

Cell Chemical Biology, Volume 28

Supplemental information

Development and pre-clinical testing

of a novel hypoxia-activated KDAC inhibitor

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SUPPLEMENTARY FIGURES

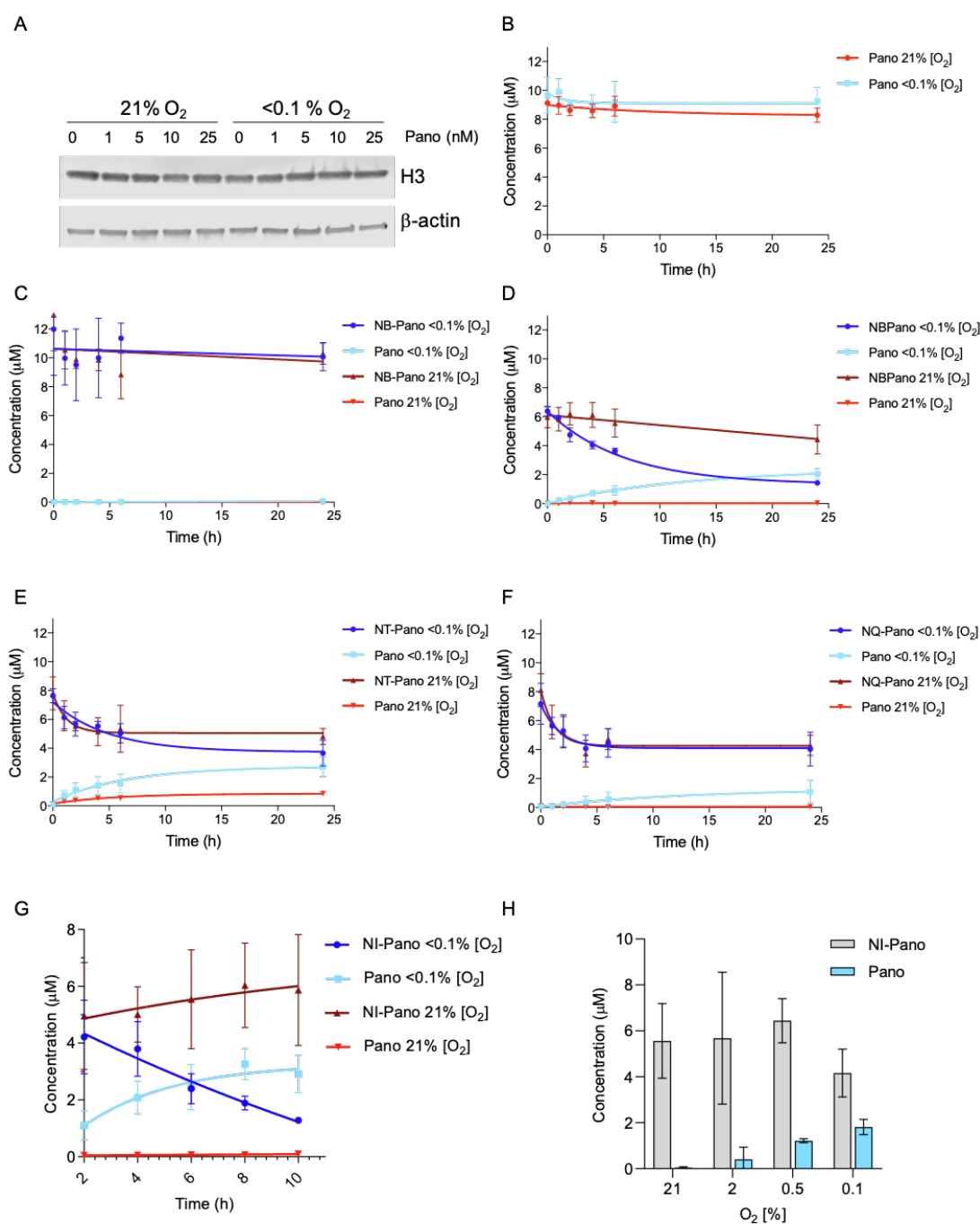


Figure S1. Oxygen dependent reduction and fragmentation of HAPs of panobinostat, related to figure 2. **A.** H3 and β -actin blots corresponding to the H3K18Ac shown in figure 2A. **B.** panobinostat (10 μ M), **C.** NB-Pano (10 μ M), **D.** NB-Pano at 92 pmol CYP004, **E.** NT-Pano (10 μ M) and **F.** NQ-Pano (10 μ M) were incubated with 9.2 pmol/mL of bacterial NADPH-CYP reductase (CYP004) in normoxic (21% O₂) or hypoxic (<0.1% O₂) conditions for 0-24 h and analyzed using LCMS. **G.** OE21 cells were treated with NI-Pano (10 μ M) in either normoxia or hypoxia (<0.1% O₂) for the indicated times and the reduction of NI-Pano to Pano was determined by LCMS. **H.** OE21 cells were treated with NI-Pano (10 μ M) for 6 h at different oxygen concentrations and analyzed by LCMS. Data are mean \pm SD, $n=3$, except **B** where $n=2$.

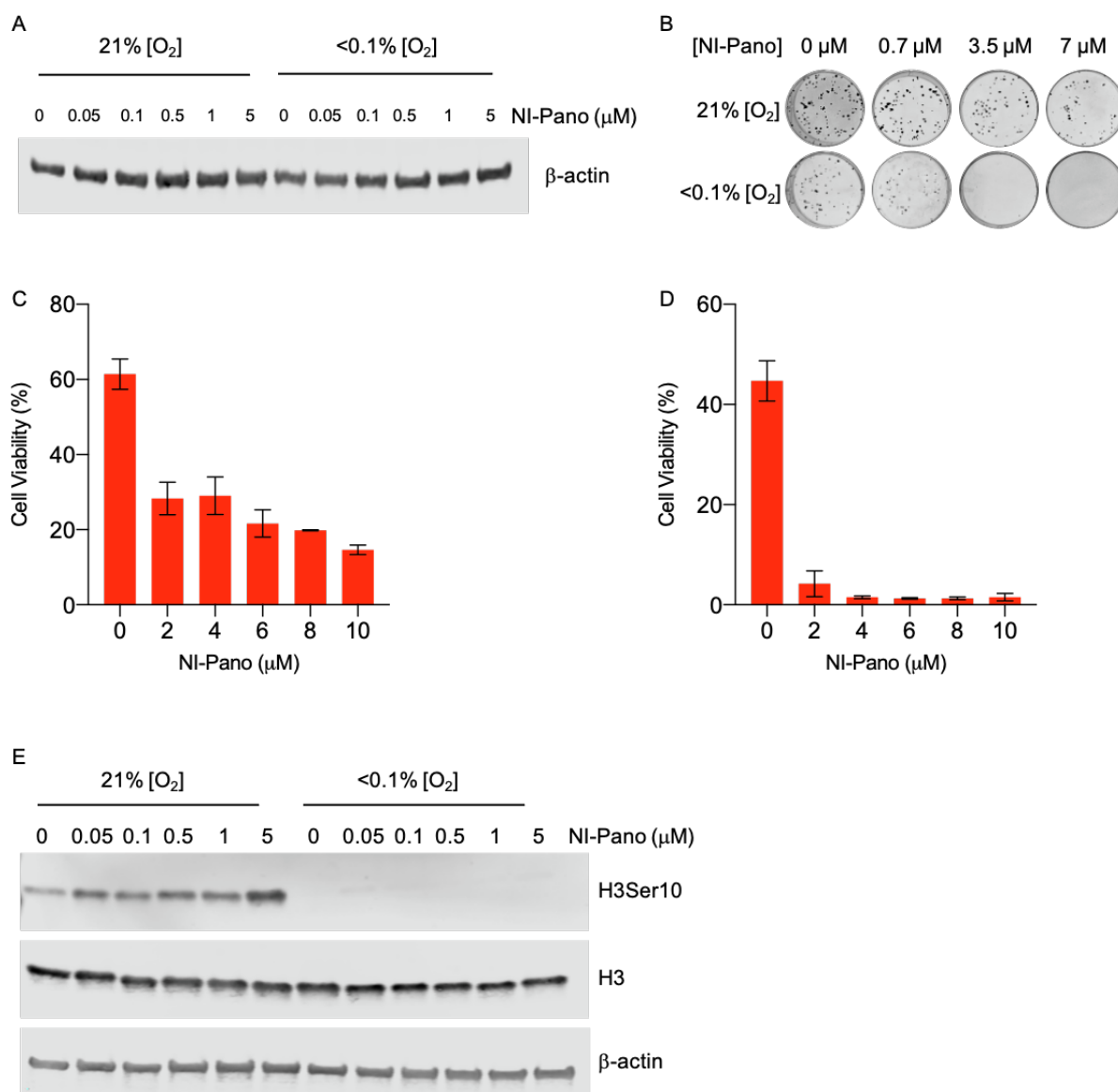


Figure S2. NI-pano decreases cancer cell survival in hypoxia, related to figure 3. **A.** Additional loading control for western blots shown in Fig. 2A. **B.** HCT116 cells were treated with NI-pano (0-7 μM) for 24 h at the oxygen concentration shown. NI-Pano was removed and cells allowed to form colonies in normoxic conditions. Representative images from figure 3D are shown. **C, D.** OE21 cells were treated with 0, 2, 4, 6, 8 or 10 μM of NI-Pano for 24 or 48 hours and exposed to <0.1% O₂. Percentage cell viability was assessed using MTT assay. **E.** OE21 cells were treated with indicated concentrations of NI-Pano and exposed to normoxia or hypoxia (<0.1% O₂) for 24 hours. Samples were probed for the phosphorylation of H3Ser10, total H3 and β-actin.

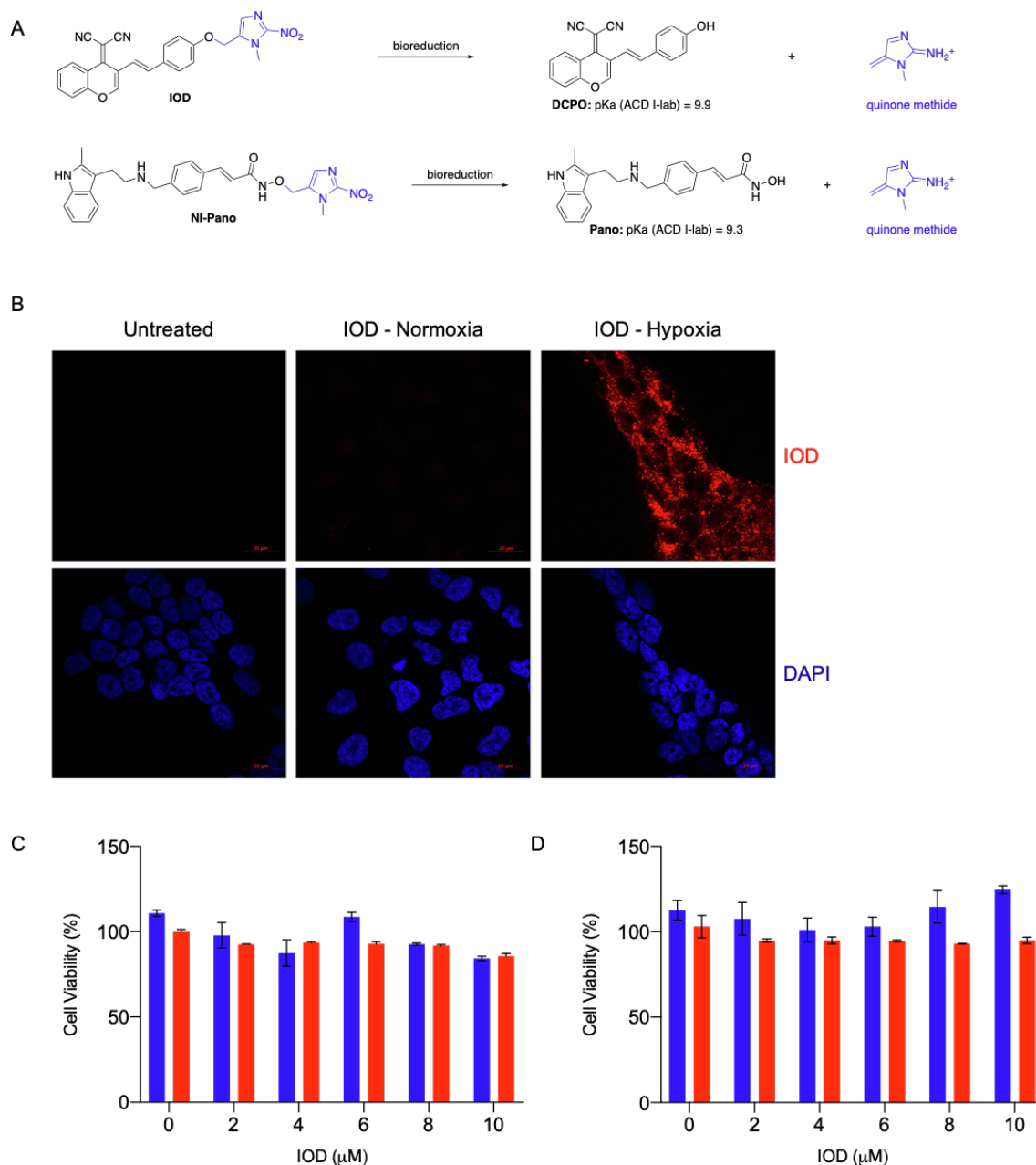


Figure S3. IOD treatment is not toxic, related to figure 2. A. A scheme showing the bioreductions of IOD and NI-Pano, which both result in the production of the same quinone-methide-like by-product. **B.** OE21 cells were treated with 10 μM of IOD and exposed to $<0.1\%$ O₂ for 16 hours. DAPI (DNA binding) was used to visualize the nucleus. Cells were fixed and imaged on a Zeiss LSM 780 confocal microscope. Images were taken at 63x magnification. Scale bar represents 20 μm . **C.** OE21 cells were treated with indicated concentrations of IOD for 24 hours and exposed to either 21% O₂ (blue) or $<0.1\%$ O₂ (red). Cell viability was measured using an MTT assay. 21% O₂ and $<0.1\%$ O₂ data are normalized to the respective 0 μM controls. **D.** OE21 cells were treated with indicated concentrations of IOD for 72 hours and exposed to either 21% O₂ or $<0.1\%$ O₂. Cell viability was measured using an MTT assay. 21% O₂ and $<0.1\%$ O₂ data are normalized to the respective 0 μM controls.

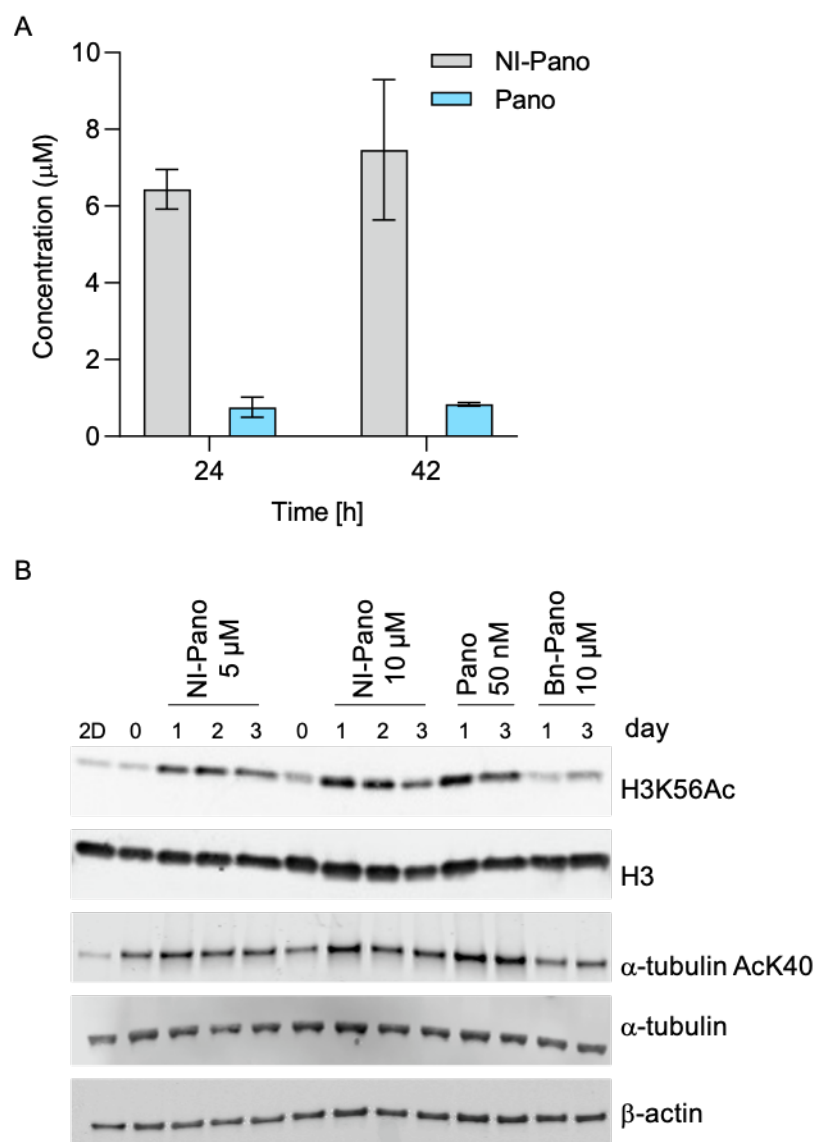


Figure S4. NI-Pano is reduced in cancer cell spheroids, related to figure 4. **A.** HCT116 cell spheroids were treated with NI-Pano (10 μ M) for the times indicated and the reduction of NI-Pano to Pano was determined by HPLC. At least 24 spheroids were analyzed per treatment condition. Data are mean \pm SD, $n=3$. **B.** Western blotting was carried out on the spheroid samples described in Fig. 4D using the antibodies indicated.

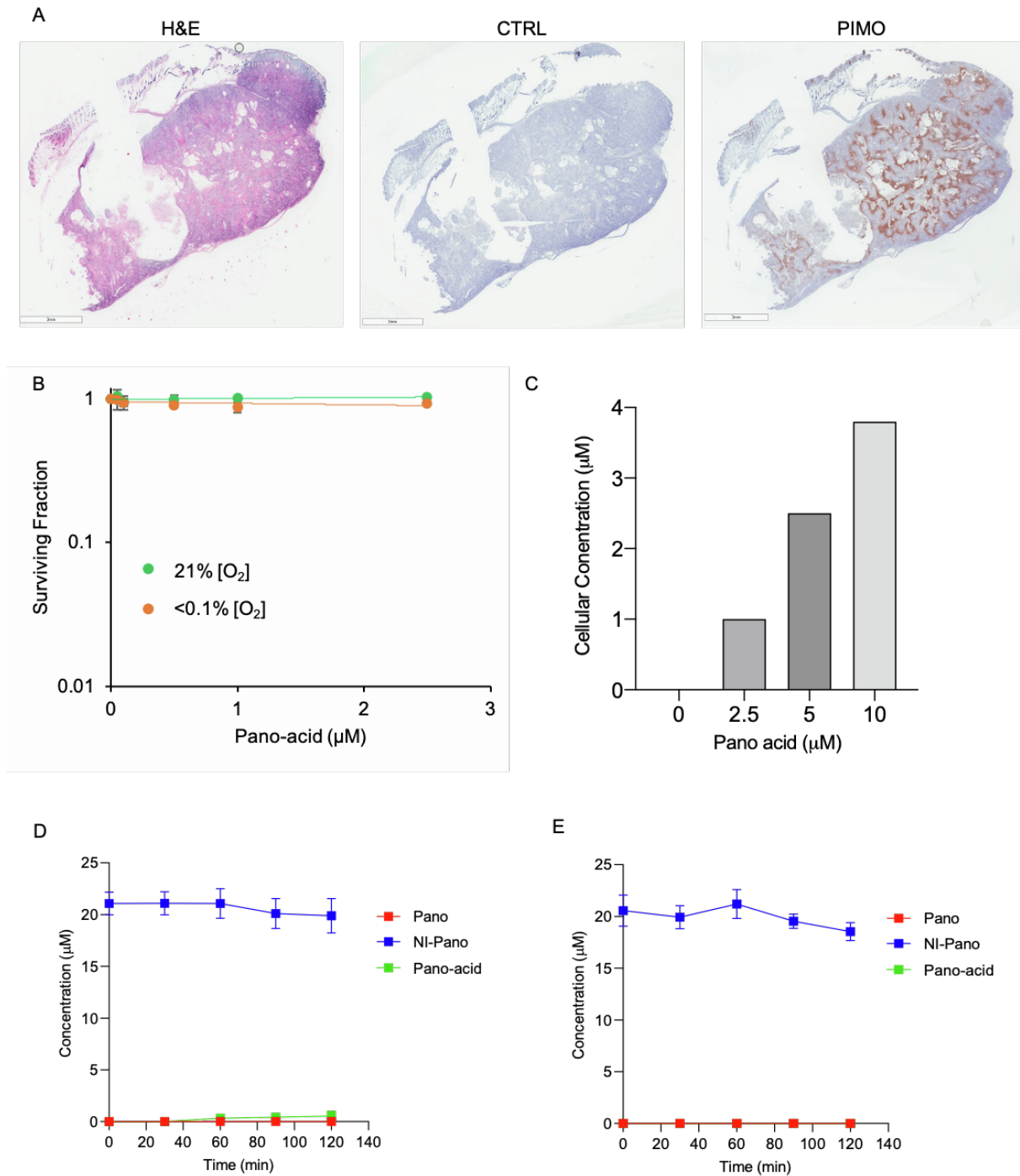


Figure S5. *In vivo* testing of NI-Pano, related to figure 5. A. OE21 xenograft tumors were stained with anti-pimonidazole antibody (PIMO) to visualize hypoxic regions and counterstained with hematoxylin and eosin (H&E). Anti-mouse secondary antibody alone was used as a negative control (CTRL). **B.** OE21 cells were treated with Pano-acid (0-2.5 μM) for 24 h at the oxygen concentrations shown. The Pano-acid was removed (media change) and cells allowed to form colonies in normoxic conditions. **C.** OE21 cells were treated with indicated doses of Pano-acid for 6 hours under normoxic conditions. Cells were collected and resulting supernatant analyzed by HPLC **D.** Plasma from healthy rats or **E.** human plasma from healthy donors was incubated with NI-Pano (20 μM) for indicated times and analyzed by LCMS. Data are mean ± SD, *n*=3.

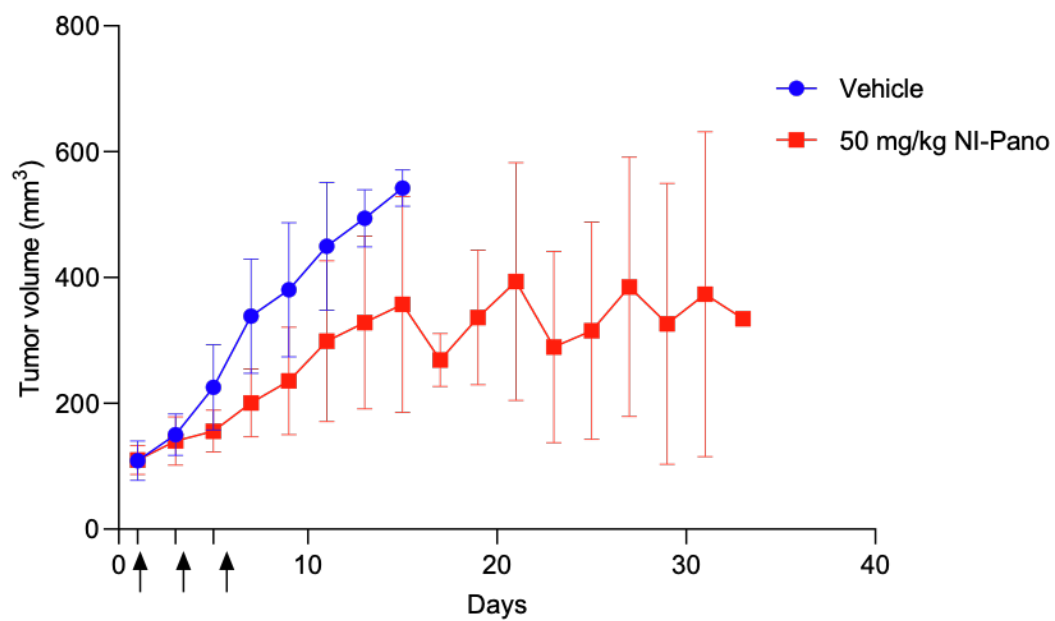
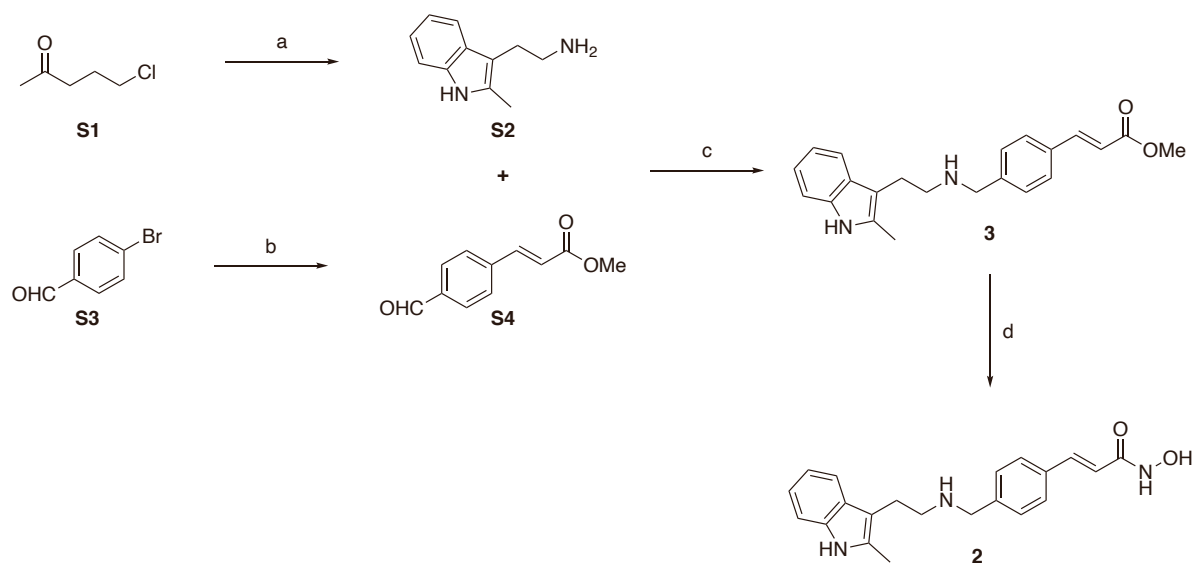


Figure S6. NI-Pano inhibits growth of OE21 xenografts, related to figure 5. Tumor volume over time in CD-1 nude mice administered with three doses of either vehicle ($n=6$) or 50 mg/kg NI-Pano ($n=7$). Arrows indicate days of treatment (days 1, 3 and 5). Results are mean \pm SD.

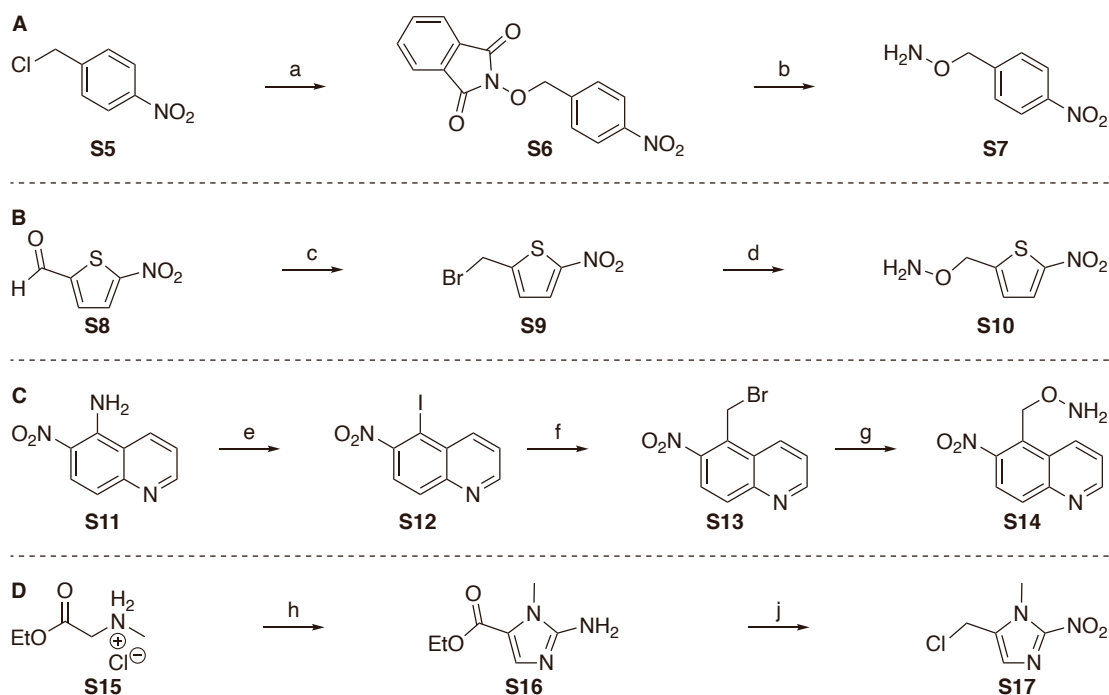
SUPPLEMENTARY SCHEMES

Scheme S1. General Synthesis of Panobinostat (2), related to figure 1



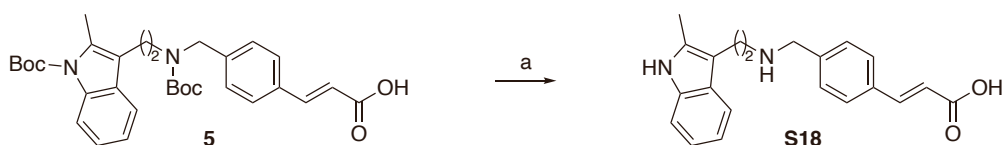
Reagents and conditions: (a) PhNHNH₂, EtOH, 80 °C, 18 h, 79-89%, n=4; (b) Methyl acrylate, Pd(OAc)₂, KOAc, DMF, 110 °C, 24 h, 94-99%, n=5; (c) ClCH₂CH₂Cl, AcOH, 3 Å molecular sieves, rt, 1 h then NaBH(OAc)₃, rt, 18 h, 52-86%, n=6; (d) KOH, HONH₂·HCl, MeOH, rt, 20 h, 63%.

Scheme S2: Synthesis the bio-reductive group precursors S7, S10, S14, S17, related to scheme 1.



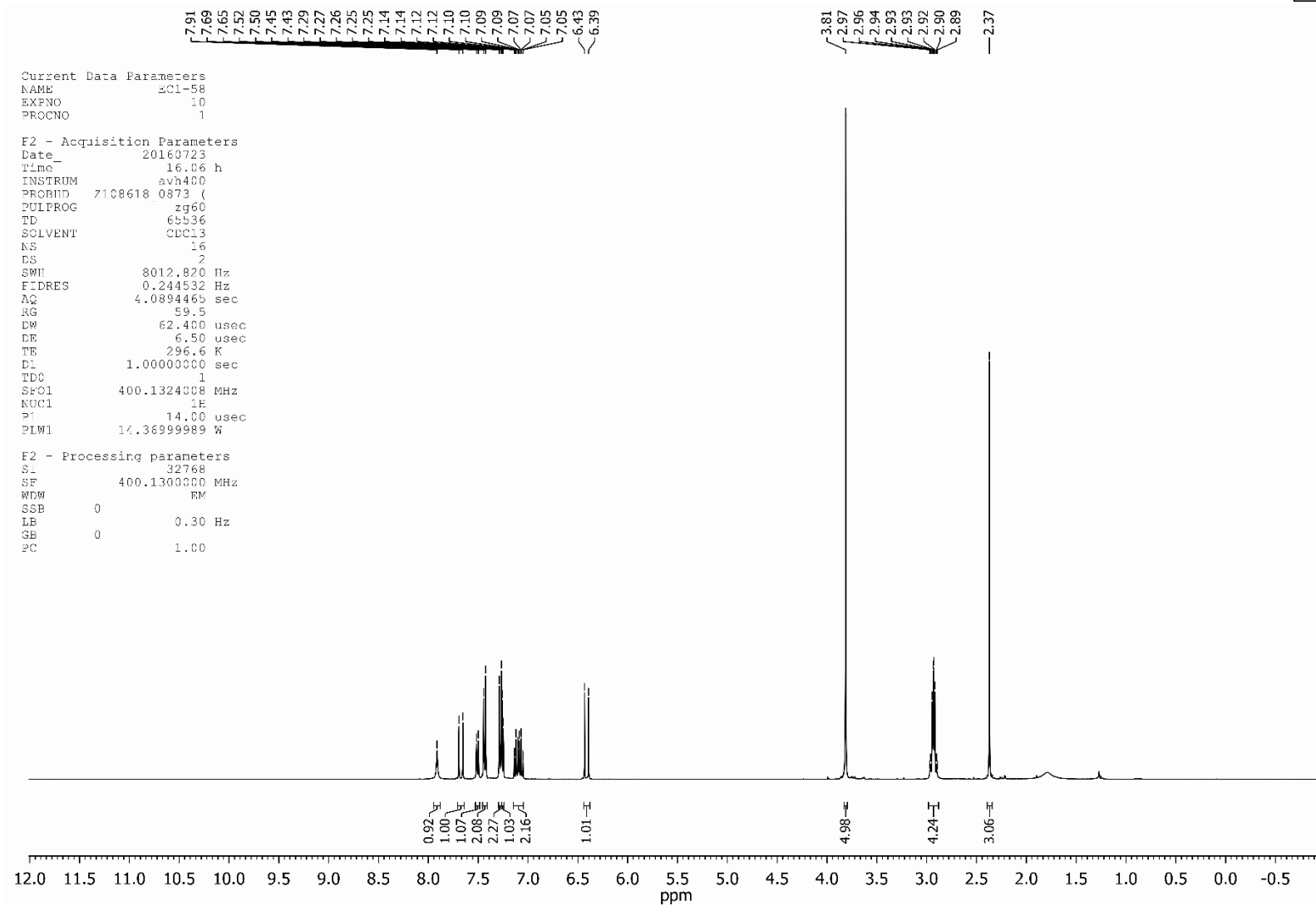
Reagents and conditions: (A) (a) *N*-Hydroxyphthalimide, DIPEA, DMF, 0 °C to rt, 2 h, 76–84%, *n*=2; (b) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, CH_2Cl_2 , rt, 2 h, 72–95%, *n*=4. (B) (c) (i) NaBH_4 , MeOH, 0 °C to rt, 2 h, 79–99%, *n*=4; (ii) PBr_3 , CH_2Cl_2 , 0 °C to rt, 5 h, 24–48%, *n*=4; (d) (i) *N*-Hydroxyphthalimide, DIPEA, DMF, 0 °C to rt, 2 h, 47–63%, *n*=2, (ii) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, CH_2Cl_2 , rt 2 h, 46%, *n*=1. (C) (e) CuI , NaNO_2 , HI (57% aq.), DMSO, 60 °C, 0.5 h, 31–60%, *n*=2; (f) (i) PhMgCl , THF, –40 °C, 10 min then $(\text{CH}_2\text{O})_n$, –40 °C to 40 °C, 18 h, 42–86%, *n*=4; (ii) HBr (48% aq.), 75 °C, 18 h, 77–99%, *n*=2; (g) (i) *N*-Hydroxyphthalimide, DIPEA, DMF, 0 °C to rt, 2 h, 88%, *n*=1; (ii) $\text{N}_2\text{H}_4\cdot\text{H}_2\text{O}$, CH_2Cl_2 , rt 2 h, 92%, *n*=1. (D) (h) (i) NaH , EtOCHO , THF, EtOH, 0 °C to rt, 18 h; (ii) HCl (37% aq.), EtOH, rt, 2 h; (iii) NCNH_2 , EtOH, H_2O , 100 °C, 2 h, 41–79% (over 3 steps), *n*=5; (j) (i) NaNO_2 , AcOH, H_2O , 0 °C to rt, 4 h, 57–81%, *n*=5; (ii) NaBH_4 , EtOH, THF, 0 °C, 3 h, 70–80%, *n*=5; (iii) $\text{CH}_3\text{SO}_2\text{Cl}$, pyridine, 0 °C to rt, 3 h, 54–67%, *n*=5.

Scheme S3. Synthesis of Pano-acid (S18), related to figure 5



Reagents and conditions: (a) TFA, TIPS-H, CH_2Cl_2 , rt, 65 min, 78%.

