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A Convergent Synthesis and Optical Properties of Near-infrared Emitting Bioluminescent Infra-luciferins

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Supplementary Information

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Biological materials and methods

Copies of ^{1}H, ^{13}C, ^{31}P NMR spectra: **8**, **9**, **10**, **11**, **12**, **6**, **4**, **13**, **14**, **15**, **16**, **5**

General Experimental Details

All manipulations were routinely carried out under an inert (Ar or N_2) atmosphere. Cooling to 0 °C was effected using an ice-water bath. Cooling to temperatures below 0 °C was effected using dry ice-acetone mixtures. For the purposes of thin layer chromatography (tlc), Merck silica-aluminium plates were used, with *uv* light (254 nm) and potassium permanganate or anisaldehyde for visualisation. For column chromatography Merck Geduran® Si 60 silica gel was used. Butyl lithium solutions were standardised with diphenyl acetic acid.

All reagents were used as received unless stated. Anhydrous solvents (THF, DCM, MeCN) were obtained from a solvent tower, where degassed solvent was passed through two columns of activated alumina and a 7 micron filter under 4 bar pressure. 6-(*β*-Methoxyethoxymethylether)benzothiazole and ethyl *O*,*O*-diethylphosphonodithioacetate (**8**) were synthesised according to the published procedures.[1, 2]

Melting points are uncorrected and were recorded on a Griffin melting point machine. All NMR data was collected using a Bruker AMX 300 MHz or a Bruker AVANCE III 600 MHz as stated. All chemical shifts (δ) are reported in parts per million (ppm) relative to residual solvent peaks. Reference values for residual solvents were taken as δ = 7.27 ($CDCl_3$), 2.51 (DMSO –*d*6), 3.30 (MeOD- *d*4) ppm for ^{1}H NMR and δ = 77.2 ($CDCl_3$), 39.5 (DMSO –*d*6), 49.0 (MeOD- *d*4) ppm for ^{13}C NMR. Coupling constants (*J*) are reported in Hertz and are recorded as observed in the spectrum without averaging. Infrared spectra were recorded using a Bruker Alpha ATR spectrometer. Mass spectrometry data was collected on a Micromass LCT Premier XE (ESI) or Thermo Finnigan Mat900xp (EI/CI) instrument.

Biological materials and methods

Optical properties

Fluorescence quantum yields of analogues (Table 1). Fluorescence spectra of 50uM analogues were measured as above and furthermore, the emission of 1uM Eosin Yellow dye (BDH Lab Supplies, Poole, UK) in basic ethanol was measured at an excitation wavelength of 490nm. Furthermore, absorption readings of 50uM analogues were also measured and used to derive fluorescence quantum yields[3] of analogues at pH 7.8 in TEM buffer.

pH dependence of fluorescence spectra of D-luciferin (1) and analogues 4 and 5 (Fig 3). 10mg/ml analogues in 50% DMSO were dissolved in 500ul TEM buffer (pH 7.8) for concentrations of 500uM

each. They were further diluted to 50uM in different solutions of TEM of differing pH values (pH 6.3, 6.8, 7.3, 7.8, 8.3, 8.8 & 9.3). The solutions were checked for pH after being made up with analogues (values displayed in data). Data were acquired in 2 instruments: the Varian Cary Eclipse Spectrofluorometer and also the Tecan Infinite M200 Spectrofluorometer in triplicate of triplicates on each machine.

Bioluminescence specific activities of luciferin analogues with wild-type firefly luciferase (Fig 4). Bioluminescence assays were conducted using the PhotonIMAGER Optima (Biospace Labs, Paris, France) at 26-28^{o}C. 50ul of 6mM ATP was dispensed onto 50ul substrates mixed with different luciferases in triplicate. Experiments were carried out several times with 0.05uM enzymes for native luciferin and 0.5uM enzymes for analogues.

Bioluminescence spectra of wild-type luciferase enzymes with luciferin and analogues. 1-10mM enzymes were used to determine bioluminescence spectra with luciferin and analogues at differing pH. The spectrum of Eosin Yellow in basic ethanol was compared to the absolute spectrum[4] to correct spectra for PMT sensitivity.

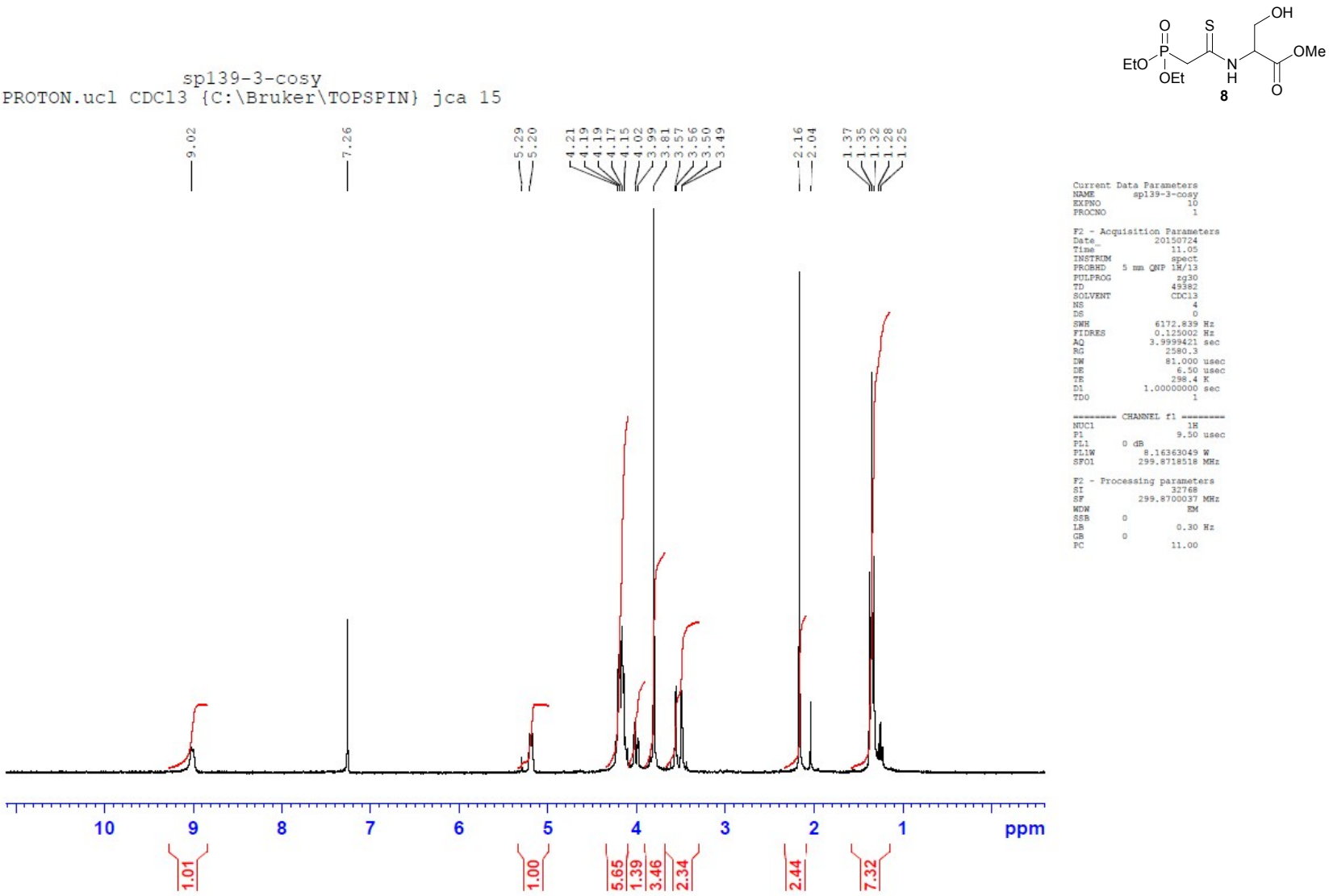
References

1) E. Pfund, T. Lequeux, S. Masson and M. Vazeux, *Org. Lett.,* 2002, **4**, 843.

2) C. S. Marvel, P. de Radzitzky and J. J. Brader, *J. Am. Chem. Soc.,* 1955, **77**, 5997.

3) *Principles of Fluorescence Spectroscopy*, Editor: Joseph R. Lakowicz, Springer US, 2006. ISBN: 978-0-387-31278-1.

4) http://omlc.org/spectra/PhotochemCAD/html/061.html (accessed June 2016)



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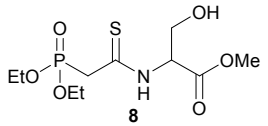

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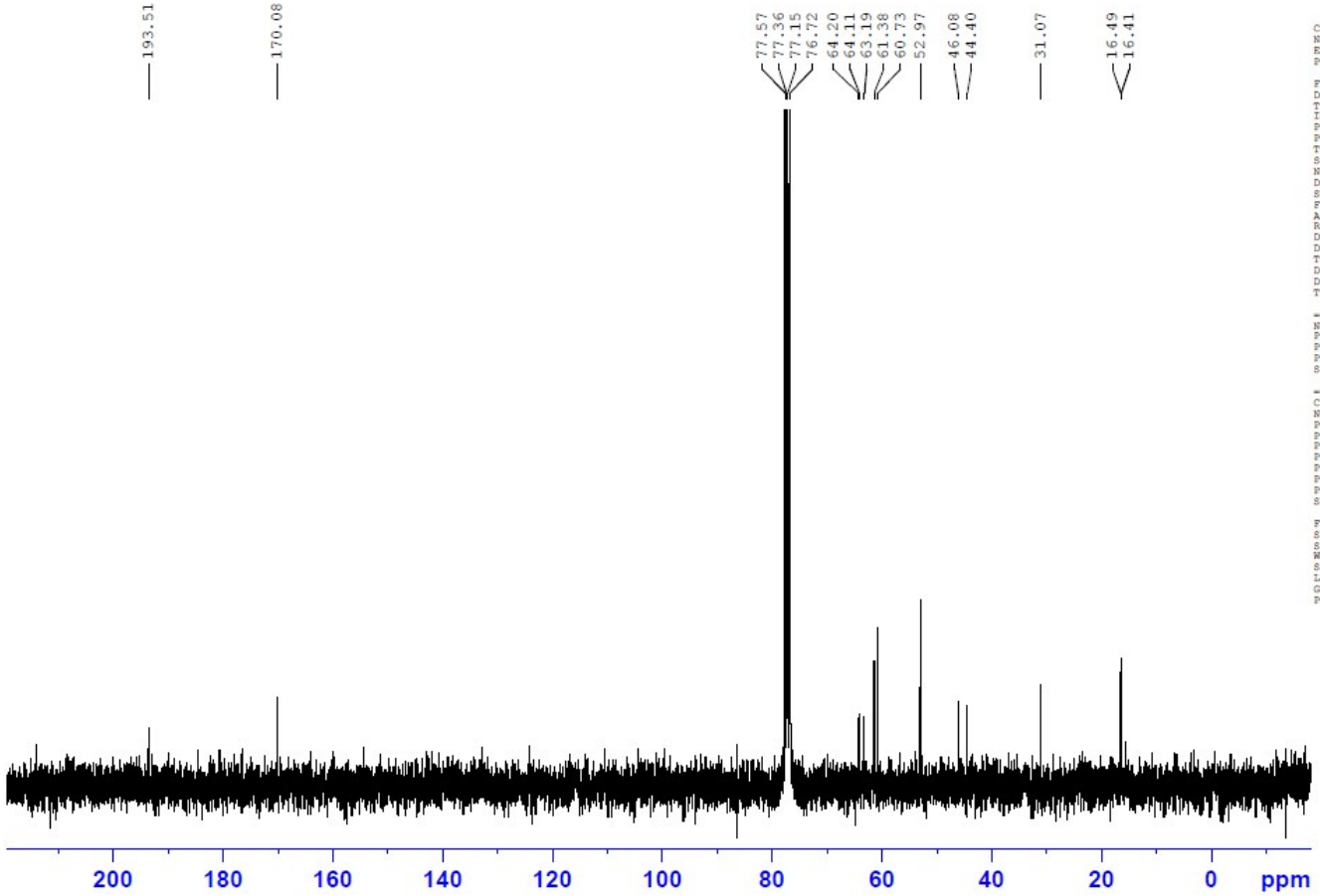


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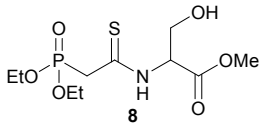

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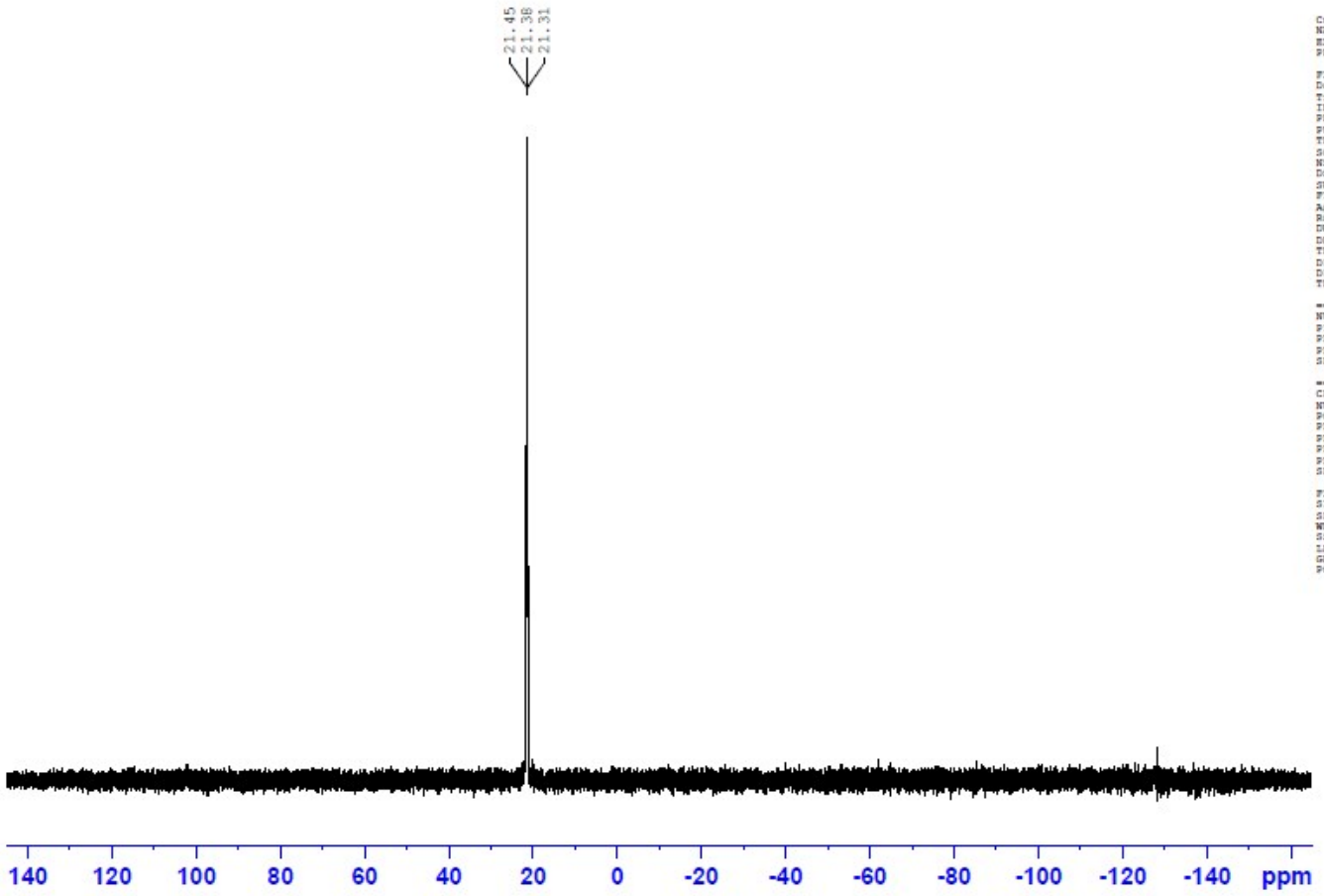


