

KJM 5300

Ш

## **ICONNMR-BACS** automation nmr

## Version 1.0

Topspin 3.2 on AVIIIHD400 and AVII400

Windows 7/Windows Vista



© F. Rise, November 2017



The combination of the BACS sample changer and the ICONNMR program that is running TopSpin for you greatly simplifies nmr acquisition, but reduces the freedom to fine tune the parameter selection. Before using the ICONNMR program you must define your password at a time when the PCs are not running experiments and your account must be activated inside the ICONNMR program by someone with administrator rights.



When this is done and the ICONNMR program is started by the user "tnmr" you will find your username in the list of users. Click on you name and enter your password in the pop up window.



Then you look for an empty position in the BACS sample changer as close as possible after the last ran sample and add you sample with a correctly positioned holder (use the adjustment tool). The holder position must also be unused in the program of course. When your experiments are done you must come back to the lab ASAP to remove your sample and also delete your entry/entries in the ICONNMR program. When you have placed your sample in the BACS you can define your experiment(s) in ICONNMR. If you do it the other way e. g. you define your experiments first and the instrument/program is idle the program might decide to activate the changing arm to grab the sample that is not there – and the program crashes completely. Click on the empty line in ICONNMR. Start filling in information from the left to the right. First you fill in the name of the molecule/sample. Then you select the solvent.

Annual A	AL RECOVERING	0.01.32	D:\uie\AV	18400-17 - New	Medicine				10	* CD	CB chief	
	NPROTONI28	n openment 128 scans										• N PROTOP
form-d	•											
	C SHSOCETGPSISP2.2	Selective HSQC										
100 Barris	C SHRBCCCTETGPL28D	selective MEBC for carbonyl region										
	N P31ZGIG20PPM N P31ZGIG											
	N CLISCPD SW240	13C experiment with decoupling, 1024 scans, 235 ppm 13C with decoupling, SW extended to 240 ppm	Cancel				-		7.0	-		
	N CI3DEPT135	13C DEPT135, CH3/CH positive, CH2 negative, 256 scans, 160 ppm		nein and	(a) 200	OK BC		Copy	1 13	1		
	N WATERSUP	1H with water supression					Add this	number of exp	enment	entries	to the curren	ity selected holder
	C COSYGPDEPRSW	Gradient selected double mustim filtered phase sensitive CDSV										and the second se
	C FASTLARE HSOC	Combined analysis of 1D1H and HSQC data		No.	Experiment	Load	ATM	Lock Shire	Aco	Proc	User	Disk
	C HMDCGP	1H-13C 1999C with gradient selection		10	PROTON	1	1	11	1	1	Tawanb	D.\uio\AVIIH00-17\data\rawar
	C HMBCETGPL3ND	1H-13C HMBC with gradient selection using 3-fold low pass filter for better 1J		10	PROTON	1	1	1. 1.	4	1	geirkild	DriviolAVIIH00-17\data\genki
1.000	C HMBCGP_15N	1H-15N HMBC with gradient selection	coupled 01	10	PROTONIZE	2	2	2 2	2	1	emilyna	Driven AVENOD-17/data/emuly
a state of the sta	C HEOC TOCSY ADIA	1H-13C HSQC-TOCSY with gradient selection BFI >= 700 HHz		11	F19CPD		1		1	1	volodyml	D:\uio\AVII400-17\data\voled
	C HSQC_TOCSY	1H-13C HSQC-TOCSY with gradient selection BP1 <= 600 HHz	and in	10	PROTON	1	1	1.1	1	1	volodymi	D:\uno\AVII400-17\data\volod
	C HSQCEDETGPSISP_AD	(A 1H-13C multiplicity edited HSQC with gradient selection Bri de 600 MEs		10	PROTON	1	1	1 1	1	1	brittpa	D:\uio\AVII400-17\data\hmmpa
Desk	C HEQCEDETGPSISP	1H-13C multiplicity edited may with gradient selection for the out has		10	PROTON	ý	2	2 2	2	5	bottpa	D:\uip\AVII400-17\data\brittea
D:\uio\	AVER ISOCHTOP 158	IN-ISS BAC WICh grantent selection	cetonitrie	10	PROTON	1	1	11	1	1	brittpa	D:\uio\AV30400-17\data\brittpa
D'lunol	AVEA00-17\data\gerkid\nmr		-30 double	10	PROTON	1	1	1, 1,	1	1	brittpa	D:\uso\AVIII400-17\data\brittpa\
Diluin	Avitago 17 data wanand port	stef: reference peak not found default calibration done	002	10	PROTON	~	-		-	4	(wearite	Division Alenano 17 data jewanni

Followed by selecting the kind of experiment(s) you need. ALWAYS select PROTON first, even if you think you do not need it. For 99 % of the two dimensional nmr experiments obtained in ICONNMR the program needs a PROTON experiment to look at. If it is missing the 2D nmr experiment will fail completely and most likely crash the program. Then you click on the Add button.



