Synthesis and Investigation of the Abiotic Formation of Pyonitrins A-D

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Supporting Information

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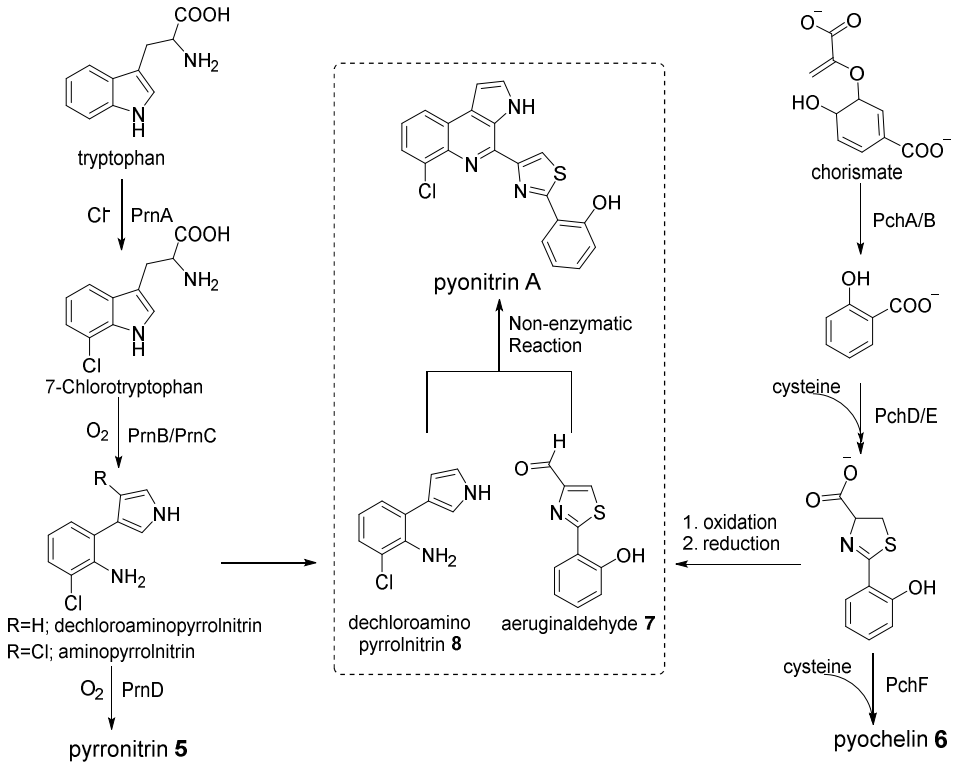
Methods and materials

Unless otherwise noted, commercially available materials were used without further purification. Reactions were performed under an atmosphere of nitrogen with magnetic stirring unless noted otherwise. Flash chromatography (FC) was performed using E. Merck silica gel 60 (240–400 mesh). Thin layer chromatography was performed using precoated plates purchased from E. Merck (silica gel 60 PF_{254}, 0.25 mm). Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker Avance III HD 800 MHz and Bruker Avance III HD 500 MHz spectrometer at operating frequencies of 800/500 MHz (^{1}H NMR) or 200/125 MHz (^{13}C NMR). Chemical shifts (δ) are given in ppm relative to residual solvent chloroform ($CDCl_3$: ^{1}H, δ = 7.26 ppm, ^{13}C, δ = 77.16 ppm), dimethyl sulfoxide ($(CD_3)_2SO$:^{1}H, δ = 2.50 ppm, ^{13}C, δ = 39.52 ppm) and coupling constants (*J*) in Hz. Multiplicity is tabulated as s for singlet, d for doublet, t for triplet, q for quadruplet, and m for multiplet and br when the signal in question is broadened. Electrospray ionization mass spectra (ESI-MS) were recorded on a LTQ-Orbitrap Velos Pro MS. Chemicals were purchased from Aldrich, Fisher, Alfa Aesar, TCI, or Oakwood chemicals and used without purification.^{15}N-N_2Na was purchased from Cambridge Isotope Laboratories, Inc. and also used without purification.

Figure S1: Proposed biosynthesis of pyonitrins A-D production



General procedures:

General procedure 1. Suzuki-Miyaura cross-coupling.

In an oven-dried round bottom flask, aromatic halide (1.0 equiv), N-(TIPS) pyrrole pinacol boronate (1.2 equiv), palladium acetate [$Pd(OAc)_2$, 0.05 equiv], 2-Dicyclohexyl-phosphino-2′,6′-dimethoxybiphenyl (SPhos, 0.10 equiv), and potassium phosphate (K_3PO_4, 2.0 equiv) was added under inert atmosphere followed by addition of the solvent system (2.0 mL/mmol aryl halide), consisting of degassed *n*-butanol (*n*-BuOH) and degassed deionized water in the ratio of 2.5:1. The resulting mixture was stirred at room temperature for 16 h. The crude reaction mixture was then filtered through a plug of silica gel using EtOAc eluent and concentrated in vacuo. Purification by column chromatography over silica gel eluting with ethyl acetate and hexanes provided the desired TIPS-protected phenylpyrrole.

General procedure 2. Removal of silyl protecting group using TBAF.

To a solution of TIPS-protected phenylpyrrole (1.0 equiv) in THF (2.0 mL/mmol SM) at 0 ^oC was added tetrabutylammonium fluoride (TBAF, 1.0 M in THF, 2.0 equiv) dropwise. Allow to stir reaction mixture for 15 min at same temperature the reaction progress monitored by TLC. The reaction mixture was quenched with saturated ammonium chloride (NH_4Cl) solution and the resulting biphasic mixture was transferred to a separatory funnel. The organic and aqueous layers were separated and the aqueous layer was extracted with ethyl acetate. The organic layers were combined and washed with saturated sodium chloride, dried with sodium sulfate and

concentrated under reduced pressure to afford the crude product. Subsequent purification by column chromatography over deactivated silica gel (5% NEt_3), eluting with ethyl acetate and hexanes afforded the desilylated product

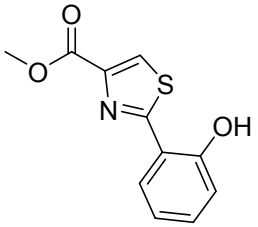
General procedure 3. Pictet-Spengler reaction.

1% solution of TFA in DMSO (1 mL), aldehyde (1 eq) and pyrrole aniline (1 eq) were added at rt. The mixture was further stirred at ambient temperature and the progress of reaction was monitored by TLC. Upon completion of the reaction, saturated aqueous $NaHCO_3$ was added to quench the acid in the reaction mixture. The product was extracted using ethyl acetate (20 mL) and the organic layer was washed with water (2 x 10 mL), brine solution (1 x 10 mL) and finally dried over anhydrous sodium sulfate. It was then evaporated in vacuo to obtain a residue and purified by column chromatography eluting with ethyl acetate and hexanes to afford pure pyonitrins.

General 2D ^{1}H-^{15}N HMBC monitoring of Pictet-Spengler condensation:

^{15}N-dechloroaminopyrrolnitrin **26**, and aeruginaldehyde **7** was added to 700 μL of 1% TFA in DMSO-d_6 in a 5 mm thin wall, 8 inch NMR tube (Wilmad) and inserted Bruker Avance III HD 800 MHz instrument. The ^{1}H-^{15}N HMBC experiment was optimized for $^{N}J_{NH}$ = 5 Hz. The ^{1}H-^{15}N HMBC data were acquired as 2048 x 172 points with 8 transients per t1 increment. A delay of 1 second was used between transients taking 27 minutes and 43 seconds per spectrum. Instrument was tuned, locked, and shimmed. NMR experiments were then queued to run in the following order: ^{1}H NMR experiment (32 scans taking 2 minutes and 15 seconds) followed by ^{1}H-^{15}N HMBC (8 scans of 86 increments taking 27 minutes and 43 seconds). Therefore, a single ^{1}H NMR spectrum was collected once every 30 min along with one HMBC spectra constantly for 48 hours.

Synthesis of methyl 2-(2-hydroxyphenyl)thiazole-4-carboxylate (12)



To the anhydrous methanol solution (50 mL) of the 2-hydroxybenzonitrile **10** (2 gm, 16.8 mmol) and anhydrous Na_2CO_3 (1.7 gm, 16.8 mmol) the L-Cysteine methyl ester hydrochloride **9** (7.1 gm 42.01 mmol) was added, then the resulting mixture was stirred at 80 ^{o}C in oil bath for 12 h, cooled to room temperature. The insoluble solid was filtered and the filtrate was then concentrated. The crude product was dissolved in water (30 mL) and extracted with ethyl acetate (2 x 50 mL). The combined organics were then washed with brine and dried over sodium sulfate and concentrated to obtain pure thiazoline product **11** (3 gm, 77% yield) was used for next step without further purification.

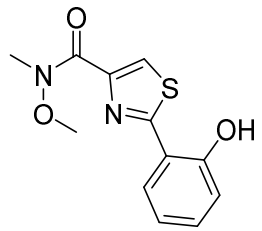
Thiazoline **11** (3 gm, 0.012 mol) was dissolved in dry CH_2Cl_2 (150 mL) and cooled to -20 °C. DBU (1.2 mL, 0.012 mol) was added and the reaction mixture was stirred for 5 min. at -20 °C, treated with $BrCCl_3$ 3 (3.8 mL, 0.025 mol) and stirred at room temperature for 12h. The reaction mixture was then diluted with ammonium chloride (50 mL) and the aqueous layer was extracted with dichloromehane (3 x 100 mL each). After drying over sodium sulfate the solvent was removed under reduced pressure, and purified by column chromatography over silica gel, eluting with ethyl acetate and hexanes to furnished 2.8 gm of methyl 2-(2-hydroxyphenyl)thiazole-4-carboxylate **12** (94% yield) as a colorless solid.

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.84 (s, 1H), 8.11 (s, 1H), 7.62 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.36 (td, *J* = 7.7, 1.2 Hz, 1H), 7.09 (d, *J* = 8.3 Hz, 1H), 6.93 (td, *J* = 7.7, 0.9 Hz, 1H), 3.97 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 169.7, 161.1, 157.2, 145.9, 132.7, 127.5, 125.3, 119.7, 118.3, 116.4, 52.6.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{11}H_{10}NO_3S$ 236.0381; Found 236.0372

Synthesis of 2-(2-hydroxyphenyl)-N-methoxy-N-methylthiazole-4-carboxamide (13)



Methyl 2-(2-hydroxyphenyl) thiazole-4-carboxylate **12** (2.8 gm, 0.011 mol) was taken into 50 ml mixture of THF:H_2O:CH_3OH (3:2:1). To this lithium hydroxide (1.0 gm, 0.025 mol) was added and the reaction mixture was stirred at room temperature for 2h. The solvent was removed via rotovap and the reaction mixture was acidified using 1N HCl. The white solid precipitate was filtered and dried over vacuum to obtain (2.6 gm, 98% yield) pure thiazole acid product.

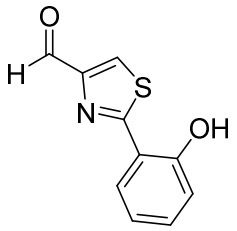
The acid product (2.5 gm, 0.011 mol) was then dissolved in dry DMF (30 mL).To this EDC (2.5 gm, 0.013 mol), HOBt (3.35 gm, 0.024 mol), *N,O*-Dimethylhydroxylamine hydrochloride (1.31 gm, 0.013 mol) and DIPEA (10 mL, 0.056 mol) were added at 0 ^oC and the reaction mixture was stirred at room temperature for 12h. The reaction mixture was diluted with water (50 mL) and extracted with ethyl acetate (100 mL x 2).The combined organic layer was washed with water (50 mL) and brine (50 mL) and dried over sodium sulfate and concentrated to obtain pure white solid product **13** (2.4 gm, 80% yield)

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.94 (s, 1H), 8.02 (s, 1H), 7.64 (dd, *J* = 7.8, 1.3 Hz, 1H), 7.35 (td, *J* = 7.9, 1.4 Hz, 1H), 7.07 (dd, *J* = 8.3, 0.7 Hz, 1H), 6.93 (td, *J* = 7.6, 1.0 Hz, 1H), 3.81 (s, 3H), 3.43 (s, 3H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 168.7, 161.7, 157.0, 147.6, 132.5, 127.5, 123.9, 119.7, 118.1, 116.7, 62.0, 34.0

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{12}H_{13}N_2O_3S$ 265.0646; Found 265.0635

Synthesis of 2-(2-hydroxyphenyl)thiazole-4-carbaldehyde (7)



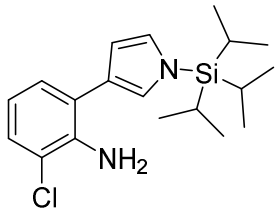
2-(2-hydroxyphenyl)-N-methoxy-N-methylthiazole-4-carboxamide **13** (100 mg, 0.37 mmol) was taken in dry THF (5 mL) and to it 2M solution of lithium aluminium hydride (0.284 mL, 0.56 mmol) was added at 0 ^{o}C. After 10 min the reaction was quenched by adding a saturated solution of ammonium chloride (10 mL) and extracted with ethyl acetate (30 mL x 2). The combined organic layer was washed with water (30 mL) and brine (30 mL) and dried over sodium sulfate and concentrated to obtain pure yellow solid product **7** (60 mg, 61% yield).

^{1}H NMR (500 MHz, $CDCl_3$) δ 11.60 (s, 1H), 10.06 (s, 1H), 8.13 (s, 1H), 7.64 (dd, *J* = 7.8, 1.2 Hz, 1H), 7.39 (td, *J* = 7.8, 1.2 Hz, 1H), 7.10 (dd, *J* = 8.3, 0.7 Hz, 1H), 6.95 (td, *J* = 7.6, 0.8 Hz, 1H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 183.5, 170.6, 157.0, 153.7, 133.0, 127.7, 125.2, 119.9, 118.3, 116.2.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_8NO_2S$ 206.0275; Found 206.0272

2-chloro-6-(1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline (18)[1]



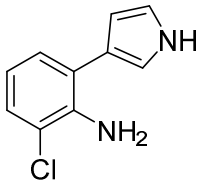
Following general procedure 1, Compound **18** was prepared from 2-chloro-6-iodoaniline **14** (250 mg, 0.99 mmol) and **17** (415 mg, 1.19 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 9/1), affording **18** 264 mg (75% yield) as colorless oil.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.17 (d, *J* = 7.8 Hz, 2H), 6.99 (t, *J* = 1.9 Hz, 1H), 6.88 (t, *J* = 2.2 Hz, 1H), 6.71 (t, *J* = 7.8 Hz, 1H), 6.52 (dd, *J* = 1.5, 2.4 Hz, 1H), 4.39 (s, 2H), 1.50 (sept, *J* = 7.4 Hz, 3H), 1.15 (d, *J* = 7.6 Hz, 18H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 140.6, 128.2, 127.1, 125.1, 124.0, 123.6, 122.7, 119.7, 118.3, 110.8, 17.9, 11.8.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{30}ClN_2Si$ 349.1866; Found 349.1869

2-chloro-6-(1H-pyrrol-3-yl)aniline (22) [1]



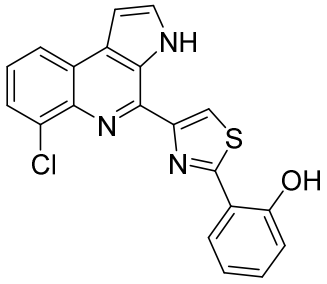
Following general procedure 2, **22** was prepared from 2-chloro-6-(1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline **18** (50 mg, 0.14 mmol). The crude residue was purified by column chromatography over deactivated silica gel (5% NEt_3) (hexane/ethyl acetate = 2/8), affording compound **22** (21 mg) as colorless oil in 76% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.19 (d, *J* = 8.0 Hz, 1H), 7.15 (d, *J* = 7.5 Hz, 1H), 6.99 (d, *J* = 1.3 Hz, 1H), 6.88 (d, *J* = 1.9 Hz, 1H), 6.71 (t, *J* = 7.8 Hz, 1H), 6.45 (d, *J* = 1.2 Hz, 1H), 4.39 (s, 2H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 140.7, 128.4, 127.3, 123.7, 121.5, 119.7, 118.8, 118.4, 116.5, 108.7.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_{10}ClN_2$ 193.0532; Found 193.0531

pyonitrin A (1)



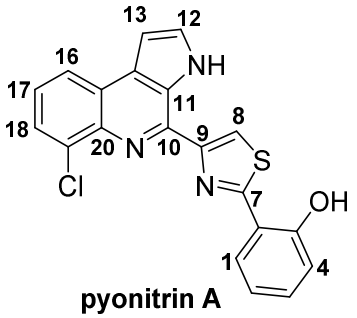
Following general procedure 3, pyonitrin A was prepared from 2-chloro-6-(1H-pyrrol-3-yl)aniline **22** (2 mg, 0.01 mmol) and **7** (2.1 mg, 0.01 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 4/6), affording pyonitrin A (2.4 mg) as a yellow solid in 61% yield.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.68 (s, 1H), 11.18 (s, 1H), 8.75 (dd, *J* = 7.8, 1.2 Hz, 1H), 8.70 (s, 1H), 8.34 (dd, *J* = 8.1, 1.1 Hz, 1H), 7.85 (t, *J* = 2.7 Hz, 1H), 7.77 (dd, *J* = 7.4, 1.0 Hz, 1H), 7.55 (t, *J* = 7.8 Hz, 1H), 7.39 (m, 1H), 7.31 (t, *J* = 2.0 Hz, 1H), 7.10 (t, *J* = 8.1 Hz, 1H), 7.08 (t, *J* = 7.2 Hz, 1H)

^{13}C NMR (125 MHz, DMSO-d_6) δ 163.5, 155.1, 152.9, 139.3, 137.7, 132.4, 131.2, 129.8, 129.6, 128.5, 126.2, 125.6, 124.7, 122.5, 121.4, 119.5, 119.4, 116.2, 101.3.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{13}ClN_3OS$ 378.0467; Found 378.0460

Table S1: Comparisons of NMR data of pyonitrin A isolated and synthetic sample in DMSO-d_6

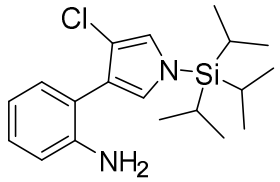


pyonitrin A

No	^{1}H NMR natural (600 MHz)	^{1}H NMR synthetic (500 MHz)	Difference in ppm	^{13}C NMR natural (600 MHz)	^{13}C NMR synthetic (500 MHz)	Difference in ppm
1	8.70 (d, 7.8)	8.75 (dd, *J* = 7.8, 1.2 Hz, 1H)	0.05	128.7 CH	128.5	0.2
2	7.00 (t, 7.5)	7.10 (t, *J* = 8.1 Hz, 1H)	0.10	118.9 CH	119.5	0.6
3	7.34 (t, 7.6)	7.39 (m, 1H),	0.05	131.6 CH	131.1	0.5
4	7.07 (t, 8.2)	7.08 (t, *J* = 7.2 Hz, 1H)	0.01	117.1 CH	116.2	0.9
5				157.3 C	155.1	2.2
6				119.9 C	119.4	0.5
7				164.4 C	163.5	0.9
8	8.64 (s)	8.70 (s, 1H)	0.06	121.4 C	121.4	-
9				153.2 C	152.9	0.3
10				139.8 C	139.2	0.6
11				126.7 C	126.2	0.5
12	7.85 (d, 2.9)	7.85 (t, *J* = 2.7 Hz, 1H)	-	130.1 CH	129.6	0.5
13	7.29 (d, 2.9)	7.31 (t, *J* = 2.0 Hz, 1H)	0.02	101.7 CH	101.3	0.4
14				130.2 C	129.6	0.6
15				125.1 C	125.5	0.4
16	8.32 (d, 8.0)	8.34 (dd, *J* = 8.1, 1.1 Hz, 1H)	0.02	122.9 CH	122.5	0.4
17	7.54 (d, 7.8)	7.55 (t, *J* = 7.8 Hz, 1H)	0.01	126.1 CH	125.6	0.5
18	7.76 (d, 7.4)	7.77 (dd, *J* = 7.4, 1.0 Hz, 1H)	0.01	126.7 CH	126.2	0.5
19				132.8	132.4	0.4
20				138.2	137.7	0.5
NH	11.69 (bs)	11.68	0.01			
OH		11.18				

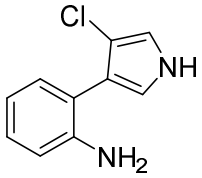
Synthesis of pyonitrin B:

2-(4-chloro-1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline (19)[1]



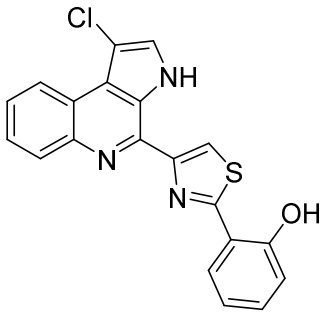
Following general procedure 1, **19** was prepared from 2-iodoaniline **15** (180mg, 0.82 mmol) and **16** (377 mg, 0.98 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 9/1), affording **19** (192 mg) as colorless oil in 67% yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.21 (d, *J* = 7.5 Hz, 1H), 7.14 (t, *J* = 7.2 Hz, 1H), 6.79 (m, 4H), 3.81 (s, 2H), 1.45 (Sept, *J* = 7.4 Hz, 3H), 1.13 (d, *J* = 7.5 Hz, 18H).
^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9, 131.6, 128.4, 123.7, 121.8, 121.7, 119.5, 118.3, 115.5, 113.8, 17.9, 11.7.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{30}ClN_2Si$ 349.1866; Found 349.1868

2-(4-chloro-1H-pyrrol-3-yl)aniline (23)[1]



Following general procedure 2, **23** was prepared from 2-(4-chloro-1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline **19** (60 mg, 0.17 mmol). The crude residue was purified by column chromatography over deactivated silica gel (5% NEt_3) (hexane/ethyl acetate = 2/8), affording **23** (28 mg) as colorless oil in 84% yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.20 (dd, *J* = 7.4, 1.0 Hz, 1H), 7.16 (td, *J* = 7.9, 1.3 Hz, 1H), 6.83 – 6.75 (m, 4H), 3.83 (s, 2H).
^{13}C NMR (125 MHz, $CDCl_3$) δ 144.9, 131.8, 128.6, 119.7, 119.3, 118.4, 117.2, 116.1, 115.5, 112.1.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_{10}ClN_2$ 193.0532; Found 193.0530

pyonitrin B (2)



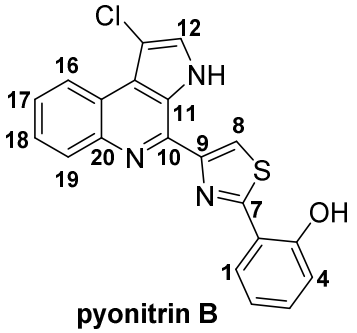
Following general procedure 3, **2** was prepared from 2-(4-chloro-1H-pyrrol-3-yl)aniline **23** (14 mg, 0.07 mmol) and **7** (15 mg, 0.07 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 4/6), affording pyonitrin B (15 mg) as a colorless oil in 55% yield.

^{1}H NMR (800 MHz, DMSO-d_6) δ 11.83 (s, 1H), 11.20 (s, 1H), 8.86 (dd, J = 7.5, 1.4 Hz, 1H), 8.71 (s, 1H), 8.70 (d, J = 6.8, 1H), 8.15 (dd, J = 7.6, 1.2 Hz, 1H), 7.89 (d, J = 2.8 Hz, 1H), 7.70 – 7.67 (m, 2H), 7.40 (dt, J = 7.5, 1.4 Hz, 1H), 7.11 (d, J = 8.1 Hz, 1H), 7.09 (t, J = 7.4 Hz, 1H).

^{13}C NMR (200 MHz, DMSO-d_6) δ 163.5, 155.1, 152.5, 142.1, 139.3, 131.2, 129.4, 128.5, 126.5, 126.2, 126.0, 125.2, 123.1, 122.3, 121.8, 121.2, 119.5, 119.4, 116.2, 105.2.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{13}ClN_3OS$ 378.0467; Found 378.0464

Table S2: Comparisons of NMR data of pyonitrin B isolated and synthetic sample in DMSO-*d*$_6$



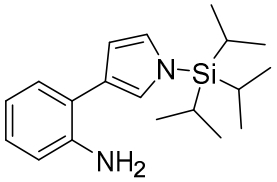
pyonitrin B

No	^{1}H NMR natural (600 MHz)	^{1}H NMR synthetic (500 MHz)	Difference in ppm	^{13}C NMR natural (600 MHz)	^{13}C NMR synthetic (500 MHz)	Difference in ppm
1	8.64 (d, 7.3)	8.70 (dd, J = 6.8, 1H)	0.06	128.6	128.5	0.1
2	7.09 (t, 7.7)	7.11 (d, J = 8.1 Hz, 1H),	0.02	117.6	116.2	1.4
3	7.30 (t, 7.3)	7.40 (dt, J = 7.5, 1.4 Hz, 1H)	0.10	131.5	131.2	0.3
4	6.93 (t, 7.1)	7.09 (t, J = 7.4 Hz, 1H)	0.16	118.1	119.4	1.3
5				158.0	155.05	2.95
6				119.8	119.5	0.3
7				164.6	163.5	1.1
8	8.62 (s)	8.71 (s, 1H),	0.09	121.1	121.2	0.1
9				152.7	152.5	0.2
10				140.0	139.3	0.7
11				123.4	121.8	1.6
12	7.88 (s)	7.88 (d, J = 2.8 Hz, 1H)	--	126.8	126.5	0.3
13				105.6	105.2	0.4

14				125.8	125.2	0.6
15				123.0	123.1	0.1
16	8.85 (d, 7.9)	8.86 (dd, *J* = 7.5, 1.4 Hz, 1H)	0.01	122.3	122.3	--
17	7.65 (m)	7.70 – 7.67 (m, 2H)	-	126.4	126.0	0.4
18	7.68 (m)		-	126.9	126.2	0.7
19	8.14 (d, 7.7)	8.16 (dd, *J* = 7.6, 1.2 Hz, 1H)	0.02	129.9	129.4	0.3
20				142.7	142.1	0.6
NH		11.83				
OH		11.20				

Synthesis of pyonitrin C:

2-(1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline (20)[1]



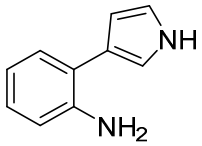
Following general procedure 1, **20** was prepared from 2-iodoaniline **15** (150 mg, 0.68 mmol) and **17** (286 mg, 0.82 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 9/1), affording **20** (180 mg) as a colorless oil in 83% yield.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.26 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.05 (td, *J* = 7.8, 1.4 Hz, 1H), 6.96 (s, 1H), 6.85 (s, 1H), 6.78 (td, *J* = 7.5, 1.0 Hz, 1H), 6.75 (dd, *J* = 7.8, 0.8 Hz, 1H), 6.51 (dd, *J* = 2.4, 1.2 Hz, 1H), 3.94 (s, 2H), 1.48 (sept, *J* = 7.6 Hz, 3H), 1.13 (d, *J* = 7.5 Hz, 18H).

