Chemical Science

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Synthesis, Broad Spectrum Antibacterial Activity, and X-ray Co crystal Structure of the Decoding Bacterial Ribosomal A-Site with 4'-Deoxy-4'-Fluoro Neomycin Analogs

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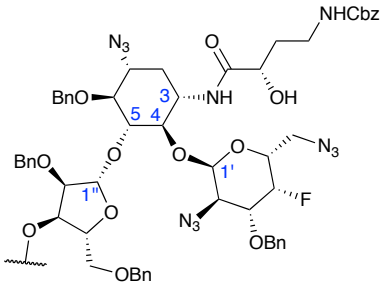
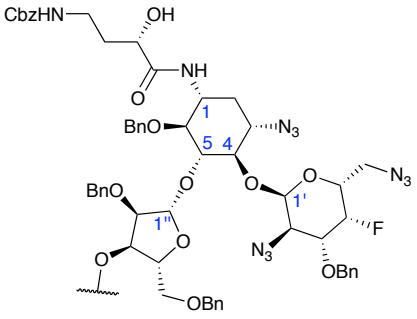
Table S1 Chemical shifts for ring B protons; compounds **16a** and **16b** *

	16a ppm	**16b** ppm
H-1	3.62	4.03
H-2ax	1.46	1.53
H-2eq	2.17	2.30
H-3	4.11	3.62
H-4	3.75	3.76
H-5	4.03	4.03
H-6	3.41	3.45
C-1	47.8	-
C-3	-	46.7

*700 MHz, $CDCl_3$.



16a



16b

Figure S1 selected HMBC correlations observed for **16a**: $^2J_{C\text{-}3,NH,}$ $^3J_{C\text{-}1',H\text{-}4,}$ $^2J_{C\text{-}1'',H\text{-}5.}$ For **16b**: $^2J_{C\text{-}1,NH,}$ $^3J_{C\text{-}1',H\text{-}4,}$ $^2J_{C\text{-}1'',H\text{-}5}$.

Table S2 ^{15}N NMR α-N's chemical shifts for compounds **9** and **10** in ppm.

Entry		**9**	**10**
2.1	α-*N*6', α-*N*2''' and α-*N*6'''	66.7, 67.2 and 67.5	66.7, 67.1 and 67.2
2.2	α-*N*2'	71.5	70.4
2.3	α-*N*1	76.9	76.7
2.4	α-*N*3	79.1	79.0

Natural abundance ^{15}N NMR spectra.

Interestingly the deoxyfluorination reaction causes a 0.9 ppm downfield displacement in the chemical shift of α-*N*2' (Table S2). So the overall effect observed by Sn_2 substitution of the 4'-OH in **10** by fluorine is to shield its α-*N*2' by 0.9 ppm.

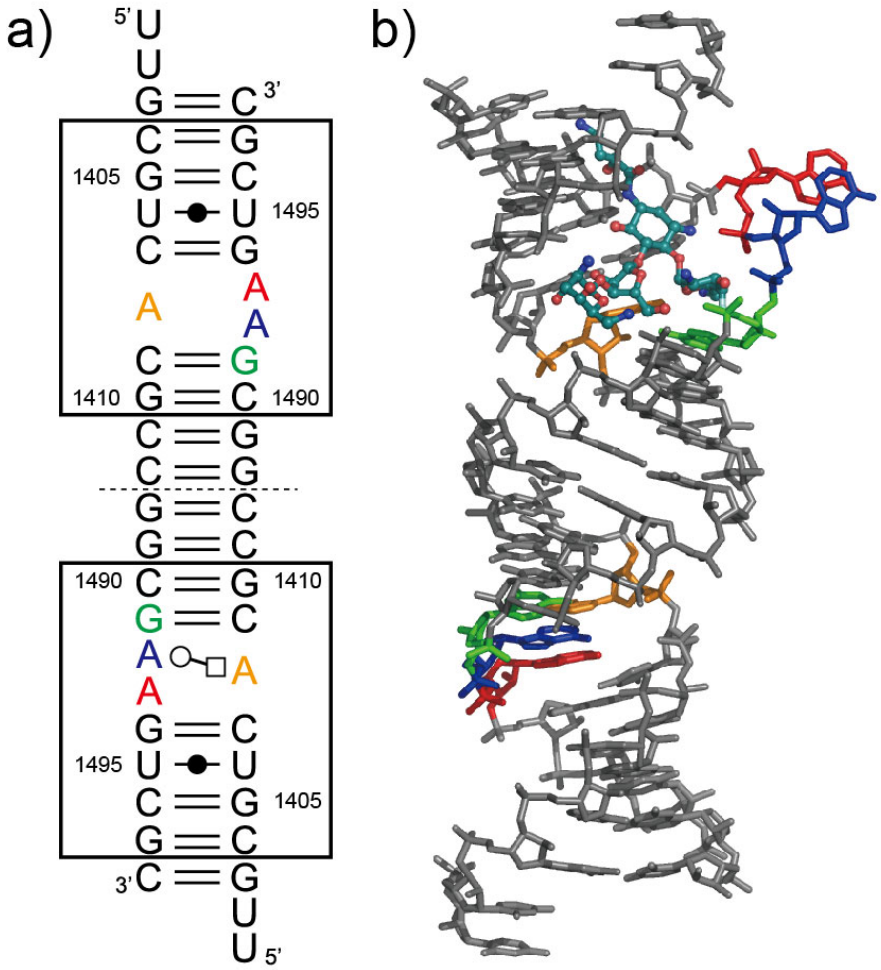


Figure S2 2D **(a)** and 3D **(b)** structures of Bact/**17**. The A1408, G1491, A1492 and A1493 residues important for aminoglycoside binding are colored in orange, green, blue and red, respectively.

Table S3. Crystallization condition

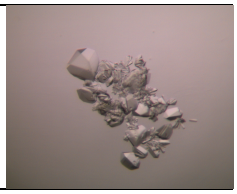
Crystal code	Bact/**17**
Temperature	293 K
RNA solution (1 µl)	
RNA	1 mM
Antibiotic **17**	2 mM
Crystallization solution (1µl)	
Sodium cacodylate (pH = 7.0)	50 mM
Spermine tetrahydrochloride	1 mM
Calcium chloride	10 mM
2-Methyl-2,4-pentanediol	1%
Reservoir solution (250 µl)	
2-Methyl-2,4-pentanediol	40%
Crystals	

Table S4. Crystal data, statistics of data collection and structure refinement

Crystal code	Bact/**17**
Crystal data	
Space group	*R*3
Unit cell (Å)	$a = b = 103.7$, $c = 44.6$
Z [a]	1
Data collection	
Beamline	BL-17A of PF
Wavelength (Å)	0.98
Resolution (Å)	19.6-3.0
of the outer shell (Å)	3.1-3.0
Unique reflections	3522
Completeness (%)	98.7
in the outer shell (%)	100.0
R_{merge} [b] (%)	8.9
in the outer shell (%)	34.3
Redundancy	5.6
in the outer shell	5.7
Structure refinement	
Resolution range (Å)	19.6-3.0
Used reflections	3522
R-factor [c] (%)	19.4
R_{free} [d] (%)	23.6
Number of antibiotic (**17**)	1
Number of Ca^{2+} ion	2
Number of water	1
R.m.s.d. bond length (Å)	0.006
R.m.s.d. bond angles (°)	1.1

[a] Number of RNA duplex in the asymmetric unit. [b] $R_{merge} = 100 \times \Sigma_{hklj}|I_{hklj} - \langle I_{hklj}\rangle| / \Sigma_{hklj}\langle I_{hklj}\rangle$. [c] R-factor $= 100 \times \Sigma||F_o| - |F_c|| / \Sigma|F_o|$, where $|F_o|$ and $|F_c|$ are optimally scaled observed and calculated structure factor amplitudes, respectively. [d] Calculated using a random set containing 10% of observations.

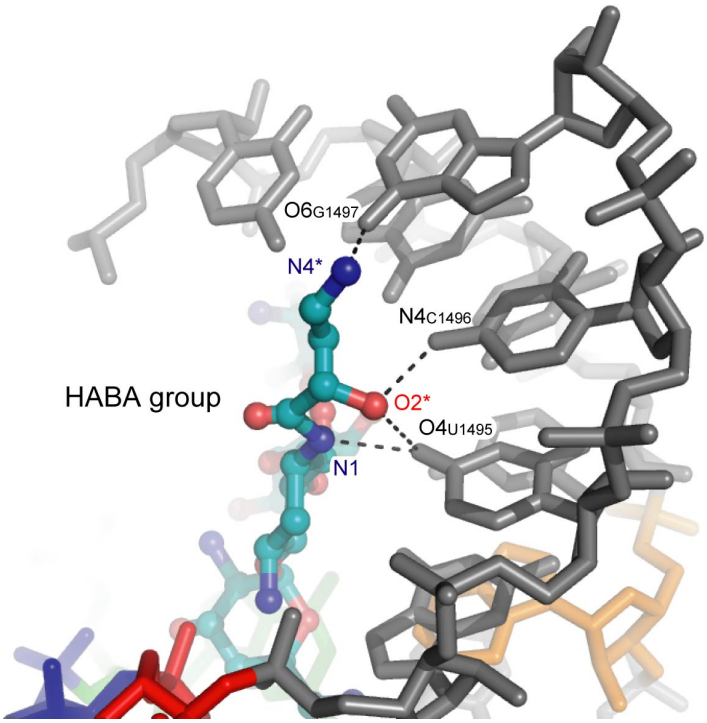
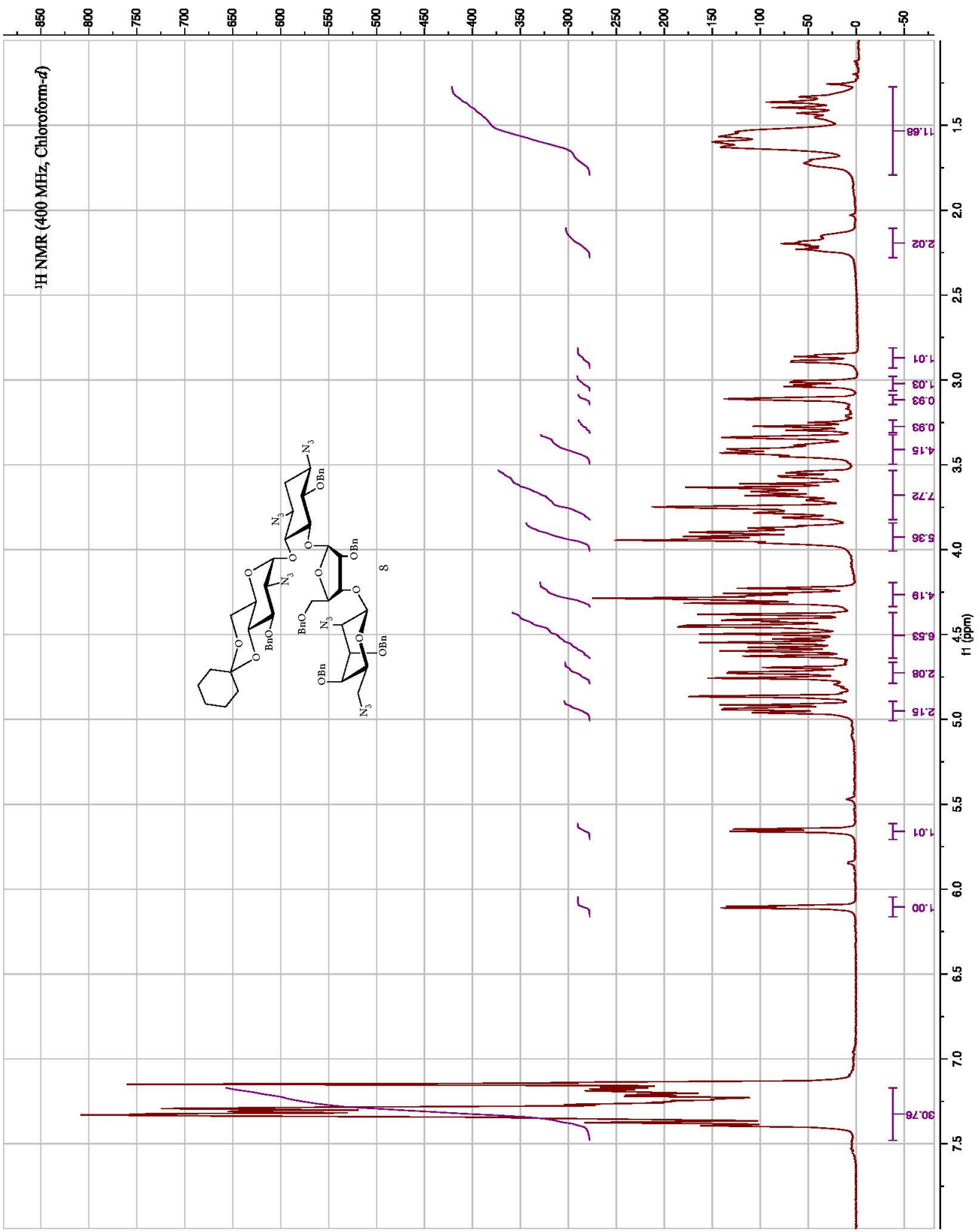
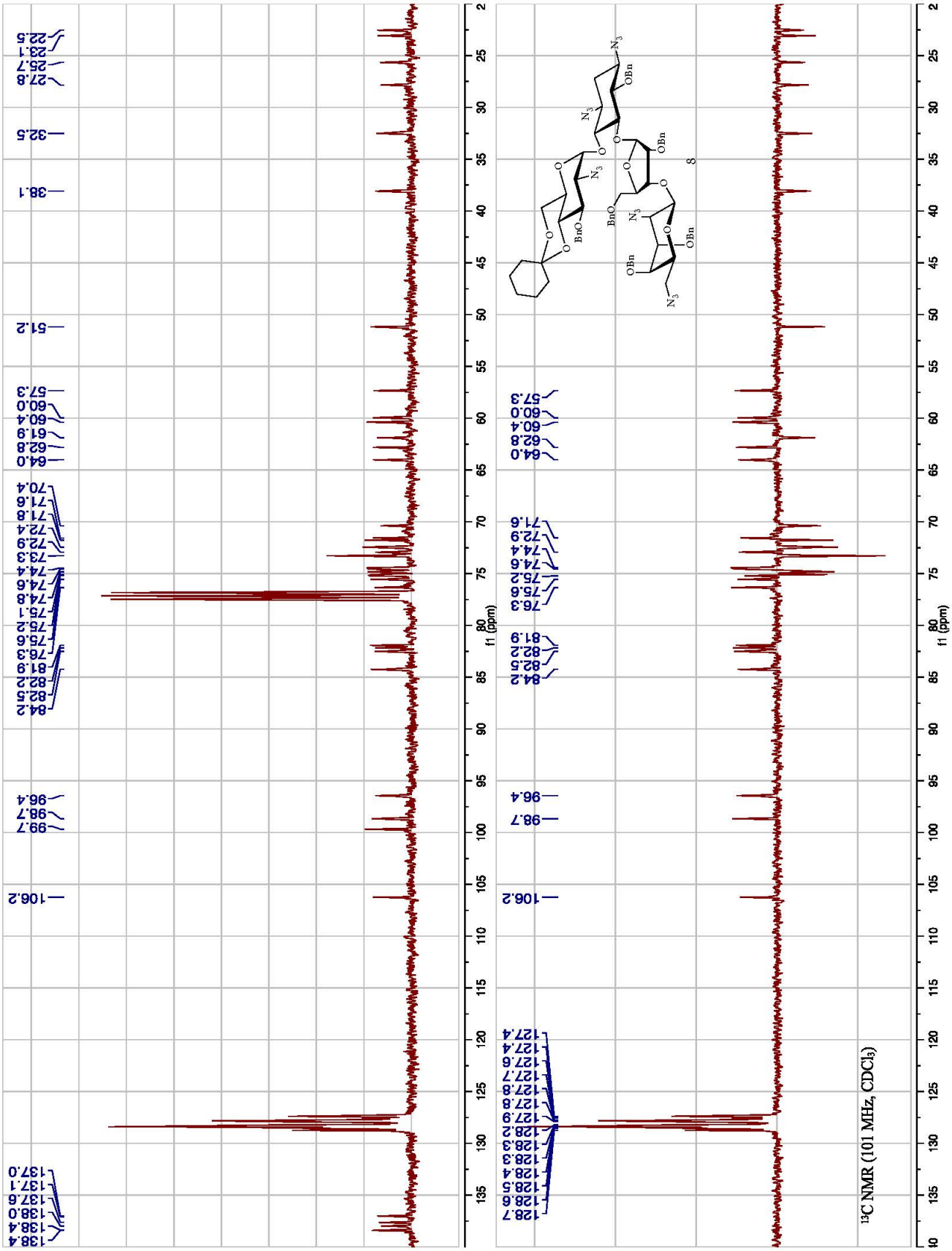
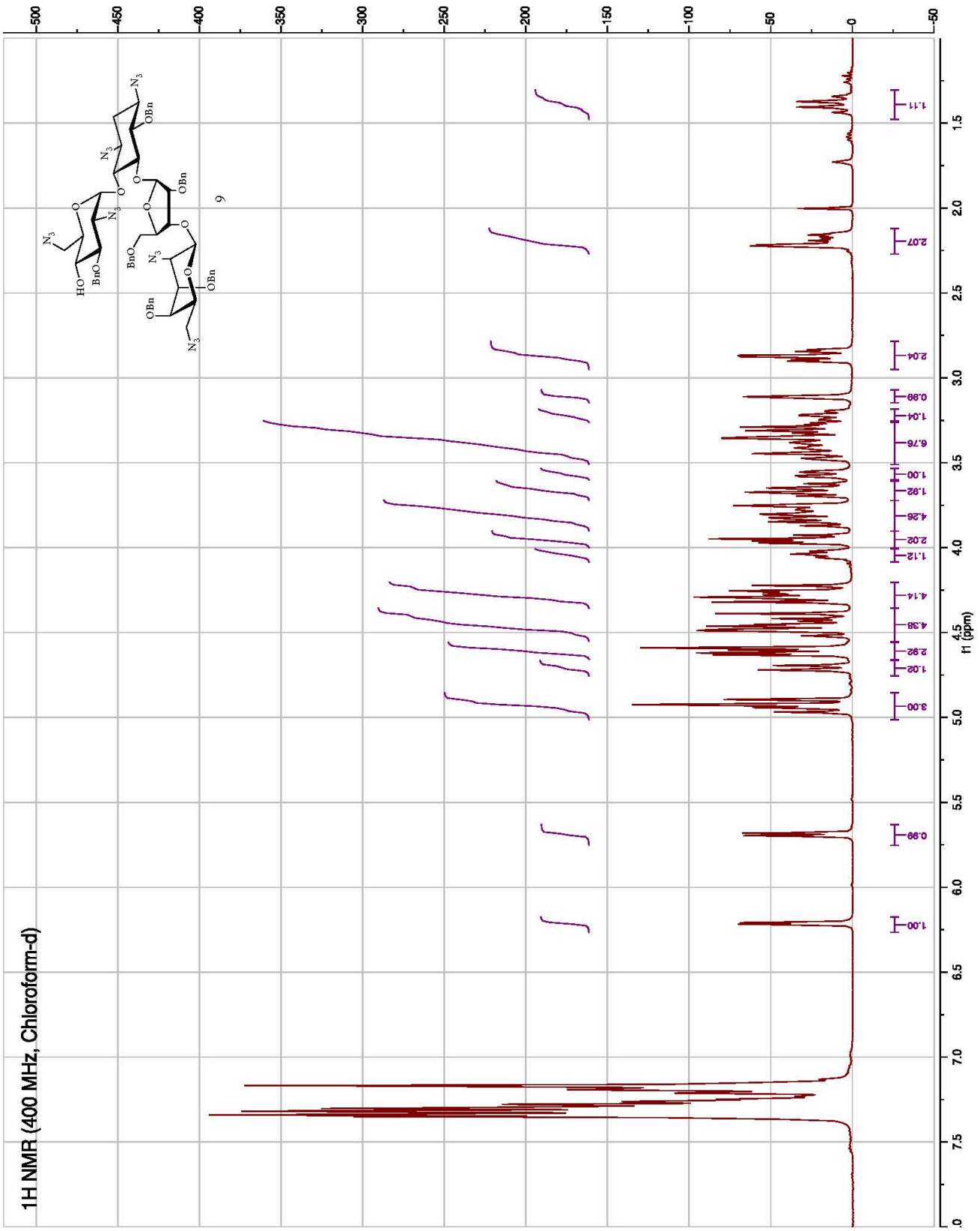
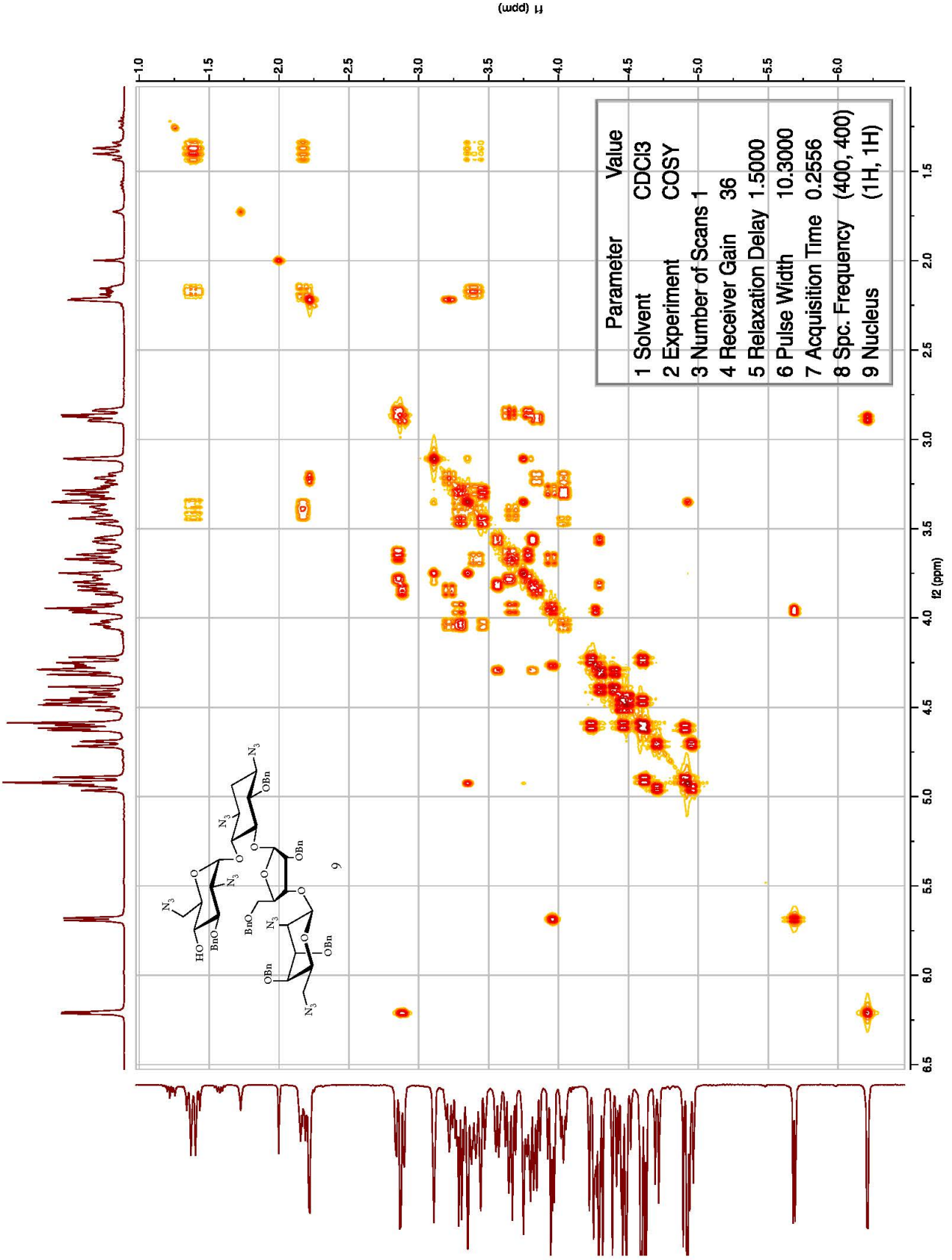


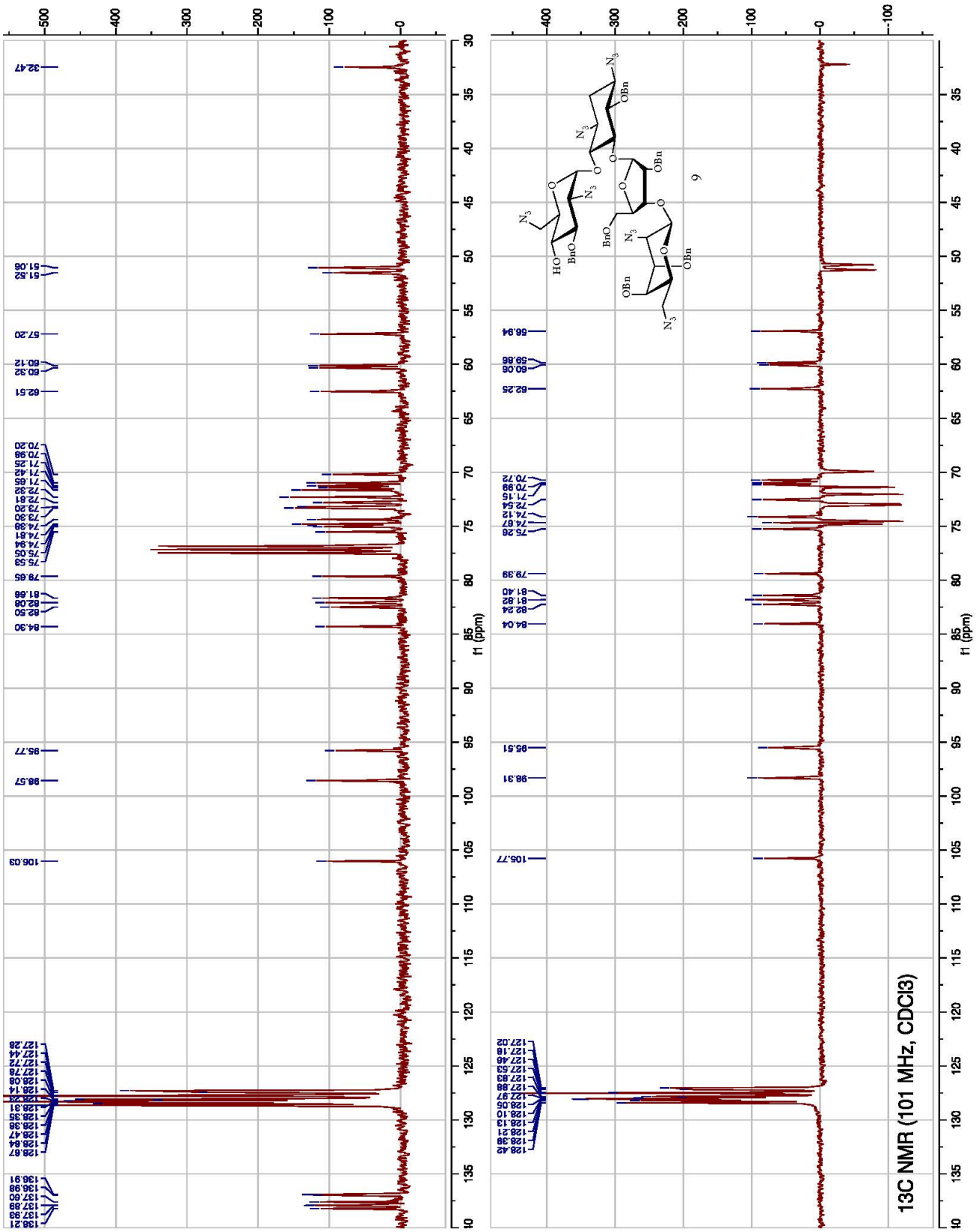
Figure S3 Interaction of the HABA group in antibiotic **17** to the bacterial A-site helix. Hydrogen bonds are shown in red dashed lines.

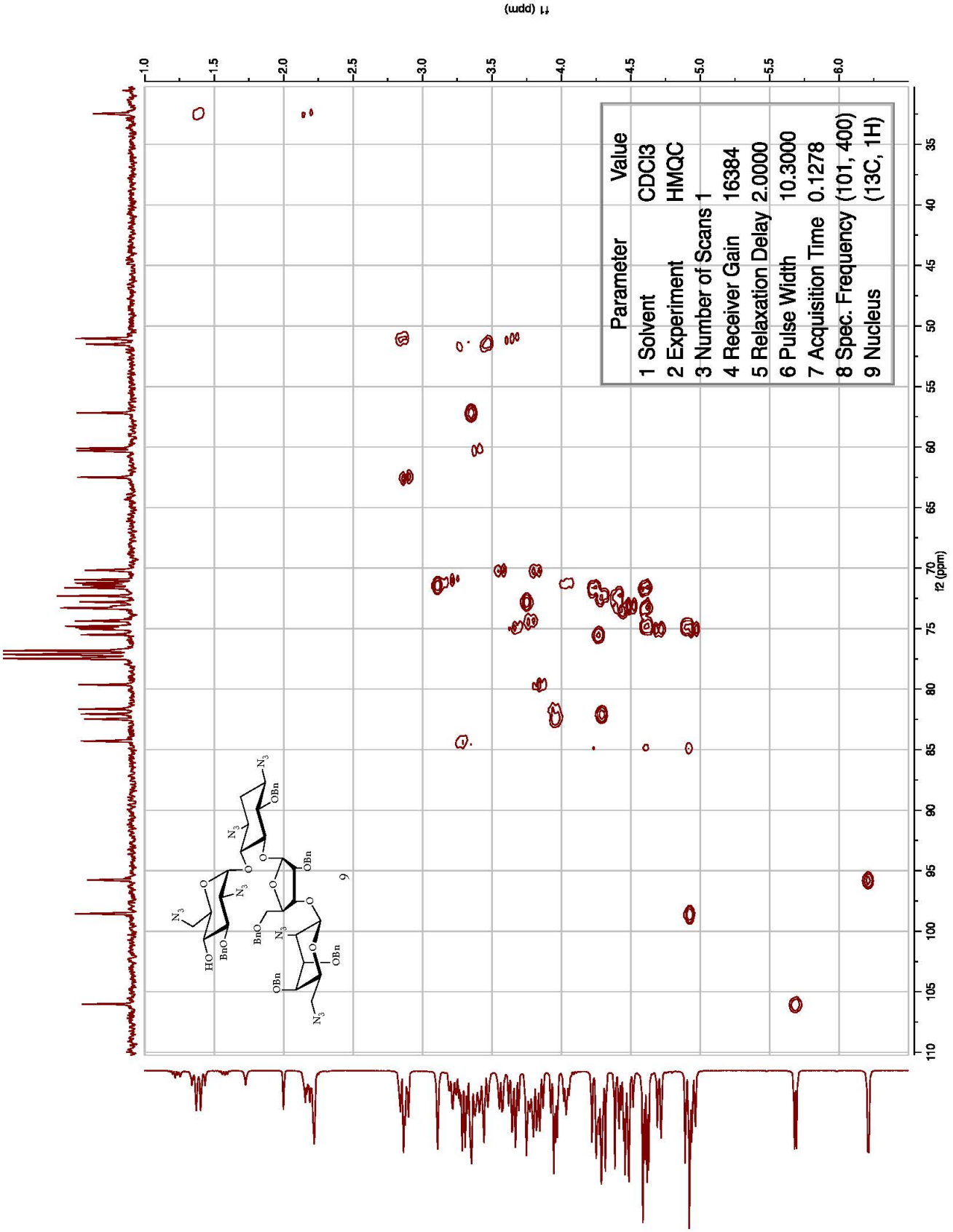

^{1}H NMR (400 MHz, Chloroform-d)
8
11.68
2.02
1.01
1.03
0.93
0.93
4.15
7.72
5.36
4.18
6.53
2.08
2.15
1.01
1.00
30.76
f1 (ppm)

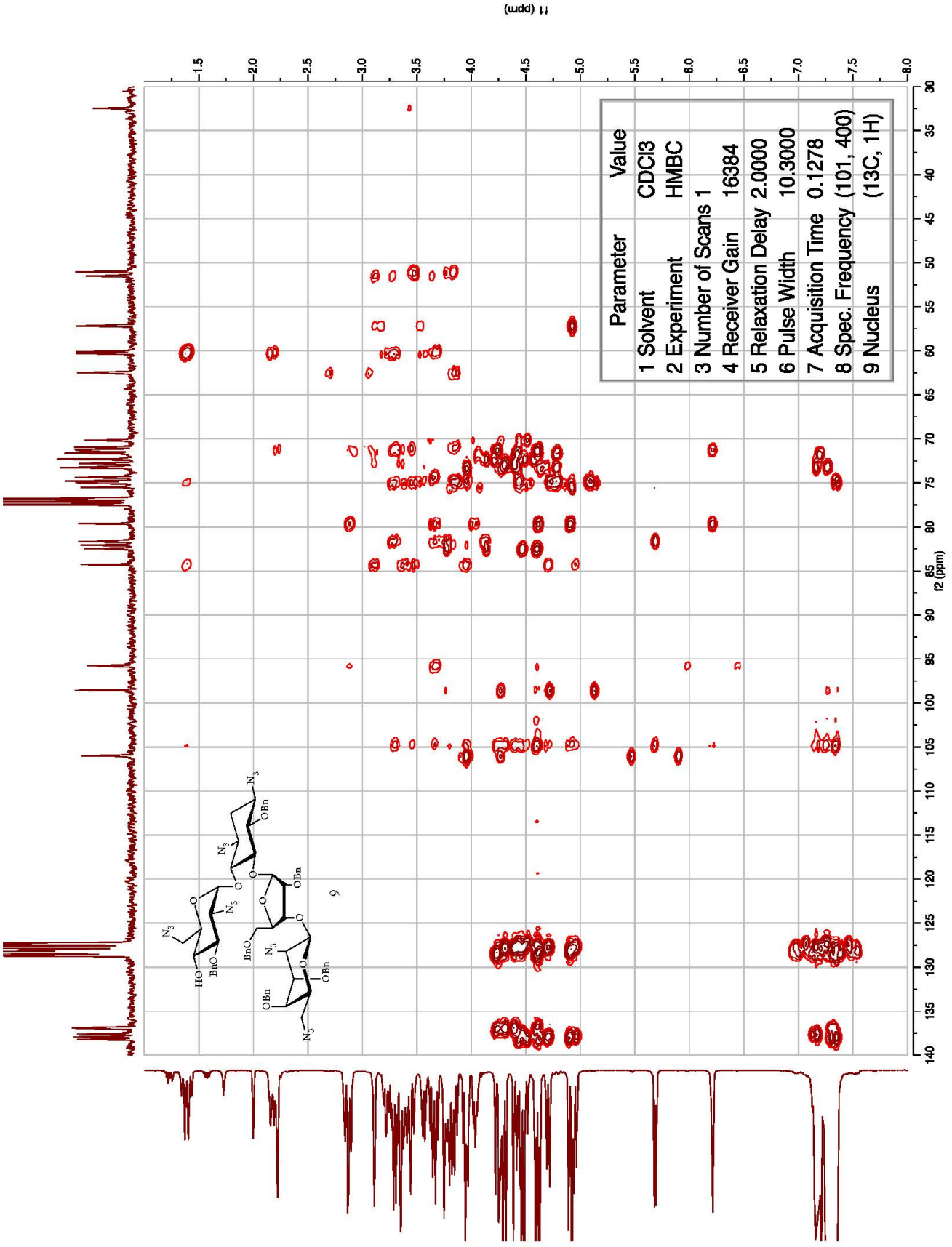



1H NMR (400 MHz, Chloroform-d)
9
f1 (ppm)




