Operating Instructions for the Varian Gemini-200

Overview of the Spectrometer

Magnet and Probe. The large white "beer keg" is the superconducting magnet with a vertical bore going through the center from top to bottom. The probe is inserted into the bottom of the bore and slides up so that the part that receives the sample tube is in the center of the magnet. The magnet is supported by two legs, and on the left leg is a gauge that indicates the spin rate as well as a green light that indicates whether or not the spin rate is regulated. To the right of the magnet is the **autosampler**, a robotic device which can sequentially insert and remove up to 50 samples into the magnet for unattended runs. The autosampler is used only by NMR facility personnel. On the top surface of the autosampler is the **tray**, which holds the spinner turbine. A sample depth gauge is printed to the right of the tray.

Console. To the left of the magnet is the console which contains all of the RF electronics and the computer. On the front panel of the console (upper left) there is a 5.25" X 5.25" floppy disk drive and a red button which will reboot the computer, as well as a digital display of the sample temperature.

Monitor. The Gemini 200 has a color monitor which is rather difficult to see - you might want to turn the lights out for better visibility. At the bottom of the monitor are five knobs which have different effects depending on the software. The definitions of the knobs will always be indicated at the bottom of the monitor display. Below the five control knobs on the monitor are knobs that adjust the brightness of the monitor and a switch that will turn the monitor on and off.

Keyboard. The keyboard on the Gemini is similar to your standard keyboard. There are 15 function keys on the keyboard (labeled *PF1 - PF15*) which control most of the commands necessary to shim, lock, and process your data. All of the commands necessary to take an nmr spectrum can be performed on the keyboard and using the five control knobs under the monitor.

Logging In

Before any operations can be performed on the spectrometer, one must first log in to the instrument. This is done by entering **LOGIN** at the keyboard; when the computer asks for the user name, enter the login name for your account. At this point the computer will ask for a password. You will be given a password when you pass the "driver's license test" and are authorized to use the instrument. This password is your secret - if you give your password to anyone else you can lose your NMR priveleges! Your account (research group) will be billed for the time starting when you log in and ending when you log out.

Sample Insertion, Lock, and Shimming

Inserting the sample. Simply enter "**E**" at the keyboard to eject the standard sample (1% $CHCl_3$ in $CDCl_3$, sealed tube). Place the standard sample and spinner turbine in one of the

slots in the autosampler tray. Insert your sample into an empty spinner turbine carefully, pushing the sample tube straight into the spinner hole (this is a pretty tight fit and NMR tubes are fragile!). Use the depth gauge printed on the sample changer to adjust the position of the sample in the spinner: start with the sample tube protruding just a little below the bottom of the spinner, position the sample and spinner with the "lip" of the spinner resting on the surface of the tray and push the sample tube gently until the bottom of the tube lines up with the mark on the gauge. This adjusts the sample depth so that the liquid in the tube will be centered inside the RF coils of the probe when the sample is inserted. Clean the bottom part of the sample tube with the ethanol and a Kim-wipe while holding the assembly by the top of the sample tube. Place your sample on the stream of air which is still coming out of the magnet, being careful not to let go of the sample until you are sure it is supported by the air. Then simply enter "**I**" to insert your sample into the magnet.

Locking. Varian's lock display is considerably different from Bruker's. Enter "**LK**" to start the interactive lock display, and *PF3* to turn the lock off. At this point one should see a display on the monitor with a straight line running horizontally across the screen, with some noise or "squiggles" in the line.   The leftmost knob (Z0) controls the magnetic field strength (a small correction to the main magnetic field ranging from -2047 to +2047), the second knob controls the lock power (maximum setting = 40), the center knob controls the lock gain (maximum setting = 30), the fourth knob controls the lock phase and the rightmost knob controls the spin rate. The lock display is the time-domain deuterium signal of your deuterated solvent. If the deuterium signal frequency (determined by Z0) is close to zero, you will see a sine wave on the lock display with a small number of cycles ("squiggles"). You may need to increase the lock power and / or lock gain to see the signal. If you still don't see a signal you need to adjust the field (Z0) to move the deuterium signal frequency into range. There should be a 3 x 5 card on the monitor which gives "ball park" values for all of these settings for each deuterated solvent. As you get closer and closer to zero frequency, the number of cycles displayed on the screen will decrease until, at the zero frequency, you just see a horizontal line which rises and falls on the screen with time. If you move past the zero frequency you will start to see the sine wave squiggles again. At the zero frequency, press function key *PF4*, which will turn the lock on, activating the feedback loop which stabilizes the field strength. The display should still be a horizontal line, but it will rise up above the reference line at the left of the display and remain stable. If the lock level (height of the horizontal line) seems to be moving up and down slowly this is an indication that you are saturating the deuterium nuclei. Turn the lock power down until the lock level stabilizes, increasing the lock gain as necessary to maintain a high lock level. The 3 x 5 card gives starting values for lock power which are quite high to help you find the signal. The final values given are more appropriate once you have locked, and turning the lock power down to this value should prevent "breathing" of the lock level. Sometimes if the shims are really far off you will have difficulty getting the system to "lock". In that case it is best to load the standard shims and try again (see below).