The next standard experiment to choose one either of the two 400 MHz instruments is the Carbon experiment C13CPD. Be aware that you must change the number of scans to 512 if you want to run this experiment on day time on AVIIIHD400 since this instrument is dedicated to fast experiments during day time. If selecting 1024 or higher for ns change to night time as shown above on this instrument. On the AVII400 instrument all experiments are running continuously so we do not distinguish between day and night experiments there. If you use ICONNMR on the AVI600 or AVII600 instruments please do not select C13CPD but use C13RESPECT instead. C13CPD gives an ugly rolling baseline in the spectrum on

the 600s since the solid state <sup>13</sup>C atoms in a polymer in an insulating material between the sample held at ~300 Kelvin/ca 25 °C and the radio sender/receiver at 15 Kelvin give a very broad <sup>13</sup>C nmr signal. This signal is removed in C13REPECT.



If your sample has low Molarity you need lots and lots of scans to get a decent 13C nmr signal. The button to the right of the day/night button brings up the list of parameters you are allowed to change. When you become experienced you might miss some parameters here. Ask and they will be added. Change for instance NS from 1024 to 4096 as shown above here. Click on OK at the bottom of the pop up window.

DCB chloroform-d	- N PROTON 1H.						
DCB chloroform-d	• N CL3CPD 13C •		chioratorm	d i	SHSOCETGPS1SP2.2 SHMRCCCTETGPL2ND	Selective HSQC	
The User Disk resets Disk Disk Disk genide Disk Disk	C 55502CRT0/911592.2 C 55802CRT0/911592.2 C 55802CRT0/91159 F 527128 F 5	Selective Hop: Selective Hop:	ber swanb pricid milyba sapar solodymi rittpa rittpa rittpa rittpa rittpa	Disk DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20 DisiolAV20	FR/TX8 F332/CIG 020FPM F332/CIG 020FPM F332/CIG 020FPM C13/CPD 5N240 C13/CPD 5N240 C13/CPD 5N2 MOTESSUP C05/CIGP/HISM FSOC FSOC/FD/FSISP FSOC FSOC/FD/FSISP ADIA RSOC/ED/FSISP	If experiment with decoupl 10 experiment with decoupl 10 extra decoupling if we are 10 extra decoupling if we are 10 extra decoupling if we are 10 extra decoupling if we are combined analysis of DUT as 10 -100 MBC with gradient as	region ling, 1024 scales, 235 pps model to 246 pps a C28 anguitw, 256 scales, 166 pps a C28 anguitw, 256 scales, 166 pps a C28 anguitw, 256 scales, 166 pps a C28 anguitw, 256 scales, 250 pps delton using 3-fold inv pass filter for better 50 Q detter mit selection MP 3-700 MPc MP villa gradient selection MP 3- 600 MPc MPC villa product selection MP 3- 600 MPc MPC villa product selection MP 3- 600 MPc MPC villa product selection MP 3- 600 MPc
velodymi DiusolAk velodymi DiusolAk heftpa DiusolAk heftpa DiusolAk heftpa DiusolAk heftpa DiusolAk heftpa DiusolAk kettpa DiusolAk velodymi DiuselAk velodymi DiuselAk	C ISOCREGESTSP C HSOCREGESTSP C HSOCREGESTSP SH400-17/udata/brittpa/nore SH400-17/udata/brittpa/nore SH400-17/udata/brittpa/nore SH400-17/udata/brittpa/nore SH400-17/udata/brittpa/nore SH400-17/udata/brittpa/nore SH400-17/udata/brittpa/nore	18-15: mittipilicity exitted RRC with gradient windertim RT - eXD Min III-100 RRC with gradient windertim and inference pairs of though default calculated data and inference pairs of found default calculated data and inference pairs of found default calculated and and inference pairs and inference calculated and and and and and inference pairs and found and and calculated and and and inference pairs and inference calculated and and and inference pairs and and and and calculated and and and inference pairs and inference calculated and and and and and and and and and and and and	srittpa kwashla crutigi volodymi otiodymi crutigi knuthy knuthy knuthy knuthy knuthy knuthy knuthy knuthy knuthy	D:\usic\AVIII4 D:\usic\AVIII4 D:\usic\AVIII4 D:\usic\AVIII4 D:\usic\AVIII4 D:\usic\AVIII4 D:\usic\AVIII6 D:\usic\AVIII6 D:\usic\AVIII6 D:\usic\AVIII6 D:\usic\AVIII6 D:\usic\AVIII6 D:\usic\AVIII6	09 17/data/bittpairms 10 17/data/bittpairms 20 17/data/violodymifums 20 17/data/violodymifums 20 17/data/violodymifums 20 17/data/violodymifums 20 17/data/violodymifums 20 17/data/viology/mms 20 17/data/viology/mms 20 17/data/viology/mms 20 17/data/viology/mms 20 17/data/viology/mms	prod far etch, 1d it wak, cdcfl prod far etch, 1d it wak, cdcfl	and information paids and found address of address on danse with INCLUS TBP innet address of the SOLVENT ENDOR of the sets to D0 with INCLUS TBP innet address of the SOLVENT ENDOR of the sets to D0 with information paids and found address of address of the with information paids and found address of address of the with information paids and found address of address of the with information paids and found address of address of the with information paids and found address of address of the with information paids and found address of address of the with information paids and found address of the sets address of the with information paids and found with address of the with information paids and found with address of the with information paids and the address of the address of the with information paids and the address of the address of the address of the with address of the address o