^{13}C NMR (125 MHz, $CDCl_3$) δ 143.8, 129.9, 127.1, 124.9, 124.0, 122.8, 122.5, 118.7, 115.6, 110.9, 18.0, 11.9.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{31}N_2Si$ 315.2256; Found 315.2274

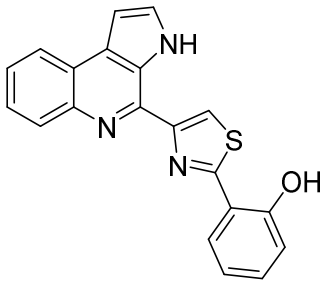
2-(1H-pyrrol-3-yl)aniline (24)[1]



Following general procedure 2, **24** was prepared from 2-(1-(triisopropylsilyl)-1H-pyrrol-3-yl) aniline **20** (50mg, 0.15 mmol). The crude residue was purified by column chromatography over deactivated silica gel (5% NEt_3) (hexane/ethyl acetate = 2/8), affording **24** (22 mg) as a colorless oil in 87% yield.

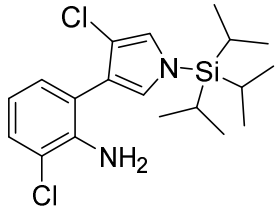
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.39 (s, 1H), 7.26 (dd, *J* = 7.4, 1.2 Hz, 1H), 7.09 (td, *J* = 7.7, 1.2 Hz, 1H), 7.01 (s, 1H), 6.88 (m, 1H), 6.82-6.77 (m, 2H), 6.47 (m, 1H), 3.73 (s, 2H).
^{13}C NMR (125 MHz, $CDCl_3$) δ 143.9, 130.0, 127.3, 122.4, 122.1, 118.8, 118.6, 116.3, 115.7, 108.9.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_{11}N_2$ 159.0922; Found 159.0917

pyonitrin C



Following general procedure 3, pyonitrin C was prepared from 2-(1H-pyrrol-3-yl)aniline **24** (10 mg, 0.06 mmol) and **7** (13 mg, 0.06 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 4/6), affording pyonitrin C (12 mg) as a yellow solid in 55% yield.
^{1}H NMR (800 MHz, DMSO-d_6) δ 11.59 (s, 1H), 8.72 (dd, *J* = 7.8, 1.6 Hz, 1H), 8.65 (s, 1H), 8.33 (dd, *J* = 7.8, 1.3 Hz, 1H), 8.09 (d,*J*= 7.5 Hz, 1H), 7.79 (t, *J* = 2.7 Hz, 1H), 7.62 – 7.56 (m, 2H), 7.37 (t, *J* = 7.3 Hz, 1H), 7.25 (s, 1H), 7.11 (d, *J* = 8.1 Hz, 1H), 7.05 (d, *J* = 7.4 Hz, 1H).
^{13}C NMR (200 MHz, DMSO-d_6) δ 163.4, 153.1, 141.9, 139.2, 131.0, 129.3, 129.0, 128.6, 128.4, 126.0, 125.9, 125.5, 123.2, 123.1, 120.3, 119.5, 119.1, 116.4, 100.8.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{14}N_3OS$ 344.0857; Found 344.0844

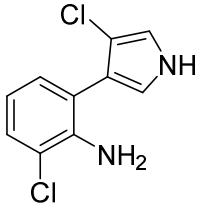
Synthesis of pyonitrin D:
2-chloro-6-(4-chloro-1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline (21)[1]



Following general procedure 1, **21** was prepared from 2-chloro-6-iodoaniline **14** (190 mg, 0.75 mmol) and **16** (345 mg, 0.90 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 9/1), affording **21** (211 mg) as a colorless oil in 73% yield.

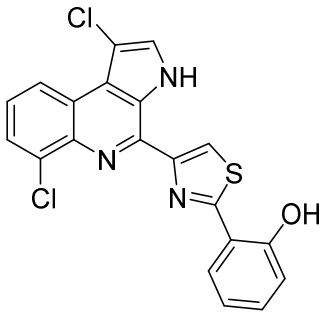
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.23 (dd, *J* = 8.0, 1.2 Hz, 1H), 7.12 (dd, *J* = 7.6, 1.2 Hz, 1H), 6.85 – 6.80 (m, 2H), 6.72 (t, *J* = 7.8 Hz, 1H), 4.23 (s, 2H), 1.45 (sept, *J* = 7.6 Hz, 3H), 1.13 (d, *J* = 7.5 Hz, 18H).
^{13}C NMR (125 MHz, $CDCl_3$) δ 141.7, 130.0, 128.4, 123.4, 122.1, 121.1, 120.7, 119.5, 118.0, 113.7, 17.9, 11.7.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{29}Cl_2N_2Si$ 383.1477; Found 383.1479

2-chloro-6-(4-chloro-1H-pyrrol-3-yl)aniline (25) [1]



Following general procedure 2, **25** was prepared from 2-chloro-6-(4-chloro-1-(triisopropylsilyl)-1H-pyrrol-3-yl) aniline **21** (100 mg, 0.26 mmol). The crude residue was purified by column chromatography over deactivated silica gel (5% NEt_3) (hexane/ethyl acetate = 2/8), affording **25** (48 mg) as a colorless oil in 81% yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.43 (s, 1H), 7.24 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.09 (dd, *J* = 7.6, 1.2 Hz, 1H), 6.86 (t, *J* = 2.6 Hz, 1H), 6.82 (t, *J* = 2.7 Hz, 1H), 6.72 (t, *J* = 7.8 Hz, 1H), 4.23 (s, 2H).
^{13}C NMR (125 MHz, $CDCl_3$) δ 141.8, 130.2, 128.6, 120.3, 119.5, 119.3, 118.0, 117.3, 116.3, 112.2.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_9Cl_2N_2$ 227.0142; Found 227.0137

pyonitrin D (4)

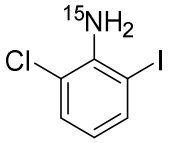


Following general procedure 2, pyonitrin D was prepared from 2-chloro-6-(4-chloro-1H-pyrrol-3-yl) aniline **25** (10 mg, 0.04 mmol) and **7** (9 mg, 0.04 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 4/6), affording pyonitrin D (12 mg) as a yellow solid in 66% yield.

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.91 (s, 1H), 11.28 (s, 1H), 8.84 (dd, J = 8.3, 1.0 Hz, 1H), 8.73 (s, 1H),8.72 (m, 1H), 7.95 (s, 1H), 7.86 (dd, J = 7.5, 1.0 Hz, 1H), 7.64 (t, J = 7.9 Hz, 1H), 7.39 (dt, J = 8.1, 1.0 Hz, 1H), 7.11 (d, J = 8.3 Hz, 1H), 7.08 (t, J = 7.5 Hz, 1H).
^{13}C NMR (125 MHz, DMSO-d_6) δ 163.8, 152.3, 139.4, 138.0, 132.7, 131.2, 128.5, 127.2, 126.9, 126.2, 125.4, 123.8, 123.4, 122.1, 121.0, 119.4, 116.3, 105.5.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{12}Cl_2N_3OS$ 412.0078; Found 412.0075

Synthesis of ^{15}N labeled pyonitrin A:

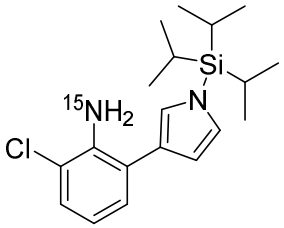
^{15}N labeled 2-chloro-6-iodoaniline (26a)



A solution of 2-chloro-6-iodobenzoic acid (281 mg 1.0 mmol) in concentrated sulfuric acid (2.5 mL) was heated to 60^{o}C in oil bath for 1 h. The solution was then cooled to rt before addition of sodium azide [^{15}N] (66 mg, 1.0 mmol). The resulting mixture was left to stir at rt for 42 h before cooling to 0^{o}C and basifying with concentrated ammonium hydroxide. The organics were extracted with ethyl acetate (2 x 20 mL). The organic layers were combined and washed with brine solution (1 x 20 mL), dried over sodium sulfate and concentrated under reduced pressure and purified by column chromatography over silica gel (hexane/ethyl acetate = 7/3), to afford the pure ^{15}N labeled 2-chloro-6-iodoaniline **26a** as brown solid (210 mg) in 82% yield.
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.54 (d, J = 7.9 Hz, 1H), 7.23 (d, J = 7.9 Hz, 1H), 6.41 (t, J = 7.9 Hz, 1H), 4.64 - 4.41 (m, 2H).
^{13}C NMR (125 MHz, $CDCl_3$) δ 143.5, 137.6, 129.7, 120.0, 118.0, 83.6.
HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_6H_6ClI^{15}N$ 254.9233; Found 254.9203

Note: We have observed multiplate for NH_2 in proton because equal possibility of the nucleophilic attack of ^{15}N labeled as well as ^{14}N nitrogen from the sodium azide ($Na^{15}NN_2$) in the reaction. The same was confirmed by high resolution mass spectroscopy showed the mass for both ^{14}N and ^{15}N products. HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for C_6H_6ClIN- 253.9233; Found 253.9232

^{15}N labeled 2-chloro-6-(1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline (26b)



Following general procedure 1, **26b** was prepared from ^{15}N labelled 2-chloro-6-iodoaniline **26a** (100 mg, 0.39 mmol) and **17** (164 mg, 0.47 mmol). The crude residue was purified by column chromatography over silica gel (hexane/ethyl acetate = 9/1), affording **26b** (111 mg) as a colorless oil in 81% yield.

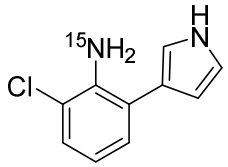
^1H NMR (500 MHz, $CDCl_3$) δ 7.16-7.14 (m, 2H), 6.96 (s, 1H), 6.86 (t, *J* = 2.2 Hz, 1H), 6.69 (t, *J* = 7.8 Hz, 1H), 6.50 (dd, *J* = 2.4, 1.2 Hz 1H), 4.37 (m, 2H), 1.48 (sept, *J* = 7.6 Hz, 3H), 1.13 (d, *J* = 7.5 Hz, 18H)

^{13}C NMR (125 MHz, $CDCl_3$) δ 140.6, 128.2, 127.1, 125.1, 124.1, 123.6, 122.7, 119.7, 118.4, 110.8, 18.0, 11.8.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{30}Cl^{15}NNSi$ 350.1866; Found 350.1847 (^{15}N product)

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{19}H_{30}ClN_2Si$ 349.1866; Found 349.1871 (^{14}N product)

^{15}N labeled 2-chloro-6-(1H-pyrrol-3-yl)aniline (26)



Following general procedure 2, **26** was prepared from ^{15}N labelled 2-chloro-6-(1-(triisopropylsilyl)-1H-pyrrol-3-yl)aniline **26b** (55 mg, 0.15 mmol). The crude residue was purified by column chromatography over deactivated silica gel (5% NEt_3) (hexane/ethyl acetate = 2/8), affording **26** (22 mg) as a colorless oil in 73% yield.

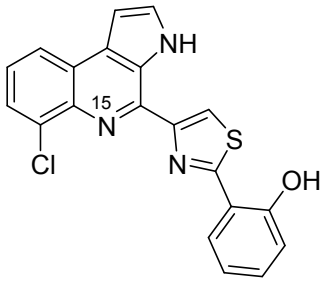
^1H NMR (800 MHz, DMSO-d_6) δ 11.05 (s, 1H), 7.10-7.08 (m, 2H), 7.01 (q, *J* = 1.8 Hz, 1H), 6.87 (q, *J* = 2.5 Hz, 1H), 6.60 (t, *J* = 7.7 Hz, 1H), 6.27 (q, *J* = 2.4 Hz, 1H), 4.87 – 4.76 (m, 2H).

^{13}C NMR (200 MHz, DMSO-d_6) δ 140.6, 127.8, 126.3, 123.6, 120.1, 118.6, 118.2, 117.3, 116.2, 107.3.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_{10}Cl^{15}NN$ 194.0532; Found 194.0499 (^{15}N product)

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{10}H_{10}ClN_2$ 193.0532; Found 193.0428 (^{14}N product)

^{15}N labeled pyonitrin A (29)



Following general procedure 3, **29** was prepared from ^{15}N labeled 2-chloro-6-(1H-pyrrol-3-yl)aniline **26** (10 mg, 0.05 mmol) and **7** (10.6 mg, 0.05 mmol). The crude residue was purified by

column chromatography over silica gel (hexane/ethyl acetate = 4/6), affording ^{15}N labeled pyonitrin A (14 mg) as a yellow solid in 71% yield.

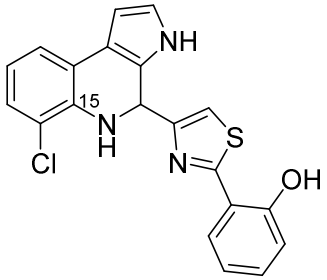
^{1}H NMR (500 MHz, DMSO-d_6) δ 11.69 (s, 1H), 11.18 (s, 1H), 8.75 (dd,*J* = 7.8, 1.1 Hz, 1H), 8.70 (s, 1H), 8.34 (dd, *J* = 8.1, 1.0 Hz, 1H), 7.85 – 7.83 (m, 1H), 7.78 (d, *J* = 7.4 Hz, 1H), 7.54 (t, *J* = 7.8 Hz, 1H), 7.39 (dt, *J* = 8.2, 1.3 Hz, 1H), 7.31 (s, 1H), 7.11 (d, *J* = 8.0 Hz, 1H), 7.09 (t, *J* = 7.4 Hz, 1H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 163.5, 155.1, 152.9, 139.3, 137.7, 132.4, 131.2, 129.8, 129.6, 128.5, 126.2, 125.6, 124.7, 122.5, 121.4, 119.5, 119.4, 116.2, 101.3.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{13}Cl^{15}NN_2OS$ 379.0467; Found 379.0438 (^{15}N product)

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{13}ClN_3OS$ 378.0467; Found 378.0457 (^{14}N product)

Synthesis and isolation of ^{15}N labeled intermediate (28)



1% solution of TFA in DMSO (1 mL), ^{15}N labeled 2-chloro-6-(1H-pyrrol-3-yl)aniline **26** (5 mg, 0.025 mmol) and 2-(2-hydroxyphenyl)thiazole-4-carbaldehyde **7** (5.31 mg, 0.025 mmol) were added at rt. The mixture was further stirred at ambient temperature for 45 min. Then saturated aqueous $NaHCO_3$ was added to quench the acid in the reaction mixture. The product was extracted using ethyl acetate (10 mL x 2) and the organic layer was washed with water (10 mL x 2), brine solution (10 mL x 1) and finally dried over anhydrous sodium sulfate. It was then evaporated in vacuo to obtain a residue and purified by flash column chromatography silica gel (hexane/ethyl acetate = 4/6), to afford pure ^{15}N labeled intermediate **28** (7 mg, 71%).

^{1}H NMR (500 MHz, DMSO-d_6) δ 11.27 (s, 1H), 10.98 (s, 1H), 7.97 (dd, *J* = 7.9, 1.5 Hz, 1H), 7.36 – 7.24 (m, 1H), 7.18 (d, *J* = 6.8 Hz, 1H), 7.12 (s, 1H), 7.00 – 6.94 (m, 2H), 6.92 (t, *J* = 7.5 Hz, 1H), 6.77 (t, *J* = 2.6 Hz, 1H), 6.58 (t, *J* = 7.7 Hz, 1H), 6.35 (t, *J* = 2.4 Hz, 1H), 6.09 (d, *J* = 2.5 Hz, 1H).

^{13}C NMR (125 MHz, DMSO-d_6) δ 165.2, 158.1, 155.3, 136.5, 131.2, 127.5, 125.1, 125.0, 121.0, 119.9, 119.4, 119.3, 118.3, 117.4, 116.8, 116.5, 114.2, 114.0, 102.0, 51.3.

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{15}Cl^{15}NN_2OS$ 381.0624; Found 381.0600 (^{15}N product)

HRMS (ESI) *m/z*: $[M + H]^+$ Calcd for $C_{20}H_{15}ClN_3OS$ 380.0624; Found 380.0622 (^{14}N product)

Reference:

1. Morrison, M. D.; Hanthorn, J. J.; Pratt, D. A. Synthesis of Pyrrolnitrin and Related Halogenated Phenylpyrroles. *Org. Lett*. **2009**, *11*, 1051–1054

Figure S2: ^{1}H NMR spectrum of **12** (500 MHz, $CDCl_3$)

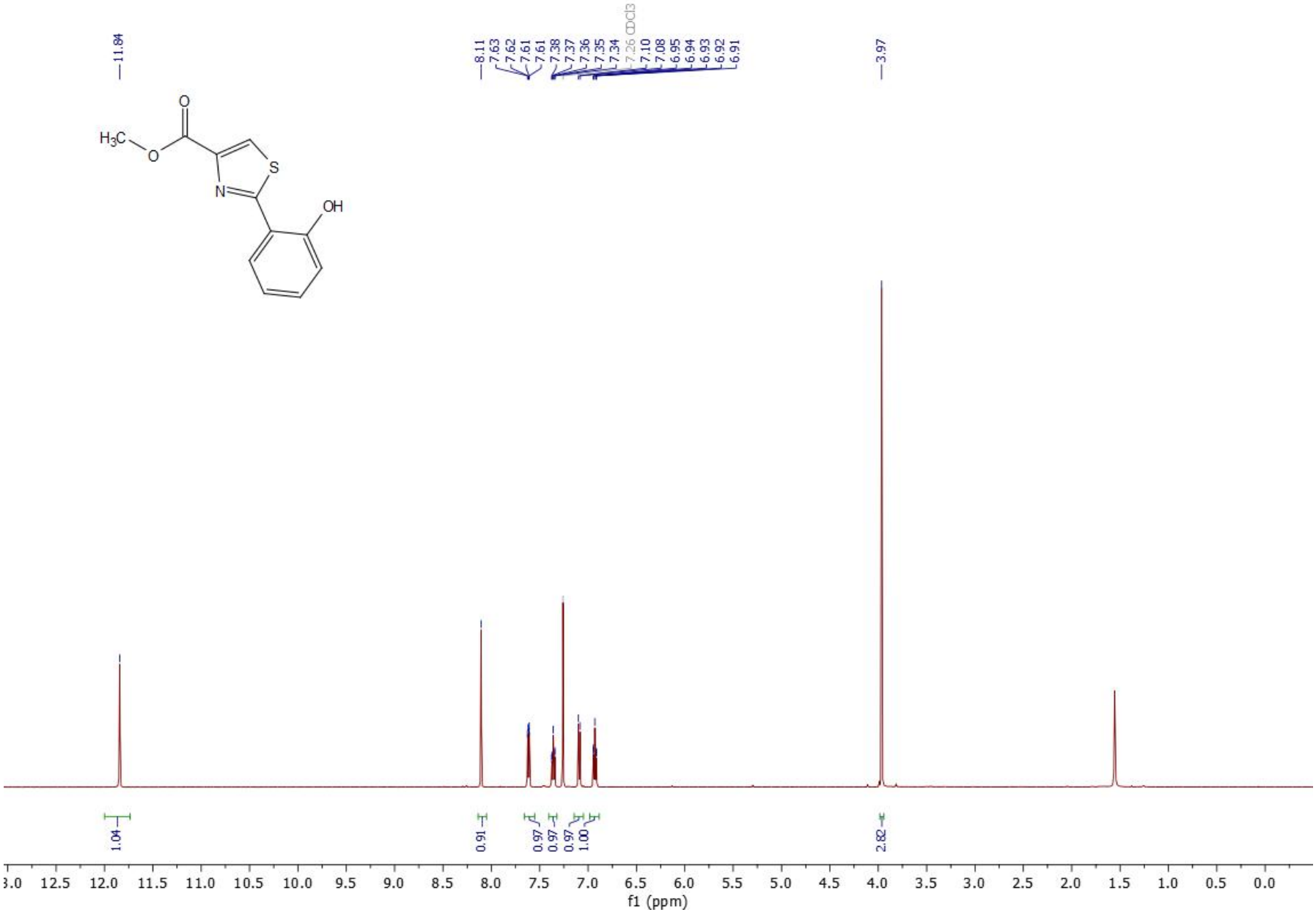


Figure S3: ^{13}C NMR spectrum of **12** (125 MHz, $CDCl_3$)

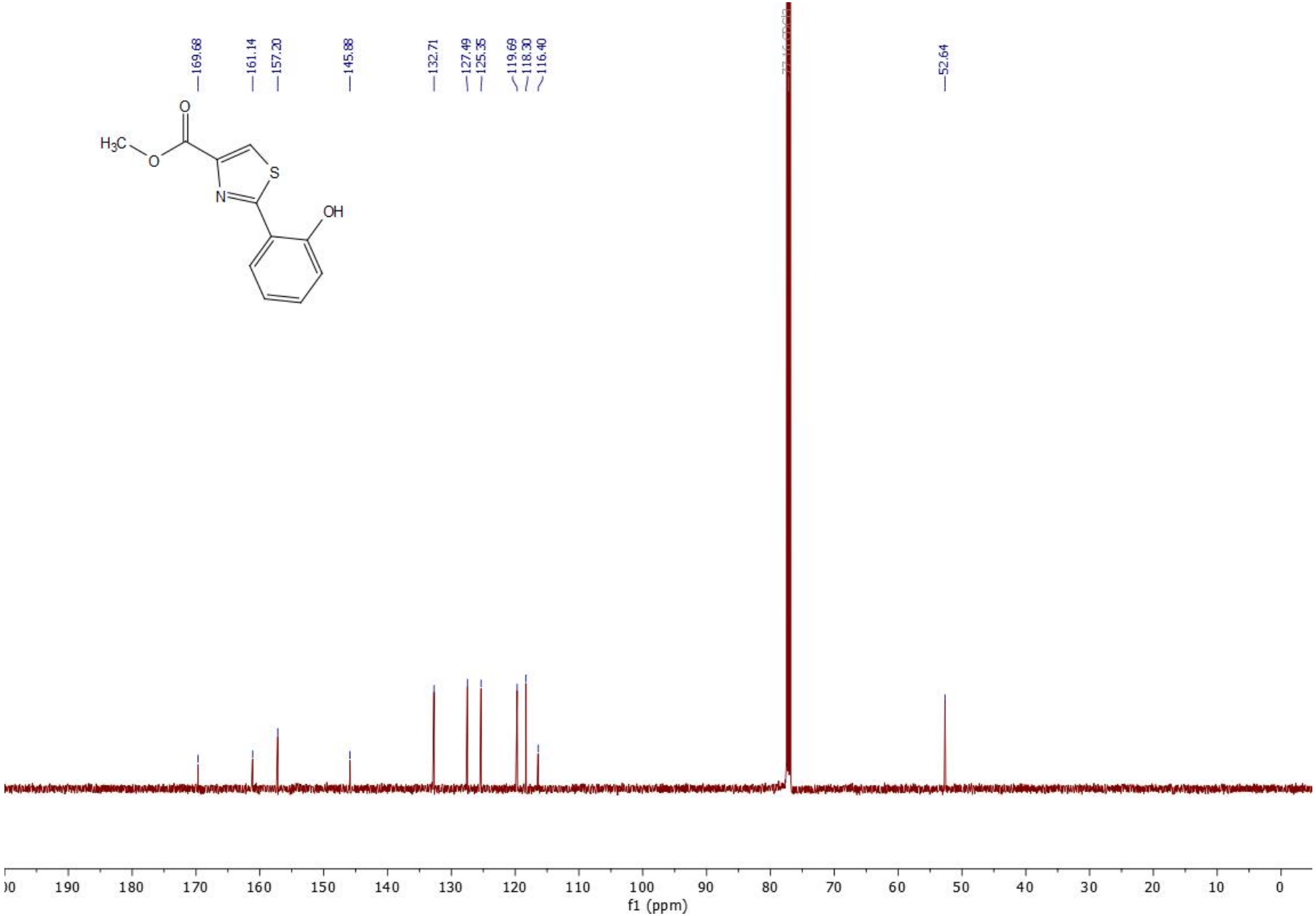


Figure S4: ^{1}H NMR spectrum of **13** (500 MHz, $CDCl_3$)

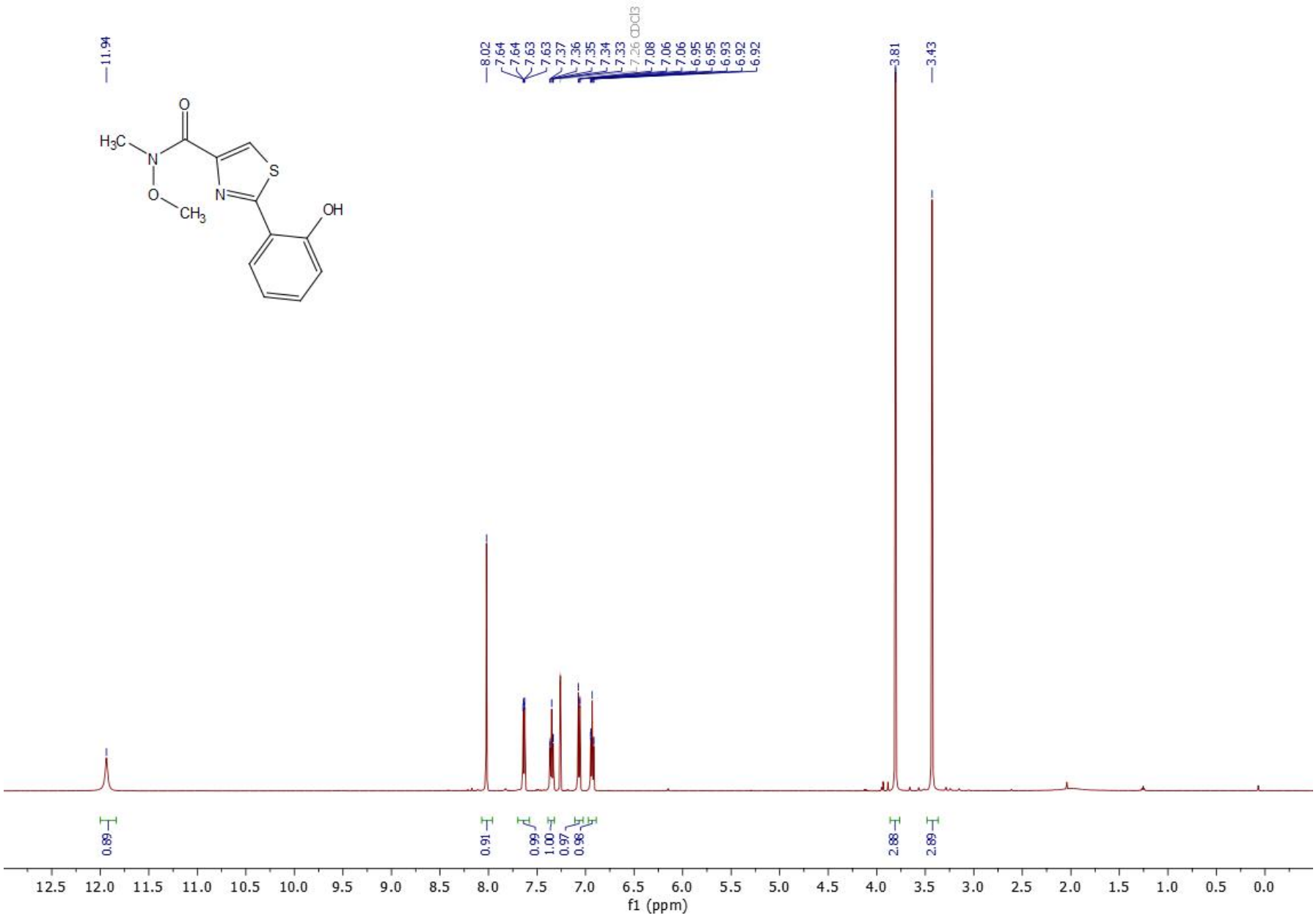


Figure S5: ^{13}C NMR spectrum of **13** (125 MHz, $CDCl_3$)

