Supplementary Material (ESI) for Organic and Biomolecular Chemistry

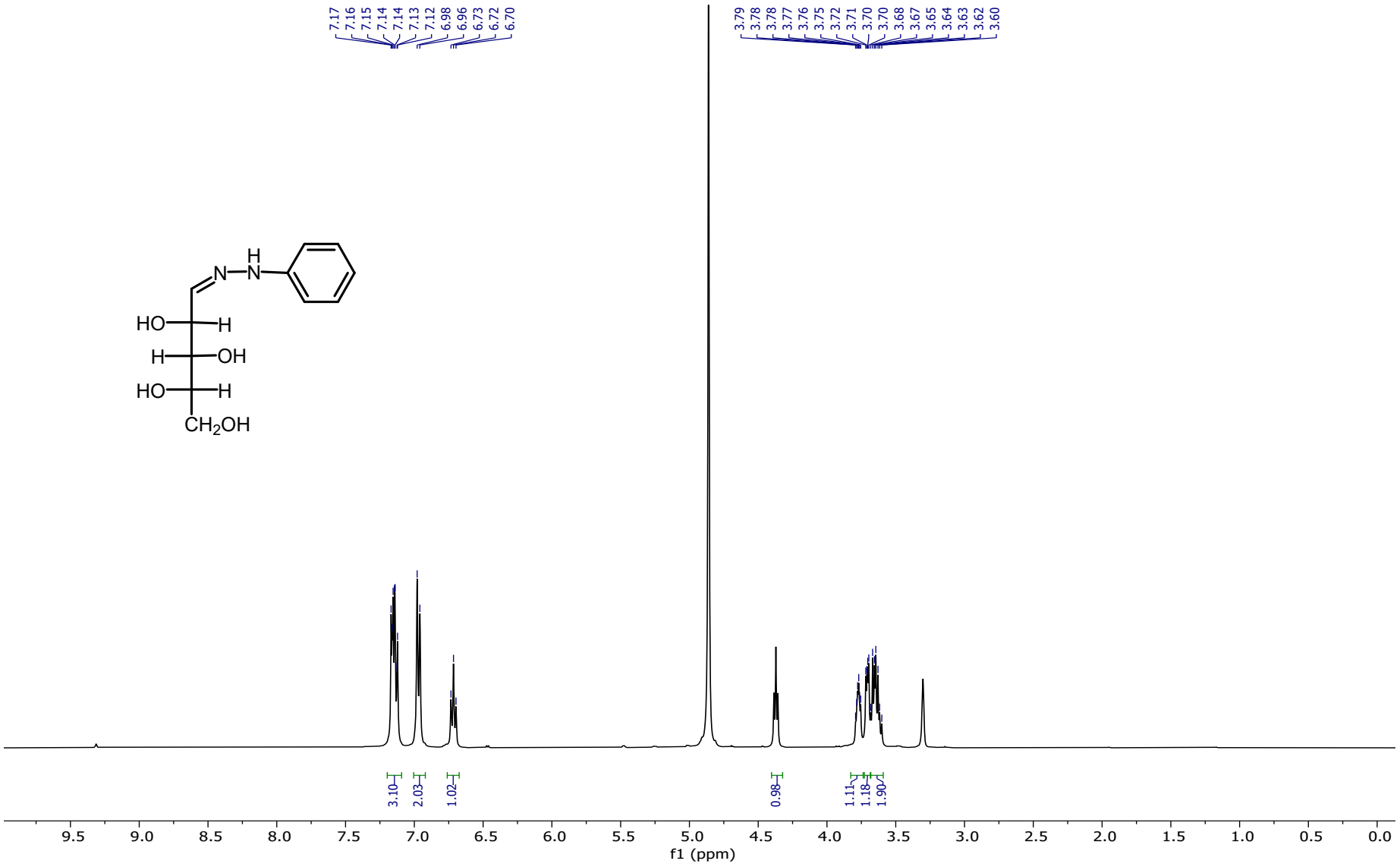
Syntheses of Optically Active Monapterin, 7,8-Dihydromonapterin, and 5,6,7,8-Tetrahydromonapterin from L-Xylose

Arun K. Ghosh*, Ashish Sharma, Satish Nagam, and Clay Fuqua

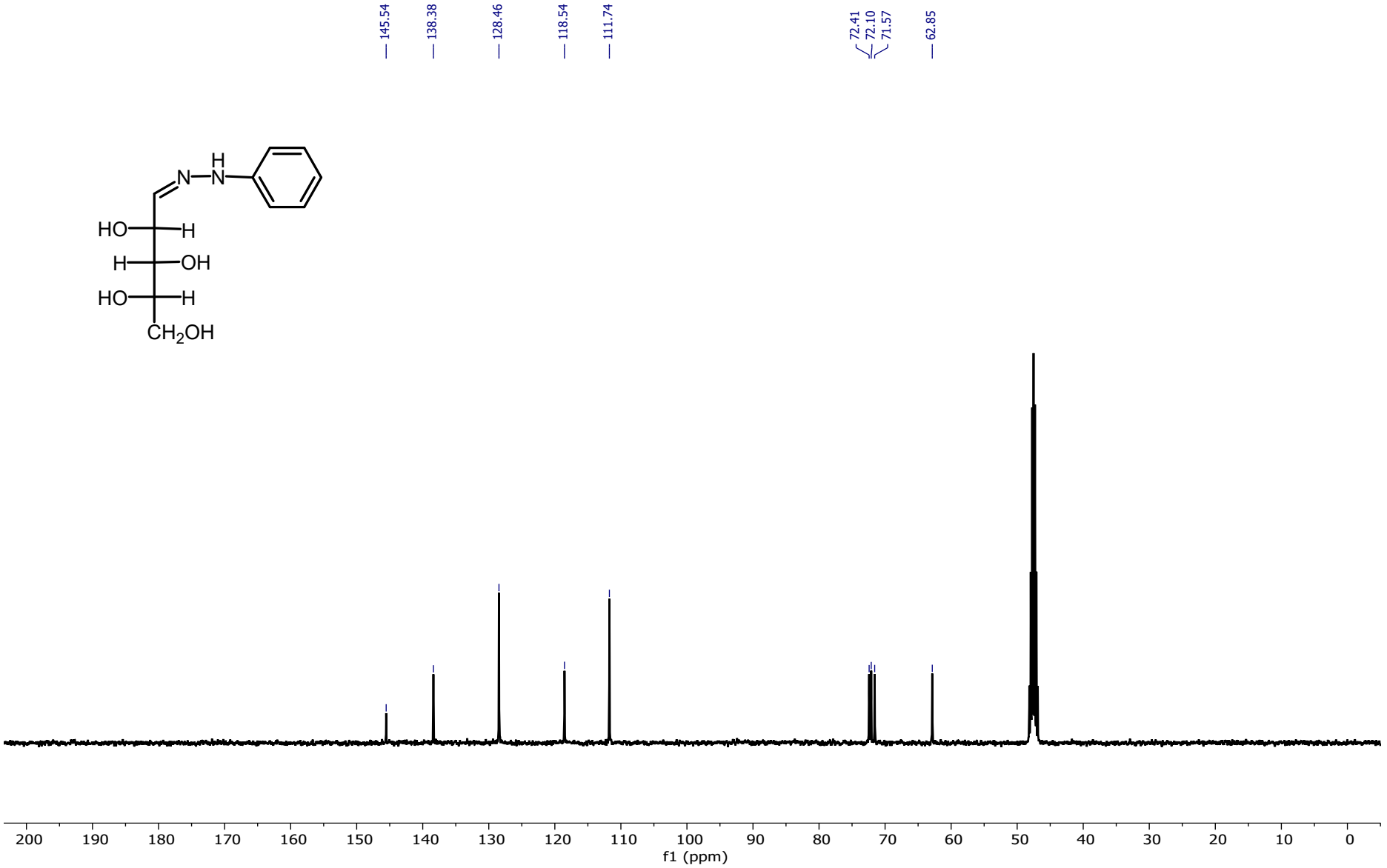
Table of Contents

General Methods.

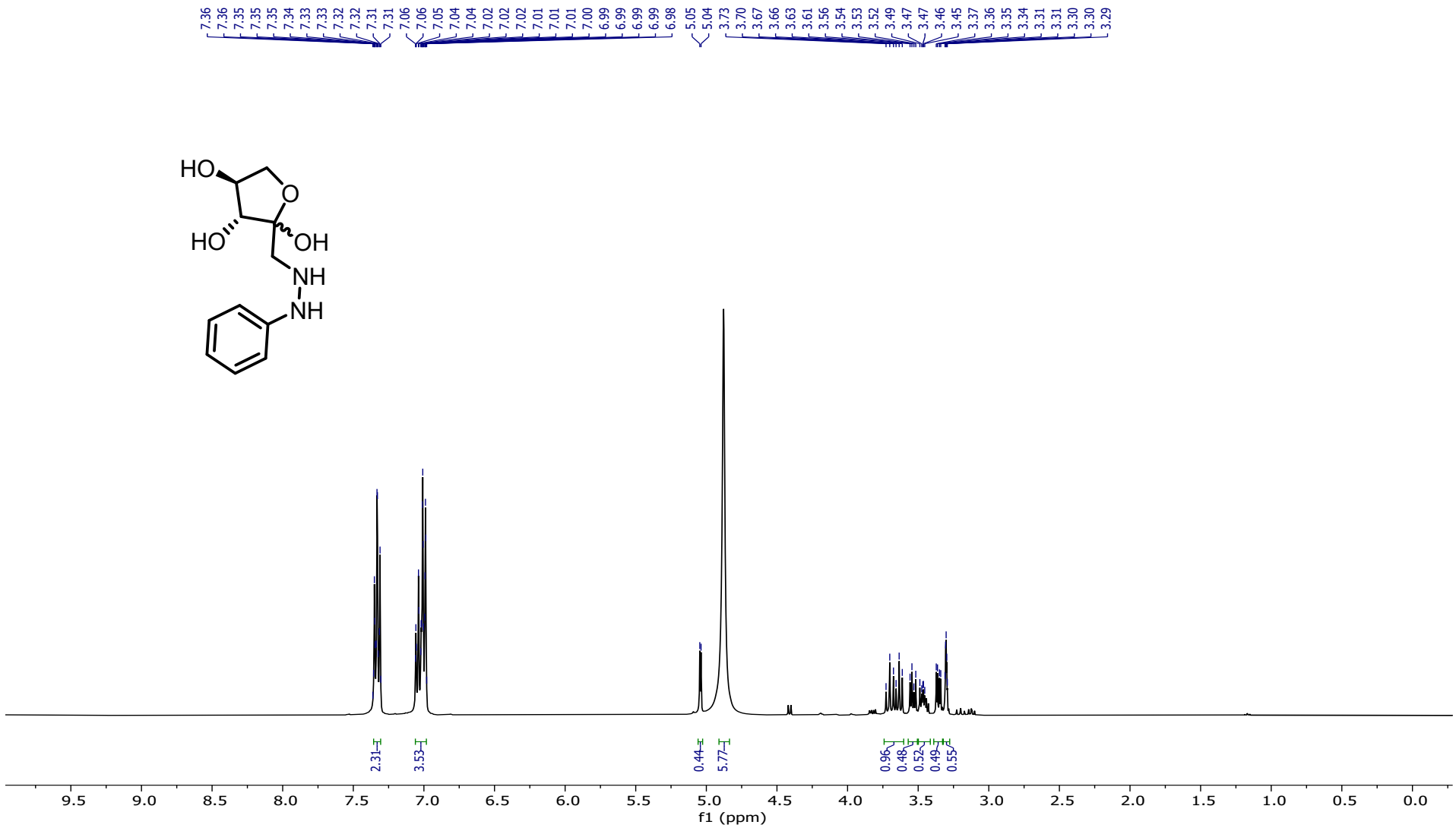
All chemicals and reagents were purchased from commercial suppliers and used without further purification unless otherwise noted. The following reaction solvents were distilled prior to use: dichloromethane from calcium hydride, diethyl ether and tetrahydrofuran from Na and benzophenone, methanol and ethanol from activated magnesium under argon. All reactions were carried out under an argon atmosphere in either flame or oven-dried (120 ^{o}C) glassware. TLC analysis was conducted using glass-backed Thin-Layer Silica Gel Chromatography Plates (60 Å, 250 μm thickness, F-254 indicator). Column chromatography was performed using 230-400 mesh, 60 Å pore diameter silica gel. ^{1}H and ^{13}C NMR spectra were recorded at room temperature on a Bruker AV800, DRX-500 and ARX-400. Chemical shifts (δ values) are reported in parts per million, and are referenced to the deuterated residual solvent peak. NMR data is reported as: δ value (chemical shift, *J*-value (Hz), integration, where s = singlet, d = doublet, t = triplet, q = quartet, brs = broad singlet). Optical rotations were recorded on a Perkin Elmer 341 polarimeter. HRMS and LRMS spectra were recorded at the Purdue University Department of Chemistry Mass Spectrometry Center. A Thermo Finnigan LCQ Classic mass was used for MS analyses. HPLC analysis and purification was done an on Agilent 1200 series instruments. The solid compound purified by reverse phase HPLC column purification using ZORBAX-NH_2, 5 μm, 4.6×250 mm column for purification. The purity of test compounds was determined by HRMS and HPLC analysis



