

# ChemMedChem

Supporting Information

## **Zap-Pano: a Photocaged Prodrug of the KDAC Inhibitor Panobinostat**

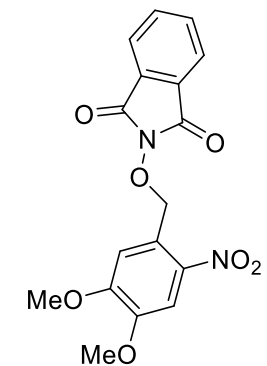
Kathrin S. Troelsen, Ewen D. D. Calder<sup>†</sup>, Anna Skwarska<sup>†</sup>, Deborah Sneddon,  
Ester M. Hammond,\* and Stuart J. Conway\*

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HPLC traces of novel and biologically tested compounds	S14
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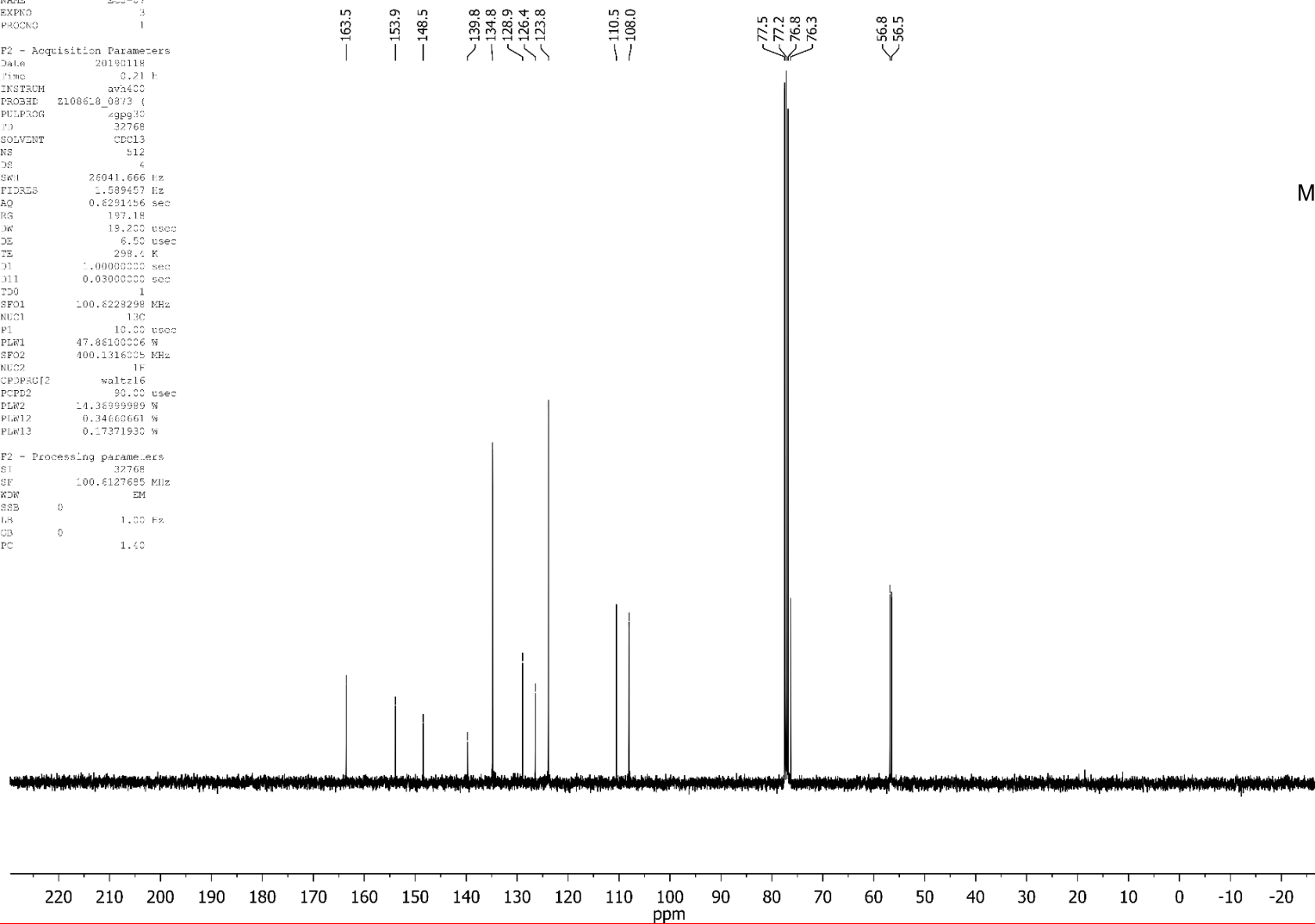
N-Phthalimido-O-(4, 5-dimethoxy-2-nitrobenzyl)-hydroxylamine (7)



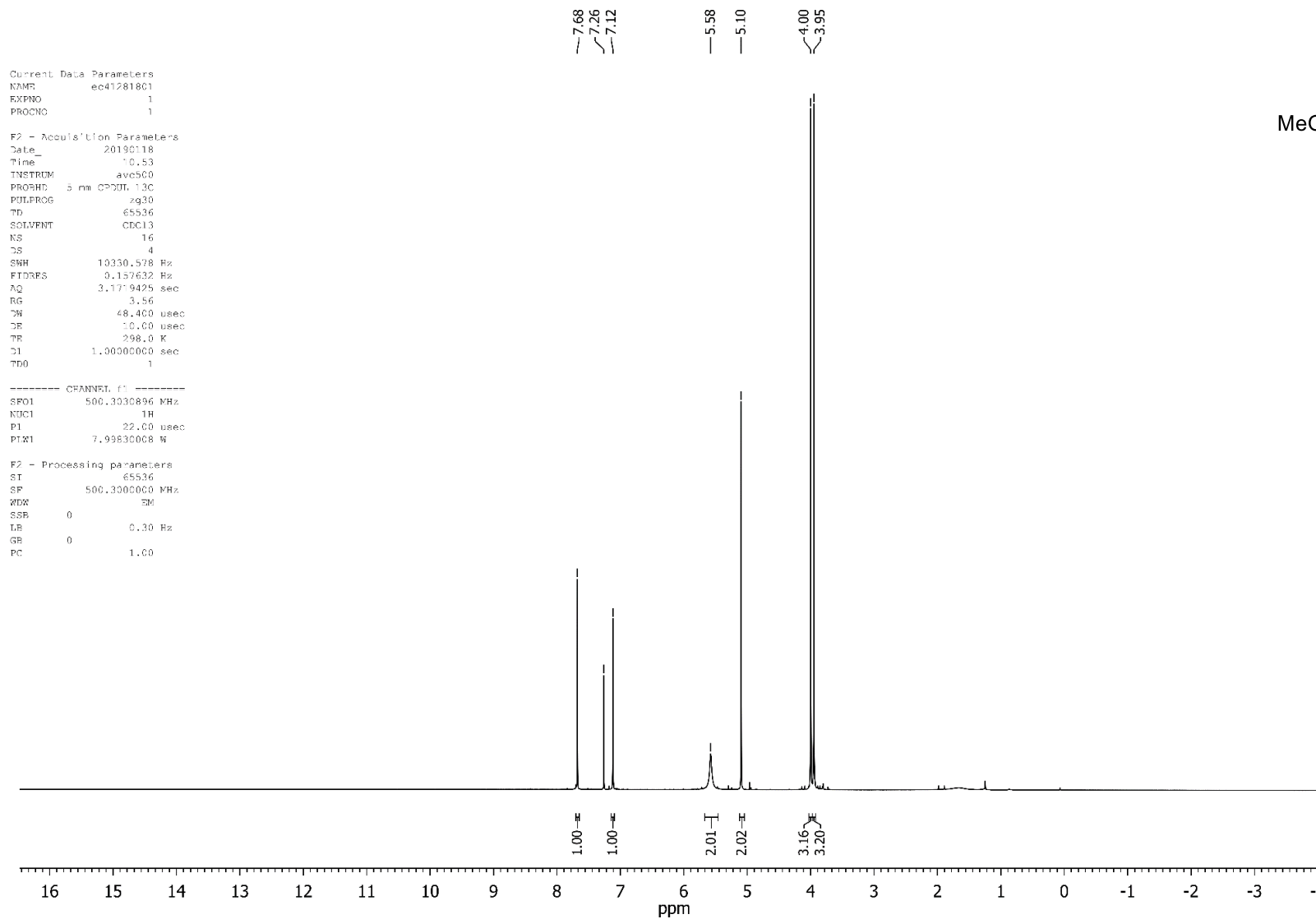
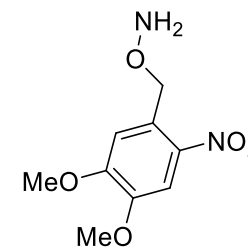
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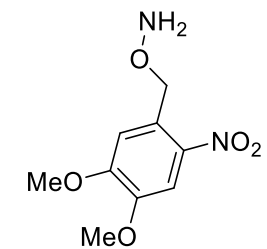
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O-(4, 5-Dimethoxy-2-nitrobenzyl)-hydroxylamine (**8**)



O-(4, 5-Dimethoxy-2-nitrobenzyl)-hydroxylamine (8)