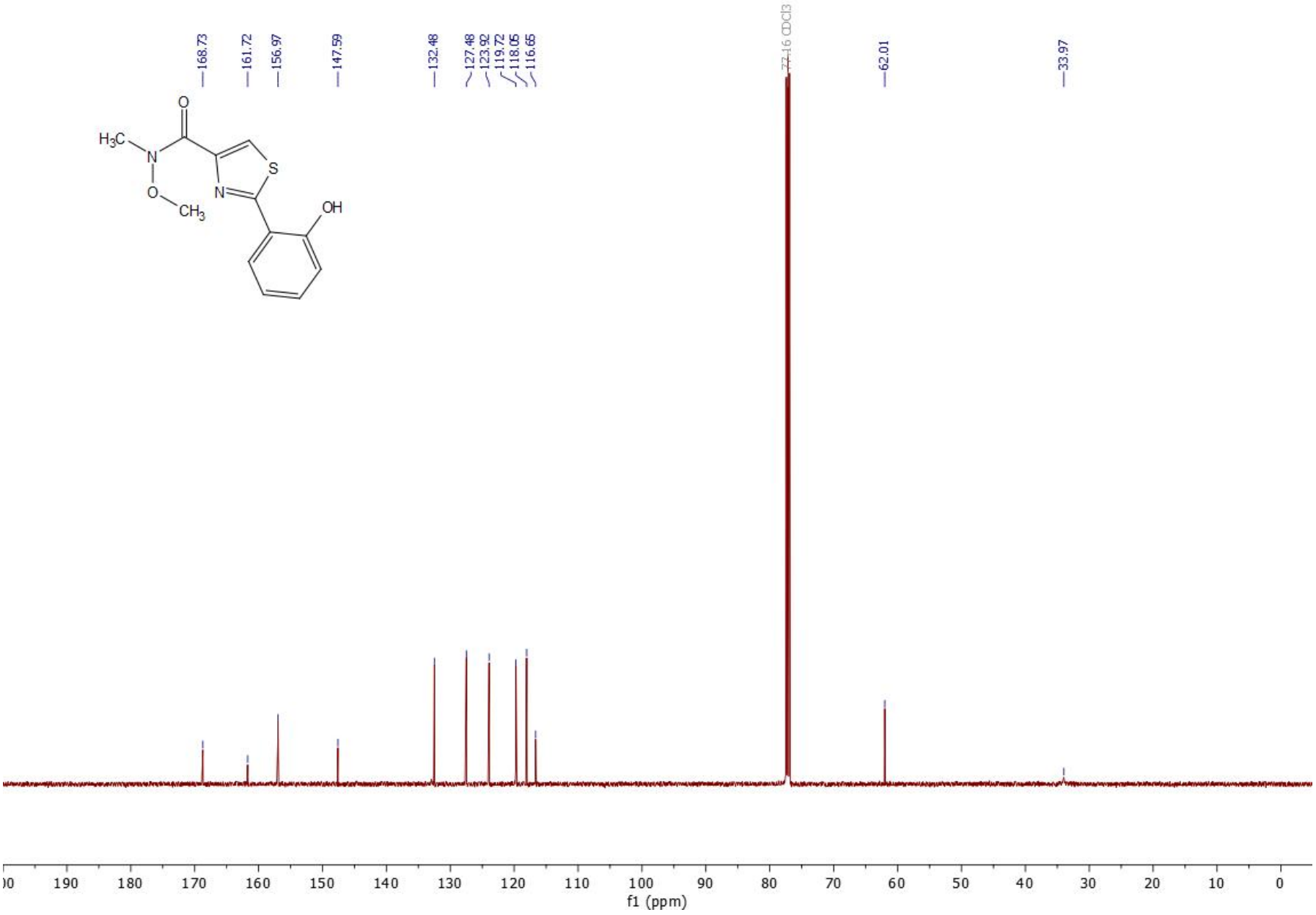


Figure S6: ^{1}H NMR spectrum of **7** (500 MHz, $CDCl_3$)

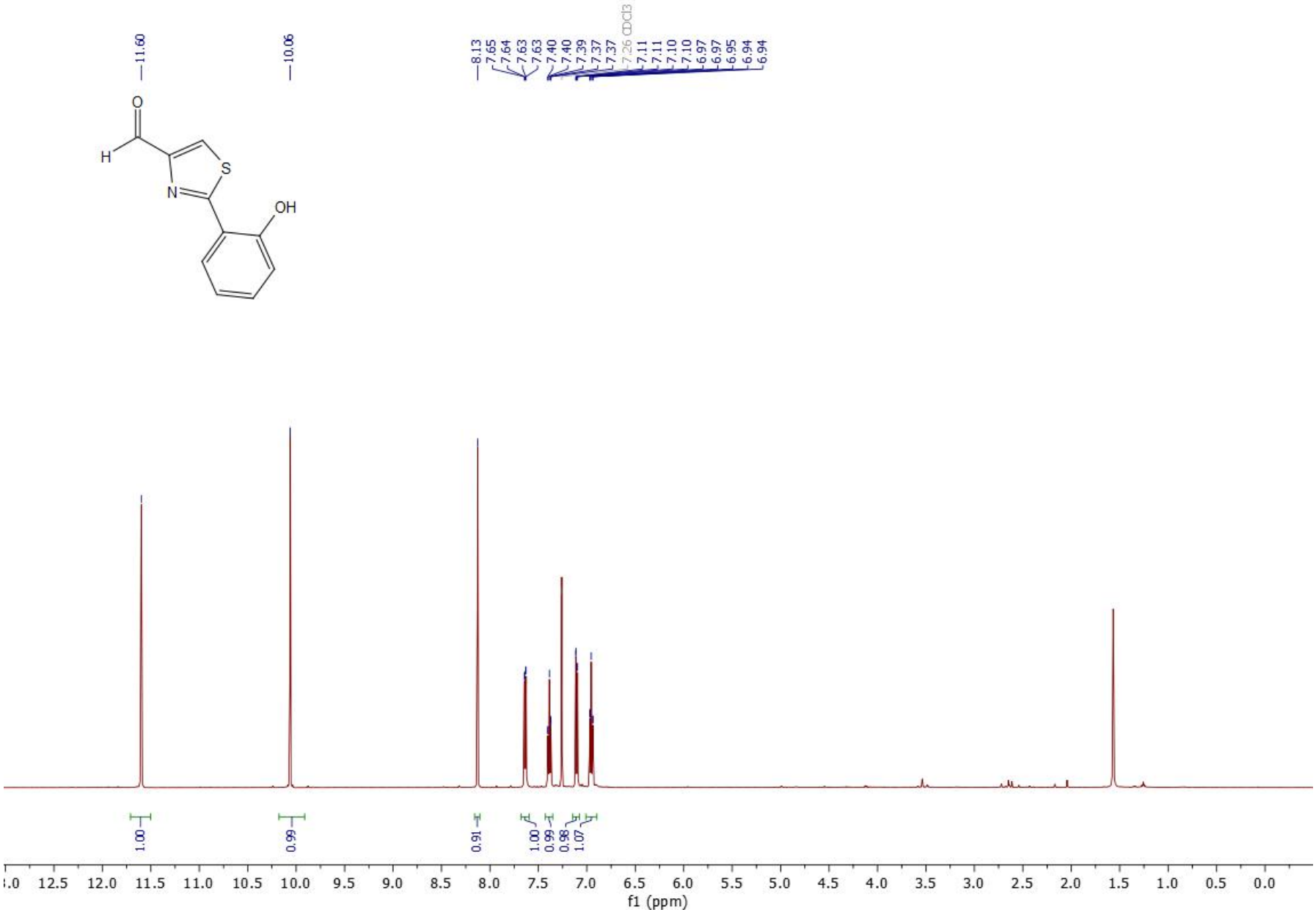


Figure S7: ^{13}C NMR spectrum of **7** (125 MHz, $CDCl_3$)

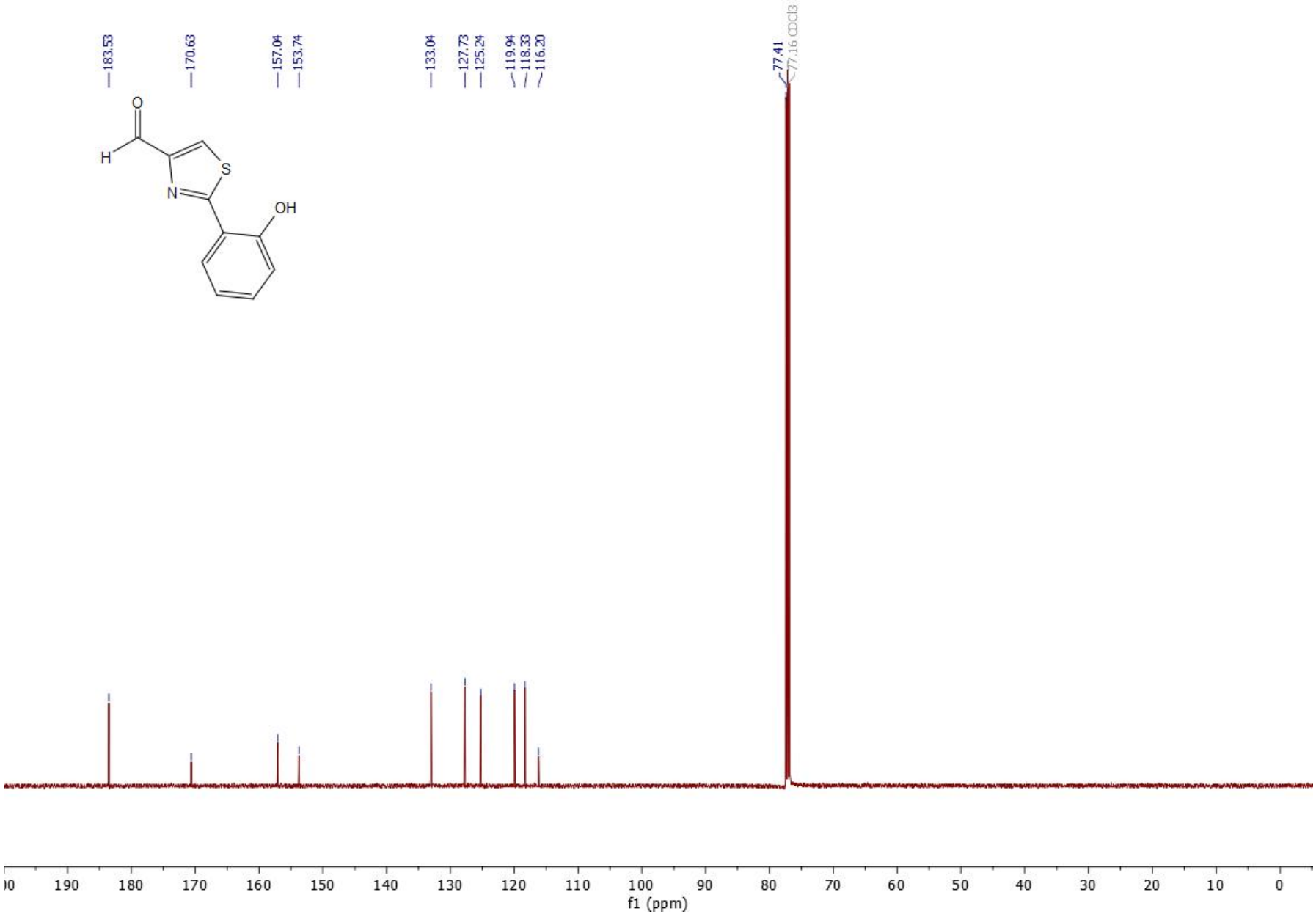


Figure S8: ^{1}H NMR spectrum of **18** (500 MHz, $CDCl_3$)

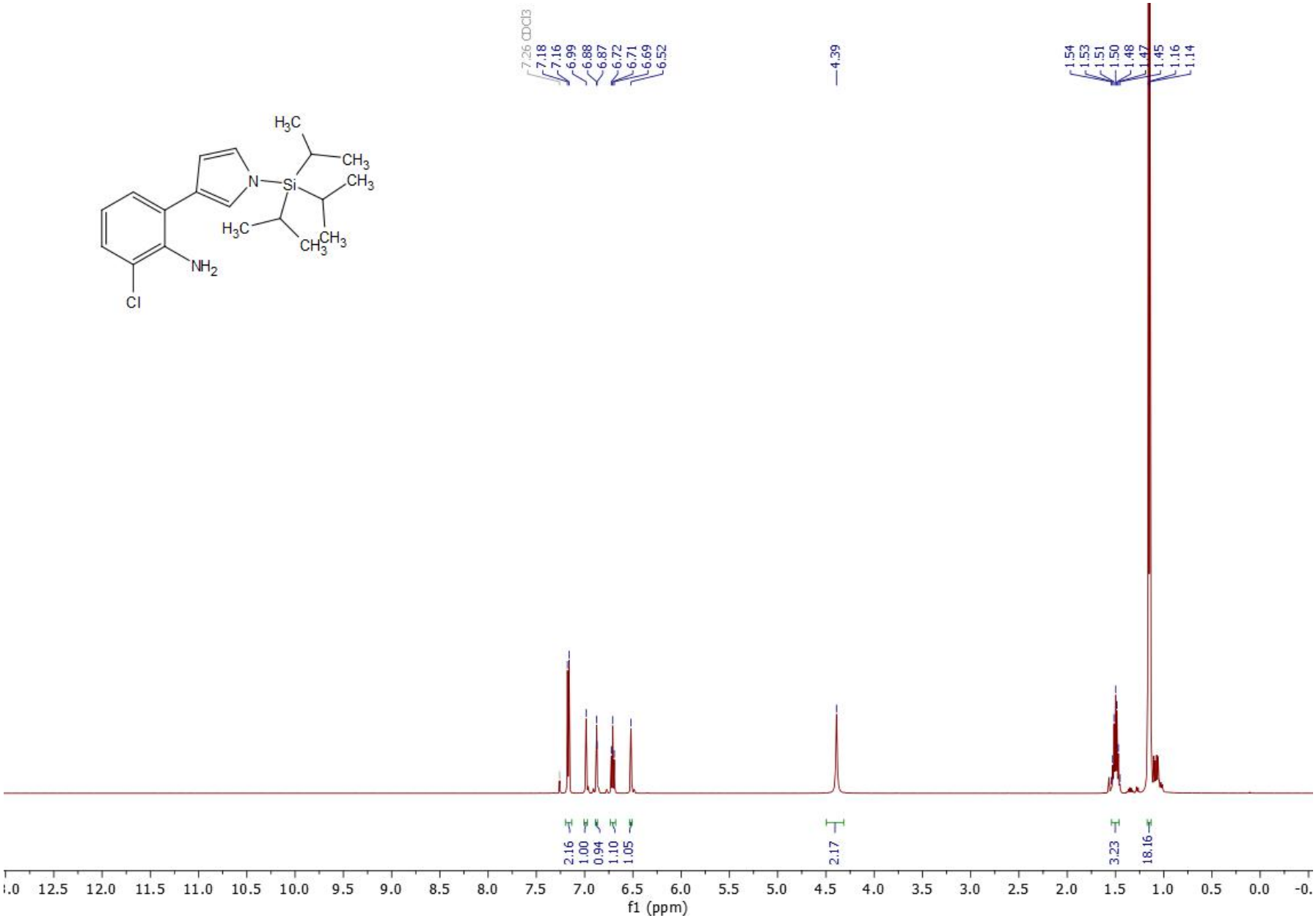


Figure S9: ^{13}C NMR spectrum of **18** (125 MHz, $CDCl_3$)

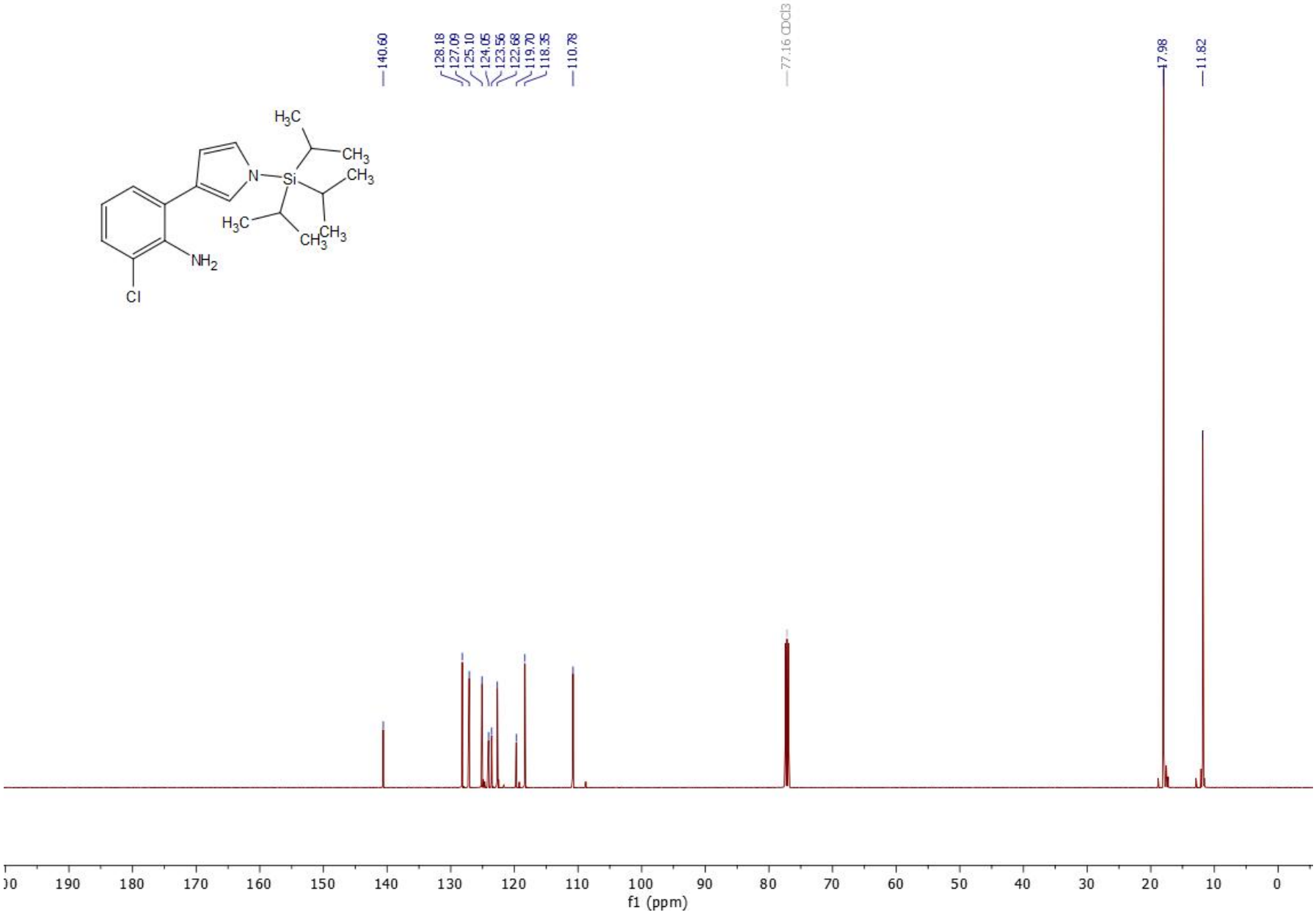


Figure S10: ^{1}H NMR spectrum of **22** (500 MHz, $CDCl_3$)

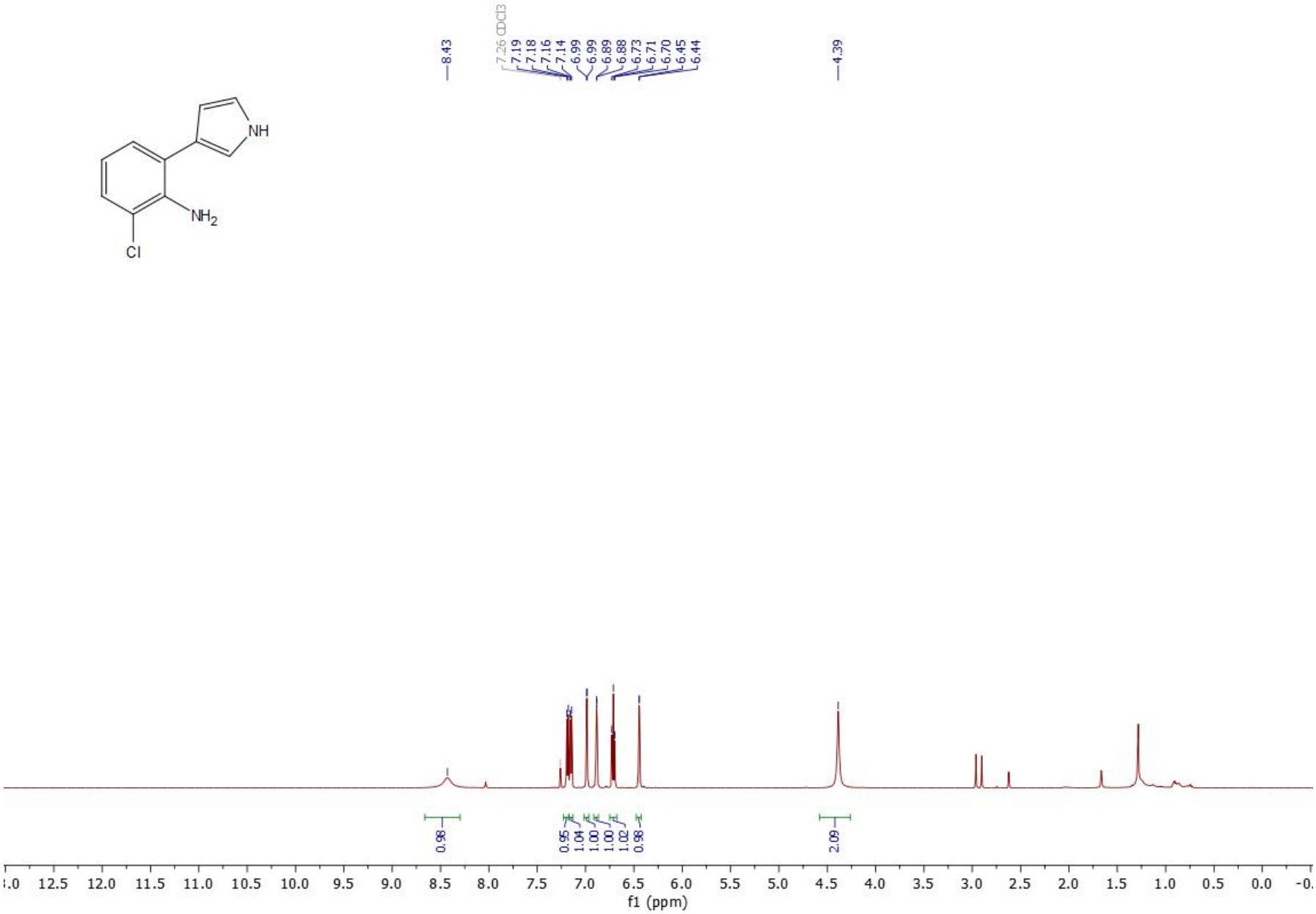


Figure S11: ^{13}C NMR spectrum of **22** (125 MHz, $CDCl_3$)