A series of two dimensional experiments and other experiments will now be selected. After clicking the ADD button (see earlier in this manual) you might select COSYGPSW. COSY shows you correlations between hydrogen atoms 2 to 4 bonds away. When this manual was made hastily the author chose to add HMBCGP, logically this is normally added last in an experiments setup, but since it is shown in the pictures it is commented here. HMBC experiments is connecting <sup>1</sup>Hs with <sup>13</sup>Cs atoms 2, 3 and 4 bonds and sometimes even 5 bonds away. The two letters GP seen here means (magnetic) gradient pulse – this is an invention that greatly speeds up your experiments when you have high Molarity samples. In old fashion 2D nmr so called phase cycling is used to suppress unwanted peaks. Often 4, 8, 16 or 32 numbers of scans had to be used for each increment in the indirect dimension even though one scan had been enough for fat samples. With the GP invention made and patented by the company Nicolet in Madison WI - 2D nmr became much faster for high Molarity samples since NS can be 1 in such cases.

woform-d • N C1	CPD 13C experiment with decoupling 1	024 ; <a -<="" th=""><th>60.01.32</th><th>or see</th><th>uradient selected COS</th><th>Y</th><th>- 🔳 🖪</th><th></th><th>froderi</th></a>	60.01.32	or see	uradient selected COS	Y	- 🔳 🖪		froderi
actorned • C CO	VGPSW Gradient selected COSY		0354.07 HMB	GP	1H-13C HMBC with gra	dient selection	-		froden •
oroform-d • CHM	BCGP 1H-13C HMBC with gradient selec	tion • 🔳 🖪 🖶 🔺 🖉	NOES	YPHSW	Phase sensitive NOES	r			froden +
Choice Choi	SYPHON     Plane sensible NOCKY       BRTOFALD     84-34-0000000000000000000000000000000000	Alter Styre with an and an an and an	0 Mits 0 Mits Provide 125 Provide 125 Pr	Arawanb'na Arawanb isa Ageidaldan Arwighala Arwighas Arwighas Arontpalan Abrittpalan Abrittpalan Abrittpalan Abrittpalan Abrittpalan Abrittpalan Abrittpalan Abrittpalan Abrittpalan Abrittpalan	Tritle/Org mr mr mr mr mr Mann Vanne Vanne Mr mr mr mr mr mr mr mr mr mr mr mr mr mr	10 AQ DI L30 17D DE DE DE DS NS 15W 15WH 25WH 01 01 01 01 01 01 01 01 01 01 01 01 01	2046 0.255 [sec] 2 [sec] 1 256 6.5 [sec] 4 9.95073 [spo] 4 9.95073 [spo] 4 0.95073 [spo] 4 0.95073 [spo] 4 0.95073 [spo] 1 4.000 [std] 1.9545 [sec] 1.95573 [spc] 1.95573	Size of Ed replaction time Balandario time Balandario time Balandario time Bandher of Langraguants Size of Ed in 12 Annanophilis and Langraguants Size of Ed in 12 Annanophilis and Langraguants Size of Ed in 12 Annanophilis and Langraguants Decky between telescophilis and Langraguants Michiko II A Annanophilis patholis and Langraguants Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 12 Annis in 14 Dala Size and Ingentificant Catter of agencanis in 14 Annis in 14 Dala Size and Ingentificant Annis Annis An	froden

