Modelling flavoenzymatic charge transfer events: development of catalytic indole deuteration strategies.

Alexander T. Murray,[†] *Jonathan D. Challinor*[†]*, Christina E. Gulácsy, Cristina Lujan,*[†] Lauren E. Hatcher, Christopher R. Pudney, [^,]* Paul R. Raithby, Matthew *P. John,*[‡] *and David R. Carbery*[*†]

[†] Department of Chemistry, University of Bath, Bath, BA2 7AY, UK,

[^] Department of Chemistry, University of Bath, Bath, BA2 7AY, UK,

[‡] GlaxoSmithKline, Gunnels Wood Road, Stevenage, SG1 2NY, UK

E-mail: d.carbery@bath.ac.uk

CONTENTS

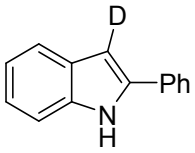
I GENERAL INFORMATION

All reagents were purchased from commercial suppliers: Acros Organics, Alfa Aesar or Sigma Aldrich and used without further purification. Flash chromatography was performed on chromatography grade, silica, 60 Å particle size 35-70 micron from Sigma Aldrich using the solvent system as stated. ^{1}H and ^{13}C NMR was performed on Brüker Avance 250 (^{1}H 250 MHz) Brüker Avance 300 (^{1}H 300 MHz and ^{13}C 75 MHz), Brüker Avance 400 (^{1}H 400 MHz and ^{13}C 100 MHz), Brüker Avance 500 (^{1}H 500 MHz and ^{13}C 125 MHz) or Agilent ProPulse 500 (^{1}H 500 MHz and ^{13}C 125 MHz) as stated. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane (TMS) (δ = 0.00). Coupling constants are reported in Hertz (Hz) and signal multiplicity is denoted as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quin.), sextet (sex.), septet (sept.), multiplet (m), and broad (br). High resolution mass spectrometry electrospray (ESI) was performed on a Brüker µTOF using electrospray ionisation (ESI) in either positive or negative ionisation. Infra-red spectroscopy was carried out using a Perkin Elmer Spectrum RX FT-IR system. All NMR data of partially deuterated compounds were compared to the authentic starting materials obtained from Sigma-Aldrich.

II EXPERIMENTAL SECTION

General Procedure for flavin-catalysed deuteration of indole (General procedure 1)

To a small vial (5 mL) of 0.5 mL CD3OD was added 0.1 mmol of indole, followed by 3 mol % (1 mg) **5a** The vial was shaken and left to stand at room temperature for one minute or alternatively capped and left to stand at room temperature for 15 minutes. The reaction was then quenched with diethyl ether before being run through a pipette column with silica gel washed with a 1:2 mixture of petroleum ether/ethyl acetate (2% triethylamine). Solvent was removed by rotary evaporation.



3-deutero-2- phenylindole (7a)

General procedure 1 (1 minute) using indole (12 mg). Full characterisation was performed on this compound and is shown below.

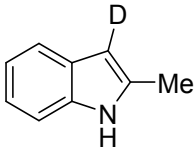
^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.70 (d, J = 7.5 Hz, 2H), 7.66 (d, J = 7.6 Hz, 1H), 7.47 Hz (apparent t, J = 7.5 Hz, 2H), 7.35 (t, J = 7.5 Hz, 1H), 7.22 (apparent t, J = 7.6 Hz, 1H), 7.15 (apparent t, J = 7.6 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 138.0, 137.0, 132.5, 129.3, 127.9, 125.3, 122.5, 120.8, 120.4, 111.0, 100.2 (d, $J_{C,D}$ = 75 Hz)

^{2}D NMR (75 MHz, CD_3OD) δ_D 6.81 (s)

IR ν_{max} (neat) 3443, 3048, 2564, 1668, 1456, 1445, 1349 cm^{-1}

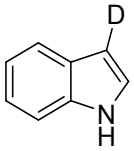
HRMS (ESI, -ve) Calcd. for $C_{14}H_9ND$, 193.0876. Found: 193.0887 $(M-H)^-$



3-deutero-2-methylindole (7b)

General procedure 1 (15 minutes) using 2-methylindole (13 mg).

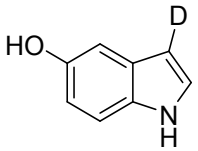
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.87 (br s, 1H), 7.56 – 7.48 (m, 1H), 7.33 – 7.25 (m, 1H), 7.17 – 7.02 (m, 2H), 6.22 (s, 0.06 H), 2.44 (s, 3H).



3-deuteroindole (7c)

General procedure 1 (15 minutes) using indole (12 mg).

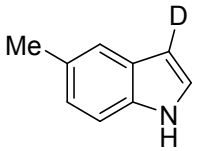
^{1}H NMR (300 MHz, $CDCl_3$) δH 7.69 (d, J = 7.9 Hz, 1 H), 7.44 (d, J = 8.0, 1 H), 7.22-7.10 (m, 4 H), 6.58 (s, 0.16 H).



3-deutero-5-hydroxyindole (7d)

General procedure 1 (1 minute) using 5-hydroxyindole (13 mg).

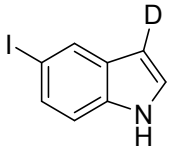
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.25 (t, *J* = 4.3 Hz, 3 H), 7.19 (s, 1 H), 7.00 (d, *J* = 2.4 Hz, 1 H), 6.78 (dd, *J* = 8.7, 2.5 Hz, 1 H), 6.43 (d, *J* = 2.4 Hz, 0.17 H).



3-deutero-5-methylindole (7e)

General procedure 1 (15 minutes) using 5-methylindole (13 mg)

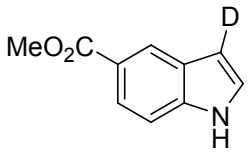
1H NMR (250 MHz, CDCl3) δ_H 8.05 (br s, 1 H), 7.44 (s, 1 H), 7.28 (m, 1 H), 7.17 (s, 1 H), 7.03 (d, *J* = 8.26 Hz, 1 H), 6.48 (d, *J* = 2.5 Hz, 0.17 H), 2.46 (s, 3 H).



3-deutero-5-iodoindole (7f)

General procedure 1 (15 minutes) using 5-iodoindole (24 mg).

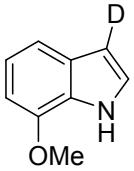
^{1}H NMR (300 MHz, $CDCl_3$) δ_H 8.19 (br s, 1 H), 7.99 (s, 1 H), 7.44 (dd, *J* = 8.5, 1.9 Hz, 1 H), 7.18 (d, *J* = 8.5 Hz, 2 H), 6.48 (d, *J* = 1.9 Hz, 0.35 H).



3-deutero-methylindole-5-carboxylate (7g)

General procedure 1 (1 minute) using methyl indole-5-carboxylate (18 mg).

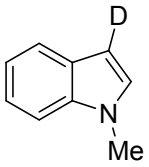
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 8.42 (s, 1 H), 7.91 (d, 8.7 Hz, 1H), 7.41 (d, *J* = 8.7 Hz, 1 H), 7.27 (m, 2 H), 6.65 (m, 0.94 H), 3.93 (s, 3 H)



3-deutero-7-methoxyindole (7j)

General procedure 1 (15 minutes) using 7-methoxyindole (15 mg)

^{1}H NMR (250 MHz, $CDCl_3$) δ_H 8.18 (br s, 1 H), 7.16-7.01 (m, 3 H), 6.67 (s, 0.67 H), 6.53 (m, 1 H), 3.97 (s, 3 H)



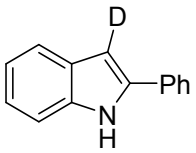
1-methylindole (7k)

General procedure 1 (15 minutes) using 1-methylindole (12 μL).

^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.64 (d, J = 8.04 Hz, 1 H), 7.35-7.05 (m, 4 H), 6.49 (d, *J* = 2.5 Hz, 0.54 H), 3.80 (s, 3 H).

General Procedure for acetyl chloride-catalysed deuteration of indole

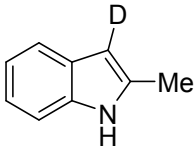
To a small vial (5 mL) of 0.5 mL CD3OD was added 0.1 mmol of indole, followed by 10 μL of acetyl chloride solution (30 μL acetyl chloride in 1 mL CD3OD). The vial was shaken and left to stand at room temperature for one minute or alternatively capped and left to stand at room temperature for 15 minutes. The reaction was then quenched with diethyl ether before being run through a pipette column with silica gel washed with a 1:2 mixture of petroleum ether/ethyl acetate (2% triethylamine). Solvent was removed by rotary evaporation.



3-deutero-2-phenylindole (7a)

General procedure 2 (1 minute) using 2-phenylindole (19 mg).

^{1}H NMR (250 MHz, $CDCl_3$) δ_H 8.37 (br s, 1 H), 7.66 (m, 3 H), 7.48-7.10 (m, 6 H), 6.84 (s, 0.07 H).

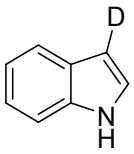


3-deutero-2-methylindole (7b)

General procedure 1 (15 minutes) using 2-methylindole (13 mg).

^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.56 – 7.48 (m, 1H), 7.33 – 7.25 (m, 1H), 7.17 – 7.02 (m, 2H), 6.15 (br s, 0.17 H), 2.44 (s, 3H).

NB. In this case it is possible, due to the broad signal the NH has been deuterated and shifted, and we do not observe residual C3. We assumed the 'worst' case that the broad peak is C3 proton.



3-deuteroindole (7c)

General procedure 2 (15 minute) using indole (12 mg). Full characterisation was performed on this compound and is shown below.

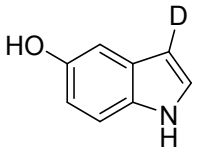
^{1}H NMR (500 MHz, $CDCl_3$) δ_H 7.67 (d, J = 8.0 Hz, 1H), 7.41 (d, J = 8.0 Hz, 1H), 7.24 – 7.19 (m, 2H), 7.16 – 7.12 (m, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ_C 135.7, 127.9, 124.0, 122.1, 120.8, 119.9, 111.1, 102.5 (t, $J_{C,D}$ = 26 Hz).

^{2}D NMR (75 MHz, CD_3OD) δ_D 6.48 (apparent s)

IR ν_{max} (neat) 3413, 2922, 2852, 1455, 1324 cm^{-1}

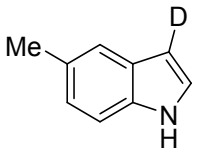
HRMS (ESI, -ve) Calcd. for C_8H_5ND 117.0563. Found: 117.0572 $(M-H)^-$



3-deutero-5-hydroxyindole (7d)

General procedure 2 (1 minute) using 5-hydroxyindole (13 mg).

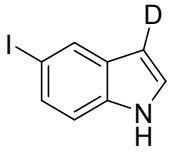
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.27-7.24 (apparent d, J = 8.5 Hz, 2 H), 7.19 (s, 1 H), 7.04 (apparent d, J = 7.0 Hz, 1 H), 6.78 (dd, J = 8.5, 2.4 Hz, 1 H), 6.43 (d, J = 2.4 Hz, 0.18 H).



3-deutero-5-methylindole (7e)

General procedure 2 (1 minute) using 5-methylindole (13 mg)

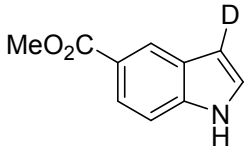
^{1}H NMR (250 MHz, $CDCl_3$) (br s, 1 H), 7.44 (s, 1 H), 7.28 (m, 1 H), 7.17 (s, 1 H), 7.03 (d, J = 8.26 Hz, 1 H), 6.48 (m, 0.57 H), 2.46 (s, 3 H).



5-iodoindole (7f)

General procedure 2 (15 minutes) using 5-iodoindole (24 mg).

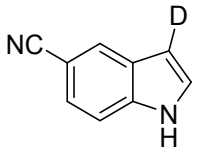
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.99 (s, 1 H), 7.44 (dd, J = 8.5, 2.0 Hz, 1 H), 7.18 (d, J = 8.5 Hz, 2 H), 6.48 (d, J = 2.0 Hz, 0.19 H).



3-deutero-methylindole-5-carboxylate (7g)

General procedure 2 (15 minutes) using methyl indole-5-carboxylate (18 mg).

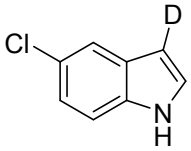
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 8.42 (s, 1 H), 7.91 (d, J = 8.7 Hz, 1H), 7.41 (d, J = 8.7 Hz, 1 H), 7.27 (m, 2 H), 6.65 (m, 0.19 H), 3.93 (s, 3 H)



3-deutero-5-cyanoindole (7h)

General procedure 2 (15 minutes) using 5-cyanoindole (14 mg)

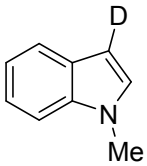
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 8.01 (s, 1 H), 7.48-7.35 (m, 3 H), 6.65 (s),0.16 H).



3-deutero-5-chloroindole (7i)

General procedure 2 (15 minutes) using 5-chloroindole (15 mg).

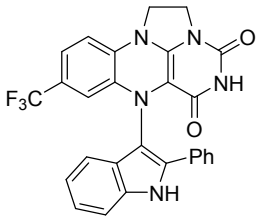
^{1}H NMR (250 MHz, $CDCl_3$) δ_H 7.54 (d, J = 2.2 Hz, 1H), 7.28 – 7.02 (m, 3H), 6.42 (d, J = 2.2 Hz, 0.06 H).



3-deutero-1-methylindole (7k)

General procedure 2 (1 minute) using 1-methylindole (13 μL) with 3 mol % acetyl chloride.

1H NMR (250 MHz, CDCl3) δ_H 7.64 (d, J = 8.1 Hz, 1 H), 7.35 (d, J = 8.1 Hz, 1 H), 7.22 (apparent d, J = 8.1 Hz, 1 H), 7.15-7.06 (m, 2 H), 6.50 (m, 0.27 H), 3.81 (s, 3 H).



