SUPPORTING INFORMATION

For

2-Difluoromethylpyridine as a bioisosteric replacement of pyridine-*N*-oxide: *the case of quorum sensing inhibitors*.

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I. Materials and methods

Chemistry

All reagents and solvents used in this work were purchased from commercially available venders including Sigma, Merck, TCI. Compounds were characterized using NMR technique including: ^{1}H-NMR (recorded at 400 or 500 MHz Bruker Avance, 300k), ^{13}C-NMR (recorded at 151 or 101 MHz). Chemical shifts (δ) are reported in ppm. *J* values are reported in Hertz. Pre-coated Silica gel 60 F_{254} plates were used for TLC and visualized under UV light. The compounds had at least 95% purity before biological screening. The electrospray (ESI) mass spectrometer system was used for record HRMS.

Biology

Quorum sensing assay. The method has been reported previously [1] without modification. Briefly, the QS reporter strain *lasB-gfp of P. aeruginosa* was used. The growth was monitered using Victor X4 multilabel plate reader. The GFP expression was recorded as fluorescence at 485 nm, 535 nm.

*Anti-violacein formation of CviR receptor***.** The method has been reported previously [1] without modification. Briefly, the *C. violaceum* 31532 was used. The absorbance was recorded at 585nm.

*Anti-biofilm biomass***.** The method has been reported previously [1] without modification. Briefly, we use *P. aeruginosa* PA14 for the assay. Negative control is DMSO.

*The protease assays***.** The assay was performed using the previously described method [2]. Briefly, growth phase of P. aeruginosa was diluted to $OD_{600} = 0.1$ in PTSB medium and incubated for 8h at 37 °C. The bacterial culture and testing compounds were added to each well of a 96 well-plate and cultured for 24h at 37 °C. The absorbance was recorded using Spectramax M4 (Molecular Devices, USA) at 440 nm. Data was calculated subtracting OD440 recorded with the final OD600 values.

I. General procedure for synthesis of library compounds.

A mixture of corresponding pyrindine (1 equiv), $K_2S_2O_8$ (2.5 equiv.), $AgNO_3$ (0.2 equiv.) and acetonitrile was added to a 25 ml RBF. Difluoromethylating CF_2HCOOH (2.5 equiv) was added, followed H_2O (1:1 to acetonitrile) and 1% trifluoroacetic acid. The mixture was stirred at 50 °C for 24h and monitored by TLC until completion. Then water (2 vol. equiv) then $NaHCO_3$ (saturated) was added and extracted with ethyl acetate (4 times). The organic layer was washed with brine, dried with anhydrous Na_2SO_4(s) and concentrated. The residue was purified by silica-gel flash column chromatography (eluent: EA/Hex = 1:3) to provide the title compound.

(4-Chlorophenyl)(2-(difluoromethyl)pyridin-4-yl)methanone (1). yield 68 %, 53 mg from (4-chlorophenyl)(pyridin-4-yl)methanone; Colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.79 (d, J = 4.8 Hz, 1H), 7.82 (s, 1H), 7.71 (d, J = 7.3 Hz, 2H), 7.61 (m, 1H), 7.45 (d, J = 7.3 Hz, 2H), 6.65 (t, J = 55.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 192.9, 153.9 (t, $J_{C\text{-}F}$ = 27.2 Hz), 150.3, 145.6, 140.6, 133.9, 131.5, 129.3, 124.5, 119.3 (t, $J_{C\text{-}F}$ = 4.2 Hz), 113.5 (t, $J_{C\text{-}F}$ = 241.6 Hz); LRMS(ESI) m/z $[M+H]^+$ found 268.0; HRMS (ESI) m/z [M+H] calculated for $C_{13}H_9ClF_2NO$ 268.0341, found 268.0342.

4-(4-Chlorobenzyl)-2-(difluoromethyl)pyridine (2). 63 %, 48 mg from 4-(4-chlorobenzyl)pyridine; Yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.48 (s, 1H), 7.37 (s, 1H), 7.24 (d, J = 7.8 Hz, 2H), 7.19 (s, 1H), 7.04 (d, J = 8.4 Hz, 2H), 6.54 (t, J = 55.8 Hz, 1H), 3.94 (s, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.3 (t, $J_{C\text{-}F}$ = 24.2 Hz), 151.3, 150.0, 136.6, 132.9, 130.4, 129.1, 125.8, 120.5, 114.2 (t, $J_{C\text{-}F}$ = 241.6 Hz), 40.7; LRMS(ESI) m/z [M+H] found 254.0; HRMS (ESI) m/z [M+H] calculated for $C_{13}H_{11}ClF_2N^+$ 254.0548, found 254.0548.

2-(Difluoromethyl)-4-phenoxypyridine (3). 62 %, 63 mg from 4-phenoxypyridine; Yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.59 (br, 1H), 7.57 (m, 2H), 7.41 (td, J = 7.3, 3.3 Hz, 1H), 7.36 (d, J = 3.6 Hz, 1H, H5), 7.22 (d, J = 4.3 Hz, 2H), 7.00 (s, 1H), 6.69 (t, J = 54.3 Hz, 2H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 166.0, 155.1 (t, $J_{C\text{-}F}$ = 26.0 Hz), 153.6, 151.2, 130.4, 125.9, 120.9, 113.6 (t, $J_{C\text{-}F}$ = 241.2 Hz), 113.5, 108.6; LRMS(ESI) m/z [M+H] found 222.1; HRMS (ESI) m/z [M+H] calculated for $C_{12}H_{10}F_2NO$ 222.0730, found 222.0732.

2-(Difluoromethyl)-4-phenylpyridine (4). 59 %, 68 mg from phenylpyridine; Colorless oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.72 (s, 1H), 7.88 (s, 1H), 7.69 (br , 2H), 7.64 (d, J = 5.0 Hz, 1H), 7.52 (br, 3H), 6.72 (t, J = 55.5 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 153.7 (t, $J_{C\text{-}F}$ = 242.4

Hz), 150.0, 137.4, 129.6, 129.3, 127.1, 123.3, 118.1 (t, J_{C-F} = 3.0 Hz), 114.1 (t, J_{C-F} = 240.6 Hz); LRMS(ESI) m/z $[M+H]^+$ found 206.1; HRMS (ESI) m/z [M+H] calculated for $C_{12}H_{10}F_2N$ 206.0781, found 206.0781.

2-(Difluoromethyl)isonicotinonitrile (5). 64 %, 61 mg from pyridine-4-carbothioamide; Yellow oil; ^{1}H NMR (600 MHz, $CDCl_3$) δ 8.80 (d, J = 4.8 Hz, 1H), 7.81 (s, 1H), 7.60 (d, J = 4.8 Hz, 1H), 6.61 (t, J = 55.2 Hz, 1H); ^{13}C NMR (151 MHz, $CDCl_3$) δ 13C NMR (151 MHz, CDCl3) δ 154.6 (t, J_{C-F} = 25.7 Hz), 150.6, 127.0, 122.11 (t, J_{C-F} = 3.0 Hz), 122.0, 115.7, 112.8 (t, J_{C-F} = 243.1 Hz, C-7); LRMS(ESI) m/z [M+H] found 155.0; HRMS (ESI) m/z [M+H] , calculated for $C_7H_5F_2N_2$ 155.0421, found 155.0422.

Methyl 2-(difluoromethyl)isonicotinate (6). 70 %, 72 mg from methyl isonicotinate; Colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 8.75 (d, J = 7.8 Hz, 1H), 8.11 (s, 1H), 7.90 (d, J = 7.2 Hz, 1H), 6.63 (t, J = 55.2 Hz, 1H), 3.92 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.7, 154.2 (t, J_{C-F} = 26.3 Hz), 150.4, 138.9, 124.7, 119.7 (t, J_{C-F} = 4.0 Hz), 113.4 (t, J_{C-F} = 242.4 Hz), 52.9; LRMS(ESI) m/z [M+H] found 188.0; HRMS (ESI) m/z [M+H] calculated for $C_8H_8F_2NO_2^+$ 188.0523, found 188.0522.

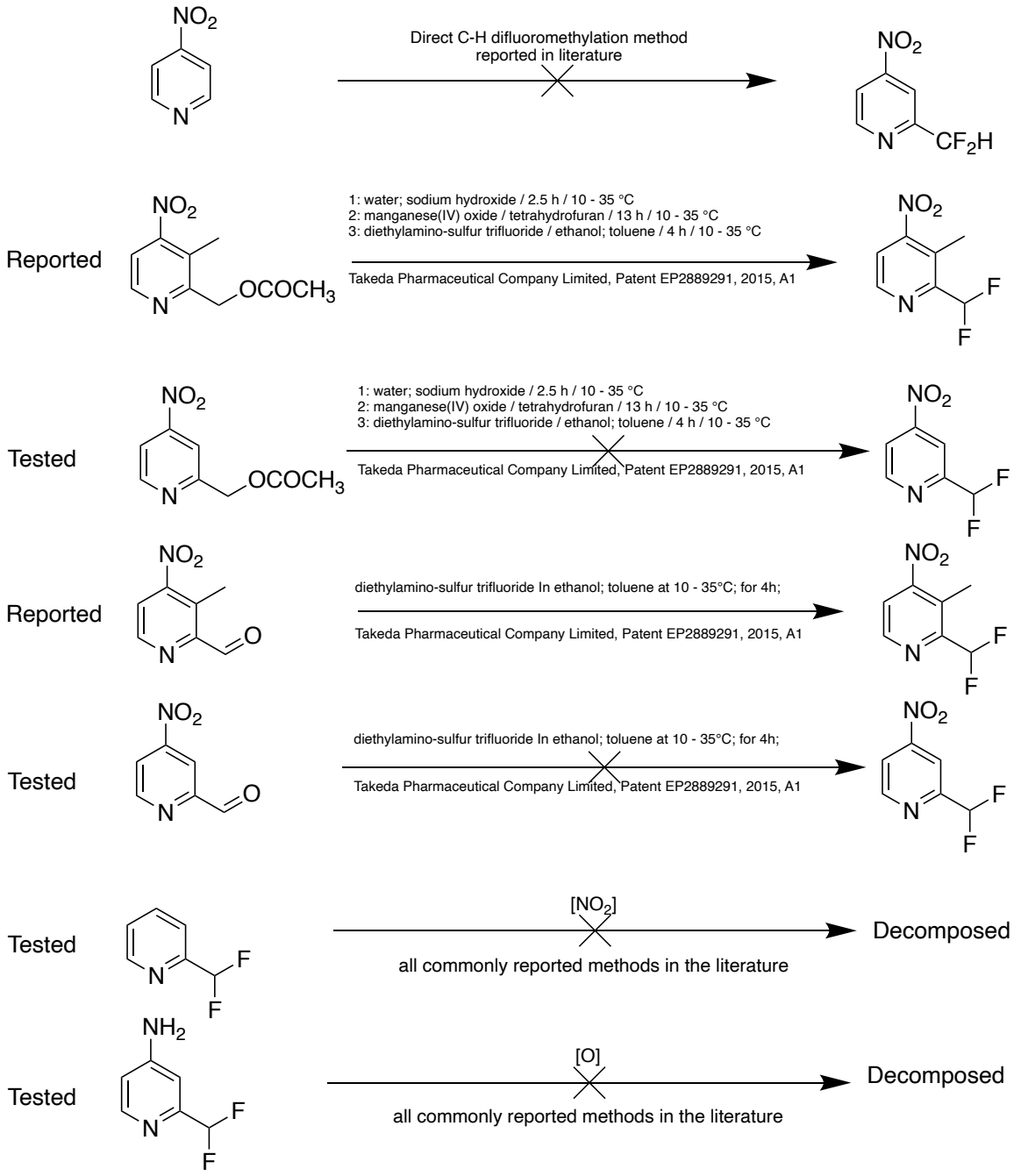
Methyl 3-(difluoromethyl)isonicotinate (**7**), using the general procedure but no TFA added, isolated as by-product of **6**. 27 %, yellow oil, 16 mg from methyl isonicotinate; ^{1}H NMR (400 MHz, $CDCl_3$) δ 9.11 (s, 1H), 8.90 (s, 1H), 7.85 (s, 1H), 7.51 (t, J=54.8, 1H), 4.00 (s, 3H); ^{13}C NMR (101 MHz, $CDCl_3$) δ 164.9, 152.4, 148.0, 136.0 (t, J = 19 Hz), 128.7 (t, J=22.0), 123.3, 111.4 (t, J=238.8 Hz), 53.1; LRMS(ESI) m/z [M+H]+ found 188.0; HRMS (ESI) m/z $[M+H]^+$ calculated for $C_8H_8F_2NO_2^+$ 188.0523, found 188.0523.

