Supplementary Information

Supplementary information on synthesis and ^{1}H NMR validation of samples.

Compound SK032•HCl

Step 1.

A mixture of benzyl bromide **1b** (724 mg, 2.90 mmol), K_2CO_3 (440 mg, 3.18 mmol), and 2,4-pentanedione (0.33 mL, 3.20 mmol) in EtOH (7.2 mL) was heated at reflux for 20 h. The reaction mixture was then cooled to room temperature, concentrated, and diluted with EtOAc (50 mL)/water (50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4, concentrated, and purified via column chromatography (10:1 to 8:1 hexanes/EtOAc) to afford product **2b** as a yellow oil (451 mg, 69%), which was used directly in the next step.[1]

Step 2.

A mixture of **2b** (451 mg, 1.99 mmol) and NH_4OAc (1.84 g, 23.9 mmol) in MeOH (10 mL) was first stirred at room temperature for 30 min, cooled to 0°C, and added $NaBH_3CN$ (624 mg, 9.93 mmol). The resulting mixture was warmed to room temperature and stirred overnight. The reaction mixture was then quenched/acidified with 2 M HCl (to pH ~1) and washed with Et_2O. The aqueous layer was separated, basified with 3 M NaOH (to pH ~14), and extracted with CH_2Cl_2 (30 mL × 3). The combined organic layers were dried over Na_2SO_4, and concentrated to afford the crude product **SK032** (278 mg, 62%), which was used directly in the next step.[2]

Step 3.

To a solution of **SK032** (278 mg, 1.22 mmol) in Et_2O (12 mL) at 0°C was added 2 M HCl in Et_2O (1.2 mL, 2.4 mmol) dropwise. The resulting solid was collected by filtration, washed with Et_2O, and dried to afford **SK032•HCl** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) for **SK032•HCl** δ 8.55 (s, 3H), 7.49 (dd, *J* = 8.0, 1.0 Hz, 1H), 7.29 (dd, *J* = 7.6, 1.5 Hz, 1H), 7.17 (td, *J* = 7.5, 1.0 Hz, 1H), 7.04 (td, *J* = 7.7, 1.6 Hz, 1H), 3.45 – 3.34 (m, 1H), 2.96 – 2.80 (m, 2H), 2.15 – 2.05 (m, 1H), 2.02 – 1.91 (m, 1H), 1.49 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for **SK032•HCl** δ 139.52, 132.89, 130.48, 128.06, 127.66, 124.24, 48.13, 35.05, 32.13, 18.92; HRMS (ES) for **SK032** *m/z* $(M+H)^+$ calculated 228.0388, observed 228.0385.

Compound SK058•HCl

Compound **SK058•HCl** was prepared following a procedure analogous to the one used for **SK032•HCl**, using benzyl bromide **1a** instead of benzyl bromide **1b** as starting material: ^{1}H NMR (500 MHz, $CDCl_3$) for **SK058•HCl** δ 8.45 (s, 3H), 7.24 (dd, *J* = 12.4, 5.0 Hz, 1H), 7.15 (q, *J* = 7.2 Hz, 1H), 6.98 (dt, *J* = 22.9, 8.0 Hz, 2H), 3.36 (s, 1H), 2.86 – 2.72 (m, 2H), 2.15 (s, 1H),

1.94 (s, 1H), 1.45 (d, *J* = 3.1 Hz, 3H); HRMS (ES) for **SK058** *m/z* $(M+H)^+$ calculated 168.1189, observed 168.1190.

Compound SK052•HCl

Compound **SK052•HCl** was prepared following a procedure analogous to the one used for **SK032•HCl**, using benzyl bromide **1c** instead of benzyl bromide **1b** as starting material: ^{1}H NMR (500 MHz, $CDCl_3$) for **SK052•HCl** δ 8.55 (s, 3H), 7.77 (d, *J* = 7.9 Hz, 1H), 7.30 – 7.26 (m, 1H), 7.20 (t, *J* = 7.4 Hz, 1H), 6.90 – 6.83 (m, 1H), 3.48 – 3.37 (m, 1H), 2.93 – 2.80 (m, 2H), 2.13 – 2.03 (m, 1H), 2.01 – 1.88 (m, 1H), 1.50 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for **SK052•HCl** δ 142.79, 139.59, 129.58, 128.60, 128.20, 100.27, 48.11, 36.70, 35.36, 18.97; HRMS (ES) for **SK052** *m/z* $(M+H)^+$ calculated 276.0249, observed 276.0247.

Compound SK041•HCl

Compound **SK041•HCl** was prepared following a procedure analogous to the one used for **SK032•HCl**, using benzyl chloride **3** instead of benzyl bromide **1b** as starting material: ^{1}H NMR (500 MHz, $CDCl_3$) for **SK041•HCl** δ 8.41 (s, 3H), 7.20 – 7.12 (m, 2H), 6.87 – 6.78 (m, 2H), 3.80 (s, 3H), 3.33 – 3.23 (m, 1H), 2.83 – 2.67 (m, 2H), 2.16 – 2.07 (m, 1H), 1.92 – 1.85 (m, 1H), 1.45 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for **SK041•HCl** δ 157.29, 130.10, 128.54, 127.54, 120.61, 110.32, 55.26, 48.04, 35.23, 26.25, 18.78; HRMS (ES) for **SK041** *m/z* $(M+H)^+$ calculated 180.1388, observed 180.1382.

Compound SK182

A mixture of **SK041•HCl** in 48% HBr in H_2O (1.80 mL) was stirred at 100°C for 5 h until completion. The reaction mixture was then concentrated, and the crude residue was purified via HPLC (MeCN/H_2O) to afford the product as a purple solid: ^{1}H NMR (500 MHz, MeOD) for **SK182** δ 7.09 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.04 (td, *J* = 7.9, 1.7 Hz, 1H), 6.81 – 6.74 (m, 2H), 3.27 – 3.18 (m, 1H), 2.78 – 2.64 (m, 2H), 2.00 – 1.92 (m, 1H), 1.86 – 1.77 (m, 1H), 1.33 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, MeOD) for **SK182** δ 156.30, 131.12, 128.61, 127.96, 120.79, 115.93, 48.62, 36.43, 27.12, 18.76; HRMS (ES) for **SK182** *m/z* $(M+H)^+$ calculated 166.1232, observed 166.1237.

Compound SK213•HCl

Step 1.

A mixture of benzyl bromide **6** (1.5 g 7.30 mmol), K_2CO_3 (1.1 g, 7.96 mmol), and 2,4-pentanedione (0.83 mL, 8.06 mmol) in EtOH (18 mL) was heated at reflux for 20 h. The reaction mixture was then cooled to room temperature, concentrated, and diluted with EtOAc (50 mL)/water (50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4,

concentrated, and purified via column chromatography (10:1 to 8:1 hexanes/EtOAc) to afford product **7** as a yellow oil (1.04 g, 78%), which was used directly in the next step.

Step 2.

A mixture of **7** (155 mg, 0.85 mmol), and $MeNH_2HCl$ (458 mg, 6.78 mmol) in MeOH (4.2 mL) was first stirred at room temperature for 30 min, cooled to 0°C, and added $NaBH_3CN$ (160 mg, 2.55 mmol). The resulting mixture was warmed to room temperature, and stirred overnight. The reaction mixture was then quenched/acidified with 2 M HCl (to pH ~1), and washed with Et_2O. The aqueous layer was separated, basified with 3 M NaOH (to pH ~14), and extracted with CH_2Cl_2 (30 mL × 3). The combined organic layers were dried over Na_2SO_4, and concentrated to afford the crude product **SK213** (126 mg, 75%), which was used directly in the next step.

Step 3.

To a solution of **SK213** (126 mg, 0.64 mmol) in Et_2O (3.2 mL) at 0°C was added 4 M HCl in 1,4-dioxane (0.32 mL, 1.28 mmol) dropwise. The resulting solid was collected by filtration, washed with Et_2O, and dried to afford **SK213•HCl** as a white solid: ^{1}H NMR (500 MHz, $CDCl_3$) for **SK213•HCl** δ 9.58 (s, 2H), 7.33 (dd, *J* = 7.5, 1.7 Hz, 1H), 7.28 (dd, *J* = 7.3, 1.9 Hz, 1H), 7.20 – 7.13 (m, 2H), 3.19 – 3.09 (m, 1H), 2.94 – 2.79 (m, 2H), 2.63 (t, *J* = 5.6 Hz, 3H), 2.28 – 2.18 (m, 1H), 2.03 – 1.93 (m, 1H), 1.50 (d, *J* = 6.6 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for the free base **SK213** δ 140.02, 133.86, 130.24, 129.47, 127.23, 126.78, 54.60, 36.55, 33.68, 30.03, 19.71; HRMS (ES) for **SK213** *m/z* $(M+H)^+$ calculated 198.1050, observed 198.1049.

Compound SK220

Step 1.

To a mixture of benzyl chloride **8** (2.0 mL, 14.4 mmol) and β-keto ester **9** (3.4 mL, 21.3 mmol) in THF (36 mL) at room temperature was addd NaH (60%, 690 mg, 17.3 mmol), followed by TBAI (531 mg, 1.44 mmol). The reaction mixture was then heated at reflux overnight until completion. The resulting mixture was cooled to room temperature, concentrated, and diluted with EtOAc (50 mL)/water (50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4, and concentrated to afford the crude intermediate **10**, which was used directly in the next step.[3]

Step 2.

The crude intermediate **10** from the previous step was dissolved in EtOH (50 mL)/H_2O (2.8 mL), and added KOH (4.0 g, 71.3 mmol). The reaction mixture was heated at reflux for 4 h, cooled to room temperature, concentrated, and diluted with EtOAc (50 mL)/water (50 mL). The organic layer was separated, washed with brine, dried over Na_2SO_4, concentrated, and purified via

column chromatography (10:1 to 8:1 hexanes/EtOAc) to afford product **11** as a yellow oil (1.51 g, 51% two steps), which was used directly in the next step.[4]

Step 3.

A mixture of **11** (576 mg, 2.80 mmol) and NH_4OAc (1.30 g, 16.9 mmol) in MeOH (10 mL) was first stirred at room temperature for 30 min, cooled to 0°C, and added $NaBH_3CN$ (396 mg, 6.30 mmol). The resulting mixture was warmed to room temperature, and stirred overnight. The reaction mixture was then quenched/acidified with 2 M HCl (to pH ~1), and washed with Et_2O. The aqueous layer was separated, basified with 3 M NaOH (to pH ~14), and extracted with CH_2Cl_2 (30 mL × 3). The combined organic layers were dried over Na_2SO_4, and concentrated to afford the crude product **12** (192 mg, 33%), which was used directly in the next step.

Step 4.

The crude material **12** from the previous step (55 mg, 0.26 mmol) was dissolved in 48% HBr in H_2O (2.6 mL), and the reaction mixture was stirred at 100°C for 5 h until completion. The reaction mixture was then cooled to room temperature, concentrated, and purified via HPLC ($MeCN/H_2O$) to afford the product **SK220** as a yellow solid: ^{1}H NMR (500 MHz, $CDCl_3$) for **SK220** δ 7.88 (s, 3H), 7.11 – 7.06 (m, 1H), 7.06 – 6.99 (m, 2H), 6.87 – 6.80 (m, 1H), 3.25 – 3.12 (m, 1H), 2.91 – 2.82 (m, 1H), 2.71 – 2.60 (m, 1H), 2.20 – 2.08 (m, 1H), 1.93 – 1.78 (m, 2H), 1.70 – 1.59 (m, 1H), 1.39 – 1.22 (m, 2H), 0.77 (t, *J* = 7.3 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for **SK220** δ 153.37, 130.27, 127.86, 126.21, 121.26, 116.12, 51.93, 35.11, 33.22, 25.29, 18.79, 13.52; HRMS (ES) for **SK220** *m/z* $(M+H)^+$ calculated 194.1545, observed 194.1548.

Compound SK232•HCl

Step 1.

To a solution of reagent **14** (1.8 mL, 7.06 mmol) in DMF (7.7 mL) at room temperature was added NaH (60%, 242 mg, 6.05 mmol). The resulting mixture was then heated to 90°C, followed by the addition of compound **13** (0.35 mL, 2.33 mmol). The reaction mixture was stirred at 90°C for another 4 h, cooled to room temperature, diluted with EtOAc (50 mL), and washed with water. The organic layer was separated, dried over Na_2SO_4, concentrated, and purified via column chromatography (8:1 to 6:1 hexanes/EtOAc) to afford the product **15** as a colorless oil (865 mg, 90%).[5]

Step 2.

