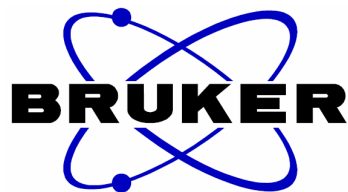# DNMR Lineshape Analysis

Software Manual

Version 1.1

Revision 071103

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CONTENTS

1. Introduction

Dynamic NMR spectroscopy (DNMR) is a well known and easy experimental method to study slow nuclear exchange processes including conformational movements like hindered rotations around bonds, ring-inversions, *cis-trans* isomerizations, pseudo-rotations, etc.[1,2,3,4,5,6,7] All these processes result in NMR signal broadening which effect exhibits strong temperature dependence. By increasing the temperature the sharp signals broaden, overlap until the coalescence point, further on sharpen at the average frequency of the signals. All these signals can be mathematically described using the common NMR spectroscopic parameters like chemical shifts, scalar coupling and relaxation time constants and the hidden chemical exchange rate parameters. The latter rate parameters and the corresponding temperatures are used to determine the activation thermodynamic parameters (activation enthalpy and entropy) using the Arrhenius or preferably the Eyring-Polányi equations[8]. The most useful method to observe the reaction rate parameters is the total line-shape analysis of the NMR spectra. This method is based on the spectrum simulation and parameter refining spectrum fitting steps. Several theoretical models are available to simulate temperature dependent NMR spectra, e.g. modified Bloch-equations for uncoupled systems[9], Monte-Carlo simulation of NMR signals in time-domain or the most efficient one the density matrix theory for coupled spin systems. Spectrum fitting and parameter refining is done usually with iterative methods[10,11]. The most widespread used DNMR simulation programs (DNMR5 [12,13,14,15,16], MEXICO [17,18,19], WinDNMR [20,21] and now Bruker's TOPSPIN DNMR module) are based on these calculation methods.

DNMR (Dynamic NMR) Lineshape Fitting module is a tool program to simulate 1D temperature dependent NMR spectra of coupled half spin nuclei, interactively set up and iteratively refine the model parameters to get the best fit of the measured and simulated 1D NMR spectra. The goal is to obtain the reaction speed parameters of exchange processes at the temperature of the NMR measurement.

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. The Hamiltonian

operator contains the interaction of the spin system with B_1 field, the isotrope chemical shifts of half spin 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 systems. These systems can be completely independent from each other or they can have common physical parameters, like natural half widths, 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.

The program is adopted from the author's program, TEDDY[22], written in FORTRAN language.

2. Theory of the calculation

The DNMR module of TopSpin is based on the *average density matrix theory*[5,7,16]. This formalism is generally used to the description of spin systems and it combines the quantum statistics of micro systems and the thermodynamic - Boltzmann - statistics of macroscopic sample.

The quantum mechanical pure state which can be described by a single wave function, ψ, can be expressed by the linear combination of a suitable complete set of orthogonal and normalized basis functions Φ_n

$$\psi = \sum_n c_n \Phi_n \tag{1}$$

where n is the number of base functions in the compete basis set, and c_n is the linear coefficient. The expectation value of a physical quantity, $\langle Q \rangle$, of a ψ state is defined by its corresponding quantum-mechanical operator $\hat{Q}$

$$\left\langle \hat{Q} \right\rangle = \left\langle \psi \left| \hat{Q} \right| \psi \right\rangle = \sum_{m,k} c_m^* c_k \left\langle \Phi_m \left| \hat{Q} \right| \Phi_k \right\rangle \tag{2}$$

where k and m are the indeces of the base functions. The density matrix is defined so that its elements, ρ_{km}, are

$$p_{km} = c_k c_m^* = \left\langle \Phi_k \left| \hat{P} \right| \Phi_m \right\rangle \tag{3}$$

By inserting the matrix elements into the Eq. (2) we obtain

$$\left\langle \hat{Q} \right\rangle = \sum_{m,k} \left\langle \Phi_k \left| \hat{P} \right| \Phi_m \right\rangle \left\langle \Phi_m \left| \hat{Q} \right| \Phi_k \right\rangle = \sum_k \left\langle \Phi_k \left| \hat{P}\hat{Q} \right| \Phi_k \right\rangle = Trace\ \hat{Q}\hat{P} = Trace\ \hat{P}\hat{Q} \tag{4}$$

The macroscopic system is a mixture of a large number of pure states with different wave functions and density matrices, but it can be described by similar formulae if we introduce the average density matrix with elements

$$\rho_{km} = \overline{c_k c_m^*} = \overline{\left\langle \Phi_k \left| \hat{P} \right| \Phi_m \right\rangle} = \left\langle \Phi_k \left| \hat{\rho} \right| \Phi_m \right\rangle \tag{5}$$

Now the expectation value of Q is

$$\left\langle \hat{Q} \right\rangle = \sum_{m,k} \left\langle \Phi_k \left| \hat{\rho} \right| \Phi_m \right\rangle \left\langle \Phi_m \left| \hat{Q} \right| \Phi_k \right\rangle = Trace\ \hat{Q}\hat{\rho} \tag{6}$$

The time-dependence of the system can be described by the time-dependent Schrödinger equation, where H is the Hamiltonian, i.e. the total energy operator of the system

$$-\frac{\hbar}{i}\frac{d\psi}{dt} = \hat{H}\psi \tag{7}$$

Using the above described connection between the ψ and ρ quantities we can get the equation which describes the time dependence of the average density matrix elements

$$\frac{d\rho_{km}}{dt} = \frac{i}{\hbar}\left\langle \Phi_k \left| \hat{\rho}\hat{H} - \hat{H}\hat{\rho} \right| \Phi_m \right\rangle \tag{8}$$

The time-dependence of the whole density matrix is described with the Liouville - von Neumann equation

$$\frac{d\hat{\rho}}{dt} = \frac{i}{\hbar}\left(\hat{\rho}\hat{H} - \hat{H}\hat{\rho}\right) = \frac{i}{\hbar}\left[\hat{\rho}, \hat{H}\right] \tag{9}$$

The Wangsness-Bloch-Redfield relaxation theory[5,15] extends this equation with phenomenological terms to describe the relaxation and chemical exchange processes as well.

$$\frac{d\hat{\rho}}{dt} = \frac{i}{\hbar}\left[\hat{\rho}, \hat{H}\right] + \frac{\rho_0 - \rho}{T_1} + \frac{\rho}{T_2} + \sum_r \frac{1}{\tau_r}\left(\rho_r^{after} - \rho_r^{before}\right) \tag{10}$$

where T_1 is the spin-lattice relaxation time, T_2 is the spin-spin relaxation time, τ_r is the correlation time constant of the chemical exchange in reaction r. The spin Hamiltonian for the liquid samples of nuclei in frequency units is

$$\hat{H} = -\sum_j \nu_j \hat{I}_{zj} + \sum_{j<k} J_{jk}\hat{I}_j \cdot \hat{I}_k + \sum_j \frac{\gamma B_1}{2\pi}\hat{I}_{xj}\cos 2\pi\nu t \tag{11}$$

where j is the number of nuclei, $\hat{\boldsymbol{I}}$, $\hat{I}_z$ and $\hat{I}_x$ are the spin operators, J is the scalar coupling, γ is the giromagnetic constant, $\boldsymbol{B}_1$ is the strength of the radiofrequency field and v is its frequency. This expression is easier to handle in the rotating frame with frequency v and rotating axis direction z-axis of the laboratory system

$$\hat{H} = -\sum_j \left(v_j - v\right)\hat{I}_{zj} + \sum_{j<k} J_{jk}\hat{I}_j \cdot \hat{I}_k + \sum_j \frac{\gamma B_1}{2\pi}\hat{I}_{xj} \tag{12}$$

The detectable total transverse magnetization is proportional to the expectation value of the operator M_+, which is

$$\hat{M}_+ = \sum_j \left(\hat{I}_{xj} + i\hat{I}_{yj}\right) = \sum_j \hat{I}_{+j} \tag{13}$$

The absorption signal is

$$Y(v) = C \cdot \mathrm{Im}\left\langle \hat{M}_+ \right\rangle = C \cdot \mathrm{Im}\left\langle Trace\ \hat{\rho}\hat{M}_+ \right\rangle \tag{14}$$

where C is a scalar amplitude factor.