13C NMR (101 MHz, CDCl3)
f1 (ppm)
9




f1 (ppm)
f2 (ppm)
Parameter Value
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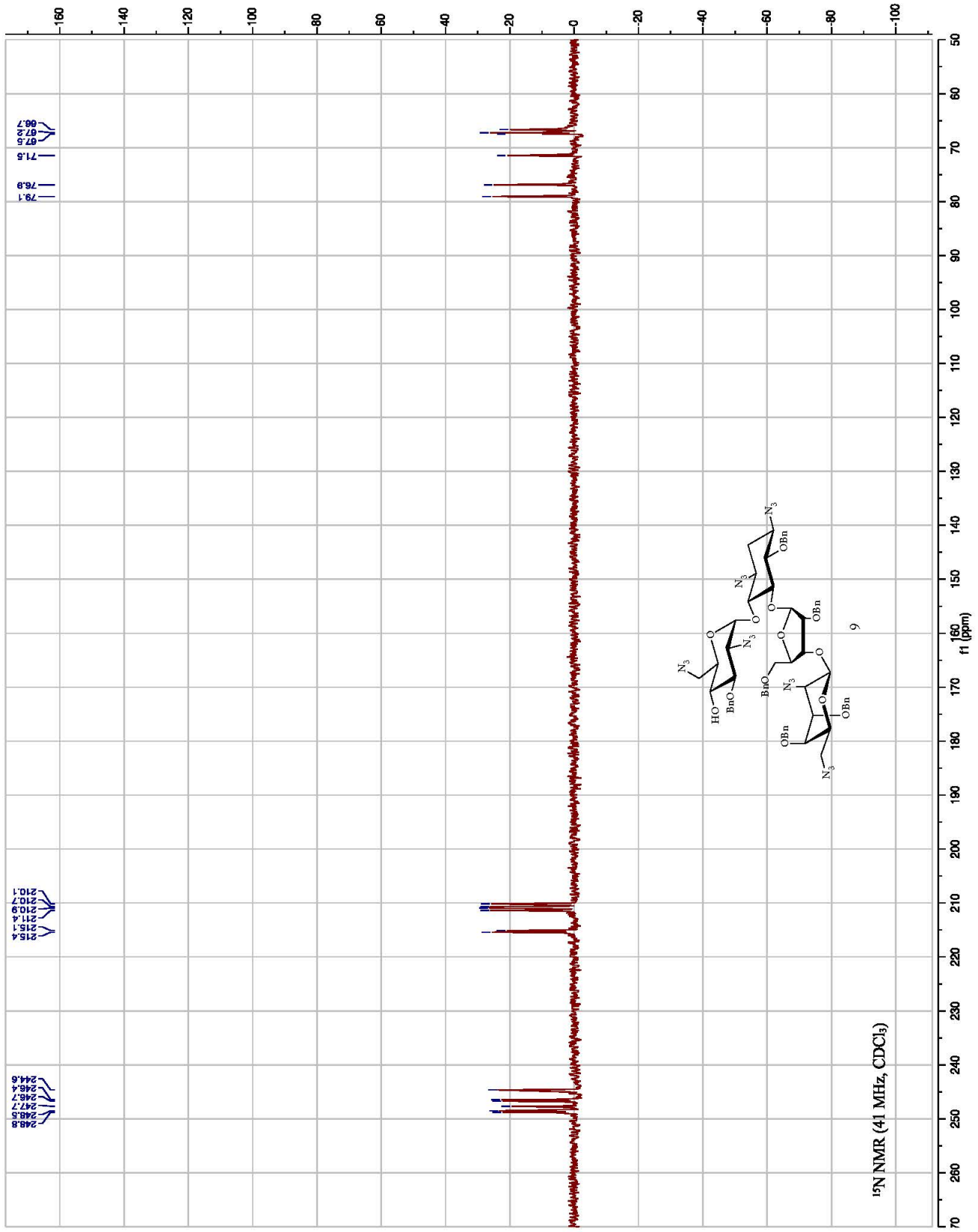
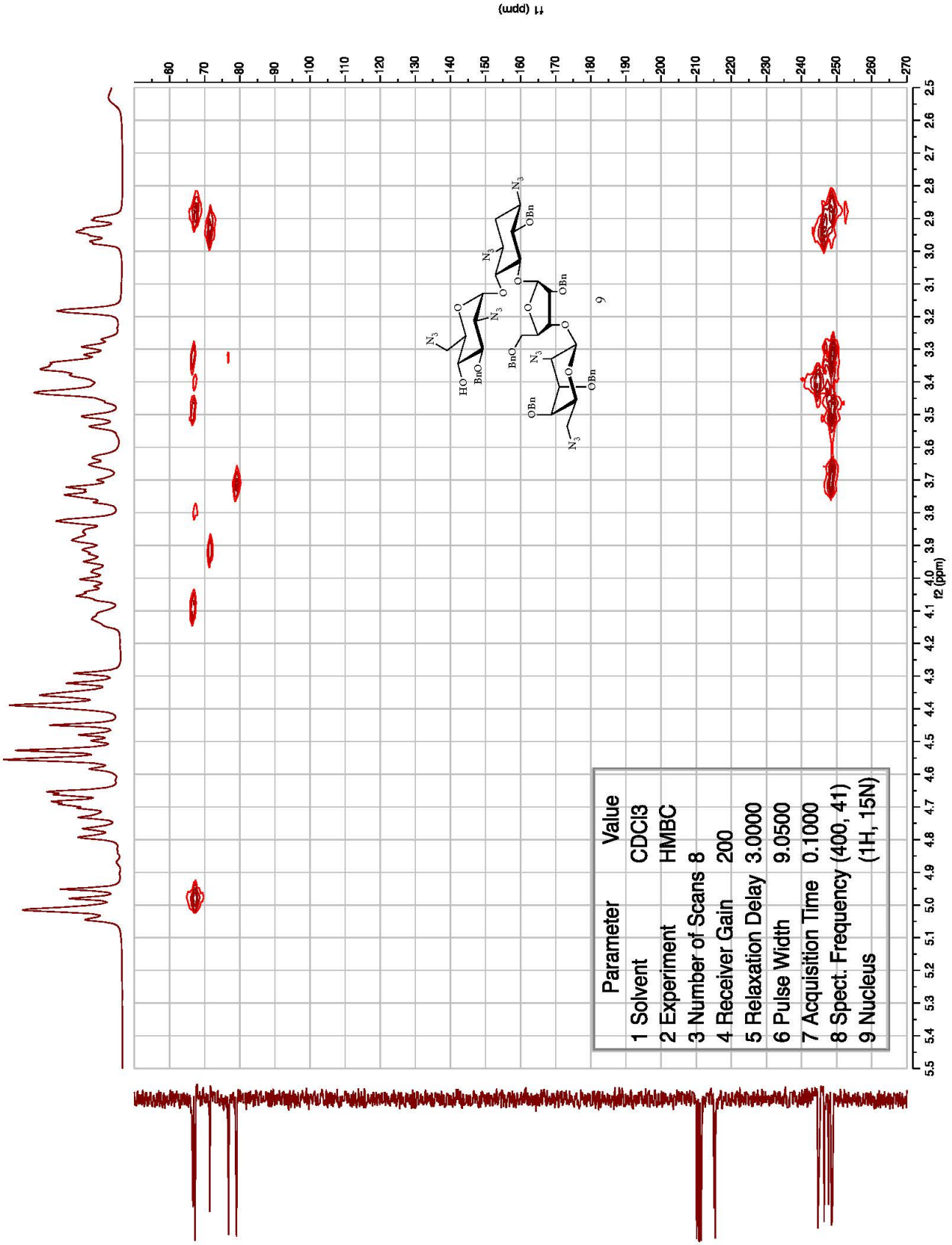
2 Experiment HMBC
3 Number of Scans 1
4 Receiver Gain 16384
5 Relaxation Delay 2.0000
6 Pulse Width 10.3000
7 Acquisition Time 0.1278
8 Spec. Frequency (101, 400)
9 Nucleus (13C, 1H)
9

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f2 (ppm)
Parameter Value
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2 Experiment HMBC
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4 Receiver Gain 200
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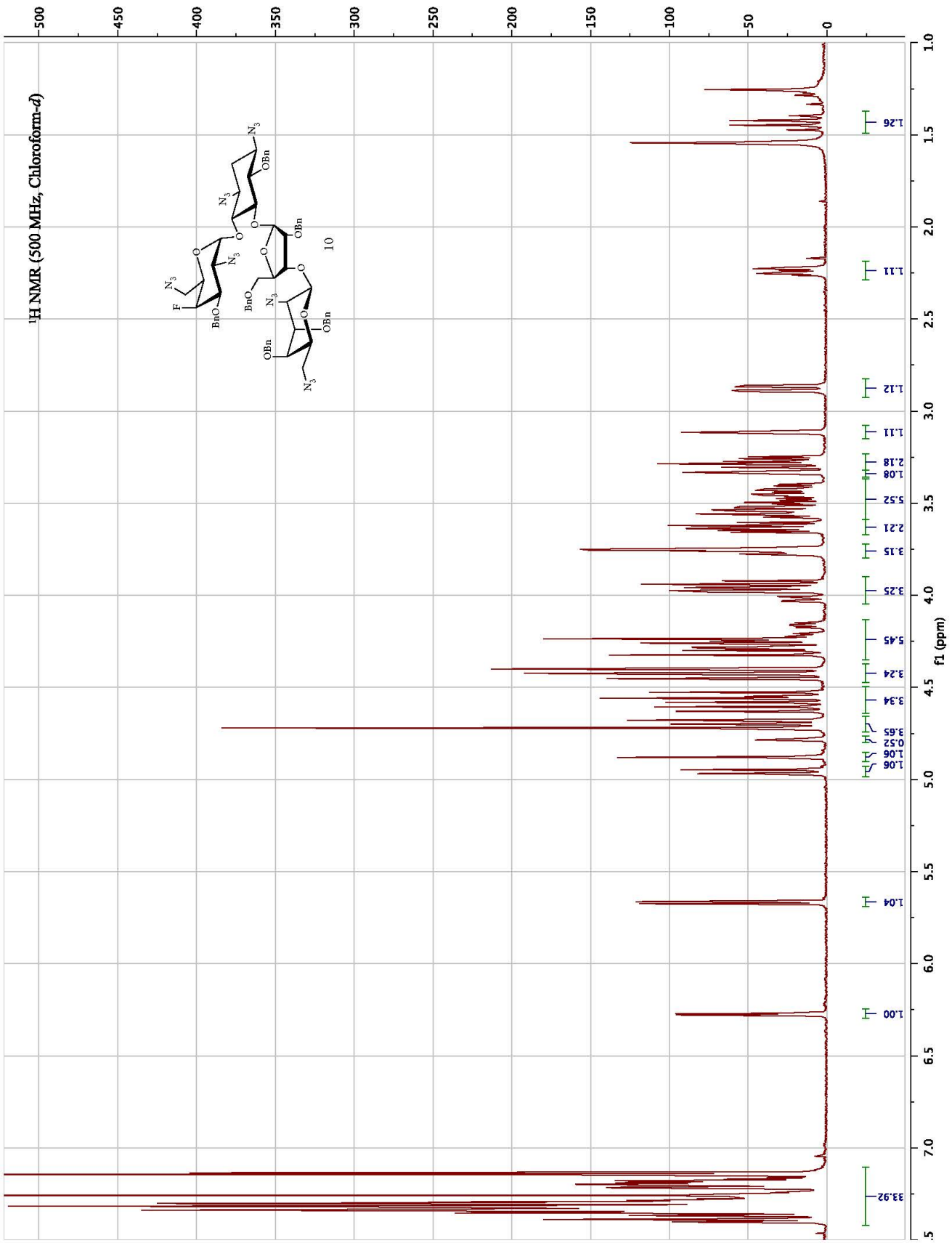
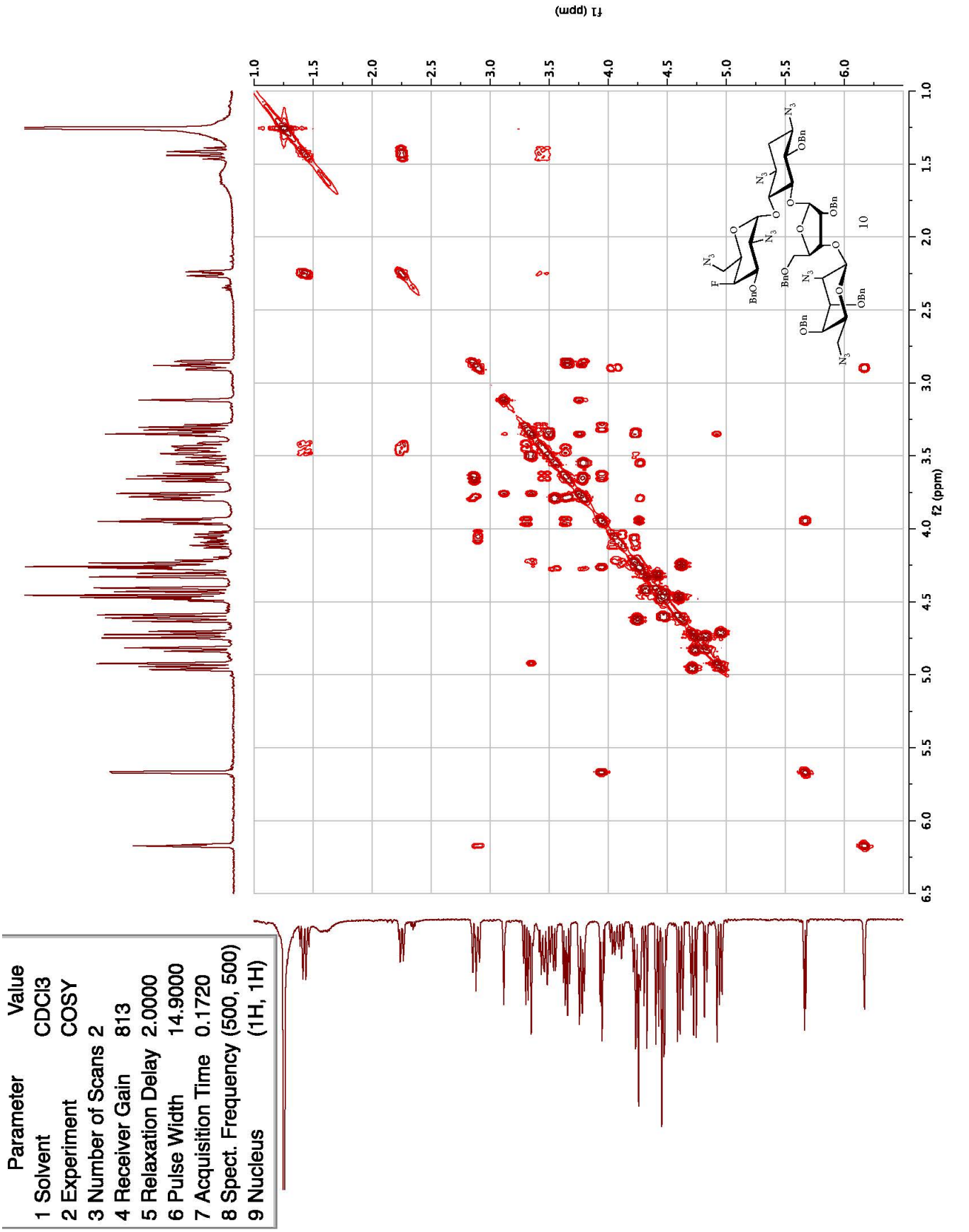
6 Pulse Width 9.0500
7 Acquisition Time 0.1000
8 Spect. Frequency (400, 41)
9 Nucleus (1H, 15N)




f1 (ppm)
f2 (ppm)
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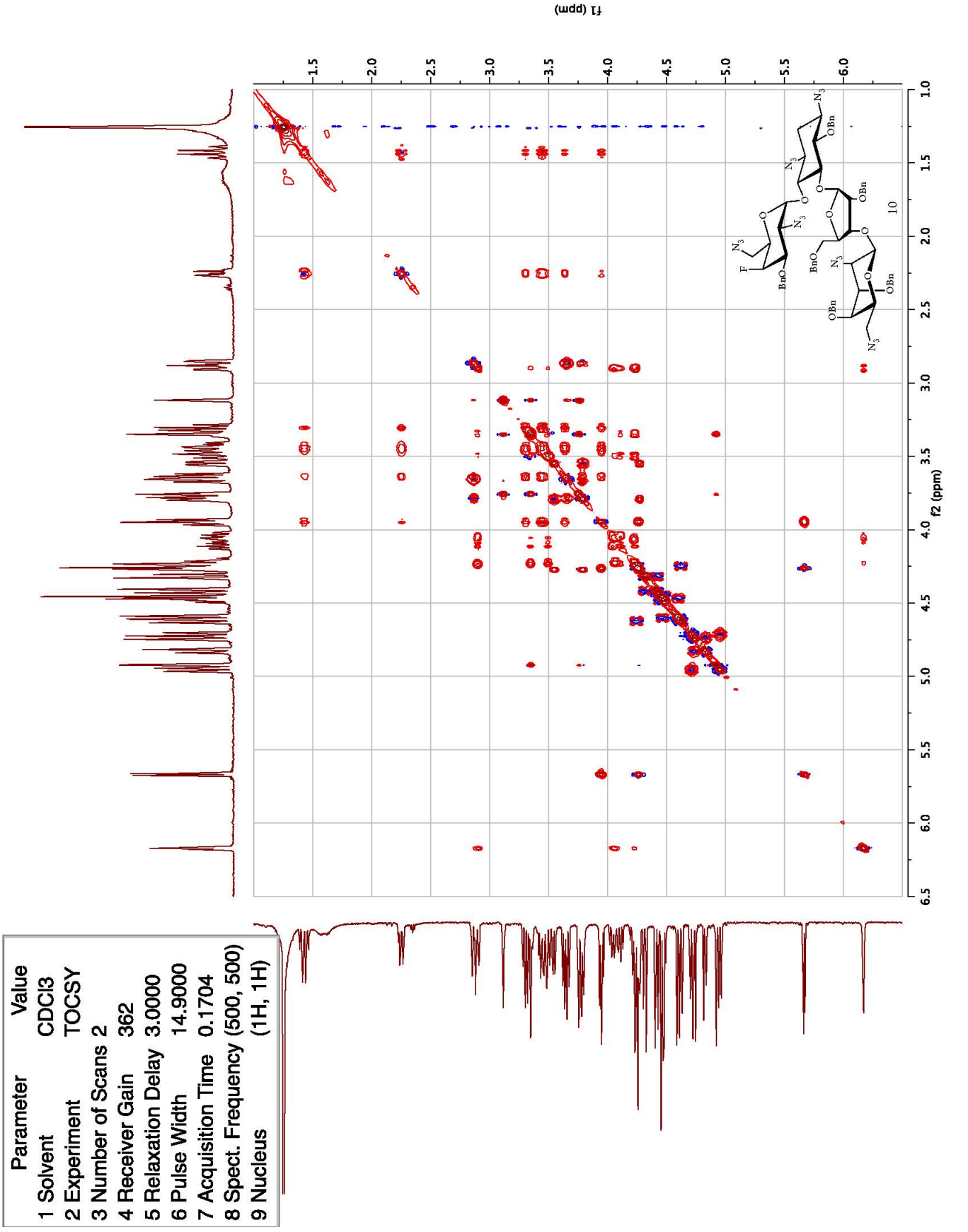
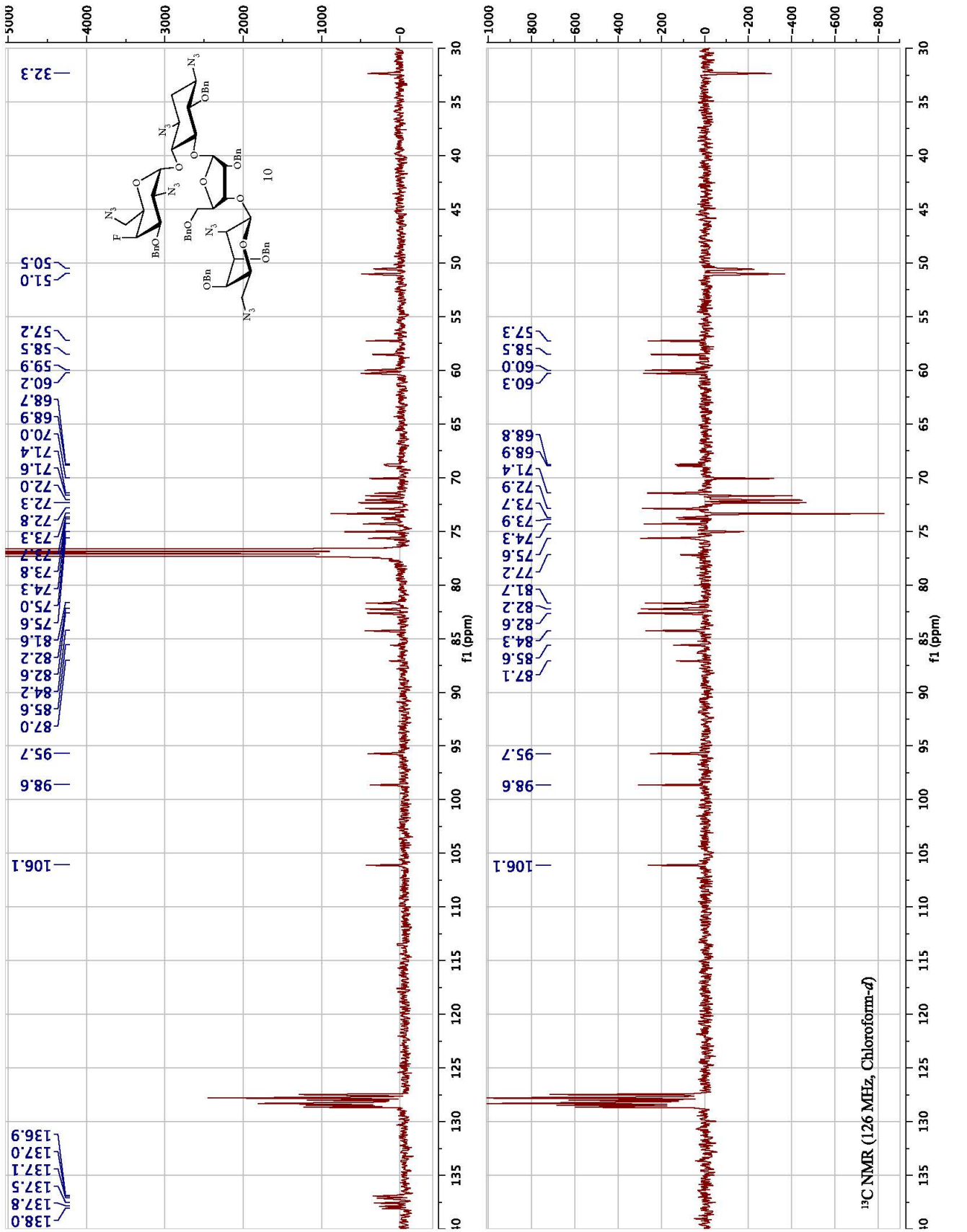
2 Experiment COSY
3 Number of Scans 2
4 Receiver Gain 813
5 Relaxation Delay 2.0000
6 Pulse Width 14.9000
7 Acquisition Time 0.1720
8 Spect. Frequency (500, 500)
9 Nucleus (1H, 1H)

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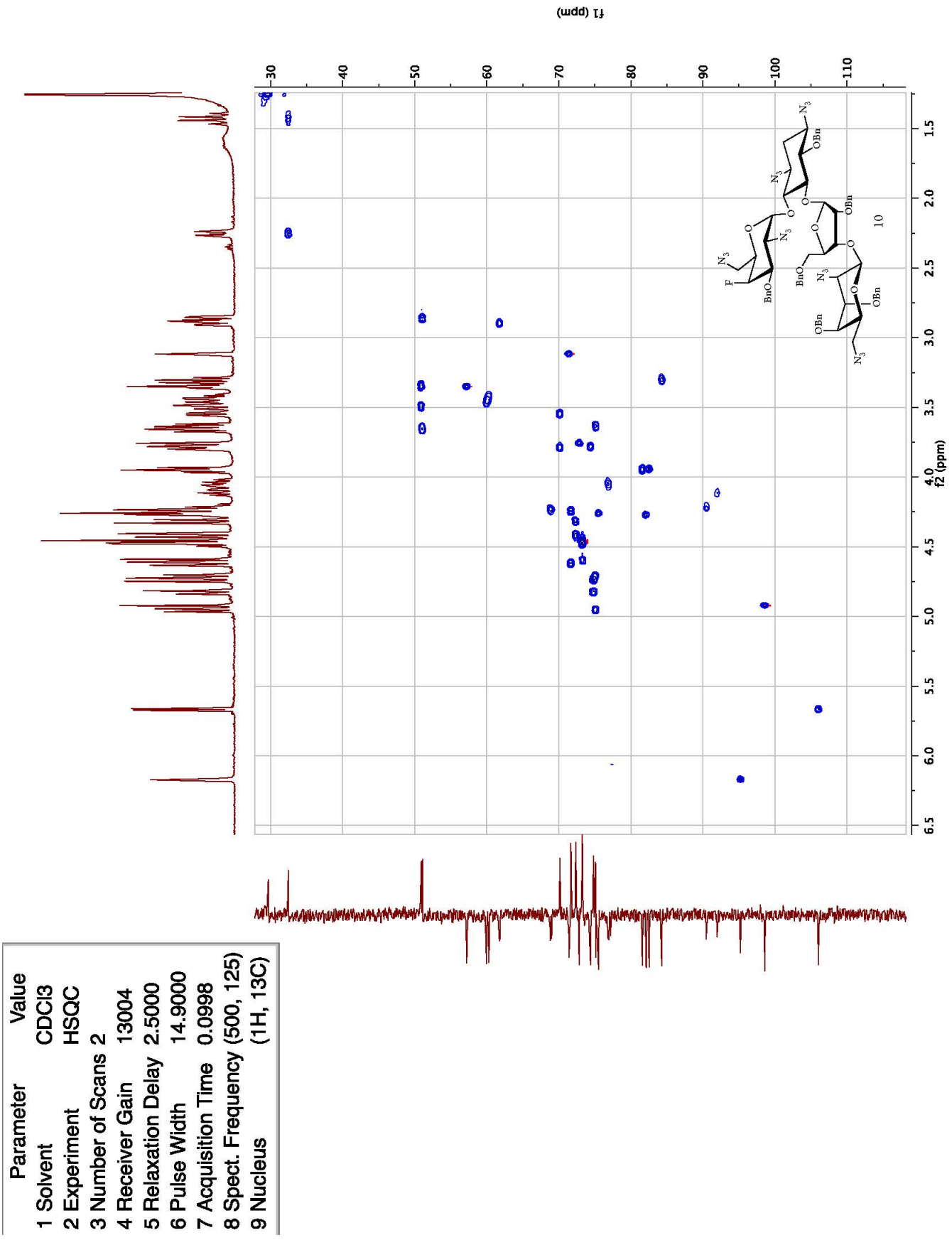
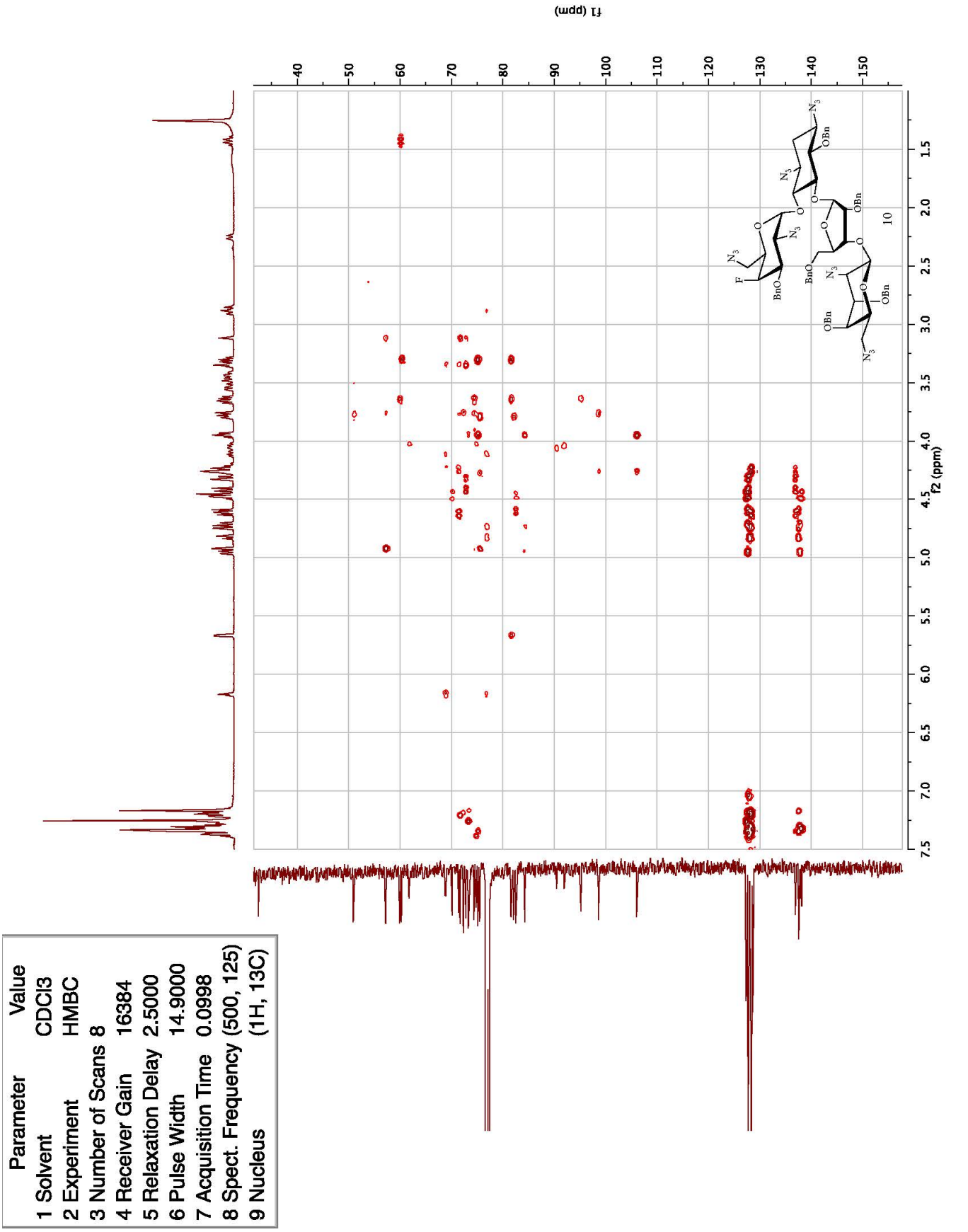
^{13}C NMR (126 MHz, Chloroform-*d*)
f1 (ppm)