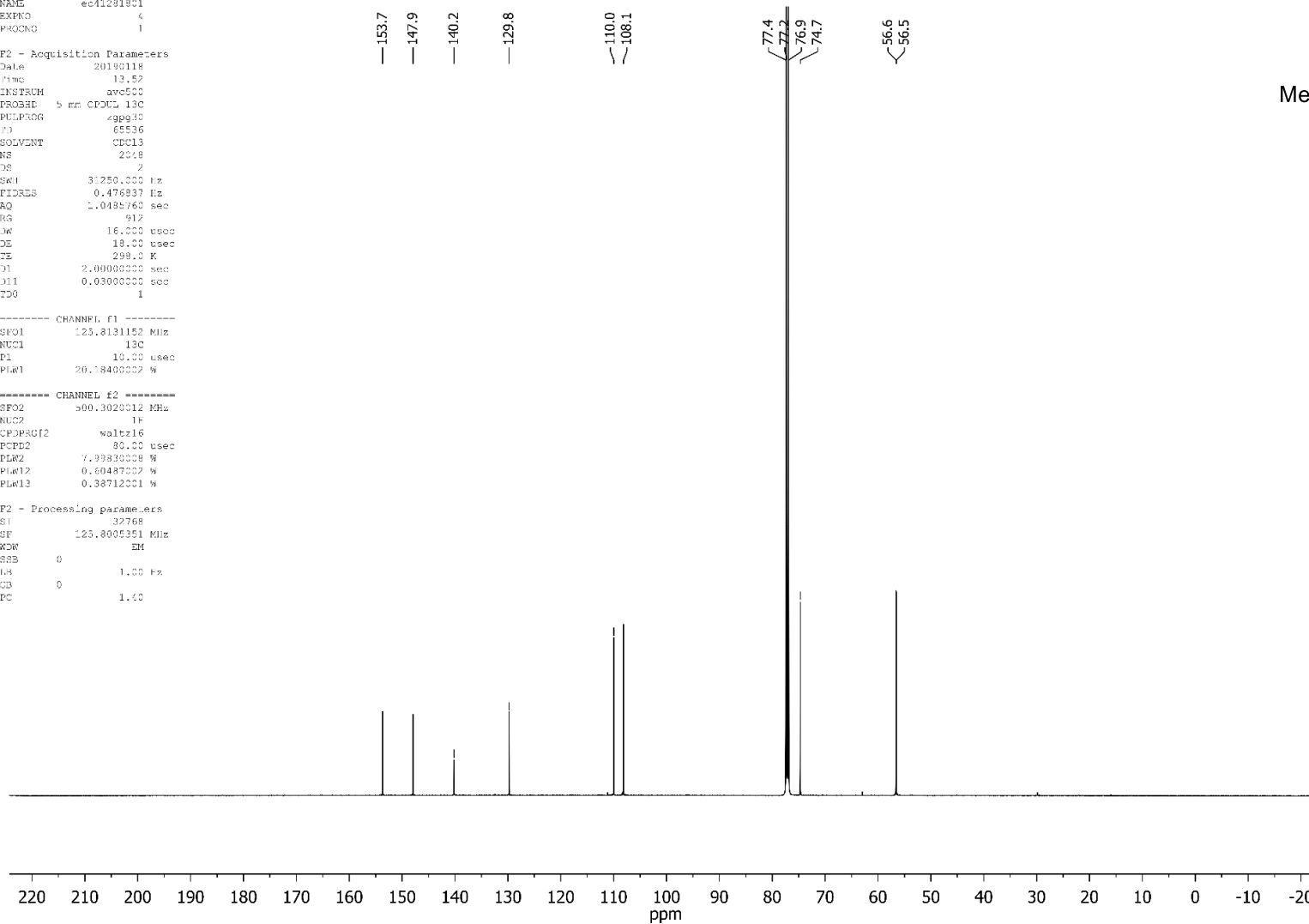
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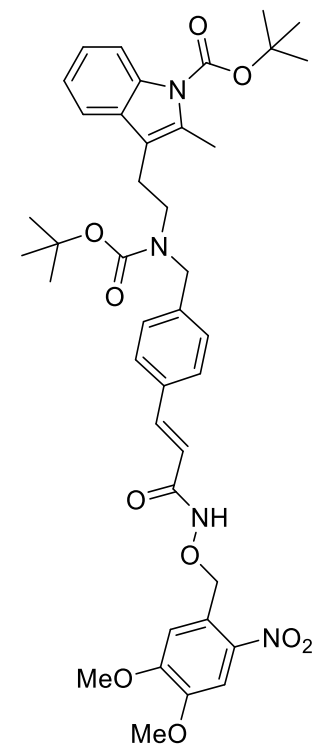
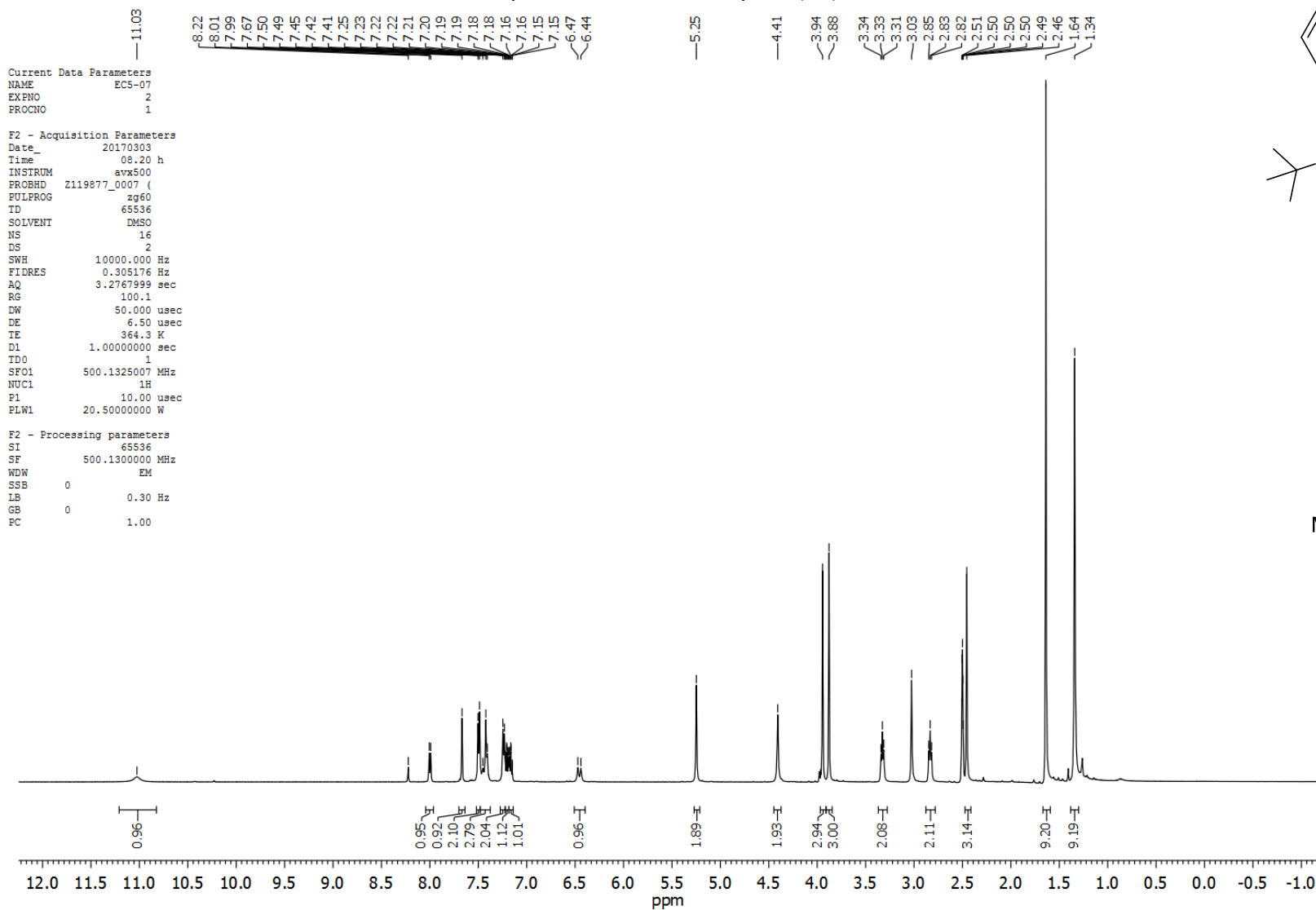
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 PLW12 0.60487000 W  
 PLW13 0.38712001 W

F2 - Processing parameters  
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 GB 0  
 PC 1.00



*tert*-Butyl-(*E*)-3-(2-((*tert*-butoxycarbonyl)(4-(3-(((4,5-dimethoxy-2-nitrobenzyl)oxy)amino)-3-oxoprop-1-en-1-yl)benzyl)amino)ethyl)-2-methyl-1*H*-indole-1-carboxylate (**10**)