To get a simulated spectrum Y the first thing to do is to solve Eq. (10) followed by the substitution of density matrix into Eq. (14). The main drawback of this calculations method is the huge size of the density matrix, however it can be reduced. M_+ transverse magnetization operator collects the single quantum transitions and it's matrix has a block structure with 0 or 1 elements. $M_{+ij} = 1$ only if $(I_{zi}{}^{total} - I_{zj}{}^{total}) = 1$. This matrix represents the spectroscopic selection rules. The Hamiltonian commutes with $I_z{}^{total}$ operator and it has similar block structure. Neither the $[H,\rho]$ commutator nor the relaxation and exchange operators mix density matrix elements of different blocks. This means that the linear equation system can be split into several smaller equation systems. The blocks of density matrix elements can be arranged into vectors and the commutator, the relaxation and exchange operators form linear super-operators L, R and X respectively. This changes the formalism from Hilbert space to Liouville space[6,12] and Eq. 10 is written as

$$\frac{d\hat{\hat{\rho}}}{dt} = -i\hat{\hat{L}}\hat{\rho} + \hat{\hat{R}}(\hat{\rho} - \hat{\rho}_0) + \hat{\hat{X}}\hat{\rho} \tag{15}$$

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In order to simplify the formalism we will omit the double hat notation over the superoperators from now. The system can be handled as steady state[23] during the detection, so the left side of the differential equation is equal to zero and the differential equation system is simplified to linear equation system which is relative easy to solve with well known matrix diagonalizations methods

$$0 = \frac{d\rho}{dt} = -(L + R + X)\rho + c \qquad (16)$$

where c vector describes interaction with the B_1 field and the spin system, L, R and X coefficients contain the chemical shift, scalar coupling, relaxation time and chemical exchange speed parameters. With the $A = L+R+X$ notation Eq. (16) is more compact

$$c = A\rho \qquad (17)$$

This equation can be solved by standard complex matrix diagonalization methods.

Since both L and the derived A depends on frequency v, ρ also exhibits frequency dependence. Thus it requires independent matrix diagonalization steps for all spectrum points v. This time consuming calculation can be simplified considering the special frequency dependence of A[24].

$$A = B - i2\pi v I \qquad (18)$$

where I is the unity matrix, and B matrix collects the frequency-independent terms. It is enough to calculate B matrix once for one spectrum. With a diagonalization algorithm[25] it is possible to get the eigenvalues D, and eigenvectors W of the B matrix.

$$D = W^{-1}BW \qquad (19)$$

The solution of the following equation results in the frequency dependent density matrix vectors

$$\rho(v) = -W(D - i2\pi v I)^{-1}W^{-1}c \qquad (20)$$

By the Liouville space equivalent F of M_+ it is convenient to calculate the spectrum points directly in the Liouville space as well.

$$Y(v) = -\text{Im}\left(F^{\dagger}W(D - i2\pi vI)^{-1}W^{-1}c\right)$$ (21)

3. Special features of the DNMR module

3.1. Spin group factorization - pseudo spins[26]

The size of density matrix blocks can be easily reduced with the introduction of the spin group factorization. In the case of several half spin nuclei with same chemical shifts it is possible to replace them with a pseudo nucleus with a spin bigger than half. For example CH_2 protons can be replaced with a pseudo-nucleus with spin I=1 and spin states α ($I_z^{total} = 1$), β ($I_z^{total} = 0$) and γ ($I_z^{total} = -1$). The quantum statistical weight of β state is twice of α or γ states. Similarly CH_3 group can be described by a nucleus with I=3/2. This factorization is based on the first order approximation of the scalar couplings and is useful for weakly coupled systems. In the case of strong coupling all spins should be described independently.

3.2. Chemical exchange operators

The last term of the extended Liouville - von Neumann equation (10) describes the chemical exchange as it is introduced by Kubo, Anderson and Sack[27,28,29].

$$X = \sum_r \frac{1}{\tau_{Br}}\left(\rho^{after} - \rho^{before}\right)$$ (22)

where τ_{Br} is the average life-time of the B species in reaction r.

The chemical exchange can be described as a chemical reaction

$$\sum_B v_B B = 0$$ (23)

where v_B is the stochiometric coefficient of compound B.

The definition of the reaction speed, v, *is*

$$v = \frac{1}{v_B}\frac{d[B]}{dt}$$ (24)

where [B] is the concentration of the species B. Focusing on the reaction in the forward direction the speed v of this reaction is given by

$$v = k \prod_B [B]^{\nu_B} \tag{25}$$

where k is the reaction rate constant which depends on the temperature.

From Eq. (24) and (25) we get

$$\frac{1}{\nu_B} \frac{d[B]}{dt} = k \prod_B [B]^{\nu_B} \tag{26}$$

The reciprocal of the life-time of species B is

$$\frac{1}{\tau_B} = \frac{d[B]}{dt} = \nu_B \cdot k \prod_B [B]^{\nu_B} \tag{27}$$

Another important question is which density matrix elements are exchanged after an exchange step. Using the *forward in time* model we can describe

$$\rho_{kl}^{after} = \hat{R} \rho_{kl}^{before} \hat{R}^\dagger \tag{28}$$

The R exchange matrix transforms all basis functions. Let as note the complete basis as P^{before} before the exchange.

$$P^{before} = \begin{matrix} \Phi_1 \\ \Phi_2 \\ \vdots \\ \Phi_n \end{matrix} = \begin{matrix} \phi_{11} \cdot \phi_{12} \cdot \phi_{13} \cdots \\ \phi_{21} \cdot \phi_{22} \cdot \phi_{23} \cdots \\ \vdots \\ \phi_{n1} \cdot \phi_{n2} \cdot \phi_{n3} \cdots \end{matrix} \tag{29}$$

The result of the chemical exchange (P^{after}), which is exchange of the atomic base functions in the product base, can be constructed without the knowledge of the elements of R matrix just with the permutation of the atomic indexes.

$$P^{after} = \begin{matrix} \Phi_1 \\ \Phi_2 \\ \vdots \\ \Phi_n \end{matrix} = \begin{matrix} \phi_{13} \cdot \phi_{12} \cdot \phi_{11} \cdots \\ \phi_{23} \cdot \phi_{22} \cdot \phi_{21} \cdots \\ \vdots \\ \phi_{n3} \cdot \phi_{n2} \cdot \phi_{n1} \cdots \end{matrix} \tag{30}$$

Using the definition of the operator matrix element we can construct any exchange R matrix.

$$R_{kl} = \left\langle \Phi_l^{before} \middle| \Phi_k^{after} \right\rangle \tag{31}$$

3.3. Fitting quality

Generally used criterion when fitting of two curves is the minimalization of the least square difference function[14]. This requirement gives the best fit result if the difference of the experimental and the calculated spectra is based on random noise. If the mathematical model is wrong (not the physical parameters are found) the calculated spectrum contains systematic error and the least square difference parameter overestimates the big differences.

Therefore an other fit criterion is introduced for DNMR module

$$FIT = 100 \cdot \left(1 - \frac{\sum_{v} \left| Y_{calc}(v) - Y_{exp}(v) \right|}{2 \sum_{v} Y_{exp}(v)} \right) \tag{33}$$

This *FIT* value = 100 if $|Y_{calc} - Y_{exp}| = 0$ at any frequency point and FIT = 0 if there is no overlap between them and the area of Y_{calc} is normalized to Y_{exp} area. A *FIT* >90 value usually gives good calculated spectrum.

The parameter set of DNMR module contains a lower and an upper spectrum limit to restrict the *FIT* calculation. The adequate spectrum components will be used for spectrum fitting only this way.

3.4. Super factorization

In order to reduce the memory requirement it is a general and efficient strategy to split the spin systems as many smaller and independent systems as it is just possible. On the other side there are several spectral and thermodynamic parameters which are used in independent spin systems but their numerical values must be the same. For example the chemical exchange speed parameter is same for the mutually exchanging two aromatic *ortho*-carbons and for the *meta*-carbons of the same ring. For this purpose super parameters are introduced. It is enough to give their values once and

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this value will be used in other spin systems as well. This method is very efficient during the iterative parameter refinement and results in realistic fitted parameters, i.e. the aromatic ring rotation is characterized with only one rotation speed value.

