

Synthesis and characterization of pyridazine-based iron chelators

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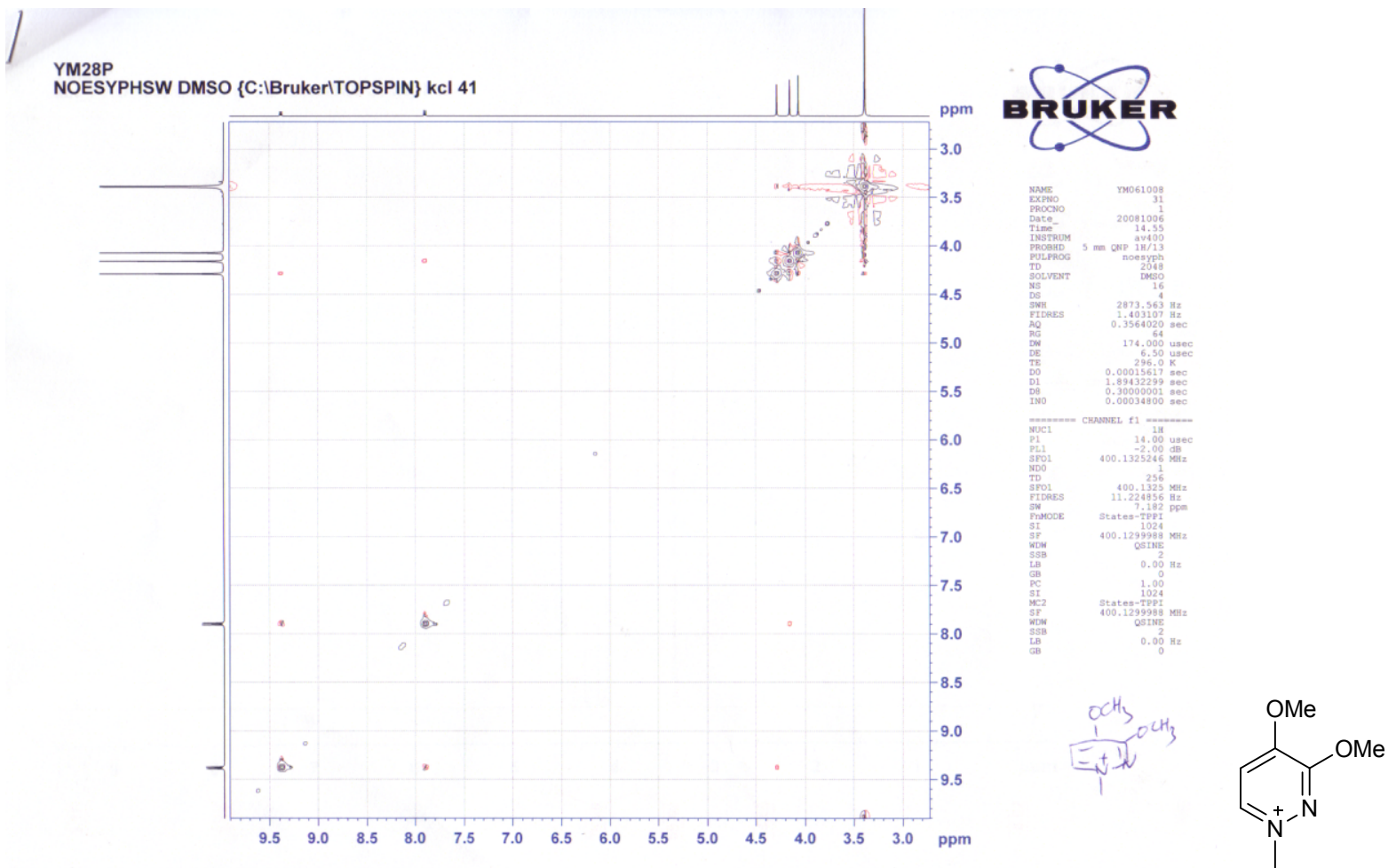
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Supporting information content:

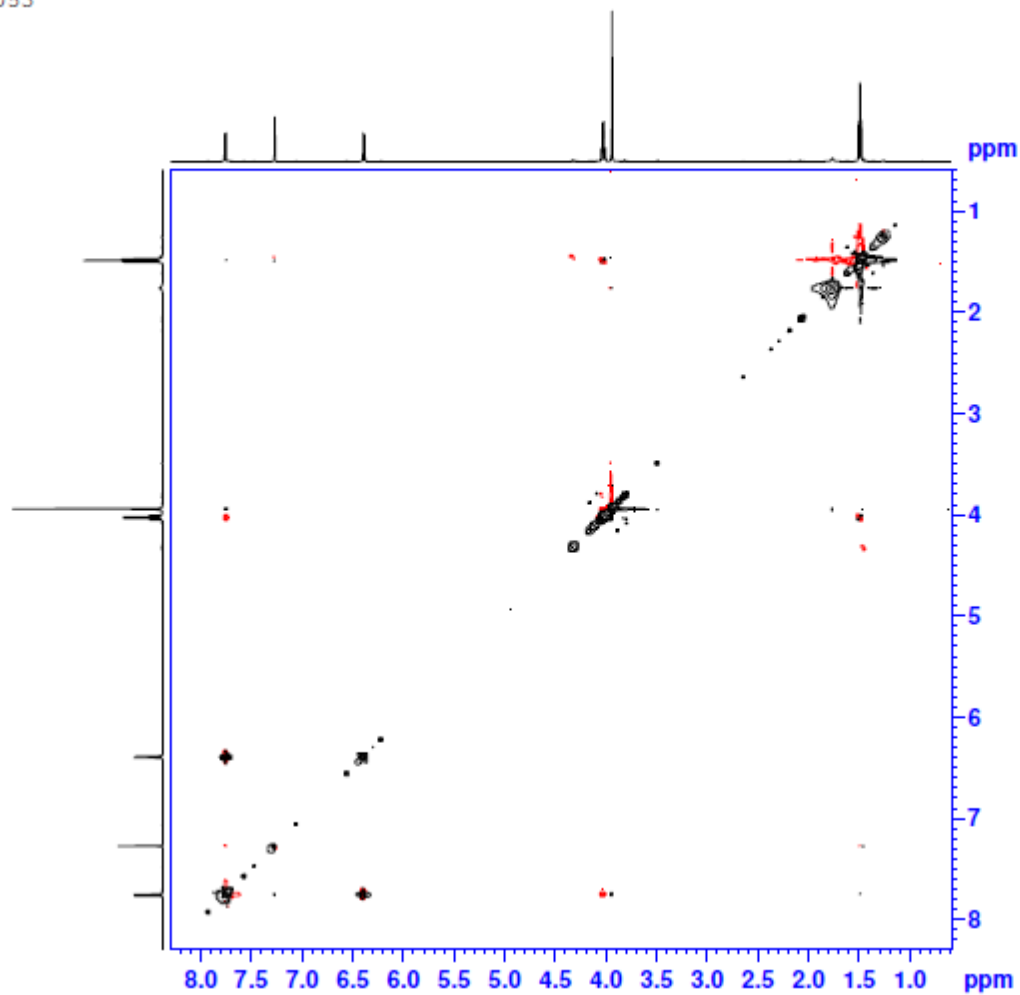
NOESY spectra of 1-substituted 3-hydroxyl protected pyridazine 16 and 18	S2-S4
¹ H and ¹³ C NMR of 20 and 21	S5-S16
HR-MS of 20 and 21	S17-S22
Speciation plot of deferiprone with iron in the presence of GSH	S23
pH-dependent UV/Vis spectra of 21a over the range of pH 1.1-2.1	S24

NOESY spectrum of 16a



NOESY spectrum of **18b**

YM1053



```

Current Data Parameters
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PROCNO   1

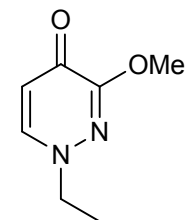
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PULPROG  noesyph
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SOLVENT  CDCl3
NS        8
DS        4
SWH       3858.025 Hz
FIDRES   1.883801 Hz
AQ        0.2656004 sec
RG        20.2
DW        129.600 usec
DE        6.00 usec
TE        298.0 K
d0        0.00011973 sec
d1        1.93364501 sec
d8        0.89999998 sec
INO       0.00025920 sec
STICNT   128

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NUC1      1H
P1        7.75 usec
PL1       1.50 dB
SFO1      500.1322305 MHz

F1 - Acquisition parameters
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FnMODE    States-TPP1

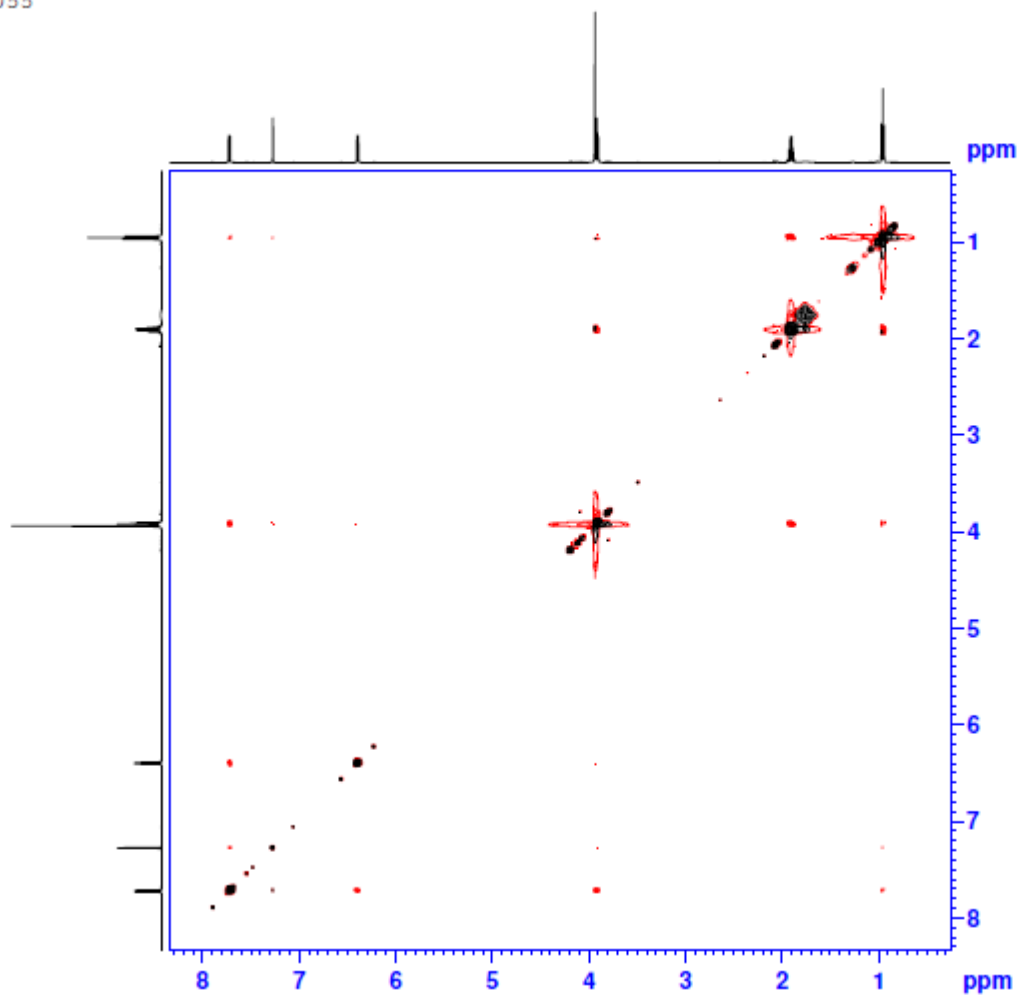
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SSB       2
LB        0.00 Hz
GB        0
PC        1.00

F1 - Processing parameters
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MC2       States-TPP1
SF        500.1300066 MHz
WDW       QSINE
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NOESY spectrum of **18c**

YM1055