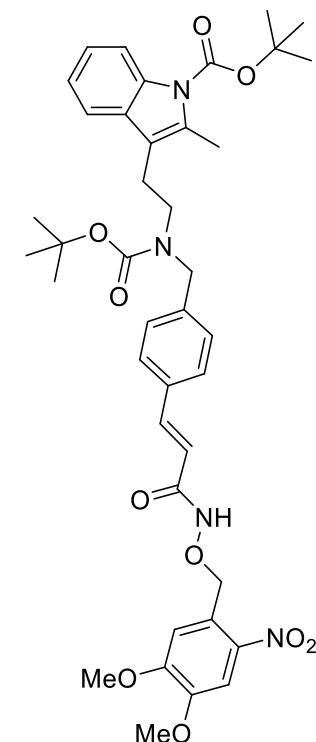
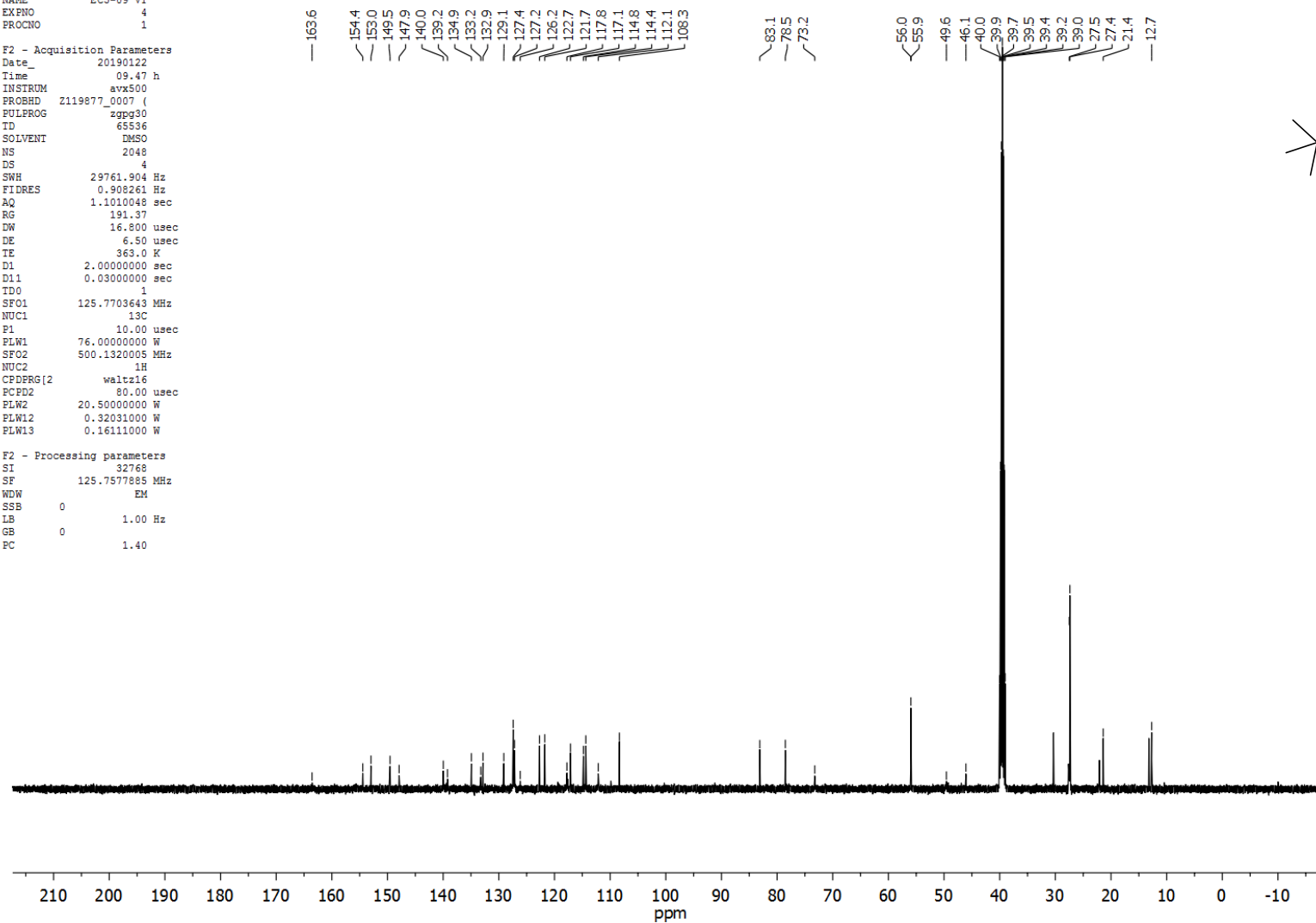


*tert*-Butyl-(*E*)-3-(2-((*tert*-butoxycarbonyl)(4-(3-(((4,5-dimethoxy-2-nitrobenzyl)oxy)amino)-3-oxoprop-1-en-1-yl)benzyl)amino)ethyl)-2-methyl-1*H*-indole-1-carboxylate (**10**)

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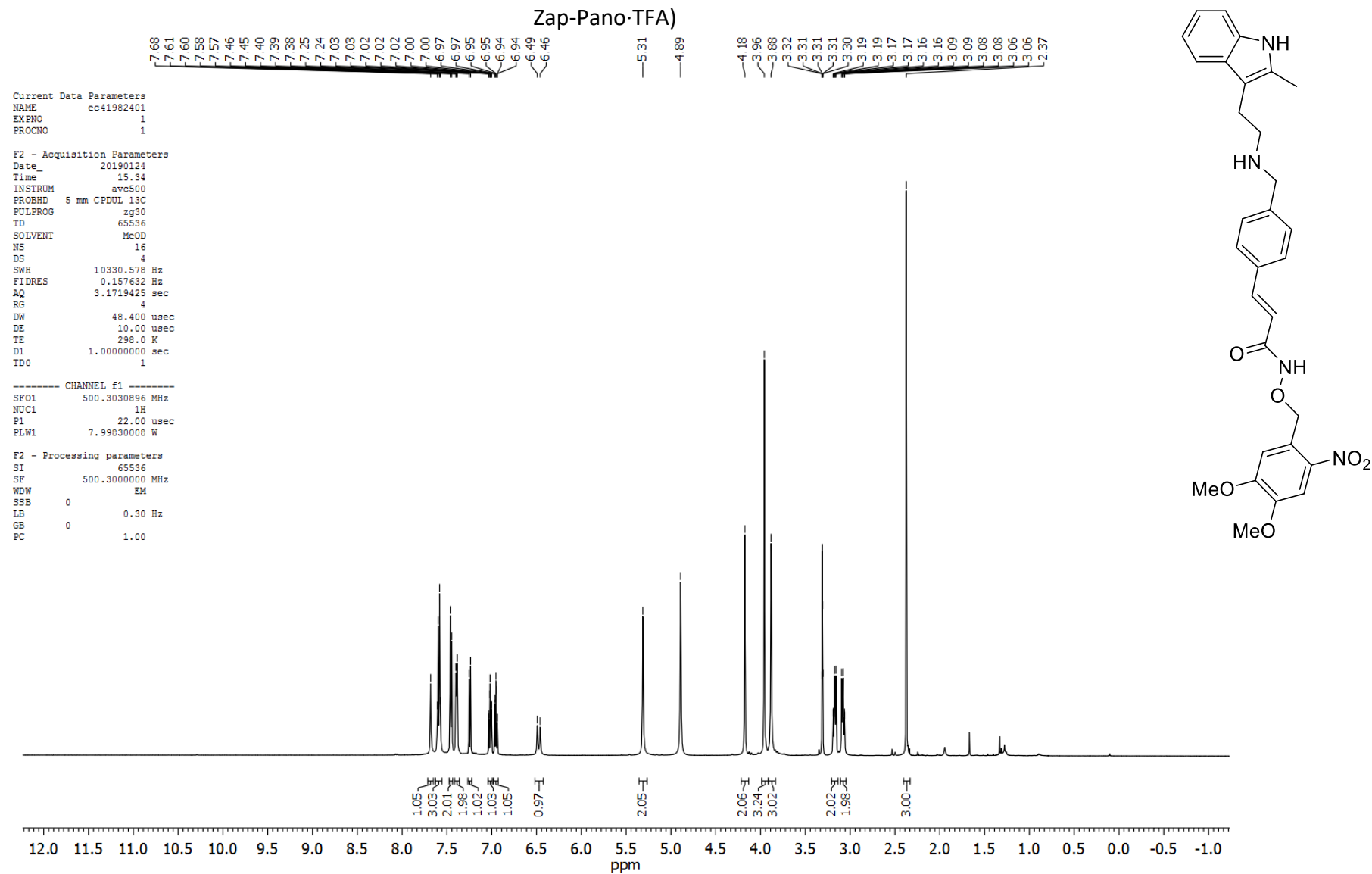
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 SOLVENT DMSO  
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 DS 4  
 SWH 29761.904 Hz  
 FIDRES 0.908261 Hz  
 AQ 1.1010048 sec  
 RG 191.37  
 DW 16.800 usec  
 DE 6.50 usec  
 TE 363.0 K  
 D1 2.0000000 sec  
 D11 0.03000000 sec  
 TD0 1  
 SFO1 125.7703643 MHz  
 NUC1 13C  
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 PLW1 76.00000000 W  
 SFO2 500.1320005 MHz  
 NUC2 1H  
 CPDPRG2 waltz16  
 PCPD2 80.00 usec  
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F2 - Processing parameters  
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 LB 1.00 Hz  
 GB 0  
 PC 1.40





(E)-N-(4-(3-((4,5-Dimethoxy-2-nitrobenzyl)oxy)-3-oxoprop-1-en-1-yl)benzyl)-2-(2-methyl-1H-indol-3-yl)ethan-1-aminium trifluoroacetate (1, Zap-Pano-TFA)