4. Program capabilities

Independent spin systems up to 5

    All have independent spectral intensity and line broadening parameters

Limits valid for each spin system, independently

   - Nuclei up to 7

      All have chemical shift, pseudo spin and molecule ID parameters

   - J coupling constants up to 6

   - Molecules up to 6

      All have molar fraction and stochiometric coefficients parameters

   - Exchange reactions up to 6

      All have reaction rate and nuclear exchange steps parameters

      All steps are defined by *from* and *to* nuclear site values

Super parameter groups

   - Line broadening up to 6

   - Reaction rate up to 6

   - Molar fraction up to 6

Spin system model

  Density matrix theory in Liouville space and

   - strong coupling approximation for half spins

   - weak coupling approximation for pesudo spin nuclei

Exchange types

   - Intramolecular and intermolecular

   - Mutual and non-mutual

Iterative methods

   - Simplex

   - Genetic algorythm - under development

5. Examples

The use of this DNMR module is demonstrated on two samples. The first sample is dimethyl acetamide (Me$_2$NCOMe) in DMSO-d$_6$, 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 (iPr$_2$SIC) in toluene-d$_8$. 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 iPr$_2$SIC 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 are hindered and two non-equally populated rotamers appear. Altogether four inequivalent methine positions result in three methine septets with different intensities.

5.1 Me$_2$NCOMe

5.1.1 Introduction

The Me$_2$NCOMe data set contains temperature dependent ^{1}H 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 (see Figure 1.). 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.
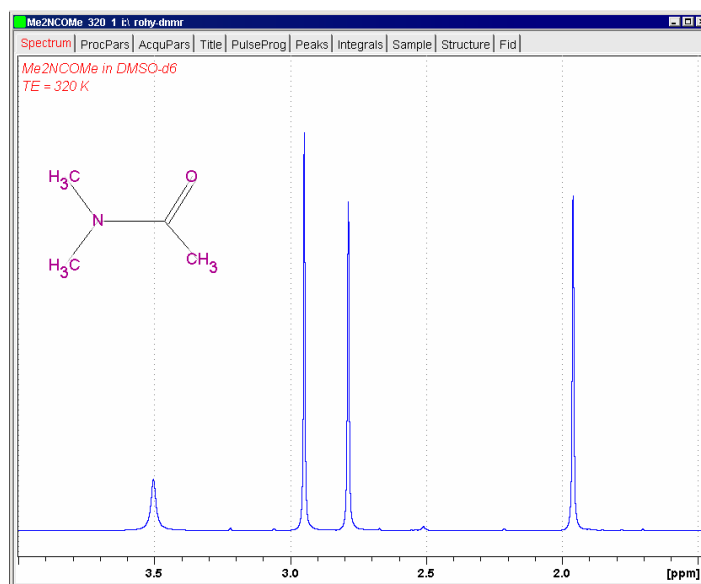
Figure 1. Dimethyl acetamide ^{1}H NMR spectrum at 320 K

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 (see Figure 2.).
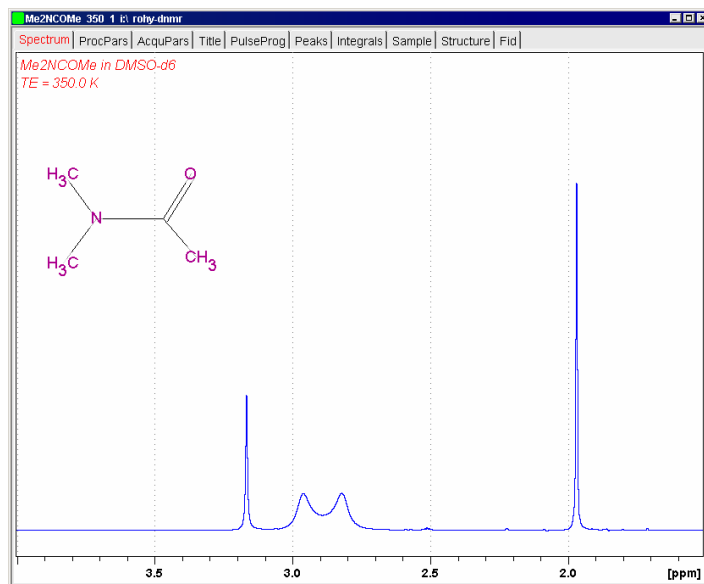
Figure 2. Dimethyl acetamide ^{1}H NMR spectrum at 350 K

Spectrum No. 370 shows only one broad N-Methyl signal. This indicates that the rotation speed is higher than in spectrum No. 350 (see Figure 3.).
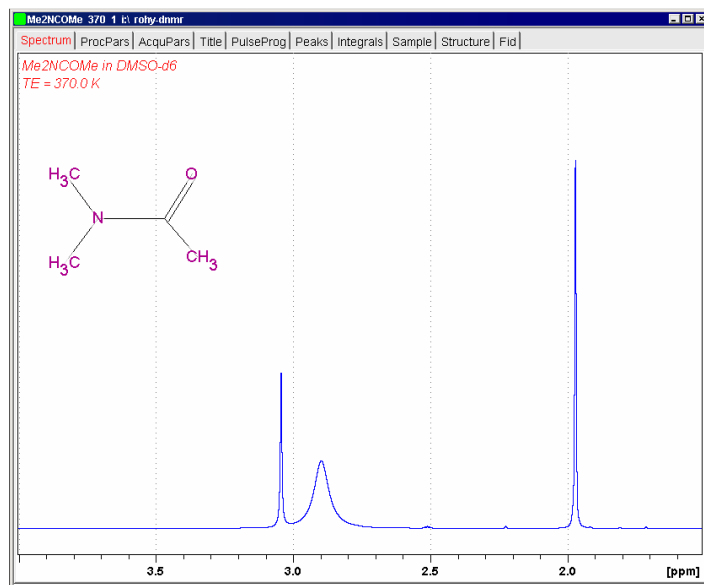
Figure 3. Dimethyl acetamide ^{1}H NMR spectrum at 370 K

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 (See Figure 4.).
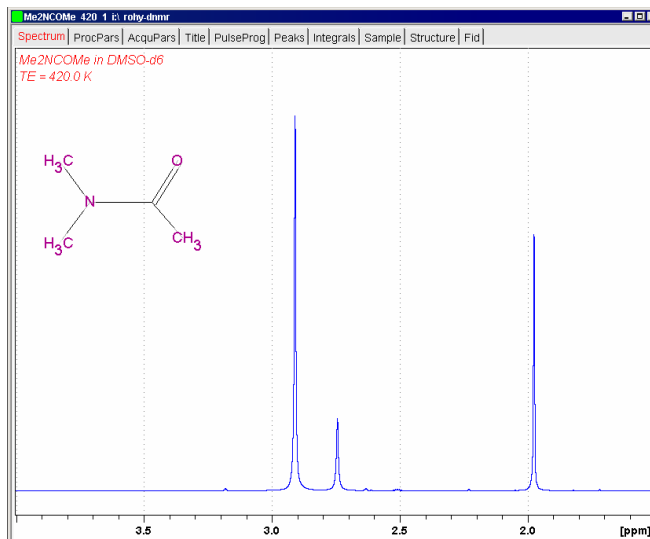
Figure 4. Dimethyl acetamide ^{1}H NMR spectrum at 420 K

5.1.2 DNMR Lineshape Analysis

Open spectrum No. 350 of Me2NCOMe. Choose "DNMR Lineshape Analysis" menu item of "Analysis" pull-down menu (See Figure 5.).
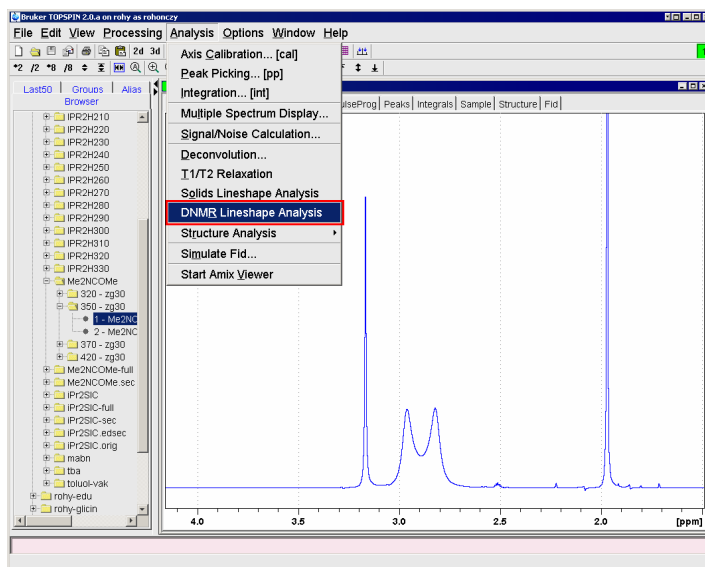
Figure 5. Open the DNMR Lineshape Analysis module

