

# **TopSpin ERETIC 2**

Electronic to Access In-Vivo Concentration
 User Manual
 Version 001

Innovation with Integrity

NMR

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### **1** General Information

The new module ERETIC2 is a quantification tool which replaces the ERETIC (Electronic to Access In Vivo Concentration) software.

This new tool is based on PULCON<sup>1</sup>, an internal standard method which correlates the absolute intensities of two different spectra. Concentration measurements with PULCON use the principle of reciprocity which indicates that the lengths of a 90° or 360° pulse are inversely proportional to the NMR signal intensity<sup>2,3</sup>. Therefore, provided that the concentration of one of the samples is known precisely and that the 90° pulse of all the samples have been well calibrated, the unknown concentrations can be obtained using the following equation<sup>1</sup>:

$$C_{UNK} = k C_{ref} \frac{A_{unk} T_{unk} \Theta_{unk} n_{ref}}{A_{ref} T_{ref} \Theta_{ref} n_{unk}}$$

Where the *unk* and *ref* indices stand for unknown and reference respectively, A is the integral value, C is the concentration, T is the temperature,  $\theta$  is the pulse length (for 90° or 30° pulse), n is the number of transients used for the experiments, and k is a correction factor taking into account the use of different receiver gains for measurement of the reference and of the unknown samples, or incomplete relaxation.

ERETIC2 can be used with internal or external standard methods, and needs 1D spectra measured under "quantitative" condition : a relaxation delay equals to at least  $5 \times T_1$  (for a 90° pulse) or  $3 \times T_1$  for a 30° pulse, an acquisition time longer than  $3 \times T_2$ , and a sufficient signal to noise (at least 10:1).

Compared to the former ERETIC software, the main advantage of this new tool is that it doesn't require any additional hardware needed to generate the electronic signal used as reference. Hence, ERETIC2 allows the user to have more flexibility when choosing the 1D NMR experiment used for quantification.

<sup>1</sup> Wider G. & Dreier L., *J. Am. Chem. Soc.*, **2006**, 128 (2571-2576).

<sup>2</sup> Hoult D. I. & Richards R. E., J. Magn. Reson., **1976** (71-85).

<sup>3</sup> Hoult D. I., Concepts in Magn. Reson., 2000, 12 (173-187).

### **2** Quantification Procedure

#### 2.1 Calibration with External Standard

Bruker does not provide any standard NMR samples for calibration. Nevertheless, it is recommended to use your own reference samples prepared in various solvents and with well-known concentrations.

#### 2.1.1 Acquisition Parameters Setting

- · Insert the reference sample into the magnet.
- Prepare a new experiment using the **New** command.

🍐 New			۲					
Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the Options.								
NAME	Calibration							
EXPNO	1							
PROCNO	1							
DIR	C:/Quantification/data/Smar	rtProbe/nmr 👻						
TITLE	BBFOsp Dioxane 10 mM Calibration							
© Use currer	nt parameters							
Experiment	t CMCQ_PROTON	Select						
<ul> <li>Options</li> </ul>								
Set so	olvent	CDCI3 -						
Execution	te 'getprosol'							
© Keep	parameters	P 1, O1, PLW 1 ▼ Change						
Show	new dataset in new window							
Receive	ers (1,2,16)	1						
		OK Cancel More Info Help						

Figure 2.1: Preparing for a New Experiment

- · Select the user and directory names, the experiment and processing numbers.
- Select the parameters set CMCQ\_PROTON.

- Lock the magnetic field (lock **solvent**).
- Tune and match the probe (atma exact).
- Shim the sample (topshim).
- Calibrate the 90° pulse either manually or with the AU program <code>pulsecal</code>. Without option for proton, or option <code>sn opt</code>:
  - Option c13 for carbone.
  - Option f19 for fluorine.
  - Option p31 for phosphorus.

In the acquisition window (eda) set the digitization mode to baseopt:

- Set D1 and NS according to your sample.
- Set the receiver gain (**rga**) (optional).
- Start the experiment (**zg**).

#### 2.2 **Processing Parameters Setting, Reference Sample**

- Select an exponential window function (EM window), with an lb=0.3.
- Use the EF command to perform an exponential window multiplication and a Fourier transform of the FID (**em**, **ft**).
- For baseopt acquisition: Automatic zero order phase correction with **apk0**.
- Otherwise: Automatic zero and first order phase correction with **apk**.
- Base line correction without automatic integration (absn).

#### 2.3 ERECTIC2 Calibration

The reference sample is a 10 mM Dioxane solution in CDCl<sub>3</sub>.