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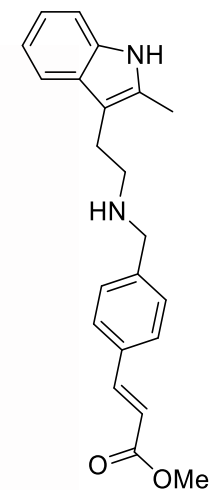
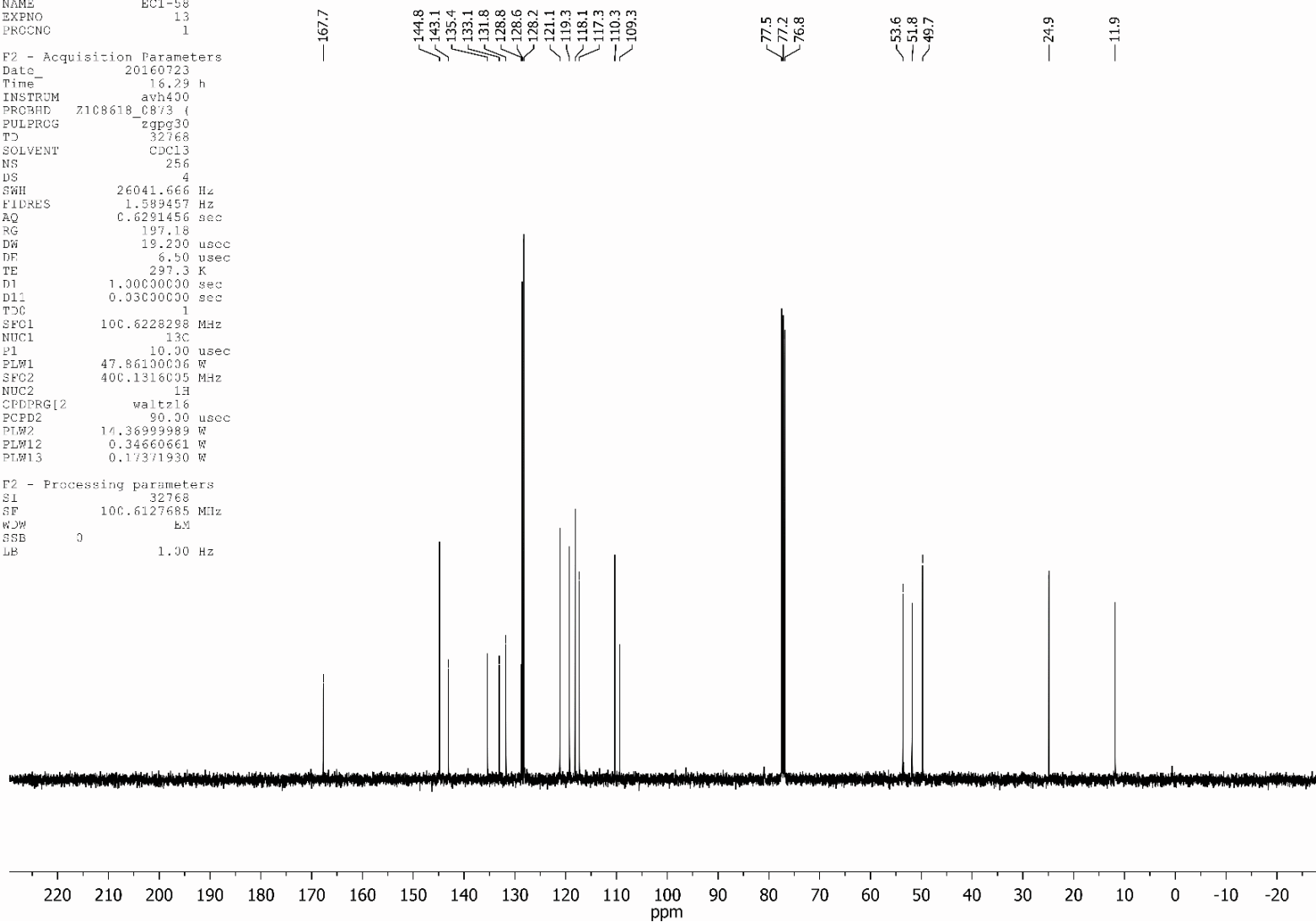


Methyl (E)-3-(4-[[2-(2-methyl-1H-indol-3-yl)ethylamino]methyl]phenyl)prop-2-enoate (3)

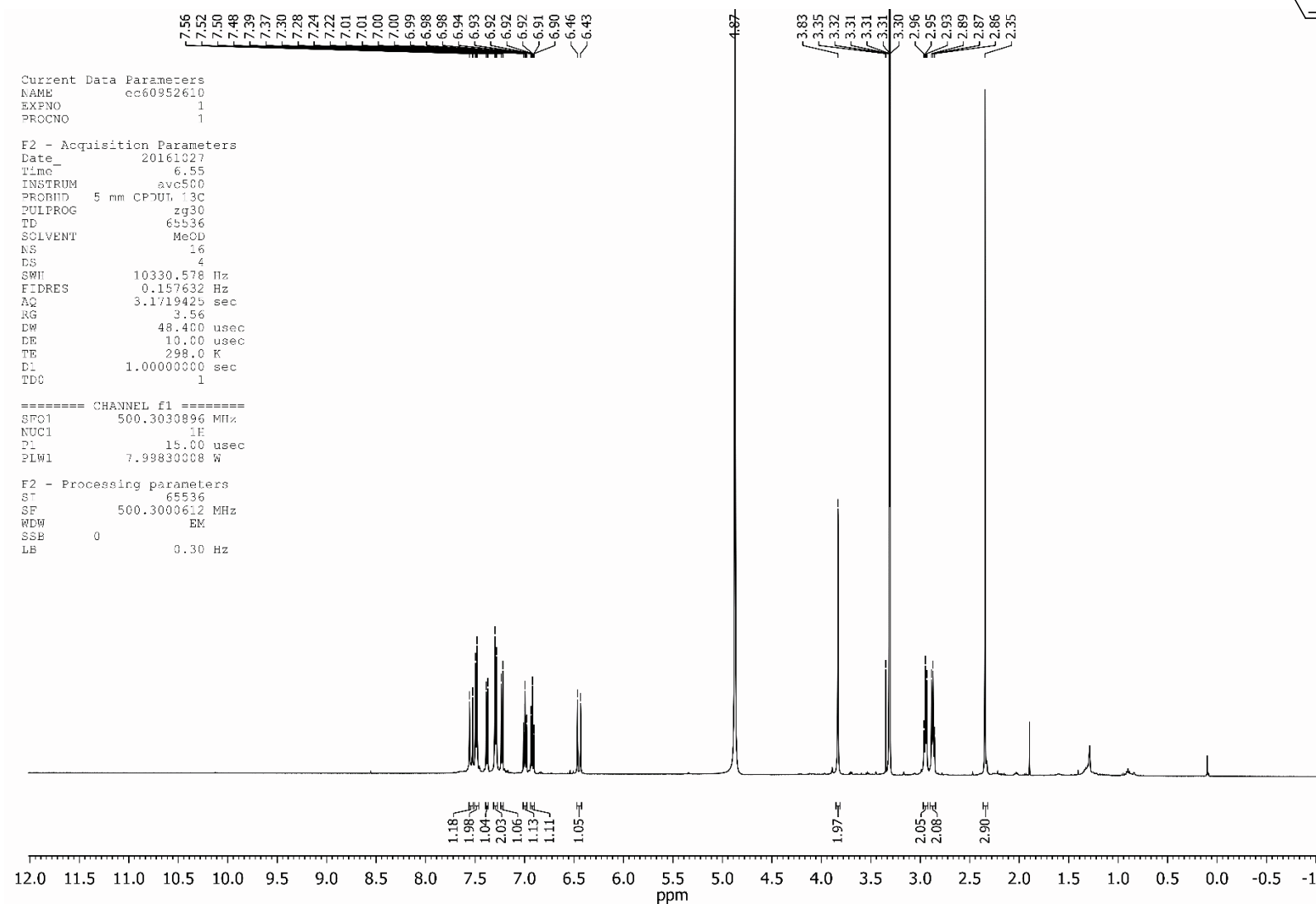
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Panobinostat (Pano, 2)



Panobinostat (Pano, 2)

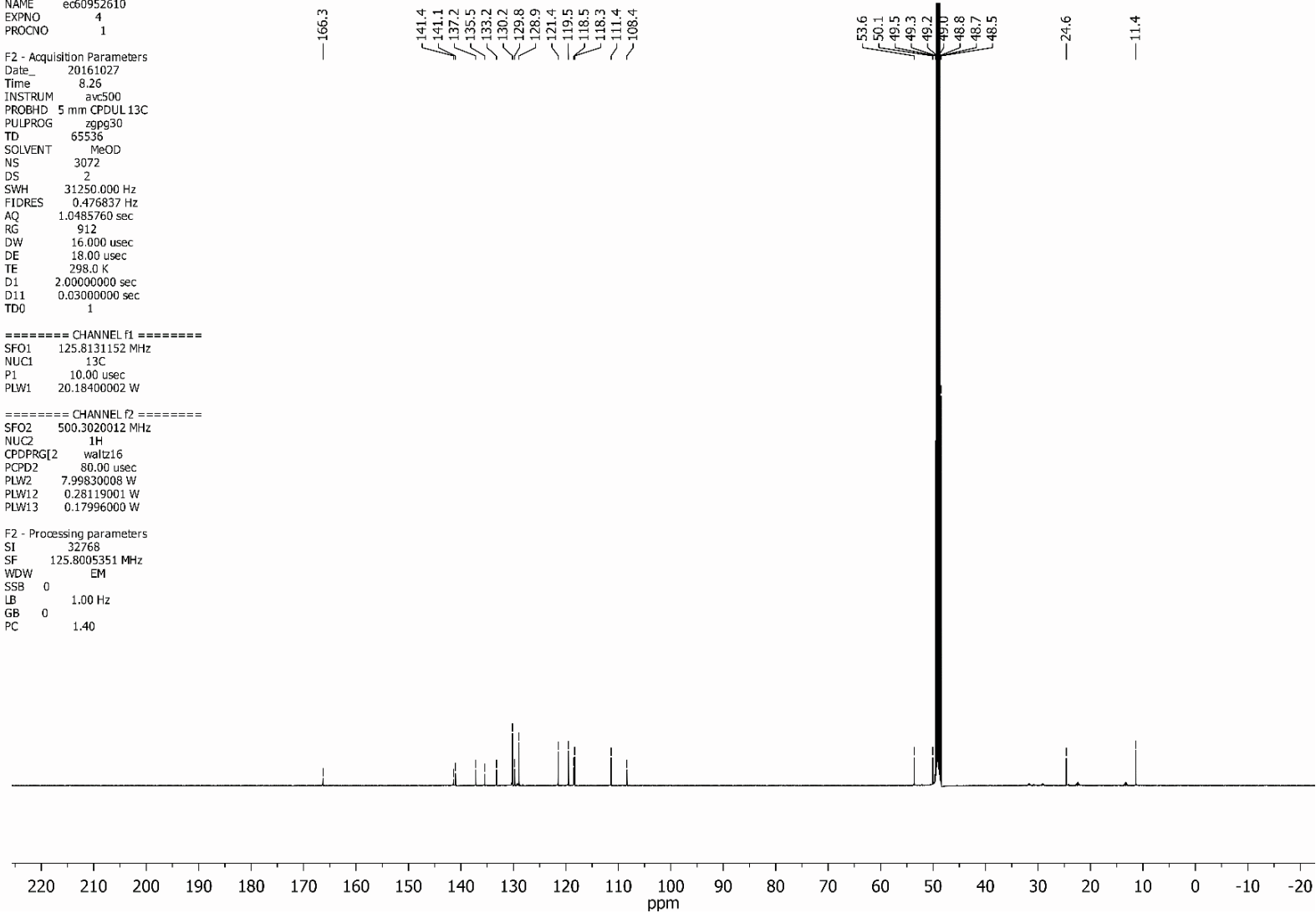
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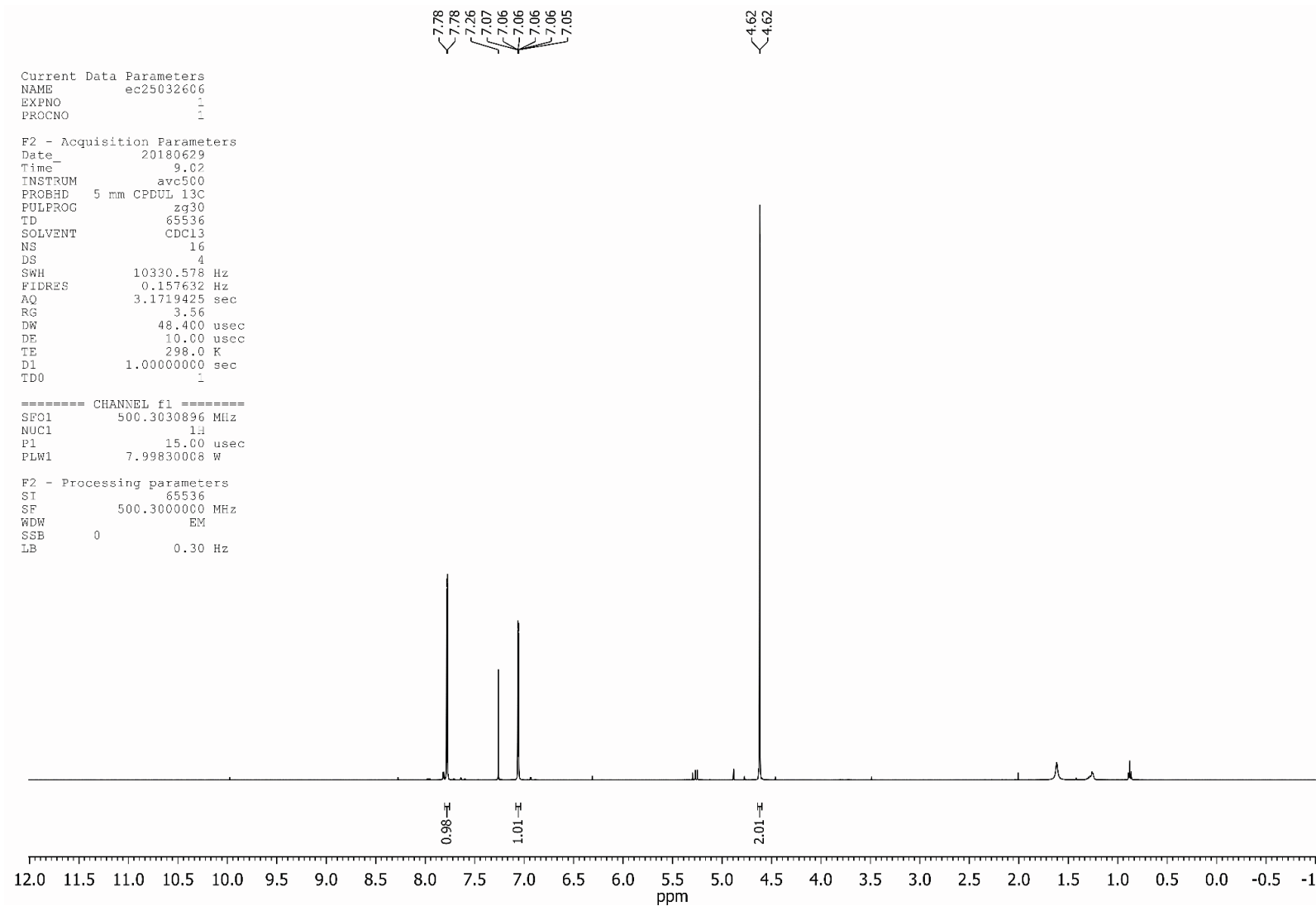
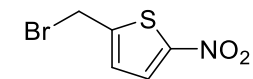
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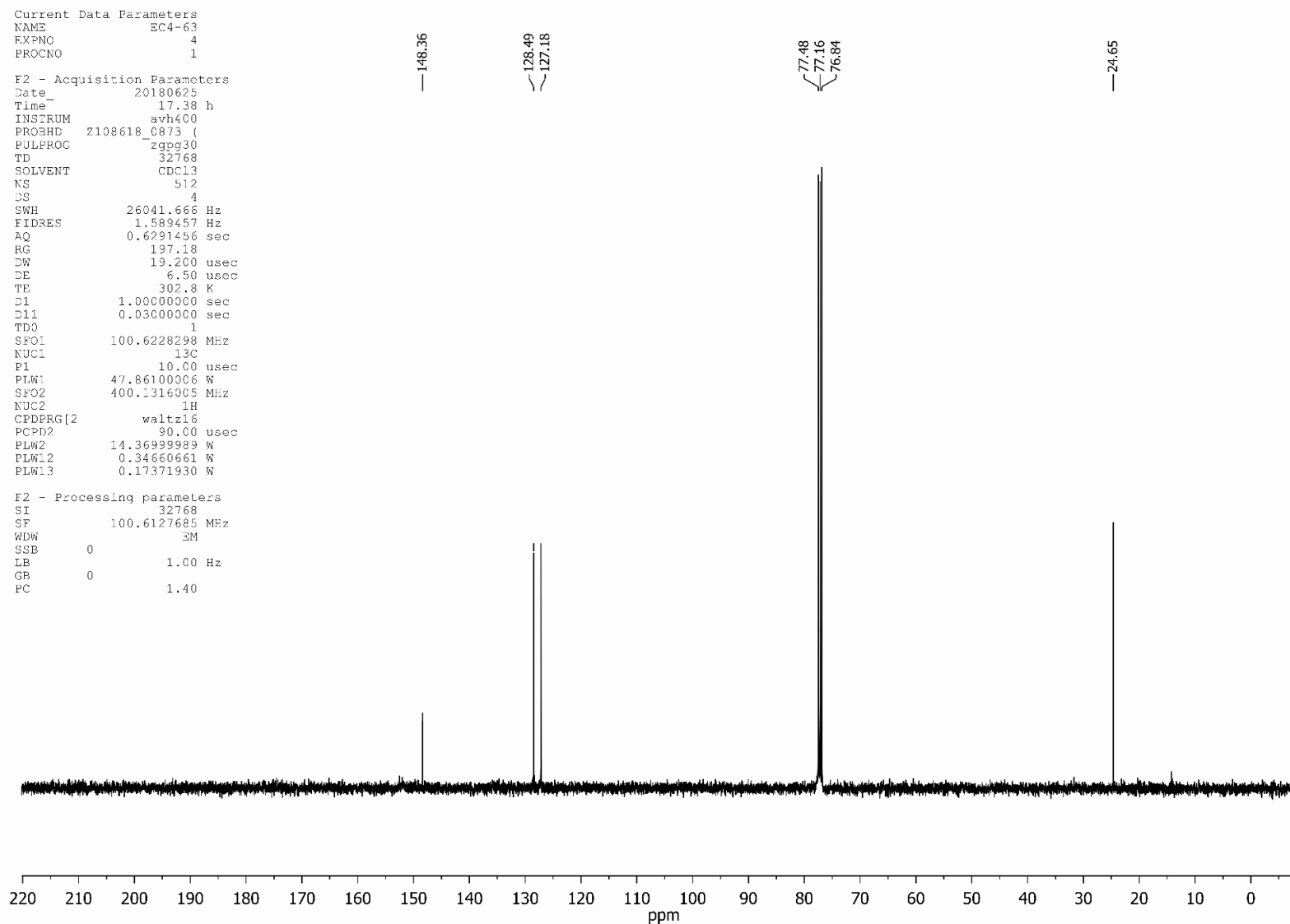
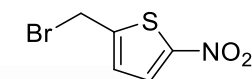
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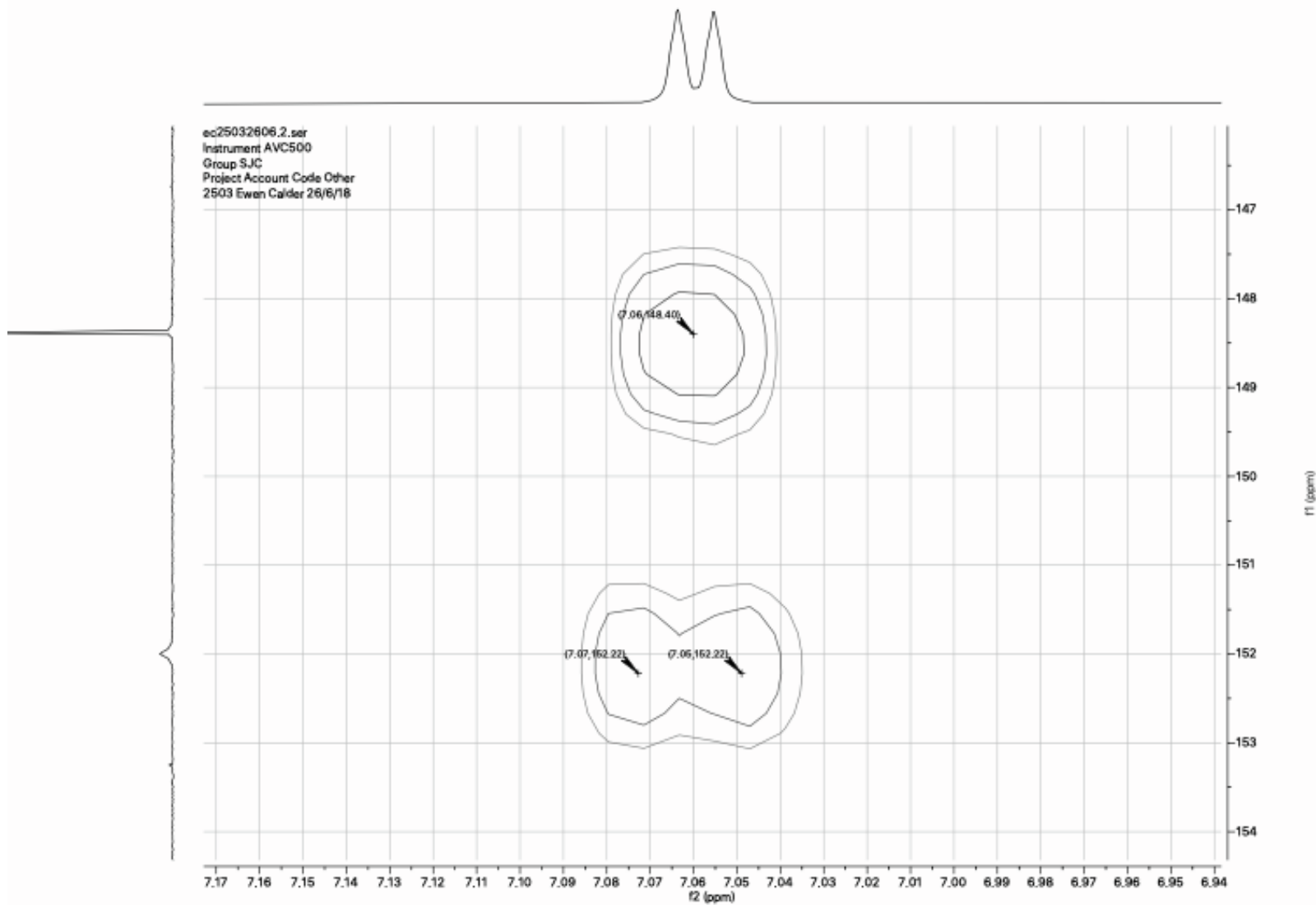
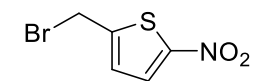
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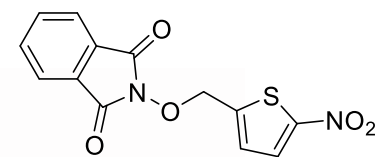
(5-Nitrothiophen-2-yl)methyl bromide (S9)



(5-Nitrothiophen-2-yl)methyl bromide (**S9**)



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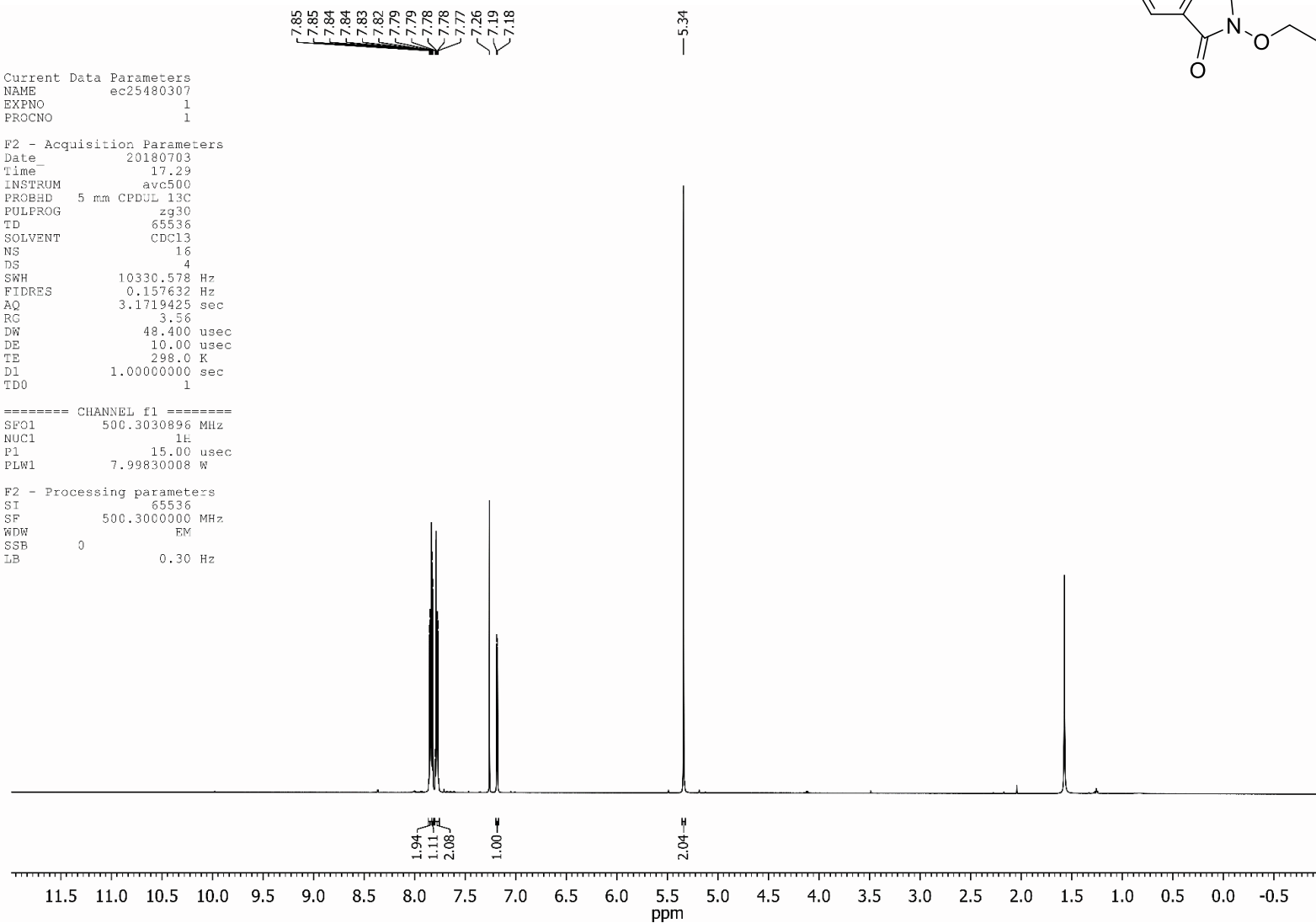


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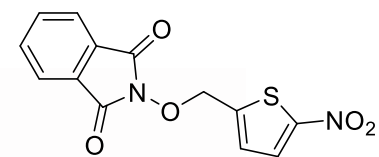
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N-Phthalimido-*O*-(5-nitrothiophen-2-yl)-hydroxylamine



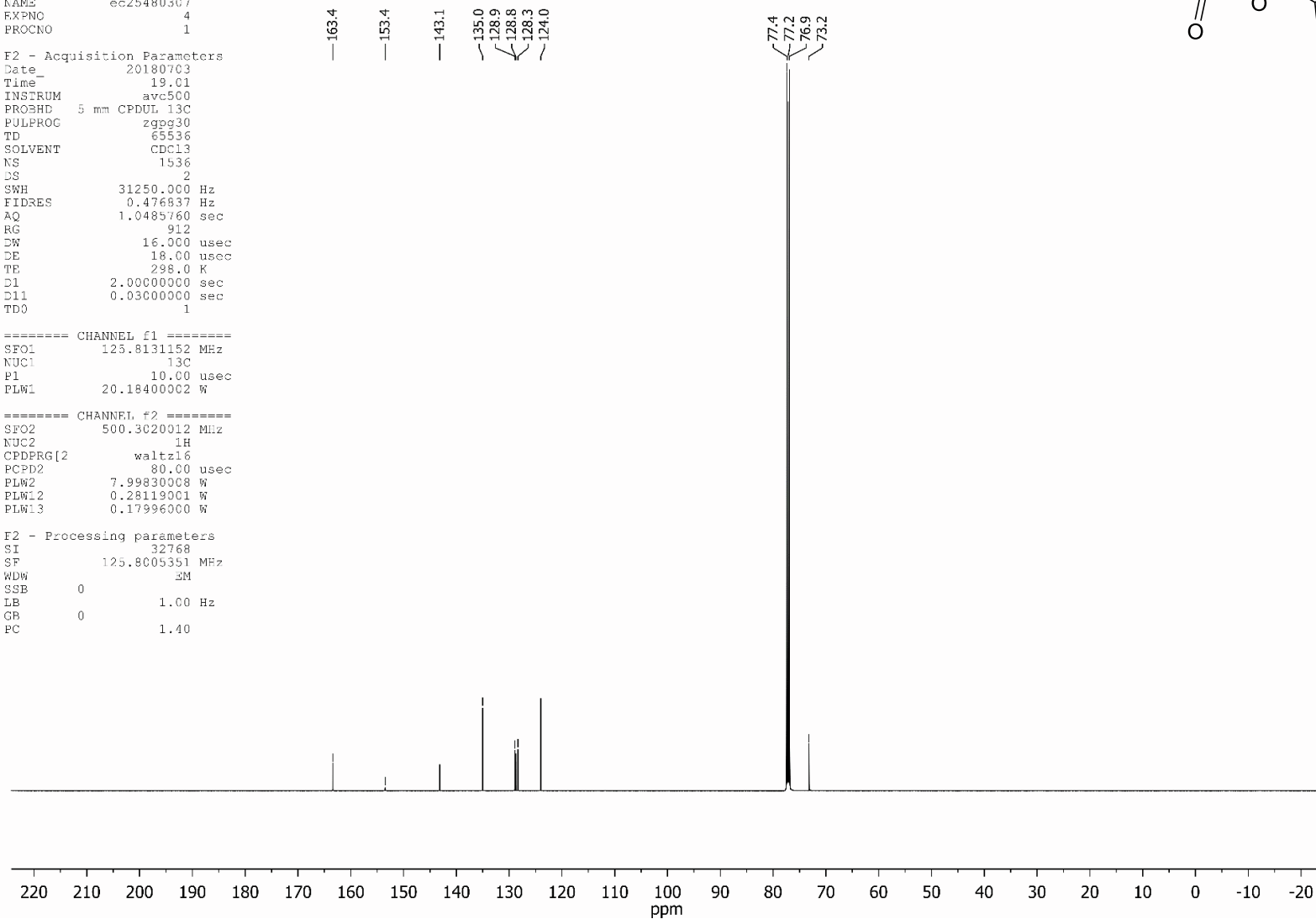
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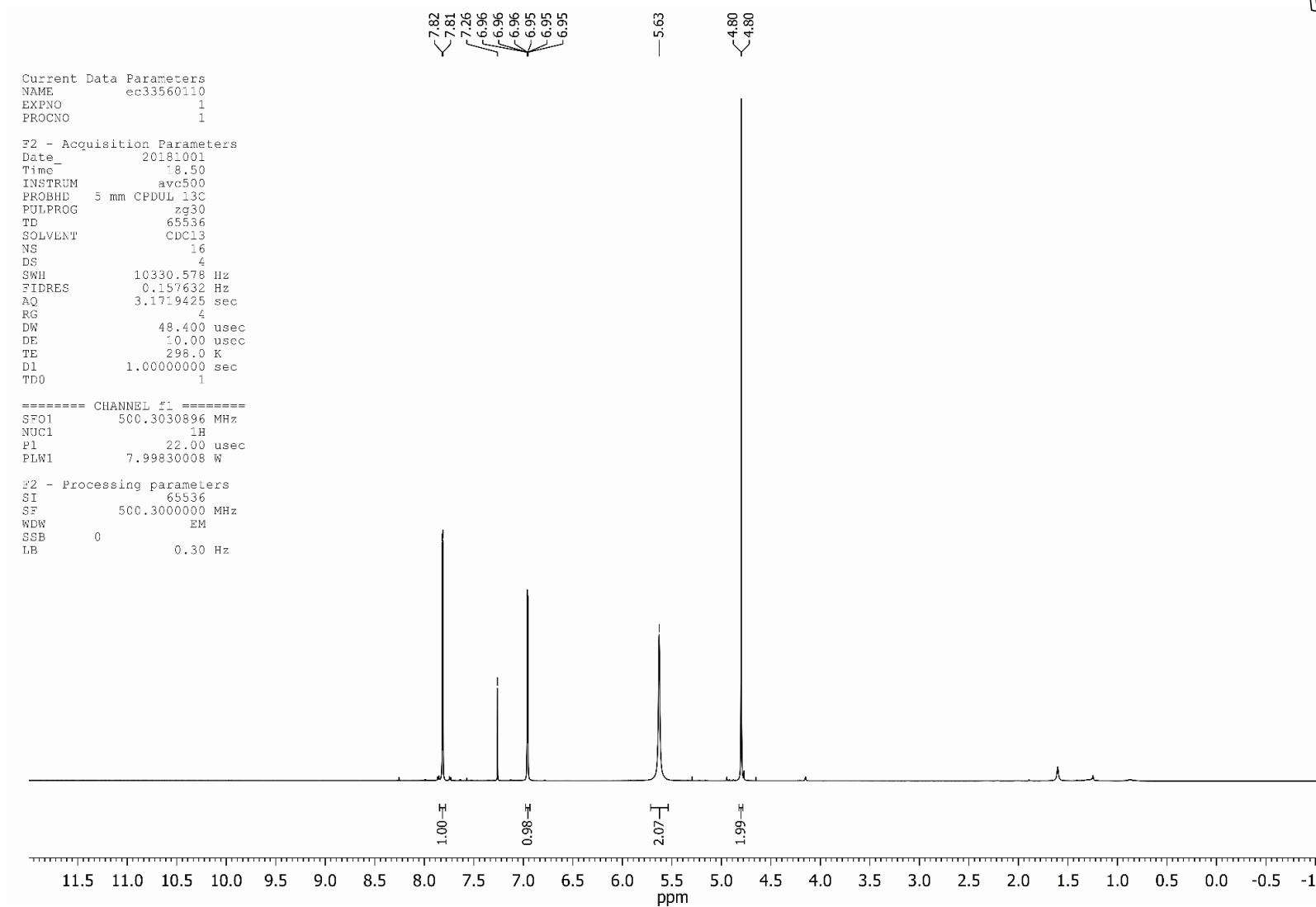
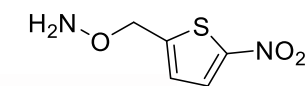
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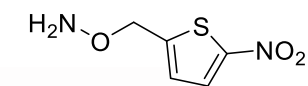
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O-(5-Nitrothiophen-2-yl)-hydroxylamine (**S10**)



O-(5-Nitrothiophen-2-yl)-hydroxylamine (S10)



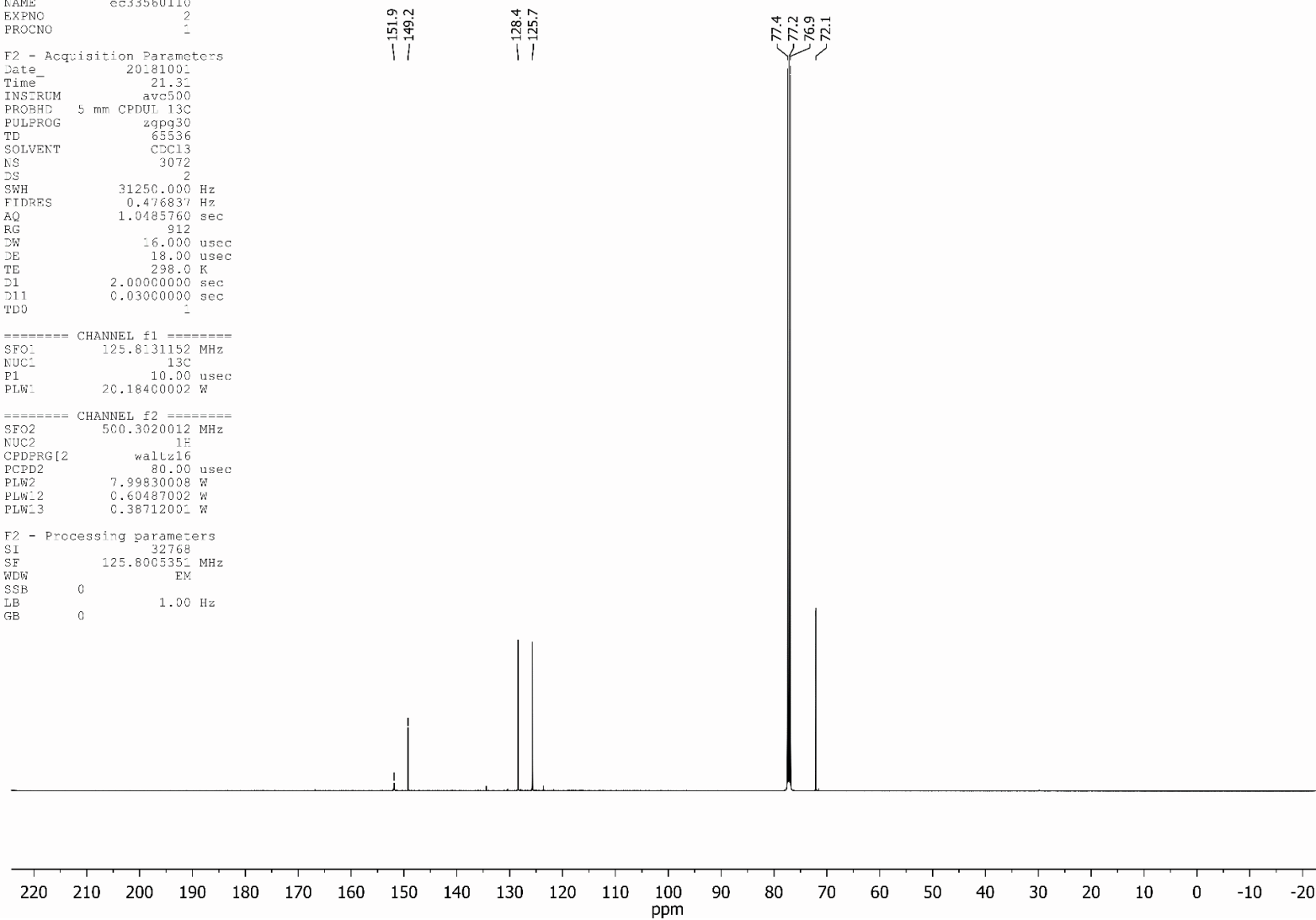
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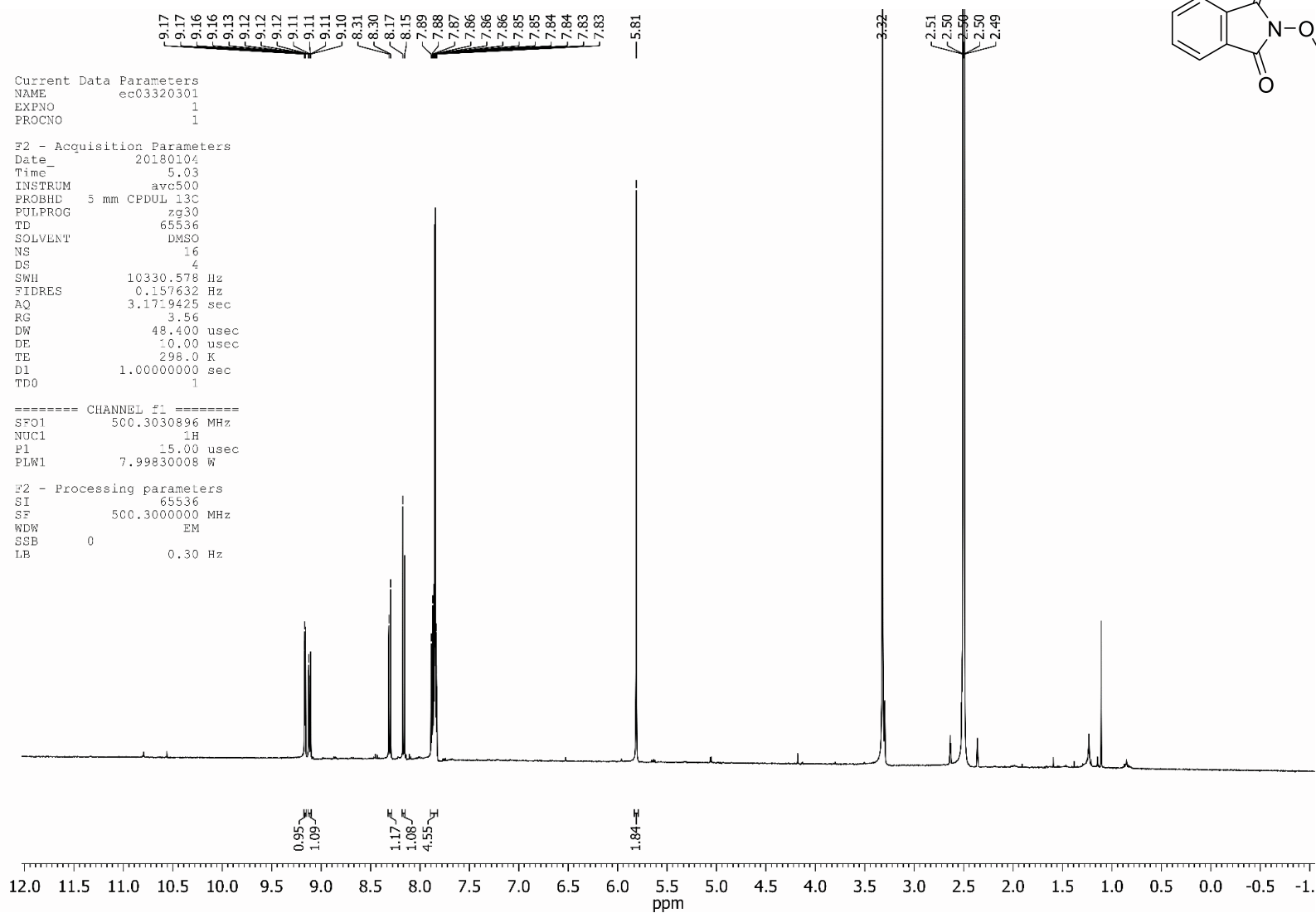
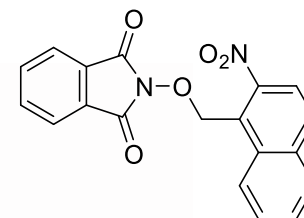
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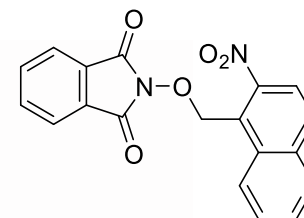
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N-Phthalimido-*O*-(6-nitroquinolin-5-yl)-hydroxylamine



N-Phthalimido-*O*-(6-nitroquinolin-5-yl)-hydroxylamine



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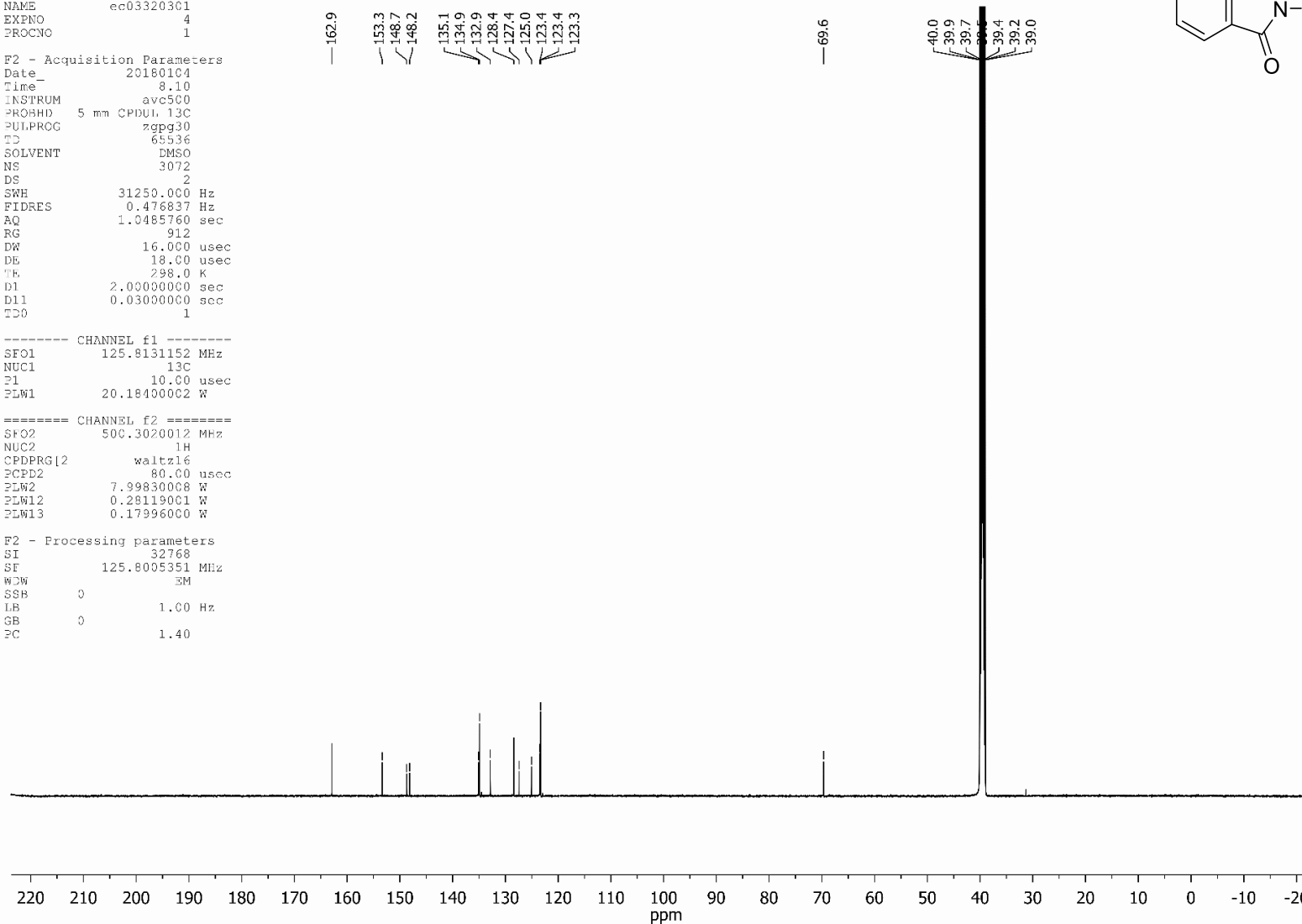
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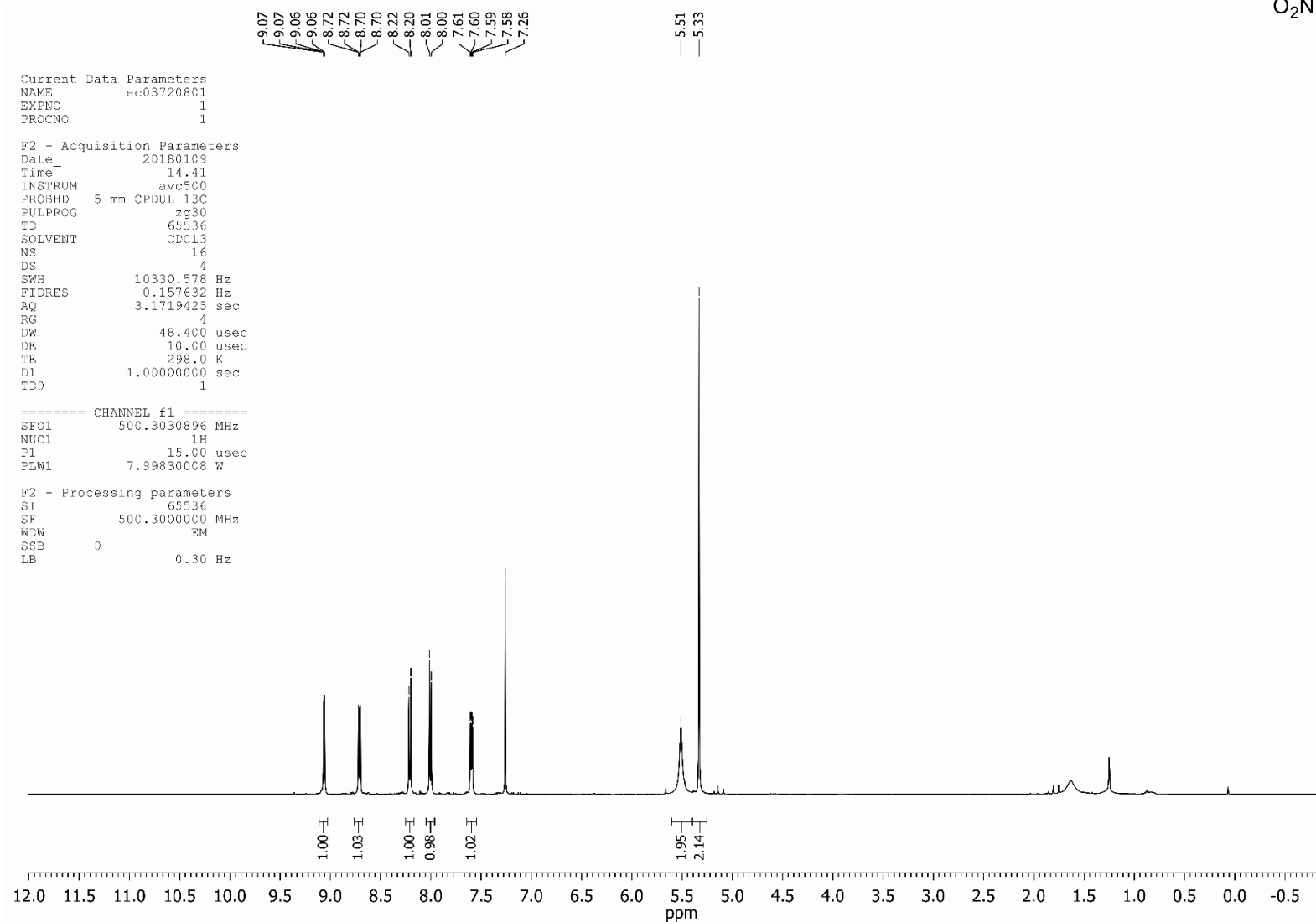
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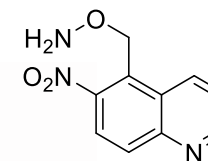
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O-(6-Nitroquinolin-5-yl)-hydroxylamine (**S14**)