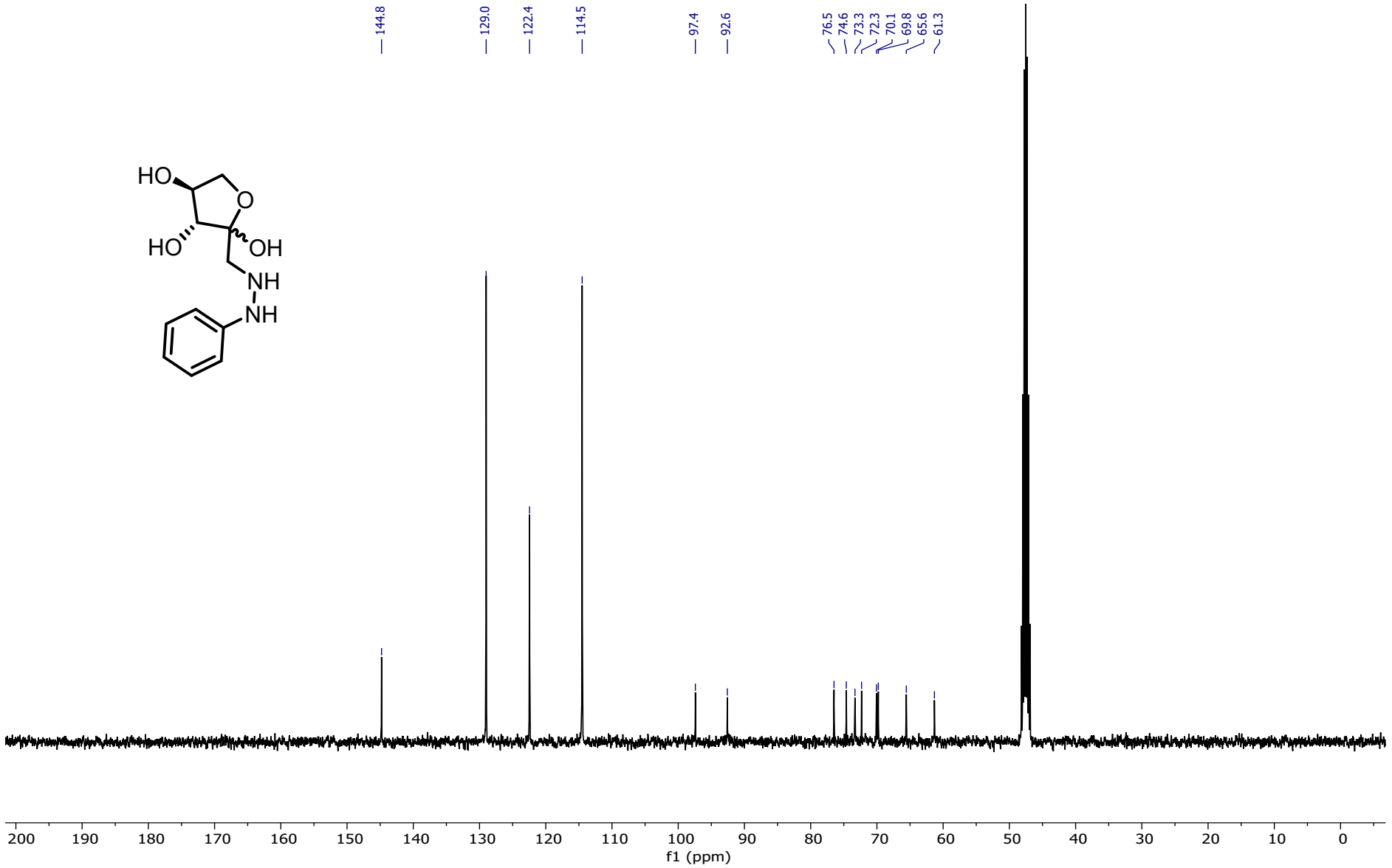
^{1}H NMR Of Compound 8 in CD_3OD (500 MHz)


145.54
138.38
128.46
118.54
111.74
72.41
72.10
71.57
62.85
HO
H
H
OH
HO
H
CH2OH
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

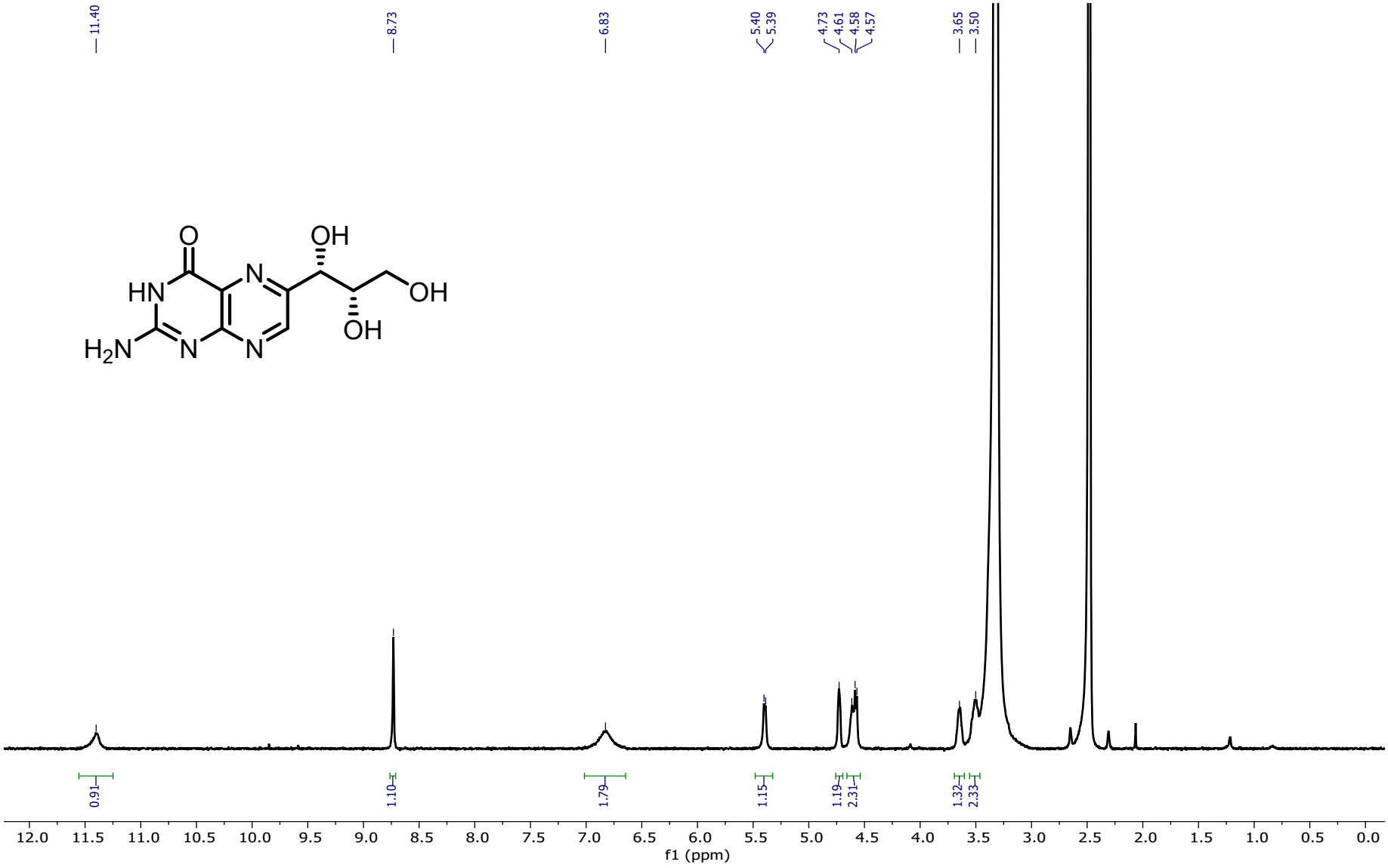
^{13}C NMR Of Compound 8 in CD_3OD (125 MHz)



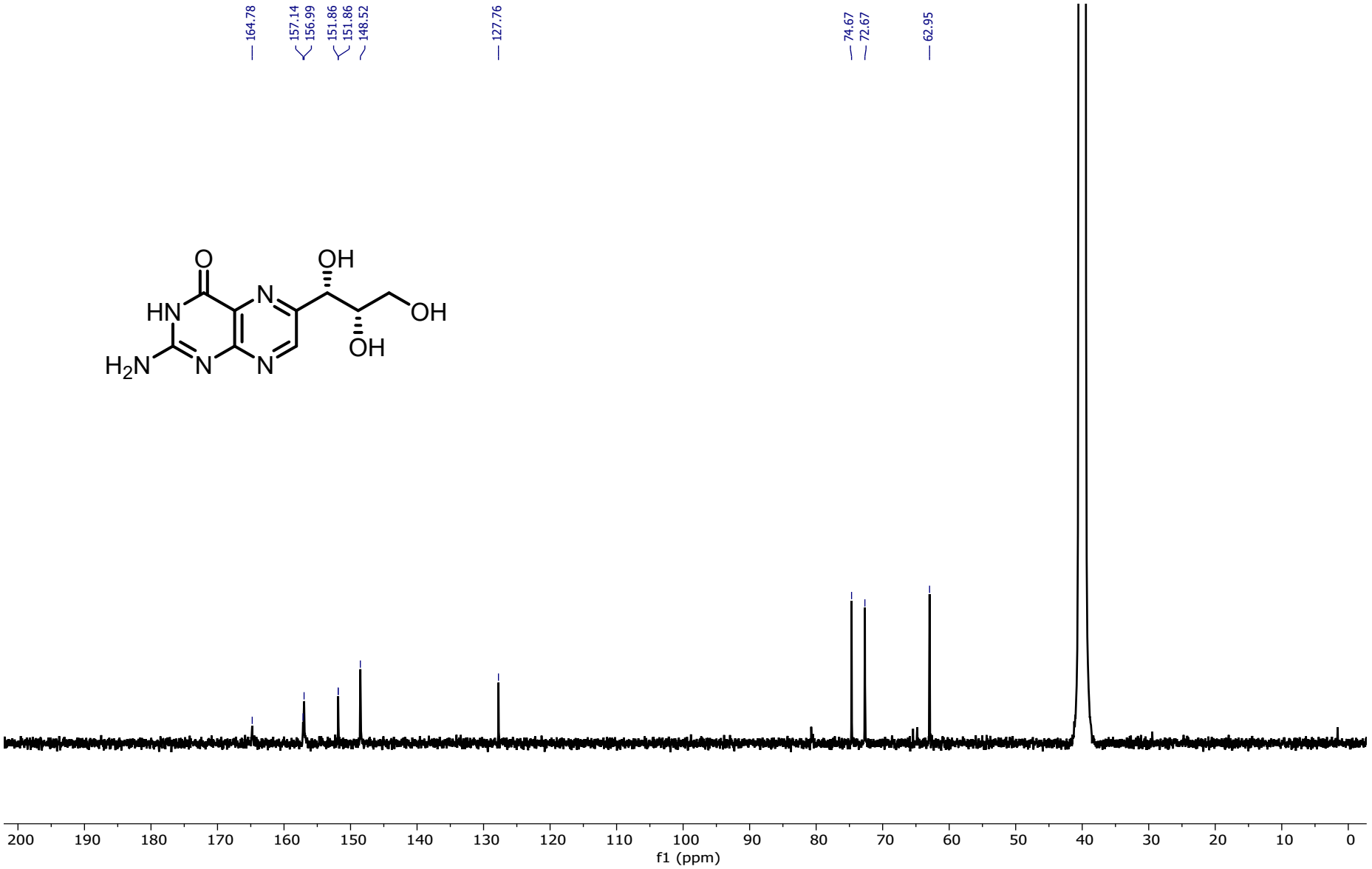
^{1}H NMR Of Compound 9 in CD_3OD (400 MHz)


