Supporting Materials

Novel sulfonamide-based carbamates as selective inhibitors of BChE

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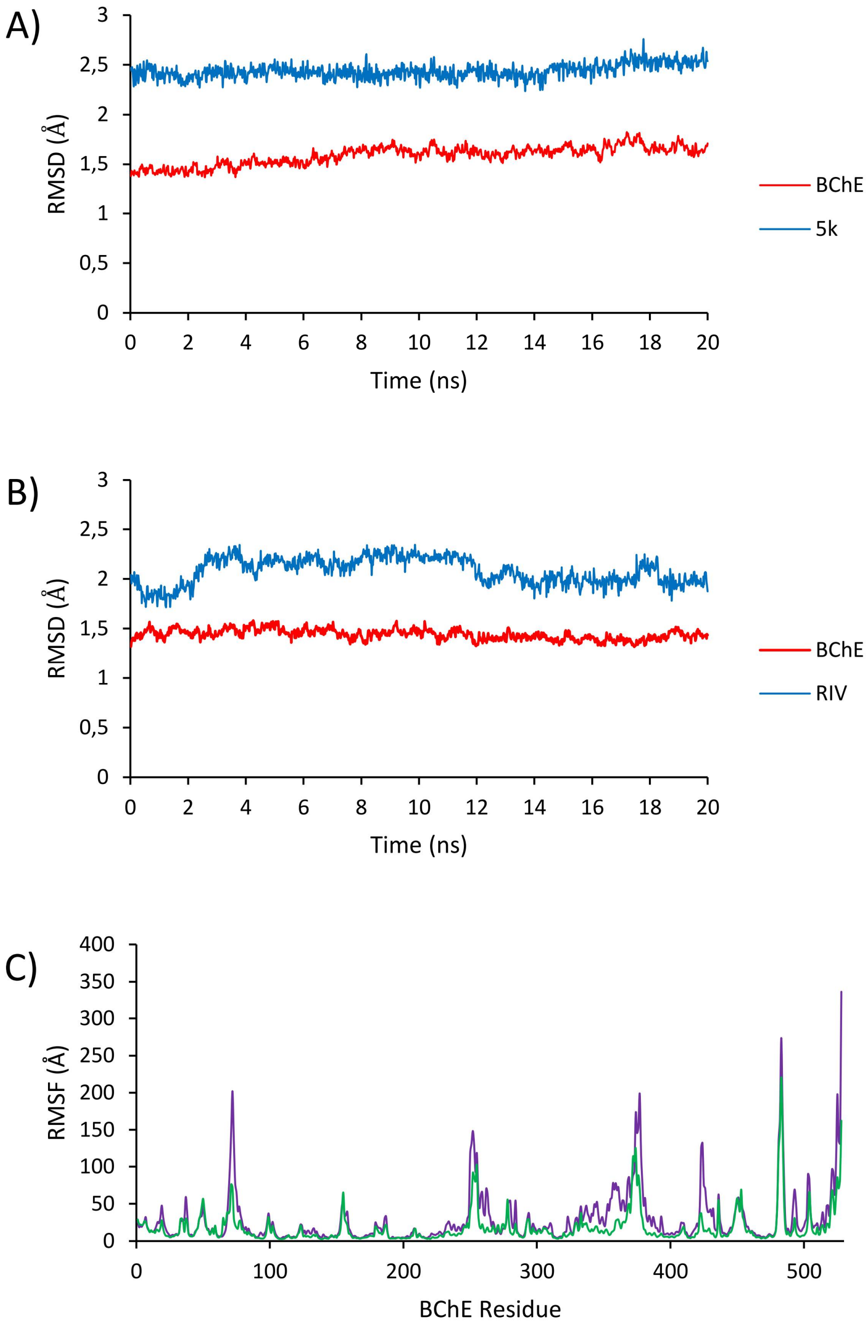


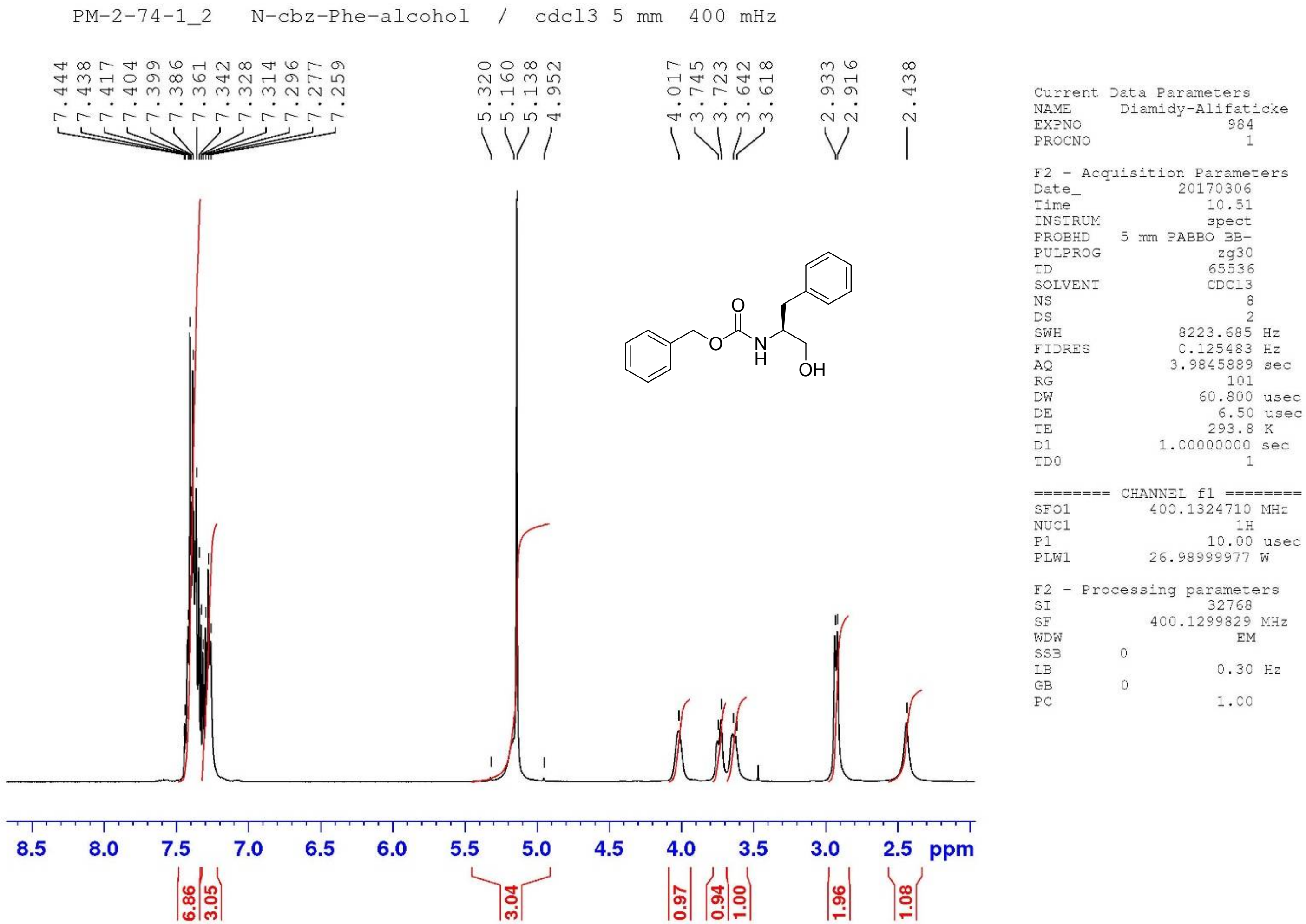
Figure S1 Root-mean-square deviations (RMSD) of (A) BChE-5k and (B) BChE-RIV complexes. (C) Comparative root-mean-square fluctuations (RSMF) of the Cα atoms of BChE when complexed with 5k (violet) and RIV (green).

Experimental part

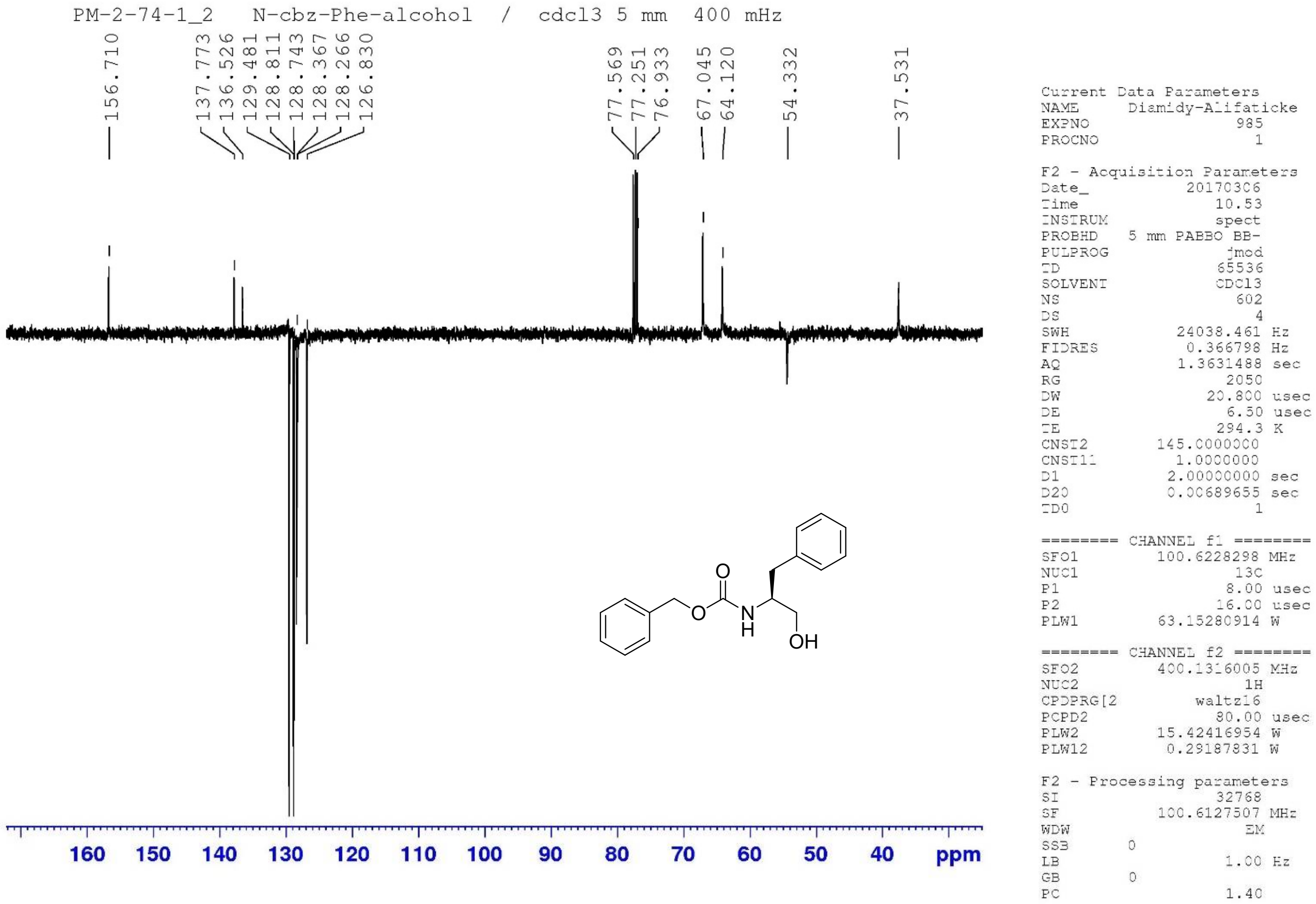
All reagents and solvents were purchased from commercial sources (TCI Europe, Sigma-Aldrich, Acros organics, fluorochem, Merck, Lach-Ner). Commercial grade reagents were used without further purification. Reactions were monitored by thin layer chromatography plates coated with 0.2 mm silica gel 60 F_{254} (Merck). TLC plates were visualized by UV irradiation (254 nm) or in a 5% solution of phosphomolybdic acid in ethanol. All melting points were determined on a Melting Point B-540 apparatus (Büchi, Switzerland) and are uncorrected. IR spectra were recorded on a Nicolet 6700 FT-IR spectrometer (Thermo Fisher Scientific, Waltham, MA, USA) over the range of 400–4000 cm^{-1} using the ATR technique. NMR spectra were measured in $CDCl_3$ solutions at ambient temperature on a Bruker Avance™ III 400 spectrometer at frequencies ^{1}H (400 MHz), ^{13}C (100.26 MHz), and ^{19}F (376.46) or a Bruker Ascend™ 500 spectrometer at frequencies ^{1}H (500.13 MHz), ^{13}C {^{1}H} (125.76 MHz), and ^{19}F (470.61 MHz). Chemical shifts, δ, are given in ppm, related to the residual solvent peak $CDCl_3$ 7.27 or to tetramethylsilane (TMS) as an internal standard. The coupling constants (*J*) are reported in [Hz]. Elemental analysis (C, H, N, S) was performed on an automatic microanalyzer Flash 2000 organic elemental analyzer. Mass spectrometry with high resolution was performed by the dried droplet method using a MALDI mass spectrometer LTQ Orbitrap XL (Thermo Fisher Scientific) equipped with a nitrogen UV laser (337 nm, 60 Hz). Spectra were measured in the positive ion mode and in regular mass extent with resolution 100,000 at m/z = 400. 2,5-Dihydroxybenzoic acid (DBH) was used as the matrix.

Characterization data of prepared compounds:

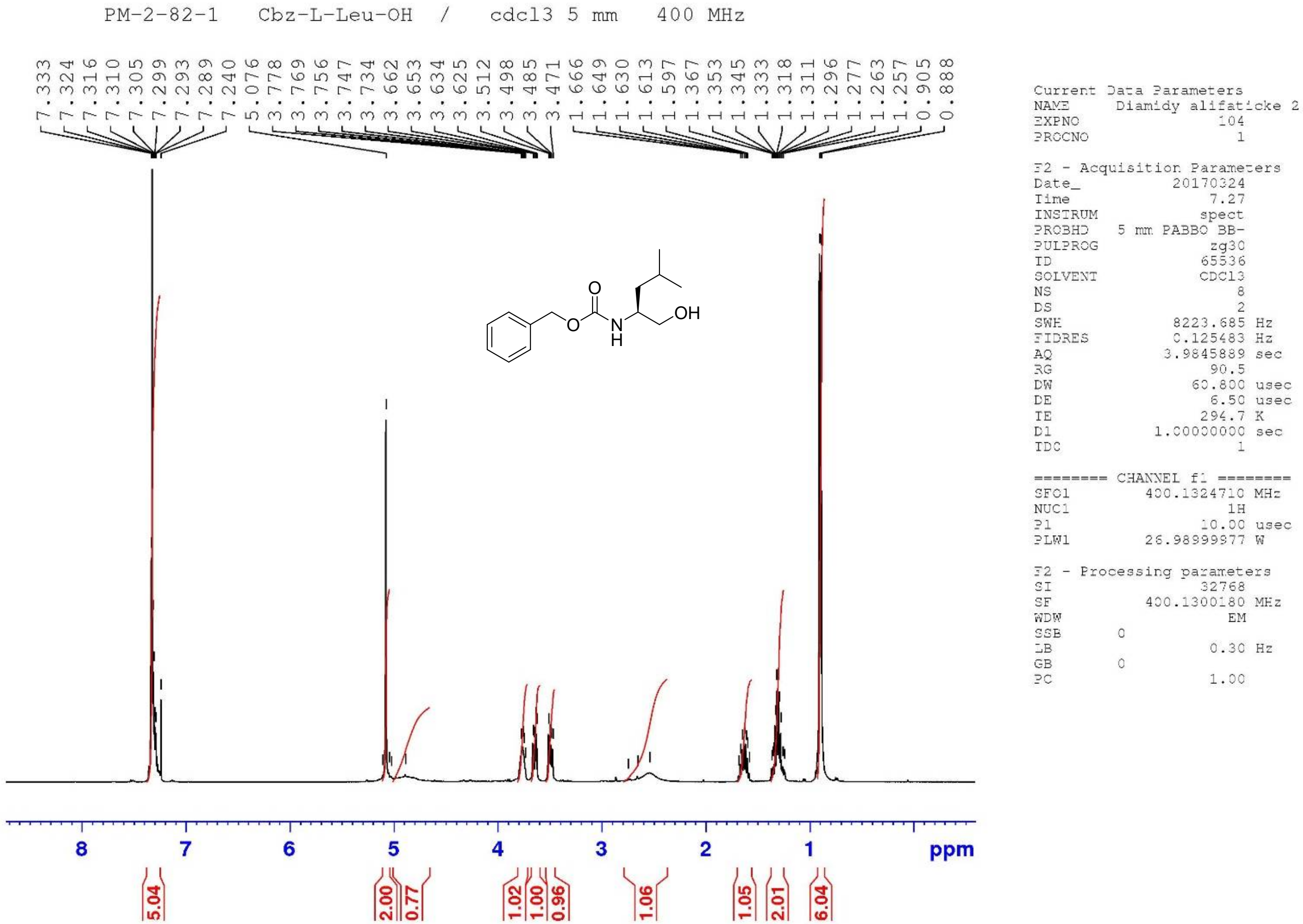
1.1. Copy of [1]H NMR of **2a**



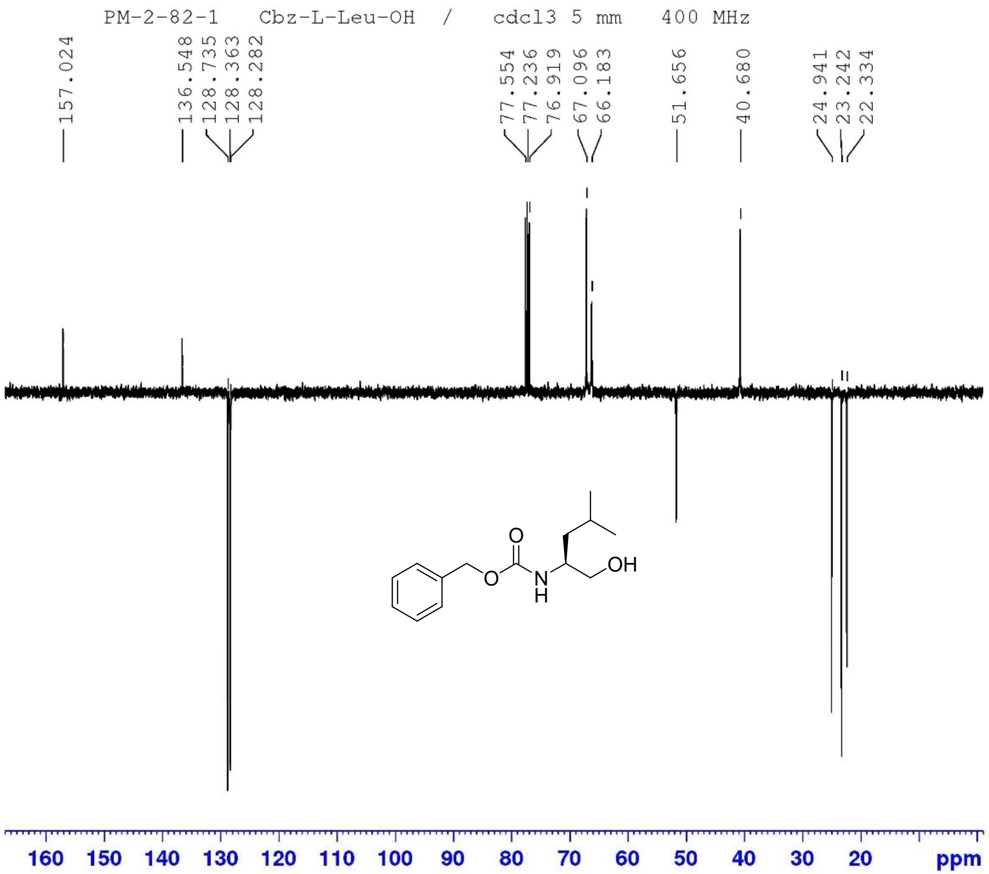
1.2. Copy of ^{13}C NMR of **2a**



1.3. Copy of ^{1}H NMR of **2b**



1.4. Copy of ^{13}C NMR of **2b**



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PROCNO 1

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PULPROG jmod
TD 65536
SOLVENT CDCl3
NS 1120
DS 4
SWH 24038.461 Hz
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 2050
DW 20.800 usec
DE 6.50 usec
TE 295.3 K
CNST2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
D20 0.00689655 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6228298 MHz
NUC1 13C
P1 8.00 usec
P2 16.00 usec
PLW1 63.15280914 W

======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 15.42416954 W
PLW12 0.29187831 W

F2 - Processing parameters
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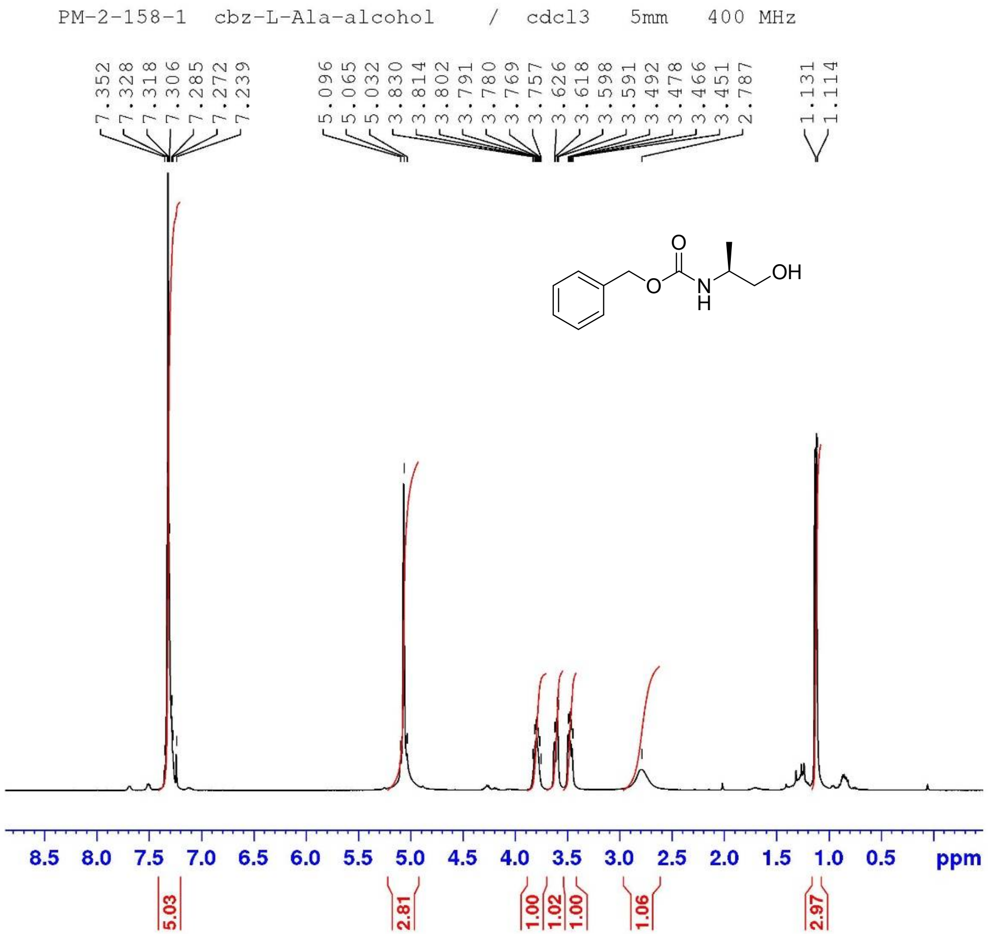
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

1.5. Copy of [1]H NMR of **2c**

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PROCNO                   1

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PULPROG               zg30
TD                   65536
SOLVENT              CDCl3
NS                      16
DS                       2
SWH               8223.685 Hz
FIDRES            0.125483 Hz
AQ               3.9845889 sec
RG                     101
DW                  60.800 usec
DE                    6.50 usec
TE                   294.5 K
D1              1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
SFO1           400.1324710 MHz
NUC1                    1H
P1                   11.00 usec
PLW1           16.15109062 W

