CMC-assist

CMC-assist

User Manual

Version 004

Innovation with Integrity

NMR

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1 Introduction

The software package CMC-assist is designed for processing and analysing acquired 1D 1H, HSQC, 1D 13C, and HMBC NMR data. Its user-friendly and well structured inter- face enables new CMC-assist users as well as experienced Bruker software users to benefit from the comprehensive range of functionalities.

1.1 About this Manual

The User Manual describes the main aspects of Bruker‘s software package CMC-assist. It intends to give an overview of the various functionalities of the CMC-assist, providing an easy way to process and analyse acquired NMR data. In order to facilitate navigation through the manual, the chapters are arranged according to a typical workflow:

- **Chapter 2** shortly describes the installation of the software package and the first steps to display and analyse a spectrum.
- **Chapter 3** addresses the handling of NMR data. This covers the different options of the *Data* menu as well as managing the data within the CMC-assist browser, including loading new data, saving, or deleting data. This chapter also addresses the creation of CMC-assist projects.
- **Chapter 4** explains all functionalities associated with the CMC-assist interface. This comprises the toolbar icons, the command line, and the data window with its specific toolbar.
- **Chapter 5** deals with the processing of NMR data. In addition to manually processing single datasets, the corresponding menu gives the opportunity to process single spectra or a batch of spectra in automation.
- **Chapter 6** describes all available functionalities regarding the analysis of NMR data. Besides carrying out a manual interpretation of the spectra, complete analysis of the spectra including integration, multiplet analysis, concentration determination, and structural consistency check can be performed automatically. This includes the joint analysis of spectra in CMC-assist projects.
- **Chapter 7** lists several possibilities to document the results, such as generating reports, printing data, or exporting results in spreadsheets.

Please note that the figures shown in this manual are designed to be general and informative and may not represent the specific version you are working with.

1.1.1 Font Conventions

- Commands that can be entered on the command line, menus, buttons and icons that can be clicked are in **Arial bold.**
- Path, File, Dataset and Experiment names are in *Arial italic.*

1.1.2 Available Documentation

- CMC-assist Manual
- Potency Determination Quick Start
- Documentation for Multiplet Analysis
- Version Info
- License Info

1.2 Functionality

CMC-assist is a software package intended for:

- Processing 1D 1H, HSQC, 13C NMR, and HMBC spectra
- Analyzing these types of NMR spectra manually
- Automated spectra analysis
- Generating NMR spectra reports and patent strings in various formats

1.3 CMC-assist License

The CMC-assist requires a license for startup. A license can be ordered online from: *https://www.bruker.com/nmr_license_requests.html*

A short instruction on installing the license will be sent together with the license. In addition, a description how to order and install a license can be found in the help menu of Topspin. Left-click on the **Help** button and select **Manuals (docs)**, afterwards click on **CodeMeter License Management**.

2 Getting Started

This chapter shortly describes the installation of the software package CMC-assist and the first steps to start the software including loading new NMR data, displaying a spectrum, printing spectra, and analyzing a spectrum in automation. For more detailed information, refer to the following chapters.

2.1 Installation

The software package CMC-assist is provided together with Bruker‘s software TopSpin. The installation always covers both programs and it is not possible to install these software packages separately. However, each program requires its own license. After successful installation, two icons will appear on the desktop  Topspin;  CMC-assist. TopSpin, and respectively CMC-assist, can be started by double-clicking the corresponding icon.

This section briefly describes the installation of CMC-assist. For more detailed information, please read the instruction available on the installation DVD or from the Bruker webpage:

https://*www.bruker.com/service/support-upgrades/software-downloads/nmr.html*

- Log in as Local Administrator
- Close all windows on the desktop
- Insert the DVD
- Start of installation

If the automatic start of the DVD is enabled, a window will automatically appear

If this window does not appear automatically, autorun is probably switched off. Open the Window Explorer, select the DVD device, and double-click *install.cmd*

- The appearing window will guide through the installation. The only decision that has to be made during the setup is the destination directory for CMC-assist and Top- Spin, respectively.
- After successful installation, the computer should be restarted

2.2 Startup CMC-assist

2.2.1 Under Windows

- Start CMC-assist using the icon on the desktop

Double-click  the CMC-assist icon on the desktop.

- Start CMC-assist from a Command Prompt

– Click *Start*, select *Run*, and enter **cmd**
– enter **cd <cmcahome>** in the Command Prompt
– enter **cmca** in the Command Prompt

2.2.2 Under Linux

- Open a Linux Shell or Terminal Window
- enter **cd <cmcahome>** in the Shell
- enter **./cmca** in the Shell

2.3 Displaying Spectra

With the CMC-assist nmr data can be located in any arbitrary directory, like in the following formats:

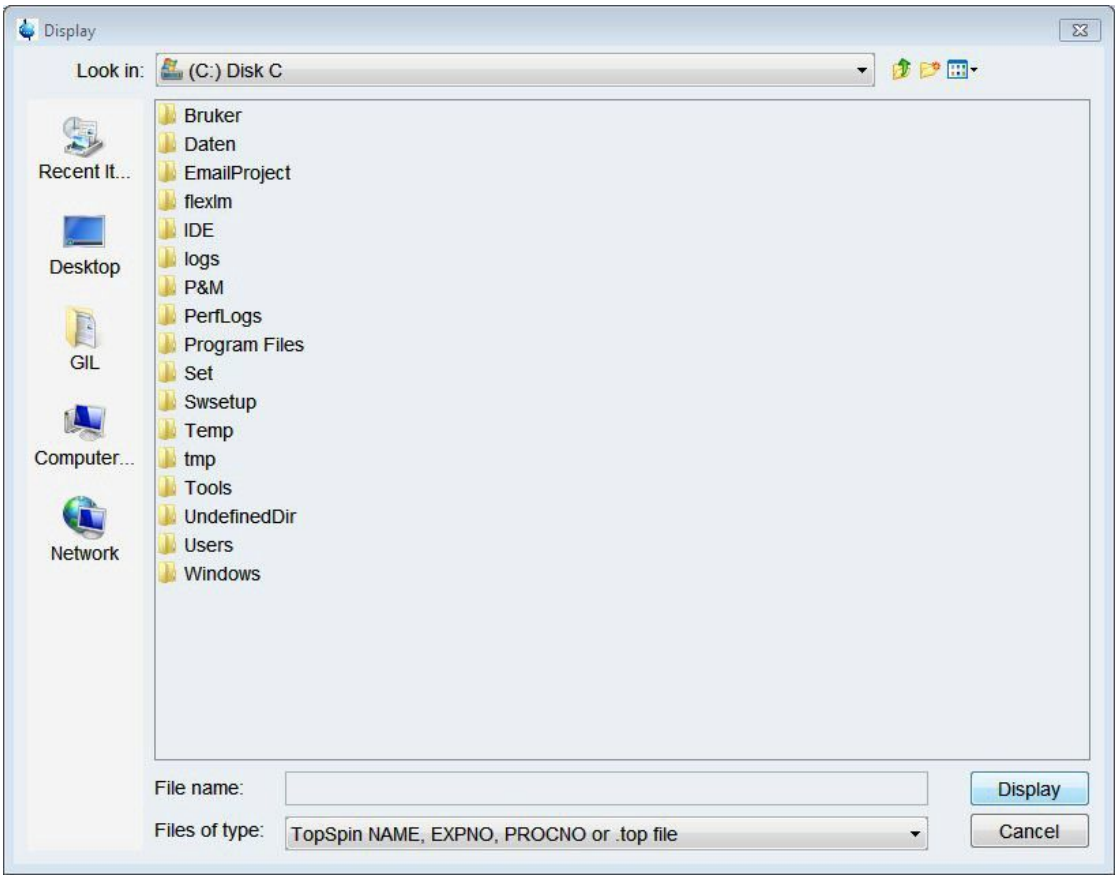
<dir>/data/<user>/nmr/<dataset name>/<expno>/pdata/<procno> e.g.: C:/Bruker/CMCa/data/guest/nmr/quinine/1/pdata/1

or

<dir>/<name>/<expno>/pdata/<procno> e.g.: C:/nmrdata/sucrose/10/pdata/1

2.3.1 Open Data from the Menu

The file menu is accessible via the tab **Data**. Select **File** which allows navigation to the desired data directory. Clicking **Display** will replace the content of the currently active window with the selected dataset. If no data window was displayed, a new one will be created.

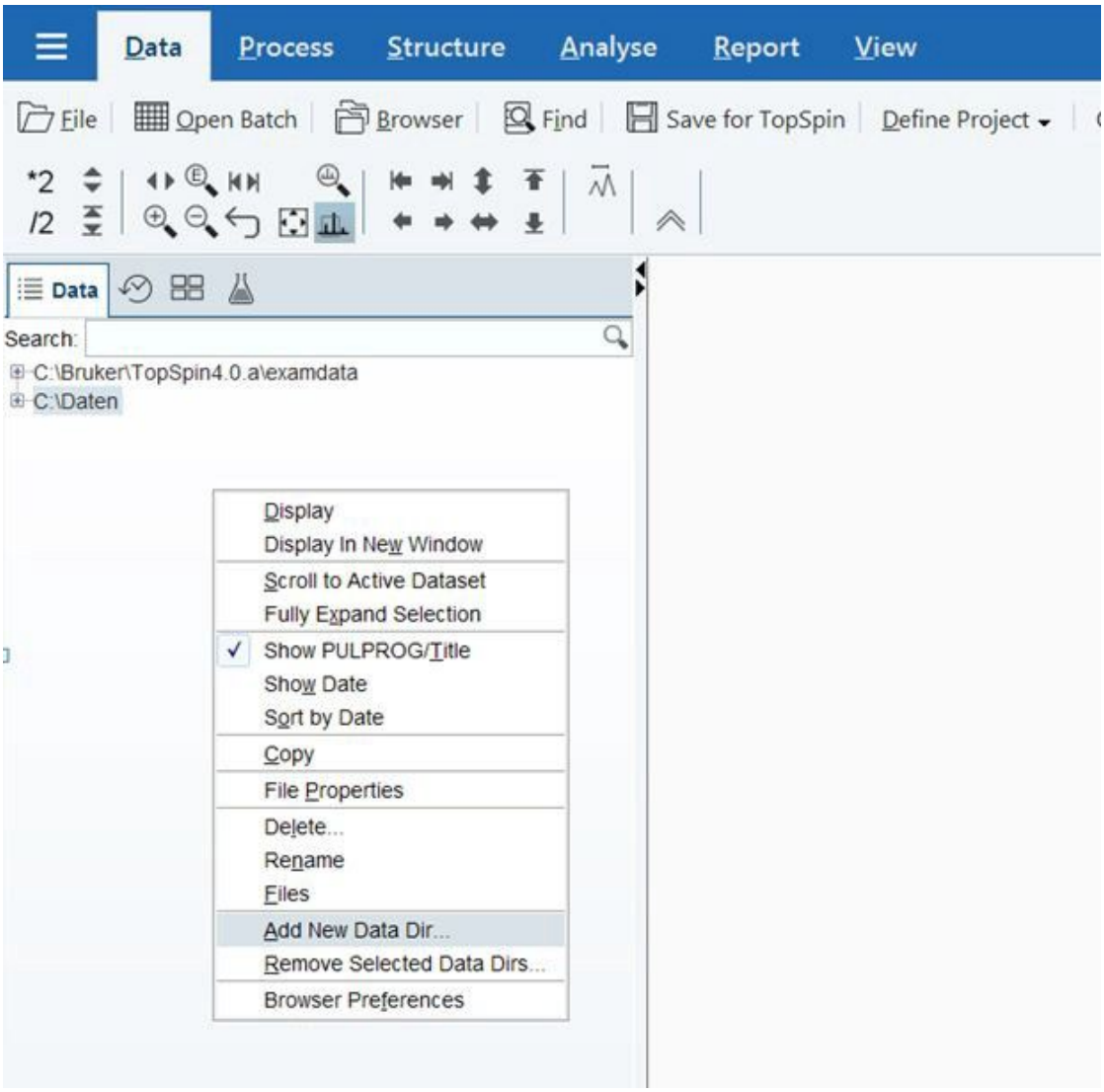


The file browser will also pop up by entering **reb** on the command line.

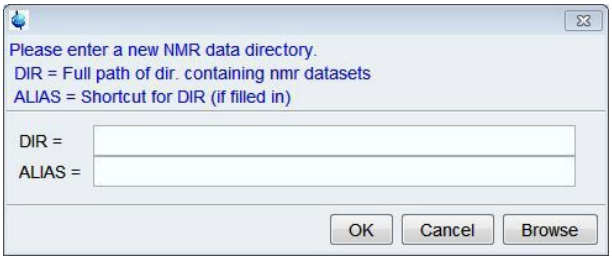
2.3.2 Open Data from the Browser

First of all, the desired data directories have to be added to the data browser.

- Move the cursor into the browser area
- Right-click and choose **Add New Data Dir** in the pop-up menu



- Browse for the desired data directory
- Optionally, a shortcut can be defined for the selected data directory



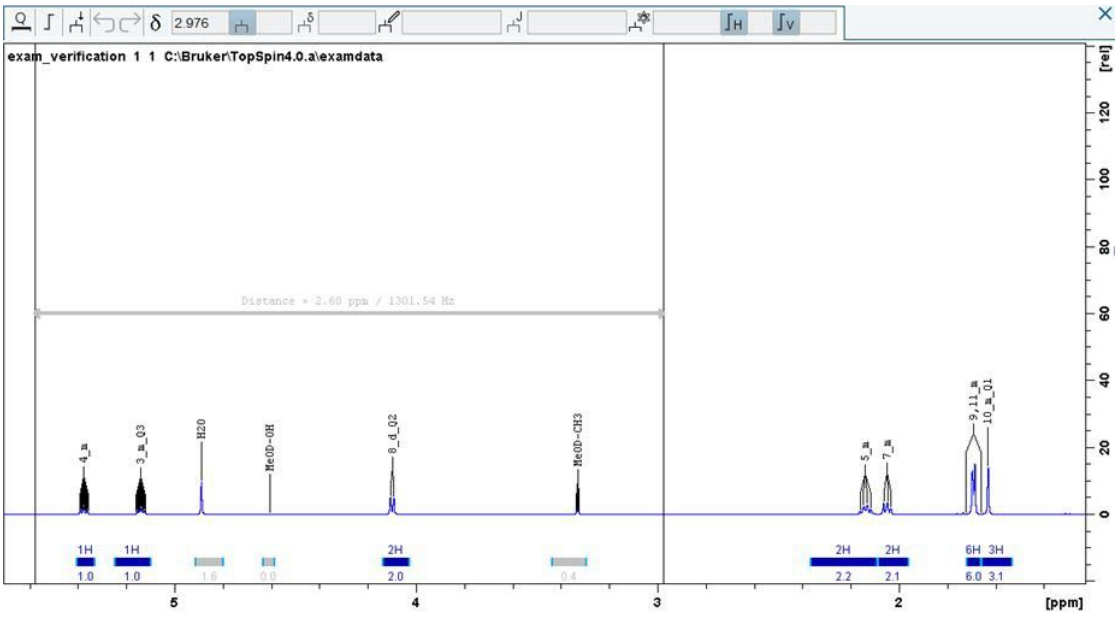
Afterwards, the data can be displayed from the browser as follows:

- Expand the directory in the browser to the level of the data name, expno, or procno
- Select the desired spectrum and drag it into the data area

2.3.3 Expand a Spectral Region

2.3.3.1 Expand a Spectral Region - 1D Data

In order to expand a certain spectral region, left-click-hold on one side of the region, drag the cursor to the other side, and release the mouse.



The expansion can be canceled by moving the cursor out of the data area while still dragging the mouse and releasing it.

Besides this mouse button functionality, the corresponding toolbar buttons for zooming can be used as described in Chapter *The CMC-assist Interface [▷ 31]*.

2.3.3.2 Expand a Spectral Region - 2D Data

To expand a certain region left-click-hold at the top corner of the desired region, drag the cursor diagonally to the bottom corner of the desired region, and release the mouse.

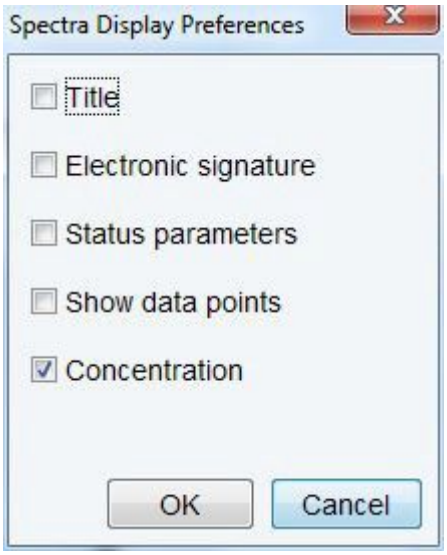
Like with 1D data, the expansion can be cancelled by moving the cursor out of the data area while still dragging the mouse and releasing it.

2.3.4 Show Integrals, Multiplet, etc.

For displayed spectra several options exist in order to show additional information together with the spectrum; for example the title, status parameters, concentration, and data points.

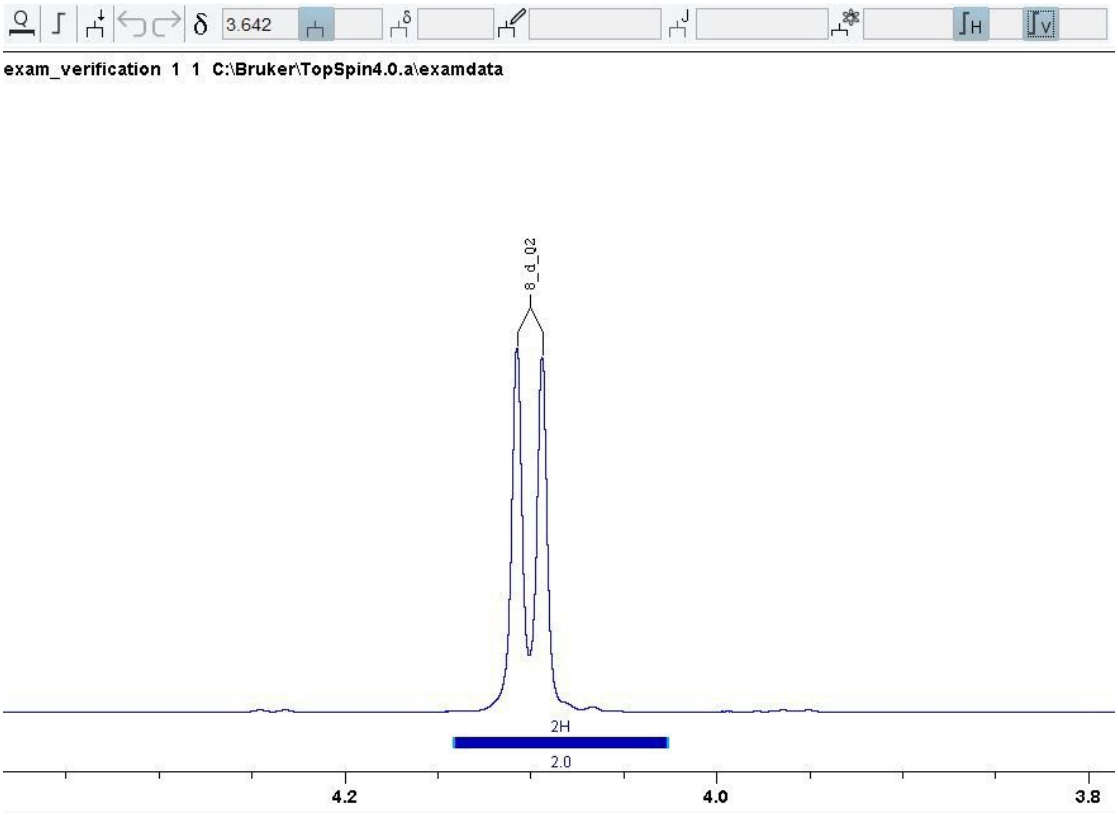
- Left-click the setup button in the upper right corner and choose the corresponding entry

- Choose the corresponding entry:

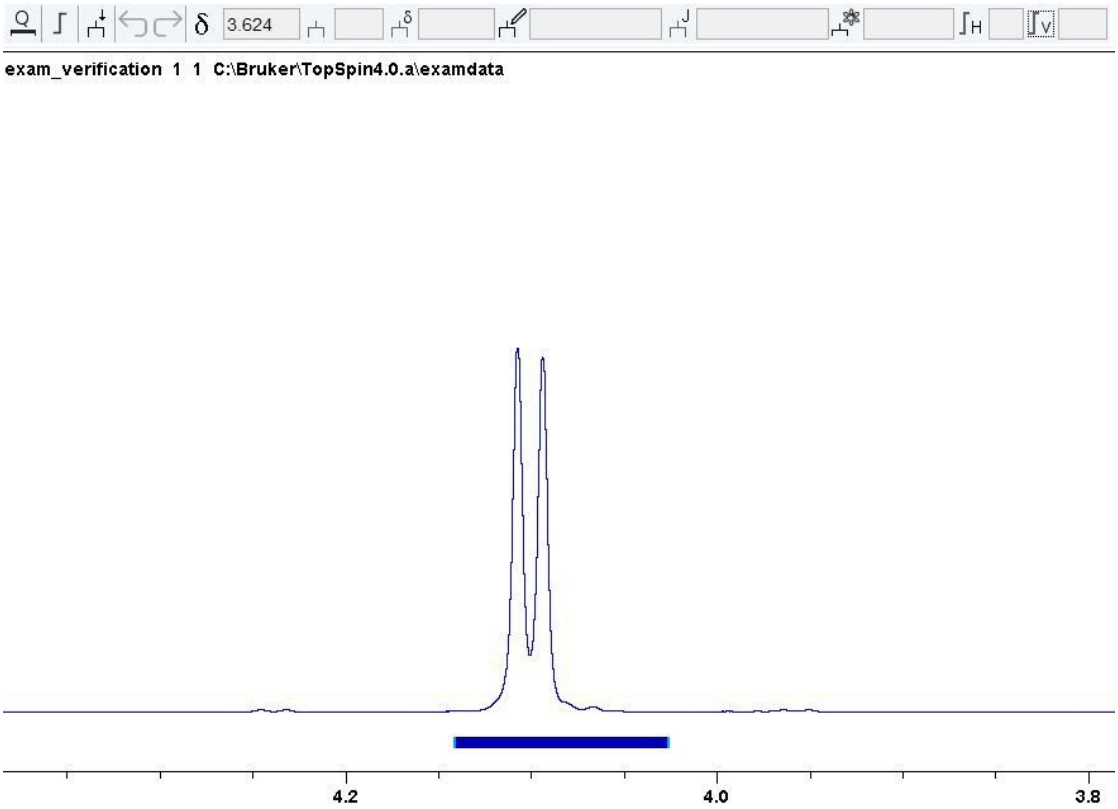


- To toggle multiplets and integral labels left-click the corresponding icons.

Activated toggle:



Deactivated toggle:

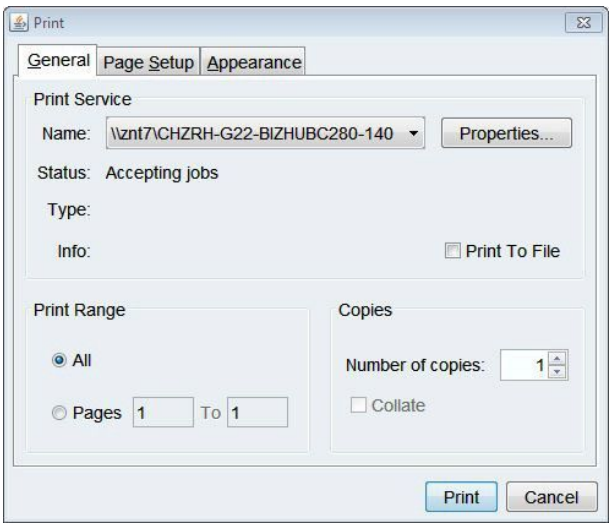


Please note, that the selected information can only be shown in the spectrum if available. For example, the concentration can only be displayed if the concentration has been determined.

2.3.5 Print or Export Contents of a Data Window

The displayed content of the active data window can be printed as follows:

- Use the shortcut [Ctrl+p]
- Click the Printer button
- Choose the **Print** option in the file menu



In addition, the displayed content of the active data window can be saved in a graphics file of selectable type (.png, .jpg, .jpeg, .bmp, .emf, and .wmf) via the **Export** option in the file menu.

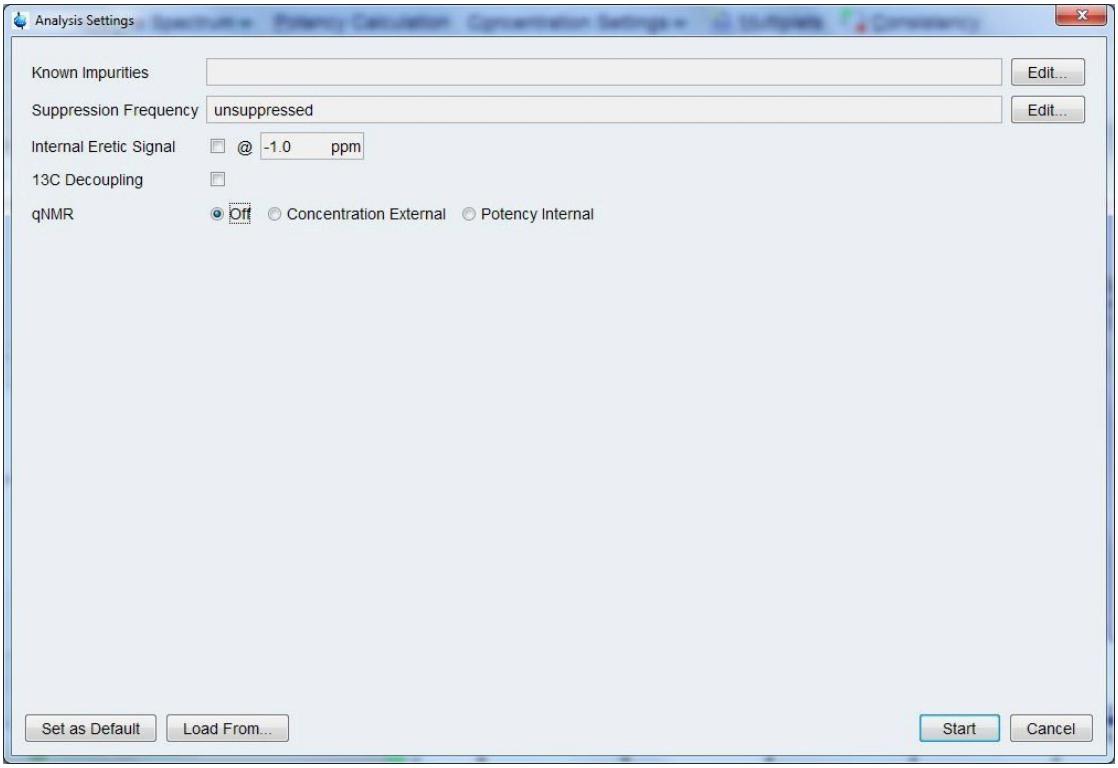
2.4 Automated Analysis of Spectra

CMC-assist offers automated spectral analysis with respect to concentration and purity determination and structure consistency check.

