

DEPT

Introduction

6.1

Distortionless **E**nhancement by **P**olarization **T**ransfer is a polarization transfer technique and so is useful for the observation of low- γ nuclei (most commonly ^{13}C) which are J-coupled to ^1H . DEPT is a spectral editing sequence, that is, it can be used to generate separate ^{13}C subspectra for methyl (CH_3), methylene (CH_2), and methine (CH) signals. DEPT makes use of the generation and manipulation of multiple quantum coherences to differentiate between the different types of ^{13}C signals. The pulse angle (θ) of the final ^1H pulse (see Figure 18) is the basis of spectral editing with DEPT. CH_3 and CH_2 groups have maximum intensity when $\theta = \pi/4$ and 0 intensity when $\theta = \pi/2$; CH groups have maximum intensity when $\theta = \pi/2$; and CH_2 groups have maximum negative intensity when $\theta = 3\pi/4$. Quaternary carbons are missing from DEPT spectra because the large one-bond heteronuclear J-coupling (J_{XH}) is used for polarization transfer. Quaternary carbons, by definition, are not directly bonded to any ^1H 's, experience only small n-bond heteronuclear J-coupling ($^nJ_{\text{XH}}$), and so undergo no polarization transfer.

DEPT may be run with or without ^1H -decoupling. In the latter case, the familiar 1:2:1 triplets and 1:3:3:1 quartets are obtained for CH_2 and CH_3 groups, respectively. DEPT is relatively insensitive to the precise matching of delays with coupling constants, and so is much easier to use than the closely related INEPT sequence. DEPT, on the other hand, is more sensitive to pulse imperfections than INEPT.

In this chapter, DEPT-45, DEPT-90, and DEPT-135 experiments with ^1H -decoupling will be described. These correspond to DEPT with $\theta = \pi/4$, $\pi/2$, and $3\pi/4$, respectively, and by appropriate adding and subtracting of the data, it is possible to obtain separate subspectra of CH , CH_2 , and CH_3 signals.

Sample

The sample used to demonstrate DEPT in this chapter is 1 g Cholesterylacetate in CDCl_3 .

Pulse Sequence Diagram

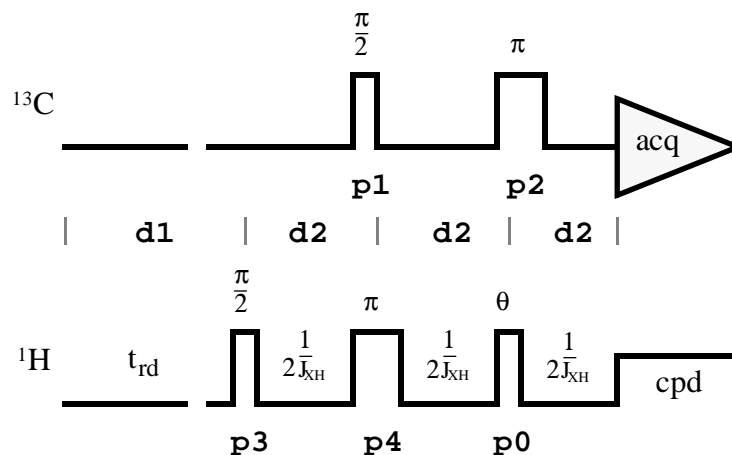
6.2

The DEPT pulse sequence is shown in Figure 18. The final ^1H pulse is shown with pulse angle θ . This angle is set to 45° in the sequence DEPT-45, which yields spectra with positive CH , CH_2 , and CH_3 signals; to 90° in DEPT-90, which yields spectra with only CH signals; and to 135° in DEPT-135, which yields spectra with positive CH and CH_3 signals and negative CH_2 signals.

Notice that the pulses **p1** and **p3** must be set to the appropriate 90° times found in Chapter 5 'Pulse Calibration'. Also, the cpd sequence used is WALTZ-16, which requires the calibrated 90° time **pcpd2**. The 180° pulse lengths **p2** and **p4** are determined by the pulse program itself.