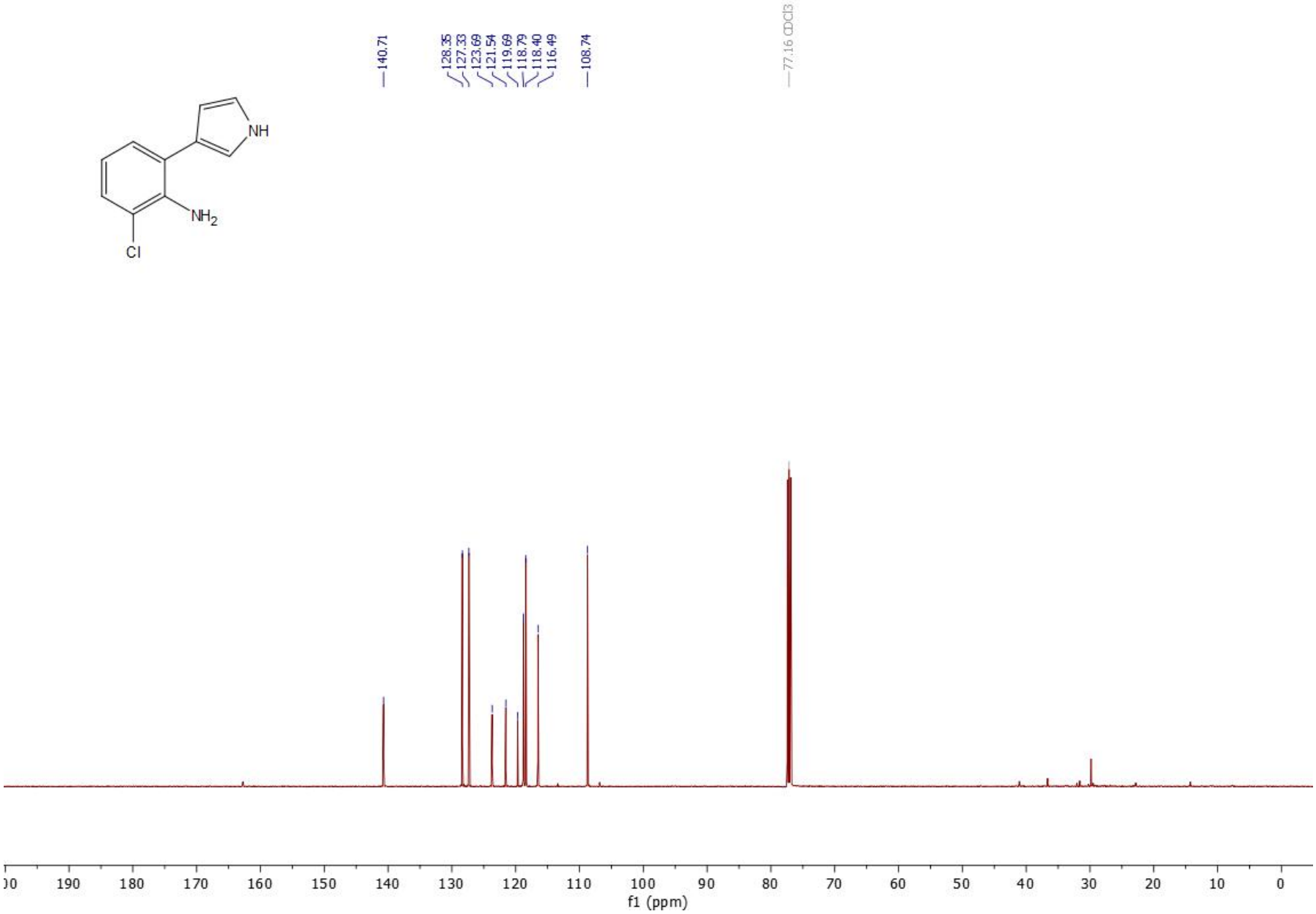


Figure S12: ^{1}H NMR spectrum of pyonitrin A **1** (500 MHz, DMSO-d_6)

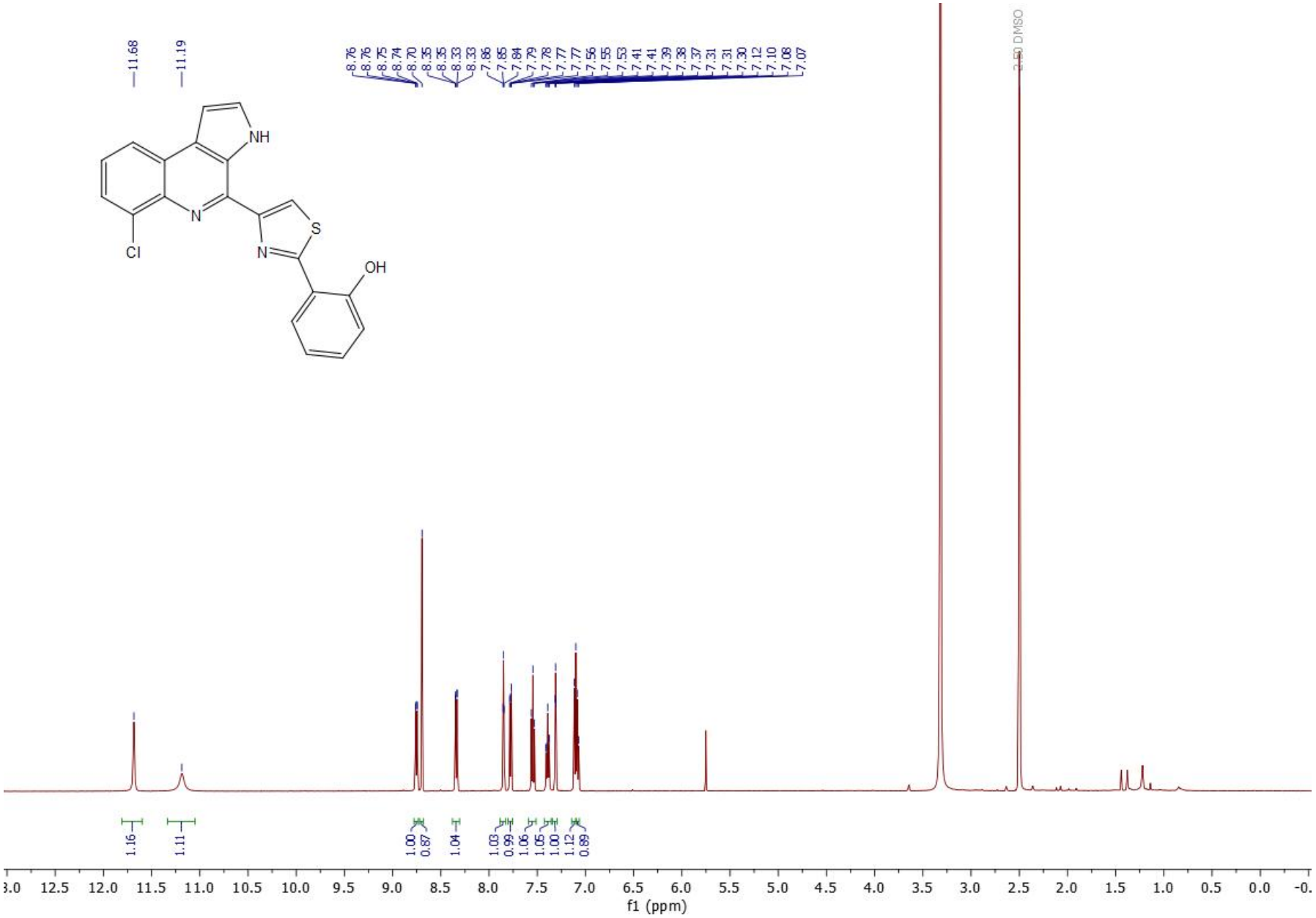


Figure S13: ^{13}C NMR spectrum of pyonitrin A **1** (125 MHz, DMSO-d_6)

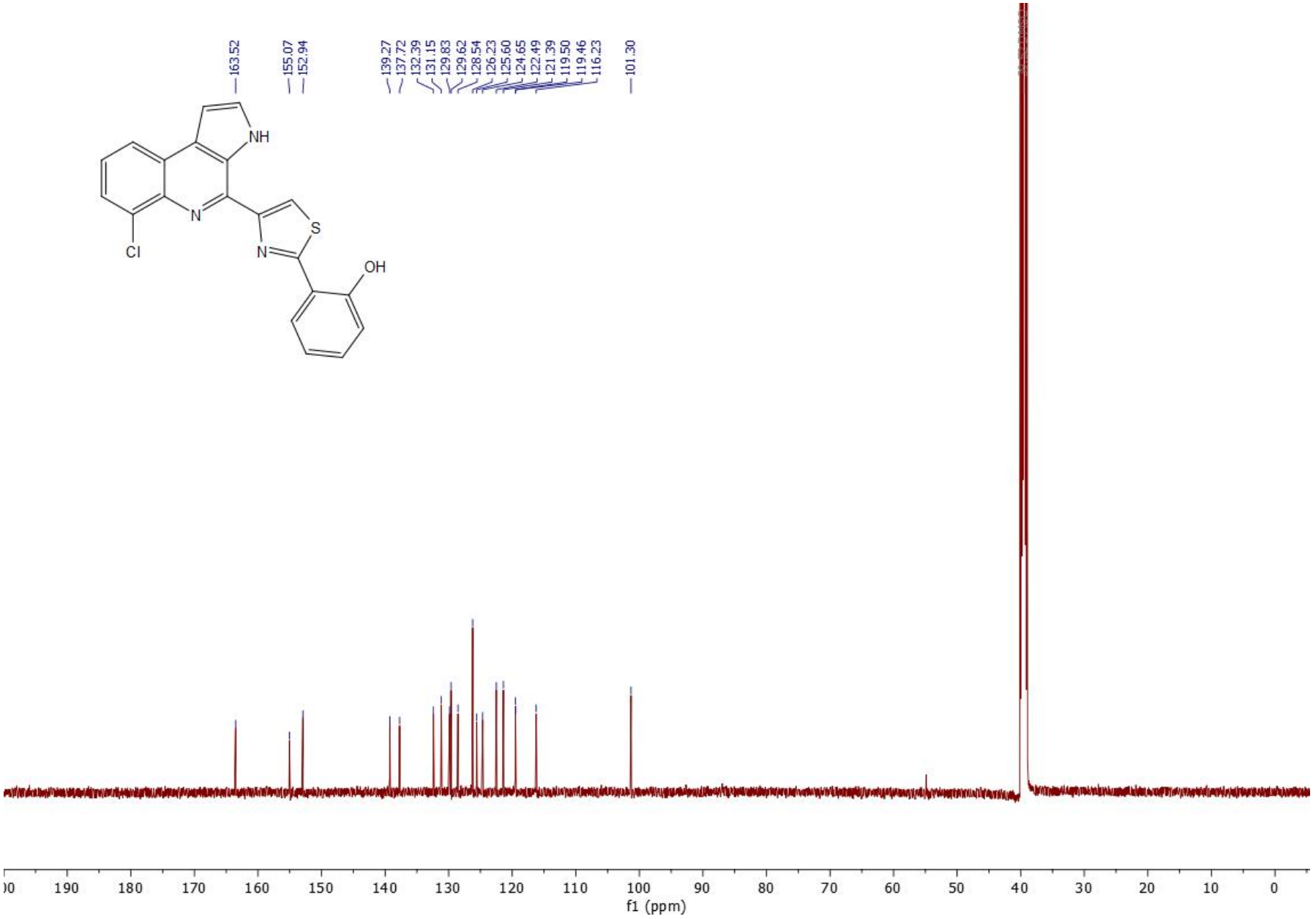


Figure S14: ^{1}H NMR spectrum of **19** (500 MHz, $CDCl_3$)

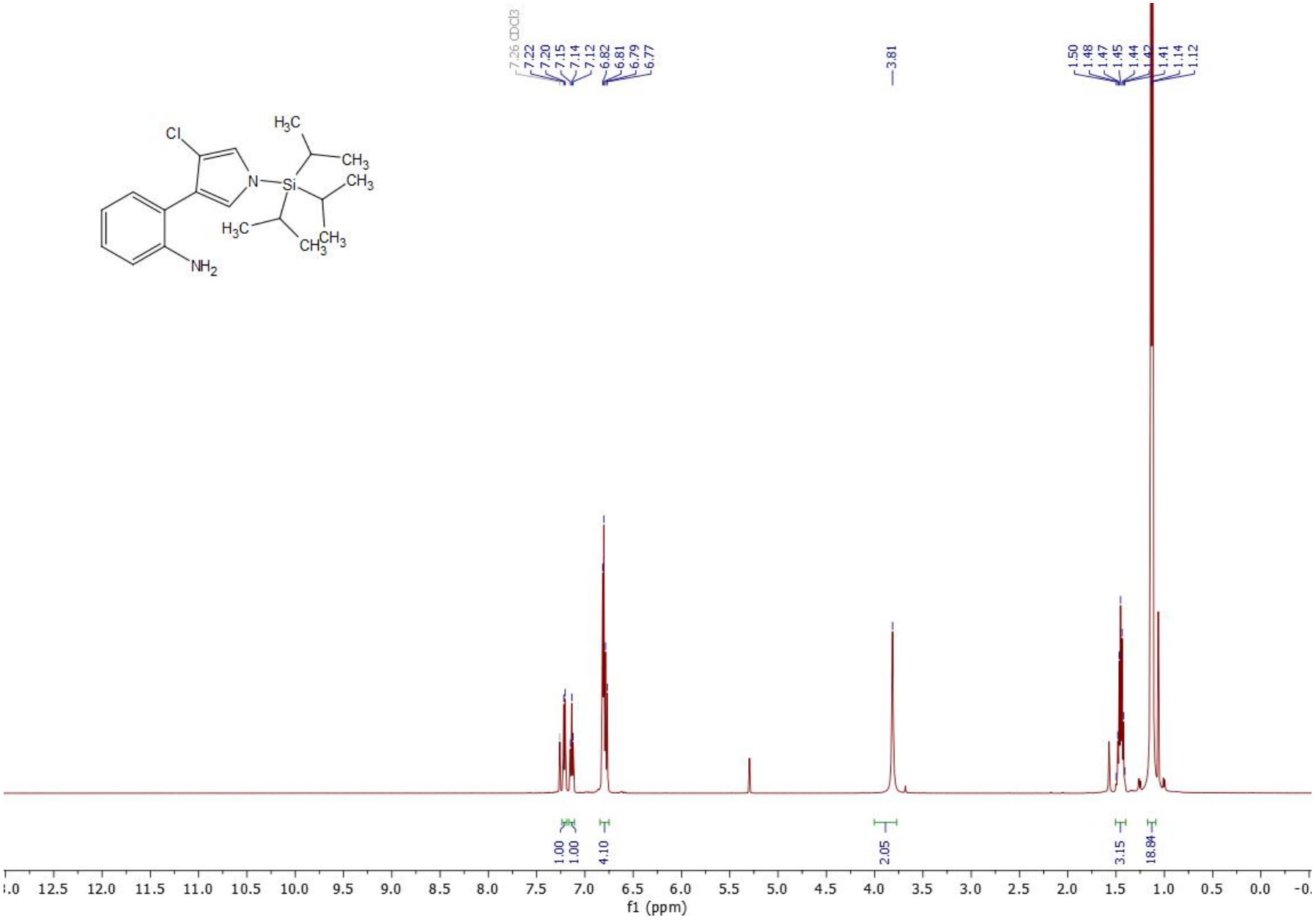


Figure S15: ^{13}C NMR spectrum of **19** (125 MHz, $CDCl_3$)

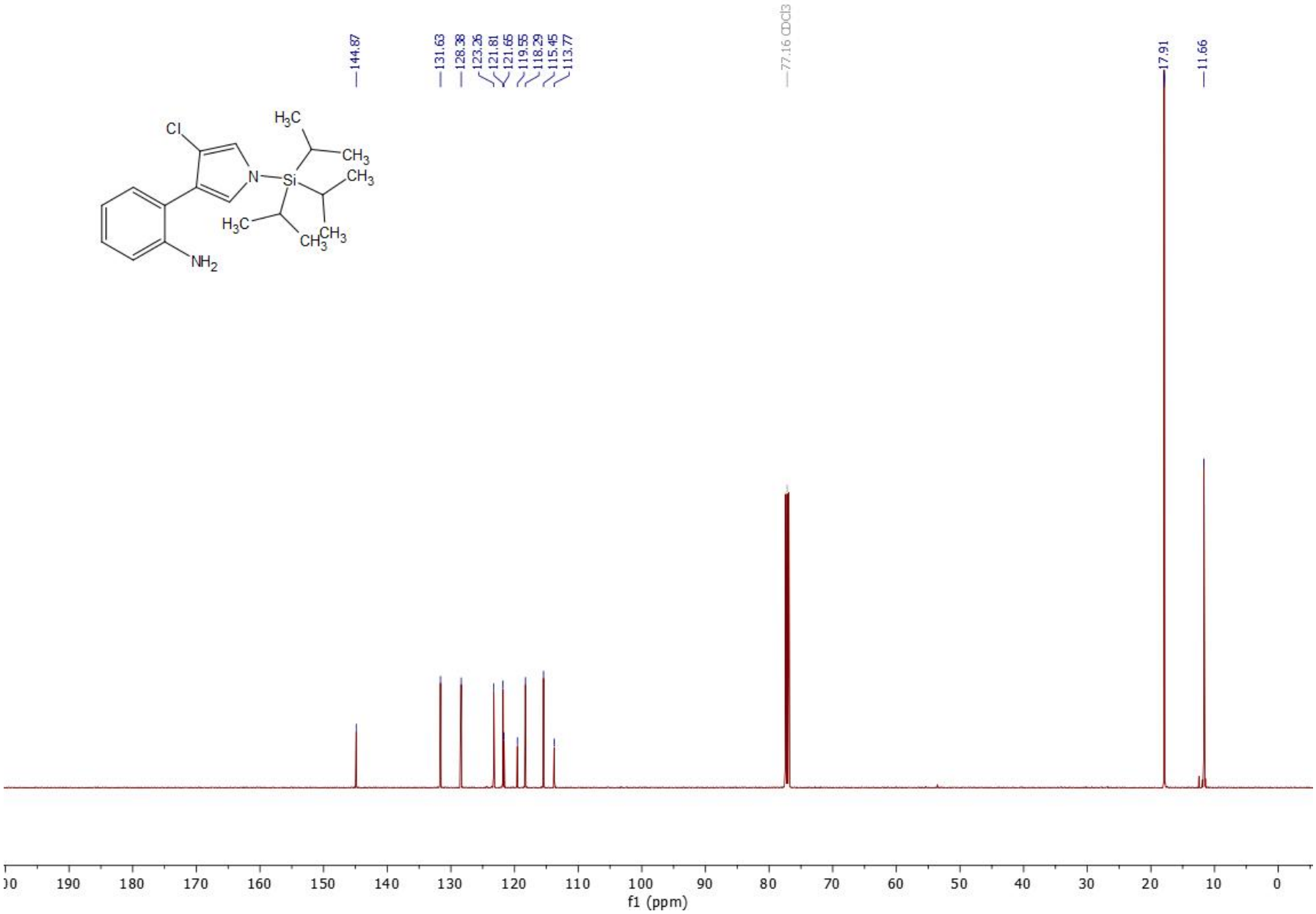


Figure S16: ^{1}H NMR spectrum of **23** (500 MHz, $CDCl_3$)

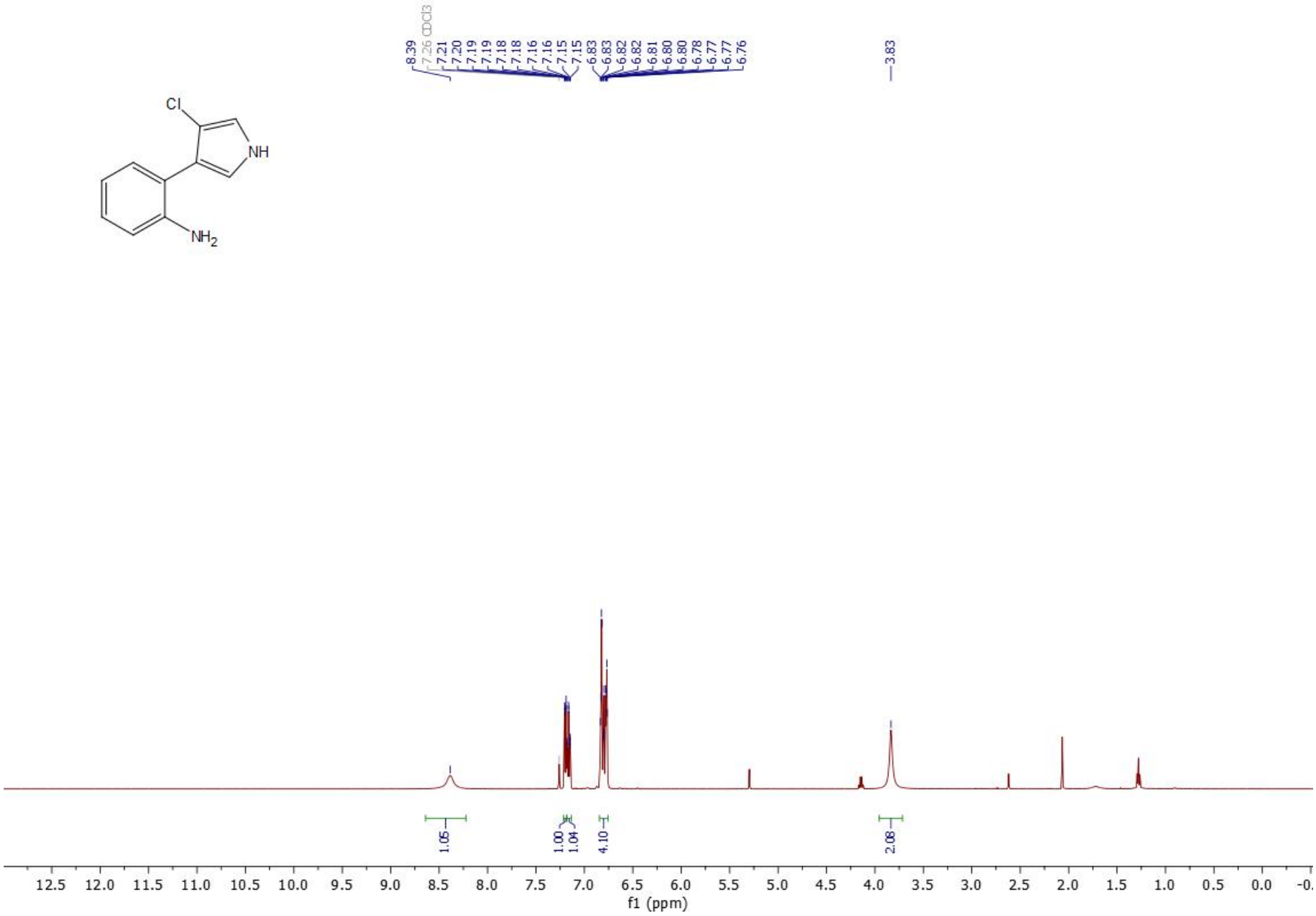


Figure S17: ^{13}C NMR spectrum of **23** (125 MHz, $CDCl_3$)

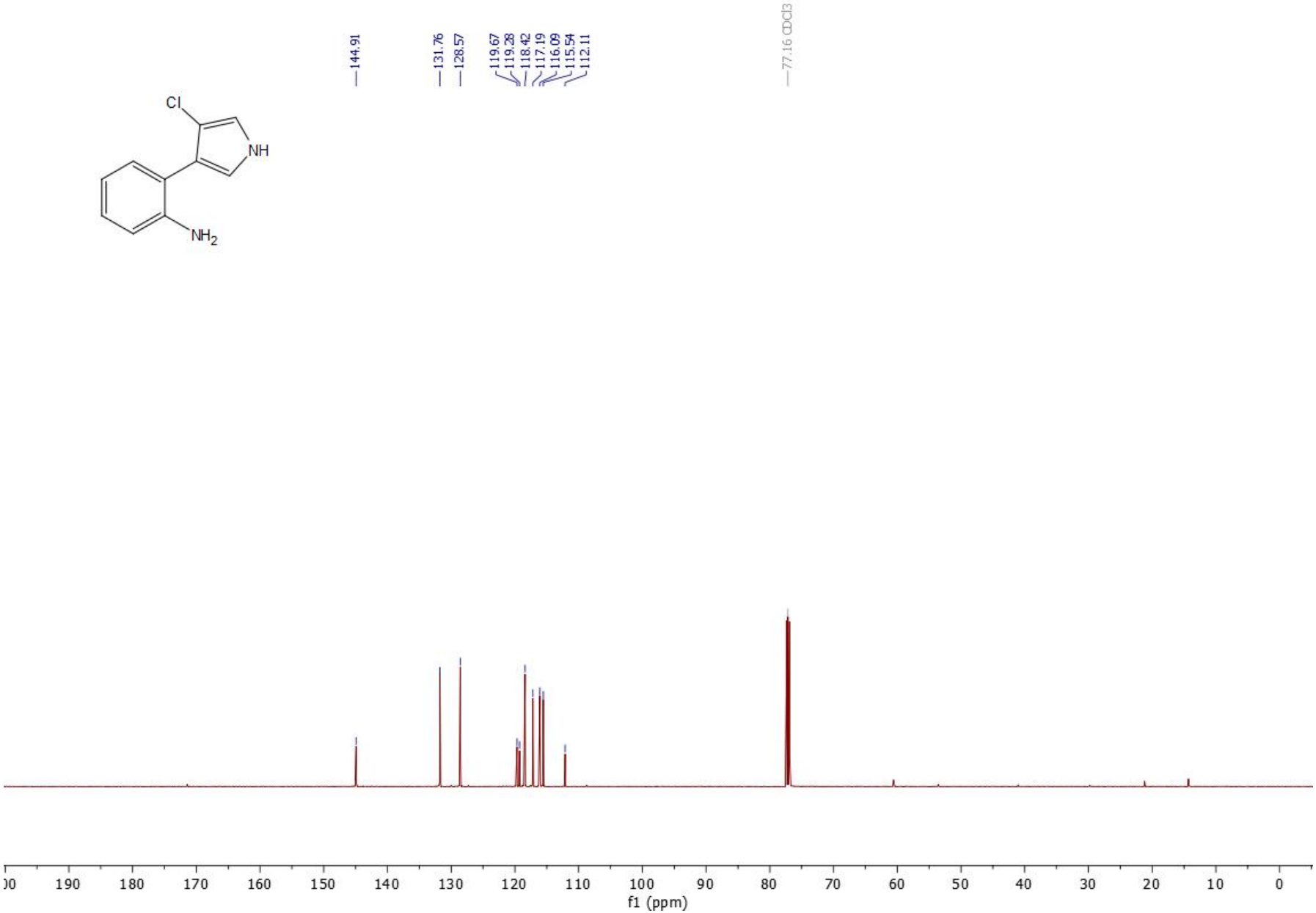


Figure S18: ^{1}H NMR spectrum of **pyonitrin B** (800 MHz, DMSO-d_6)