2.4.1 Automated Analysis - 1D 1H Spectra only

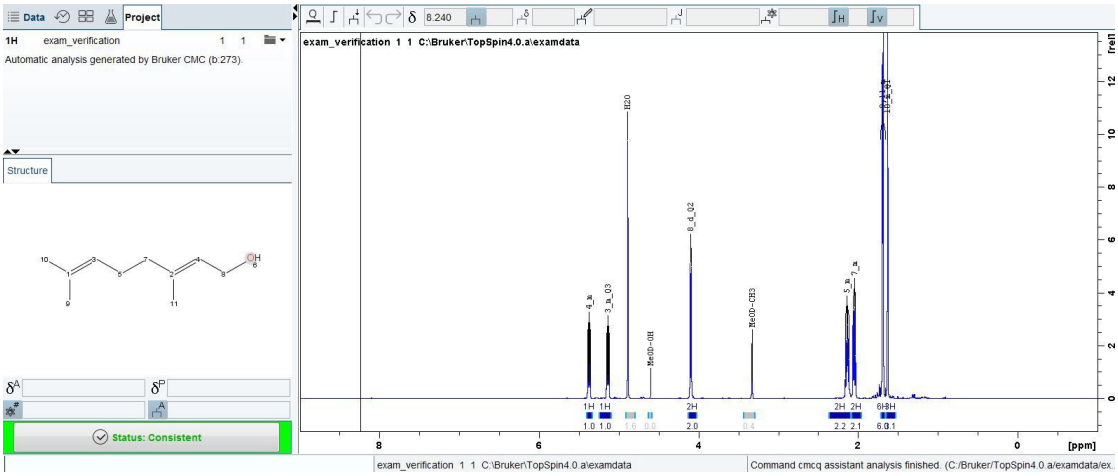
Clicking on the tab **Analyse Spectrum** starts this automated interpretation of the currently displayed spectrum.

If the analysis runs for the first time on a dataset, it may be necessary to give some additional information. The appearing dialog shows the required information, that consists of the number of suppression frequencies, any known impurities that should be excluded from the analysis, possible 13C decoupling, the existence of an eretic signal including its position, and some options for quantification purposes.



The consistency result of the automated spectrum analysis will be displayed underneath the dedicated molecular structure viewer and color coded according to the result (green meaning the spectrum and structure are in good agreement, red indicating a mismatch between the structure and the spectrum, and blue meaning the consistency could not be determined for technical reasons).

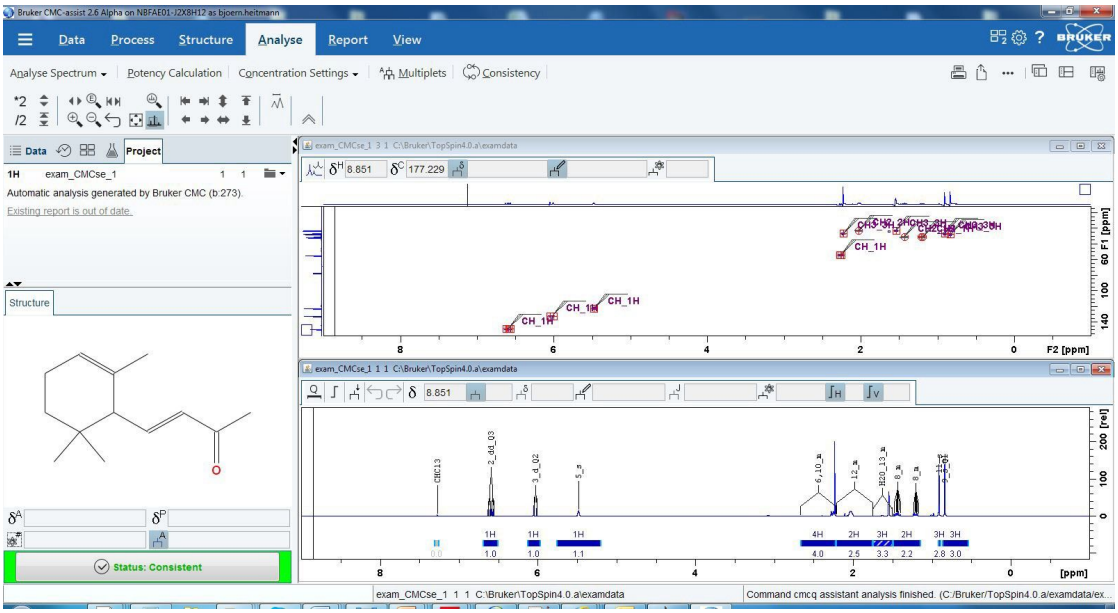
In the case that the molecular structure coincides with the spectrum, the software provides an interpretation of each peak including integration, proton number, multiplicity, and assignment. Besides the assignment, all the available information is readily displayed together with the spectrum. In order to visualize the assignment, move the cursor line on the desired peak and the corresponding atom will be highlighted in yellow within the molecular structure.



2.4.2 Automated Analysis - CMC-assist Projects

CMC-assist can also perform a joint analysis of the 1D1H spectrum with, e.g., an HSQC spectrum in automation. To perform this joint analysis the data must be linked within a CMC-assist project. For information about creating a project see Chapter *Define Project [▷ 28]*.

After a project has been created, clicking **Analyse Spectrum** will perform a joint automated analysis of the spectra in the project. The result of this analysis will be shown in the data windows and the consistency decision will be based on the joint analysis.

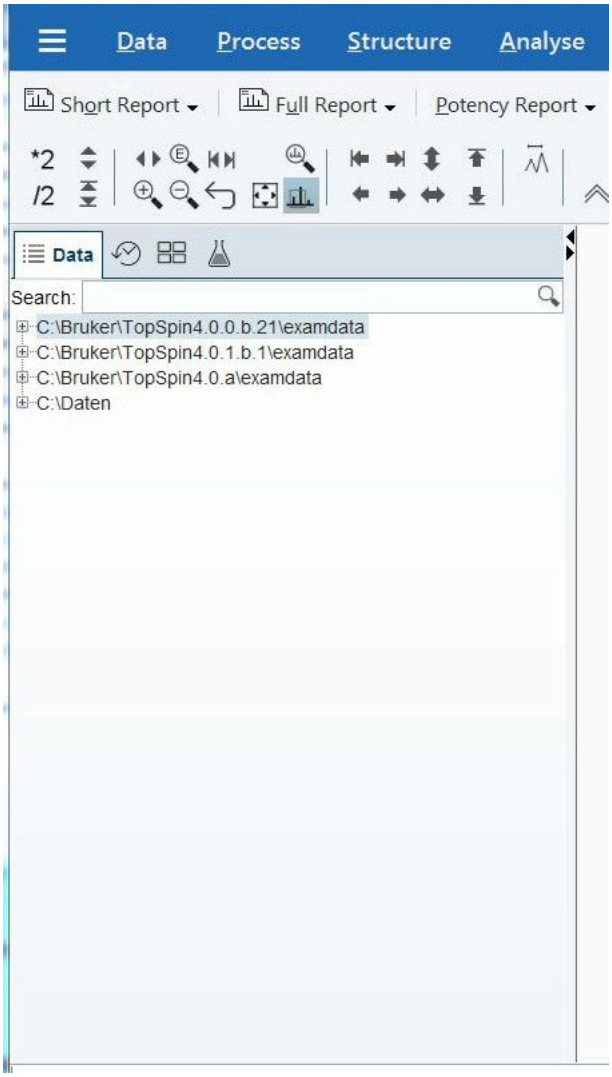


3 Data Handling

The following chapter addresses the handling of NMR data. This covers the different options of the **Data** menu as well as managing the data within the CMC-assist browser, like loading new data, saving, or deleting data.

3.1 CMC-assist Browser

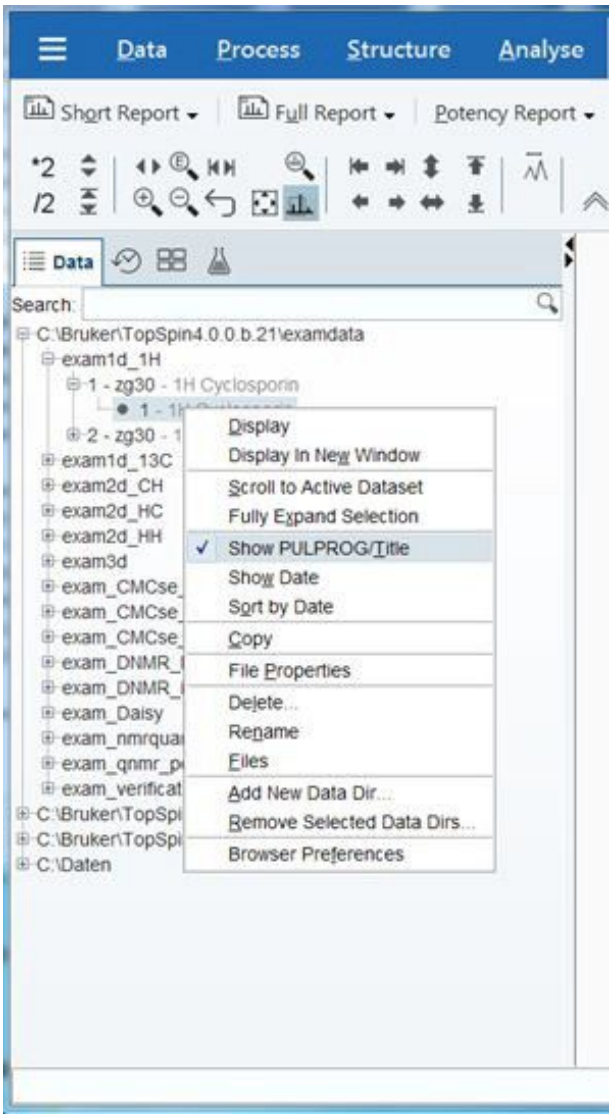
The browser appears at the left of the CMC-assist window and can be shown or hidden with [Ctrl+d] or by clicking the arrow buttons at the upper right corner of the browser. Its main tab is labeled **Data**:



3.1.1 The DataTab

The data browser shows data directory trees that can be expanded or collapsed.

Furthermore, various additional information can be displayed together with the data directory, like the pulse program, the title of the spectrum, or the acquisition date. Just right-click on the respective directory and select the desired option from the appearing dialog.



- **Display**: display dataset in current data window
- **Display In New Window**: display dataset in new data window
- **Scroll to Active Dataset:** scroll to procno of active data window
- **Fully Expand Selection**: fully expand selected data directory
- **Show PULPROG/Title**: switch pulse program/title display on/off
- **Show Date**: show acq. date (expno) or last mod. date (name)
- **Sort by Date**: sort data by last modified date
- **Copy**: copy dataset to clipboard
- **File Properties**: show dataset properties

- **Delete**: delete selected entry (name, expno, or procno)
- **Rename**: rename dataset name, expno, or procno
- **Files**: list files in selected entry (expno or procno)
- **Add New Data Dir**: add new top level data directory
- **Edit Selected Data Dir**: edit selected top level data directory
- **Remove Selected Data Dir:** remove selected top level data directory
- **Browser Preferences**: set display options
- **Close All Group Windows**: close all data window(s) belonging to this group
- **Remove Selected Groups**: remove selected group(s) from the list

3.1.2 Put Focus in the Browser

Hit the F2 key or click inside the browser.

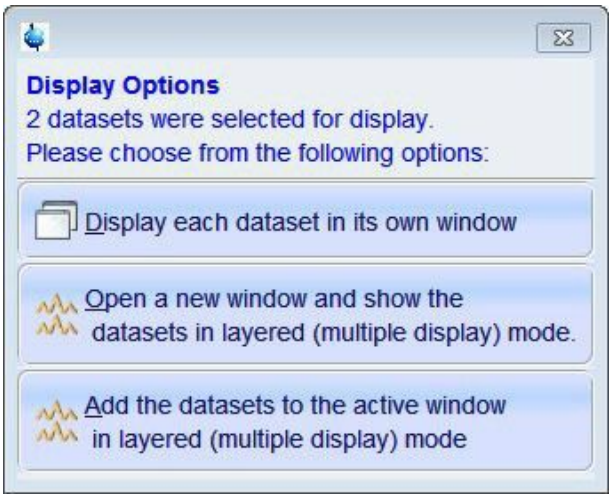
3.1.3 Expand/Collapse a Folder in the Browser

- Expanding a collapsed folder:
  - Left-click the + button to the left of the folder
  - Double-click the folder
  - Hit the right-arrow key while the folder is highlighted
- Fully expanding a collapsed folder:
  - Right-click the folder and choose **Fully Expand Selection**
- Collapsing an expanded folder:
  - Left-click the - button to the left of the folder
  - Double-click the folder
  - Hit the left-arrow key while the folder is highlighted

3.1.4 Open Data from the Browser

- Double-click on a dataset will result in the display of the dataset in data area
- Left-click-hold a dataset and drag it into the data area (the new dataset will replace the currently displayed dataset)
- Right-click on a dataset and choose *Display* from the pop-up menu (the new dataset will replace the currently displayed dataset)
- Right-click on a dataset and select *Display in New Window* from the appearing dialog (the dataset will be opened in a new data window)

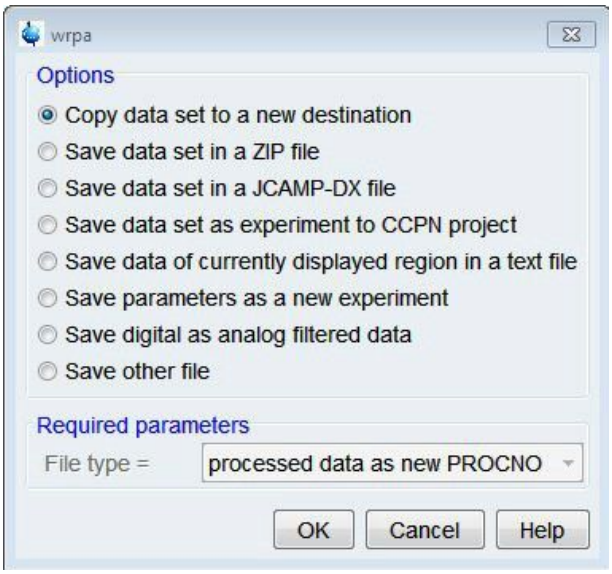
In order to load multiple spectra, hold the [Ctrl] key and left-click on the desired datasets to select them or hold the [Shift] key and left-click two datasets to select these two and all in between. Executing any of the options described above will open a new dialog that offers three different ways of presenting the selected multiple datasets.



3.2 Saving Data

In principle, all modifications of the data (like processing, integration, multiplet analysis, etc.) made within the CMC-assist are saved automatically.

Apart from that, the option **Save As** in the file menu provides the opportunity to save the dataset in several different formats.



The choices of the pop-up dialog correspond to the following command line commands:

- Copy the current dataset to a new destination (name or expno): **wrpa**
- Save the selected dataset in ZIP format: **tozip**
- Convert the current dataset to JCAMP-DX format: **tojdx**
- Save the selected dataset in CCPN format: **toccpn**
- Convert the current dataset to text format: **totxt**
- Write parameterset: **wpar**
- Save digitally filtered data as analog filtered data: **convdta**

3.2.1 Save an Entire Dataset

- Select **Save As** in the file menu or use the shortcut [Crtl+s]

- Choose **Copy dataset to a new destination**
- Specify dataset variable (name or expno)

3.2.2 Save Processed Data

- Select **Save As** in the file menu or use the shortcut [Crtl+s]
- Tick **Save other file**
- Choose as **File type** processed data as new PROCNO
- Enter a processing number (procno)

3.2.3 Save Acquisition Data

- Select **Save As** in the file menu or use the shortcut [Crtl+s]
- Tick **Save other file**
- Choose as **File type** acqu. data as new EXPNO
- Enter a experiment number (expno)

3.2.4 Save Processed Data as Pseudo Raw Data

- Select **Save As** in the file menu or use the shortcut [Crtl+s]
- Tick **Save other file**
- Choose as **File type** 1r/1i as fid
- Enter a experiment number (expno)

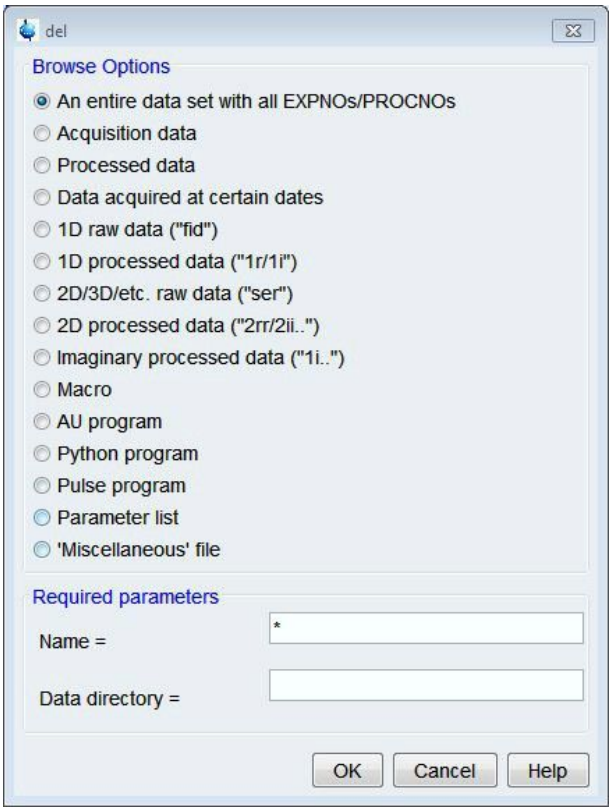
3.3 Deleting Data

3.3.1 Delete a Specific Dataset

- Right-click on the dataset name, expno, or procno and select **Delete**
- Right-click on a data directory and choose **Remove Selected Data Dir**

3.3.2 Delete Types of Datasets

- Select the option **Delete** in the file menu or enter **delete** on the command line
- Define the type of data for removing via the various criteria of the appearing window



- Specify the **Required parameters** (note that question mark (?) can be used for any single character and asterisk (*) for any character and any number of characters)
- Select dataset entries for deletion from the pop-up dialog showing the matching datasets

3.4 Data Menubar

3.4.1 File

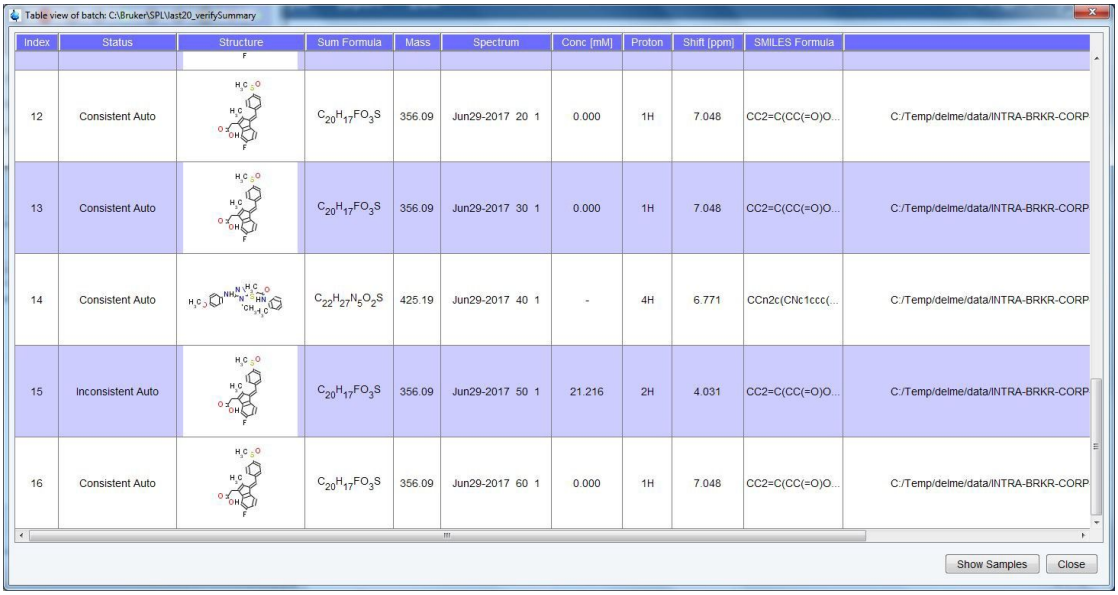
In order to open a new dataset in the data window, click on the tab **File** and browse for the desired dataset (the new dataset will replace the currently displayed dataset).

3.4.2 Open Batch

Apart from single spectra, a whole collection of spectra can be displayed and analyzed. Opening a batch of spectra requires a serial processing list that can be generated by the command **serial** (see Chapter *Serial [▶ 57]*). Furthermore, this feature is intended for manual inspection of automatically analyzed batches of datasets (for batch analysis see

Chapter *Automatic Spectra Analysis [▶ 61]*). To show the batch data, click the tab **Open Batch** and browse for the appropriate serial processing list. Besides the standard CMC-assist interface, two additional windows will appear.

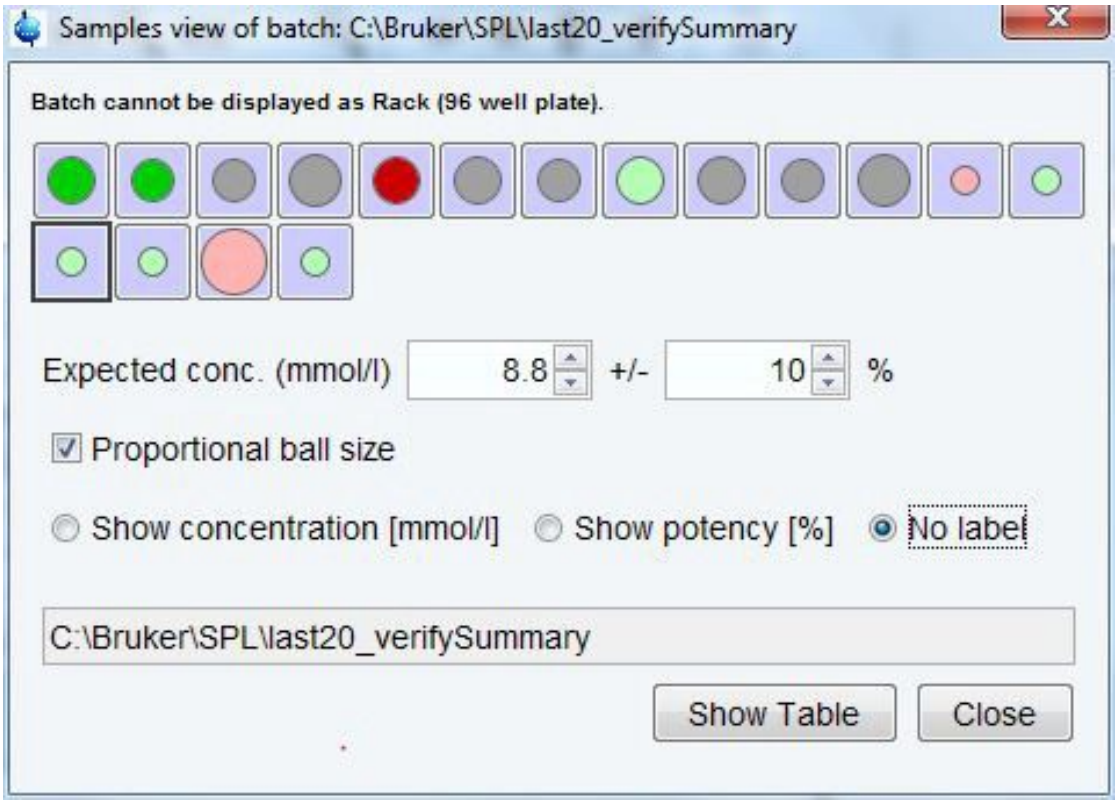
One window lists all molecules with the available information from the automatic analysis, like concentration, consistency status, water content, etc. The content of this table can be formatted: Move the mouse over the column header and right-click to get all display options. To export the table move the mouse over any cell below the header and right-click to get to the option **Export...** . Supported formats are: spreadsheet (.csv, .xls, .xlsx) and document (.pdf, .html).



The second window represents the data form the serial processing list in a compact format, graphically displaying the determined concentration, potency, and structural consistency:

- green means consistent
- red means inconsistent
- blue indicates technical complications
- light colors: results are obtained in automation
- intense colors: results are set by the user

Moreover, it offers the option to additionally show the values of the concentration or potency and to size the displayed balls proportional to the value. If the fields for the expected concentration and its expected deviation are adjusted, samples within this concentration range have a white background, whereas the ones outside this range are highlighted with a blue background.



Clicking on any of the colored balls will open the corresponding spectrum in the data window of the CMC-assist and highlight the respective entry in the table of the other batch window. The same applies for selecting an entry from the table.

3.4.3 Browser

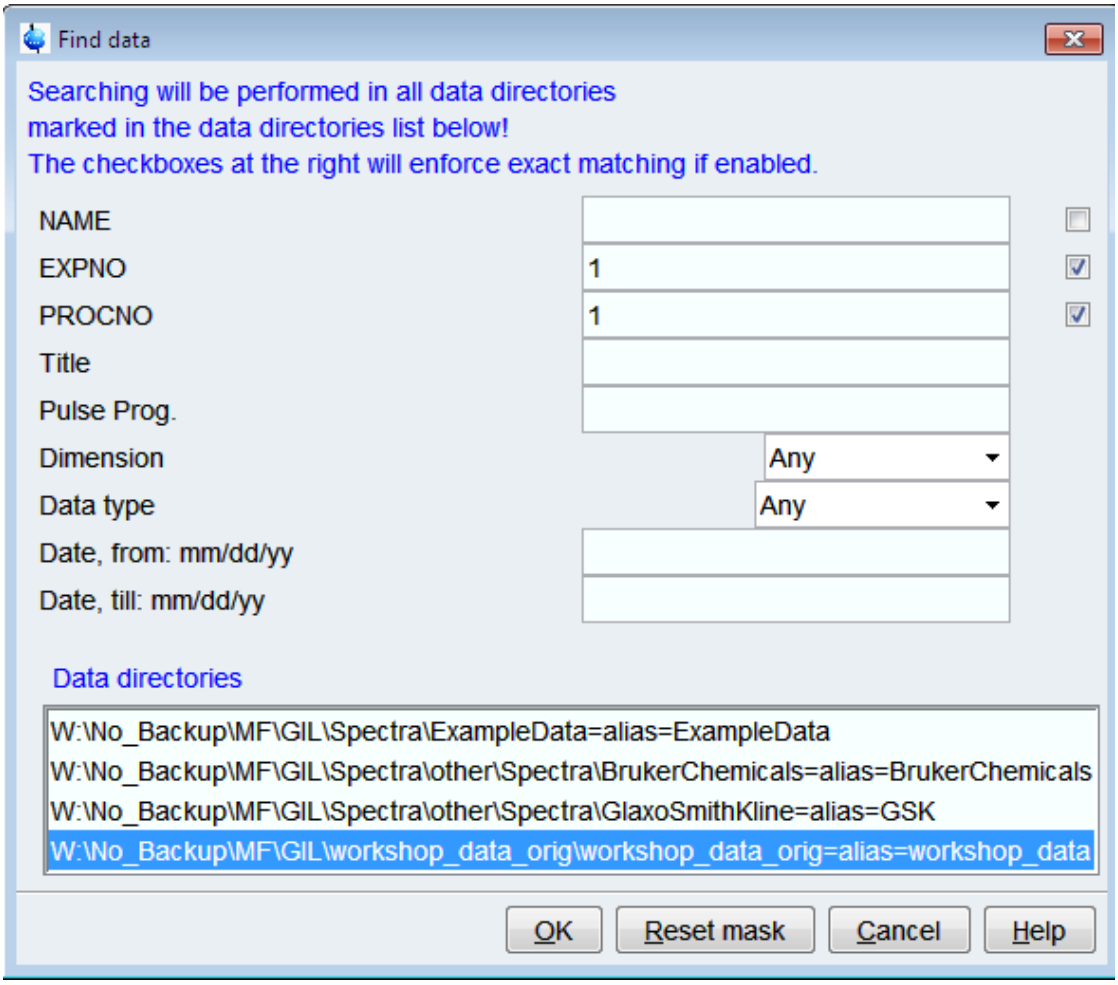
Clicking on the tab **Browser** displays and hides the CMC-assist browser, respectively.