O-(6-Nitroquinolin-5-yl)-hydroxylamine (**S14**)



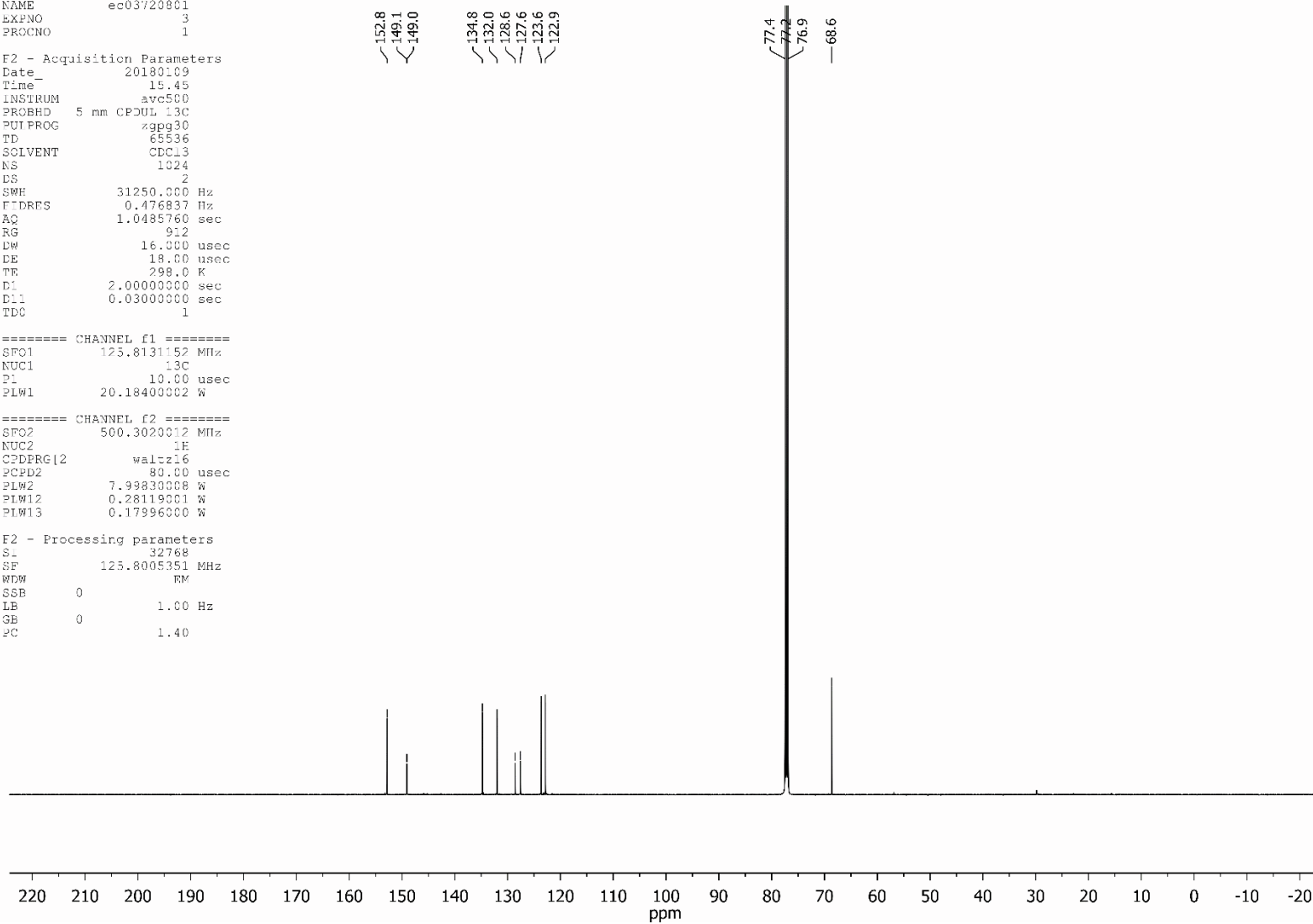
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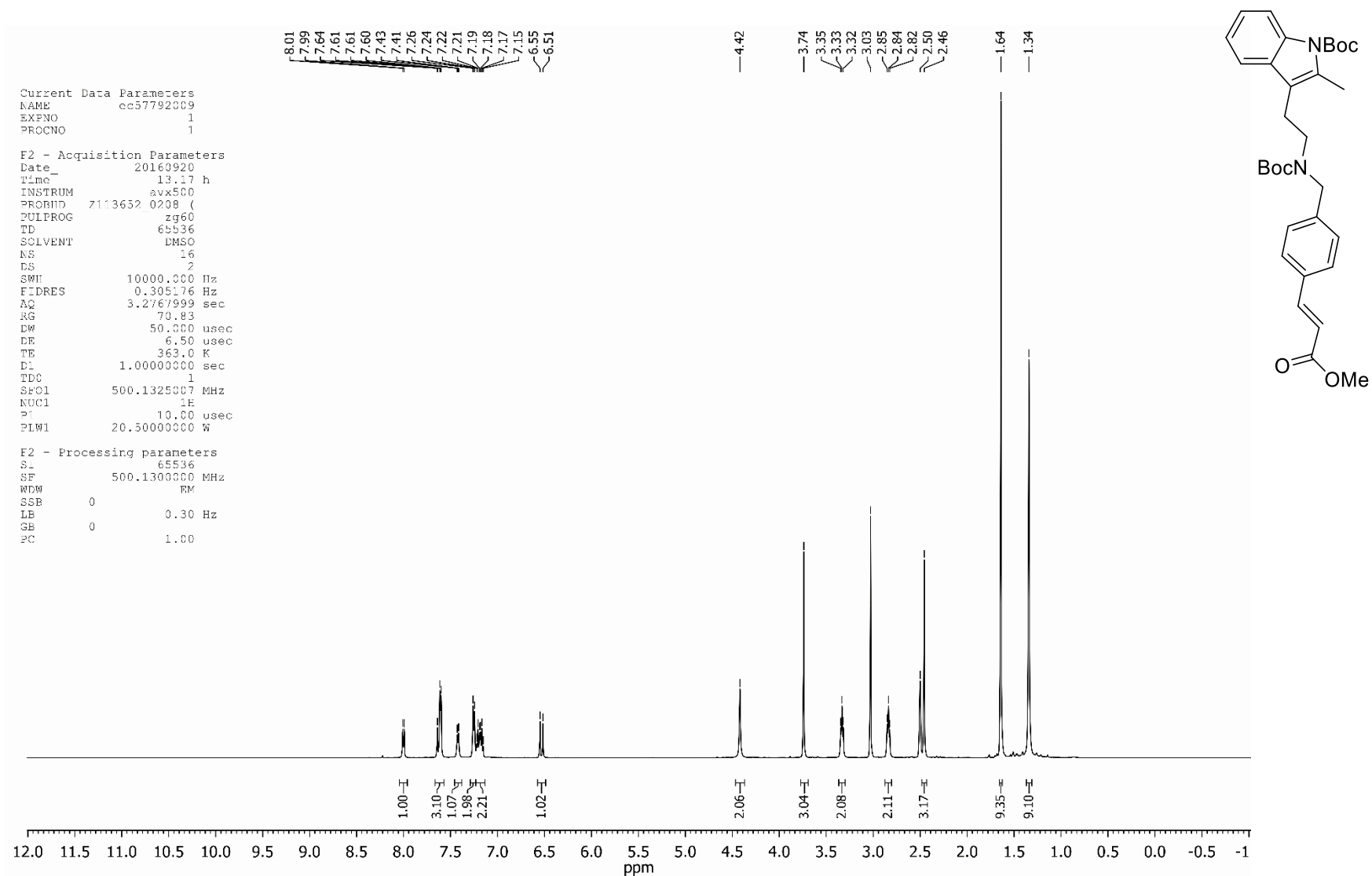
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Methyl (E)-3-(4-[[*tert*-butoxycarbonyl-(2-[1-(*tert*-butoxycarbonyl)-2-methyl-1*H*-indol-3-yl]ethyl)amino]methyl]phenyl)prop-2-enoate (**4**)

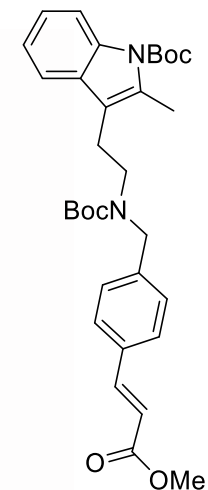
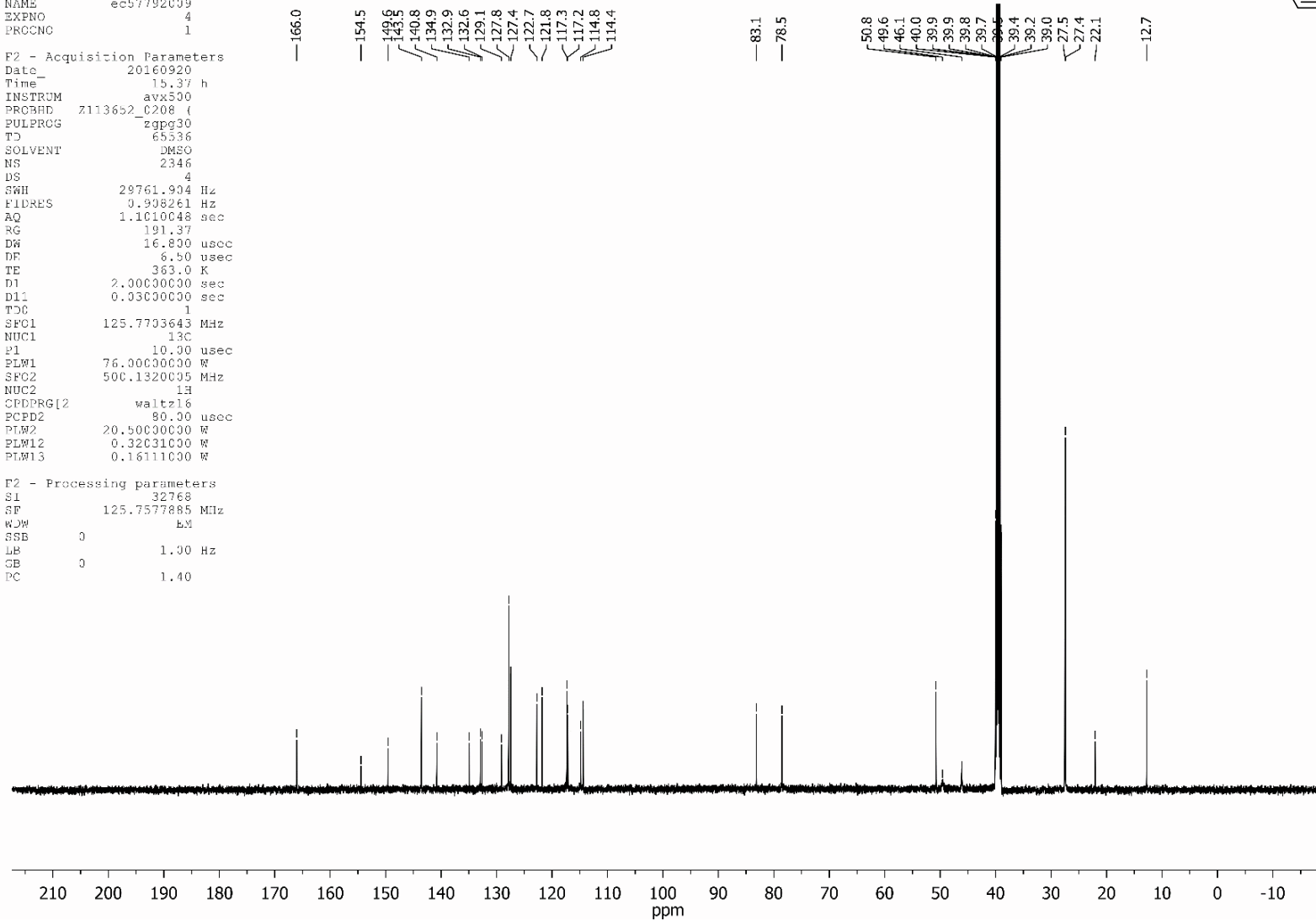


Methyl (E)-3-(4-[[*tert*-butyloxycarbonyl-(2-{1-[*tert*-butyloxycarbonyl]-2-methyl-1*H*-indol-3-yl}ethyl)amino]methyl]phenyl)prop-2-enoate (**4**)

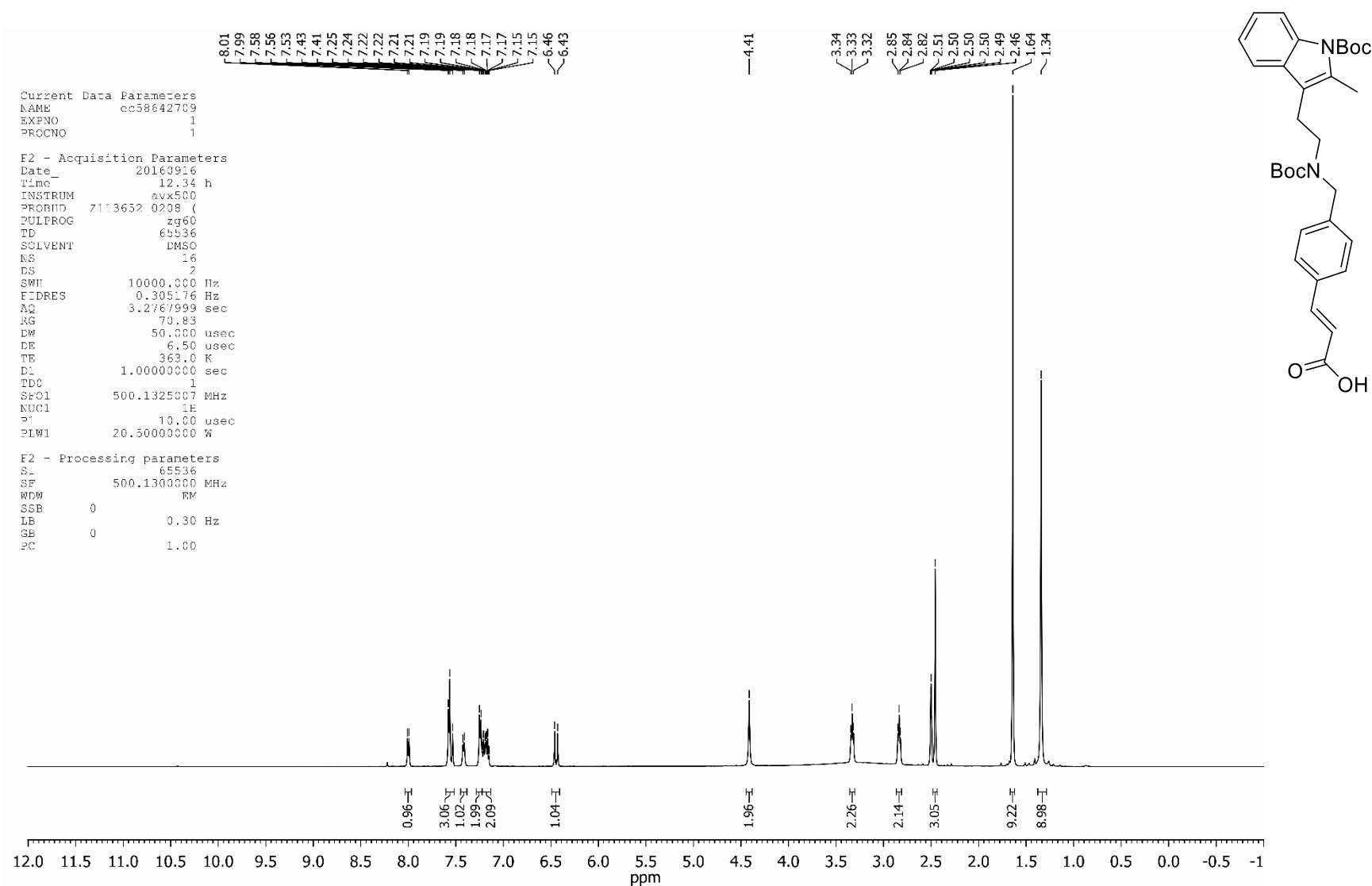
Current Data Parameters
NAME ec5779209
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160920
Time_ 15.37 h
INSTRUM avx500
PROBHD z113652_C208 (4
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2346
DS 4
SWH 29761.904 Hz
FIDRES 0.998261 Hz
AQ 1.1010048 sec
RG 191.37
DW 16.800 usec
DE 6.50 usec
TE 363.0 K
D1 2.00030030 sec
D11 0.03030030 sec
TDC 1
SFO1 125.7703643 MHz
NUC1 13C
P1 10.00 usec
PLW1 76.00030030 W
SFO2 500.1320035 MHz
NUC2 1H
CPDPRG12 waltz16
PCPD2 80.00 usec
PLW2 20.50030030 W
PLW12 0.32031030 W
PLW13 0.16111030 W

F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW BM
SSE 0
LB 1.00 Hz
GB 0
PC 1.40



(*E*)-3-(4-[[*tert*-Butyloxycarbonyl-(2-{1-[*tert*-butyloxycarbonyl]-2-methyl-1*H*-indol-3-yl)ethyl]amino)methyl}phenyl)prop-2-enoic acid (**5**)

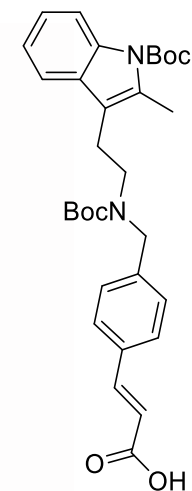
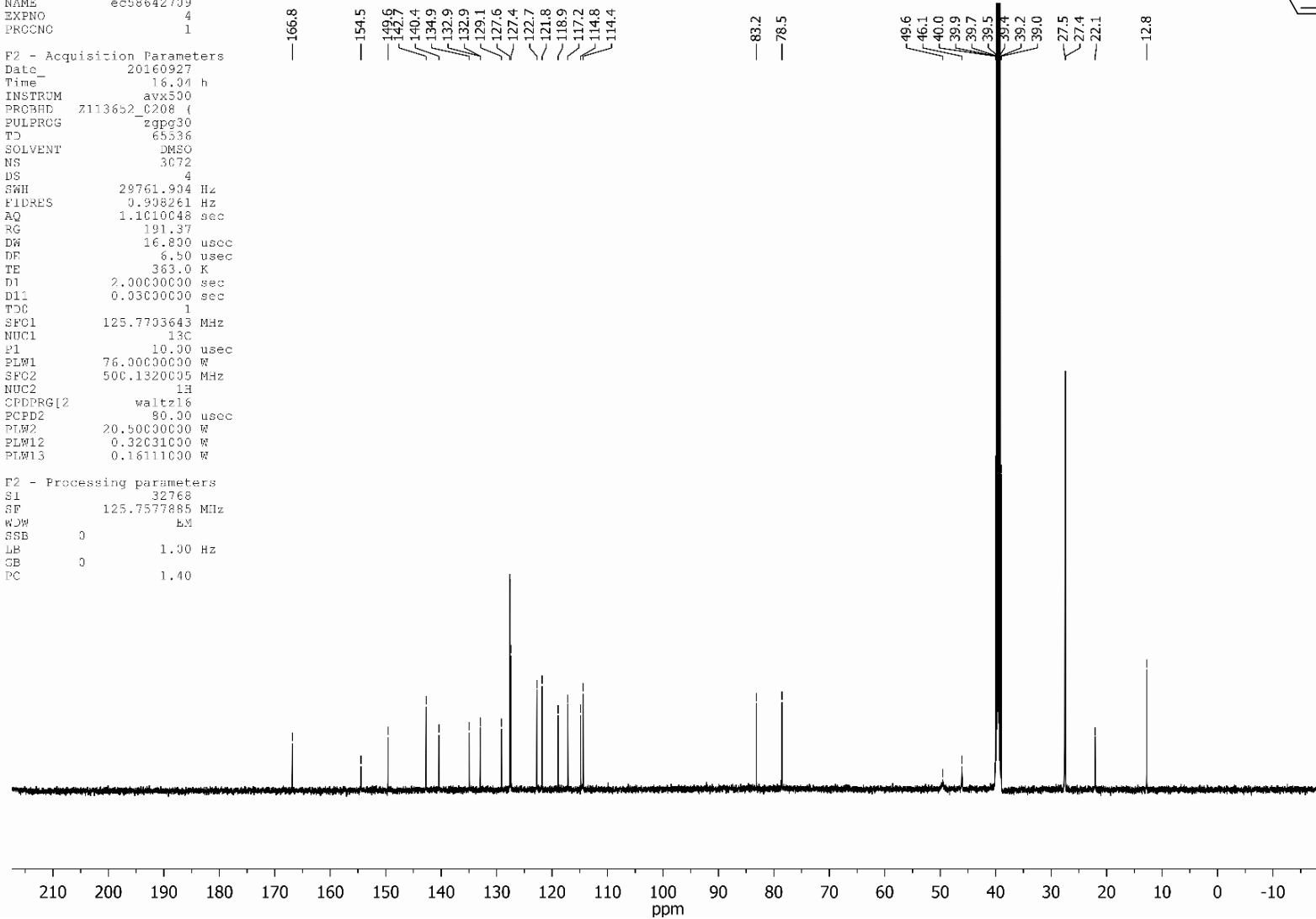


(E)-3-(4-([*tert*-Butyloxycarbonyl-(2-{1-[*tert*-butyloxycarbonyl]-2-methyl-1*H*-indol-3-yl)ethyl]amino)methyl}phenyl)prop-2-enoic acid (5)

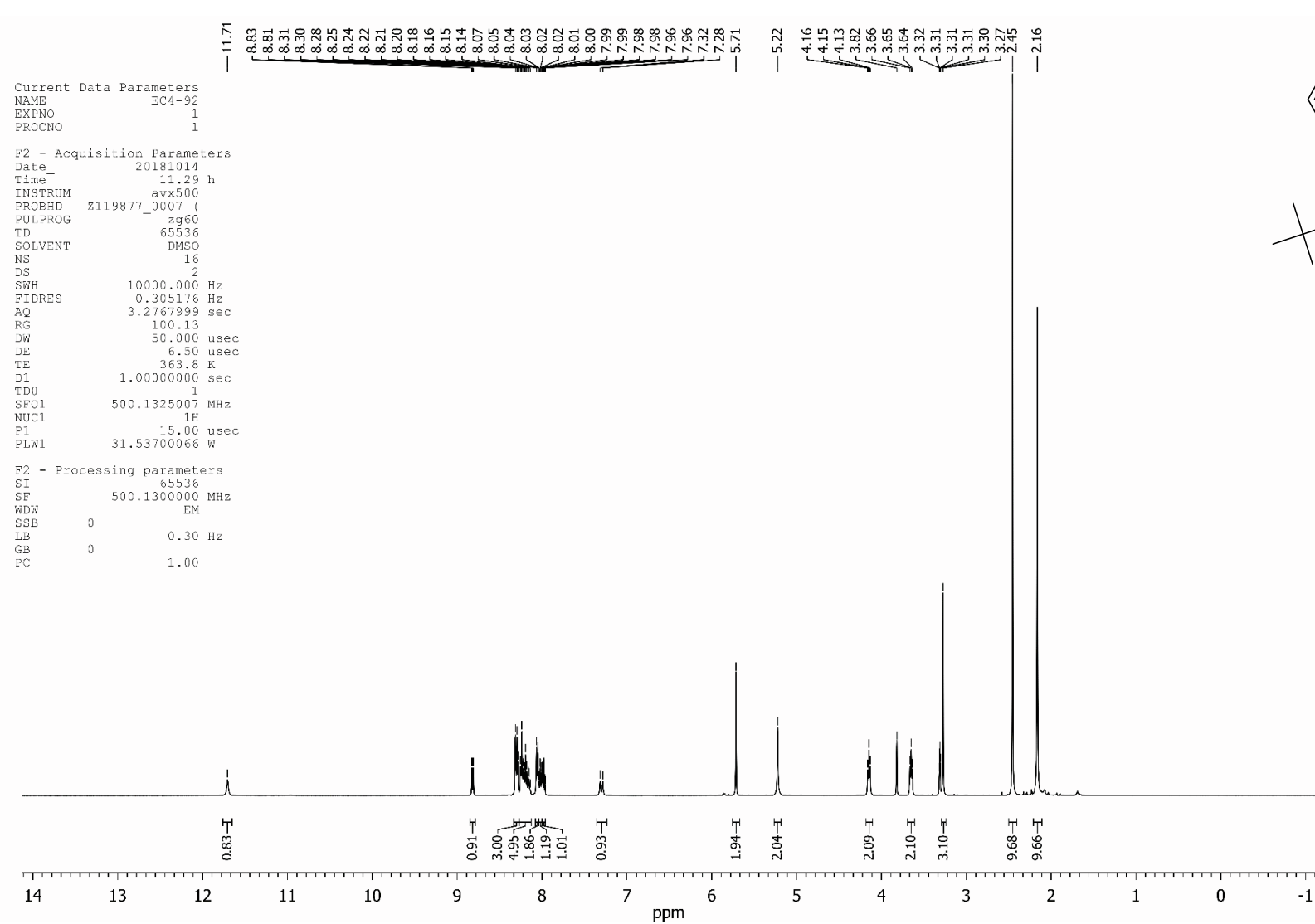
Current Data Parameters
NAME ec58642709
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20160927
Time_ 16.04 h
INSTRUM avx500
PROBHD z1136b2_0208 {
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 3072
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 191.37
DW 16.800 usec
DE 6.50 usec
TE 363.0 K
D1 2.0000000 sec
D11 0.0300000 sec
TDC 1
SFO1 125.7703643 MHz
NUC1 13C
P1 10.00 usec
PLW1 76.0000000 W
SFO2 500.1320035 MHz
NUC2 1H
CPDPRG12 waltz16
PCPD2 80.00 usec
PLW2 20.5000000 W
PLW12 0.3203100 W
PLW13 0.1611100 W

F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



N,N-Di-Boc-*O*-benzyl-Panobinostat (**6**)

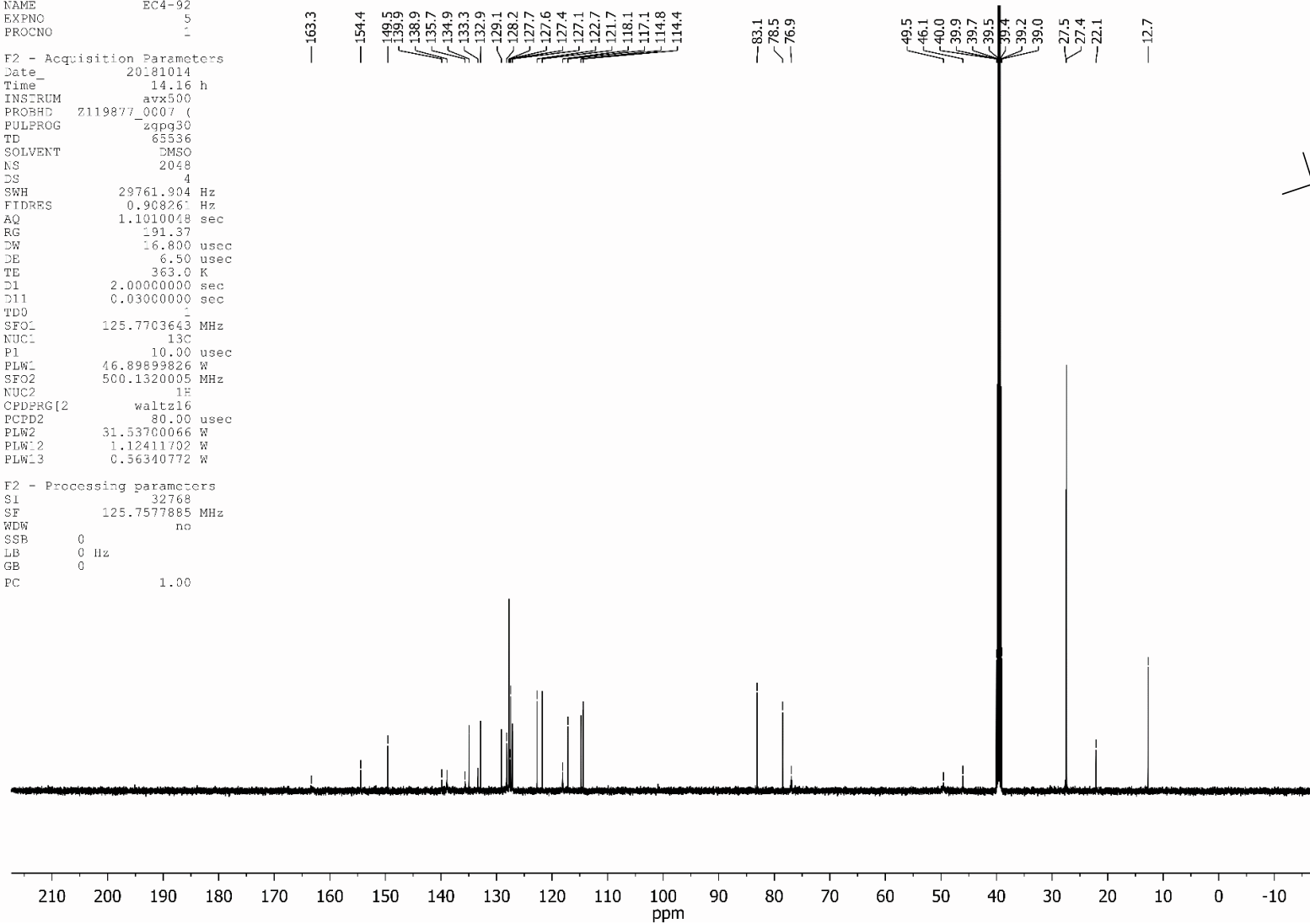


N,N-DiBoc-*O*-benzyl-Panobinostat (**6**)

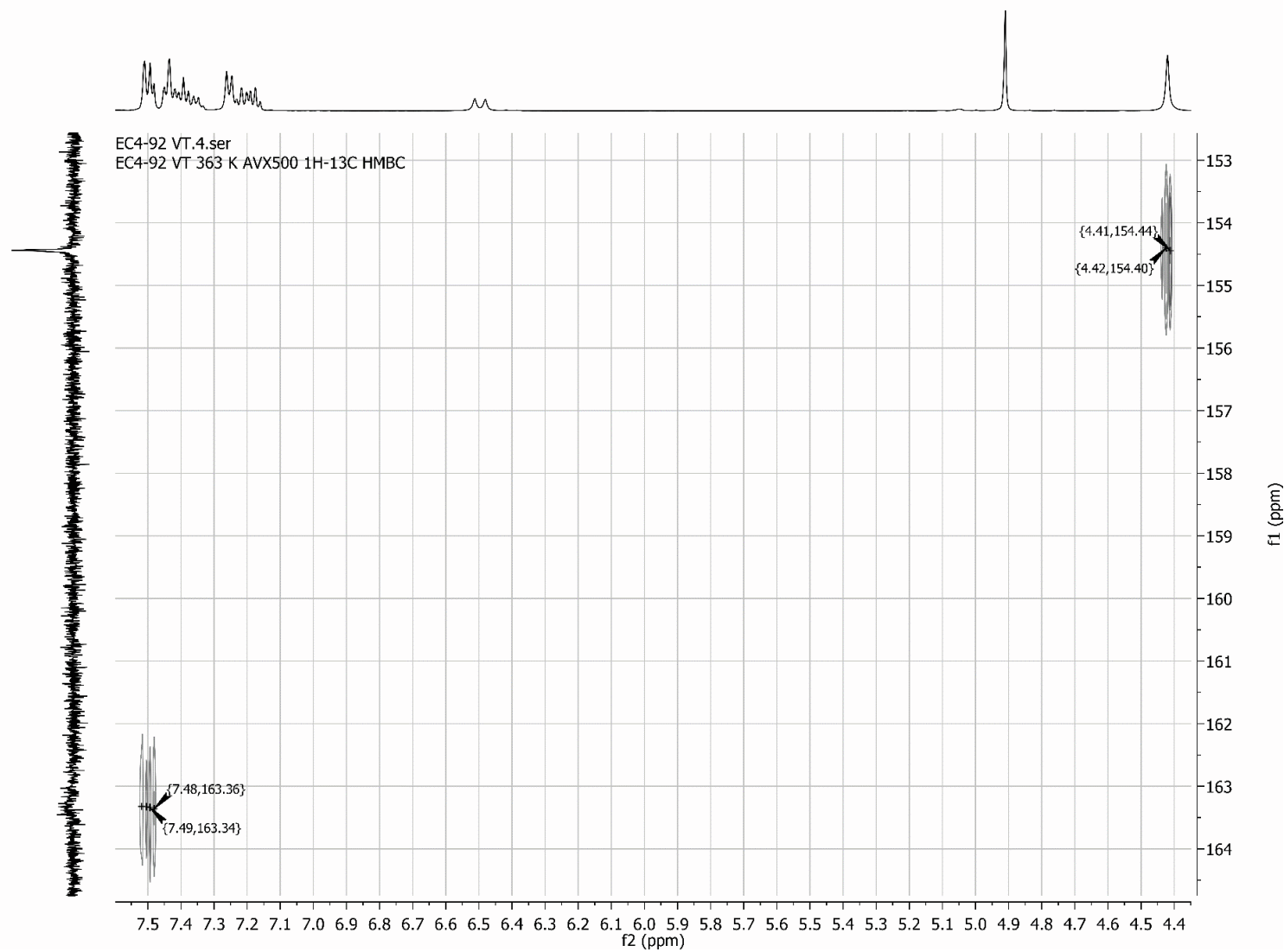
Current Data Parameters
NAME EC4-92
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20181014
Time 14.16 h
INSTRUM avx500
PROBHD Z119877_0001 (
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2048
DS 4
SWH 29761.904 Hz
FIDRES 0.90826 Hz
AQ 1.1010048 sec
RG 191.37
DW 16.800 usec
DE 6.50 usec
TE 363.0 K
D1 2.0000000 sec
D11 0.0300000 sec
TD0 -
SFO1 125.7703643 MHz
NUC1 13C
P1 10.00 usec
PLW1 46.89899826 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 31.53700066 W
PLW12 1.12411702 W
PLW13 0.56340772 W

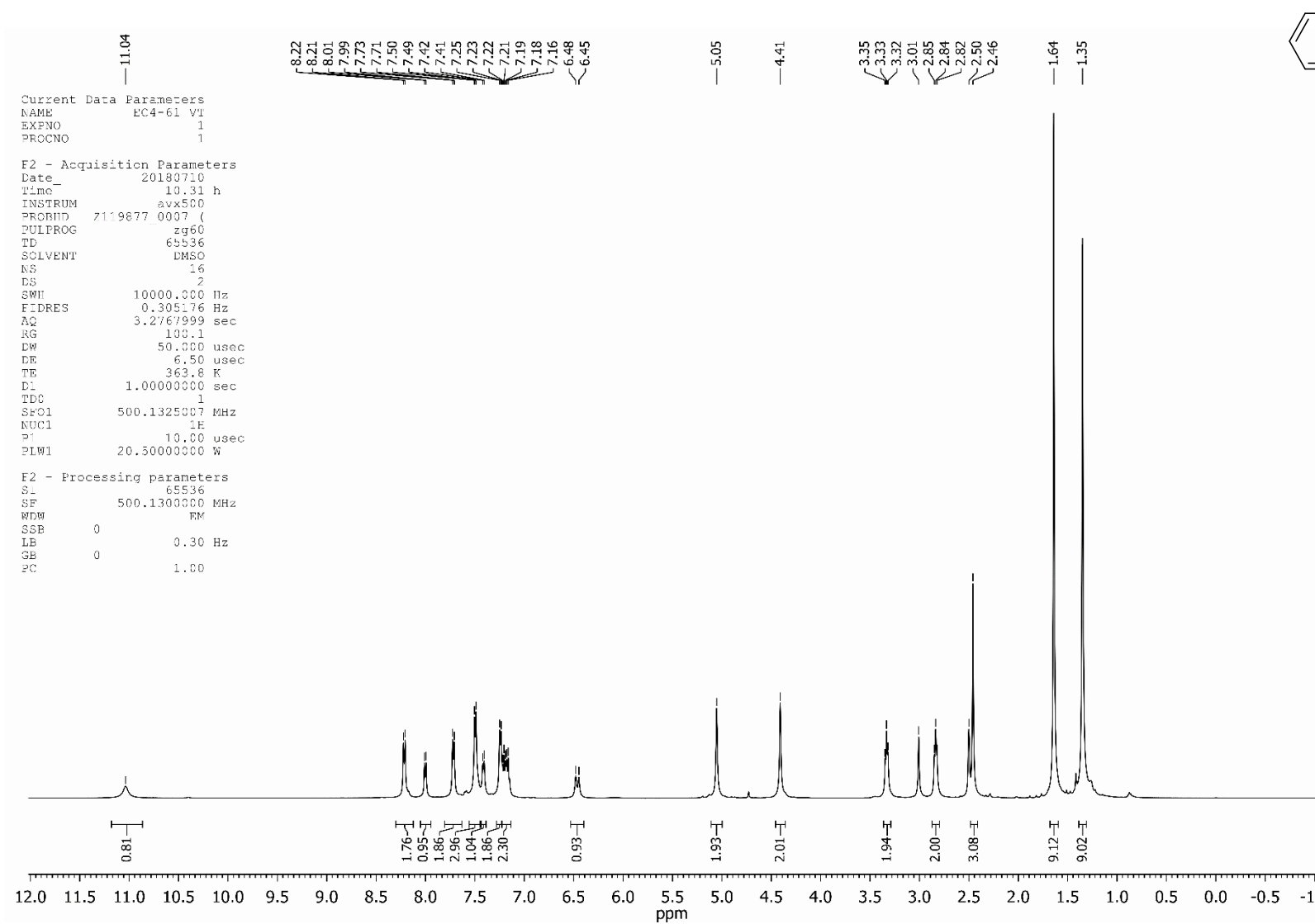
F2 - Processing parameters
S1 32768
SF 125.7577885 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00



N,N-Di-Boc-*O*-benzyl-Panobinostat (**6**)



N,N-Di-Boc-*O*-(4-nitrobenzyl)-Panobinostat (**7**)