To a solution of **15** (342 mg, 0.83 mmol) in DMF (2.1 mL) at room temperature was added LiBr (79 mg, 0.91 mmol) and H_2O (30 μL, 1.67 mmol). The reaction mixture was then stirred and heated to 150°C overnight until completion. The resulting mixture was then cooled to room temperature, diluted with EtOAc (50 mL), and washed with water. The organic layer was

separated, dried over Na_2SO_4, concentrated, and purified via column chromatography (6:1 hexanes/EtOAc) to afford the product **16** as a colorless oil (178 mg, 63%).[6]

Step 3.

To a solution of **16** (178 mg, 0.43 mmol) in Et_2O (8.6 mL) at 0°C was added $LiAlH_4$ (33 mg, 0.87 mmol). The reaction mixture was warmed to room temperature, and stirred for 1 h until completion. The reaction mixture was then quenched by successive addition of H_2O (34 μL), 15% NaOH in H_2O (34 μL), and H_2O (102 μL). The resulting solid was removed by filtration, and the filtrate solution was concentrated, and purified via column chromatography (2:1 hexanes/EtOAc with 1% EtOH to 4:3 hexanes/EtOAc with 1% EtOH) to afford the product **Boc-SK232** as a slightly orange oil (122 mg, 95%).

Step 4.

A mixture of **Boc-SK232** (122 mg, 0.41 mmol) in 4.0 M HCl in 1,4-dioxane (4.1 mL) was stirred at room temperature for 30 min until completion. The reaction mixture was then concentrated, and the resulting solid was washed with Et_2O to afford **SK232•HCl** as a white solid: ^{1}H NMR (500 MHz, MeOD) for **SK232•HCl** δ 7.36 (d, *J* = 17.4, 7.6, 1.5 Hz, 2H), 7.30 – 7.18 (m, 2H), 3.84 (dd, *J* = 11.7, 3.7 Hz, 1H), 3.65 (dd, *J* = 11.7, 6.6 Hz, 1H), 3.31 – 3.22 (m, 1H), 2.87 (m, 2H), 2.03 – 1.86 (m, 2H); ^{13}C NMR (125 MHz, MeOD) for **SK232•HCl** δ 139.42, 134.75, 131.61, 130.66, 129.25, 128.43, 61.91, 54.31, 30.75, 30.43; HRMS (ES) for **SK232** *m/z* $(M+H)^+$ calculated 200.0842, observed 200.0844.

Compound SK609•HCl

Compound **SK609•HCl** was prepared following a procedure analogous to the one used for **SK032•HCl**, step 3, using commercially available compound **SK609** as starting material: ^{1}H NMR (500 MHz, $CDCl_3$) for **SK609•HCl** δ 8.54 (s, 3H), 7.35 – 7.25 (m, 2H), 7.16 – 7.04 (m, 2H), 3.44 – 3.30 (m, 1H), 2.98 – 2.76 (m, 2H), 2.15 – 2.03 (m, 1H), 2.01 – 1.88 (m, 1H), 1.48 (d, *J* = 6.5 Hz, 3H); ^{13}C NMR (125 MHz, $CDCl_3$) for **SK609•HCl** δ 137.78, 133.77, 130.49, 129.57, 127.82, 126.99, 48.13, 34.91, 29.61, 18.91; HRMS (ES) for **SK609** *m/z* $(M+H)^+$ calculated 184.0893, observed 184.0893.

References:

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3. Kuroita, T.; Sakamoto, H.; Igawa, H.; Sasaki, M.; Asano, K.; Maekawa, T.; Fuji, K. PCT Int. Appl. Crystalline compound WO 2009133970 A1

4. Cordes, J.; Wessel, C.; Harms, K.; Koert, U. (2008) meta-Selective Aromatic Borylation as Key Step in the Synthesis of Poipuol Synthesis, 14, 2217 - 2220