F2 - Processing parameters
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WDW                     EM
SSB      0
LB                    0.30 Hz
GB       0
PC                    1.00
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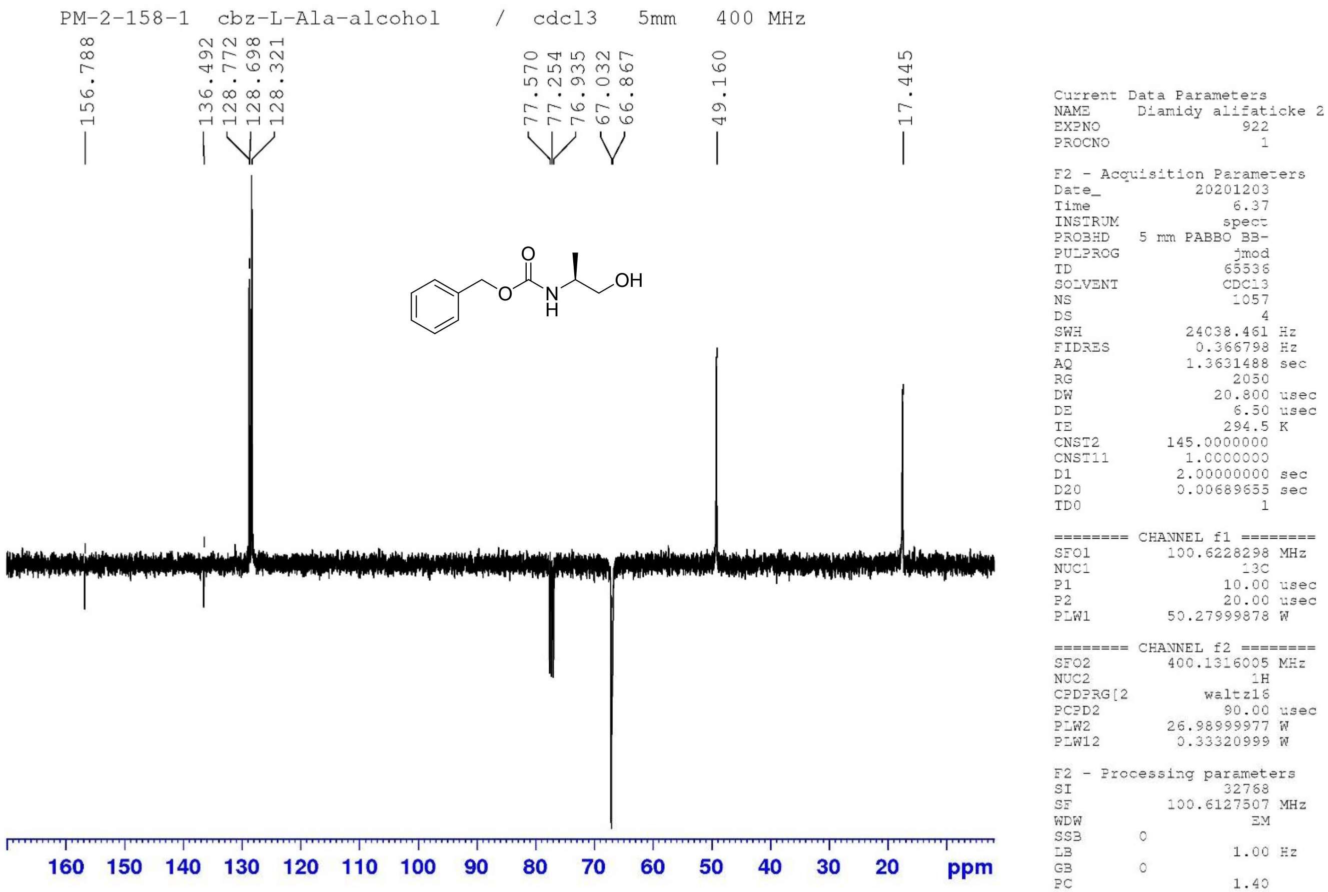
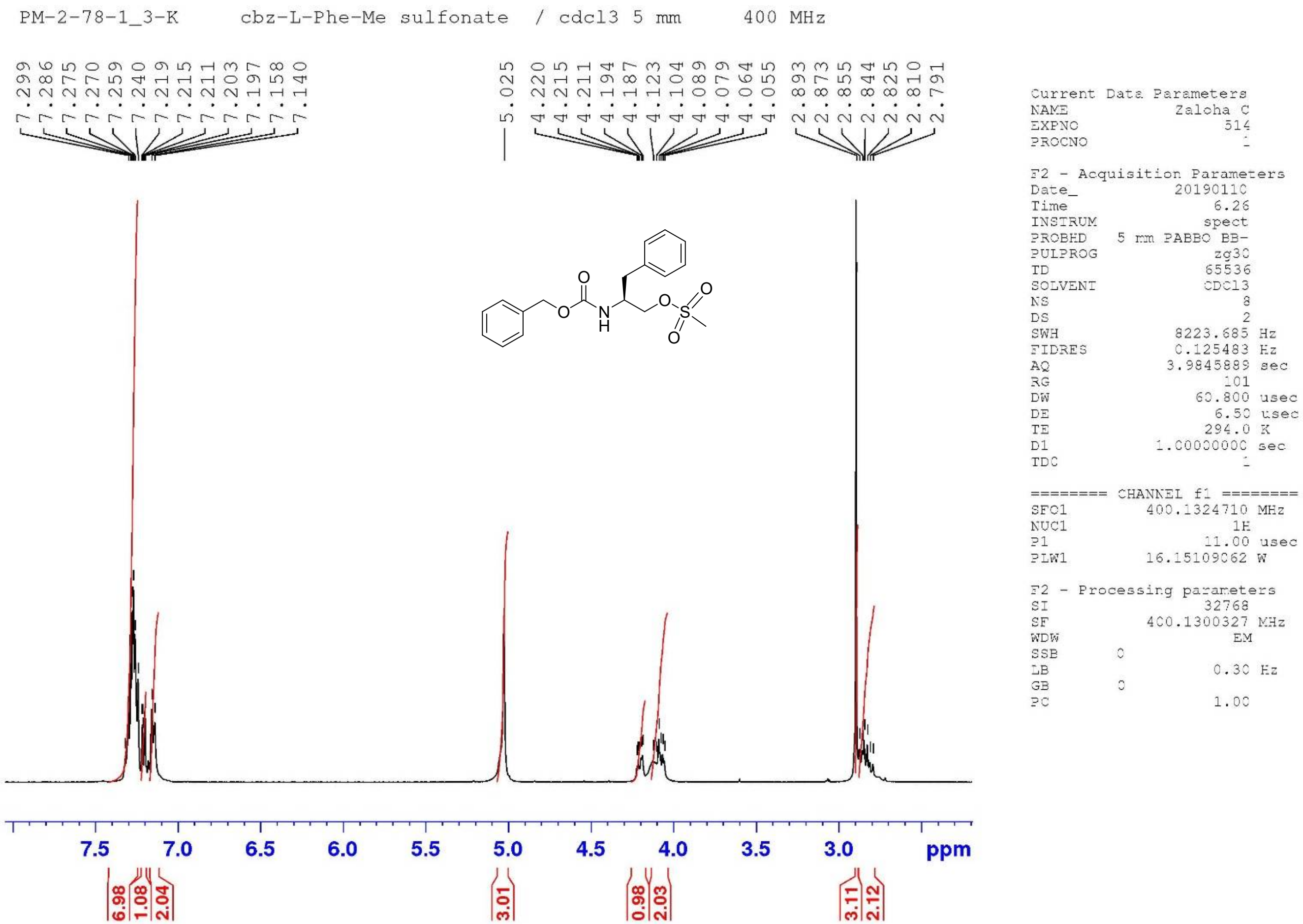
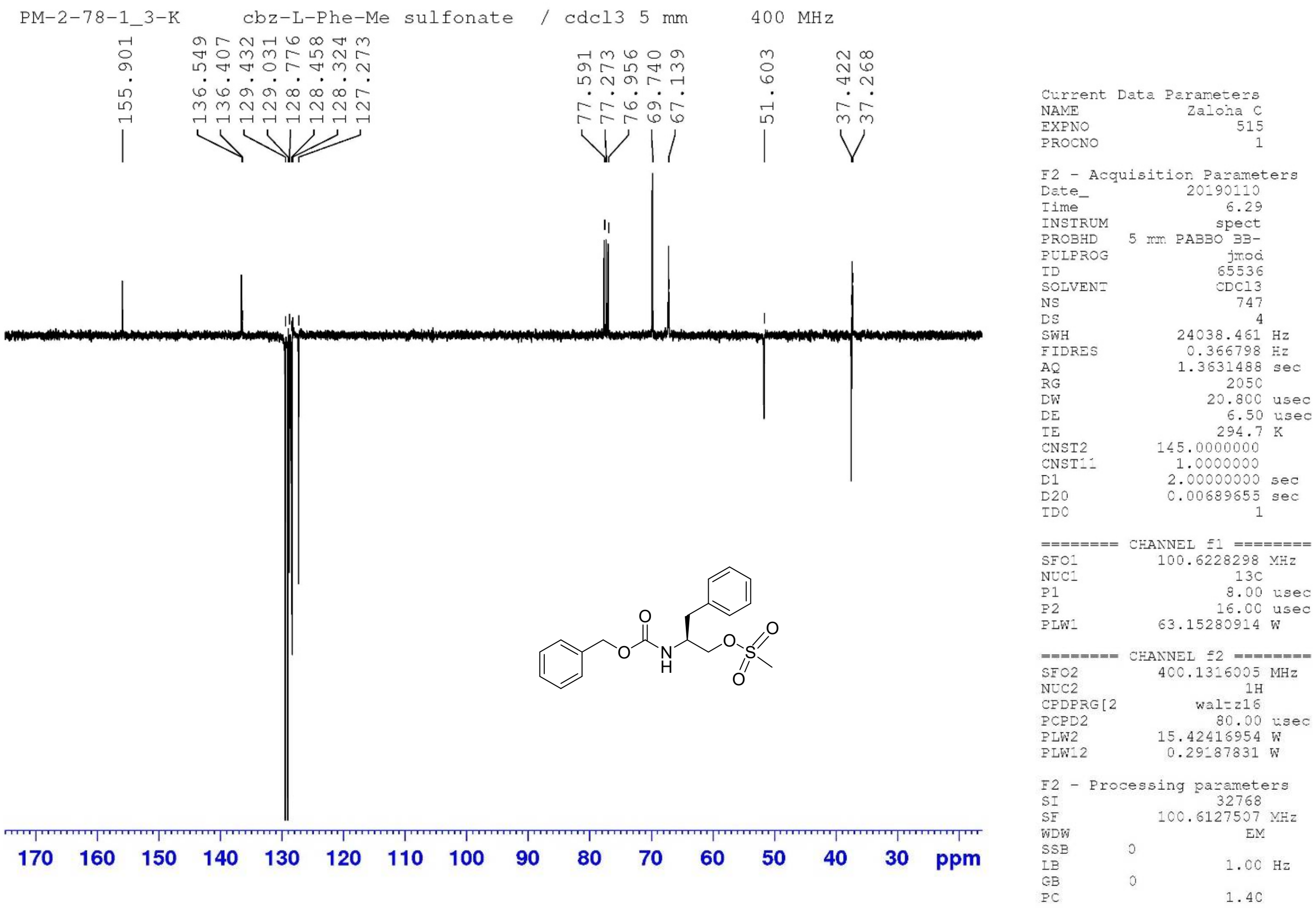
1.6. Copy of ^{13}C NMR of **2c**



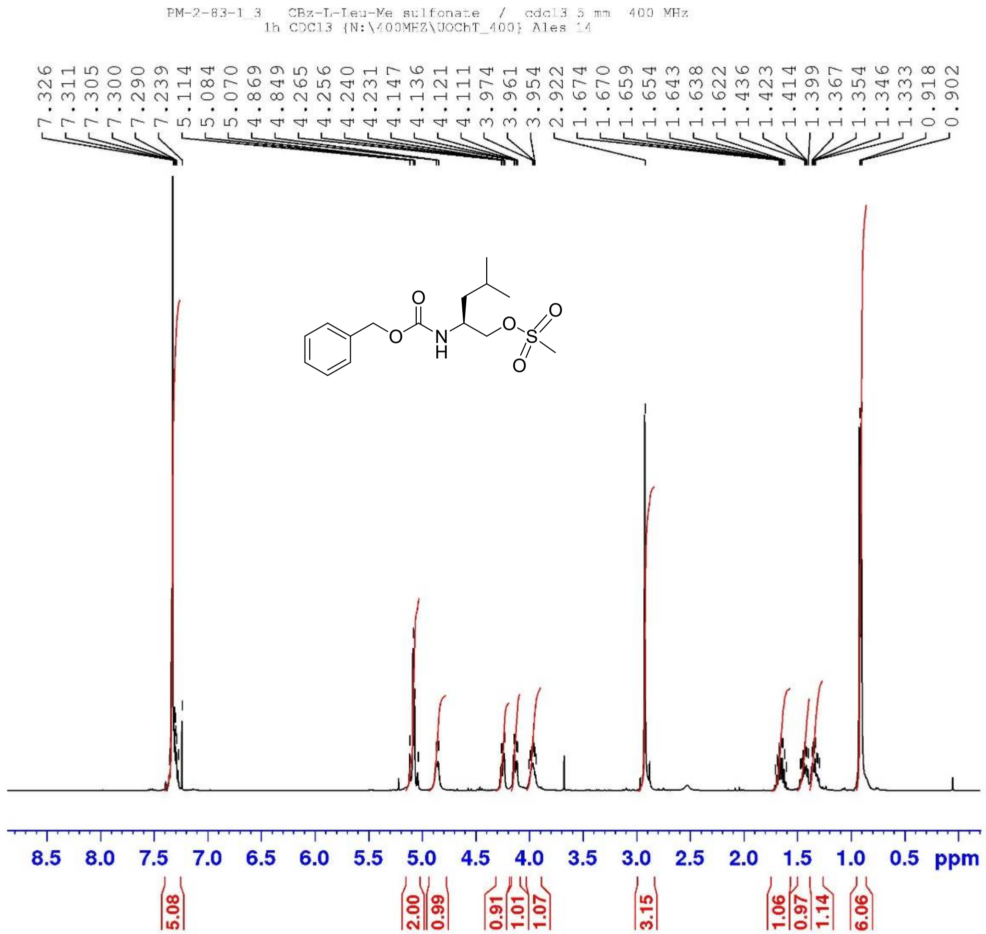
1.7. Copy of ^{1}H NMR of **3a**



1.8. Copy of ^{13}C NMR of **3a**



1.9. Copy of ^{1}H NMR of **3b**

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PROCNO                    1

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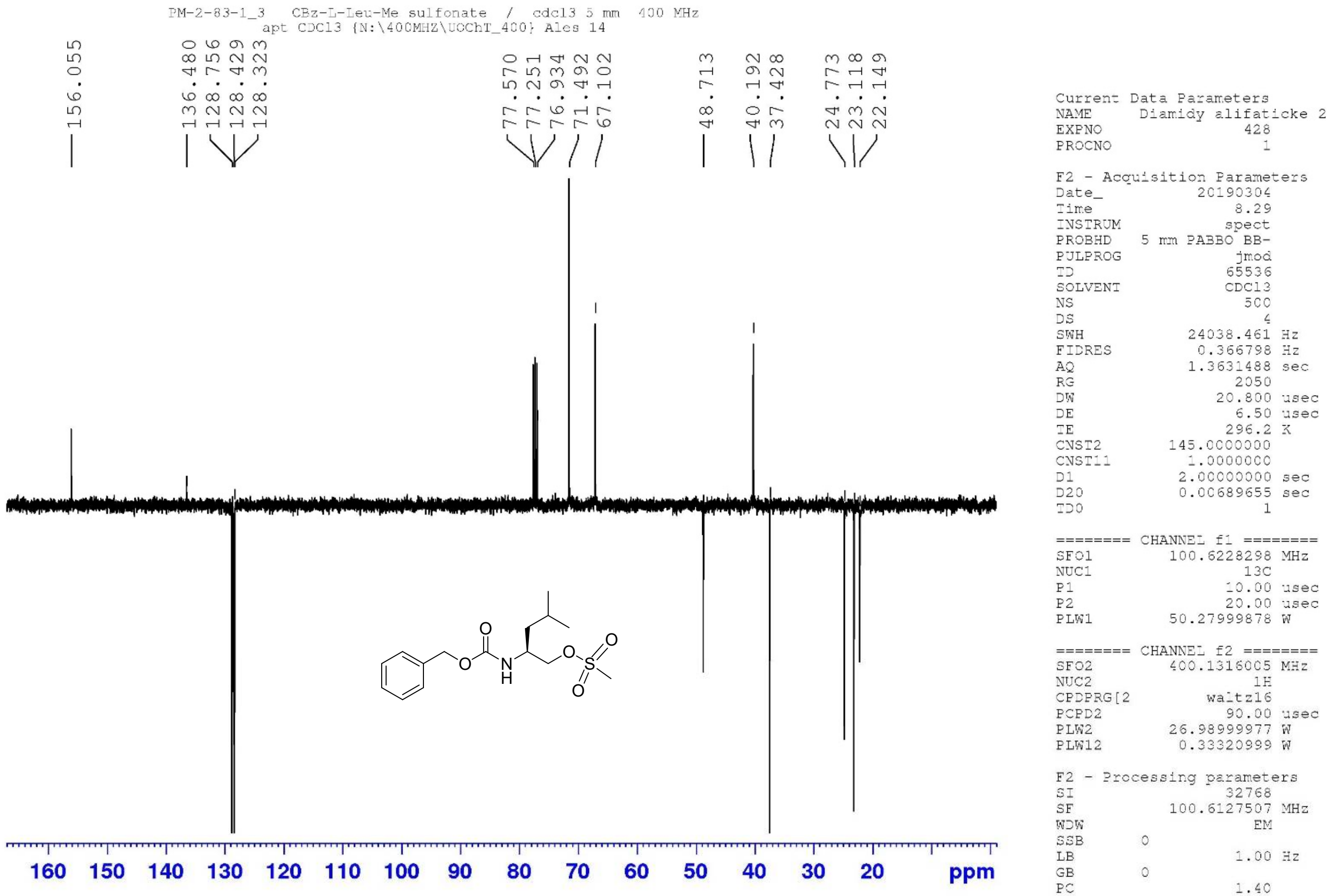
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        8
DS                        2
SWH             8223.685 Hz
FIDRES          0.125483 Hz
AQ             3.9845889 sec
RG                     90.5
DW               60.800 usec
DE                 6.50 usec
TE                295.2 K
D1           1.00000000 sec
TD0                       1

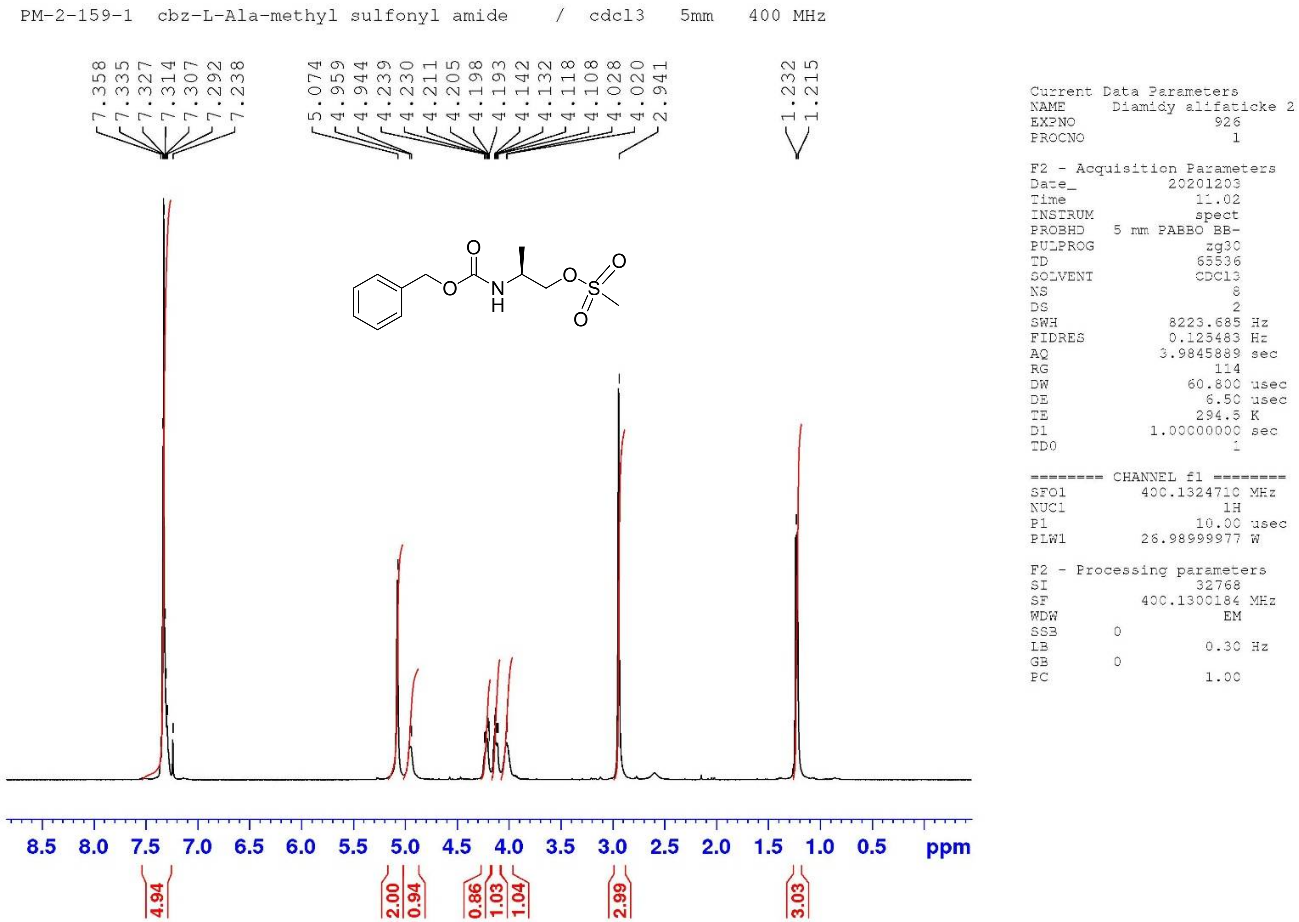
======== CHANNEL f1 ========
SFO1        400.1324710 MHz
NUC1                     1H
P1                10.00 usec
PLW1        26.98999977 W