### **Quantification Procedure**



Figure 2.2: Integrating a Spectrum Manually

• In the reference sample, go into the integrate menu.

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<u>Start Process Analyse Publish View M</u> anage	0	1
A Proc. Spectrum - Adjust Phase - A Calib. As	is w Pick Peaks w I Integrate w Advanced w	
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10 mM		
Calibration		
Mouse Sensitivity: 9.765625E-4		
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		- 2
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		- 10
		-
		-
		- 1
	Save & Ouit	
	Ouit	
	Select / Deselect	
	Cut Current Integral	
	Delete Current Integral	-
	Calibrate Current Integral	
3.40 3.38 3.36	Normalize Sum Of Integrals Use Eretic Reference for Calibration	[ppm]
	Use Lastscale For Calibration Calculate Concentration	
	Eretic Define as Eretic Reference	
	Deconvolution •	

Figure 2.3: Defining the ERETIC Reference

- Integrate the reference signals.
- Select the signals you want to use for calibration.
- Click on the right mouse button, and choose the option **Define as Eretic Reference**.

### **Quantification Procedure**

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BBFOsp Dioxane 10 mM Calibration						Lei Lei				
ERETIC2 Calibration						×				
Reference dataset										
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Concentration [mmol/l]	10.0000									
Number of atoms	Region start [ppm]	Region end [ppm]	Molecule name	Sample volume [ml]	Molar mass [g/mol]					
8	3.380000	3.310000	Dioxane	0.6000	88.1100	+ - *				
						OK Cancel				
4			//							
						- - - -				
			8		<b>_</b>	-				
3.40	3.38	3.36	3.34	3.32	3.30	[ppm]				

Figure 2.4: Defining the concentration of the reference sample (mM)

- Define the concentration of the reference sample (mM).
- Define the number of nuclei per signal.
- Molecule name, sample volume as well as molar mass could be defined.



Figure 2.5: The Number of Nuclei, the Concentration and Corresponding Weight Appear on the Spectrum

🖕 Bruker TopSpin 3.5.a on INTRA-BRKR-CORP as Jerome.Coutant											
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崎 S 1,2, M E 🖤	# <b>3</b>										
Reference	Reference				*						
Window											
Phase	SI	65536		Size of real spectrum	E						
Baseline	SF [MHz]	500.1600000		Spectrometer frequency							
Integration	OFFSET [ppm]	14.69680		Low field limit of spectrum							
Peak	SR [Hz]	0		Spectrum reference frequency							
Automation	HZpPT [Hz]	0.152588		Spectral resolution							
Miscellaneous	SPECTYP	UNDEFINED	•	Type of spectrum e.g. COSY, HMQC,							
User	💿 Window functi	on									
	WDW	EM -		Window functions for trf, xfb,							
	LB [Hz]	0.30		Line broadening for em							
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	TM2	0		Right limit for tm 0 <tm2<1< th=""><th></th></tm2<1<>							
	Phase correcti	on									
	PHC0 [degrees]	-24.750		0th order correction for pk							
	PHC1 [degrees]	0		1st order correction for pk	~						
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<u></u>											
I											

Figure 2.6: The E Button

In the processing menu, the **E** button leads to a sub-menu where you can find a summary of the acquisition parameters of the calibration experiment.

👙 Bruker TopSpin 3.5.a	a on INTRA-BRKR-CORP as Jer	ome.Coutant				
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Л	Pro <u>c</u> . Spectrum <del>▼</del>	Adjust Phase 🗢 🌖	👌 Calib. A <u>x</u> is 🗢	🎌 Pick P <u>e</u> aks <del>→</del>	∫ <u>I</u> ntegrate <del>→</del> Ag	<u>d</u> vanced <del>▼</del>
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	NS	8		Number of	scans	
	P1 [µsec]	9.82		Pulse lengt	h	
	PL1		Power level			
	REINT	2.76436e+007		Integral		
	RECONC	10		Concentrat	ion represented by I	Eretic reference signal
	RECONCU	mmol/l		Unit of refe	erence concentration	1
	REPOS [ppm]	0		Position of	Eretic signal	
	RELW [Hz]	1		Line with		
	REREG	Show	]	Integral reg	gions of reference s	pectrum
	RG	181		Receiver ga	in	
	RPROBHD	5 mm PABBO BB-1H	I/D Z-GRD Z1136	52/C Probe		
Show eretic par	rameters					

Figure 2.7: The "NS", "P1", "RG", and "PROBE" Parameters

#### 3.1 Acquisition

From the calibration spectrum, create a new experiment with the **New** command. As the calibration and quantification should be as close as possible, use the option **Use current params** in the experiment line.