144.8
129.0
122.4
114.5
97.4
92.6
76.5
74.6
73.3
72.3
70.1
69.8
65.6
61.3
HO
O
HO
OH
NH
NH
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

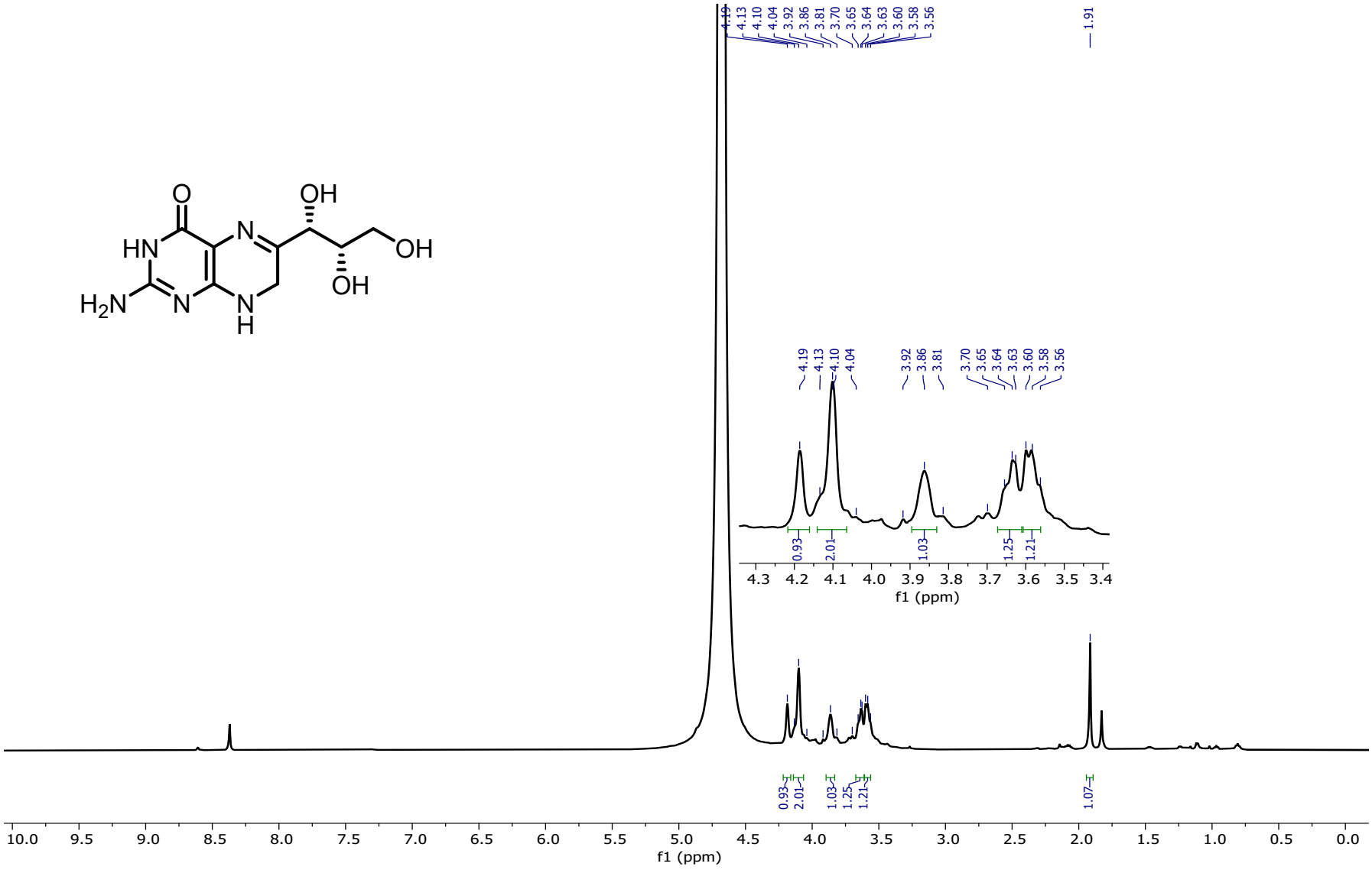
^{13} C NMR Of Compound 9 in CD_3OD (100 MHz)



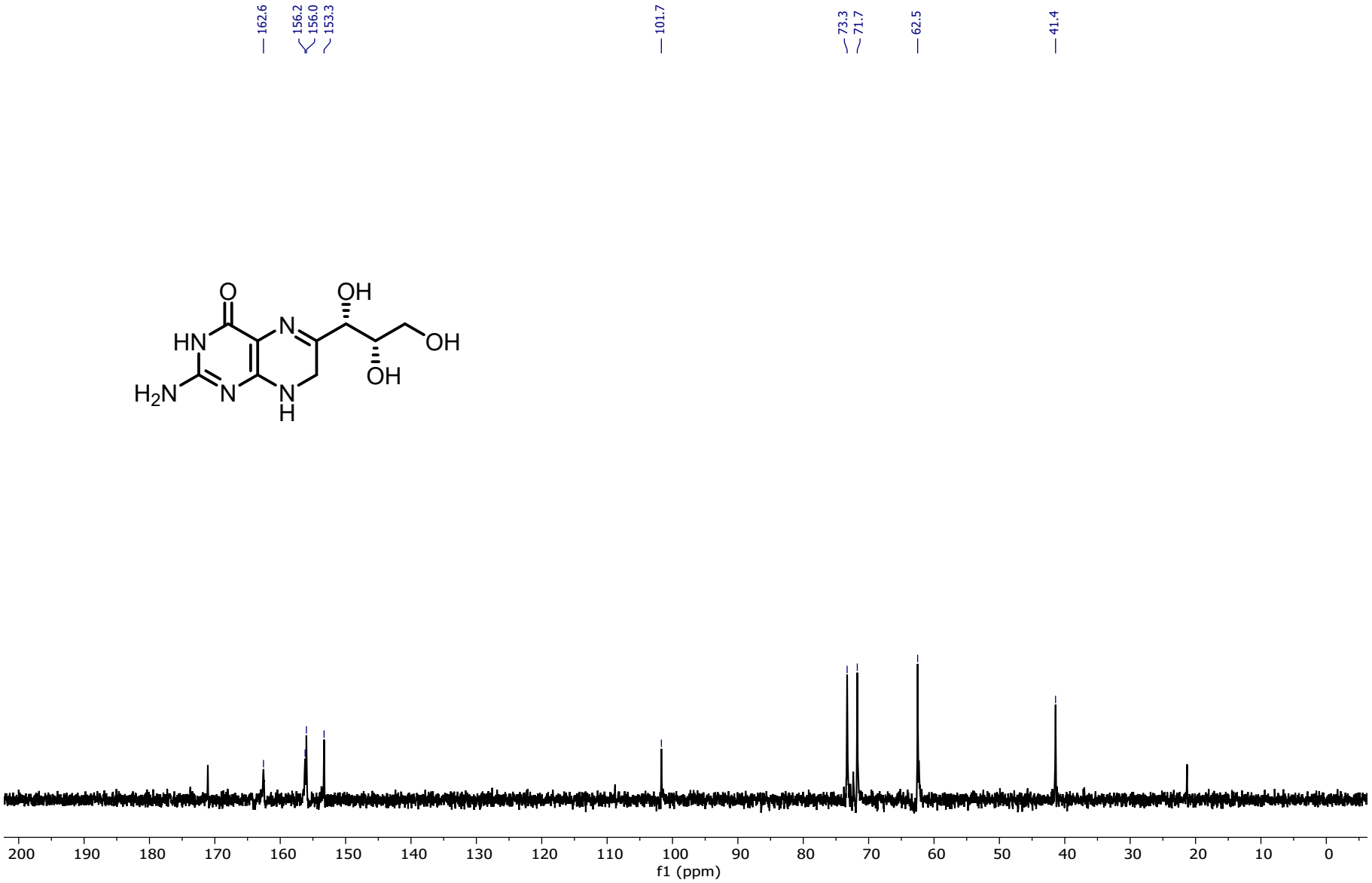
^{1}H NMR Of Compound 4 in DMSO-d6 (125 MHz)


164.78
157.14
156.99
151.86
151.86
148.52
127.76
74.67
72.67
62.95
O
HN
N
OH
OH
OH
H2N
N
N
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

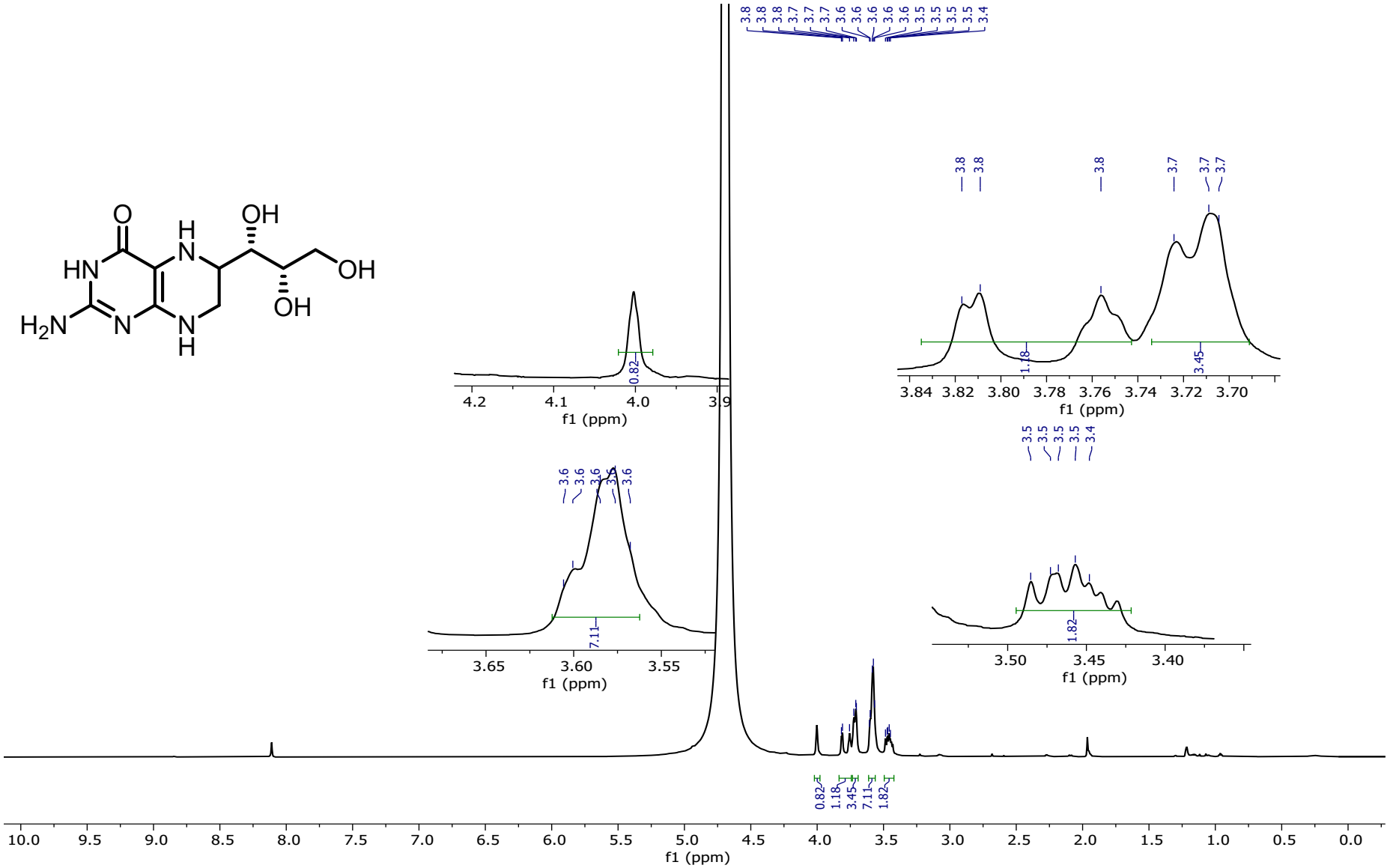
^{13} C NMR Of Compound 4 in DMSO-d6 (125 MHz)



