Norepinephrine and its metabolites are involved in the synthesis of neuromelanin derived from the *locus coeruleus*

Kazumasa Wakamatsu, Keisuke Tabuchi, Makoto Ojika, Fabio A. Zucca, Luigi Zecca and Shosuke Ito

Supporting information

Additional Supporting Information may be found in the online version of this article.

Figure S1. ^{1}H-NMR spectra of CMT-DA.

Figure S2. ^{13}C-NMR spectra of CMT-DA.

Figure S3. HRMS spectra of CMT-DA.

Figure S4. ^{1}H-NMR spectra of 5-*S*-Cys-CMT-DA.

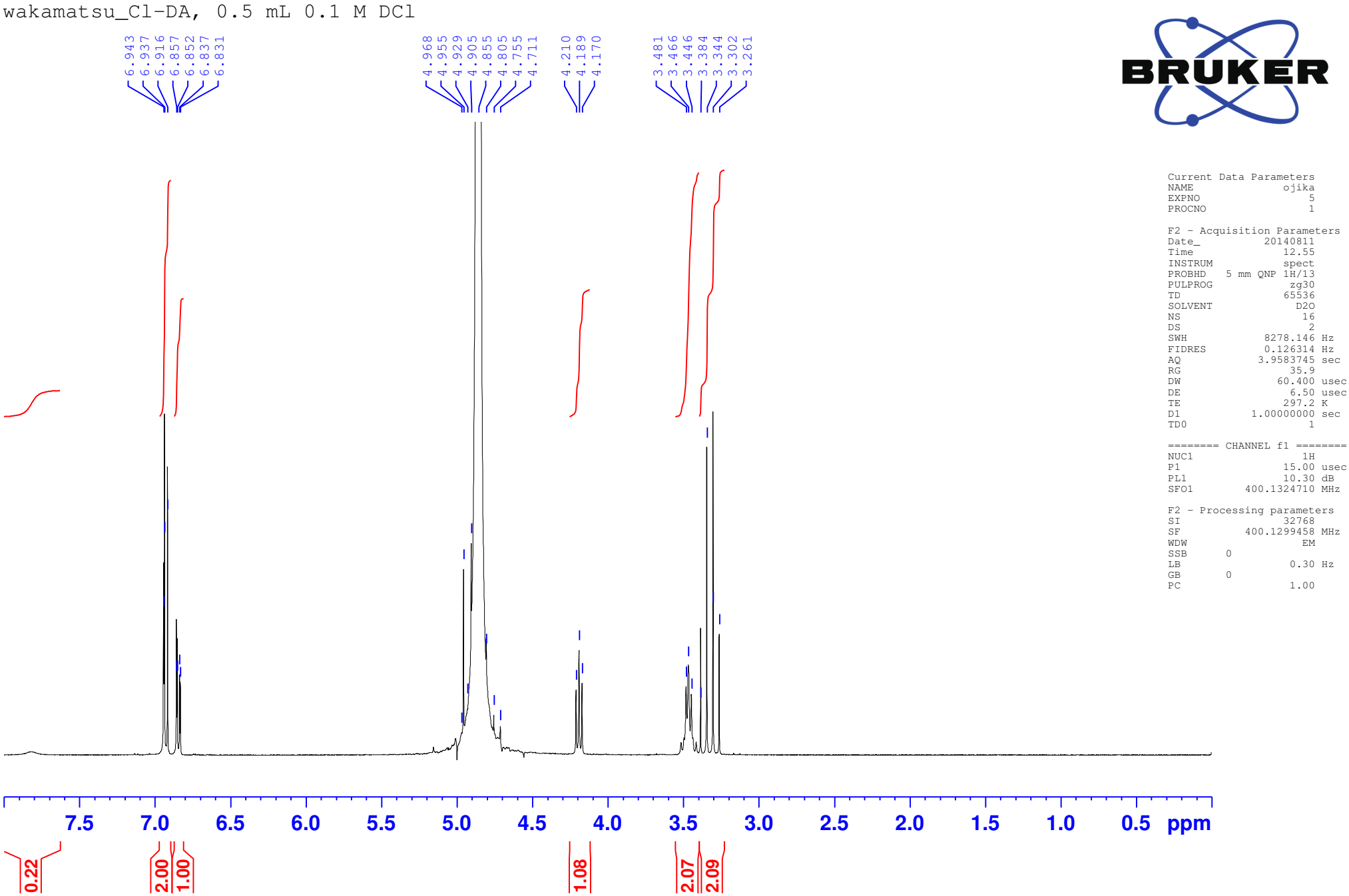
Figure S5. ^{13}C-NMR spectra of 5-*S*-Cys-CMT-DA.

Figure S6. HRMS spectra of 5-*S*-Cys-CMT-DA.