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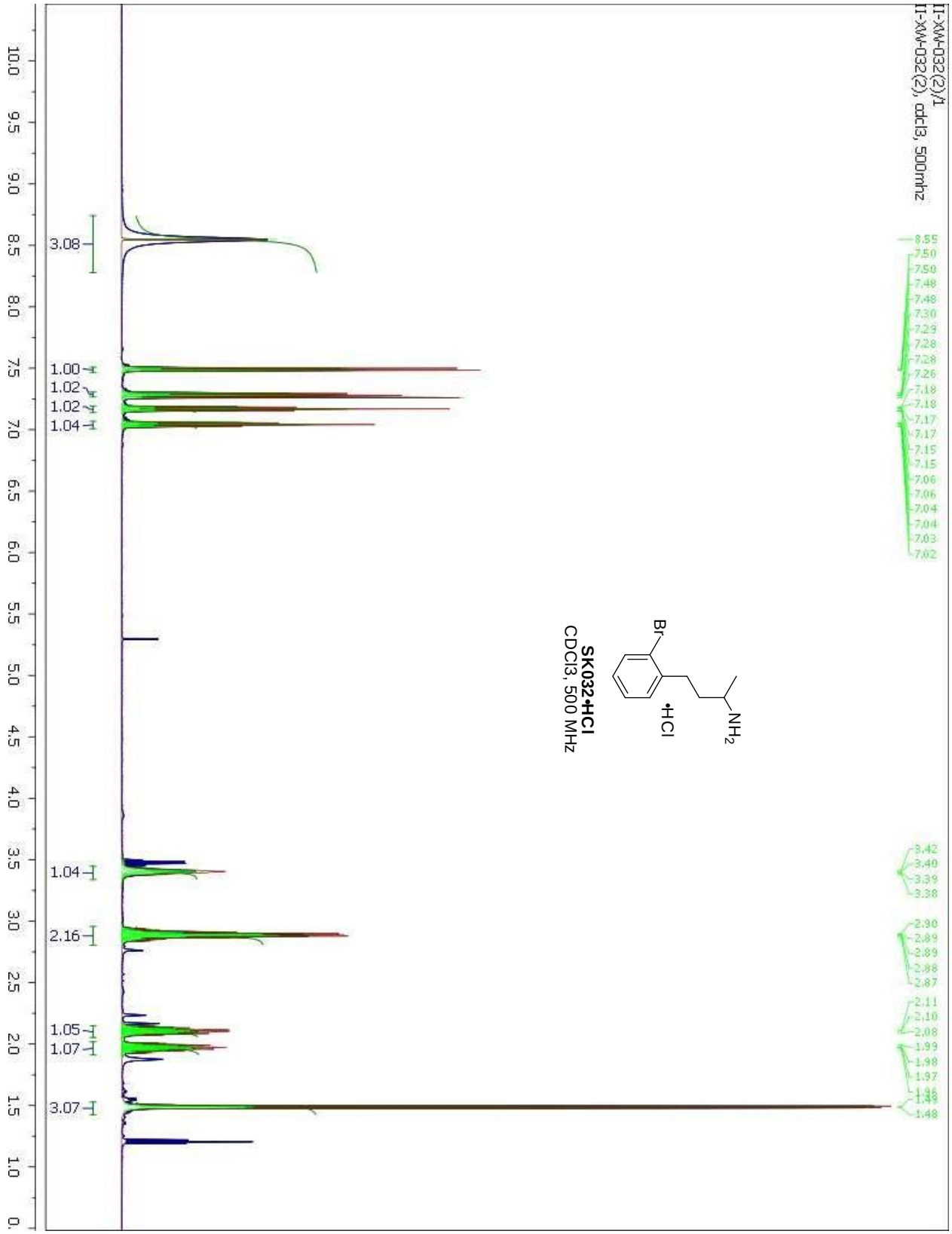
6. Chen, R.; Lee, V.; Adlington, R. M.; Baldwin, J. E. (2007); A Facile Synthesis of Ethyl 2-Acetamido-4-methylenehex-5-enoate, a Versatile Diels-Alder Synthon for the Parallel Synthesis of Novel α-Amino Acid Derivatives; *Synthesis*, *1*, 113-117.