N,N-Di-Boc-*O*-(4-nitrobenzyl)-Panobinostat (**7**)

```
Current Data Parameters
NAME          EC4-61 VI
EXPNO         5
PROCNO        1
```

```

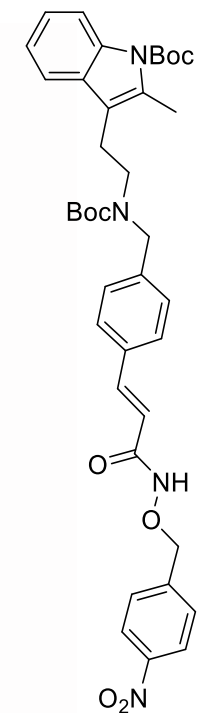
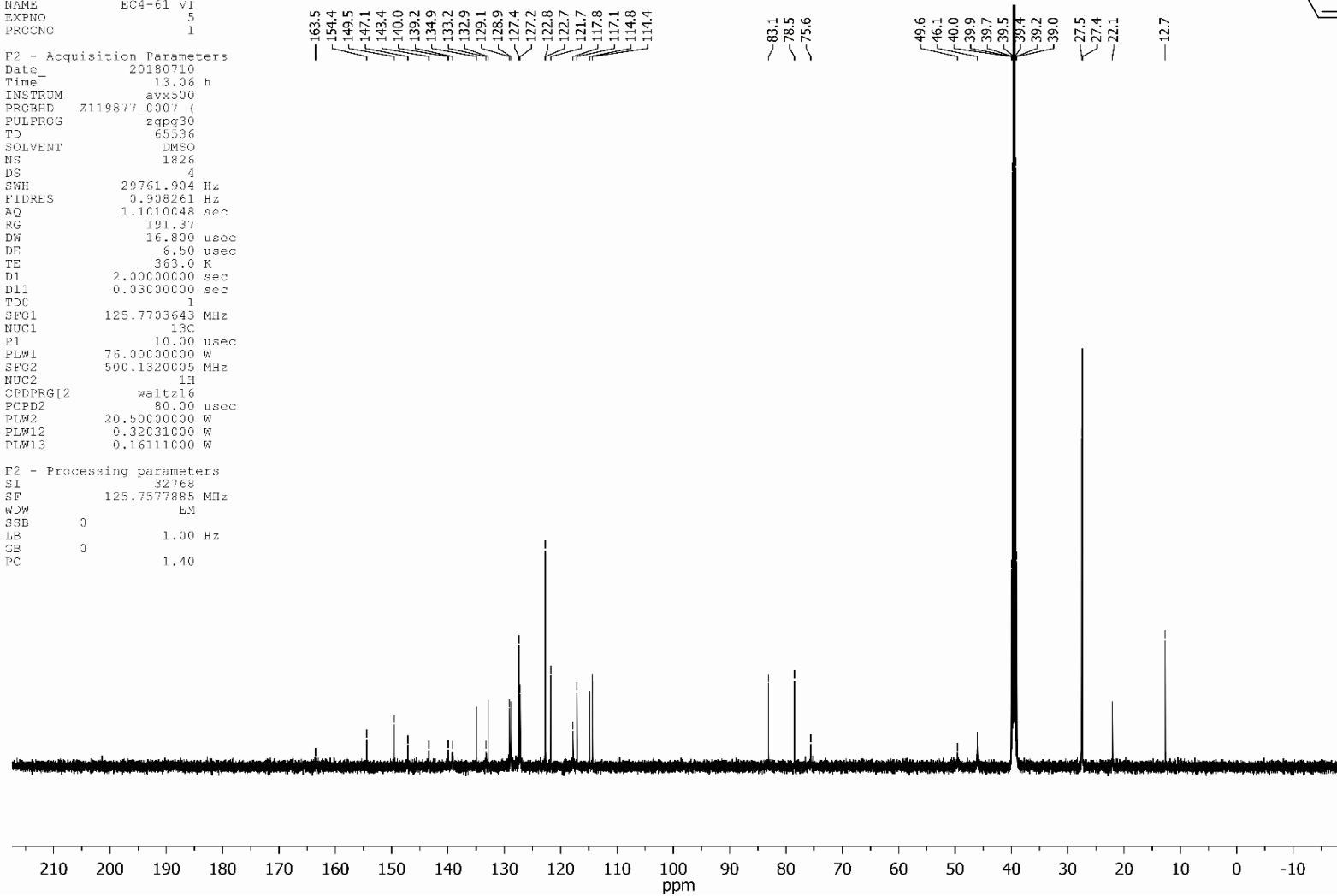
P2 - Acquisition Parameters
Date_      20180710
Time_      13.36 h
INSTRUM    avx500
PROBHD     Z118977_0007 f
PULPRG     zgpg30
TD         65536
SOLVENT    DMSO
NS         1826
DS         4
SWH        29761.904 Hz
FIDRES     0.908261 Hz
AQ         1.1010048 sec
RG         191.37
DW         16.800 usec
DE         6.50 usec
TE         363.0 K
D1         2.3003000 sec
d11        0.3003000 sec
T2        206
SFG1       125.7703643 MHz
NUC1       13C
P1         10.00 usec
PLW1       76.00000000 W
SFG2       500.1320035 MHz
NUC2       1H
CPDPRG2    wait216
PCPD2      80.00 usec
PLW2       20.50000000 W
PLW12     0.32031000 W
PLW13     0.16110000 W

```

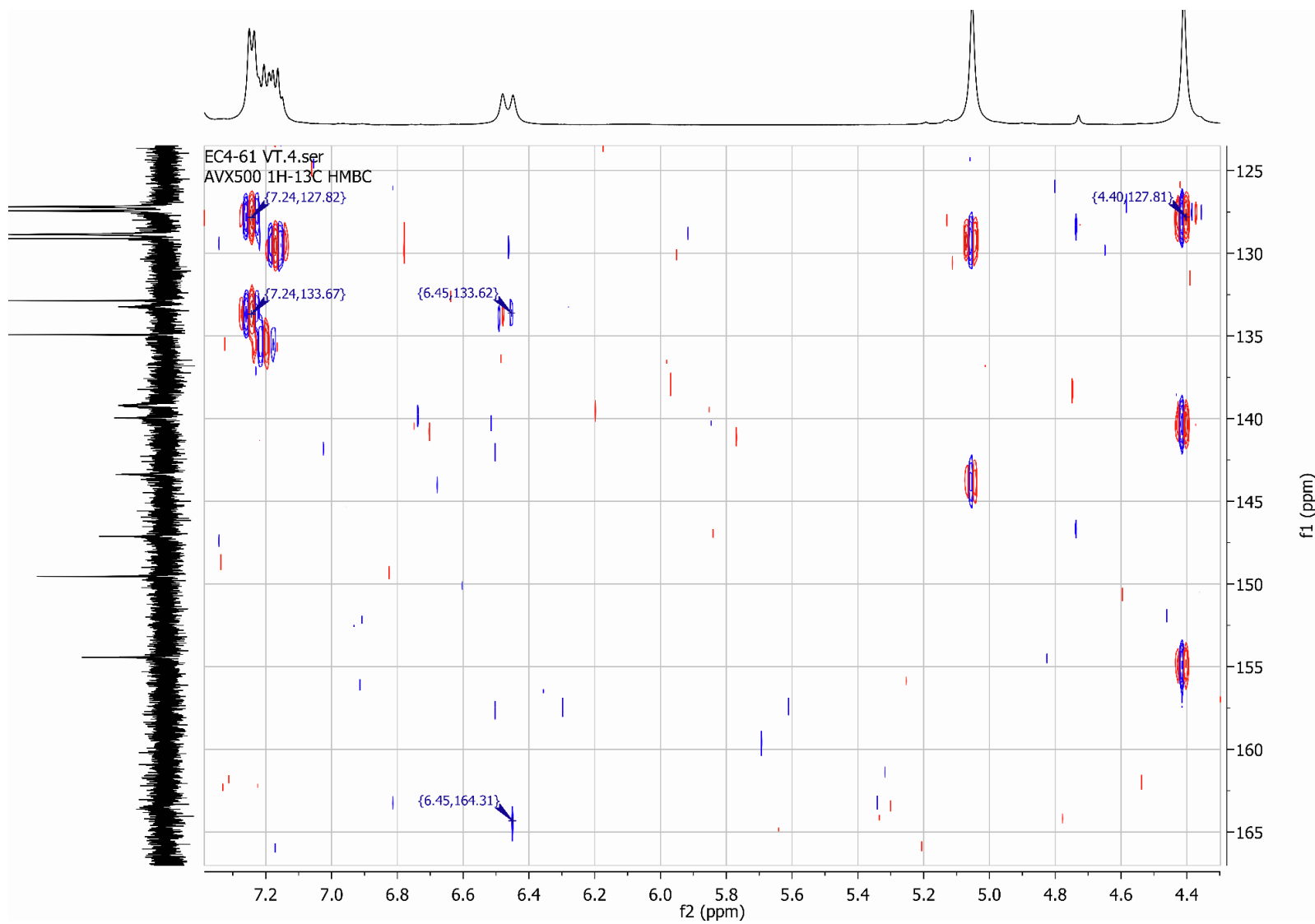
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F2 - Processing parameters
SI                      32768
SF                      125.7577885 MHz
WDW                      EM
SSB                      0
LB                      1.00 Hz
CB                      0
PC                      1.40

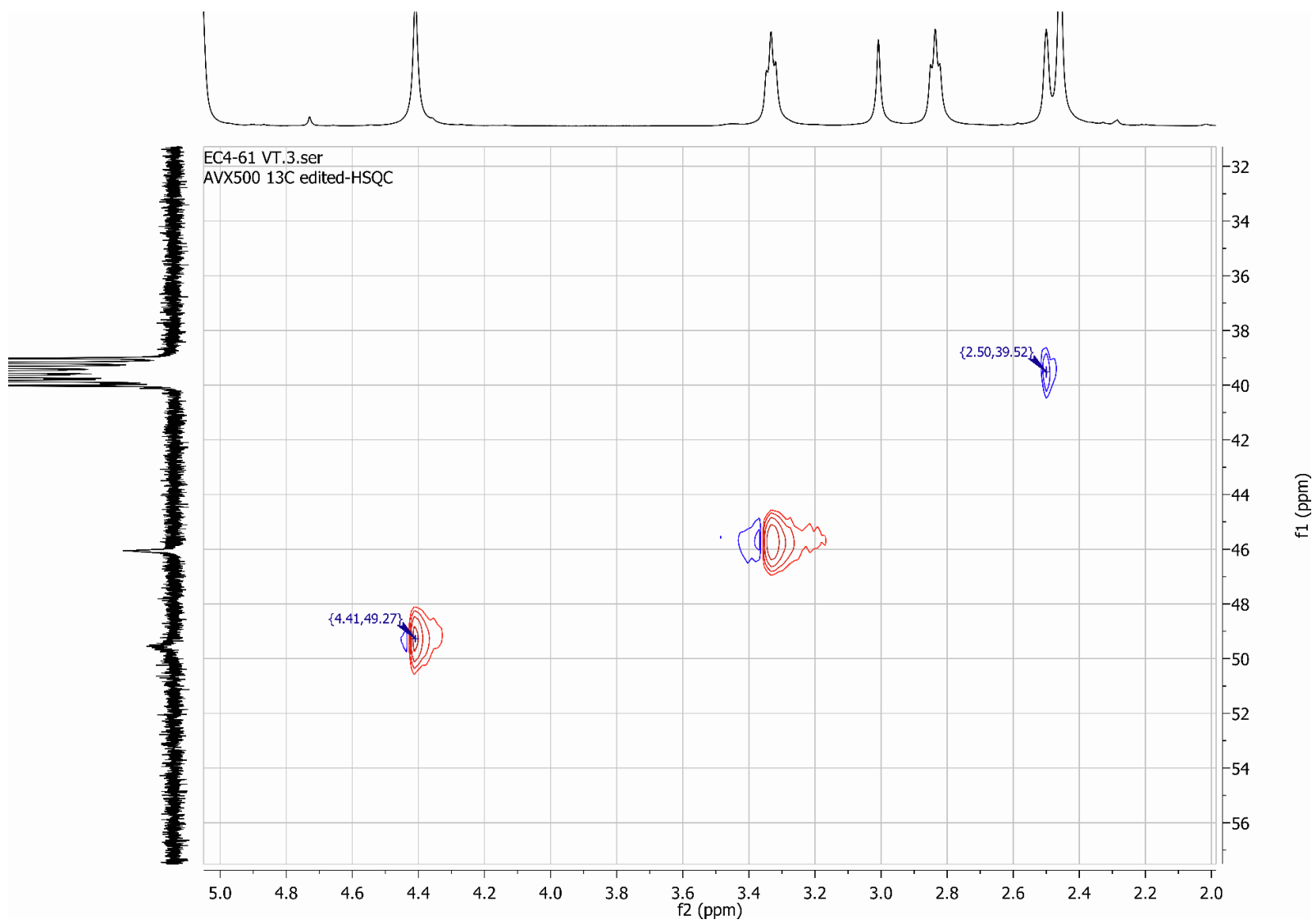
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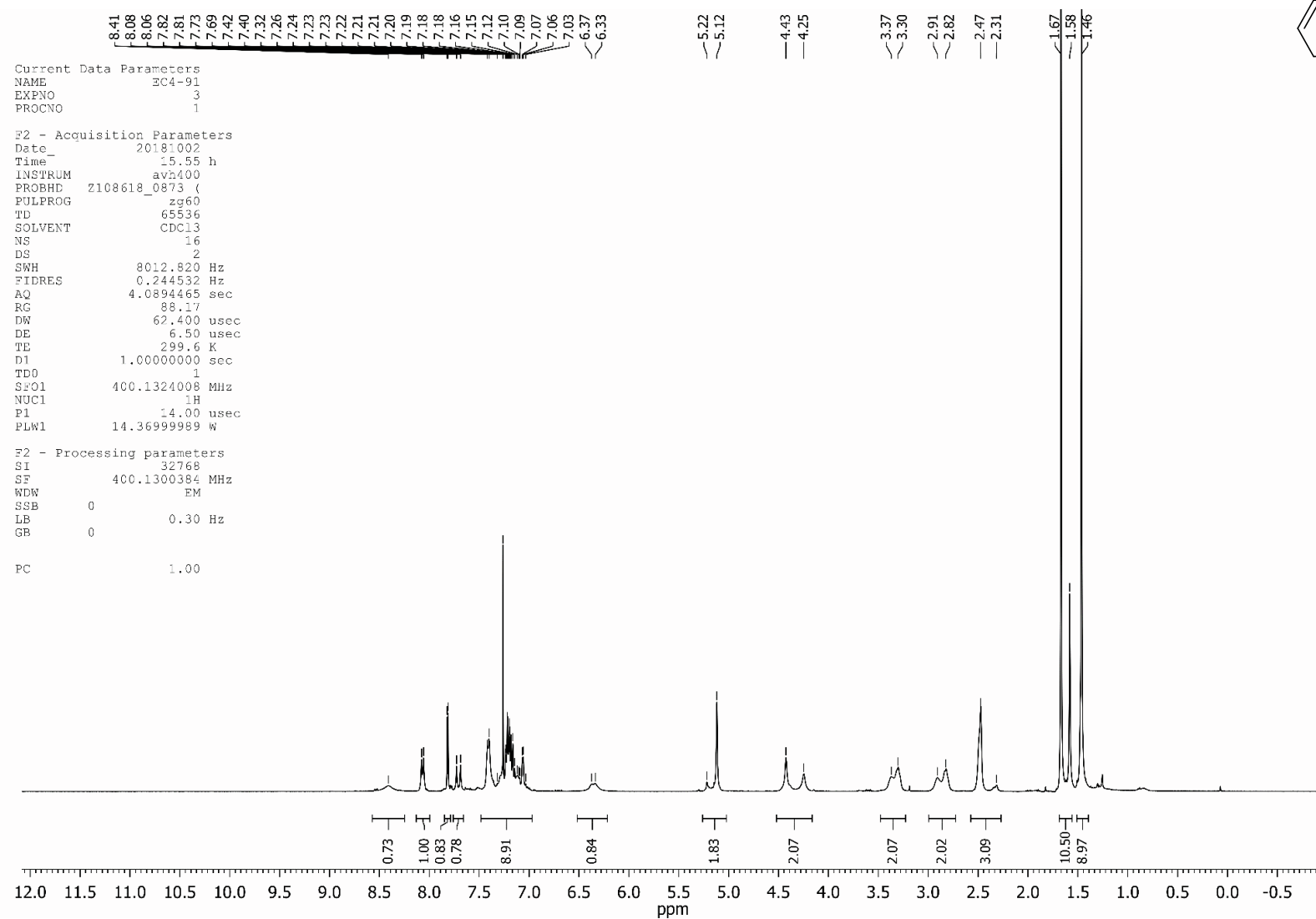
N,N-Di-Boc-O-(4-nitrobenzyl)-Panobinostat (**7**)



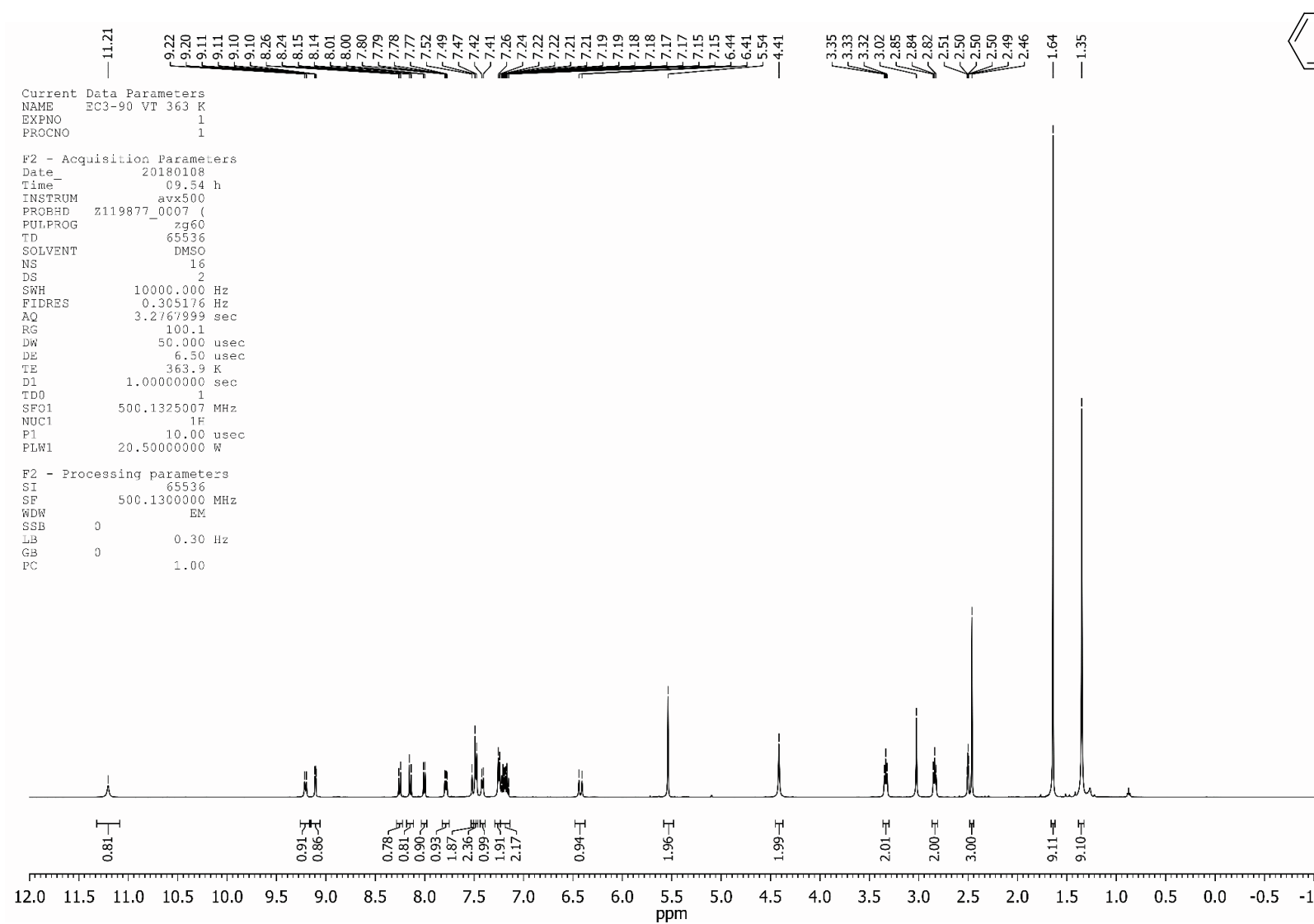
N,N-Di-Boc-*O*-(4-nitrobenzyl)-Panobinostat (**7**)



N,N-Di-Boc-*O*-(2-nitrothiophen-5-yl)methyl)-Panobinostat (**8**)



N,N-Di-Boc-*O*-(6-nitroquinolin-5-yl)methyl)-Panobinostat (**9**)

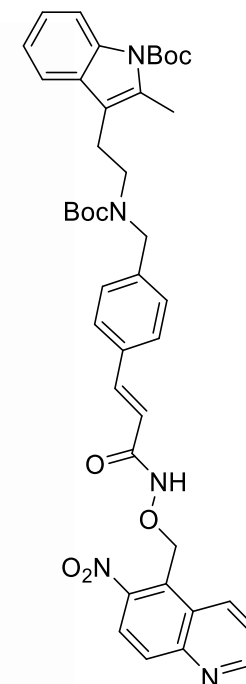
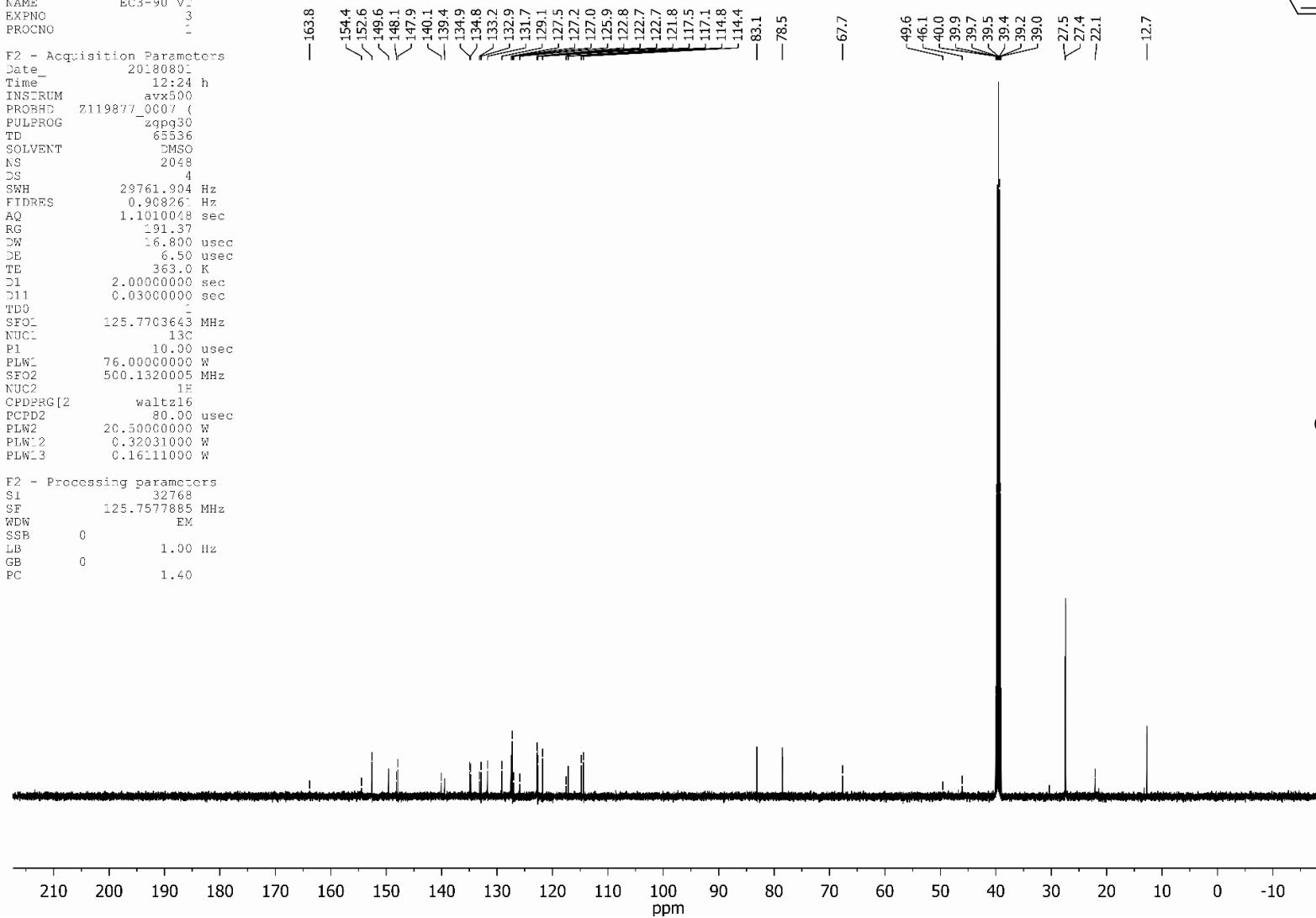


N,N-Di-Boc-*O*-((6-nitroquinolin-5-yl)methyl)-Panobinostat (**9**)

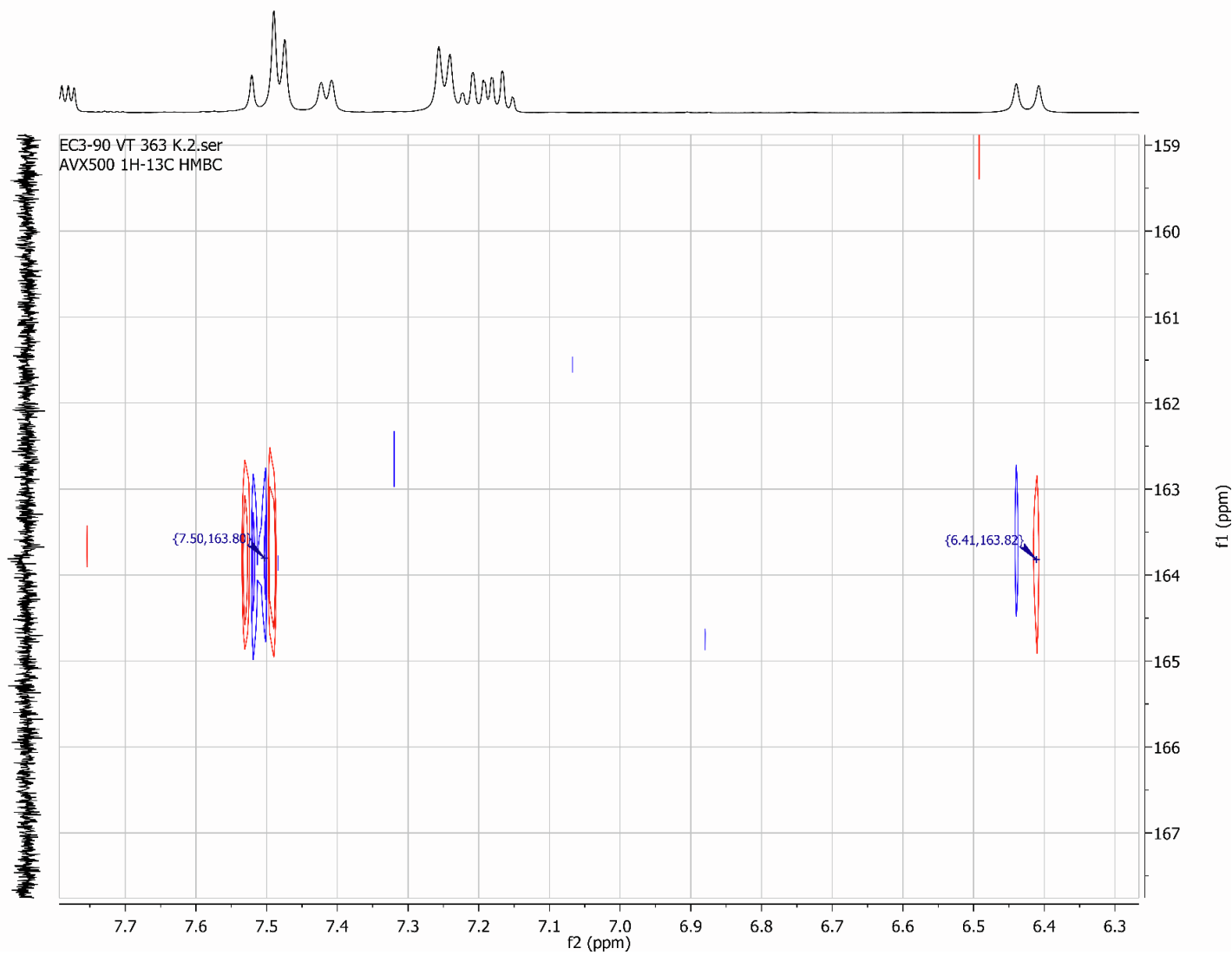
Current Data Parameters
NAME EC3-90 V
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180801
Time_ 12:24 h
INSTRUM avx500
PROBHD Z119877_0007 (
PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 2048
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 191.37
DW 16.800 usec
DE 6.50 usec
TE 363.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 -
SFO1 125.7703643 MHz
NUC1 13C
P1 10.00 usec
PLW1 76.00000000 W
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 20.50000000 W
PLW12 0.32031000 W
PLW13 0.16111000 W

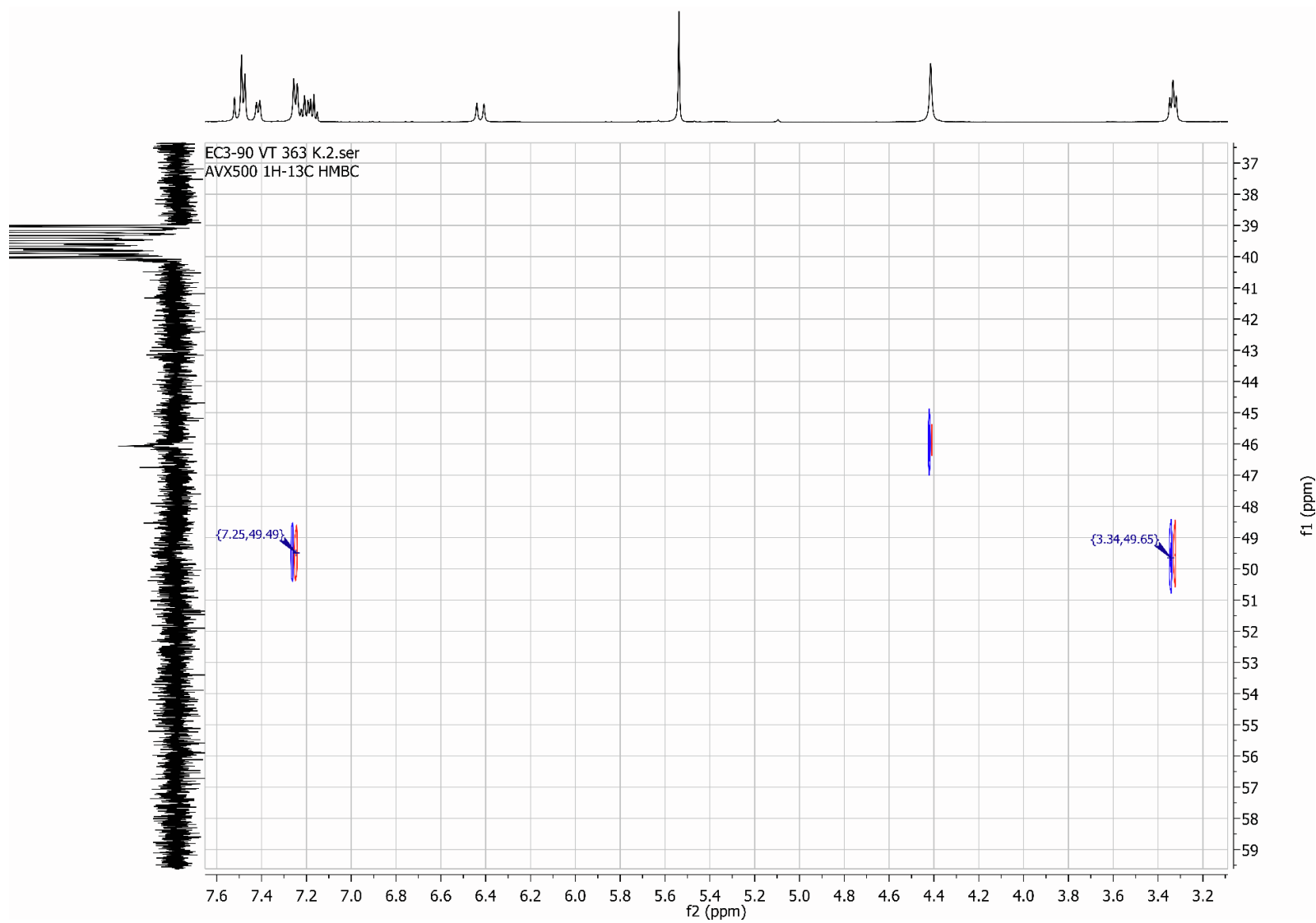
F2 - Processing parameters
SI 32768
SF 125.7577885 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



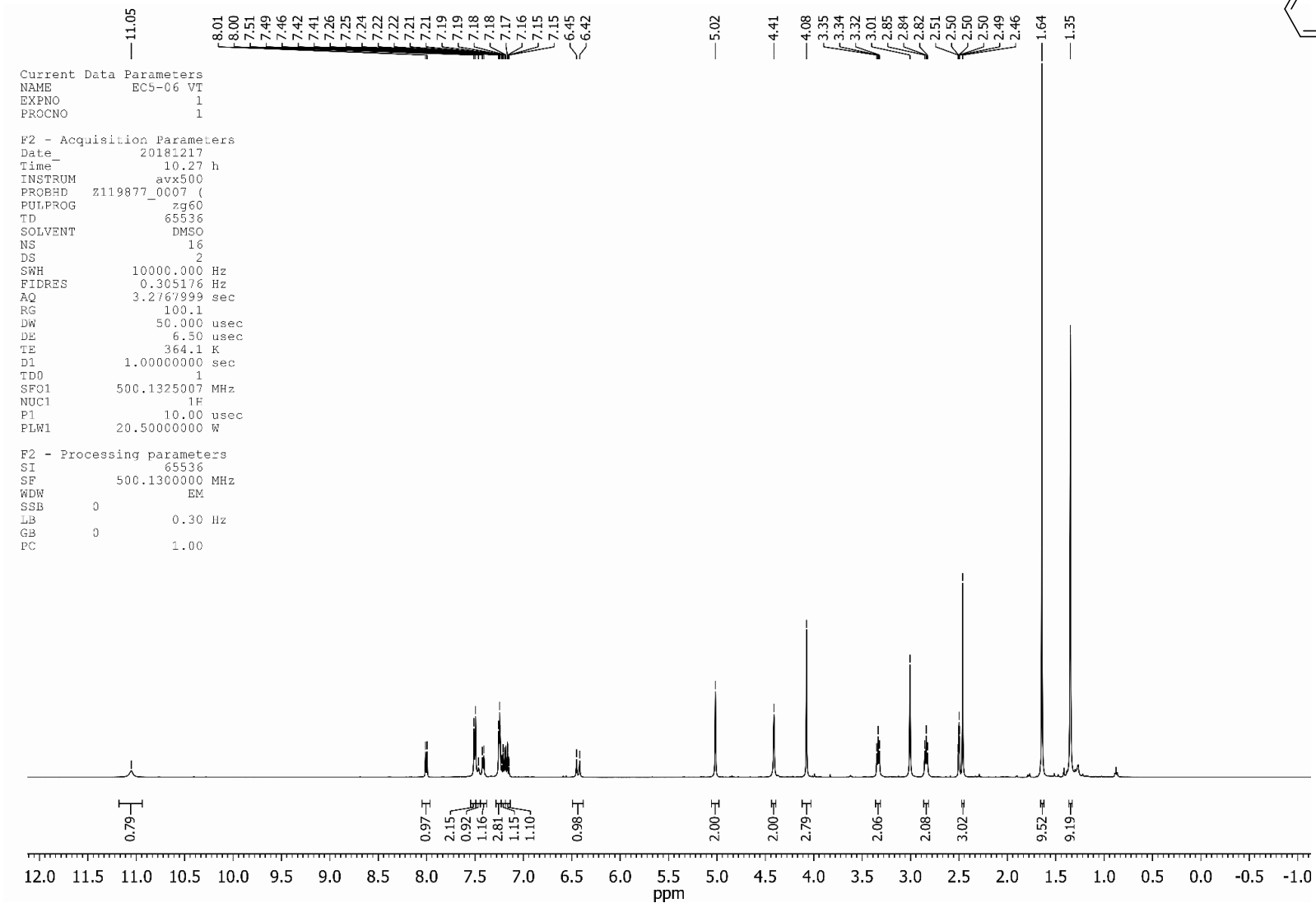
N,N-Di-Boc-*O*-(6-nitroquinolin-5-yl)methyl)-Panobinostat (**9**)



N,N-Di-Boc-*O*-((6-nitroquinolin-5-yl)methyl)-Panobinostat (**9**)



N,N-Di-Boc-O-(1-methyl-2-nitroimidazol-5-yl)methyl)-panobinostat

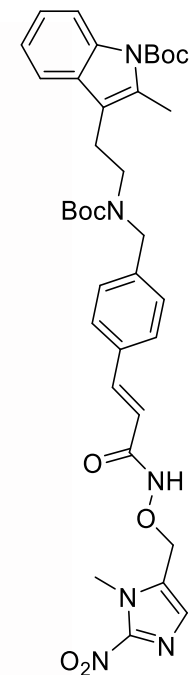
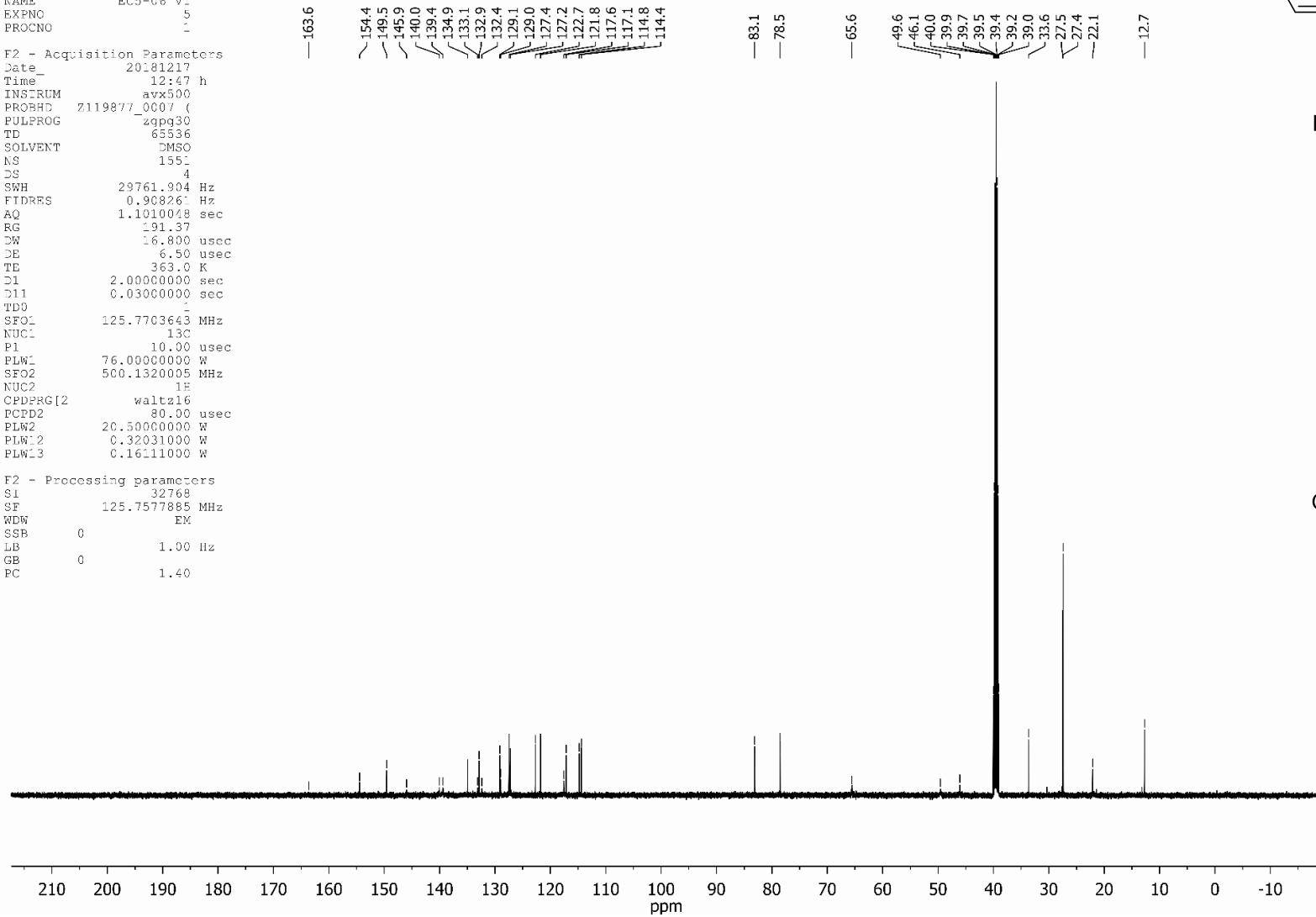


N,N-Di-Boc-*O*-((1-methyl-2-nitroimidazol-5-yl)methyl)-panobinostat

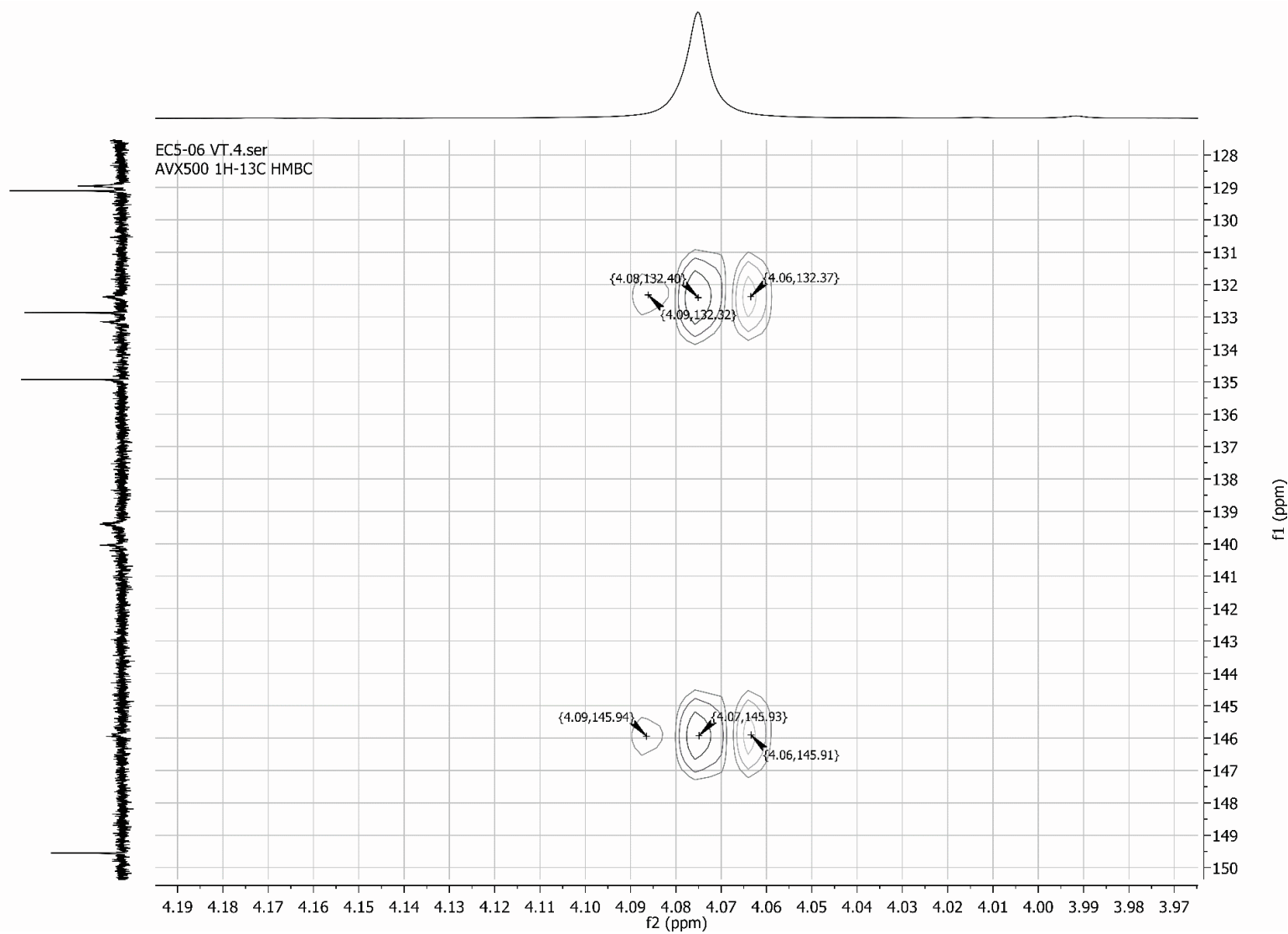
```
Current Data Parameters
NAME          EC5-06 VT
EXPNO          5
PROCNO         1
```