Figure S7. 5-*S*-Cys-CMT-DA by Ion Trap LC-MS

Figure S8. LC-NM by Ion Trap LC-MS

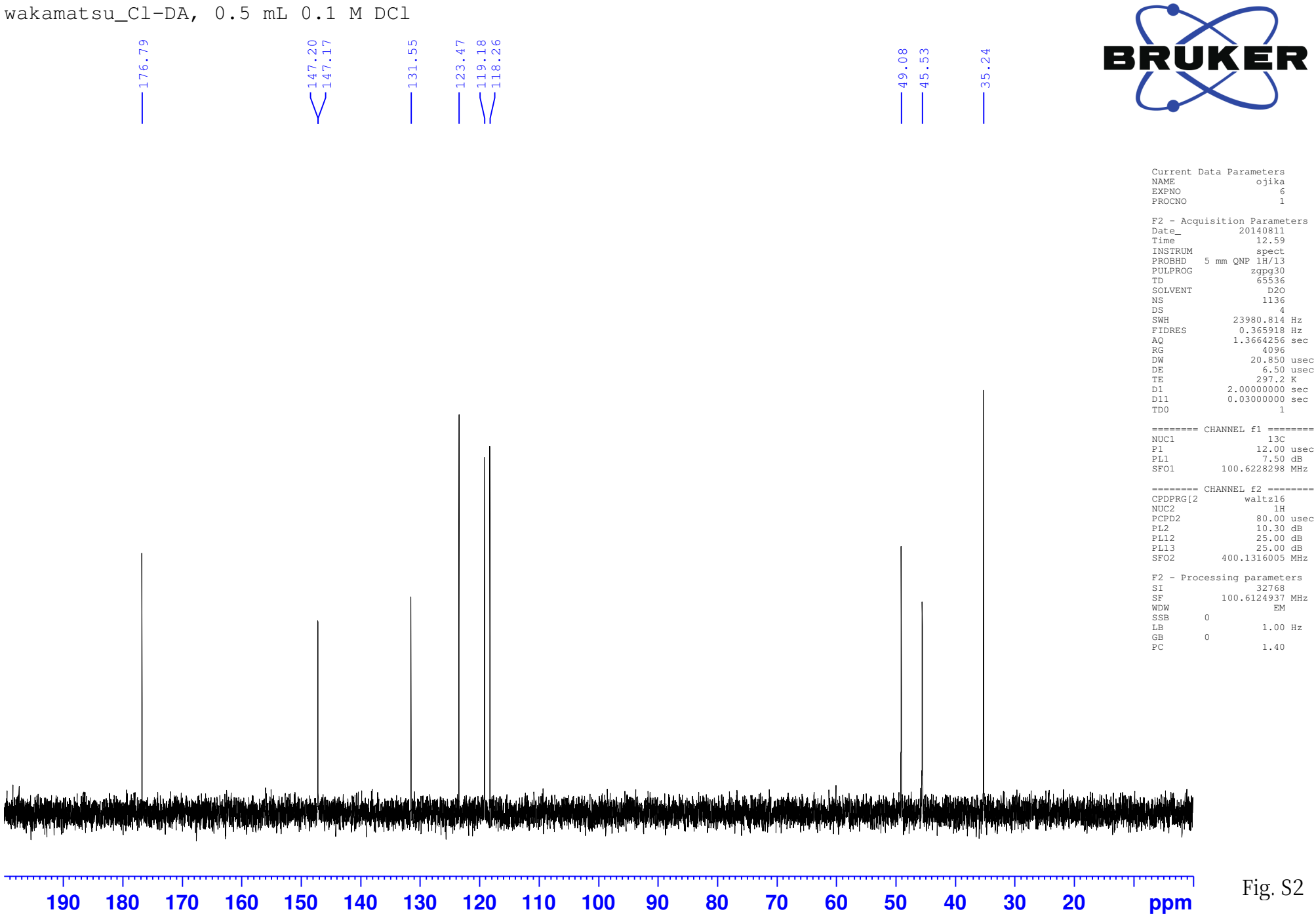
Figure S9. Proposed mechanism for the production of CMT-DA by hydrolysis of NE with 6 M HCl in the presence of thioglycolic acid. DA-thioether derivative CMT-DA may be produced from NE via the quinonemethide intermediate.




```
Current Data Parameters
NAME                  ojika
EXPNO                    5
PROCNO                   1

F2 - Acquisition Parameters
Date_              20140811
Time                 12.55
INSTRUM              spect
PROBHD    5 mm QNP 1H/13
PULPROG              zg30
TD                  65536
SOLVENT               D2O
NS                     16
DS                      2
SWH             8278.146 Hz
FIDRES          0.126314 Hz
AQ              3.9583745 sec
RG                   35.9
DW                60.400 usec
DE                 6.50 usec
TE                297.2 K
D1             1.00000000 sec
TD0                      1

======== CHANNEL f1 ========
NUC1                    1H
P1                   15.00 usec
PL1                  10.30 dB
SFO1          400.1324710 MHz

F2 - Processing parameters
SI                   32768
SF            400.1299458 MHz
WDW                     EM
SSB          0
LB                    0.30 Hz
GB           0
PC                    1.00
```