```

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EXPNO    31
PROCNO   1

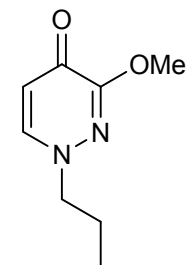
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Time     15.26
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PULPROG  noesyph
TD        2048
SOLVENT  CDCl3
NS        8
DS        4
SWH       4045.307 Hz
FIDRES    1.975248 Hz
AQ        0.2533064 sec
RG        20.2
DW        123.600 usec
DE        6.00 usec
TE        298.0 K
d0        0.00011373 sec
D1        1.94593298 sec
D8        0.89999998 sec
INO       0.00024720 sec
STICNT    128

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NUC1      1H
P1        7.75 usec
PL1       1.50 dB
SFO1      500.1321580 MHz

F1 - Acquisition parameters
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TD        256
SFO1      500.1322 MHz
FIDRES    15.801982 Hz
SW        8.088 ppm
FnMODE    States-IPPI

F2 - Processing parameters
SI        1024
SF        500.1300087 MHz
WDW       QSINE
SSB       2
LB        0.00 Hz
GB        0
PC        1.00

F1 - Processing parameters
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MC2       States-IPPI
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SSB       2
LB        0.00 Hz
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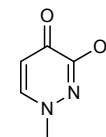
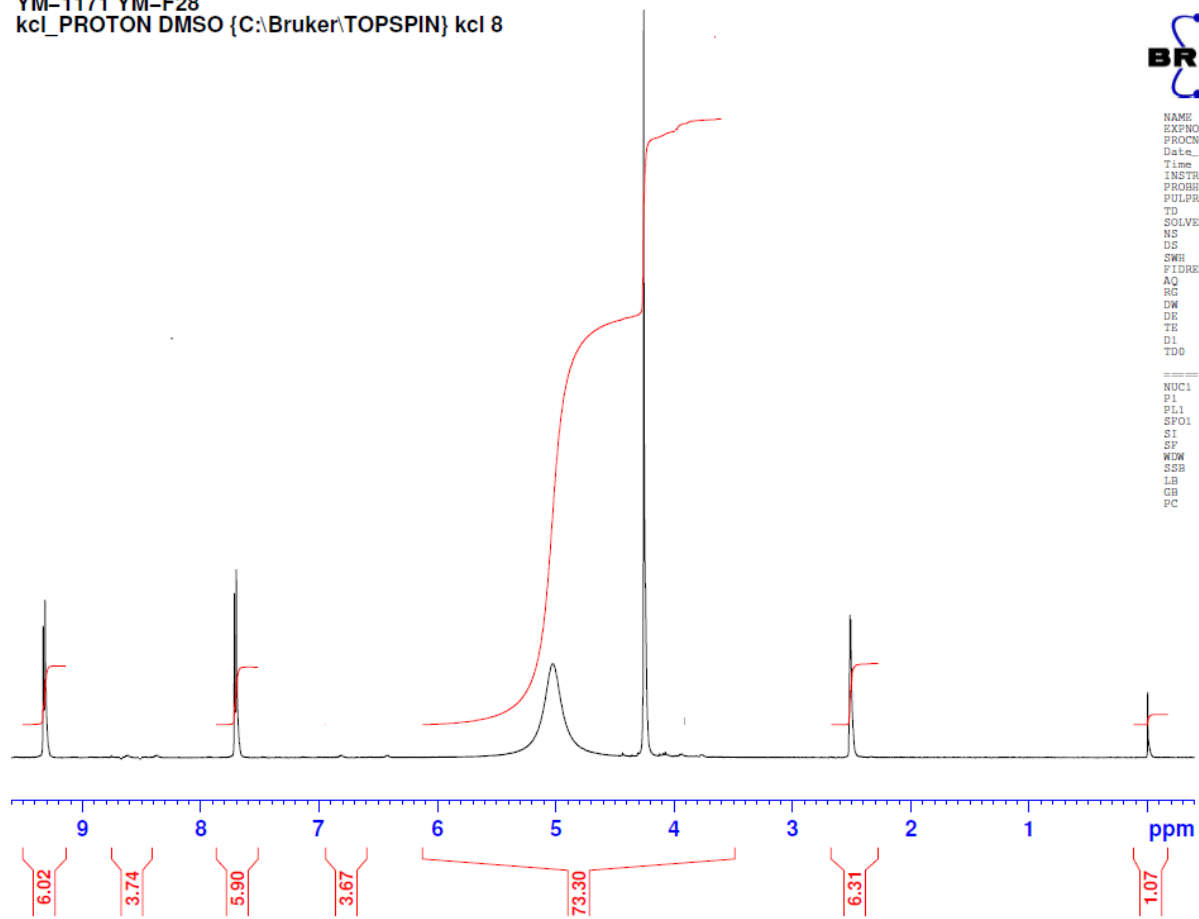


