

## Supporting Information

for

# Hydrolysis, polarity, and conformational impact of C-terminal partially fluorinated ethyl esters in peptide models

Vladimir Kubyshkin\* and Nediljko Budisa\*

Address: Biocatalysis group, Institute of Chemistry, Technical University of Berlin, Müller-Breslau-Strasse 10, Berlin 10623, Germany

Email: Vladimir Kubyshkin - kubyshkin@win.tu-berlin.de; Nediljko Budisa - nediljko.budisa@tu-berlin.de

\*Corresponding author

### Amide equilibrium constants (Table S1) and copies of the NMR and CD spectra

Table of contents

Table S1	S2
Copies of the NMR spectra for compounds <b>1–5</b>	S3–S25
<sup>1</sup> H NMR spectrum of compound <b>7</b>	S26
NMR spectra of <b>3–5</b> with the europium shift reagent	S27–S29
NMR spectra of the peptides	S30–S36
Circular dichroism spectra for the peptides	S37–S39
Hydrolysis of the peptides	S40–S44

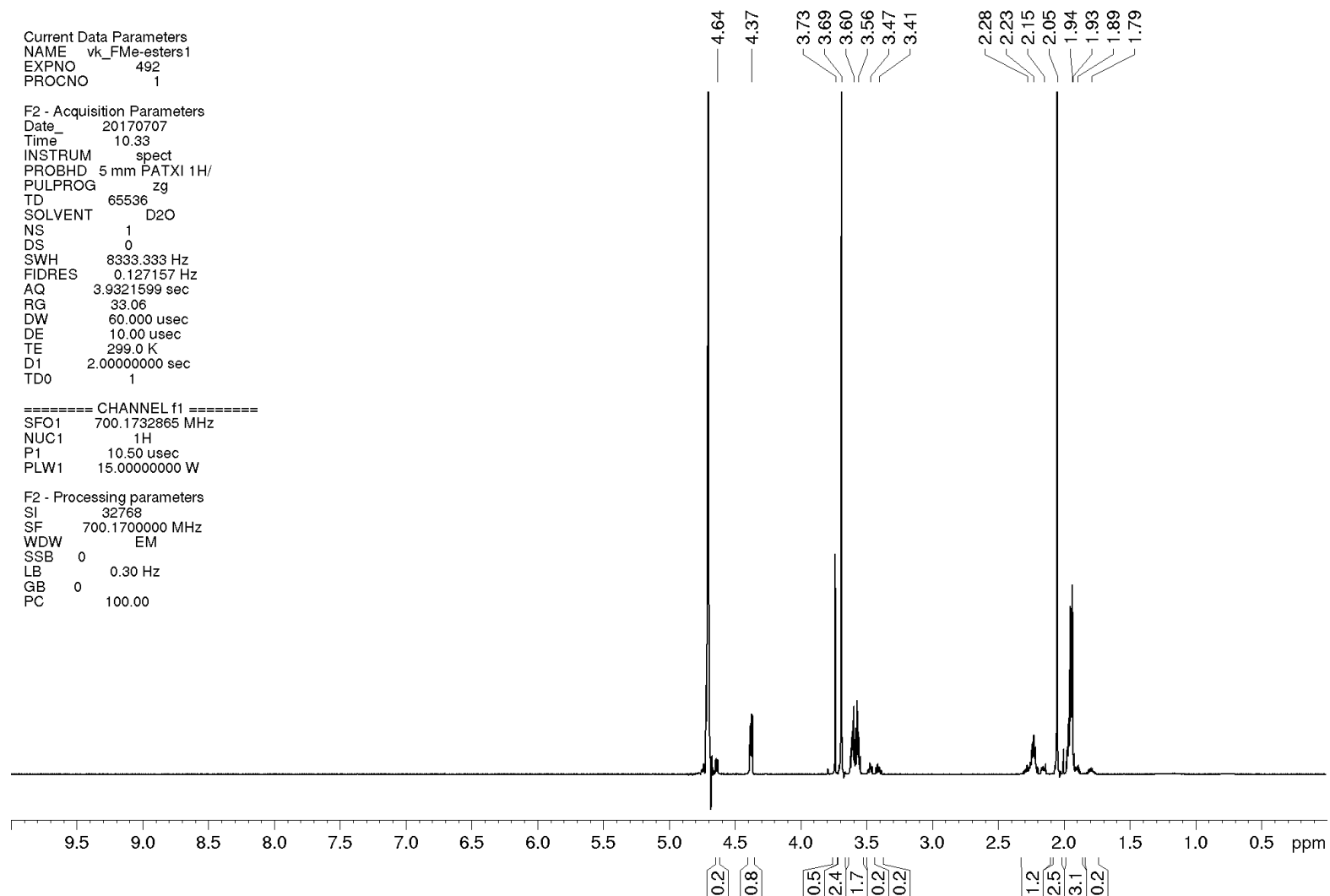
**Remark:** We use the notation ✓ (checkmark)-shape for description of the log*P* tendencies. On our opinion, this should be distinguished from more simple notation 'V-shape' due to the asymmetry of both the dip position and the edge highs.

**Table S1:** Amide equilibrium constants for compounds **1–5** as determined by <sup>1</sup>H and <sup>19</sup>F NMR at 298 K in different solvents.

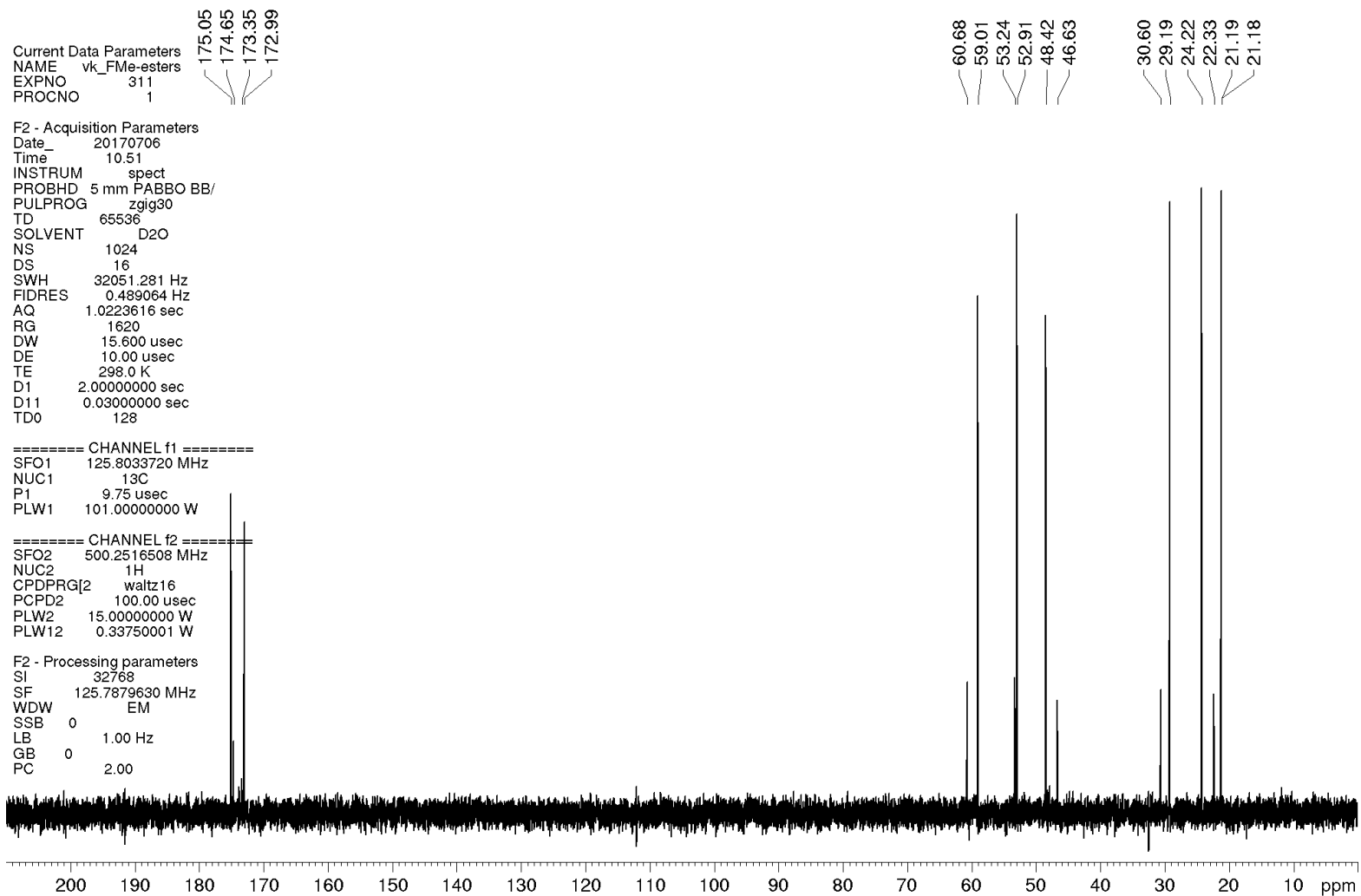
compound	<i>K</i> <sub>trans/cis</sub>					
	D <sub>2</sub> O ε 80.1	CD <sub>3</sub> CN ε 32.7	CD <sub>3</sub> OD ε 32.7	CD <sub>2</sub> Cl <sub>2</sub> ε 8.93	CDCl <sub>3</sub> ε 4.81	C <sub>6</sub> D <sub>6</sub> ε 2.27
<b>1</b>	4.95±0.05	4.08±0.04	3.78±0.03	4.18±0.03	3.84±0.04	5.10±0.10
<b>2</b>	4.60±0.08	3.58±0.04	3.49±0.04	3.52±0.03	3.20±0.04	4.67±0.03
<b>3</b>	4.74±0.04	4.12±0.17	3.61±0.03	4.77±0.10	4.44±0.20	6.80±0.04
<b>4</b>	4.95±0.05	4.85±0.07	4.12±0.03	6.57±0.14	6.29±0.22	9.06±0.26
<b>5</b>	5.48±0.14	5.65±0.05	4.49±0.05	7.07±0.11	6.65±0.18	10.04±0.15

# Copies of the NMR spectra for compounds 1–5

<sup>1</sup>H NMR spectrum of 1 in deuterium oxide at 700 MHz



$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **1** in deuterium oxide at 126 MHz



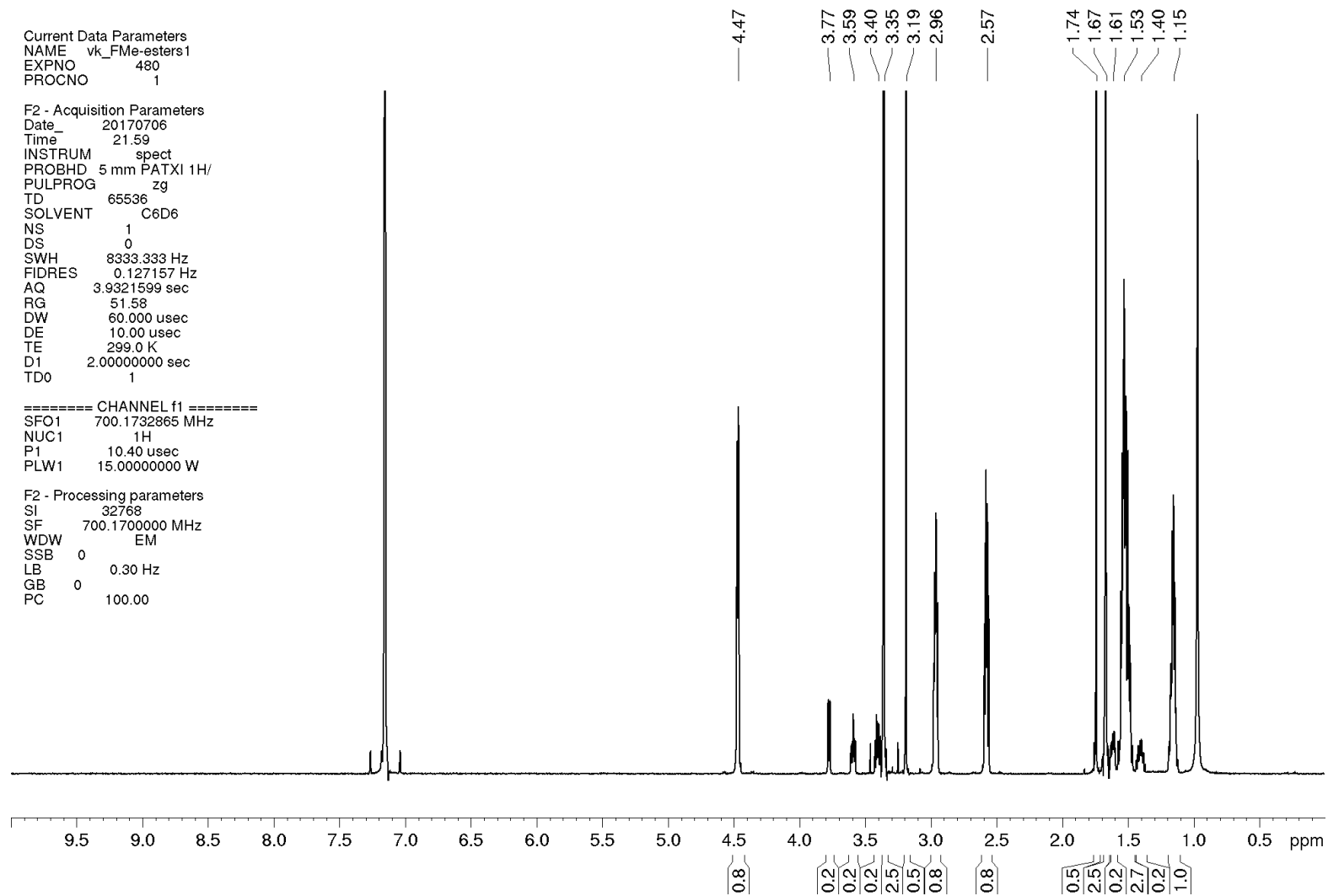
<sup>1</sup>H NMR spectrum of **1** in benzene-d<sub>6</sub> at 700 MHz

Current Data Parameters  
NAME vk\_FMe-esters1  
EXPNO 480  
PROCNO 1

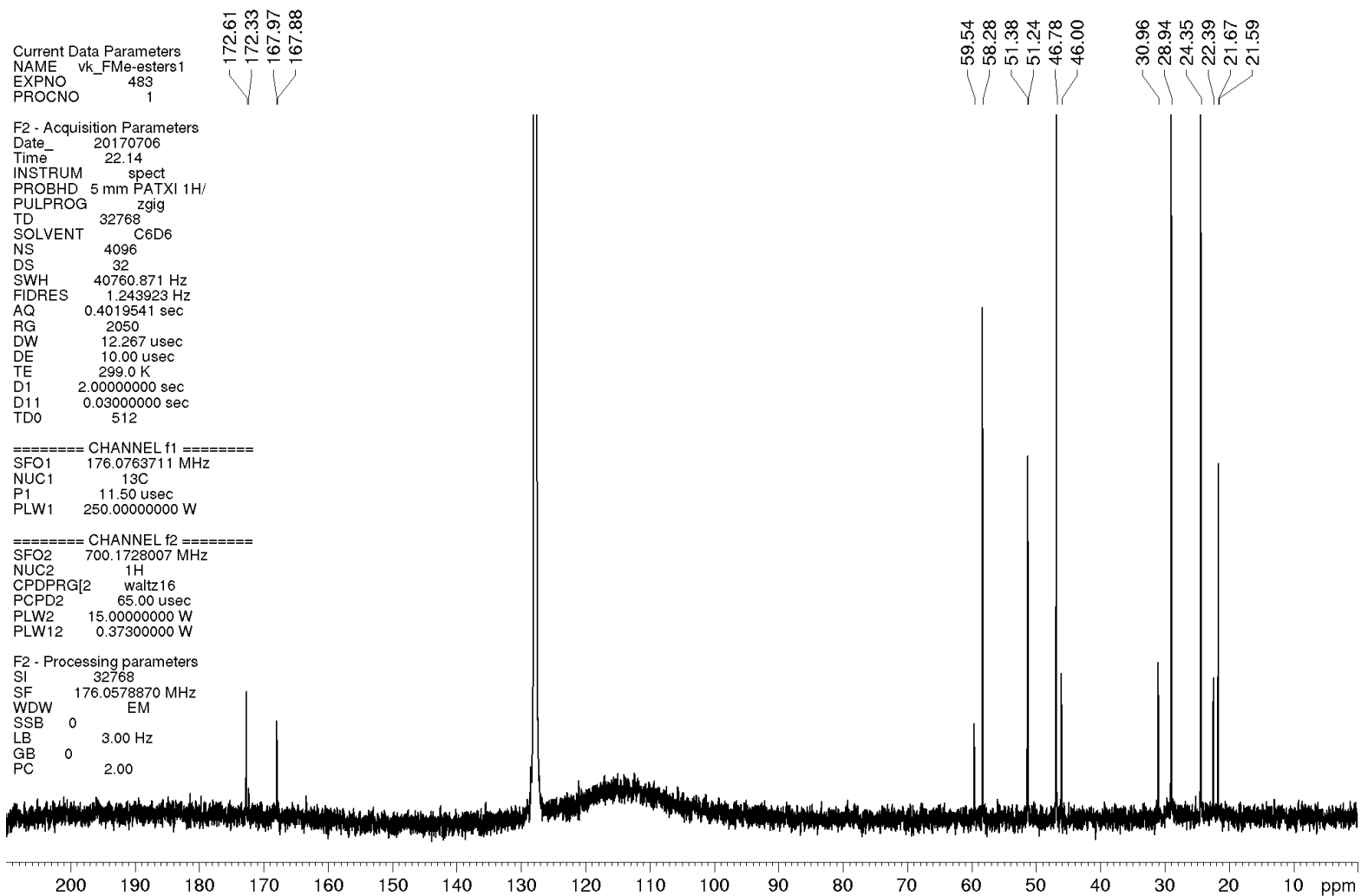
F2 - Acquisition Parameters  
Date\_ 20170706  
Time 21.59  
INSTRUM spect  
PROBHD 5 mm PATXI 1H/  
PULPROG zg  
TD 65536  
SOLVENT C6D6  
NS 1  
DS 0  
SWH 8333.333 Hz  
FIDRES 0.127157 Hz  
AQ 3.9321599 sec  
RG 51.58  
DW 60.000 usec  
DE 10.00 usec  
TE 299.0 K  
D1 2.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 700.1732865 MHz  
NUC1 1H  
P1 10.40 usec  
PLW1 15.00000000 W

F2 - Processing parameters  
SI 32768  
SF 700.1700000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 100.00



$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **1** in benzene- $\text{d}_6$  at 176 MHz