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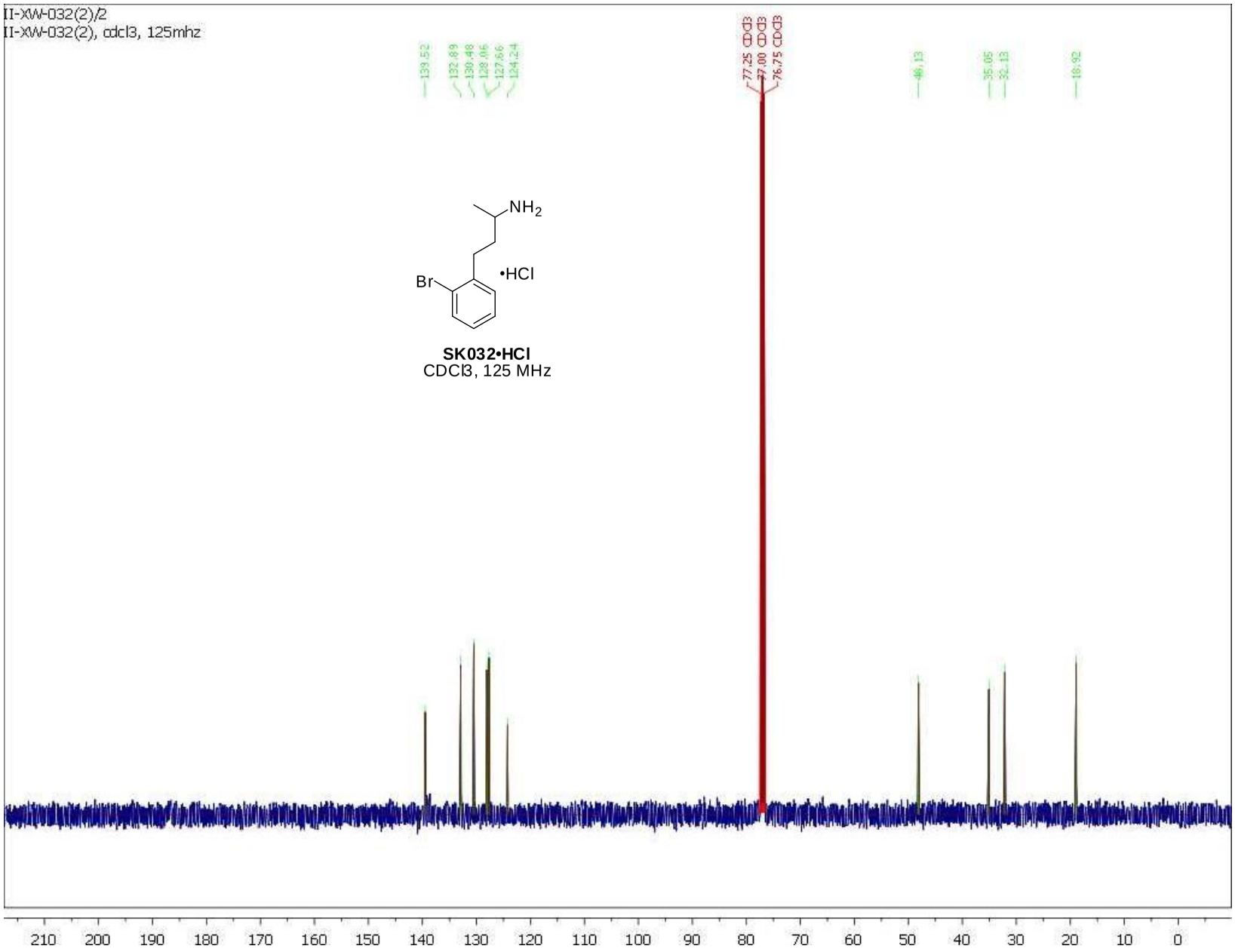
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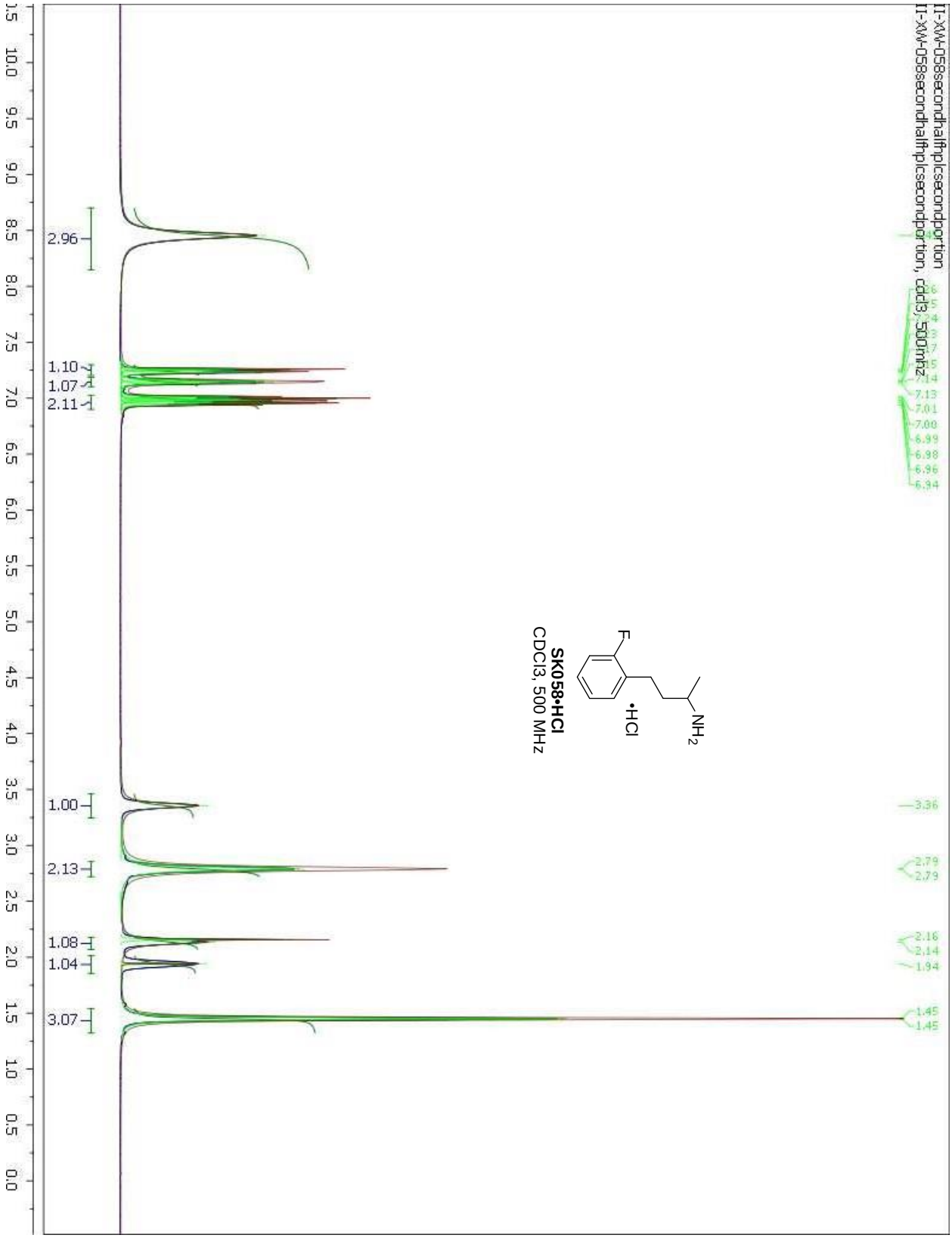
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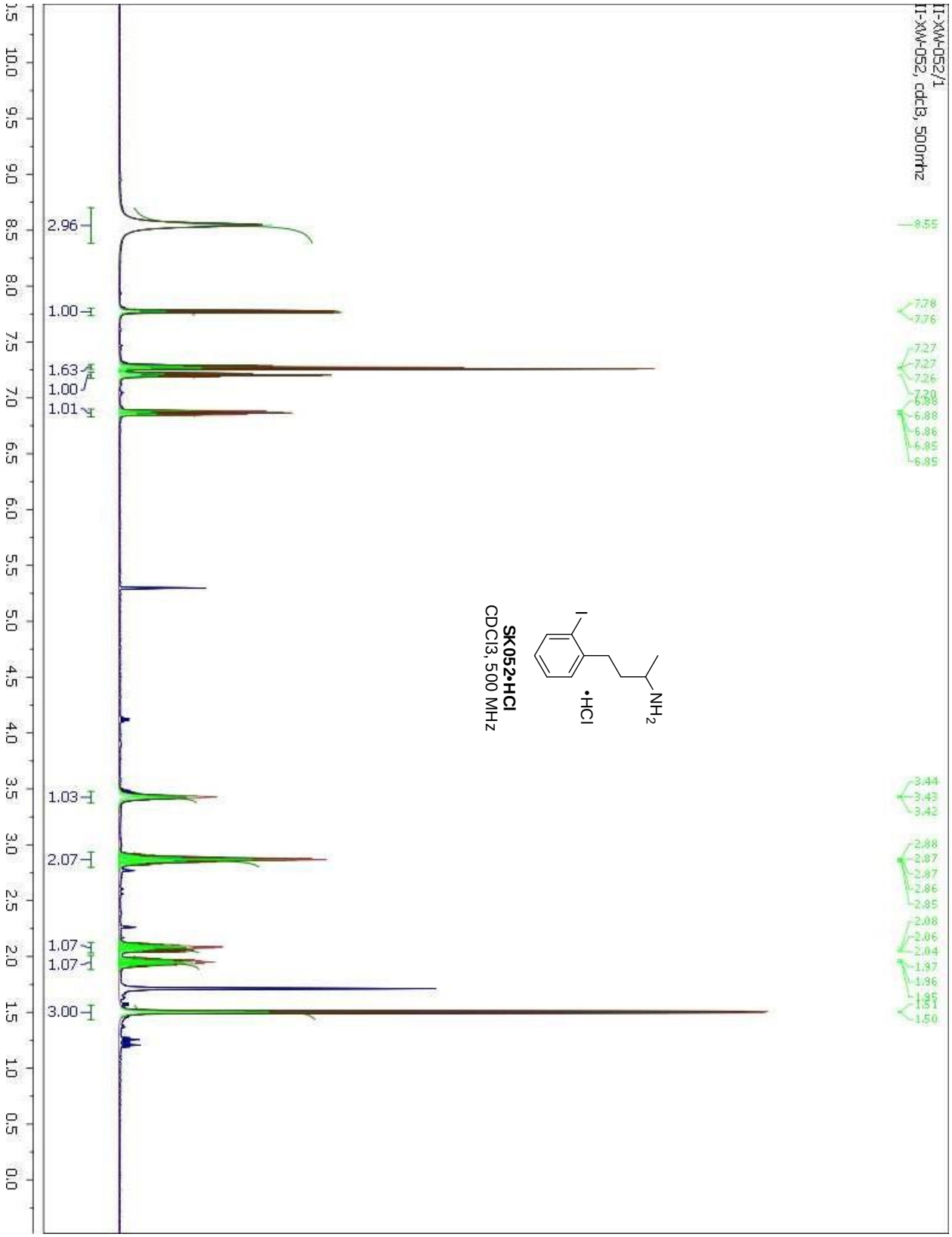
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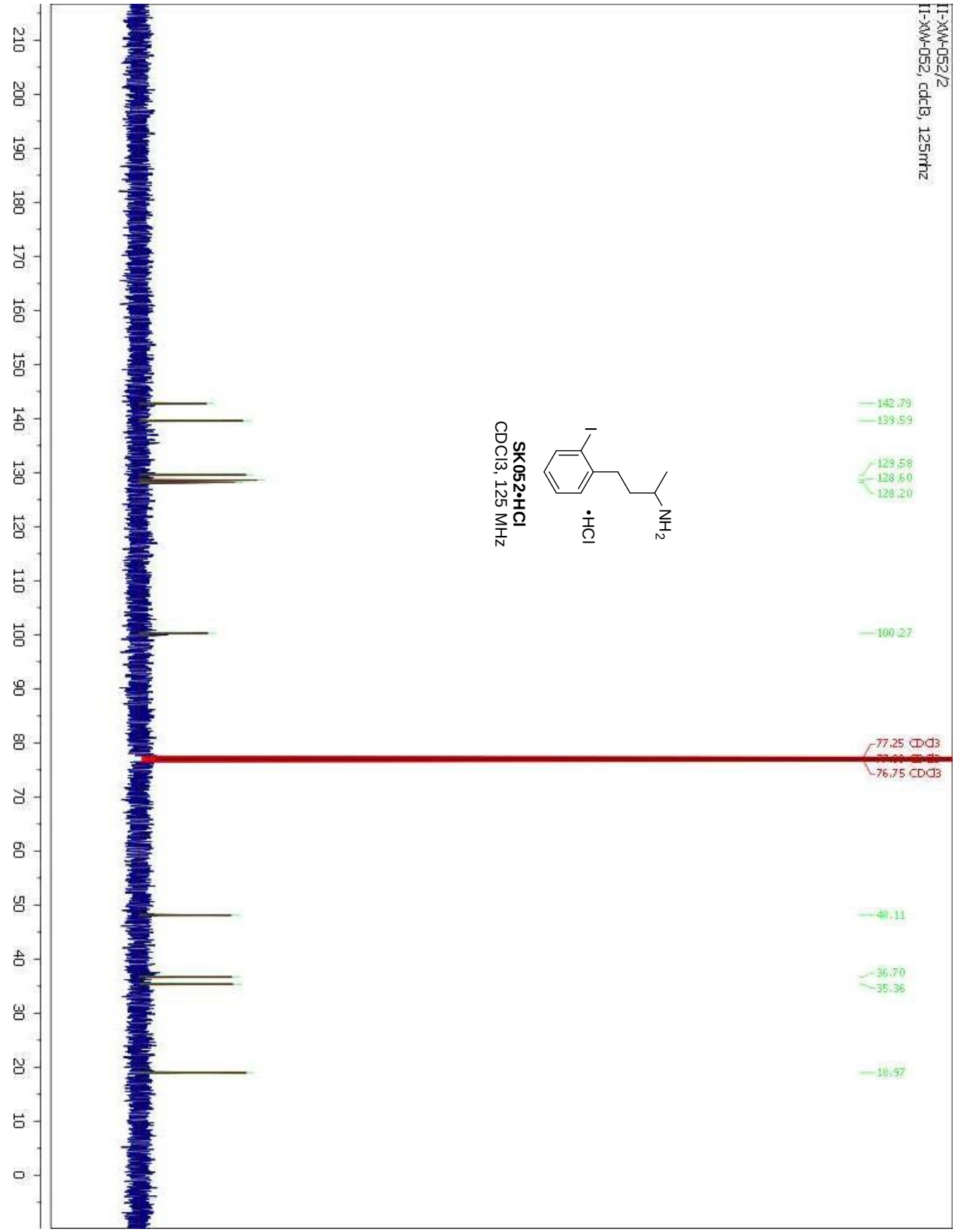
