# Supporting Information. Part 2.

## Efficient Functionalization of Oligonucleotides by New Achiral Nonnucleosidic Monomers

**Maxim S. Kupryushkin,[1] Mikhail D. Nekrasov,[1,2] Dmitry A. Stetsenko,[1] and Dmitrii V. Pyshnyi[1,2]***

[1] *Institute of Chemical Biology and Fundamental Medicine, Siberian Branch of the Russian Academy of Sciences, 8 Lavrentiev Ave, Novosibirsk 630090, Russia*

[2] *Novosibirsk State University, 2 Pirogova St, Novosibirsk 630090, Russia*

*pyshnyi@niboch.nsc.ru*

## Content

## NMR spectra.

### Notes

It has been observed that in $^{1}$H NMR spectra of compounds **4a-g** the expected signals in some cases were accompanied by additional signals of lesser intensity, or the expected signals had more complex structure (higher multiplicity). Careful analysis showed that in all cases the ratio between the expected and the additional signals was constant and equal to 5:3. In the cases of more complex structure (higher multiplicity) the additional signal overlapped with the expected signal. Integration of the spectra by combining the expected and the additional peaks produced values that correlate well with the expected structure of the compound.

$^{13}$C NMR spectra demonstrated similar splitting of the signals; the same ratio of 5:3 was maintained. We ascribe such a splitting of the signals to the presence of two rotamers in the ratio of 5:3 due to restricted rotation around a rigid oxalamide fragment in the presence of bulky substituents such as dimethoxytrityl group. In phosphoramidites **5a-g** the effect is even more pronounced due to the presence of another bulky group *N*,*N*-diisopropylamino-2-cyanoethoxyphosphinyl.

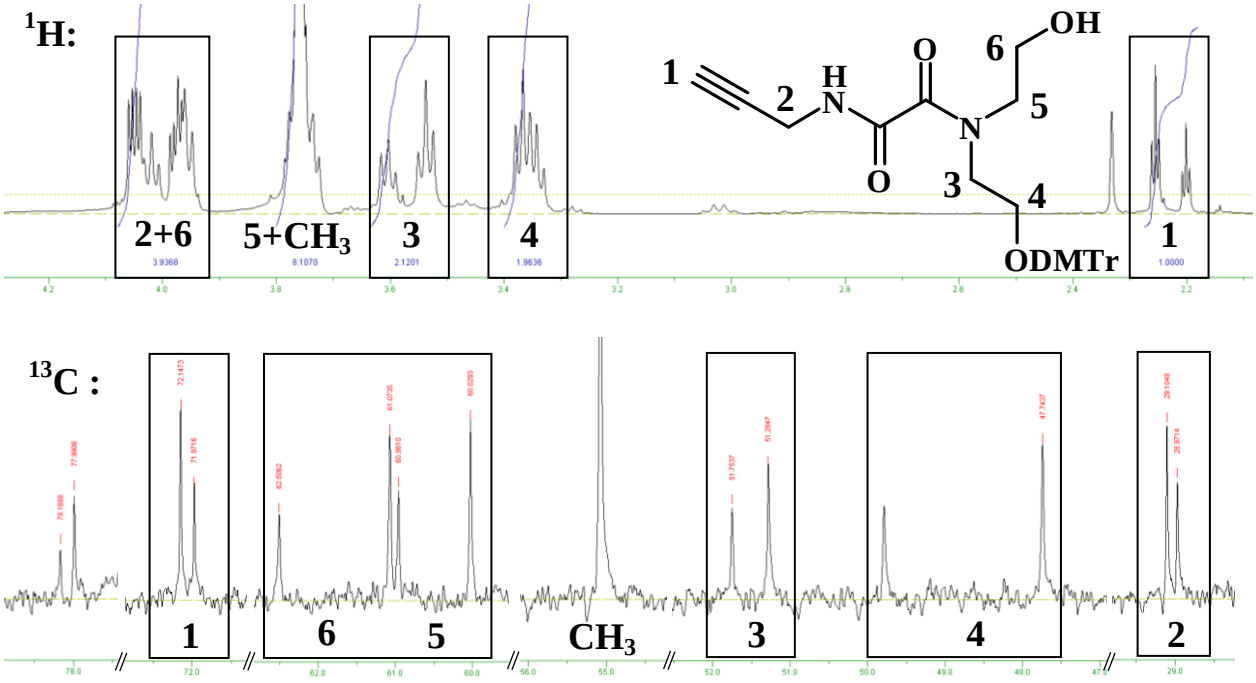



**Fig. 1**. Splitting of signals in $^{1}$H (top) and $^{13}$C (bottom) NMR spectra of **4a**.

An example of such a splitting is shown in Fig. 1. In the $^{1}$H NMR spectrum of **4a** (top) the peaks 1 and 3 double up; in the peak 4 there is overlap of two signals resulting in higher multiplicity. In the $^{13}$C spectrum (bottom) all the main signals are accompanied by additional minor peaks.

Analysis of the reaction mixtures of early oligonucleotide syntheses has revealed the presence of truncated sequences with the 5'-terminal ethyl phosphate group. The same byproducts were present in the reaction mixtures from different phosphoramidites such as **5b**, **5c** or **5g**. In $^{31}$P NMR spectra of phosphoramidites **5b**, **5c** and **5g** an additional peak at 140.0-146.6 ppm was observed that we have identified as *N*,*N*-diisopropyl-*O*-ethyl phosphoramidite. In some initial batches of the phosphoramidites the content of the byproduct was quite substantial and this could have resulted in the formation of oligonucleotide byproducts as ethyl phosphoramidite coelutes with the monomer on column. We have traced the origin of the admixture to the work-up of phosphitylation reaction with dichloromethane stabilized with 0.05% ethanol. After we have uncovered the fact, we have introduced quenching of phosphitylation reaction with small volume of 0.3 M aq $KH_2PO_4$ followed by extraction with dichloromethane. After quenching the amount of ethyl phosphoramidite in $^{31}$P spectra (e.g. **5a**, **5d** and **5e**) was significantly reduced, which has resulted in elimination of oligonucleotide byproducts with ethyl phosphate group.

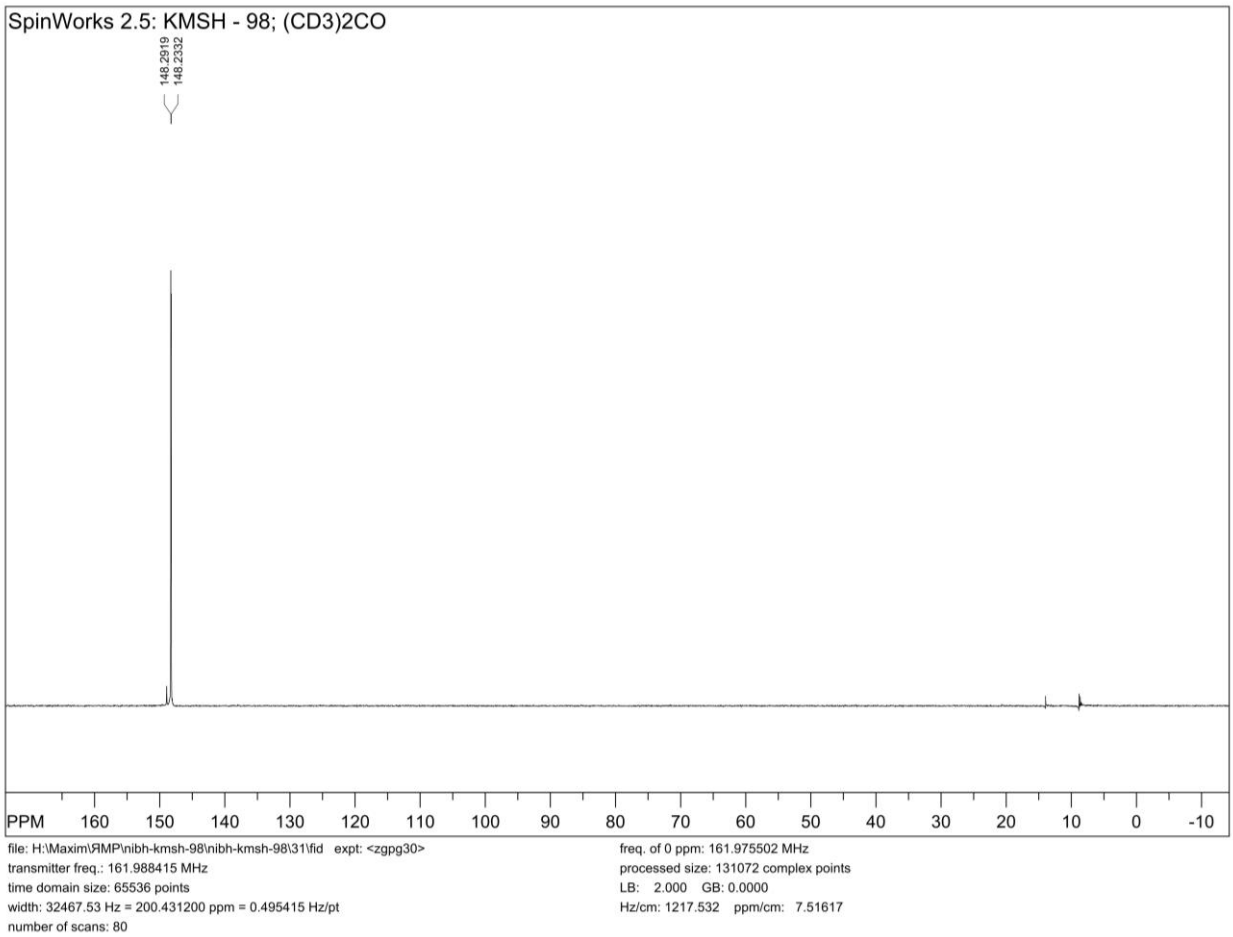
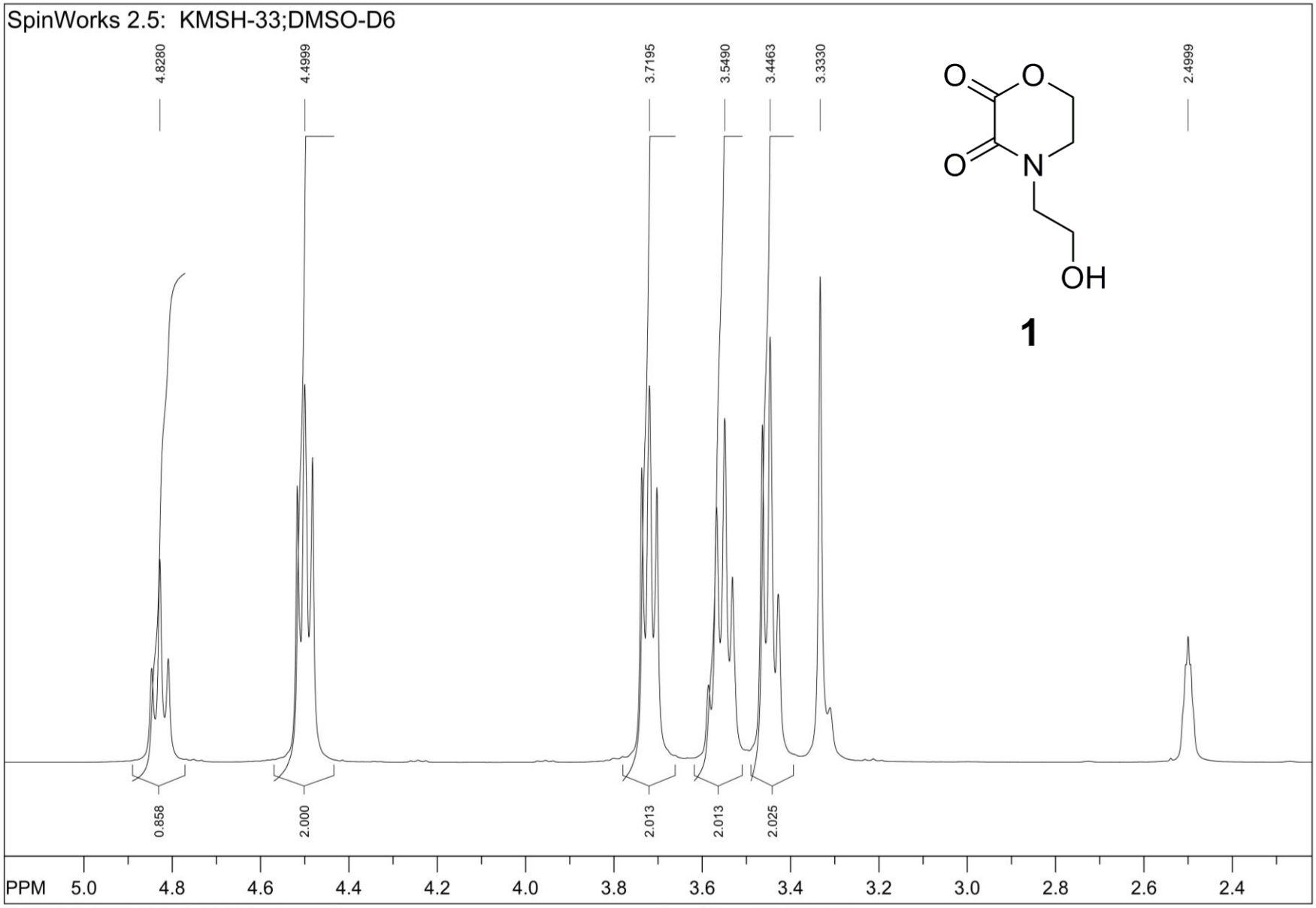



**Fig. 2**. $^{31}$P NMR spectrum of phosphoramidite **5b** purified by gel filtration on Sephadex LH-20.

Analytically pure samples of phosphoramidites **5** could be obtained after gel filtration on a Sephadex LH-20 column eluted with dichloromethane (Fig. 2).

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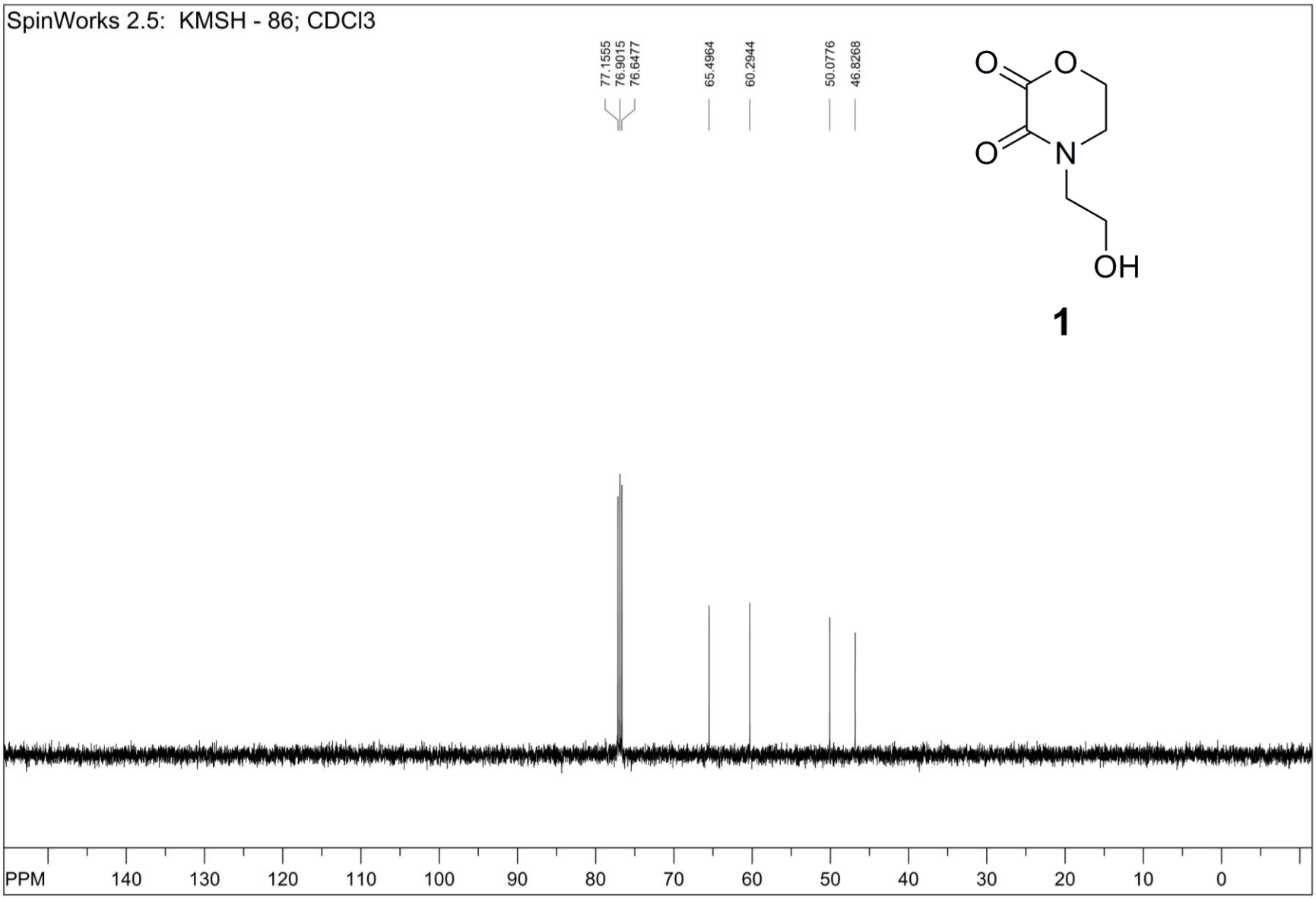


