SUPPORTING INFORMATION

Reactions of 1,3-diketones with a dipeptide isothiazolidin-3-one: toward agents that covalently capture oxidized protein tyrosine phosphatase 1B (PTP1B)

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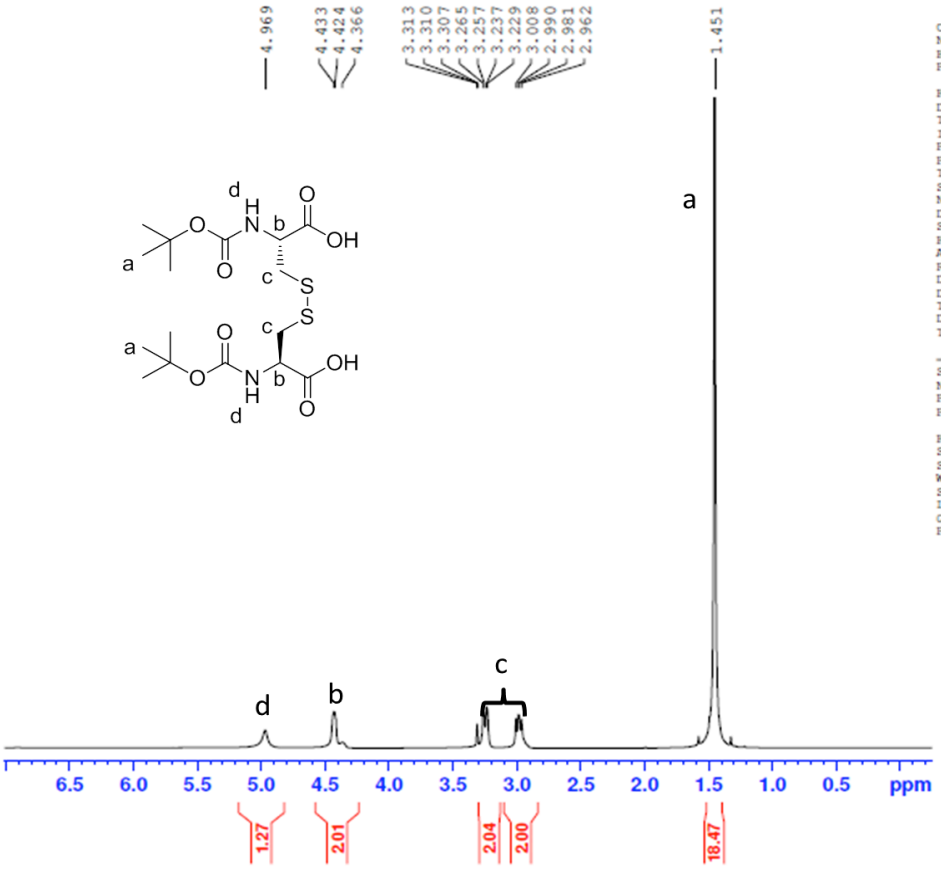
^{1}H & ^{13}C NMR of 2

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PROCNO                       1

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Date_                 20150506
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PULPROG                  zg30
TD                      65536
SOLVENT                  MeOD
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NUC1                        1H
P1                         8.00 usec
PLW1                4.30000019 W

F2 - Processing parameters
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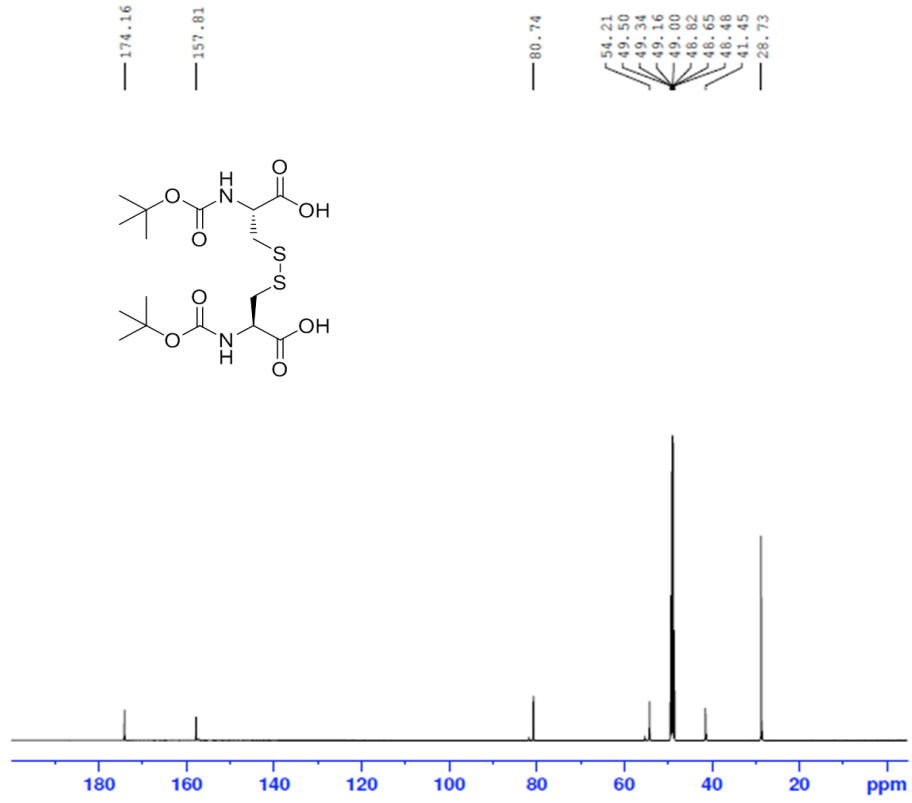


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PROCNO                       1

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PROBHD    5 mm CPTCI 1H-
PULPROG                zgpg30
TD                      65536
SOLVENT                  MeOD
NS                         2058
DS                            4
SWH                29761.904 Hz
FIDRES                 0.454131 Hz
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RG                       184.57
DW                        16.800 usec
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TE                        300.0 K
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D11                0.03000000 sec
TD0                          1

======== CHANNEL f1 ========
SFO1               125.7703637 MHz
NUC1                        13C
P1                        12.00 usec
PLW1               69.00000000 W

======== CHANNEL f2 ========
SFO2               500.1320005 MHz
NUC2                        1H
CPDPRG[2                waltz16
PCPD2                     80.00 usec
PLW2                4.30000019 W
PLW12               0.04300000 W
PLW13               0.02752000 W

F2 - Processing parameters
SI                          32768
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WDW                         EM
SSB                  0
LB                         1.00 Hz
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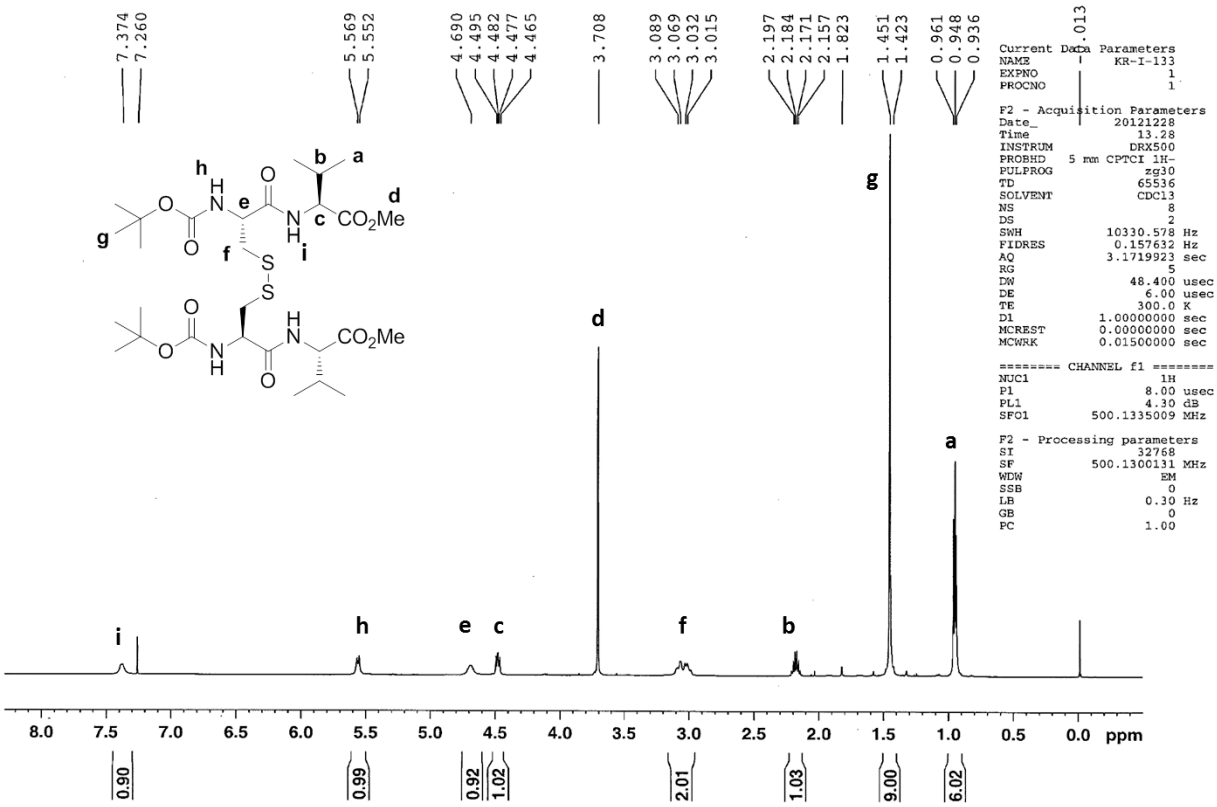
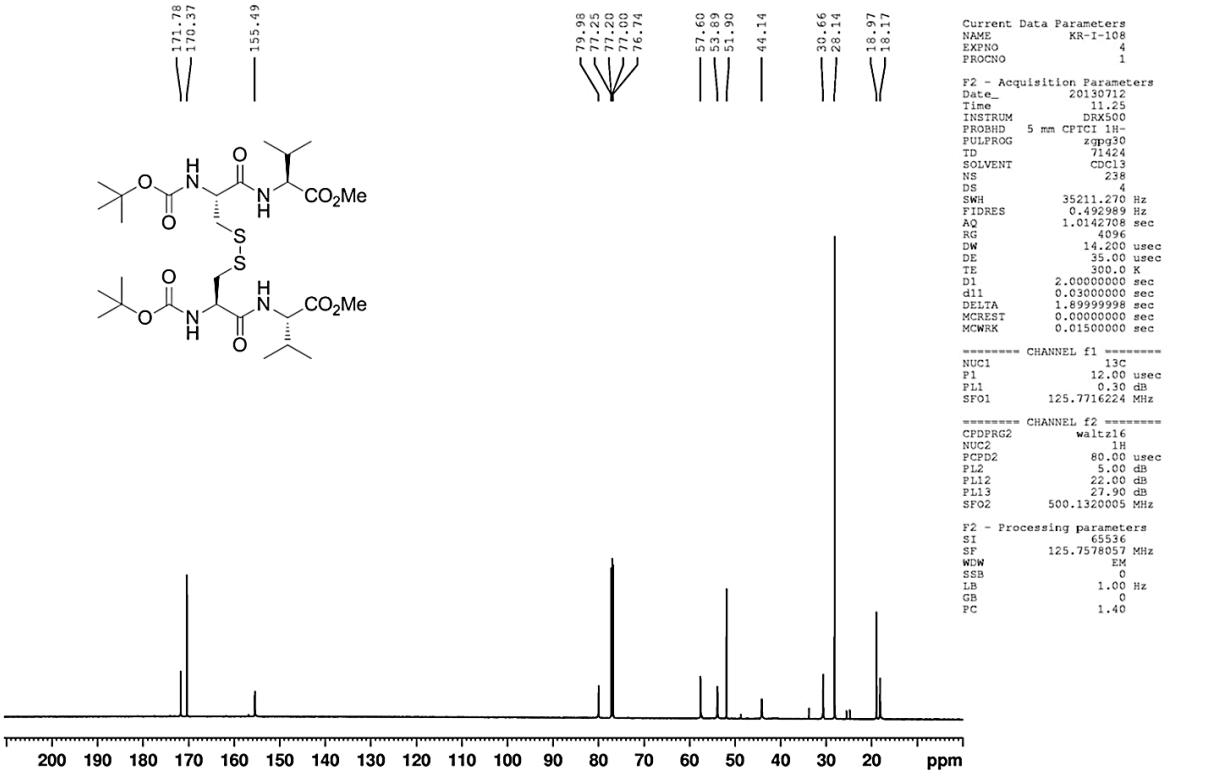
^{1}H & ^{13}C NMR of 3





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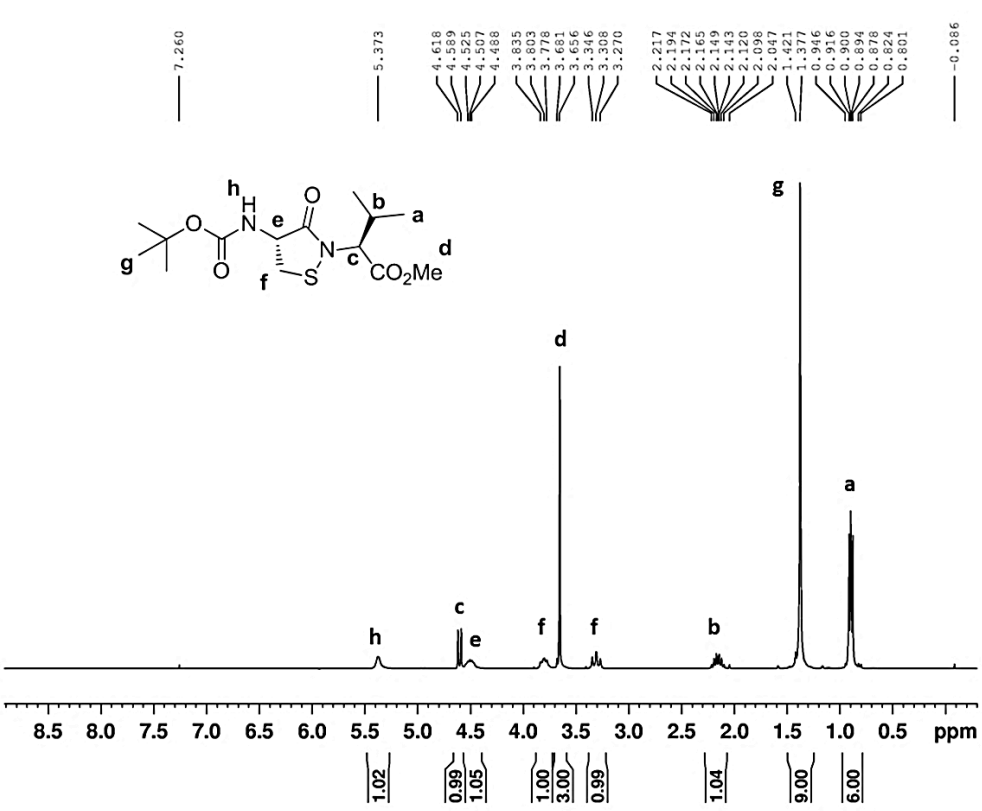


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EXPNO                   3
PROCNO                  1

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PULPROG          zg30pad
TD                 32768
SOLVENT             CDC13
NS                     16
DS                      2
SWH              6172.839 Hz
FIDRES           0.188380 Hz
AQ               2.6542580 sec
RG                   45.3
DW                 81.000 usec
DE                  6.00 usec
TE                 300.0 K
D1             1.00000000 sec
D31            0.00000000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                   7.05 usec
PL1                  0.00 dB
SFO1          300.1318534 MHz

F2 - Processing parameters
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WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.30
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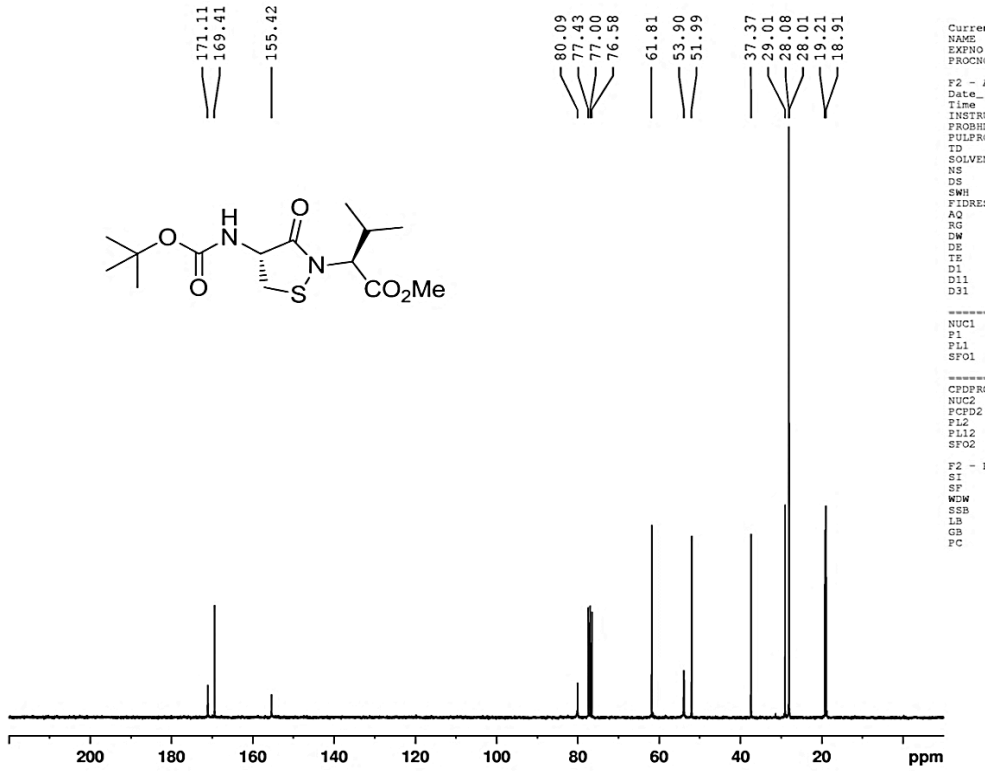


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Current Data Parameters
NAME        sulfenylamide
EXPNO                   4
PROCNO                  1

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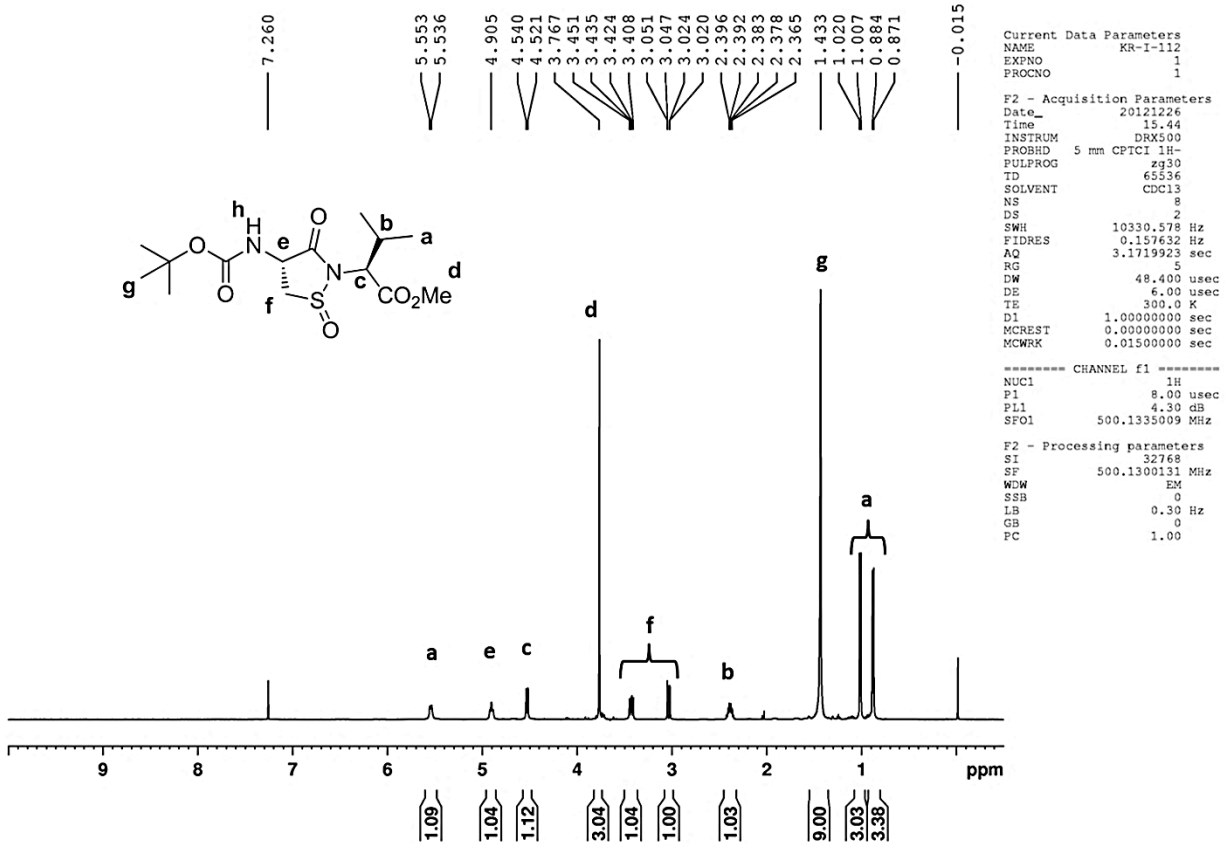
PULPROG        zgdc30pad
TD                 65536
SOLVENT             CDC13
NS                    463
DS                      4
SWH             18832.393 Hz
FIDRES           0.287360 Hz
AQ               1.7400308 sec
RG                   22528
DW                 26.550 usec
DE                  6.00 usec
TE                 300.0 K
D1             2.00000000 sec
D11            0.03000000 sec
D31            0.00000000 sec