F2 - Acquisition Parameters		
Date_	20181217	
Time_	12:47 h	
INSTRUM	avx500	
PROBHD	Z119877-0007 Q	
PULPROG	zgpg30	
TD	65536	
SOLVENT	DMSO	
NS	1551	
DS	4	
SWH	29761.904	Hz
FIDRES	0.908266	Hz
AQ	1.10100048	sec
RG	191.37	
DW	16.800	usec
DE	6.50	usec
TE	363.0	K
D1	2.00000000	sec
D11	0.03000000	sec
TD0	-	
SFO1	125.7703643	MHz
NUC1	13C	
P1	10.00	usec
PLW1	76.00000000	W
SFO2	500.1320005	MHz
NUC2	1H	
CPDPRG2	waltz16	
PCPD2	80.00	usec
PLW2	20.50000000	W
PLW2	0.32031000	W
PLW3	0.16111000	W

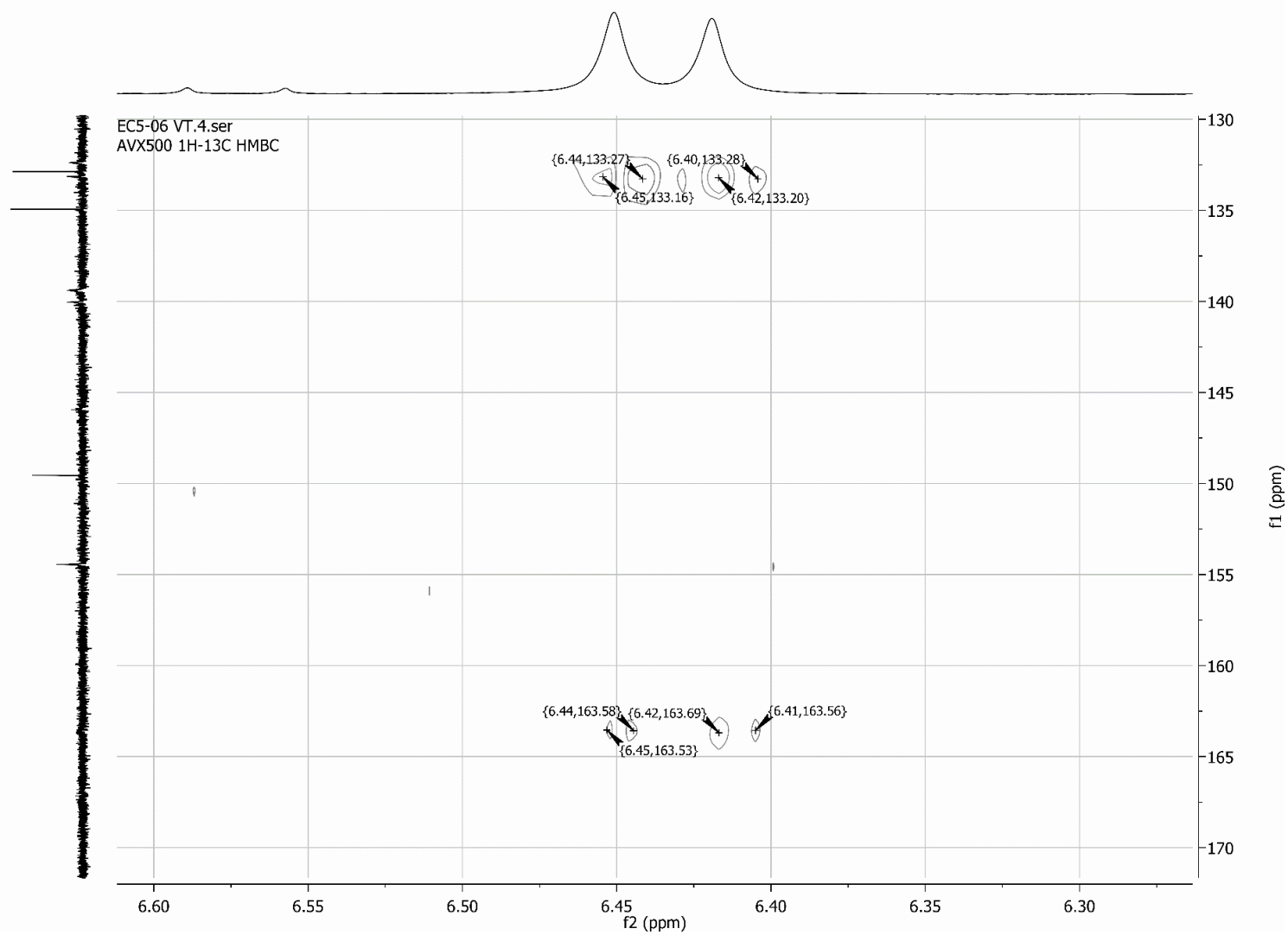
```
F2 - Processing parameters
SI              32768
SF              125.7577885 MHz
WDW              EM
SSB             0
LB              1.00 Hz
GB             0
PC              1.40
```



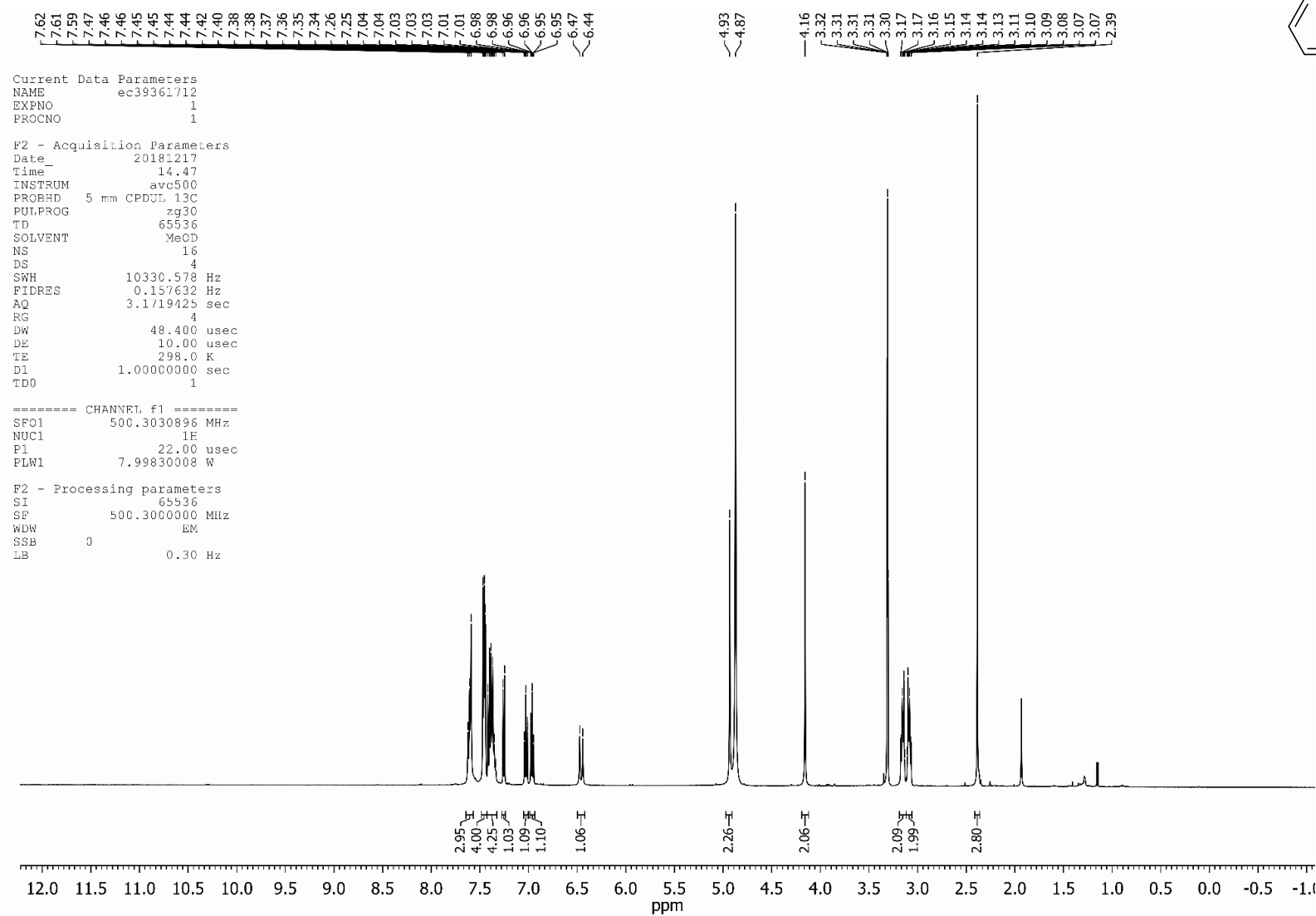
N,N-Di-Boc-*O*-((1-methyl-2-nitroimidazol-5-yl)methyl)-panobinostat



N,N-Di-Boc-*O*-((1-methyl-2-nitroimidazol-5-yl)methyl)-panobinostat



O-Benzyl-Panobinostat (Bn-Pano, **10**)



O-Benzyl-Panobinostat (Bn-Pano, **10**)

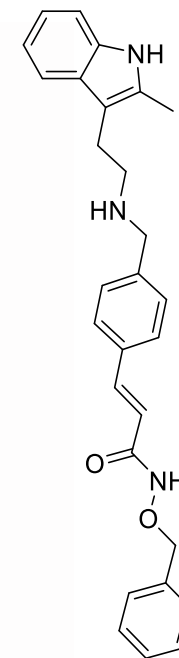
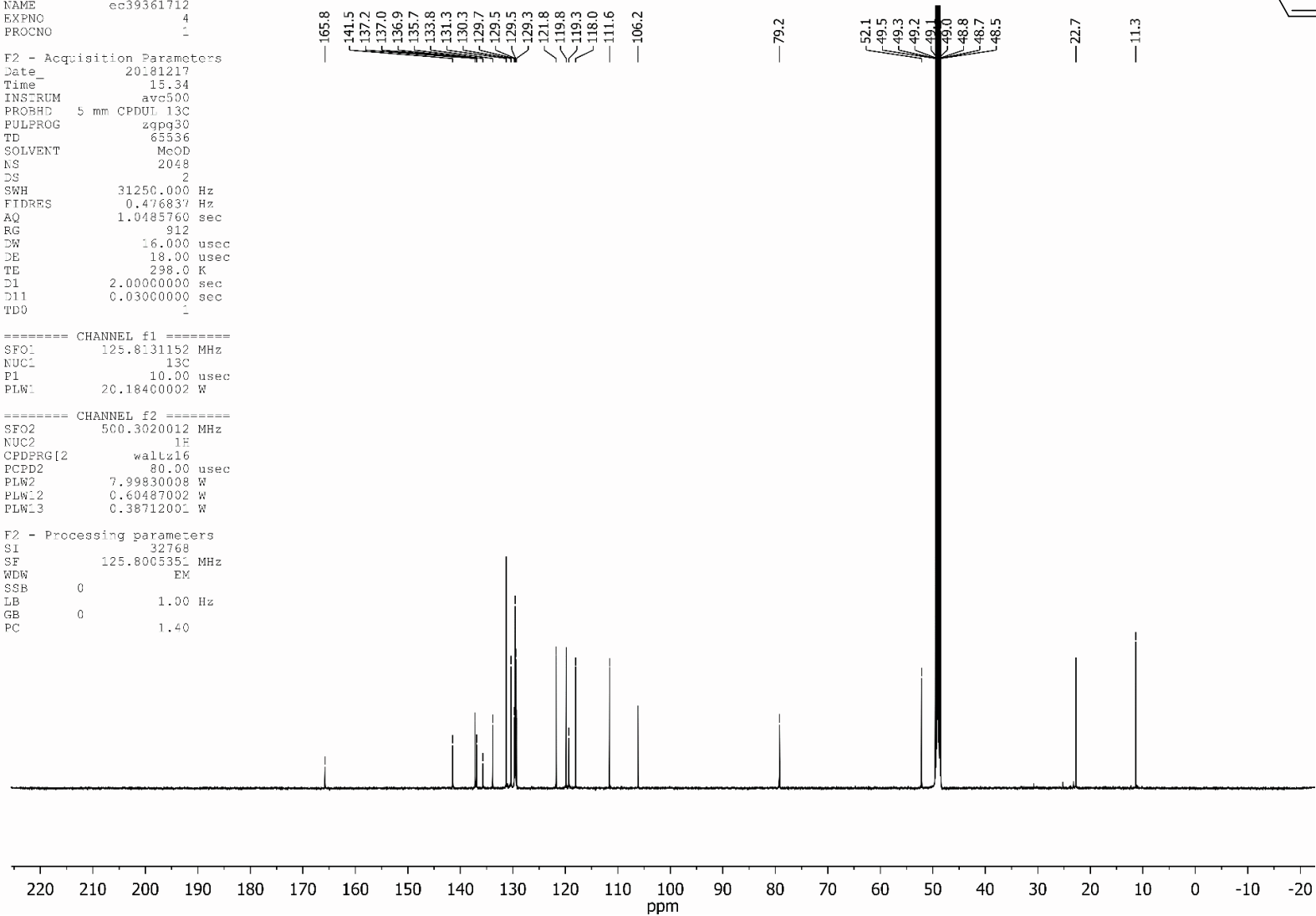
Current Data Parameters
NAME ec39361712
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20181217
Time_ 15.34
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 2048
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.000 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

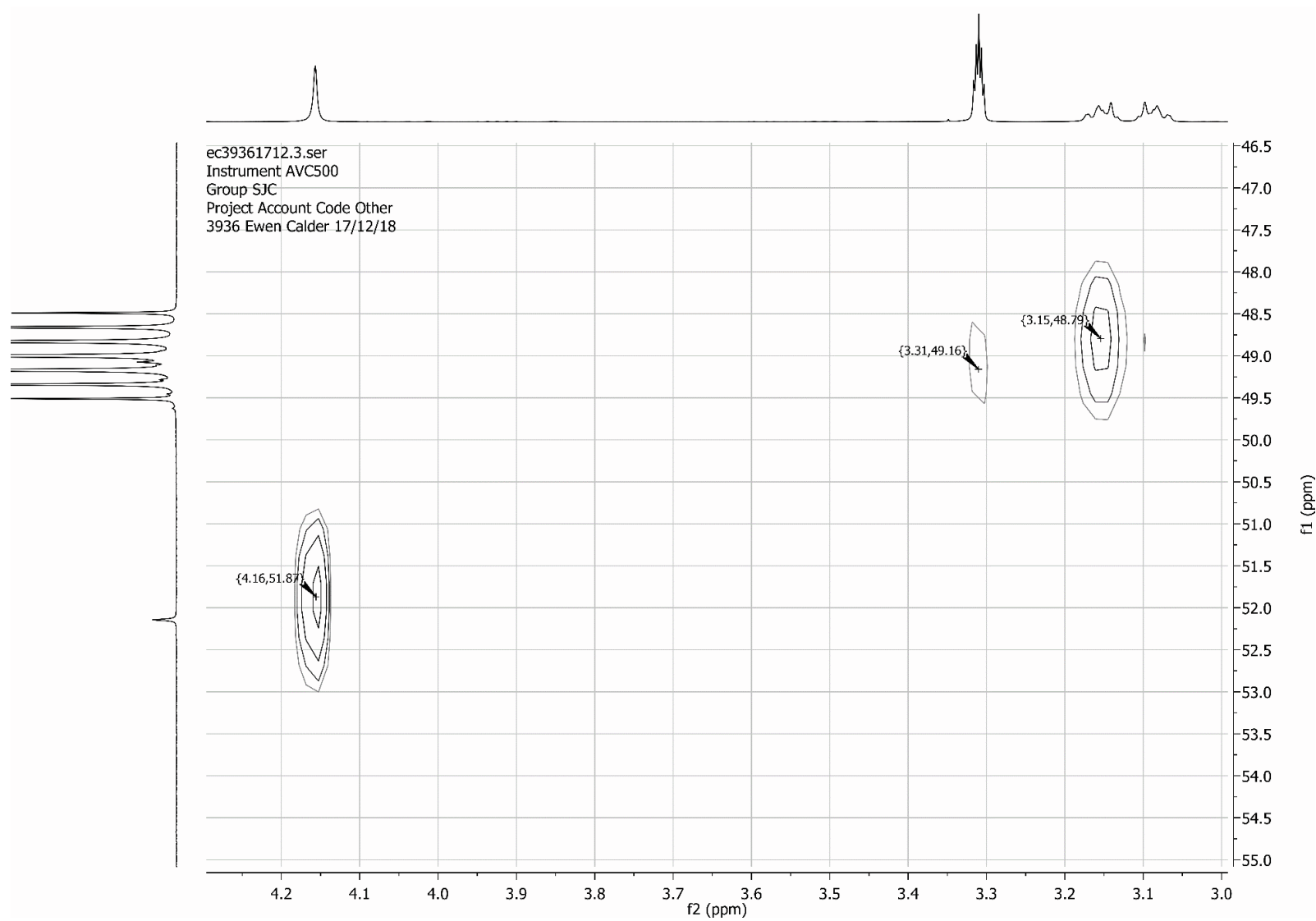
===== CHANNEL f1 =====
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W

===== CHANNEL f2 =====
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG12 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.60487002 W
PLW13 0.38712001 W

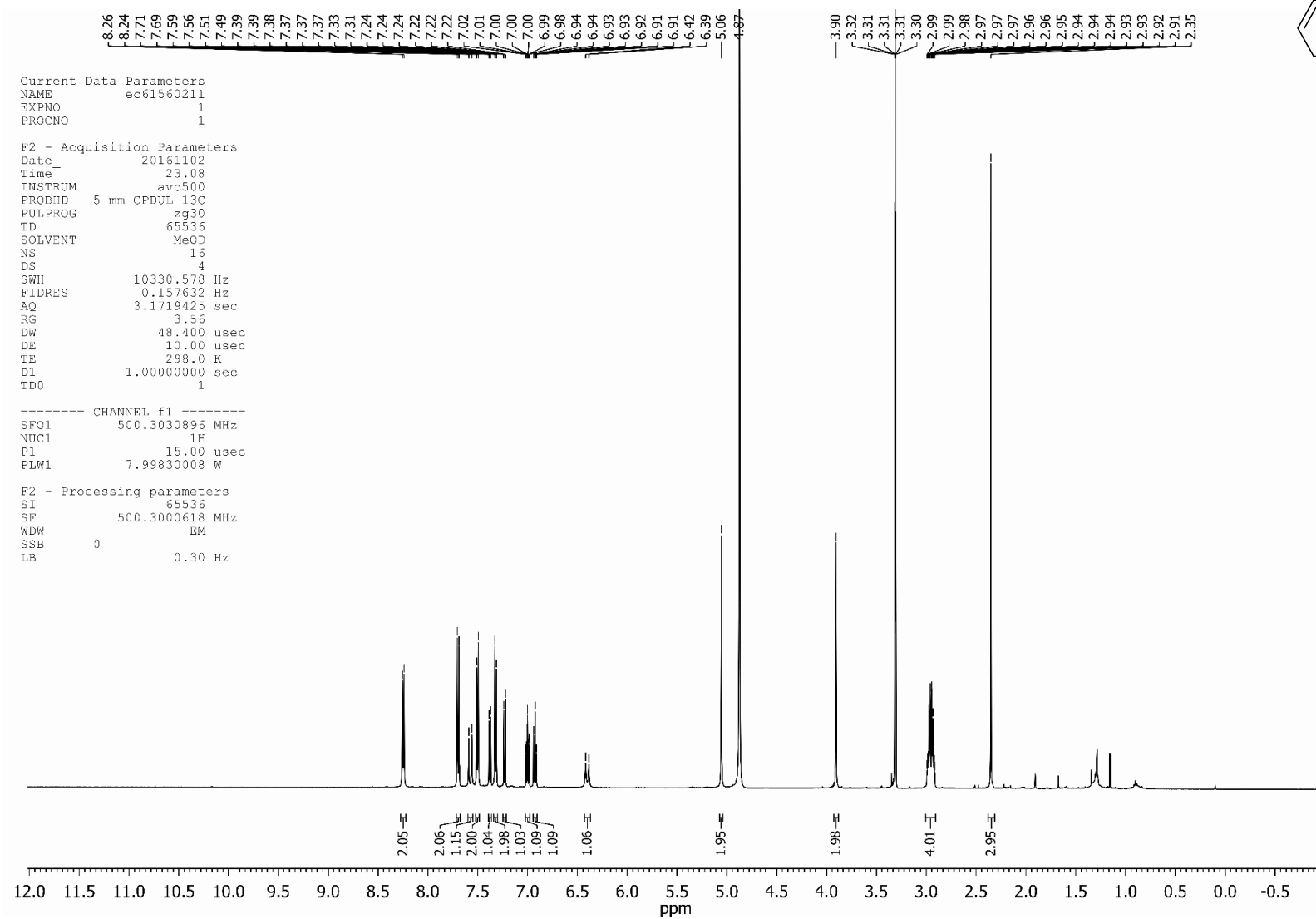
F2 - Processing parameters
SI 32768
SF 125.8005351 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



O-Benzyl-Panobinostat (Bn-Pano, **10**)



O-(4-Nitrobenzyl)-panobinostat trifluoroacetate (NB-Pano, **11**)



O-(4-Nitrobenzyl)-panobinostat trifluoroacetate (NB-Pano, **11**)

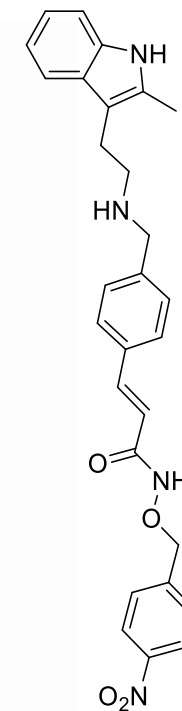
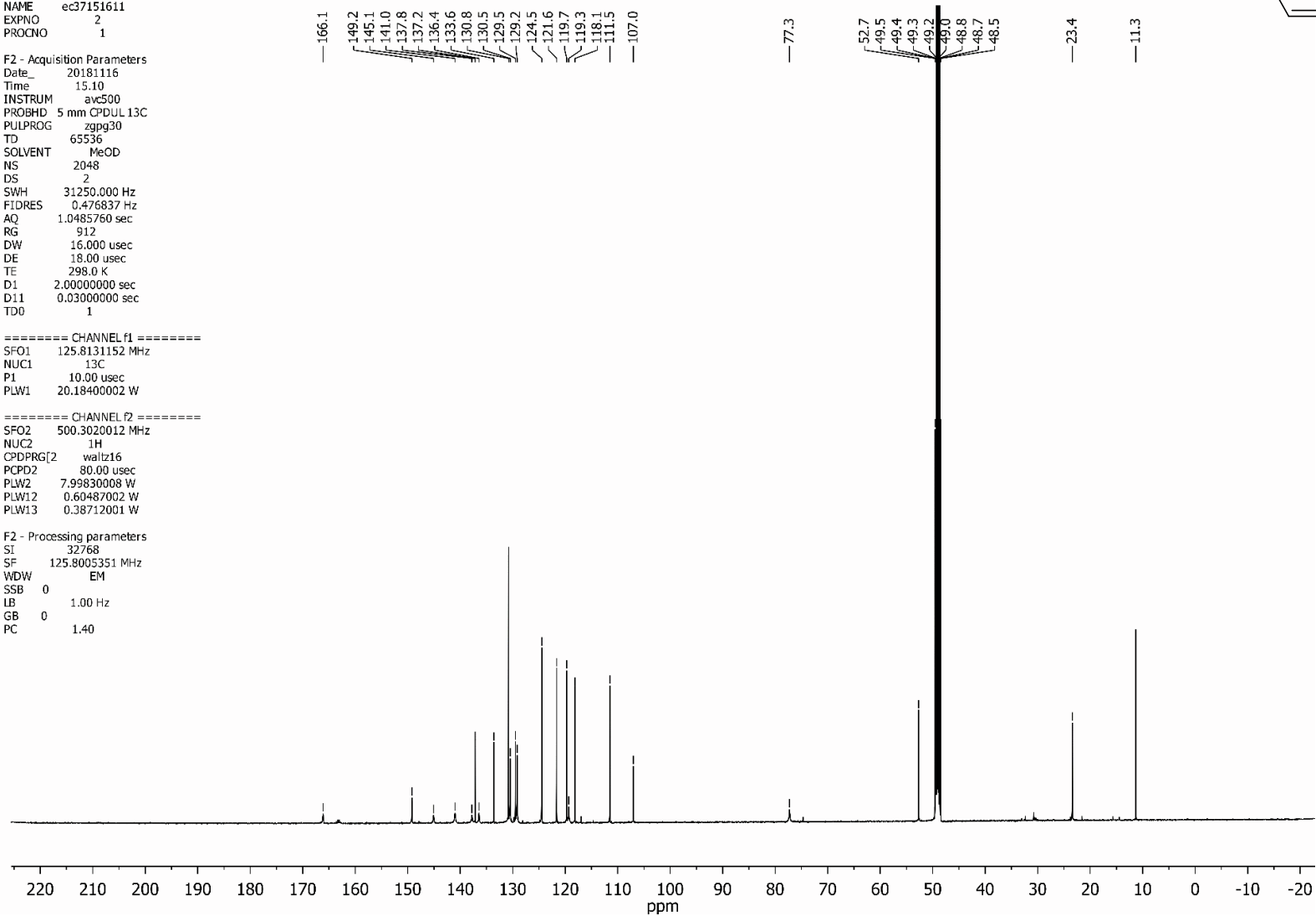
Current Data Parameters
 NAME ec37151611
 EXPNO 2
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20181116
 Time 15.10
 INSTRUM avc500
 PROBHD 5 mm CPDUL 13C
 PULPROG zgpg30
 TD 65536
 SOLVENT MeOD
 NS 2048
 DS 2
 SWH 31250.000 Hz
 FIDRES 0.476837 Hz
 AQ 1.0485760 sec
 RG 912
 DW 16.000 usec
 DE 18.00 usec
 TE 298.0 K
 D1 2.00000000 sec
 D11 0.03000000 sec
 TD0 1

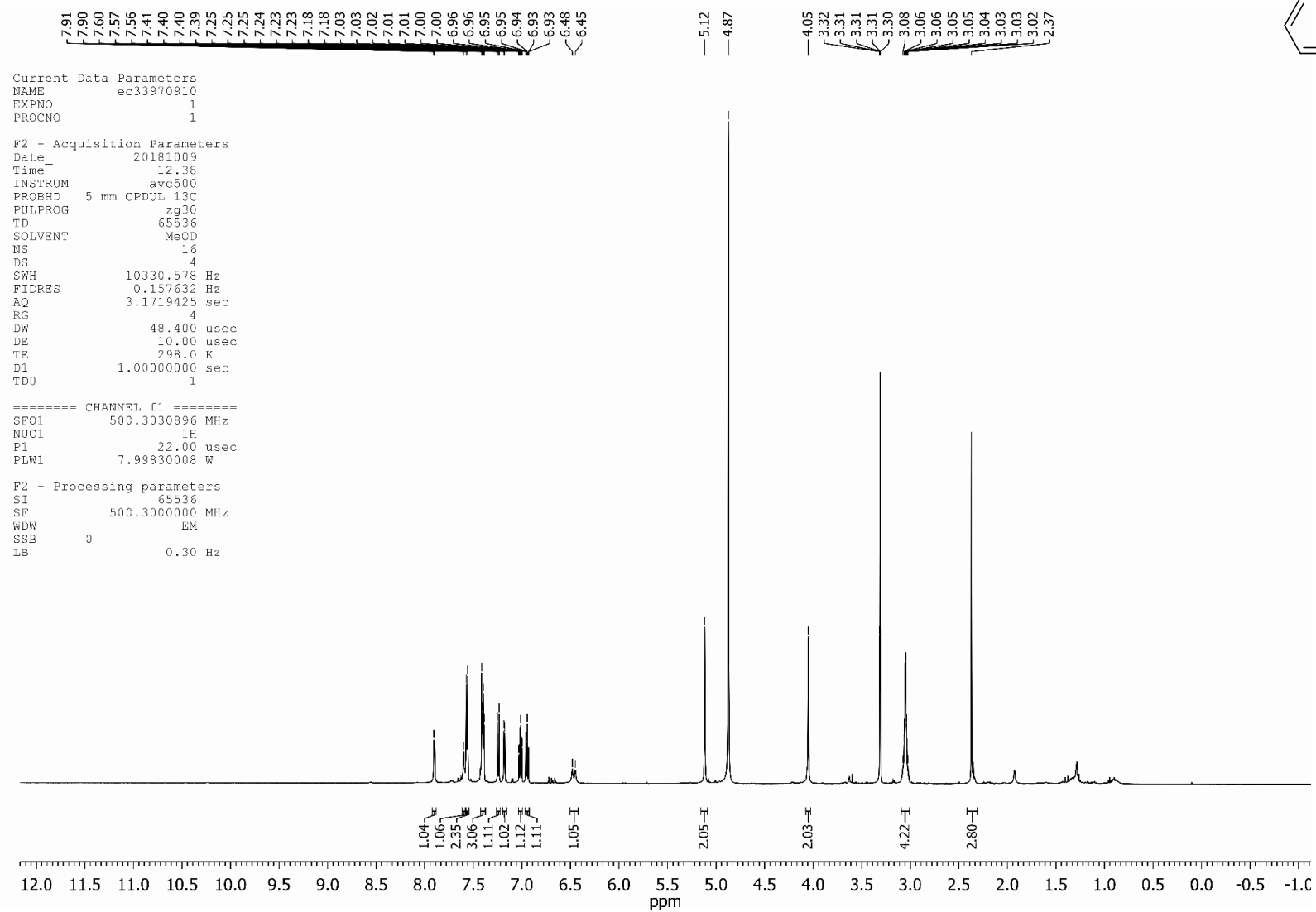
===== CHANNEL f1 =====
 SFO1 125.8131152 MHz
 NUC1 ¹³C
 P1 10.00 usec
 PLW1 20.18400002 W

===== CHANNEL f2 =====
 SFO2 500.3020012 MHz
 NUC2 ¹H
 CPDPRG2 waltz16
 PCPD2 80.00 usec
 PLW2 7.998300008 W
 PLW12 0.60487002 W
 PLW13 0.38712001 W

F2 - Processing parameters
 SI 32768
 SF 125.8005351 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40



O-(5-Nitrothiophen-2-yl)methyl)-panobinostat trifluoroacetate (NT-Pano, **12**)



O-(5-Nitrothiophen-2-yl)methyl)-panobinostat trifluoroacetate (NT-Pano, **12**)

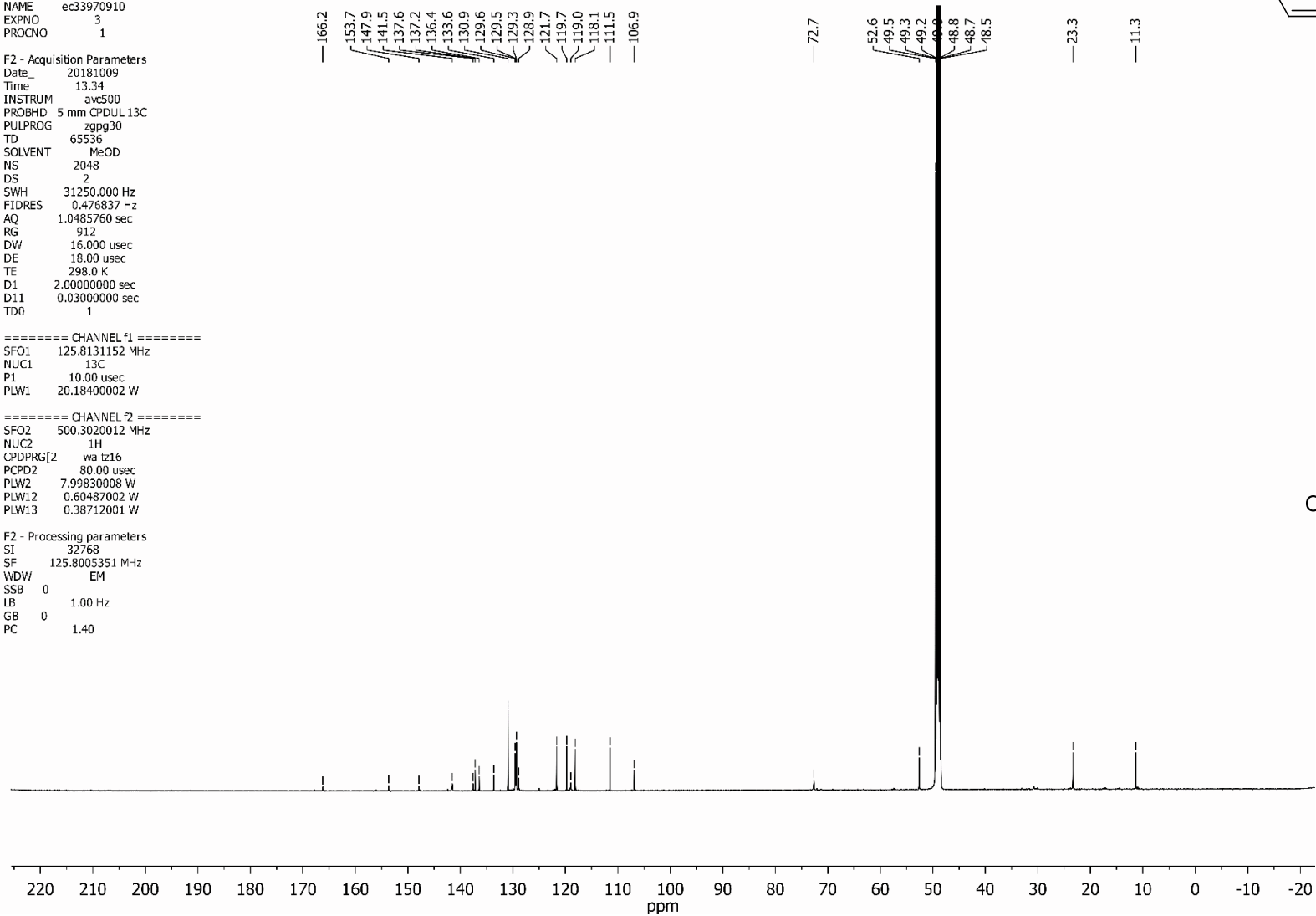
Current Data Parameters
NAME ec33970910
EXPNO 3
PROCNO 1

F2 - Acquisition Parameters
Date_ 20181009
Time 13.34
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 2048
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

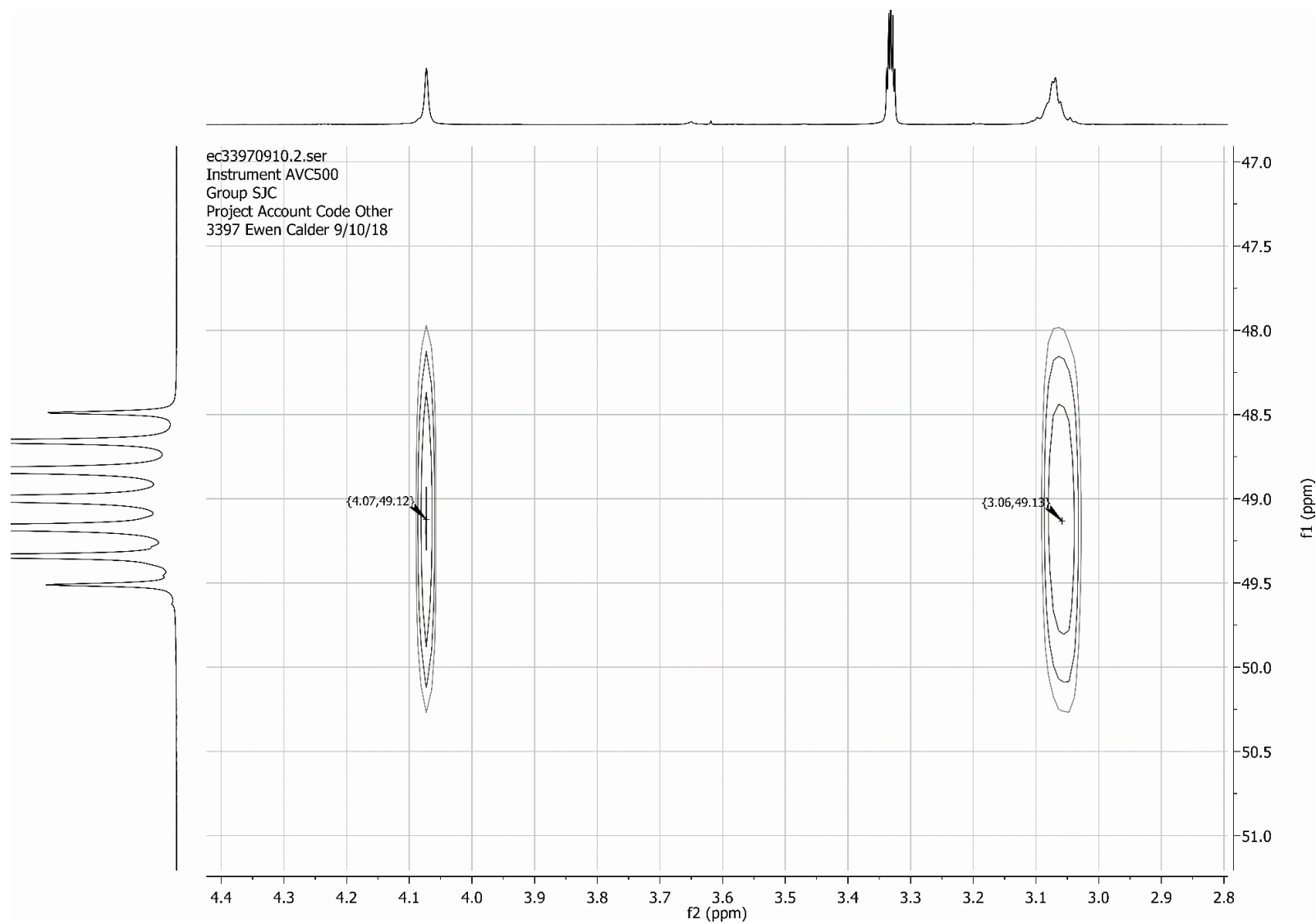
===== CHANNEL f1 =====
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W

===== CHANNEL f2 =====
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.998300008 W
PLW12 0.60487002 W
PLW13 0.38712001 W

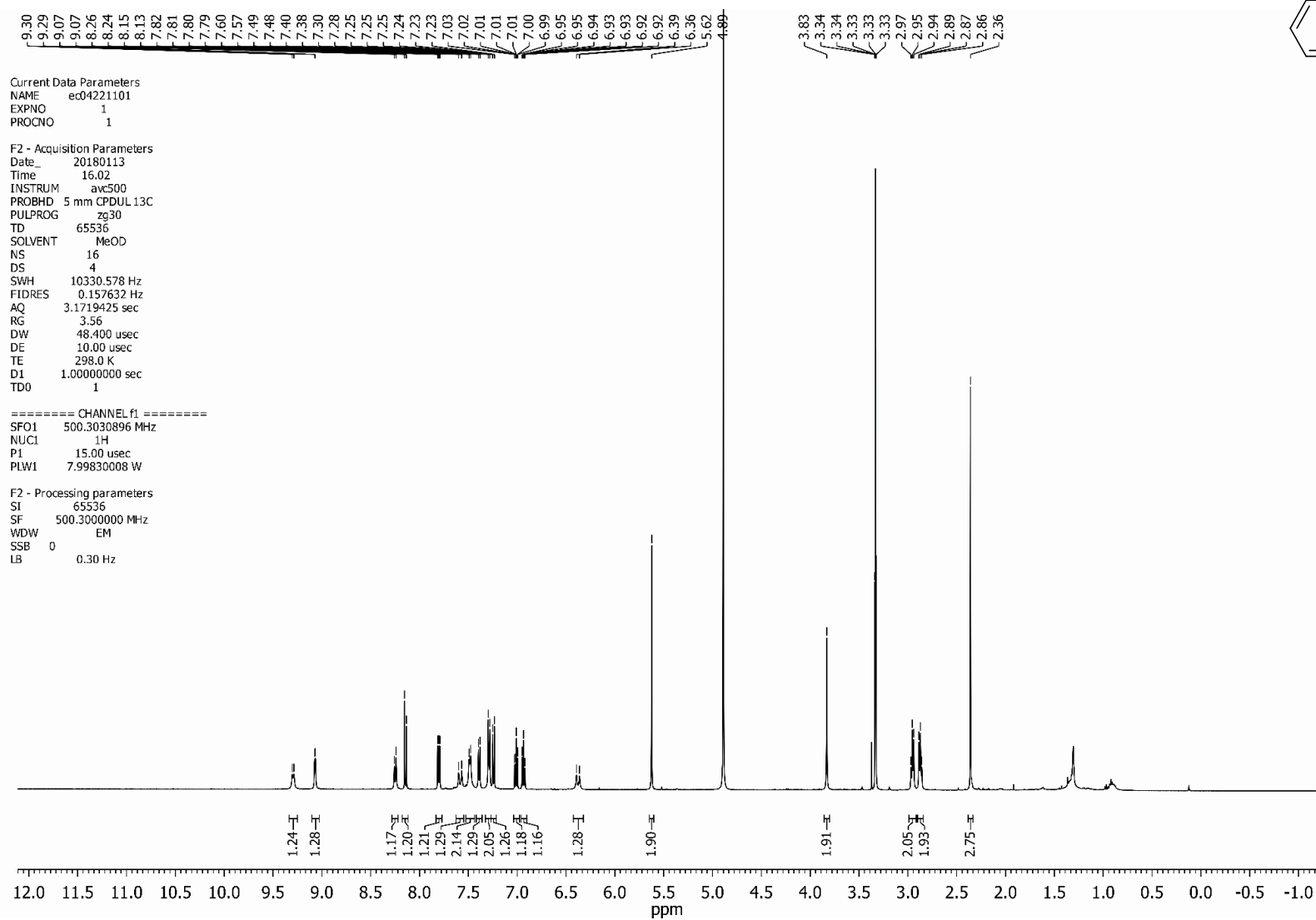
F2 - Processing parameters
SI 32768
SF 125.8005351 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