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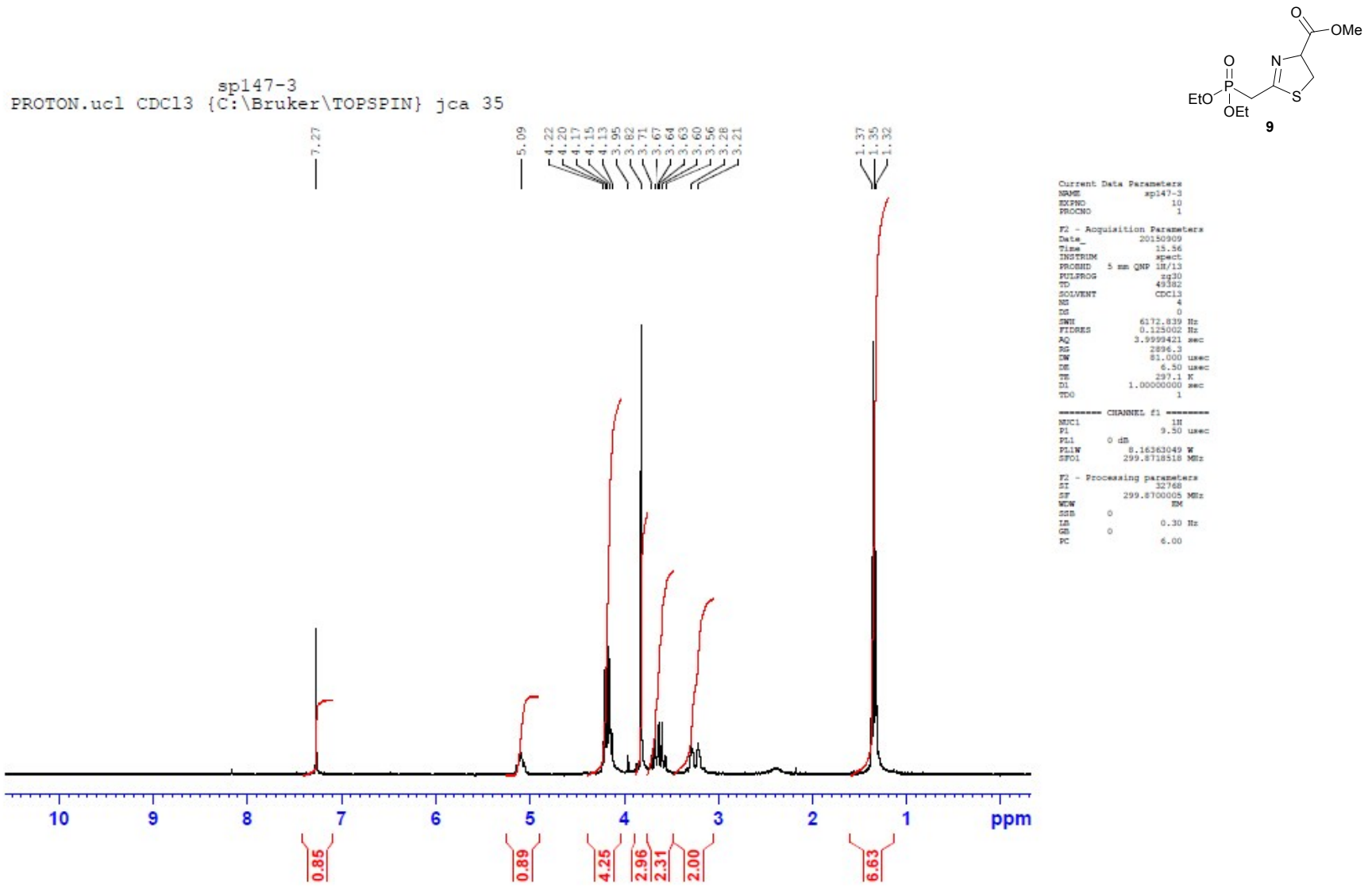

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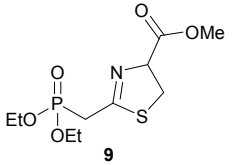

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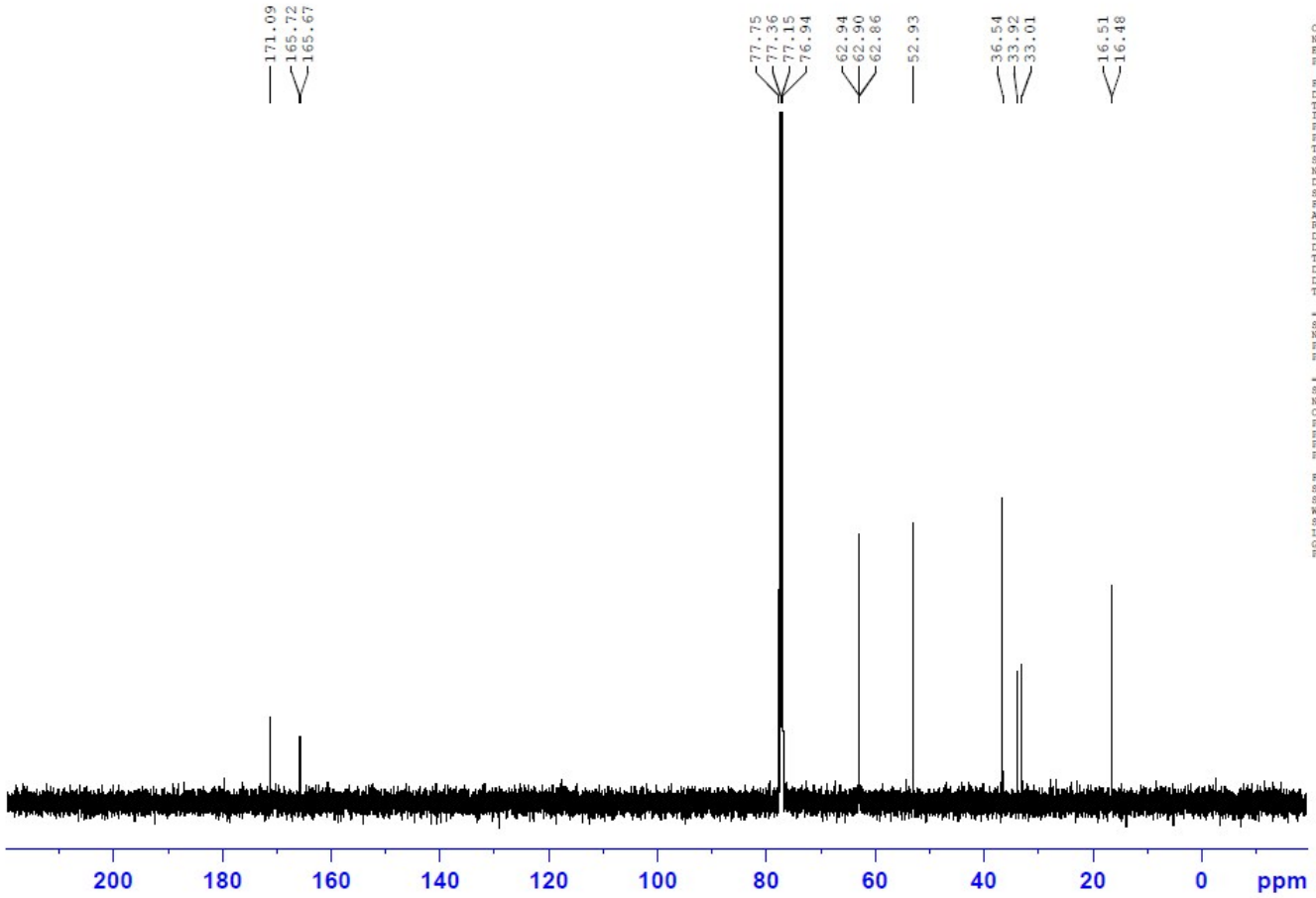


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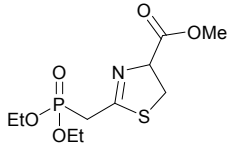

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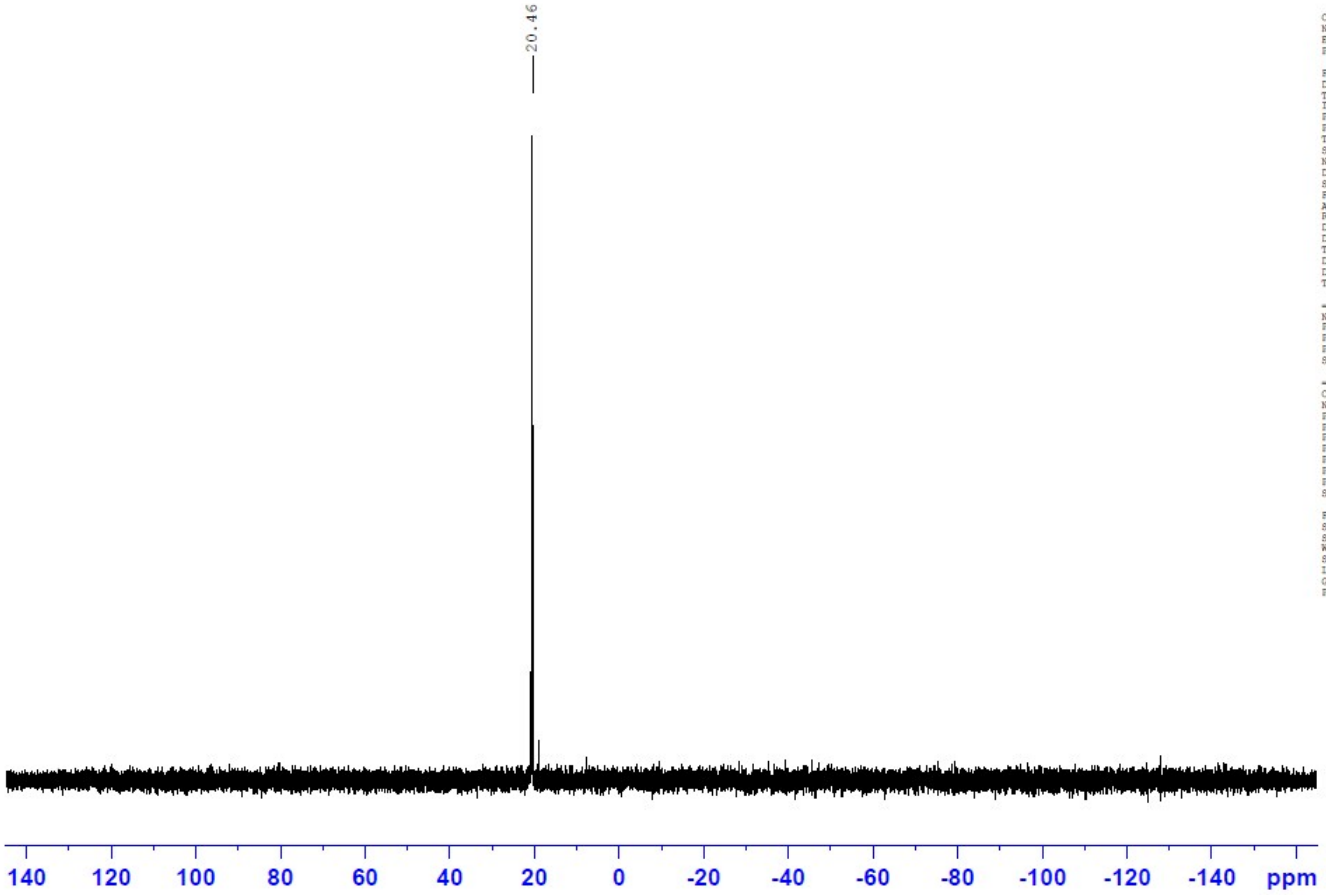


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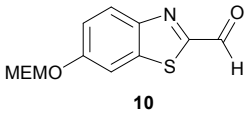

