Article

4-amino-1,2,4-triazole-3-thione as a promising scaffold for the inhibition of serine and metallo β-lactamases

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Figure S1. Catalytic site in VIM-1 apo form (PDB code 5n5g). Protein structure is reported in cartoon representation, zinc atoms are shown as grey spheres, bridging water Wat1 and coordinating water Wat2 have been depicted as red spheres and labelled, significant residues coordinating the zinc atoms or lining the binding pocket are shown as sticks, coordination bonds are reported as dashed lines. Loops L3 (red) and L10 (green) are highlighted.

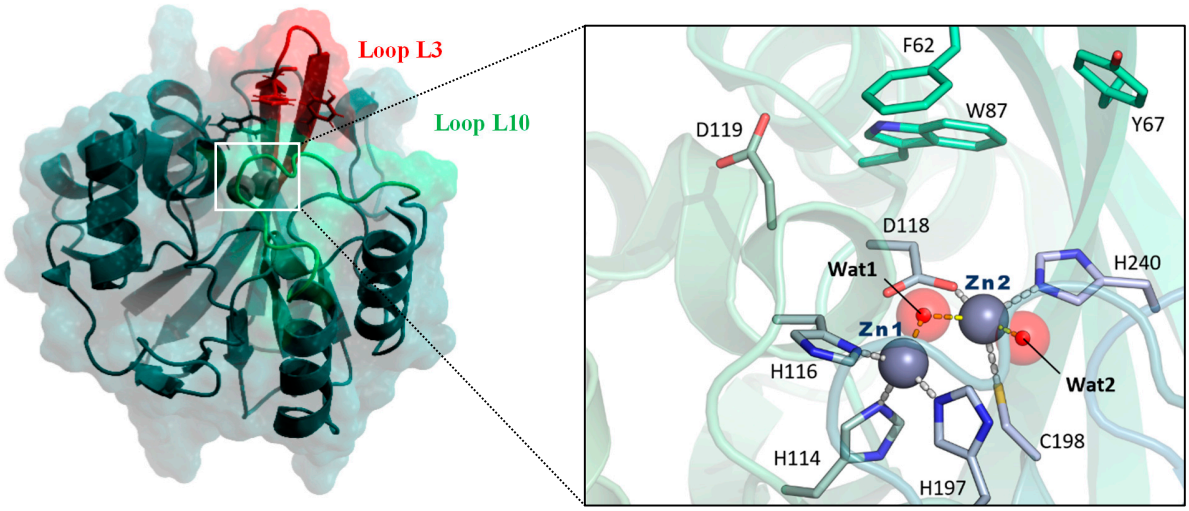

Figure S2. Comparison of VIM-1 (a) and IMP-1 (b) binding site. Protein structure is reported in cartoon representation, zinc atoms are shown as grey spheres, significant residues coordinating the zinc atoms or lining the binding pocket are shown as sticks, coordination bonds are reported as dashed lines.

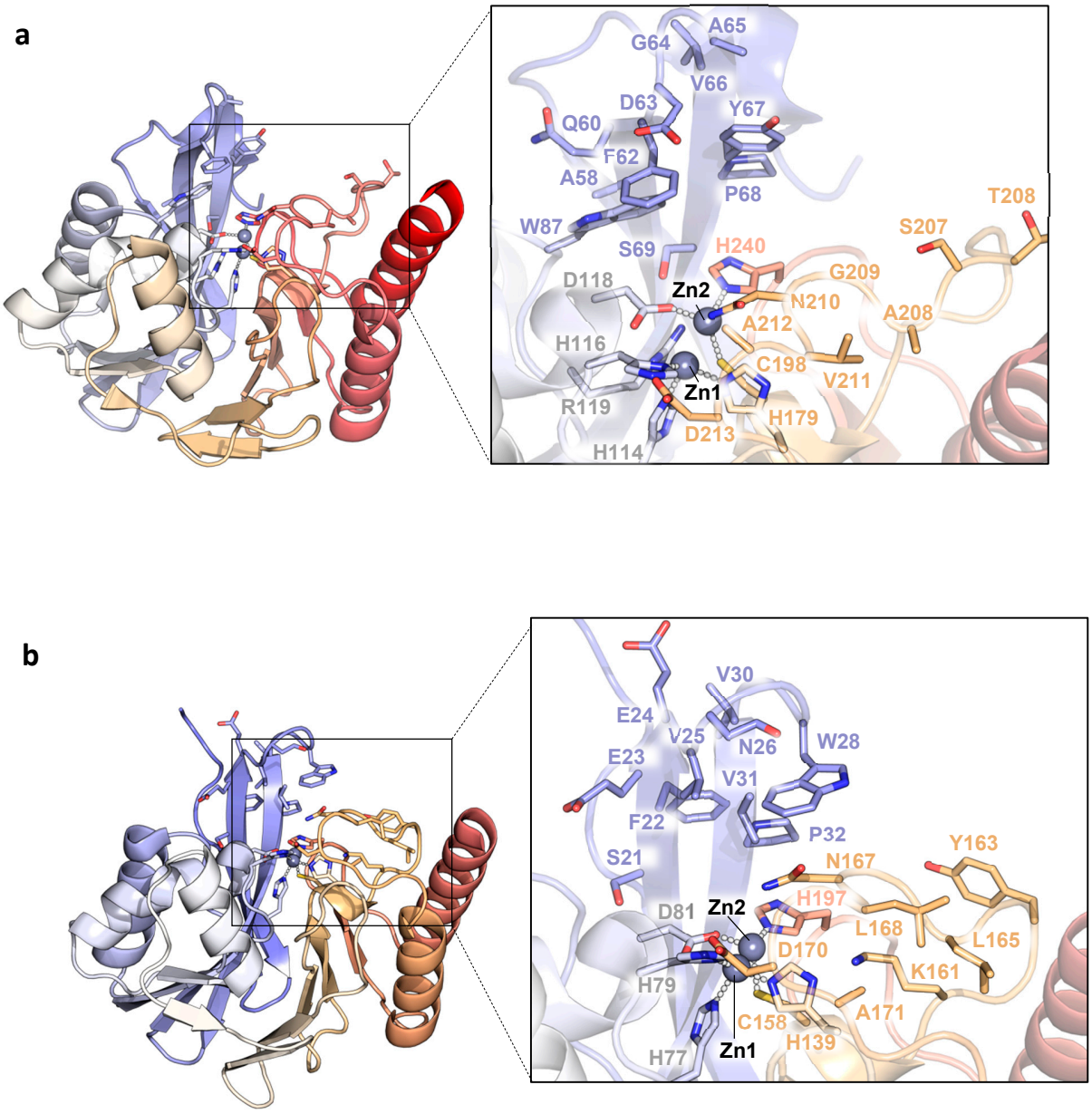

Figure S3. Crystallographic orientation of a 1,2,4-triazole-3-thiol compound in L1 MBL (PDB CODE 5dpx). Protein structure is reported in cartoon representation, zinc atoms are shown as grey spheres, significant residues coordinating the zinc atoms or lining the binding pocket are shown as sticks, coordination bonds are reported as dashed lines.

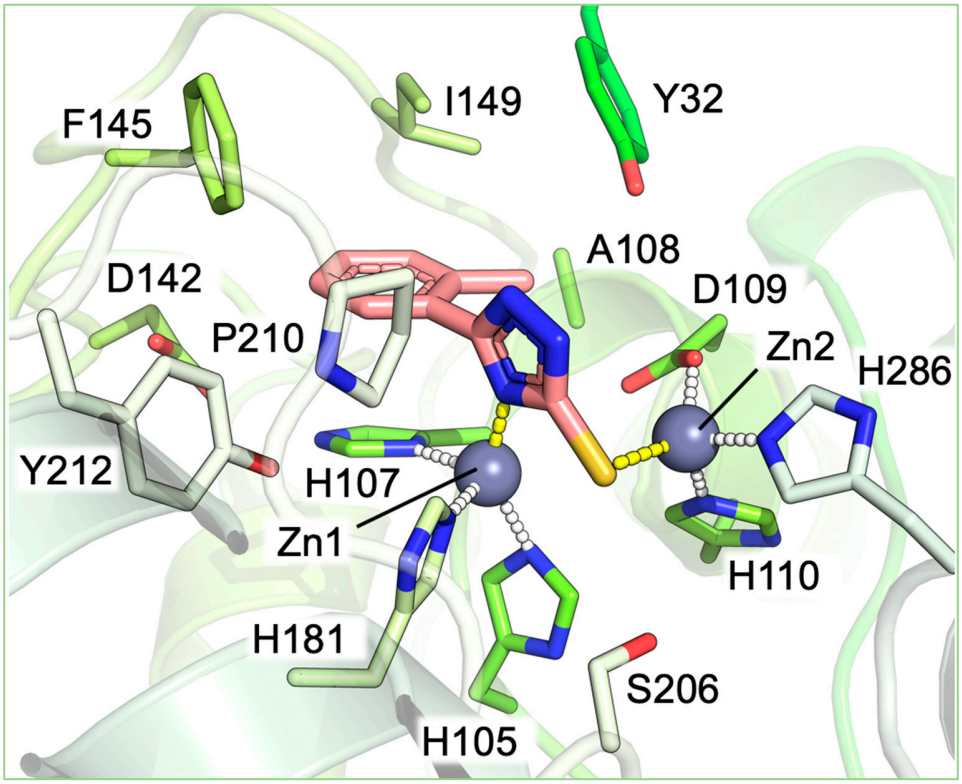

Table S1. Enzymatic Inhibitor Activity of compounds 1a-g and 2a-g against VIM-1, IMP-1 and KPC-2 not included in Table 1.

Code	VIM-1 K_i (μM)	IMP-1 % of inhibition at 200 μM	KPC-2 % of inhibition at 200 μM
1a	601	37	20
1b	137	51	17
1c	182	44	6
1e	204	44	15
1g	n.t.	n.t	n.t
2a	50,3	46	18
2c	124	45	4
2d	66,9	45	33
2e	142	46	7
2f	145	40	21

Table S2. MICs of meropenem in combination with compounds 1a-g and 2a-g

Clinical strains of *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *P. aeruginosa* producing NDM-1, VIM-2, IMP-28 and KPC-2 were used. The MIC was calculated through the microdilution method using 96-microwell plates. The bacterial inoculum in each well was adjusted at a final dilution of 1:1000 from an overnight culture. Meropenem (MEM) was mixed with every compound maintaining a mole ratio of 1:1 in all the wells.

