Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry.

γ-Trifluoromethyl proline: Evaluation as a structural substitute of proline for solid state ^{19}F-NMR peptide studies

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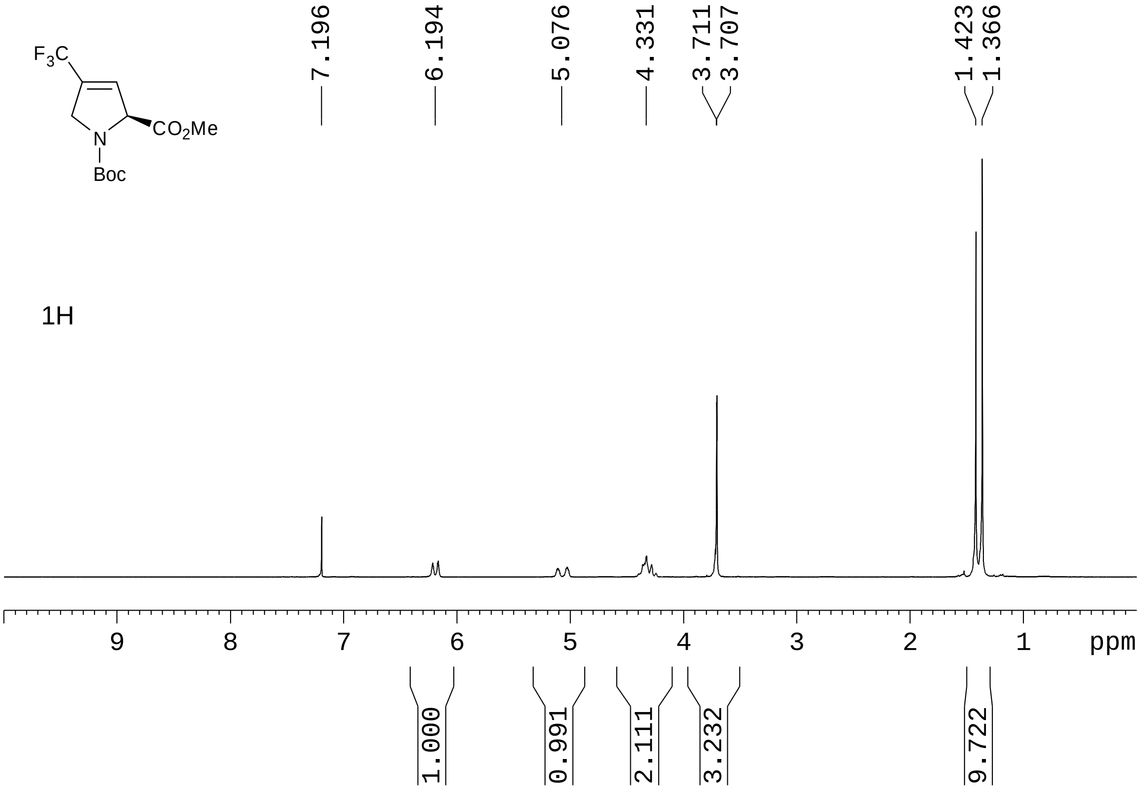
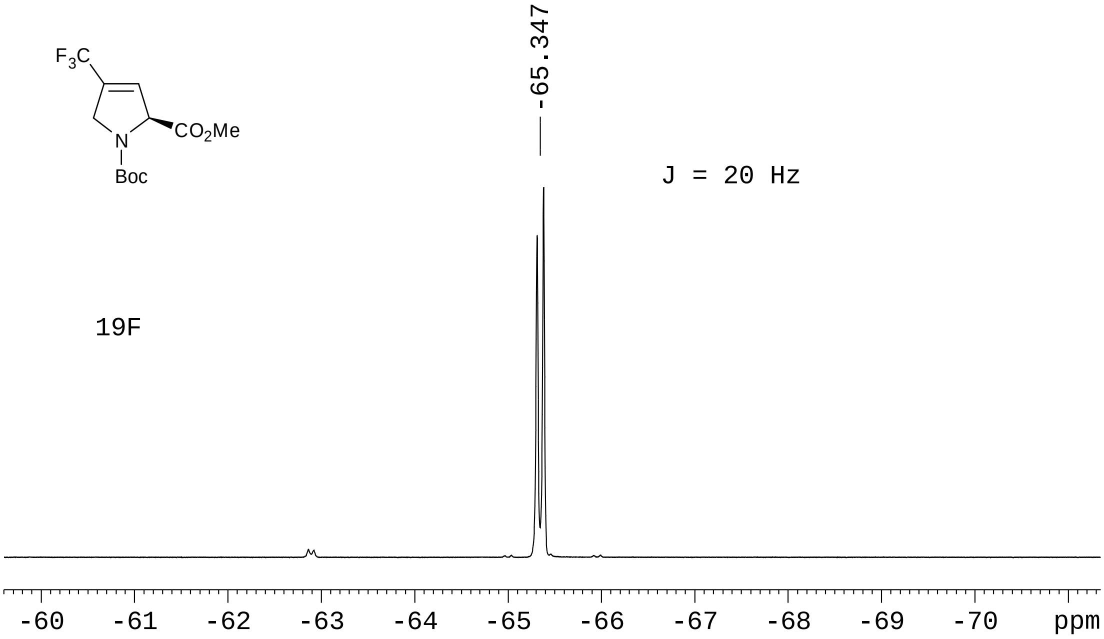
Contents

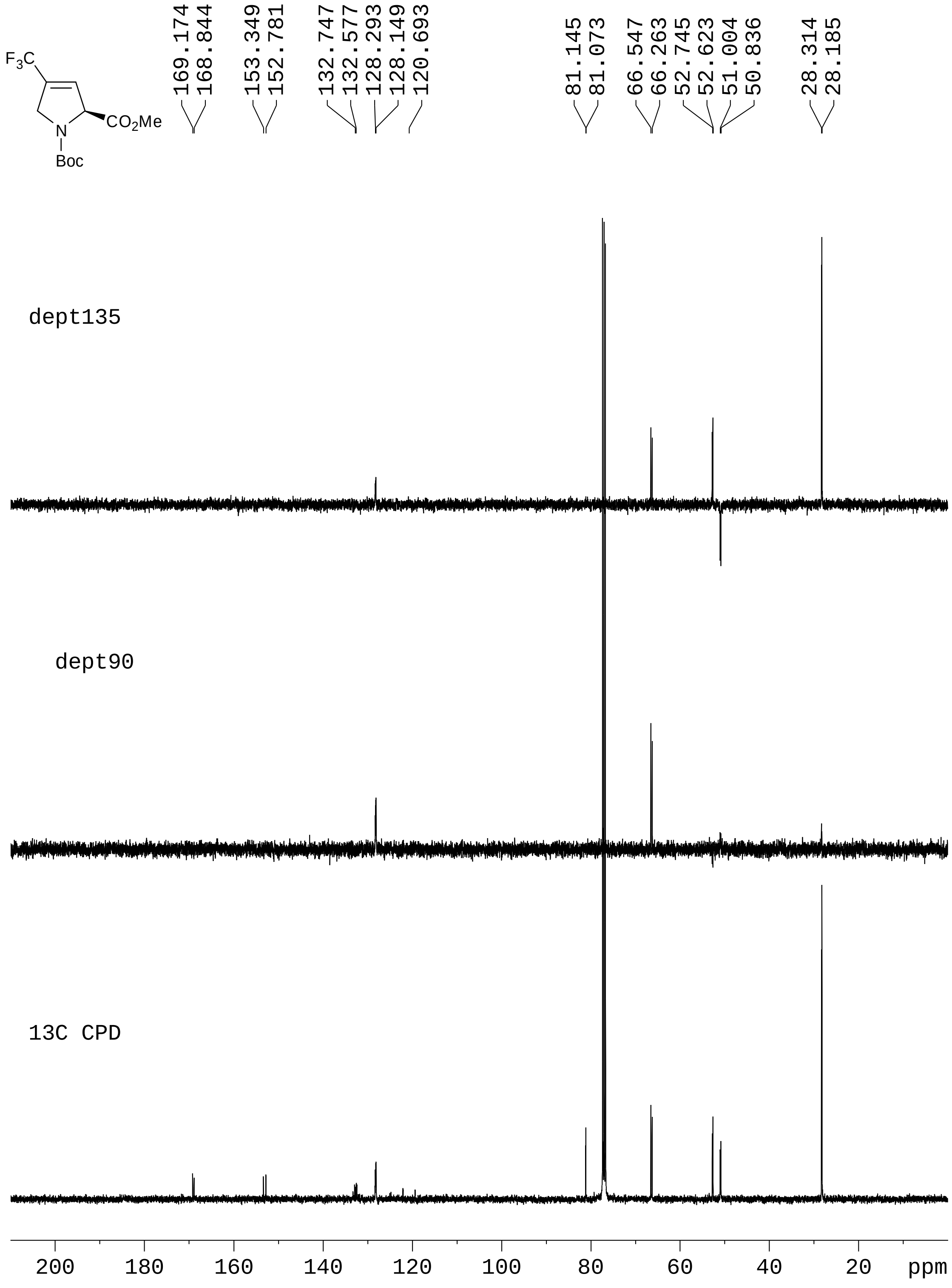
Copies of the NMR spectra for the compounds

Copies of the NMR spectra for the compounds

2-*tert*-butyl 2-methyl (2*S*)-4-trifluoromethyl-3-pyrrolin-1,2-dicarboxylate (**6**)