f1 (ppm)
f2 (ppm)
10
Parameter Value
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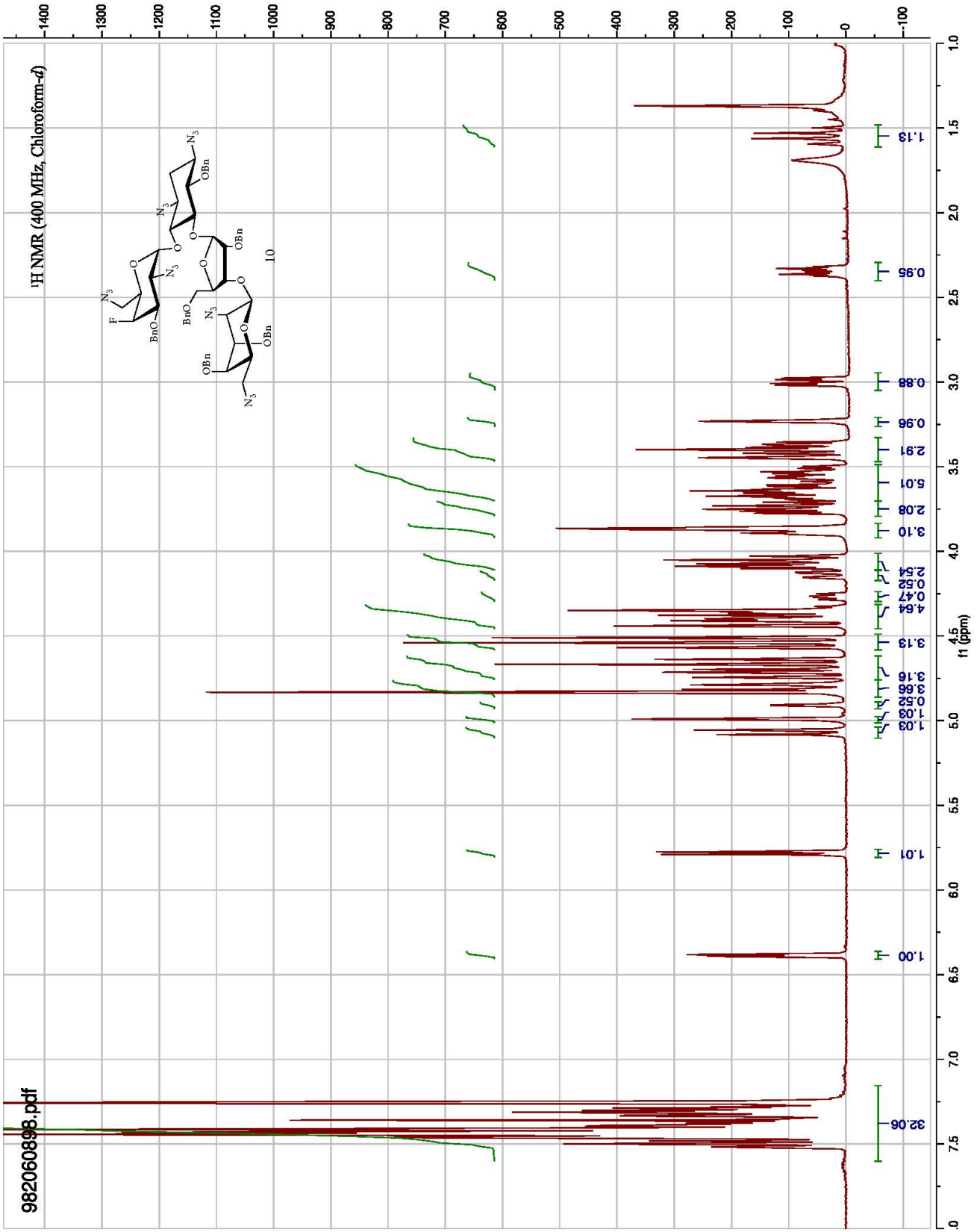
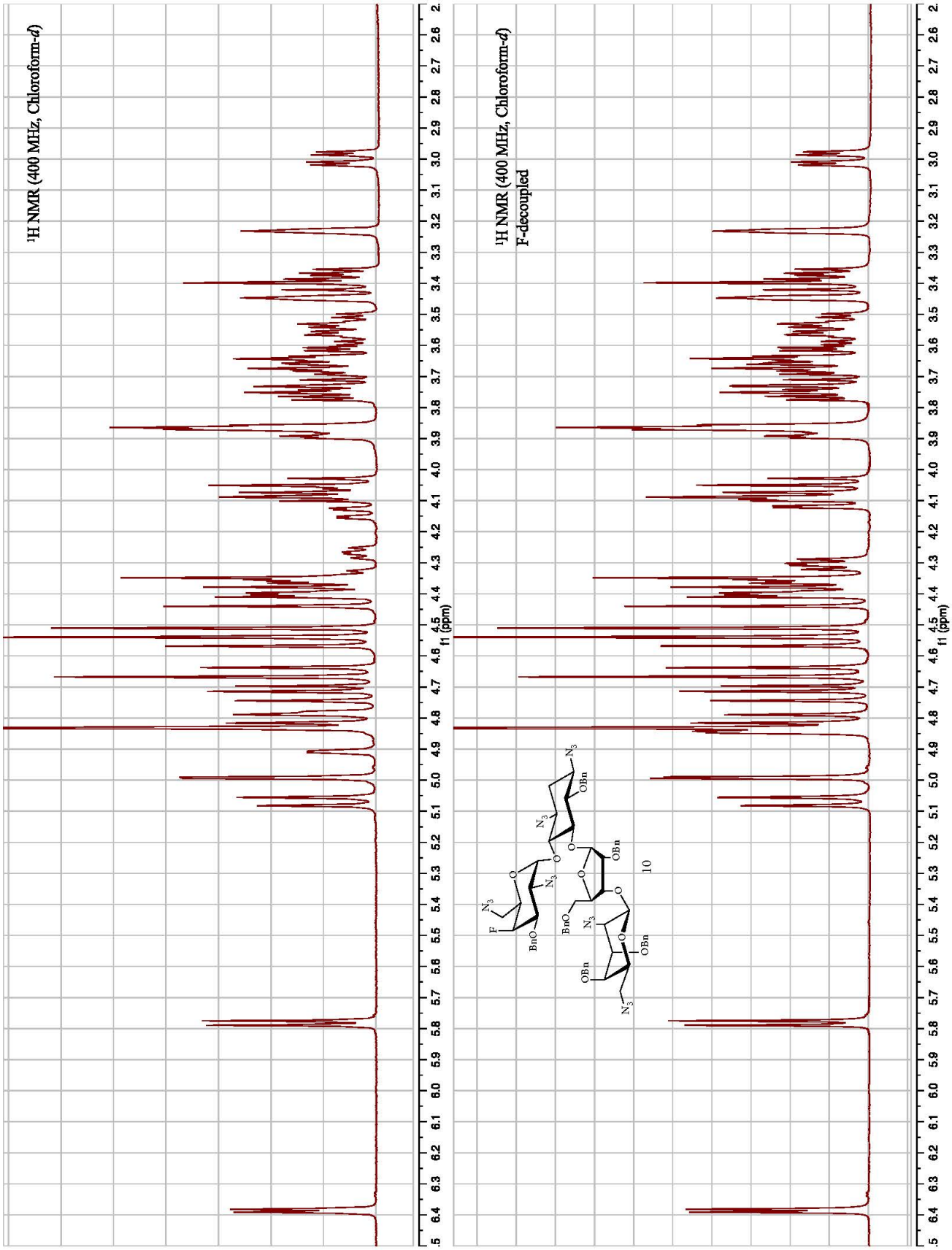
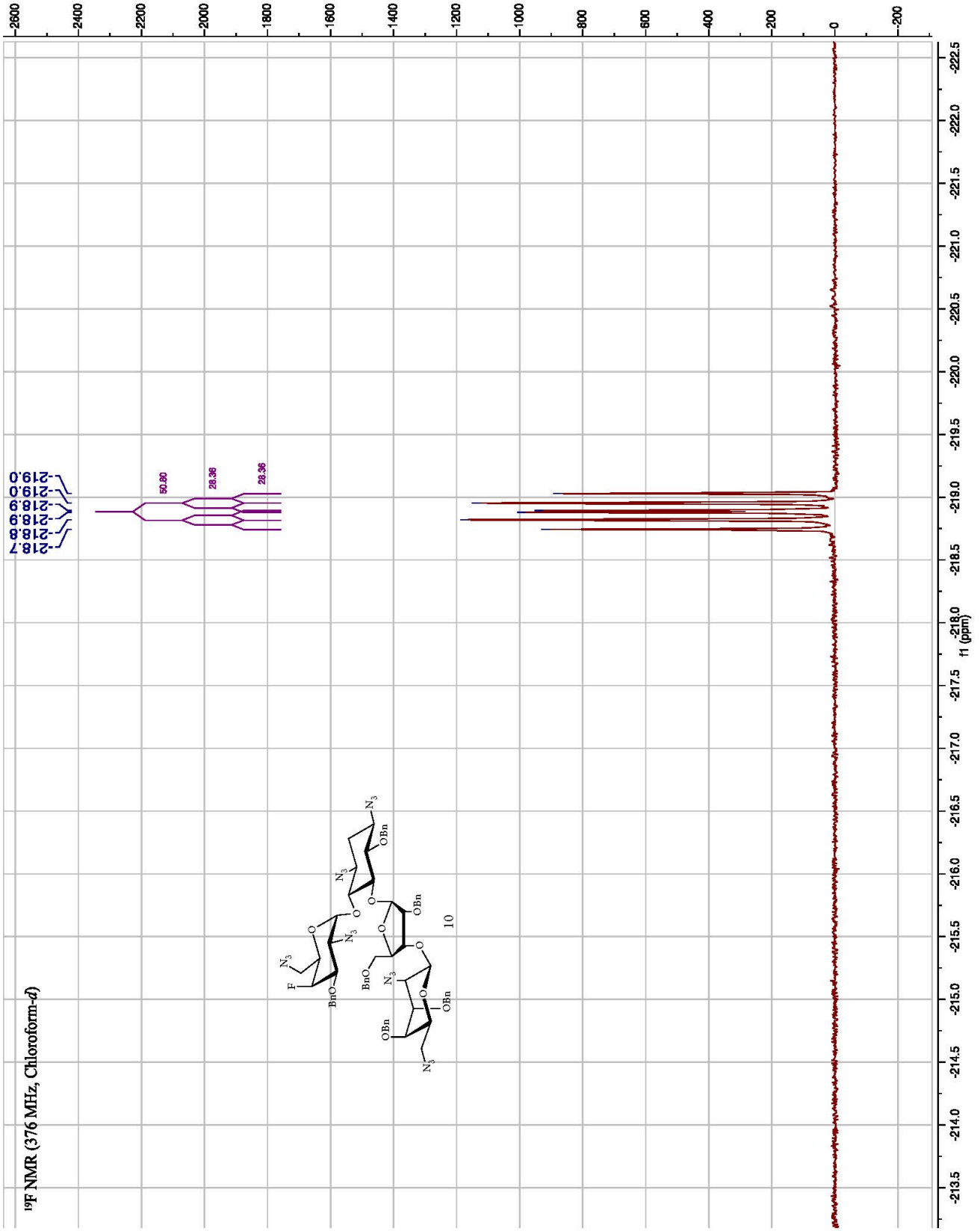
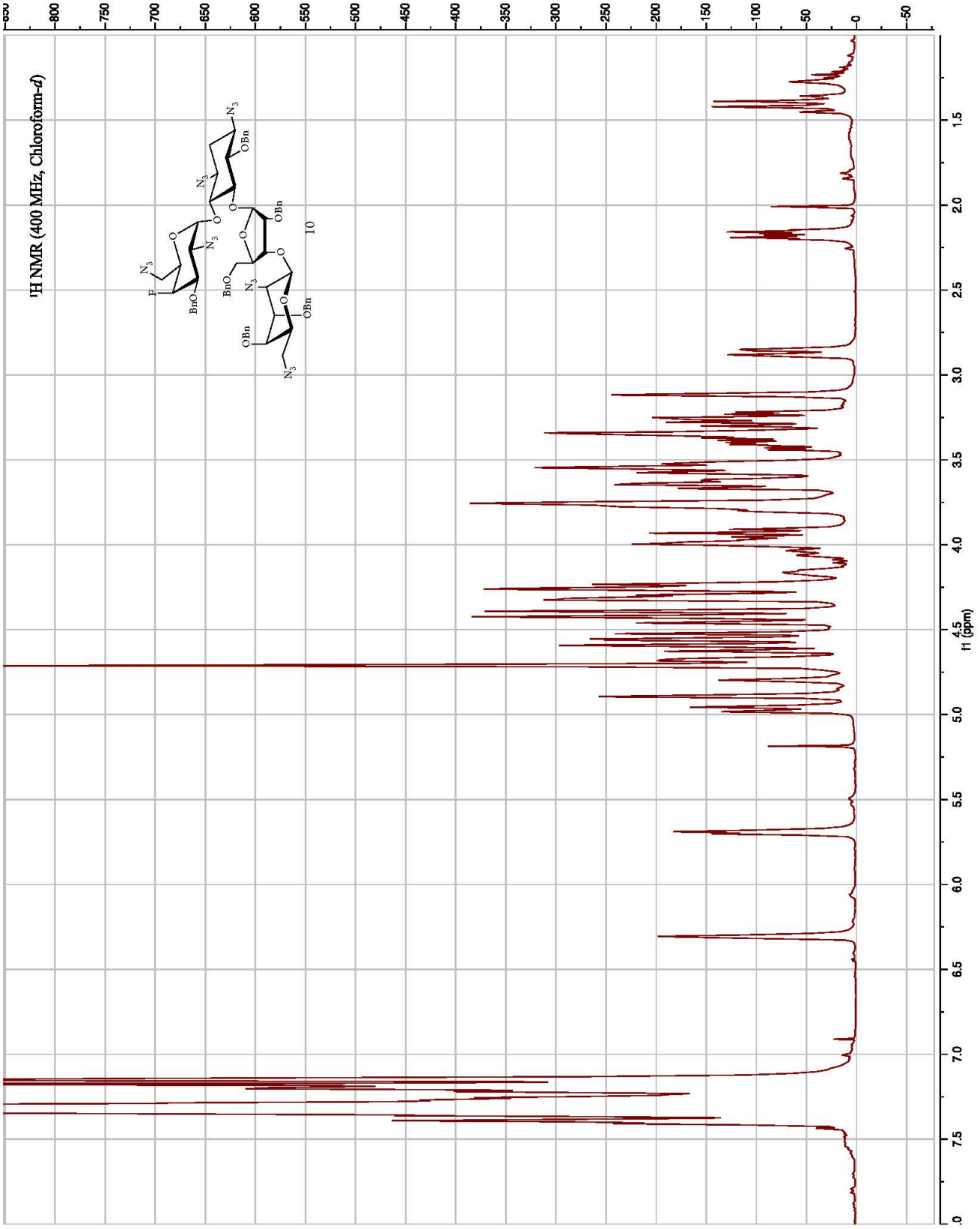
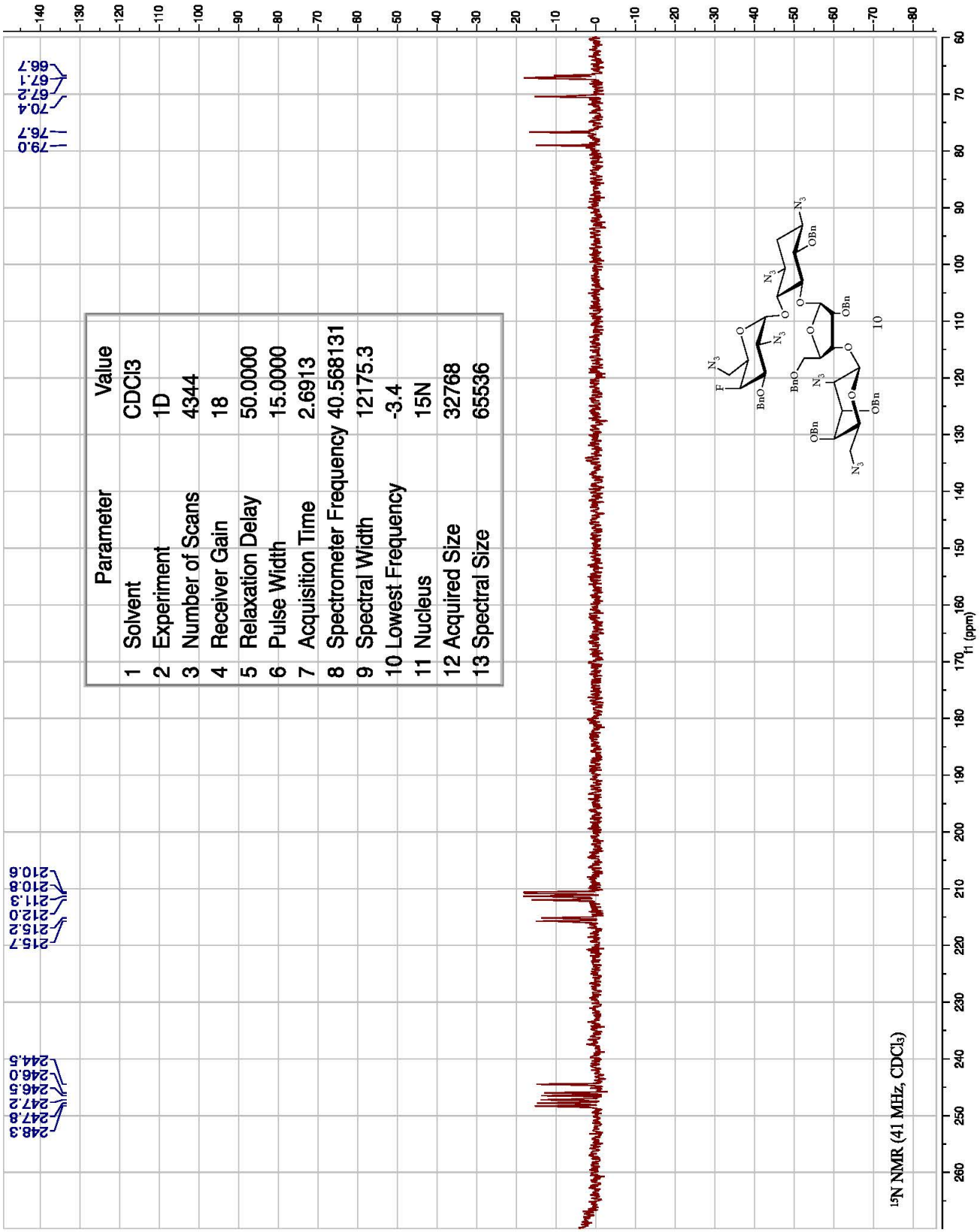
2 Experiment HMBC
3 Number of Scans 8
4 Receiver Gain 16384
5 Relaxation Delay 2.5000
6 Pulse Width 14.9000
7 Acquisition Time 0.0998
8 Spect. Frequency (500, 125)
9 Nucleus (1H, 13C)

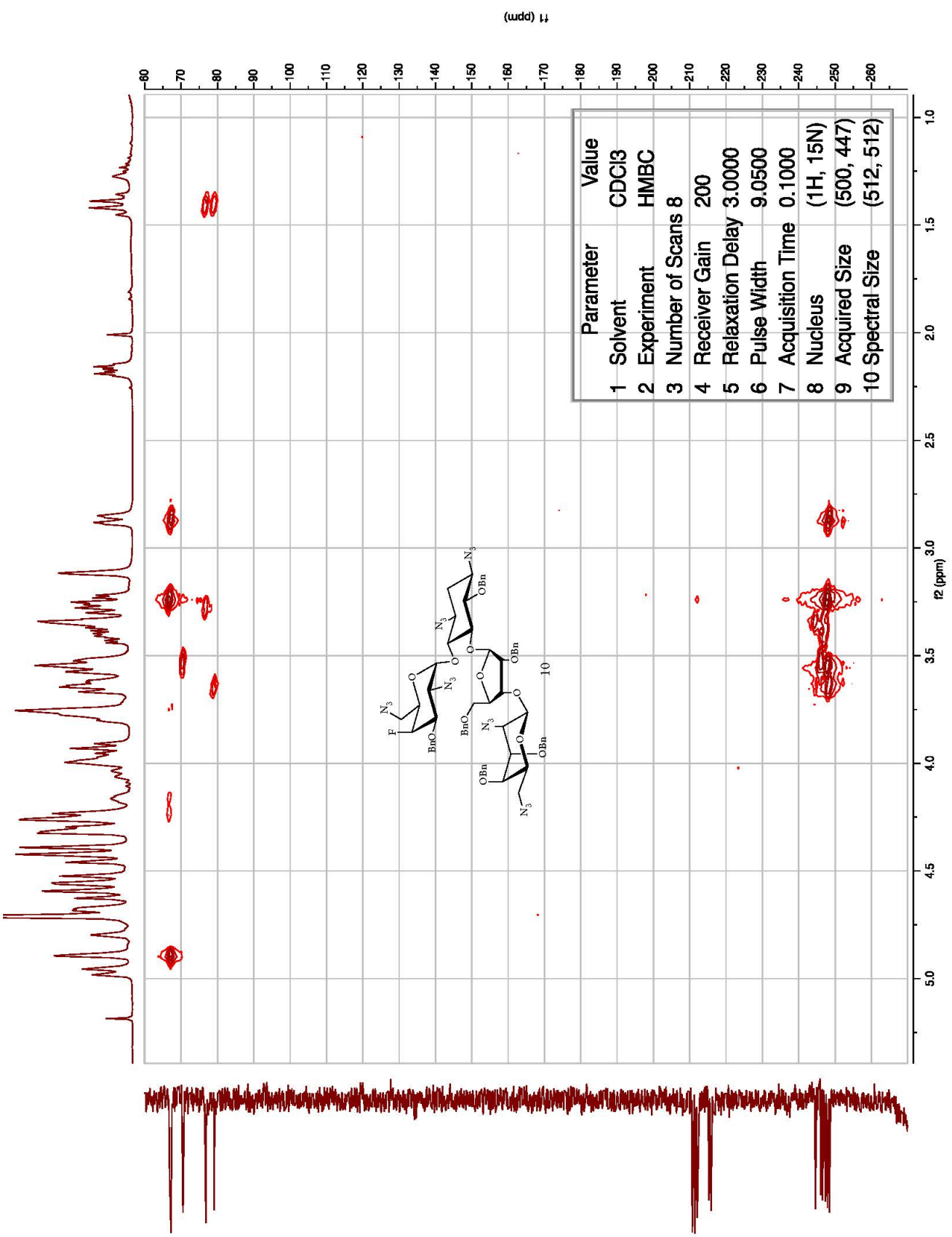



^{1}H NMR (400 MHz, Chloroform-*d*)
^{1}H NMR (400 MHz, Chloroform-*d*)
F-decoupled
f1 (ppm)
10




^{1}H NMR (400 MHz, Chloroform-*d*)
f1 (ppm)

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f2 (ppm)
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2 Experiment HMBC
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4 Receiver Gain 200
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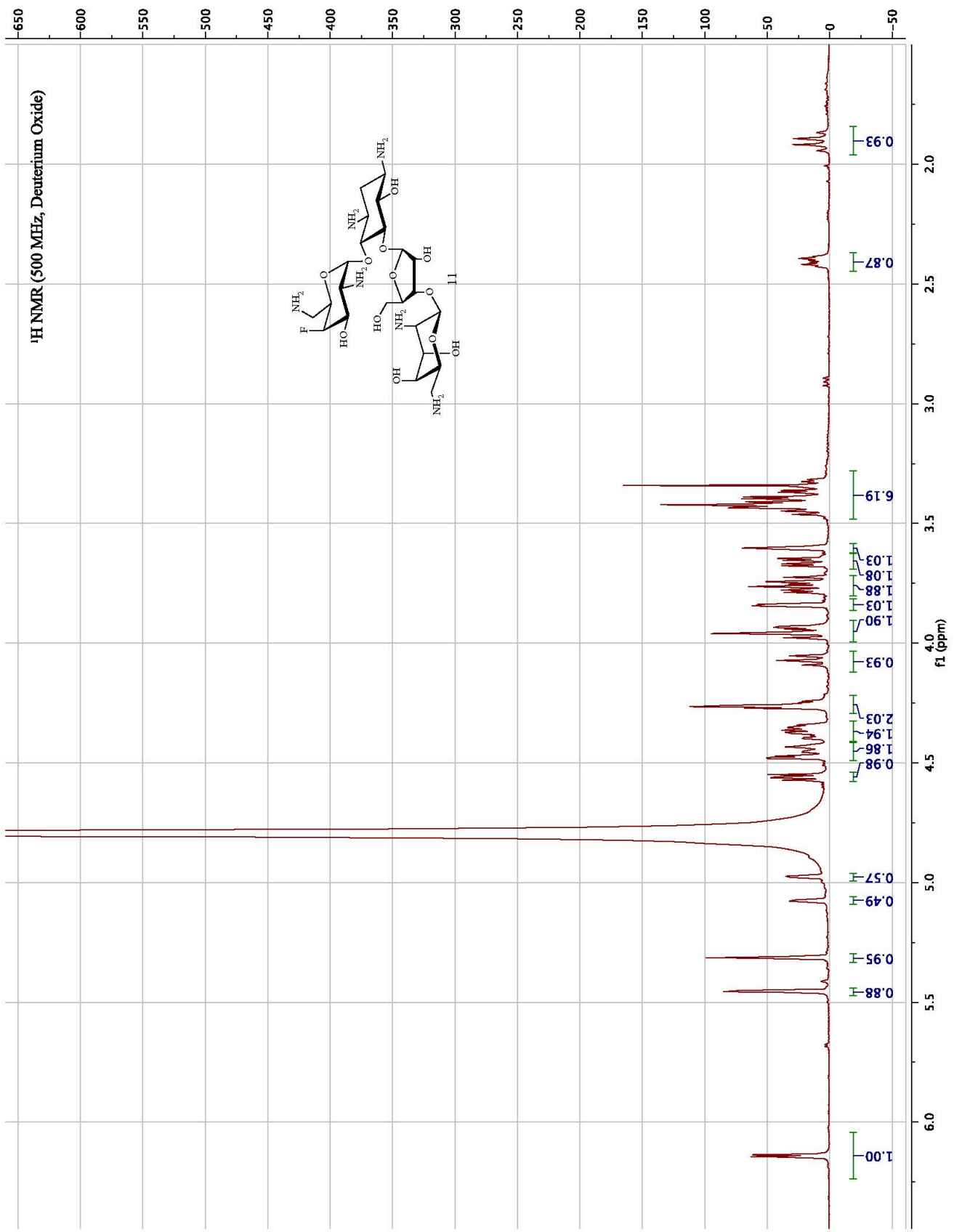
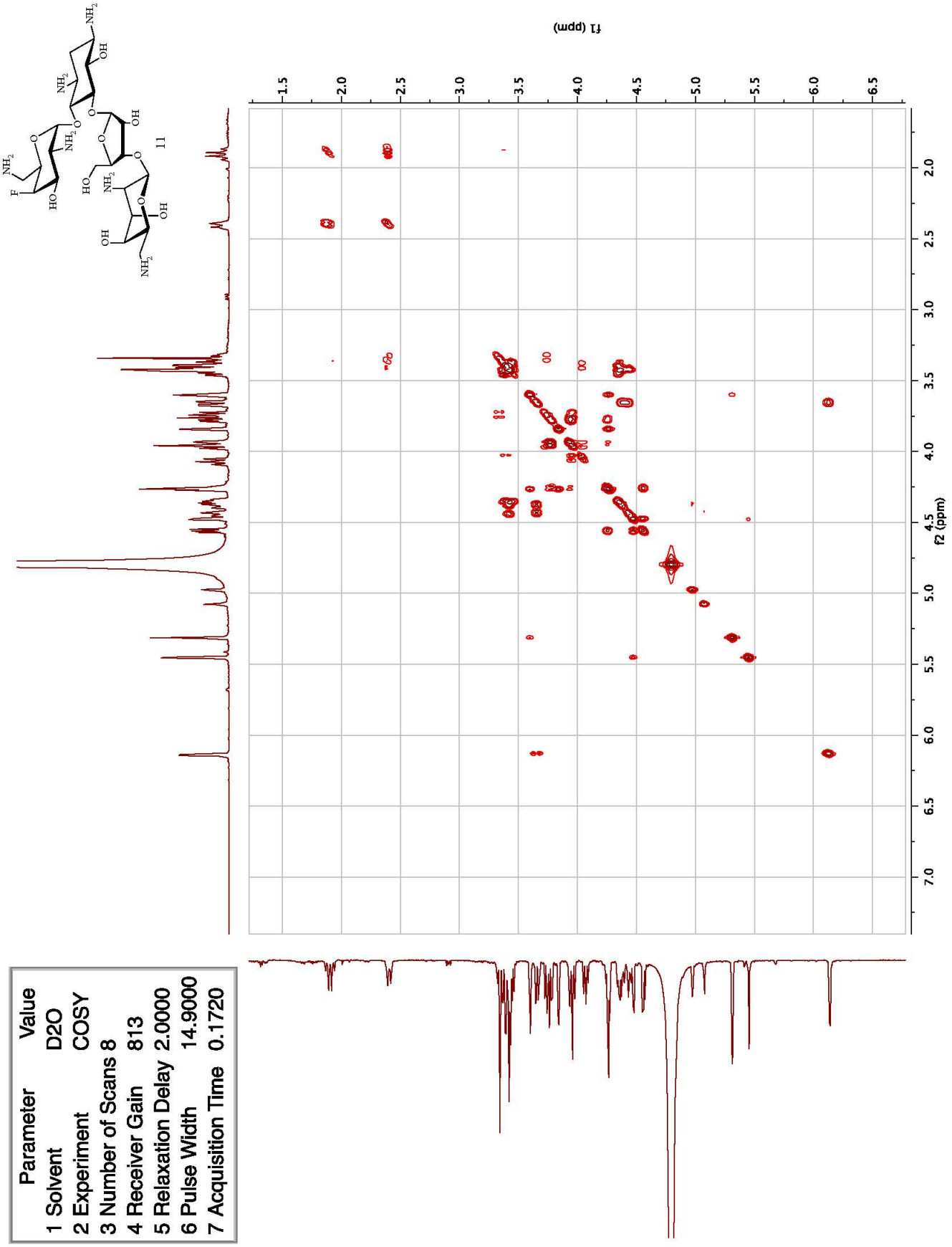
6 Pulse Width 9.0500
7 Acquisition Time 0.1000
8 Nucleus (1H, 15N)
9 Acquired Size (500, 447)
10 Spectral Size (512, 512)
10




11
f1 (ppm)
f2 (ppm)
Parameter Value
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5 Relaxation Delay 2.0000
6 Pulse Width 14.9000
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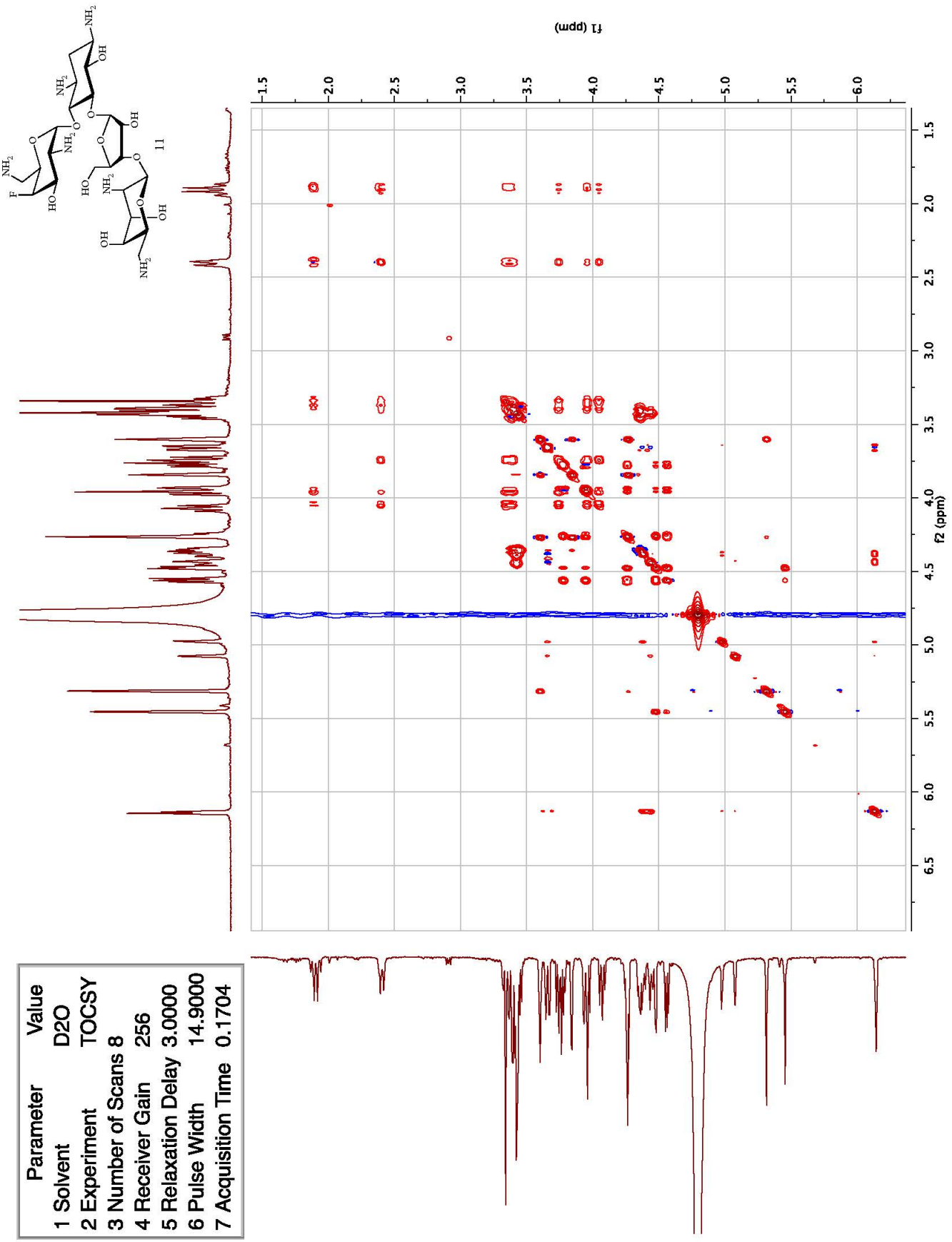
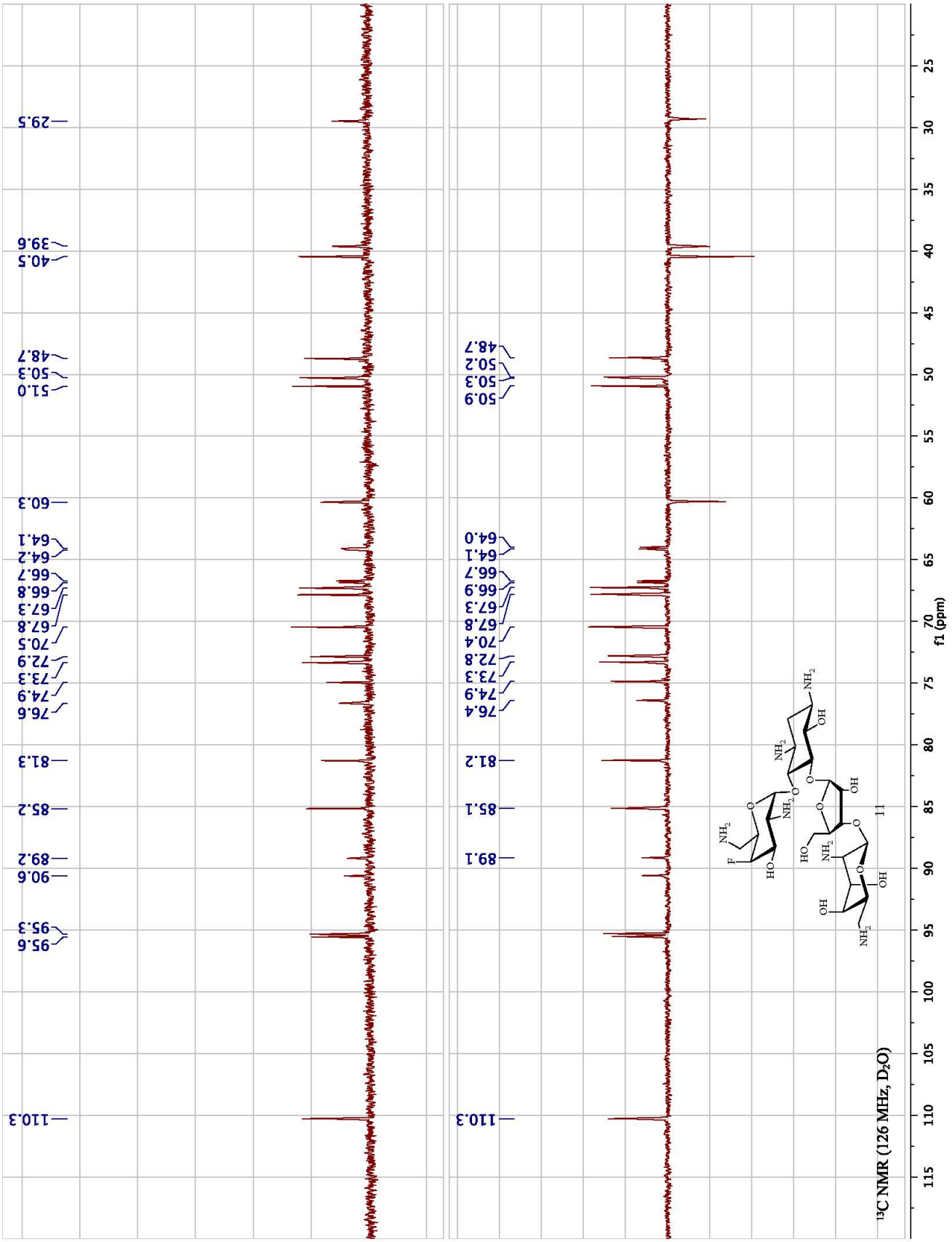
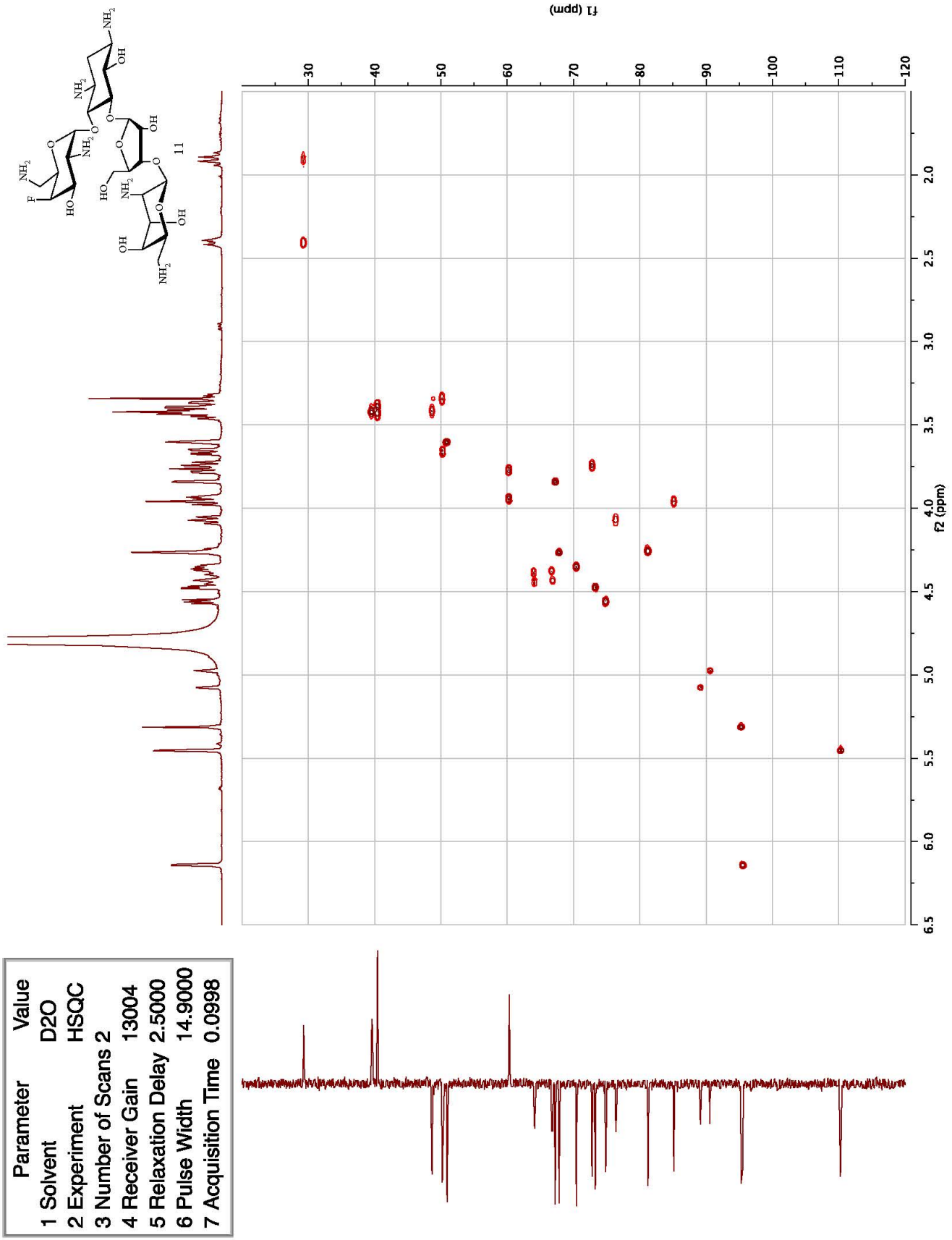
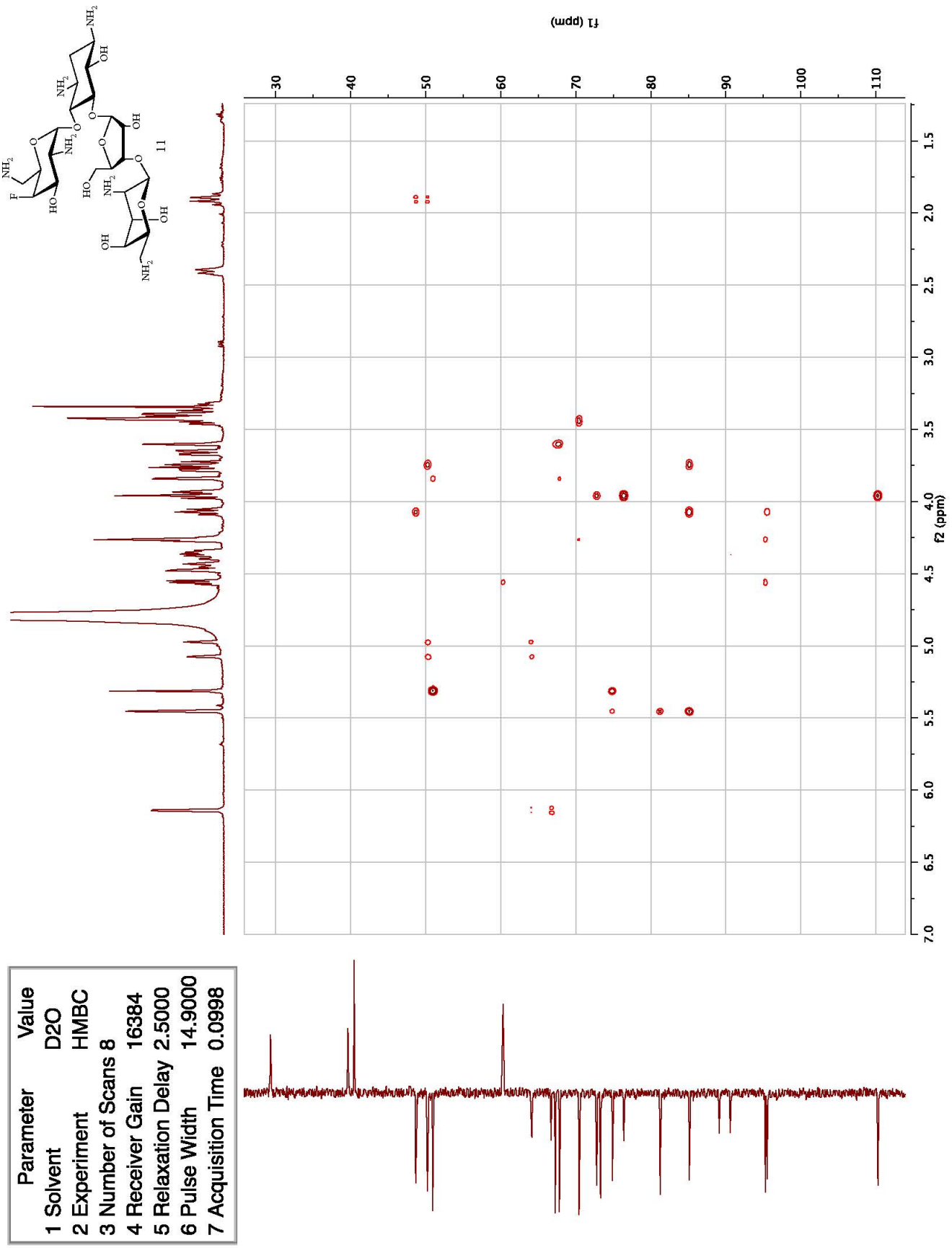