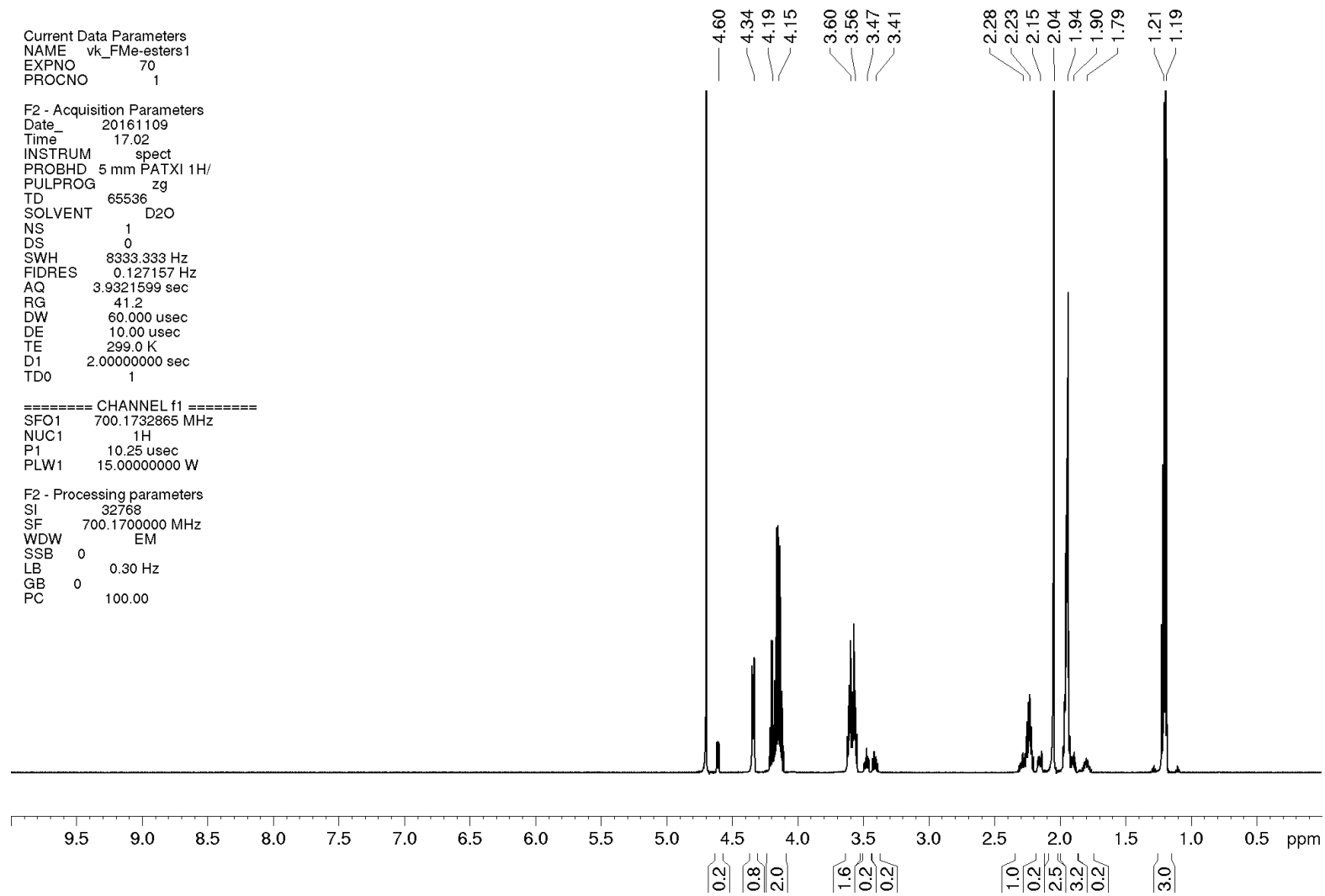
<sup>1</sup>H NMR spectrum of **2** in deuterium oxide at 700 MHz

Current Data Parameters  
 NAME vk\_FMe-esters1  
 EXPNO 70  
 PROCNO 1

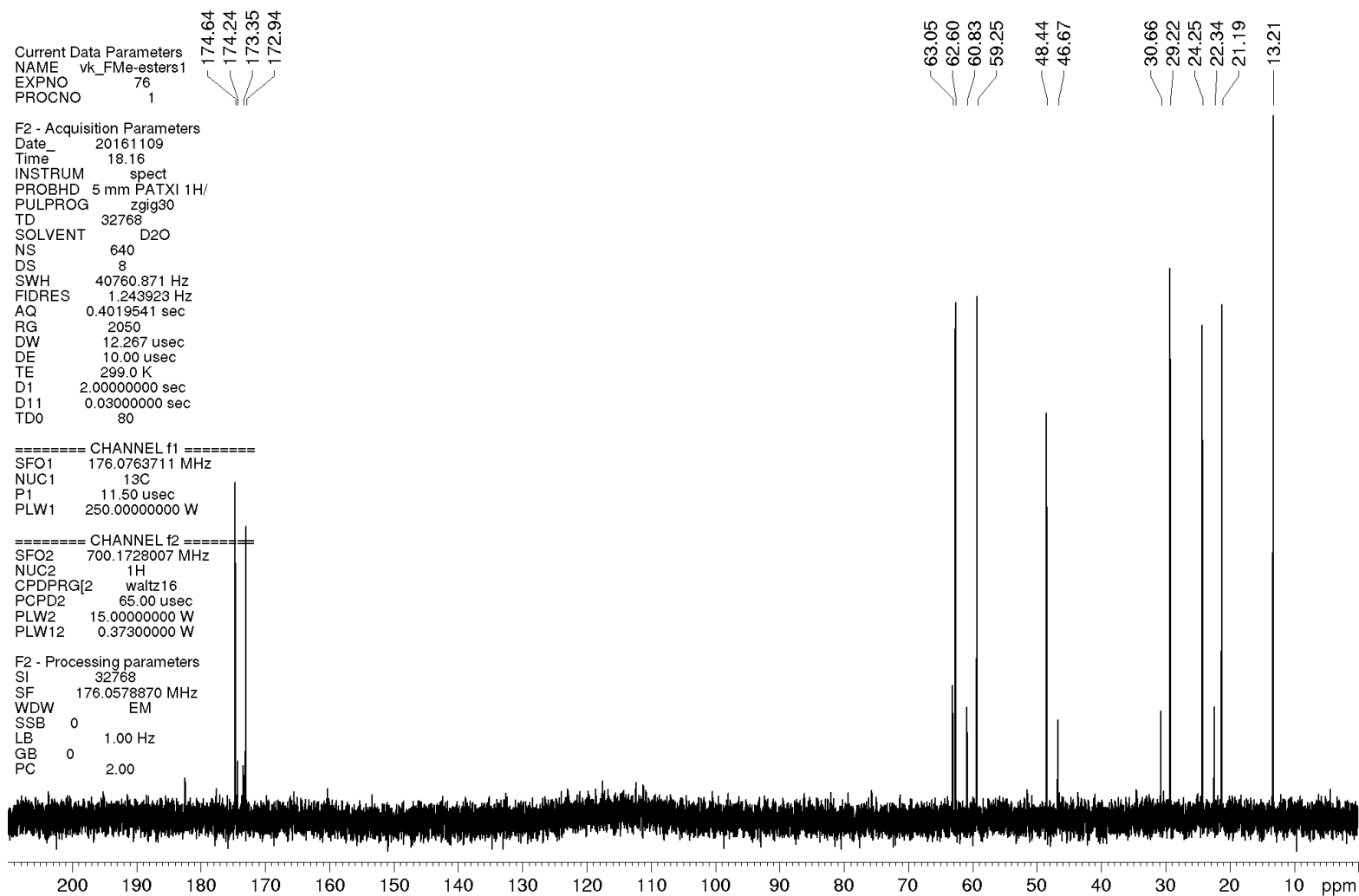
F2 - Acquisition Parameters  
 Date\_ 20161109  
 Time 17.02  
 INSTRUM spect  
 PROBHD 5 mm PATXI 1H/  
 PULPROG zg  
 TD 65536  
 SOLVENT D2O  
 NS 1  
 DS 0  
 SWH 8333.333 Hz  
 FIDRES 0.127157 Hz  
 AQ 3.9321599 sec  
 RG 41.2  
 DW 60.000 usec  
 DE 10.00 usec  
 TE 299.0 K  
 D1 2.0000000 sec  
 TD0 1

===== CHANNEL f1 =====  
 SFO1 700.1732865 MHz  
 NUC1 1H  
 P1 10.25 usec  
 PLW1 15.0000000 W

F2 - Processing parameters  
 SI 32768  
 SF 700.1700000 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 100.00



$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **2** in deuterium oxide at 176 MHz





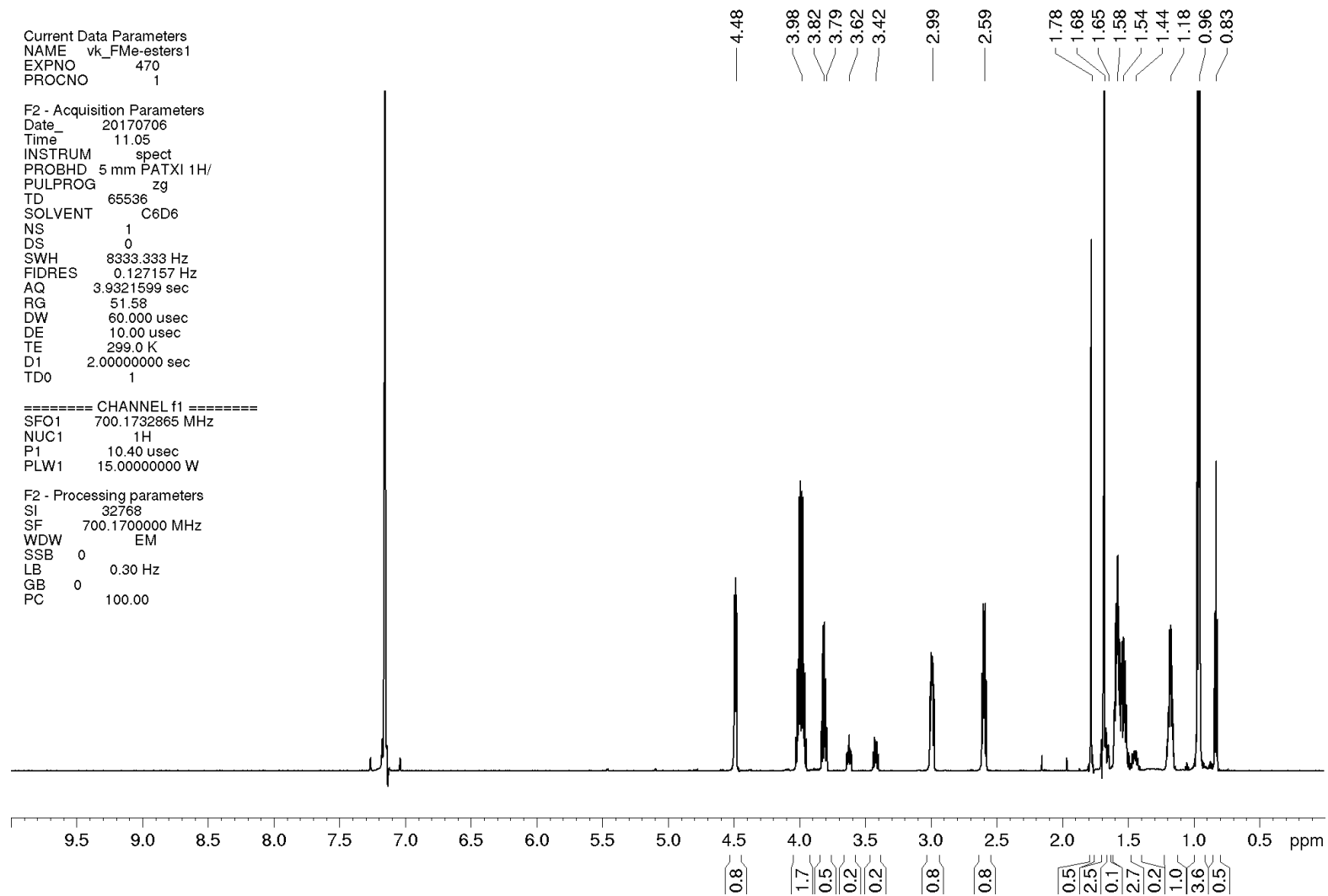
<sup>1</sup>H NMR spectrum of **2** in benzene-d<sub>6</sub> at 700 MHz

Current Data Parameters  
 NAME vk\_FMe-esters1  
 EXPNO 470  
 PROCNO 1

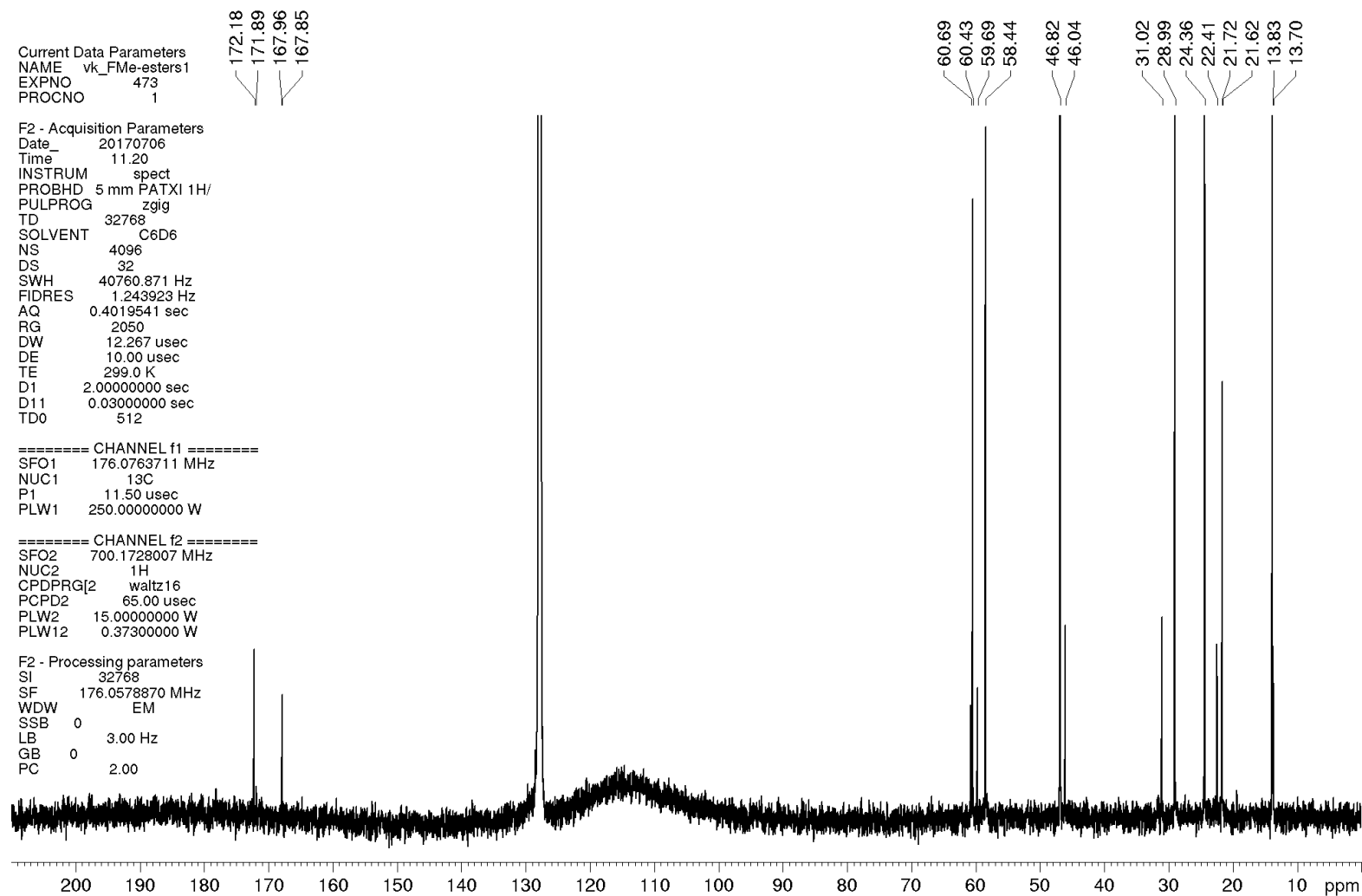
F2 - Acquisition Parameters  
 Date\_ 20170706  
 Time 11.05  
 INSTRUM spect  
 PROBHD 5 mm PATXI 1H/  
 PULPROG zg  
 TD 65536  
 SOLVENT C6D6  
 NS 1  
 DS 0  
 SWH 8333.333 Hz  
 FIDRES 0.127157 Hz  
 AQ 3.9321599 sec  
 RG 51.58  
 DW 60.000 usec  
 DE 10.00 usec  
 TE 299.0 K  
 D1 2.00000000 sec  
 TD0 1

===== CHANNEL f1 =====  
 SFO1 700.1732865 MHz  
 NUC1 1H  
 P1 10.40 usec  
 PLW1 15.00000000 W

F2 - Processing parameters  
 SI 32768  
 SF 700.1700000 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 100.00



$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **2** in benzene- $d_6$  at 176 MHz



$^1\text{H}$  and  $^1\text{H}\{^{19}\text{F}\}$  NMR spectra of **3** in deuterium oxide at 500 MHz

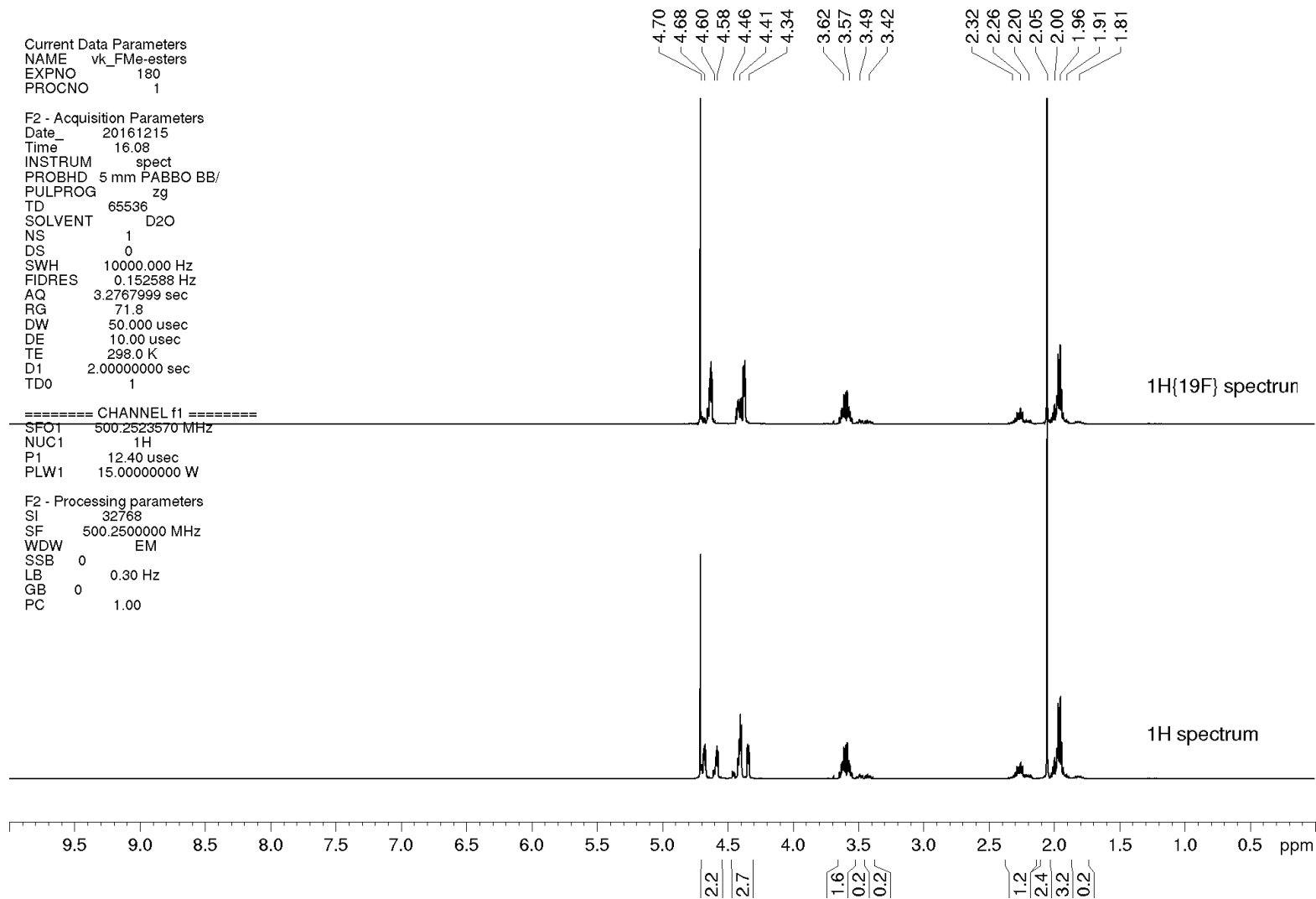
Current Data Parameters  
 NAME vk\_FMe-esters  
 EXPNO 180  
 PROCNO 1