K. pneumoniae **NDM-1**	**MIC (µg/ml)**
Meropenem (MEM)	512
MEM + 2f	512
MEM + 1a	256 – 512
MEM + 1b	256 – 512
MEM + 1c	512
MEM + 1d	512
MEM + 1e	512
MEM + 1g	512
MEM + 2b	512
MEM + 2c	256
MEM + 2e	256 – 512
MEM + 2g	512

K. pneumoniae 53A8 **KPC-2**	**MIC (µg/ml)**
Meropenem (MEM)	64
MEM + 1a	32
MEM + 1b	64
MEM + 1c	32
MEM + 1d	64
MEM + 1e	64
MEM + 1g	64
MEM + 2b	64
MEM + 2c	64
MEM + 2e	64
MEM + 2f	64
MEM + 2g	64

P. aeruginosa **VIM-2**	**MIC (µg/ml)**
Meropenem (MEM)	8
MEM + 2f	8
MEM + 1a	8
MEM + 1b	8
MEM + 1c	8
MEM + 1d	8
MEM + 1e	8
MEM + 1g	8
MEM + 2b	8
MEM + 2c	8
MEM + 2e	8
MEM + 2g	8

K. pneumoniae 53A9 **KPC-2**	**MIC (µg/ml)**
Meropenem (MEM)	64
MEM + 1a	64
MEM + 1b	64
MEM + 1c	64
MEM + 1d	64
MEM + 1e	64
MEM + 1g	64
MEM + 2b	64
MEM + 2c	64
MEM + 2e	64
MEM + 2f	64
MEM + 2g	32

P. aeruginosa **IMP-28**	**MIC (µg/ml)**
Meropenem (MEM)	16
MEM + 2f	16
MEM + 1a	16
MEM + 1b	16
MEM + 1c	16
MEM + 1d	16
MEM + 1e	16
MEM + 1g	16
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MEM + 2e	16
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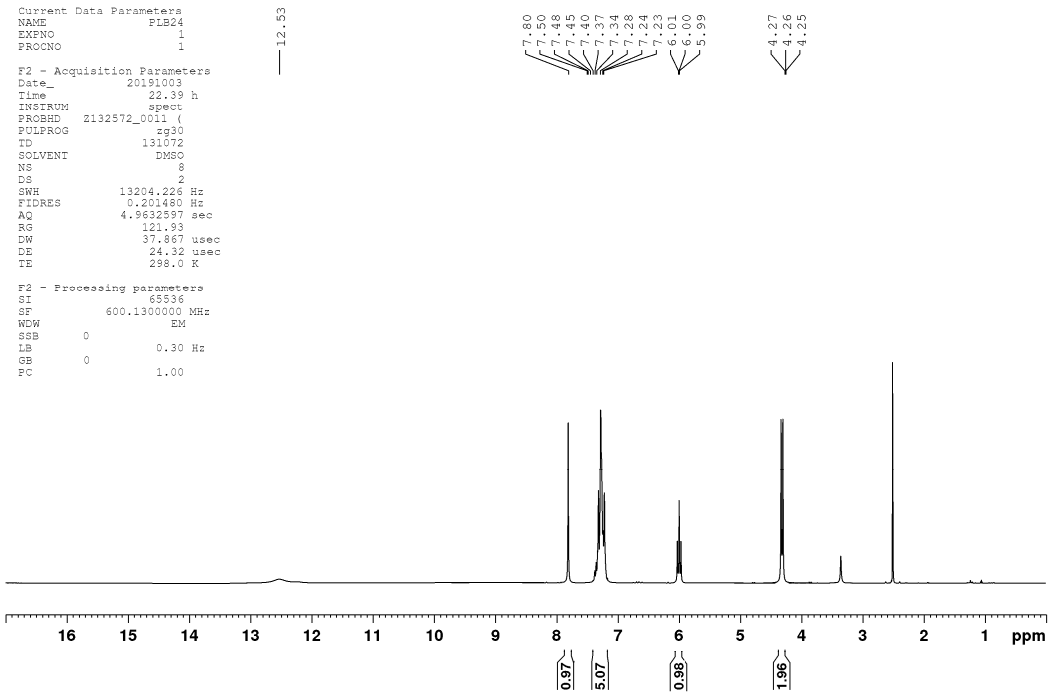
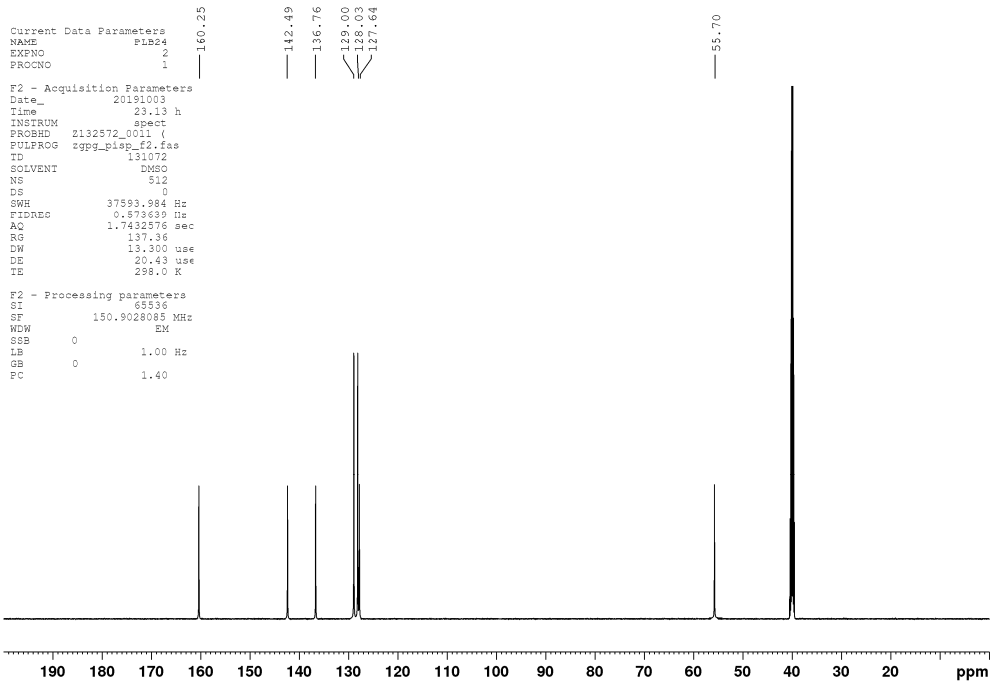
^{1}H and ^{13}C NMR spectra of compounds 1a-g and 2a-g