3.4.4 Find

Searching for certain data can be performed according to various criteria. The respective dialog will pop up when clicking the tab **Find**, entering the command **find** on the com- mand line, or hitting the shortcut [Ctrl+f].

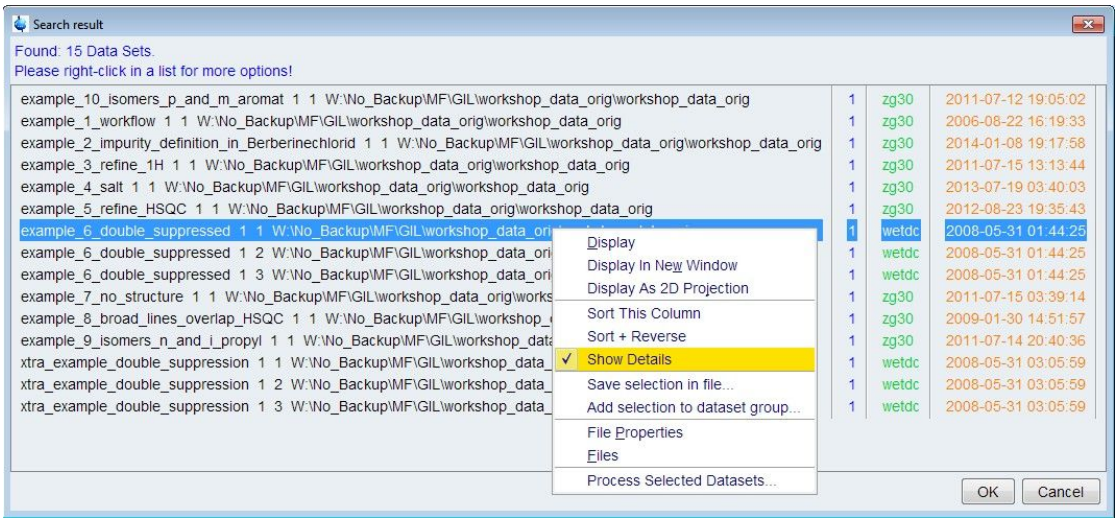
- Enter the search items in the upper part of the dialog
- Exact matching is performed for the dataset variables NAME, EXPNO, and PROCNO, if the corresponding checkbox at the right is ticked
- Entries in the fields **Title** and **Pulse Prog**. cause searching for items containing these specified strings
- The search can be restricted to data created between specified dates, referring to the acquisition dates
- Select the **Data directories** to be searched in the lower part of the dialog (if no directories are selected, all directories will be used for search)

- Clicking **OK** starts the search and a list of data that fulfill the defined criteria will appear



Right-click on a list entry offers the following options:

- **Display:** display the selected dataset(s) in the current data window
- **Display In New Window**: show selected dataset(s) in a new data window
- **Sort This Column**: sort the column in ascending alphabetical order in relation to the dataset title (numbers before letters)
- **Sort + Reverse**: sort the column in descending alphabetical order in relation to the dataset title (numbers after letters)
- **Show Details**: switch display of expno, pulse program, and acquisition date on/off



- **Save Selection In File**: save the list of selected datasets in a text file
- **Add Selection To Dataset Group**: add selected datasets to an existing dataset group or define a new group for the selected datasets
- **File Properties**: show main dataset parameters like dimension, pulse program, acquisition date, nuclei, spectrometer frequency, and solvent
- **Files**: display the files within the processed data directory (procno) of the selected dataset
- **Process Selected Datasets**: perform serial processing on the selected datasets (see Chapter *Serial [▷ 57]*)

3.4.5 Save for TopSpin

The tab **Save for TopSpin** exports assignment information and spectrum analysis features to standard Bruker TopSpin files. This will overwrite any existing information previously generated by TopSpin, for example peak, integral, and multiplet lists created after data acquisition.

3.4.6 Define Project

In order for a joint analysis of a 1D 1H spectrum with something else (HSQC, HMBC, 1D 13C), the datasets must be linked in a CMC-assist Project.

To create a Project:

- Open each dataset in a separate window
  - Right click on the dataset in the CMC-assist Browser and **select Display in New Window**
- Select **Define Project** from the **Data** menu
- In the resulting dialog, click ok if the listed datasets are correct

To remove an unwanted dataset from a project:

- Activate the window of the unwanted dataset by clicking on it
- Click the small arrow on the **Define Project** button and select **Remove dataset from project**
- In the resulting dialog, click ok

3.4.7 Open Project

Clicking this button will open all datasets in a project alongside the currently selected dataset. Each of the linked datasets will be opened in their own window.

4 CMC-assist Interface

CMC-assist uses a workflow based interface with its available functionalities arranged according to diverse working processes, like data handling, data processing, data analysis, etc.

4.1 The CMC-assist Window

The CMC-assist window consists of a data area, data browser, molecular structure viewer, toolbars, and menubar. The data browser can be inactive ([Ctrl+d] or clicking the tab **Data i**n the menu **Data**) or displayed as a separate window. Part of the browser is composed of the molecular viewer and the consistency decision when a dataset is open in the data window.

4.1.1 Menubar

The menubar includes the following menus:

- **Data:** performing data/file handling tasks
- **Process**: data processing
- **Structure**: adding and modifying chemical structures
- **Analyse**: automatic and manual data analysis
- **Report:** generating various types of reports and patent strings
- **View:** display properties

Clicking any of these menus opens its sub-menu.

4.1.2 Toolbar - General

Experienced users might prefer to work with the command line rather than the toolbar buttons. A mouse contact of the toolbar buttons opens a balloon help, that shows the corresponding commands and shortcuts in square brackets behind the explanation of the button‘s functionality.

 Increase intensity by a factor of 2 (***2**)

 Decrease intensity by a factor of 2 (**/2**)

 smaller/larger

 Reset intensity scale (baseline position remains unchanged) (**.vr**, [Ctrl+Alt+PgUp])

 Reset zooming to full spectrum, leave intensity scale (**.hr**)

 Show last zoom (**.zl**)

 Show full spectrum, reset intensity scale (**.all**)

 Retain expansion and scale when changing dataset (**.keep**)

 Shift to left end of spectrum (**.sl0**)

 Shift to right end of spectrum (**.sr0**)

 Shift spectrum to the left, half of the displayed region (**.sl**)

 Shift spectrum to the right, half of the displayed region (**.sr**)

 Shift baseline up/down while pressing left mouse button

 Shift spectrum left/right while pressing left mouse button

 Shift baseline to center of displayed region (**.su**, [Alt+UpArr])

 Shift baseline to bottom of displayed region (**.sd**, [Alt+DwnArr])

 Start distance measurement

 Less Icons (.**onerow 1d**)(**.onerow 2d**) More Icons (.**onerow 1d**)(**.onerow 2d**)

4.1.2.1 Toolbar - Active HSQC window

Below are additional toolbar buttons available while an HSQC window is active.

 Toggle positive and negative levels (**.lt**)

 Show maximum vertical regionin the f1 dimension (**.f1r**)

 Move the spectrum around

 Contour mode (**.co**)

4.1.2.2 Chemical Shift Distance Measurement

As long as the icon  **Start distance measurement** is highlighted in yellow, the functionality of the cursor line changes in order to measure chemical shift distances. Left-clicking at one peak position and moving the mouse to another peak position will measure the distance in ppm and Hz. Right-clicking in the data window or moving the cursor out of the data window will leave the distance measurement mode.

4.1.3 Command Line

To execute a command via the command line, the command line has to be activated first by the [Esc] key or clicking inside the command line. All commands that have been entered on the command line since CMC-assist was started are stored and can be retrieved by the [Up-Arrow] key to go back to previously entered commands and the [Down-Arrow] key to scroll forward to recently entered commands. The [Left-Arrow] and [Right-Arrow] keys move the cursor within the command line.

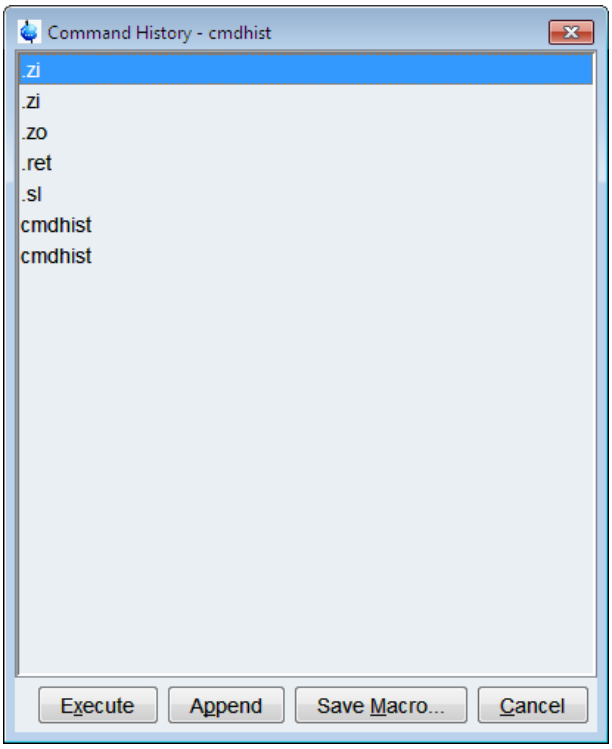
4.1.3.1 Series of Commands

Besides single commands a series of commands, separated by semicolons, can also be entered on the command line (e.g. em; ft; apk). If this series of commands will be used regularly, it can be stored as a macro by right-clicking in the command line and selecting **Save As A Macro** (to edit this macro, enter **edmac <macro-name>**; to execute it, enter its name on the command line).

4.1.3.2 Command Line History

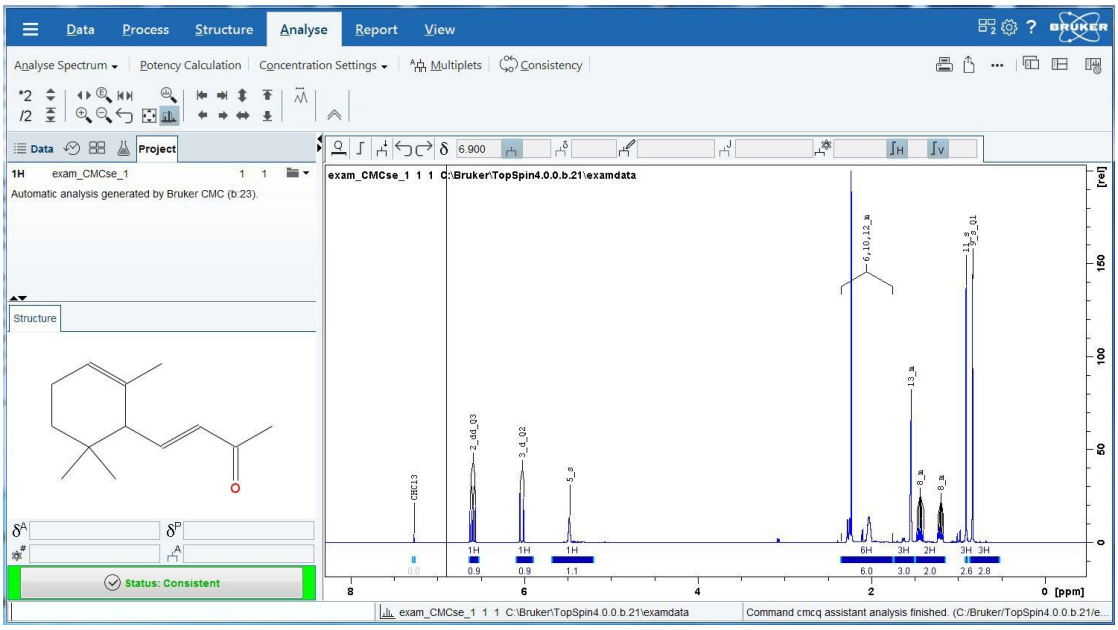
To open a command history control window, right-click in the command line and choose **Command Line History** or type **cmdhist** on the command line. This history shows all commands that have been entered on the command line since CMC-assist was started. After marking one or several commands, the following functions can be applied:

- **Execute:** execute the selected command(s)
- **Append:** append the (first) selected command to the command line
- **Save Macro:** the selected command(s) are stored as macro (to edit this macro, enter **edmac <macro-name>**; to execute it, enter its name on the command line)



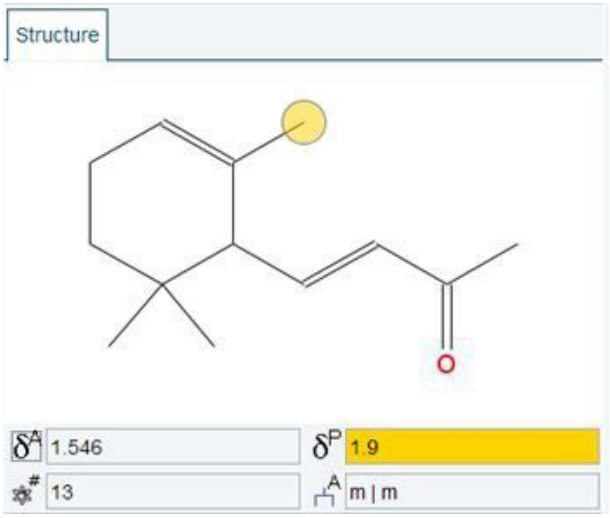
4.1.4 Molecular Structure Viewer

The molecular structure viewer is located at the bottom of the browser. This is where the molecular structure will be displayed. If an analysis has previously been run and the mouse hovers over an atom, it will be highlighted yellow along with any assigned multiplet in the spectrum. Also, when the mouse hovers over a multiplet in the spectrum, any assigned atom will be highlighted in the molecular structure viewer.



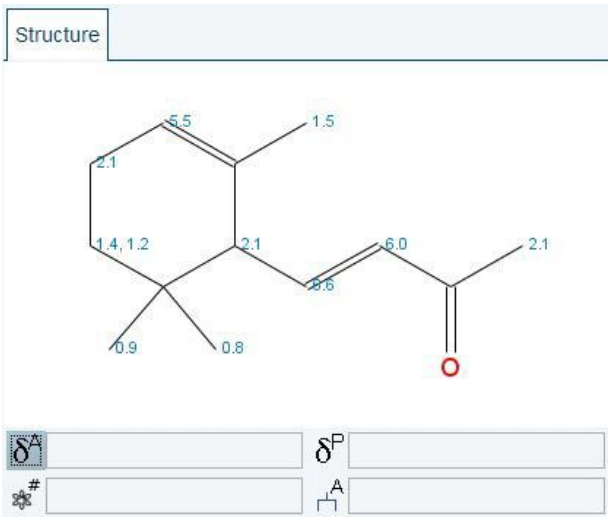
4.1.4.1 Molecular Structure Viewer Options

Moving the mouse over an atom will display the properties listed below.



- **Assigned Shifts:** This displays the chemical shifts of the multiplets assigned to each atom.
- **Predicted Shifts***:* This will display the predicted chemical shift for each atom.
- **Multiplets***:* This displays the annotation that is displayed on the screen for the multiplet assigned to each atom.
- **Numbers***:* If desired, the atom number (from the mol file) can be displayed.
- **Names***:* Selecting this option will display the user defined name of any atom with a name defined via the **Edit Atom Name** option.

The properties can also be displayed for all atoms be left-click the corresponding icons. As in example the assigned shifts are turned on in the picture below:

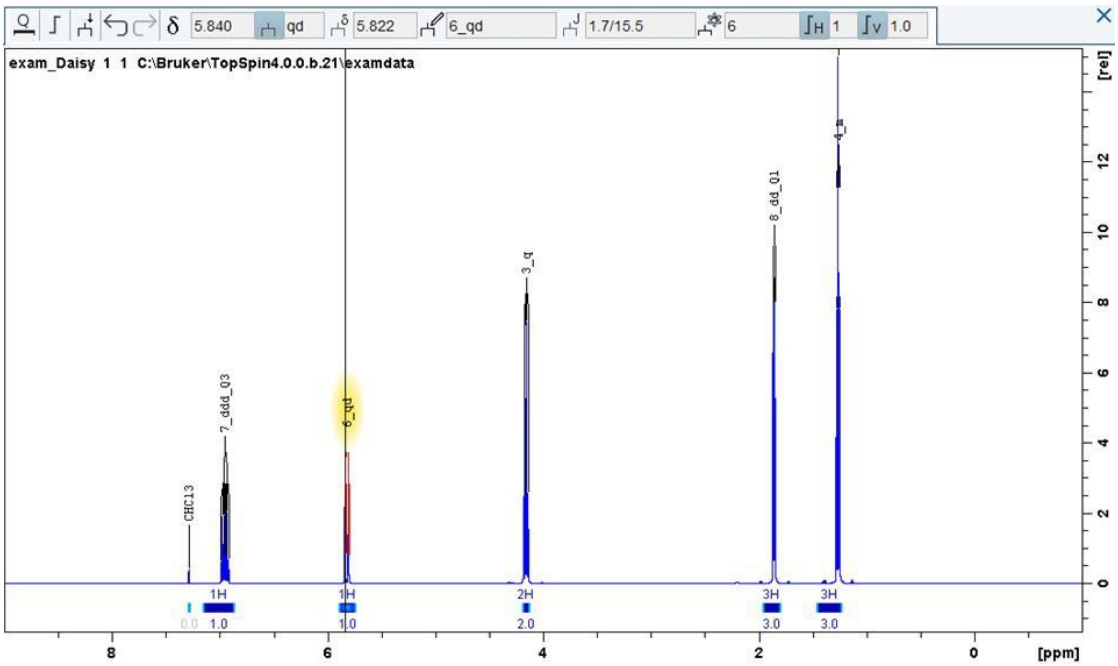


Further options are available when right-clicking on a highlighted atom in the molecular viewer:

- **Clear Assignment(s):** This will remove the connection between an atom and any assigned multiplet.
- **Edit structure...:** This will open the structure editor to alter the molecule
- **Edit Atom Name***:* Clicking this option will bring up a dialog where one can set the name of an atom. This will not be displayed on the spectrum but can be displayed, if desired, in the molecular viewer via the **Toggle Names** option.

4.1.5 4.1.5 Data Window

The CMC-assist data window consists of data field, title bar, and toolbar. Besides the name of the dataset currently displayed in the data field, the entire data path of the spectrum is written in the title bar. Furthermore, for the location of the cursor different proper- ties are displayed (e.g. the multiplet label).



4.1.5.1 Move and Resize Data Windows

Several data windows can be opened within the CMC-assist data field by selecting **Display** *in* **New Window** every time a new dataset is loaded. These data windows can be arranged individually by moving or resizing the windows.

- Move a data window
  - Left-click-hold the title bar and move the mouse
- Resize a data window
  - Move the cursor to the window edge until it becomes a double-headed arrow
  - Left-click-hold that position and move the mouse

Depending on the position of the double-headed arrow, the window can be changed in height, width, or both.

4.1.5.2 Activate a Data Window

All functionalities of the toolbar and commands from the command line only correspond to and act on the active data window, readily identifiable by the highlighted title bar.

- Activate a data window
  - Left-click in the desired data window or left-click its title bar
  - Left-click one of the colored buttons above the data area (the pressed button indicates the currently activated data window)
  - Hit the [F6] key to activate the next window and repeat hitting the key until the desired window is the active one

4.2 CMC-assist Dataset Toolbar

4.2.1 CMC-assist Toolbar Buttons

All of the buttons listen below are available when a 1D1H window is active. When an HSQC window is active these options are not available.

Zoom to quantification range on dataset opening

Define new integrals

Define new multiplets

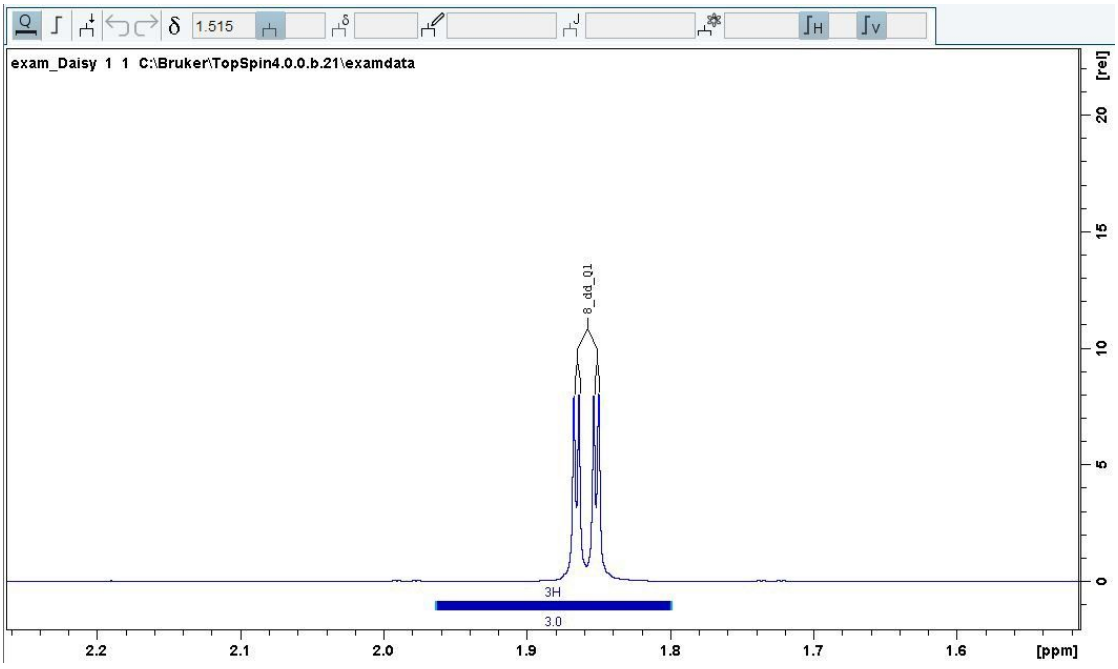
Undo last action

Redo last action

4.2.2 CMC-assist Toolbar Functionalities

4.2.2.1 Zoom to Quantification Range

In order to activate this zoom mode, click the button (the zoom mode is active as long as the corresponding button appears yellow). Another click on this button deactivates the zoom mode again.



This special zoom mode becomes effective for every dataset that is newly loaded in the data window and was analyzed in automation previously. Instead of the entire spectrum, only a certain region of the spectrum is displayed. This zoomed region shows the first multiplet used

for quantification including the calculated concentration at the center of the data window. In the case no quantification information exists for the opened dataset, the entire spectrum will be displayed.

4.2.2.2 Define New Integrals

If the integration mode is active, the corresponding toolbar icon is highlighted.

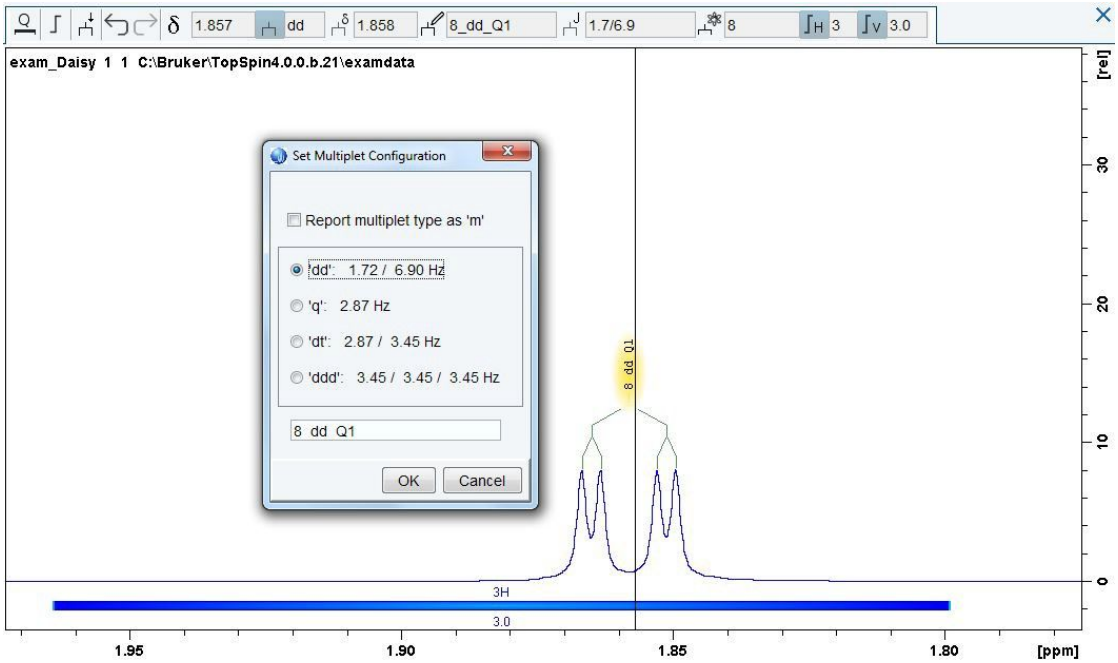
- Define integral

  Left-click-hold and move the mouse
- There are two alternative options to exit the integration mode
  - Left-click the yellow highlighted icon
  - Right-click in the data window and select **End Integral Definition**

4.2.2.3 Define New Multiplet

This highlighted toolbar icon indicates that the multiplet analysis mode is active and the cursor line becomes dashed.

- Define multiplet
  - Left-click on the peaks which belong to one multiplet
  - Right-click in the data window and choose *Finish Multiplet Definition*
  - Depending on the number of peaks which define the multiplet, a dialog appears that lists all possible multiplet configurations. Selecting any of the listed options will display the corresponding multiplicity in the data window. If none of the suggested multiplet configurations is appropriate, it still leaves the possibility to tick **Report multiplet type as 'm',** which results in reporting no multiplicity and no coupling constant. In addition, the labeling of the multiplet can be changed.