F2 - Processing parameters
SI                    32768
SF          400.1300186 MHz
WDW                      EM
SSB      0
LB                  0.30 Hz
GB       0
PC                     1.00
```

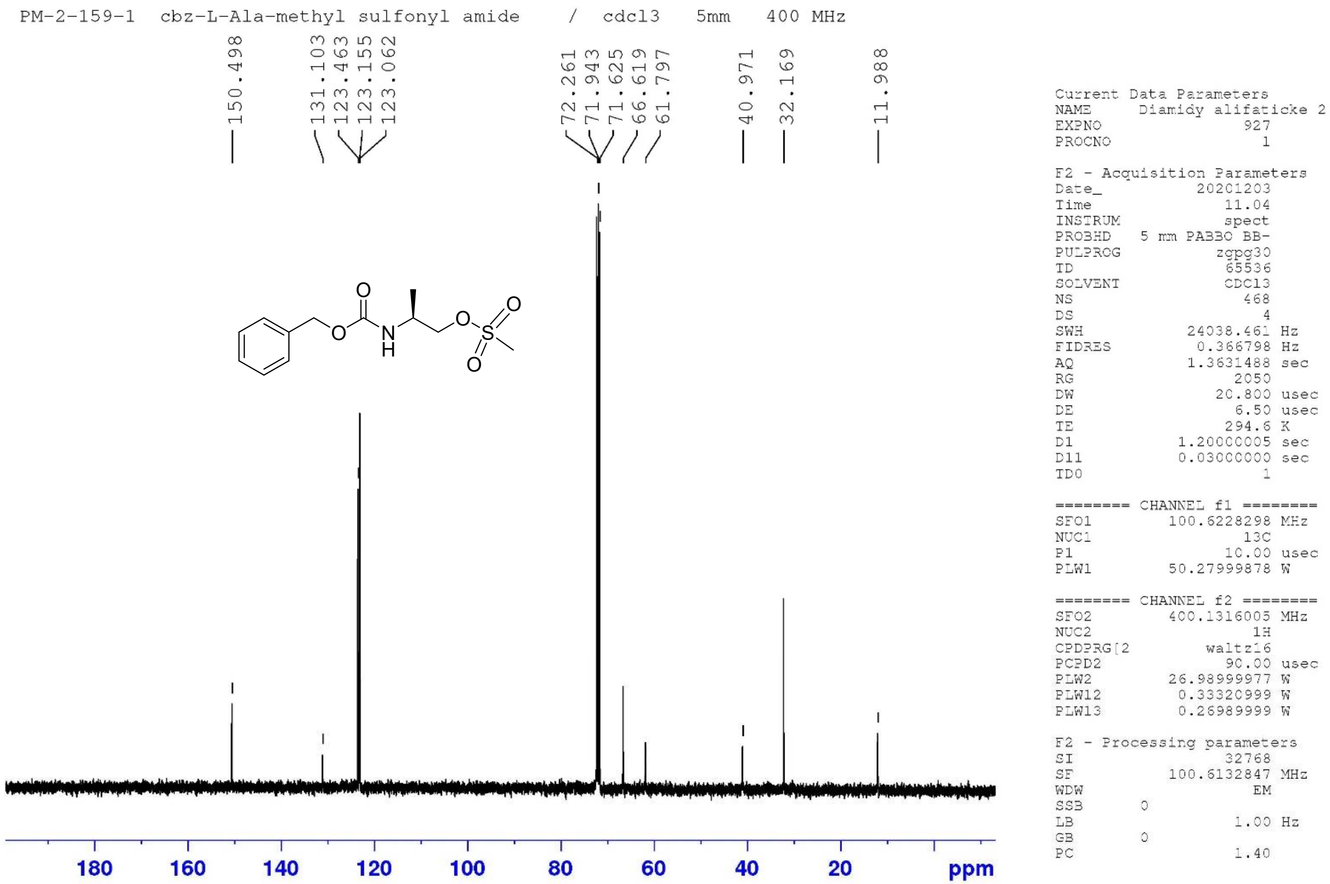
1.10. Copy of ^{13}C NMR of **3b**



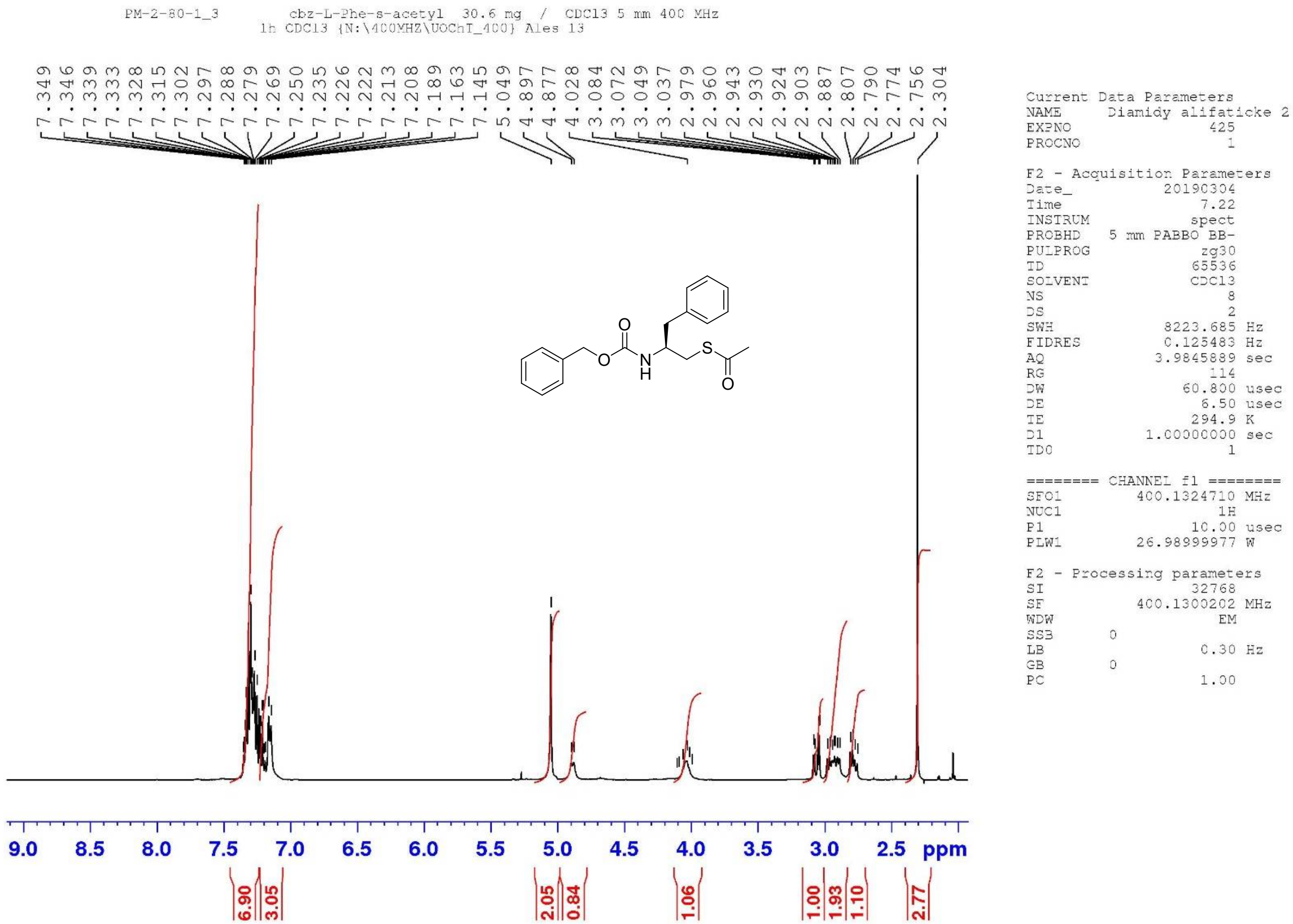
1.11. Copy of [1]H NMR of **3c**



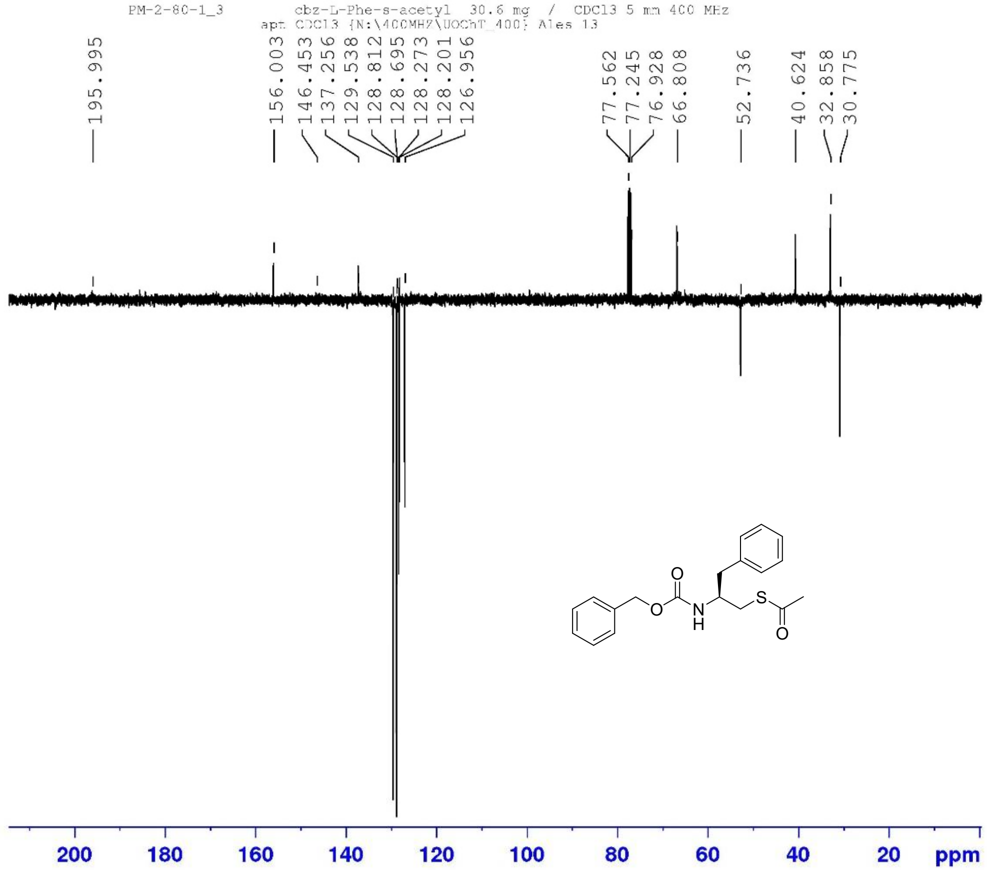
1.12. Copy of ^{13}C NMR of **3c**



1.13. Copy of [1]H NMR of **4a**



1.14. Copy of ^{13}C NMR of **4a**

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PROCNO                  1

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PULPROG              jmod
TD                  65536
SOLVENT             CDCl3
NS                    500
DS                      4
SWH             24038.461 Hz
FIDRES           0.366798 Hz
AQ              1.3631488 sec
RG                   2050
DW                 20.800 usec
DE                   6.50 usec
TE                  296.1 K
CNST2         145.0000000
CNST11          1.0000000
D1             2.00000000 sec
D20            0.00689655 sec
TD0                     1

======== CHANNEL f1 ========
SFO1          100.6228298 MHz
NUC1                  13C
P1                  10.00 usec
P2                  20.00 usec
PLW1          50.27999878 W

======== CHANNEL f2 ========
SFO2          400.1316005 MHz
NUC2                   1H
CPDPRG[2          waltz16
PCPD2               90.00 usec
PLW2          26.98999977 W
PLW12          0.33320999 W

F2 - Processing parameters
SI                  32768
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SSB      0
LB                   1.00 Hz