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CO2Me
N
Boc
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168.844
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152.781
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128.293
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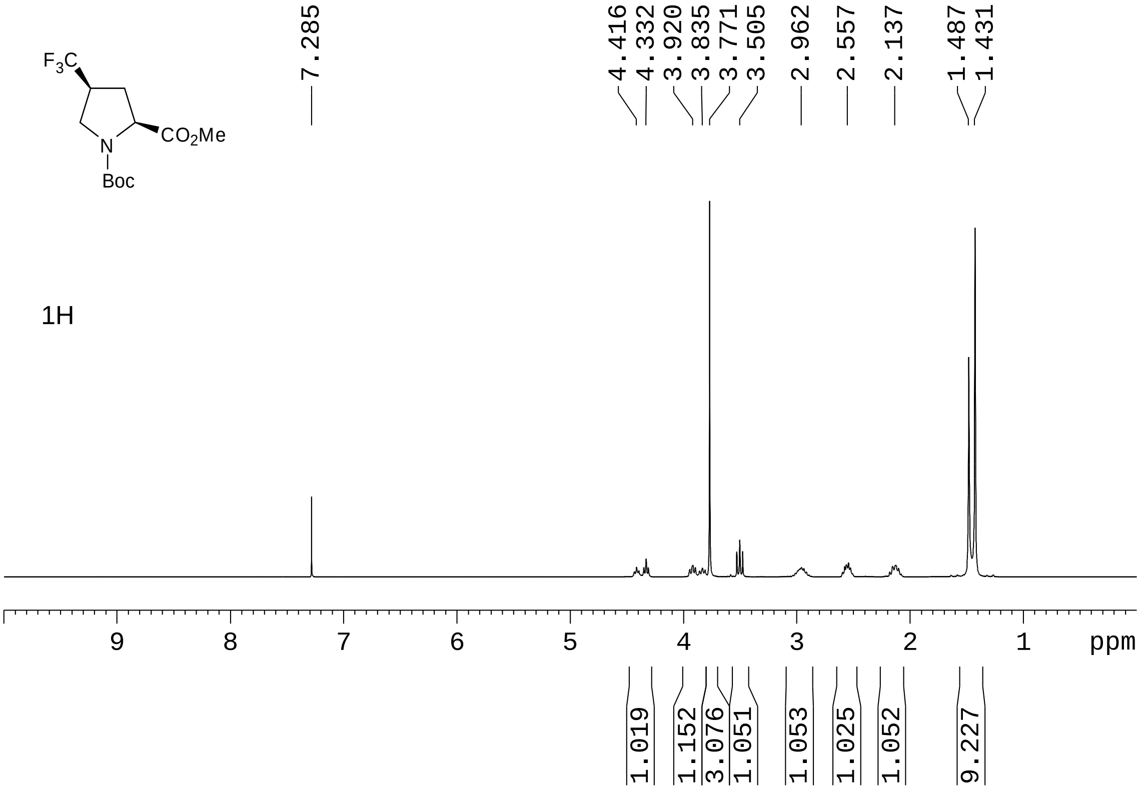
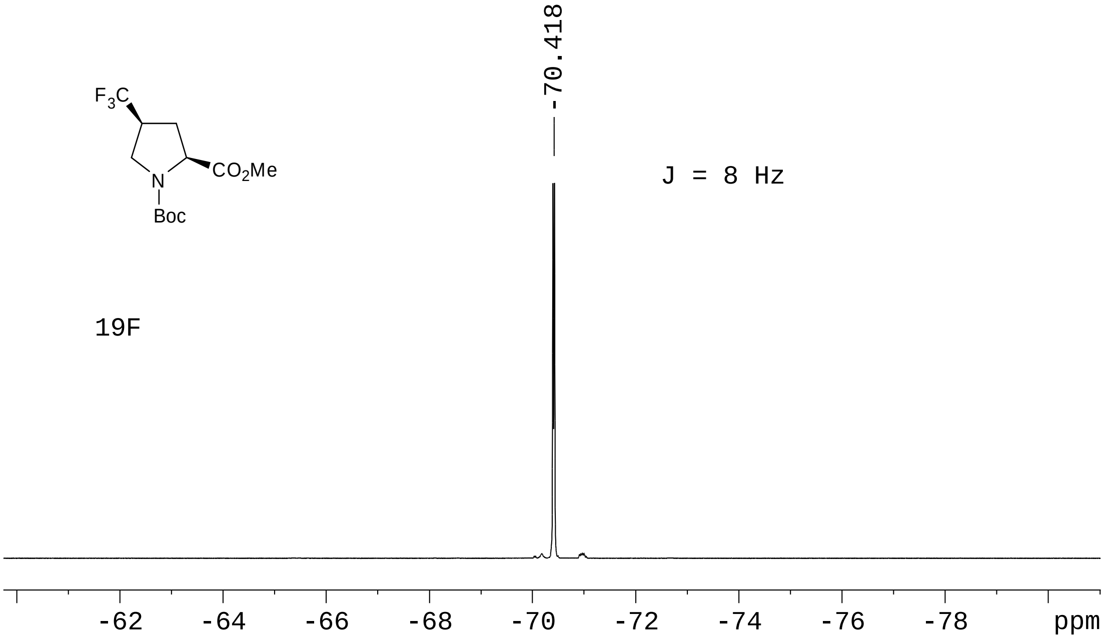
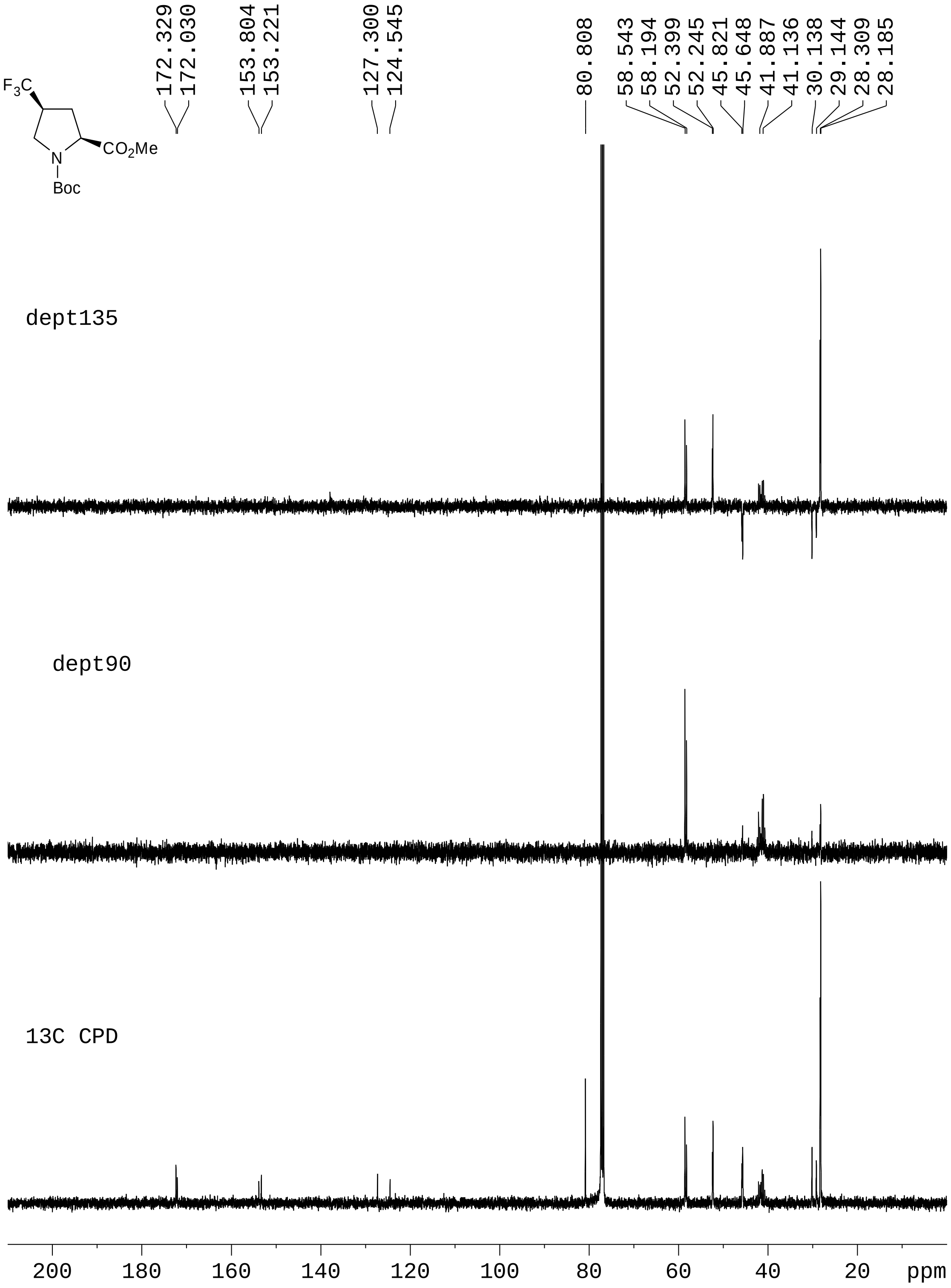
120.693
81.145
81.073
66.547
66.263
52.745
52.623
51.004
50.836
28.314
28.185
dept135
dept90
13C CPD
200
180
160
140
120
100
80
60
40
20
ppm

Methyl (2*S*,4*S*)-N-tert-buthoxycarbonyl-4-trifluoromethylprolinate (**7**)