(E)-N-(4-(3-((4,5-Dimethoxy-2-nitrobenzyl)oxy)-3-oxoprop-1-en-1-yl)benzyl)-2-(2-methyl-1H-indol-3-yl)ethan-1-aminium trifluoroacetate (1, Zap-Pano-TFA)

```

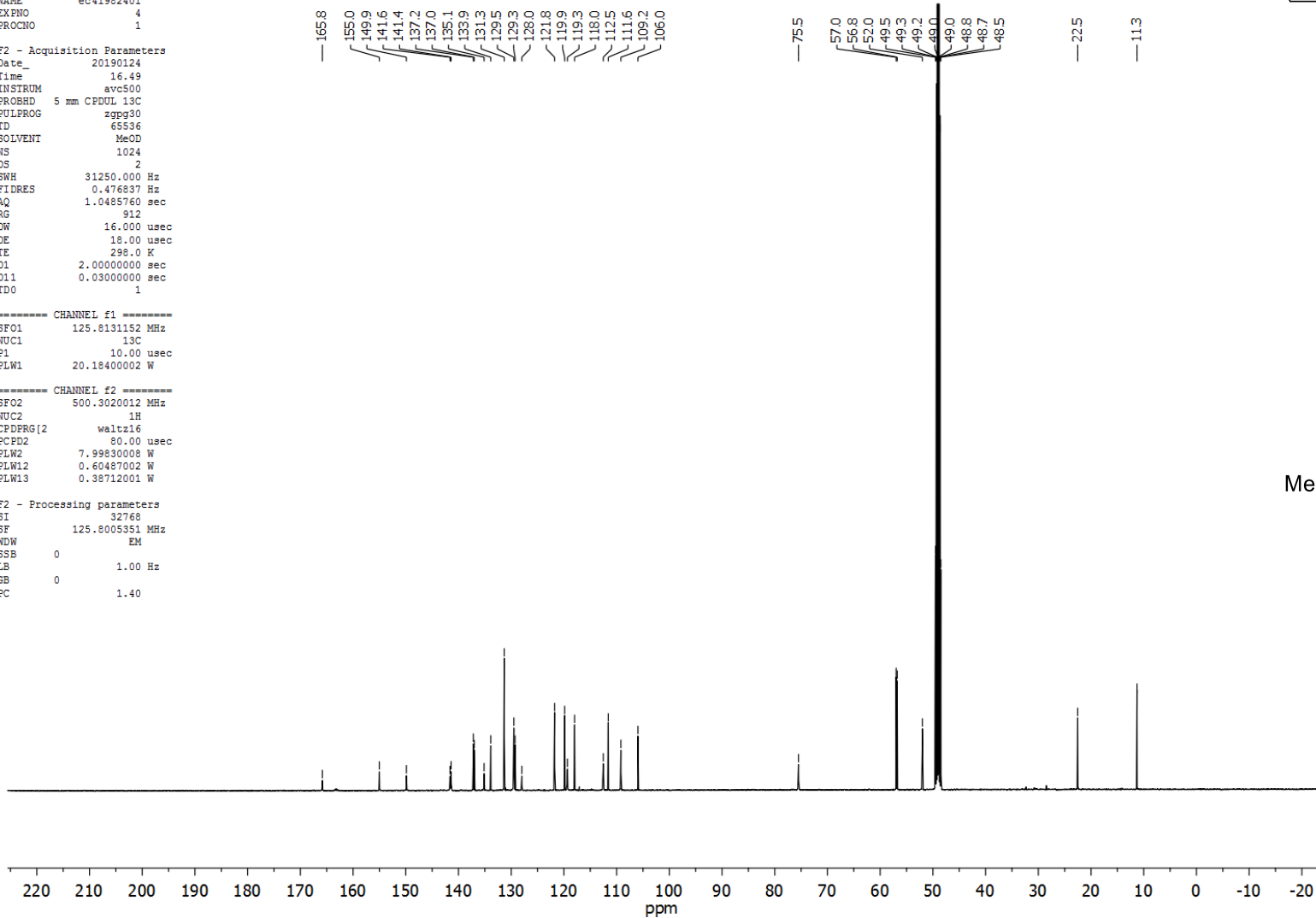
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SOLVENT   MeOD
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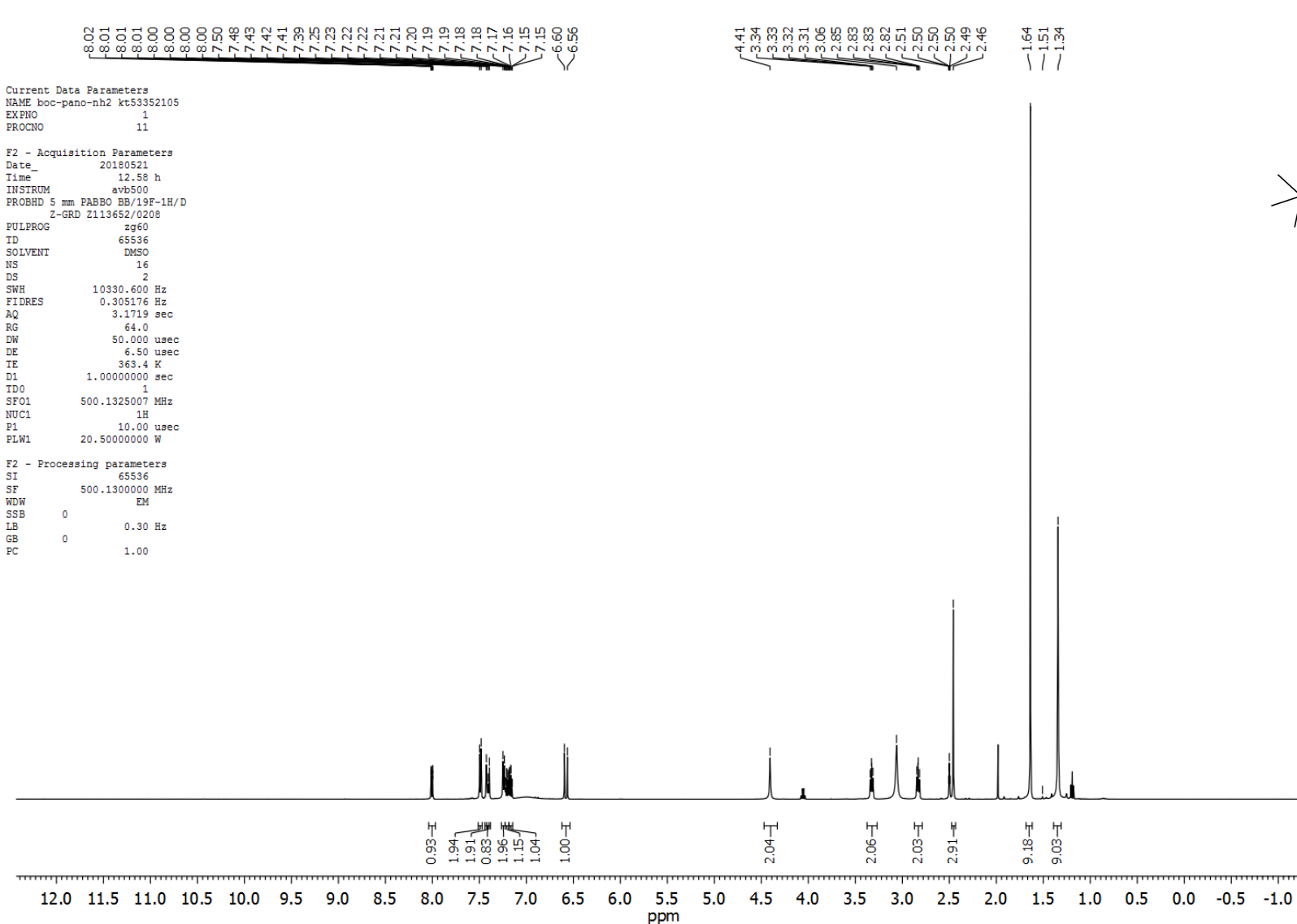
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PLW12      0.60487002 W
PLW13      0.38712001 W

F2 - Processing parameters
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LB         1.00 Hz
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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-enamide (S1)



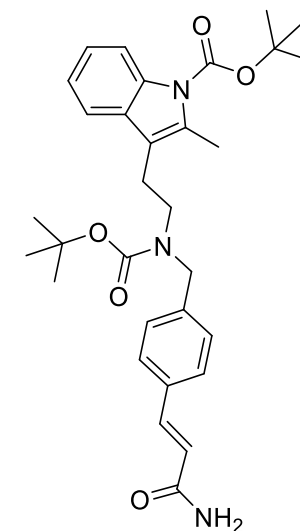
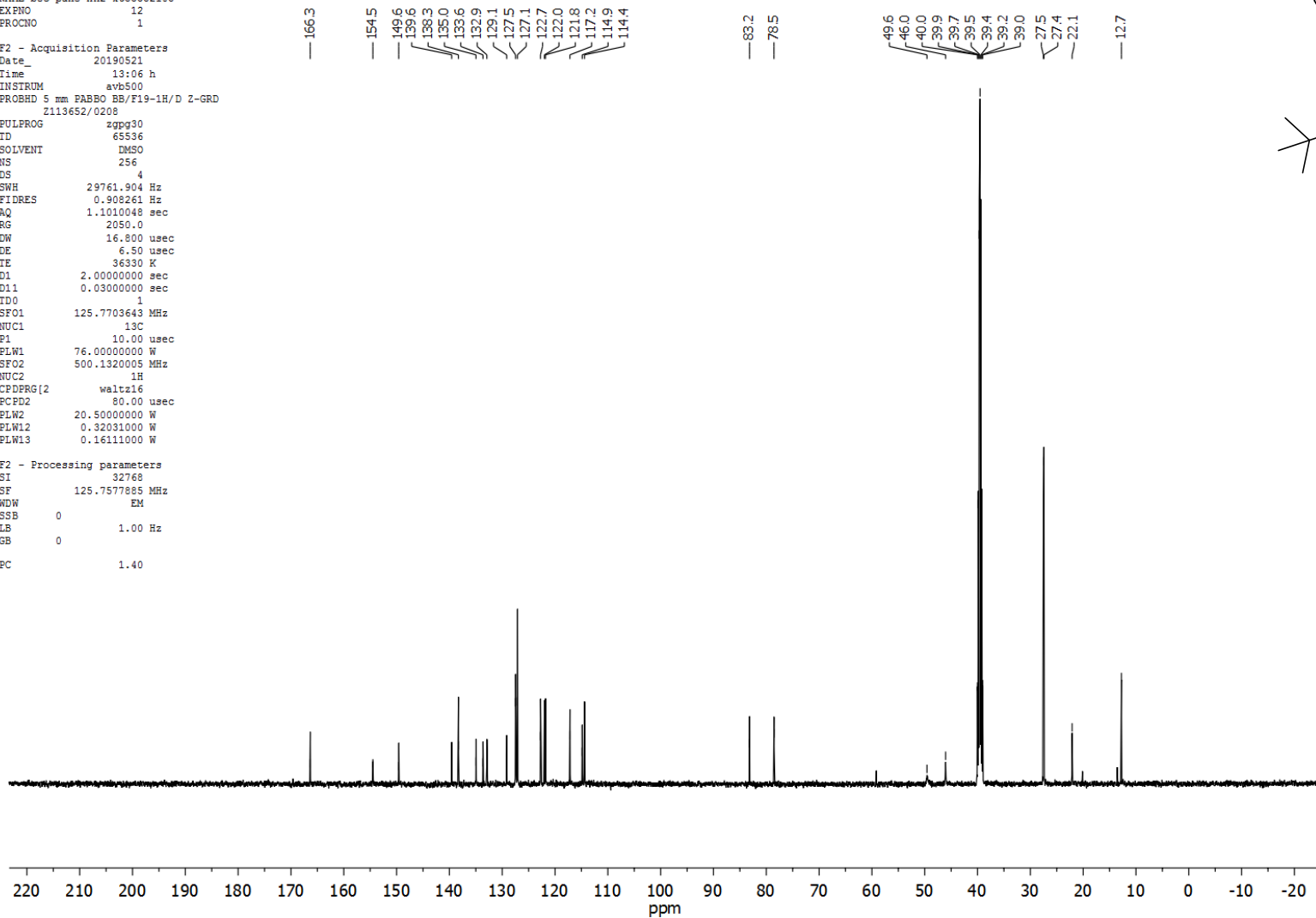
(E)-3-(4-{{tert-Butyloxycarbonyl-(2-{1-[tert-butyloxycarbonyl]-2-methyl-1H-indol-3-yl}ethyl)amino)methyl}phenyl)prop-2-enamide (S1)