8-(2-phenyl-1H-indol-3-yl)-5-(trifluoromethyl)-1,8,11,13-tetraazatetracyclo[7.6.1.0^{2},^{7}.0^{13},^{16}]hexadeca-2,4,6,9(16)-tetraene-10,12-dione (9)

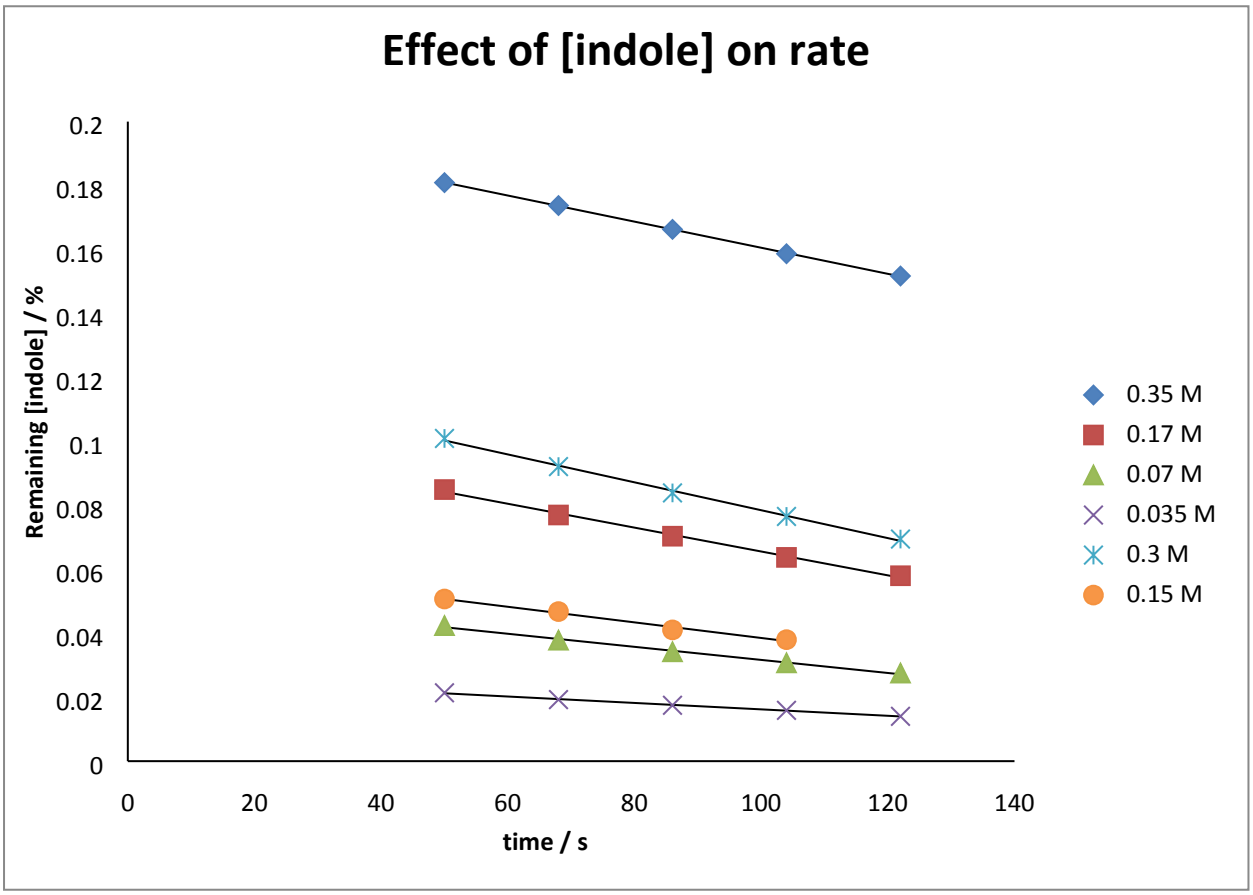
Flavin **5a** (17 mg, 50 μmol) was dissolved in methanol (0.5 mL) and 2-phenylindole **6a** (10 mg, 50 μmol) was added, followed by water (100 μL). After six days, the yellow crystals were washed and decanted with 5:1 MeOH/water (5 x 1 mL), the the product was isolated by column chromatography (100% CH_2Cl_2 → 100% EtOAc + 2% AcOH). **9** was isolated as a yellow solid (1 mg, 4%). ^{1}H NMR (300 MHz, CD_3CN) δ_H 9.67 (s, 9.67, 1H), 8.09 (d, J = 7.1 Hz, 2H), 7.95 (d, J = 8.0 Hz, 1H), 7.58 – 7.36 (m, 4H), 7.19 (t, J = 7.8 Hz, 1H), 7.09 (t, J = 7.8 Hz, 1H), 6.96 – 6.89 (m, 1H), 6.50 (d, J = 8.0 Hz, 1H), 6.26- 6.22 (m, 1H), 4.15 – 4.04 (m, 2H), 4.02 – 3.70 (br, 2H). ^{13}C NMR (125 MHz, d^{6}-Acetone) δ_C 147.5, 139.0, 138.2, 136.1, 135.0, 132.2, 128.9, 128.5, 128.1, 127.7, 125.5, 125.2 (m), 122.9, 122.2, 120.3, 119.7 (q, $J_{C,F}$ = 4 Hz), 119.6, 117.9, 113.8 (q, $J_{C,F}$ = 4 Hz), 112.7, 111.7, 111.6, 98.3, 45.9, 43.0 (not all C,F coupling resolvable). ^{19}F NMR (471 Hz, d^{6}-Acetone) δ_C -63.4. IR ν_{max} (neat) 3221, 3060, 1718, 1654, 1499, 1331, 1304, 1118. MP 199 – 204 ˚C. HRMS (ESI, -ve) *m/z* calcd. for $C_{27}H_{14}F_3N_5O_2$ 500.1329, found: 500.1331 $(M-H)^-$

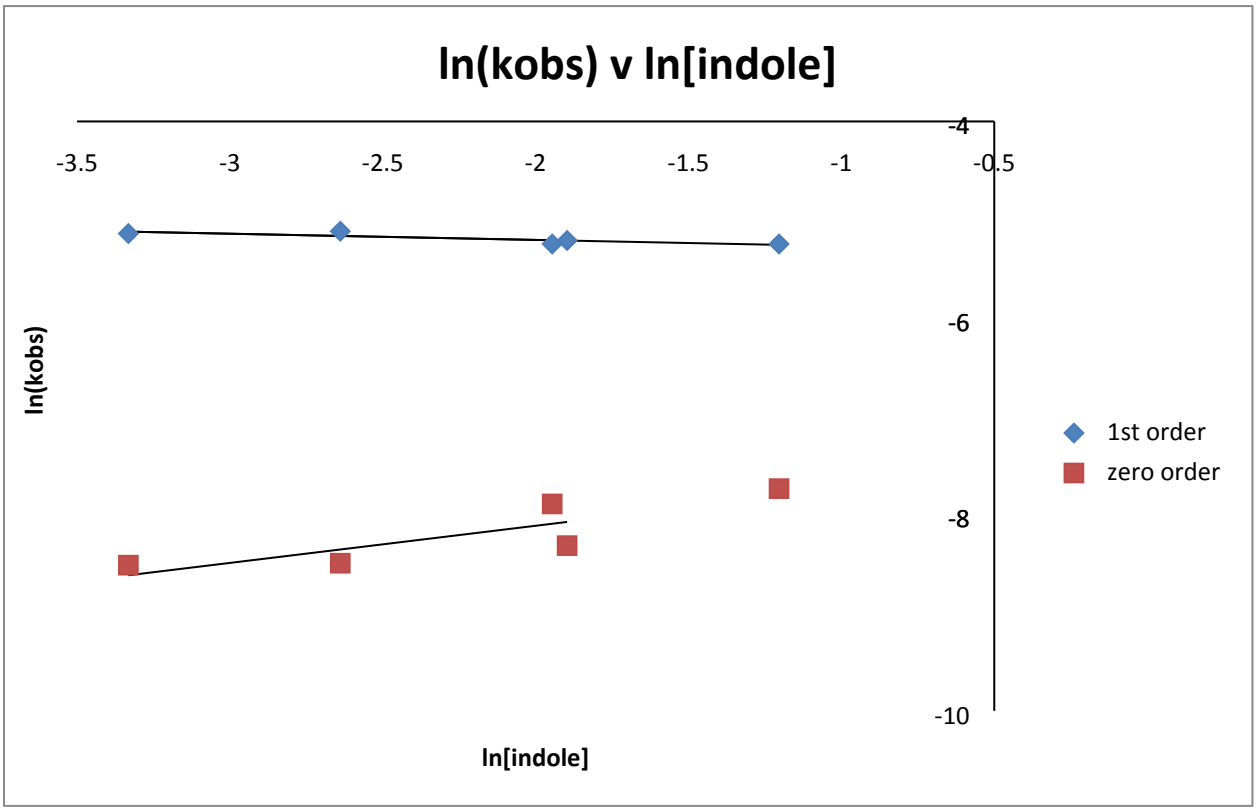
Kinetic experiments

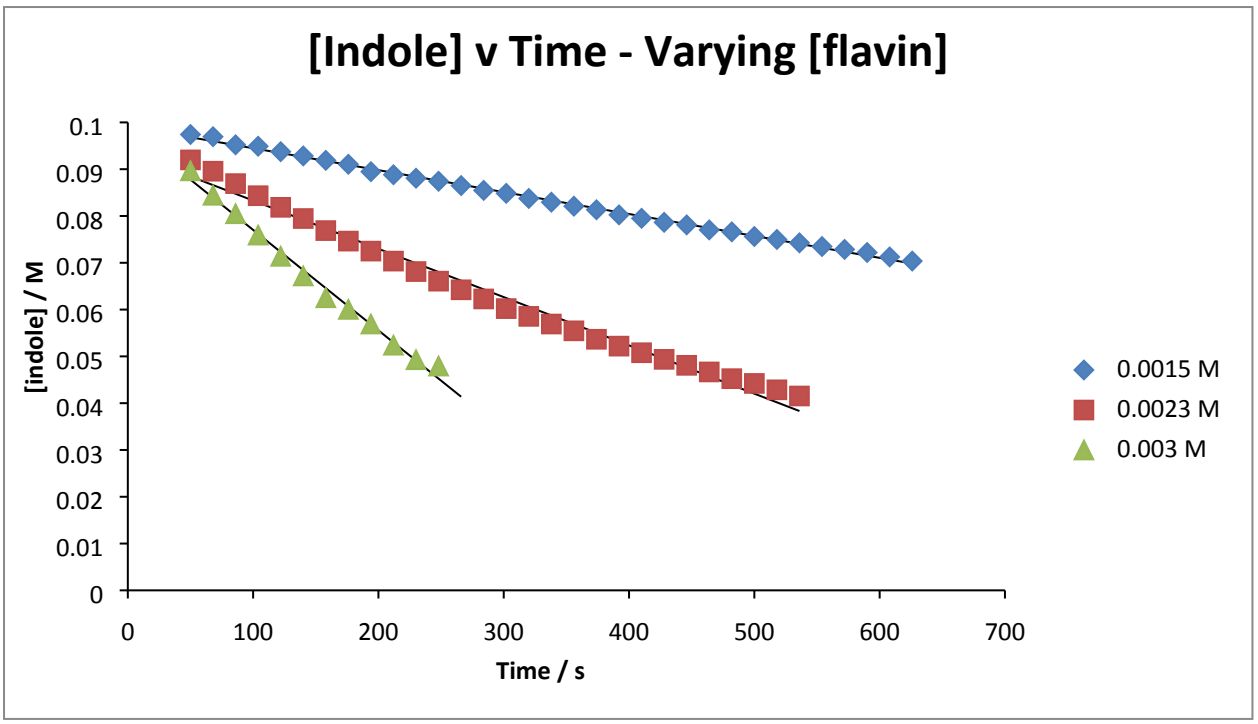
General procedure for kinetic experiments was as those for isolation of deuteroindoles except the contents were placed in an NMR tube and multiple ^{1}H NMR spectra were automatically gathered without delay using an automation procedure, and ratios of deuteration were measured by integration of the C3 proton relative to another peak. Indole was used for flavin experiments, and 5-chloroindole for those using AcCl.

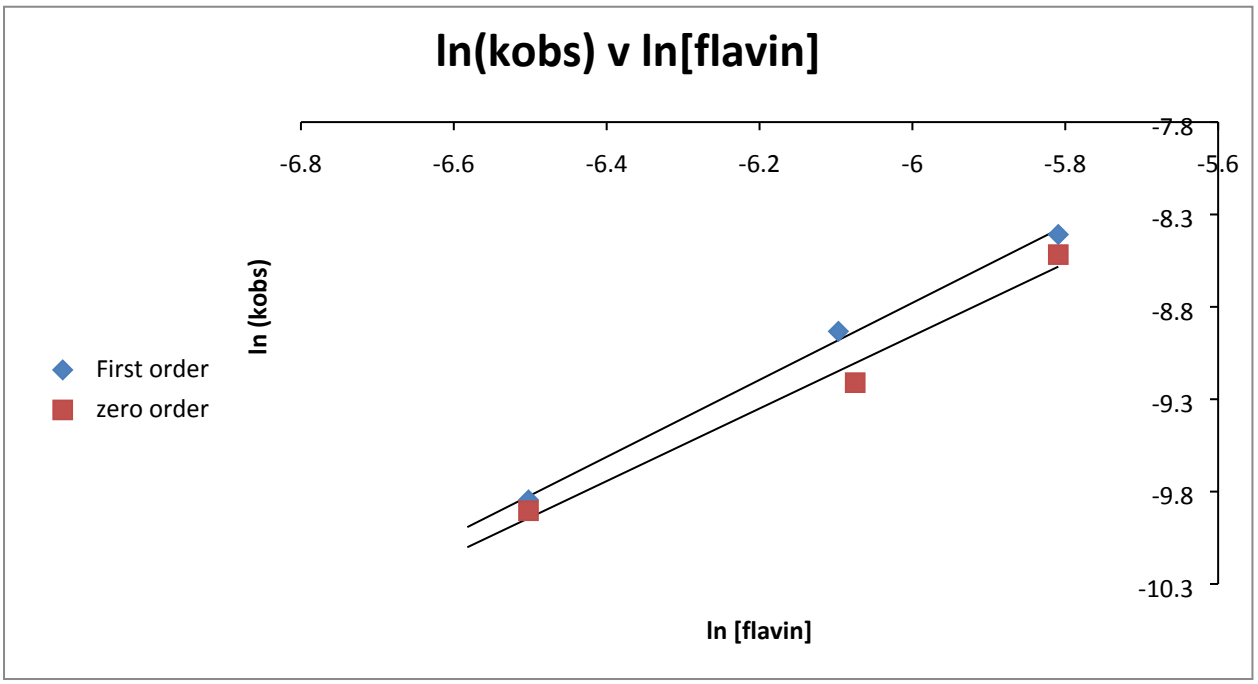
Unless deviated from, standard conditions were 0.1 mmol indole, 0.5 mL MeOD, 3 mol% flavin and 1.5 mol% AcCl.