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N
Boc
CO2Me
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153.221
127.300
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80.808
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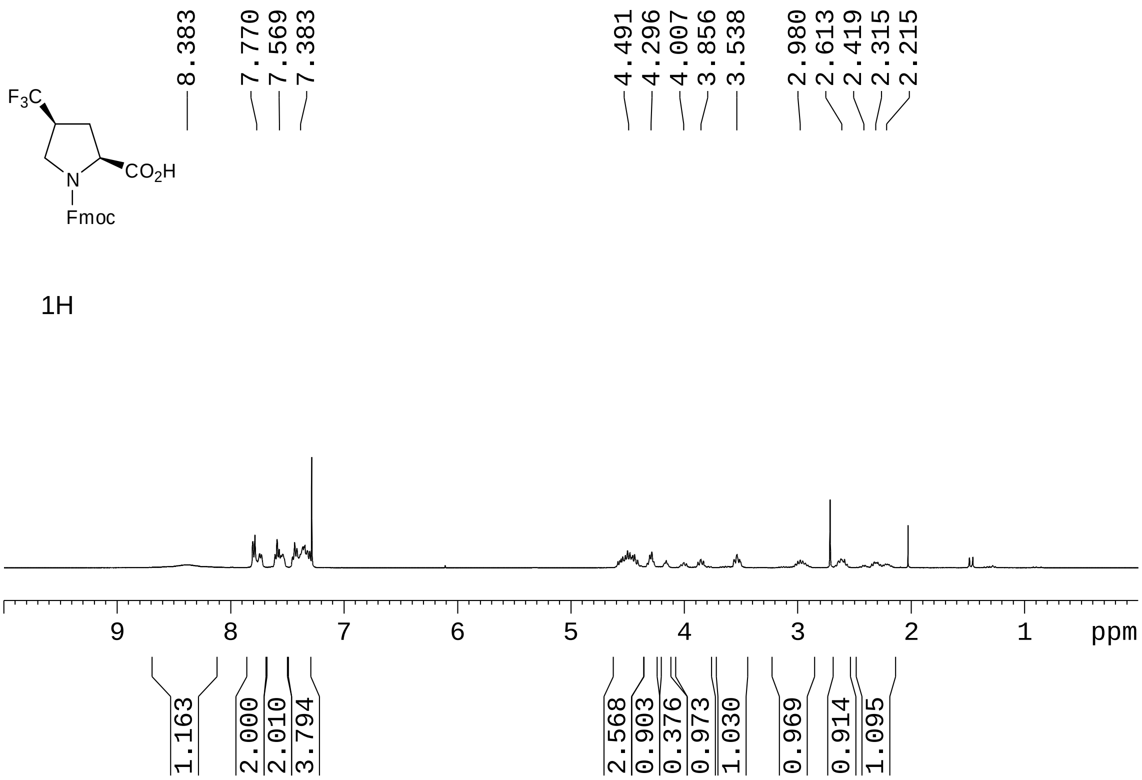
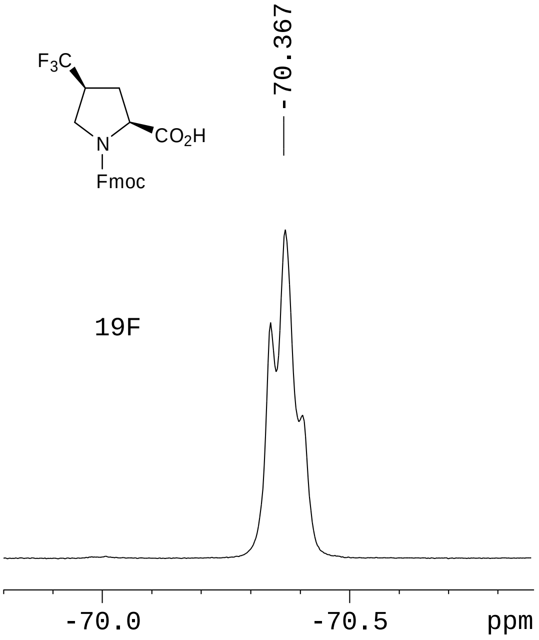
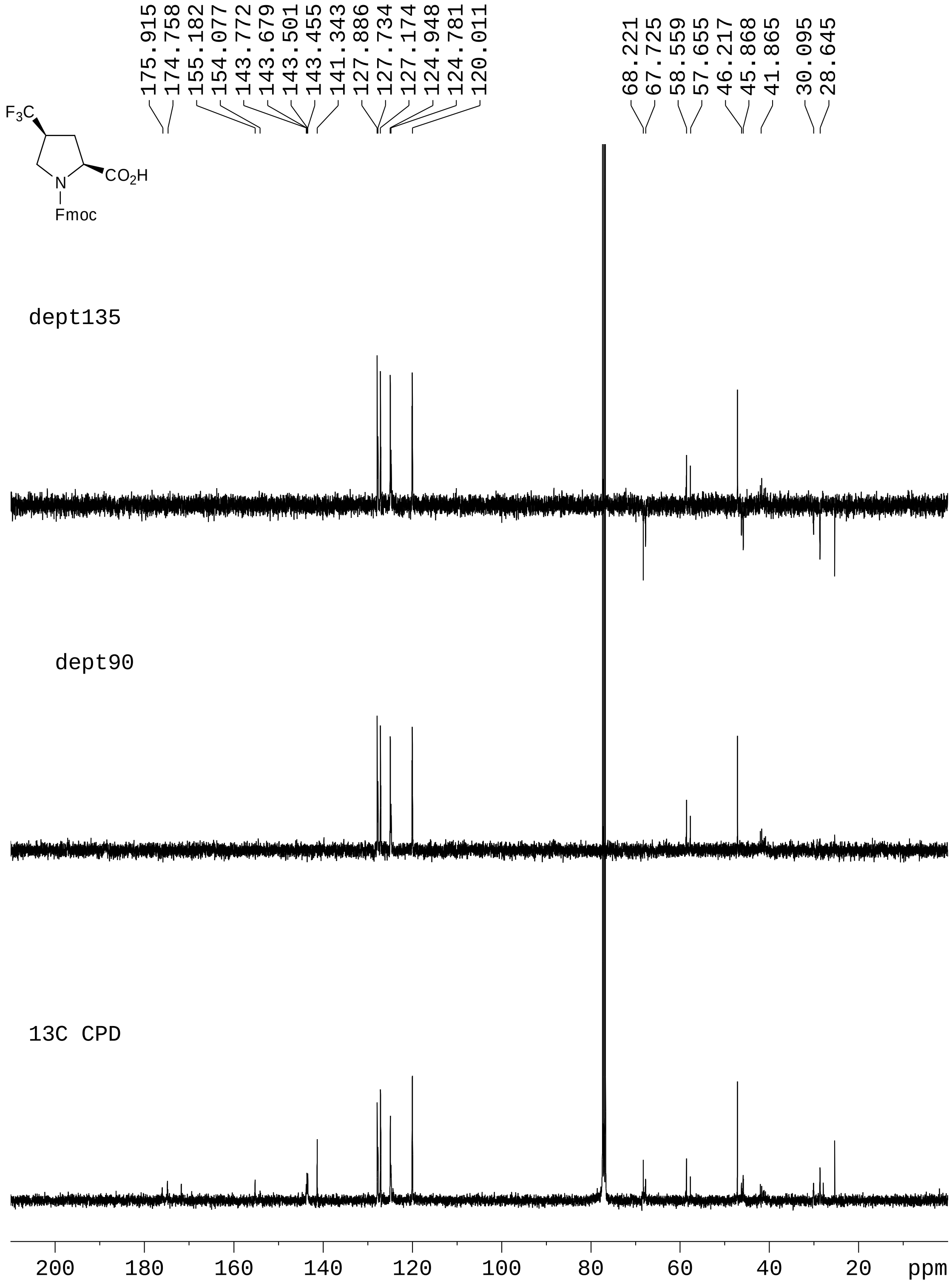
58.194
52.399
52.245
45.821
45.648
41.887
41.136
30.138
29.144
28.309
28.185
dept135
dept90
13C CPD
200
180
160
140
120
100
80
60
40
20
ppm

(2*S*,4*S*)-N-(9-fluorenylmethoxycarbonyl)-4-trifluoromethylproline (**9**)



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174.758
155.182
154.077
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143.679
143.501
143.455
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127.886
127.734
127.174
124.948
124.781
120.011
68.221
67.725
58.559
57.655
46.217
45.868
41.865
30.095
28.645
F_3C
N
CO_2H
Fmoc
dept135
dept90
13C CPD
200
180
160
140
120
100
80
60
40
20
ppm

