Biomimetic Reagents for Selective Free Radical and Acid-Base Chemistry of Glycans: Application to Glycan Structure Determination by Mass Spectrometry

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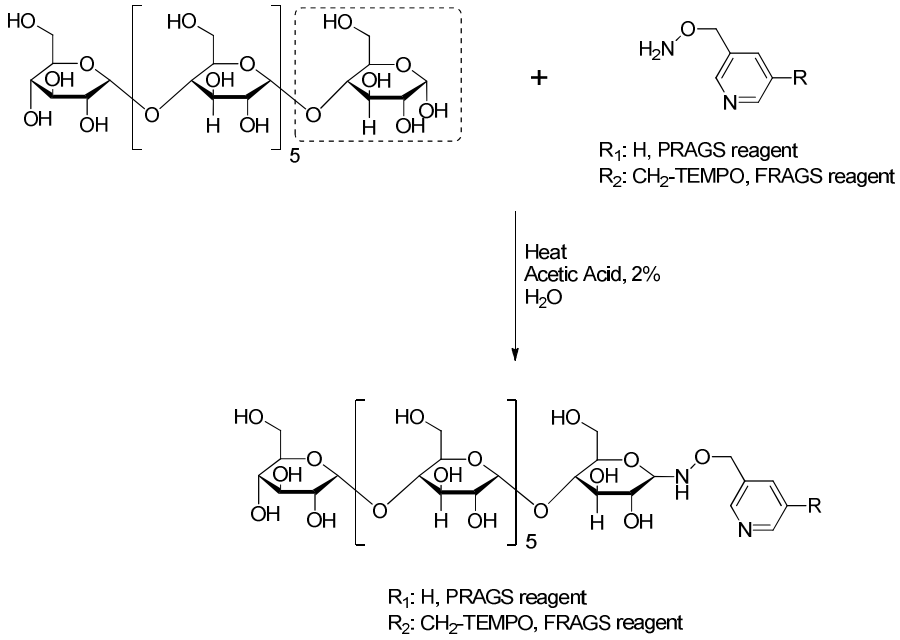
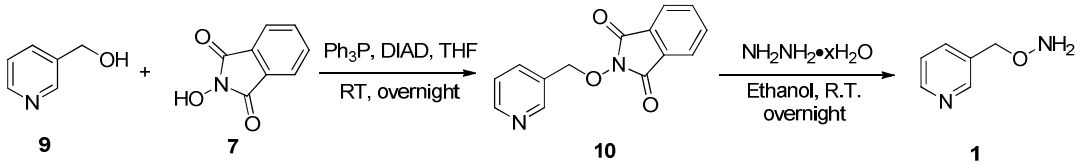
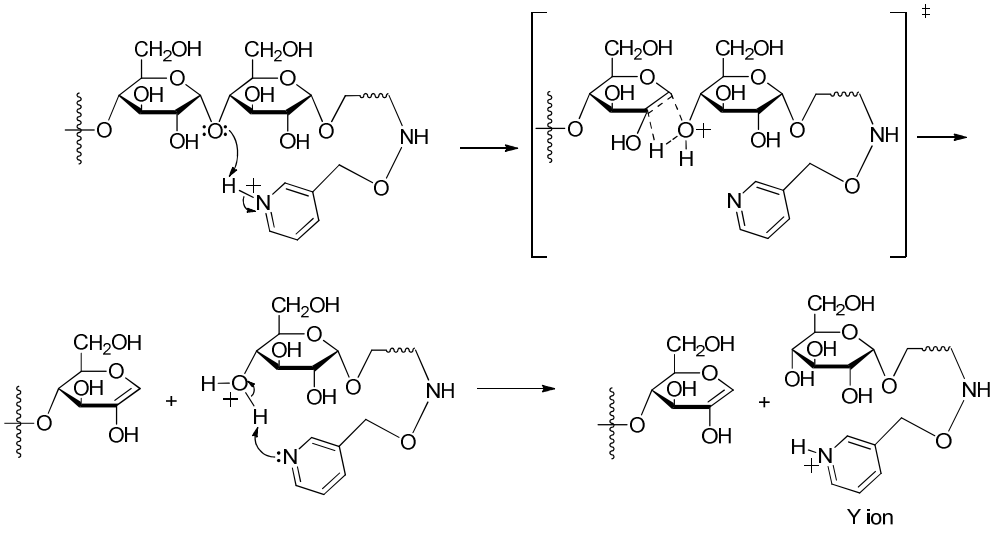


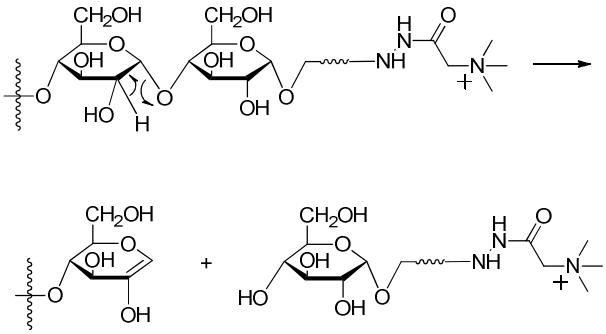
Scheme S1. The derivatization reaction employing maltoheptaose as an example. Portions in the dotted rectangle are the terminal Glucose (reducing terminus) chemical structure undergoing reaction.



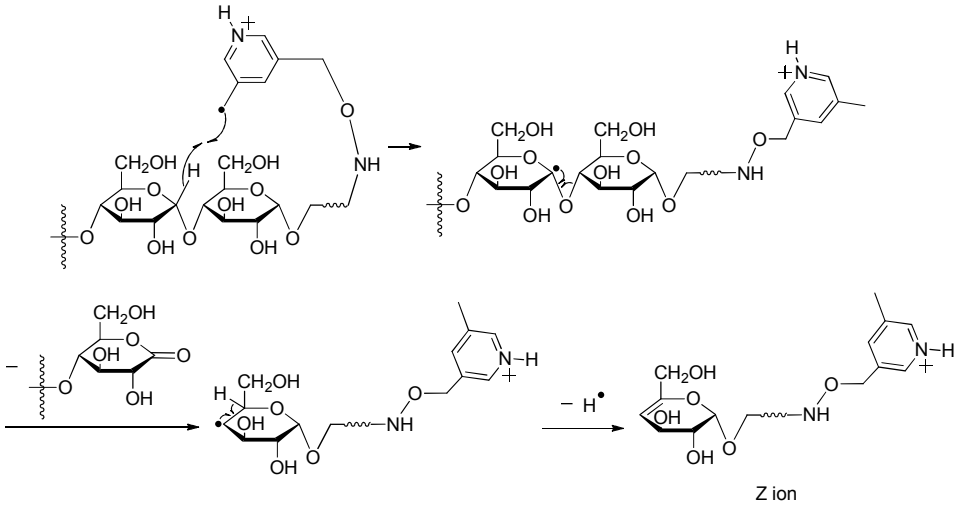
Scheme S2. Synthesis of proton reagent for acid-catalyzed glycan sequencing (PRAGS).



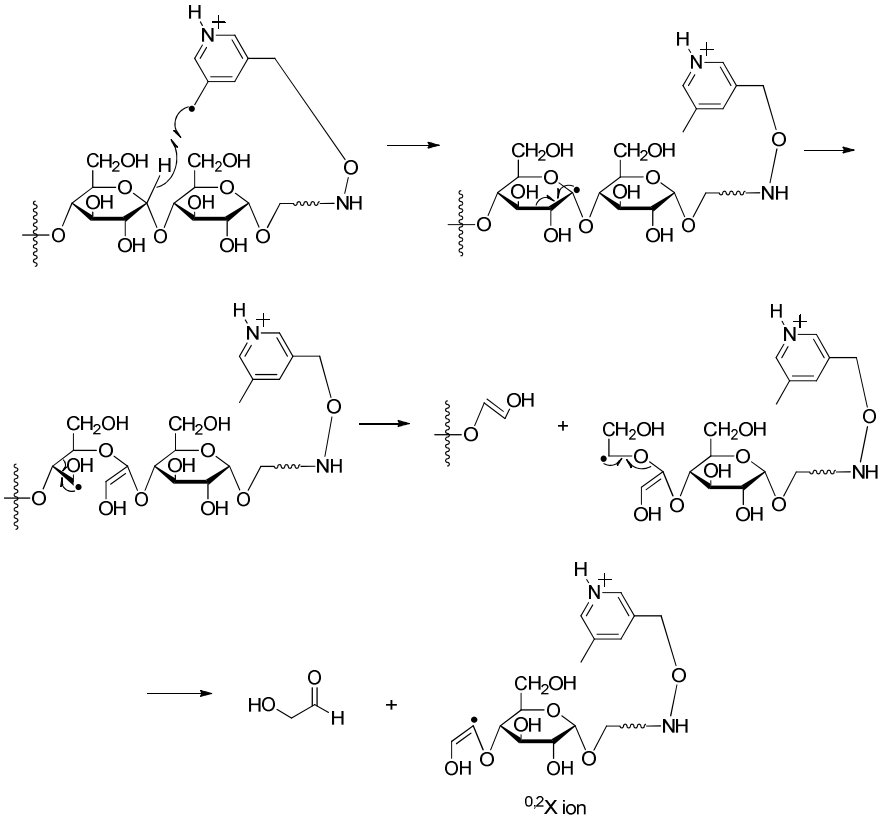
Scheme S3. Alternative mechanism for the formation of Y ions upon CID of singly-protonated PRAGS-derivatized maltoheptaose.



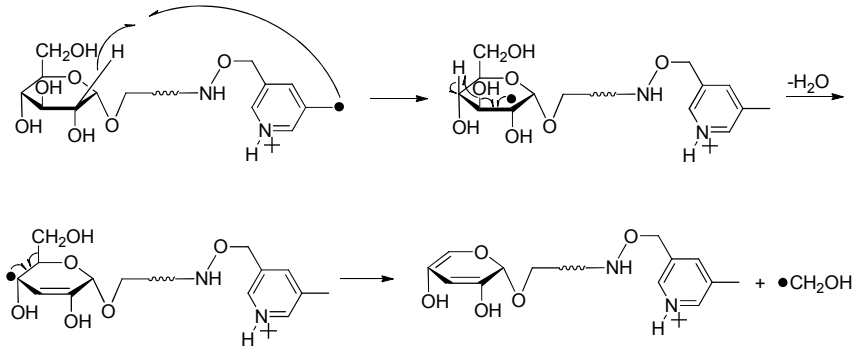
Scheme S4. Proposed mechanism for the formation of Y ions upon CID of GT-derivatized maltoheptaose.



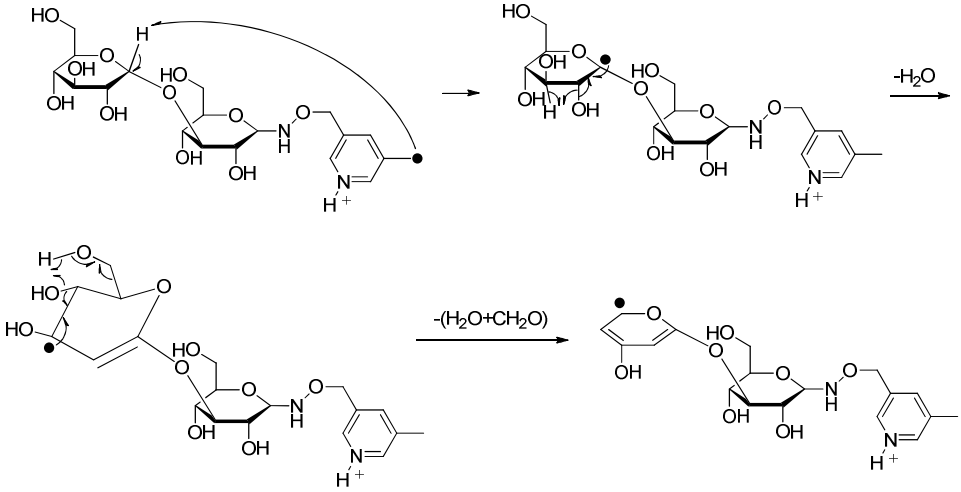
Scheme S5. Proposed alternative mechanism for the formation of Z ions upon CID of singly-protonated FRAGS-derivatized maltoheptaose.



