

SUPPLEMENTARY INFORMATION

Structure revision and chemical synthesis of ligandrol's main bishydroxylated long-term metabolic marker

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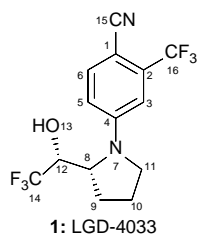
I. Experimental Section

Ia. Chemical Synthesis

General methods

All reactions were carried out under a dry argon atmosphere with anhydrous solvents (freshly distilled over the appropriate desiccant or dried over 3 Å molecular sieves)¹ under anhydrous conditions, unless otherwise noted. All reactions were magnetically stirred with Teflon stir bars, and temperatures were measured externally. Reactions requiring anhydrous conditions were carried out in oven dried (120 °C, 24 h) or flame dried (vacuum < 0.5 Torr) glassware. All reagents were purchased at the highest commercial quality and used without further purification, unless otherwise stated. Yields refer to chromatographically and spectroscopically homogeneous materials, unless otherwise noted. All reactions were monitored by Thin Layer Chromatography (TLC) carried out on 0.25 mm E. Merck silica gel plates (60F₂₅₄). UV light was used for visualization and an acidified ethanolic solution of *p*-anisaldehyde or an acidified aqueous solution of ceric ammonium molybdate and heat were used as developing agents.² E. Merck silica gel (60 Å, particle size 0.040–0.063 mm) or Acros Organics silica gel (60 Å, particle size 0.035–0.070 mm) were used for flash column chromatography.³ Optical rotations were recorded using a Perkin-Elmer 241 polarimeter at the sodium D line (589 nm) using a 10 cm path-length cell in the solvent and concentration indicated. Nuclear Magnetic Resonance (NMR) spectra were recorded using a Bruker Avance DRX 500 MHz or Bruker Avance III 250 MHz instrument and were calibrated using as internal reference the residual nondeuterated solvent for ¹H-NMR and the deuterated solvent for ¹³C-NMR, respectively (*e.g.*, CDCl₃: δ_H = 7.26 ppm, δ_C = 77.16 ppm; CD₃OD: δ_H = 3.31 ppm, δ_C = 49.00 ppm).^{4,5} Multiplicities are designated as singlet (s), doublet (d), triplet (t), quartet (q), quintet (quint.) or multiplet (m). Broad or obscured peaks are indicated as “br” or “obs”, respectively. To facilitate NMR spectra comparisons, the established LGD-4033 skeleton numbering⁷ has been used for all compounds when assigning signals. High resolution mass spectra (HRMS) were acquired on a LC qExactive plus HRMS (Thermo Scientific, Bremen, Germany) instrument.

4-{{(2R)-2-[(1R)-2,2,2-trifluoro-1-hydroxyethyl]pyrrolidin-1-yl}-2-(trifluoromethyl)benzonitrile (1: LGD-4033):

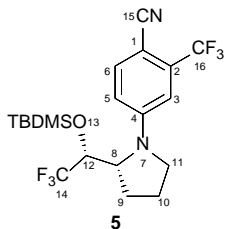


capsules of ligandrol sold over the Internet with the warning that the material is “intended for laboratory and research purposes only” and stated to contain 8 mg of LGD-4033 (>97% purity) / capsule were obtained *via* an online order. The content of ten capsules (3.8 g) was placed in a centrifuge tube, suspended in methanol (10 mL) and sonicated for 15 min. Upon centrifugation, the supernatant was filtered through a short pad of Celite. The solids remaining in the centrifuge tube were subjected four more times to the above procedure and the unified filtrates were evaporated under reduced pressure to dryness. The amorphous white solid thus obtained was dissolved in 10% MeOH in dichloromethane (10 mL), silica gel (0.5 g) was added, and volatiles were removed

under reduced pressure. The residue was loaded on top of a chromatography column (silica gel) and eluted with dichloromethane to provide LGD-4033 (32.6 mg) as amorphous white solid that was used without further purification. R_f = 0.12 (silica gel, CH₂Cl₂); ¹H NMR (500 MHz, CD₃OD): δ = 7.64 (d, J = 8.8 Hz, 1 H, H-6), 7.14 (s, 1 H, H-3), 6.97 (dd, J = 8.8, 2.5 Hz, 1 H, H-5), 4.25 (t, J = 7.8 Hz, 1 H, H-8), 3.97 (quint., J = 7.2 Hz, 1 H, H-12), 3.59 (ddd, J = 9.8, 6.8, 2.9 Hz, 1 H, H-11), 3.33–3.28 (obs, 1 H, H-11'), 2.19–2.04 (m, 4 H, H-10 & H-10' & H-9 & H-9') ppm; ¹H NMR (250 MHz, CDCl₃): δ = 7.52 (d, J = 8.7 Hz, 1 H, H-6), 7.06 (d, J = 2.5 Hz, 1 H, H-3), 6.90 (dd, J = 8.7, 2.5 Hz, 1 H, H-5), 4.26–4.20 (m, 1 H, H-8), 3.98–3.85 (m, 1 H, H-12), 3.65–3.58 & 3.33–3.23 (2 m, 2 H, H-11 & H-11'), 2.94 (d, J = 4.5 Hz, 1 H, OH), 2.22–2.00 (m, 4 H, H-10 & H-10' & H-9 & H-9') ppm; ¹³C NMR (62.5 MHz, CDCl₃): δ = 151.3 (s), 135.7 (s, C-6), 133.8 (q, J = 32 Hz), 124.7 (q, J = 283 Hz), 122.7 (q, J = 274 Hz), 117.3 (s), 115.4 (s, C-5), 111.1 (q, J = 5 Hz, C-3), 95.6 (s), 72.3 (q, J =

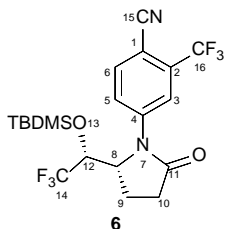
29 Hz, C-12), 58.6 (s, C-8), 49.4 (s, C-11), 29.2 & 23.0 (2 s, C-9 & C-10) ppm. The above data are in accordance with the ones previously reported.⁶⁻⁸

4-((2R)-2-((1R)-1-((tert-butyldimethylsilyloxy)-2,2-trifluoroethyl)pyrrolidin-1-yl)-2-(trifluoromethyl)benzonitrile (5): The above partially purified LGD-4033 (32.6 mg, 96.4 μ mol) was placed in a 5 mL pear-shaped flask and imidazole (195 mg, 2.86 mmol), TBDMSCl (241 mg, 1.60 mmol), and DMF (0.15 mL) were added sequentially under an argon atmosphere. The mixture was stirred at ambient temperature for 48 h. Volatiles were removed under reduced pressure and the residue was dissolved in dichloromethane (2 mL). Silica gel (0.5 g) was added, volatiles were removed under reduced pressure, the residue was loaded on top of a chromatography column (silica gel), and eluted with *n*-hexane/dichloromethane 75:25 to provide TBS-protected LGD-4033 (**5**) as amorphous white solid (41.4 mg, 91.5 μ mol, 94.9% yield). R_f = 0.71 (silica gel, CH₂Cl₂);



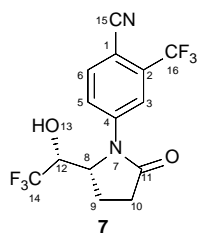
$[\alpha]_D^{25}$ = +20 (c = 0.97, CH₂Cl₂); **¹H NMR** (250 MHz, CDCl₃): δ = 7.57 (d, J = 8.8 Hz, 1 H, H-6), 7.02 (br s, 1 H, H-3), 6.79 (dd, J = 8.8, 2.7 Hz, 1 H, H-5), 4.20–4.14 (m, 1 H, H-8), 3.81 (dq, J = 8.8, 6.0 Hz, 1 H, H-12), 3.55–3.48 (m, 1 H, H-11), 3.30–3.19 (m, 1 H, H-11'), 2.23–1.98 (m, 4 H, H-9 & H-10), 0.66 (s, 9 H, (CH₃)₃CSi), 0.05 & –0.23 (2 s, 6 H, CH₃SiCH₃) ppm; **¹³C NMR** (62.5 MHz, CDCl₃): δ = 151.1 (s), 135.6 (s, C-6), 133.8 (q, J = 32 Hz), 124.9 (q, J = 284 Hz), 122.8 (q, J = 274 Hz), 117.4 (s), 115.0 (s, C-5), 111.3 (s, C-3), 95.4 (s), 73.4 (q, J = 28 Hz, C-12), 58.6 (s, C-8), 49.0 (s, C-11), 29.4 (s, C-10), 25.3 (s, CH₃)₃CSi, 23.0 (s, C-9), 17.8 (s, (CH₃)₃CSi), –4.7, –5.5 (2 s, CH₃SiCH₃) ppm; **HRMS** (ESI +): calculated for C₂₀H₂₇F₆N₂O₃Si⁺ [M+H]⁺: 453.1791, found 453.1780; **HRMS** (ESI –): m/z calculated for C₂₁H₂₇F₆N₂O₃Si[–] [M+HCOO][–]: 497.1690, found 497.1705.

4-((2R)-2-((1R)-1-((tert-butyldimethylsilyloxy)-2,2-trifluoroethyl)-5-oxo-pyrrolidin-1-yl)-2-(trifluoromethyl)benzonitrile (6): A 5 mL round bottom flask equipped with an efficient magnetic stirring bar was charged with RuCl₃ (5.4 mg, 26 μ mol) and 10% w/v aqueous NaIO₄ solution (0.25 mL, 12 $\times 10^{-5}$ mol) was added. To the stirred black solution that ensued was added dropwise a solution of **6** (18.5 mg, 40.9 μ mol) in ethyl acetate (0.9 mL), the flask was sealed, and the mixture was vigorously stirred at ambient temperature for 3 h. Water (1 mL) and ethyl acetate (3 mL) was added and the organic phase was separated. The aqueous layer was extracted with ethyl acetate (2 \times 3 mL). To the combined organic layers was added isopropanol (0.5 mL), the mixture was stirred for 1 h, and the black precipitate formed was removed by filtration through a short pad



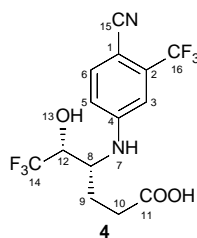
of Celite. The filtrate was washed with brine (2 \times 3 mL), dried over Na₂SO₄, and concentrated. Flash column chromatography (silica gel, *n*-hexane/EtOAc 9:1 to 8:2) gave pyrrolidone **6** as light brown oil (13.7 mg, 29.4 μ mol, 71.9% yield). R_f = 0.52 (silica gel, hexane/EtOAc 1:1); $[\alpha]_D^{25}$ = +3.3 (c = 0.95, CHCl₃); **¹H NMR** (500 MHz, CDCl₃): δ = 8.12 (dd, J = 8.7, 2.3 Hz, 1 H, H-5), 8.03 (d, J = 2.3 Hz, 1 H, H-3), 7.81 (d, J = 8.6 Hz, 1 H, H-6), 4.66 (t, J = 7.2 Hz, 1 H, H-8), 4.07 (quint., J = 6.3 Hz, 1 H, H-12), 2.76 (ddd, J = 17.8, 11.4, 9.4, 1 H, H-10), 2.60 (ddd, J = 17.9, 9.7, 1.9 Hz, 1 H, H-10'), 2.45–2.29 (m, 2 H, H-9 & H-9'), 0.75 (s, 9 H, (CH₃)₃CSi), –0.08 & –0.04 (2 s, 6 H, CH₃SiCH₃) ppm; **¹³C NMR** (125 MHz, CDCl₃): δ = 174.8 (s, C-11), 143.0 (s, C-4), 135.6 (s, C-6), 133.8 (q, J = 32.8 Hz, C-2), 124.5 (q, J = 284.4 Hz, C-14), 123.8 (s, C-5), 122.3 (q, J = 274.1 Hz, C-16), 118.6 (q, J = 5.0 Hz, C-3), 115.5 (s, C-15), 105.1 (br d, J = 2.3 Hz, C-1), 70.7 (q, J = 29.3 Hz, C-12), 58.7 (s, C-8), 30.7 (s, C-10), 25.3 (s, CH₃)₃CSi, 21.3 (s, C-9), 17.9 (s, (CH₃)₃CSi), –5.0 & –5.3 (2 s, CH₃SiCH₃) ppm; **HRMS** (ESI –): m/z calculated for C₂₁H₂₅F₆N₂O₄Si[–] [M+HCOO][–]: 511.1482, found 511.1488.

4-((5R)-2-oxo-5-((1R)-2,2,2-trifluoro-1-hydroxyethyl)pyrrolidin-1-yl)-2-(trifluoromethyl)benzonitrile (7):⁹ A solution of TBS-protected pyrrolidone **6** (12.4 mg, 26.6 μ mol) in THF (0.5 mL) was treated at ambient temperature and under an atmosphere of argon with 1.0 M solution of TBAF in THF (0.04 mL, 4 $\times 10^{-5}$ mol). After 0.5 h, half saturated aqueous NH₄Cl solution (0.5 mL) was added and the mixture was



extracted with ethyl acetate (3 × 5 mL). The combined organic layers were washed with brine (5 mL), dried over Na₂SO₄, and concentrated. Flash column chromatography (silica gel, CH₂Cl₂/EtOAc 9:1) gave pyrrolidone **7** as colorless solid (8.8 mg, 25 μmol, 94% yield). *R_f* = 0.26 (silica gel, CH₂Cl₂/EtOAc 8:2); [α]_D²⁵ = -3.2 (*c* = 0.99, MeOH); ¹H NMR (500 MHz, CD₃OD): δ = 8.19 (s, 1 H, H-3), 7.97–7.93 (m, 2 H, H-5 & H-6), 4.92 (ddt, *J* = 6.8, 5.2, 1.7 Hz, 1 H, H-8), 4.19 (qd, *J* = 7.5, 5.2 Hz, 1 H, H-12), 2.89–2.79 (m, 1 H, H-10), 2.54–2.45 (m, 2 H, H-10' & H-9), 2.20 (td, *J* = 10.4, 2.2 Hz, 1 H, H-9') ppm; ¹³C NMR (125 MHz, CD₃OD): δ = 177.7 (s, C-11), 145.0 (s, C-4), 136.6 (s, C-6), 133.8 (q, *J* = 32.6 Hz, C-2), 127.6 (s, C-5), 126.1 (q, *J* = 282.9 Hz, C-14), 123.9 (q, *J* = 273.0 Hz, C-16), 122.6 (q, *J* = 5.1 Hz, C-3), 116.5 (s, C-15), 106.2 (br d, *J* = 2.0 Hz, C-1), 72.3 (q, *J* = 29.5 Hz, C-12), 60.1 (s, C-8), 31.8 (s, C-10), 23.4 (s, C-9) ppm; HRMS (ESI -): *m/z* calculated for C₁₅H₁₁F₆N₂O₄⁻ [M+HCOO]⁻: 397.0618, found 397.0624.