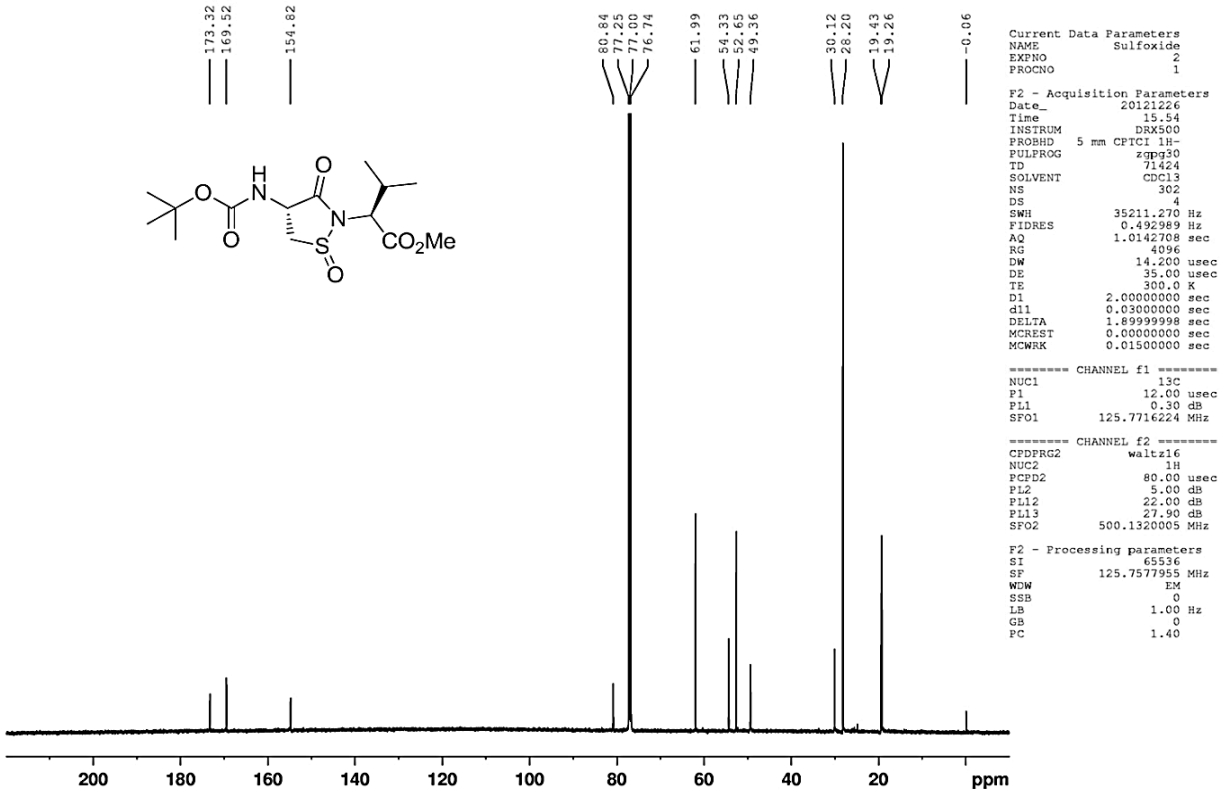
======== CHANNEL f1 ========
NUC1                  13C
P1                  10.00 usec
PL1                  5.00 dB
SFO1          75.4760107 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                   1H
PCPD2              100.00 usec
PL2                120.00 dB
PL12                21.41 dB
SFO2          300.1312005 MHz

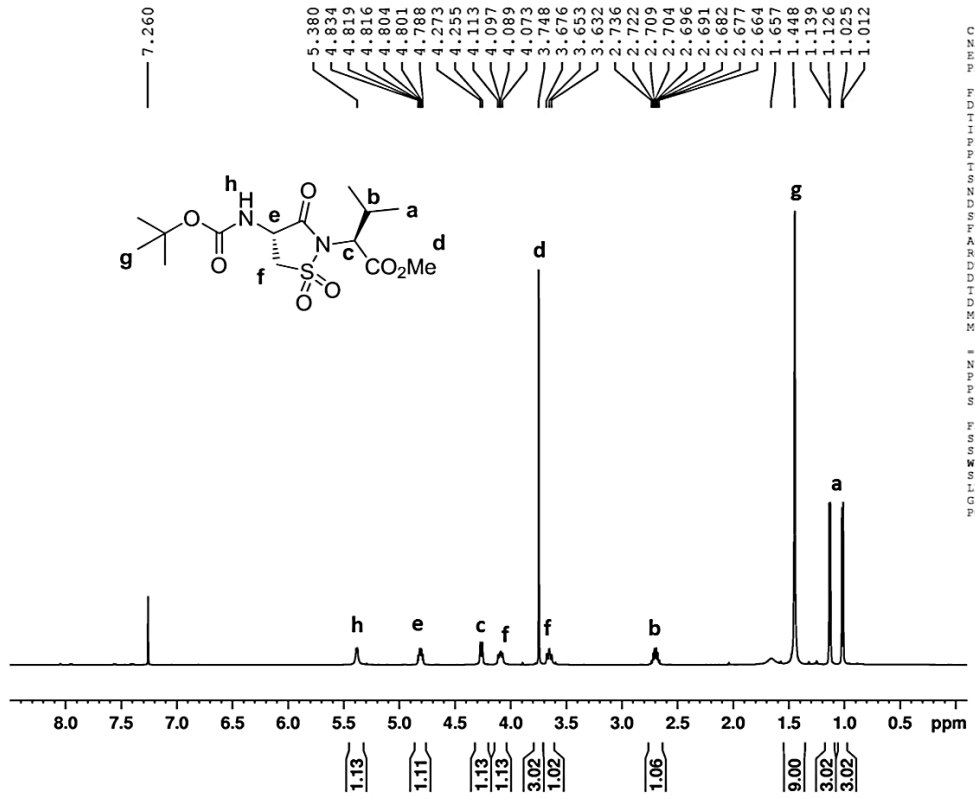
F2 - Processing parameters
SI                 32768
SF            75.4677594 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
```

^{1}H & ^{13}C NMR of 5





^{1}H & ^{13}C NMR of 6

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EXPNO                       1
PROCNO                      1

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Time                   17.37
INSTRUM                DRX500
PROBHD   5 mm CPTCI 1H-
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                          8
DS                          2
SWH                10330.578 Hz
FIDRES             0.157632 Hz
AQ                 3.1719923 sec
RG                       11.3
DW                     48.400 usec
DE                      6.00 usec
TE                     300.0 K
D1                 1.00000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                       8.00 usec
PL1                      4.30 dB
SFO1               500.1335009 MHz

F2 - Processing parameters
SI                         32768
SF                 500.1300140 MHz
WDW                           EM
SSB                            0
LB                          0.30 Hz
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PC                          1.40
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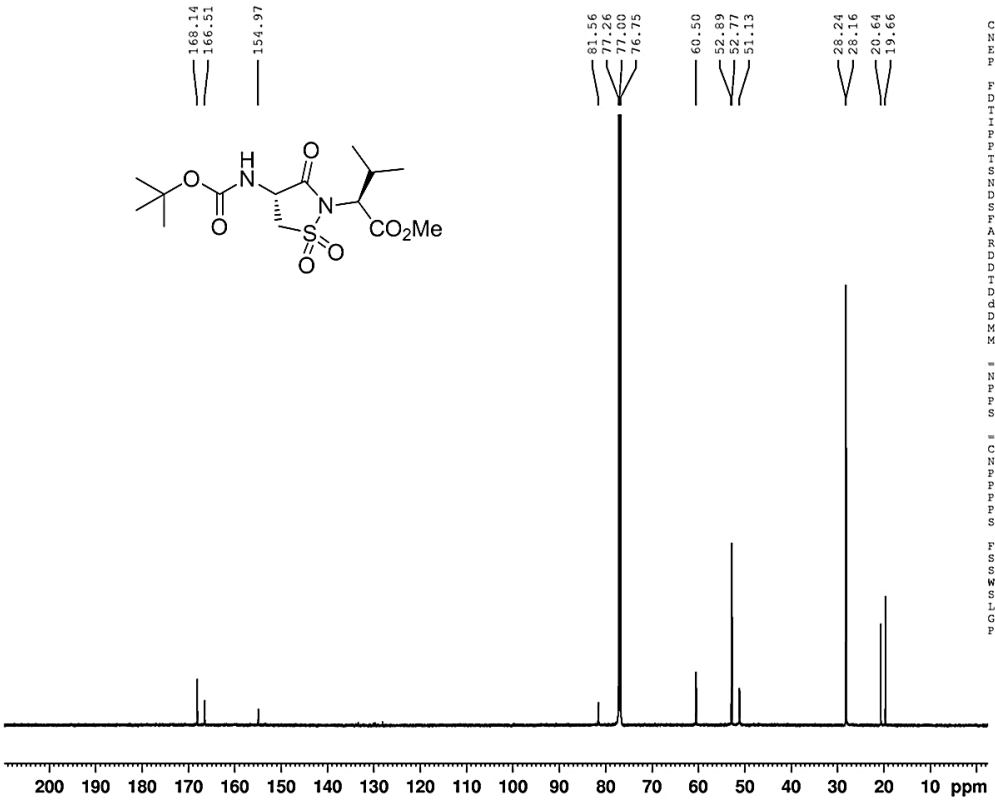


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Current Data Parameters
NAME                 sulfone1
EXPNO                       2
PROCNO                      1

F2 - Acquisition Parameters
Date_               20130709
Time                   17.46
INSTRUM                DRX500
PROBHD   5 mm CPTCI 1H-
PULPROG              zgpg30
TD                     71424
SOLVENT                CDCl3
NS                       918
DS                         4
SWH                35211.270 Hz
FIDRES             0.492989 Hz
AQ                 1.0142708 sec
RG                      4096
DW                     14.200 usec
DE                      35.00 usec
TE                     300.0 K
D1                 2.00000000 sec
d11                0.03000000 sec
DELTA              1.89999998 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                       13C
P1                      12.00 usec
PL1                      0.30 dB
SFO1               125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                   80.00 usec
PL2                      5.00 dB
PL12                    22.00 dB
PL13                    27.90 dB
SFO2               500.1320005 MHz

F2 - Processing parameters
SI                         65536
SF                 125.7577928 MHz
WDW                           EM
SSB                            0
LB                          1.00 Hz
GB                             0
PC                          1.00
```
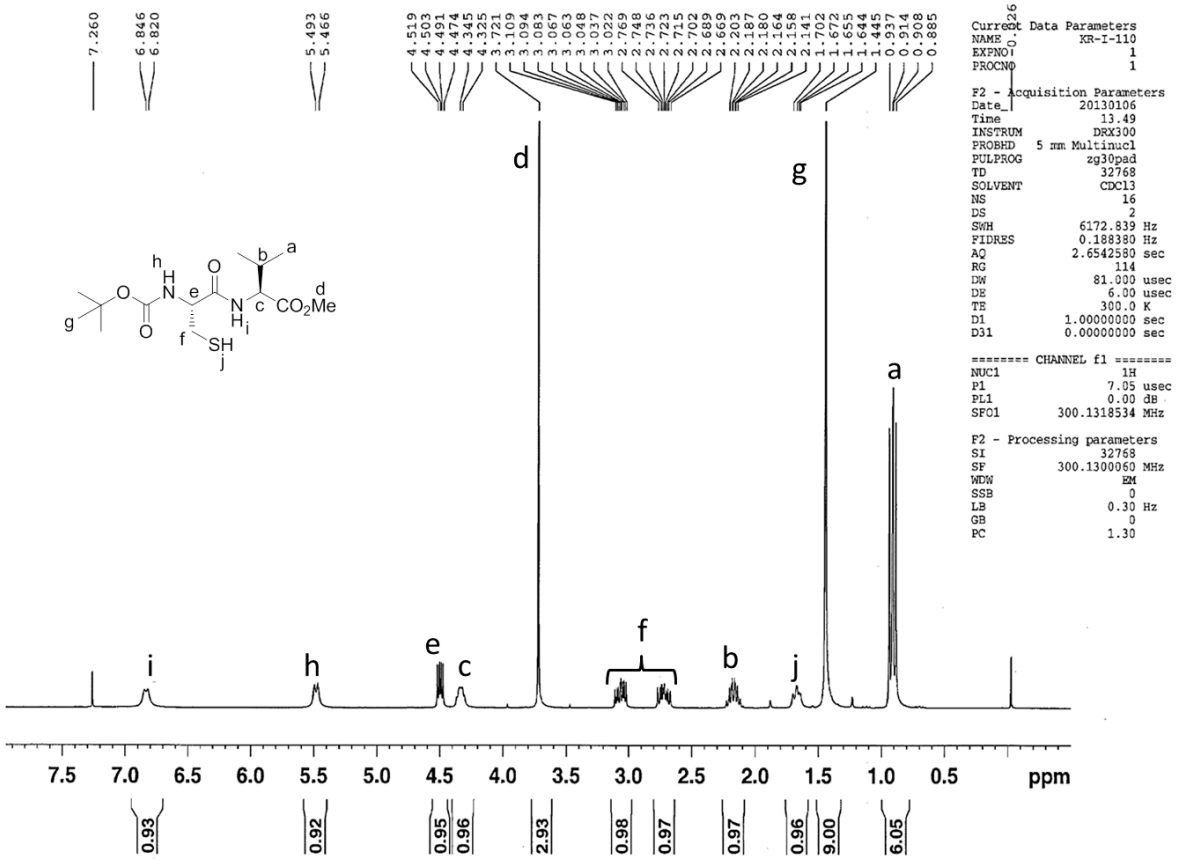

^{1}H & ^{13}C NMR of 7

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EXPNO                        1
PROCNO                       1

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Time                     13.49
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PROBHD    5 mm Multinucl
PULPROG              zg30pad
TD                       32768
SOLVENT                  CDCl3
NS                          16
DS                           2
SWH                 6172.839 Hz
FIDRES              0.188380 Hz
AQ                  2.6542580 sec
RG                         114
DW                    81.000 usec
DE                     6.00 usec
TE                     300.0 K
D1                 1.00000000 sec
D31                0.00000000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                     7.05 usec
PL1                    0.00 dB
SFO1           300.1318534 MHz

F2 - Processing parameters
SI                       32768
SF              300.1300060 MHz
WDW                         EM
SSB                          0
LB                     0.30 Hz
GB                           0
PC                        1.30
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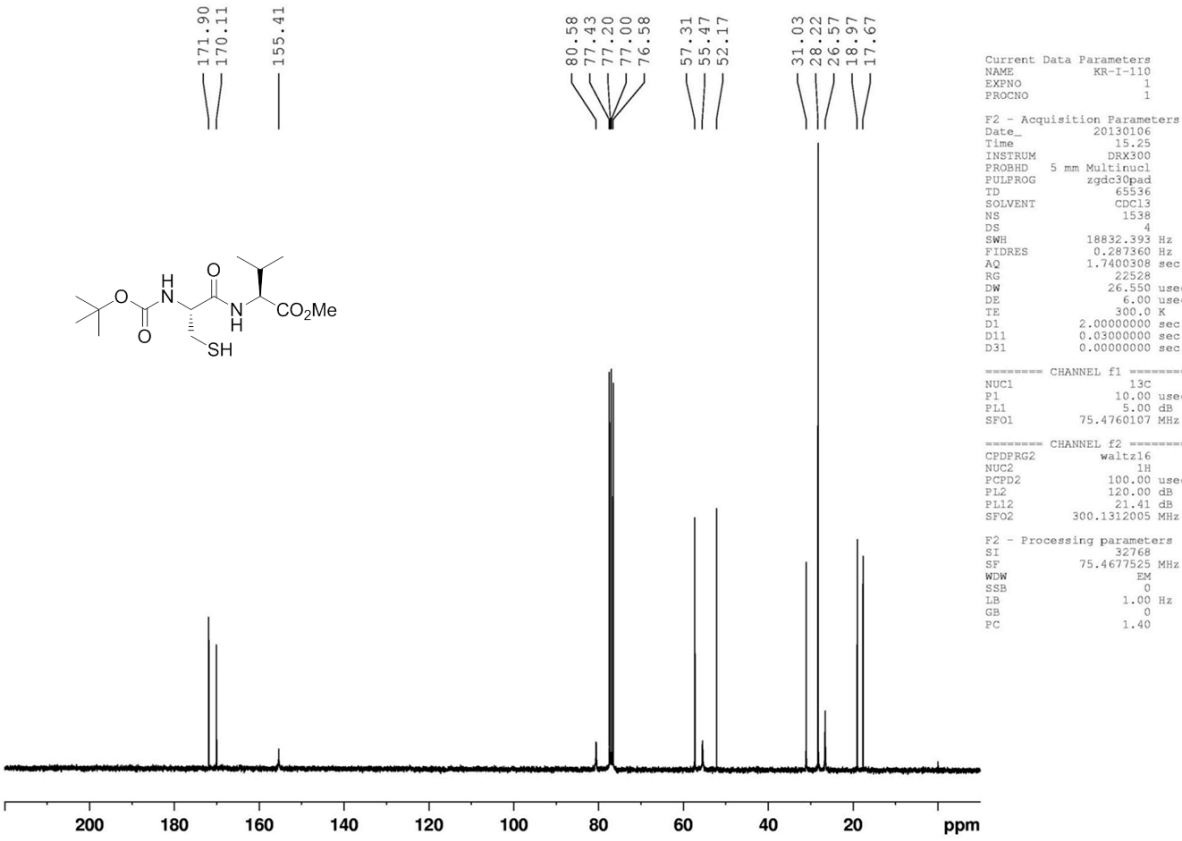


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Current Data Parameters
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EXPNO                        1
PROCNO                       1

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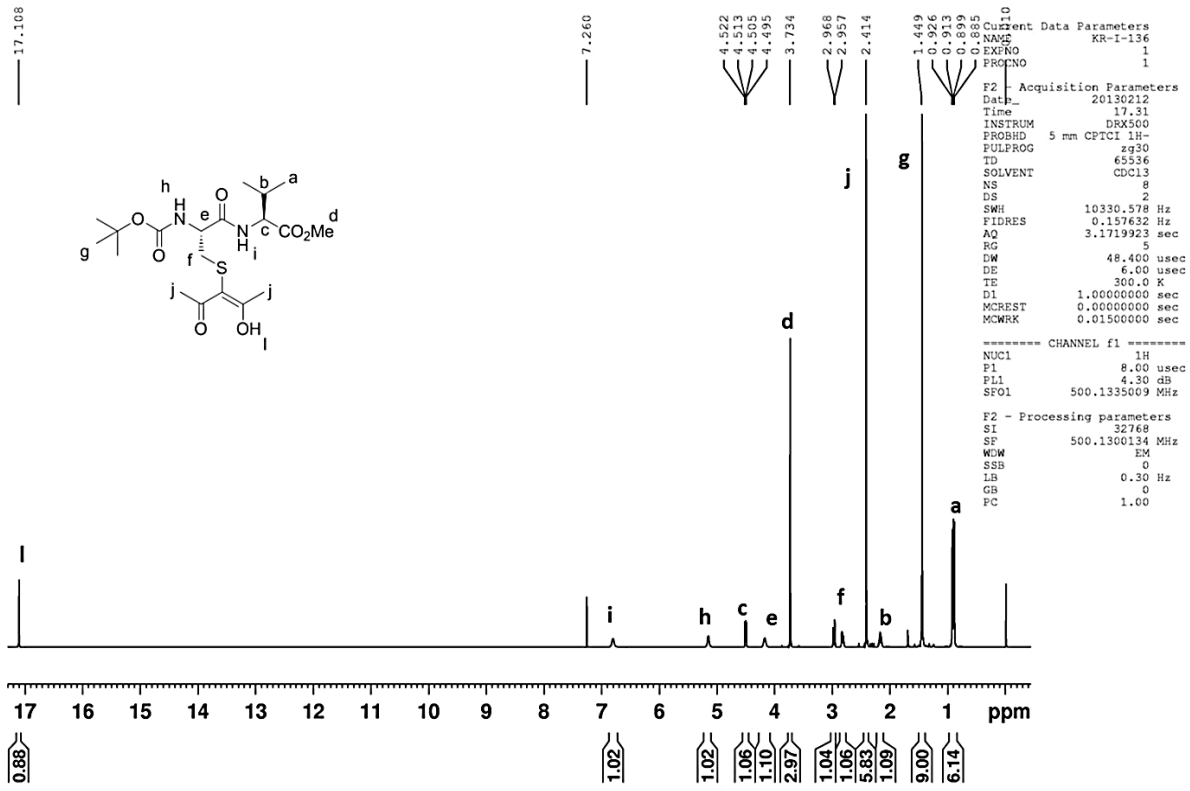
INSTRUM                DRX300
PROBHD    5 mm Multinucl
PULPROG            zgdc30pad
TD                       65536
SOLVENT                  CDCl3
NS                        1538
DS                           4
SWH                18832.393 Hz
FIDRES              0.287360 Hz
AQ                  1.7400308 sec
RG                       22528
DW                    26.550 usec
DE                     6.00 usec
TE                     300.0 K
D1                 2.00000000 sec
D11                0.03000000 sec
D31                0.00000000 sec

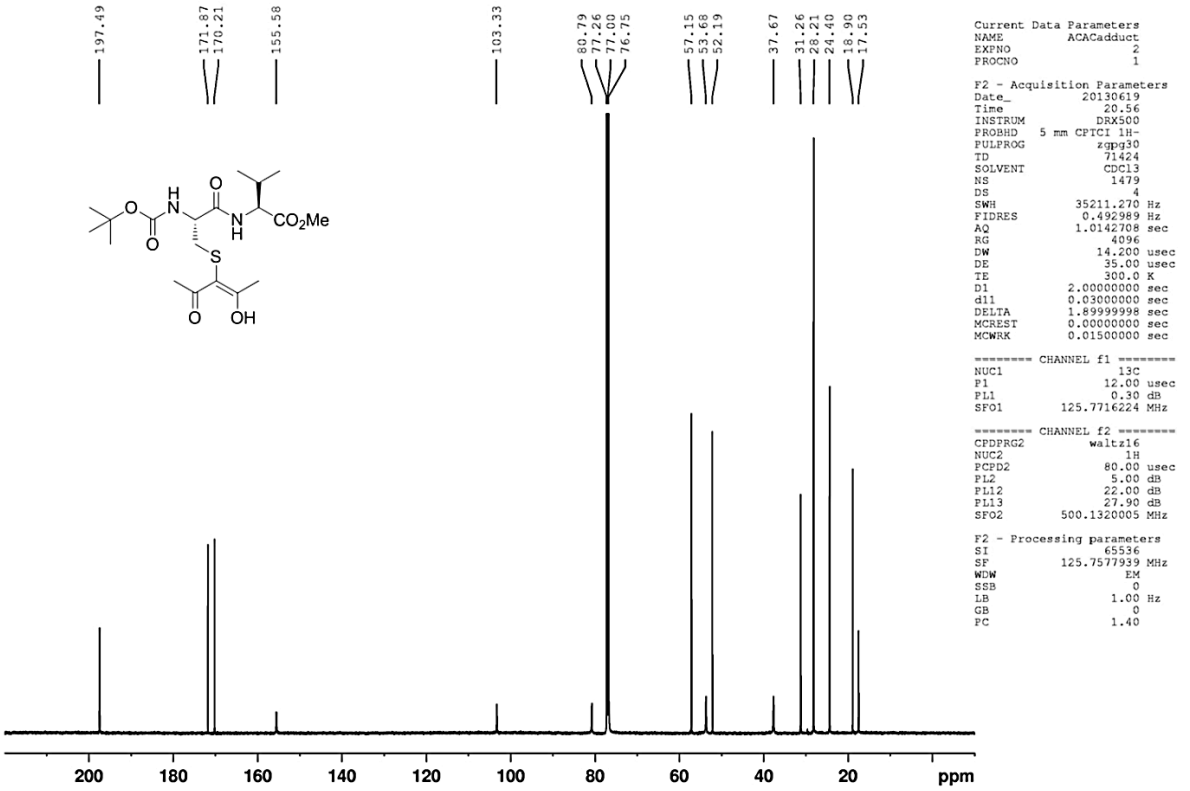
======== CHANNEL f1 ========
NUC1                      13C
P1                    10.00 usec
PL1                    5.00 dB
SFO1           75.4760107 MHz