1a





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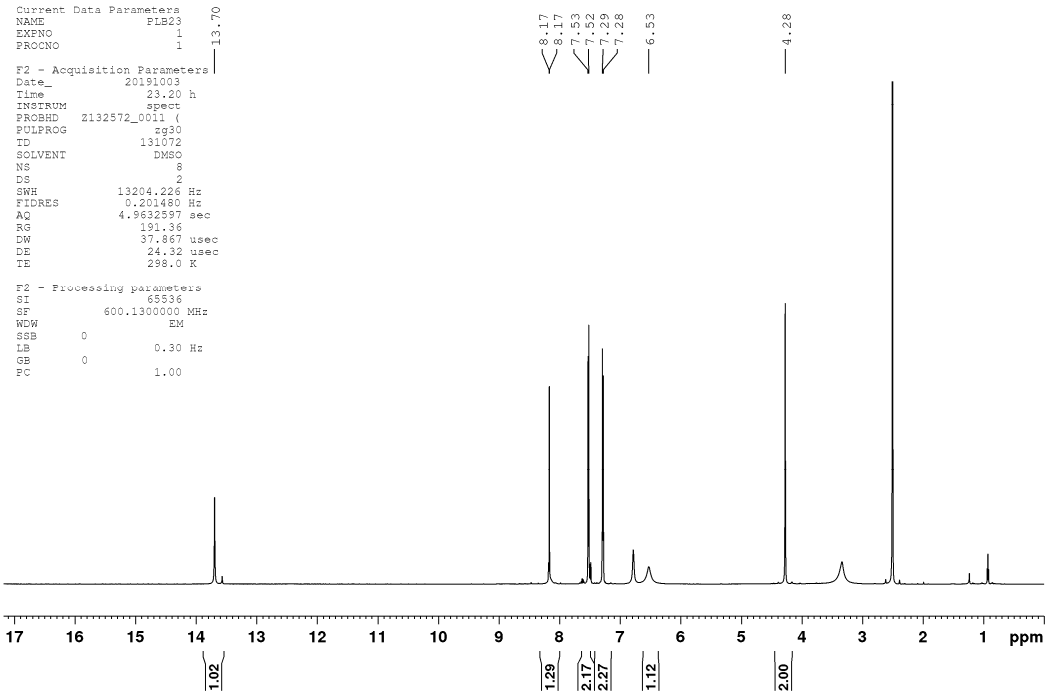
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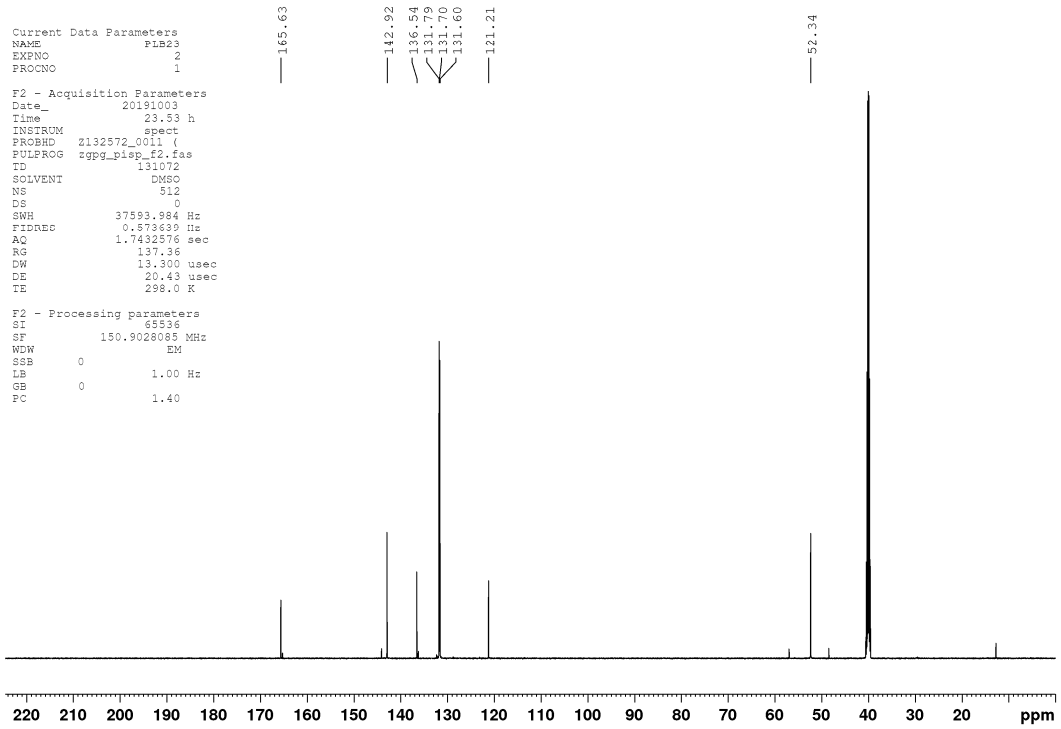
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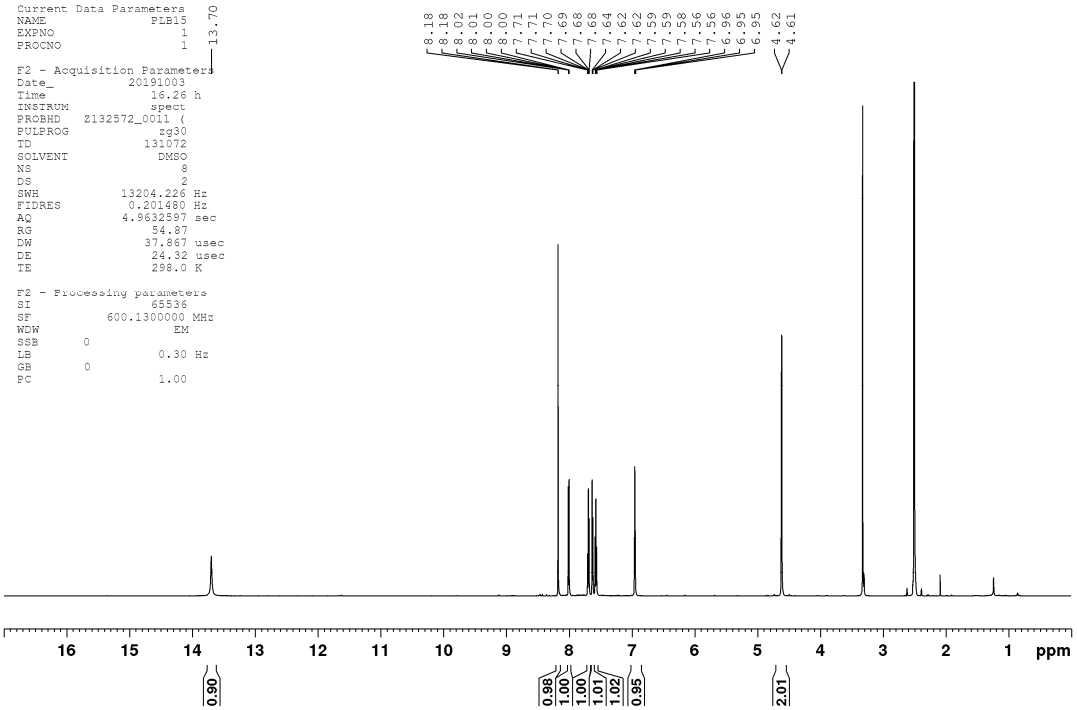
1c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6.95
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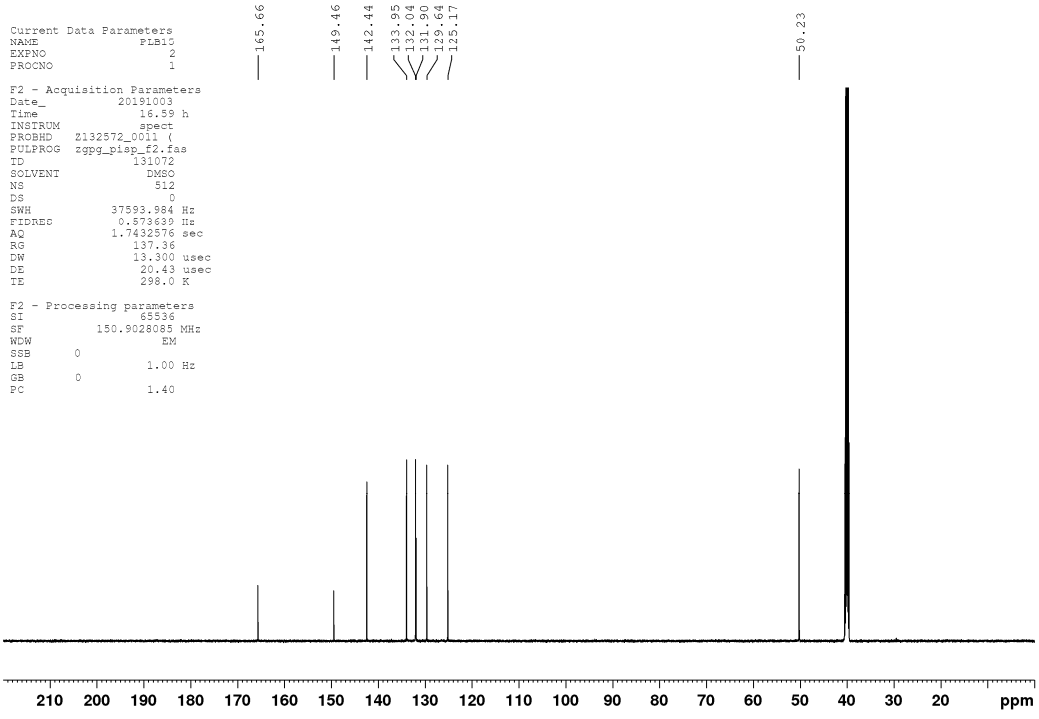
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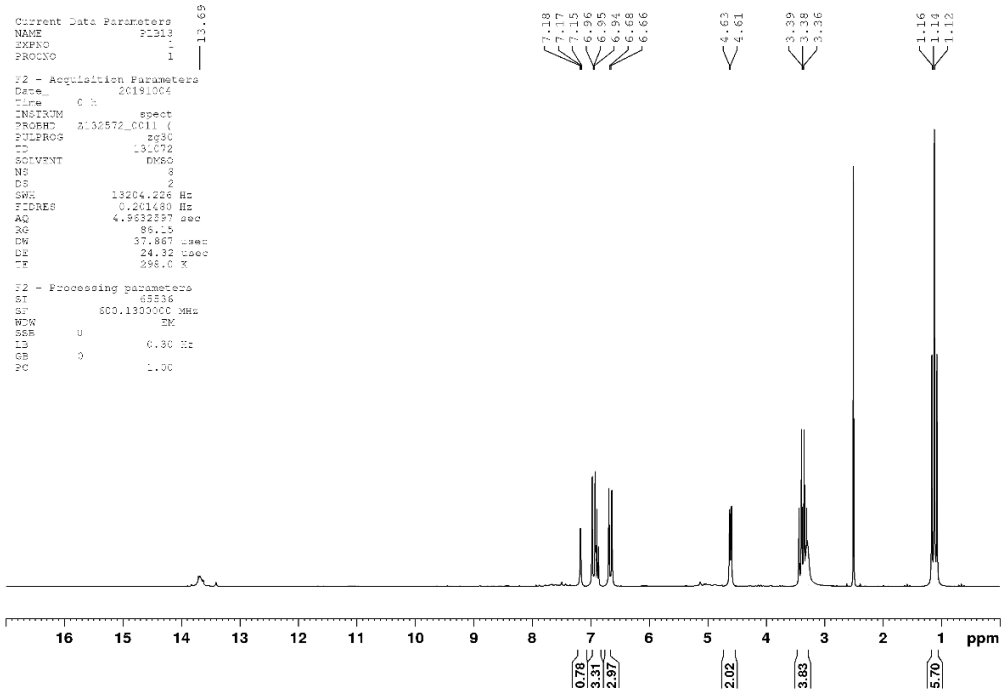
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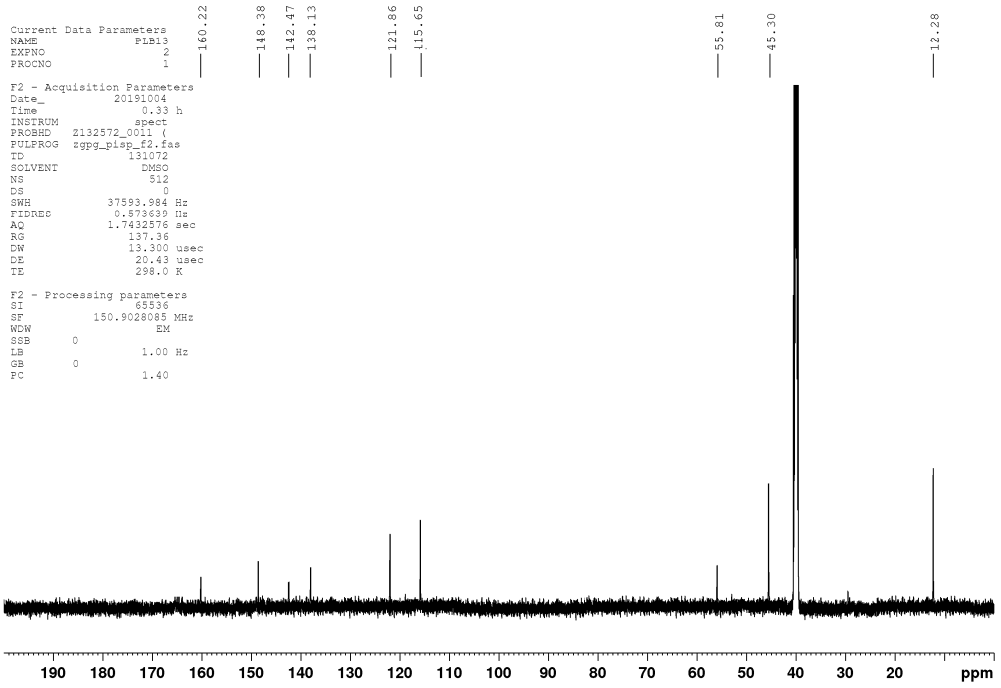