(4*R*,5*R*)-4-[[4-cyano-3-(trifluoromethyl)phenyl]amino]-6,6,6-trifluoro-5-hydroxyhexanoic acid (4): To a



stirred solution of pyrrolidone **5** (7.5 mg, 21 μmol) in THF/MeOH/H₂O 8:1:4 (0.4 mL) was added at 0 °C LiOH·H₂O (11.0 mg, 262 μmol). The mixture was allowed to reach ambient temperature and it was then stirred at 30 °C for 4 h. The reaction was quenched at 0 °C with the dropwise addition of AcOH/H₂O 1:1 (1 mL). The mixture was diluted with EtOAc (5 mL) and washed with half saturated brine (2 × 5 mL). The aqueous washings were extracted with EtOAc (3 × 5 mL) and the combined organic layers were dried over Na₂SO₄, and concentrated under reduced pressure. Benzene (3 × 5 mL) was added to the residue and volatiles were removed under reduced pressure.

The light-yellow solid thus obtained was dissolved in 2% MeOH in dichloromethane (2 mL), silica gel (0.1 g) was added, volatiles were removed under reduced pressure, and the residue was loaded on top of a chromatography column (silica gel, CH₂Cl₂/EtOAc 8:2). Elution with CH₂Cl₂/EtOAc 8:2 to CH₂Cl₂/EtOAc/AcOH 80:20:1 provided carboxylic acid **4** as colorless glass (7.1 mg, 19 μmol, 90% yield). *R_f* = 0.34 (silica gel, CH₂Cl₂/EtOAc/AcOH 80:20:1); [α]_D²⁵ = +12 (*c* = 0.14, MeOH); ¹H NMR (500 MHz, CD₃OD): δ = 7.58 (d, *J* = 8.7 Hz, 1 H, H-6), 7.08 (d, *J* = 2.5 Hz, 1 H, H-3), 6.89 (dd, *J* = 8.7, 2.5 Hz, 1 H, H-5), 4.10–4.04 (m, 2 H, H-8 & H-12), 2.40 (t, *J* = 7.2 Hz, 2 H, H-10 & H-10'), 1.99 (q, *J* = 7.2 Hz, 2 H, H-9 & H-9') ppm; ¹³C NMR (125 MHz, CD₃OD): δ = 176.8 (s, C-11), 153.4 (s, C-4), 137.4 (s, C-6), 134.9 (q, *J* = 31.6 Hz, C-2), 126.4 (q, *J* = 282.9 Hz, C-14), 124.3 (q, *J* = 272.9 Hz, C-16), 118.3 (s, C-15), 114.8 (br s, C-5), 111.5 (br s, C-3), 94.5 (br d, *J* = 2.4 Hz, C-1), 71.3 (q, *J* = 29.7 Hz, C-12), 52.0 (s, C-8), 30.9 (s, C-10), 28.7 (s, C-9) ppm; HRMS (ESI -): *m/z* calculated for C₁₄H₁₁F₆N₂O₃⁻ [M-H]⁻: 369.0668, found 369.0671.

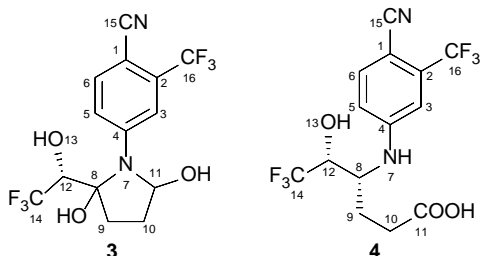
Table S1. ^1H NMR (600 MHz, CD₃OD) data reported⁷ for compound **3** vs. ^1H NMR (500 MHz, CD₃OD) signals observed for compound **4**.

Position	Compound 3 $\delta_{\text{H}}^{\ddagger, \S}$	Position	Compound 4 (this work) δ_{H} (mult., J in Hz)	Difference ($\delta_4 - \delta_3$)
1	–	1	–	–
2	–	2	–	–
3	7.07	3	7.08 (d, $J = 2.5$ Hz)	0.01
4	–	4	–	–
5	6.89	5	6.89 (dd, $J = 8.7, 2.5$ Hz)	0.00
6	7.56	6	7.58 (d, $J = 8.7$ Hz)	0.02
7	–	7	–	–
8	–	11	–	–
9	2.24	10	2.40 (t, $J = 7.2$ Hz)	0.16 ^{\S\S}
10	1.94	9	1.99 (q, $J = 7.2$ Hz)	0.05
11	4.00	8	4.10–4.04 (m)	
12	4.07	12	4.10–4.04 (m)	
13	–	13	–	–
14	–	14	–	–
15	–	15	–	–
16	–	16	–	–

‡ No information regarding signal multiplicities / coupling constants was reported.

§ The actual spectrum was not reported.

\S\S A difference of -0.02 ppm is calculated assuming that the reported chemical shift of 2.24 ppm was actually 2.42 ppm.



Ib. LC-HRMS Analysis

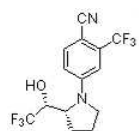
A Dionex UHPLC system (Thermo Scientific, Bremen, Germany) was used for the chromatographic separation. The system consisted of a vacuum degasser, a high-pressure binary pump, an autosampler with a temperature-controlled sample tray set at 7 °C and a column oven set at 30 °C. Chromatographic separation was performed at 30 °C using a Zorbax Eclipse Plus C18 column (100 × 2.1 mm i.d., 1.8 μm particle size; Agilent Technologies). The mobile phase consisted of 5 mM ammonium formate in 0.02% formic acid (solvent A) and a mixture of acetonitrile/water (90:10 v/v) containing 5 mM ammonium formate and 0.01% formic acid (solvent B). A gradient elution program was employed at a constant flow rate of 0.2 mL min⁻¹ with solvent B starting at 5% for 3 min, increasing to 30% in 4 min, increasing to 90% in 11 min and then, set back to 5% in 11.5 min. Post-run equilibrium time was 3.5 min. The injection volume was 5 μL.

The mass spectrometer was a QExactive plus benchtop Orbitrap-based mass spectrometer (ThermoScientific, Bremen, Germany) operated in the negative polarity mode and equipped with a heated electro-spray ionization (HESI) source. Source parameters were: sheath gas (nitrogen) flow rate, auxiliary gas (nitrogen) flow rate and sweep gas flow rate: 40, 10 and 1 arbitrary units respectively, capillary temperature: 300 °C, heater temperature: 30 °C, spray voltage: +4.0 kV (positive polarity). The instrument operated in FS mode from *m/z* 100–1000 at 17,500 resolving power and duty cycle of 100 ms and in MS/MS mode from *m/z* 100–1000 at 17,500 resolving power and duty cycle of 62 ms (product ion mode). The automatic gain control (AGC) was set to 106. The mass calibration of the Orbitrap instrument was evaluated in both positive and negative modes daily and external calibration was performed prior to use following the manufacturer's calibration protocol.

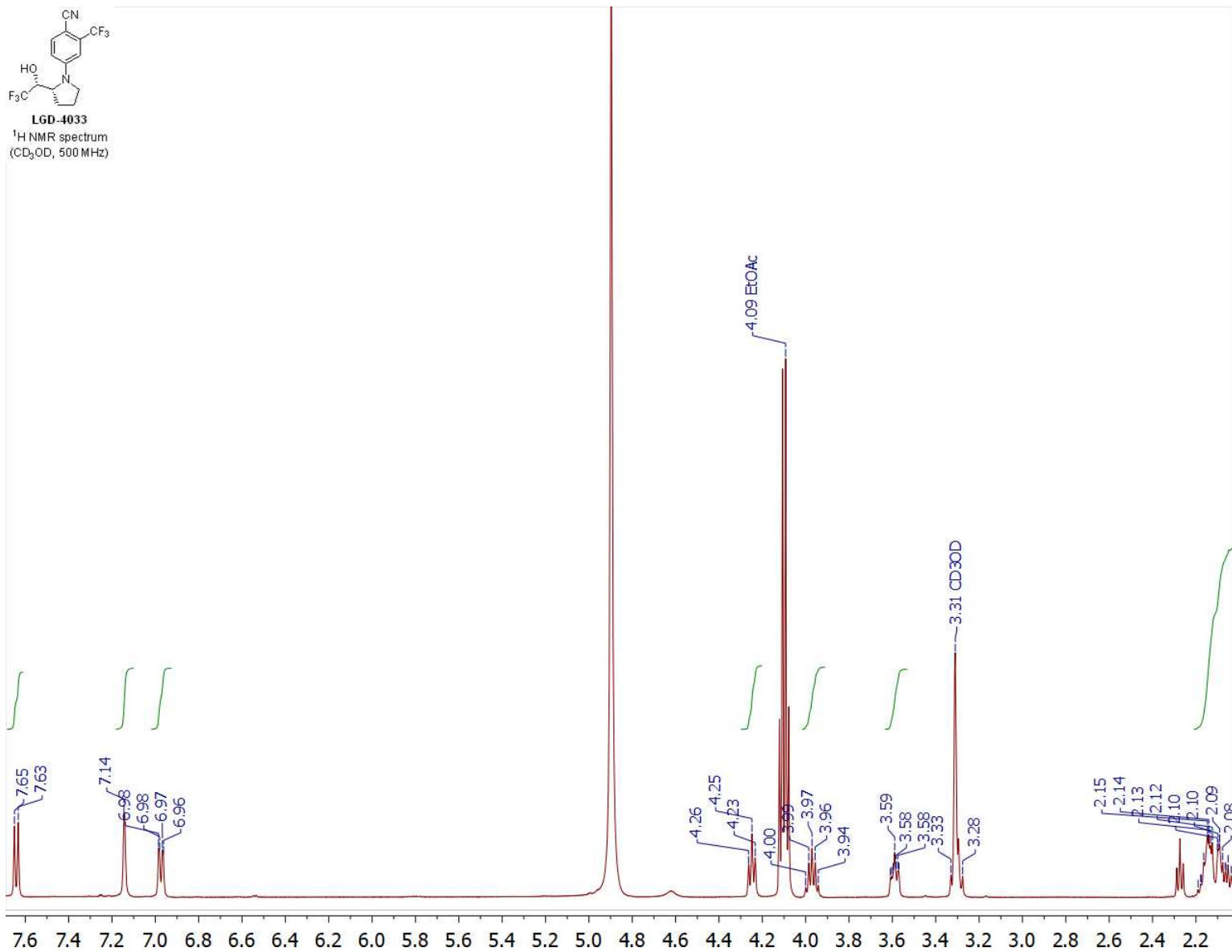
II. References

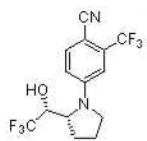
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III. NMR Spectra of Compounds



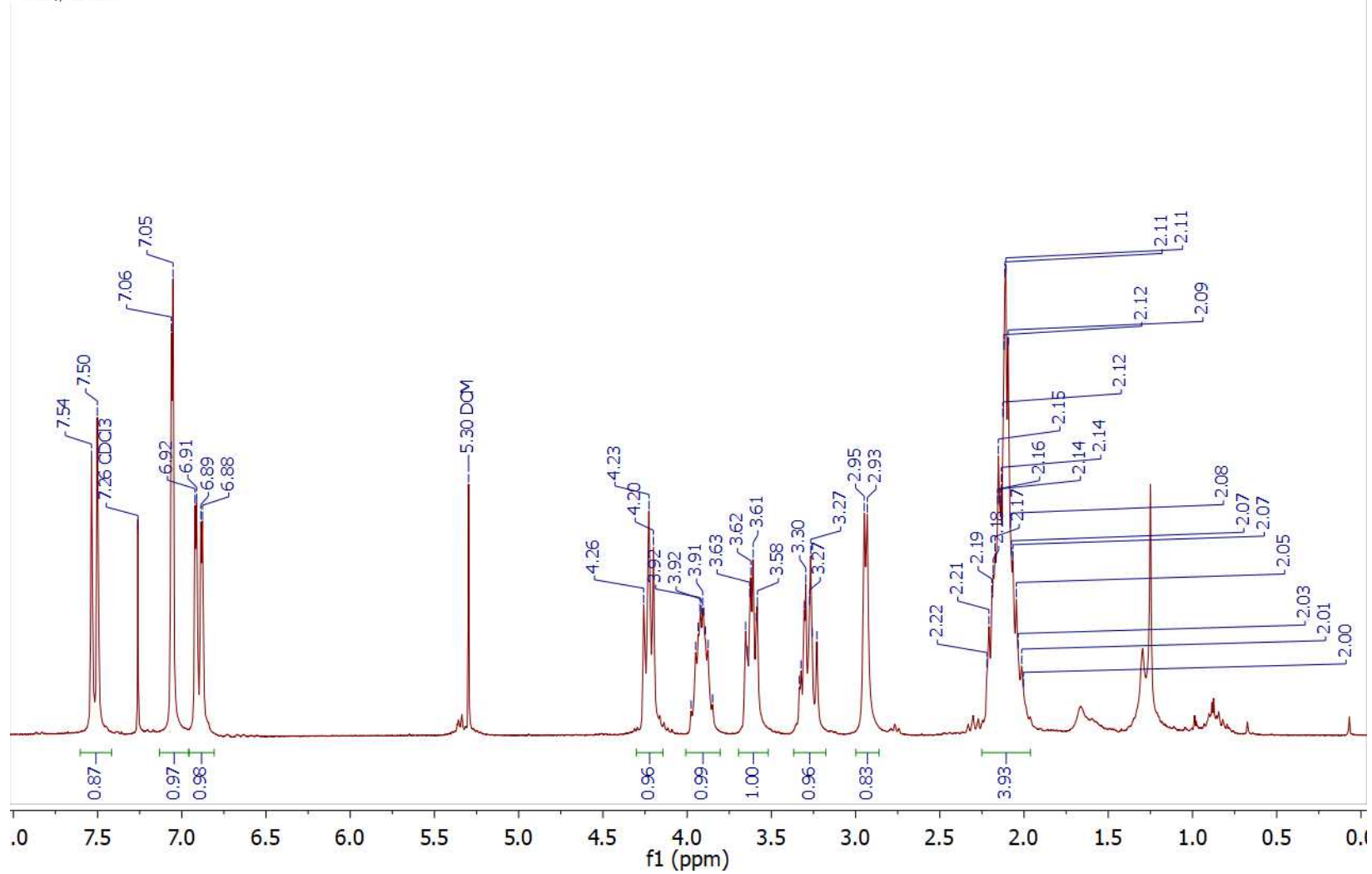
LGD-4033
¹H NMR spectrum
(CD₃OD, 500 MHz)

