Synthesis and Characterization of Oligodeoxyribonucleotides Modified with 2'-Amino-α-L-LNA Adenine Monomers: High-affinity Targeting of Single-Stranded DNA

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SUPPORTING INFORMATION

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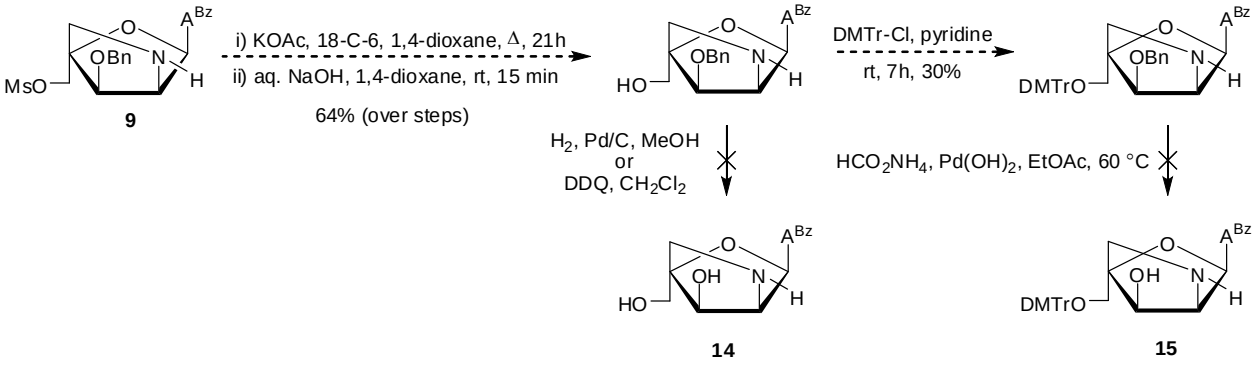
General experimental section. All solvents and reagents were obtained from commercial suppliers and used without further purification. Petroleum ether of the distillation range 60-80 °C was used. Anhydrous DMF and pyridine were used directly as obtained from commercial suppliers. Dichloromethane, 1,2-dichloroethane and *N*,*N*'-diisopropylethylamine were dried through storage over activated 4Å molecular sieves. Reactions were conducted under an atmosphere of argon whenever anhydrous solvents were used. All reactions were monitored by thin-layer chromatography (TLC) using silica gel coated plates with fluorescence indicator (SiO_2-60, F-254), which were visualized (a) under UV light, (b) by dipping in 5% concd. sulfuric acid in absolute ethanol (v/v) followed by heating, or (c) by dipping in a solution of molybdate phosphoric acid (12.5 g/L) and cerium(IV)sulfate (5 g/L) followed by heating. Silica gel column chromatography using moderate pressure (pressure ball) was performed using Silica gel 60 (particle size 0.040-0.063 mm). Evaporation of solvents was carried out under reduced pressure with a temperature not exceeding 45 °C. After column chromatography, appropriate fractions were pooled, evaporated, and dried at high vacuum for at least 8h to give products in high purity (>95% by 1D NMR techniques), unless stated otherwise. ^{1}H NMR and/or ^{13}C NMR ascertained sample purity. ^{1}H NMR and ^{13}C NMR spectra were recorded at either 500/125 MHz, or 300/75.5 MHz as specified. Chemical shifts are reported in parts per million (ppm) relative to tetramethylsilane or deuterated solvent as the internal standard (δ_H: $CDCl_3$ 7.26 ppm, DMSO-d_6 2.50 ppm; δ_C: $CDCl_3$ 77.00 ppm, DMSO-d_6 39.43 ppm). Exchangeable (ex) protons were identified through disappearance of signals upon D_2O addition. Assignments of NMR spectra are based on COSY, HSQC and DEPT and follow standard carbohydrate/nucleoside nomenclature. The carbon atom of C4 substituents is numbered C5" in nucleoside derivatives. Similar conventions apply for the corresponding hydrogen atoms. Quaternary carbons were not assigned

in ^{13}C NMR but their presence was verified through absence of signals in HSQC and DEPT. MALDI-HRMS were recorded in positive ion mode on a mass spectrometer using 2,5-dihydroxybenzoic acid or 3-hydroxypicolinic acid as a matrix and mixture of polyethylene glycol (PEG 600) and (PEG 1000) as internal calibration standards. ESI-HRMS were recorded in positive ion mode on a Q-TOF mass spectrometer using NaCl in MeCN:H_2O and PEG 600 and PEG 1000 as internal calibration standards.



Scheme S1. Additional attempted synthetic strategies toward key intermediates **14**/**15**. 18-C-6: 18-crown-6.

Table S1. MS-data for **X**/**Y**-modified ONs used in the current study.

ONs	Sequence	Calc. *m*/*z* [M-H]	Found *m*/*z* [M-H]
X1	5'-GTG **X**TA TGC	2995	2995
X2	5'-GTG AT**X** TGC	2995	2993
X3	5'-GTG **X**T**X** TGC	3236	3236
X4	3'-CAC T**X**T ACG	2924	2923
X5	3'-CAC TAT **X**CG	2924	2923
X6	3'-CAC T**X**T **X**CG	3165	3163
Y1[a]	5'-GTG **Y**TA TGC	3009	3008
Y2	5'-GTG AT**Y** TGC	3009	3009
Y3	5'-GTG **Y**T**Y** TGC	3264	3264
Y4	3'-CAC T**Y**T ACG	2937	2937
Y5	3'-CAC TAT **Y**CG	2937	2936
Y6	3'-CAC T**Y**T **Y**CG	3192	3192
Y7[b]	3'-CAC T**Y**A ACG	2947	2948
Y8	3'-CAC T**Y**C ACG	2923	2923
Y9[c]	3'-CAC T**Y**G ACG	2963	2962

Purity: [a]70%, [b]84% [c]75% purity.

Table S2. MS-data for **X**/**Y**-modified ONs used in the current study.

ONs	Sequence	Calc. *m*/*z* [M]	Found *m*/*z* [M+H]
W1[a]	5'-GTG **W**TA TGC	2780	2781
W2[b]	5'-GTG AT**W** TGC	2780	2781
W3[c]	5'-GTG **W**T**W** TGC	2807	2808
W4[d]	3'-CAC T**W**T ACG	2709	2710
W5[e]	3'-CAC TAT **W**CG	2709	2710
W6[f]	3'-CAC T**W**T **W**CG	2736	2737
Z1	5'-GTG **Z**TA TGC	3022	3023
Z2	5'-GTG AT**Z** TGC	3022	3023
Z3	5'-GTG **Z**T**Z** TGC	3291	3292
Z4	3'-CAC T**Z**T ACG	2951	2952
Z5	3'-CAC TAT **Z**CG	2951	2952
Z6	3'-CAC T**Z**T **Z**CG	3220	3221

Purity: [a]72%, [b]58%, [c]73%, [d]71%, [e]63%, [f]58% - we hypothesize that the low purities are due to the presence of 'skipmers' (i.e., ONs without the **W** monomer), which arose due to inadequate capping during fabrication of **W**-modified ONs.

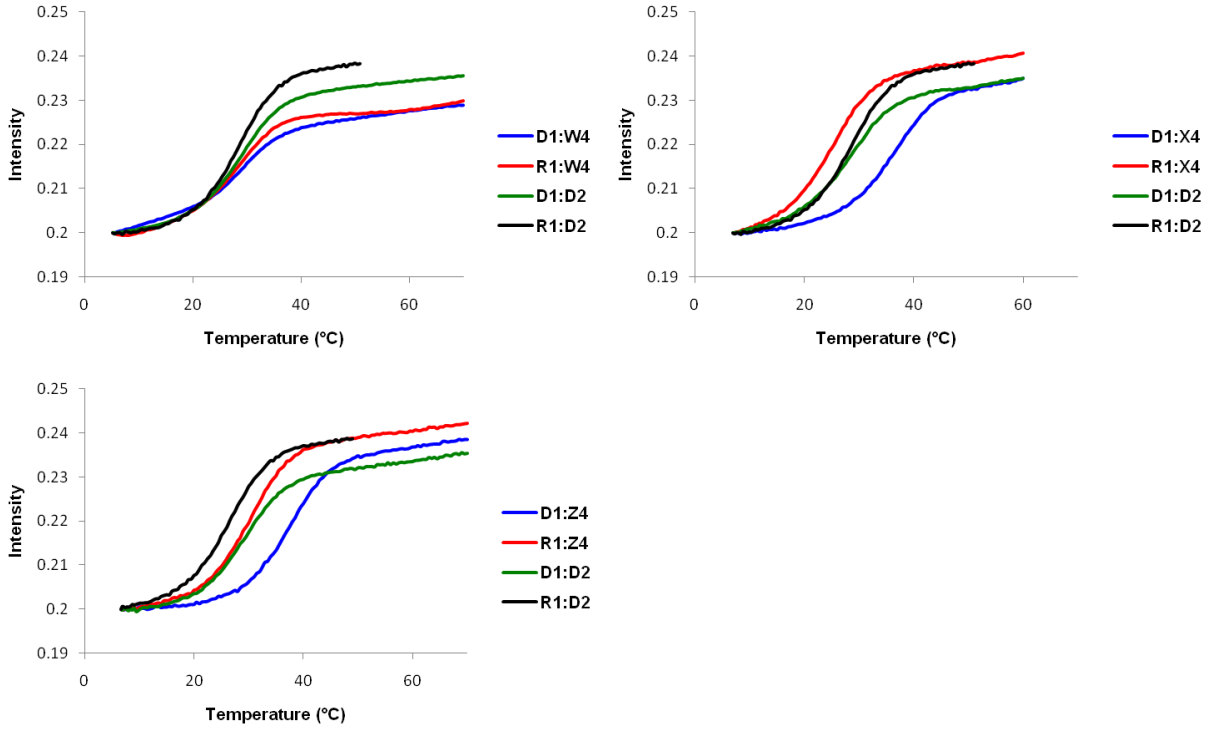


Figure S1. Representative thermal denaturation curves for modified and reference duplexes.

Table S3. DNA-selectivity of **B1-B6**.[a]

			$\Delta\Delta T_m$ (DNA-RNA)/°C			
ON	**Sequence**	**B =**	**W**	**X**	**Y**	**Z**
B1	5'-GTG **B**TA TGC		-3.0	+6.0	+9.5	+2.5
B2	5'-GTG AT**B** TGC		-2.0	+6.0	+9.0	+3.0
B3	5'-GTG **B**T**B** TGC		-4.0	+6.5	+9.0	+5.0
B4	3'-CAC T**B**T ACG		-4.5	+7.0	+9.0	+1.0
B5	3'-CAC TAT **B**CG		-7.0	+2.0	+5.5	-2.0
B6	3'-CAC T**B**T **B**CG		-9.0	+5.0	+6.5	±0.0

[a] DNA selectivity defined as $\Delta\Delta T_m$ (DNA-RNA) = ΔT_m (vs DNA) - ΔT_m (vs RNA).

Additional discussion – mismatch discrimination of B3-series ONs. The specificity of ONs with two modifications positioned as next-nearest neighbors (**B3** series) was evaluated against DNA targets with a single central mismatched nucleotide opposite of the central thymidine (Table S4). **X3** and **Y3** display very poor binding specificity, whereas **Z3** displays binding specificity that is comparable to unmodified reference strand **D1**.

Table S4. Discrimination of mismatched DNA targets by **B3**-series and reference ONs.[a]

			DNA : 3'-CAC T**B**T ACG			
			T_m/°C	ΔT_m/°C		
ON	Sequence	B =	A	C	G	T
D1	5'-GTG ATA TGC		**27.5**	-16.5	-9.5	-17.0
X3	5'-GTG **X**T**X** TGC		**32.5**	+2.0	+0.5	+2.0
Y3	5'-GTG **Y**T**Y** TGC		**41.0**	-5.0	-2.0	-2.0
Z3	5'-GTG **Z**T**Z** TGC		**43.5**	-14.5	-11.5	-9.5

[a] For conditions of thermal denaturation experiments, see Table 1. T_m's of fully matched duplexes are shown in bold. ΔT_m = change in T_m relative to fully matched DNA:DNA duplex.

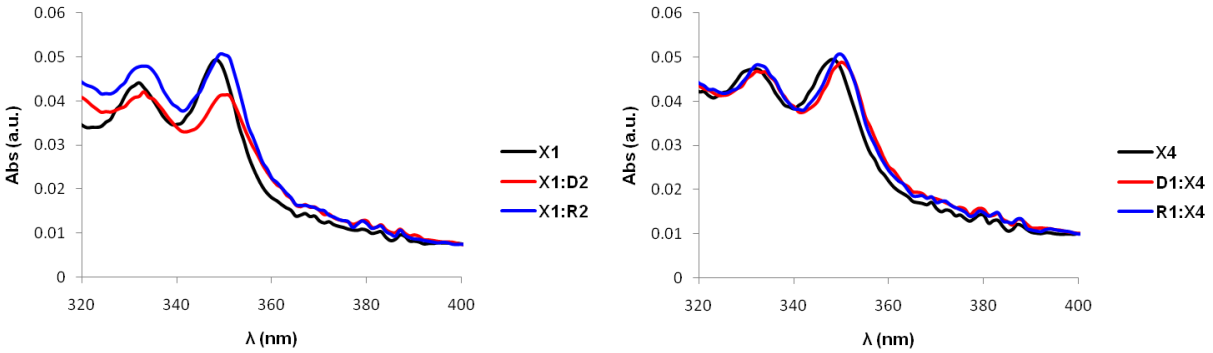


Figure S2. Representative absorption spectra of **X**-modified ONs and the corresponding duplexes with complementary DNA/RNA.

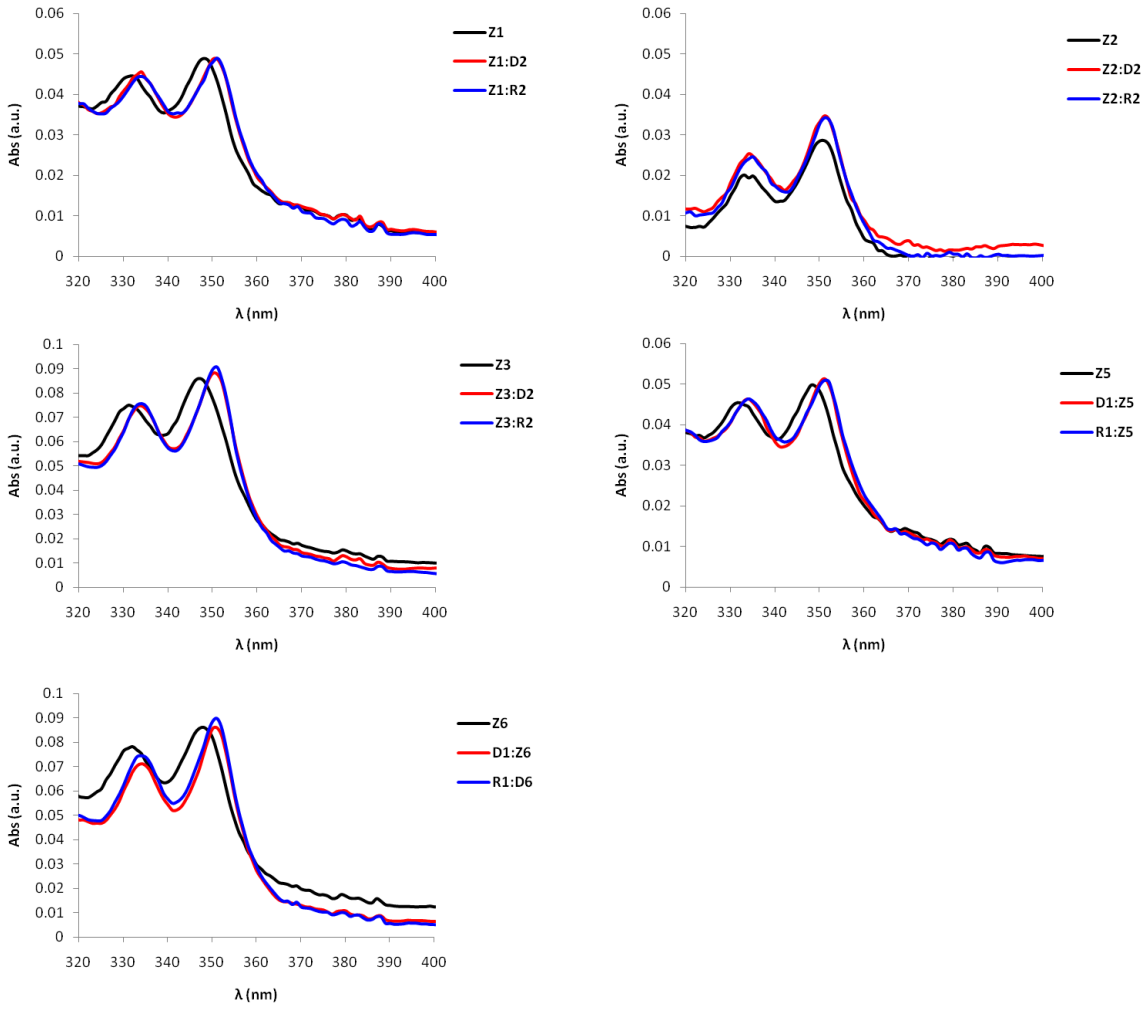


Figure S3. Representative absorption spectra of **Z**-modified ONs and the corresponding duplexes with complementary DNA/RNA.

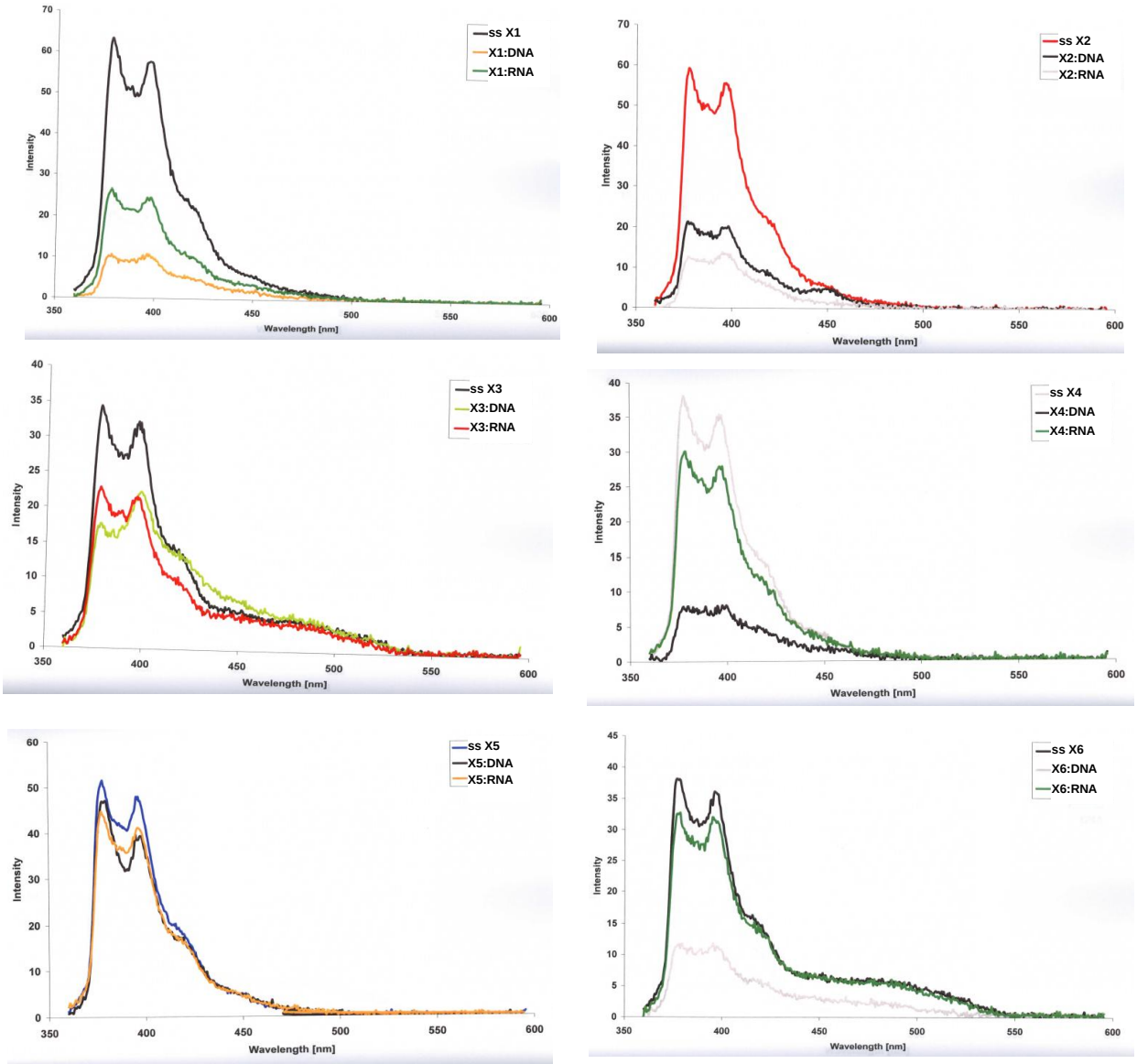


Figure S4. Steady-state fluorescence emission spectra of **X1**-**X6** in the presence of absence of complementary DNA/RNA. T_{exp} = 5 °C; λ_{ex} = 350 nm; each ON used at 1 μM concentration in T_m buffer.

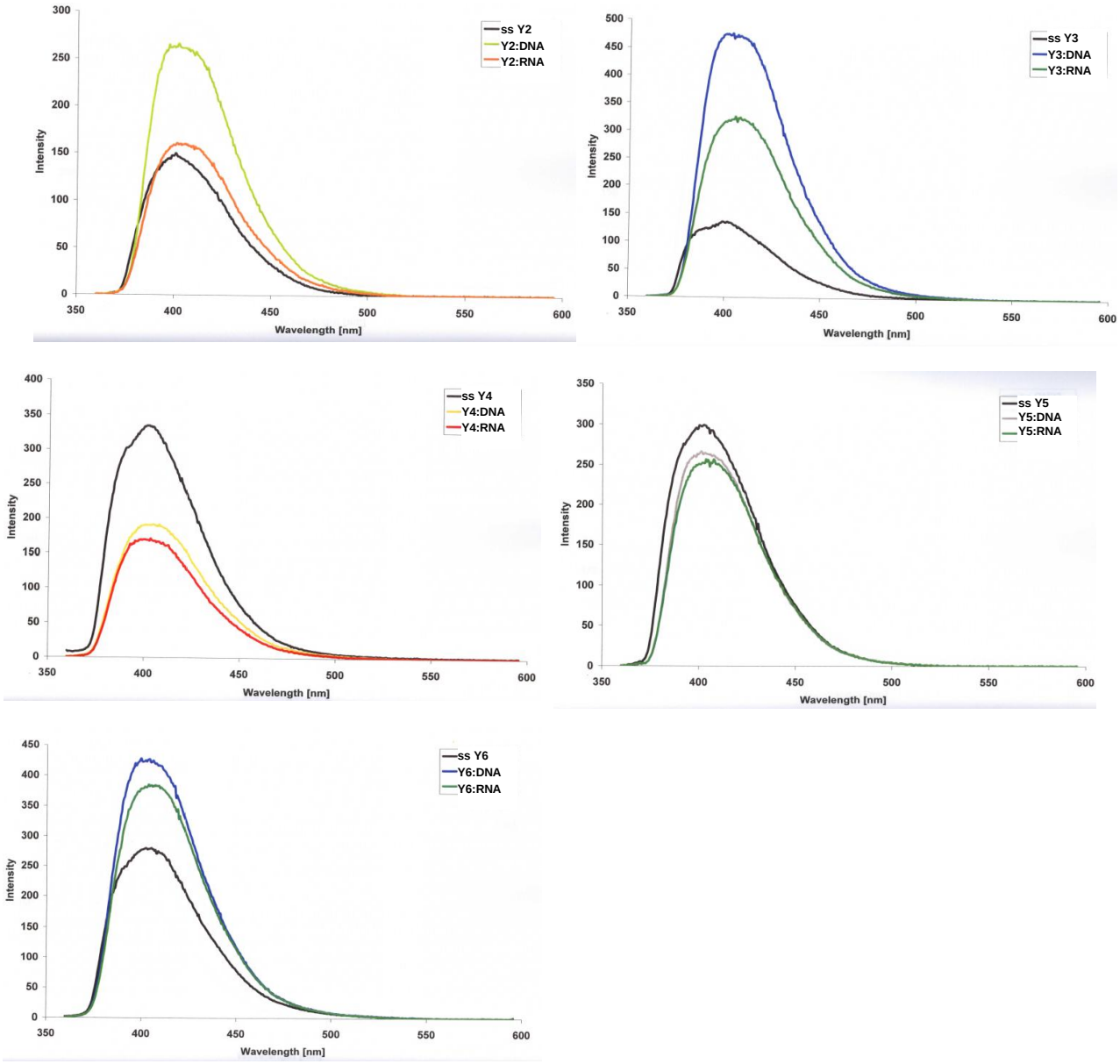


Figure S5. Steady-state fluorescence emission spectra of **Y1**-**Y6** in the presence of absence of complementary DNA/RNA. T_{exp} = 5 °C; λ_{ex} = 350 nm; each ON used at 0.15 μM concentration in T_m buffer.

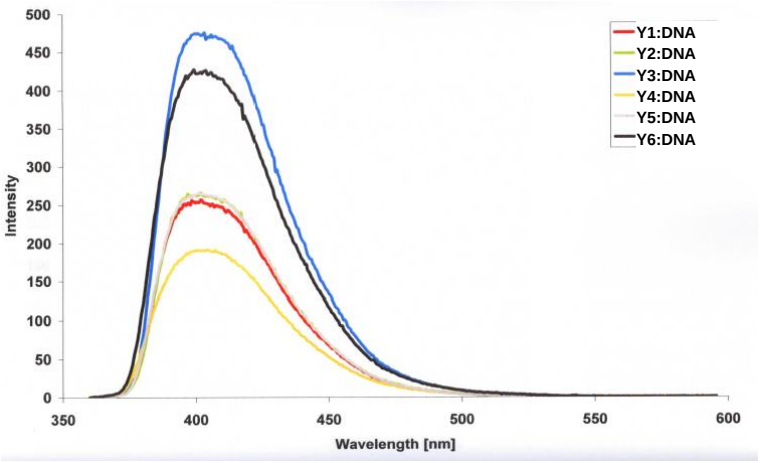


Figure S6. Steady-state fluorescence emission spectra of duplexes between **Y1**-**Y6** and complementary DNA. The spectra for **Y2**:DNA and **Y5**:DNA are overlapping. T_{exp} = 5 °C; λ_{ex} = 350 nm; each ON used at 0.15 μM concentration in T_m buffer.

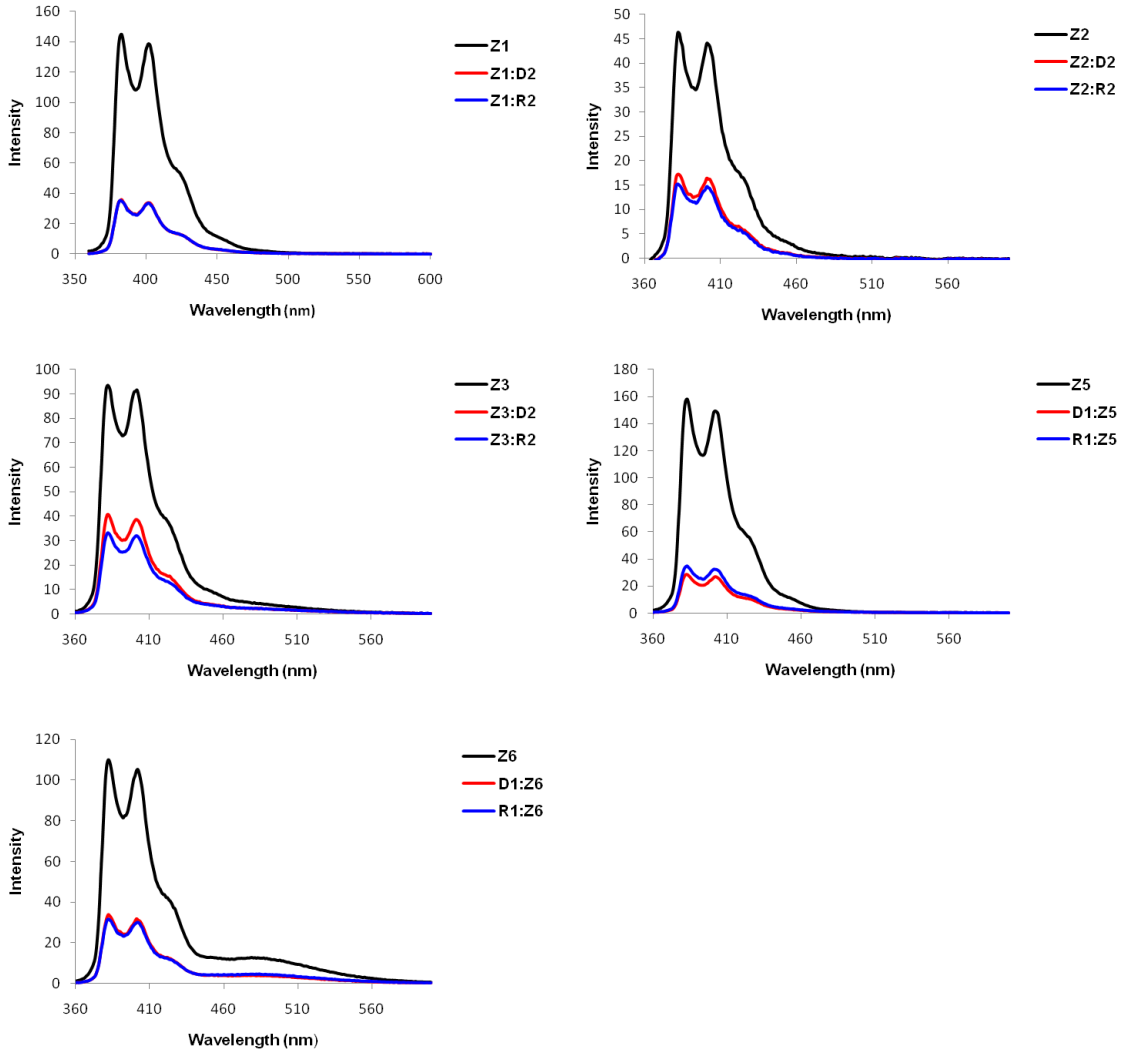


Figure S7. Steady-state fluorescence emission spectra of **Z**-modified ONs in the presence or absence of complementary DNA/RNA. T_{exp} = 5 °C; λ_{ex} = 350 nm; each ON used at 1 μM concentration in T_m buffer.

Additional discussion – mismatch discrimination via fluorescence. Probes **X4**/**Y4**/**Z4** were evaluated as probes for potential discrimination of single nucleotide polymorphisms (SNPs). Thus, these probes were hybridized with DNA strands with mismatched nucleotides opposite of the **X**-**Z** monomers. Although differences in fluorescence emission are observed, the differences are too small to be practically useful (Fig. S8-S10).

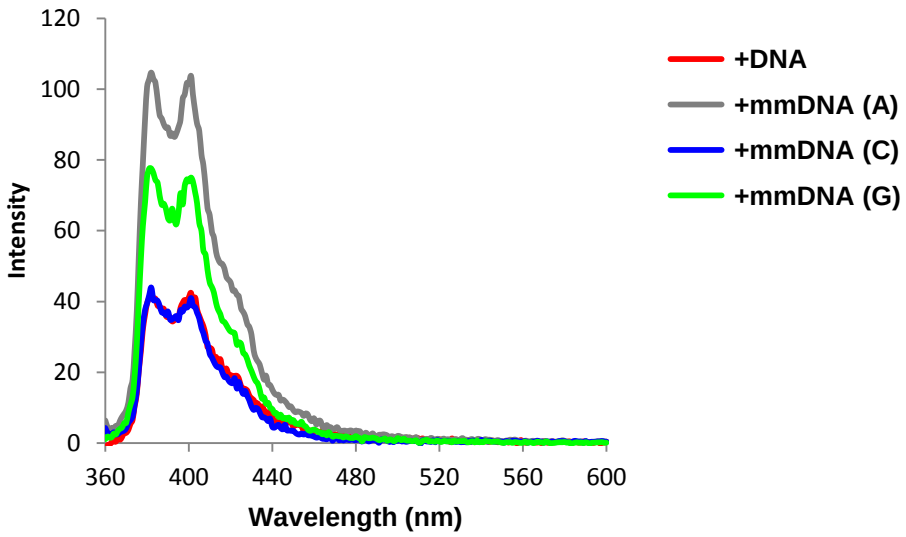


Figure S8. Steady-state fluorescence emission spectra of duplexes between **X4** and matched or mismatched DNA targets (mismatched nucleotide listed in parenthesis). Spectra were recorded at 5 °C using λ_{ex} = 350 nm and 1 μM concentration of each ON.

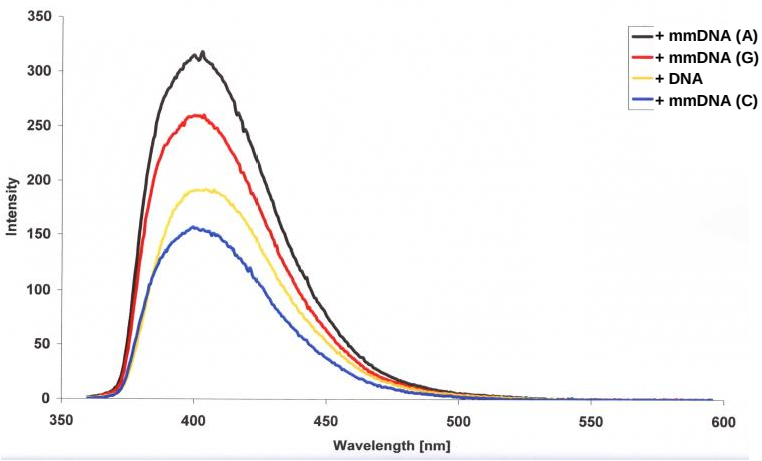


Figure S9. Steady-state fluorescence emission spectra of duplexes between **Y4** and matched or mismatched DNA targets (mismatched nucleotide listed in parenthesis). Spectra were recorded at 5 °C using λ_{ex} = 350 nm and 0.15 μM concentration of each ON.

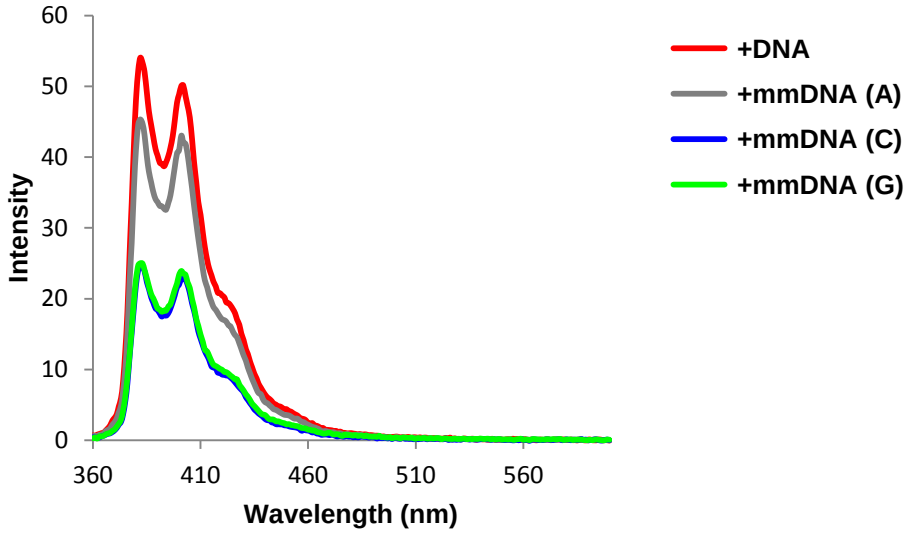
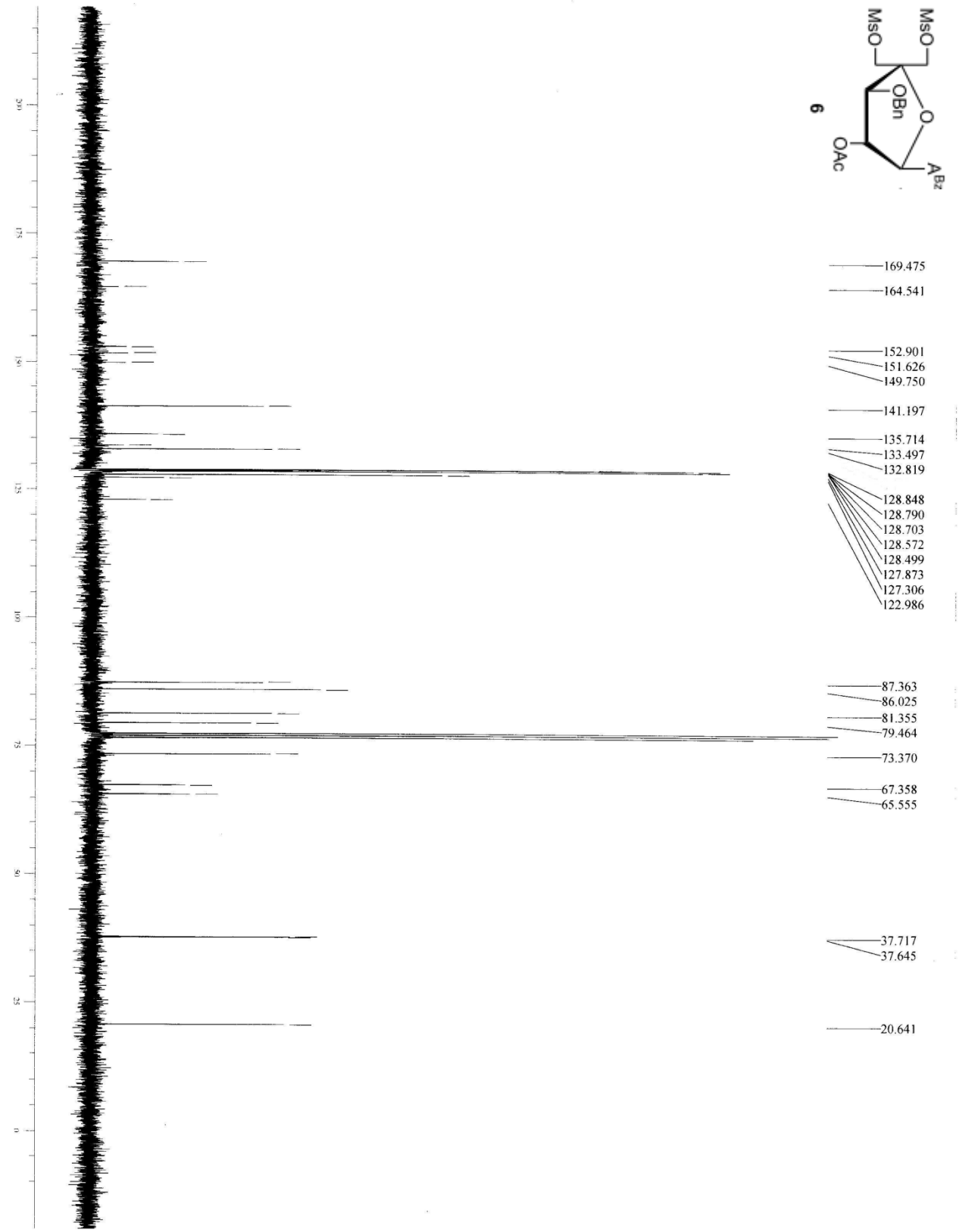


Figure S10. Steady-state fluorescence emission spectra of duplexes between **Z4** and matched or mismatched DNA targets (mismatched nucleotide listed in parenthesis). Spectra were recorded at 5 °C using λ_{ex} = 350 nm and 1 μM concentration of each ON.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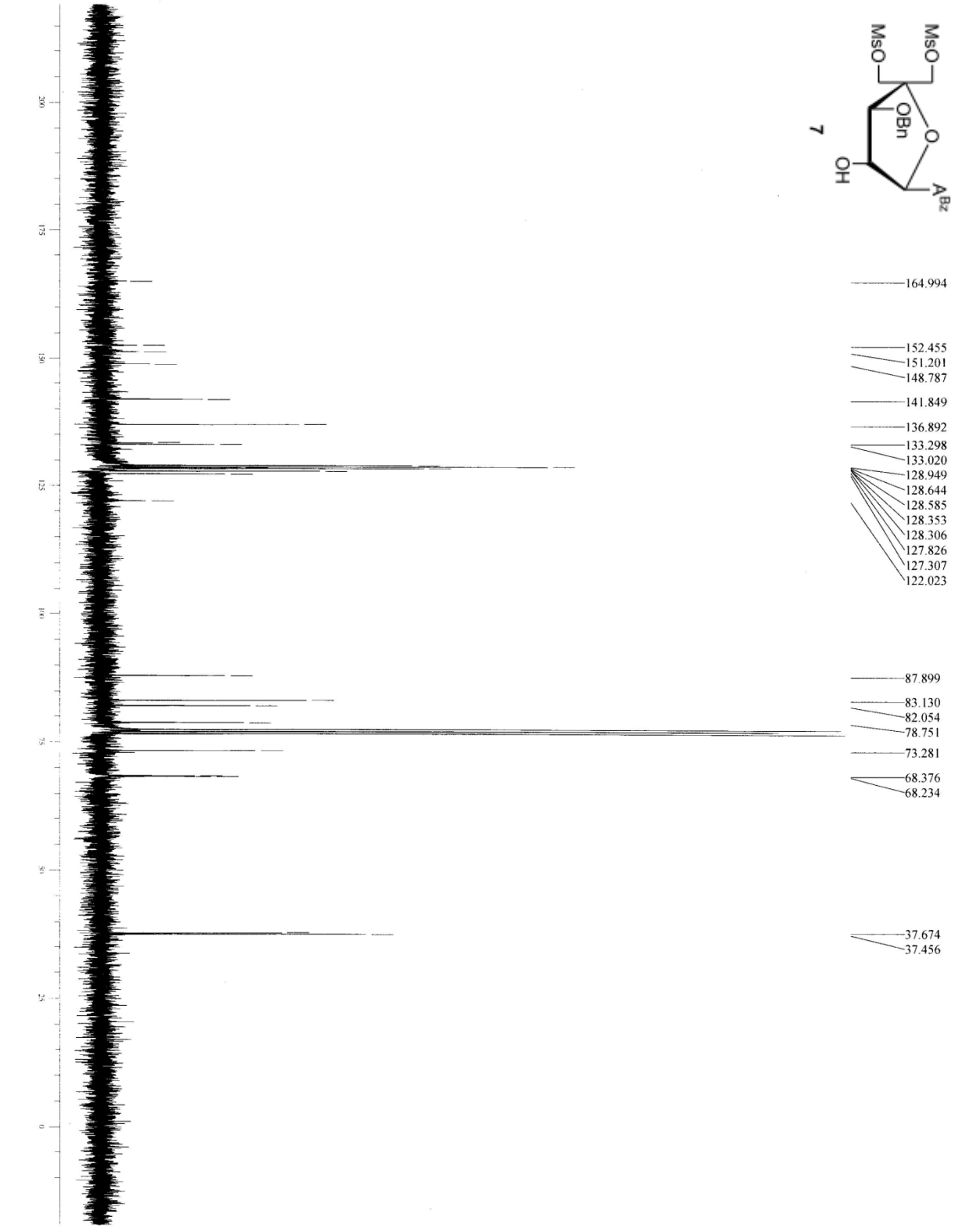
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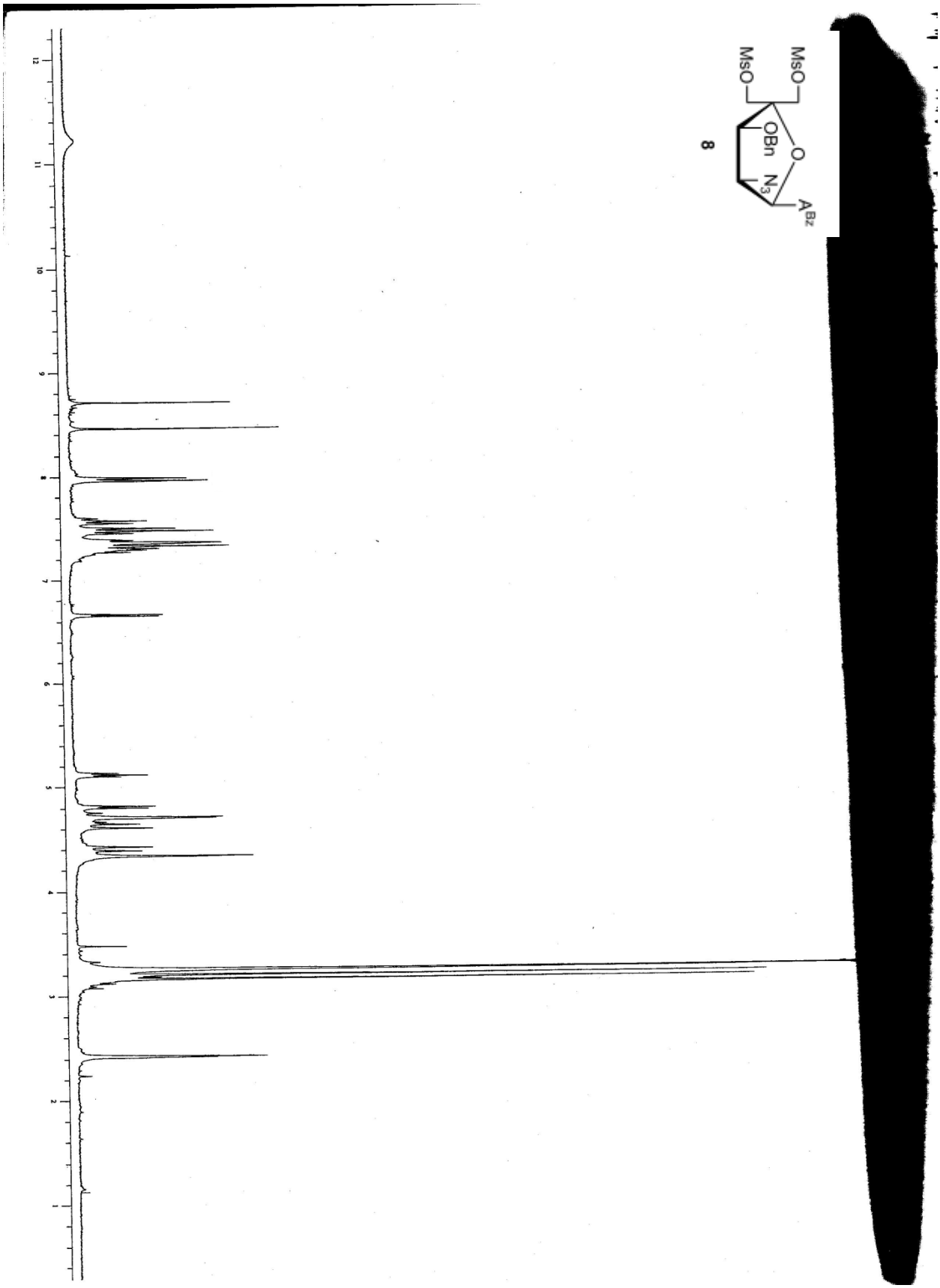
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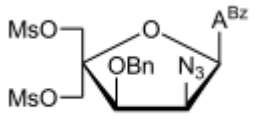