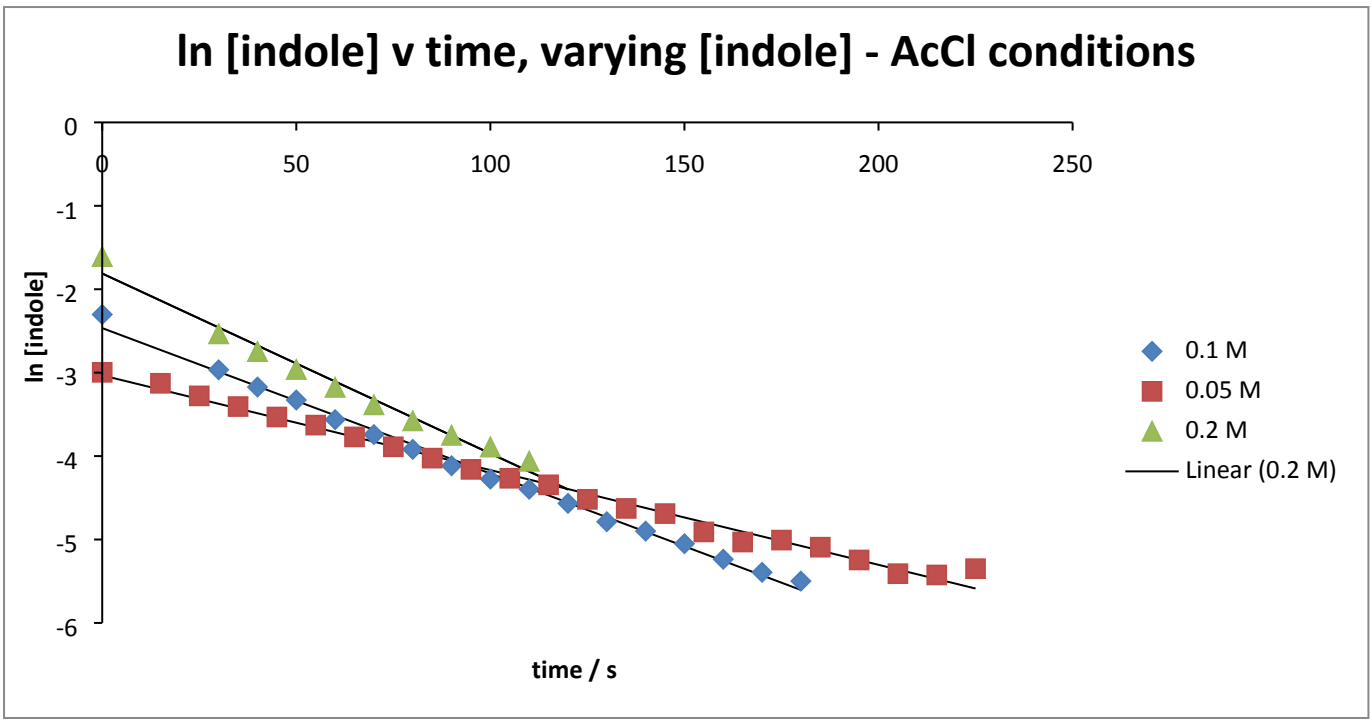
Method A – flavin catalyis

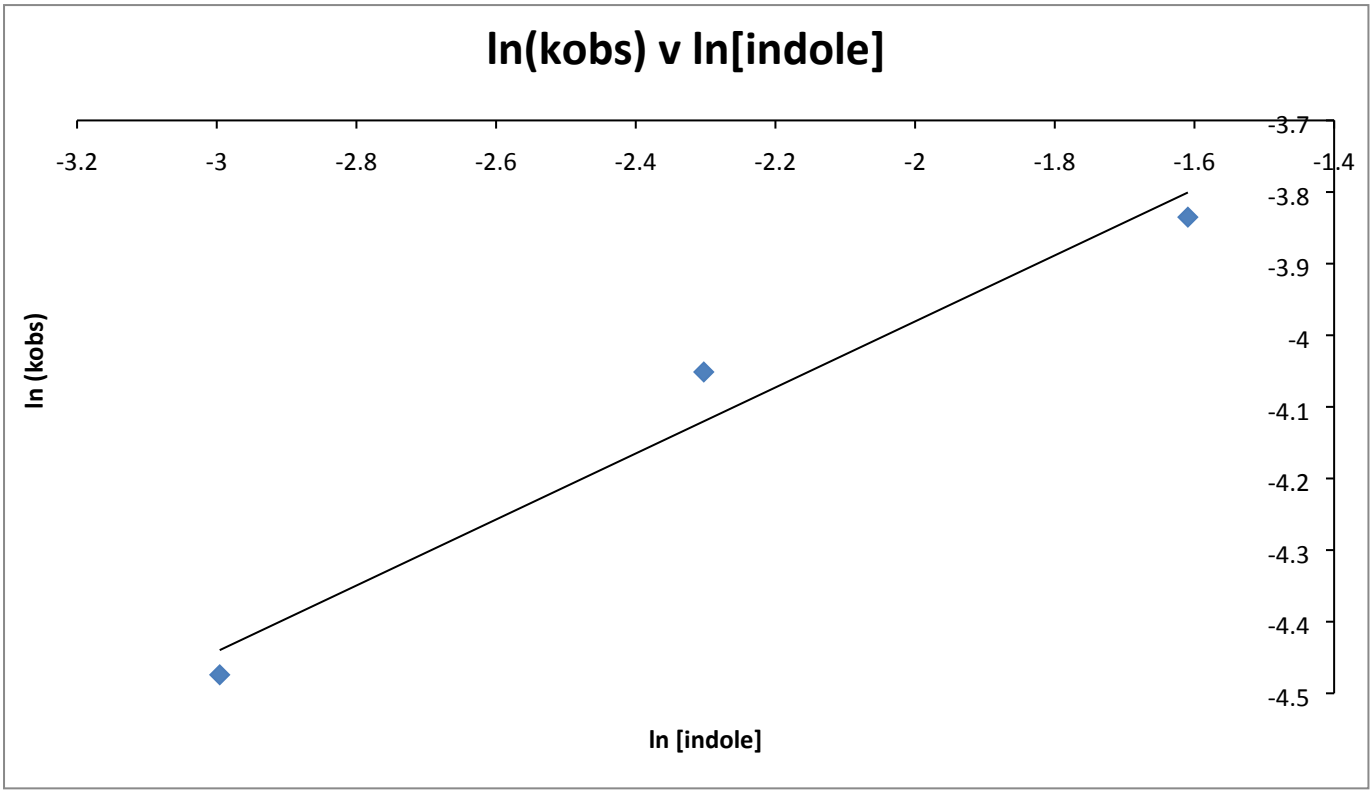


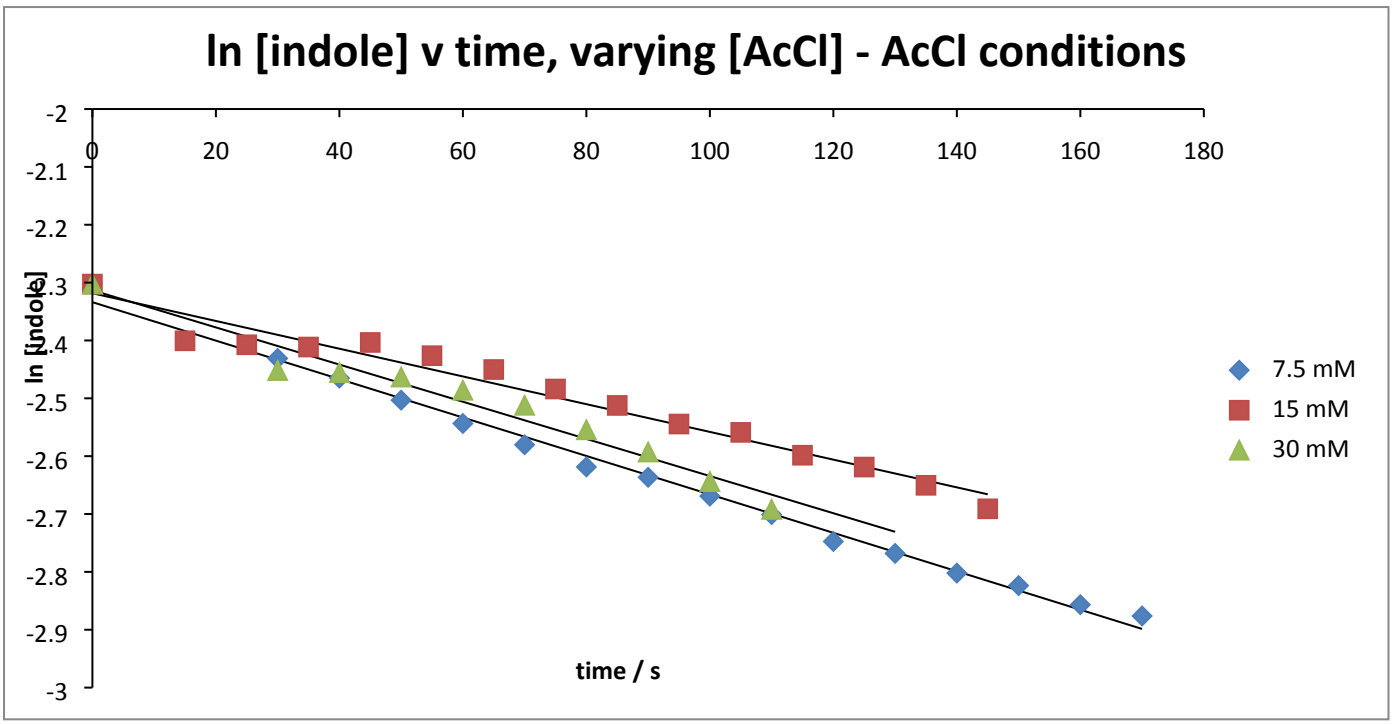


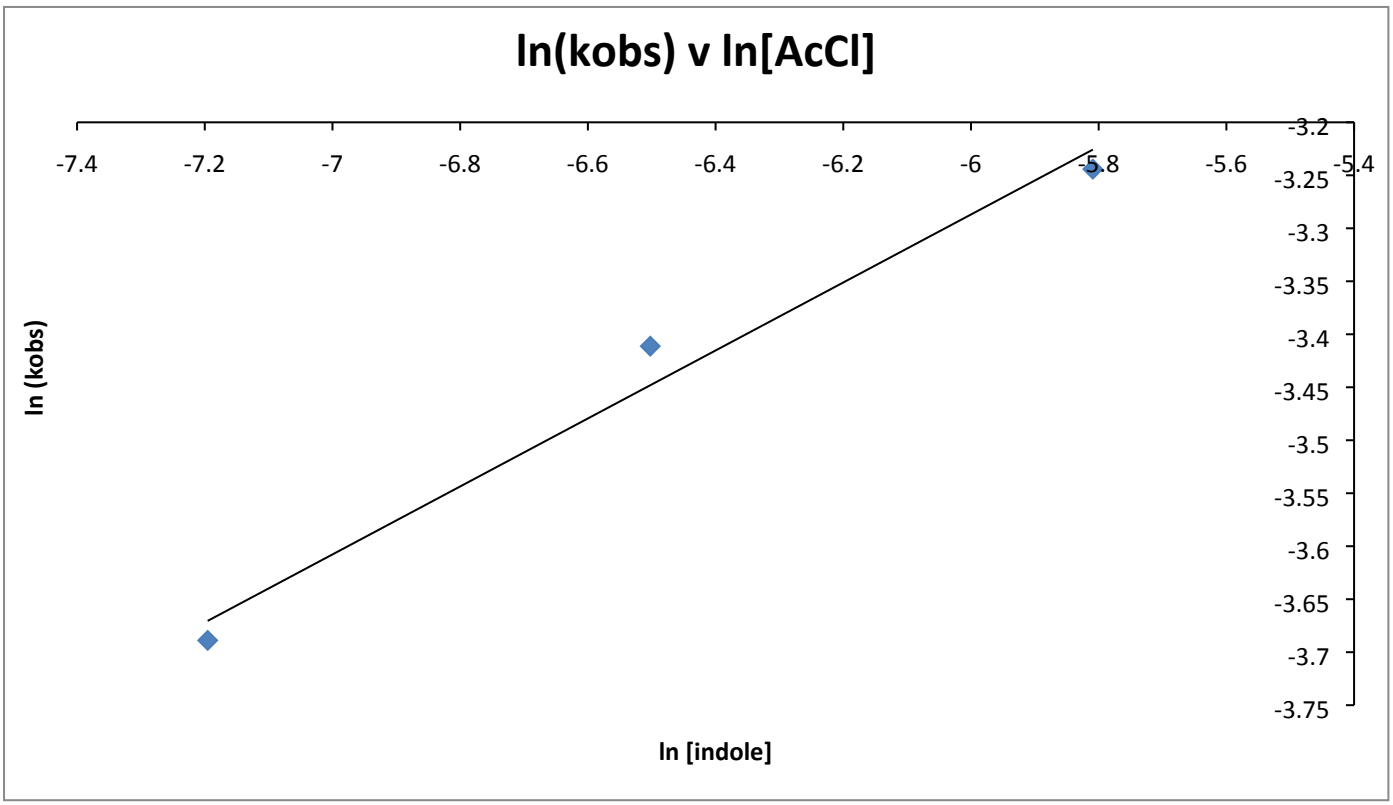

[Indole] v Time - Varying [flavin]
[indole] / M
0.1
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0
0
100
200
300
400
500
600
700
Time / s
0.0015 M
0.0023 M
0.003 M


ln(kobs) v ln[flavin]
-6.8
-6.6
-6.4
-6.2
-6
-5.8
-5.6
-7.8
-8.3
-8.8
-9.3
-9.8
-10.3
ln (kobs)
ln [flavin]
First order
zero order

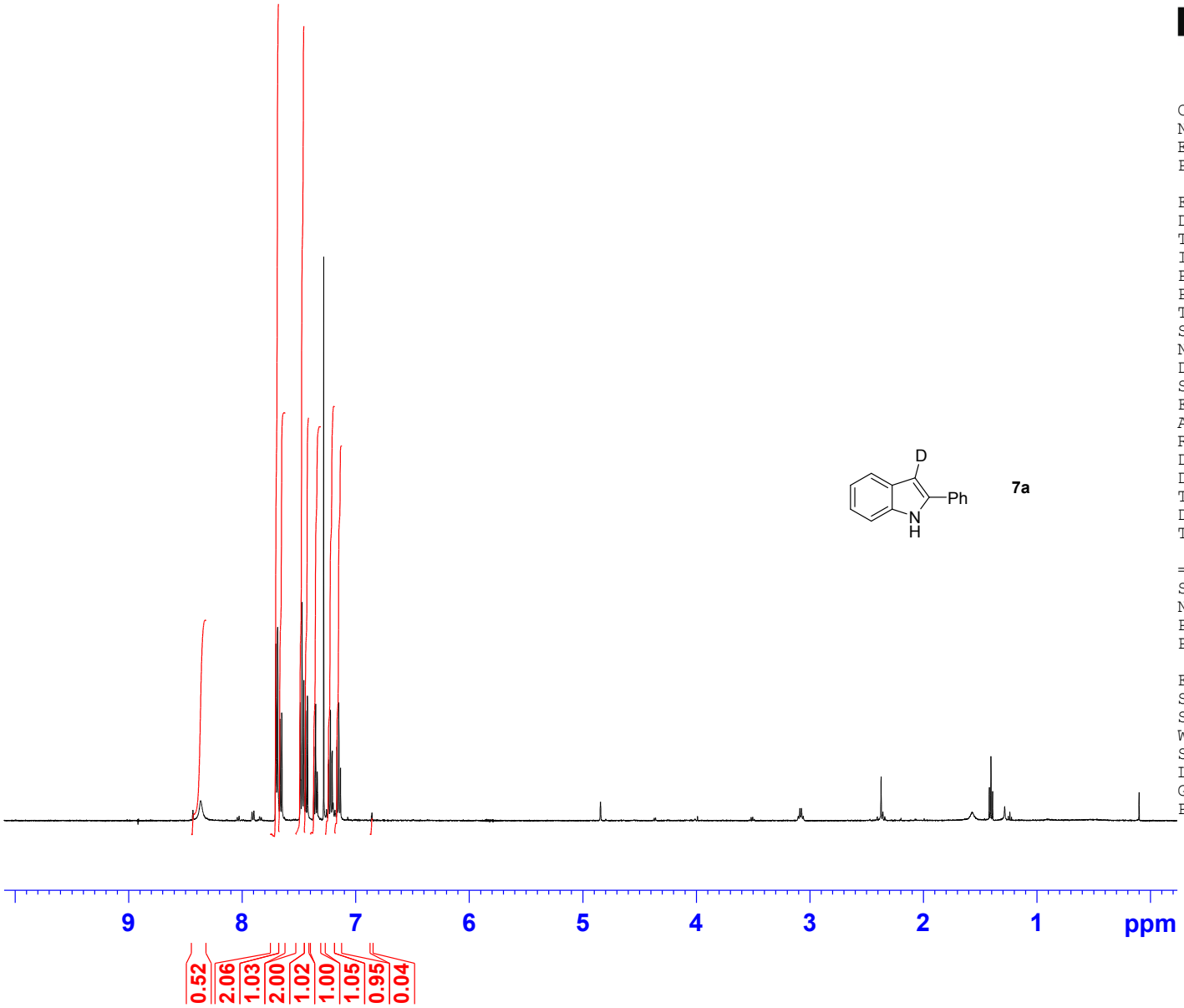

ln [indole] v time, varying [indole] - AcCl conditions
0
-1
-2
-3
-4
-5
-6
0
50
100
150
200
250
ln [indole]
time / s
0.1 M
0.05 M
0.2 M
Linear (0.2 M)


ln(kobs) v ln[indole]
-3.2
-3
-2.8
-2.6
-2.4
-2.2
-2
-1.8
-1.6
-1.4
-3.7
-3.8
-3.9
-4
-4.1
-4.2
-4.3
-4.4
-4.5
ln (kobs)
ln [indole]


ln [indole] v time, varying [AcCl] - AcCl conditions
-2
-2.1
-2.2
-2.3
-2.4
-2.5
-2.6
-2.7
-2.8
-2.9
-3
0
20
40
60
80
100
120
140
160
180
ln [indole]
time / s
7.5 mM
15 mM
30 mM