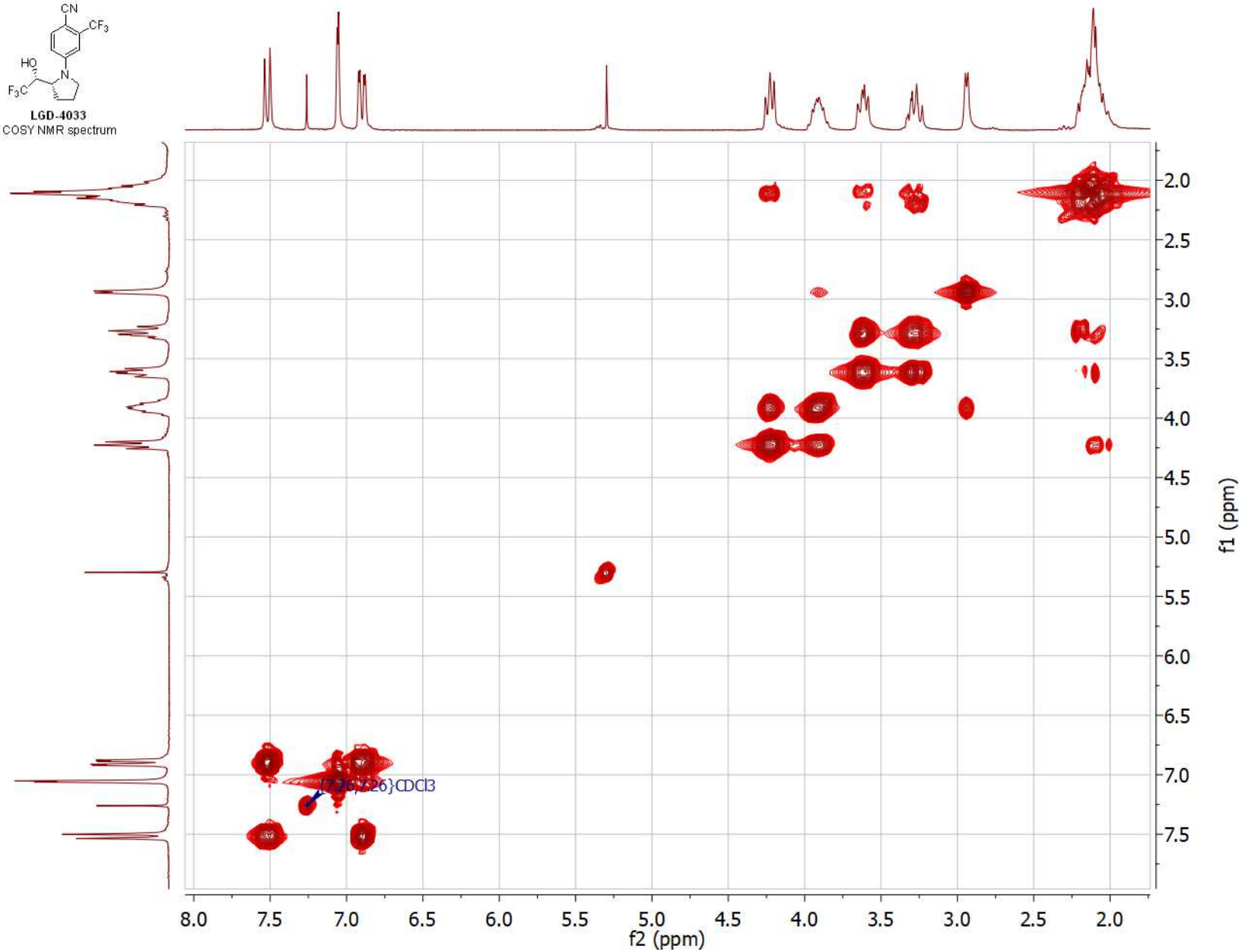
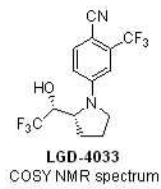


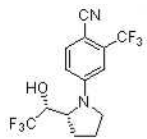


LGD-4033

¹H NMR spectrum
(CDCl₃, 250 MHz)

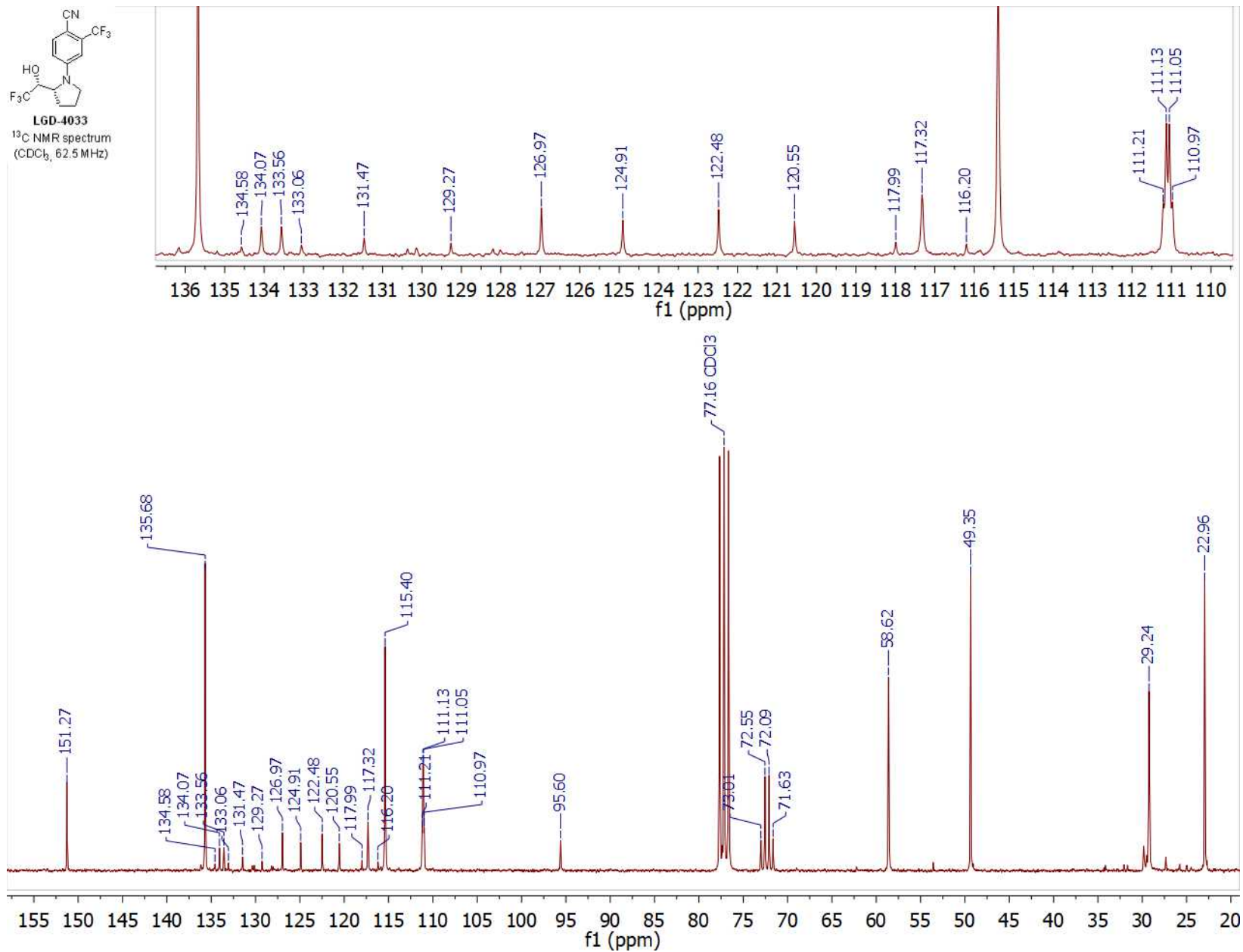


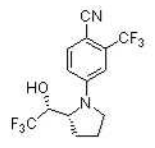




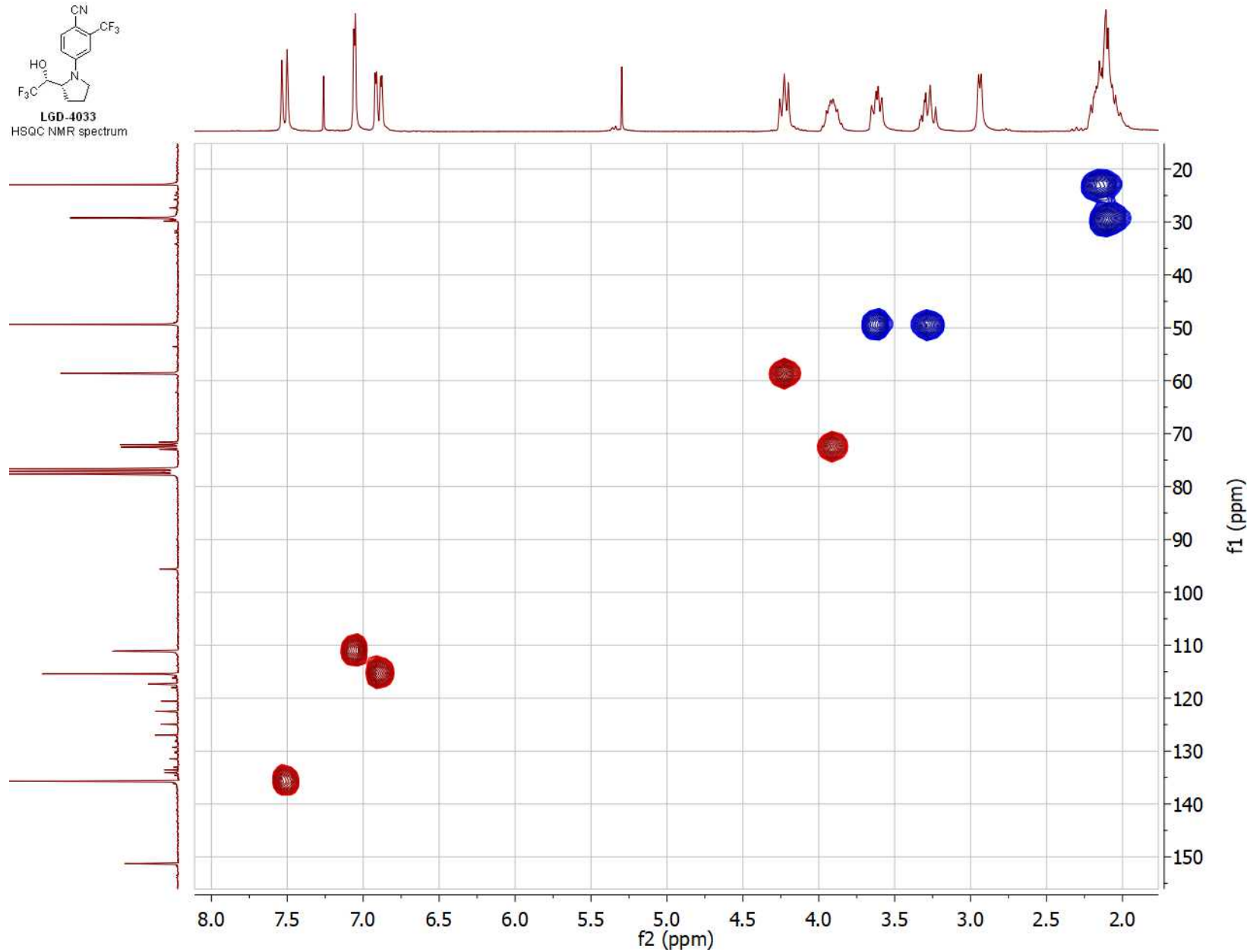
LGD-4033

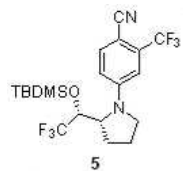
¹³C NMR spectrum
(CDCl₃, 62.5 MHz)