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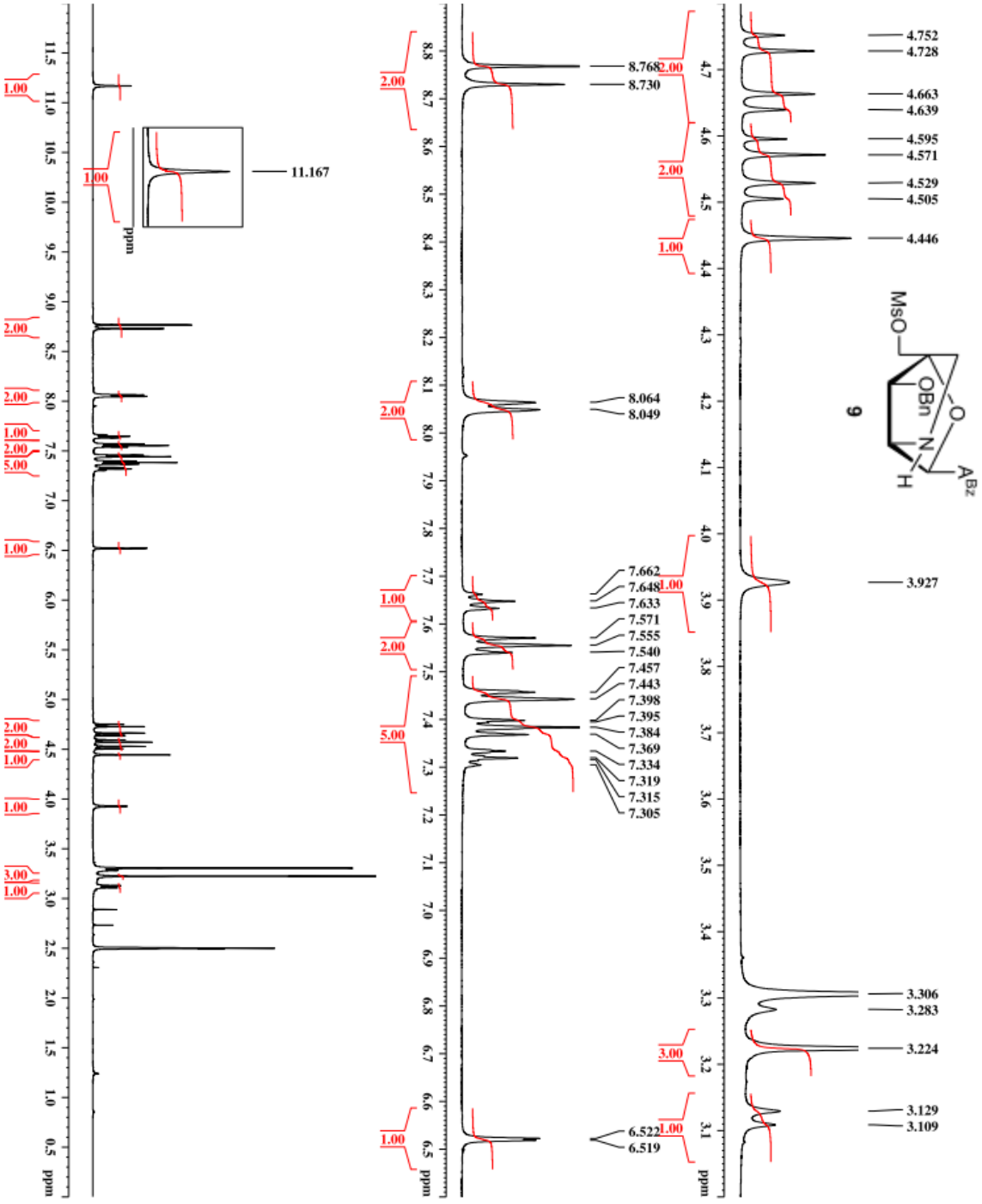
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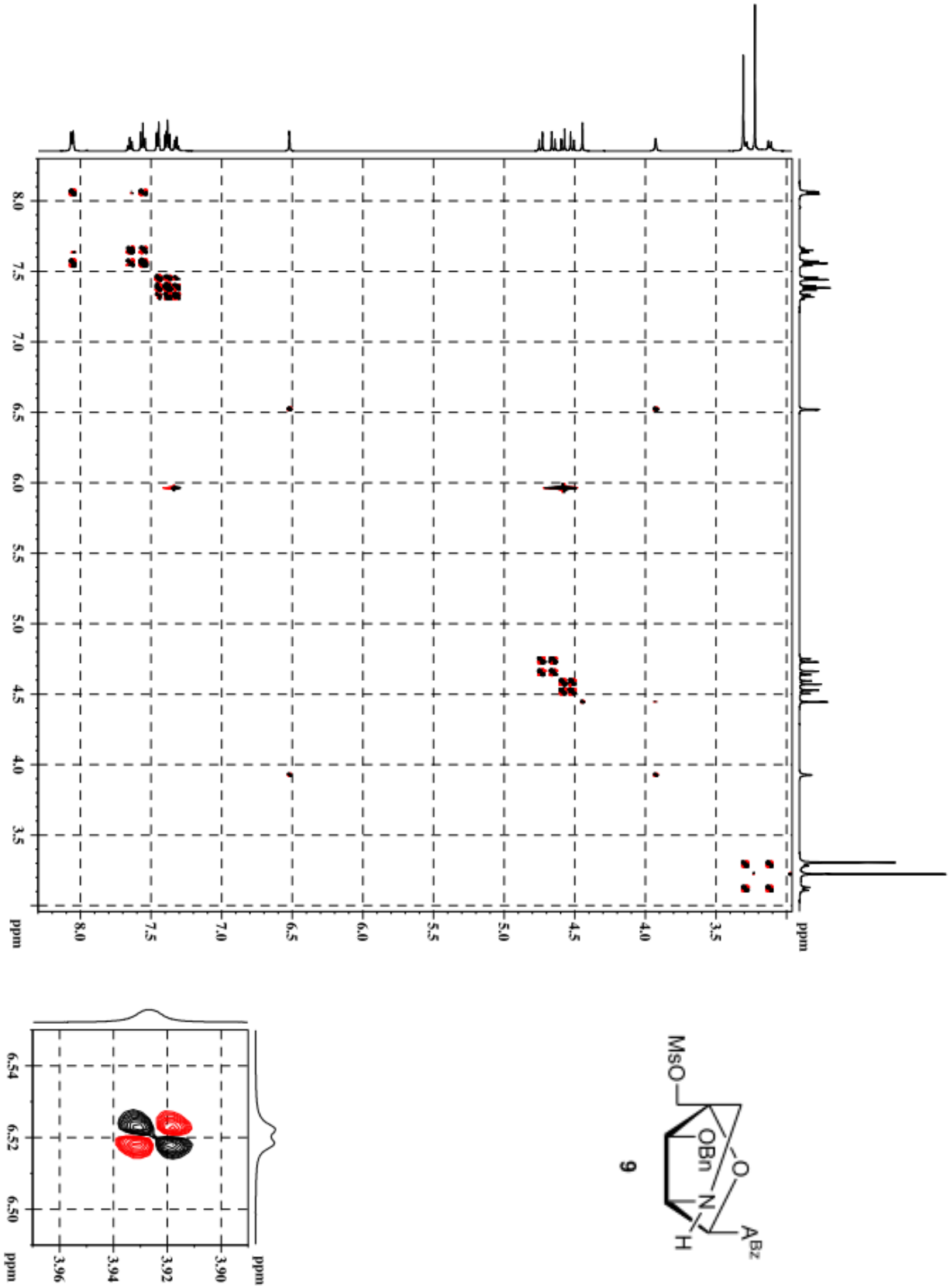
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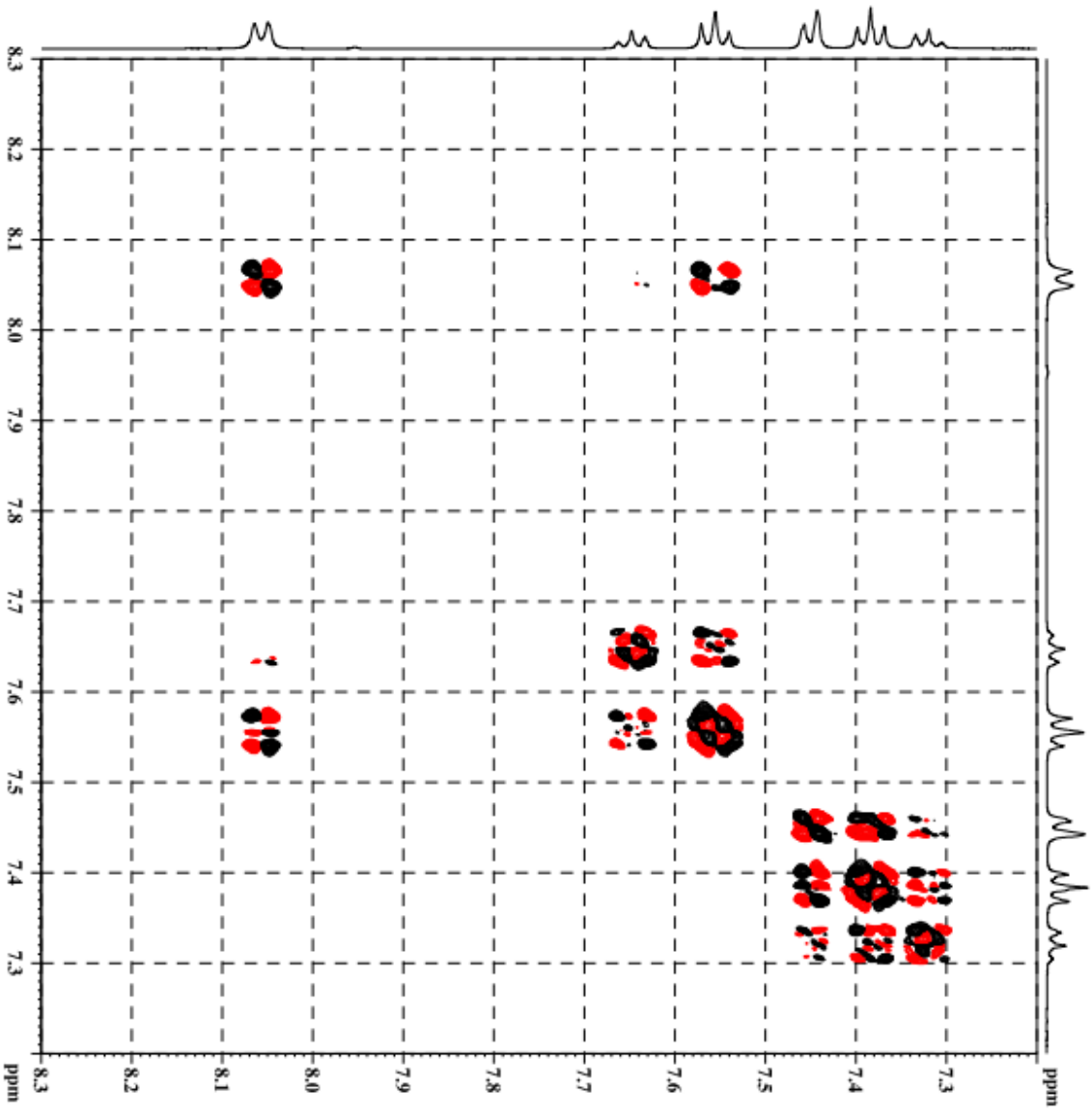

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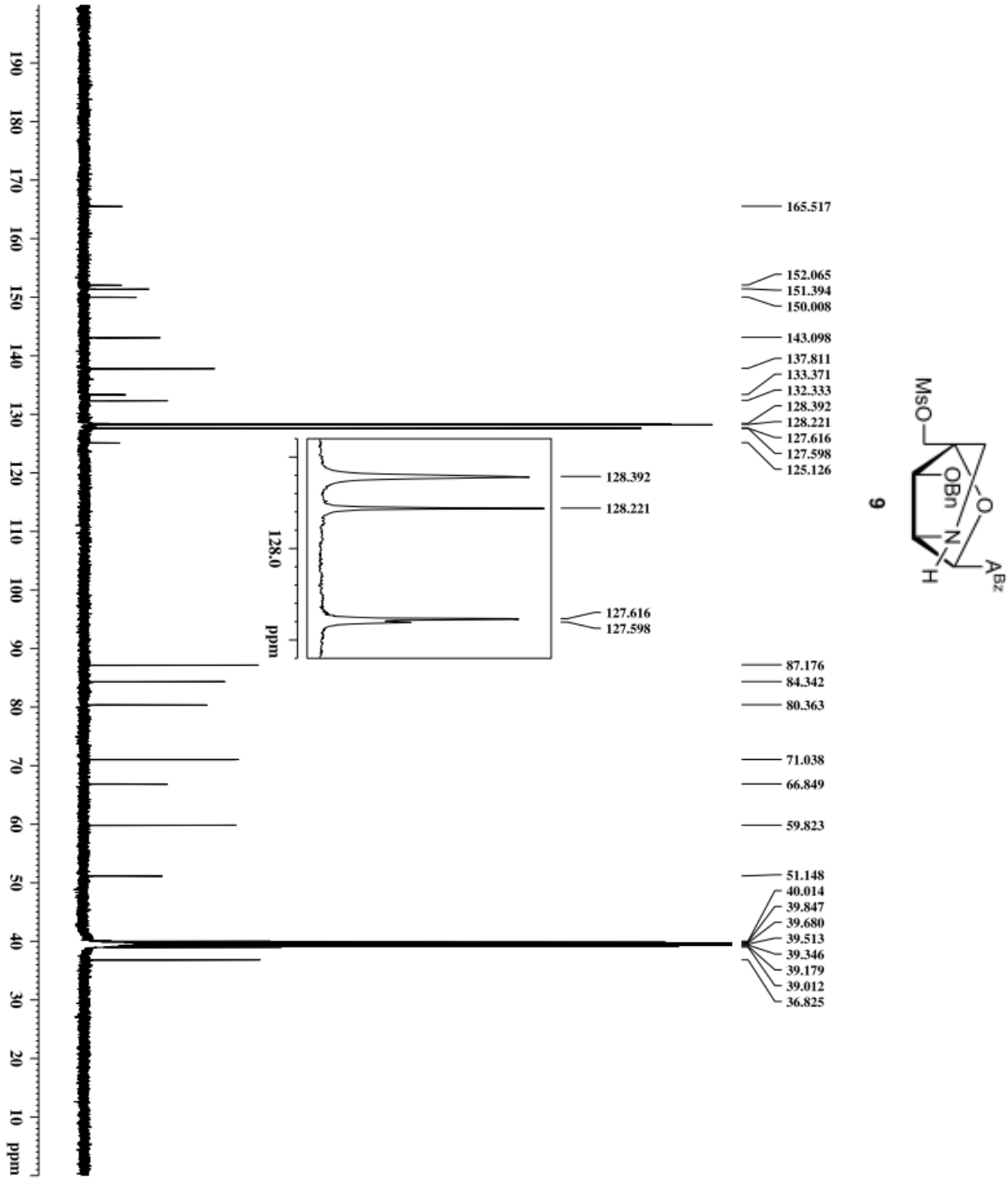
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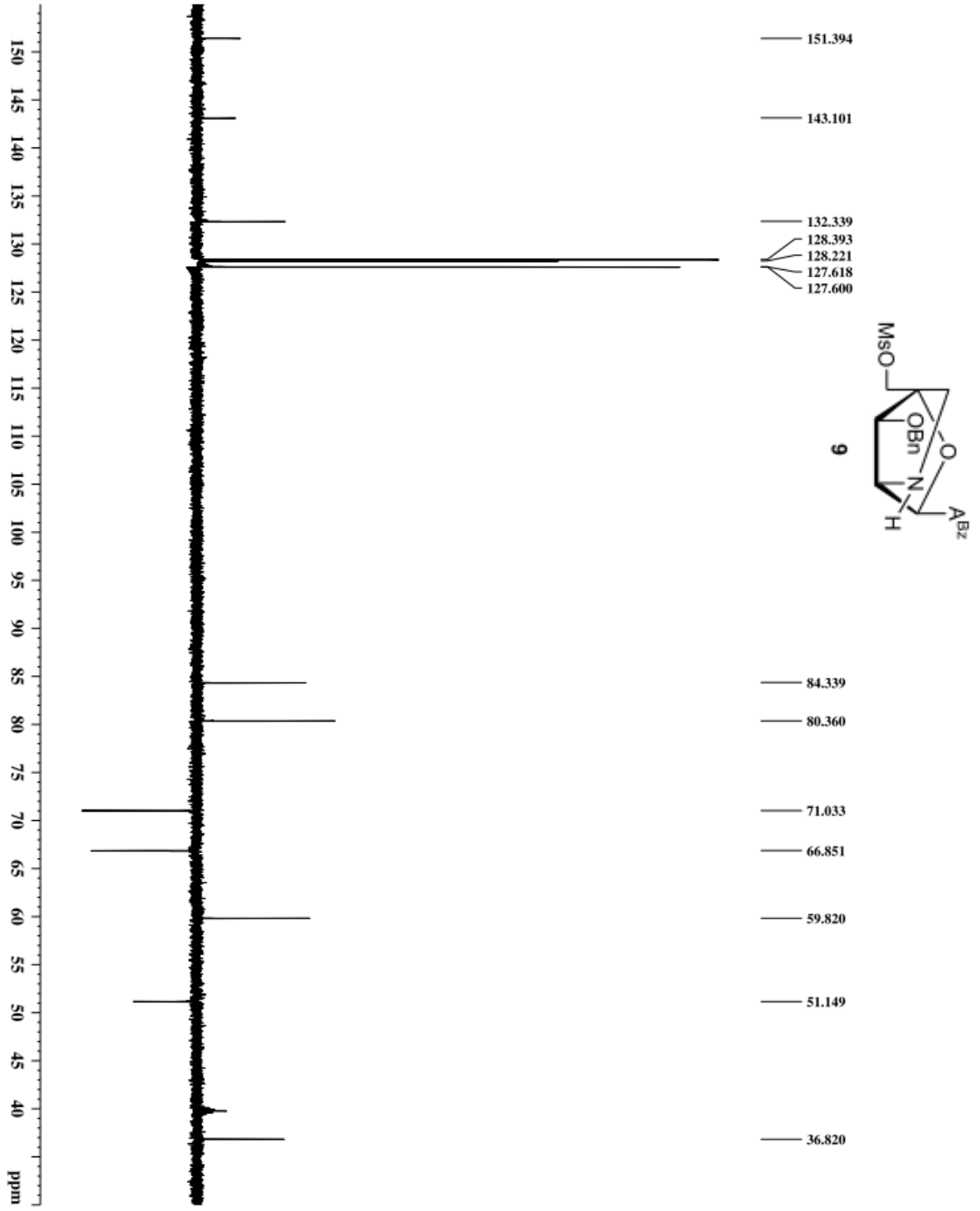
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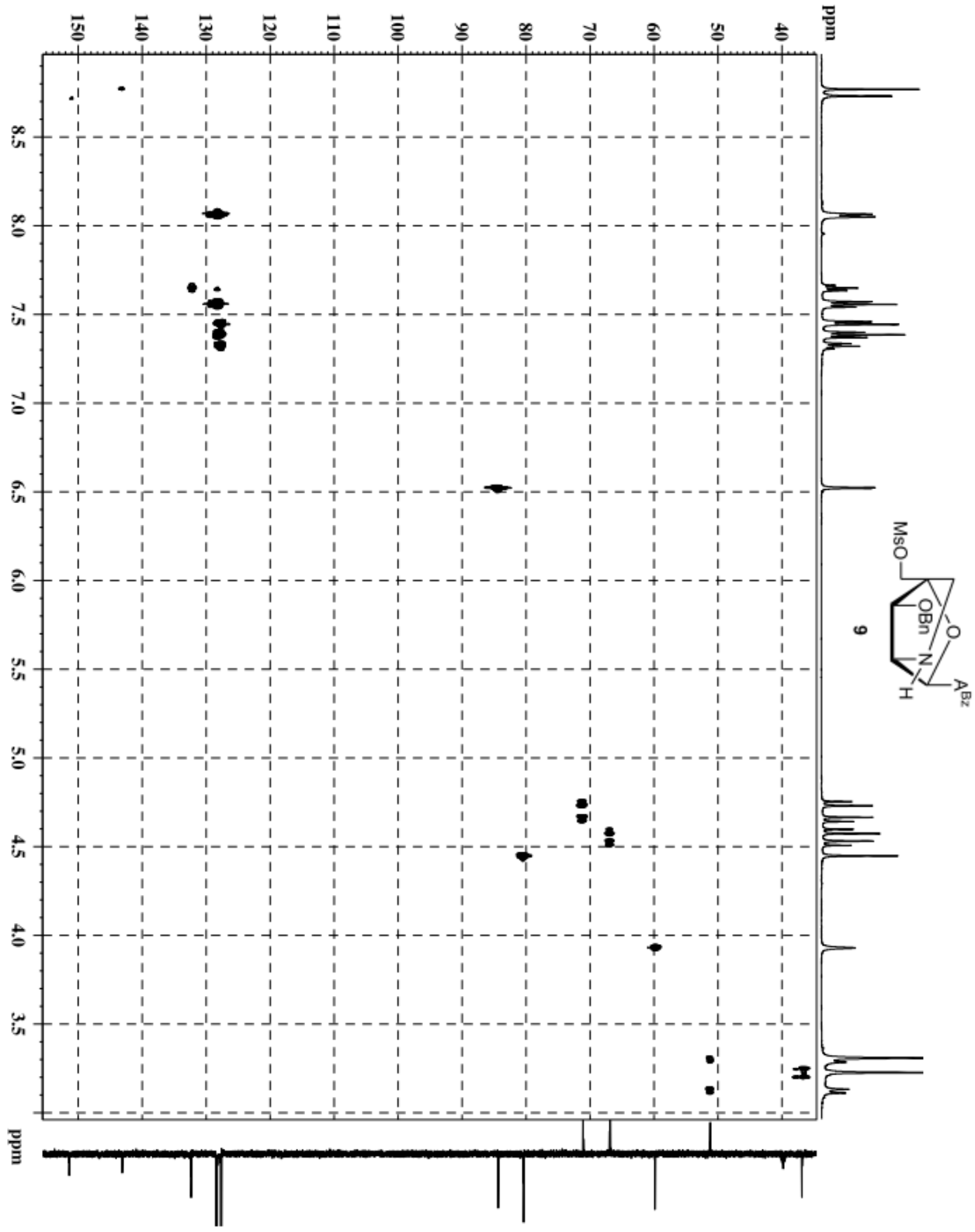
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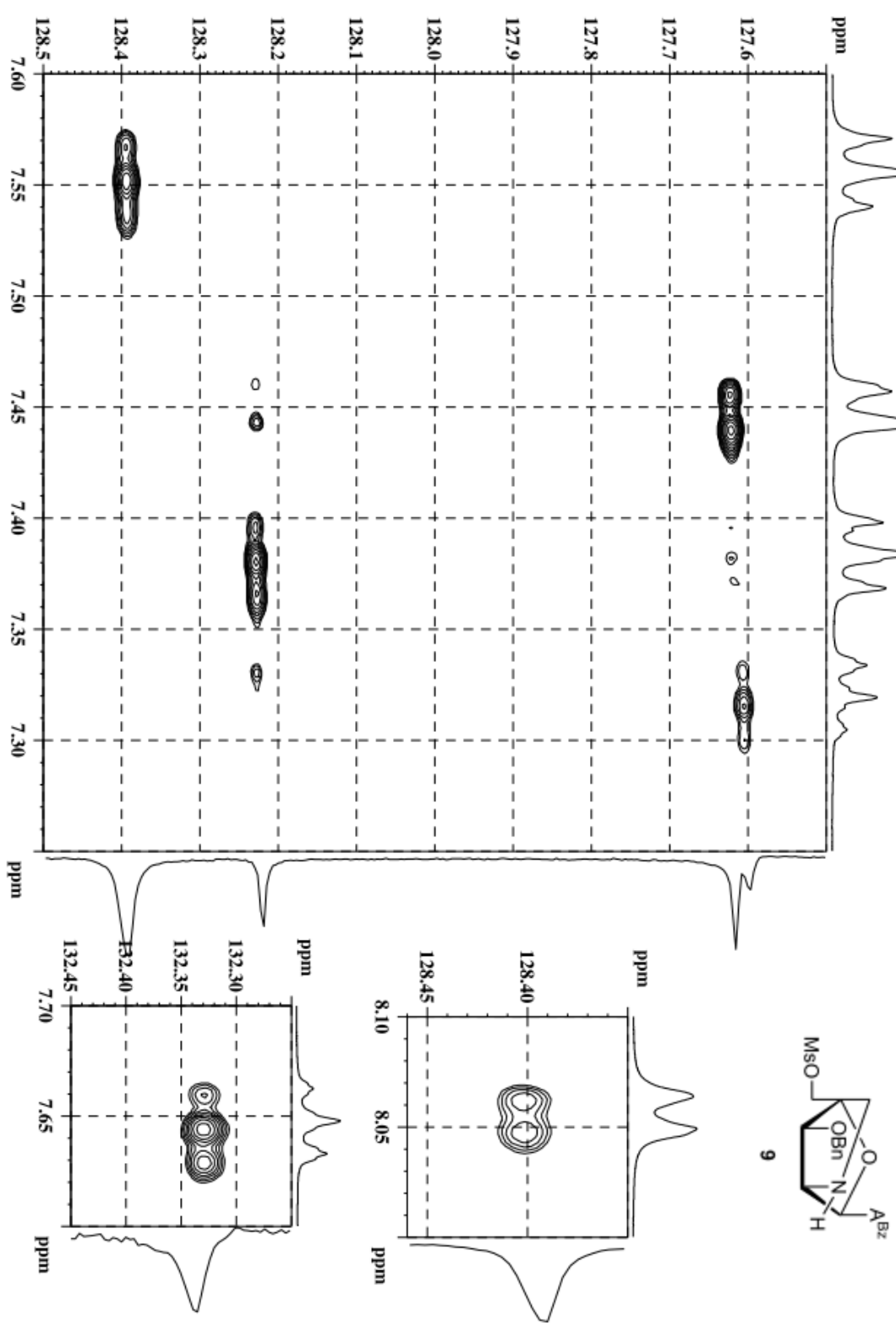
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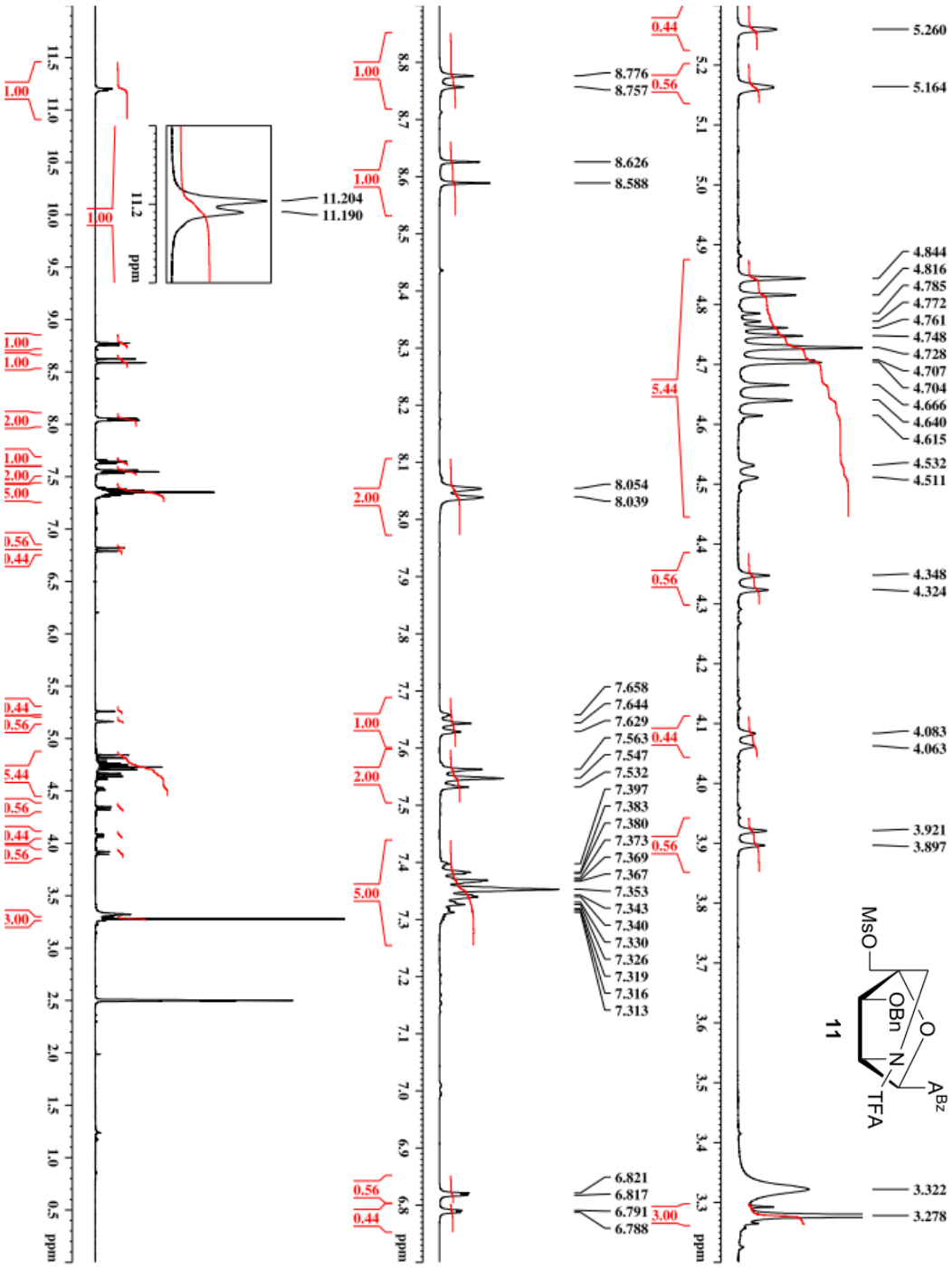
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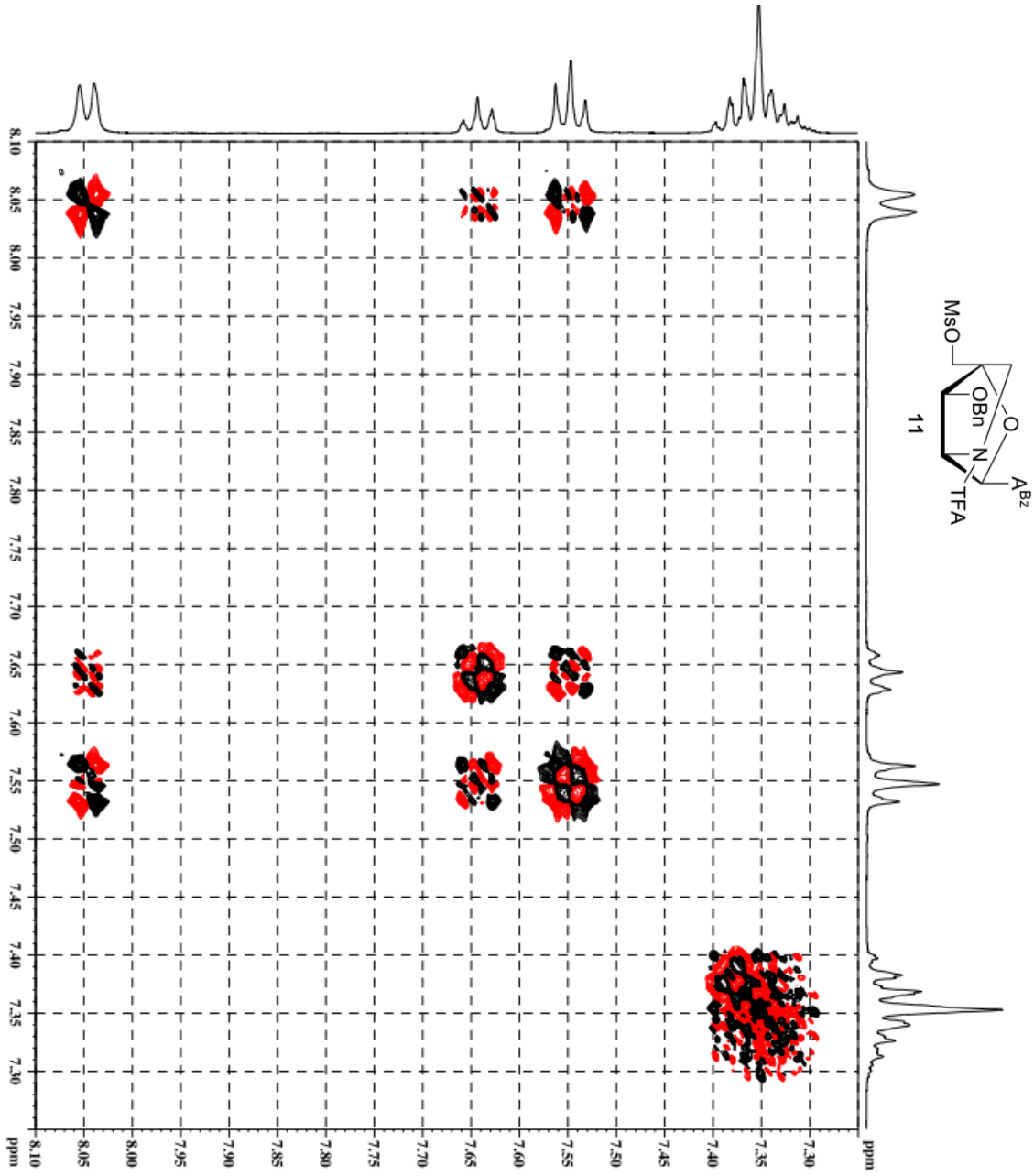
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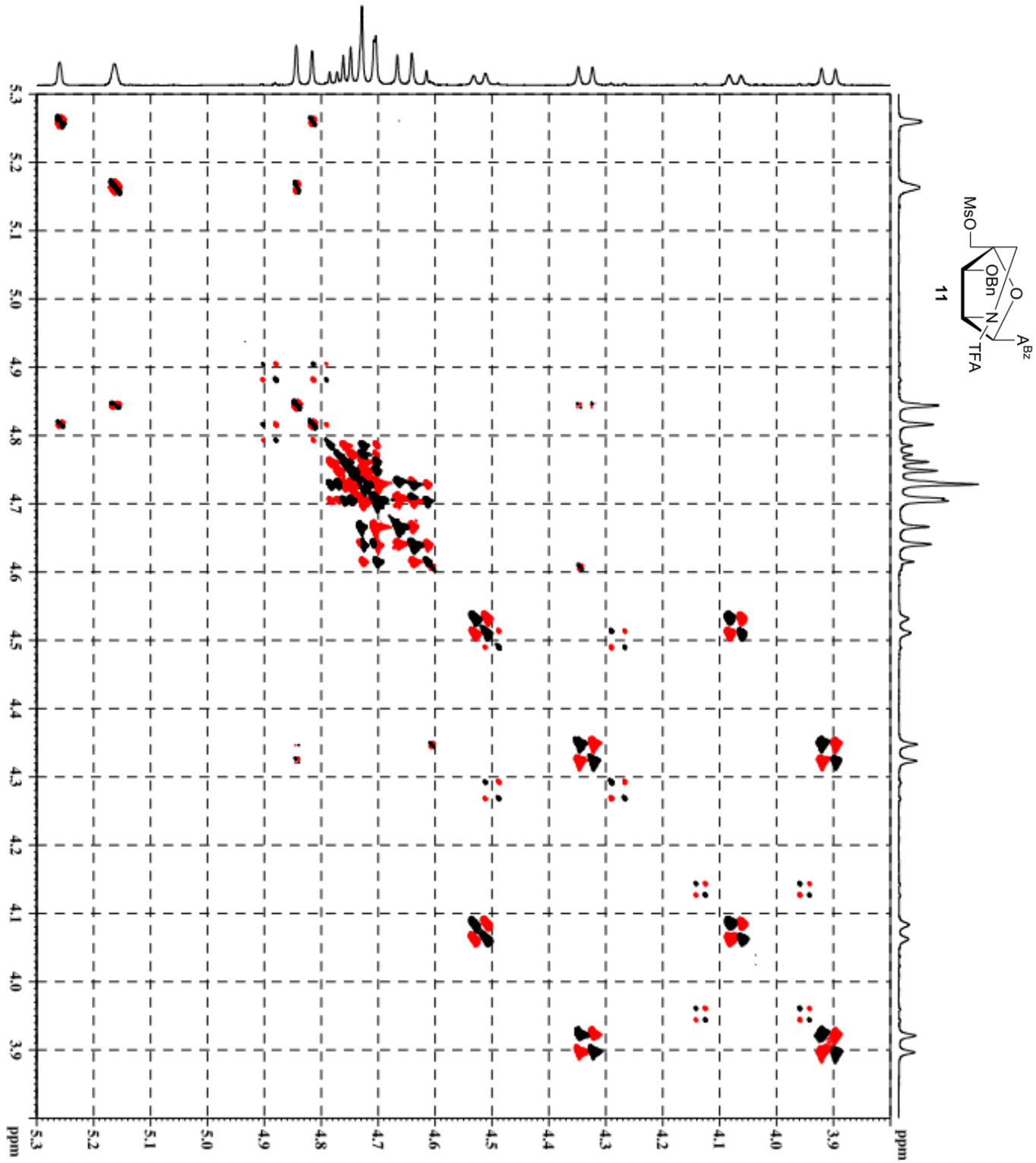
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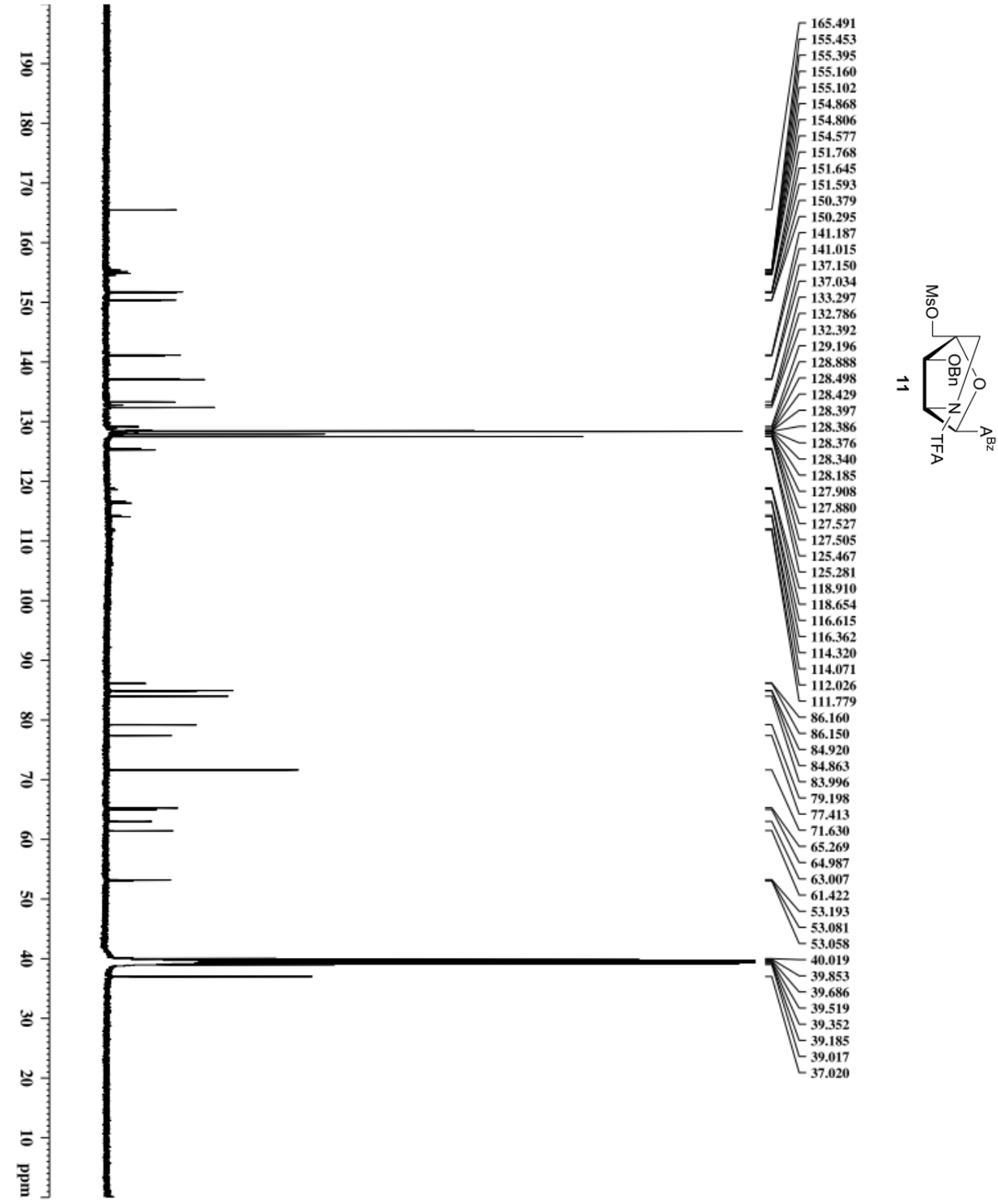
3.9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155.395
155.160
155.102
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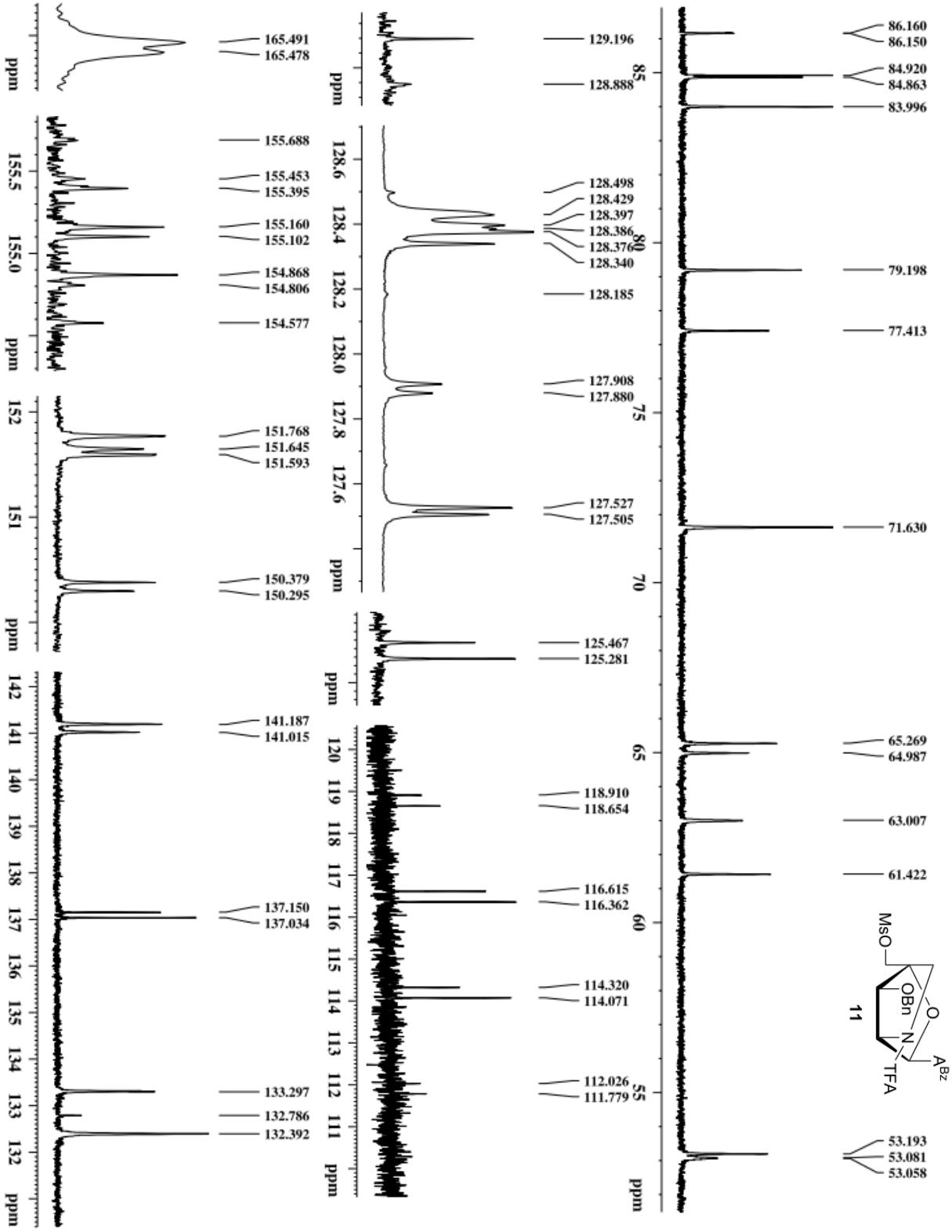
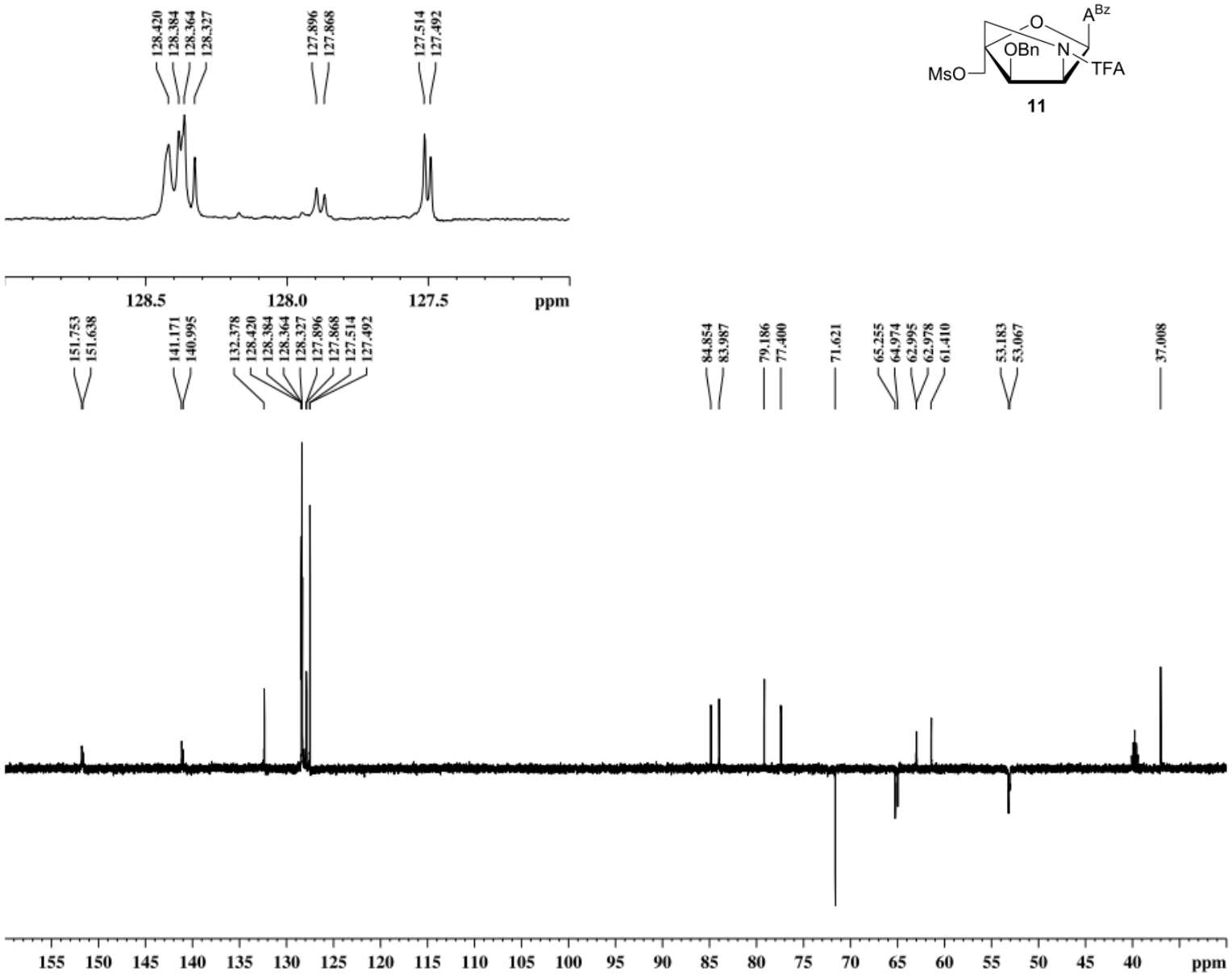
154.806
154.577
151.768
151.645
151.593
150.379
150.295
141.187
141.015
137.150
137.034
133.297
132.786
132.392
129.196
128.888
128.498
128.429
128.397
128.386
128.376
128.340
128.185
127.908
127.880
127.527
127.505
125.467
125.281
118.910
118.654
116.615
116.362
114.320
114.071
112.026
111.779
86.160
86.150
84.920
84.863
83.996
79.198
77.413
71.630
65.269
64.987
63.007
61.422
53.193
53.081
53.058
40.019
39.853
39.686
39.519
39.352
39.185
39.017
37.020
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm
MsO
OBn
O
N
N
TFA
ABz
11