The next experiment selected here is the NOESY experiment. This experiment correlates <sup>1</sup>Hs that are close in space. Usually this experiment is used by Biochemists and one important parameter the so called mixing time is set to a default value that is suitable for large MW molecules. The D8 parameter must be changed to a higher number than what the program selects for you in order get meaningful results. Ideally you should have done a so called T1 inversion recovery experiment and found relaxation times for the hydrogens in your molecule and then used a formula to calculate D8. This is beyond the scope of KJM 5300. For small molecules MW 300-700 you simply change D8 to 0.75 seconds and hope for the best. One other problem with NOESY for small molecules is that you might have both COSY and NOESY correlations between the same hydrogens at the same time. The COSY correlations might infest or pollute your NOESY spectra. How to distinguish them is not explained here.

ATTACK PRODUCTION	n gradient sele	rction .	•	* & Ø	frodeni			and matter cosy		
NOESYPHOW Phase sensitive N 5 7 Adda (search) and 17 Adda (searc	VOESY	TD AQ D1 L30 1TD 2TD DE D8 D8 NS 1SW 1SW 1SW 1SW 2SW 2SWH 2SW 2SWH 01	2048 0.256 [sec] 2 [sec] 1 256 2048 6.5 [µsec] 0.257 4 4 4 9.99671 [ppm] 4000 [Hz] 9.99671 [ppm] 4000 [Hz] 1381.86 [Hz]	See of fel anguitos trais: Number of supersating pairs. See of fel 17 dimension See of fel 18 Z dimension See of fel 18 Z dimension NOIX mining time (set 2 to 2 stor small molecules) days between schematic pairs of the second NOIX mining time (set 4 to 22 stor small molecules) days Number of z dimension pairs within F2 Z dimension in His within F2 Z dimension in His supersation 18 Z dimension in His	froder •	d Disk Diskov Diskov Diskov Diskov Avg Diskov Avg Diskov Avg	C. BROOP C. BROOP C. BROOP C. BROOP C. BROOTH C.BROOTH C.BROOT	aw opt. HEC with p w opt. HEC with p w opt. doi:10.1000 ar opt. HEC with p w opt. doi:10.1000 ar opt. HEC with p w opt. HEC with p ar opt. HEC with p a	The set of sectors and a set of a sector a sector a sector a set of a sector a set of a sector a secto	
9-17-Udata jewashikivnin 6-17-Udata (costigilumi 10-17-Udata (colodymilumi 10-17-Udata (colodymilumi 10-17-Udata (knudhylumi 10-17-Udata (knu	sil fra etoh, 1: sil fra etoh, 1: sil fra etoh, 1:	O2 O2P P1 PLW1 TE	4.803 [ppm] 1841.86 Hz 4.603 [ppm] 9.6 [usec] 15.9 [W] 300 [K]	Control of spectrum in z and, in piper in z liper, can be cannot a spectrum in E1 dim, in the taken second name Contex of spectrum in E1 dim, in pain (also second suppor Public Power level in Wett Required Sample temperature	on point manually in 2 page point manually -	Draiolavi Draiolavi Draiolavi Draiolavi Draiolavi Draiolavi Draiolavi Draiolavi	pr. Cristol 17-124-10 400-17/data/brittpa/nmv 400-17/data/brittpa/nmv 400-17/data/brittpa/nmv 400-17/data/brittpa/nmv 400-17/data/brittpa/nmv 400-17/data/cristig/nmv	u = 10	stell: reference peak not found default calibration done stell: reference peak not found default calibration done sort: reference peak not found default calibration done unt: reference peak not found default calibration done	

After adding a new experiment line HSQCETGP is selected here. This experiment gives correlation peaks between all pairs of <sup>1</sup>H and <sup>13</sup>C that are directly correlated (one bond).

a shareform	d .	CHOCETOP THE	ngit. HSQC with gradients (e/a TPD) - 🔡 🔯 🗐 👘 🚖 🗭
II chipshana	d .	MOOOP TEQOOP	av opt. HQC with gratients (maps. mode) av opt. HQC sens. improved with gratients (son TPF)
		INCOMPANY INCOMPANY INCOMPANY INCOMPANY INCOMPANY	av     07-01-01160     Unit MDC with gradients (asyn, mong)       av     07-101     Units SID palae (asyn, mong)       av     07-100     Units SID palae (base, mong)
		NECCEND.	av opt. BDC with graduents
tawardo rawardo gorkita serilijita wegav voledjeni voledjeni bettga bettga bettga	Disk Drawn AVE Drawn AVE	INDECTION ISOCENTARE. ISOCENTARE. ISOCENTARE. ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCOLOSIS ISOCENTARE. ISOCEN	exp (etc. IBE with low pass - full ter range, non) (and the second se
brittps jitnozačkia cristinji volicodymi volicodymi cristinji kristinji	DrawnAVE DrawnAVE DrawnAVE DrawnAVE DrawnAVE DrawnAVE DrawnAVE	00-17 idata/dorittpa/usere 00-17 idata/dorista/orene 00-17 idata/condigRorene 00-17 idata/condigRorene 00-17 idata/condigRorene 00-17 idata/condigRorene 00-17 idata/condigRorene 00-17 idata/kondigRorene	with respect to the set of the se

You might also choose to compare the results from HMBCGP with HMBCGPND (ND – no decoupling).