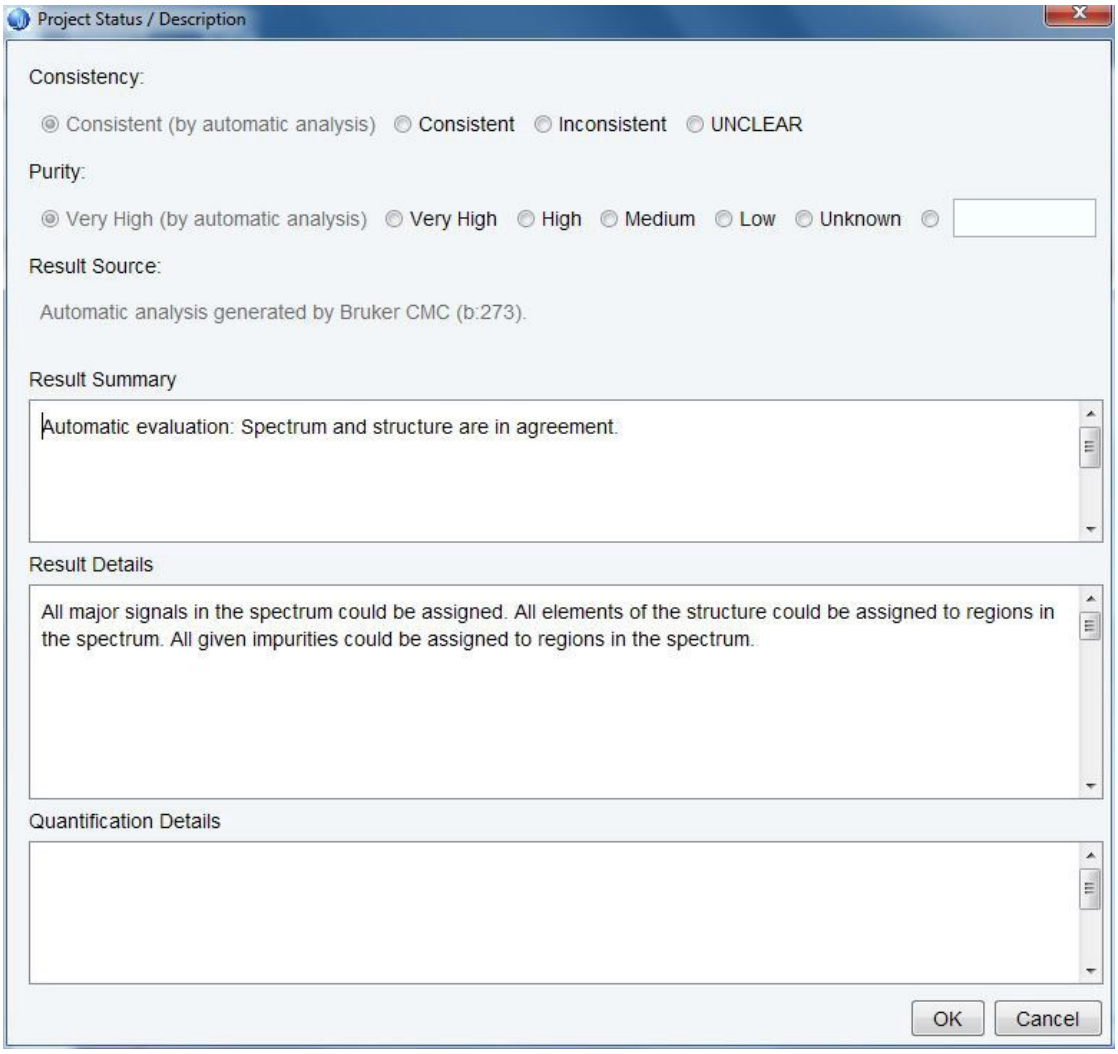


Right-clicking in the data window after peak picking gives the opportunity to delete all the peaks by choosing *Cancel*.

- Exit multiplet analysis - Left-click the yellow highlighted button

4.2.2.4 Show and Edit Project Status Information

Description and status of the current project can be modified or generated via the dialog that pops up when clicking on the corresponding tab *Consistency*. Along with the consistency status, the purity as well as statements regarding the result can be viewed and edited in the resulting window.



The project status information can also be accessed by clicking the color-coded consistency button below the molecular viewer.

In addition to the definition of the consistency status, statements concerning the results can be edited. All the entered information will be taken over and marked as manually modified if creating a short or full report for this dataset.

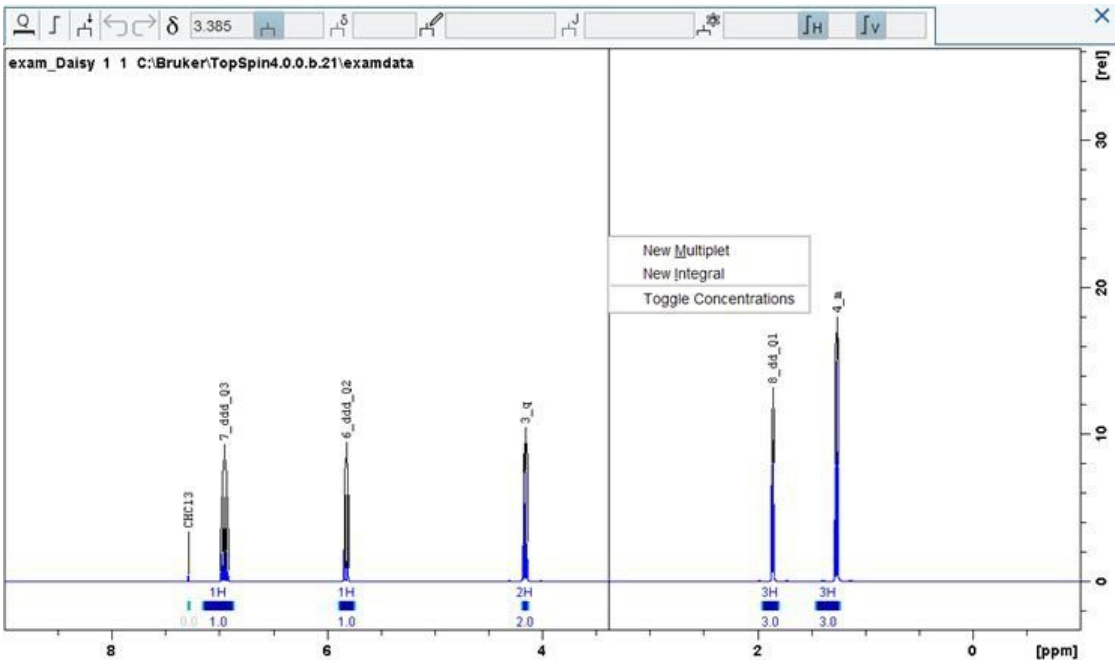
4.3 CMC-assist Data Window - 1D Data

It is also possible to analyse a spectrum just using the mouse button functionalities. Depending on the position of the cursor within the data window different actions are executable.

4.3.1 Basic Functionalities

When the cursor line resides next to any integral or multiplet without any highlighted region right-clicking offers the following basic functionalities:

- **New Multiplet:** activates the button **Define new multiplet** (multiplet analysis as mentioned above) and automatically exits the mode after defining one multiplet
- **New Integral:** enters the integration mode (integration as described for the button **Define new integrals**) and automatically quits the mode after a single integration
- **Toggle Concentration:** switches between displaying and hiding the calculated concentration



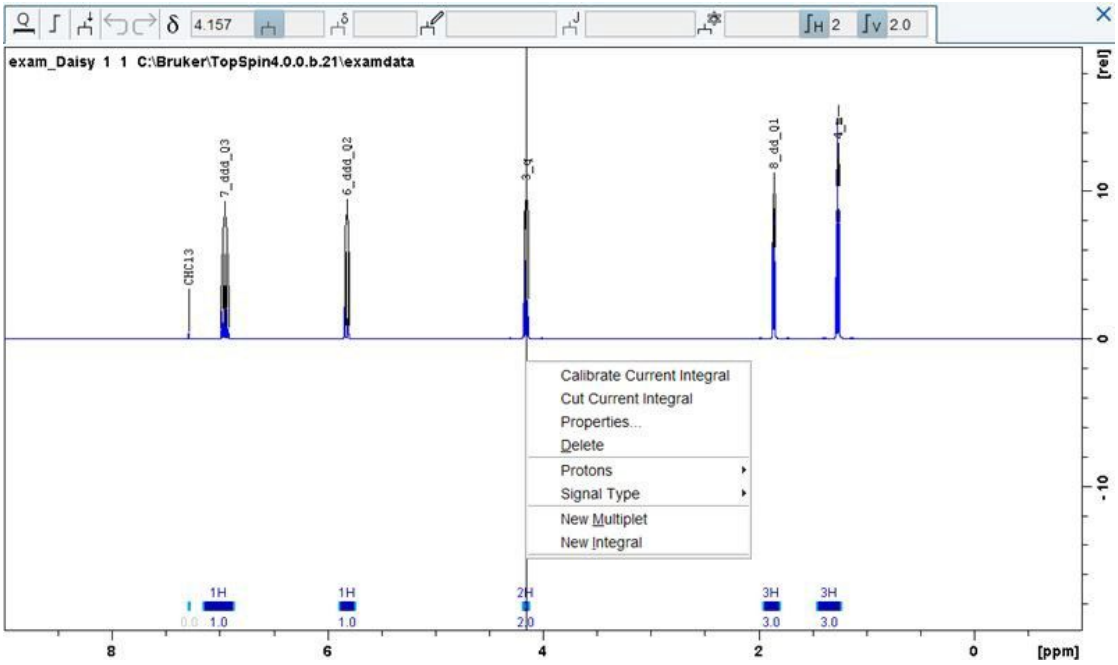
4.3.2 Integration

If the signals of the spectrum have been analyzed with respect to integral and multiplicity, the functionalities available via the mouse buttons depend on the position of the cur- sor within the data window. When the cursor line highlights a multiplet/integration region, right-clicking with the cursor below the baseline opens the integration menu, whereas right-clicking above the baseline enters the multiplet menu.

Right-clicking below the baseline opens a pop-up window with the following choices:

- **Calibrate Current Integral:** enable calibration of the selected integral
- **Cut Current Integral:** cut the integral exactly at the corresponding cursor position

- **Properties:** allow editing of several properties, like definition of the status (substance, mixture, or impurity) and the appropriate proton number for the selected integral. Furthermore, an annotation can be made and the concentration can be calculated based on this integral.
- **Delete:** delete the selected integral
- **Protons:** list proton numbers in order to set a new proton content for the selected integral
- **Signal Type:** another way to define the status of the selected integral as substance, mixture, or impurity
- **New Multiplet:** single multiplet definition as mentioned above
- **New Integral:** single integration as described above



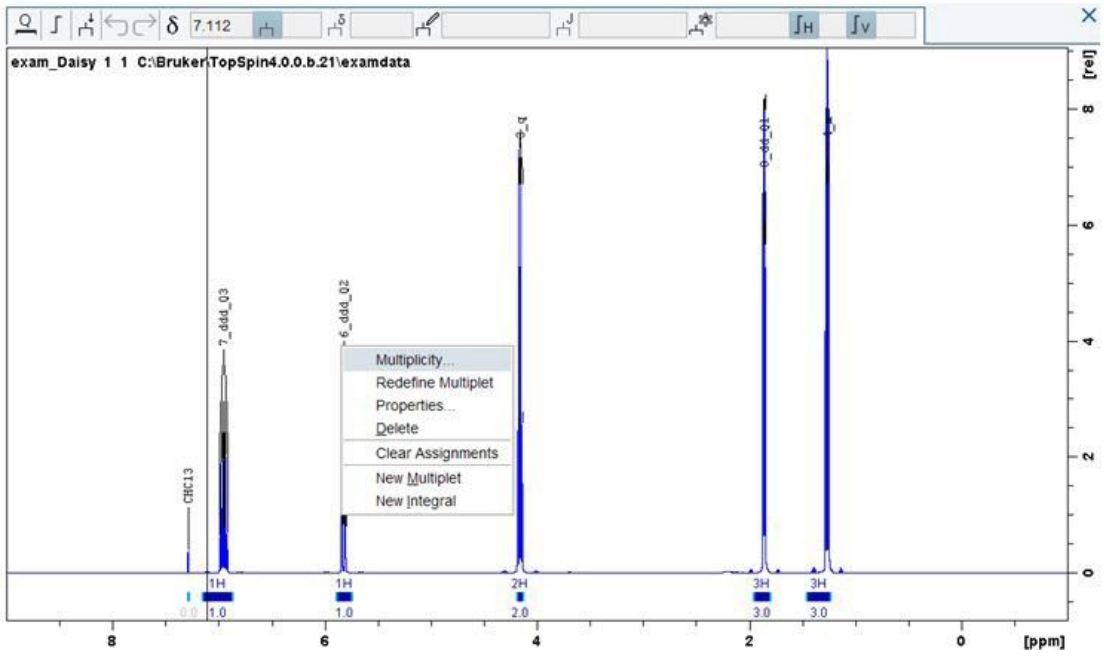
4.3.3 Multiplet Analysis

In the case that integral and multiplicity are defined for one signal, the cursor line has to highlight a multiplet/integration region and the cursor has to be positioned anywhere above the baseline in order to access the multiplet menu. Otherwise, right-clicking underneath the baseline will open the integration menu.

Right-clicking above the baseline makes the following functionalities available:

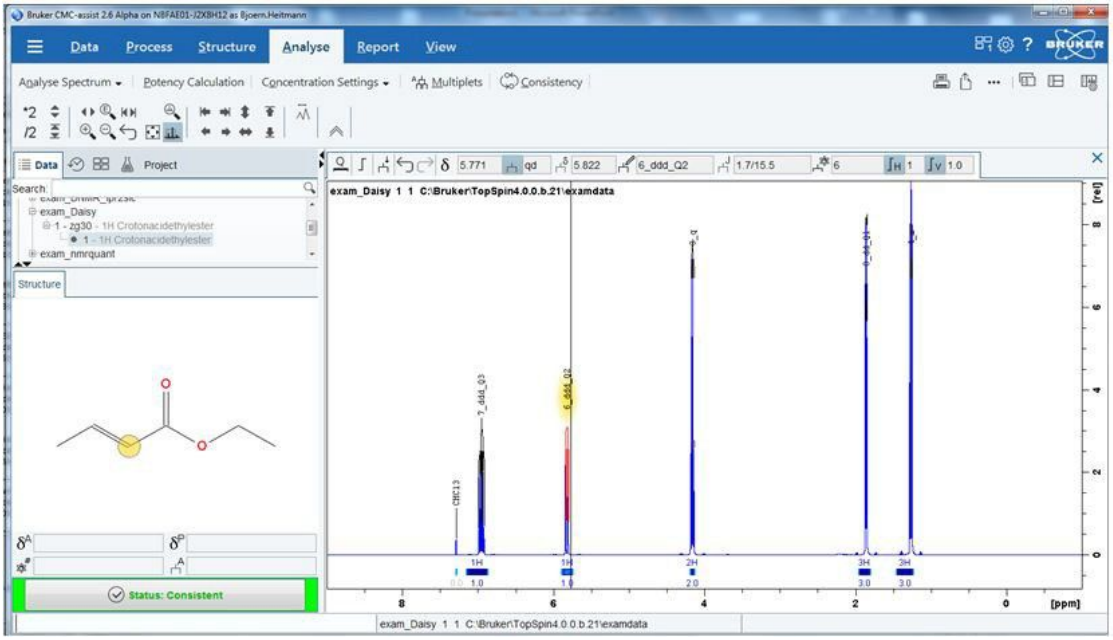
- **Multiplicity:** open a dialog that lists all possible multiplet configurations, depending on the number of peaks which define the multiplet. Selecting any of the listed options will display the corresponding multiplicity in the data window. If none of the suggested multiplet configurations are appropriate, it still leaves the possibility to tick **Report multiplet type as 'm',** which results in reporting no multiplicity and no coupling constant. In addition, the labeling of the multiplet can be changed.
- **Redefine Multiplet:** delete the current multiplet and enter the multiplet analysis mode to newly define this multiplet
- **Properties***:* allow editing of several properties, like redefining the multiplicity and the assigned atoms for the selected multiplet. Furthermore, an annotation can be made and the label can be changed.

- **Delete:** delete the selected multiplet
- **Clear Assignments:** clear the connection of the selected multiplet to any atom
- **New Multiplet:** single multiplet definition as mentioned above
- **New Integral:** single integration as described above



4.3.4 Assignment

If an assignment already exists, moving the cursor to any atom of the displayed structure will highlight this atom as well as the corresponding multiplet. The same holds true the other way around, moving the cursor line to any multiplet will highlight the atom(s) of the molecule assigned to this multiplet.



There are two different ways to connect a signal in the spectrum with the respective atom(s) of the molecular structure, depending on the cursor position.

- Cursor at highlighted atom of the molecule

  Left-click-hold on any atom of the molecule and move the mouse to the corresponding multiplet. This will assign this atom to the selected multiplet.

- Cursor at highlighted multiplet

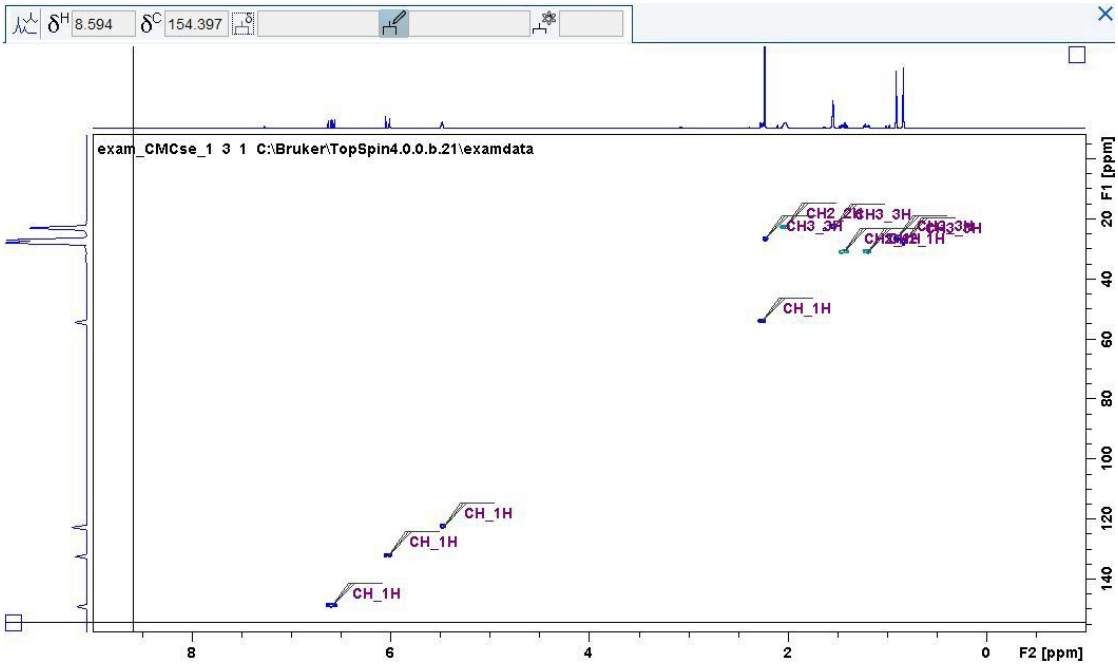
  Left-click-hold on any multiplet and move the mouse to the appropriate atom of the displayed structure. This will connect the selected multiplet with this atom.

4.3.5 Increasing Horizontal Scaling

[Shift+left-click-hold] always enables increasing the horizontal scaling (zoom in).

Besides, only left-click-hold also allows increasing the horizontal scaling (zoom in), if the cursor is positioned below the baseline or above any multiplet.

4.4 CMC-assist Data Window - HSQC



The data window consists of 3 sub-locations: the spectrum, the F1 projection, and the F2 projection. Depending on where the cursor is within these sub-locations, different functionalities are available.

4.4.1 Basic Functionalities

When the cursor resides on top of the F1 or F2 projection, right-clicking allows the following viewing option for the respective dimension:

- **External Projection***...:* Allows the projection of a different spectrum instead of the respective projection
- **Internal Projection***:* Will switch the projection back to the internal one if it had previously been altered
- **Baseline at Center:** This option centers the baseline of the projection in its respective sub-location
- **Baseline at Bottom:** Puts the baseline of the projection at the bottom edge of its respective sub-location (at the edge of the 2D spectrum).

4.4.2 Multiplet Analysis

When the cursor lies over the main part of the spectrum, right-clicking brings up the option **New Multiplet.** Selecting this option manually creates a multiplet at the location of the click. The resulting dialog allows the setting of the multiplet label.

If the spectrum has been analyzed previously, right-clicking when the cursor lies over a multiplet, it will be highlighted and right-clicking offers the following options:

- **Properties***:* Brings up a window where the multiplet label can be edited
- **Delete:** Deletes the highlighted multiplet
- **Clear Assignment:** Disconnects the multiplet from its assignment to in the structure
- **New Multiplet:** Creates a new multiplet

4.4.3 Multiplet Assignment

Each multiplet in the spectrum can be assigned to the structure. When the spectrum has been automatically analyzed, each multiplet has been assigned to the structure. To view this assignment, move the mouse over the desired multiplet. This will highlight the multiplet, and the atom it is assigned to, in yellow.

To manually assign a multiplet to the structure, left-click on it and drag to the desired atom in the structure. This can be repeated for the same multiplet to more than one atom in the structure.

5 Data Processing

The software package CMC-assist is designed to view, process, and analyse 1D 1H, 1D 13C, HSQC, and HMBC NMR spectra. Consequently, only this type of spectra can be processed with the software package CMC-assist. The corresponding *Process* menu gives the opportunity to manually process the acquired data; or to automatically process single spectra as well as batches of spectra in automation.

5.1 Automatic Processing

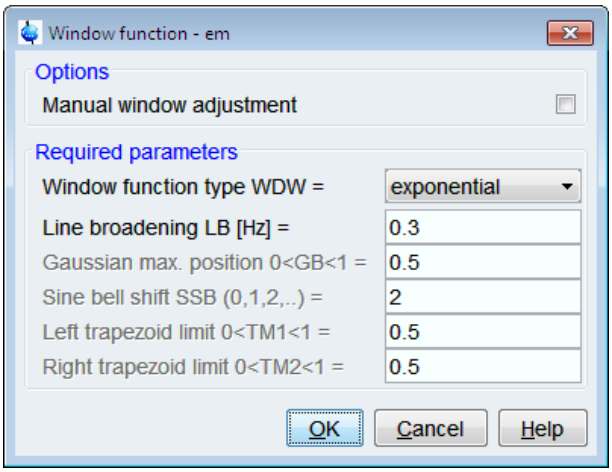
Bruker 1D 1H, 1D 13C, HSQC, and HMBC NMR spectra can be processed in a fully automated way. After opening appropriate NMR data, clicking the tab **Process Spectrum** will execute the standard processing routine for the selected spectrum. For 1D data, this includes an exponential window function, fourier transformation, automated phase correction, and automated baseline correction.

5.2 Interactive Processing - 1D Data

Besides the automatic processing, it is also possible to interactively process the NMR data. Advanced users, who are familiar with the software package TopSpin, can enter all the known single commands for processing 1D spectra on the command line.

5.2.1 Window Function

By clicking the down arrow on the right side of the tab **Process Spectrum** one can choose any window function for multiplication together with their required parameters.



5.2.2 Phase Correction

Subsequently, the phase can be either corrected manually by clicking the tab **Phase** or automatically by choosing one of the various modes accessible via the down arrow on the right side of the **Phase** tab.

5.2.2.1 Automatic Phase Correction

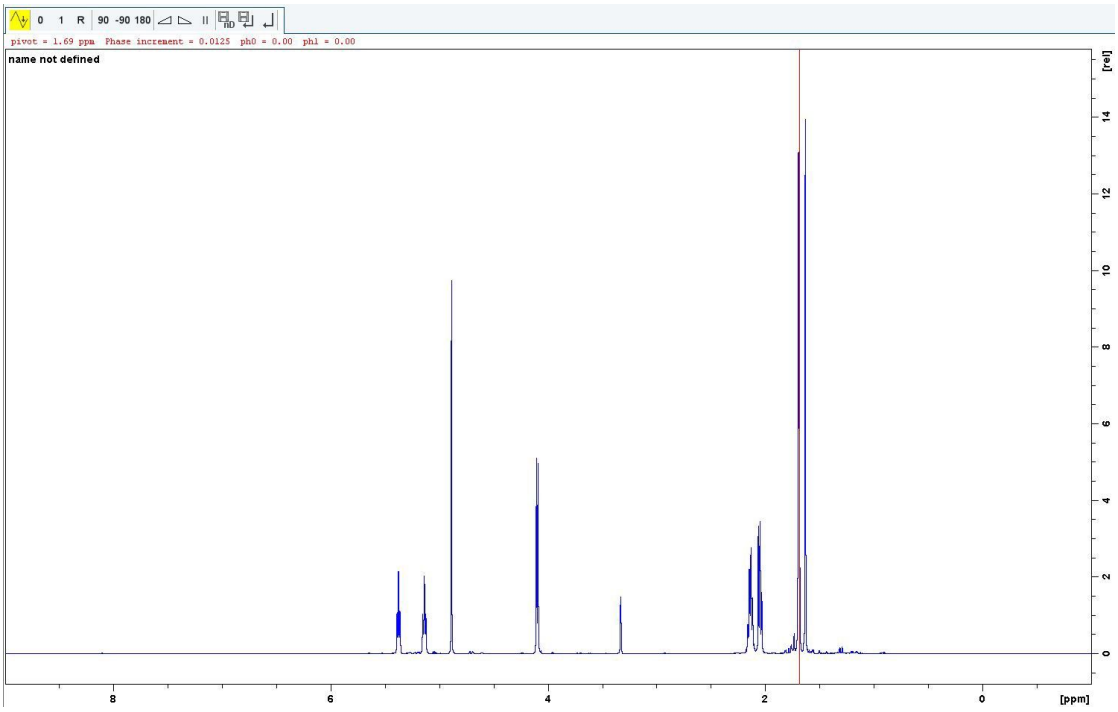
- **Phase Spectrum Using PHC0/PHC1** (**pk**): perform phase correction with existing values for 0th and 1st order
- **0th + 1st Order Correction** (**apk**): adjust phase with automatically determined values for 0th and 1st order
- **0th Order Correction Only** (**apk0**): phase spectrum automatically using only 0th order phase correction
- **Alternate Algorithm 1** (**apks**): apply an alternate algorithm for 0th and 1st order phase correction
- **Alternate Algorithm 2** (**apkm**): 0th and 1st order phase correction using another alternate algorithm

5.2.2.2 Manual Phase Correction

Besides clicking the *Phase* tab, entering **.ph** on the command line also switches to the phase correction mode and replaces the standard CMC-assist toolbar by the toolbar specific for interactive phase correction.

The yellow button  indicates that the phase correction mode is active.

- Left-click-hold the button  and move the mouse until the reference peak is in pure absorption mode
- Left-click-hold the button  and move the mouse until the entire spectrum is in pure absorption mode
- To save the corrected phase and return to the standard CMC-assist toolbar click one of the buttons with the disc



By default, the pivot point is set to the peak with the highest intensity of the displayed region of the spectrum (recognizable by the red line). In order to change the pivot point, right-click on the desired position and select **Set Pivot Point** from the pop-up menu.

Right-clicking in the data window opens a dialog with the option **Calculate ph0**, that executes an automatic phase correction of zero order according to the calculated value.