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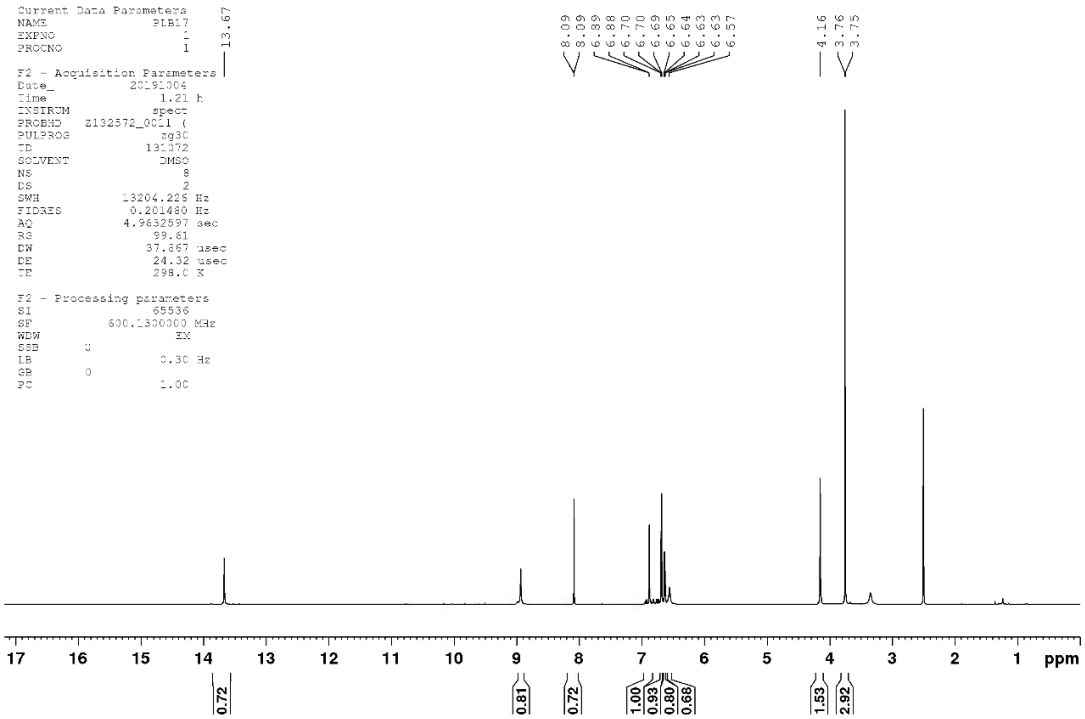
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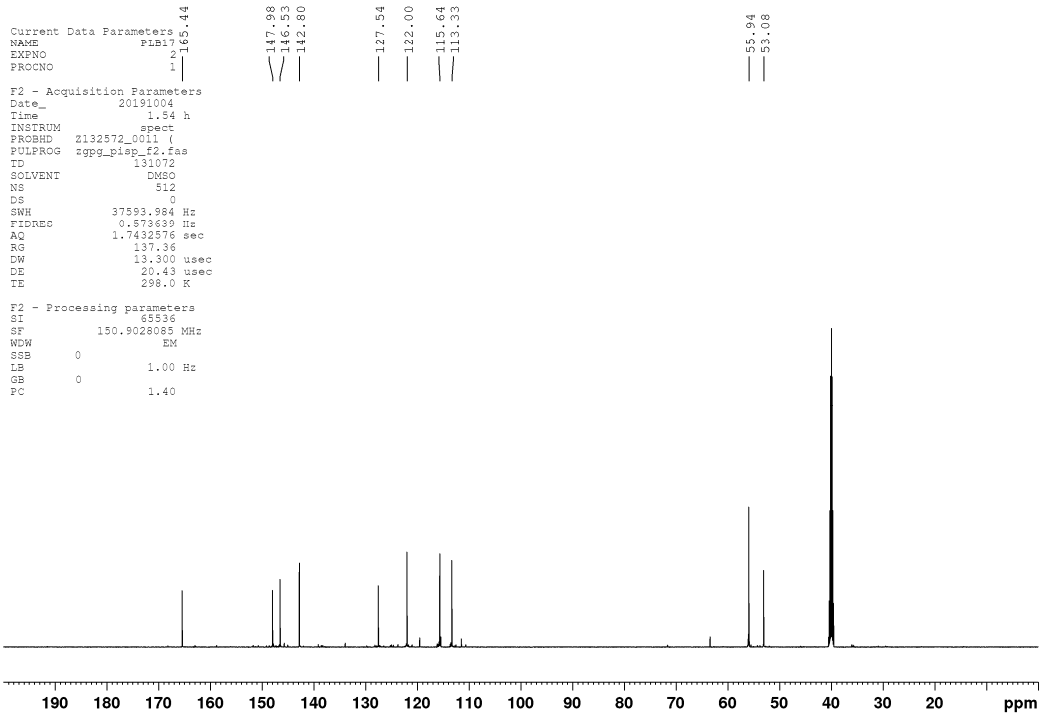
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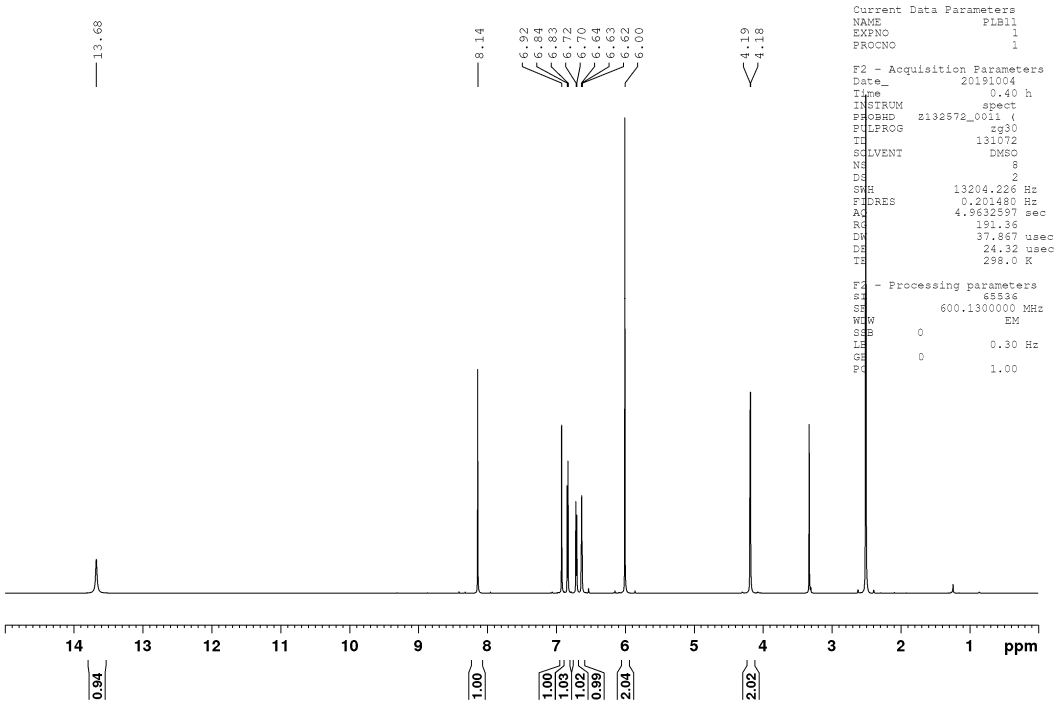
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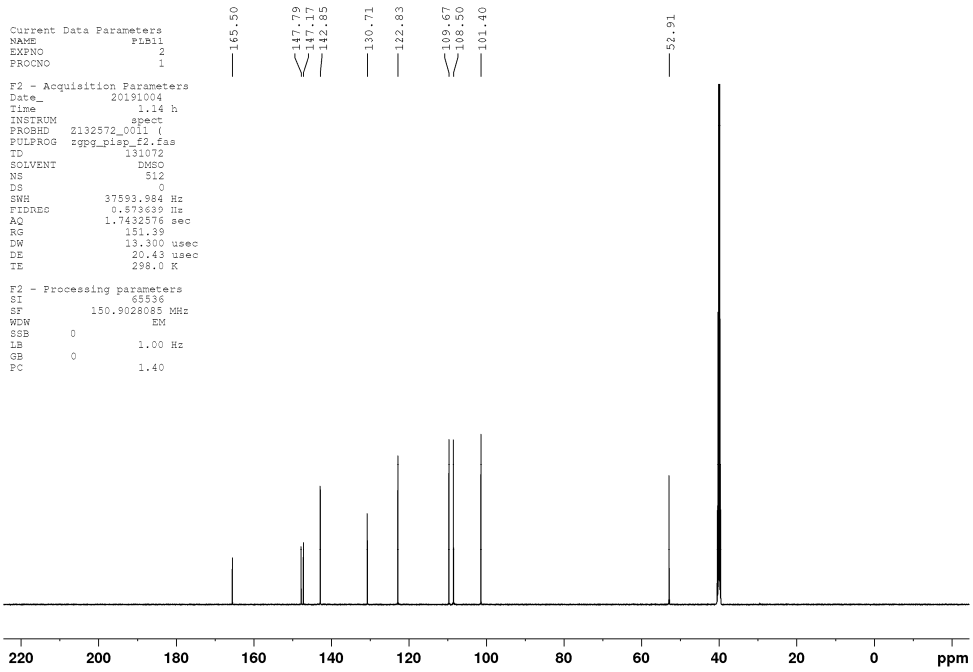
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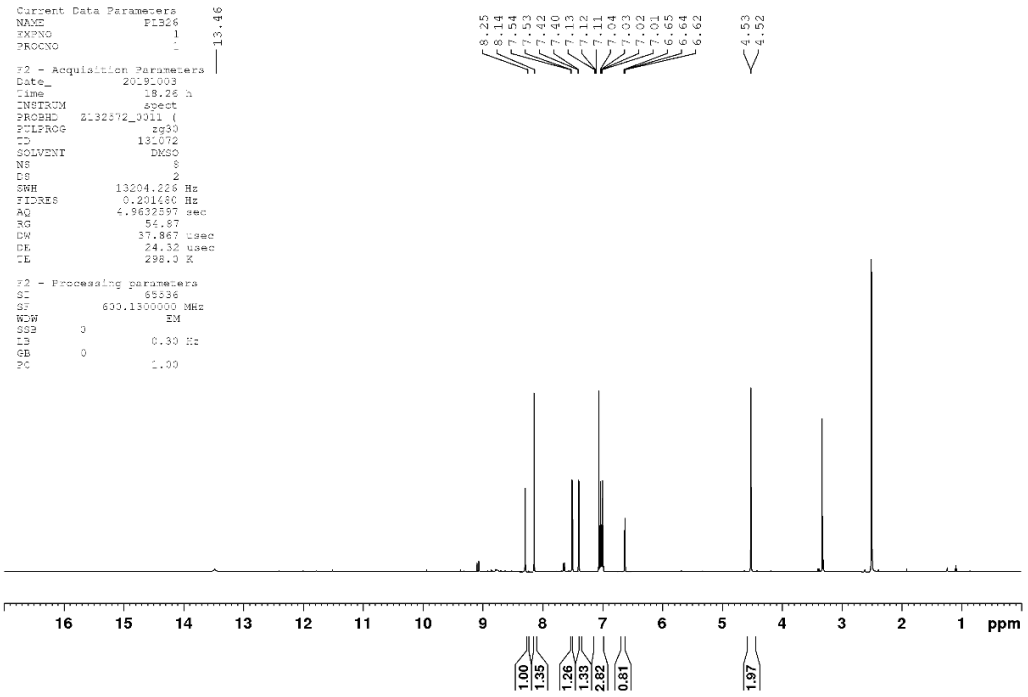
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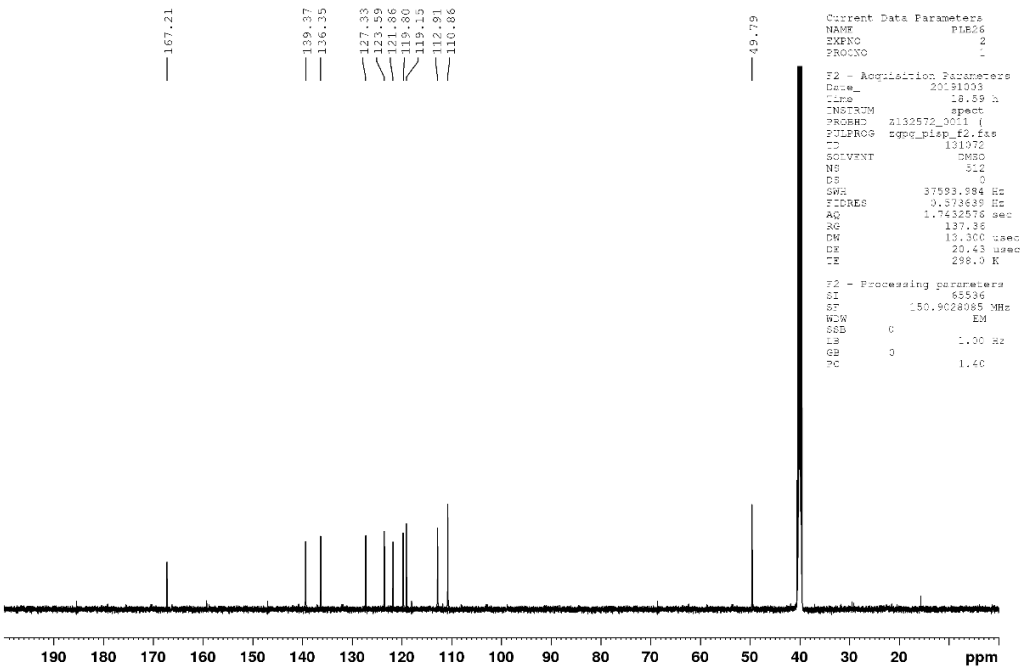
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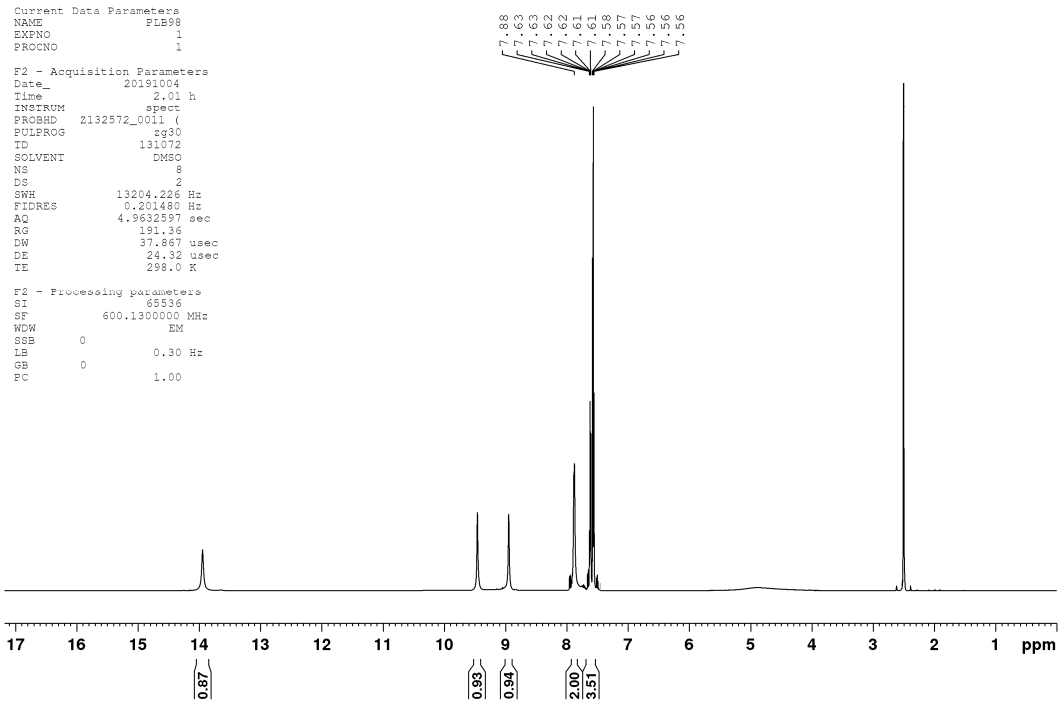
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DE 24.32 usec