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Figure 18: DEPT Pulse Sequence



Acquisition and Processing

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Insert the sample in the magnet. Lock the spectrometer. Readjust the Z and Z^2 shims until the lock level is optimized. Tune and match the probehead for ^{13}C observation, ^1H decoupling.

Reference spectra

Since DEPT is a ^{13}C -observe, ^1H -decouple experiment, the first step would be to obtain a reference ^1H spectrum of the sample to determine the correct **o2** for ^1H decoupling. The second step would then be to obtain a ^1H -decoupled ^{13}C spectrum to determine the correct **o1** and **sw** for the DEPT experiments. However, both of these steps were already carried out in Section 4.3 starting on page 37. So, a ^1H -decoupled ^{13}C reference spectrum of this sample can be found in carbon/3/1. (The one thing to be aware of is that broadband decoupling was used in carbon/3/1, but here the cpd sequence WALTZ-16 will be used).

Create a new file directory for the data set

Enter **re carbon 3 1** to call up the reference spectrum. Enter **edc** and change the following parameters:

NAME	dept
EXPNO	1
PROCNO	1 .

Click **SAVE** to create the data set dept/1/1.

Set up the acquisition parameters

Enter **eda** and set the acquisition parameters as shown in Table 22. Use the values determined in Chapter 5 'Pulse Calibration' for the parameters **p11** and **p1** (^{13}C observe high power level and 90° pulse time), **p12** and **p3** (^1H decouple high power level and 90° pulse time), and **p112** and **pcpd2** (^1H decouple low power level and

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90° pulse time). Also be sure that CPDPRG2 is now set to “waltz16” and not “bb” as it was for carbon/3/1. Note that the dept, dept45, dept90, and dept135 pulse programs call an include file in which **cnst2** (defined to be J_{XH}) is used to calculate **d2** ($d2 = 1/(2 * cnst2)$). Thus, it is only necessary for the user to set the value of **cnst2**.

Table 22. DEPT Acquisition Parameters

Parameter	Value	Comments
PULPROG	dept	(or dept45, dept90, or dept135) see Figure 18 for pulse sequence diagram.
TD	32k	
NS	4	the number of scans must be 4 * n for the phase cycling to work correctly.
DS	8	number of dummy scans.
PL1		high power level on f1 channel (see “An Important Note on Power Levels” on page 7).
PL2		high power level on f2 channel (see “An Important Note on Power Levels” on page 7).
PL12		power level for cpd on f2 channel.
P0		θ = high power pulse on f1 channel (only necessary for dept pulse program): 45°: all positive; 90°: XH only; 135°: XH and XH ₃ positive, XH ₂ negative.
P1		90° ¹³ C high power pulse on f1 channel.
P2		180° ¹³ C high power pulse on f1 channel; calculated internally.
P3		90° ¹ H high power pulse on f2 channel.
P4		180° ¹ H high power pulse on f2 channel; calculated internally.
PCPD2		90° ¹ H pulse for cpd sequence.
D1	2sec	relaxation delay; should be 1–5 * T ₁ (¹³ C).
D2	3.45msec	delay for creation of anti-phase magnetization (1/(2J _{XH})); calculated internally.
D12	20µsec	delay for power switching; predefined.
CNST2	145Hz	one-bond heteronuclear J-coupling (J _{XH}); 145Hz is a good intermediate value for ¹³ C.
CPDPRG2	waltz16	composite pulse decoupling sequence.

Acquire the spectrum

First acquire a DEPT-45 spectrum. Either select the pulse program dept45 or set **p0** to the length of a 45° pulse (i.e., one half of **p1**). Enter **zg** to acquire the time domain data.

Notice that the receiver gain should already be set correctly if this data set was created from carbon/3/1. Also, this sample is quite concentrated; that is why an **ns** value of only 4 is sufficient. If the user wishes to try DEPT on another, less concentrated sample, an **ns** value of 64 may be more appropriate.

Set up the processing parameters

Enter **edp** and set the processing parameters as shown in Table 23.

Table 23. DEPT Processing Parameters

Parameter	Value	Comments
SI	16 k	
WDW	EM	exponential multiply.
LB	2	2Hz line broadening.
PKNL	TRUE	necessary when using the digital filter.