```

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PROCNO 1

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PULPROG zgpg30
TD 65536
SOLVENT DMSO
NS 256
DS 4
SWH 29761.904 Hz
FIDRES 0.908261 Hz
AQ 1.1010048 sec
RG 2050.0
DW 16.800 usec
DE 6.50 usec
TE 36330 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
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
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PLW12 0.32031000 W
PLW13 0.16111000 W

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LB 1.00 Hz
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(E)-3-(4-[(2-{2-Methyl-1H-indol-3-yl}ethyl)amino]methyl)phenyl)prop-2-enamide trifluoroacetate (**15**)

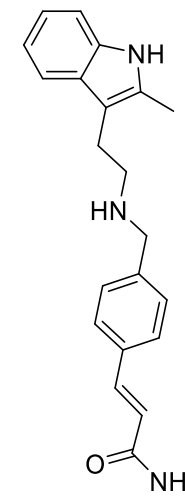
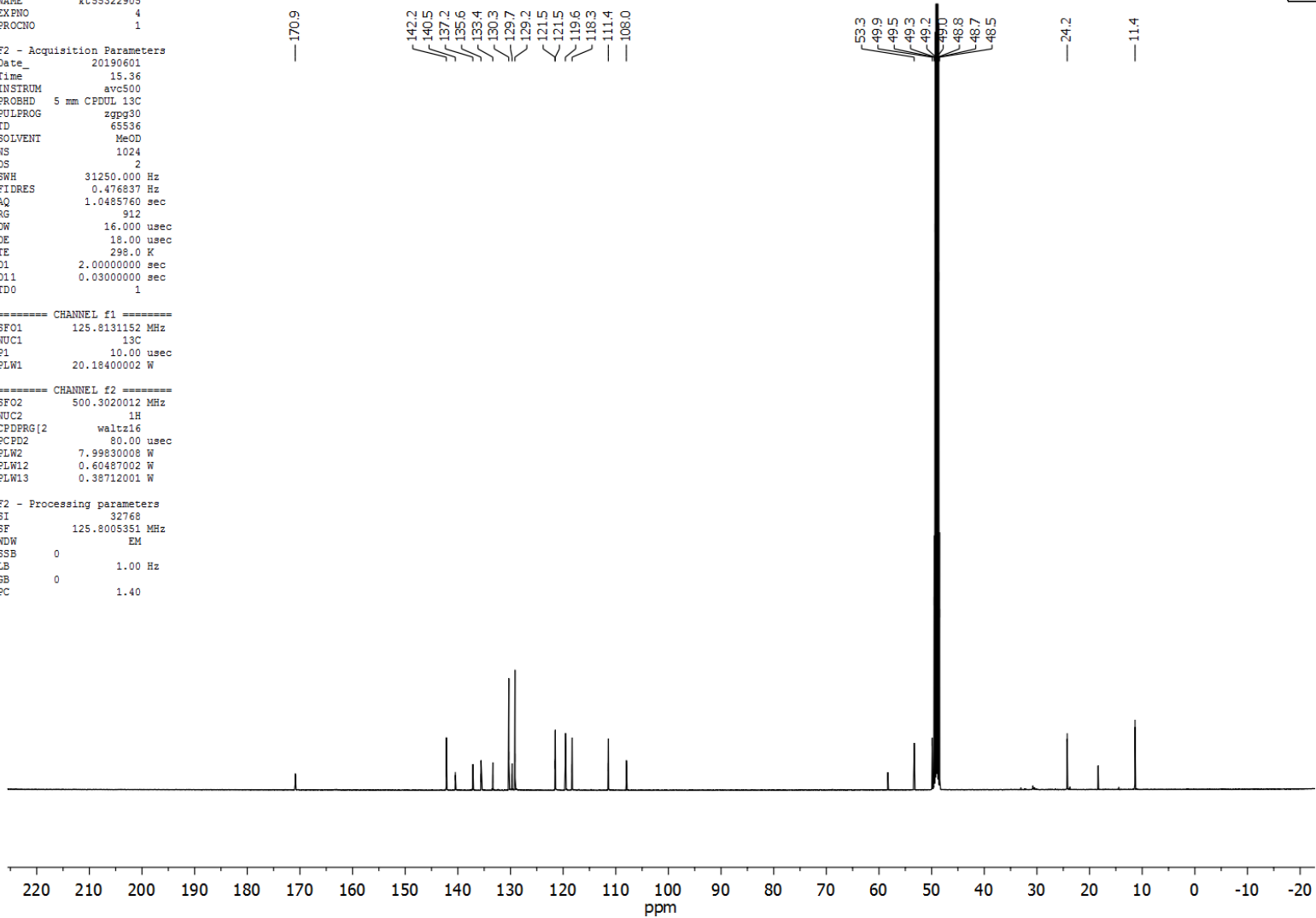
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FIDRES 0.476837 Hz  
AQ 1.0485760 sec  
RG 912  
DW 16.000 usec  
DE 18.00 usec  
TE 296.0 K  
D1 2.0000000 sec  
D11 0.0300000 sec  
TD0 1

==== CHANNEL f1 =====  
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NUC2 1H  
CPDPRG2 waltz16  
PCPD2 80.00 usec  
PLW2 7.99830008 W  
PLW12 0.60487002 W  
PLW13 0.38712001 W

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LB 1.00 Hz  
GB 0  
PC 1.40



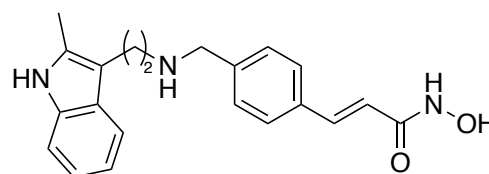
# Panobinostat (2)

EC4-14 Pano

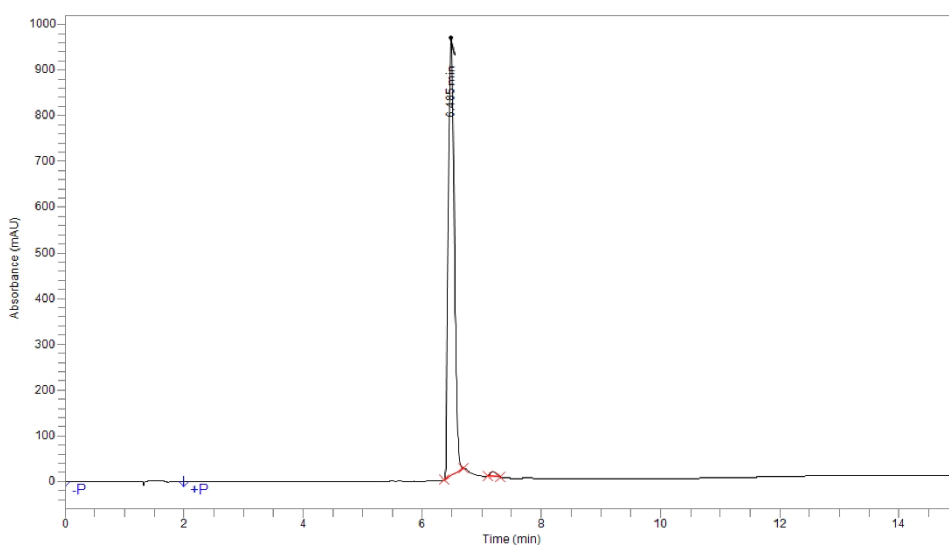
2/26/2018



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
<b>Total</b>		6,698,440.7	100.00