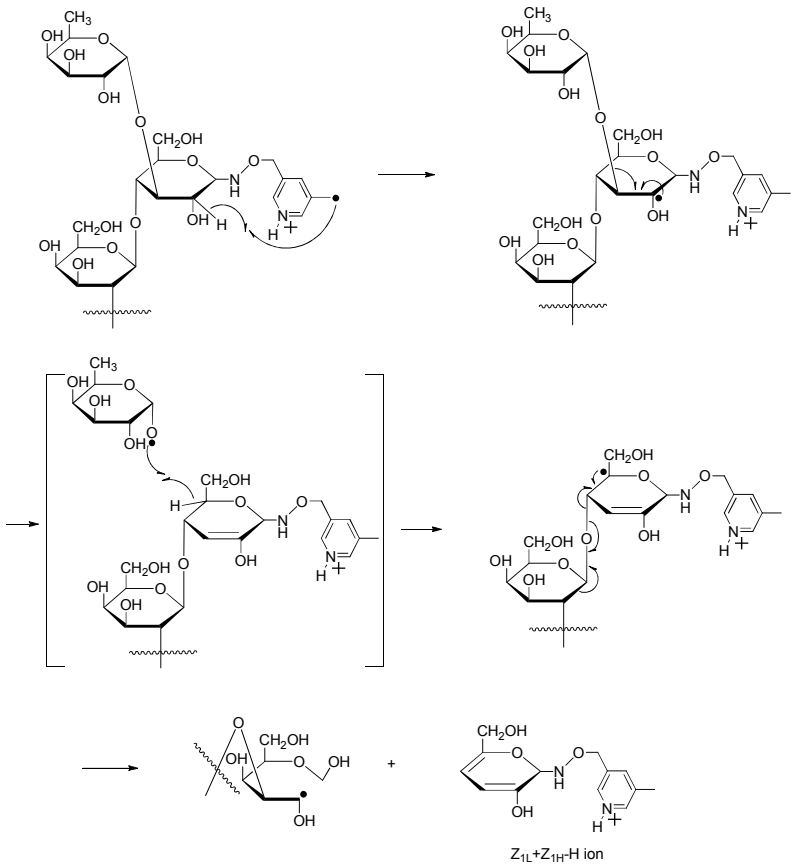
Scheme S6. Proposed mechanism for the formation of ^{0,2}X ions upon CID of singly-protonated FRAGS-derivatized maltoheptaose.



Scheme S7. Proposed mechanism for the formation of n ions upon CID of singly-protonated FRAGS-derivatized maltoheptaose.



Scheme S8. Proposed mechanism for the formation of $–CH_6O_3$ ions upon CID of singly-protonated FRAGS-derivatized nigerose.



Scheme S9. Proposed mechanism for the formation of Z_{1H}+Z_{1L}-H ion upon CID of singly-protonated FRAGS-derivatized LNDFH II.

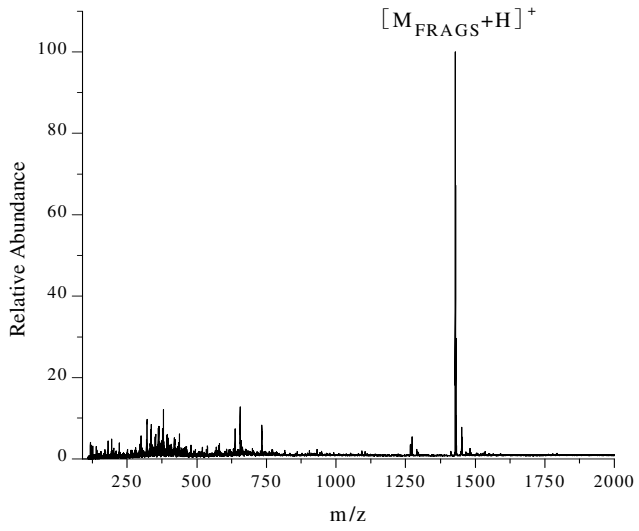


Figure S1. MS^1 spectrum of FRAGS-derivatized maltoheptaose.

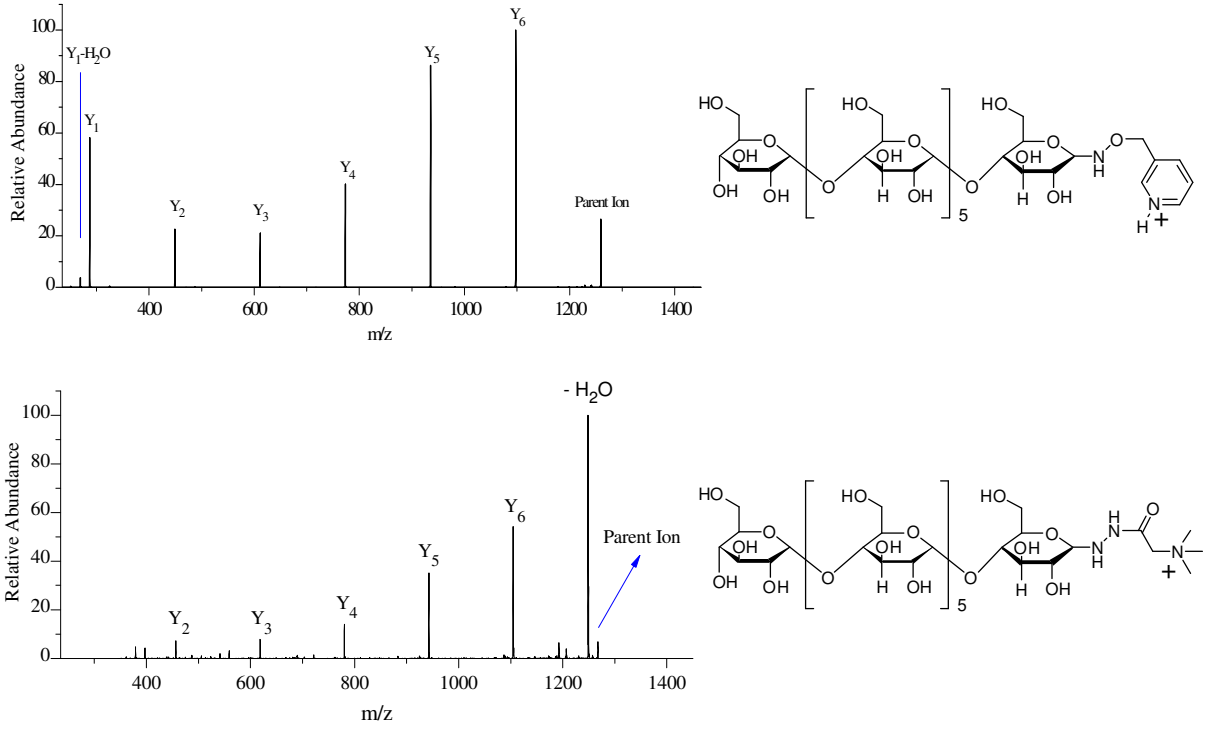


Figure S2. CID spectra for PRAGS- (top) and GT- (bottom) derivatized maltoheptaose.

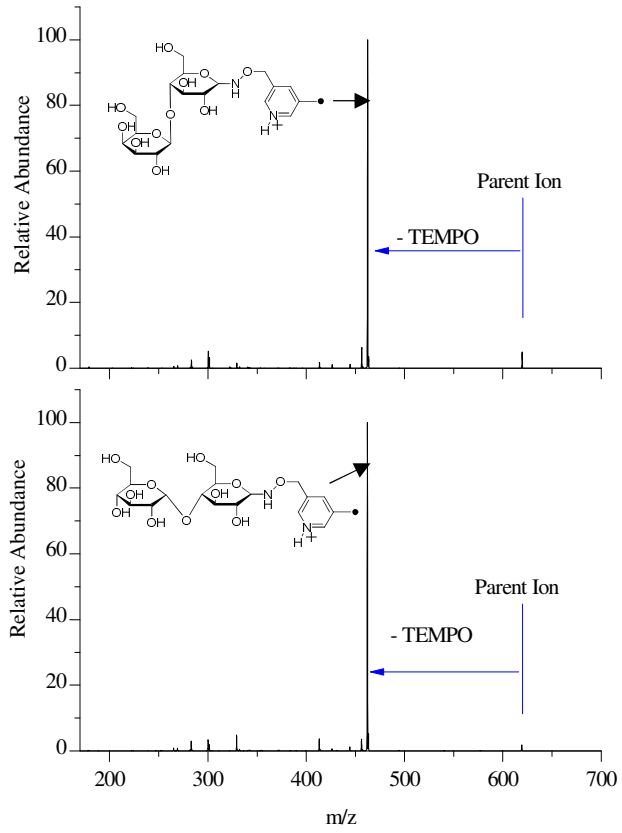


Figure S3: CID of the singly-protonated FRAGS-derivatized lactose (top) and maltose (bottom).

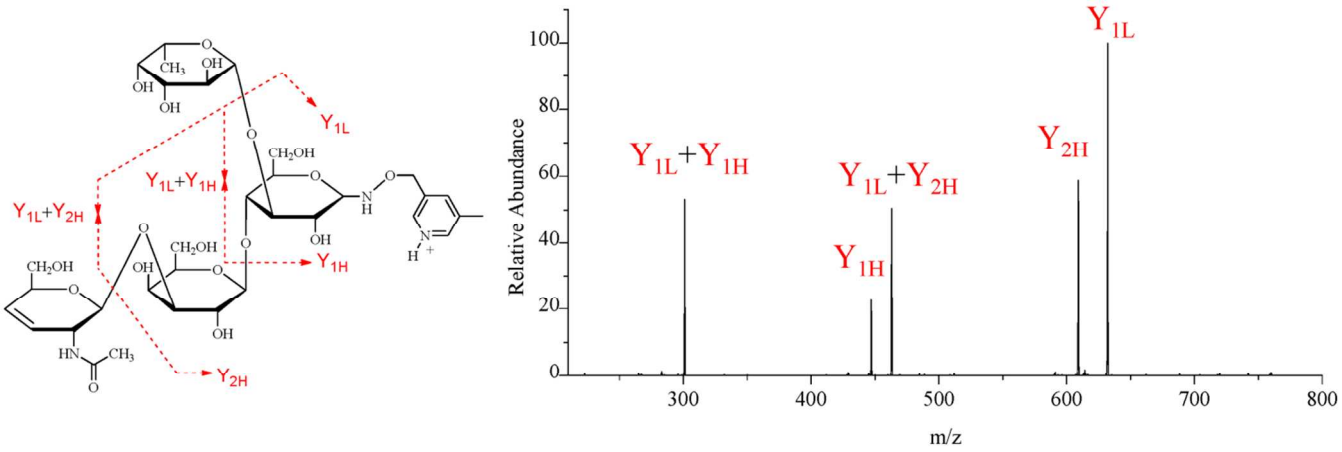


Figure S4. The fragmentation patterns observed following CID of Z_{3HH}+Z_{3HL}+H ion of LNDFH II (left), and the CID spectrum of Z_{3HH}+Z_{3HL}+H ion (right).