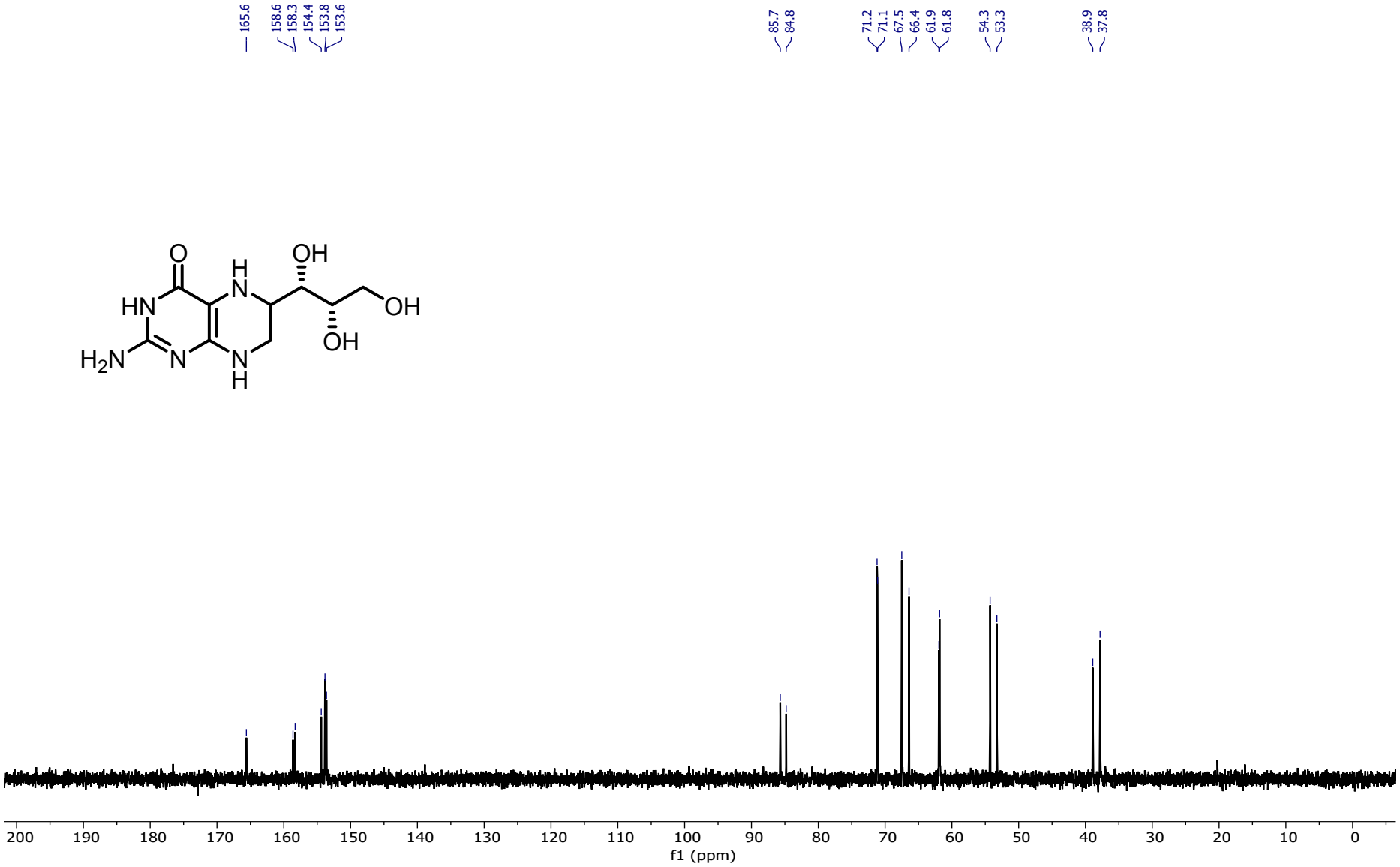
^{1}H NMR Of Compound 5 in D_2O (500 MHz)


162.6
156.2
156.0
153.3
101.7
73.3
71.7
62.5
41.4
O
HN
N
OH
OH
OH
H_2N
N
N
H
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

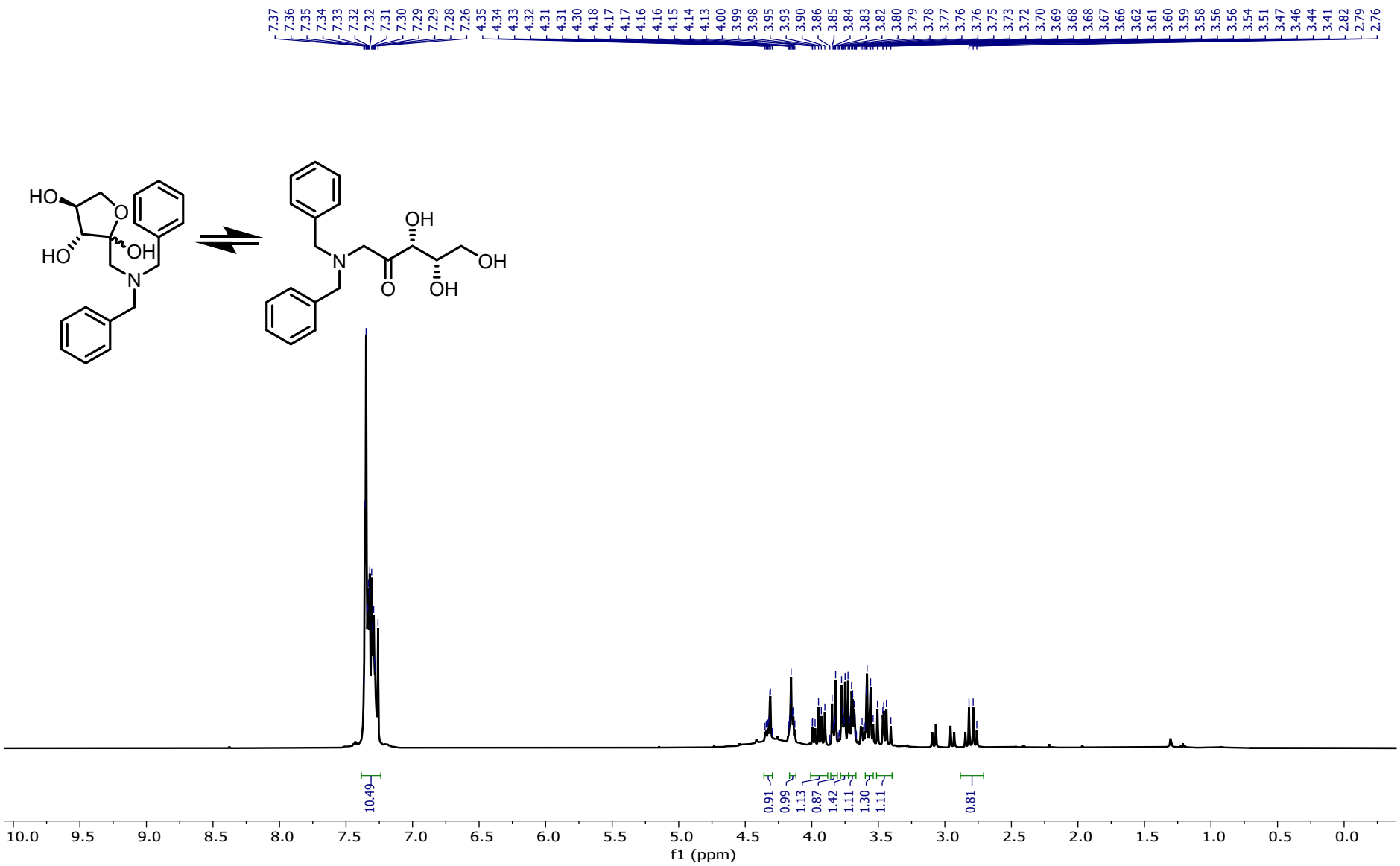
^{13}C NMR Of Compound 5 in D_2O (125 MHz)


O
HN
H
N
OH
OH
OH
H_2N
N
N
H
3.8 3.8 3.8 3.7 3.7 3.6 3.6 3.6 3.6 3.5 3.5 3.5 3.4
0.82
4.2 4.1 4.0 3.9
f1 (ppm)
3.8 3.8 3.8 3.7 3.7 3.7
1.18
3.45
3.84 3.82 3.80 3.78 3.76 3.74 3.72 3.70
f1 (ppm)
3.6 3.6 3.6 3.6 3.6
7.11
3.65 3.60 3.55
f1 (ppm)
3.5 3.5 3.5 3.5 3.4
1.82
3.50 3.45 3.40
f1 (ppm)
0.82 1.18 3.45 7.11 1.82
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

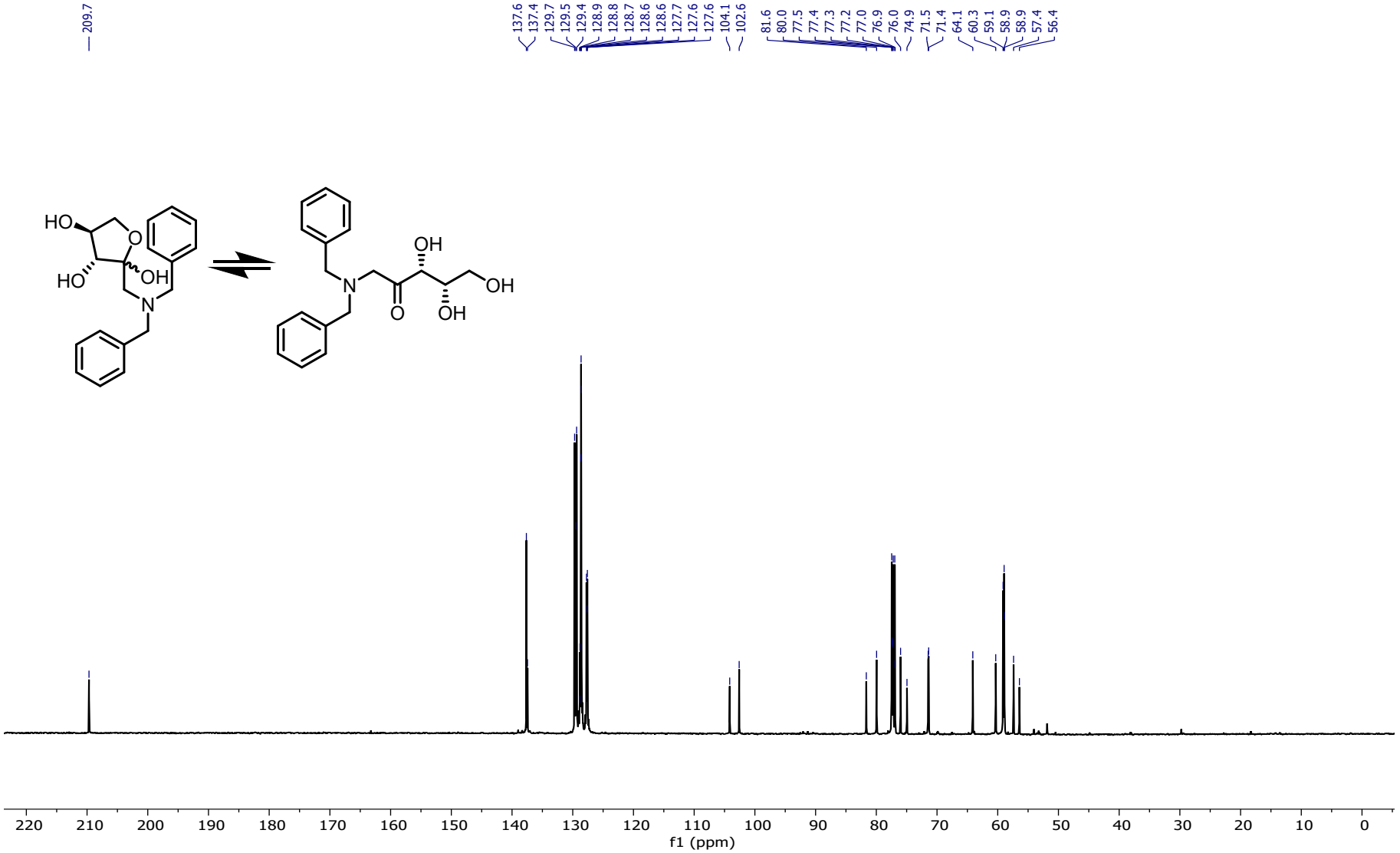
^{1}H NMR Of Compound 6 in D_2O (800 MHz)