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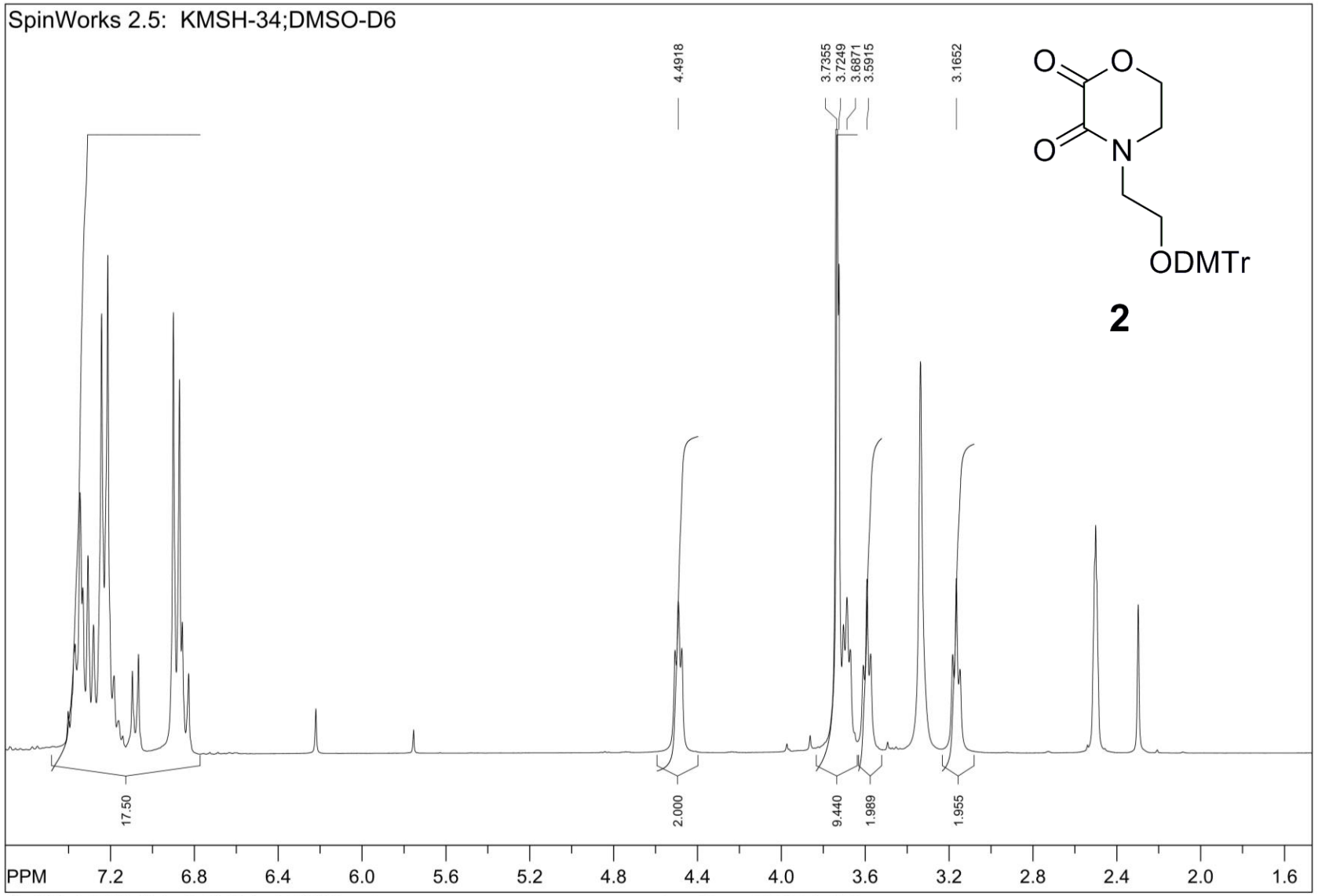


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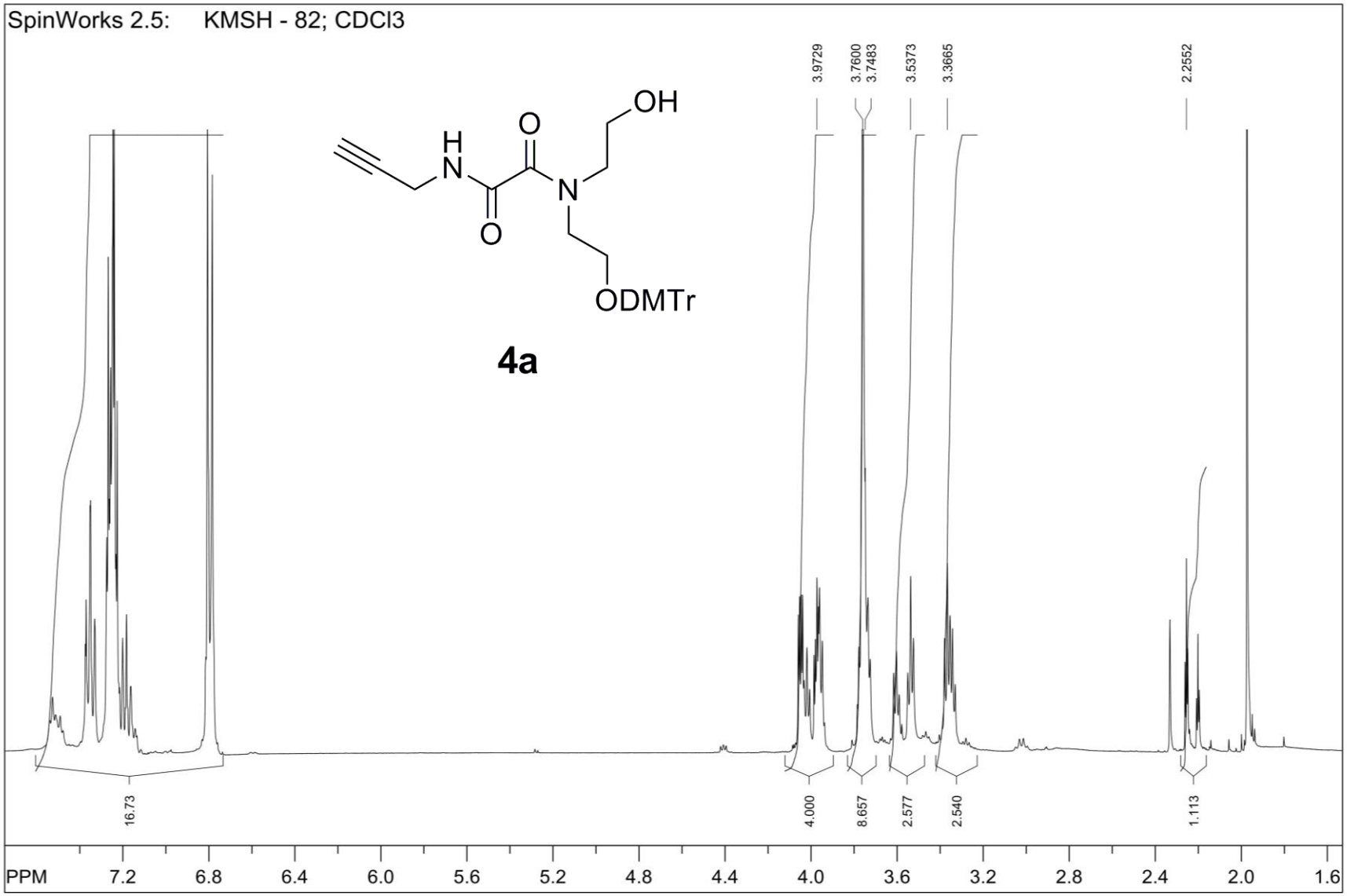


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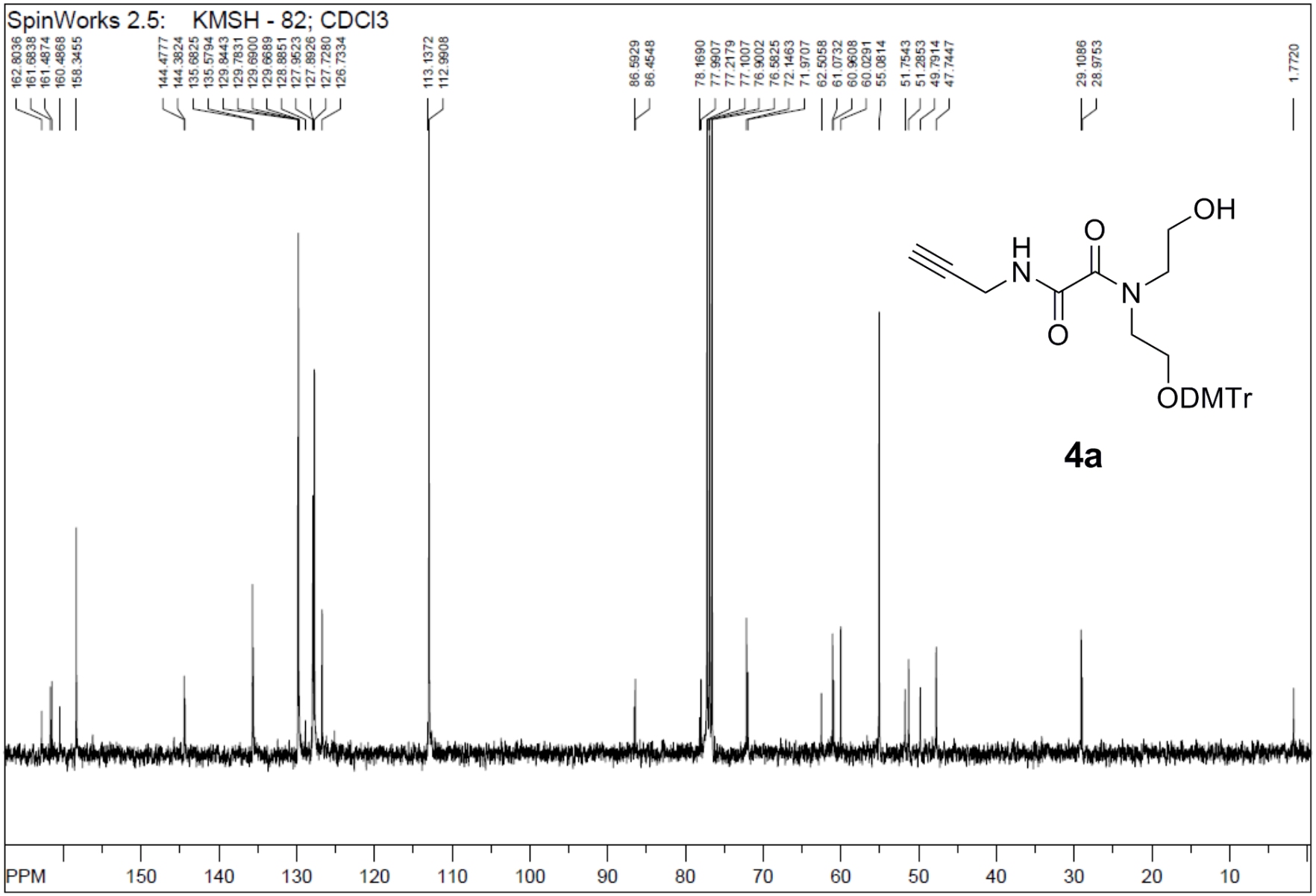


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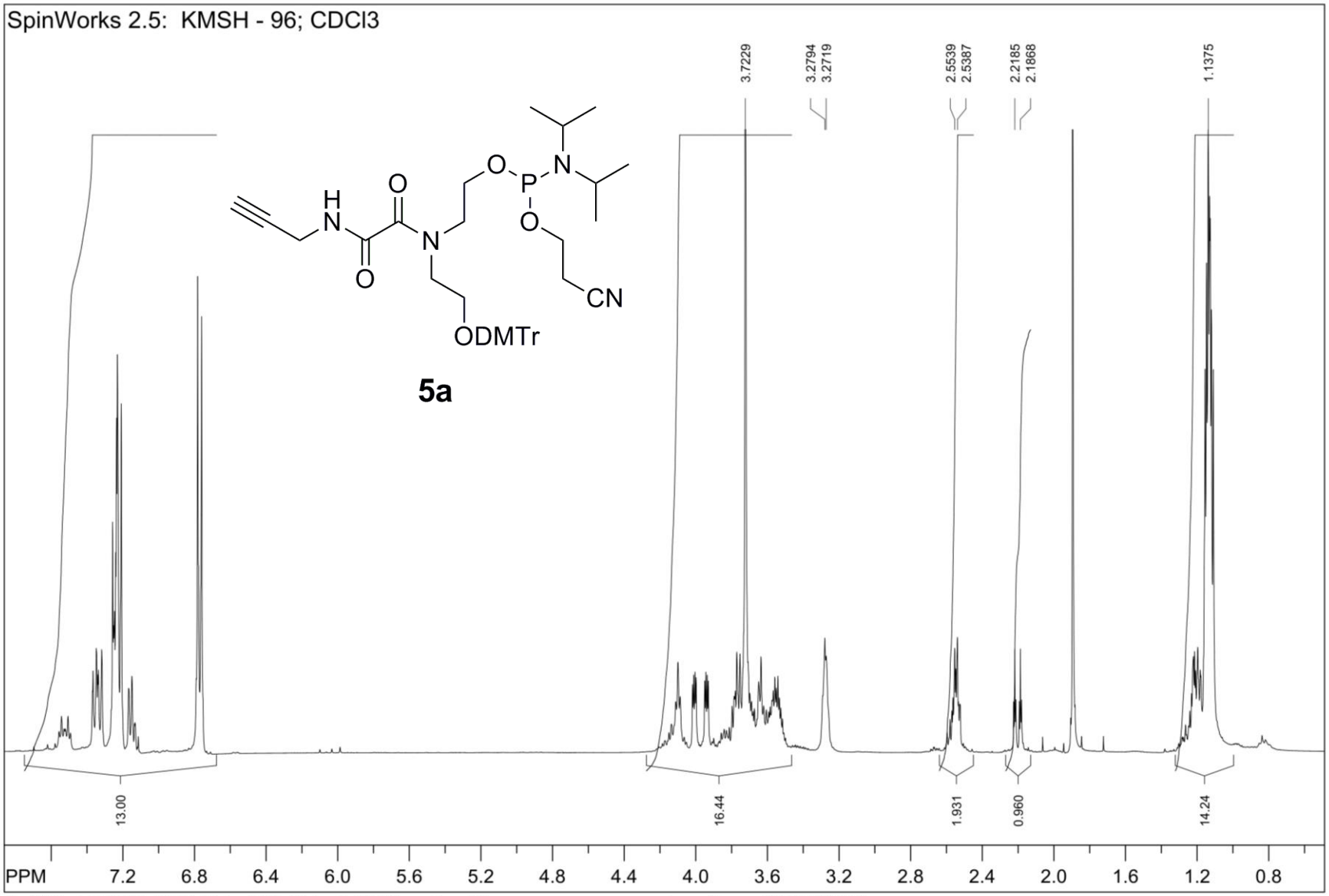


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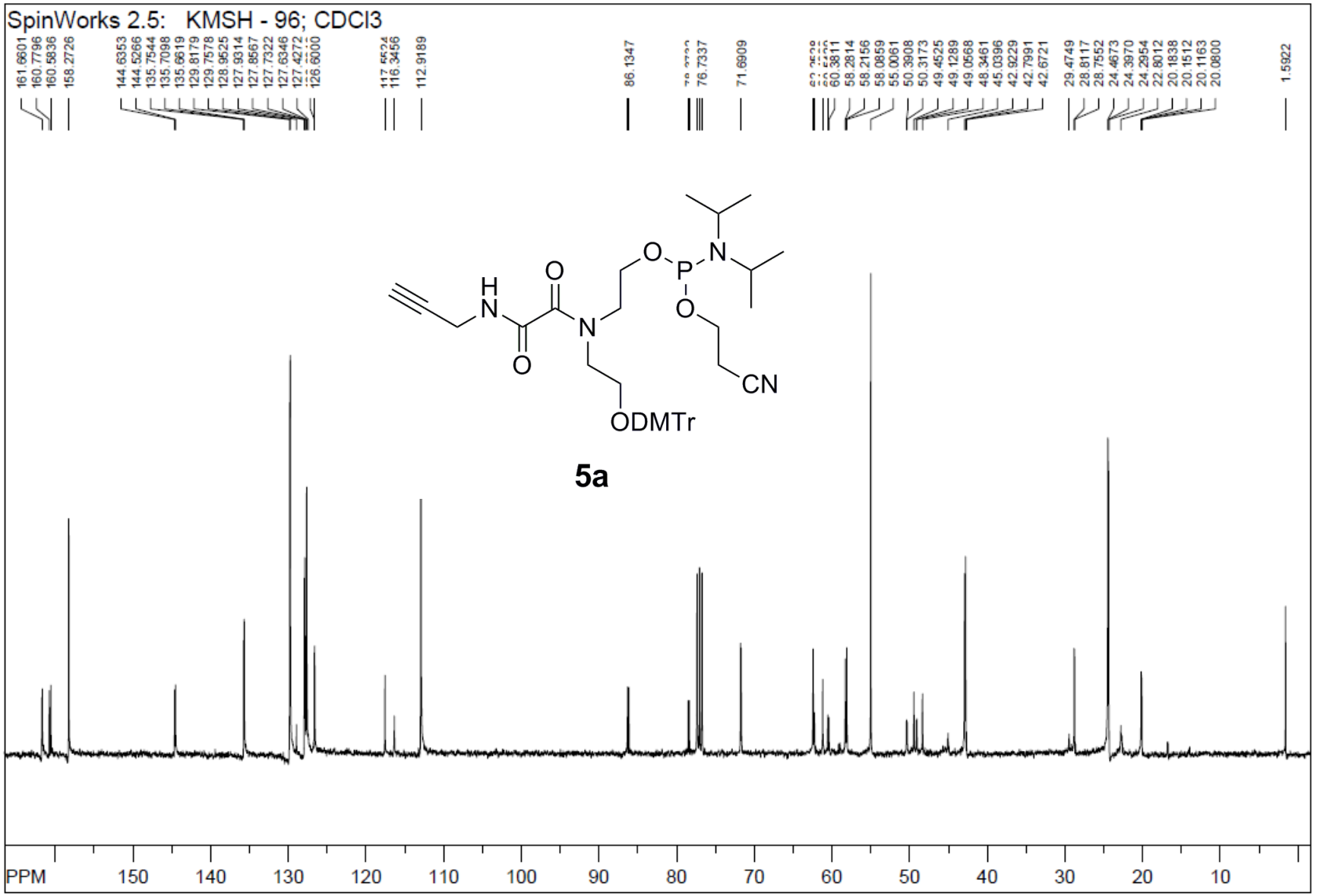


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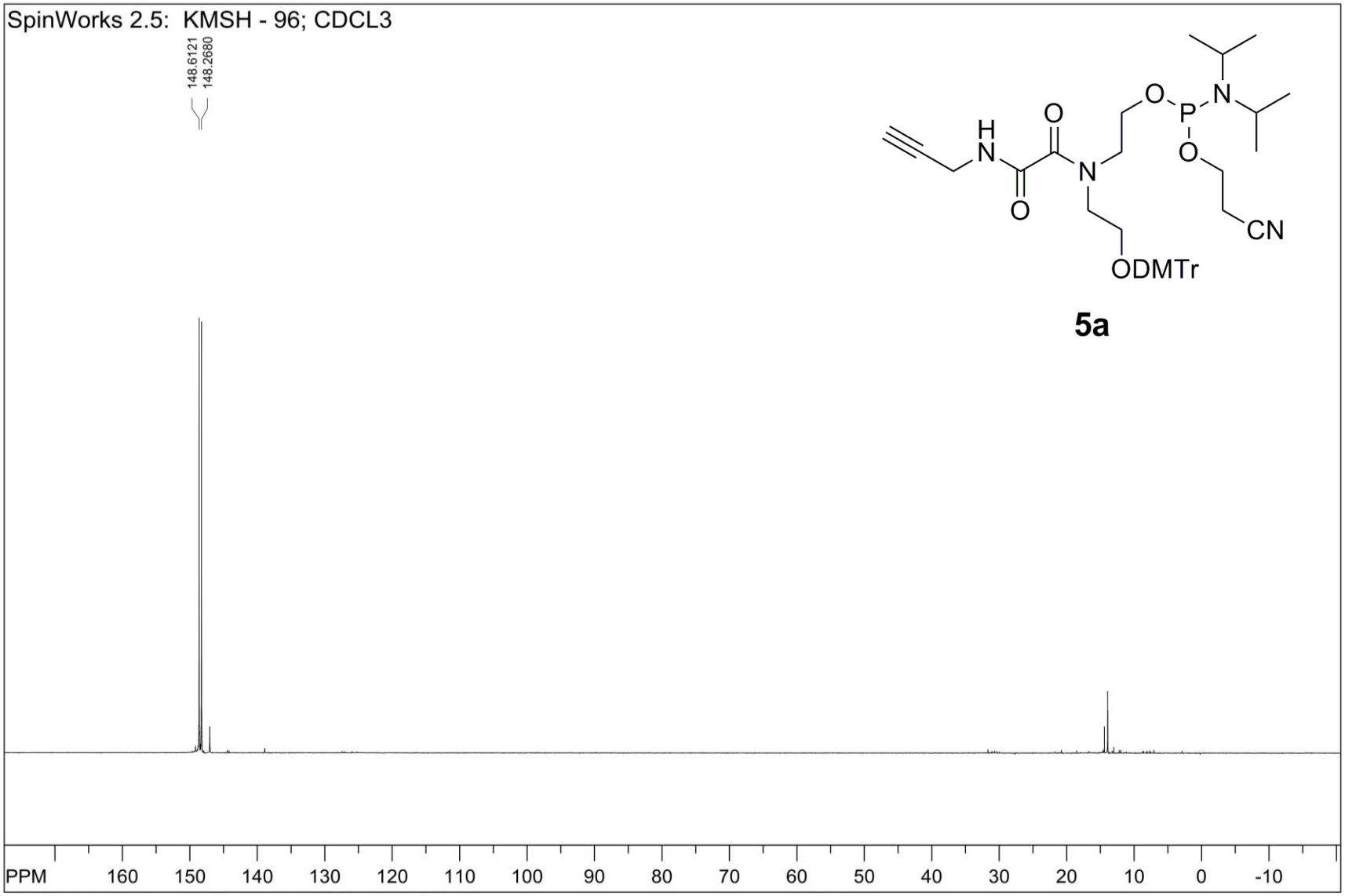