86.160
86.150
84.920
84.863
83.996
79.198
77.413
71.630
65.269
64.987
63.007
61.422
53.193
53.081
53.058
129.196
128.888
128.498
128.429
128.397
128.386
128.376
128.340
128.185
127.908
127.880
127.527
127.505
125.467
125.281
118.910
118.654
116.615
116.362
114.320
114.071
112.026
111.779
165.491
165.478
155.688
155.453
155.395
155.160
155.102
154.868
154.806
154.577
151.768
151.645
151.593
150.379
150.295
141.187
141.015
137.150
137.034
133.297
132.786
132.392
MsO
OBn
O
N
TFA
A^Bz
11
ppm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128.364
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127.896
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127.492
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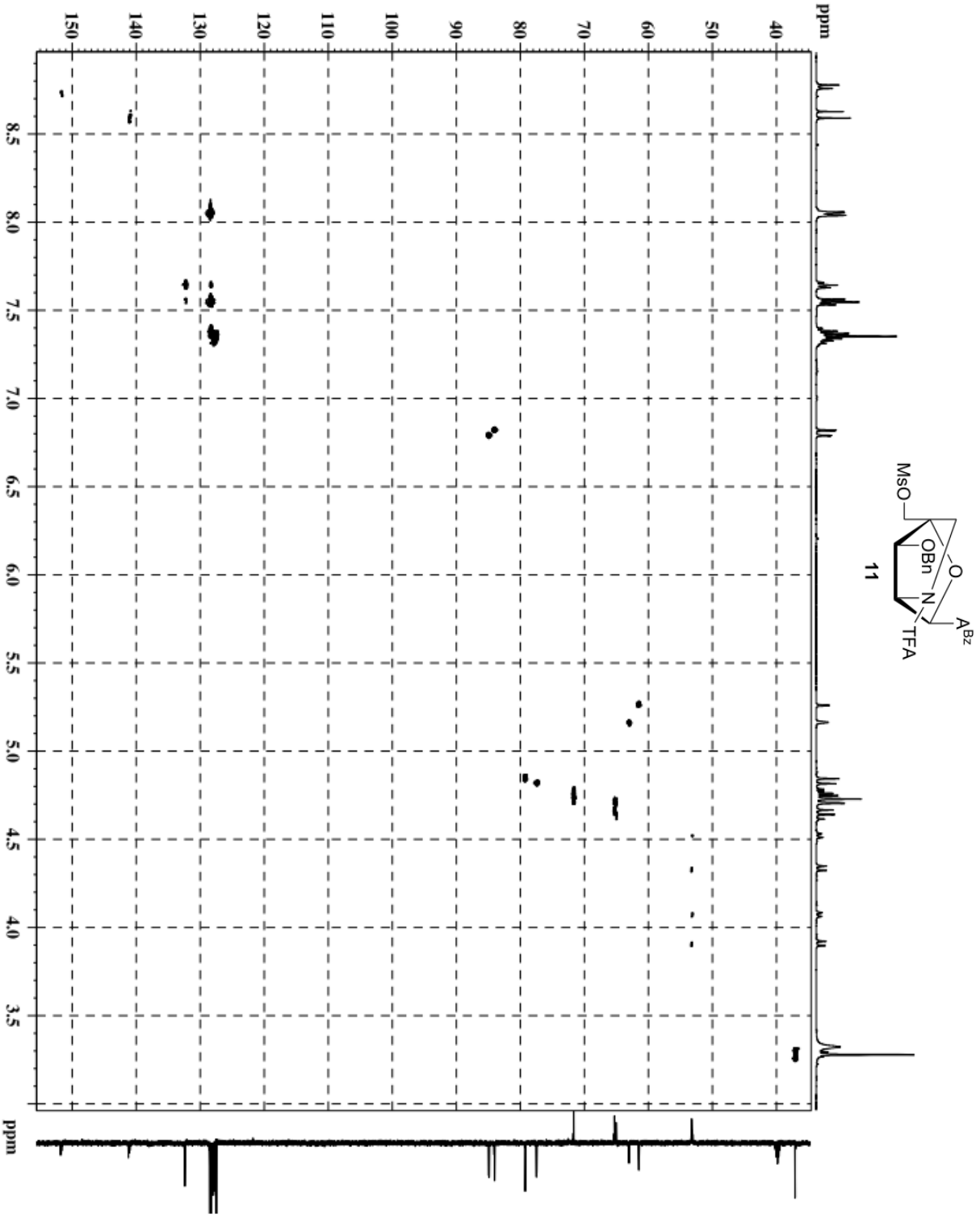
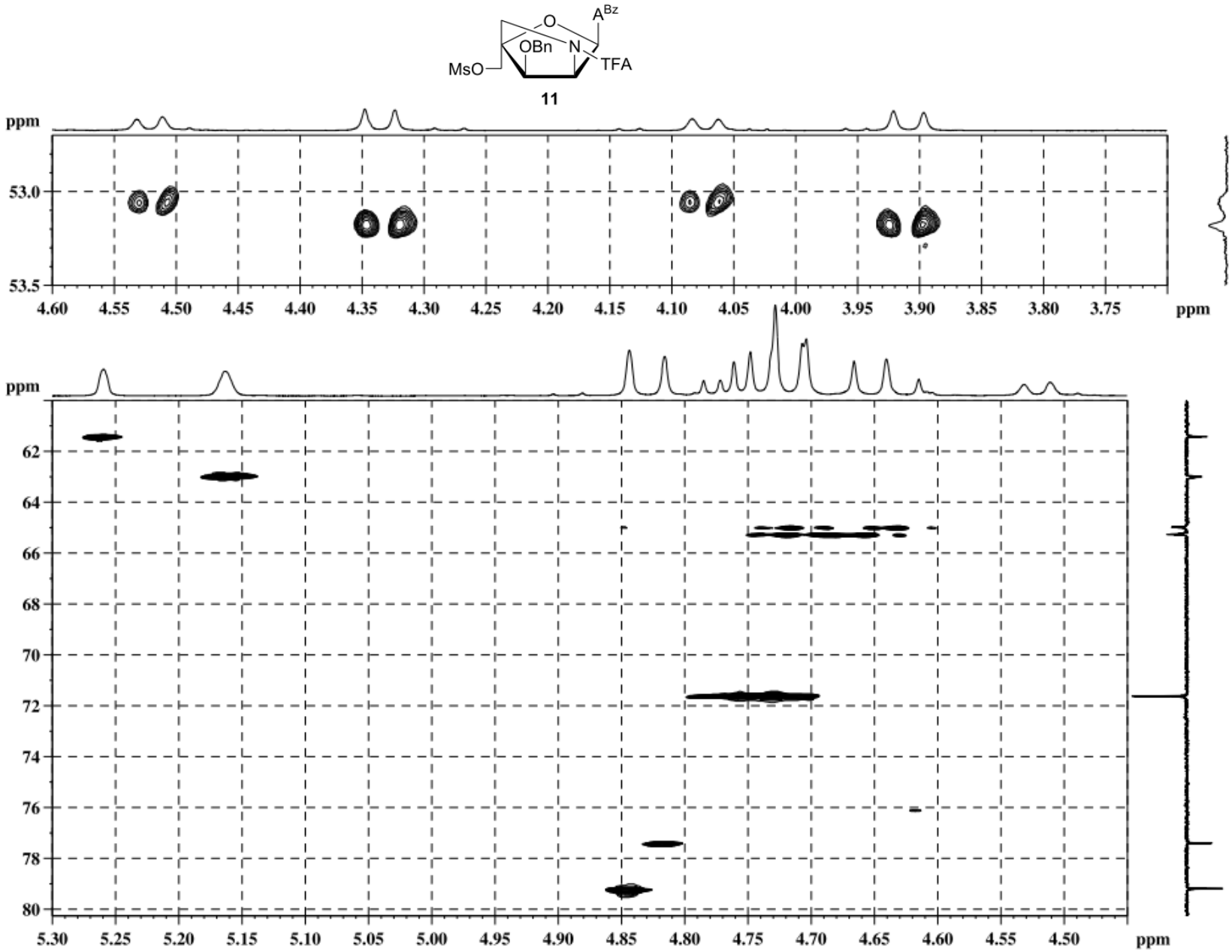
128.0
127.5
ppm
151.753
151.638
141.171
140.995
132.378
128.420
128.384
128.364
128.327
127.896
127.868
127.514
127.492
84.854
83.987
79.186
77.400
71.621
65.255
64.974
62.995
62.978
61.410
53.183
53.067
37.008
155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 ppm

O
A^Bz
OBn
N
MsO
TFA

11


MsO
OBn
O
N
TFA
ABz
11
ppm
ppm


O
A^Bz
OBn
N
MsO
TFA
11
ppm
53.0
53.5
4.60
4.55
4.50
4.45
4.40
4.35
4.30
4.25
4.20
4.15
4.10
4.05
4.00
3.95
3.90
3.85
3.80
3.75
ppm
ppm
62
64
66
68
70
72
74
76
78
80
5.30
5.25
5.20
5.15
5.10
5.05
5.00
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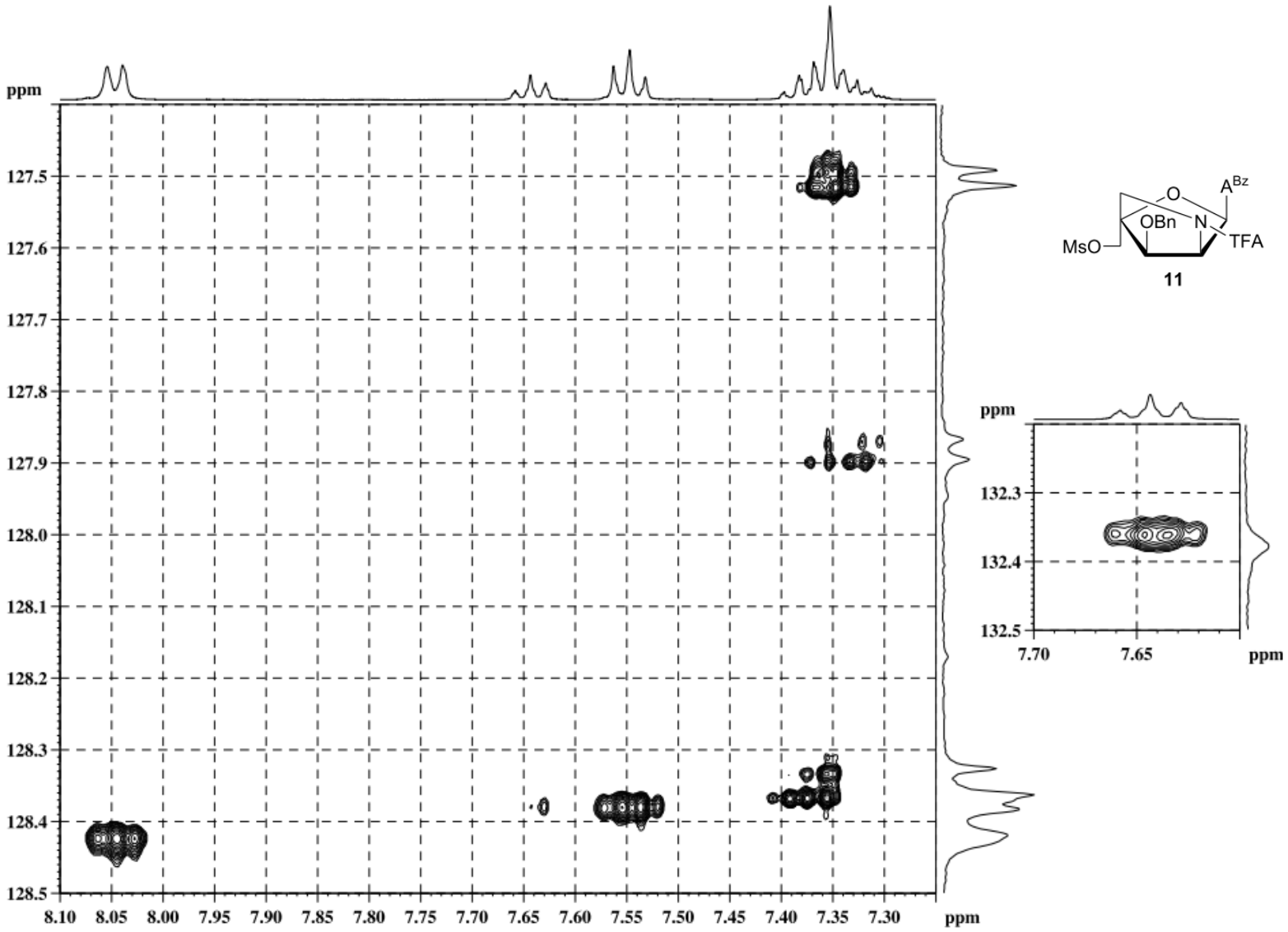
4.90
4.85
4.80
4.75
4.70
4.65
4.60
4.55
4.50
ppm


ppm
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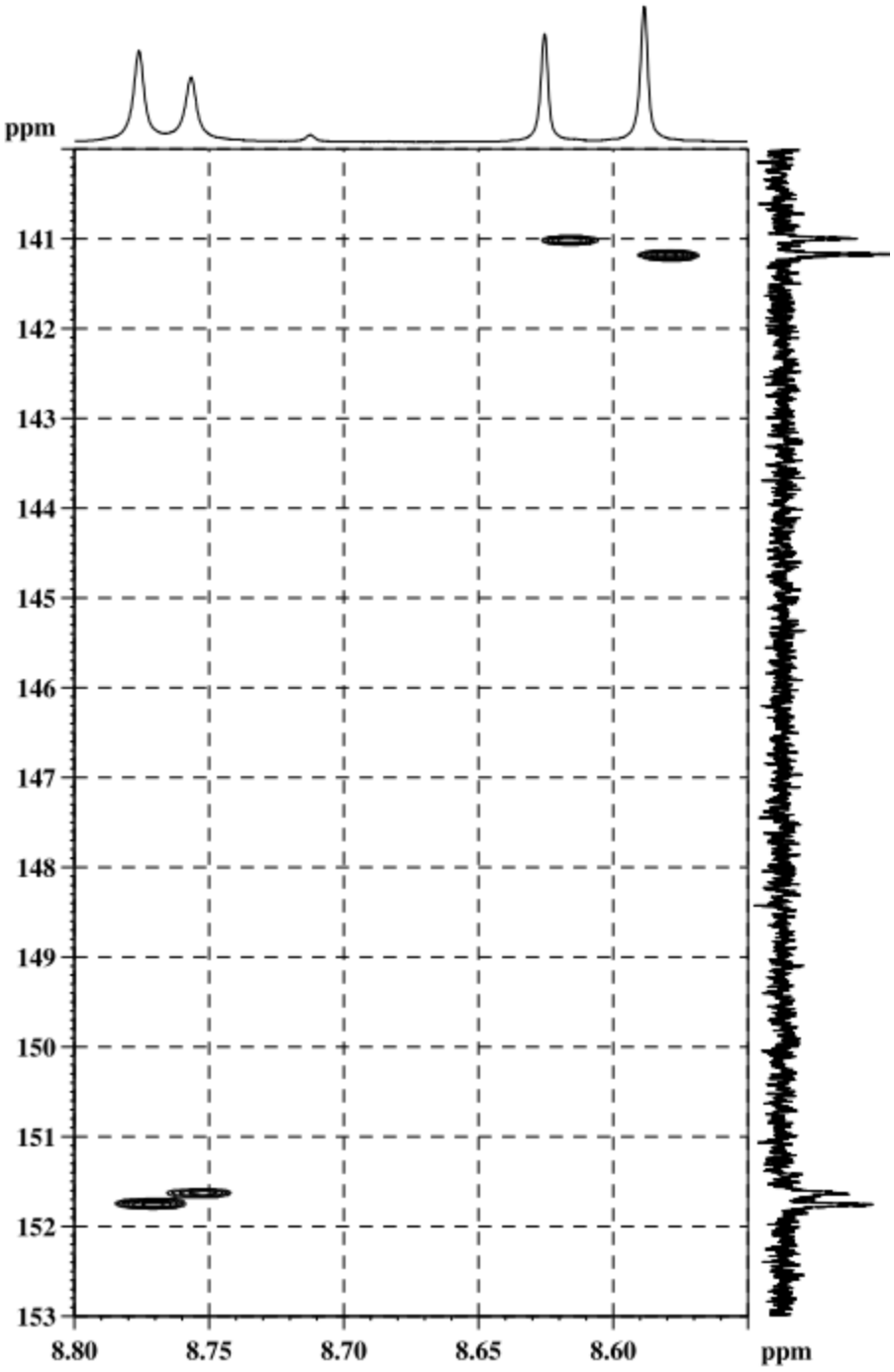
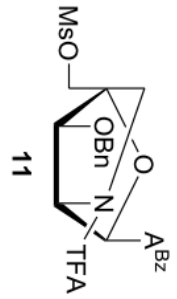
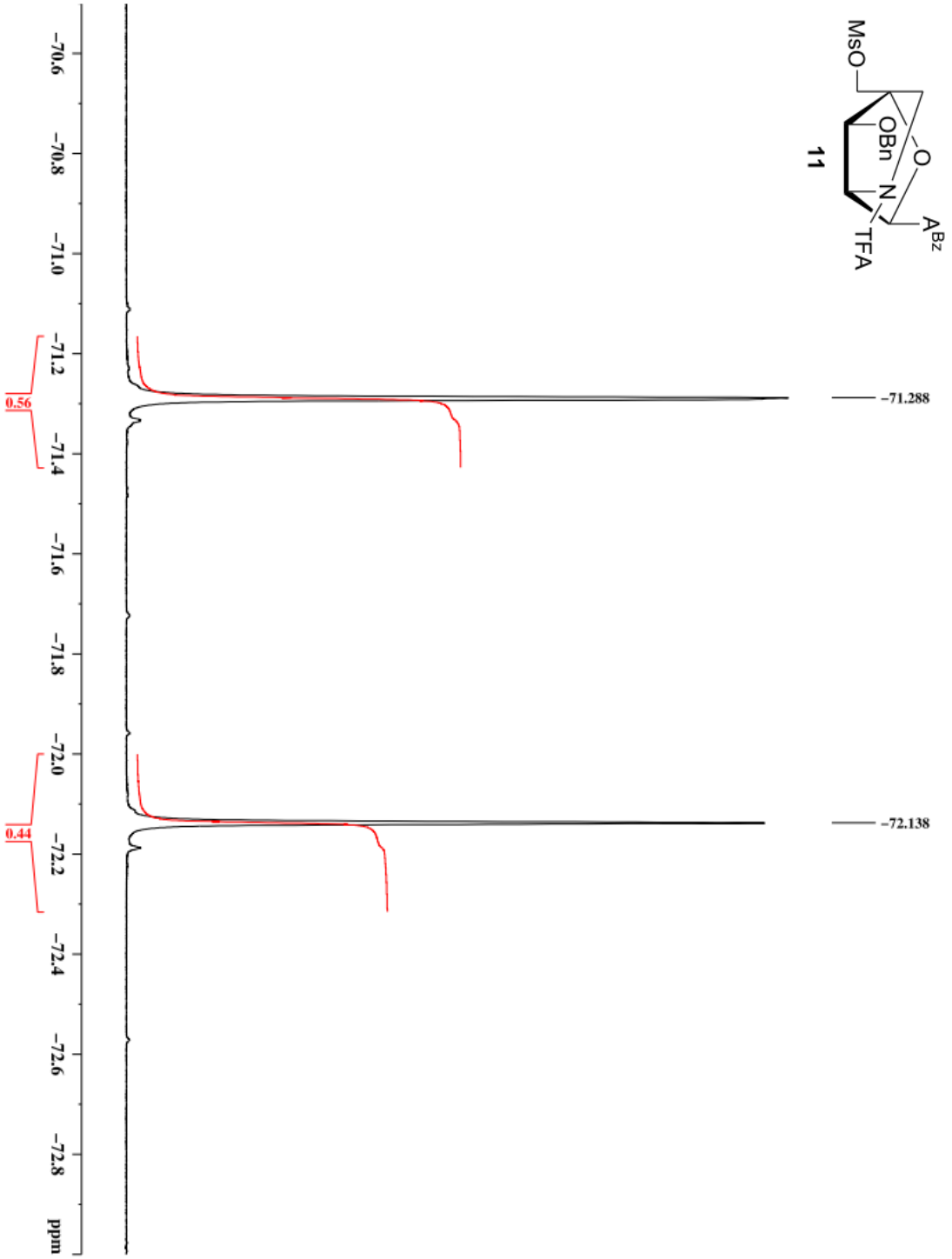
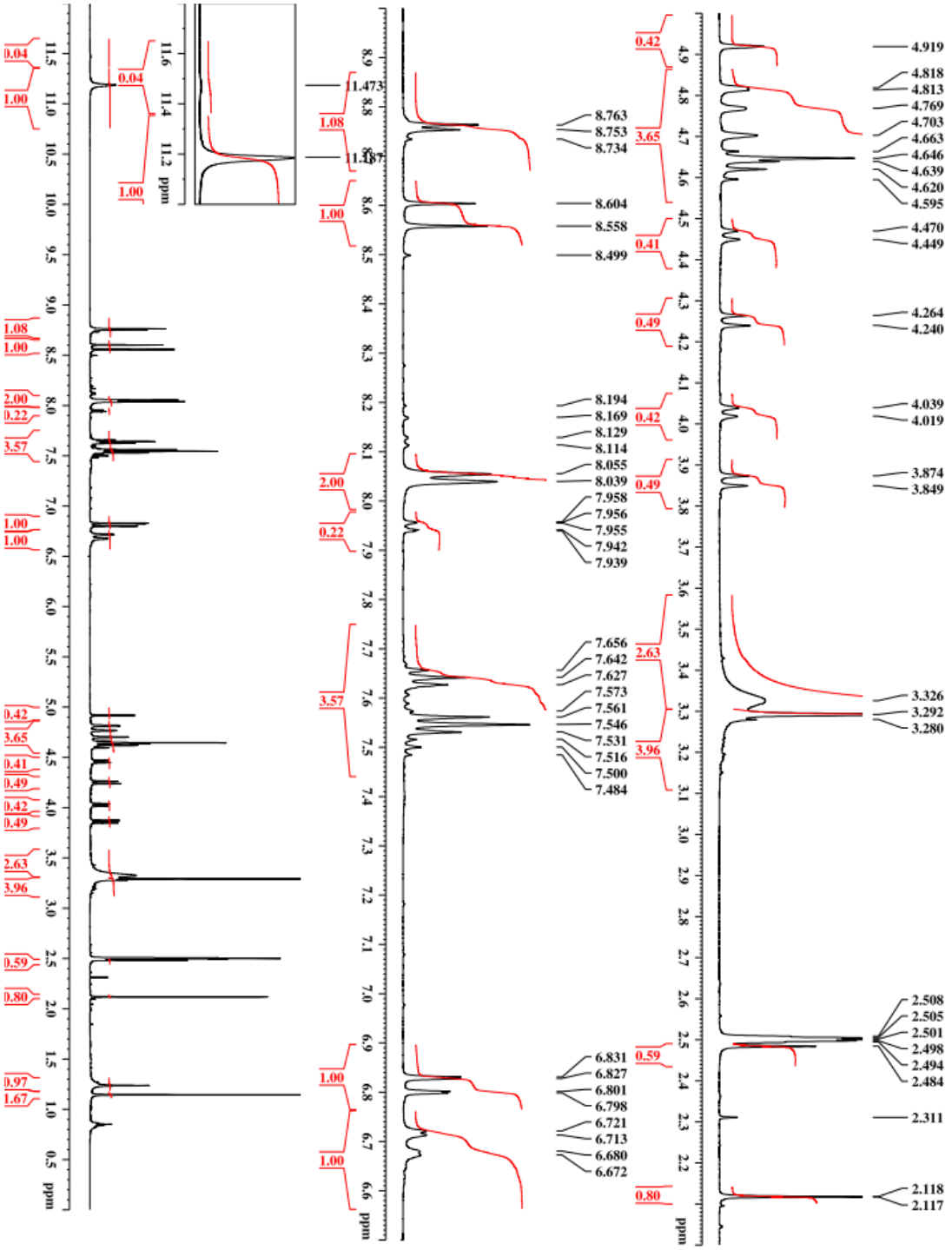
127.6
127.7
127.8
127.9
128.0
128.1
128.2
128.3
128.4
128.5
8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30
ppm
O
A^Bz
OBn
N
MsO
TFA
11
ppm
132.3
132.4
132.5
7.70 7.65
ppm

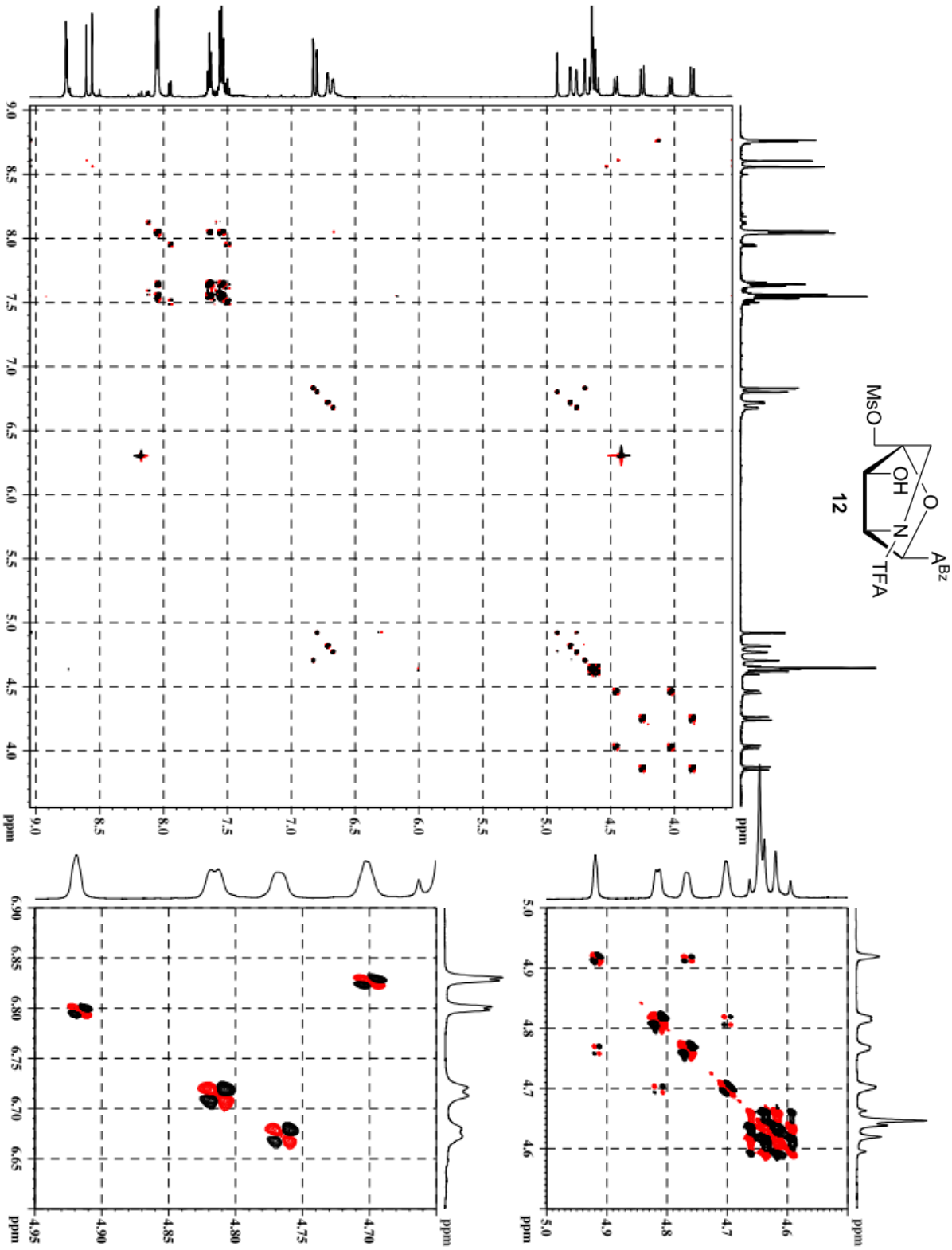

ppm
141
142
143
144
145
146
147
148
149
150
151
152
153
8.80
8.75
8.70
8.65
8.60
ppm

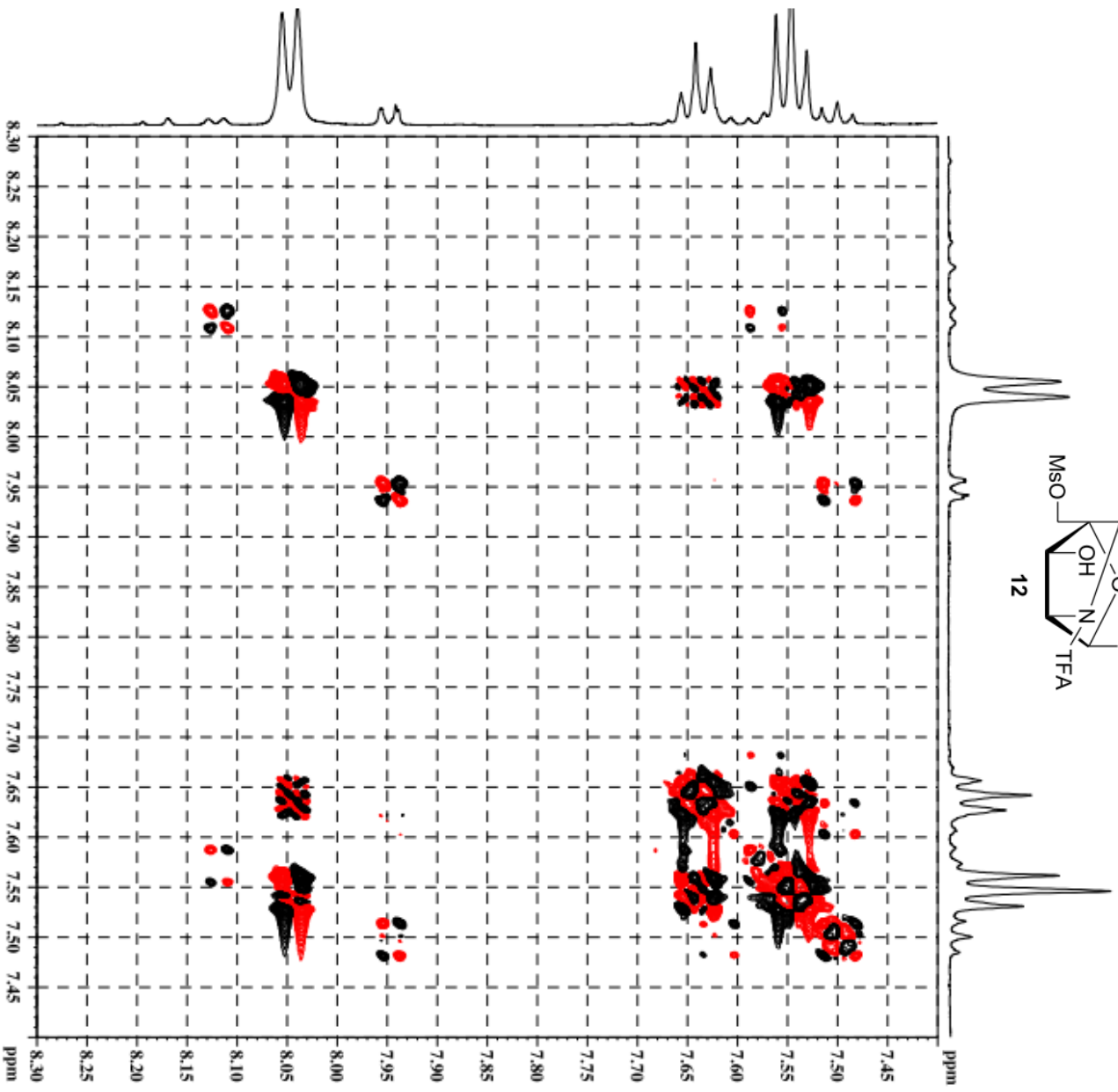

MsO
OBn
O
N
TFA
ABz

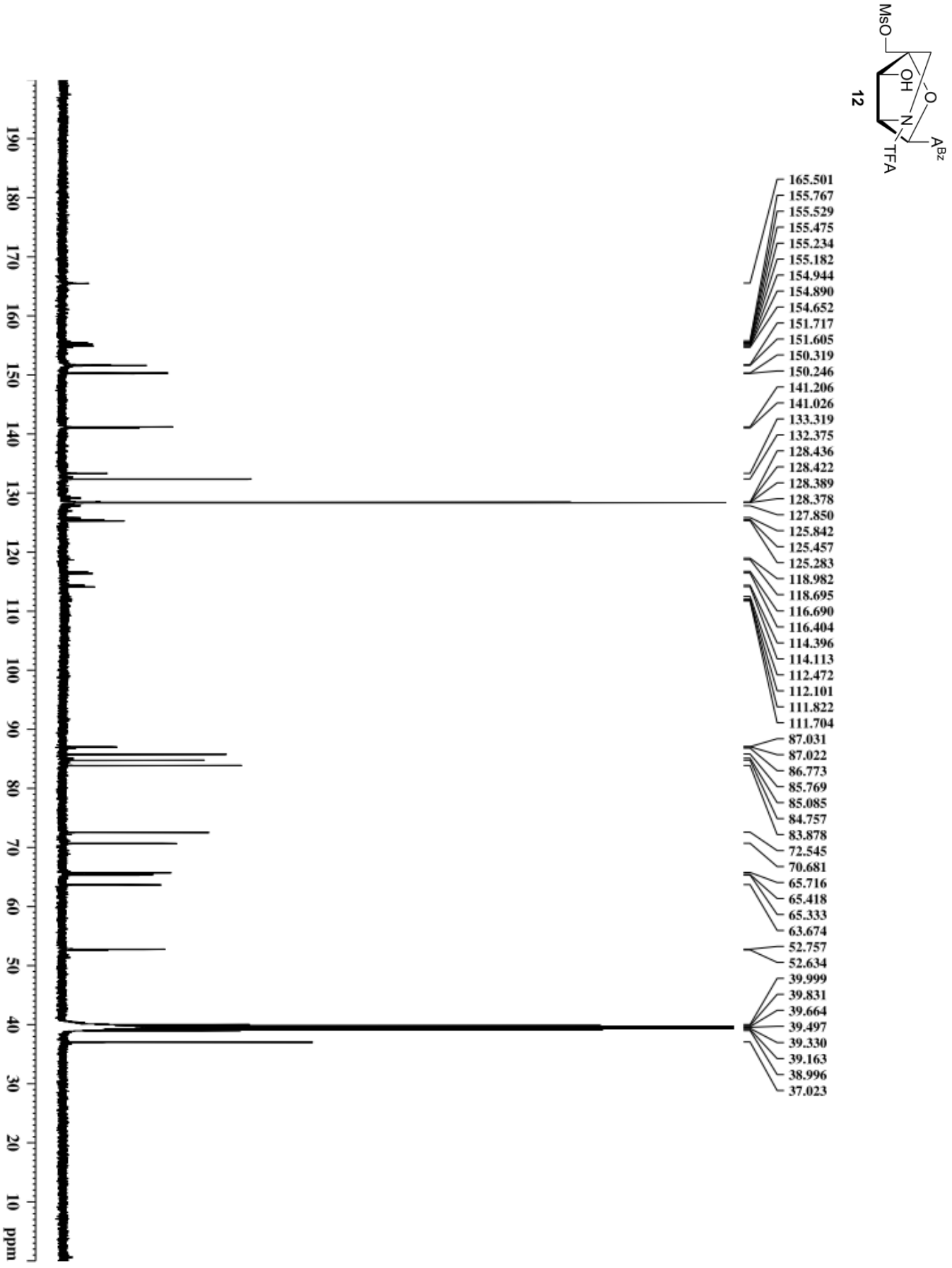
11

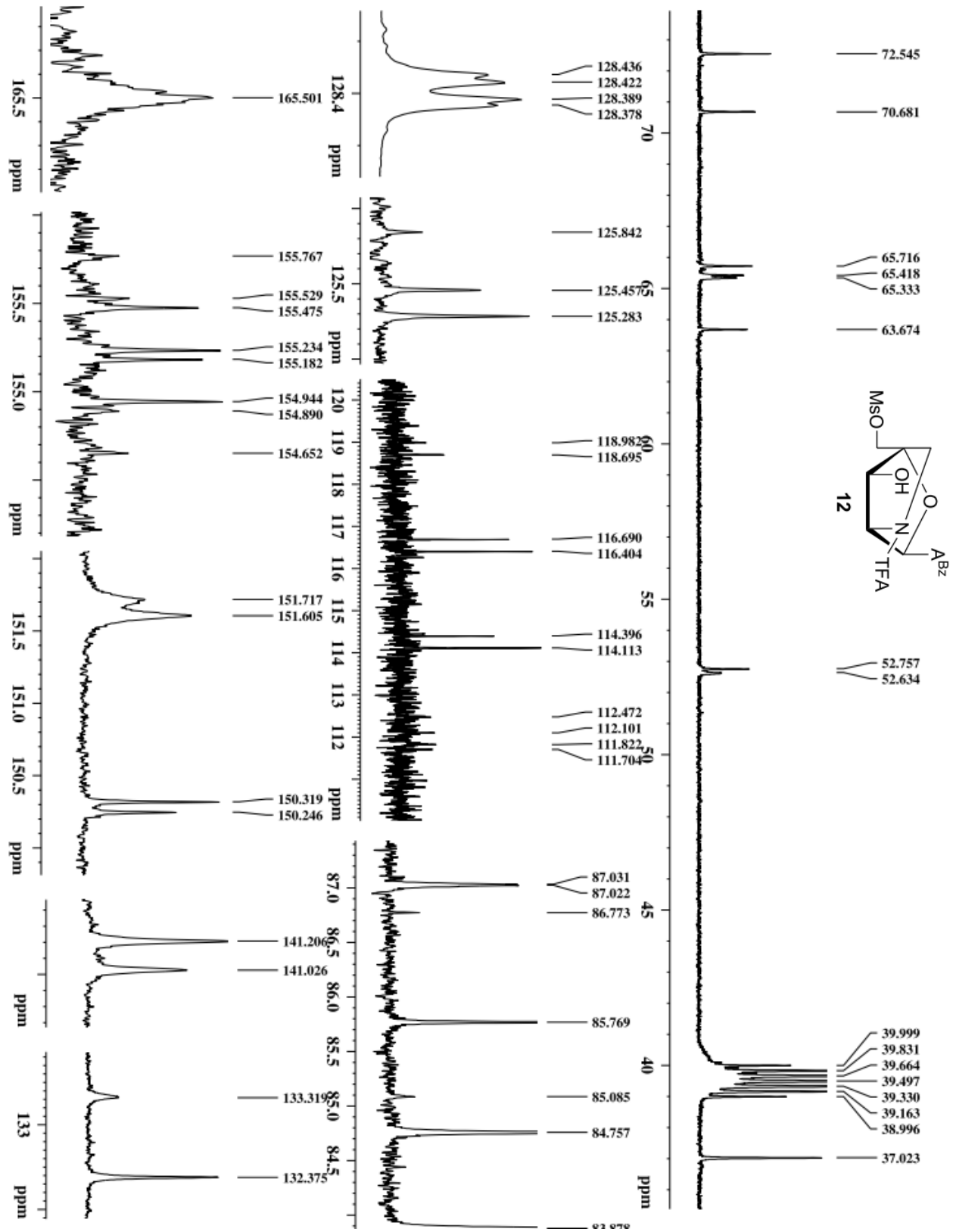

MsO
OBn
O
N
N
TFA
ABz
11
−71.288
−72.138
0.56
0.44
−70.6
−70.8
−71.0
−71.2
−71.4
−71.6
−71.8
−72.0
−72.2
−72.4
−72.6
−72.8
ppm


MsO
OH
O
N
TFA
ABz
12


MsO
OH
O
N
TFA
ABz
12
ppm


MsO
OH
O
N
TFA
ABz
12
ppm
8.30 8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45


MsO
OH
O
N
TFA
ABz
12
165.501
155.767
155.529
155.475
155.234
155.182
154.944
154.890
154.652
151.717
151.605
150.319
150.246
141.206
141.026
133.319
132.375
128.436
128.422
128.389
128.378
127.850
125.842
125.457
125.283
118.982
118.695
116.690
116.404
114.396
114.113
112.472
112.101
111.822
111.704
87.031
87.022
86.773
85.769
85.085
84.757
83.878
72.545
70.681
65.716
65.418
65.333
63.674
52.757
52.634
39.999
39.831
39.664
39.497
39.330
39.163
38.996
37.023
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm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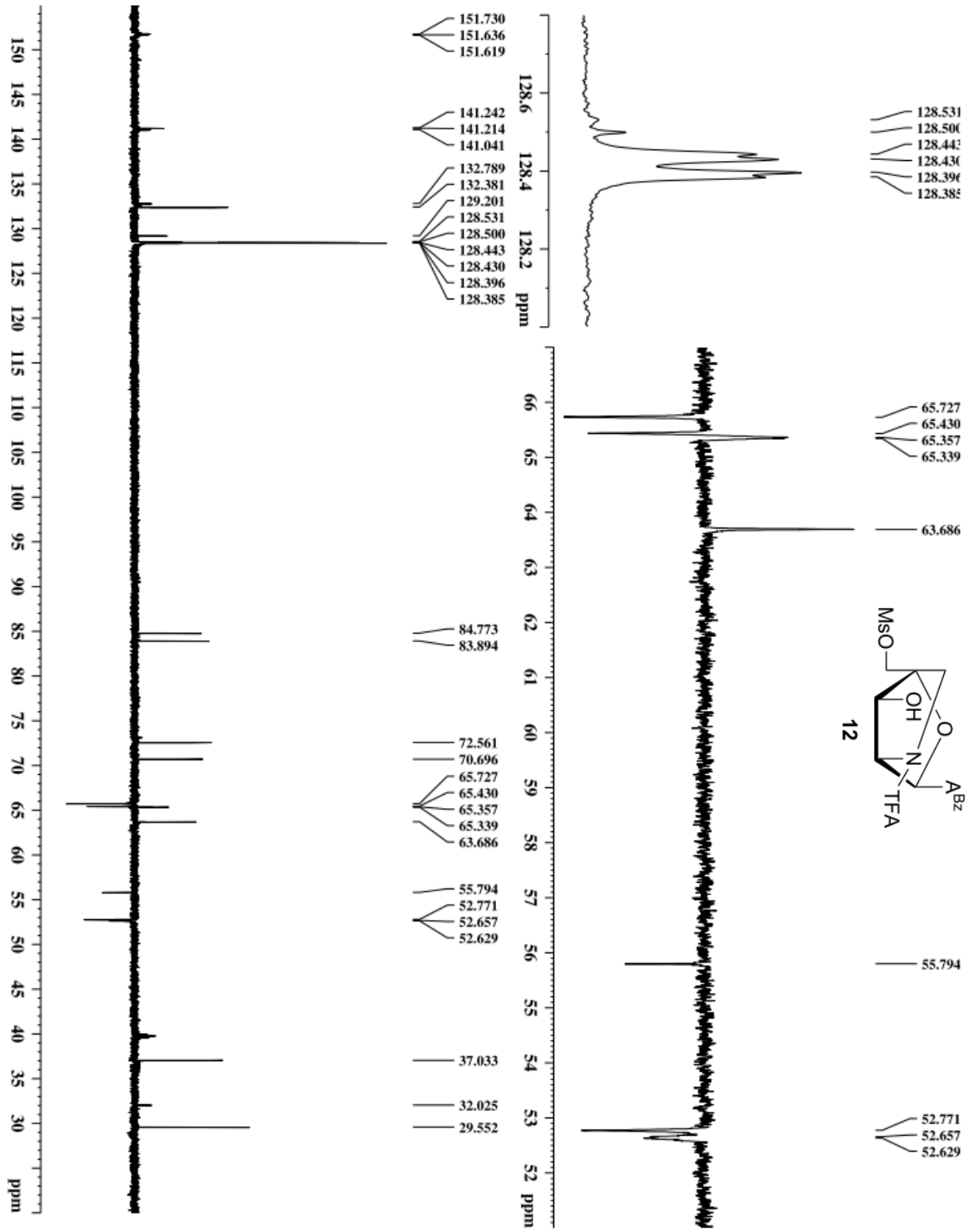
65.716
65.418
65.333
63.674
52.757
52.634
39.999
39.831
39.664
39.497
39.330
39.163
38.996
37.023
MsO
OH
O
N
TFA
ABz
12
128.436
128.422
128.389
128.378
125.842
125.457
125.283
118.982
118.695
116.690
116.404
114.396
114.113
112.472
112.101
111.822
111.704
87.031
87.022
86.773
85.769
85.085
84.757
165.501
155.767
155.529
155.475
155.234
155.182
154.944
154.890
154.652
151.717
151.605
150.319
150.246
141.206
141.026
133.319
132.375
ppm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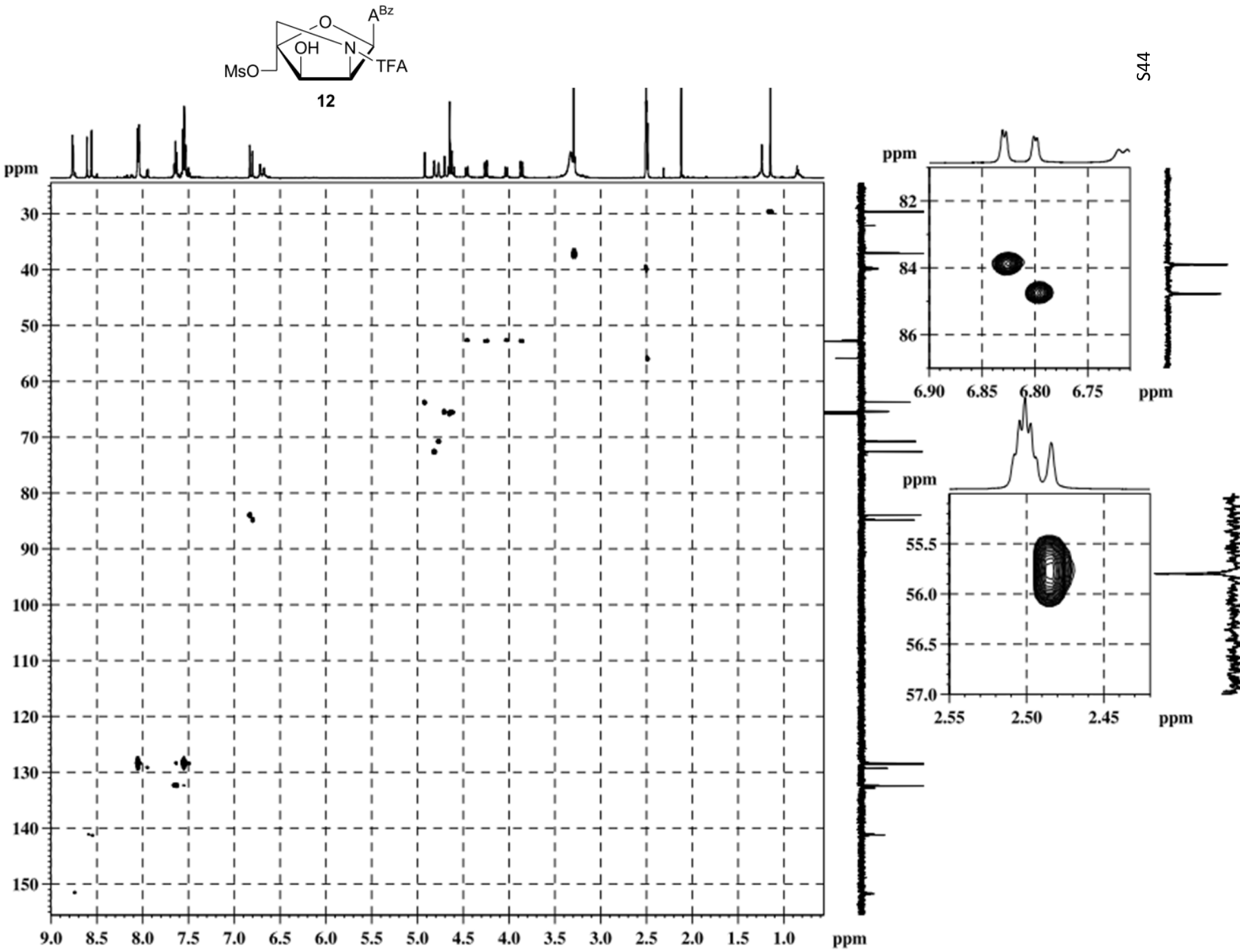
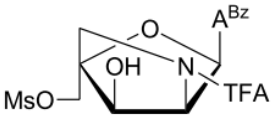
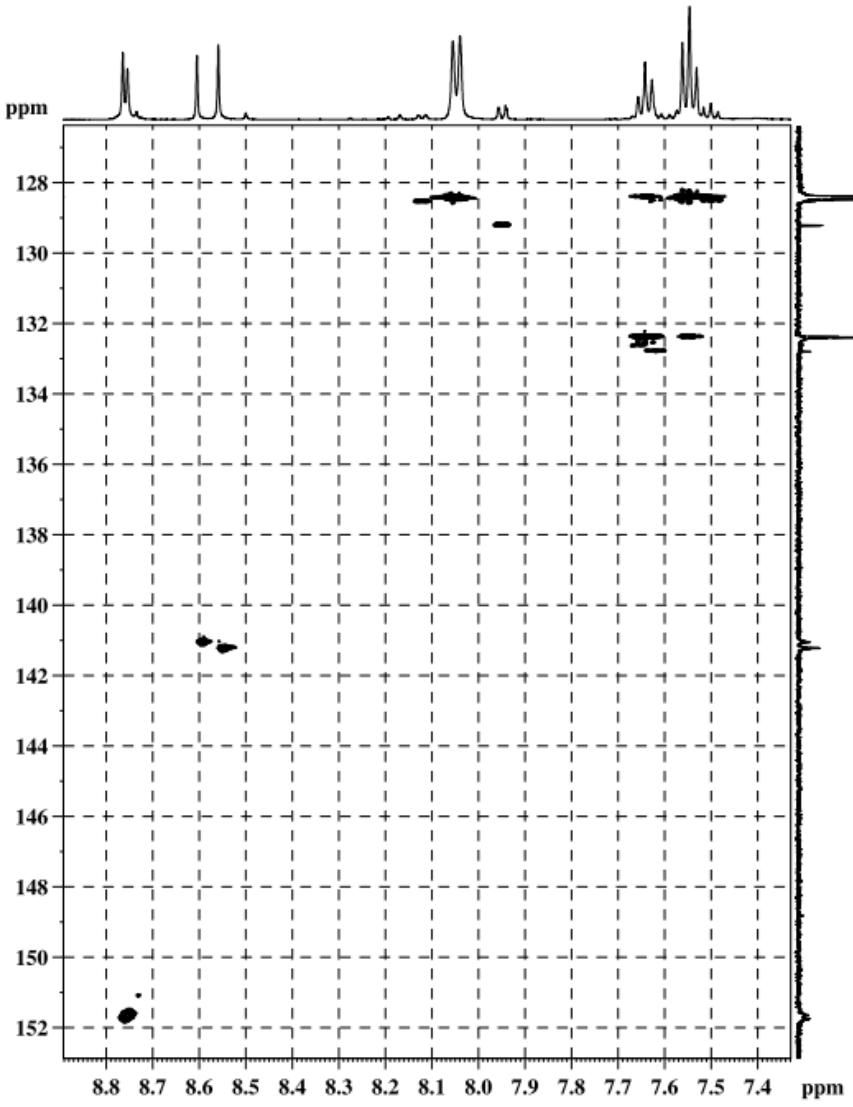
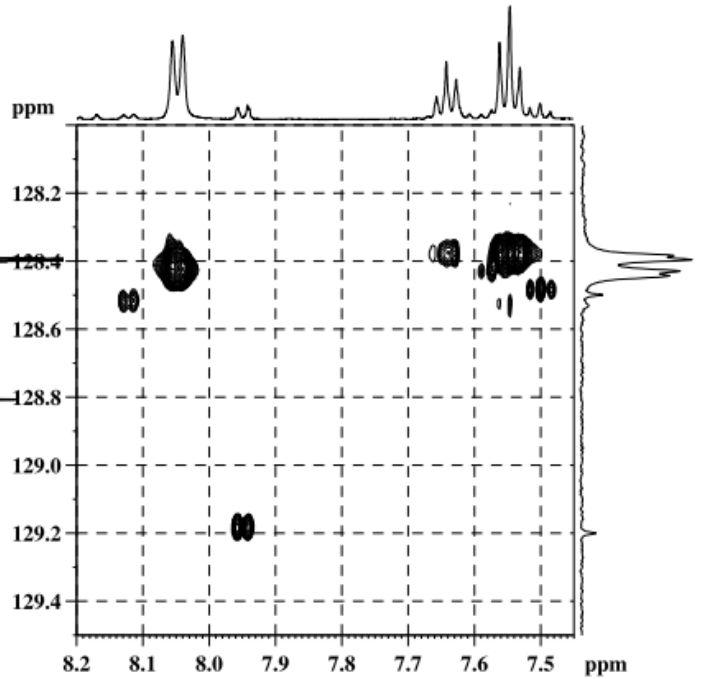
151.636
151.619
141.242
141.214
141.041
132.789
132.381
129.201
128.531
128.500
128.443
128.430
128.396
128.385
128.6
128.4
128.2
ppm
84.773
83.894
72.561
70.696
65.727
65.430
65.357
65.339
63.686
55.794
52.771
52.657
52.629
37.033
32.025
29.552
66
65
64
63
62
61
60
59
58
57
56
55
54
53
52
ppm
MsO
OH
O
N
TFA
ABz
12
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40
35
30
ppm