165.6
158.6
158.3
154.4
153.8
153.6
85.7
84.8
71.2
71.1
67.5
66.4
61.9
61.8
54.3
53.3
38.9
37.8
O
HN
H2N
N
H
N
H
OH
OH
OH
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

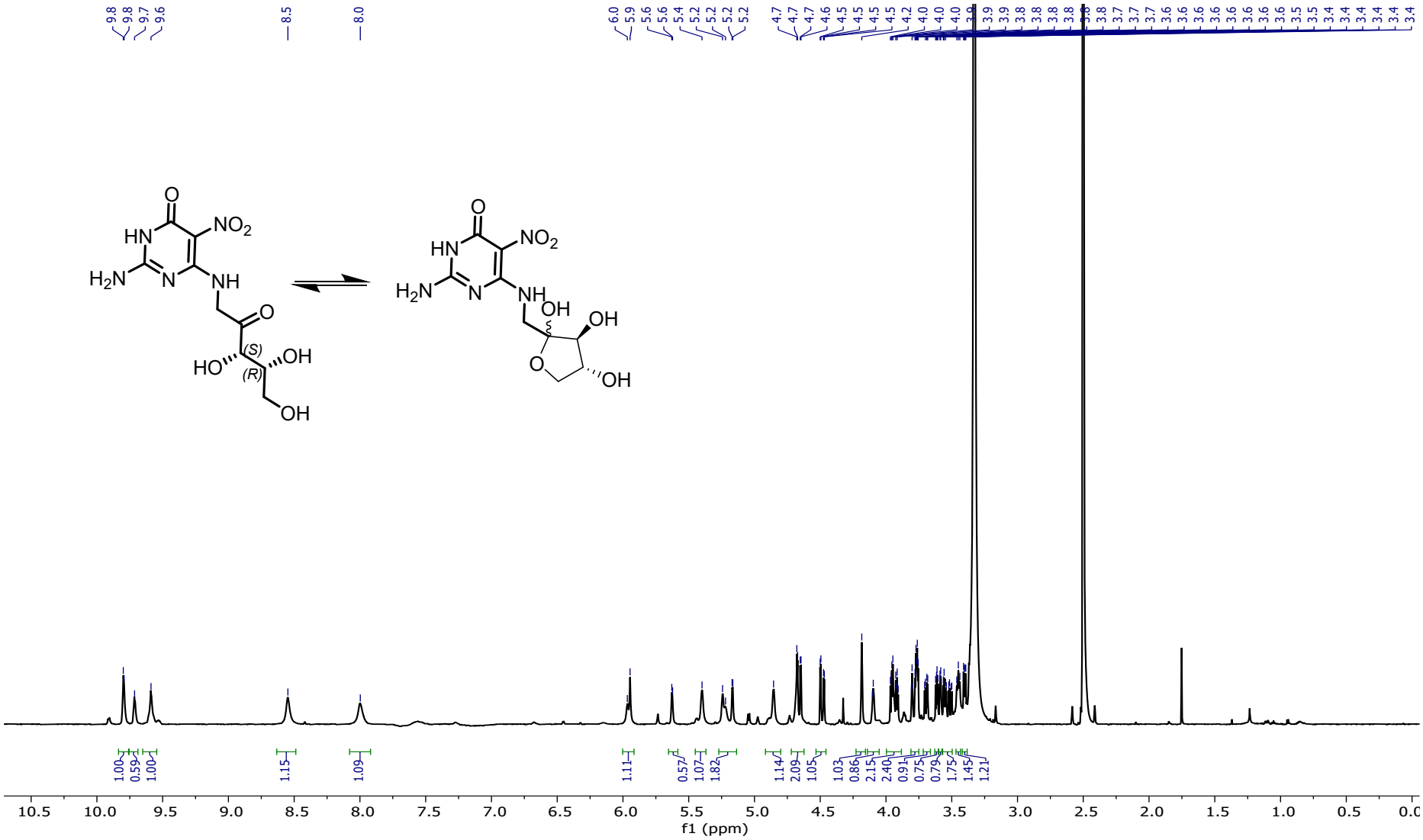
^{13}C NMR Of Compound 6 in D_2O (200 MHz)


7.37 7.36 7.35 7.34 7.33 7.32 7.32 7.31 7.30 7.29 7.29 7.28 7.26 4.35 4.34 4.33 4.32 4.31 4.31 4.30 4.18 4.17 4.17 4.16 4.16 4.15 4.14 4.13 4.00 3.99 3.98 3.95 3.93 3.90 3.86 3.85 3.84 3.83 3.82 3.80 3.79 3.78 3.77 3.76 3.76 3.75 3.73 3.72 3.70 3.69 3.68 3.68 3.67 3.66 3.62 3.61 3.60 3.59 3.58 3.56 3.56 3.54 3.51 3.47 3.46 3.44 3.41 2.82 2.79 2.76
HO
O
HO
OH
N
OH
N
O
OH
OH
10.49
0.91
0.99
1.13
0.87
1.42
1.11
1.30
1.11
0.81
10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0
f1 (ppm)

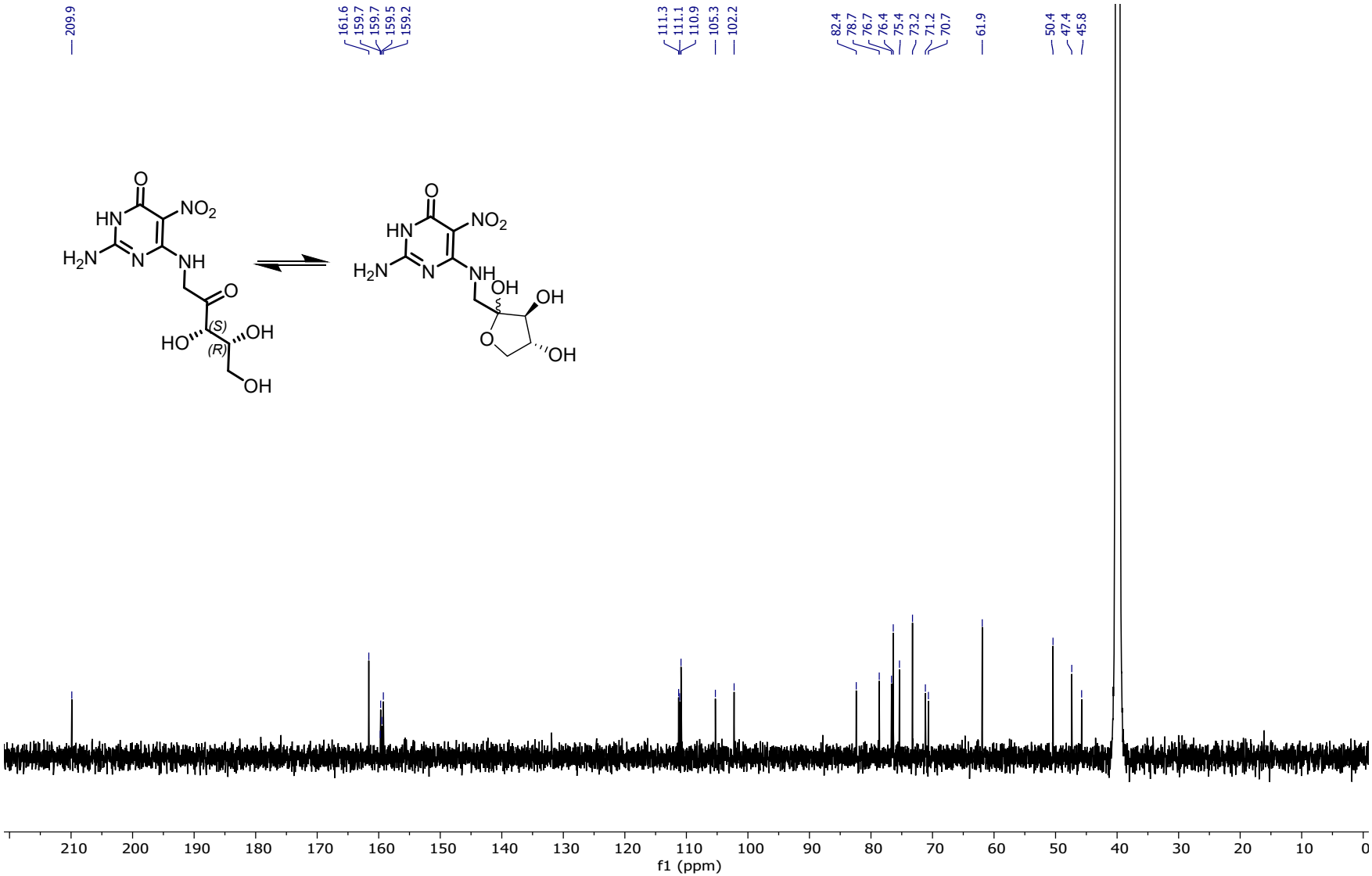
^{1}H NMR Of Compound 13 in $CDCl_3$ (500 MHz)



^{13}C NMR Of Compound 13 in $CDCl_3$ (125 MHz)



^{1}H NMR Of Compound 15 in DMSO-d6 (800 MHz)



^{13}C NMR Of Compound 15 in DMSO-d6 (200 MHz)