Shimming. The shimming mode can be entered by entering "**SHIMI**" (shim interactive). Simply entering "SHIM" tells instrument to automatically shim for you, but the instrument generally shims much more slowly than you can. The SHIMI display shows a list of function keys which activate different sets of shims you can control with the five control knobs, and two

vertical lock level bars. The bar one on the right is a 5X expansion of the one on the left. First adjust the lock phase by pressing function key *PF5* and then adjusting the fourth knob from the left to maximize the lock level. If lock phase is improperly set, you can maximize lock level with the shims and still get a lousy spectrum! Then press function key *PF2* to access the Z shims. Generally for routine samples you will only need to adjust the Z^1 and Z^2 shims. Use the lock gain adjustment to set the lock level to around 80 and then adjust the Z^1 shim to maximize the lock level. Then adjust the Z^2 shim to maximize the lock level. Shimming is an iterative process, and this maximization routine must be repeated several times. If the instrument is very poorly shimmed and adjusting the Z^1 and the Z^2 doesn't dramatically improve the quality of the spectrum, you can read in a shim file from the disk. This can be done by entering "**RTS**(*DATE*)" and then "**SU**". A list up on the bulletin board behind the monitor shows the dates when shim files were stored. By using the most recent date (e.g., MAR08) in place of *DATE*, you will get a set of shims which should be close to optimal. This can then be used as a starting point for manual shimming using **SHIMI**.

Setting Up for Data Acquisition

Entering "**DG**" (display group of parameters) will display a list of the parameters. Any parameter can be changed by entering "*NAME=value*" where *NAME* is the name of the parameter (e.g., NT) and *value* is the new value (e.g., 32). The easy way to set up an experiment is simply to type "**SETUP**(*nucleus*,*solvent*)" which will set up the parameters for a simple 1D spectrum of the *nucleus* (H for proton, C for carbon) in the indicated deuterated *solvent* (CDCL3, D2O, DMSO, ACETON, or CD2CL2). The parameters are listed under headings of Acquisition, Decoupling, Flags, Processing, and Special. Under Acquisition:

SFRQ	Spectrometer Frequency (in MHz)
TN	Transmitter Nucleus (^1H = 1.000, ^{13}C = 13.000)
SW	Spectral Window (in Hertz)
AT	Acquisition Time (in seconds)
NP	Number of Data Points (real + imaginary)
FB	Filter Bandwidth (Hertz, calc. from SW automatically)
BS	Block Size (Number of Transients between Disk Saves)
SS	Number of Steady State (Dummy) Transients
PW	Pulse Width (in microseconds)
P1	Width of Second Pulse (not used in simple 1-D)
D1	Relaxation Delay (in seconds)
D2	Second Delay (not used in simple 1-D)
TO	Transmitter Offset (in Hertz) from the basic frequency SFRQ
NT	Number of Transients Planned
CT	Completed Transients at the Present Time

The decoupling parameters are only relevant for ^{13}C acquisition or for homonuclear (proton) decoupling experiments such as NOE difference:

DN	Decoupler Nucleus (^1H = 1.000, ^{13}C = 13.000)

DO	Decoupler Offset (in Hertz)
DM	Decoupler Mode (Y = on, N = off; for relaxation, excitation, acquisition)
DHP	Decoupler High Power Level
DLP	Decoupler Low Power Level
HOMO	Homonuclear Decoupling (Y = Yes, N = No)

Flags which are important are:

IN	Interlock (stops acquisition if lock is lost)
DP	Double Precision (uses a more precise data value)
ALOCK	Automatic Lock