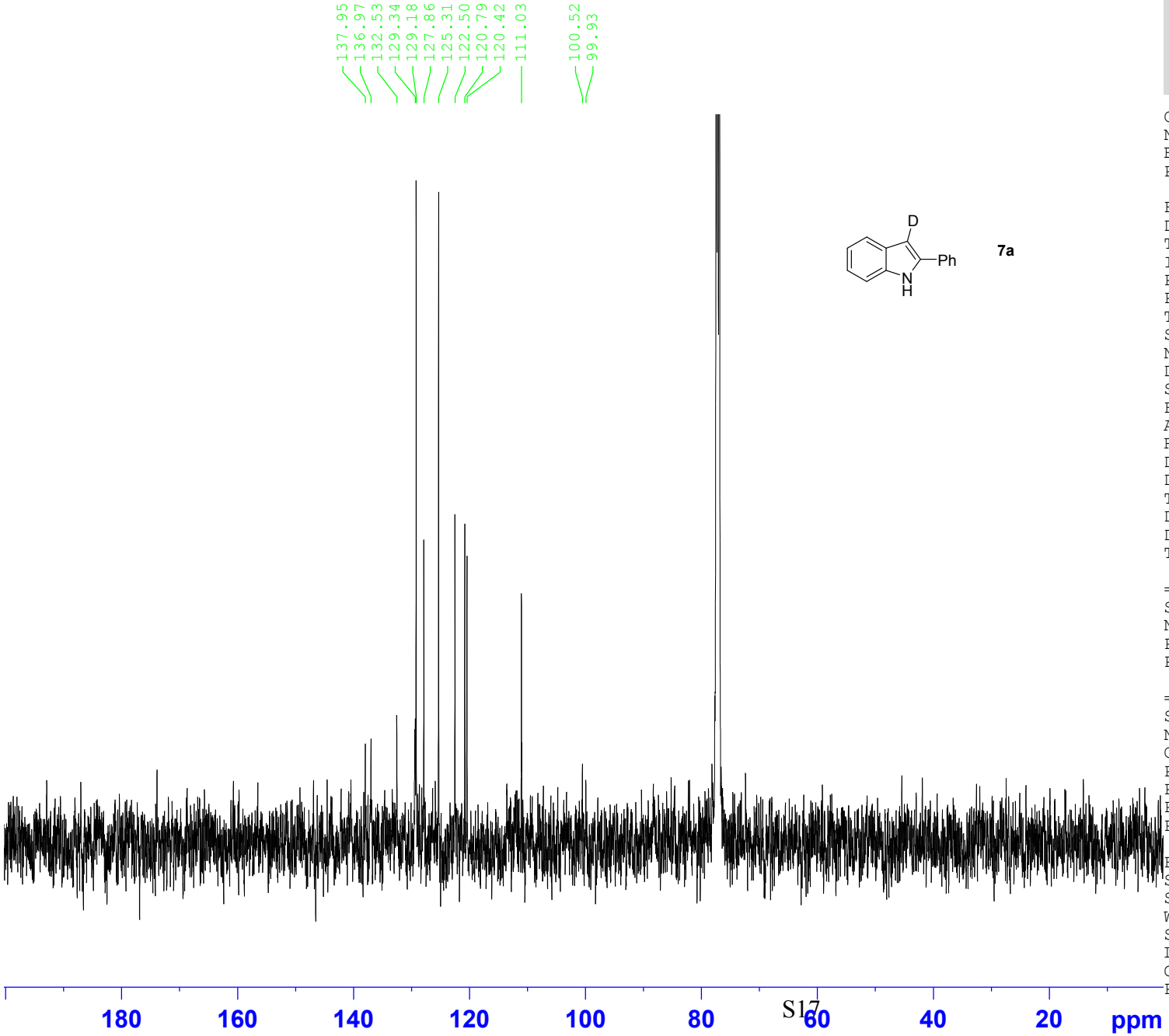

ln(kobs) v ln[AcCl]
-7.4
-7.2
-7
-6.8
-6.6
-6.4
-6.2
-6
-5.8
-5.6
-5.4
-3.2
-3.25
-3.3
-3.35
-3.4
-3.45
-3.5
-3.55
-3.6
-3.65
-3.7
-3.75
ln (kobs)
ln [indole]

METHOD A



7a

```
Current Data Parameters
NAME            AM1415 r
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150226
Time              16.53
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG            zg30
TD                65536
SOLVENT           CDCl3
NS                    8
DS                    0
SWH           10330.578 Hz
FIDRES         0.157632 Hz
AQ            3.1719425 sec
RG                  456
DW                48.400 usec
DE                 6.50 usec
TE                298.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
SFO1        500.1330885 MHz
NUC1                1H
P1                 9.50 usec
PLW1        27.19599915 W

F2 - Processing parameters
SI                32768
SF          500.1300000 MHz
WDW                  no
SSB       0
LB        0 Hz
GB        0
PC                1.00
```



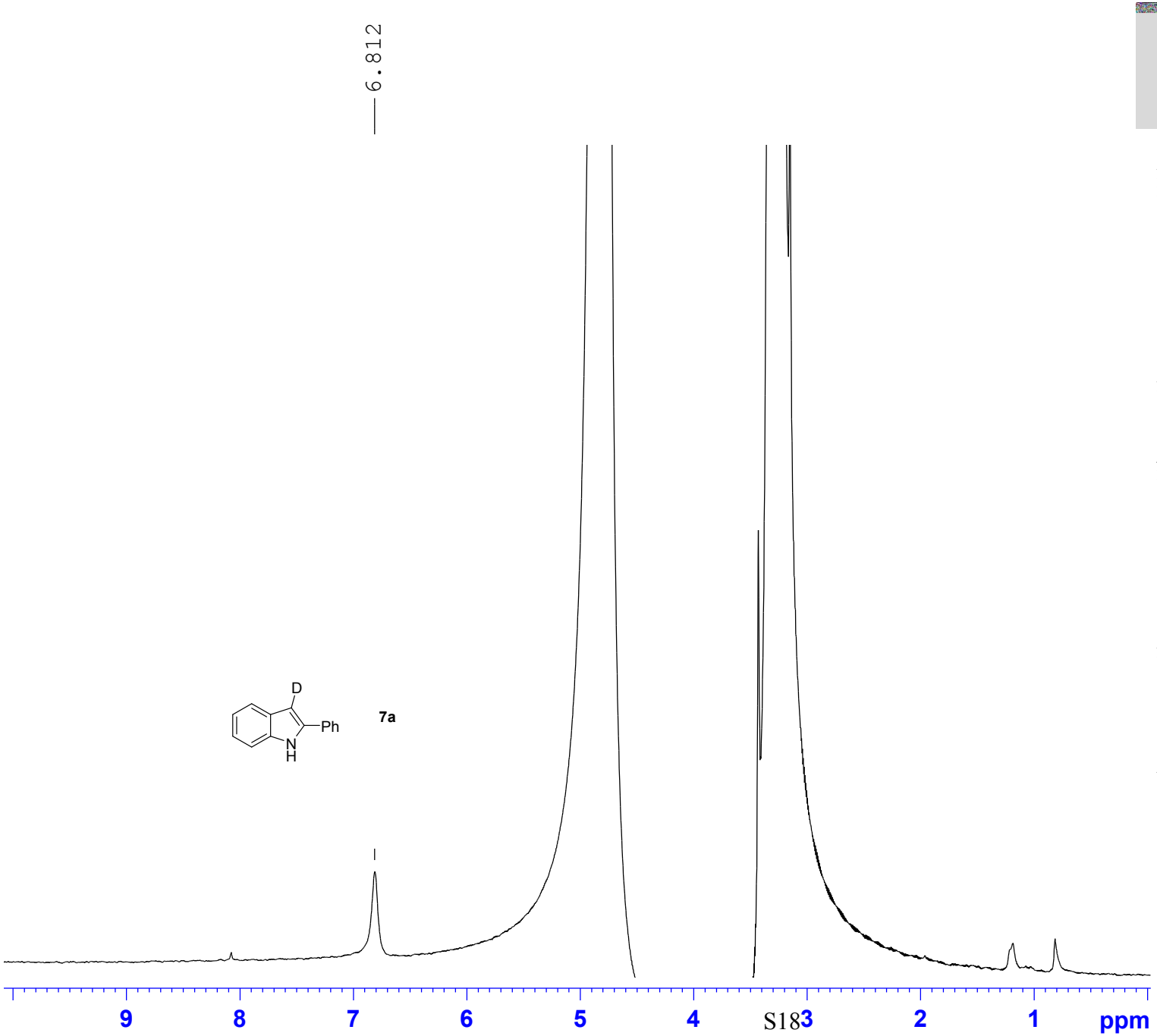
Current Data Parameters
NAME AM1415 r
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150226
Time 16.58
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgpg30
TD 65536
SOLVENT CDCl3
NS 166
DS 4
SWH 29761.904 Hz
FIDRES 0.454131 Hz
AQ 1.1010048 sec
RG 912
DW 16.800 usec
DE 6.50 usec
TE 298.0 K
D1 6.00000000 sec
D11 0.03000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 125.7703643 MHz
NUC1 13C
P1 7.50 usec
PLW1 63.66600037 W

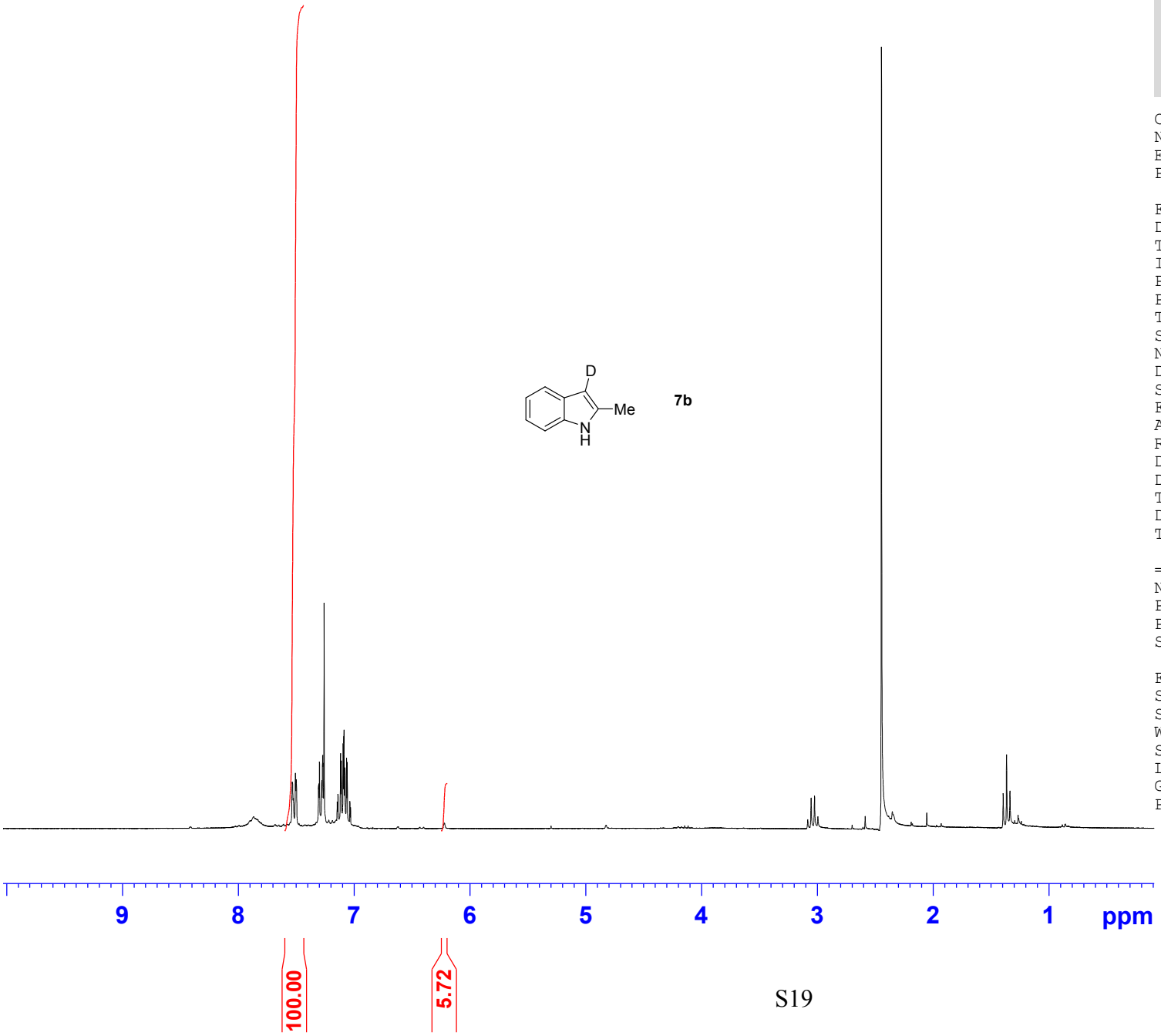
======== CHANNEL f2 ========
SFO2 500.1320005 MHz
NUC2 1H
CPDPRG[2 waltz16
PCPD2 80.00 usec
PLW2 27.19599915 W
PLW12 0.34419000 W
PLW13 0.22028001 W

F2 - Processing parameters
SI 32768
SF 125.7577707 MHz
WDW EM
SSB 0
LB 4.00 Hz
GB 0
PC 1.40