RIC-NS-13270_ESI+_MEOH_SN-03-67 #1-36 RT: 0.00-0.50 AV: 36 NL: 8.19E6
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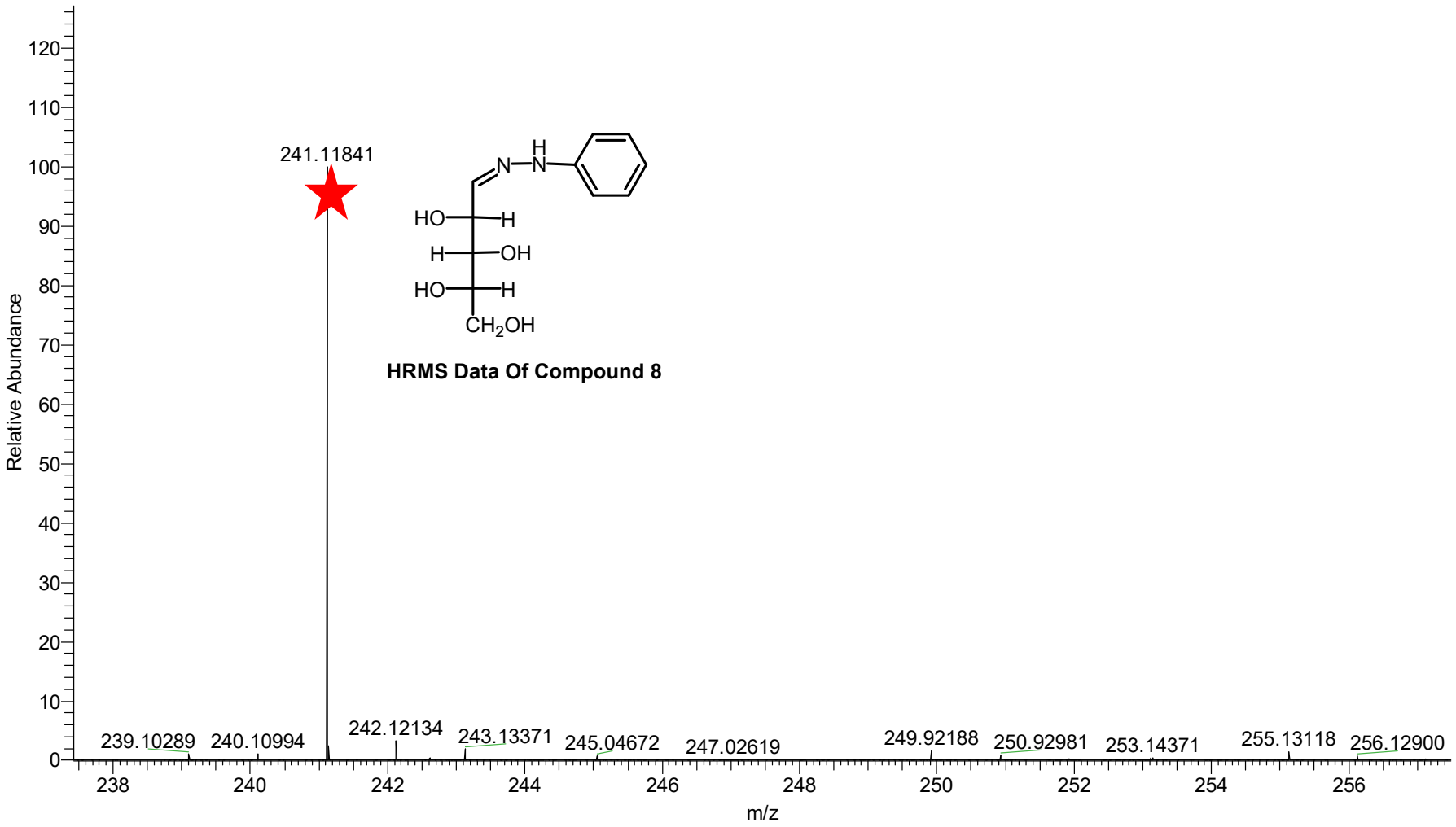
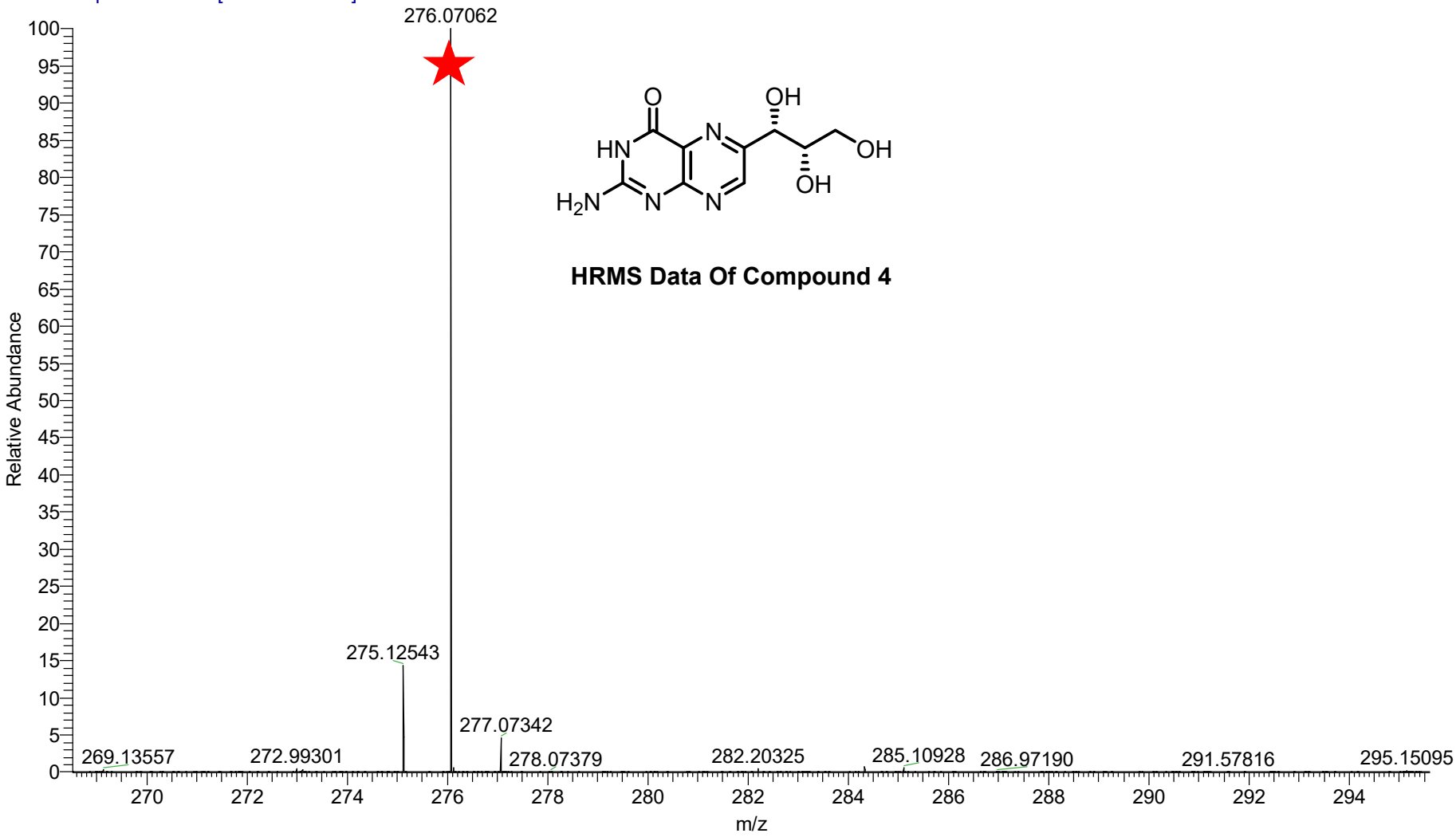


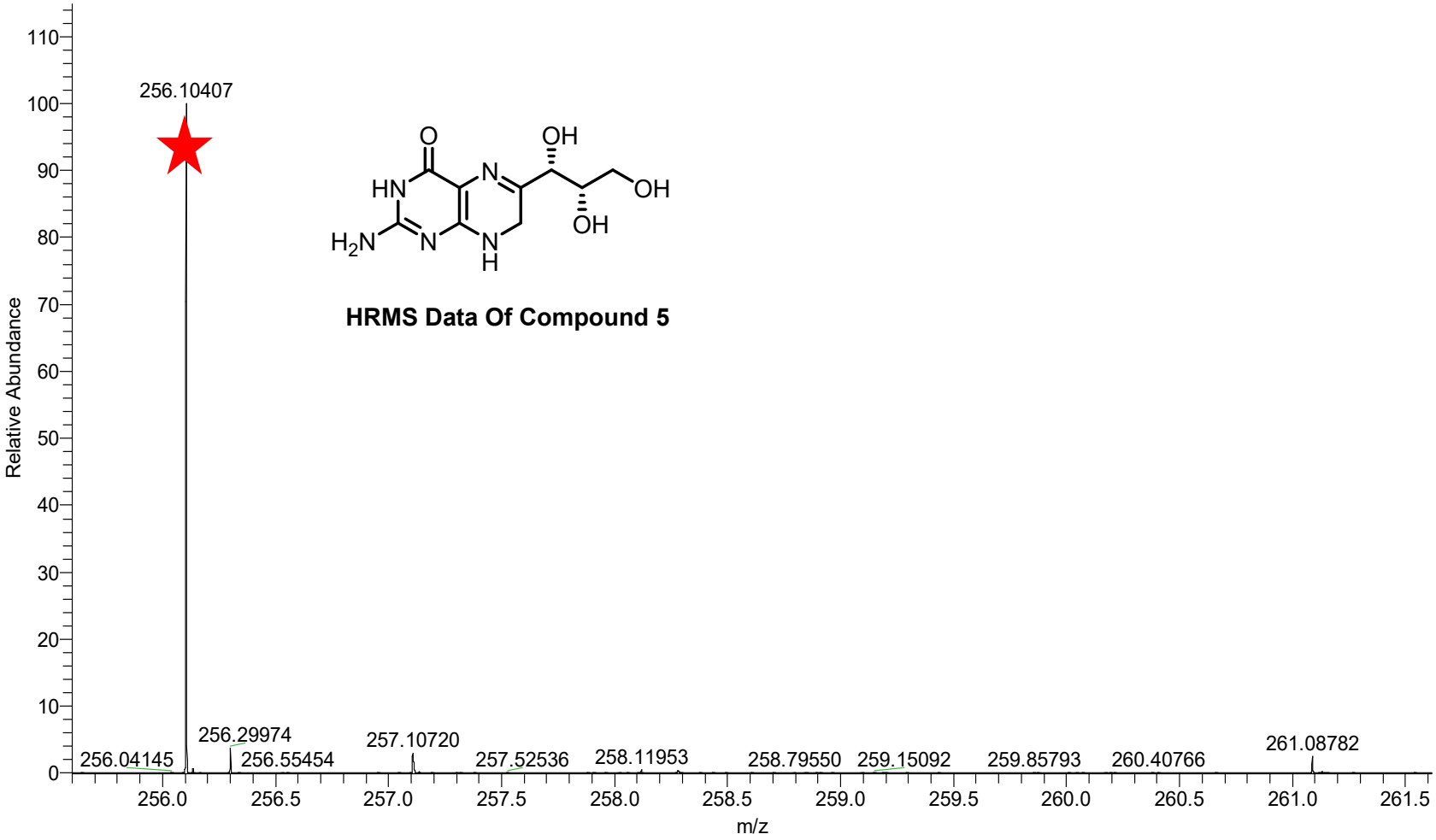
HRMS Data Of Compound 8

RIC-AS-13266_ESI+_MEOH_253 #1-36 RT: 0.00-0.50 AV: 36 NL: 3.87E7
T: FTMS + p ESI Full ms [100.00-800.00]