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. (See Figure 6.)
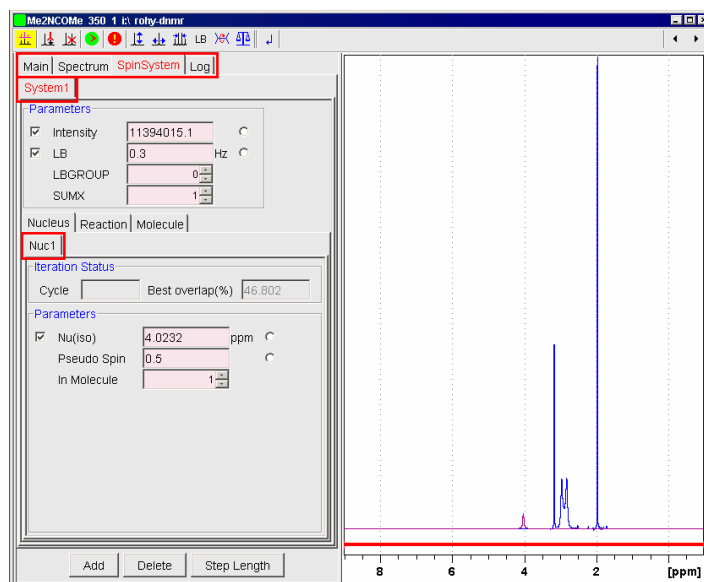
Figure 6. The DNMR Lineshape Analysis window

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".

5.1.3 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. A red horizontal bar on the spectrum display shows this restricted spectrum range. Only this range will be used in the lineshape fitting calculation. (See Figure 7.)
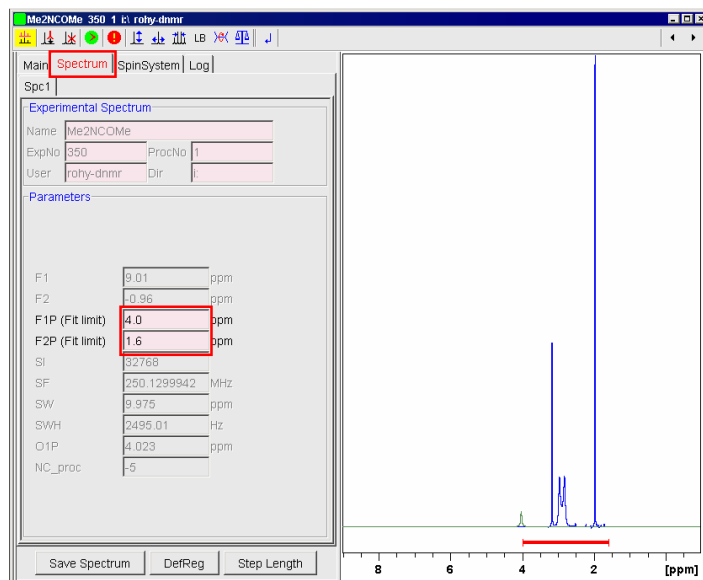
Figure 7. The Spectrum panel is used to set the fit range.

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 LB 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 (See Figure 8.).
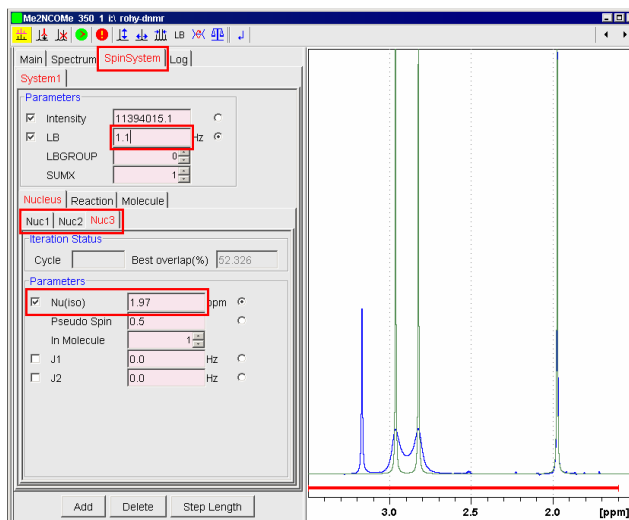
Figure 8. Define the Nu and LB parameters of the three methyl groups.

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. (See Figure 9.)
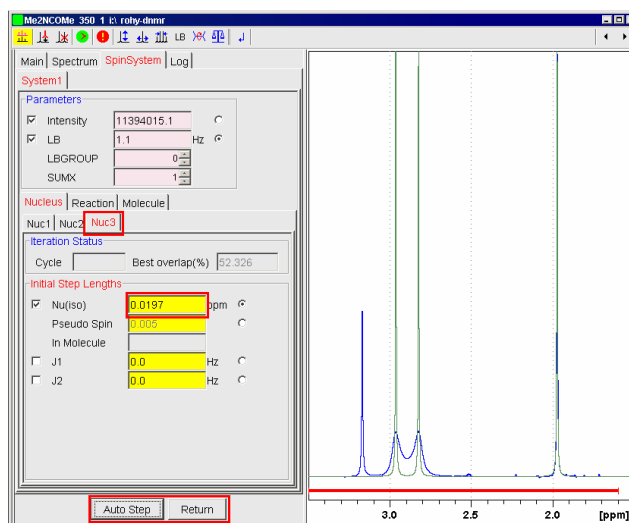

Figure 9. Define the step length of the Nu parameter.

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.

5.1.4 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 ($1 \rightarrow 2$ and $2 \rightarrow 1$). 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 ![icon] toolbar icon. Do not forget to mark the parameter and set its difference value as well. (See Figure 10.).
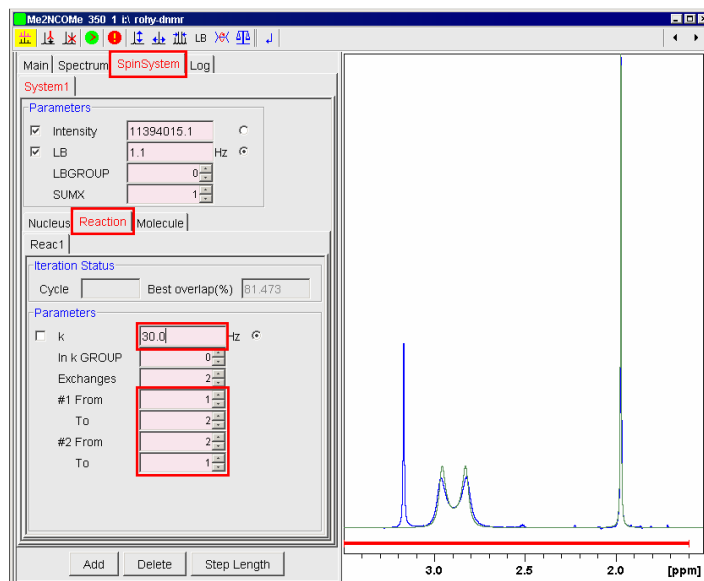
Figure 10. Define the exchanges and the speed parameter.

5.1.5 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 (See Figure 11.).
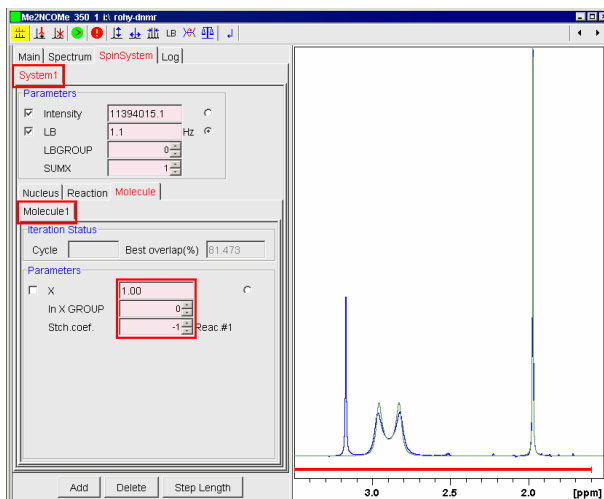
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Figure 11. The Molecule panel contains the molar fraction
and stoichiometric coefficients of a molecule.