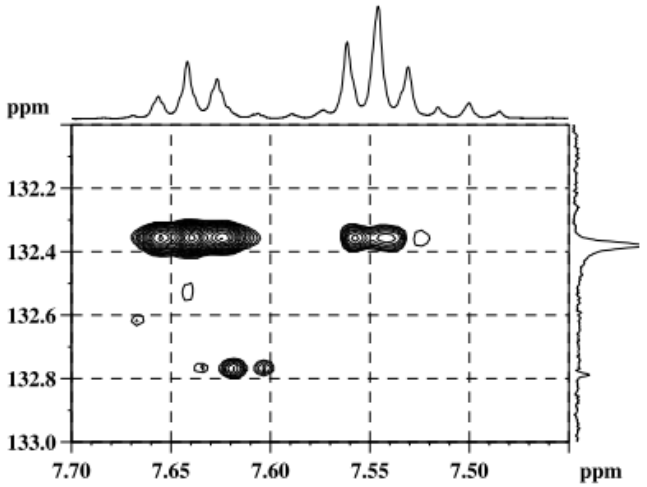

O
A^Bz
OH
N
MsO
TFA
12
ppm
30
40
50
60
70
80
90
100
110
120
130
140
150
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
ppm
ppm
82
84
86
6.90
6.85
6.80
6.75
ppm
ppm
55.5
56.0
56.5
57.0
2.55
2.50
2.45
ppm

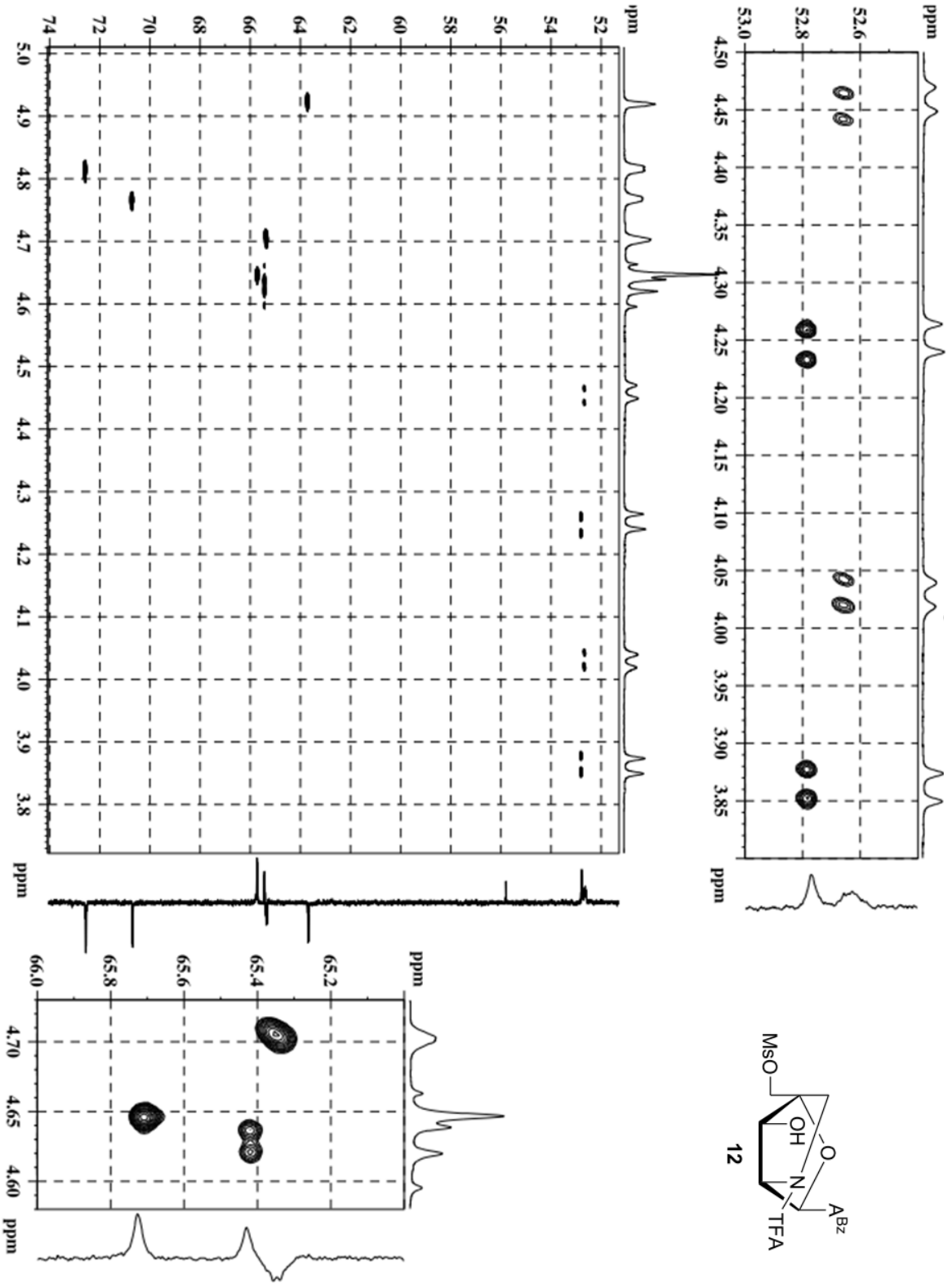

O
A^Bz
OH
N
MsO
TFA

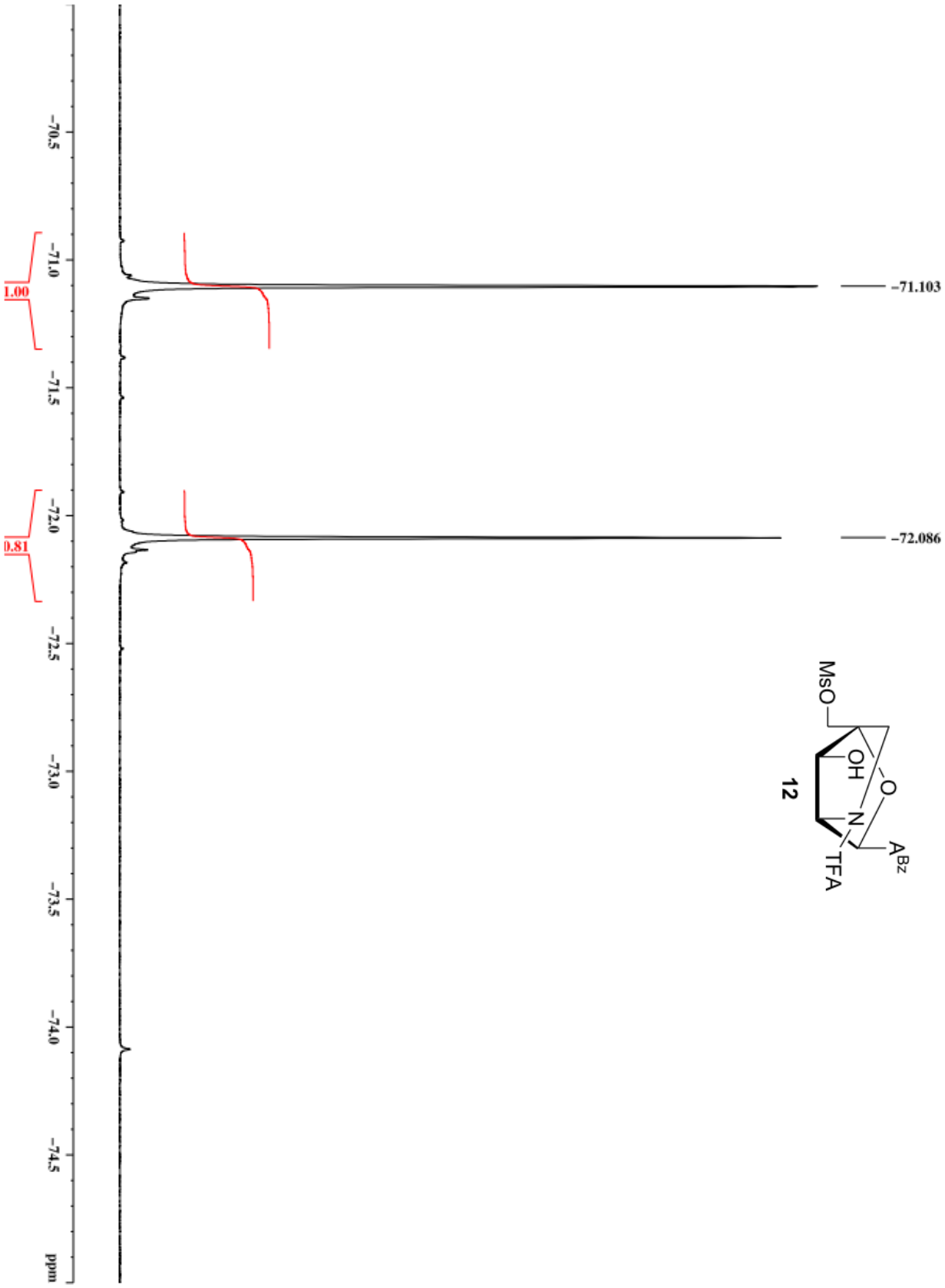
12

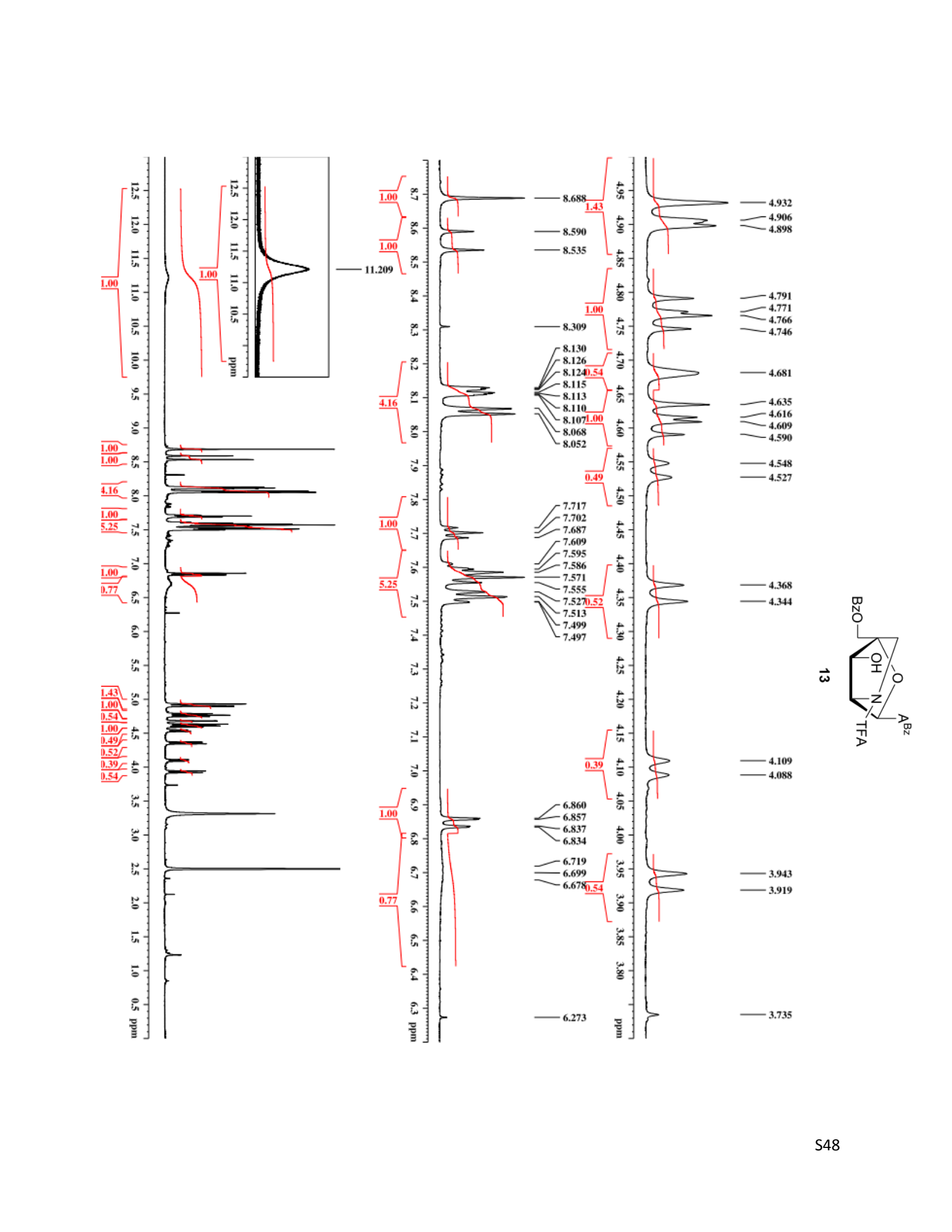

ppm
128
130
132
134
136
138
140
142
144
146
148
150
152
8.8 8.7 8.6 8.5 8.4 8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4
ppm


ppm
128.2
128.4
128.6
128.8
129.0
129.2
129.4
8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5
ppm


ppm
132.2
132.4
132.6
132.8
133.0
7.70 7.65 7.60 7.55 7.50
ppm


MsO
OH
O
N
A^Bz
TFA
12


-70.5
-71.0
-71.5
-72.0
-72.5
-73.0
-73.5
-74.0
-74.5
ppm
1.00
0.81
-71.103
-72.086
MsO
OH
O
N
TFA
ABz
12


BzO
OH
O
N
ABz
TFA
13
4.932
4.906
4.898
4.791
4.771
4.766
4.746
4.681
4.635
4.616
4.609
4.590
4.548
4.527
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3.943
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3.735
8.688
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8.535
8.309
8.130
8.126
8.124
8.115
8.113
8.110
8.107
8.068
8.052
7.717
7.702
7.687
7.609
7.595
7.586
7.571
7.555
7.527
7.513
7.499
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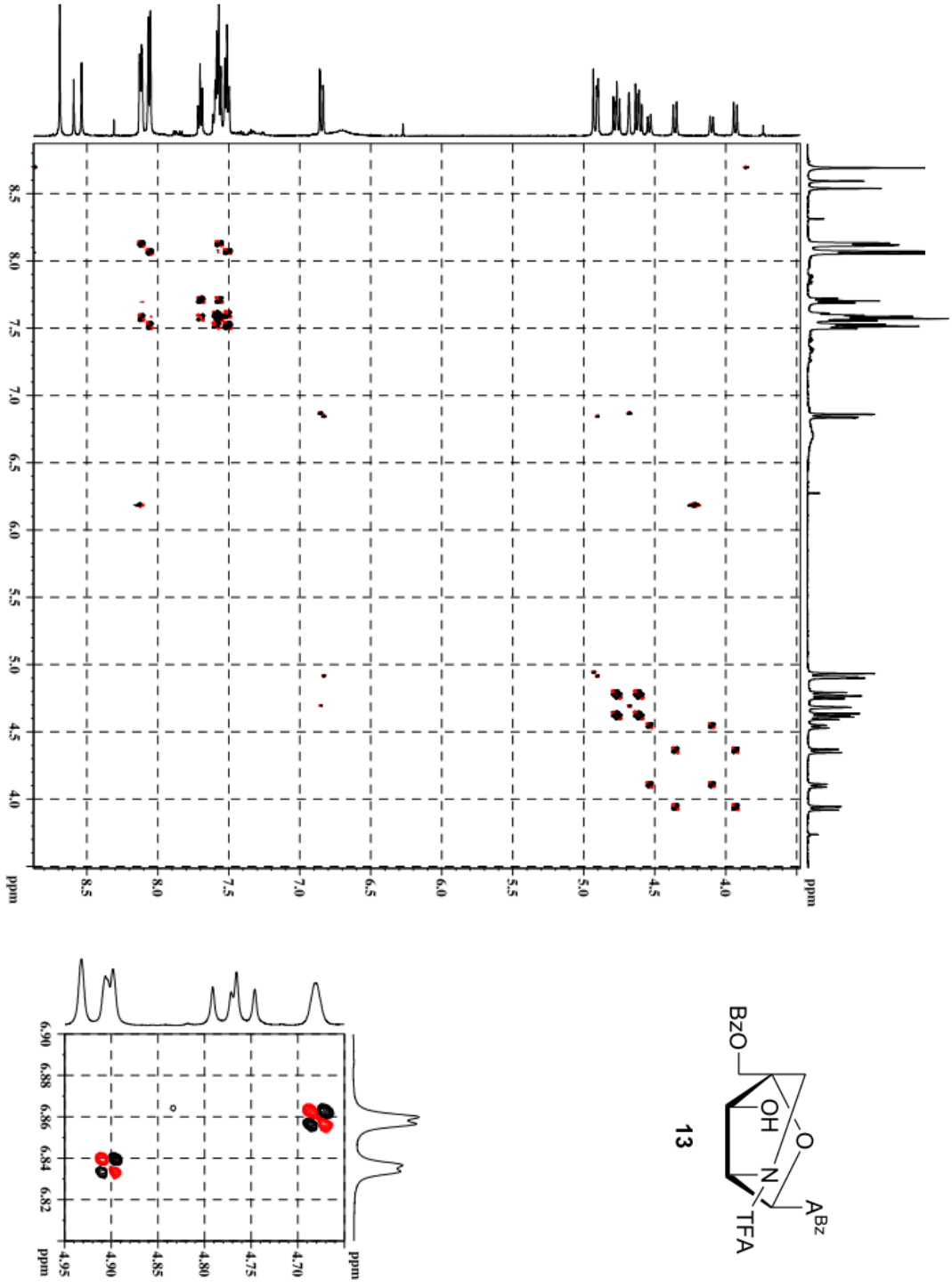
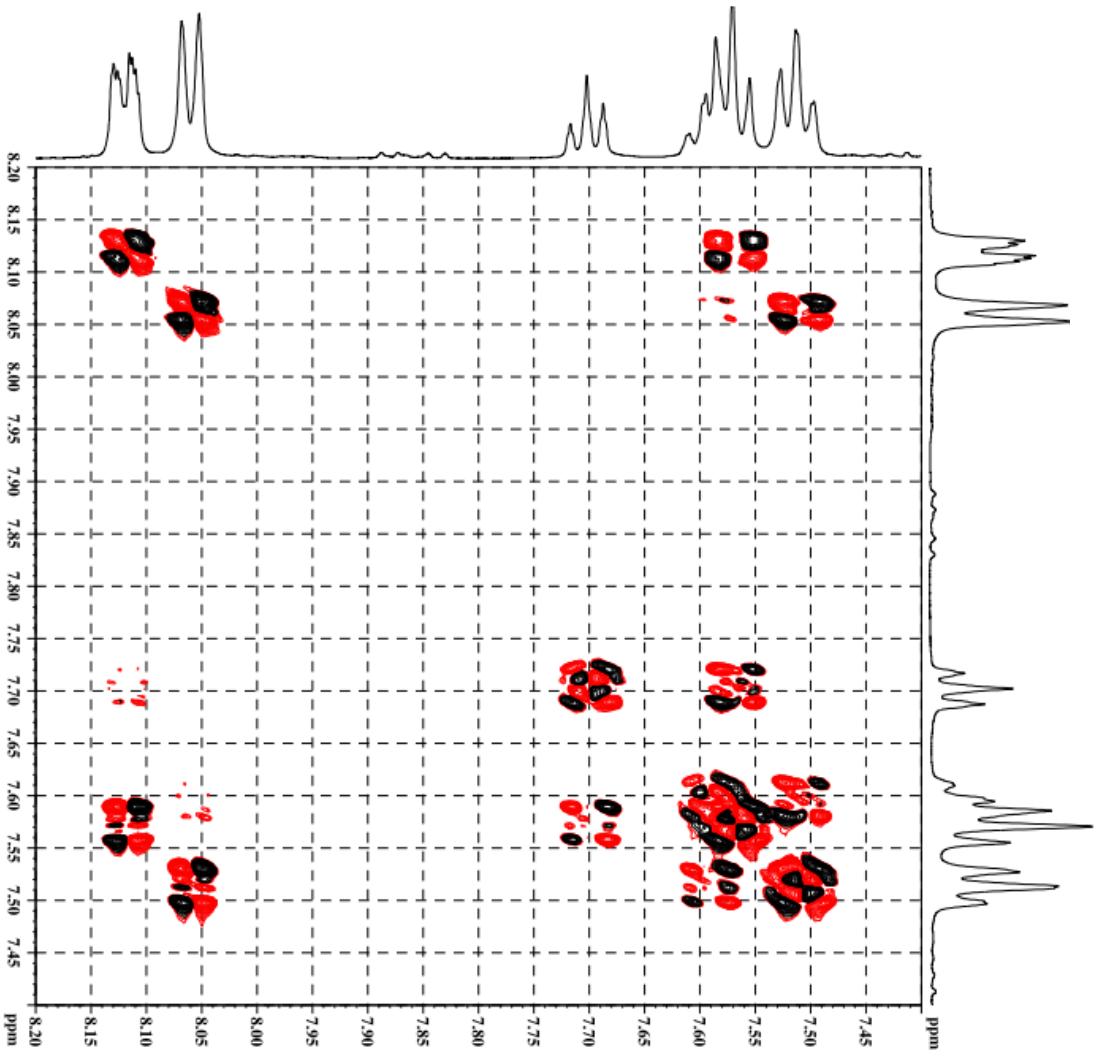
ppm


BzO
OH
O
N
TFA
ABz
13


8.20
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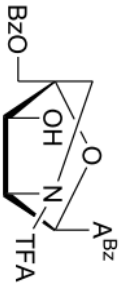
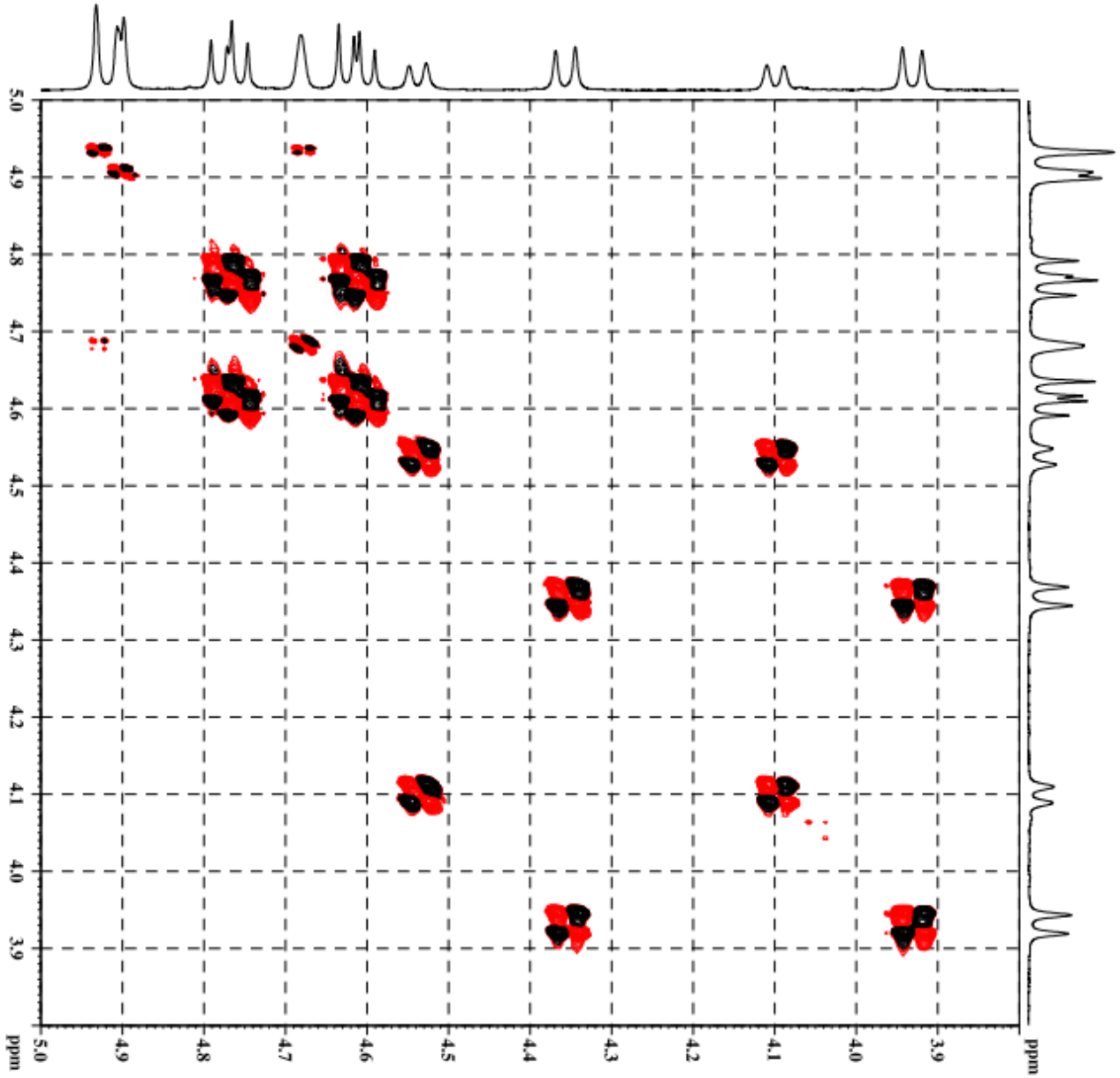
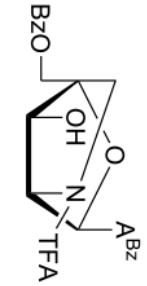
8.10
8.05
8.00
7.95
7.90
7.85
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
ppm


BzO
OH
O
N
TFA
ABz

13





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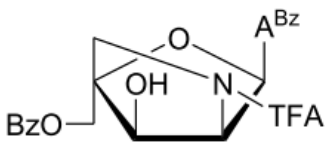
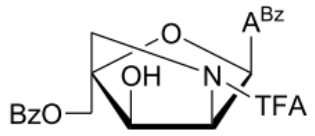
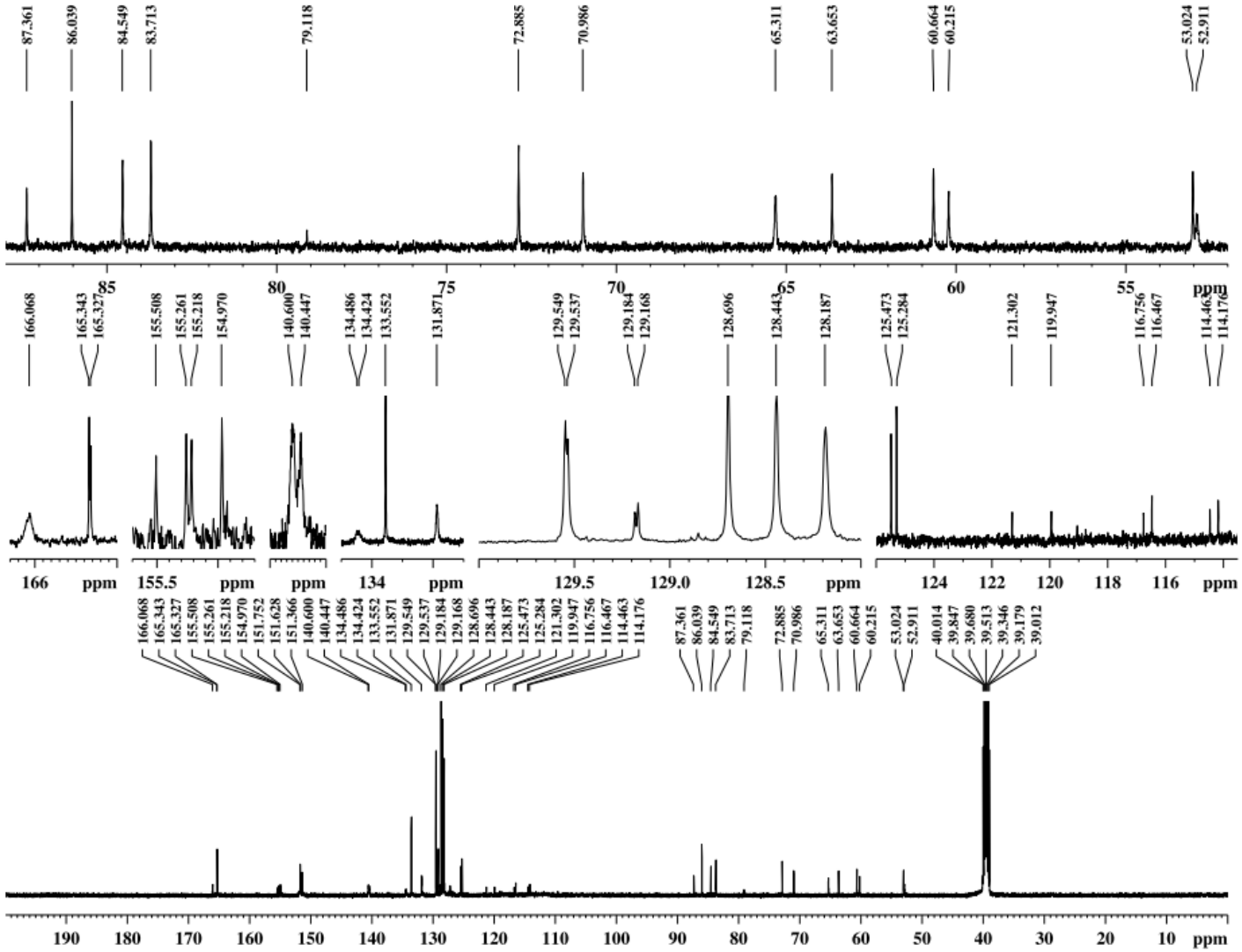


13


O
A^Bz
OH
N
BzO
TFA

13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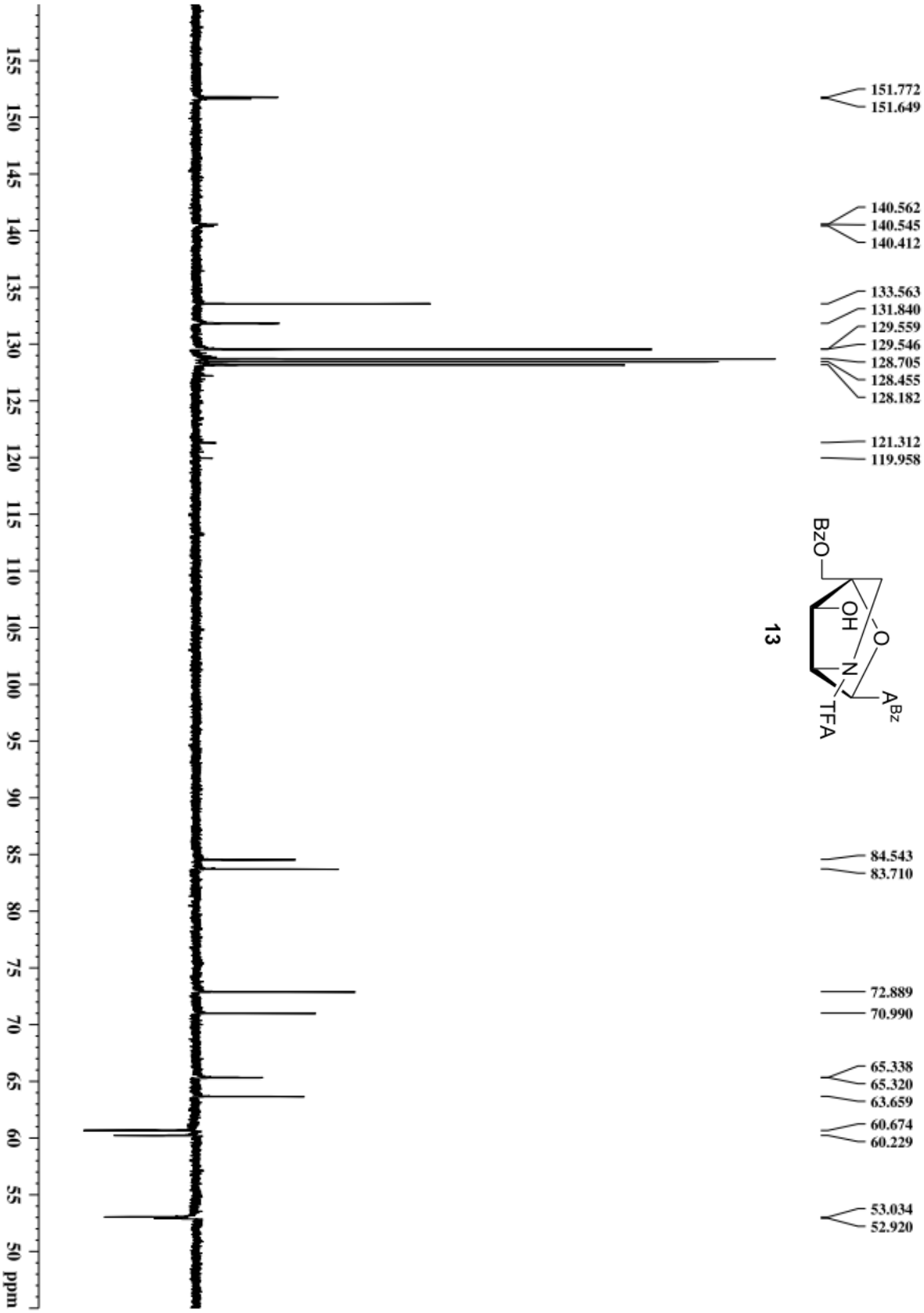
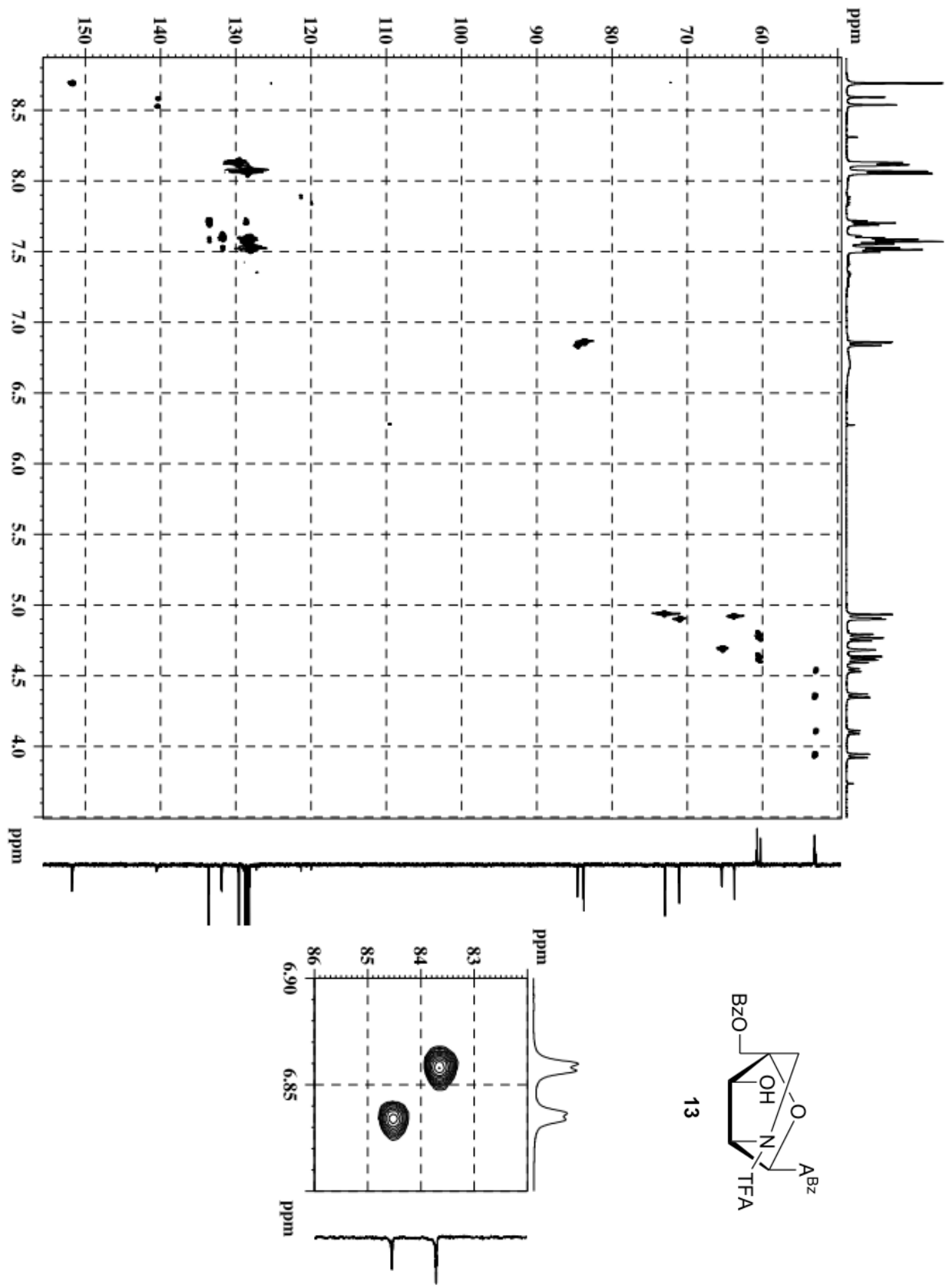
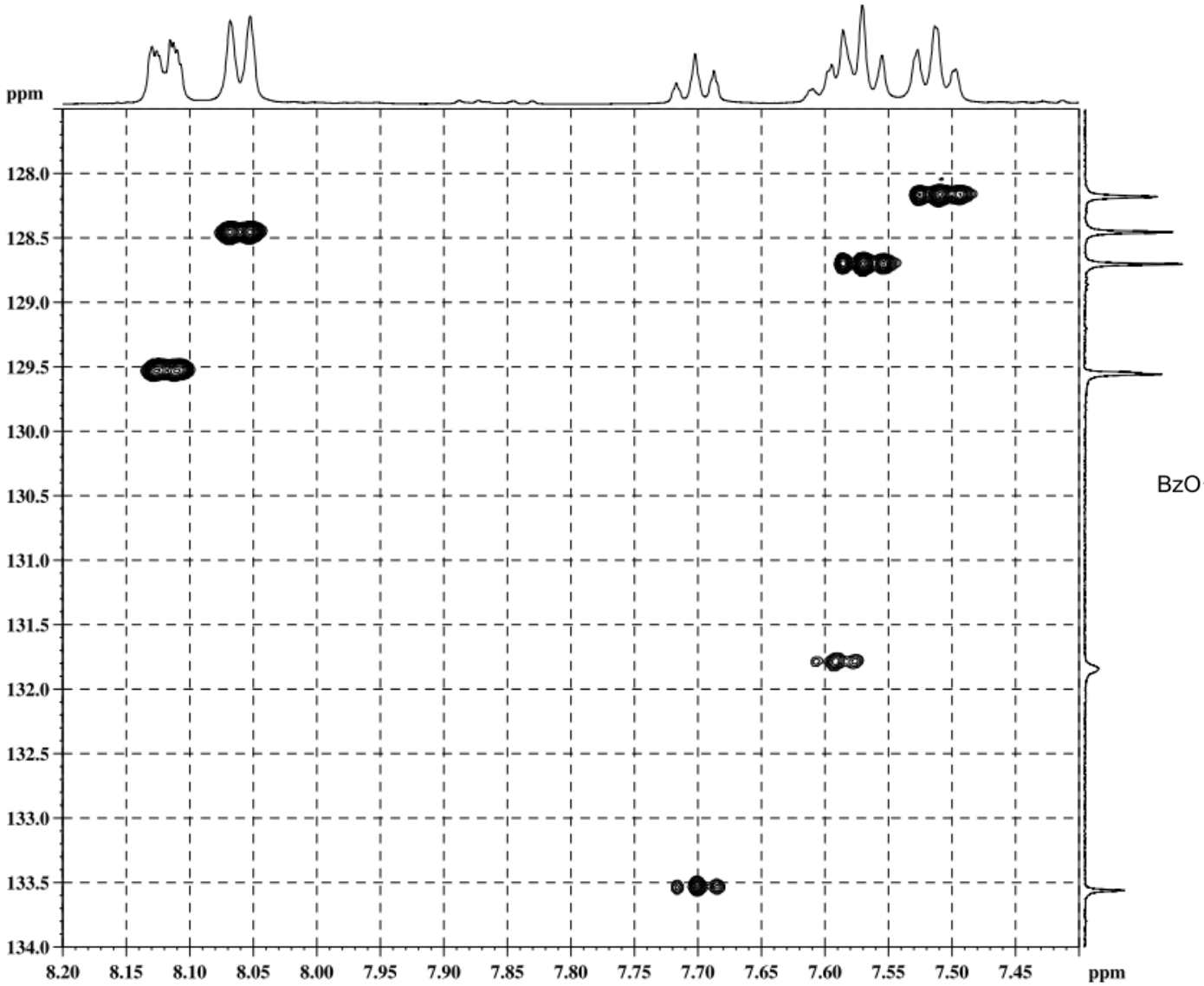
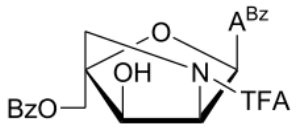
86.039
84.549
83.713
79.118
72.885
70.986
65.311
63.653
60.664
60.215
53.024
52.911
85
80
75
70
65
60
55
ppm
166.068
165.343
165.327
155.508
155.261
155.218
154.970
140.600
140.447
134.486
134.424
133.552
131.871
129.549
129.537
129.184
129.168
128.696
128.443
128.187
125.473
125.284
121.302
119.947
116.756
116.467
114.463
114.176
166
ppm
155.5
ppm
ppm
134
ppm
129.5
129.0
128.5
ppm
124
122
120
118
116
ppm
166.068
165.343
165.327
155.508
155.261
155.218
154.970
151.752
151.628
151.366
140.600
140.447
134.486
134.424
133.552
131.871
129.549
129.537
129.184
129.168
128.696
128.443
128.187
125.473
125.284
121.302
119.947
116.756
116.467
114.463
114.176
87.361
86.039
84.549
83.713
79.118
72.885
70.986
65.311
63.653
60.664
60.215
53.024
52.911
40.014
39.847
39.680
39.513
39.346
39.179
39.012
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
ppm

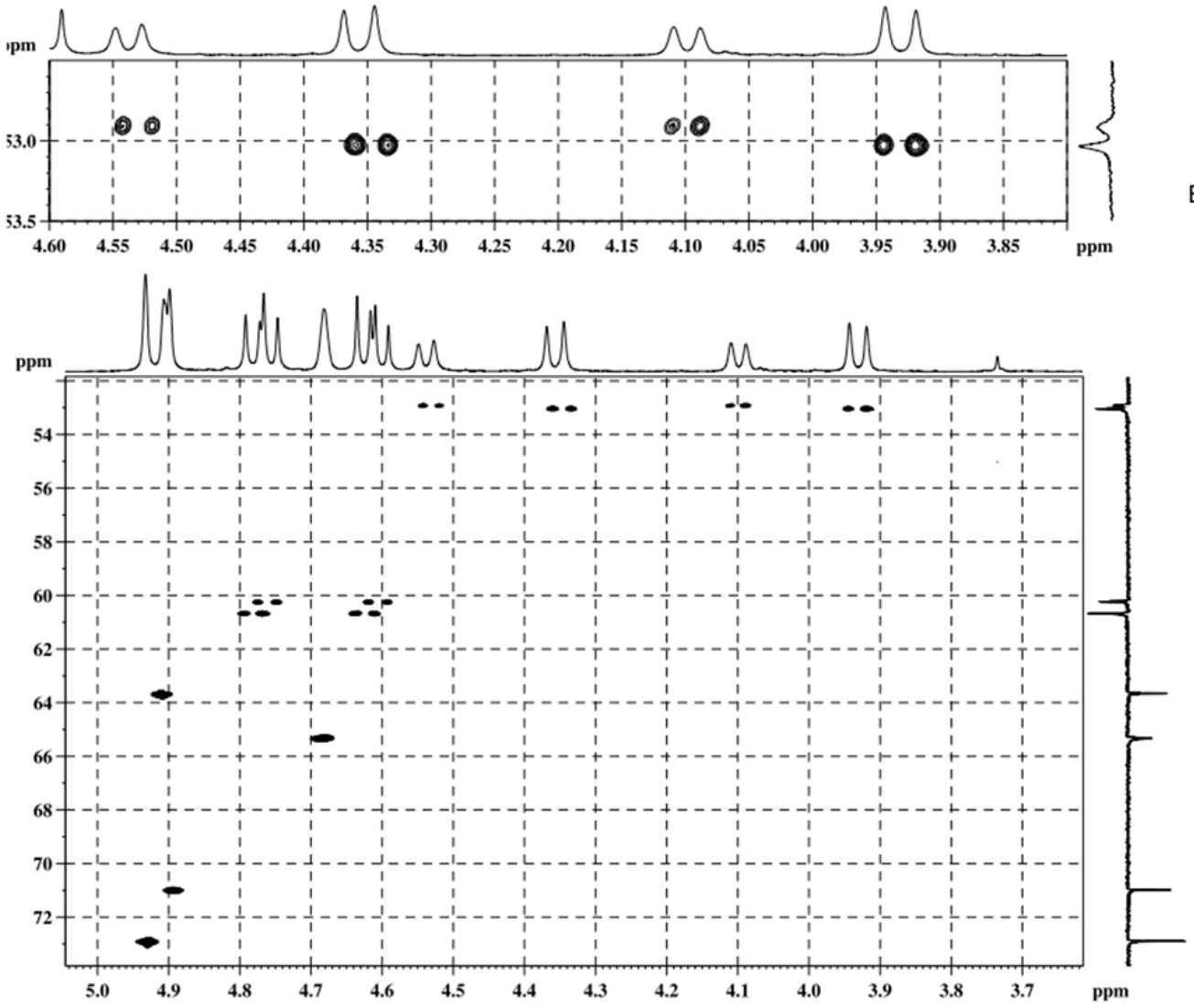

155 150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 ppm
151.772
151.649
140.562
140.545
140.412
133.563
131.840
129.559
129.546
128.705
128.455
128.182
121.312
119.958
84.543
83.710
72.889
70.990
65.338
65.320
63.659
60.674
60.229
53.034
52.920
BzO
OH
O
N
ABz
TFA
13