Procedure for purification of PRAGS and FRAGS derivatized glycans using C18 pipet tip

1. Attach a C18 pipet tips to a micropipettor and condition twice by aspirating 10 µl of 1:1 acetonitrile/water.

2. Wash pipet tip twice with 10 µl water.

3. Draw up 10 µl of aqueous glycan derivatization solution and return to the main solution. Repeat approximately ten times to saturate the C18 pipet tip with the derivatized glycan.

4. Wash pipet tip twice with 10 µl water.

5. Draw up 10 µl of 1:1 acetonitrile/water and return to the main solution. Repeat approximately ten times to elute glycans.

The solution can be used directly for ESI-MS.

Lewis-Y tetrasaccharide. Lewis-Y tetrasaccharide was used as another model glycan, in which a centralized reducing terminus exists on the second sugar, yielding a branched site following derivatization, as shown in Figure S5. Similar to collisional activation of singly-protonated PRAGS-derivatized maltohelpaose (Figure 2), collisional activation of singly-protonated PRAGS-derivatized Lewis-Y tetrasaccharide generates exclusively C1–O glycosidic bond cleavages retaining charge on the reducing terminus. However, the latter also generates ions with two C1–O glycosidic bond cleavages from each branch retaining charge on the reducing terminus (Y+Y ions, Figure S5). Two product ions, denoted Y_{1H} (m/z 474.3, Figure S5) and $Y_{1L}+Y_{2H}$ (m/z 490.3, Figure S5), can be employed to illustrate the site of reducing terminus as well as the branch structure information of PRAGS-derivatized Lewis-Y tetrasaccharide. The mass difference of Y_{1H} and $Y_{1L}+Y_{2H}$ is due to the mass difference between Fucose (164.2) and galactose (180.2). Therefore, CID of singly-protonated PRAGS-derivatized Lewis-Y tetrasaccharide provides information not only for composition and sequence analysis but also for the branch structure analysis. However, if the branched ions Y_{1H} and $Y_{1L}+Y_{2H}$ ions have the same m/z value, one cannot unambiguously identify the existence of a branched structure. In this case, the FRAGS reagent can be utilized to yield much more branching information as explained below.

Unlike collisional activation of singly-protonated FRAGS-derivatized maltoheptaose, collisional activation of singly-protonated FRAGS-derivatized Lewis-Y tetrasaccharide generates not only a systematic series of $^{1,5}X$, $^{0,2}X$, Y and Z ions but also a systematic series of $Y+^{1,5}X$ ions and Y+Y ions (Figure S5). It needs to be pointed out that there are no n ions formed. The Y and Y+Y ions have been observed in the CID of singly-protonated PRAGS-derivatized Lewis-Y tetrasaccharide. Two product ions, denoted $^{1,5}X_{2H}+Y_{1L}$ or $^{1,5}X_{1L}+Y_{2H}$ (m/z 532.3, Figure S5), $^{1,5}X_{1H}+Y_{1L}$ or $^{1,5}X_{1L}+Y_{1H}$ (m/z 370.2, Figure S5), can be employed to illustrate the existence of the branch structure. Moreover, two more characteristic ions, $Z_{1H}+Z_{1L}-H$ (m/z 306.2, Figure S5) and $Z_{1H}+Y_{1L}$ or $Z_{1L}+Y_{1H}$ (m/z 324.2, Figure S5), which are produced only at the branched site, provide additional evidence for the existence of the branch structure. Furthermore, $Z_{1H}+Z_{1L}-H$ ion provides information for the site of reducing terminus.

FRAGS is capable of identifying branched sites in even more complicated glycans, as outlined in the analysis of the CID spectrum of FRAGS-derivatized Lacto-N-difucohexaose II (Figure 6).

The formation of Z_{1H}+Z_{1L}-H ion is proposed to occur-via a radical driven mechanism (Scheme S7). Briefly, dissociation is proposed to occur via hydrogen atom abstraction by the nascent free radical from C2 of the central saccharide unit, followed by homolytic cleavage of the α-glycosidic bond to form a double bond between C2 and C3. The resulting oxygen-centered radical then abstracts a hydrogen atom from C5, followed by homolytic cleavage of the β-glycosidic bond to form a double bond between C4 and C5.

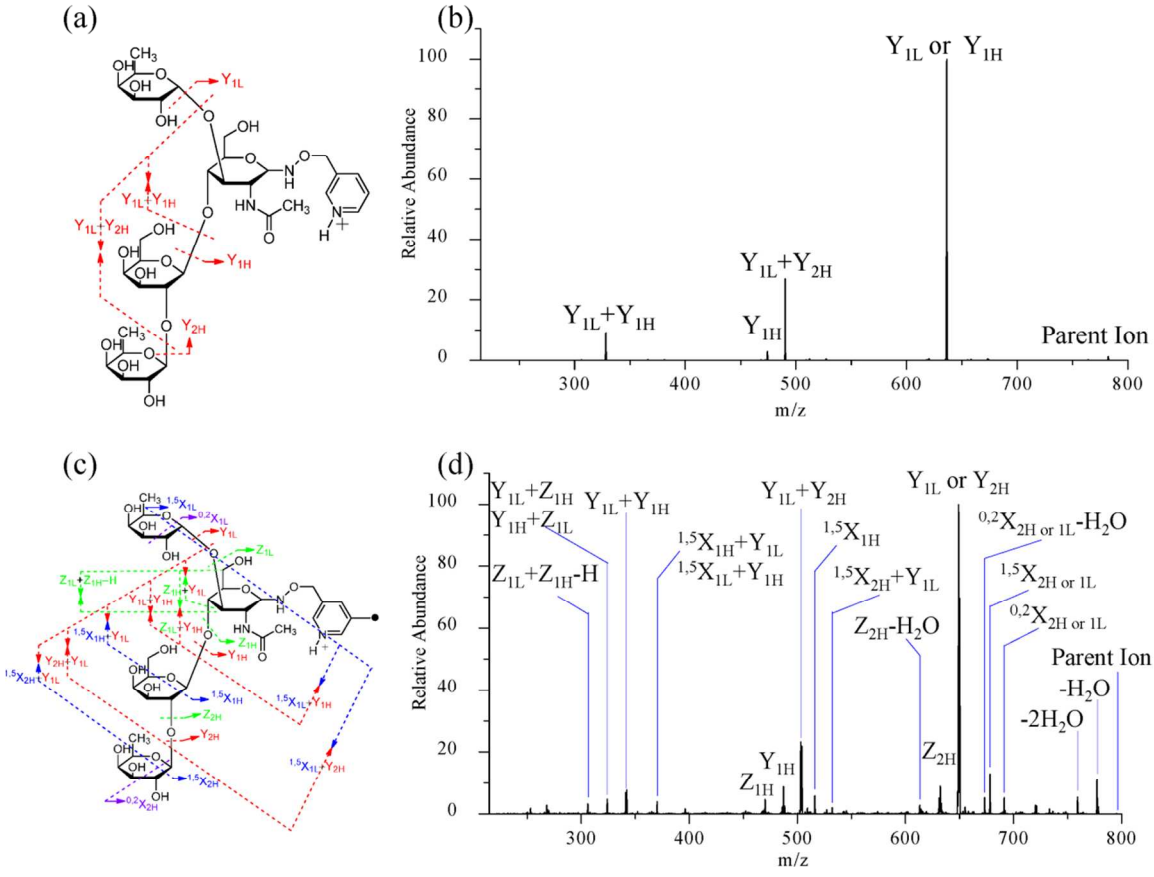
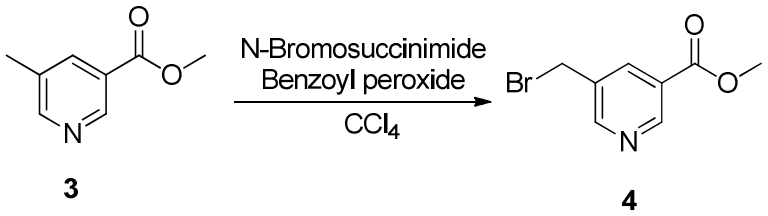


Figure S5. The the fragmentation patterns observed following CID of singly-protonated PRAGS-derivatized (a) and FRAGS-derivatized (c) Lewis-Y tetrasaccharide, and CID specta of singly-protonated PRAGS-derivatized (b) and FRAGS-derivatized (d) Lewis-Y tetrasaccharide.

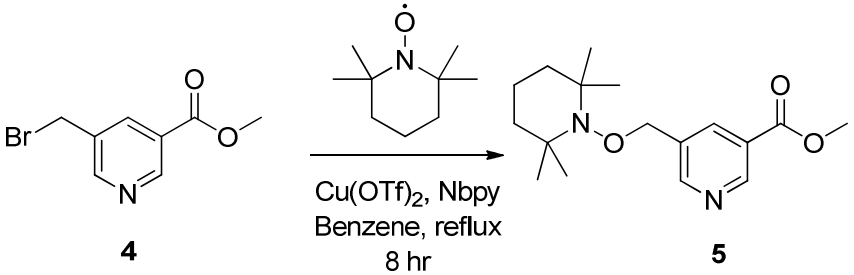
Synthesis of FRAGS and PRAGS reagents

Methyl 5-(bromomethyl)nicotinate (4)



To a solution of methyl 5-methylnicotinate (**3**) (1.51 g, 10 mmol) and *N*-bromosuccinimide (NBS) (2.13 g, 12 mmol) in CCl_4 (50 mL) was added benzoyl peroxide (24.2 mg, 0.1 mmol) under argon.[1] The reaction mixture was stirred under reflux for 10 h. After cooling to room temperature, the reaction mixture was extracted with CH_2Cl_2 (×3). The extract was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (hexane–EtOAc 1 : 1) to give methyl 5-(bromomethyl)nicotinate (**4**) as a white solid (1.17 g, 51%). ^{1}HNMR (500 MHz, $CDCl_3$, δ ppm): 9.13 (d, J = 2.0 Hz, 1H), 8.78 (d, J = 2.2 Hz, 1H), 8.32 (t, J = 2.2 Hz, 1H), 4.50 (s, 2H), 3.96 (s, 3H).); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm), 28.6, 52.5, 126.0, 133.6, 137.5, 150.4, 153.3, 165.1. ESI-MS: $[M+H]^+$, 230.0, 232.0.

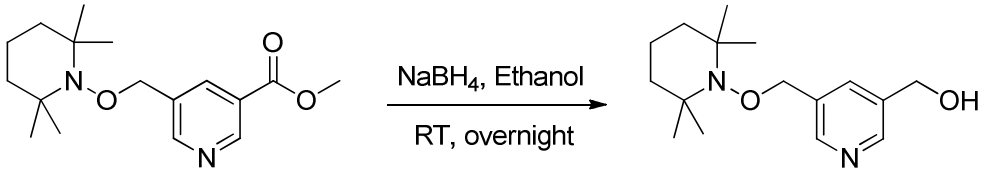
Methyl 5-(((2,2,6,6-tetramethylpiperidin-1-yl)oxy)methyl)nicotinate (5)



To a Schlenk flask was added **4** (1.15 g, 5mmol), (2,2,6,6-tetramethylpiperidin-1-yl)oxyl (TEMPO, 936 mg, 6 mmol), $Cu(OTf)_2$ (217 mg, 0.6 mmol), copper powder (32.0 mg, 5 mmol), 4,4'-dinonyl-2,2'-bipyridyl (Nbpy, 818 mg, 2 mmol), and benzene (15 mL).[1] The reaction mixture was degassed by bubbling argon for 5 min and heated at 80 ˚C overnight. After cooling the reaction mixture to room

temperature, it was filtered through a short pad of silica gel using EtOAc. The filtrate was concentrated in vacuo and the residue was purified by flash column chromatography using hexane–EtOAc (3 : 1). The desired product **5** was obtained as a white solid (1.16 g, 76%). ^{1}HNMR (500 MHz, $CDCl_3$, δ ppm): 9.09 (d, J = 2.1 Hz, 1H), 8.70 (d, J = 2.0 Hz, 1H), 8.20 (m, 1H), 4.84 (s, 2H), 3.92 (s, 3H), 1.53 (m, 1H), 1.45 (m, 4H), 1.30 (m, 1H), 1.19 (s, 6H), 1.10 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm), 16.9, 20.1, 32.7, 39.5, 52.3, 60.0, 75.7, 125.6, 133.4, 135.8, 149.7, 152.5, 165.7. ESI-MS: $[M+H]^+$, 307.3.