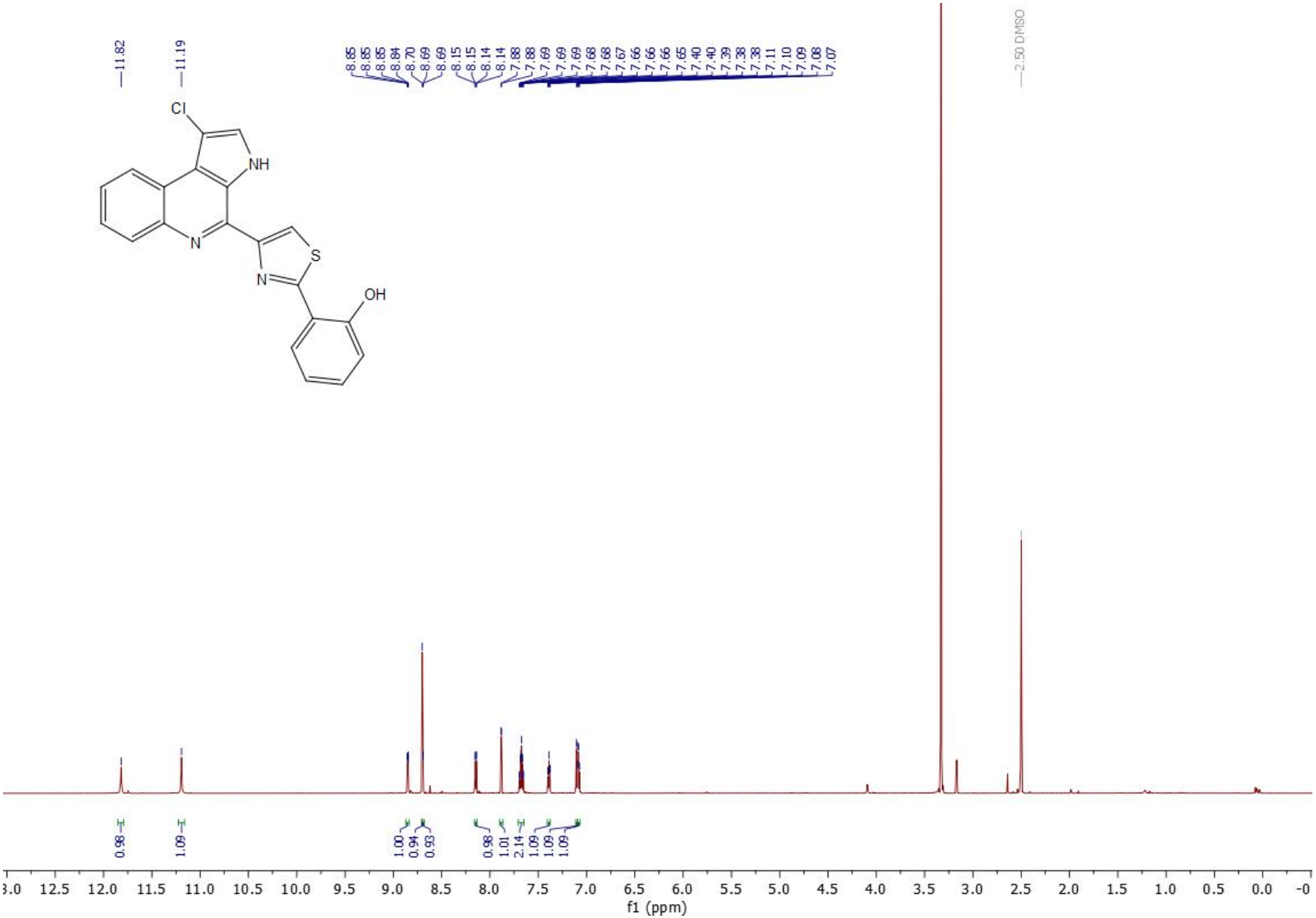


Figure S19: ^{13}C NMR spectrum of **pyonitrin B** (200 MHz, DMSO-d_6)

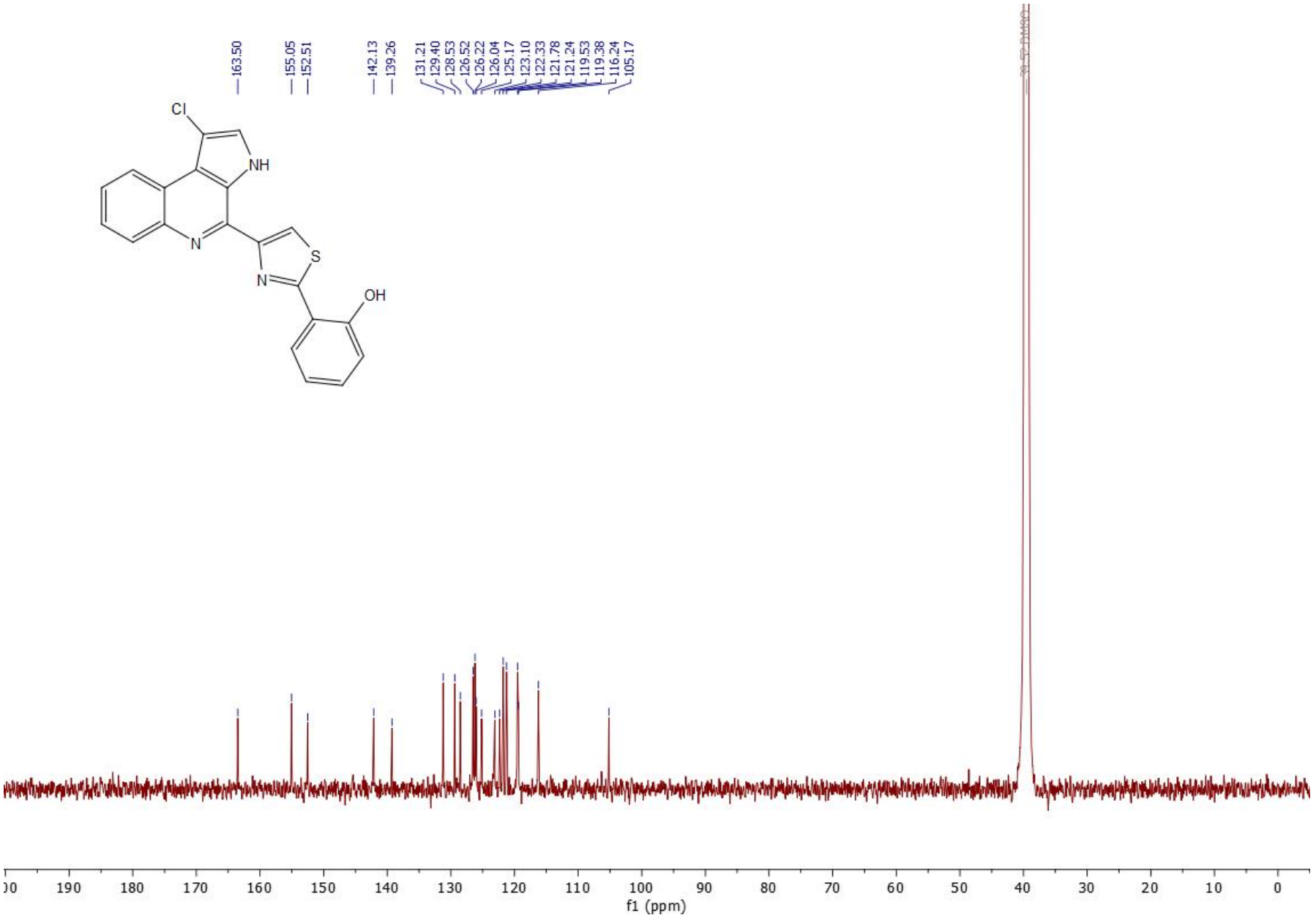


Figure S20: ^{1}H NMR spectrum of **20** (500 MHz, $CDCl_3$)

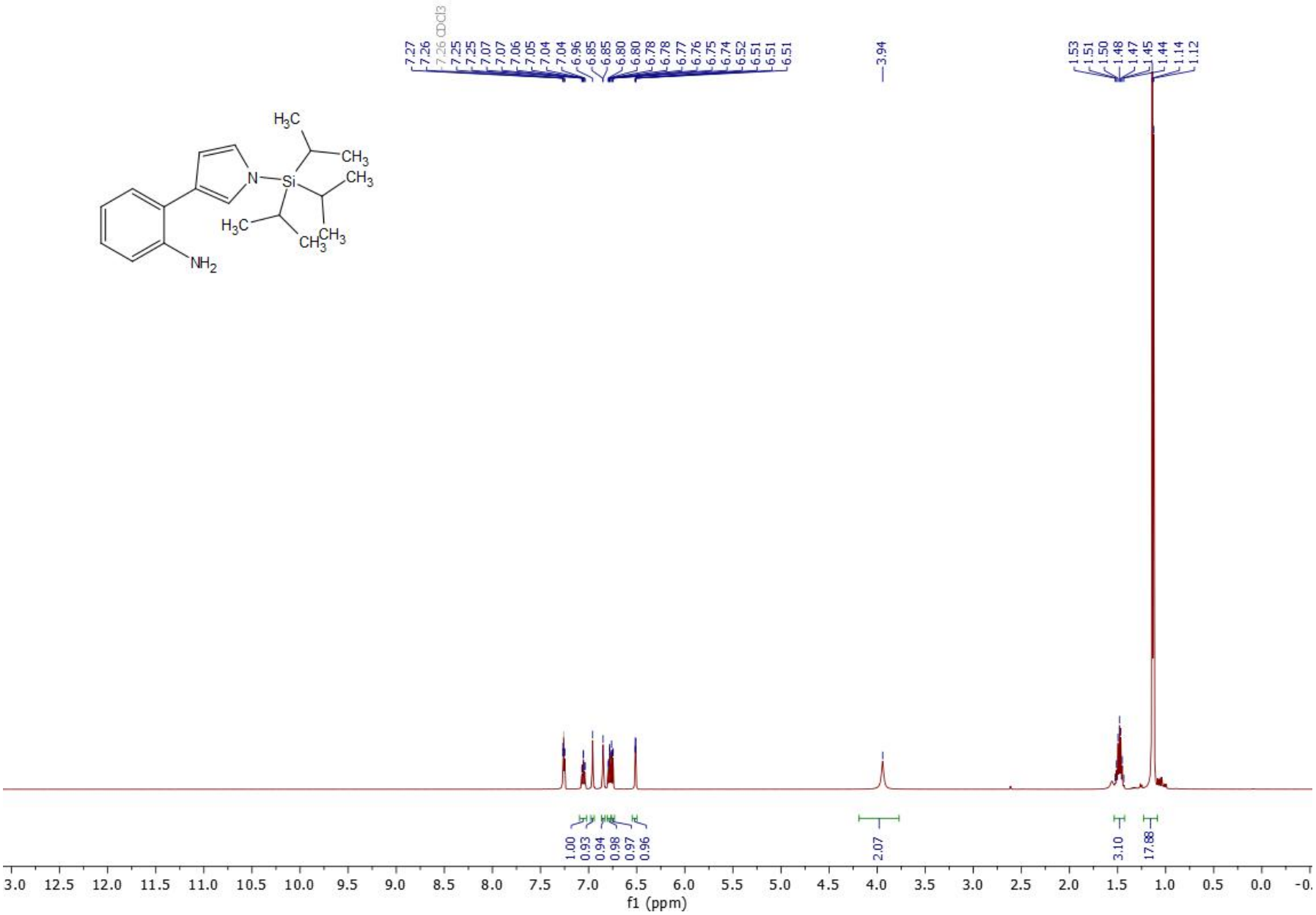


Figure S21: ^{13}C NMR spectrum of **20** (125 MHz, $CDCl_3$)

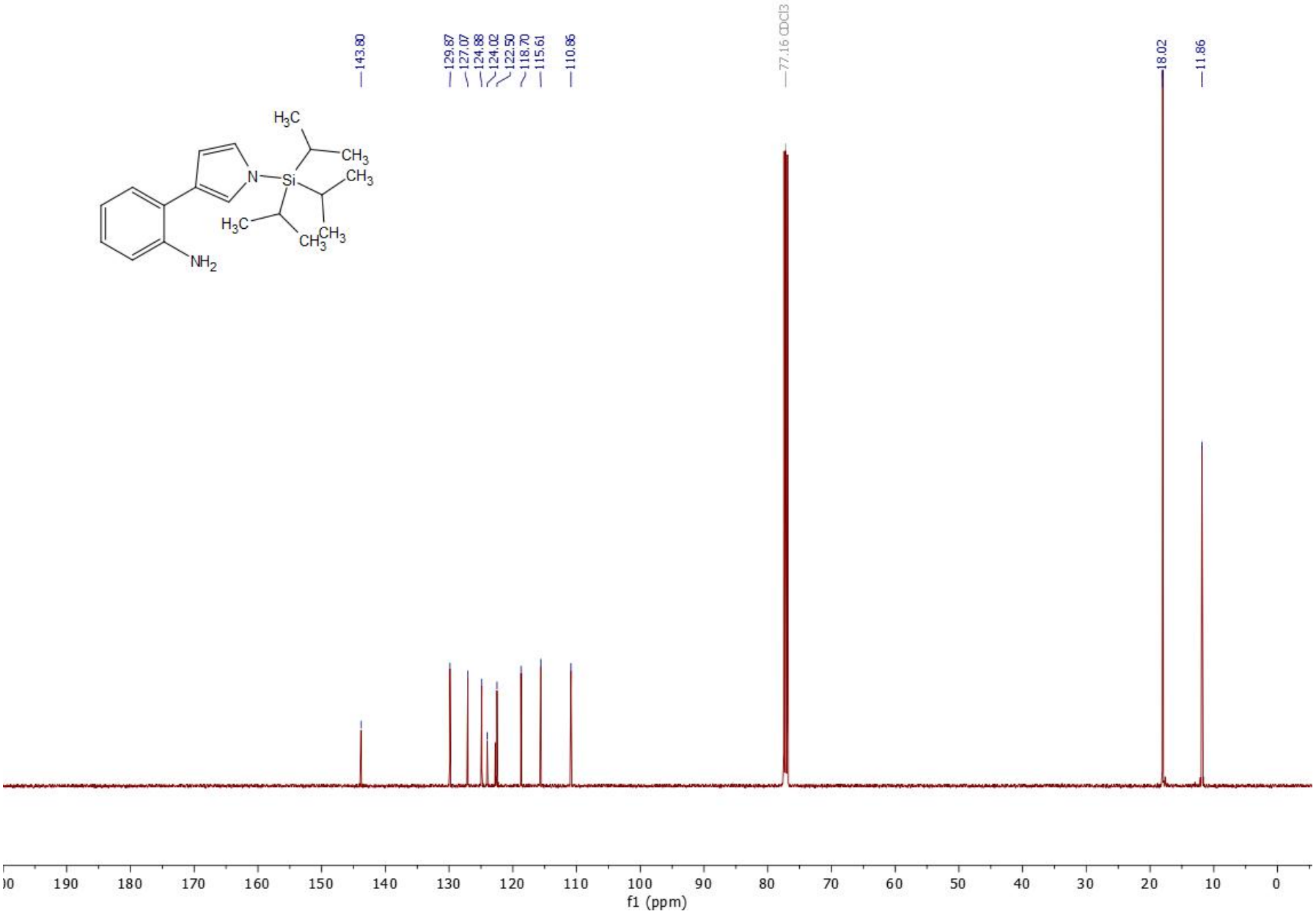


Figure S22: ^{1}H NMR spectrum of **24** (500 MHz, $CDCl_3$)

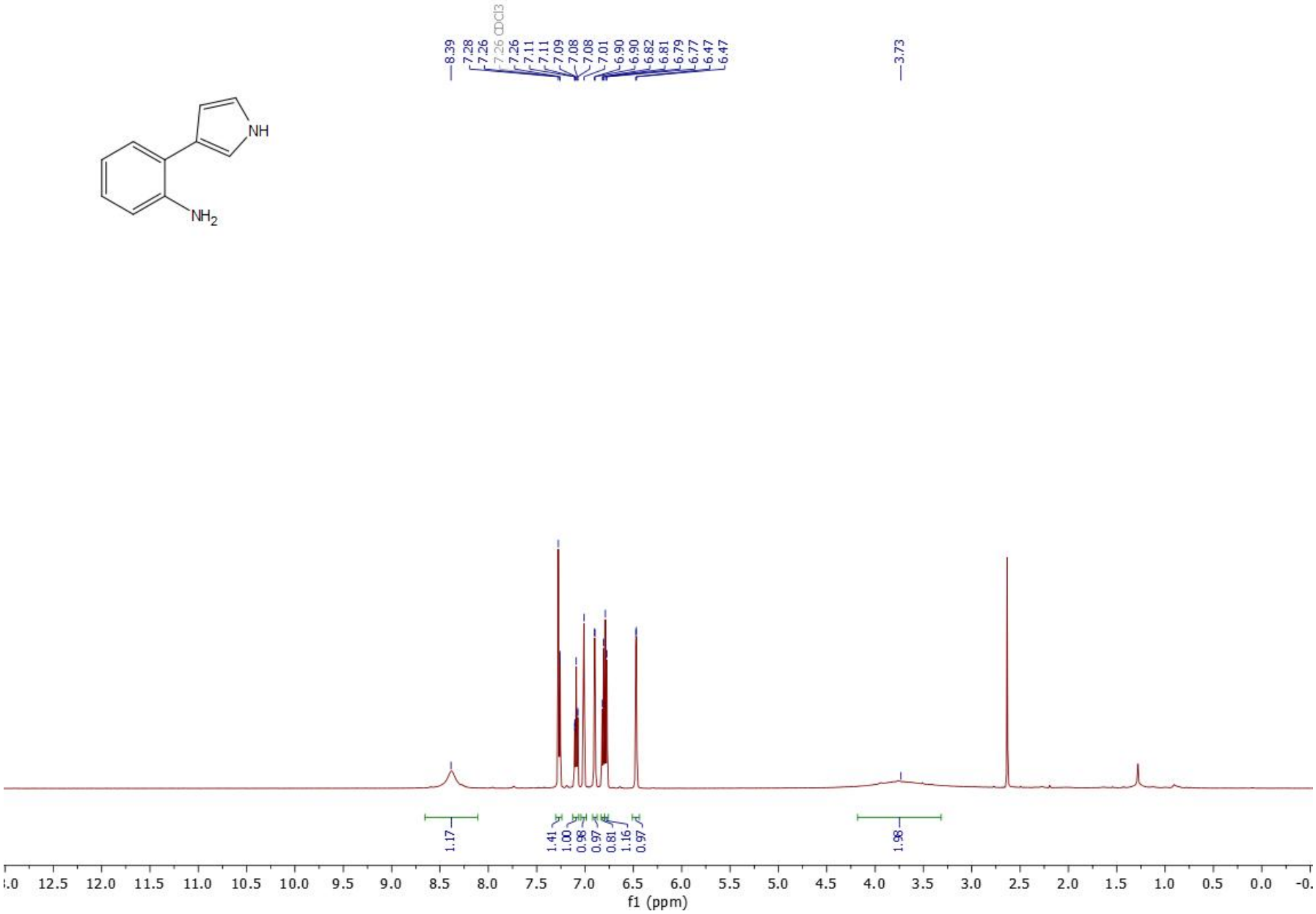


Figure S23: ^{13}C NMR spectrum of **24** (125 MHz, $CDCl_3$)

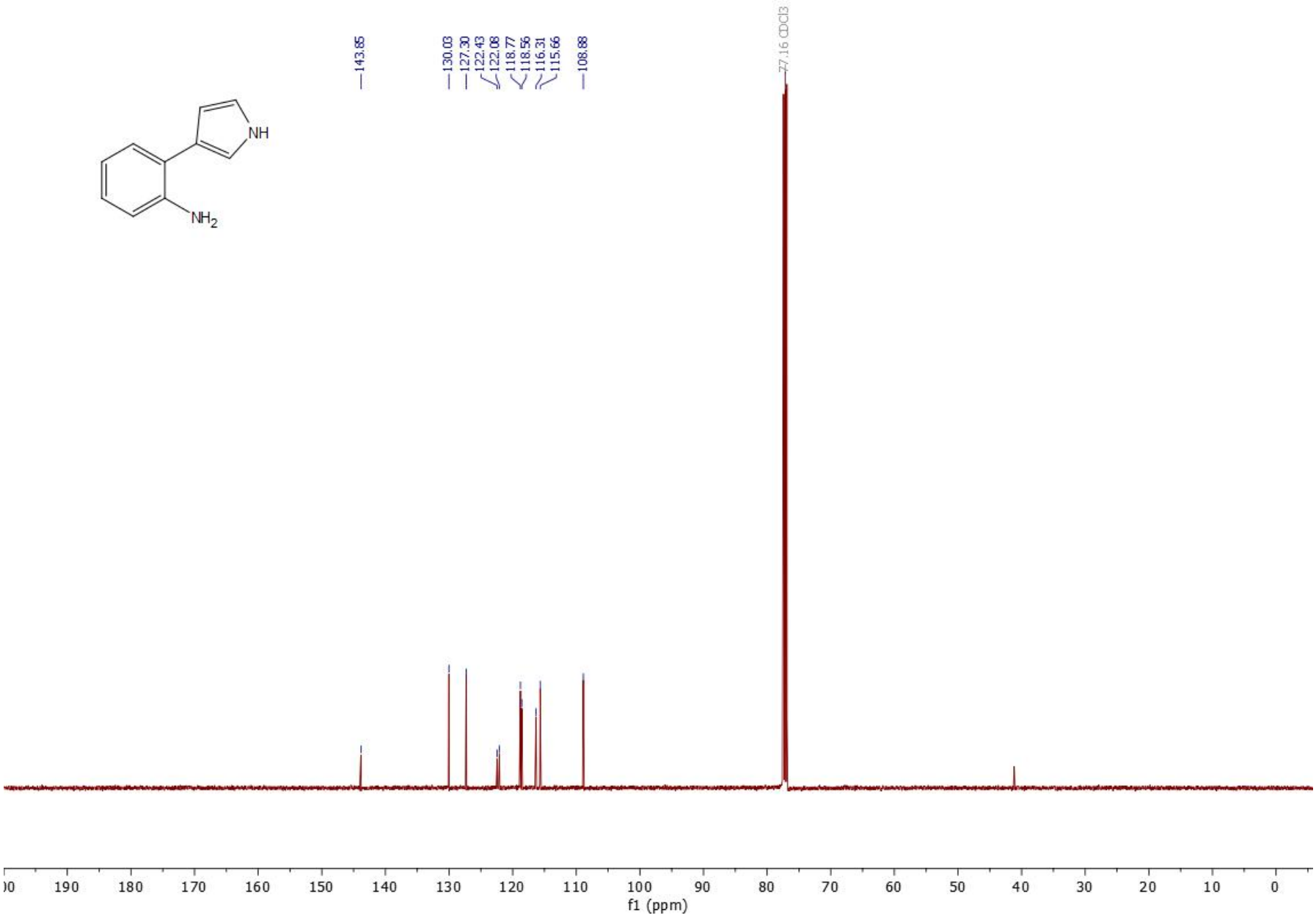


Figure S24: ^{1}H NMR spectrum of **pyonitrin C** (800 MHz, DMDO-d_6)

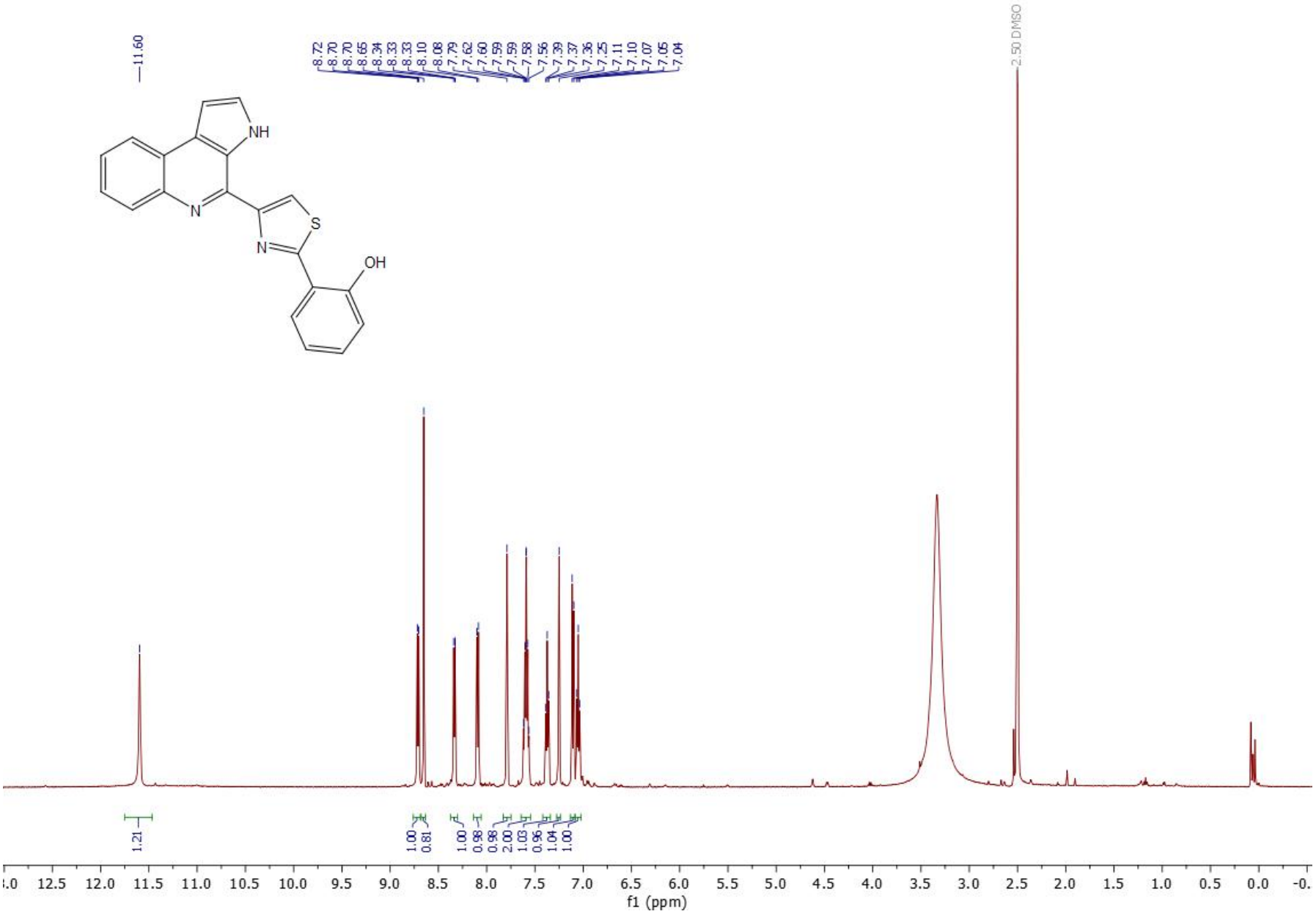


Figure S25: ^{13}C NMR spectrum of **pyonitrin C** (200 MHz, DMDO-d_6)