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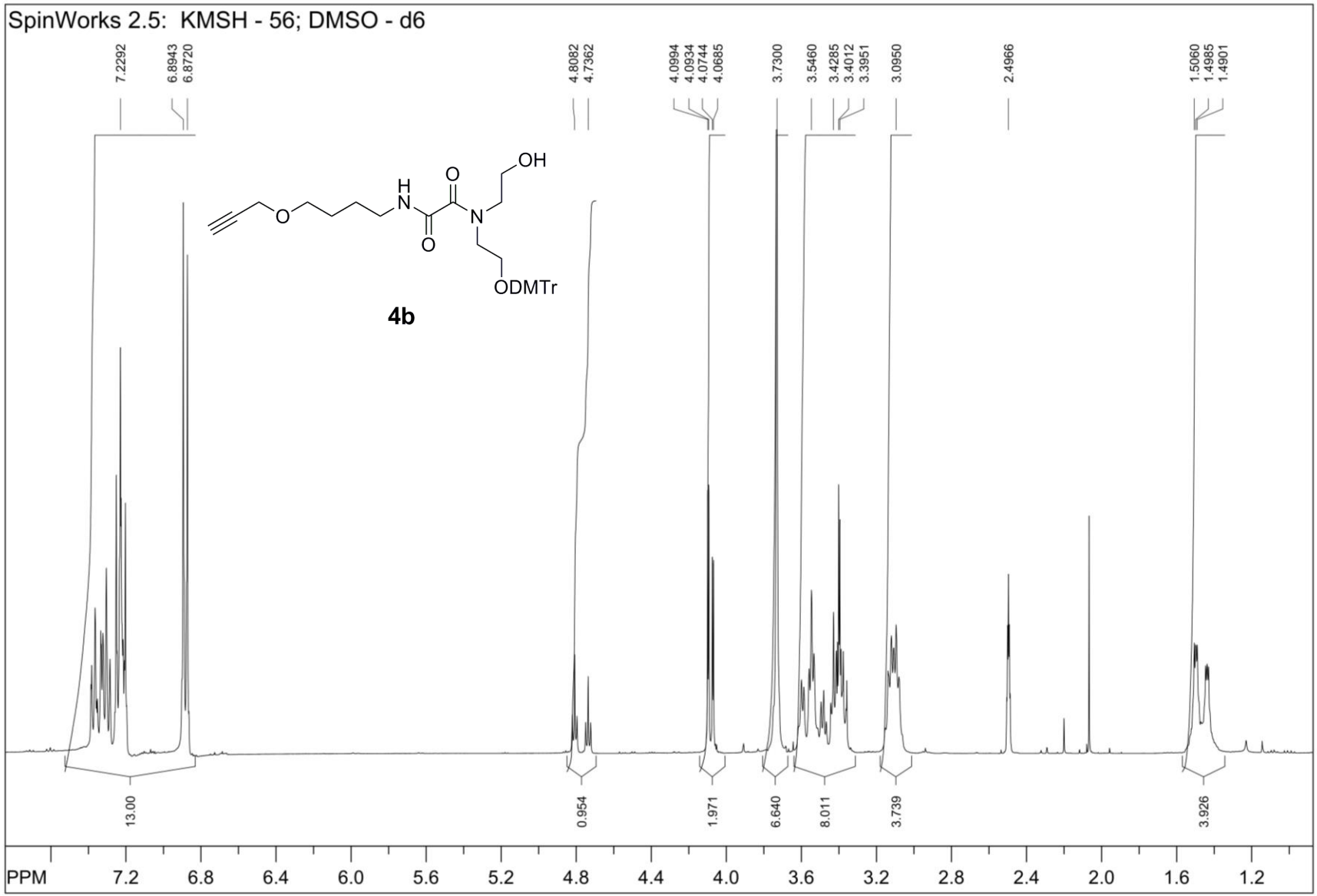


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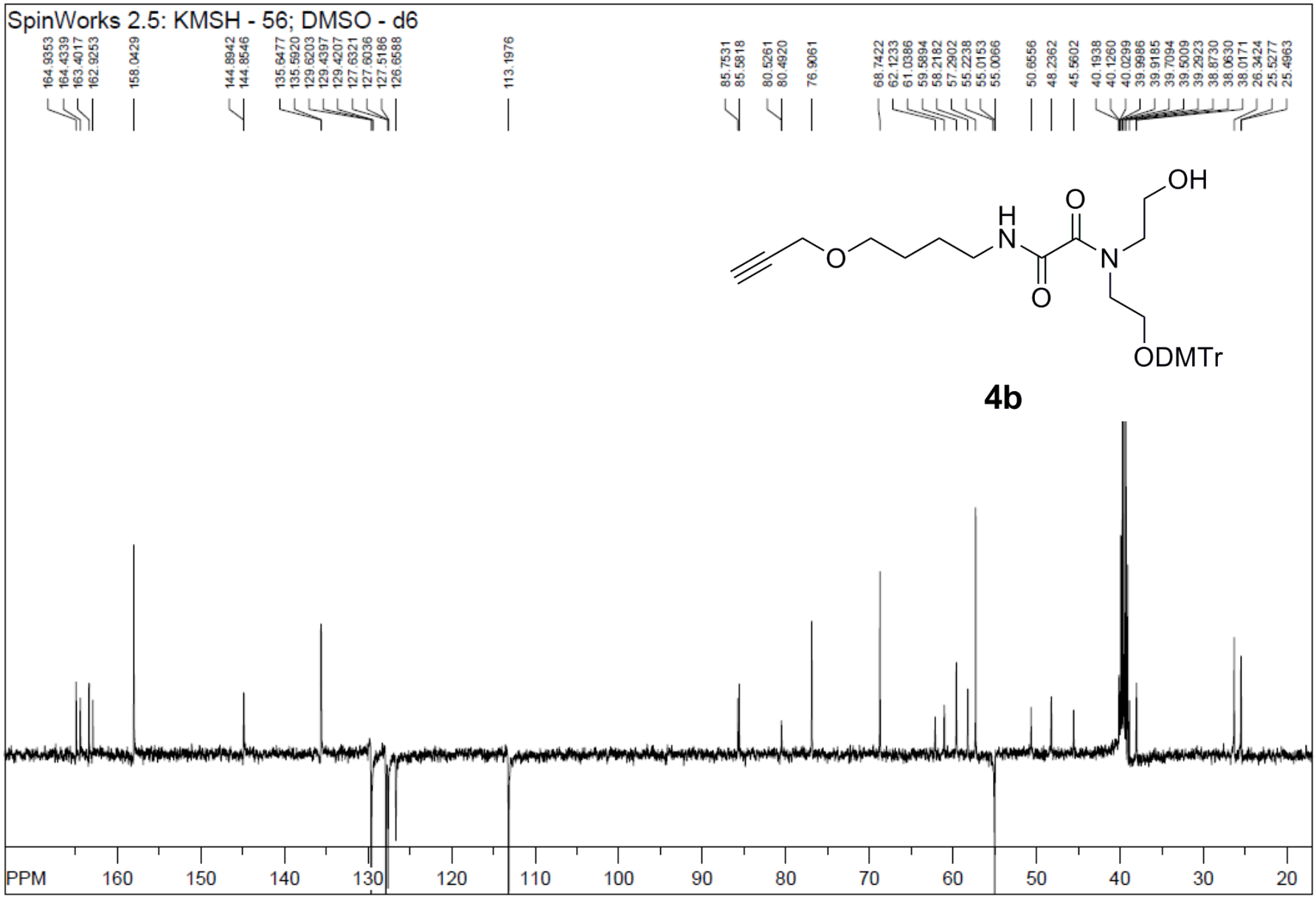
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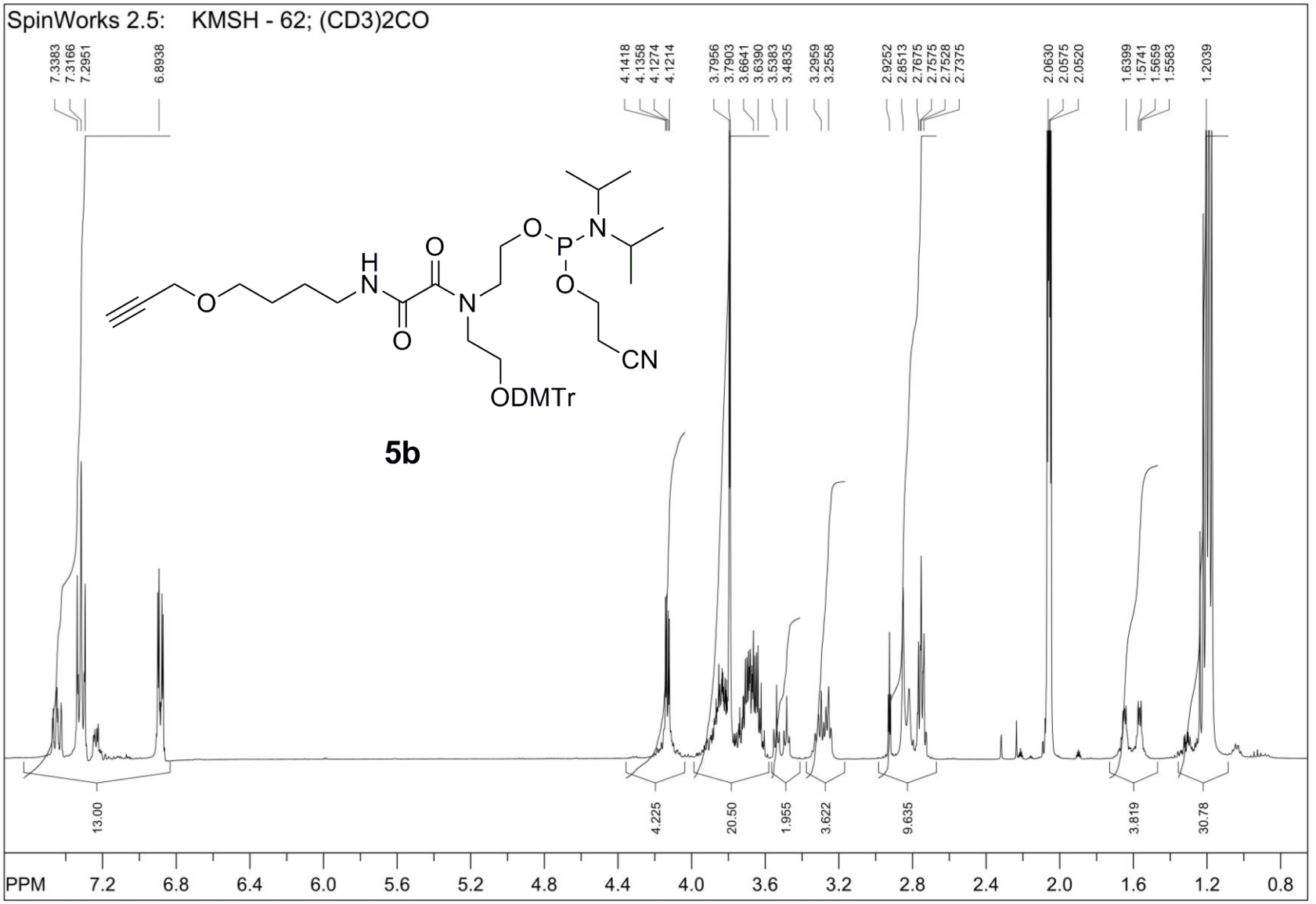


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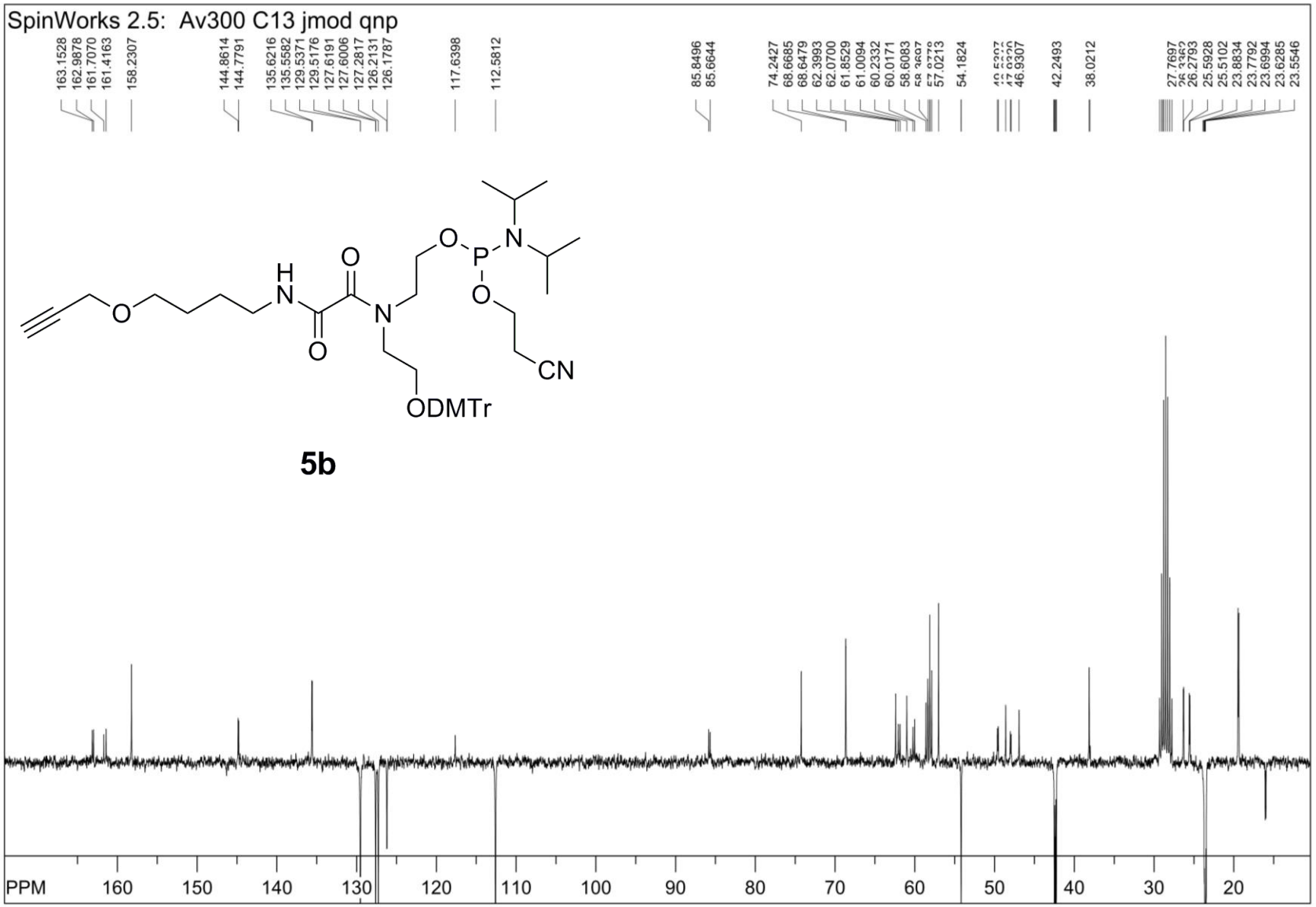


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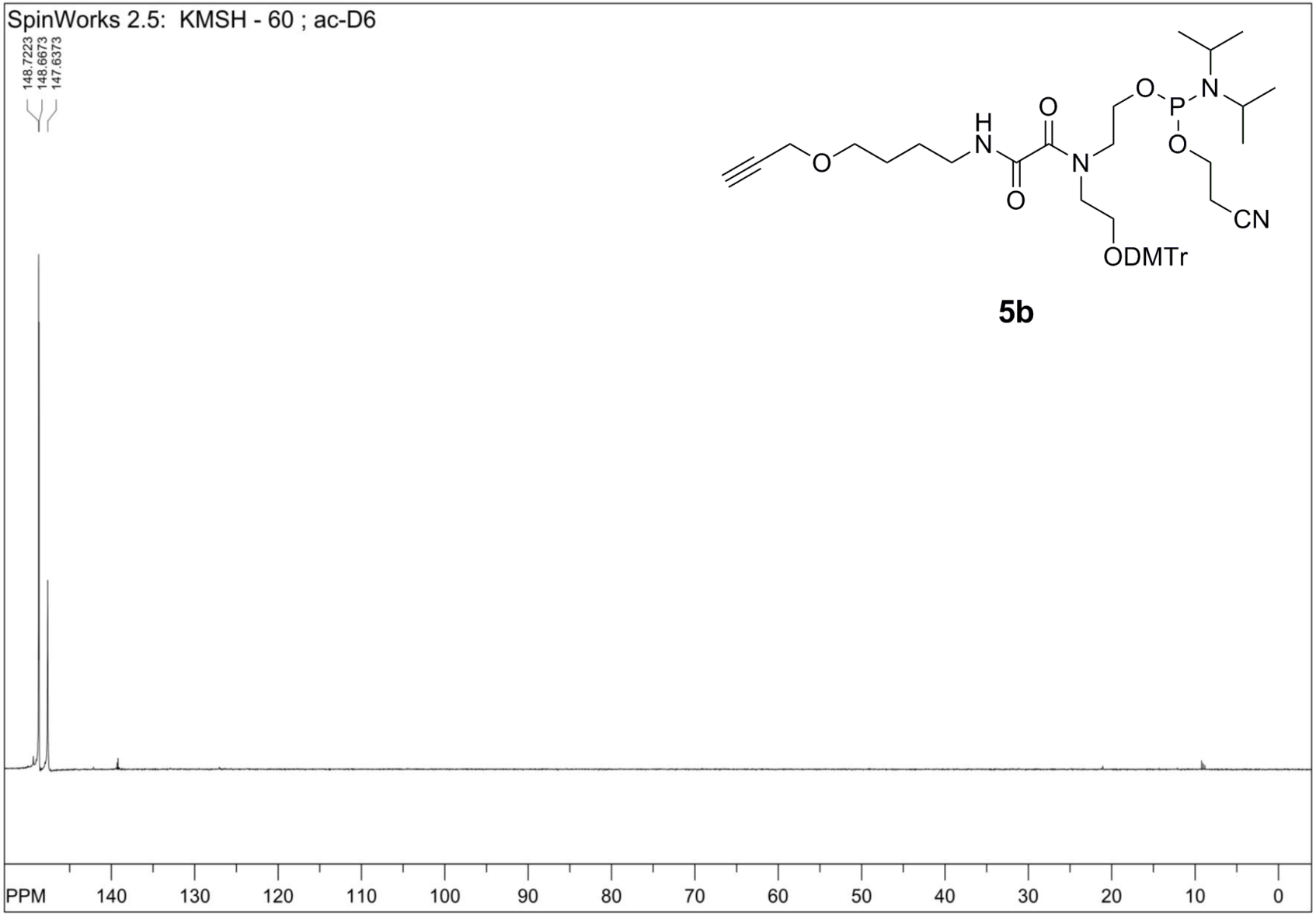