5.1.6 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] 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 (See Figure 12.).
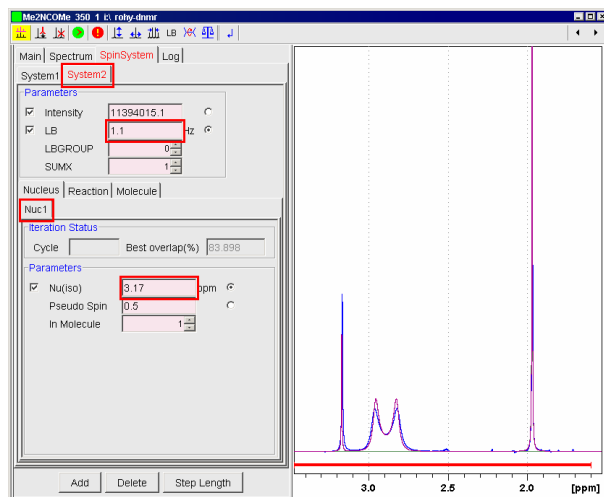

Figure 12. Define the second spin system to simulate the water signal.

5.1.7 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 (See Figure 13.).
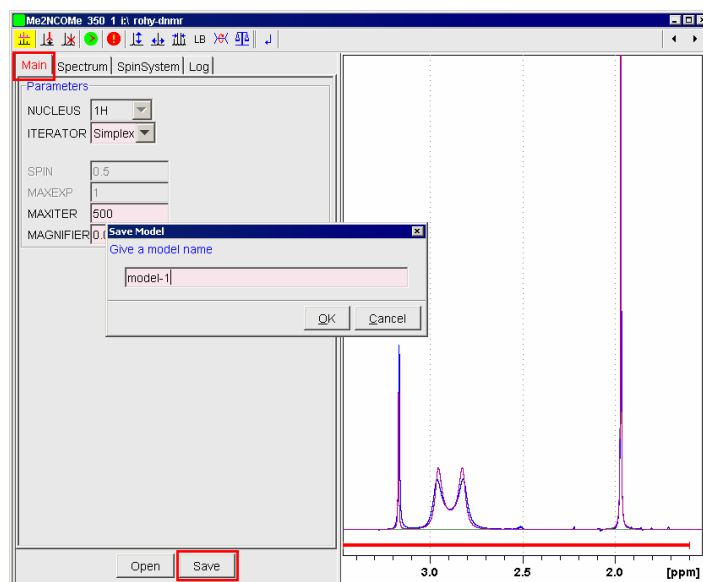

Figure 13. Save the model.

Now the model is ready to refine its variable parameters.

5.1.8 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 (See Figure 14.).

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.
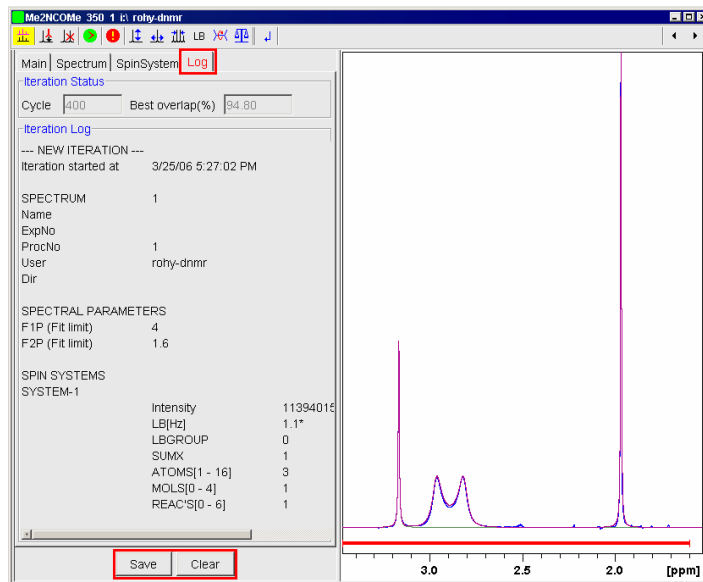
Figure 14. The Log panel with the "Save" and "Clear" buttons.

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 (See Figure 15.).
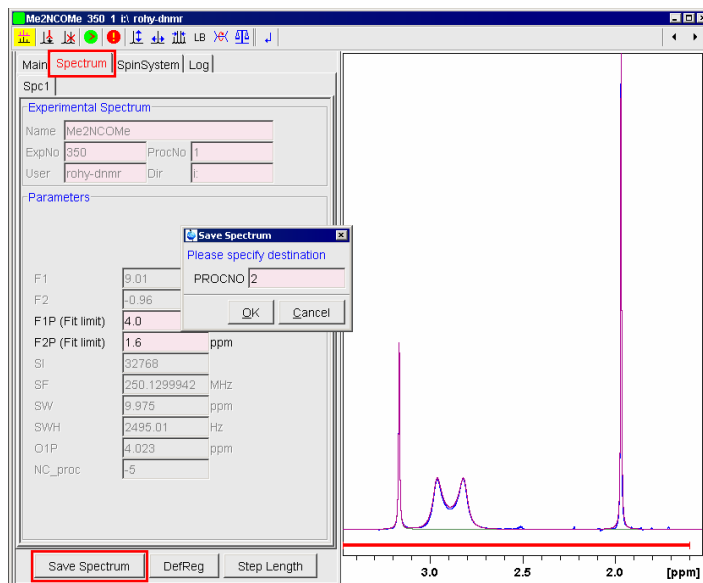

Figure 15. Save the simulated spectrum with an unused procno.

5.1.9 Exit the DNMR Module

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

5.1.10 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" (See Figure 15.). After that you can modify the fit parameters (at least the reaction speed parameter) and start the parameter refinement at this temperature.
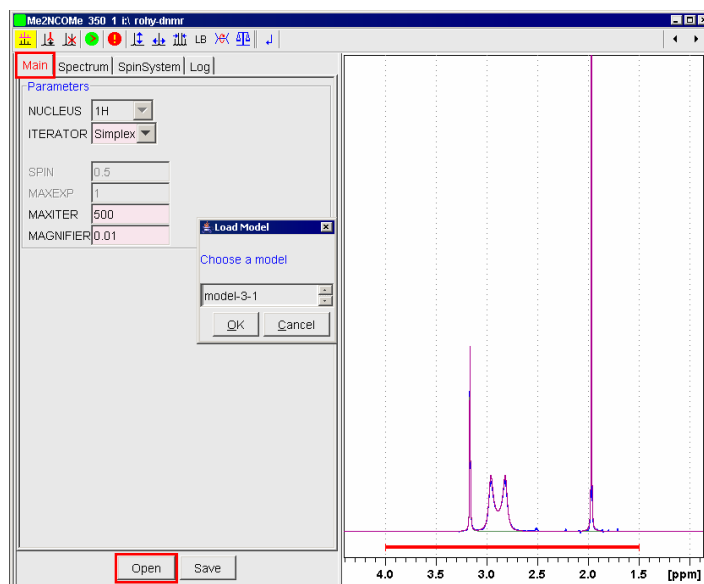

Figure 15. Open a model.

5.2 iPr$_2$SIC – Example for Mutually Exchanging Spin System

5.2.1 Introduction

The iPr$_2$SIC compound is diisopropyl carbamic acid trimethyl silyl ester[30] (formula: (iPr)$_2$NC(O)OSiMe$_3$) which was solved in toluene-d$_8$ and its ^{1}H NMR spectrum was measured. Methine septets of iPr-groups show very strong and complex temperature dependence.

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 unequivalent 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 (See Figure 16.).
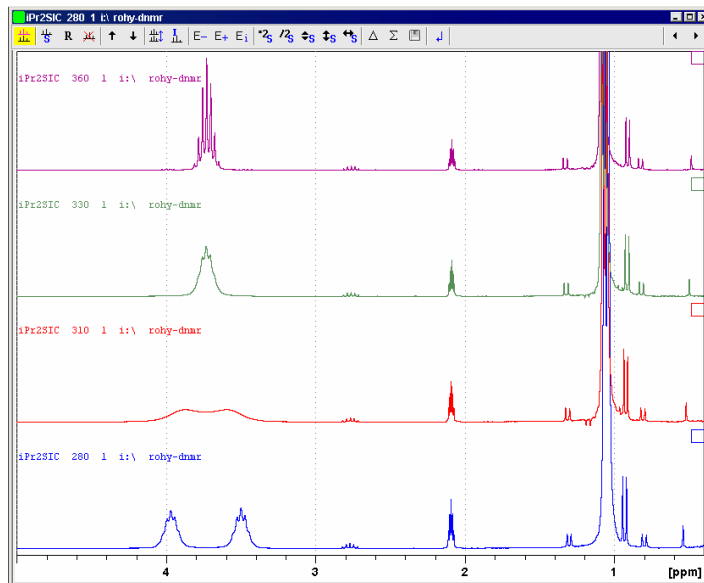

Figure 16. Temperature dependent ^{1}H NMR spectra of iPr2SIC
in toluene-d$_8$ in the temperature range of 280 - 360 K.