90 Perform 90° zero order phase correction (**.ph90**)

-90 Perform -90° zero oder phase correction (**.phm90**)

180 Perform 180° zero order phase correction (**.ph180**)

R Reset zero and first order phase values (**.phr**)

Increase (double) mouse sensitivity (**.inc**)

Decrease (halve) mouse sensitivity (**.dec**)

Reset mouse sensitivity

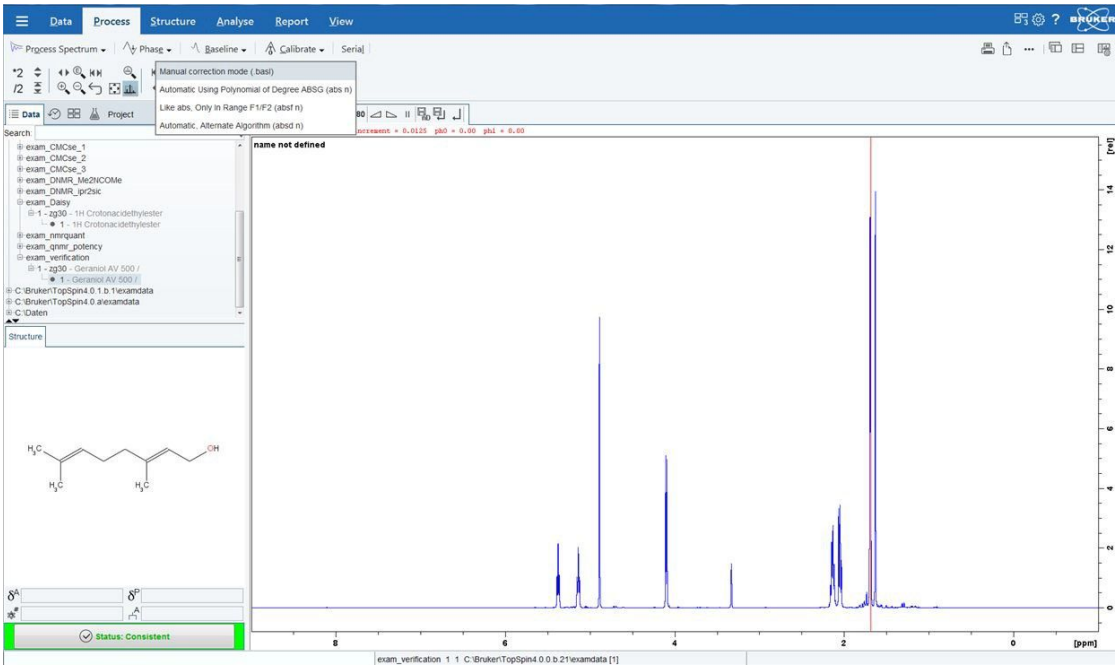
Execute phase correction, save current phase correction values, and leave the phase correction mode

Return and discard any changes

5.2.3 Baseline Correction

For the baseline correction all available options, manual and automatic, are listed in the submenu, whereas the tab *Baseline* itself executes an automatic baseline correction using polynomial degree (**absn**).

5.2.3.1 Automatic Baseline Correction

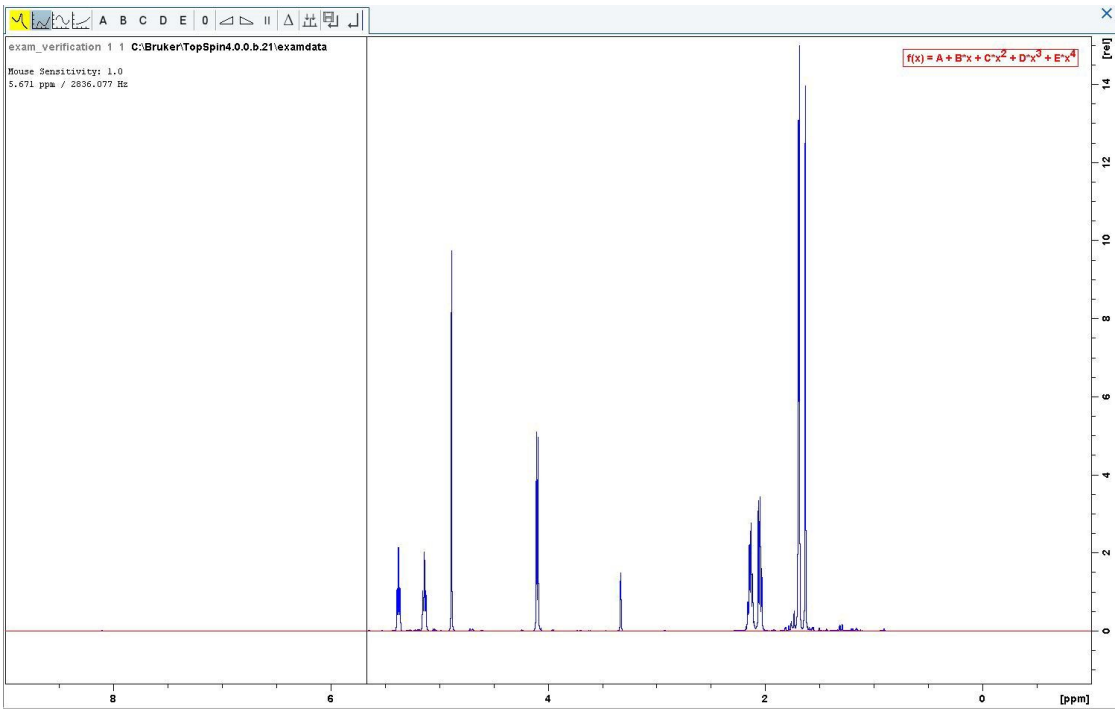


- **Automatic Using Polynomial of Degree ABSG** (**abs n**): perform automatic baseline correction using a polynomial function of degree ABSG
- **Like abs, Only In Range F1/F2** (**absf n**): adjust baseline the same way as **abs n** just within the defined range F1/F2
- **Automatic, Alternate Algorithm** (**absd n**): apply an alternate algorithm for automatic baseline correction

5.2.3.2 Manual Baseline Correction

Besides clicking the submenu **Manual correction mode,** entering **.basl** on the command line also switches to the baseline correction mode and replaces the standard CMC- assist toolbar by the toolbar specific for baseline correction.

 The yellow button indicates that the baseline correction mode is active.



- Left-click the following button  for polynomial baseline correction

  In the data window a red horizontal line will appear as well as the equation that describes the polynomial function f(x) = A + Bx + Cx2 + Dx3 + Ex4.
- Left-click-hold the button for variable A and move the mouse until the red line coin- cides with the first point of the spectrum
- Repeat the procedure of variable A for all the other variables (B, C, D, and E) until the red line matches the entire baseline of the spectrum
- Left-click the following button  for sine baseline correction

  In the data window a red horizontal line will appear as well as the equation that describes the sine function f(x) = A + Bsin(Cx+D).
- Left-click-hold the button for variable A and move the mouse until the red line coin- cides with the first point of the spectrum
- Repeat the procedure of variable A for all the other variables (B, C, and D) until the red line matches the entire baseline of the spectrum
- Left-click the following button  for exponential baseline correction

  In the data window a red horizontal line will appear as well as the equation that describes the exponential function f(x) = A + Bexp(Cx).
- Left-click-hold the button for variable A and move the mouse until the red line coincides with the first point of the spectrum
- Repeat the procedure of variable A for the other variables (B and C) until the red line matches the entire baseline of the spectrum

Before actually performing the baseline correction, the result can be previewed by clicking the following button:

 Preview corrected spectrum

The corrected spectrum will be displayed in red. If the baseline is correct, click the save button  . If further correction is needed, click the yellow highlighted preview button  again to show the original spectrum and the red correction line.

 Reset red correction line to zero

 Increase (double) mouse sensitivity (**.inc**)

 Decrease (halve) mouse sensitivity (**.dec**)

 Reset the mouse sensitivity

 Execute baseline correction, save current baseline correction values, and leave the baseline correction mode

 Return and discard any changes

Click the following button  to define points for the cubic spline baseline correction.

The toolbar of the data window will change and the cursor line turns red. If a list of baseline points already exists, a pop-up dialog gives the opportunity to either overwrite or append to these points. **Append** displays the labels of the existing points on the screen, whereas **Overwrite** does not show any labels. However, the existing points are only overwritten, when new baseline points are defined and saved. In order to define new baseline points, move the cursor line to an appropriate baseline position and left-click at that position. This has to be done for at least five baseline points.

The actual baseline correction is only performed when clicking the save button in the spline baseline and the interactive baseline mode. Existing points can also be deleted by right-clicking on the corresponding point position and choosing **Delete Current** from the pop-up menu. The same applies for removing all existing points, just select **Delete All** from the appearing window.

Apart from the buttons for returning from the cubic spline baseline mode with or without saving (mentioned above), right-clicking in the data window offers the options **Save&Quit** or **Quit**, respectively.

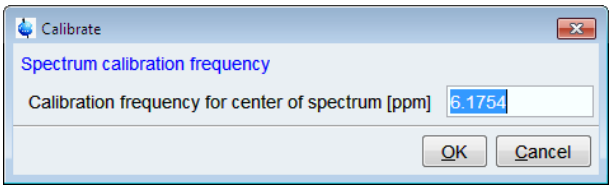
5.2.4 Calibrate Axis

Clicking the  *Calibrate* or entering **.cal** on the command line replaces the standard CMC-assist toolbar by the toolbar of the calibration mode.This calibration mode enables manual definition of the reference frequency. When the calibration button  is yellow it indicates that the calibration mode is active.

5.2.4.1 Calibrate to the Center of the Spectrum

On the one hand, the reference frequency can be changed by redefining the frequency of the center of the spectrum.

Clicking on the button  opens a dialog where the new frequency for the center of the spectrum can be entered.



5.2.4.2 Redefining the Reference Frequency

On the other hand, the reference frequency can be modified by positioning the red cursor line at the desired peak, left-clicking at that position, and entering the new frequency of this peak in the upcoming dialog.

In both cases, the spectrum will be calibrated and the calibration mode will be closed automatically.

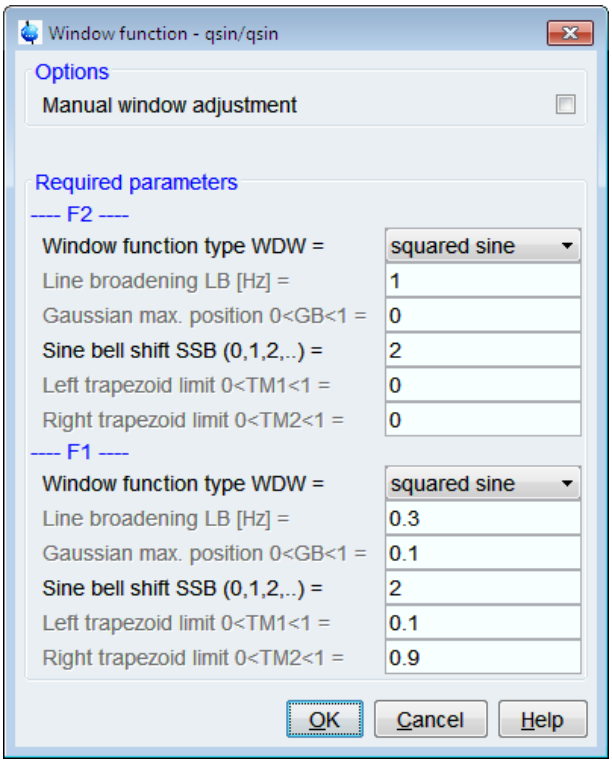
5.3 Interactive Processing - 2D Data

Along with the option to automatically process 2D data, CMC-assist offers also the possibility for interactive data processing.

5.3.1 General Processing

5.3.1.1 Window Function

To set the desired window function, click the down arrow on the tab  **Process Spectrum** and select **Window Multiplication**. From the resulting dialog, one can set the desired window functions and parameters for each dimension. After setting the parameters, click **OK** to apply them.



5.3.1.2 Magnitude Spectrum in F2

To access this option, click the down arrow on the tab  **Process Spectrum** and select **Magnitude Spectrum in F2**. Selecting this option converts the spectrum in the F2 dimension to a magnitude spectrum.

5.3.2 Phase Correction

The phase of a 2D spectrum can be corrected either automatically or manually via the options available in the tab **Phase**.

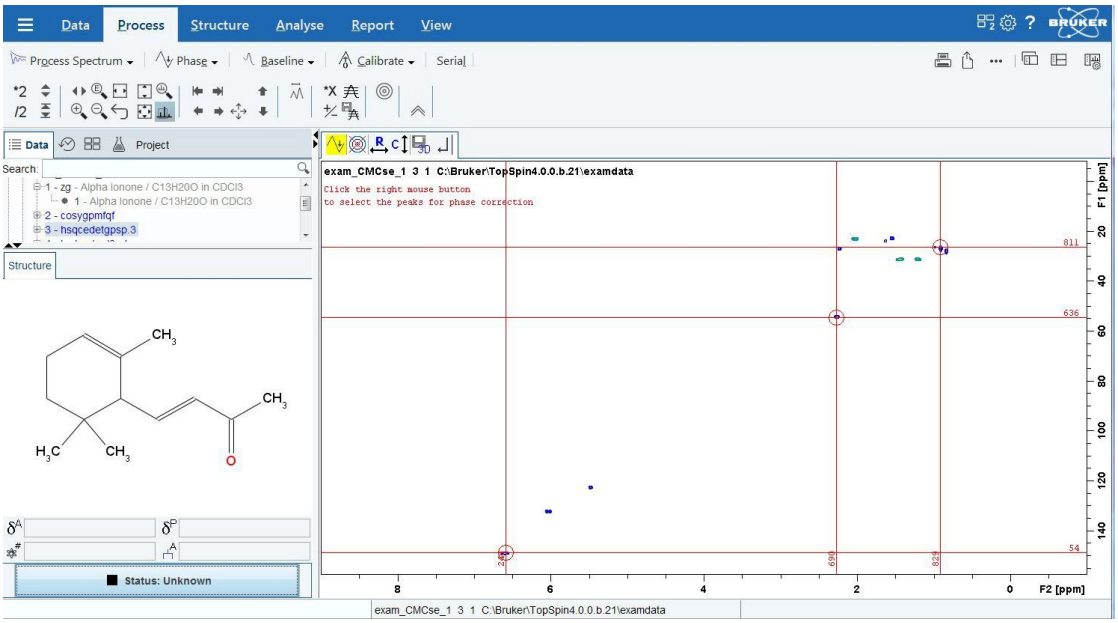
5.3.2.1 Automatic Phase Correction

Clicking the down arrow on the tab **Phase** and selecting **Automatic Phase Correction** will perform an automatic phase correction of the 2D spectrum using the **apk2d** routine.

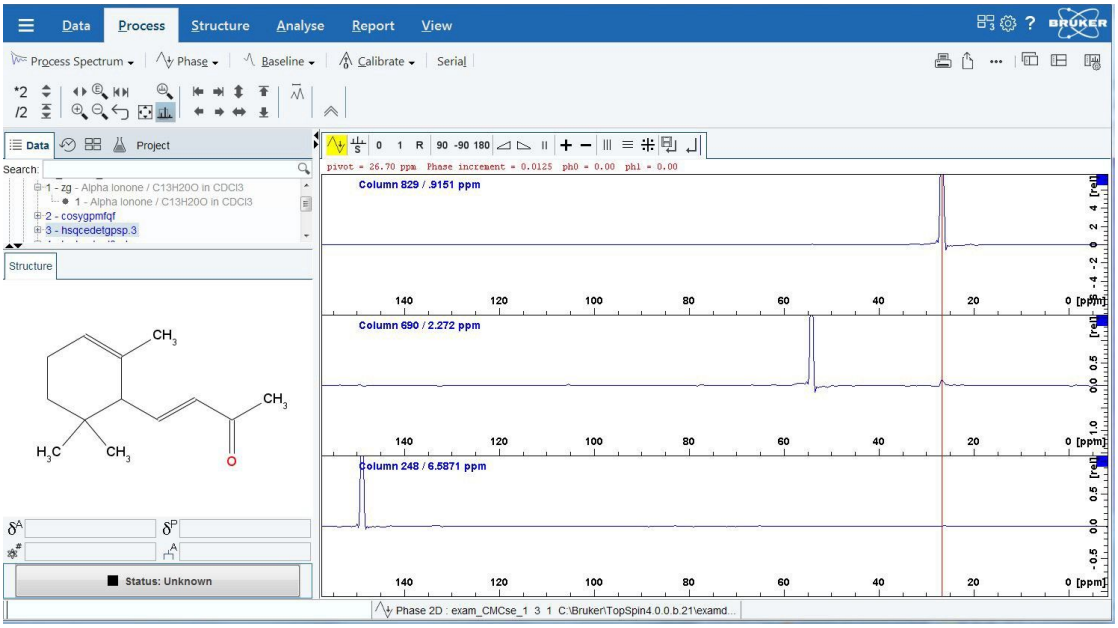
5.3.2.2 Manual Phase Correction

To access the manual phase correction mode, click the tab **Phase** or enter **.ph** on the command line. The steps to manually phase a 2D spectrum are described below.

- At least two peaks must be picked from different parts of the spectrum
- Zoom into the area of the chosen peak
- Right-click in the center of the chosen peak and select **Add**
- Repeat for all peaks desired



- Click the button which will open a new phasing mode



The toolbar and the right-click pop up menu offer all of the same capabilities as the 1D phase correction mode plus the following additional button capabilities:

+ Show the next row/column

− Show the previous row/column

||| Arrange rows/columns horizontally

≡ Arrange rows/columns vertically

⁜ Arrange rows/columns vertically in a split window

- By default all columns are selected as indicated by the filled blue squares. The red vertical line indicates the pivot point in the upper column.
- Click-hold the 0 button to perform a zero order correction
- Click-hold the 1 button to perform a first order phase correction
- Click the  button to execute the changes, save, and return
- Click the  button which will open the further phasing mode
- Adjust the phase in the same manner as for the columns

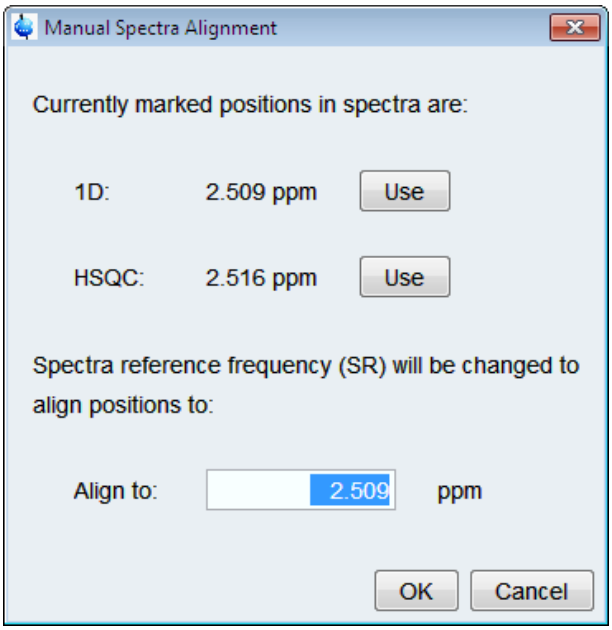
5.3.3 Calibrate Axis

As with 1D data, clicking the tab  **Calibrate** or entering **.cal** on the command line replaces the standard CMC-assist toolbar by the toolbar of the calibration mode. When the calibration button  is yellow it indicates that the calibration mode is active. The calibration functions work the same as with 1D data but the shift must be entered for both F1 and F2 dimensions.

5.3.3.1 Align 1D1H Spectrum with an HSQC Spectrum

It is also possible to align a 1D1H spectrum with e.g. an HSQC spectrum that is within the same CMC-assist project. To do this

- Open both spectra in different windows.
- Click the down arrow on the  **Calibrate** tab and select *Manual* **Spectra Alignment** or by typing **cmca_salign** on the command line. Click ok in the resulting dialog.
- Left-click on the signal to use a reference in the 1D1H spectrum and also in the HSQC spectrum.
- Select this menu item again to bring up the **Manual Spectra Alignment** dialog from here the reference frequency can be set for both spectra.



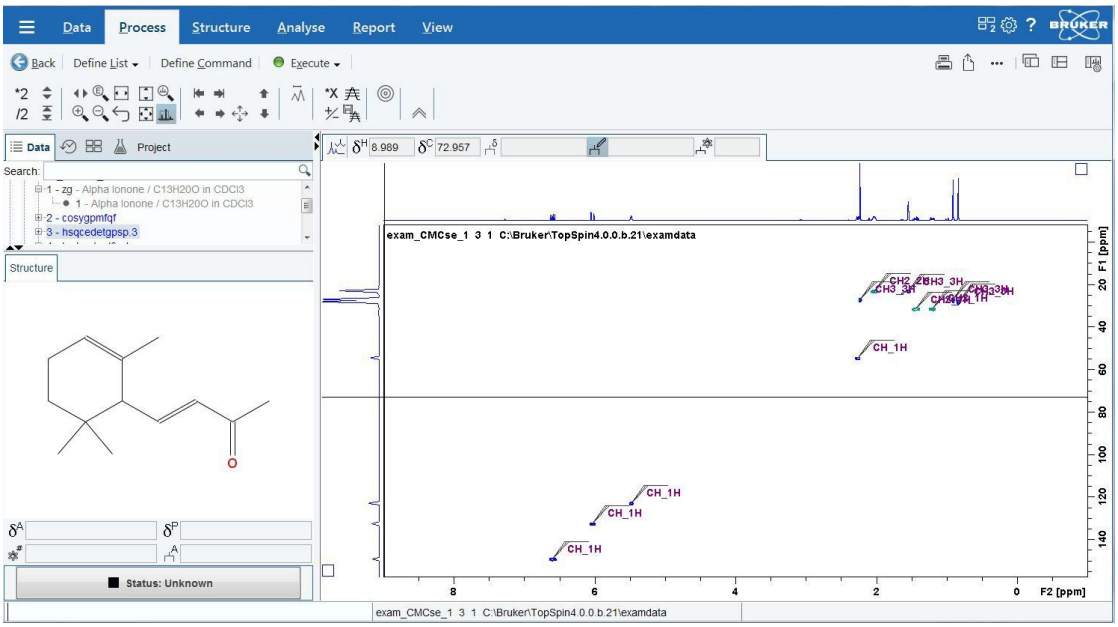
5.4 Serial

The command **serial** allows the handling of whole batches of datasets. The Serial mode can be accessed by typing **serial** on the command line or by clicking the **Serial** tab in the Data menu.

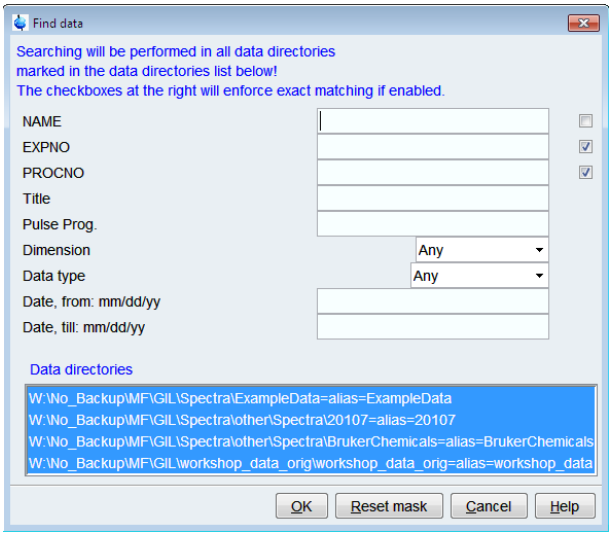
5.4.1 Generating a Serial Processing List

First of all, a serial processing list has to be created in order to define the different paths of the spectra that should be processed or analyzed the same way. This serial processing list can be generated as follows:

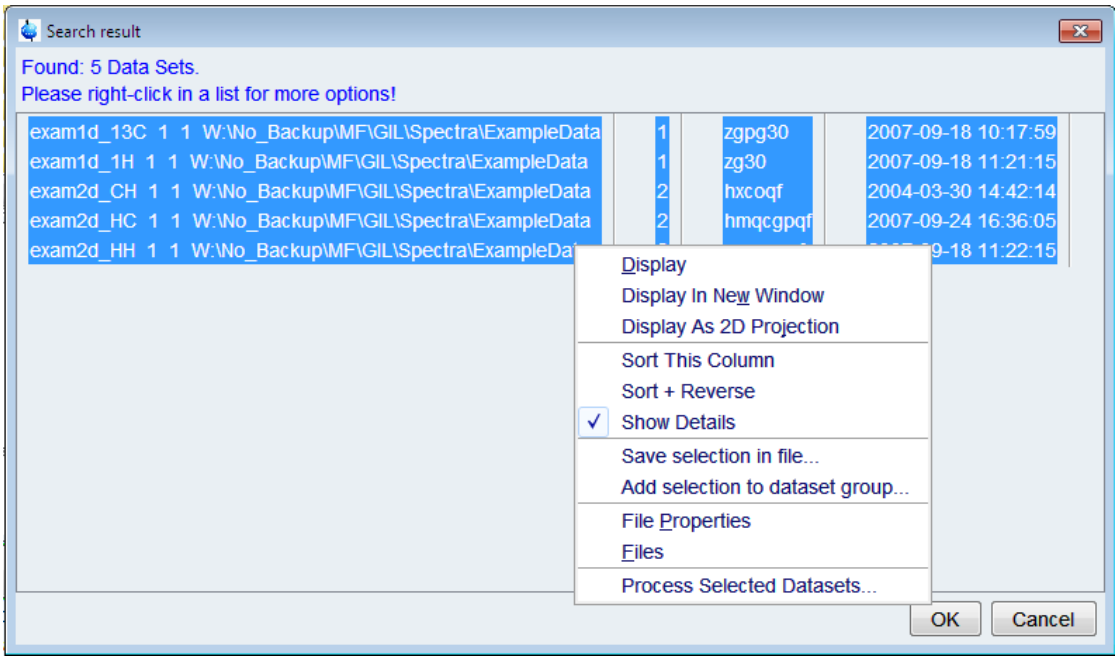
- Clicking on the tab **Serial** opens a new submenu with the tabs **Define List, Define Command,** and **Execute**
- The down arrow on the right side of the tab **Define List** offers the option **Build dataset list using ”find”,** which opens a dialog



- The search will be performed in all data directories marked in the data directories list and can be further specified by certain entries for EXPNO, PROCNO, Dimension, etc.