New				×			
Prepare for a new experiment by creating a new data set and initializing its NMR parameters according to the selected experiment type. For multi-receiver experiments several datasets are created. Please define the number of receivers in the Options.							
NAME	NAME Ethoxycinnamic Acid						
EXPNO		1					
PROCNO		1					
DIR	C:\Quantifi	cation\data\SmartI	Probe\nmr	•			
TITLE		BBFOsp Ethoxycinnamic acid Quantification					
• Use curren	it paramete	rs					
© Experimen	t CMCQ_PR	OTON		Select			
<ul> <li>Options</li> </ul>							
🗹 Set so	lvent		CDCI3 •				
Execut	te 'getproso	I					
© Кеер ∣	parameters		P 1, O1, PLW 1 👻 Char	ige			
Show new dataset in new window							
Receive	ers (1,2,1	.6)	1				
			OK Cancel More In	fo Help			

Figure 3.1: Preparing for a New Experiment

- Lock the magnetic field (lock **solvent**).
- Tune and match the probe (atma exact).
- Shim the sample (topshim).
- Calibrate the 90° pulse either manually or with the AU program pulsecal. Without option for proton, or option **sn opt**.
  - Option c13 for carbone.

- Option f19 for fluorine.
- Option p31 for phosphorus.

In the acquisition window (eda) set the digitization mode to baseopt.

- Set D1 and NS according to your sample.
- Set the receiver gain (**rga**) (optional).
- Start the experiment (zg).

#### 3.2 Processing

Keep the same processing parameters that the one used for the calibration experiment.

#### 3.3 Quantification of the Sample

The sample used in the example is a cinnamic acid solution in CDCl3. The 1D 1H spectrum (pulse sequence **zg**) of this sample is represented in the figure below.



Figure 3.2: Quantification of the Sample

Once the spectrum has been recorded and processed, go into the integrate menu, integrate the signals to be quantified, select all integrals and then right click on the mouse and select the option **Calculate Concentration**.

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r (		-
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Quit		
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Cut Current Integ	rat CO	
Calibrate Current		-
8 Normalize Sum Of	Integrals Use Eretic Reference for Calibration	0 [nnm]
Use Lastscale For	Calibration Calculate Concentration	<ul> <li>[bbuil</li> </ul>
Eretic	Define as Eretic Reference	
Deconvolution	<b>&gt;</b>	

Figure 3.3: The Calculate Concentration Option

- Go into the integration menu, integrate the signals you want to quantify.
- Right click on the mouse, and select Calculate Concentration.

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BBFOsp Ethoxycinnamic acid Quantification						[rei]				
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Concentration 10 mmo	ol/I									
Quantified dataset										
Name C:\Quantification	on\data\SmartProbe\nm	r\Ethoxycinnamic Acid\1	\pdata\1							
Number of atoms	Region start [ppm]	Region end [ppm]	Molecule name	Sample volume [ml]	Molar mass [g/mol]					
1	7.809147	7.648134	Ethoxycinnamic acid	0.6000	192.2100	+ - *				
3	7.490475	7.342879	Ethoxycinnamic acid	0.6000	192.2100	+ -				
1	6.581420	6.346609	Ethoxycinnamic acid	0.6000	192.2100	+ -				
2	4.488247	4.136030	Ethoxycinnamic acid	0.6000	192.2100	+ -				
						-				
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41 54	8	1	12			-				
0.95	<b>6</b>		ŏ							
8	6		4	2	0	[ppm]				

Figure 3.4: Defining the Reference File Used for Quantification

- Define the reference file used for quantification.
- Define the number of nuclei that are included in each integrals used for quantification.
- Molecule name, sample volume and molar mass can be defined as well, in order to get the amount (mg) of sample in the NMR tube.

Once this information has been entered in the window, the concentrations appear on the right. Then, click on **OK**. The results will now appear in the spectrum.



Figure 3.5: The Concentrations and Corresponding Weights Appear in the Spectrum

The concentrations and corresponding weights appear in the spectrum.

You can also have a display of the quantification results in the integral tab:

- Move the mouse in one of the cells of the first line of the table.
- Then click on the right mouse button, and select **Concentration (eretic)** and **Atoms (eretic)**.