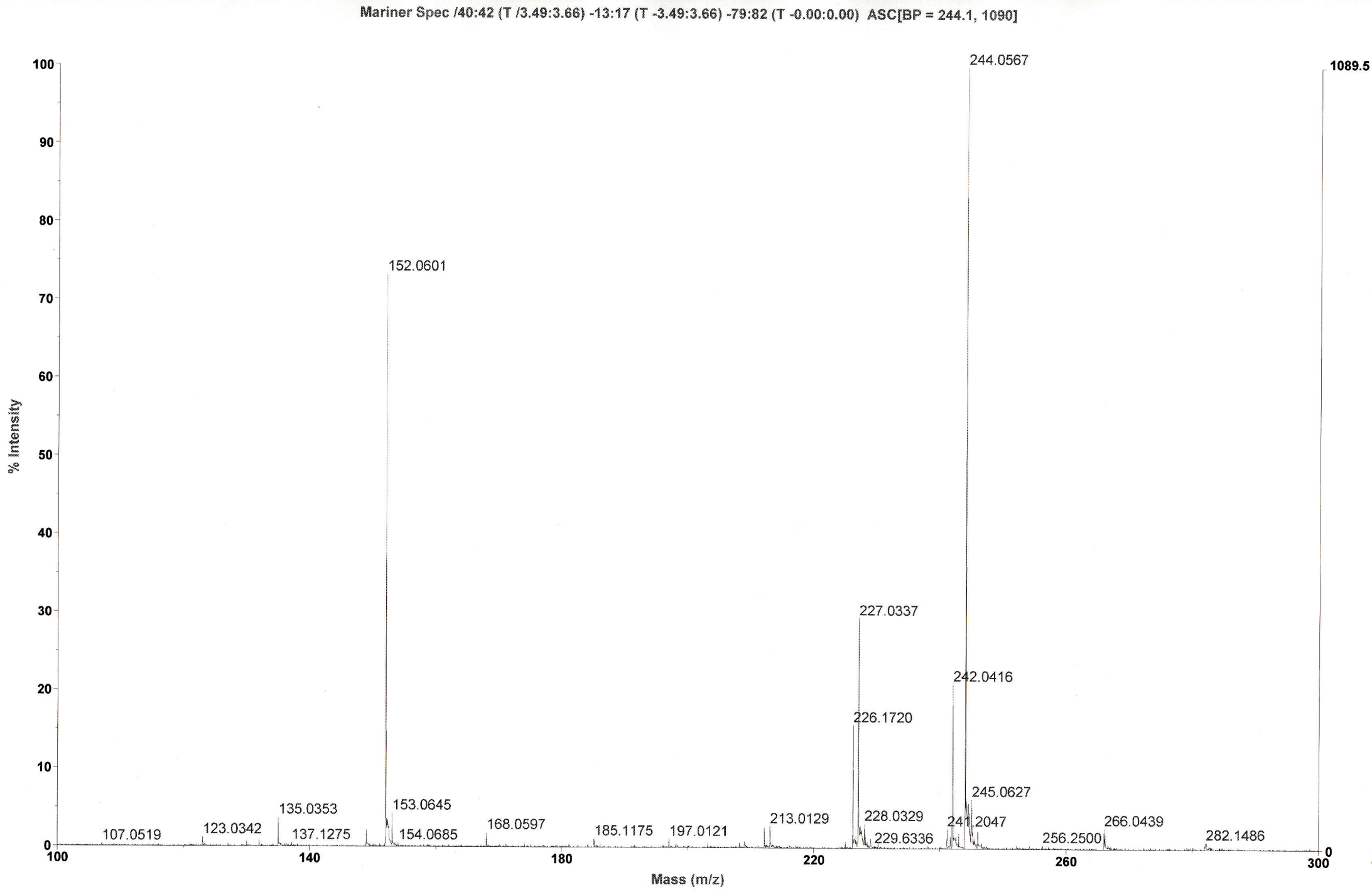
Fig. S1

wakamatsu_Cl-DA, 0.5 mL 0.1 M DCl




```
Current Data Parameters
NAME                 ojika
EXPNO                   6
PROCNO                  1

F2 - Acquisition Parameters
Date_             20140811
Time                 12.59
INSTRUM              spect
PROBHD    5 mm QNP 1H/13
PULPROG           zgpg30
TD                  65536
SOLVENT               D2O
NS                   1136
DS                      4
SWH              23980.814 Hz
FIDRES           0.365918 Hz
AQ               1.3664256 sec
RG                   4096
DW                 20.850 usec
DE                  6.50 usec
TE                 297.2 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                 12.00 usec
PL1                 7.50 dB
SFO1          100.6228298 MHz

======== CHANNEL f2 ========
CPDPRG[2          waltz16
NUC2                  1H
PCPD2              80.00 usec
PL2                10.30 dB
PL12               25.00 dB
PL13               25.00 dB
SFO2          400.1316005 MHz

F2 - Processing parameters
SI                   32768
SF            100.6124937 MHz
WDW                     EM
SSB       0
LB                  1.00 Hz
GB        0
PC                  1.40
```