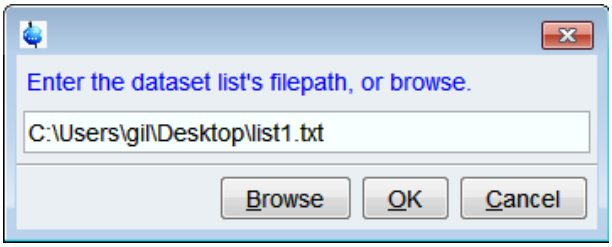
- Enter the search items in the upper part of the dialog
  - Exact matching is performed for the dataset variables NAME, EXPNO, and PROCNO, if the corresponding checkbox at the right is ticked
  - Entries in the fields **Title** and **Pulse Prog**. cause searching for items containing these specified strings
  - The search can be restricted to data created between specified dates, referring to the acquisition dates
- Select the **Data directories** to be searched in the lower part of the dialog (if no directories are selected, all directories will be used for search)
- **OK** starts the search and the results will be displayed within a new dialog



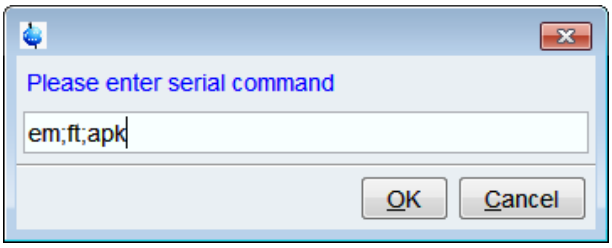
- In order to save these search results as a serial processing list, all entries of the list have to be marked using [Ctrl+a]. Right-clicking on the marked list provides the opportunity **Save Selection In File** to save the selection in a text file.

5.4.2 Executing Serial Commands

If a serial processing list already exists, clicking on the tab **Define List** enables browsing for the respective text file.



Subsequently, the serial processing command has to be entered via the tab **Define Command** and executed via the corresponding tab.

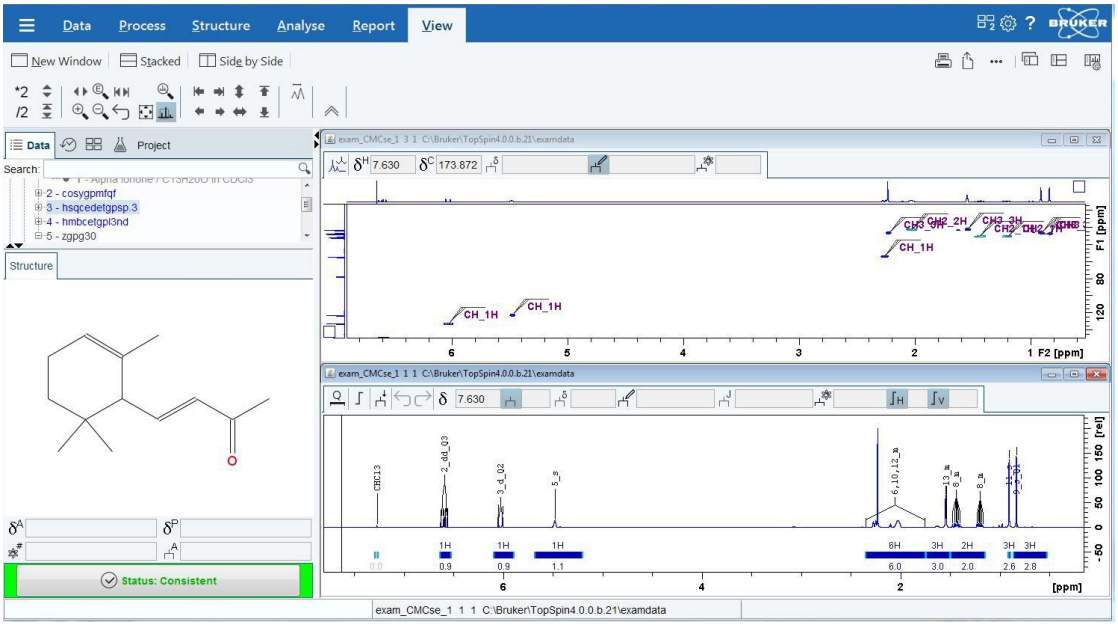


The defined serial command can consist of just a single command (e.g., efp) as well as several commands separated by semicolons (e.g., em;ft;apk) or macros (e.g., xmac my-macro).

After finishing the serial processing a feedback dialog pops up. Finally, the **Back** button will exit the **Serial** submenu.

5.5 Viewing Spectra

If more than one internal window is opened, all windows can be arranged  **Side by Side** or  **Stacked** by clicking the corresponding tabs in the menu **View**.



Further viewing options can be chosen from the menus in the upper right corner of CMC-assist.

6 Data Analysis

The software package CMC-assist offers the possibility to run a fully automated spectra analysis as well as to interpret the NMR data manually including integration, multiplet analysis, and quantification. All the features associated with the spectra interpretation are combined in the **Analyse** menu.

6.1 Automatic Spectra Analysis

The CMC-assist offers an automatic analysis of 1D 1H NMR spectra with respect to integration regions, proton numbers, multiplet interpretation, assignment, concentration determination, purity, and structure consistency check.

In conjunction with the 1D1H spectrum, HSQC, 1D13C, and HMBC data may also be analysed. The analysis of the HSQC includes multiplet interpretation and assignment. The analysis of the 13C spectrum involves marking of the solvent (and any impurities) along with peak picking and the assignment of each peak in the spectrum to the mole- cule.

6.1.1 Automatic Analysis of Single Spectra

Clicking on the tab **Analyse Spectrum** starts this automated interpretation of the currently displayed spectrum.

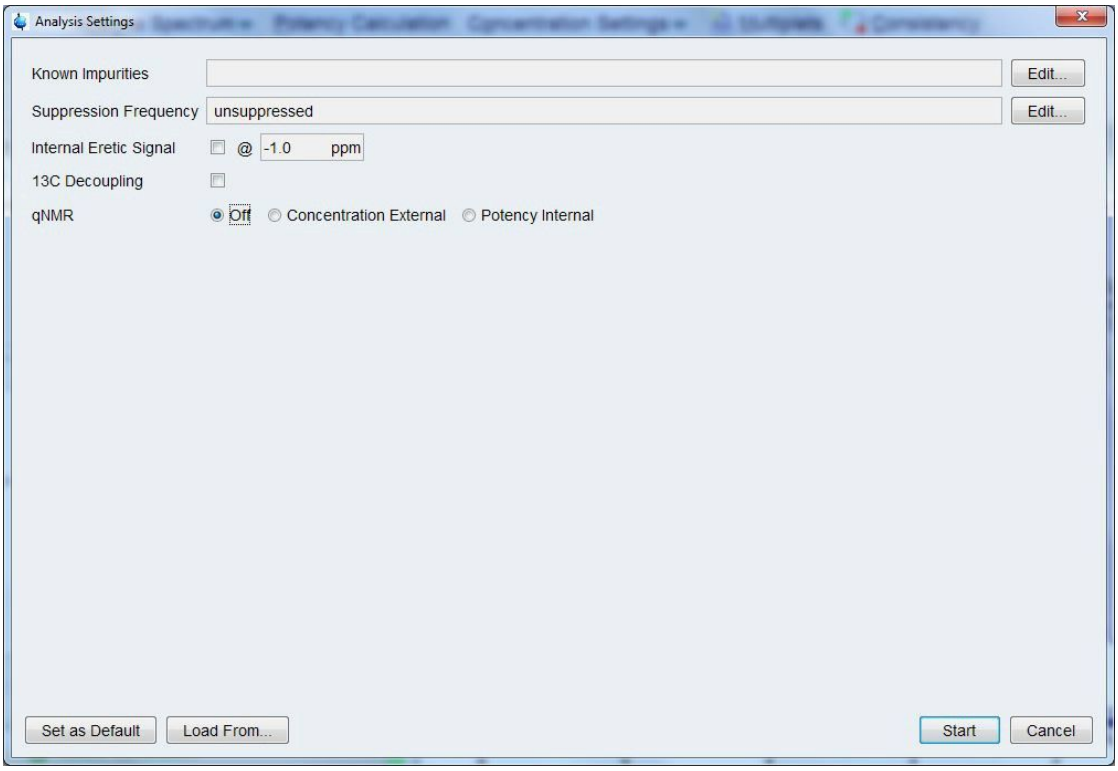
In the case the data have also been acquired in automation using the software package CMC-q available under TopSpin or the feature SmartDriveNMR integrated within IconNMR, all information needed for the automated analysis already exists within the dataset. Thus, clicking the tab **Analyse Spectrum** directly interprets the current dataset.

The automatic analysis of a single spectrum can only be done for 1D1H spectra. If an analysis of an HSQC or 1D13C spectrum is desired, it must be performed along with the corresponding 1D1H spectrum as a CMC-assist Project. For the analysis of the HMBC the spectra 1D1H and HSQC have to be present as well in the CMC-assist Project.

6.1.1.1 Analysis Settings

In the case the data have not been acquired with respective Bruker software packages and the analysis is being run for the first time on a dataset, some basic parameters have to be specified for the automatic analysis.

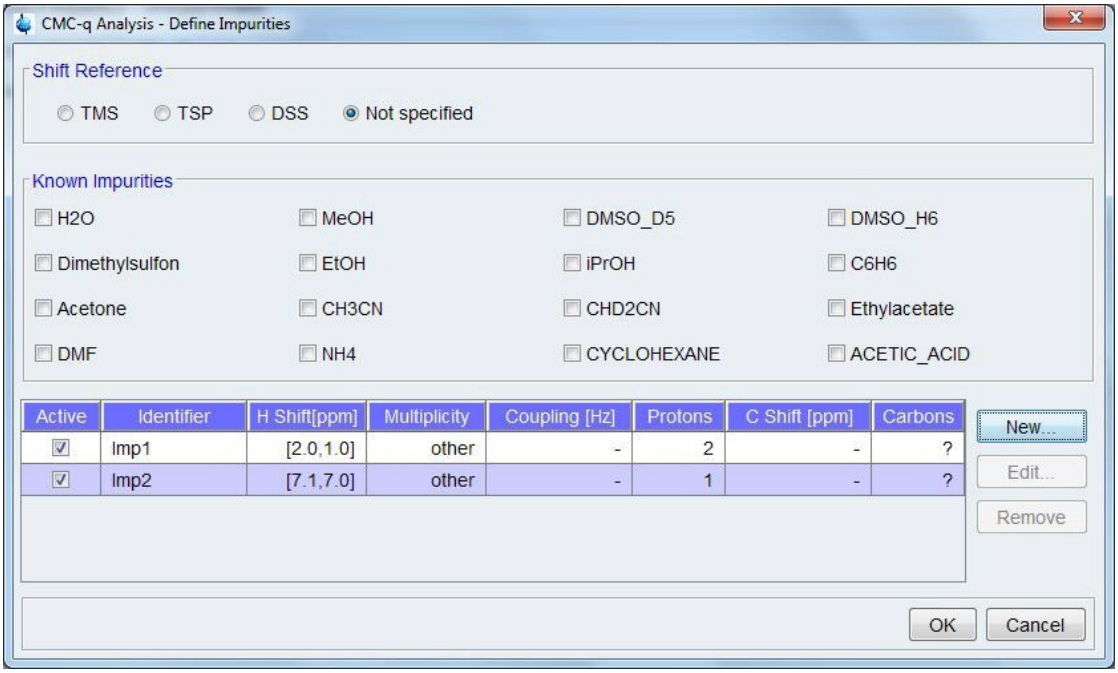
Clicking the tab **Analyse Spectrum** opens a dialog that shows the required information. These consist of the number of suppression frequencies, any known impurities that should be excluded from the analysis, possible 13C decoupling, existence of an eretic signal including its position, and for quantification purposes the path to the required calibration file.



All these settings can be changed any time by the submenu **Analysis Settings**, accessible by the down arrow on the right side of the tab **Analyse Spectrum**.

Besides substance signals, the spectrum might also be comprised of peaks originating from impurities. If any impurities are known to occur within the acquired dataset, these should be declared for the spectrum interpretation.

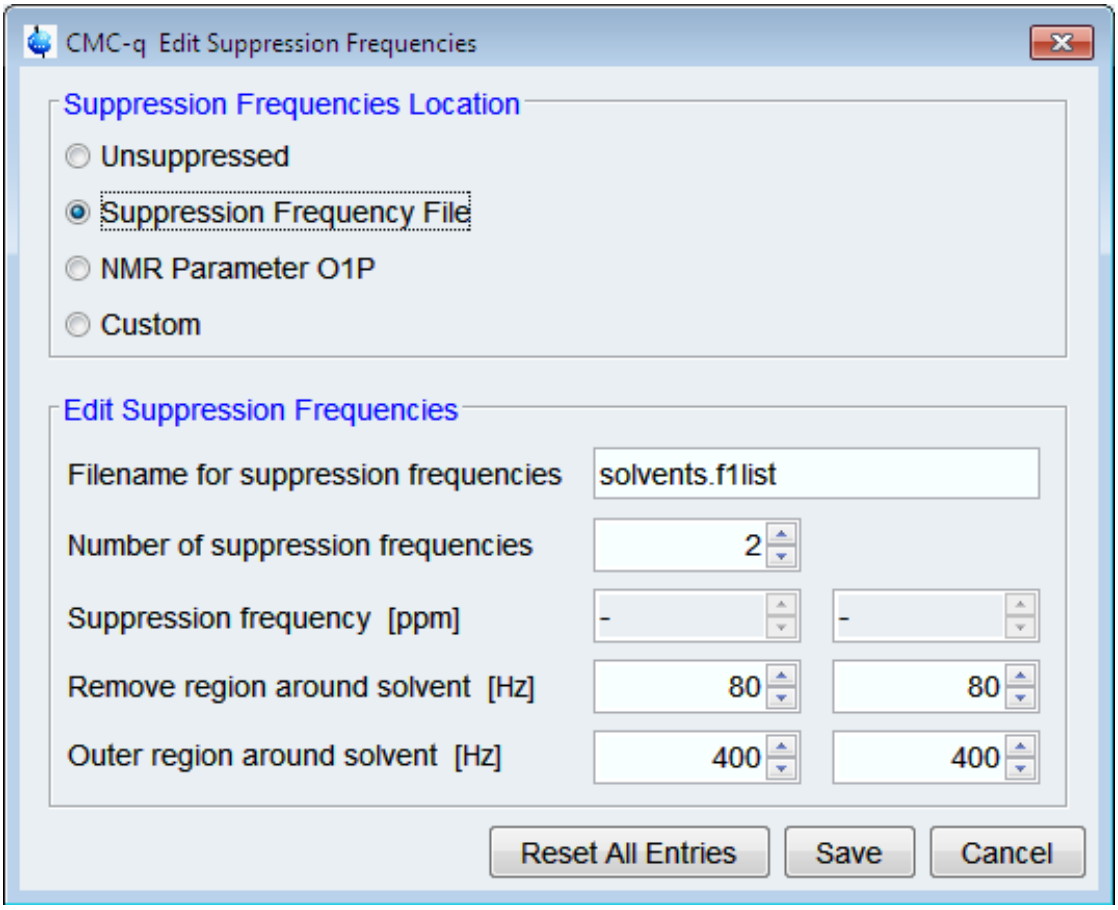
Clicking the button **Edit** on the right side of the field **Known Impurities** opens a new window where these impurities can be defined.



Some typical impurities regularly found within spectra of synthesized chemical compounds can be selected by just ticking the corresponding boxes. In addition, special or rarely appearing impurities can be defined individually. In order to add an impurity, click the tab **Create Impurity** and fill in any known information. Please note that the individually entered impurities have to be activated the same way as the predefined ones by ticking the respective boxes. Before leaving this dialog any changes should always be saved. If a single impurity will show more than one multiplet in any spectrum, each multiplet should be marked as its own impurity.

For automatic spectra analysis, information about the number of suppression frequencies is essential. In the case the data were acquired unsuppressed all expected signals should be visible within the spectrum. Whereas the suppression of solvent signals might result in missing peaks that should appear within the suppression region.

All the required information on the suppression frequencies can be edited in another dialog that will pop up when clicking the tab **Edit** on the right side of the field **Suppression Frequency.**



- Unsuppressed

  In the case the data were acquired unsuppressed just select this option and no further specification is required.

- Suppression Frequency File

  Suppressed spectra can be acquired in various ways, depending on the number and position of the solvent signal. Consequently, the information about the suppression frequencies can be stored in different parameters or files.

If several solvent signals have to be suppressed or the suppression frequency is off-resonant, all the information about number and position of the suppression frequencies is typically stored in a frequency file. In this case, the filename has to be specified in the lower part of the dialog (this file should be automatically generated during acquisition and located at the level of the expno within the dataset). In addition the field **Number of suppression frequencies** has to be edited and must match with the number of entries in the frequency list.

- NMR Parameter O1P

  Choose this option for on-resonant solvent suppression. By definition, this type of suppressed spectra possesses only one suppression frequency whose frequency corresponds to the NMR parameter O1P.

- Custom

  In the unlikely case that none of the above mentioned variations of suppressed spectra matches the type of acquired data, number and position of the suppression frequencies can be specified individually.

- Remove region around solvent

  The range in Hertz (Hz) defined in this field is completely ignored for spectra analysis. Typically, it covers the suppression frequency itself including the nearby suppression artefacts. Please note that a setting of 60 Hz means a region of ±30 Hz around the suppression frequency. Besides, each suppression frequency requires one entry, where the left entry in the dialog is related to the suppression frequency with the lower ppm value.

- Outer region around solvent

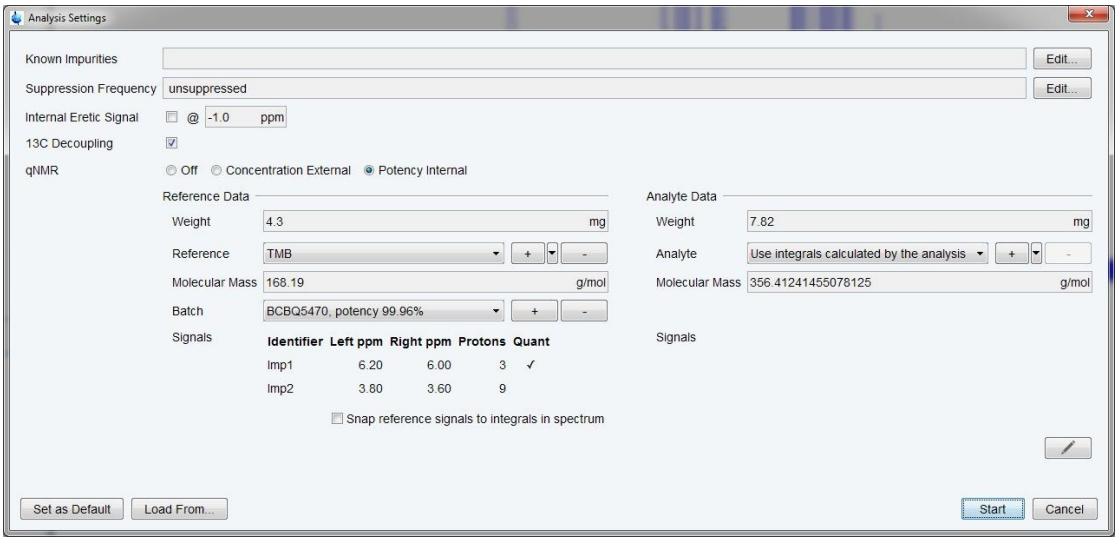
  Peaks within this outer region only experience minor disturbances from the suppression. For the automated spectra interpretation, these peaks are analyzed with respect to their multiplicity, however their integrated proton numbers are classified as not reliable.

If the spectrum contains an artificial signal for quantification, this box must be ticked and its position within the spectrum has to be stated. The artificial signal may either be a “real“ hardware ERETIC signal or it may be a signal introduced digitally after the data acquisition.

This box indicates whether the spectra were acquired with or without 13C decoupling.

Quantification of a dataset requires a reference spectrum of a molecule with known concentration. The data path to this reference spectrum has to be specified in order to calculate the concentration of the compound of interest. Please note, that an already existing calibration file will not be overwritten, and the concentration determination will be based on the previously defined reference.

Potency (also termed absolute NMR purity) of a compound can be determined by using an internal standard together with the weights of the used materials.



6.1.2 Automatic Analysis of Batch Spectra

Besides the interpretation of a single spectrum, a batch of datasets can be automatically analysed.

6.1.2.1 Import Expert Settings

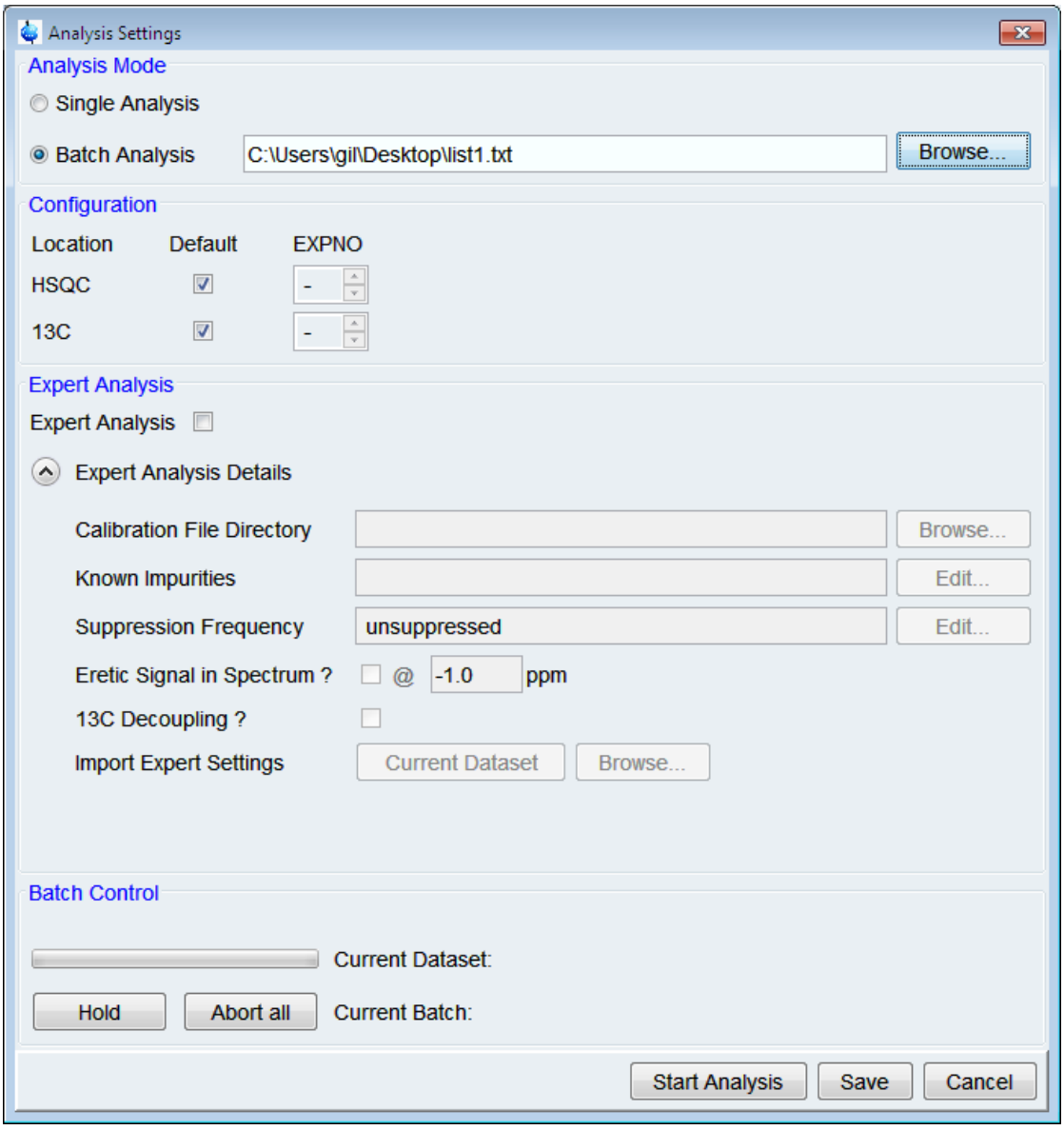
In the case the settings have already been adjusted according to the individual conditions previously or for different data, these settings can be imported for the current data- set.

- Current Dataset

  This option imports the settings of the last automated analysis of the current dataset. This functionality is intended for minor changes of the previous settings and avoids editing of the parameters from scratch. If the spectrum interpretation should run again with the same settings as before except for an additional impurity, one can import the previous settings and add the new impurity information.

- Browse

  In the case the desired settings for the current dataset have already been adjusted for a different dataset, they can be imported via the *Browse* function (select the cmcq file in the procno of the automatically analyzed spectrum in order to apply the same settings for the automated interpretation of the current dataset).



Clicking the submenu **Batch Analysis** opens a dialog where a serial processing list has to be defined before starting the analysis in the batch mode. How to generate this serial processing list (a text file made up of the data path for every single spectrum of the desired batch dataset) is described in Chapter *Serial [▶ 57]*.

After starting the batch analysis, the *Batch Control* in the lower part of the window shows the path of the dataset currently analyzed, the file name of the specified serial processing list, and the number of all datasets defined in the list as well as the number of data- sets for which the spectra interpretation is completed. In addition, it provides the opportunity to hold and resume or abort the batch analysis. Please note, that the maxi- mum capacity of one batch is restricted to 96 datasets in order to enable visual inspection of the batch result.

6.1.3 Automated Analysis of CMC-assist Projects

CMC-assist can perform an automated analysis of HSQC and 1D13C spectra in con- junction with the 1D1H analysis. To do so, these spectra must be defined as part of a CMC-assist project. For detailed information on creating a CMC-assist project see Chapter *Define Project [▶ 28]*.

After the spectra have been linked as a project, clicking the tab **Analyse Spectrum** will start a joint automated analysis of all spectra in the project. As a default, the most recently set analysis settings will be used.

To change the analysis settings, including the configuration information, click the down arrow on the right side of the **Analyse Spectrum** tab and select **Analysis Settings**. This is only possible when the 1D1H spectrum is the active window. From here, all of the set- tings can be modified as for the single 1D1H analysis. In addition, if the HSQC spectrum and 13C spectrum are not located in the consecutive expnos, then this should be set in the configuration block. Furthermore, 13C infomation should be provided, if known, for any custom impurities to aid in impurity identification by the analysis.

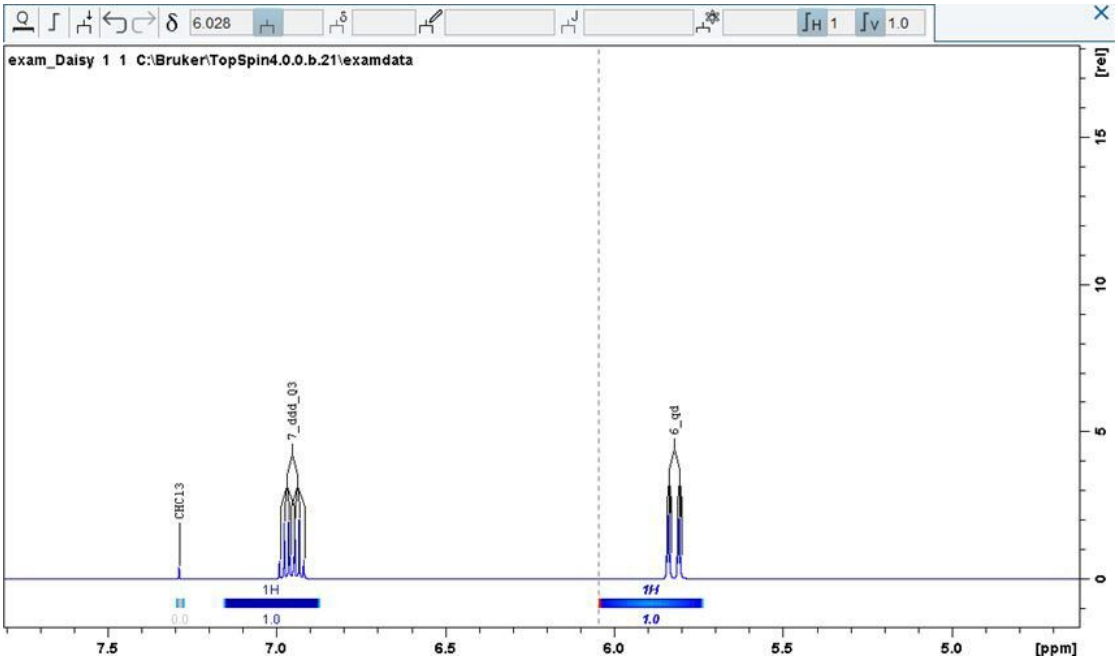
6.2 Modifying Results from Automated Analysis

Any results of the automated analysis can be modified manually. After modifying the automatically generated results, any report will indicate that the results were edited manually. This will also be indicated in the consistency section of the browser.