References

[1] Truong T. Tung, Thang N. Quoc, Discovery of novel β-turn mimetic-based peptides as novel quorum sensing inhibitors of gram-negative bacteria, *Bioorganic & Medicinal Chemistry Letters*, **2021**,46, 128170

[2] P.J. Petersen, P. Labthavikul, C.H. Jones, P.A. Bradford, J. Antimicrob. Chemother. 2006, 57, 573-576.

II. Attempt to synthesis the 2-(difluoromethyl)-4-nitropyridine



Scheme S1. Attempt to synthesis the 2-(difluoromethyl)-4-nitropyridine

Direct methods:

[3] Truong T. Tung, Søren B. Christensen, John Nielsen, Difluoroacetic Acid as a New Reagent for Direct C−H Difluoromethylation of Heteroaromatic Compounds, *Chem. Eur. J*. **2017**, 23, 18125-18128.

[4] Yuta Fujiwara, Janice A Dixon, Rodrigo A Rodriguez, Ryan D Baxter, Darryl D Dixon, Michael R Collins, Donna G Blackmond, Phil S Baran, A New Reagent for Direct Difluoromethylation, *J. Am. Chem. Soc*. **2012**, 134(3), 1494–1497.

[5] Sheng-Qing Zhu, Yin-Li Liu, Huan Li, Xiu-Hua Xu, Feng-Ling Qing, Direct and Regioselective C–H Oxidative Difluoromethylation of Heteroarenes, *J. Am. Chem. Soc.* **2018**, 140, 37, 11613–11617.

[6] Claudio F Meyer, Sandrine M Hell, Antonio Misale, Andrés A Trabanco, Véronique Gouverneur, Hydrodifluoromethylation of Alkenes with Difluoroacetic Acid, *Angew Chem Int Ed Engl* . **2019**, 58(26), 8829-8833.

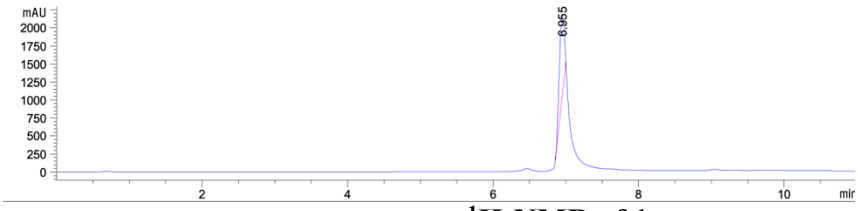
[7] Alexandra C Sun, Edward J McClain, Joel W Beatty, Corey R J Stephenson, *Organic Letters* **2018**, 20, 3487 – 3490.

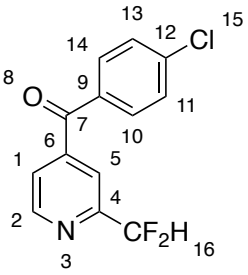
Indirect method:

[8] Takeda Pharmaceutical Company Limited, Patent EP2889291, 2015, A1

III. Copy of NMR-spectra and chromatographic analysis

Compound **1**





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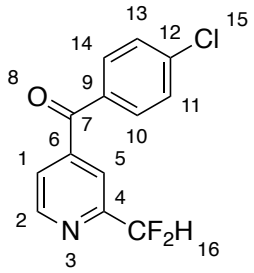

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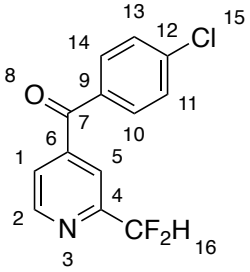
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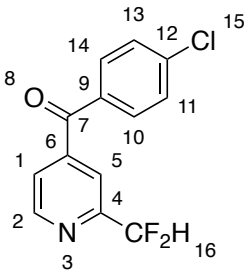
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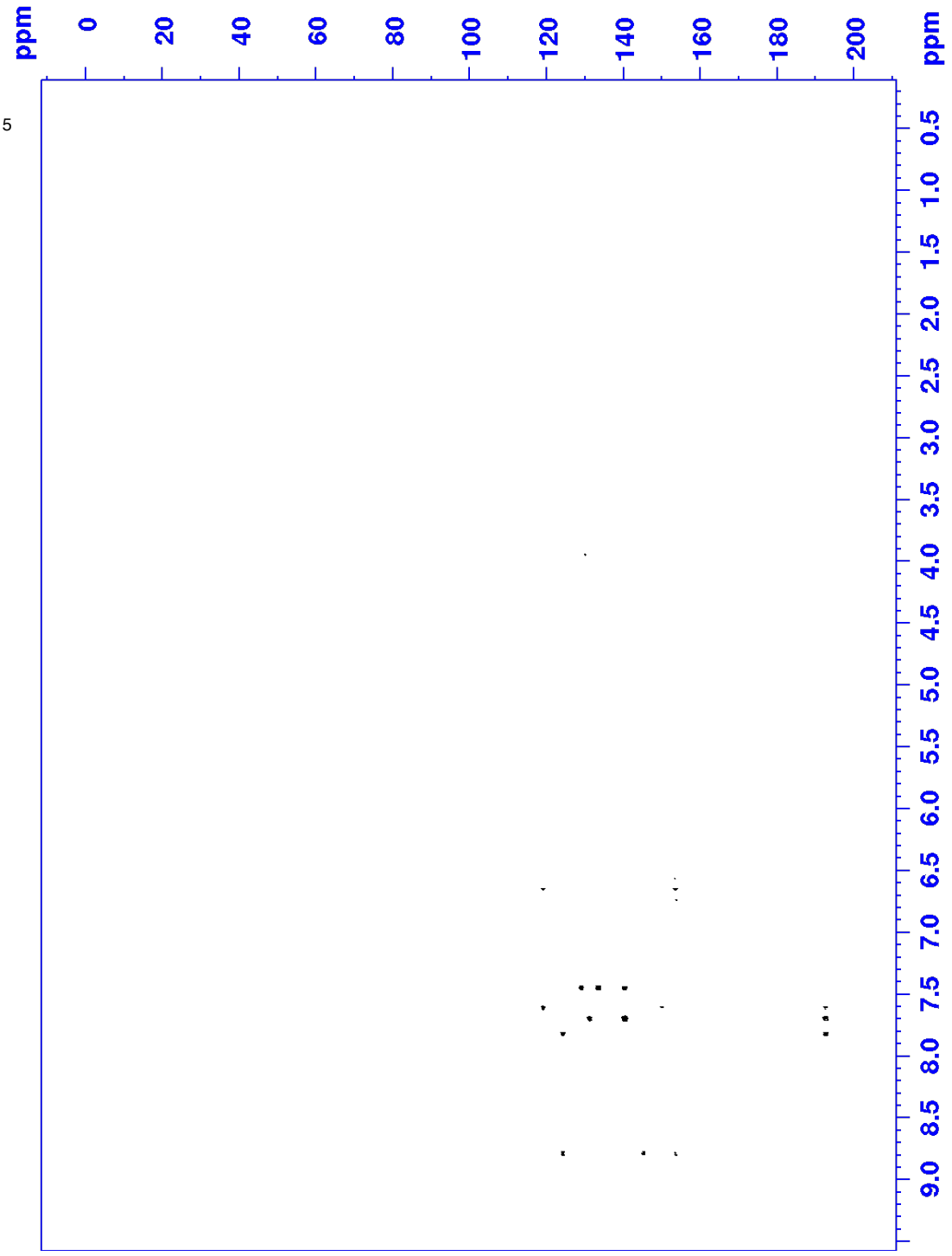
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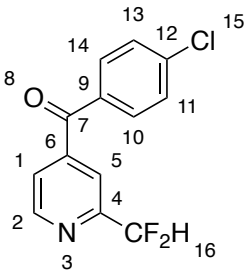
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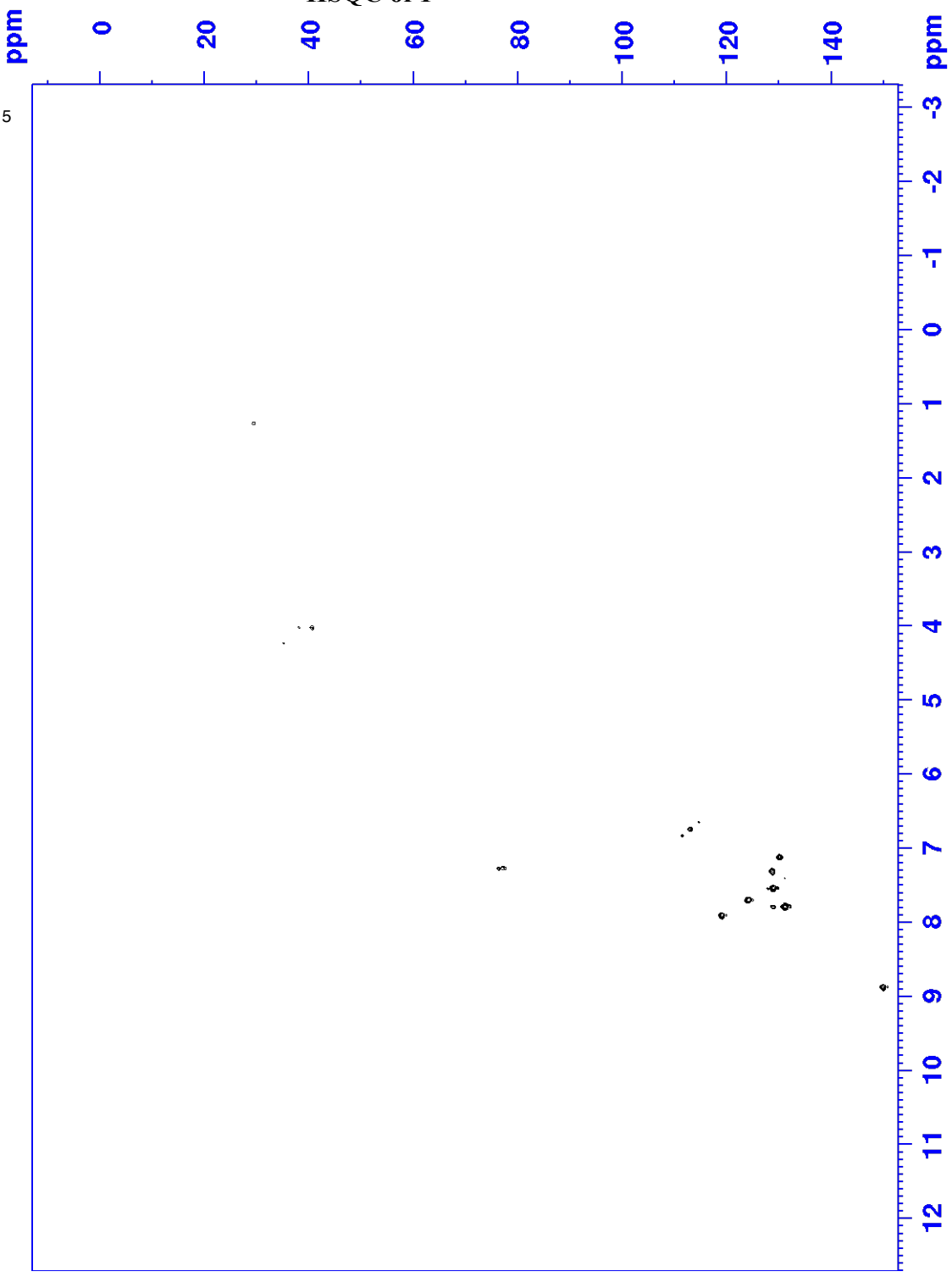
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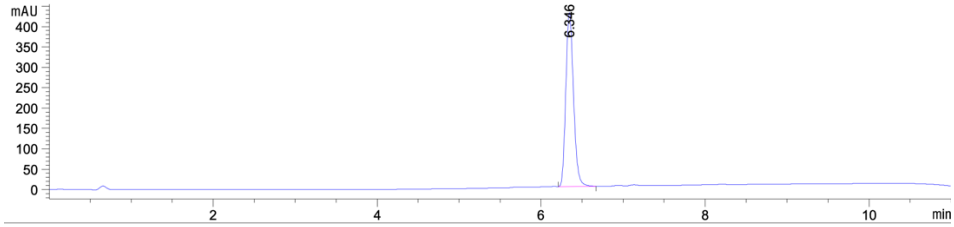
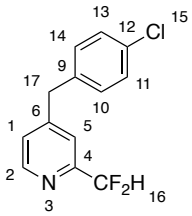
Compound **2**





