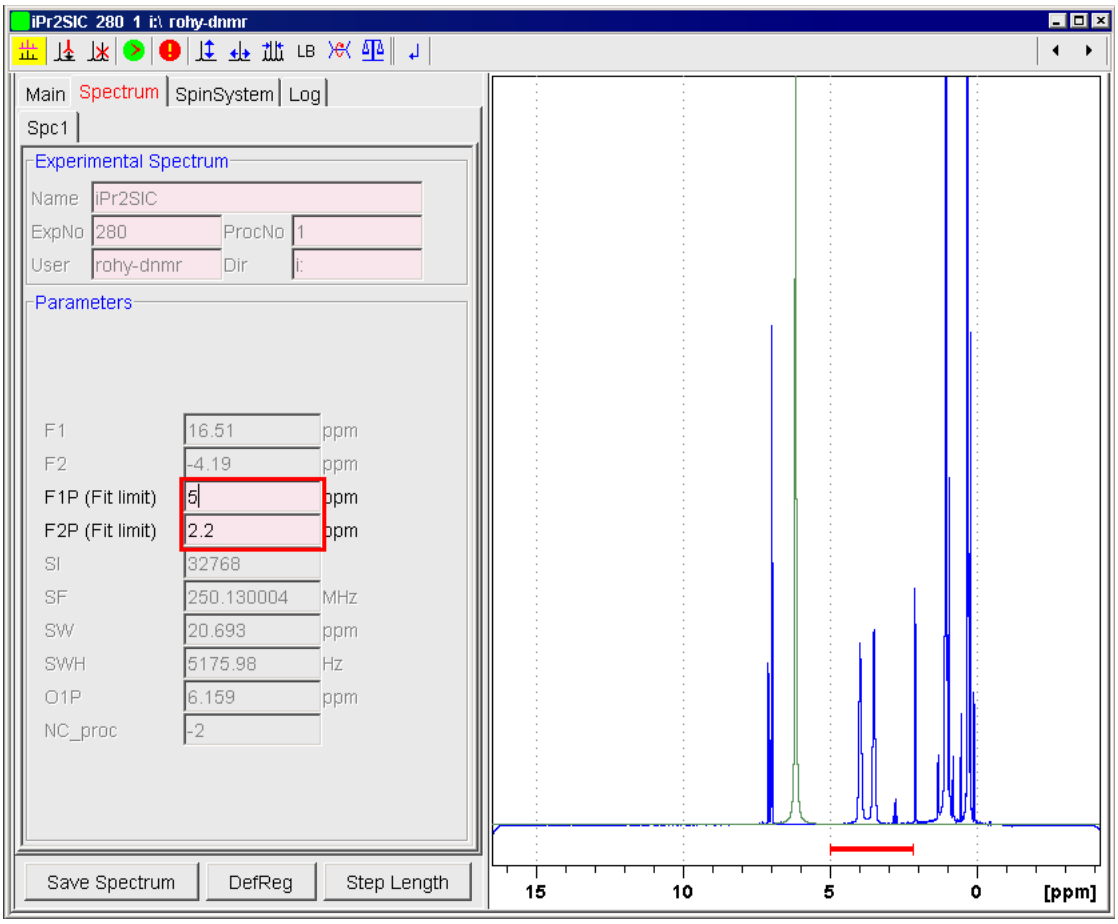


Figure 4.18: Set the fit limits.

Select **Nuc1** on **SpinSystem** tab and set Nu = 3.97 ppm. This is one of the two methine protons. You can set its chemical shift value numerically or by dragging (horizontal movement) icon in the toolbar.

Click on **Add** button underneath or on icon in the toolbar. Set Nu = 1.05 ppm. Nuc2 defines the six equivalent isopropyl methyl protons. Set its Pseudo Spin parameter to 3.0 (6 x 1/2 = 6/2) and J1 = 6.6 Hz. This pseudo spin model results in a first order approximation of the scalar coupled spin system which is suitable to describe the methine signal with septet multiplicity. On the other hand this model is much more efficient (faster calculation and smaller memory requirement) than a model of a spin system with seven 1/2 spin nuclei coupled in higher order.

Mark the check box of J1 and remove the mark of Nu. It means that during the parameter refinement J1 will be refined while Nu of Nuc2 remains fixed (Nuc2 peak is out of the defined fit range). Click on **Step Length** button and type J1 = 0.1 Hz into the yellow box (see Figure 6.9 to study the setup of initial step length.). Click on **Return** button.

Expand the spectrum range into the 5.2 - 2.0 ppm, set LB=1.1 Hz and Intensity = 120000 values on the System1 panel. see the green fit curve with septet multiplicity at 3.97 ppm on:

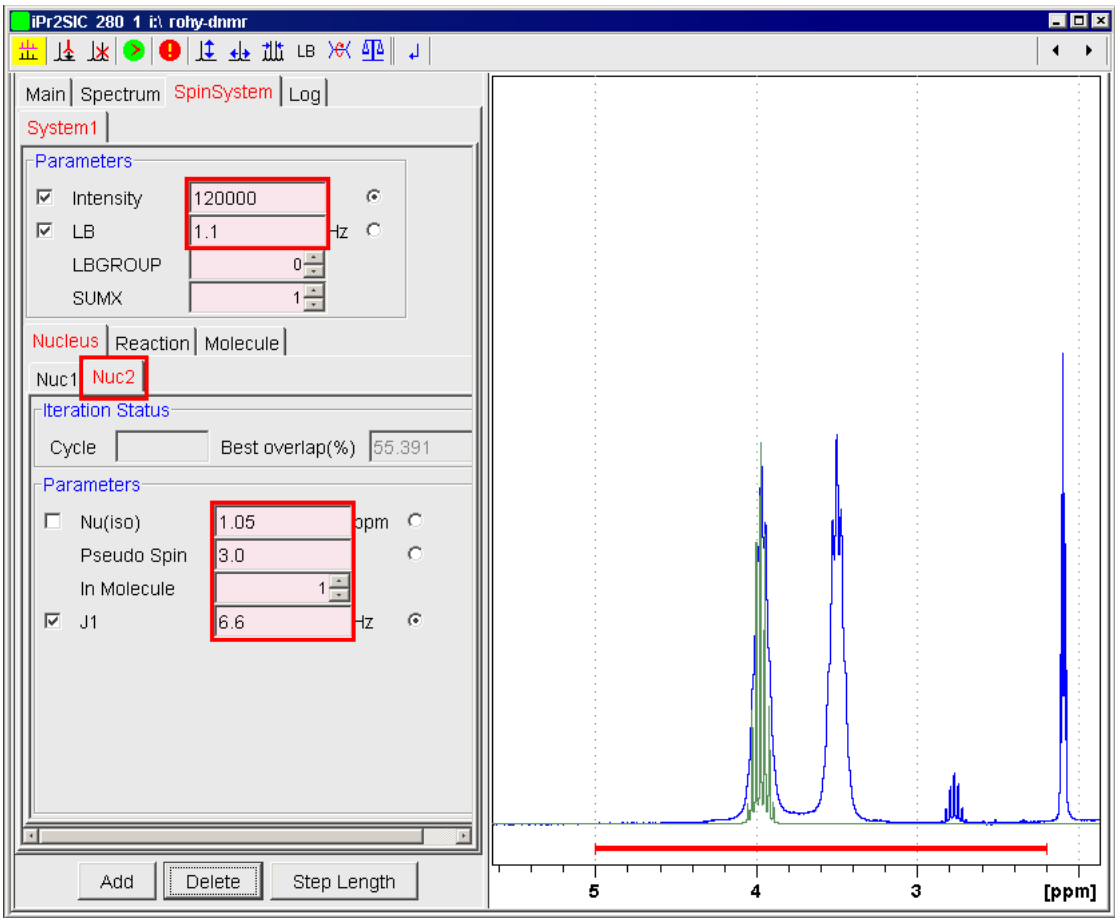


Figure 4.19: The methine spectrum region of the experimental iPr2SIC

The figure above shoes a methine spectrum region of the experimental iPr2SIC spectrum with one fitting curve (green curve with septet multiplicity).

4.3.3 Define the Other Isopropyl Methine Signal

Now you can enlarge the spin system with another isopropyl group. Due to the slow chemical exchange between the isopropyl groups, the half width of iPr2SIC methine signals are bigger than the half width of the methine signal at 2.77 ppm. (The latter septet is coming from the (iPr)2NH which is a minor hydrolysis product of iPr2SIC.)

This chemical exchange requires a spin system model containing both isopropyl methine protons of the iPr2SIC. The simplest way would be to add two nuclei – Nuc3 with 1/2 spin and Nuc4 with 6/2 spin – to the spin system. The fitting range defined earlier excludes all methyl protons from the parameter refinement. So it is possible to replace the second 6/2 pseudo spin nucleus (Nuc4) – which is only a coupling partner of the observed second methine proton (Nuc3) – with Nuc2. In this case both Nuc1 and Nuc3 couple to Nuc2. This approximation results in a wrong triplet line shape at 1.05 ppm, but a good one in the fitting range.