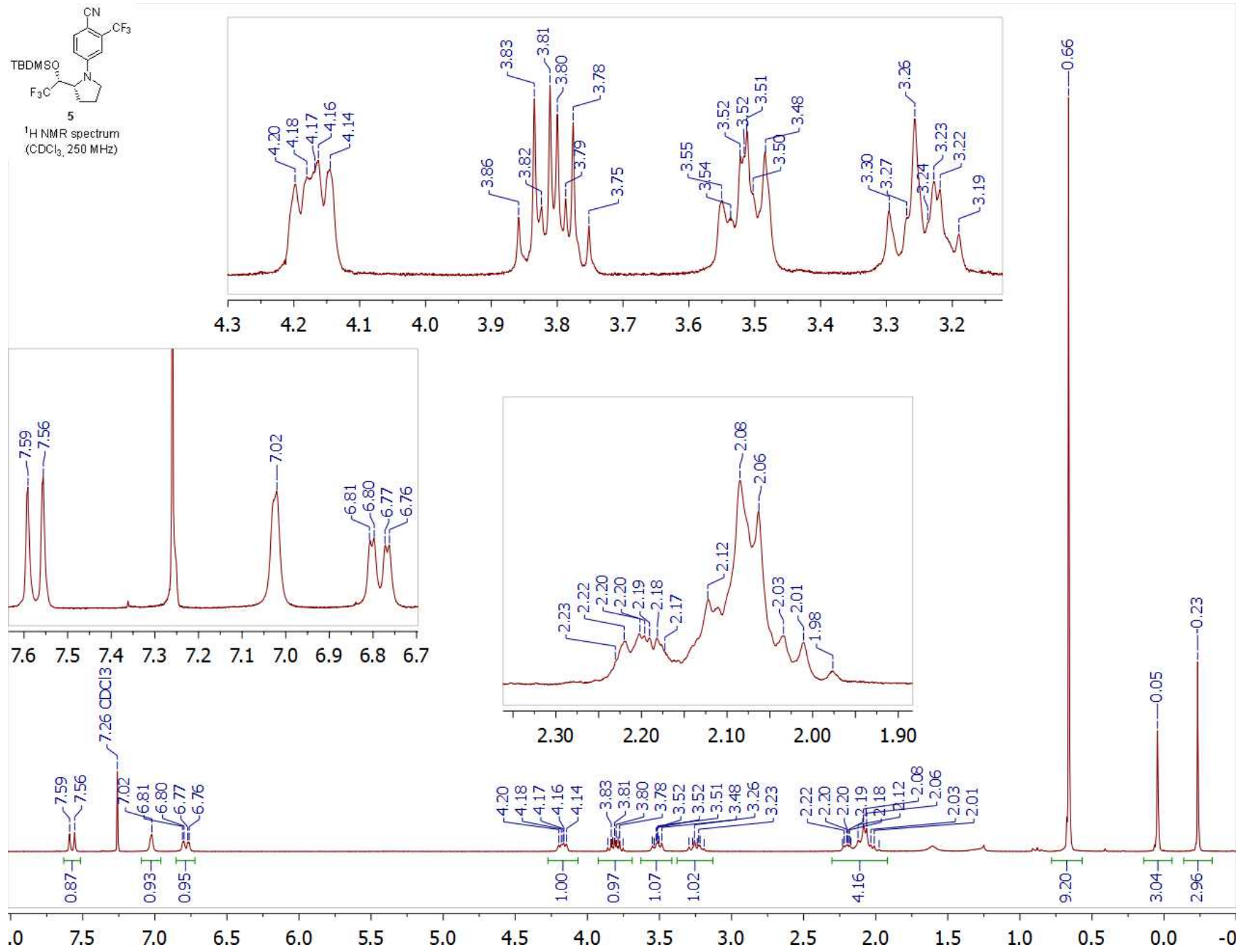


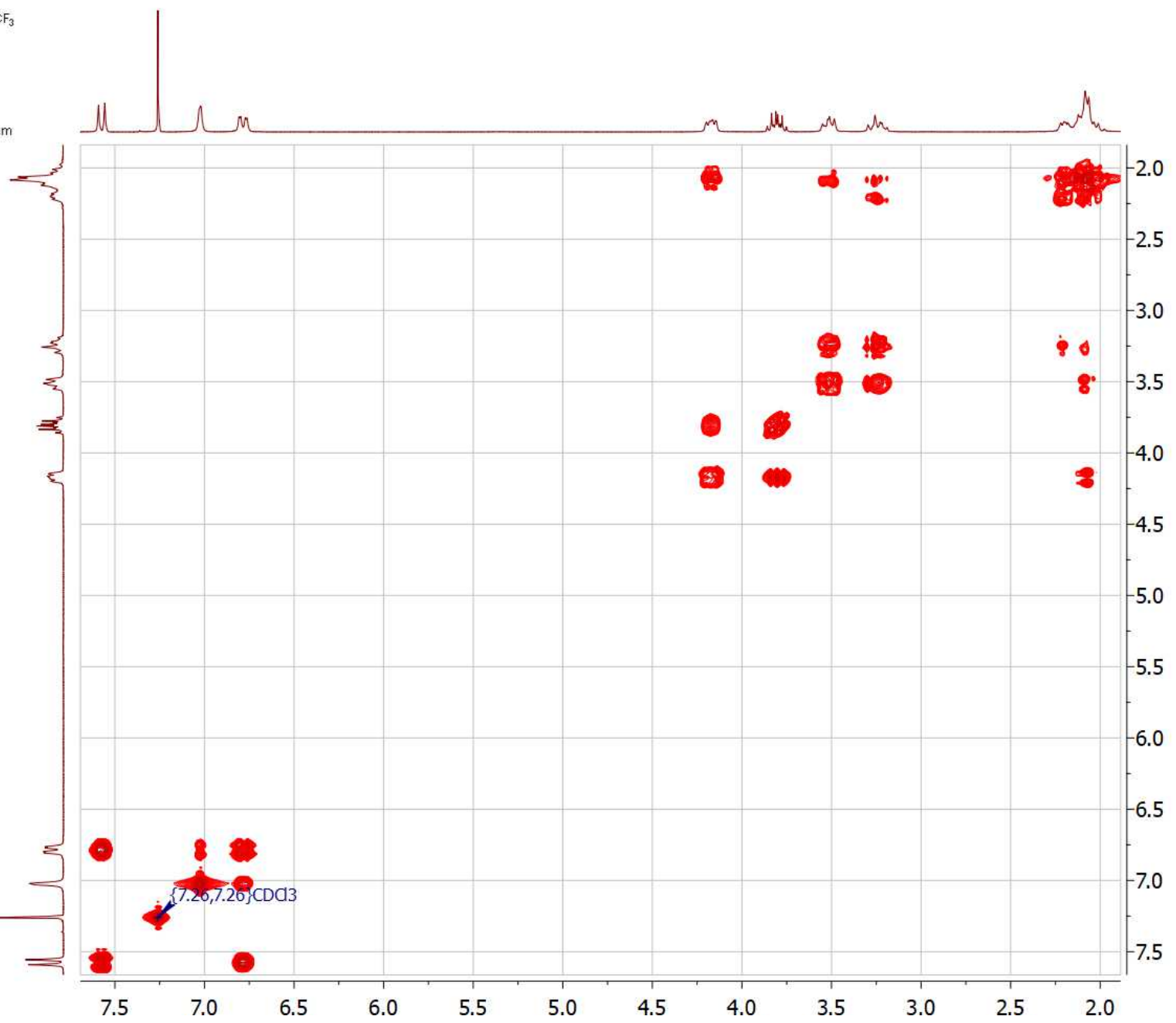
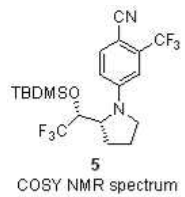
LGD-4033
HSQC NMR spectrum





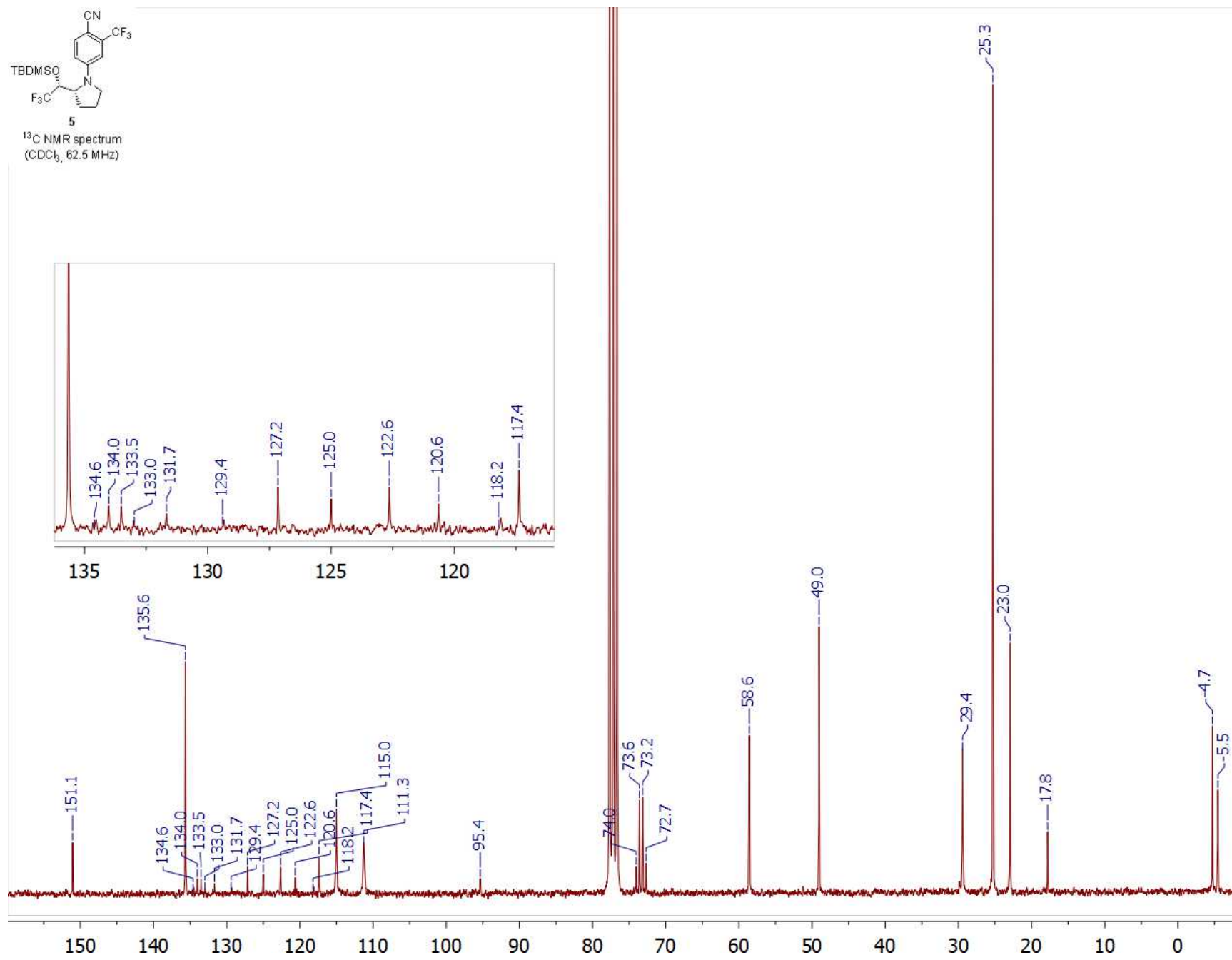
¹H NMR spectrum
(CDCl₃, 250 MHz)

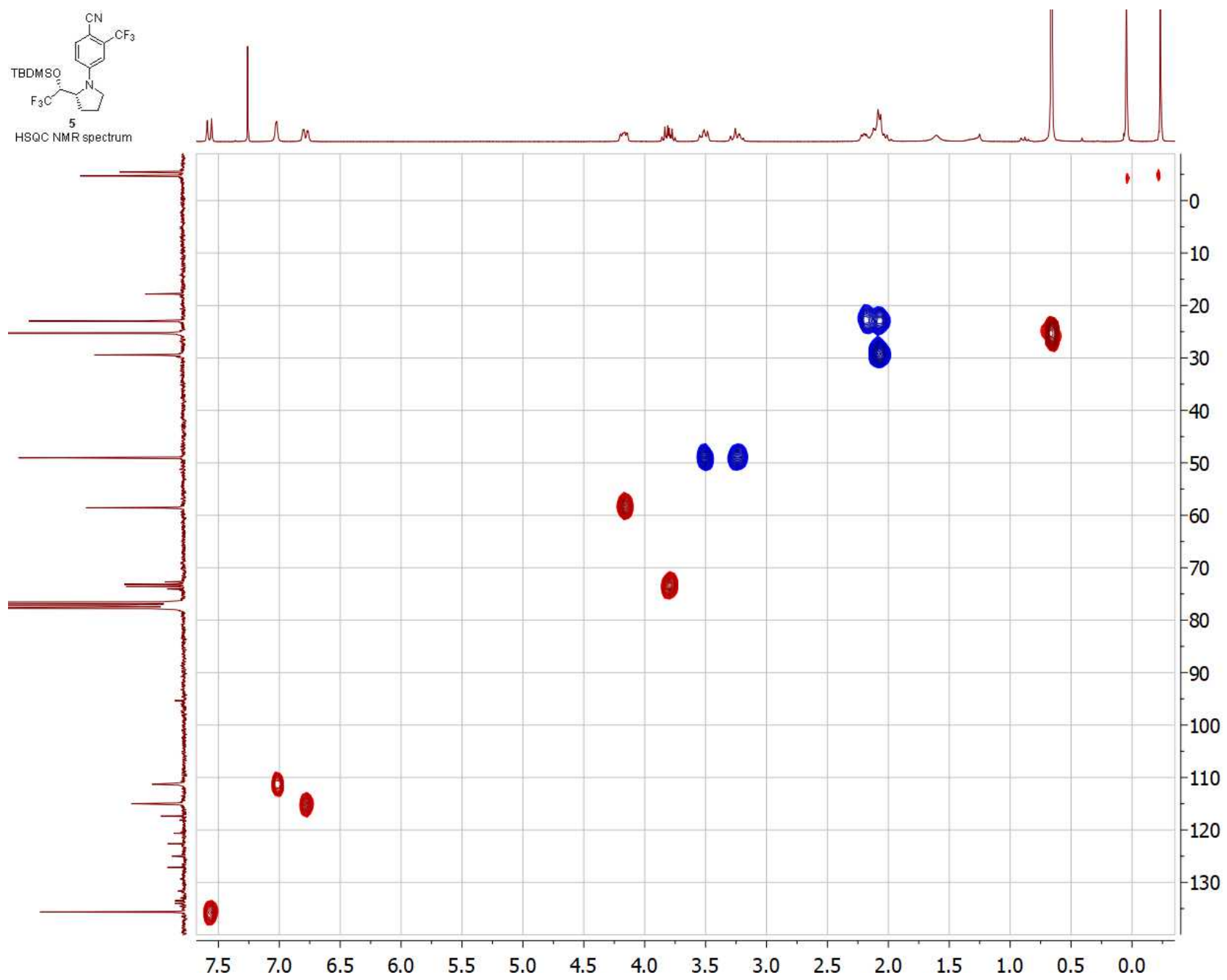
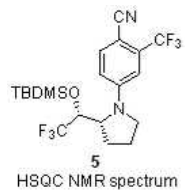