======== CHANNEL f2 ========
CPDPRG2               waltz16
NUC2                       1H
PCPD2                100.00 usec
PL2                   120.00 dB
PL12                   21.41 dB
SFO2           300.1312005 MHz

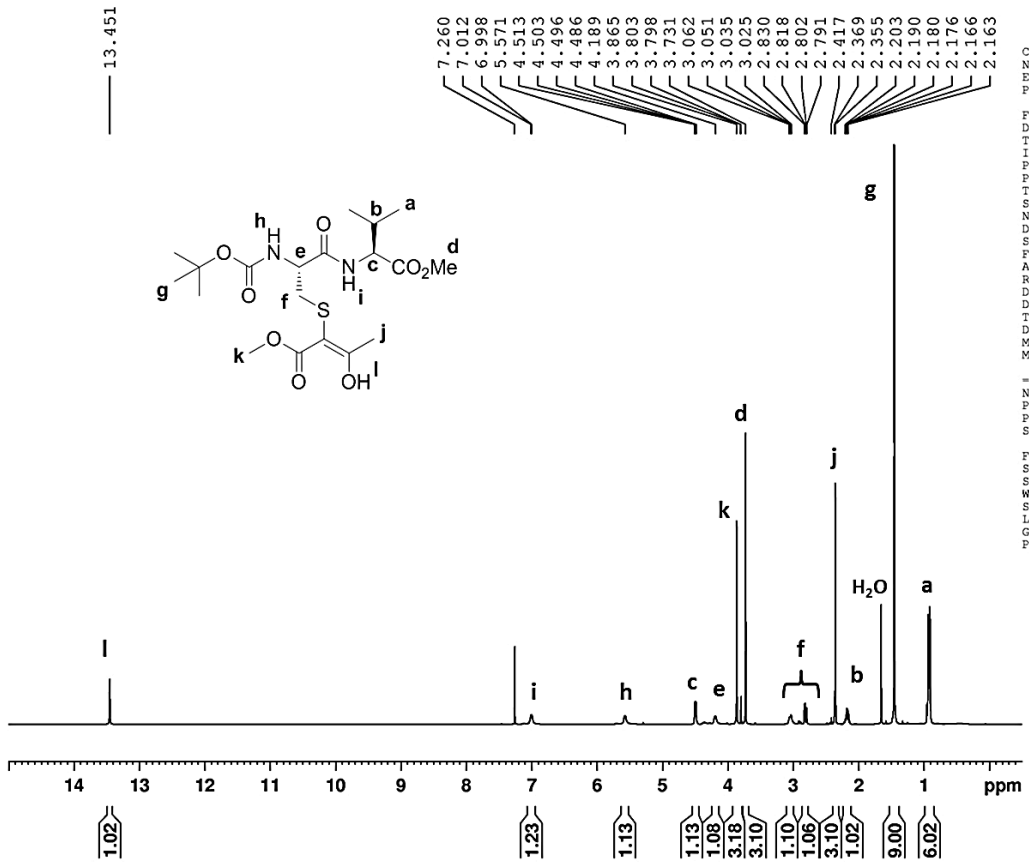
F2 - Processing parameters
SI                       32768
SF              75.4677525 MHz
WDW                         EM
SSB                          0
LB                     1.00 Hz
GB                           0
PC                        1.40
```

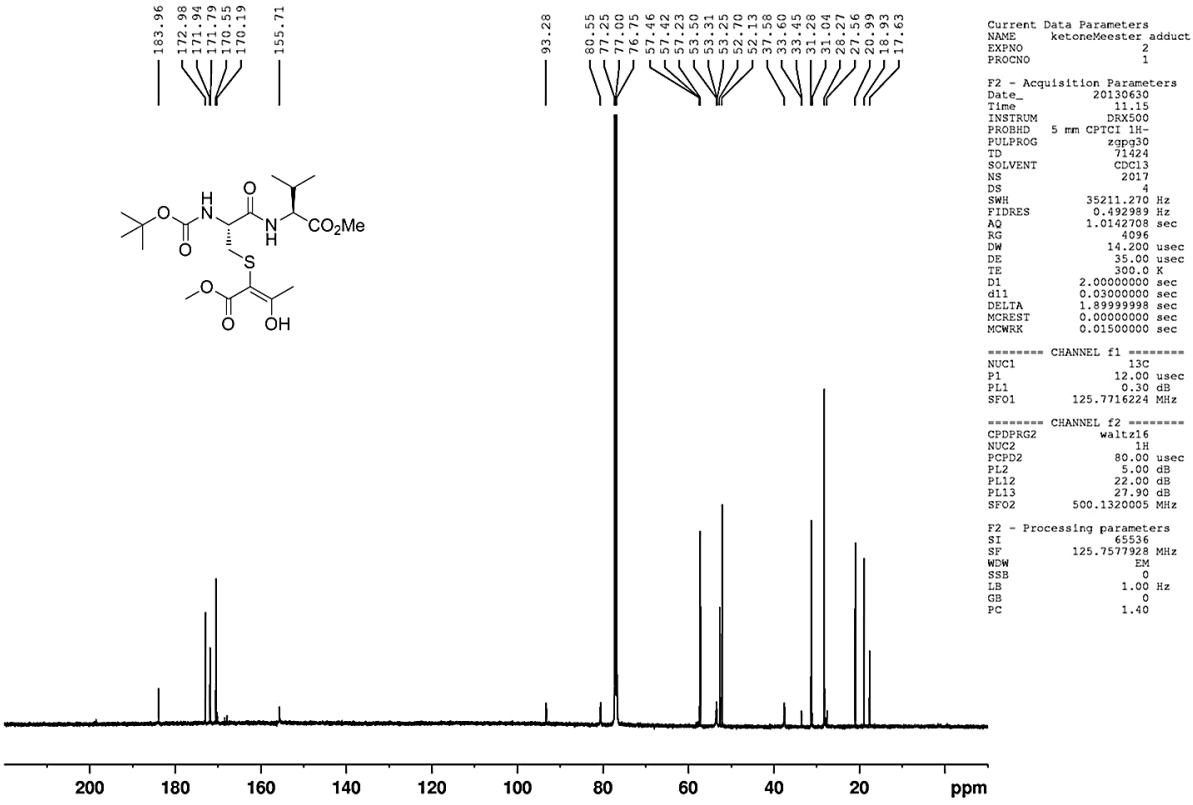
^{1}H & ^{13}C NMR of 8a



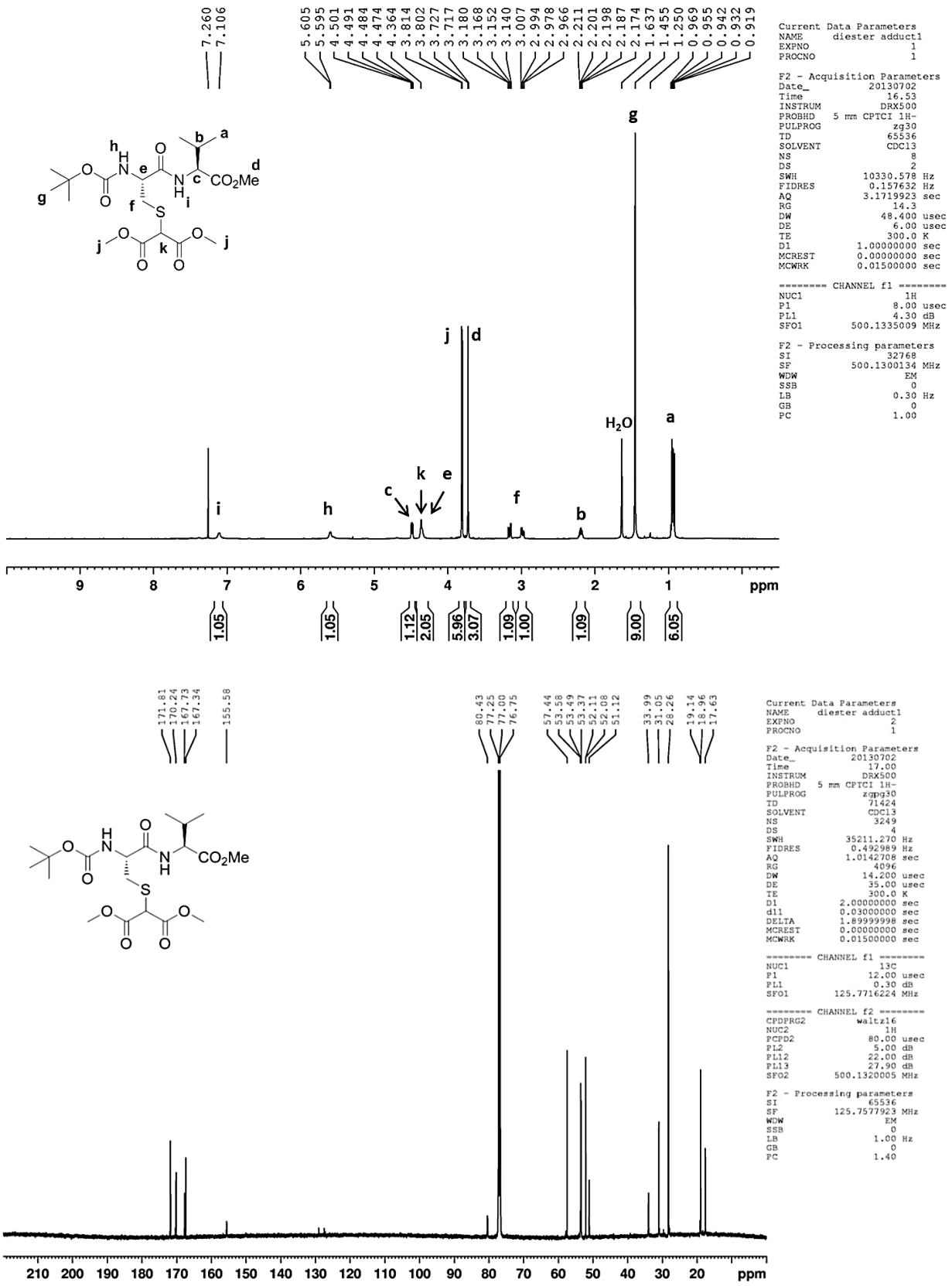


^{1}H & ^{13}C NMR of 8b

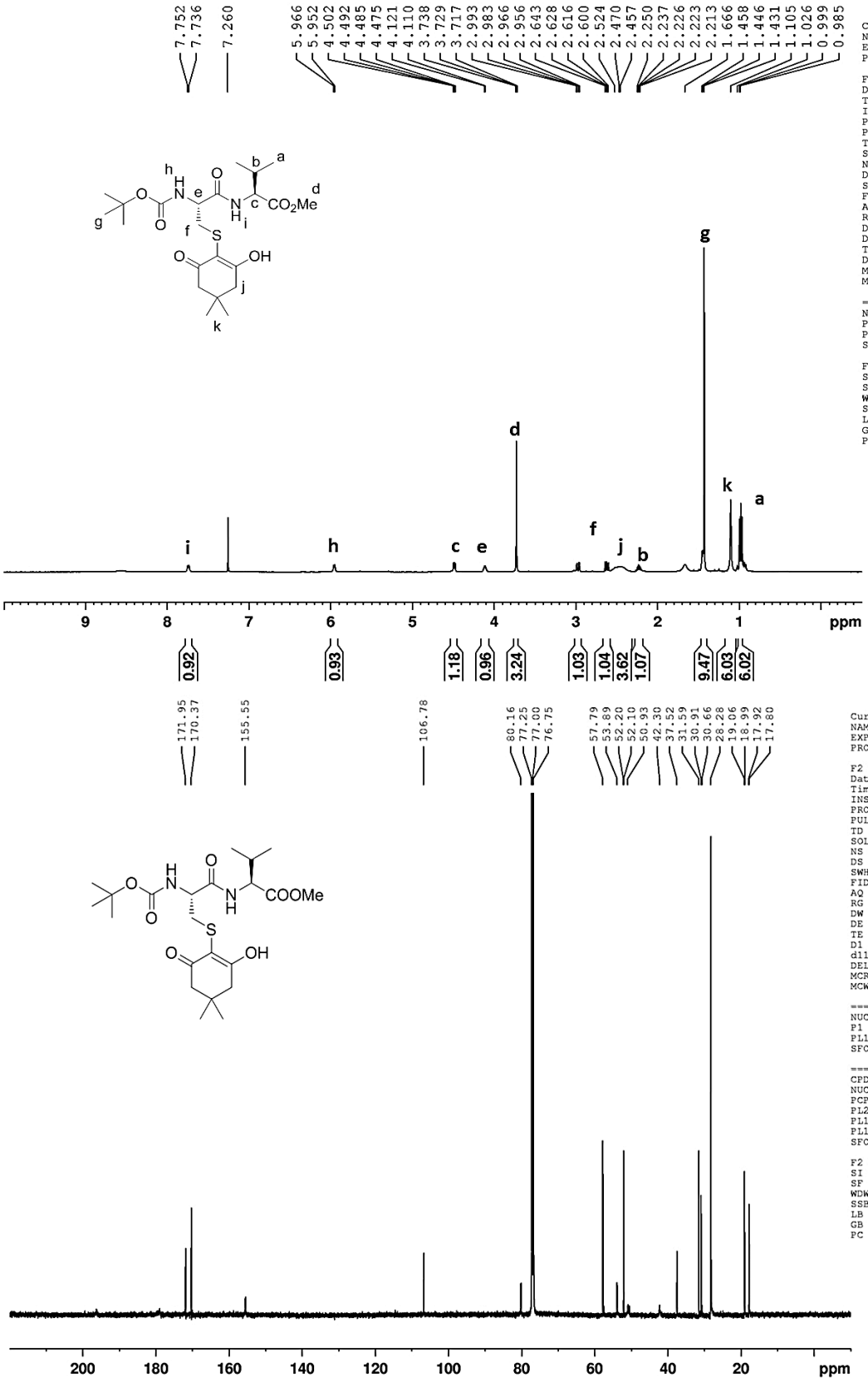




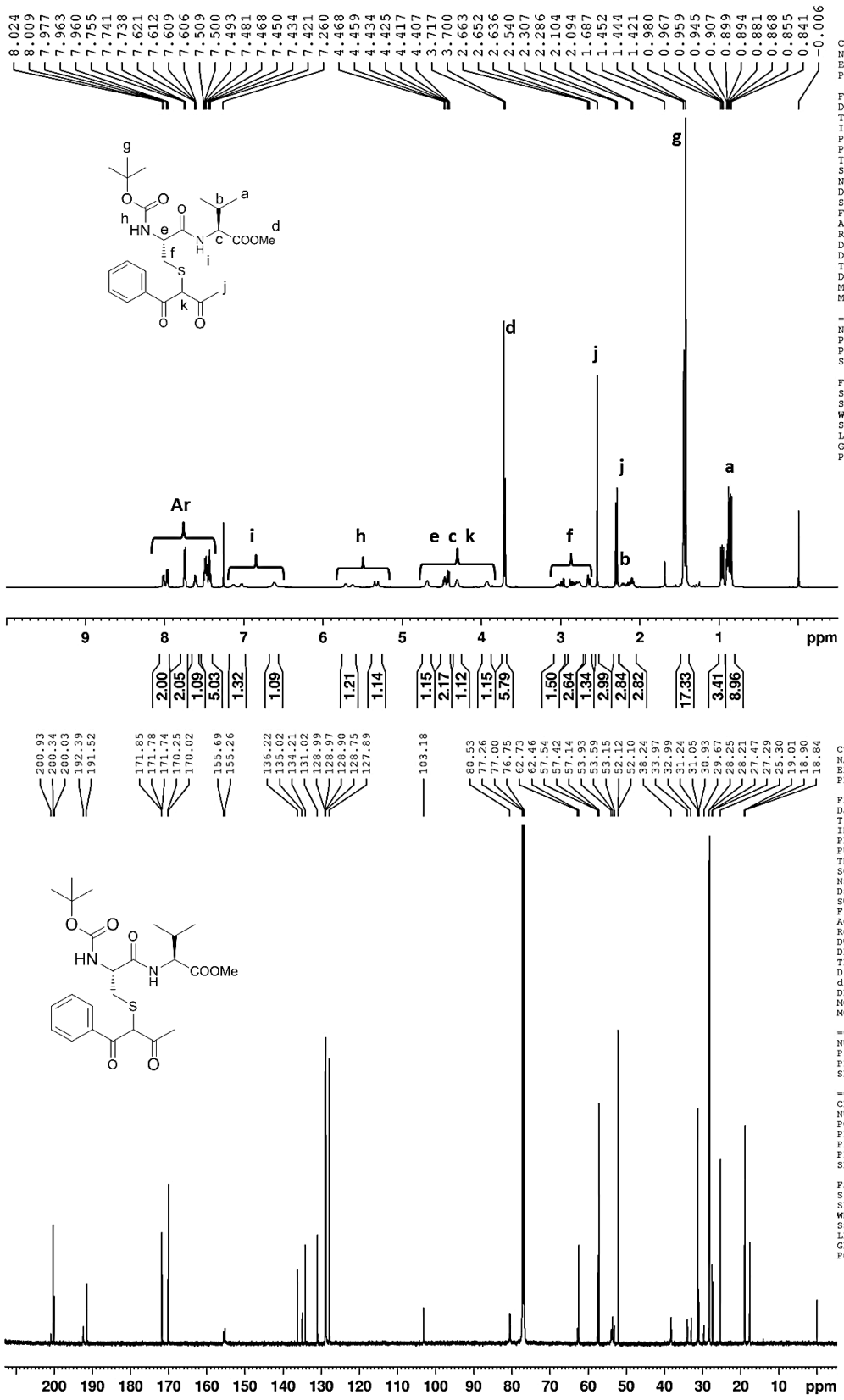
^{1}H & ^{13}C NMR of 8c



^{1}H & ^{13}C NMR of 8d



^{1}H & ^{13}C NMR of 8e

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INSTRUM           DRX500
PROBHD   5 mm CPTCI 1H-
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                    8
DS                    2
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ             3.1719923 sec
RG                    5
DW                48.400 usec
DE                 6.00 usec
TE                300.0 K
D1            1.00000000 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                  1H
P1                 8.00 usec
PL1                4.30 dB
SFO1        500.1335009 MHz

F2 - Processing parameters
SI                 32768
SF          500.1300131 MHz
WDW                   EM
SSB                    0
LB                  0.30 Hz
GB                     0
PC                  1.00
```

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Current Data Parameters
NAME           KR-I-139
EXPNO                 5
PROCNO                1

F2 - Acquisition Parameters
Date_           20130817
Time               21.54
INSTRUM           DRX500
PROBHD   5 mm CPTCI 1H-
PULPROG          zgpg30
TD                71424
SOLVENT           CDCl3
NS                 3139
DS                    4
SWH           35211.270 Hz
FIDRES         0.492989 Hz
AQ             1.0142708 sec
RG                 4096
DW                14.200 usec
DE                35.00 usec
TE                300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                 13C
P1                12.00 usec
PL1                0.00 dB
SFO1        125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                  1H
PCPD2             80.00 usec
PL2                5.00 dB
PL12              22.00 dB
PL13              27.90 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                 65536
SF          125.7577933 MHz
WDW                   EM
SSB                    0
LB                  1.00 Hz
GB                     0
PC                  1.40
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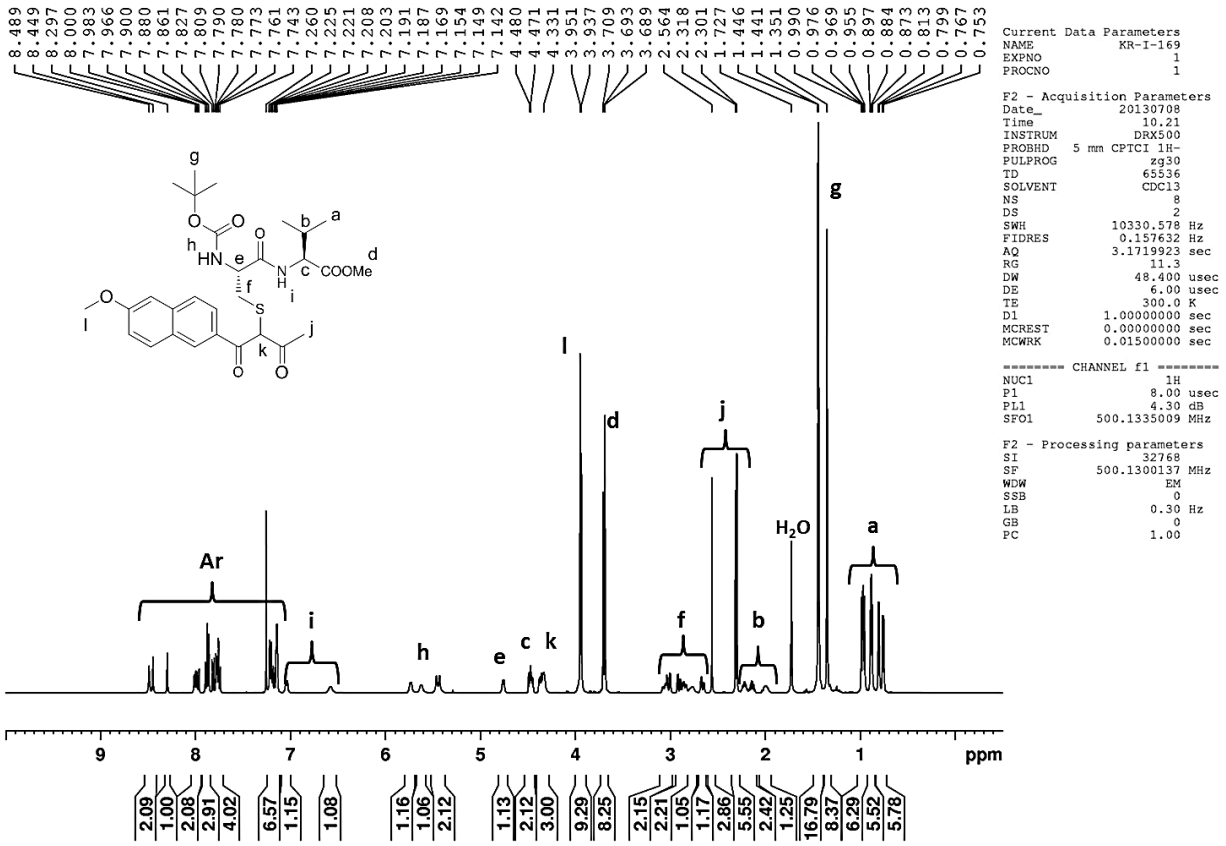
^{1}H & ^{13}C NMR of 8f