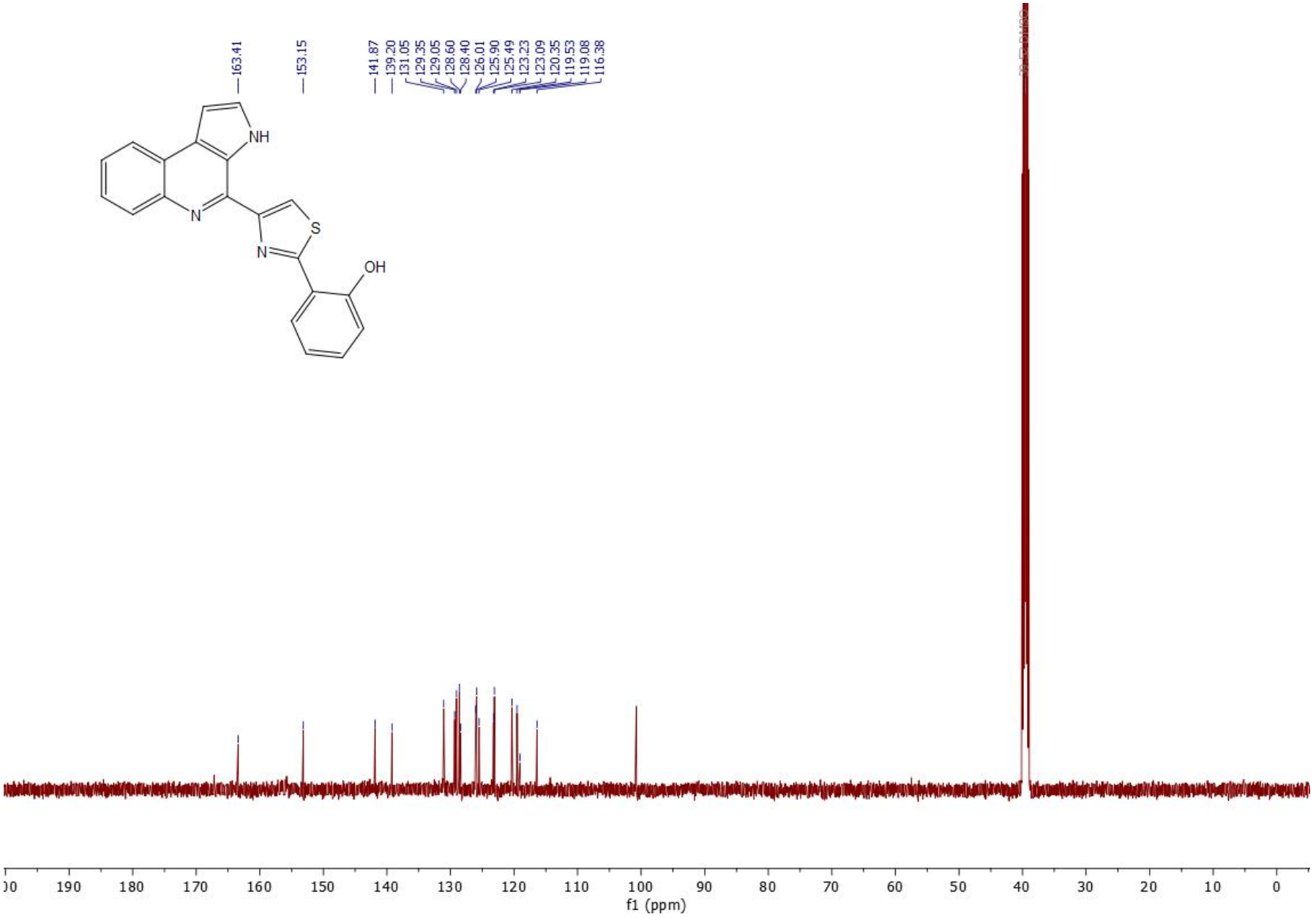


Figure S26: ^{1}H NMR spectrum of **21** (500 MHz, $CDCl_3$)

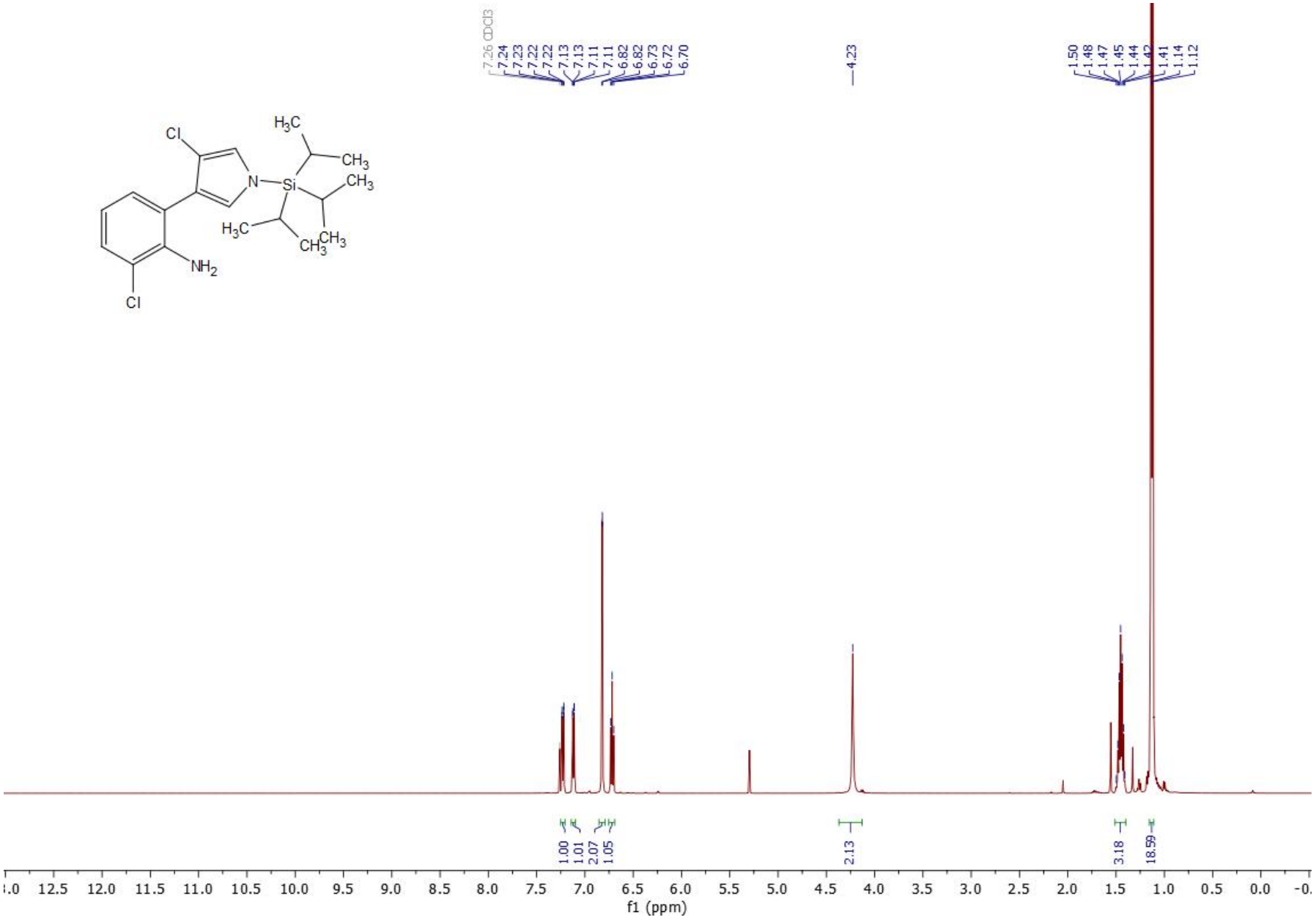


Figure S27: ^{13}C NMR spectrum of **21** (125 MHz, $CDCl_3$)

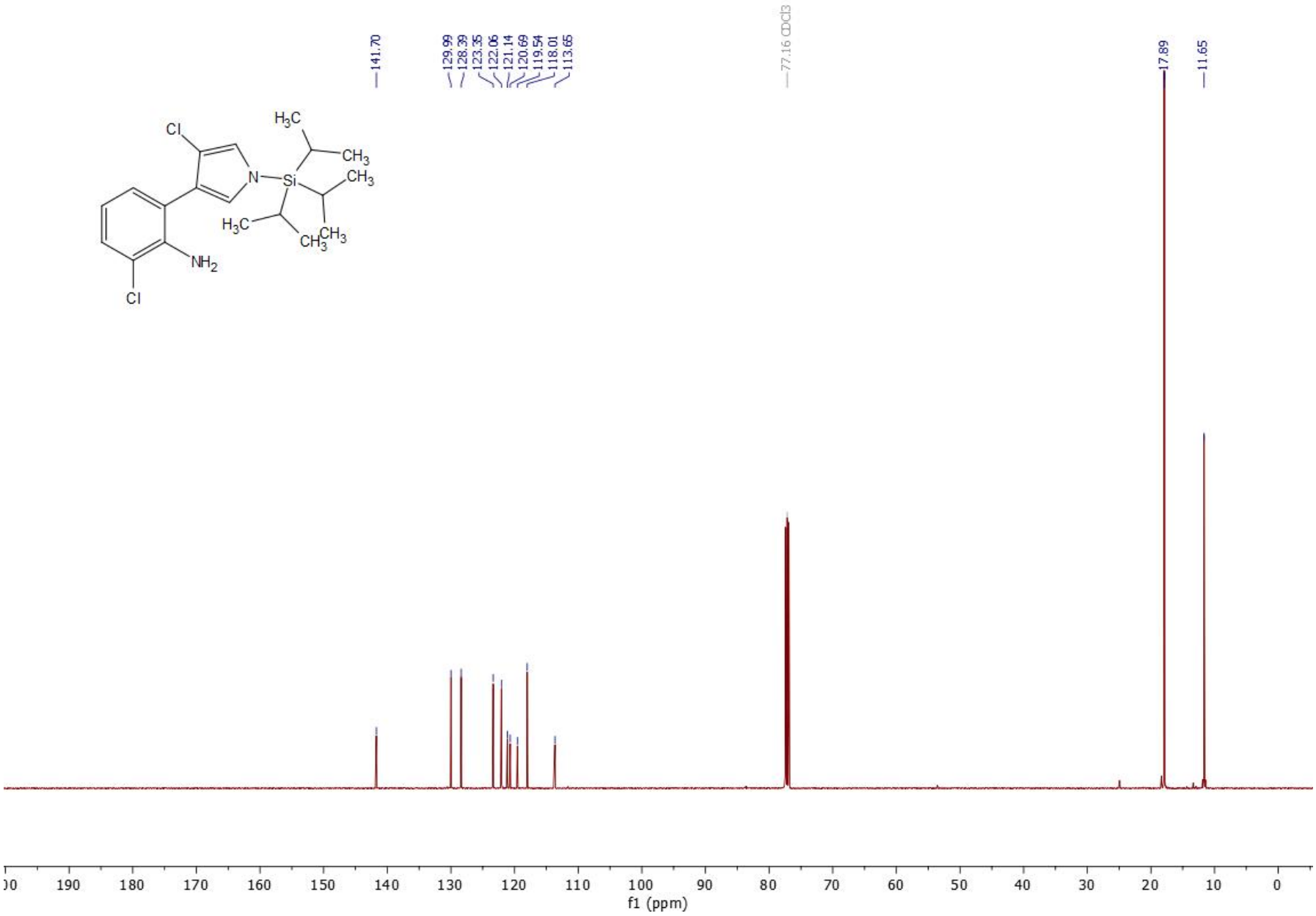


Figure S28: ^{1}H NMR spectrum of **25** (500 MHz, $CDCl_3$)

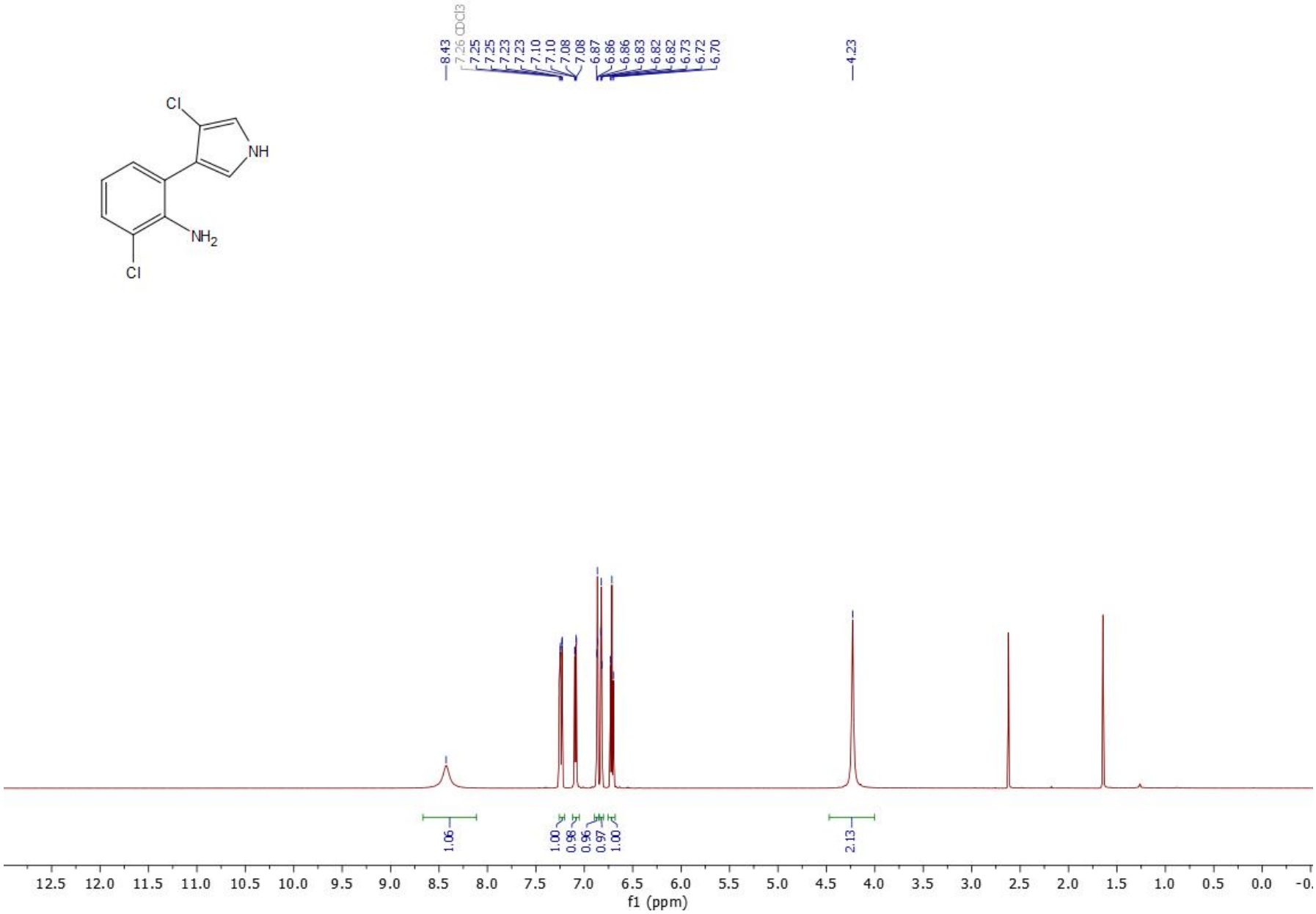


Figure S29: ^{13}C NMR spectrum of **25** (125 MHz, $CDCl_3$)

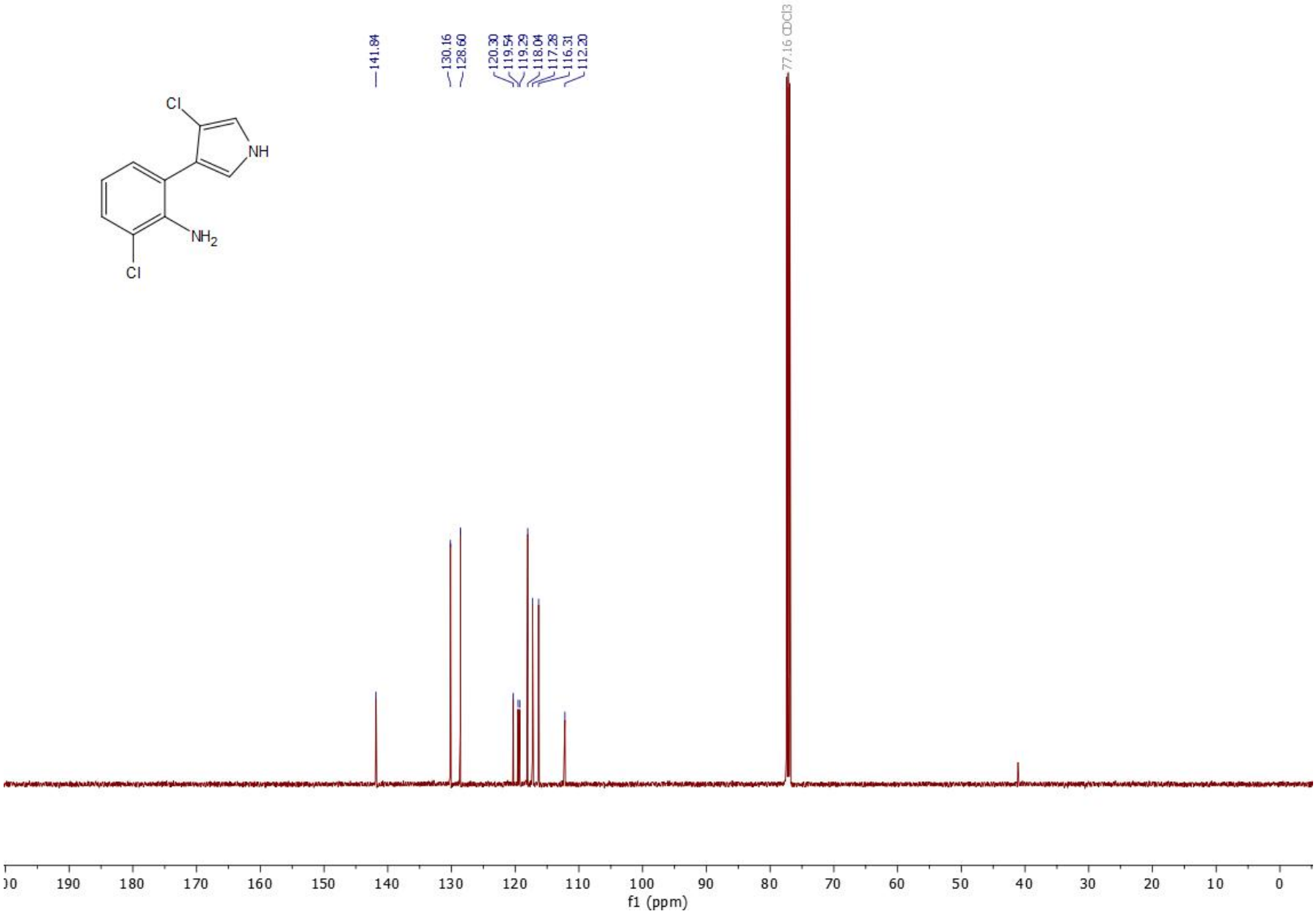


Figure S30: ^{1}H NMR spectrum of **pyonitrin D** (500 MHz, DMSO-d_6)

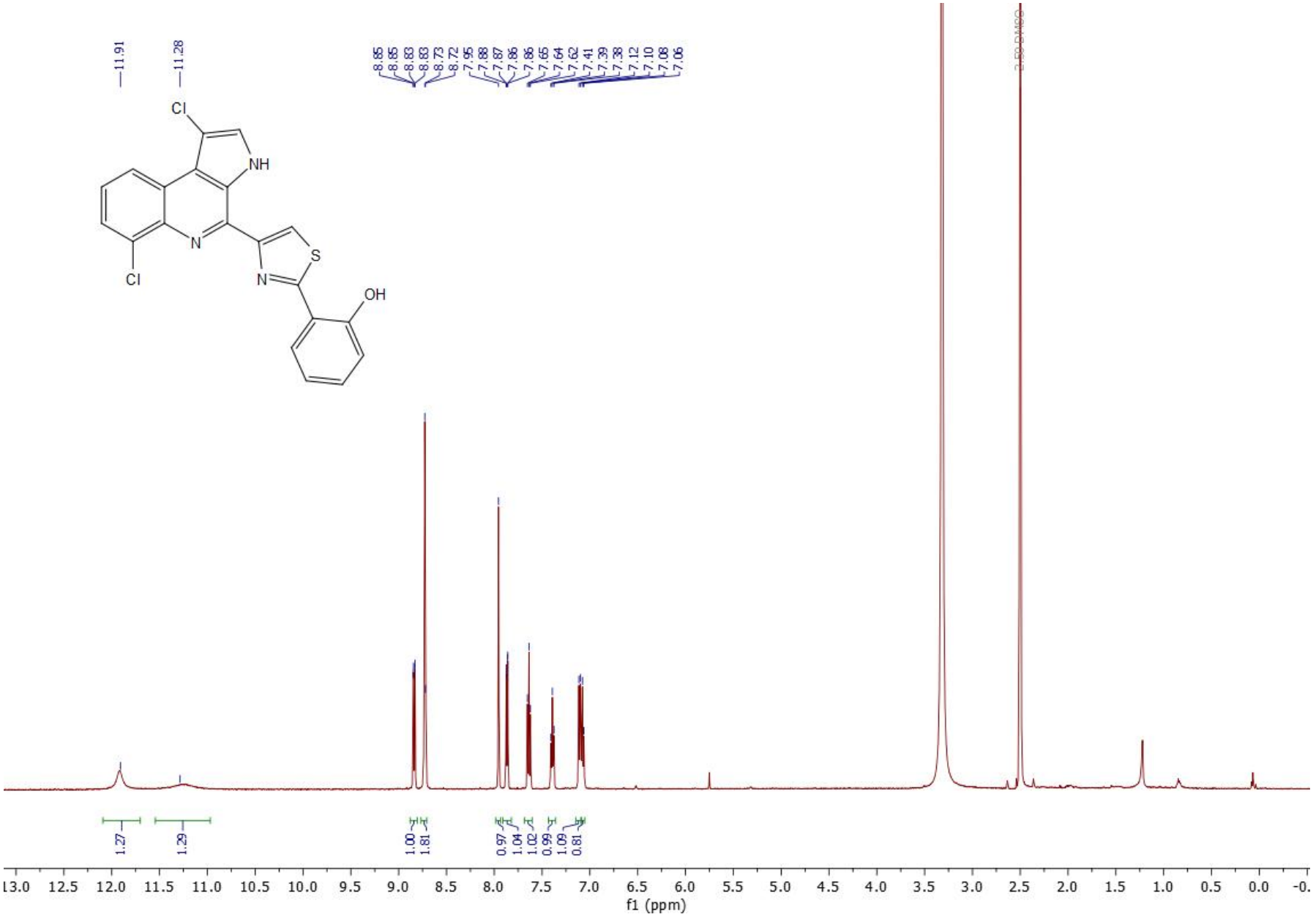


Figure S31: ^{13}C NMR spectrum of **pyonitrin D** (200 MHz, DMSO-d_6)

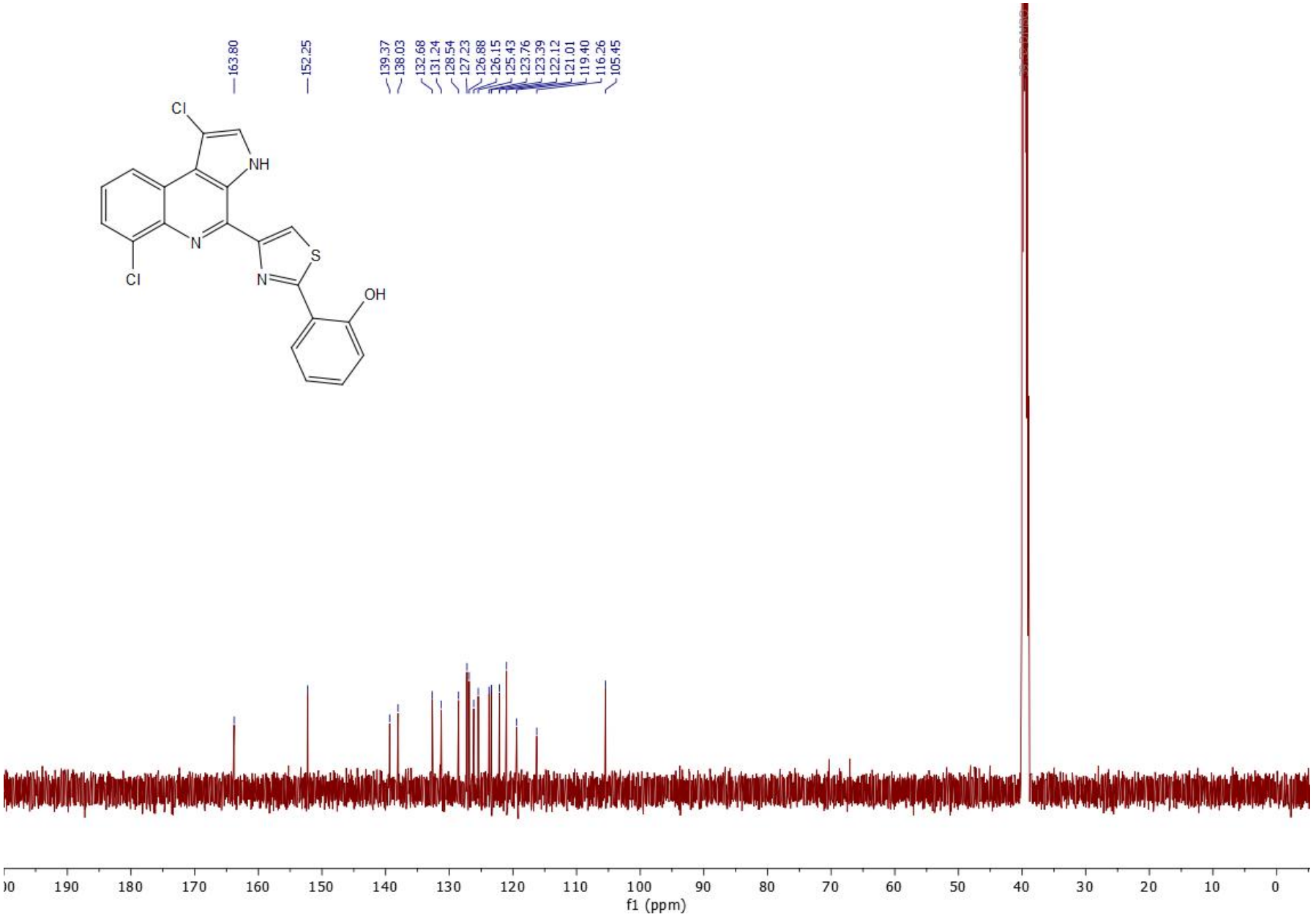


Figure S32: ^{1}H NMR spectrum of **26a** (500 MHz, $CDCl_3$)