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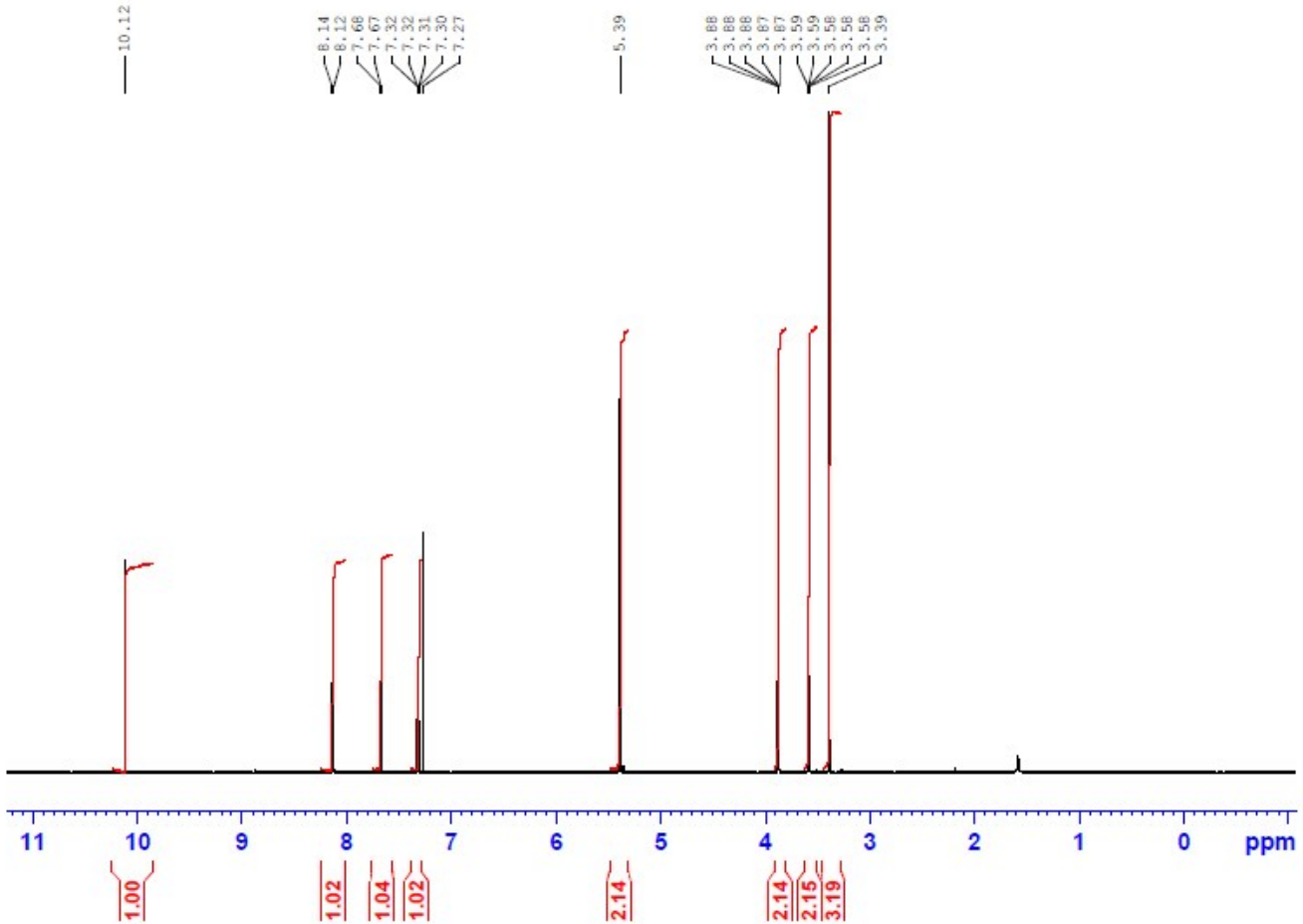


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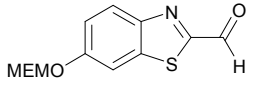

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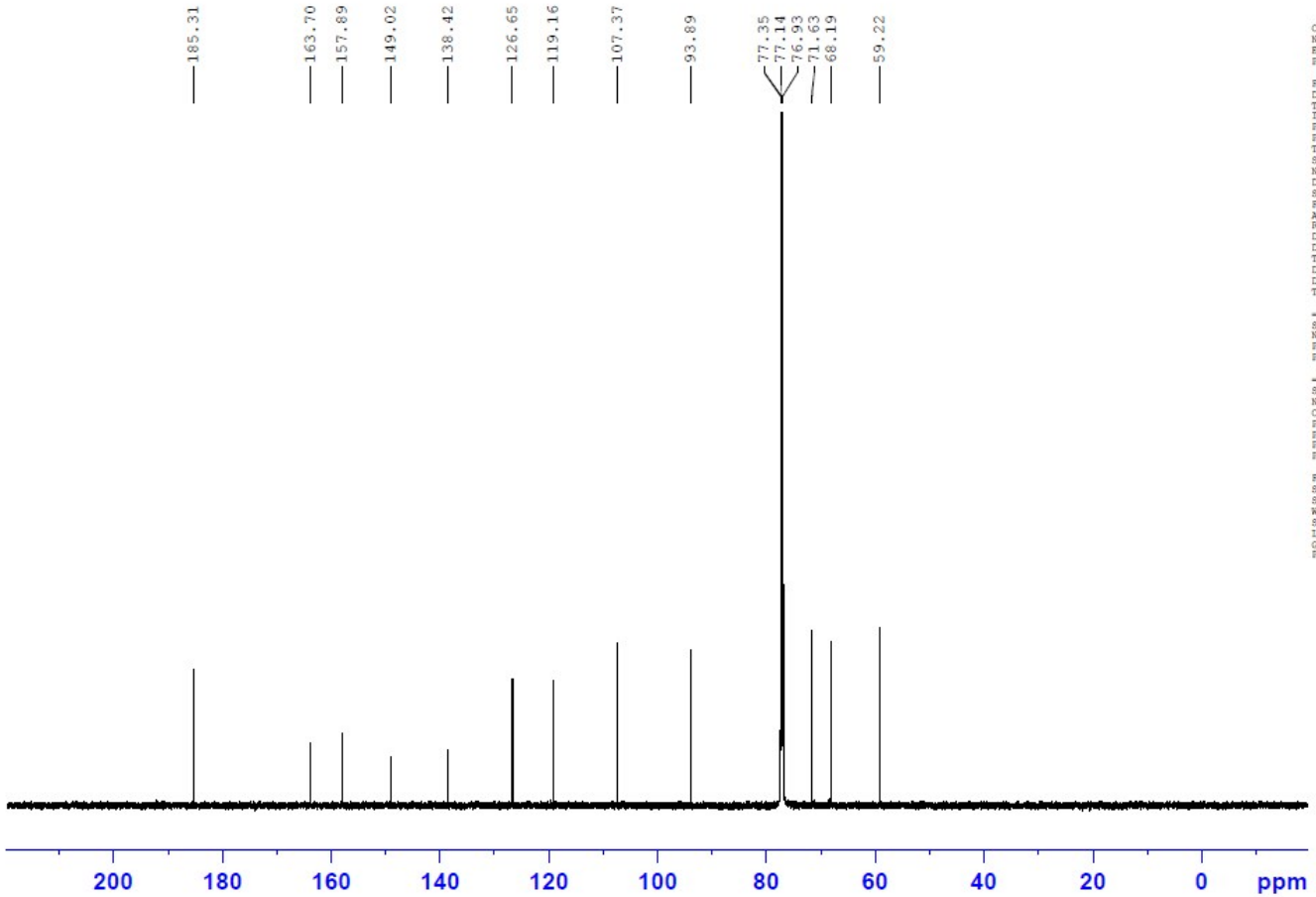


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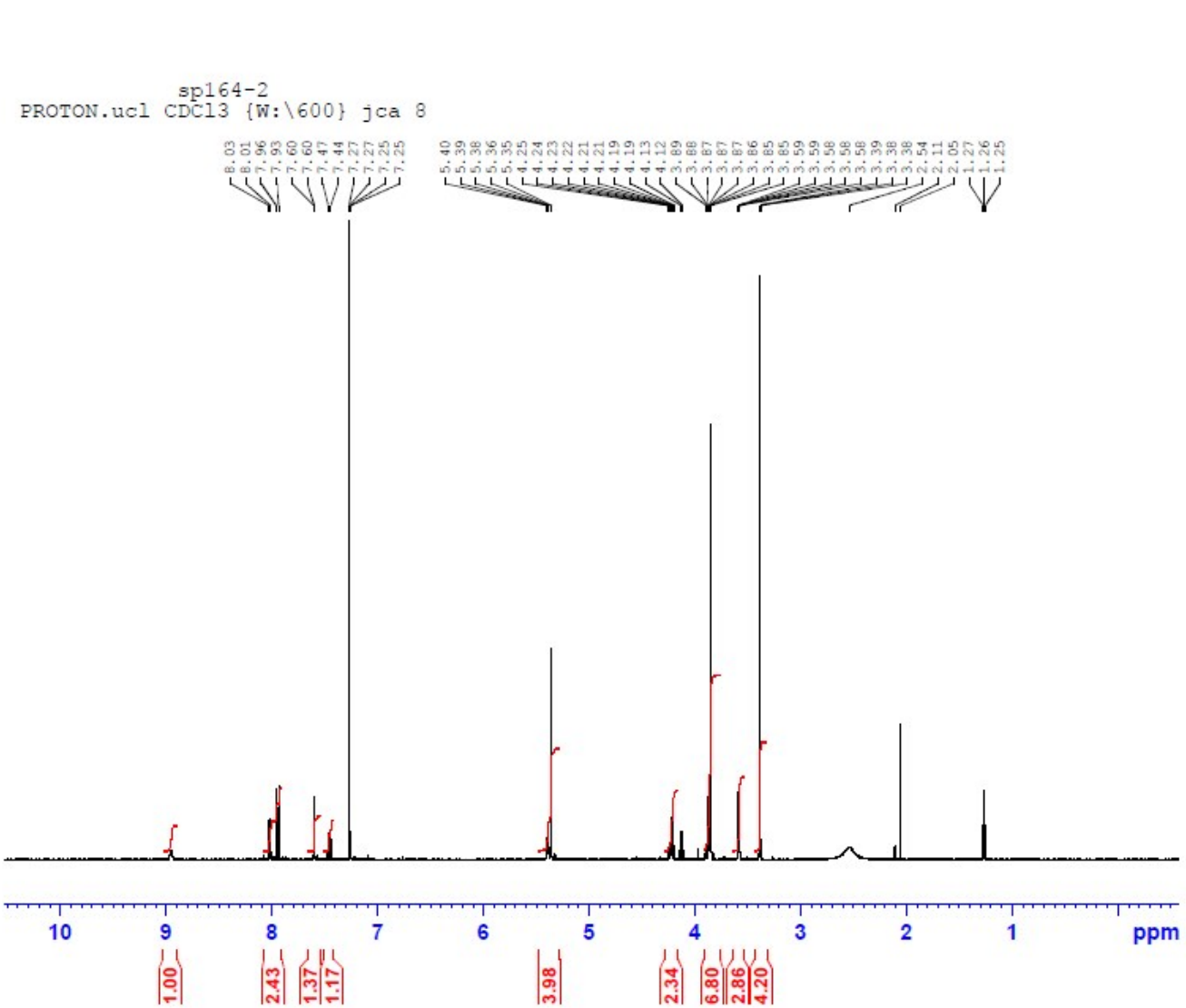

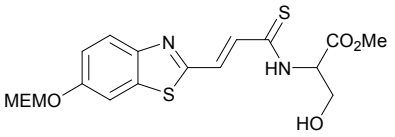

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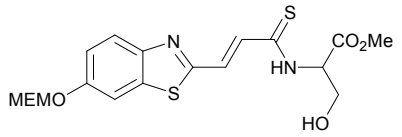

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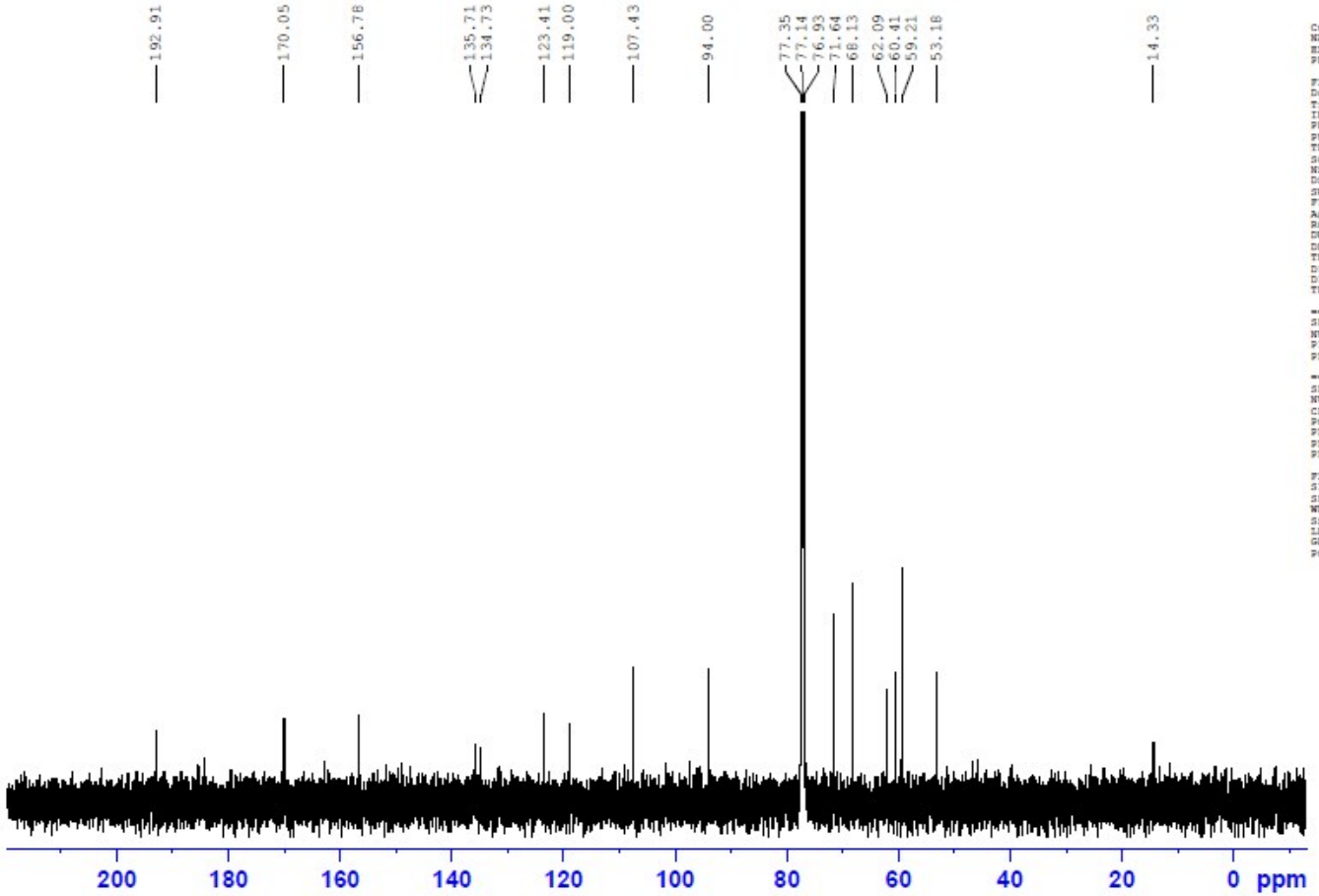