GB       0
PC                   1.40
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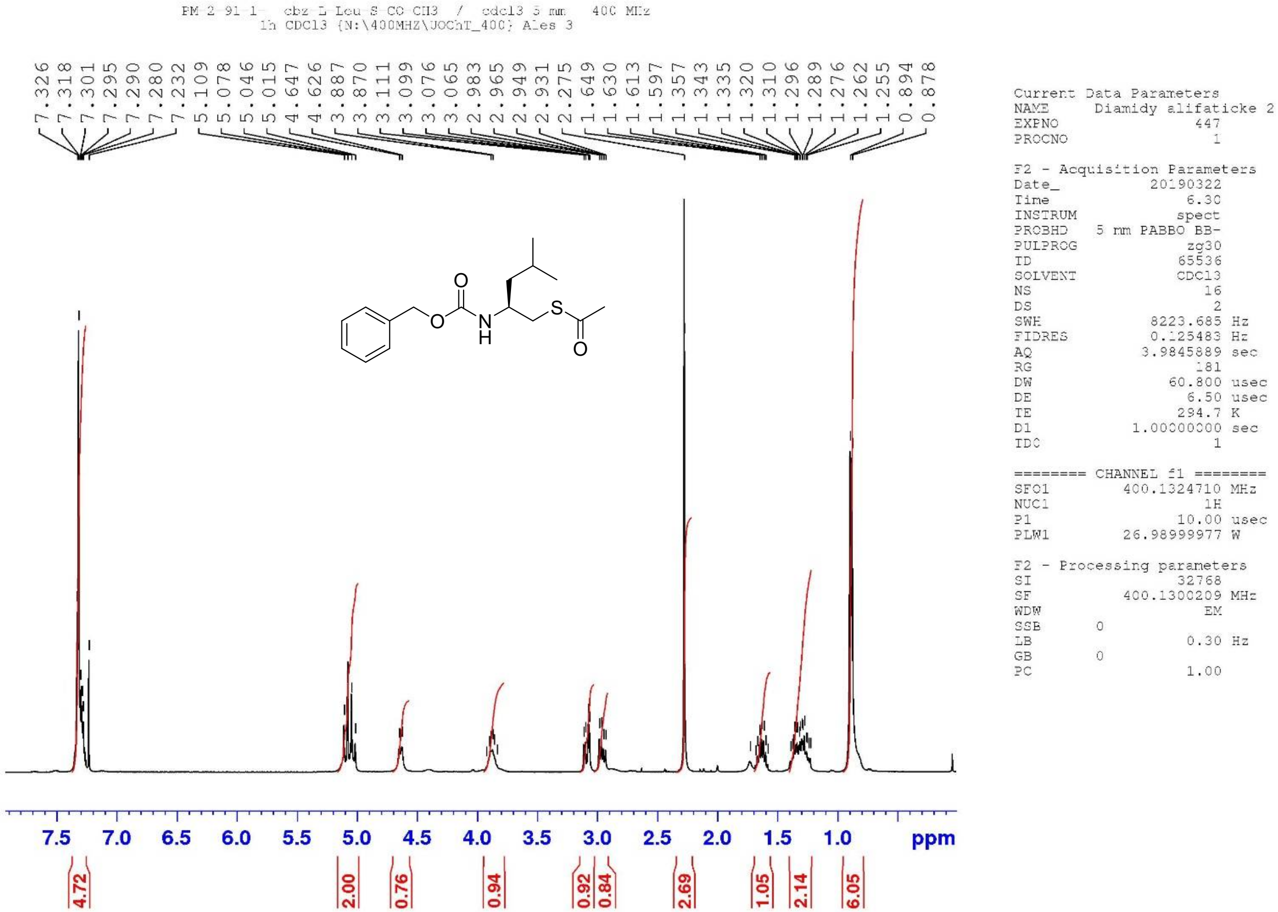
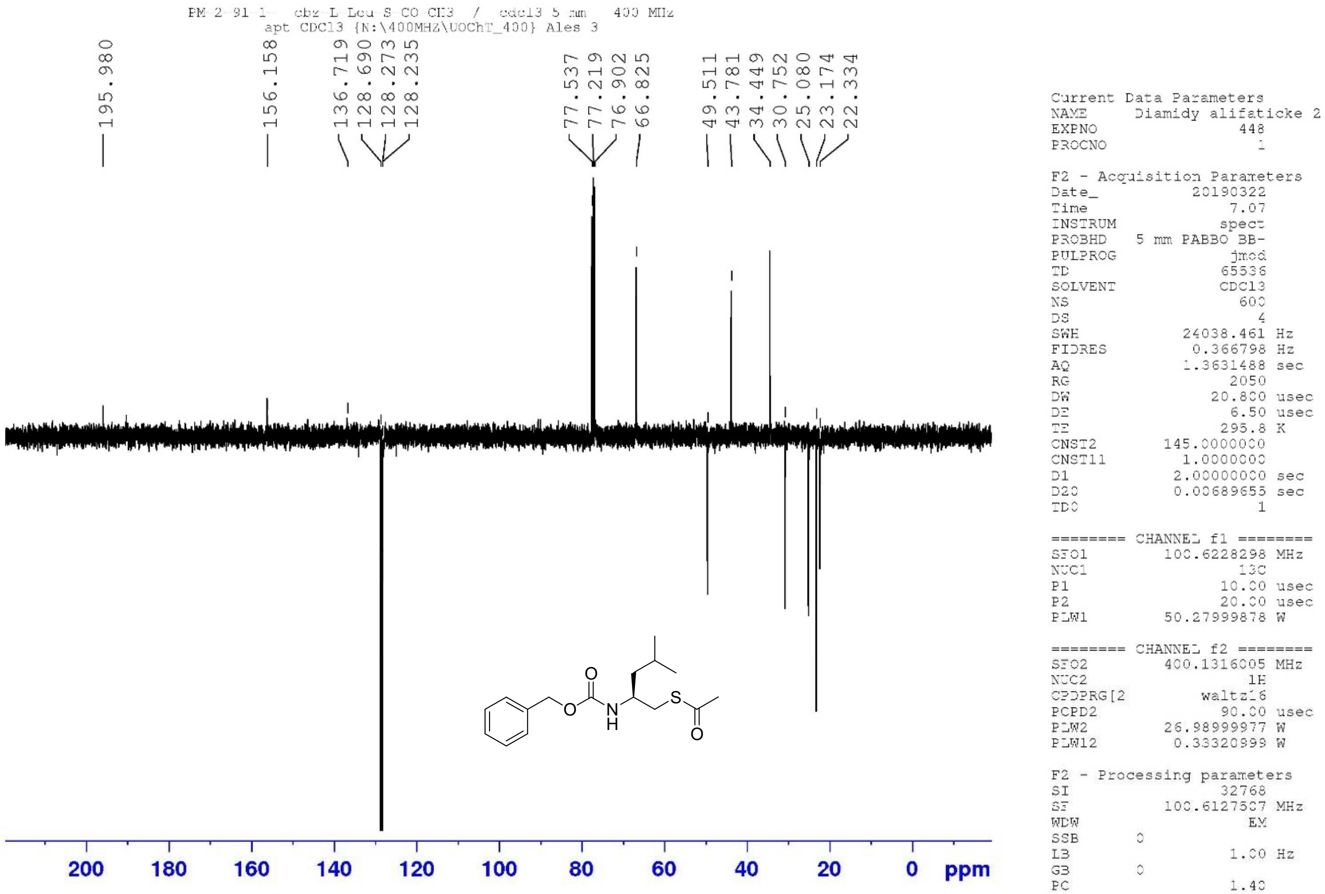
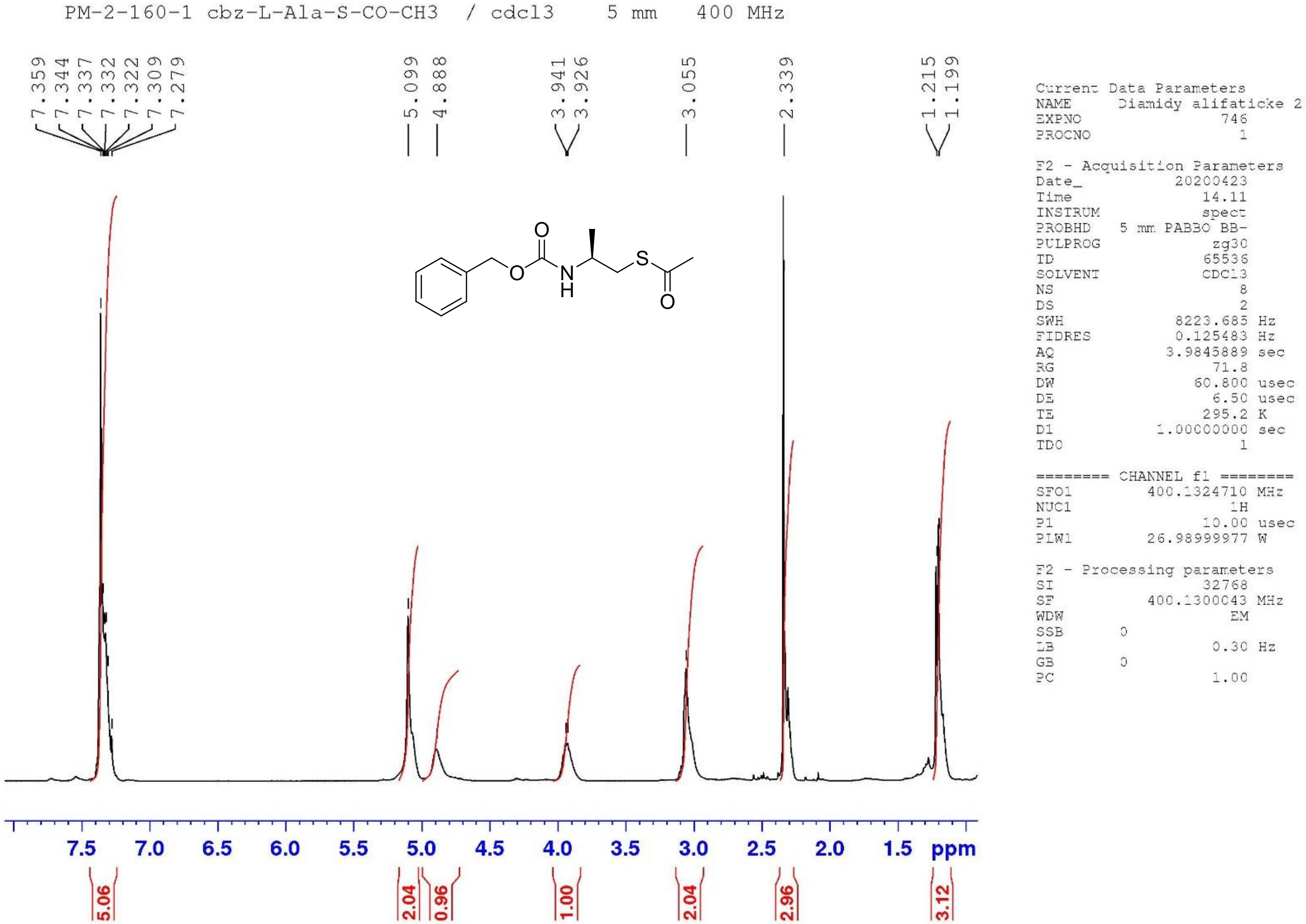
1.15. Copy of [1]H NMR of **4b**



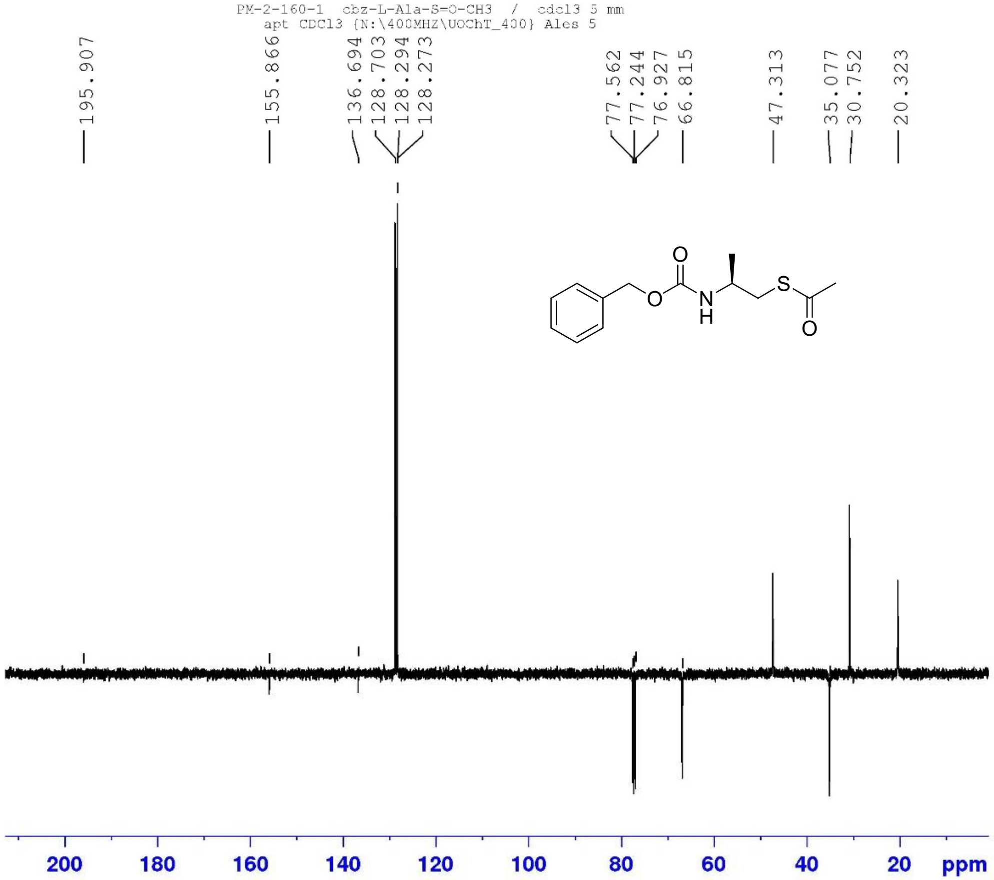
1.16. Copy of ^{13}C NMR of **4b**



1.17. Copy of [1]H NMR of **4c**



1.18. Copy of ^{13}C NMR of **4c**



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PROCNO 1

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TD 65536
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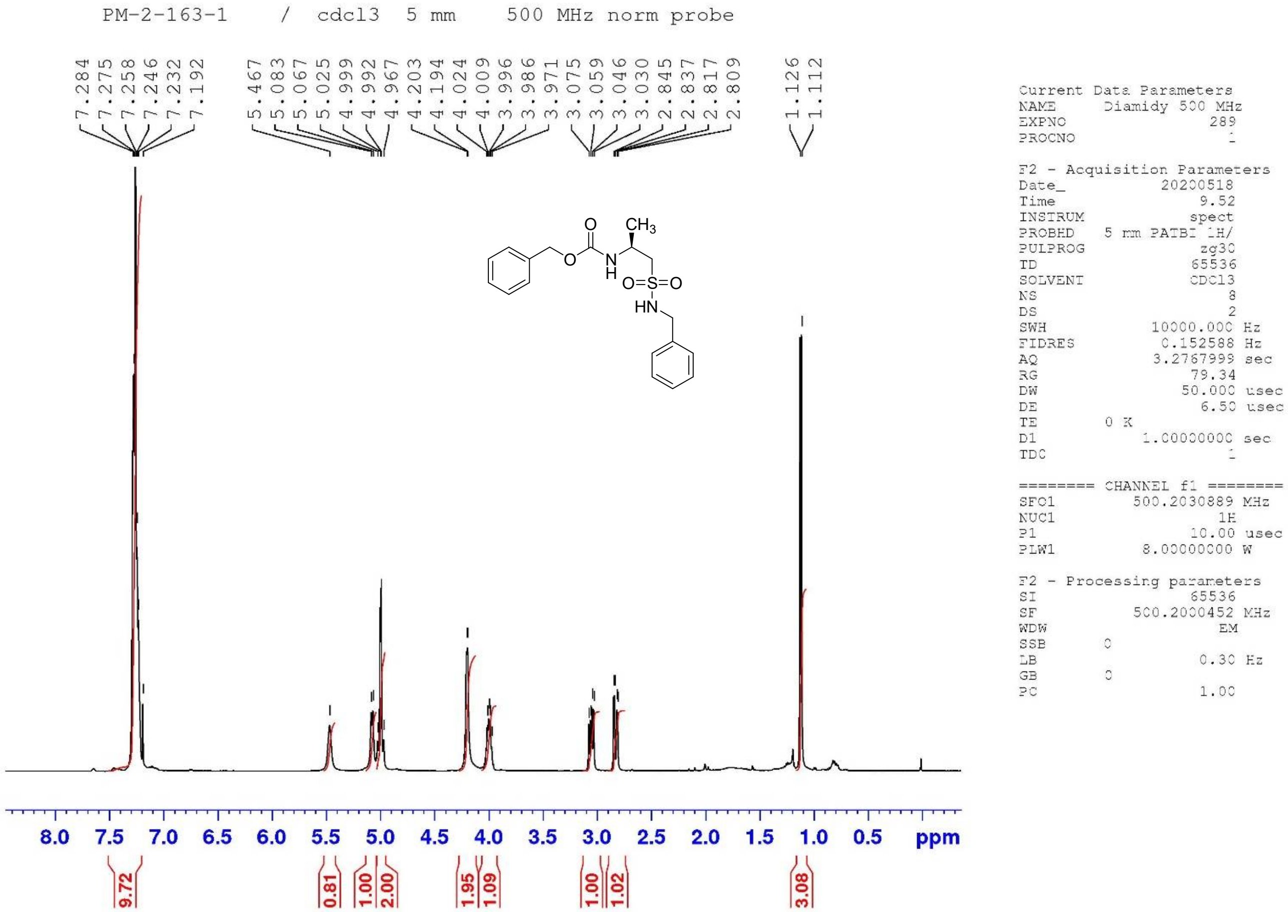
FIDRES 0.366798 Hz
AQ 1.3631488 sec
RG 2050
DW 20.800 usec
DE 6.50 usec
TE 295.6 K
CNST2 145.0000000
CNST11 1.0000000
D1 2.00000000 sec
D20 0.00689655 sec
TD0 1

======== CHANNEL f1 ========
SFO1 100.6228298 MHz
NUC1 13C
P1 10.00 usec
P2 20.00 usec
PLW1 50.27999878 W

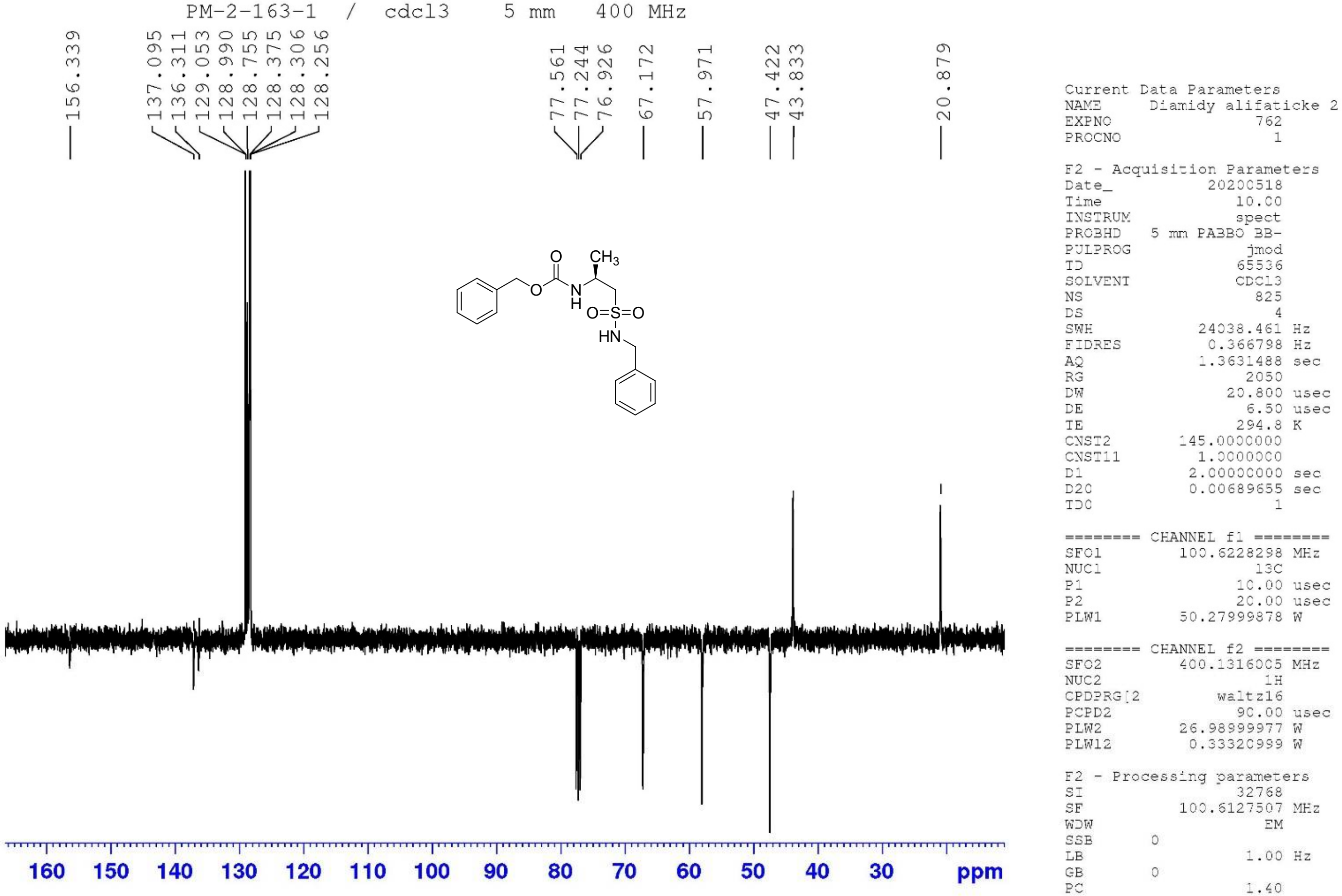
======== CHANNEL f2 ========
SFO2 400.1316005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 90.00 usec
PLW2 26.98999977 W
PLW12 0.33320999 W

F2 - Processing parameters
SI 32768
SF 100.6127507 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40

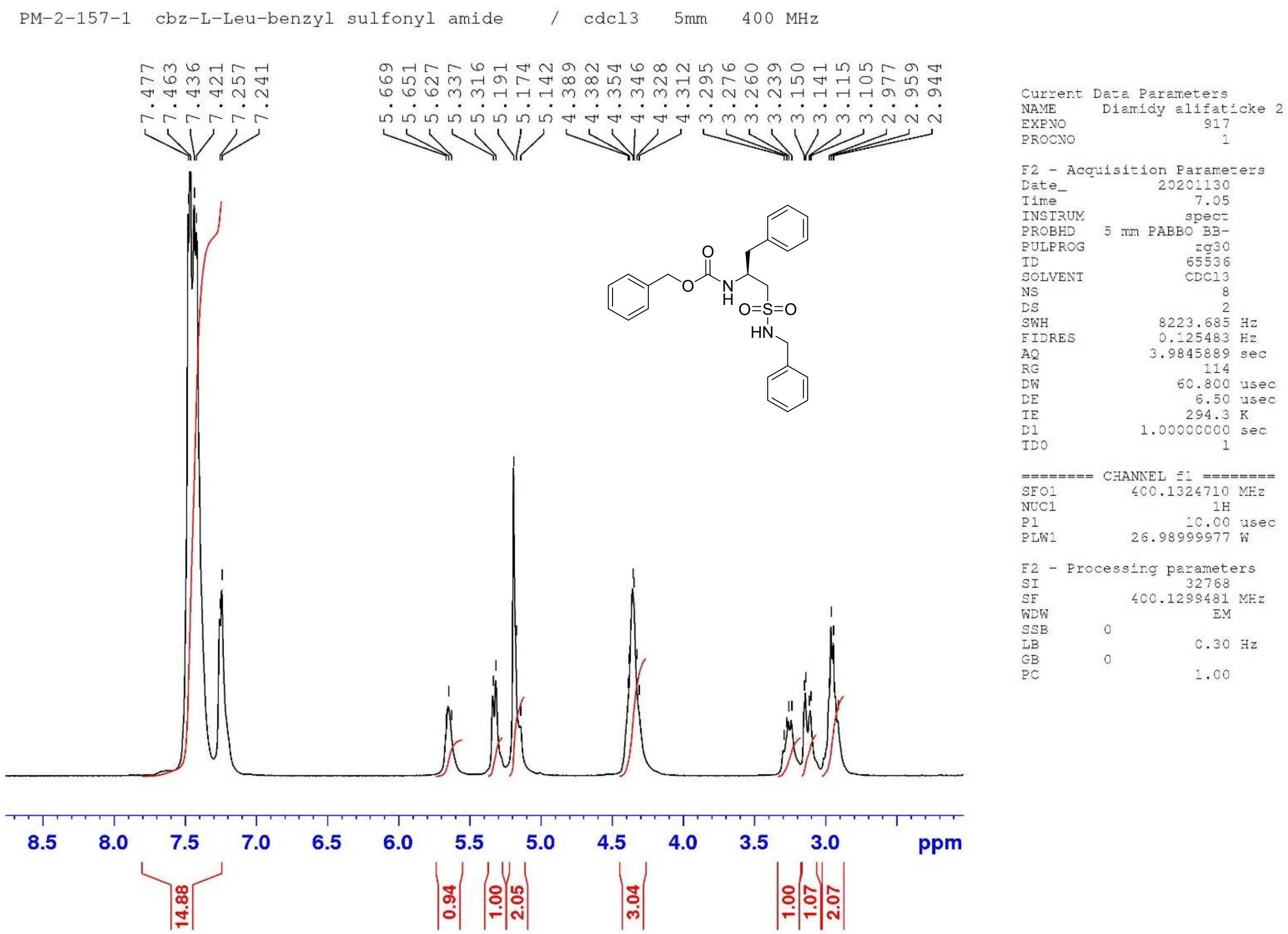
1.19. Copy of ^{1}H NMR of **5a**



1.20. Copy of ^{13}C NMR of **5a**



1.21. Copy of ^{1}H NMR of **5b**



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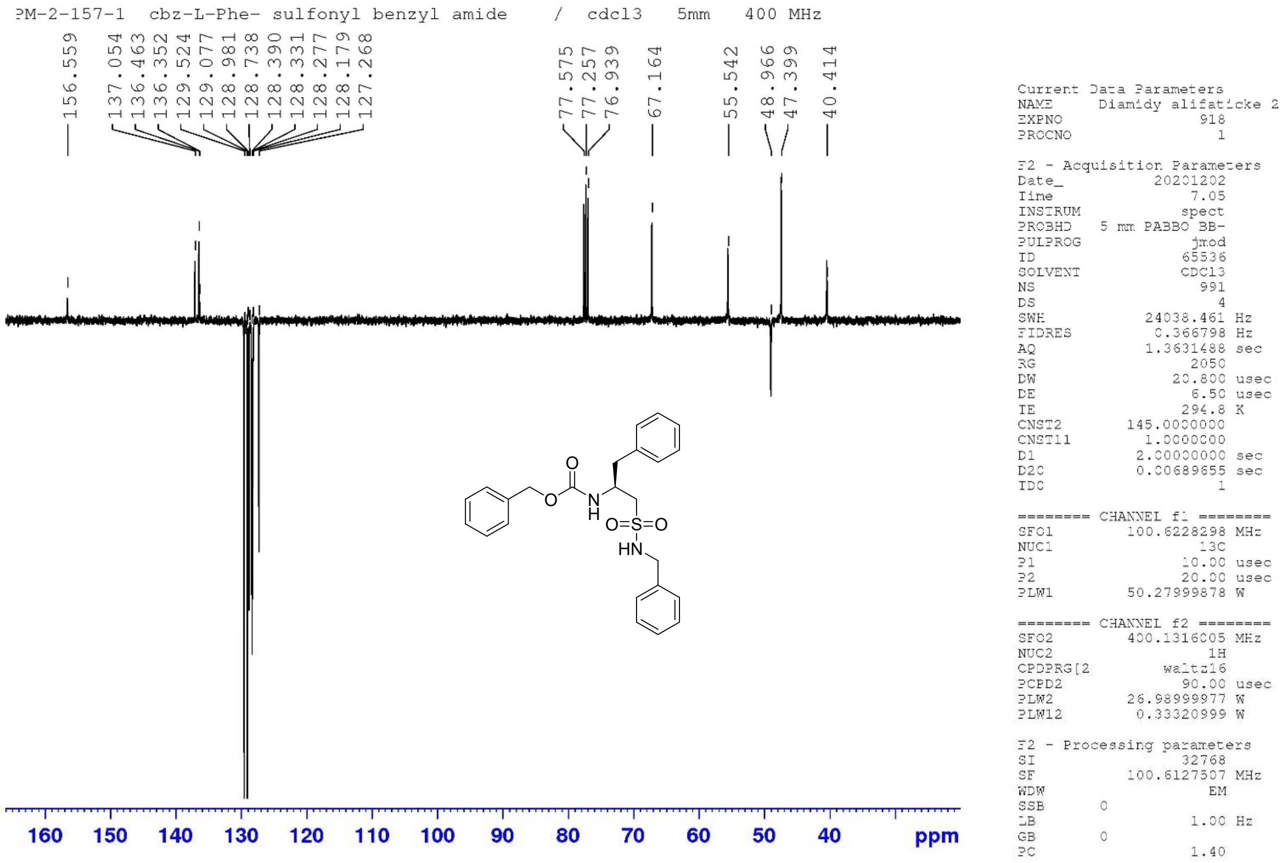
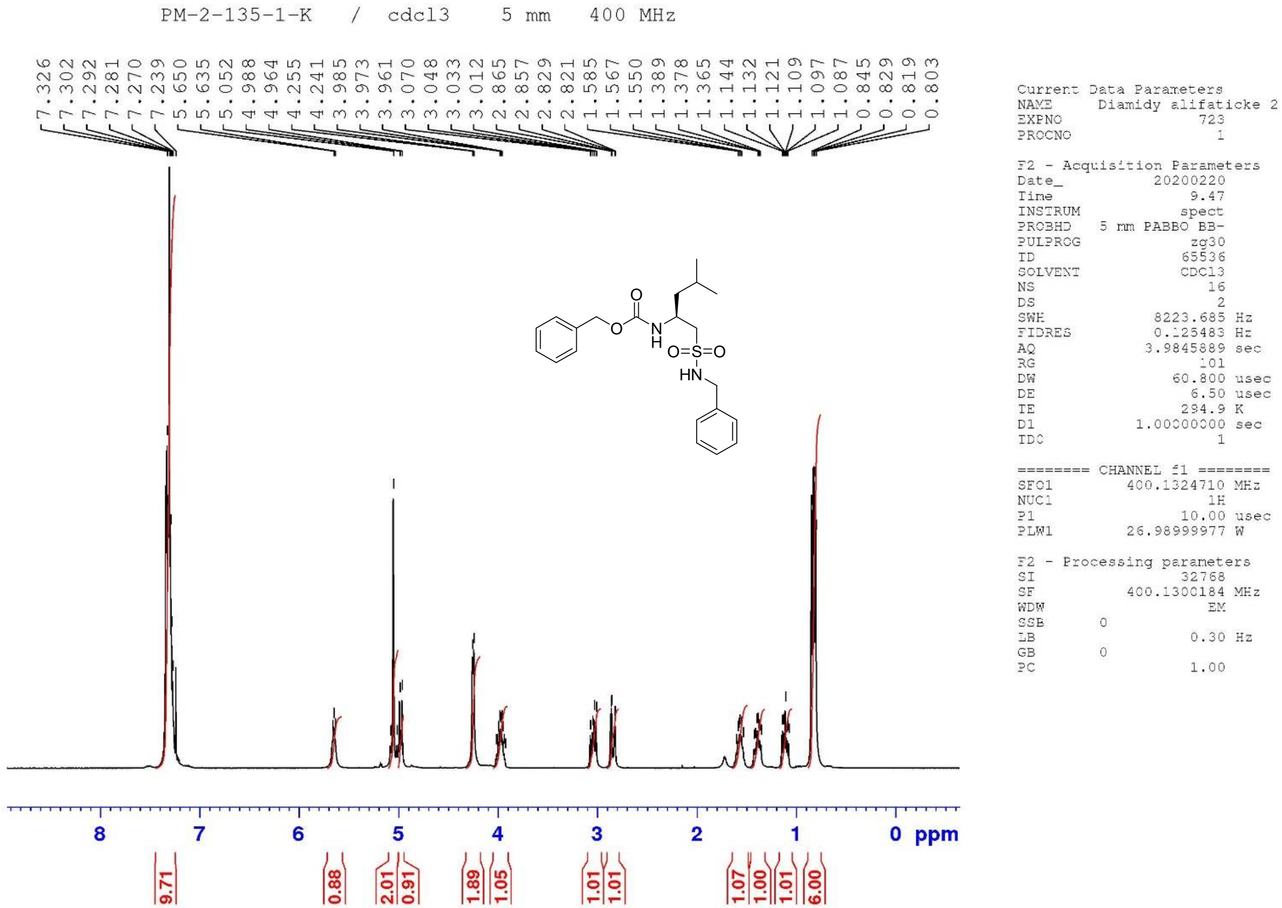
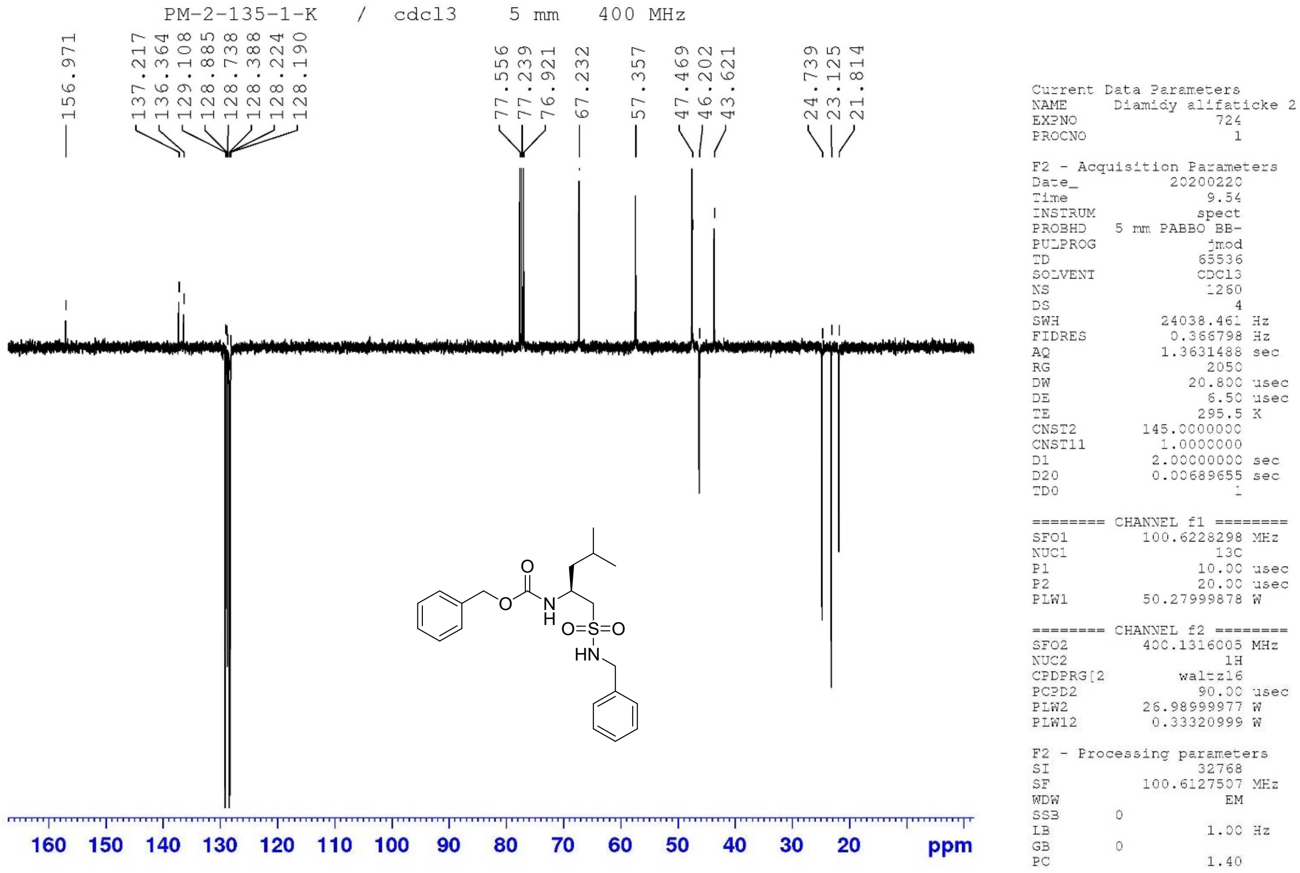


1.23. Copy of ^{1}H NMR of **5c**



1.24. Copy of ^{13}C NMR of **5c**