Spe	ectrum	ProcPars	AcquPars	Title	PulseProg	Peaks	Integrals	Sample	Structure	Plot	Fid
0	bject	Integr	al [abs]	Inte	egral [rel]	v(F1) [pp	om] Conce	ntration (	Eretic) Ator	ns (Ere	etic)
I	integral	2	6413726.3	5	0.9954	7.72	286	19	.2275		1
I	integral	8	1573629.5	3	3.0741	7.41	.67	19	.7935		3
I	integral	2	5998715.32	2	0.9798	6.46	540	18	.9254		1
I	integral	5	64694291.8	)	2.0612	4.31	.21	19	.9070		2

## 4 Miscellanous

#### 4.1 ERETIC Signal

In the quantification procedure, ERETIC signal insert is not necessary. However, you still have the possibility to add this synthetic signal in your spectrum:

• Go into the processing menu (edp) and click on the E button.

In this sub-menu, you have the possibility of manually defining the line width (ELW parameter) and chemical shift (EPOS parameter) of the ERETIC signal.

You can also find some of the acquisition parameters of the reference spectrum (especially those used in the quantification calculation):

- Number of scans
- Pulse length
- · Concentration of the reference sample
- Probe
- · Receiver gain

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	A Proc. Spectrum <del>-</del>	Adjust Phase 👻 🚴 Calib. Axis 👻 🎊	Pick Peaks → ∫ Integrate → Advanced →
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1 "Ethoxycinnar	mic Acid" 1 1 Ci\Quantificatio	n\data\SmartProbe\nmr	
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PCA	L 🐨 🚜		
Current			
Reference	Current spec	crum	
	ECONC	10	Concentration represented by Eretic signal
	ECONCU	mmol/l	Unit of concentration
	EPOS [ppm]	0	Position of Eretic signal
	ELW [Hz]	1	Line width of Eretic signal
	EINT	2.76436e+007	Integral of Eretic signal
	ECORR	1	Additional concentration correction factor
	RORIGIN	C:/Quantification/data/SmartProbe/r	Full path of originating Eretic file
	Reference Sp	pectrum	
	NS	8	Number of scans
	P1 [µsec]	9.82	Pulse length
	PL1	Power level	
	REINT	2.76436e+007	Integral
	RECONC	10	Concentration represented by Eretic reference signal
	RECONCU	mmol/l	Unit of reference concentration
	REPOS [ppm]	0	Position of Eretic signal
	RELW [Hz]	1	Line with
	REREG	Show	Integral regions of reference spectrum
	RG	181	Receiver gain
	RPROBHD	5 mm PABBO BB-1H/D Z-GRD Z113652	Probe

Figure 4.1: Inserting the Synthetic Signal in the Spectrum

The **adderetic** command will insert the synthetic signal in the spectrum, with the user-defined line width and chemical shift. Clicking on the **A** button will do the same. It should be pointed out that the integral value of this signal is weighted by the P90, NS and RG ratios between calibration and quantification.

Bruker TopSpin 3.5.a on INTRA-BRKR-CORP as Jerome.Coutant	
<u>Start</u> Process A <u>n</u> alyse P <u>u</u> blish <u>V</u> iew <u>M</u> anage	1
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1 "Ethoxycinnamic Acid" 1 1 C:\Quantification\data\SmartProbe\nmr	- • 💌
Spectrum ProcPars AcquPars Title PulseProg Peaks Integrals Sample Structure Plot Fid	
BBFOsp Ethoxycinnamic acid Quantification	[lei]
	-
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	- - - <b>4</b>
	- 70
	-
	[ppm]

Figure 4.2: Eretic signal

### 4.2 Modification of the Reference Dataset

ERETIC2 offers the possibility to modify the dataset used as calibration reference. You simply have to use the **Full path of originating Eretic file** button.

### Miscellanous

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Current	Current Spectr	um							
Reference	ECONC	10	Concentration represented by Eretic signal						
	ECONCU	mmol/l	Unit of concentration						
	EPOS [ppm]	0	Position of Eretic signal						
	ELW [Hz]	1	Line width of Eretic signal						
	EINT	2.76436e+007	Integral of Eretic signal						
	ECORR	1	Additional concentration correction factor						
	RORIGIN	C:/Quantification/data/SmartProber	Full path of originating Eretic file						
	Reference Spe	ectrum							
	NS	8	Number of scans						
	P1 [µsec]	9.82	Pulse length						
	PL1	Power level							
	REINT	2.76436e+007	Integral						
	RECONC	10	Concentration represented by Eretic reference signal						
	RECONCU	mmol/l	Unit of reference concentration						
	REPOS [ppm]	0	Position of Eretic signal						
	RELW [Hz]	1	Line with						
	REREG	Show	Integral regions of reference spectrum						
	RG	181	Receiver gain						
	RPROBHD	5 mm PABBO BB-1H/D Z-GRD Z113652	Probe						

Figure 4.3: Using the Full Path of Originating ERETIC File Button

A new dialog window will open.