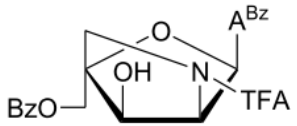

ppm
60
70
80
90
100
110
120
130
140
150
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm
BzO
OH
O
N
TFA
ABz
13
ppm
83
84
85
86
6.90
6.85
ppm


ppm
128.0
128.5
129.0
129.5
130.0
130.5
131.0
131.5
132.0
132.5
133.0
133.5
134.0
8.20
8.15
8.10
8.05
8.00
7.95
7.90
7.85
7.80
7.75
7.70
7.65
7.60
7.55
7.50
7.45
ppm

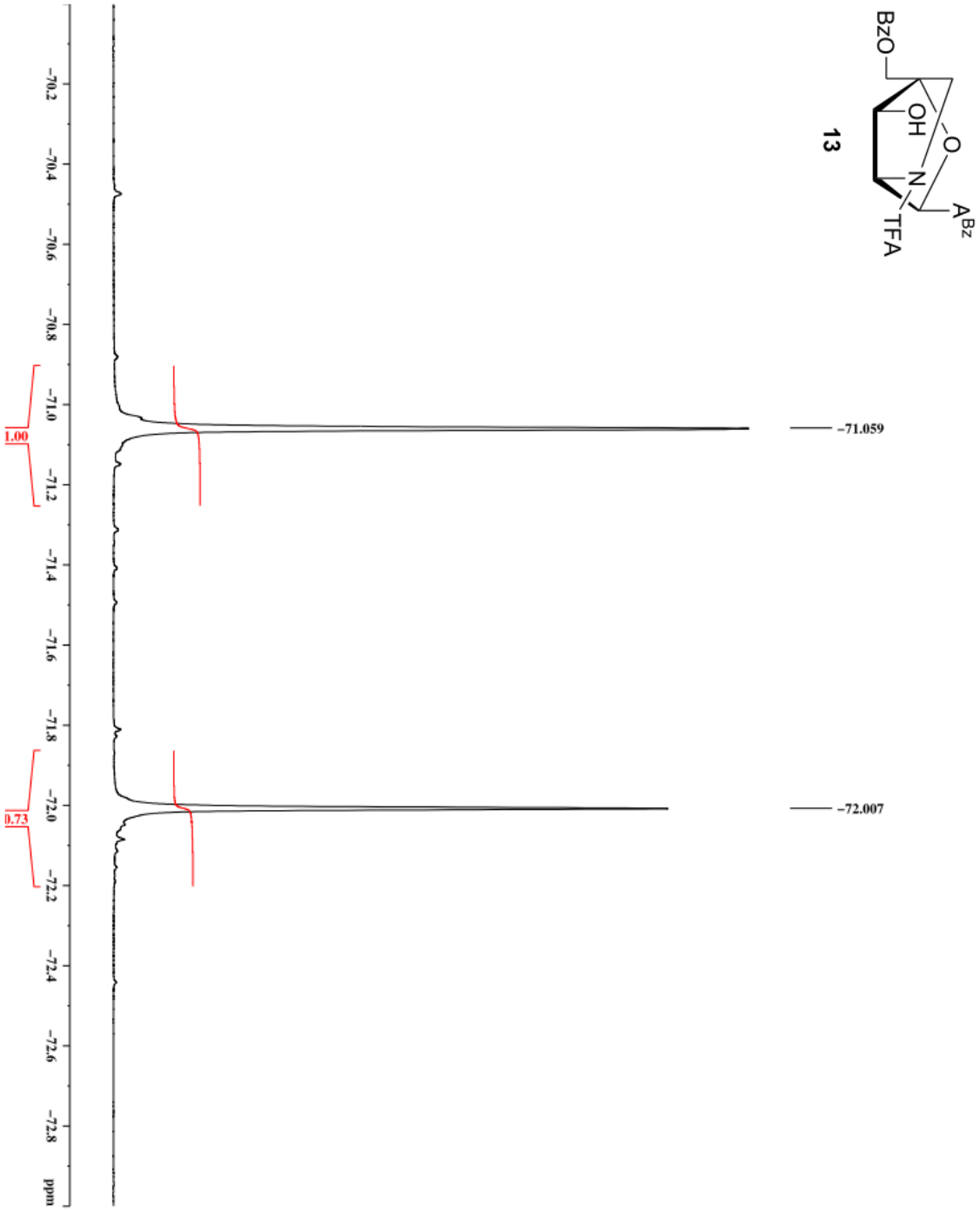

O
A^Bz
OH
N
BzO
TFA

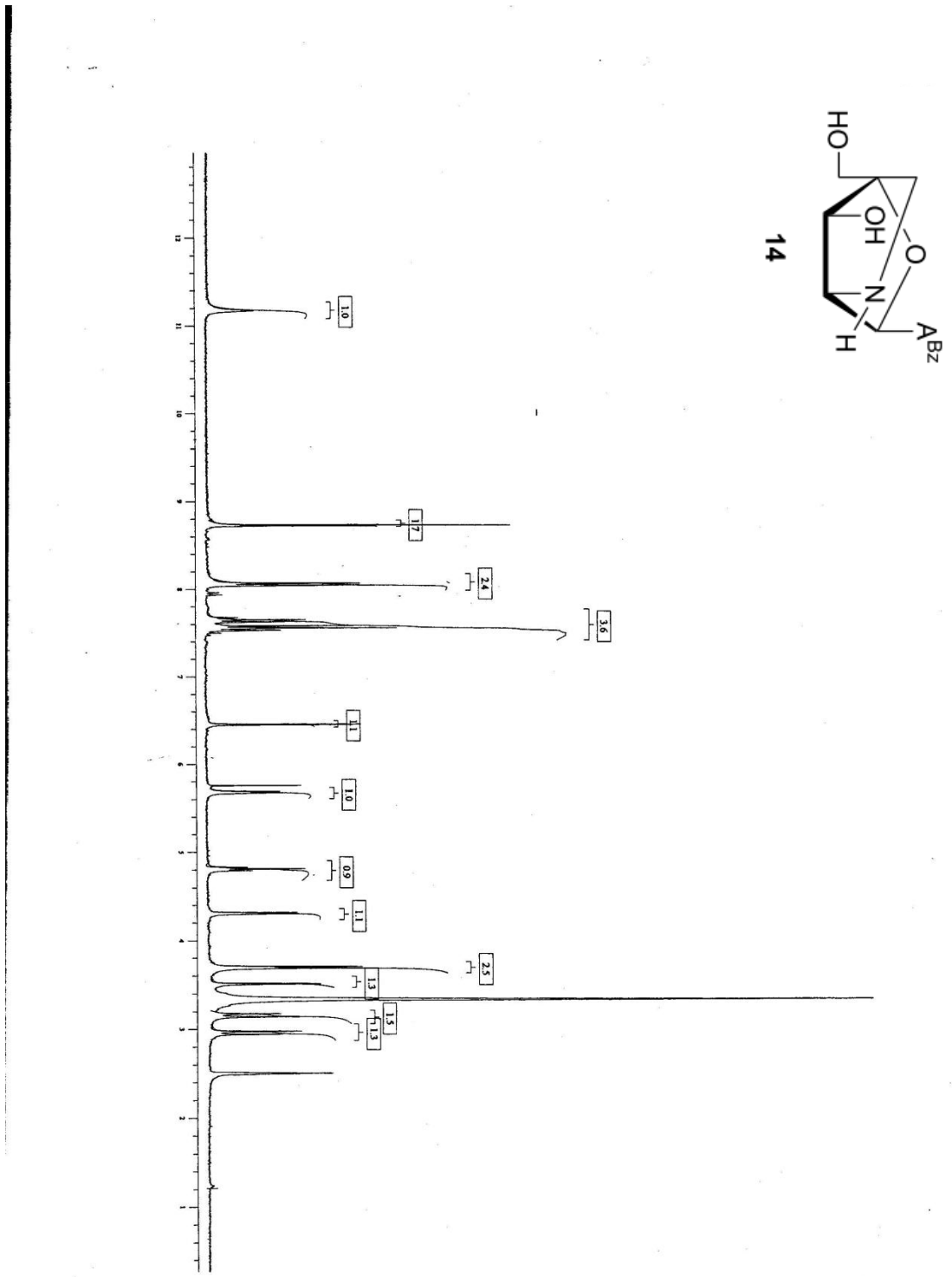
13


ppm
53.0
53.5
4.60 4.55 4.50 4.45 4.40 4.35 4.30 4.25 4.20 4.15 4.10 4.05 4.00 3.95 3.90 3.85 ppm
ppm
54
56
58
60
62
64
66
68
70
72
5.0 4.9 4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 ppm

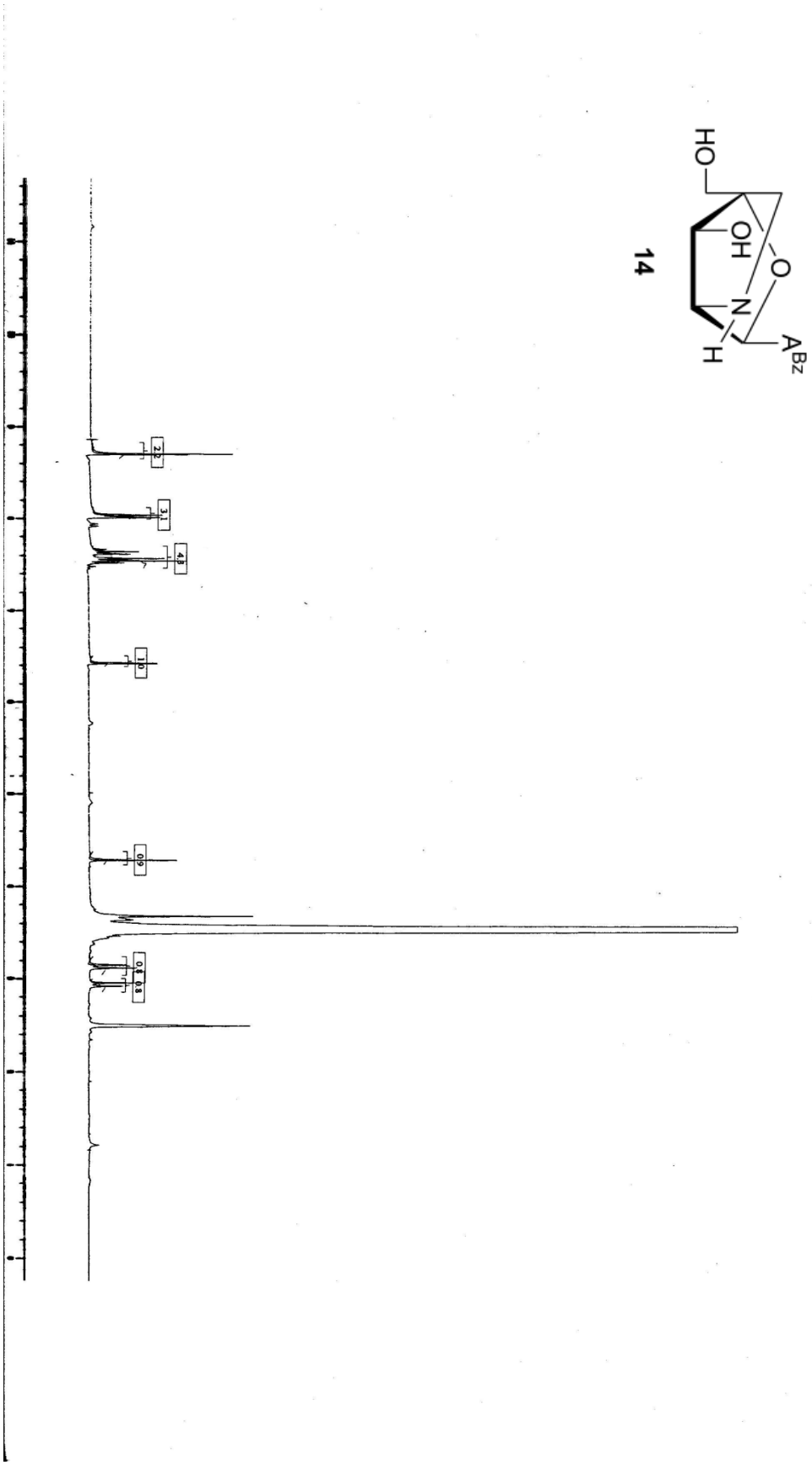

O
A^Bz
OH
N
BzO
TFA

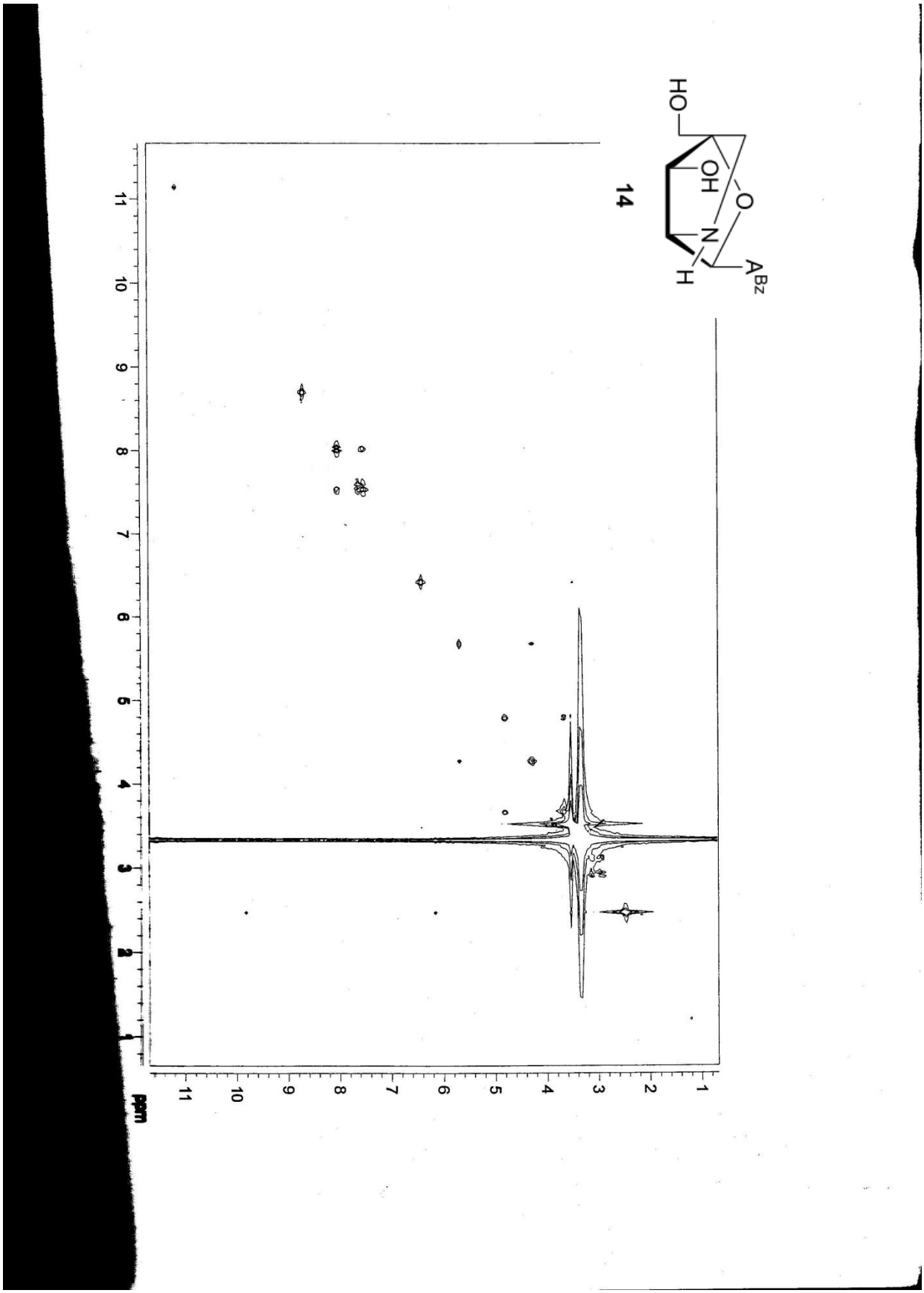
13

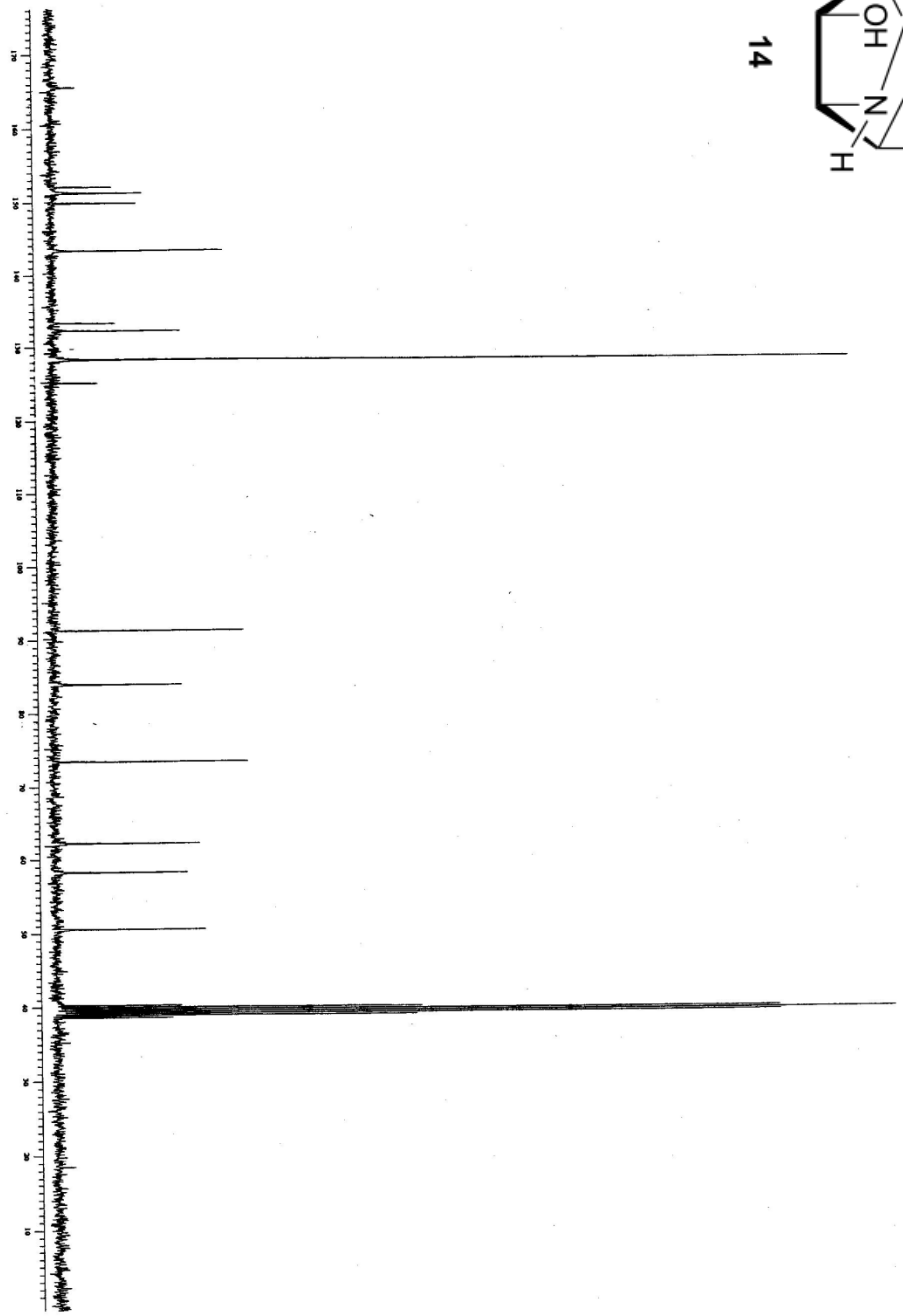

BzO
OH
O
N
TFA
ABz
13
-71.059
-72.007
1.00
0.73
-70.2
-70.4
-70.6
-70.8
-71.0
-71.2
-71.4
-71.6
-71.8
-72.0
-72.2
-72.4
-72.6
-72.8
ppm

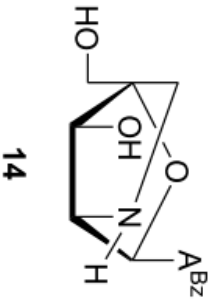

HO
OH
O
N
H
ABz
14
1.0
1.7
2.4
3.6
1.1
1.0
0.9
1.1
2.5
1.3
1.5
1.3

D_2O exchange

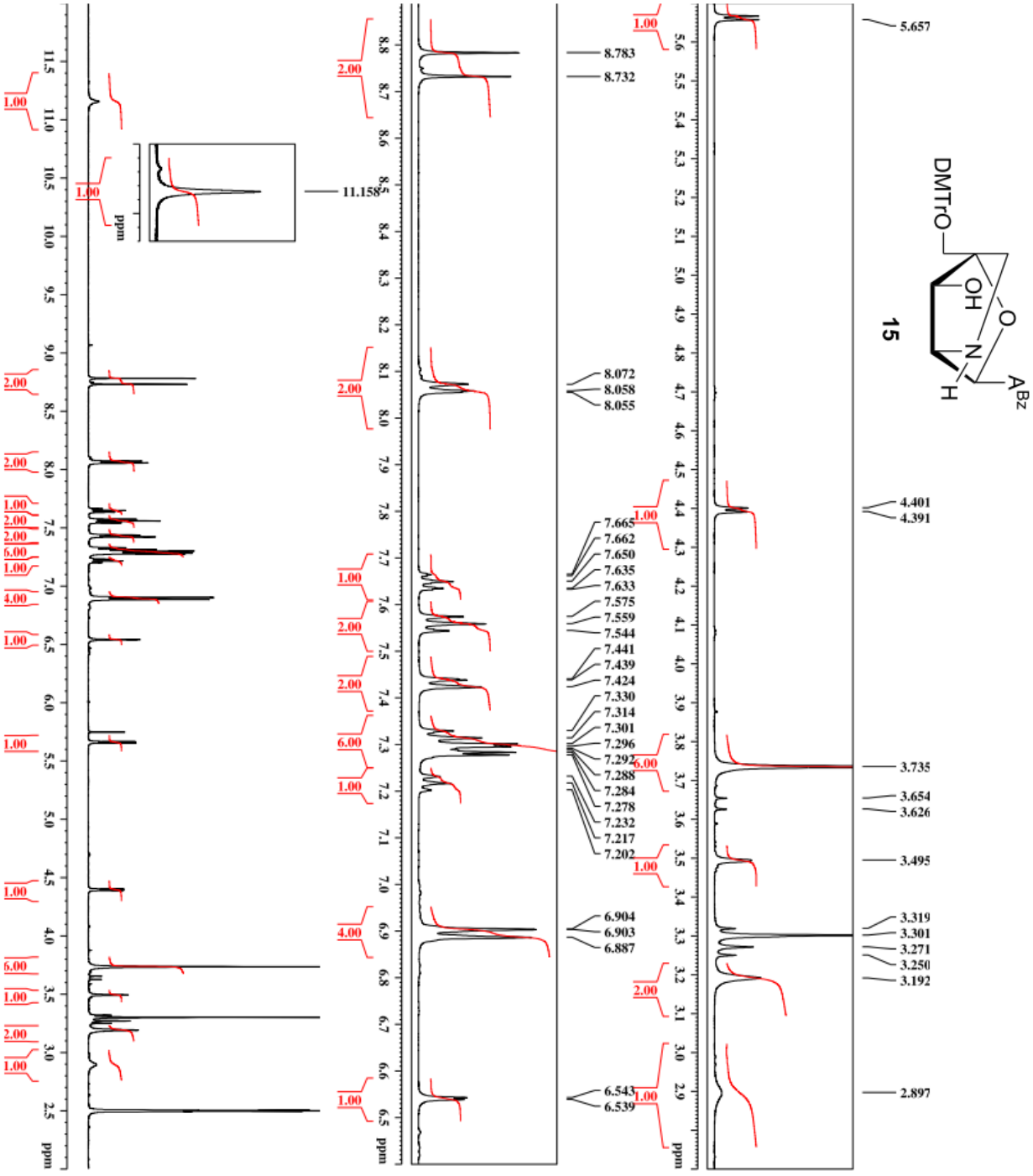


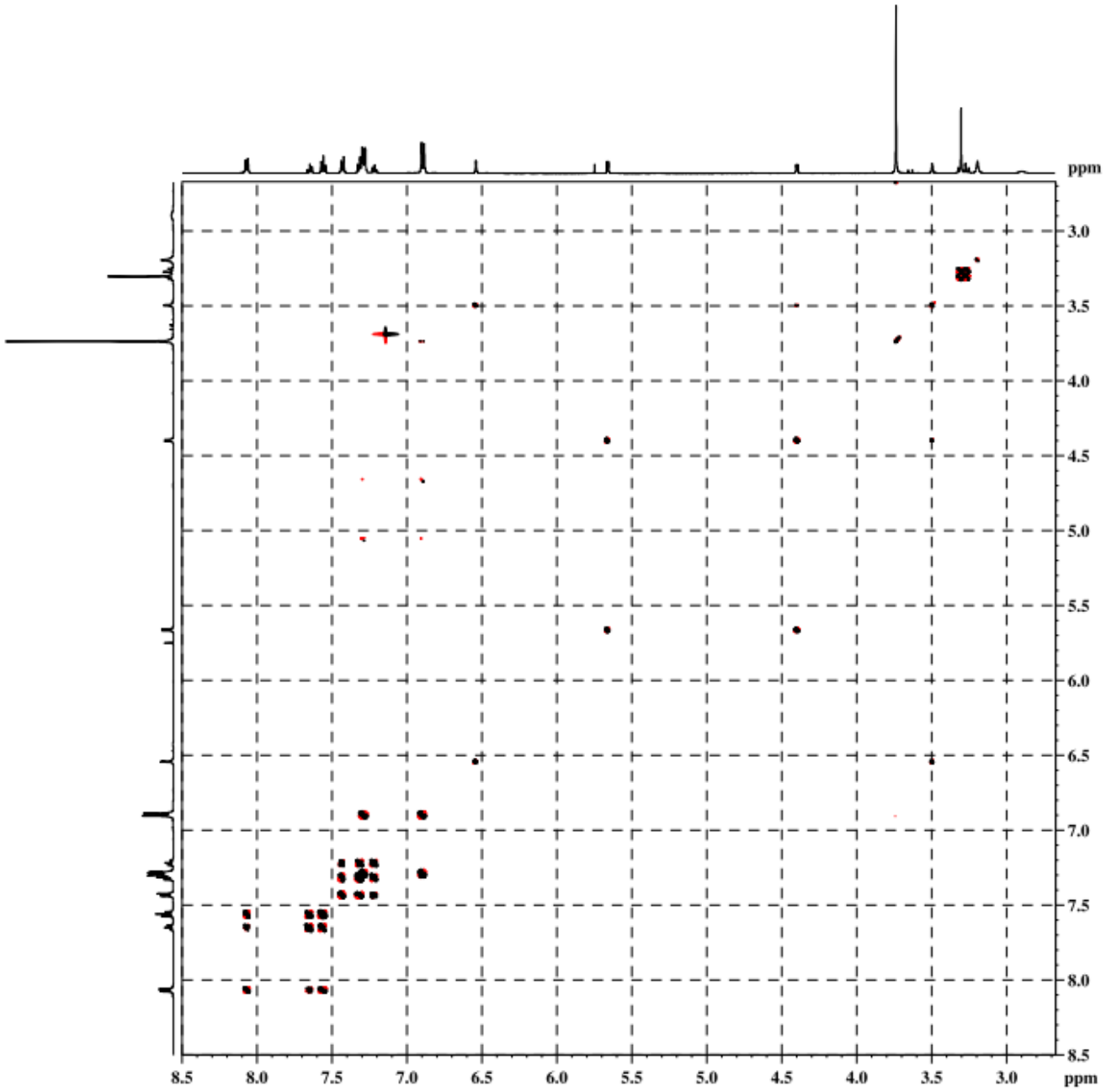

HO
OH
O
N
H
ABz
14
ppm

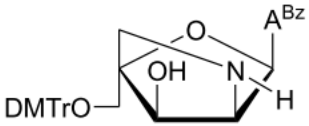




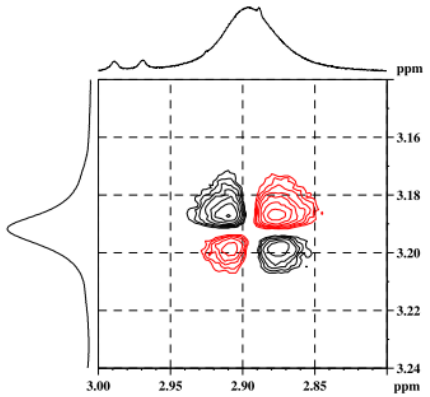
14

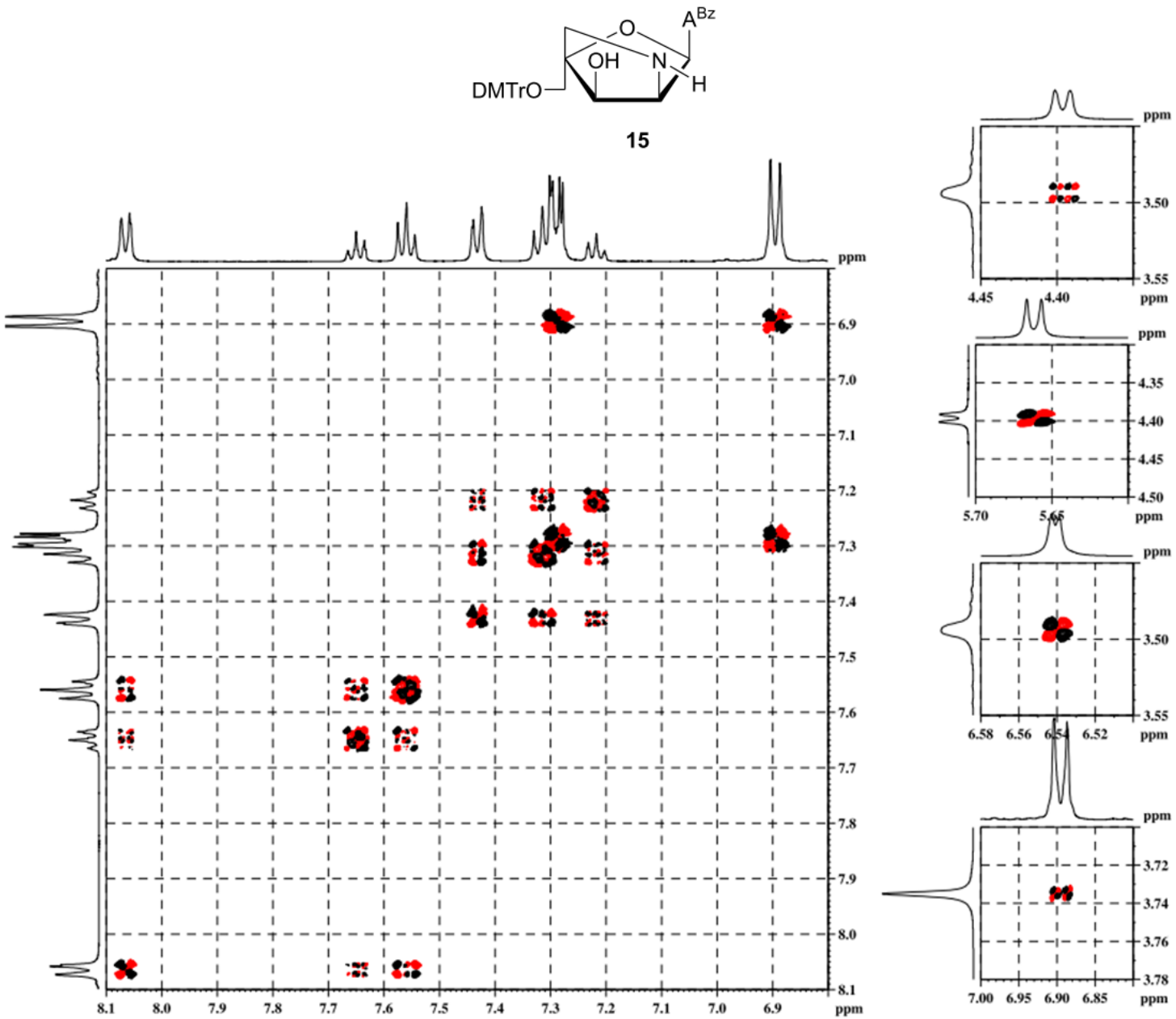

DMTrO
OH
O
N
H
ABz
15
5.657
4.401
4.391
3.735
3.654
3.626
3.495
3.319
3.301
3.271
3.250
3.192
2.897
8.783
8.732
8.072
8.058
8.055
7.665
7.662
7.650
7.635
7.633
7.575
7.559
7.544
7.441
7.439
7.424
7.330
7.314
7.301
7.296
7.292
7.288
7.284
7.278
7.232
7.217
7.202
6.904
6.903
6.887
6.543
6.539
11.158
ppm


ppm
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm


O
A^Bz
OH
N
H
DMTrO

15


ppm
3.16
3.18
3.20
3.22
3.24
3.00
2.95
2.90
2.85
ppm


O
A^Bz
OH
N
H
DMTrO
15
ppm
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
4.45
4.40
3.50
3.55
5.70
5.65
4.35
4.40
4.45
4.50
6.58
6.56
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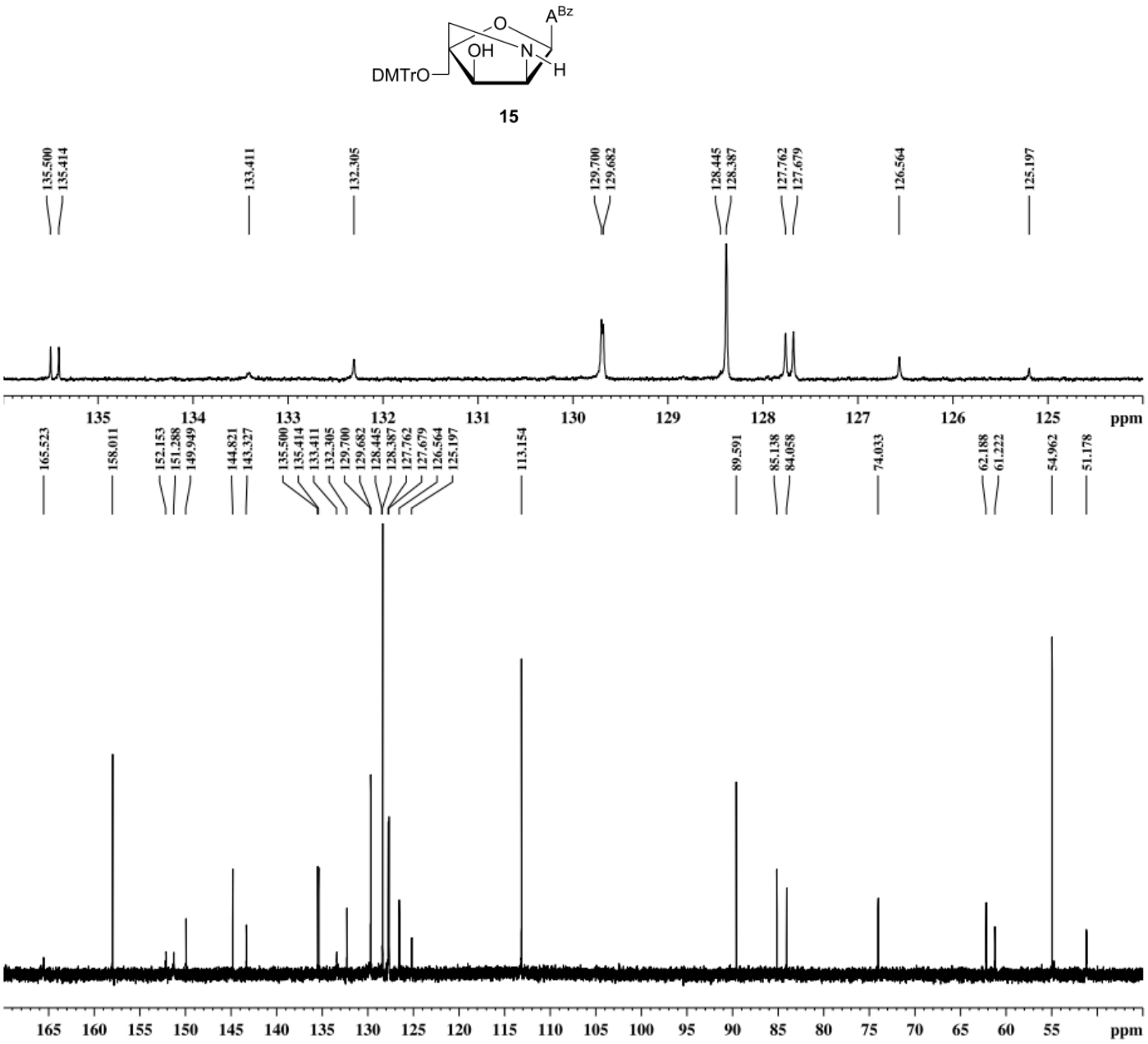
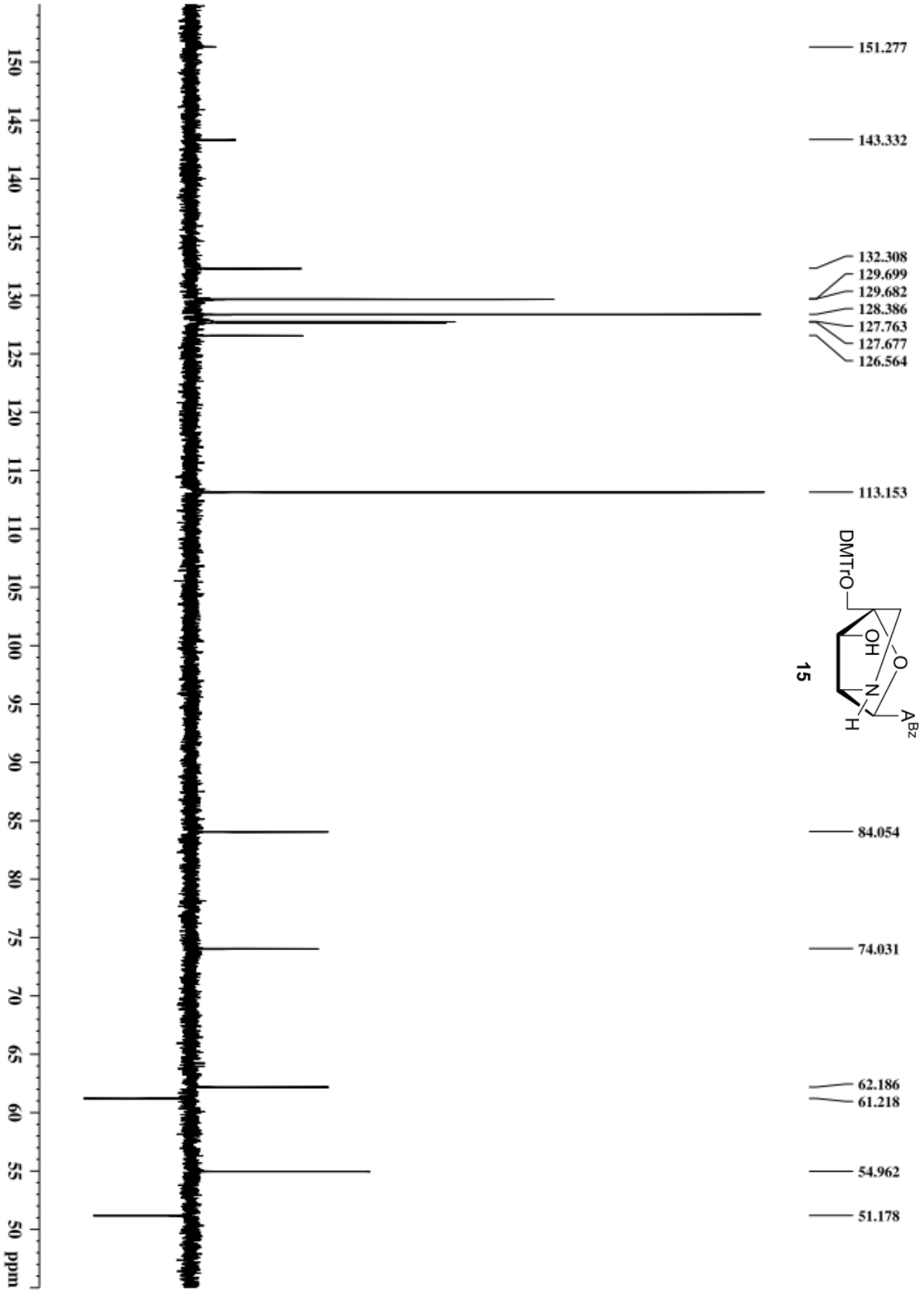
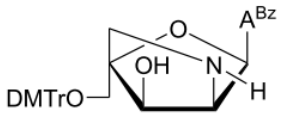
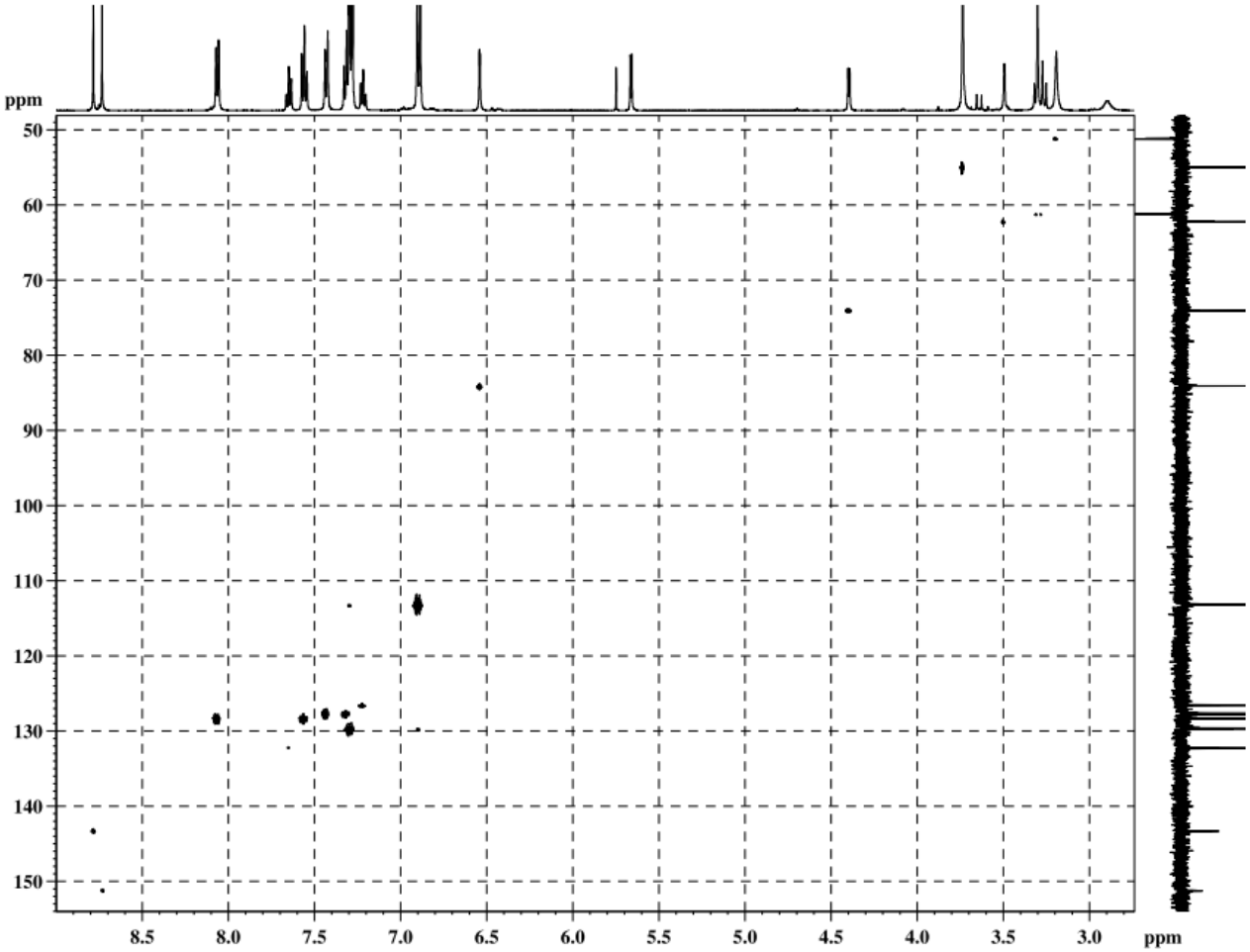
3.76
3.78
7.00
6.95
6.90
6.85