¹H NMR of 20a

YM-1171 YM-F28
kcl_PROTON DMSO {C:\Bruker\TOPSPIN} kcl 8

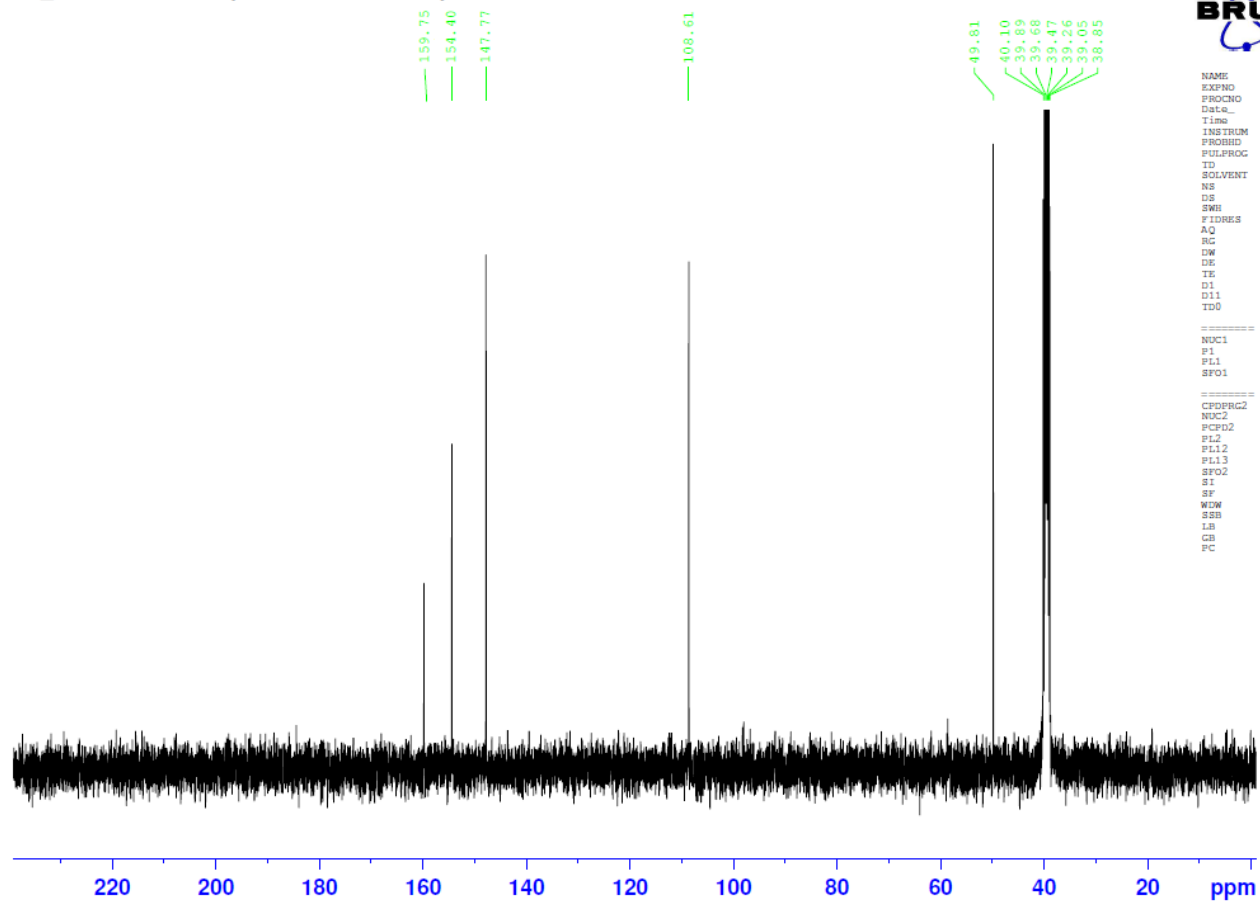


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EXPNO         80
PROCNO        1
Date_         20091010
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PROBHD        5 mm QNP 1H/13
PULPROG       zg30
TD            65536
SOLVENT       DMSO
NS            16
DS            0
SWH           8278.146 Hz
FIDRES        0.126314 Hz
AQ            3.9584243 sec
RG            256
DW            60.400 usec
DE            6.50 usec
TE            298.0 K
D1            1.00000000 sec
TDO           1
===== CHANNEL f1 =====
NUC1          1H
P1            10.30 usec
PL1           -3.00 dB
SFO1          400.1324710 MHz
SI            32768
SF            400.1299978 MHz
WDW           EM
SSB           0
LB            0.30 Hz
GB            0
PC            4.00
```



¹³C NMR of 20a

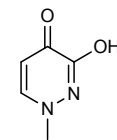
YM-1171, YM-F28
kcl_CARBO DMSO {C:\Bruker\TOPSPIN} kcl 8



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PROCNO    1
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INSTRUM   av400
PROBHD    5 mm QNP 1H/13
PULPROG   zgpg30
TD         65536
SOLVENT   DMSO
NS         1024
DS         1
SWH        27173.912 Hz
FIDRES     0.414641 Hz
AQ         1.2059124 sec
RG         14596.5
DM         18.400 usec
DE         6.50 usec
TE         298.0 K
D1         0.1000000 sec
D11        0.0300000 sec
TD0        1

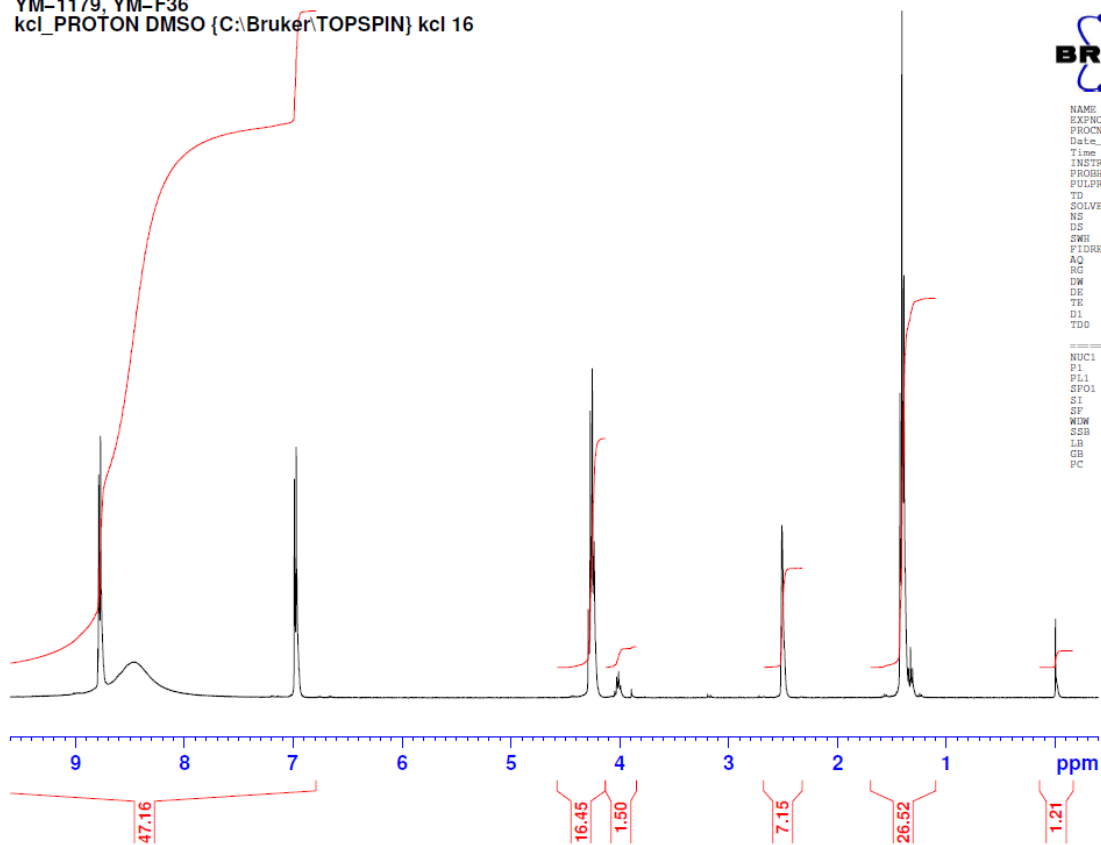
===== CHANNEL f1 =====
NUC1       13c
P1         10.00 usec
PL1        0.00 dB
SFO1       100.6233333 MHz

===== CHANNEL f2 =====
CPDPRG2    waltz16
NUC2       1H
PCPD2      100.00 usec
PL2        -3.00 dB
PL12       16.74 dB
PL13       18.00 dB
SFO2       400.1316005 MHz
SI         32768
SF         100.6128193 MHz
WFW        DM
SSB        0
LB         1.00 Hz
GB         0
PC         1.40
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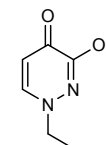
¹H NMR spectrum of **20b**

YM-1179, YM-F36
kcl_PROTON DMSO {C:\Bruker\TOPSPIN} kcl 16



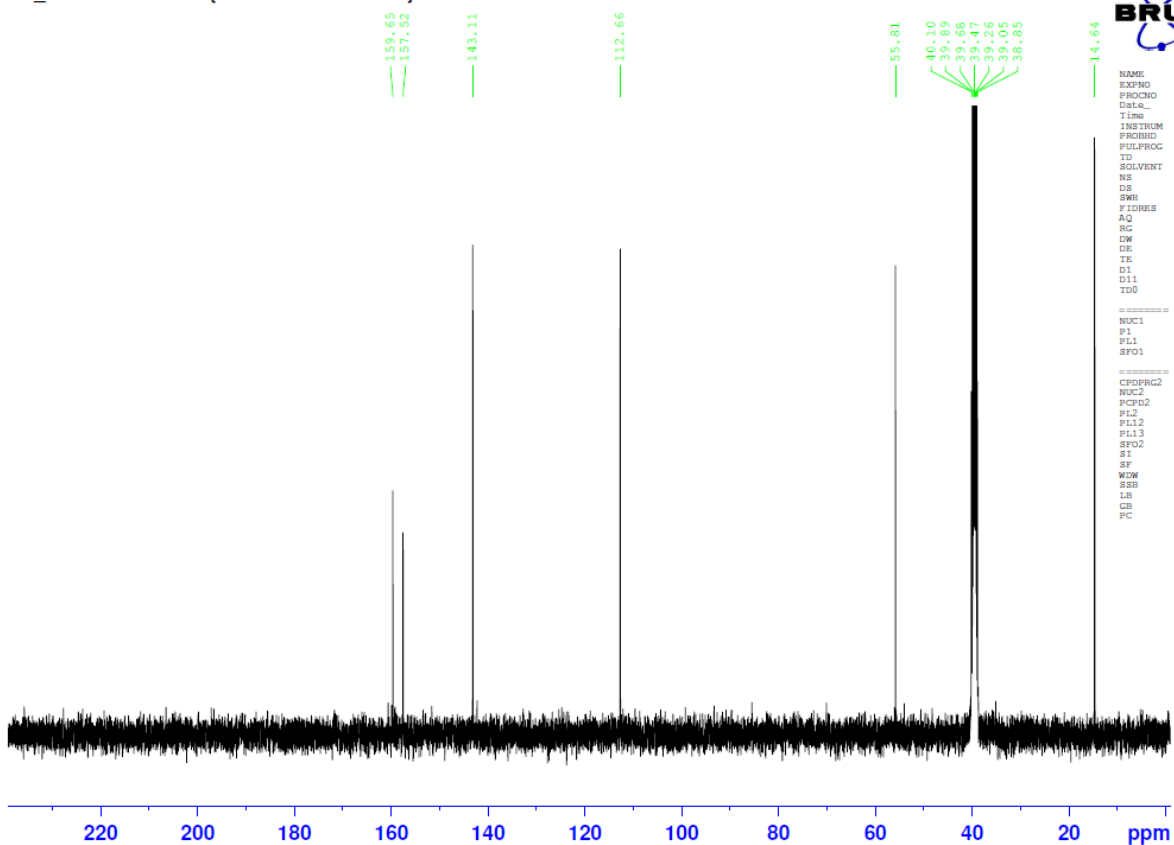
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EXPNO     1
PROCNO    1
Date_     20091010
Time      9.44
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PROBHD    5 mm QNP 1H/13
PULPROG   zg30
TD         65536
SOLVENT   DMSO
NS         16
DS         0
SWH        8278.146 Hz
FIDRES     0.126314 Hz
AQ         3.9584243 sec
RG         256
DW         60.400 usec
DE         6.50 usec
TE         298.0 K
D1         1.00000000 sec
TDO        1
```

```
===== CHANNEL f1 =====
NUC1      1H
P1         10.30 usec
PL1        -3.00 dB
SFO1      400.1324710 MHz
SI         32768
SF         400.1299984 MHz
WDW        EM
SSB         0
LR         0.30 Hz
GB         0
PC         4.00
```



¹³C NMR spectrum of **20b**

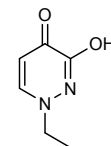
YM-1179, YM-F36
kcl_CARBO DMSO {C:\Bruker\TOPSPIN} kcl 16



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EXPNO     1
PROCNO    1
Date_     20091010
Time      10.08
INSTRUM   av400
PROBHD    5 mm QNP 1H/13
PULPROG   zgpg30
TD         65536
SOLVENT   DMSO
NS         1024
DS         1
SWH        27173.912 Hz
FIDRES     0.414641 Hz
AQ         1.2059124 sec
RG         16384
DW         18.400 usec
DE         6.50 usec
TE         299.9 K
D1         0.10000000 sec
D11        0.03000000 sec
TD0        1