6.2.1 1D 1H Data

6.2.1.1 Integrals

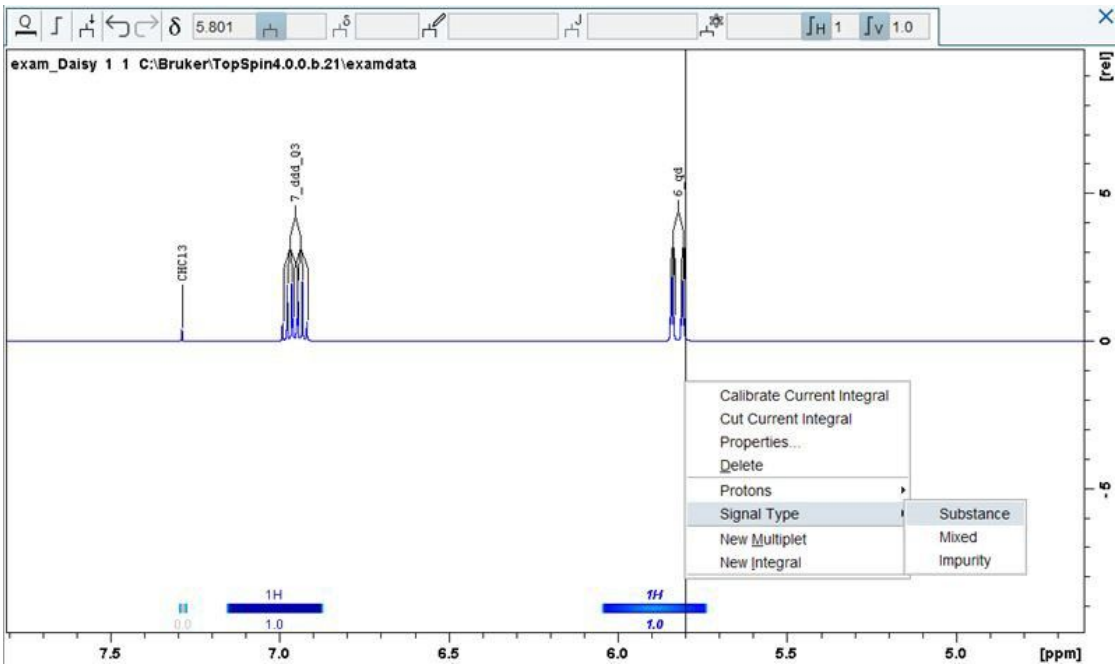
Integration regions can be easily changed, by moving the cursor to the edge of the blue bar until the vertical boundary line turns red. Left-click-hold and move the mouse in order to expand or reduce the integration region.



The bars of the integration regions are colored differently depending on their signal type. Blue bars correspond to signals originating from the investigated compound, grey bars label impurities, and striped bars indicate a mixture of substance and impurity.

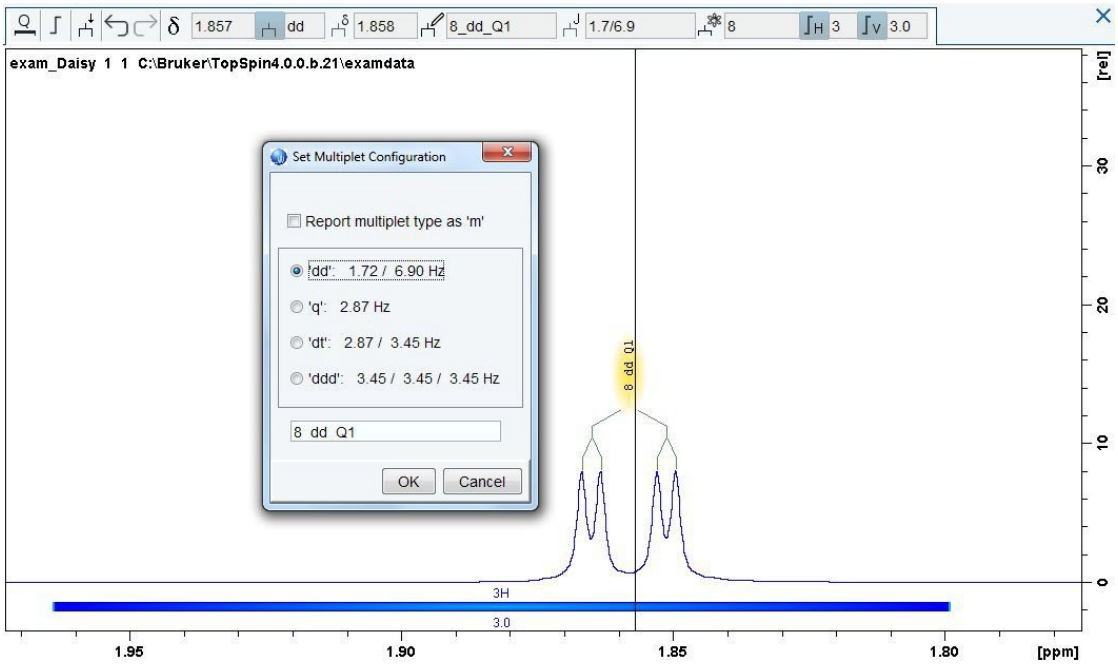
The status of the signal type can be altered by right-clicking on the integral and choosing the appropriate status via the submenu **Signal Type**. Furthermore, the dialog that appears when right-clicking on the integral offers additional options like calibrating the integral, cutting the integral, and changing the correlated proton number.

As soon as an integral has been modified in some way, the integral and the proton number are shown in italic, indicating that the displayed results were created manually.



6.2.1.2 Multiplets

In the case the automatically analyzed multiplicity needs to be changed, right-click on the multiplet and select *Multiplicity*.



The pop-up window lists all possible multiplet configurations, depending on the number of peaks which define the multiplet. Selecting any of the listed options will display the corresponding multiplicity in the data window.

In addition, right-clicking on the multiplet provides the opportunity to redefine the multiplet, which means that the currently displayed multiplet will be deleted and the multiplet analysis mode will be entered in order to newly define the multiplicity.

6.2.1.3 Assignment

Moving the cursor line to any multiplet will highlight the atom(s) of the molecule assigned to this multiplet. The same applies the other way around, positioning the cursor at any atom of the displayed structure will highlight the connected multiplet.

Before any signal in the spectrum can be newly assigned, the existing assignment has to be cleared. This option can be selected from the dialog available by right-clicking on the multiplet or the atom.

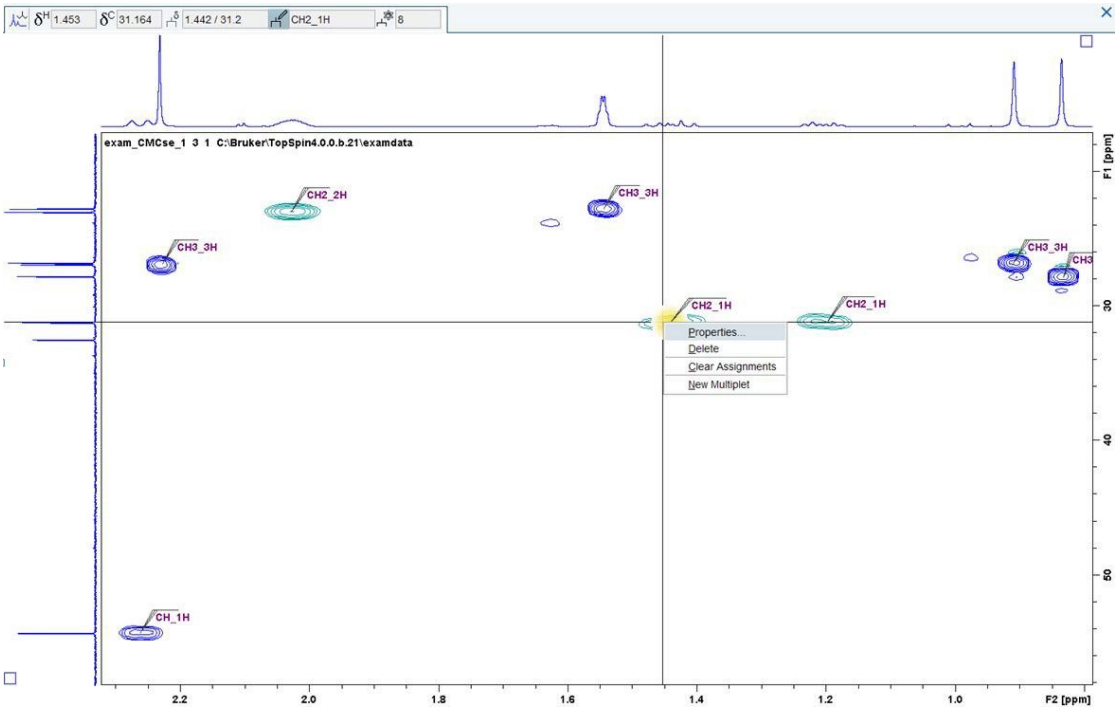
Subsequently, there are two different ways to connect a signal in the spectrum with the corresponding atom(s) of the molecular structure:

- Left-click-hold on any atom of the molecule and move the mouse to the respective multiplet will assign this atom to the selected multiplet
- Left-click-hold on any multiplet and move the mouse to the appropriate atom of the displayed structure will assign the selected multiplet with this atom

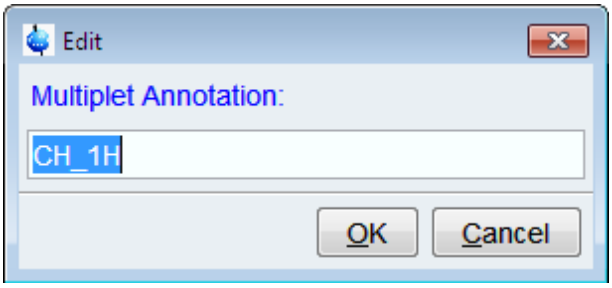
6.2.2 HSQC Data

6.2.2.1 Multiplet Annotation

The automatically generated results of the HSQC analysis include an annotation for each defined multiplet. This annotation, or label, can be seen on the spectrum. The automatically generated annotation includes the type of CH group (-CH3, -CH2, or -CH) along with the number of protons assigned to this peak.



To edit this label, right-click on the multiplet when it is highlighted in yellow and select *Properties* from the pull down menu.



In the resulting dialog, enter the desired label and click ok.

6.2.2.2 Multiplet Assignment

As in the 1D1H spectra, moving the cursor over any multiplet will highlight the atom(s) of the molecule assigned to the multiplet and vice versa.

Before changing the multiplet assignment, the current assignment should be removed. To do this, right-click on the multiplet and choose *Clear Assignment*.

To then assign the multiplet to the corresponding atom(s) in the structure:

- Left-click-hold on any atom of the molecule and move the mouse to the respective multiplet to assign this atom to the selected multiplet
- Left-click-hold on any multiplet and move the mouse to the appropriate atom of the displayed structure to assign the selected multiplet to this atom

6.2.3 1D 13C Data

6.2.3.1 Multiplet Annotation

The automatically generated results of the 1D13C analysis include an annotation for each defined multiplet similar to the annotation of the multiplets in the HSQC spectrum. This annotation, or label, can be seen on the spectrum. The automatically generated annotation includes the type of carbon (-CH3, -CH2, -CH, or -C) along with the number of carbon atoms assigned to this peak.

6.2.3.2 Multiplet Assignment

After an automatic analysis, each peak in the spectrum will be assigned to one or more carbon atoms in the structure. When the cursor is hovering over a multiplet in the spectrum, it will be highlighted yellow along with the assigned carbon atoms in the structure.

To alter this assignement one must first clear the existing assignment by right-clicking on the multiplet and choosing *Clear Assignment.* After the existing assignment is cleared, left-click and hold on the desired multiplet then drag and drop onto the desired atom in the structure.

6.3 Manual Spectra Analysis

If an automated interpretation is not desired, CMC-assist also offers the option for manual spectra interpretation.

6.3.1 Integration

To define new integrals, click the corresponding toolbar icon  . The integration mode is active, when the icon is highlighted in yellow and the cursor line turns red.

- Define integral

  Left-click-hold and move the mouse
- Exit integration mode
  - Left-click the yellow highlighted button 
  - Right-click in the data window and select **End Integral Definition**

After integration it might be necessary to adjust the integrals and proton numbers. Right-clicking on the integration region provides several options:

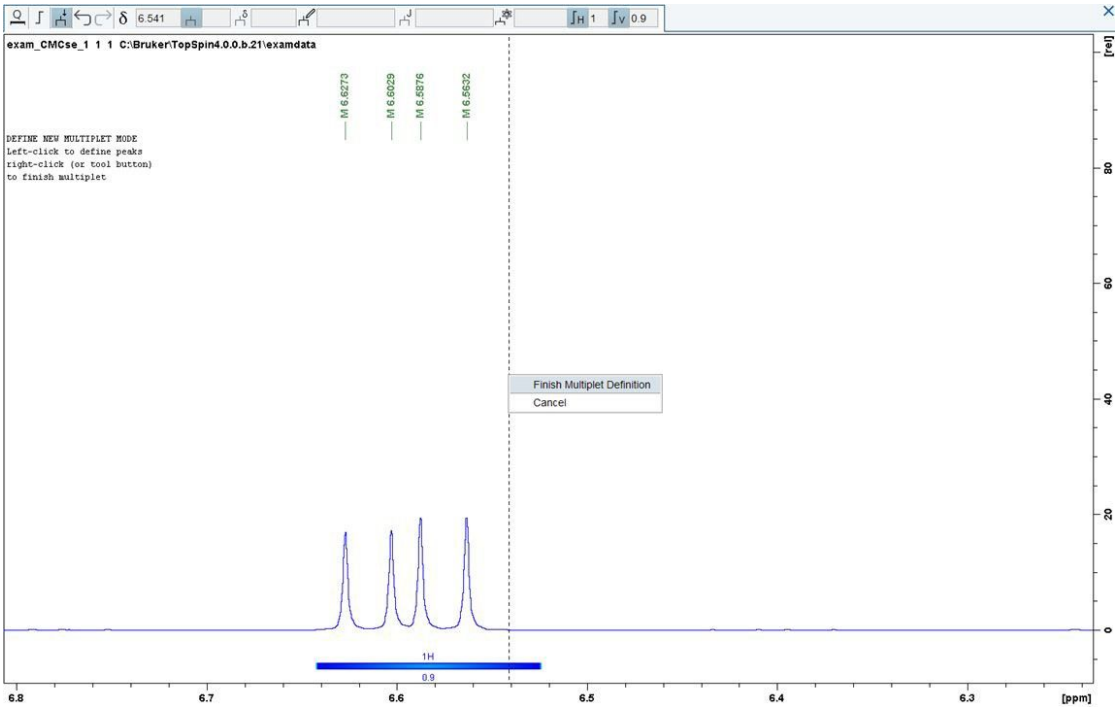
- **Calibrate Current Integral:** enable calibration of the selected integral
- **Cut Current Integral:** cut the integral according to the cursor position
- **Properties:** allow editing of several properties, like definition of the status (substance, mixture, or impurity) and the appropriate proton number for the selected integral. Furthermore, an annotation can be made and the concentration can be calculated based on this integral.
- **Delete:** delete the selected integral
- **Protons:** list proton numbers in order to set a new proton content for the selected integral
- **Signal Type***:* enable setting of the status of the selected integral as substance, mixture, or impurity

6.3.2 Multiplet Analysis

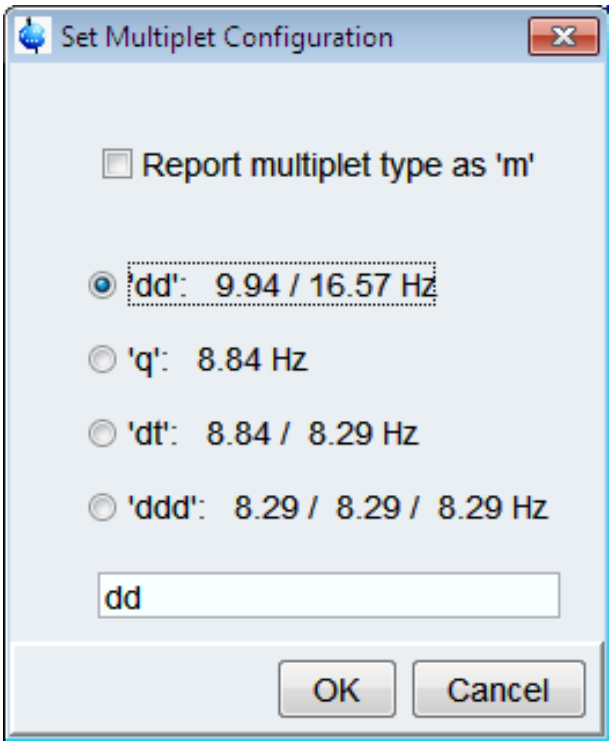
6.3.2.1 1D Data

Clicking on the toolbar icon  **Define new multiplets** activates the multiplet analysis mode and the cursor line becomes dashed.

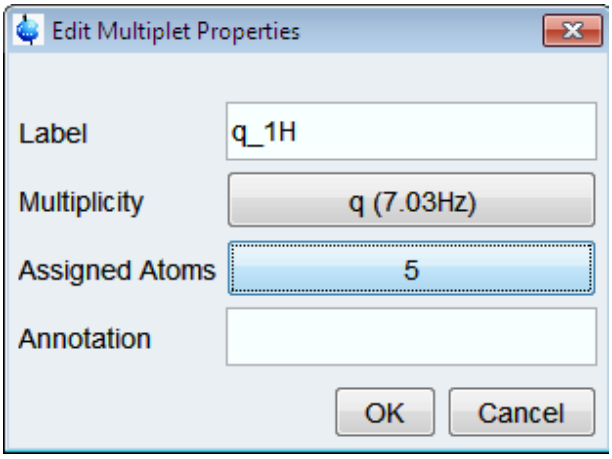
- Define multiplet
  - Left-click on the peaks which belong to one multiplet
  - Right-click in the data window and choose **Finish Multiplet Definition**



  - Depending on the number of peaks which define the multiplet, a dialog appears that lists all possible multiplet configurations. Selecting any of the listed options will display the corresponding multiplicity in the data window. If none of the suggested multiplet configurations is appropriate, it still leaves the possibility to tick **Report multiplet type as 'm'** which results in reporting no multiplicity and no coupling constant. In addition, the labeling of the multiplet can be changed.



- To edit the multiplet label and further properties of the multiplet, right-click on the multiplet and choose **Properties**. From the resulting dialog one can alter the multiplet label, multiplicity, assigned atoms, and annotation.



- Exit multiplet analysis, left-click the yellow highlighted button 

If the created multiplet needs to be deleted again, leave the multiplet analysis mode, right-click on the multiplet and choose **Delete** from the appearing window.

6.3.2.2 HSQC Data

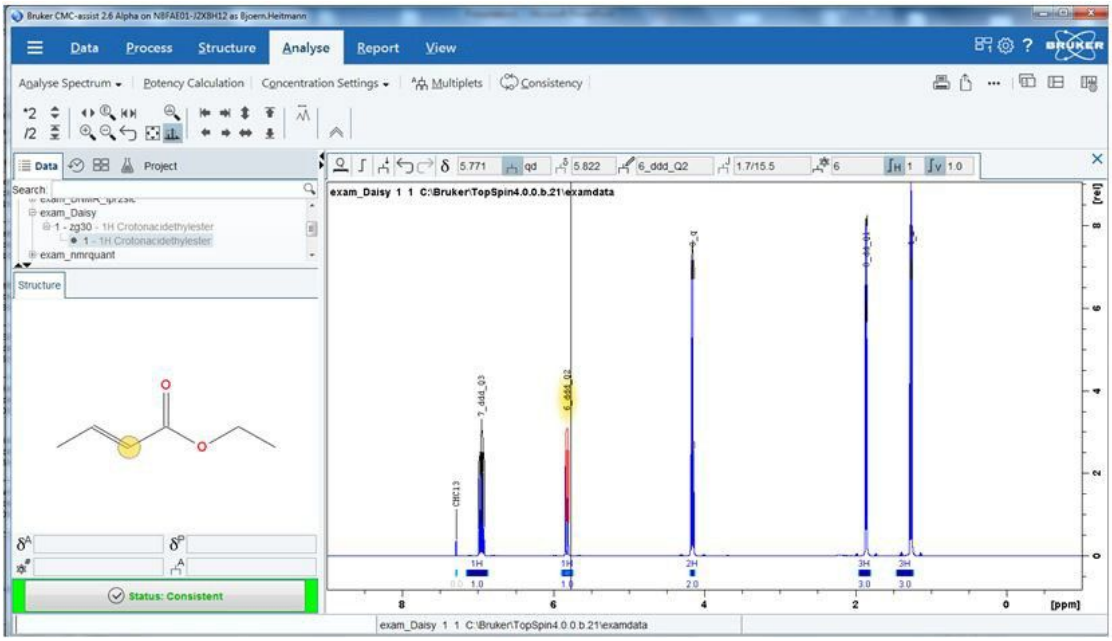
To define a new multiplet in an HSQC spectrum right-click when the cursor is over the center of the desired multiplet. In the resulting pull-down menu select **New Multiplet.** This will open a dialog where the multiplet label can be entered. This multiplet label will be displayed on the spectrum.

6.3.3 Assignment

Connecting a multiplet to the corresponding atom(s) in the structure works the same way for all three spectra types.

There are two different ways to connect a signal in the spectrum with the corresponding atom(s) of the molecular structure:

- Left-click-hold on any atom of the molecule and move the mouse to the respective multiplet to assign this atom to the selected multiplet
- Left-click-hold on any multiplet and move the mouse to the appropriate atom of the displayed structure to assign the selected multiplet with this atom



Moving the cursor line to any multiplet will highlight the atom(s) of the molecule assigned to this multiplet. The same applies the other way around, positioning the cursor at any atom of the displayed structure will highlight the connected multiplet.

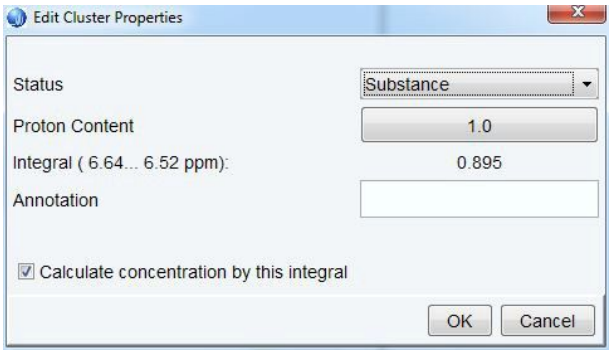
6.4 Potency Calculation

Clicking the tab **Potency Calculation** performs a potency calculation on the current dataset if all needed settings are available. If they are not - a dialog pops up which prompts for the missing information. For further information about potency please refer to the manual **Potency Determination Quick Start** available via the help button.

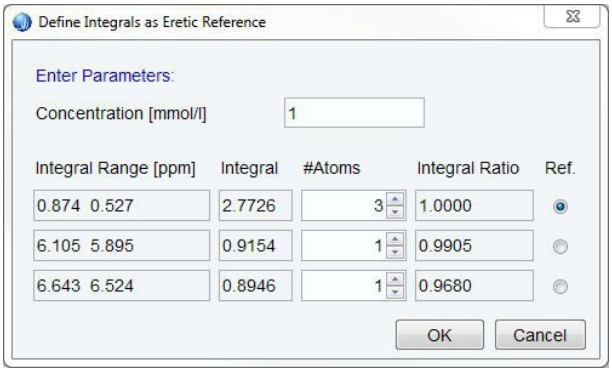
6.5 Concentration Settings

The tab **Concentration Settings** enables concentration determination of the measured substance. For precise analysis, a reference spectrum is essential. This spectrum should be acquired preferably under the same conditions as the molecule of interest. In addition, the reference substance should be fully soluble in the solvent used and the exact amount of compound has to be known. Please note, that the most suitable window function for spectra processing for quantification purposes is an exponential function. After appropriate acquisition and processing, this reference spectrum has to be defined as reference.

First of all, at least one signal within the reference spectrum has to be integrated. Subsequently, it has to be stated on which signal(s) the reference concentration is based. To do this, right-click on the respective integral(s) and selecting **Properties** from the result- ing pull-down menu. This will bring up a dialog where one can tick the option **Calculate concentration by this integral.**

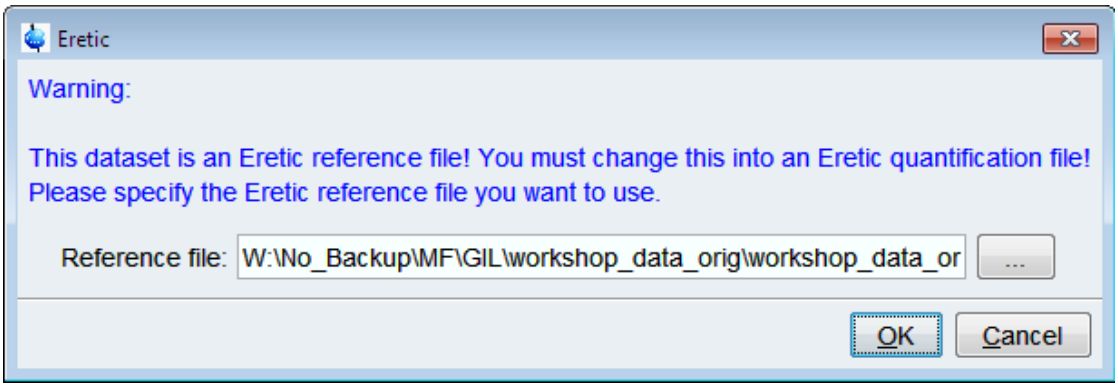


Afterwards, the down arrow on the right side of the tab **Concentration Settings** offers the possibility to define the marked and integrated signal(s) as reference by choosing **Define as Eretic Reference**.



Clicking the submenu **Define as Eretic Reference** opens up a dialog, where the assigned proton number for each labeled and integrated signal has to be entered as well as the exact concentration of the reference substance (all the given information will be saved in a file called eretic).