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NUC1                       13C
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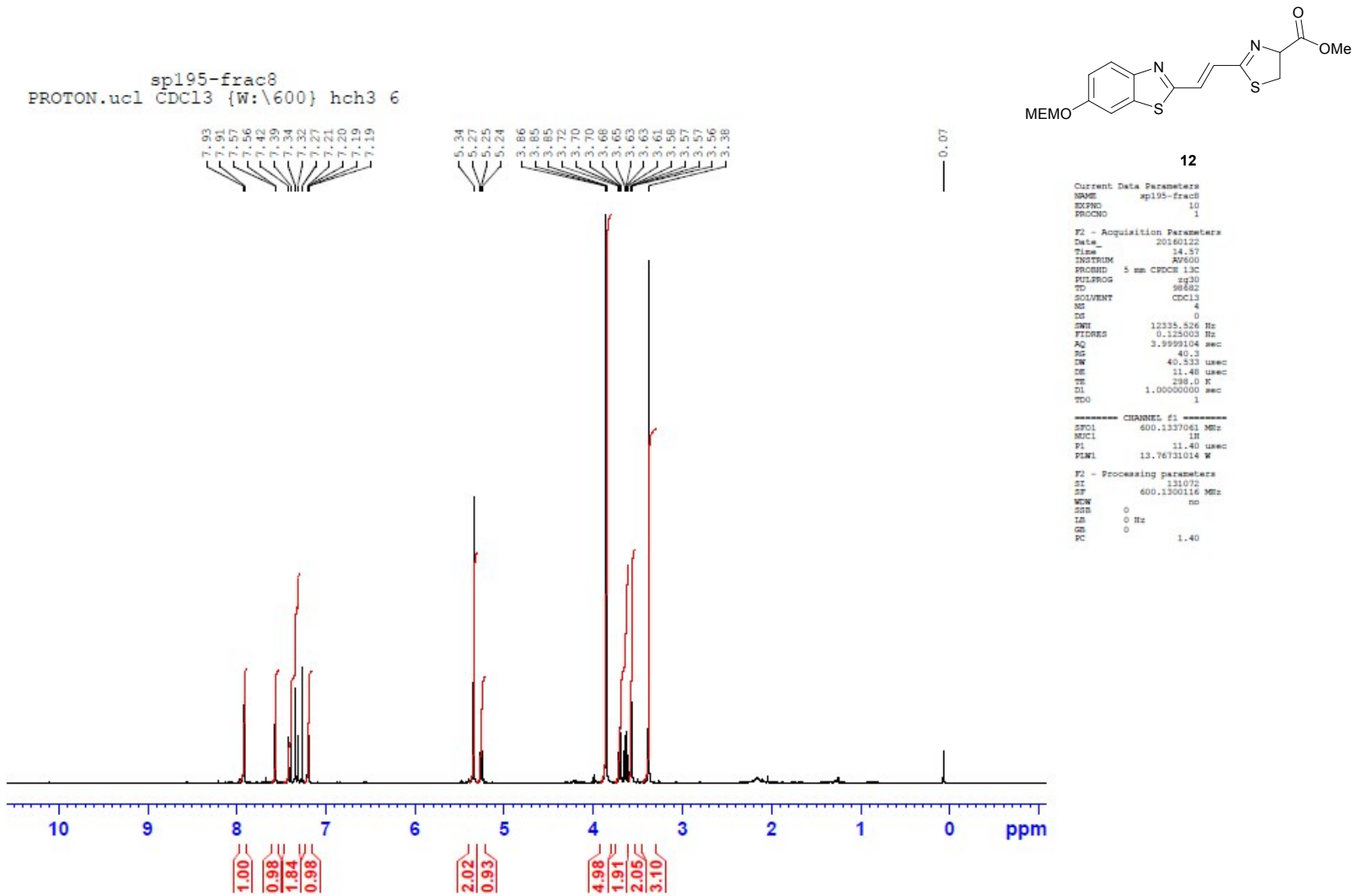

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NUC1                   1H
P1                 11.40 usec
PLW1          13.76731014 W

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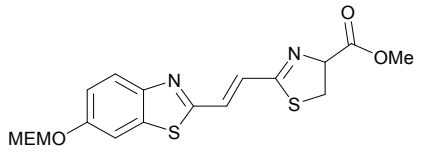
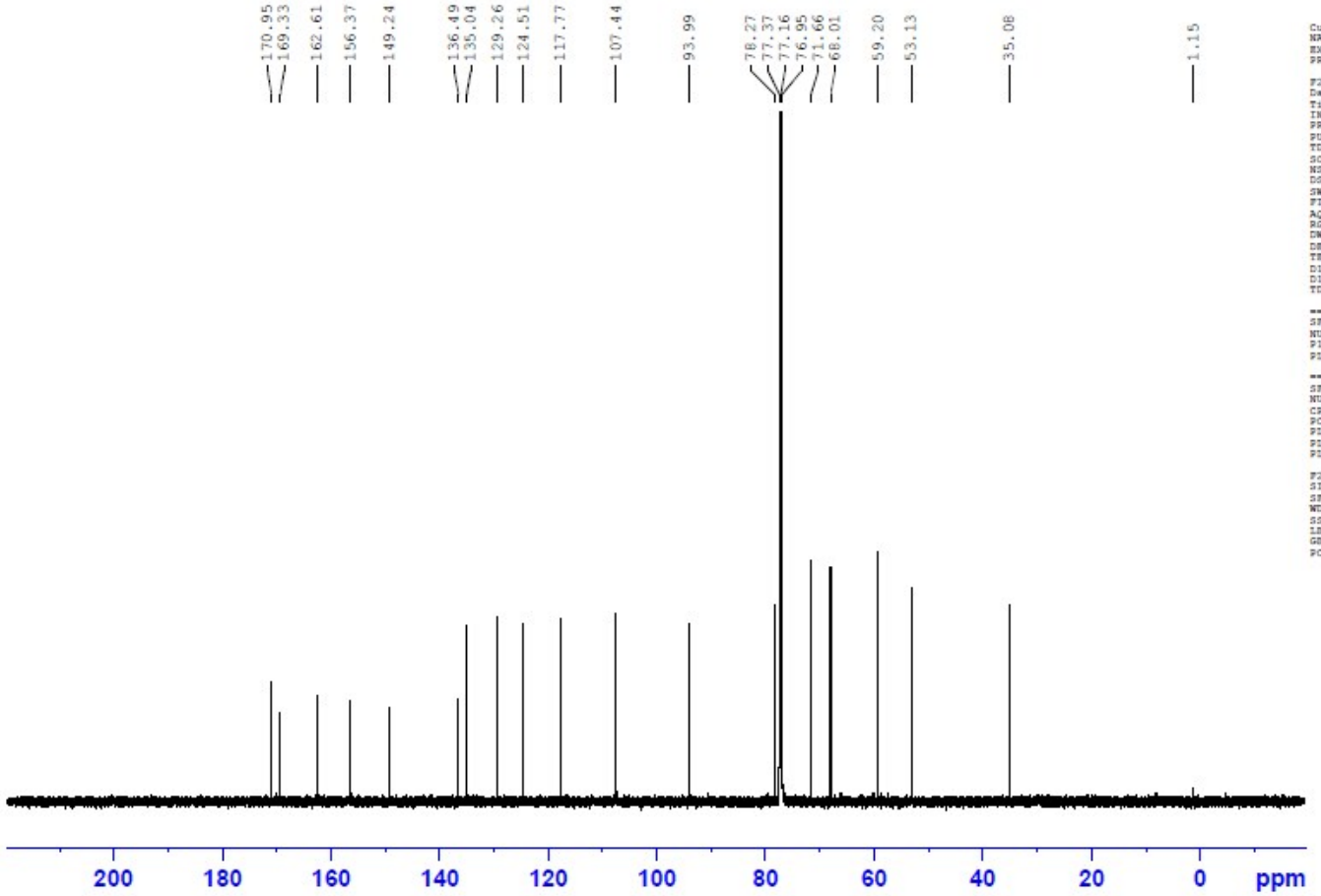
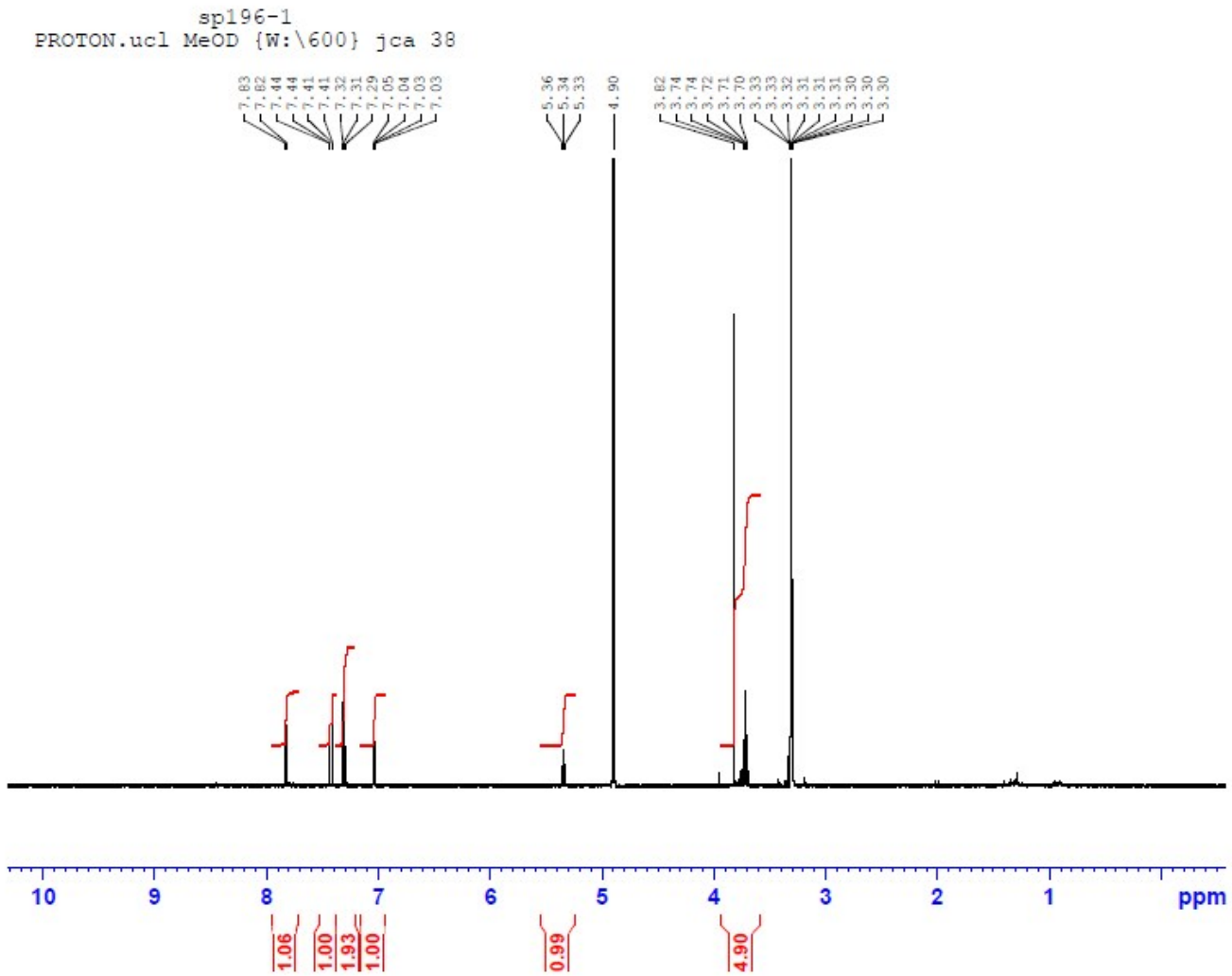
sp195-frac8
C13_DayTime.ucl CDCl3 {W:\600} hch3 6

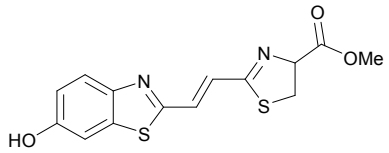


12



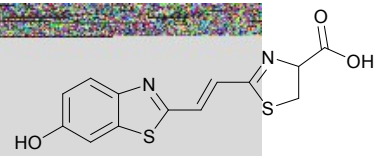

```
Current Data Parameters
NAME          sp195-frac8
EXPNO                  11
PROCNO                  1

F2 - Acquisition Parameters
Date_            20160122
Time                15.00
INSTRUM            AV600
PROBHD  5 mm CPDCH 13C
PULPROG          zgpg30
TD                 71914
SOLVENT             CDCl3
NS                     32
DS                      4
SWH             36057.691 Hz
FIDRES          0.501400 Hz
AQ              0.9972075 sec
RG                    203
DW                13.867 usec
DE                19.08 usec
TE                298.0 K
D1              1.79999995 sec
D11             0.03000000 sec
TD0                     2

======== CHANNEL f1 ========
SFO1          150.9178981 MHz
NUC1                   13C
P1                    9.80 usec
PLW1          26.76899910 W

======== CHANNEL f2 ========
SFO2          600.1324005 MHz
NUC2                    1H
CPDPRG[2           waltz16
PCPD2                70.00 usec
PLW2          13.76700020 W
PLW12          0.36513001 W
PLW13          0.17892000 W

F2 - Processing parameters
SI                    65536
SF            150.9027930 MHz
WDW                     EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```



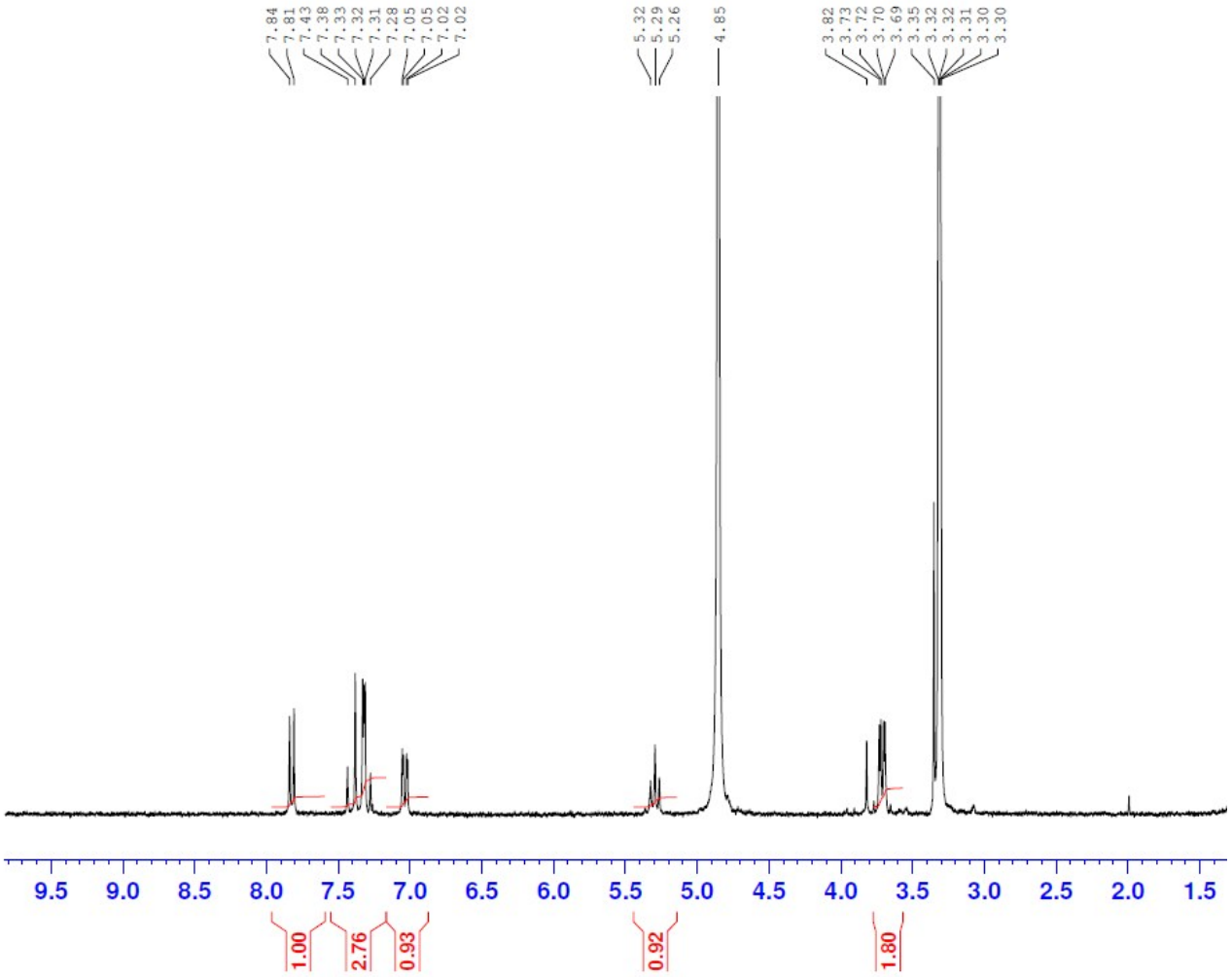


6