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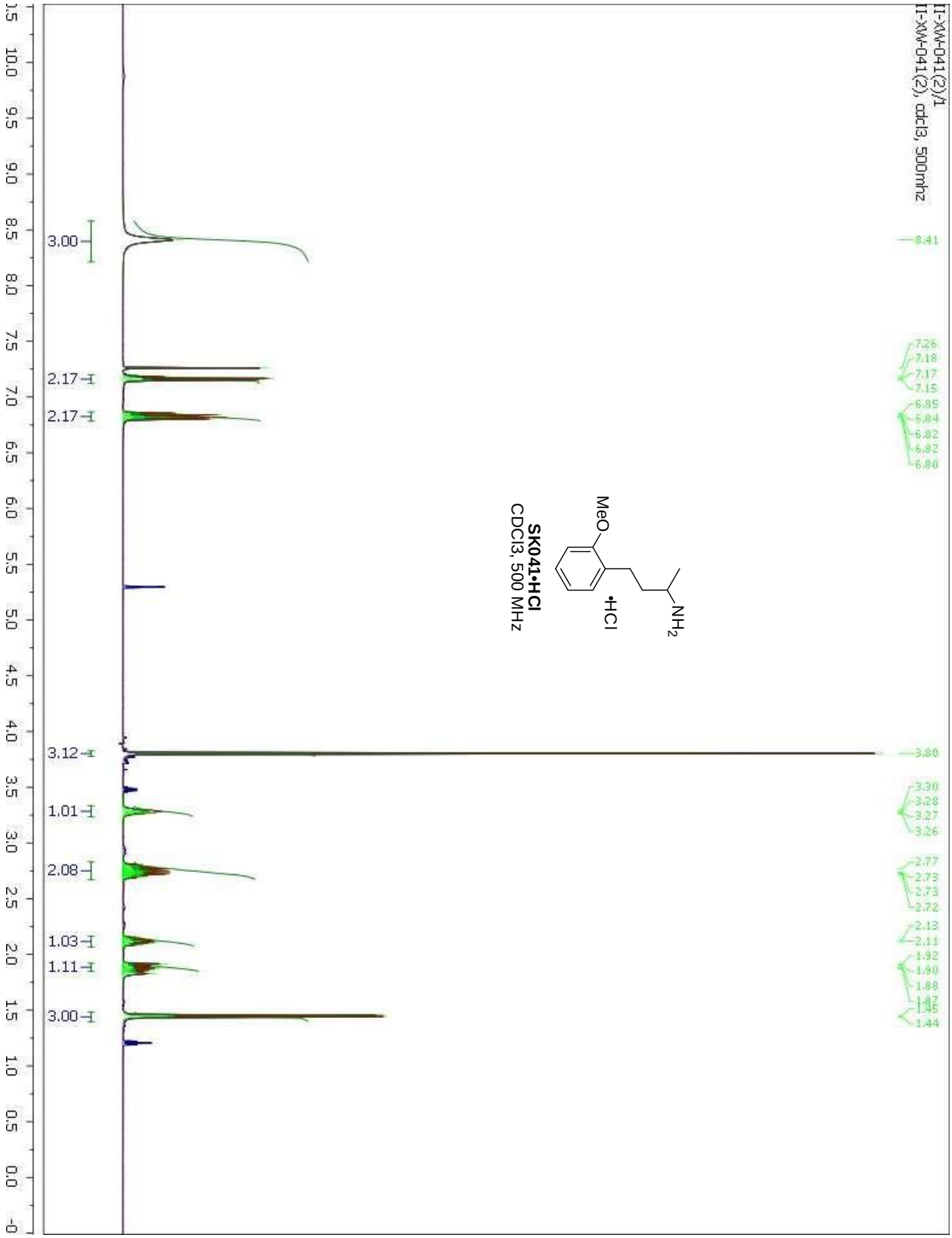
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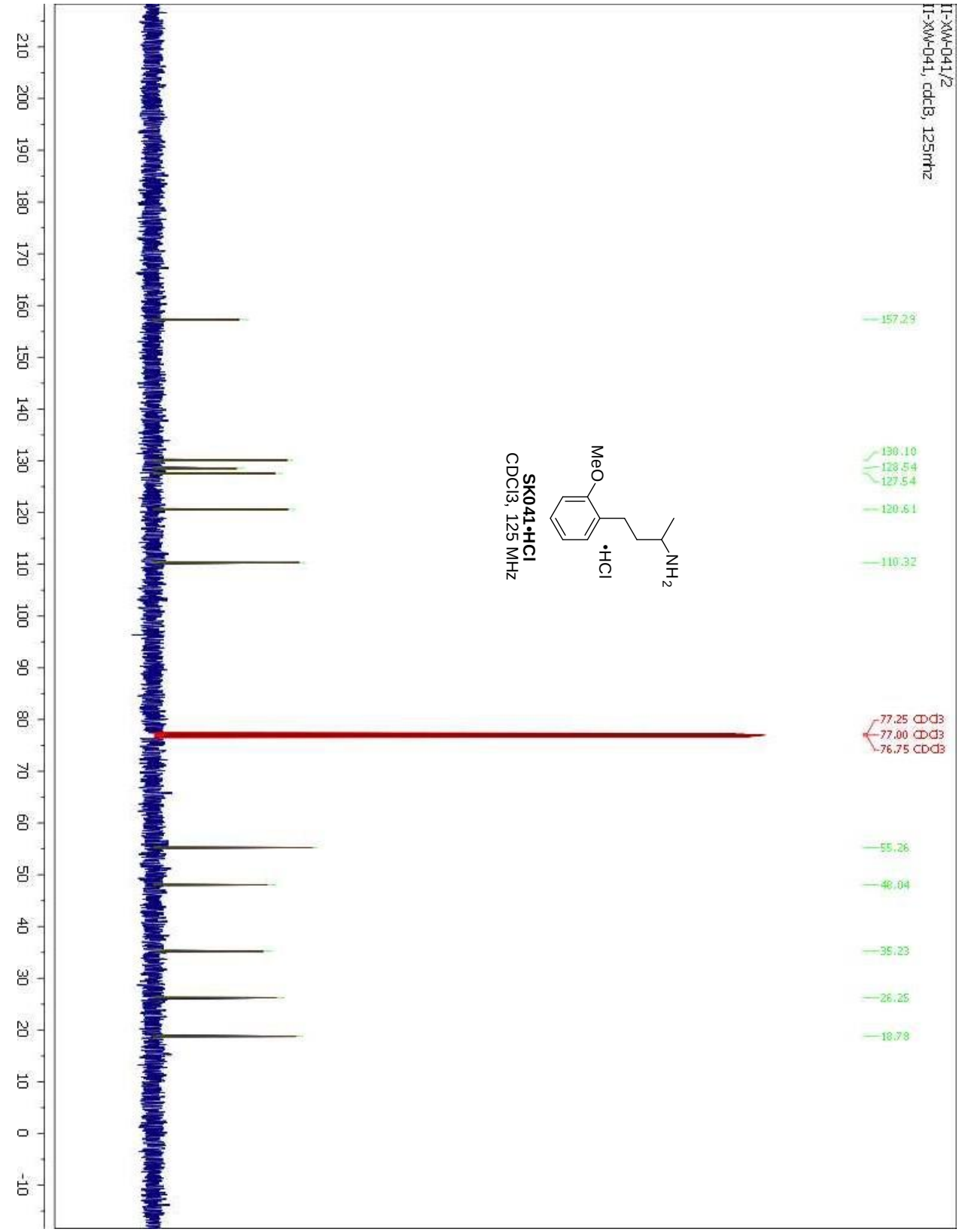
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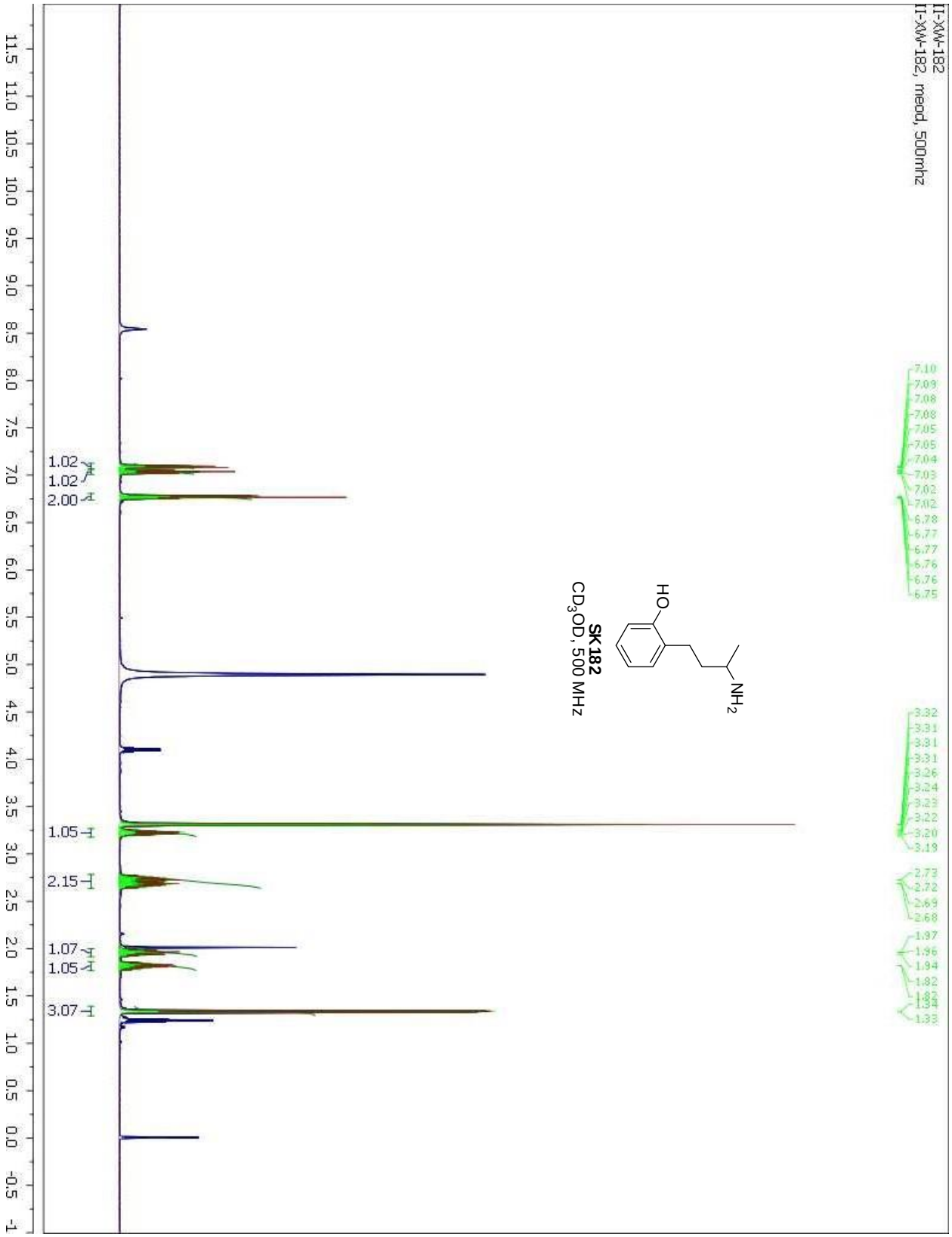
