DEPT on the U400

PRELIMINARY INFORMATION

1) The DEPT (Distortionless Enhancement by Polarization Transfer) experiment is frequently used to identify the types of carbons (*i.e.*, C, CH, CH_2, and CH_3) in a molecule. Depending on the setting of the parameter *mult*, a different DEPT spectrum (or spectra if *mult* is arrayed), and consequently, different results can be obtained:

   mult=0.5 → DEPT-45 (gives all protonated carbons)

   mult=1.0 → DEPT-90 (gives CH's only)

   mult=1.5 → DEPT-135 (gives CH's and CH_3's up and CH_2's down)

   Quaternary carbon signals are suppressed in the DEPT experiment, and therefore, can be easily identified upon comparison of any DEPT spectrum with the regular ^{13}C spectrum. Routinely, only a DEPT-135 is needed to identify all the carbons since CH's and CH_3's usually have quite different chemical shifts. Occasionally, a DEPT-90 may be needed to distinguish CH's from CH_3's. The DEPT experiment can also be set up to acquire a set of four spectra (mult=0.5,1,1,1.5 for one DEPT-45, two DEPT-90 and one DEPT-135) for use in automatic spectral analysis and/or editing (see details in Part 4 below). Finally, the DEPT experiment is somewhat more sensitive than the APT or normal ^{13}C experiment. In addition, the setting of the parameter *d1* is governed by the T1's of the proton nucleus instead of the carbon nucleus. This could be advantageous if your carbons' T1's are longer than those of the protons, which is the case for most molecules.

2) ***pw90*** and ***pp***: The default *pw90* and *pp* are usually good enough for this experiment, unless the physical properties of your sample are very different from those of the standard due to, for example, the presence of ionic or paramagnetic materials or the use of a very different solvent. Talk to MSL staff if you are not sure if you need to calibrate these parameters for your sample or don't know how. If new values are determined, remember to change the parameter *pw90* before setting up the DEPT experiment, and change the parameter *pp* before starting the DEPT experiment.

3) ***d1***: By default, *d1* is set to 1.5 seconds. In general, *d1* is set to 1 to 2 times the value of T1 for the PROTONS of interest.

4) ***nt***: By default, *nt* is set to half the value used in your regular ^{13}C spectrum due to its higher sensitivity.

1. COLLECT A ^{13}C SPECTRUM

† If you have already collected a ^{13}C spectrum previously, load the data into an experiment (*e.g.*, exp1) and process it as usual. Note that when set up this way, the DEPT spectrum may have to be phased manually.

† If no ^{13}C spectrum has been acquired, acquire one and phase and reference the spectrum as usual.

2. SET UP THE DEPT EXPERIMENT — dodept

INTRODUCTION: **dodept** is an interactive macro that will take you through the setup of a DEPT experiment. In this handout, the dialog will appear **`in this font`**, and the symbol ¶ signifies that a response is required. Most messages will appear in the Status Window, while inputs are entered *via* the Input Window. Window arrangement on the left side of the screen is, from top to bottom, Status, Input, Menu, Graphics, and Text.

2A. Enter the Macro

In the experiment that contains the ^{13}C spectrum, enter:
dodept <rtn> macro to set up a DEPT experiment in exp2

NOTE: If you wish to do the DEPT in a different experiment, enter **dodept(*exp#*)**, where ***exp#*** is the experiment number for the DEPT. Experiment number must be between 1 and 9, excluding 5.

If the DEPT exp# is the same as the current experiment, the following dialog will appear:
Do dept in current experiment? (y/n) ¶

If answered no:
Enter dept exp#: ¶

Then the following menu will be displayed in the text window at the bottom of the screen:

```
DEPT MENU

1)  DEPT-135
2)  DEPT-90
3)  DEPT-135 and DEPT-90
4)  DEPT for spectral editing
```

2B. Choose a Setup

Enter your choice of setup at the following prompt in the Input Window:
Enter your choice (1-4) from the menu below: ¶

† If 1 is chosen, a DEPT-135 experiment will be set up (*i.e.*, mult=1.5).
† If 2 is chosen, a DEPT-90 experiment will be set up (*i.e.*, mult=1).
† If 3 is chosen, an array will be set up for a DEPT-135 and a DEPT-90 (*i.e.*, mult=1.5,1).
† If 4 is chosen, an array will be set up to collect a set of four spectra: one DEPT-45, two DEPT-90, and one DEPT-135 (*i.e.*, mult=0.5,1,1,1.5). This setup is recommended for spectral editing and/or analysis only, because the data will take four times as long to acquire as that in choice #1 and 2 and twice as long as choice #3.

By default, *d1* will be set to 1.5 seconds and *nt* to half of that of the regular ^{13}C spectrum. However, you may change these parameters by answering the following question accordingly:
Do you wish to change nt and/or d1? (y/n) ¶

If answered yes, enter the new values at the following prompts:
nt= ¶
d1= ¶

The **dodept** macro will terminate at this point. Be sure to check the parameters before starting the experiment.

2C. Start the Experiment

You may start the DEPT experiment with the **au** *or* **ga** *command:*

au — start DEPT experiment and process the data automatically with the **deptproc** macro when acquisition is complete. NOTE: If the experiment is terminated with **aa** or **sa** (see below), no data processing will occur.
ga — start DEPT experiment and do a **wft** when acquisition is complete.