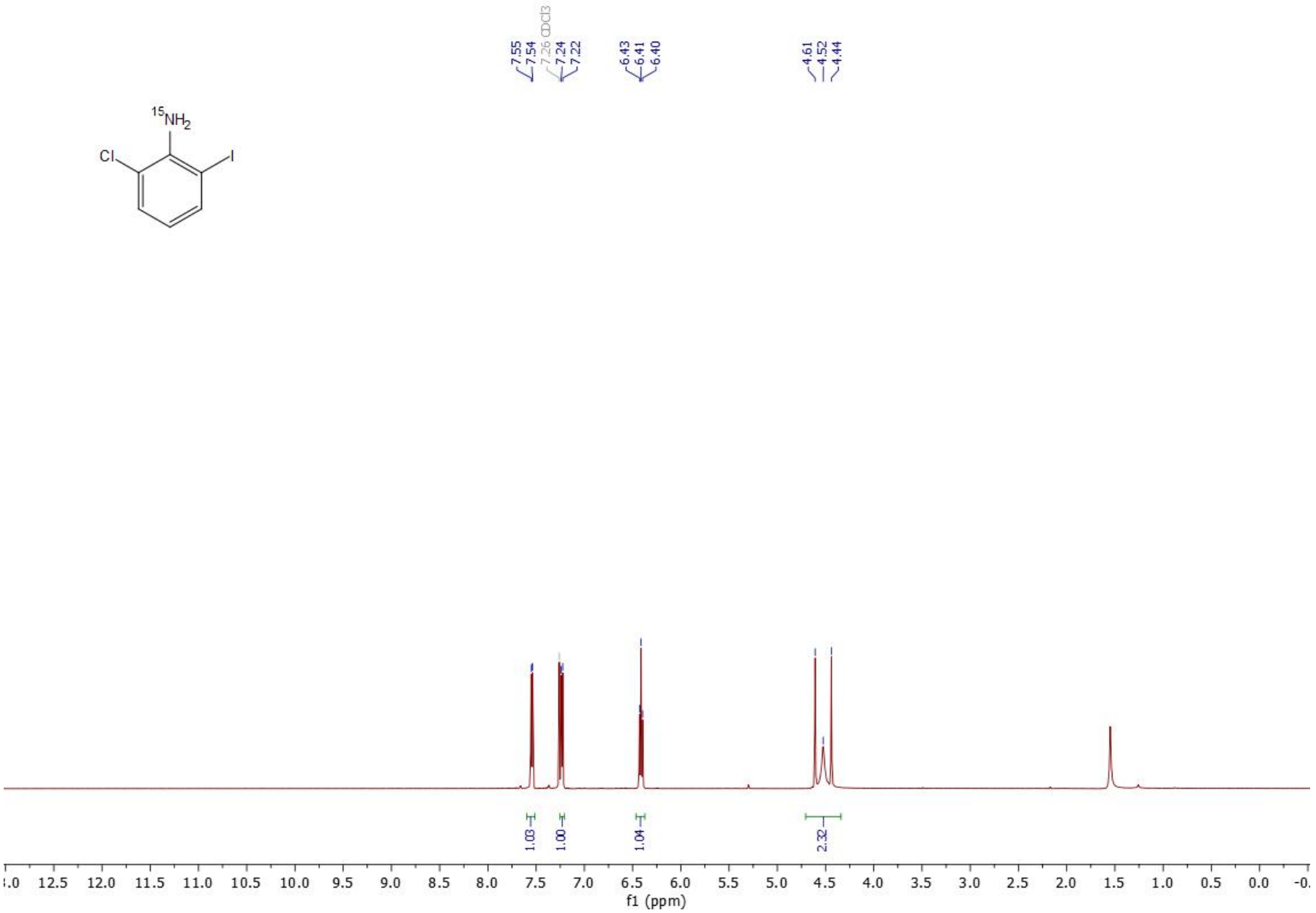


Figure S33: ^{13}C NMR spectrum of **26a** (125 MHz, $CDCl_3$)

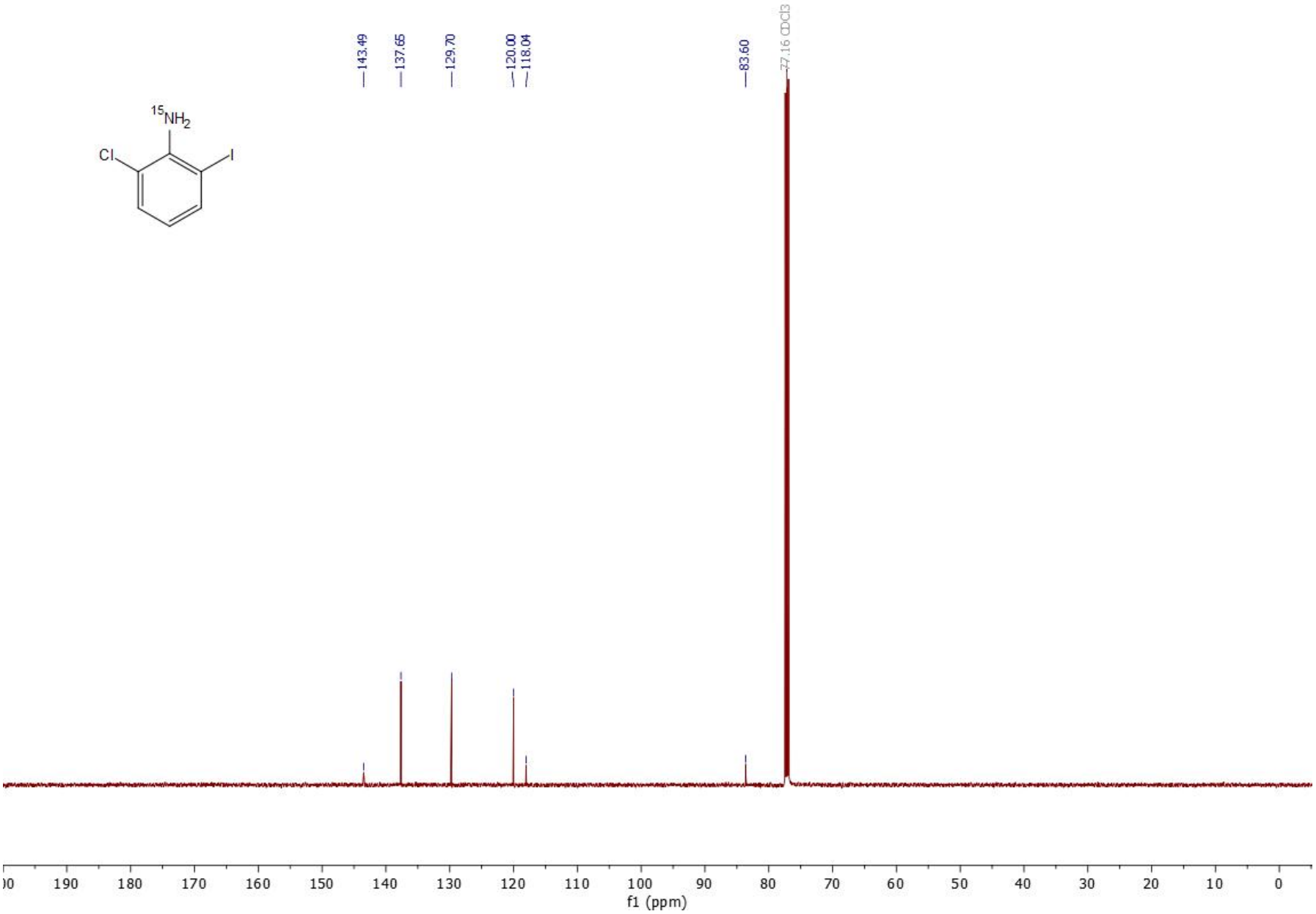


Figure S34: ^{1}H NMR spectrum of **26b** (500 MHz, $CDCl_3$)

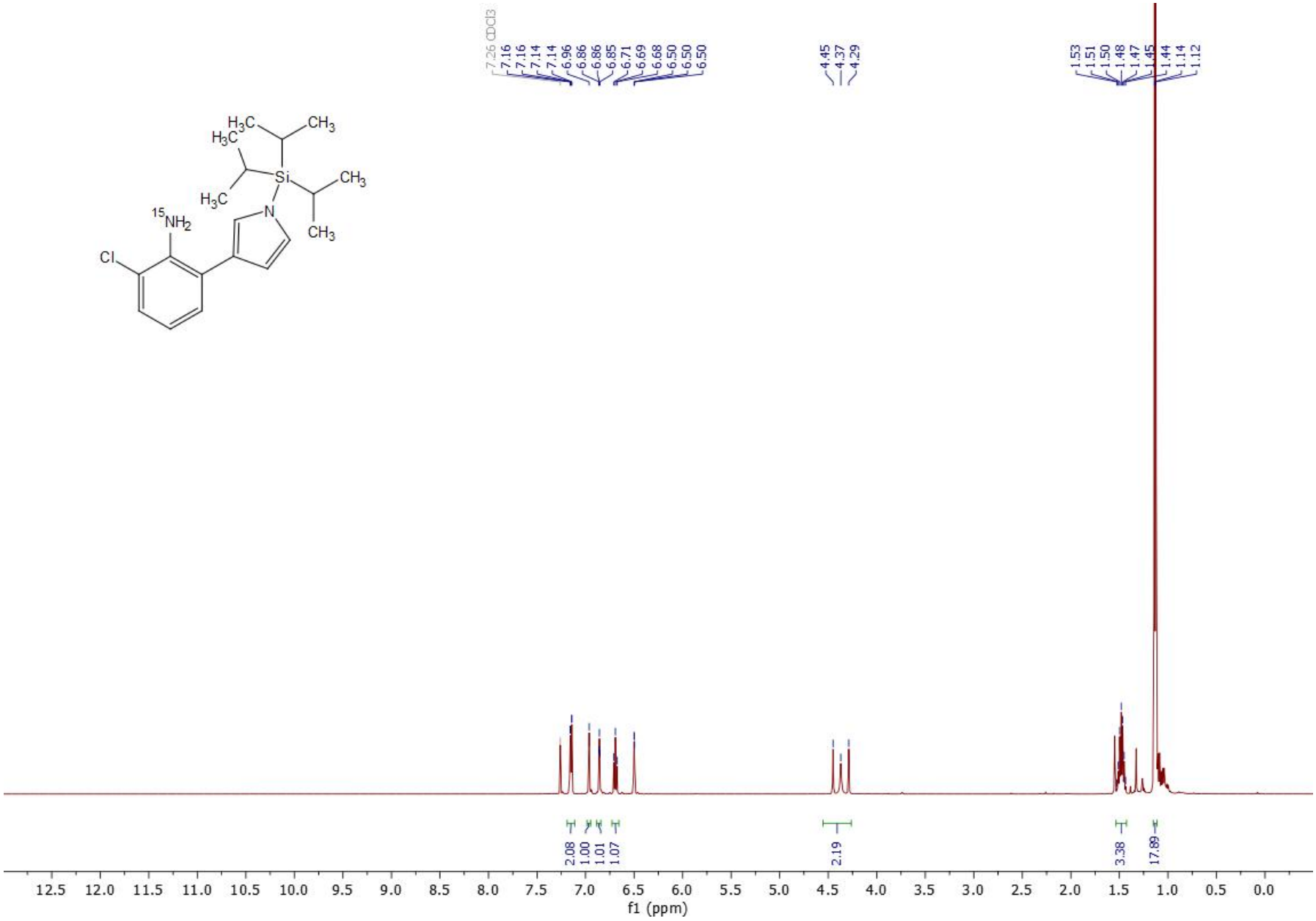


Figure S35: ^{13}C NMR spectrum of **26b** (125 MHz, $CDCl_3$)

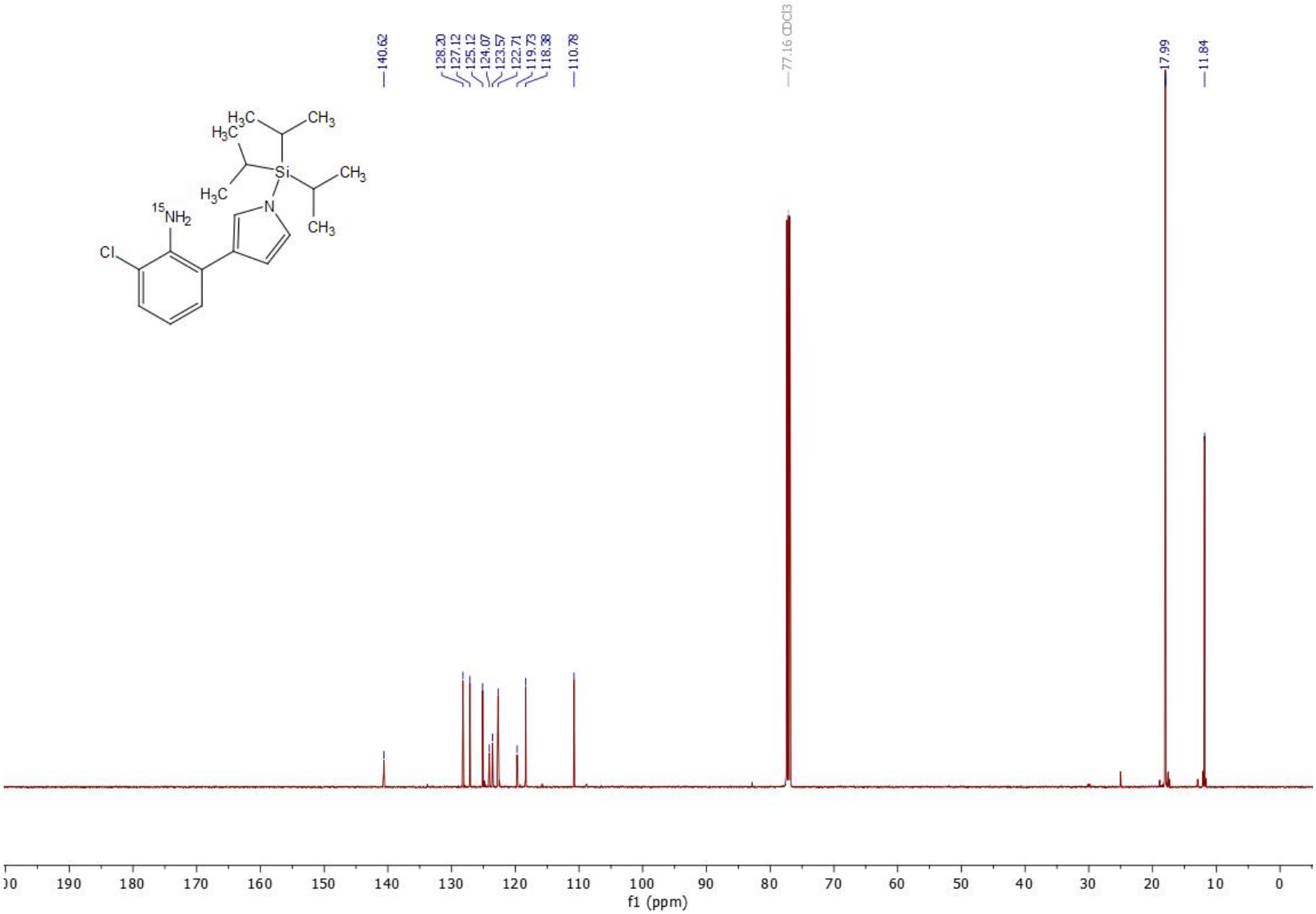


Figure S36: ^{1}H NMR spectrum of **26** (800 MHz, DMSO-d_6)

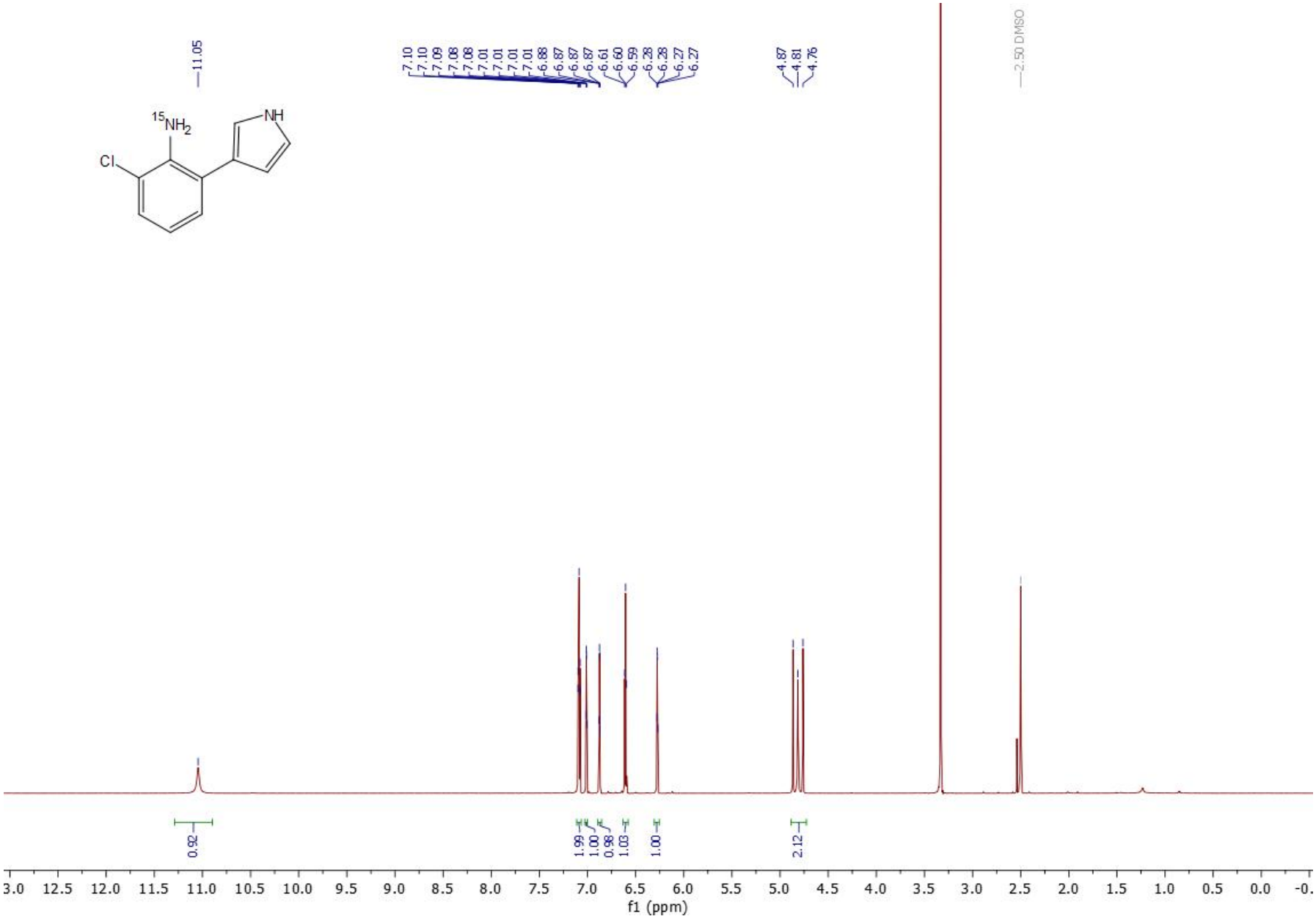


Figure S37: ^{13}C NMR spectrum of **26** (200 MHz, DMSO-d_6)

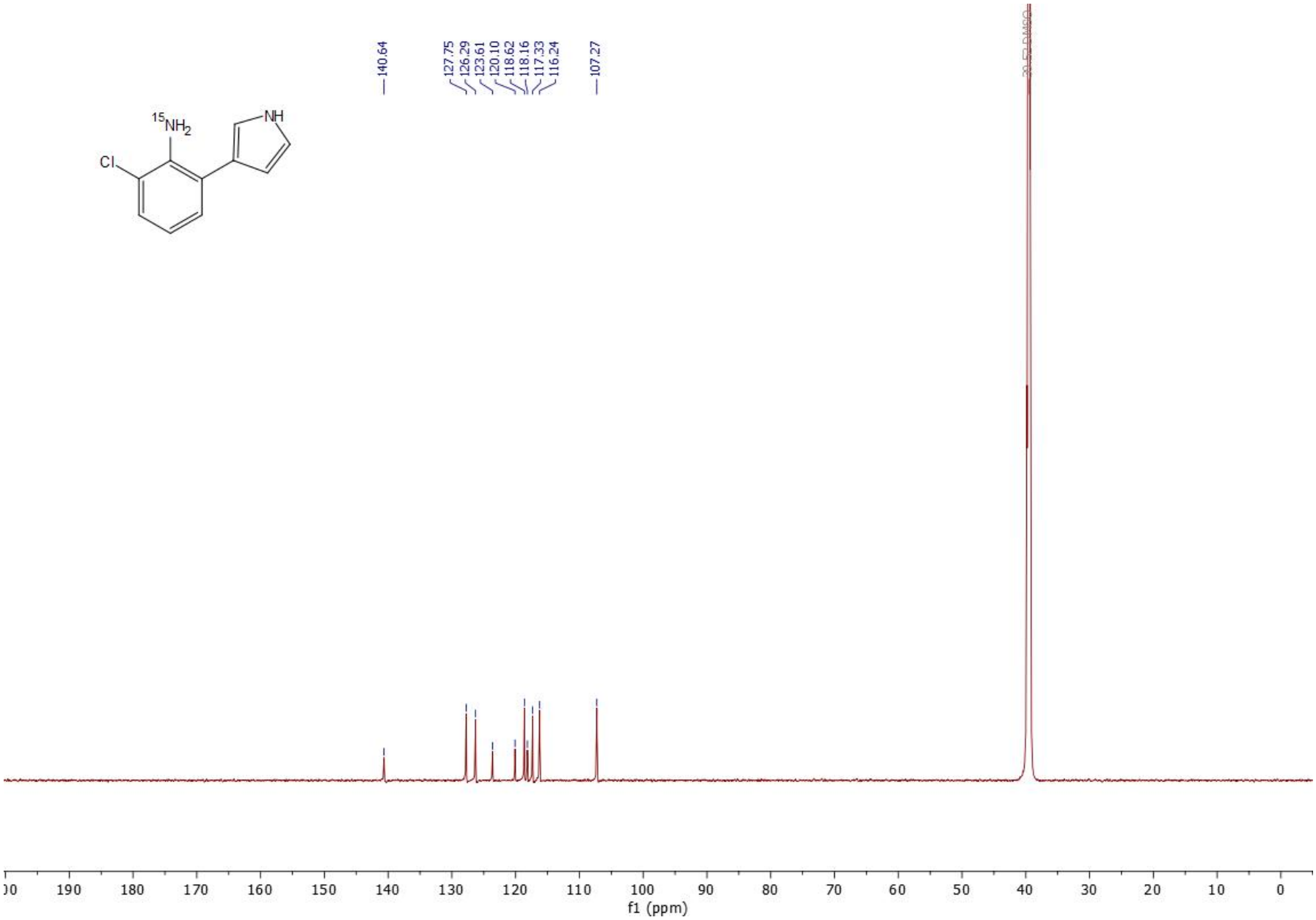


Figure S38: ^{1}H NMR spectrum of ^{15}N pyonitrin A **29** (500 MHz, DMSO-d_6)

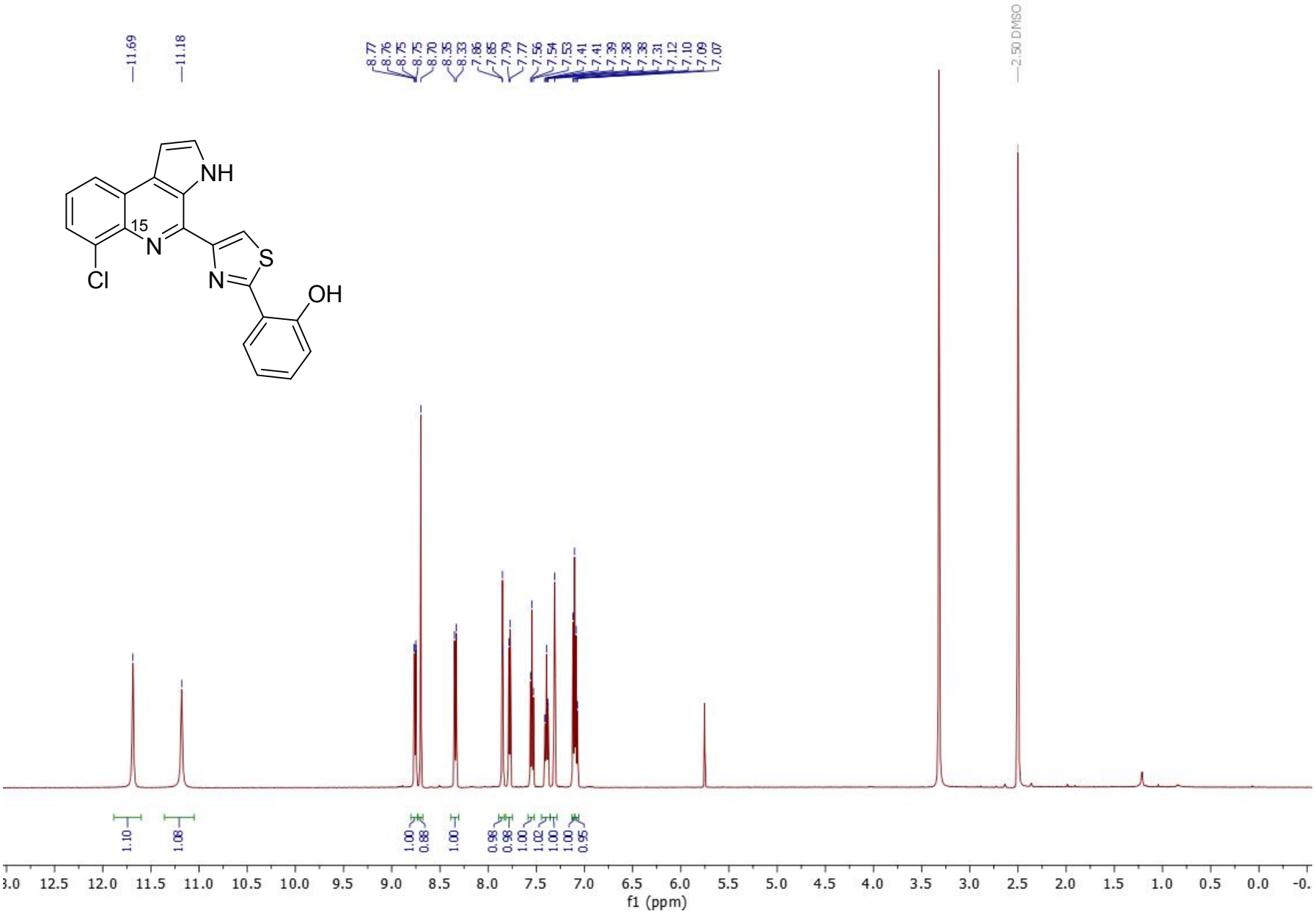


Figure S39: ^{13}C NMR spectrum of ^{15}N pyonitrin A **29** (125 MHz, DMSO-d_6)