Process the spectrum

Add line broadening and Fourier transform the time domain signal with the command **ef**. Manually phase correct the spectrum and store the correction. If all parameters have been set correctly, it will be possible to phase the spectrum so that all peaks are positive. The signals that appear in this spectrum are from the ¹³C's in CH, CH₂, and CH₃ groups.

Other spectra

To obtain a DEPT-90 spectrum, create the data set dept/2/1, either select the pulse program dept90 or set **p0** to the length of a 90° pulse (i.e., equal to **p1**), acquire and process the data (**zg**, **efp**). This spectrum also has all positive peaks; however, only CH signals are visible here, so there should be fewer peaks in this spectrum than in the DEPT-45 spectrum.

To obtain a DEPT-135 spectrum, create the data set dept/3/1, either select the pulse program dept135 or set **p0** to the length of a 135° pulse (i.e., 1.5 times **p1**), acquire and process the data (**zg**, **efp**). This spectrum has both positive and negative peaks. The positive peaks arise from the ¹³C's of CH and CH₃ groups and the negative peaks from the ¹³C's of CH₂ groups.

Plot the spectra

A straightforward way to plot 1D ¹³C spectra, such as the DEPT spectra acquired here, is by using most of the plotting parameters found in the plot parameter file standard1D. Read in the file standard1D by entering **rpar**, selecting **standard1D** from the menu of parameter file names, and then selecting **plot** from the menu of parameter file types that appears. Equivalently, simply enter **rpar standard1D plot**. This sets most of the plotting parameters to values which are appropriate for

these 1D spectra, assuming that the paper size to be used here is the same as the default paper size defined when the spectrometer was configured.

More information about plotting parameters and the file standard1D can be found in Appendix C '1D and 2D Plotting Parameters'.

To select the spectral region (full or expanded) to be plotted, first make sure the spectrum appears as desired on the screen, and then click **DP1** and simply hit return in response to the following three (3) questions:

```
F1 =                <return>
F2 =                <return>
Change y-scaling on display according to PSCAL?<return>
```

For ^{13}C spectra, it is a good idea to change the separation between tic marks on the x-axis. Enter **edg** to edit the plotting parameters. Click the **ed** next to the parameter EDAXIS to enter the X- and Y-axis parameters submenu. Change the value of the parameter XTICDIS from 0.1 to 5. This value is appropriate for a basic ^{13}C spectrum with a large **sw** as described in this chapter. For optimized spectra with narrower **sw**'s (e.g., less than 150 ppm), a value of 2.5 may be more appropriate. Click **SAVE** to save this change and return to the **edg** menu.

In addition, unless special precautions are taken to deal with the long ^{13}C T_1 relaxation times and potential NOE build-up during ^1H decoupling, the integrated intensities will not faithfully reflect the numbers of different types of ^{13}C nuclei in a given molecule. Thus, it is best not to integrate standard ^{13}C spectra. Within **edg**, click the **yes** next to the parameter INTEGR so that it toggles to **no**.

Click **SAVE** to save all the above changes and exit the **edg** menu.

Next create a title for the spectrum. Enter **setti** to use the editor to open the title file. Write a title and save the file.

To plot the spectrum, simply enter **plot** (provided the correct plotter is selected in **edo**).

DEPT-45, DEPT-90, and DEPT-135 spectra of 1 g Cholesterylacetate in CDCl_3 are shown in Figure 19.

The DEPT results can be compared with the standard ^1H -decoupled ^{13}C spectrum in carbon/3/1. Notice that some of the peaks that appear in carbon/3/1 do not appear in any of the DEPT spectra. These are the signals from the quaternary ^{13}C 's (i.e., those not directly bonded to any ^1H 's). From the combination of standard ^1H -decoupled, and DEPT-45, -90, and -135 spectra, it is possible to determine which signals are from primary, secondary, tertiary, and quaternary ^{13}C 's.

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Figure 19: DEPT Spectra of 1 g Cholesterylacetate in CDCl₃