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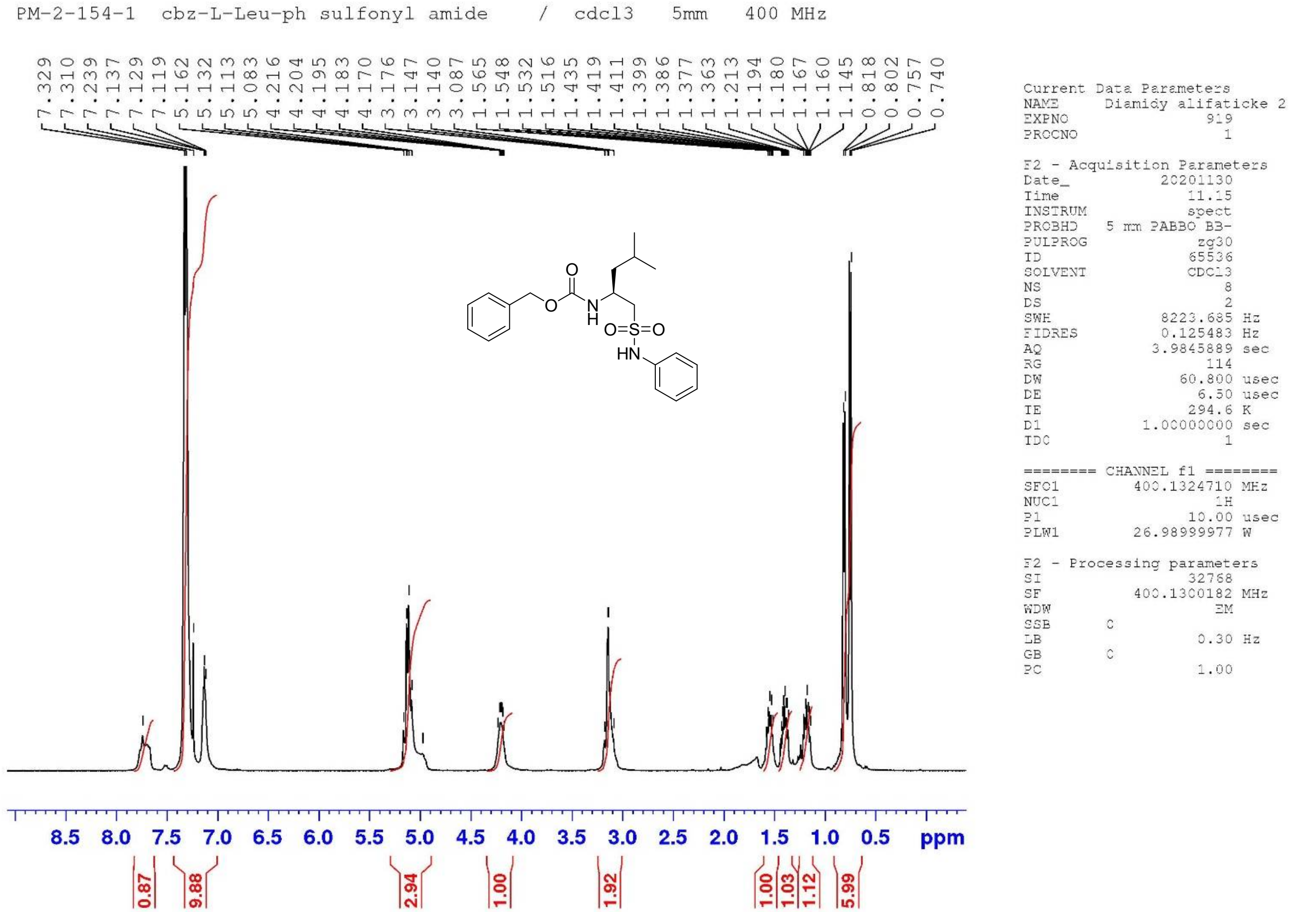
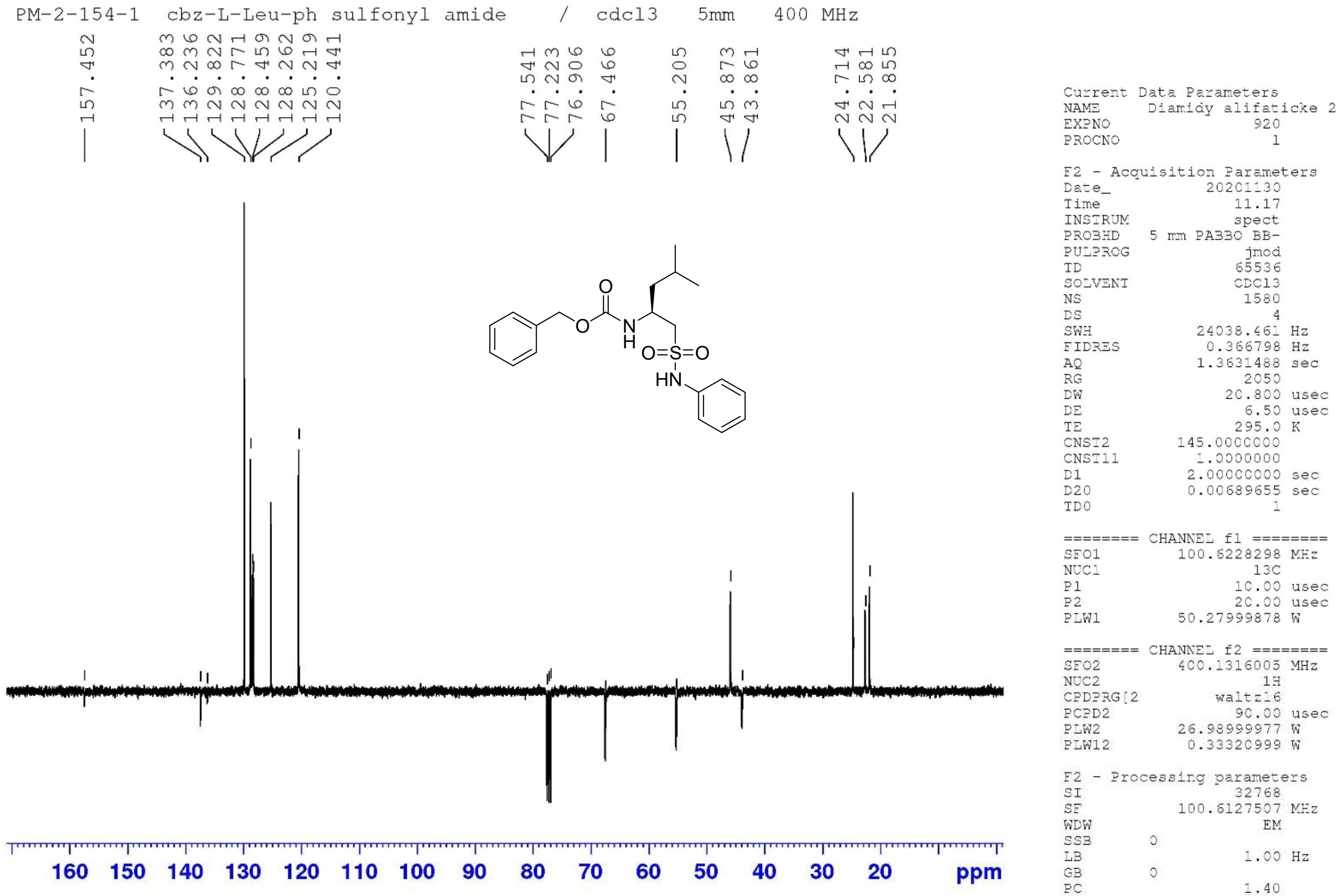
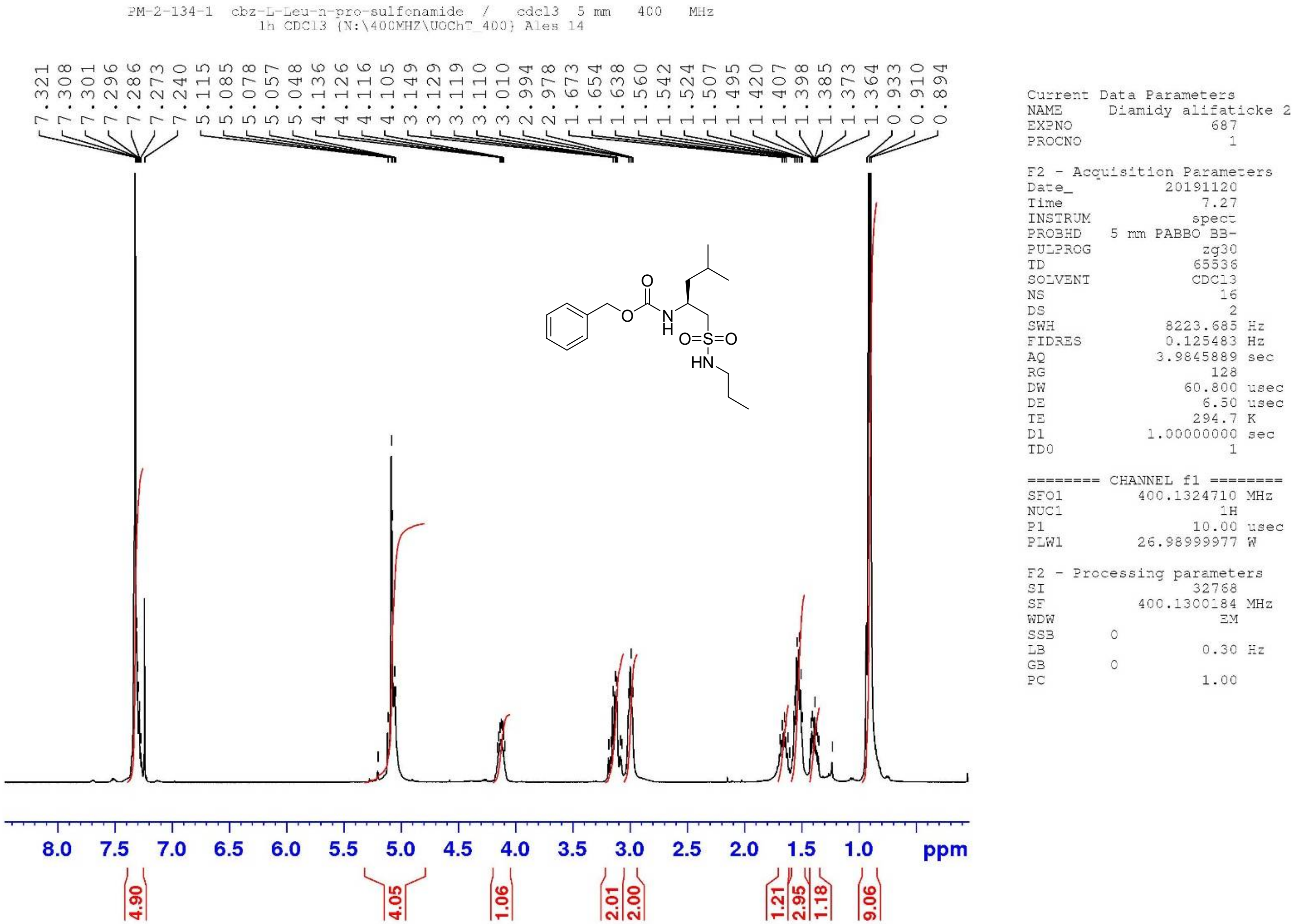


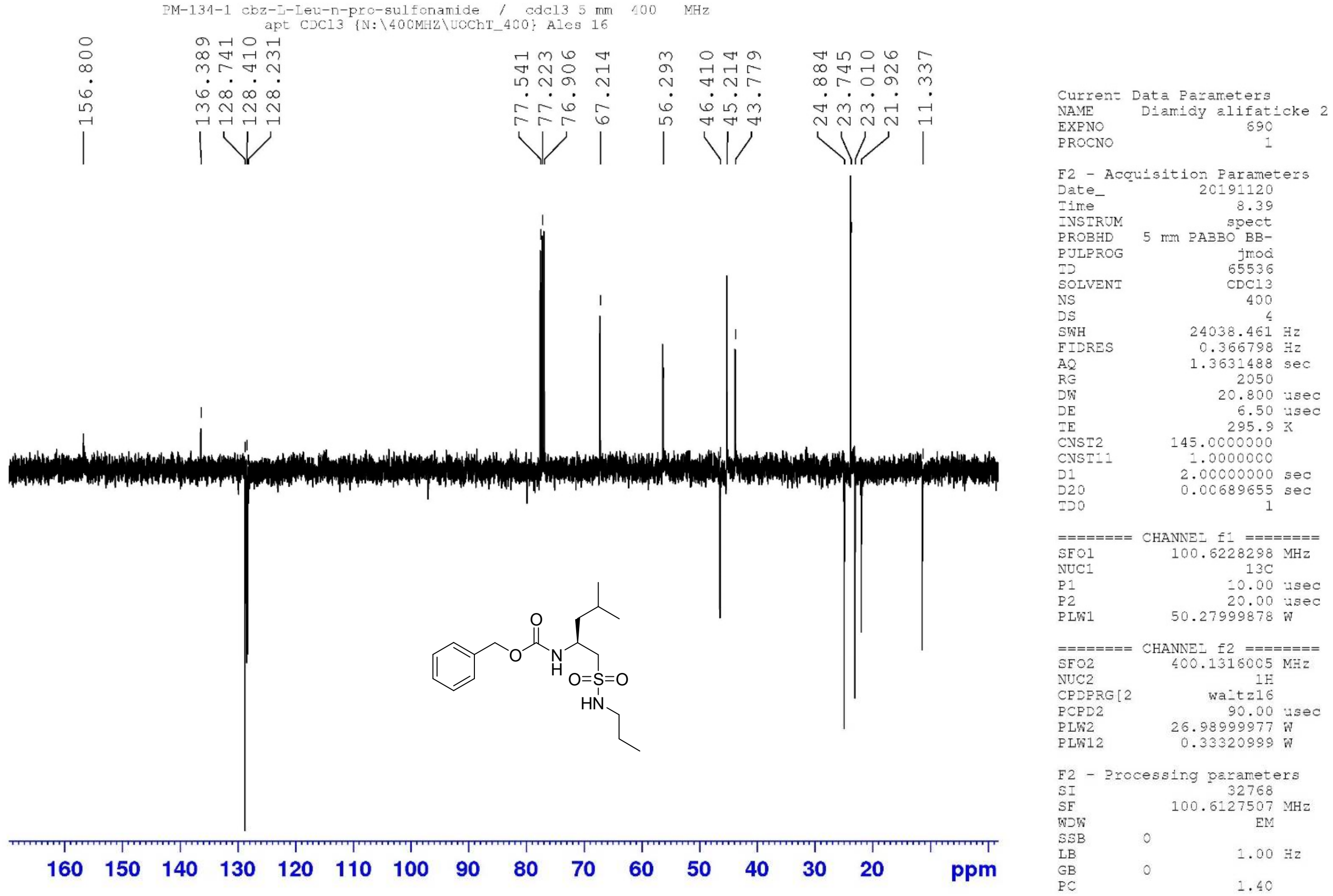
1.26. Copy of ^{13}C NMR of **5d**



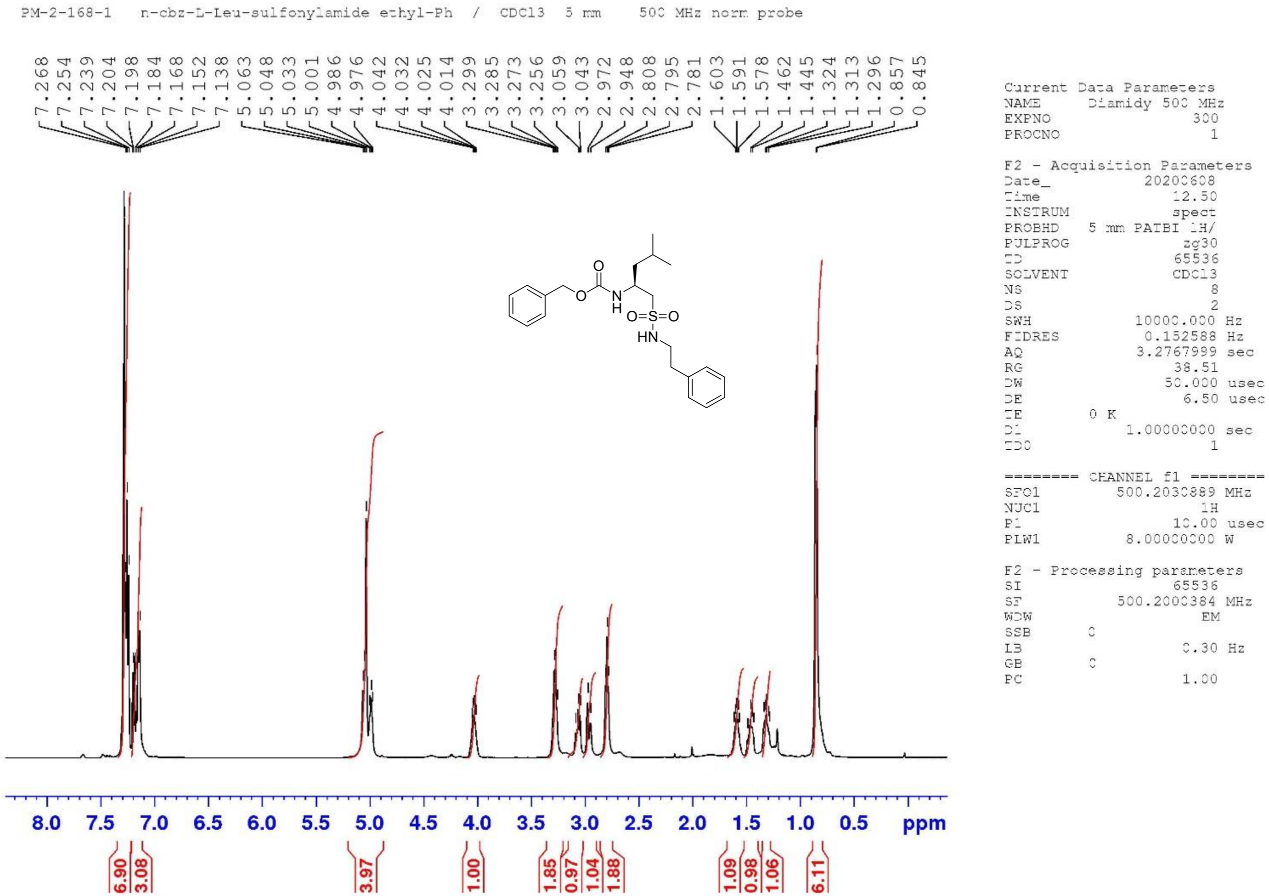
1.27. Copy of ^{1}H NMR of **5e**



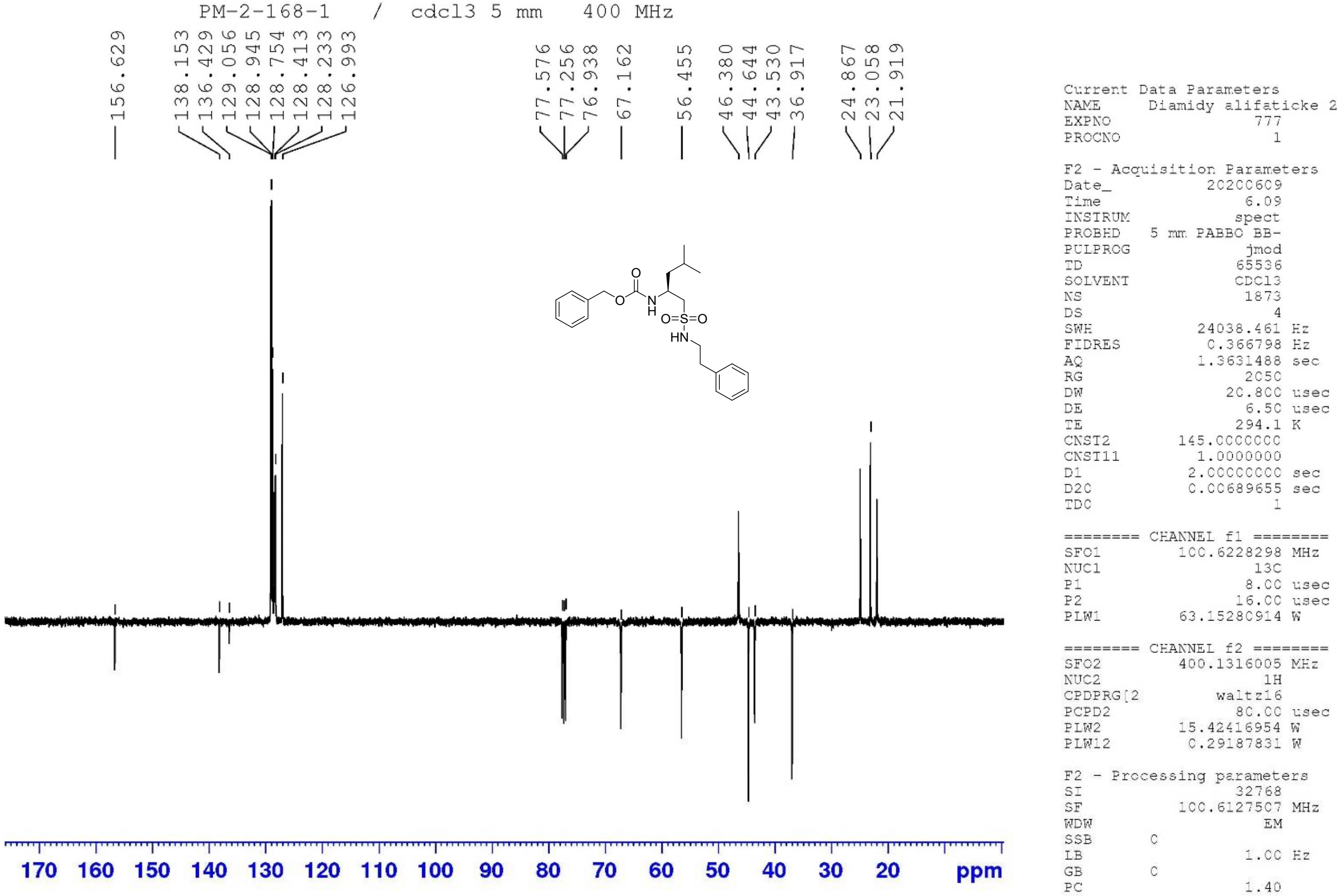
1.28. Copy of ^{13}C NMR of **5e**



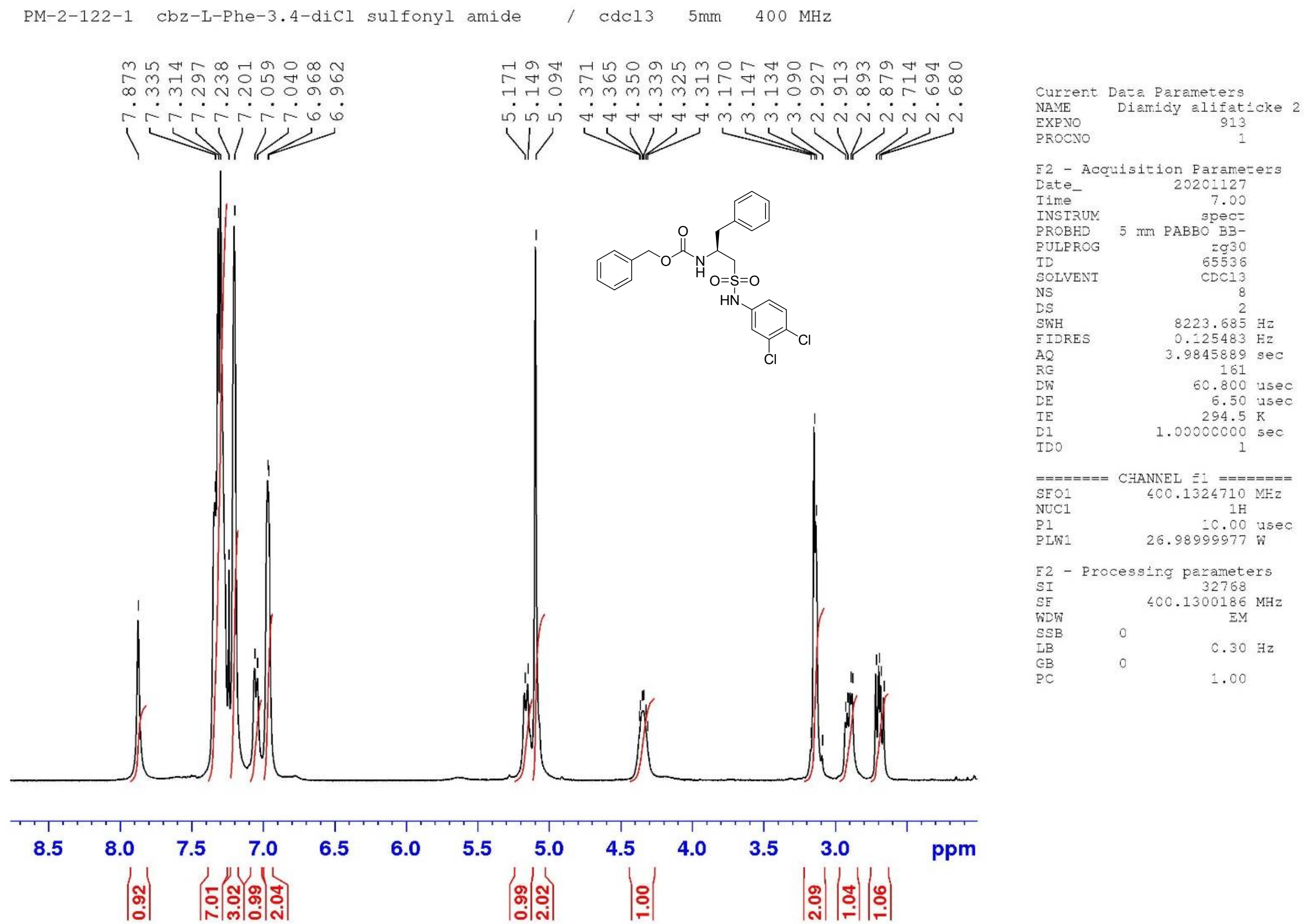
1.29. Copy of ^{1}H NMR of **5f**



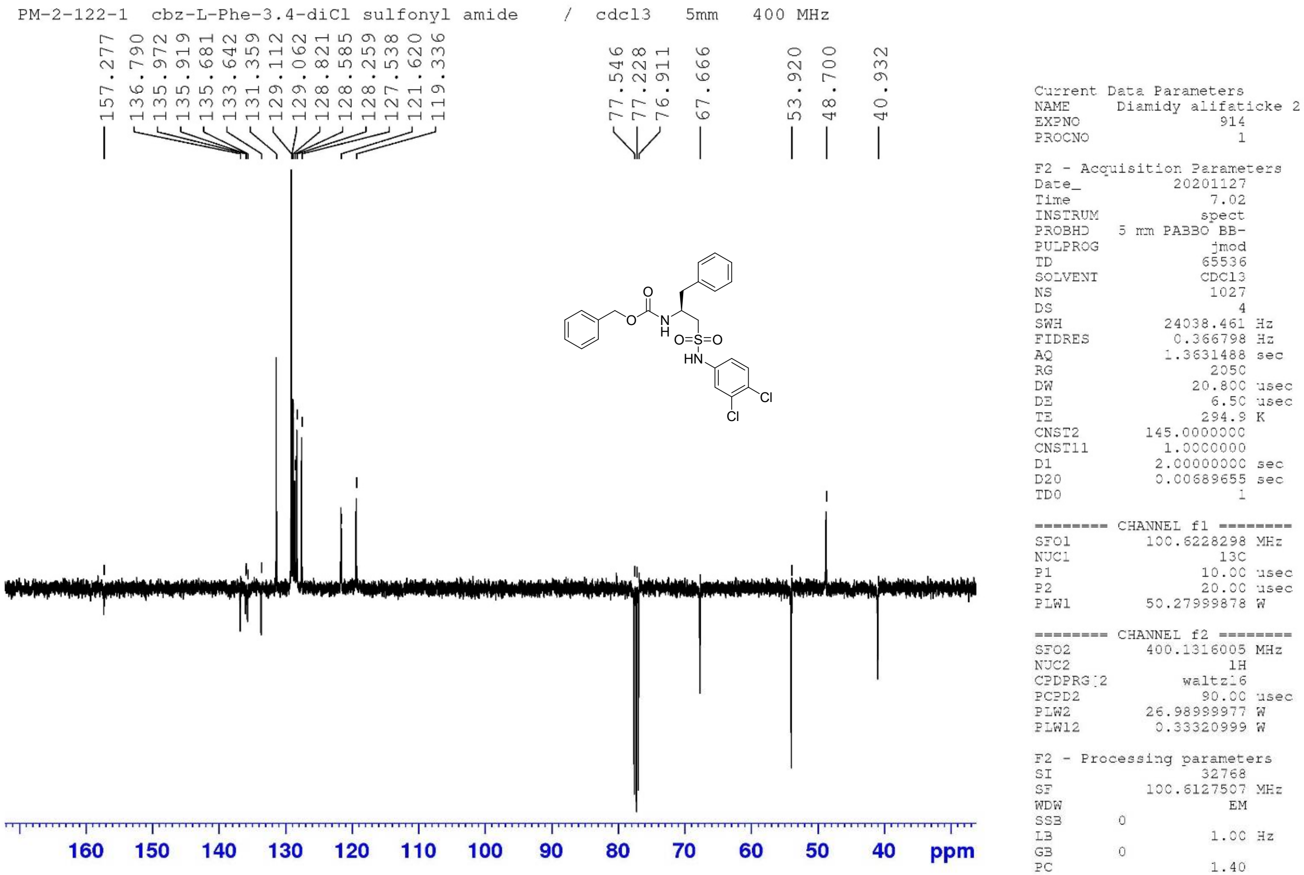
1.30. Copy of ^{13}C NMR of **5f**



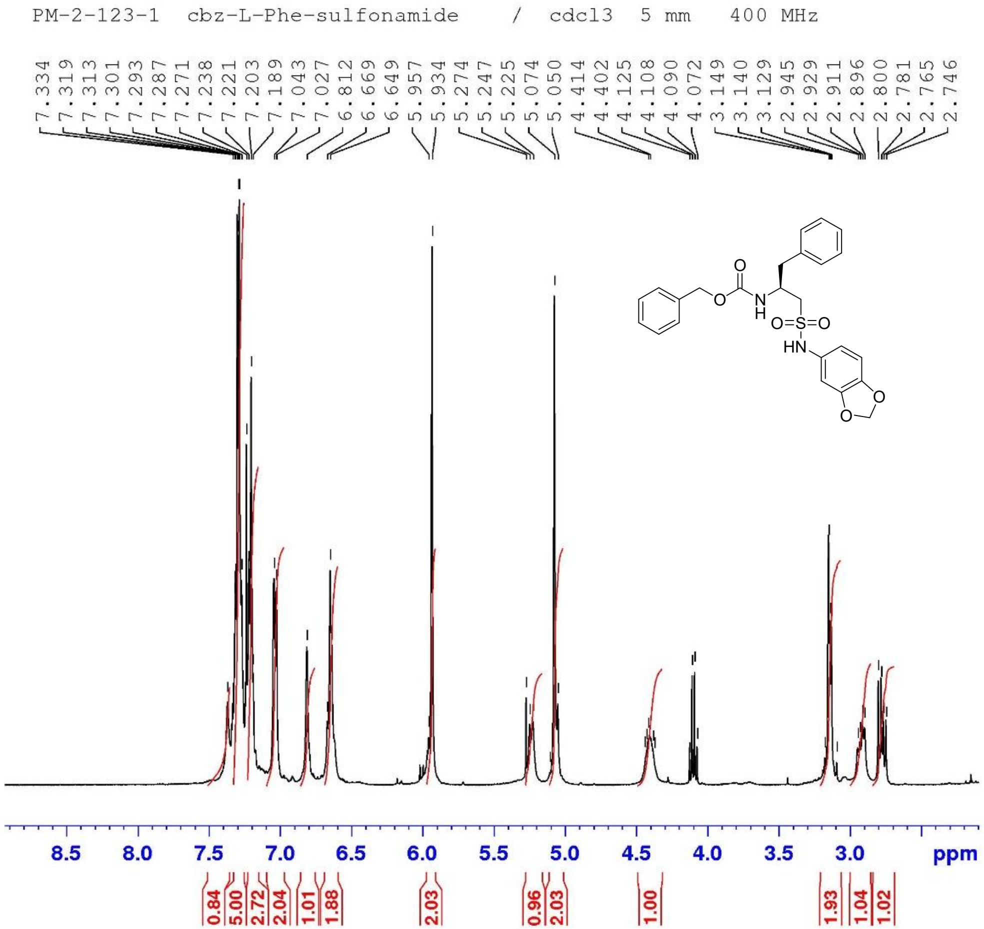
1.31. Copy of ^{1}H NMR of **5g**



1.32. Copy of ^{13}C NMR of **5g**



1.33. Copy of ^{1}H NMR of **5h**



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PROCNO 1

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SOLVENT CDCl3
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DS 2
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FIDRES 0.125483 Hz
AQ 3.9845889 sec
RG 128
DW 60.800 usec
DE 6.50 usec
TE 294.9 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 400.1324710 MHz
NUC1 1H
P1 10.00 usec
PLW1 26.98999977 W

F2 - Processing parameters
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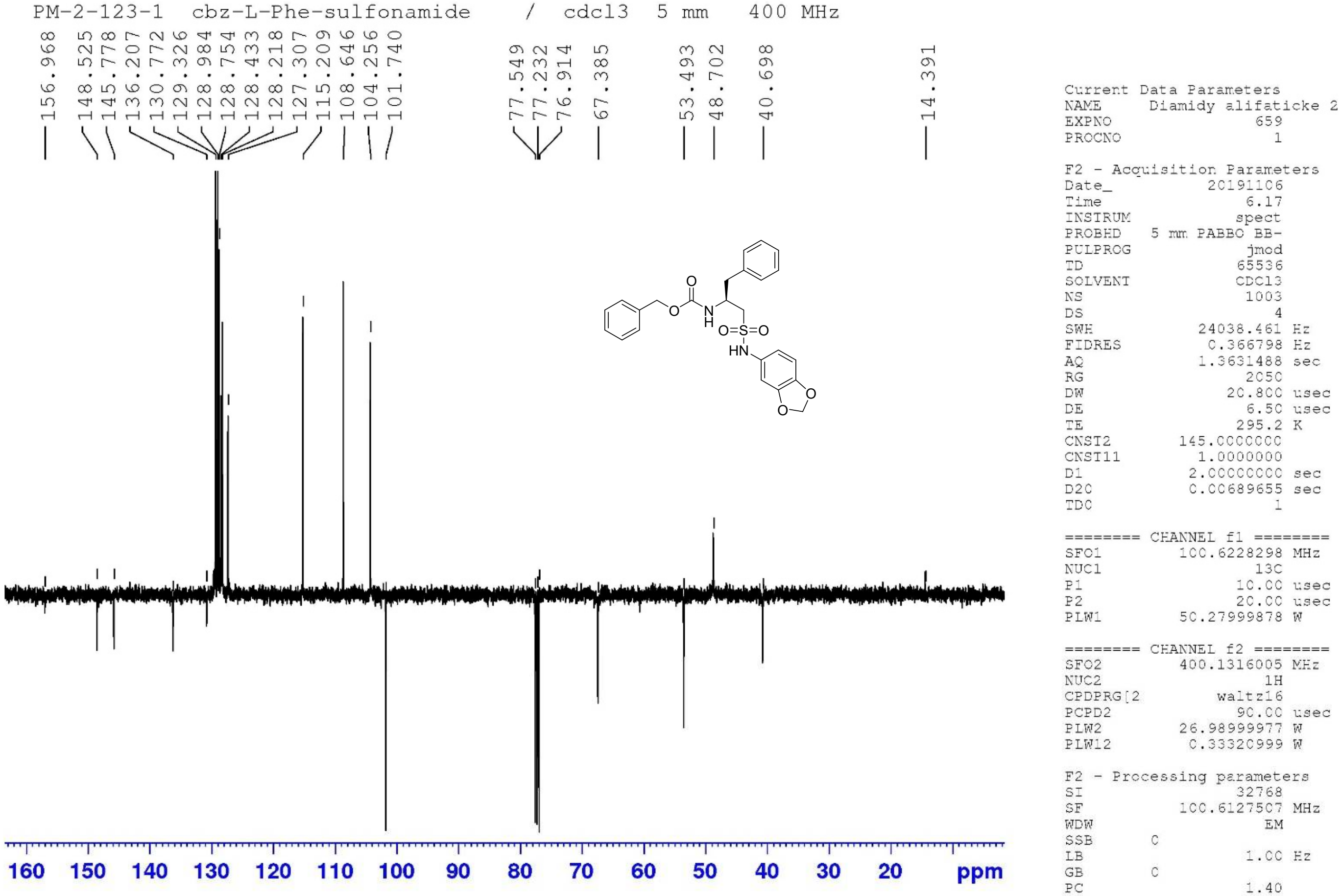
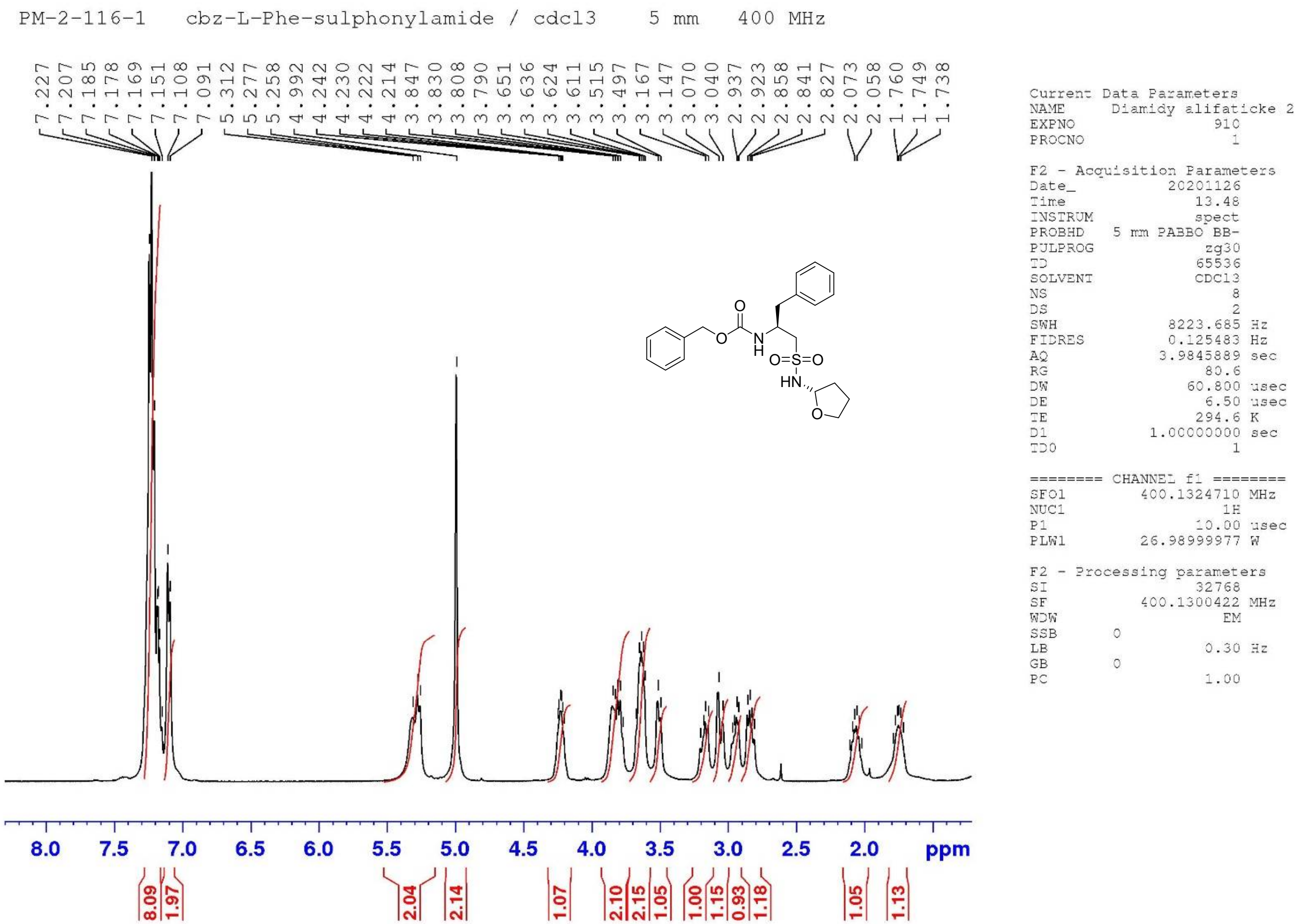
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

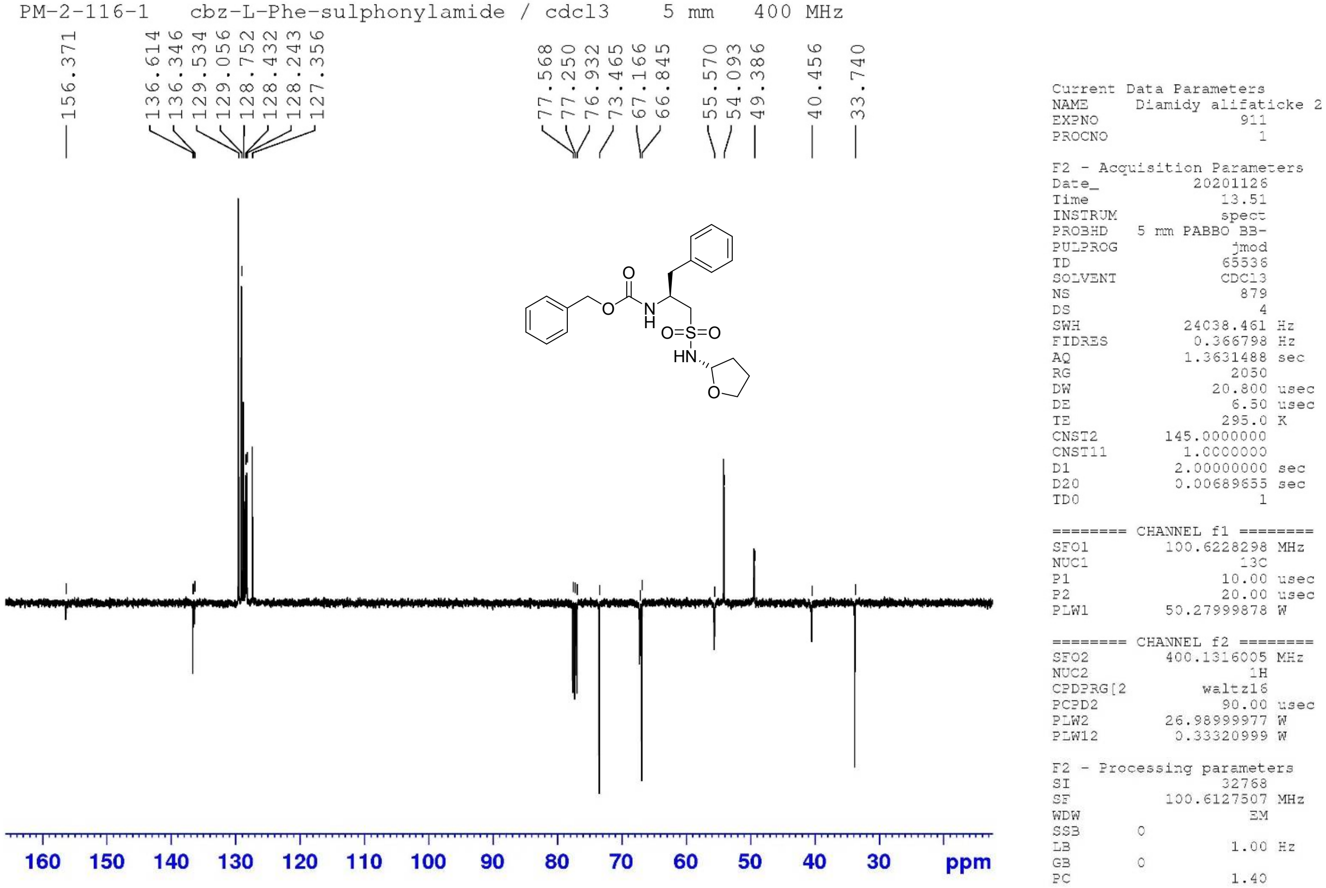
1.34. Copy of ^{13}C NMR of **5h**



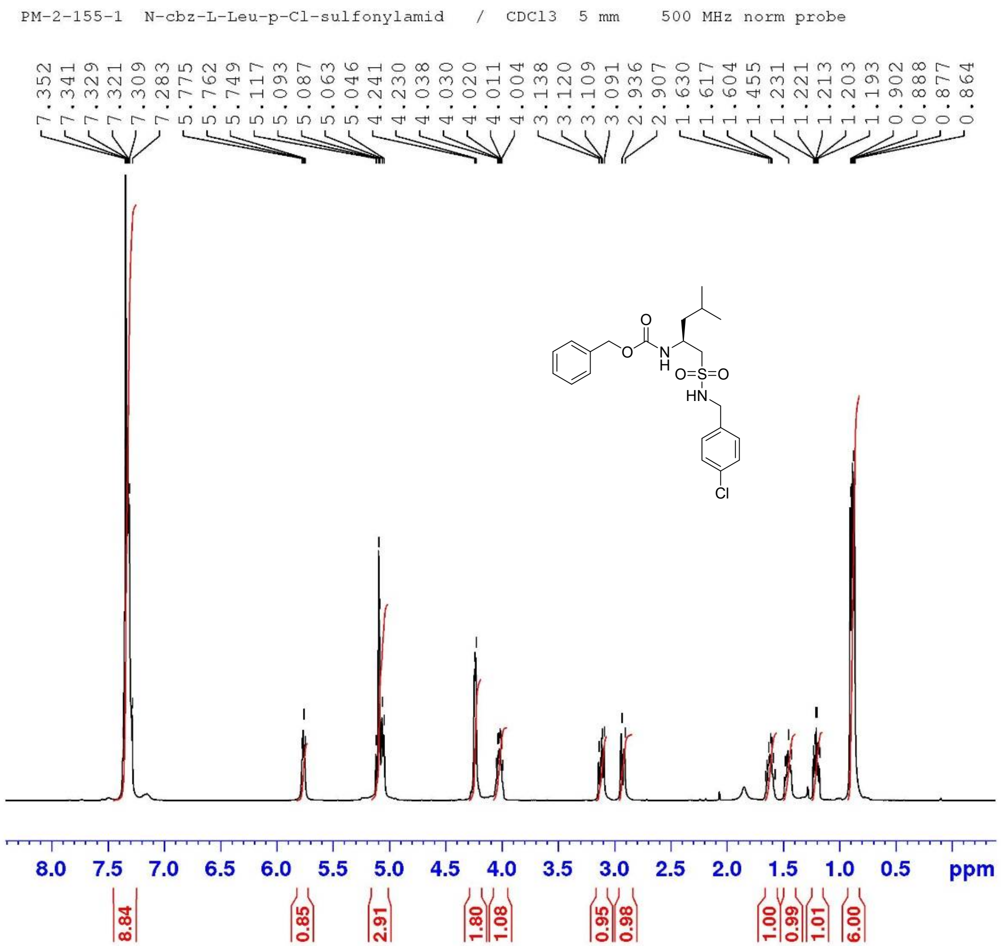
1.35. Copy of ^{1}H NMR of **5i**



1.36. Copy of ^{13}C NMR of **5i**



1.37. Copy of [1]H NMR of **5j**