F2 - Acquisition Parameters  
 Date\_ 20161215  
 Time 16.08  
 INSTRUM spect  
 PROBHD 5 mm PABBO BB/  
 PULPROG zg  
 TD 65536  
 SOLVENT D2O  
 NS 1  
 DS 0  
 SWH 10000.000 Hz  
 FIDRES 0.152588 Hz  
 AQ 3.2767999 sec  
 RG 71.8  
 DW 50.000 usec  
 DE 10.00 usec  
 TE 298.0 K  
 D1 2.00000000 sec  
 TD0 1

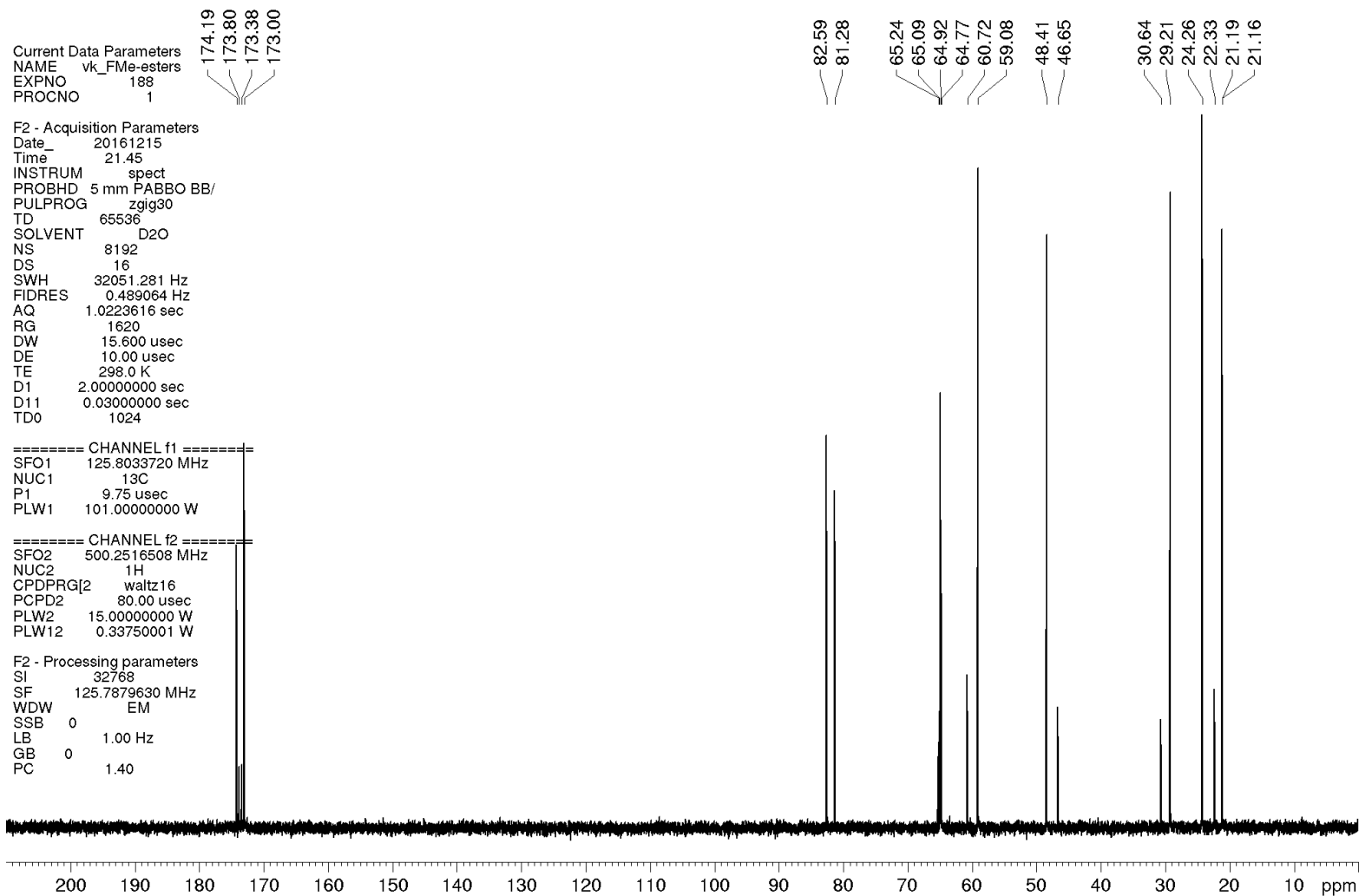
===== CHANNEL f1 =====

SFO1 500.2523570 MHz  
 NUC1  $^1\text{H}$   
 P1 12.40 usec  
 PLW1 15.00000000 W

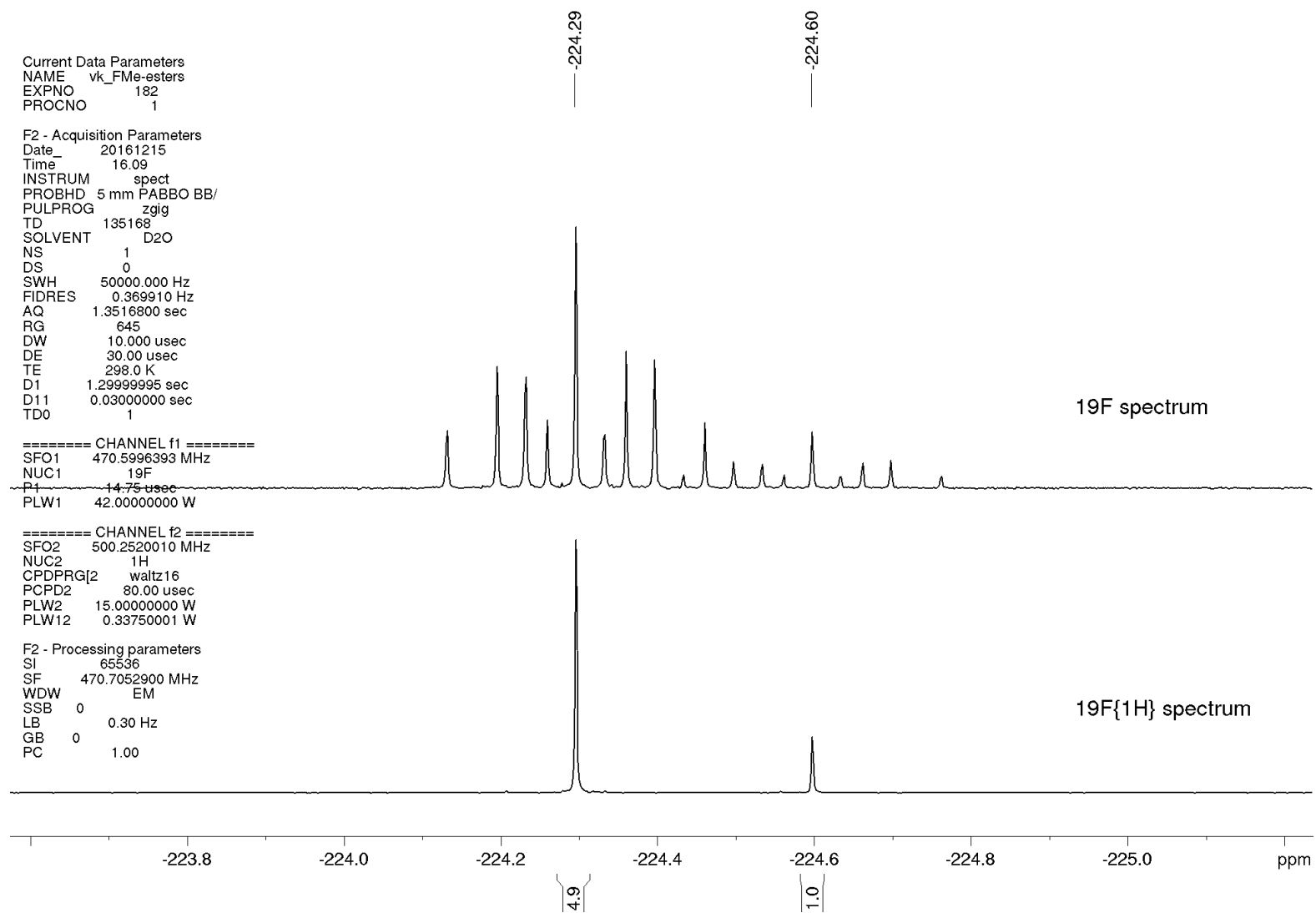
F2 - Processing parameters  
 SI 32768  
 SF 500.2500000 MHz  
 WDW EM  
 SSB 0  
 LB 0.30 Hz  
 GB 0  
 PC 1.00



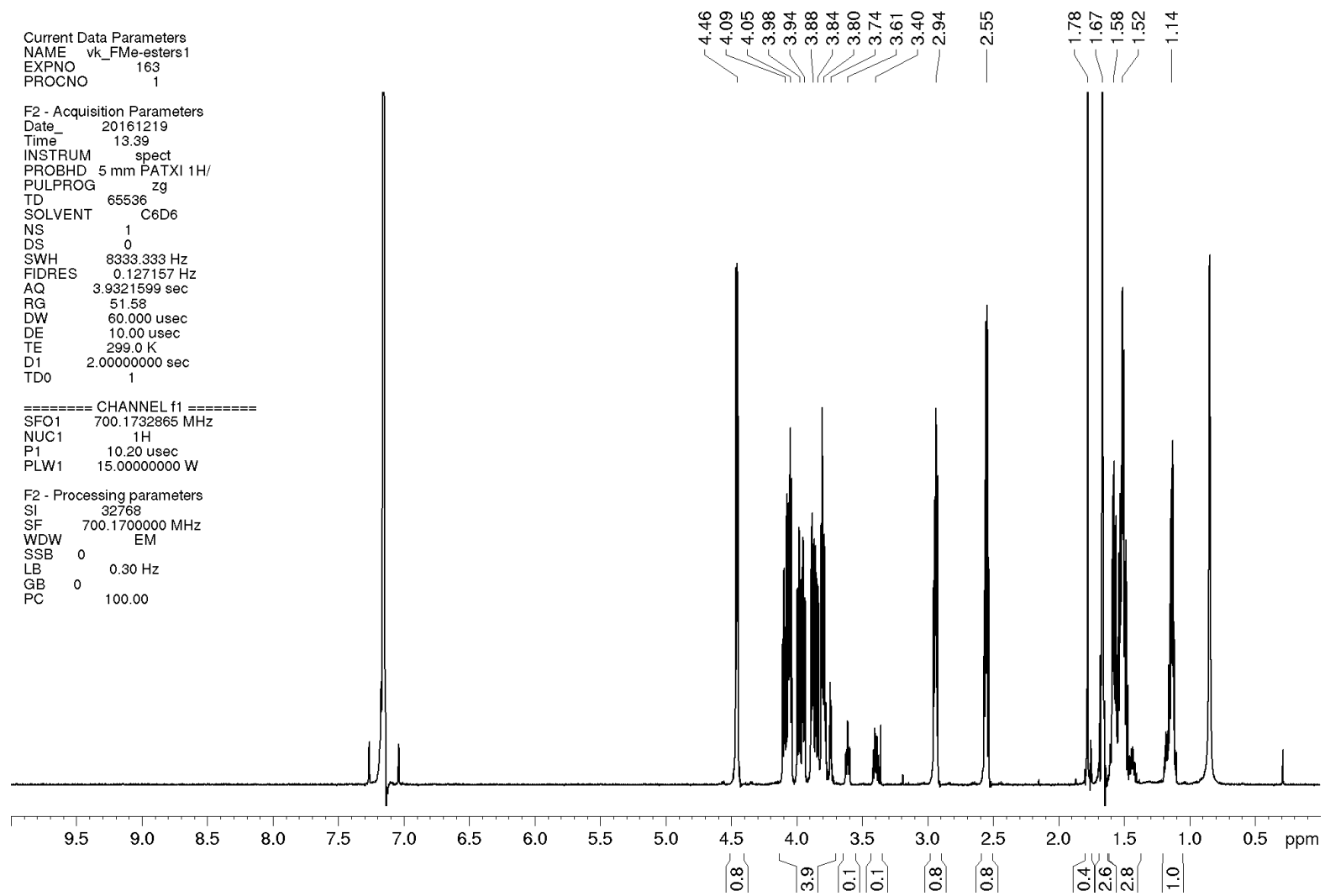
$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **3** in deuterium oxide at 126 MHz



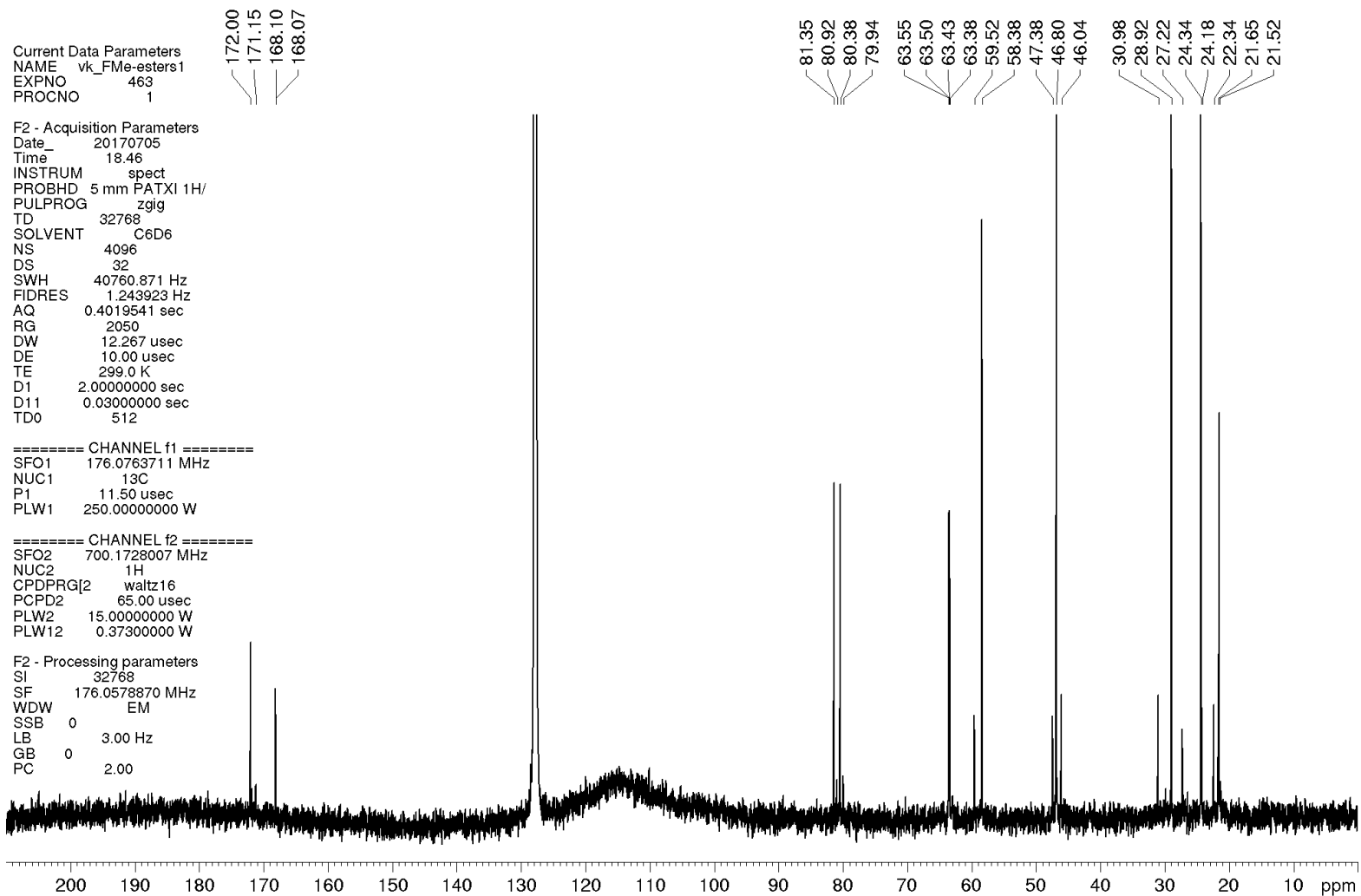
$^{19}\text{F}$  and  $^{19}\text{F}\{^1\text{H}\}$  NMR spectra of **3** in deuterium oxide at 471 MHz



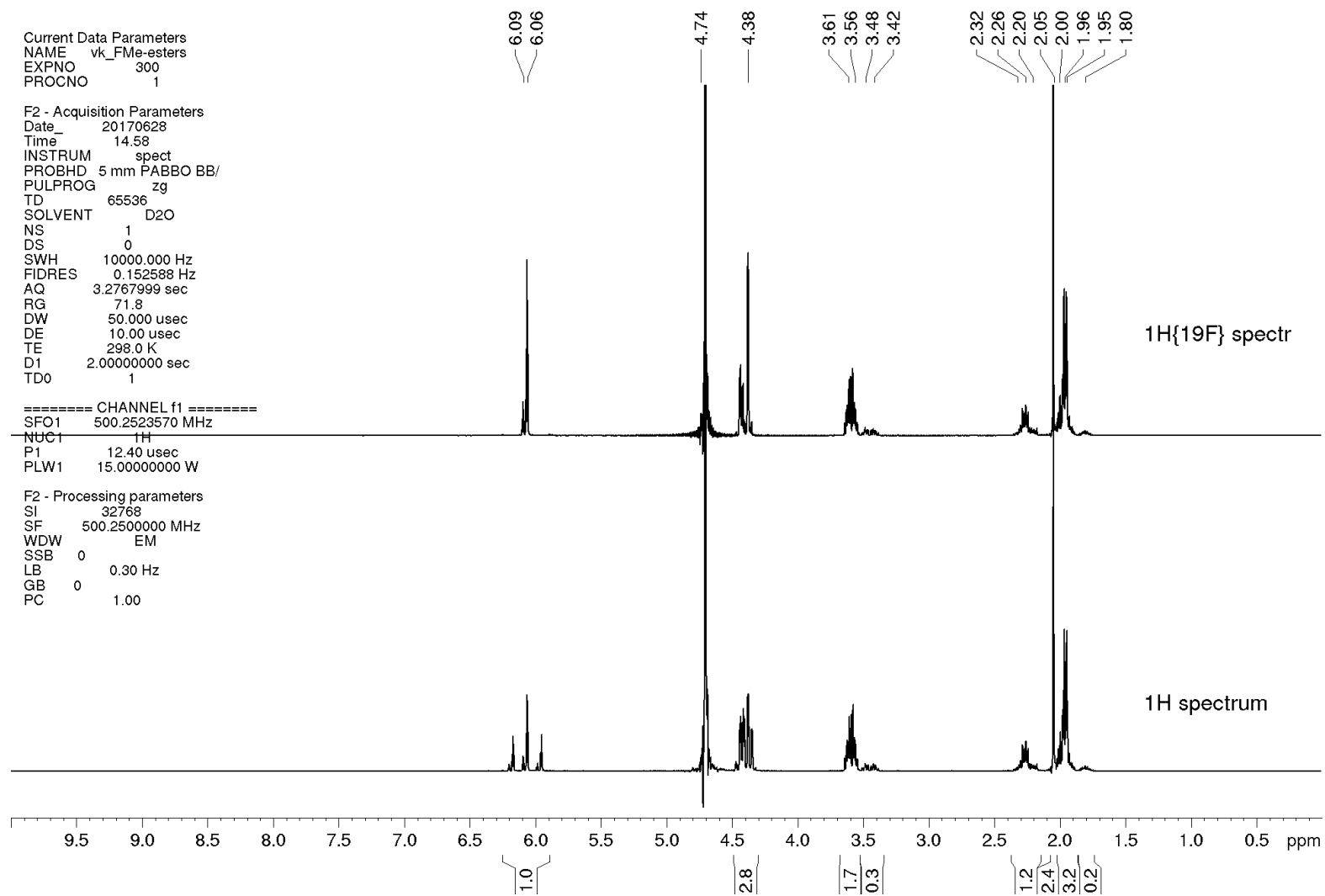
<sup>1</sup>H NMR spectrum of **3** in benzene-d<sub>6</sub> at 700 MHz



$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **3** in benzene- $\text{d}_6$  at 176 MHz