Click on **Nuc2** tab and on **Add** button. **Nuc3** tab appears. Set Nu = 3.50 ppm, J2 = 6.6 Hz. Mark Nu and J2 as variable parameters. Set their initial step length values using **Step Length** button. (Nu=0.0616 ppm and J2=0.1 Hz are the suggested values). Click on **Return** button. Finally set Intensity = 80000 on **System1** tab:

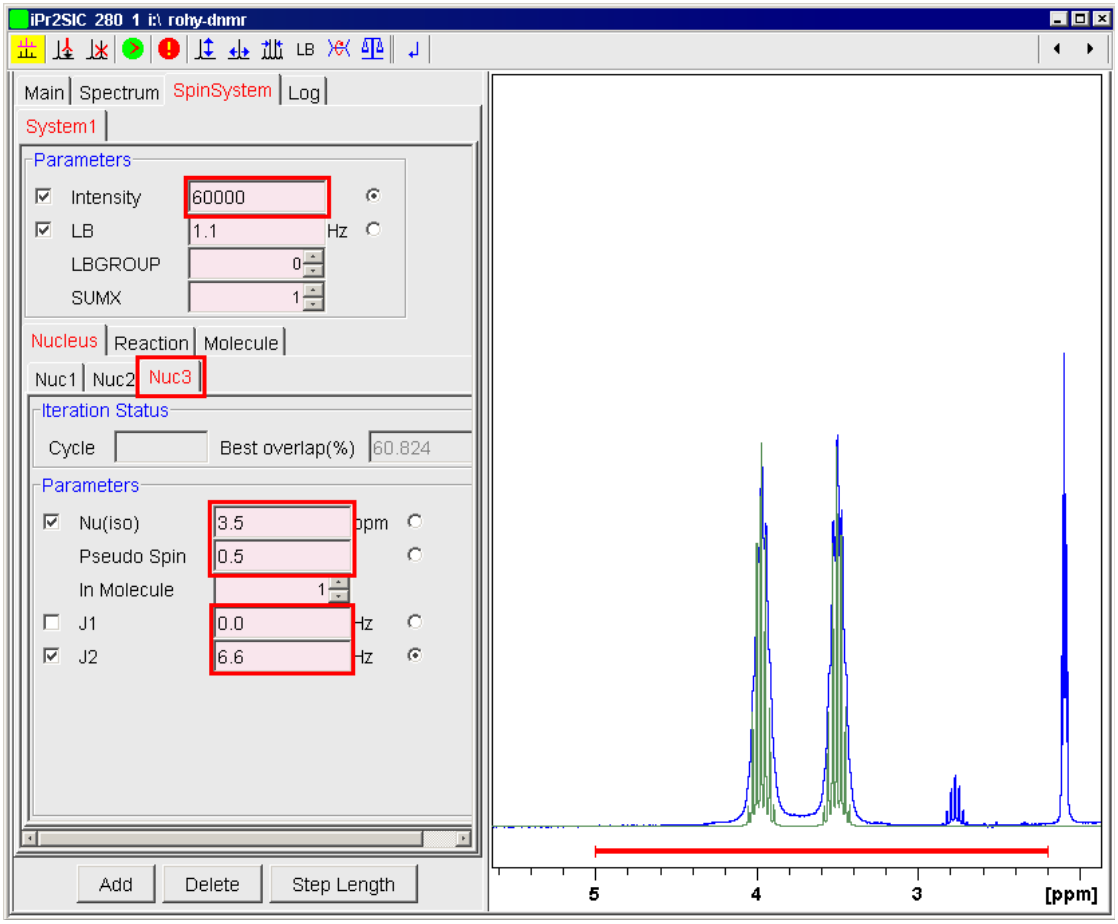


Figure 4.20: The methine spectrum region of the experimental iPr2SIC spectrum with two fitting septets.

4.3.4 Define the Chemical Exchange

Click on **Reaction** tab. Set the "#1 To" = 3 and "#2 From" = 3 values in respect to the Nuc1 → Nuc3 and Nuc3 → Nuc1 exchange. This describes the exchanges of the two methine protons. Set k = 15.0 Hz. Septets are broader but still have splittings. Mark the checkbox of "k" parameter to allow this parameter to be fitted. Click on **Step Length** button and set k = 1.5 Hz as step length. Click on **Return**. Set Intensity = 235000. Now the overlap of the experimental and the simulated spectra is better than 91 %.

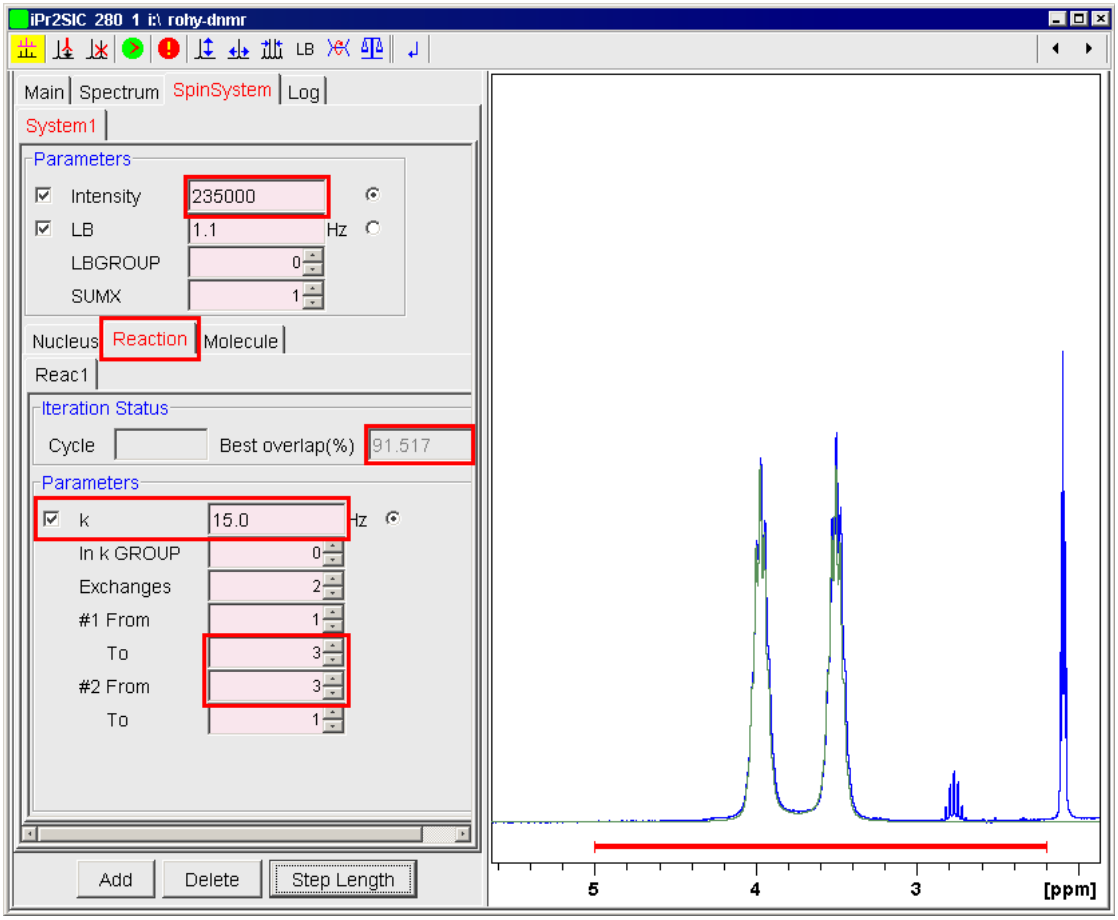


Figure 4.21: Define the 1->3 and 3->1 exchange with k = 15 Hz speed.

4.3.5 Define a Second Spin System

For the first approximation the spin system model defined above would be a good starting point for the parameter refinement. But we cannot distinguish between the broadening effects of the LB and k parameters. We can involve the septet signal of the di-isopropyl amine contamination into the parameter refinement. Its natural half width (LB) is very similar to the LB of the iPr2SIC but its shape is independent of k.

Click on **SpinSystem** tab. A **System2** appears with **Nuc1**. Set Nu = 2.77 ppm and mark it to fit. Click on **Nuc1** tab and **Step Length** button. Set Initial Step Length Nu = 0.06 ppm. Click on **Return** button.

Click on **Add** button. Set Nu = 0.93 ppm and J1 = 6 Hz. Mark J1 and unmark Nu. Set Initial Step Length of J1 = 0.01 Hz. Click on **System2** tab and set LB=1.1 Hz and Intensity = 18000. Mark both of these parameters to be variable and check their step length values as well. Now the second spin system is defined as well.

4.3.6 Define an LB Group

Till now we did not care about the group parameters. Their default value is 0 which means that the parameter is refined independently from other similar parameters. If you set a group number different from 0, all parameters with the same group number will be refined together. In this case the LB of System2 and System1 can be the same.