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PROCNO                   1

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PULPROG             zg30
TD                 65536
SOLVENT            CDCl3
NS                     8
DS                     2
SWH          10000.000 Hz
FIDRES        0.152588 Hz
AQ           3.2767999 sec
RG               48.36
DW              50.000 usec
DE                6.50 usec
TE          0 K
D1         1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1       500.2030889 MHz
NUC1                  1H
P1                 10.00 usec
PLW1          8.00000000 W

F2 - Processing parameters
SI                 65536
SF         500.2000000 MHz
WDW                   EM
SSB       0
LB                  0.30 Hz
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PC                  1.00
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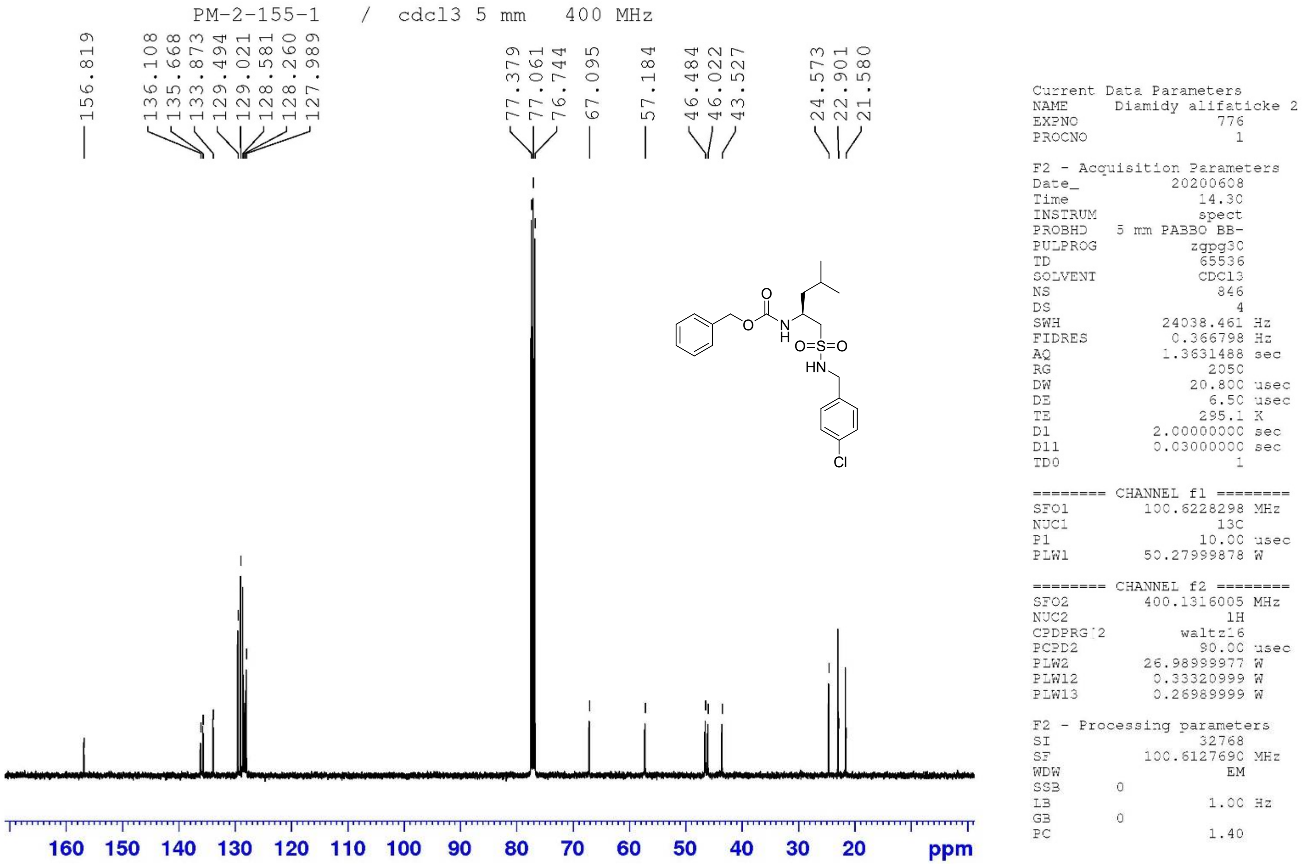
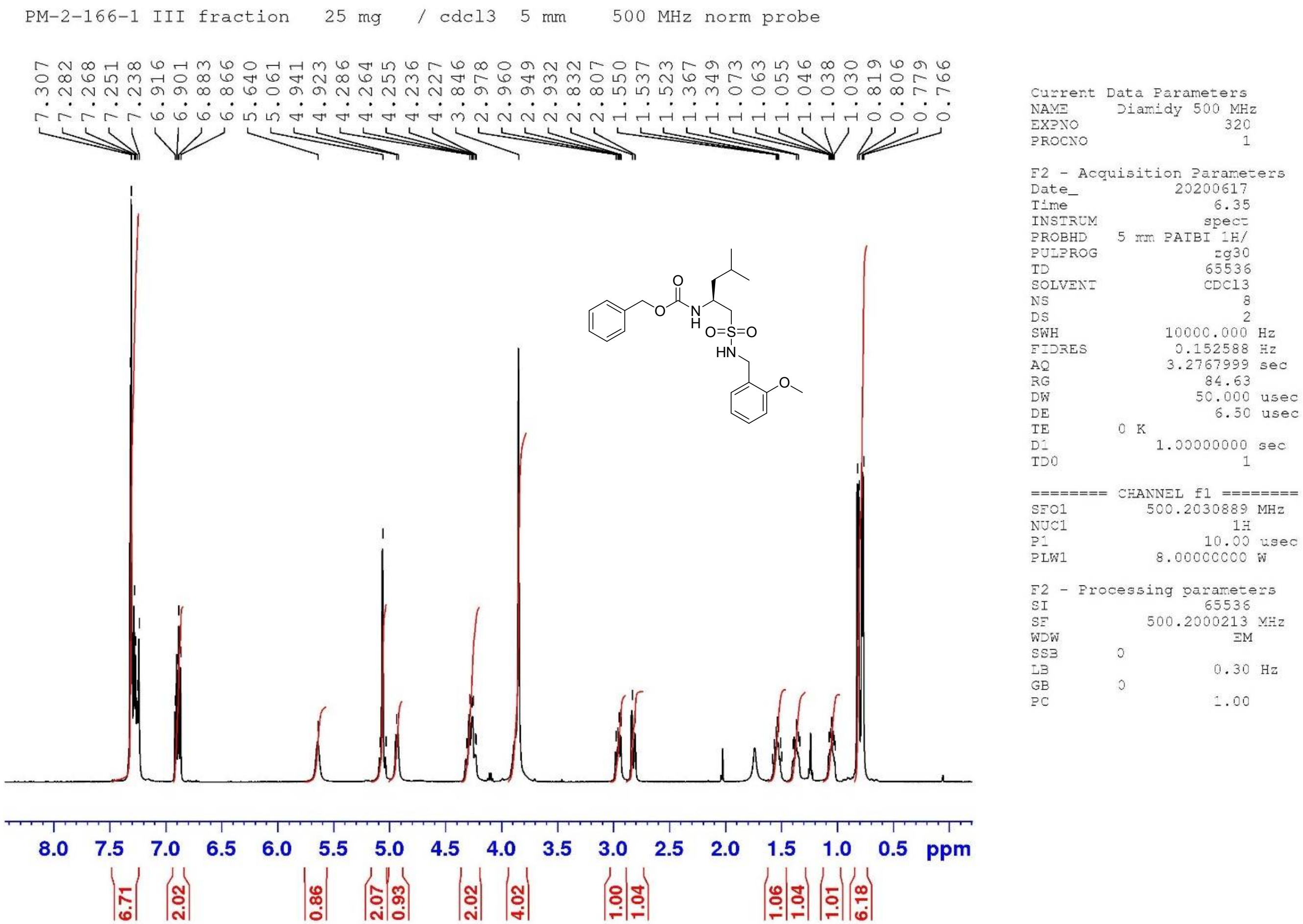
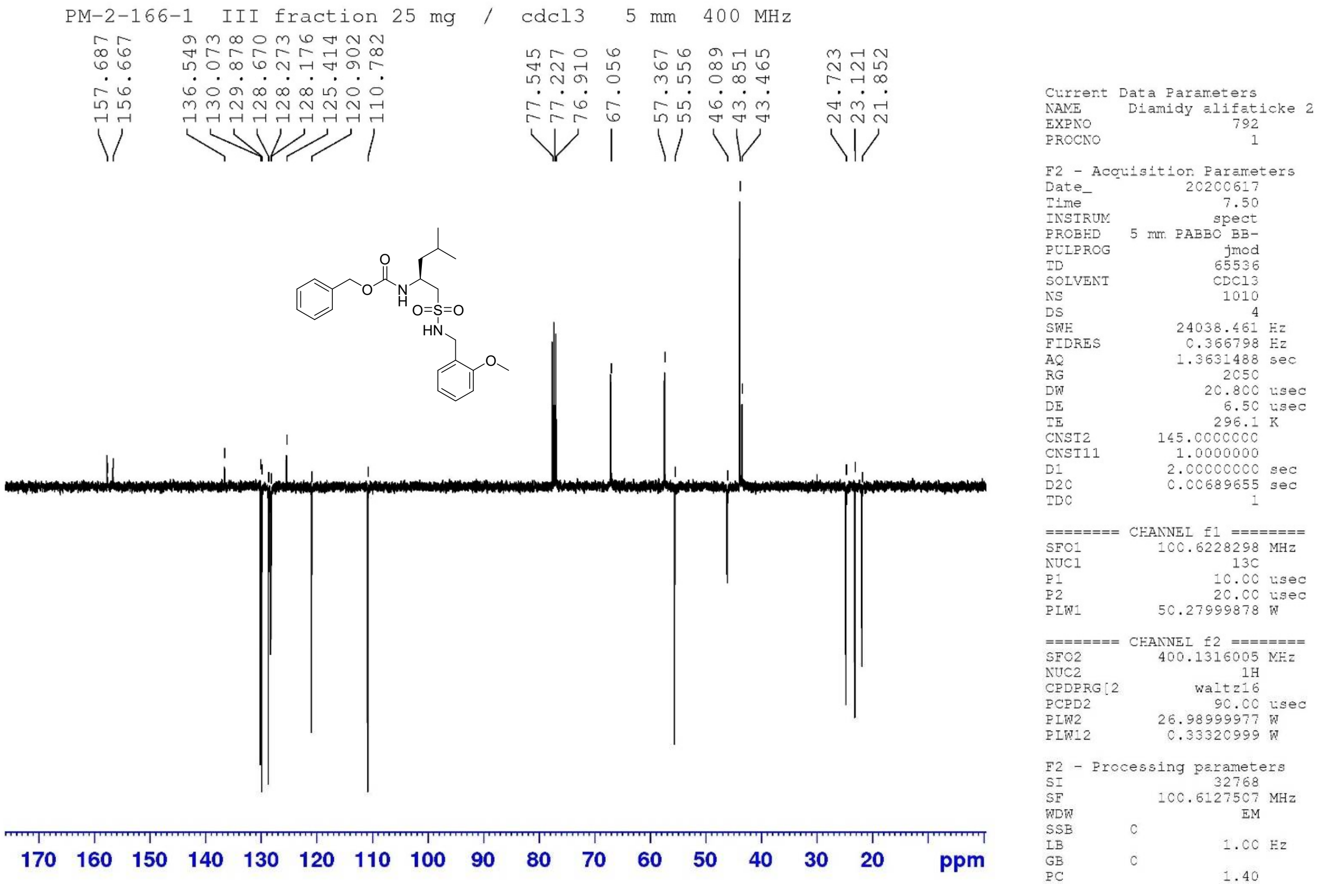
1.38. Copy of ^{13}C NMR of **5j**



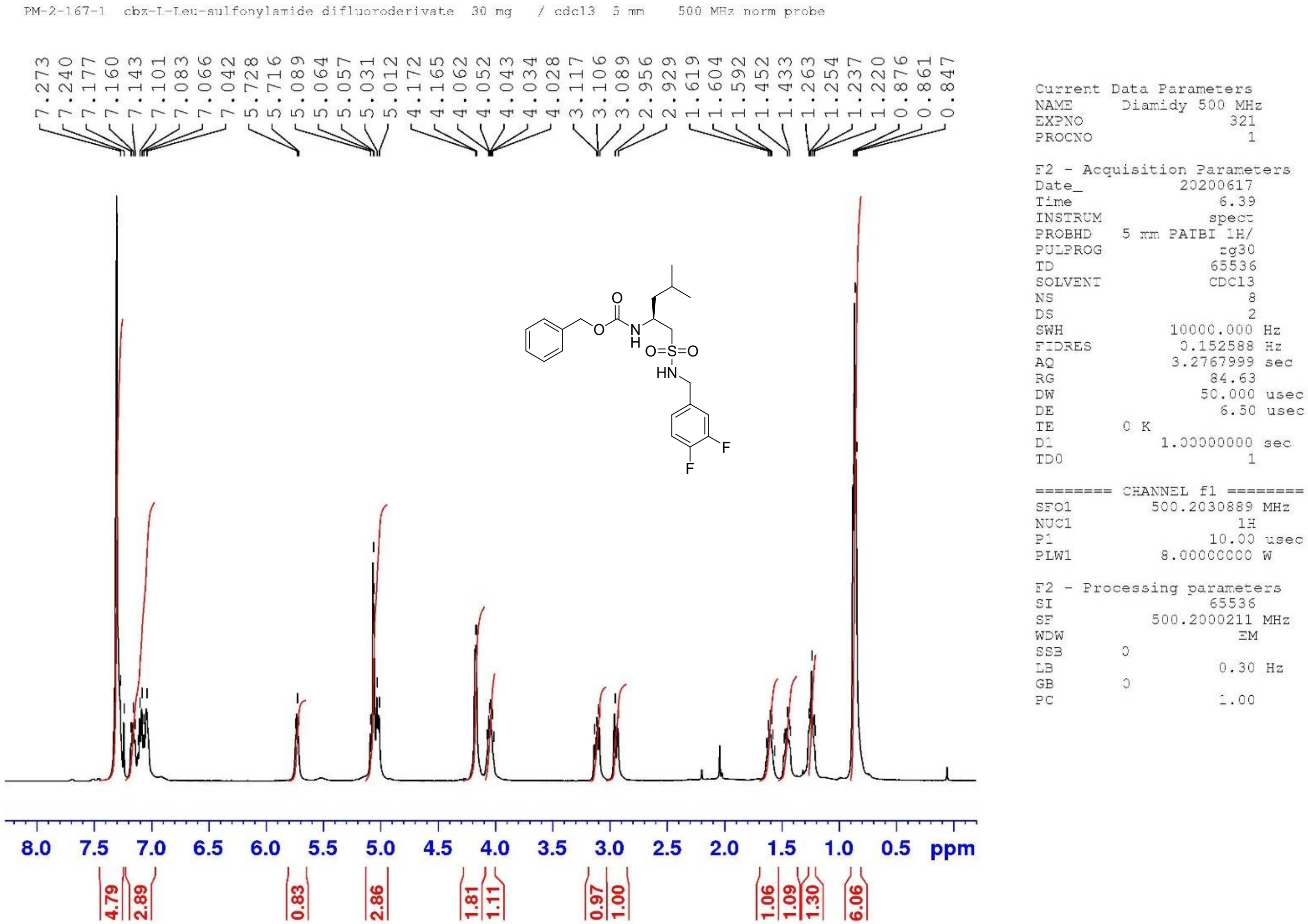
1.39. Copy of ^{1}H NMR of **5k**



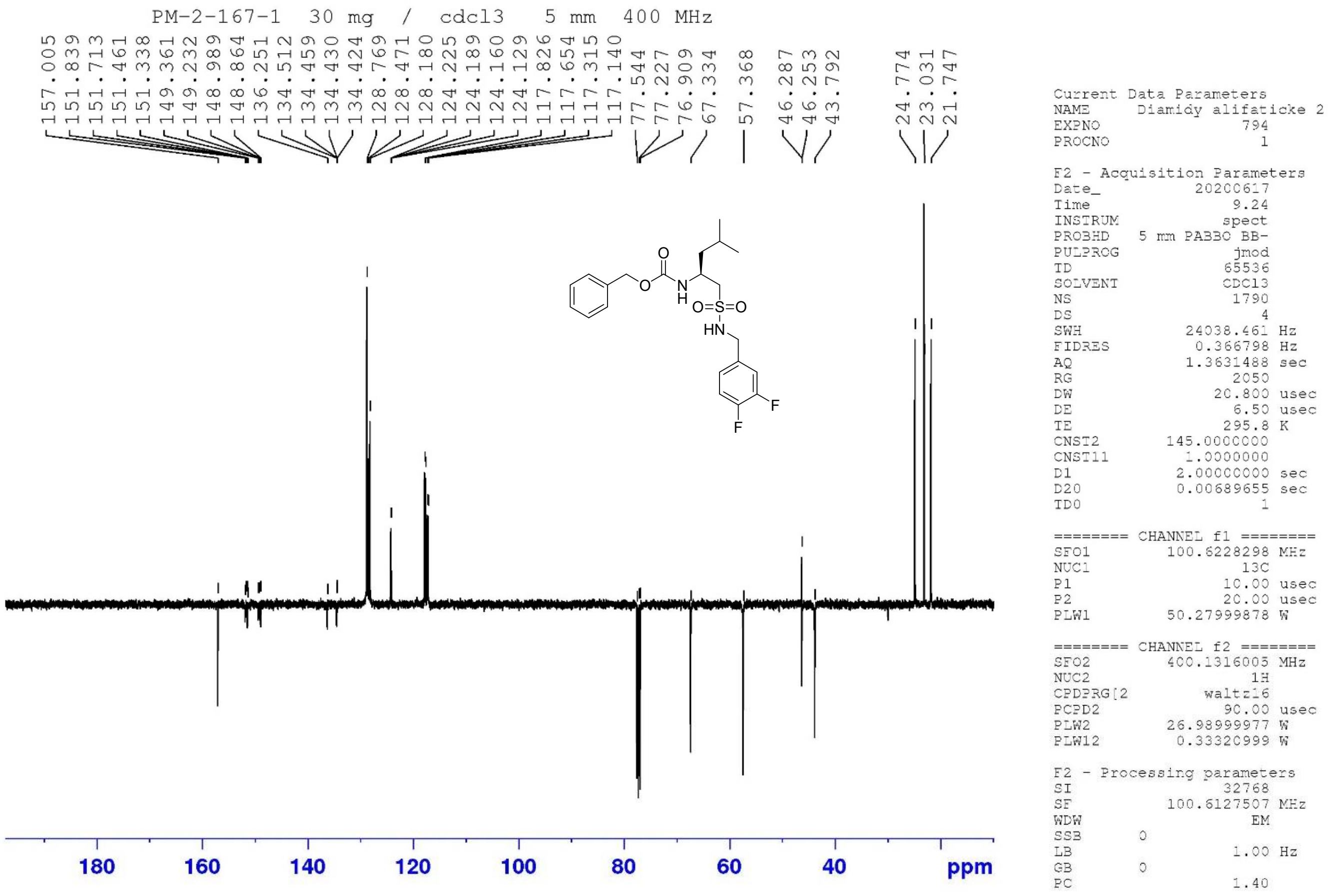
1.40. Copy of ^{13}C NMR of **5k**



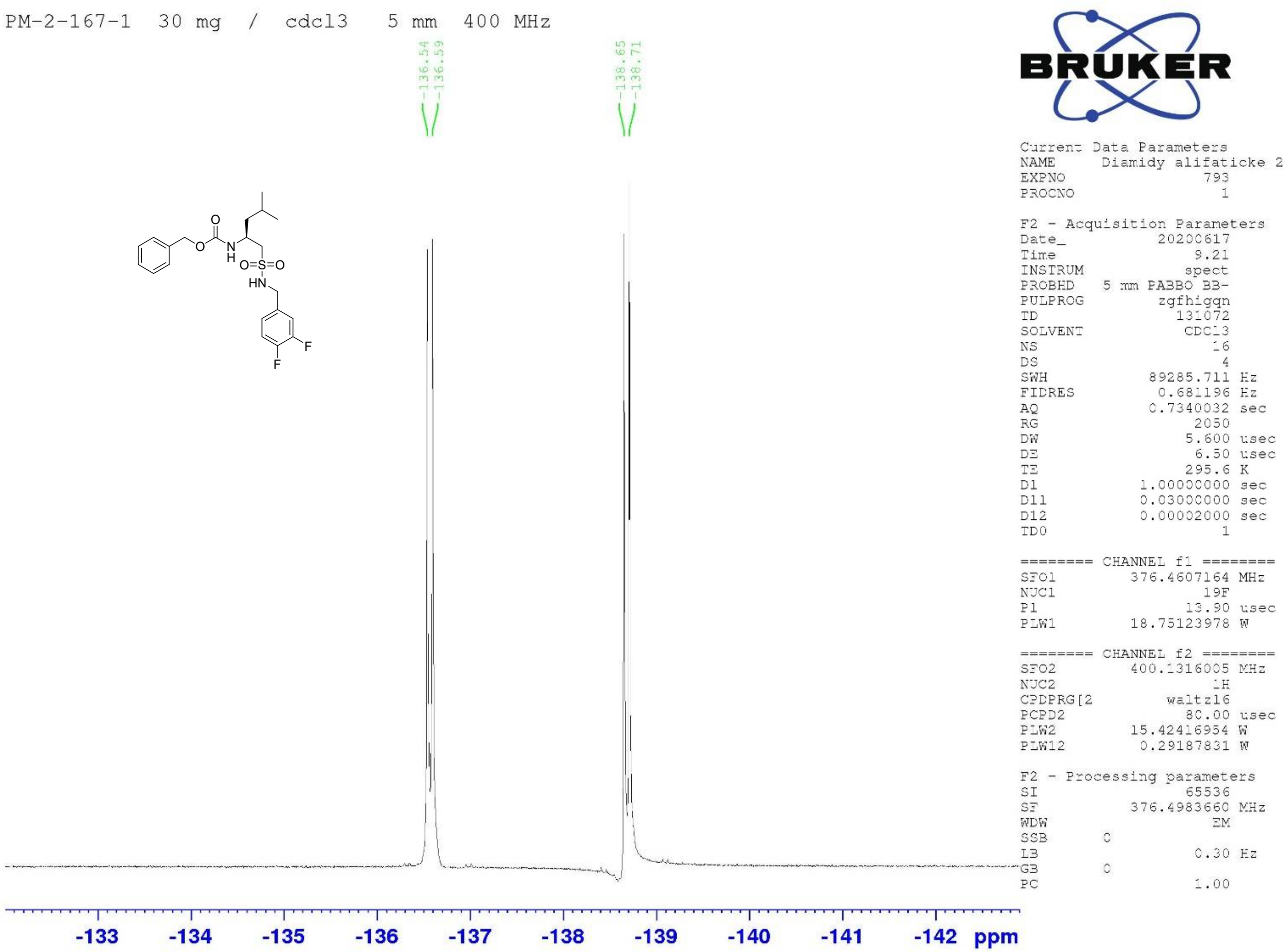
1.41. Copy of ^{1}H NMR of **5l**



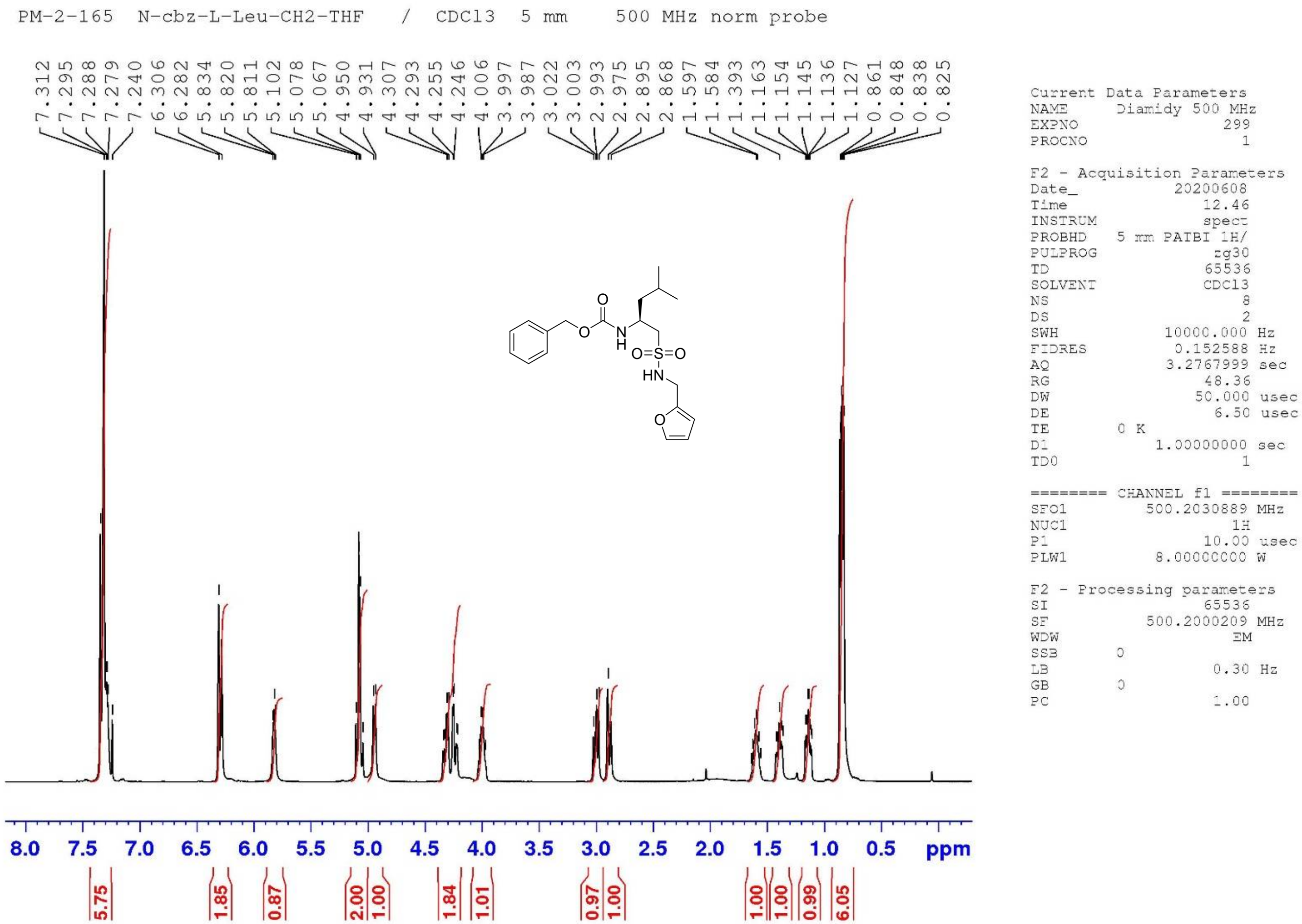
1.42. Copy of ^{13}C NMR of **5l**



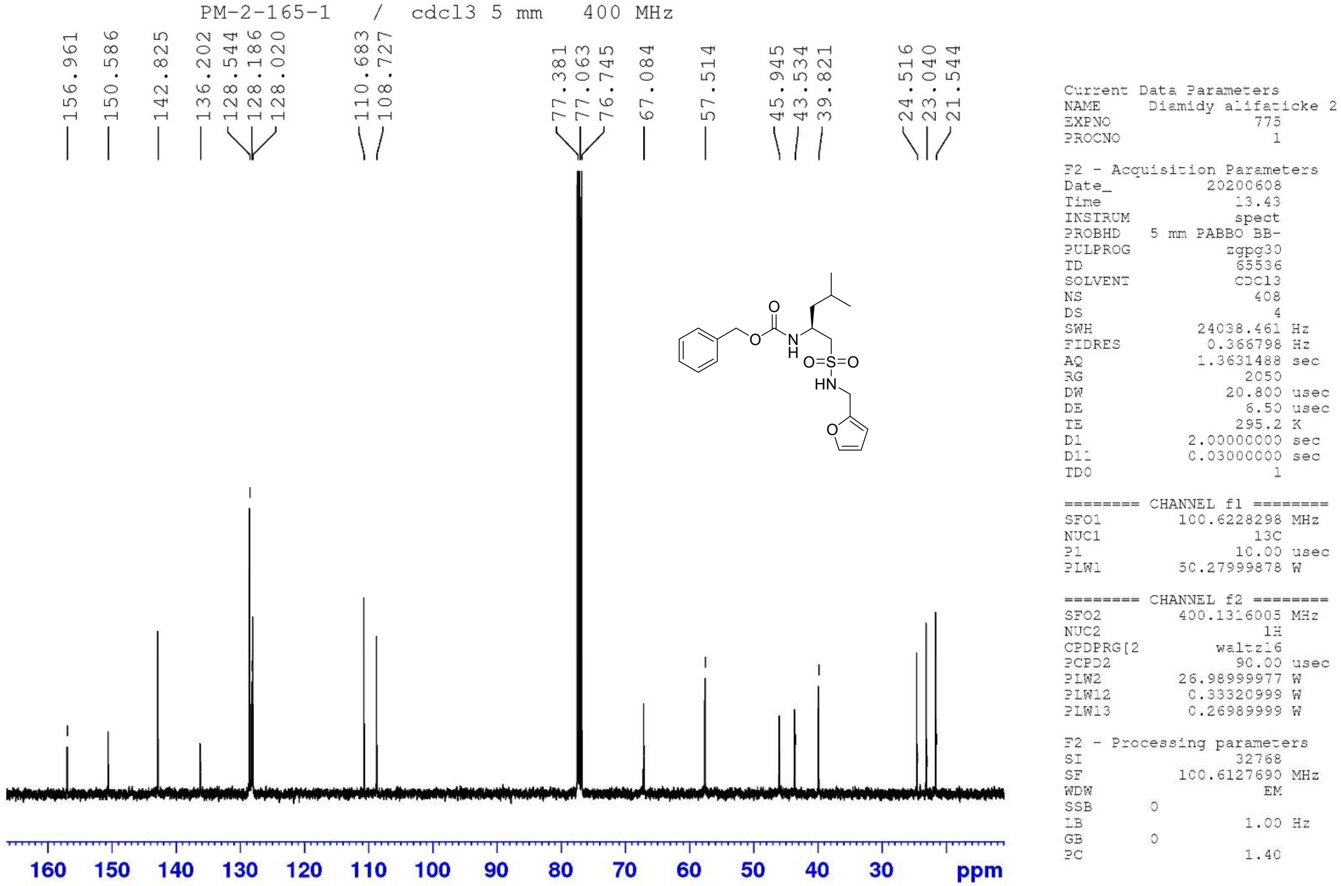
1.43. Copy of ^{19}F NMR of **5l**



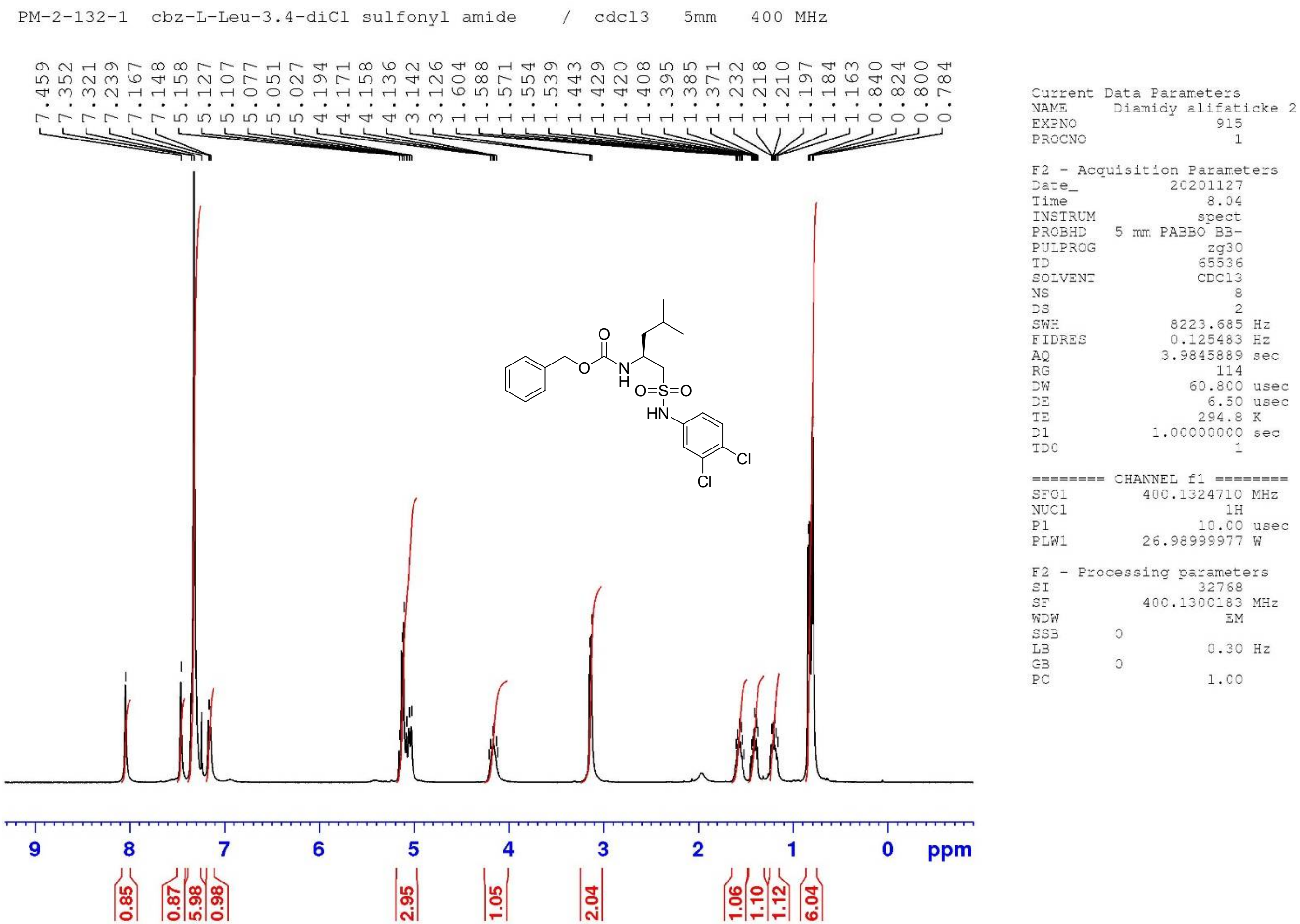
1.44. Copy of [1]H NMR of **5m**



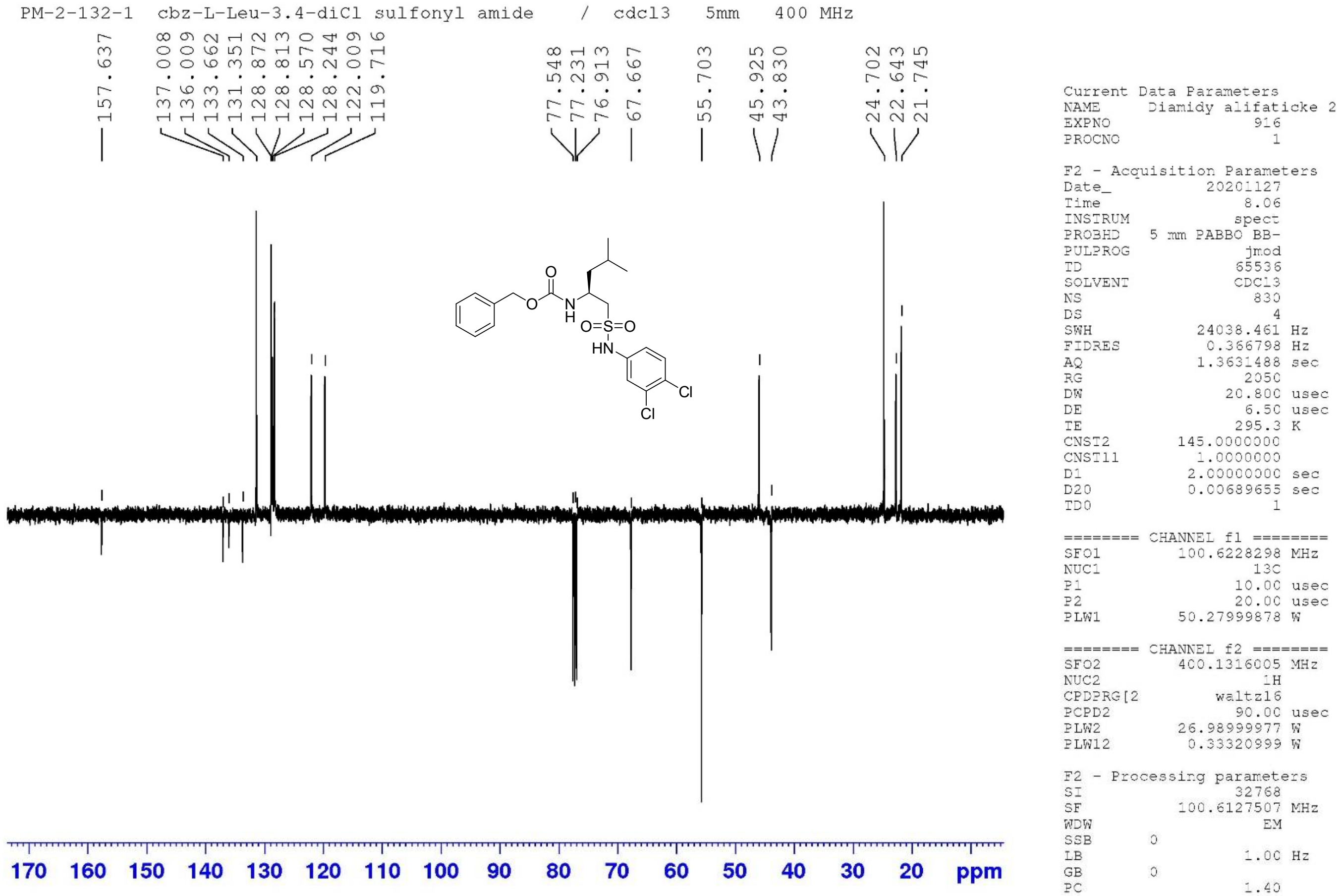
1.45. Copy of ^{13}C NMR of **5m**



1.46. Copy of ^{1}H NMR of **5n**



1.47. Copy of ^{13}C NMR of **5n**