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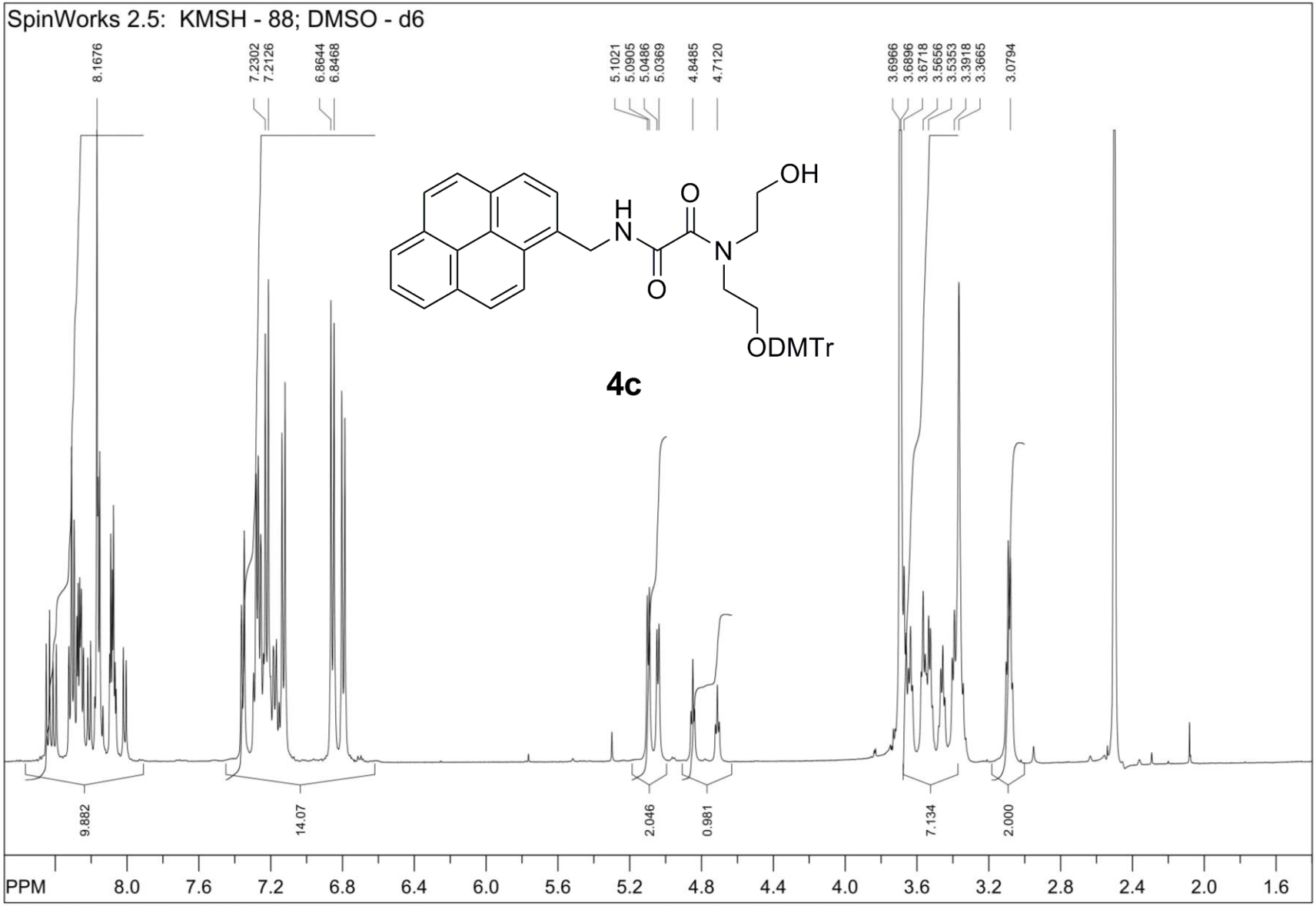


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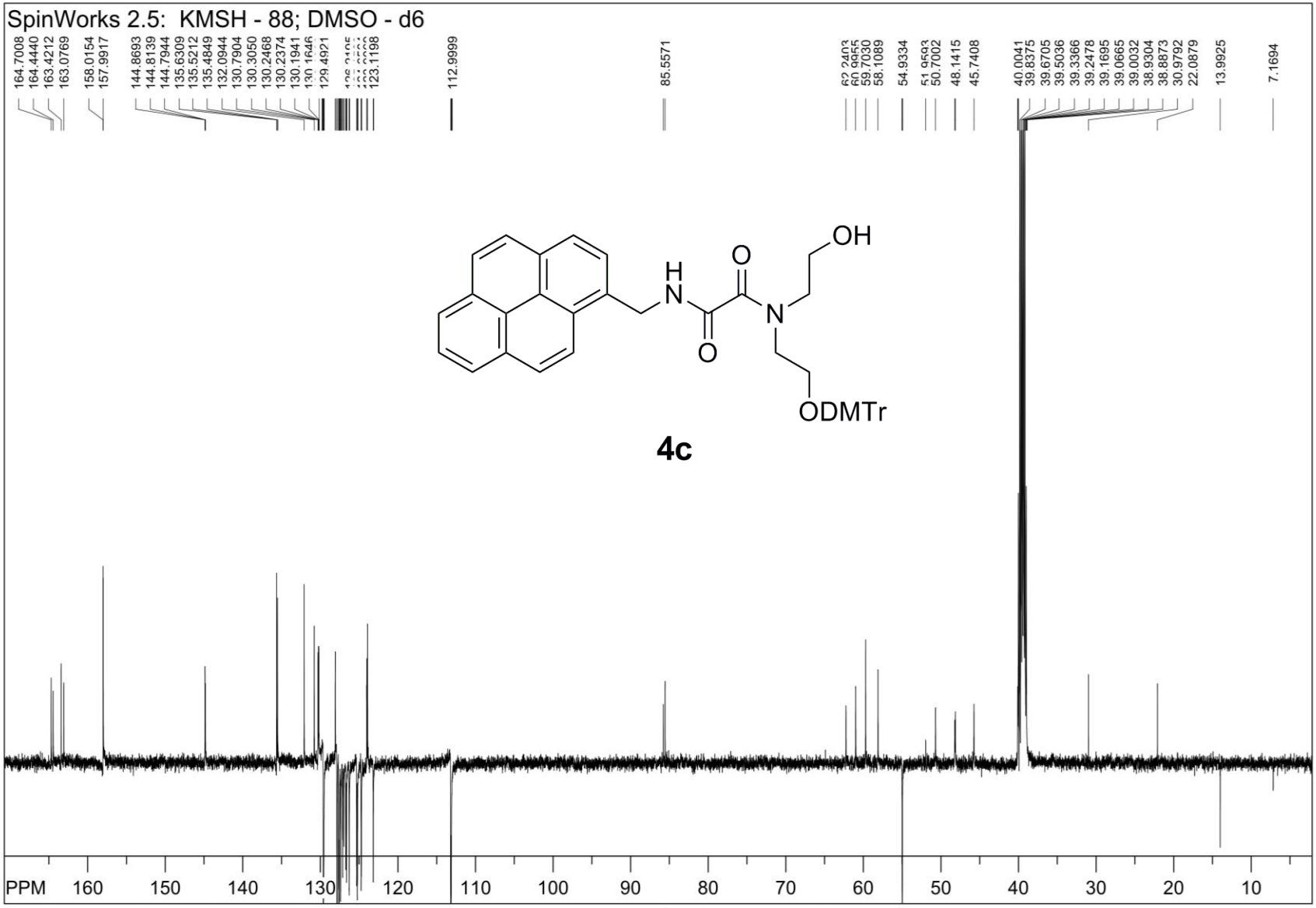


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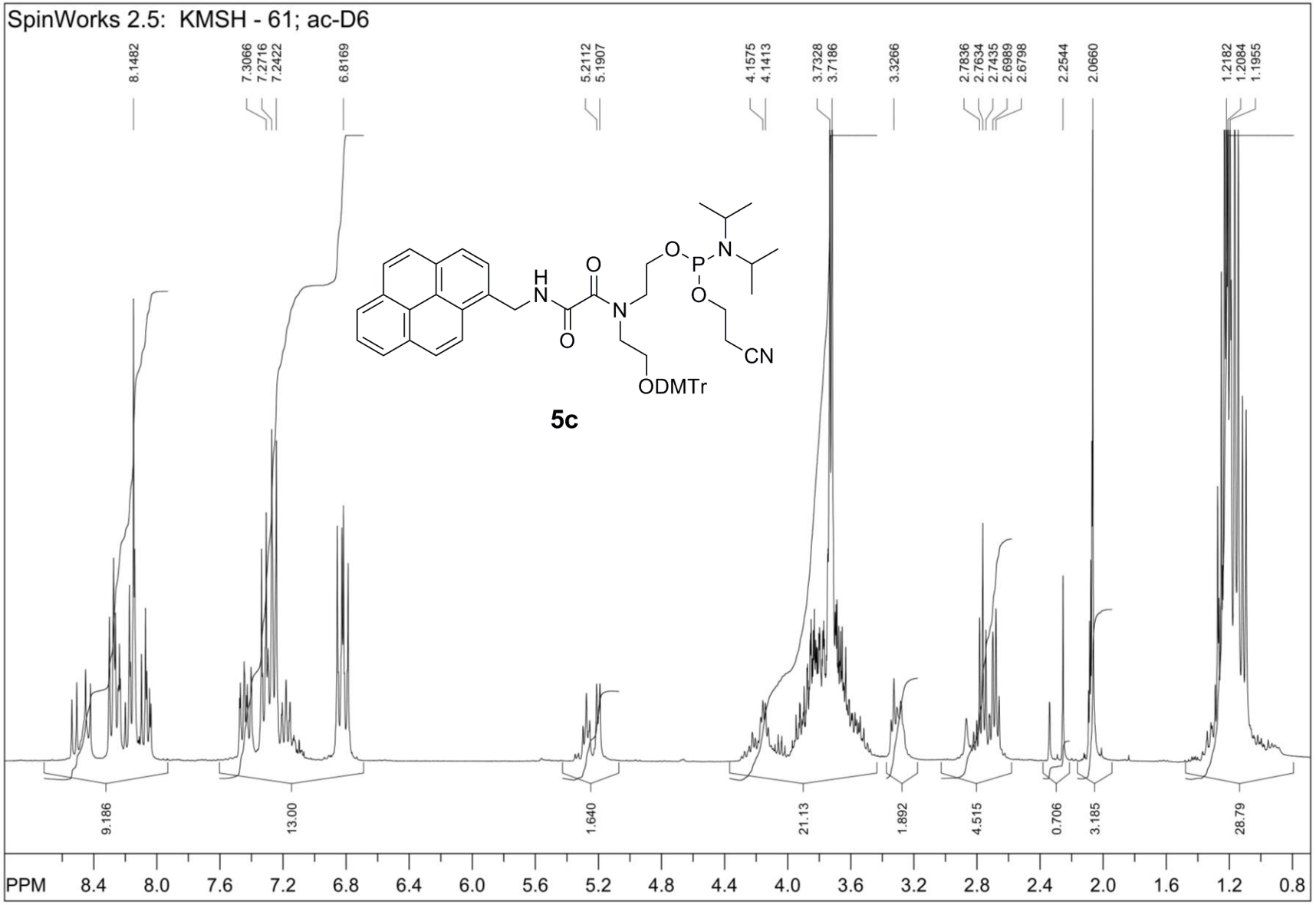


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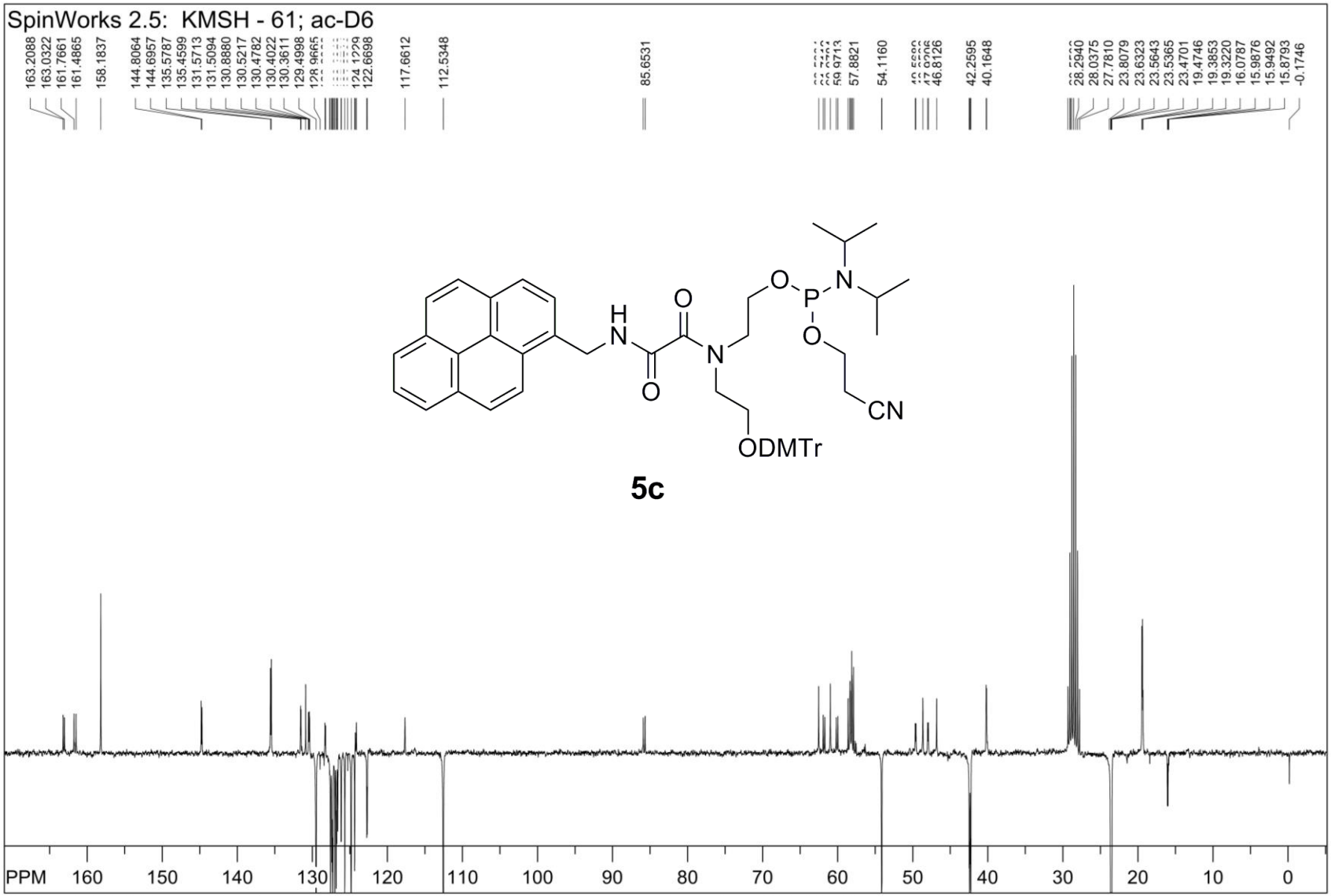


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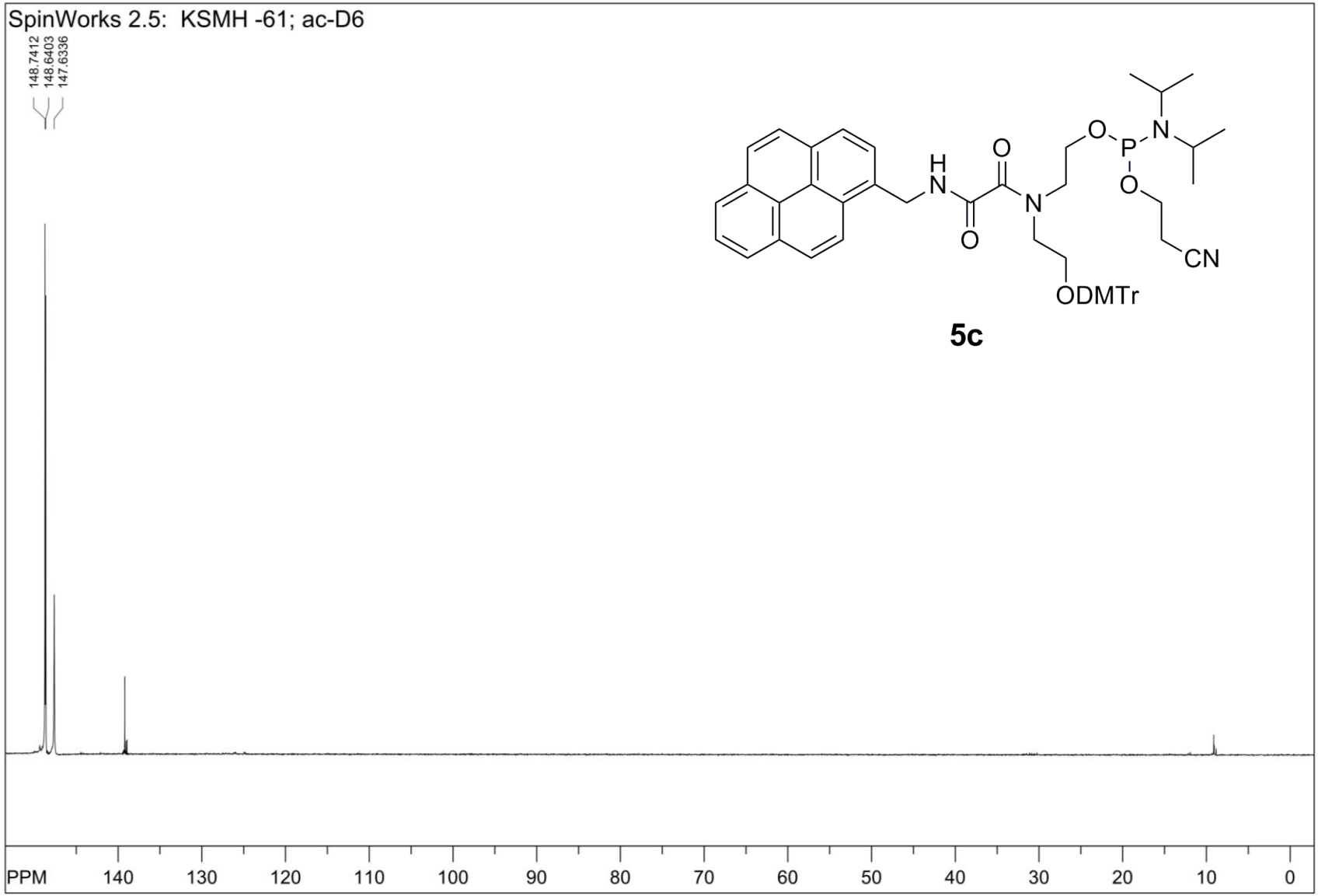