IN is usually set to "N". **DP** should be set to "**N**" except for long acquisitions, for example overnight ^{13}C acquisitions. The FID file size will be twice as big with **DP**, so it is only used in situations where the repeated summing of a large number of FIDs could overflow the precision of the data values. If this happens, acquisition will stop with the message "Maximum Number of Transients". ALOCK is a time-consuming automatic locking process which is executed at the beginning of acquisition; it should be disabled ("**N**"). The important Processing parameters include:

LB	Line Broadening Parameter in Hertz
FN	Number of Data Points after Zero Filling

The following parameters are not listed by the command **DG**:

GAIN	receiver gain
NI	number of experiments in a 2D acquisition

Acquiring Data

The Gemini will automatically adjust the receiver gain if the parameter GAIN is set to N (not used). For proton acquisition on very concentrated samples you will sometimes get the error message "*Gain at a Minimum, reduce pulse width*". This means the automatic gain adjustment has reduced the receiver gain as much as possible and the receiver signal is still too large for the analog-to-digital converter (ADC). In this case decrease the pulse width (PW) to a small value like 3 (microseconds) and start the acquisition again. The small pulse width gives a much less than 90 degree "flip" of the spins, creating a much smaller magnetization in the X-Y plane.

To start the acquisition, enter "GO"; this starts the acquisition, which will continue for the number of transients (**NT**) shown in the parameter list. The computer will beep at you and display the message "Acquisition Complete" when the requested number of scans are finished.

Processing Data While Acquiring. The FID is not normally displayed in the acquisition mode on the Gemini.   To see the FID, simply enter "FID" and the current sum of FIDs will be displayed. This sum is only updated on the screen if you enter "FID" again. When the number of transients collected (CT) equals the size of a block (defined by the parameter BS), the data

collected up to that point can be processed. When the value of CT reaches any multiple of BS, the summed FID up to that point is stored to disk and is available for processing. Simply typing "**DF**" at this point will display the sum of FIDs. Then the FID can be transformed as described below, and acquisition will continue in the background until **NT** scans are acquired. Unlike the Bruker, the number of scans cannot be set to infinity, but you can make **NT** sufficiently large (e.g., 100,000) that it is almost the same thing. In this way you can follow the progress of a long acquisition such as a carbon spectrum, monitoring the signal to noise ratio of the spectrum as the acquisition progresses. To stop acquisition at any point simply enter "**SA**" (stop acquisition). Alternatively one can type "**/A**" or "**/AA**" which will also stop the acquisition. The difference between these three commands is that after entering "**SA**", one can reset the "**NT**" to a new value and then resume the acquisition using the command "**RA**". "**/A**" stops the acquisition without the possibility of resuming, and "**/AA**" aborts all acquisitions (including any that may be queued).

Processing the Data

The Fourier Transform. When acquisition is complete, enter "**DF**" to view the FID. Use the *VF* knob to adjust the vertical scale so you can examine the FID. Check the line-broadening parameter "**LB**" (enter **LB?**) to check the steepness of the exponential multiplier. I generally use 0.2 Hz for proton spectra and 1.0 Hz for carbon spectra. Entering the command "**WFT**" then applies the weighting function and executes a Fourier Transform. Enter "**F**" to display the full width of the spectrum.

Expanding Regions of a Spectrum. The region of a spectrum displayed on the screen can be expanded by by adjusting the leftmost control knob (*WP*): the smaller the value of *WP* (width of plot), the smaller the region one is observing. The "display window" can be moved left or right by adjusting the second control knob from the left (*SP*). The value of **SP** (start of plot) indicates the value of the rightmost point which is presently being displayed on the monitor. Another way to expand a region is to display both cursors on the screen (press *PF3* twice). The *PF3* fuction key serves as a toggle bgetween having one cursor on the screen, two cursors, or no cursors. Use the *LCR* (left cursor) and *RCR* (right cursor) knobs to position the cursors around the region to be expanded and pressing function key *PF4* to expand the region. *PF4* serves as a toggle between displaying the region of interest (between the two cursors) or the full spectrum. Entering "**F**" at any time will redisplay the full spectrum.