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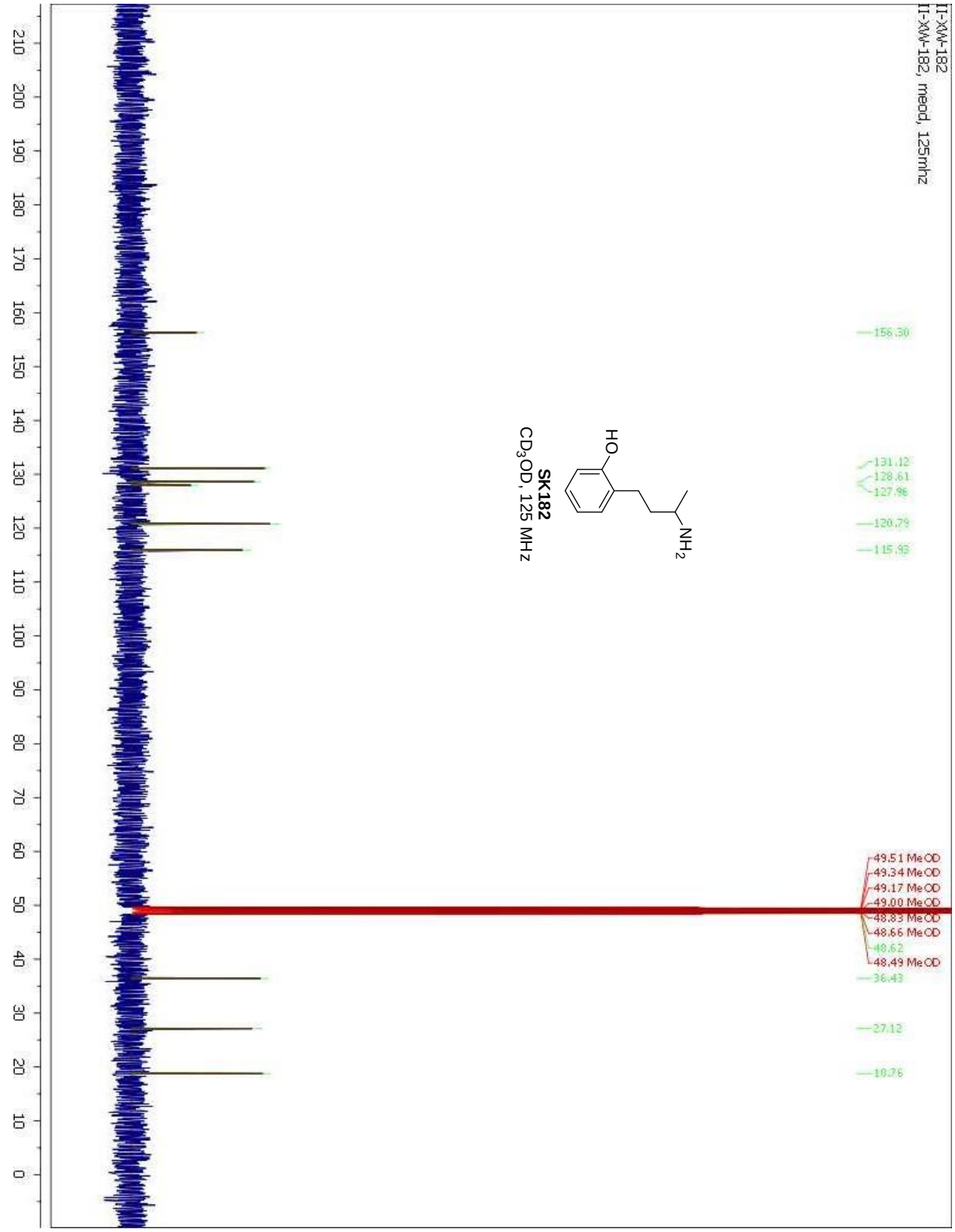
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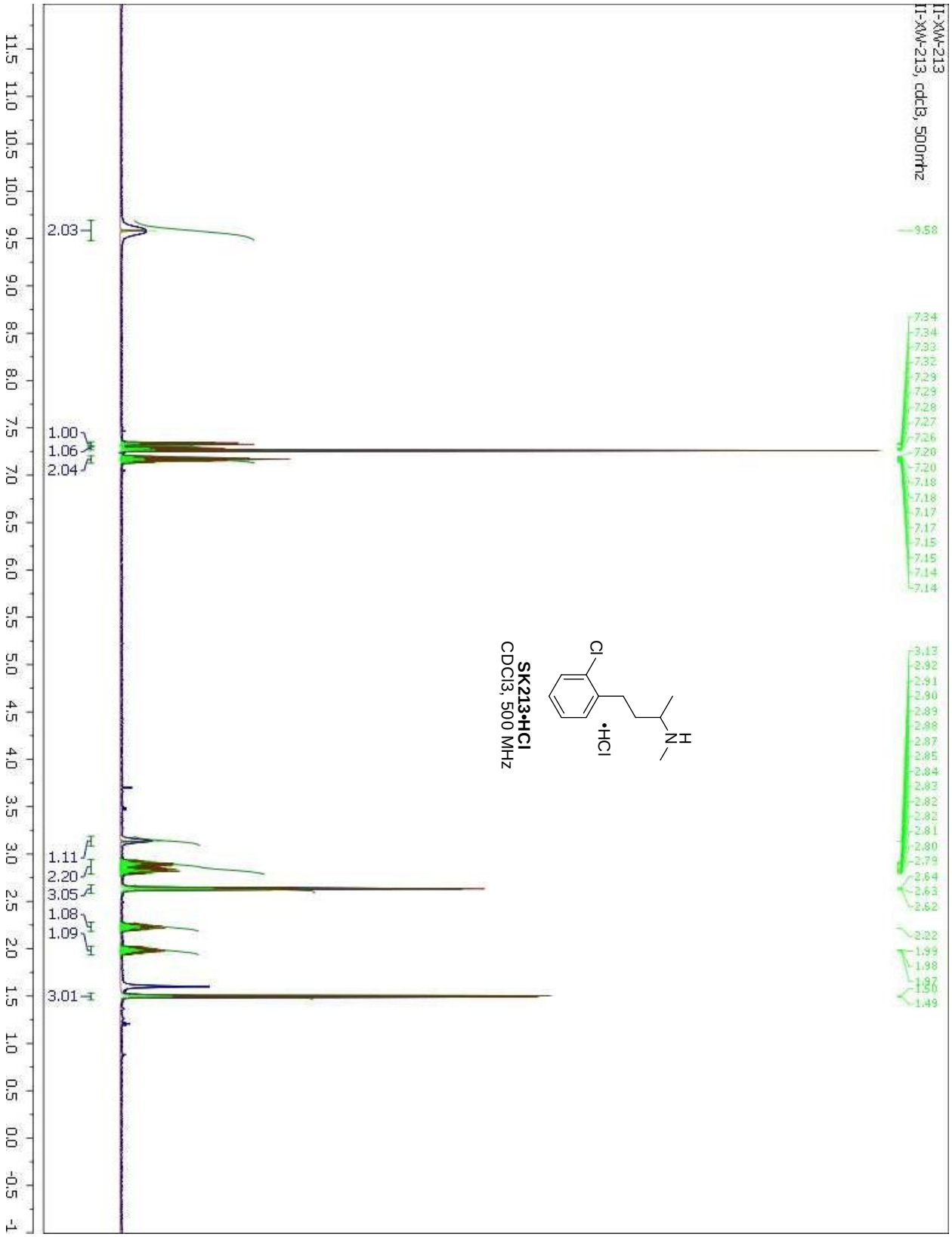
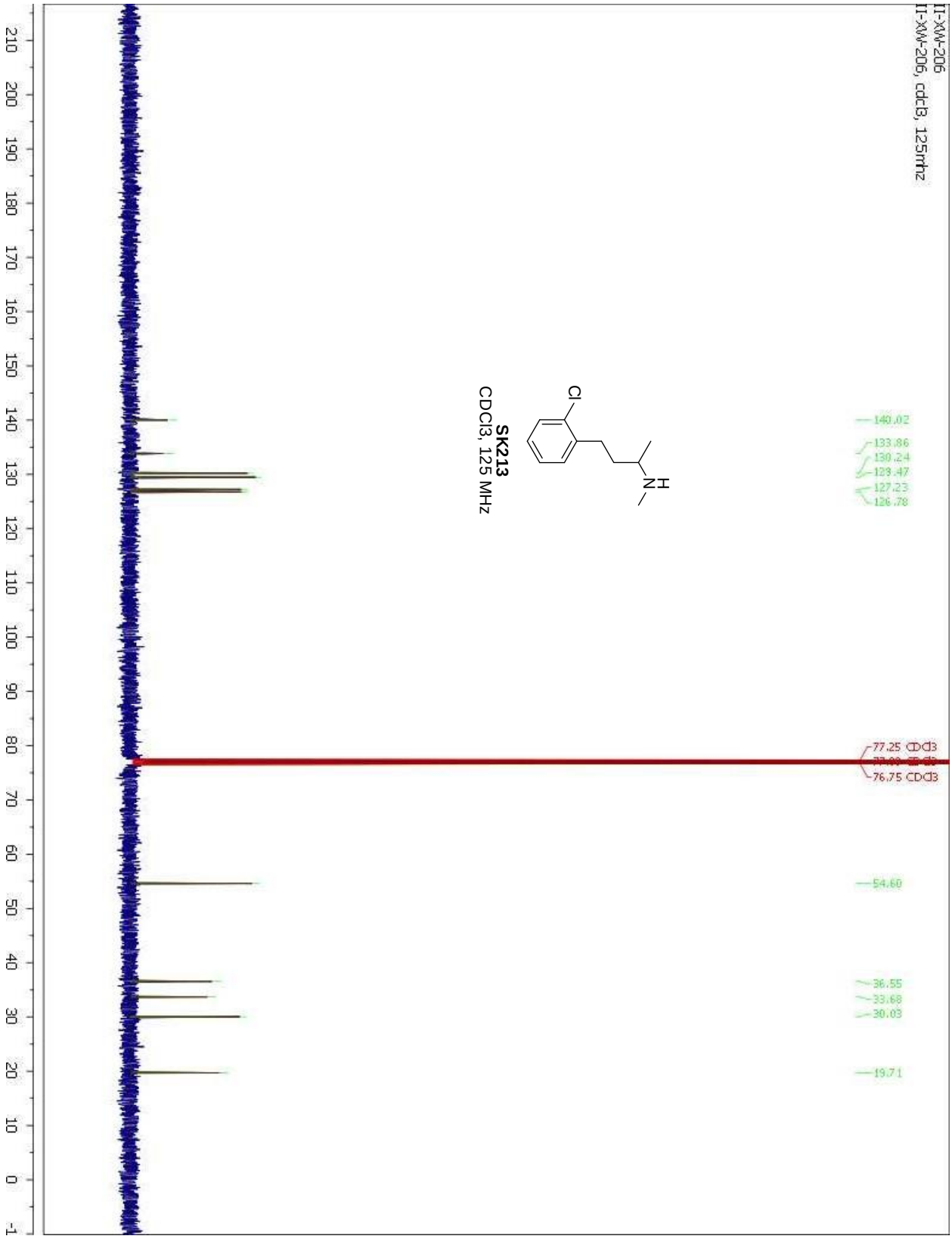
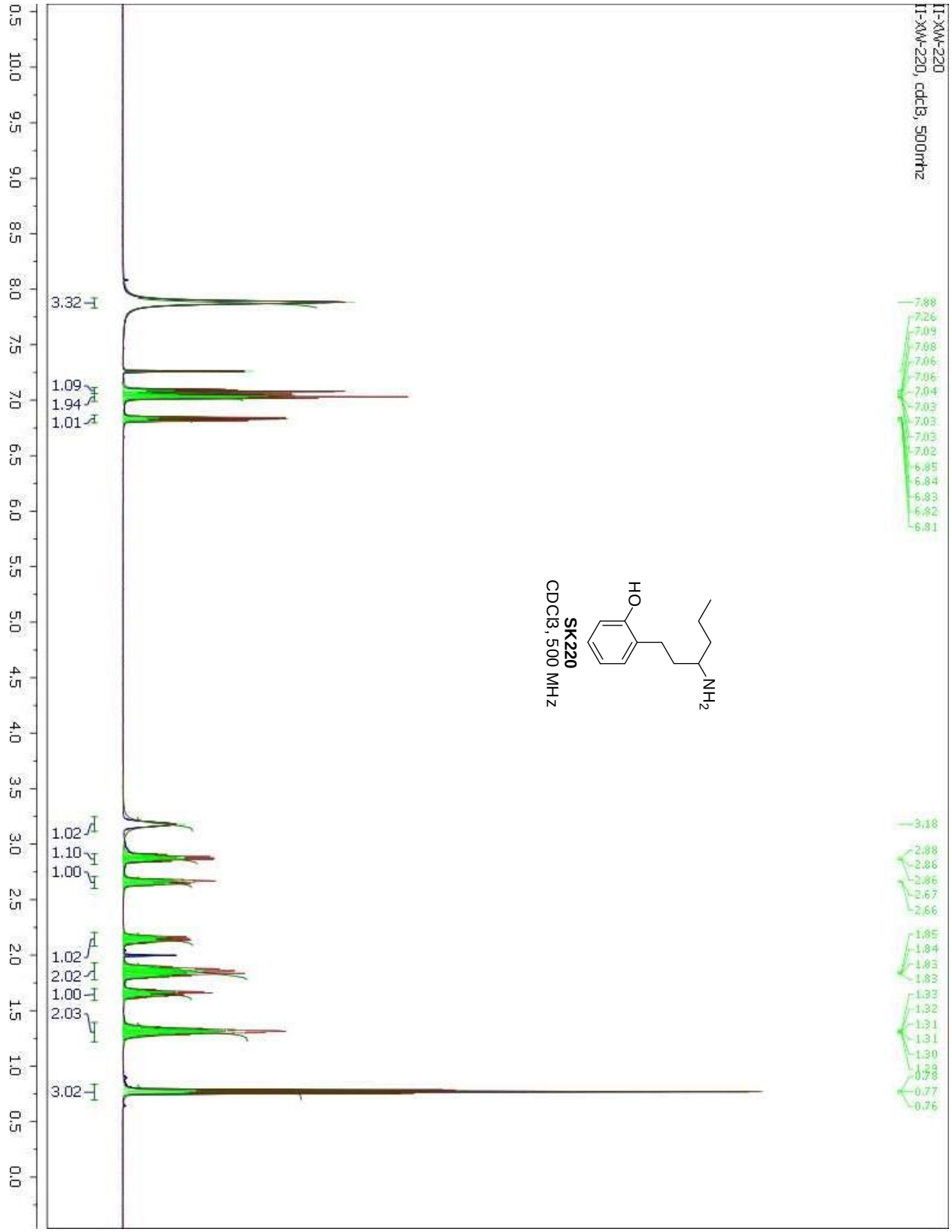
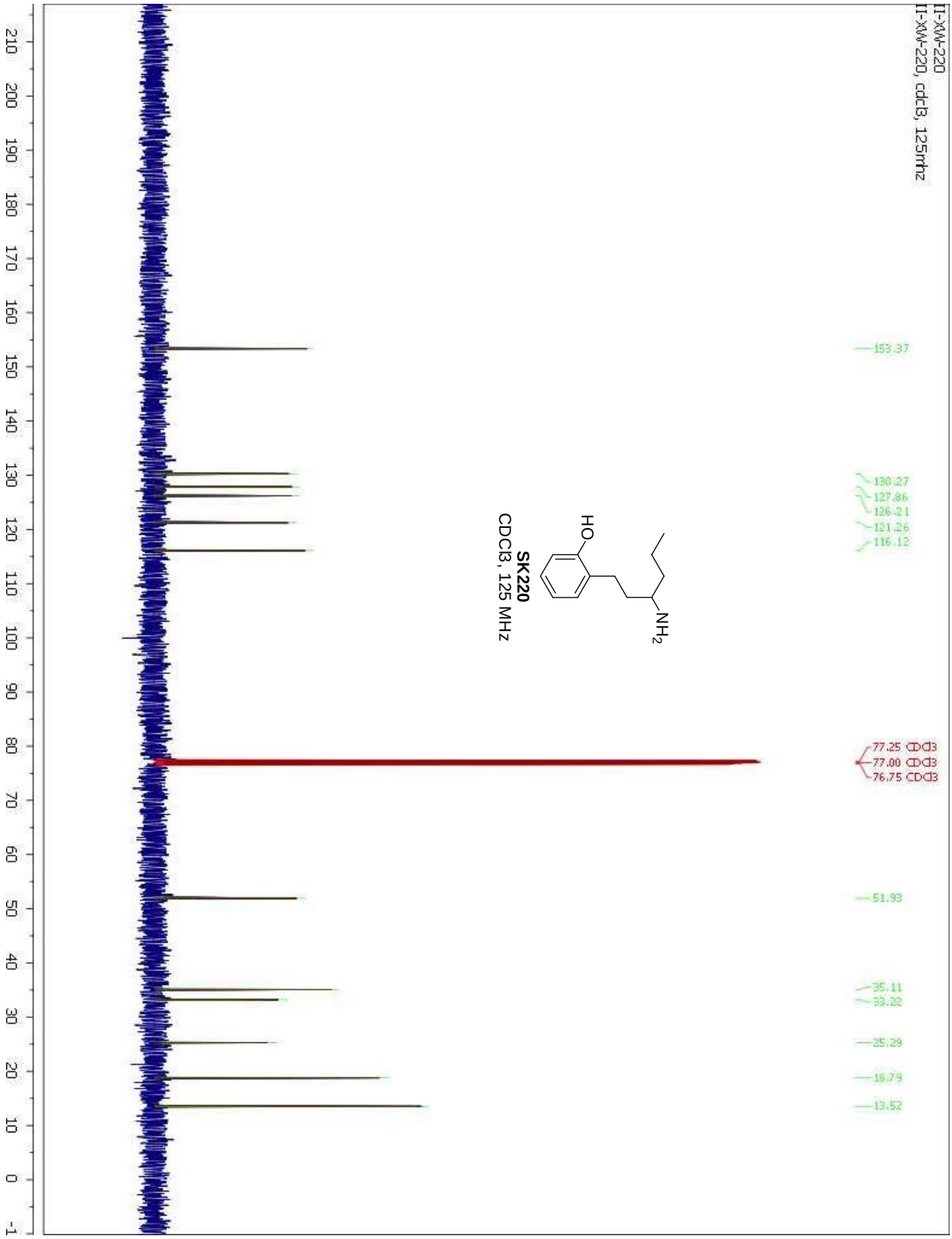
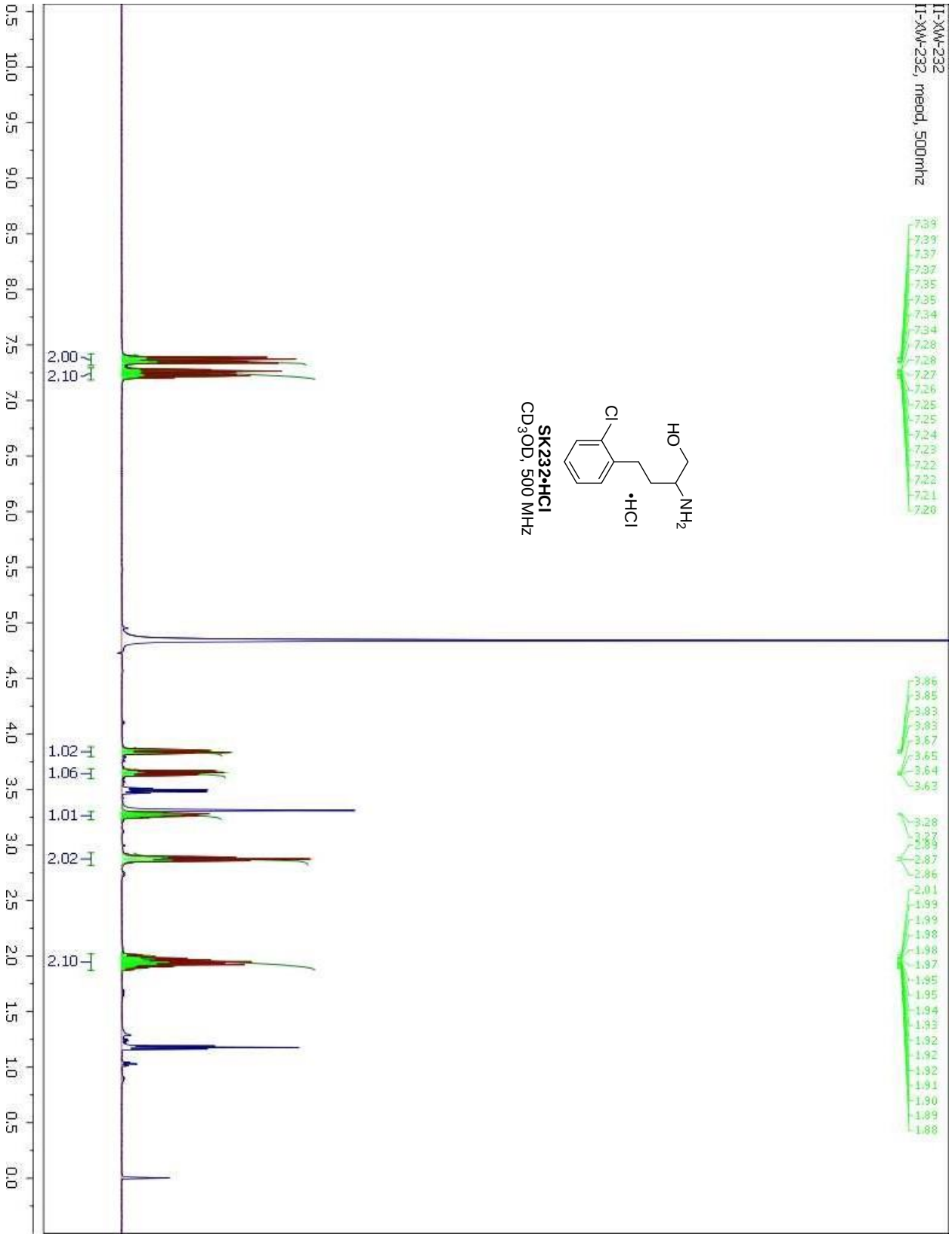
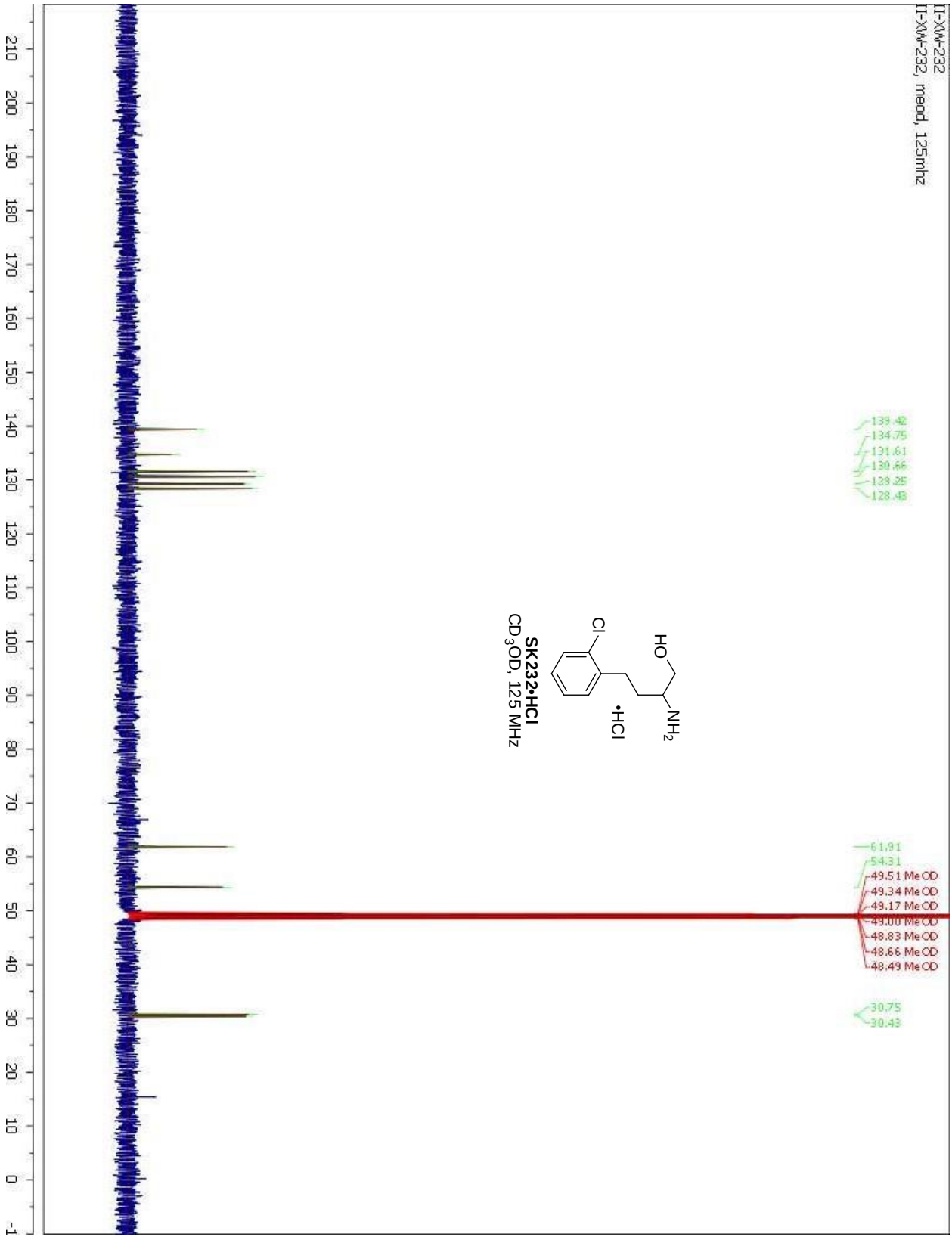