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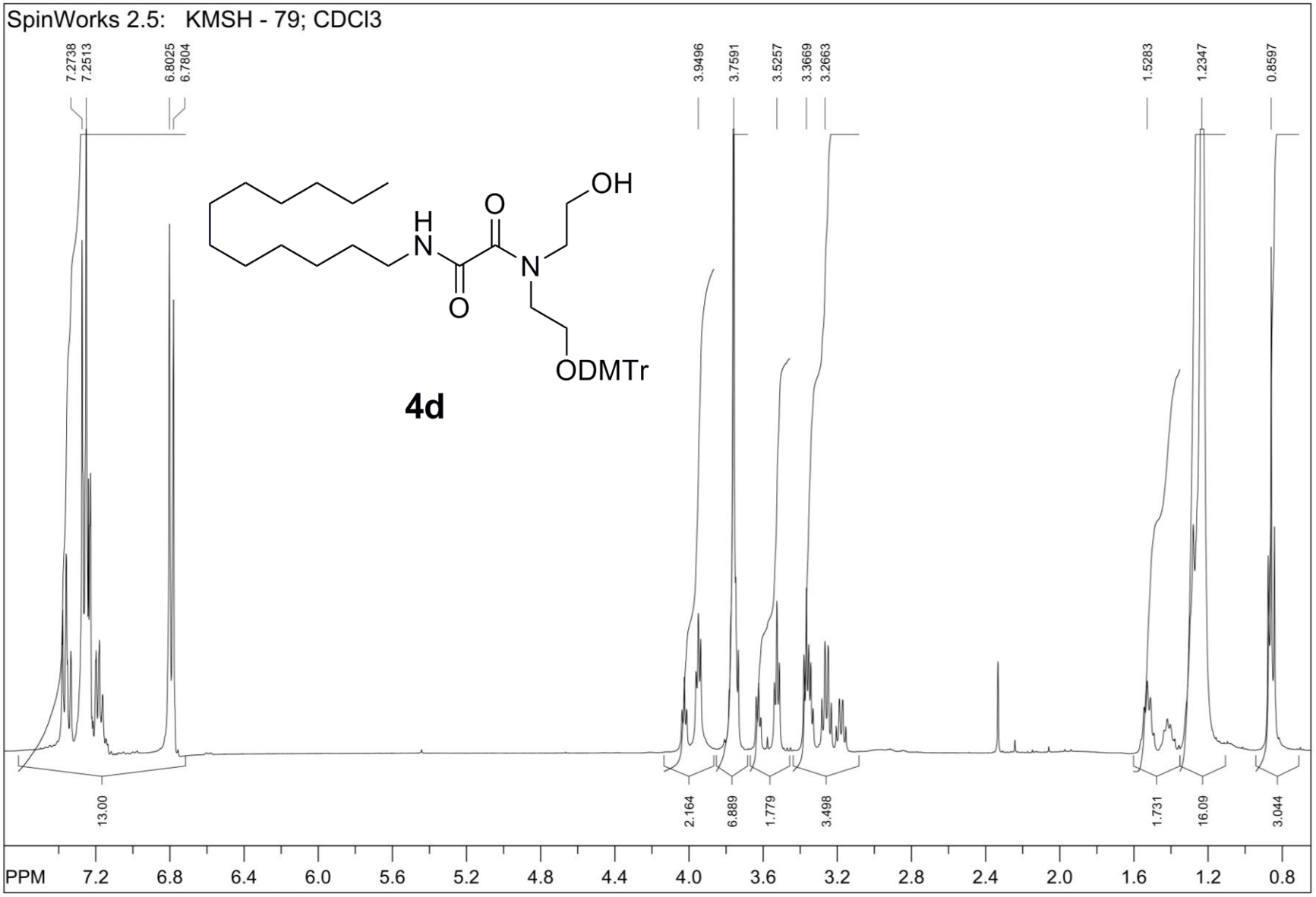


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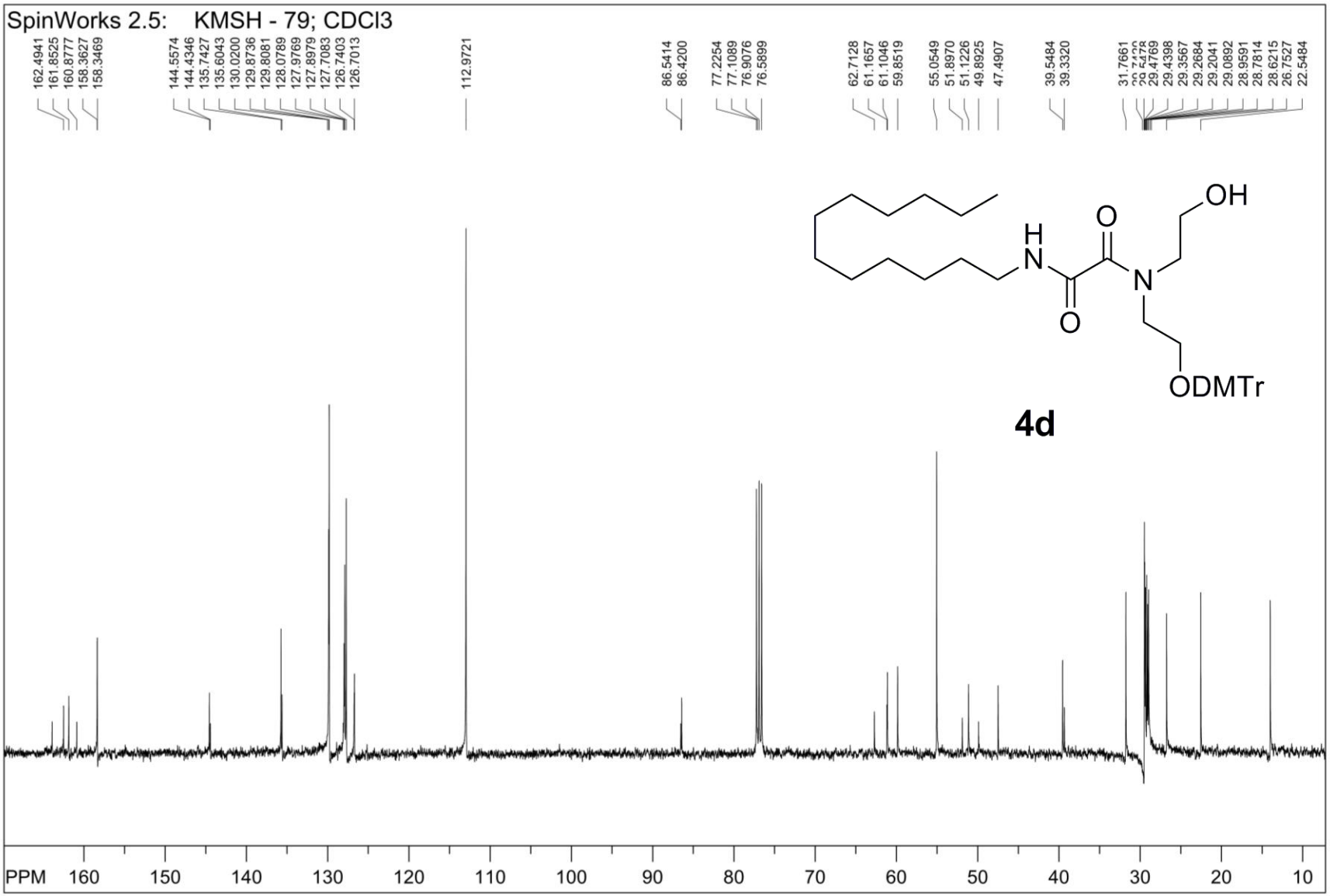


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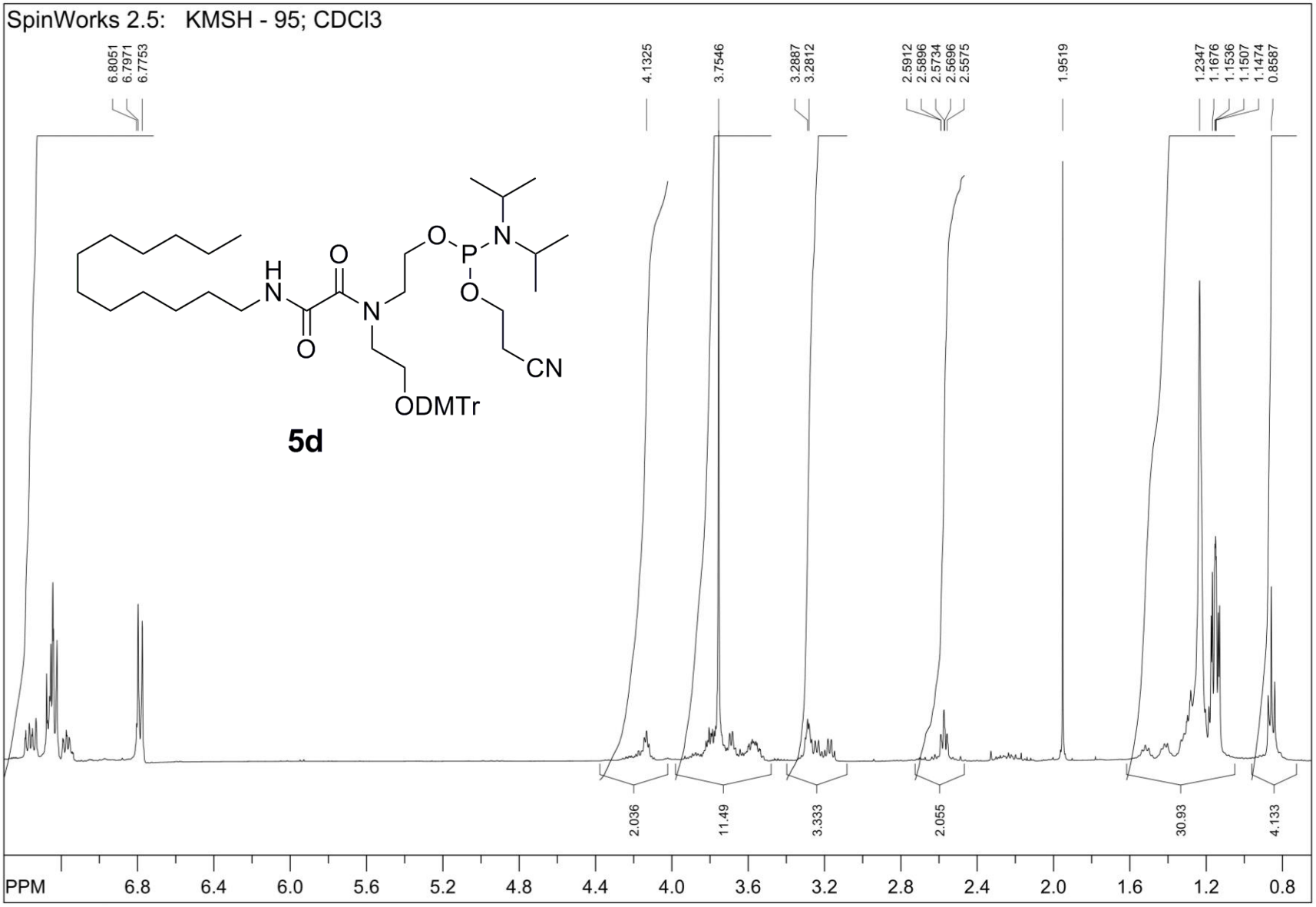


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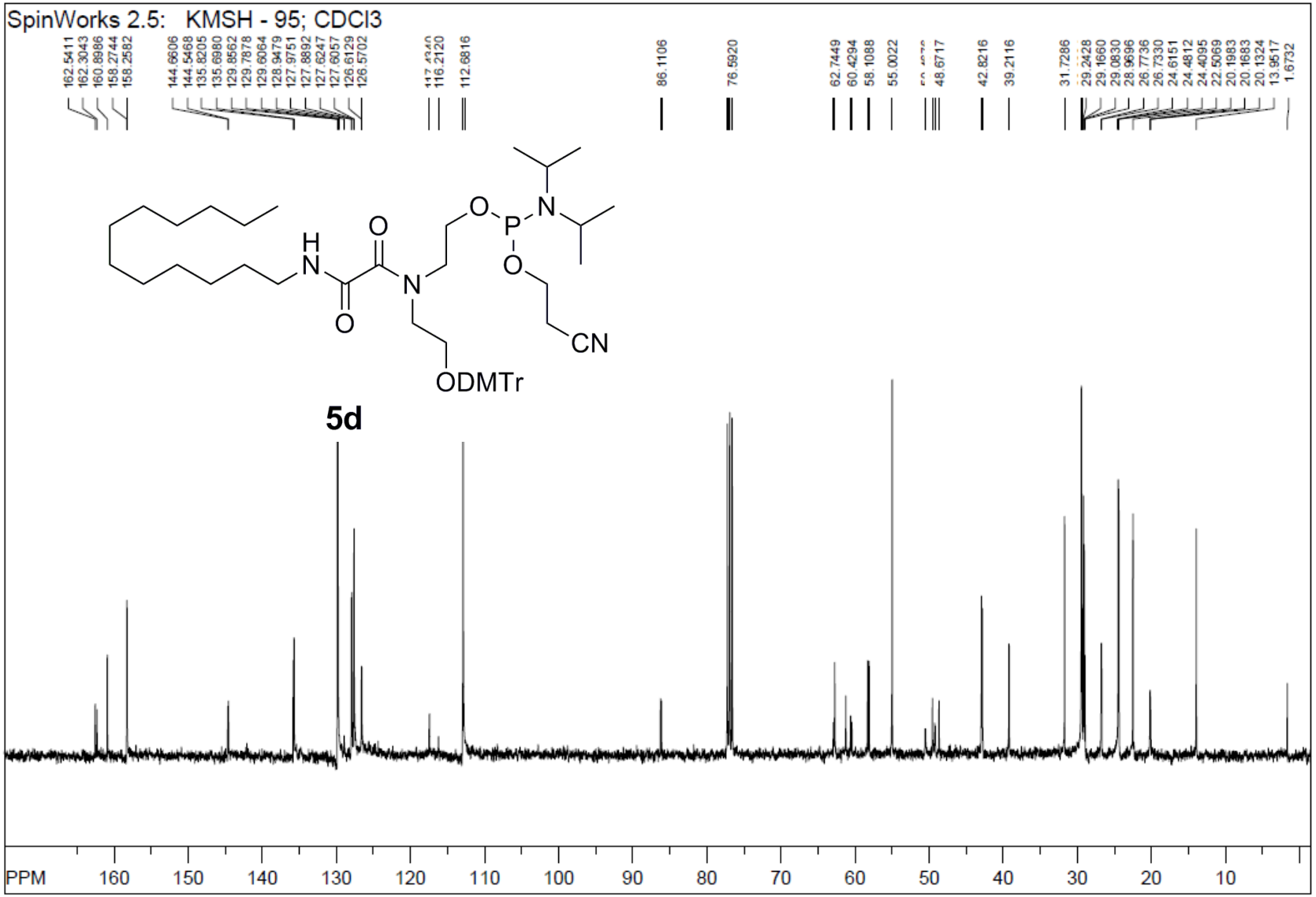


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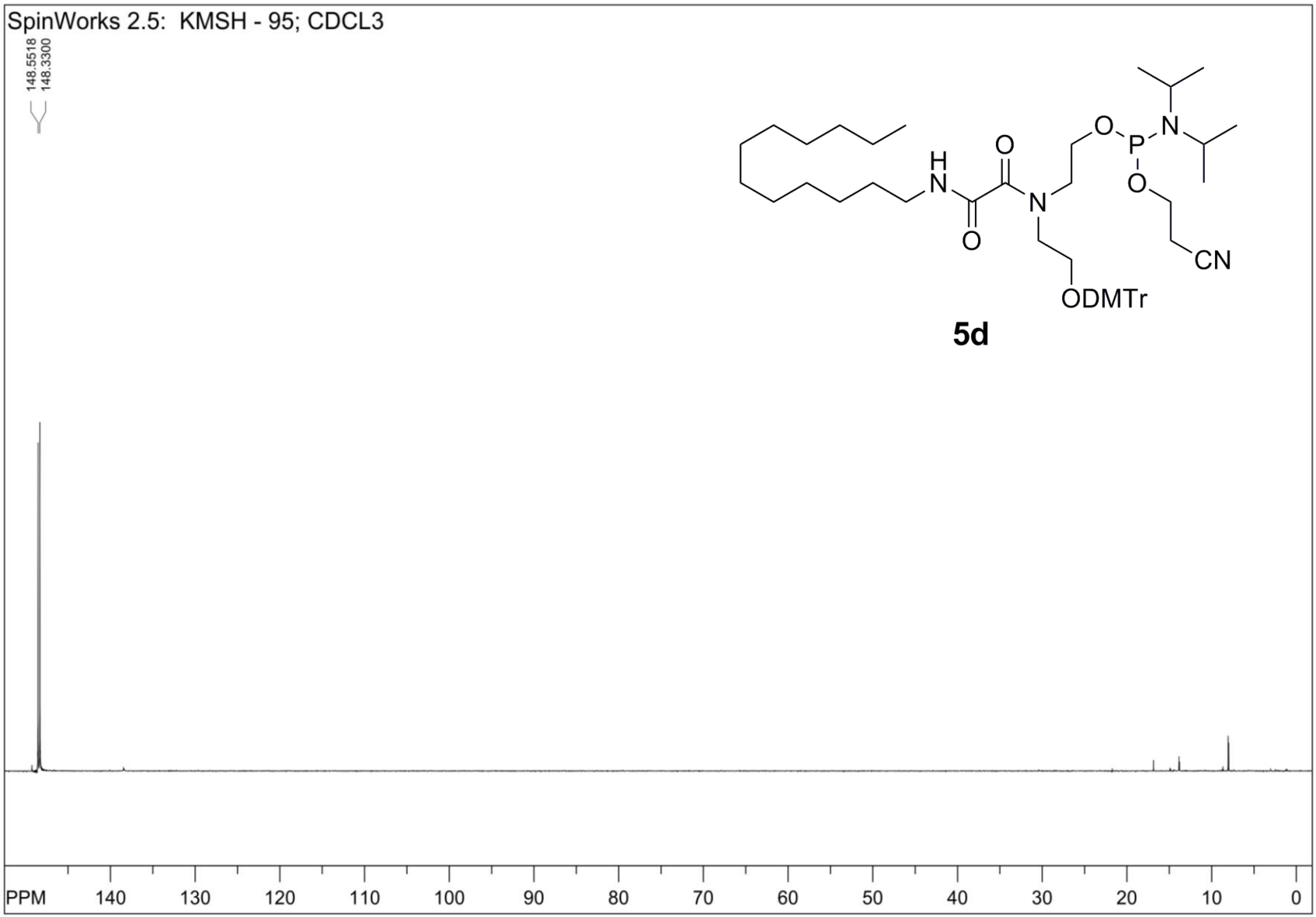