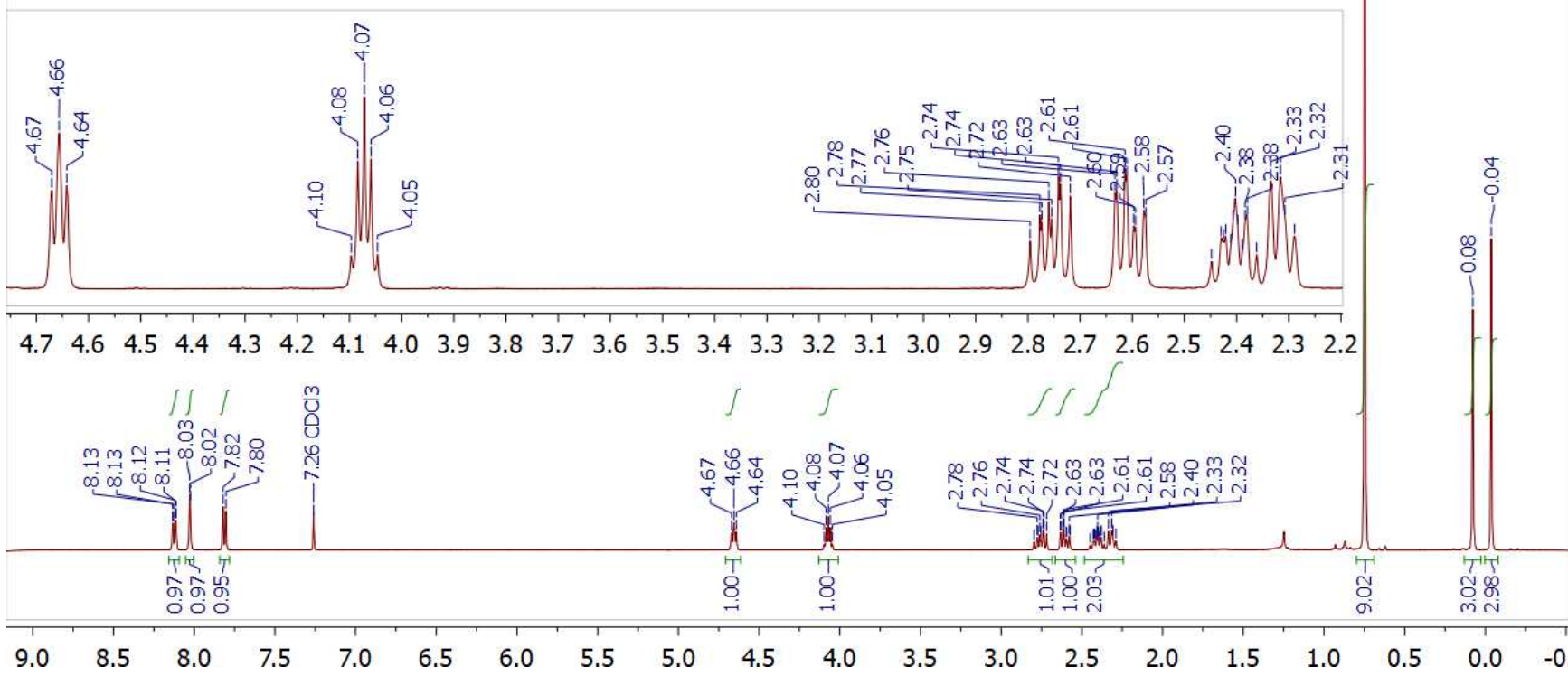
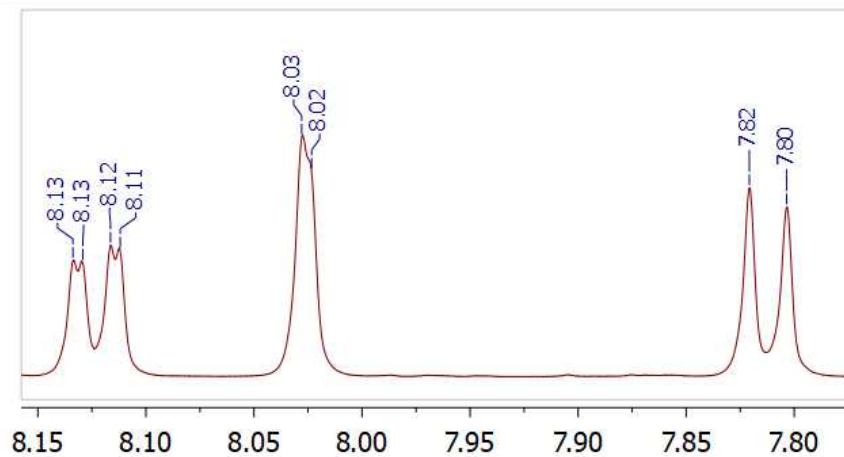
^{13}C NMR spectrum
(CDCl_3 , 62.5 MHz)

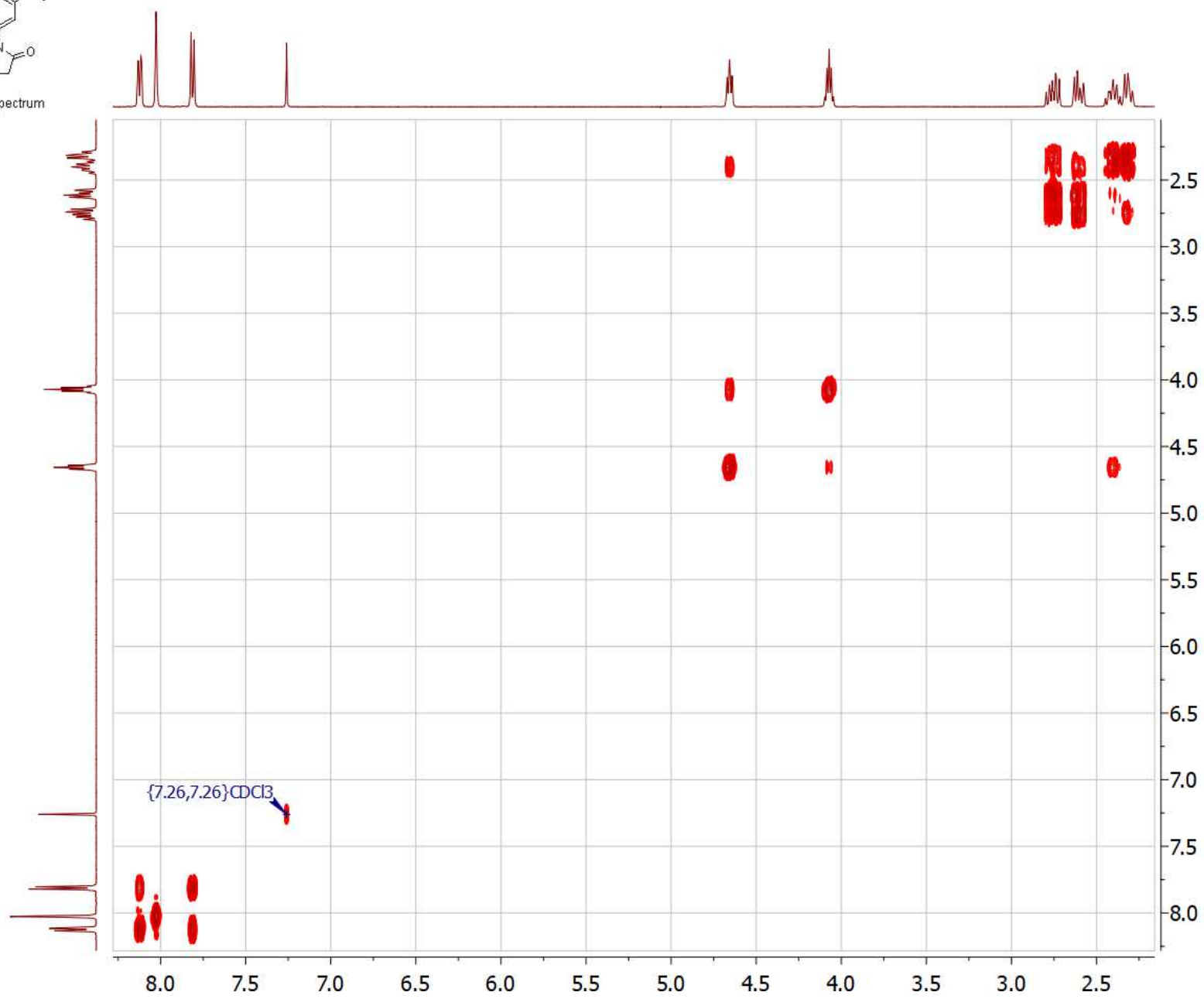
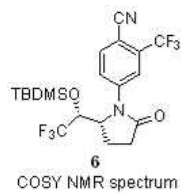


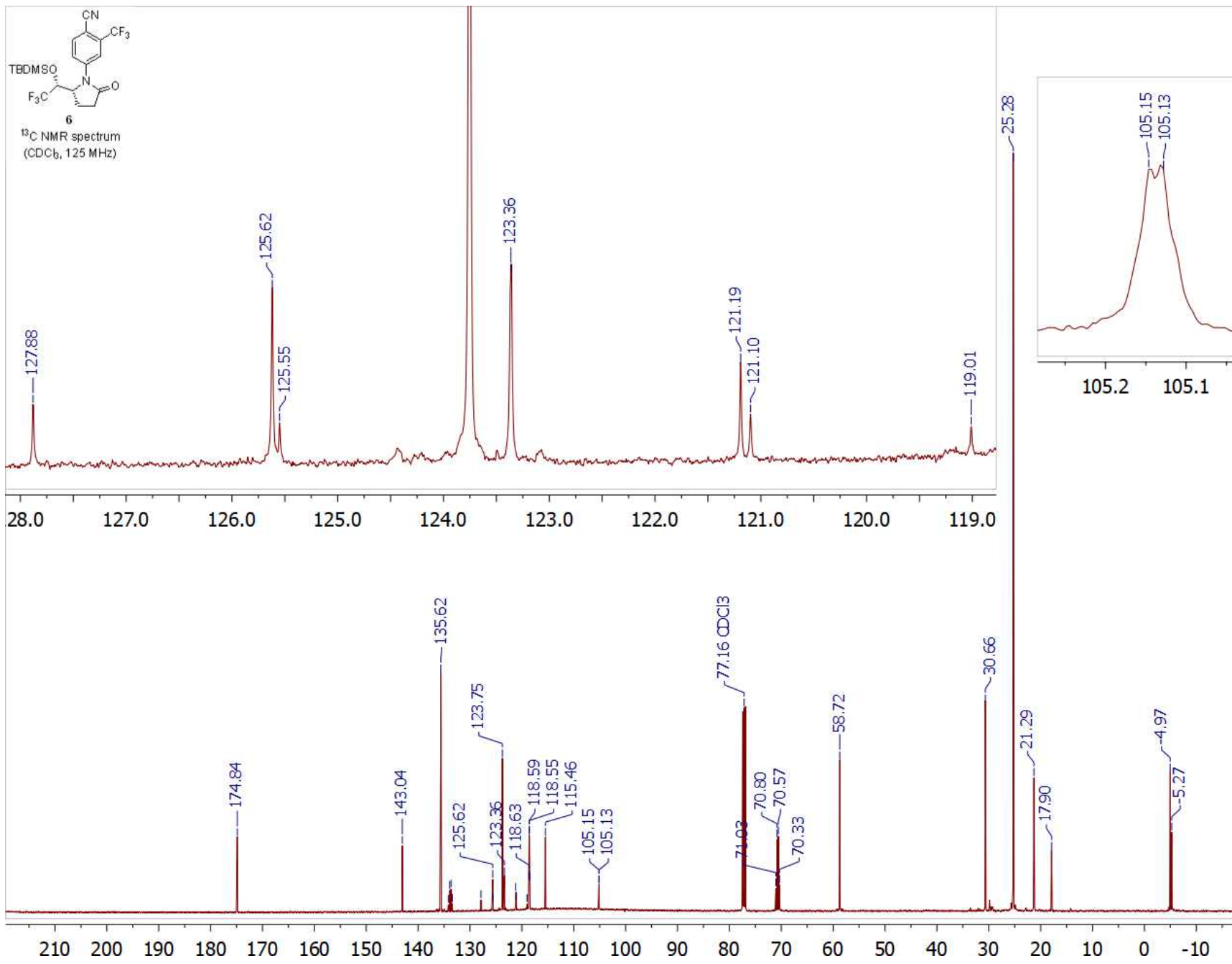




¹H NMR spectrum
(CDCl₃, 500 MHz)