29.5
39.6
40.5
48.7
50.3
51.0
60.3
64.1
64.2
66.7
66.8
67.3
67.8
70.5
72.9
73.3
74.9
76.6
81.3
85.2
89.2
90.6
95.3
95.6
110.3
48.7
50.2
50.3
50.9
64.0
64.1
66.7
66.9
67.3
67.8
70.4
72.8
73.3
74.9
76.4
81.2
85.1
89.1
110.3
f1 (ppm)
25
30
35
40
45
50
55
60
65
70
75
80
85
90
95
100
105
110
115
^{13}C NMR (126 MHz, D_2O)

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f1 (ppm)
f2 (ppm)
Parameter Value
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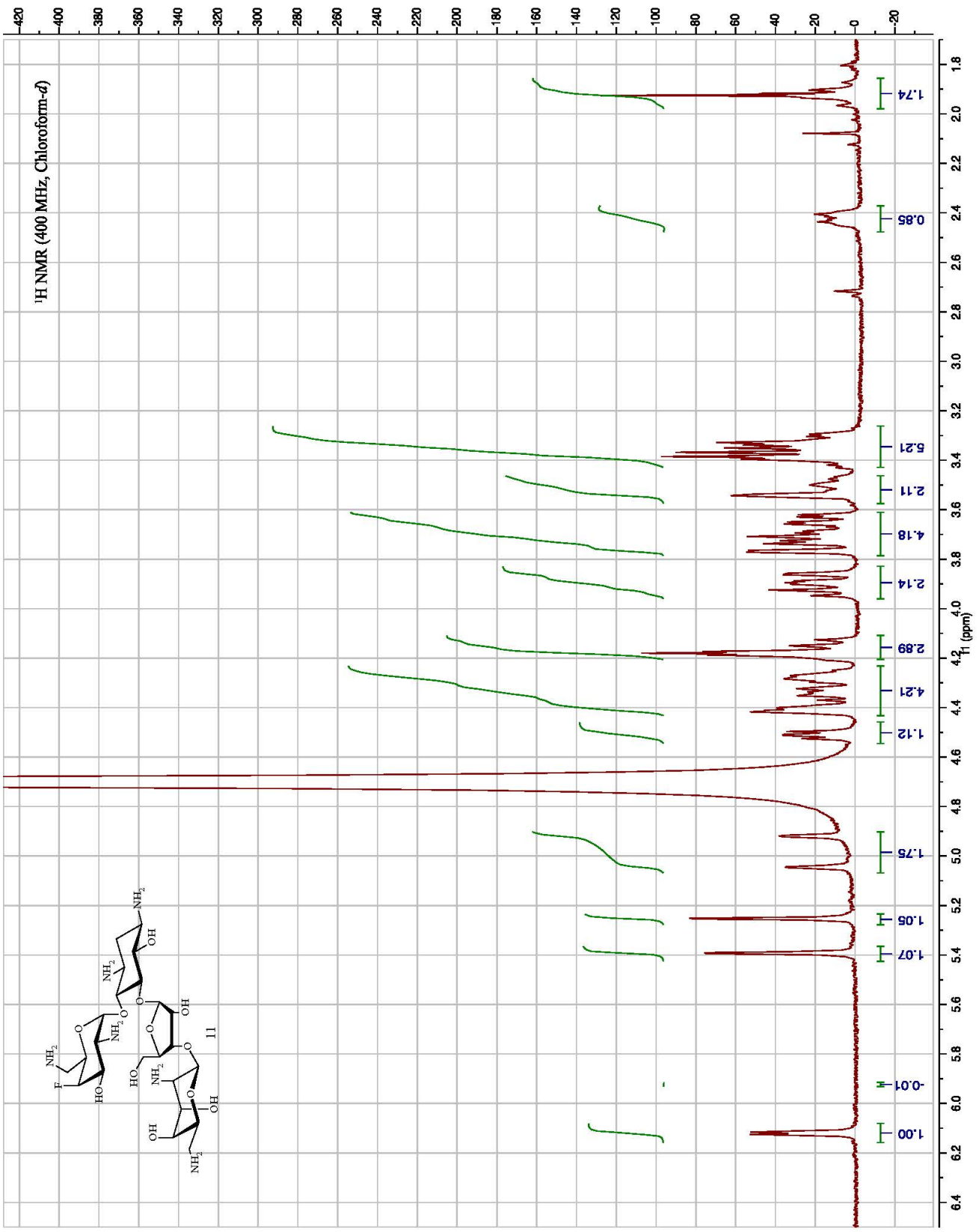
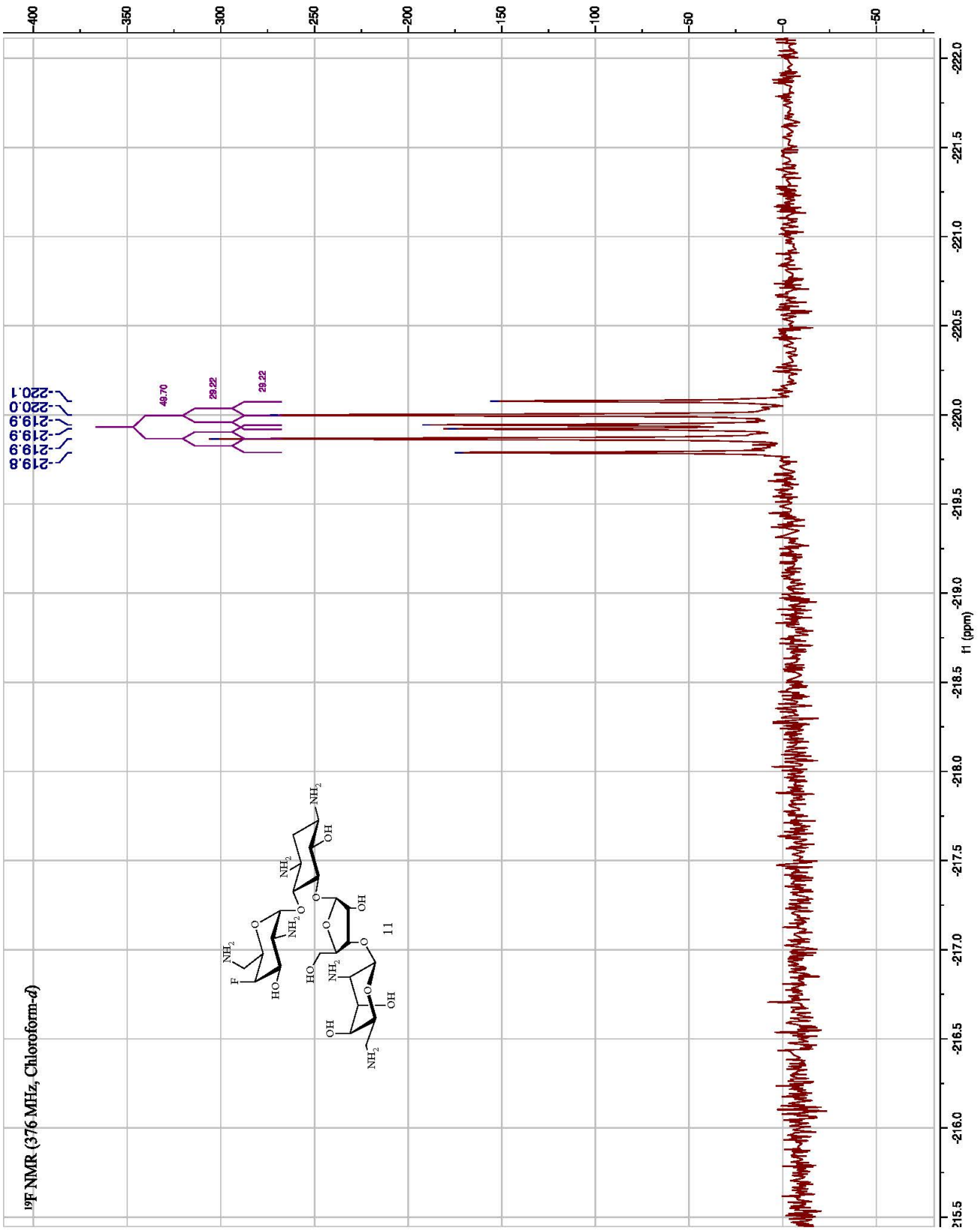
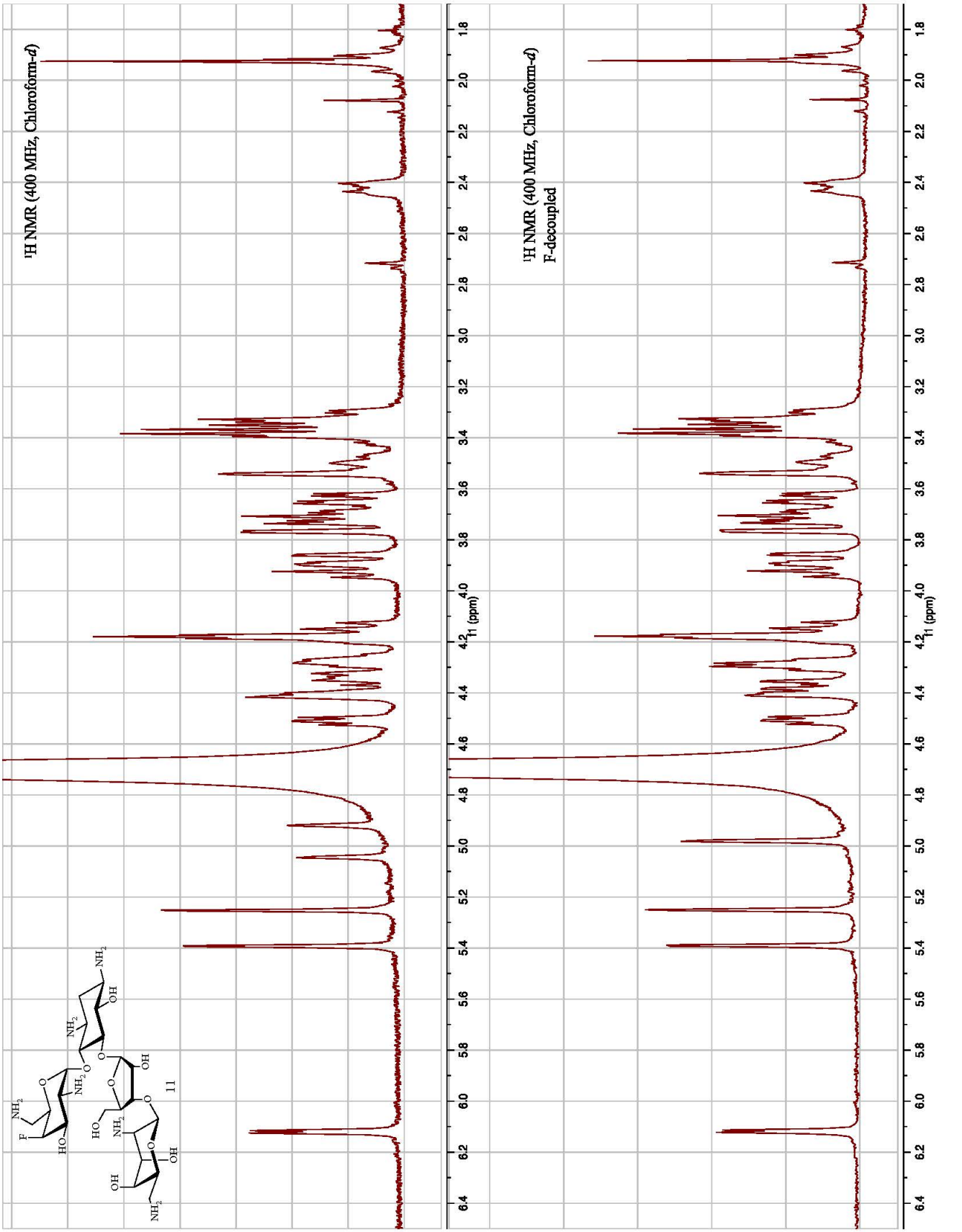
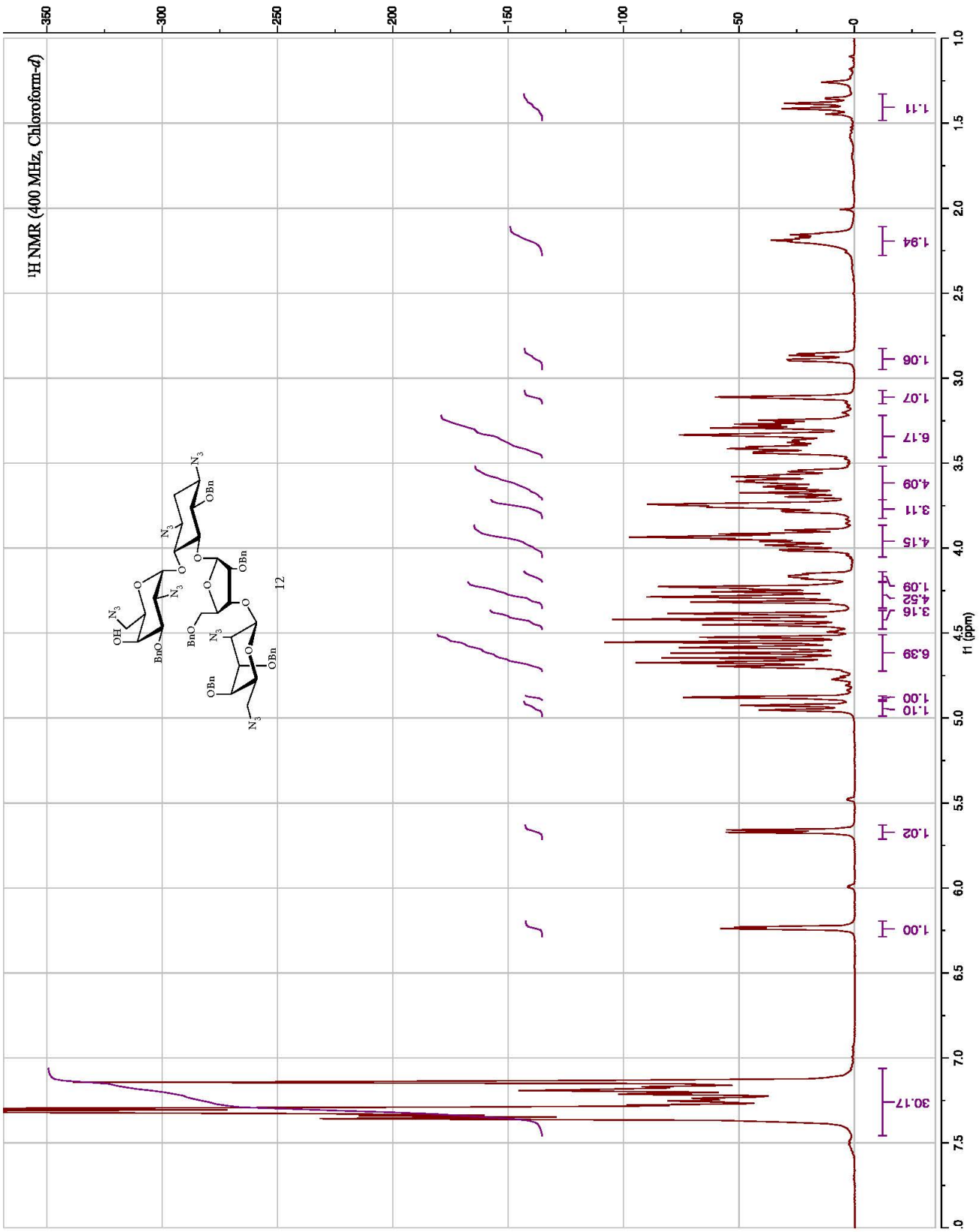
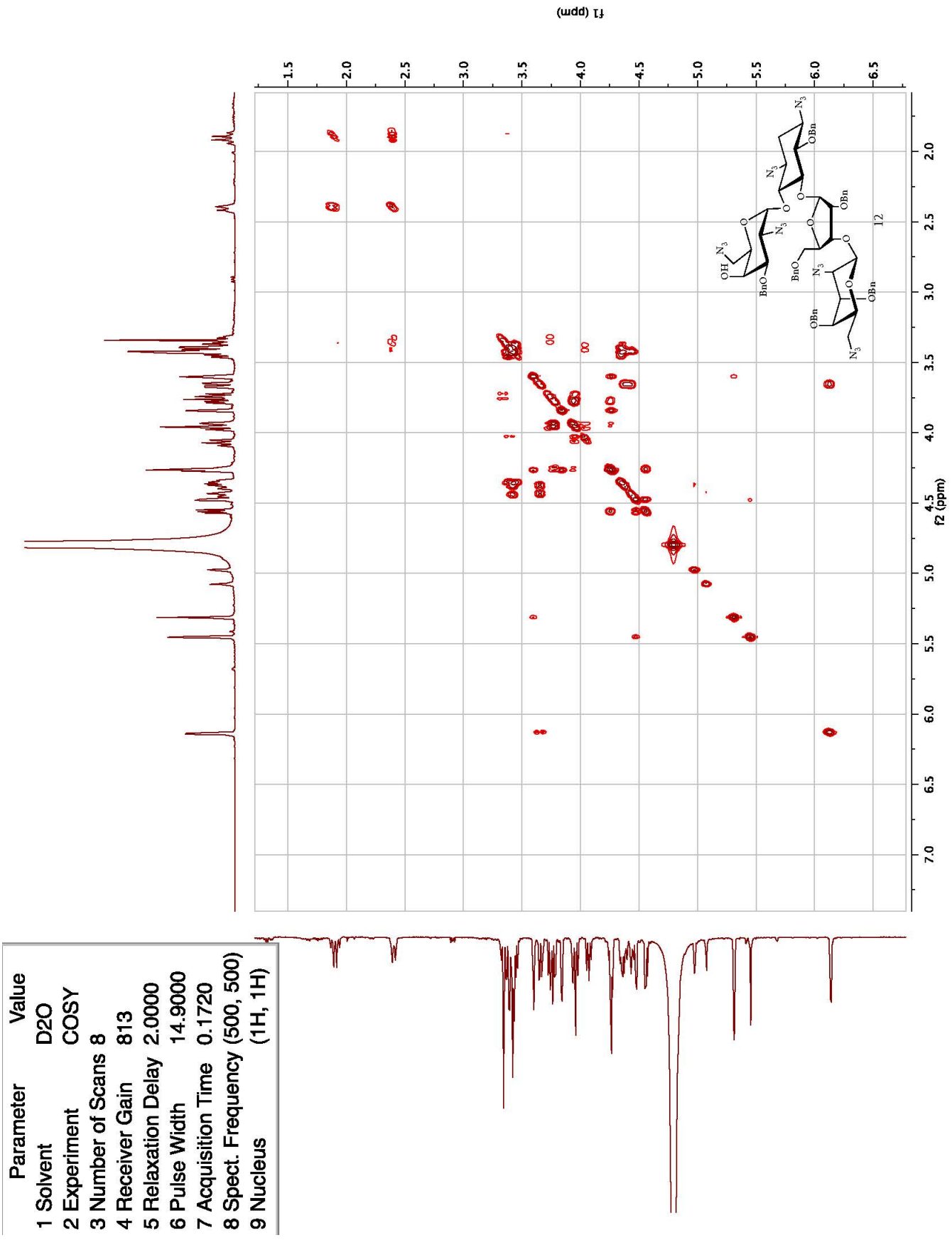
2 Experiment HMBC
3 Number of Scans 8
4 Receiver Gain 16384
5 Relaxation Delay 2.5000
6 Pulse Width 14.9000
7 Acquisition Time 0.0998

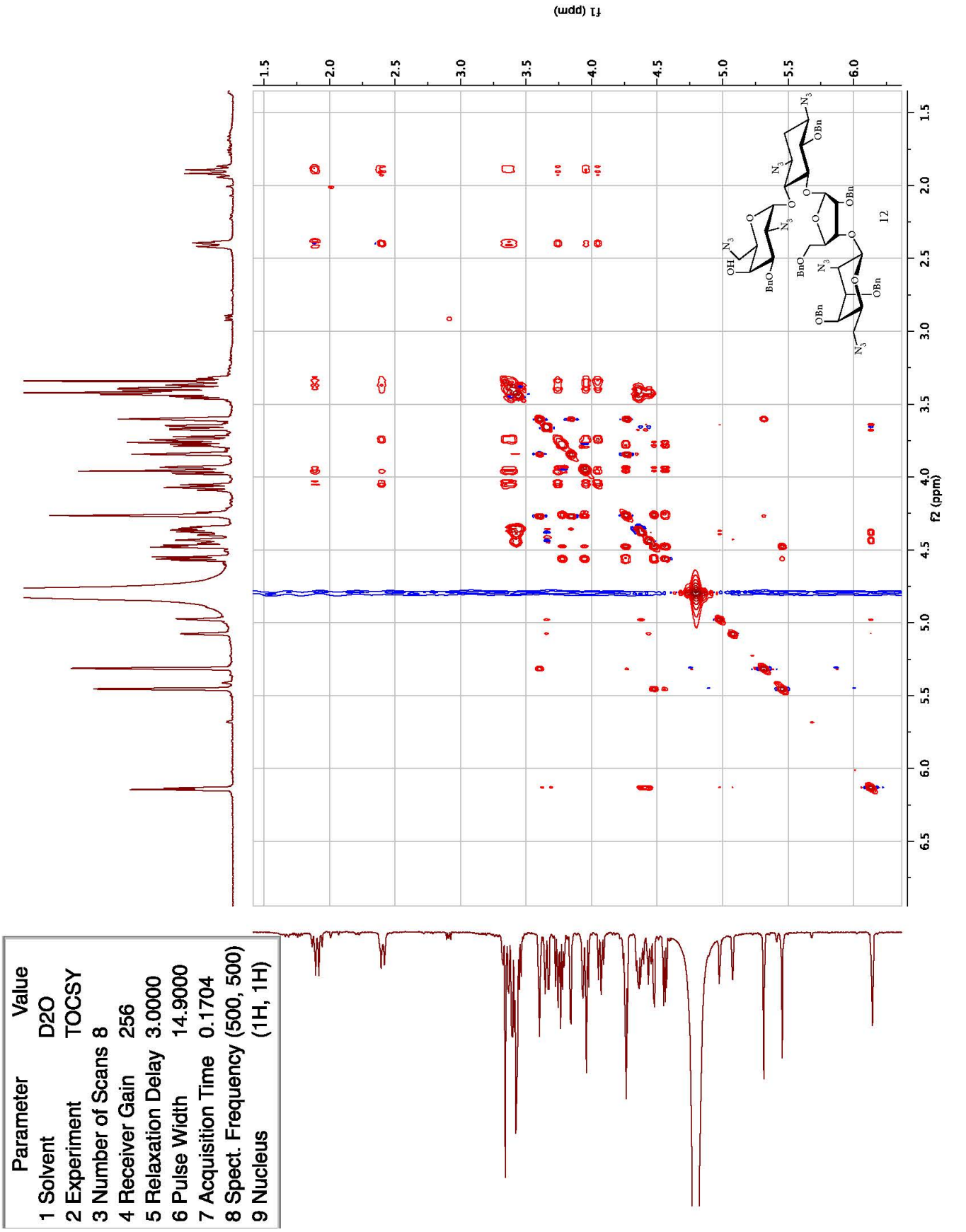



^{19}F NMR (376 MHz, Chloroform-d)
49.70
29.22
29.22
-219.8
-219.9
-219.9
-219.9
-220.0
-220.1
f1 (ppm)
11




^{1}H NMR (400 MHz, Chloroform-d)
12
f1 (ppm)




f1 (ppm)
f2 (ppm)
12
Parameter Value
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2 Experiment TOCSY
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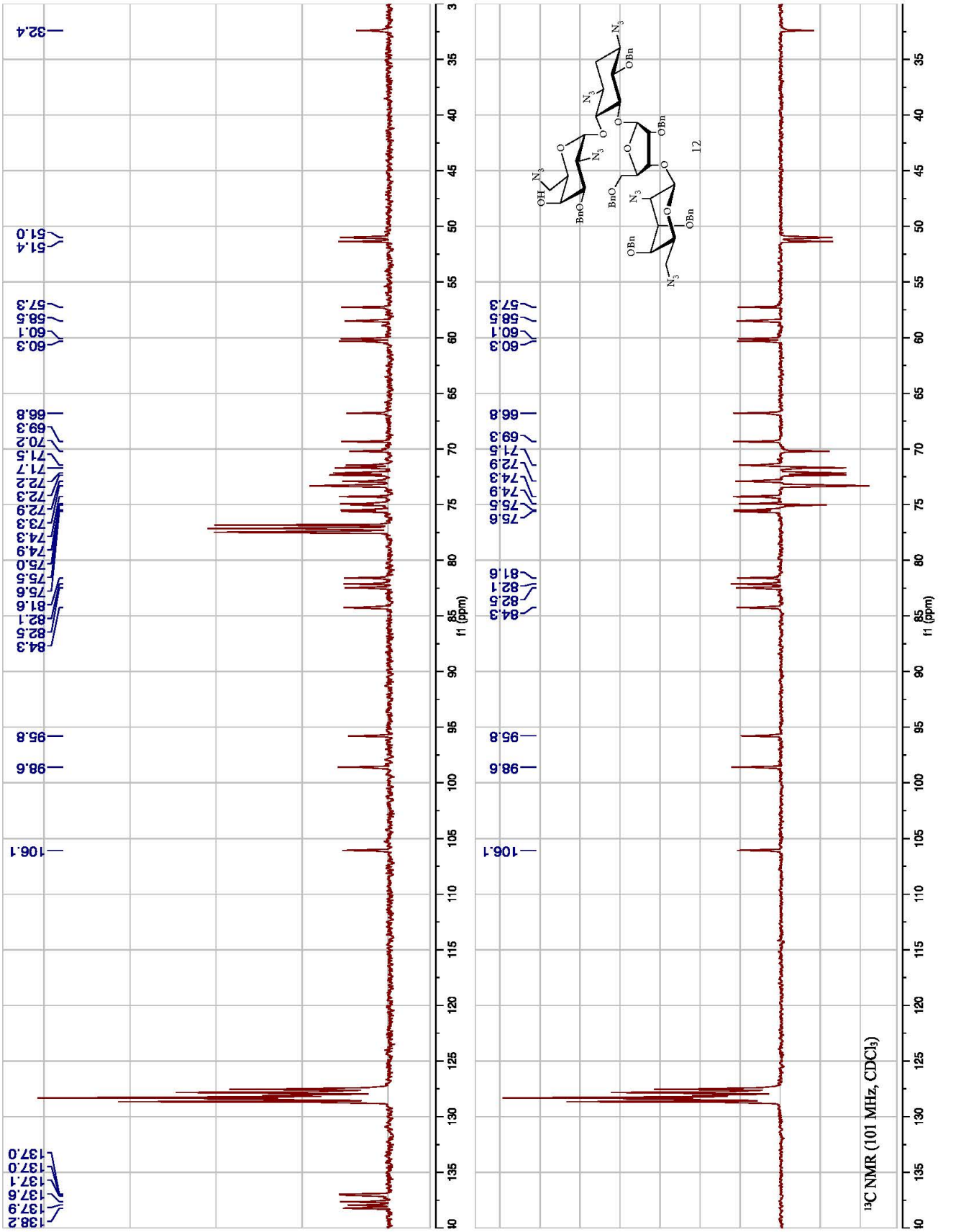
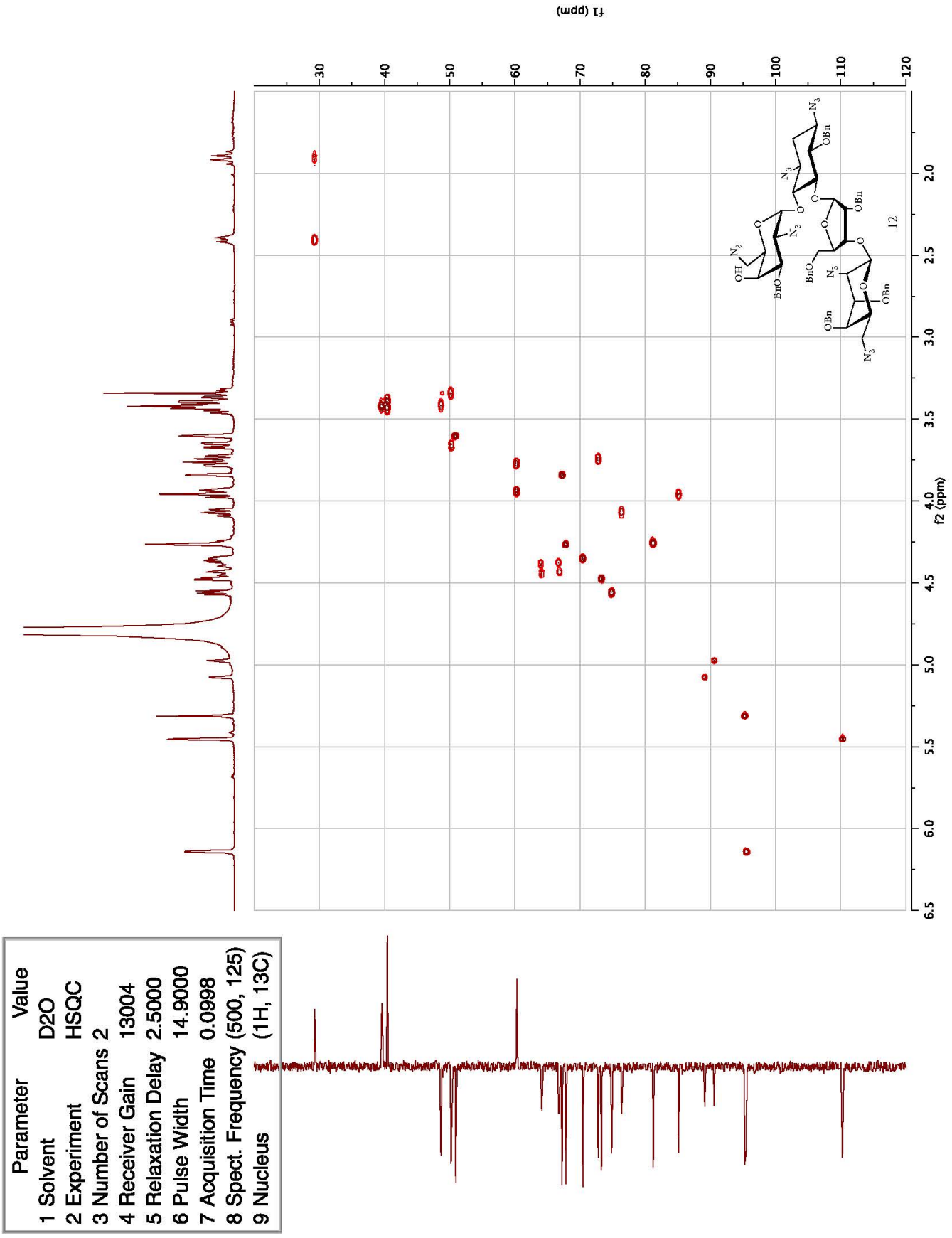
4 Receiver Gain 256
5 Relaxation Delay 3.0000
6 Pulse Width 14.9000
7 Acquisition Time 0.1704
8 Spect. Frequency (500, 500)
9 Nucleus (1H, 1H)