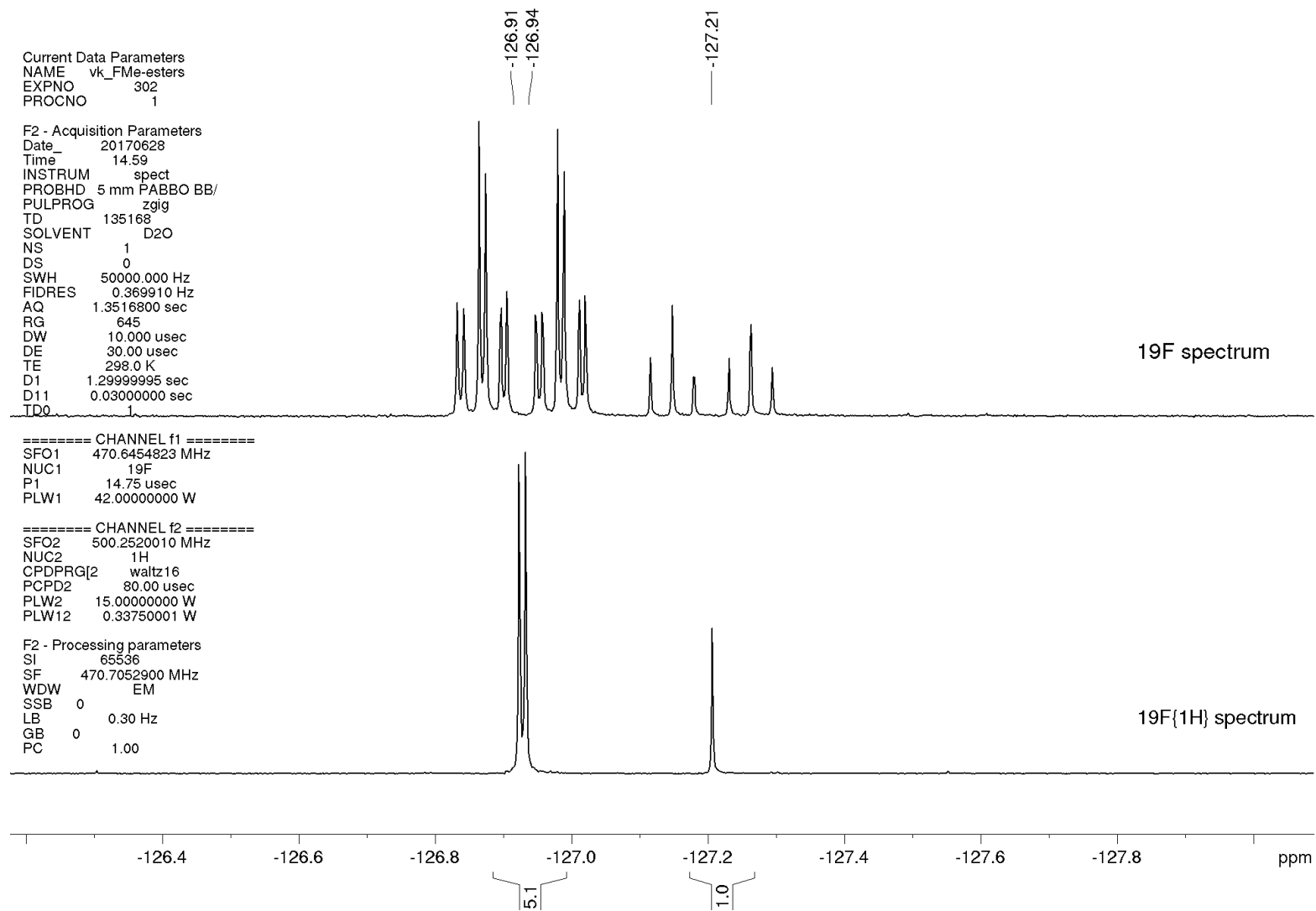


$^1\text{H}$  and  $^1\text{H}\{^{19}\text{F}\}$  NMR spectra of **4** in deuterium oxide at 500 MHz

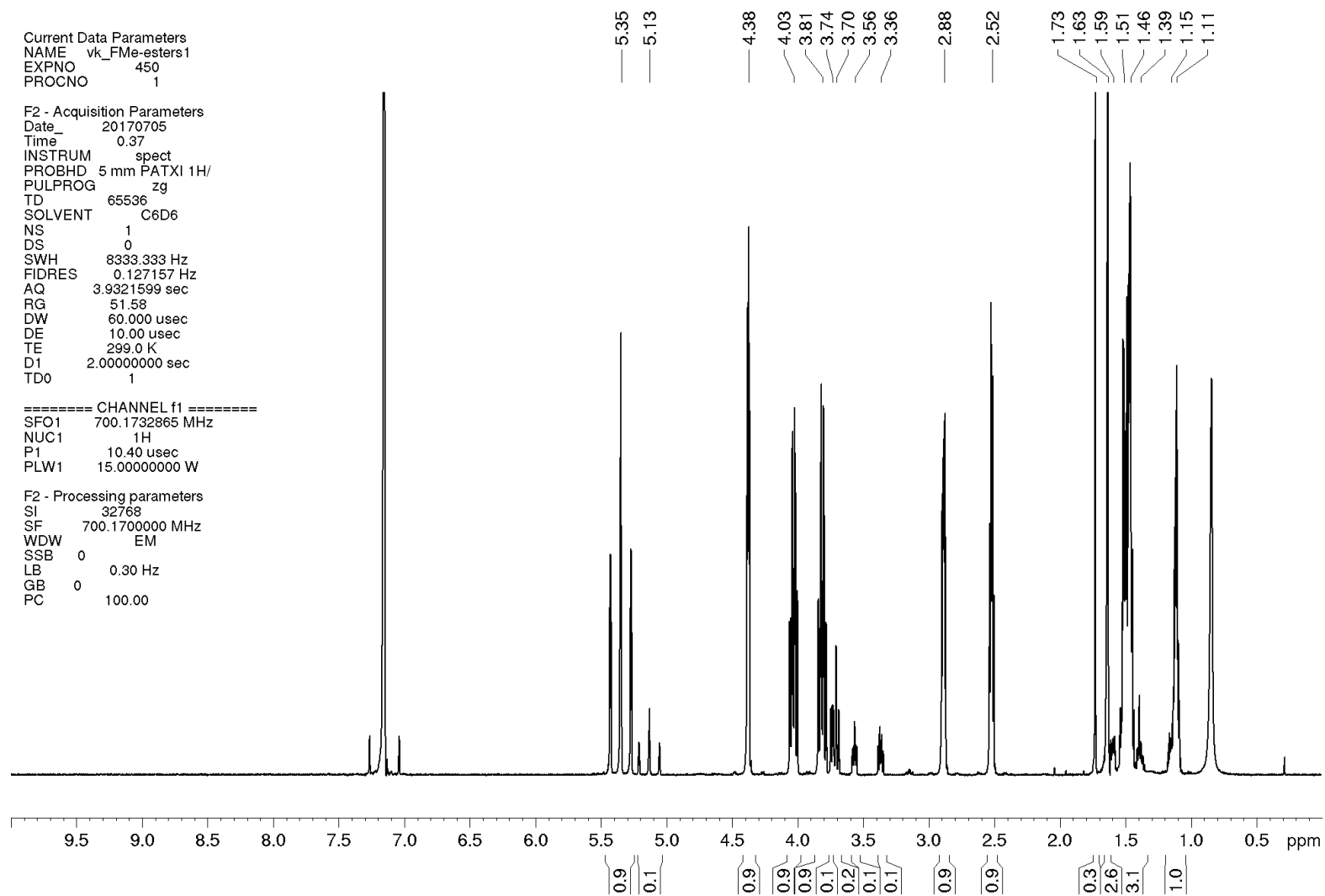




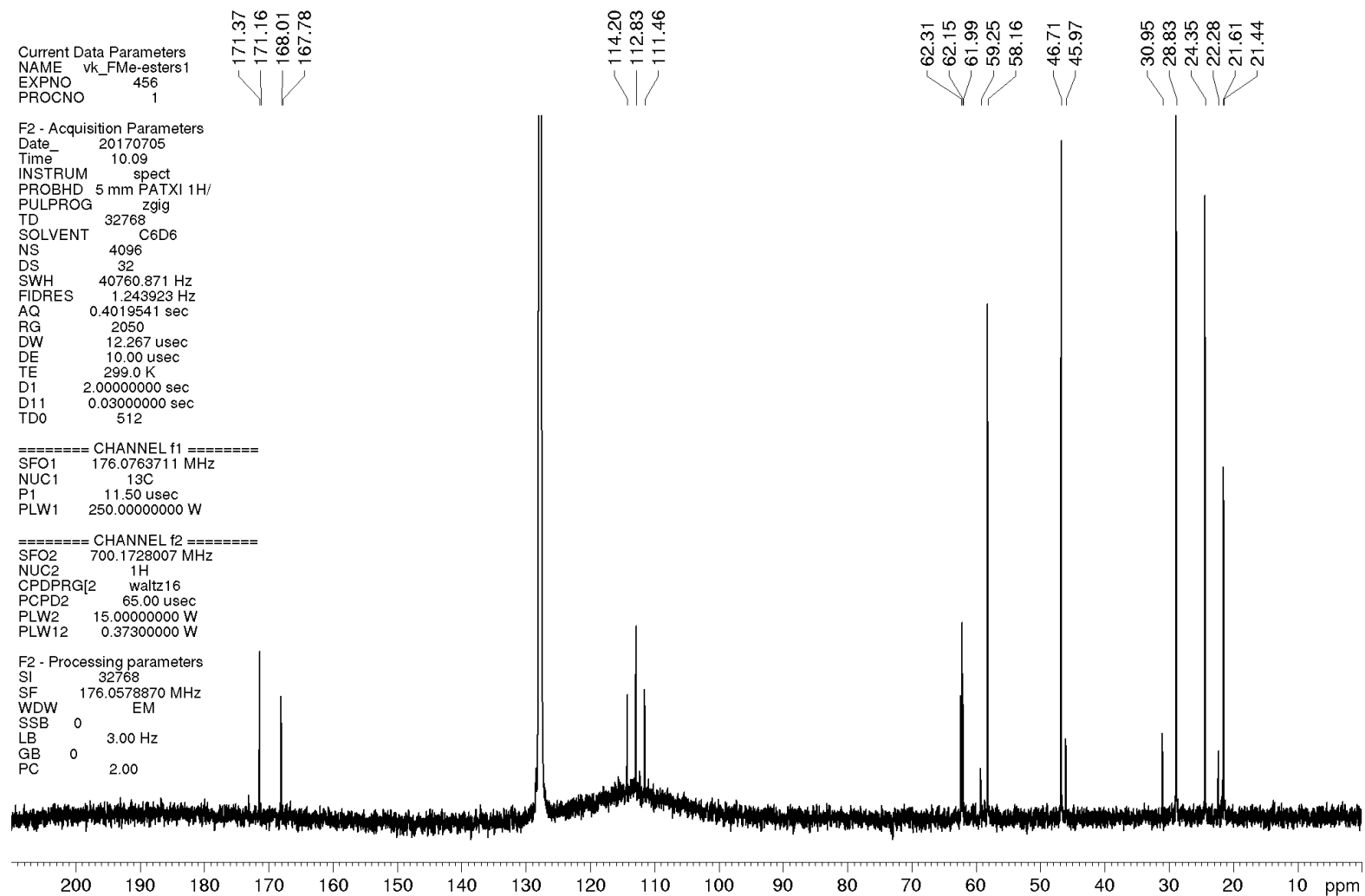
$^{19}\text{F}$  and  $^{19}\text{F}\{^1\text{H}\}$  NMR spectra of **4** in deuterium oxide at 471 MHz



<sup>1</sup>H NMR spectrum of **4** in benzene-d<sub>6</sub> at 700 MHz



$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **4** in benzene- $\text{d}_6$  at 176 MHz



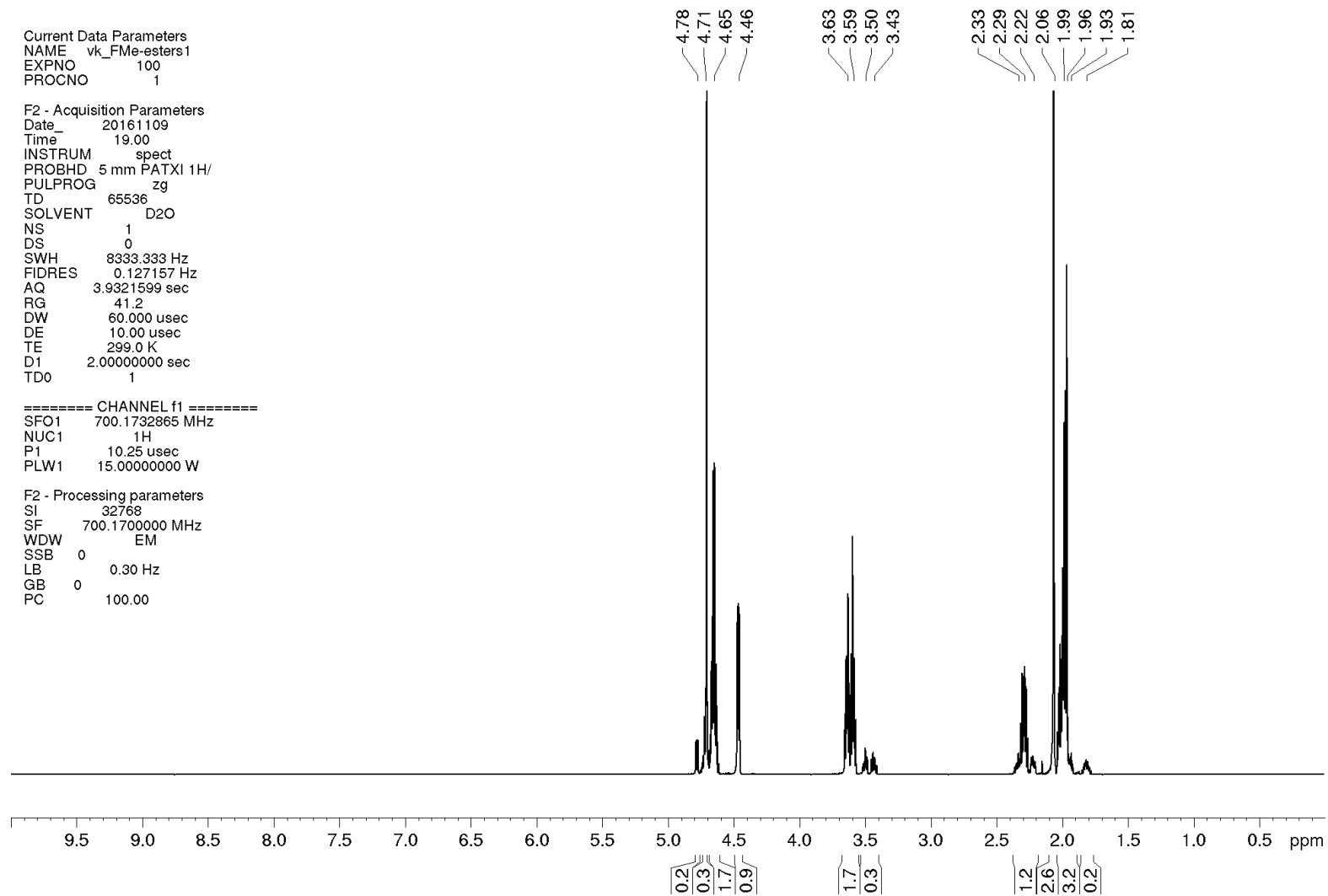
<sup>1</sup>H NMR spectrum of **5** in deuterium oxide at 700 MHz

Current Data Parameters  
NAME vk\_FMe-esters1  
EXPNO 100  
PROCNO 1

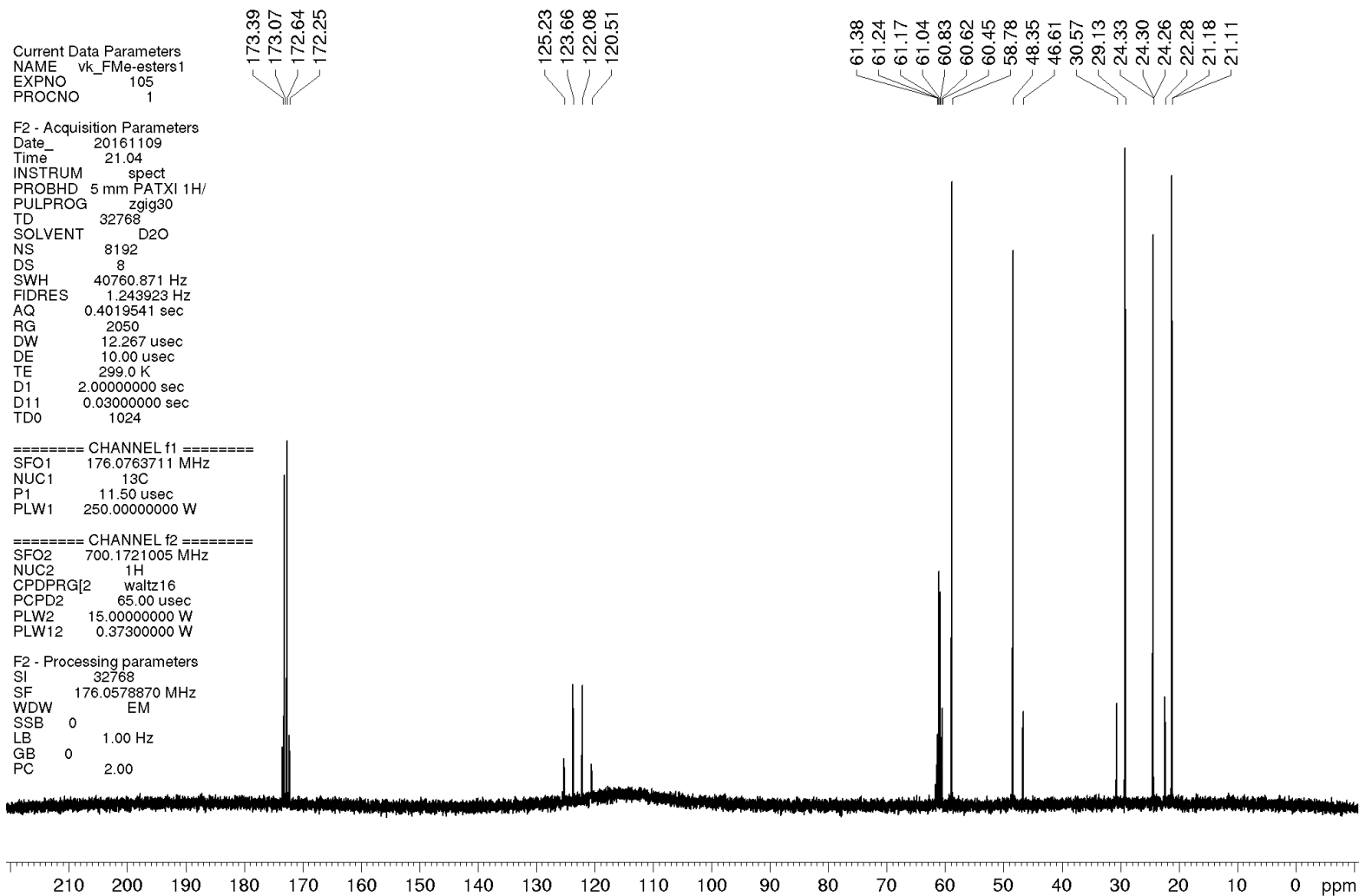
F2 - Acquisition Parameters  
Date\_ 20161109  
Time 19.00  
INSTRUM spect  
PROBHD 5 mm PATXI 1H/  
PULPROG zg  
TD 65536  
SOLVENT D2O  
NS 1  
DS 0  
SWH 8333.333 Hz  
FIDRES 0.127157 Hz  
AQ 3.9321599 sec  
RG 41.2  
DW 60.000 usec  
DE 10.00 usec  
TE 299.0 K  
D1 2.0000000 sec  
TD0 1

===== CHANNEL f1 =====  
SFO1 700.1732865 MHz  
NUC1 1H  
P1 10.25 usec  
PLW1 15.00000000 W