TE 298.0 K
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GB 0
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7.61
7.58
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7.56
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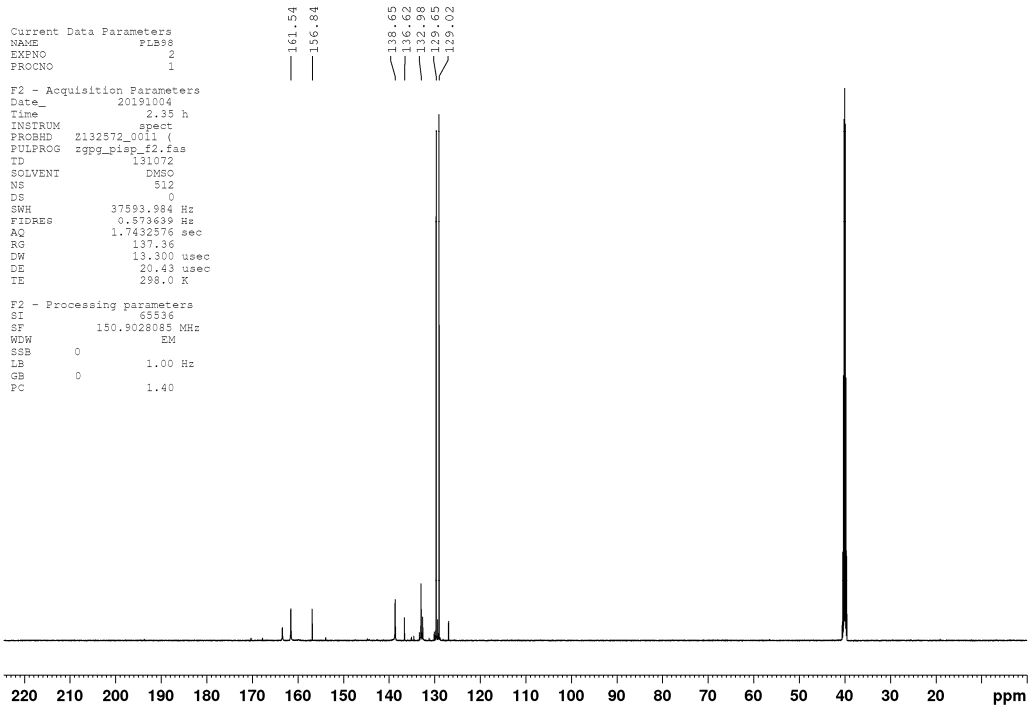
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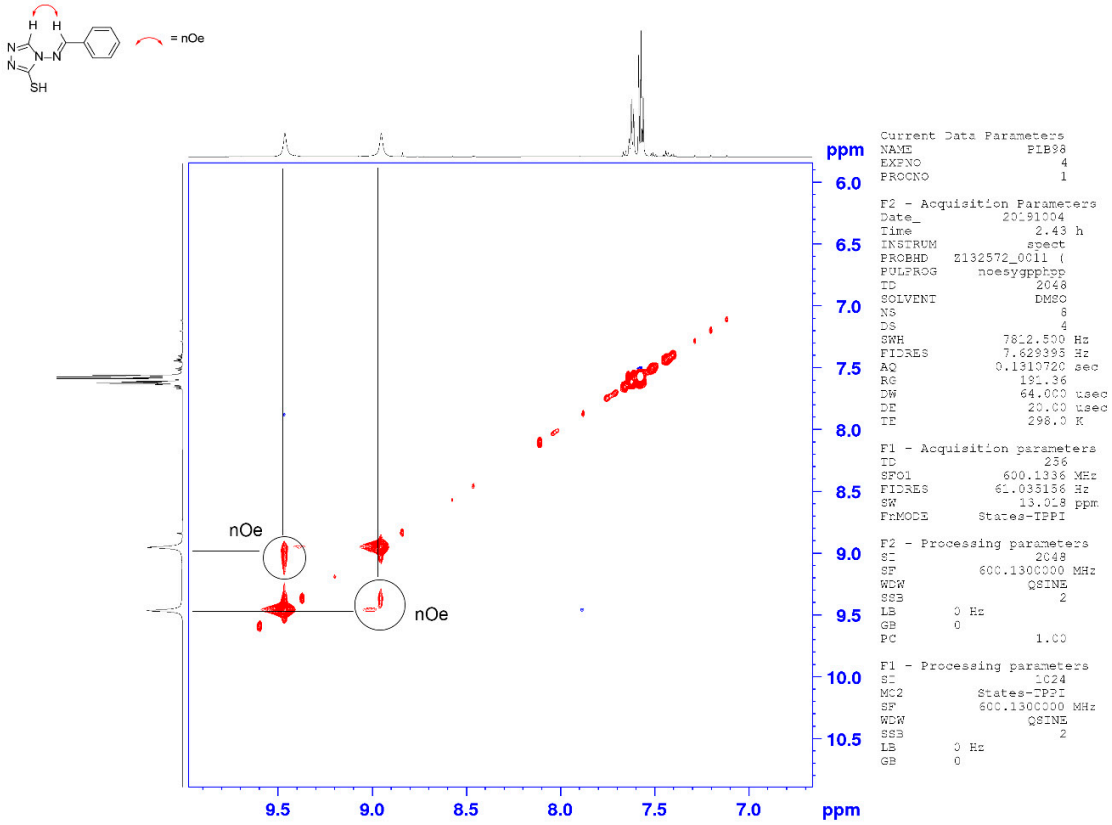
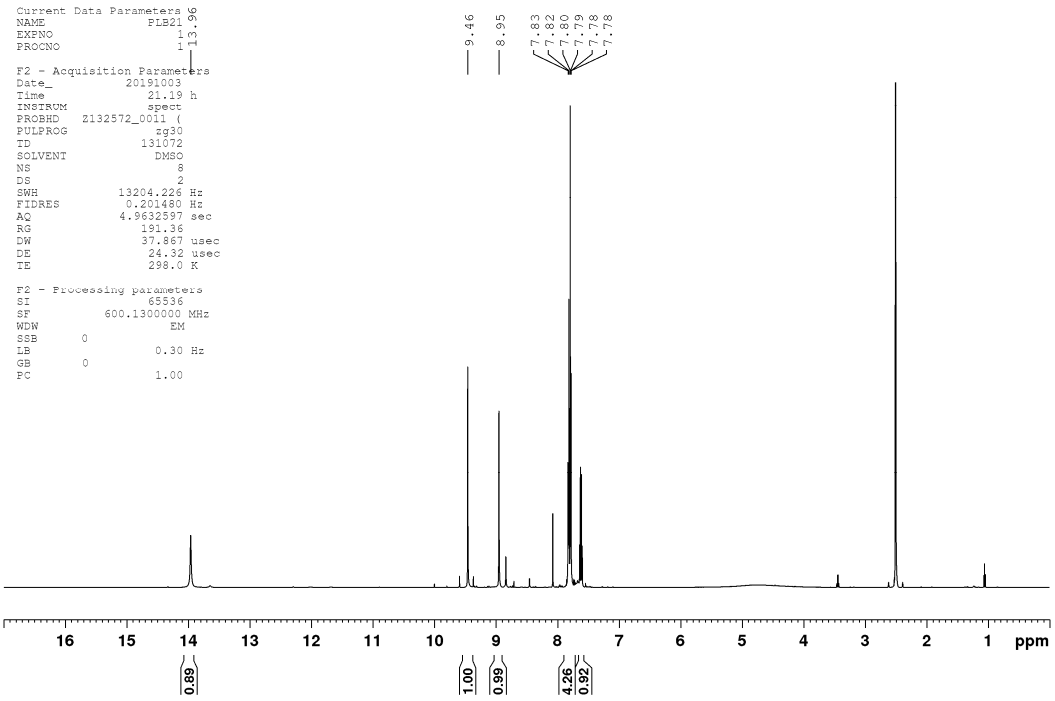
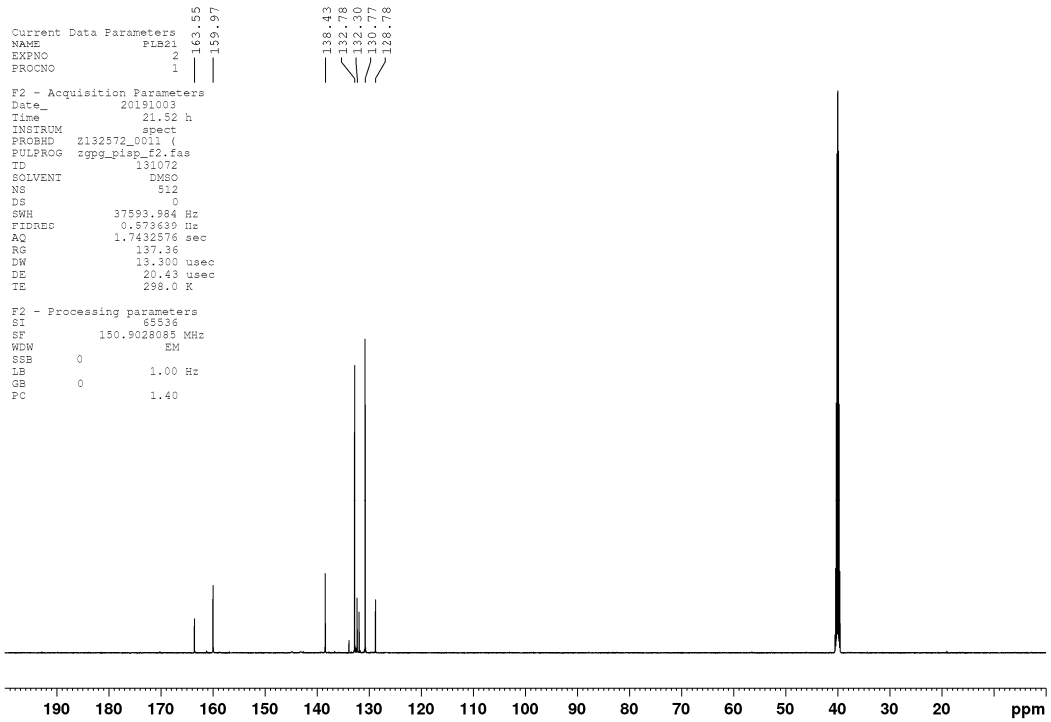
F2 - Processing parameters
SI 65536
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
161.54
156.84
138.65
136.62
132.98
129.65
129.02
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