Create Dataset       Index in the index inde		<u>S</u> tart	<u>P</u> rocess	A <u>n</u> alyse	P <u>u</u> blish	<u>V</u> iew	<u>M</u> anage	ascom	0						1
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You will have to browse through the directory to the new calibration dataset, and load the "eretic" file.

#### Miscellanous



Once the file has been changed, you have to reload the acquisition parameters of the new calibration dataset by clicking on the  $\bf{C}$  button.

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1 "Ethoxycinnamic	Acid" 1 1 C:\Quantification	h\data\SmartProbe\nmr							
Spectrum	ProcPars AcquPa	rs Title PulseProg Peaks Integrals San	nple Structure Plot Fid						
PCAL	V #3								
Current		trum							
Reference		10	Concentration concentration frontia signal						
	ECONC	10	Concentration represented by Eretic signal						
	ECONCU	mmoi/i	Unit of concentration						
	EPOS [ppm]								
		1	Line width of Eretic signal						
	ELINI	1	Additional concentration correction factor						
	POPICIN	1 C:\Quantification\data\SmartDroho\r	Full path of originating Frotic file						
	Reference Sp	ectrum							
	NS	8	Number of scans						
	P1 [µsec]	9.82	Pulse length						
	PL1	Power level							
	REINT	2.78136e+007	Integral						
	RECONC	10	Concentration represented by Eretic reference signal						
	RECONCU	mmol/l	Unit of reference concentration						
	REPOS [ppm]	0	Position of Eretic signal						
	RELW [Hz]	1	Line with						
	REREG	Show	Integral regions of reference spectrum						
	RG	181	Receiver gain						
	RPROBHD	5 mm PABBO BB-1H/D Z-GRD Z113652	Probe						
1.									
-									

Figure 4.4: Reloading the Acquisition Parameters of the Calibration Dataset Using the "C" Button

The acquisition parameters of the new reference spectrum have been loaded.

#### 4.3 List of Commands

Adderetic : Add an ERETIC peak in the current data set.

**Create\_eretic\_ref** : Define the concentration (mM), the integral regions and nucleus/region used for the setting of the reference ERETIC peak.

tererence dataset										
Name	C:\Quantification\dat	C:\Quantification\data\SmartProbe\nmr\Ethoxycinnamic Acid\1\pdata\1								
Concentration [mmol/	/ ] 10.0000									
Number of atoms	Region start [ppm]	Region end [ppm]	Molecule name	Sample volume [ml]	Molar mass [g/mol]					
1	7.809147	7.648134	Ethoxycinnamic acid	0.6000	192.2100	+ -				
3	7.490475	7.342879	Ethoxycinnamic acid	0.6000	192.2100	+ -				
1	6.581420	6.346609	Ethoxycinnamic acid	0.6000	192.2100	+ -				
2	4.488247	4.136030	Ethoxycinnamic acid	0.6000	192.2100	+ -				

Calc\_eretic: Calculate the concentration (mM).

ERETIC2 Quantification							×	
Reference dataset								
Name	Name C:\Quantification\data\SmartProbe\nmr\Calibration\1\pdata\1							
Concentration	10 mmol/l							
Quantified data	aset htification\data\SmartProbe\nm	r\Ethoxycinnamic Acid\:	l\pdata\1					
Number of ato	ms Region start [ppm]	Region end [ppm]	Molecule name	Sample volume [ml]	Molar mass [g/mol]			
1	7.809147	7.648134	Ethoxycinnamic acid	0.6000	192.2100	+ -	•	
3	7.490475	7.342879	Ethoxycinnamic acid	0.6000	192.2100	+ -		
1	6.581420	6.346609	Ethoxycinnamic acid	0.6000	192.2100	+ -		
2	4.488247	4.136030	Ethoxycinnamic acid	0.6000	192.2100	+ -		
							-	
						OK Cano	el	

**wpar eretic**: If an eretic file has been created, the option **eretic** added to the command wpar will write the eretic file in the parameter set file.

**rpar eretic**: This command will write in the current dataset the eretic file contained in the parameter set file which has been read.

# 5 Contact

#### Manufacturer

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WEEE DE43181702

#### **NMR Hotlines**

Contact our NMR service centers.

Bruker BioSpin NMR provides dedicated hotlines and service centers, so that our specialists can respond as quickly as possible to all your service requests, applications questions, software or technical needs.

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https://www.bruker.com/service/information-communication/helpdesk.html

Phone: +49 721-5161-6155 E-mail: nmr-support@bruker.com

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