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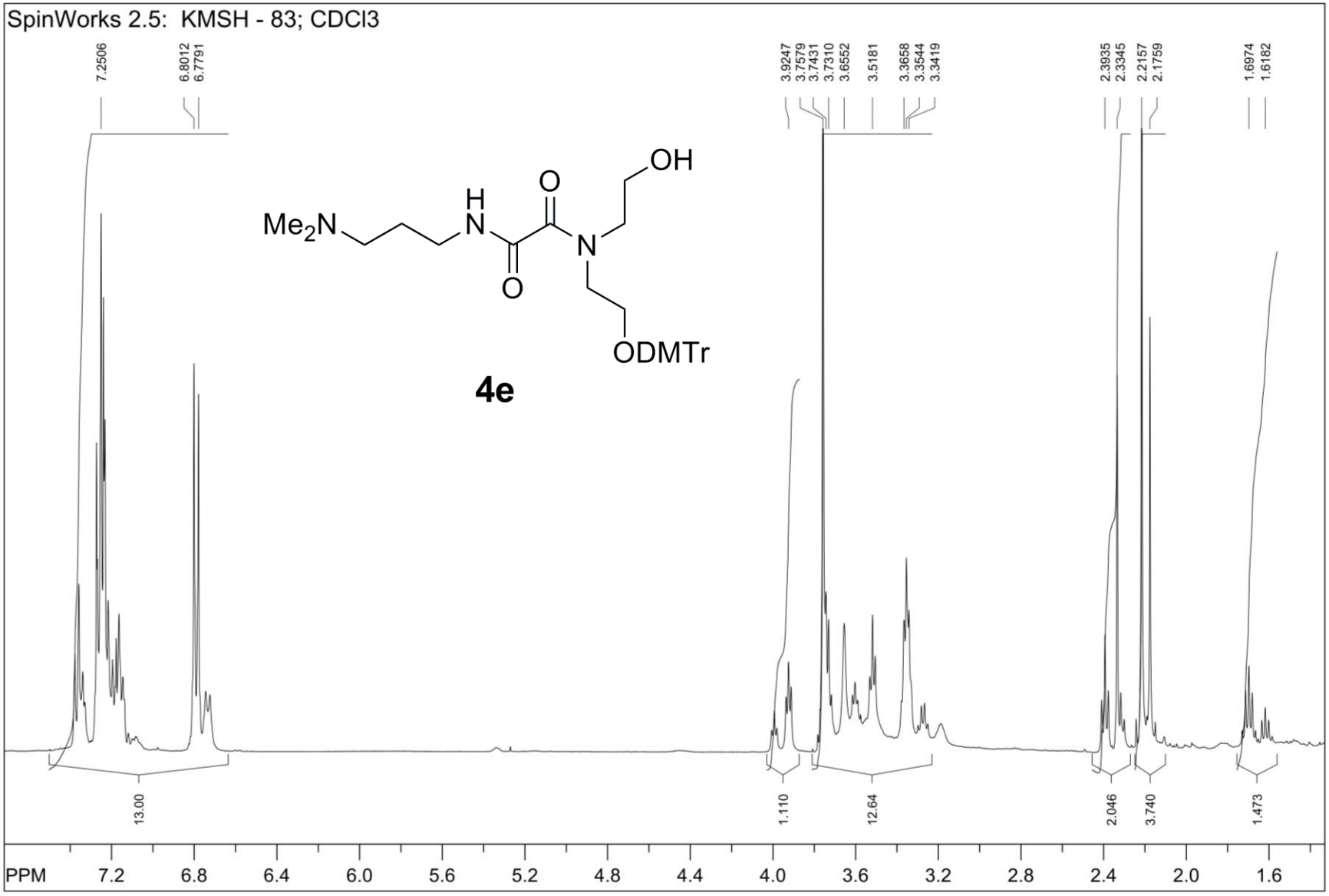


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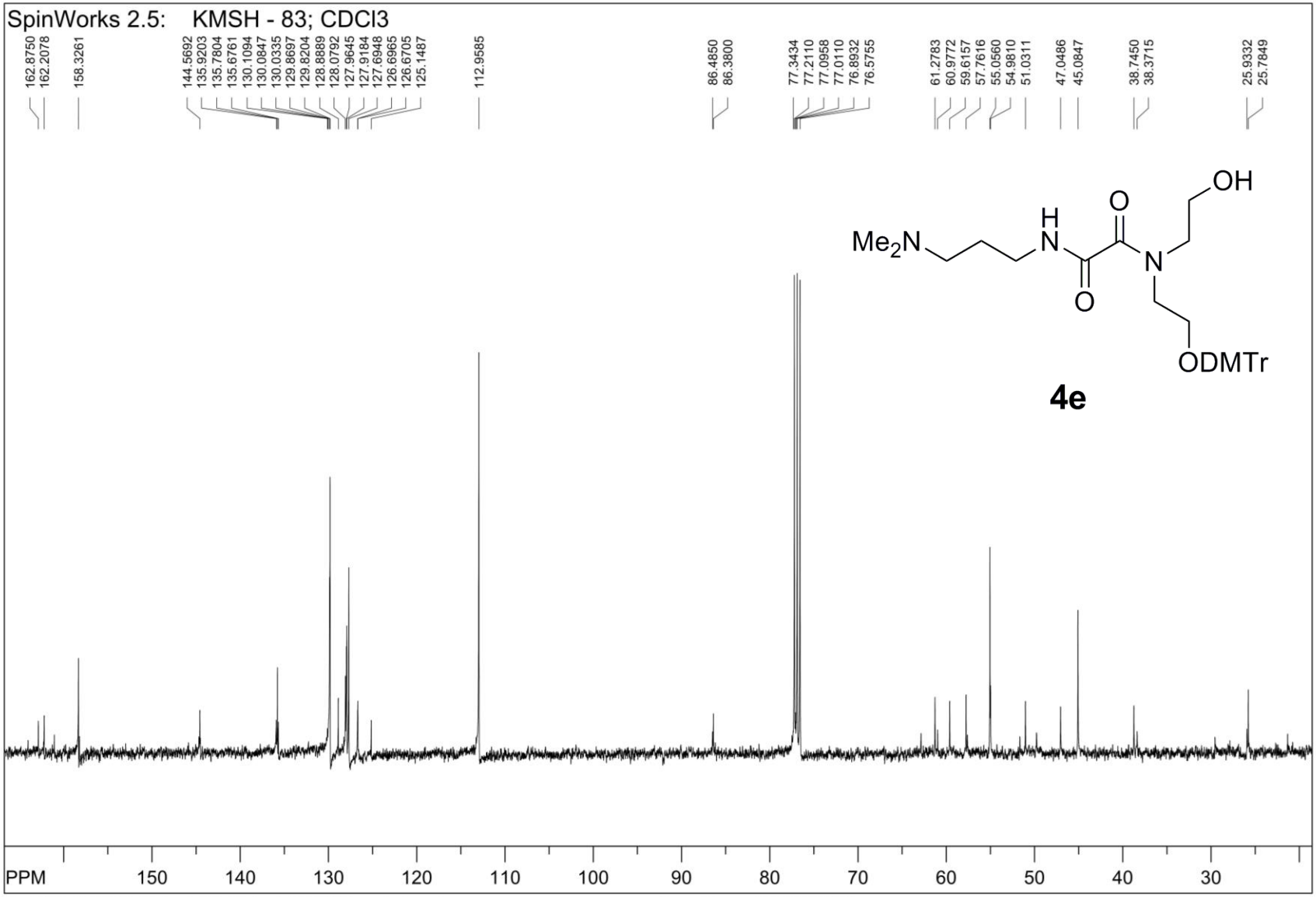
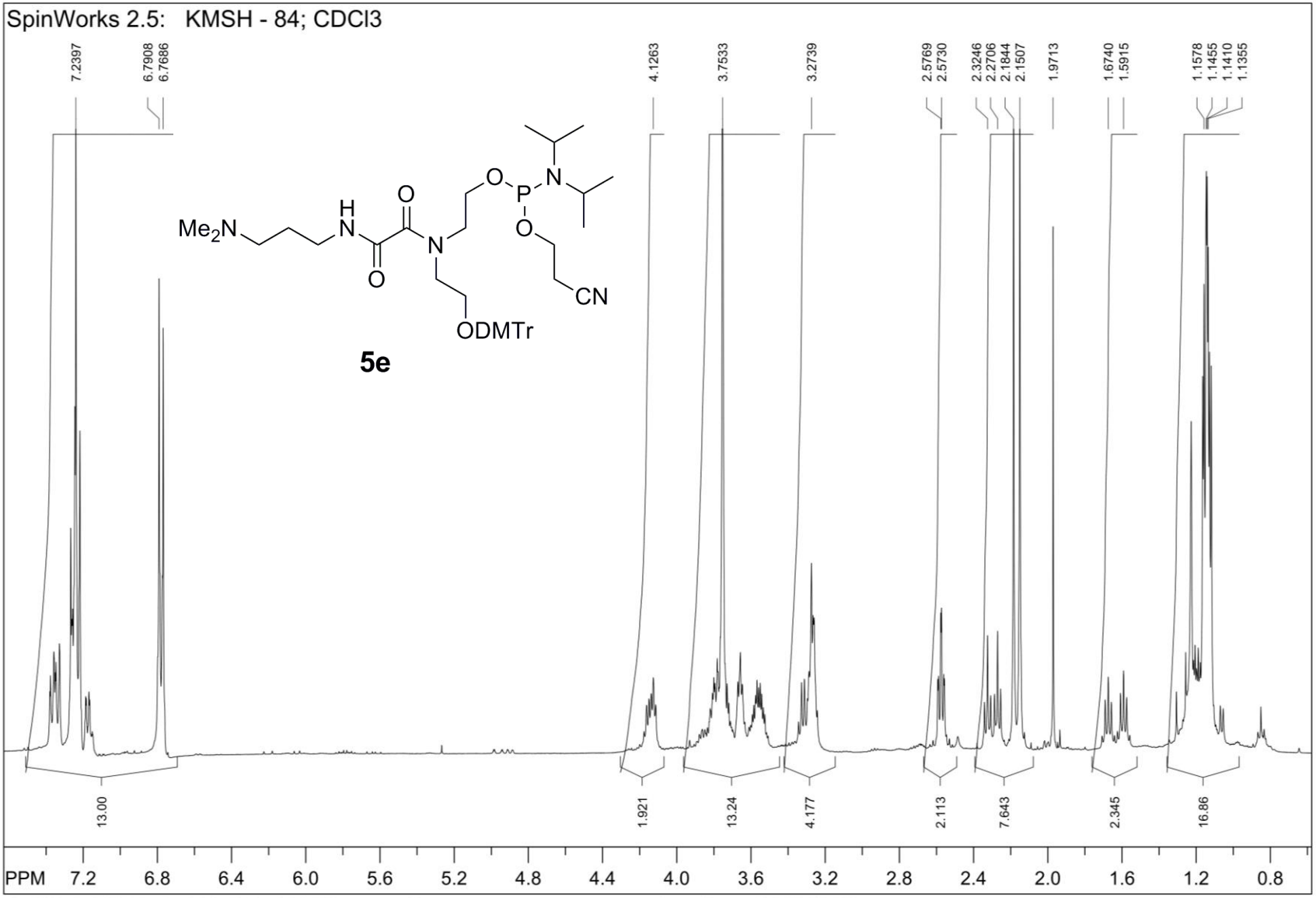
time domain size: 65536 points
width: 6203.47 Hz = 15.503573 ppm = 0.094658 Hz/pt
number of scans: 32

freq. of 0 ppm: 400.130018 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 102.251 ppm/cm: 0.25554



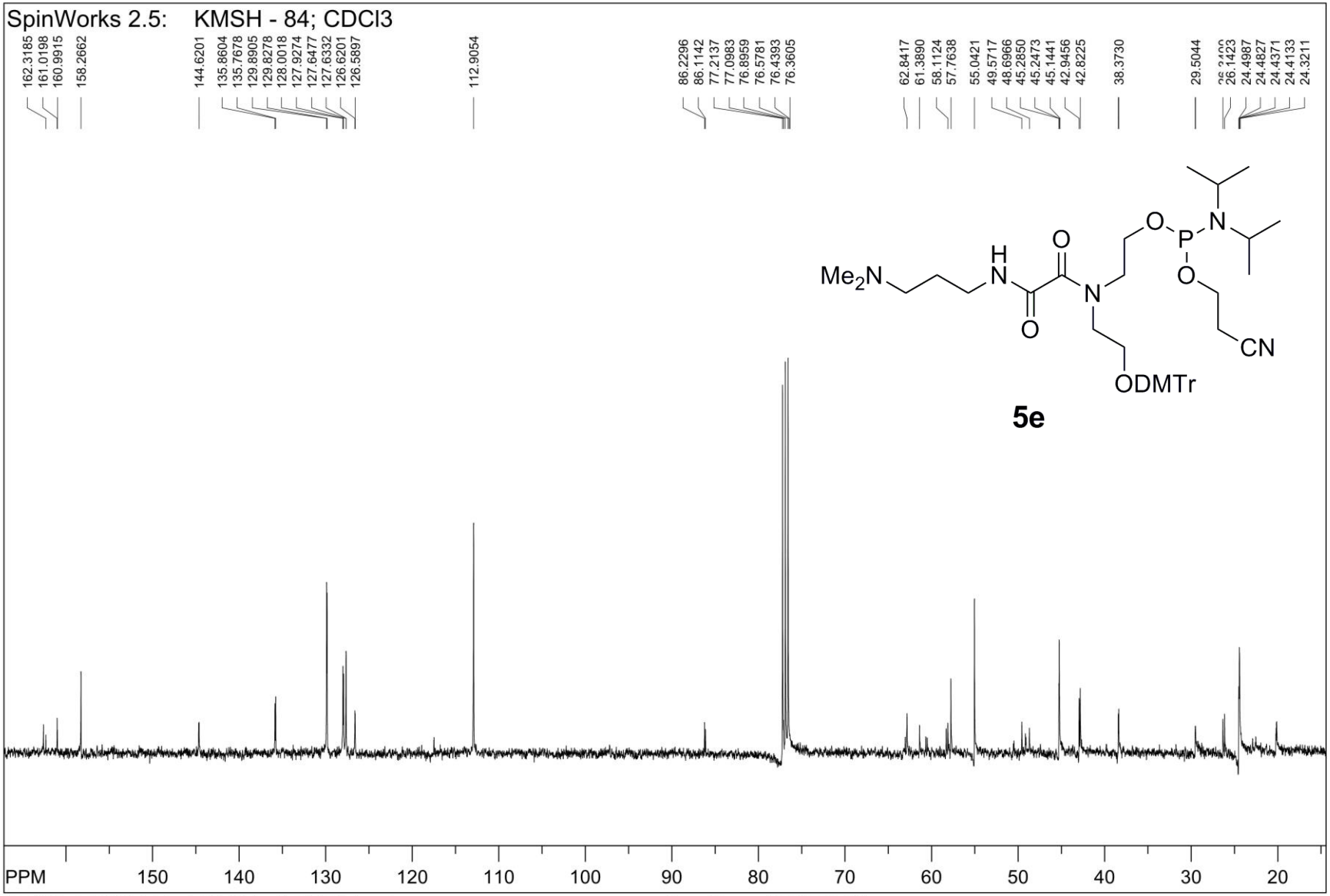

file: H:\Maxim\ЯМР\nibh-kmsh-83\nibh-kmsh-83\136\fid expt: <zgpg>
transmitter freq.: 100.624820 MHz
time domain size: 65536 points
width: 28409.09 Hz = 282.326875 ppm = 0.433488 Hz/pt
number of scans: 128

freq. of 0 ppm: 100.612786 MHz
processed size: 131072 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 596.297 ppm/cm: 5.92594



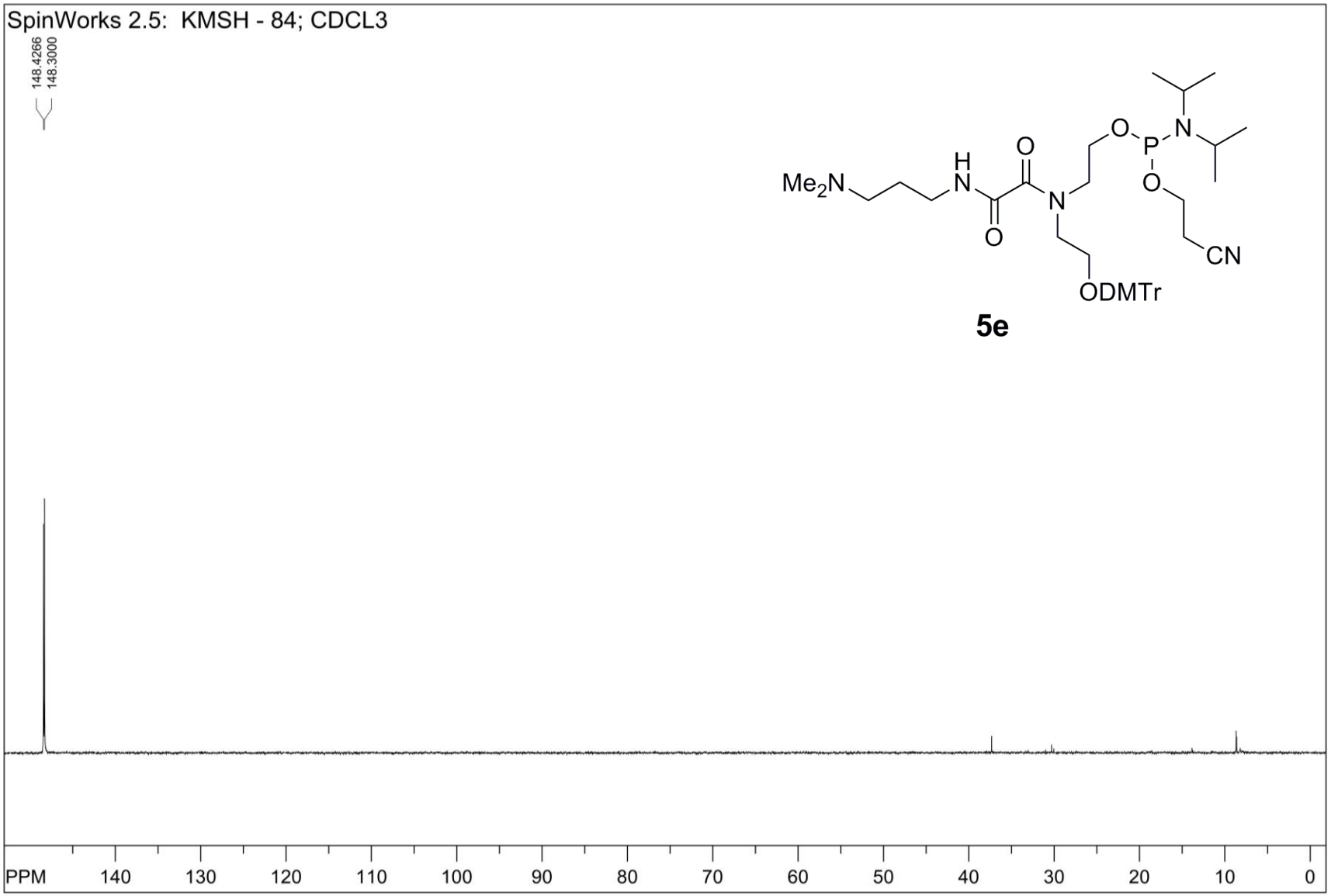

file: H:\Maxim\ЯМР\nibh-kmsh-84\nibh-kmsh-84\1\fid expt: <zg30>
transmitter freq.: 400.131884 MHz
time domain size: 65536 points
width: 6203.47 Hz = 15.503573 ppm = 0.094658 Hz/pt
number of scans: 32

freq. of 0 ppm: 400.130018 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 112.836 ppm/cm: 0.28200




file: H:\Maxim\ЯМР\nibh-kmsh-84\nibh-kmsh-84\136\fid expt: <zgpg>
transmitter freq.: 100.624820 MHz
time domain size: 65536 points
width: 28409.09 Hz = 282.326875 ppm = 0.433488 Hz/pt
number of scans: 112