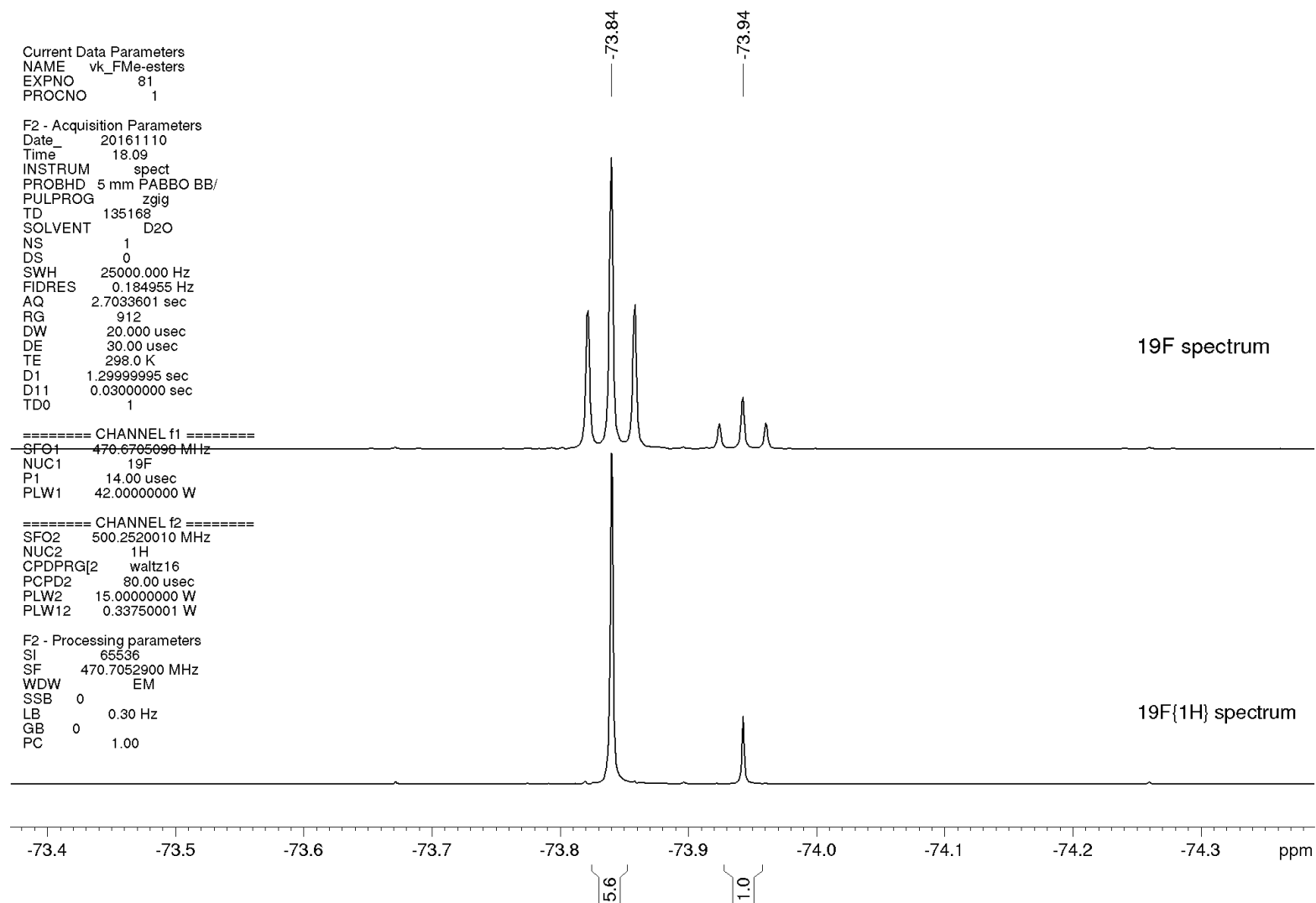
F2 - Processing parameters  
SI 32768  
SF 700.1700000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 100.00



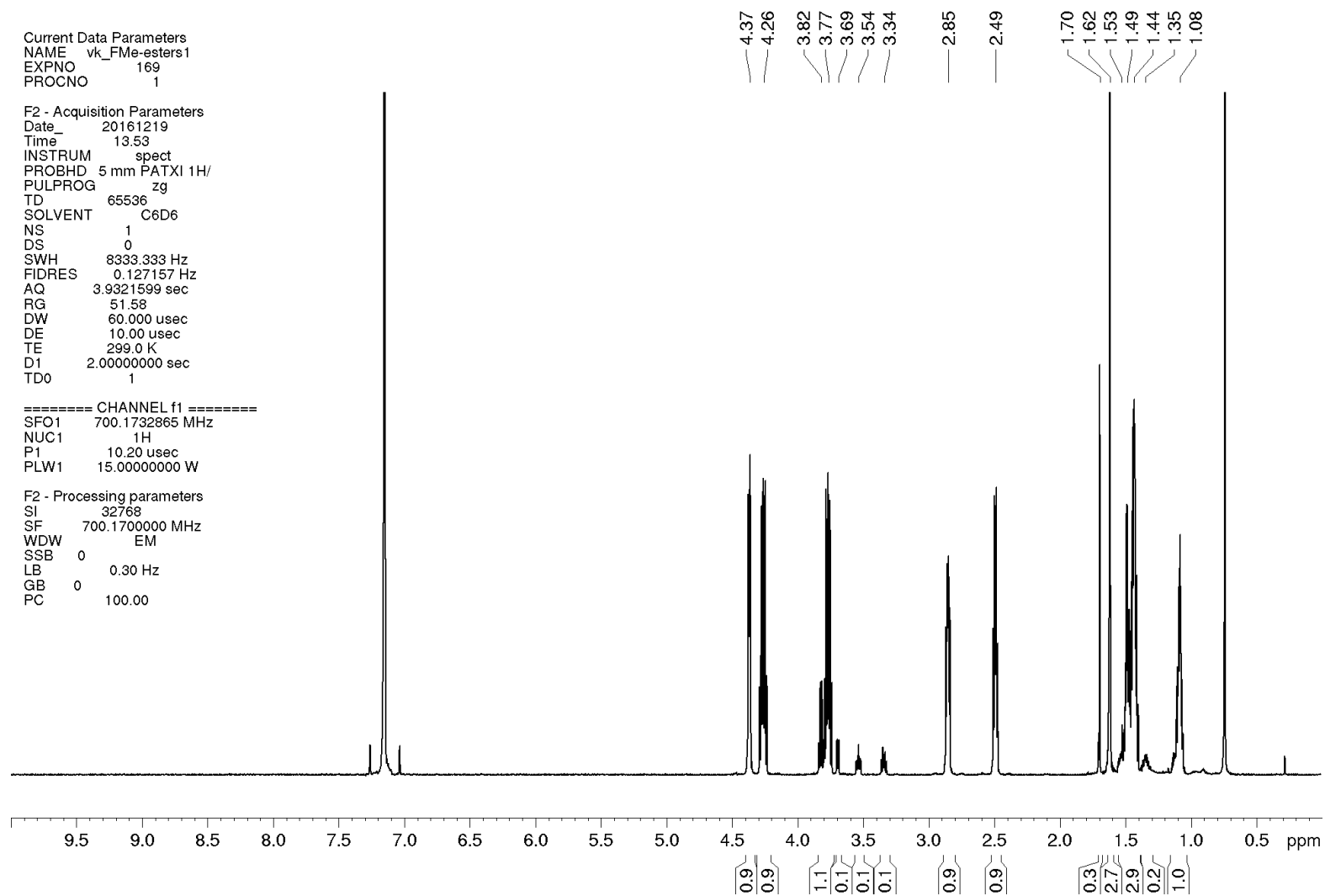
$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **5** in deuterium oxide at 176 MHz



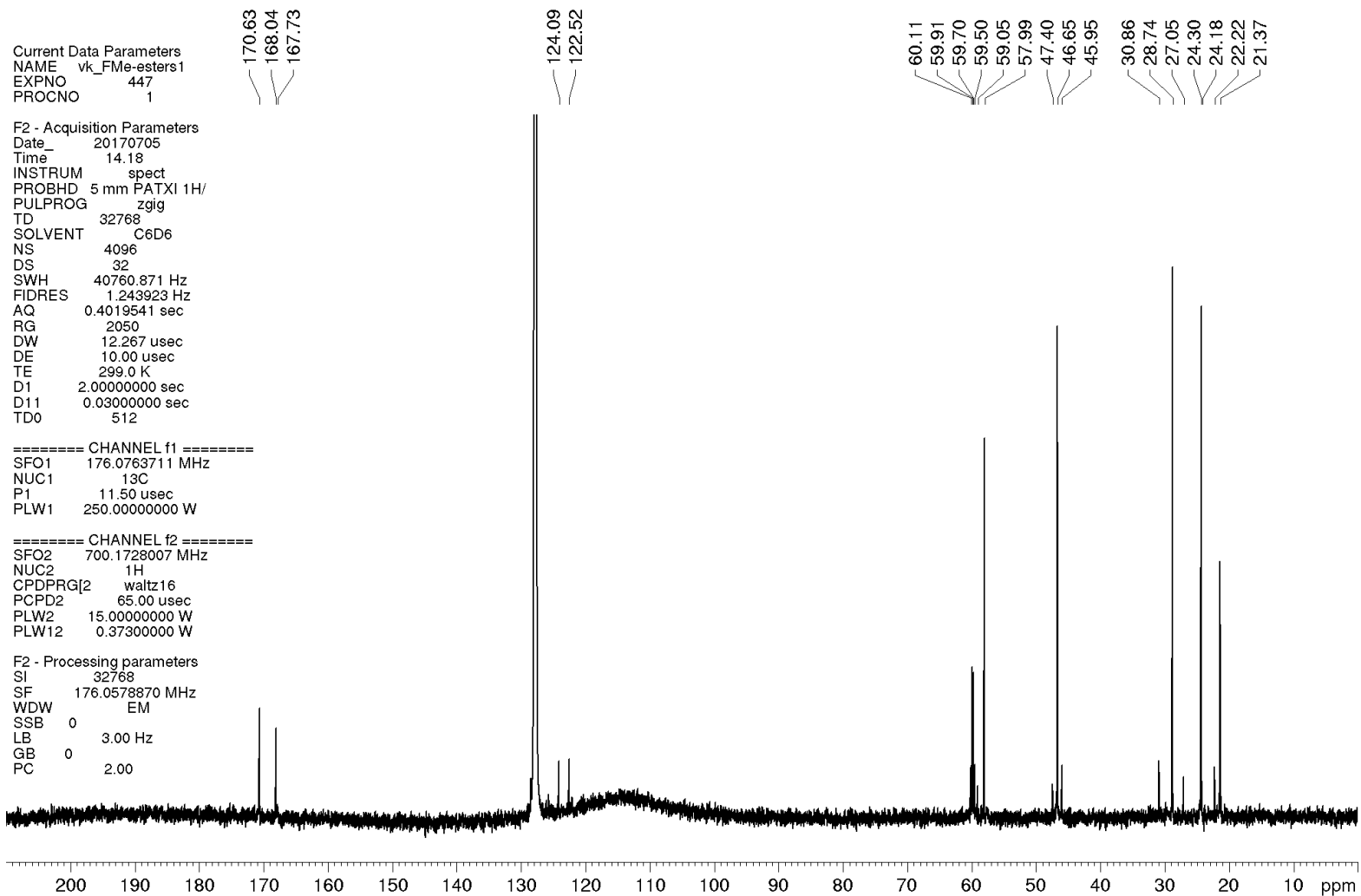
$^{19}\text{F}$  and  $^{19}\text{F}\{^1\text{H}\}$  NMR spectra of **5** in deuterium oxide at 471 MHz



$^1\text{H}$  NMR spectrum of **5** in benzene- $d_6$  at 700 MHz

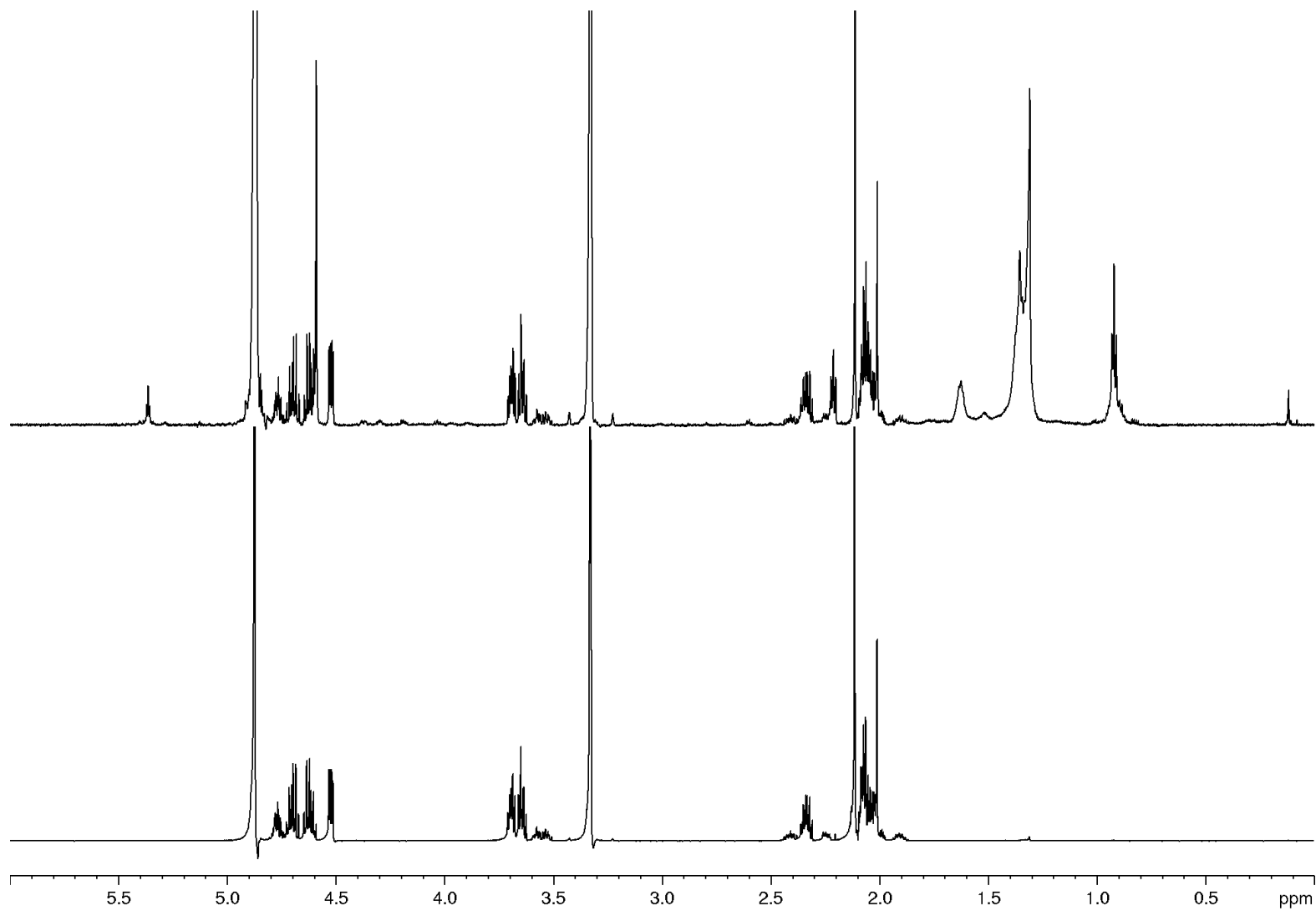


$^{13}\text{C}\{^1\text{H}\}$  NMR spectrum of **5** in benzene- $d_6$  at 176 MHz



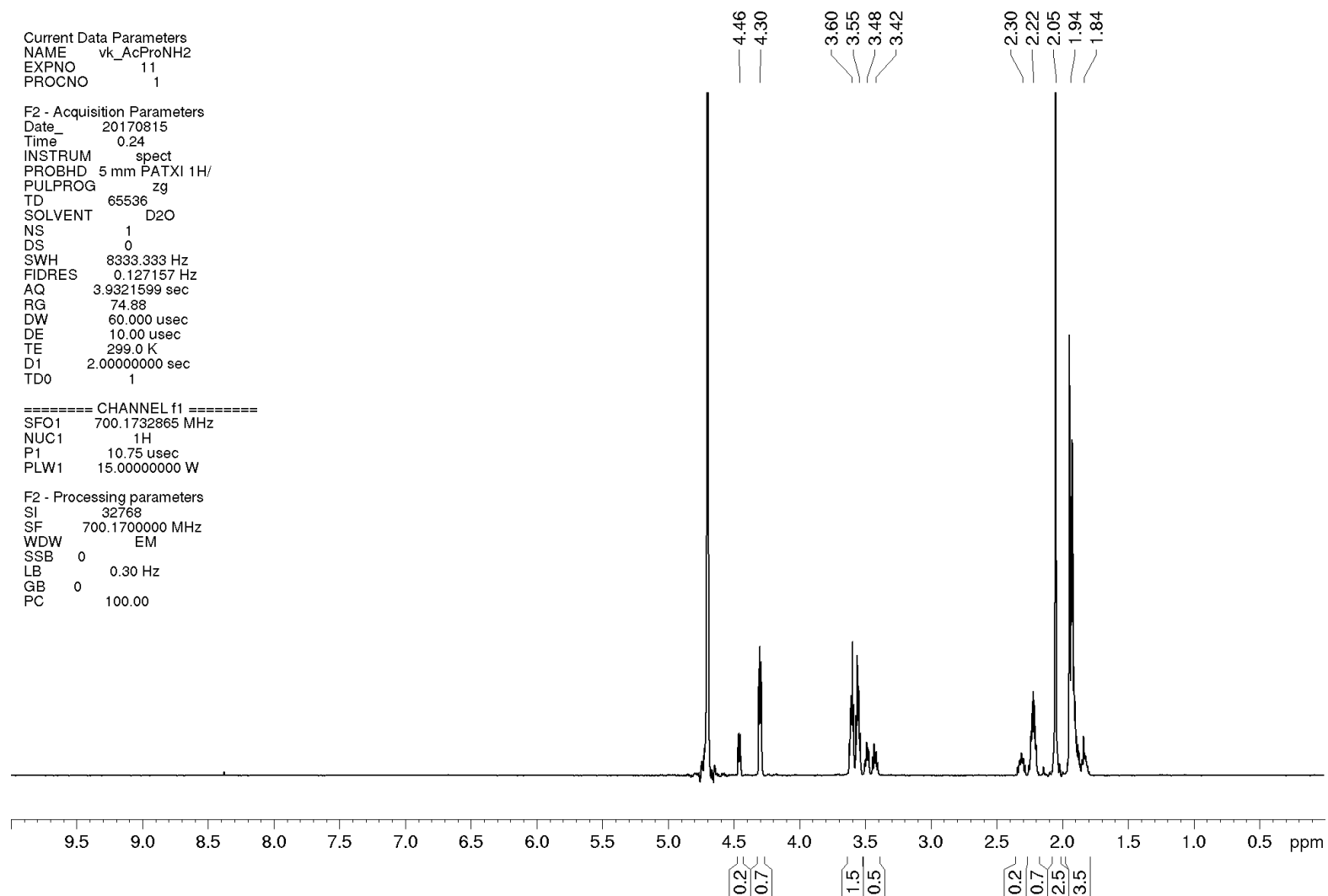


Spectra of **5** (in methanol- $d_4$ , 700 MHz) obtained after esterification via chloranhydride (bottom) and in acidic trifluoroethanol (top)