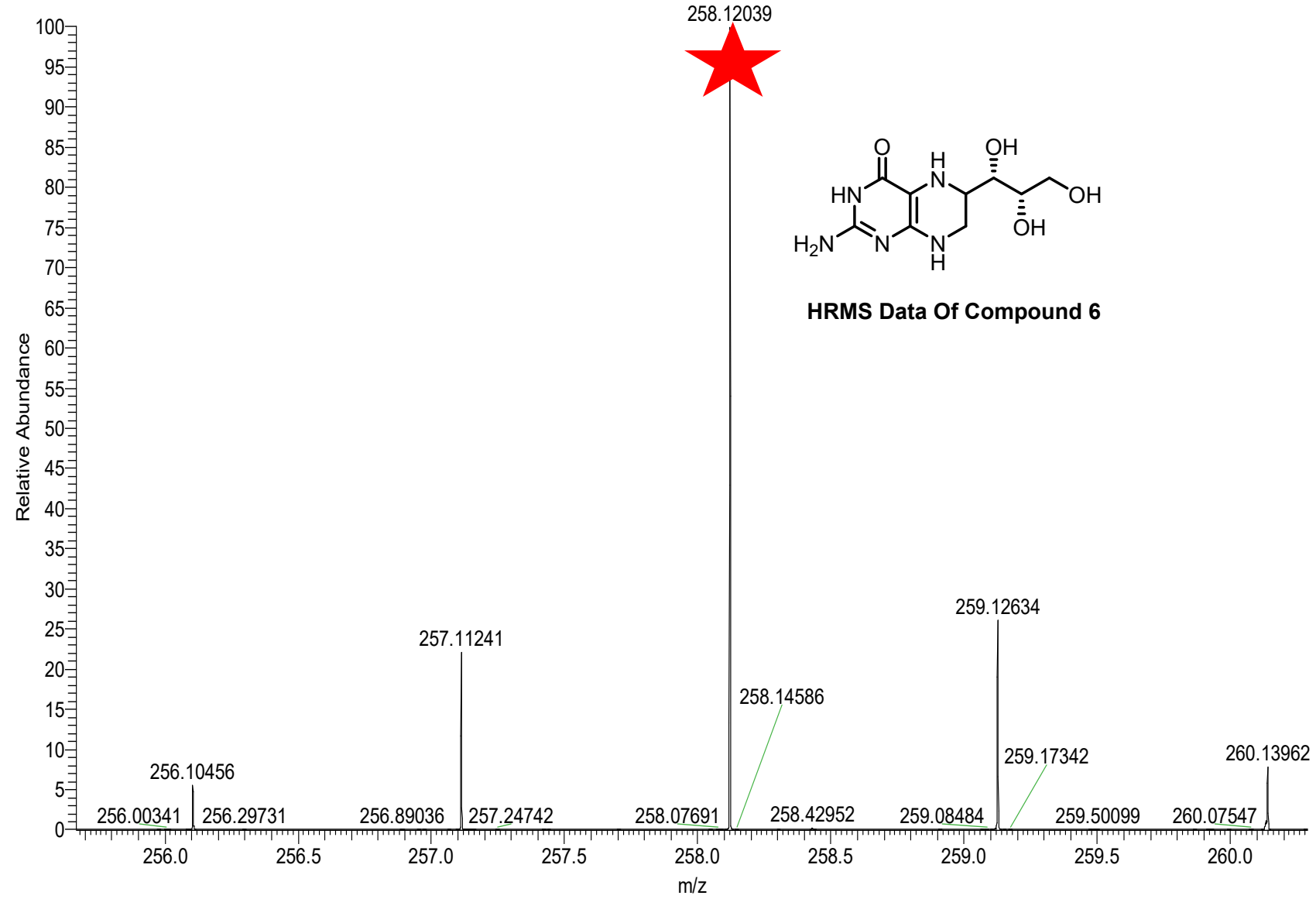


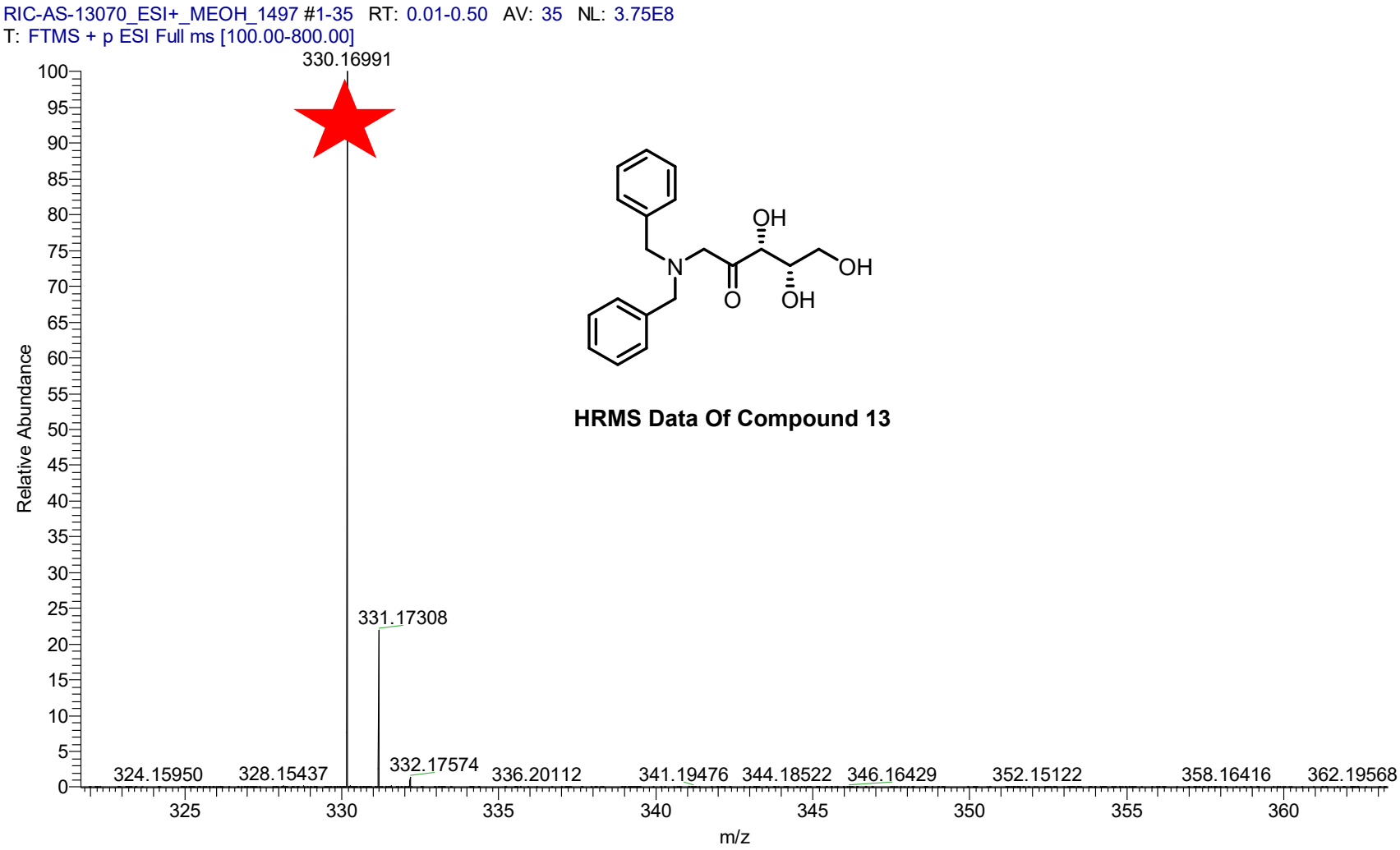
HRMS Data Of Compound 4

RIC-AS-13266_ESI+_MEOH_255 #1-35 RT: 0.01-0.49 AV: 35 NL: 1.96E6
T: FTMS + p ESI Full ms [100.00-800.00]



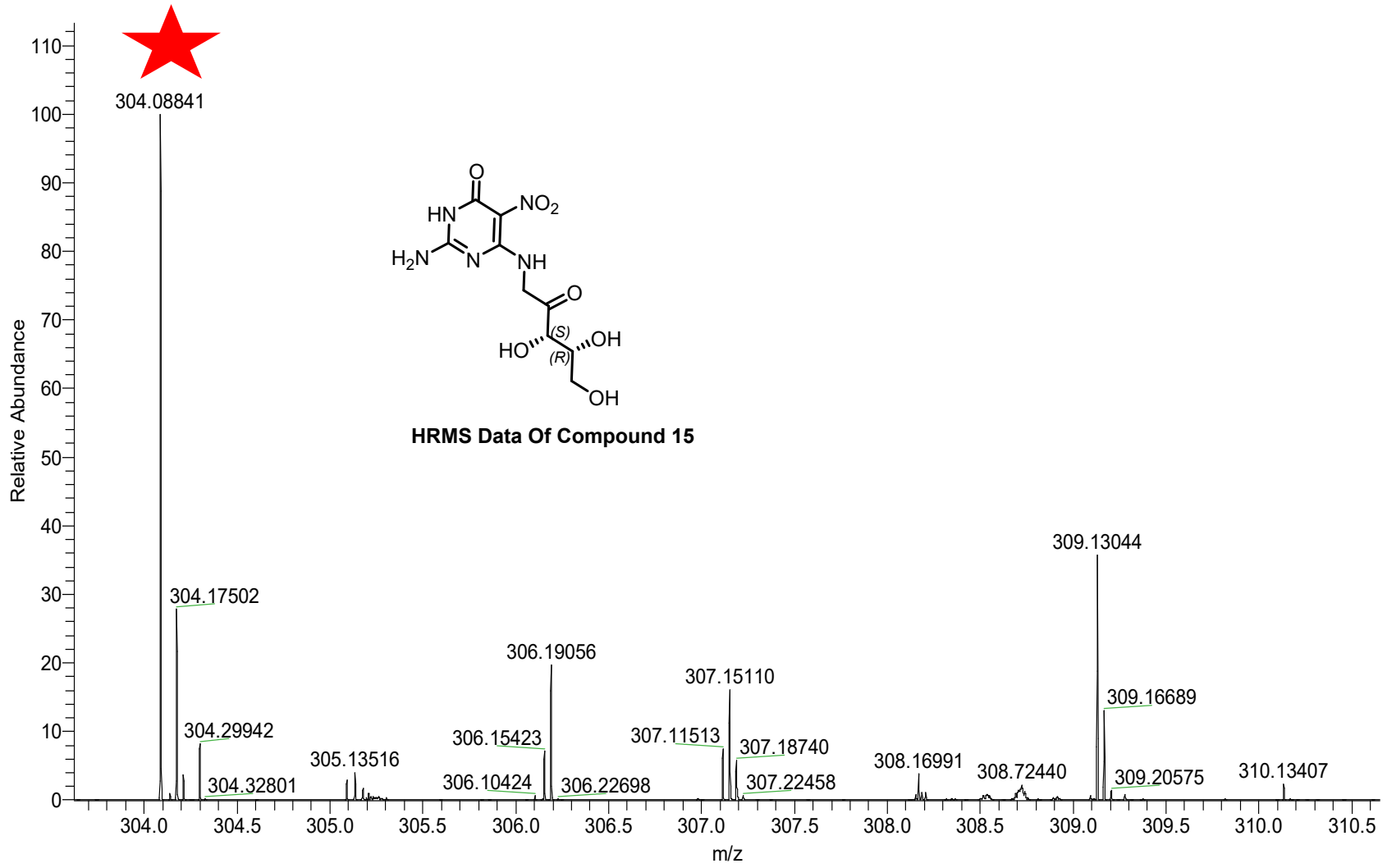
HRMS Data Of Compound 5


RIC-AS-13042_ESI+_direct_ASH-09-1515 #1-30 RT: 0.01-0.45 AV: 30 NL: 1.47E6
T: FTMS + p ESI Full ms [150.00-300.00]
258.12039
HRMS Data Of Compound 6
Relative Abundance
m/z
256.10456
257.11241
258.14586
259.12634
259.17342
260.13962
256.00341
256.29731
256.89036
257.24742
258.07691
258.42952
259.08484
259.50099
260.07547



HRMS Data Of Compound 13

RIC-AS-13070_ESI+_MEOH_1516 #1-35 RT: 0.01-0.49 AV: 35 NL: 1.90E6
T: FTMS + p ESI Full ms [100.00-800.00]



HRMS Data Of Compound 15

Analytical HPLC data of Monapterin (HPLC Purified):