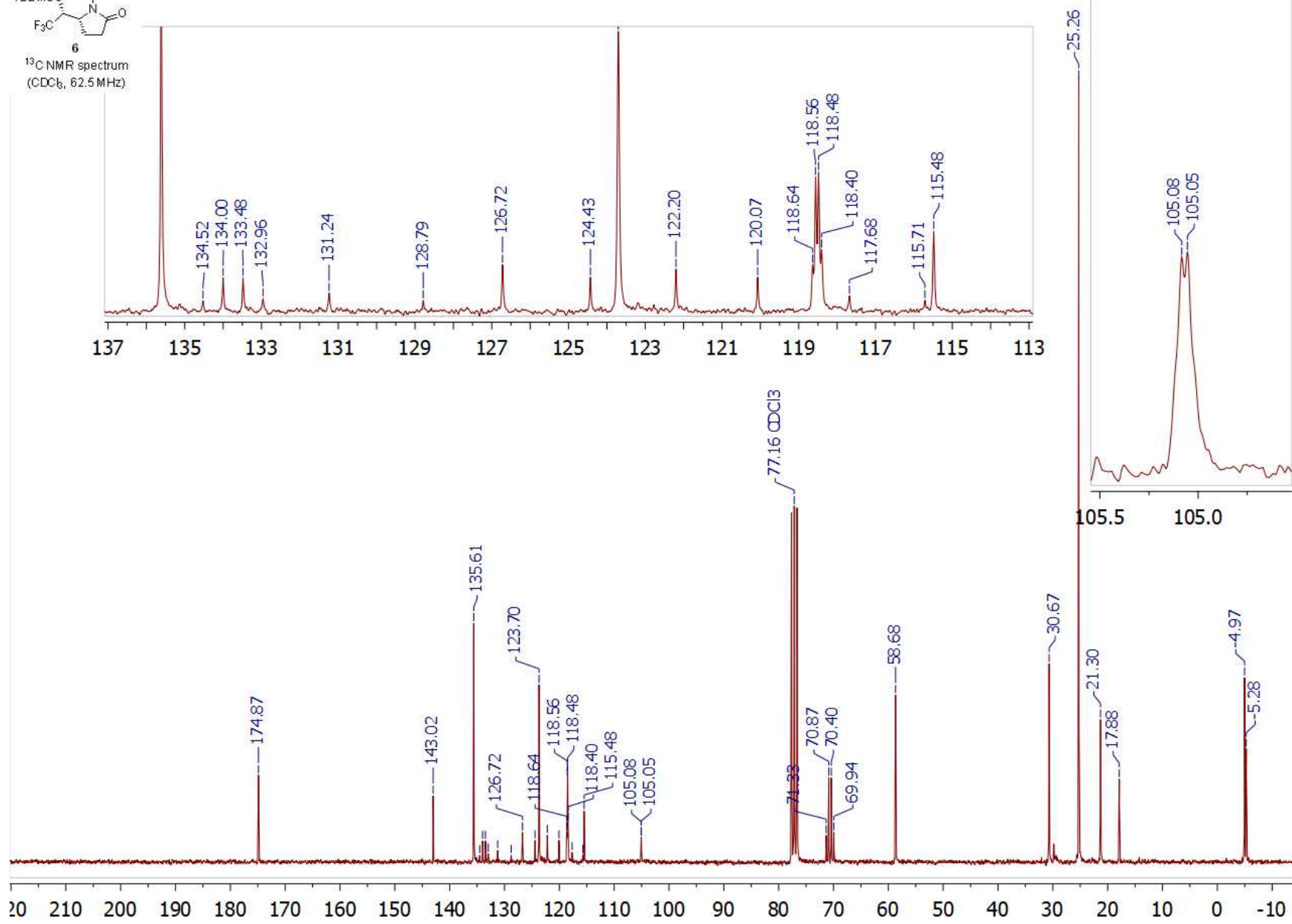


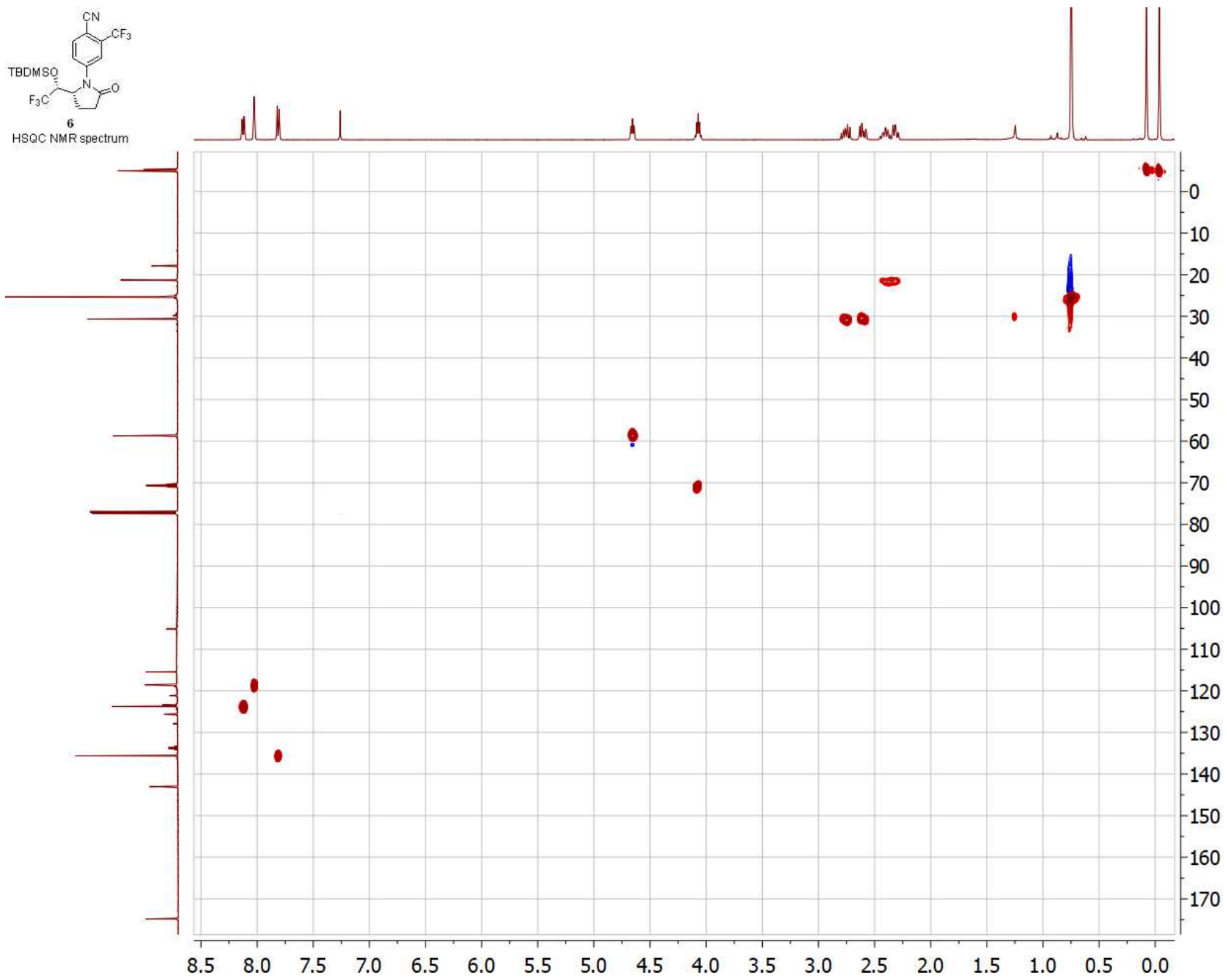
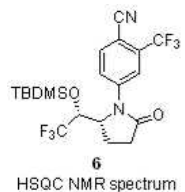


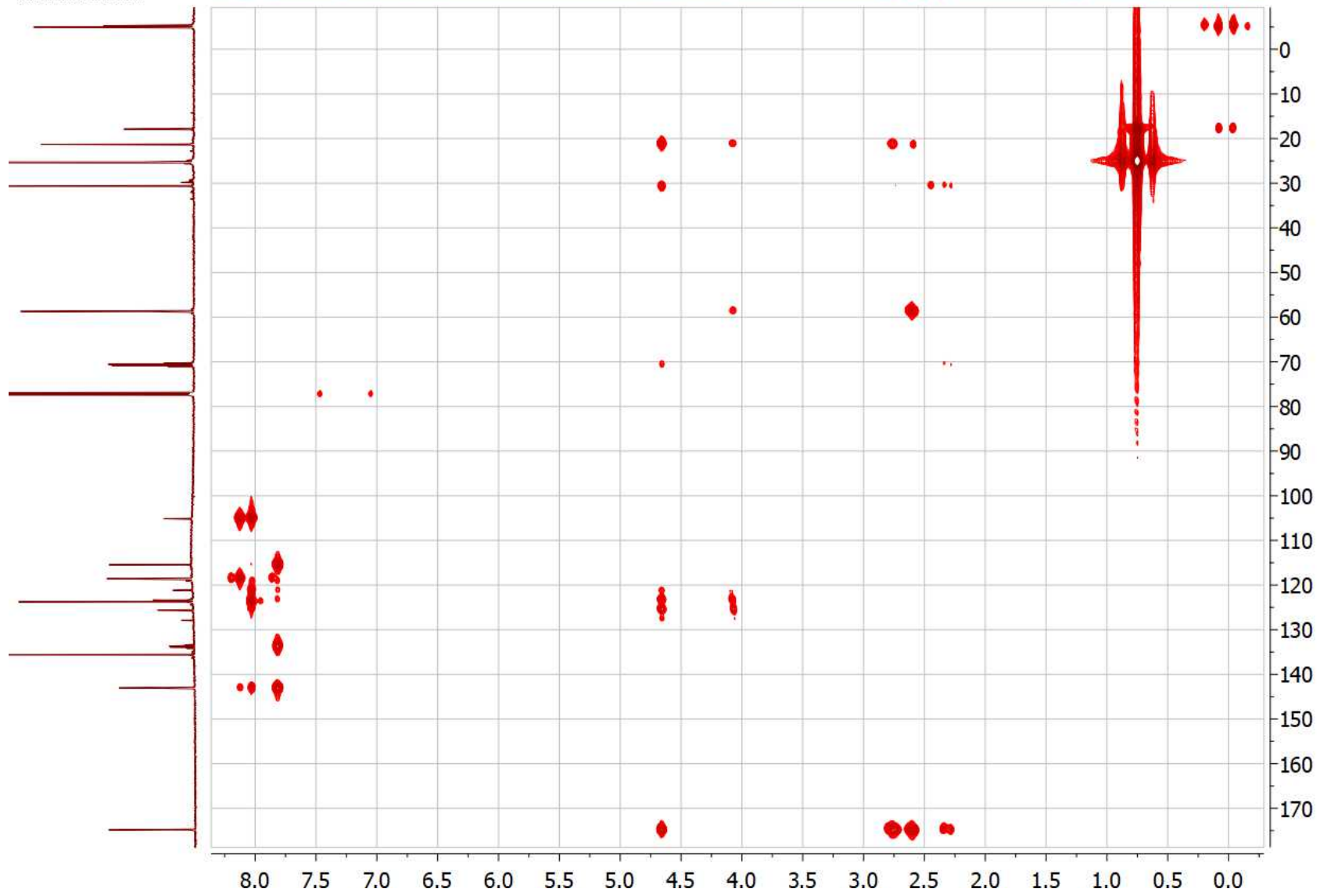
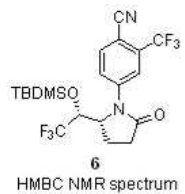


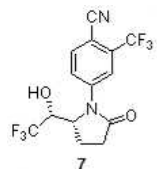


¹³C NMR spectrum
 (CDCl₃, 62.5 MHz)

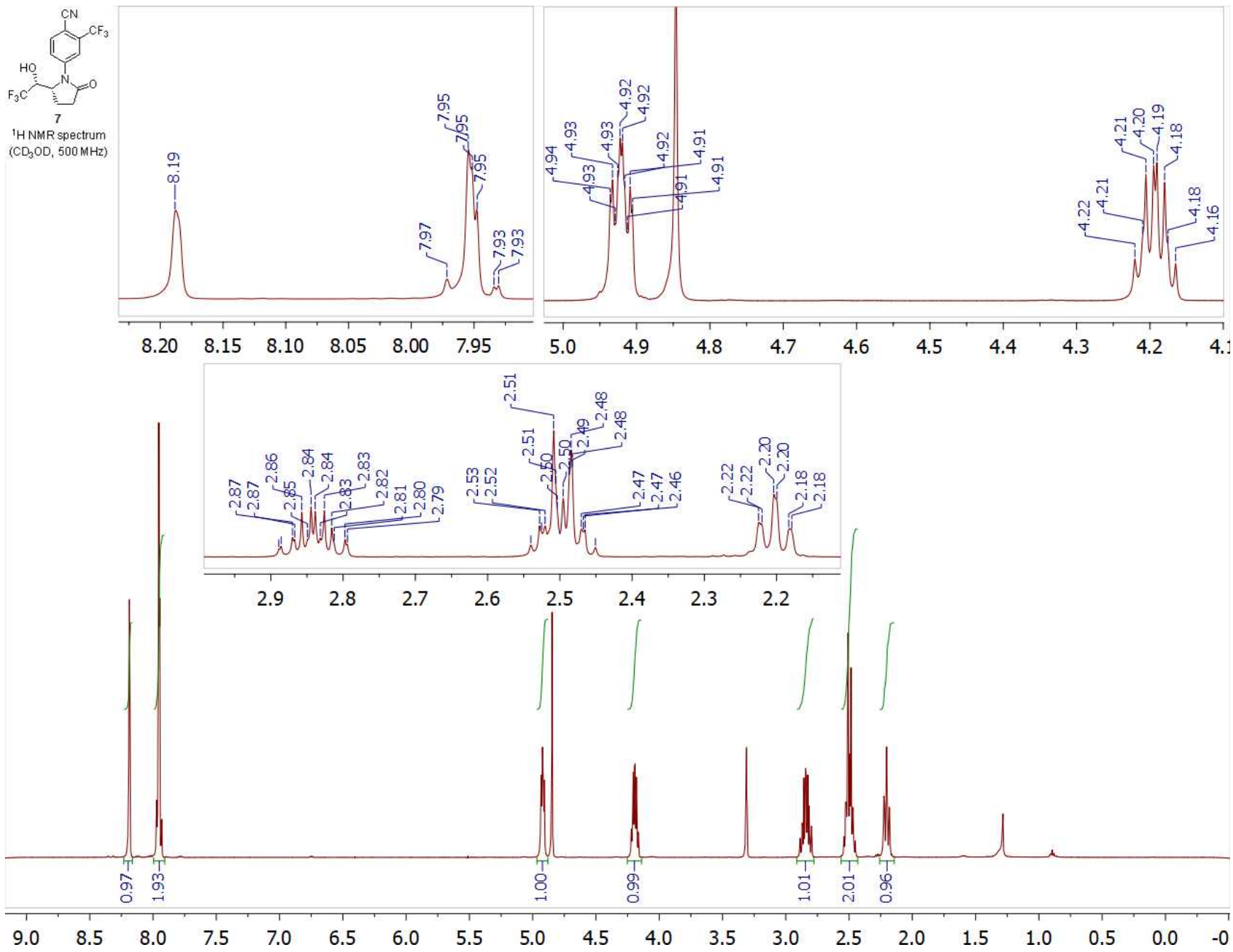


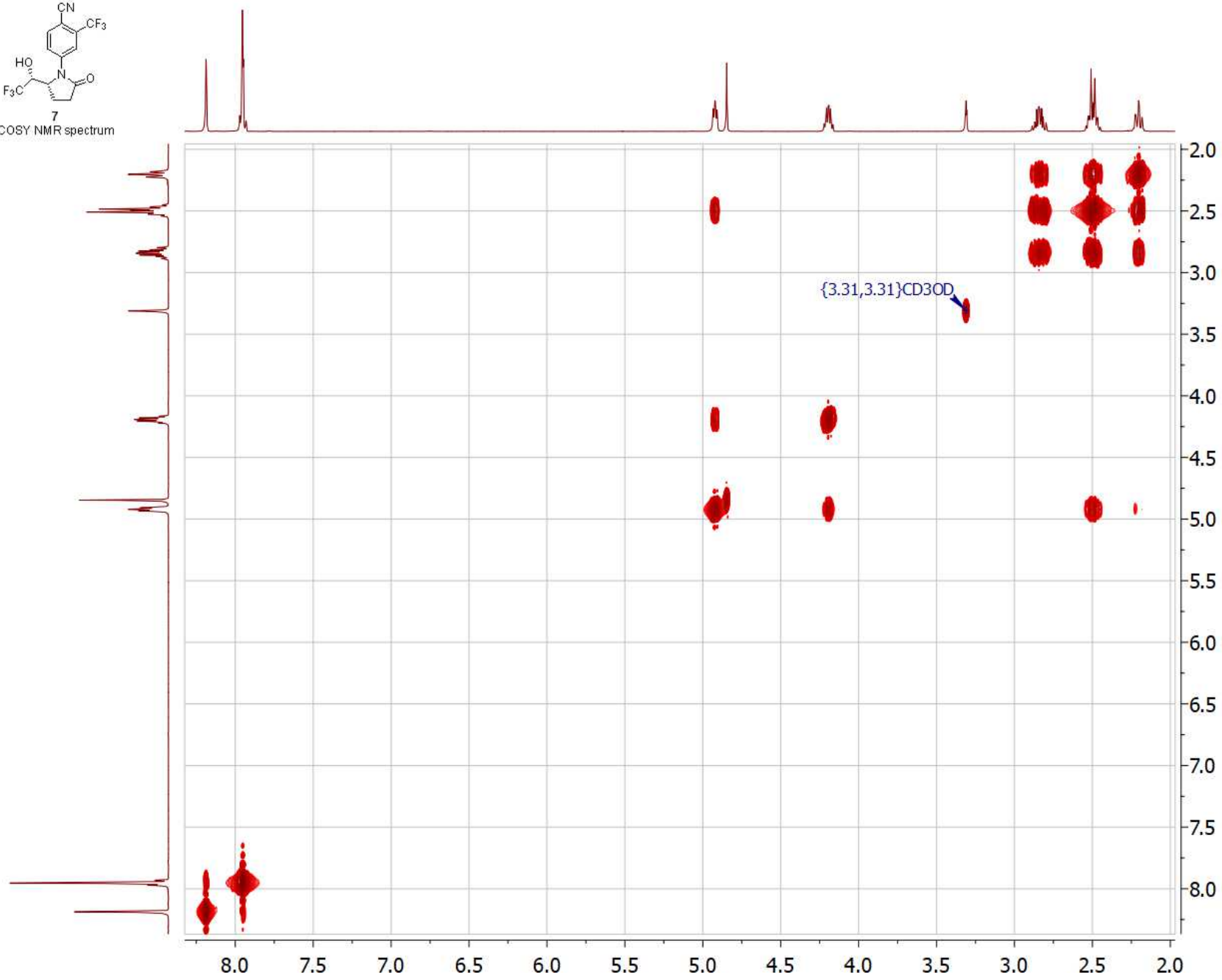
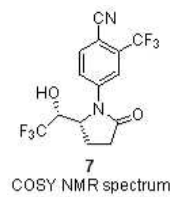