Parameter Value
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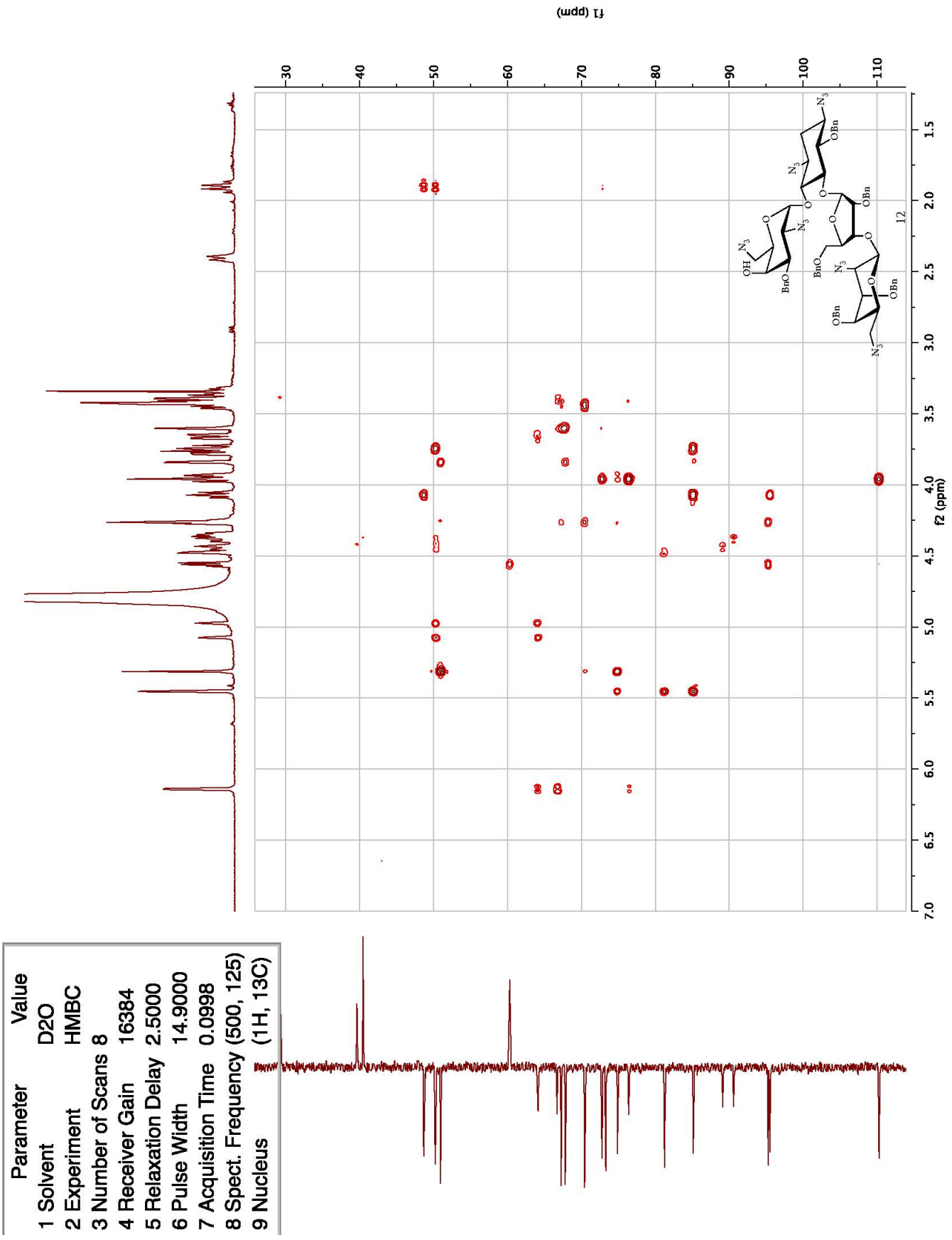
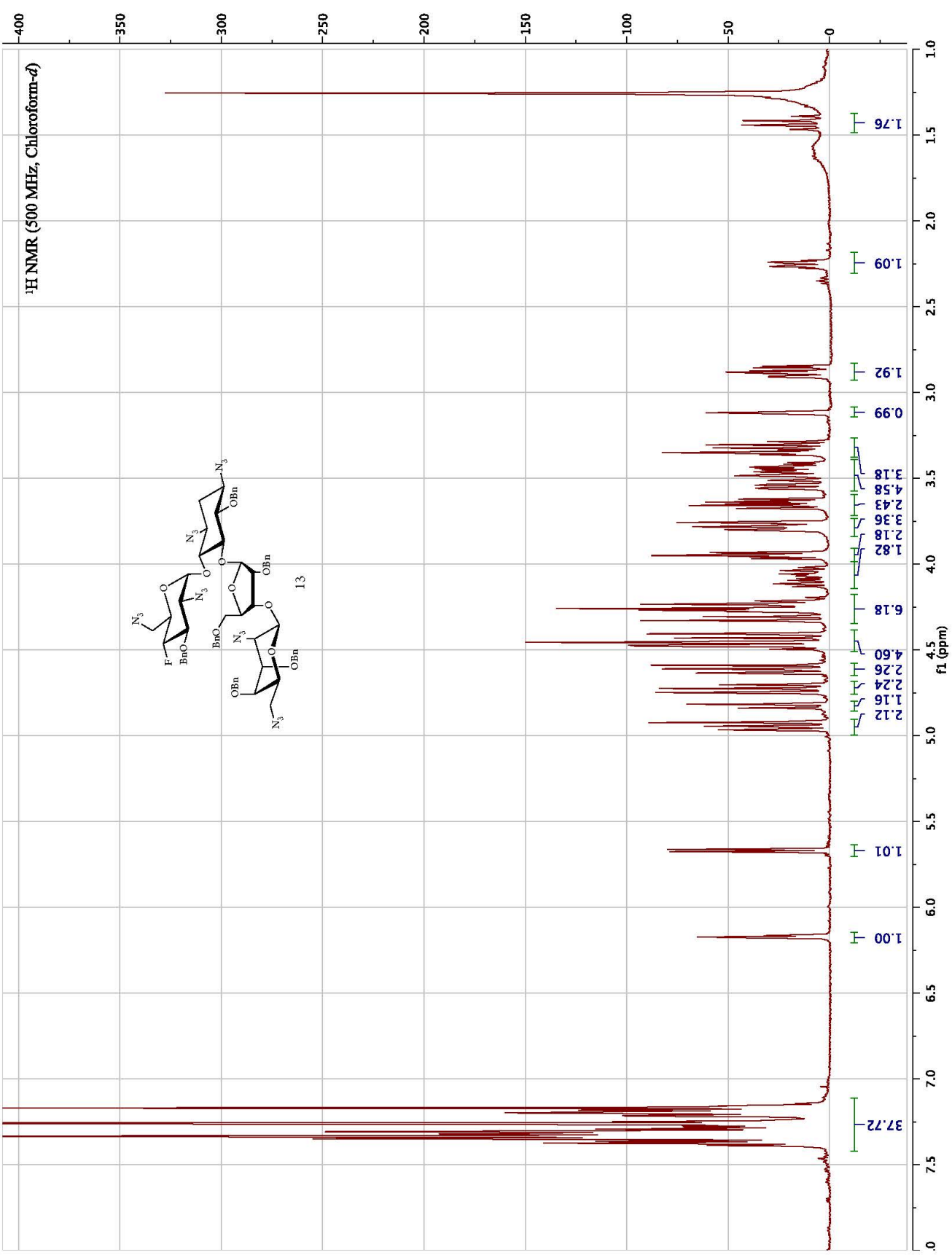
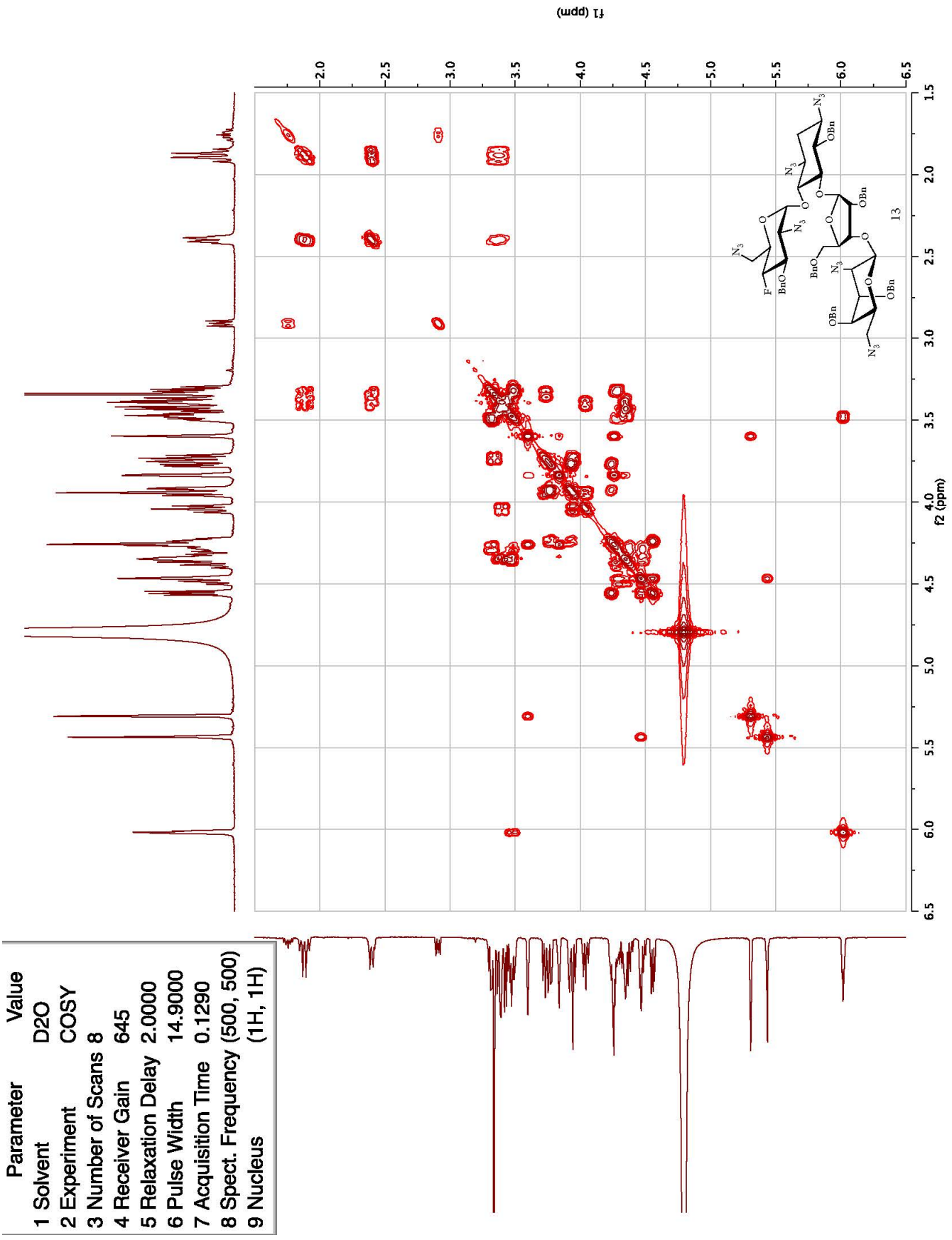
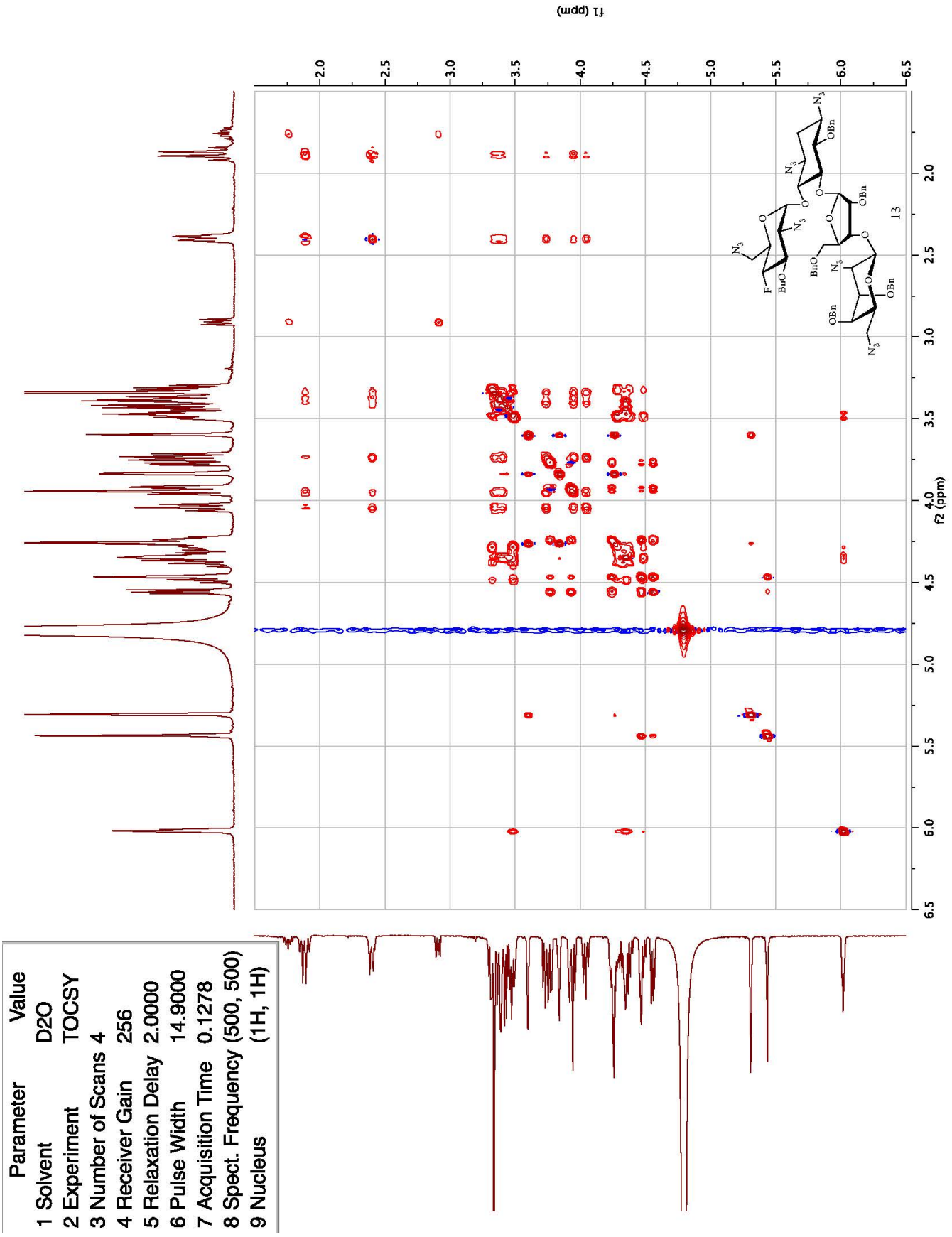
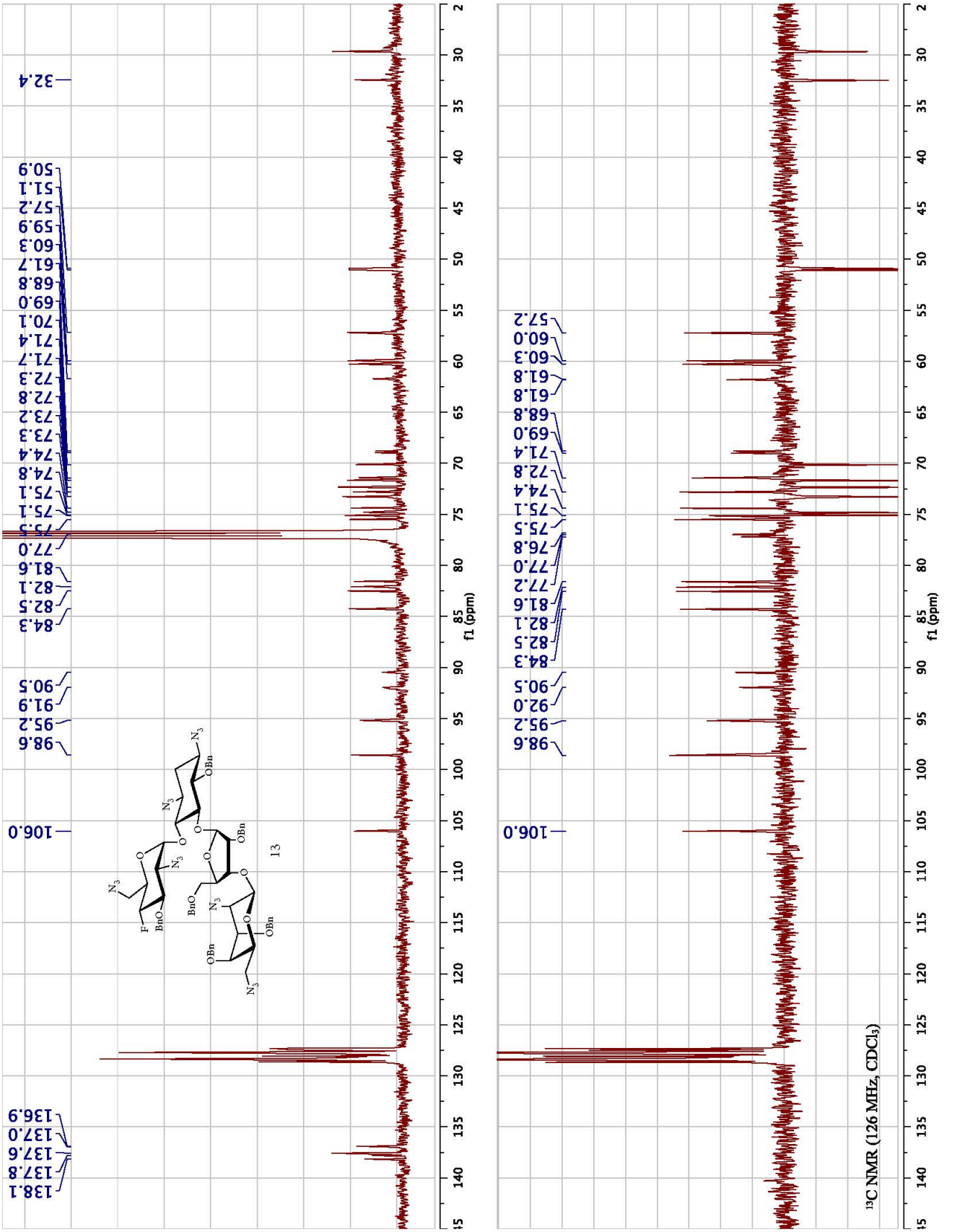
2 Experiment HSQC
3 Number of Scans 2
4 Receiver Gain 13004
5 Relaxation Delay 2.5000
6 Pulse Width 14.9000
7 Acquisition Time 0.0998
8 Spect. Frequency (500, 125)
9 Nucleus (1H, 13C)
f1 (ppm)
f2 (ppm)
12

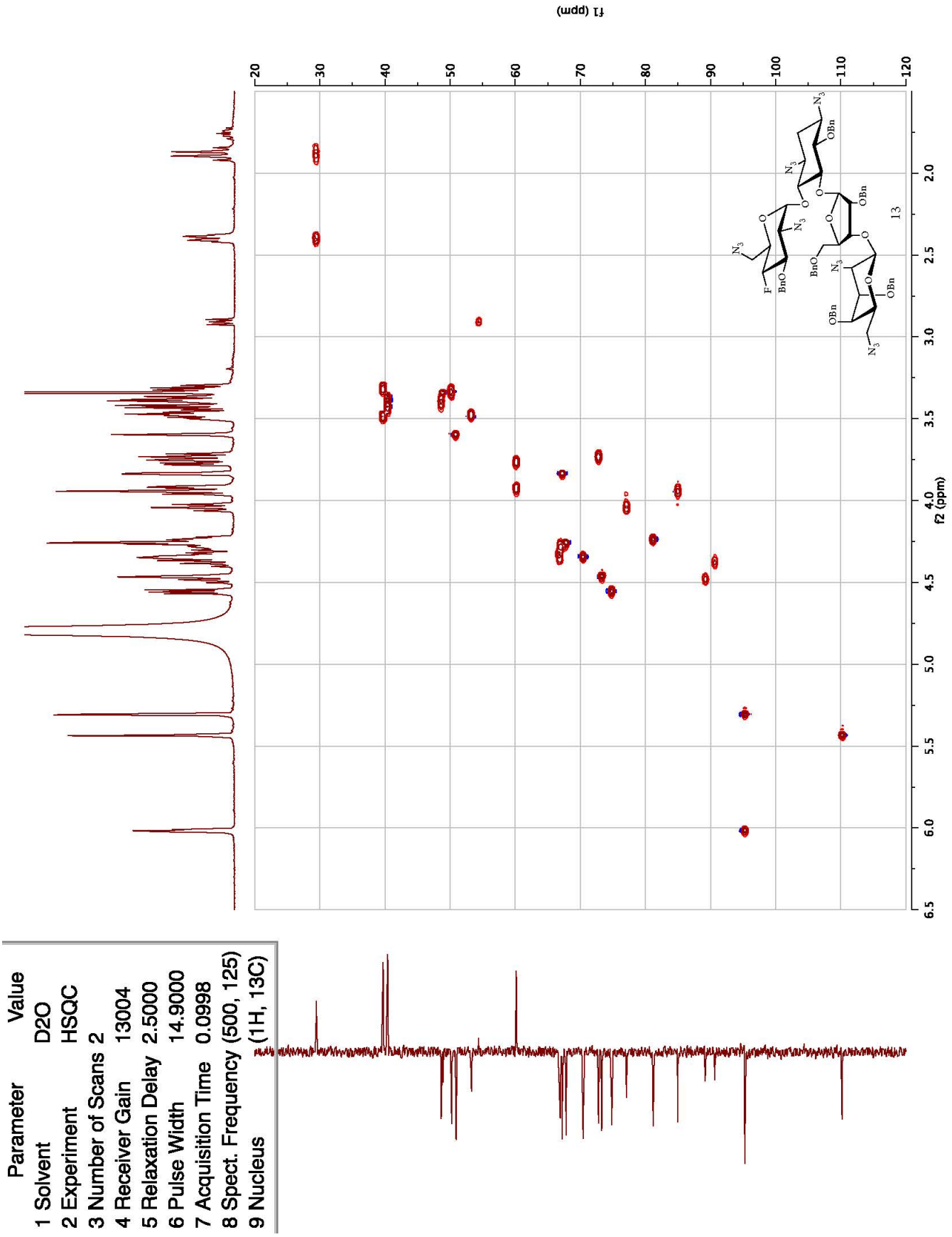


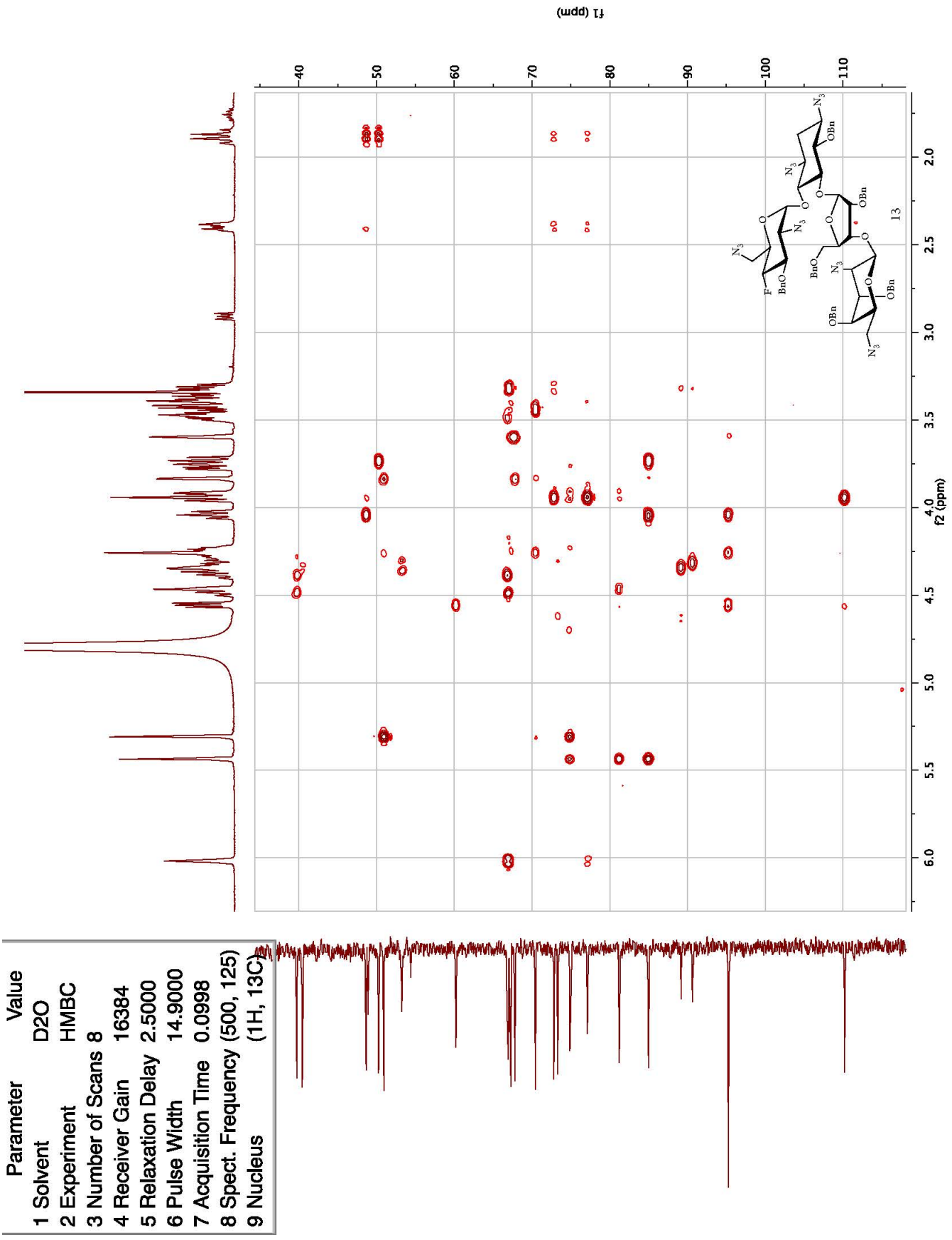

^{1}H NMR (500 MHz, Chloroform-d)
13
1.76
1.09
1.92
0.99
3.18
4.58
2.43
3.36
2.18
1.82
6.18
4.60
2.26
2.24
1.16
2.12
1.01
1.00
37.72
f1 (ppm)

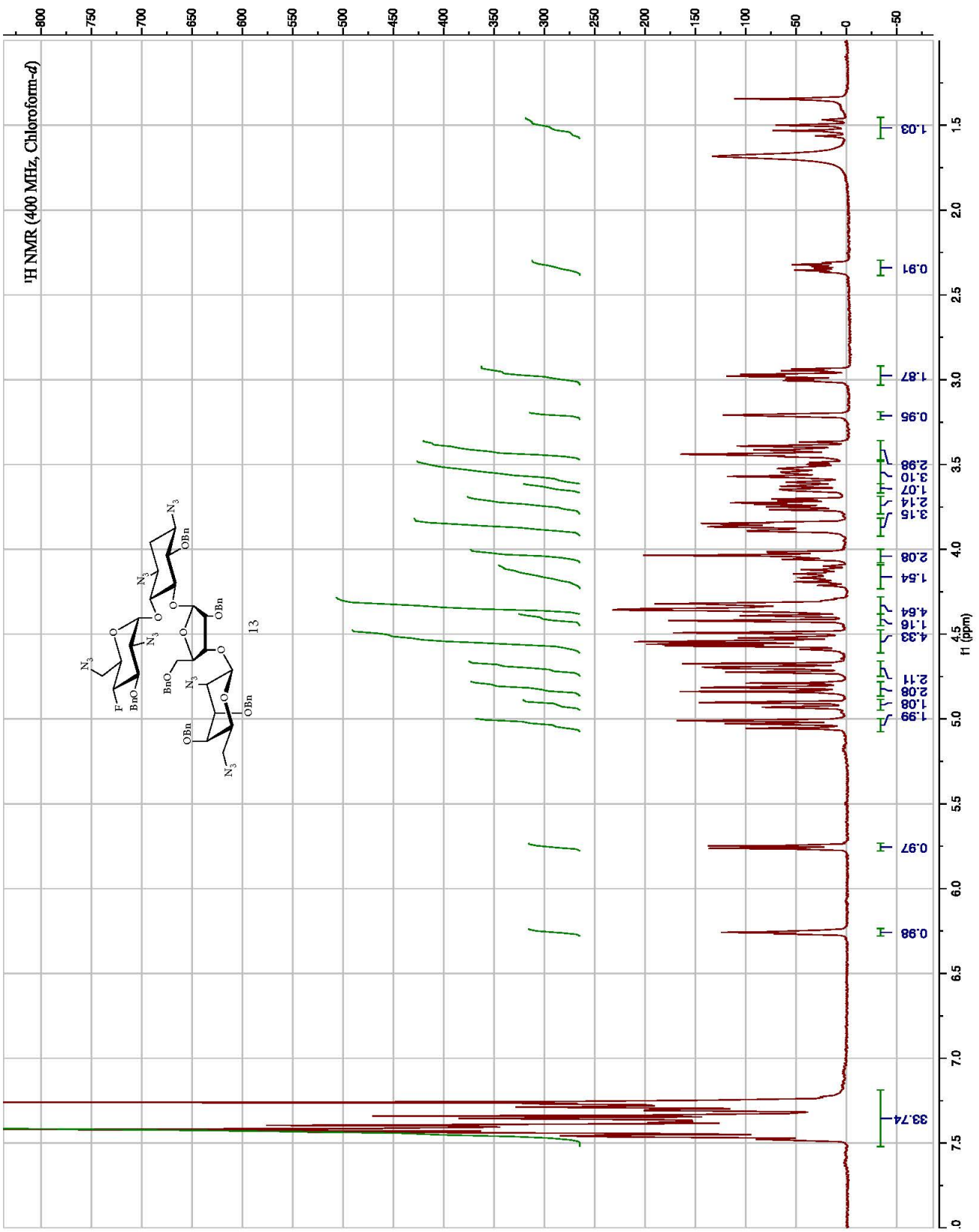


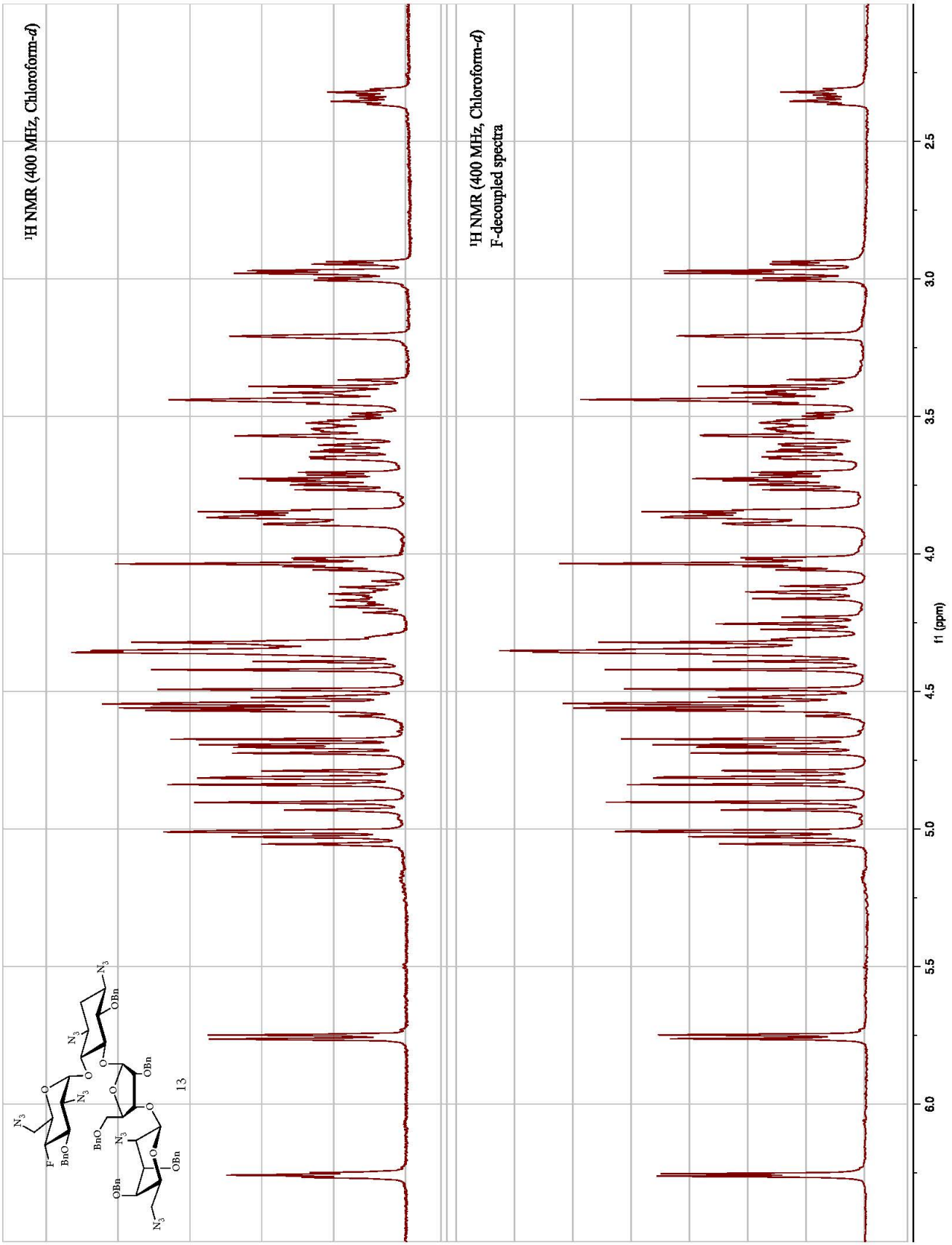

f1 (ppm)
f2 (ppm)
13
Parameter Value
1 Solvent D2O
2 Experiment TOCSY
3 Number of Scans 4
4 Receiver Gain 256
5 Relaxation Delay 2.0000
6 Pulse Width 14.9000
7 Acquisition Time 0.1278
8 Spect. Frequency (500, 500)
9 Nucleus (1H, 1H)

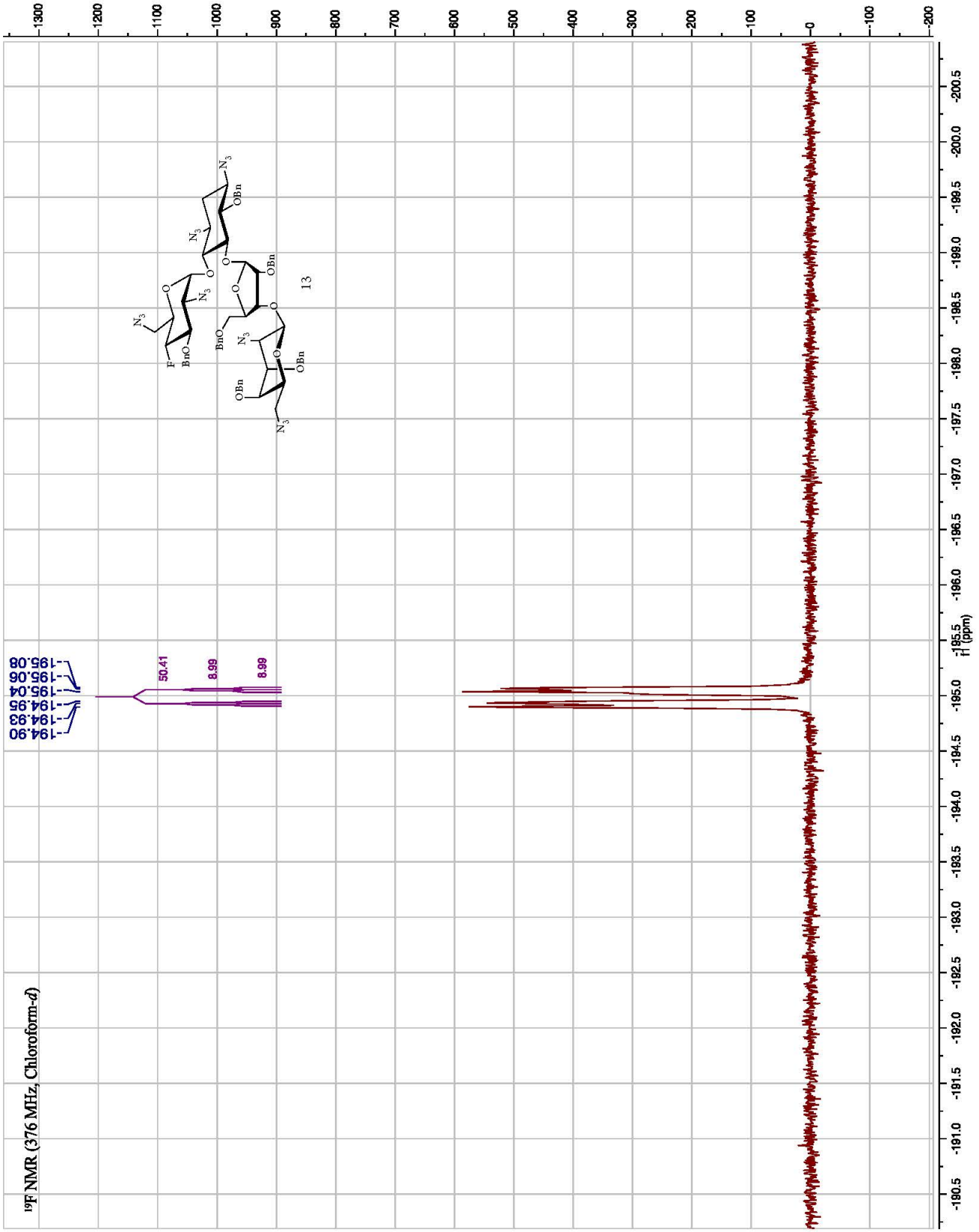


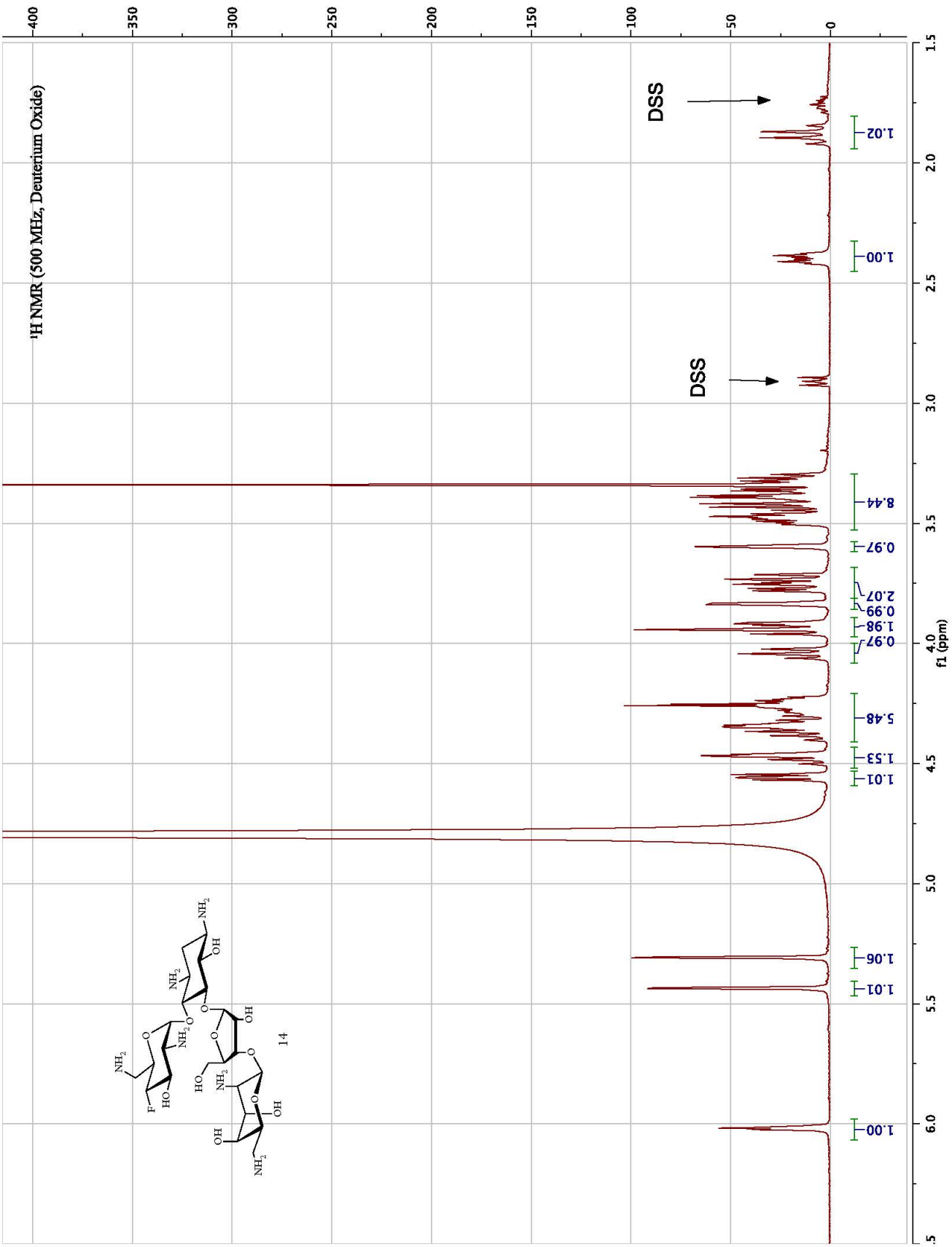

Parameter Value
1 Solvent D2O
2 Experiment HSQC
3 Number of Scans 2
4 Receiver Gain 13004
5 Relaxation Delay 2.5000
6 Pulse Width 14.9000
7 Acquisition Time 0.0998
8 Spect. Frequency (500, 125)
9 Nucleus (1H, 13C)
f1 (ppm)
f2 (ppm)
13