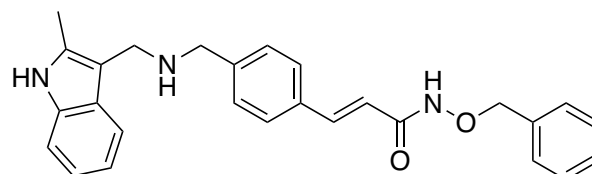
(*E*)-*N*-(4-(3-((Benzyloxy)amino)-3-oxoprop-1-en-1-yl)benzyl)-2-(2-methyl-1*H*-indol-3-yl)ethan-1-aminium 2,2,2-trifluoroacetate (**14**)

EC5-03

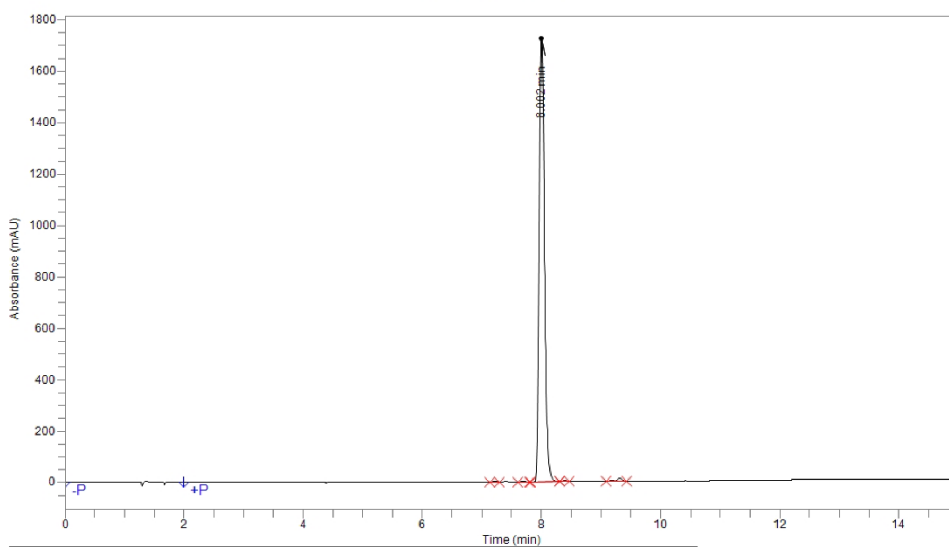
11/15/2018



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
<b>Total</b>		10,361,839.6	100.00



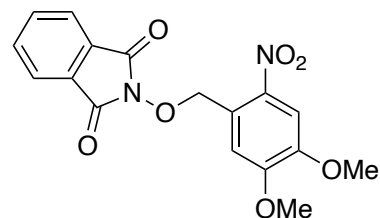
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EC5-07

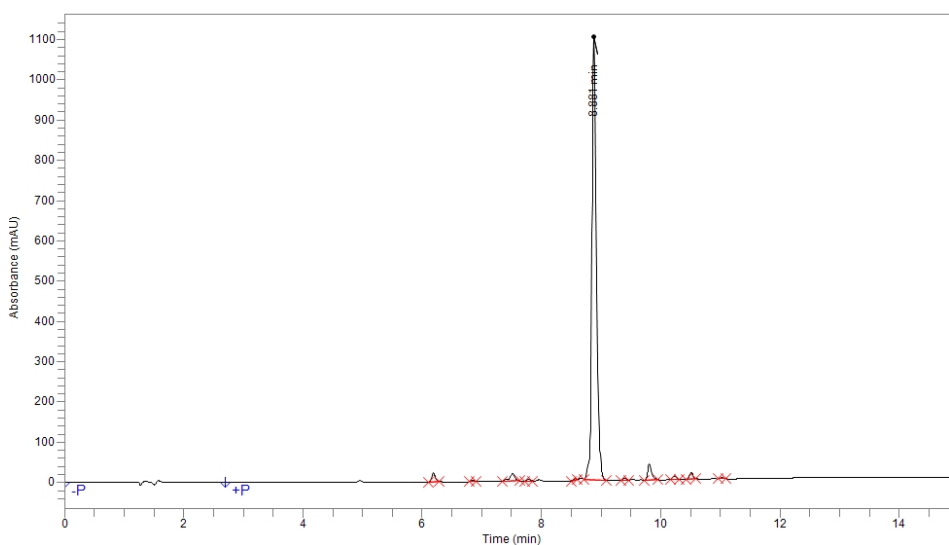
2/4/2019



Acquisition Method      Purity short run @254 nm  
 Acquisition Date/Time    2/4/2019 2:56 pm  
 Injection Volume          15  
 Sample Name                EC5-07  
 Sample Description  
 Batch Description         For start up



EC5-07 : Injection 1



Time	Height	Area	Area %
6.187	22,291.7	95,260.7	1.56
6.849	3,125.7	10,874.1	0.18
7.515	19,069.4	121,228.5	1.98
7.786	4,729.3	18,356.1	0.30
8.568	3,096.0	9,847.7	0.16
8.881	1,100,867.4	5,542,449.0	90.73
9.396	5,132.9	19,542.5	0.32
9.812	40,597.3	180,004.5	2.95
10.230	7,652.0	28,388.1	0.46
10.512	16,526.1	67,792.6	1.11
11.025	3,921.4	14,825.9	0.24
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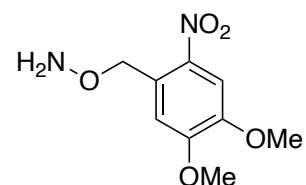
# O-(4, 5-Dimethoxy-2-nitrobenzyl)-hydroxylamine (8)

EC5-08

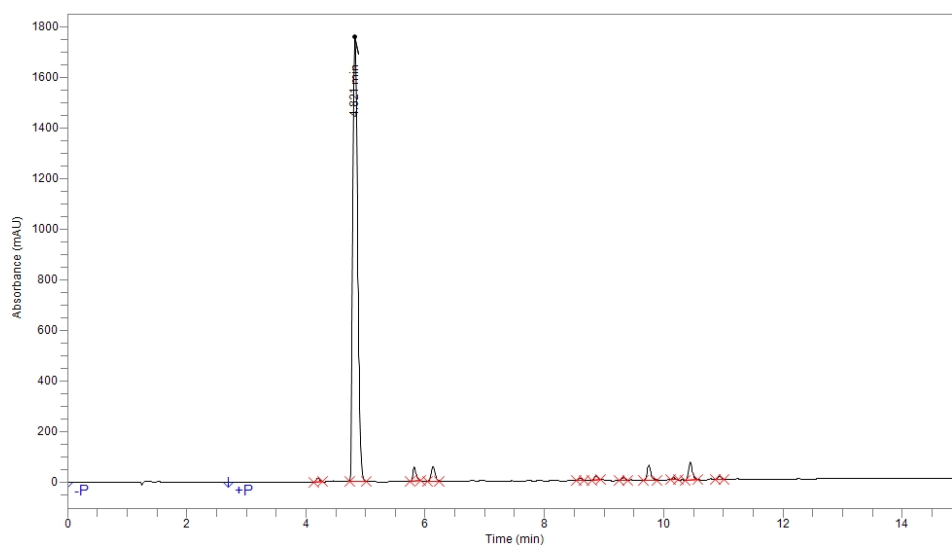
2/4/2019



Acquisition Method Purity short run @254 nm  
 Acquisition Date/Time 2/4/2019 3:31 pm  
 Injection Volume 15  
 Sample Name EC5-08  
 Sample Description  
 Batch Description For start up



EC5-08 : Injection 1



Time	Height	Area	Area %
4.203	14,582.9	56,795.7	0.51
4.821	1,758,260.8	9,880,647.6	88.18
5.818	55,634.5	228,306.6	2.04
6.129	57,109.0	245,435.0	2.19
8.598	9,865.6	41,665.7	0.37
8.868	17,319.8	69,371.1	0.62
9.326	10,320.8	40,380.4	0.36
9.750	59,452.5	268,264.5	2.39
10.176	7,656.6	26,268.1	0.23
10.446	69,466.2	294,405.6	2.63
10.940	13,207.9	53,766.9	0.48
<b>Total</b>		11,205,307.1	100.00

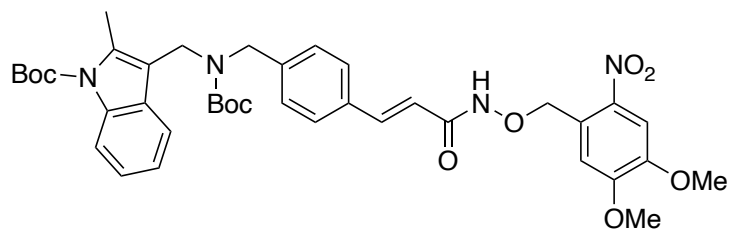
*tert*-Butyl-(*E*)-3-(2-((*tert*-butoxycarbonyl)(4-(3-(((4,5-dimethoxy-2-nitrobenzyl)oxy)amino)-3-oxoprop-1-en-1-yl)benzyl)amino)ethyl)-2-methyl-1*H*-indole-1-carboxylate (**10**)