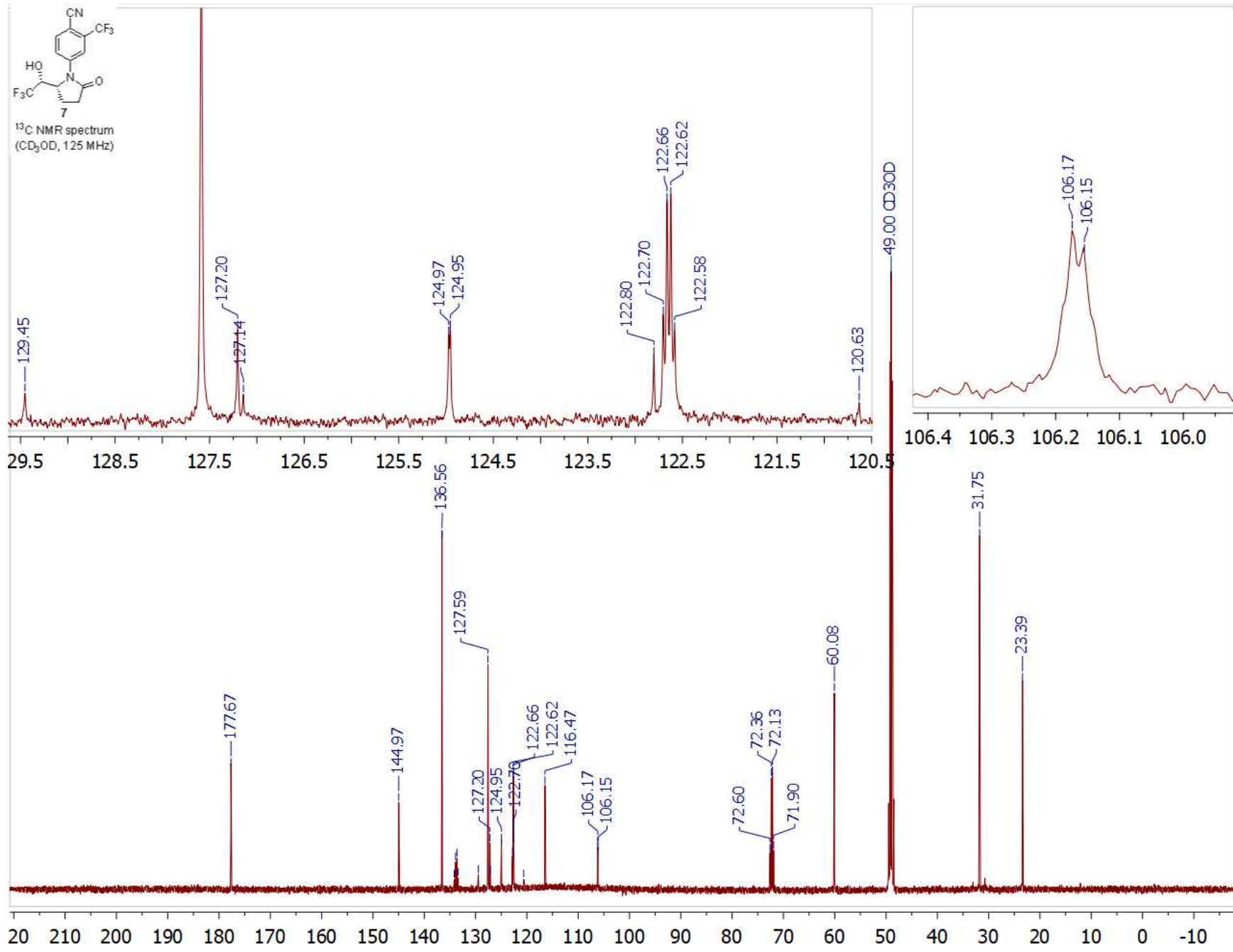


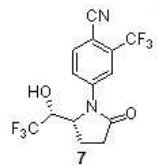


¹H NMR spectrum
(CD₃OD, 500 MHz)

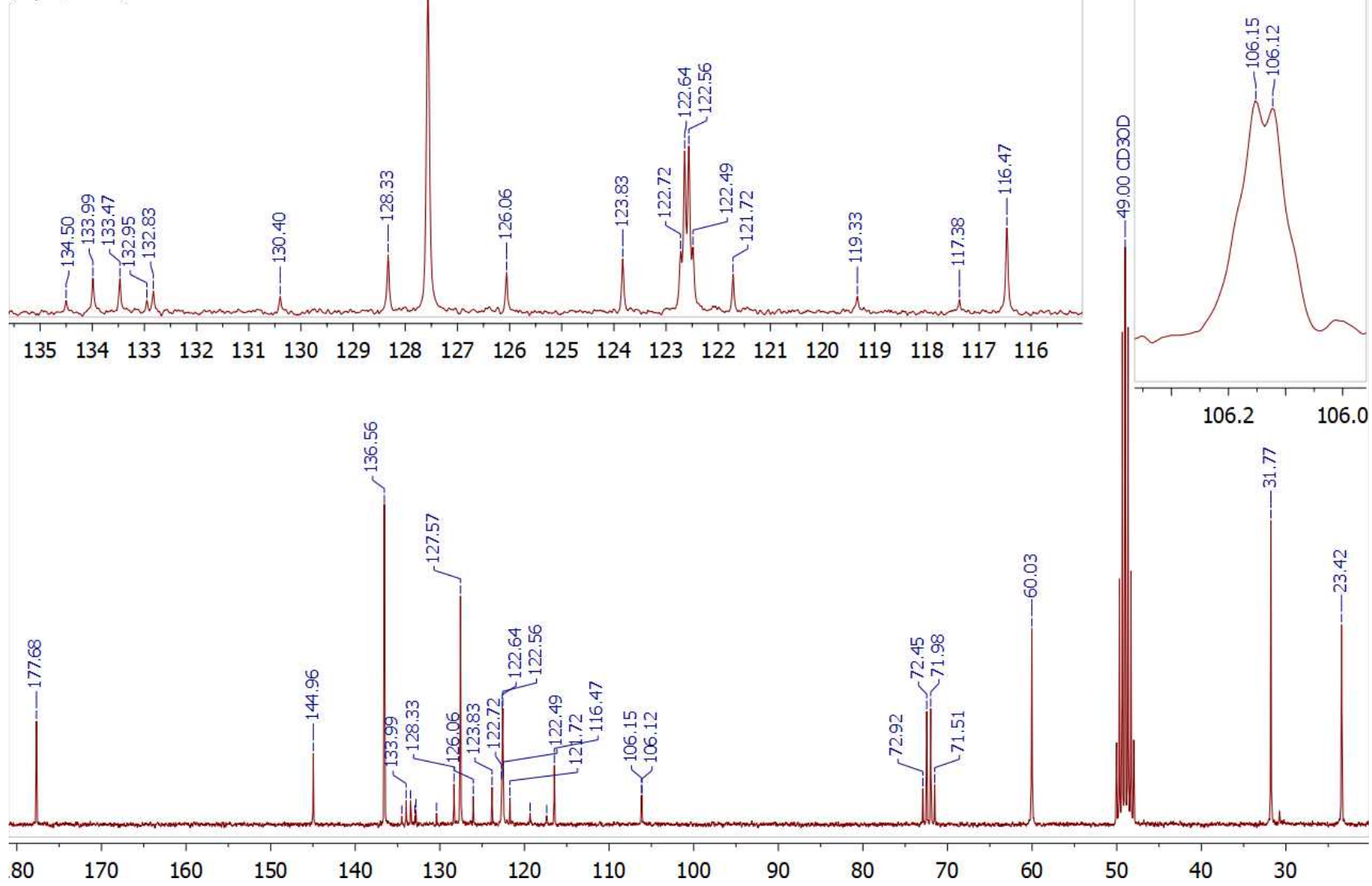


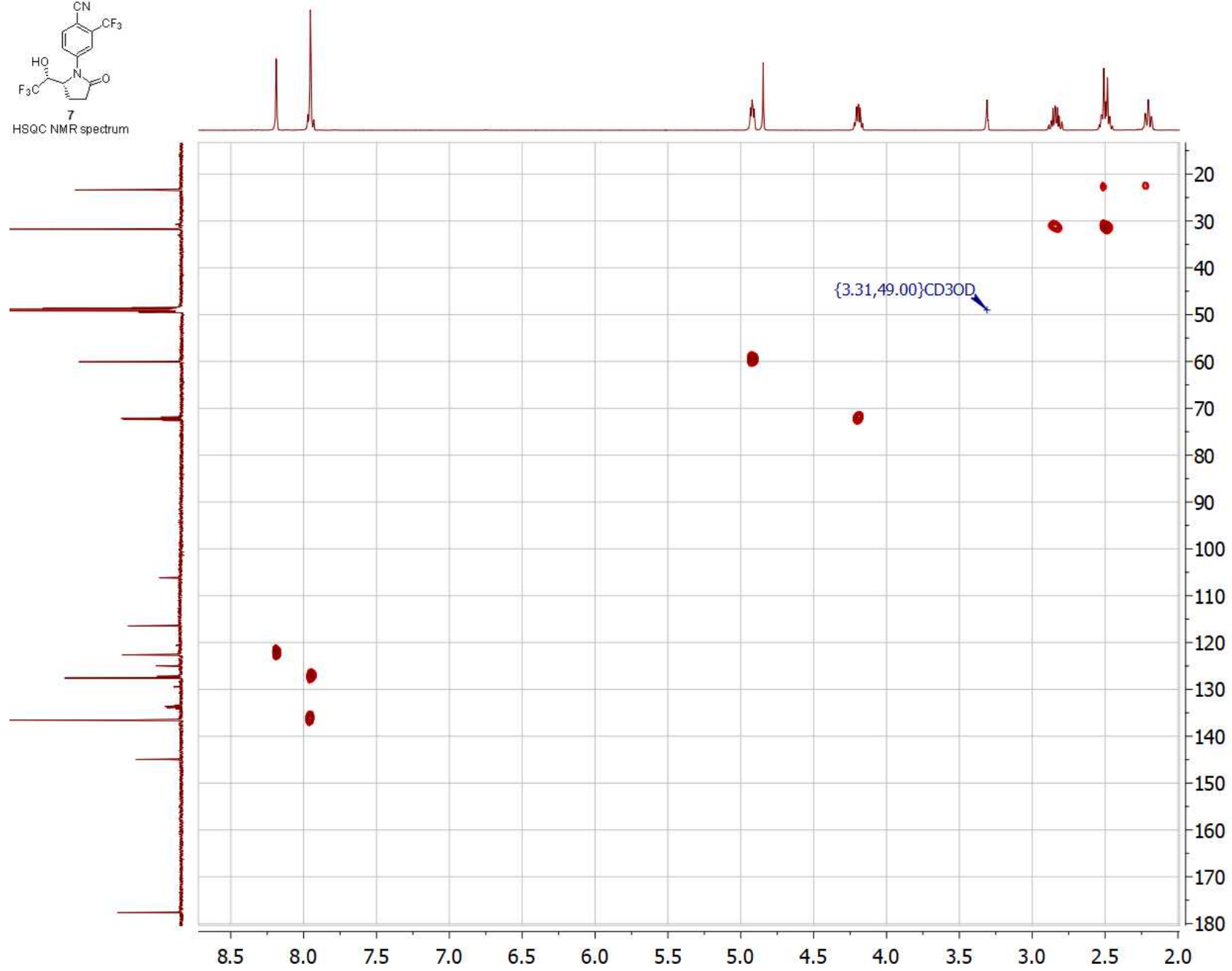
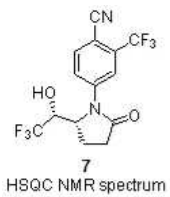