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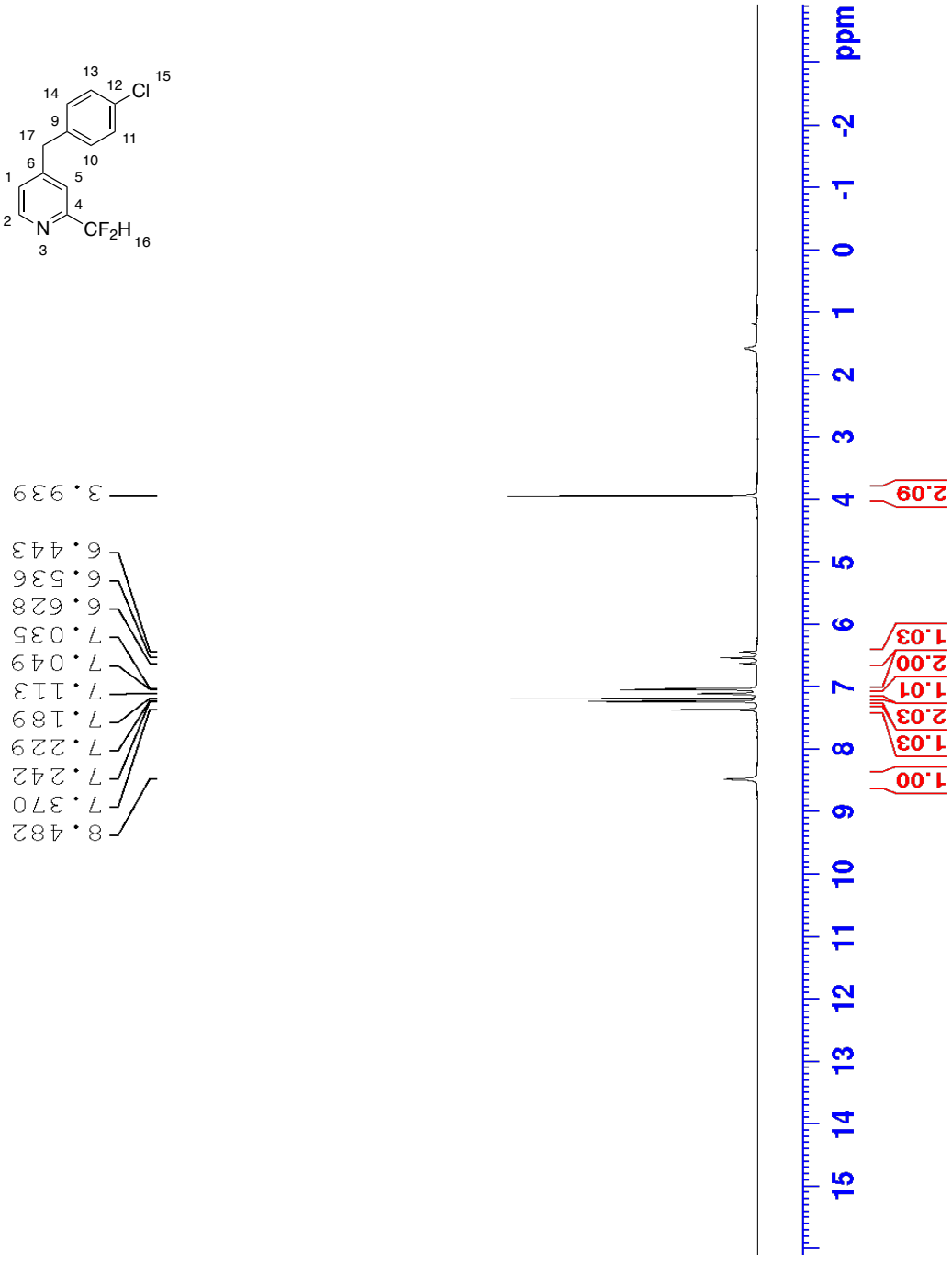

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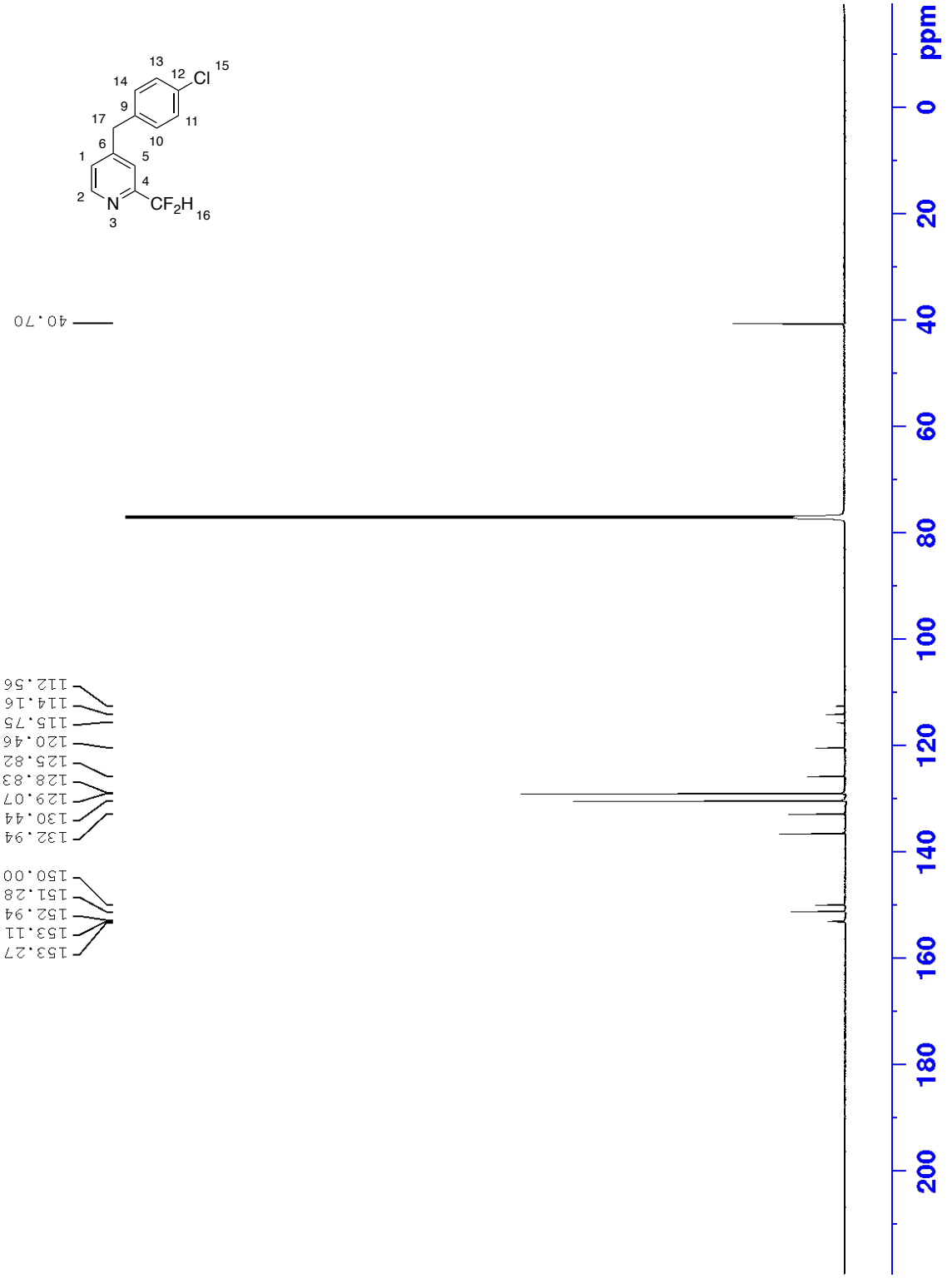
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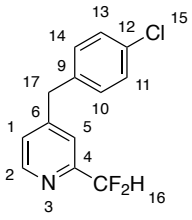
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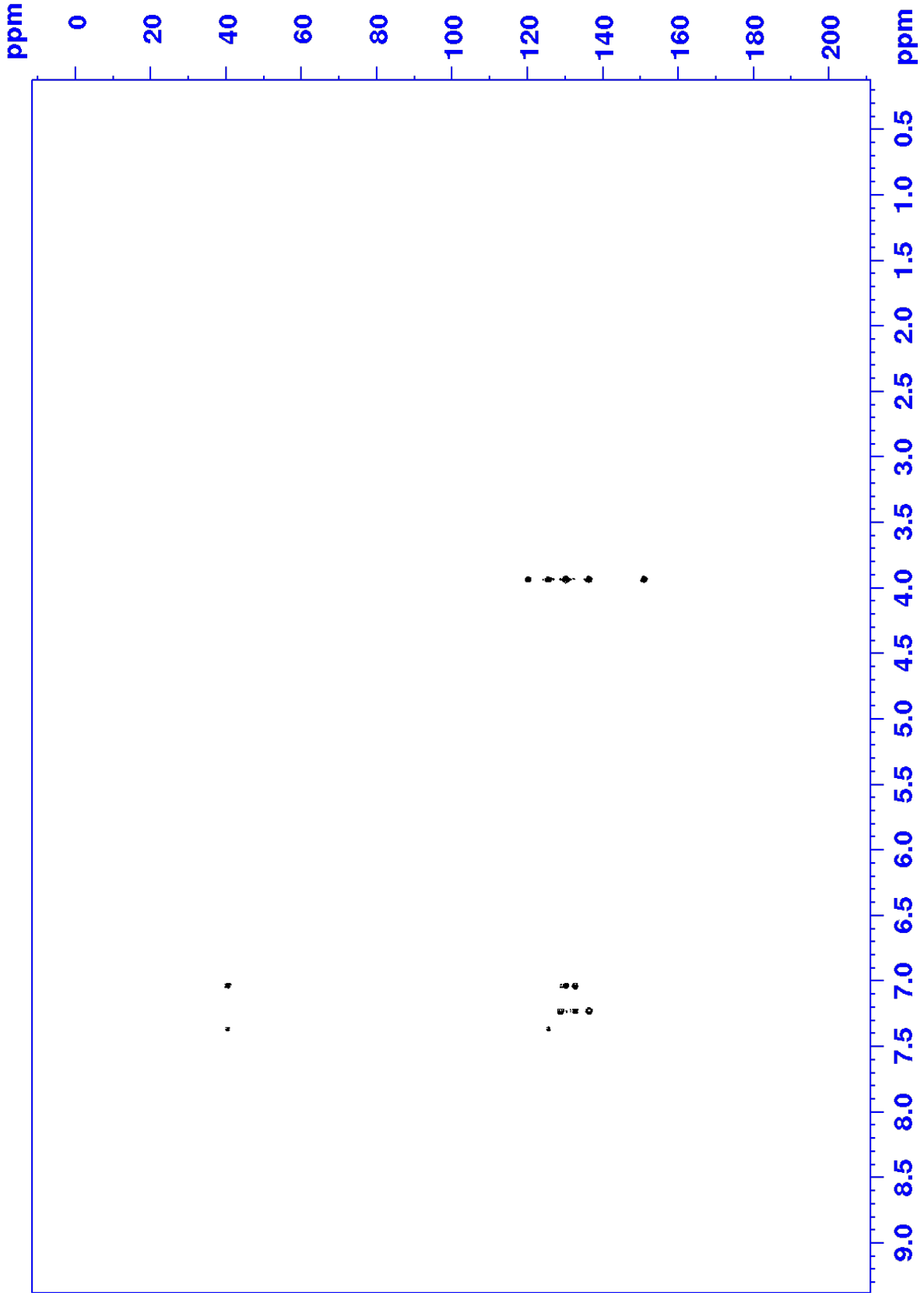
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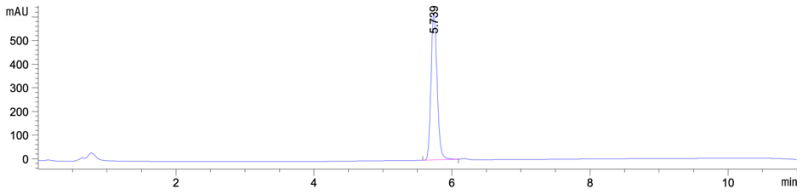
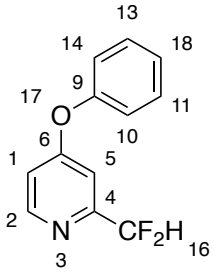
Compound **3**