EC2-46

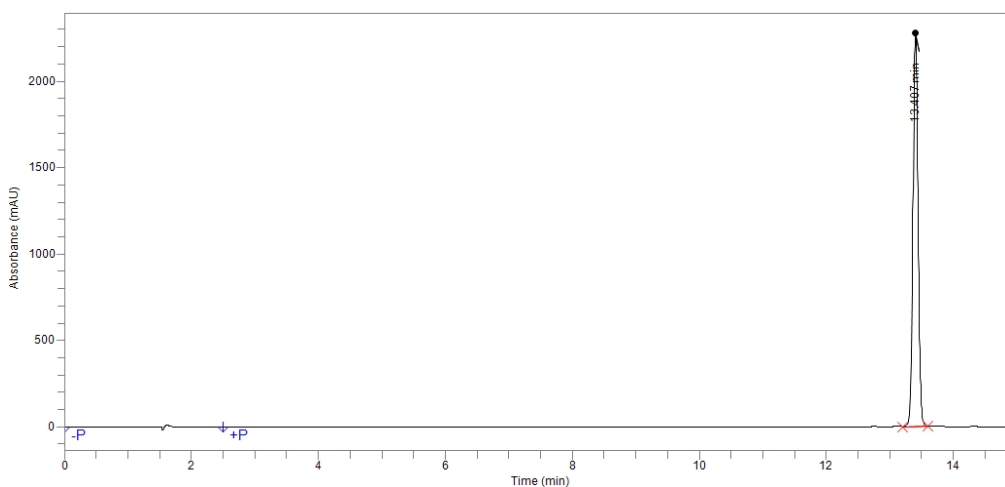
Purity short run @254 nm

2/26/2017 4:01 pm

Sample Name EC2-46  
 Vial Number 1  
 Injection Volume 10  
 Acquisition Date/Time 2/26/2017 3:42 pm  
 Acquisition Method Purity short run @254 nm  
 Processing Method Purity short run @254 nm



EC2-46 : Injection 1



Time	Area	Area %
13.407	13,108,258.3	100.00
<b>Total</b>	13,108,258.3	100.00

2/26/2017 4:01 pm

Flexar HPLC 2

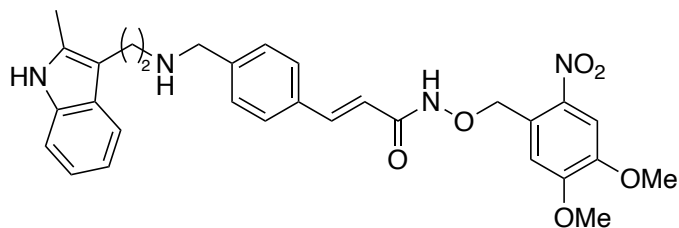
(E)-N-(4-(3-((4,5-Dimethoxy-2-nitrobenzyl)oxy)-3-oxoprop-1-en-1-yl)benzyl)-2-(2-methyl-1H-indol-3-yl)ethan-1-aminium trifluoroacetate (**1**, Zap-Pano·TFA)

EC5-16

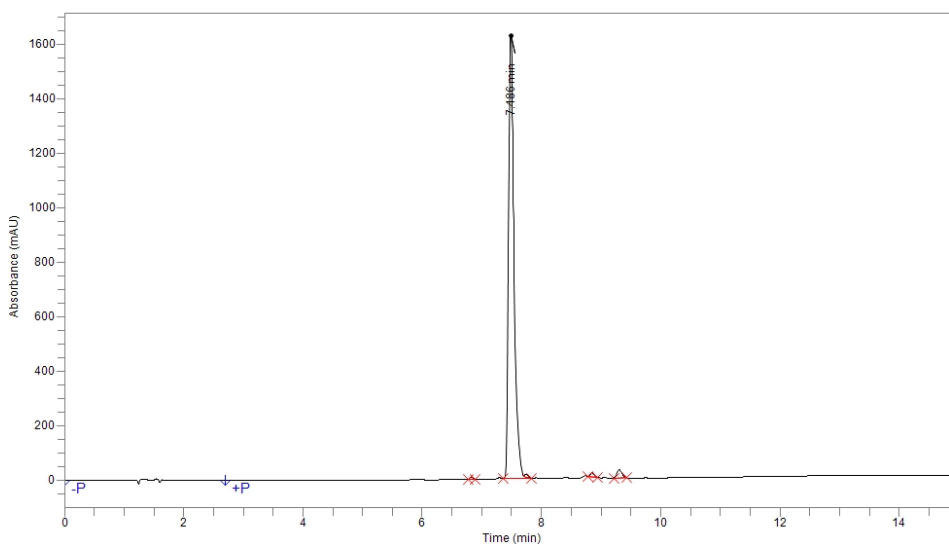
2/4/2019



Acquisition Method Purity short run @254 nm  
 Acquisition Date/Time 2/4/2019 4:27 pm  
 Injection Volume 10  
 Sample Name EC5-16  
 Sample Description  
 Batch Description For start up



EC5-16 : Injection 1



Time	Height	Area	Area %
6.826	6,818.0	25,051.6	0.23
7.486	1,624,206.1	10,592,235.0	97.20
7.746	12,212.0	51,254.2	0.47
8.849	15,436.2	67,951.5	0.62
9.303	29,285.4	160,642.4	1.47
<b>Total</b>		10,897,134.7	100.00

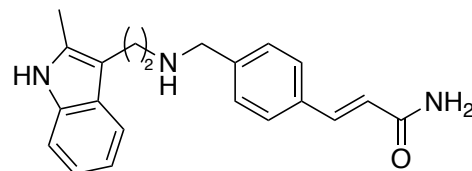
(E)-3-(4-(((2-(2-Methyl-1H-indol-3-yl)ethyl)amino)methyl)phenyl)prop-2-enamide (**15**)

EC2-49

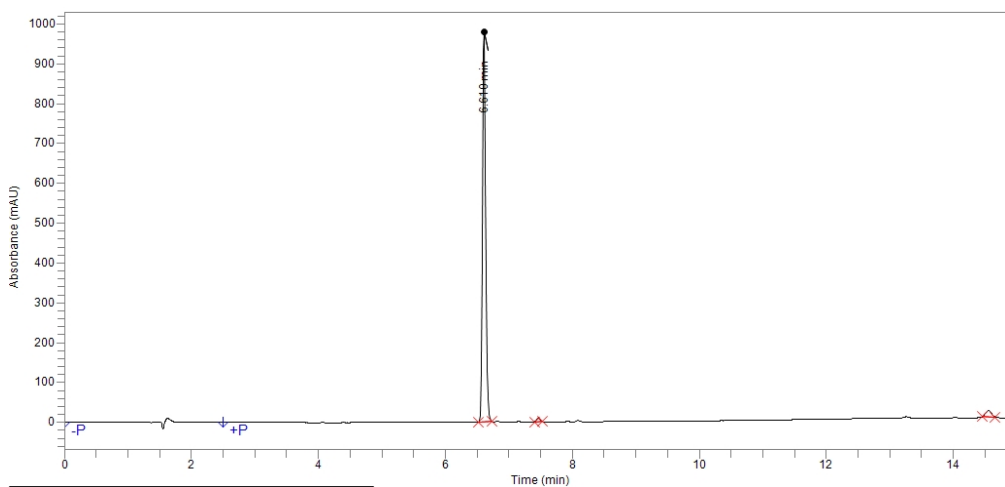
Purity short run @254 nm

3/7/2017 10:13 am

Sample Name EC2-49  
Vial Number 1  
Injection Volume 10  
Acquisition Date/Time 3/7/2017 9:56 am  
Acquisition Method Purity short run @254 nm  
Processing Method Purity short run @254 nm



EC2-49 : Injection 1



Time	Area	Area %
6.610	3,324,959.2	96.21
7.462	33,716.5	0.98
14.555	97,152.7	2.81
<b>Total</b>	<b>3,455,828.4</b>	<b>100.00</b>

3/7/2017 10:13 am

Flexar HPLC 2

# LCMS comparison of Pano-NH<sub>2</sub> (15) and Pano acid

## Preparative LC - With MS Spectra

PANO acid\_PANO amide SPIKE

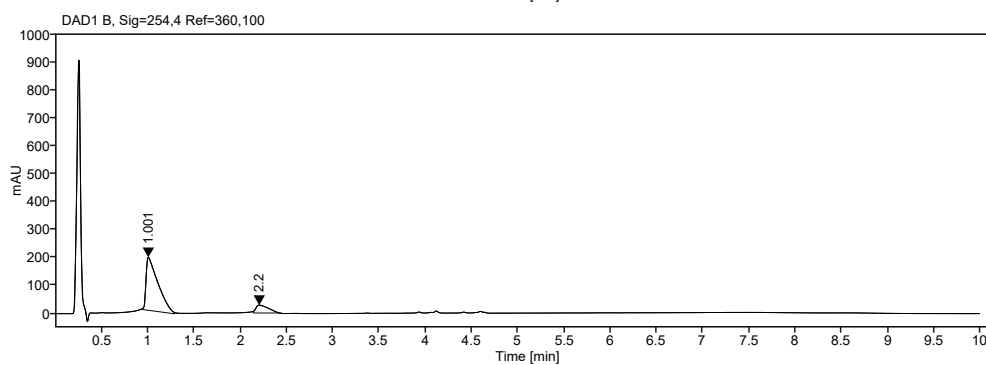
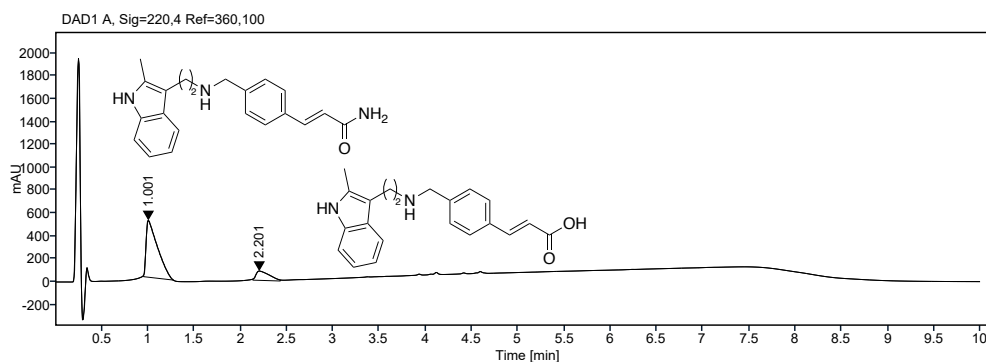