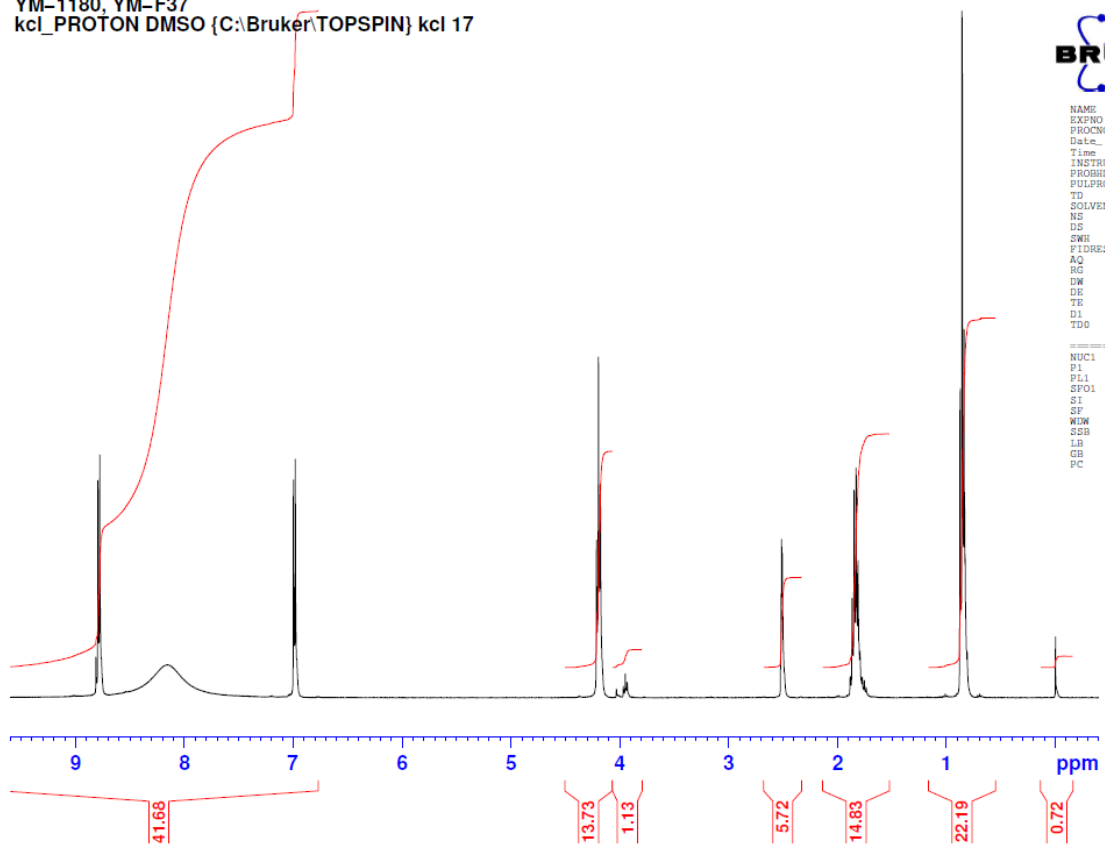
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PL1        0.00 dB
SFO1       100.6233333 MHz

===== CHANNEL f2 =====
CPDPRG2    waltz16
NUC2       1H
PCPD2      100.00 usec
PL2        -3.00 dB
PL12       16.74 dB
PL13       19.00 dB
SFO2       400.1316005 MHz
SI         32768
SF         100.6128193 MHz
WDW        EM
SSB        0
LB         1.00 Hz
GB         0
PC         1.40
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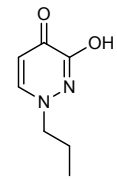


¹H NMR spectrum of **20c**

YM-1180, YM-F37
kcl_PROTON DMSO {C:\Bruker\TOPSPIN} kcl 17

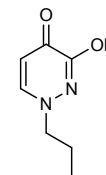
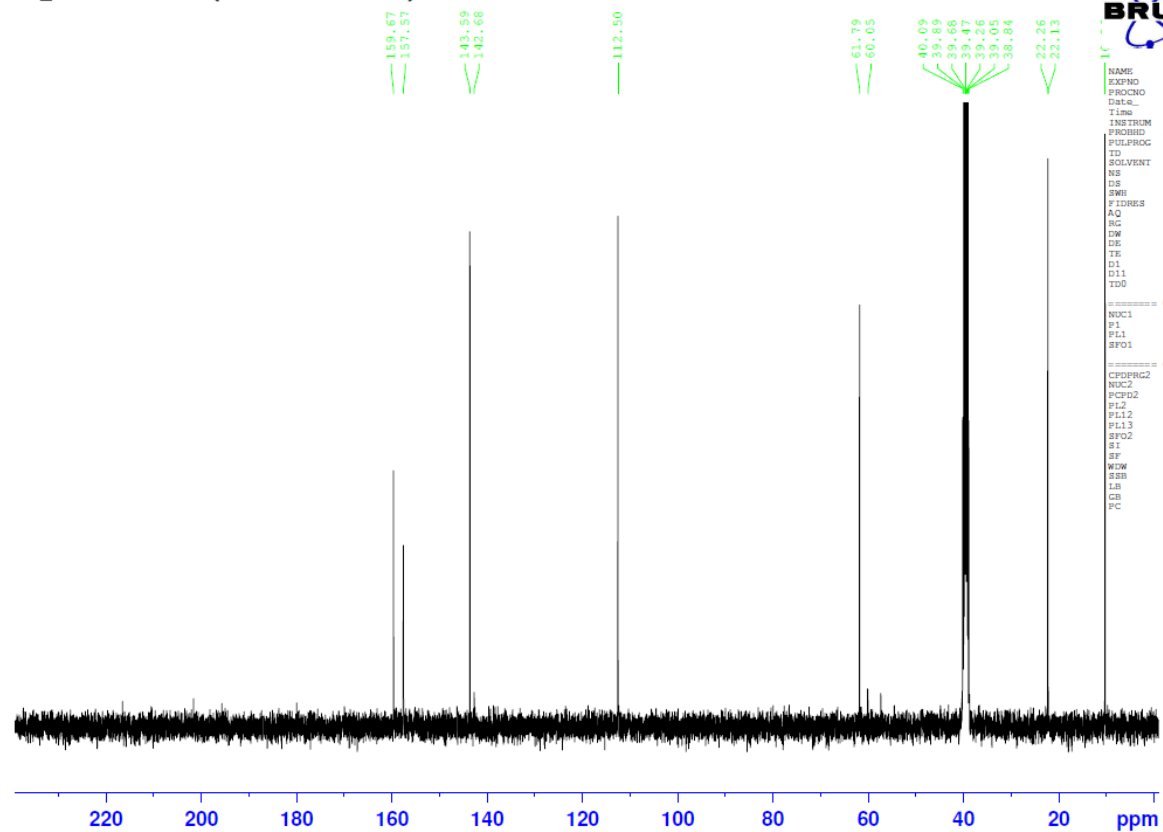