O-(5-Nitrothiophen-2-yl)methyl)-panobinostat trifluoroacetate (NT-Pano, **12**)



O-(6-Nitroquinolin-5-yl)methyl)-panobinostat di-trifluoroacetate (NQ-Pano, **13**)



O-(6-Nitroquinolin-5-yl)methyl)-panobinostat di-trifluoroacetate (NQ-Pano, **13**)

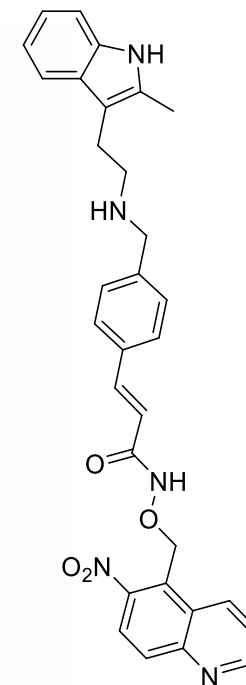
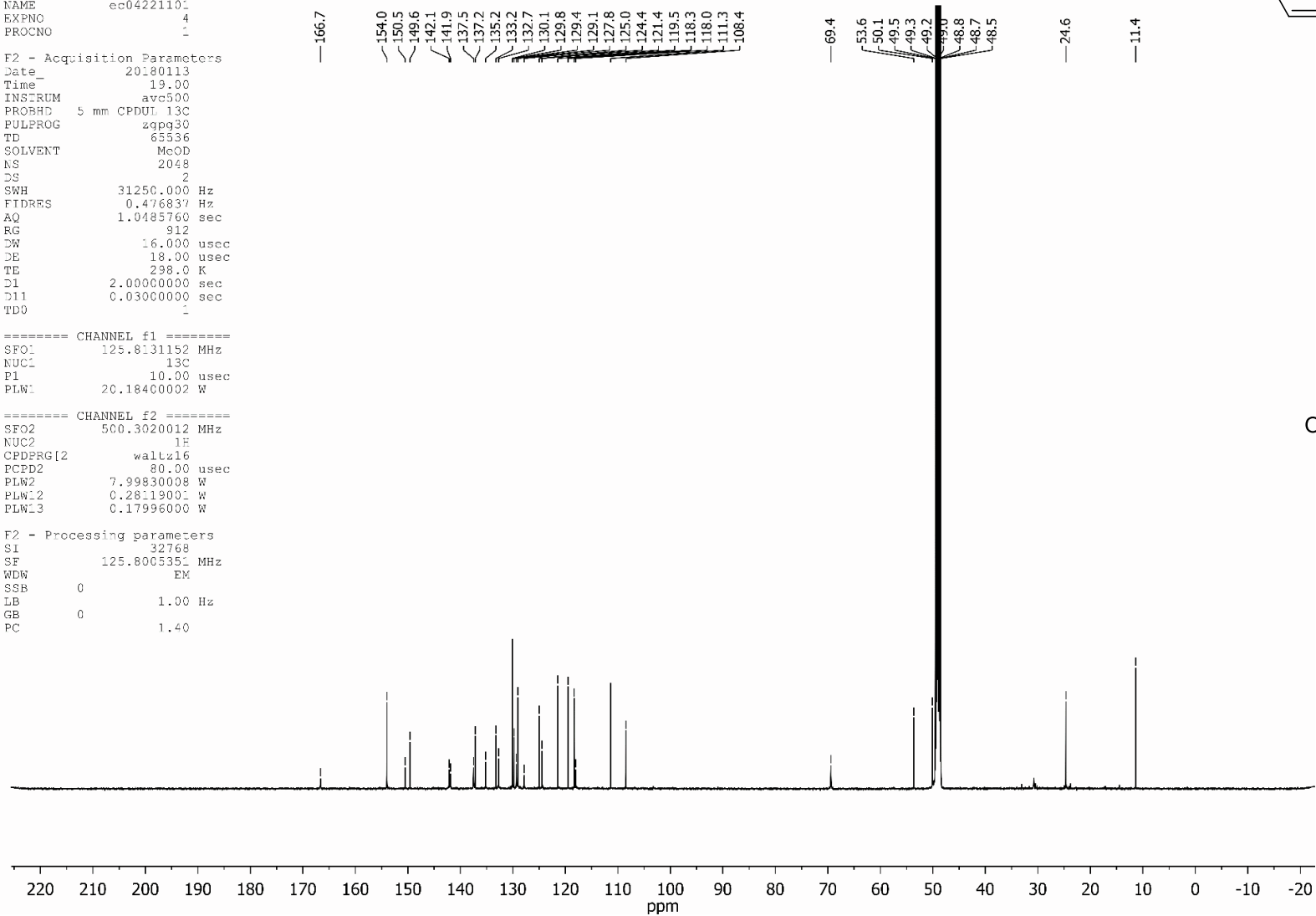
Current Data Parameters
NAME ec04221101
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180113
Time_ 19.00
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 2048
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.000 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 -

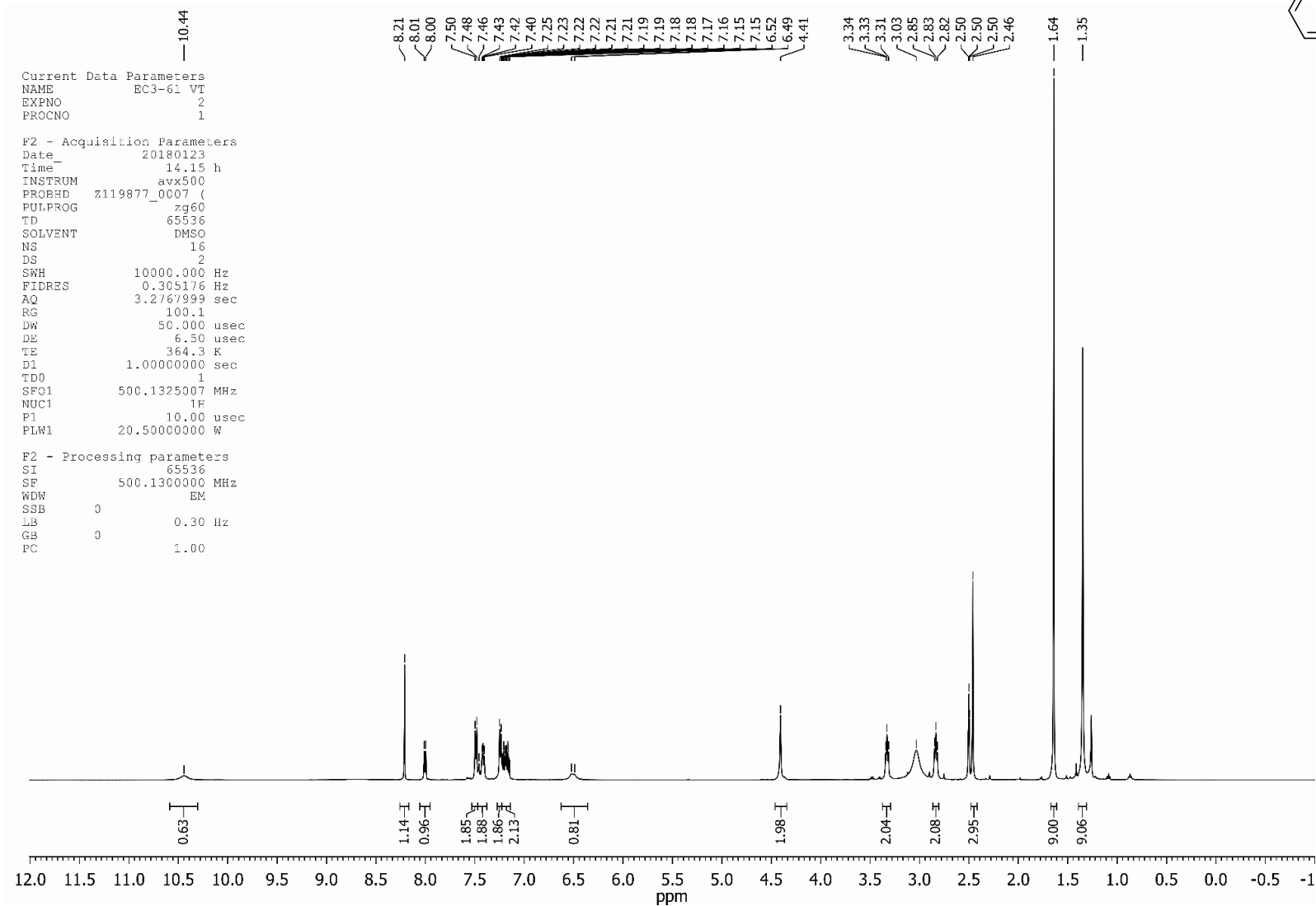
===== CHANNEL f1 =====
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W

===== CHANNEL f2 =====
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.28119001 W
PLW13 0.17996000 W

F2 - Processing parameters
SI 32768
SF 125.8005351 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



N,N-Di-Boc-Panobinostat (**14**)

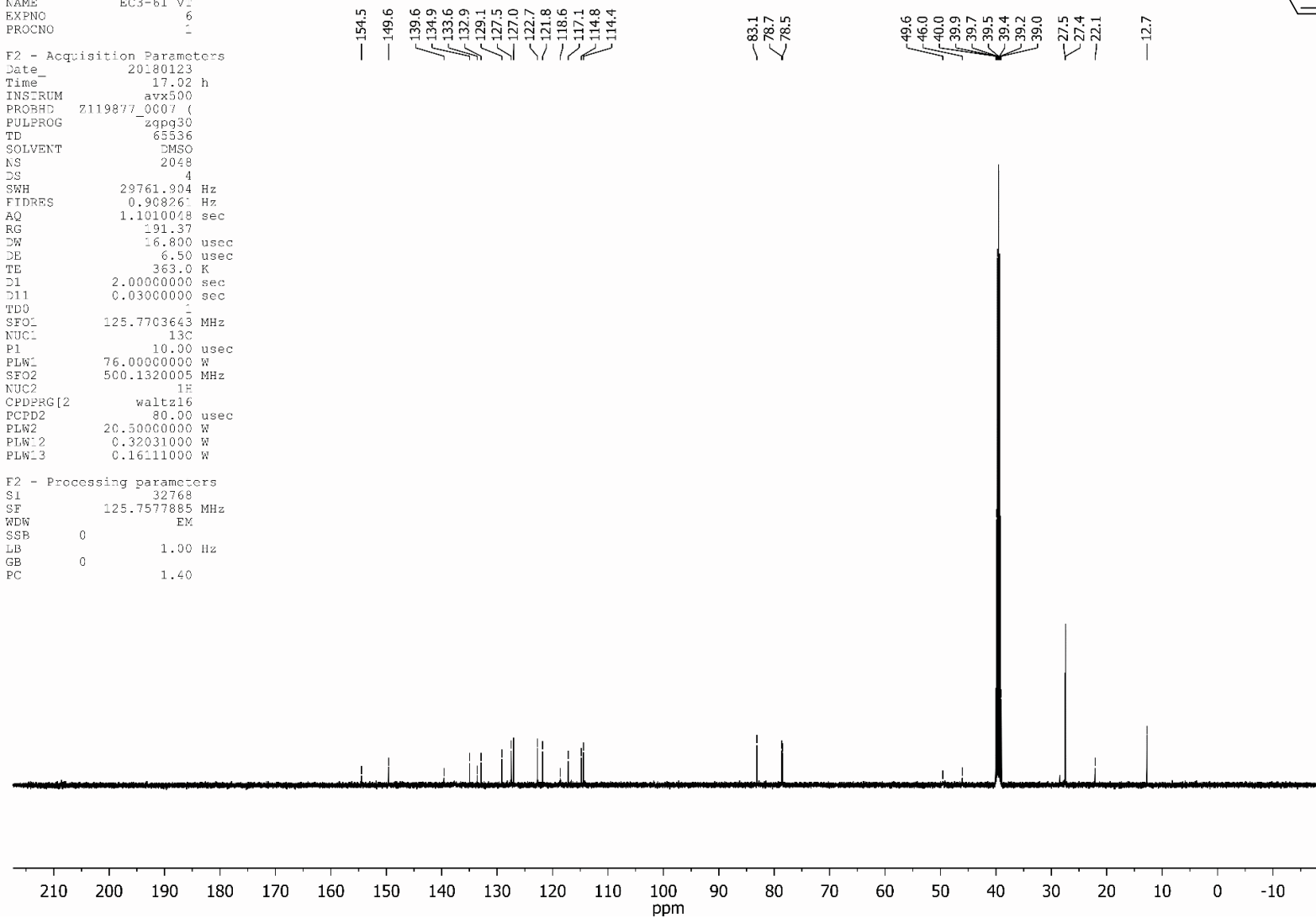


N,N-Di-Boc-Panobinostat (**14**)

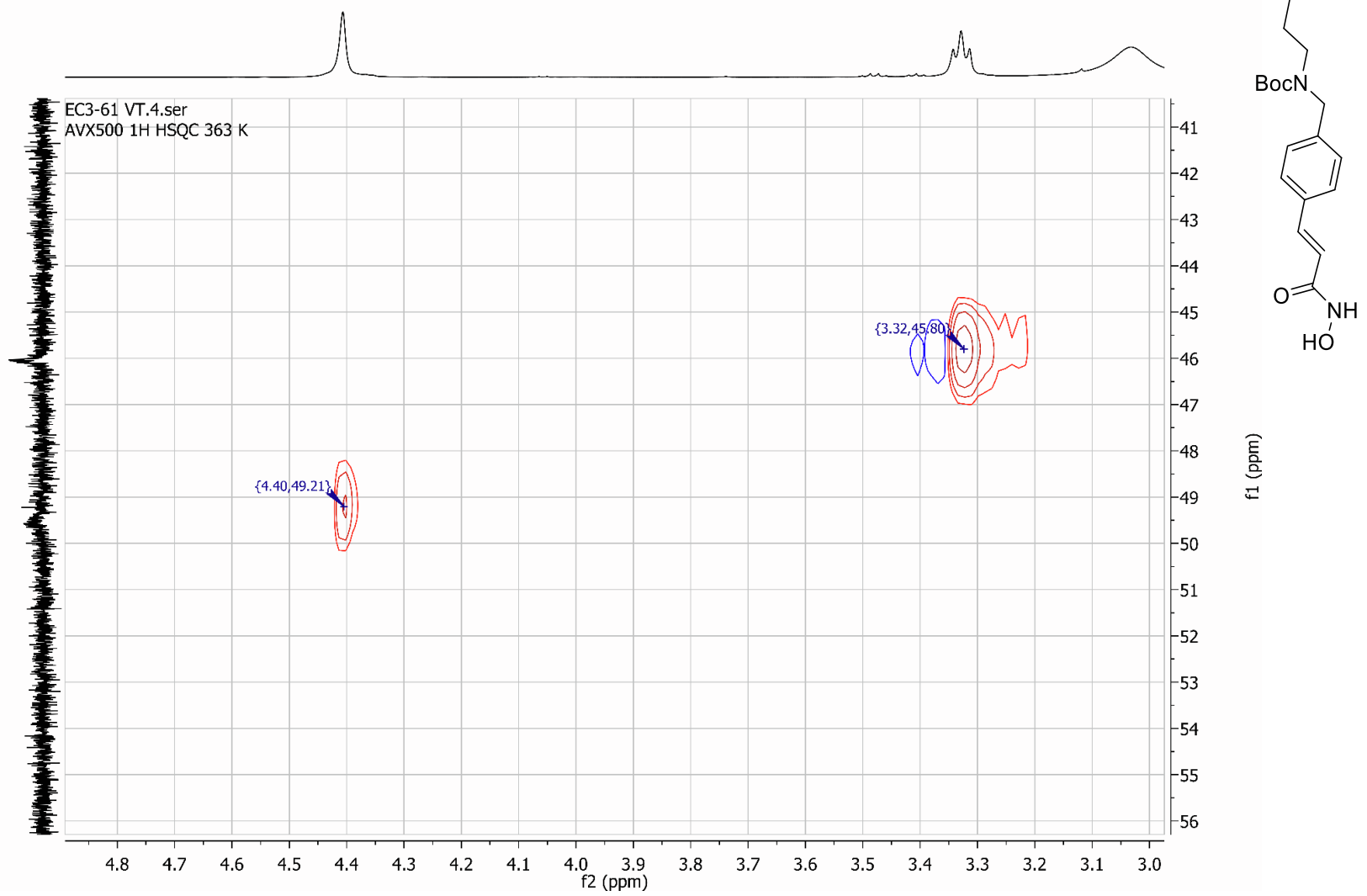
Current Data Parameters
 NAME EC3-61 V
 EXPNO 6
 PROCNO 1

F2 - Acquisition Parameters
 Date_ 20180123
 Time_ 17.02 h
 INSTRUM avx500
 PROBHD Z119877_0007 (
 PULPROG zgpg30
 TD 65536
 SOLVENT DMSO
 NS 2048
 DS 4
 SWH 29761.904 Hz
 FIDRES 0.908261 Hz
 AQ 1.1010048 sec
 RG 191.37
 DW 16.800 usec
 DE 6.50 usec
 TE 363.0 K
 D1 2.00000000 sec
 D11 0.03000000 sec
 TD0 -
 SFO1 125.7703643 MHz
 NUC1 13C
 P1 10.00 usec
 PLW1 76.00000000 W
 SFO2 500.1320005 MHz
 NUC2 1H
 CPDPRG[2] waltz16
 PCPD2 80.00 usec
 PLW2 20.50000000 W
 PLW12 0.32031000 W
 PLW13 0.1611000 W

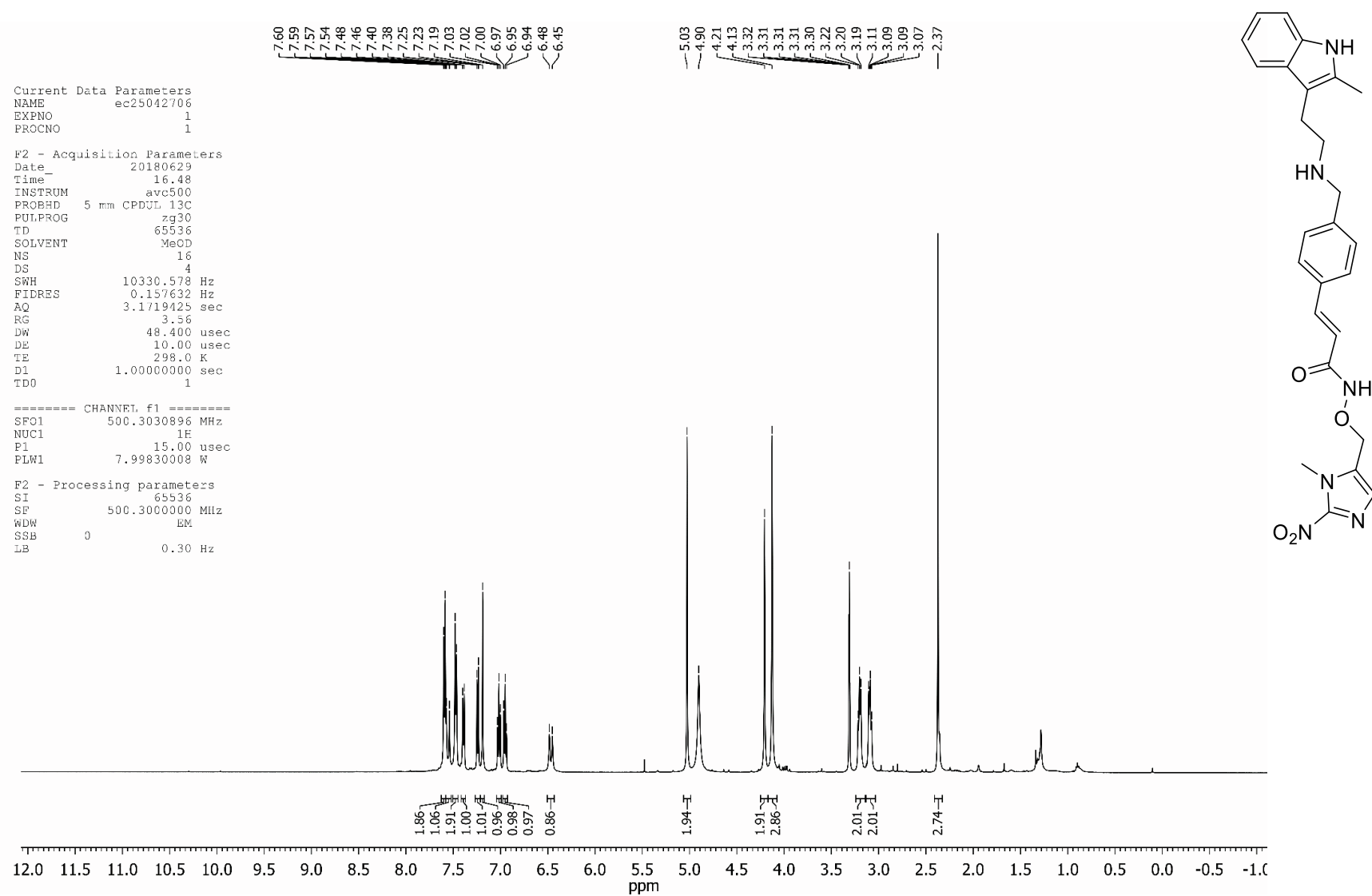
F2 - Processing parameters
 SI 32768
 SF 125.7577885 MHz
 WDW EM
 SSB 0
 LB 1.00 Hz
 GB 0
 PC 1.40



N,N-Di-Boc-Panobinostat (**14**)



O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, 1)



O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, 1)

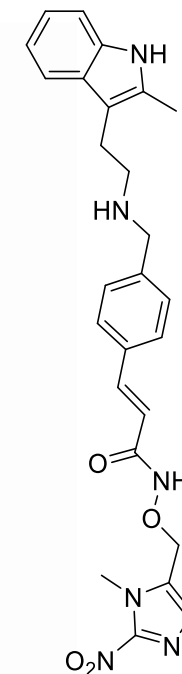
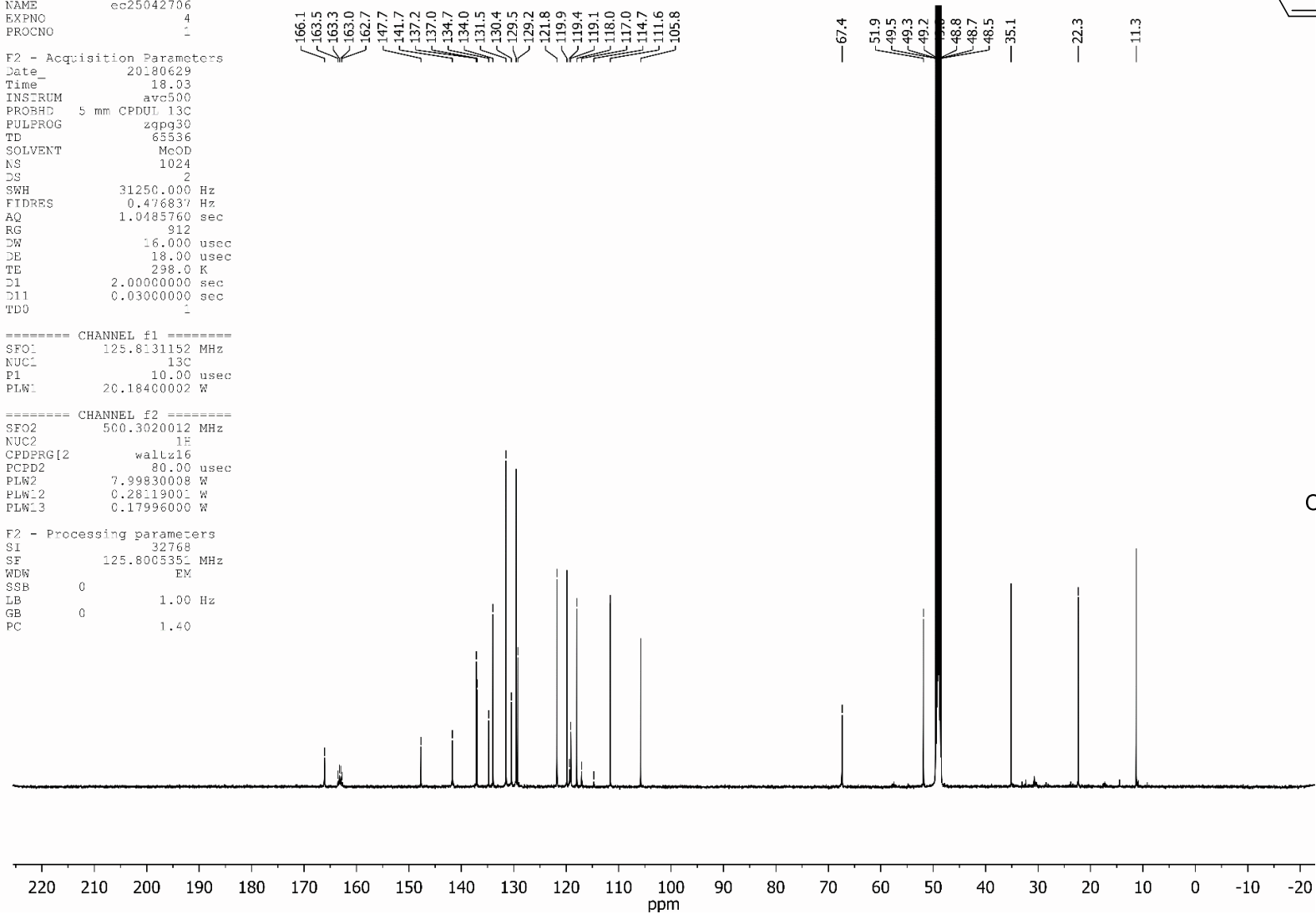
Current Data Parameters
NAME ec25042706
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20180629
Time_ 18.03
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 1024
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 -

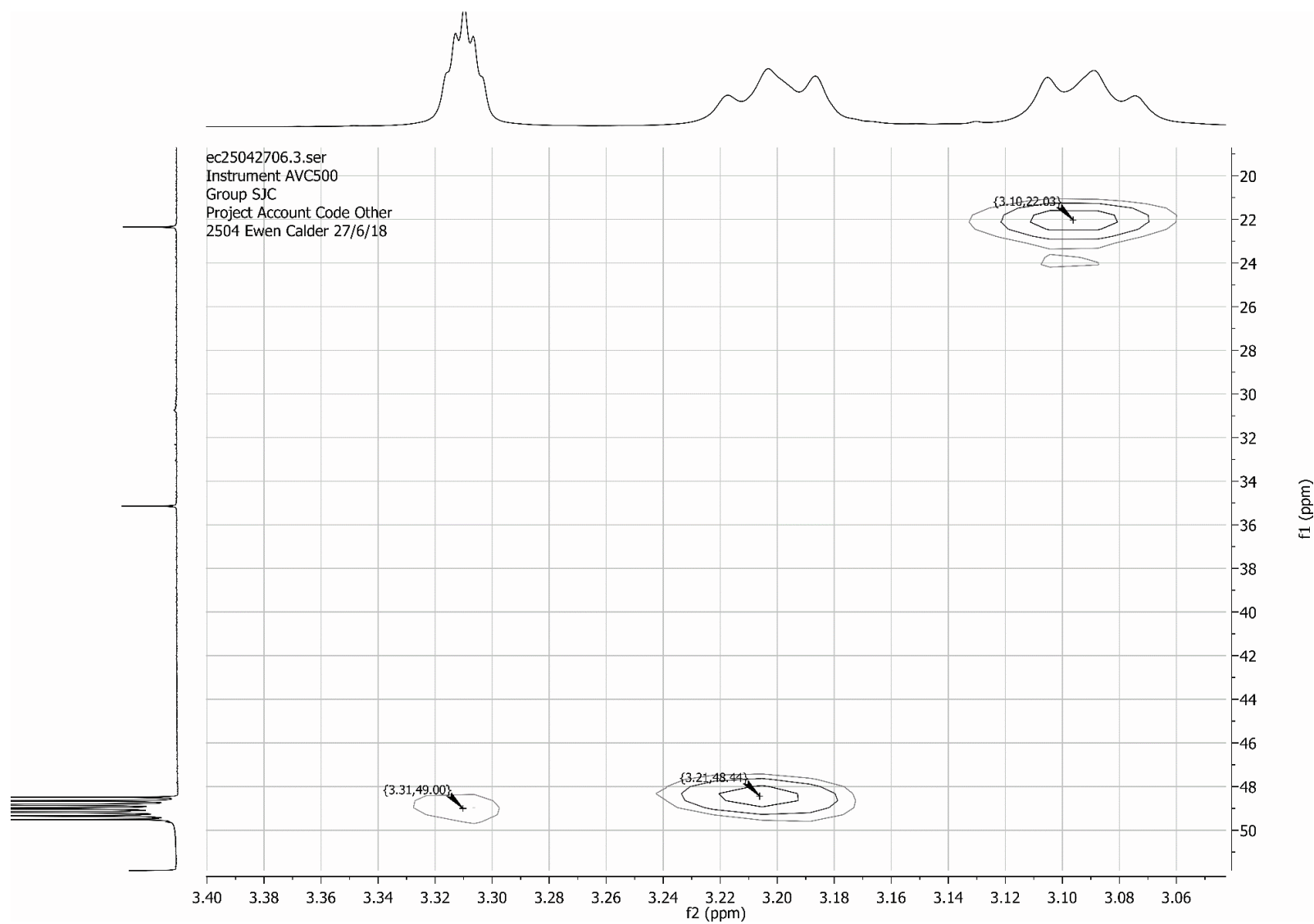
===== CHANNEL f1 =====
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W

===== CHANNEL f2 =====
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.28119001 W
PLW13 0.17996000 W

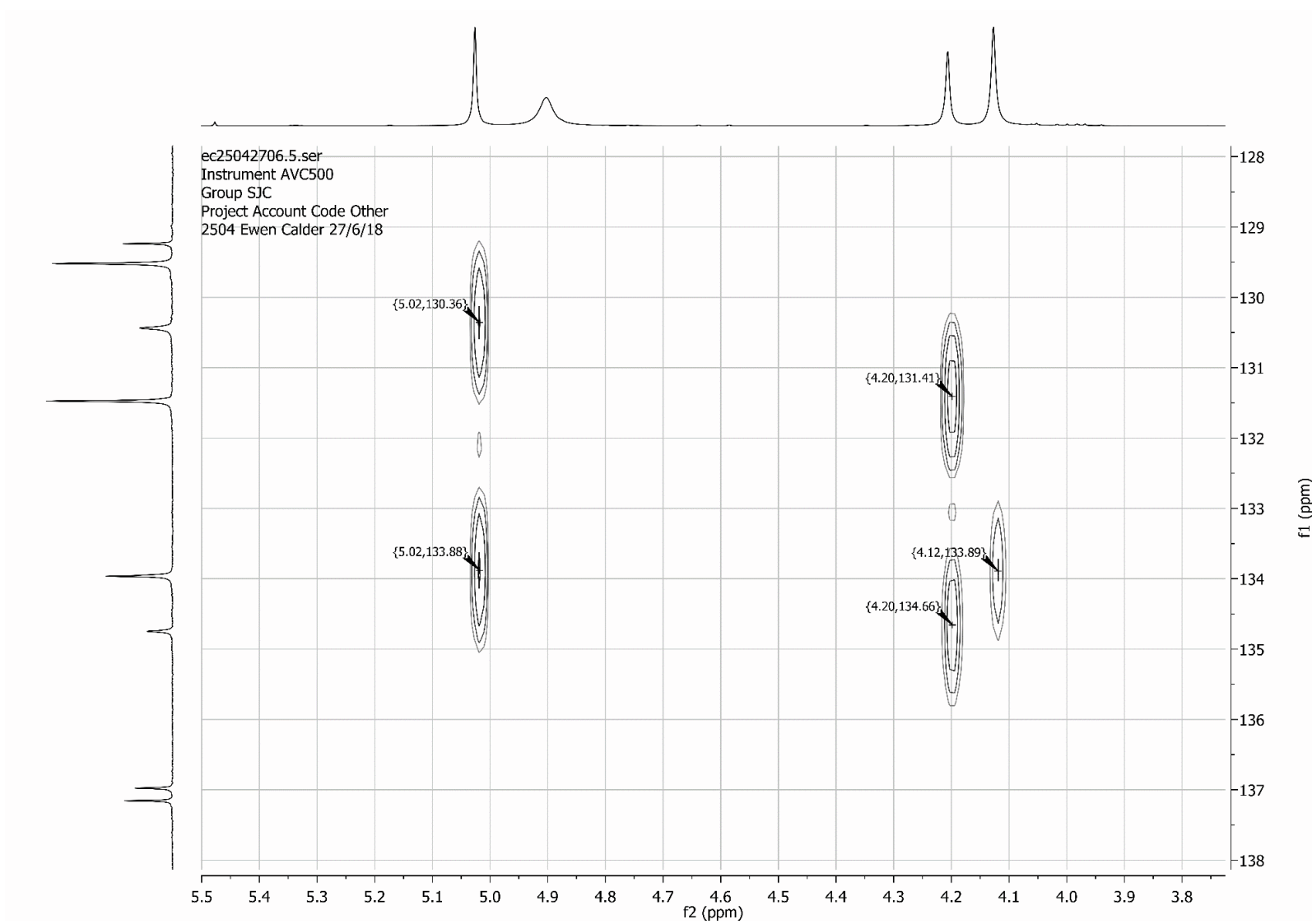
F2 - Processing parameters
SI 32768
SF 125.8005351 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



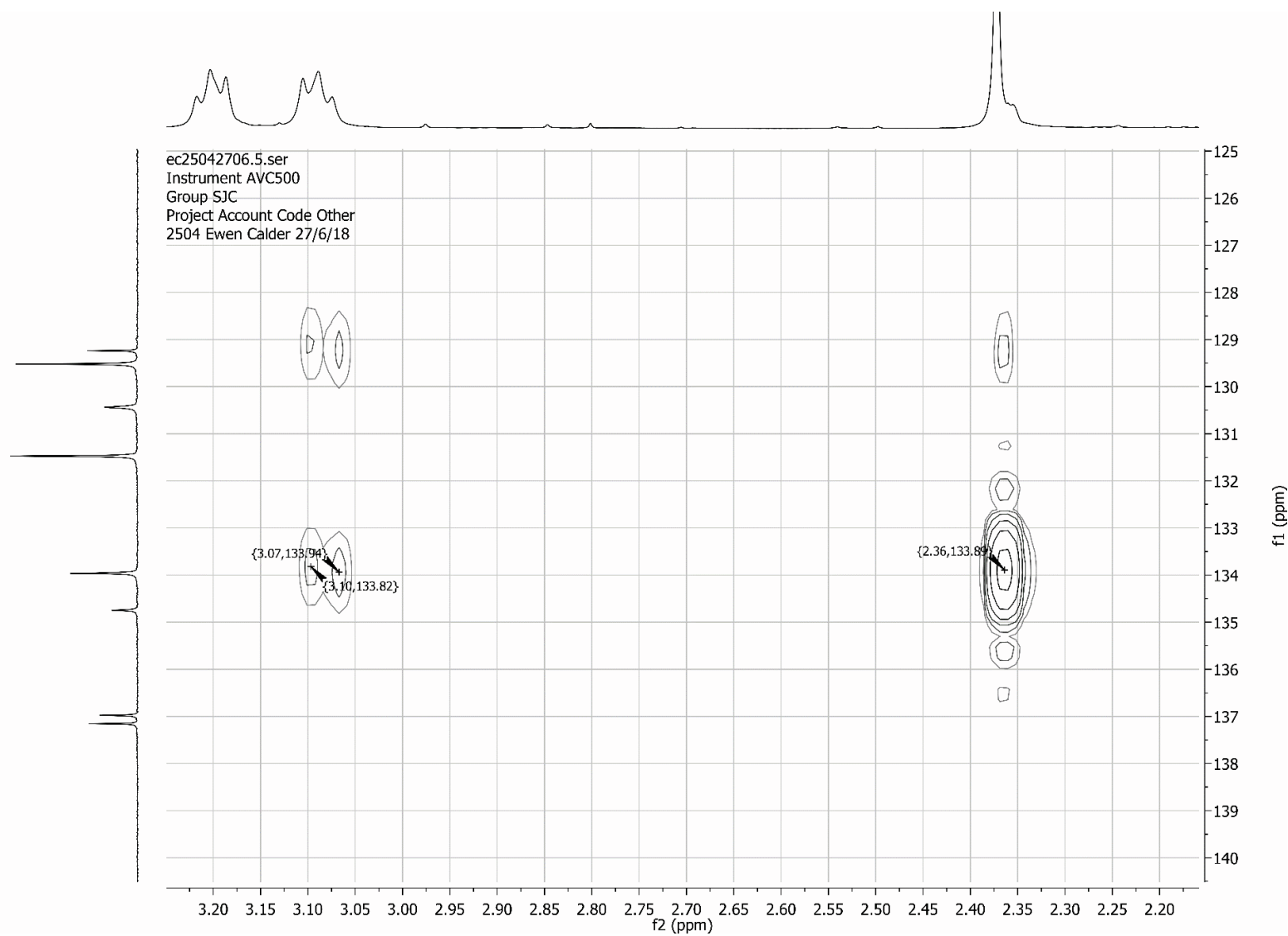
O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, 1)



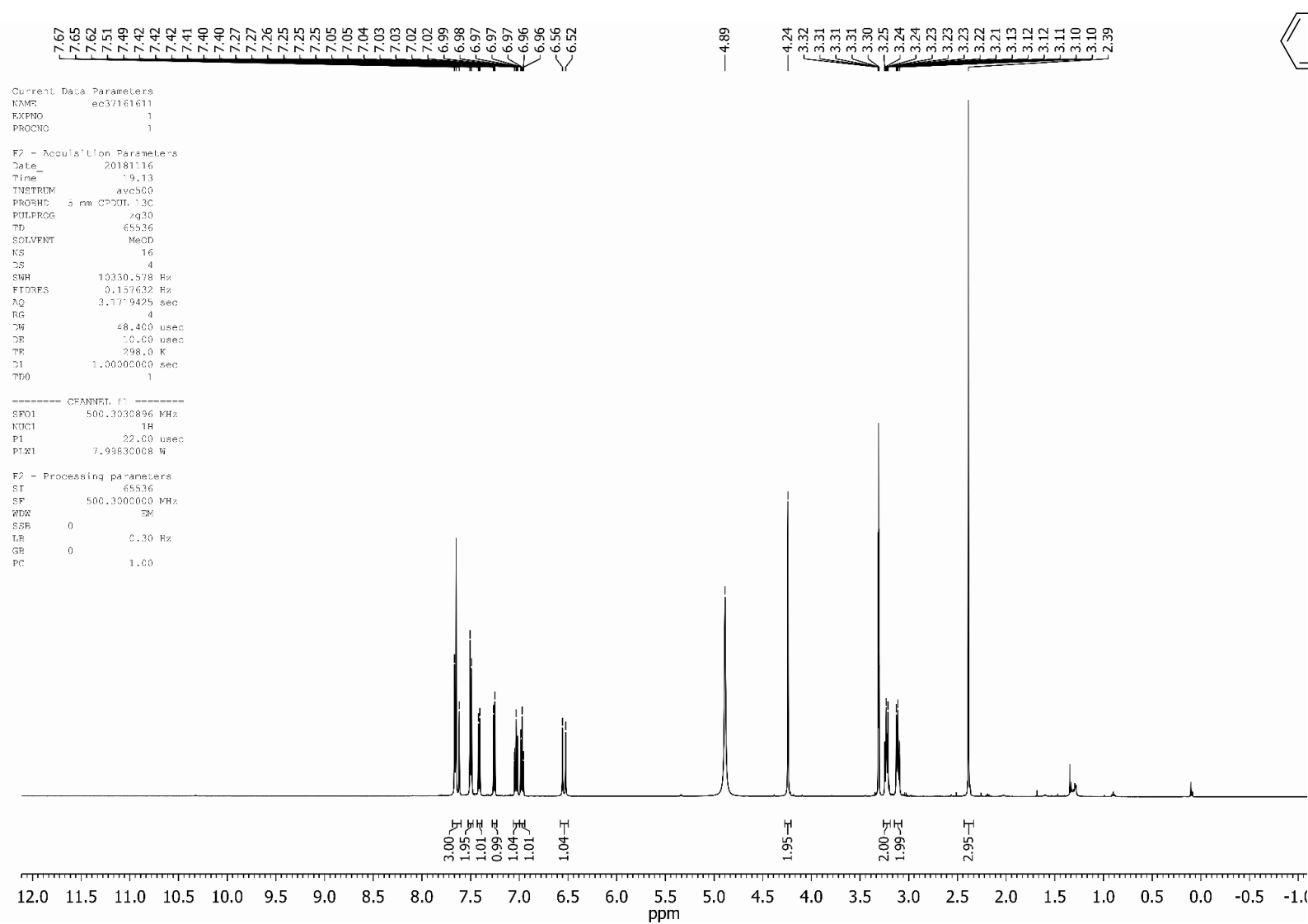
O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, **1**)



O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, 1)



(E)-3-(4-([2-(2-methyl-1H-indol-3-yl)ethylamino)methyl]phenyl)prop-2-enoic acid trifluoroacetate (**S18**)



(E)-3-(4-([2-(2-methyl-1H-indol-3-yl)ethylamino]methyl)phenyl)prop-2-enoic acid trifluoroacetate (**S18**)