5.2.2 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 (See Figure 17.).
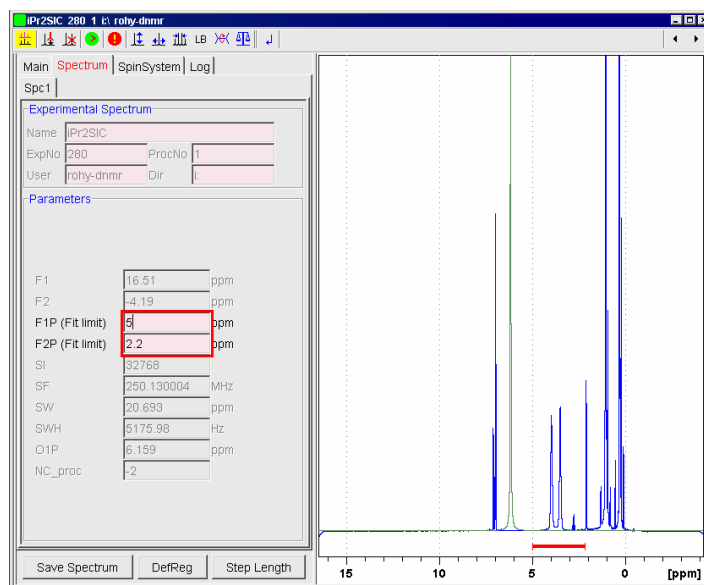

Figure 17. Set the fit limits.

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 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 18.
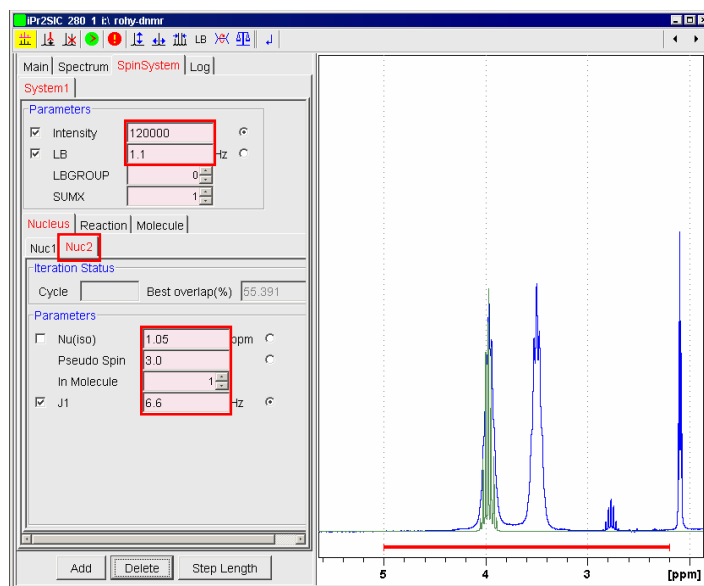

Figure 18. The methine spectrum region of the experimental iPr2SIC
spectrum with one fitting curve. (Green curve with septet multiplicity.)

5.2.3 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)$_2$NH 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 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. (See Figure 19.)
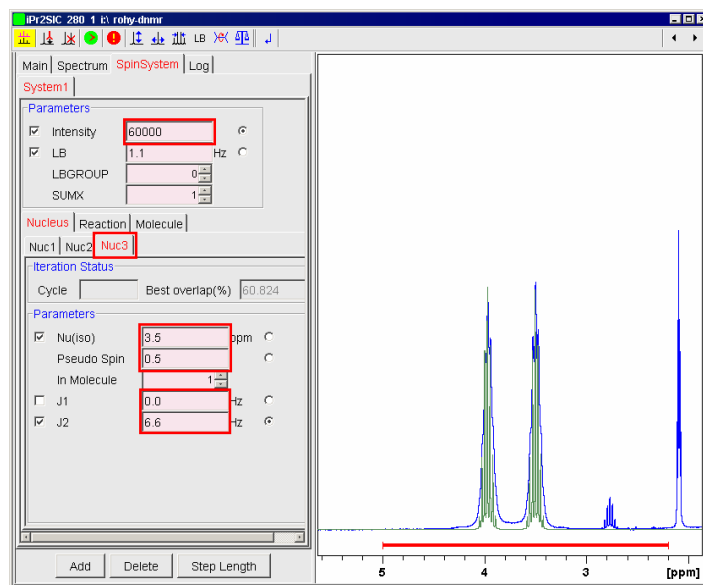

Figure 19. The methine spectrum region of the experimental
iPr2SIC spectrum with two fitting septets.

5.2.4 Define the Chemical Exchange

Click on "Reaction" tab. Set the "#1 To" = 3 and "#2 From" = 3 values in respect to the Nuc1 $\rightarrow$ Nuc3 and Nuc3 $\rightarrow$ 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 %.
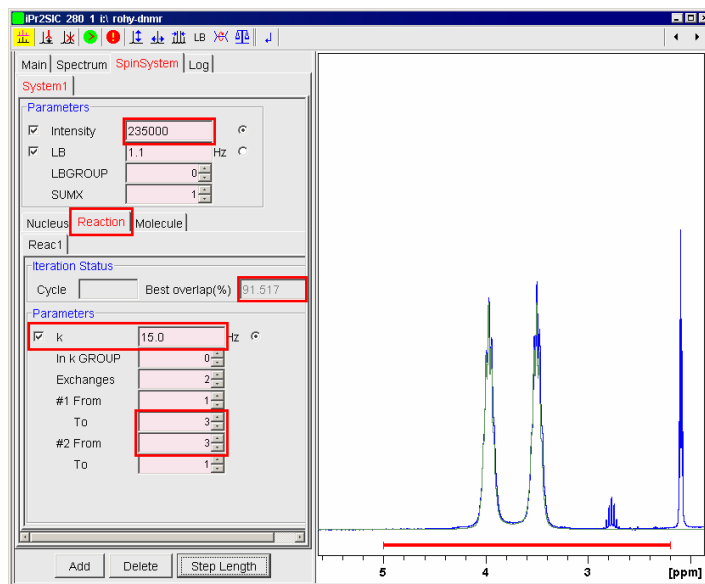
Figure 20. Define the 1->3 and 3->1 exchange with k = 15 Hz speed.

5.2.5 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.

5.2.6 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.

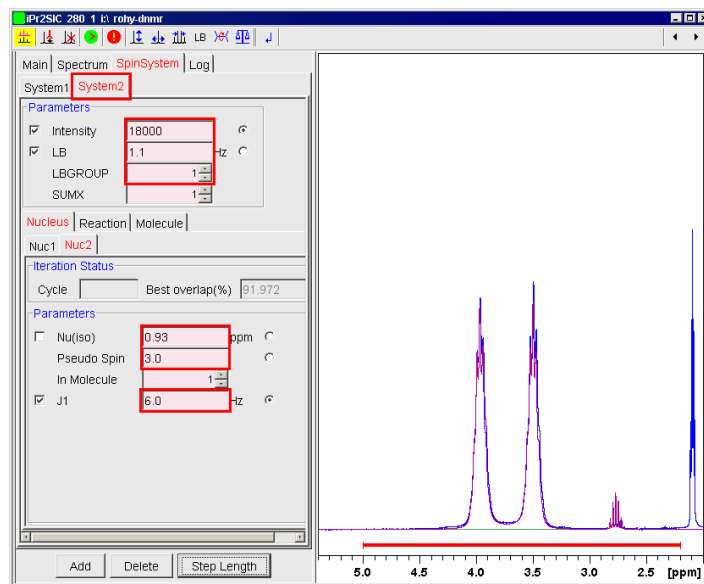Set LBGROUP = 1 of "System2" (See Figure 21.).

Figure 21. Define second spin system and set its LB to LBGROUP=1.

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

5.2.7 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.

5.2.8 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".