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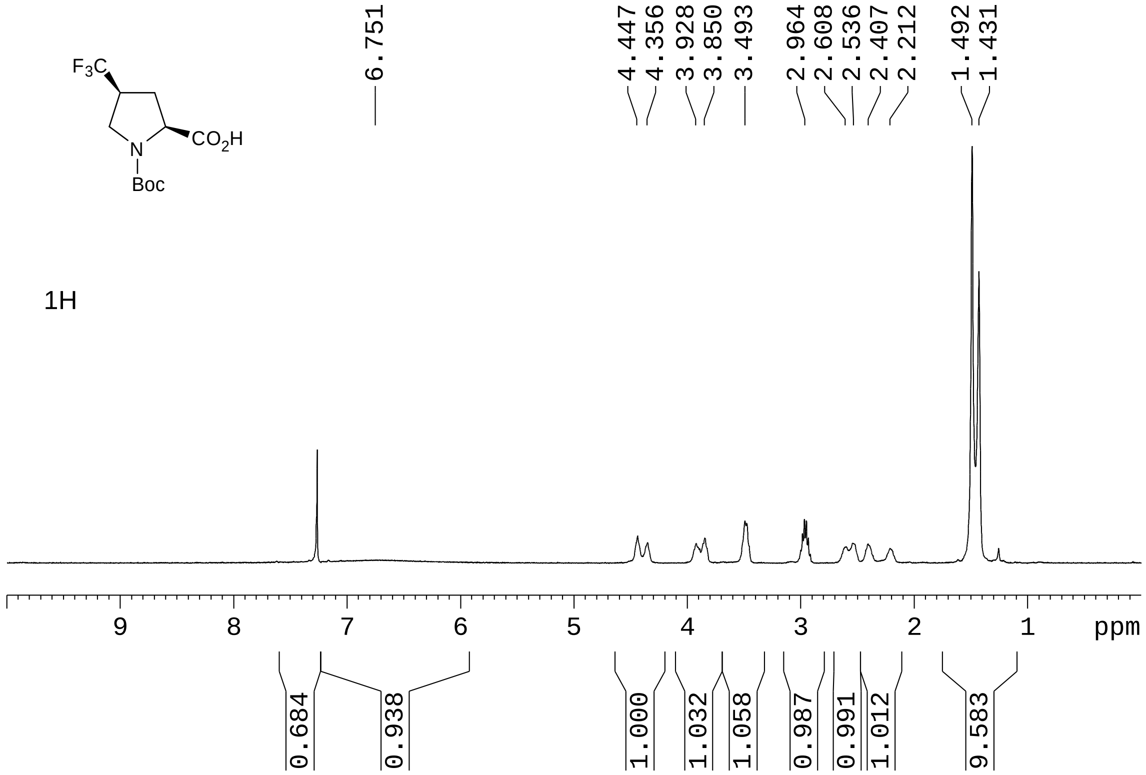
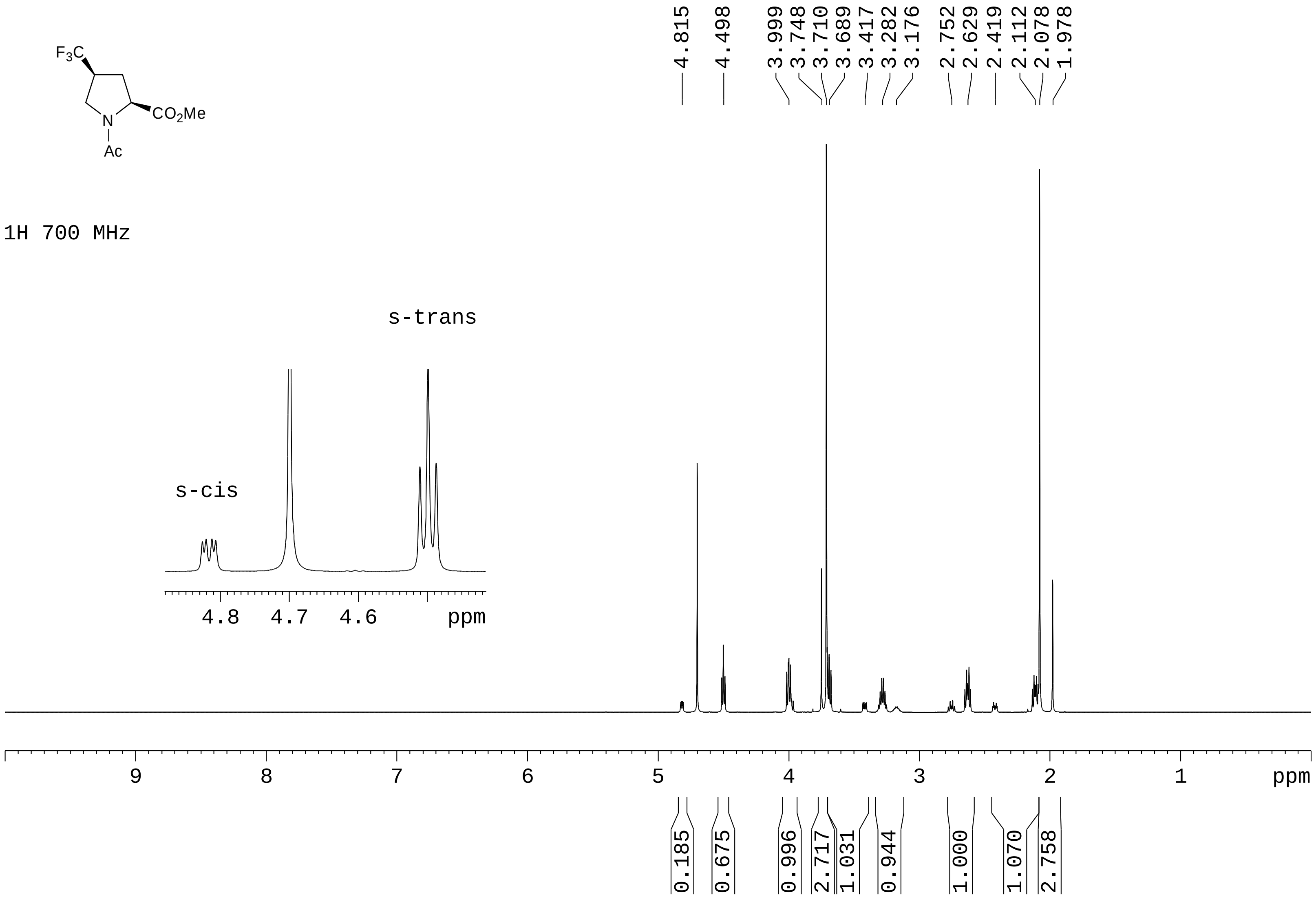
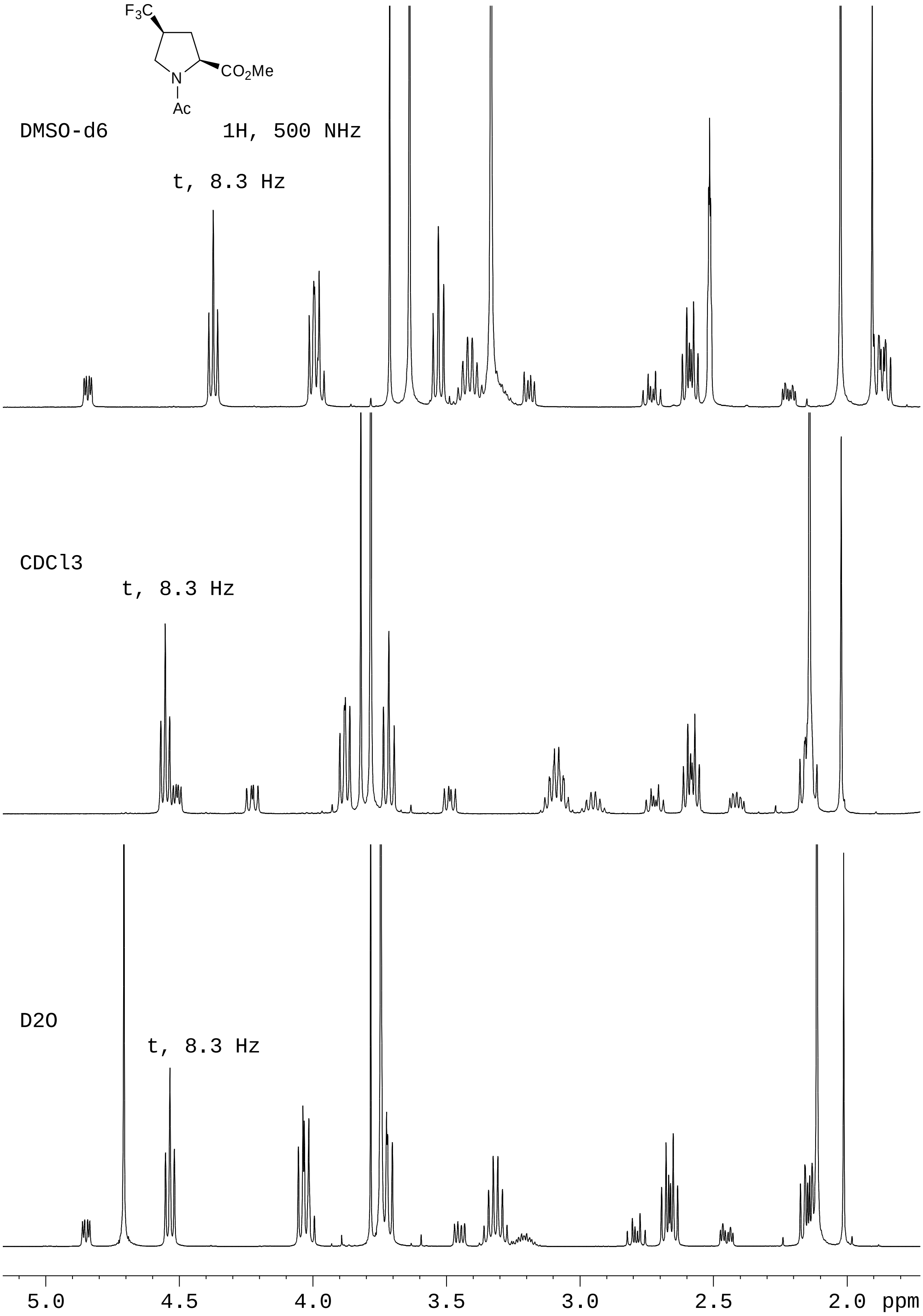
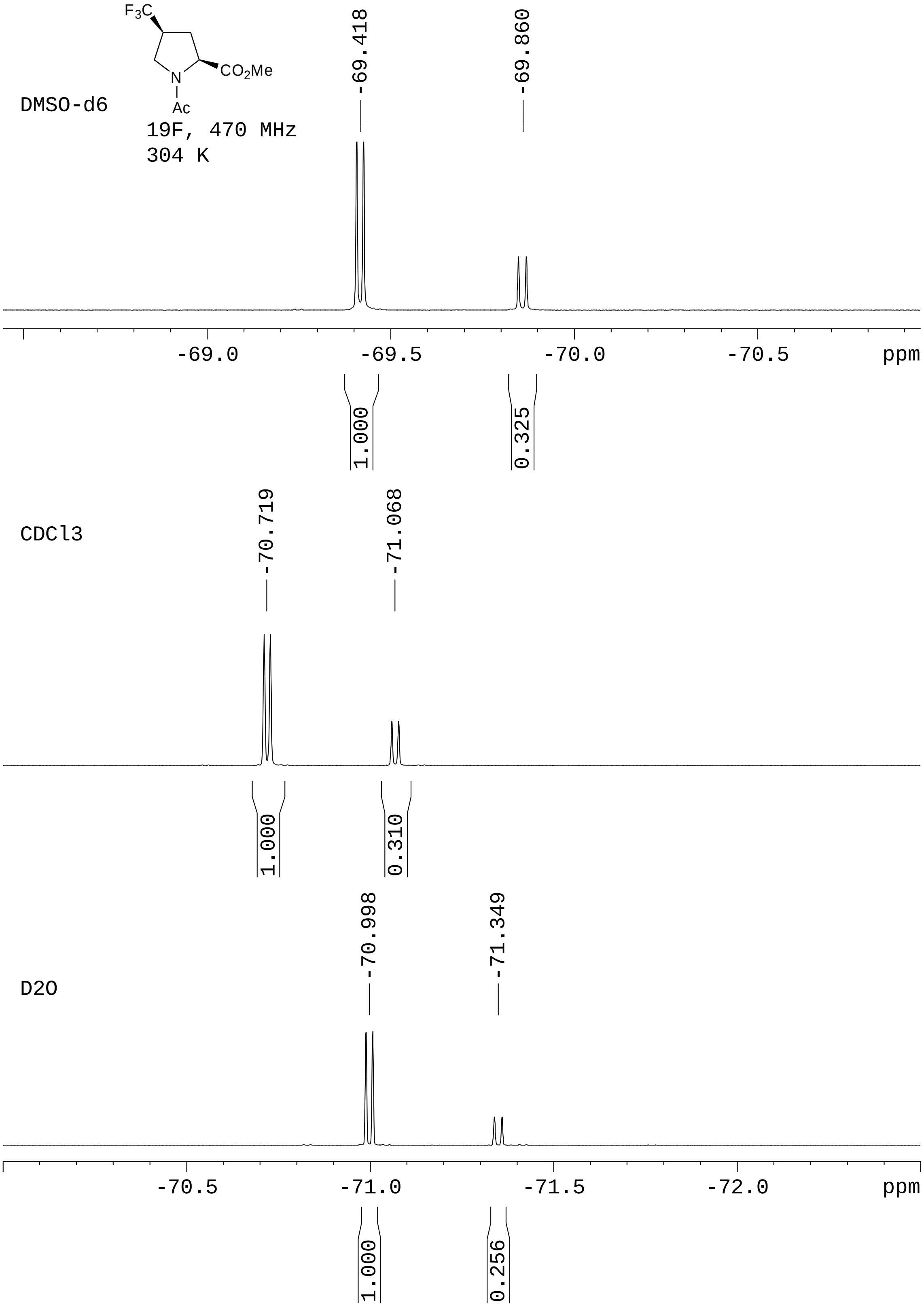