In order to calculate the concentration of the compound of interest, this reference has to be specified for the respective dataset via the submenu **Browse for Reference** accessible by the down arrow on the right side of the tab **Concentration Settings**. Please note, that the required path of the reference spectrum has to consist of all levels including procno.



For quantification of the investigated compound, right-click on the desired integral(s) on which the concentration determination should be based, select **Properties** and tick the box for **Calculate concentration by this integral.** The calculated concentration will be displayed in green numbers above the selected integral(s).

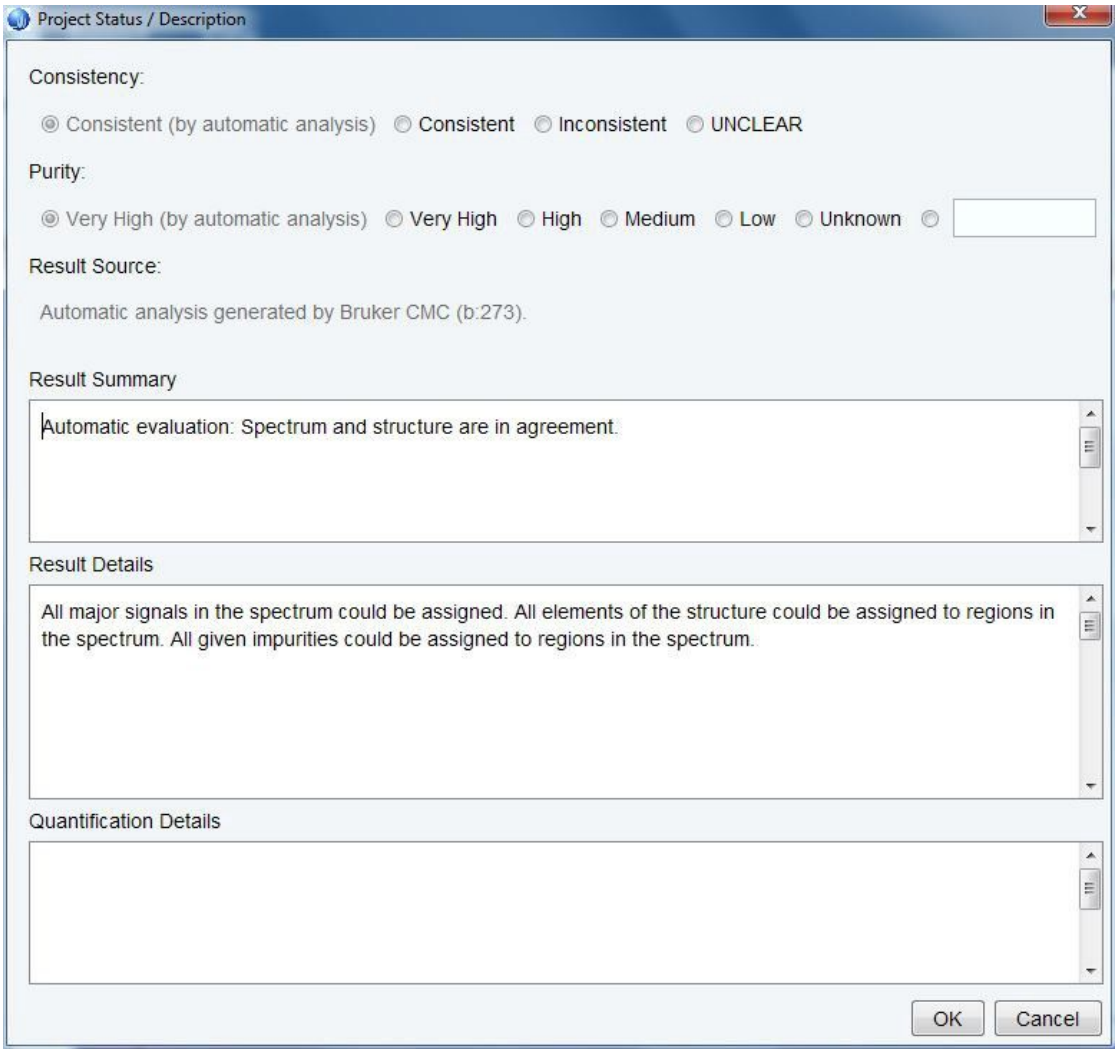
6.6 Analysis of Complex Multiplets

Besides the multiplet analysis mode accessible via the toolbar icon  **Define new multiplets**, another multiplet tool is offered that allows creation of multiplets for more complex coupling systems.

Clicking on the tab  **Multiplets** starts this multiplet tool. For further information about the handling of this tool please refer to the manual **Documentation for Multiplet Analysis** available via the  help button.

6.7 Consistency and Purity

The **Consistency** tab opens a dialog that enables the viewing and changing of the consistency status and purity information, as well as the addition of individual comments in the fields below. This dialog can also be accessed via the color-coded **Consistency** but- ton in the browser field.



Any manual changes or comments, as well as statements from the automated spectra analysis, are printed on the first page of the report (generating the report see Chapter *Short Report [▶ 81]*).

6.8 Molecular Structure

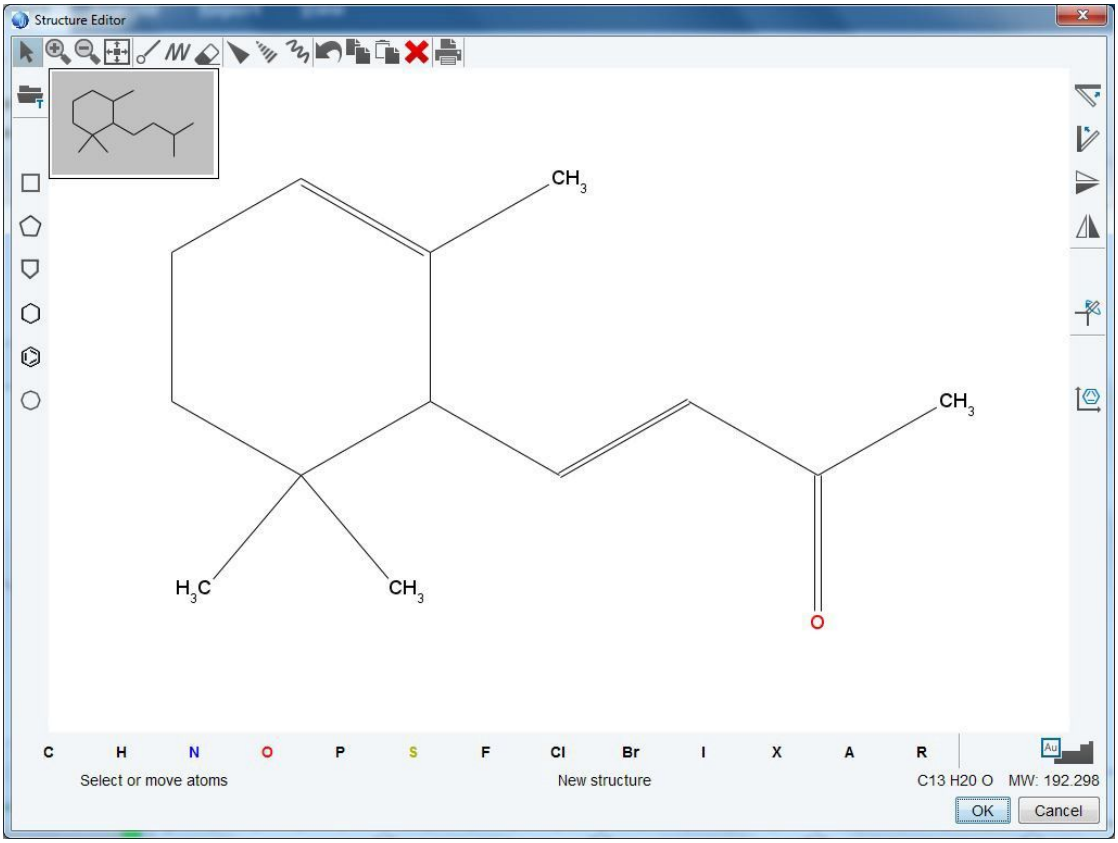
The structure editor is an integrated part of the software package CMC-assist that enables drawing and modifying chemical structures. Besides this editor the **Structure** menu also offers the option to view predicted chemical shift ranges for the given molecu- lar structure.

6.8.1 Adding .mol Files

The submenu **Add MOL file** within the **Structure** menu allows loading a new structure in the format of a .mol file into the current dataset.

6.8.2 Editing Structures

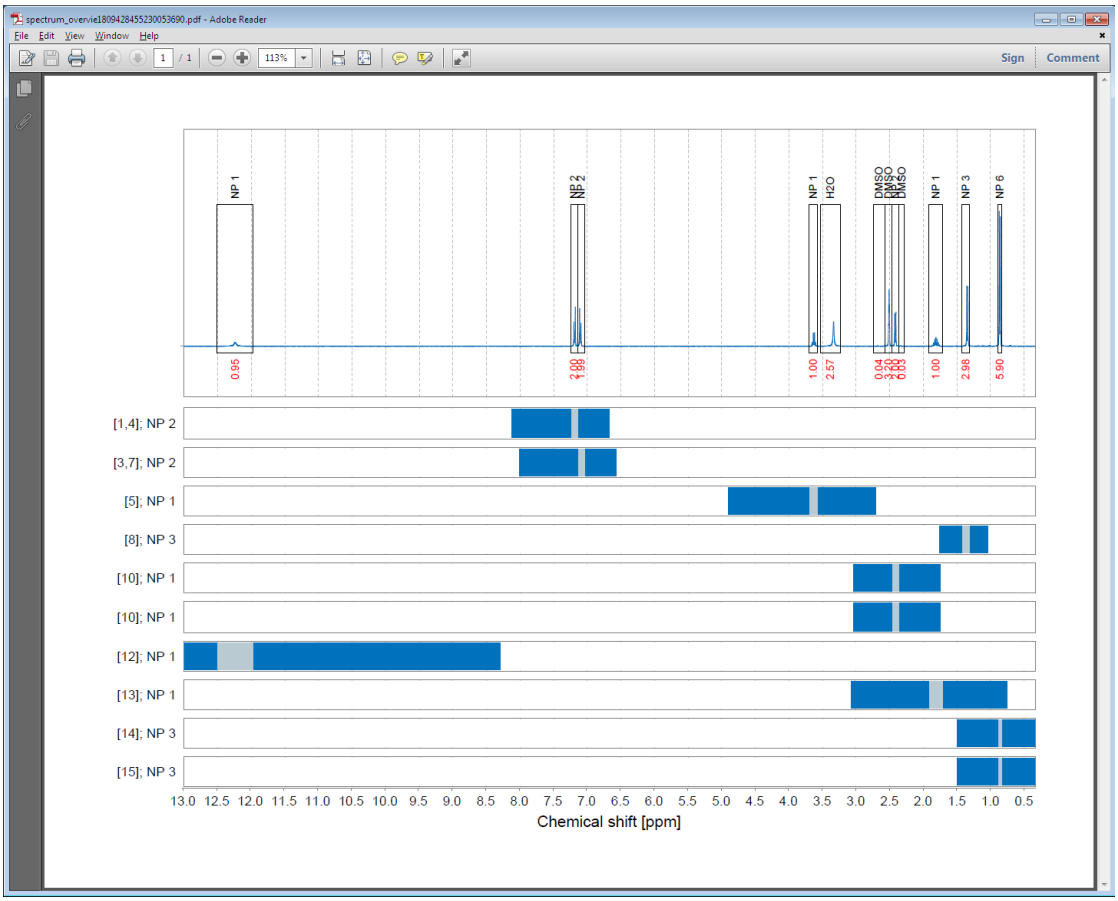
The tab **Structure Editor** starts the structure editing module, an editor for 2D molecular structures, in a separate window. For detailed information on creating or modifying molecular structures, please consult the Topspin Structure Analysis Tools manual available via the **Help** button in the upper right corner of the Topspin window.



Moreover, please note that in case a dataset has no existing .mol file, the 2D Structure Editor will open a dummy file consisting of an ethane molecule. If no changes are made, the 2D Structure Editor should be closed without saving otherwise the ethane molecule will be copied into the current dataset. Saving newly created molecules or modified structures will overwrite already existing .mol files.

6.9 Prediction

Clicking the tab **Prediction** opens a .pdf document that shows the prediction information used for the automated spectra analysis of the 1D1H spectrum. The upper part displays the spectrum including the integrals (red numbers below the baseline) and proton numbers (abbreviation nP above the signals) for each peak cluster. The lower part of this graphic indicates the predicted chemical shift ranges. The grey bars in the first line correspond to the integration regions of the peak clusters of the acquired spectrum. Each of the following lines represents the predicted chemical shift ranges (depicted as blue bars) for each atom (specified in squared brackets).



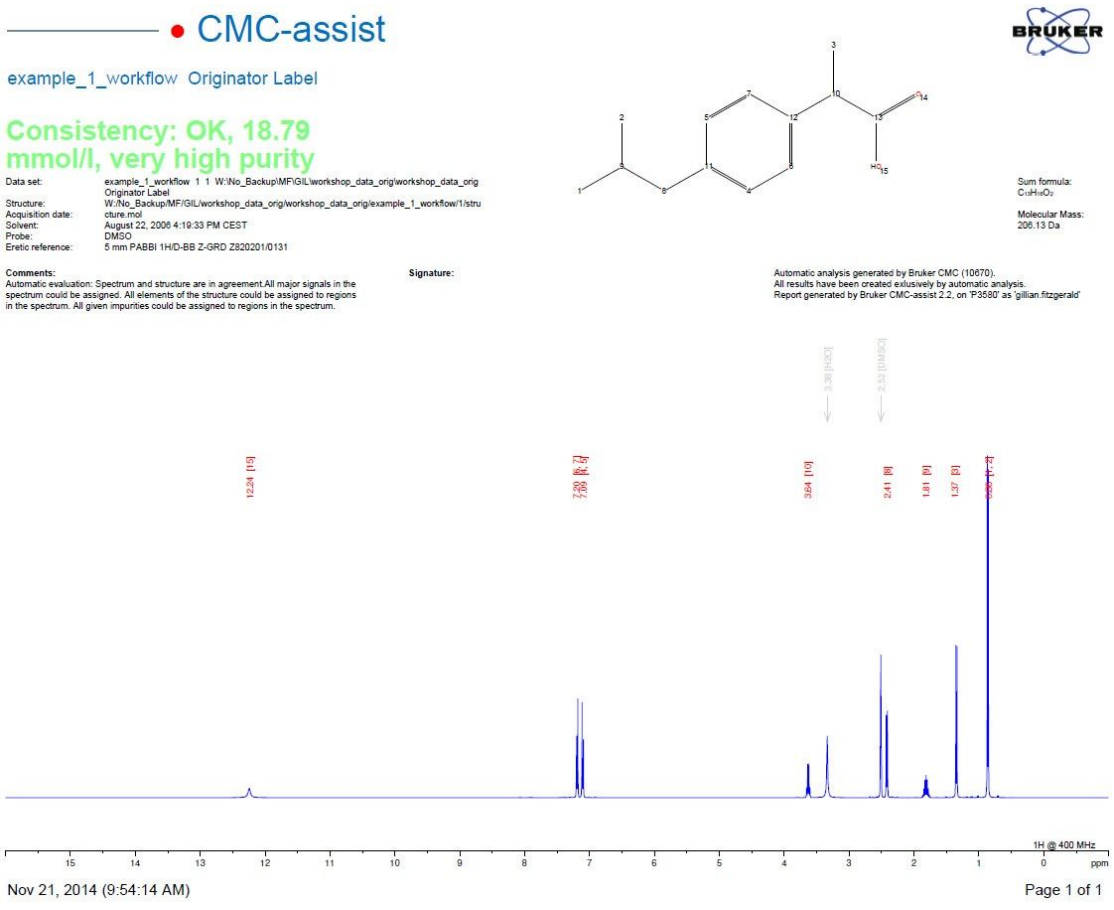
The predicted shift information can also be viewed in the molecular structure viewer. To view the predicted shift for each atom in the structure, click the corresponding icon in the molecular structure viewer or move the mouse over the atom of interest.

7 Reporting

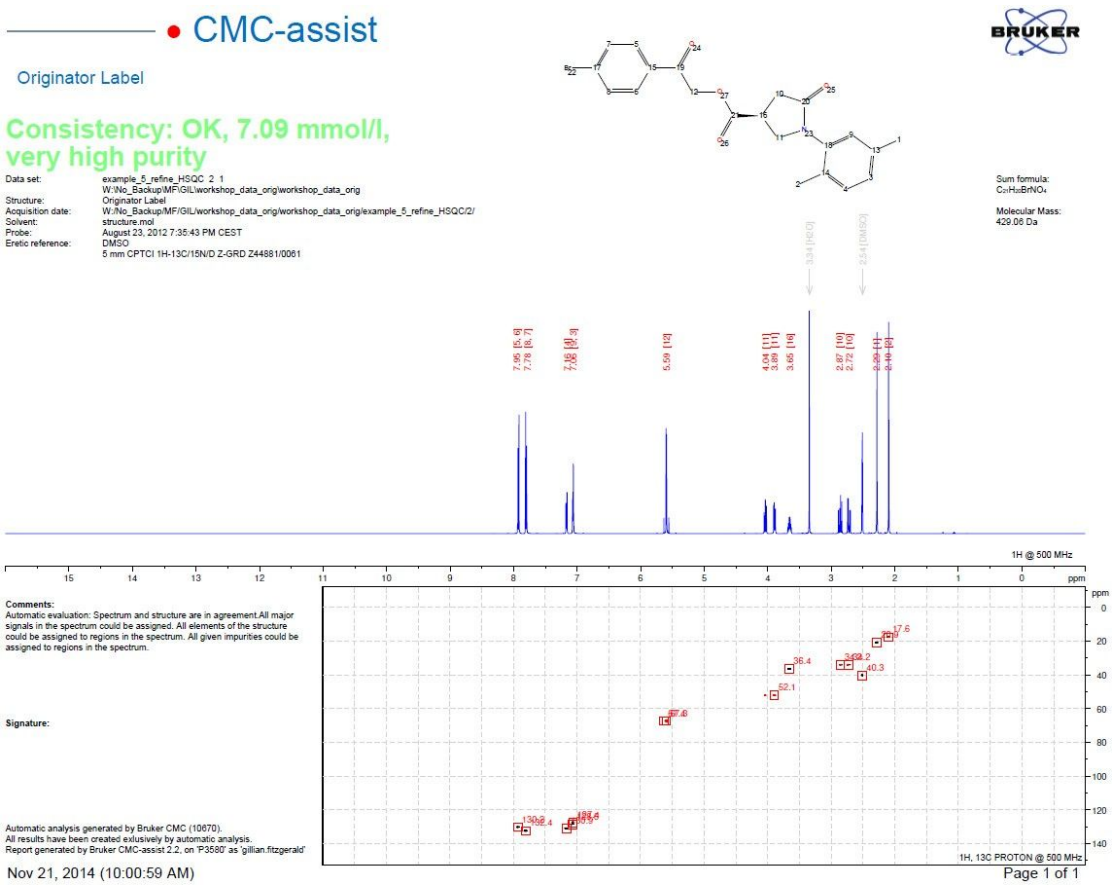
The software package CMC-assist enables generation of a short report of one page, a detailed report of several pages, and also a text file including patent strings in various formats. All these options are available via the **Report** menu.

7.1 Short Report

The tab **Short Report** creates a one page assignment report displaying the consistency status, the molecular structure, the spectrum interpretation (based on the automatically generated assignment or the manually edited assignment), the determined concentration and purity, and any comments made regarding the summary of results or relevant details.



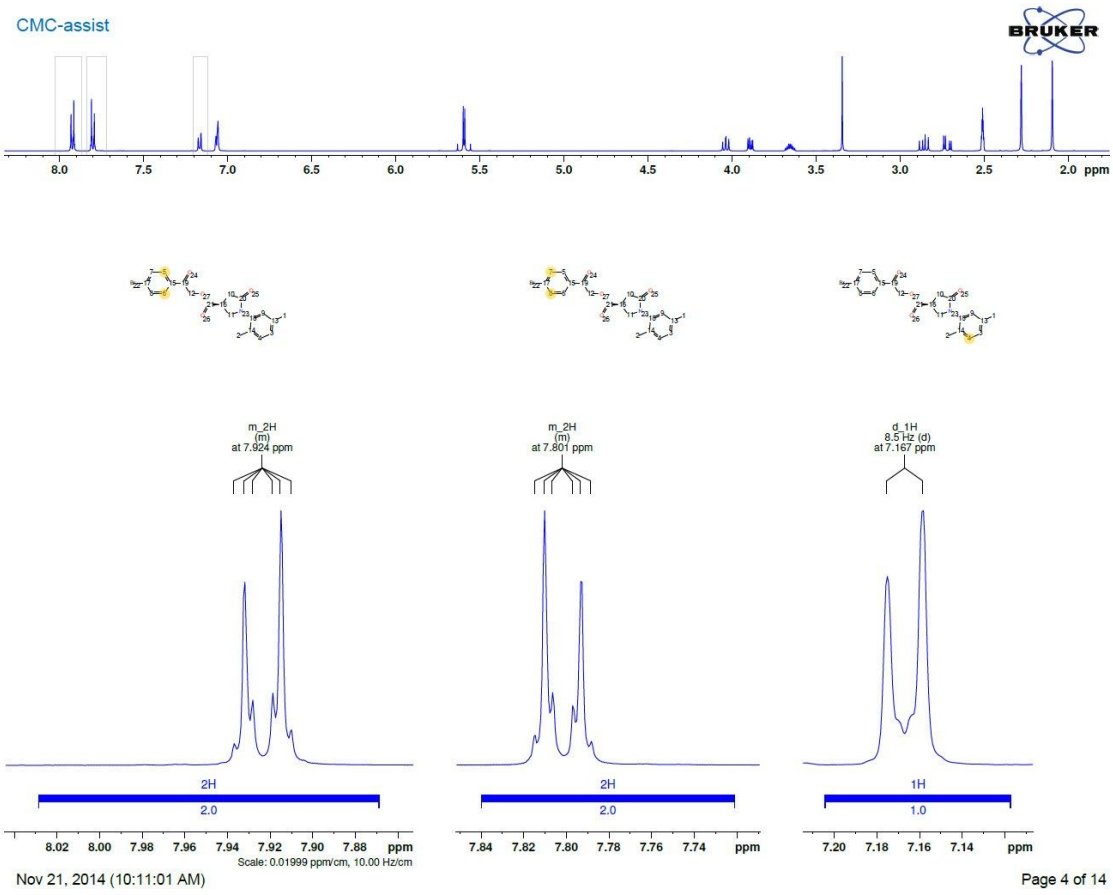
Furthermore, if the report is for a CMC-a project, all of the relevant spectra and their interpretation will be displayed along with the aforementioned information and data.



7.2 Full Report

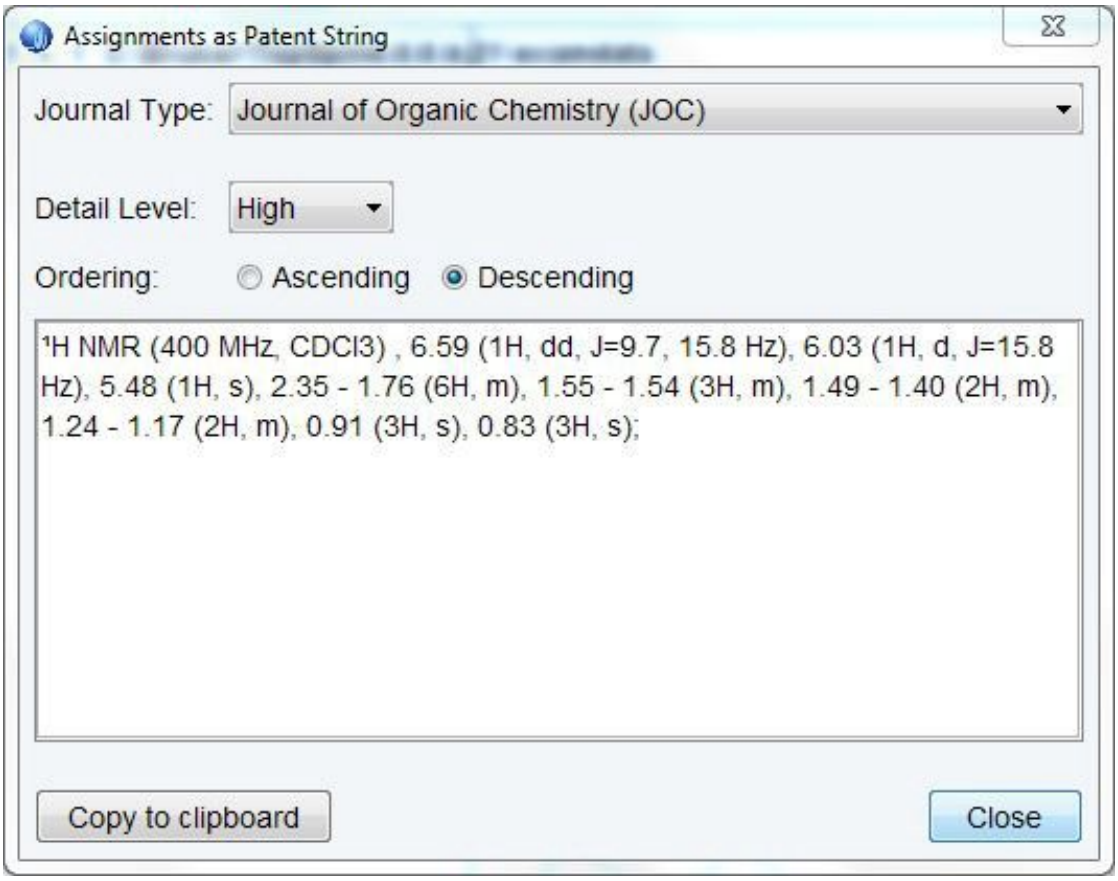
The first page of the full report, available by the corresponding tab **Full Report,** is identical to the short report. The following pages show a more detailed interpretation of the spectrum including integration, multiplicity, and assignment for each peak. Finally, the last page presents the complete assignment in various journal formats.

Furthermore, via the down arrow on the right side of the tab the **Report Settings** can be altered. This includes adding an originator label to the first page of the report, setting the report logo, or switching the page size between letter and A4.



7.3 Patent String

Clicking the tab **Patent String** opens a dialog which gives the opportunity to select different journal types and copy the appropriate patent string.



Along with changing the journal format, the level of detail provided in the string can be changed, as well as the order in which the peaks appear.

7.4 Print

Clicking on the tab **Print** will print the currently active data window. Alternatively, the down arrow on the right side of the tab offers several file types for saving of the dis- played data window (e.g., pdf, png, or jpg file). Note: When choosing any of the direct saving options, the molecular structure will not be included in the window.

7.5 Copy

The selected spectrum can also be copied to the clipboard by clicking on the tab **Copy** and pasted into a word processor or a presentation program.

7.6 Mobile

The mobile function is provided to enable data sharing on a certain workstation. This can be accessed via the **Mobile** tab. For further documentation on how to use this function please see the the manual in Topspin.

8 Contact

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