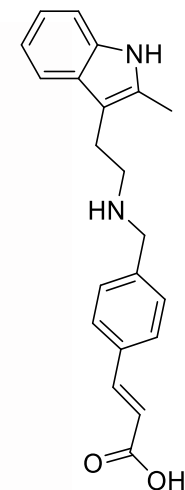
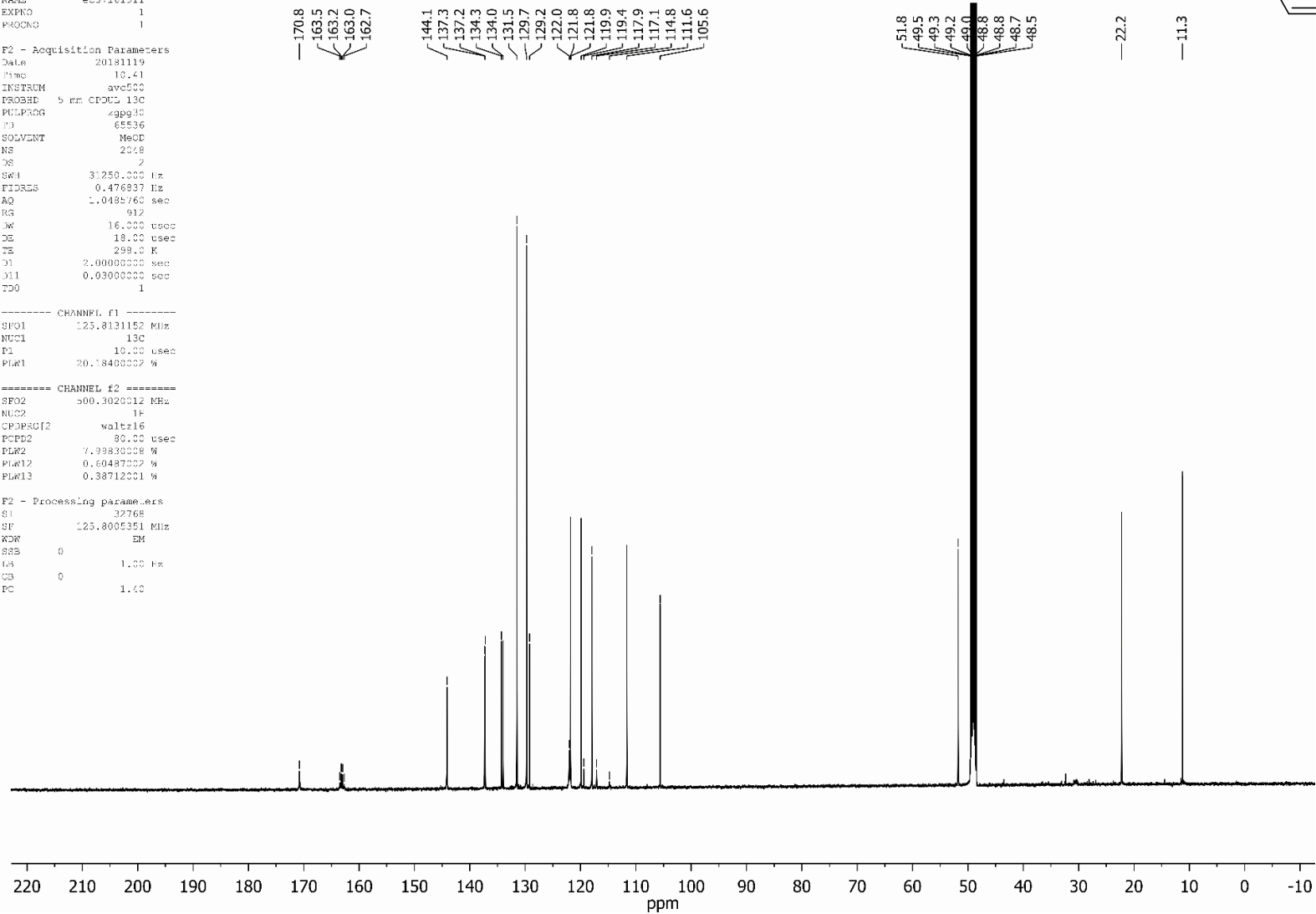
Current Data Parameters
NAME ec37161911
EXPRO 1
PROCNO 1

F2 - Acquisition Parameters
Date 20181119
Time 10.41
INSTRUM avc500
PROBHD 5 mm CPDCL 13C
PULPROG zgpg30
TD 65536
SOLVENT MeOD
NS 2248
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
SW 16.000 usec
DE 18.00 usec
TE 298.3 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

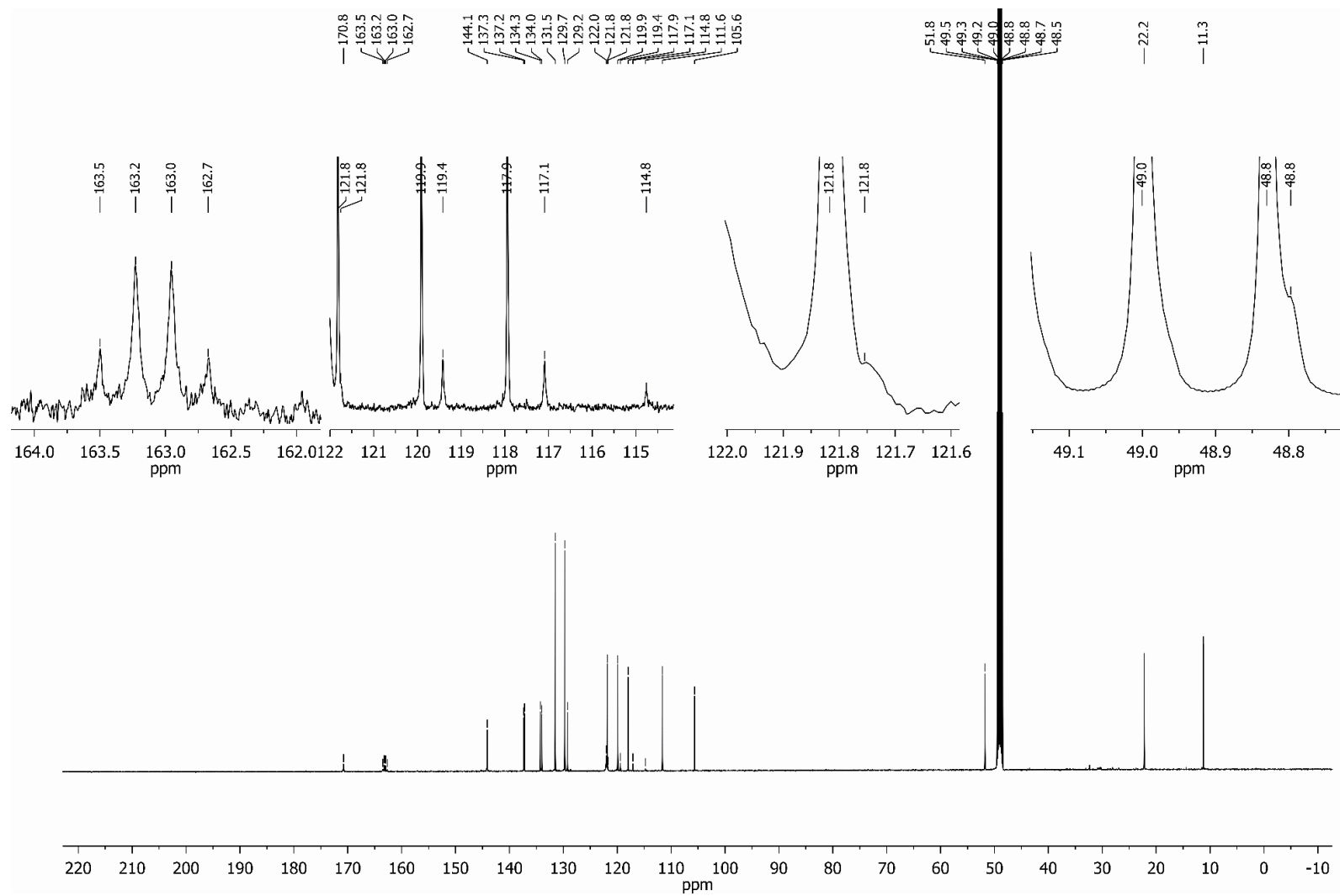
----- CHANNEL f1 -----
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W

===== CHANNEL f2 =====
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99830000 W
PLW12 0.60487002 W
PLW13 0.38712001 W

F2 - Processing parameters
SI 32768
SF 125.8005351 MHz
WDW EM
SSB 0
LA 1.00 Hz
GB 0
PC 1.40



(*E*)-3-(4-([2-(2-methyl-1*H*-indol-3-yl)ethylamino]methyl)phenyl)prop-2-enoic acid trifluoroacetate (**S18**)



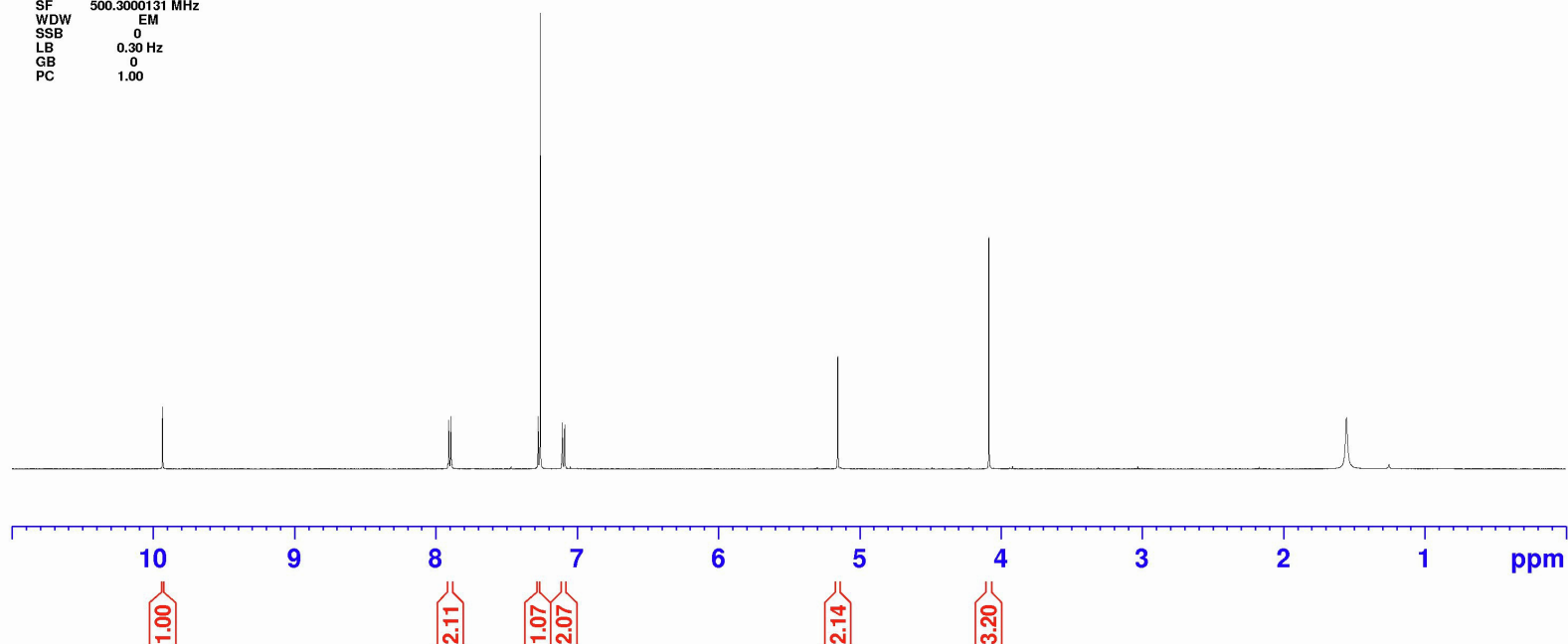
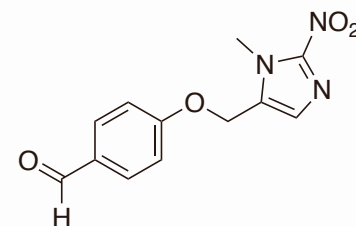
4-((1-Methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)benzaldehyde

Current Data Parameters
NAME mo604701501 MLOx120
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210117
Time 9.11
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1

===== CHANNEL f1 =====
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99630008 W

F2 - Processing parameters
SI 65536
SF 500.3000131 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



4-((1-Methyl-2-nitro-1*H*-imidazol-5-yl)methoxy)benzaldehyde

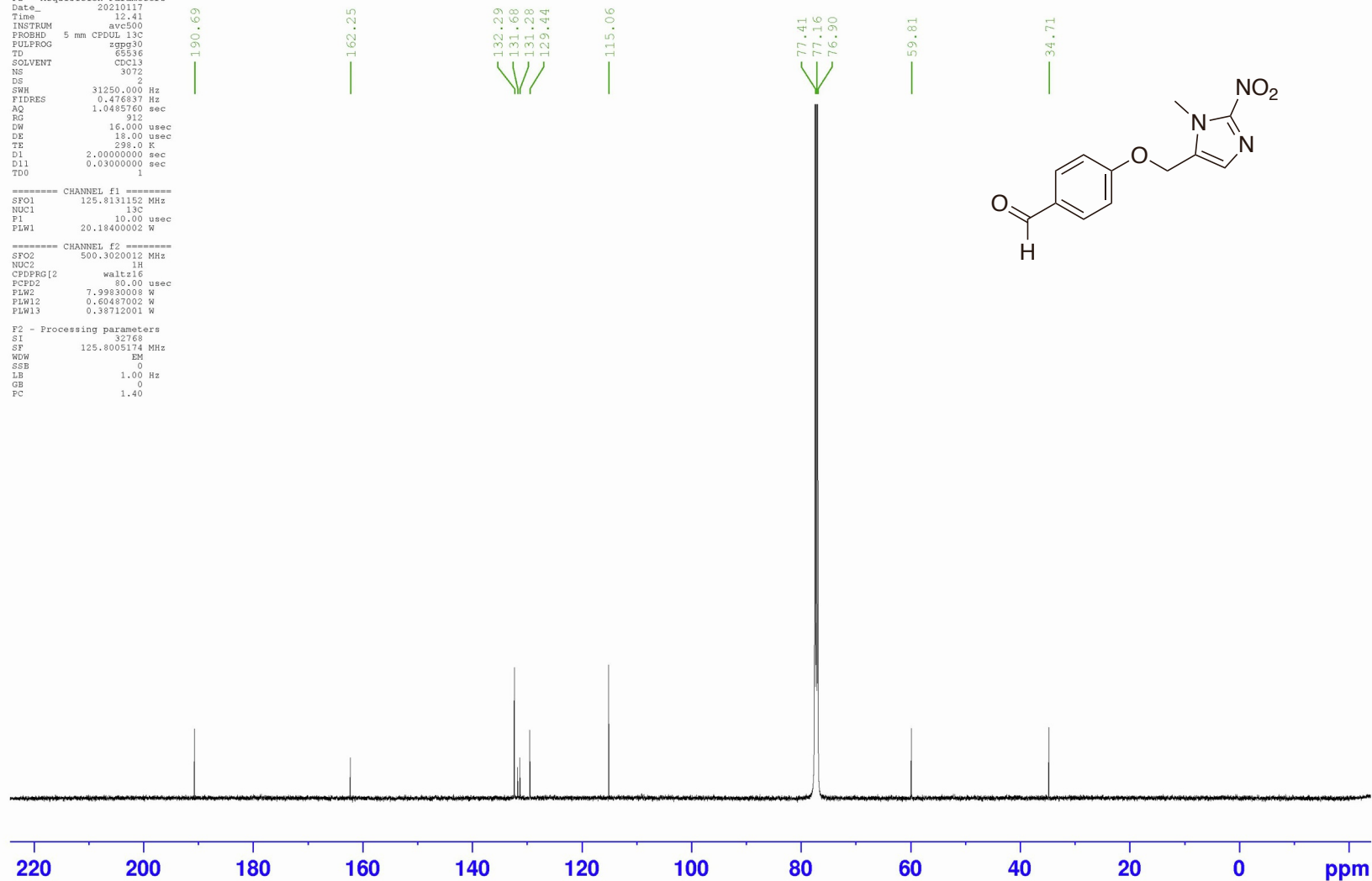
Current Data Parameters
NAME mo604701501 MLOx120
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210117
Time_ 12.41
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 2
SWH 31250.000 Hz
FIDRES 0.476637 Hz
AQ 1.0485760 sec
RG 912
DW 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

===== CHANNEL f1 =====
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W

===== CHANNEL f2 =====
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.60487002 W
PLW13 0.38712001 W

F2 - Processing parameters
SI 32768
SF 125.8005174 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



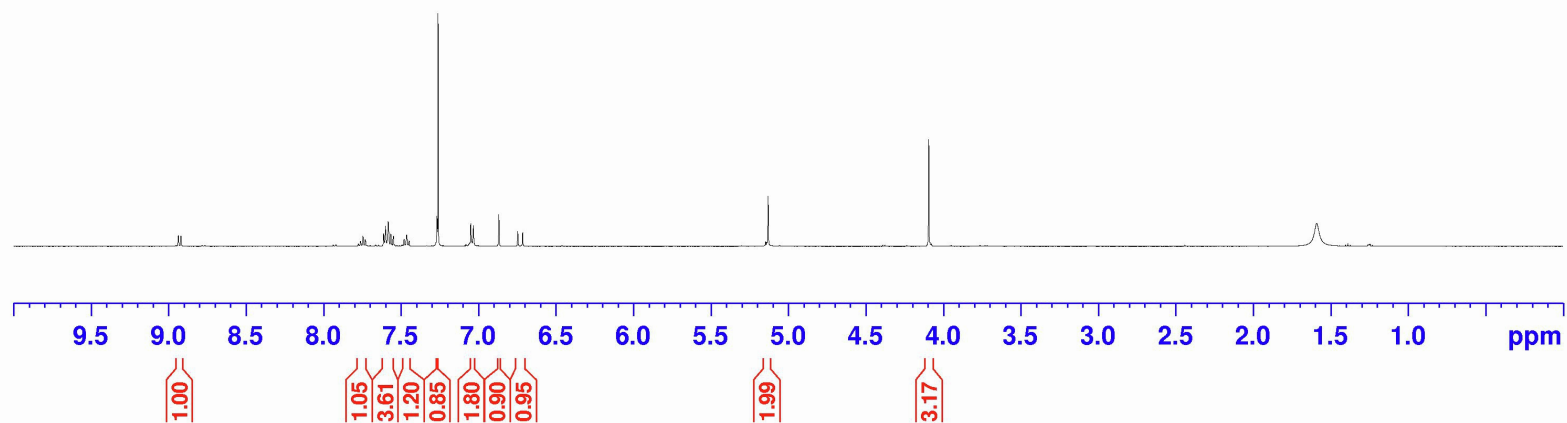
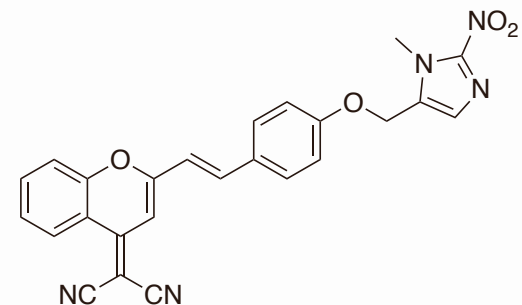
(E)-2-(2-(4-((1-Methyl-2-nitro-1H-imidazol-5-yl)methoxy)styryl)-4H-chromen-4-ylidene)malononitrile

Current Data Parameters
NAME mo605021801 - MLOx121
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210119
Time 22.51
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zg30
TD 65536
SOLVENT CDCl3
NS 16
DS 4
SWH 10330.578 Hz
FIDRES 0.157632 Hz
AQ 3.1719425 sec
RG 4.5
DW 48.400 usec
DE 10.00 usec
TE 298.0 K
D1 1.0000000 sec
TD0 1

===== CHANNEL f1 =====
SFO1 500.3030896 MHz
NUC1 1H
P1 22.00 usec
PLW1 7.99830008 W

F2 - Processing parameters
SI 65536
SF 500.3000129 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00



(E)-2-(2-(4-((1-Methyl-2-nitro-1H-imidazol-5-yl)methoxy)styryl)-4H-chromen-4-ylidene)malononitrile

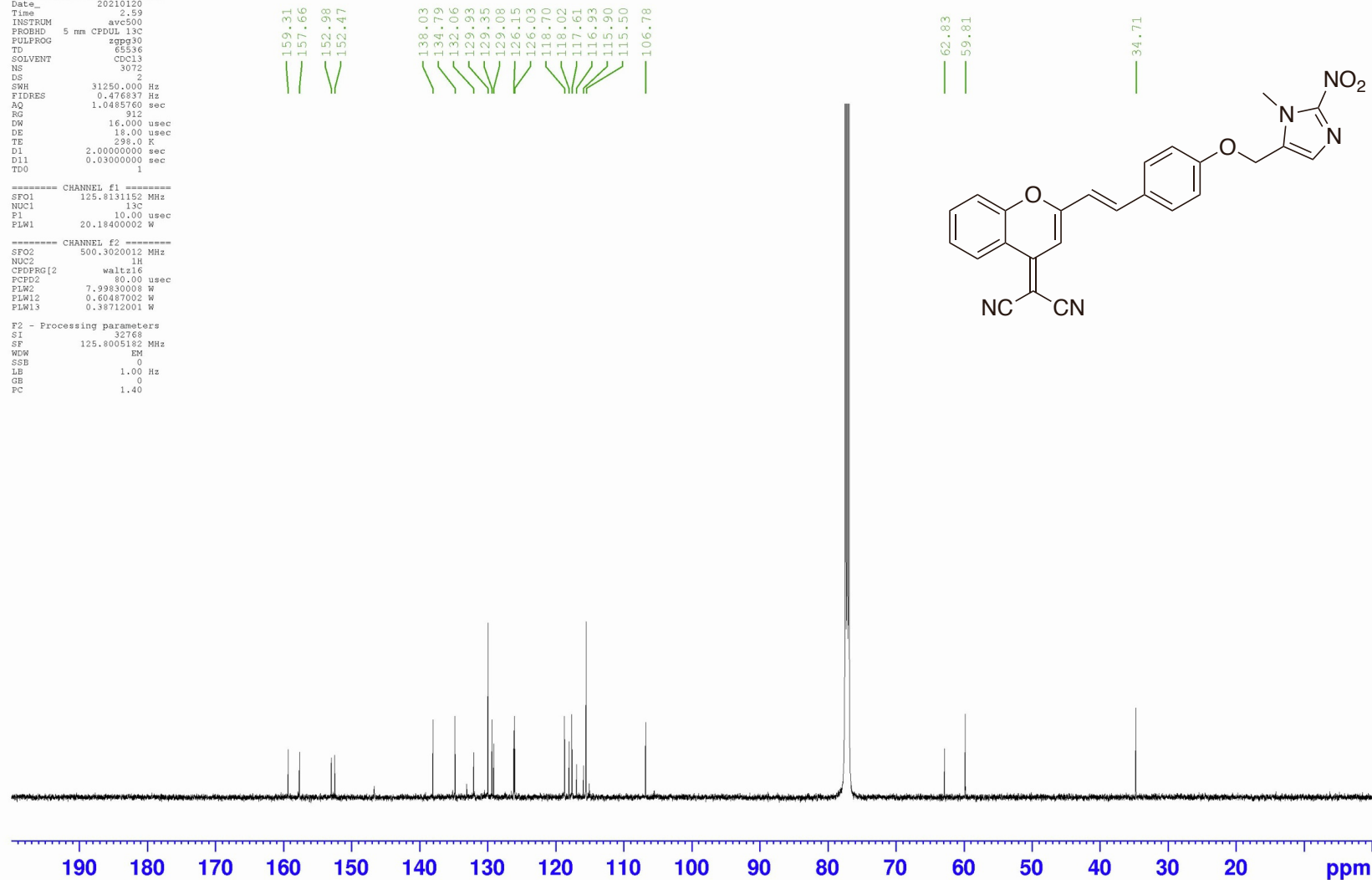
Current Data Parameters
NAME mo605021801 - MLOx121
EXPNO 4
PROCNO 1

F2 - Acquisition Parameters
Date_ 20210120
Time 2.59
INSTRUM avc500
PROBHD 5 mm CPDUL 13C
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 3072
DS 2
SWH 31250.000 Hz
FIDRES 0.476837 Hz
AQ 1.0485760 sec
RG 912
DM 16.000 usec
DE 18.00 usec
TE 298.0 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1

===== CHANNEL f1 =====
SFO1 125.8131152 MHz
NUC1 13C
P1 10.00 usec
PLW1 20.18400002 W

===== CHANNEL f2 =====
SFO2 500.3020012 MHz
NUC2 1H
CPDPRG2 waltz16
PCPD2 80.00 usec
PLW2 7.99830008 W
PLW12 0.60487002 W
PLW13 0.38712001 W

F2 - Processing parameters
SI 32768
SF 125.8005182 MHz
WDW EM
SSB 0
LB 1.00 Hz
GB 0
PC 1.40



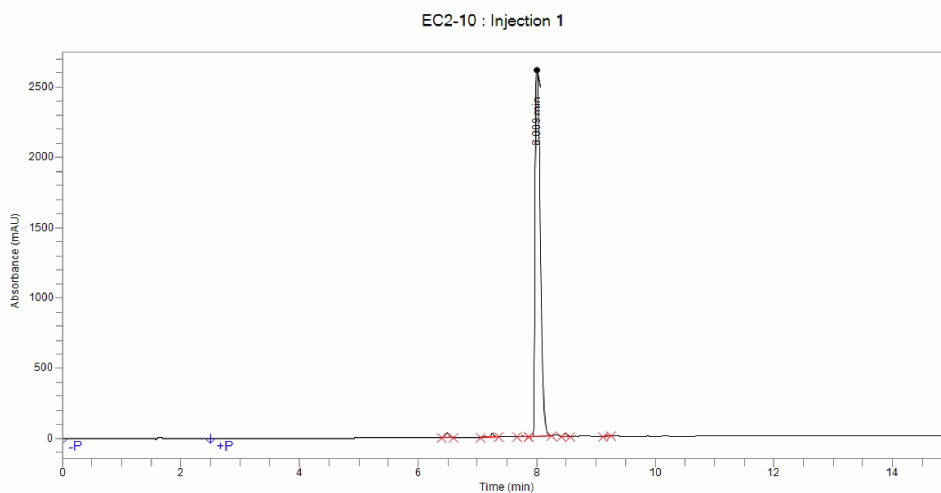
Methyl (E)-3-(4-([2-(2-methyl-1H-indol-3-yl)ethylamino]methyl)phenyl)prop-2-enoate (3)

EC2-10

Purity short run @254 nm

2/1/2017 8:37 pm

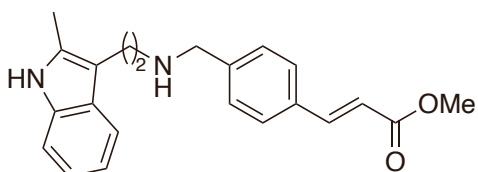
Sample Name EC2-10
Vial Number 6
Injection Volume 5
Acquisition Date/Time 1/31/2017 6:02 pm
Acquisition Method Purity short run @254 nm
Processing Method Purity short run @254 nm



Time	Area	Area %
6.492	142,182.4	0.86
7.131	27,766.8	0.17
7.257	110,631.7	0.67
7.753	39,724.4	0.24
8.009	16,026,271.6	97.35
8.475	88,047.3	0.53
9.188	28,156.0	0.17
Total	16,462,780.3	100.00

2/1/2017 8:37 pm

Flexar HPLC 2



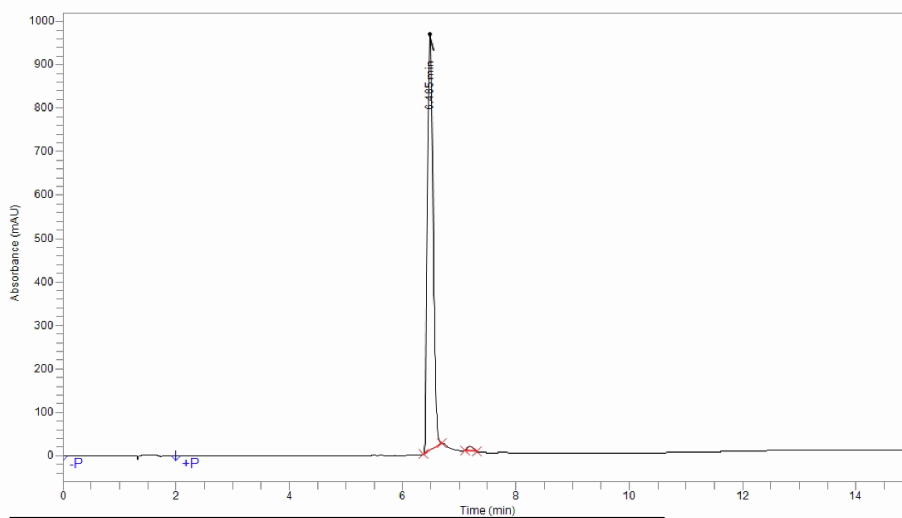
Panobinostat (2)

EC4-14 Pano

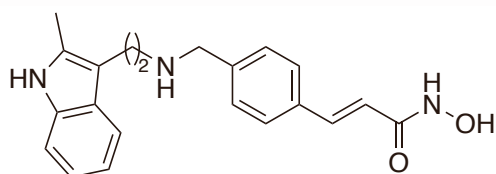
2/26/2018

Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 2/26/2018 6:56 pm
 Injection Volume 10
 Sample Name EC4-14 Pano
 Sample Description

EC4-14 Pano : Injection 1



Time	Height	Area	Area %
6.485	958,967.0	6,619,001.0	98.81
7.183	11,494.8	79,439.7	1.19
Total		6,698,440.7	100.00



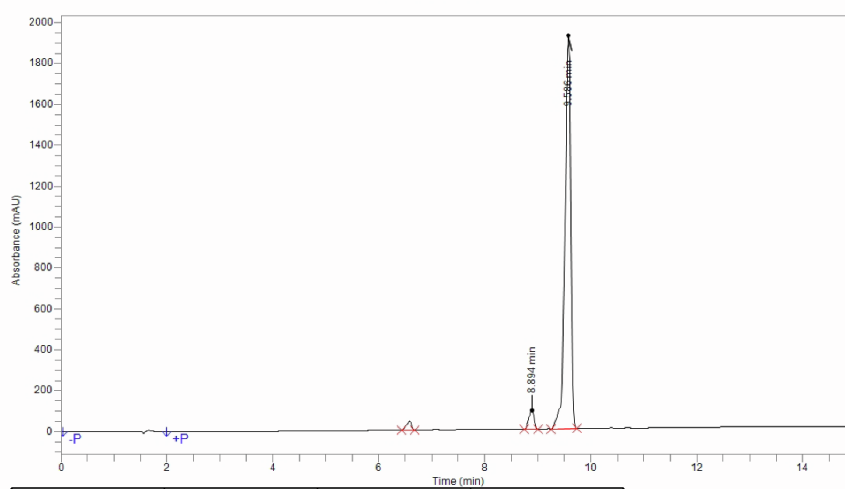
(5-Nitrothiophen-2-yl)methyl bromide (S9)

EC4-63

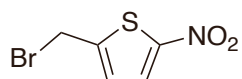
9/11/2018

Acquisition Method Purity short run @254 nm
Acquisition Date/Time 6/25/2018 4:14 pm
Injection Volume 10
Sample Name EC4-63
Sample Description
Batch Description

EC4-63 : Injection 1



Time	Height	Area	Area %
6.585	43,937.1	298,598.4	1.88
8.894	91,542.3	599,907.0	3.78
9.586	1,923,605.5	14,976,498.3	94.34
Total		15,875,003.6	100.00



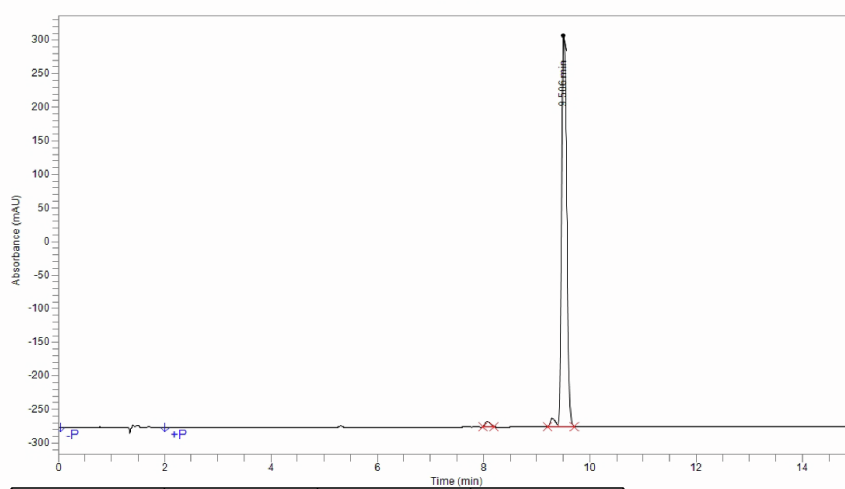
N-Phthalimido-*O*-(5-nitrothiophen-2-yl)-hydroxylamine

EC4-68

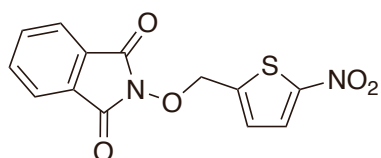
9/11/2018

Acquisition Method Purity short run @254 nm
Acquisition Date/Time 7/3/2018 5:41 pm
Injection Volume 10
Sample Name EC4-68
Sample Description
Batch Description

EC4-68 : Injection 1



Time	Height	Area	Area %
8.071	8,336.6	51,781.2	1.29
9.292	12,498.6	81,327.4	2.03
9.506	582,893.5	3,865,476.7	96.67
Total		3,998,585.4	100.00



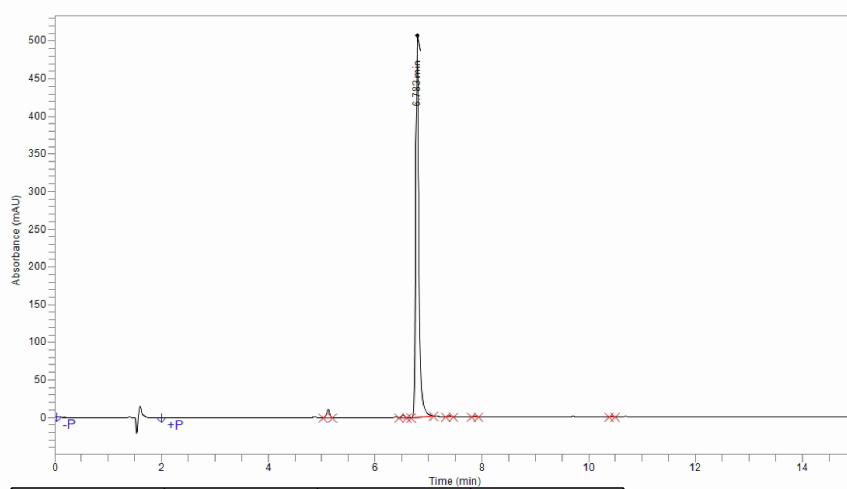
O-(5-Nitrothiophen-2-yl)-hydroxylamine (**S10**)

EC4-90

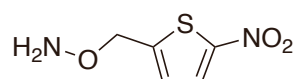
10/1/2018

Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 10/1/2018 2:41 pm
 Injection Volume 10
 Sample Name EC4-90
 Sample Description
 Batch Description

EC4-90 : Injection 1



Time	Height	Area	Area %
5.119	11,409.7	44,269.3	1.93
6.518	3,648.0	13,869.0	0.61
6.783	507,580.5	2,210,708.1	96.60
7.387	2,336.8	8,811.9	0.39
7.870	1,697.5	6,964.7	0.30
10.436	1,216.3	3,794.2	0.17
Total		2,288,417.3	100.00



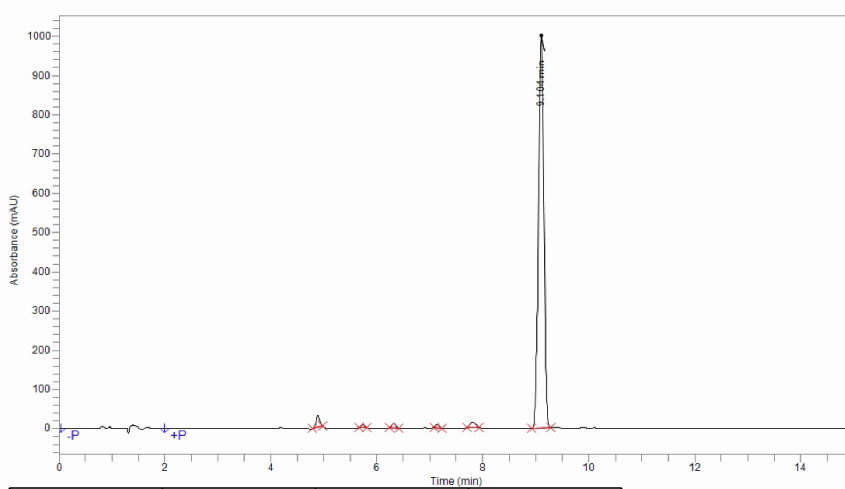
N-Phthalimido-O-(6-nitroquinolin-5-yl)-hydroxylamine

EC3-88

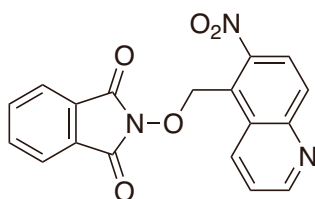
9/12/2018

Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 1/8/2018 1:50 pm
 Injection Volume 20
 Sample Name EC3-88
 Sample Description
 Batch Description

EC3-88 : Injection 1



Time	Height	Area	Area %
4.891	29,133.4	145,138.3	1.98
5.735	6,556.7	31,610.1	0.43
6.319	11,319.6	57,009.5	0.78
7.138	6,535.2	32,462.8	0.44
7.807	13,451.0	94,303.7	1.28
9.104	1,001,339.3	6,982,876.3	95.09
Total		7,343,400.6	100.00



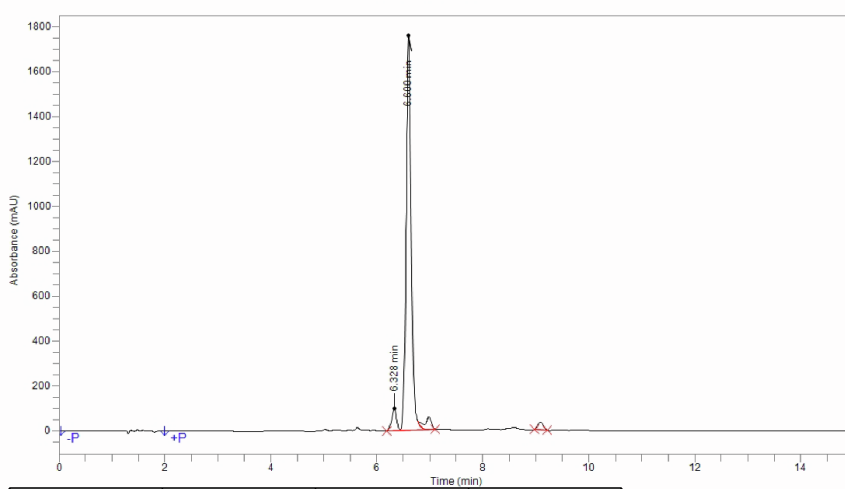
O-(6-Nitroquinolin-5-yl)-hydroxylamine (S14)

EC3-89

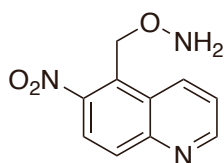
9/12/2018

Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 1/8/2018 2:25 pm
 Injection Volume 20
 Sample Name EC3-89
 Sample Description
 Batch Description

EC3-89 : Injection 1



Time	Height	Area	Area %
6.328	99,800.0	620,898.6	4.75
6.600	1,756,176.4	11,754,905.5	89.95
6.986	53,118.9	455,530.4	3.49
9.092	34,047.3	236,249.0	1.81
Total		13,067,583.6	100.00



Methyl (E)-3-(4-([*tert*-butyloxycarbonyl-(2-{1-[*tert*-butyloxycarbonyl]-2-methyl-1*H*-indol-3-yl)ethyl]amino)methyl}phenyl)prop-2-enoate (**4**)

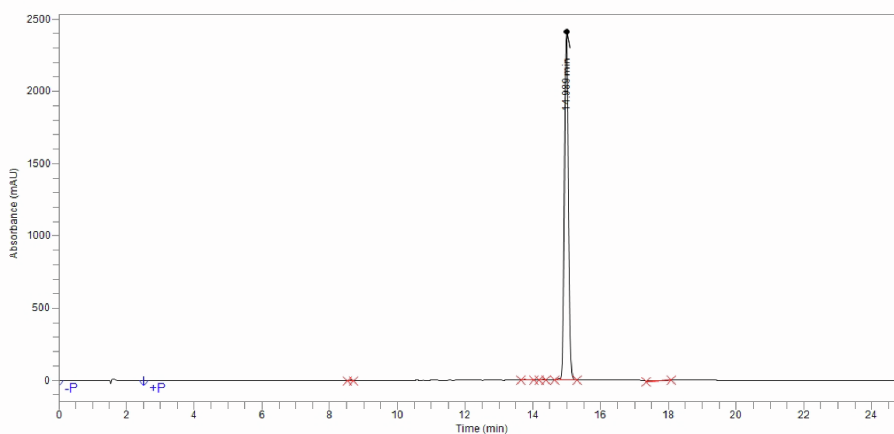
EC2-38

Purity short run @254 nm

2/1/2017 1:48 pm

Sample Name EC2-38
Vial Number 1
Injection Volume 10
Acquisition Date/Time 2/1/2017 9:45 am
Acquisition Method Purity short run @254 nm
Processing Method Purity short run @254 nm

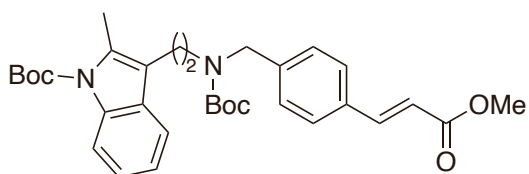
EC2-38 : Injection 1



Time	Area	Area %
8.624	8,997.7	0.05
13.759	34,580.5	0.18
13.941	13,625.3	0.07
14.296	33,348.9	0.17
14.789	82,617.3	0.43
14.989	18,982,727.6	98.24
18.018	167,802.4	0.87
Total	19,323,699.6	100.00

2/1/2017 1:48 pm

Flexar HPLC 2



(E)-3-(4-([*tert*-Butyloxycarbonyl-(2-{1-[*tert*-butyloxycarbonyl]-2-methyl-1*H*-indol-3-yl}ethyl)amino]methyl)phenyl)prop-2-enoic acid (5)