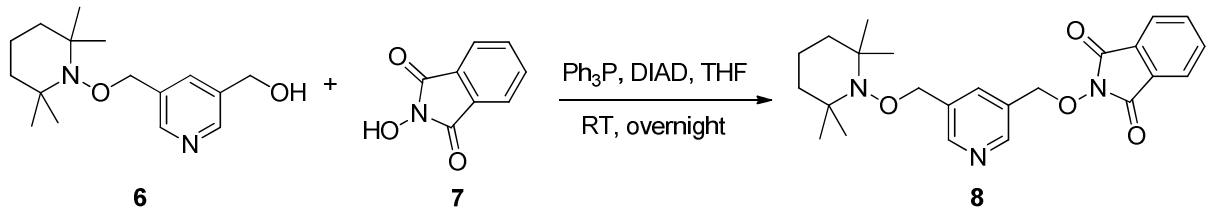
(5-(((2,2,6,6-Tetramethylpiperidin-1-yl)oxy)methyl)pyridin-3-yl)methanol (6)



5 **6**

To a solution of **5** (1.07 g, 3.5 mmol) in anhydrous ethanol (20 mL) was added sodium borohydride (266 mg, 7 mmol) portionally under argon.[2] The reaction mixture was stirred under reflux overnight. After cooling to room temperature, the reaction mixture was diluted by adding 50 ml EtOAc, washed by water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (hexane–EtOAc 1 : 1 to 1 : 5) to give the desired product **6** as a white solid (731 mg, 75%). ^{1}HNMR (500 MHz, CD_3OD, δ ppm): 8.45 (d, J = 2.1 Hz, 1H), 8.43 (d, J = 2.1 Hz, 1H), 7.83 (m, 1H), 4.89 (s, 2H), 4.68 (m, 2H), 1.66 (m, 1H), 1.54 (m, 4H), 1.38 (m, 1H), 1.26 (s, 6H), 1.16 (s, 6H); ^{13}C NMR (125 MHz, CD_3OD, δ ppm), 18.2, 20.8, 33.7, 40.9, 61.4, 62.5, 77.3, 135.5, 136.1, 139.1, 148.0. ESI-MS: $[M+H]^+$, 279.3.

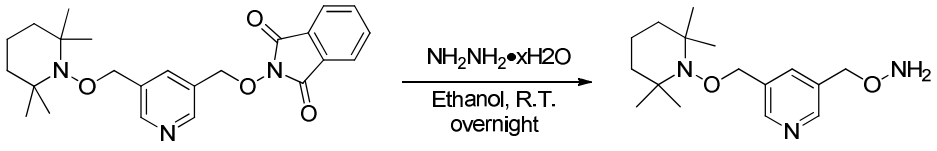
2-((5-(((2,2,6,6-Tetramethylpiperidin-1-yl)oxy)methyl)pyridin-3-yl)methoxy)isoindoline-1,3-dione (8)



6 7 8

To a solution of **6** (557 mg, 2 mmol), 2-hydroxyisoindoline-1,3-dione (**7**, 391mg, 2.4 mmol), and triphenylphosphine (629 mg, 2.4 mmol) in anhydrous THF (20 mL) was added a solution of diisopropyl azodicarboxylate (DIAD, 525 mg, 2.6 mmol) in 5 ml anhydrous THF at 0 ˚C under argon.[3] The reaction mixture was stirred at 0 ˚C for 30 minutes and then warmed to room temperature and stirred overnight. The reaction mixture was washed by water and brine, dried over Na_2SO_4, filtered, and concentrated in vacuo. The crude product was purified by flash chromatography on silica gel (hexane–EtOAc 1 : 2) to give the desired product **8** as a white solid (695 mg, 82%). ^{1}HNMR (500 MHz, $CDCl_3$, δ ppm): 8.60 (d, J = 2.1 Hz, 1H), 8.58 (d, J = 2.0 Hz, 1H), 7.86 (t, J = 2.1 Hz, 1H), 7.78 (dd, J_1 = 2.1 Hz, J_2 = 5.5 Hz, 2H), 7.71 (dd, J_1 = 2.1 Hz, J_2 = 5.5 Hz, 2H), 5.22 (s, 2H), 4.83 (s, 2H), 1.55 (m, 1H), 1.45 (m, 4H), 1.32 (m, 1H), 1.26 (s, 6H), 1.15 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm), 16.9, 20.1, 32.9, 39.5, 60.0, 75.8, 77.0, 123.5, 128.6, 128.9, 133.5, 134.5, 136.3, 149.5, 149.7, 163.2. ESI-MS: $[M+H]^+$, 424.4.

***O*-((5-(((2,2,6,6-tetramethylpiperidin-1-yl)oxy)methyl)pyridin-3-yl)methyl)hydroxylamine (FRAGS, 2)**

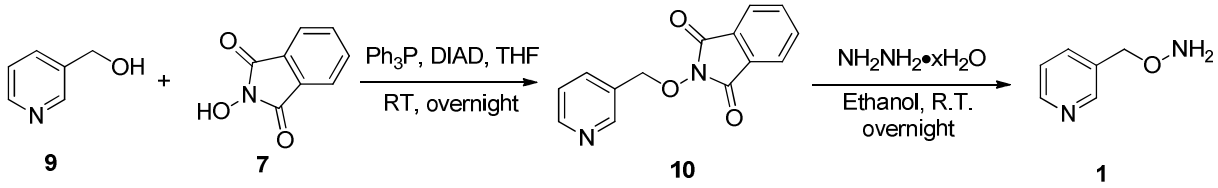


8 2

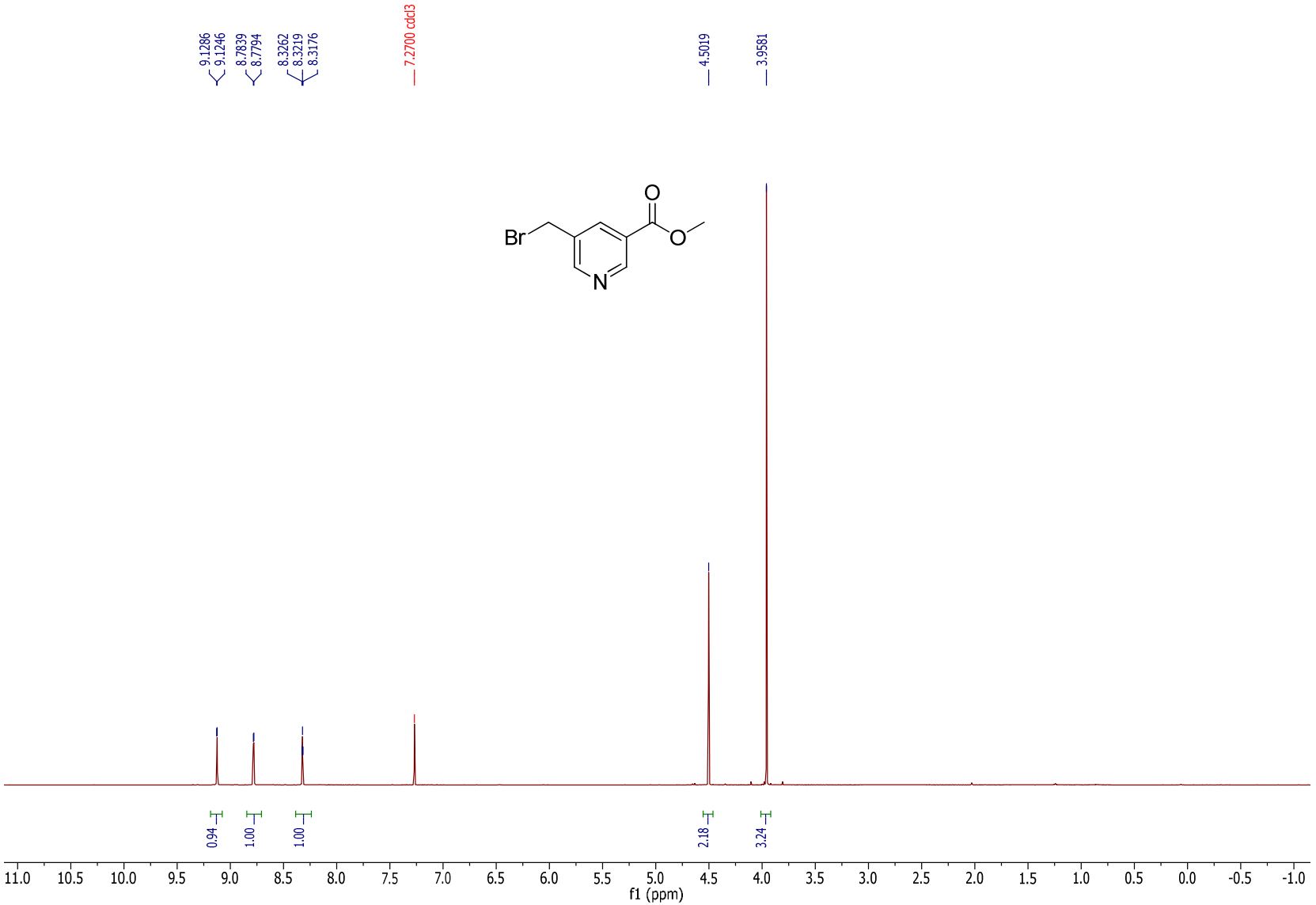
To a solution of **8** (1.07 g, 1.5 mmol) in anhydrous ethanol (20 mL) was added hydrazine hydrate (N_2H_4, 50-60%, 0.86 mL, 15 mmol) portionally under argon.[3] The reaction mixture was stirred under

reflux overnight. After cooling to room temperature, the reaction mixture was concentrated in vacuo. The crude product was purified by flash chromatography on silica gel ($CHCl_3$–Hexane 3 : 1) to give the desired product **2** as a white solid (378 mg, 86%). ^{1}HNMR (500 MHz, $CDCl_3$, δ ppm): 8.57 (s, 1H), 8.54 (s, 1H), 7.67 (t, J = 2.1 Hz, 1H), 4.86 (s, 2H), 4.72 (s, 2H), 1.59 (m, 1H), 1.50 (m, 4H), 1.37 (m, 1H), 1.26 (s, 6H), 1.15 (s, 6H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm), 17.0, 20.3, 33.0, 39.6, 60.1, 75.2, 76.2, 132.6, 133.4, 135.3, 148.5, 148.8. ESI-MS: $[M+H]^+$, 294.4.