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Current Data Parameters
NAME                  KR-I-169
EXPNO                        1
PROCNO                       1

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Time                   10.21
INSTRUM                DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                         8
DS                         2
SWH                10330.578 Hz
FIDRES             0.157632 Hz
AQ                 3.1719923 sec
RG                      11.3
DW                    48.400 usec
DE                     6.00 usec
TE                    300.0 K
D1                 1.00000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                      8.00 usec
PL1                     4.30 dB
SFO1               500.1335009 MHz

F2 - Processing parameters
SI                     32768
SF                 500.1300137 MHz
WDW                        EM
SSB                         0
LB                       0.30 Hz
GB                          0
PC                       1.00
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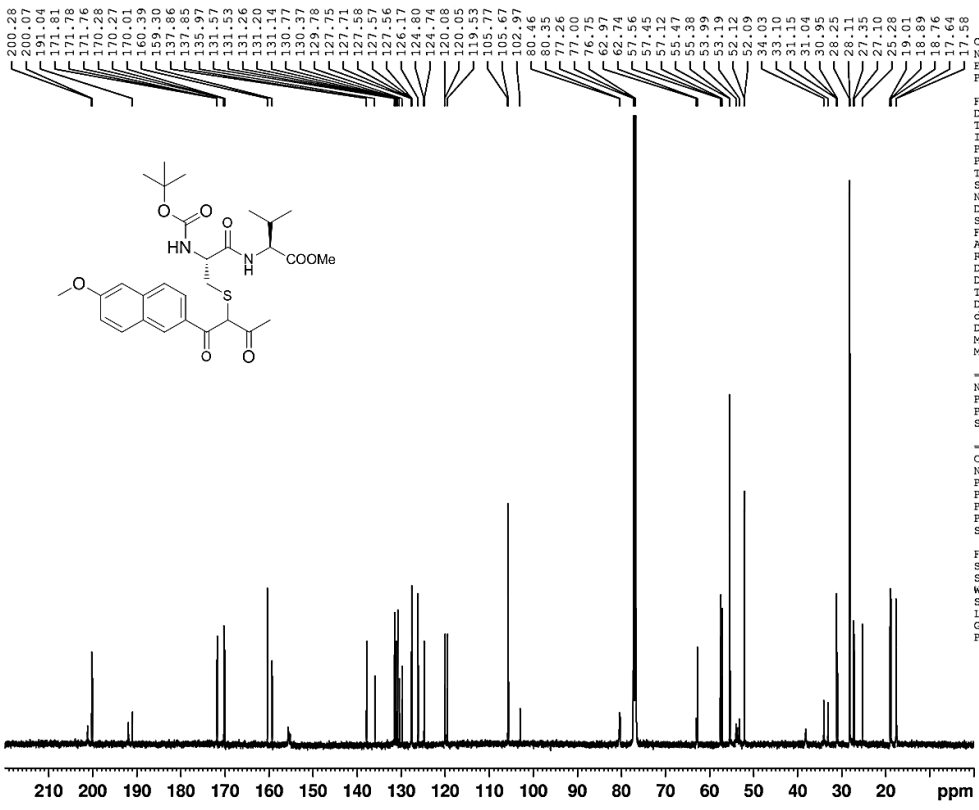


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Current Data Parameters
NAME                  KR-I-169
EXPNO                        2
PROCNO                       1

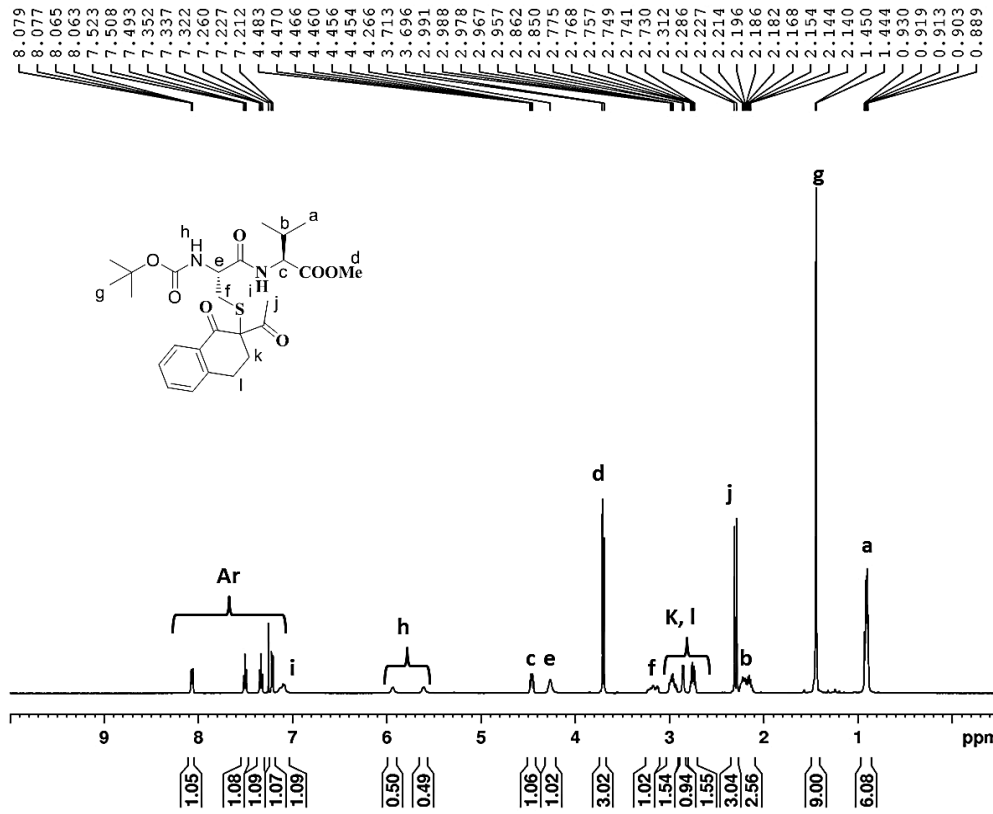
F2 - Acquisition Parameters
Date_               20130708
Time                   10.28
INSTRUM                DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG                zgpg30
TD                     71424
SOLVENT                CDCl3
NS                      1283
DS                         4
SWH                35211.270 Hz
FIDRES             0.492989 Hz
AQ                 1.0142708 sec
RG                      4096
DW                    14.200 usec
DE                     35.00 usec
TE                    300.0 K
D1                 2.00000000 sec
d11                0.03000000 sec
DELTA              1.89999998 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                      13C
P1                     12.00 usec
PL1                     0.30 dB
SFO1               125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                  80.00 usec
PL2                     5.00 dB
PL12                   22.00 dB
PL13                   27.90 dB
SFO2               500.1320005 MHz

F2 - Processing parameters
SI                     65536
SF                 125.7577939 MHz
WDW                        EM
SSB                         0
LB                       1.00 Hz
GB                          0
PC                       1.40
```

^{1}H & ^{13}C NMR of 8g

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EXPNO                    1
PROCNO                   1

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PROBHD    5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT             CDCl3
NS                      8
DS                      2
SWH             10330.578 Hz
FIDRES          0.157632 Hz
AQ              3.1719923 sec
RG                   11.3
DW                 48.400 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                   8.00 usec
PL1                  4.30 dB
SFO1          500.1335009 MHz

F2 - Processing parameters
SI                    32768
SF            500.1300137 MHz
WDW                      EM
SSB                       0
LB                     0.30 Hz
GB                        0
PC                     1.40
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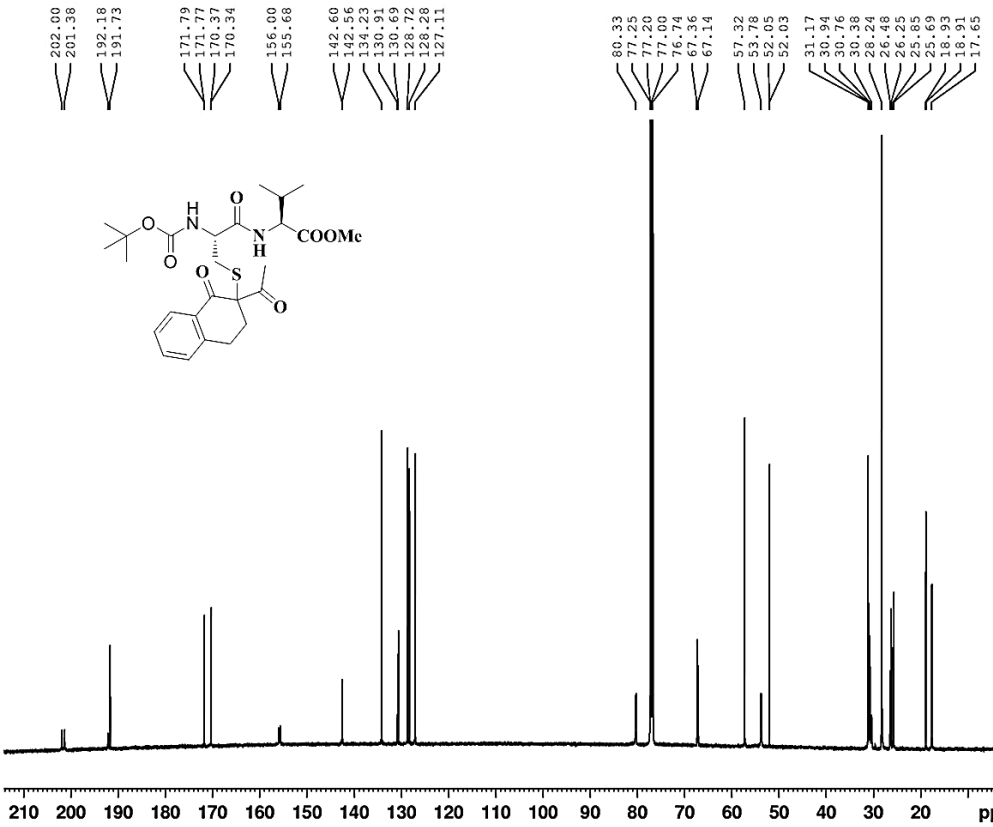



```
Current Data Parameters
NAME      tetralone adduct
EXPNO                    2
PROCNO                   1

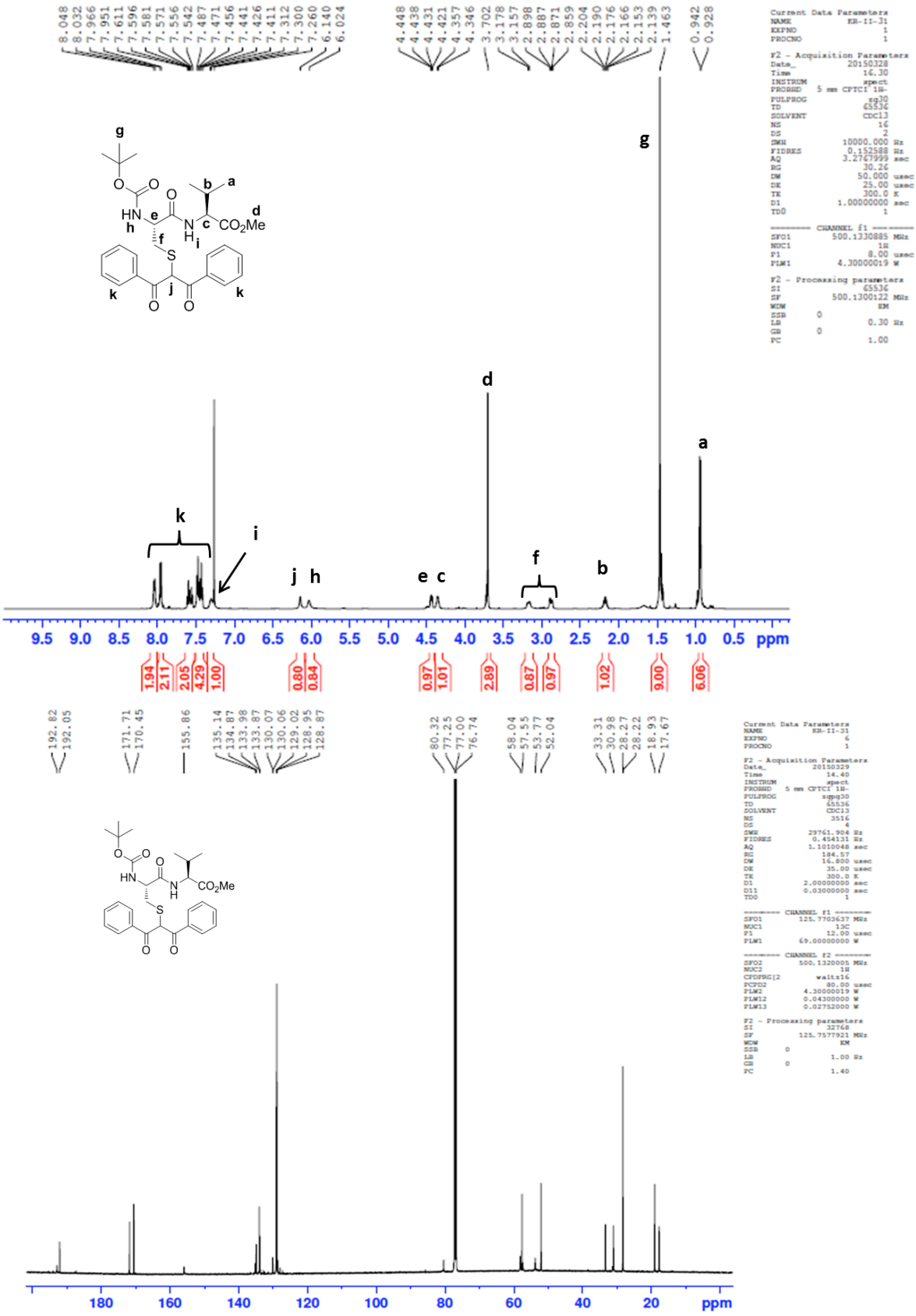
F2 - Acquisition Parameters
Date_             20130702
Time                 20.07
INSTRUM              DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG           zgpg30
TD                  71424
SOLVENT             CDCl3
NS                   2324
DS                      4
SWH             35211.270 Hz
FIDRES          0.492989 Hz
AQ              1.0142708 sec
RG                   4096
DW                 14.200 usec
DE                 35.00 usec
TE                 300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                  13C
P1                  12.00 usec
PL1                  0.30 dB
SFO1          125.7716224 MHz

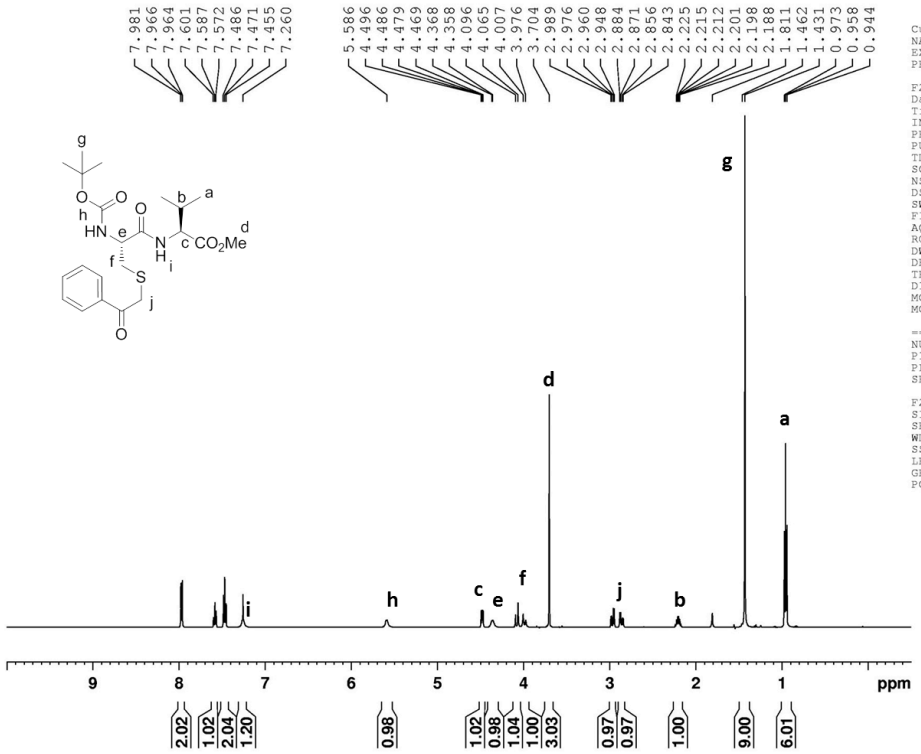
======== CHANNEL f2 ========
CPDPRG2           waltz16
NUC2                   1H
PCPD2              80.00 usec
PL2                 5.00 dB
PL12               22.00 dB
PL13               27.90 dB
SFO2          500.1320005 MHz