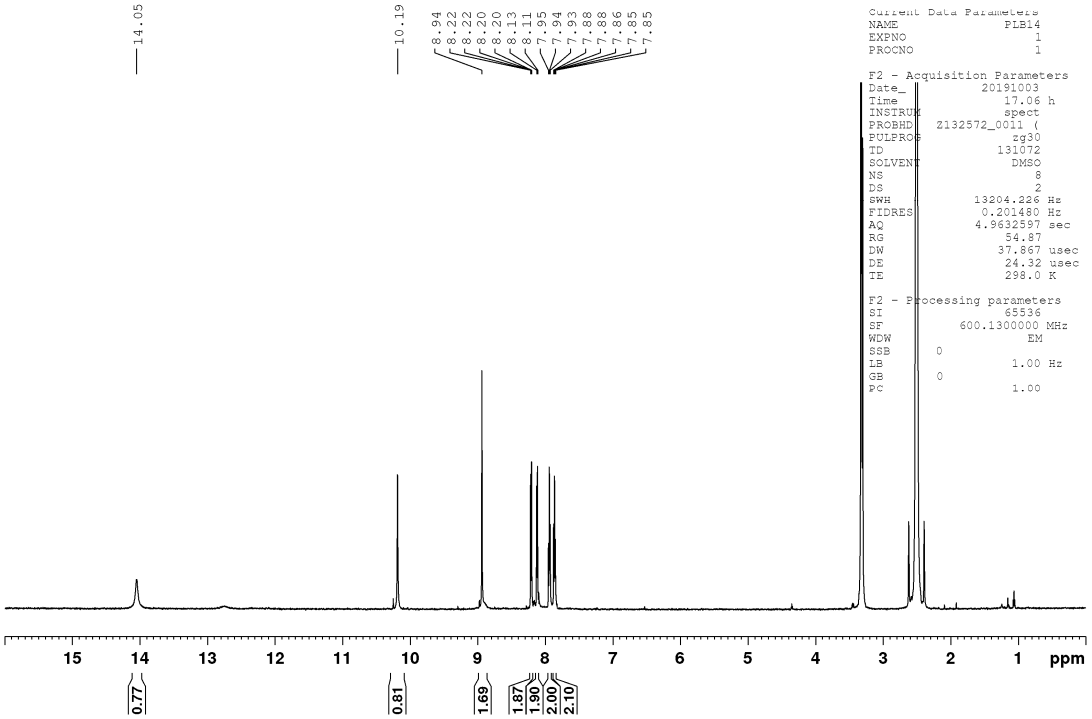

= nOe
nOe
nOe
ppm
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5
10.0
10.5
9.5
9.0
8.5
8.0
7.5
7.0
ppm
Current Data Parameters
NAME PLB98
EXPNO 4
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191004
Time 2.43 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG noesygpphpp
TD 2048
SOLVENT DMSO
NS 8
DS 4
SWH 7812.500 Hz
FIDRES 7.629395 Hz
AQ 0.1310720 sec
RG 181.36
DW 64.000 usec
DE 20.00 usec
TE 298.0 K
F1 - Acquisition parameters
TD 256
SFO1 600.1336 MHz
FIDRES 61.035156 Hz
SW 13.018 ppm
FnMODE States-TPPI
F2 - Processing parameters
SI 2048
SF 600.1300000 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0
PC 1.00
F1 - Processing parameters
SI 1024
MC2 States-TPPI
SF 600.1300000 MHz
WDW QSINE
SSB 2
LB 0 Hz
GB 0

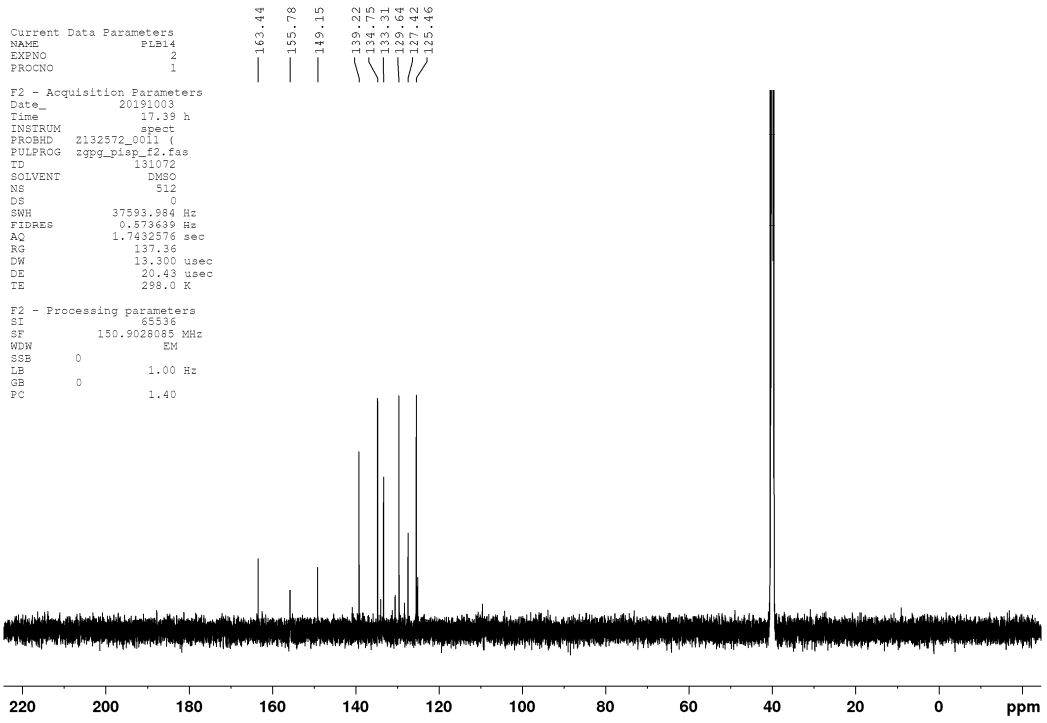
2b


Current Data Parameters
NAME PLB21
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 21.19 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 8
DS 2
SWH 13204.226 Hz
FIDRES 0.201480 Hz
AQ 4.9632597 sec
RG 191.36
DW 37.867 usec
DE 24.32 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 600.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.96
9.46
8.95
7.83
7.82
7.80
7.79
7.78
7.78
16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.89
1.00
0.99
4.26
0.92

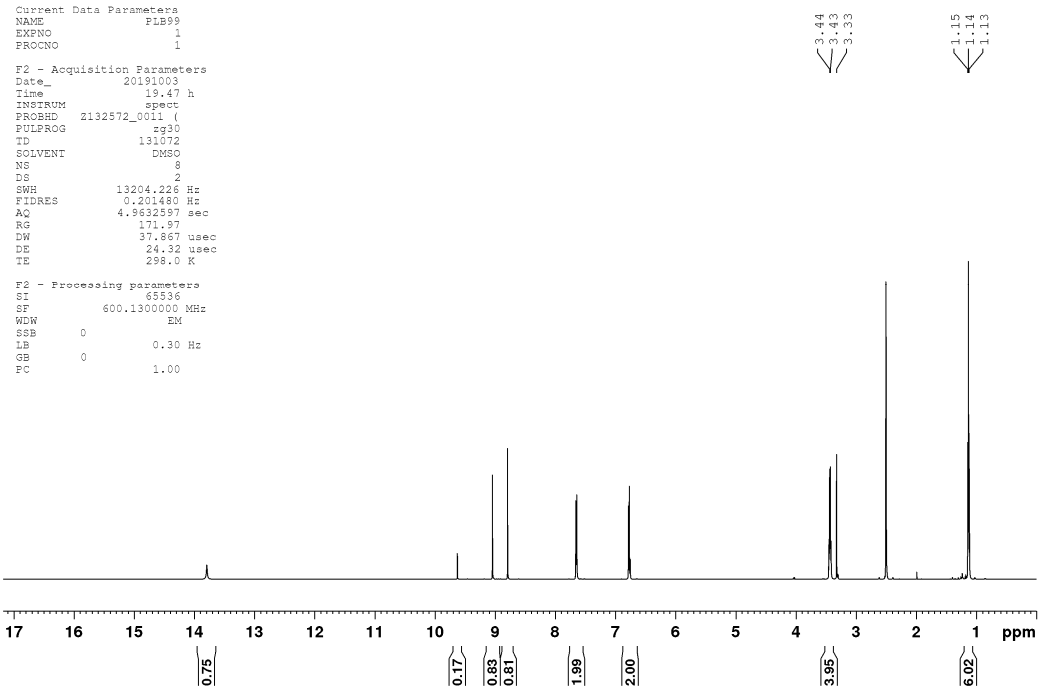

Current Data Parameters
NAME PLB21
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 21.52 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zgpg_pisp_f2.fas
TD 131072
SOLVENT DMSO
NS 512
DS 0
SWH 37593.984 Hz
FIDRES 0.573639 Hz
AQ 1.7432576 sec
RG 137.36
DW 13.300 usec
DE 20.43 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
163.55
159.97
138.43
132.78
132.30
130.77
128.78
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

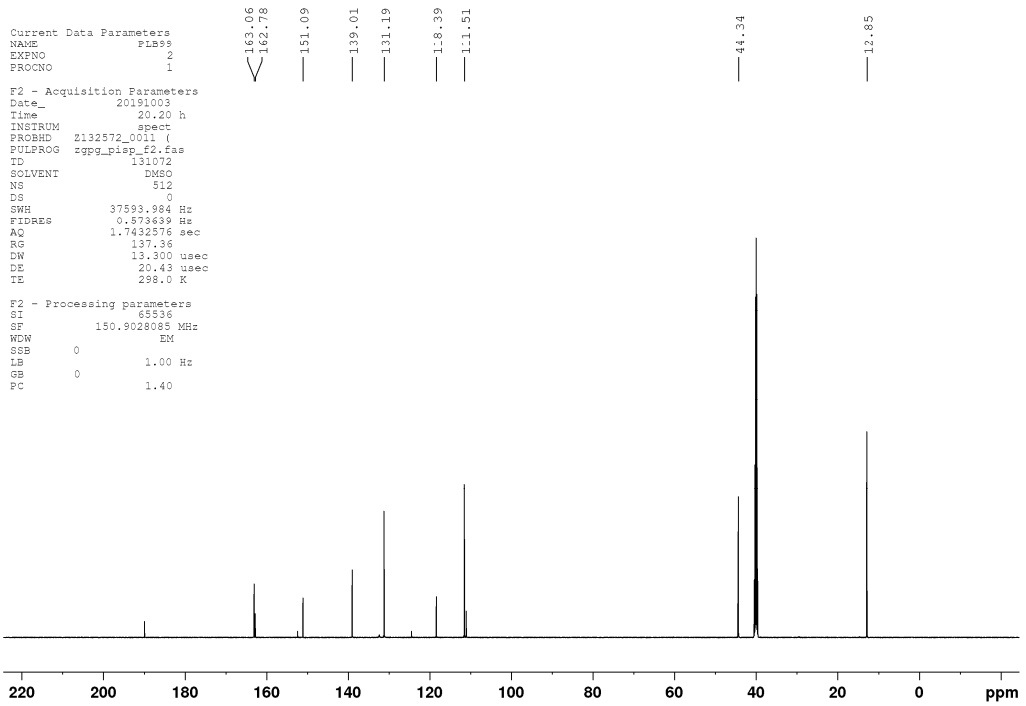
2c

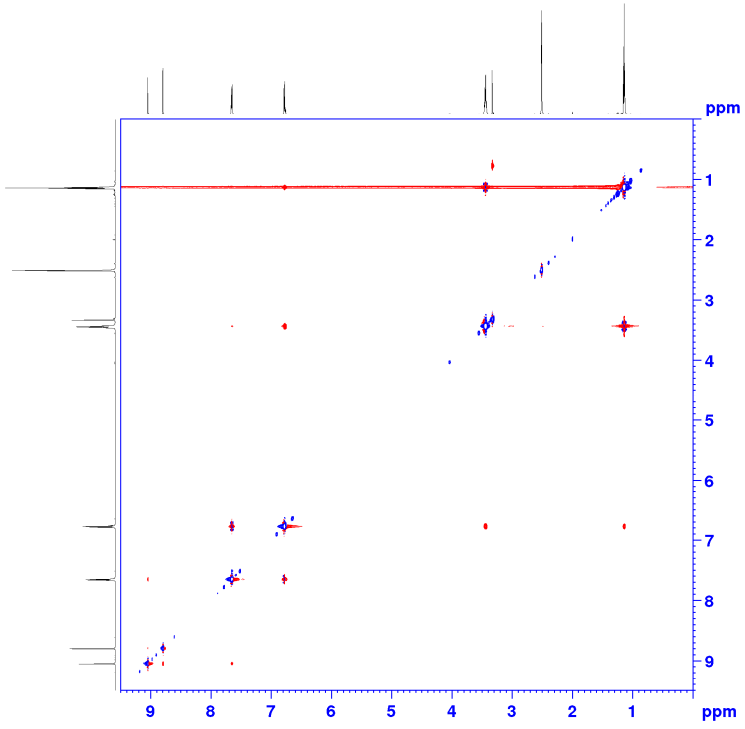

14.05
10.19
8.94
8.22
8.22
8.20
8.20
8.13
8.11
7.95
7.94
7.93
7.88
7.88
7.86
7.85
7.85
Current Data Parameters
NAME PLB14
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 17.06 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 8
DS 2
SWH 13204.226 Hz
FIDRES 0.201480 Hz
AQ 4.9632597 sec
RG 54.87
DW 37.867 usec
DE 24.32 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 600.1300000 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.00
15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.77 0.81 1.69 1.87 1.90 2.00 2.10