***O*-(pyridin-3-ylmethyl)hydroxylamine (PRAGS, 1)**



O-(pyridin-3-ylmethyl)hydroxylamine (PRAGS, **1**) was synthesized following the procedure for synthesis of FRAGS reagent (**2**).[3] Overall yield 46%. ^{1}HNMR (500 MHz, CD_3OD, δ ppm): 8.54 (d, 1H), 8.47 (dd, J_1 = 1.6 Hz, J_2 = 5.0 Hz, 1H), 7.85 (m, 1H), 7.44 (m, 1H), 4.71 (s, 2H); ^{13}C NMR (125 MHz, $CDCl_3$, δ ppm), 75.4, 125.3, 135.7, 138.4, 149.5, 150.1. ESI-MS: $[M+H]^+$, 125.1.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O
O
N
0.94
1.00
1.00
2.18
3.24
11.0
10.5
10.0
9.5
9.0
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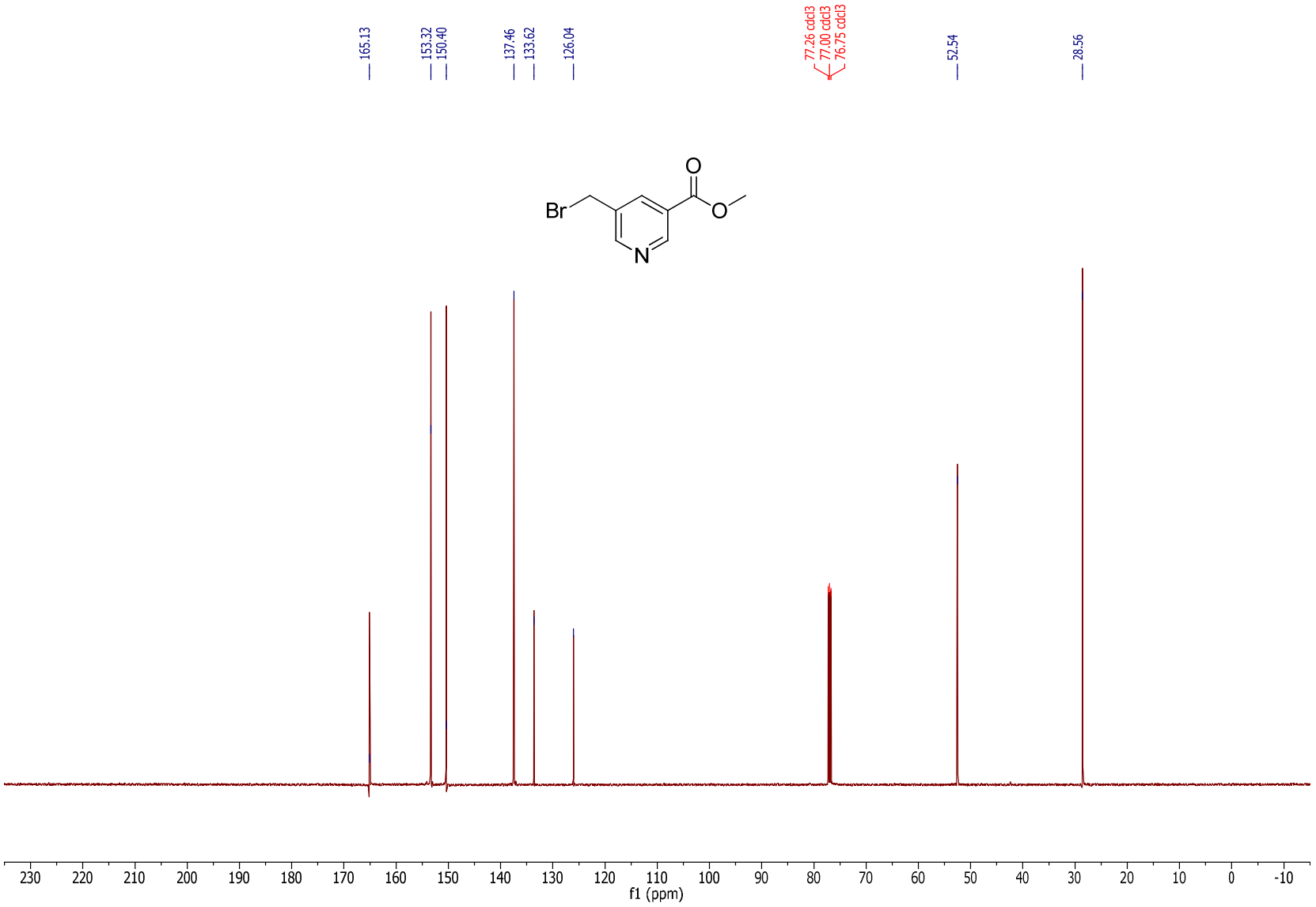
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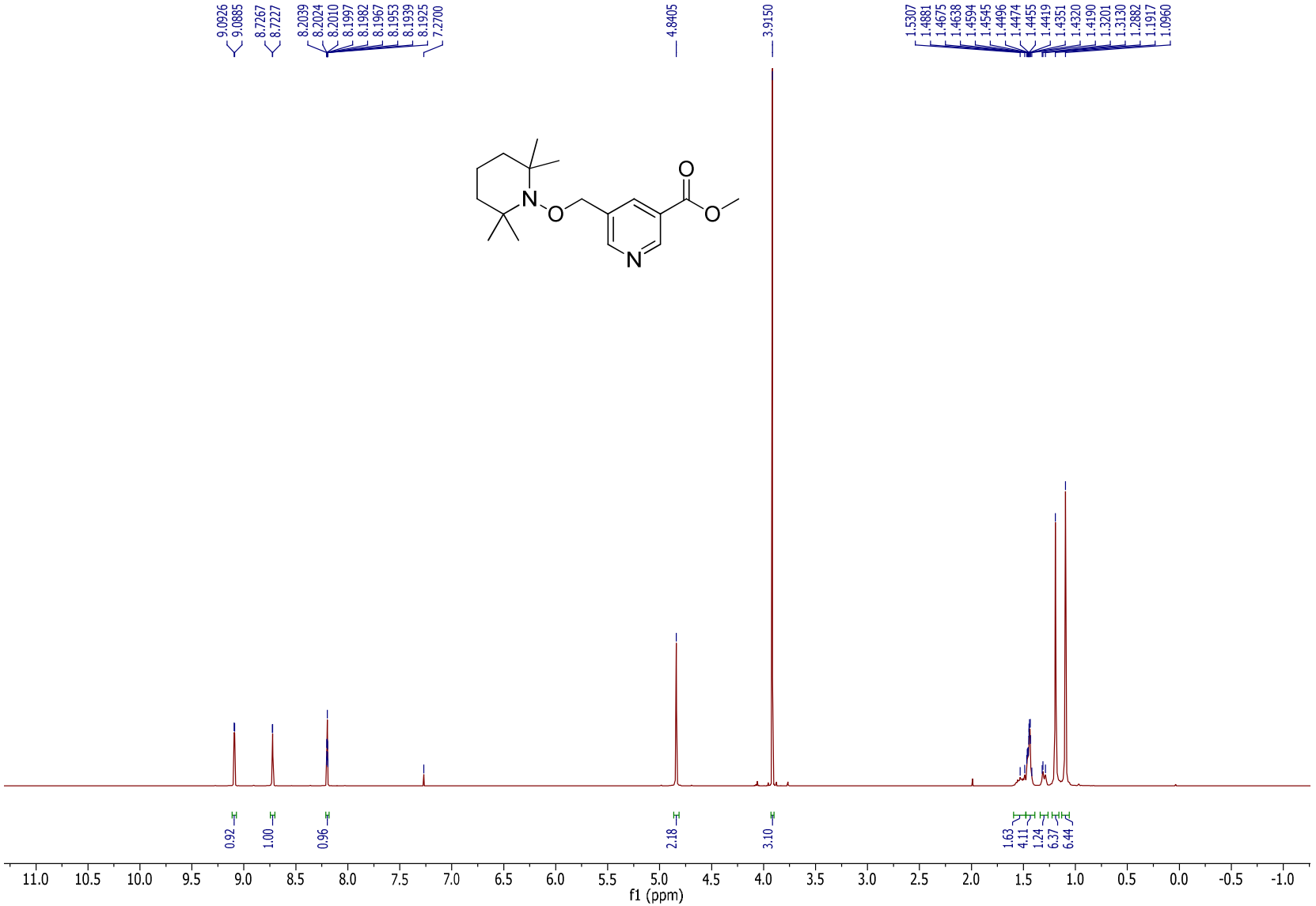
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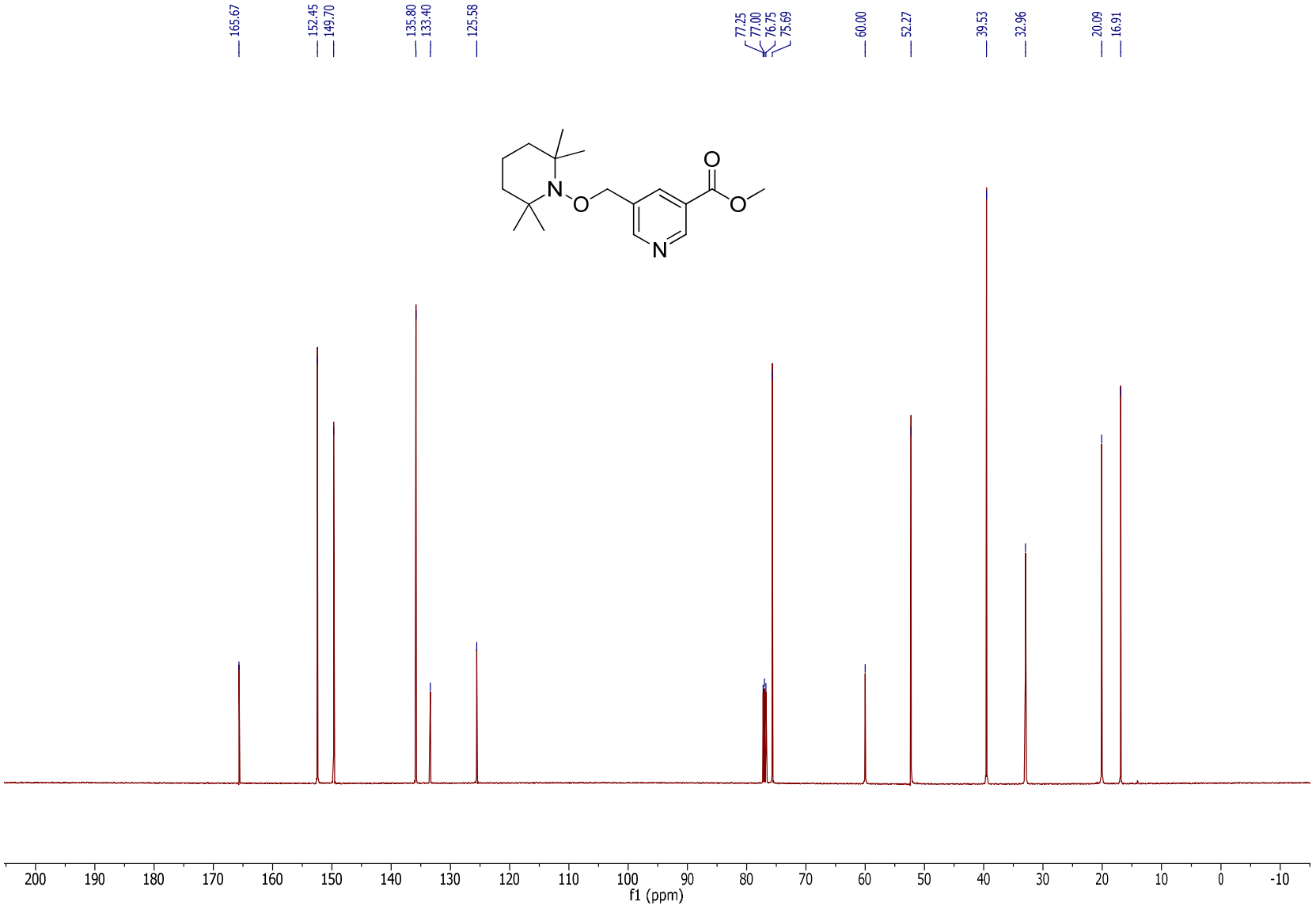
f1 (ppm)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30
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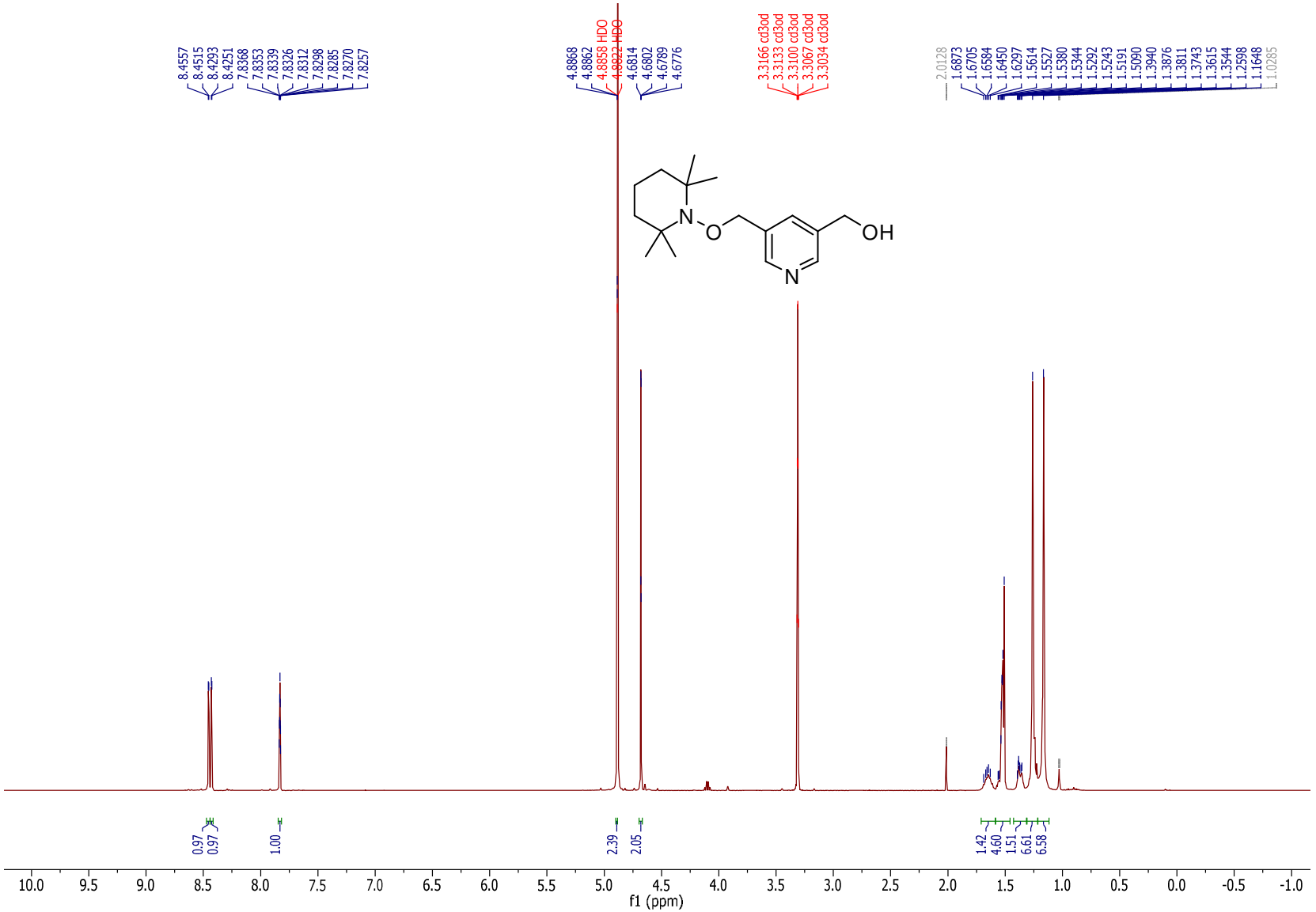
-10
f1 (ppm)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7.8285
7.8270
7.8257
4.8868
4.8862
4.8858 HDO
4.8822 HDO
4.6814
4.6802
4.6789
4.6776
3.3166 cd3od
3.3133 cd3od
3.3100 cd3od
3.3067 cd3od
3.3034 cd3od
2.0128
1.6873