```
Current Data Parameters
NAME                  sp196-1
EXPNO                      10
PROCNO                      1

F2 - Acquisition Parameters
Date_               20160126
Time                   13.28
INSTRUM                AV600
PROBHD   5 mm CPDCH 13C
PULPROG                zg30
TD                    98682
SOLVENT                MeOD
NS                         4
DS                         0
SWH               12335.526 Hz
FIDRES             0.125003 Hz
AQ                3.9999104 sec
RG                      40.3
DW                    40.533 usec
DE                    11.48 usec
TE                    298.0 K
D1                1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1           600.1337061 MHz
NUC1                    1H
P1                    11.40 usec
PLW1          13.76731014 W

F2 - Processing parameters
SI                    131072
SF             600.1300141 MHz
WDW                       no
SSB                    0
LB                     0 Hz
GB                     0
PC                     1.40
```



4


7.84
7.81
7.43
7.38
7.33
7.32
7.31
7.28
7.05
7.05
7.02
7.02
5.32
5.29
5.26
4.85
3.82
3.73
3.72
3.70
3.69
3.35
3.32
3.32
3.31
3.30
3.30
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
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1.00
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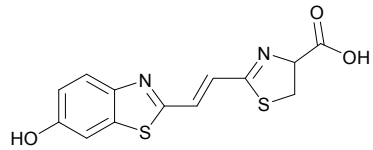
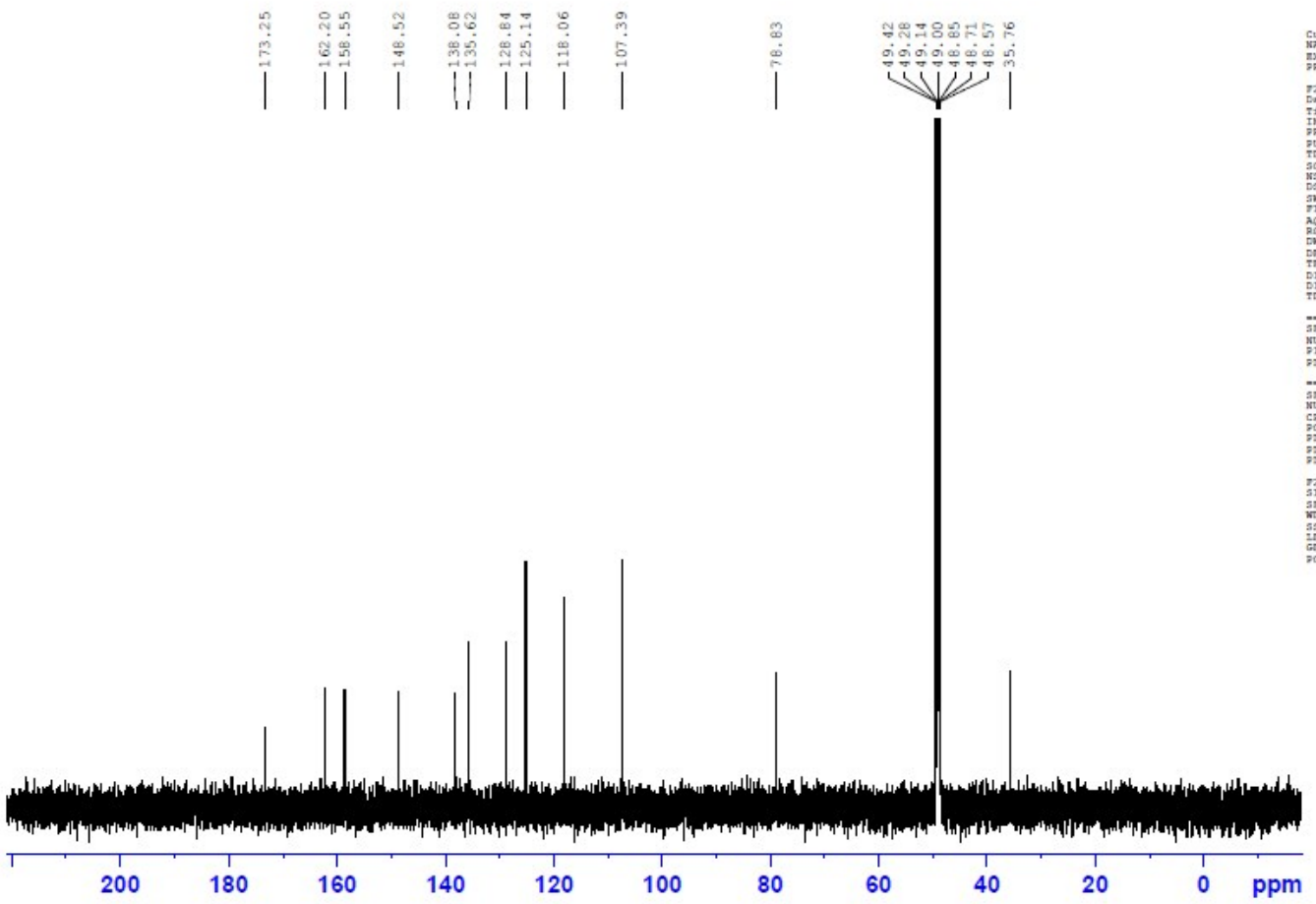
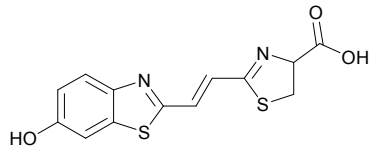
0.93
0.92
1.80


HO
N
S
N
S
O
OH

4

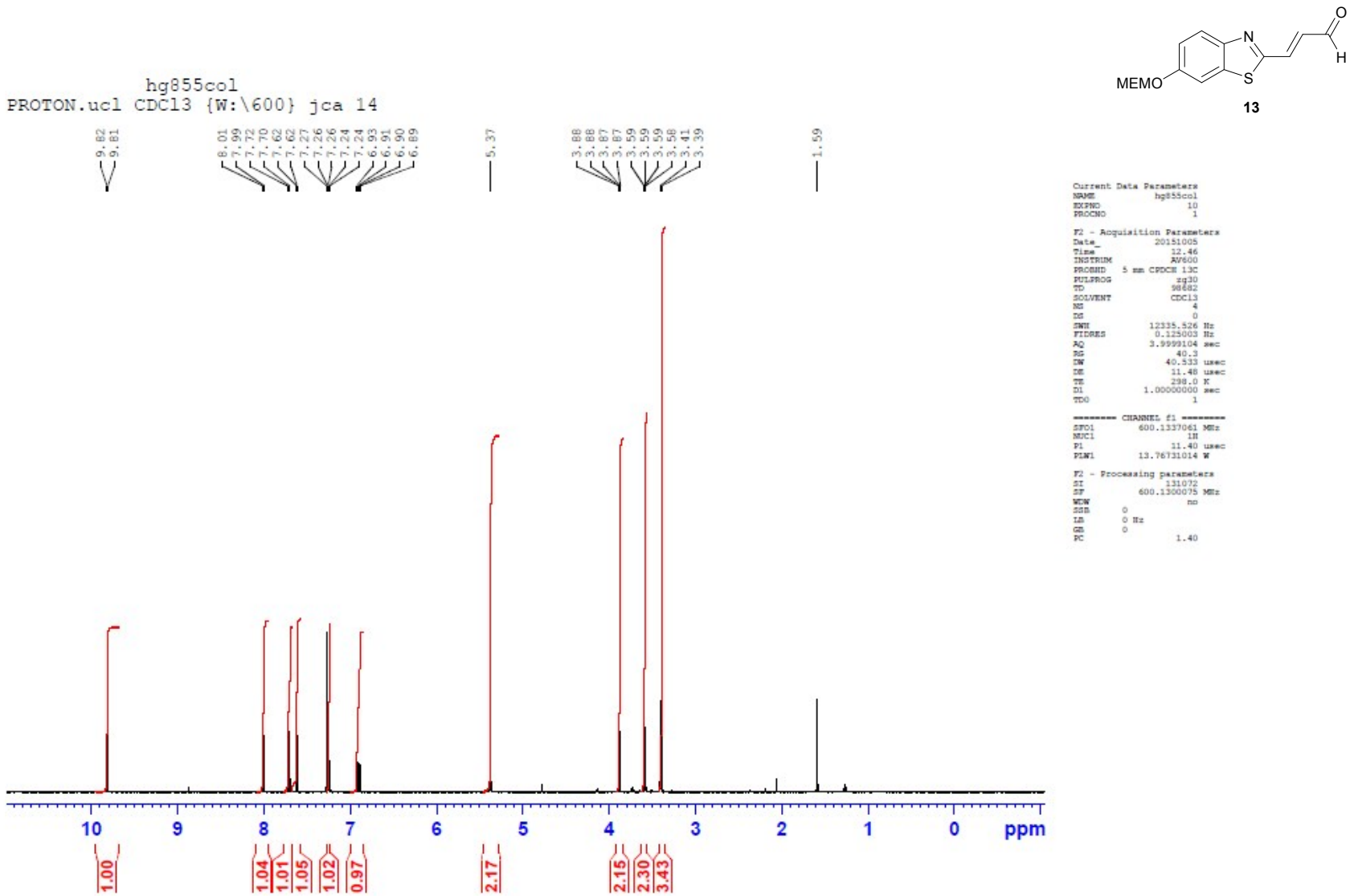
sp215
C13_DayTime.ucl MeOD {W:\600} jca 26





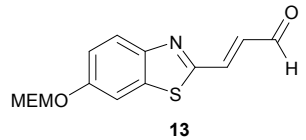
4

```
Current Data Parameters
NAME      sp215-full data
EXPNO              12
PROCNO              1

F2 - Acquisition Parameters
Date_          20160420
Time              14.48
INSTRUM           AV600
PROBHD   5 mm CPDCH 13C
PULPROG          zgpg30
TD               71914
SOLVENT           MeOD
NS                  32
DS                   4
SWH           36057.691 Hz
FIDRES        0.501400 Hz
AQ            0.9972075 sec
RG                 203
DW               13.867 usec
DE               19.08 usec
TE               298.0 K
D1            1.79999995 sec
D11           0.03000000 sec
TD0                  2

======== CHANNEL f1 ========
SFO1         150.9178981 MHz
NUC1               13C
P1                9.80 usec
PLW1         26.76899910 W

======== CHANNEL f2 ========
SFO2         600.1324005 MHz
NUC2               1H
CPDPRG[2         waltz16
PCPD2             70.00 usec
PLW2         13.76700020 W
PLW12         0.36513001 W
PLW13         0.17892000 W

F2 - Processing parameters
SI                65536
SF           150.9025980 MHz
WDW                EM
SSB       0
LB                1.00 Hz
GB        0
PC                1.40
```



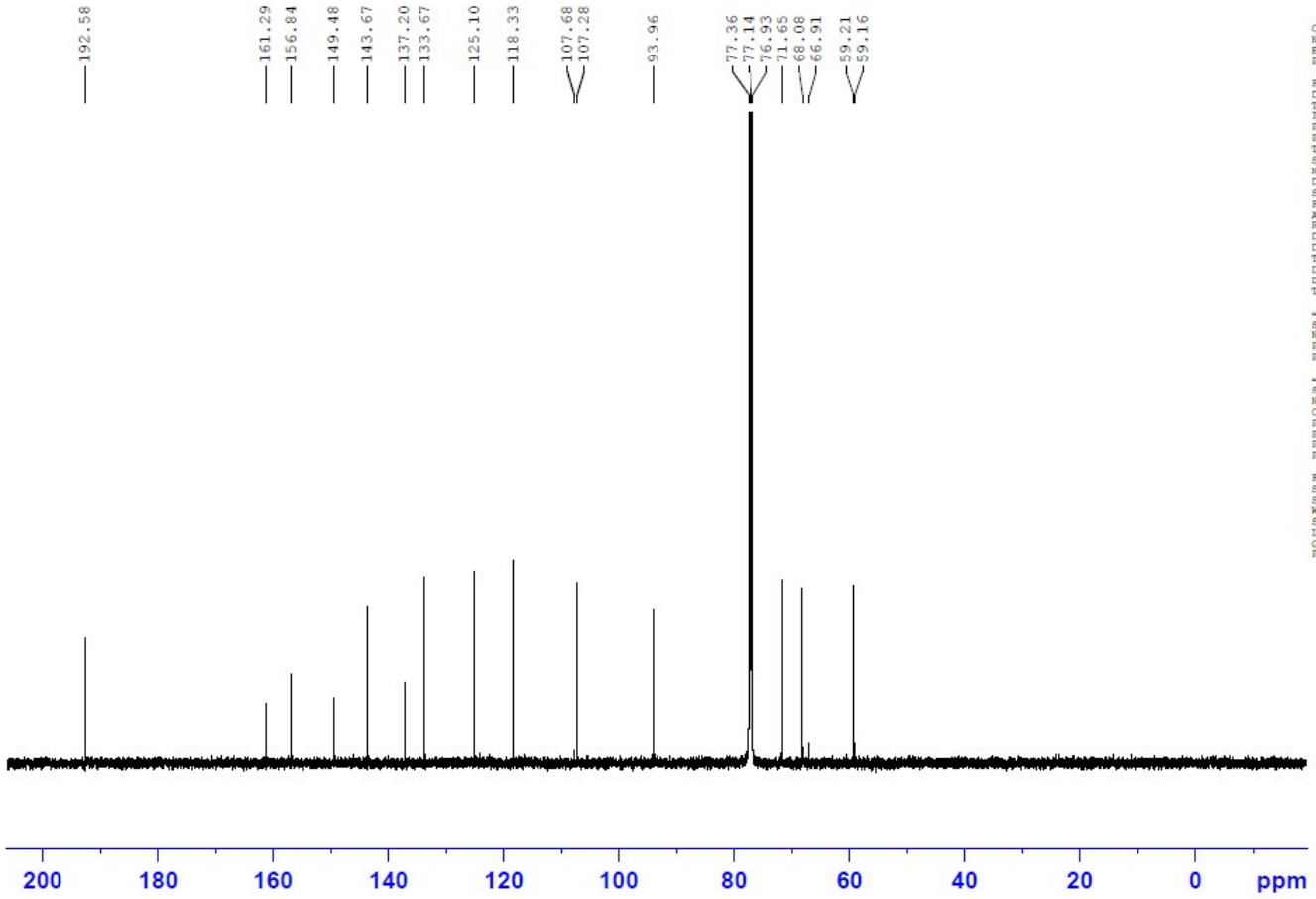
13