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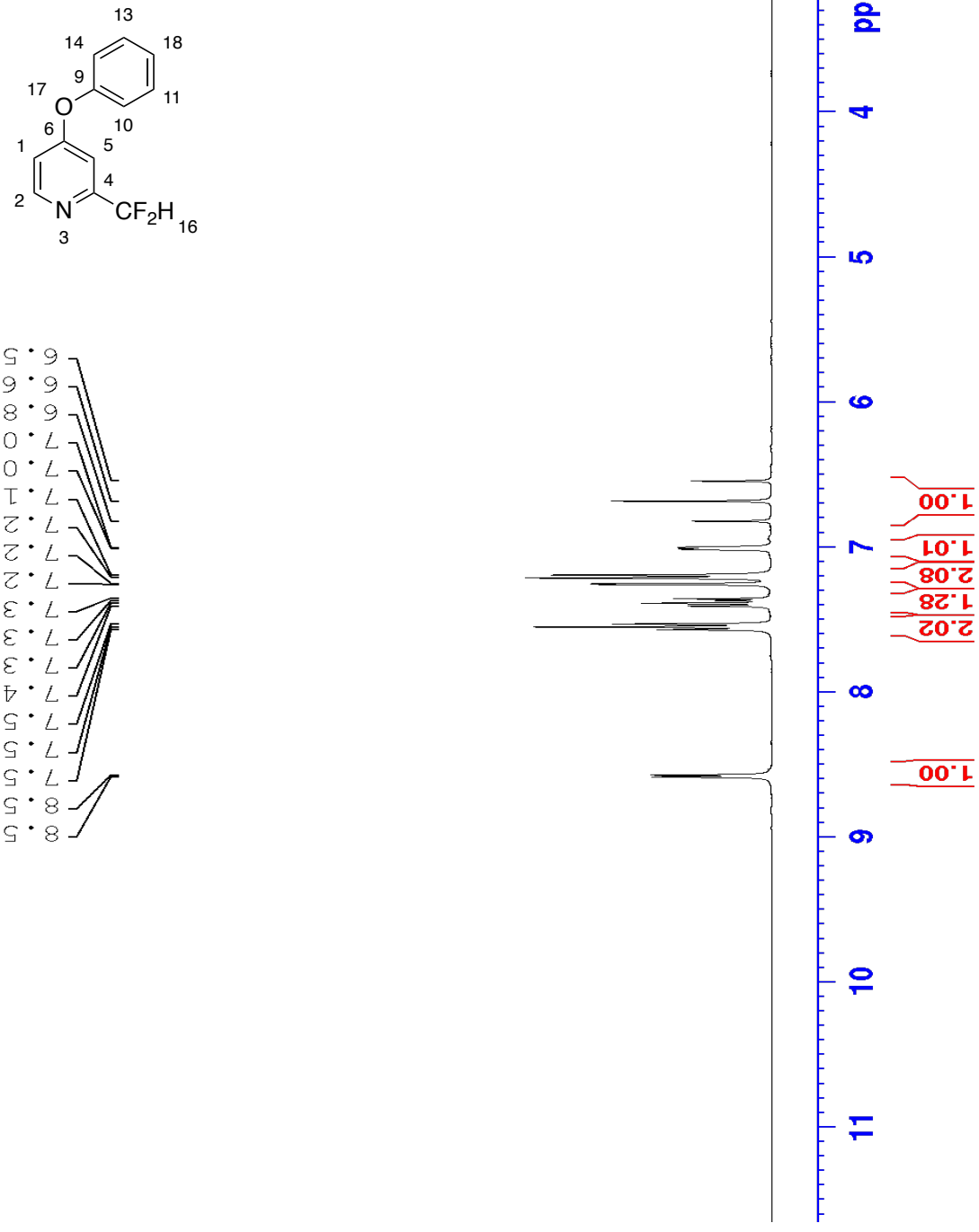

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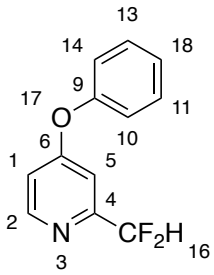
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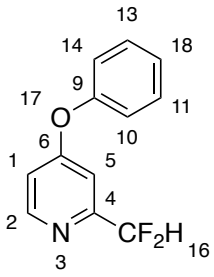
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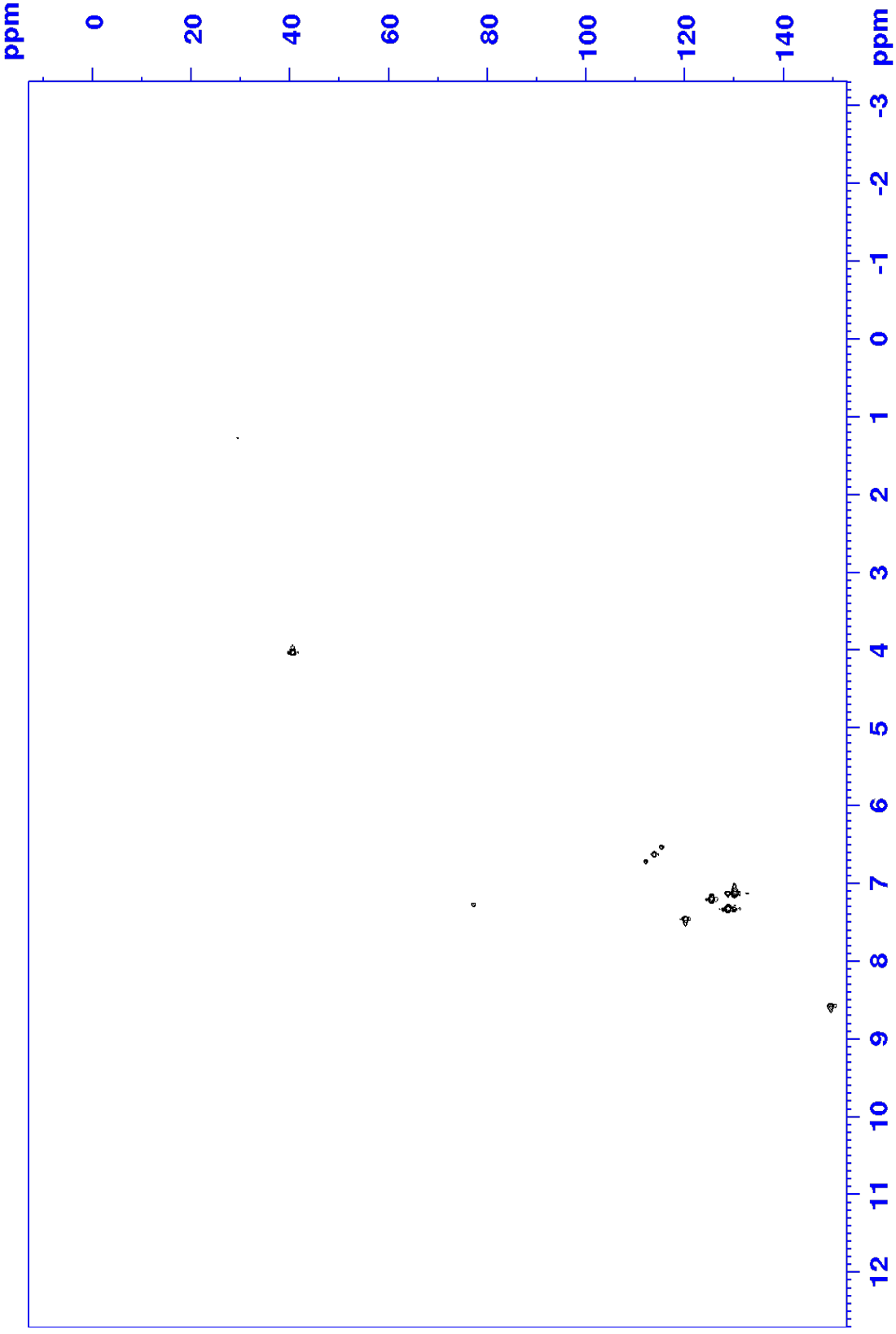
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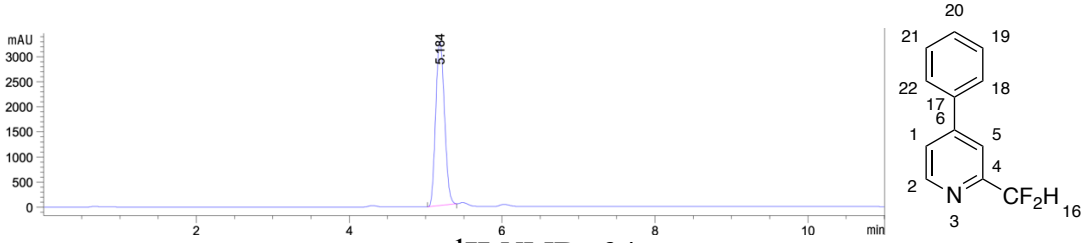
Compound **4**