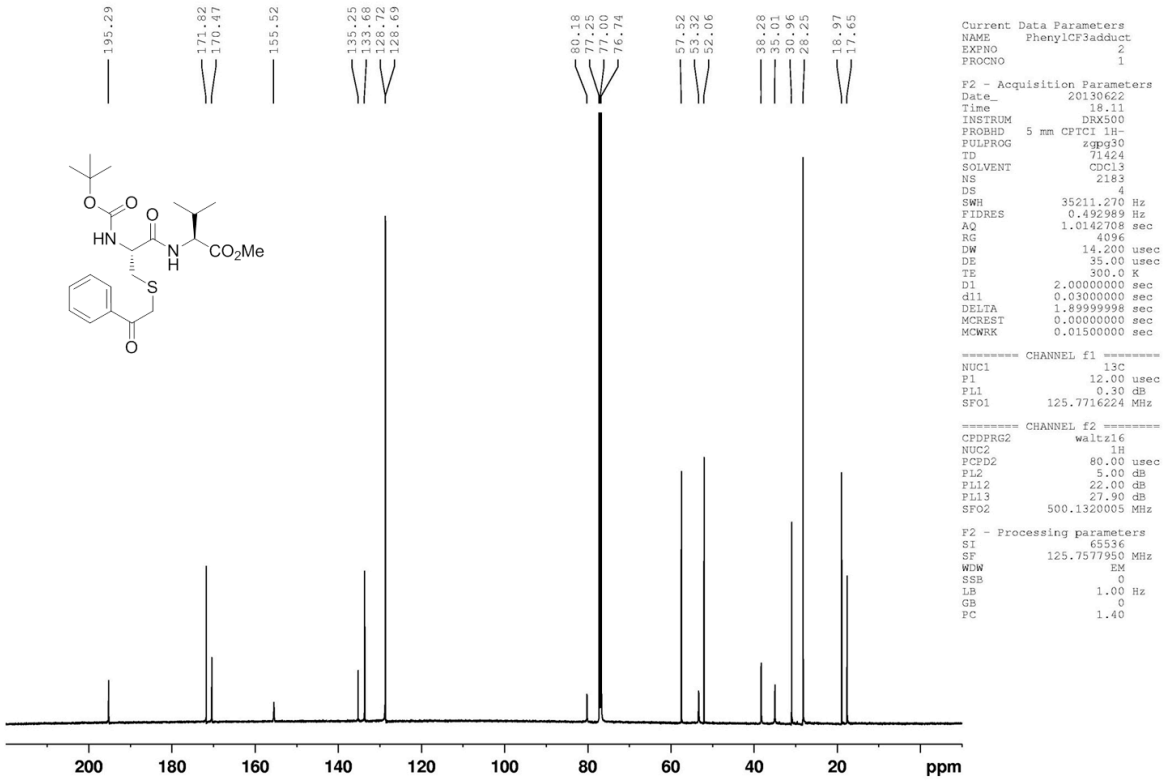
F2 - Processing parameters
SI                    65536
SF            125.7577955 MHz
WDW                      EM
SSB                       0
LB                     1.00 Hz
GB                        0
PC                     1.00
```

^{1}H & ^{13}C NMR of 8h

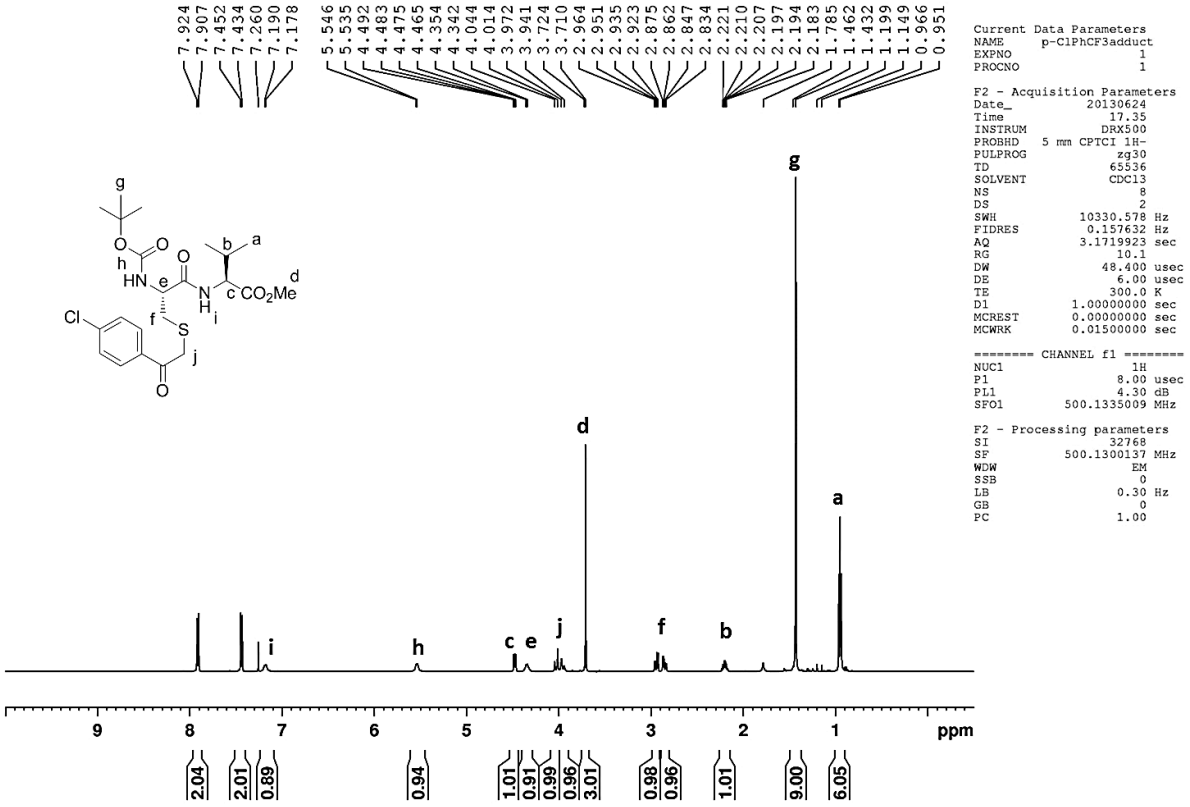


^{1}H & ^{13}C NMR of 8i





^{1}H & ^{13}C NMR of 8j

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Current Data Parameters
NAME       p-ClPhCF3adduct
EXPNO                    1
PROCNO                   1

F2 - Acquisition Parameters
Date_              20130624
Time                  17.35
INSTRUM              DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        8
DS                        2
SWH               10330.578 Hz
FIDRES             0.157632 Hz
AQ                 3.1719923 sec
RG                      10.1
DW                    48.400 usec
DE                     6.00 usec
TE                    300.0 K
D1                 1.00000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     8.00 usec
PL1                    4.30 dB
SFO1           500.1335009 MHz

F2 - Processing parameters
SI                        32768
SF             500.1300137 MHz
WDW                          EM
SSB                           0
LB                         0.30 Hz
GB                            0
PC                         1.00
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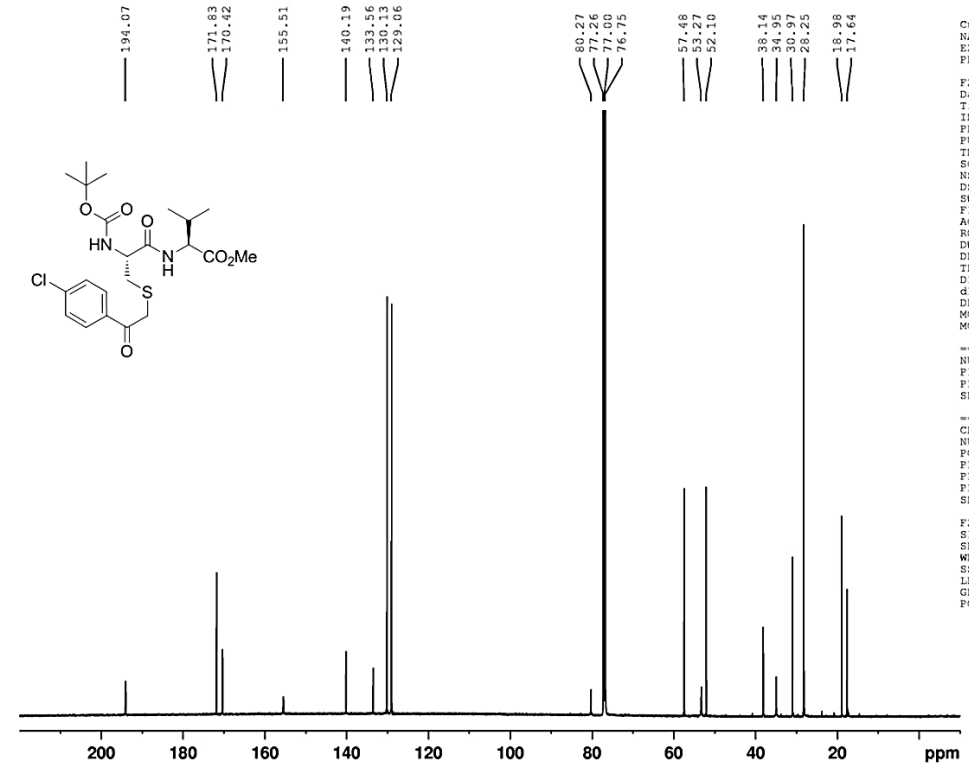



```
Current Data Parameters
NAME       p-ClPhCF3adduct
EXPNO                    2
PROCNO                   1

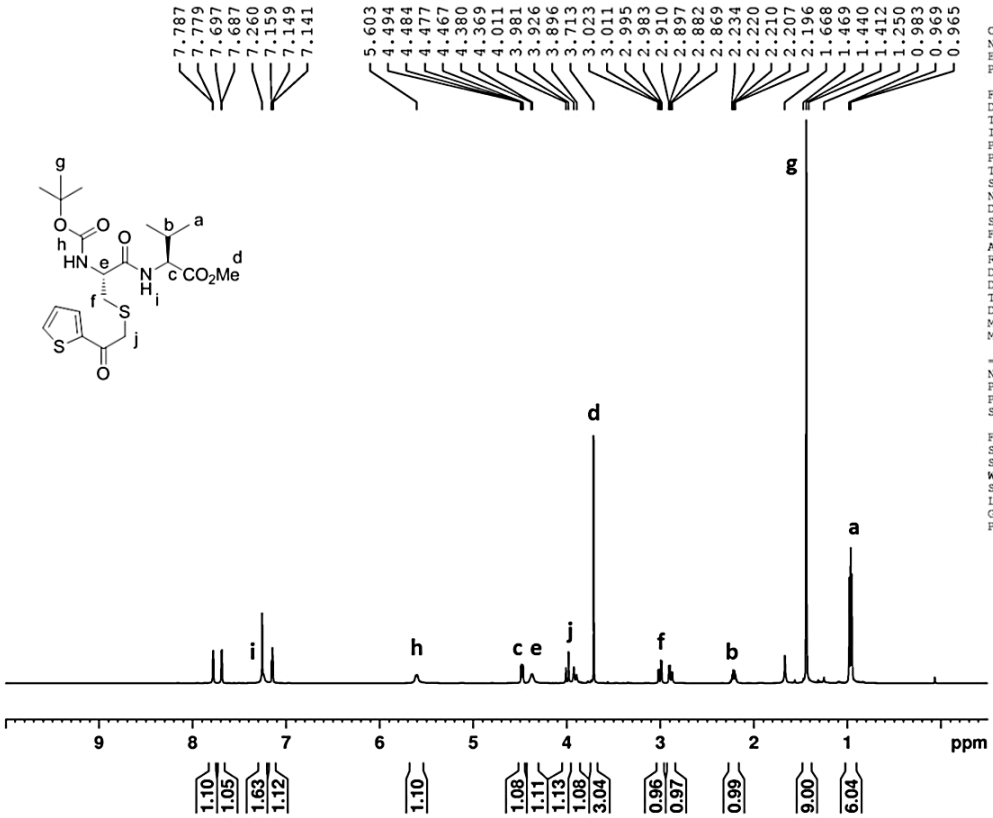
F2 - Acquisition Parameters
Date_              20130624
Time                  18.38
INSTRUM              DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG              zgpg30
TD                    71424
SOLVENT               CDCl3
NS                     2535
DS                        4
SWH               35211.270 Hz
FIDRES             0.492989 Hz
AQ                 1.0142708 sec
RG                     4096
DW                    14.200 usec
DE                    35.00 usec
TE                    300.0 K
D1                 2.00000000 sec
d11                0.03000000 sec
DELTA              1.89999998 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                      13C
P1                    12.00 usec
PL1                    0.30 dB
SFO1           125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                      1H
PCPD2                 80.00 usec
PL2                    5.00 dB
PL12                  22.00 dB
PL13                  27.90 dB
SFO2           500.1320005 MHz

F2 - Processing parameters
SI                        65536
SF             125.7577939 MHz
WDW                          EM
SSB                           0
LB                         1.00 Hz
GB                            0
PC                         1.40
```

^{1}H & ^{13}C NMR of 8k

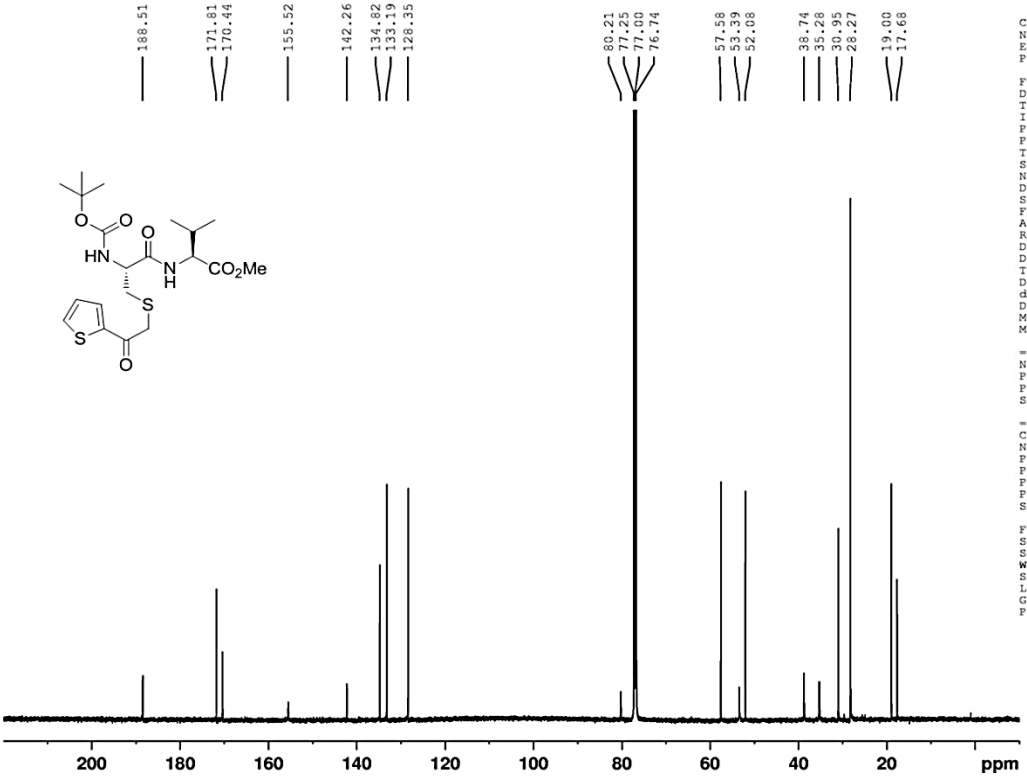



```
Current Data Parameters
NAME      ThiopheneCF3adduct
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20130622
Time                  14.37
INSTRUM              DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        8
DS                        2
SWH               10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719923 sec
RG                     28.5
DW                   48.400 usec
DE                    6.00 usec
TE                   300.0 K
D1                1.00000000 sec
MCREST            0.00000000 sec
MCWRK             0.01500000 sec

======== CHANNEL f1 ========
NUC1                      1H
P1                     8.00 usec
PL1                    4.30 dB
SFO1           500.1335009 MHz

F2 - Processing parameters
SI                        32768
SF             500.1300140 MHz
WDW                          EM
SSB                           0
LB                     0.30 Hz
GB                            0
PC                     1.00
```




```
Current Data Parameters
NAME      ThiopheneCF3adduct
EXPNO                      2
PROCNO                     1

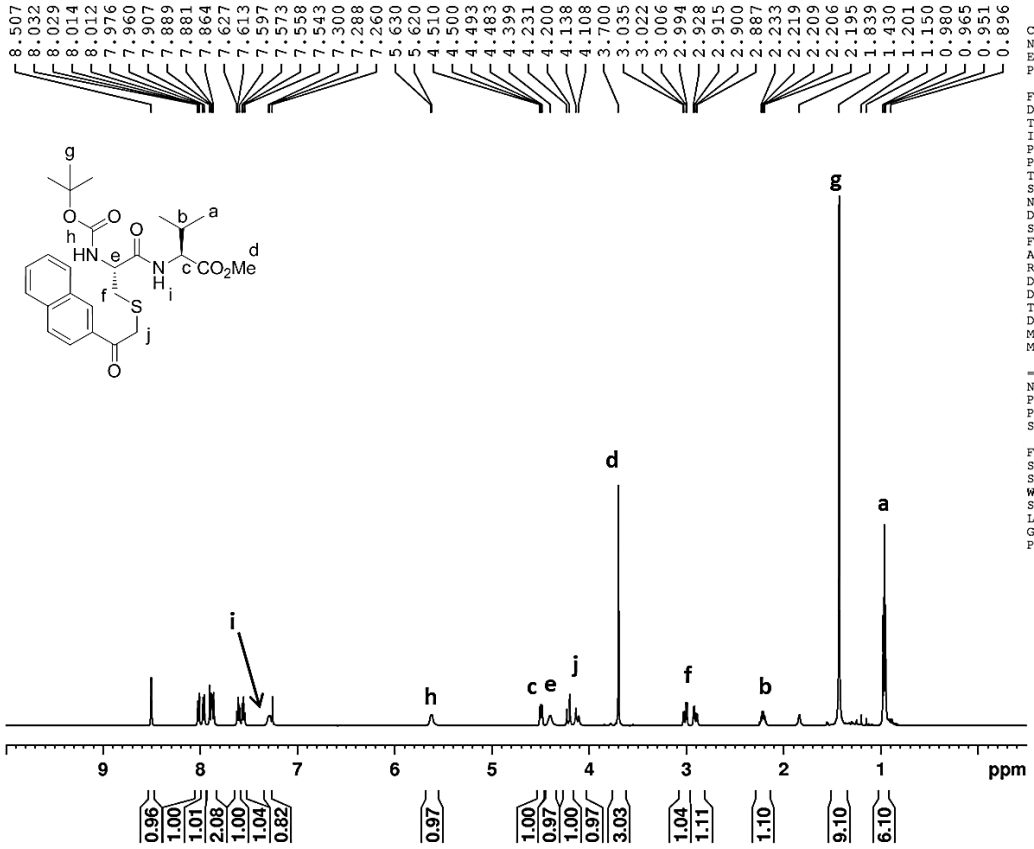
F2 - Acquisition Parameters
Date_              20130622
Time                  14.48
INSTRUM              DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG             zgpg30
TD                    71424
SOLVENT               CDCl3
NS                     1710
DS                        4
SWH               35211.270 Hz
FIDRES            0.492989 Hz
AQ                1.0142708 sec
RG                     4096
DW                   14.200 usec
DE                   35.00 usec
TE                   300.0 K
D1                2.00000000 sec
d11               0.03000000 sec
DELTA             1.89999998 sec
MCREST            0.00000000 sec
MCWRK             0.01500000 sec

======== CHANNEL f1 ========
NUC1                      13C
P1                    12.00 usec
PL1                    0.30 dB
SFO1           125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                      1H
PCPD2                 80.00 usec
PL2                    5.00 dB
PL12                  22.00 dB
PL13                  27.90 dB
SFO2           500.1320005 MHz

F2 - Processing parameters
SI                        65536
SF             125.7577928 MHz
WDW                          EM
SSB                           0
LB                     1.00 Hz
GB                            0
PC                     1.40
```

^{1}H & ^{13}C NMR of 8l

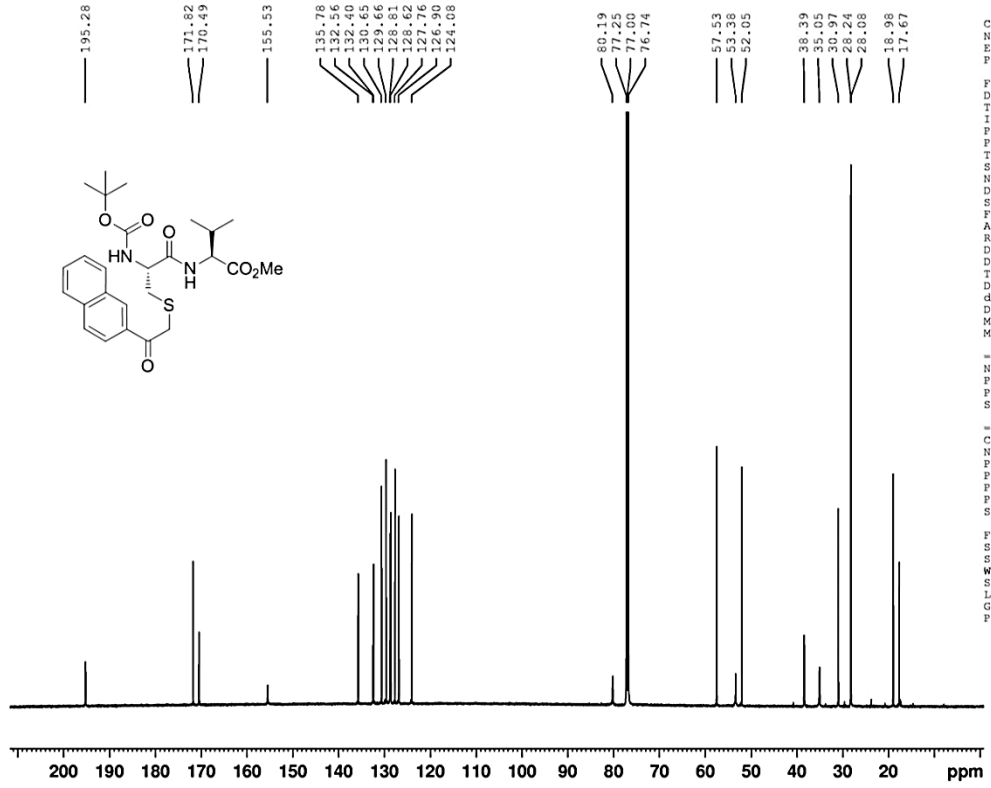



```
Current Data Parameters
NAME       NaphCF3adduct
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130624
Time               19.58
INSTRUM           DRX500
PROBHD   5 mm CPTCI 1H-
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                    8
DS                    2
SWH           10330.578 Hz
FIDRES        0.157632 Hz
AQ            3.1719923 sec
RG                 10.1
DW               48.400 usec
DE                6.00 usec
TE               300.0 K
D1            1.00000000 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                 1H
P1                8.00 usec
PL1               4.30 dB
SFO1        500.1335009 MHz

F2 - Processing parameters
SI                32768
SF          500.1300134 MHz
WDW                  EM
SSB                   0
LB                 0.30 Hz
GB                    0
PC                 1.00
```




```
Current Data Parameters
NAME       NaphCF3adduct
EXPNO                  2
PROCNO                 1

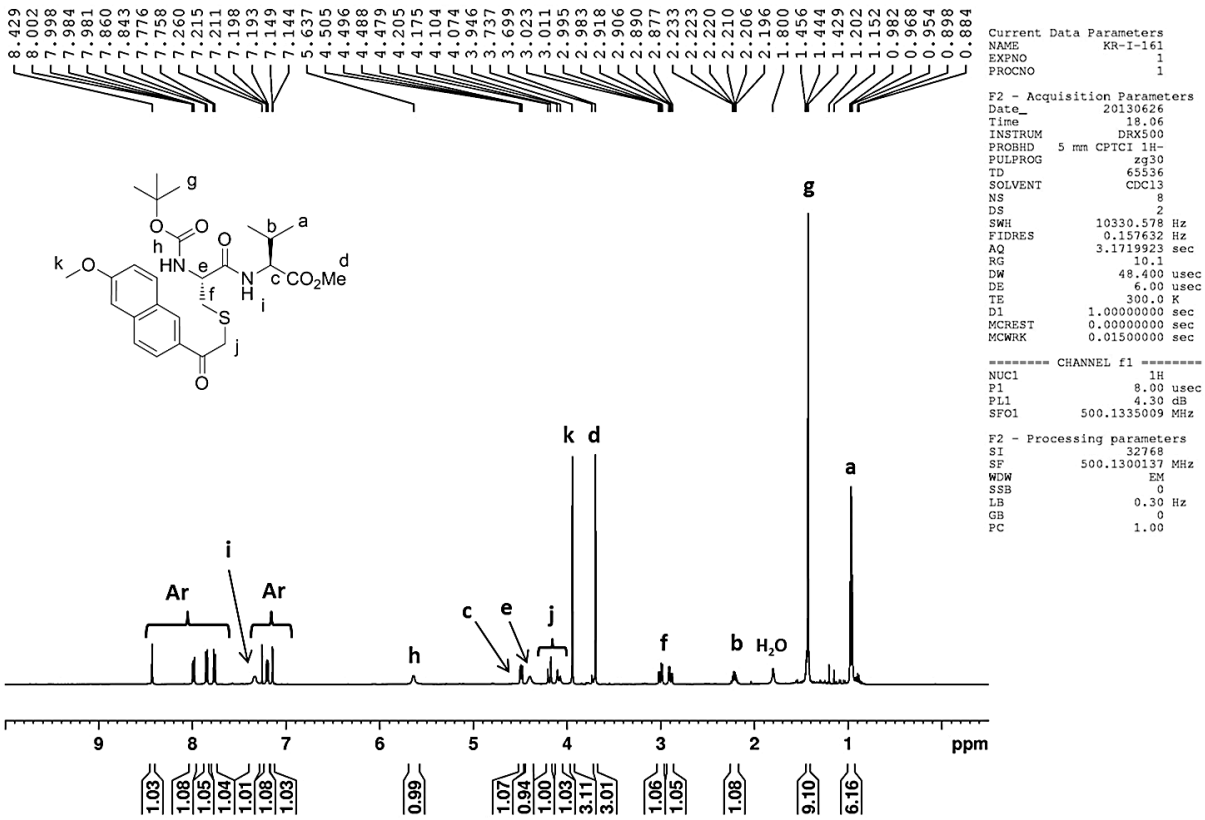
F2 - Acquisition Parameters
Date_           20130624
Time               21.29
INSTRUM           DRX500
PROBHD   5 mm CPTCI 1H-
PULPROG          zgpg30
TD                71424
SOLVENT           CDCl3
NS                 1716
DS                    4
SWH           35211.270 Hz
FIDRES        0.492989 Hz
AQ            1.0142708 sec
RG                 4096
DW               14.200 usec
DE               35.00 usec
TE               300.0 K
D1            2.00000000 sec
d11           0.03000000 sec
DELTA         1.89999998 sec
MCREST        0.00000000 sec
MCWRK         0.01500000 sec