13	-										
						Contract of Contract of Contract			-		
a line beauting of	and that the										
0- # 1							-	-	Paking	CENTRA	COLUMN PROV
ma	later .				Sec		_			UULE	日日キョ
								199	1	the	feer lines
	Section of Contemport	3	100	attendent a	Notesta State	International Addression (12) and	-	B14	-	-	
A Description	Sumaire Sumaire	1.1	100	-	Cristonia	Instant strend 1000	100	S+s		11	Station of the
	Walking of		1213	-	CHARLING	- or the an paint	-	Bra		-	1000000
Contraction of	Section of Concession, Name		-12-12		CHARDIP	In the same we prove strength	-	-		11	-
Toronto	Succession of the local division of the loca		1000		ALCORE	10), dividing product street. (11), multiple product street.		24.8 24.8	10.00 A	H	And in face of the
T-IN.	No. of Concession, Name	2	003	distant of	ATEX PE	100 cop comes paint becausing 200 cop comes paint becausing		014 014		H	Contract (197)
Santan I	and a state of the	8 H R	000	china d	NULLINATE NULLINATE NULLINATE	Di Maninistrano San, COUTE protest Di manurati, se incensiva		514 514		1	Calmination (Calmin State)
Contraction of	Party of the local division of the local div	2	000	ittention d	AUXILIARY NO.	COLUMN STATES AND ADDRESS AND ADDRESS		10+4 10+4	-	1	This is the State
			1000	Contrast of Contra	AUG 100 FUT	SAL METERS, Deliving passing, Drawing	- 36	Bra	-	-	ALL DO NO. OF CO.
2											
3											
2											
			-	and the second	-	Illingenet	-	-	-	-	A Designation of the
Survey of	5700 Tot 1		Contra La C	and the second		(Anternet Martin Trans	15	Bis min	A LOCAL OF BRIDE		States 1

An extensive list of experiments is shown here. In a real scientific setting we select the experiments needed and not a lot of unnecessary experiments. To get the PC to actually add your experiments in the queue you must mark the experiments as shown above with the help of the mouse. Then you click on the Submit button.

The 29Si, 19FCPD, 31PCPD experiments are hopefully self-explanatory. LC1D12 is a <sup>1</sup>H experiment with 2 solvent suppression points, the parameter L30 can be set to either 1 or 2 solvent points to be suppressed. The program is finding the largest peak(s) and kills it/them. LC1DCWPS is a program that suppresses multiple peaks. Change the parameter L30 to the number of peaks to be killed. C13APT sorts CH/ CH<sub>3</sub> (positive) from C /CH<sub>2</sub> (negative). C13GD is a 1D <sup>13</sup>C experiment with no 1H decoupling, it takes time to run since the intensity of the signals seen in C13CPD experiments are split in many peaks. C13IG is a <sup>13</sup>C experiment with no nuclear Overhauser enhancements, but with decoupling during acquisition to give singlets and not multiple signals. C13DEPT90 give CH carbons only. C13DEP45 give you all Cs except carbons with no protons on them. C13 DEPT135 is giving you CH<sub>3</sub> and CH as positive peaks and CH<sub>2</sub> negative.



If you at some point you might realize that a parameter is in need of change, mark the corresponding line with the mouse that contains the experiment and click on Cancel and then on Edit.



Then you do the necessary changes in the pop up window. Click OK. Be sure the line is blue marked. and click Submit.



Be aware that this experiment then will be the last experiment to be run. You cannot change anything in an experiment that is needed for another experiment later in the list. The experiment(s) that need the information from the one you changed will fail since the needed experiment will run later and after three failures the program will crash. If unsure cancel all experiments and submit the whole list again.



Bring up the Change user window (button located far to the right in the IconNmr window and click on Lock IconNMR. If you forget this and another distracted user arrives shortly after you left the instrument the screen is still open and that absent minded user might add her/his experiments with your identity.

Wait for e-mails telling you that your experiments are finished. See another manual for how to obtain your data for processing.