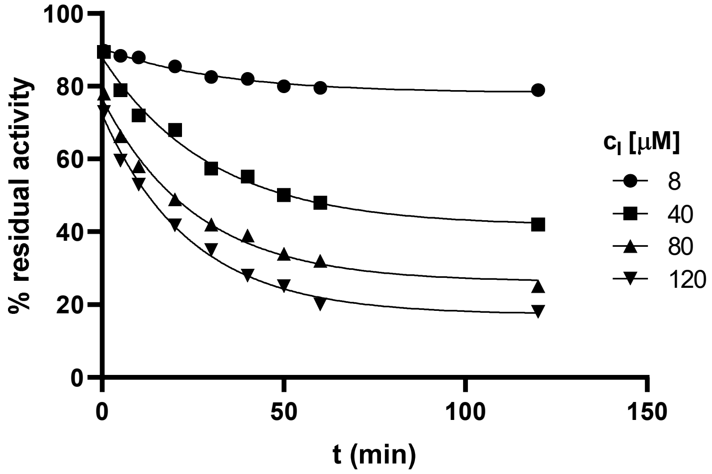


Figure S2 The dependence % residual BChE activity vs. time in the presence of **5k**. The points are expressed as the mean of at least two separate experiments.

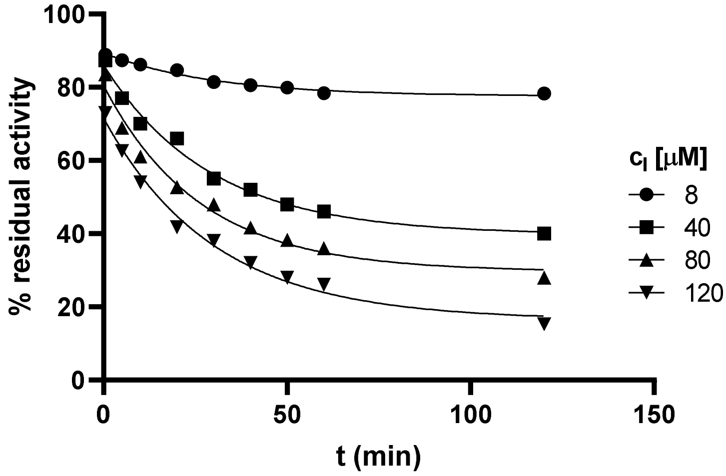


Figure S3 The dependence % residual BChE activity vs. time in the presence of **5j**. The points are expressed as the mean of at least two separate experiments.

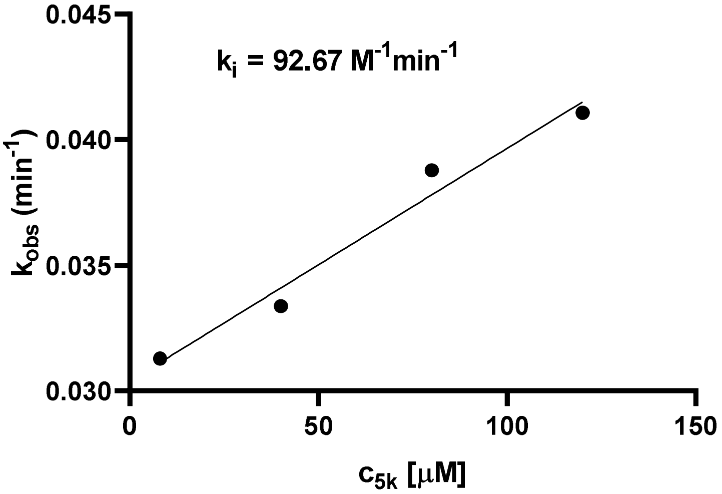


Figure S4 The dependence pseudo-first-order inhibition rates vs. concentration of **5k.**

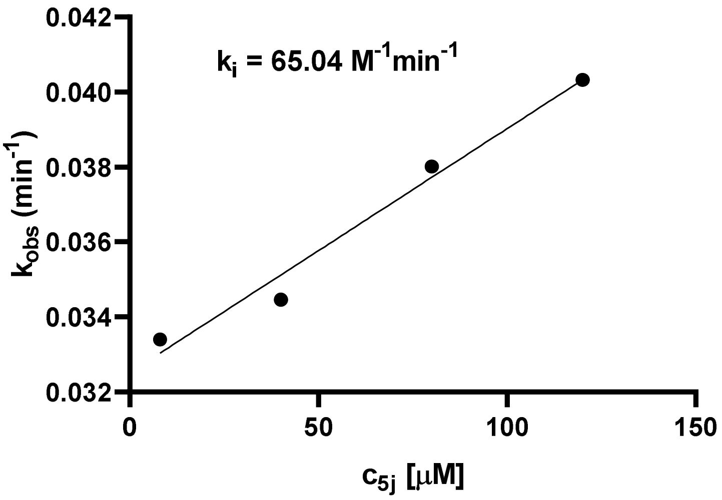


Figure S5 The dependence pseudo-first-order inhibition rates vs. concentration of **5j.**

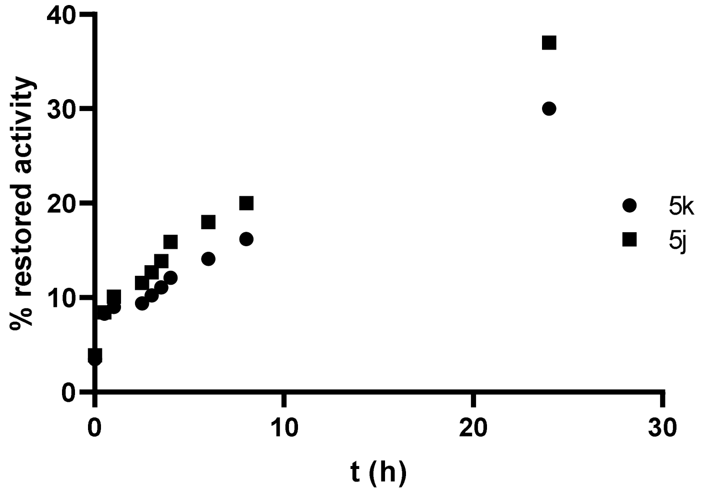


Figure S6 Reactivation of BChE inhibited by **5k** and **5j.** The points are expressed as the mean of at least two separate experiments.

Preliminary stability test of compound **5k** (HPLC results)

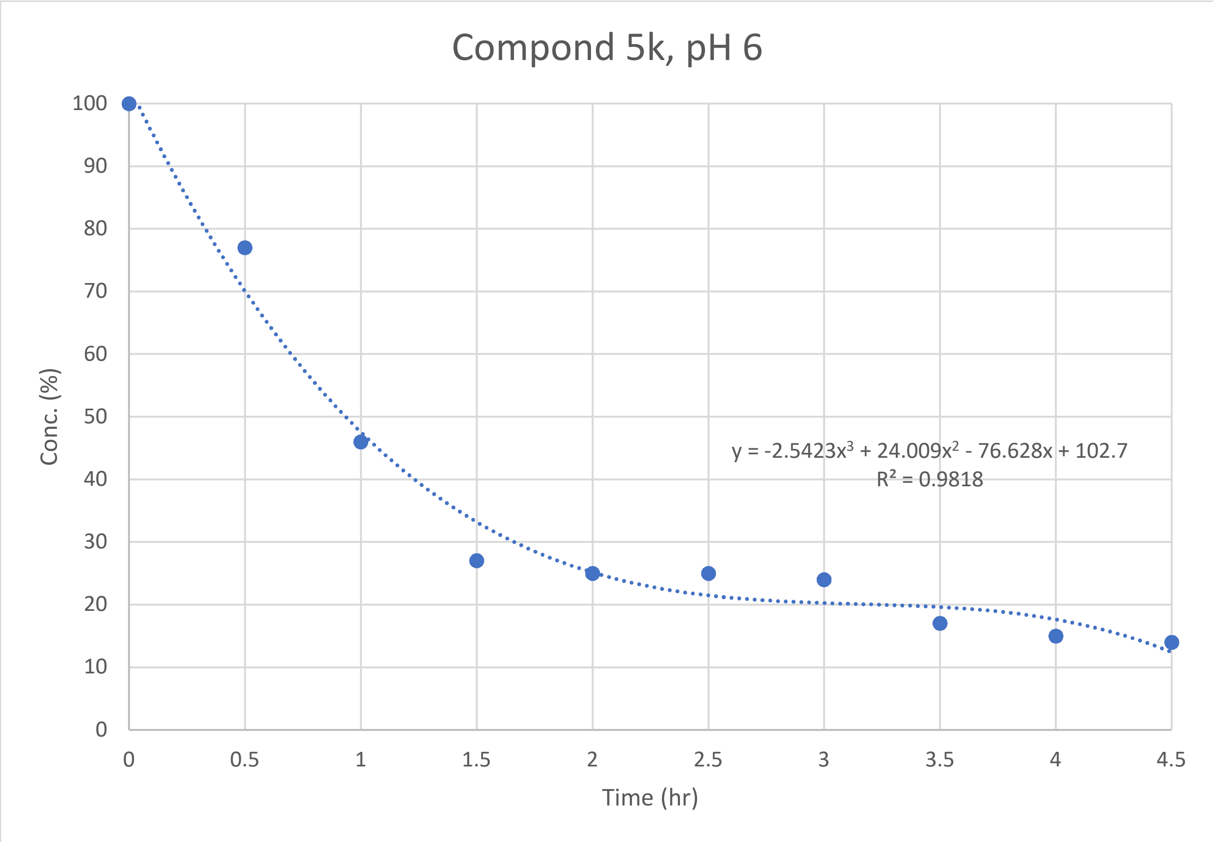
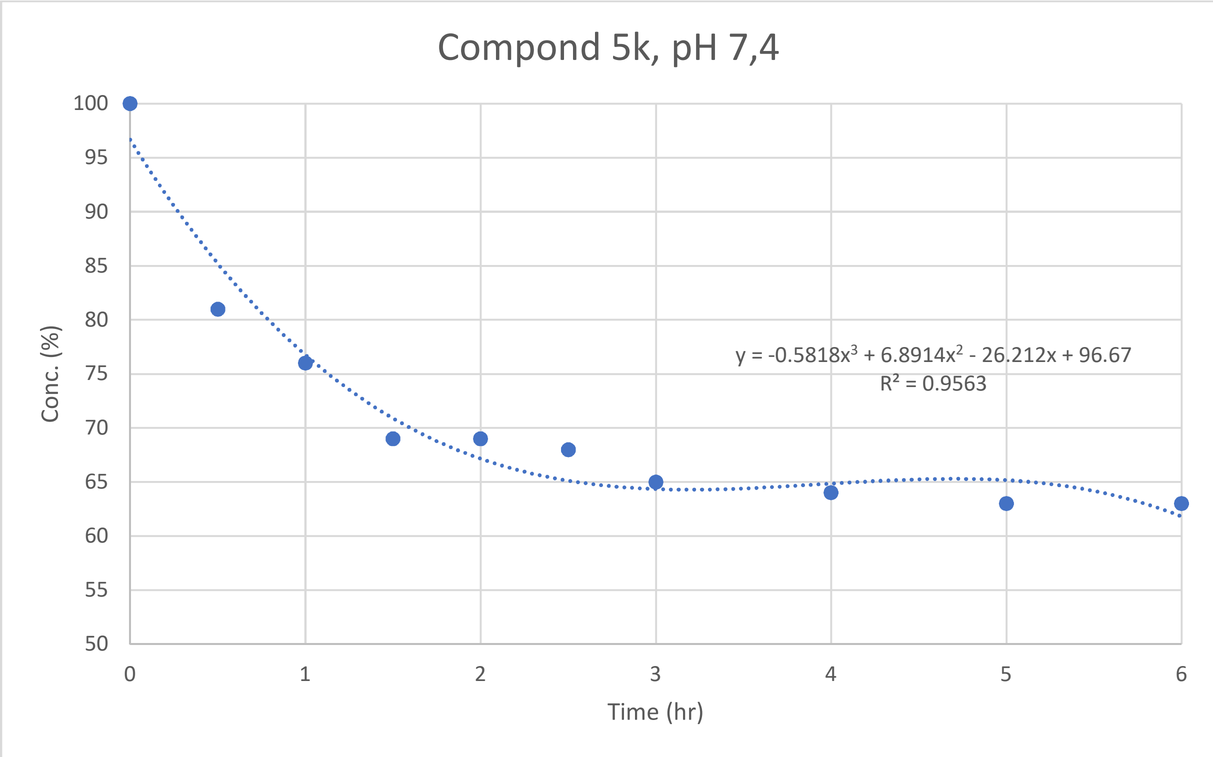


Fig. 1. Decomposition study of compound **5k** at pH 6



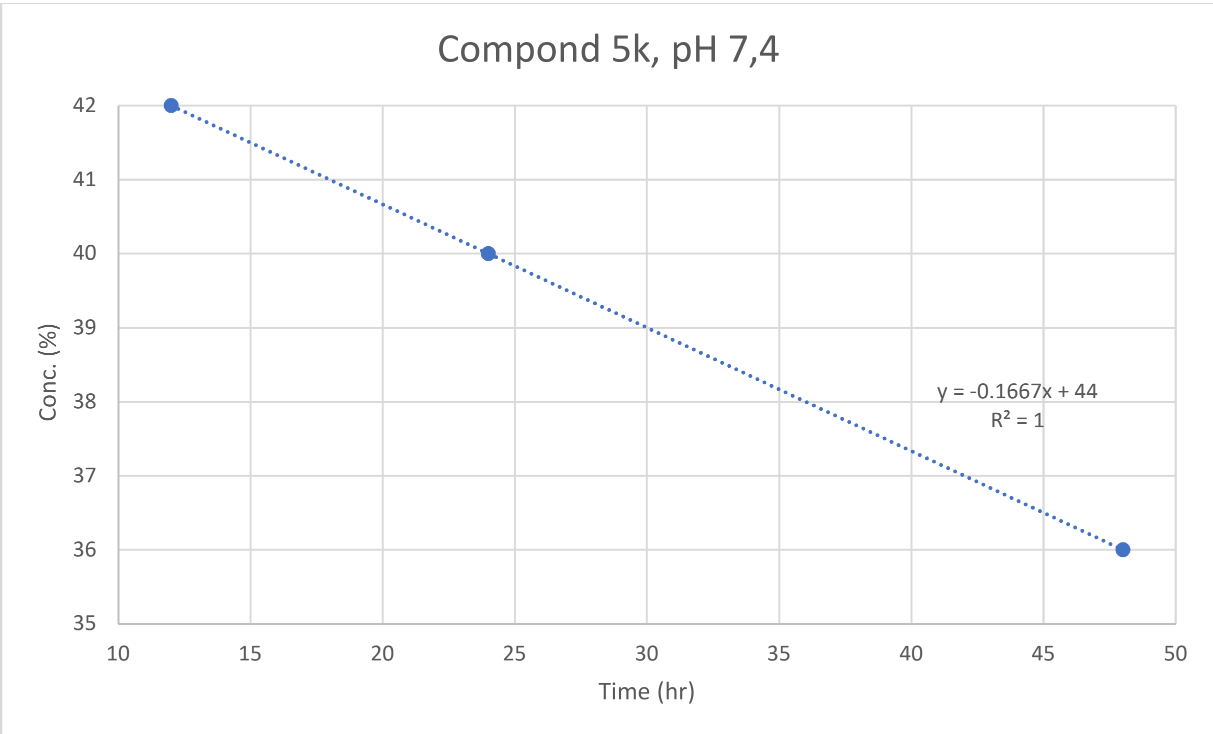


Fig. 2. Decomposition study of compound **5k** at pH 7,4.

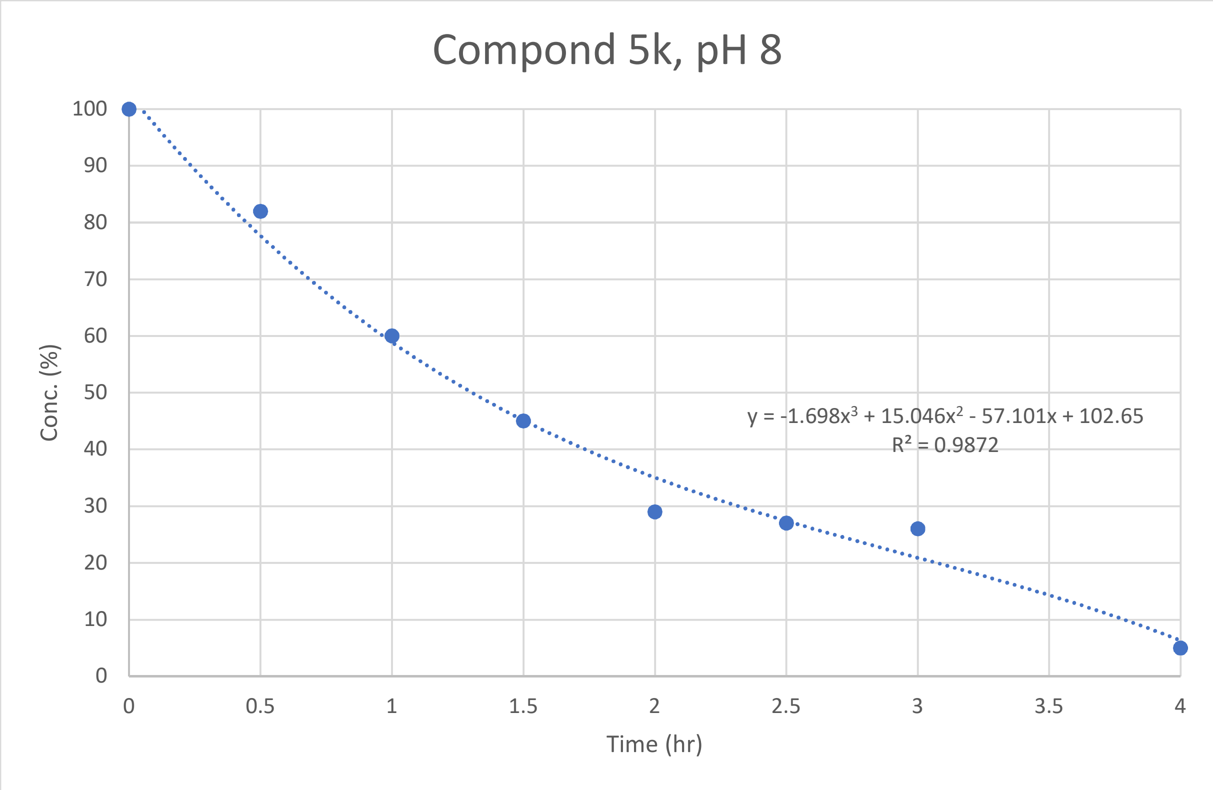


Fig. 3. Decomposition study of compound **5k** at pH 8.

The compound **5k** was chosen for the preliminary study of degradation in three different pH (6, 7.4, 8). Measurement of these degradations was performed by HPLC in reverse phase acetonitrile/water (98:2). The compound was stirred in a tempered buffer (37 °C) of appropriate pH and a sample was taken for HPLC analysis at a specified time. The individual graphs show a clear decomposition of the studied substance as a function of time. In all cases, it is always a pseudo-first order, where one component was in excess and its concentration does not change in time.