======== CHANNEL f1 ========
NUC1                13C
P1               12.00 usec
PL1               0.30 dB
SFO1        125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2          waltz16
NUC2                 1H
PCPD2             80.00 usec
PL2                5.00 dB
PL12              22.00 dB
PL13              27.90 dB
SFO2        500.1320005 MHz

F2 - Processing parameters
SI                65536
SF          125.7577960 MHz
WDW                  EM
SSB                   0
LB                 1.00 Hz
GB                    0
PC                 1.40
```

^{1}H & ^{13}C NMR of 8m

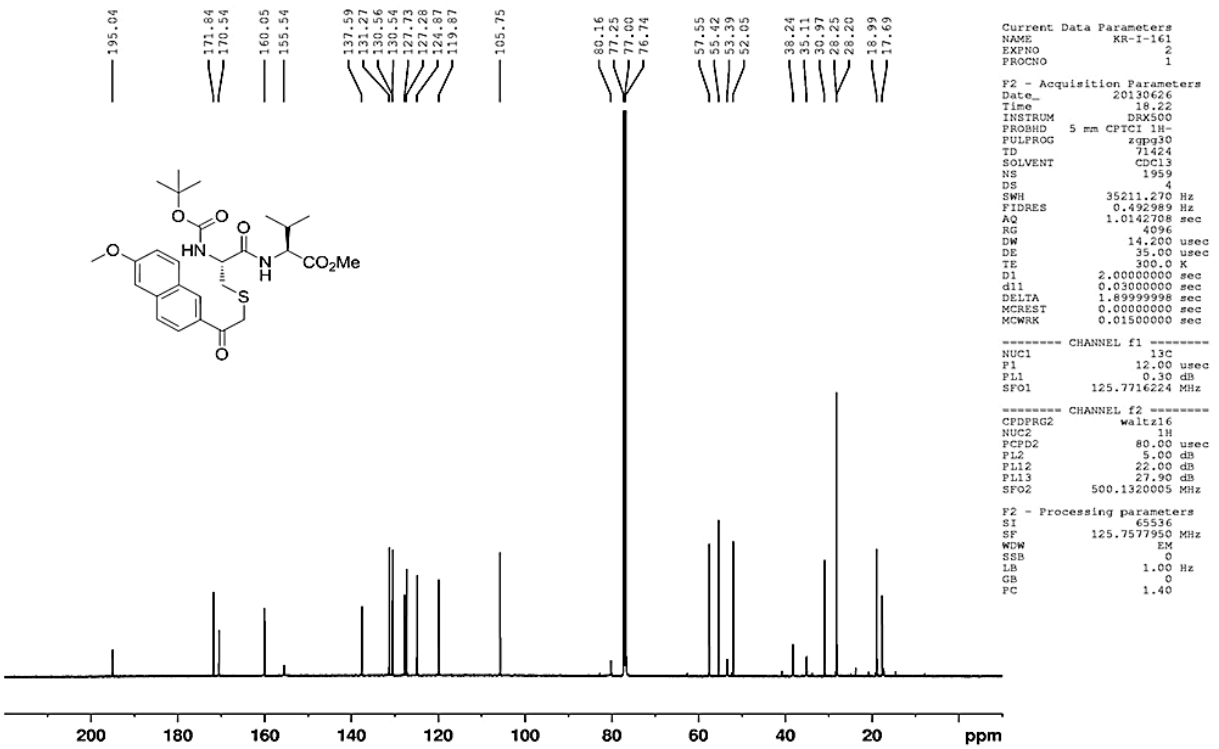



```
Current Data Parameters
NAME                  KR-I-161
EXPNO                        1
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20130626
Time                     18.06
INSTRUM                DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG                  zg30
TD                      65536
SOLVENT                 CDCl3
NS                           8
DS                           2
SWH                 10330.578 Hz
FIDRES                0.157632 Hz
AQ                    3.1719923 sec
RG                         10.1
DW                       48.400 usec
DE                        6.00 usec
TE                       300.0 K
D1                    1.00000000 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

======== CHANNEL f1 ========
NUC1                         1H
P1                        8.00 usec
PL1                       4.30 dB
SFO1                500.1335009 MHz

F2 - Processing parameters
SI                       32768
SF                  500.1300137 MHz
WDW                         EM
SSB                          0
LB                        0.30 Hz
GB                           0
PC                        1.00
```




```
Current Data Parameters
NAME                  KR-I-161
EXPNO                        2
PROCNO                       1

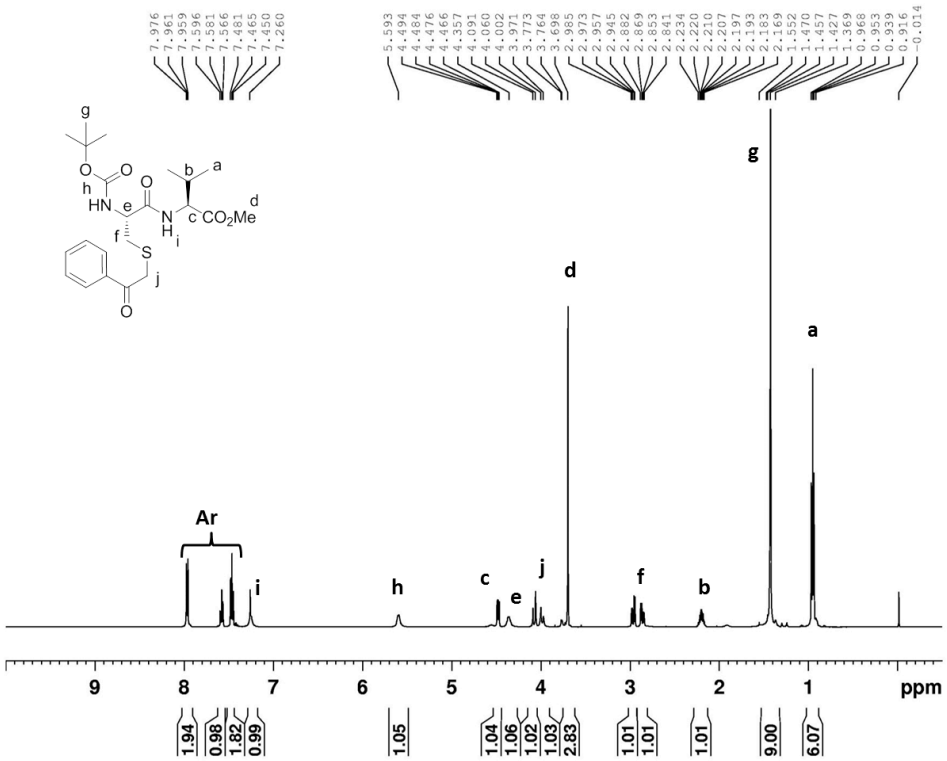
F2 - Acquisition Parameters
Date_                 20130626
Time                     18.22
INSTRUM                DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG                zgpg30
TD                      71424
SOLVENT                 CDCl3
NS                        1959
DS                           4
SWH                 35211.270 Hz
FIDRES                0.492989 Hz
AQ                    1.0142708 sec
RG                        4096
DW                       14.200 usec
DE                       35.00 usec
TE                       300.0 K
D1                    2.00000000 sec
d11                   0.03000000 sec
DELTA                 1.89999998 sec
MCREST                0.00000000 sec
MCWRK                 0.01500000 sec

======== CHANNEL f1 ========
NUC1                        13C
P1                       12.00 usec
PL1                       0.30 dB
SFO1               125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2                 waltz16
NUC2                         1H
PCPD2                    80.00 usec
PL2                       5.00 dB
PL12                     22.00 dB
PL13                     27.90 dB
SFO2                500.1320005 MHz

F2 - Processing parameters
SI                       65536
SF                 125.7577950 MHz
WDW                         EM
SSB                          0
LB                        1.00 Hz
GB                           0
PC                        1.40
```

^{1}H NMR of 8n

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Current Data Parameters
NAME          KR-I-125-A1
EXPNO                   1
PROCNO                  1

F2 - Acquisition Parameters
Date_            20130217
Time                16.47
INSTRUM           DRX500
PROBHD   5 mm CPTCI 1H-
PULPROG              zg30
TD                  65536
SOLVENT              CDCl3
NS                      8
DS                      2
SWH             10330.578 Hz
FIDRES          0.157632 Hz
AQ              3.1719923 sec
RG                      8
DW                 48.400 usec
DE                  6.00 usec
TE                 300.0 K
D1              1.00000000 sec
MCREST          0.00000000 sec
MCWRK           0.01500000 sec

======== CHANNEL f1 ========
NUC1                   1H
P1                  8.00 usec
PL1                 4.30 dB
SFO1          500.1335009 MHz

F2 - Processing parameters
SI                  32768
SF            500.1300134 MHz
WDW                    EM
SSB                     0
LB                 0.30 Hz
GB                      0
PC                   1.00
```
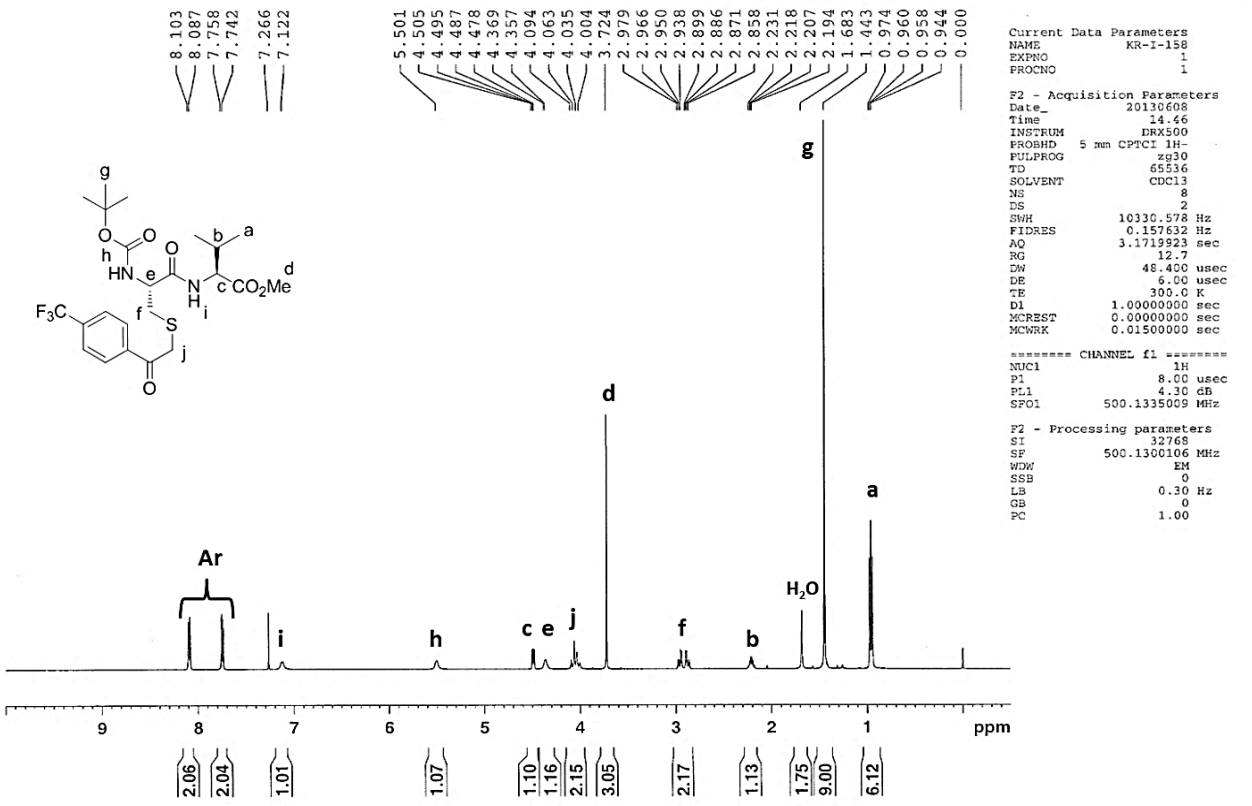
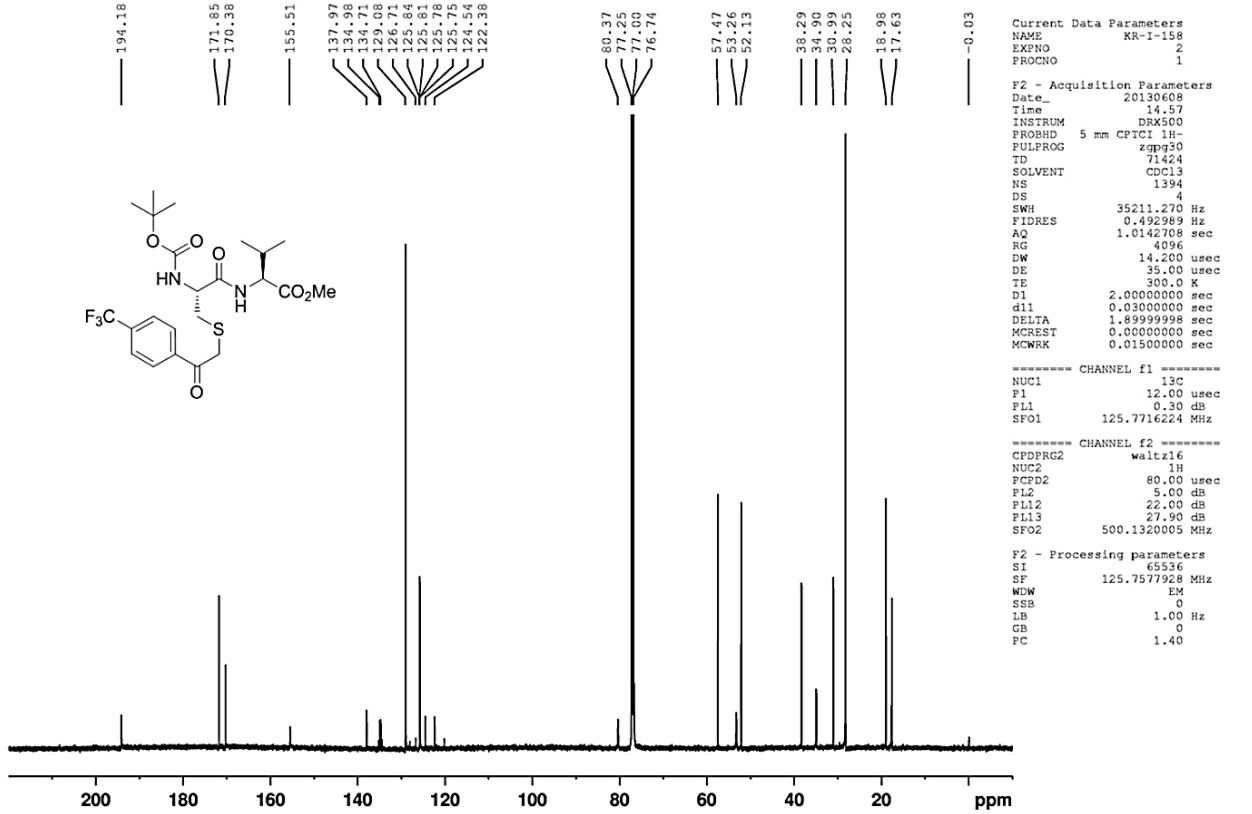

^{1}H & ^{13}C NMR of 8o

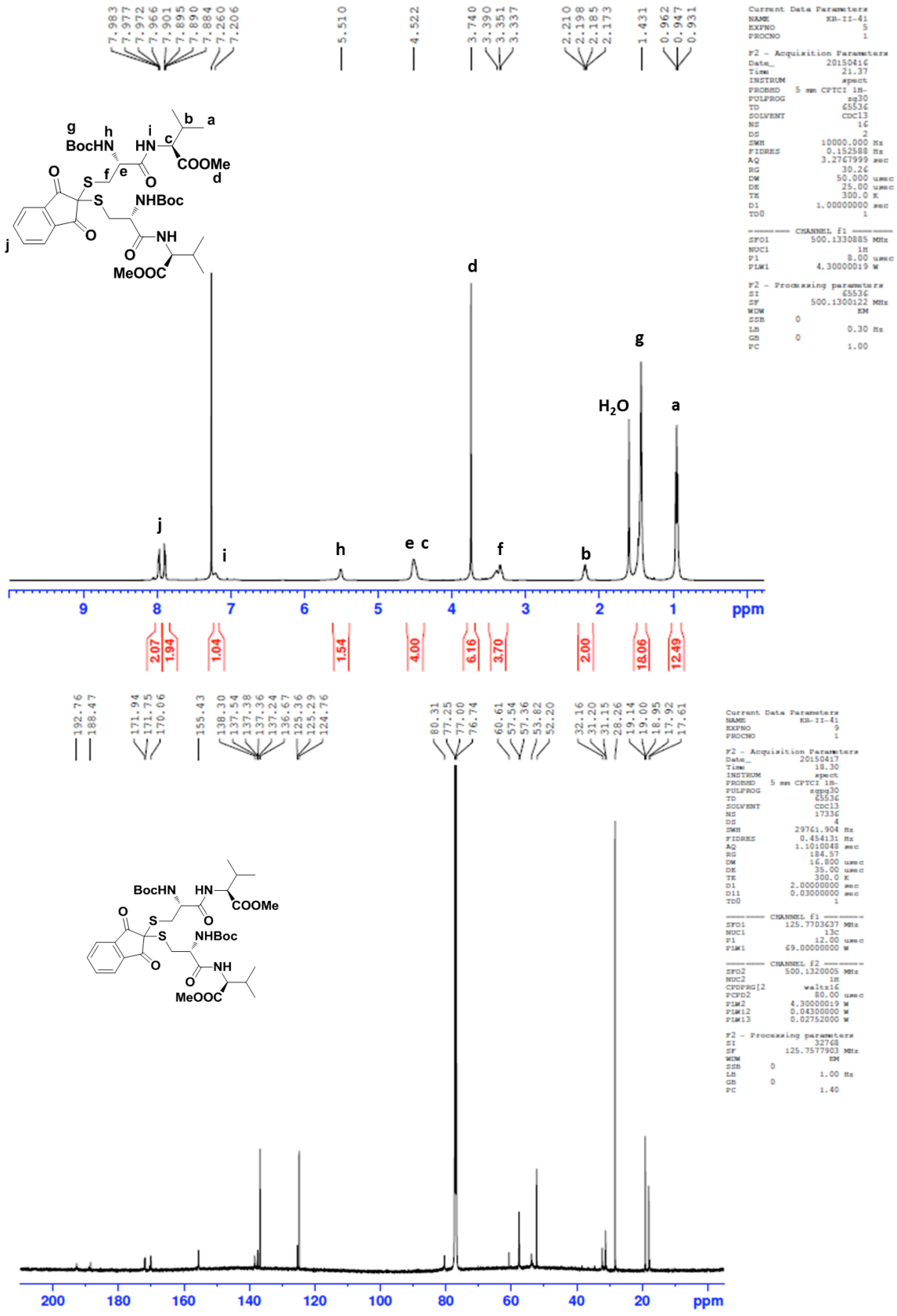



```
Current Data Parameters
NAME                  KR-I-158
EXPNO                        1
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20130608
Time                     14.46
INSTRUM                DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG                 zg30
TD                     65536
SOLVENT                CDCl3
NS                           8
DS                           2
SWH                10330.578 Hz
FIDRES             0.157632 Hz
AQ                 3.1719923 sec
RG                        12.7
DW                    48.400 usec
DE                     6.00 usec
TE                     300.0 K
D1                 1.00000000 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                       1H
P1                     8.00 usec
PL1                    4.30 dB
SFO1           500.1335009 MHz

F2 - Processing parameters
SI                       32768
SF             500.1300106 MHz
WDW                         EM
SSB                          0
LB                      0.30 Hz
GB                           0
PC                        1.00
```




```
Current Data Parameters
NAME                  KR-I-158
EXPNO                        2
PROCNO                       1

F2 - Acquisition Parameters
Date_                 20130608
Time                     14.57
INSTRUM                DRX500
PROBHD    5 mm CPTCI 1H-
PULPROG              zgpg30
TD                     71424
SOLVENT                CDCl3
NS                        1394
DS                           4
SWH                35211.270 Hz
FIDRES             0.492989 Hz
AQ                 1.0142708 sec
RG                        4096
DW                    14.200 usec
DE                     35.00 usec
TE                     300.0 K
D1                 2.00000000 sec
d11                0.03000000 sec
DELTA              1.89999998 sec
MCREST             0.00000000 sec
MCWRK              0.01500000 sec

======== CHANNEL f1 ========
NUC1                      13C
P1                    12.00 usec
PL1                    0.30 dB
SFO1           125.7716224 MHz

======== CHANNEL f2 ========
CPDPRG2                waltz16
NUC2                       1H
PCPD2                 80.00 usec
PL2                    5.00 dB
PL12                  22.00 dB
PL13                  27.90 dB
SFO2           500.1320005 MHz

F2 - Processing parameters
SI                       65536
SF             125.7577928 MHz
WDW                         EM
SSB                          0
LB                      1.00 Hz
GB                           0
PC                        1.40
```

^{1}H & ^{13}C NMR of 11



^{1}H & ^{13}C NMR of 12

Spectrophotometric Determination of Carbon Acid Ionization Constants. A buffer containing glycine, sodium acetate, and sodium phosphate (all 50 mM, final concentrations) was prepared in distillied, deionized H_2O. Portions of the buffer were prepared at pH 3 and pH 13, and at half pH unit intervals between. UV-vis measurements were made at room temperature (23 ± 2°C). The approximate ionic strength of the buffer was 0.2 M (that due to buffer component contributions). Concentrated stock solutions of the carbon acids were prepared in DMSO (typically 1-10 mM) and stored at –20 °C until used. Because molar absorptivity may vary greatly as a function of molecular structure, final concentrations of carbon acids suitable for use in the spectrophotometer were determined separately for each molecule, but typically ranged from 50 – 250 μM.