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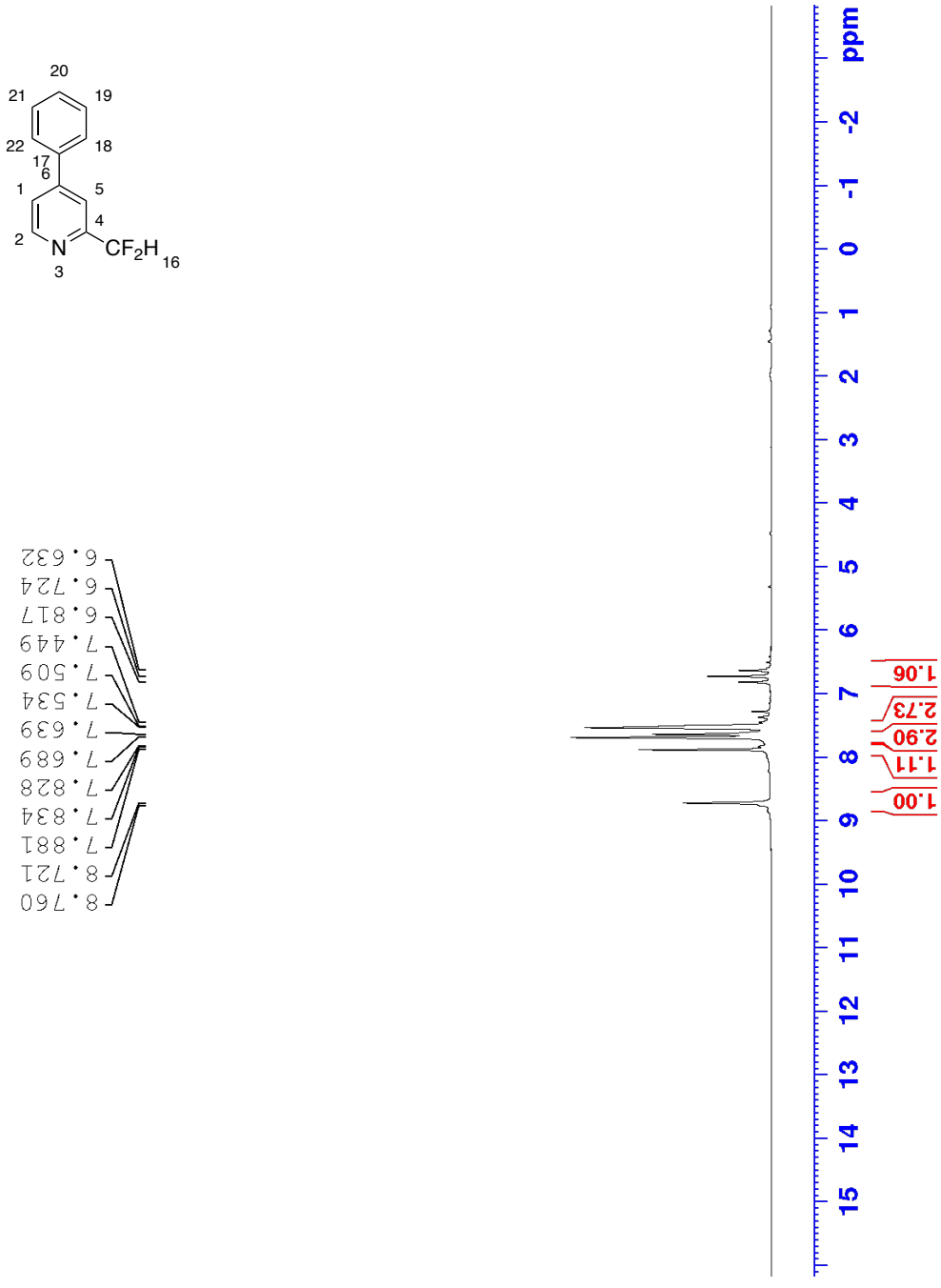

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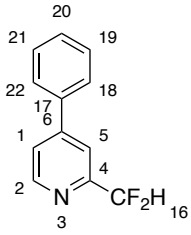
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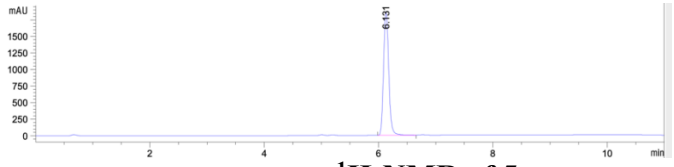
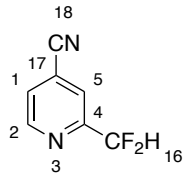
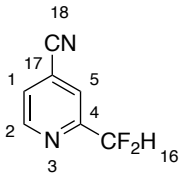
Compound **5**

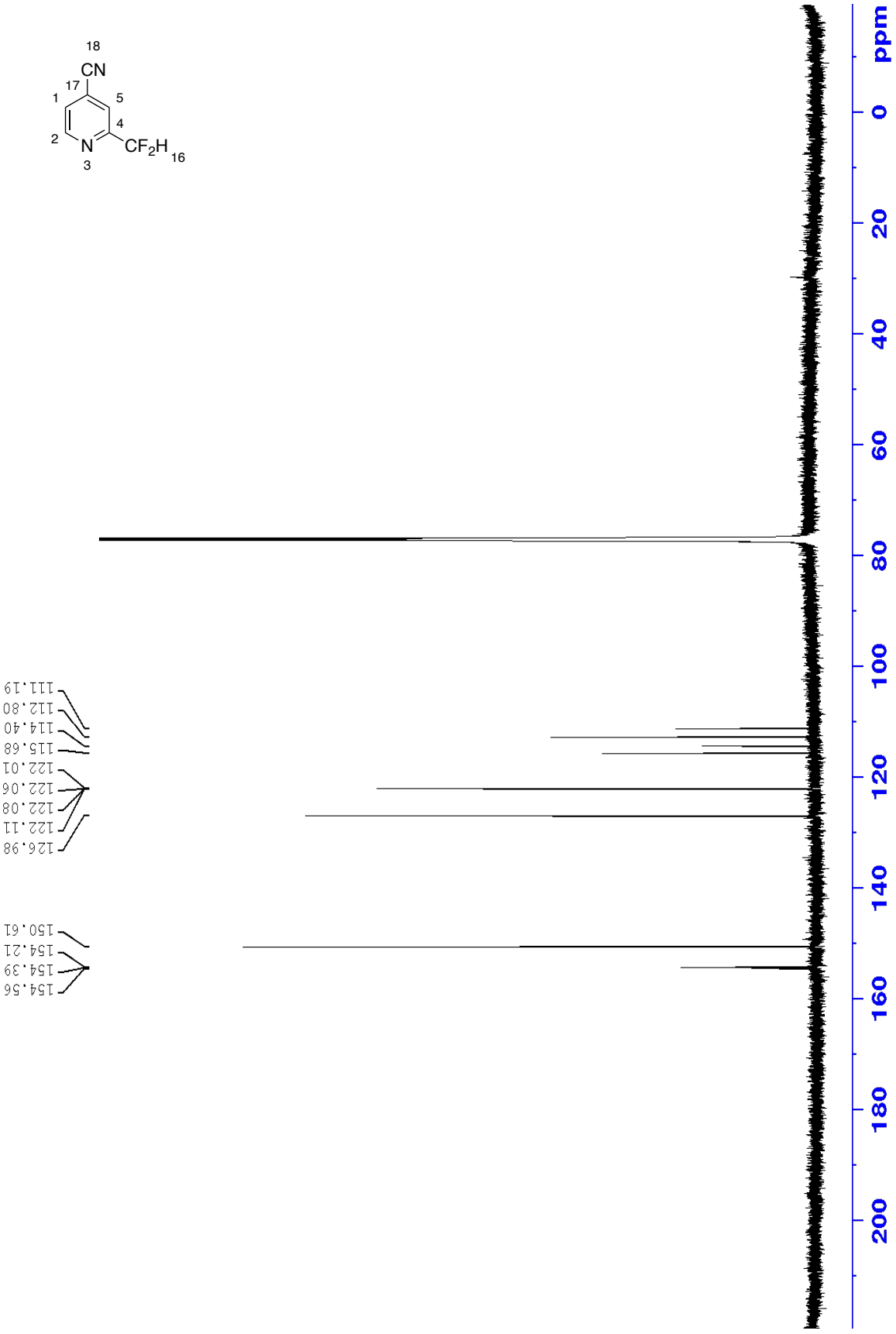




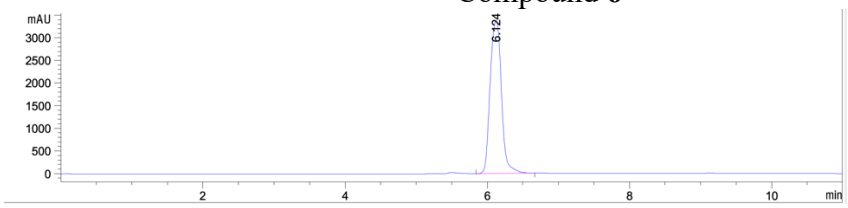
^{1}H-NMR of 5

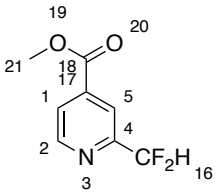


^{13}C-NMR of 5

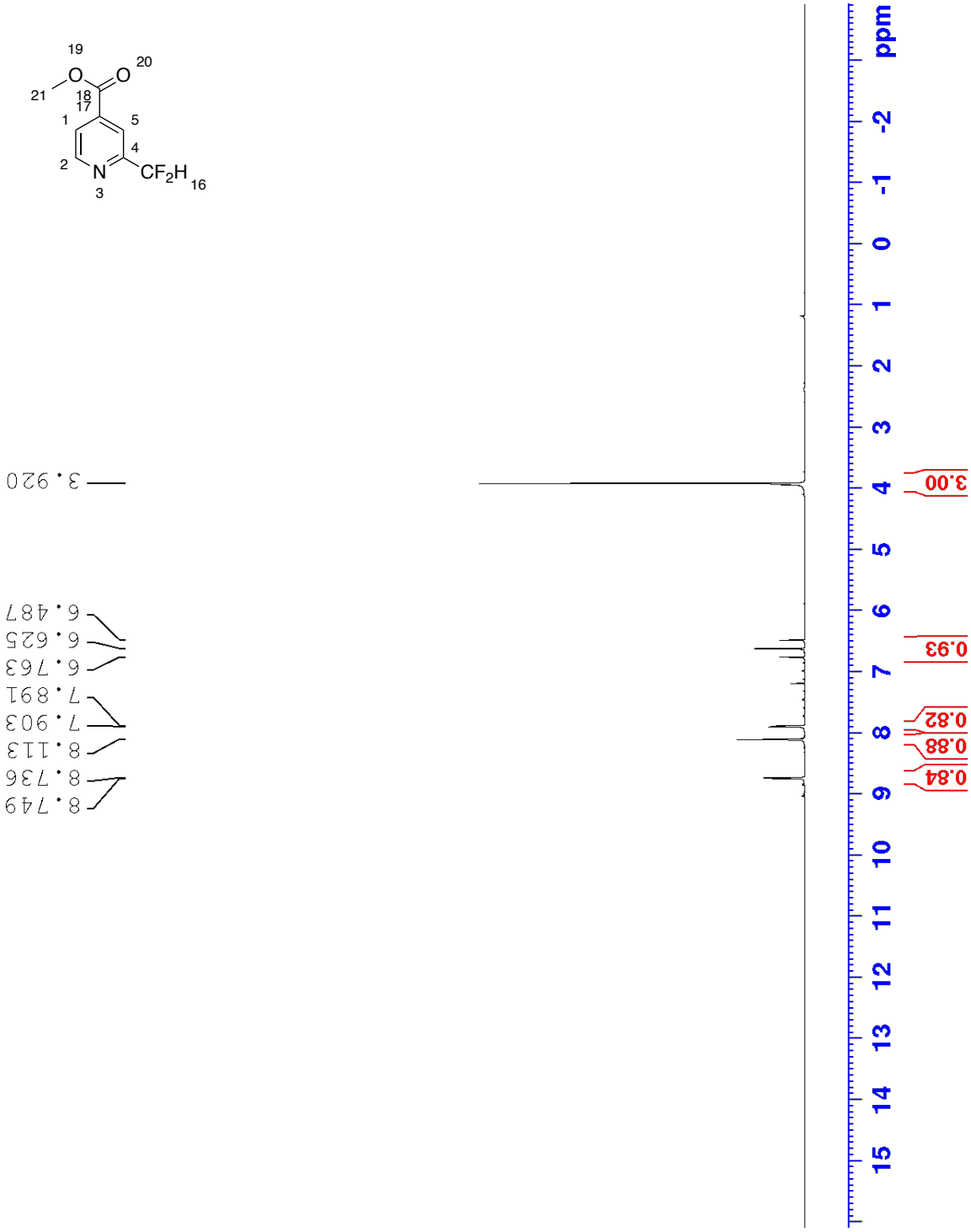

18
CN
17
1
5
4
2
N
3
CF2H
16
111.19
112.80
114.40
115.68
122.01
122.06
122.08
122.11
126.98
150.61
154.21
154.39
154.56
ppm
0
20
40
60
80
100
120
140
160
180
200