5.2.9 Start Parameter Refinement

Click on ![icon] "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 ![stop icon] 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 (See Figure 22.).
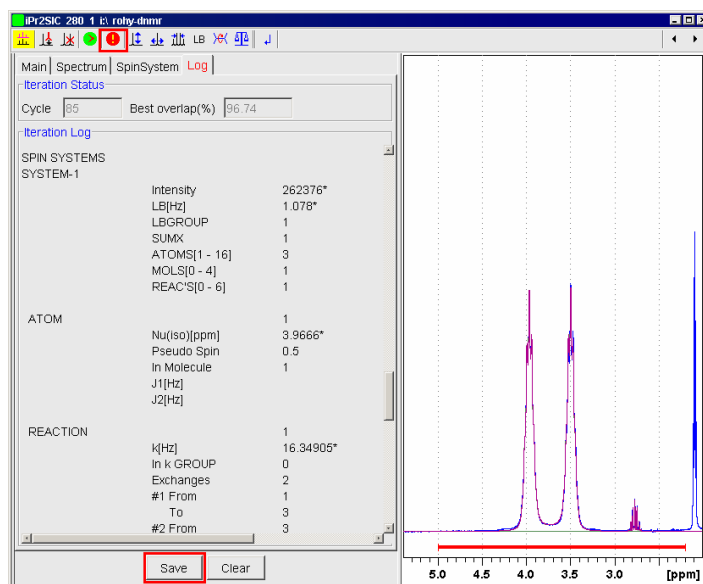

Figure 22. Stop the iteration and save the iteration log.

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

5.2.10 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.

5.3 iPr$_2$SIC – Example for Non-mutually Exchanging System

5.3.1 Introduction

The ^{1}H NMR spectra of the iPr$_2$SIC 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 Chapter 2.1) 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. (See Figure 23.) (At 200 K the solubility of iPr2SIC is low in toluene-d8 and the poor field homogeneity results in broad lineshapes.)
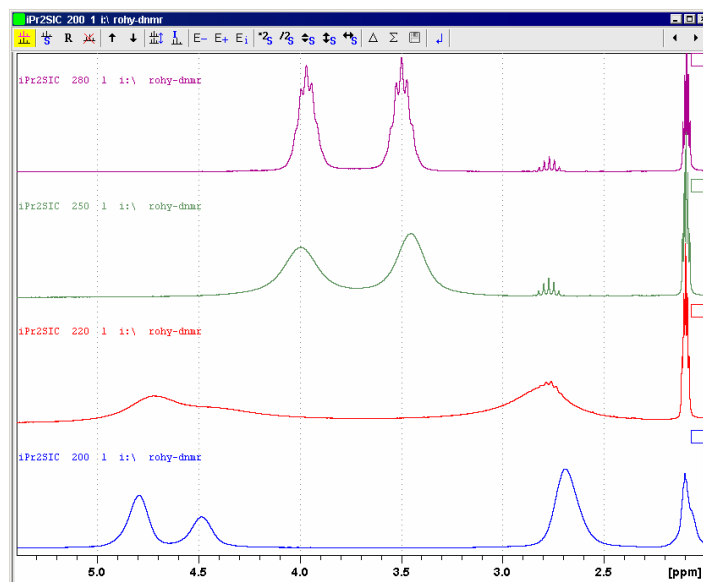

Figure 23. Methine signals of iPr2SIC in toluene-d8
in the temperature range of 200 K – 280 K.

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 24.). 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

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not distinguish them. We simply use an atomic position 5 with pseudo spin 6/2 to describe the expected septet multiplicities. See Figure 24. with the conformers I and II, and the atomic positions 1-5.
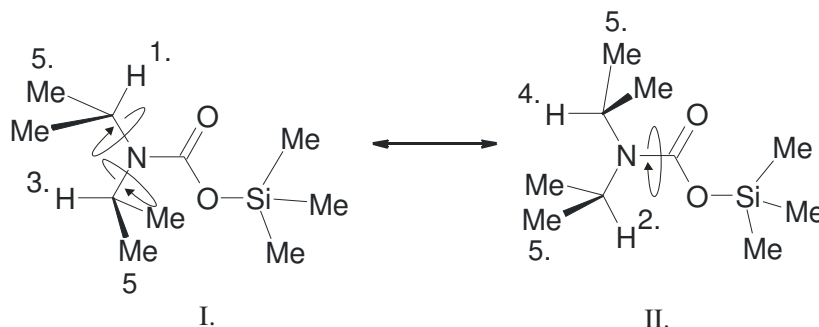

Figure 24. Two planar conformers of iPr2SIC with positions 1-4 of methine protons
and an averaged position 5 of iPr-methyl protons. Rotational arrows in conformer I
show the "gear effect", while the arrow in conformer II shows the frozen amide-bond rotation.

5.3.2 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.

5.3.3 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

[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 (See Figure 25.).
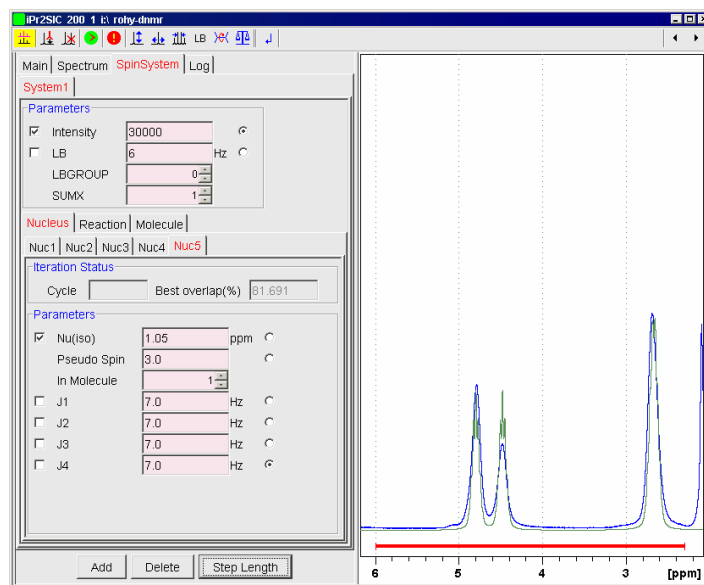

Figure 25. Set a five nuclei model.

5.3.4 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 $\rightarrow$ Nuc4, Nuc4 $\rightarrow$ Nuc1, Nuc2 $\rightarrow$ Nuc3 and Nuc3 $\rightarrow$ 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". See Figure 26. 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.
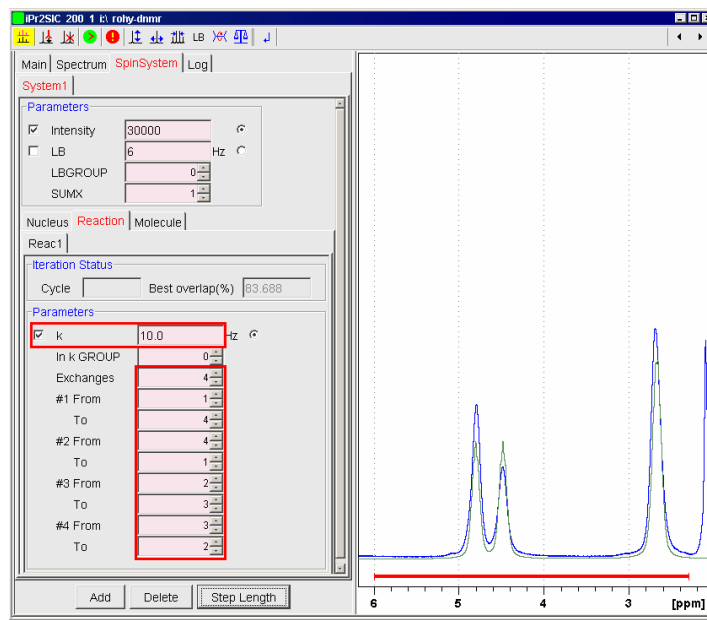
Figure 26. Define the four site exchange reaction.

5.3.5 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 (See Figure 24.). 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. (See Figure 27.).
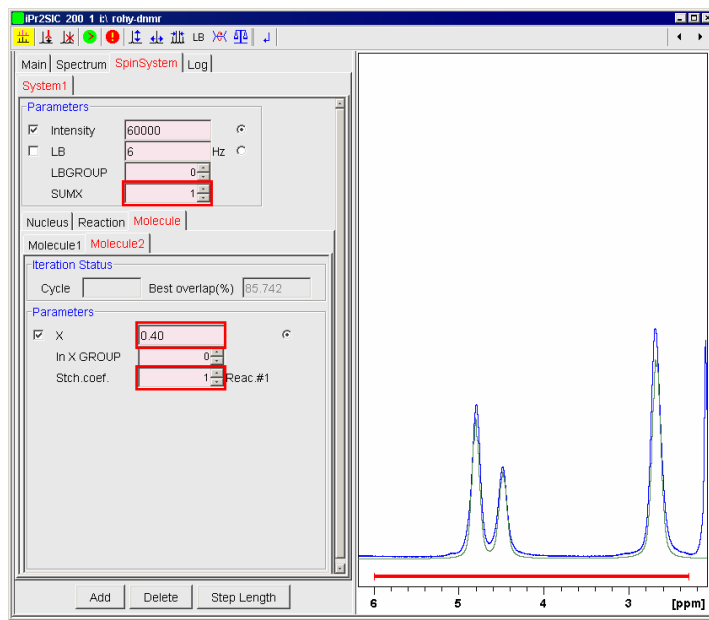
Figure 27. Define the molar fractions and the stoichiometric coefficients.