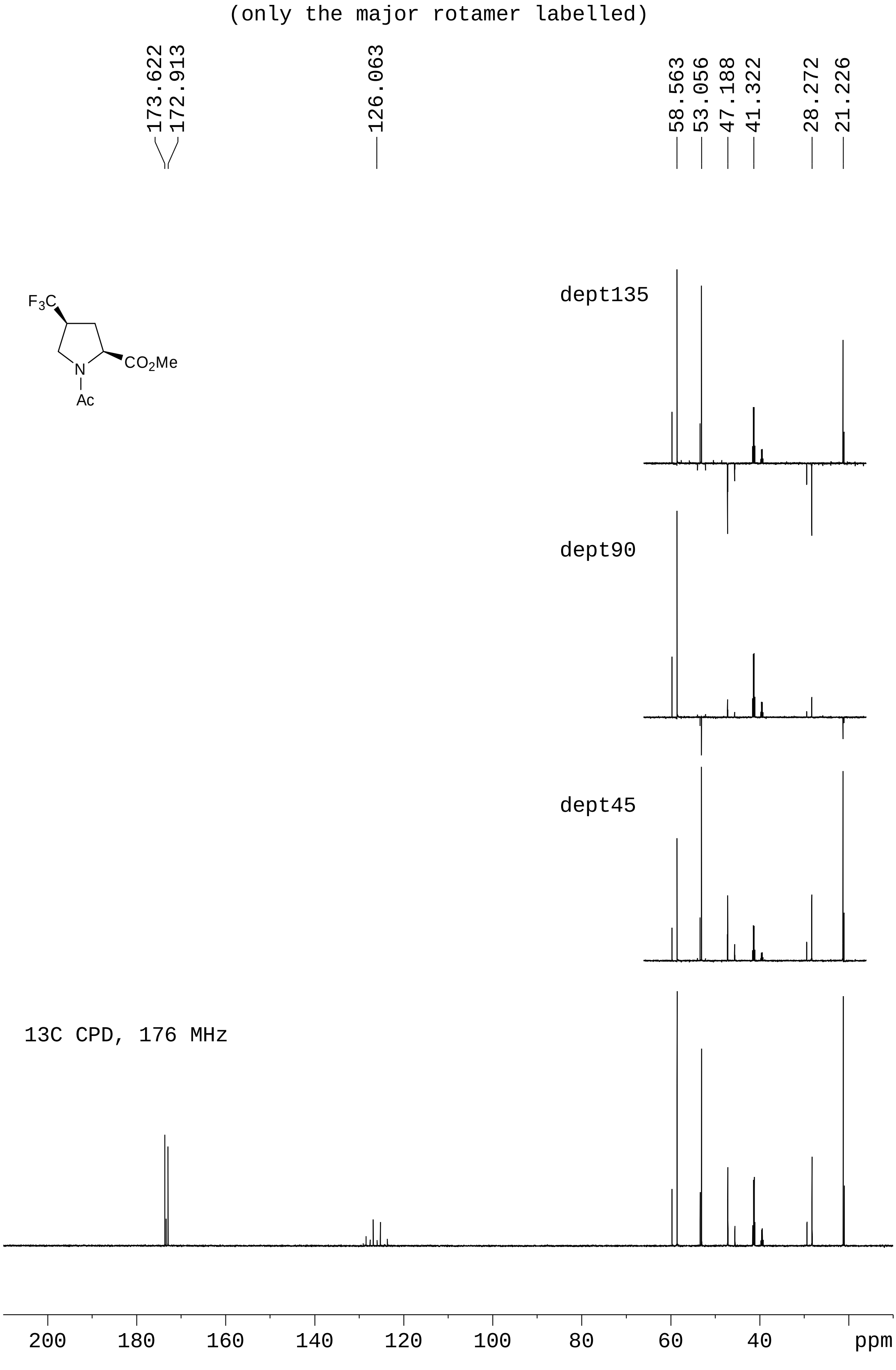
Methyl (2*S*,4*S*)-N-acetyl-4-trifluoromethylprolinate (**8**)

1H 700 MHz

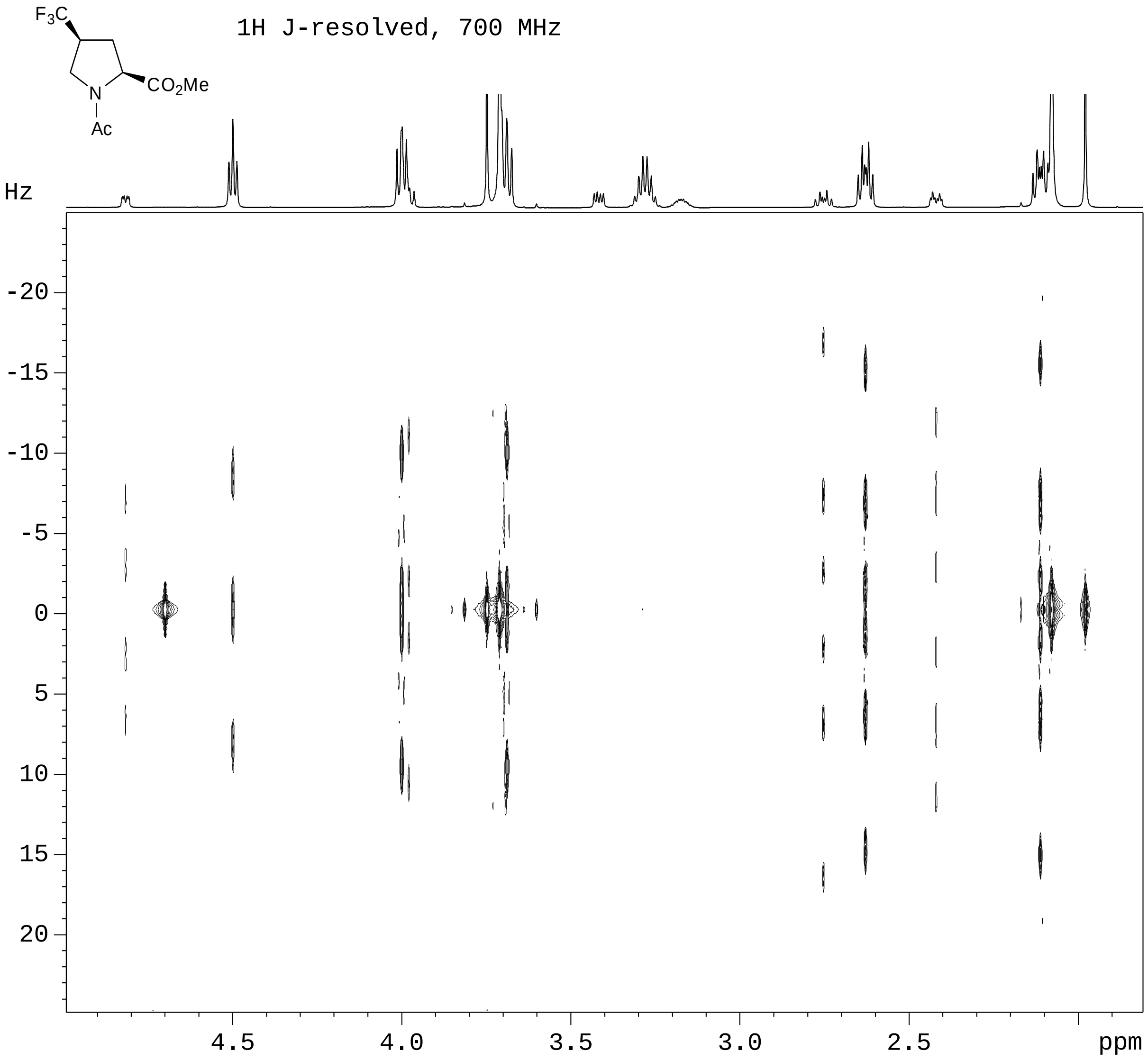



F3C
N
CO2Me
Ac
DMSO-d6
1H, 500 NHz
t, 8.3 Hz
CDCl3
t, 8.3 Hz
D2O
t, 8.3 Hz
5.0
4.5
4.0
3.5
3.0
2.5
2.0 ppm


F3C
CO2Me
N
Ac
DMSO-d6
19F, 470 MHz
304 K
-69.418
-69.860
-69.0
-69.5
-70.0
-70.5
ppm
1.000
0.325
CDCl3
-70.719
-71.068
1.000
0.310
D2O
-70.998
-71.349
-70.5
-71.0
-71.5
-72.0
ppm
1.000
0.256


(only the major rotamer labelled)
173.622
172.913
126.063
58.563
53.056
47.188
41.322
28.272
21.226
F_3C
N
CO_2Me
Ac
dept135
dept90
dept45
13C CPD, 176 MHz
200
180
160
140
120
100
80
60
40
ppm

1H J-resolved, 700 MHz



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PROCNO                  1

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PULPROG            jresqf
TD                   4096
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NS                      4
DS                     16
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RG                  33.06
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DE                  10.00 usec
TE                  297.8 K
D0             0.00000300 sec
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NUC1                   1H
P1                  17.00 usec
P2                  34.00 usec
PLW1          22.00000000 W

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FIDRES           0.781250 Hz
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WDW                  SINE
SSB      0
LB       0 Hz
GB       0
PC                  10.00