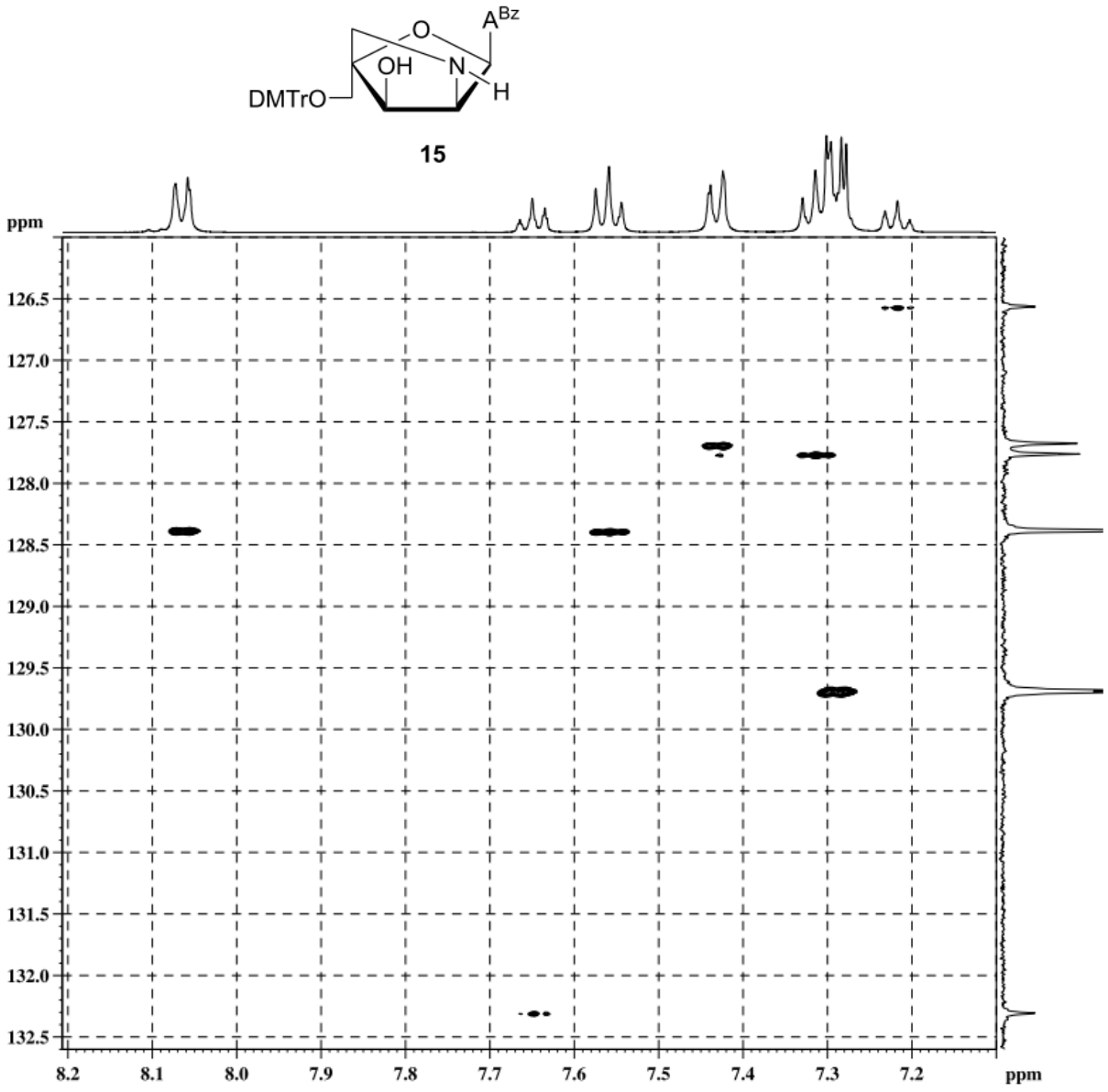

A^Bz
O
OH
N
H
DMTrO
15
135.500
135.414
133.411
132.305
129.700
129.682
128.445
128.387
127.762
127.679
126.564
125.197
135
134
133
132
131
130
129
128
127
126
125
ppm
165.523
158.011
152.153
151.288
149.949
144.821
143.327
135.500
135.414
133.411
132.305
129.700
129.682
128.445
128.387
127.762
127.679
126.564
125.197
113.154
89.591
85.138
84.058
74.033
62.188
61.222
54.962
51.178
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
ppm


151.277
143.332
132.308
129.699
129.682
128.386
127.763
127.677
126.564
113.153
84.054
74.031
62.186
61.218
54.962
51.178
150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 50 ppm
DMTrO
OH
O
N
H
ABz
15

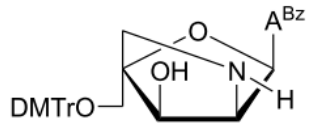

O
A^Bz
OH
N
H
DMTrO

15

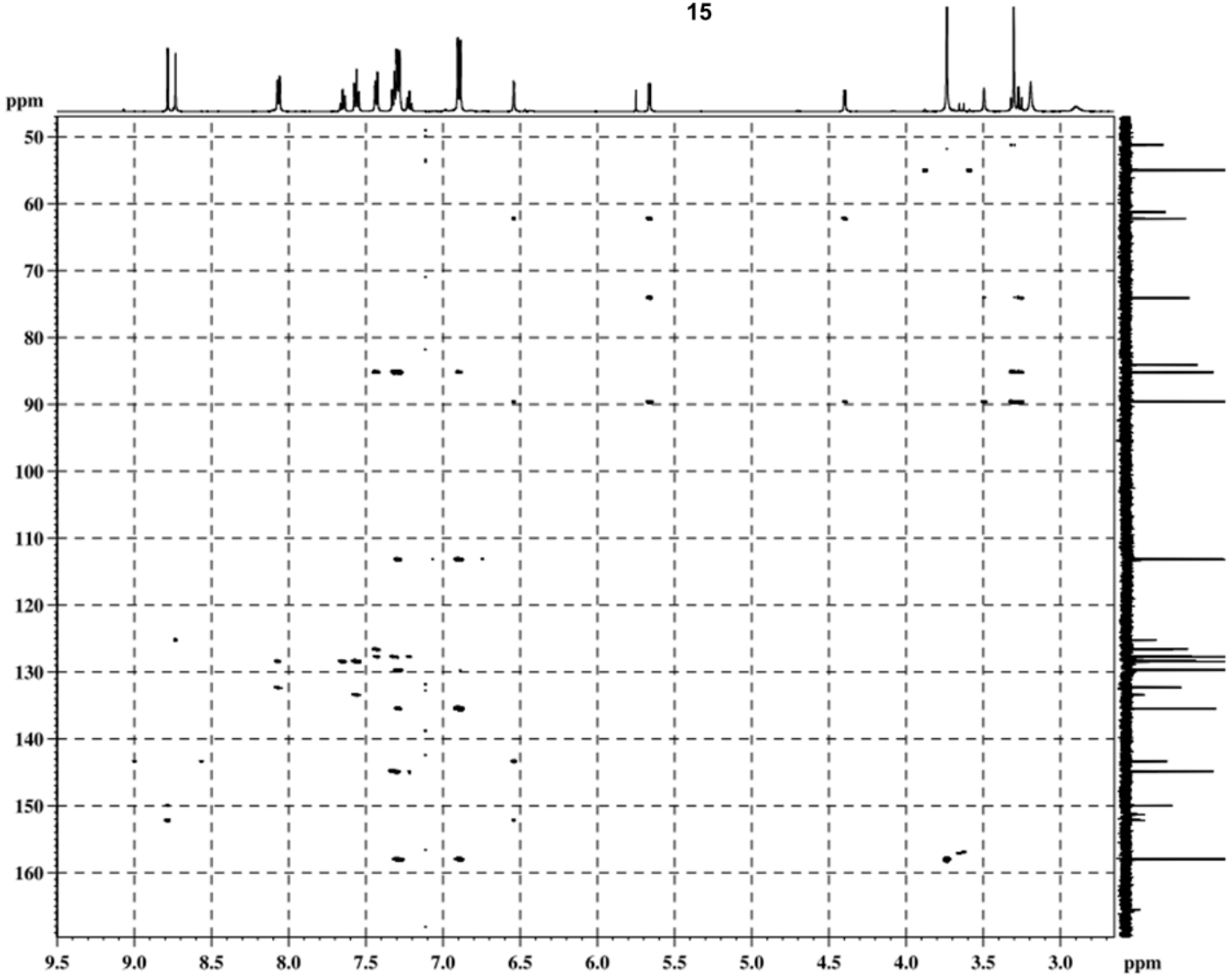

ppm
50
60
70
80
90
100
110
120
130
140
150
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm

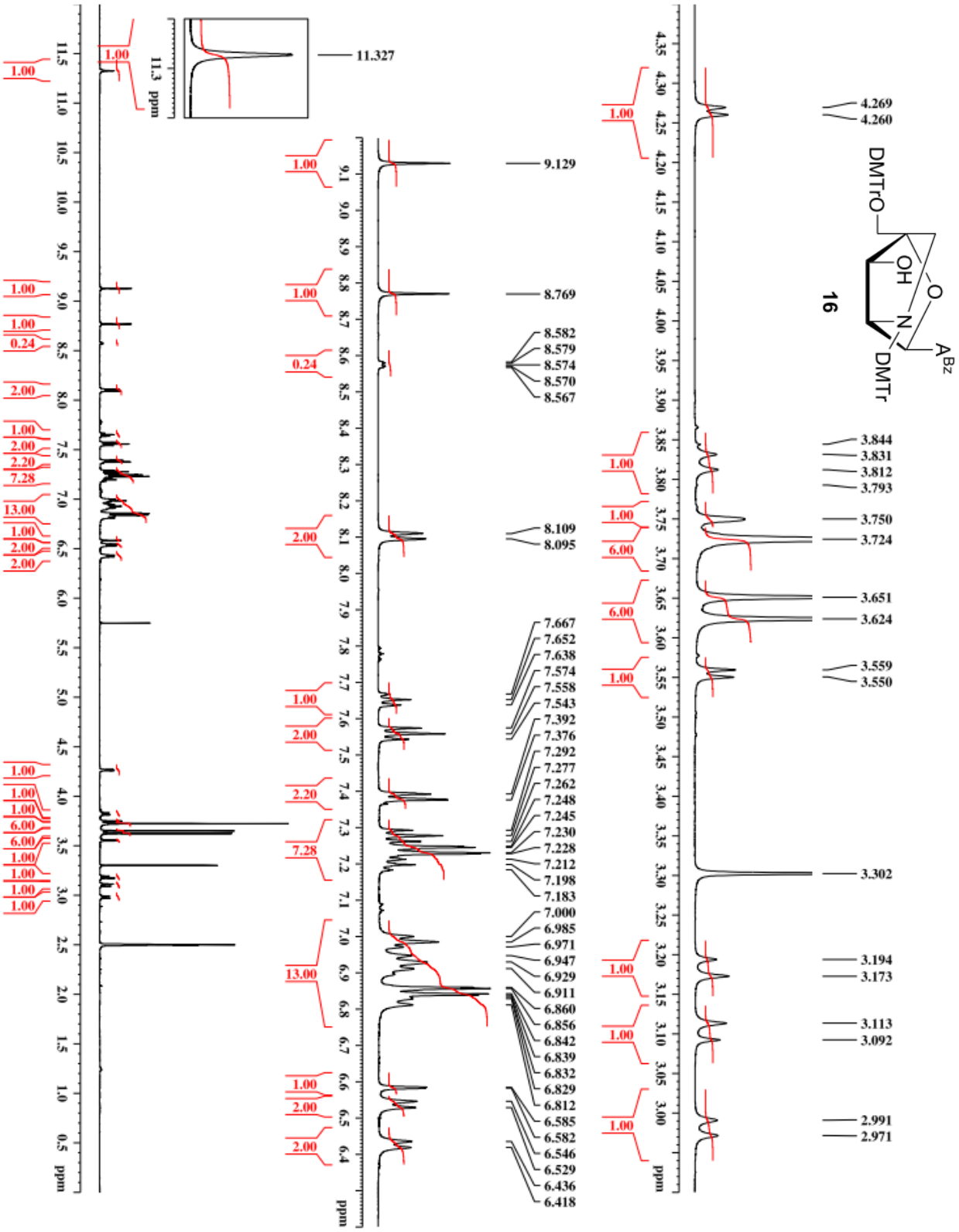

O
A^Bz
OH
N
H
DMTrO
ppm
126.5
127.0
127.5
128.0
128.5
129.0
129.5
130.0
130.5
131.0
131.5
132.0
132.5
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
ppm

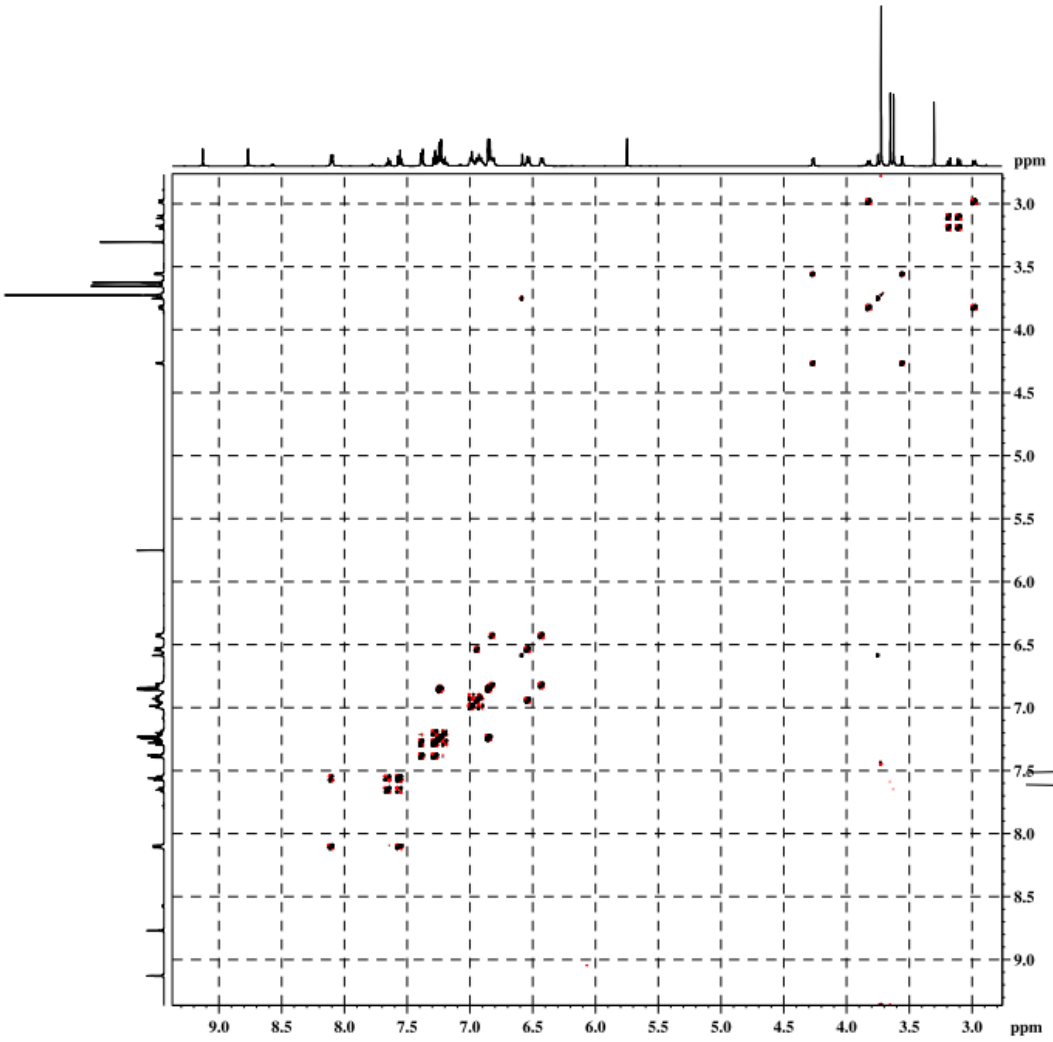
15


O
A^Bz
OH
N
H
DMTrO

15


ppm
50
60
70
80
90
100
110
120
130
140
150
160
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
ppm


DMTrO
OH
O
N
ABz
DMTr
16
4.269
4.260
3.844
3.831
3.812
3.793
3.750
3.724
3.651
3.624
3.559
3.550
3.302
3.194
3.173
3.113
3.092
2.991
2.971
9.129
8.769
8.582
8.579
8.574
8.570
8.567
8.109
8.095
7.667
7.652
7.638
7.574
7.558
7.543
7.392
7.376
7.292
7.277
7.262
7.248
7.245
7.230
7.228
7.212
7.198
7.183
7.000
6.985
6.971
6.947
6.929
6.911
6.860
6.856
6.842
6.839
6.832
6.829
6.812
6.585
6.582
6.546
6.529
6.436
6.418
11.327
11.3 ppm
ppm


ppm
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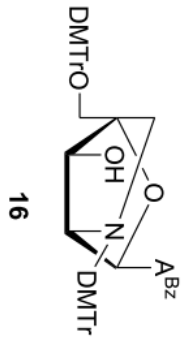
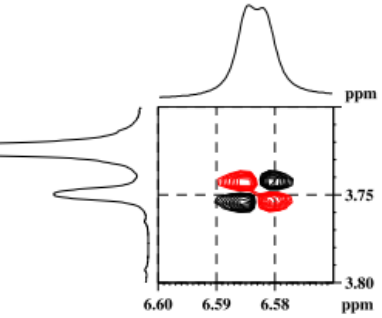
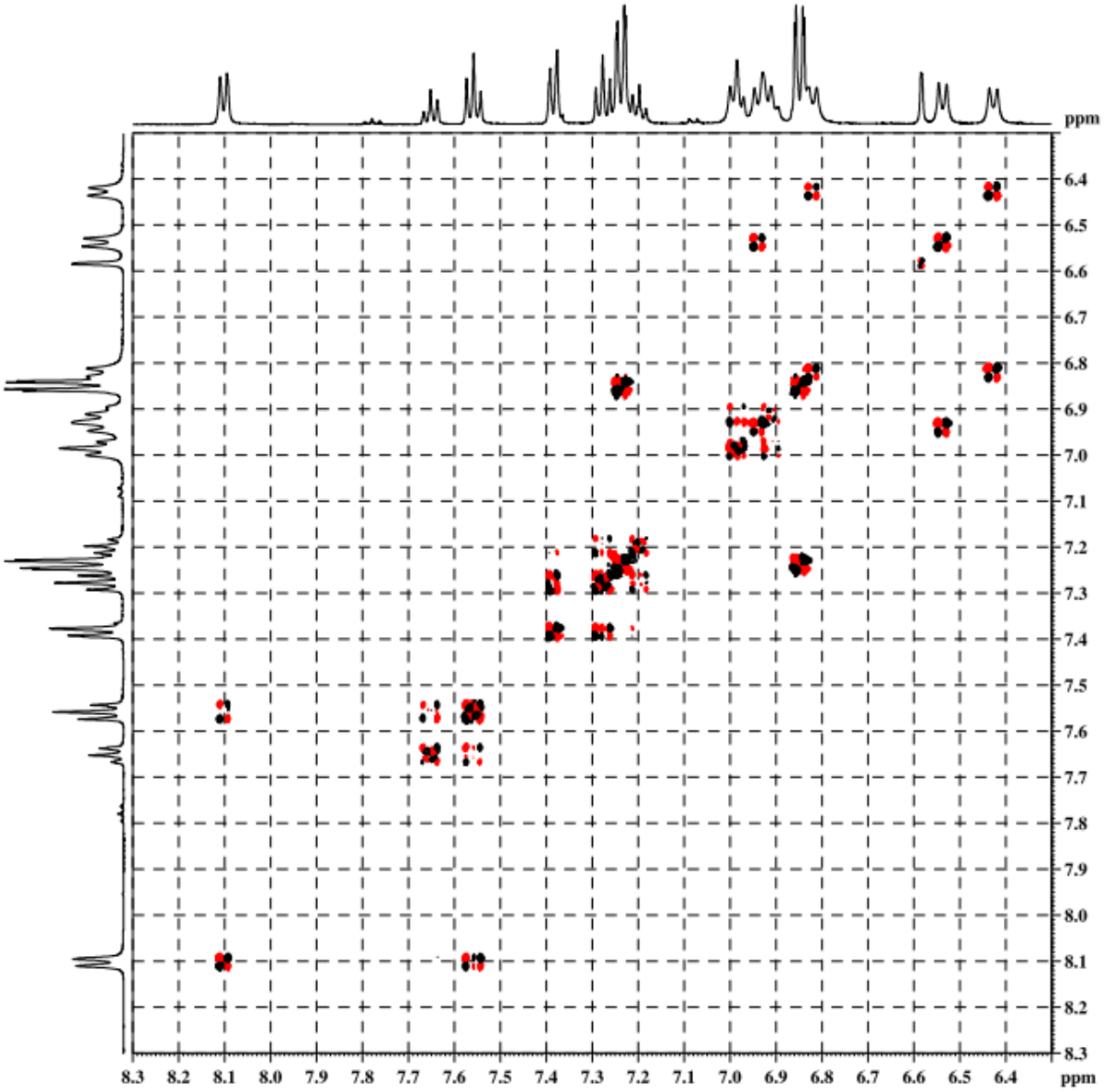
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm

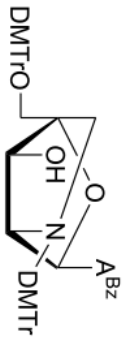

DMTrO
OH
O
N
ABz
DMTr

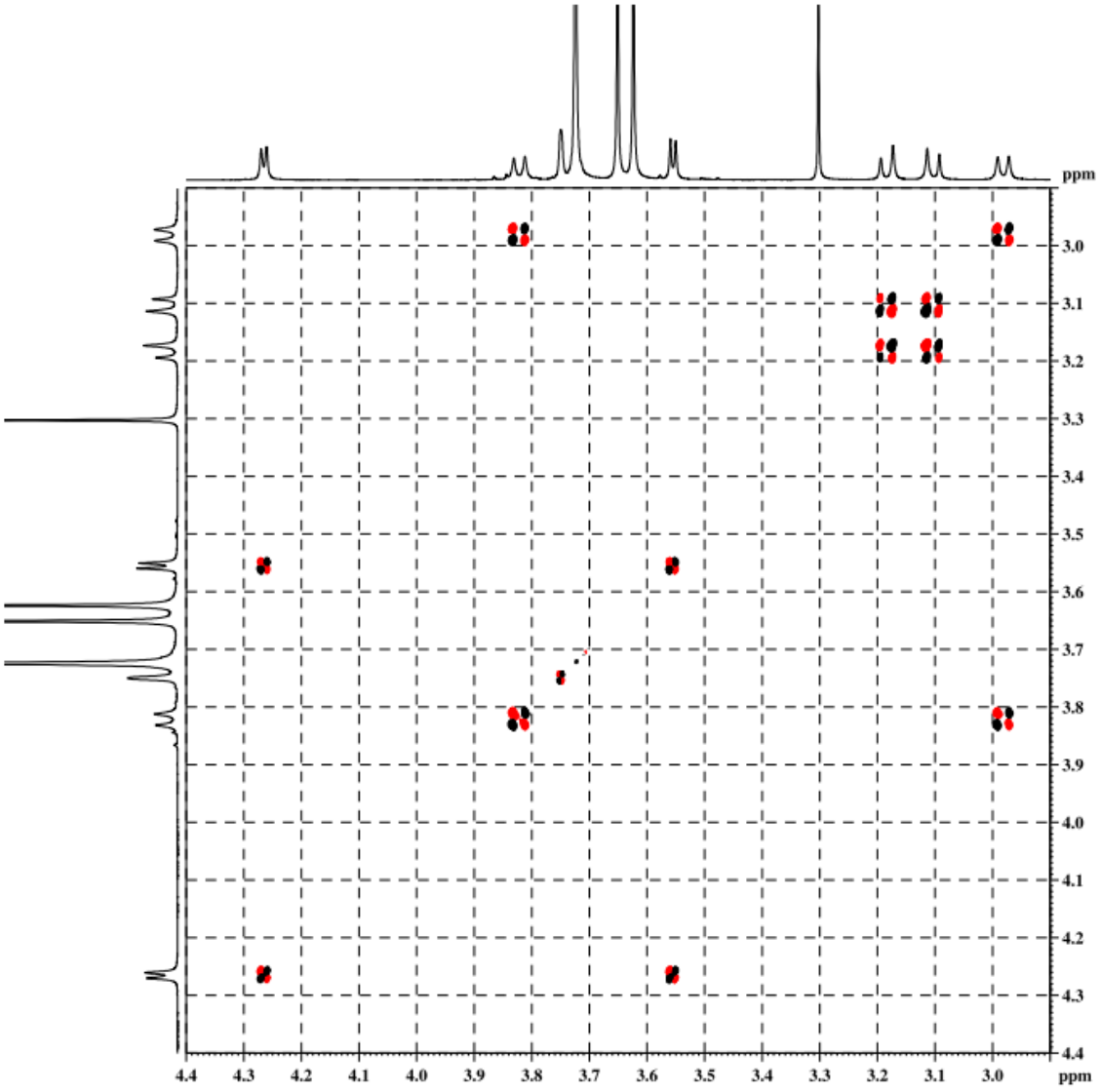
16

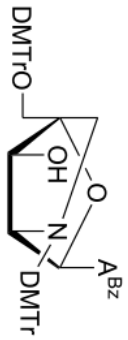

ppm
3.75
3.80
6.60
6.59
6.58
ppm


ppm
6.4
6.5
6.6
6.7
6.8
6.9
7.0
7.1
7.2
7.3
7.4
7.5
7.6
7.7
7.8
7.9
8.0
8.1
8.2
8.3
8.3 8.2 8.1 8.0 7.9 7.8 7.7 7.6 7.5 7.4 7.3 7.2 7.1 7.0 6.9 6.8 6.7 6.6 6.5 6.4 ppm

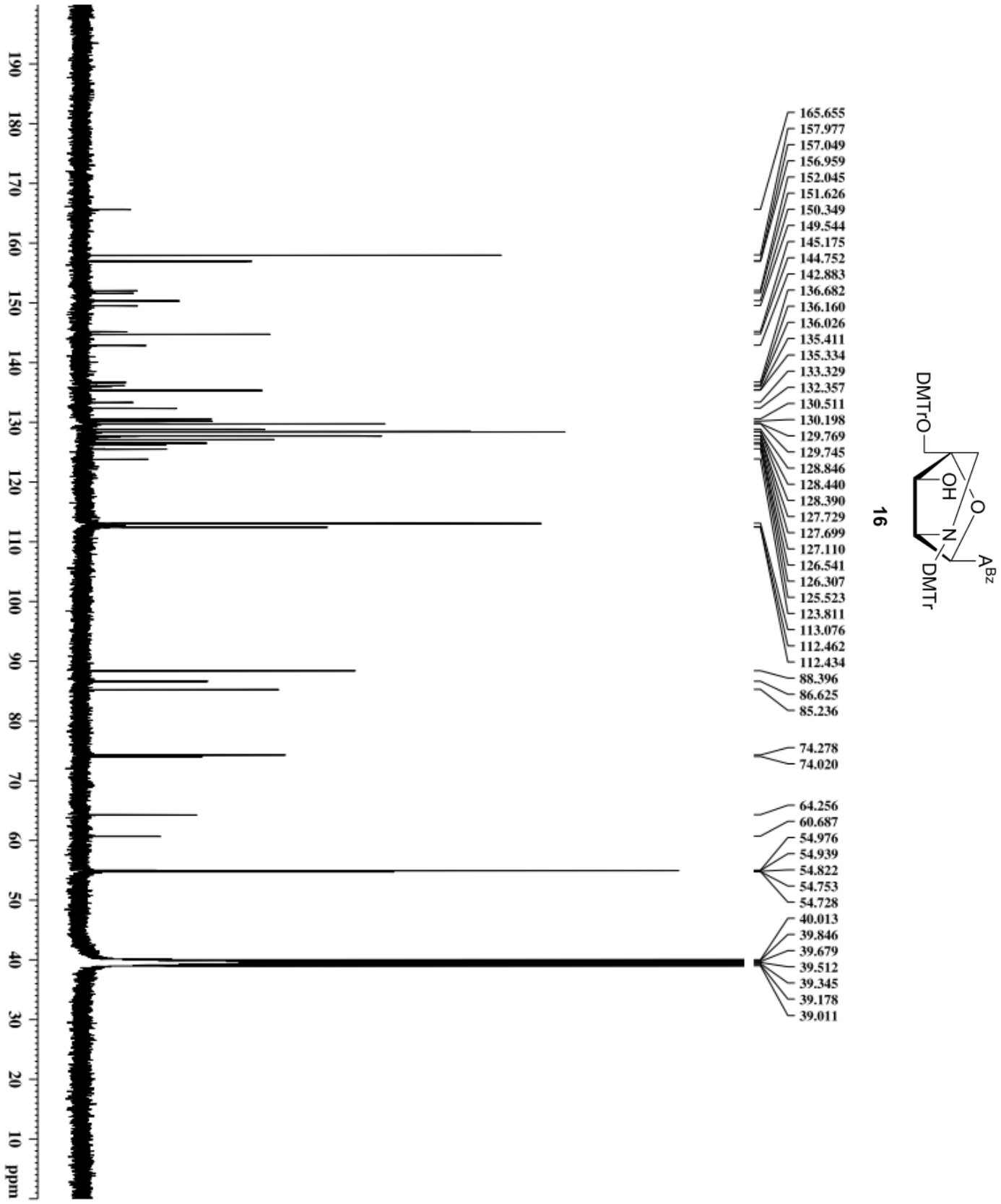
16


DMTrO
OH
O
N
ABz
DMTr


ppm
3.0
3.1
3.2
3.3
3.4
3.5
3.6
3.7
3.8
3.9
4.0
4.1
4.2
4.3
4.4
4.4
4.3
4.2
4.1
4.0
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
ppm


DMTrO
OH
O
N
ABz
DMTr

16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156.959
152.045
151.626
150.349
149.544
145.175
144.752
142.883
136.682
136.160
136.026
135.411
135.334
133.329
132.357
130.511
130.198
129.769
129.745
128.846
128.440
128.390
127.729
127.699
127.110
126.541
126.307
125.523
123.811
113.076
112.462
112.434
88.396
86.625
85.236
74.278
74.020
64.256
60.687
54.976
54.939
54.822
54.753
54.728
40.013
39.846
39.679
39.512
39.345
39.178
39.011
190
180
170
160
150
140
130
120
110
100
90
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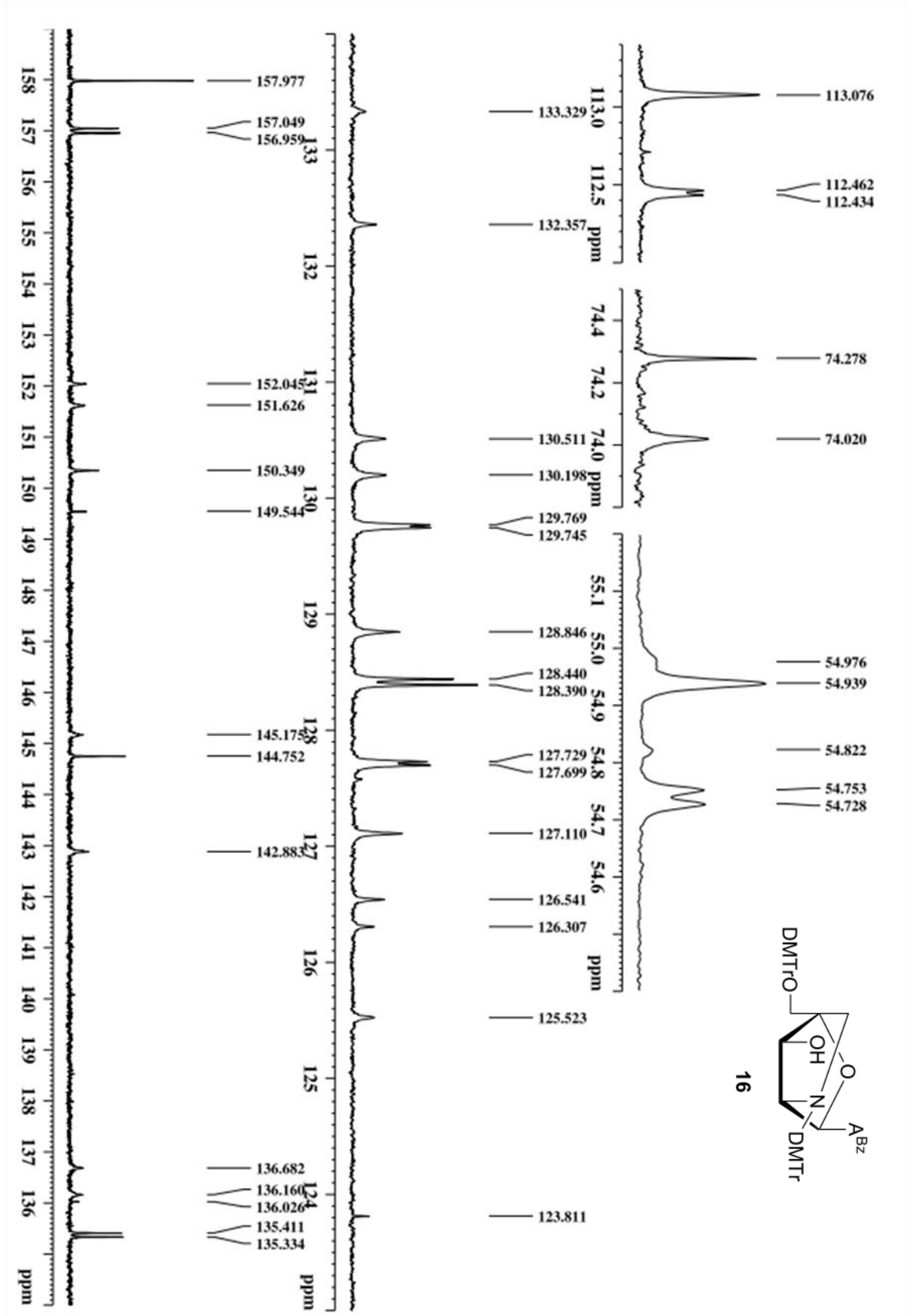
70
60
50
40
30
20
10
ppm
DMTrO
OH
O
N
ABz
DMTr
16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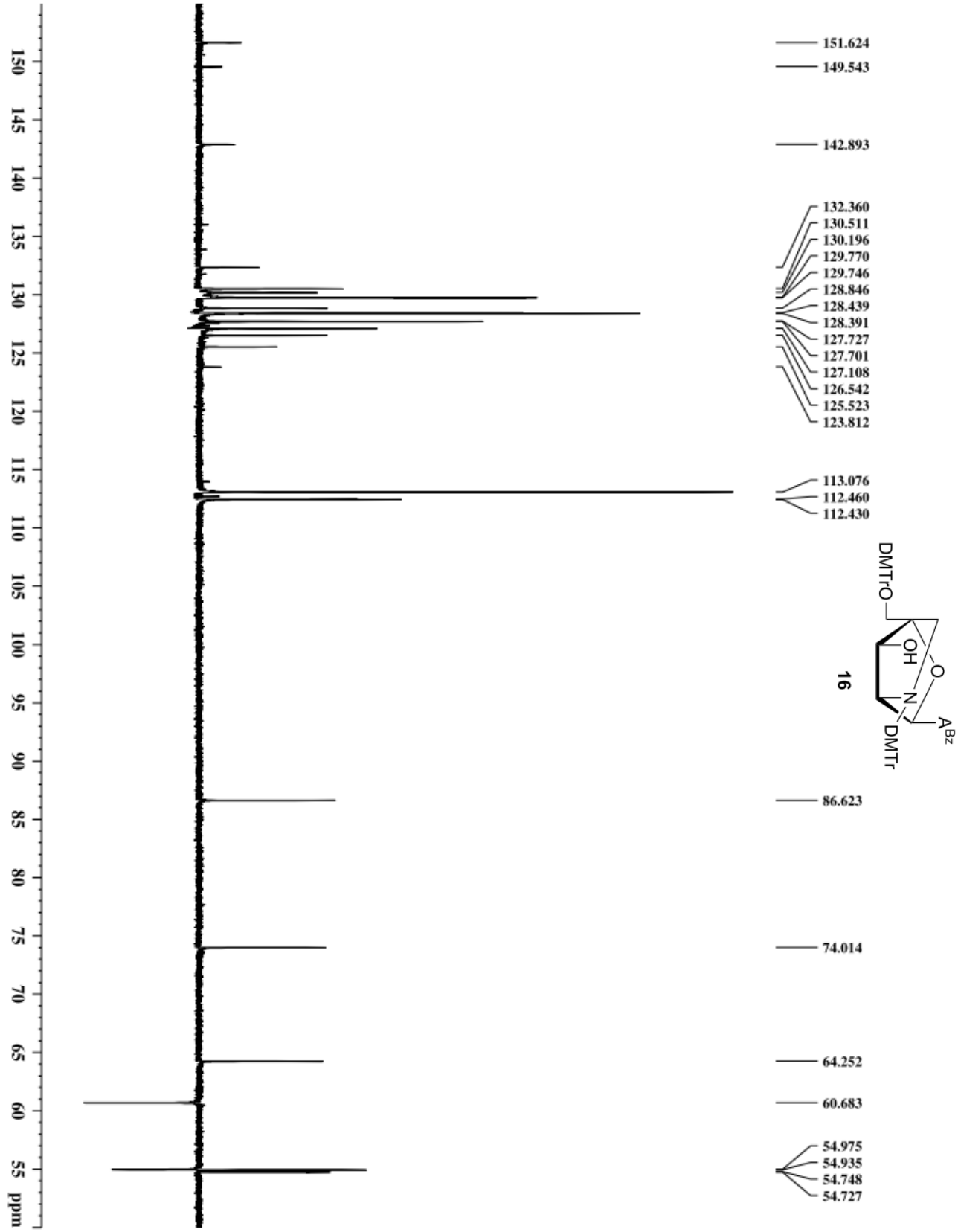
112.462
112.434
74.278
74.020
54.976
54.939
54.822
54.753
54.728
133.329
132.357
130.511
130.198
129.769
129.745
128.846
128.440
128.390
127.729
127.699
127.110
126.541
126.307
125.523
123.811
157.977
157.049
156.959
152.045
151.626
150.349
149.544
145.175
144.752
142.883
136.682
136.160
136.026
135.411
135.334
DMTrO
OH
O
N
A^Bz
DMTr
16
ppm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142.893
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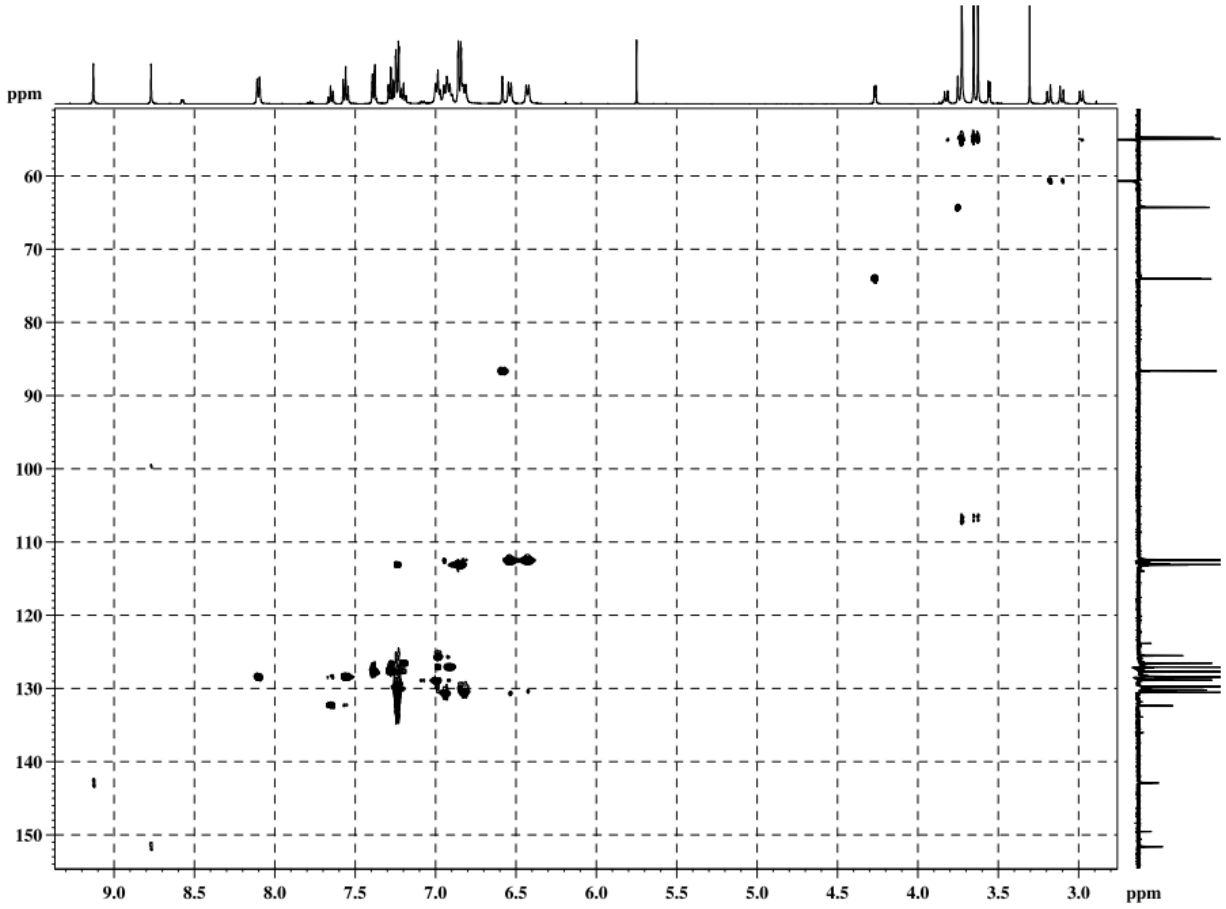
130.196
129.770
129.746
128.846
128.439
128.391
127.727
127.701
127.108
126.542
125.523
123.812
113.076
112.460
112.430
86.623
74.014
64.252
60.683
54.975
54.935
54.748
54.727
150 145 140 135 130 125 120 115 110 105 100 95 90 85 80 75 70 65 60 55 ppm
DMTrO
OH
O
N
ABz
DMTr
16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70
80
90
100
110
120
130
140
150
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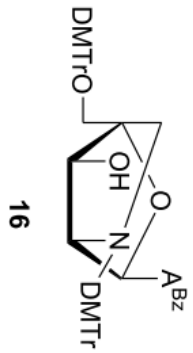
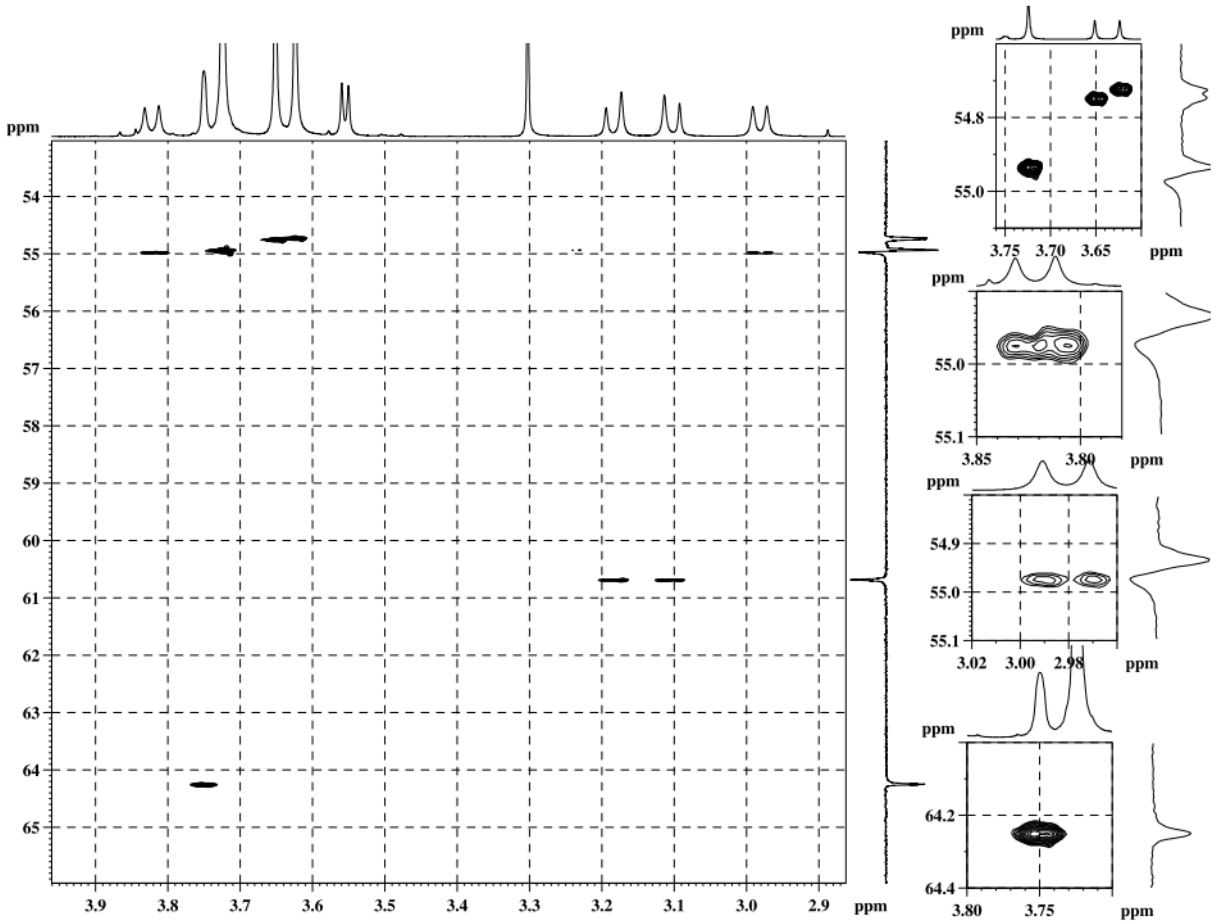
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm


DMTrO
OH
O
N
ABz
DMTr

16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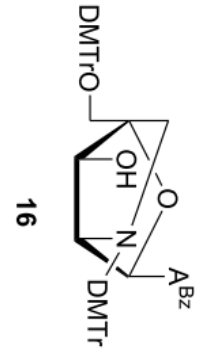
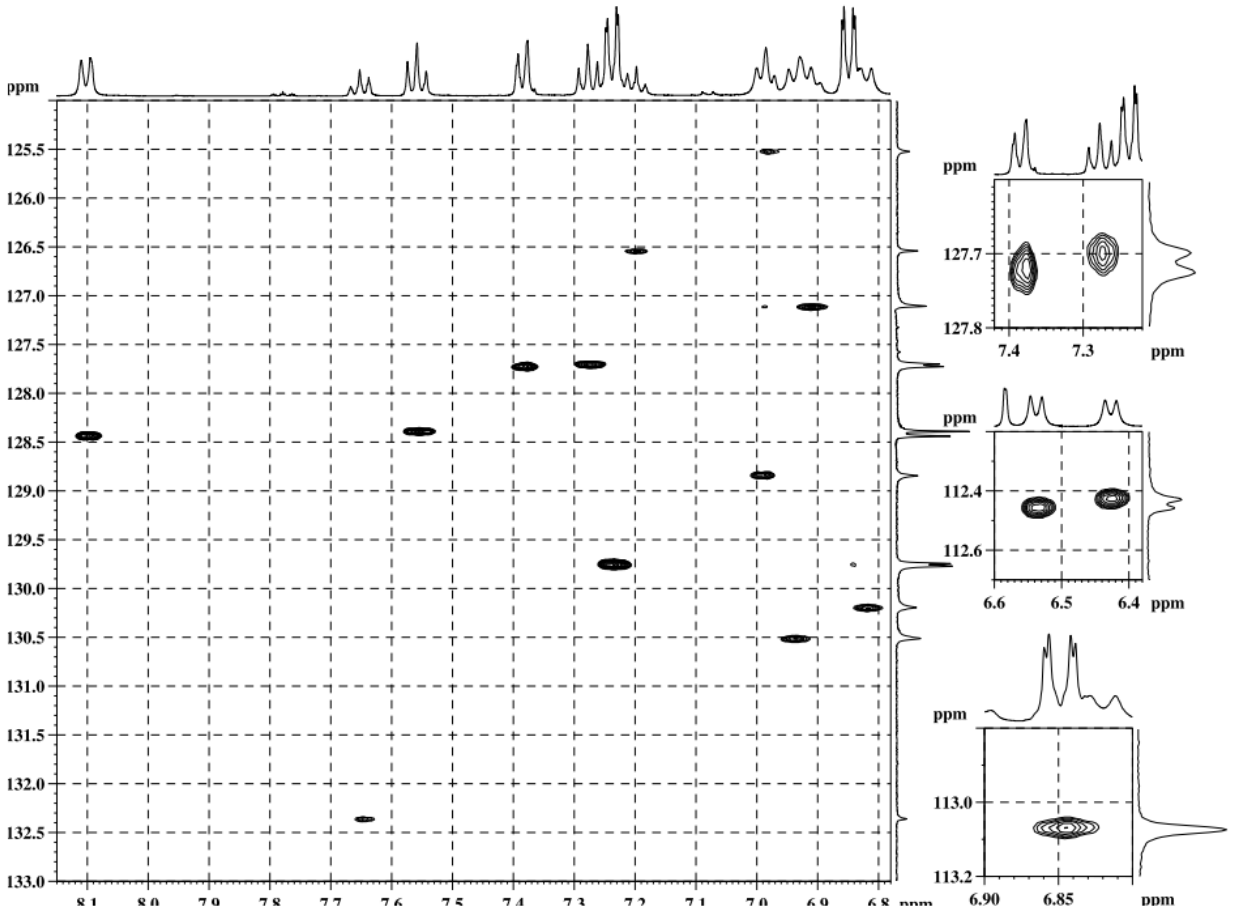
54
55
56
57
58
59
60
61
62
63
64
65
3.9
3.8
3.7
3.6
3.5
3.4
3.3
3.2
3.1
3.0
2.9
ppm
54.8
55.0
3.75
3.70
3.65
55.1
3.85
3.80
54.9
3.02
3.00
2.98
64.2
64.4