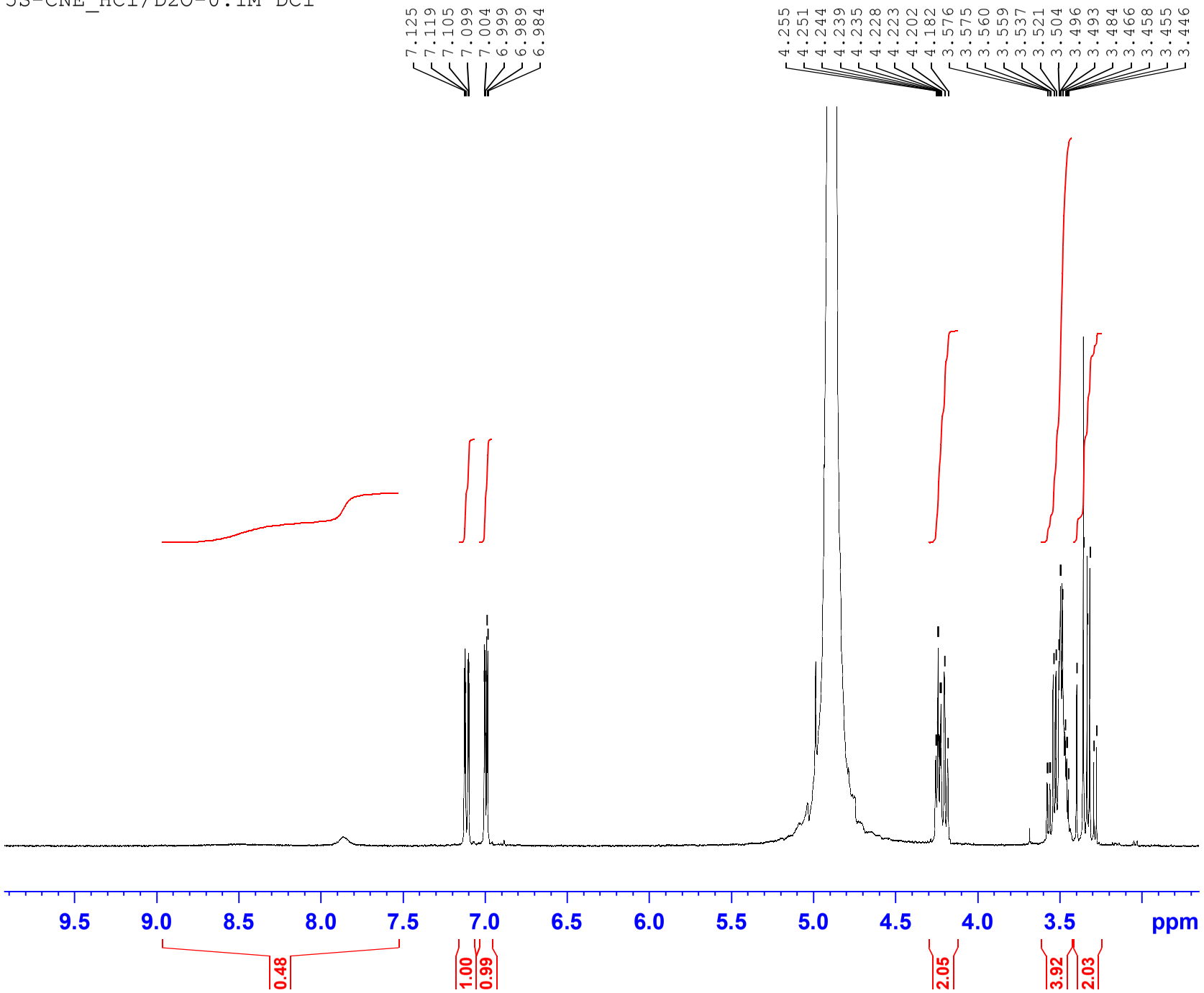
Fig. S2



10 uM, 50% MeCN-0.1% HCOOH
D:\...\CI-DA002.dat
Acquired: Aug 11 13:24:00 2014

Fig. S3

5S-CNE_HCl/D2O-0.1M DCl



Current Data Parameters
NAME ojika_irai
EXPNO 1
PROCNO 1

F2 - Acquisition Parameters
Date_ 20141024
Time 9.51
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zg30
TD 65536
SOLVENT D2O
NS 16
DS 2
SWH 8278.146 Hz
FIDRES 0.126314 Hz
AQ 3.9583745 sec
RG 35.9
DW 60.400 usec
DE 6.50 usec
TE 296.2 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
NUC1 1H
P1 15.00 usec
PL1 10.30 dB
SFO1 400.1324710 MHz

F2 - Processing parameters
SI 32768
SF 400.1299342 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00