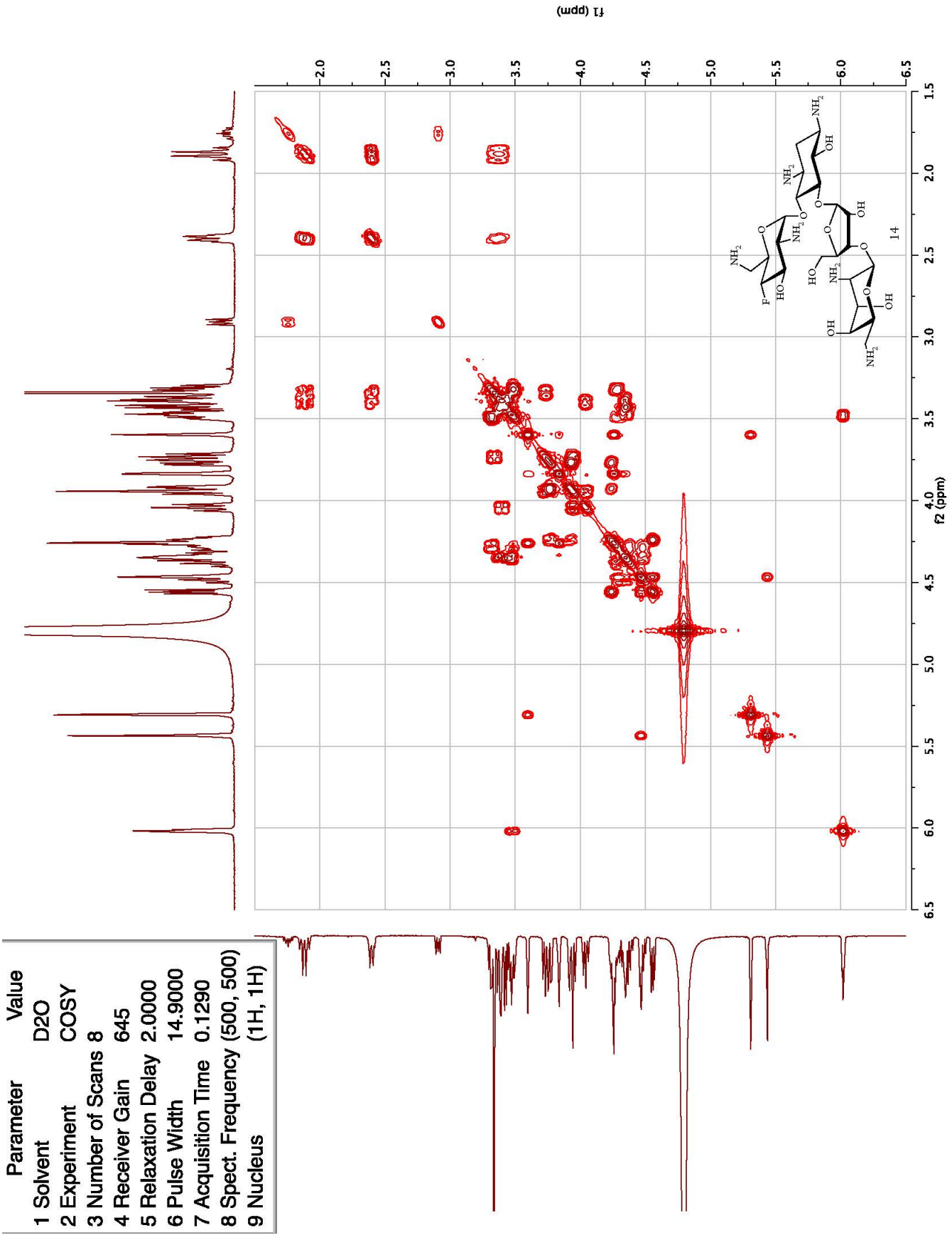


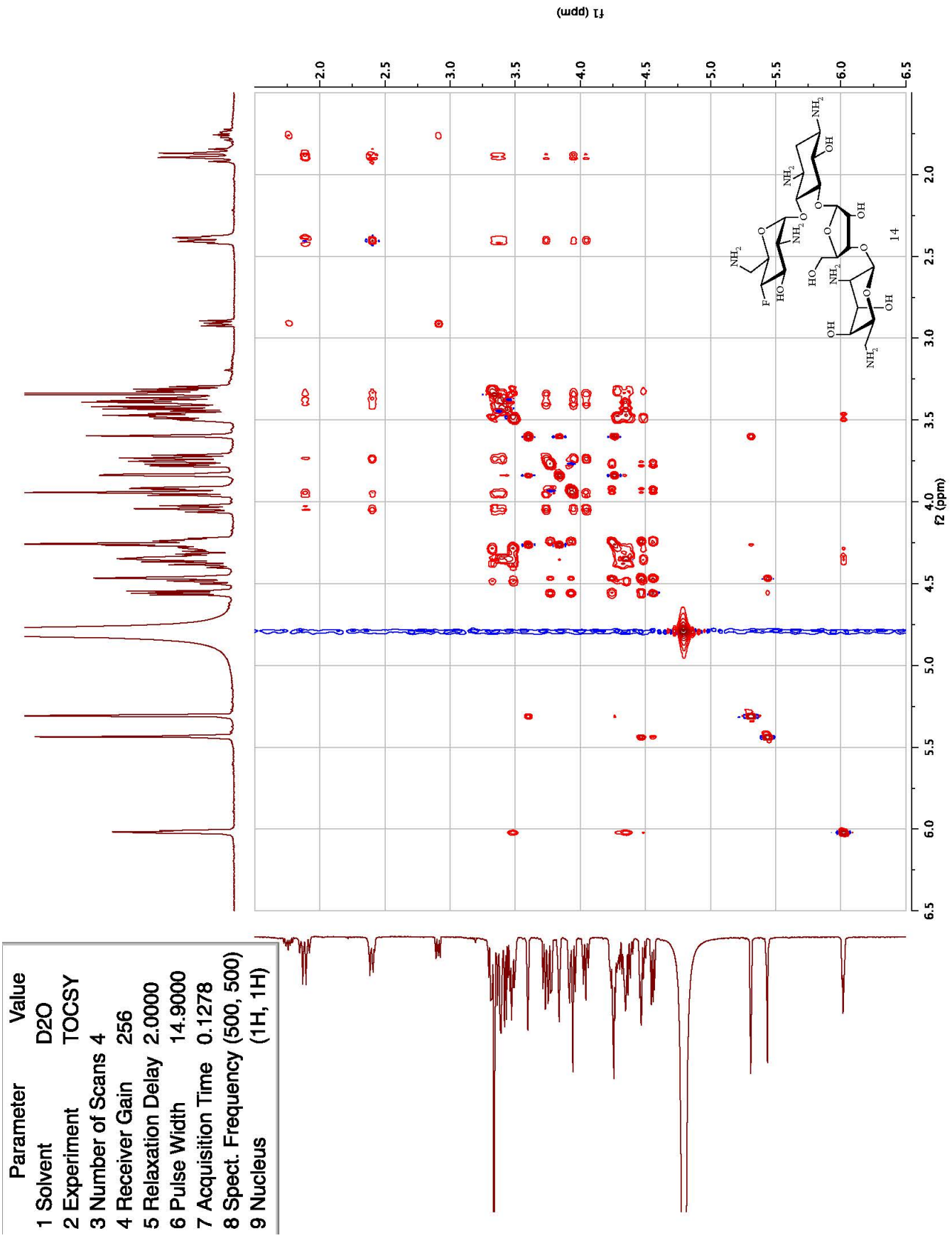

^{1}H NMR (400 MHz, Chloroform-d)
13
f1 (ppm)

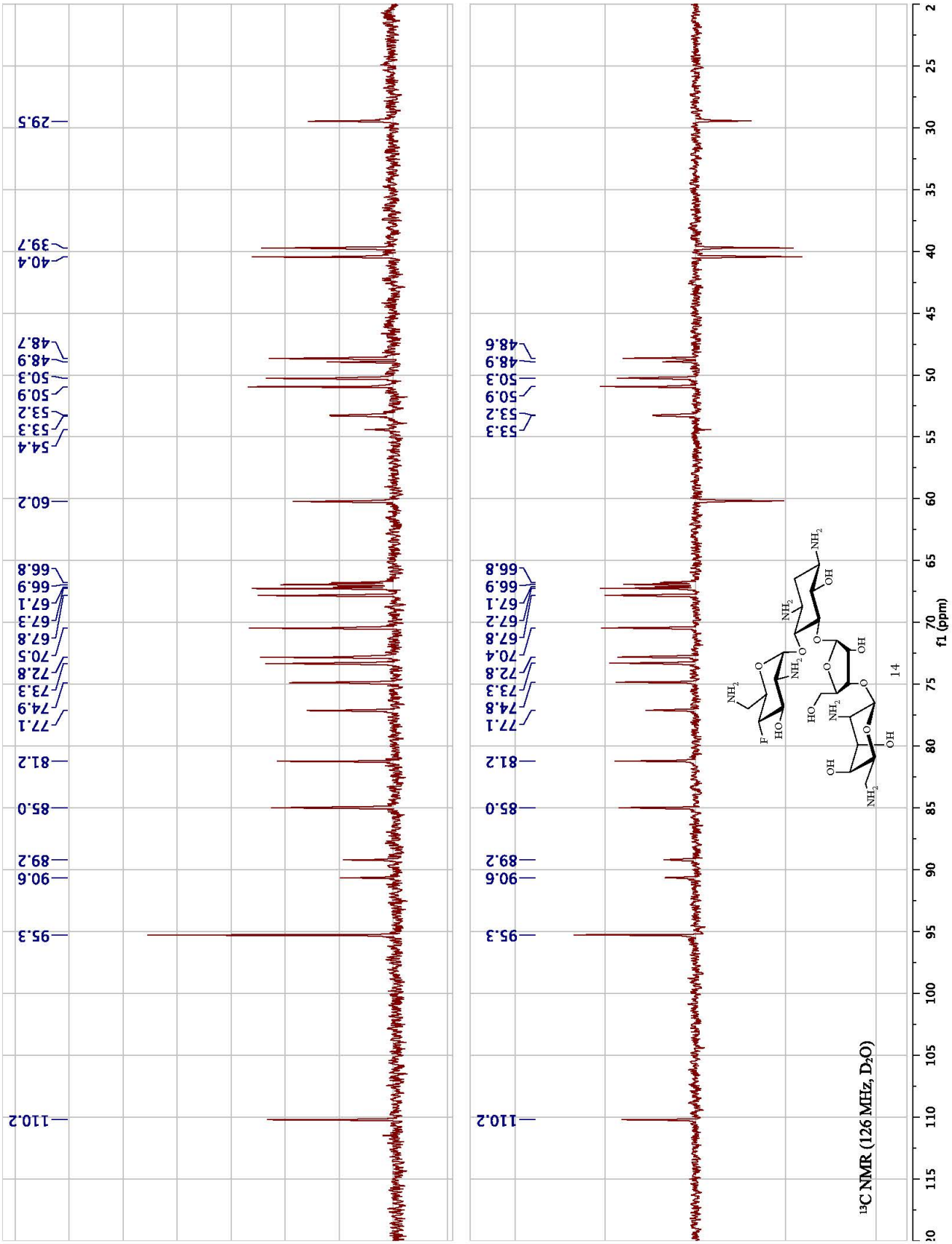


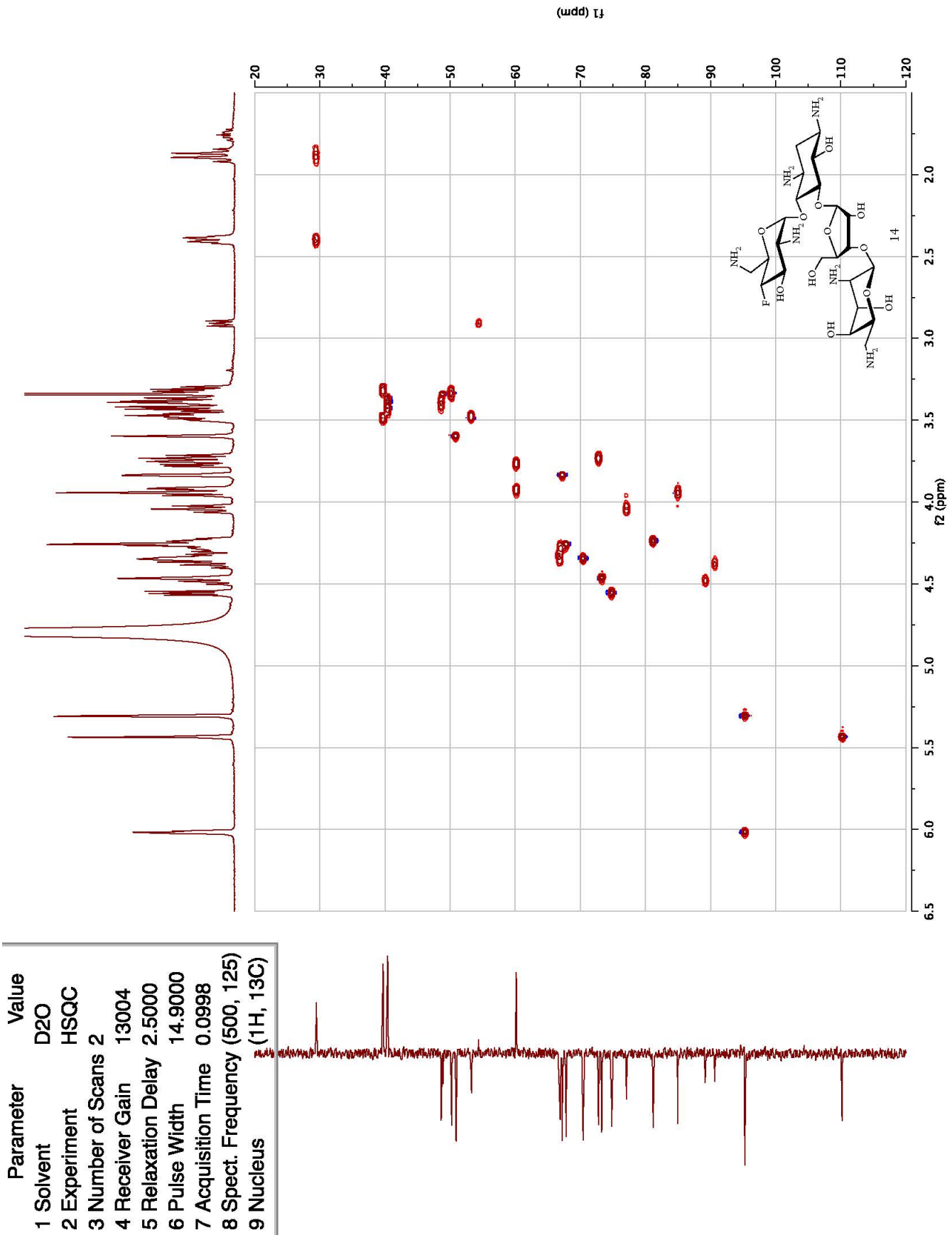

19F NMR (376 MHz, Chloroform-d)
13
50.41
8.99
8.99
-194.90
-194.93
-194.95
-195.04
-195.06
-195.08
f1 (ppm)

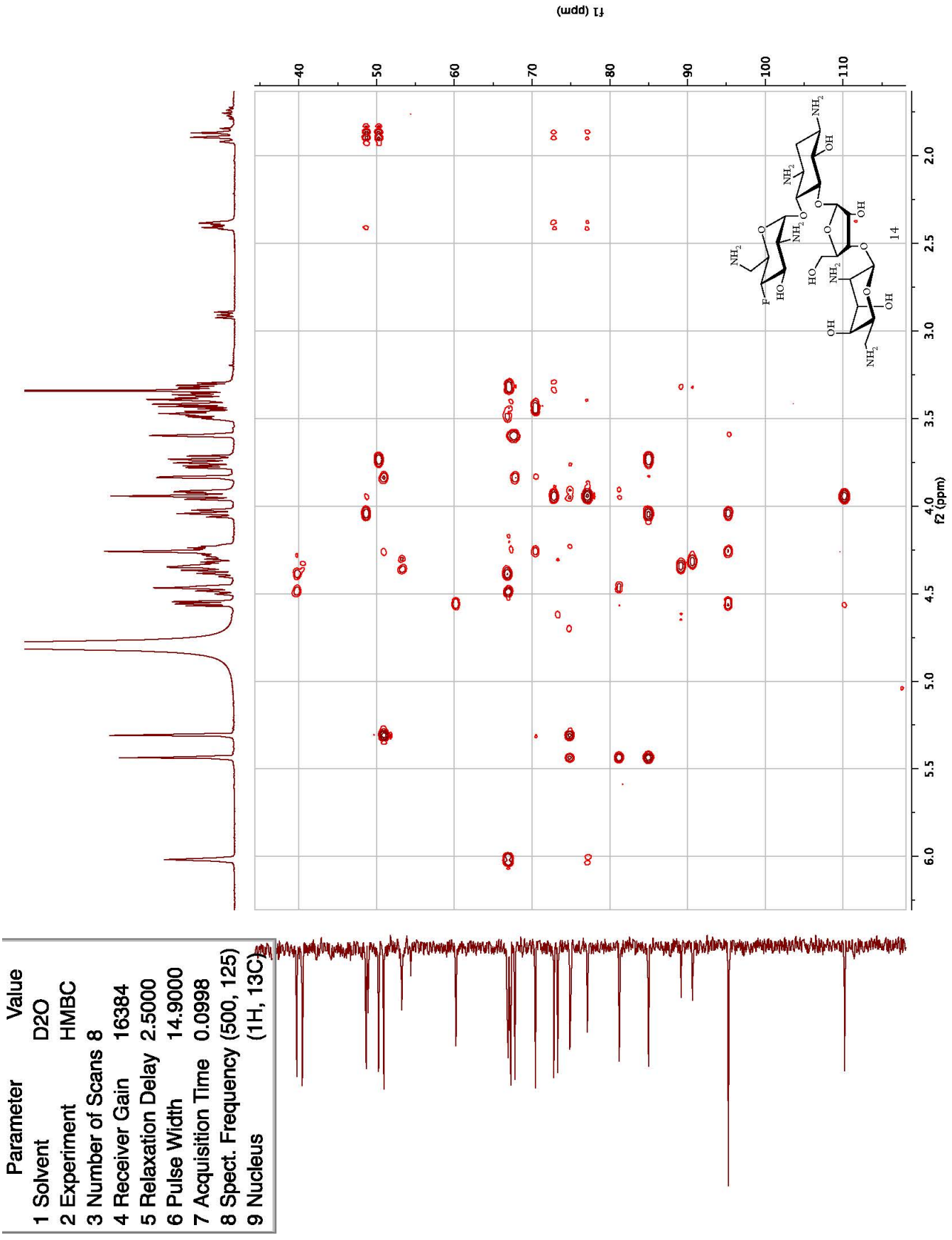


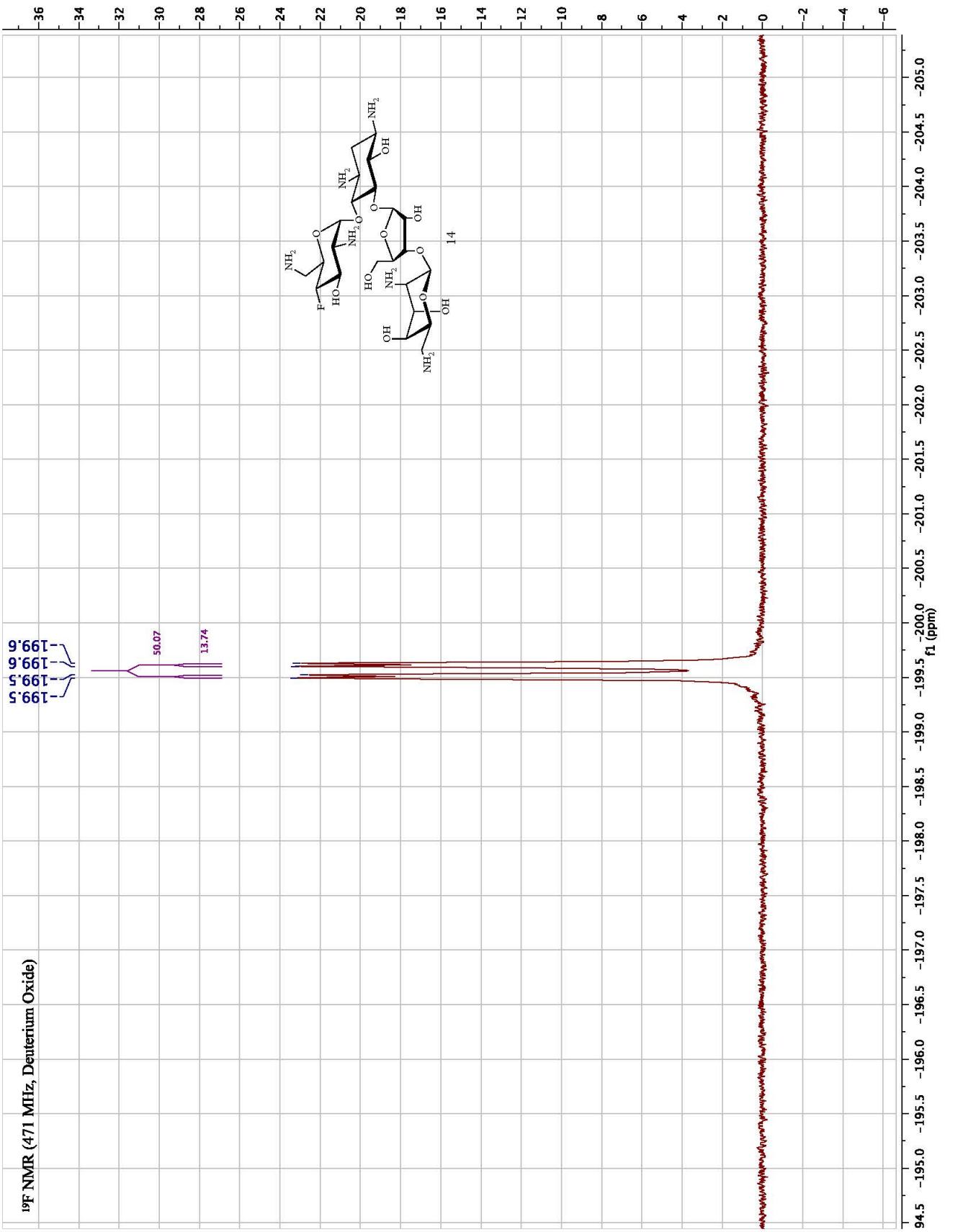

f1 (ppm)
f2 (ppm)
14
Parameter Value
1 Solvent D2O
2 Experiment COSY
3 Number of Scans 8
4 Receiver Gain 645
5 Relaxation Delay 2.0000
6 Pulse Width 14.9000
7 Acquisition Time 0.1290
8 Spect. Frequency (500, 500)
9 Nucleus (1H, 1H)

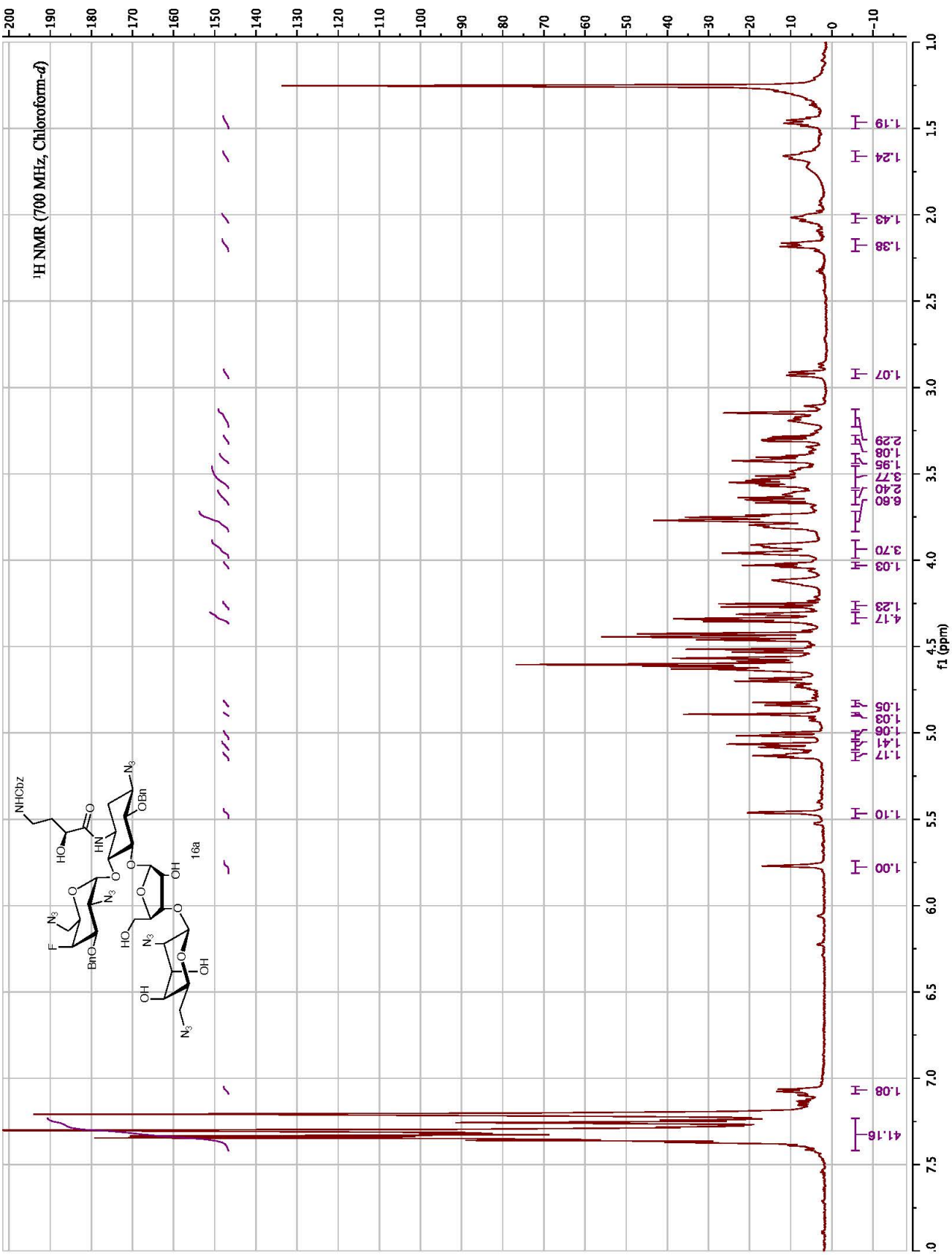


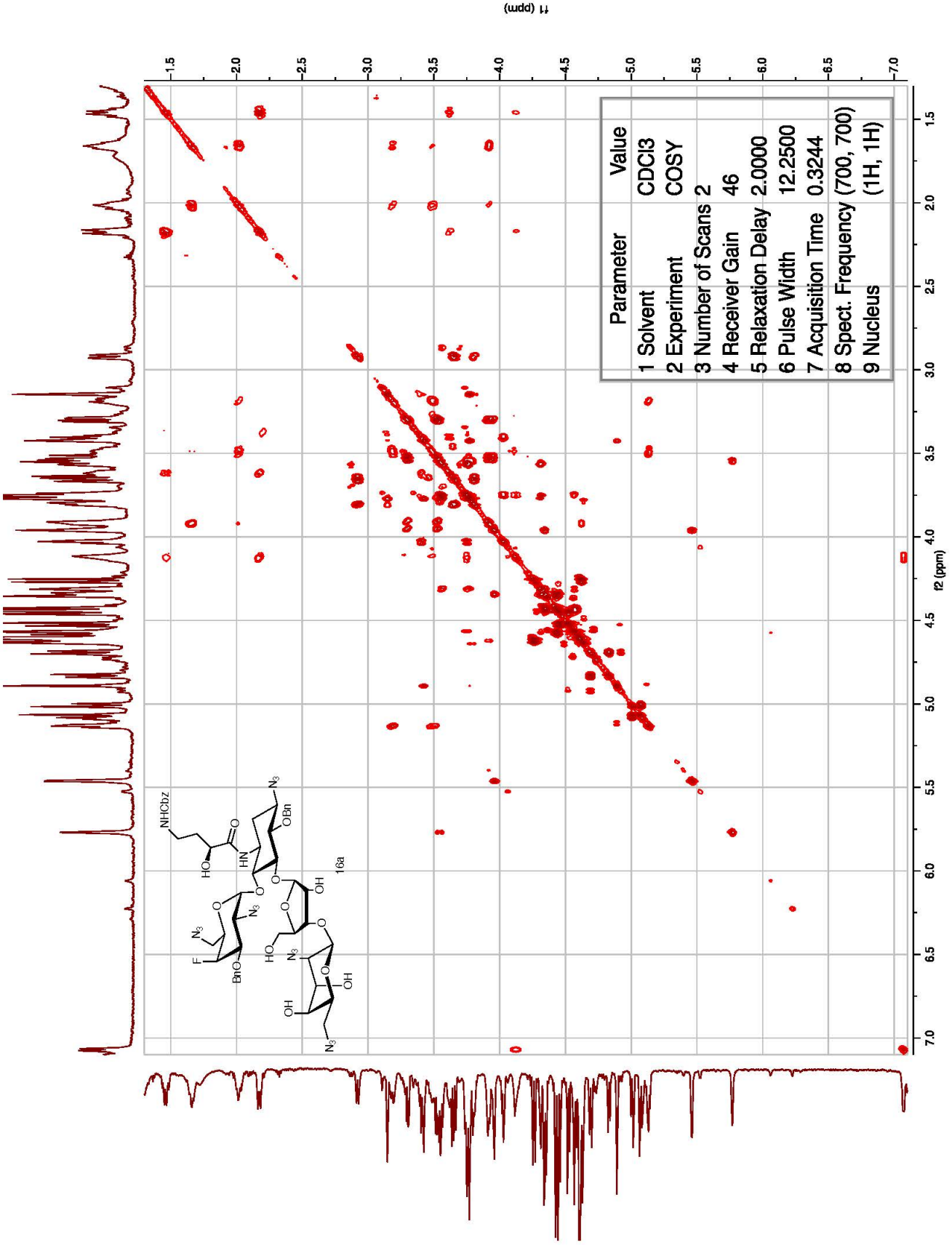

29.5
39.7
40.4
48.7
48.9
50.3
50.9
53.2
53.3
54.4
60.2
66.8
66.9
67.1
67.3
67.8
70.5
72.8
73.3
74.9
77.1
81.2
85.0
89.2
90.6
95.3
110.2
48.6
48.9
50.3
50.9
53.2
53.3
66.8
66.9
67.1
67.2
67.8
70.4
72.8
73.3
74.8
77.1
81.2
85.0
89.2
90.6
95.3
110.2
f1 (ppm)
^{13}C NMR (126 MHz, D$_2$O)
14