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    Sample Operator : SYSTEM
    Acq. Instrument : LC2                          Location :   1
    Injection Date  : 9/28/2024 1:23:15 PM
                                                   Inj Volume : 5.000 µl
    Acq. Method     : C:\Chem32\1\Methods\CURRENT.M
    Last changed    : 9/28/2024 1:10:08 PM by SYSTEM
                      (modified after loading)
    Analysis Method : C:\Chem32\1\Data\ASH-09-MONAPTERI-HPLC-PURITY 772.D\DA.M (CURRENT.M, From
                      Data File)
    Last changed    : 9/28/2024 2:42:37 PM by SYSTEM
    Sample Info     : YMC-ODSA
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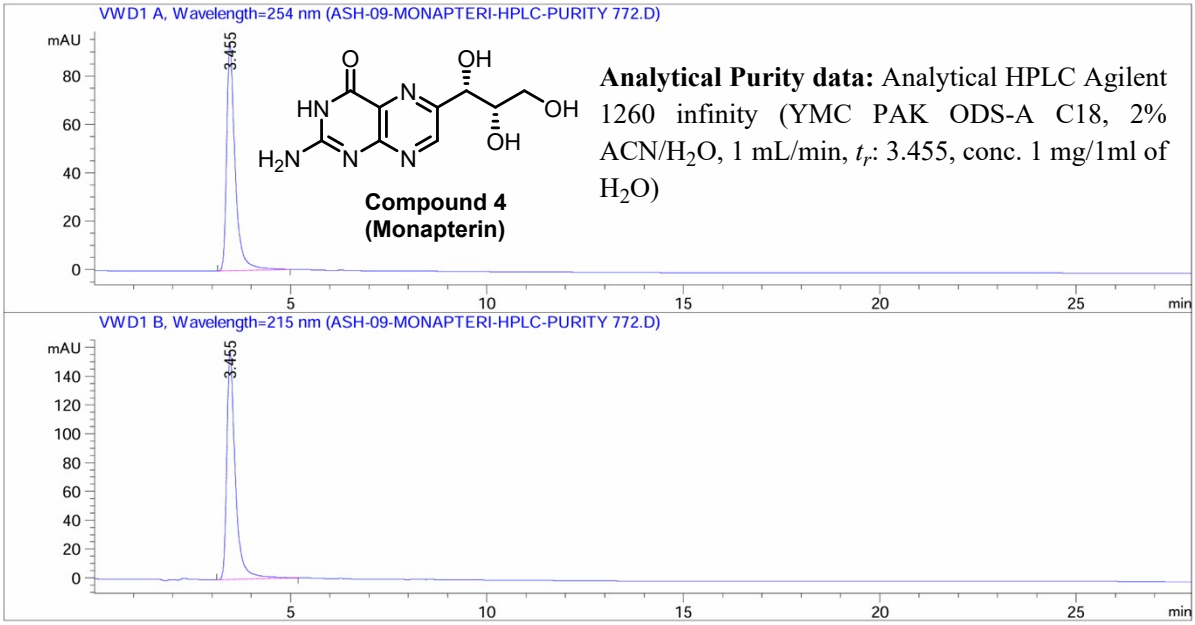
                      1.0 mL/min
                      215  nm, 254 nm
                      5uL inj., 1.0 mg/1.0 ml of water
```



Compound 4 (Monapterin)

Analytical Purity data: Analytical HPLC Agilent 1260 infinity (YMC PAK ODS-A C18, 2% ACN/H_2O, 1 mL/min, t_r: 3.455, conc. 1 mg/1ml of H_2O)

```
    ================================================================================
                              Fraction Information
    ================================================================================
    No Fractions found.
    ================================================================================
    ================================================================================
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Data File C:\Chem32\1\Data\ASH-09-MONAPTERI-HPLC-PURITY 772.D
Sample Name: ASH-09-MONAPTERI-HPLC-PURITY

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Area Percent Report

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Sorted By : Signal
Multiplier : 1.0000
Dilution : 1.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.455	BB	0.2214	1415.17456	94.10886	100.0000

Totals : 1415.17456 94.10886

Signal 2: VWD1 B, Wavelength=215 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.455	VB	0.2231	2405.98389	158.47923	100.0000

Totals : 2405.98389 158.47923

==========

*** End of Report ***

Analytical HPLC data of Dihydromonapterin:

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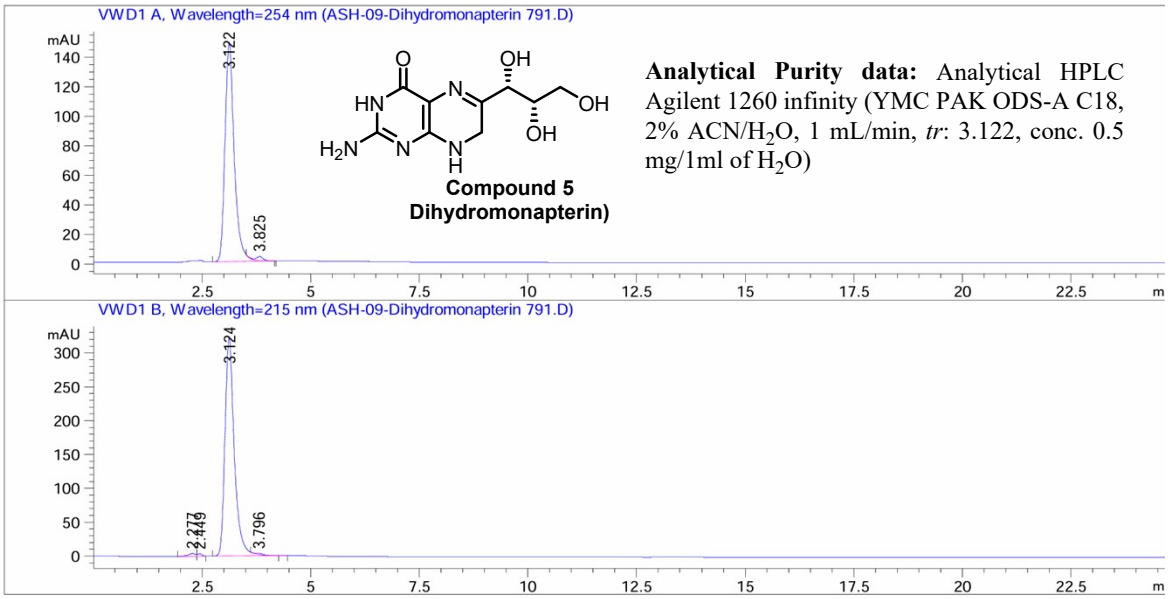
======================================================================
Acq. Operator   : SYSTEM
Sample Operator : SYSTEM
Acq. Instrument : LC2                          Location :   1
Injection Date  : 9/30/2024 5:52:03 PM
                                              Inj Volume : 5.000 µl
Different Inj Volume from Sample Entry!  Actual Inj Volume : 20.000 µl
Method          : C:\Chem32\1\Methods\CURRENT.M
Last changed    : 9/30/2024 5:28:44 PM by SYSTEM
                  (modified after loading)
Sample Info     : YMC-ODSA
                  2% ACN/98%H2O
                  1.0 mL/min
                  215  nm, 254 nm
                  5uL inj., 0.5 mg/1.0 ml of water
```



Compound 5
Dihydromonapterin)

Analytical Purity data: Analytical HPLC Agilent 1260 infinity (YMC PAK ODS-A C18, 2% ACN/H_2O, 1 mL/min, *tr*: 3.122, conc. 0.5 mg/1ml of H_2O)

```
================================================================================
                          Fraction Information
================================================================================
No Fractions found.
================================================================================
================================================================================
======================================================================
                        Area Percent Report
======================================================================

Sorted By             :      Signal
Multiplier            :      1.0000
Dilution              :      1.0000
Sample Amount:        :      1.00000  [ng/ul]   (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs
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Data File C:\Chem32\1\Data\ASH-09-Dihydromonapterin 791.D
Sample Name: ASH-09-Dihydromonapterin

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.122	BV R	0.2247	2196.68066	148.36096	98.4279
2	3.825	VB E	0.1669	35.08458	2.98938	1.5721
Totals :				2231.76525	151.35034	

Signal 2: VWD1 B, Wavelength=215 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	2.277	BV	0.1634	49.97865	4.30703	1.0125
2	2.449	VB	0.1057	24.98795	3.56917	0.5062
3	3.124	BV R	0.2245	4839.72412	323.63531	98.0491
4	3.796	VB E	0.1607	21.33151	1.90399	0.4322
Totals :				4936.02223	333.41552	

*** End of Report ***

Analytical HPLC data of compound 15 (HPLC Purified):

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Sample Operator  : SYSTEM
Acq. Instrument  : LC2                          Location :   1
Injection Date   : 9/28/2024 6:58:35 PM
                                                Inj Volume : 20.000 µl
Acq. Method      : C:\Chem32\1\Methods\CURRENT.M
Last changed     : 9/28/2024 6:57:13 PM by SYSTEM
                   (modified after loading)
Analysis Method  : C:\Chem32\1\Data\ASH-09-1515-INTERMEDIATE-PURITY 783.D\DA.M (CURRENT.M,
                   From Data File)
Last changed     : 9/28/2024 7:19:40 PM by SYSTEM
Sample Info      : YMC-ODSA
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                   1.0 mL/min
                   215  nm, 254 nm
                   5uL inj., 0.5 mg/1.0 ml of water
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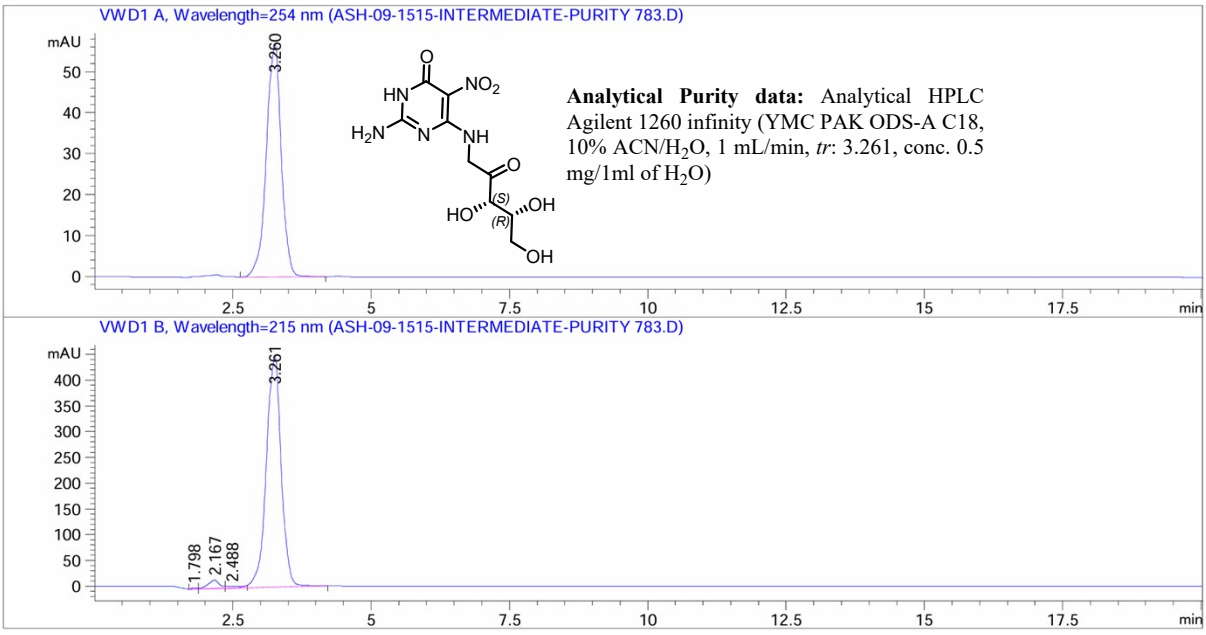

Analytical Purity data: Analytical HPLC Agilent 1260 infinity (YMC PAK ODS-A C18, 10% ACN/H_2O, 1 mL/min, *tr*: 3.261, conc. 0.5 mg/1ml of H_2O)

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No Fractions found.
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Data File C:\Chem32\1\Data\ASH-09-1515-INTERMEDIATE-PURITY 783.D
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Area Percent Report

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Sorted By : Signal
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Dilution : 1.0000
Sample Amount: : 1.00000 [ng/ul] (not used in calc.)
Use Multiplier & Dilution Factor with ISTDs

Signal 1: VWD1 A, Wavelength=254 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	3.260	BB	0.2899	1069.18726	56.71944	100.0000
Totals :				1069.18726	56.71944	

Signal 2: VWD1 B, Wavelength=215 nm

Peak #	RetTime [min]	Type	Width [min]	Area [mAU*s]	Height [mAU]	Area %
1	1.798	BV E	0.0928	15.63502	2.65137	0.1792
2	2.167	VV E	0.2067	227.08672	16.28590	2.6033
3	2.488	VV E	0.2292	62.42081	3.65579	0.7156
4	3.261	VB R	0.2892	8417.91699	448.08588	96.5019
Totals :				8723.05954	470.67894	

==========

*** End of Report ***