Fig. S4

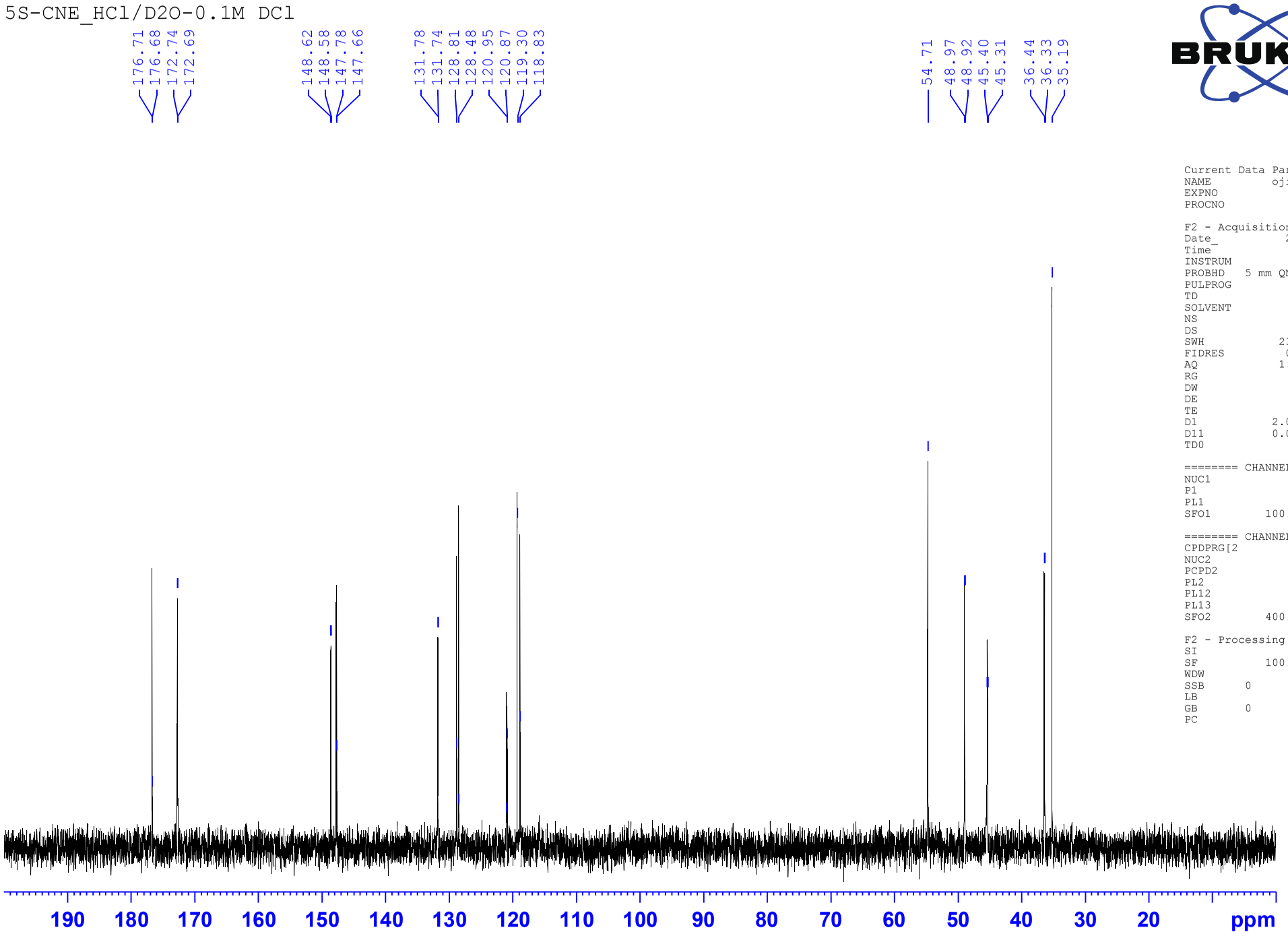
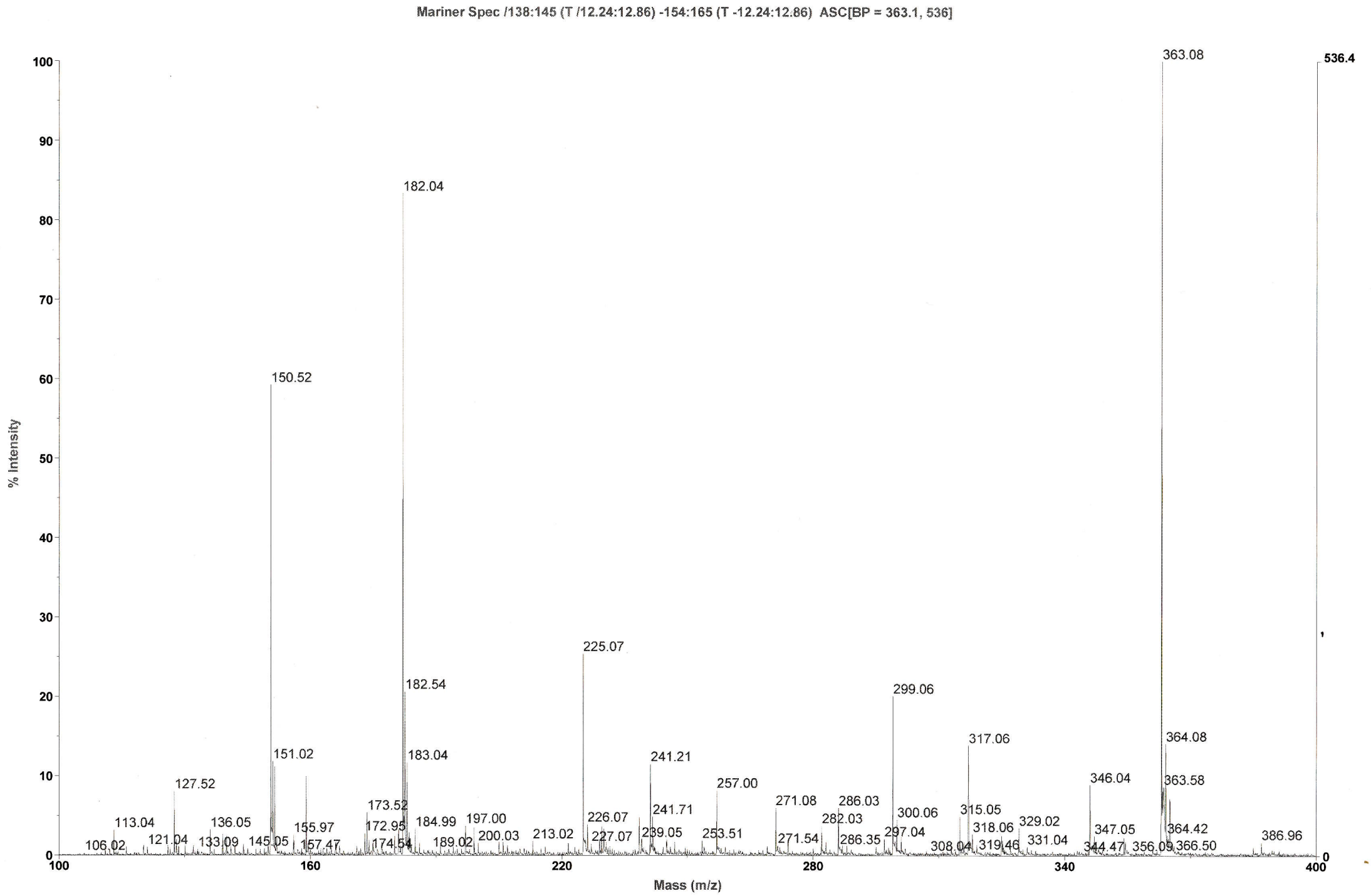