Figure 1 represents decomposition study of compound **5k** at pH **6**. The graph shows a significant decomposition in the first hour, when 75% of the substance is rapidly decomposed. However after 6 hrs remains only 13% of starting material in a sample.

In second case (**Figure 1**) If the 5k substance was stirred in a pH 7.4 buffer, its decomposition took twice as long as in the case of pH 6 as it can be seen from the graph. Then there is a gradual decomposition, when only 36% of the starting material remains in 48 hours.

A more interesting situation occurs when the compound 5k is stirred in a buffer of pH 8. Very rapid decomposition occurs as early as 4 hours, when only 5% of the starting material is present in the mixture.

As already mentioned, this is a preliminary study, which was repeated only twice. This is statistically low and it will require further follow-up experiments.

Procedure:

The substance (5k) was dissolved in a buffer of desired pH in Erlenmayer flask and it was placed to tempered water bath (37 °C). At zero time, a sample is taken immediately from solution (1 ml) and analyzed by HPLC (column C18 for reverse phase, mobil phase AcCN/Water 98/2, column temp. 40 °C, flow rate 1 m l/min, injection 10 µl, detection at 214 and 254 nm). Another samples which were taken from solution were at times: 0,5h, 1h, 1,5h, 2h, 2,5h, 3h, 4h, 5h, 6h, 12h, 24h, 48h, 72h and 96h.