Phasing. To automatically correct the phase of the spectrum, enter "**APH**". If this does not phase perfectly minor adjustments can be made by adjusting the two rightmost knobs (*RP* can be viewed as the zero-order correction with the pivot at the right edge of the spectrum, and *LP* can be viewed as the first-order correction). If this still doesn't do the trick, enter **QP** to start the quick phase mode. Place the cursor (knob labelled "*CR*") on the pivot peak (major peak on the right side of the spectrum) and adjust *RP* to correct its phase, then use the *LP* knob to correct the phase of a major peak on the left side of the spectrum. Enter "**DS**" (display spectrum) will get you out of this mode. If there are phase errors, your integrals will not be accurate.

Referencing a Peak. To reference a peak bring one cursor onto the screen (function key *PF3*) and position the cursor close to the reference peak. Enter "**NL**" to position the cursor

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exactly on the top of the peak. Then enter "**RL(#P)**" (reference line, ppm) where # is the reference chemical shift value in ppm of the peak. For TMS, for example, you would enter "**RL(0P)**" to set the TMS peak chemical shift to zero. Enter "**DSCALE**" to display a ppm scale at the bottom of the spectrum. If you have trouble finding the reference peak, remember that if you correctly set the SOLVNT parameter to the deuterated solvent you are using, the ppm value displayed for the cursor position should be approximately correct. Posted on the bulletin board there is a chart of chemical shift values for ^{13}C and residual ^{1}H peaks from the solvent.

Setting a Peak Picking Threshold. If you wish to have a printed list of peak chemical shifts, or if you want the chemical shifts indicated on the plot next to each peak, you must first set a threshold value. Press function key *PF6* and adjust the height of the horizontal yellow line using the *TH* knob. Any of the peaks which are above this line will be selected for peak picking. Enter "**LL**" to send the peak list to the printer, located to the right of the monitor. Enter "**DPF**" (display peak frequencies) to display the chemical shifts next to the peaks on the screen. To get rid of these chemical shift labels simply type "**DS**" and the spectrum will be redisplayed. To redisplay the integrals press the function key *PF1*.

Integration. Unlike the Bruker, one does not need to plot the spectrum before integrating; the "**PL**" (plot command) plots both integrals and spectrum if both are displayed on the screen. Press function key *PF1* to display the integrals on top of the spectrum. *PF1* serves as a toggle between having integrals displayed on the spectrum and displaying the spectrum alone. The integral will initially be displayed as a single curve, moving up vertically as it passes each peak in the spectrum horizontally. To reset the integral to the baseline after each peak and turn off the display of integral segments in the noise regions between the peaks, you need to define the integral regions of interest. To clear the integral zero markers, enter "**CZ**". Bring one cursor on the screen using function key *PF3*. Position the cursor just to the left of the leftmost peak, and press function key *PF7* to mark the beginning of the first integral region. Position the cursor to the right of this peak and push function key *PF7* again. Repeat this process for all of the peaks to be integrated. If the integral is still displayed in the regions between integral segments, press *PF10* or enter **INTBL** to "blank" these regions. You can now increase the integral vertical scale by adjusting the *IS* (integral scale) knob until the largest integral is near the top of the screen.

Numerical integral values can also be included on the plot. Enter **INS=1 DLI** to display a list of integral values on the screen. **INS** is the total value of all integral areas, and **DLI** stands for "display list of integrals". The integral regions are listed in order from the most downfield (farthest left) to the most upfield region, with the area of each integral shown. Find the entry in the list which corresponds to a peak for which you know the number of protons (e.g., 3 for a methyl group). Set **INS** to the area displayed for this peak divided by the theoretical number of hydrogens. You don't need a calculator for this: just enter **INS=**(area)/(number of H's). This will normalize the integral values, and you can check the list by entering **DLI** again. If you change the integral scale at any point (knob labelled *IS*), you will need to repeat the normalization process. When you are ready to plot, include **PIRN** (plot normalized integral regions) in your string of plot commands (see below). To re-display the spectrum and integrals, enter **DS** and hit function key *PF1*.

Adding Text.   To enter a short text description of your sample and experiment which can be plotted on the spectrum, enter "**TEXT('**blah blah blah**')**", where "blah blah blah" stands for your message (note the single quotes). The text will then be displayed on a nearly blank screen. To redisplay the spectrum enter "**DS**". Integral regions can be redisplayed by pressing function key *PF1*.