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**Sample name:** PANO acid\_PANO amide SPIKE

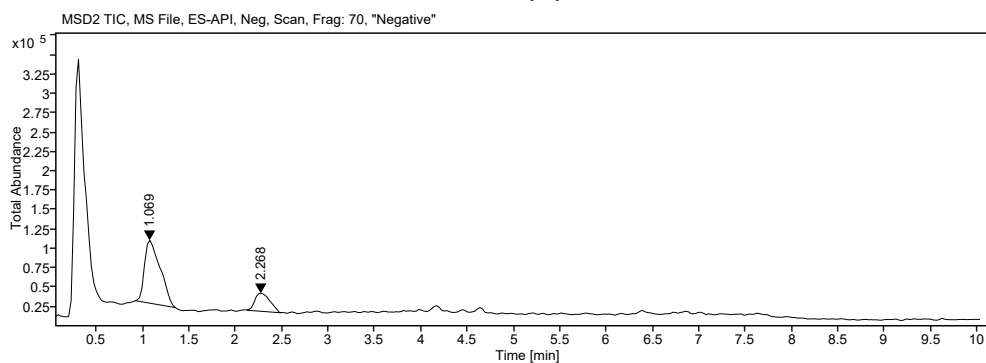
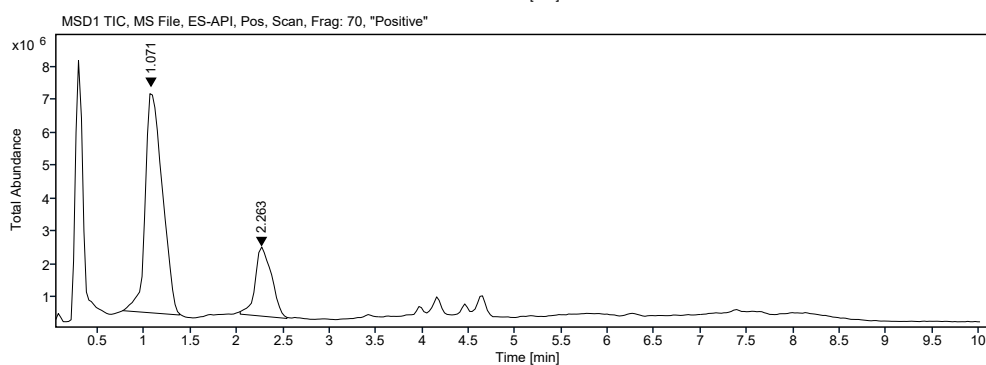
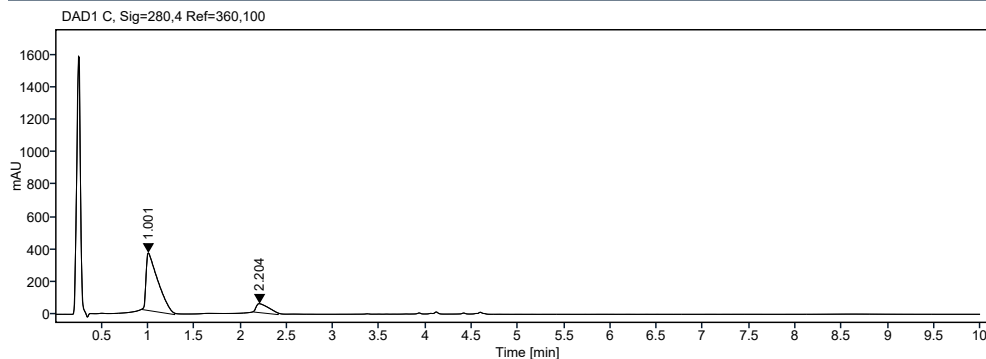
**Description:**

<b>Injection:</b> 1 of 1	<b>Location:</b> P1-E11
<b>Injection volume:</b> 10.000	<b>Sample amount:</b> 0.000
<b>Instrument:</b> LCMS	<b>Injection date:</b> 2021-07-02 07:20:50+00:00
<b>Acq. operator:</b> SYSTEM	<b>Acq. method:</b> ZAP-PANO_MECN_20_80_H2O_FA_100_1000_POS_NEG.FULL.M
<b>Analysis method:</b> ZAP-PANO_MECN_20_80_H2O_FA_100_1000_POS_NEG.FULL.M	<b>Last changed:</b> 2021-07-02 07:11:00+00:00



# Preparative LC - With MS Spectra

PANO acid\_PANO amide SPIKE

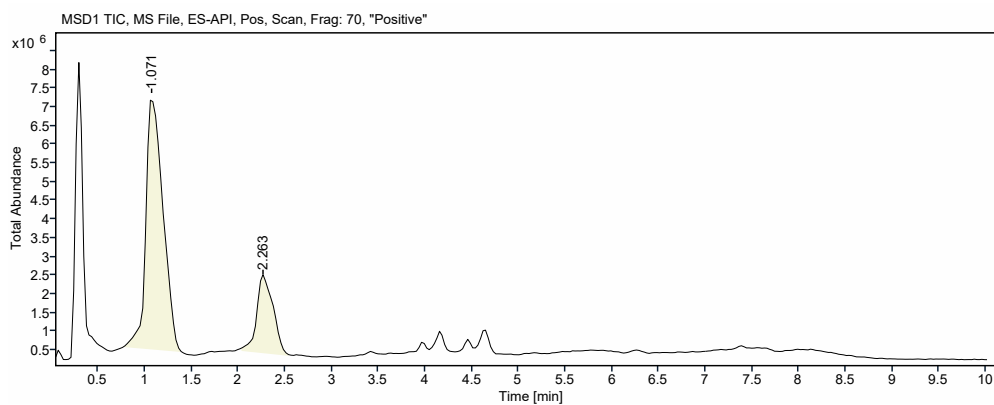


# Preparative LC - With MS Spectra

PANO acid\_PANO amide SPIKE



Signal details: MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"



RT [min]	Signal Description	Symmetry	Resolution	Height [count]	Area [count*min]	Rel. Area [%]
1.071	MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"	0.51739		6858366	86063536	77.0
2.263	MSD1 TIC, MS File, ES-API, Pos, Scan, Frag: 70, "Positive"	0.62214	3.72645	2113574	25744076	23.0

Filtered on peak height > 100000 counts

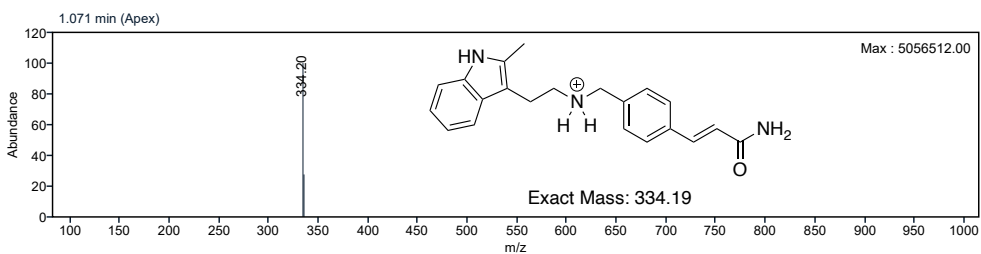


## Preparative LC - With MS Spectra

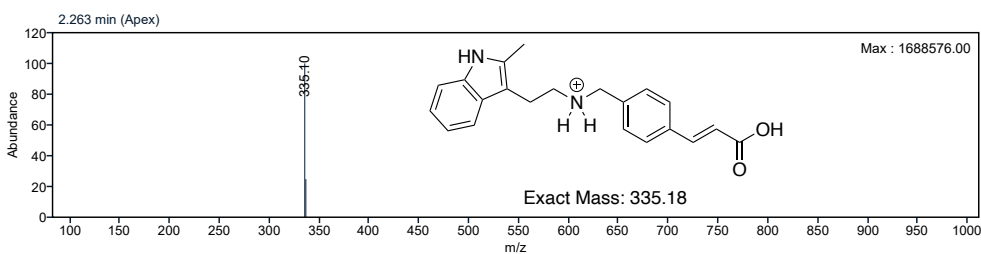
PANO acid\_PANO amide SPIKE



Retention time: 1.071 min Area Percent: 77%



Retention time: 2.263 min Area Percent: 23%



Acquisition Method: ZAP-PANO\_MECN\_20\_80\_H2O\_FA\_100\_1000\_POS\_NEG.FULL.M  
Path: C:\Users\Public\Documents\ChemStation\1\Data\PANO acid\_DSCH05051 2021-07-02 06-47-46  
Acquisition Method: ZAP-PANO\_MECN\_20\_80\_H2O\_FA\_100\_1000\_POS\_NEG.FULL.M  
Path: C:\Users\Public\Documents\ChemStation\1\Data\PANO acid\_DSCH05051 2021-07-02 06-47-46  
Data Analysis Method: ZAP-PANO\_MECN\_20\_80\_H2O\_FA\_100\_1000\_POS\_NEG.FULL.M  
Path: C:\Users\Public\Documents\ChemStation\1\Data\PANO acid\_DSCH05051 2021-07-02 06-47-46

### Method Information

Method: C:\Users\Public\Documents\ChemStation\1\Data\PANO acid\_DSCH05051 2021-07-02 06-47-46\ZAP-PANO\_MECN\_20\_80\_H2O\_FA\_100\_1000\_POS\_NEG.FULL.M  
Modified: 1/1/1970 at 12:00:00 AM  
Description: 80%H2O/20%MeCN +0.1% FA to 30%H2O/70%MeCN + 0.1% FA over 6 minutes, 40 °C