II-XM-213
II-XM-213, cdcl3, 500mhz
SK213•HCl
CDCl3, 500 MHz
Cl
•HCl
H
N
2.03
1.00
1.06
2.04
1.11
2.20
3.05
1.08
1.09
3.01

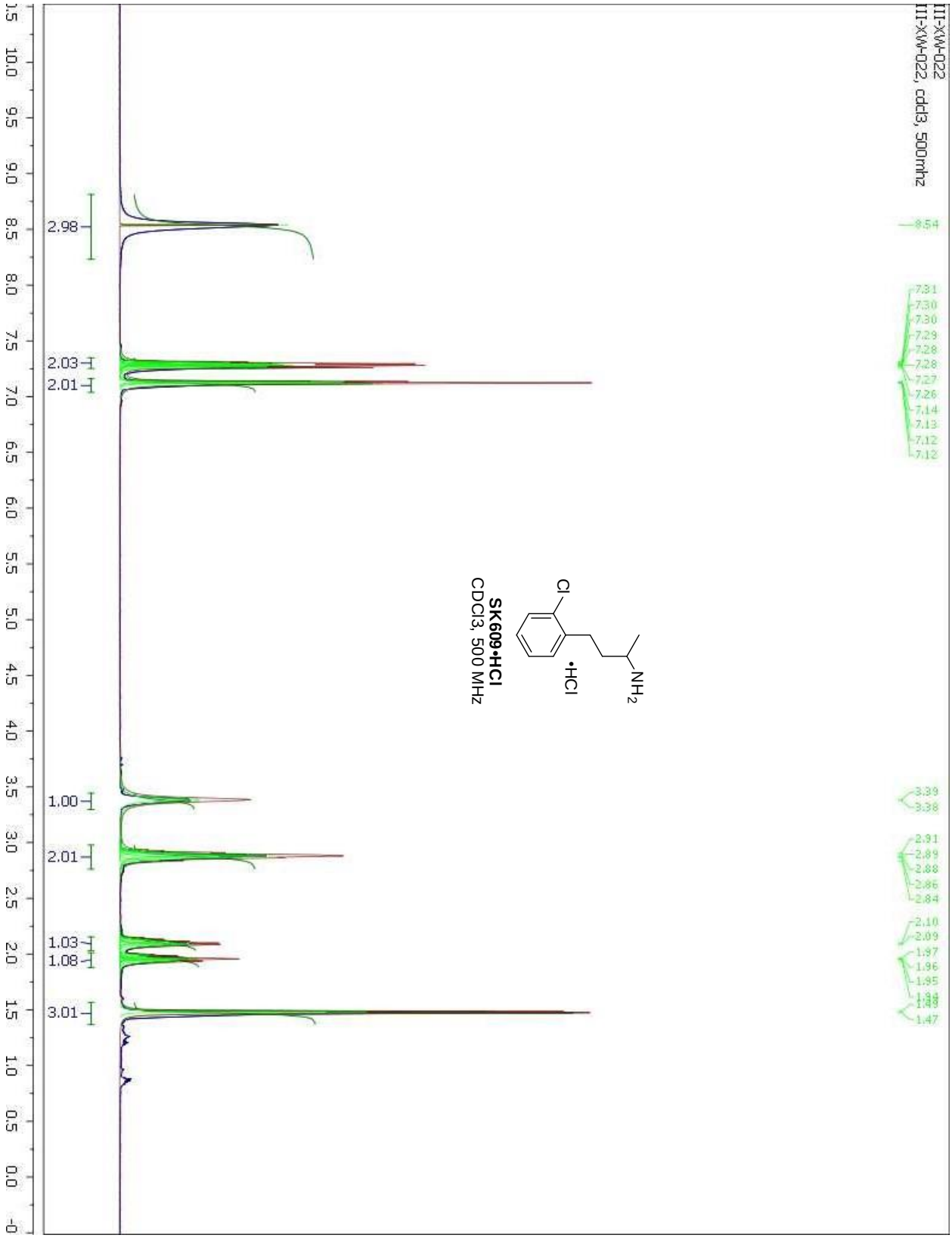

II-XM-206
II-XM-206, cdcl3, 125mhz
SK213
CDCl3, 125 MHz
Cl
H
N
140.02
133.86
130.24
129.47
127.23
126.78
77.25 CDCl3
76.75 CDCl3
54.60
36.55
33.68
30.03
19.71

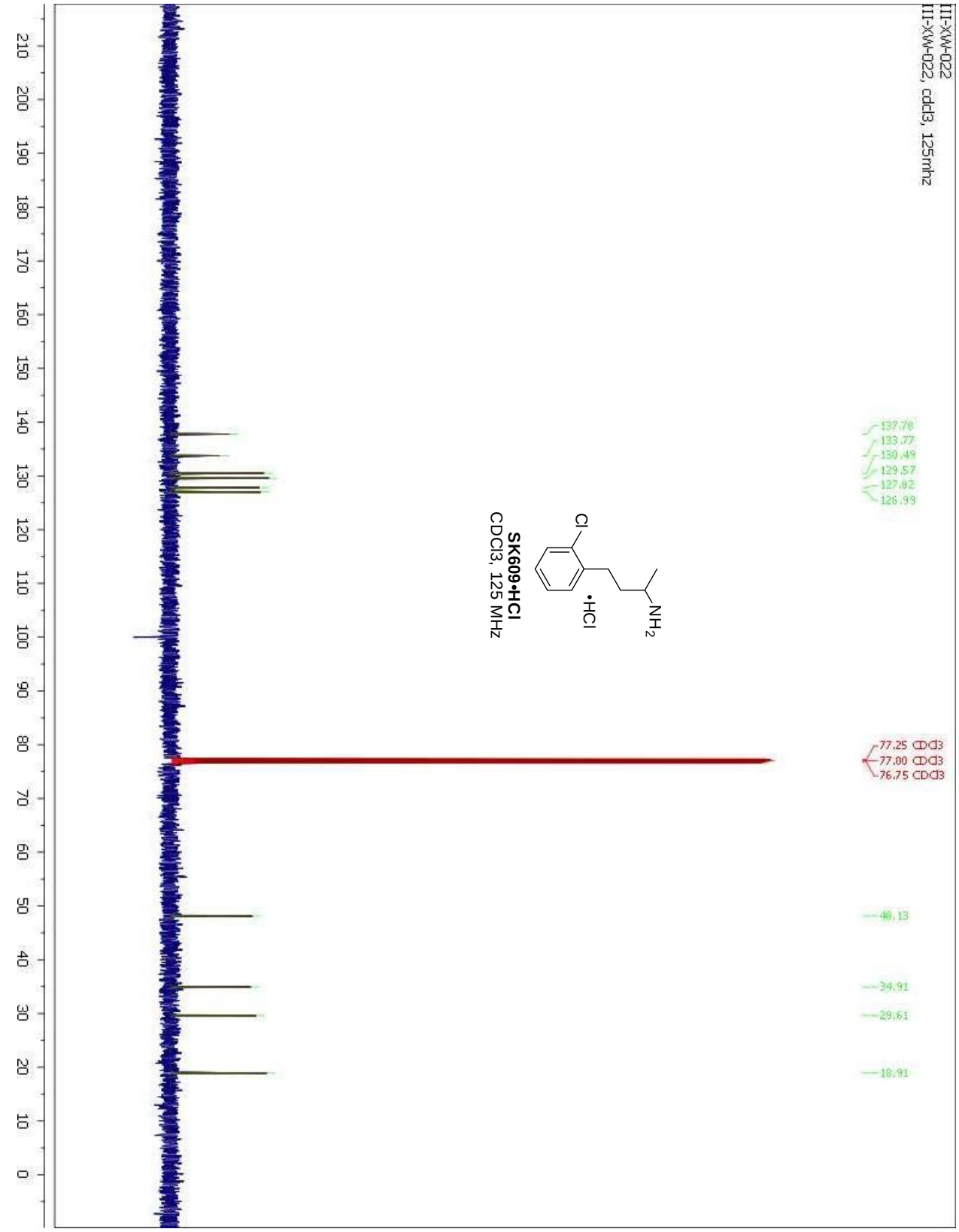

II-XM-220
II-XM-220, cdcl3, 500mhz
SK220
CDCl3, 500 MHz
HO
NH2
3.32
1.09
1.94
1.01
1.02
1.10
1.00
1.02
2.02
1.00
2.03
3.02
7.88
7.26
7.09
7.08
7.06
7.06
7.04
7.03
7.03
7.03
7.02
6.85
6.84
6.83
6.82
6.81
3.18
2.88
2.86
2.86
2.67
2.66
1.85
1.84
1.83
1.83
1.33
1.32
1.31
1.31
1.30
0.78
0.77
0.76


II-XM-220
II-XM-220, cdcl3, 125mhz
153.37
130.27
127.86
126.21
121.26
116.12
77.25 CDCl3
77.00 CDCl3
76.75 CDCl3
51.93
35.11
33.22
25.29
18.79
13.52
SK220
CDCl3, 125 MHz
HO
NH2

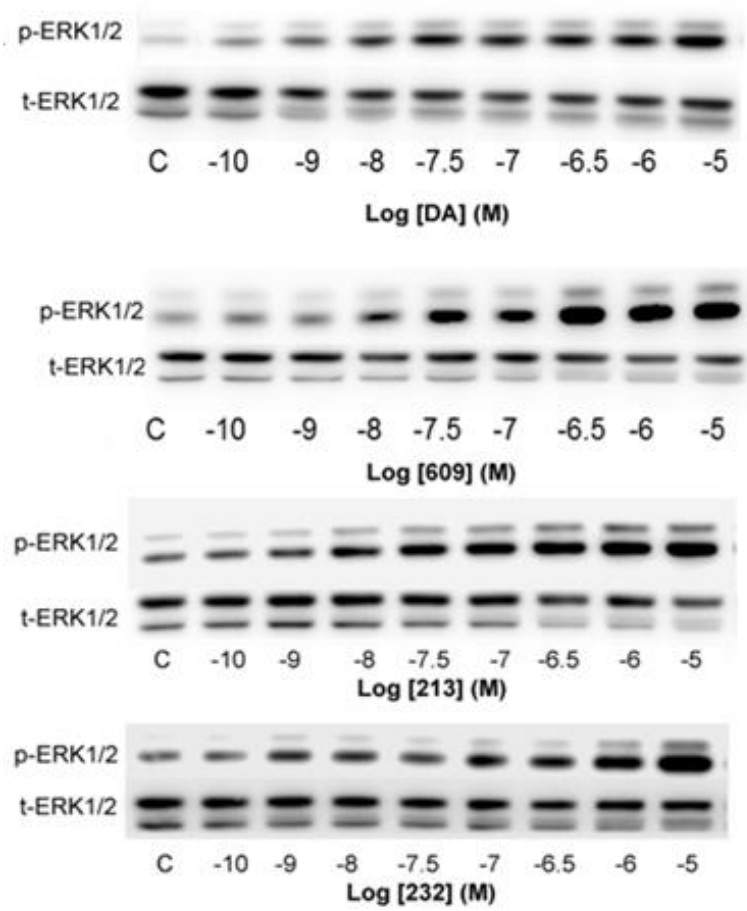

II-XM-232
II-XM-232, meod, 500mhz
SK232•HCl
CD3OD, 500 MHz
Cl
HO
NH2
•HCl
2.00
2.10
1.02
1.06
1.01
2.02
2.10


II-xM-232
II-xM-232, meod, 125mhz
SK232•HCl
CD3OD, 125 MHz
Cl
HO
•HCl
NH2
139.42
134.75
131.61
130.66
129.25
128.43
61.91
54.31
49.51 MeOD
49.34 MeOD
49.17 MeOD
49.00 MeOD
48.83 MeOD
48.66 MeOD
48.49 MeOD
30.75
30.43
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
-1