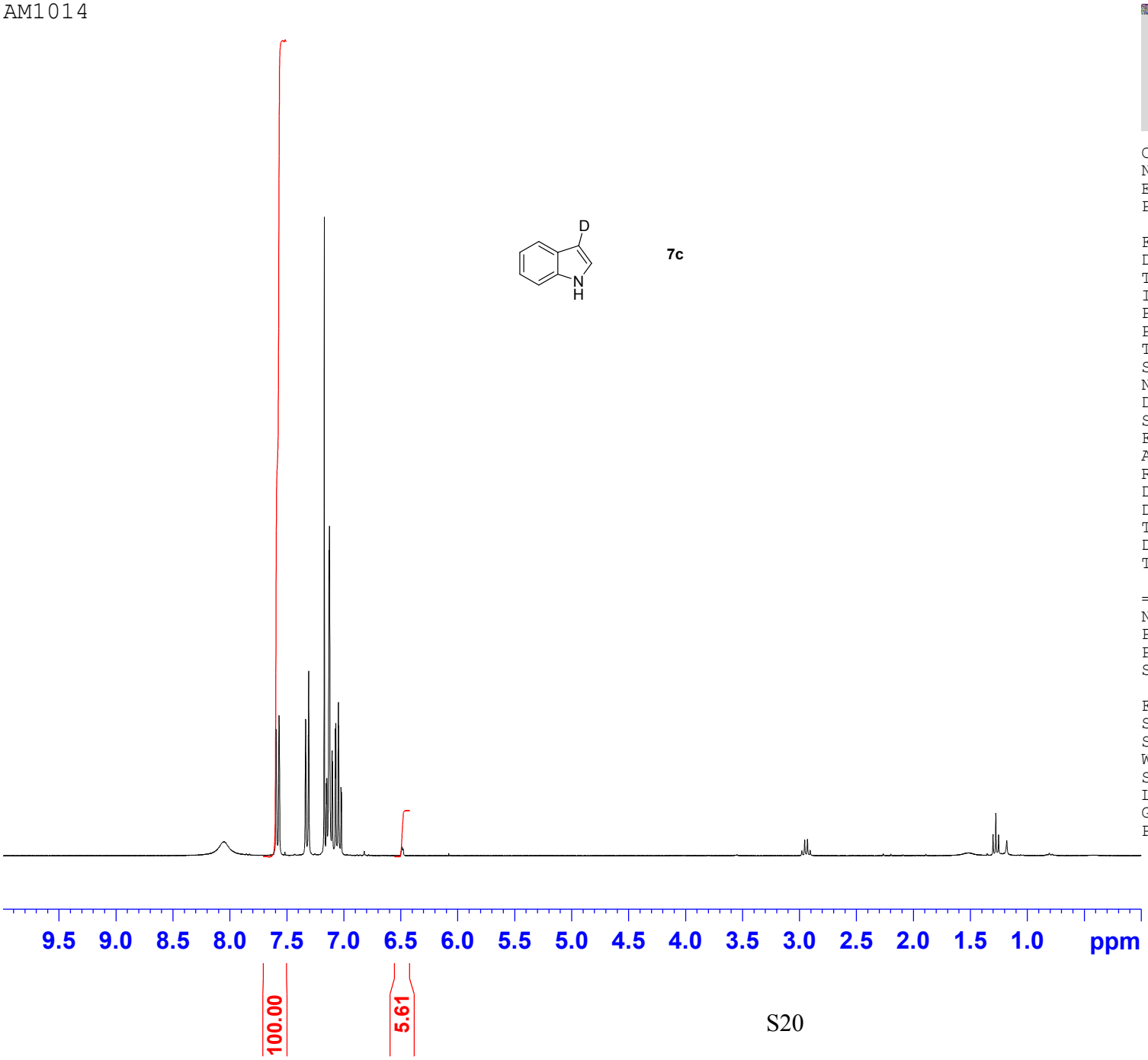

```
Current Data Parameters
NAME         AM843 rerun
EXPNO                  2
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130612
Time              13.44
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG           zg2h
TD                4096
SOLVENT           MeOD
NS                 528
DS                   0
SWH           1151.543 Hz
FIDRES        0.281138 Hz
AQ           1.7784832 sec
RG                3.56
DW             434.200 usec
DE               6.50 usec
TE              298.0 K
D1          0.05000000 sec
D11         0.03000000 sec
TD0                  1

======== CHANNEL f1 ========
SFO1         76.7732534 MHz
NUC1                2H
P1             230.00 usec
PLW1         2.79320002 W

F2 - Processing parameters
SI                4096
SF           76.7730000 MHz
WDW                EM
SSB       0
LB              1.00 Hz
GB        0
PC              1.00
```

AM1424v



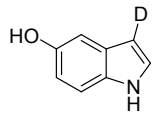

```
Current Data Parameters
NAME            02272015
EXPNO                 40
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150227
Time               18.01
INSTRUM            spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    32
DS                     2
SWH             5175.983 Hz
FIDRES          0.157958 Hz
AQ              3.1653888 sec
RG                 228.1
DW                96.600 usec
DE                 6.00 usec
TE                300.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300077 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

AM1014




```
Current Data Parameters
NAME          Nov28-2013
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131128
Time               18.30
INSTRUM            av300
PROBHD   5 mm BBO BB-1H
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    32
DS                     0
SWH             6172.839 Hz
FIDRES          0.188380 Hz
AQ              2.6542079 sec
RG                   512
DW                81.000 usec
DE                 6.00 usec
TE                294.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.40 usec
PL1               -1.50 dB
SFO1        300.2218540 MHz

F2 - Processing parameters
SI                 32768
SF          300.2200284 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

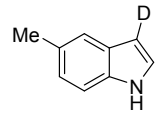
AM1017



7d


```
Current Data Parameters
NAME            11282013
EXPNO                 40
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131128
Time                16.47
INSTRUM             spect
PROBHD   5 mm DUL 1H-13
PULPROG              zg30
TD                  32768
SOLVENT             CDCl3
NS                     32
DS                      2
SWH              5175.983 Hz
FIDRES           0.157958 Hz
AQ               3.1653888 sec
RG                  228.1
DW                 96.600 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                  32768
SF           250.1300000 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

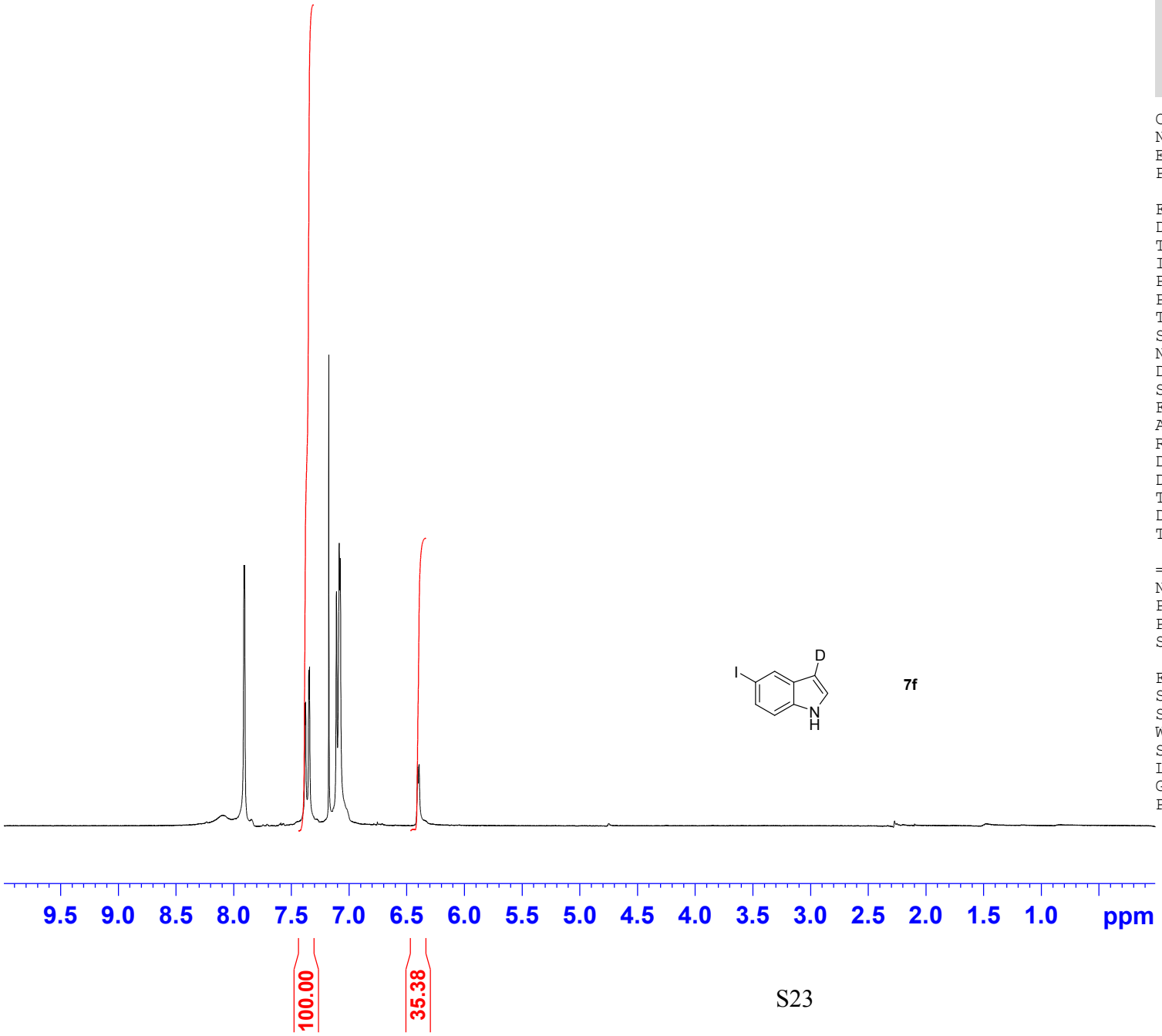
AM1017



7e


```
Current Data Parameters
NAME            11282013
EXPNO                 40
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131128
Time                16.47
INSTRUM             spect
PROBHD   5 mm DUL 1H-13
PULPROG              zg30
TD                  32768
SOLVENT             CDCl3
NS                     32
DS                      2
SWH              5175.983 Hz
FIDRES           0.157958 Hz
AQ               3.1653888 sec
RG                  228.1
DW                 96.600 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                  32768
SF           250.1300000 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

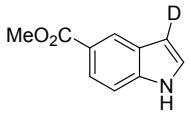
AM1437



7f

```
Current Data Parameters
NAME            03022015
EXPNO                 40
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150302
Time                15.47
INSTRUM             spect
PROBHD   5 mm DUL 1H-13
PULPROG              zg30
TD                  32768
SOLVENT             CDCl3
NS                     32
DS                      2
SWH              5175.983 Hz
FIDRES           0.157958 Hz
AQ               3.1653888 sec
RG                  228.1
DW                 96.600 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                  32768
SF           250.1300286 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

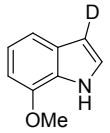
jc20B



7g

```
Current Data Parameters
NAME          Jul15-2013
EXPNO                 20
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130715
Time              11.19
INSTRUM           spect
PROBHD   5 mm DUL 1H-13
PULPROG            zg30
TD                32768
SOLVENT           CDCl3
NS                   32
DS                    2
SWH            5175.983 Hz
FIDRES        0.157958 Hz
AQ            3.1653888 sec
RG                  256
DW               96.600 usec
DE                6.00 usec
TE               300.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1               11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                32768
SF          250.1300082 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

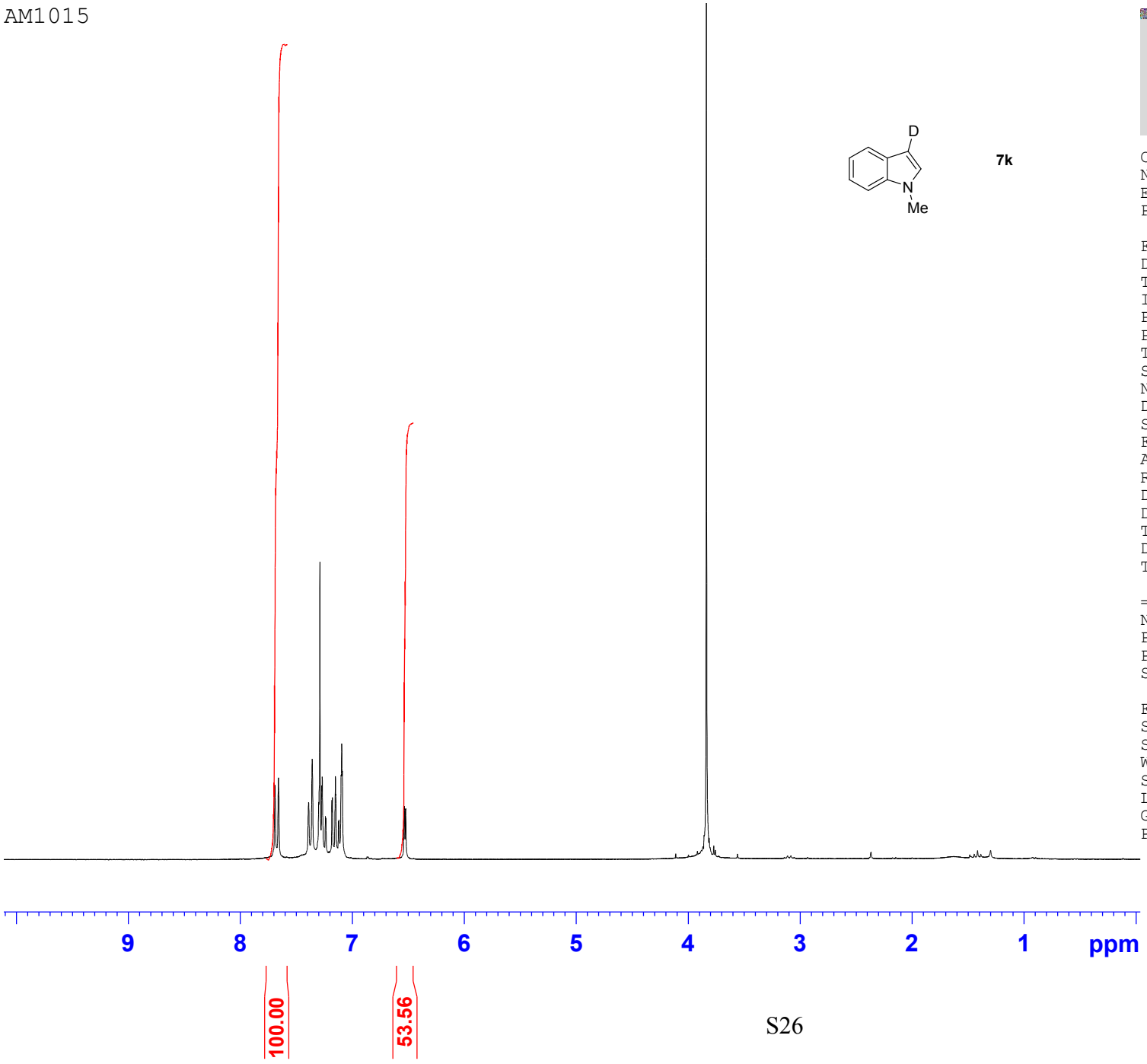
AM1436



7j

```
Current Data Parameters
NAME            03022015
EXPNO                 30
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150302
Time               15.38
INSTRUM            spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    32
DS                     2
SWH             5175.983 Hz
FIDRES          0.157958 Hz
AQ              3.1653888 sec
RG                 203.2
DW                96.600 usec
DE                 6.00 usec
TE                300.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300307 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

AM1015

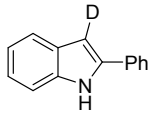


7k

```
Current Data Parameters
NAME            11282013
EXPNO                 20
PROCNO                 1

F2 - Acquisition Parameters
Date_           20131128
Time                13.04
INSTRUM             spect
PROBHD   5 mm DUL 1H-13
PULPROG              zg30
TD                  32768
SOLVENT             CDCl3
NS                     32
DS                      2
SWH              5175.983 Hz
FIDRES           0.157958 Hz
AQ               3.1653888 sec
RG                  228.1
DW                 96.600 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                  32768
SF           250.1300000 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