NOTE: Once the experiment is started, it may be aborted by clicking the Abort Acq button or typing the **aa** command *if no data is to be retained*, such as when you discover that a mistake has been made in the setup of the experiment, or when it has become apparent that the experiment is useless. *If data retention is desired*, such as when sufficient signal-to-noise has been obtained or when you have run out of time, you should use the **sa**

command to stop the acquisition. At this point, you should process the data manually because automation will not work when the experiment is terminated before its completion.

When the experiment is finished, save the data and proceed with its processing and plotting as described below.

3. PROCESSING AND DISPLAYING

NOTE: If a regular ^{13}C spectrum is acquired together with the DEPT data, it is most likely that no further phasing of the DEPT spectra will be necessary. In that case, you should use the **deptproc** macro to process the data. On the other hand, if the ^{13}C spectrum is collected at another time, the DEPT spectra will probably require some phasing, either auto or manual, depending on the type of data. If your data contains a DEPT-45 (preferred) or a DEPT-90 spectrum (may or may not work), you may use the macro **deptpaph** for processing. Otherwise, use **deptproc** to process the data and phase the spectrum manually if necessary.

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A. To process and display DEPT data — without autophasing:
deptproc <rtn>

B. To process and display DEPT data — with autophasing:
deptpaph <rtn>

C. To display DEPT data — without any processing:
deptds <rtn>

4. PLOTTING

A. deptplot — for data acquired with menu item #1 - 3 above. It will plot DEPT data, with or without the regular ^{13}C spectrum on the same page. See examples in Fig. 1 - 2.

deptplot(C13exp#) <rtn> *C13exp#* is the experiment number that contains the ^{13}C spectrum

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If no argument is provided with the **deptplot** *macro, the following dialog will appear:*

```
Plot without 13C spectrum? (y/n) ¶
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If answered yes, the macro will terminate here and only the DEPT spectrum (or spectra) will be plotted (see example in Fig. 1).

If answered no, the dialog will continue:

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Enter exp# containing 13C spectrum: ¶
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Finally, you are provided with the option to plot the text:

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Plot with text? ¶
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Then the plotting will begin.

B. adeptpp — for data acquired with menu item #4 only. It will plot the edited spectra and print the analysis table, OR plot the unedited spectra. See examples in Fig. 3 - 4.

adeptpp <rtn> enter the plotting macro

Then enter your choice of plotting option at the following prompt:

```
Plot 1)edited or 2)unedited spectra? ¶
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† If option 1 is chosen, spectral editing will be performed and the resulting four new spectra and a spectral analysis table will be plotted and printed respectively. The edited spectra will contain, from bottom to top, all protonated carbons, CH carbons, CH_2 carbons, and CH_3 carbons respectively.

† If option 2 is chosen, the unedited spectra will be plotted (*i.e.*, from bottom to top, DEPT-45, -90, and -135).

You will also have a choice of plotting with the parameters, text only, or none at all:

`Plot with 1)parameters, 2)text only, or 3)none? ¶`

NOTE: If option 1 is chosen, **w c** (width of chart) will be adjusted accordingly to accommodate the parameters.

Next, the macro will determine if the data have been edited, and if it has, the data will be retransformed.

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If you choose plotting option 1, the threshold will be adjusted and a line list will be printed in the text window. The number of lines found will also be shown in the status window. Check the threshold setting by answering the following question accordingly (NOTE: only protonated carbons should be and is counted!):

`Is the number of peaks picked correct? ¶`

If you answer no to the above question, enter 1 if too many peaks are picked or 2 if too few:

`1)increase or 2)decrease the threshold? ¶`

The threshold will then be increased or decreased by 20% and the above dialog will be repeated until the correct number of lines are picked.

Then, spectral editing and analysis will be performed on the data and the analysis table will be printed.

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Finally, the DEPT spectra, edited or not, will be plotted.

C. adeptplot — *for data acquired with menu item #4 only. It will plot the edited spectra and the spectrum analysis table, all on the same page. See example in Fig. 5.*

adeptplot <rtn> enter the plotting macro

First, the macro will determine if the data have been edited, and if it has, the data will be retransformed.

Then, the threshold will be adjusted and a line list will be printed in the text window. The number of lines found will also be shown in the status window. Check the threshold setting by answering the following question accordingly (NOTE: only protonated carbons should be and is counted!):

`Is the number of peaks picked correct? ¶`

If answered no, enter 1 if too many peaks are picked or 2 if too few:

`1)increase or 2)decrease the threshold? ¶`

The threshold will then be increased or decreased by 20% and the above dialog will be repeated until the correct number of lines are picked. Then, spectral editing and analysis will be performed on the data, and the resulting edited spectra and analysis table will be plotted on the same page.

D. adeptprint — *for data acquired with menu item #4 only. Prints spectrum analysis table.*

After the macro is entered, it will determine if the data has been edited. If it has, the results will be printed. If not, a dialog similar to the one for **adeptplot** will appear, and at the end, a spectral analysis table will be printed.

Fig. 1. deptplot: ***DEPT-135, with ^{13}C spectrum***

Fig. 2. deptplot: ***DEPT-135 and DEPT-90, with ^{13}C spectrum***

Fig. 3. adeptpp: ***edited spectra, with parameters***
(spectrum analysis table on next page)

Fig. 4. adeptpp: ***unedited spectra, with text only***

Fig. 5. adeptplot: ***typical output, with text***