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PROCNO    1
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Time      10.34
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PROBHD    5 mm QNP 1H/13
PULPROG   zg30
TD         65536
SOLVENT   DMSO
NS         16
DS         0
SWH        8278.146 Hz
FIDRES     0.126314 Hz
AQ         3.9584243 sec
RG         256
DW         60.400 usec
DE         6.50 usec
TE         298.0 K
D1         1.00000000 sec
TDO        1
===== CHANNEL f1 =====
NUC1       1H
P1         10.30 usec
PL1        -3.00 dB
SFO1       400.1324710 MHz
SI         32768
SF         400.1299974 MHz
WDW        EM
SSB        0
LR         0.30 Hz
GB         0
PC         4.00
```



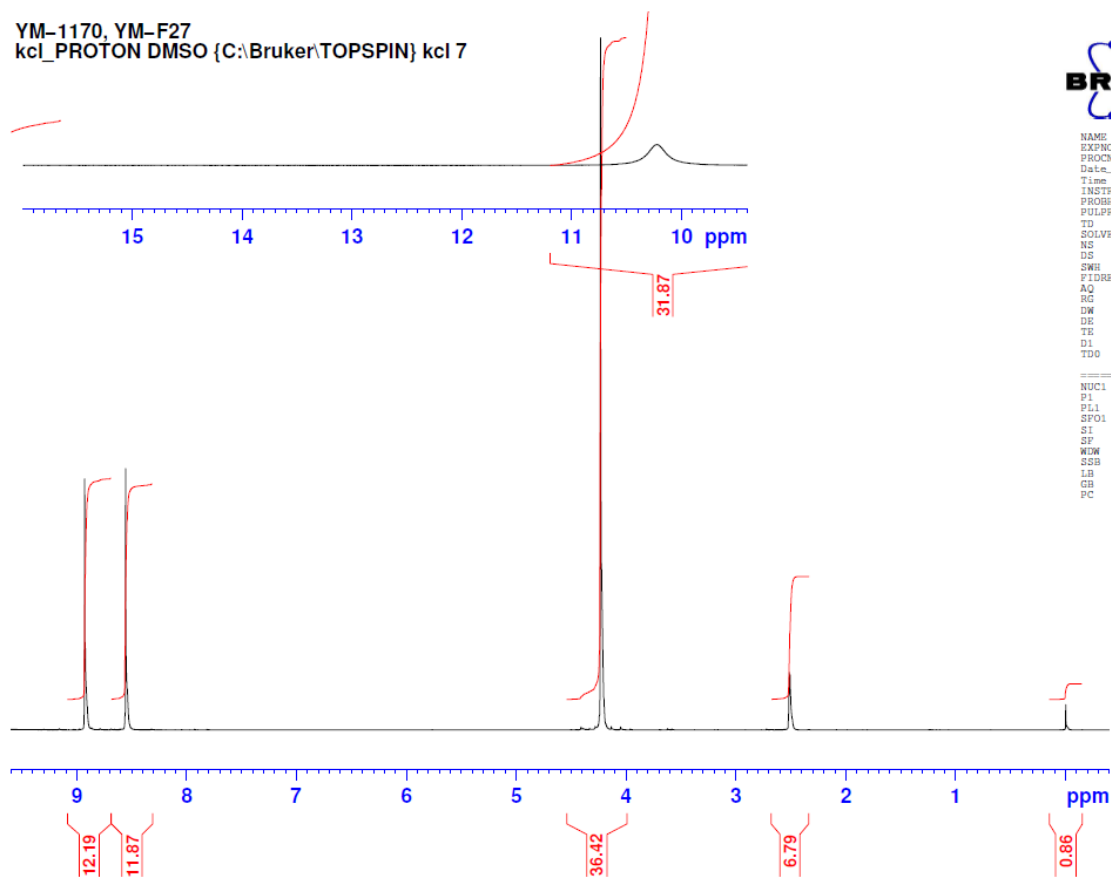
¹³C NMR spectrum of **20c**

YM-1180, YM-F37
kcl_CARBO DMSO {C:\Bruker\TOPSPIN} kcl 17



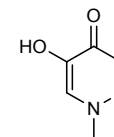
¹H NMR spectrum of **21a**

YM-1170, YM-F27
kcl_PROTON DMSO {C:\Bruker\TOPSPIN} kcl 7



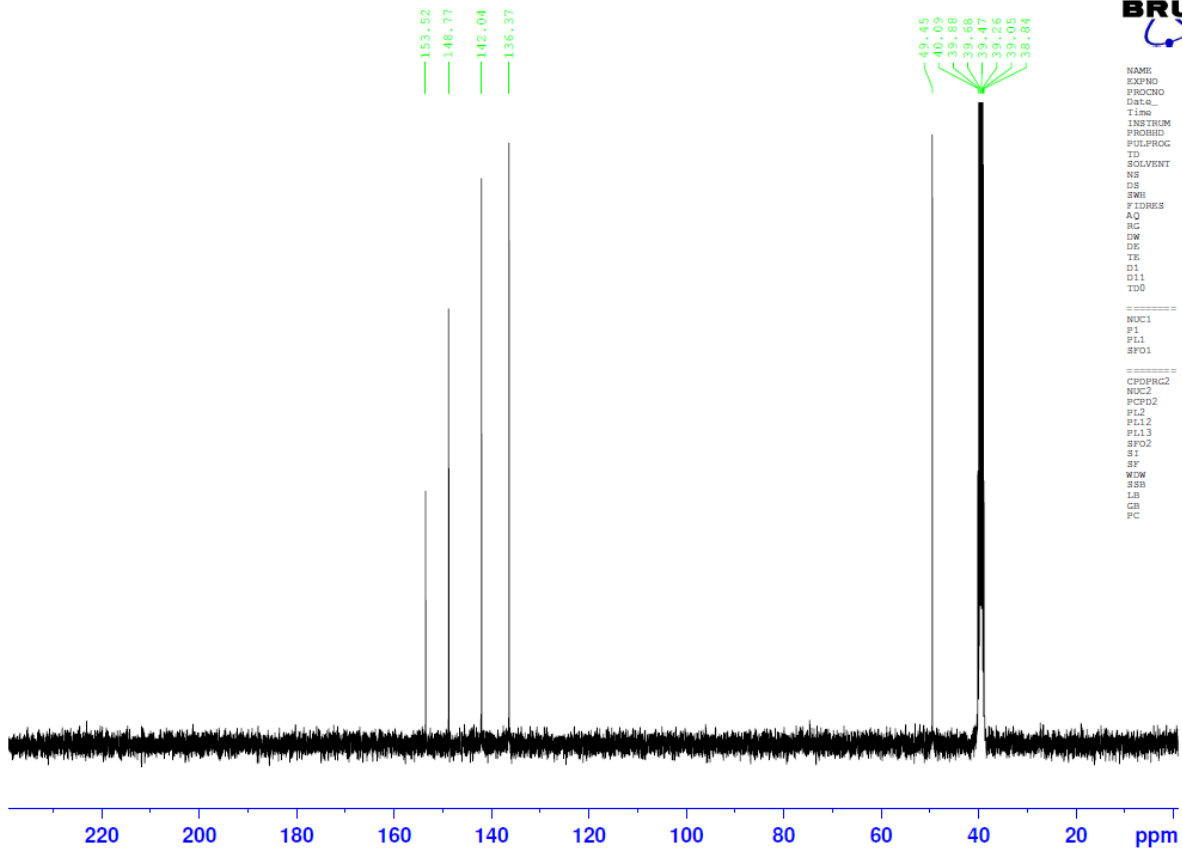
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EXPNO     1
PROCNO    1
Date_     20091010
Time      2.06
INSTRUM   av400
PROBHD    5 mm QNP 1H/13
PULPROG   zg30
TD         65536
SOLVENT   DMSO
NS         16
DS         0
SWH        8278.146 Hz
FIDRES     0.126314 Hz
AQ         3.9584243 sec
RG         256
DW         60.400 usec
DE         6.50 usec
TE         298.0 K
D1         1.00000000 sec
TDD        1

===== CHANNEL f1 =====
NUC1       1H
P1         10.30 usec
PL1        -3.00 dB
SFO1       400.1324710 MHz
SI         32768
SF         400.1299977 MHz
WDW        EM
SSB        0
LB         0.30 Hz
GB         0
PC         4.00
```



¹³C NMR spectrum of **21a**

YM-1170, YM-F27
kcl_CARBO DMSO {C:\Bruker\TOPSPIN} kcl 7

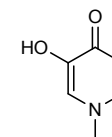


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PROCNO        1
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PULPROG       zgpg30
TD            65536
SOLVENT       DMSO
NS            1024
DS            1
SWH           27173.912 Hz
FIDRES        0.414641 Hz
AQ            1.2059124 sec
RG            14596.5
SW           18.400 usec
DE            6.50 usec
TE            298.0 K
D1            0.10000000 sec
D11           0.03000000 sec
TD0           1

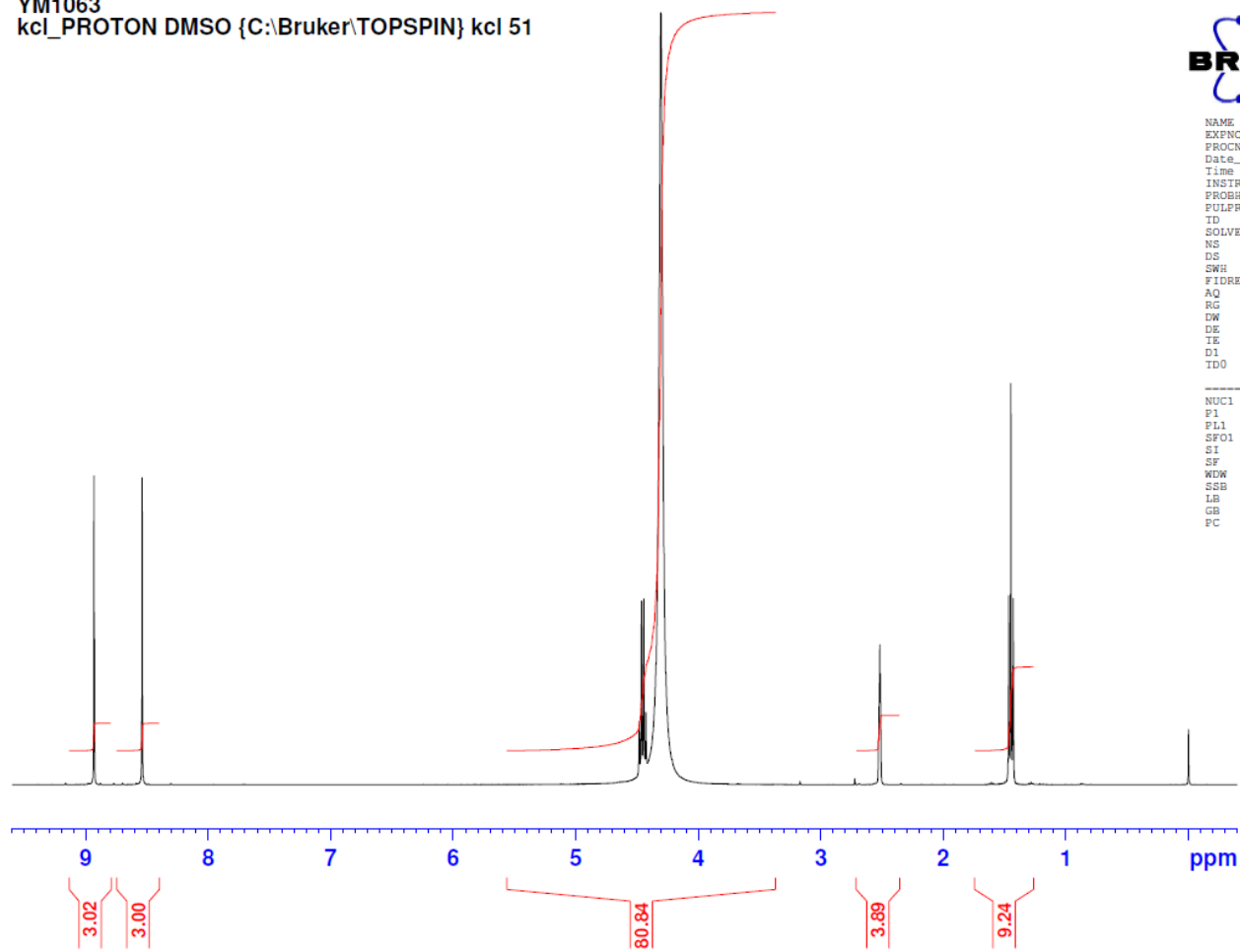
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NUC1          13C
P1            10.00 usec
PL1           0.00 dB
SFO1          100.6233333 MHz

===== CHANNEL f2 =====
CPCPRG2       waltz16
NUC2          1H
PCPD2         100.00 usec
PL2           -3.00 dB
PL12          16.74 dB
PL13          18.00 dB
SFO2          400.1316005 MHz
SI            32768
SF            100.6128193 MHz
WDW           EM
SSB           0
LB            1.00 Hz
GB            0
PC            1.40
    