```
Current Data Parameters
NAME                  hg855col
EXPNO                       10
PROCNO                       1

F2 - Acquisition Parameters
Date_              20151005
Time                   12.46
INSTRUM                AV600
PROBHD   5 mm CPDCH 13C
PULPROG                 zg30
TD                     98682
SOLVENT                CDCl3
NS                         4
DS                         0
SWH               12335.526 Hz
FIDRES             0.125003 Hz
AQ                3.9999104 sec
RG                      40.3
DW                    40.533 usec
DE                    11.48 usec
TE                    298.0 K
D1                1.00000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1            600.1337061 MHz
NUC1                      1H
P1                     11.40 usec
PLW1           13.76731014 W

F2 - Processing parameters
SI                    131072
SF              600.1300075 MHz
WDW                       no
SSB                        0
LB                     0 Hz
GB                         0
PC                      1.40
```

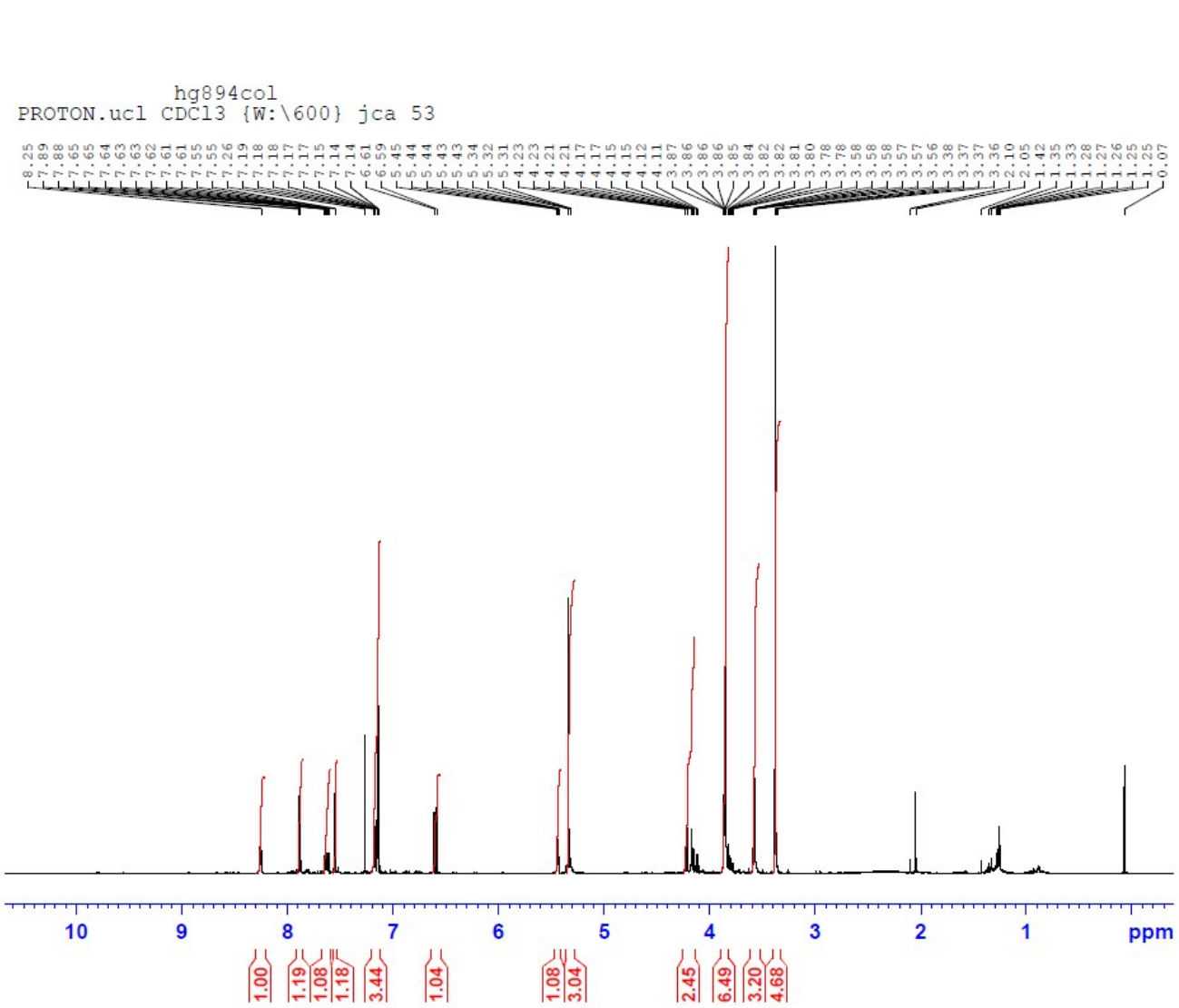


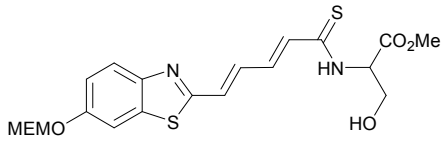
13

hg855col
C13CPD.ucl CDCl3 {W:\600} jca 14




```
Current Data Parameters
NAME                  hg855col
EXPNO                       12
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20151005
Time                     13.01
INSTRM                   AV600
PROBHD    5 mm CPDCH 13C
PULPROG                zgpg30
TD                        65340
SOLVENT                   CDCl3
NS                          128
DS                            0
SWH                   36057.691 Hz
FIDRES                0.551847 Hz
AQ                    0.9060480 sec
RG                         1030
DW                       13.867 usec
DE                       20.82 usec
TE                       298.0 K
D1                    2.00000000 sec
D11                   0.03000000 sec
TD0                           1

======== CHANNEL f1 ========
SFO1                150.9178981 MHz
NUC1                        13C
P1                         9.80 usec
PLW1                26.76885986 W

======== CHANNEL f2 ========
SFO2                600.1324005 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                     70.00 usec
PLW2                13.76731014 W
PLW12                0.32798141 W
PLW13                0.17332020 W

F2 - Processing parameters
SI                         32768
SF                  150.9027930 MHz
WDW                           EM
SSB                 0
LB                         1.00 Hz
GB                  0
PC                         1.40
```

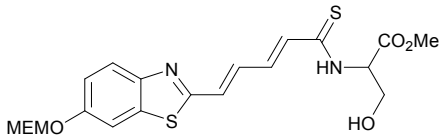




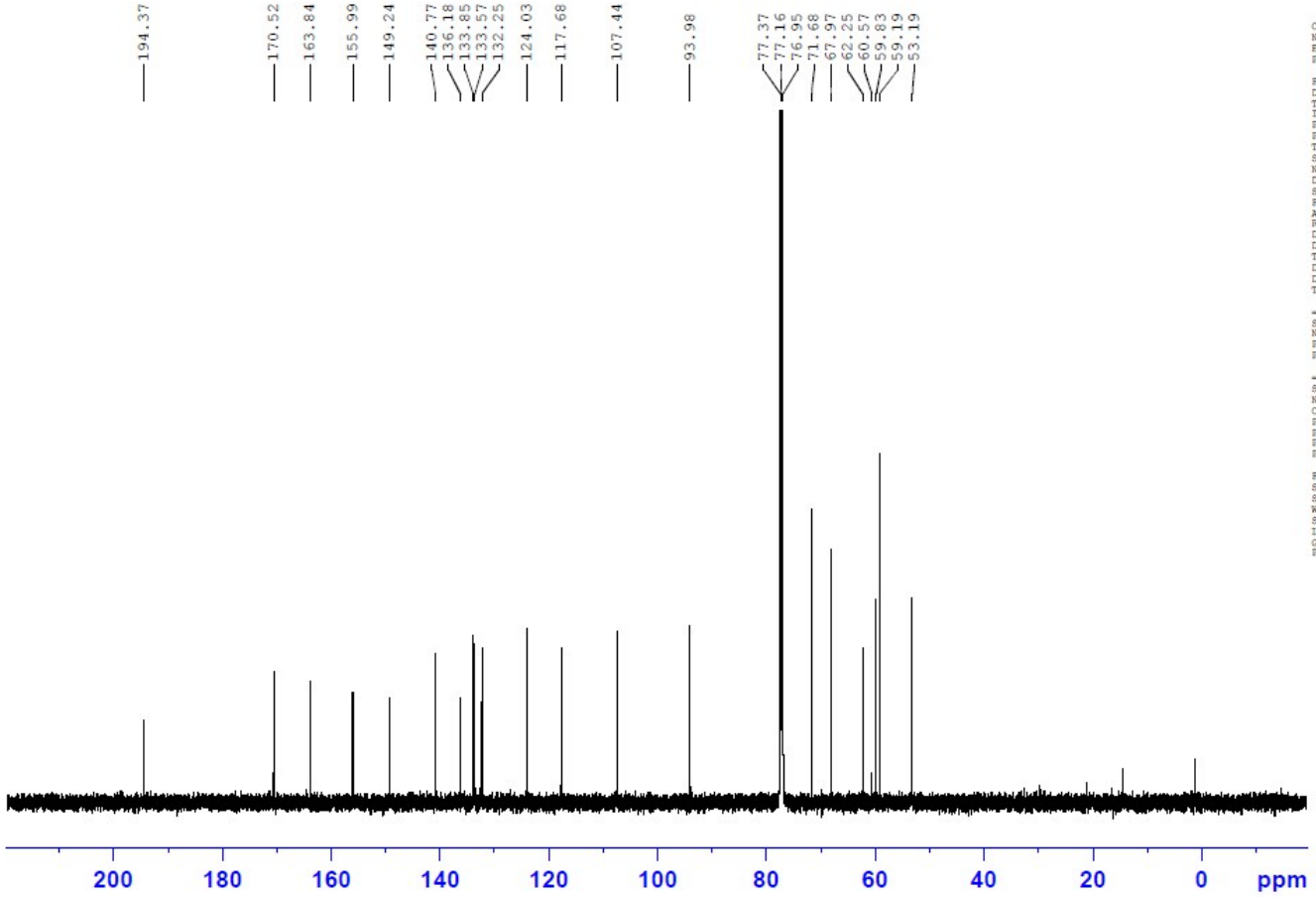
14

```
Current Data Parameters
NAME                  hg894col
EXPNO                       10
PROCNO                       1

F2 - Acquisition Parameters
Date_               20160120
Time                    12.53
INSTRUM                 AV600
PROBHD    5 mm CPDCH 13C
PULPROG                 zg30
TD                      98682
SOLVENT                 CDCl3
NS                          4
DS                          0
SWH                 12335.526 Hz
FIDRES              0.125003 Hz
AQ                  3.9999104 sec
RG                       40.3
DW                     40.533 usec
DE                     11.48 usec
TE                     298.0 K
D1                  1.00000000 sec
TD0                         1

======== CHANNEL f1 ========
SFO1             600.1337061 MHz
NUC1                       1H
P1                      11.40 usec
PLW1             13.76731014 W

F2 - Processing parameters
SI                     131072
SF               600.1300116 MHz
WDW                        no
SSB          0
LB           0 Hz
GB           0
PC                       1.40
```

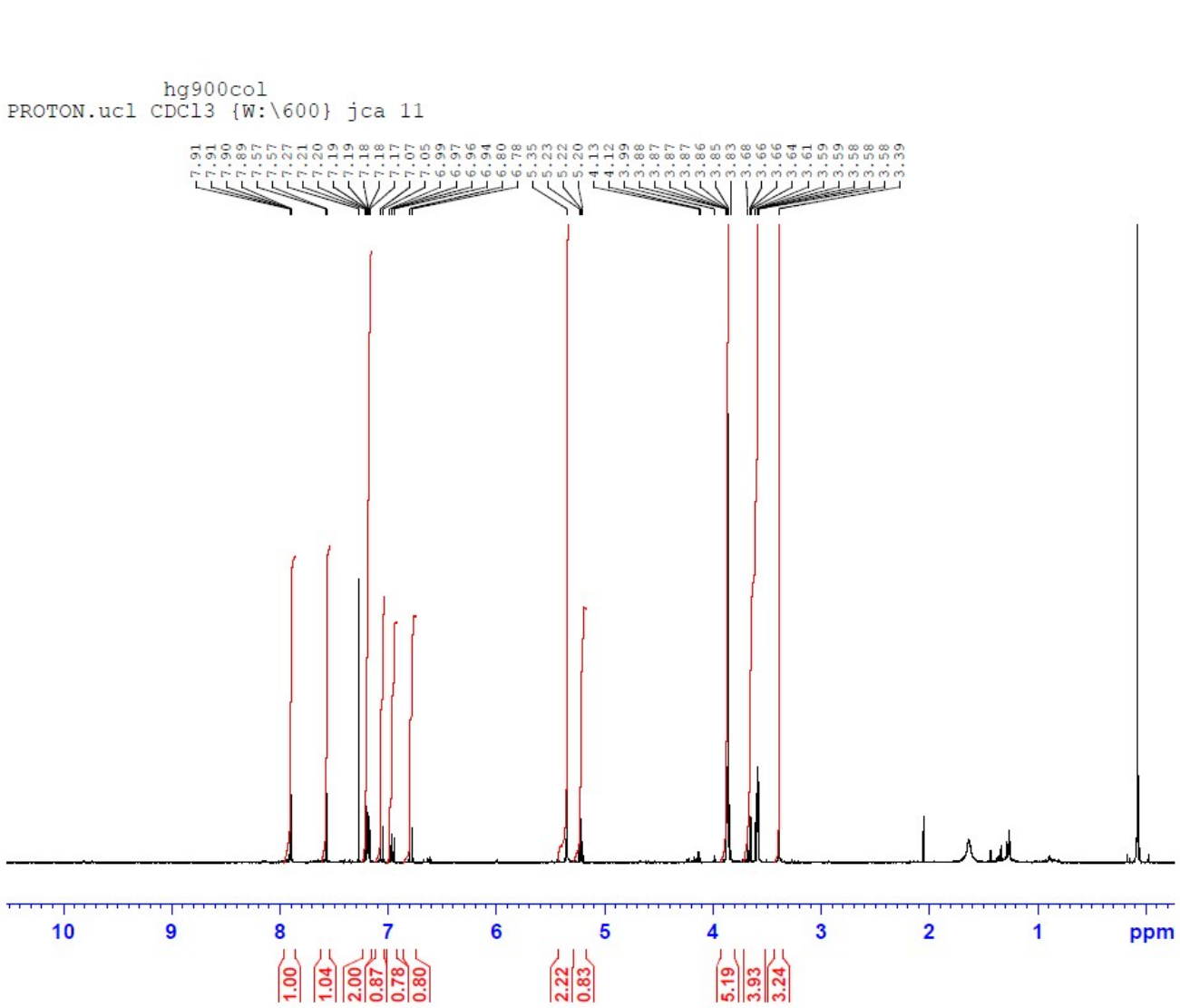
hg894col
C13_DayTime.ucl CDCl3 {W:\600} jca 53

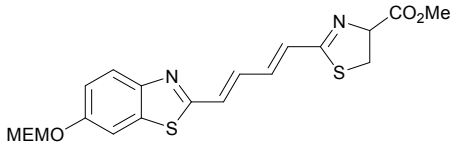


14