1.6705
1.6584
1.6450
1.6297
1.5614
1.5527
1.5380
1.5344
1.5292
1.5243
1.5191
1.5090
1.3940
1.3876
1.3811
1.3743
1.3615
1.3544
1.2598
1.1648
1.0265
N
O
OH
N
0.97
0.97
1.00
2.39
2.05
1.42
4.60
1.51
6.61
6.58
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9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
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2.0
1.5
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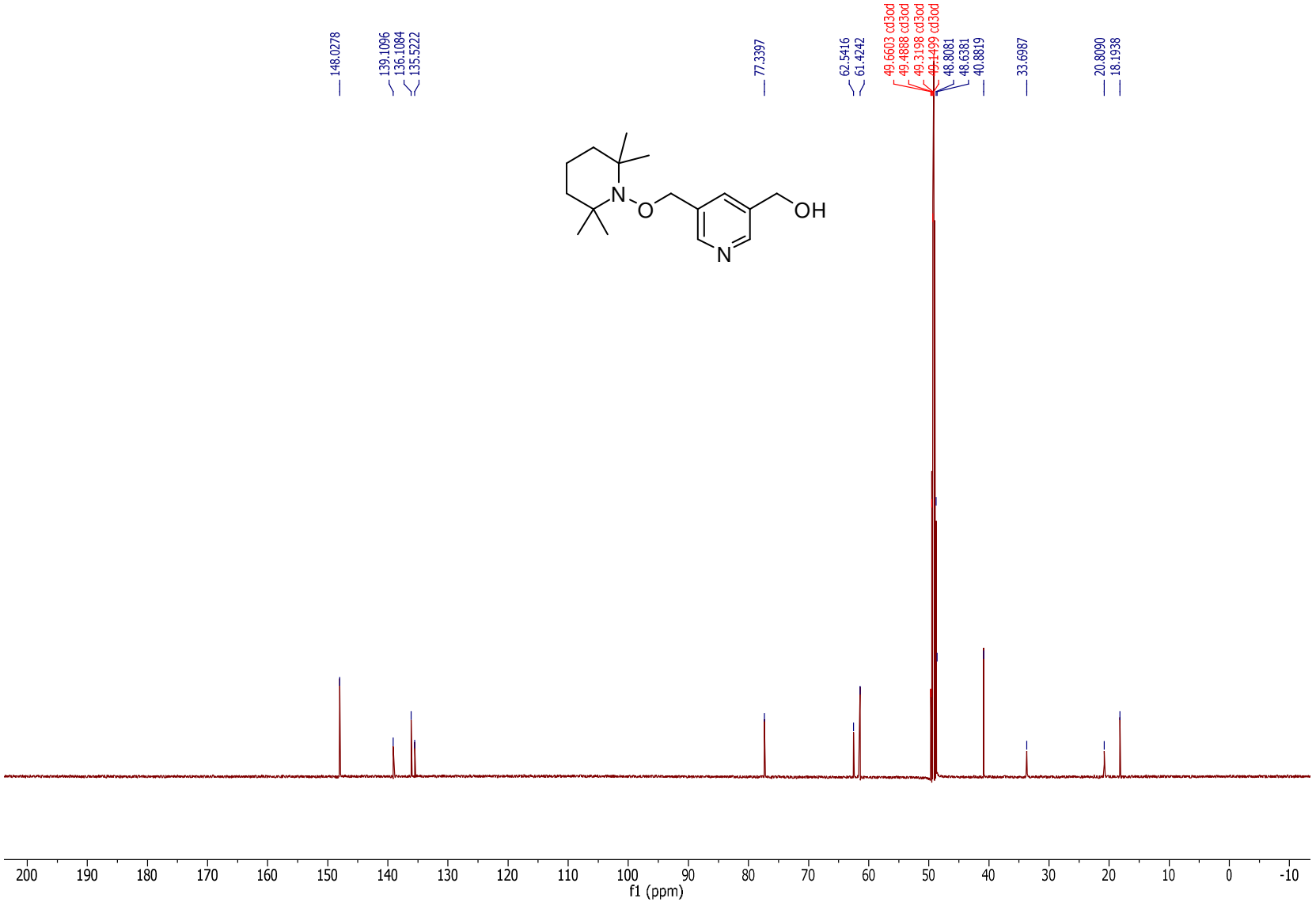
-1.0
f1 (ppm)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136.1084
135.5222
77.3397
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61.4242
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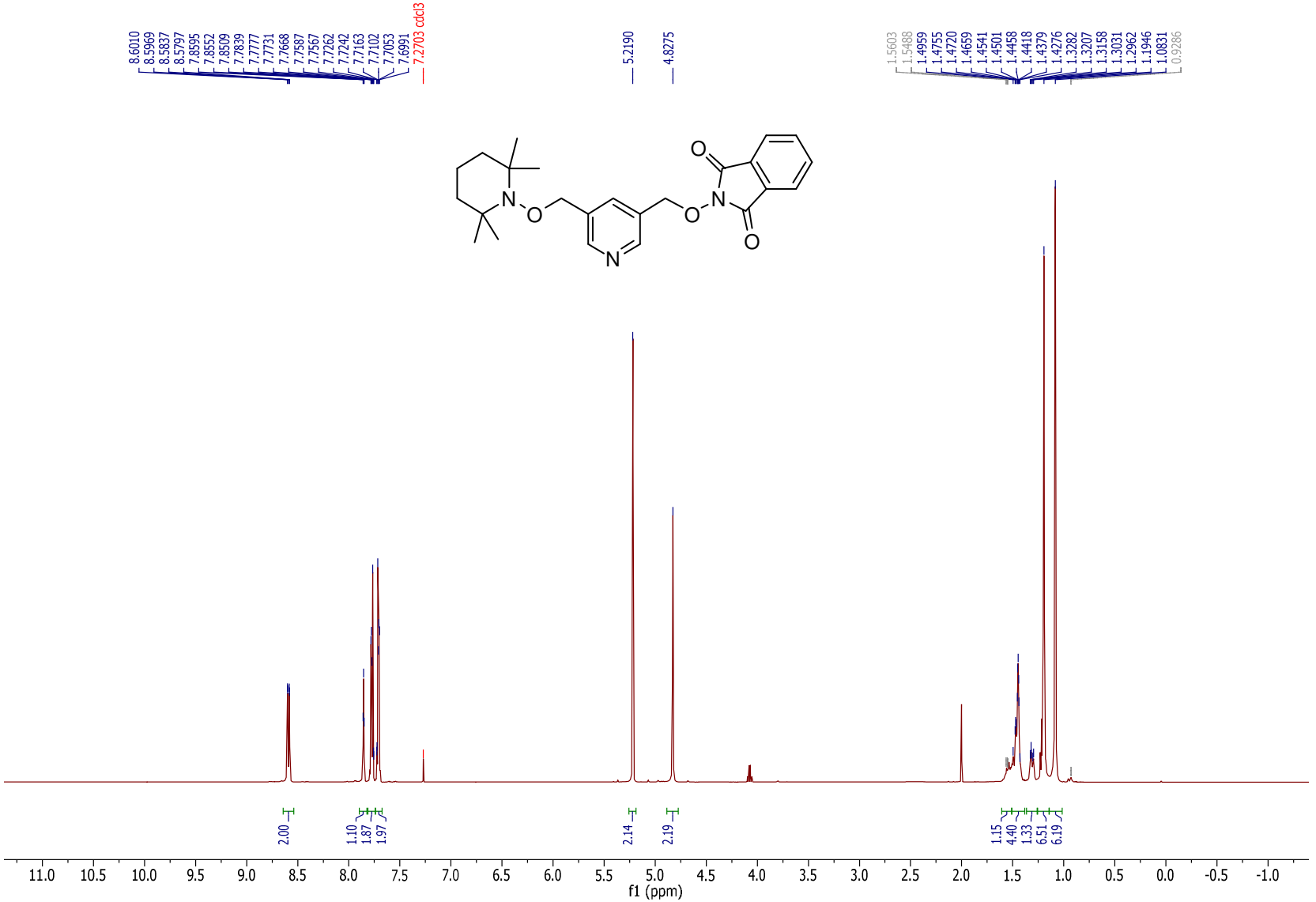
49.4888 cd3od
49.3198 cd3od
49.1499 cd3od
48.8081
48.6381
40.8819
33.6987
20.8090
18.1938
N
O
OH
N
200 190 180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10 0 -10
f1 (ppm)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7.7587
7.7567
7.7262
7.7242
7.7163
7.7102
7.7053
7.6991
7.2703 cdcl3
5.2190
4.8275
1.5603
1.5488
1.4959
1.4755
1.4720
1.4659
1.4541
1.4501
1.4458
1.4418
1.4379
1.4276
1.3282
1.3207
1.3158
1.3031
1.2962
1.1946
1.0831
0.9286
O
N
O
N
O
O
N
2.00
1.10
1.87
1.97
2.14
2.19
1.15
4.40
1.33