Compound **6**

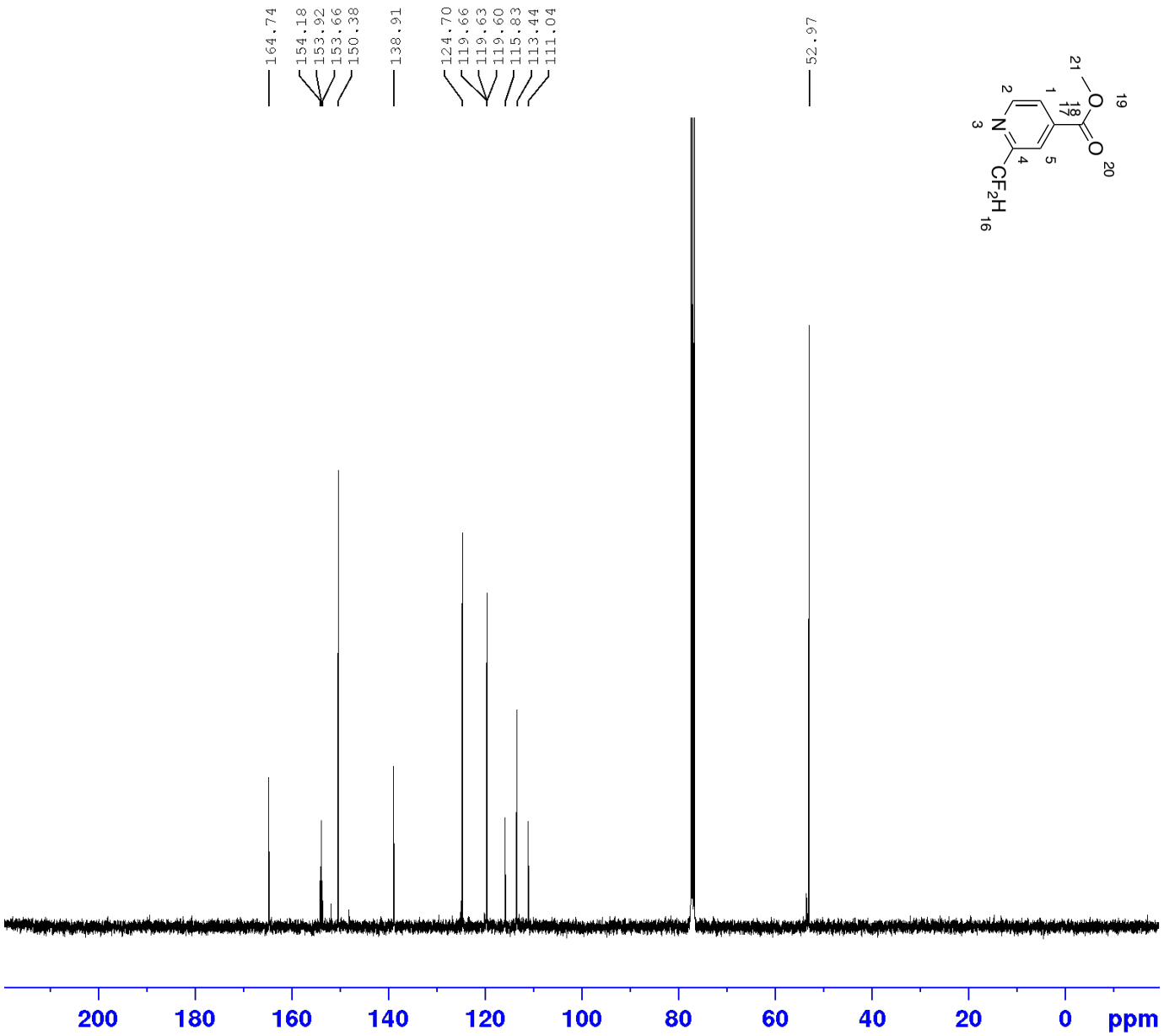




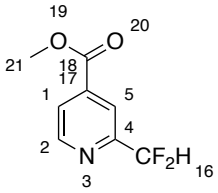
^{1}H-NMR of 6

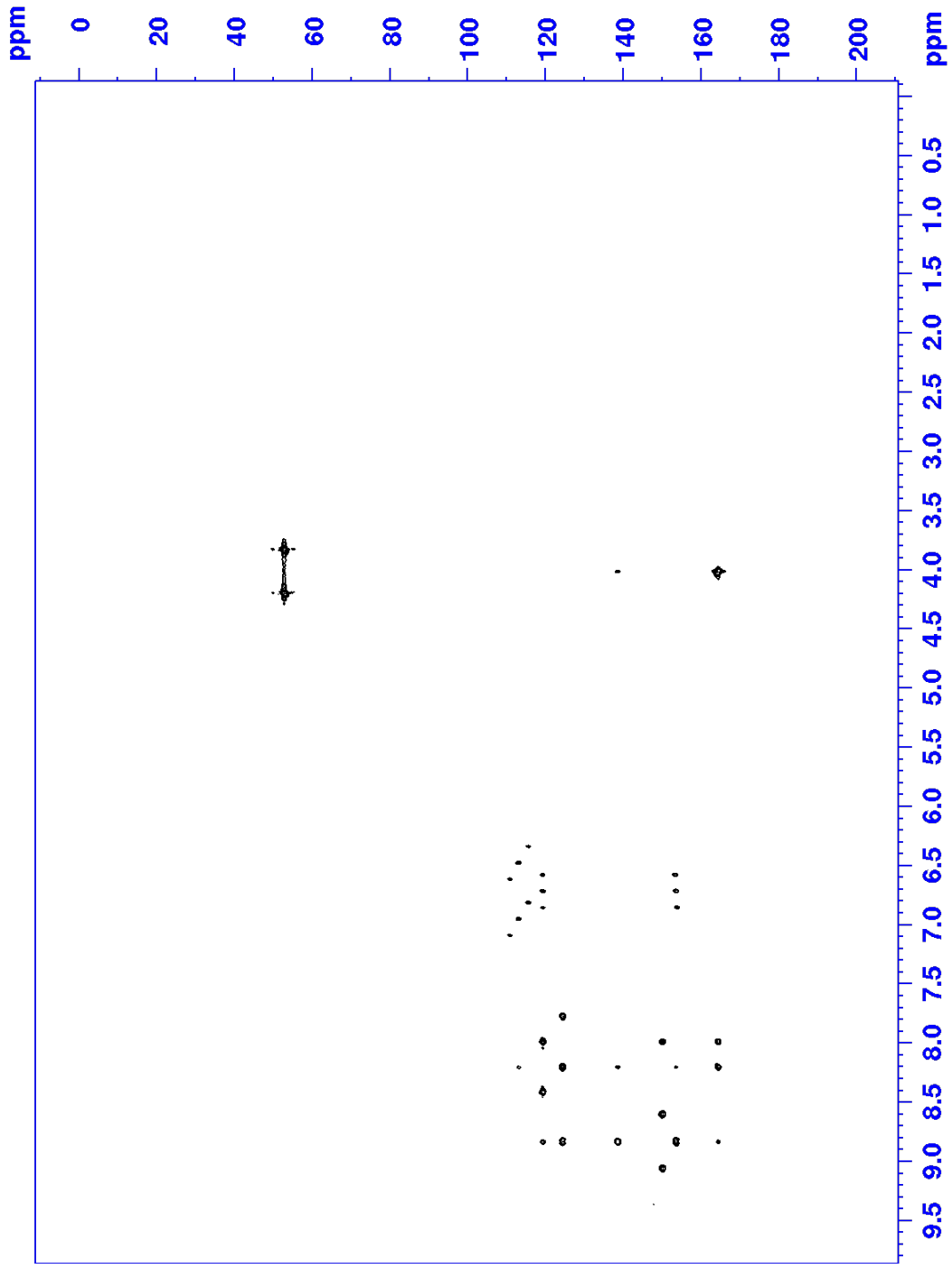


^{13}C-NMR of 6

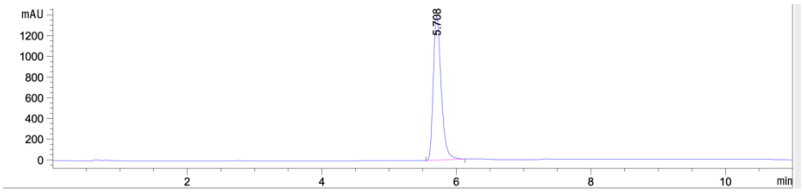

164.74
154.18
153.92
153.66
150.38
138.91
124.70
119.66
119.63
119.60
115.83
113.44
111.04
52.97
200
180
160
140
120
100
80
60
40
20
0
ppm

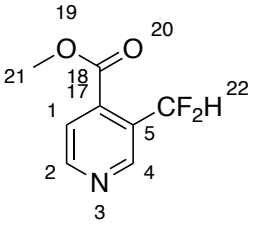
HMBC of 6


19
O
20
O
21
18
17
1
5
4
2
N
3
CF2H
16

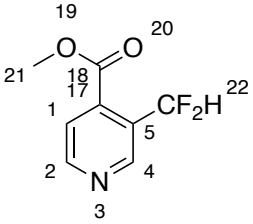

ppm
0
20
40
60
80
100
120
140
160
180
200
ppm
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
8.5
9.0
9.5