```
Current Data Parameters
NAME                  hg894col
EXPNO                       11
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20160120
Time                     12.56
INSTRM                   AV600
PROBHD     5 mm CPDCH 13C
PULPROG                zgpg30
TD                      71914
SOLVENT                 CDCl3
NS                         32
DS                          4
SWH                 36057.691 Hz
FIDRES              0.501400 Hz
AQ                  0.9972075 sec
RG                        203
DW                     13.867 usec
DE                     19.08 usec
TE                     298.0 K
D1                  1.79999995 sec
D11                 0.03000000 sec
TD0                         2

======== CHANNEL f1 ========
SFO1               150.9178981 MHz
NUC1                       13C
P1                        9.80 usec
PLW1               26.76899910 W

======== CHANNEL f2 ========
SFO2               600.1324005 MHz
NUC2                       1H
CPDPRG[2                waltz16
PCPD2                    70.00 usec
PLW2               13.76700020 W
PLW12               0.36513001 W
PLW13               0.17892000 W

F2 - Processing parameters
SI                         65536
SF                 150.9027930 MHz
WDW                           EM
SSB          0
LB                        1.00 Hz
GB           0
PC                        1.40
```

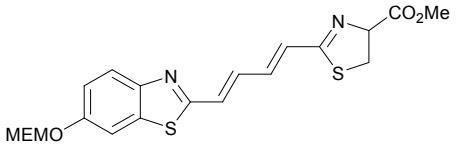




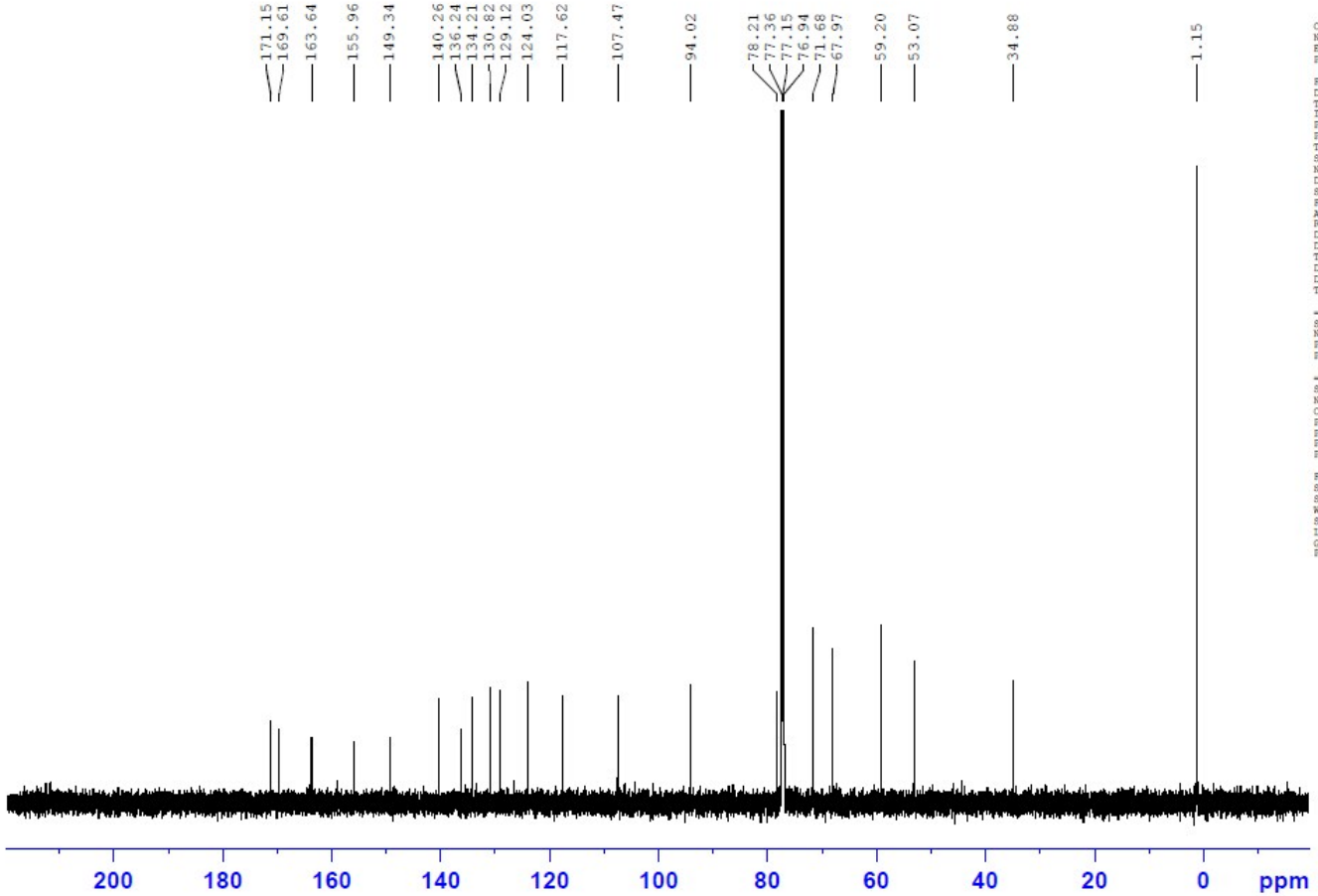
15

```
Current Data Parameters
NAME                  HG900col
EXPNO                       10
PROCNO                       1

F2 - Acquisition Parameters
Date_               20160128
Time                    11.28
INSTRUM                 AV600
PROBHD     5 mm CPDCH 13C
PULPROG                 zg30
TD                      98682
SOLVENT                 CDCl3
NS                          4
DS                          0
SWH                 12335.526 Hz
FIDRES                0.125003 Hz
AQ                  3.9999104 sec
RG                       40.3
DW                     40.533 usec
DE                     11.48 usec
TE                     298.0 K
D1                  1.00000000 sec
TD0                         1

======== CHANNEL f1 ========
SFO1               600.1337061 MHz
NUC1                       1H
P1                      11.40 usec
PLW1               13.76731014 W

F2 - Processing parameters
SI                      131072
SF                 600.1300096 MHz
WDW                        no
SSB                 0
LB                  0 Hz
GB                  0
PC                       1.40
```

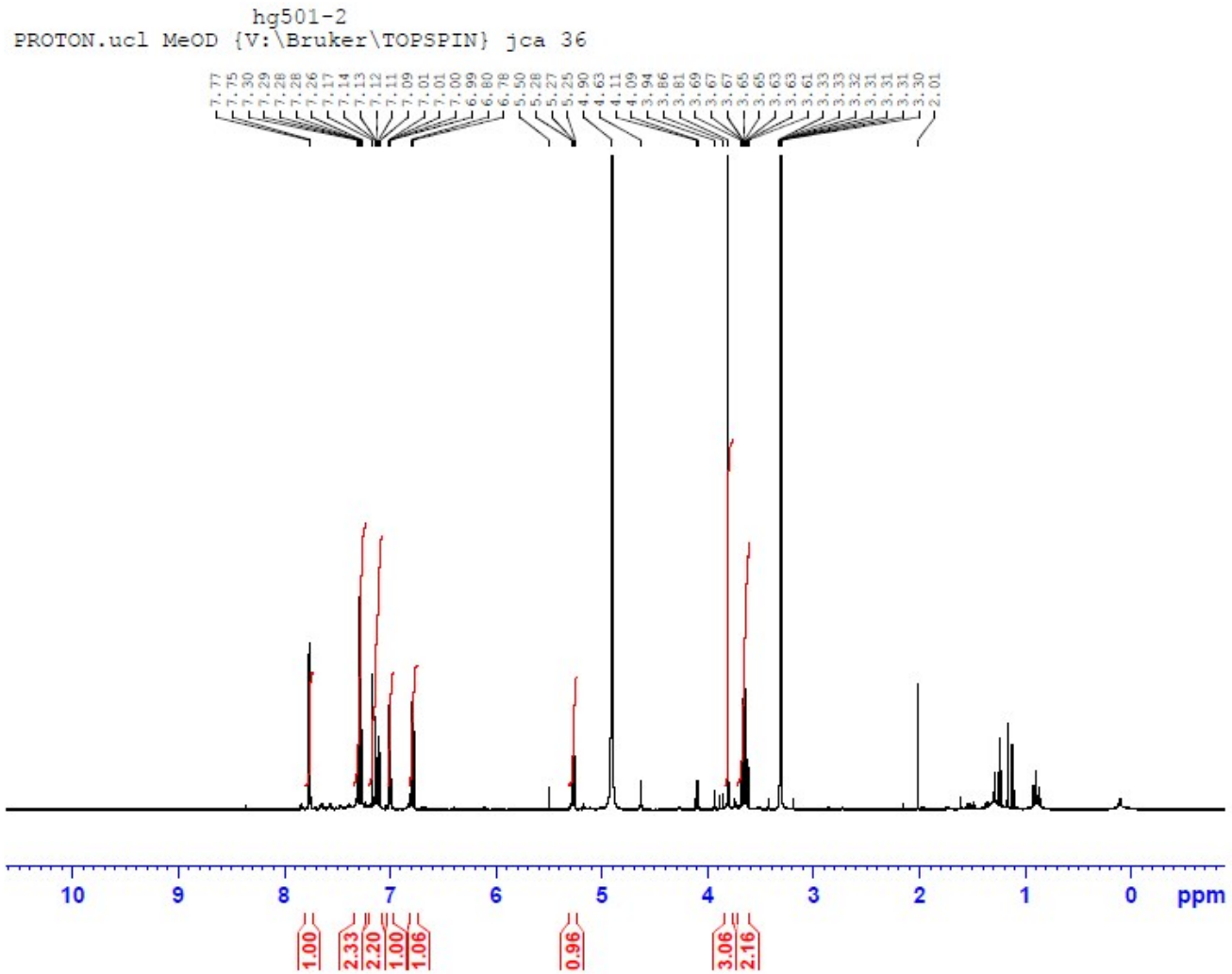
hg900col
C13_DayTime.ucl CDCl3 {W:\600} jca 11

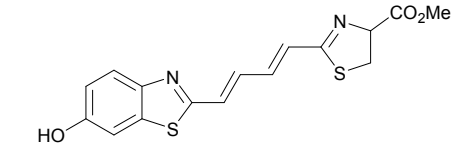


15



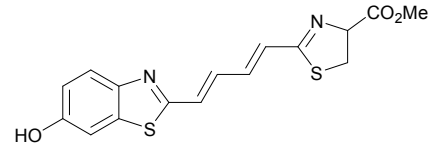

```
Current Data Parameters
NAME                  HG900col
EXPNO                       11
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20160128
Time                     11.32
INSTRUM                  AV600
PROBHD    5 mm CPDCH 13C
PULPROG                zgpg30
TD                        71914
SOLVENT                 CDCl3
NS                           16
DS                            4
SWH                  36057.691 Hz
FIDRES                0.501400 Hz
AQ                    0.9972075 sec
RG                          203
DW                       13.867 usec
DE                       19.08 usec
TE                       298.0 K
D1                    1.79999995 sec
D11                   0.03000000 sec
TD0                          2

======== CHANNEL f1 ========
SFO1                150.9178981 MHz
NUC1                        13C
P1                        9.80 usec
PLW1                26.76899910 W

======== CHANNEL f2 ========
SFO2                600.1324005 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                    70.00 usec
PLW2                13.76700020 W
PLW12                0.36513001 W
PLW13                0.17892000 W