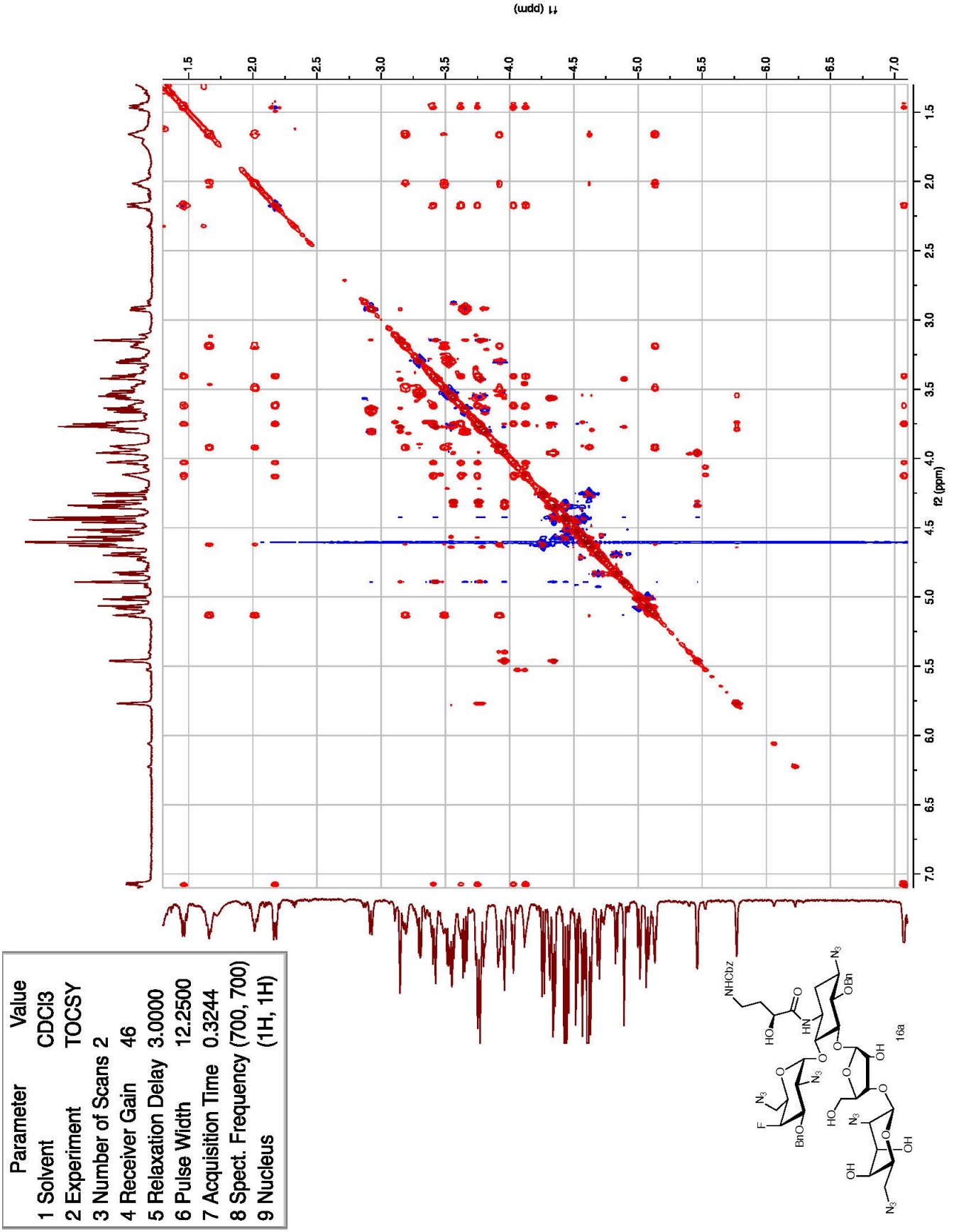


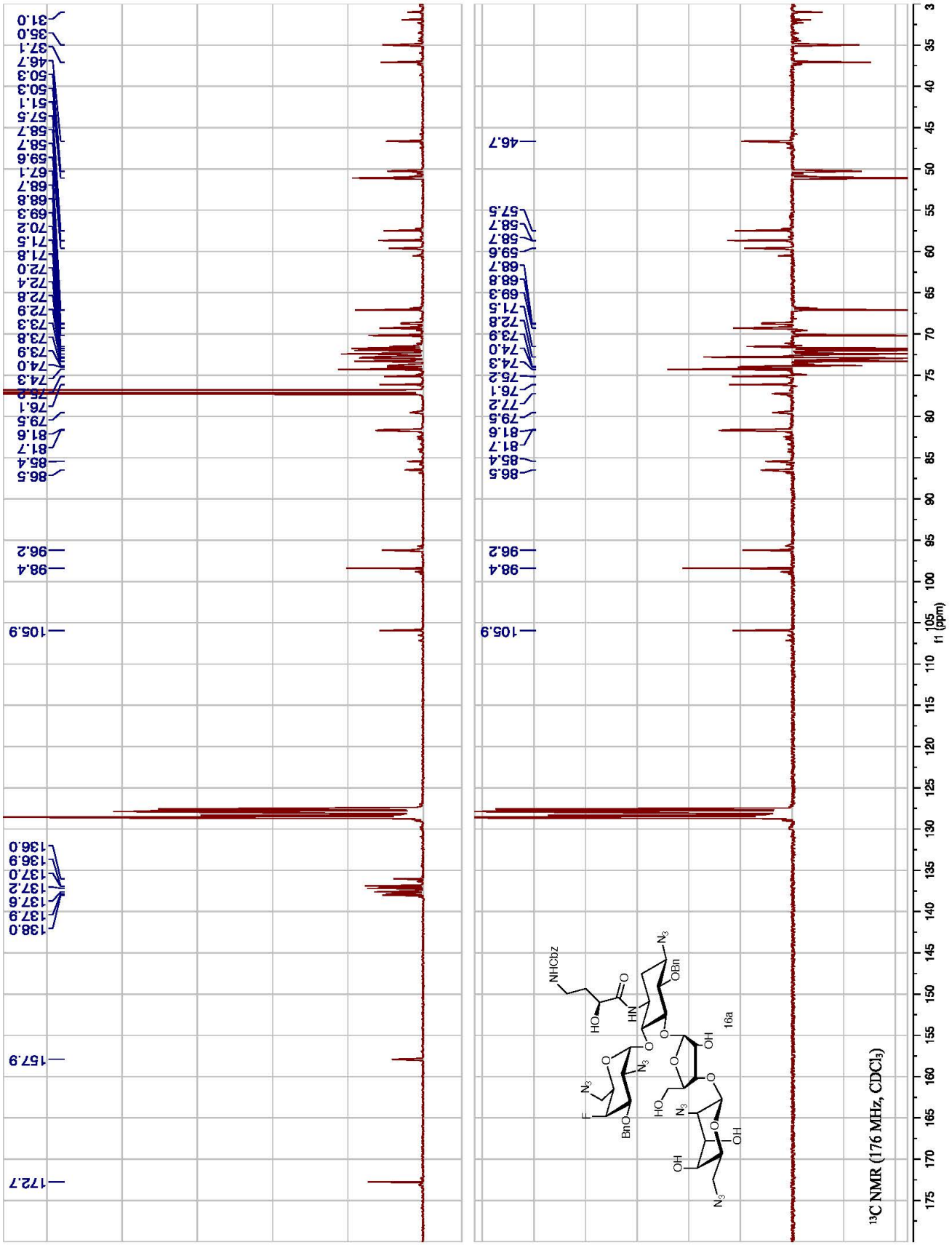

f1 (ppm)
f2 (ppm)
14
Parameter Value
1 Solvent D2O
2 Experiment HMBC
3 Number of Scans 8
4 Receiver Gain 16384
5 Relaxation Delay 2.5000
6 Pulse Width 14.9000
7 Acquisition Time 0.0998
8 Spect. Frequency (500, 125)
9 Nucleus (1H, 13C)

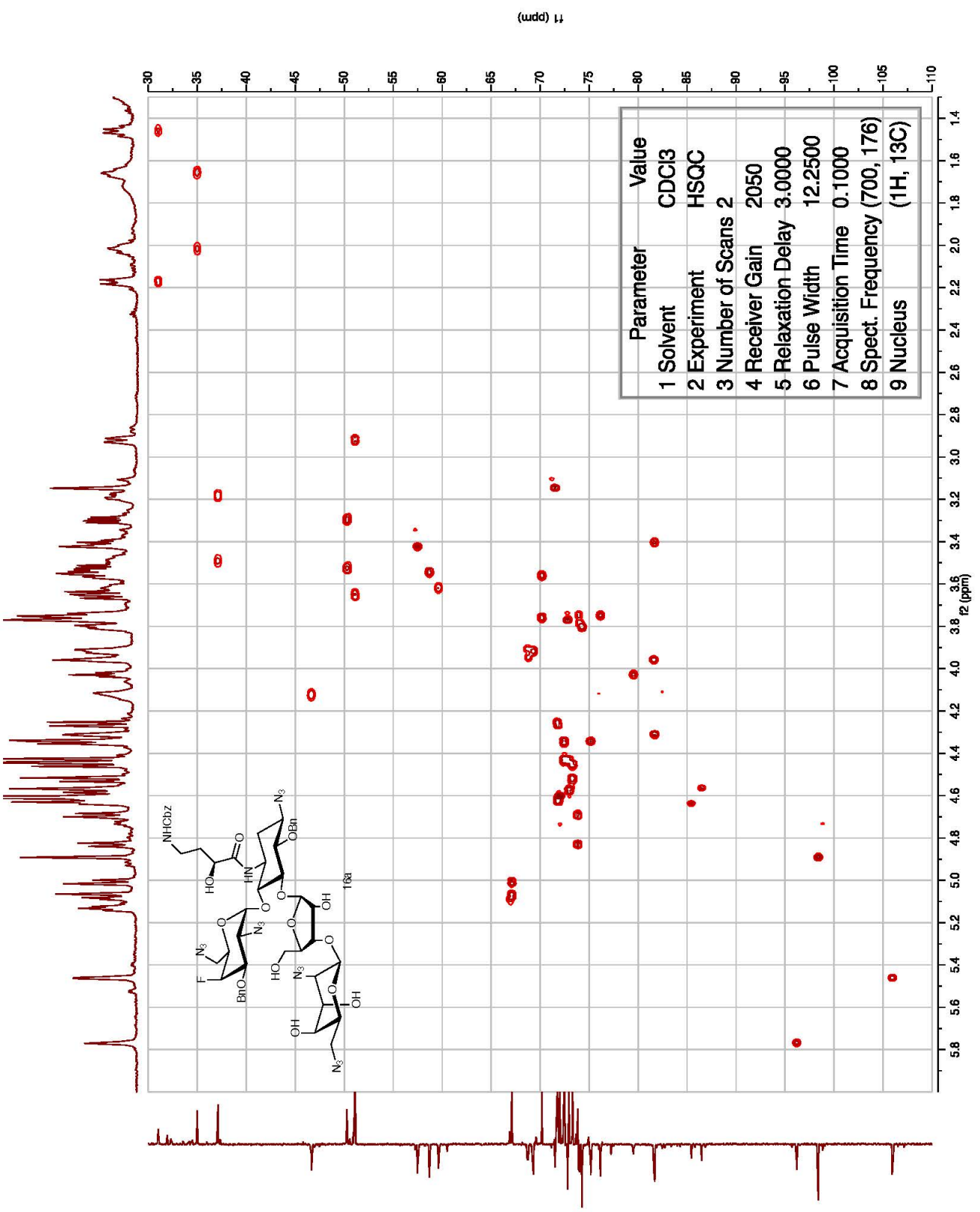


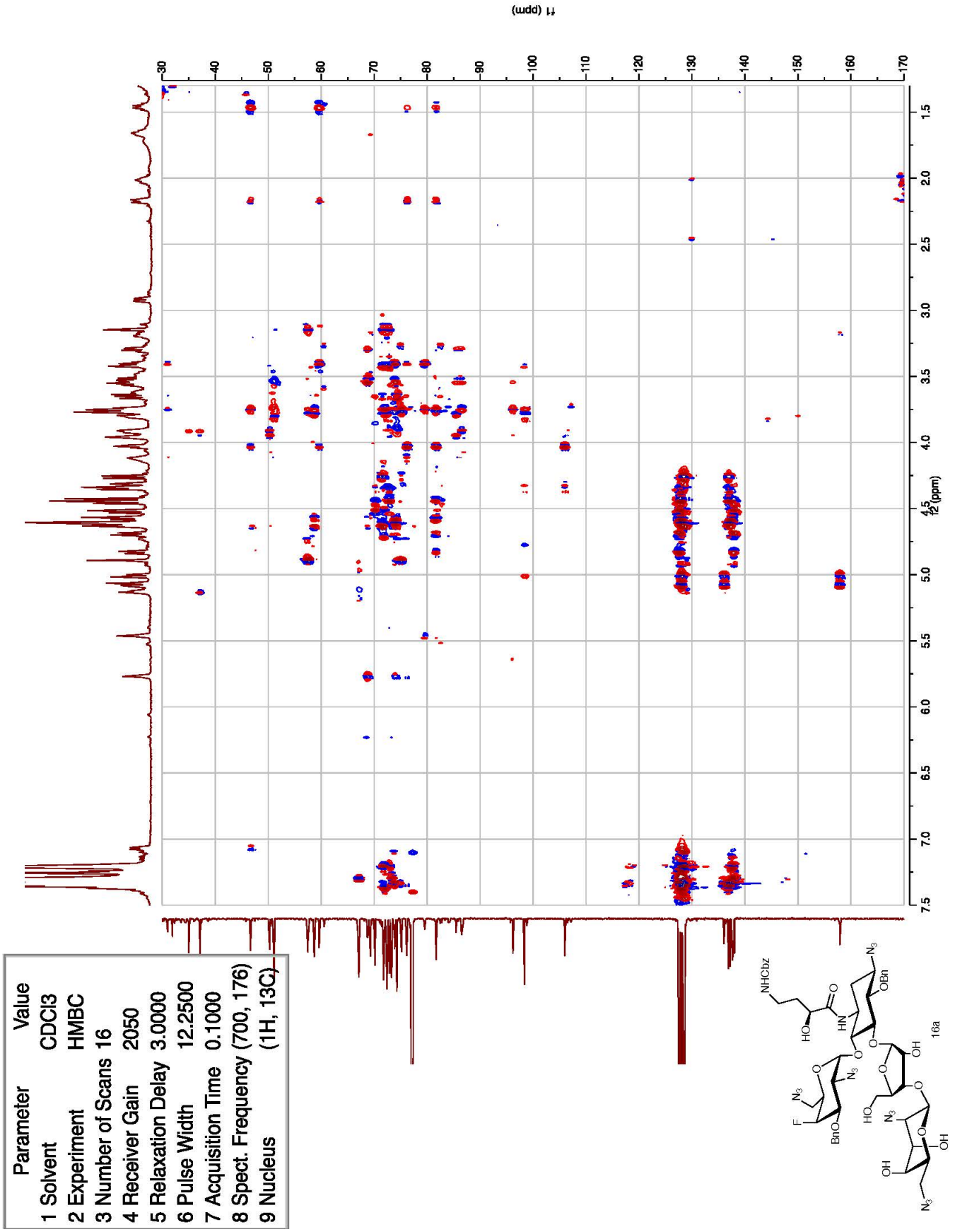

^{1}H NMR (700 MHz, Chloroform-*d*)
16a
f1 (ppm)

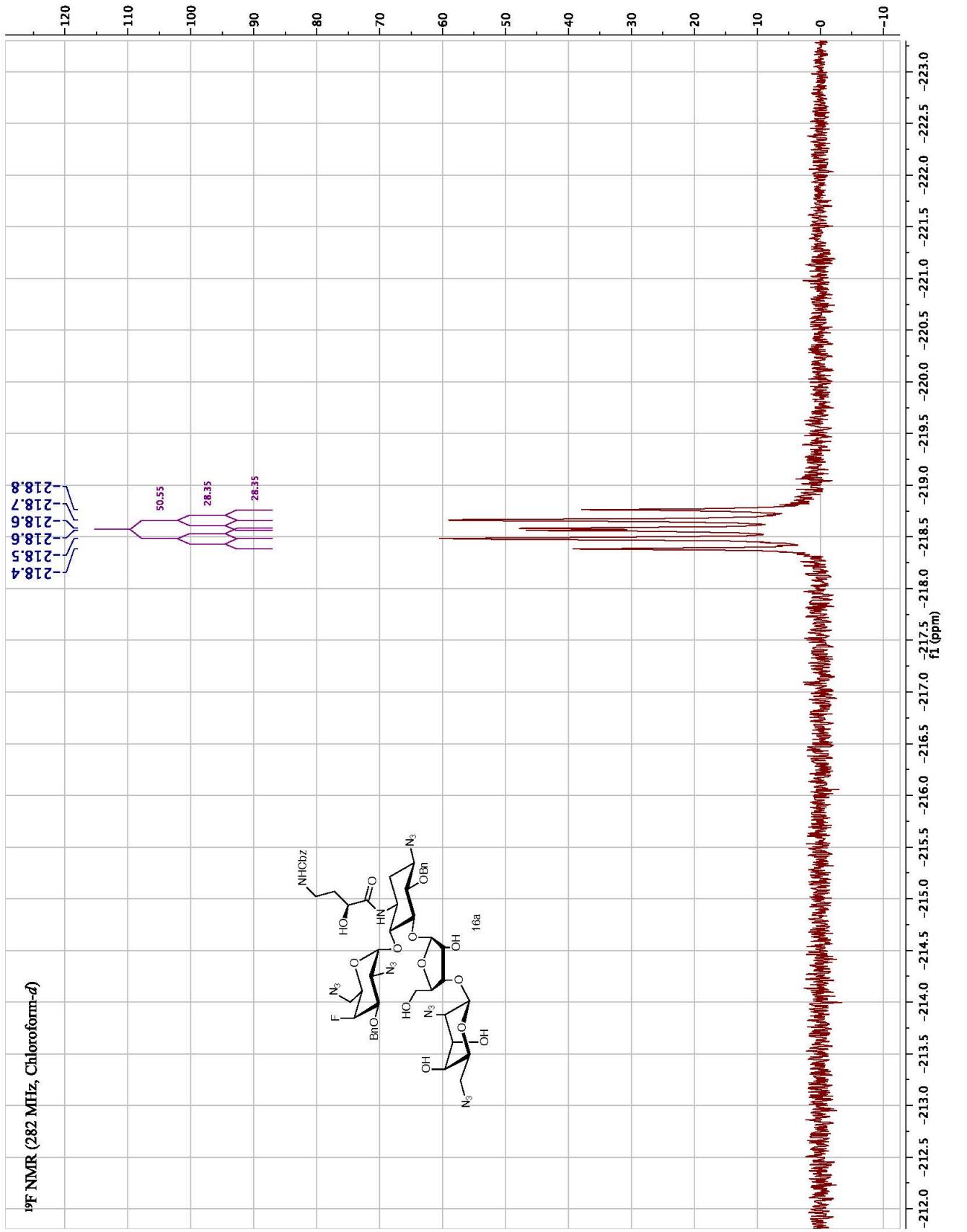


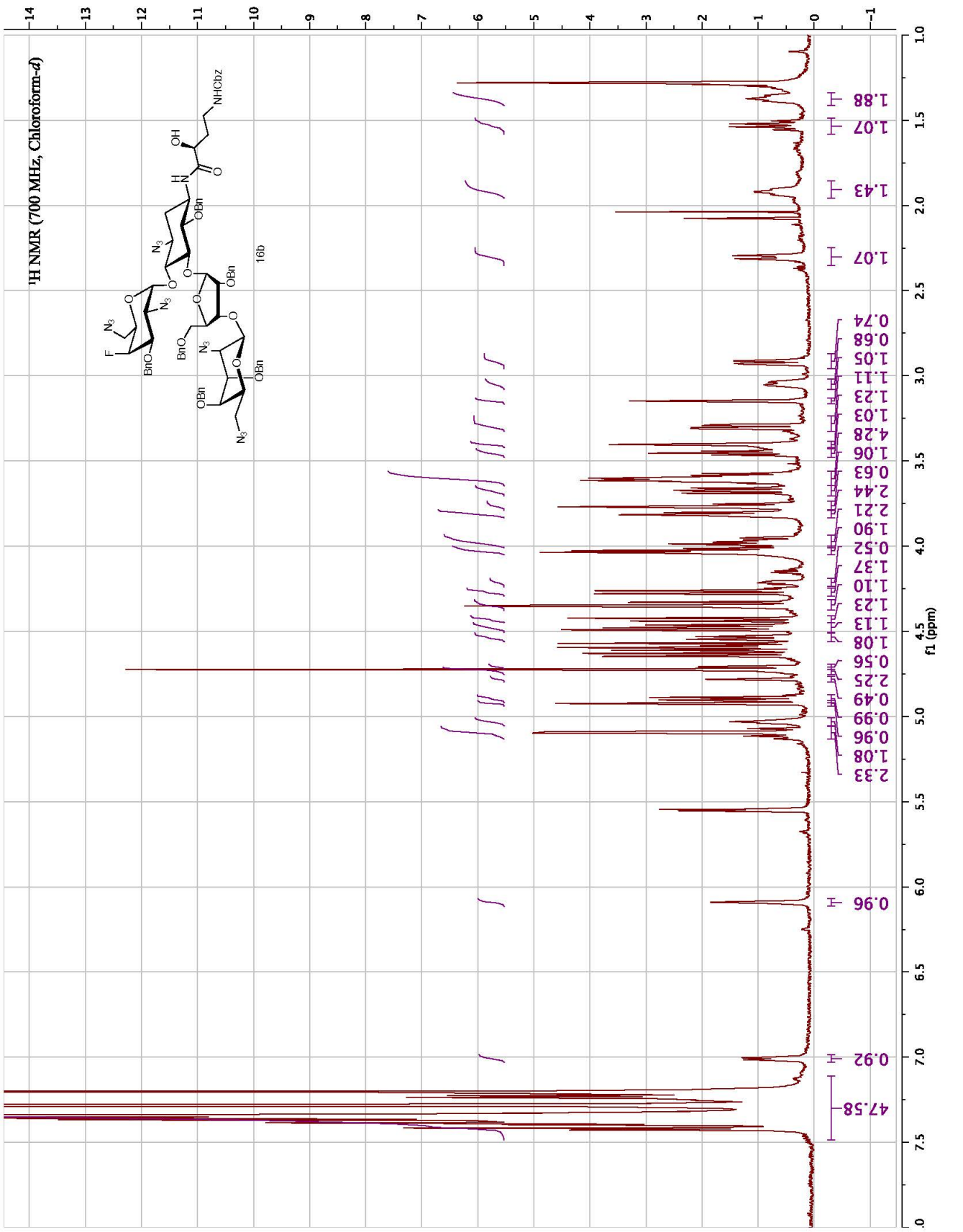

f1 (ppm)
f2 (ppm)
Parameter Value
1 Solvent CDCl3
2 Experiment TOCSY
3 Number of Scans 2
4 Receiver Gain 46
5 Relaxation Delay 3.0000
6 Pulse Width 12.2500
7 Acquisition Time 0.3244
8 Spect. Frequency (700, 700)
9 Nucleus (1H, 1H)
16a

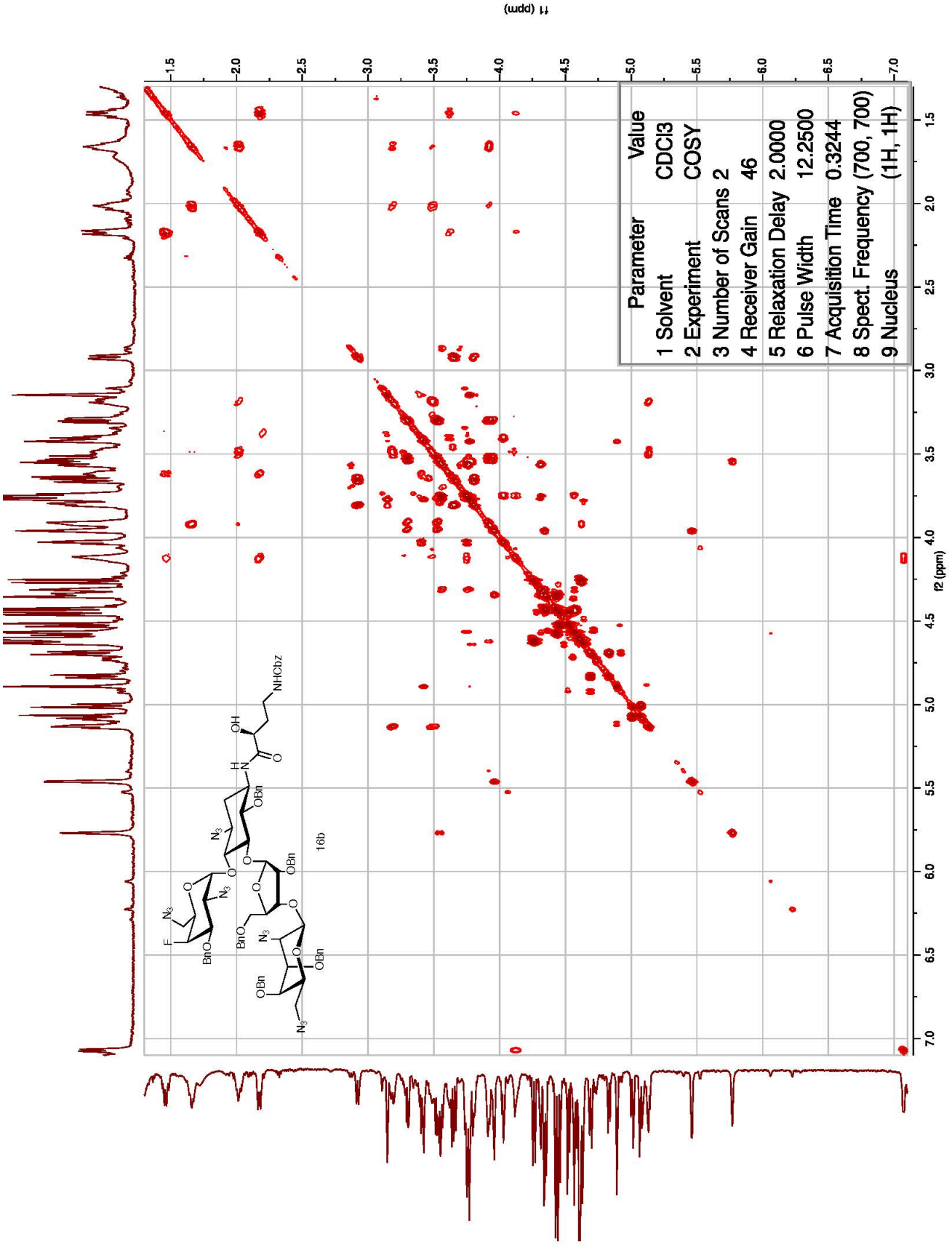


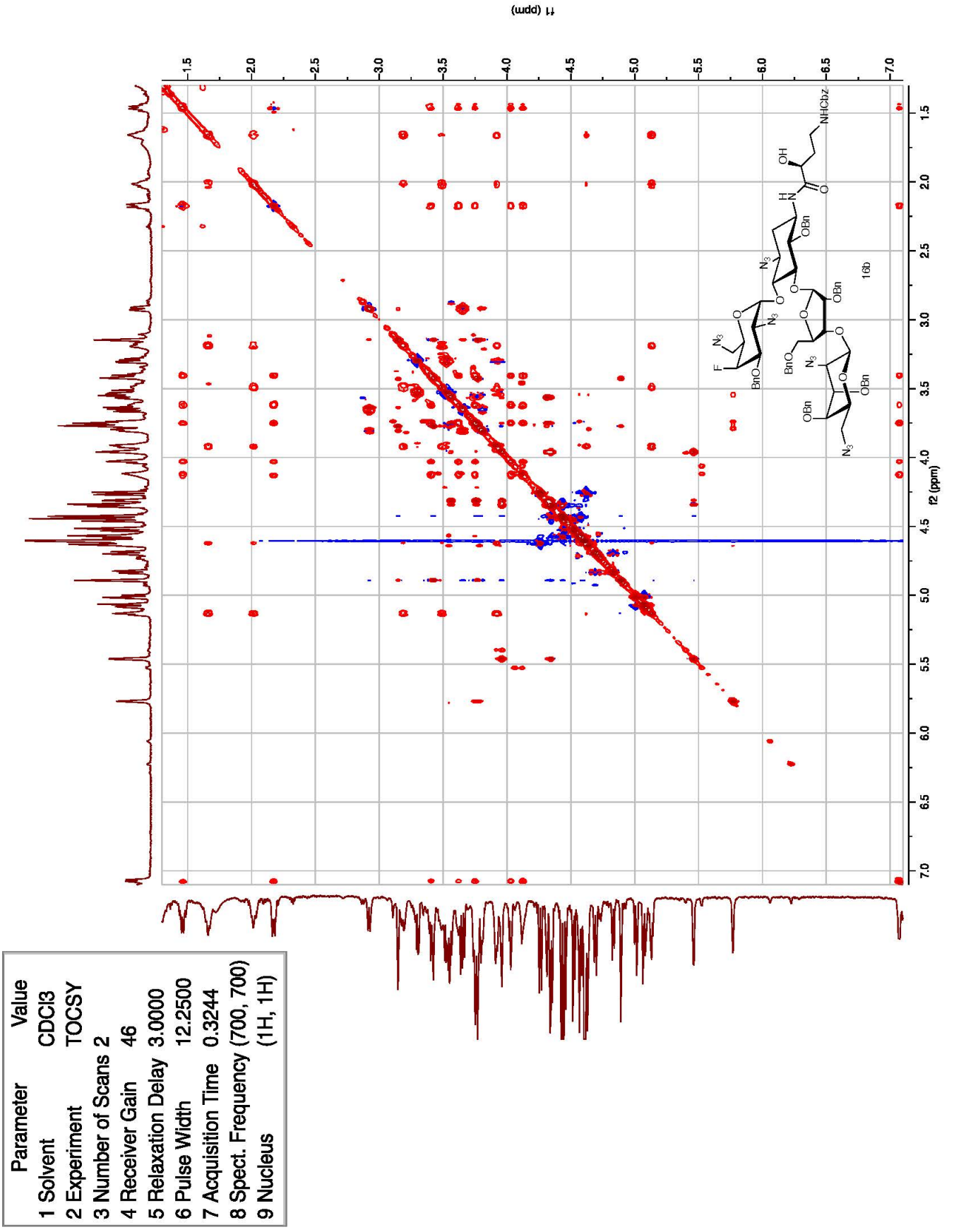

f1 (ppm)
f2 (ppm)
Parameter Value
1 Solvent CDCl3
2 Experiment HSQC
3 Number of Scans 2
4 Receiver Gain 2050
5 Relaxation Delay 3.0000
6 Pulse Width 12.2500
7 Acquisition Time 0.1000
8 Spect. Frequency (700, 176)
9 Nucleus (1H, 13C)
NHCbz
HO
HN
OBn
N3
BnO
F
HO
OH
16a

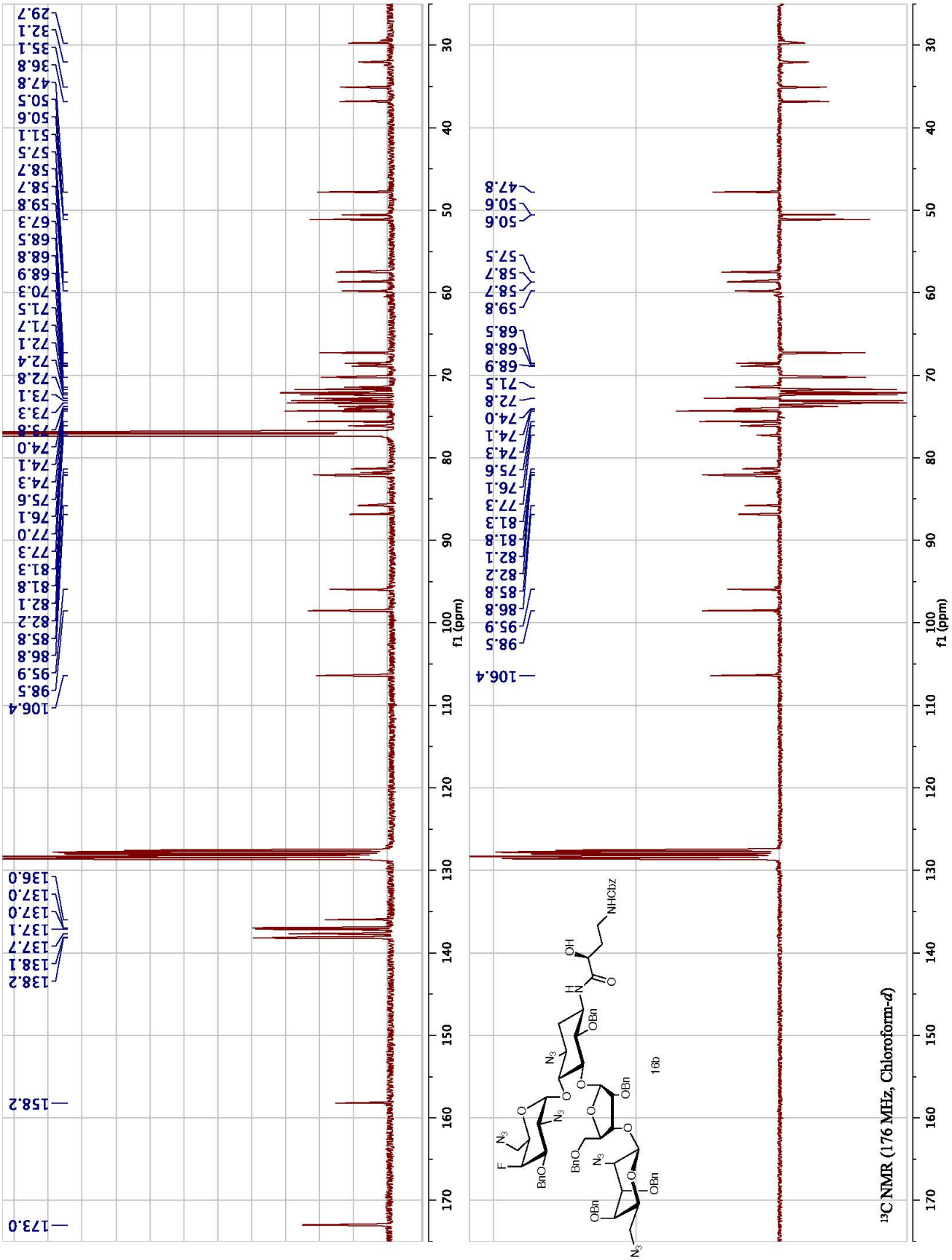


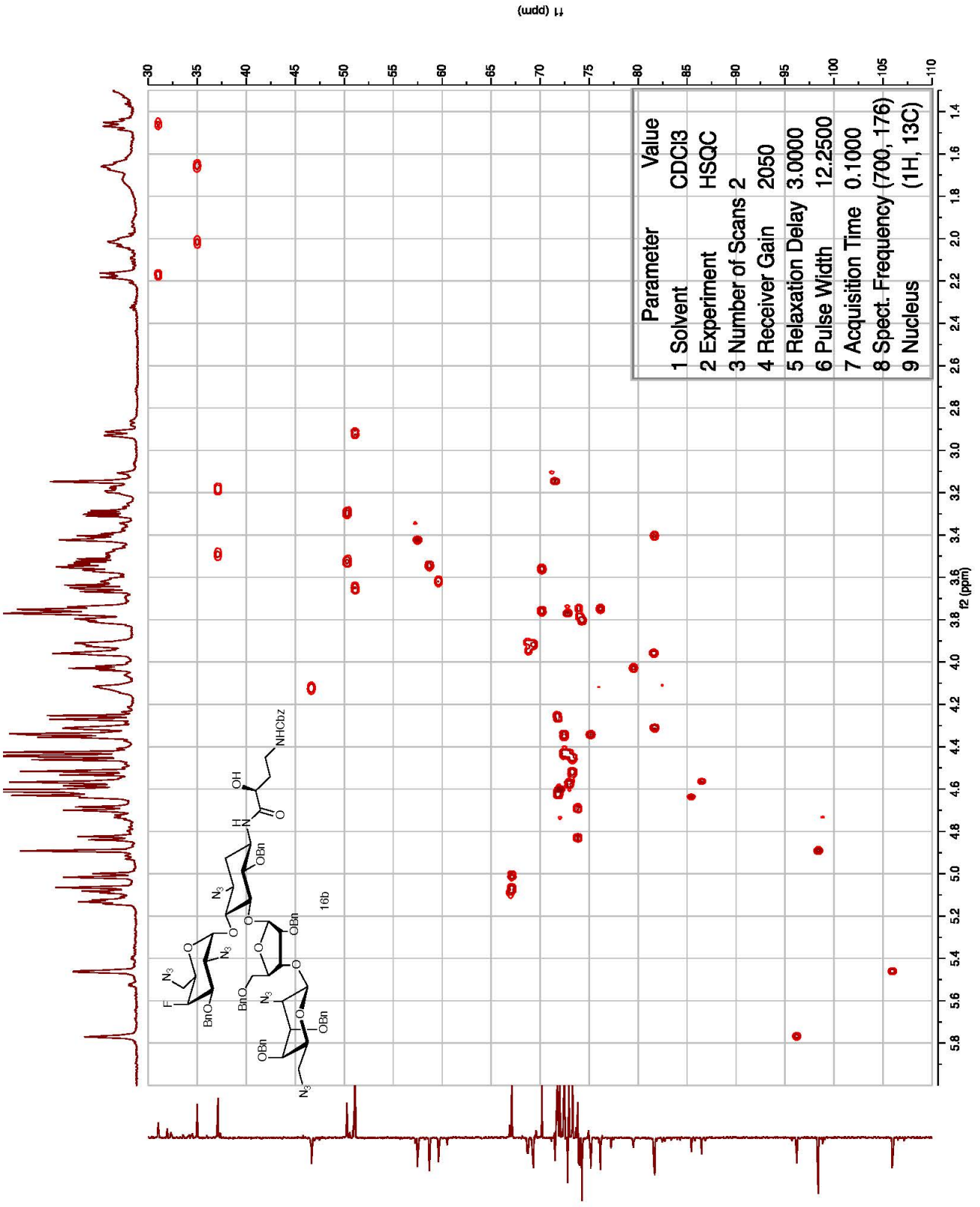

^{19}F NMR (282 MHz, Chloroform-*d*)
50.55
28.35
28.35
-218.4
-218.5
-218.6
-218.6
-218.6
-218.7
-218.8
16a
f1 (ppm)