Set LBGROUP = 1 of **System2**:

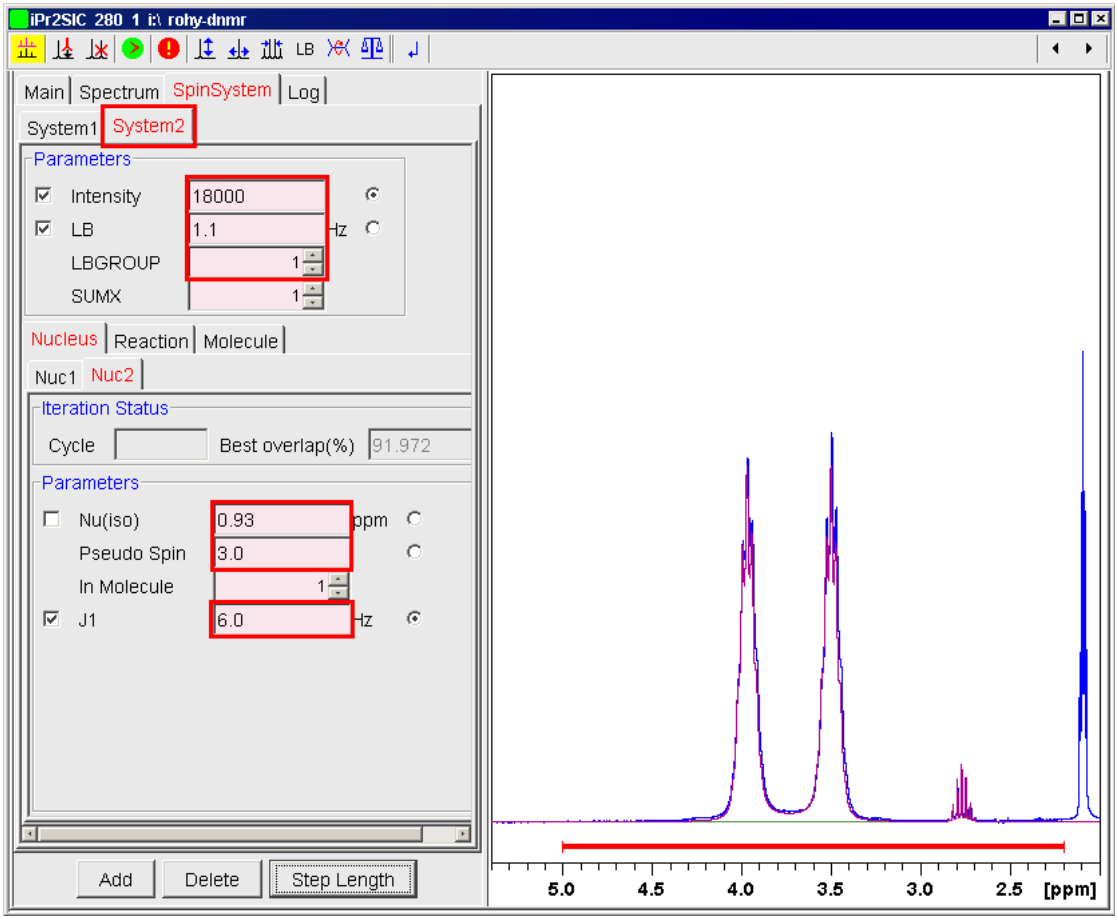


Figure 4.22: Define second spin system and set its LB to LBGROUP=1.

Click on **System1** and set its LBGROUP = 1 as well.

4.3.7 Define Molecules

Both systems contain one molecule. After the rotation the iPr2SIC molecule remains the same. Its molar fraction x =1.0 and its stoichiometric coefficient is equal to -1 These are the default parameter of the Molecule panel. So it is not necessary to change any value on Molecule panel in this case.

4.3.8 Save the Model

Before the refinement, save the parameter set. Click on **Main** tab to enter Main panel. Click on **Save** button underneath of the panel. A "Save model" dialog window pops up. For example, give a model name, "model-1".

4.3.9 Start Parameter Refinement

Click on "Start" icon in the toolbar. "Log" window appears and you can see the starting parameters. In "Cycle" and "Best overlap(%)" boxes you can see the actual parameters of the iteration.

During the iteration you can switch to **SpinSystem** panel and select **Nucleus**, **Reaction** and **Molecule** panels where you can see the best fit values of the variable parameters. You can stop the iteration by pressing icon in the toolbar.

It is possible to save the iteration log as a *fitlog.txt* file in the processing directory by clicking **Save** button on the "Log" panel:

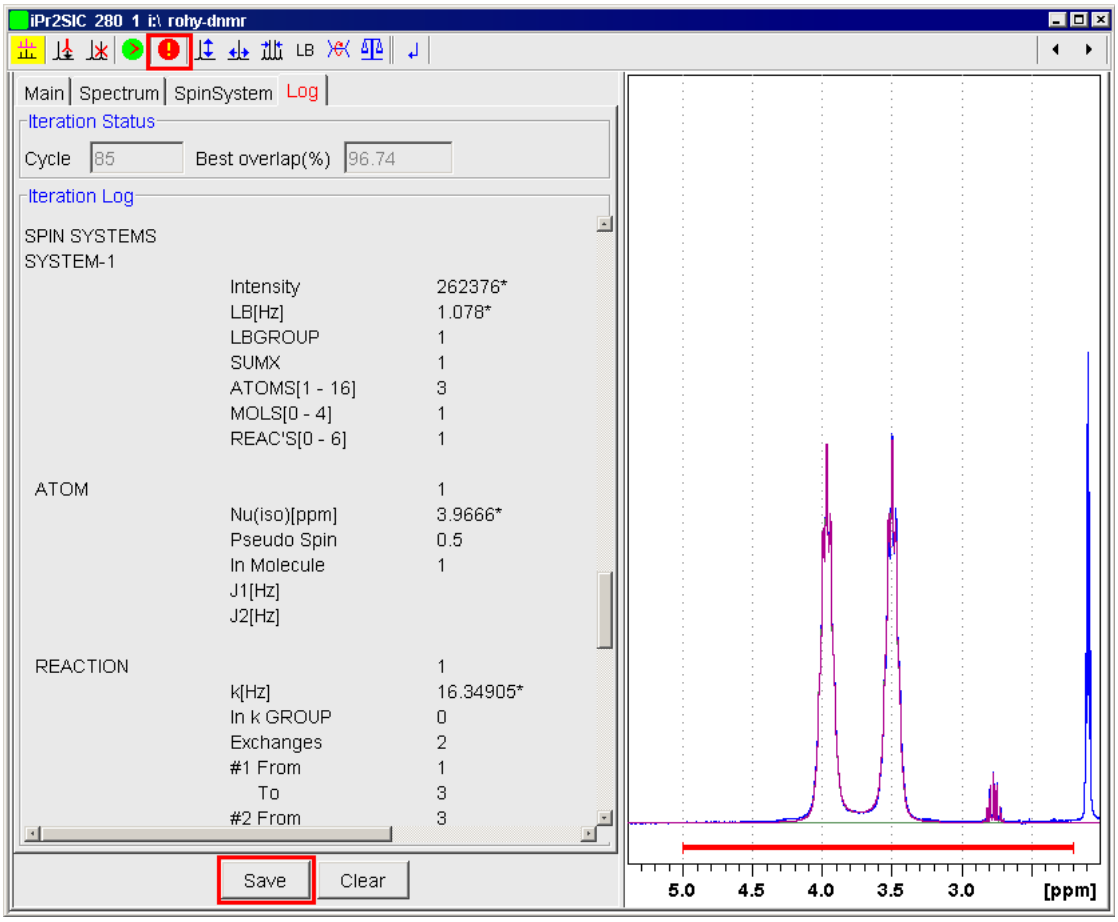


Figure 4.23: Stop the iteration and save the iteration log.

Finally select **Spectrum** panel and click on **Save Spectrum** button. Enter an empty procno to save the simulated spectrum.

4.3.10 Use the Model in an Other Experiment

You can find fitparfix-model-1 and fitparfine-model-1 files in the original processing directory. You can copy them into the processing directories of expno 310, 330 and 360 respectively. If you open one of these experiments and enter to the DNMR module, you can read the previously saved model parameter set. Go to **Main** panel and click on **Open** button. Select the required model and click on **OK**. After that you can modify the fit parameters (at least the reaction speed parameter) and start the parameter refinement at this temperature.