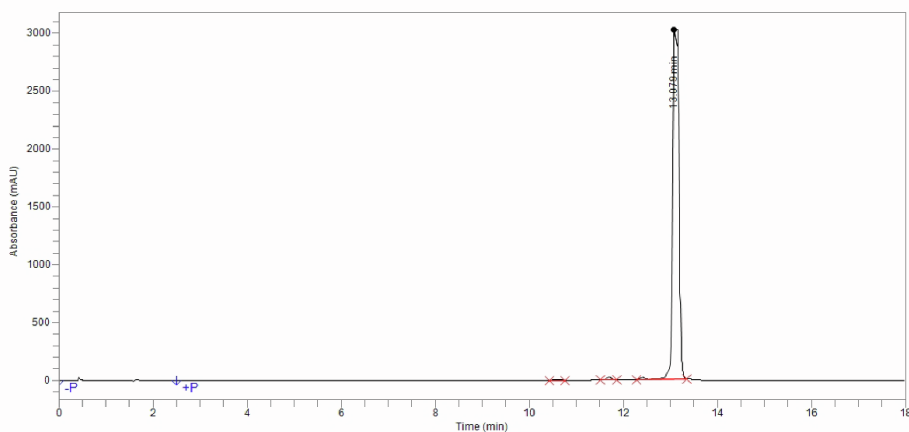
EC2-39

EC Purity short run @254 nm longer

2/2/2017 5:15 pm

Sample Name EC2-39
 Vial Number 7
 Injection Volume 5
 Acquisition Date/Time 2/2/2017 4:55 pm
 Acquisition Method EC Purity short run @254 nm longer
 Processing Method EC Purity short run @254 nm longer

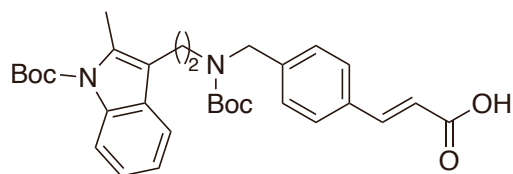
EC2-39 : Injection 1



Time	Area	Area %
10.601	49,007.8	0.17
11.713	182,154.1	0.63
12.427	140,338.8	0.48
13.079	28,606,702.2	98.72
Total	28,978,202.8	100.00

2/2/2017 5:15 pm

Flexar HPLC 2



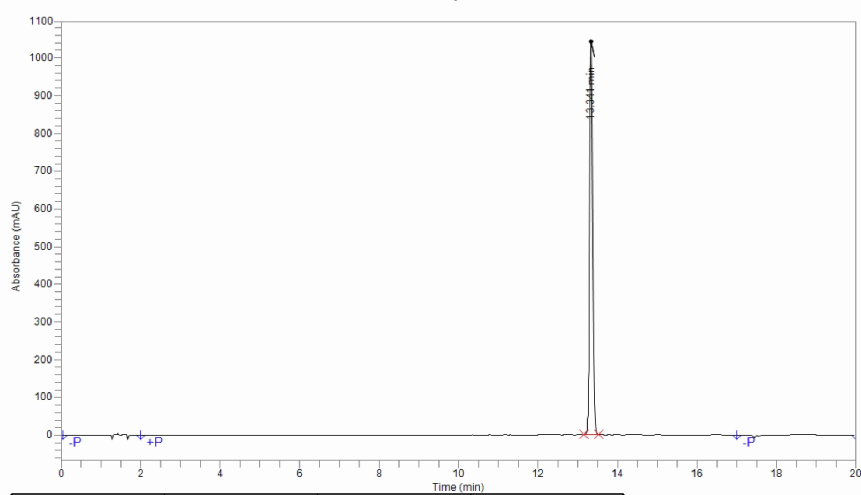
N,N-Di-Boc-*O*-benzyl-panobinostat (**6**)

EC4-92

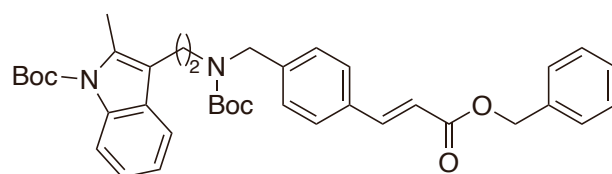
10/10/2018

Acquisition Method	Purity short run @254 nm
Acquisition Date/Time	10/10/2018 12:27 pm
Injection Volume	10
Sample Name	EC4-92
Sample Description	
Batch Description	

EC4-92 : Injection 1



Time (min)			
Time	Height	Area	Area %
13.341	1,044,426.9	5,146,725.1	100.00
Total		5,146,725.1	100.00



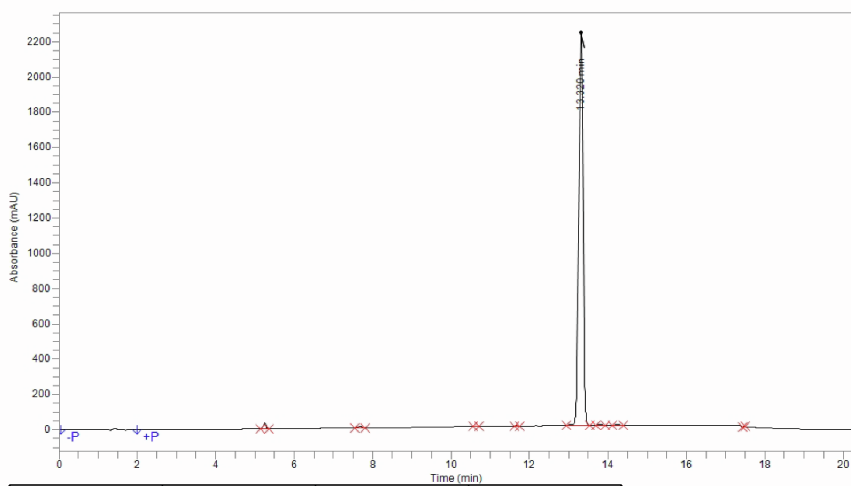
N,N-Di-Boc-*O*-(4-nitrobenzyl)-panobinostat (**7**)

EC4-61

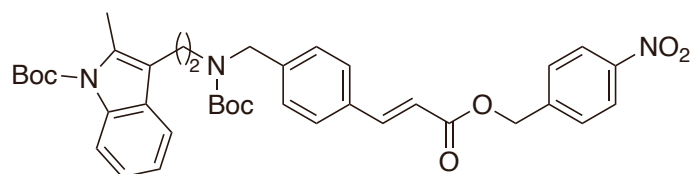
9/19/2018

Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 6/22/2018 4:51 pm
 Injection Volume 10
 Sample Name EC4-61
 Sample Description
 Batch Description

EC4-61 : Injection 1



Time	Height	Area	Area %
5.258	33,504.5	167,128.0	0.96
7.687	10,754.0	76,820.7	0.44
10.632	2,487.5	12,000.8	0.07
11.671	2,152.6	9,633.4	0.06
13.320	2,229,257.0	17,107,225.8	98.04
13.802	3,814.4	30,947.5	0.18
14.244	4,433.2	38,467.9	0.22
17.455	2,460.5	6,740.1	0.04
Total		17,448,964.1	100.00



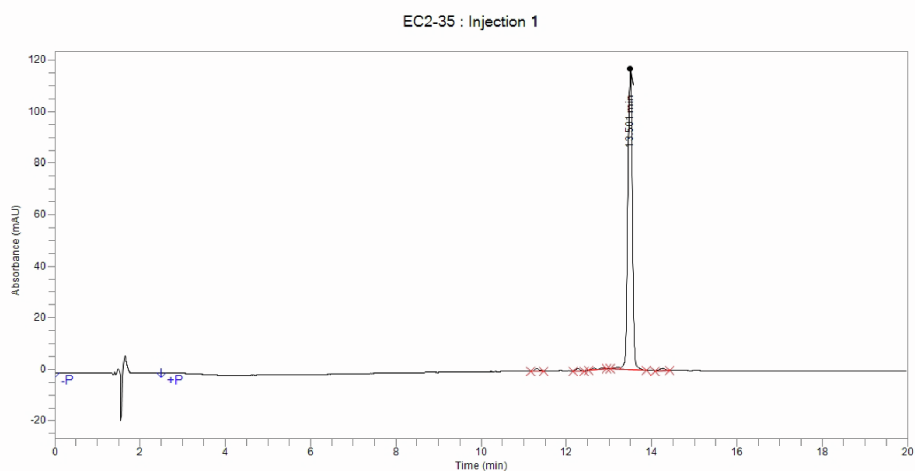
N,N-Di-Boc-*O*-(2-nitrothiophen-5-yl)methyl)-panobinostat (**8**)

EC2-35

EC Purity short run @254 nm longer

2/1/2017 6:23 pm

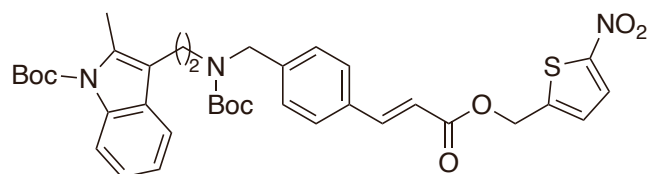
Sample Name	EC2-35
Vial Number	12
Injection Volume	10
Acquisition Date/Time	2/1/2017 5:38 pm
Acquisition Method	EC Purity short run @254 nm longer
Processing Method	EC Purity short run @254 nm longer



Time	Area	Area %
11.309	7,739.2	0.88
12.284	5,856.3	0.67
12.649	5,843.2	0.66
12.853	3,870.5	0.44
13.185	6,107.3	0.69
13.501	840,590.3	95.64
14.270	8,871.3	1.01
Total	878,878.1	100.00

2/1/2017 6:23 pm

Flexar HPLC 2



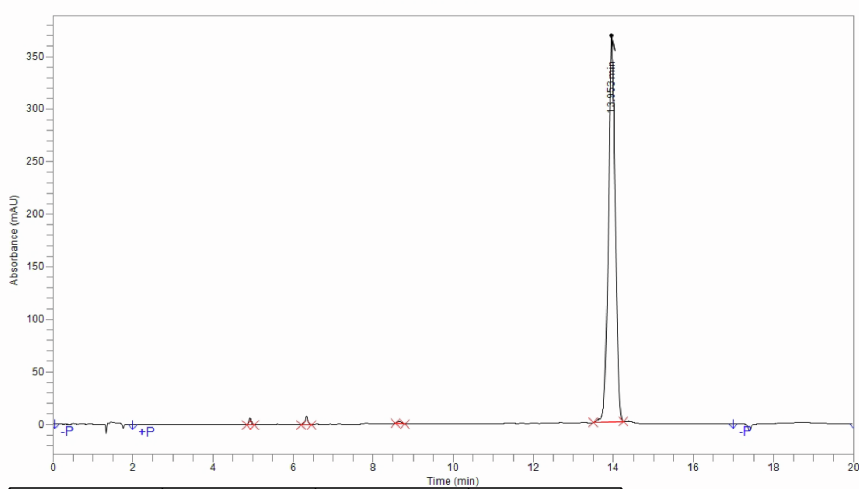
N,N-Di-Boc-*O*-((6-nitroquinolin-5-yl)methyl)-panobinostat (9)

EC3-90

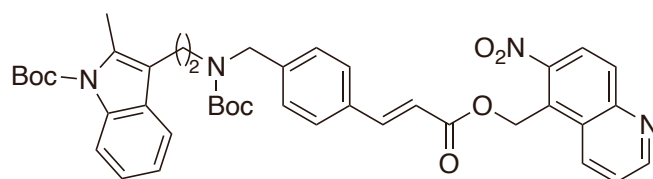
9/20/2018

Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 1/8/2018 3:01 pm
 Injection Volume 10
 Sample Name EC3-90
 Sample Description
 Batch Description

EC3-90 : Injection 1



Time	Height	Area	Area %
4.927	6,738.4	31,633.7	0.70
6.332	7,877.7	48,338.7	1.07
8.658	2,341.0	16,158.8	0.36
13.953	368,327.9	4,423,922.2	97.87
Total		4,520,053.3	100.00



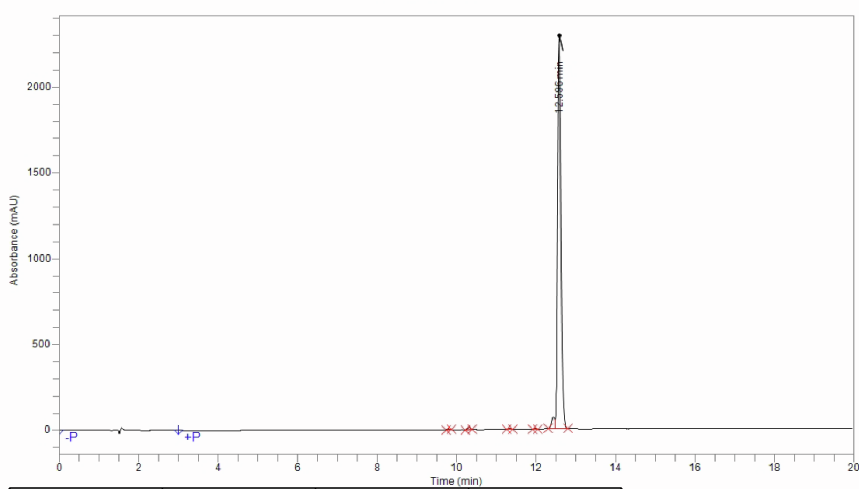
N,N-Di-Boc-*O*-((1-methyl-2-nitroimidazol-5-yl)methyl)-panobinostat

EC5-06

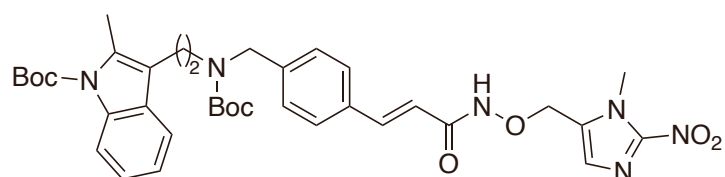
12/11/2018

Acquisition Method Purity short run @254 nm with long hold
 Acquisition Date/Time 12/11/2018 2:40 pm
 Injection Volume 10
 Sample Name EC5-06
 Sample Description
 Batch Description

EC5-06 : Injection 1



Time	Height	Area	Area %
9.809	5,860.4	23,457.5	0.18
10.319	16,539.6	69,084.1	0.54
11.351	5,998.6	25,916.7	0.20
11.990	3,952.6	15,955.0	0.12
12.440	68,462.0	327,459.7	2.54
12.596	2,293,450.6	12,416,674.7	96.41
Total		12,878,547.7	100.00



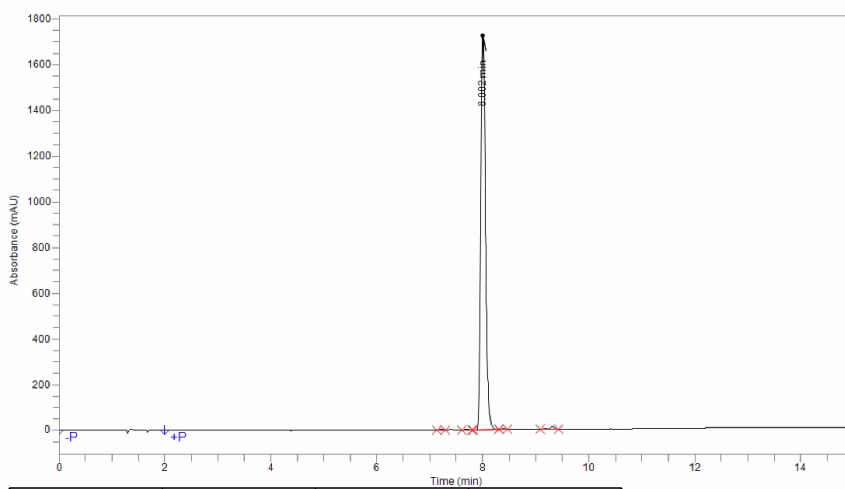
O-Benzyl-panobinostat (Bn-Pano, **10**)

EC5-03

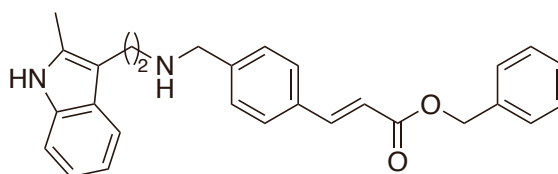
11/15/2018

Acquisition Method Purity short run @254 nm
Acquisition Date/Time 11/15/2018 10:41 am
Injection Volume 10
Sample Name EC5-03
Sample Description
Batch Description

EC5-03 : Injection 1



Time	Height	Area	Area %
7.223	3,653.0	16,559.7	0.16
7.696	3,057.3	14,995.5	0.14
8.002	1,727,141.3	10,237,501.0	98.80
8.393	4,153.3	17,709.5	0.17
9.185	3,472.0	16,907.9	0.16
9.317	11,731.4	58,166.0	0.56
Total		10,361,839.6	100.00



O-(4-Nitrobenzyl)-panobinostat trifluoroacetate (NB-Pano, **11**)

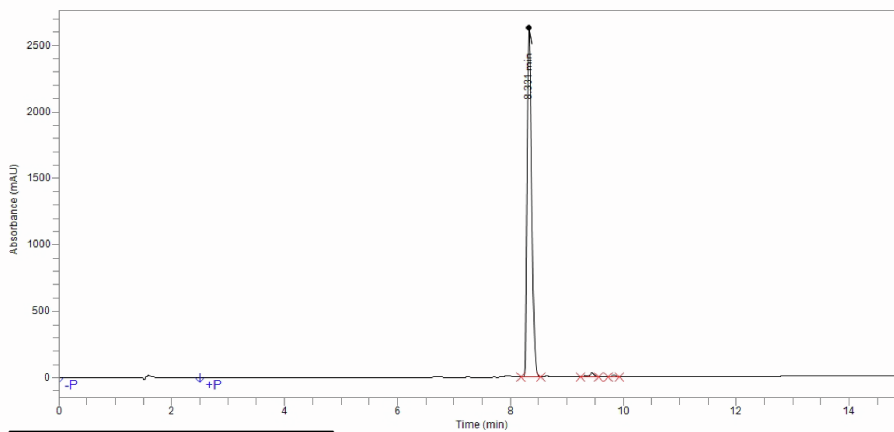
EC2-18

Purity short run @254 nm

11/4/2016 3:54 pm

Sample Name EC2-18
Vial Number 3
Injection Volume 20
Acquisition Date/Time 11/4/2016 1:24 pm
Acquisition Method Purity short run @254 nm
Processing Method Purity short run @254 nm

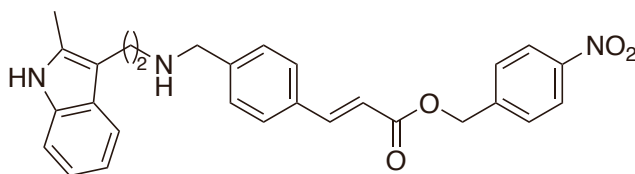
EC2-18 : Injection 1



Time	Area	Area %
8.331	14,908,280.8	98.07
9.321	49,133.2	0.32
9.444	168,073.8	1.11
9.829	76,090.2	0.50
Total	15,201,577.9	100.00

11/4/2016 3:54 pm

Flexar HPLC 2



O-(5-Nitrothiophen-2-yl)methyl))-panobinostat trifluoroacetate (NT-Pano, **12**)

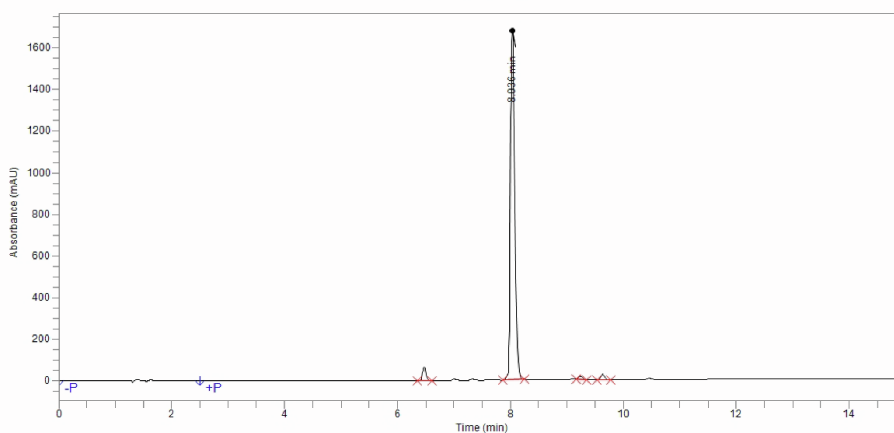
EC2-37

Purity short run @254 nm

1/11/2017 2:40 pm

Sample Name EC2-37
Vial Number 1
Injection Volume 10
Acquisition Date/Time 1/11/2017 2:22 pm
Acquisition Method Purity short run @254 nm
Processing Method Purity short run @254 nm

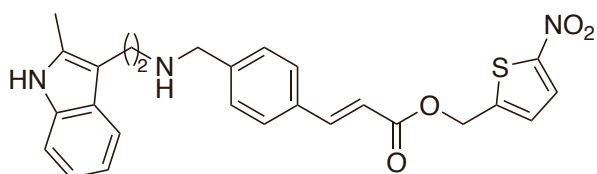
EC2-37 : Injection 1



Time	Area	Area %
6.474	267,632.6	2.77
8.036	9,169,783.6	95.06
9.237	76,595.7	0.79
9.635	132,733.0	1.38
Total	9,646,744.8	100.00

1/11/2017 2:40 pm

Flexar HPLC 2



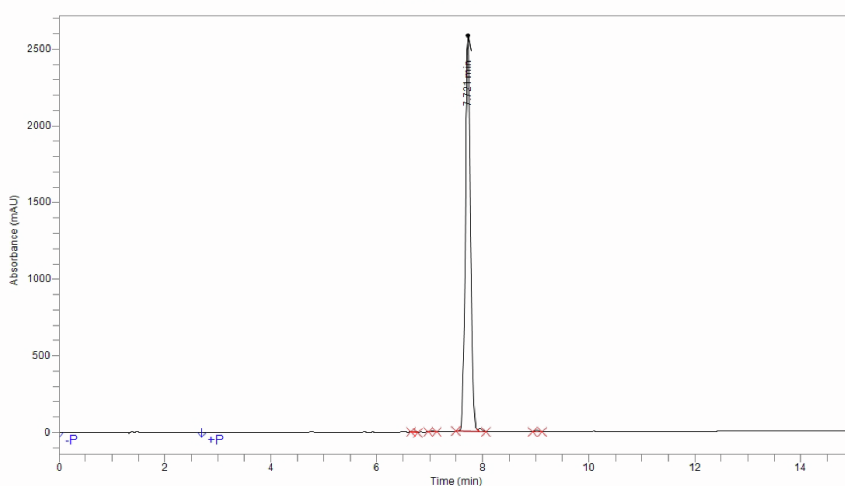
O-(6-Nitroquinolin-5-ylmethyl))-panobinostat di-trifluoroacetate (NQ-Pano, **13**)

EC3-91

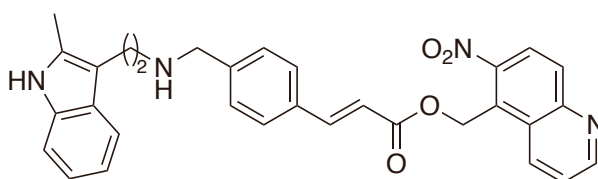
1/4/2019

Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 1/10/2018 12:56 pm
 Injection Volume 10
 Sample Name EC3-91
 Sample Description
 Batch Description EC

EC3-91 : Injection 1



Time	Height	Area	Area %
6.703	5,278.4	22,946.9	0.13
7.053	5,771.8	27,979.5	0.16
7.721	2,585,241.8	17,486,575.7	98.82
7.952	20,853.4	105,450.1	0.60
9.023	10,176.2	52,548.6	0.30
Total		17,695,500.7	100.00

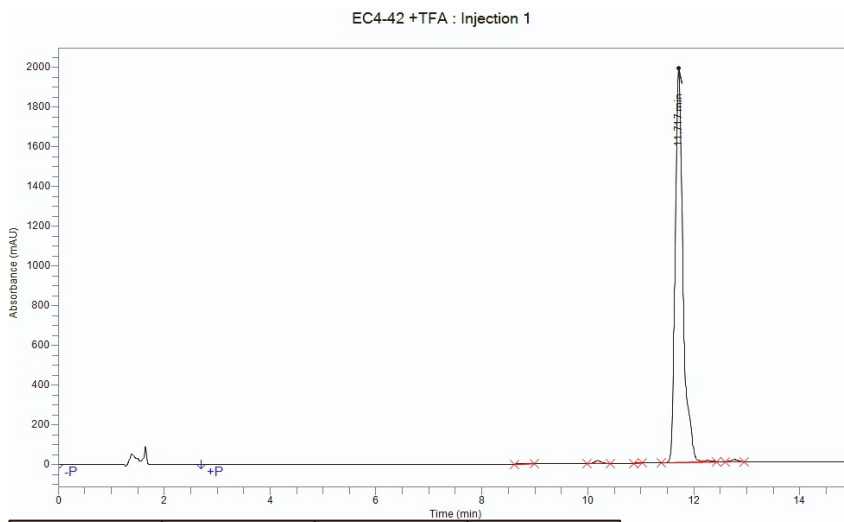


N,N-Di-Boc-panobinostat (**14**)

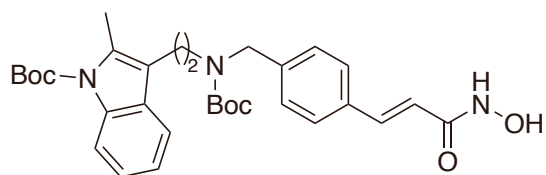
EC4-42 +TFA

1/4/2019

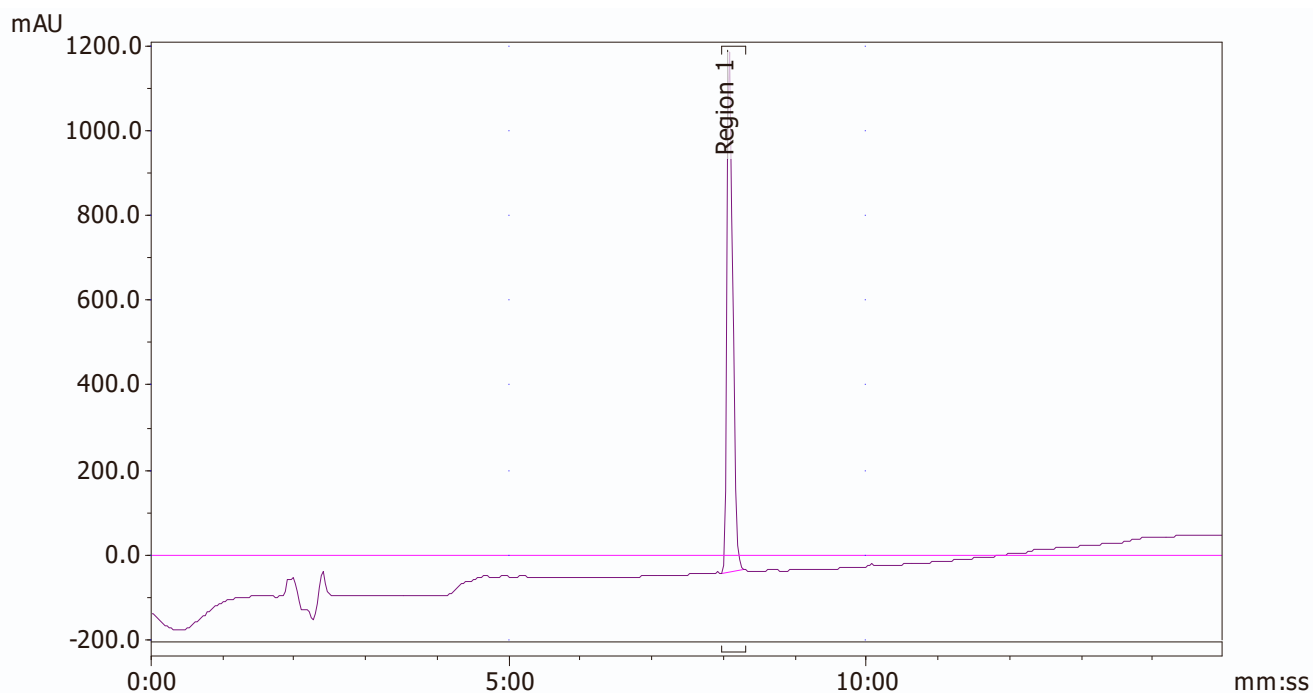
Acquisition Method Purity short run @254 nm
 Acquisition Date/Time 1/4/2019 11:13 am
 Injection Volume 10
 Sample Name EC4-42 +TFA
 Sample Description
 Batch Description



Time	Height	Area	Area %
8.752	3,563.8	31,672.7	0.15
10.185	14,619.4	115,771.7	0.57
11.006	193.5	1,350.5	0.01
11.717	1,986,041.2	20,146,471.9	98.52
12.264	5,418.6	54,861.0	0.27
12.770	11,253.5	97,984.8	0.48
Total		20,448,112.5	100.00



O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, 1)



Regions: DA-C@220nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)	%Total (%)
Region 1	7:58	8:18	8:04	6361.4	100.00	N/A
1 Peak				6361.4	100.00	N/A

Total Area: -33335.6 mAU
Average Background: N/A mAU

Method: Xbridge_nonRadio_default

Instrument: N/A Serial no FR1A/0217/389

Run Length: 15m

Dwell: 1s

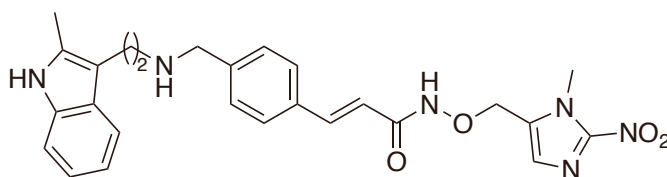
Cell Volume: 10 µL

Cell Type: Solid

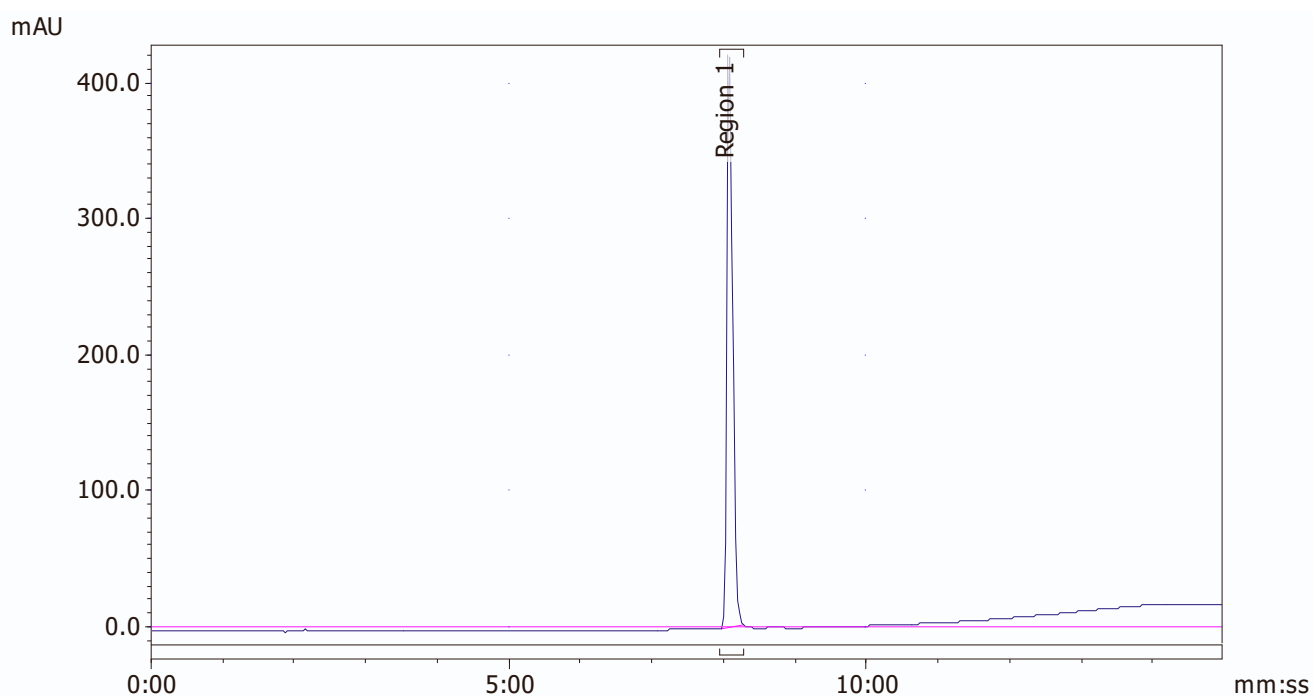
Eluate Flow: 1.00 mL/min

Residence Time: 0.6s

Injection Volume: 10 µL



O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, 1)



Regions: DA-B@254nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)	%Total (%)
Region 1	7:57	8:16	8:04	2147.8	100.00	62.27
1 Peak				2147.8	100.00	62.27

Total Area: 3448.9 mAU
Average Background: N/A mAU

Method: Xbridge nonRadio default

Instrument: N/A Serial no FR1A/0217/389

Run Length: 15m

Dwell: 1s

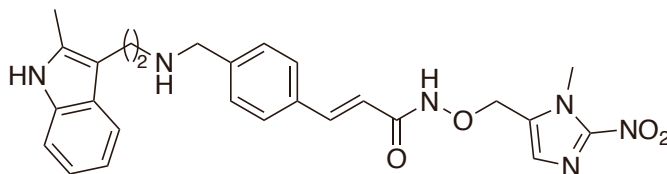
Cell Volume: 10 µL

Cell Type: Solid

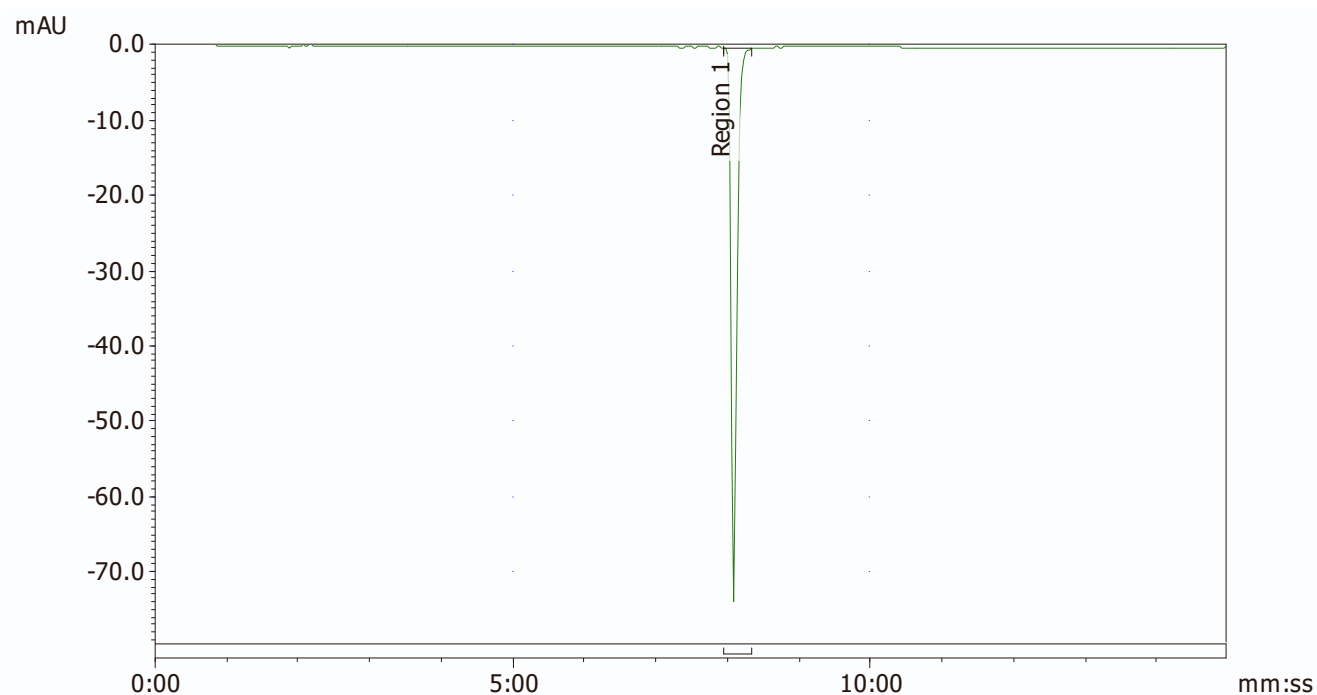
Eluate Flow: 1.00 mL/min

Residence Time: 0.6s

Injection Volume: 10 µL



O-((1-Methyl-2-nitroimidazol-5-yl)methyl)-Panobinostat di-trifluoroacetate (CH-03, NI-Pano, 1)



Regions: DA-D@365nm Detector:

Name	Start (mm:ss)	End (mm:ss)	Retention (mm:ss)	Area (mAU·s)	%ROI (%)	%Total (%)
Region 1	7:57	8:20	7:57	-400.8		N/A
1 Peak				-400.8	100.00	N/A

Total Area: -635.4 mAU

Average Background: N/A mAU

Method: Xbridge nonRadio default

Instrument: N/A Serial no FR1A/0217/389

Run Length: 15m

Dwell: 1s

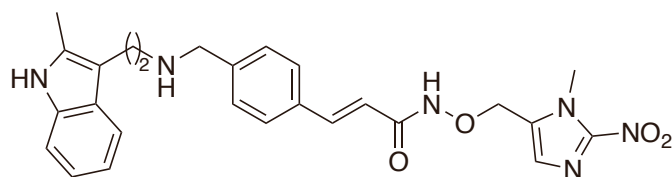
Cell Volume: 10 µL

Cell Type: Solid

Eluate Flow: 1.00 mL/min

Residence Time: 0.6s

Injection Volume: 10 µL



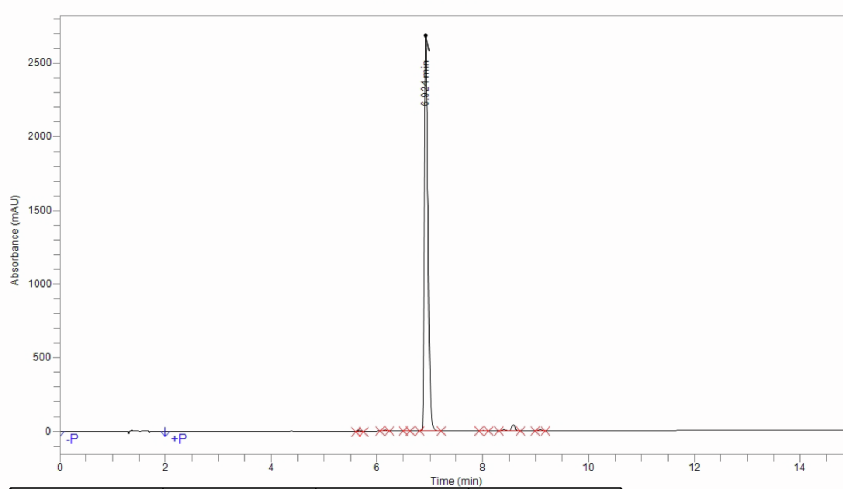
(*E*)-3-(4-[[2-(2-Methyl-1*H*-indol-3-yl)ethylamino]methyl]phenyl)prop-2-enoic acid trifluoroacetate (**S18**)

EC5-05

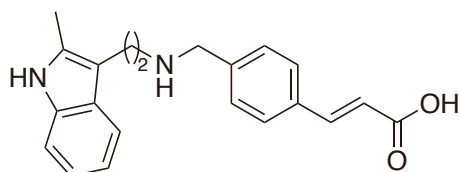
11/15/2018

Acquisition Method Purity short run @254 nm
Acquisition Date/Time 11/13/2018 5:31 pm
Injection Volume 10
Sample Name EC5-05
Sample Description
Batch Description

EC5-05 : Injection 1



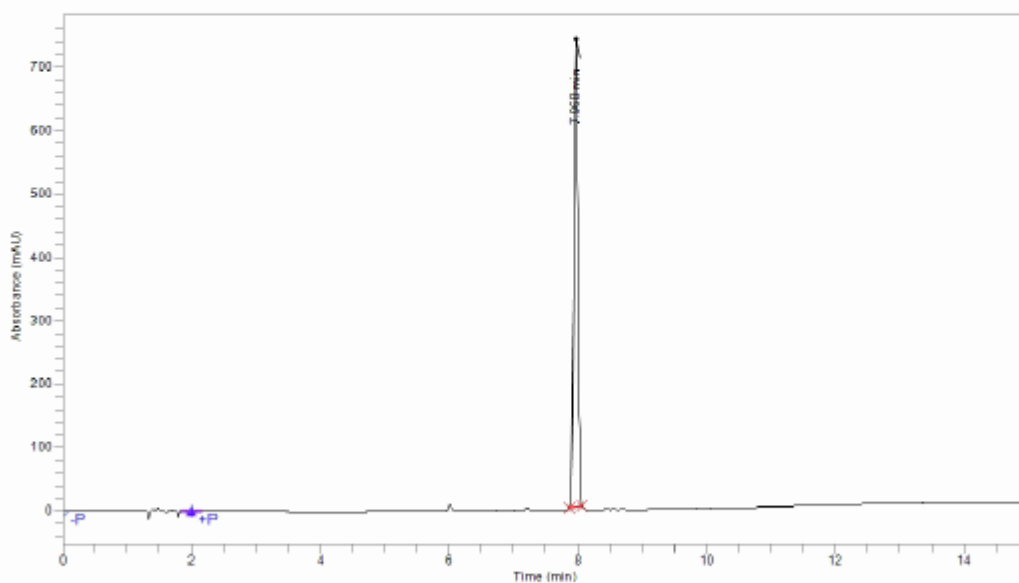
Time	Height	Area	Area %
5.666	7,378.7	24,641.2	0.18
6.151	12,629.0	48,704.8	0.35
6.560	4,172.3	14,854.3	0.11
6.924	2,689,301.6	13,536,942.6	96.97
8.011	4,110.7	19,689.0	0.14
8.397	12,240.3	59,987.8	0.43
8.578	41,434.1	197,179.3	1.41
9.088	12,533.8	57,532.1	0.41
Total		13,959,531.1	100.00



(E)-2-(2-(4-((1-Methyl-2-nitro-1H-imidazol-5-yl)methoxy)styryl)-4H-chromen-4-ylidene)malononitrile

Acquisition Method Purity short run @254 nm
Acquisition Date/Time 1/15/2021 12:40 pm
Injection Volume 10
Sample Name MLOx120
Sample Description
Batch Description

MLOx120 : Injection 1



Time	Height	Area	Area %
7.968	738,461.0	3,213,778.3	100.00
Total		3,213,778.3	100.00