METHOD B

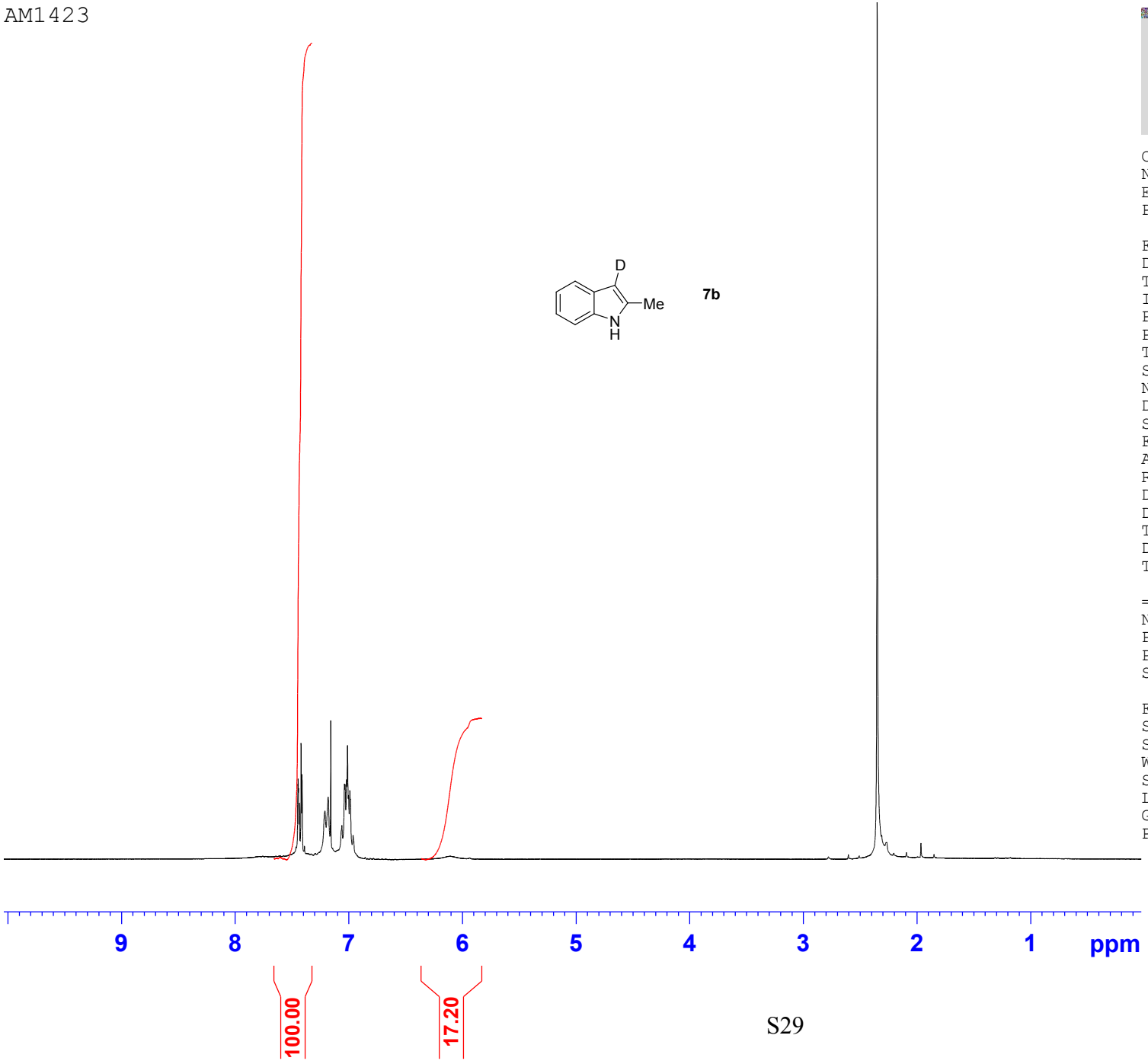
AM1432



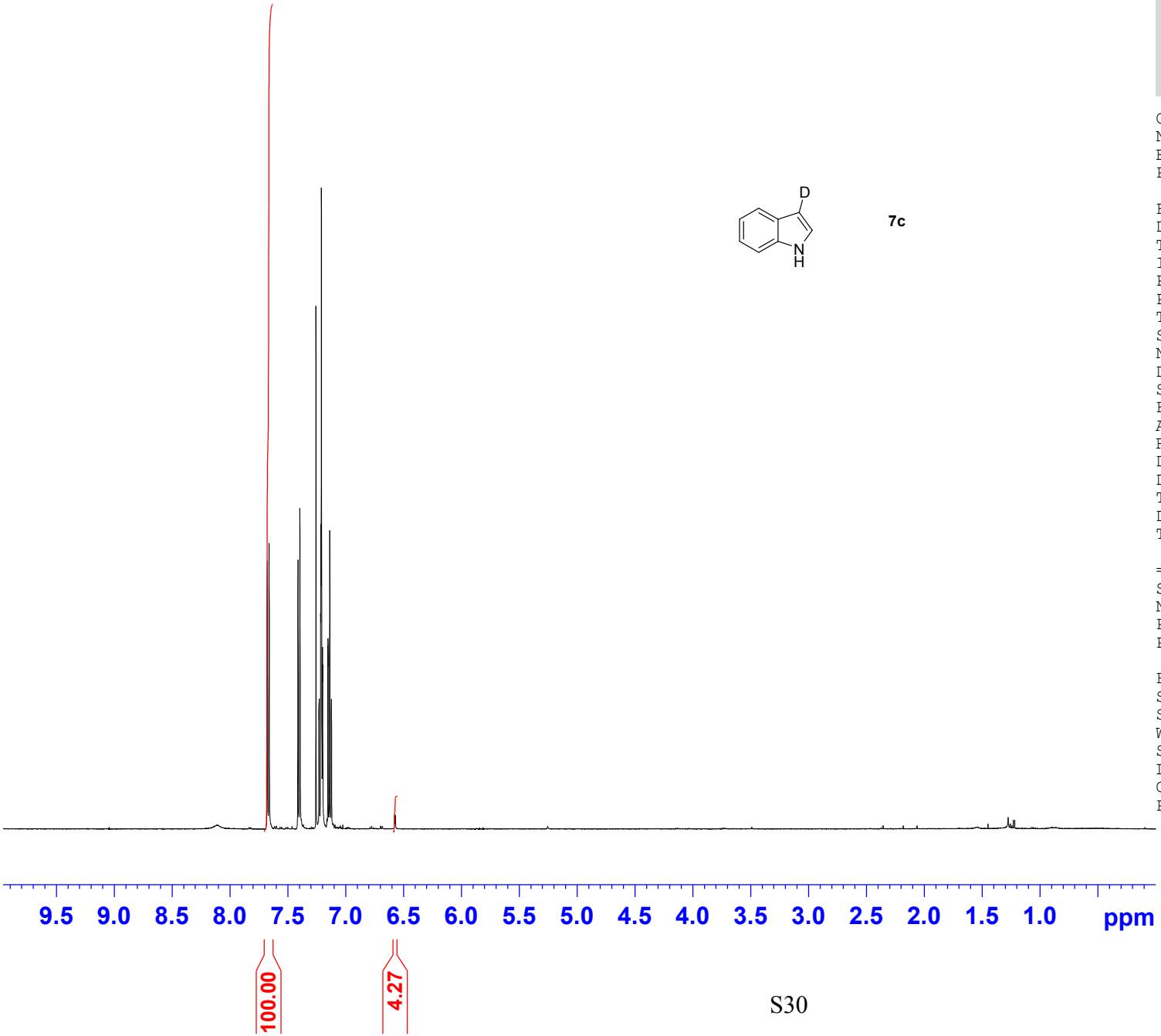
7a


```
Current Data Parameters
NAME            02282015
EXPNO                 30
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150228
Time               18.49
INSTRUM            spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    32
DS                     2
SWH             5175.983 Hz
FIDRES          0.157958 Hz
AQ              3.1653888 sec
RG                 203.2
DW                96.600 usec
DE                 6.00 usec
TE                 300.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300331 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

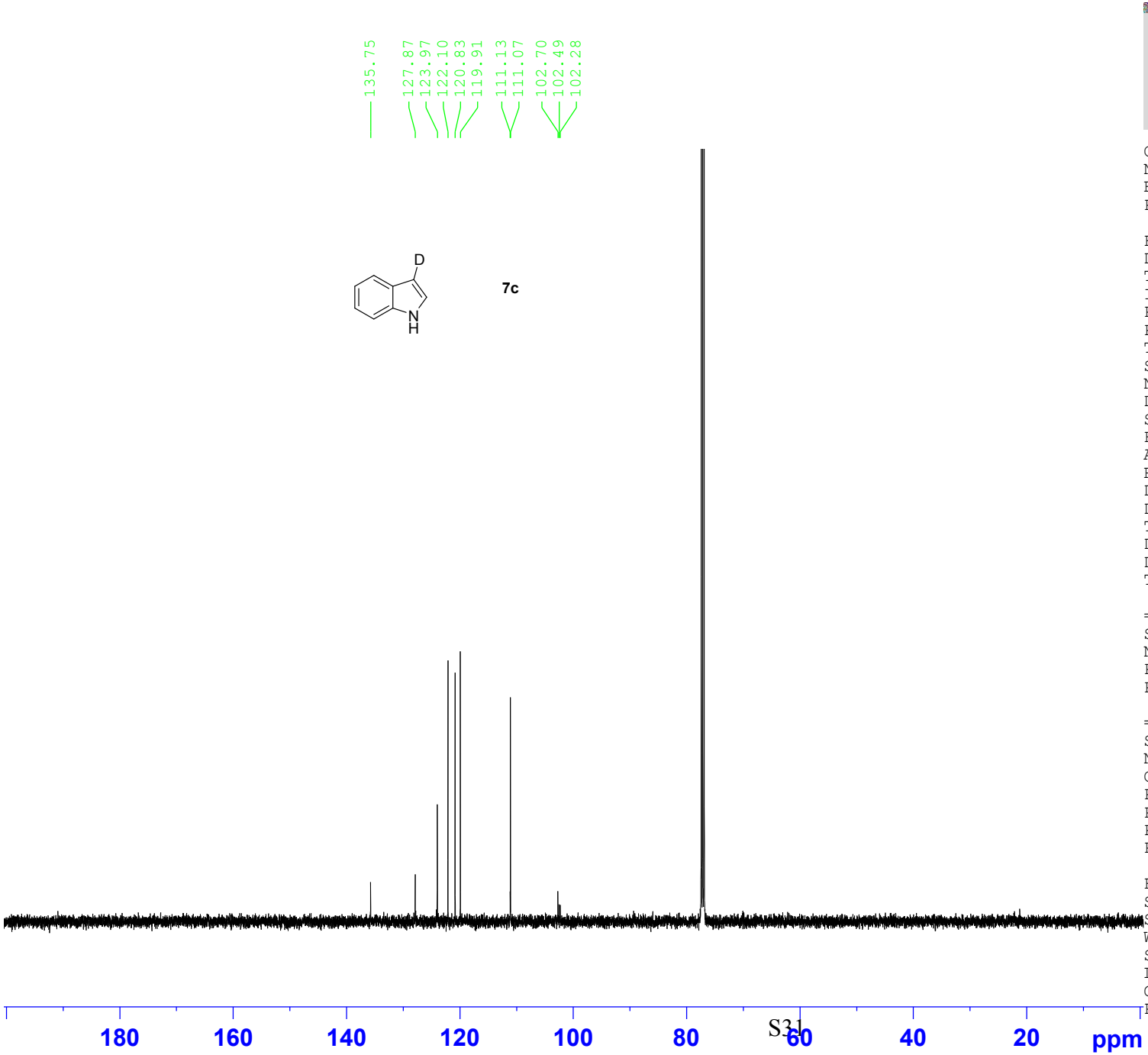
AM1423



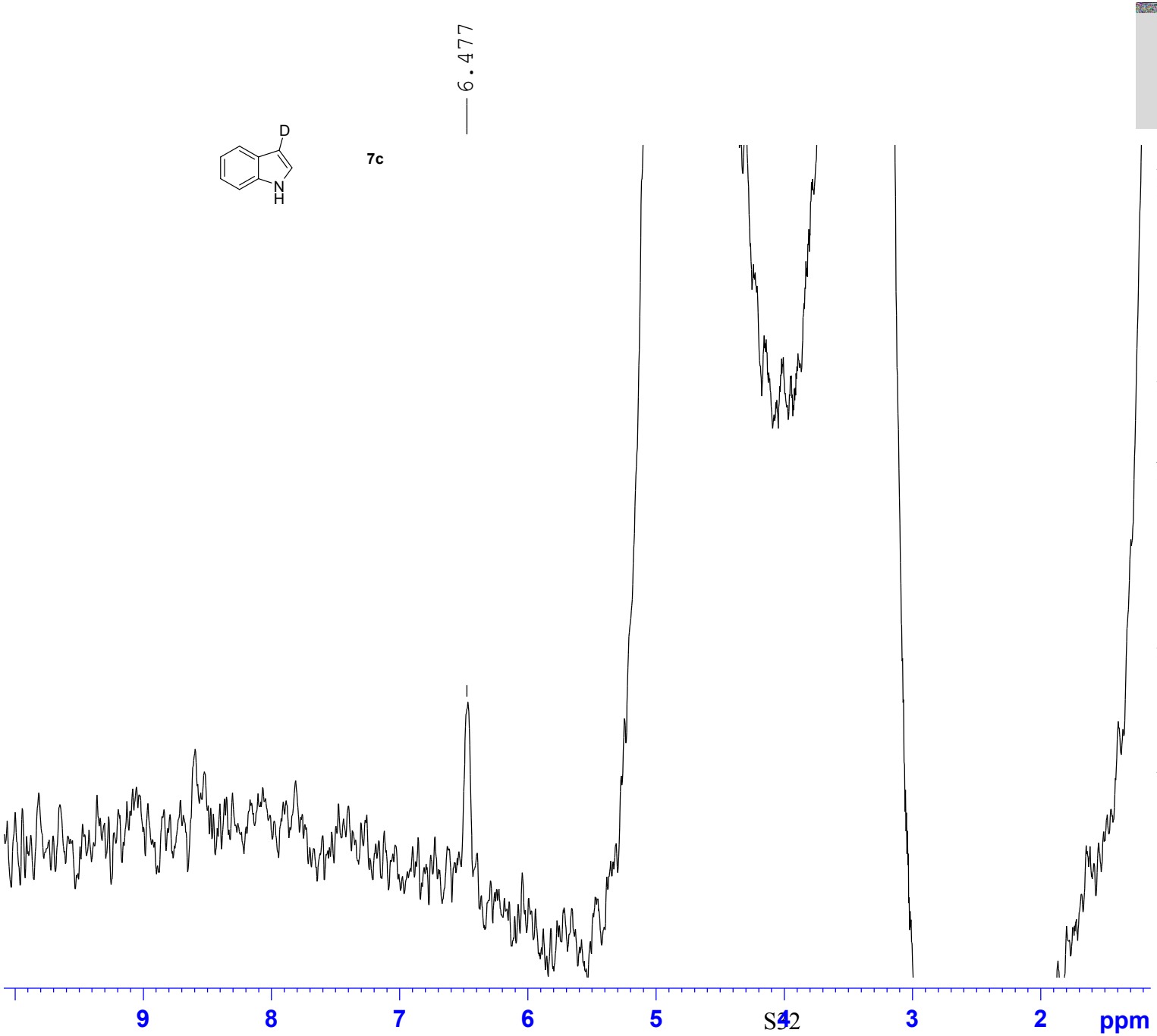

```
Current Data Parameters
NAME            02272015
EXPNO                 20
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150227
Time              13.11
INSTRUM           spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                32768
SOLVENT           CDCl3
NS                   32
DS                    2
SWH            5175.983 Hz
FIDRES        0.157958 Hz
AQ            3.1653888 sec
RG                203.2
DW               96.600 usec
DE                6.00 usec
TE               300.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                32768
SF           250.1300327 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```




```
Current Data Parameters
NAME                  AM1449
EXPNO                      1
PROCNO                     1

F2 - Acquisition Parameters
Date_              20150306
Time                  11.10
INSTRUM               spect
PROBHD    5 mm PABBO BB/
PULPROG                zg30
TD                    65536
SOLVENT               CDCl3
NS                        8
DS                        0
SWH              10330.578 Hz
FIDRES            0.157632 Hz
AQ                3.1719425 sec
RG                      406
DW                   48.400 usec
DE                    6.50 usec
TE                   298.0 K
D1              1.00000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1           500.1330885 MHz
NUC1                    1H
P1                    9.50 usec
PLW1           27.19599915 W

F2 - Processing parameters
SI                    32768
SF             500.1300127 MHz
WDW                     no
SSB       0
LB        0 Hz
GB        0
PC                    1.00
```