Compound **7**

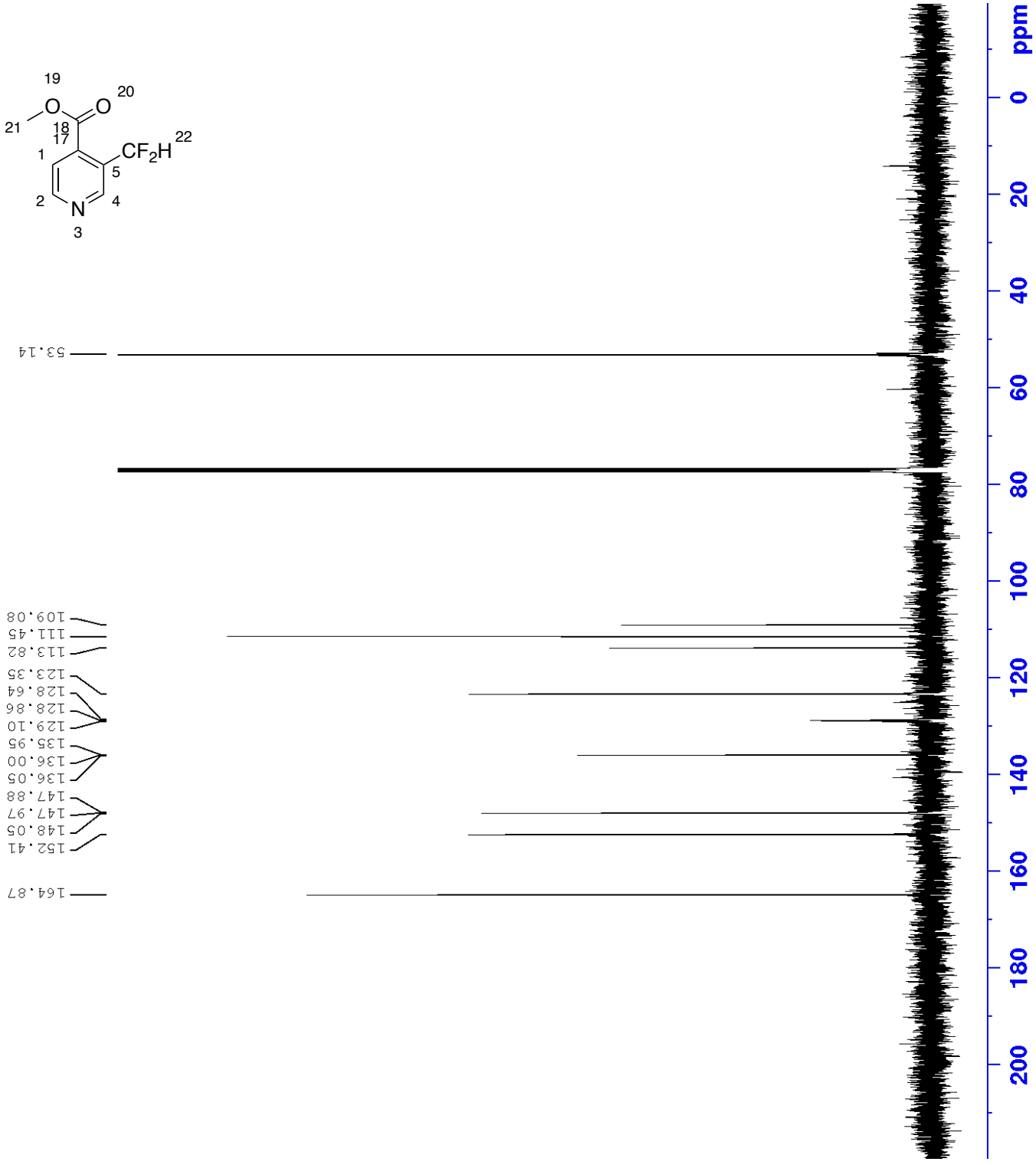




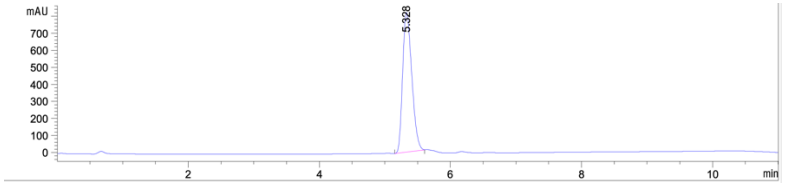
^{1}H-NMR of 7

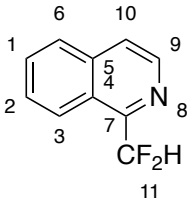


^{13}C-NMR of 7

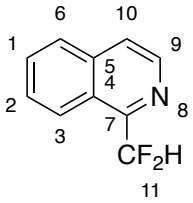

19
20
O
O
21
18
17
1
CF2H
22
5
2
4
N
3
53.14
109.08
111.45
113.82
123.35
128.64
128.86
129.10
135.95
136.00
136.05
147.88
147.97
148.05
152.41
164.87
ppm
0
20
40
60
80
100
120
140
160
180
200

Compound **11**

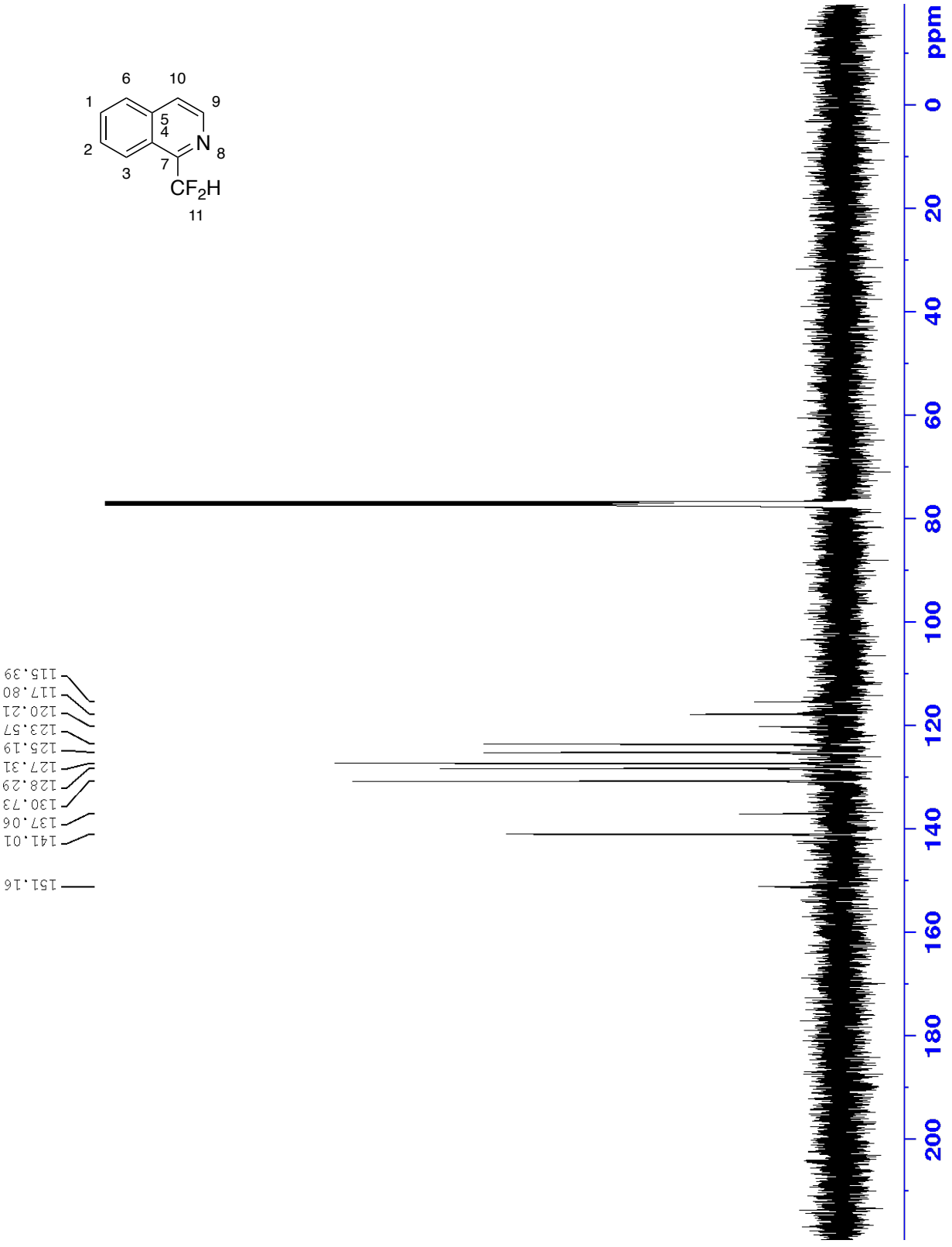




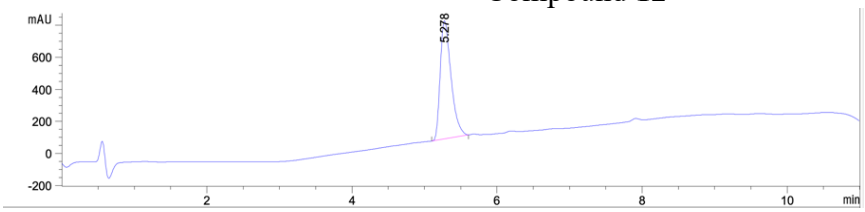
^{1}H-NMR of 11

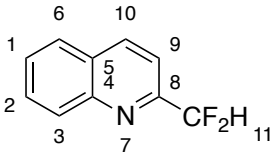


^{13}C-NMR of 11

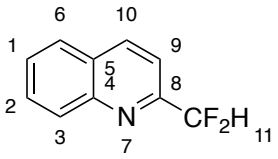

6
10
1
9
5
4
2
N
8
3
7
CF2H
11
115.39
117.80
120.21
123.57
125.19
127.31
128.29
130.73
137.06
141.01
151.16
ppm
0
20
40
60
80
100
120
140
160
180
200