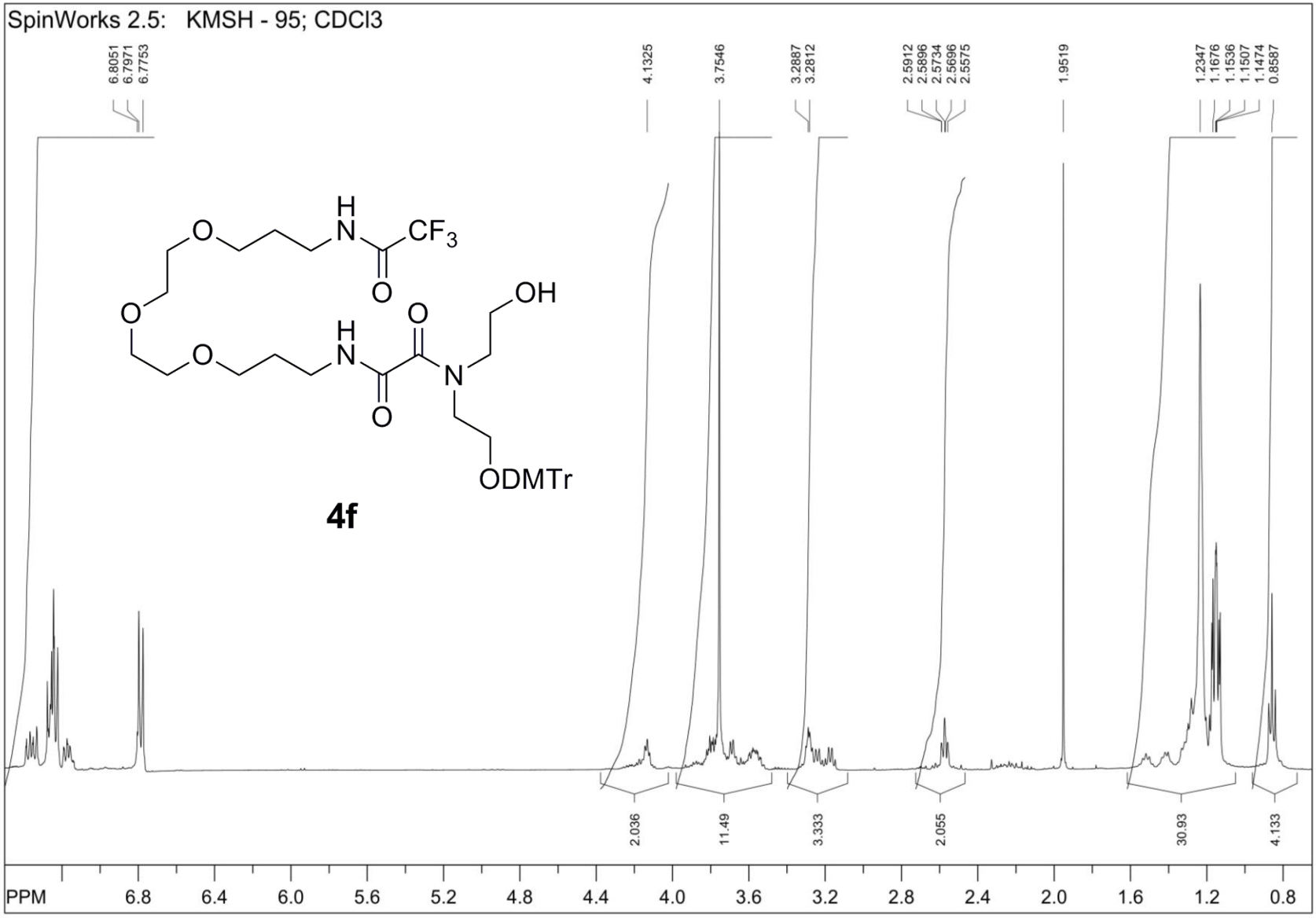
freq. of 0 ppm: 100.612786 MHz
processed size: 131072 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 614.690 ppm/cm: 6.10873




**5e**

file: H:\Maxim\ЯМР\nibh-kmsh-84\nibh-kmsh-84\31\fid expt: <zgpg>
transmitter freq.: 121.502141 MHz
time domain size: 32768 points
width: 31645.57 Hz = 260.452774 ppm = 0.965746 Hz/pt
number of scans: 200

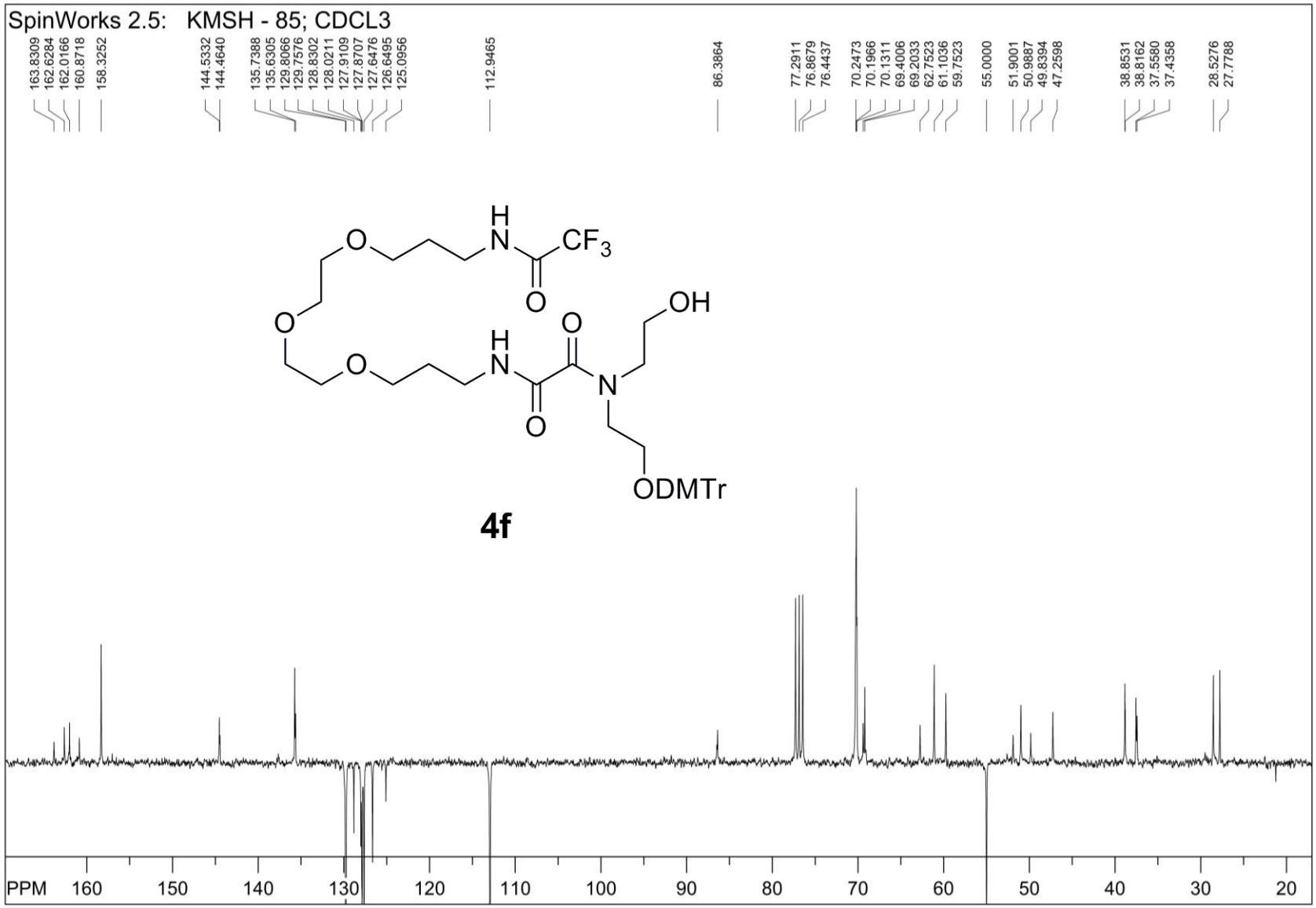
freq. of 0 ppm: 121.494824 MHz
processed size: 65536 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 752.638 ppm/cm: 6.19444




**4f**

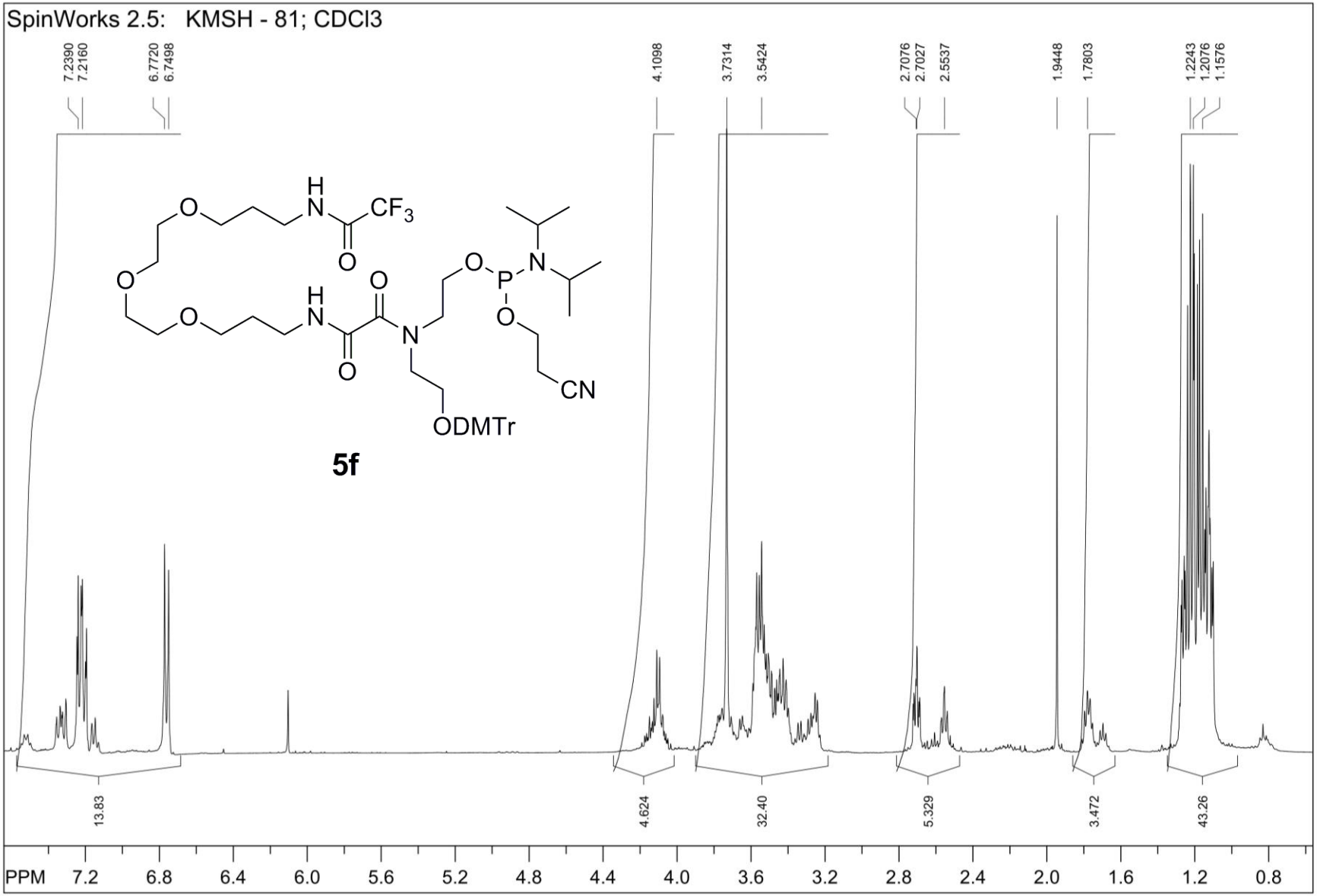
file: H:\Maxim\ЯМР\94-95-96\nibh-kmsh-95\nibh-kmsh-95\1\fid expt: <zg30>
transmitter freq.: 400.131884 MHz
time domain size: 65536 points
width: 6203.47 Hz = 15.503573 ppm = 0.094658 Hz/pt
number of scans: 64

freq. of 0 ppm: 400.130017 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 109.664 ppm/cm: 0.27407




file: H:\Maxim\ЯМР\nibh-kmsh-85\nibh-kmsh-85\137\fid expt: <jmod>
transmitter freq.: 75.475975 MHz
time domain size: 32768 points
width: 19607.84 Hz = 259.789200 ppm = 0.598384 Hz/pt
number of scans: 1248

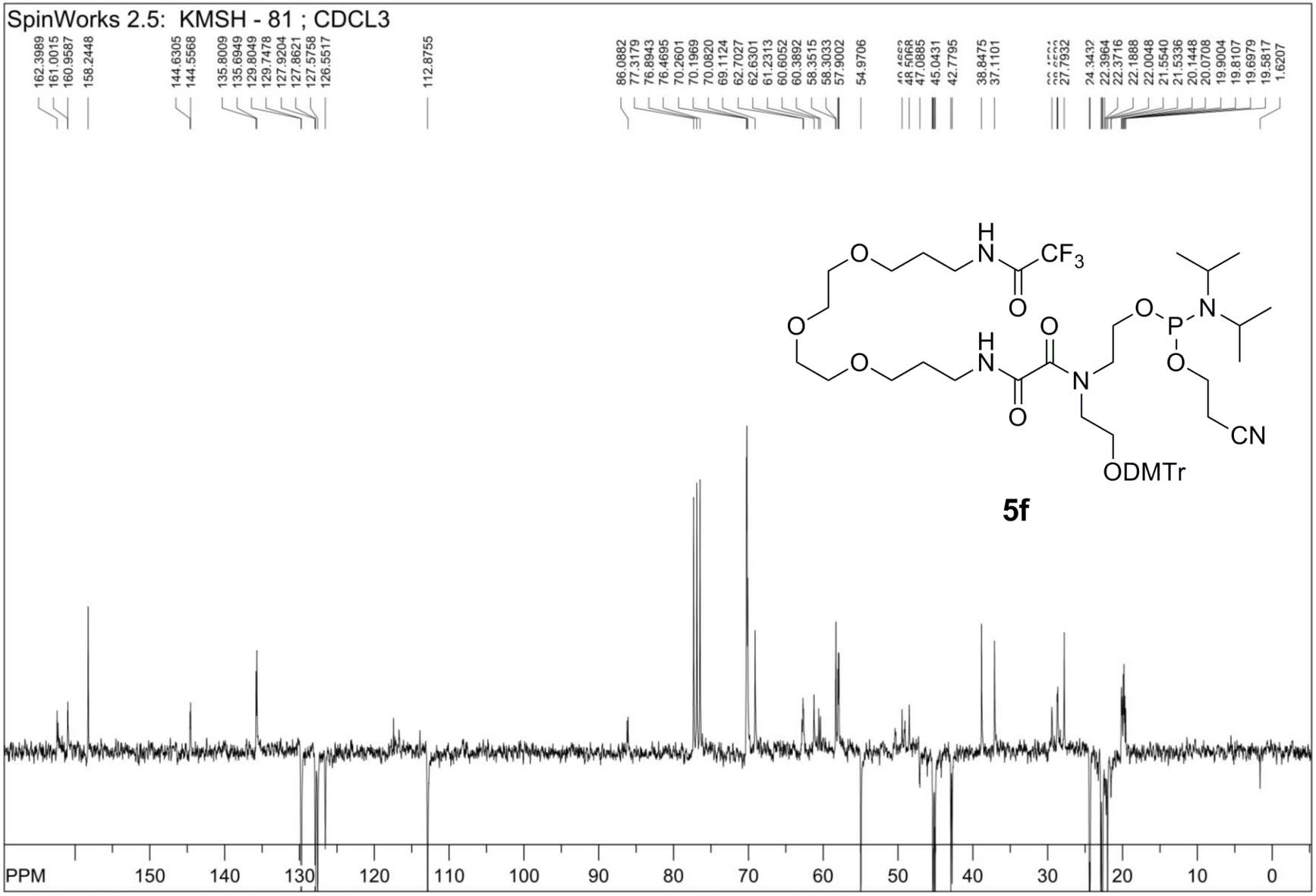
freq. of 0 ppm: 75.467765 MHz
processed size: 65536 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 460.299 ppm/cm: 6.09862




**5f**

file: H:\Maxim\ЯМР\nibh-kmsh-81\nibh-kmsh-81\1\fid expt: <zg30>
transmitter freq.: 400.131884 MHz
time domain size: 65536 points
width: 6203.47 Hz = 15.503573 ppm = 0.094658 Hz/pt
number of scans: 32

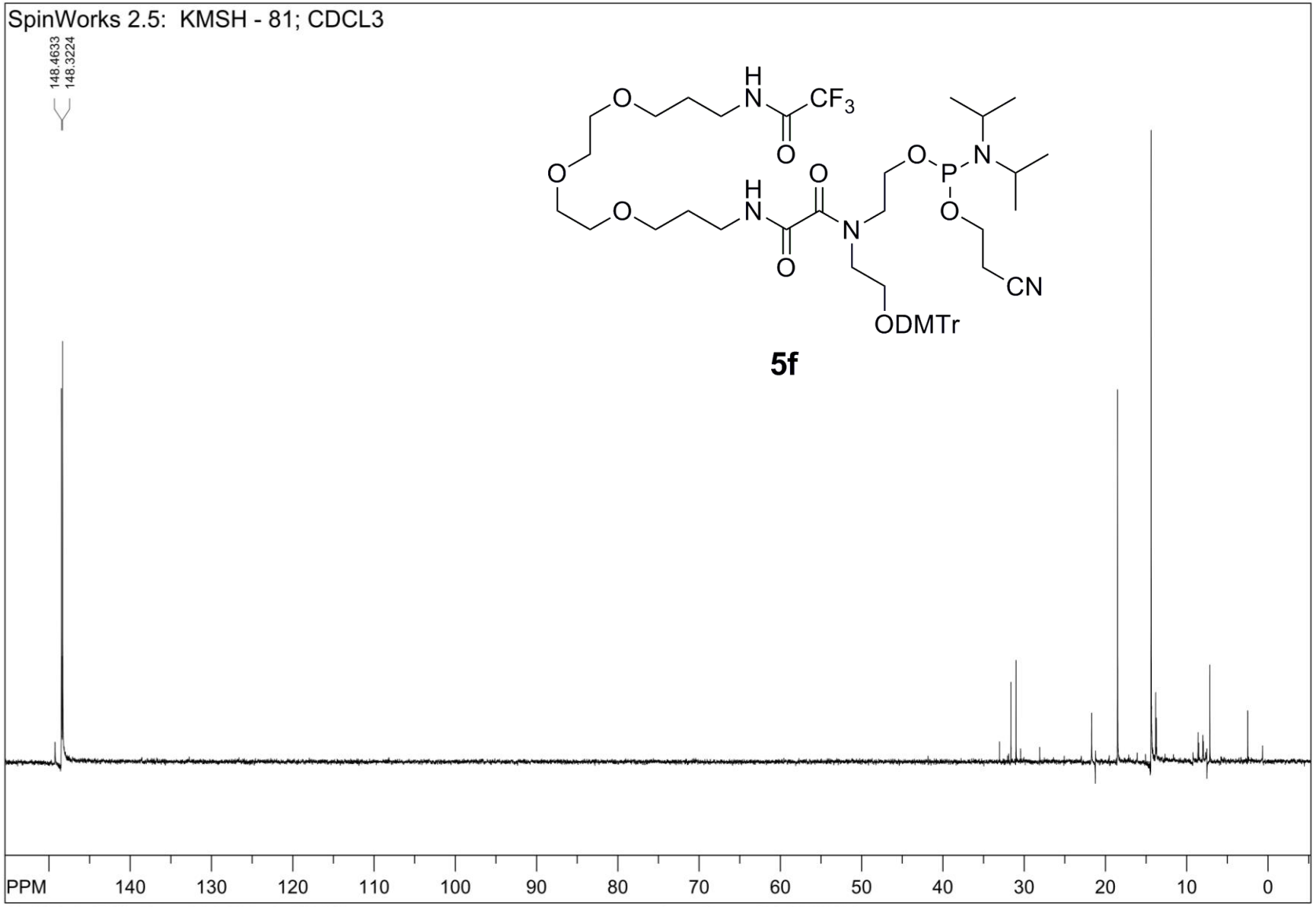
freq. of 0 ppm: 400.130018 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 113.303 ppm/cm: 0.28316