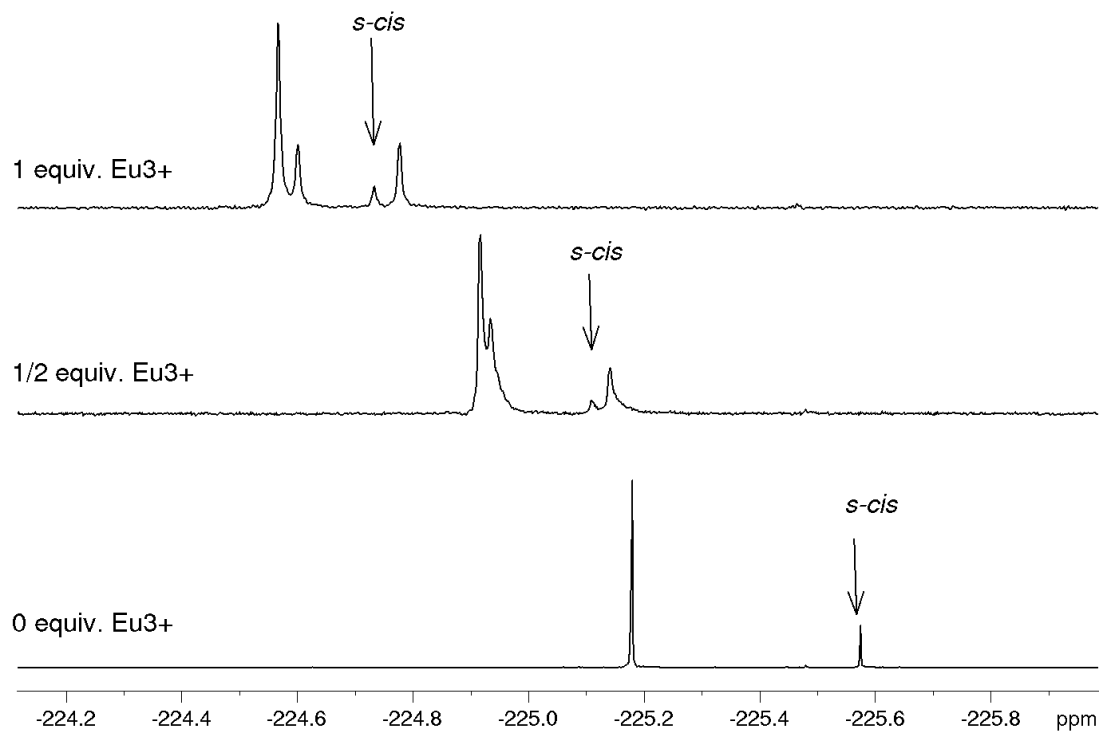
# <sup>1</sup>H NMR spectrum of compound 7

<sup>1</sup>H NMR spectrum of compound 7 in deuterium oxide at 700 MHz

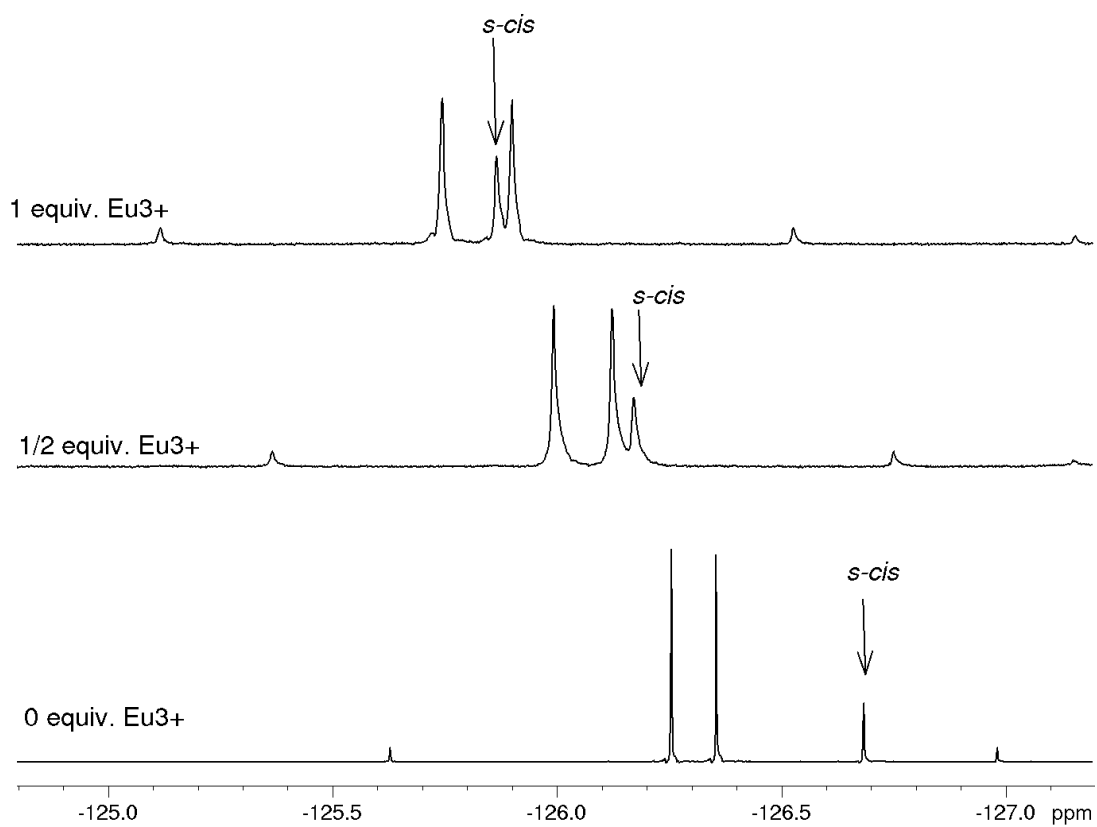


## NMR spectra of 3-5 with the europium shift reagent

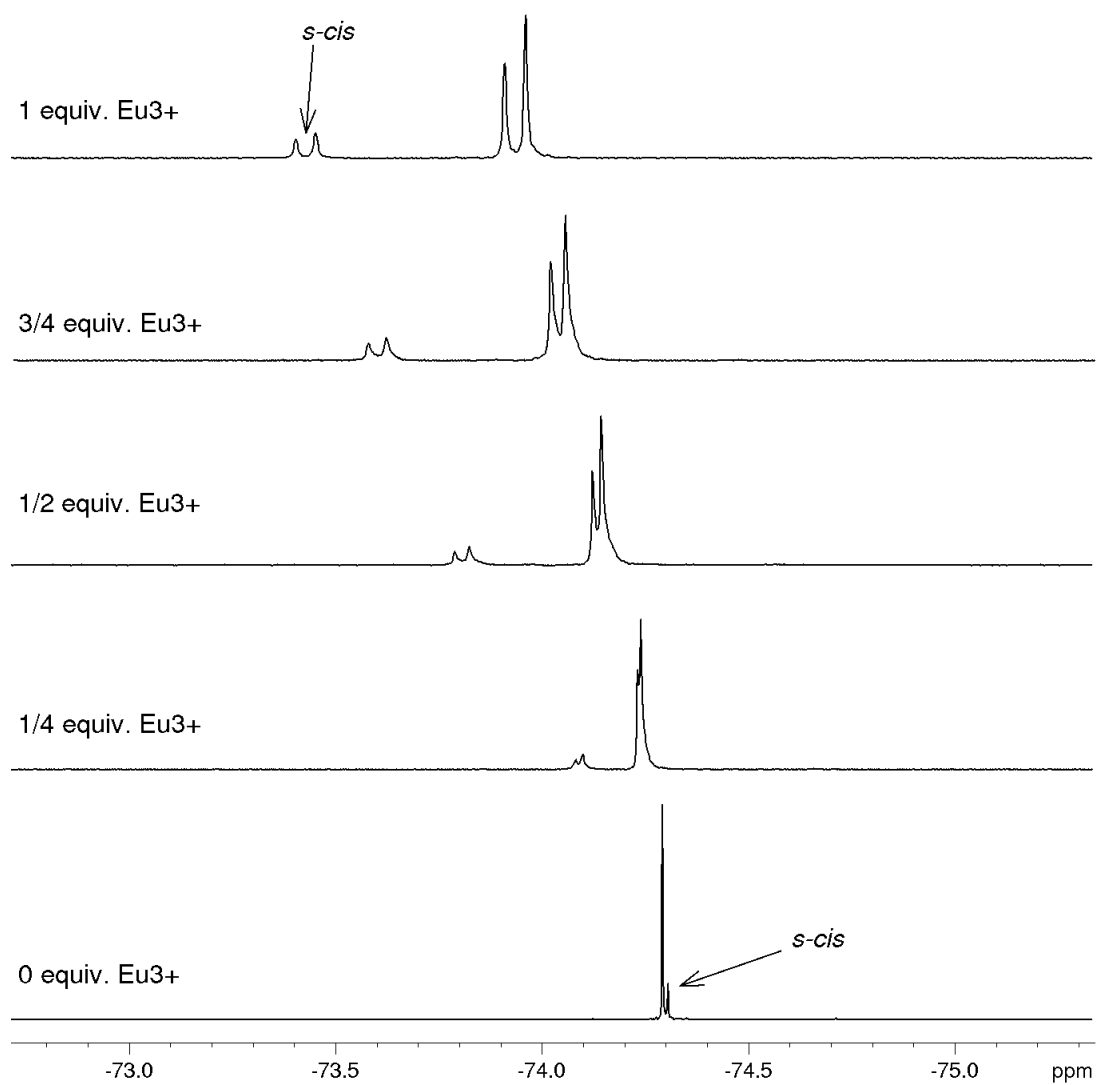
$^{19}\text{F}\{^1\text{H}\}$  NMR spectra (inverse-gated decoupling) of **3** in dichloromethane- $d_2$  upon addition of  $\text{Eu}^{3+}$  shifting reagent (two enantiomers)



$^{19}\text{F}\{^1\text{H}\}$  NMR spectra (inverse-gated decoupling) of **4** in dichloromethane- $\text{d}_2$  upon addition of  $\text{Eu}^{3+}$  shifting reagent (single enantiomer)

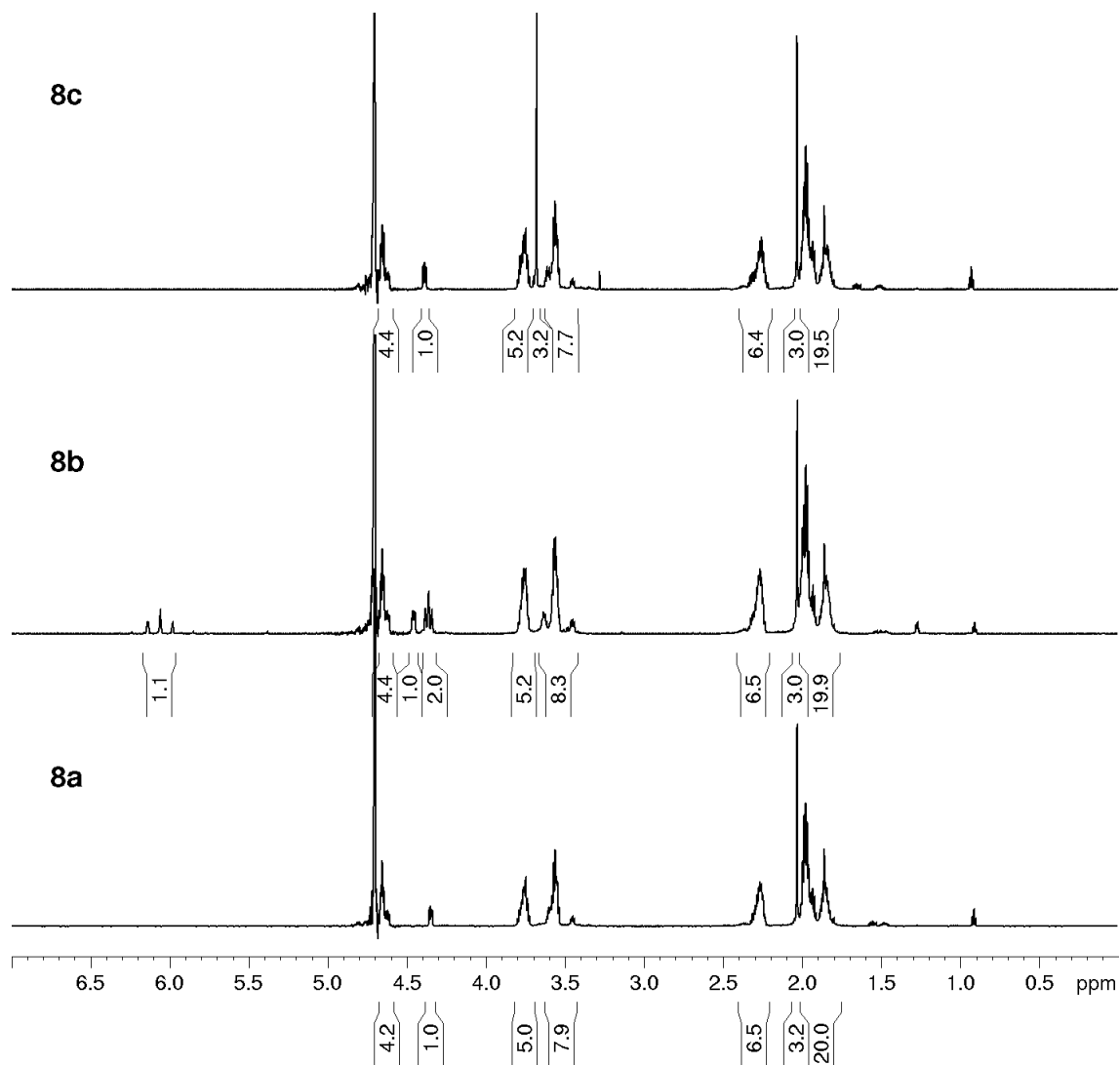


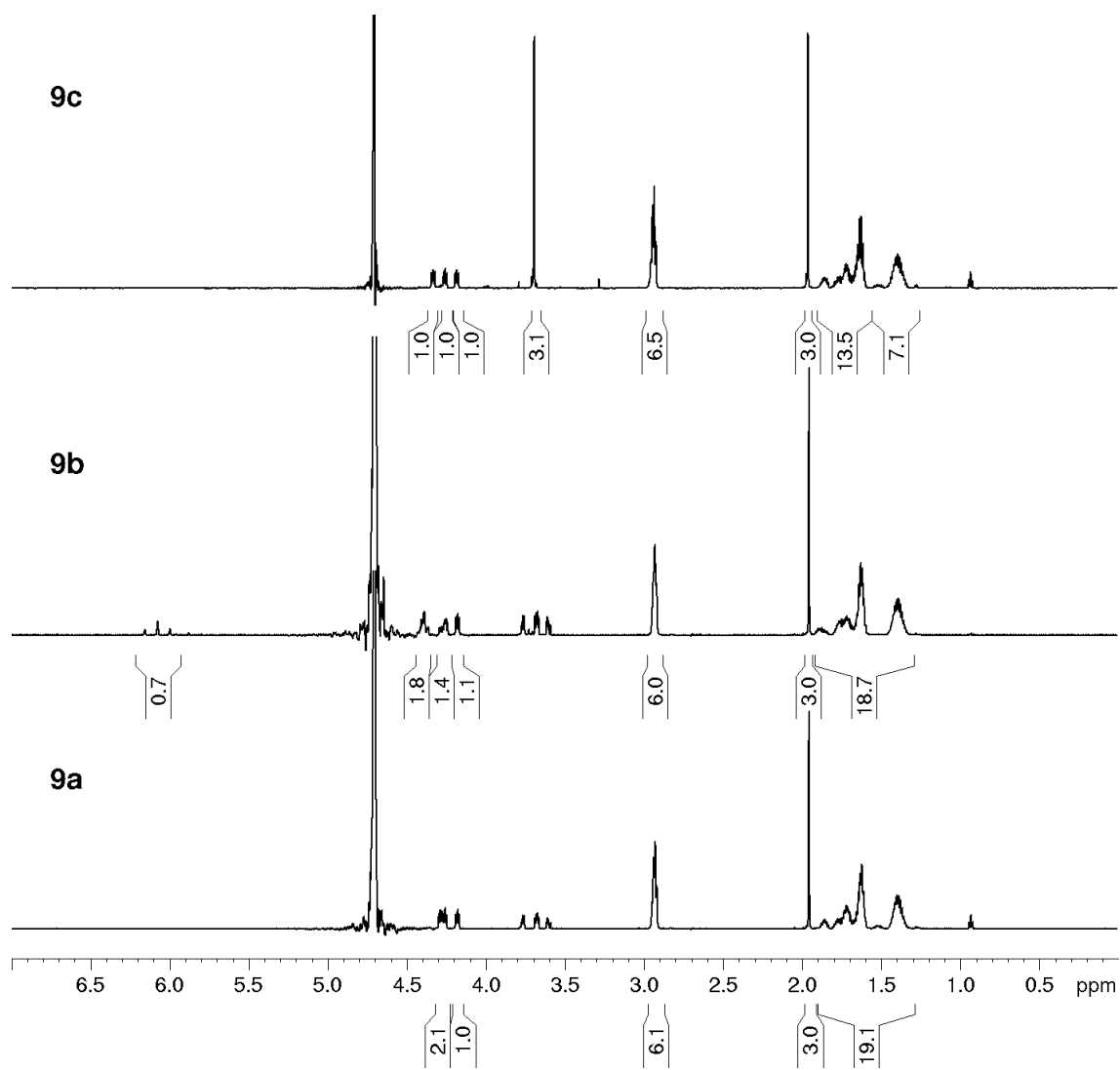
$^{19}\text{F}\{^1\text{H}\}$  NMR spectra (inverse-gated decoupling) of **5** in dichloromethane- $\text{d}_2$  upon addition of  $\text{Eu}^{3+}$  shifting reagent (two enantiomers)