5S-CNE_HCl/D2O-0.1M DCl
176.71
176.68
172.74
172.69
148.62
148.58
147.78
147.66
131.78
131.74
128.81
128.48
120.95
120.87
119.30
118.83
54.71
48.97
48.92
45.40
45.31
36.44
36.33
35.19
Current Data Parameters
NAME ojika_irai
EXPNO 2
PROCNO 1
F2 - Acquisition Parameters
Date_ 20141024
Time 12.04
INSTRUM spect
PROBHD 5 mm QNP 1H/13
PULPROG zgpg30
TD 65536
SOLVENT D2O
NS 2308
DS 2
SWH 23980.814 Hz
FIDRES 0.365918 Hz
AQ 1.3664256 sec
RG 18390.4
DW 20.850 usec
DE 6.50 usec
TE 297.2 K
D1 2.00000000 sec
D11 0.03000000 sec
TD0 1
======== CHANNEL f1 ========
NUC1 13C
P1 12.00 usec
PL1 7.50 dB
SFO1 100.6228298 MHz
======== CHANNEL f2 ========
CPDPRG[2 waltz16
NUC2 1H
PCPD2 80.00 usec
PL2 10.30 dB
PL12 25.00 dB
PL13 25.00 dB
SFO2 400.1316005 MHz
F2 - Processing parameters
SI 32768
SF 100.6124938 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

Fig. S5



10 uM, 40% MeCN-0.1% HCOOH
D:\...\5S-Cys-NA001.dat
Acquired: Oct 24 19:01:00 2014

Fig. S6

CMT-DA (Synthetic compound)

Display Report

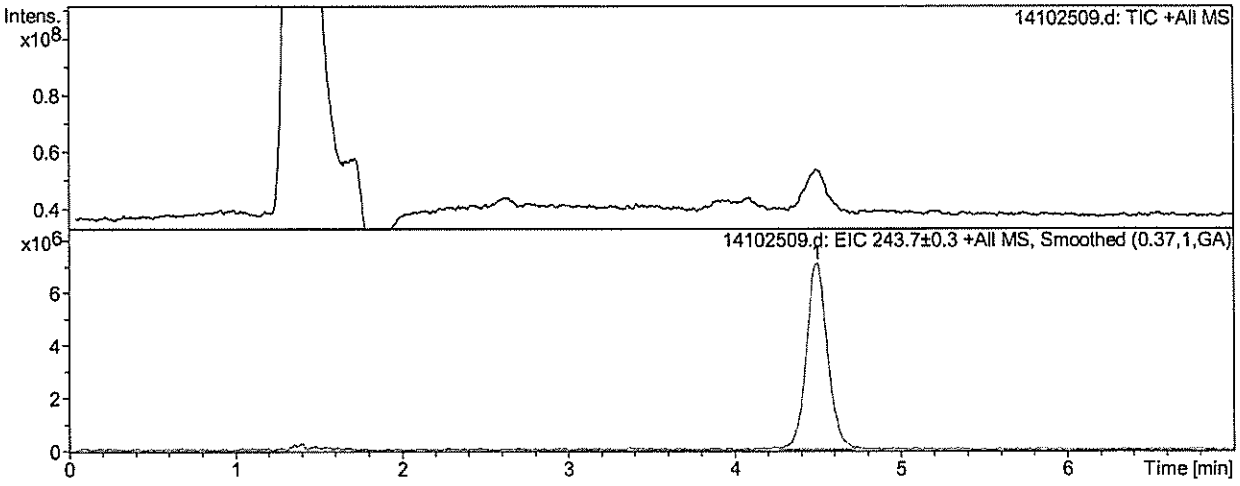
Analysis Info

Analysis Name	D:\Chem32\DATA\bioact-ojika\14102509.d	Acquisition Date	10/25/2014 19:59:40
Method	ojikaLC.m	Operator	HCT-Plus
Sample Name	b-CMT-DA_std	Instrument	HCTultra
Comment	sample#1, 1 uL of 100 times dil, 10% MeOH-0.1% HCOOH, ODS (2*75mm)		

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Ultra Scan	Scan Begin	50 m/z	Scan End	400 m/z
Capillary Exit	106.0 Volt	Skimmer	40.0 Volt	Trap Drive	38.5
Accumulation Time	5286 æs	Averages	8 Spectra	Auto MS/MS	off