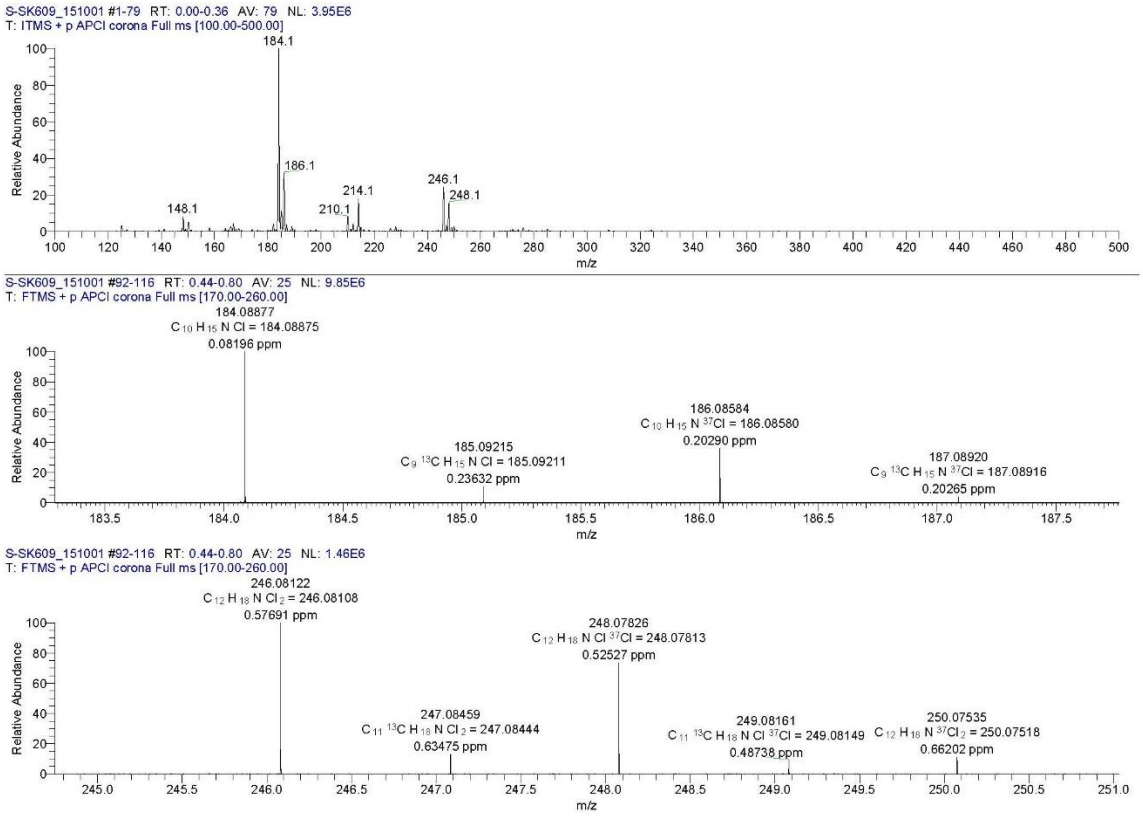

III-XW-022
III-XW-022, cdcl3, 500mhz
SK609•HCl
CDCl3, 500 MHz
Cl
•HCl
NH2
2.98
2.03
2.01
1.00
2.01
1.03
1.08
3.01
8.54
7.31
7.30
7.30
7.29
7.28
7.28
7.27
7.26
7.14
7.13
7.12
7.12
3.39
3.38
2.91
2.89
2.88
2.86
2.84
2.10
2.09
1.97
1.96
1.95
1.47

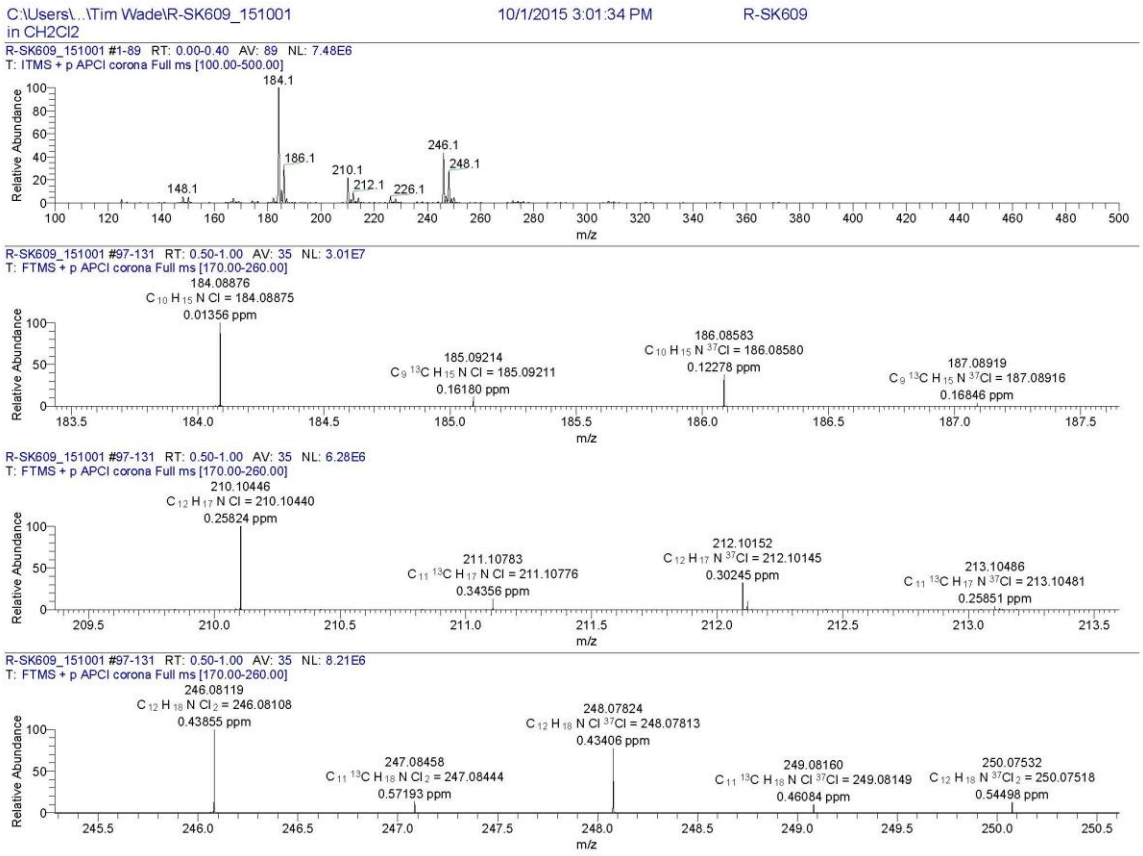

III-XW-022
III-XW-022, cdcl3, 125mhz
137.78
133.77
130.49
129.57
127.82
126.99
77.25 CDCl3
77.00 CDCl3
76.75 CDCl3
48.13
34.91
29.61
18.91
SK609•HCl
CDCl3, 125 MHz
Cl
•HCl
NH2
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0

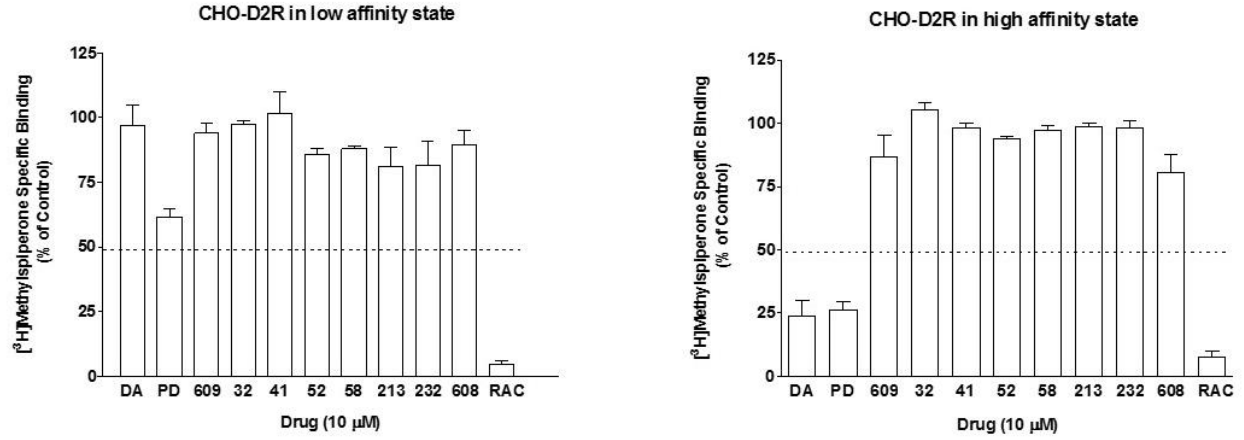
Supplementary Figure 1: Dose-response curves of ERK1/2 phosphorylation induced by DA, SK609, SK213 and SK232 at CHO-D3R. CHO cells overexpressing D3R were pretreated with vehicle, indicated concentrations of DA, SK609, SK213 or SK232 for 5 min, washed once with PBS and lysed for western blots.



Supplementary Figure 2: LC-MS profiles of the enantiomeric forms S-SK609 and R-SK609 are shown confirming the mass and enantiomeric profiles.


S-SK609_151001 #1-79 RT: 0.00-0.36 AV: 79 NL: 3.95E6
T: ITMS + p APCI corona Full ms [100.00-500.00]
184.1
186.1
148.1
214.1
210.1
246.1
248.1
Relative Abundance
m/z
S-SK609_151001 #92-116 RT: 0.44-0.80 AV: 25 NL: 9.85E6
T: FTMS + p APCI corona Full ms [170.00-260.00]
184.08877
C10 H15 N Cl = 184.08875
0.08196 ppm
185.09215
C9 13C H15 N Cl = 185.09211
0.23632 ppm
186.08584
C10 H15 N 37Cl = 186.08580
0.20290 ppm
187.08920
C9 13C H15 N 37Cl = 187.08916
0.20265 ppm
Relative Abundance
m/z
S-SK609_151001 #92-116 RT: 0.44-0.80 AV: 25 NL: 1.46E6
T: FTMS + p APCI corona Full ms [170.00-260.00]
246.08122
C12 H18 N Cl2 = 246.08108
0.57691 ppm
247.08459
C11 13C H18 N Cl2 = 247.08444
0.63475 ppm
248.07826
C12 H18 N Cl 37Cl = 248.07813
0.52527 ppm
249.08161
C11 13C H18 N Cl 37Cl = 249.08149
0.48738 ppm
250.07535
C12 H18 N 37Cl2 = 250.07518
0.66202 ppm
Relative Abundance
m/z


C:\Users\...\Tim Wade\R-SK609_151001 10/1/2015 3:01:34 PM R-SK609
in CH2Cl2
R-SK609_151001 #1-89 RT: 0.00-0.40 AV: 89 NL: 7.48E6
T: ITMS + p APCI corona Full ms [100.00-500.00]
184.1
186.1
246.1
248.1
210.1
212.1
226.1
148.1
Relative Abundance
m/z
R-SK609_151001 #97-131 RT: 0.50-1.00 AV: 35 NL: 3.01E7
T: FTMS + p APCI corona Full ms [170.00-260.00]
184.08876
C10 H15 N Cl = 184.08875
0.01356 ppm
185.09214
C9 13C H15 N Cl = 185.09211
0.16180 ppm
186.08583
C10 H15 N 37Cl = 186.08580
0.12278 ppm
187.08919
C9 13C H15 N 37Cl = 187.08916
0.16846 ppm
Relative Abundance
m/z
R-SK609_151001 #97-131 RT: 0.50-1.00 AV: 35 NL: 6.28E6
T: FTMS + p APCI corona Full ms [170.00-260.00]
210.10446
C12 H17 N Cl = 210.10440
0.25824 ppm
211.10783
C11 13C H17 N Cl = 211.10776
0.34356 ppm
212.10152
C12 H17 N 37Cl = 212.10145
0.30245 ppm
213.10486
C11 13C H17 N 37Cl = 213.10481
0.25851 ppm
Relative Abundance
m/z
R-SK609_151001 #97-131 RT: 0.50-1.00 AV: 35 NL: 8.21E6
T: FTMS + p APCI corona Full ms [170.00-260.00]
246.08119
C12 H18 N Cl2 = 246.08108
0.43855 ppm
247.08458
C11 13C H18 N Cl2 = 247.08444
0.57193 ppm
248.07824
C12 H18 N Cl 37Cl = 248.07813
0.43406 ppm
249.08160
C11 13C H18 N Cl 37Cl = 249.08149
0.46084 ppm
250.07532
C12 H18 N 37Cl2 = 250.07518
0.54498 ppm
Relative Abundance
m/z



Supplementary Figure 3: Comparison of binding affinity of SK609 and its analogues to CHO-D2R in the low affinity state and the high affinity state. Two binding buffers were used to determine the affinity of reference compounds DA and PD128907 and D2-like antagonist raclopride and test compounds in competing for the binding of [^{3}H]methylspiperone to CHO-D2R: Na^+-GTPγS binding buffer (50 mM Tris-HCl, pH 7.4, with 154 mM NaCl, 0.025% ascorbic acid, 10 μM GTPγS, and 0.001% bovine serum albumin), which converts the receptors to a low affinity state for agonists, and Mg^{2+} binding buffer (50 mM Tris-HCl, pH 7.4, 6 mM $MgCl_2$, 0.025% ascorbic acid, and 0.001% bovine serum albumin) for high-affinity state agonist binding. For converting the receptors to a high affinity state, membranes were preincubated with 100 μM GDP and 100 mM NaCl for 30 min at room temperature and washed and centrifuged at 40,000 × *g* for 15 min to remove excess Na^+ and GDP from membranes, which promotes the receptors to a high affinity state for agonists in the presence of Mg^{2+}. [^{3}H]methylspiperone (about 0.25 nM) binding to D2R was conducted with vehicle or 10 μM of DA and PD128907 and raclopride (RAC) or test compounds for 1 h at 30^{0}C in the

two binding buffer, respectively.10 μM haloperidol was used to define nonspecific binding.