**5f**

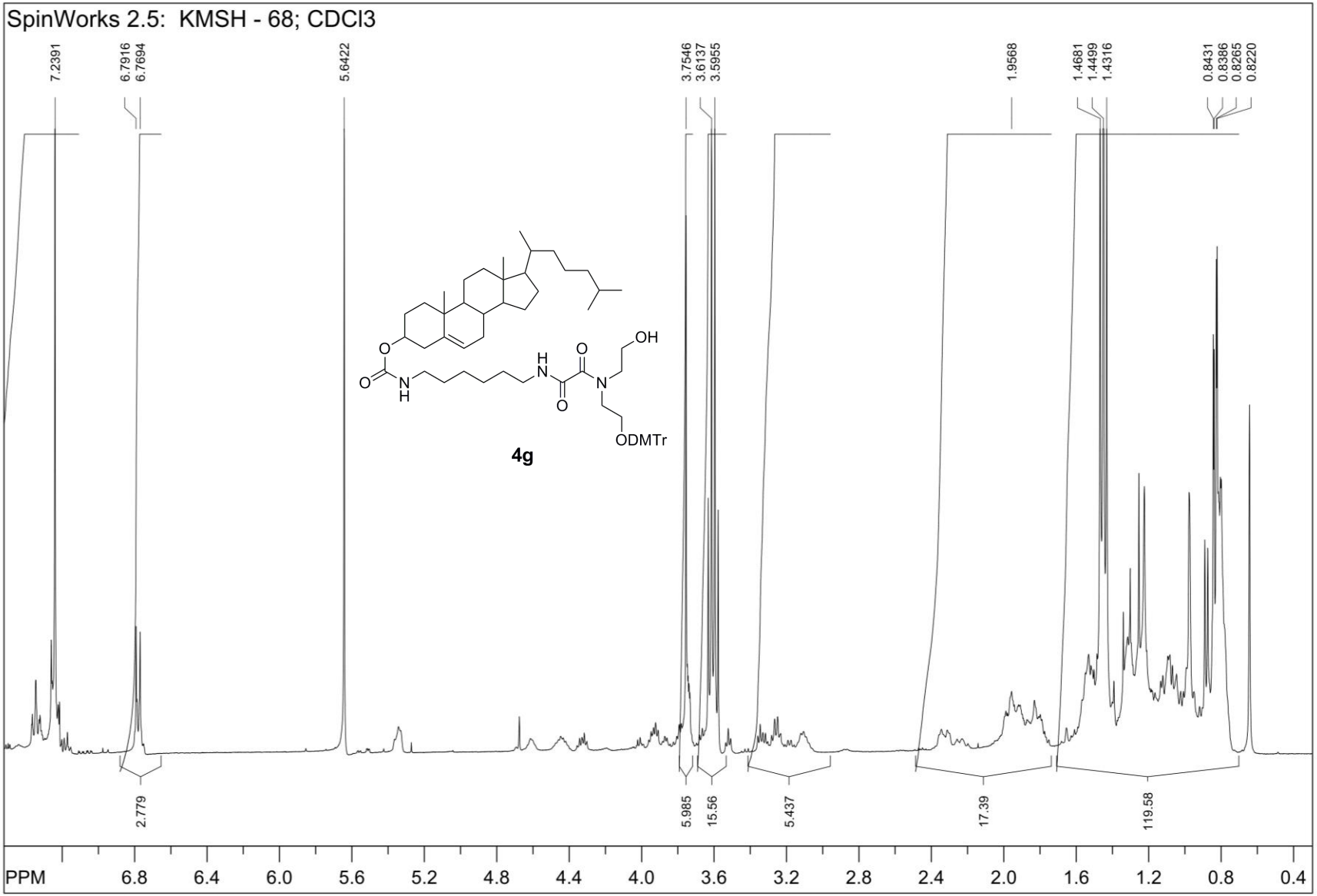
file: H:\Maxim\ЯМР\nibh-kmsh-81\nibh-kmsh-81\137\fid expt: <jmod>
transmitter freq.: 75.475975 MHz
time domain size: 32768 points
width: 19607.84 Hz = 259.789200 ppm = 0.598384 Hz/pt
number of scans: 800

freq. of 0 ppm: 75.467765 MHz
processed size: 65536 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 527.395 ppm/cm: 6.98759



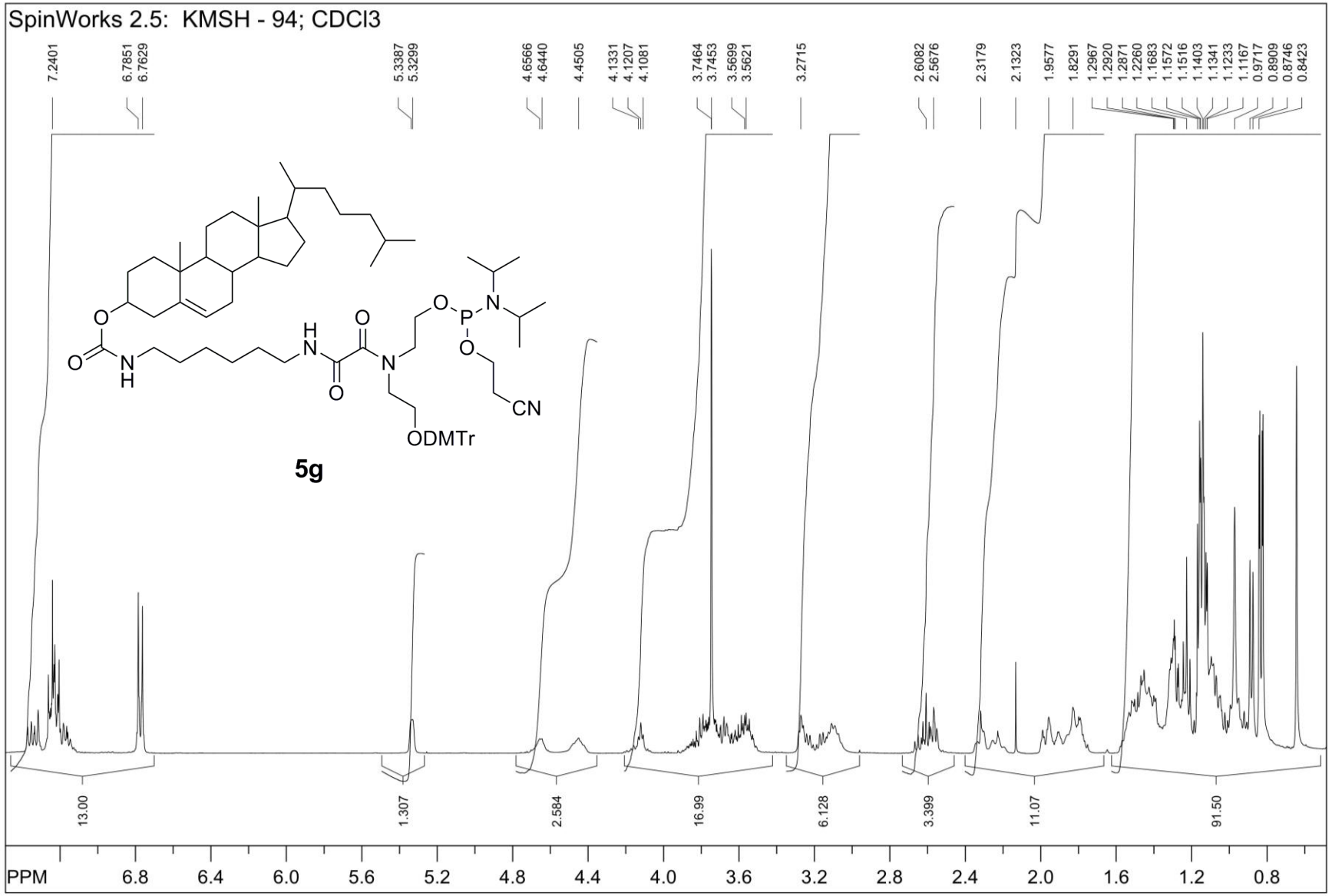

file: H:\Maxim\ЯМР\nibh-kmsh-81\nibh-kmsh-81\31\fid expt: <zgpg>
transmitter freq.: 121.502141 MHz
time domain size: 32768 points
width: 31645.57 Hz = 260.452774 ppm = 0.965746 Hz/pt
number of scans: 80

freq. of 0 ppm: 121.494824 MHz
processed size: 65536 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 780.836 ppm/cm: 6.42652



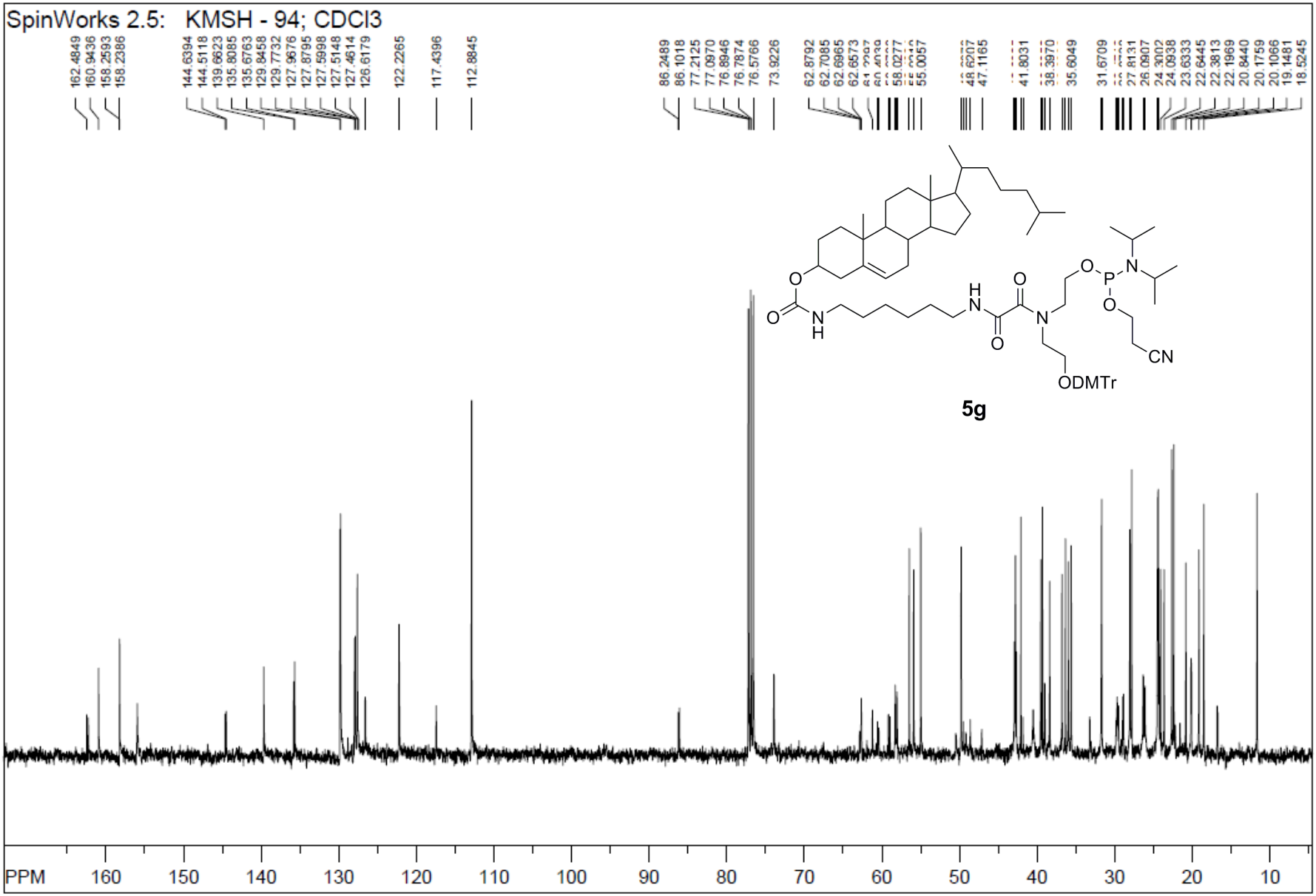

file: H:\Maxim\ЯМР\nibh-kmsh-68\nibh-kmsh-68\1\fid expt: <zg30>
transmitter freq.: 400.131635 MHz
time domain size: 65536 points
width: 5630.63 Hz = 14.071946 ppm = 0.085917 Hz/pt
number of scans: 32

freq. of 0 ppm: 400.130018 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 115.643 ppm/cm: 0.28901



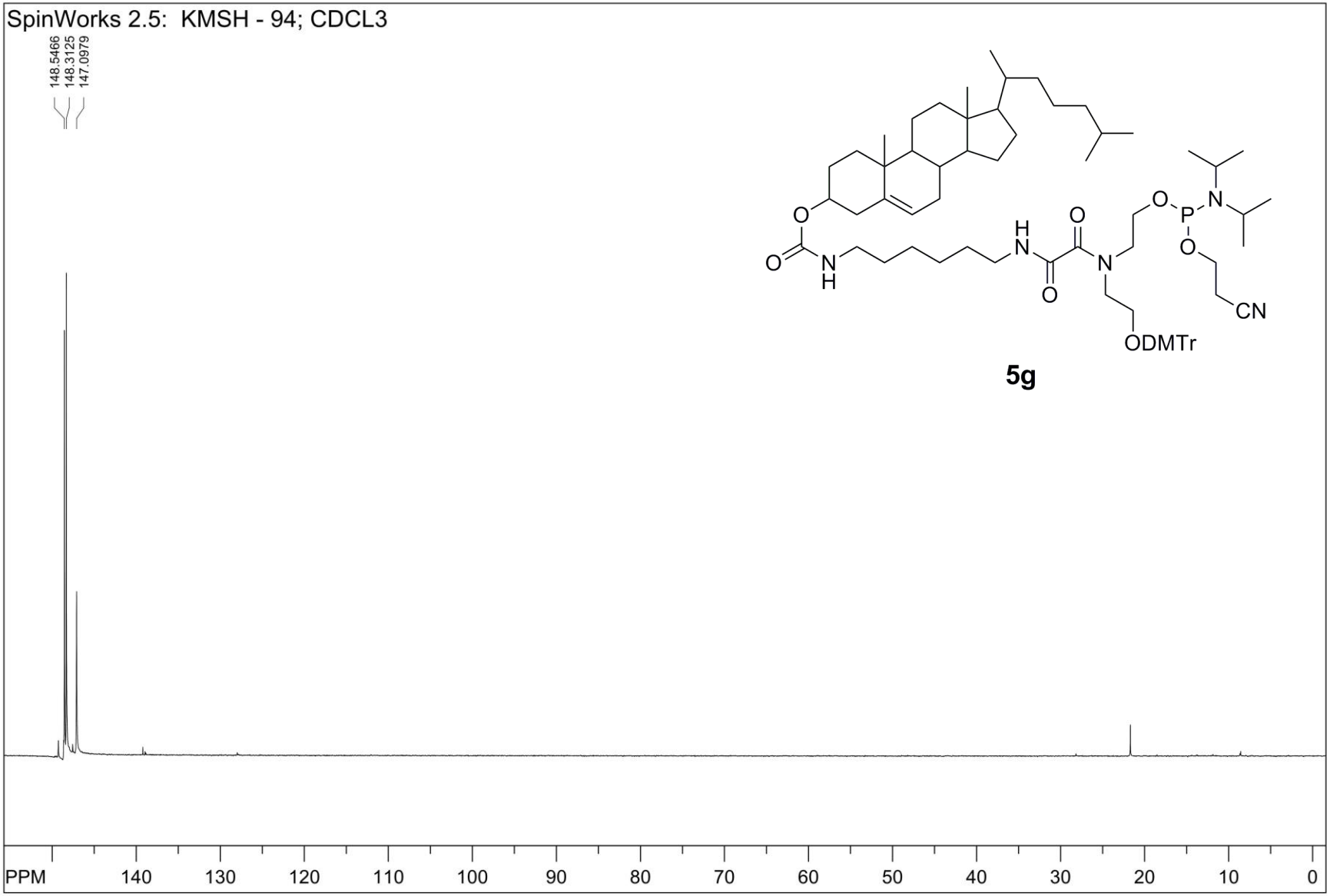

file: H:\Maxim\ЯМР\94-95-96\nibh-kmsh-94\nibh-kmsh-94\1\fid expt: <zg30>
transmitter freq.: 400.131884 MHz
time domain size: 65536 points
width: 6203.47 Hz = 15.503573 ppm = 0.094658 Hz/pt
number of scans: 16

freq. of 0 ppm: 400.130017 MHz
processed size: 32768 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 112.100 ppm/cm: 0.28016




file: H:\Maxim\RMP\94-95-96\nibh-kmsh-94\nibh-kmsh-94\136\fid expt: <zgpg>
transmitter freq.: 100.624820 MHz
time domain size: 65536 points
width: 28409.09 Hz = 282.326875 ppm = 0.433488 Hz/pt

freq. of 0 ppm: 100.612789 MHz
processed size: 131072 complex points
LB: 2.000 GB: 0.0000
Hz/cm: 678.770 ppm/cm: 6.74555




**5g**

file: H:\Maxim\ЯМР\94-95-96\P31\nibh-kmsh-94\nibh-kmsh-94\31\fid expt: <zgpg>
transmitter freq.: 121.502141 MHz
time domain size: 32768 points
width: 31645.57 Hz = 260.452774 ppm = 0.965746 Hz/pt
number of scans: 120

freq. of 0 ppm: 121.494824 MHz
processed size: 65536 complex points
LB: 0.000 GB: 0.0000
Hz/cm: 764.473 ppm/cm: 6.29185