#	RT [min]	Area
1	4.5	63237079
n.a.	4.5	n.a.



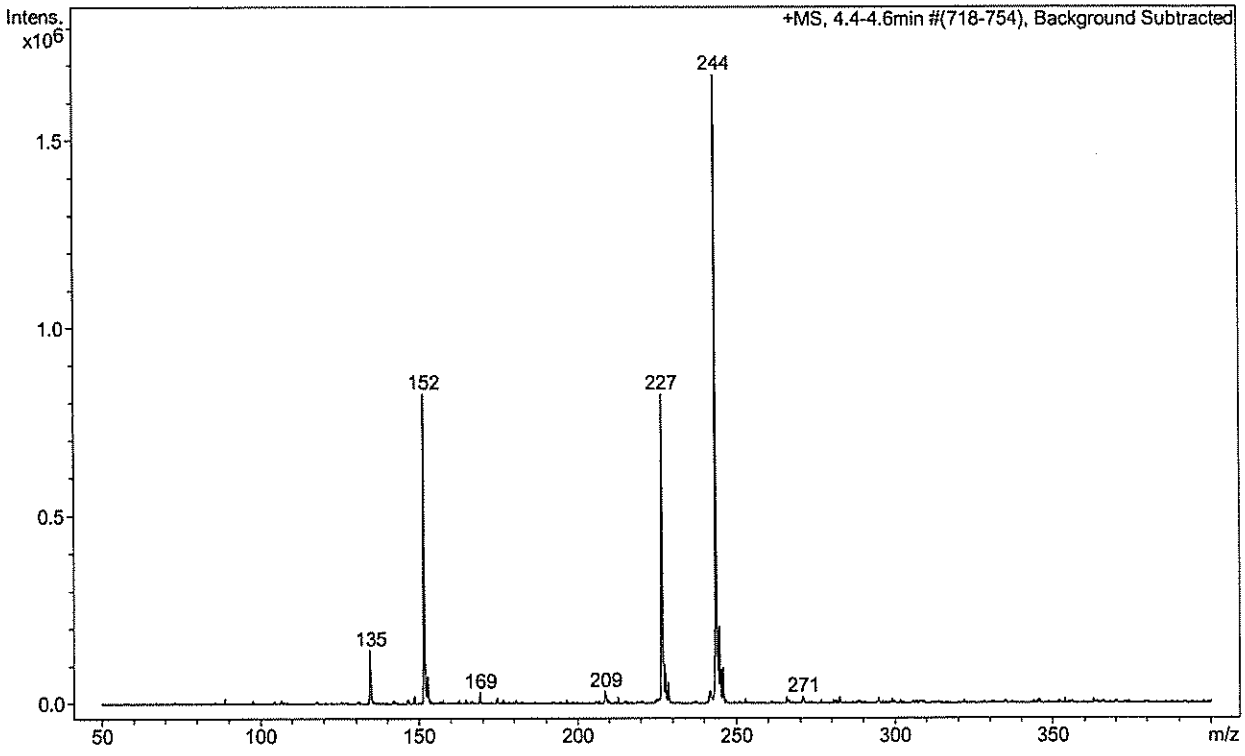


Fig. S7

LC-NM

Display Report

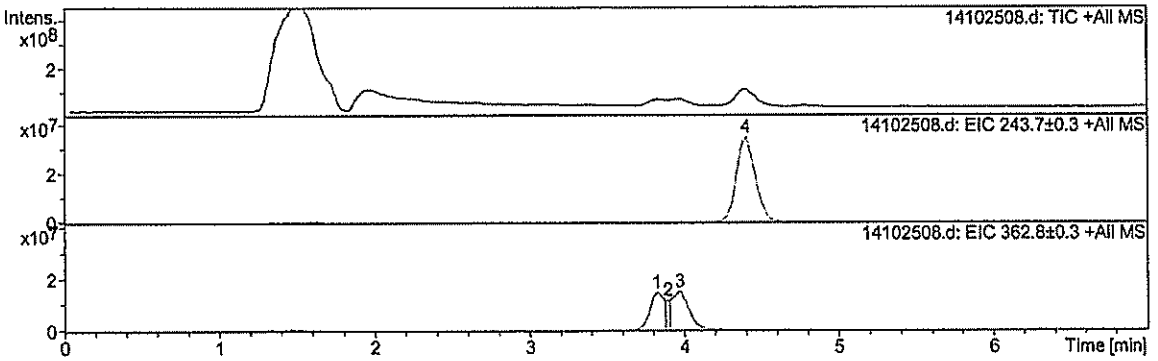
Analysis Info

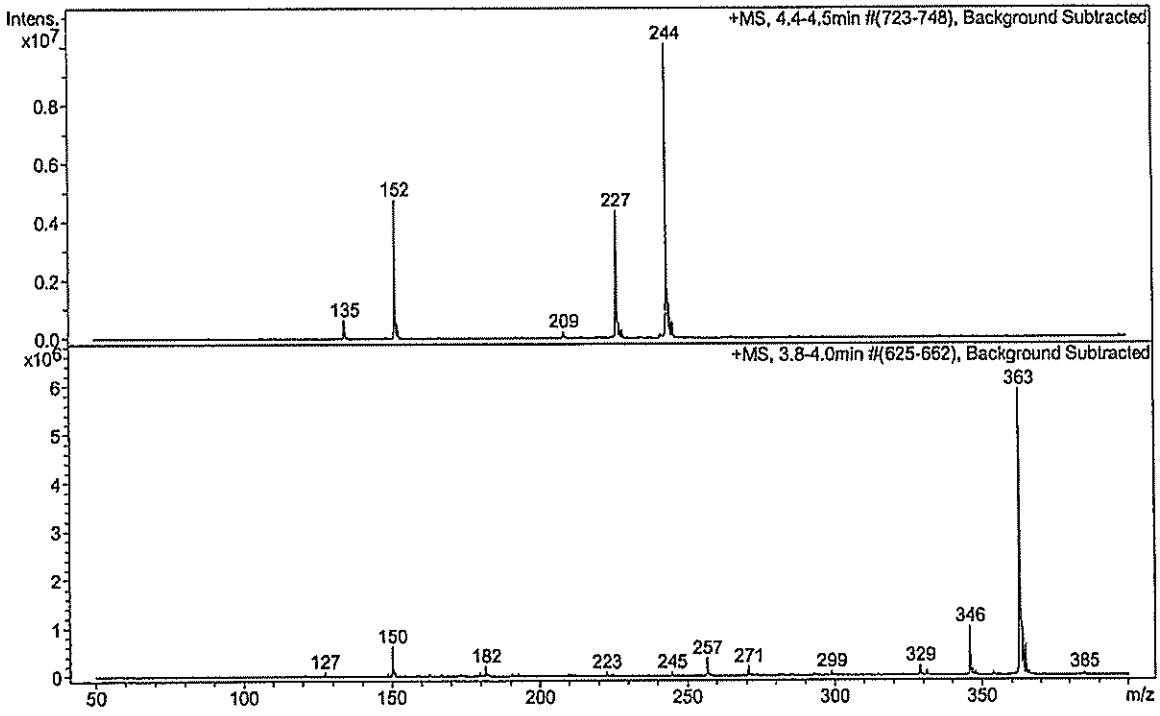
Acquisition Date	10/25/2014 19:47:26		
Analysis Name	D:\Chem32\DATA\bioact-ojika\14102508.d		
Method	ojikaLC.m	Operator	HCT-Plus
Sample Name	LC-NM	Instrument	HCTultra
Comment	Sample 2μl injection 10% MeOH-0.1% HCOOH, ODS (2*75mm)		