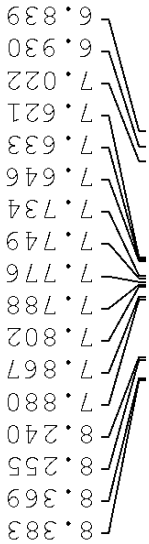
Compound **12**

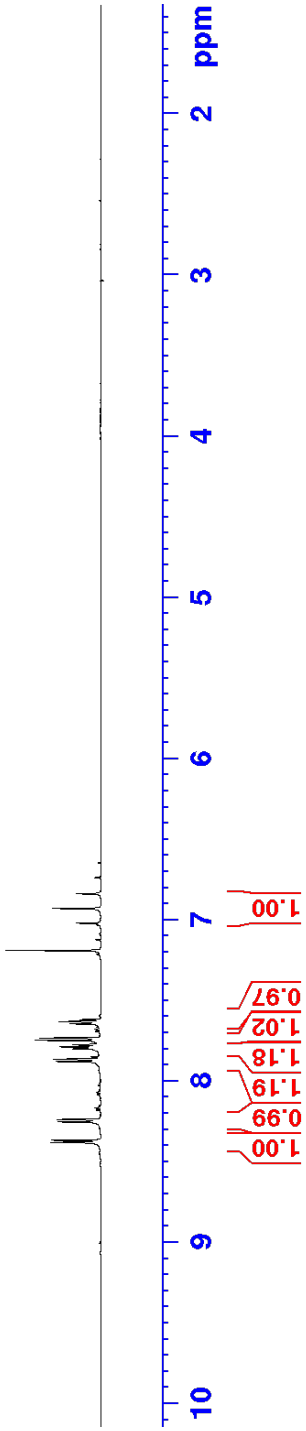




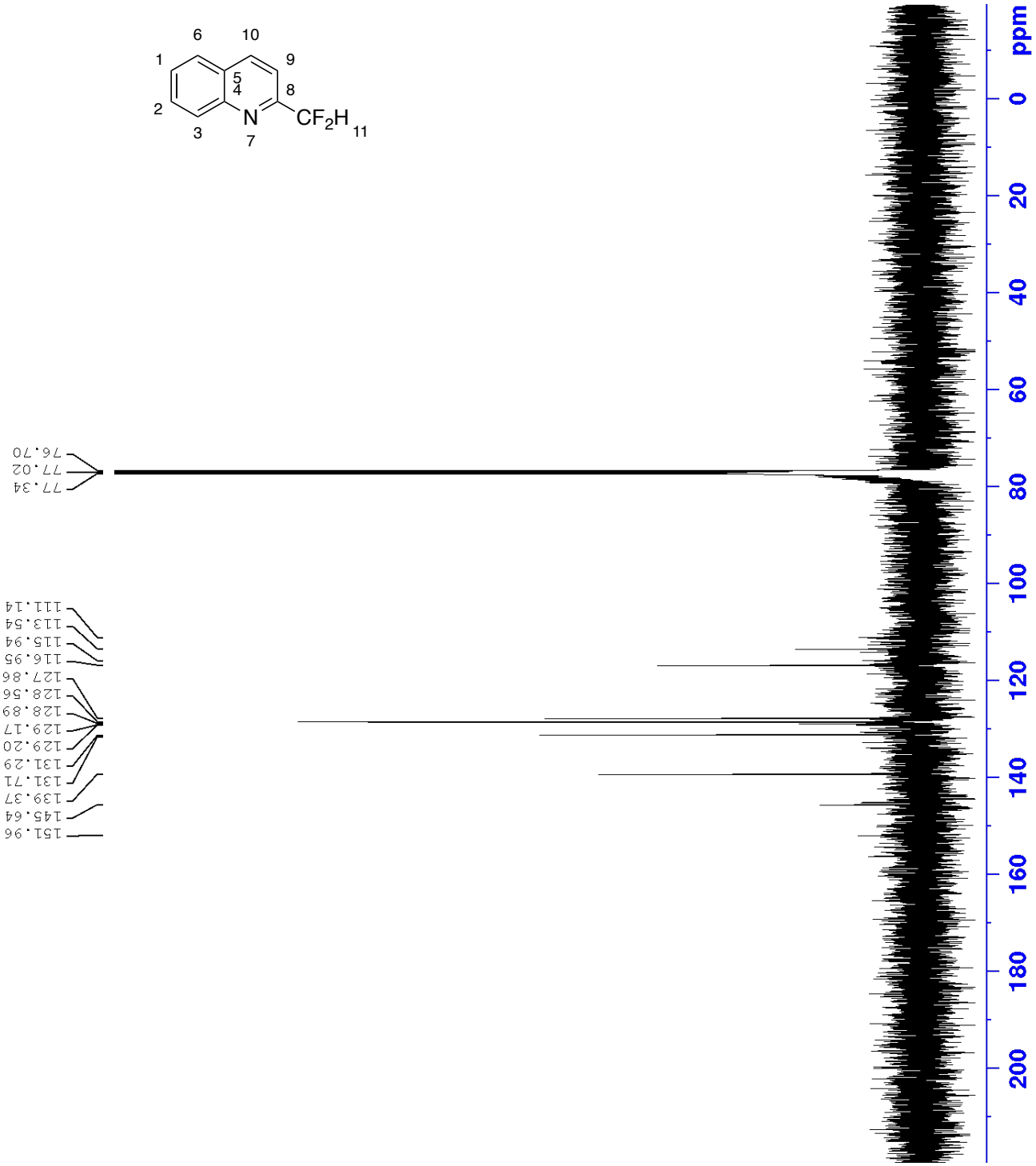
^{1}H-NMR of 12



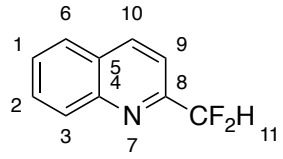


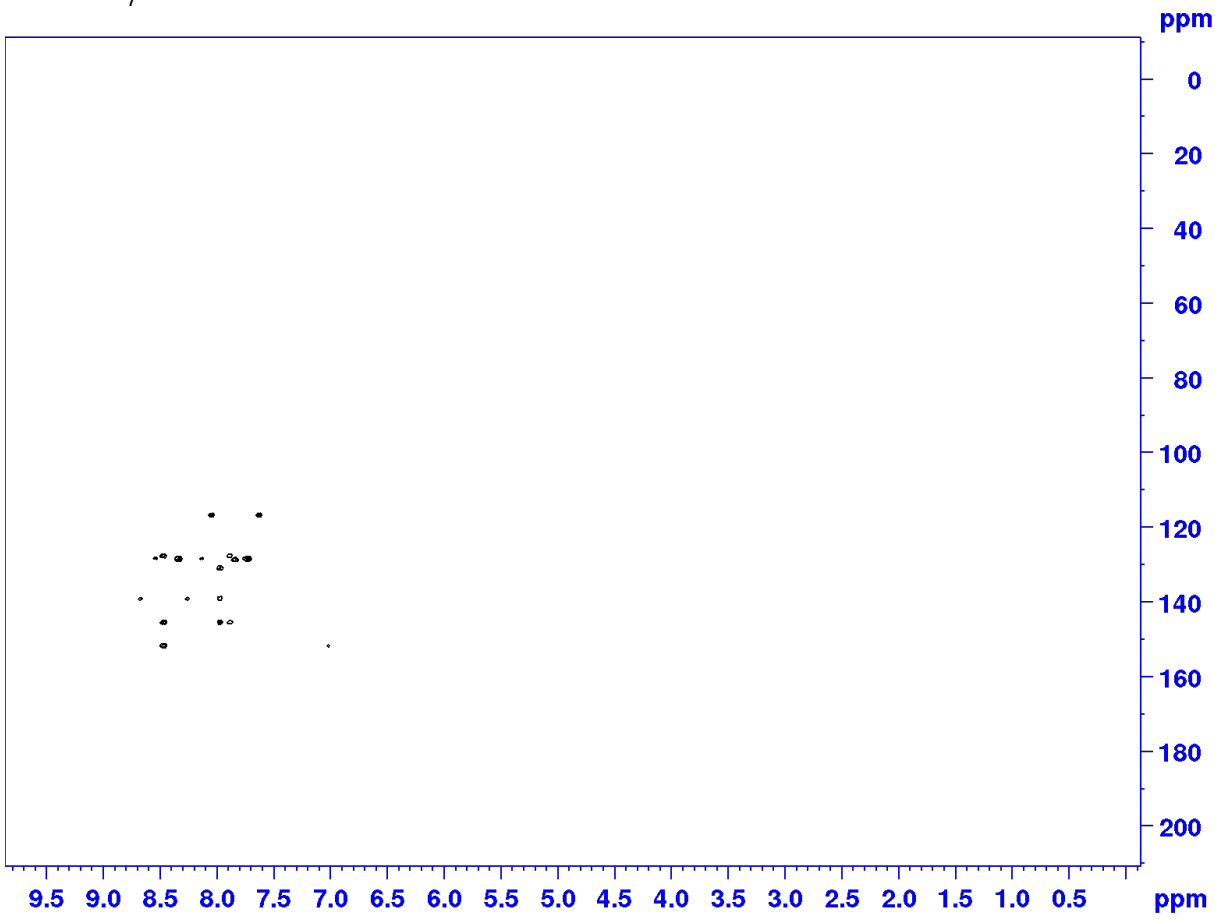


^{13}C-NMR of 12

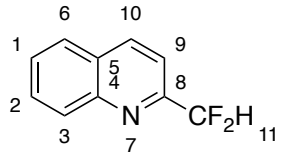

6
10
1
9
5
4
8
2
N
CF2H
11
3
7
76.70
77.02
77.34
111.14
113.54
115.94
116.95
127.86
128.56
128.89
129.17
129.20
131.29
131.71
139.37
145.64
151.96
ppm
0
20
40
60
80
100
120
140
160
180
200

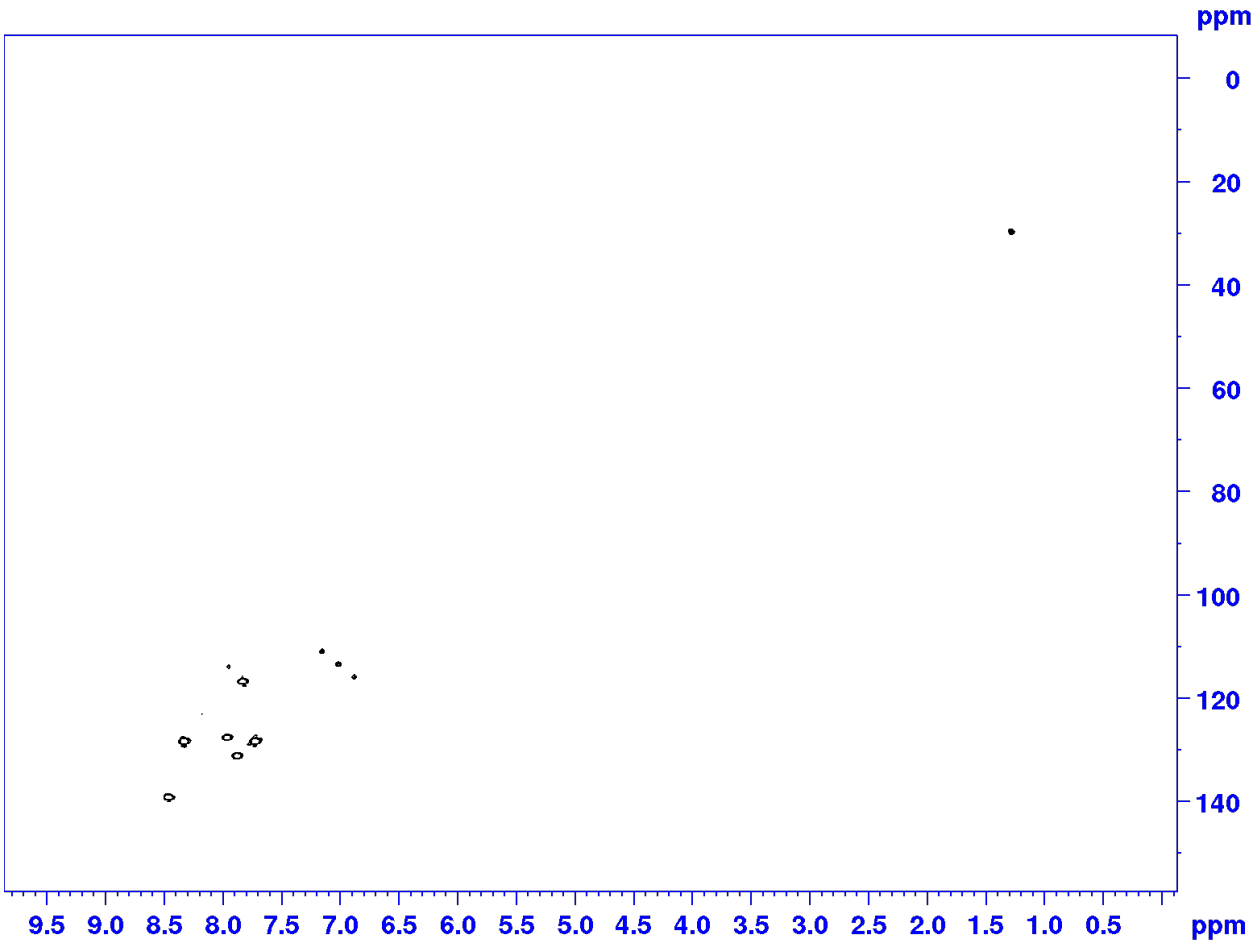
HBMC of **12**


6
10
1
9
5
4
8
2
N
CF2H
11
3
7


ppm
0
20
40
60
80
100
120
140
160
180
200
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm

HSQC of **12**


6
10
1
9
5
4
8
2
N
CF2H
11
3
7


ppm
0
20
40
60
80
100
120
140
9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5
ppm