In order to determine which carbon acid UV-Vis absorption maxima might be suitable for use in pK_a determination, we first examined the absorption spectra of a fixed concentration of carbon acid at the pH extremes (3 and 13), and looked for spectral changes as a function of pH. Generally, spectral peaks were blue-shifted and/or attenuated upon acidification (at pH 3), relative to those appearing at pH 13, consistent with anions being the relevant absorbing species. Having thus determined suitable concentrations of carbon acids and the associated wavelength(s) to monitor during titration experiments, fixed concentrations of each carbon acid were introduced to each buffer (pH 3 to 13), briefly allowed to equilibrate, and the pH–dependent spectral peak intensities recorded. In each case, the final composition of the mixture assayed in the cuvette was 4% DMSO in buffer (v/v; 40 μL carbon acid in DMSO, 960 μL buffer). Finally, absorption at λ_{max} for the wavelengths monitored was plotted as a function of pH, and the pK_a determined by fitting to the equation:

$$A_{pH} = A_{\min} + \frac{(A_{\max} - A_{\min})}{1 + 10^{pK_a - pH}}$$

where A_{min}, A_{max}, and A_{pH} are the absorbances at the pH minimum (pH 3), maximum pH (pH 13), and intermediate pH values, respectively, at the wavelength being monitored for a given carbon acid (Figure 4B). To validate both the solvent system employed (aqueous buffer components + 4% DMSO, μ = 0.2 M) and the method used in data analysis, a set of benchmark carbon acids and heteroatom-based acids of known aqueous pK_a were examined, and the ionization constants determined in our hands matched excellently those reported in the literature, determined by various means.

Spectrophotometric determination of acid dissociation constants over a pH range 3-13.

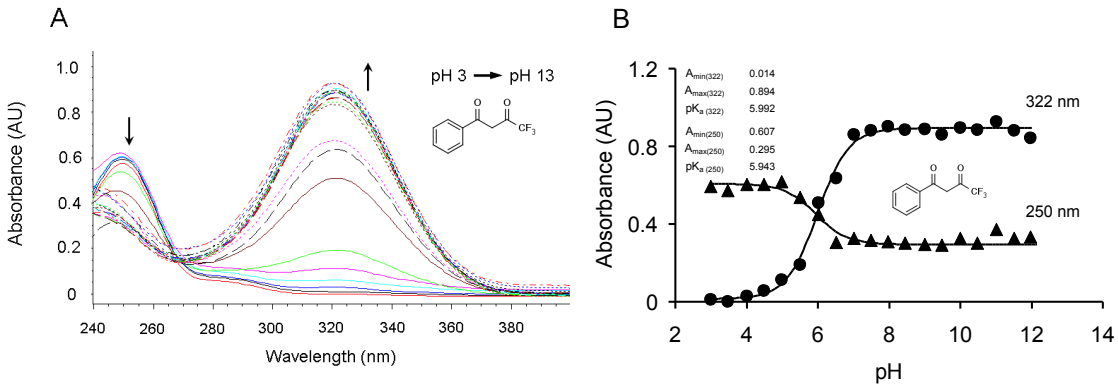
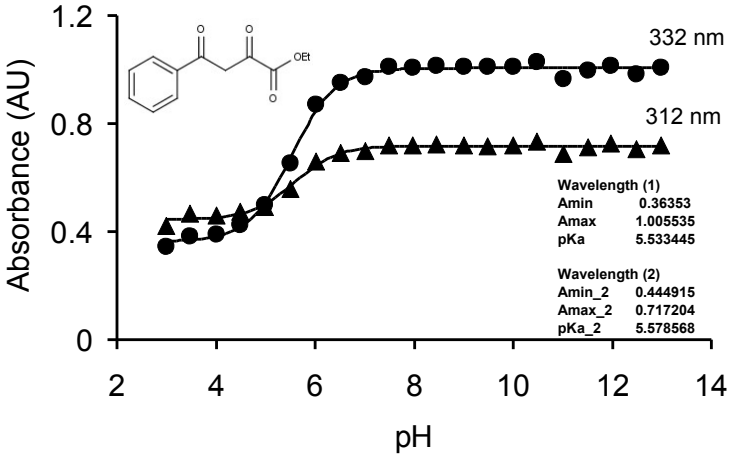
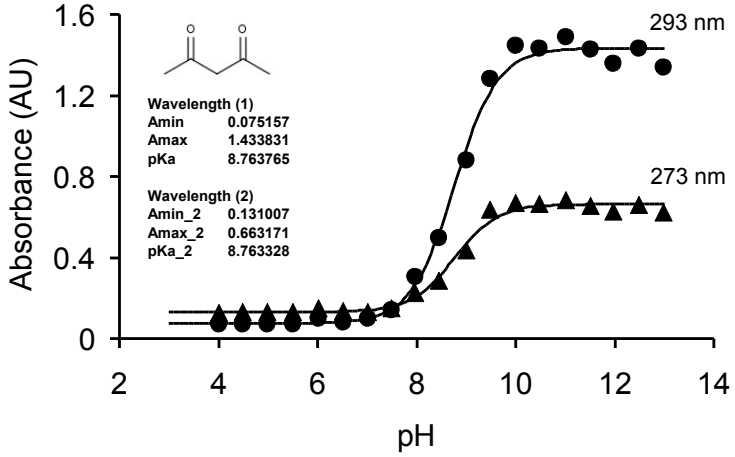
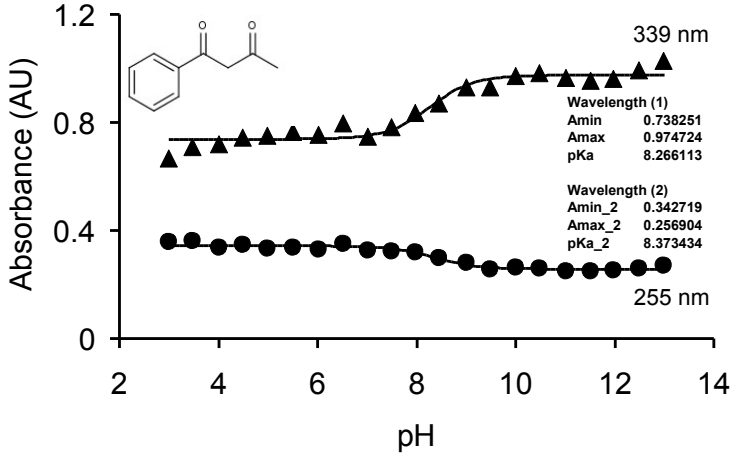


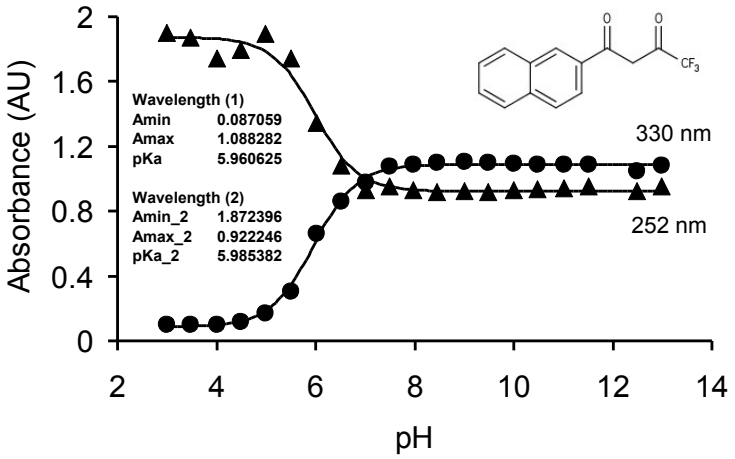
Figure S1. Spectrophotometric determination of carbon acid ionization constants. **A.** To 960 μL samples of "universal buffer" pH 3 – 13 (see text) were added 40 μL of 1.25 mM compound **H** in dmso to final concentration 50 μM. The solution was briefly allowed to equilibrate and the absorption spectrum versus buffer blank recorded. **B.** The absorbances of spectral peaks that varied as a function of pH were plotted versus the corresponding pH and the data fitted to afford the apparent pK_a.

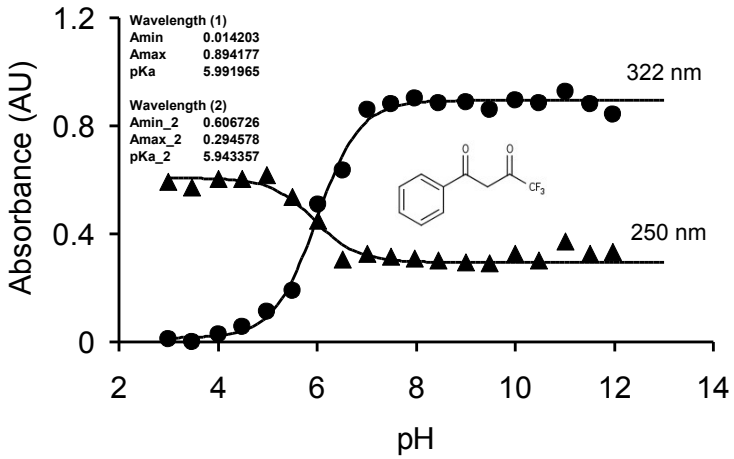
Carbon acid pK_a determination: titrimetric data and associated fits.

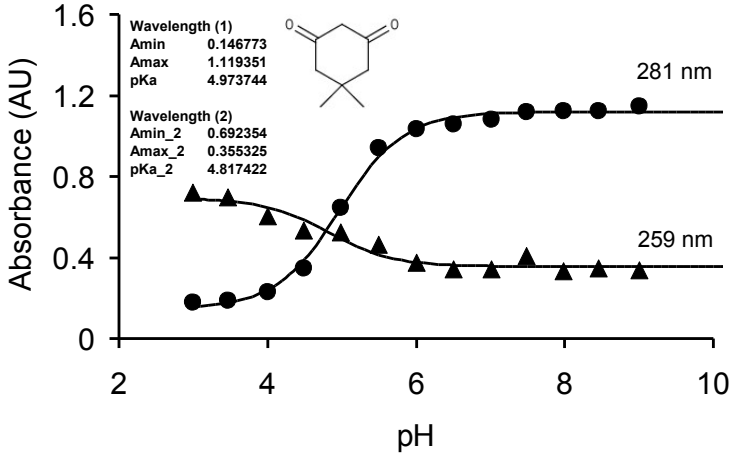


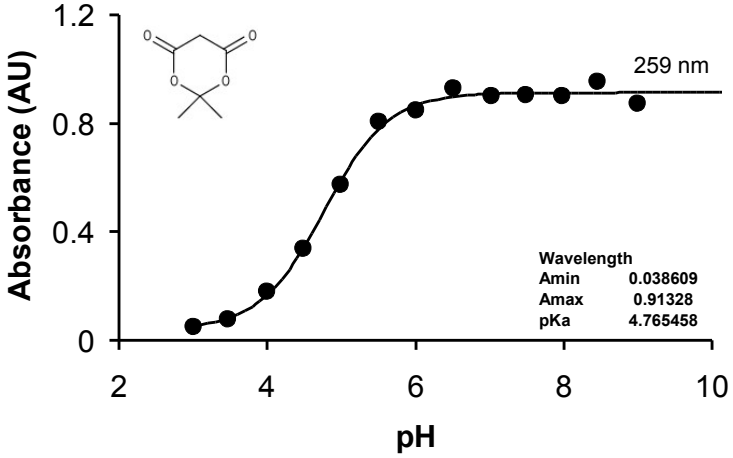




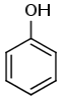
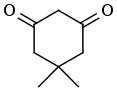
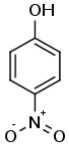
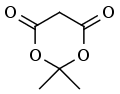
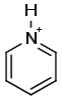
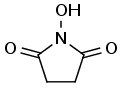
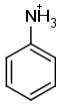

Absorbance (AU)
pH
Wavelength (1)
Amin 0.087059
Amax 1.088282
pKa 5.960625
Wavelength (2)
Amin_2 1.872396
Amax_2 0.922246
pKa_2 5.985382
330 nm
252 nm


Absorbance (AU)
pH
Wavelength (1)
Amin 0.014203
Amax 0.894177
pKa 5.991965
Wavelength (2)
Amin_2 0.606726
Amax_2 0.294578
pKa_2 5.943357
322 nm
250 nm


Absorbance (AU)
pH
Wavelength (1)
Amin 0.146773
Amax 1.119351
pKa 4.973744
Wavelength (2)
Amin_2 0.692354
Amax_2 0.355325
pKa_2 4.817422
281 nm
259 nm



Benchmark set for pK_a determination methodology: all pK_a values determined in this work, listed below as "pK_a (det.)", were determined using the methodology detailed in the Methods section of the manuscript.

	pK_a (*det.*)	pK_a *(lit.)*		pK_a (*det.*)	pK_a *(lit.)*
	10.0	10.0 [a]		5.0	4.8 [b]
	6.9	7.2 [a]		4.8	4.9 [b]
	5.0	5.2 [a]		5.9	6.0 [c]
	4.5	4.6 [a]			

[a] Harris, D. C. (2010) *Quantitative Chemical Analysis*, 8th ed.

[b] Mofaddel, N., *et al. Anal. Biochem.* (2004), 380, 664-68

[c] Cline, G. W. *J. Org. Chem.* (1988), 53, 3583-3586

Development of Assay Methodology for the Selective Detection of Sulfenyl Amide via Spectrophotometry. In order to follow the reaction of diketones with **4** spectrophotometrically, we first prepared the colorimetric reagent 2-nitro-5-sulfanylbenzoic acid (TNB, Abs_{410nm} in aqueous media). Synthesis of TNB was accomplished by reduction of Ellman's reagent (5,5′-dithiobis(2-nitrobenzoic acid)) with 8 equiv of cysteamine hydrochloride in water brought to pH 8 with $NaHCO_3$. The reaction was stirred at room temperature for 2 h, acidified to pH 1 with 12 M HCl, and then extracted with ethyl acetate. The organic extract was dried over Na_2SO_4, filtered, the solvent removed by rotary evaporation to afford pure TNB as an orange solid. NMR spectral data matched that previously reported in the literature.[2]

We determined that TNB reacts selectively and stoichiometrically (1:1) with **4**; that is, loss of absorbance at 410 nm from a solution of TNB was observed upon addition of sulfenyl amide to the solution, but not when disulfides such as 2-mercaptoethanol disulfide or thioethers such as **8a** were added (Supporting Information). Stoichiometric (1:1) reaction of TNB with **4** suggests the process proceeds to the mixed disulfide, but further reduction of the mixed disulfide to the cysteine dipeptide **7**, accompanied by formation of DTNB, does not occur. Failure of TNB to fully reduce **4** to **7** is in agreement with precedented thiol-disulfide reaction equilibria involving low-pK_a aryl thiols, wherein equilibrium heavily favors the free aryl thiol.[3] Thus, we established that (a) the assay reading (Abs_{410}) changes solely as a function of concentration of **4** in solution, in 1:1 fashion, and (b) that instrument response is linearly dependent on analyte (**4**) concentration within the concentration regime considered. Accordingly, the assay employed a slight molar excess (1.25 equiv) of TNB over sulfenyl amide.

A concentrated stock of TNB (50 mM) in ethanol was prepared and stored at –20 ^{o}C until use. During use, the solution was stored on ice. When handled this way, the stock thiol was found to be stable for weeks (no appreciable loss of Abs_{410} at a fixed concentration of TNB). *Note: DMSO was found to be unsuitable for storage of TNB, as the solution rapidly decolored, presumably due to DMSO-mediated oxidation of TNB to colorless DTNB*.[4] Stock solutions of all carbon nucleophiles and of the sulfenyl amide were prepared in DMSO and stored at –20 ^{o}C before use.

Determination of Rate Constants for Reaction of 1,3-Diketo Nucleophiles with the Model Sulfenyl Amide. Approximately 10 min prior to the start of the assay, an aliquot (1–120 μL) of concentrated stock (250–500 mM) of carbon nucleophile in DMSO was added to the reaction buffer (1:1 methanol:buffer B – 50 mM Tris, 50 mM Bis-Tris, 10 mM DTPA, 100 mM NaOAc, pH 7.0, 11.88 mL total volume). To this solution was added additional DMSO, as needed, to bring the final volume of the reaction buffer to 12.0 mL total (1% DMSO v/v, final). This afforded final diketone nucleophile concentrations of 500 μM–5 mM. The 10 min pre-incubation period was intended to allow for ionization of the carbon acid to proceed toward equilibrium.[5] Following the pre-incubation period, 2 mL of the mixture (now containing diketone), were removed and set aside for determination of initial (A_o) and endpoint (A_∞) measurements for the reaction.

Assay buffer solutions were prepared via addition of 10 μL of TNB (50 mM) in ethanol to 1990 μL of Buffer C (500 mM sodium acetate, 100 mM Bis-Tris, 75 mM DTPA, pH 5.0), affording 2 mL final volume and 250 μM TNB final concentration. The 2 mL of assay buffer solutions were subsequently divided into nine 200 μL aliquots and stored on ice until used; 7 aliquots were dedicated to reaction kinetics time point measurements, and the last 2 to endpoint determinations (A_o and A_∞, *vide supra*).

To the remaining 10 mL of reaction buffer were added 10 μL of the model sulfenyl amide (50 mM in DMSO), to give a final concentration of 50 μM. Immediately upon introduction of the **4** to the reaction mixture, a timer was started and aliquots (800 μL) were periodically removed from the reaction mixture and assayed for remaining (unreacted) **4** by dilution into ready-made 200 μL aliquots of assay buffer containing TNB (*vide supra*). Reaction progress was typically assayed at 0.5, 1, 2, 3.5, 5, 8, and 11 min time points. The assay mixture, strongly buffered at pH 5, permitted simultaneous quenching of the enolate-**4** reaction and quantitative measurement of unreacted **4**.[6] The quenched time point mixtures (1 mL total; 800 μL reaction mixture, 200 μL assay buffer + TNB) afforded 50 μM TNB and 40 μM **4** formal concentrations (final). Prior to measuring Abs_{410} of these samples, they were allowed to thermally equilibrate by standing at room temperature for 5-10 min. The Abs_{410} at time zero (A_o) and infinite time (A_∞) were determined separately by preparation of two independent endpoint samples; 800 μL of the set-aside 2 mL of reaction mixture were added to 200 μL assay buffer (done for two samples). Then, to the initial reading sample (A_o), 0.8 μL of DMSO were added, and to the endpoint sample (A_∞) 0.8 μL of **4** (50 mM) in DMSO were added. Use of these samples for determination of A_o and A_∞ account for any contribution the diketone/enolate might have to Abs_{410}.

Plots of Abs_{410} with respect to reaction time afforded monophasic rising curves, consistent with consumption of **4** by the carbon nucleophiles. Because the concentration of carbon nucleophile in the kinetics experiment was at least 10-fold higher than that of **4**, the data were analyzed using pseudo first-order kinetics by fitting to the equation:

$$A_t = [(A_o - A_\infty) \times e^{-k_\psi \times t}] + A_\infty$$

where A_o, A_∞, and A_t are the Abs_{410} initially, finally, and intermediately during the reaction, and k_ψ is the pseudo first-order rate constant for reaction at a given concentration of carbon nucleophile. Note that the expression above is merely a rearranged form of the equation that describes any (pseudo) first-order process:

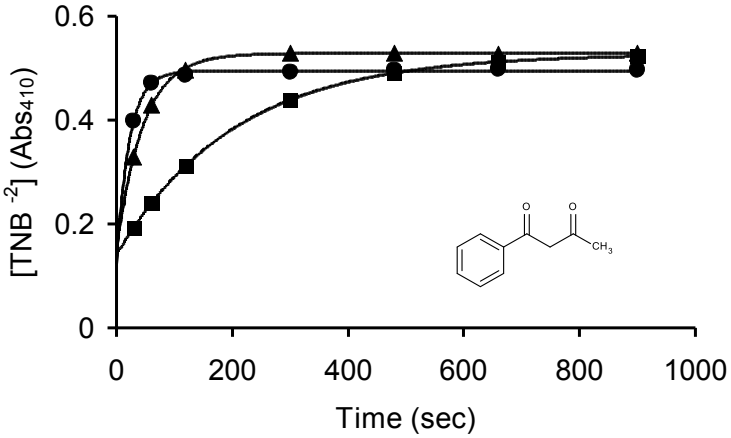
$$\frac{A_t - A_\infty}{A_o - A_\infty} = e^{-k*t}$$

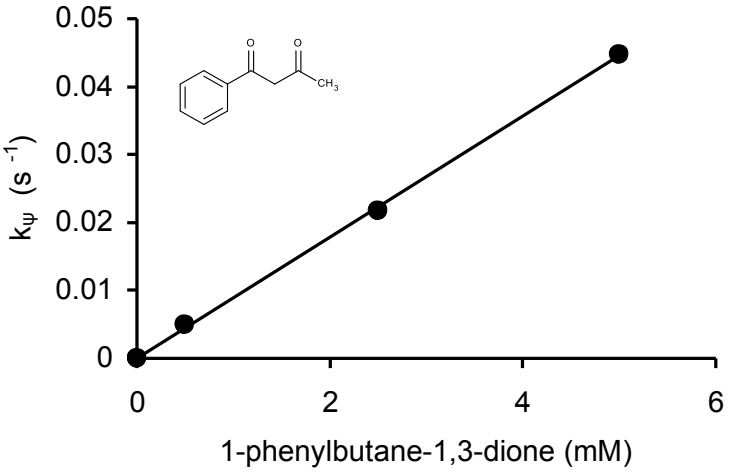
Indeed, first-order kinetic models fit the data quite well, and plotting the observed pseudo first-order rate constants versus concentration of the carbon nucleophile in excess afforded a straight line passing through the origin. This result is consistent with a simple bimolecular process being involved in the rate-determining step, described by rate law:

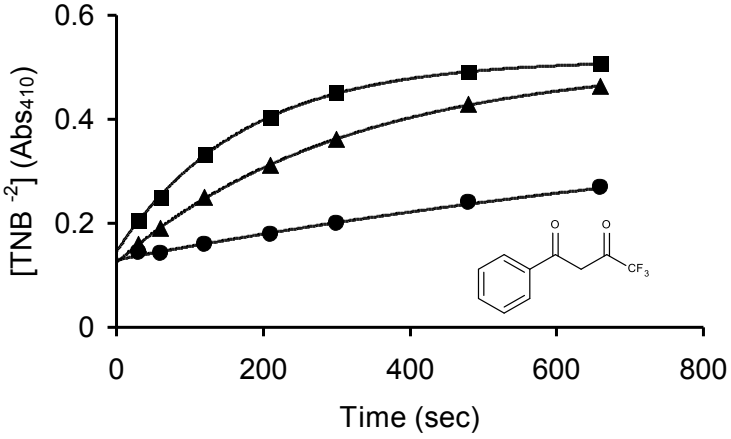
$$Rate = -\frac{d[SA]}{dt} = +\frac{d[P]}{dt} = k_{obs}[SA][Nu]$$

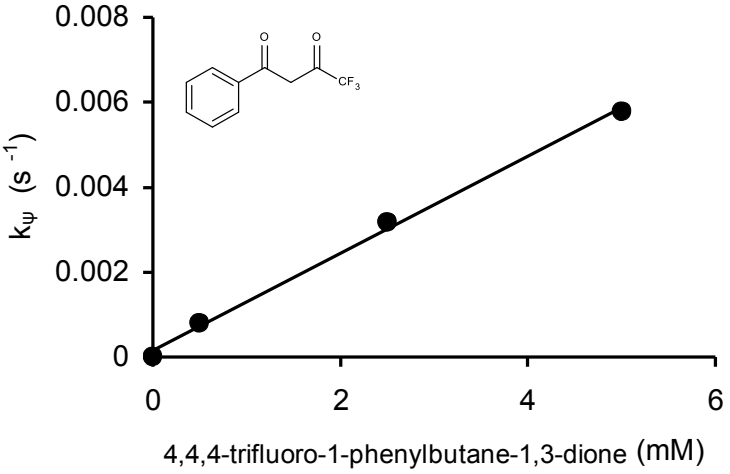
where k_{obs} is the second-order rate constant (also referred to as k_{trap} herein), and [SA], [P], and [Nu] are the concentrations of **4**, product thioether, and enolate nucleophile, respectively.