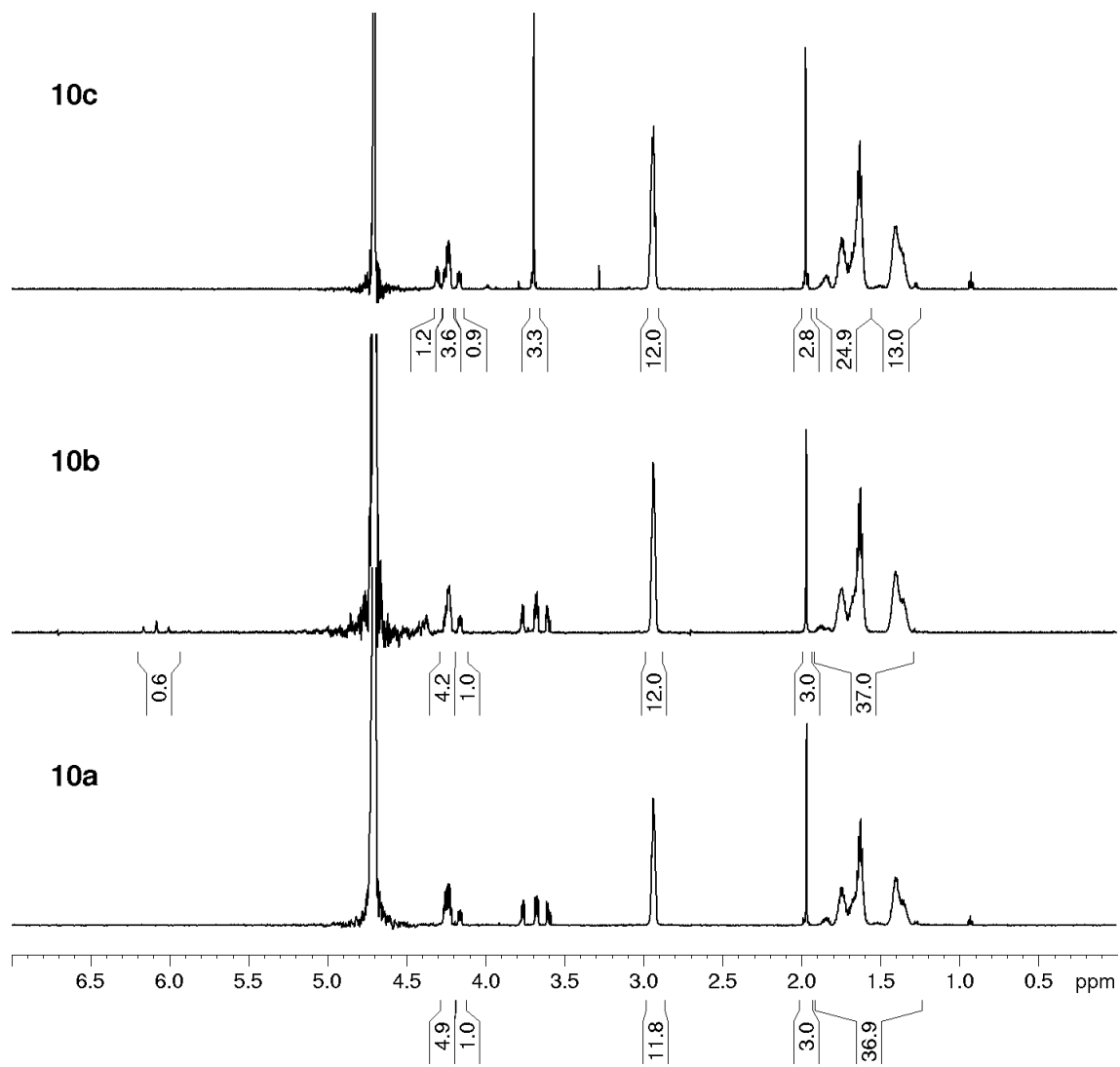


# NMR spectra of the peptides

$^1\text{H}$  90-pulse NMR spectra in deuterium oxide

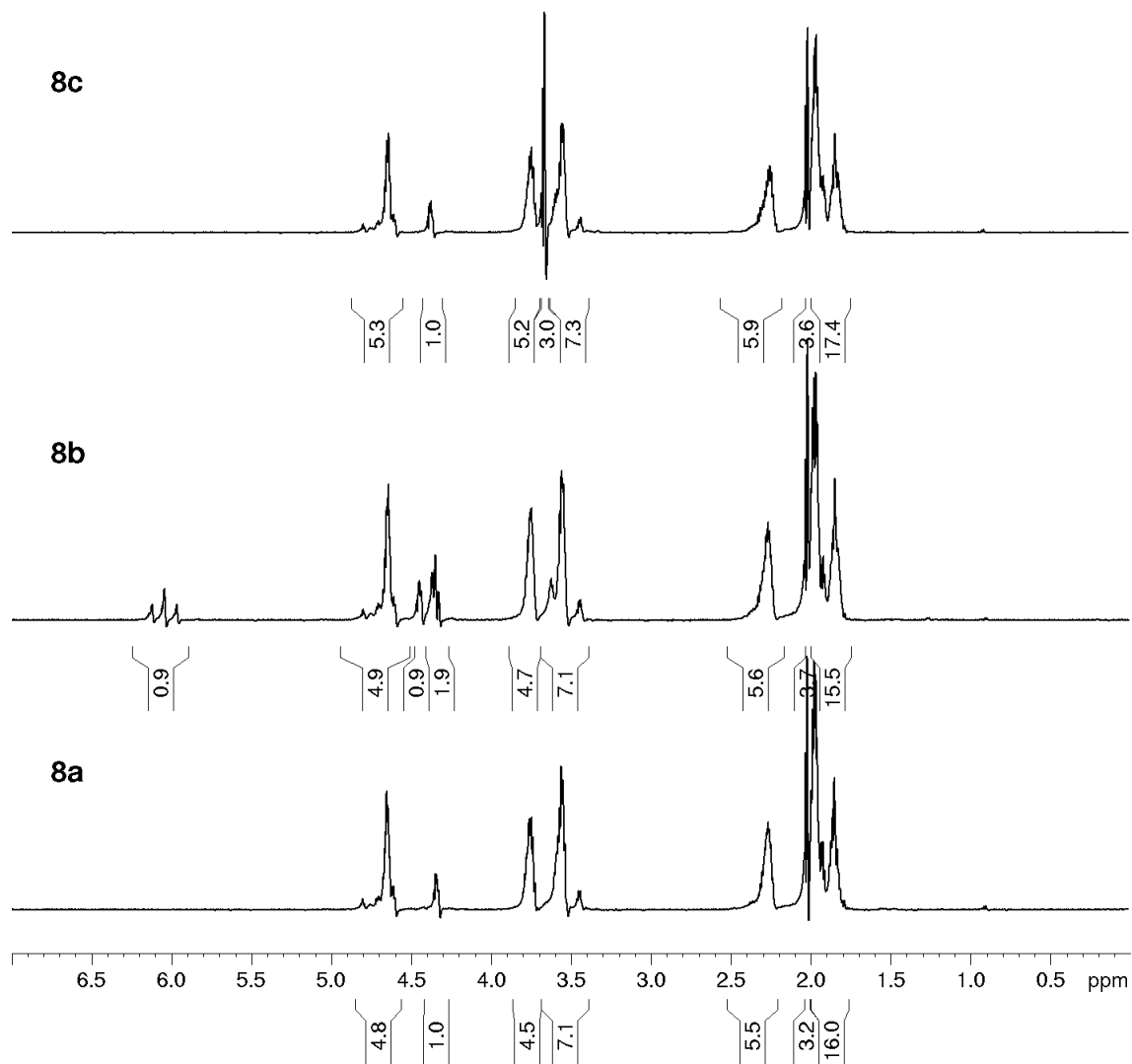


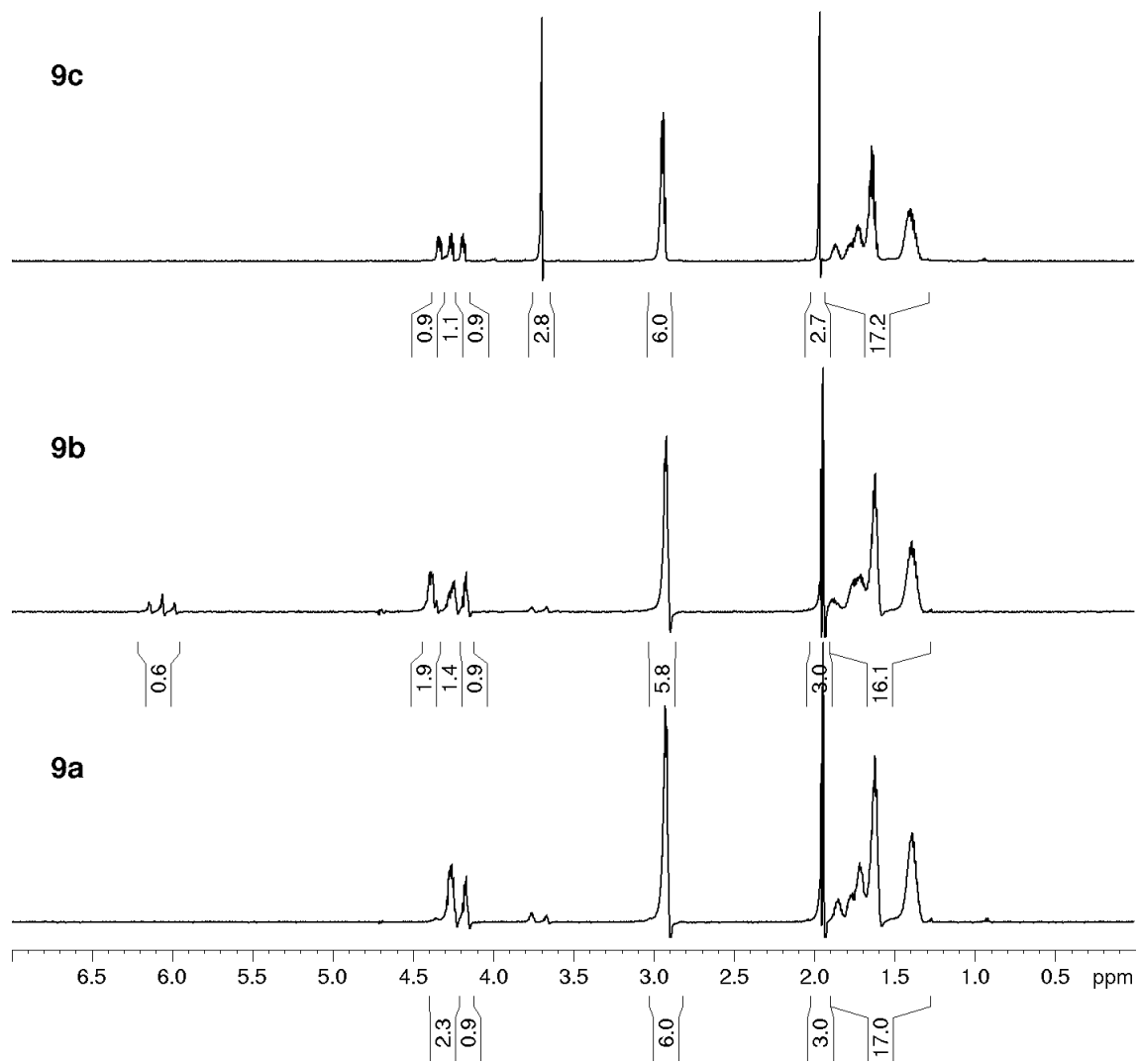


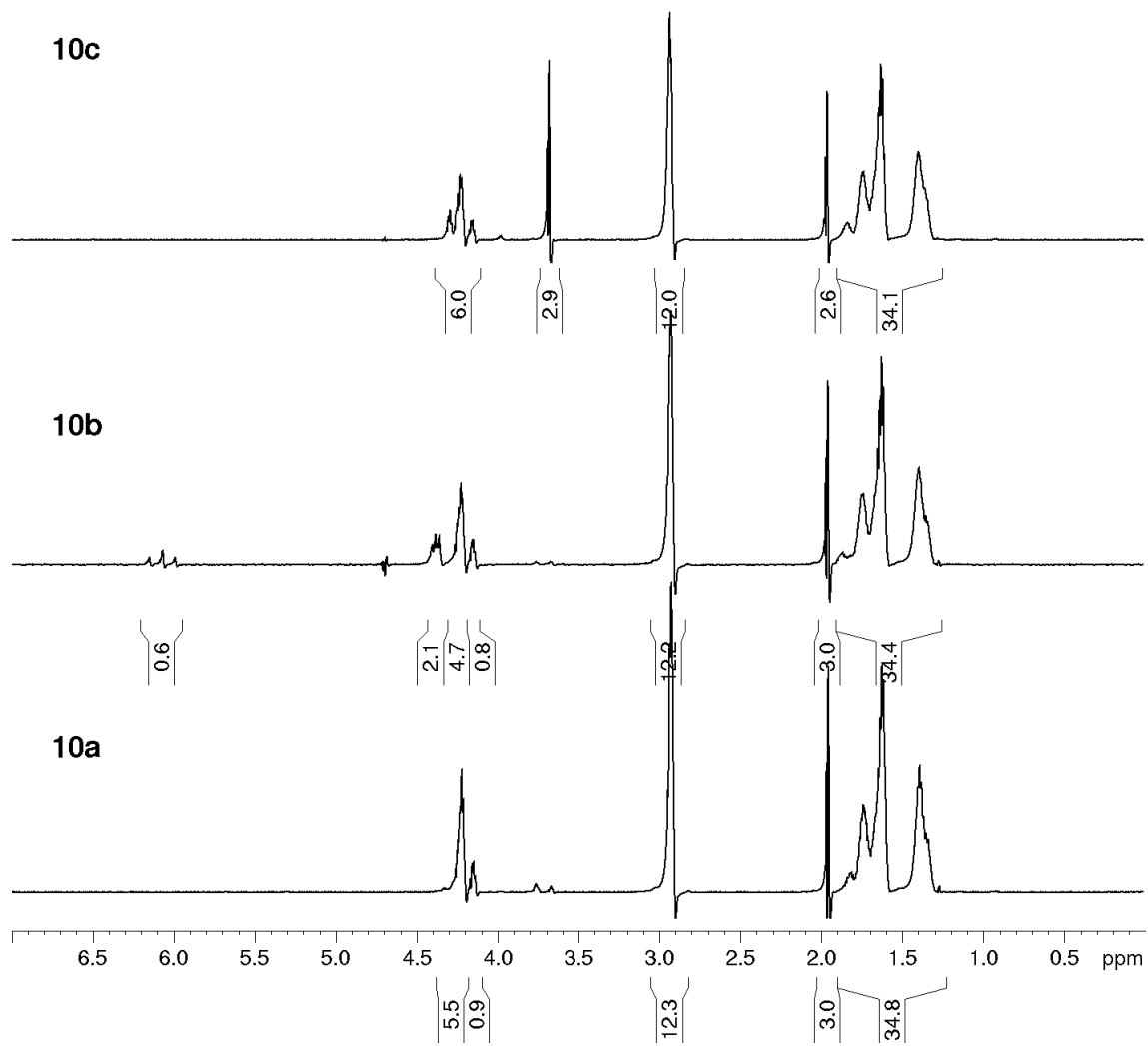




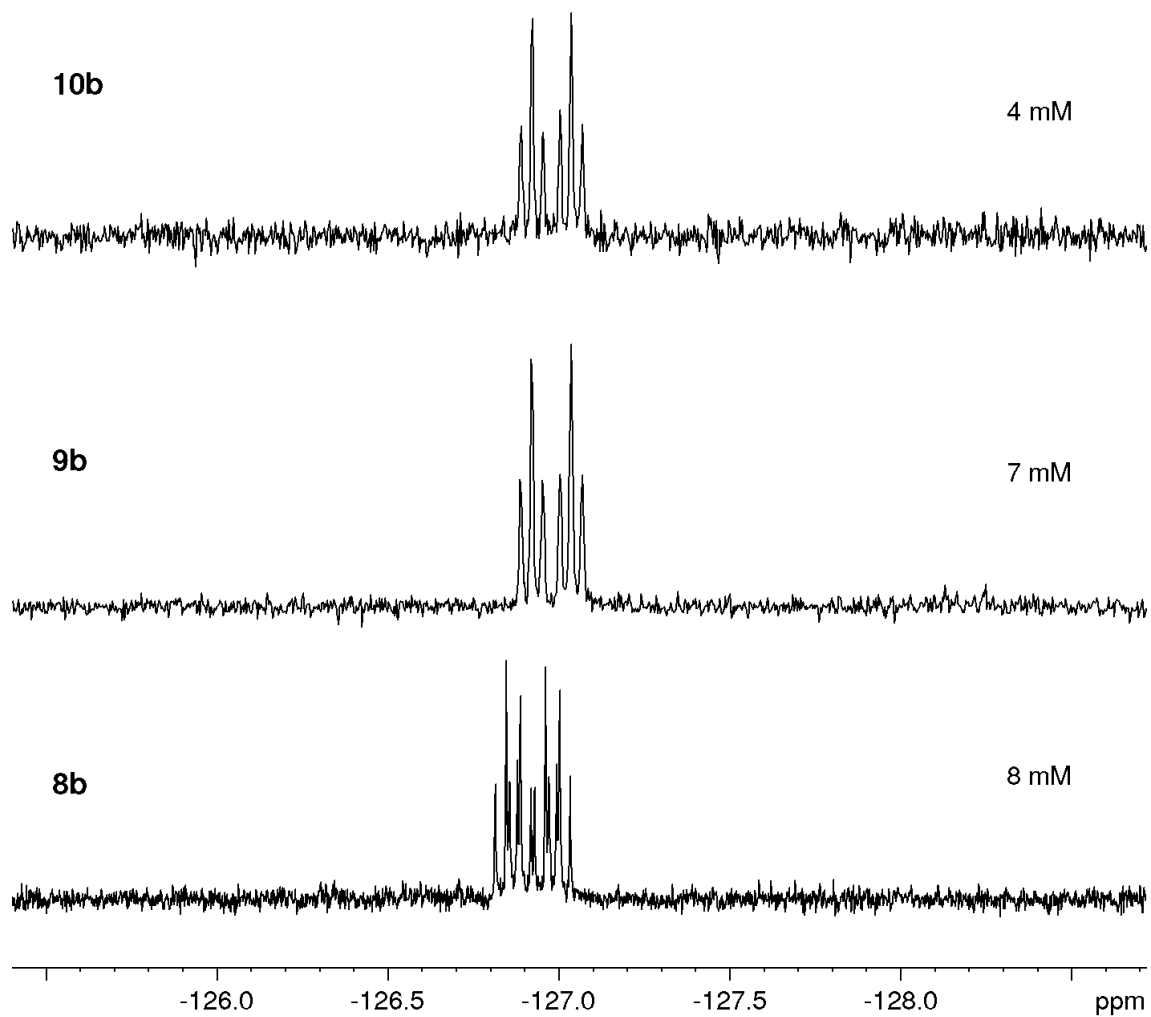
<sup>1</sup>H 1D stimulated echo NMR spectra in deuterium oxide at 700 MHz