Plotting The Spectrum

Make sure that the desired paper size is loaded in the plotter. To change paper size simply eject the current page by pushing the lower left button next to the liquid crystal display (LCD) on the plotter, position the new paper at the appropriate line, and push the load button again. At the keyboard enter "**PLOT=HPA8**" for small paper (8 1/2" x 11") and "**PLOT=HPA**" for large paper (11" x 17"). The rule for plotting is "what you see is what you get", as far as the spectrum and integral are concerned. Whatever region of the spectrum is displayed on the monitor will be the region that is plotted, and the integrals will appear exactly as they appear on the screen. Enter "**PL**" to plot the spectrum and integral (if the integral is displayed on the screen). Enter "**PSCALE**" to plot the chemical shift scale below the spectrum. Enter "**PIRN**" to plot the normalized numerical values of the integral areas below each integral. Enter "**PPA**" to plot an abbreviated version of the parameters on your spectrum including the text that you entered previously using the **TEXT** command. Alternatively, you can enter "**PAP**" to plot a longer version of the parameters. Enter "**PPF**" (plot peak frequencies) to plot the peak chemical shift values on your plot. If the peak threshold has not already been set using the *PF6* function key (see above) you might end up with thousands of chemical shifts listed on your spectrum, or none. At this point you may have noticed that most of the commands which display things on the spectrum on the monitor screen start with a **D** ("display") and have equivalent commands for plotting on the spectrum which start with a **P** ("plot"). None of these plot commands will have any effect until you enter "**PAGE**", which sends all the plot information to the plotter, completes the plot, and ejects the page from the plotter. All of the above plot commands can be performed as a string of commands. For example, entering "**PL PSCALE PIRN PAP PPF PAGE**" will plot your spectrum and integrals with a chemical shift scale, integral areas, parameters (long version), and peak chemical shifts and will then eject the page from the plotter.

Saving Data to Disk

The FID data can be saved to a floppy disk simply by entering "**SVF(DSK5.myfile)**" where "myfile" is the filename you have chosen. This will save your file on the floppy, which is designated as disk partition 5. **If you don't have a floppy disk loaded in the disk drive when you enter this command, the computer will crash and you will have to re-boot!** Filenames can be 1 to 6 characters in length, consisting of any combination of letters and numbers as long as it starts with a letter. Unlike DOS, there is no extension. The Gemini accepts the old, $5^{1/4}$" x $5^{1/4}$" floppies, which must be formatted on the spectrometer since the disk operating system is unique to the Gemini. To format a diskette, insert it into the floppy disk drive and enter "**FORMAT(DSK5)**". Data saved on the diskette cannot be read on a PC or a Mac without special software. This software, called "V_HELPER", is now available on the PC next to the AM-250. To display a list of the FID files saved on your floppy disk, enter "**DLIST(DSK5)**" or

"**CAT(DSK5)**". The two commands give slightly different display formats. To retrieve your FID from a diskette simply enter "**RT(DSK5.myfile)**". You can now process the data as if you had just acquired it.

To save your file to the hard disk, use a different disk partition: you can use any partition except DSK1, which is reserved for system programs. I usually used DSK3 to save data on the hard disk. Data on the hard disk is not secure - it can be erased by anyone at any time. Data can also be transferred to the Sun workstations. Save the FID on the hard disk and note which partition you used. From the Sun workstation, the following commands can be entered in the input window of VNMR:

elist('gem200','dsk#')   List the files on disk partition # of the
                                                                     Gemini

eread('*newname***','gem200','dsk#.***oldname***')**   Copy FID file *oldname* from the
        Gemini hard disk partition # to the Sun workstation, giving it filename
        "*newname.5*".

convert('*newname***.5','***newname***')**   Converts the Gemini file "*newname.5*" into VNMR
        format and loads it into the present VNMR experiment. Use **svf('***filename***')** in
        VNMR to create the VNMR directory "*filename.fid*", containing files fid,
        procpar, text, and log.

The same commands can be entered in a UNIX shell, leaving out the parentheses, commas and single quotes and replacing them with spaces, except that the UNIX equivalent of **convert** is **cpos_cvt**.

Re-booting the Computer

Sometimes the computer "freezes up" and will not accept any input from the keyboard. This usually happens when the message WAIT is displayed more or less permanently in the upper right corner of the monitor screen. To re-boot the computer, press and hold the red button on the console and count to 10 slowly. Then release the button and the computer will re-boot. If you go to the same experiment you were working in (command **JEXP#**), your FID data should still be intact. The 10-second wait is to allow the ethernet card to initialize before the computer checks it.