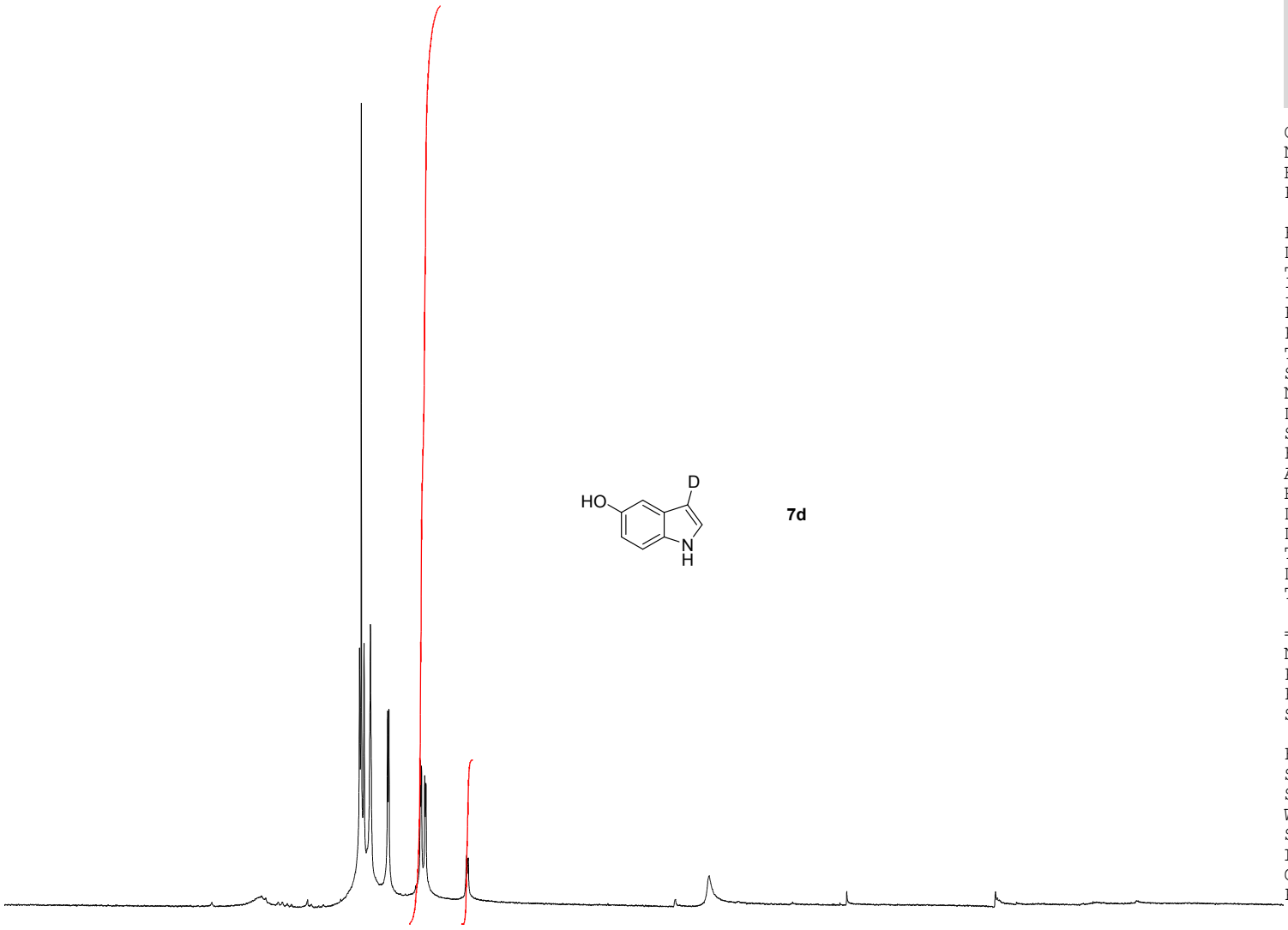



```
Current Data Parameters
NAME                 AM1449
EXPNO                     2
PROCNO                    1

F2 - Acquisition Parameters
Date_              20150306
Time                  11.35
INSTRUM               spect
PROBHD   5 mm PABBO BB/
PULPROG              zgpg30
TD                    65536
SOLVENT               CDCl3
NS                      204
DS                        4
SWH              29761.904 Hz
FIDRES            0.454131 Hz
AQ               1.1010048 sec
RG                     1820
DW                  16.800 usec
DE                   6.50 usec
TE                   298.0 K
D1               5.00000000 sec
D11              0.03000000 sec
TD0                       1

======== CHANNEL f1 ========
SFO1           125.7703643 MHz
NUC1                    13C
P1                    7.50 usec
PLW1           63.66600037 W

======== CHANNEL f2 ========
SFO2           500.1320005 MHz
NUC2                    1H
CPDPRG[2           waltz16
PCPD2                80.00 usec
PLW2           27.19599915 W
PLW12           0.34419000 W
PLW13           0.22028001 W

F2 - Processing parameters
SI                    32768
SF           125.7577752 MHz
WDW                      EM
SSB       0
LB                    1.00 Hz
GB        0
PC                    1.40
```



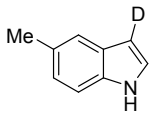

```
Current Data Parameters
NAME            AM1447 r
EXPNO                  1
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150306
Time              10.50
INSTRUM           spect
PROBHD   5 mm PABBO BB/
PULPROG            zg2h
TD                 4096
SOLVENT            MeOD
NS                   16
DS                    0
SWH            1151.543 Hz
FIDRES        0.281138 Hz
AQ            1.7784832 sec
RG                 6.35
DW              434.200 usec
DE                6.50 usec
TE               298.0 K
D1           0.05000000 sec
D11          0.03000000 sec
TD0                    1

======== CHANNEL f1 ========
SFO1         76.7733839 MHz
NUC1                  2H
P1              230.00 usec
PLW1         2.79320002 W

F2 - Processing parameters
SI                  4096
SF           76.7727213 MHz
WDW                   EM
SSB       0
LB                 1.00 Hz
GB        0
PC                 1.00
```

AM1434




```
Current Data Parameters
NAME            03022015
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150302
Time                12.10
INSTRUM             spect
PROBHD   5 mm DUL 1H-13
PULPROG              zg30
TD                  32768
SOLVENT             CDC13
NS                     32
DS                      2
SWH              5175.983 Hz
FIDRES           0.157958 Hz
AQ               3.1653888 sec
RG                    256
DW                 96.600 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                  32768
SF           250.1300260 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```

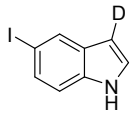
jc23A



7e

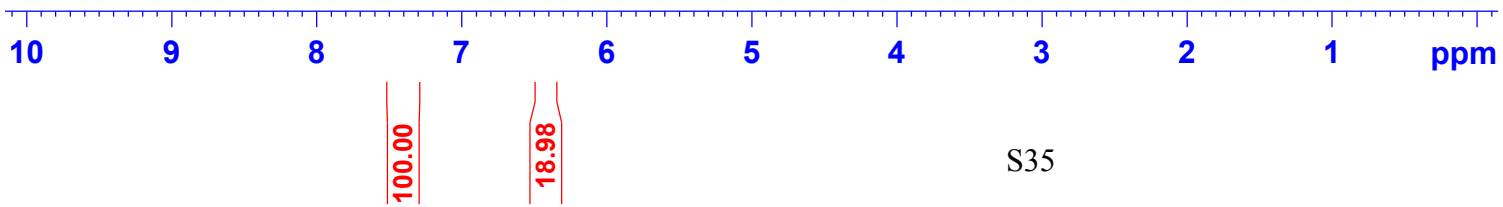

```
Current Data Parameters
NAME          Jul16-2013
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130716
Time               11.59
INSTRUM            spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                 32768
SOLVENT            CDC13
NS                    32
DS                     2
SWH             5175.983 Hz
FIDRES          0.157958 Hz
AQ              3.1653888 sec
RG                   256
DW                96.600 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300000 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

AM1444

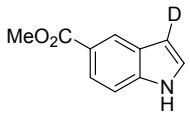


7f

```
Current Data Parameters
NAME            03052015
EXPNO                 20
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150305
Time              17.54
INSTRUM           spect
PROBHD   5 mm DUL 1H-13
PULPROG            zg30
TD                32768
SOLVENT           CDCl3
NS                   32
DS                    2
SWH            5175.983 Hz
FIDRES        0.157958 Hz
AQ            3.1653888 sec
RG                203.2
DW               96.600 usec
DE                6.00 usec
TE                300.0 K
D1           1.00000000 sec
TD0                   1

======== CHANNEL f1 ========
NUC1                  1H
P1                11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                32768
SF           250.1300294 MHz
WDW                  EM
SSB       0
LB                0.30 Hz
GB        0
PC                1.00
```