```



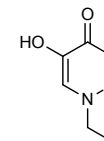
¹H NMR spectrum of **21b**

YM1063
kcl_PROTON DMSO {C:\Bruker\TOPSPIN} kcl 51



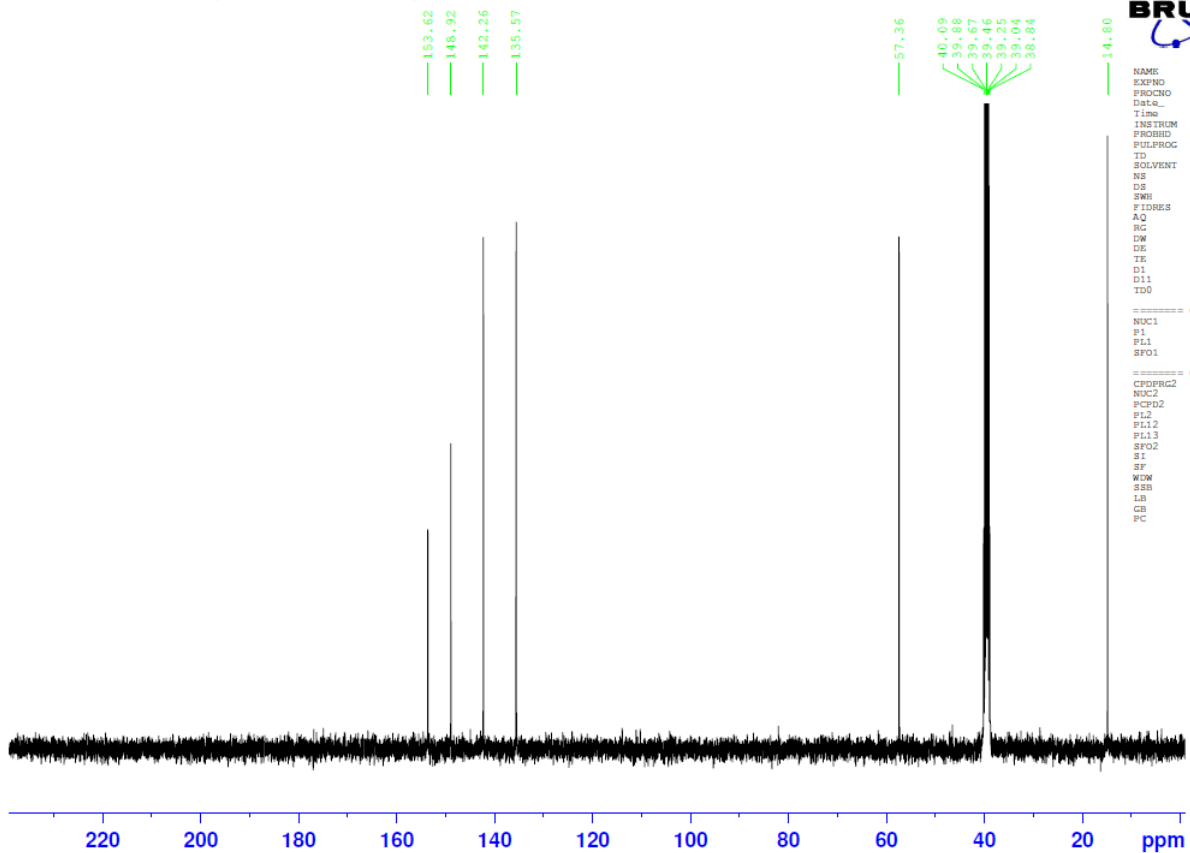
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TD         65536
SOLVENT   DMSO
NS         16
DS         0
SWH        8278.146 Hz
FIDRES     0.126314 Hz
AQ         3.9584243 sec
RG         114
DW         60.400 usec
DE         6.50 usec
TE         295.5 K
D1         1.00000000 sec
TD0        1
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```
----- CHANNEL f1 -----
NUC1      1H
P1         14.00 usec
PL1        -2.00 dB
SFO1      400.1324710 MHz
SI         32768
SF         400.1299954 MHz
WDW        EM
SSB        0
LB         0.30 Hz
GB         0
PC         4.00
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¹³C NMR spectrum of **21b**

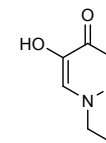
YM-1177, YM-F34
kcl_CARBO DMSO {C:\Bruker\TOPSPIN} kcl 14



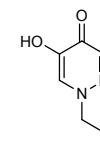
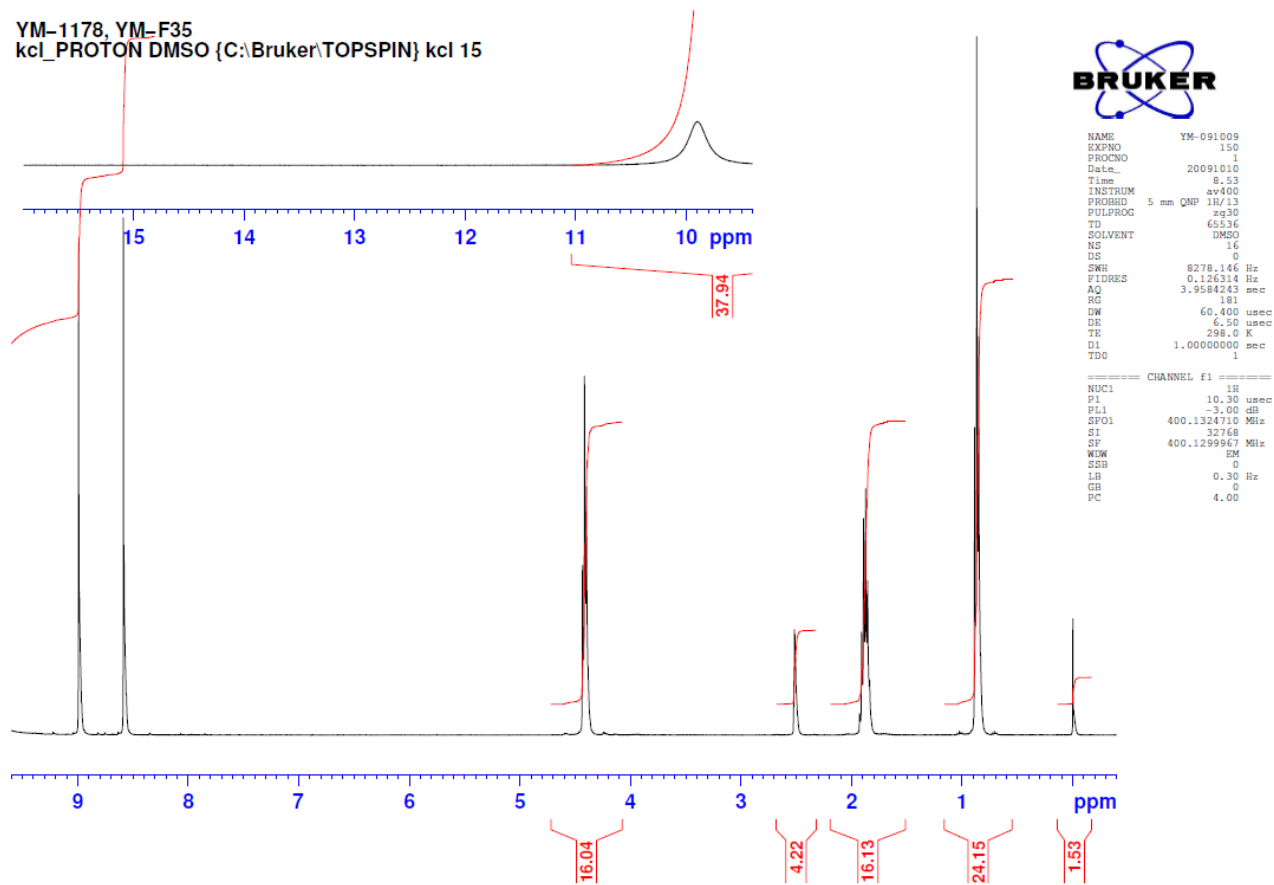
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PULPROG   zgpg30
TD        65536
SOLVENT   DMSO
NS        1024
DS        4
SWH        27173.912 Hz
FIDRES    0.414641 Hz
AQ        1.2059124 sec
RG        16384
DW        18.400 usec
DE        6.50 usec
TE        298.0 K
D1        0.10000000 sec
D11       0.03000000 sec
TD0       1

===== CHANNEL f1 =====
NUC1      13C
P1        10.00 usec
PL1       0.00 dB
SFO1     100.6233333 MHz

===== CHANNEL f2 =====
CPDPRG2   waltz16
NUC2      1H
PCPD2     100.00 usec
PL2       -3.00 dB
PL12      16.74 dB
PL13      18.00 dB
SFO2     400.1316005 MHz
S1        32768
SF        100.6128193 MHz
WDW       EM
SSB       0
LB        1.00 Hz
GB        0
PC        1.40
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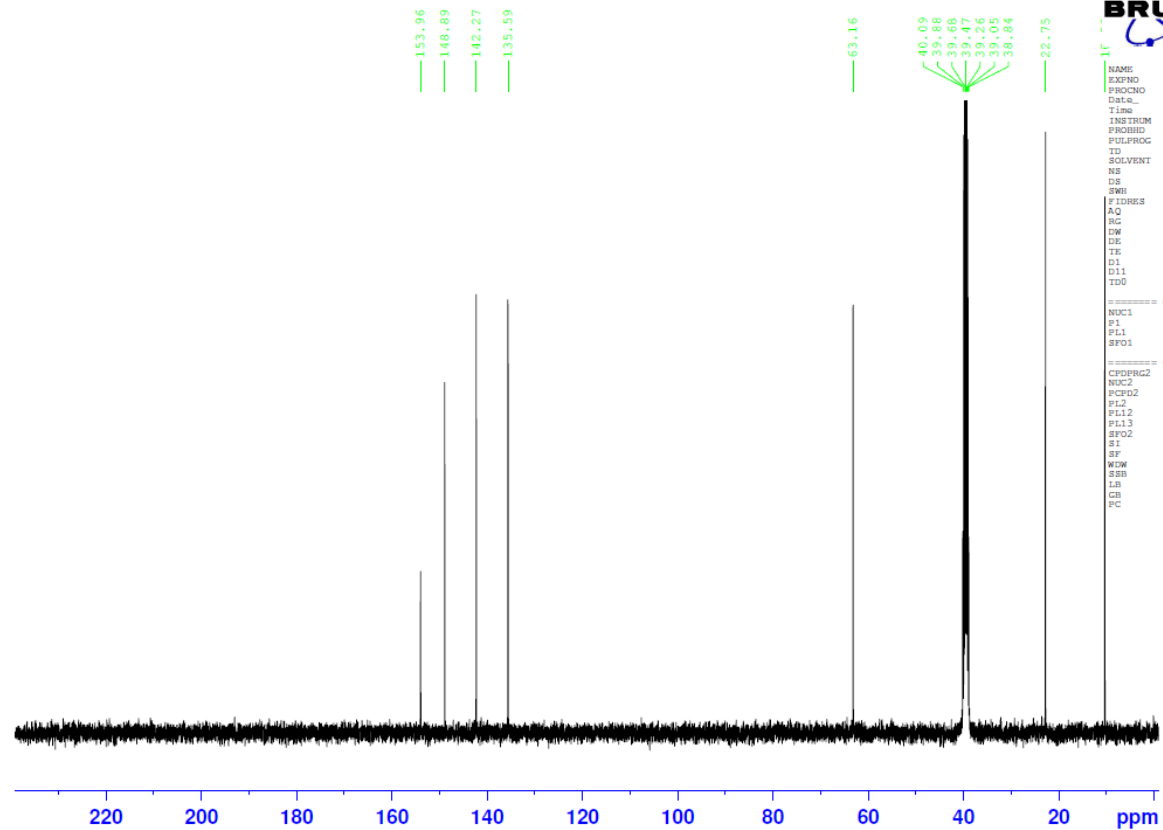


¹H NMR spectrum of **21c**



¹³C NMR spectrum of **21c**

YM-1178, YM-F35
kcl_CARBO DMSO {C:\Bruker\TOPSPIN} kcl 15

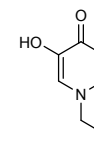


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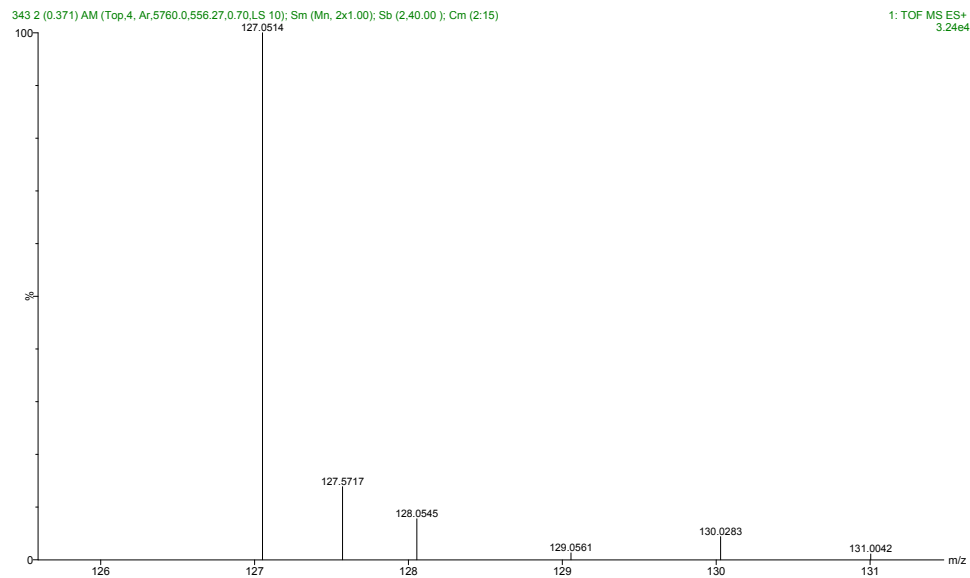
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PULPROG       zgpg30
TD            65536
SOLVENT       DMSO
NS            1024
DS            1
SWH           27173.912 Hz
FIDRES        0.414641 Hz
AQ            1.2059124 sec
RG            16384
DMW           18.400 usec
DE            6.50 usec
TE            298.0 K
D1            0.10000000 sec
D11           0.33000000 sec
TD0           1

===== CHANNEL F1 =====
NUC1           13C
P1            10.00 usec
PT1           0.00 dB
SFO1          100.6233333 MHz

===== CHANNEL F2 =====
CPDPRG2       waltz16
NUC2           1H
PCPD2         100.00 usec
PL2           -3.00 dB
PT2           16.74 dB
PL13          18.00 dB
SFO2          400.1360005 MHz
S1            32768
SF            100.6128193 MHz
WDW           EM
SSB           0
LB            1.00 Hz
GB            0
PC            1.40
    
```