F1 - Processing parameters
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GB       0
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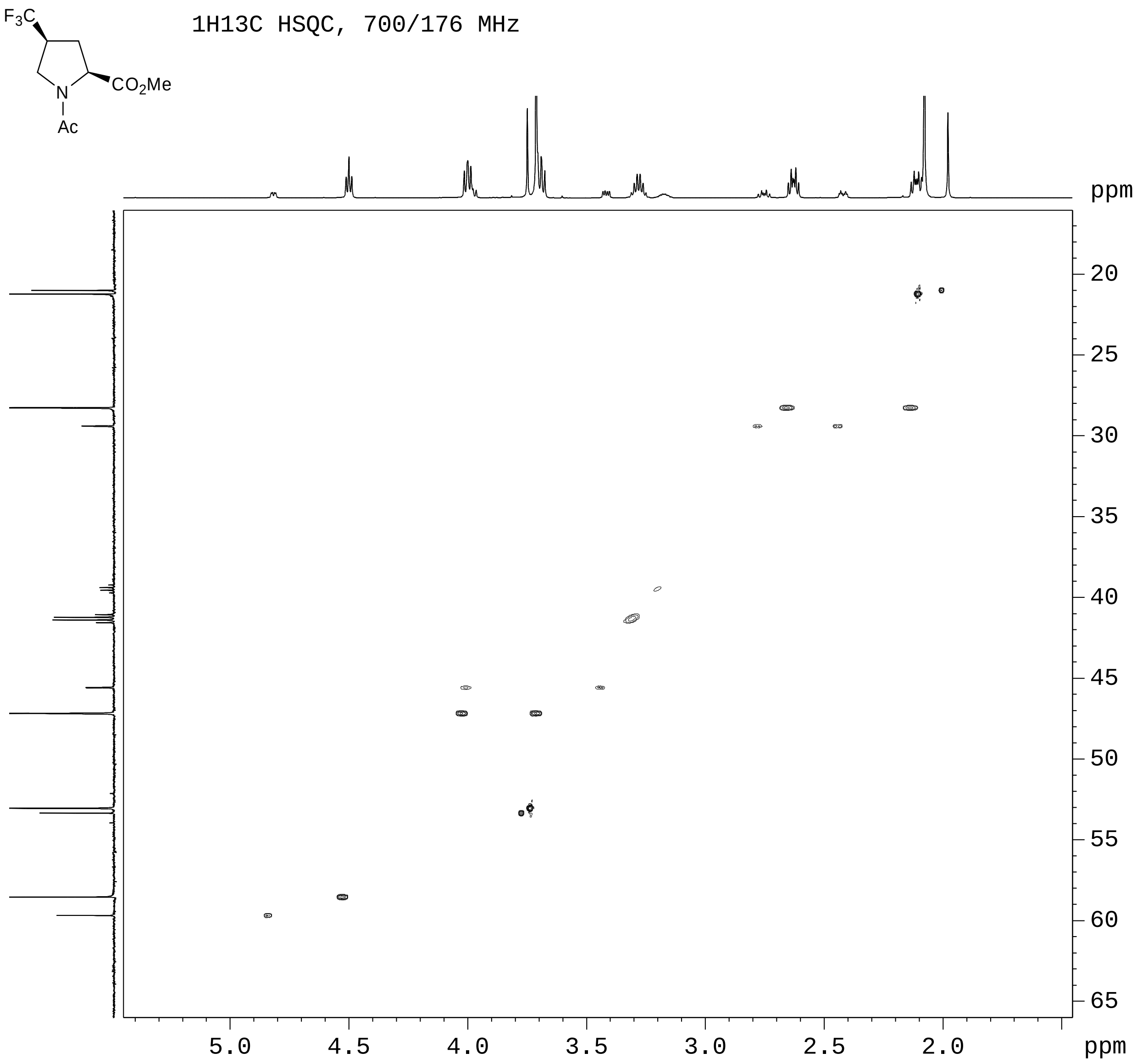
1H13C HSQC, 700/176 MHz



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PROCNO                1

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DS                   16
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FIDRES         1.366969 Hz
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RG                 2050
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DE                10.00 usec
TE                298.3 K
CNST2       145.0000000
D0           0.00000300 sec
D1           2.00000000 sec
D4           0.00172414 sec
D11          0.03000000 sec
D16          0.00020000 sec
D24          0.00086207 sec
IN0          0.00005680 sec
ZGOPTNS

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NUC1                 1H
P1                17.00 usec
P2                34.00 usec
P28             1000.00 usec
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======== CHANNEL f2 ========
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CPDPRG[2           garp
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PCPD2             55.00 usec
PLW2        67.00000000 W
PLW12        2.92919993 W

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GPNAM[3]     SMSQ10.100
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GPZ2              20.10 %
GPZ3              11.00 %
GPZ4              -5.00 %
P16             1000.00 usec
P19              600.00 usec

F1 - Acquisition parameters
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FIDRES        34.386005 Hz
SW               49.998 ppm
FnMODE    Echo-Antiecho

F2 - Processing parameters
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SF          700.1700000 MHz
WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
PC                10.00

F1 - Processing parameters
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WDW               QSINE
SSB                   2
LB       0 Hz
GB       0
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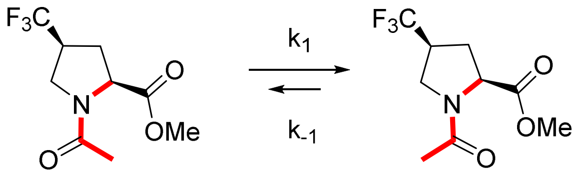
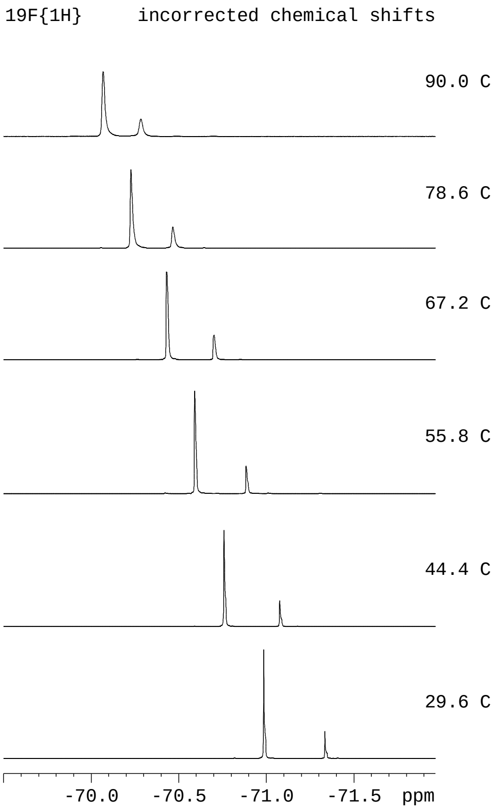
Thermodynamic parameters of the *trans-cis* rotation in 8



s-*cis* $K_{\textit{trans-cis}} = k_1/k_{-1}$ s-*trans*

Basic experimental procedure is described in the main text. ^{19}F NMR 30-deg flip angle spectra were collected on 471 MHz resonance frequency without or with ^{1}H decoupling (inverse gated, no NOE enhancement).

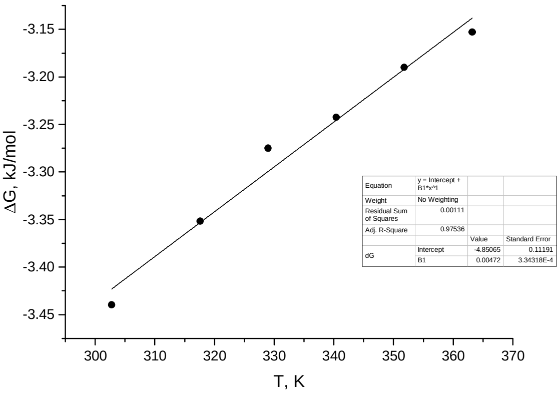
The 1D time domain contained 131072 data-points making 2.8 s acquisition time. Recycling delay was 1 s. The spectra were collected from 4 steady state and 32 recorded transients. Time domain spectra were processed with Gaussian windowing (gb = 0.05, lb = -1 Hz) in a phase sensitive fashion, baseline corrected and integrated. The K values were calculated directly from the integrals and converted into ΔG for plotting.



- measured in deuterium oxide. The shift due to the lock (water) resonance temperature shift was not corrected.

$T_{calibrated}$, K	T, °C	K_{tc}	ΔG, kJ/mol
302.8	29.6	3.92	-3.44
317.6	44.4	3.56	-3.35
329.0	55.8	3.31	-3.27
340.4	67.2	3.14	-3.24
351.8	78.6	2.98	-3.19
363.2	90.0	2.84	-3.15

linearization:



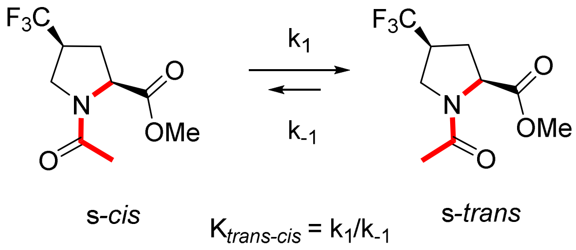
Equation	y = Intercept + B1*x^1		
Weight	No Weighting		
Residual Sum of Squares	0.00111		
Adj. R-Square	0.97536		
		Value	Standard Error
dG	Intercept	-4.85065	0.11191
	B1	0.00472	3.34318E-4

Determined parameters of the rotation:

ΔH = -4.85±0.11 kJ/mol

ΔS = -4.72±0.33 J/mol·K

Kinetic parameters of the *trans-cis* rotation in 8

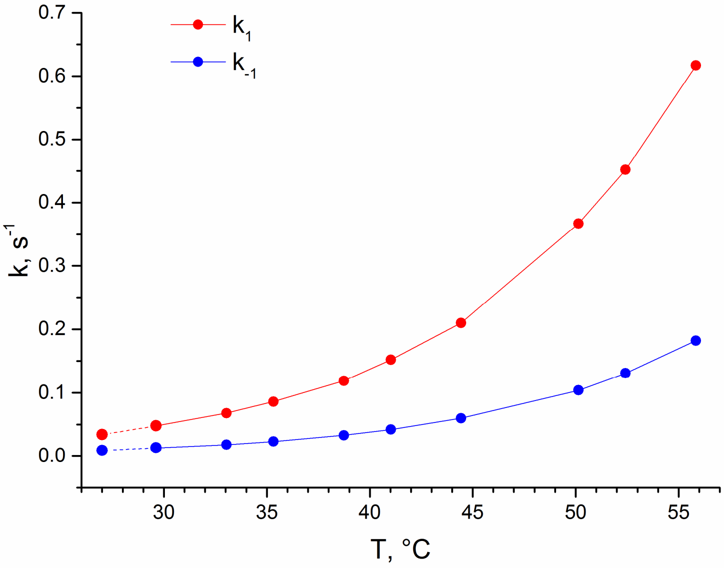


Basic experimental procedure is described in the main text. The ^{19}F EXSY spectra were collected on 471 MHz resonance frequency without decoupling.

The spectral width was 2.0x2.0 ppm, time domain array 2048x512 data-points, making acquisition times of 1.11x0.26 s. Recycling delay was 1 s, number of scans 2. The spectra were processed with sinc squared windowing on both dimensions in a phase sensitive mode, baseline corrected and integrated. 128 data-points were linearly predicted in the indirect dimension. The difference to a standard NOESY processing setup was the use of squared sinc function instead of conventional squared sine bell. This change was done in order to make additional smoothening of the peaks for integration and reduce truncation noise.