4.4 Example 2: iPr2SIC – Non-mutually Exchanging System

4.4.1 Introduction

The 1H NMR spectra of the iPr2SIC show additional temperature dependent splittings of the isopropyl methine protons in the temperature range of 200 K to 250 K. In this temperature region the rotation around the amide-bond (see paragraph 6.2) is very slow, practically

frozen. Rotations around the N-iPr bonds also slow down. This hindered simultaneous rotation around the N-iPr bonds is known as "gear effect", results in further splitting of the isopropyl septets due to four molecular positions at 200 K:

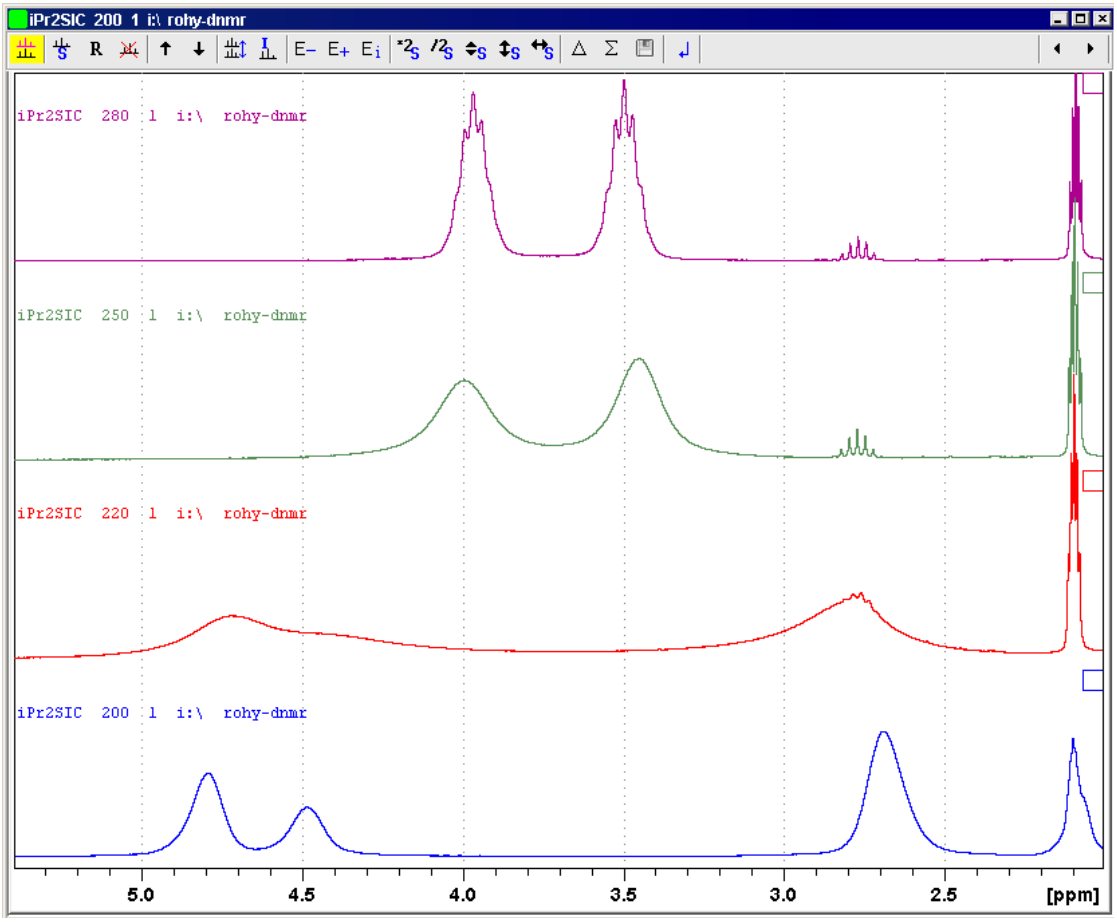


Figure 4.24: Methine signals of iPr2SIC in toluene-d8 in the temperature range of 200 K – 280 K.

At 200 K the solubility of iPr2SIC is low in toluene-d8 and the poor field homogeneity results in broad lineshapes.

There are two conformers of iPr2SIC (I. and II.) with molar ratio of 0.6 : 0.4 at 200 K. The broad signal at 4.80 ppm (with 0.6 intensity) belongs to position 1 of conformer I. The smaller signal at 4.48 ppm (with 0.4 intensity) belongs to the position 2 of conformer II (see Figure 6.25). The chemical shift difference is much smaller between the methine protons in position 3 of I and 4 of II. These signals overlap to each other at 2.69 ppm with sum intensity of 1.0. The isopropyl methyl protons show similar splitting as well but their chemical shift range is much smaller. In this model we do not distinguish them. We simply use an atomic position 5 with pseudo spin 6/2 to describe the expected septet multiplicities.

In the following figure are two planar conformers of iPr2SIC with positions 1-4 of methine protons and an averaged position 5 of iPr-methyl protons. Rotational arrows in conformer I show the "gear effect", while the arrow in conformer II shows the frozen amide-bond rotation

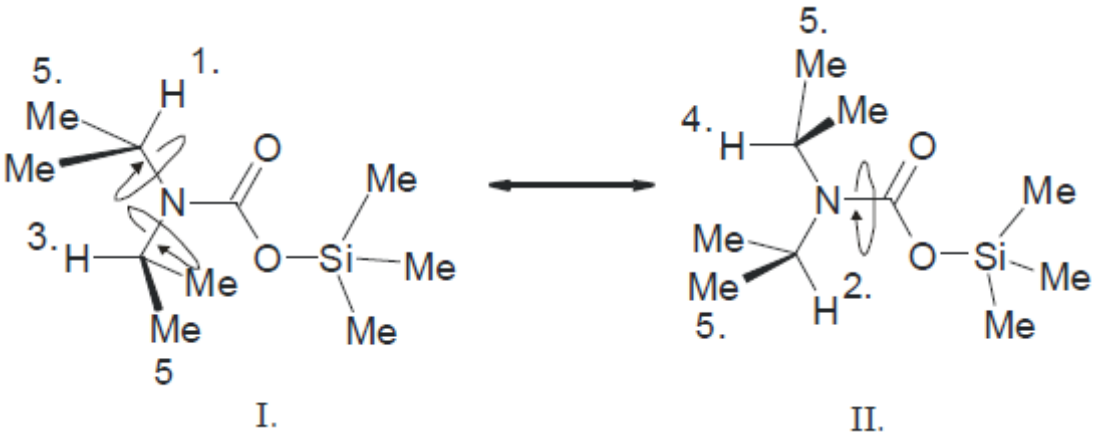


Figure 4.25: Two planar conformers of iPr2SIC

4.4.2 Open the DNMR Module and Set Fit Range Limits

Open spectrum No. 200 of iPr2SIC. Choose the **DNMR Lineshape Analysis** menu item of **Analysis** pull-down menu. Select the **Spectrum** tab and set F1P = 6.0 ppm and F2P = 2.3 ppm as fit range limits.

4.4.3 Define Nuclei

Go to the **SpinSystem** panel and click on the **Nuc1** tab. Add four new nuclei.

Set the following values:

Nuc1 x Nu = 4.80 ppm (0.5 spin)

Nuc2 x Nu = 4.48 ppm (0.5 spin) J1 = 0

Nuc3 x Nu = 2.70 ppm (0.5 spin) J1 = 0 J2 = 0

Nuc4 x Nu = 2.65 ppm (0.5 spin) J1 = 0 J2 = 0 J3 = 0

Nuc5 Nu = 1.05 ppm (3.0 spin) J1 = 7 J2 = 7 J3 = 7 J4 = 7

where x means a marked parameter to the iterative parameter refinement. Do not forget to check the initial step length parameters. Use the **Step Length** and **Return** buttons. 0.06 ppm is a good initial step length for Nu parameter in the proton spectrum.

Finally set LB = 6 Hz, which is the half width of the trimethyl-silyl group at 0.40 ppm. Unmark LB parameter and set Intensity = 30000:

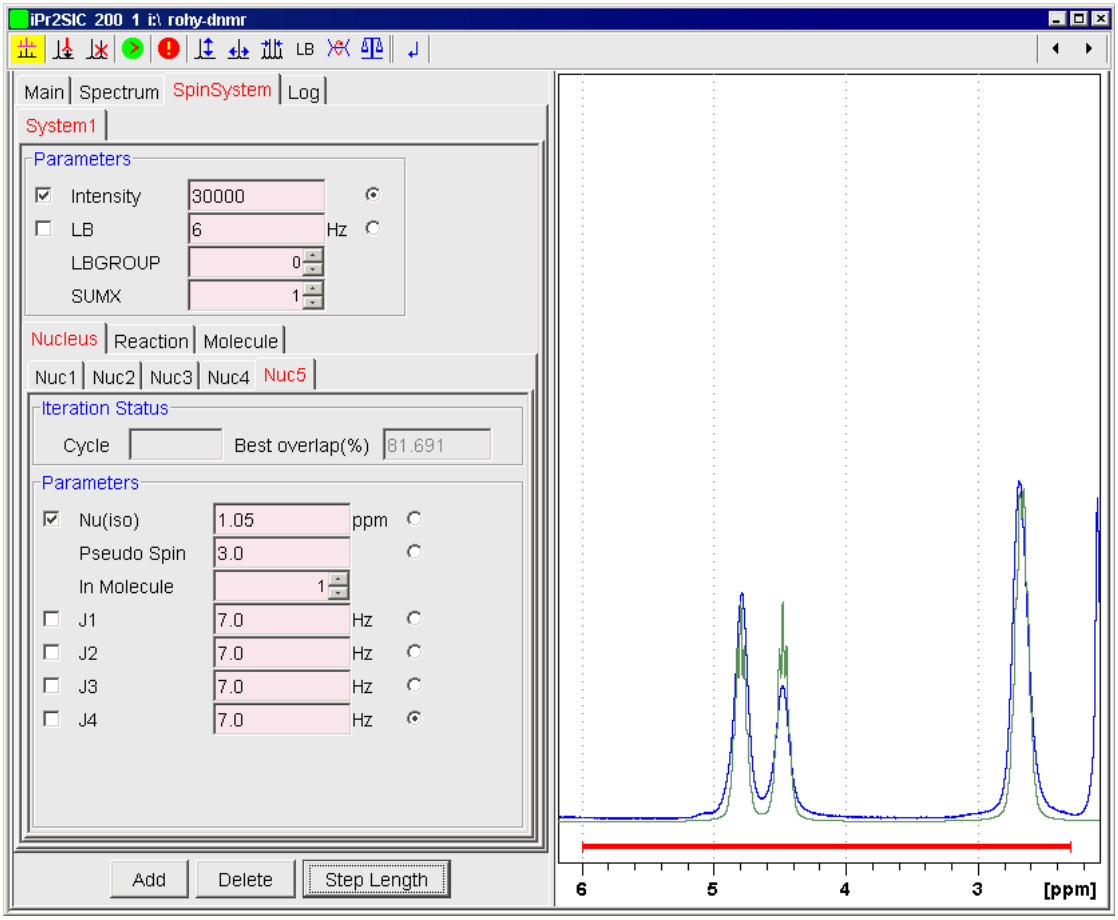


Figure 4.26: Set a five nuclei model.

4.4.4 Define the Chemical Exchange

Click on **Reaction** tab. Set Exchanges = 4 and the exchange partners:

#1 From = 1 To = 4

#2 From = 4 To = 1

#3 From = 2 To = 3

#4 From = 3 To = 2

values in respect to the Nuc1 → Nuc4, Nuc4 → Nuc1, Nuc2 → Nuc3 and Nuc3 → Nuc2 simultaneous exchanges. This parameter set describes the exchanges of the four methine protons. Set k = 10.0 Hz. Septets are broader and do not show splittings. Mark the checkbox of "k" parameter to allow this parameter to be fitted. Click on **Step Length** button and set k = 0.1 Hz as step length. Click on **Return**. The following figure which shows still wrong 1:1 intensity ratio. To correct this we have to define the conformers as molecules and the conformational changes as chemical reaction.

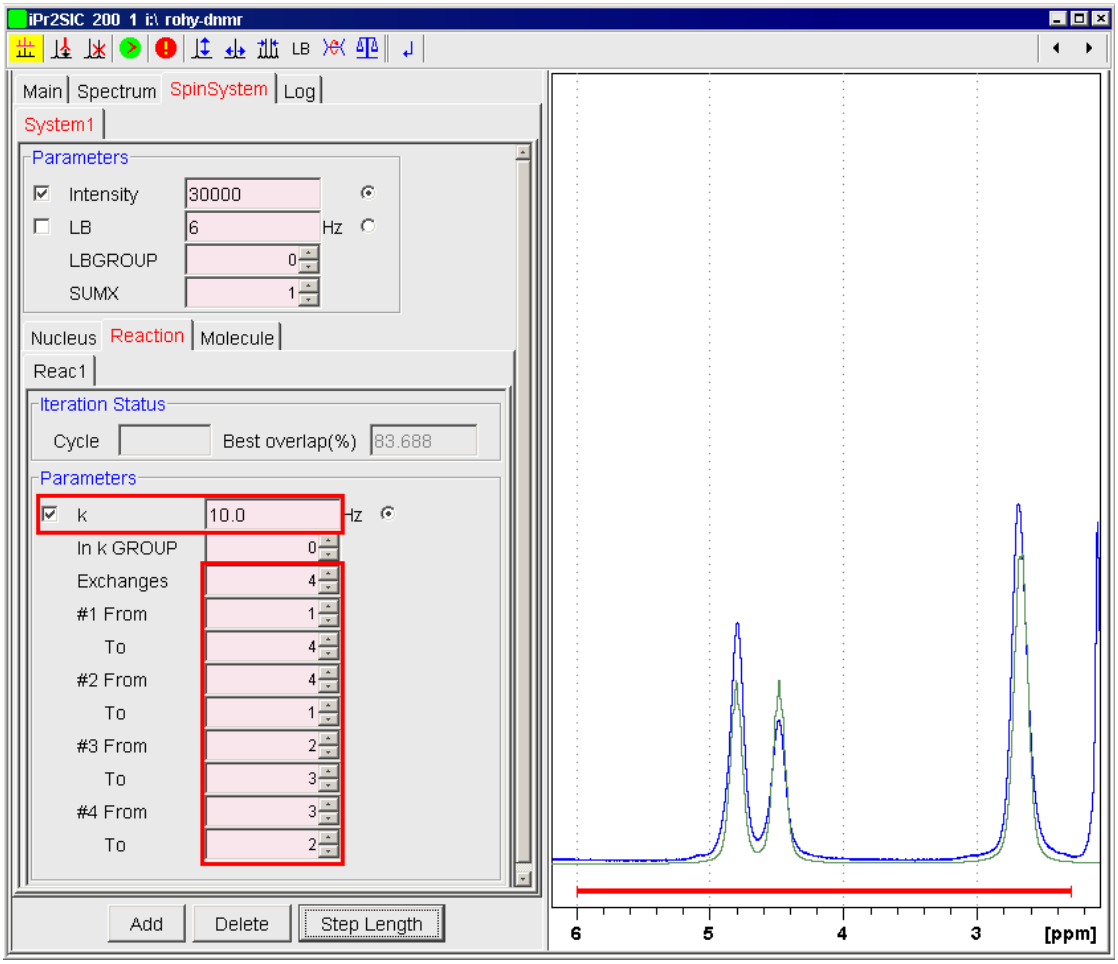


Figure 4.27: Define the four site exchange reaction.

4.4.5 Define Molecules

Nuclei of this spin system belong to two molecules with 0.6 and 0.4 molar fraction ratio. After the exchange process conformer I of iPr2SIC molecule goes to conformer II. The stoichiometric coefficient of the conformer I is equal to –1 (reactant), while the coefficient of the conformer II is +1 (product).

Click on **Molecule** panel and on the **Add** button. **Molecule2** appears.

Click on **Molecule1** and set X = 0.6. Mark X to set it fit parameter. Set initial step length = 0.006. Leave the Stch.coef.= -1.

Click on **Molecule2**. X should be equal to 0.4. The SUMX parameter = 1, so the last molar fraction value is calculated automatically (This is a normalized calculation mode.). Mark X to set it fit parameter and set its initial step length = 0.004. Set the Stch.coef.= +1:

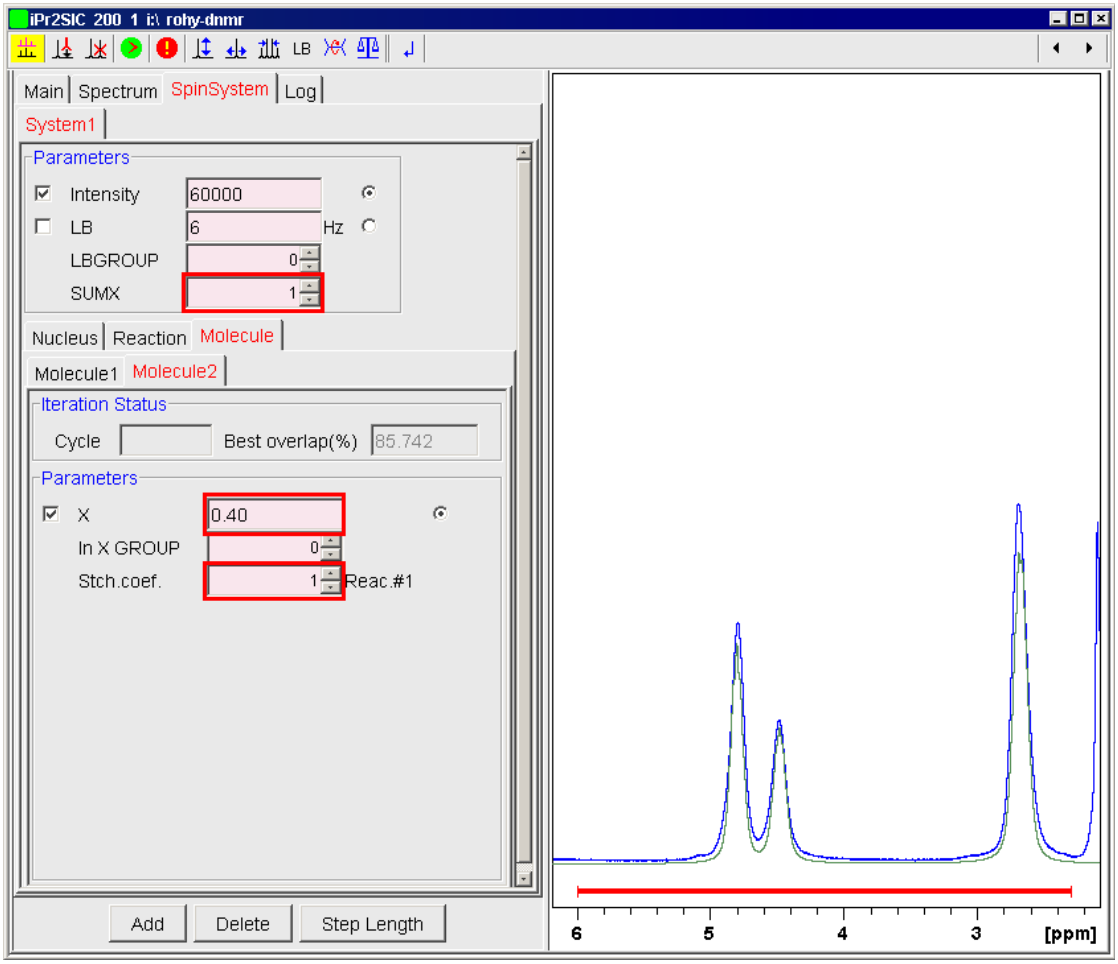


Figure 4.28: Define the molar fractions and stoiciometric coefficients.