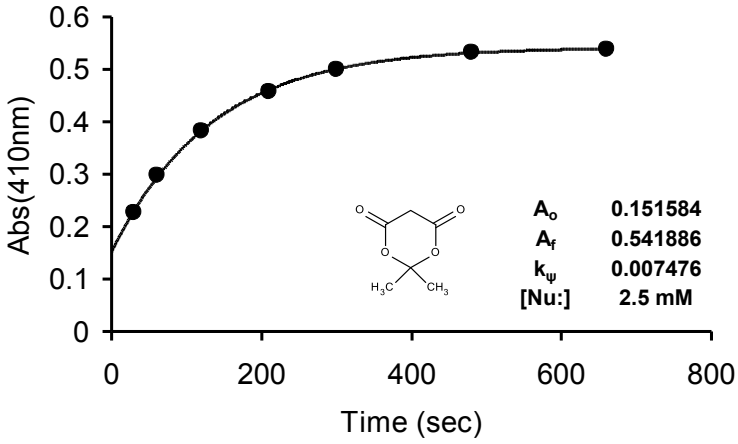
Kinetics data: determination of rate constants for reaction of carbon nucleophile with model sulfenyl amide *via* TNB assay methodology, associated fitting to kinetic data, and determination of reaction order with respect to various carbon nucleophiles.

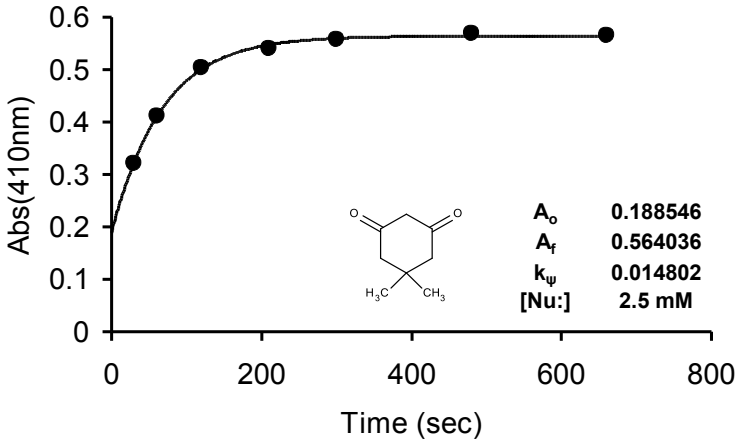







0.008
0.006
0.004
0.002
0
k_ψ (s^{-1})
0
2
4
6
4,4,4-trifluoro-1-phenylbutane-1,3-dione (mM)


0.6
0.5
0.4
0.3
0.2
0.1
0
Abs(410nm)
0
200
400
600
800
Time (sec)
A_o 0.151584
A_f 0.541886
k_ψ 0.007476
[Nu:] 2.5 mM


0.6
0.5
0.4
0.3
0.2
0.1
0
Abs(410nm)
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600
800
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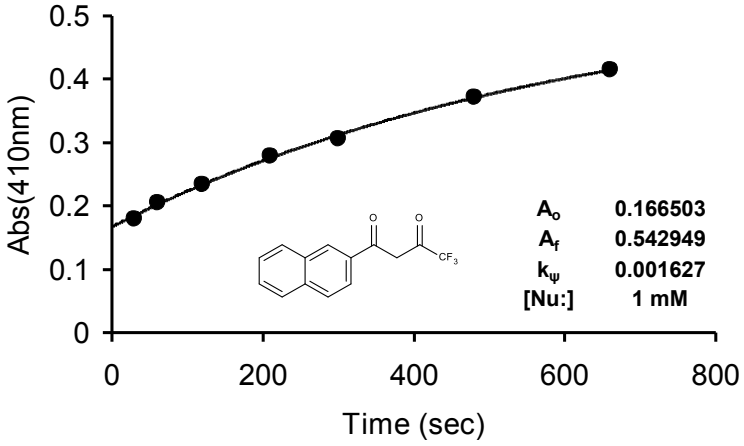
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0.3
0.2
0.1
0
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400
600
800
Time (sec)
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k_ψ 0.001627
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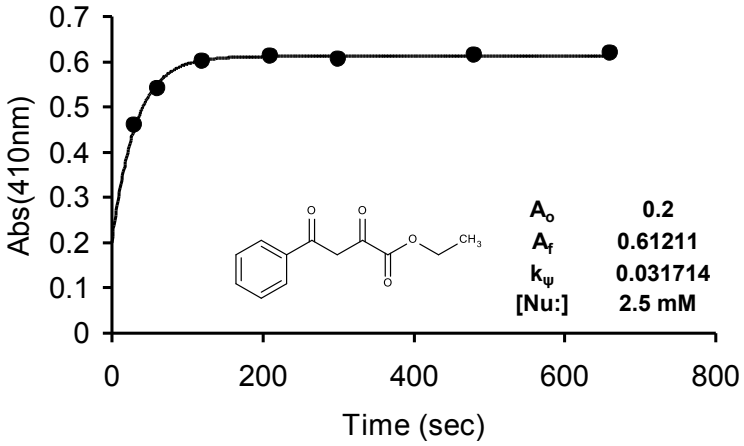

0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
Abs(410nm)
0
200
400
600
800
Time (sec)
A_o 0.2
A_f 0.61211
k_ψ 0.031714
[Nu:] 2.5 mM

TNB assay methodology development and "calibration": determination of instrument response, reaction selectivity, and stoichiometry of the assay.

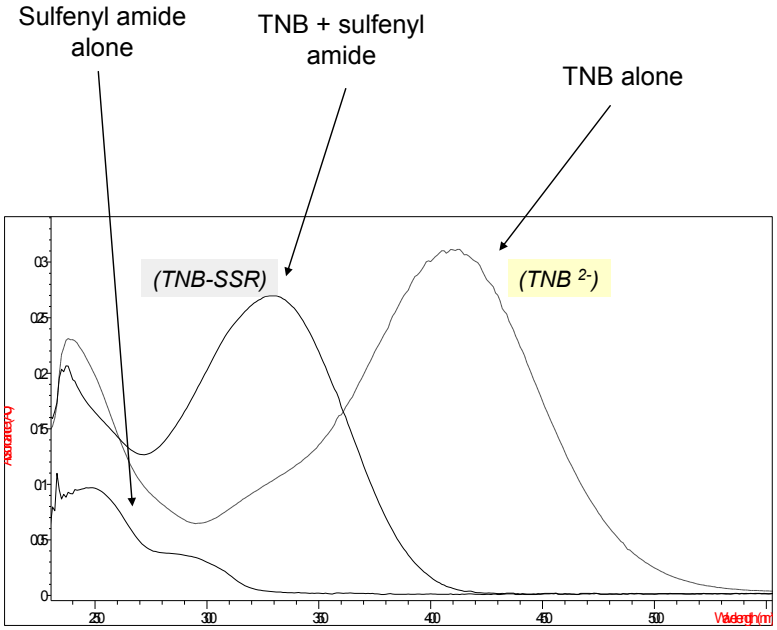


Figure S2. An equimolar concentration of the model sulfenyl amide to the TNB^{-2} ion under the TNB assay conditions completely converts TNB^{-2} to TNB-SSR. To 1:1 MeOH : pH 5 Buffer C, 50 μM sulfenyl amide or 50 μM TNB^{-2} were added (leftmost and rightmost traces/peaks, respectively). The absorption spectra reveal that TNB^{-2} absorbs strongly at 410 nm in the assay mixture and that the sulfenyl amide contributes no appreciable absorbance at this wavelength. When 50 μM sulfenyl amide was added to an assay mixture containing 50 μM TNB^{-2}, immediate loss in yellow color of the solution (due to A_{410} of the TNB^{-2} ion) was observed; the absorption spectrum of the resulting mixture reveals strong absorbance at 325 nm, consistent with TNB-disulfide formation. Because of the reaction stoichiometry, it may be safely assumed the disulfide is a mixed disulfide of the TNB thiol and sulfenyl amide sulfur. Importantly, the mixed disulfide does not contribute to A_{410}.

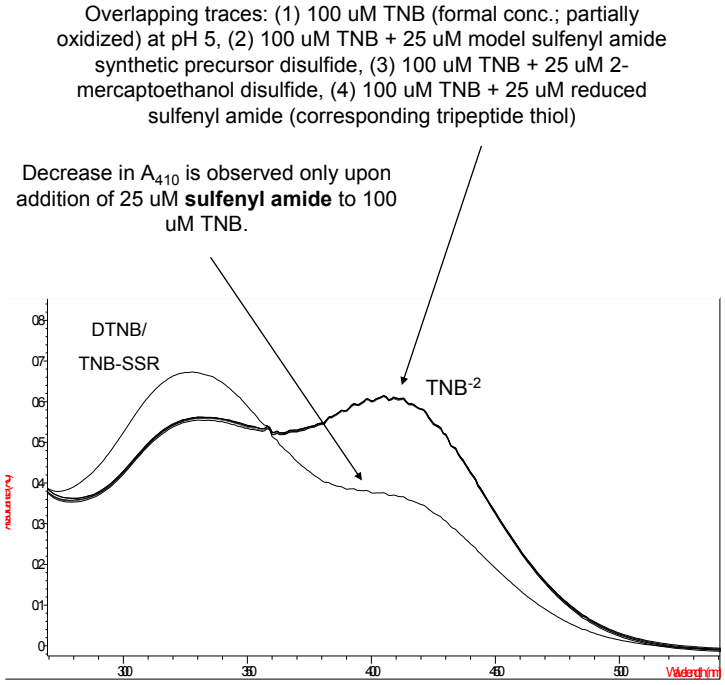


Figure S3. Determination of selectivity of the TNB assay to other potentially-interfering redox-active species. Oxidation of TNB^{-2} (to TNB-SSR or DTNB) was observed **only** upon addition of sulfenyl amide to the mixture; neither the disulfide synthetic precursor to the sulfenyl amide (see synthetic route), nor β-mercaptoethanol disulfide effected conversion of TNB^{-2} to the corresponding mixed disulfide (final concentrations indicated in Figure; all spectra collected in 1:1 MeOH : pH 5 Buffer C). Additionally, addition of thiol agent (the thiol corresponding to fully-reduced sulfenyl amide) did not convert any DTNB present to the TNB^{-2} ion. Finally, only the addition of sulfenyl amide (25 μM) afforded a decrease in A_{410}, corresponding to the conversion of TNB^{-2} to the TNB-model sulfenyl amide mixed disulfide ("TNB-SSR").

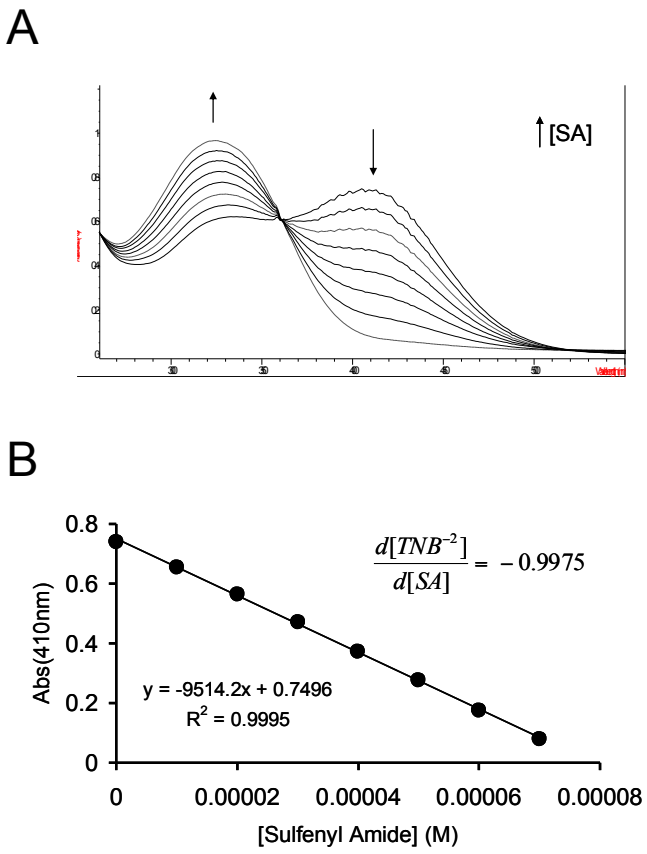


Figure S4. Determination of reaction stoichiometry between theTNB^{-2} ion and the model sulfenyl amide under the assay conditions employed (1:1 MeOH : Buffer C, pH 5.0). **A.** To a 1 mL solution of 1:1 MeOH : Buffer C containing 100 μM TNB^{-2} (formal conc.; partially oxidized by air) were added aliquots (2 μL) of 5 mM sulfenyl amide in dmso, from 0 μM to 70 μM, final. As sulfenyl amide was titrated in, A_{410} smoothly decreased with concomitant increase in A_{325}, corresponding to conversion of TNB^{-2} to the TNB-model sulfenyl amide mixed disulfide ("TNB-SSR"). **B.** A plot of the absorbences at 410 nm versus molar concentration of the sulfenyl amide titrated in affords excellent linear correlation with a slope of -9514. In a separate experiment, wherein TNB^{-2} was maintained in fully reduced form by the presence of an excess (2 equiv) of known thiol-reducing agent TCEP (tris(2-carboxyethyl)phosphine), the approximate extinction coefficient of the TNB^{-2} ion under these conditions was found to be ~ 9538 M^{-1} cm^{-1}. Thus, using the established extinction coefficient and slope of the line relating [TNB^{-2}] to molar concentration of sulfenyl amide titrated in, we find that the reaction proceeds stoichiometrically (1:1, as d[TNB^{-2}]/d[SA] ≈ 1.0, inset in panel **B**). Furthermore, because instrument response is linear with respect to [SA], monitoring A_{410} is a suitable means by which to track [SA] in kinetics experiments under these conditions.

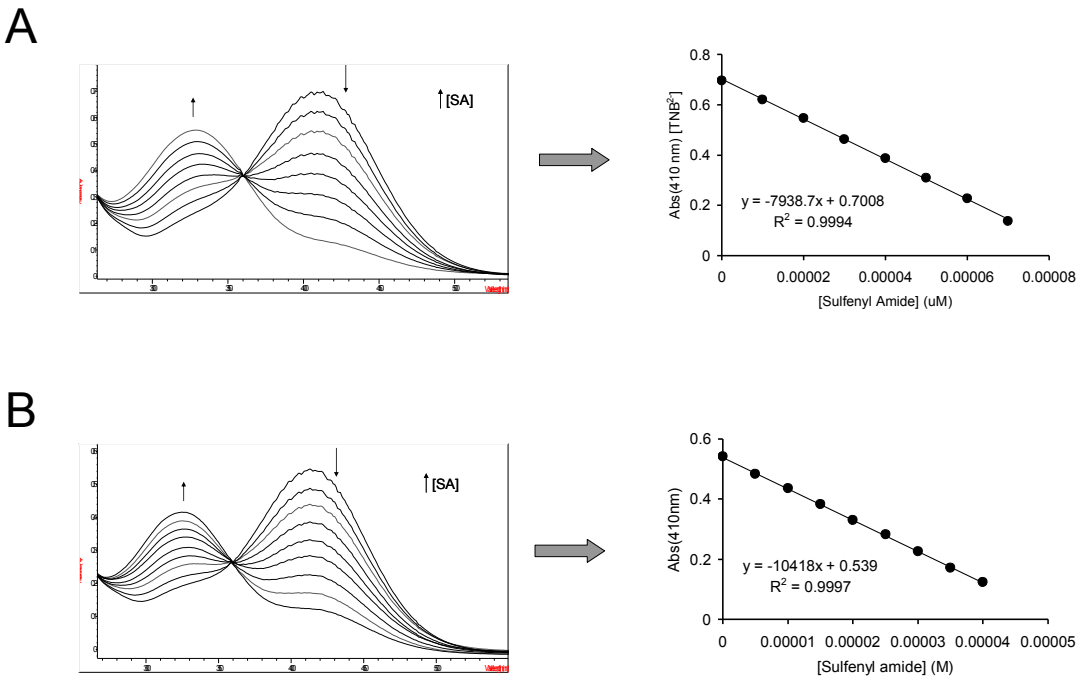
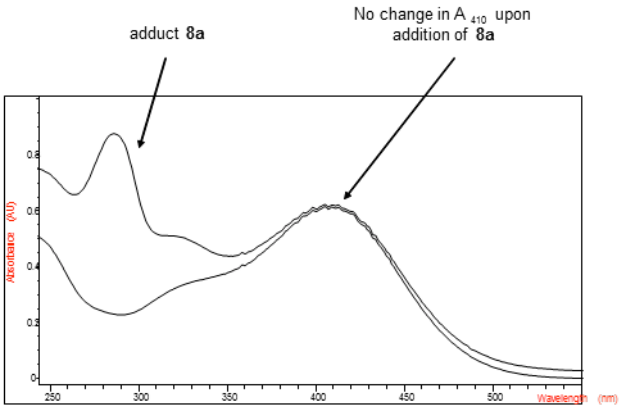


Figure S5. The "TNB assay" is robust with respect to presence or absence of organic cosolvent (methanol) and pH of the assay mixture. Essentially identical methodology to that described above in sulfenyl amide titrations was used here to determine instrument response to increasing concentrations of secondary analyte (sulfenyl amide). **A.** Increasing concentrations of sulfenyl amide were titrated into a solution of TNB^{-2} in pure aqueous buffer, pH 5.0 (Buffer C). Excellent linear dependence of instrument response on molar concentration is observed in the absence of organic cosolvent (methanol). **B.** Again, excellent linear dependence of A_{410} on molar concentration of sulfenyl amide is observed when the assay solution pH is changed (1:1 MeOH : Buffer B (see text)). Note that the extinction coefficient is undoubtedly larger at pH 7, relative to that at pH 5 (due to an increased fraction of TNB thiolate at higher pH); hence the increased negative slope at pH 7 relative to that at pH 5.

A



B

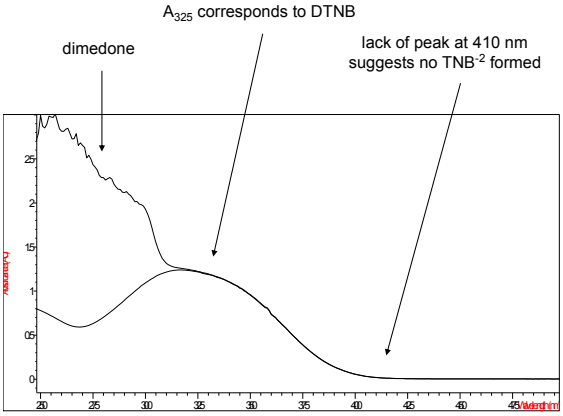


Figure S6. Thioether products and carbon nucleophiles do not interfere with the TNB assay. **A.** To confirm the sulfide products formed during kinetics experiments would not react with the TNB^{-2} ion, to a solution of 100 μM TNB^{-2} in 1:1 MeOH : Buffer C ("standard assay conditions") was added product 8**a** to a final concentration of 100 μM and allowed to stand for 5 minutes. No change in A_{410} resulted from introduction of the sulfide product. **B.** Because kinetics assays were performed under pseudo first-order conditions (large molar excess of carbon nucleophile to sulfenyl amide), confirmation of lack of cross-reactivity was crucial. Namely, in principle, carbon nucleophiles could attack electrophilic disulfides (such as TNB-SSR or DTNB), releasing chromaphoric TNB^{-2}. However, when dimedone (**D**, 1 mM final concentration) was added to a solution of 100 μM DTNB (the disulfide of TNB^{-2}) in 1:1 MeOH : Buffer C and allowed to stand for 2 minutes, no release of TNB^{-2} (at A_{410}) was observed, confirming the "inertness" of excess nucleophilic carbon acid to the TNB assay under the conditions employed.

References.

1. Kedika, B.; Patri, S. V.; *Molecular Pharmaceutics*. **2012**, 9, 1146−1162.
2. Van Berkel, S. S.; Brem, J.; Rydzik, A. M.; Salimraj, R.; Cain, R.; Verma, A.; Owens, R. J.; Fishwick, C. W. G.; Spencer, J.; Schofield, C. J. *J. Med. Chem.* **2013**, *56*, 6945–6953.
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4. Yiannios, C. N.; Karabinos, J. V. *J. Org. Chem.* **1963**, *28*, 3246–3248.
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