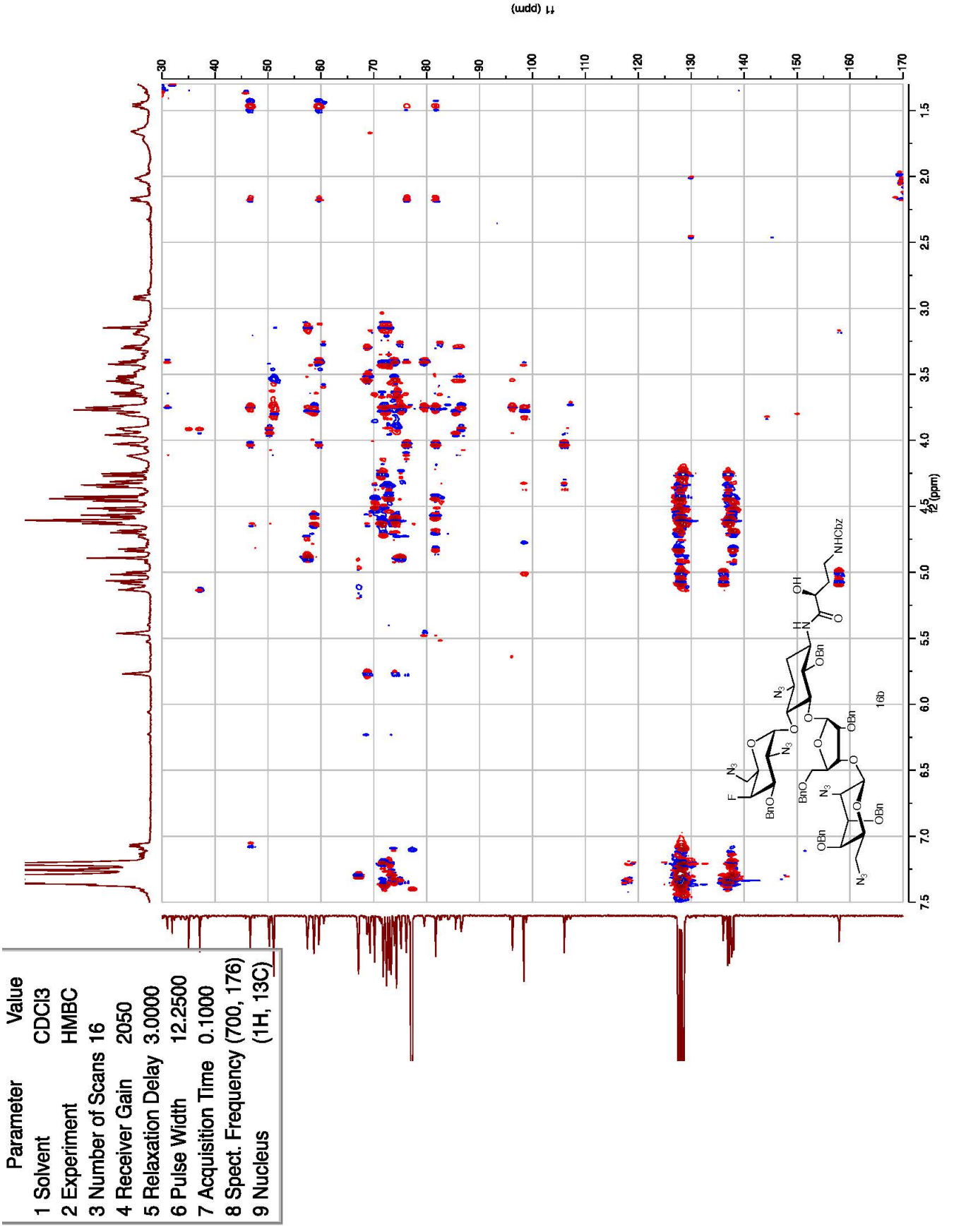


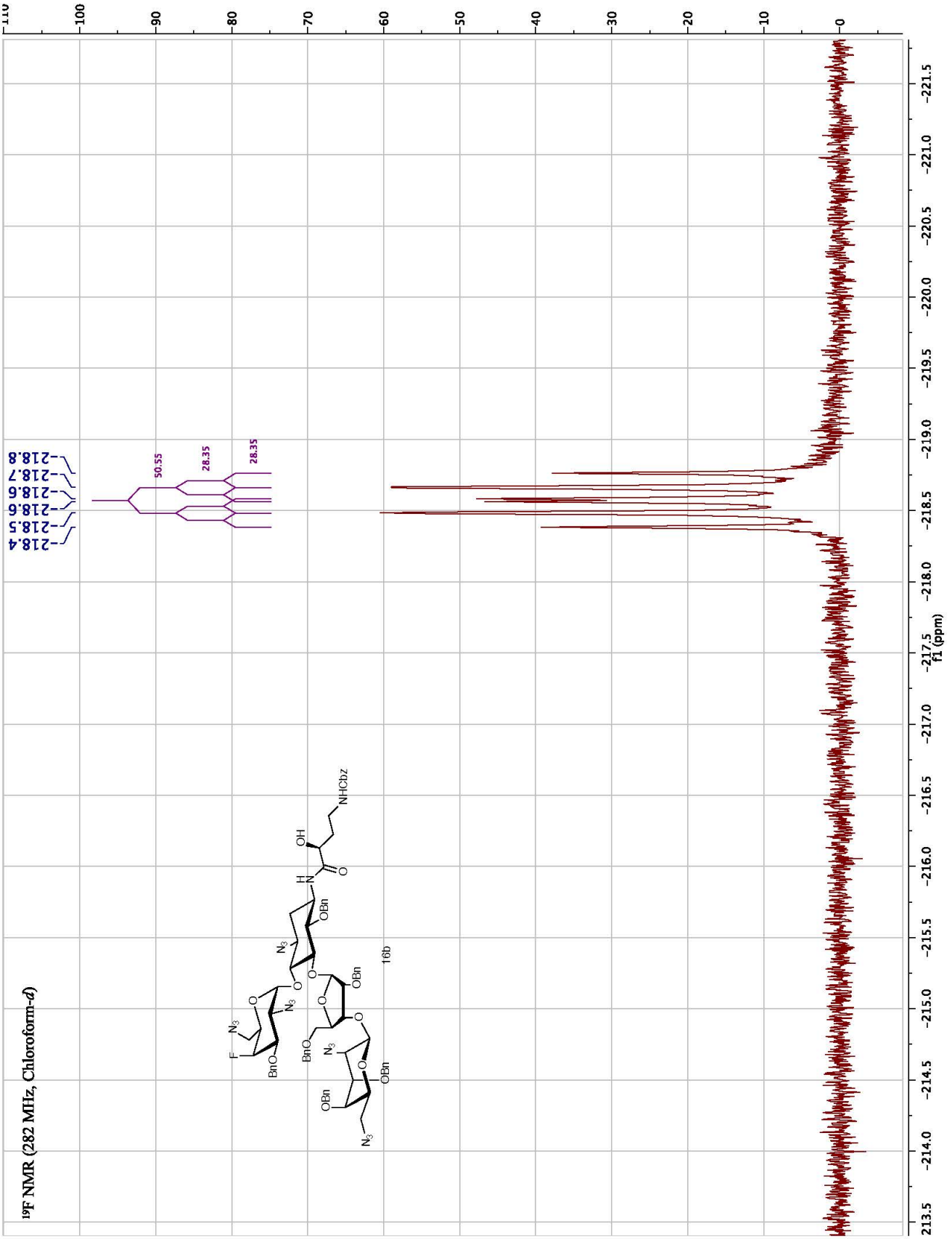

f1 (ppm)
f2 (ppm)
Parameter Value
1 Solvent CDCl3
2 Experiment COSY
3 Number of Scans 2
4 Receiver Gain 46
5 Relaxation Delay 2.0000
6 Pulse Width 12.2500
7 Acquisition Time 0.3244
8 Spect. Frequency (700, 700)
9 Nucleus (1H, 1H)
16b

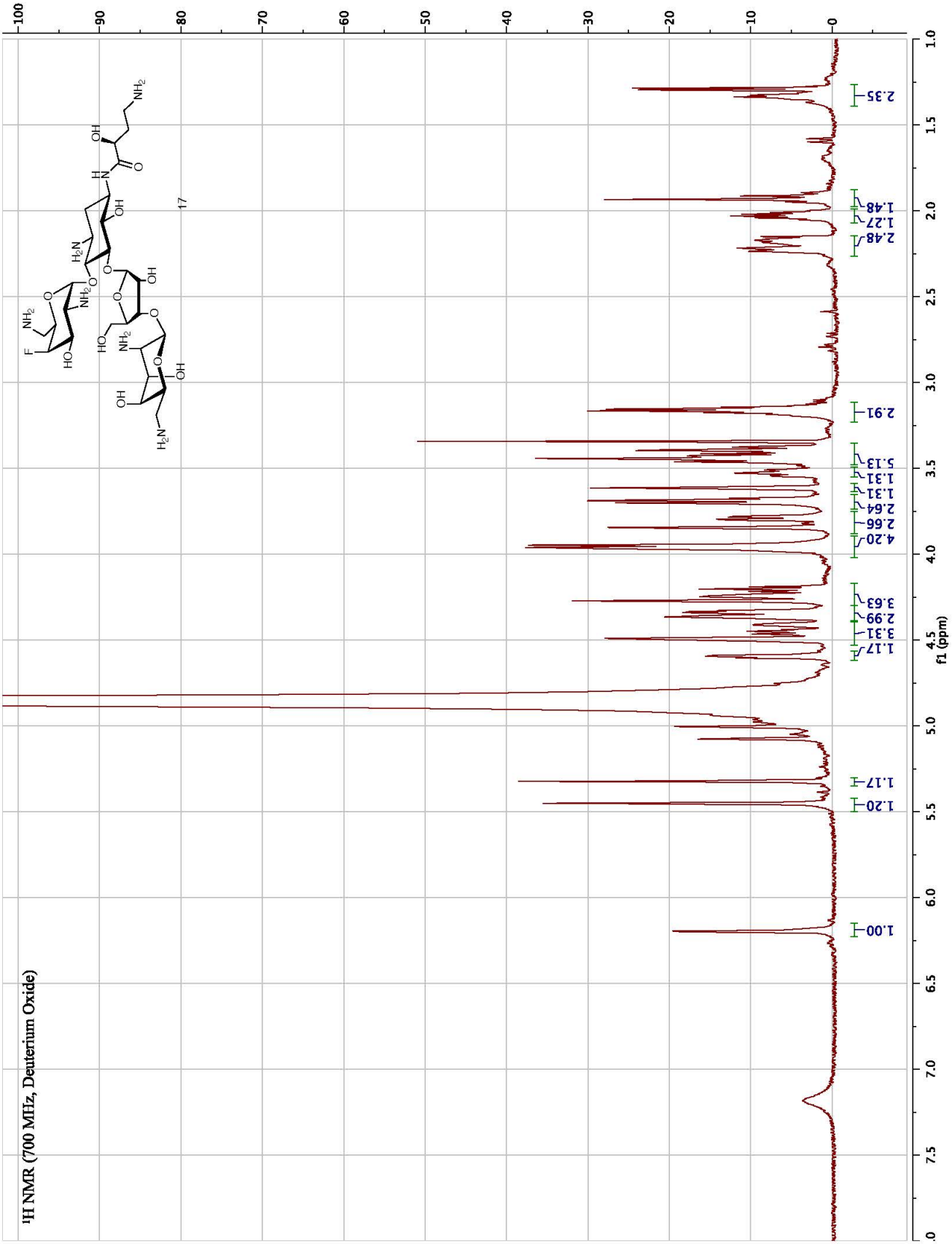


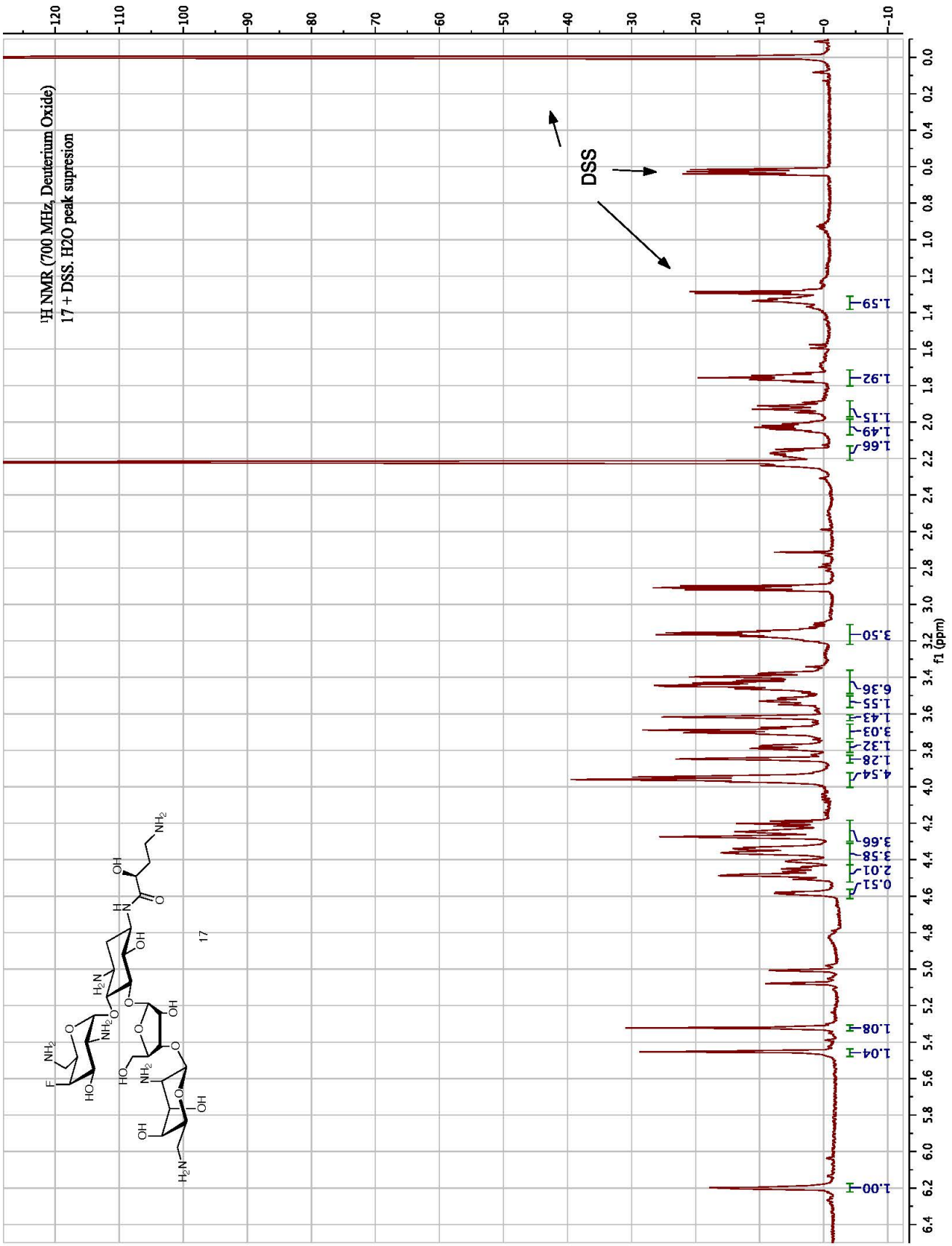

^{13}C NMR (176 MHz, Chloroform-*d*)
16b
f1 (ppm)

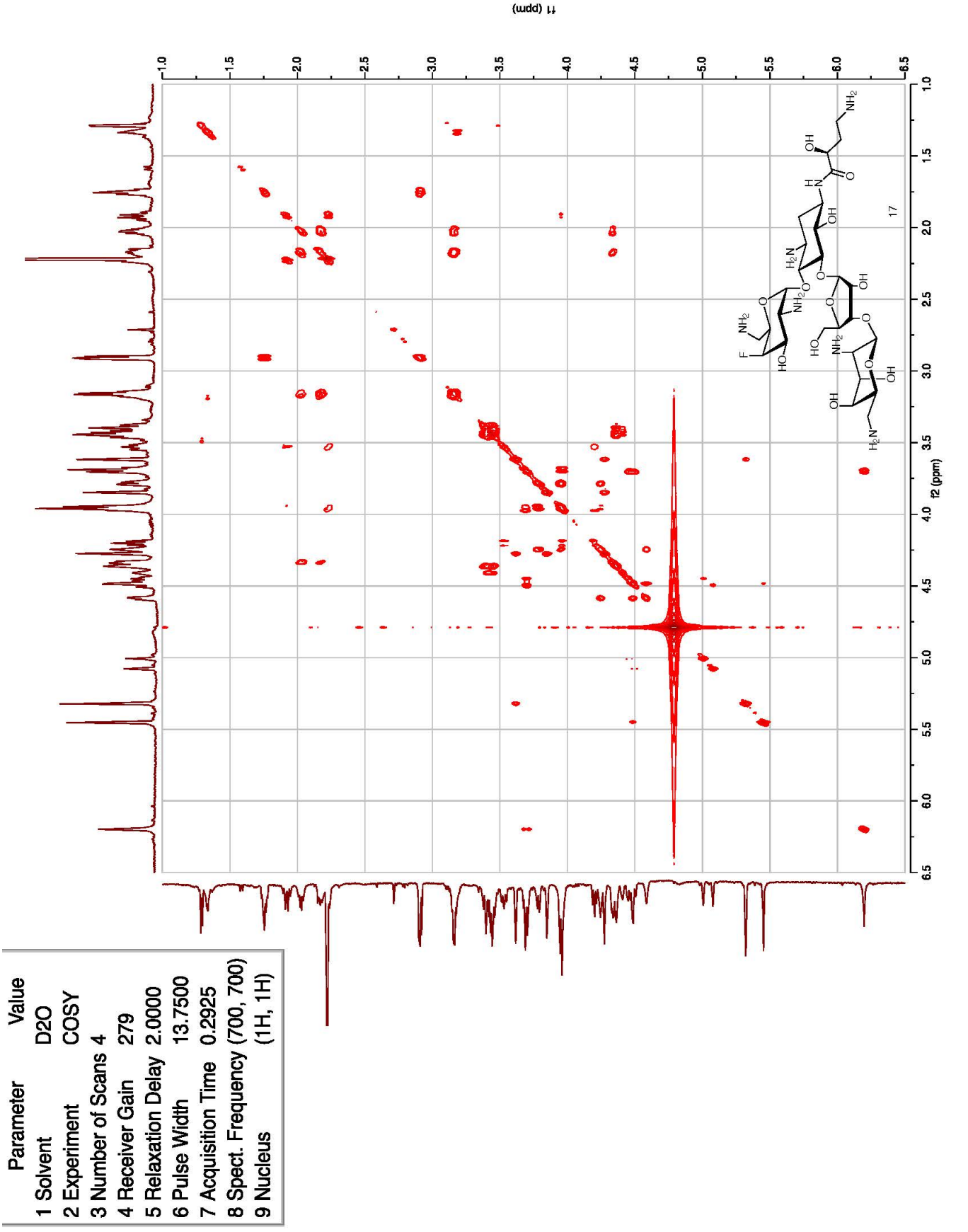


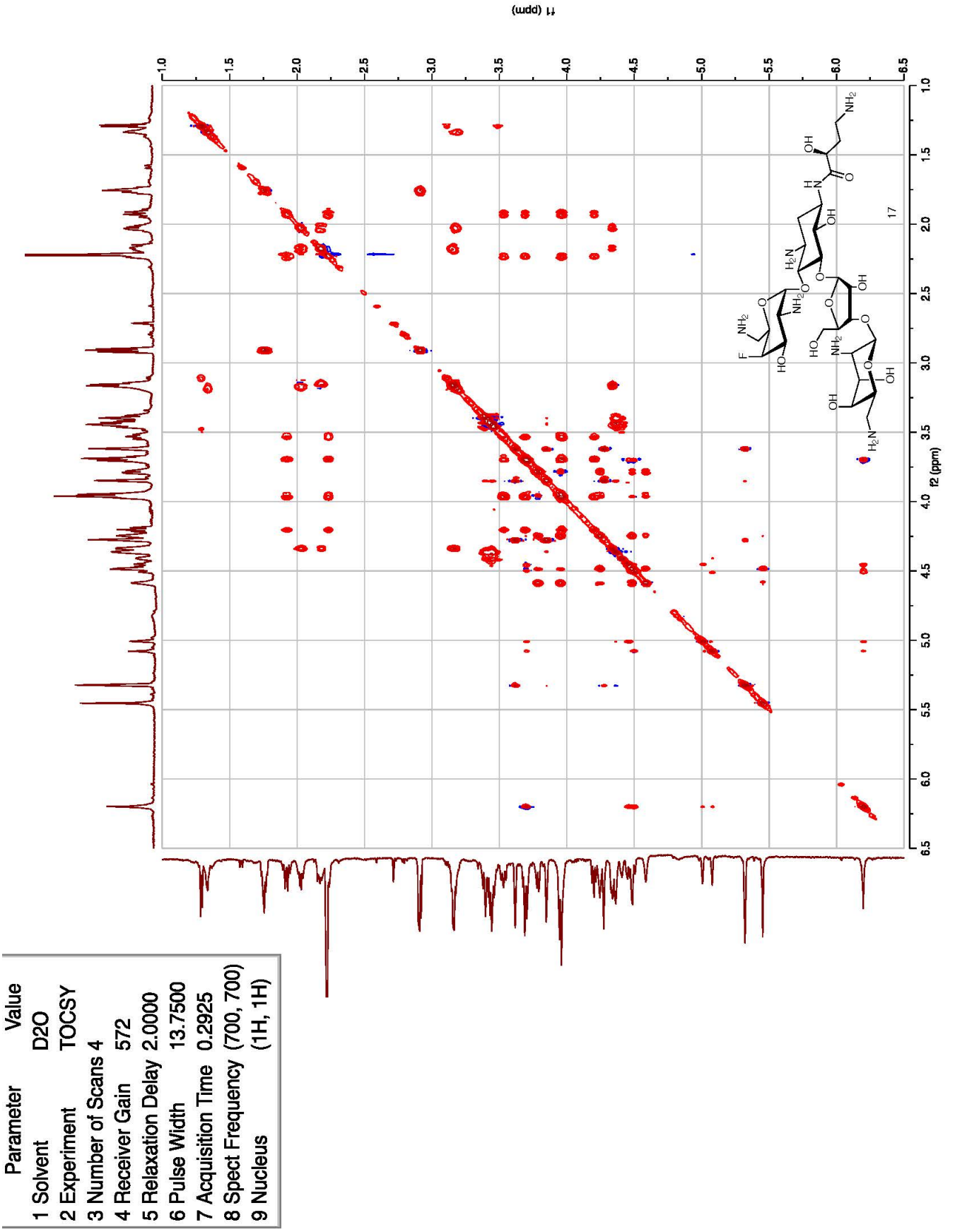

Parameter Value
1 Solvent CDCl3
2 Experiment HMBC
3 Number of Scans 16
4 Receiver Gain 2050
5 Relaxation Delay 3.0000
6 Pulse Width 12.2500
7 Acquisition Time 0.1000
8 Spect. Frequency (700, 176)
9 Nucleus (1H, 13C)
f1 (ppm)
f2 (ppm)
16b

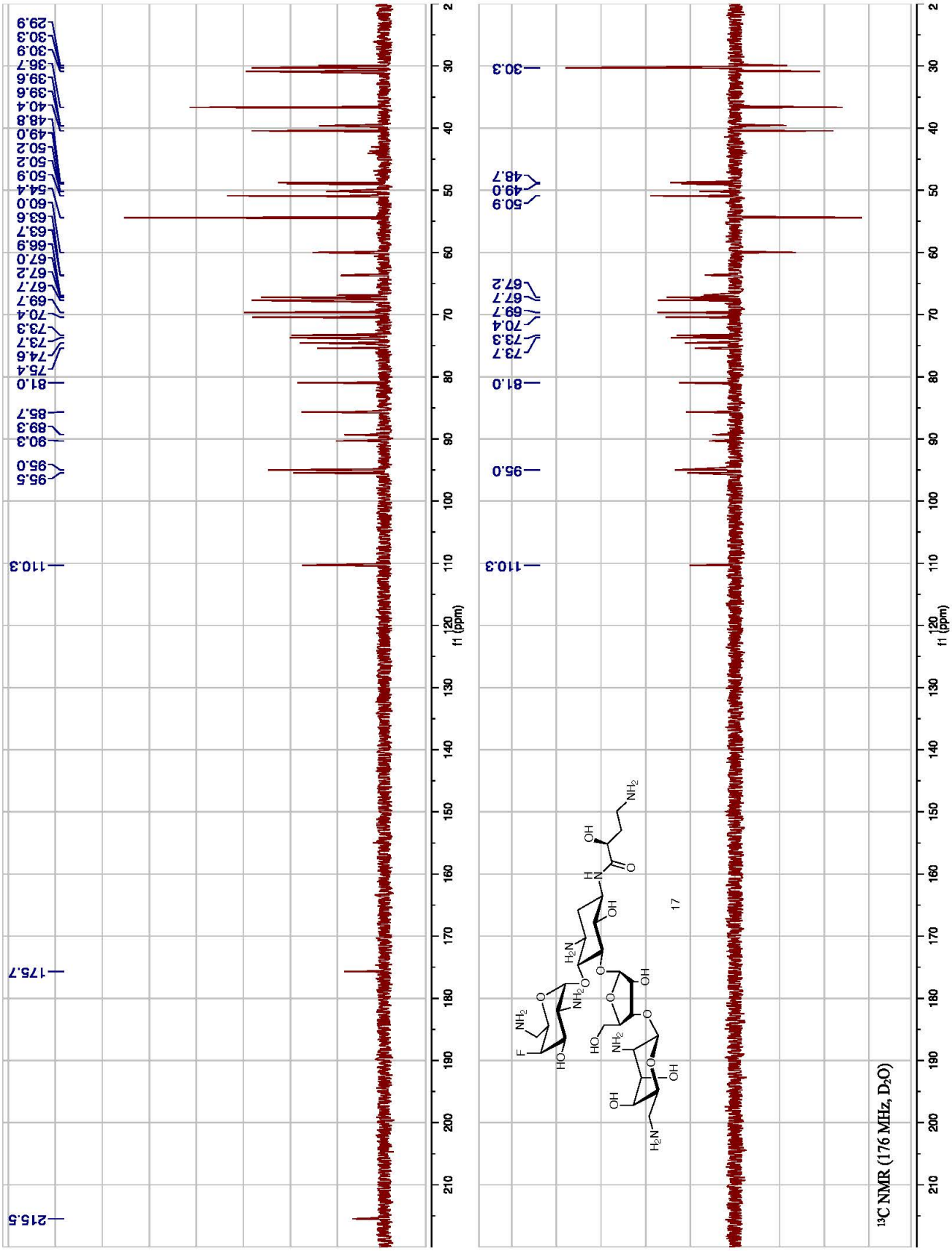


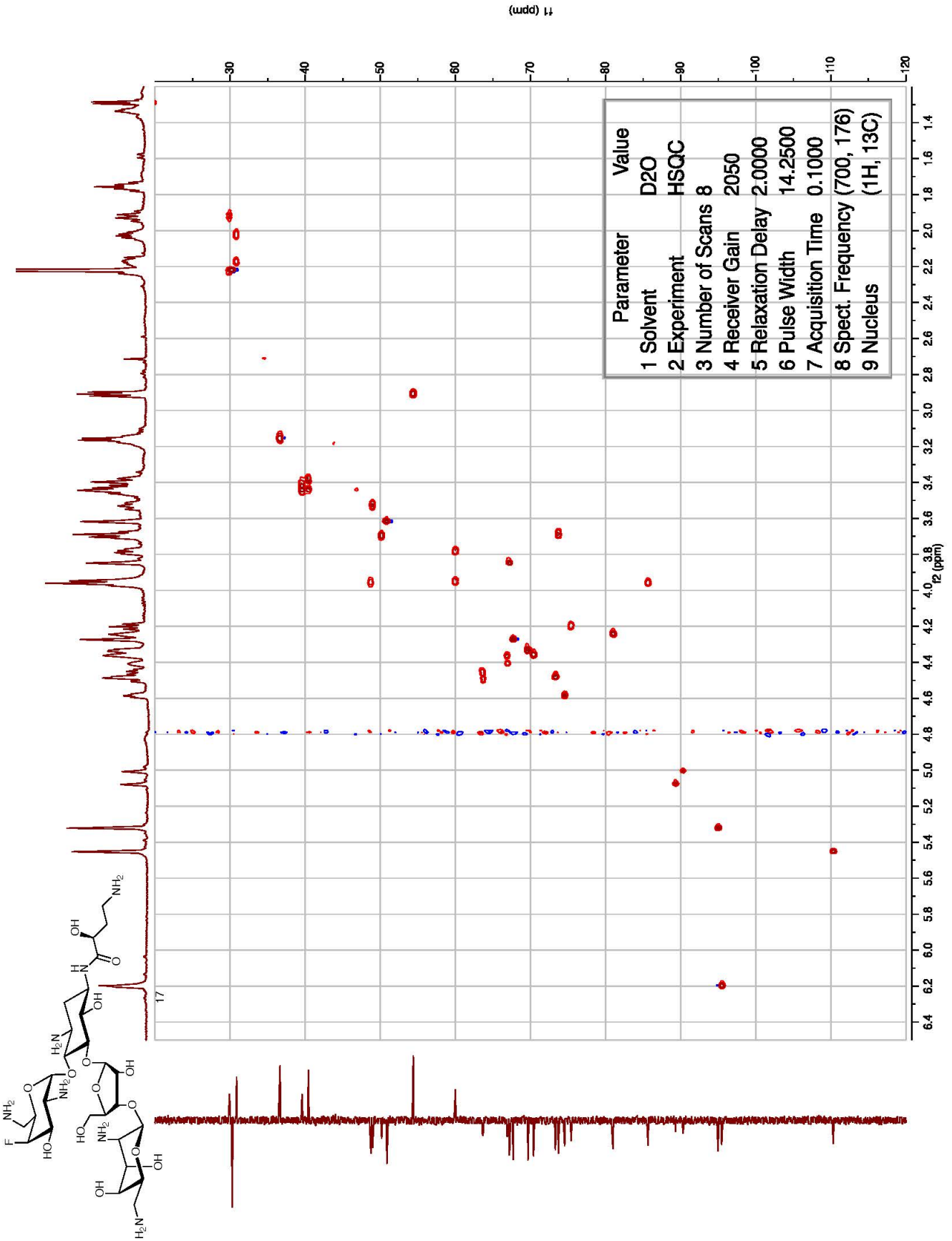

17
¹H NMR (700 MHz, Deuterium Oxide)
f1 (ppm)
2.35
1.48
1.27
2.48
2.91
5.13
1.31
1.31
2.64
2.66
4.20
3.63
2.99
3.31
1.17
1.17
1.20
1.00

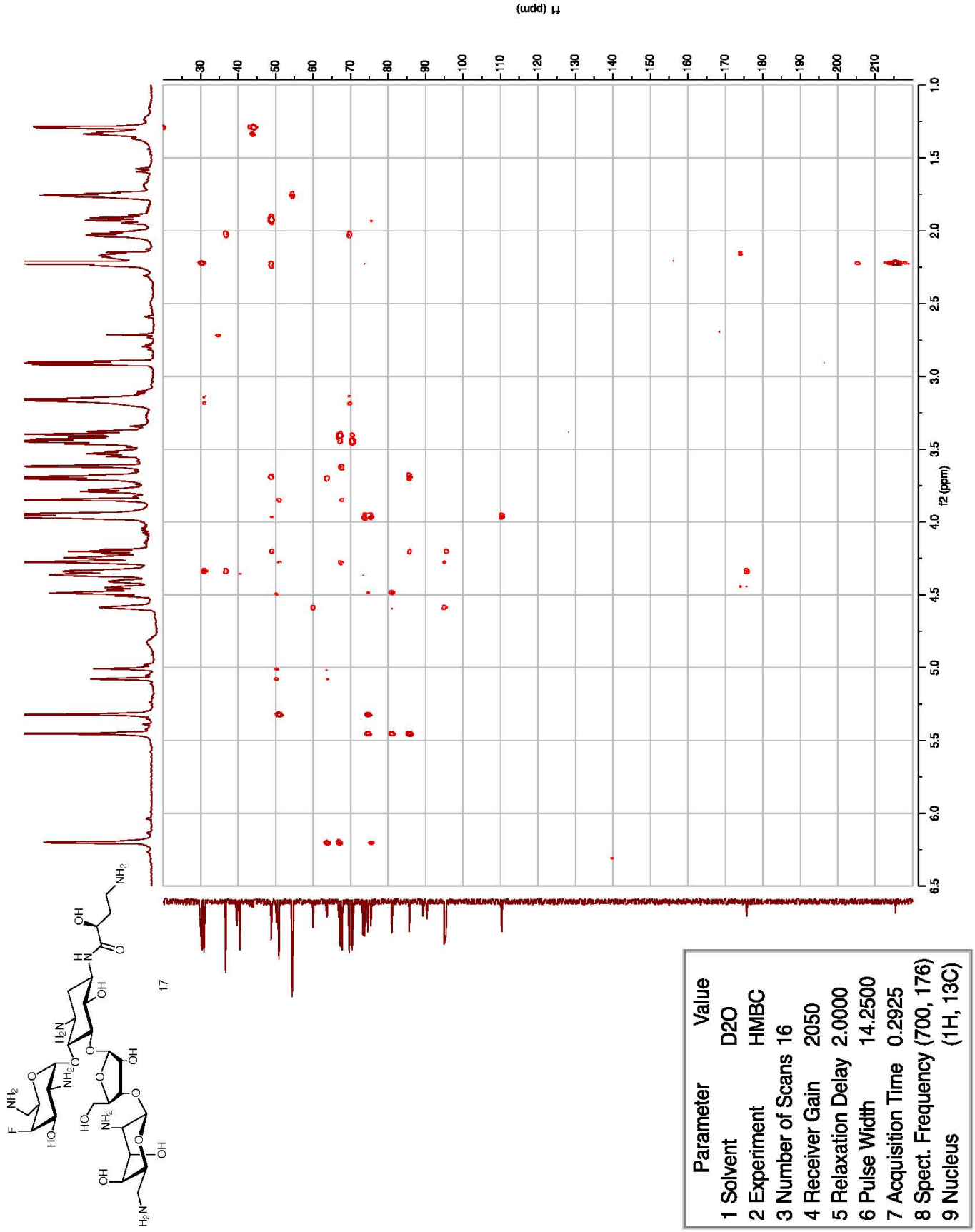



f1 (ppm)
f2 (ppm)
17
Parameter Value
1 Solvent D2O
2 Experiment COSY
3 Number of Scans 4
4 Receiver Gain 279
5 Relaxation Delay 2.0000
6 Pulse Width 13.7500
7 Acquisition Time 0.2925
8 Spect. Frequency (700, 700)
9 Nucleus (1H, 1H)




29.9
30.3
30.9
36.7
39.6
39.6
40.4
48.8
49.0
50.2
50.2
50.9
54.4
60.0
63.6
63.7
66.9
67.0
67.2
67.7
69.7
70.4
73.3
73.7
74.6
75.4
81.0
85.7
89.3
90.3
95.0
95.5
110.3
175.7
215.5
f1 (ppm)
30.3
48.7
49.0
50.9
67.2
67.7
69.7
70.4
73.3
73.7
81.0
95.0
110.3
f1 (ppm)
17
^{13}C NMR (176 MHz, D_2O)




f1 (ppm)
f2 (ppm)
17
Parameter Value
1 Solvent D2O
2 Experiment HMBC
3 Number of Scans 16
4 Receiver Gain 2050
5 Relaxation Delay 2.0000
6 Pulse Width 14.2500
7 Acquisition Time 0.2925
8 Spect. Frequency (700, 176)
9 Nucleus (1H, 13C)