Now click on **Nucleus** panel and after that on **Nuc2** tab. Set " *In Molecule*" parameter = 2. Finally click on **Nuc4** tab and set the " *In Molecule*" parameter = 2.

Modify Nu value slightly to see the intensity changes in the spectrum. Set Intensity = 60000. Now the five nuclei model is ready to the parameter refinement:

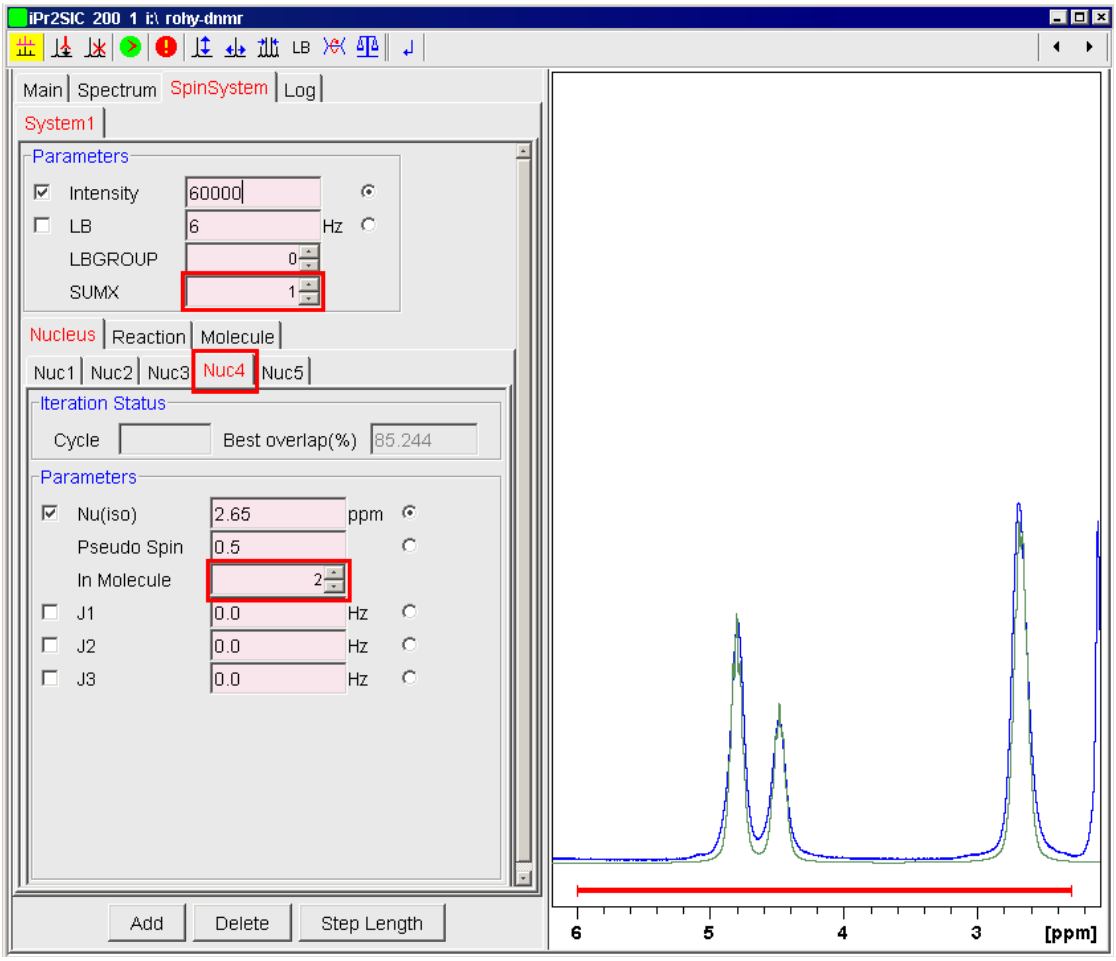


Figure 4.29: Define the four site exchange reaction.

4.4.6 Perform the Parameter Refinement

First save the spin system model on **Main** panel. Start the refinement and check the parameters and the quality of the simulation (Best overlap). If you stop the estimation save the contents of "Log" panel. Save the model with a new name (for example, model-2) and save the simulated spectrum with an unused procno. (see the parameter refinement result in the following figure:

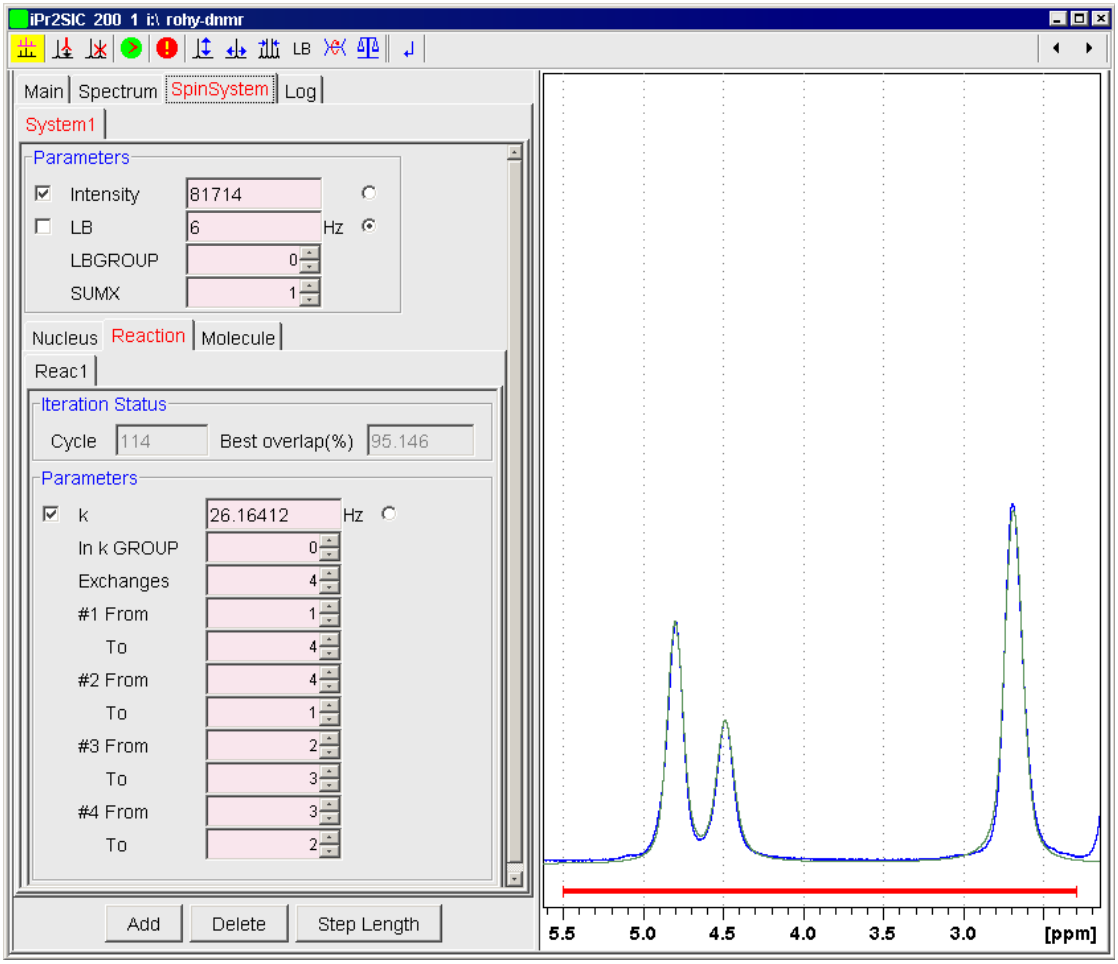


Figure 4.30: Experimental and simulated spectra after parameter refinement.