6.51
6.19
11.0
10.5
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
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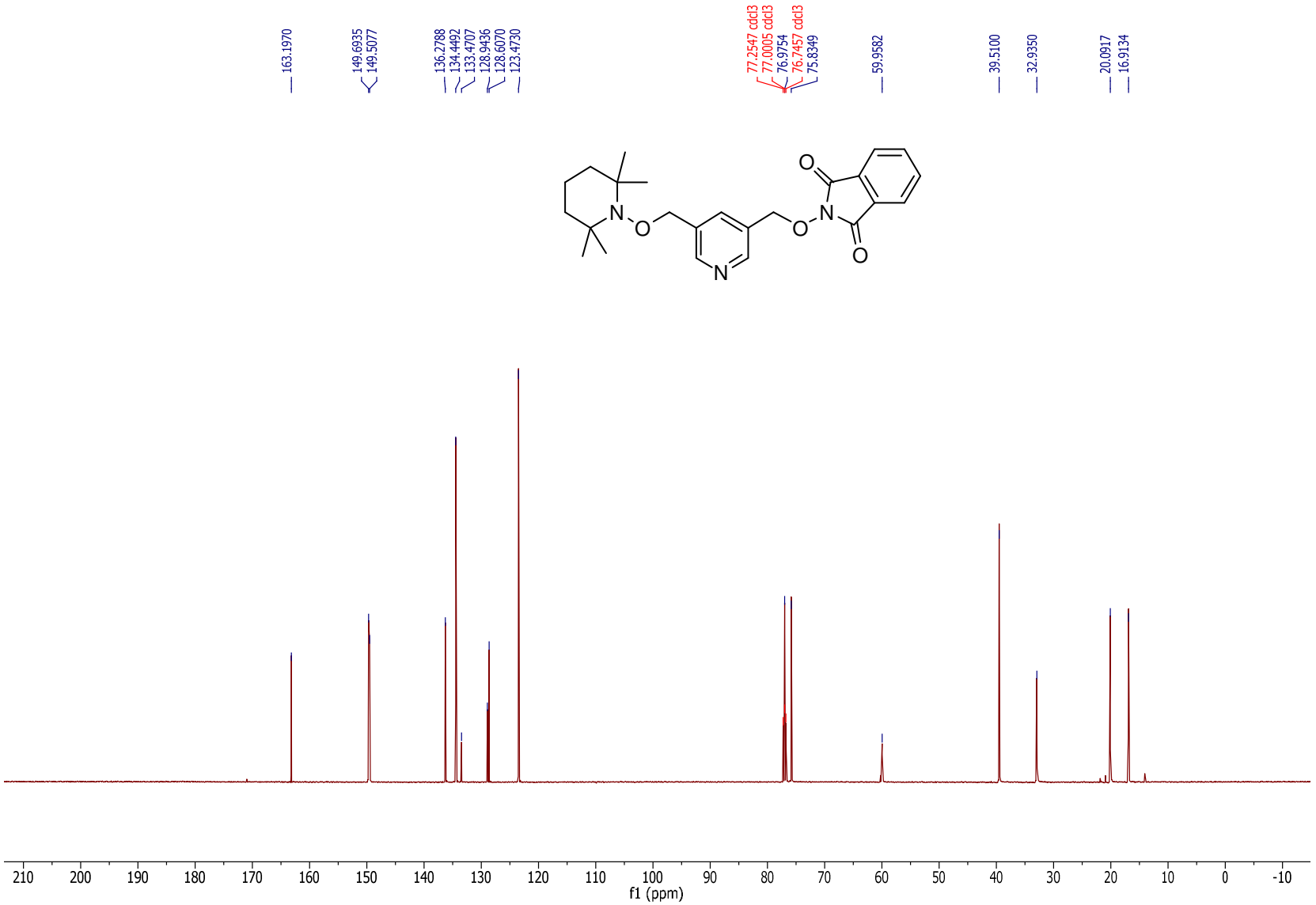
1.0
0.5
0.0
-0.5
-1.0
f1 (ppm)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128.9436
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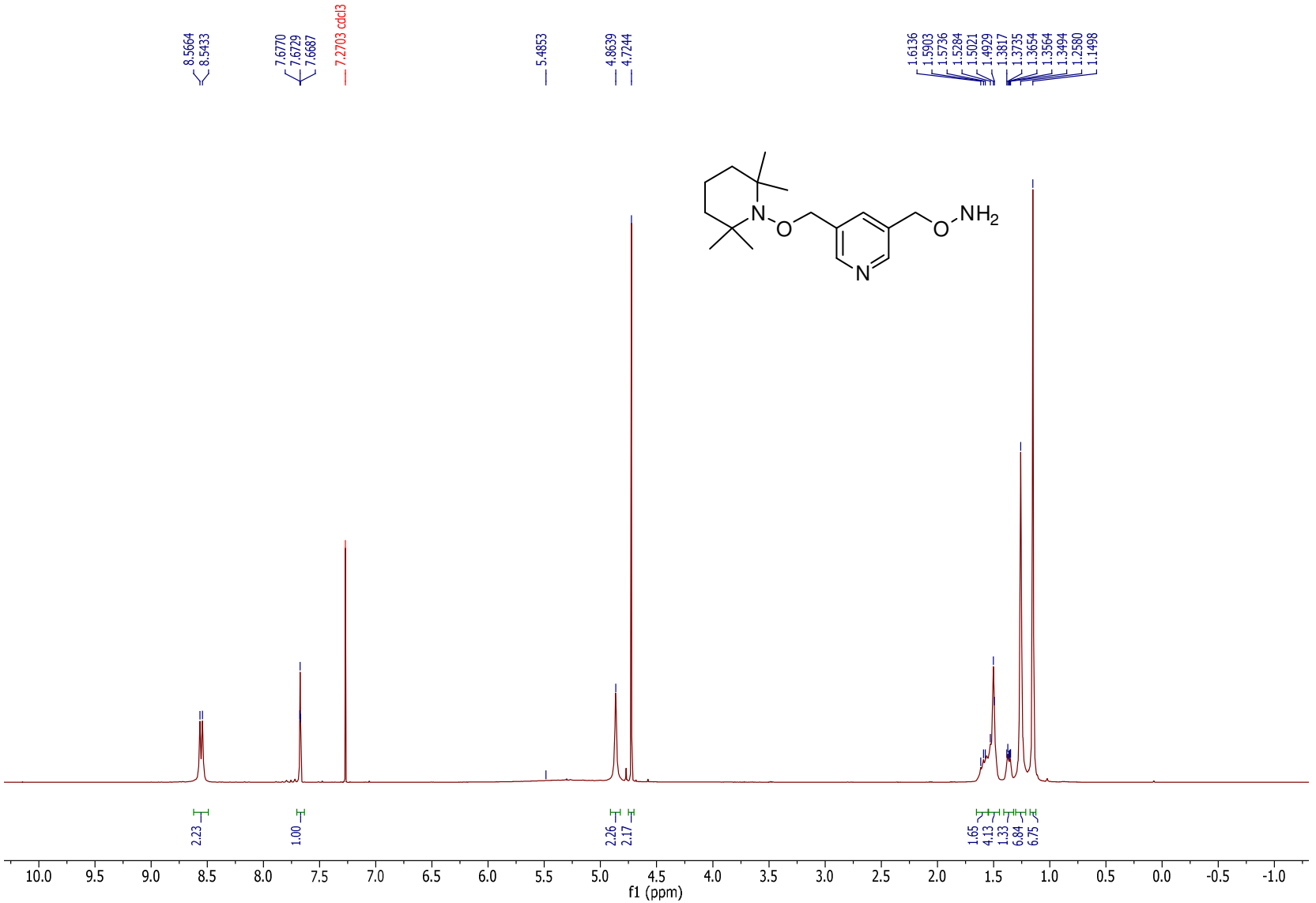
123.4730
77.2547 cdcl3
77.0005 cdcl3
76.9754
76.7457 cdcl3
75.8349
59.9582
39.5100
32.9350
20.0917
16.9134
O
N
O
N
O
O
N
f1 (ppm)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1.5903
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1.5021
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1.3817
1.3735
1.3654
1.3564
1.3494
1.2580
1.1498
N
O
O
NH2
N
2.23
1.00
2.26
2.17
1.65
4.13
1.33
6.84
6.75
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
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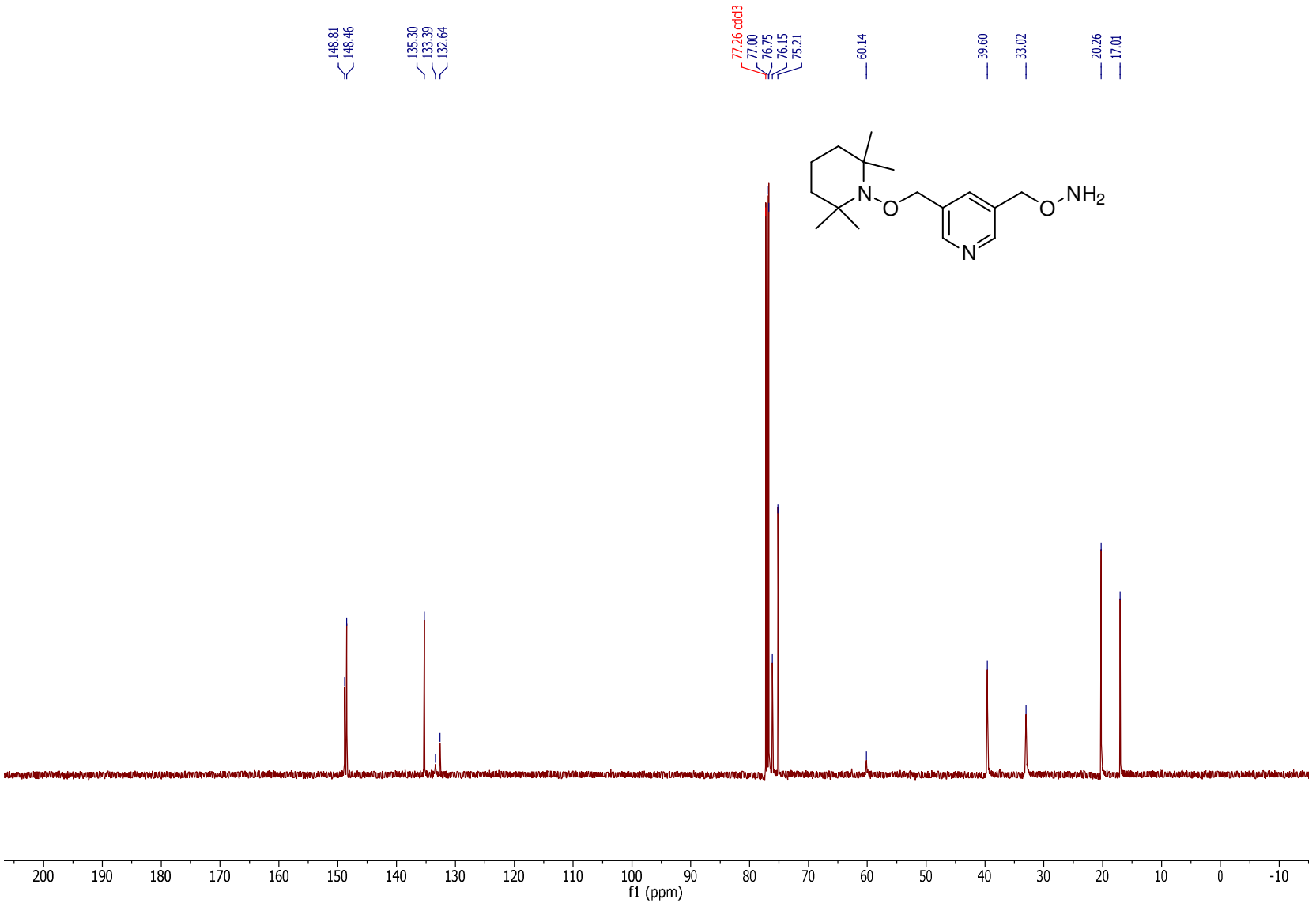
0.0
-0.5
-1.0
f1 (ppm)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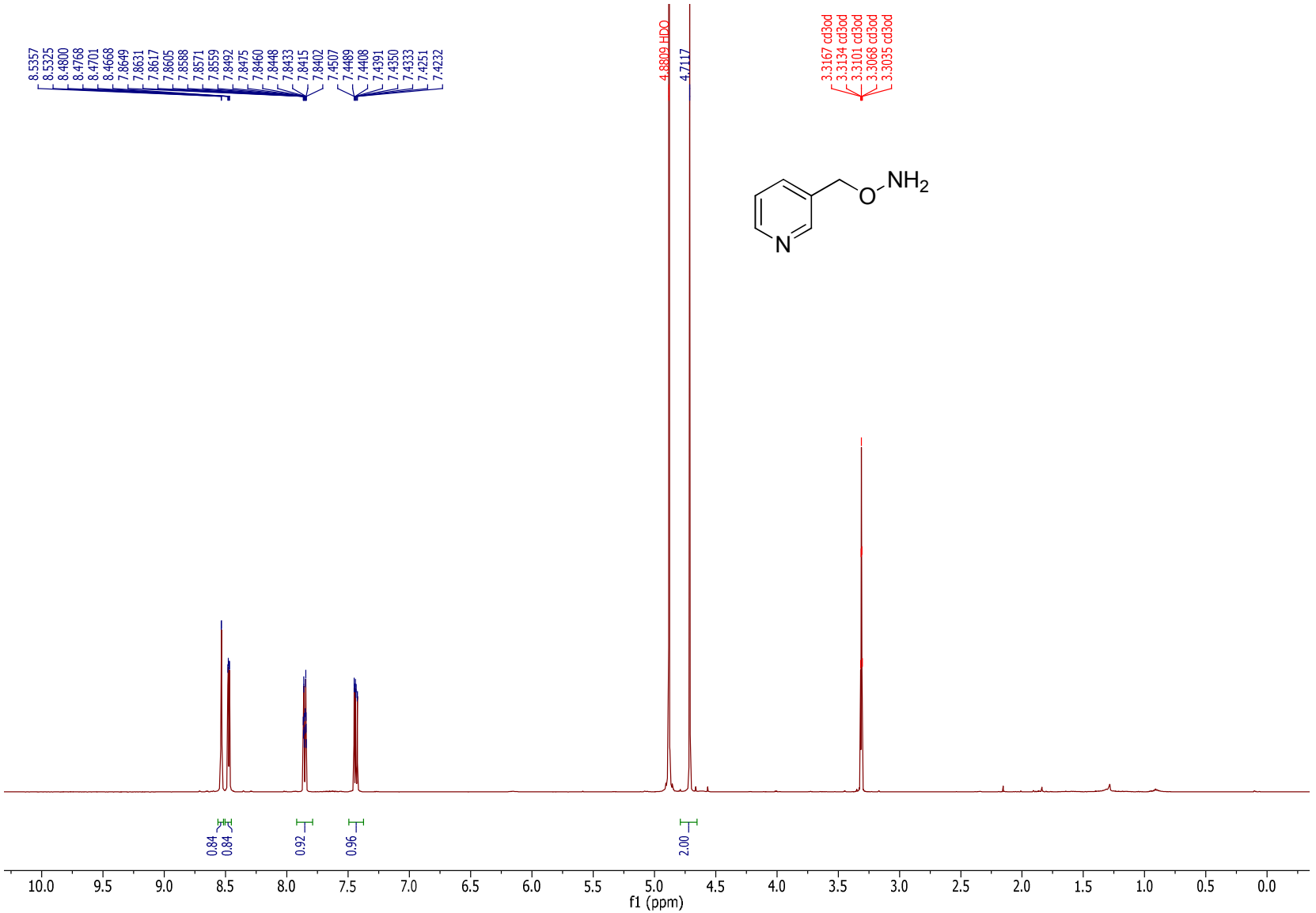
135.30
133.39
132.64
77.26 cdcl3
77.00
76.75
76.15
75.21
60.14
39.60
33.02
20.26
17.01
N
O
O
NH2
N
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)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7.8571