DMTrO
OH
O
N
ABz
DMTr

16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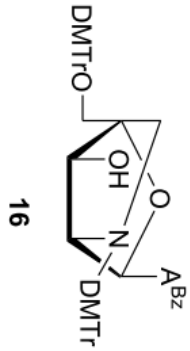
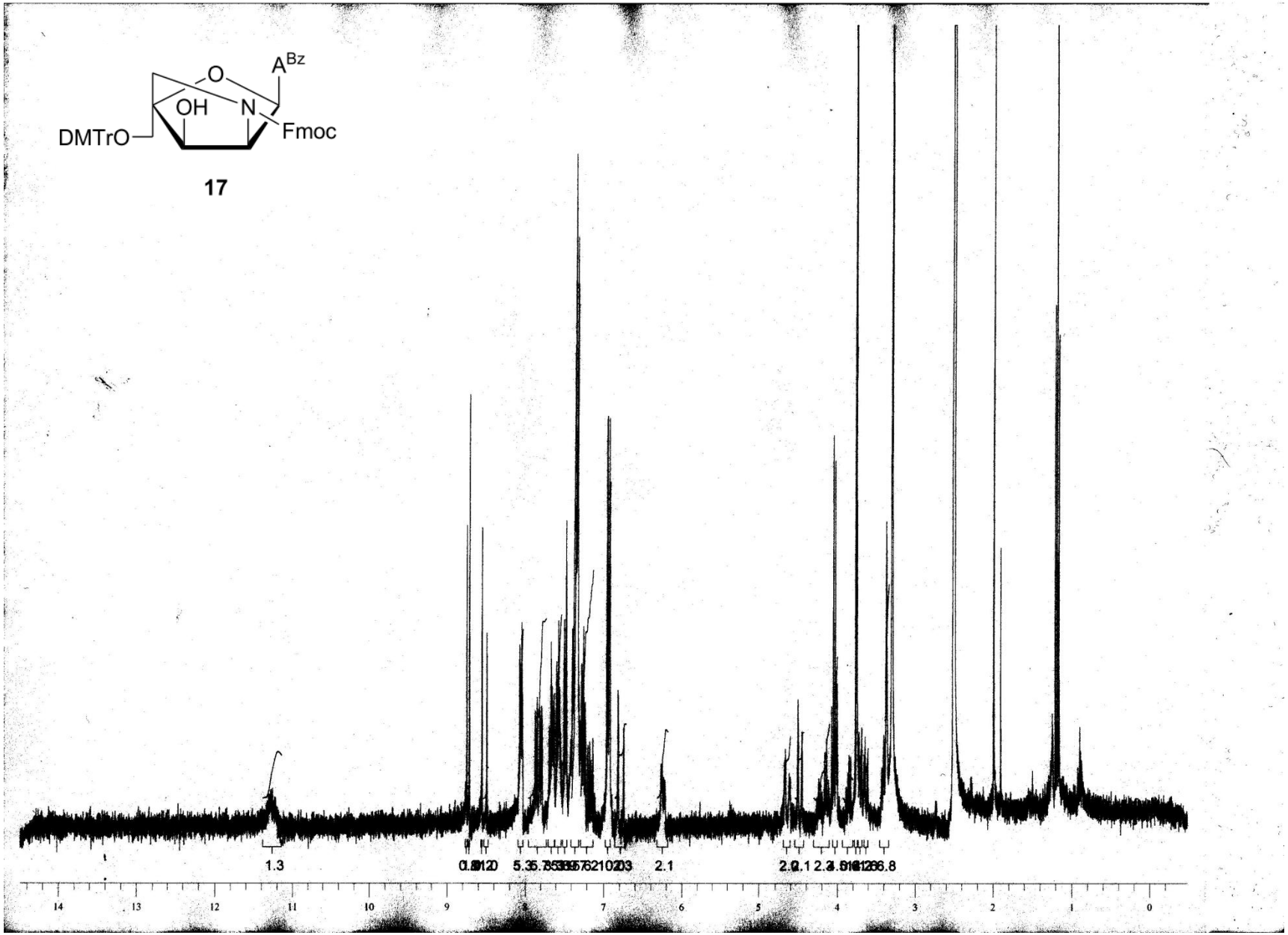
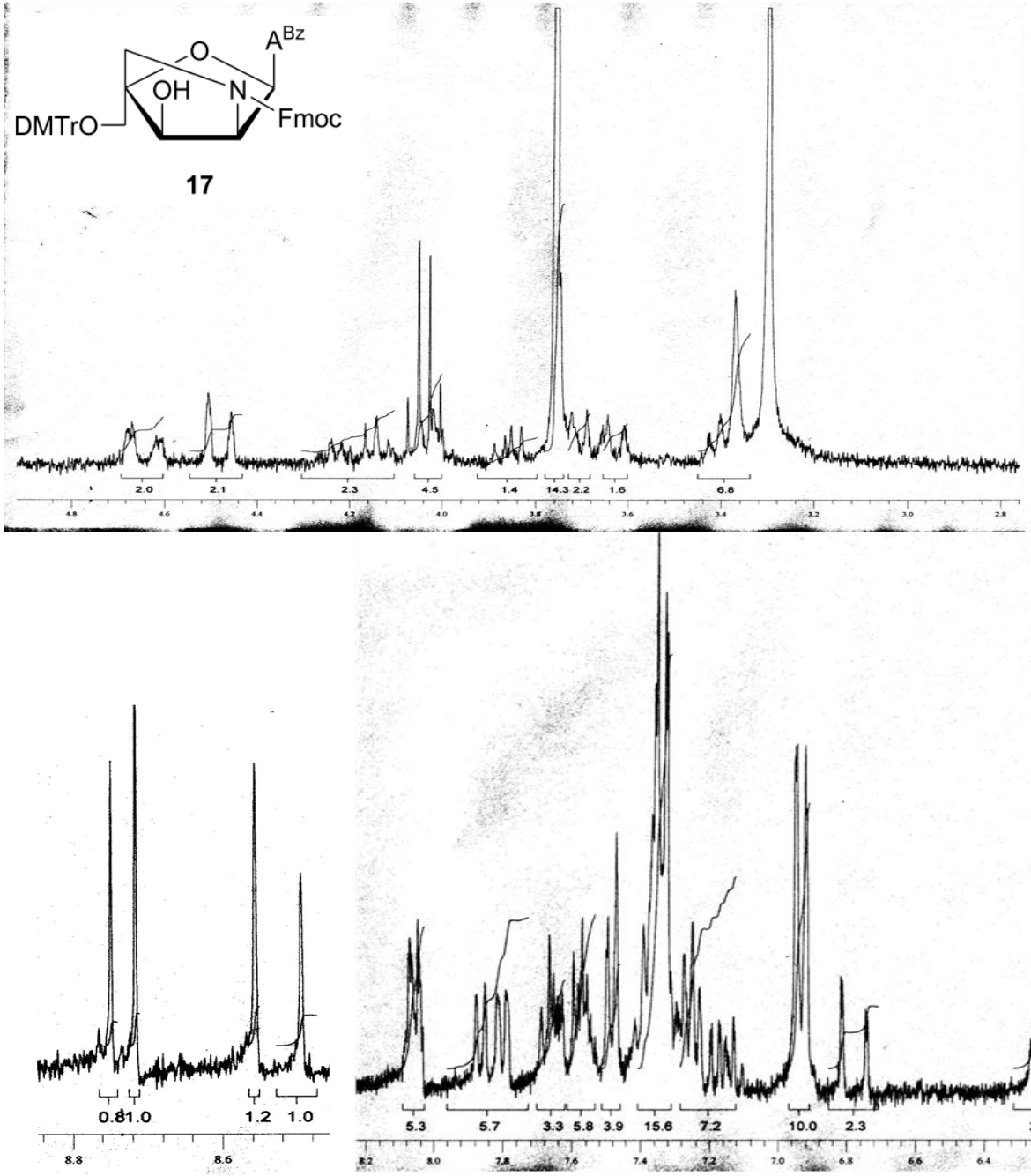
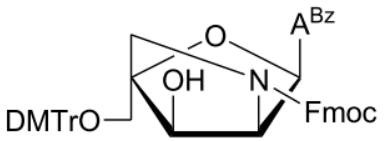
127.5
128.0
128.5
129.0
129.5
130.0
130.5
131.0
131.5
132.0
132.5
133.0
ppm
127.7
127.8
7.4
7.3
ppm
ppm
112.4
112.6
6.6
6.5
6.4
ppm
ppm
113.0
113.2
6.90
6.85
ppm


DMTrO
OH
O
N
ABz
DMTr

16


O
A^Bz
OH
N
DMTrO
Fmoc
17
1.3
2.1
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0


O
A^Bz
OH
N
DMTrO
Fmoc
17
2.0
2.1
2.3
4.5
1.4
14.3
2.2
1.6
6.8
0.8
1.0
1.2
1.0
5.3
5.7
3.3
5.8
3.9
15.6
7.2
10.0
2.3
2.1


O
A^Bz
OH
N
DMTrO
Fmoc

17

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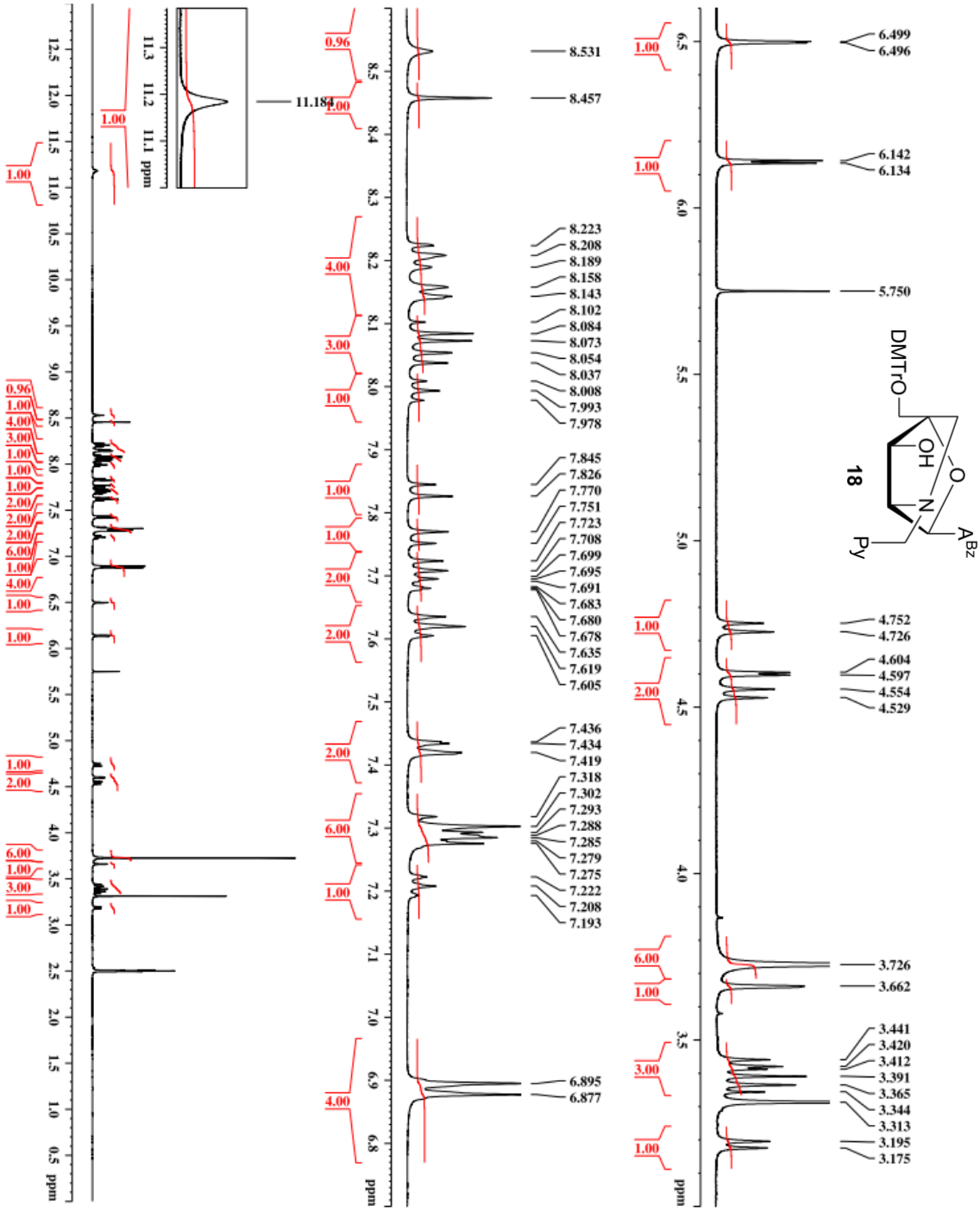
154.610
151.856
151.530
150.309
150.188
144.665
143.701
143.586
142.791
141.350
141.235
140.613
140.436
140.310
135.301
135.173
133.373
132.395
129.763
128.835
128.406
127.852
127.677
127.616
127.517
127.198
127.086
126.850
126.660
125.386
125.159
125.105
124.946
124.626
121.289
119.991
119.936
113.206
88.871
88.411
85.371
84.787
84.663
84.649
72.488
72.047
66.797
66.736
63.907
63.420
60.613
60.521
54.961
52.731
52.594
46.447
45.861
200
175
150
125
100
75
50
25
0


DMTrO
OH
O
N
ABz
Py
18
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6.496
6.142
6.134
5.750
4.752
4.726
4.604
4.597
4.554
4.529
3.726
3.662
3.441
3.420
3.412
3.391
3.365
3.344
3.313
3.195
3.175
8.531
8.457
8.223
8.208
8.189
8.158
8.143
8.102
8.084
8.073
8.054
8.037
8.008
7.993
7.978
7.845
7.826
7.770
7.751
7.723
7.708
7.699
7.695
7.691
7.683
7.680
7.678
7.635
7.619
7.605
7.436
7.434
7.419
7.318
7.302
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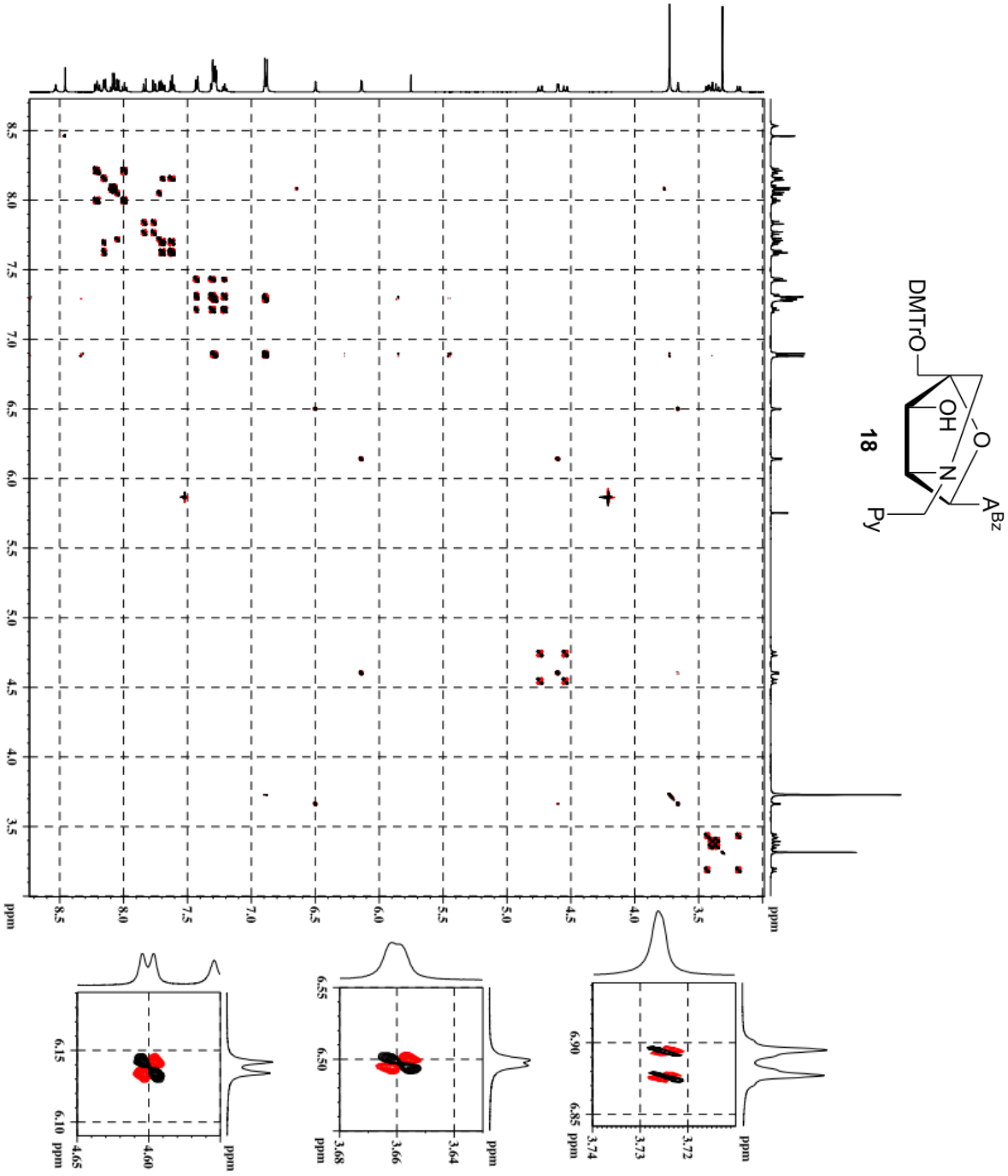
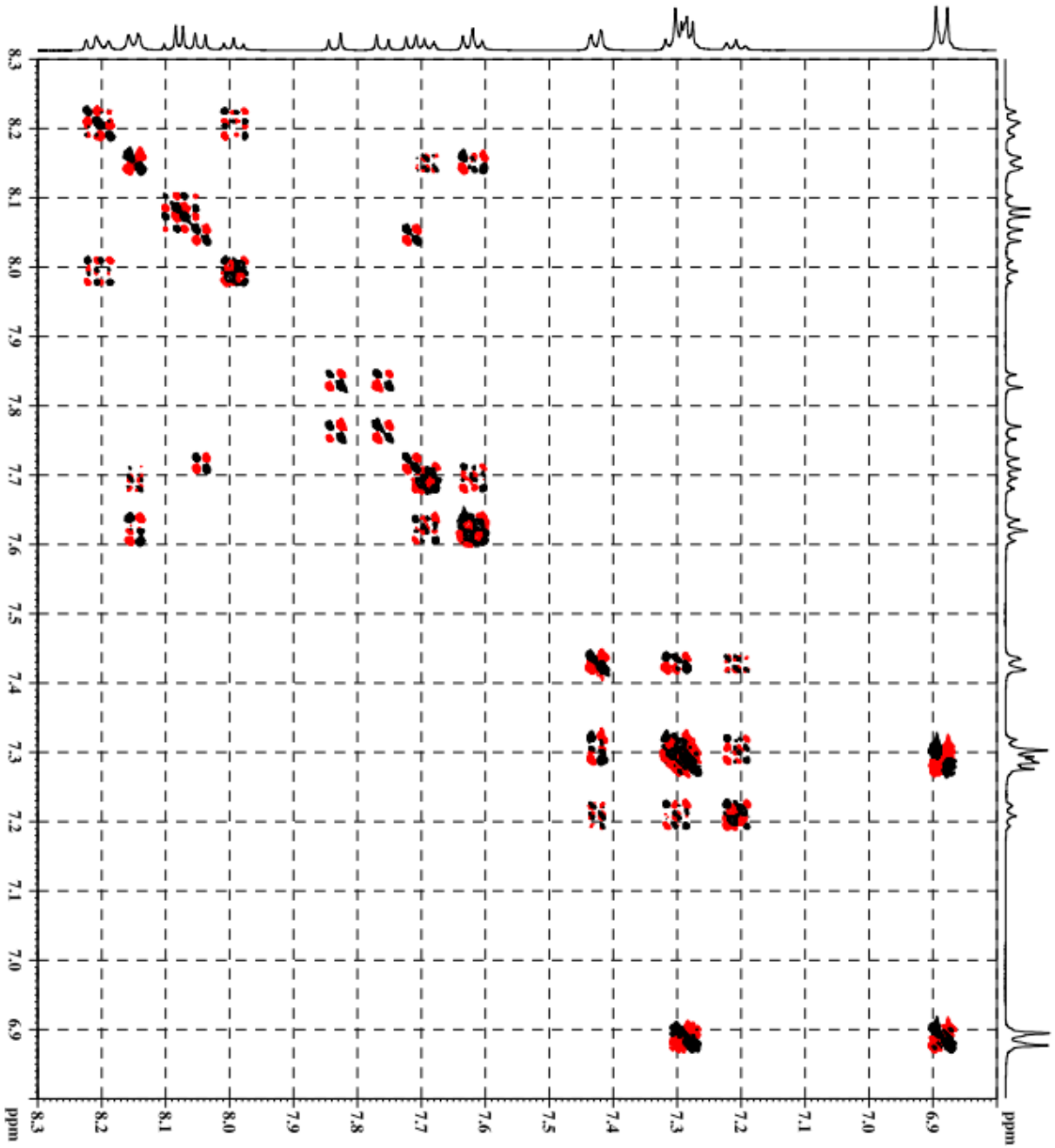
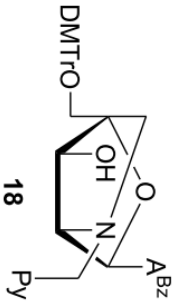

DMTrO
OH
O
N
Abz
Py
18
ppm





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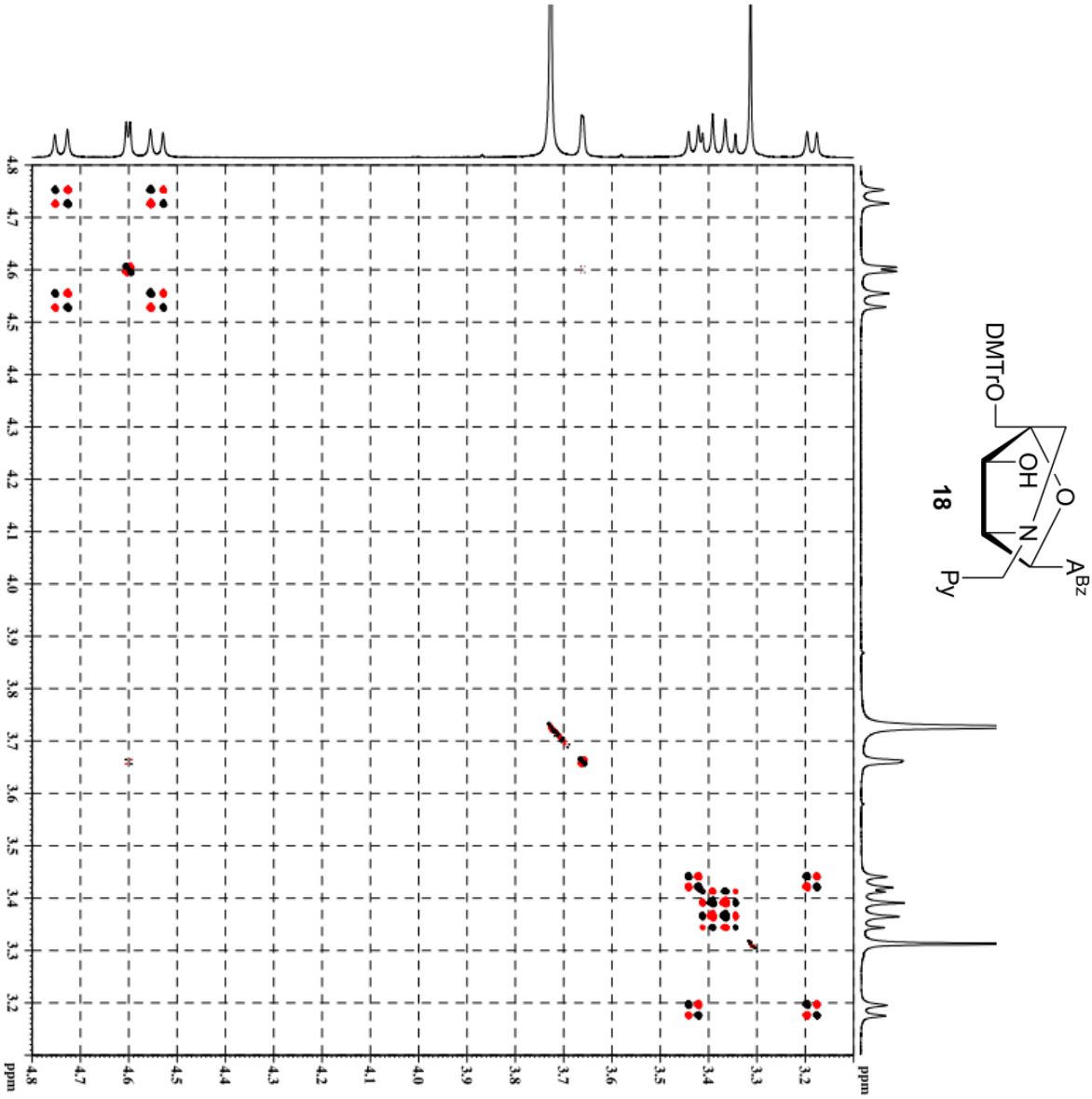
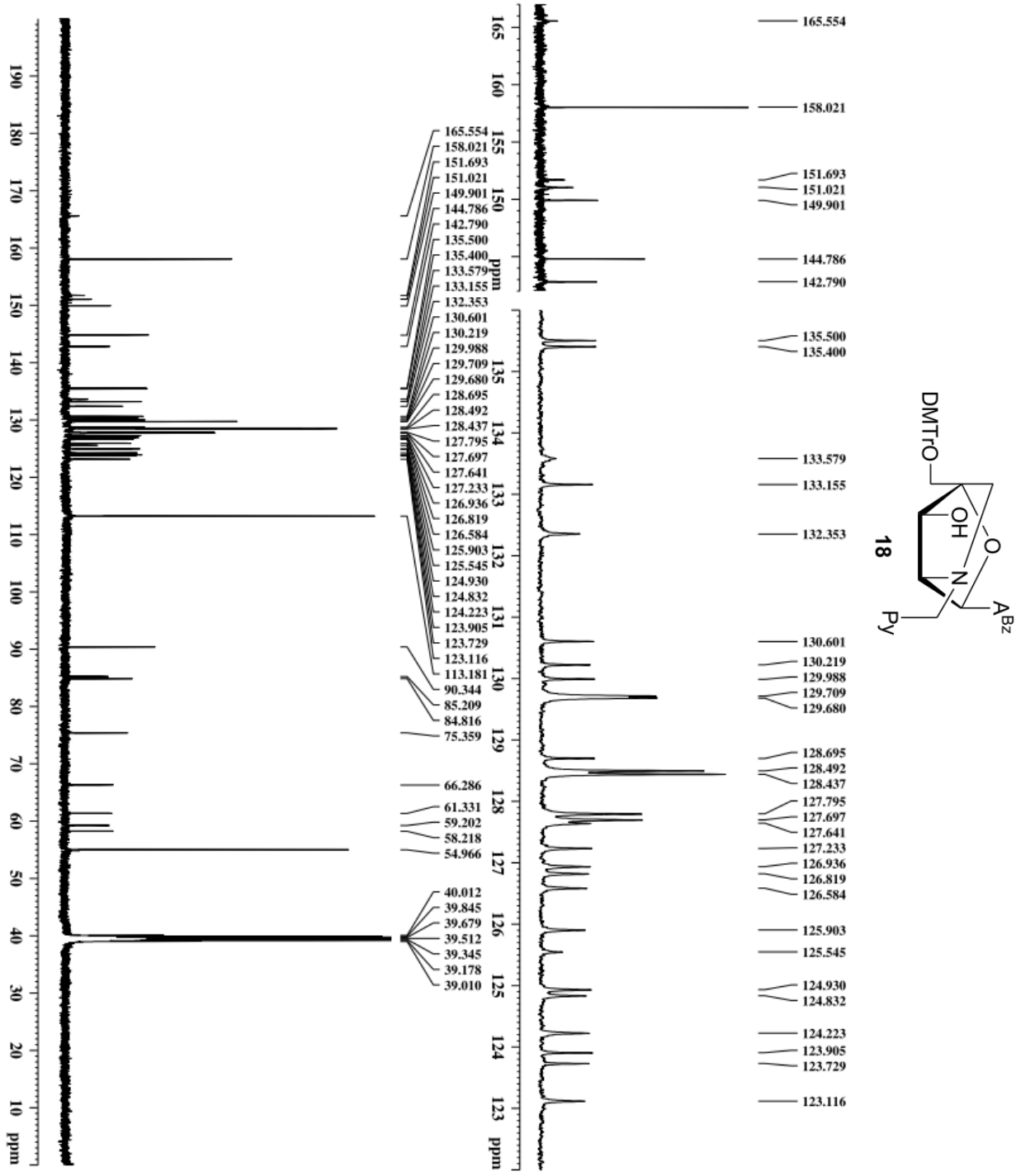

DMTrO
OH
O
N
ABz
Py
18
ppm
ppm


DMTrO
OH
O
N
Py
ABz
18
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151.021
149.901
144.786
142.790
135.500
135.400
133.579
133.155
132.353
130.601
130.219
129.988
129.709
129.680
128.695
128.492
128.437
127.795
127.697
127.641
127.233
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124.223
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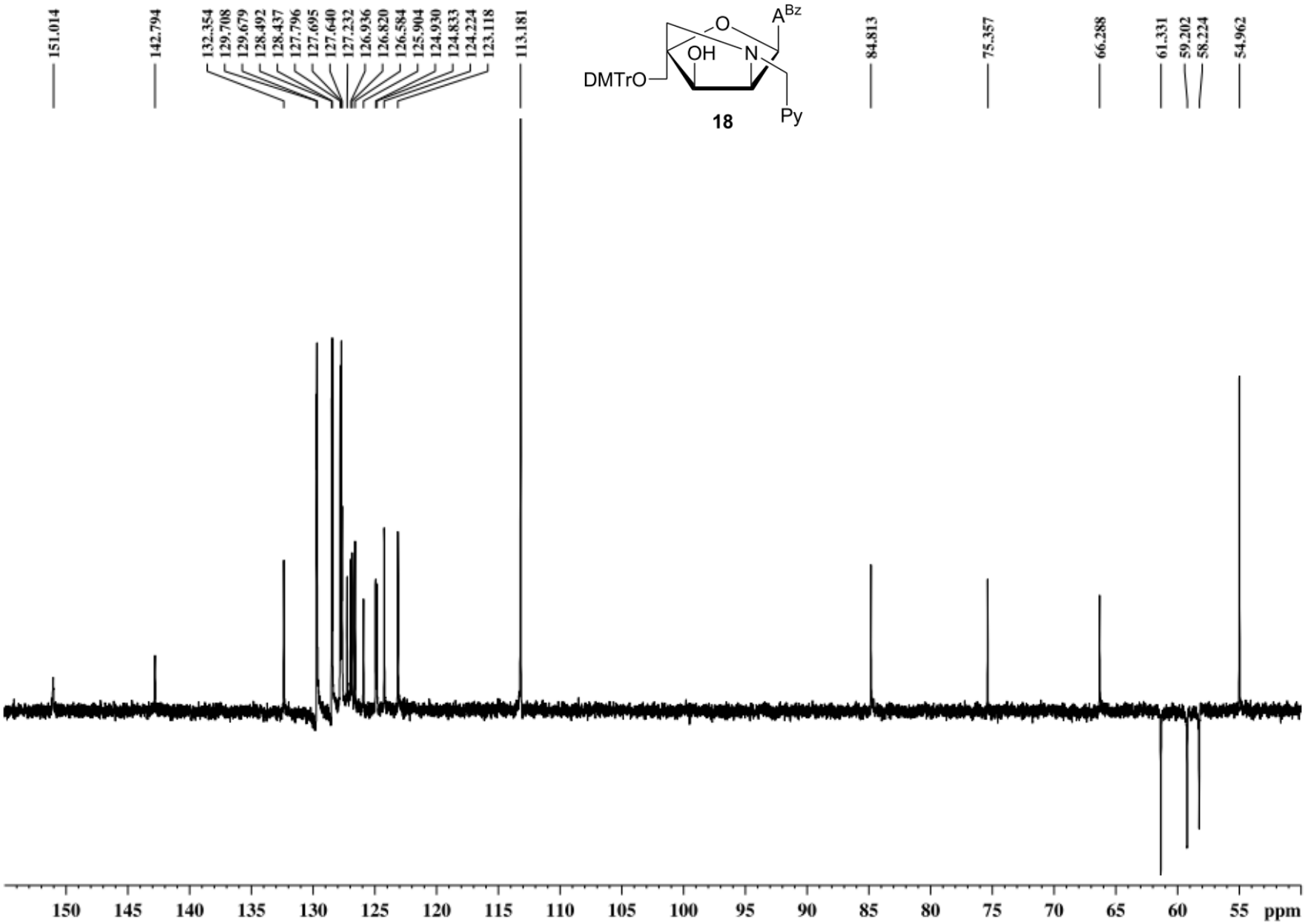
123.116
ppm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127.796
127.695
127.640
127.232
126.936
126.820
126.584
125.904
124.930
124.833
124.224
123.118
113.181
84.813
75.357
66.288
61.331
59.202
58.224
54.962
DMTrO
OH
O
N
A^Bz
Py
18
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120
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105
100
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85
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75
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65
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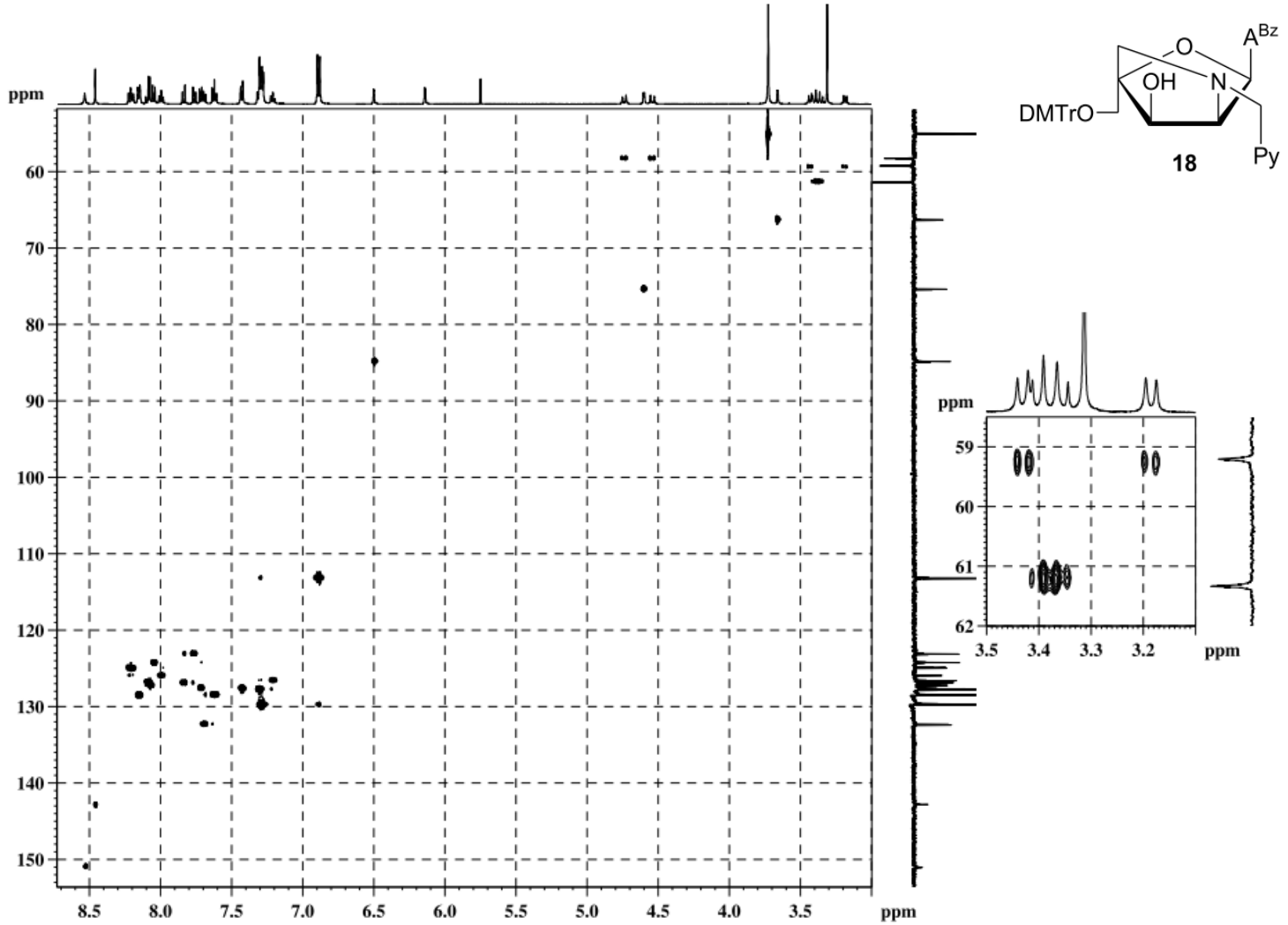
55
ppm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90
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130
140
150
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7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm
O
A^Bz
OH
N
DMTrO
Py
18
ppm
59
60
61
62
3.5
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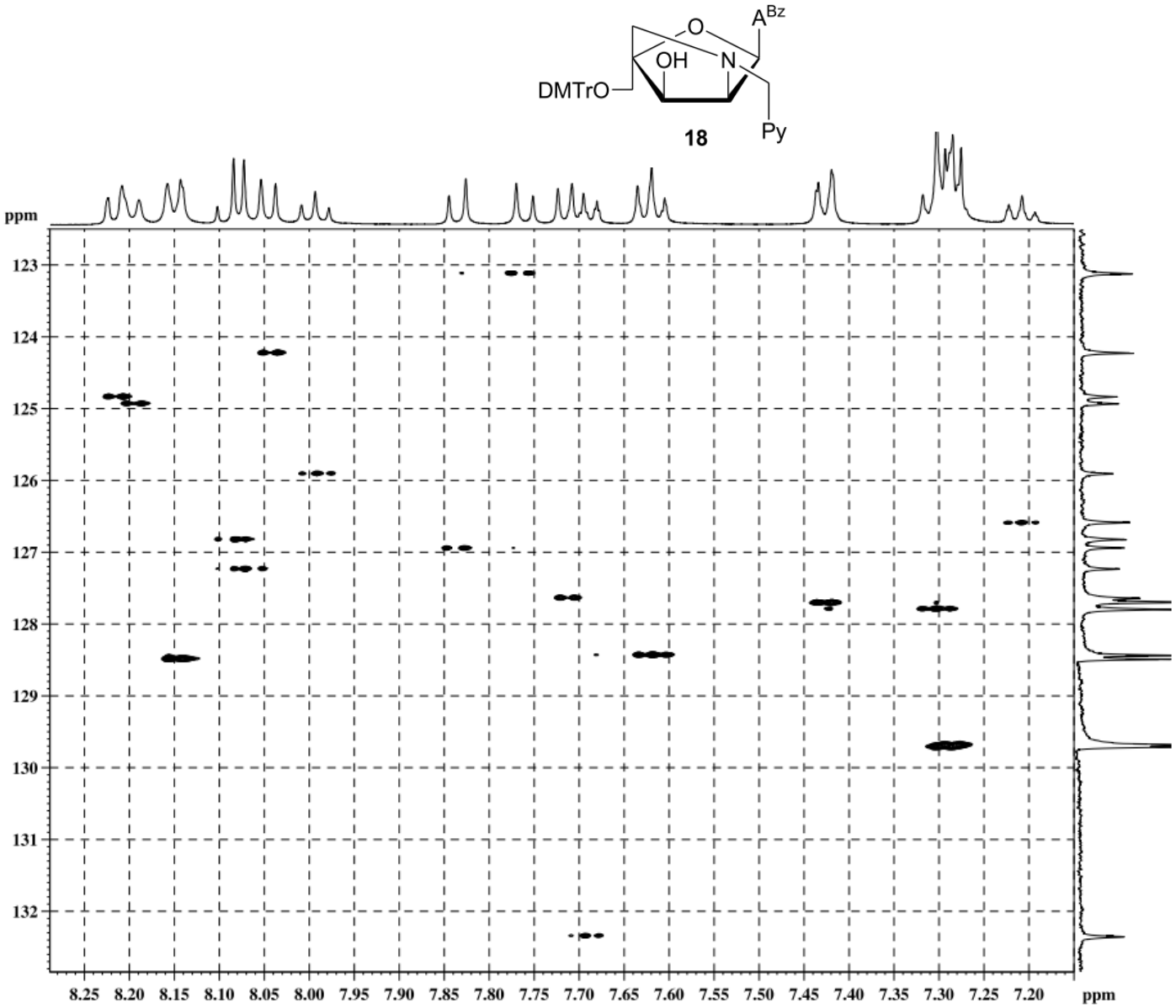
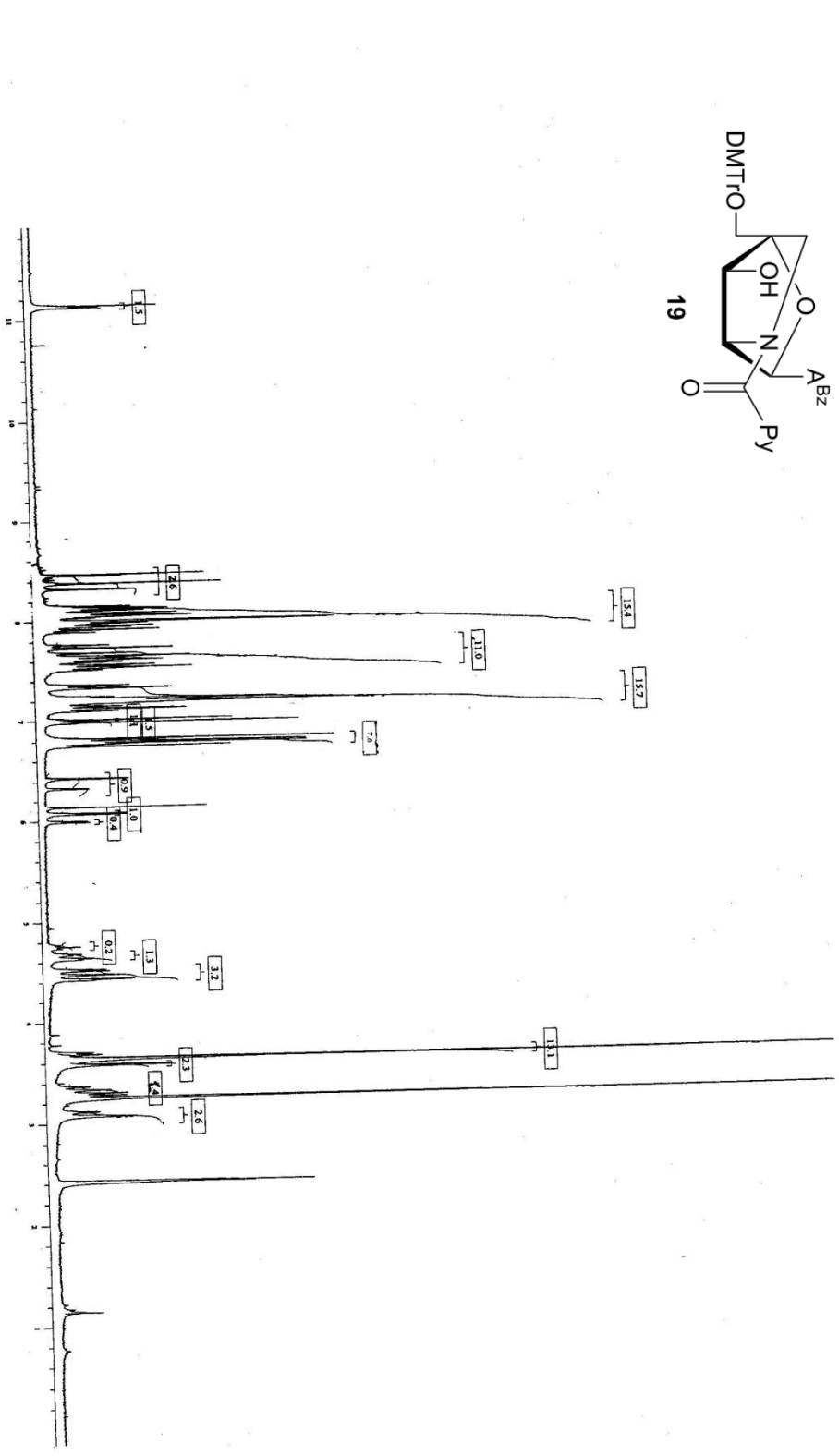
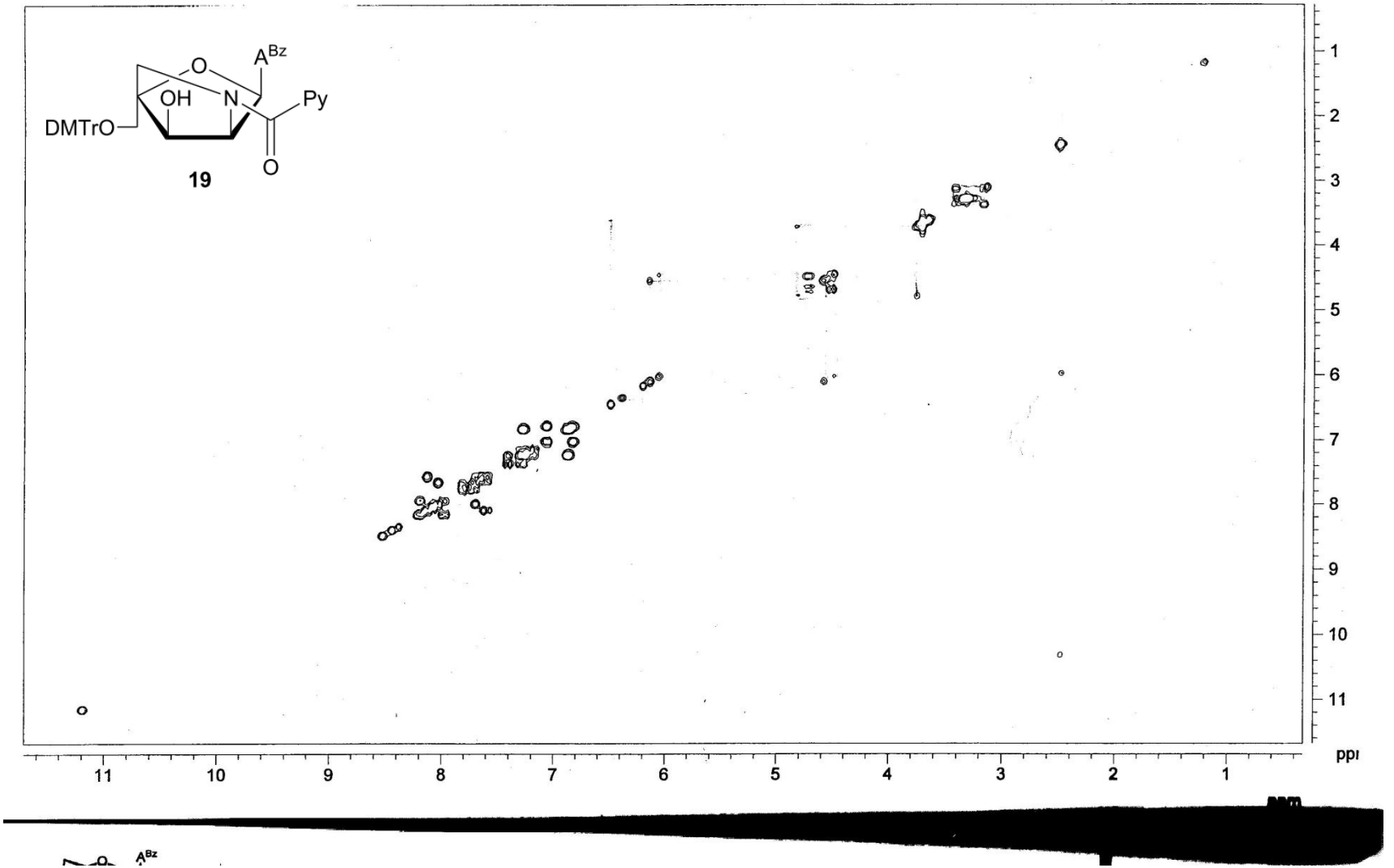
3.3
3.2
ppm


O
A^Bz
OH
N
DMTrO
Py
18
ppm
123
124
125
126
127
128
129
130
131
132
8.25 8.20 8.15 8.10 8.05 8.00 7.95 7.90 7.85 7.80 7.75 7.70 7.65 7.60 7.55 7.50 7.45 7.40 7.35 7.30 7.25 7.20
ppm