Now click on "Nucleus" panel and after that on "Nuc2" tab. Set "In Molecule" parameter = 2. Finally click on "Nuc4" tab and set "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 (See Figure 28.).
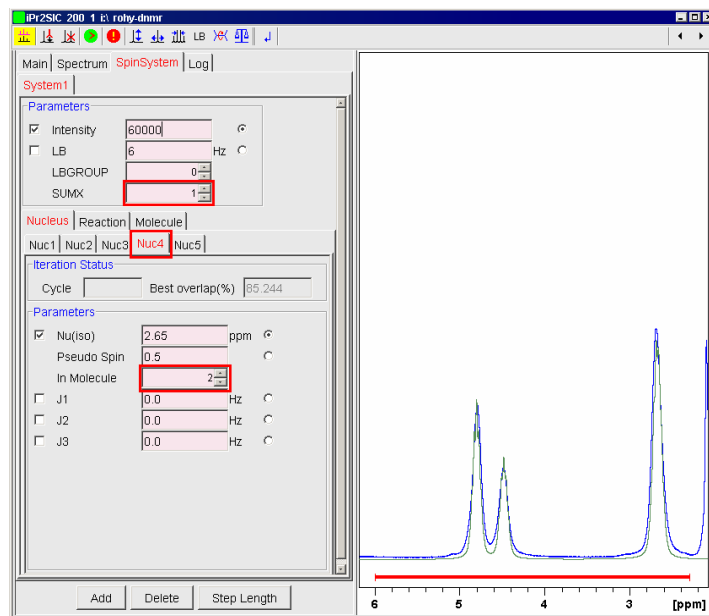

Figure 28. Define the four site exchange reaction.

5.3.6 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 on Figure 29.) Finally exit the DNMR module.
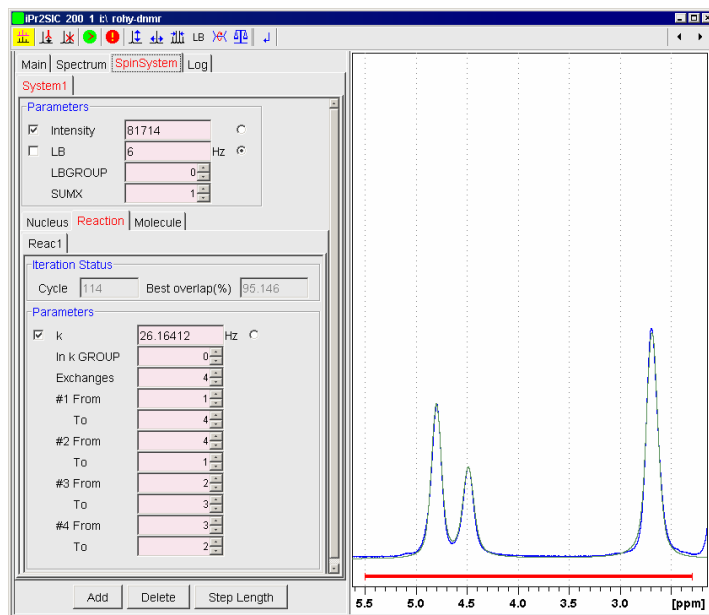

Figure 29. The experimental and the simulated spectra after the iterative parameter refinement.

5.3.7 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. (See Figure 30.)

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

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for both spin systems. (See Figure 31.) Save the model with a new name (for example, model-3).
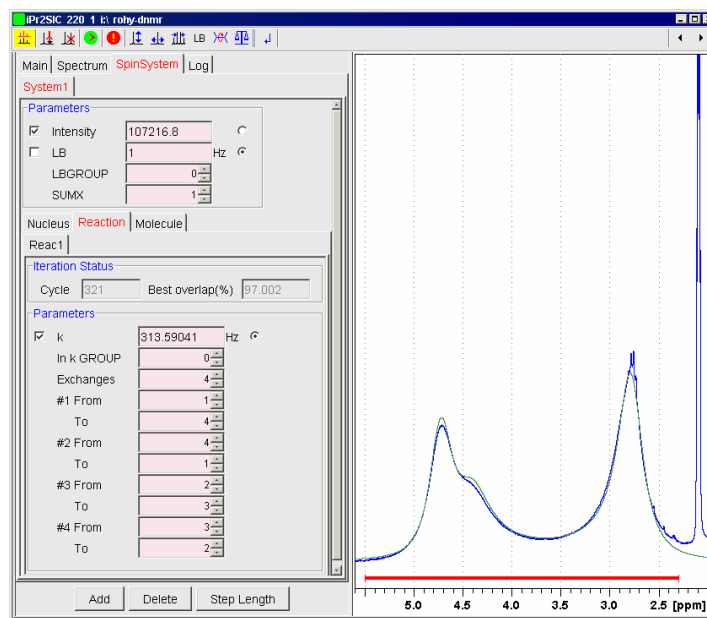

Figure 30. The experimental and the simulated spectra after the iterative parameter refinement with a five nuclei model at 220 K.
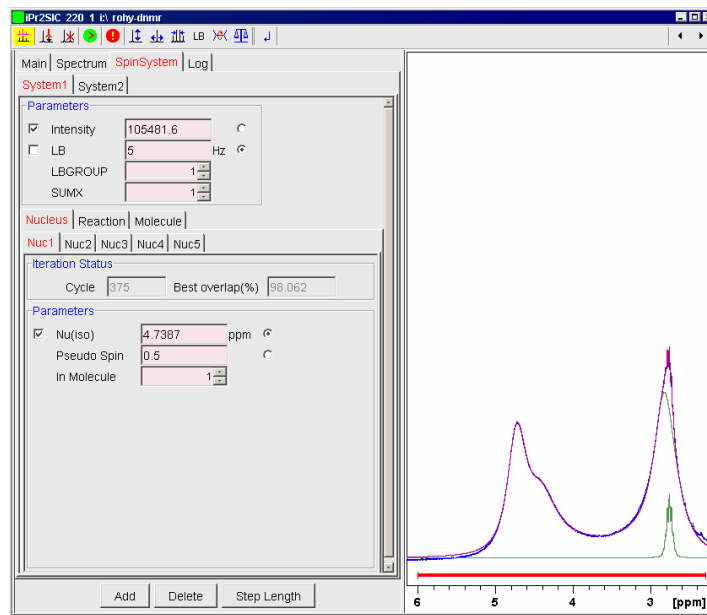

Figure 31. The experimental and the simulated spectra after the iterative parameter refinement with a five+two nuclei model at 220 K.

5.3.8 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.

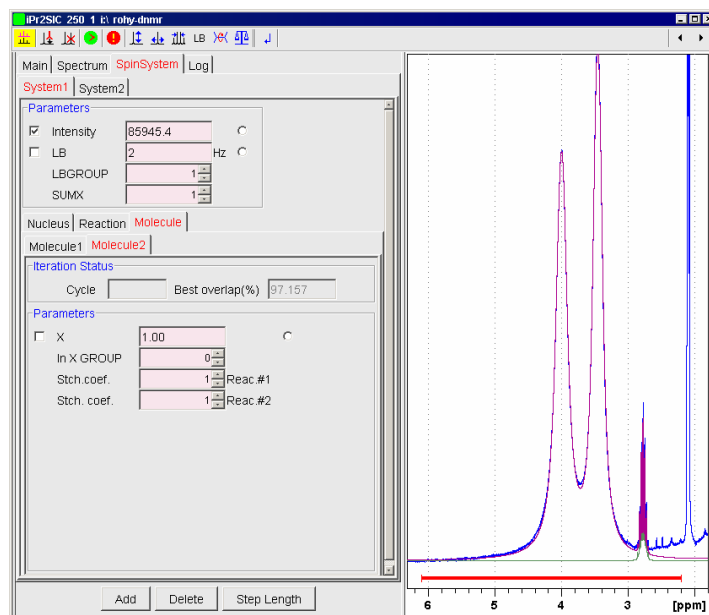Save the model, start the iteration and save the results. (See Figure 32.)

Figure 32. The experimental and the simulated spectra after the iterative parameter refinement
with a five+two nuclei and two exchange reactions model at 250 K.

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