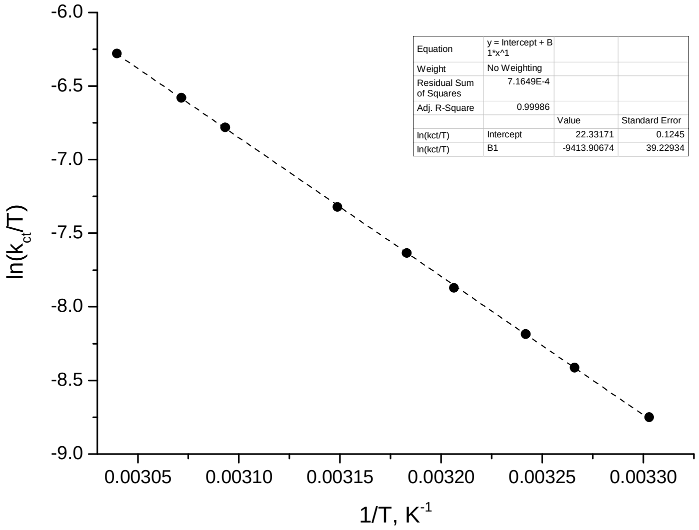
Exchange rate matrices calculations were performed in EXSYCalc® (Mestrec), where EXSY integral values were inset. Secondary diagonal elements were the k values given in the table below:

$T_{calibrated}$, K	T, °C	k_1, s^{-1}	k_{-1}, s^{-1}
302.8	29.6	0.048	0.013
306.2	33.0	0.068	0.018
308.5	35.3	0.086	0.023
311.9	38.7	0.119	0.033
314.2	41.0	0.152	0.042
317.6	44.4	0.21	0.06
323.3	50.1	0.367	0.104
325.6	52.4	0.452	0.131
329.0	55.8	0.617	0.182



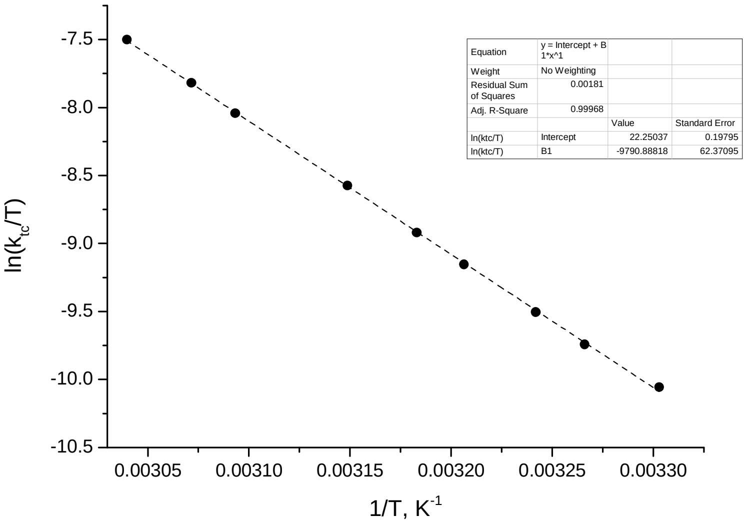
Eyring plots:

for the k_1



Equation	y = Intercept + B 1*x^1		
Weight	No Weighting		
Residual Sum of Squares	7.1649E-4		
Adj. R-Square	0.99986		
		Value	Standard Error
ln(kct/T)	Intercept	22.33171	0.1245
ln(kct/T)	B1	-9413.90674	39.22934

for the k_{-1}



Equation	y = Intercept + B 1*x^1		
Weight	No Weighting		
Residual Sum of Squares	0.00181		
Adj. R-Square	0.99968		
		Value	Standard Error
ln(ktc/T)	Intercept	22.25037	0.19795
ln(ktc/T)	B1	-9790.88818	62.37095

Determined parameters of the process:

for k_1:

$\Delta H = 78.3 \pm 0.3$ kJ/mol

$\Delta S = -11.9 \pm 1.0$ J/mol·K

For 300 K $E_a = 81.9 \pm 0.6$ kJ/mol

for k_{-1}:

$\Delta H = 81.4 \pm 0.5$ kJ/mol

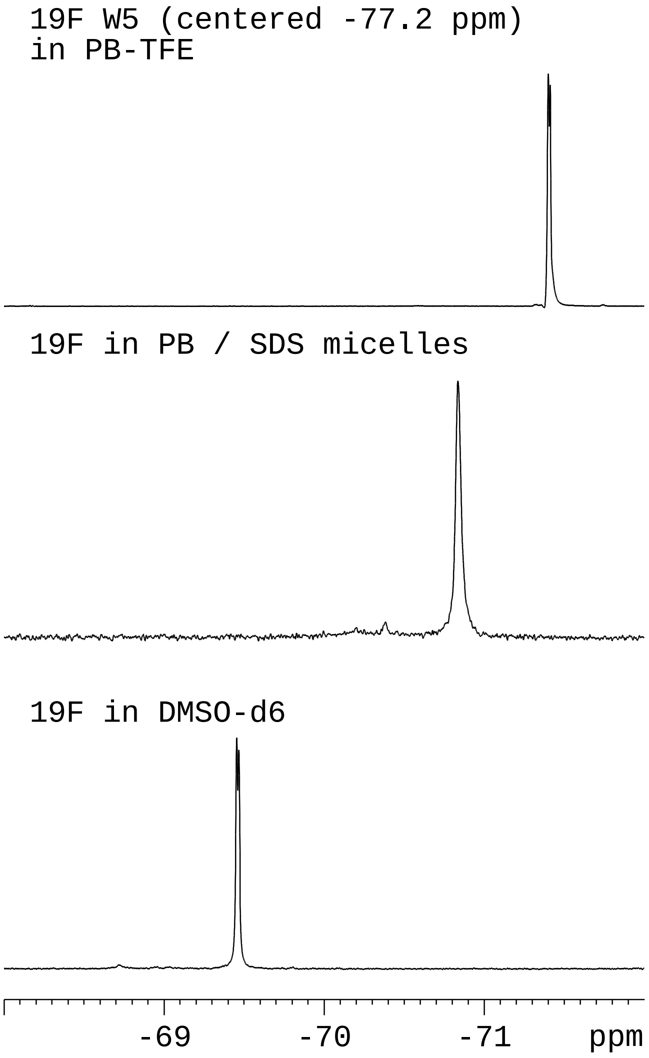
$\Delta S = -12.6 \pm 1.6$ J/mol·K

For 300 K $E_a = 85.2 \pm 1.0$ kJ/mol

ΔE_a (*cis/trans* – *trans/cis*)= -3.4 kJ/mol

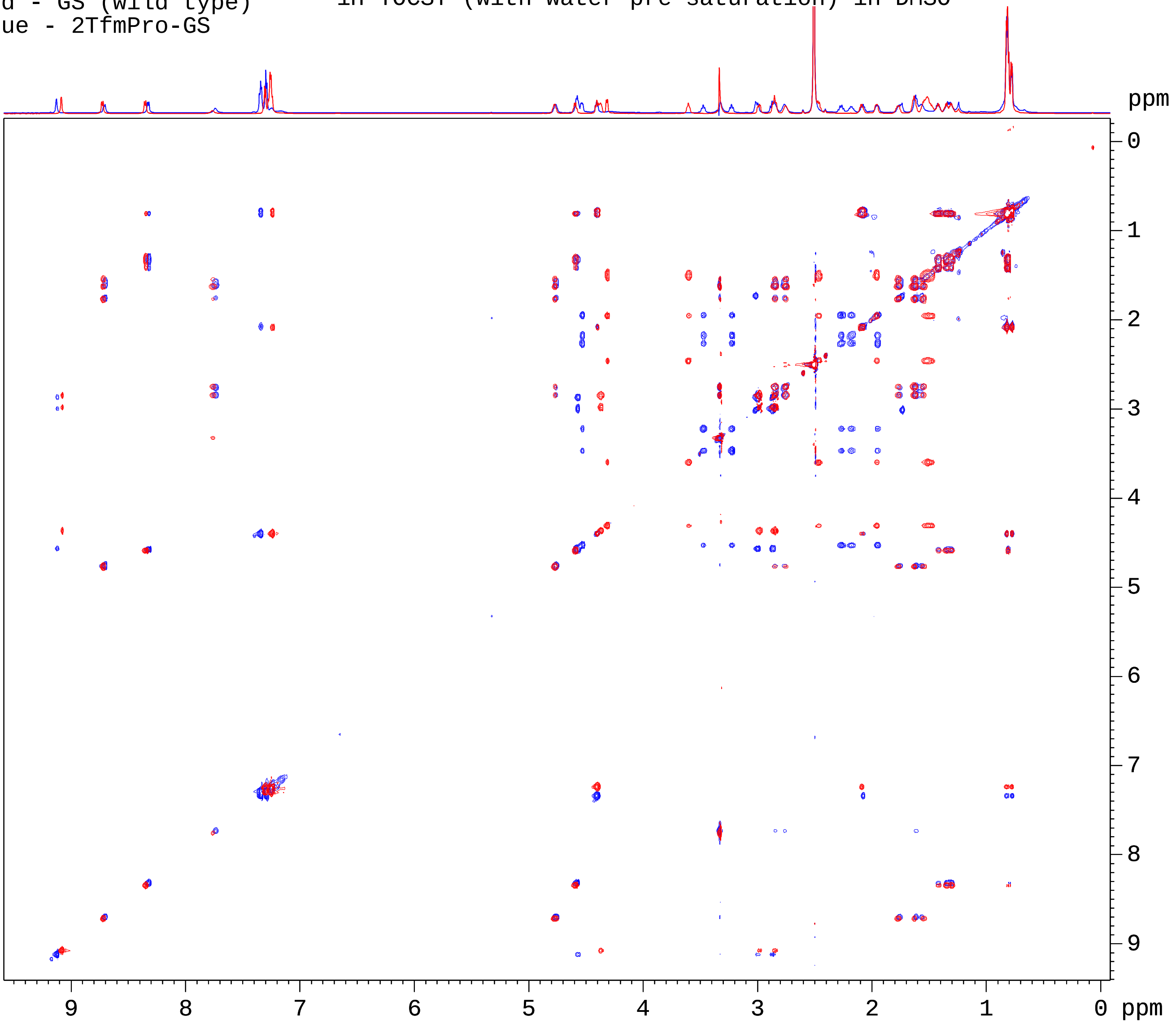
Solution NMR spectra of the peptides

^{19}F NMR spectra of **2TfmPro-GS** in different media (at 298 K):