7.8559
7.8492
7.8475
7.8460
7.8448
7.8433
7.8415
7.8402
7.4507
7.4489
7.4408
7.4391
7.4350
7.4333
7.4251
7.4232
4.8809 HDO
4.7117
3.3167 cd3od
3.3134 cd3od
3.3101 cd3od
3.3068 cd3od
3.3035 cd3od
NH2
O
N
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0.84
0.92
0.96
2.00
10.0
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
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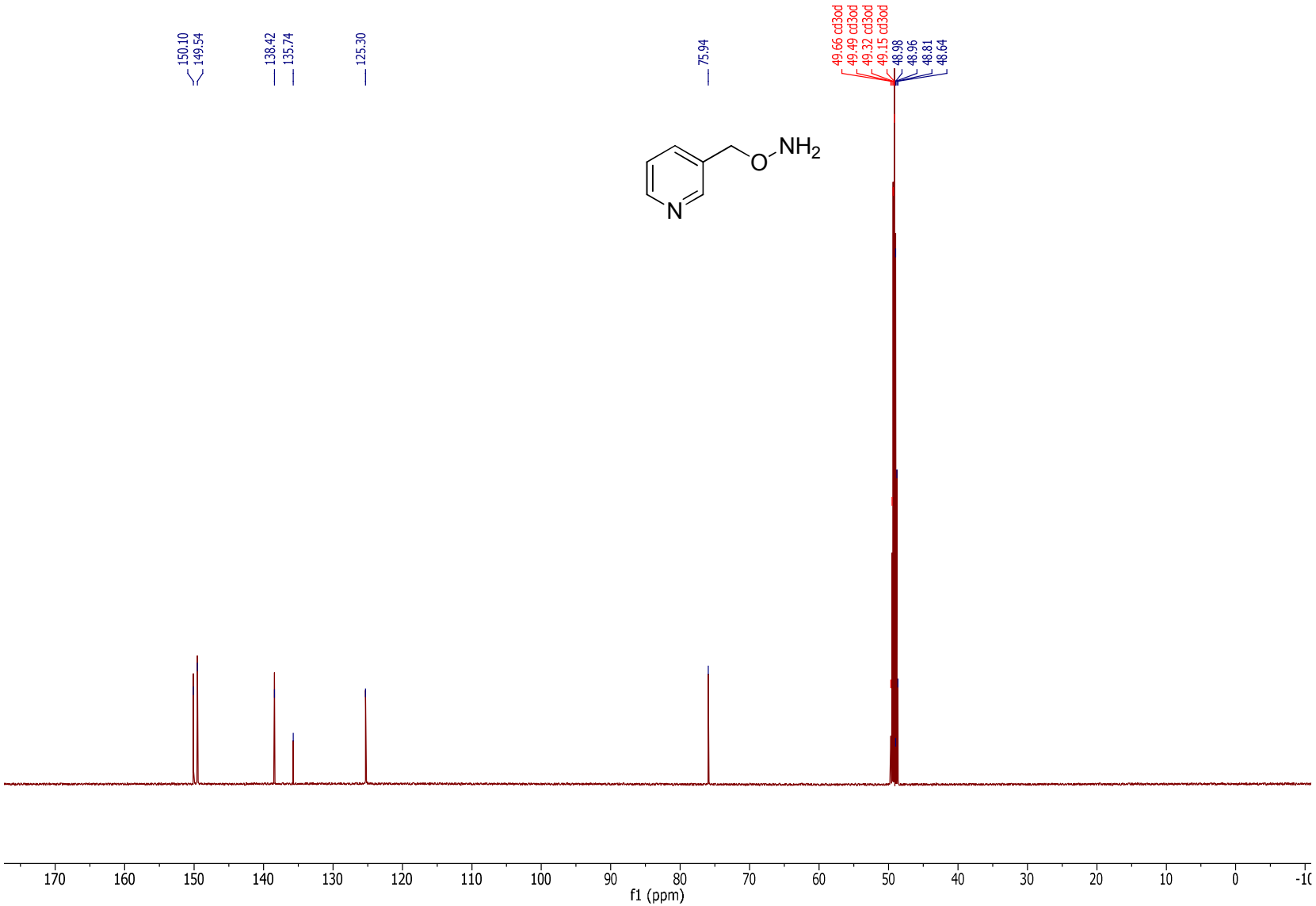
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
f1 (ppm)


150.10
149.54
138.42
135.74
125.30
75.94
49.66 cd3od
49.49 cd3od
49.32 cd3od
49.15 cd3od
48.98
48.96
48.81
48.64
NH2
O
N
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-10
f1 (ppm)

REFERENCES

(1) Lee, M.; Kang, M.; Moon, B.; Oh, H. B. *Analyst* **2009**, *134*, 1706-1712.

(2) Lind, K. E.; Lin, E. Y.; Nguyen, T. B.; Tangonan, B. T.; Erlanson, D. A.; Guckian, K.; Simmons, R. L.; Lee, W.; Sun, L.; Hansen, S.; Pathan, N.; Zhang, L. Patent WO/2008/005457, January 10, 2008.

(3) Deraeve, C.; Guo, Z.; Bon, R. S.; Blankenfeldt, W.; DiLucrezia, R.; Wolf, A.; Menninger, S.; Stigter, E. A.; Wetzel, S.; Choidas, A.; Alexandrov, K.; Waldmann, H.; Goody, R. S.; Wu, Y. W. *J. Am. Chem. Soc.* **2012**, *134*, 7384-7391.