F2 - Processing parameters
SI                        65536
SF                  150.9027930 MHz
WDW                           EM
SSB                  0
LB                        1.00 Hz
GB                   0
PC                        1.40
```





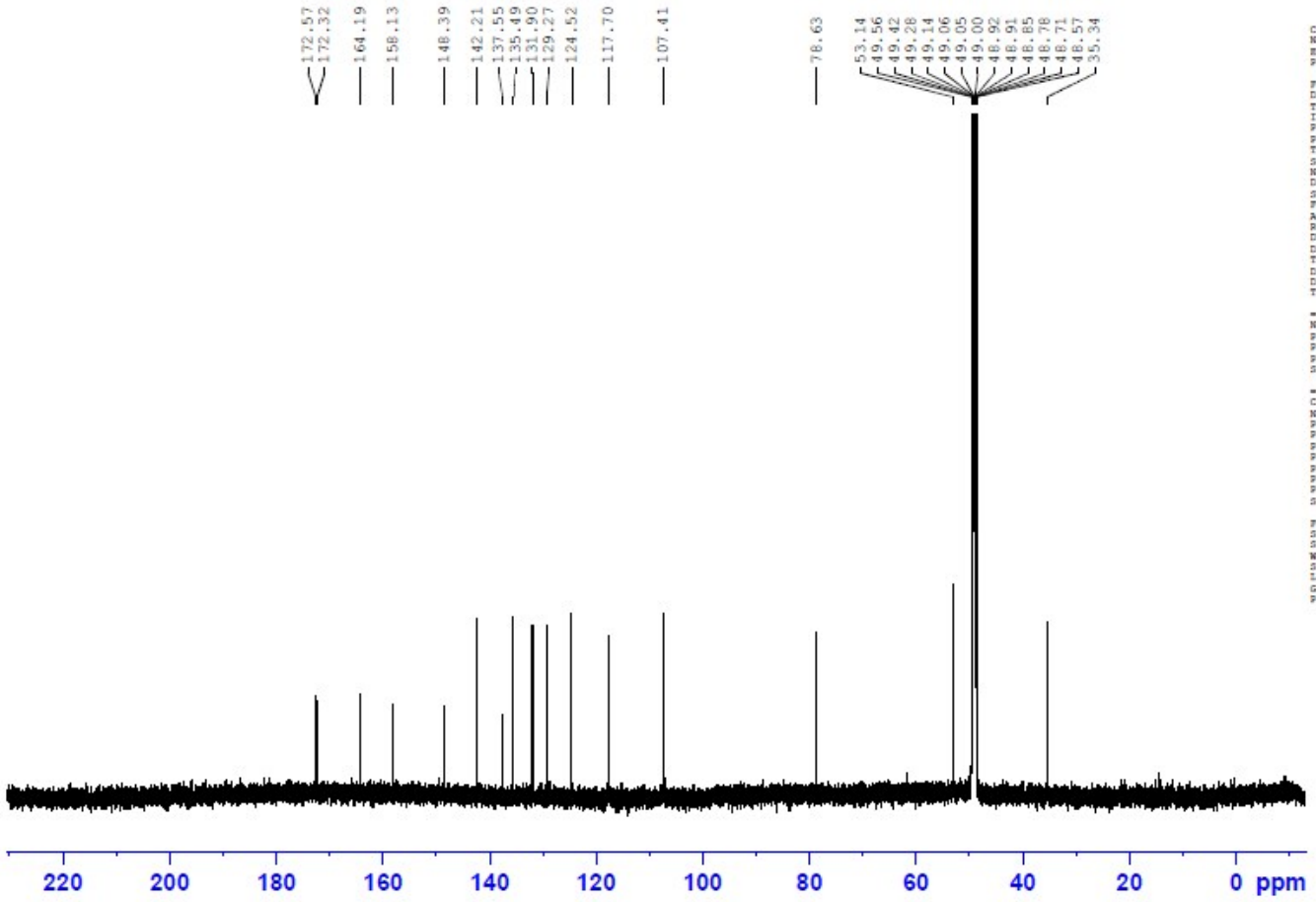
16

```
Current Data Parameters
NAME                  NMR
EXPNO                  60
PROCNO                  1

F2 - Acquisition Parameters
Date_              20120713
Time                  11.50
INSTRUM              AV600
PROBHD   5 mm CPDCH 13C
PULPROG                zg30
TD                    98682
SOLVENT                MeOD
NS                        8
DS                        0
SWH               12335.526 Hz
FIDRES             0.125003 Hz
AQ                 3.9999104 sec
RG                      40.3
DW                   40.533 usec
DE                   10.48 usec
TE                   298.0 K
D1                1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
NUC1                    1H
P1                   11.40 usec
PL1                   1.00 dB
PL1W           13.76731014 W
SFO1           600.1337061 MHz

F2 - Processing parameters
SI                    32768
SF             600.1300141 MHz
WDW                      EM
SSB          0
LB                    0.30 Hz
GB           0
PC                    1.40
```



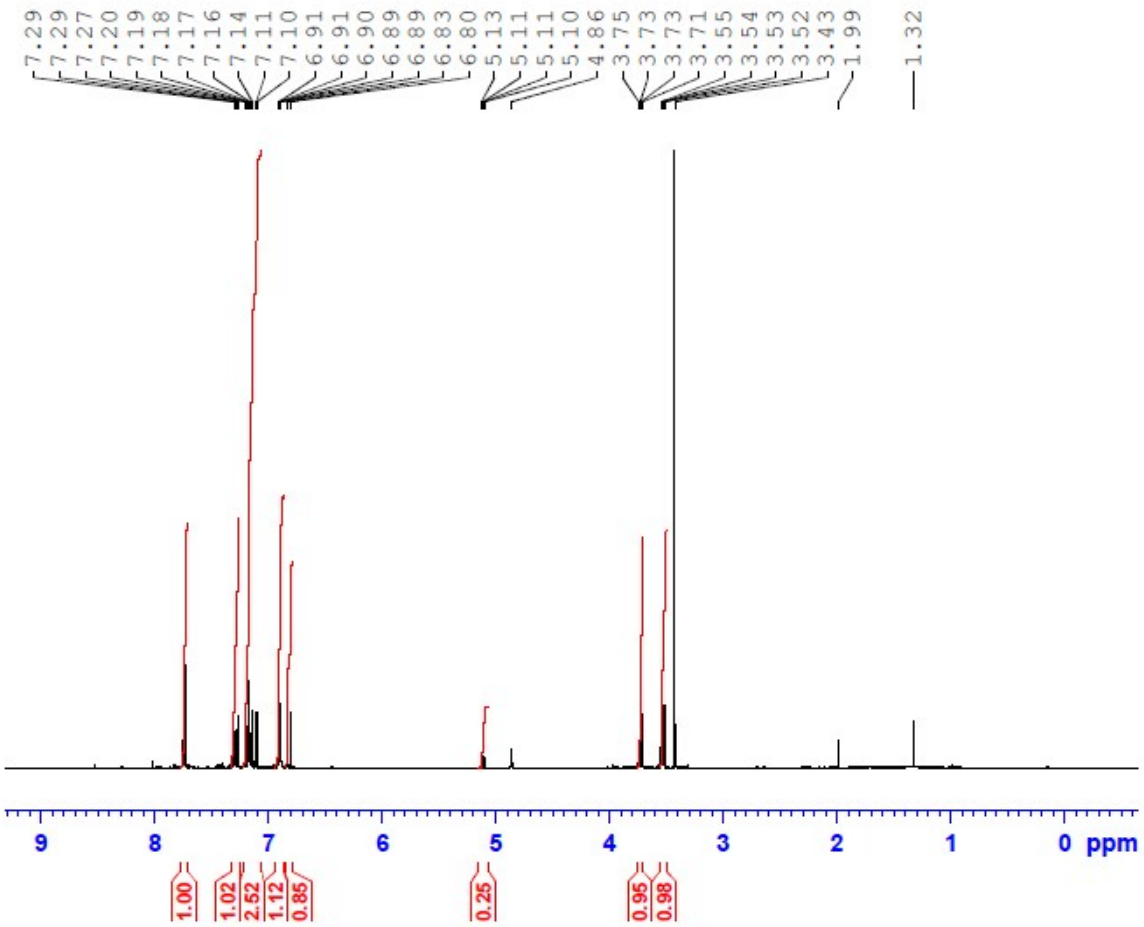
16

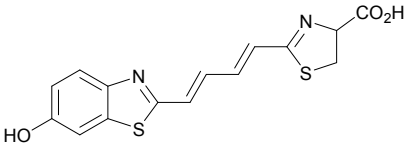
hg501-2
C13CPD.ucl MeOD {V:\Bruker\TOPSPIN} jca 36




```
Current Data Parameters
NAME                  NMR
EXPNO                  62
PROCNO                  1

F2 - Acquisition Parameters
Date_              20120713
Time                  13.30
INSTRUM               AV600
PROBHD   5 mm CPDCH 13C
PULPROG             zgpg30
TD                   70308
SOLVENT               MeOD
NS                     128
DS                       0
SWH              39062.500 Hz
FIDRES           0.555591 Hz
AQ               0.8999424 sec
RG                    1030
DW                 12.800 usec
DE                 21.12 usec
TE                 298.0 K
D1              2.00000000 sec
D11             0.03000000 sec
TD0                     1

======== CHANNEL f1 ========
NUC1                  13C
P1                   9.80 usec
PL1                  5.00 dB
PL1W         26.76886177 W
SFO1         150.9201628 MHz

======== CHANNEL f2 ========
CPDPRG[2           waltz16
NUC2                   1H
PCPD2                70.00 usec
PL2                   1.00 dB
PL12                 17.23 dB
PL13                 20.00 dB
PL2W         13.76731014 W
PL12W         0.32798135 W
PL13W         0.17332016 W
SFO2         600.1324005 MHz

F2 - Processing parameters
SI                    65536
SF           150.9025980 MHz
WDW                     EM
SSB      0
LB                    1.00 Hz
GB       0
PC                    1.40
```

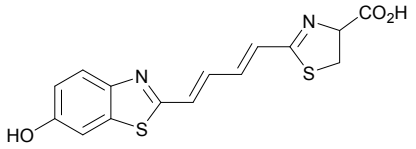
Solvent suppression of largest peak (H_2O).





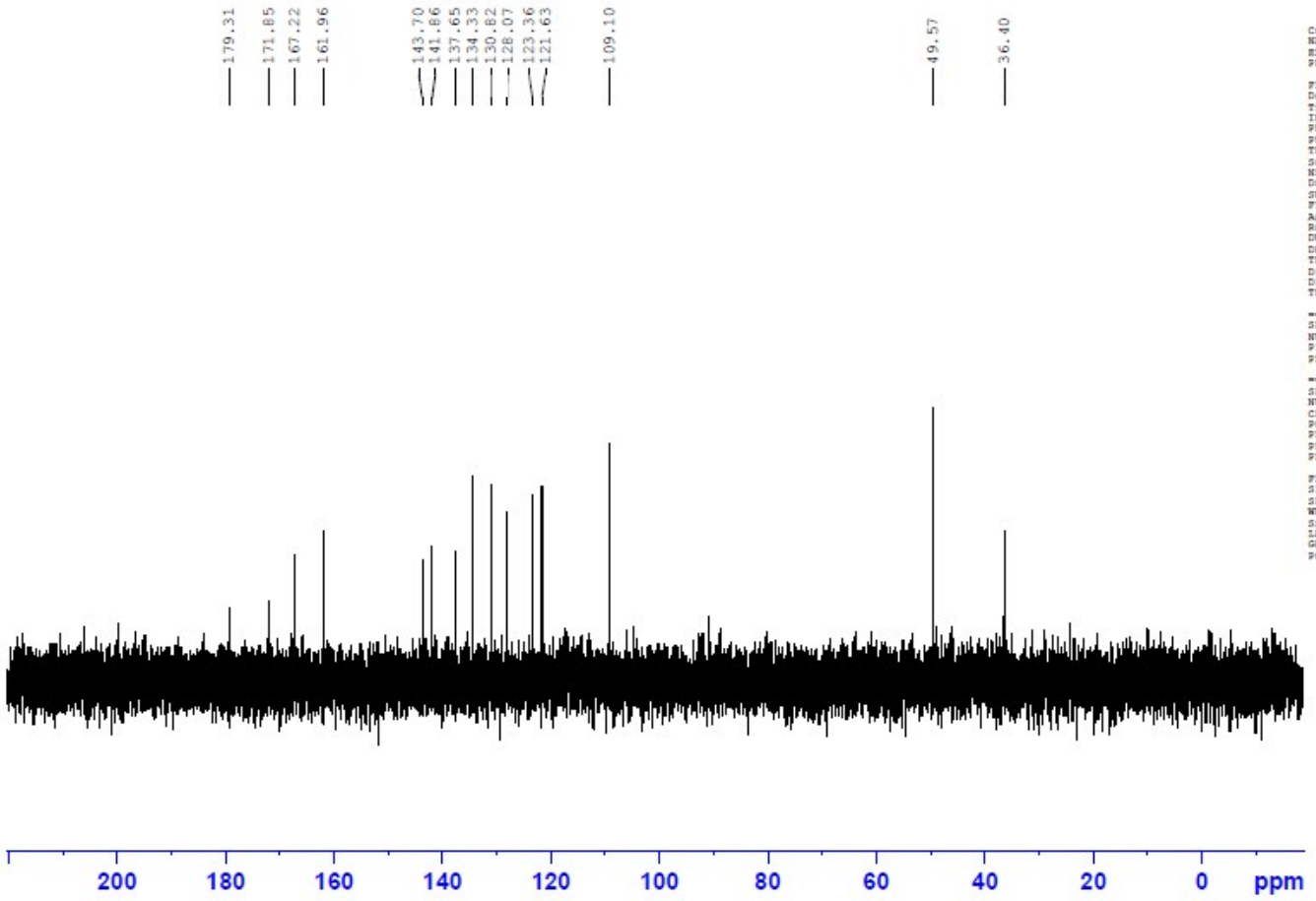
5

```
Current Data Parameters
NAME                  hg932
EXPNO                    10
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160608
Time                  11.41
INSTRUM               AV600
PROBHD   5 mm CPDCH 13C
PULPROG      noesygpprld
TD                   144228
SOLVENT                D2O
NS                         8
DS                         0
SWH               18028.846 Hz
FIDRES            0.125002 Hz
AQ                3.9999232 sec
RG                      1030
DW                   27.733 usec
DE                   40.73 usec
TE                   303.0 K
D1                4.00000000 sec
D8                0.01000000 sec
D12               0.00002000 sec
D16               0.00020000 sec
TD0                        1
ZGOPTNS         -DFLAG_BLK

======== CHANNEL f1 ========
SFO1           600.1328217 MHz
NUC1                   1H
P0                   11.43 usec
P1                   11.43 usec
PLW1          13.76700020 W
PLW9           0.00001800 W

====== GRADIENT CHANNEL =====
GPNAM[1]       SMSQ10.100
GPNAM[2]       SMSQ10.100
GPZ1                50.00 %
GPZ2               -10.00 %
P16               1000.00 usec

F2 - Processing parameters
SI                    131072
SF             600.1299052 MHz
WDW                       EM
SSB       0
LB                     0.30 Hz
GB        0
PC                     1.00
```



5

C13_DayTime.ucl D2O {W:\600} jca 44




```
Current Data Parameters
NAME                  hg932
EXPNO                    14
PROCNO                    1

F2 - Acquisition Parameters
Date_              20160608
Time                  13.11
INSTRUM               AV600
PROBHD   5 mm CPDCH 13C
PULPROG             zgpg30
TD                   71914
SOLVENT                D2O
NS                       512
DS                         4
SWH               36057.691 Hz
FIDRES             0.501400 Hz
AQ                 0.9972075 sec
RG                      1150
DW                   13.867 usec
DE                    19.08 usec
TE                   303.0 K
D1                1.79999995 sec
D11               0.03000000 sec
TD0                        1

======== CHANNEL f1 ========
SFO1             150.9178981 MHz
NUC1                     13C
P1                      9.80 usec
PLW1             26.76899910 W

======== CHANNEL f2 ========
SFO2             600.1324005 MHz
NUC2                     1H
CPDPRG[2              waltz16
PCPD2                  70.00 usec
PLW2             13.76700020 W
PLW12             0.36513001 W
PLW13             0.17892000 W

F2 - Processing parameters
SI                       65536
SF               150.9026985 MHz
WDW                        EM
SSB       0
LB                      1.00 Hz
GB        0
PC                      1.40
```