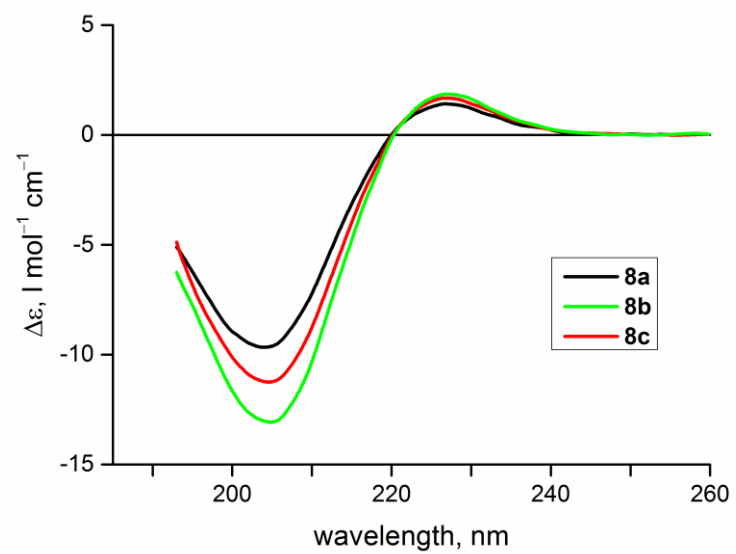


$^{19}\text{F}$  NMR spectra of the peptides in deuterium oxide at 471 MHz

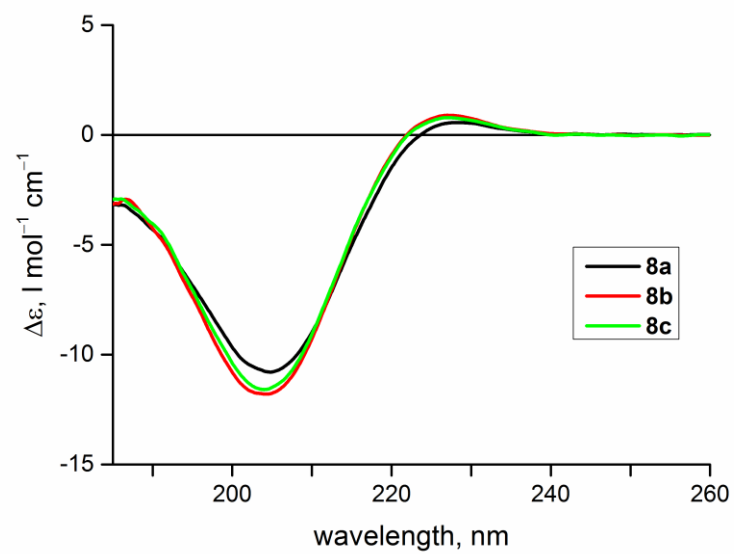


## Circular dichroism spectra for the peptides

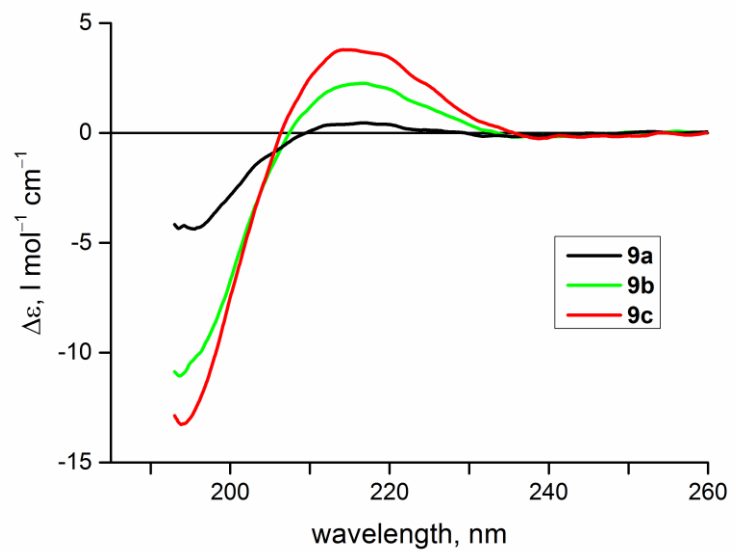
*in methanol:*



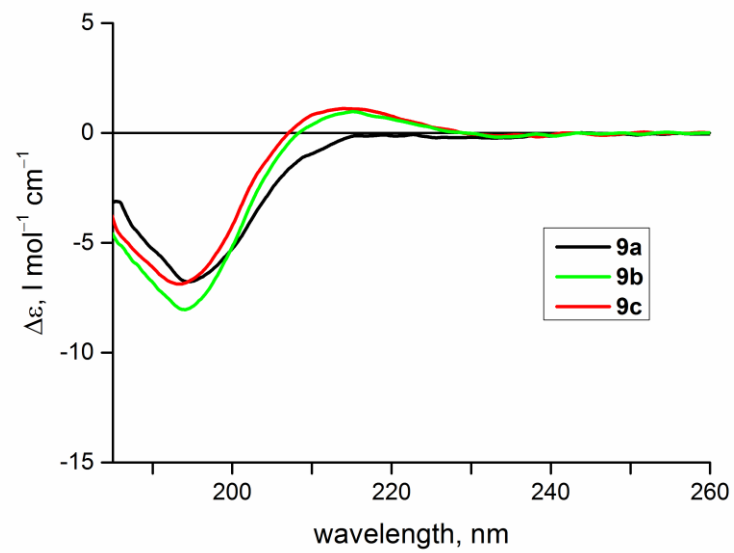
*in aqueous buffer:*



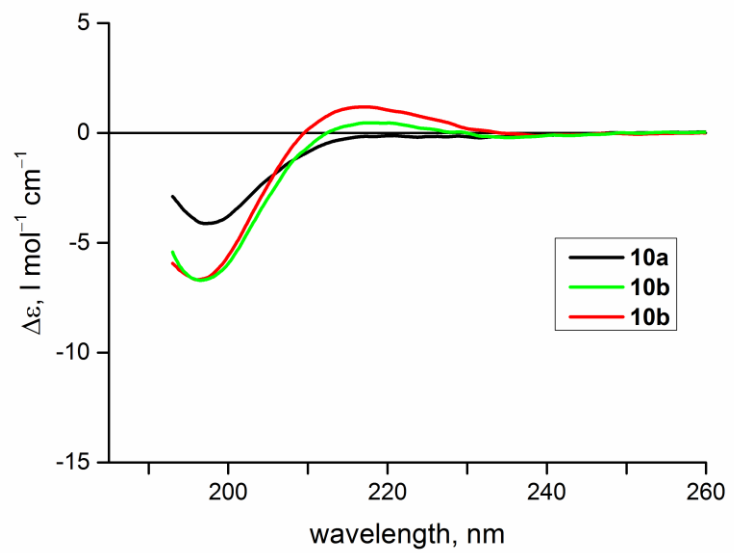
*in methanol:*



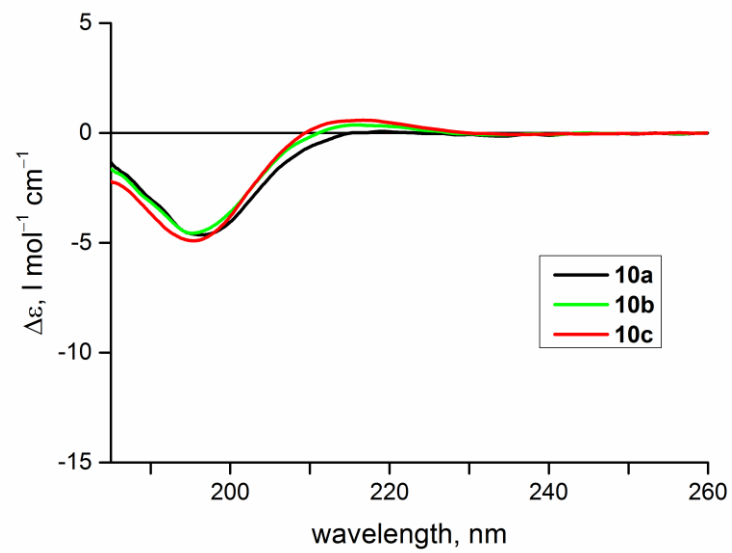
*in aqueous buffer:*



*in methanol:*



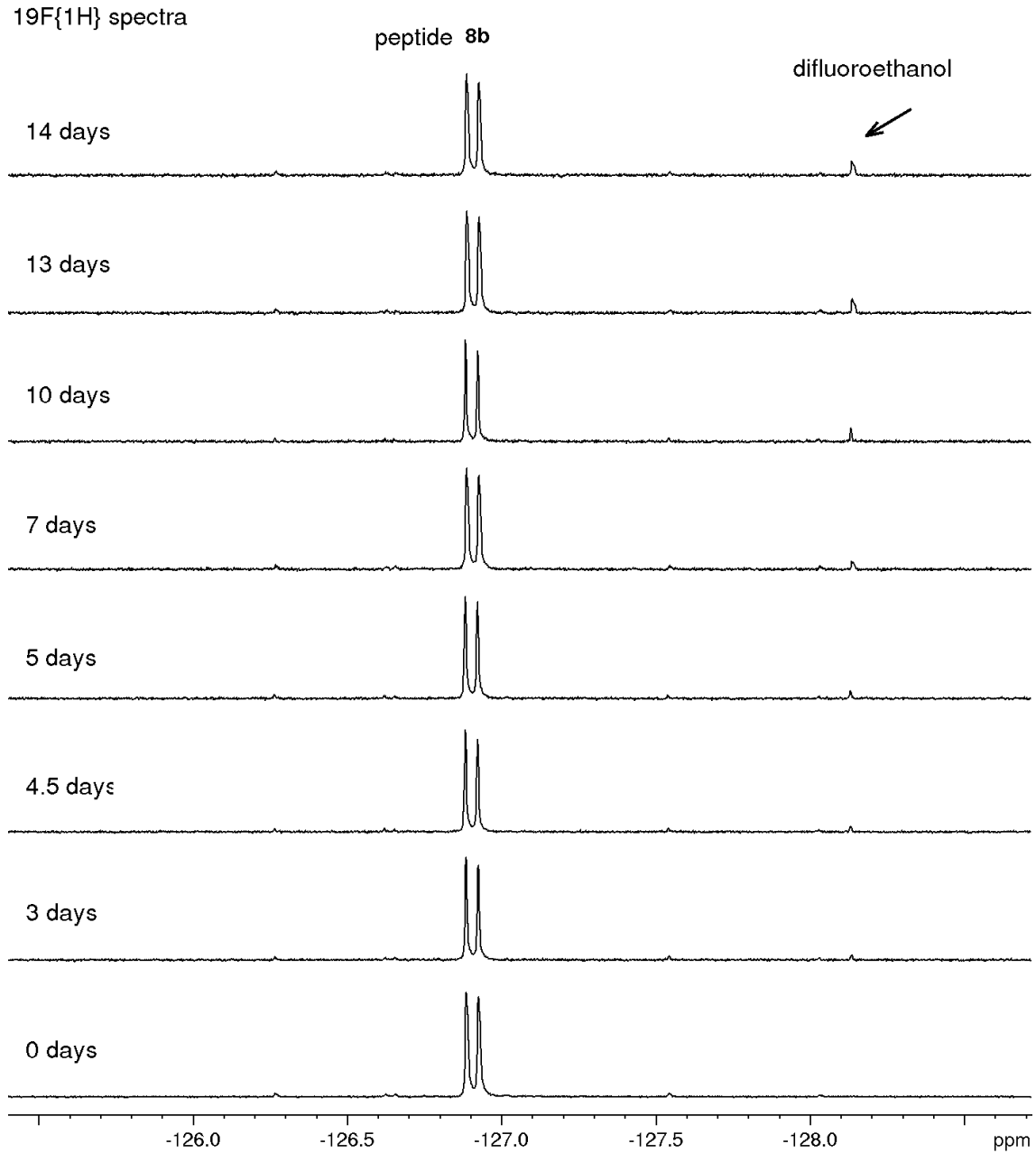
*in aqueous buffer:*



## Hydrolysis of the peptides

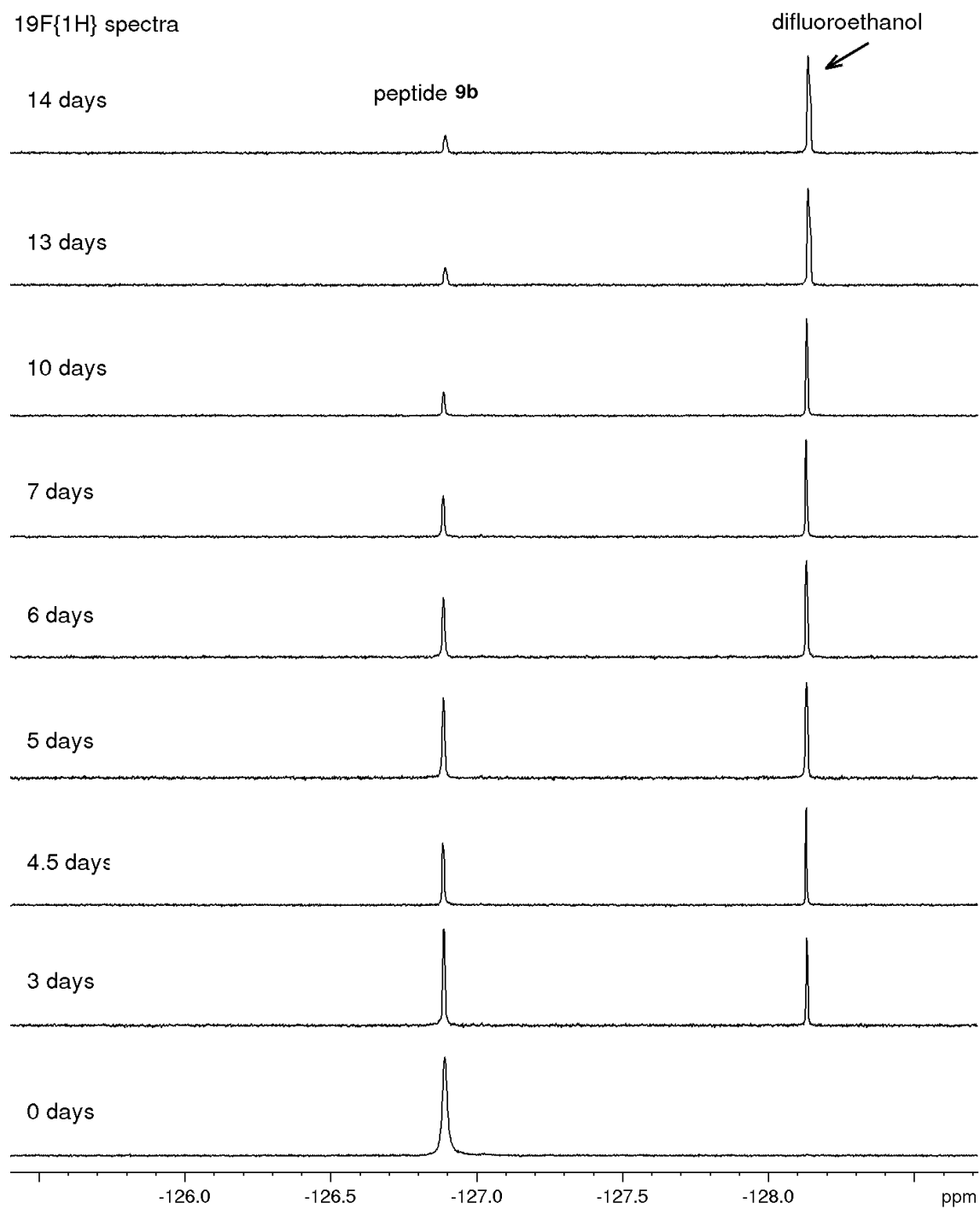
Starting peptide concentrations **8b** – 5 mM, **9b** – 5 mM, **10b** – 2.5 mM

peptide **8b**, series # 1

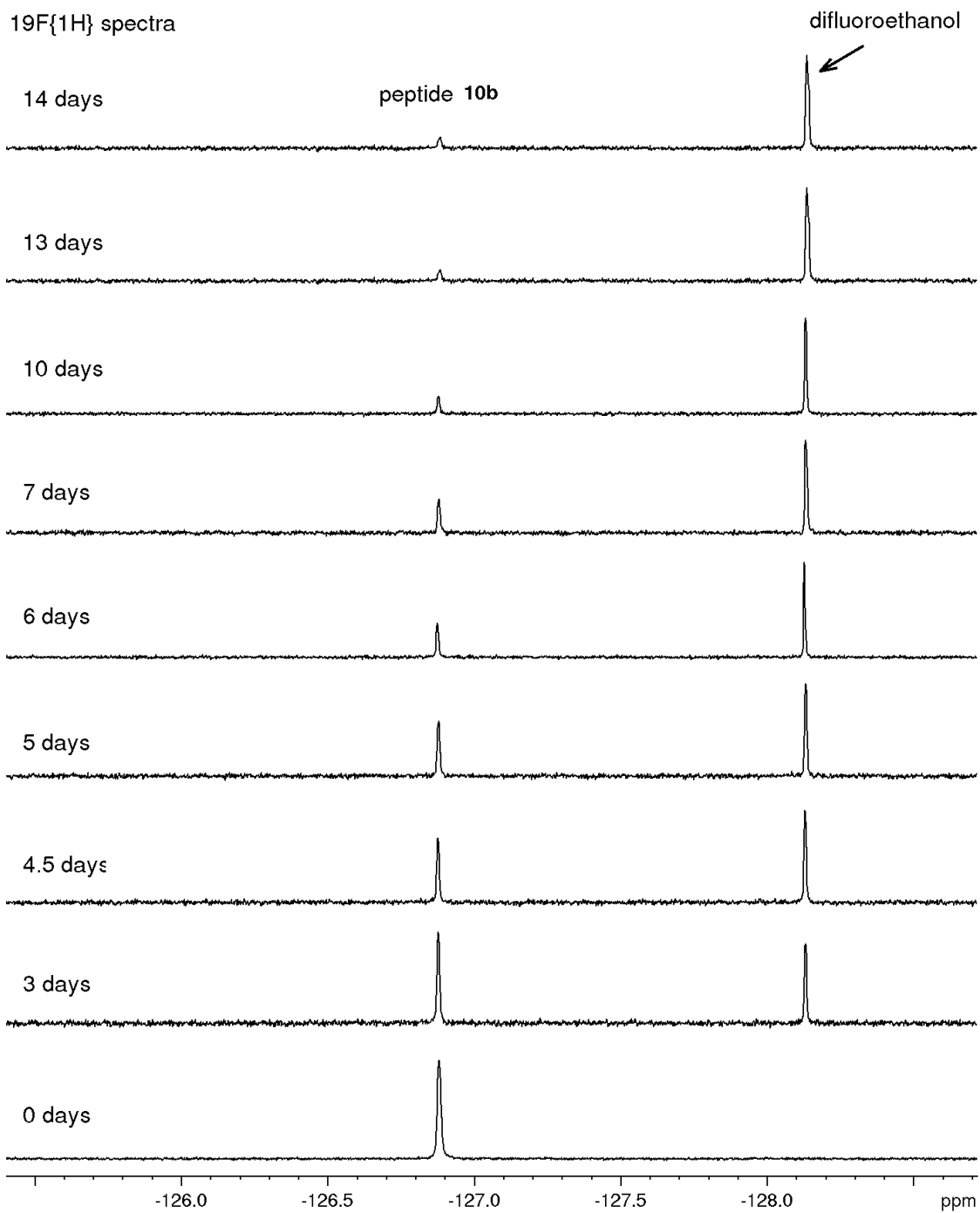




peptide **9b**, series # 1

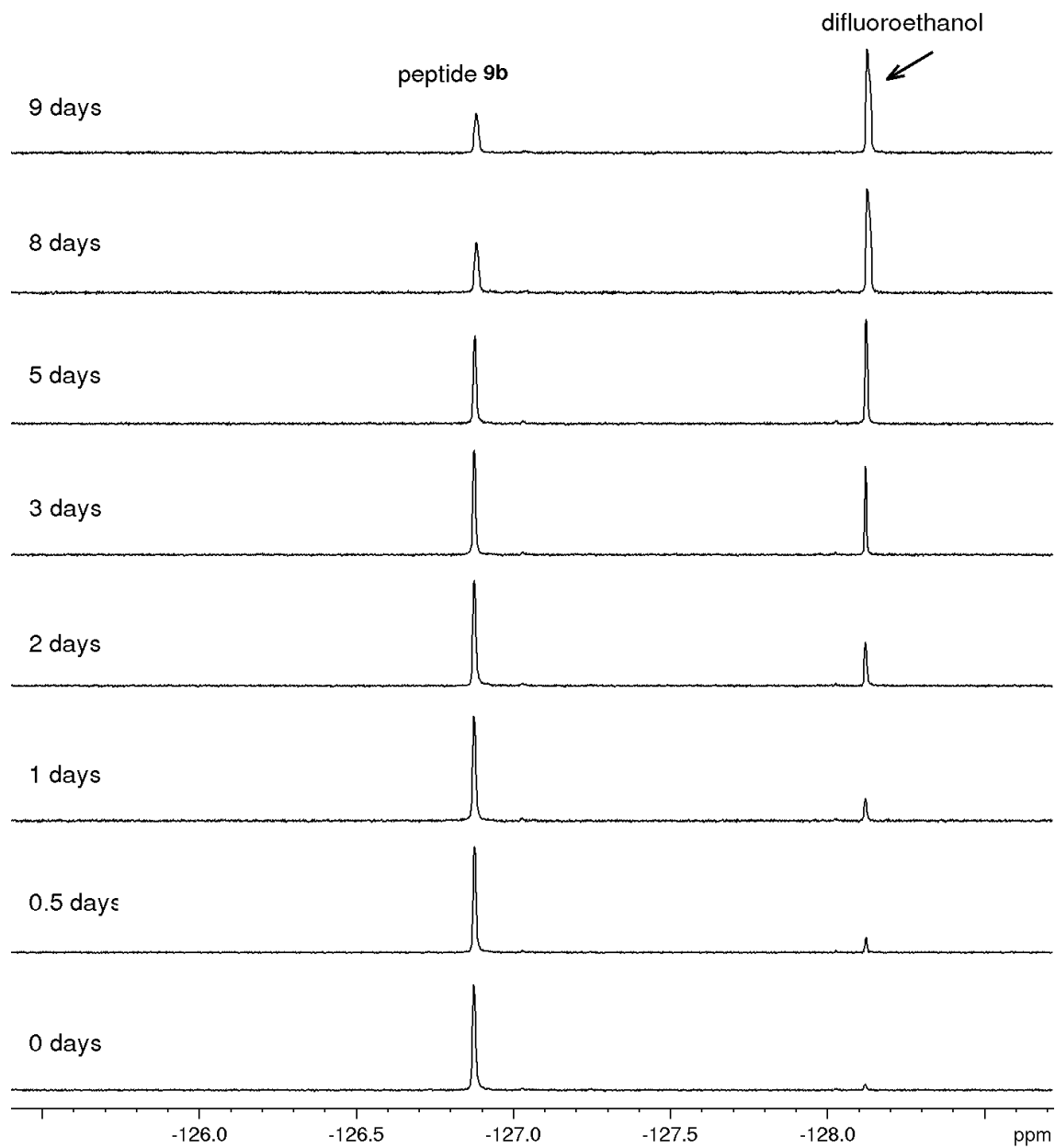


peptide **10b**, series # 1



peptide **9b**, series # 2

$^{19}\text{F}\{^1\text{H}\}$  spectra



peptide **10b**, series # 2

$^{19}\text{F}\{^1\text{H}\}$  spectra