HRMS of Sample 20a



Single Mass Analysis

Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

112 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 2-25 H: 2-50 N: 0-5 O: 0-5 F: 0-2

Minimum:

-1.5

Maximum:

5.0

20.0

50.0

Mass

Calc. Mass

mDa

PPM

DBE

i-FIT

Formula

127.0514

127.0519

-0.5

-3.9

-0.5

483.5

C2 H8 N2 O3 F

127.0508

0.6

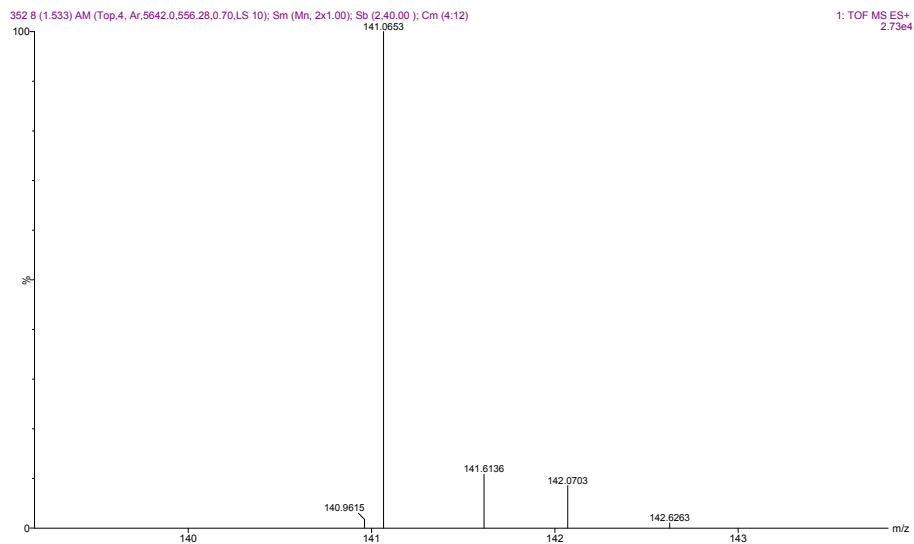
4.7

3.5

98.1

C5 H7 N2 O2

HRMS of Sample 20b



Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

166 formula(e) evaluated with 2 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 2-25 H: 2-50 N: 0-5 O: 0-5 F: 0-4

Minimum:

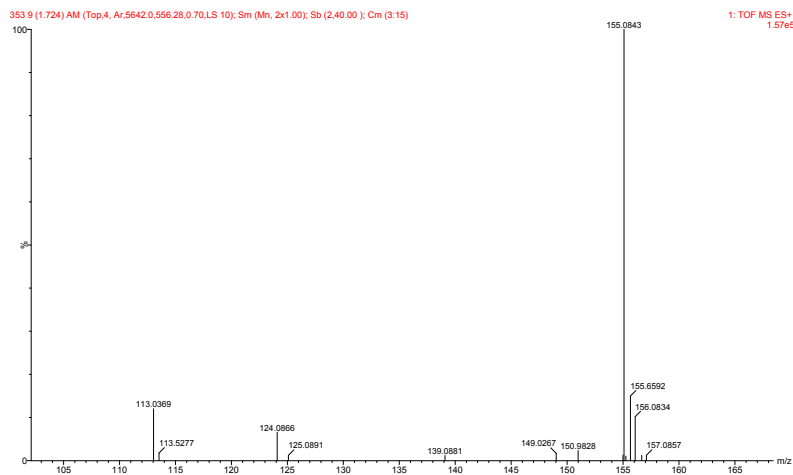
-1.5

Maximum:

5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
141.0653	141.0664	-1.1	-7.8	3.5	n/a	C6 H9 N2 O2
	141.0640	1.3	9.2	0.5	n/a	C4 H8 N2 F3

HRMS of Sample 20c



Single Mass Analysis

Tolerance = 100.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

223 formula(e) evaluated with 10 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 2-50 H: 2-50 N: 0-5 O: 0-11 32S: 0-6 79Br: 0-2 81Br: 0-2

Minimum:

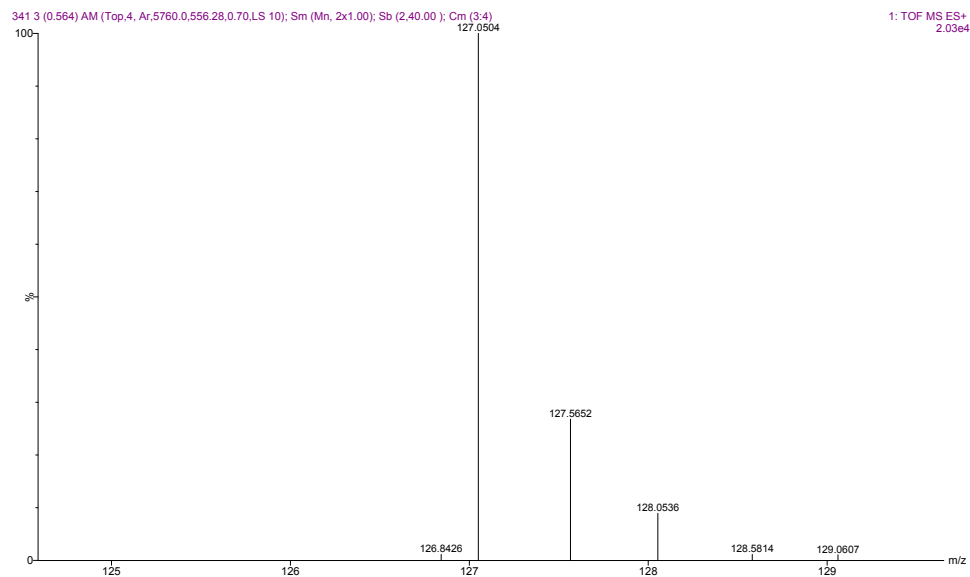
-1.5

Maximum:

5.0 100.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
155.0843	155.0854	-1.1	-7.1	-1.5	2026.5	C4 H15 N2 O2 32S
	155.0861	-1.8	-11.6	7.5	979.7	C12 H11
	155.0821	2.2	14.2	3.5	393.8	C7 H11 N2 O2
	155.0894	-5.1	-32.9	2.5	1032.6	C9 H15 32S
	155.0780	6.3	40.6	-0.5	3726.4	C2 H11 N4 O4

HRMS of Sample 21a



Single Mass Analysis

Tolerance = 20.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

61 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 2-25 H: 2-50 N: 0-7 O: 0-9

Minimum:

-1.5

Maximum:

5.0

20.0

50.0

Mass

Calc. Mass

mDa

PPM

DBE

i-FIT

Formula

127.0504

127.0508

-0.4

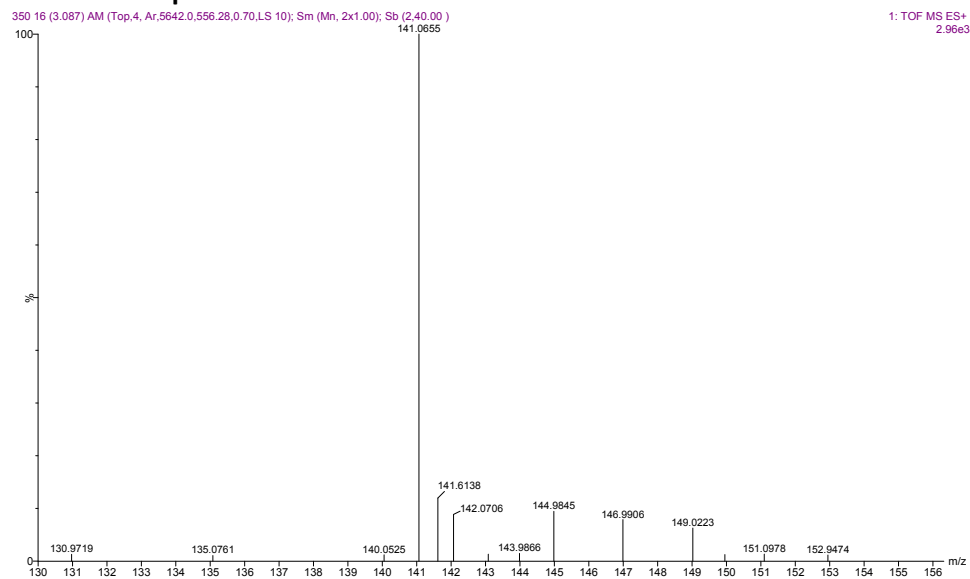
-3.1

3.5

87.0

C5 H7 N2 O2

HRMS of Sample 21b



Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

166 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

C: 2-25 H: 2-50 N: 0-5 O: 0-5 F: 0-4

Minimum:

-1.5

Maximum:

5.0

10.0

50.0

Mass Calc. Mass

mDa

PPM

DBE

i-FIT

Formula

141.0655 141.0664

-0.9

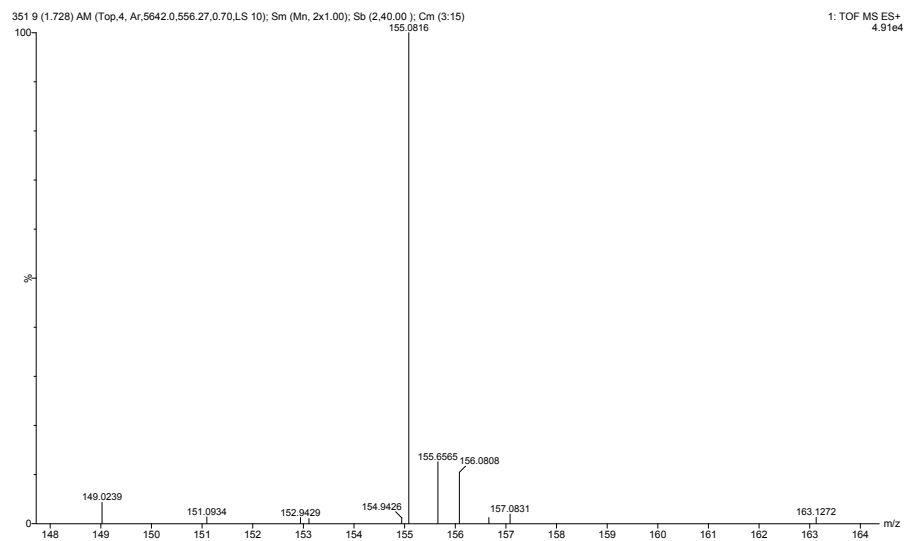
-6.4

3.5

7.8

C6 H9 N2 O2

HRMS of Sample 21c



Single Mass Analysis

Tolerance = 10.0 PPM / DBE: min = -1.5, max = 50.0

Element prediction: Off

Number of isotope peaks used for i-FIT = 3

Monoisotopic Mass, Even Electron Ions

200 formula(e) evaluated with 1 results within limits (up to 50 closest results for each mass)

Elements Used:

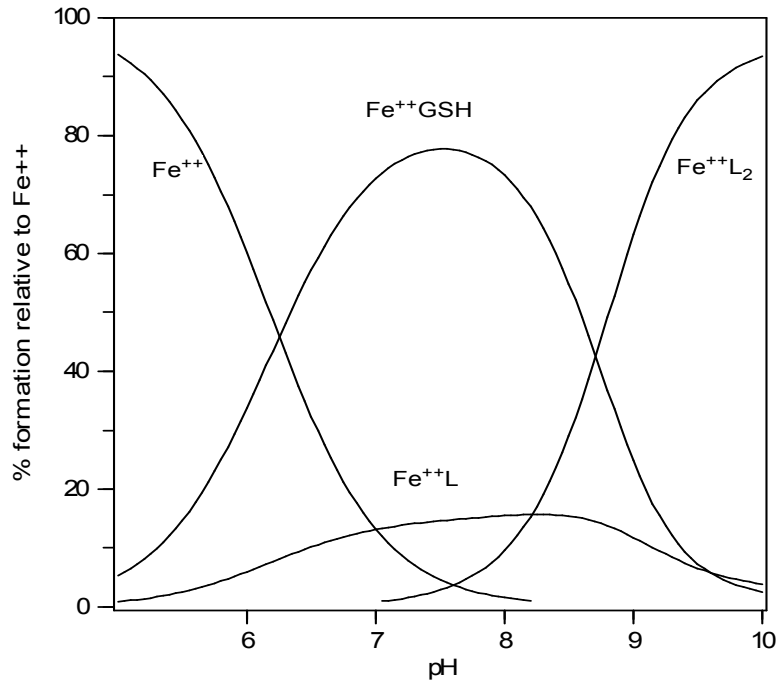
C: 2-25 H: 2-50 N: 0-5 O: 0-5 F: 0-4

Minimum: -1.5

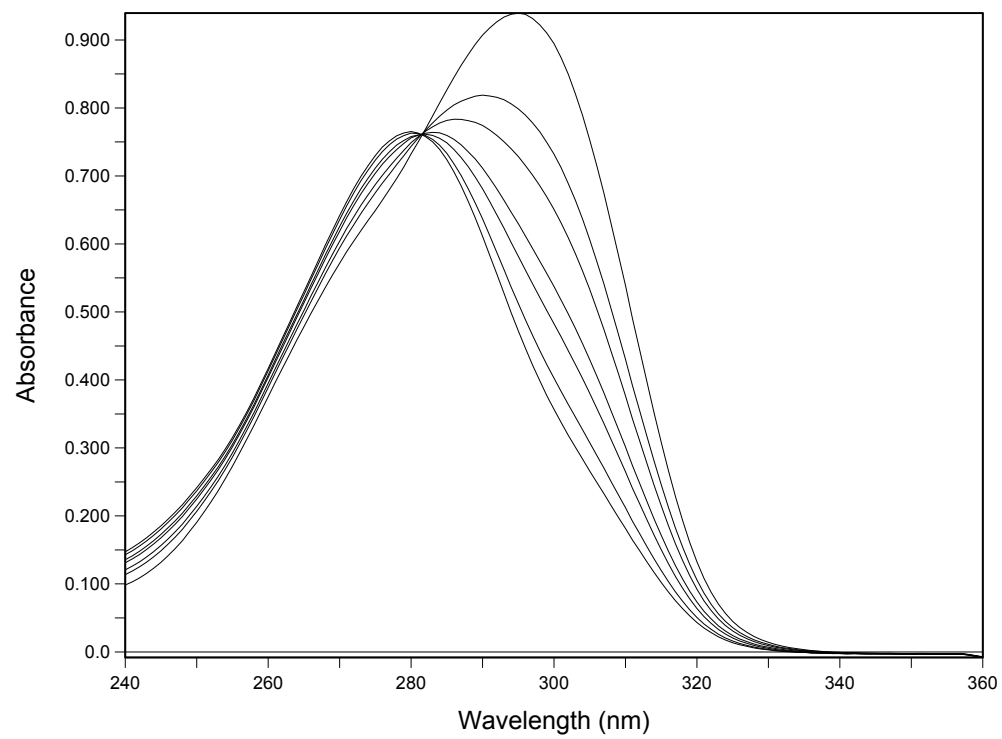
Maximum: 5.0 10.0 50.0

Mass	Calc. Mass	mDa	PPM	DBE	i-FIT	Formula
155.0816	155.0821	-0.5	-3.2	3.5	252.8	C7 H11 N2 O2

Iron complex speciation plot in the presence of GSH.



Speciation plot: $[\text{Fe}]_{\text{total}} = 1 \mu\text{M}$ and $[\text{Deferiprone}]_{\text{total}} = 100 \mu\text{M}$ $[\text{GSH}] = 2 \text{mM}$



pH-dependent UV/Vis spectra of **21a** over the range of pH 1.1-2.1.