Finally exit the DNMR module.

4.4.7 Simulate the Spectrum at 220 K

Copy the fitparfix-model-2 and fitparfine-model-2 files into the experiments expno 220 and 250.

Enter the iPr2SIC experiment expno 220. Click on **Main** tab and open the model.

Click on **SpinSystem** tab and on **Reaction** tab. Set k = 300 Hz and LB=1 Hz. Start the iteration and save the result:

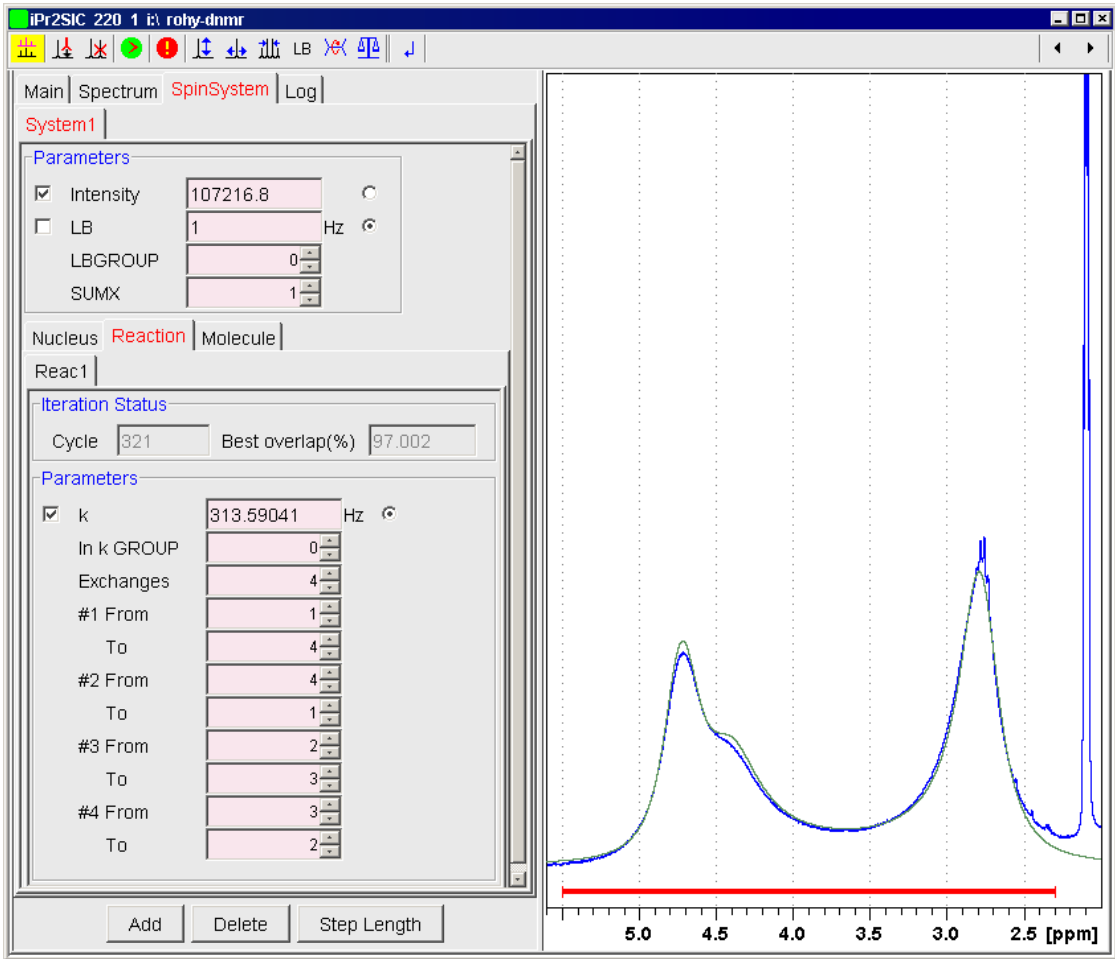


Figure 4.31: Experimental and simulated spectra after parameter refinement with five nuclei model at 220 K

You can get a better result if you add a second spin system with Nuc1= 2.756 ppm (0.5 spin) and Nuc2=0.928 ppm (3.0 spin), J1=6.6 Hz, LB=5 Hz, which describes the diisopropyl amine contamination. You can use the LBGROUP=1 to set common LB for both spin systems:

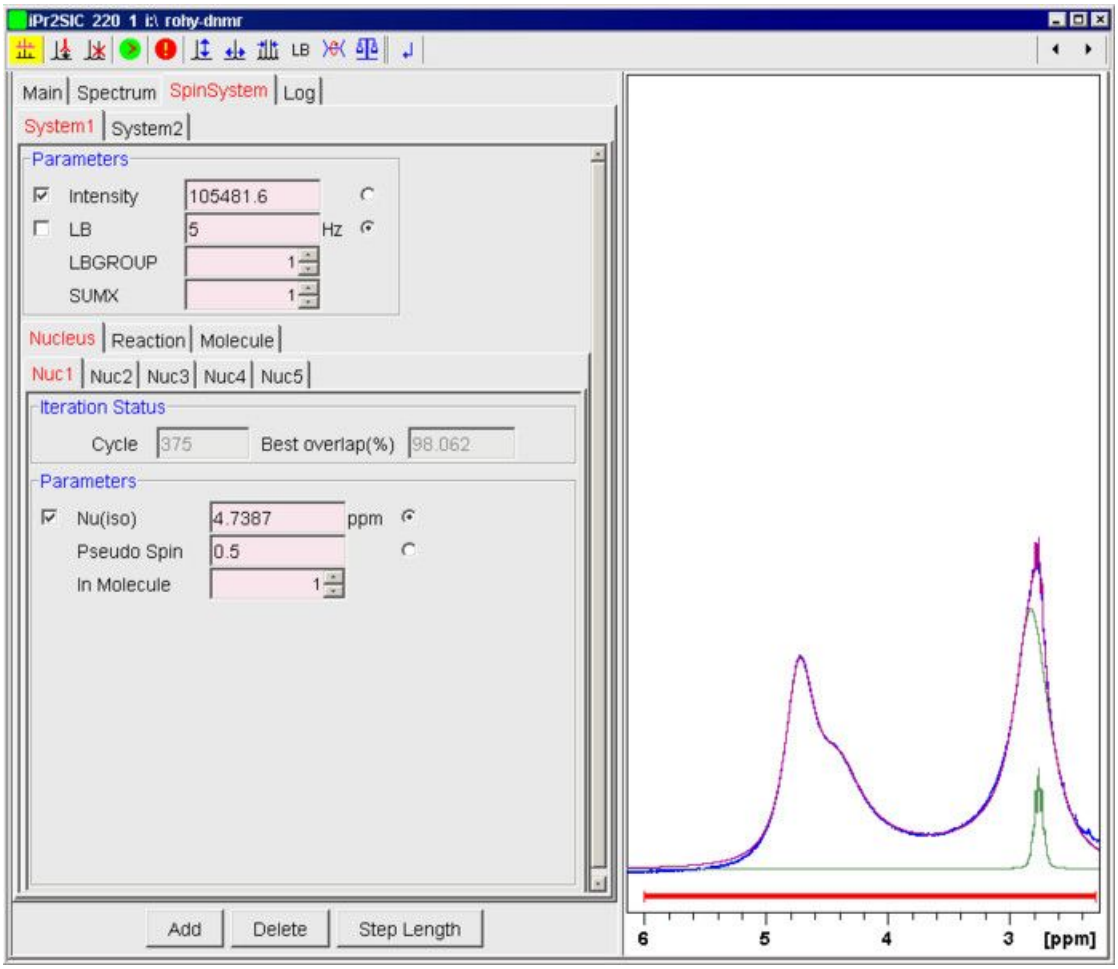


Figure 4.32: Experimental and simulated spectra after parameter refinement with five+two nuclei model at 220 K

Save the model with a new name (for example, model-3).

4.4.8 Simulate the Spectrum at 250 K

Copy the fitparfix-model-3 and fitparfine-model-3 files into the processing directory of expno 250. Enter the iPr2SIC experiment expno 250. Click on **Main** tab and open the model.

Click on **SpinSystem** and **System1** tabs and finally on **Reac1** tab. Set k = 7000 Hz and LB = 2 Hz.

Define a Reac2 with Exchanges = 4 and

#1 From = 1 To = 2

#2 From = 2 To = 1

#3 From = 3 To = 4

#4 From = 4 To = 3

values in respect to the Nuc1 → Nuc2, Nuc2 → Nuc1, Nuc3 → Nuc4 and Nuc4 → Nuc3 simultaneous exchanges. This parameter set describes the exchanges of the four methine protons in the rotation around the amide bond. Set k = 0.01 Hz. Enter **Molecule2** tab and change the Stch.coef. to +1 for both reactions. Set the intensities.