Acquisition Parameter

Ion Source Type	ESI	Ion Polarity	Positive	Alternating Ion Polarity	off
Mass Range Mode	Ultra Scan	Scan Begin	50 m/z	Scan End	400 m/z
Capillary Exit	106.0 Volt	Skimmer	40.0 Volt	Trap Drive	38.5
Accumulation Time	7507 æs	Averages	8 Spectra	Auto MS/MS	off

#	RT [min]	Area	
1	3.8	90039846	5-S-cys-CMT-DA
2	3.9	19849868	
3	4.0	117001329	
4	4.4	291970453	← CMT-DA
n.a.	4.4	n.a.	
n.a.	3.9	n.a.	





Bruker Daltonics DataAnalysis 3.4 printed: 10/25/2014 20:36:55 Page 1 of 1

Fig. S8

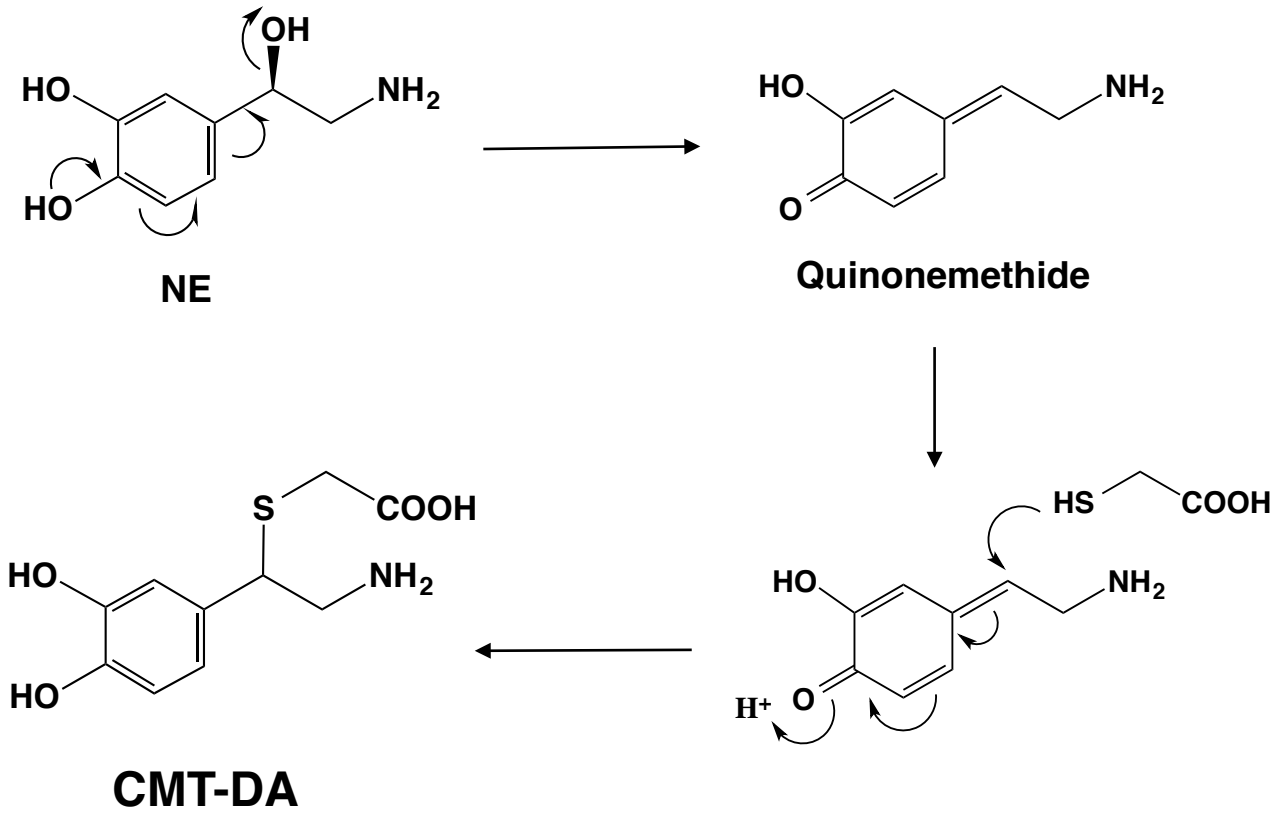

OH
HO
NH2
HO
NE
HO
NH2
O
Quinonemethide
HS
COOH
HO
NH2
H+
O
S
COOH
HO
NH2
HO
CMT-DA

Fig. S9