Current Data Parameters
NAME PLB14
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 17.39 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zgpg_pisp_f2.fas
TD 131072
SOLVENT DMSO
NS 512
DS 0
SWH 37593.984 Hz
FIDRES 0.573639 Hz
AQ 1.7432576 sec
RG 137.36
DW 13.300 usec
DE 20.43 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
163.44
155.78
149.15
139.22
134.75
133.31
129.64
127.42
125.46
220 200 180 160 140 120 100 80 60 40 20 0 ppm

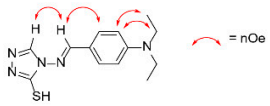
2d

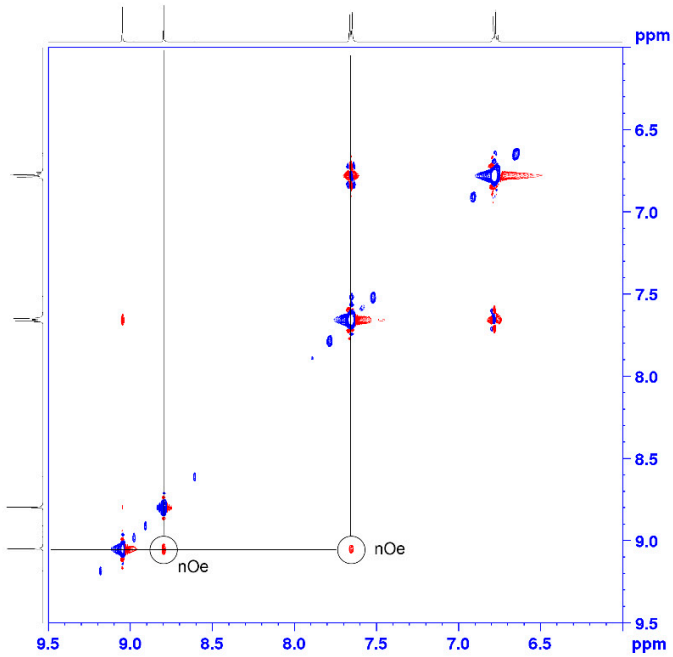

Current Data Parameters
NAME PLB99
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 19.47 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 8
DS 2
SWH 13204.226 Hz
FIDRES 0.201480 Hz
AQ 4.9632597 sec
RG 171.97
DW 37.867 usec
DE 24.32 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 600.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
3.44
3.43
3.33
1.15
1.14
1.13
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.75
0.17
0.83
0.81
1.99
2.00
3.95
6.02


Current Data Parameters
NAME PLB99
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 20.20 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zgpg_pisp_f2.fas
TD 131072
SOLVENT DMSO
NS 512
DS 0
SWH 37593.984 Hz
FIDRES 0.573639 Hz
AQ 1.7432576 sec
RG 137.36
DW 13.300 usec
DE 20.43 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
163.06
162.78
151.09
139.01
131.19
118.39
111.51
44.34
12.85
220 200 180 160 140 120 100 80 60 40 20 0 ppm



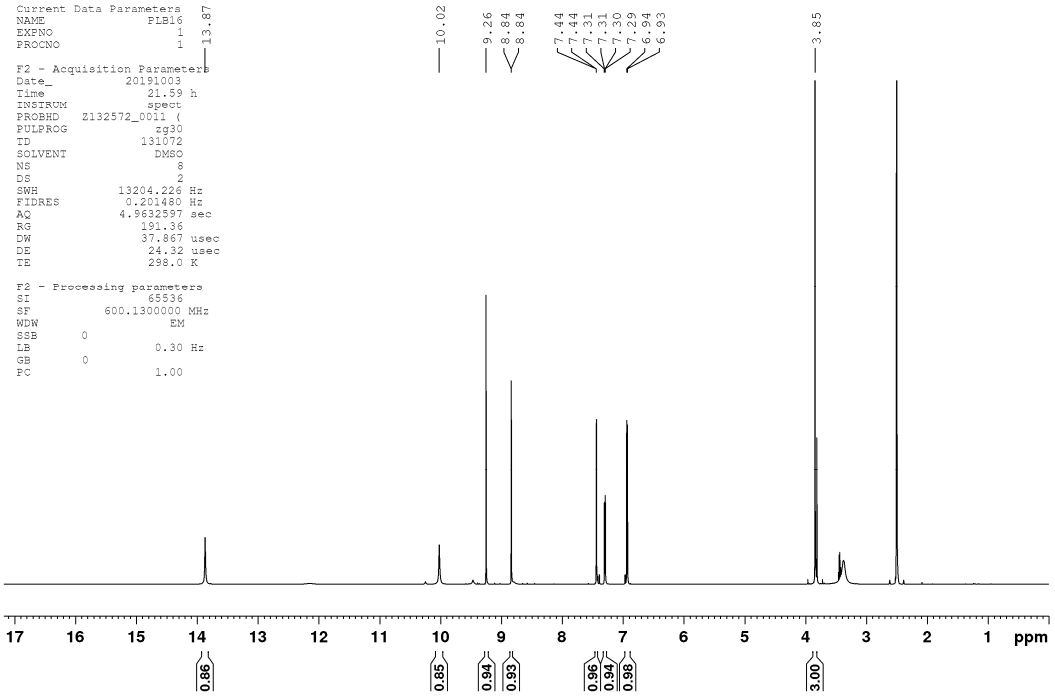

```
Current Data Parameters
NAME                  PLB99
EXPNO                     3
PROCNO                    1

F2 - Acquisition Parameters
Date_              20191003
Time                 20.21 h
INSTRUM                spect
PROBHD    Z132572_0011 (
PULPROG       noesygpphpp
TD                     2048
SOLVENT                DMSO
NS                        8
DS                        4
SWH               7812.500 Hz
FIDRES            7.629395 Hz
AQ                0.1310720 sec
RG                   191.36
DW                  64.000 usec
DE                  20.00 usec
TE                  298.0 K

F1 - Acquisition parameters
TD                      256
SFO1              600.1336 MHz
FIDRES           61.035156 Hz
SW                   13.018 ppm
FnMODE        States-TPPI

F2 - Processing parameters
SI                     2048
SF             600.1300000 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
PC                     1.00

F1 - Processing parameters
SI                     1024
MC2           States-TPPI
SF             600.1300000 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
```

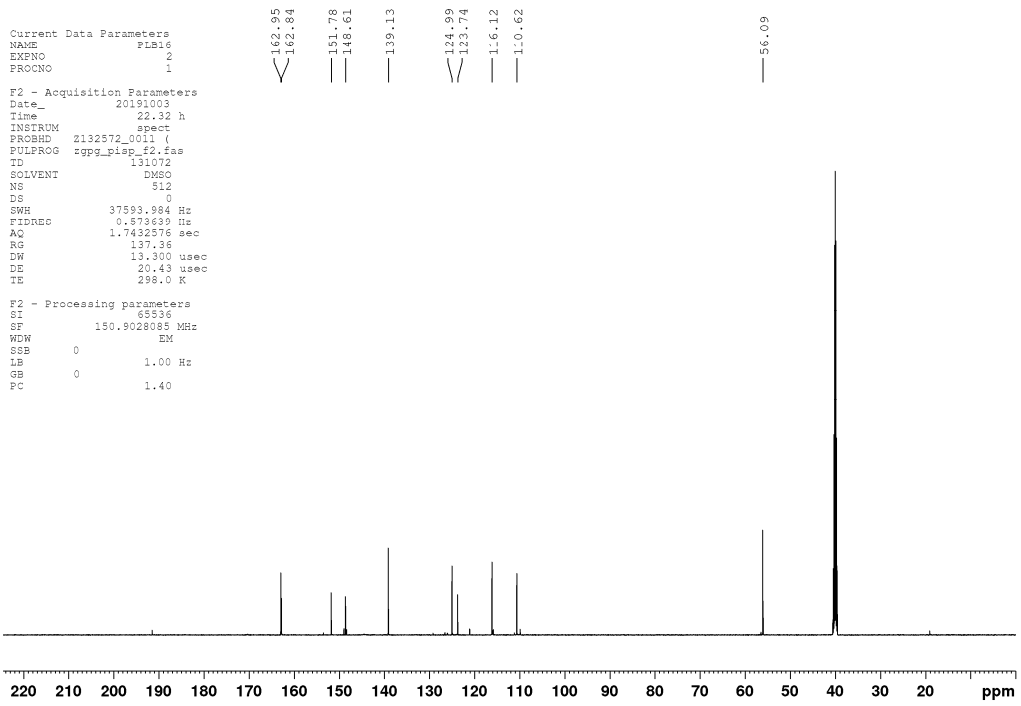




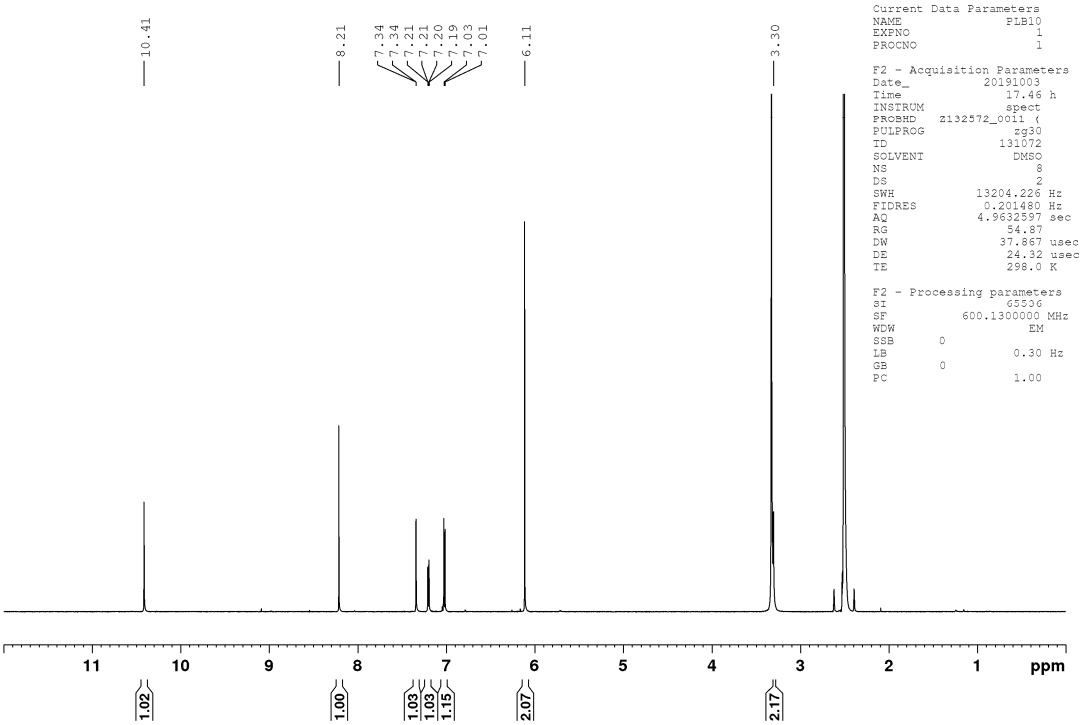

```
Current Data Parameters
NAME                  PLB99
EXPNO                     3
PROCNO                    1

F2 - Acquisition Parameters
Date_              20191003
Time                 20.21 h
INSTRUM                spect
PROBHD    Z132572_0011 (
PULPROG       noesygpphpp
TD                     2048
SOLVENT                DMSO
NS                        8
DS                        4
SWH               7812.500 Hz
FIDRES            7.629395 Hz
AQ                0.1310720 sec
RG                   191.36
DW                  64.000 usec
DE                  20.00 usec
TE                  298.0 K

F1 - Acquisition parameters
TD                      256
SFO1              600.1336 MHz
FIDRES           61.035156 Hz
SW                   13.018 ppm
FnMODE        States-TPPI

F2 - Processing parameters
SI                     2048
SF             600.1300000 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
PC                     1.00

F1 - Processing parameters
SI                     1024
MC2           States-TPPI
SF             600.1300000 MHz
WDW                   QSINE
SSB                       2
LB         0 Hz
GB         0
```