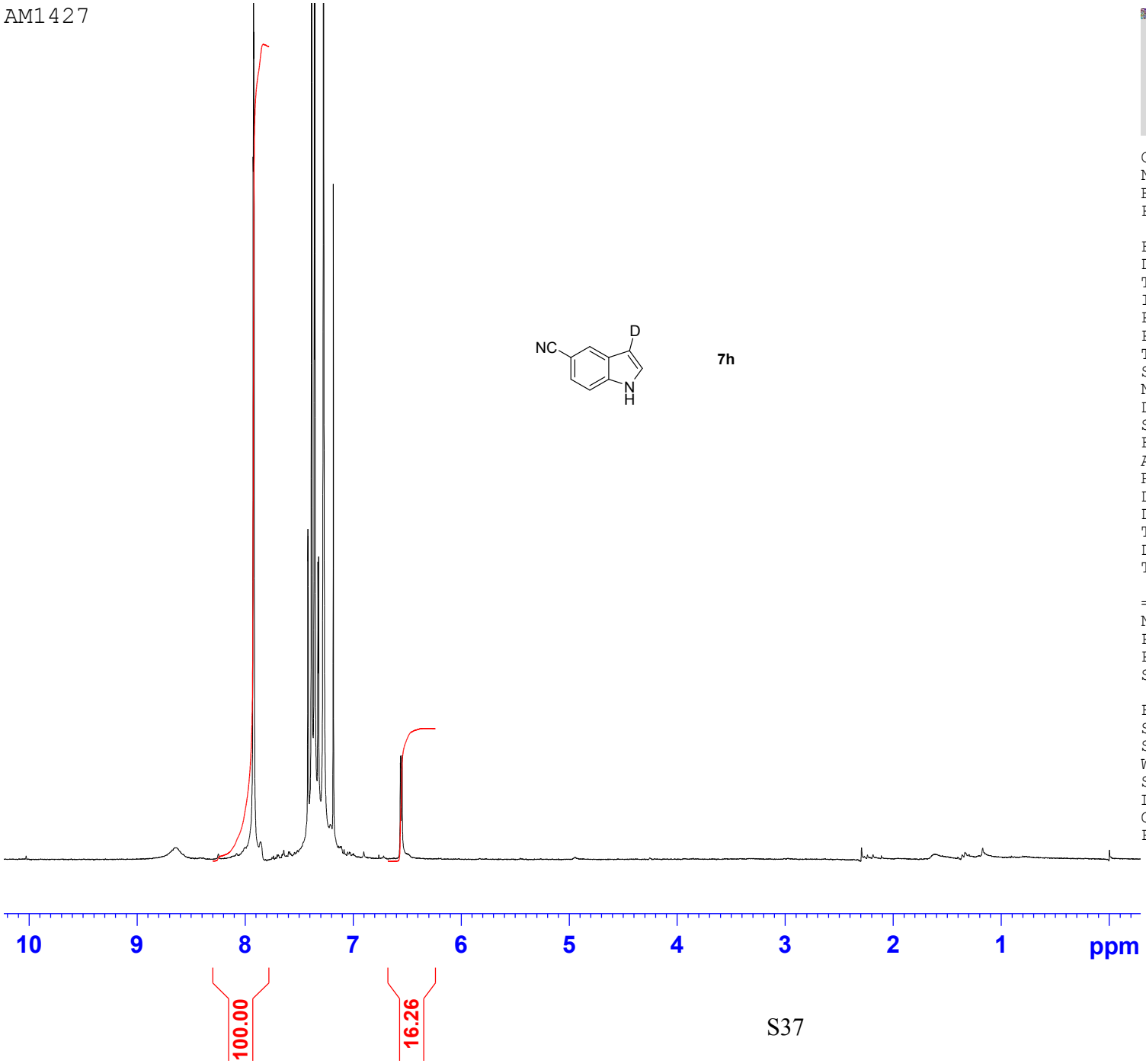


AM1428



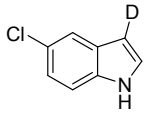
7g


```
Current Data Parameters
NAME            02282015
EXPNO                 20
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150228
Time                16.27
INSTRUM             spect
PROBHD   5 mm DUL 1H-13
PULPROG              zg30
TD                  32768
SOLVENT             CDC13
NS                     32
DS                      2
SWH              5175.983 Hz
FIDRES           0.157958 Hz
AQ               3.1653888 sec
RG                  228.1
DW                 96.600 usec
DE                  6.00 usec
TE                 300.0 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                   1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                  32768
SF           250.1300266 MHz
WDW                    EM
SSB       0
LB                  0.30 Hz
GB        0
PC                  1.00
```




```
Current Data Parameters
NAME            02282015
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150228
Time               16.19
INSTRUM            spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                 32768
SOLVENT            CDC13
NS                    32
DS                     2
SWH             5175.983 Hz
FIDRES          0.157958 Hz
AQ              3.1653888 sec
RG                 228.1
DW                96.600 usec
DE                 6.00 usec
TE                 300.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300265 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

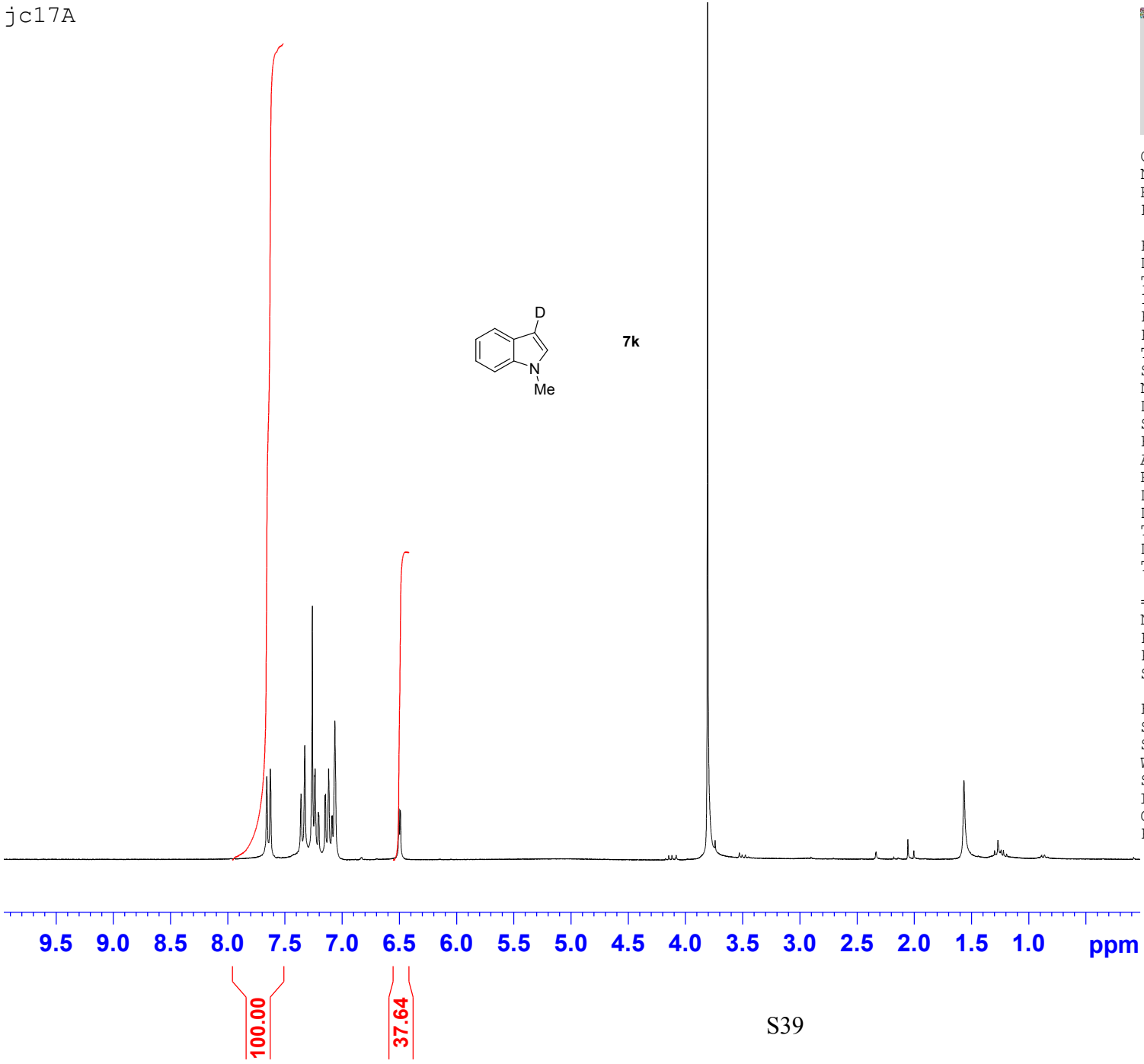
AM1418



7i

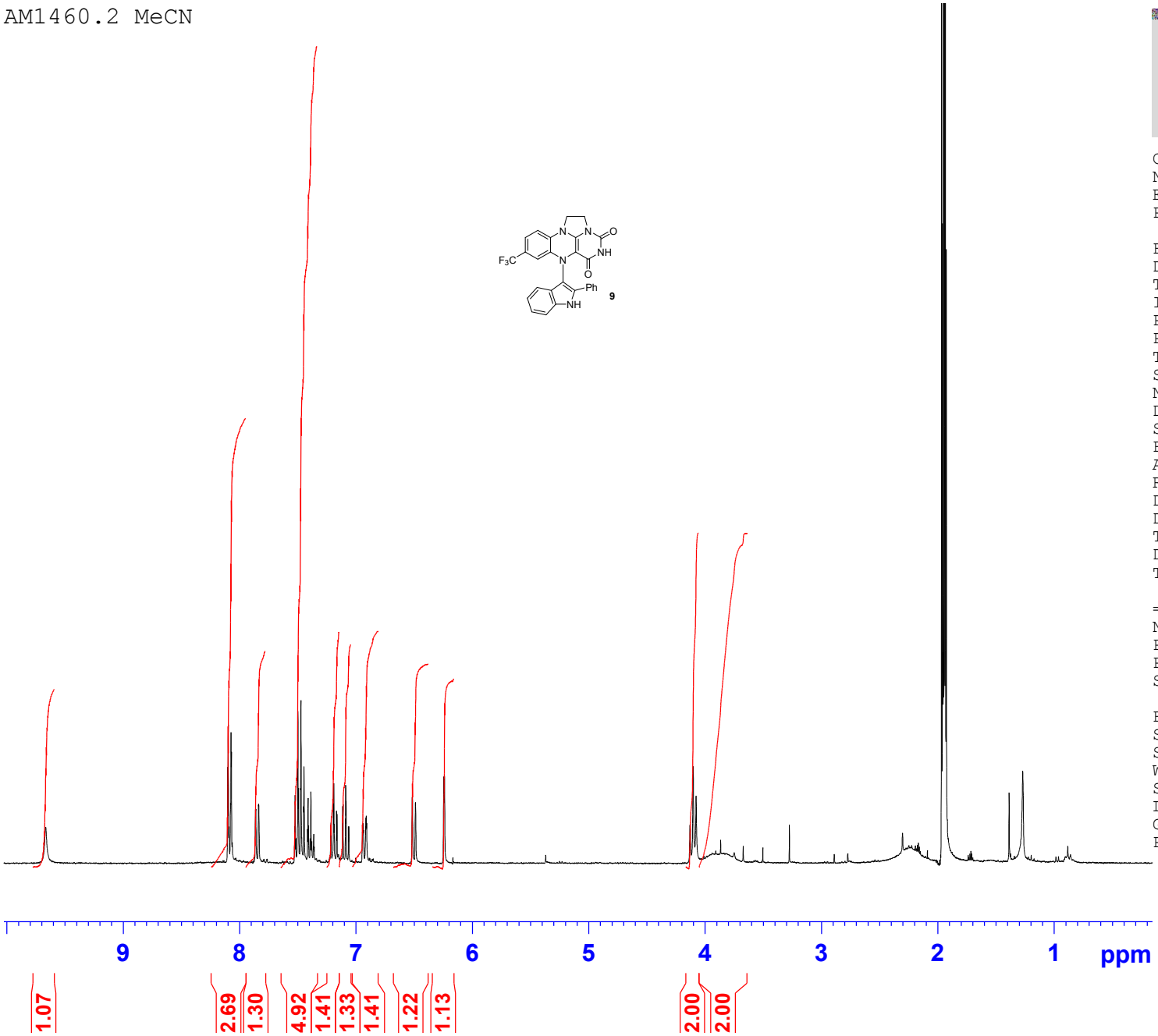

```
Current Data Parameters
NAME            02262015
EXPNO                 40
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150226
Time               17.58
INSTRUM            spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    32
DS                     2
SWH             5175.983 Hz
FIDRES          0.157958 Hz
AQ              3.1653888 sec
RG                 228.1
DW                96.600 usec
DE                 6.00 usec
TE                 300.0 K
D1             1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300276 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

jc17A

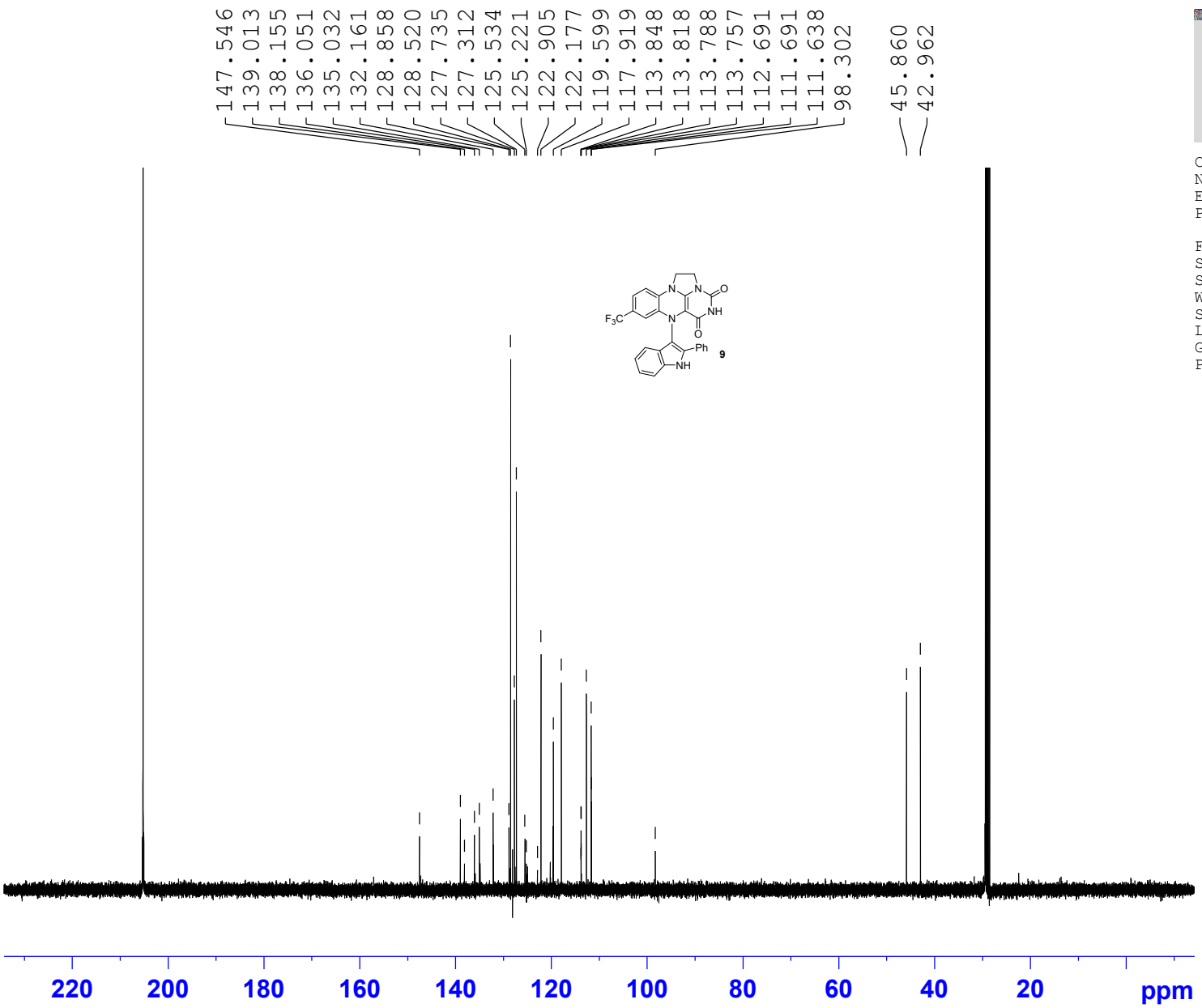



```
Current Data Parameters
NAME          Jul10-2013
EXPNO                 30
PROCNO                 1

F2 - Acquisition Parameters
Date_           20130710
Time               15.58
INSTRUM            spect
PROBHD   5 mm DUL 1H-13
PULPROG             zg30
TD                 32768
SOLVENT            CDCl3
NS                    32
DS                     2
SWH             5175.983 Hz
FIDRES          0.157958 Hz
AQ              3.1653888 sec
RG                 228.1
DW                96.600 usec
DE                 6.00 usec
TE                300.0 K
D1           1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 11.00 usec
PL1       0 dB
SFO1         250.1315450 MHz

F2 - Processing parameters
SI                 32768
SF           250.1300078 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```

AM1460.2 MeCN



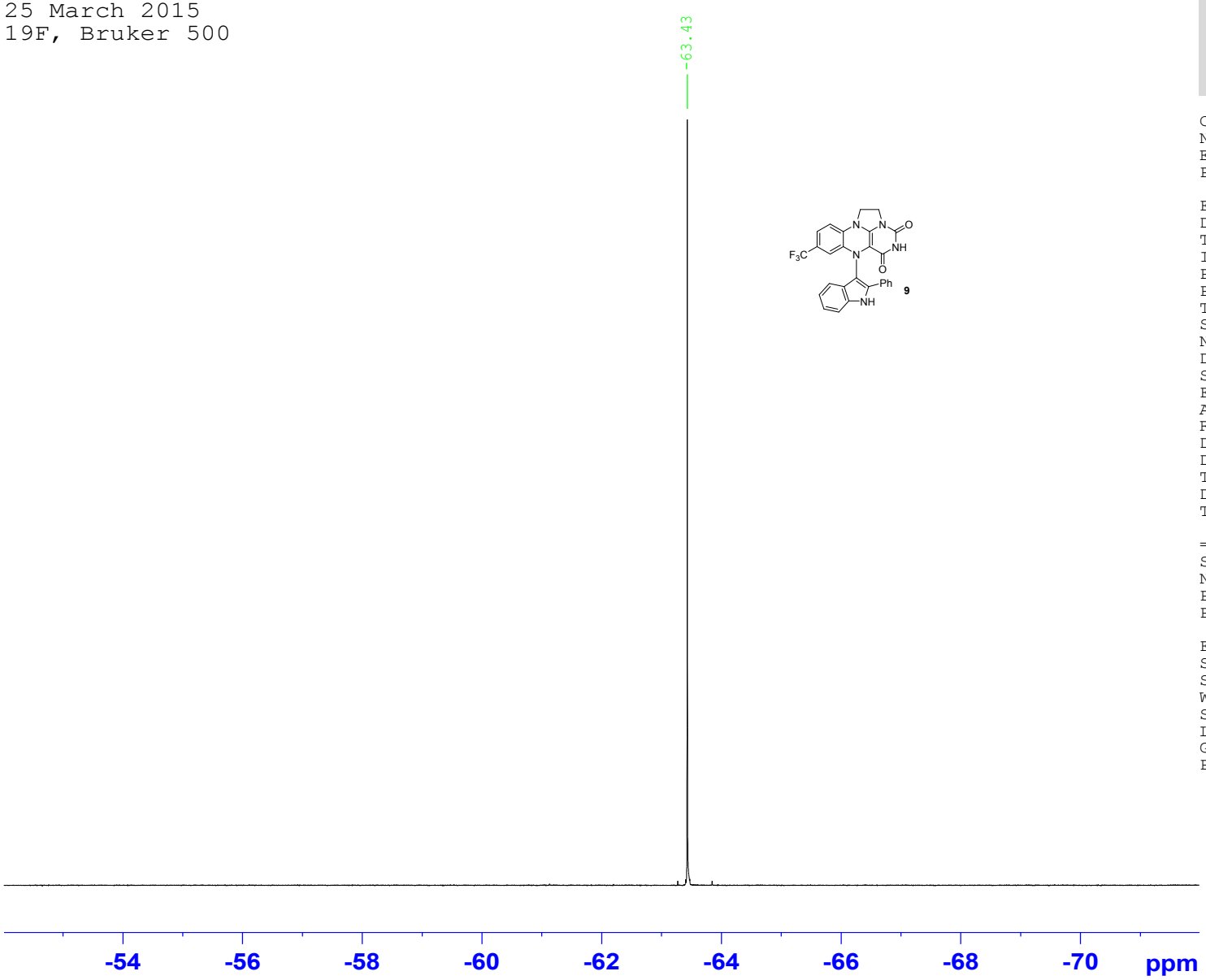

```
Current Data Parameters
NAME          Mar25-2015
EXPNO                 10
PROCNO                 1

F2 - Acquisition Parameters
Date_           20150325
Time               17.55
INSTRUM            av300
PROBHD   5 mm BBO BB-1H
PULPROG             zg30
TD                 32768
SOLVENT            CD3CN
NS                    32
DS                     0
SWH             6172.839 Hz
FIDRES          0.188380 Hz
AQ              2.6542079 sec
RG                 574.7
DW                81.000 usec
DE                 6.00 usec
TE                 673.2 K
D1            1.00000000 sec
TD0                    1

======== CHANNEL f1 ========
NUC1                  1H
P1                 9.40 usec
PL1               -1.50 dB
SFO1         300.2218540 MHz

F2 - Processing parameters
SI                 32768
SF           300.2200023 MHz
WDW                   EM
SSB       0
LB                 0.30 Hz
GB        0
PC                 1.00
```



Current Data Parameters
NAME AM1460.2 CARBON_01.fid
EXPNO 1
PROCNO 1

F2 - Processing parameters
SI 65536
SF 125.7422525 MHz
WDW no
SSB 0
LB 0 Hz
GB 0
PC 1.00

AM1410-2
25 March 2015
19F, Bruker 500



9

Current Data Parameters
NAME AM1410_2
EXPNO 2
PROCNO 1

F2 - Acquisition Parameters
Date_ 20150325
Time 15.52
INSTRUM spect
PROBHD 5 mm PABBO BB/
PULPROG zgflqn
TD 32768
SOLVENT Acetone
NS 16
DS 0
SWH 9398.496 Hz
FIDRES 0.286819 Hz
AQ 1.7432576 sec
RG 114
DW 53.200 usec
DE 6.50 usec
TE 298.0 K
D1 1.00000000 sec
TD0 1

======== CHANNEL f1 ========
SFO1 470.5632003 MHz
NUC1 19F
P1 13.97 usec
PLW1 21.85300064 W

F2 - Processing parameters
SI 32768
SF 470.5923770 MHz
WDW EM
SSB 0
LB 0.30 Hz
GB 0
PC 1.00