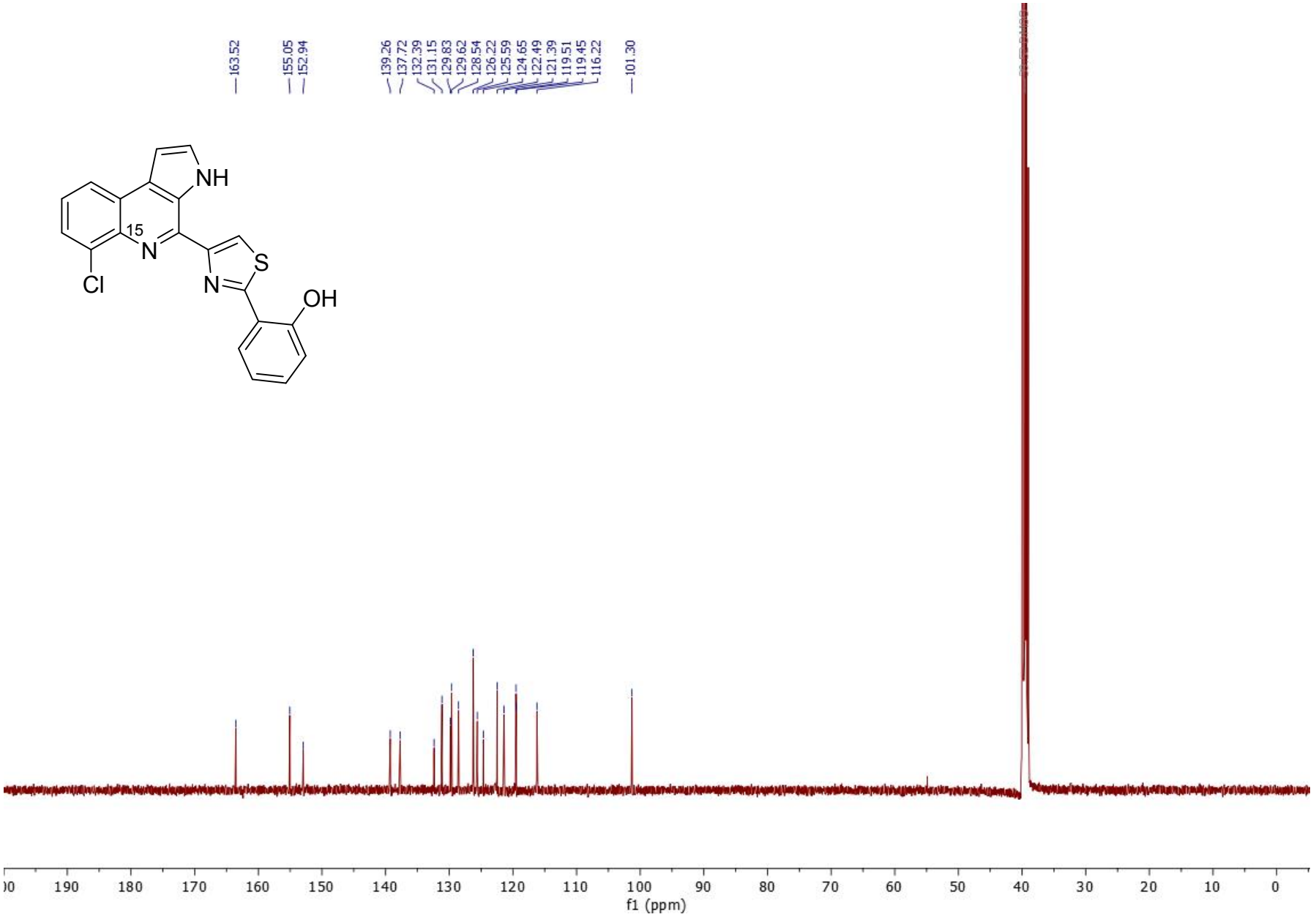


Figure S40: ^{1}H NMR spectrum of **28** (500 MHz, DMSO-d_6)

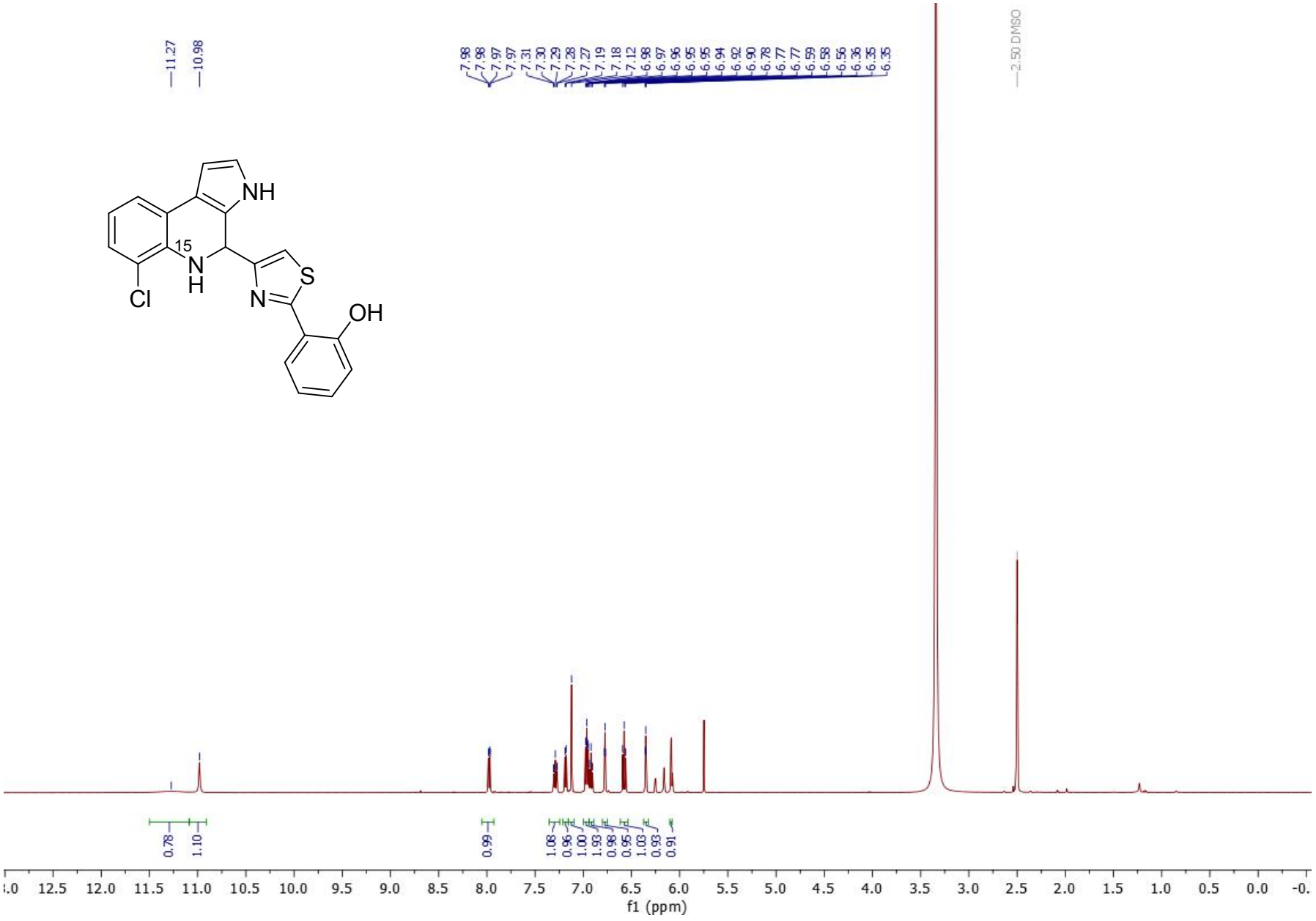


Figure S41: ^{13}C NMR spectrum of **28** (125 MHz, DMSO-d_6)

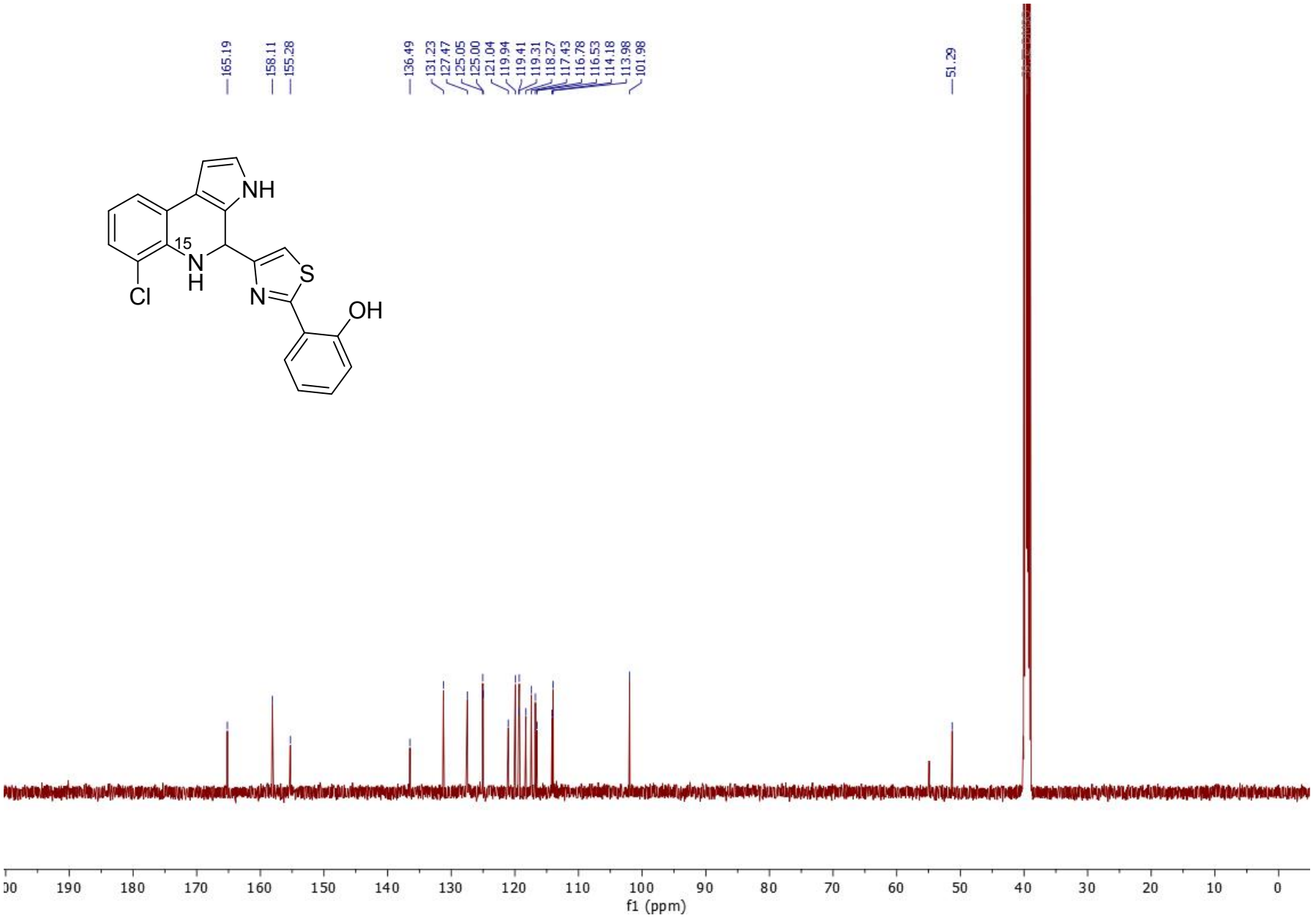


Figure S42: ^{1}H-^{15}N HMBC NMR spectrum of **26** (500 MHz, DMSO-d_6)

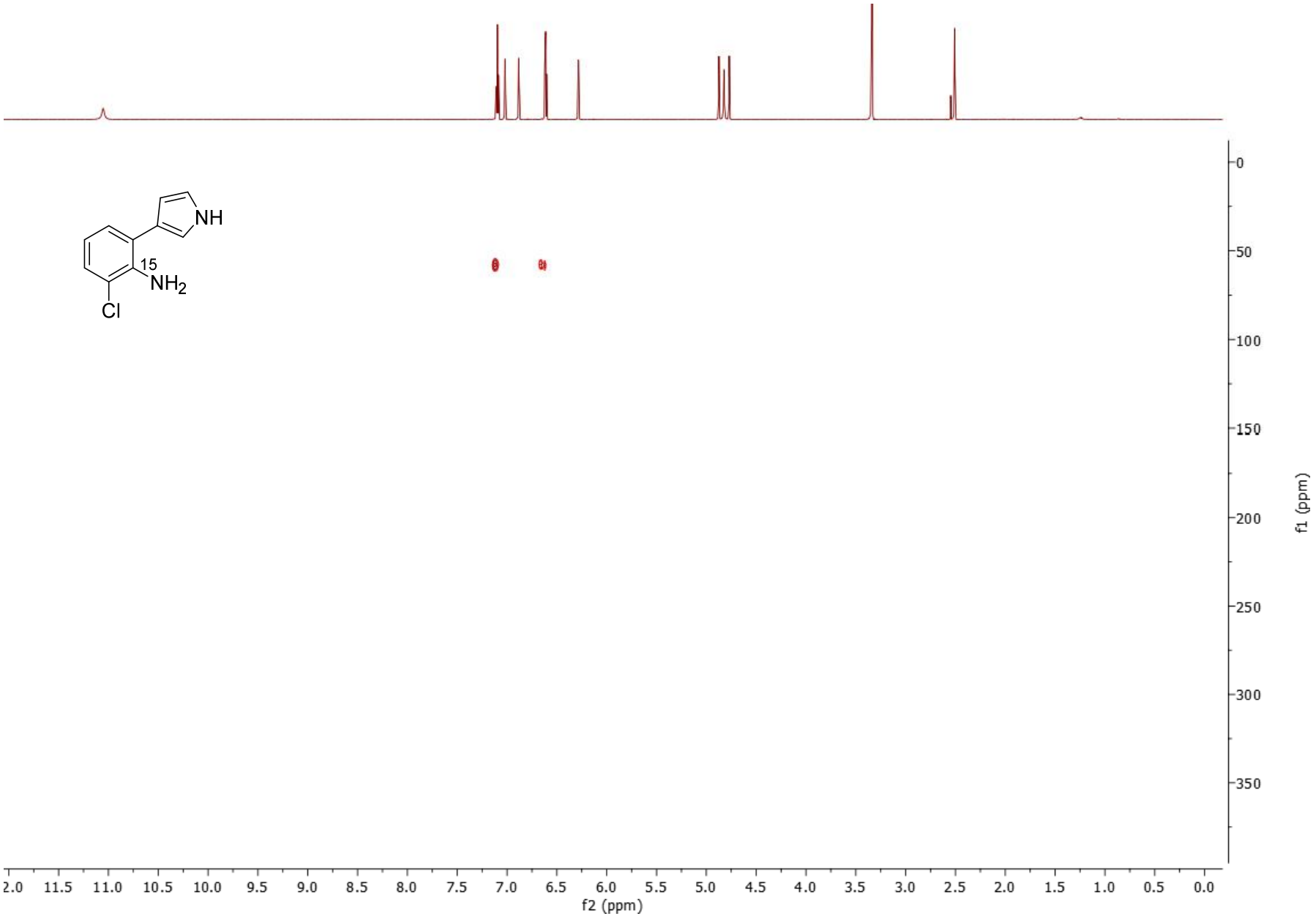


Figure S43: ^{1}H-^{15}N HMBC NMR spectrum of **28** (500 MHz, DMSO-d_6)

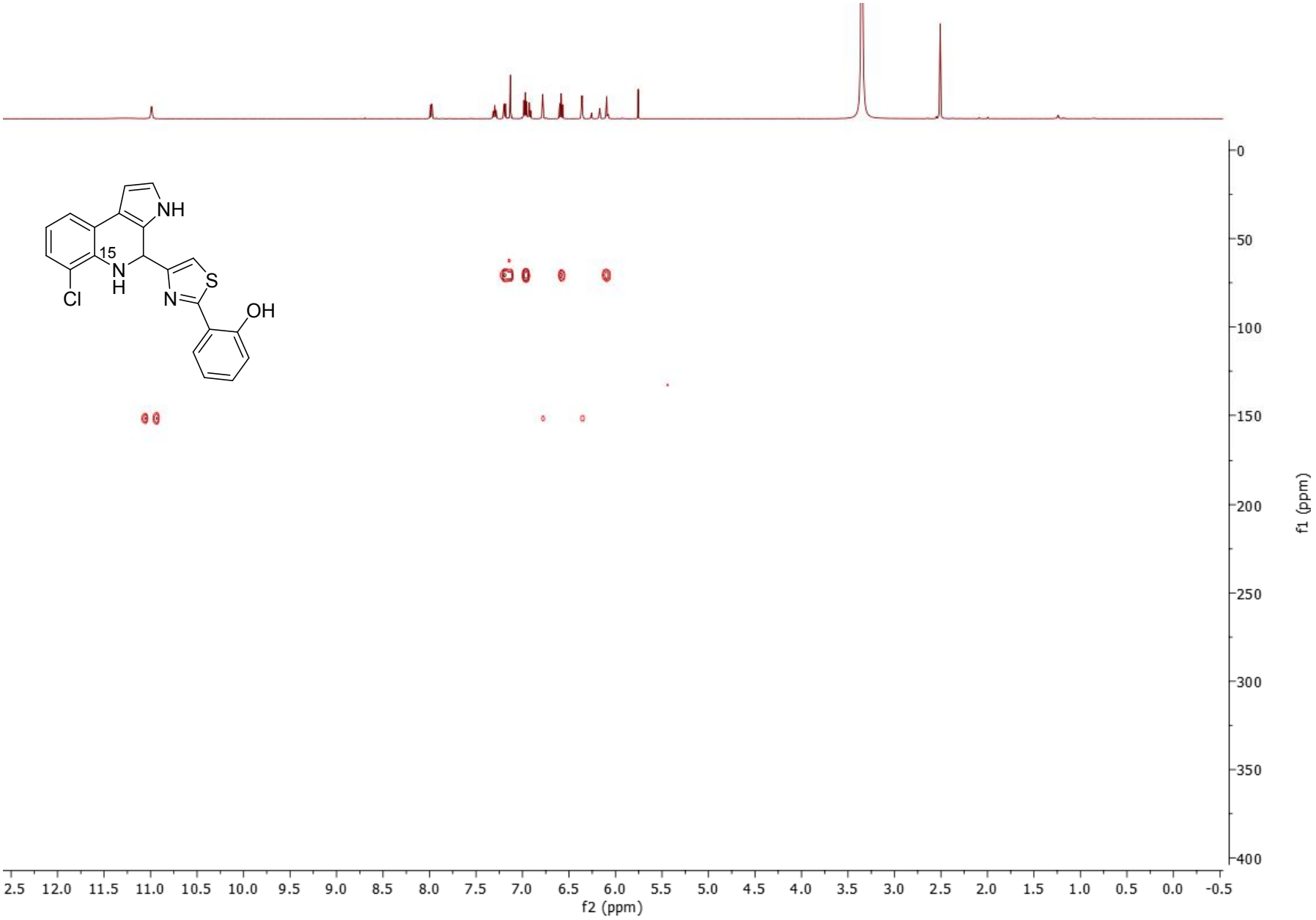


Figure S44: ^{1}H-^{15}N HMBC NMR spectrum of **29** (500 MHz, DMSO-d_6)

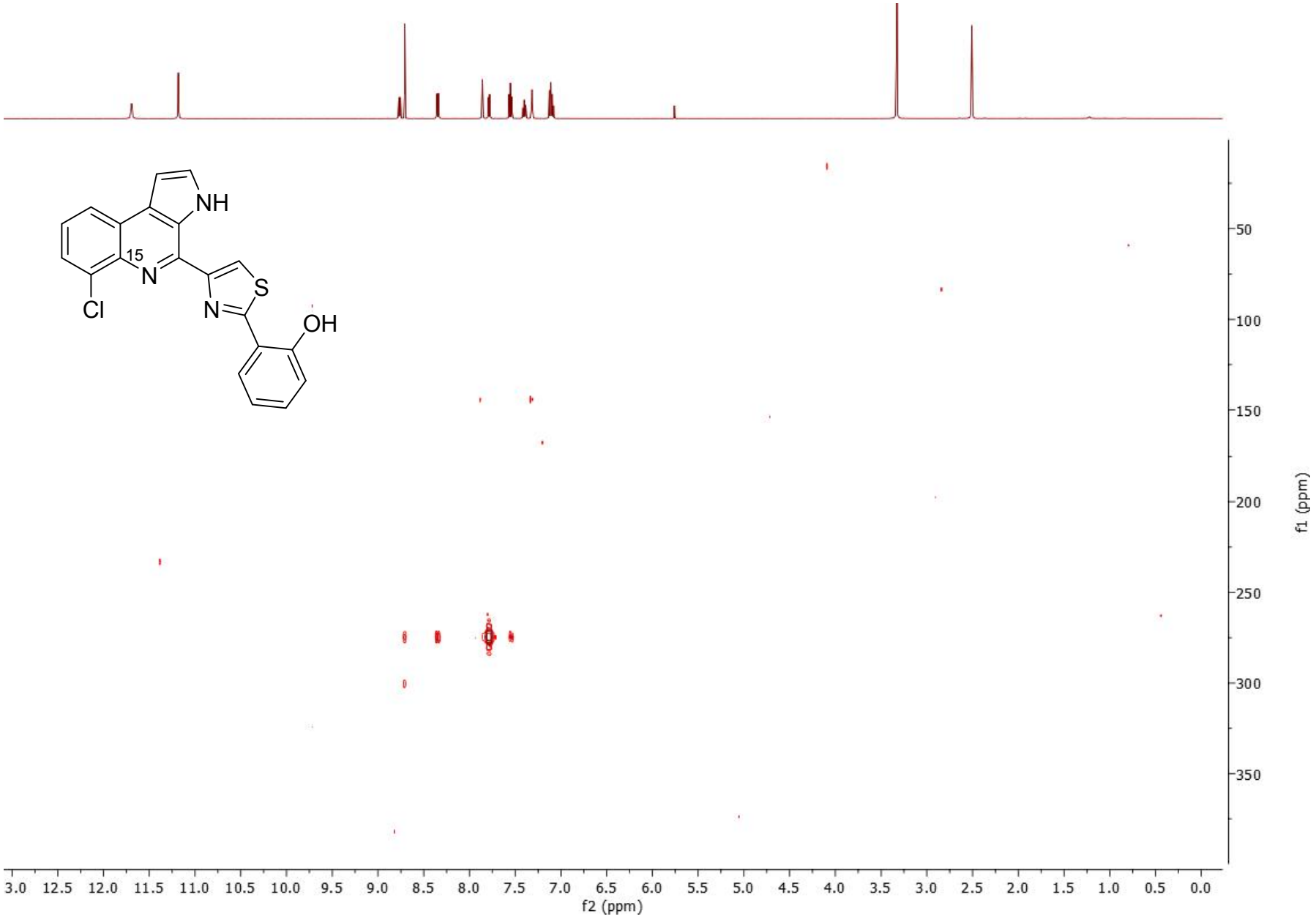
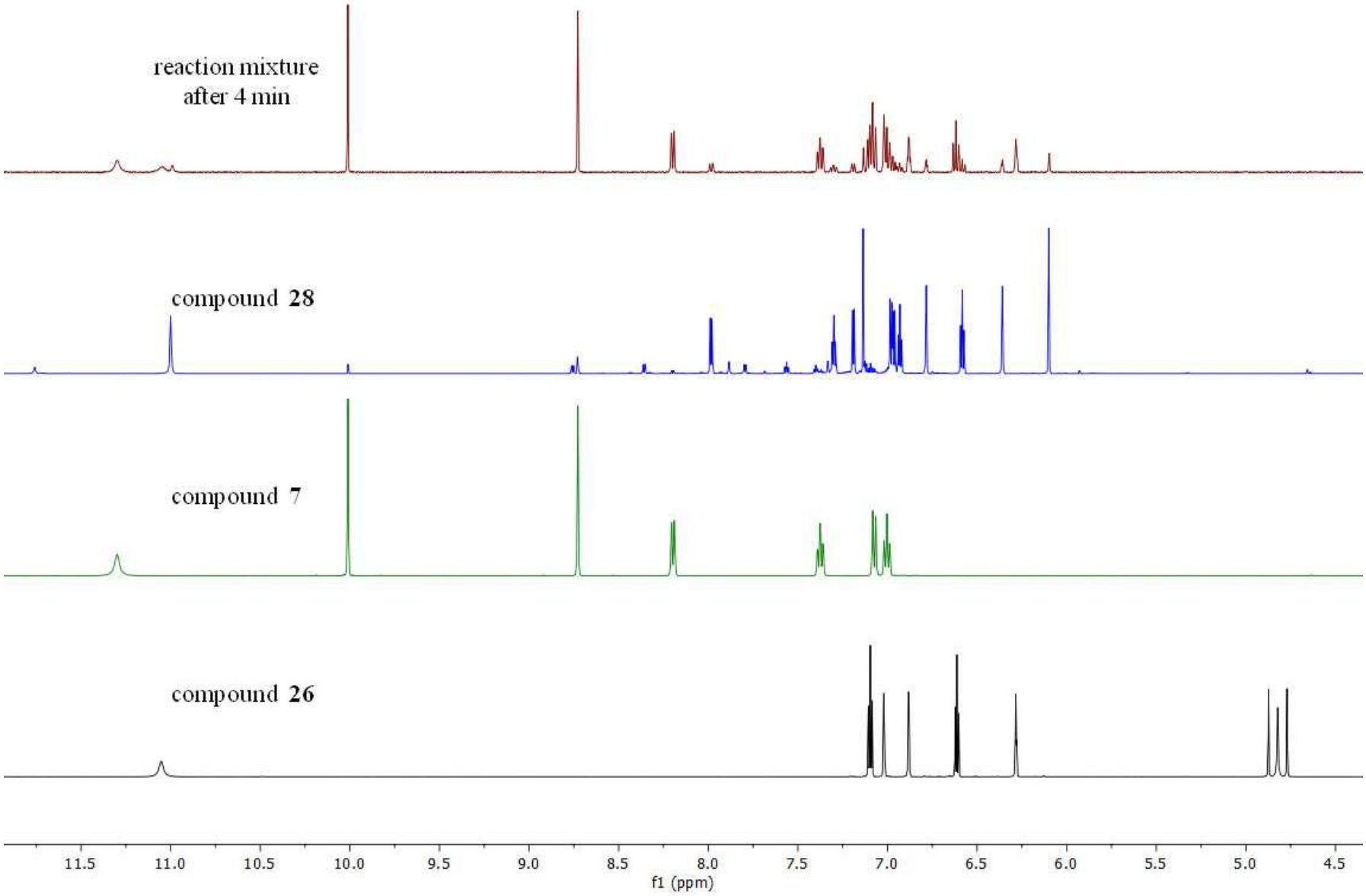


Figure S45: Overlay of the ^{1}H NMR of **26, 7, 28** and reaction mixture



1H NMR