DMTrO
OH
O
N
O
Py
ABz
19


DMTrO
OH
O
N
A^Bz
Py
O
19
1
2
3
4
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6
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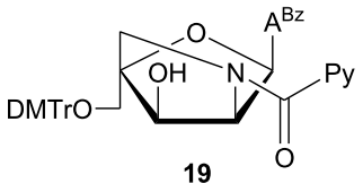
8
9
10
11
11
10
9
8
7
6
5
4
3
2
1
ppm


O
A^Bz
OH
N
Py
DMTrO
O

19

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160
150
140
130
120
110
100
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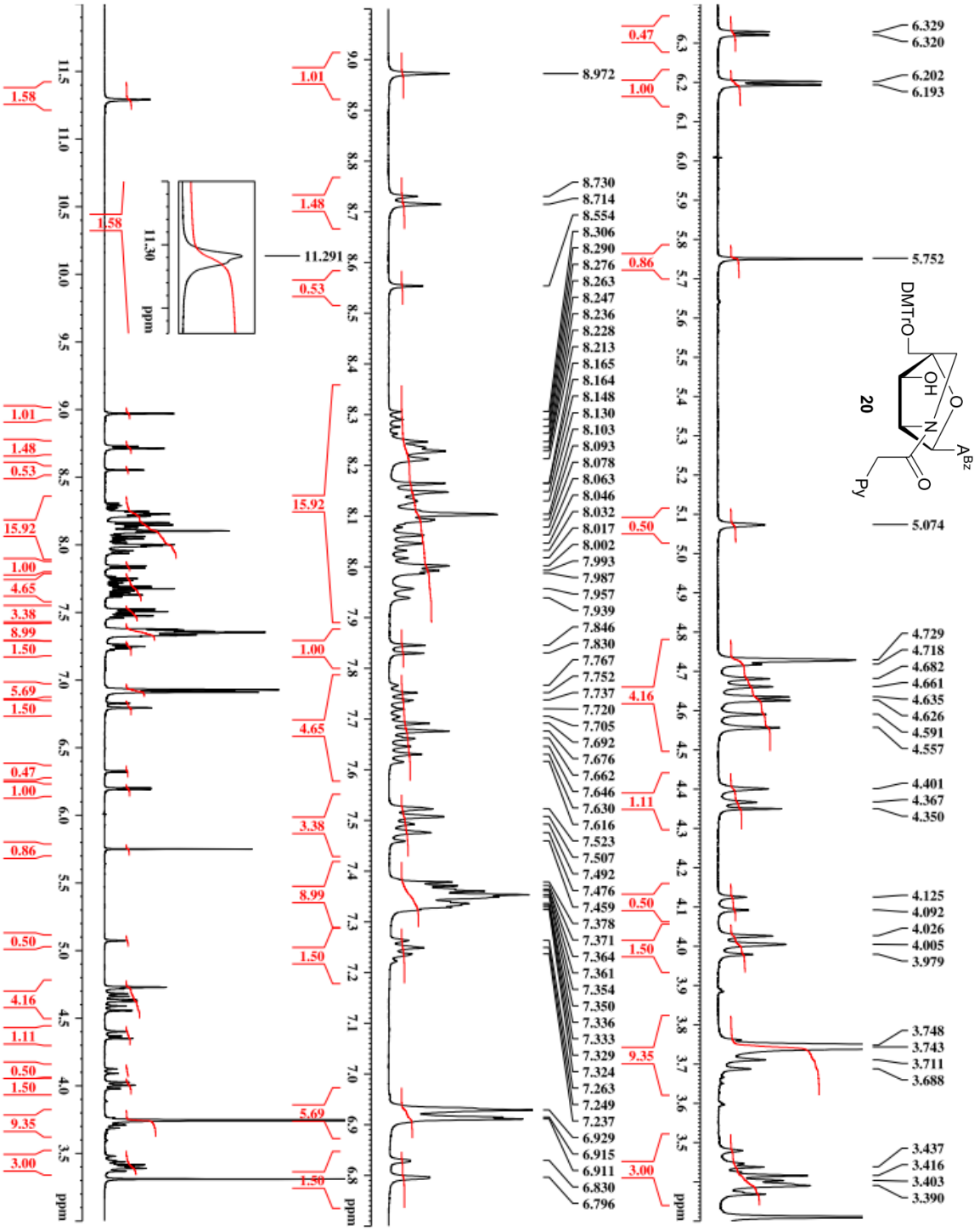
80
70
60
50
40
30
20
10


20
DMTrO
OH
O
N
O
Py
ABz
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5.074
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4.718
4.682
4.661
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4.626
4.591
4.557
4.401
4.367
4.350
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4.026
4.005
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3.748
3.743
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3.390
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8.276
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8.247
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7.767
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7.523
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7.371
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7.354
7.350
7.336
7.333
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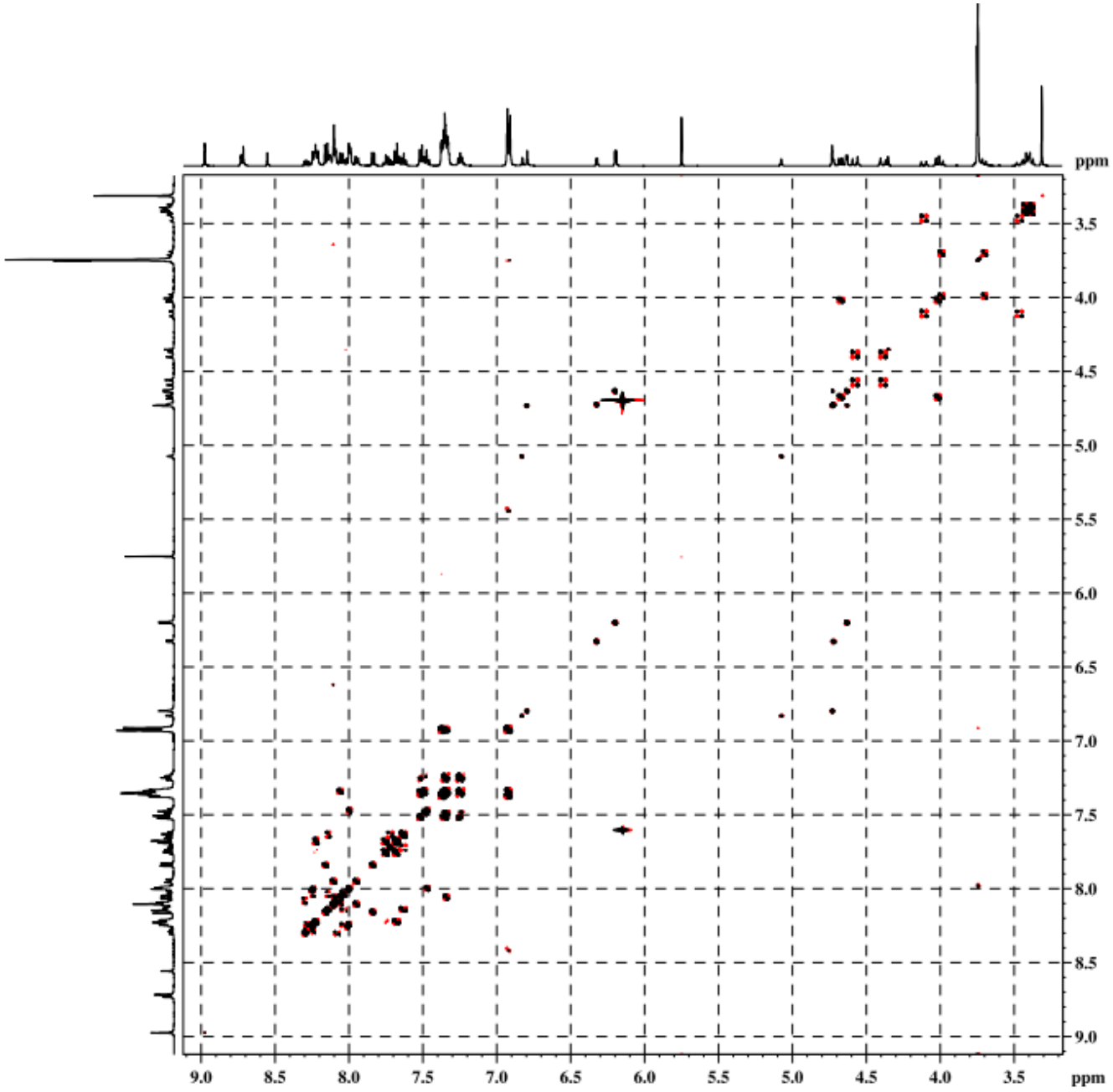
ppm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7.5
8.0
8.5
9.0
9.0
8.5
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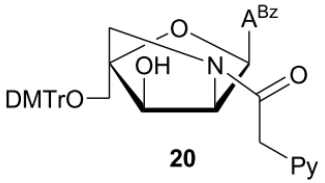
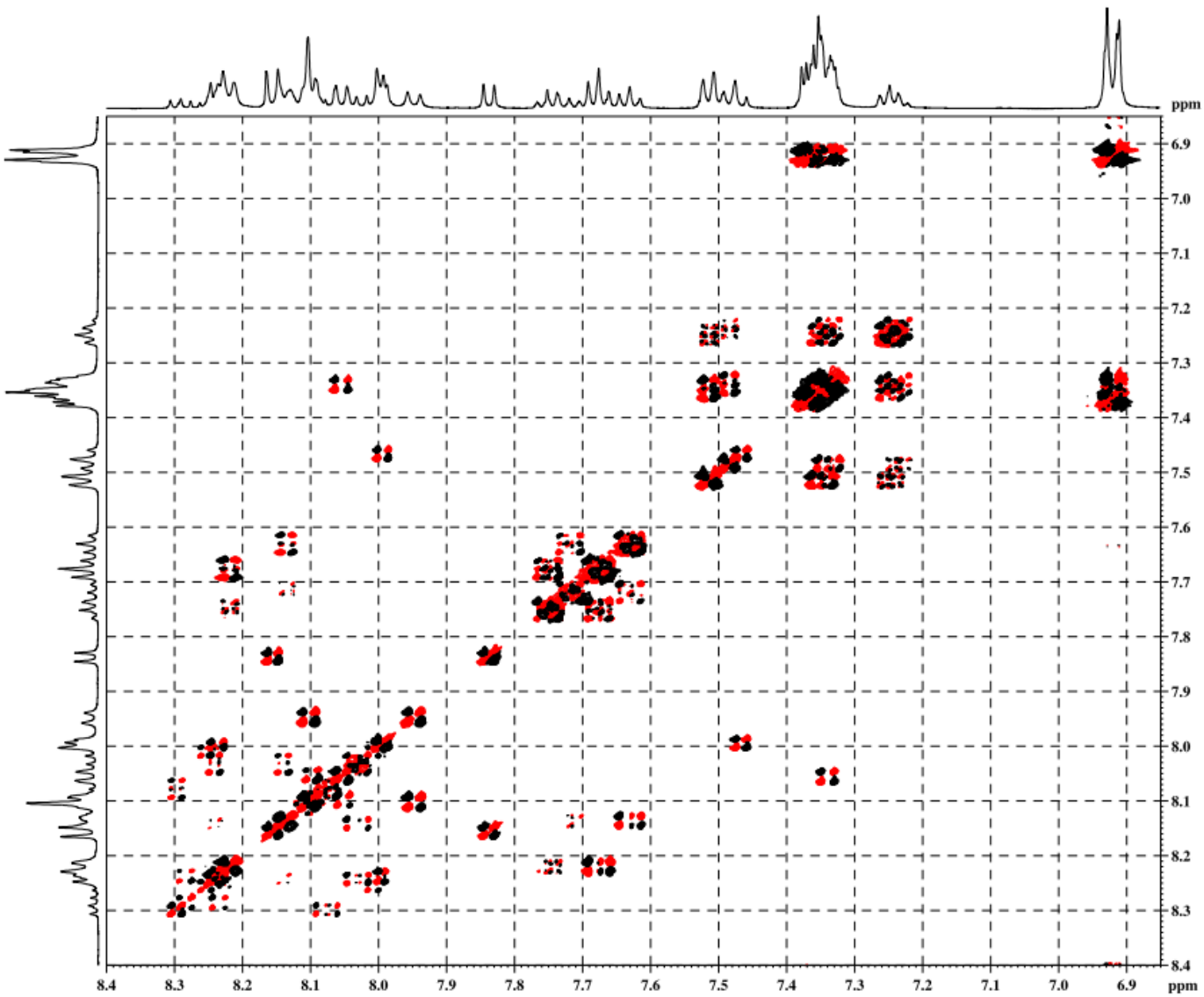
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm


O
A^Bz
OH
N
O
DMTrO
Py

20


ppm
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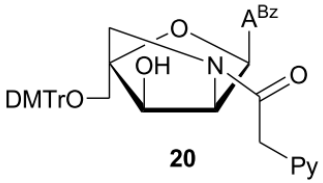
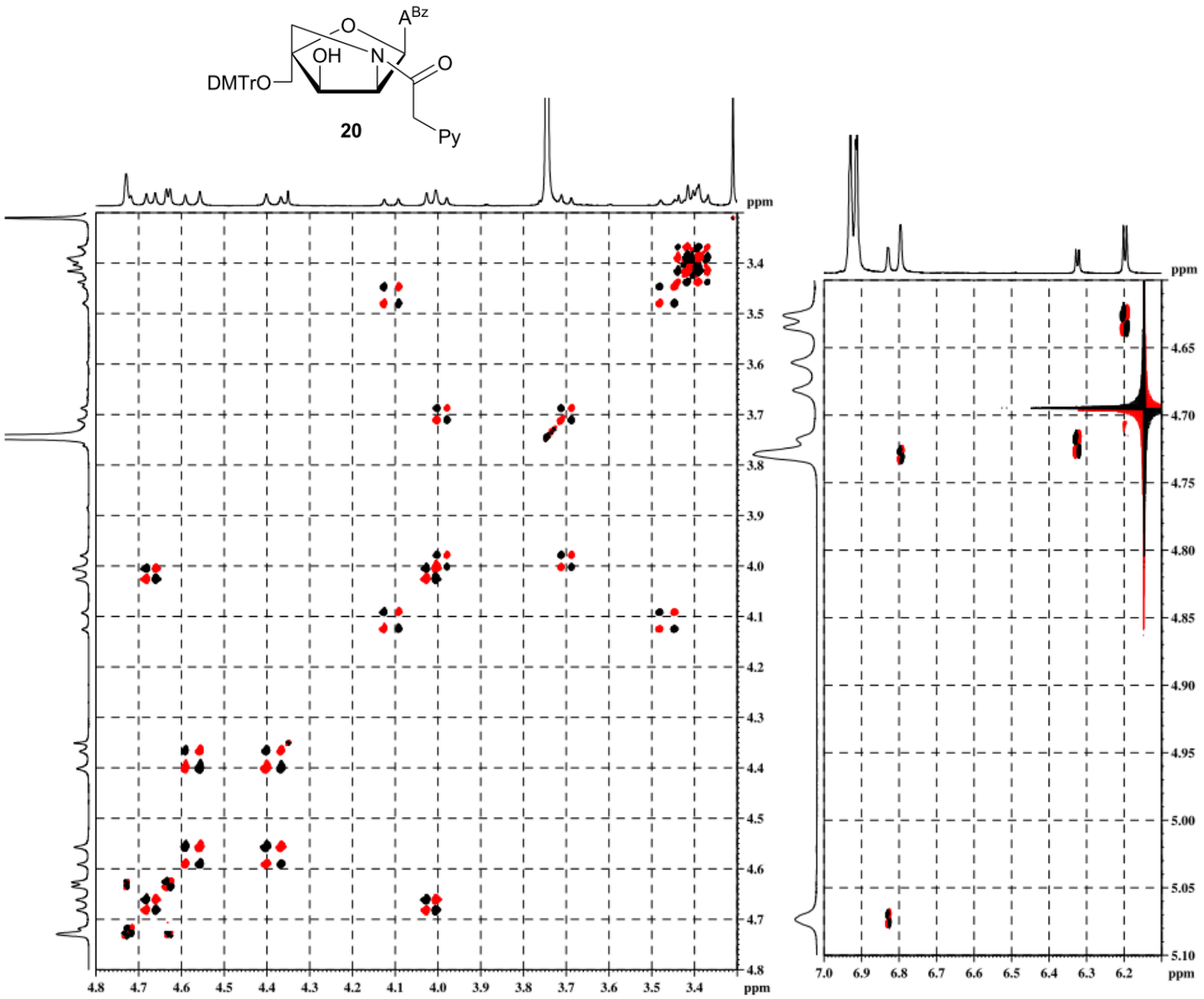
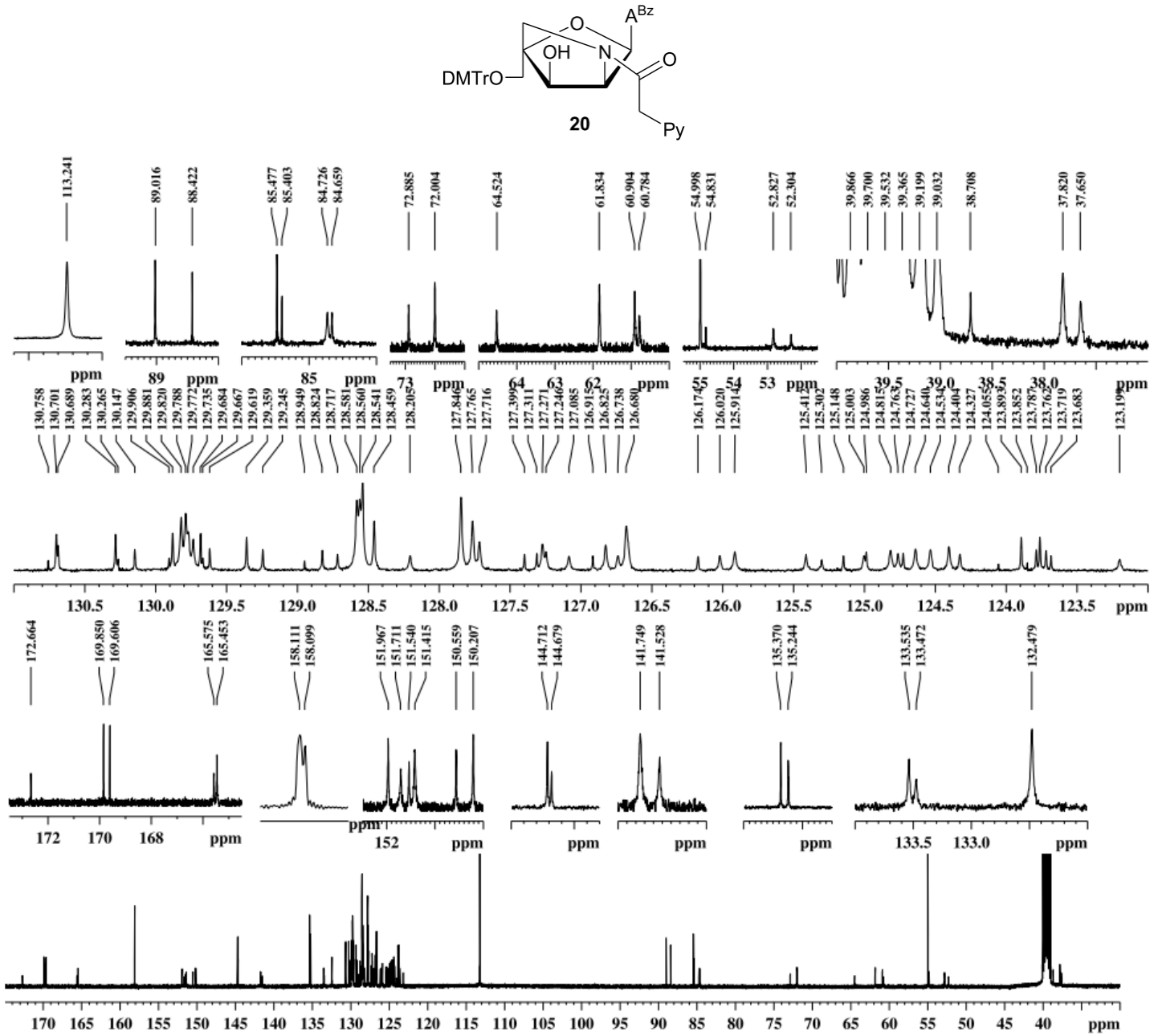
7.2
7.3
7.4
7.5
7.6
7.7
7.8
7.9
8.0
8.1
8.2
8.3
8.4
8.4
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
7.2
7.1
7.0
6.9
ppm


O
A^Bz
OH
N
O
DMTrO
Py

20


DMTrO
OH
O
N
A^Bz
O
Py
20
ppm
4.8 4.7 4.6 4.5 4.4 4.3 4.2 4.1 4.0 3.9 3.8 3.7 3.6 3.5 3.4
3.4 3.5 3.6 3.7 3.8 3.9 4.0 4.1 4.2 4.3 4.4 4.5 4.6 4.7 4.8
7.0 6.9 6.8 6.7 6.6 6.5 6.4 6.3 6.2
4.65 4.70 4.75 4.80 4.85 4.90 4.95 5.00 5.05 5.10


A^Bz
O
OH
N
O
DMTrO
Py
20
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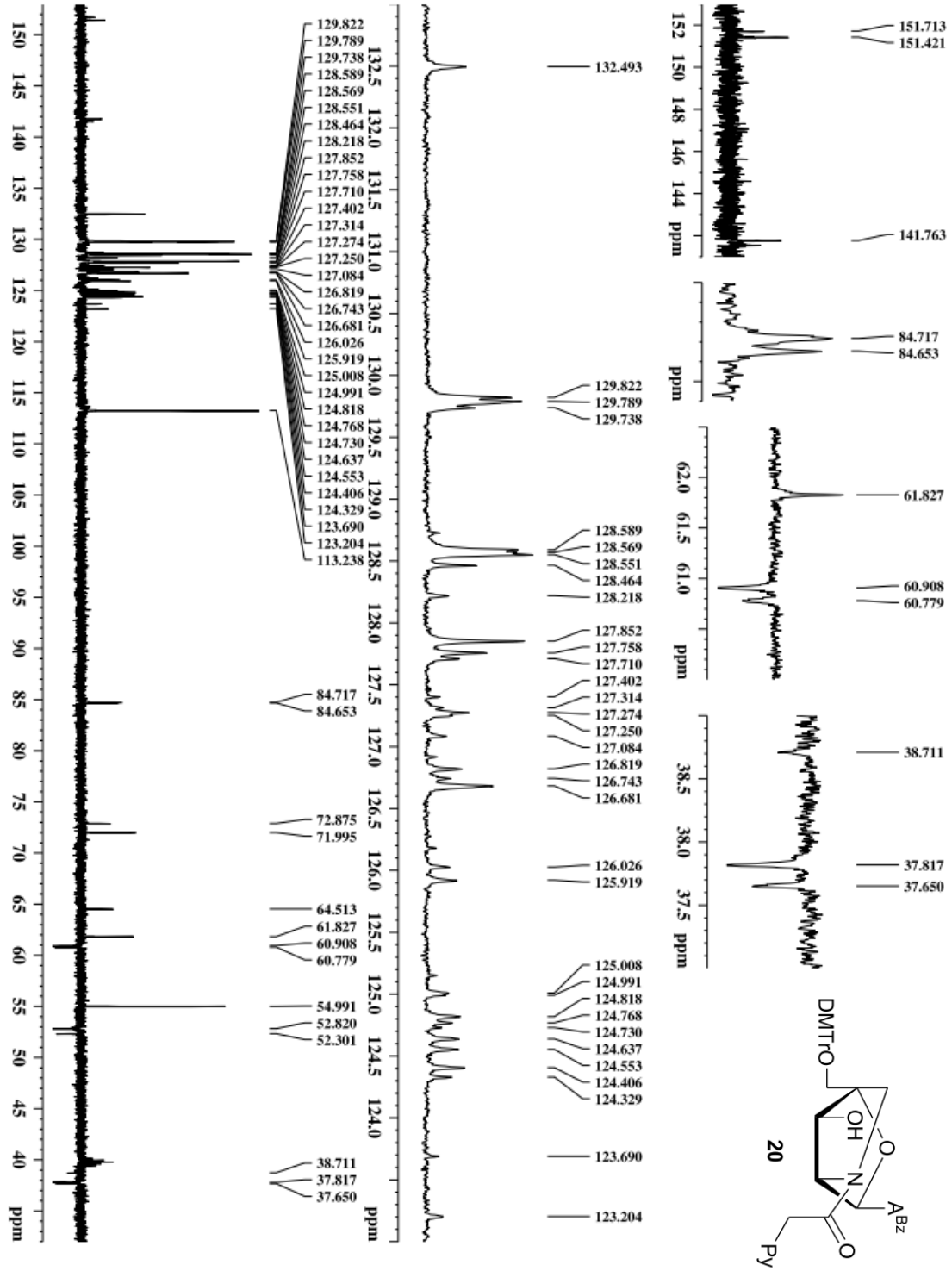
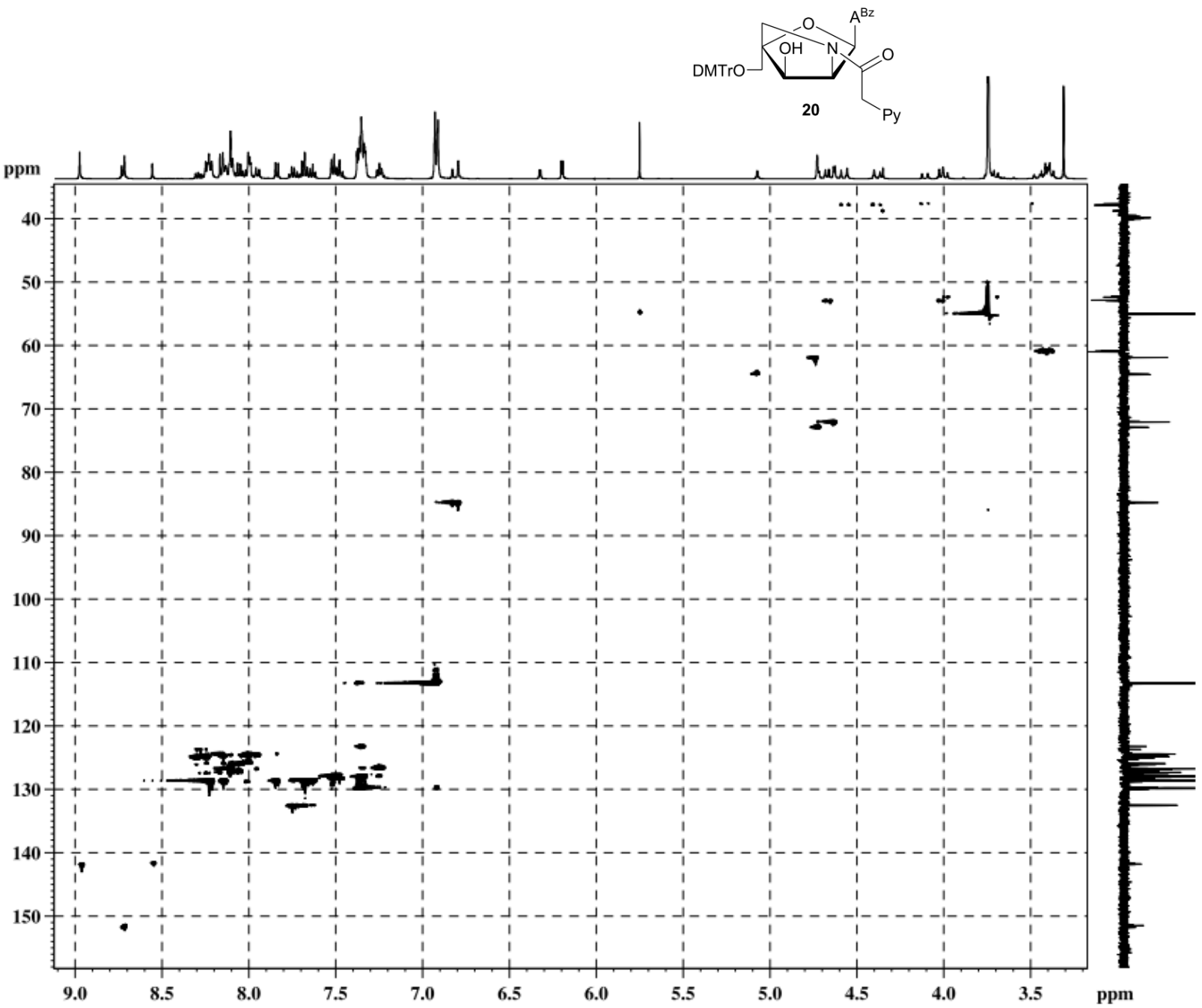
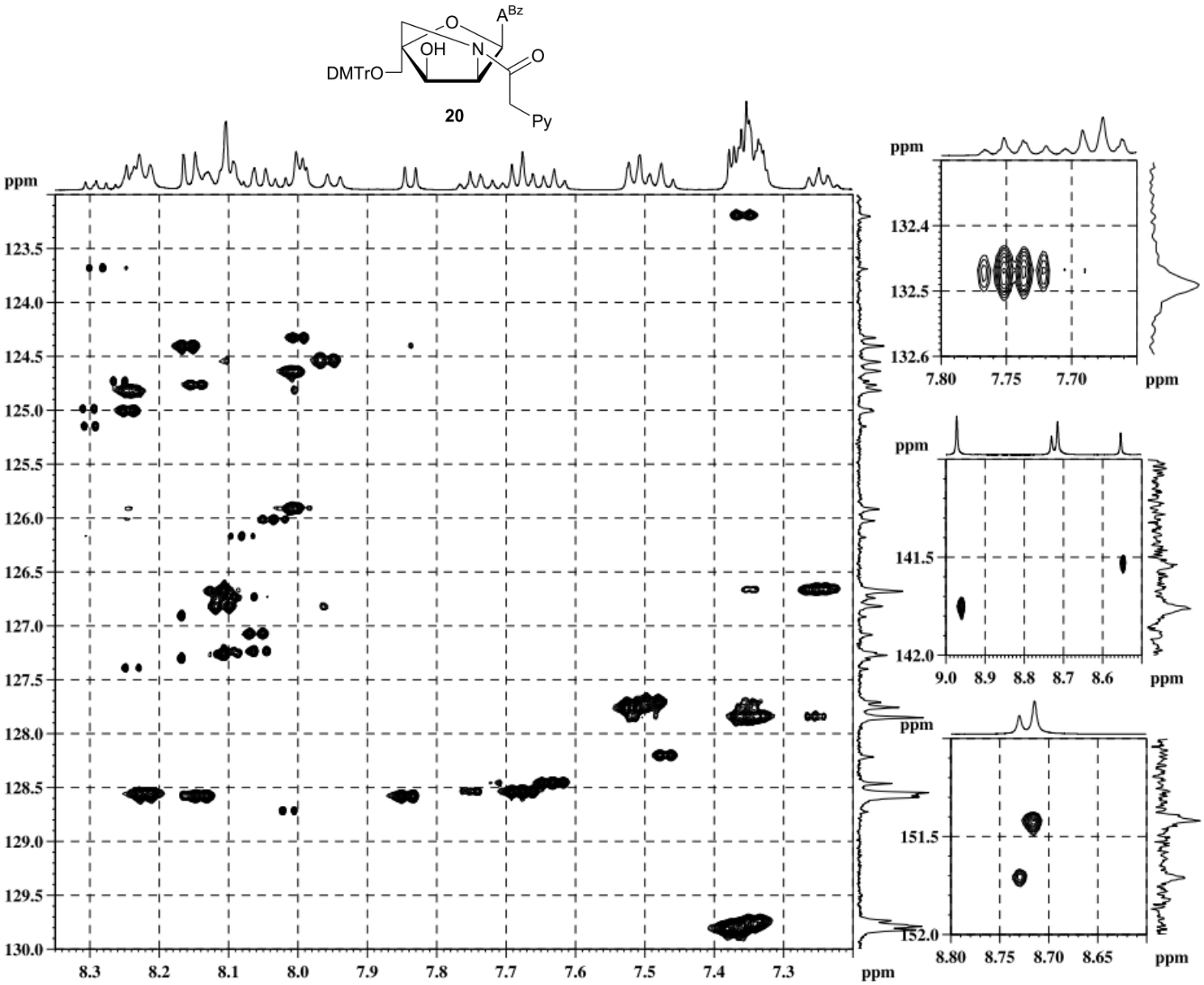
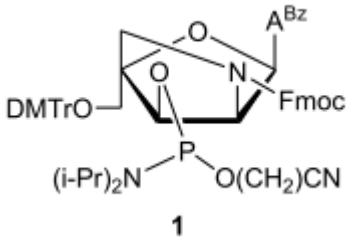
89.016
88.422
85.477
85.403
84.726
84.659
72.885
72.004
64.524
61.834
60.904
60.784
54.998
54.831
52.827
52.304
39.866
39.700
39.532
39.365
39.199
39.032
38.708
37.820
37.650
130.758
130.701
130.689
130.283
130.265
130.147
129.906
129.881
129.820
129.788
129.772
129.735
129.684
129.667
129.619
129.359
129.245
128.949
128.824
128.717
128.581
128.560
128.541
128.459
128.205
127.846
127.765
127.716
127.399
127.311
127.271
127.246
127.085
126.915
126.825
126.738
126.680
126.174
126.020
125.914
125.417
125.302
125.148
125.003
124.986
124.815
124.763
124.727
124.640
124.534
124.404
124.327
124.055
123.893
123.852
123.787
123.763
123.719
123.683
123.199
172.664
169.850
169.606
165.575
165.453
158.111
158.099
151.967
151.711
151.540
151.415
150.559
150.207
144.712
144.679
141.749
141.528
135.370
135.244
133.535
133.472
132.479
ppm
130.5
130.0
129.5
129.0
128.5
128.0
127.5
127.0
126.5
126.0
125.5
125.0
124.5
124.0
123.5
172
170
168
152
133.5
133.0
170
165
160
155
150
145
140
135
130
125
120
115
110
105
100
95
90
85
80
75
70
65
60
55
50
45
40

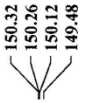

151.713
151.421
141.763
84.717
84.653
61.827
60.908
60.779
38.711
37.817
37.650
DMTrO
OH
O
N
ABz
O
Py
20
132.493
129.822
129.789
129.738
128.589
128.569
128.551
128.464
128.218
127.852
127.758
127.710
127.402
127.314
127.274
127.250
127.084
126.819
126.743
126.681
126.026
125.919
125.008
124.991
124.818
124.768
124.730
124.637
124.553
124.406
124.329
123.690
123.204
113.238
72.875
71.995
64.513
54.991
52.820
52.301
ppm

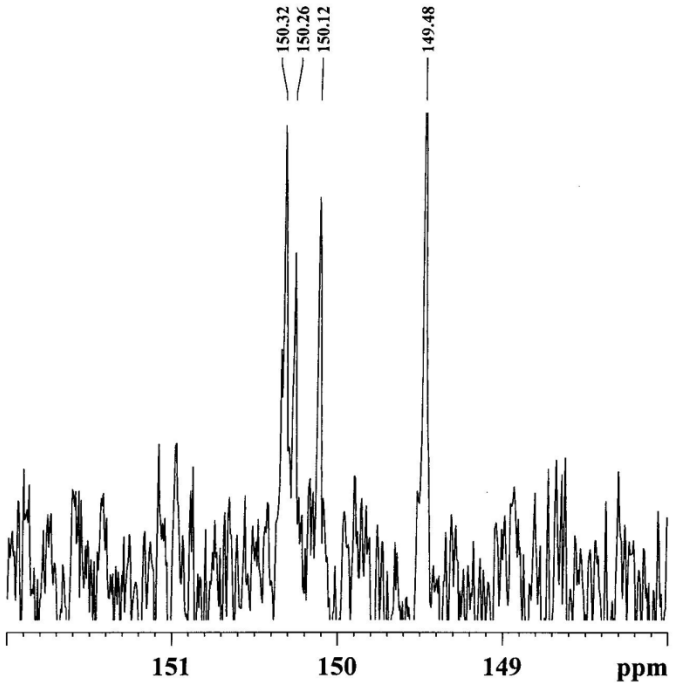

DMTrO
OH
O
N
A^Bz
O
Py
20
ppm
40
50
60
70
80
90
100
110
120
130
140
150
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm

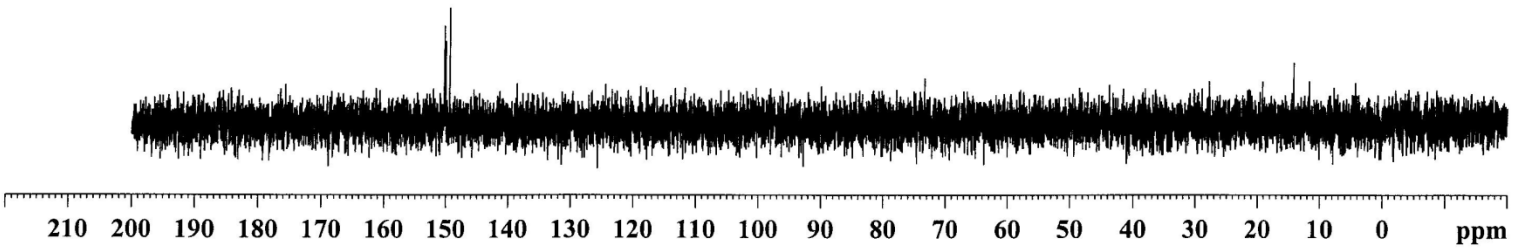

DMTrO
OH
O
N
A^Bz
O
Py
20
ppm
123.5
124.0
124.5
125.0
125.5
126.0
126.5
127.0
127.5
128.0
128.5
129.0
129.5
130.0
8.3
8.2
8.1
8.0
7.9
7.8
7.7
7.6
7.5
7.4
7.3
ppm
ppm
132.4
132.5
132.6
7.80
7.75
7.70
ppm
ppm
141.5
142.0
9.0
8.9
8.8
8.7
8.6
ppm
ppm
151.5
152.0
8.80
8.75
8.70
8.65
ppm

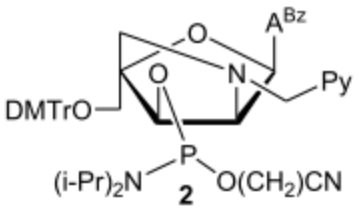

O
A^Bz
N
O
DMTrO
Fmoc
P
(i-Pr)2N
O(CH2)CN

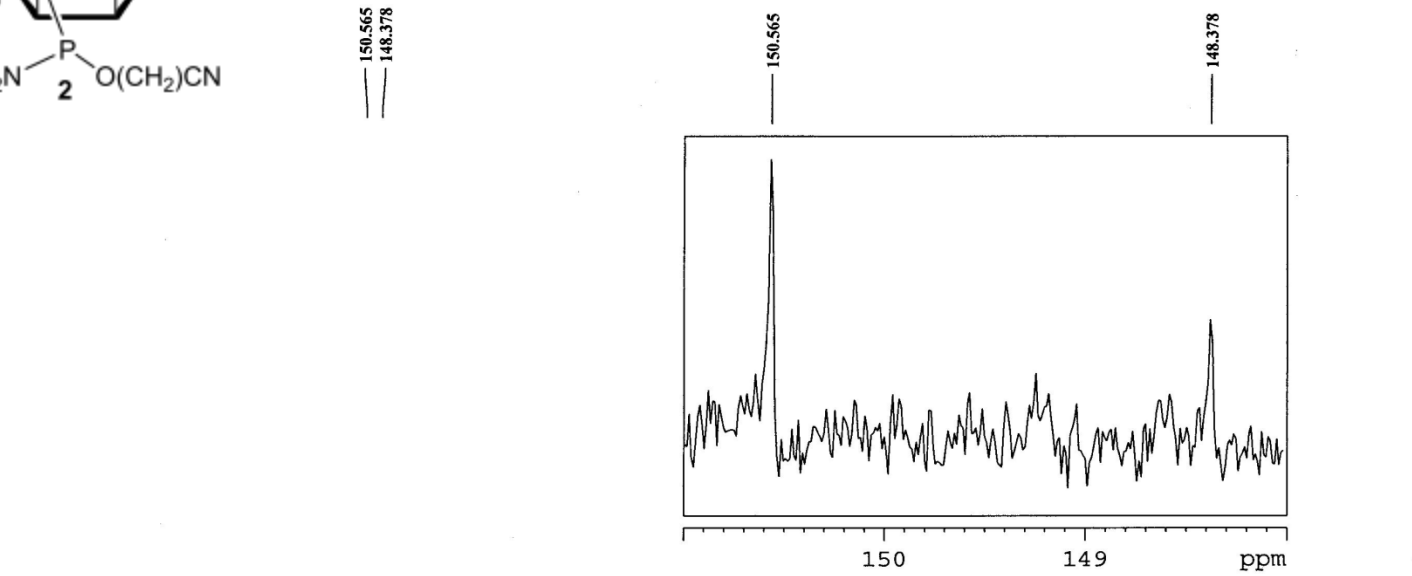
1

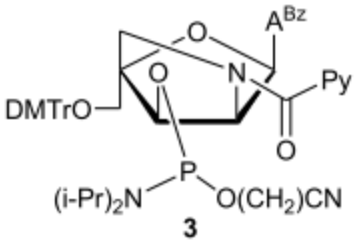

150.32
150.26
150.12
149.48


150.32
150.26
150.12
149.48
151
150
149
ppm

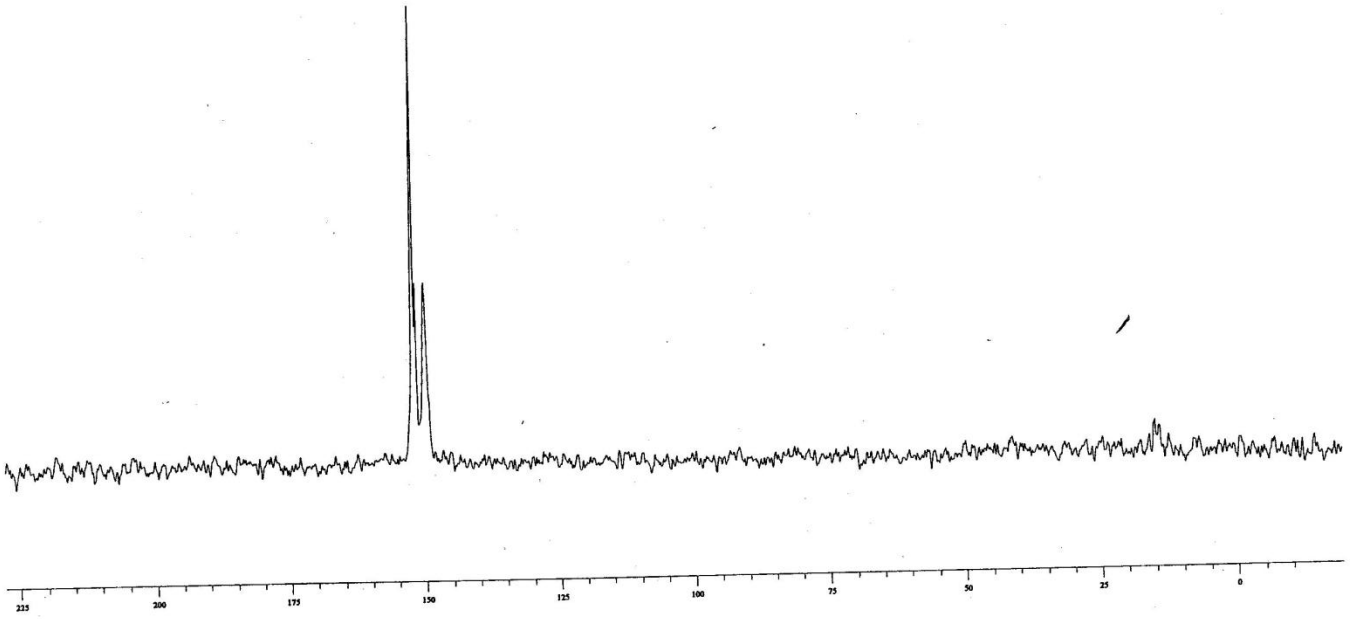

210 200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0
ppm

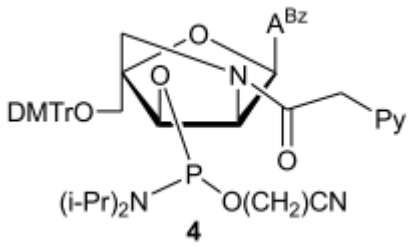

O
A^Bz
O
N
Py
DMTrO
P
(i-Pr)2N
O(CH2)CN
2


150.565
148.378
150.565
148.378
150
149
ppm
180
160
140
120
100
80
60
40
20
ppm

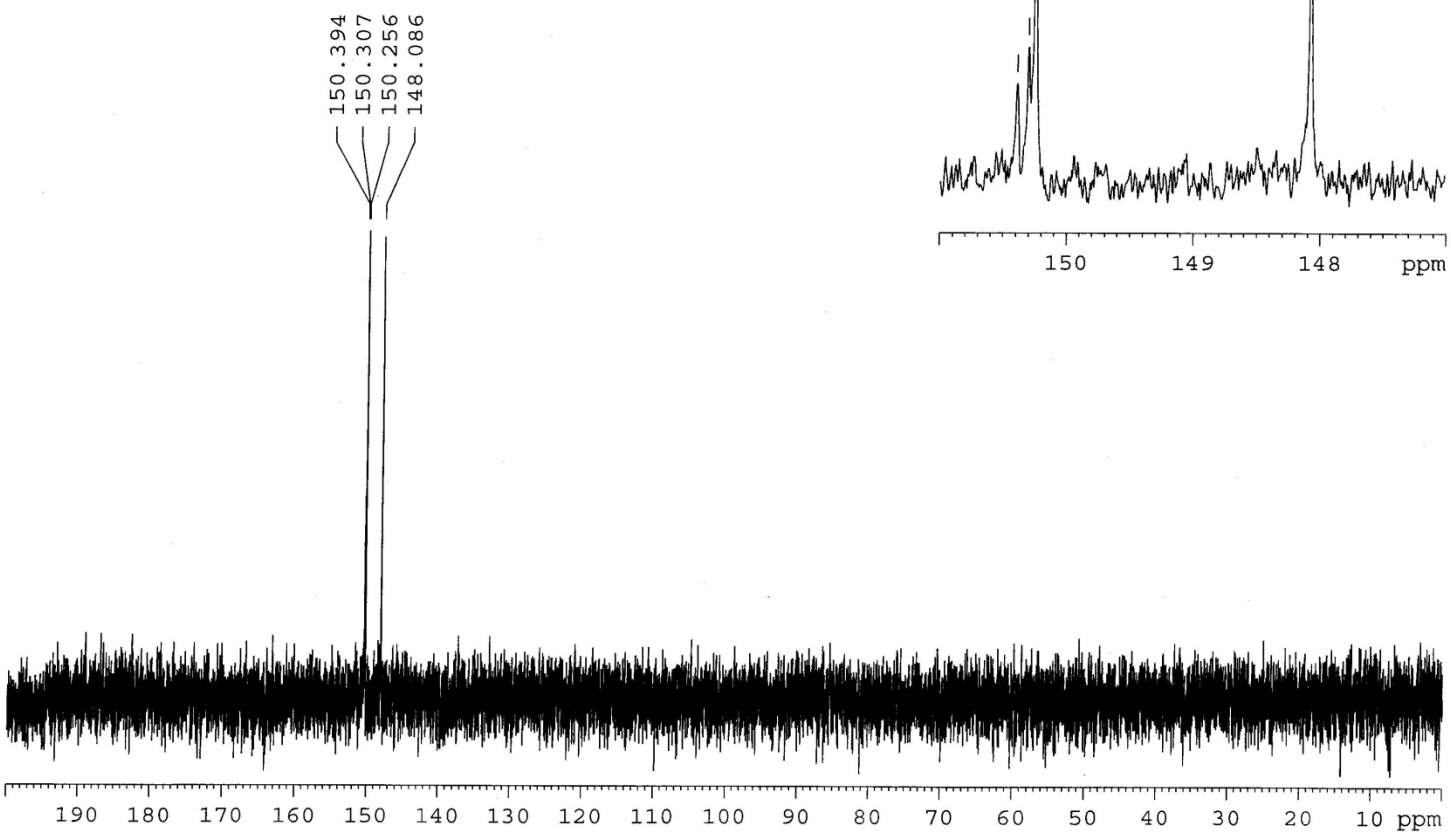

A^Bz
O
N
Py
DMTrO
O
O
P
(i-Pr)2N
O(CH2)CN

3


225
200
175
150
125
100
75
50
25
0


A^Bz
O
N
O
DMTrO
Py
O
P
(i-Pr)2N
O(CH2)CN

4


150.394
150.307
150.256
148.086
150
149
148
ppm
190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 ppm