Save the model, start the iteration and save the result:

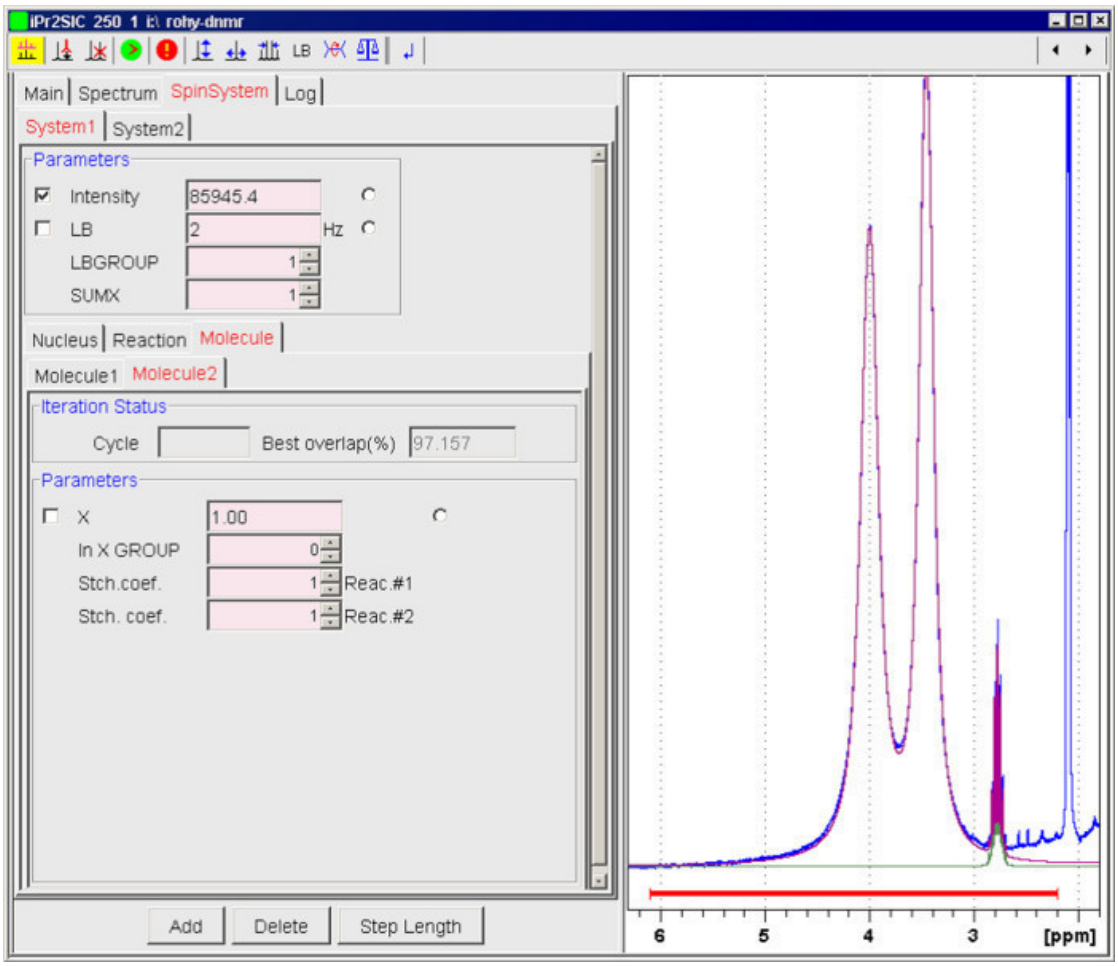


Figure 4.33: Experimental and simulated spectra after parameter refinement with five+two nuclei model and two exchange reactions model at 250 K

4.5 References

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- Rohonczy, J., Knausz, D., Csákvári, B., Sohár, P., Pelczer, I., Párkányi, L.: "Structure of trimethylsilyl-N,N-diisopropyl carbamate in solid phase and in solution" J. Organometallic Chem. 340 (1988) 293-302.

5 Identify C13 Spectra with CSEARCH

For a single C13 spectrum it is possible to search in the CSEARCH database and try to match all possible structures. TopSpin automatically generates a request file with the information about the experimental c13 shifts and optional knowledge like the chemical formula and the mass.

For more information about CSEARCH use the following link:

http://nmrpredict.orc.univie.ac.at/

With the menu item **Analyse -> Structures -> Identify structures with CSEARCH** or with the command **csearch_ident** the following dialog for the CSEARCH request opens.

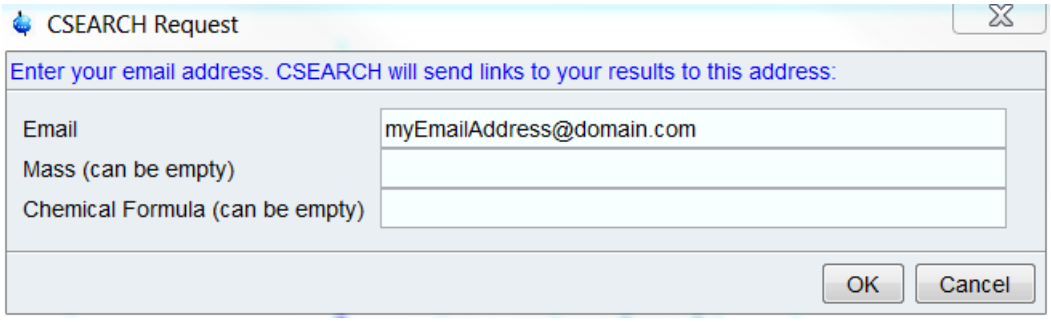

Figure 5.1: CSEARCH Request Dialog

In a next step the default email program starts with an automatically filled email. Following the instruction in the email text, it is necessary to attach the data file located in the given file path. With the paste keyboard shortcut (e.g. Control V) the filename entry can be pasted in the mail client attachment browser, because TopSpin copied the filename into the clipboard. The email subject is always the project name, ending with a number coding the current date and a time.

The result will be send to the given email address after a short time. There could be a handful of emails including links to possible results. The following figure shows an example for the structure matching:

Identify C13 Spectra with CSEARCH

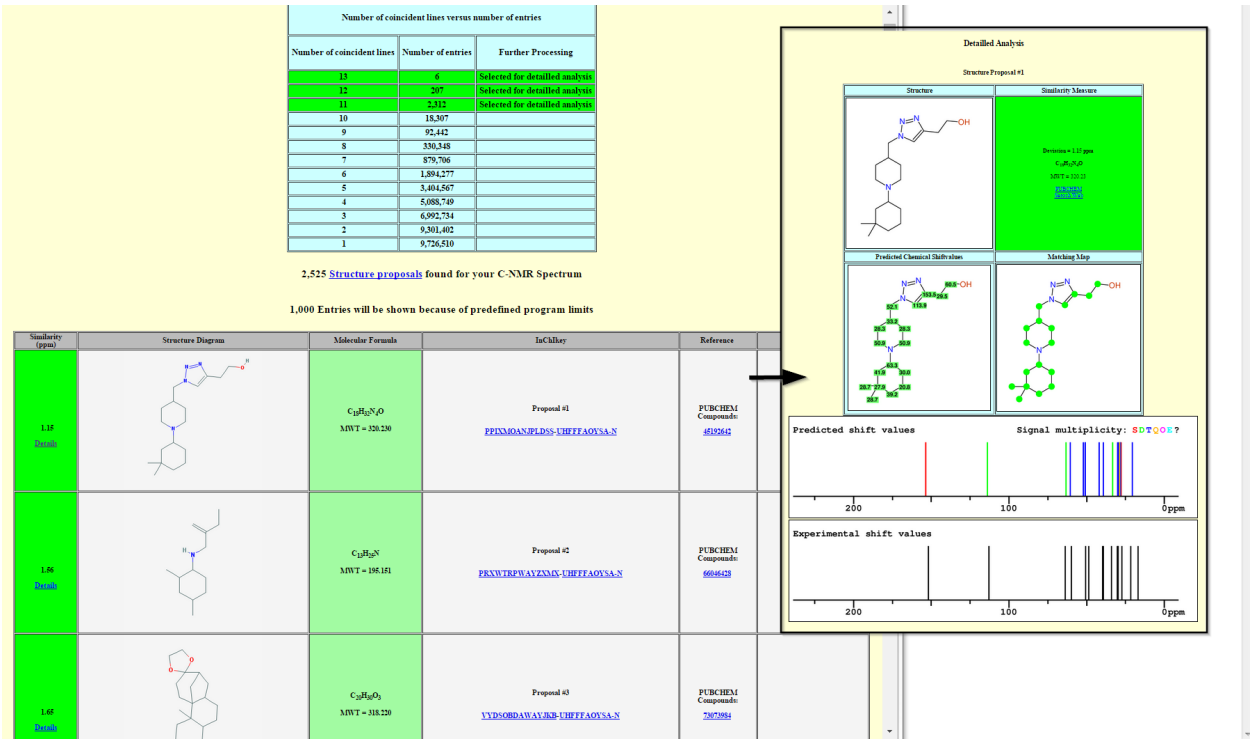

Figure 5.2: CSEARCH result example

6 Contact

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