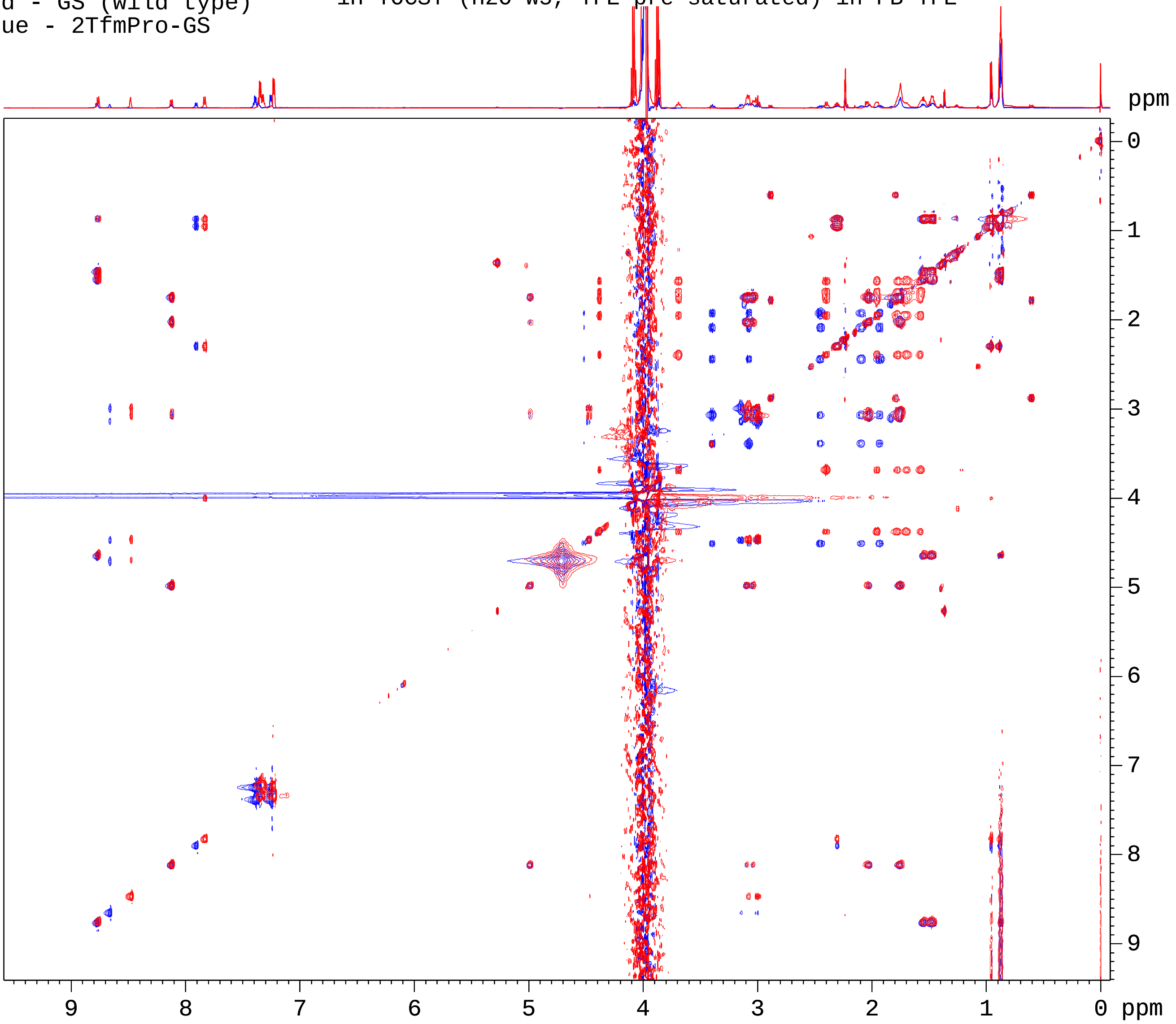
red - GS (wild type)
blue - 2TfmPro-GS

1H TOCSY (with water pre-saturation) in DMSO



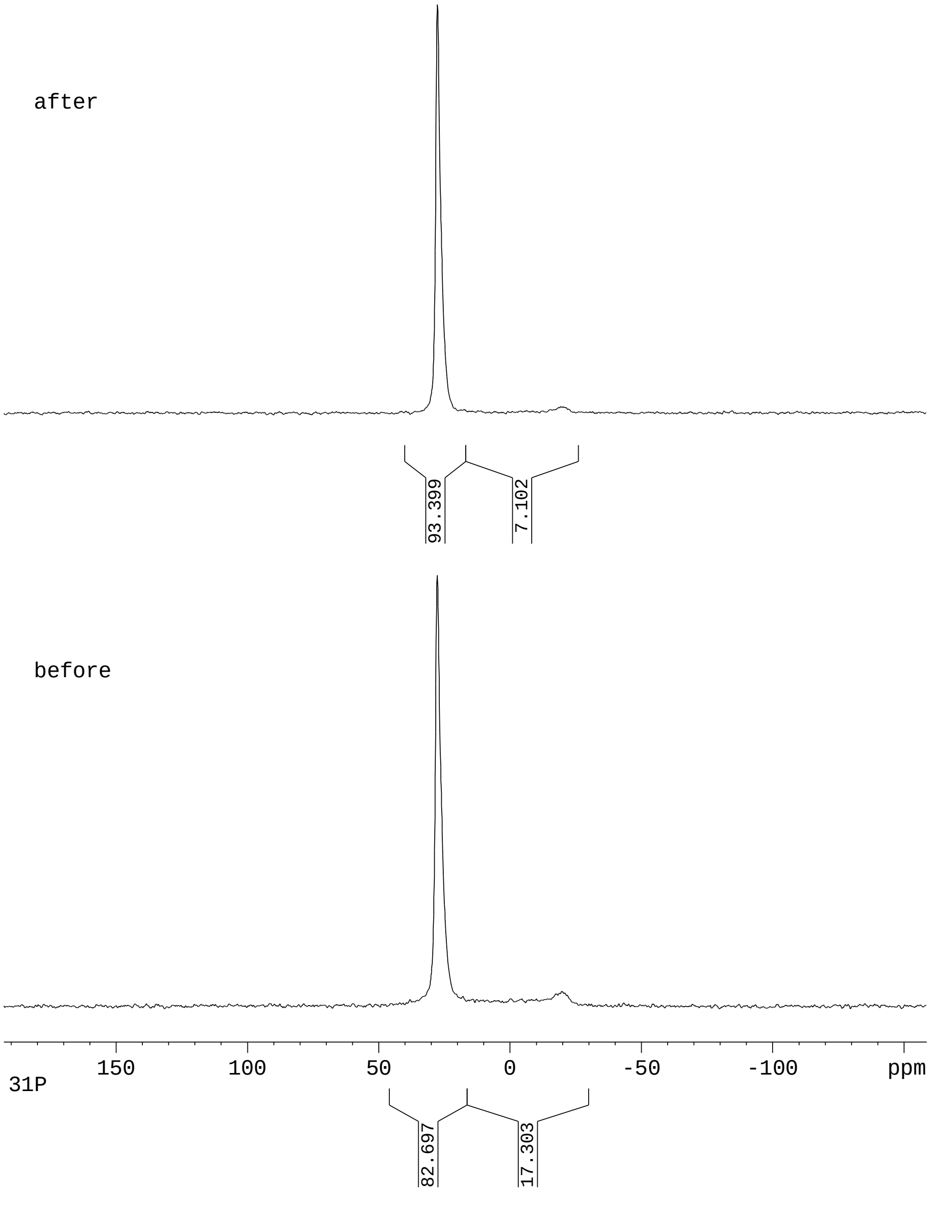
red - GS (wild type)
blue - 2TfmPro-GS

1H TOCSY (H2O W5, TFE pre-saturated) in PB-TFE

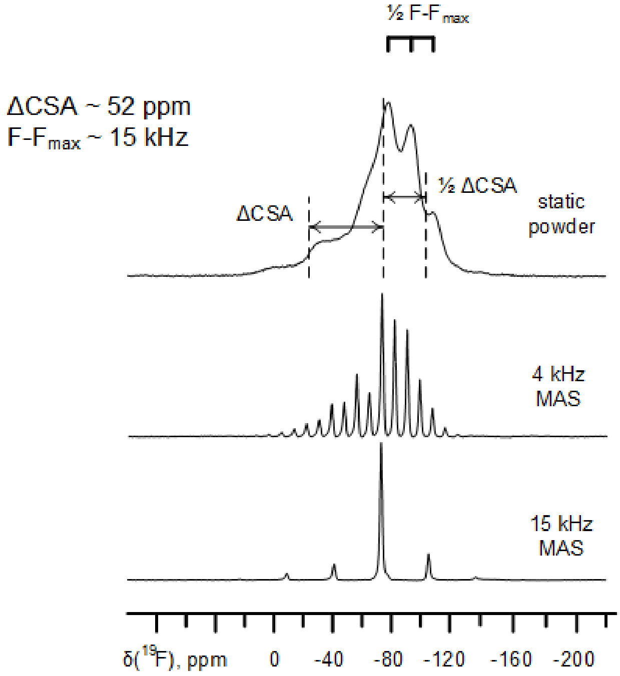


Supporting solid state NMR spectra

^{31}P NMR of the **2TfmPro-GS**/DLPC oriented sample before and after the ^{19}F NMR temperature series reported in the main text. The spectra were measured well above the phase transition of DLPC – at 303 K. Sample macroscopic placement was such that the membrane normal was parallel to the magnetic field. The percentage of the desired macroscopic alignment was found to be higher than 80 %.



^{19}F NMR powder static and magic angle spinning (MAS) spectra of **2TfmPro-GS** for estimation of the maximal anisotropic parameters. The spectra were taken at 298 K and 470.5 MHz. The isotropic chemical shift was found to be -70.4 ppm.



pK_a determination for the amino group of TfmPro

25 mg of **1** was dissolved in 1 ml dichloromethane and 0.5 ml trifluoroacetic was added. The solution was stirred at the room temperature for an hour and the volatiles were blown off by excessive nitrogen current. The rest was dissolved in 50 µl of water and resulting solution was aliquotized 2µl in the NMR tubes containing 550 µl of 150 mM sodium phosphate buffer. The final concentration of TfmPro in the buffered systems was therefore ~ 6 mM. The last data-point at pH 13 was measured in 0.1 M NaOH.

^{19}F NMR spectra were measured in inverse gated decoupled 30-degree flip angle experiments at 471 MHz frequency and 298 K. The residual TFA signal was observed at -75.39±0.01 ppm in all samples and the ratio between TfmPro and TFA was found as 1.3.

From these measurements several points deviated from the sigmoidal behavior in the range 8.5 – 10.1. In this range the buffer capacity of phosphate buffer is too low to quench 16 mM acidic additive.

Therefore we collected additional dataset with diluted TfmPro*TFA. Such that the final concentration was 0.6 and 0.16 mM.

All datapoints were collected together except of the ones rejected where preference was given to the more diluted samples. The found ^{19}F chemical shifts of TfmPro were plotted against pH as depicted, fitted according to the Boltzman fit (OrifinPro 9.1, alternatively logistic fit with similar outcome) and the first derivative of the fit delivered the maximum point 8.5. Apparent standard error was 0.1 (electrode).

^{31}P NMR chemical shifts were used for control. Two series showed after all a good consistency to one another. The bending point for the second ionization constant was found at 6.8 in both series.