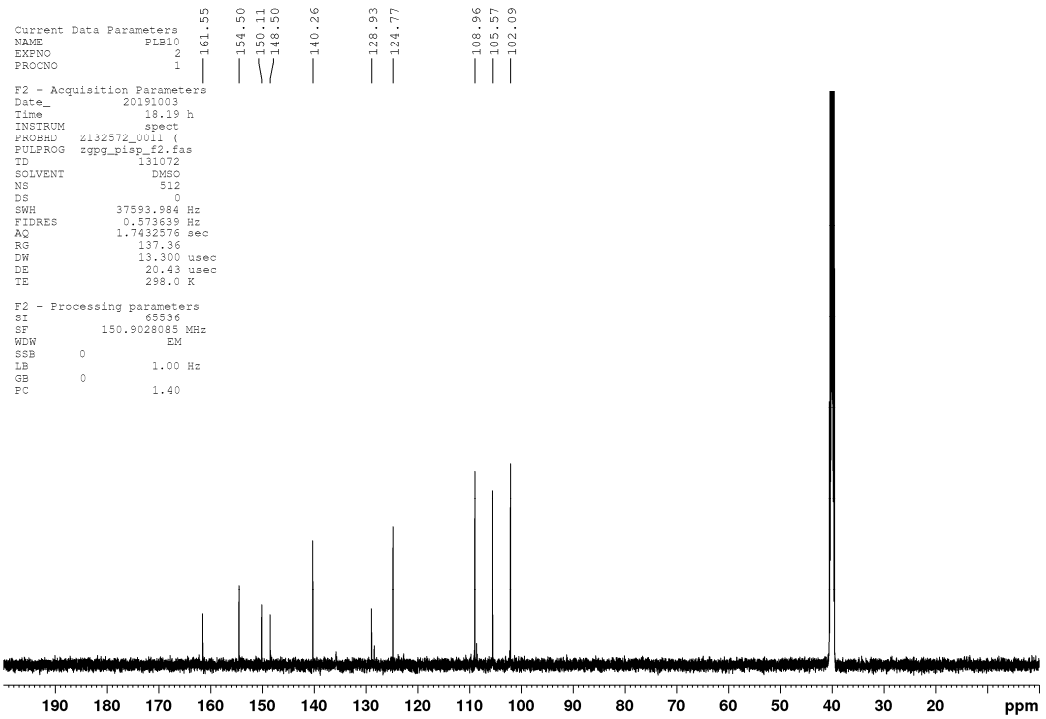
2e


Current Data Parameters
NAME PLB16
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 21.59 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 8
DS 2
SWH 13204.226 Hz
FIDRES 0.201480 Hz
AQ 4.9632597 sec
RG 191.36
DW 37.867 usec
DE 24.32 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 600.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.87
10.02
9.26
8.84
8.84
7.44
7.44
7.31
7.31
7.30
7.29
6.94
6.93
3.85
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.86
0.85
0.94
0.93
0.96
0.94
0.98
3.00

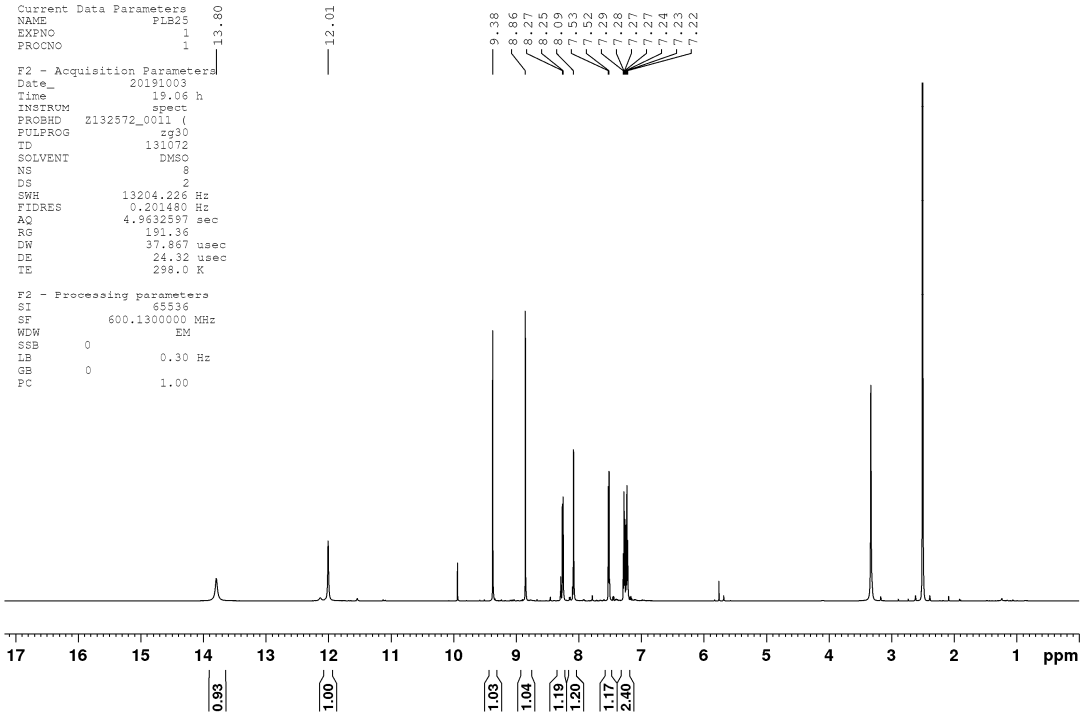

Current Data Parameters
NAME PLB16
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 22.32 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zgpg_pisp_f2.fas
TD 131072
SOLVENT DMSO
NS 512
DS 0
SWH 37593.984 Hz
FIDRES 0.573639 Hz
AQ 1.7432576 sec
RG 137.36
DW 13.300 usec
DE 20.43 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
162.95
162.84
151.78
148.61
139.13
124.99
123.74
116.12
110.62
56.09
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

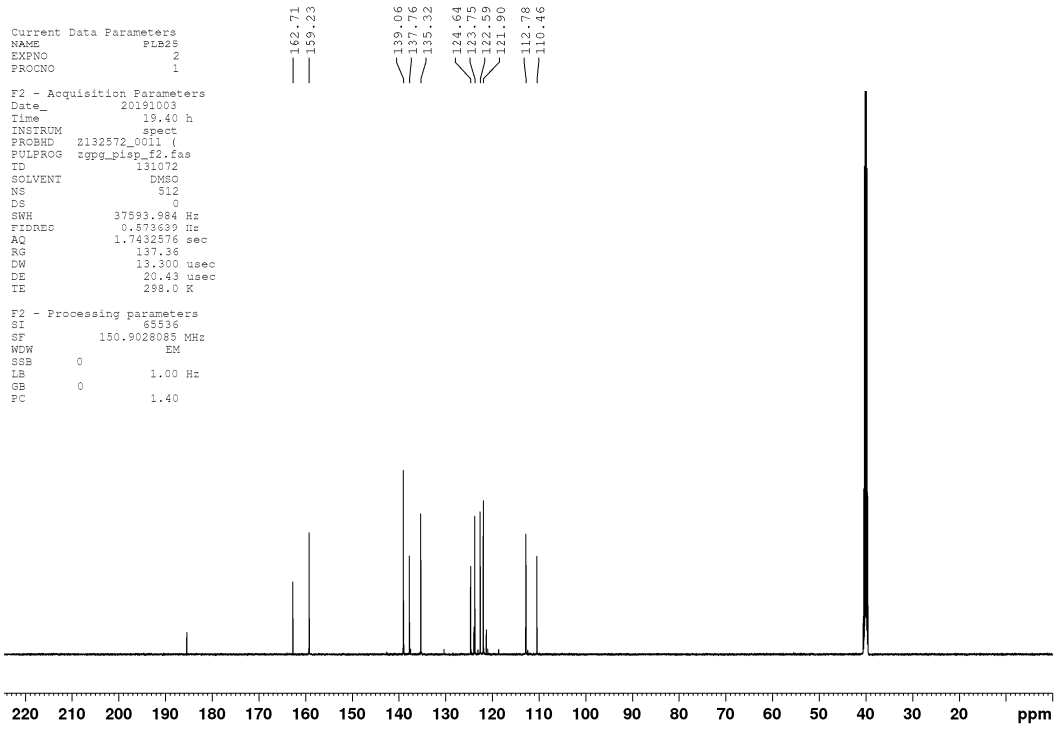
2f


10.41
8.21
7.34
7.34
7.21
7.21
7.20
7.19
7.03
7.01
6.11
3.30
Current Data Parameters
NAME PLB10
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 17.46 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 8
DS 2
SWH 13204.226 Hz
FIDRES 0.201480 Hz
AQ 4.9632597 sec
RG 54.87
DW 37.867 usec
DE 24.32 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 600.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
11 10 9 8 7 6 5 4 3 2 1 ppm
1.02 1.00 1.03 1.03 1.15 2.07 2.17


161.55
154.50
150.11
148.50
140.26
128.93
124.77
108.96
105.57
102.09
Current Data Parameters
NAME PLB10
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 18.19 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zgpg_pisp_f2.fas
TD 131072
SOLVENT DMSO
NS 512
DS 0
SWH 37593.984 Hz
FIDRES 0.573639 Hz
AQ 1.7432576 sec
RG 137.36
DW 13.300 usec
DE 20.43 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm

2g


Current Data Parameters
NAME PLB25
EXPNO 1
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 19.06 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zg30
TD 131072
SOLVENT DMSO
NS 8
DS 2
SWH 13204.226 Hz
FIDRES 0.201480 Hz
AQ 4.9632597 sec
RG 191.36
DW 37.867 usec
DE 24.32 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 600.1300000 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00
13.80
12.01
9.38
8.86
8.27
8.25
8.09
7.53
7.52
7.29
7.28
7.27
7.27
7.24
7.23
7.22
17 16 15 14 13 12 11 10 9 8 7 6 5 4 3 2 1 ppm
0.93
1.00
1.03
1.04
1.19
1.20
1.17
2.40


Current Data Parameters
NAME PLB25
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20191003
Time 19.40 h
INSTRUM spect
PROBHD Z132572_0011 (
PULPROG zgpg_plsp_f2.fas
TD 131072
SOLVENT DMSO
NS 512
DS 0
SWH 37593.984 Hz
FIDRES 0.573639 Hz
AQ 1.7432576 sec
RG 137.36
DW 13.300 usec
DE 20.43 usec
TE 298.0 K
F2 - Processing parameters
SI 65536
SF 150.9028085 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40
162.71
159.23
139.06
137.76
135.32
124.64
123.75
122.59
121.90
112.78
110.46
220 210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 ppm