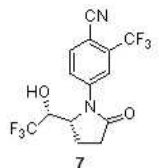




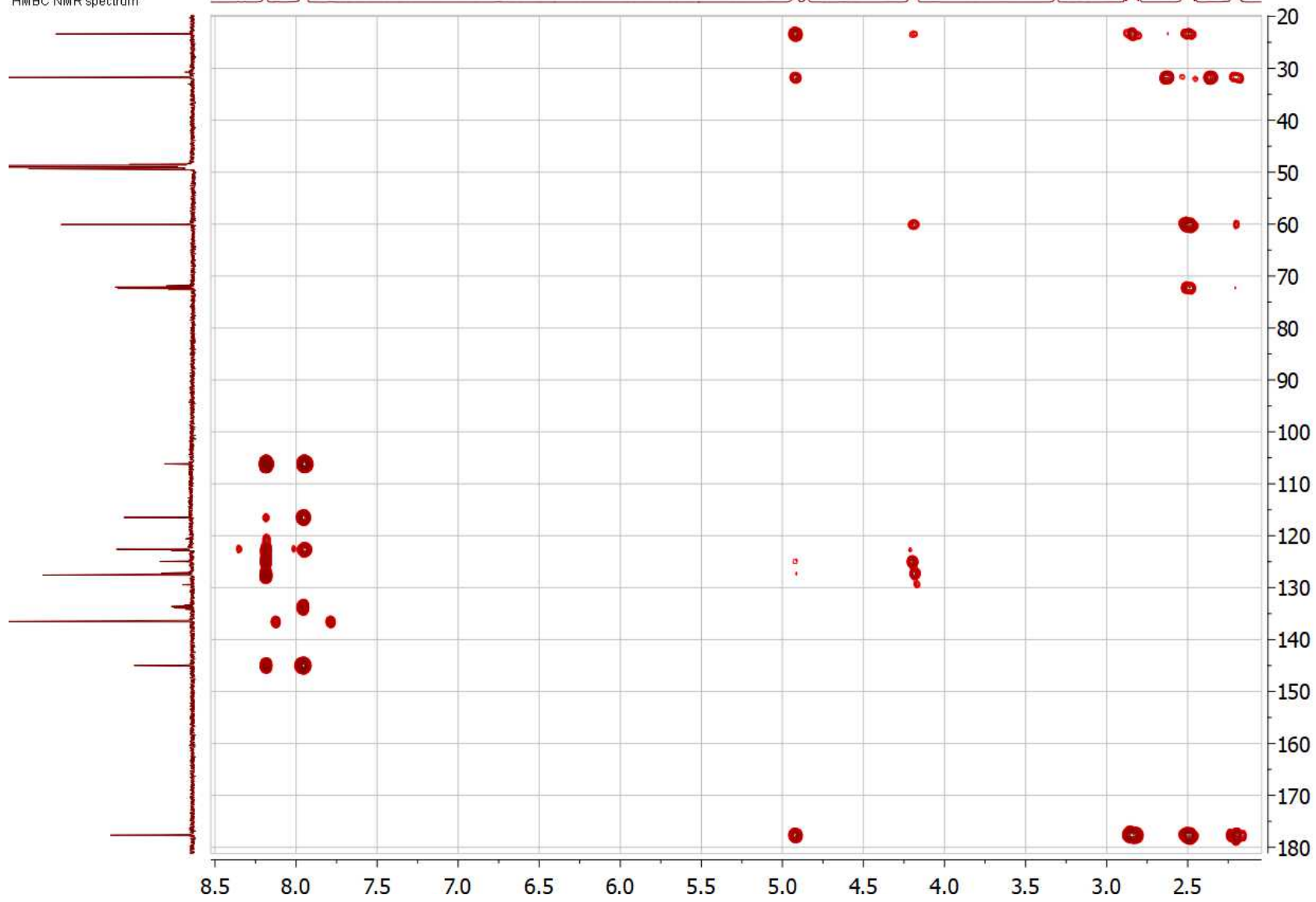
^{13}C NMR spectrum
(CD_3OD , 62.5 MHz)

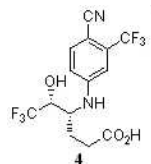




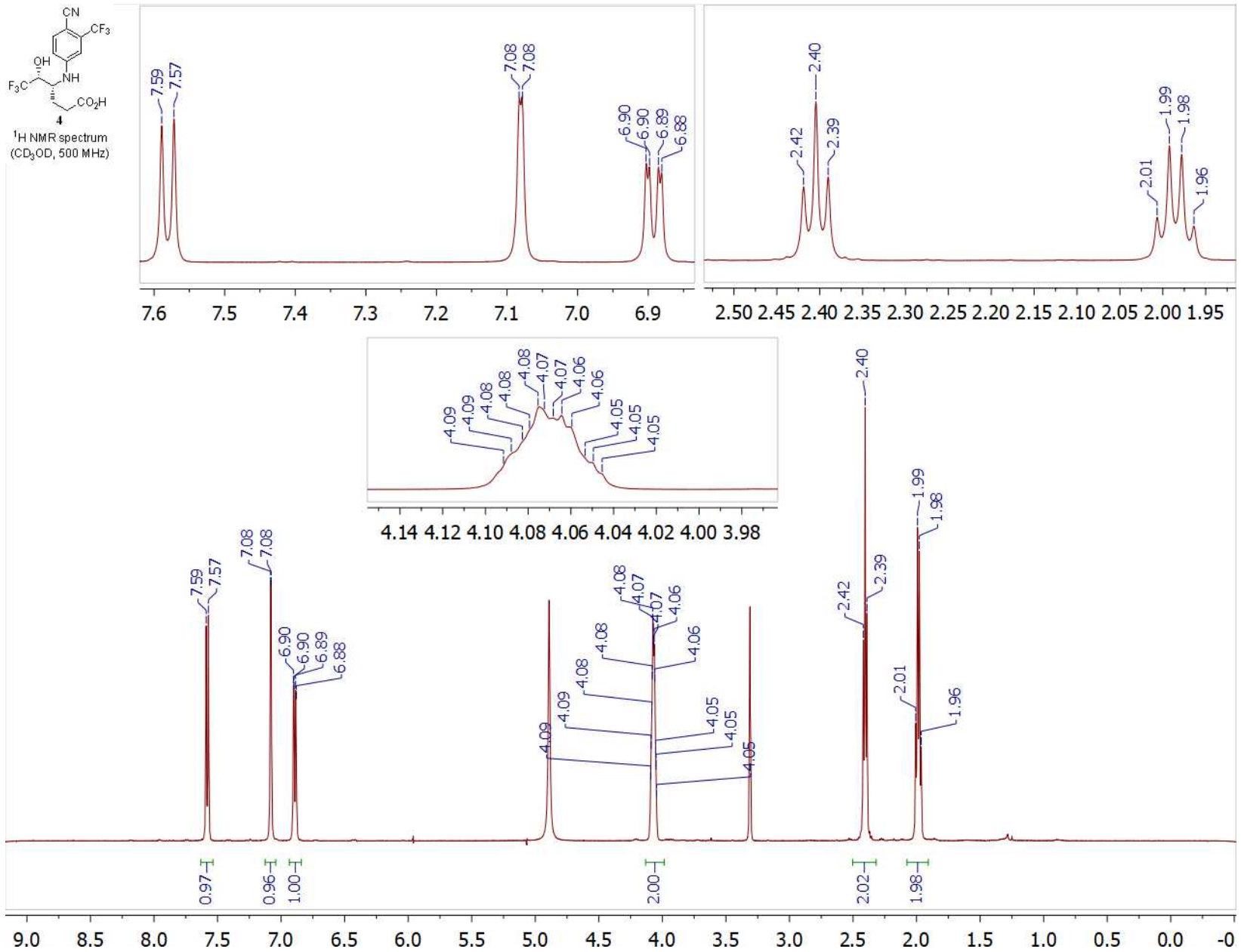


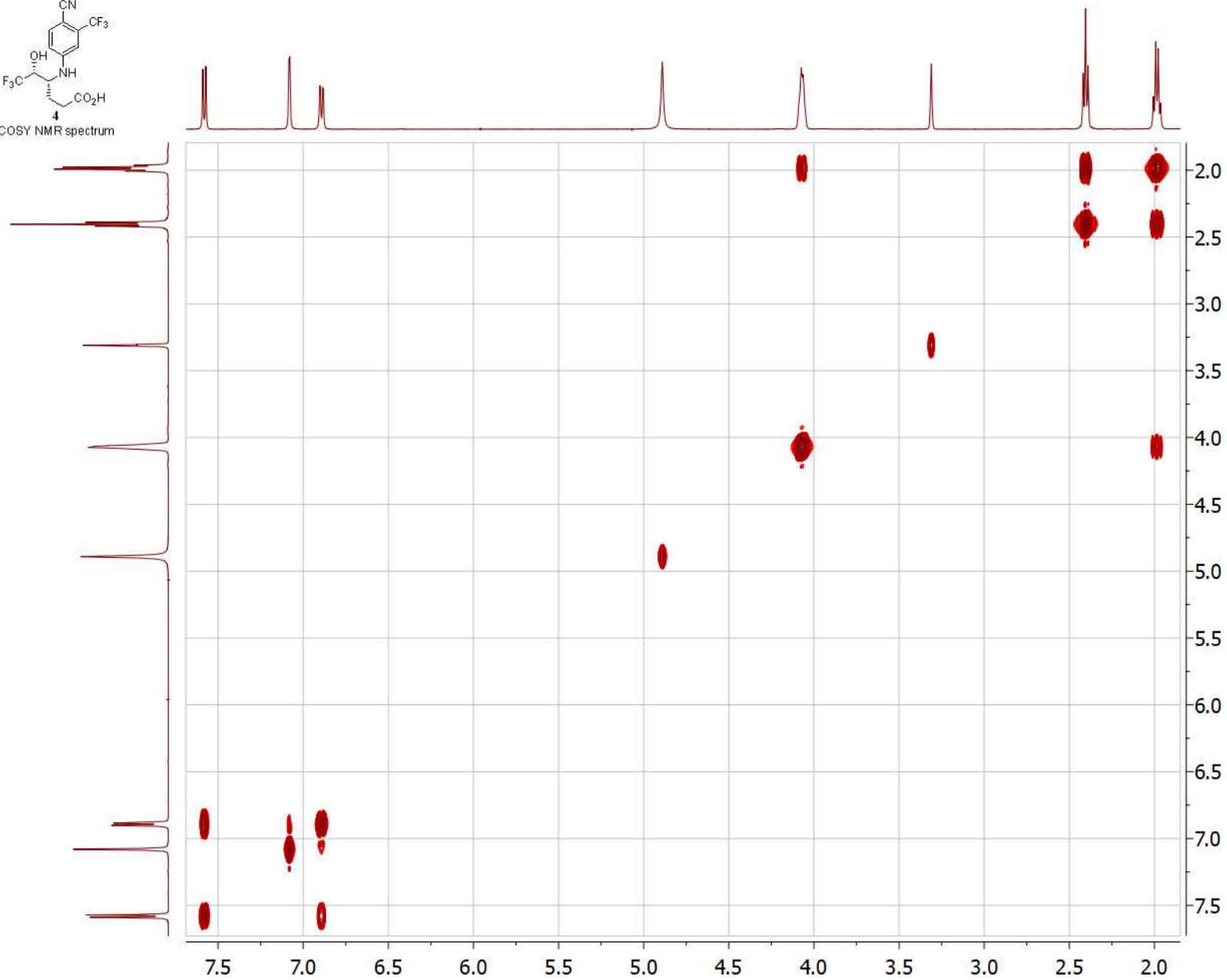
HMBC NMR spectrum

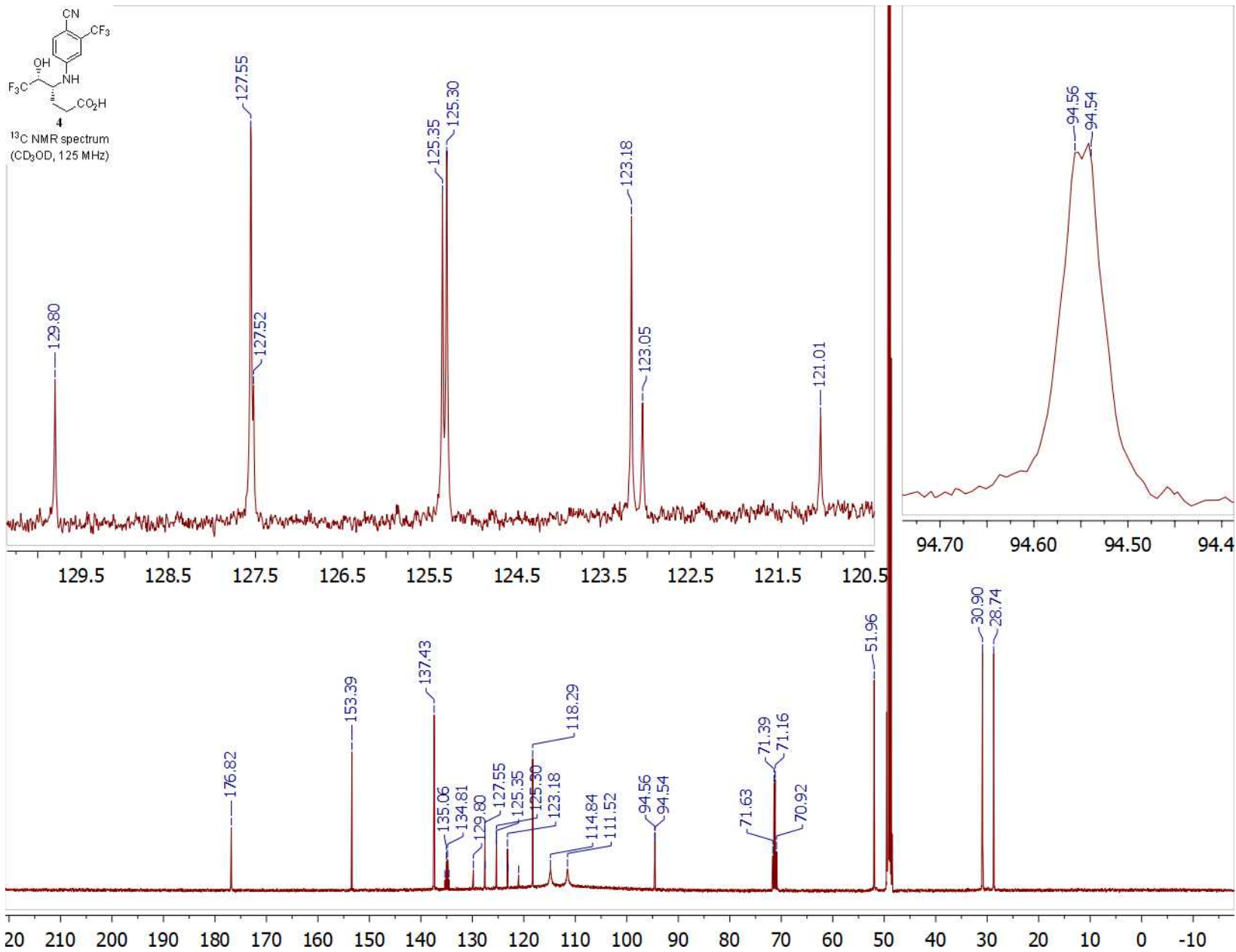


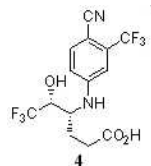


¹H NMR spectrum
(CD₃OD, 500 MHz)

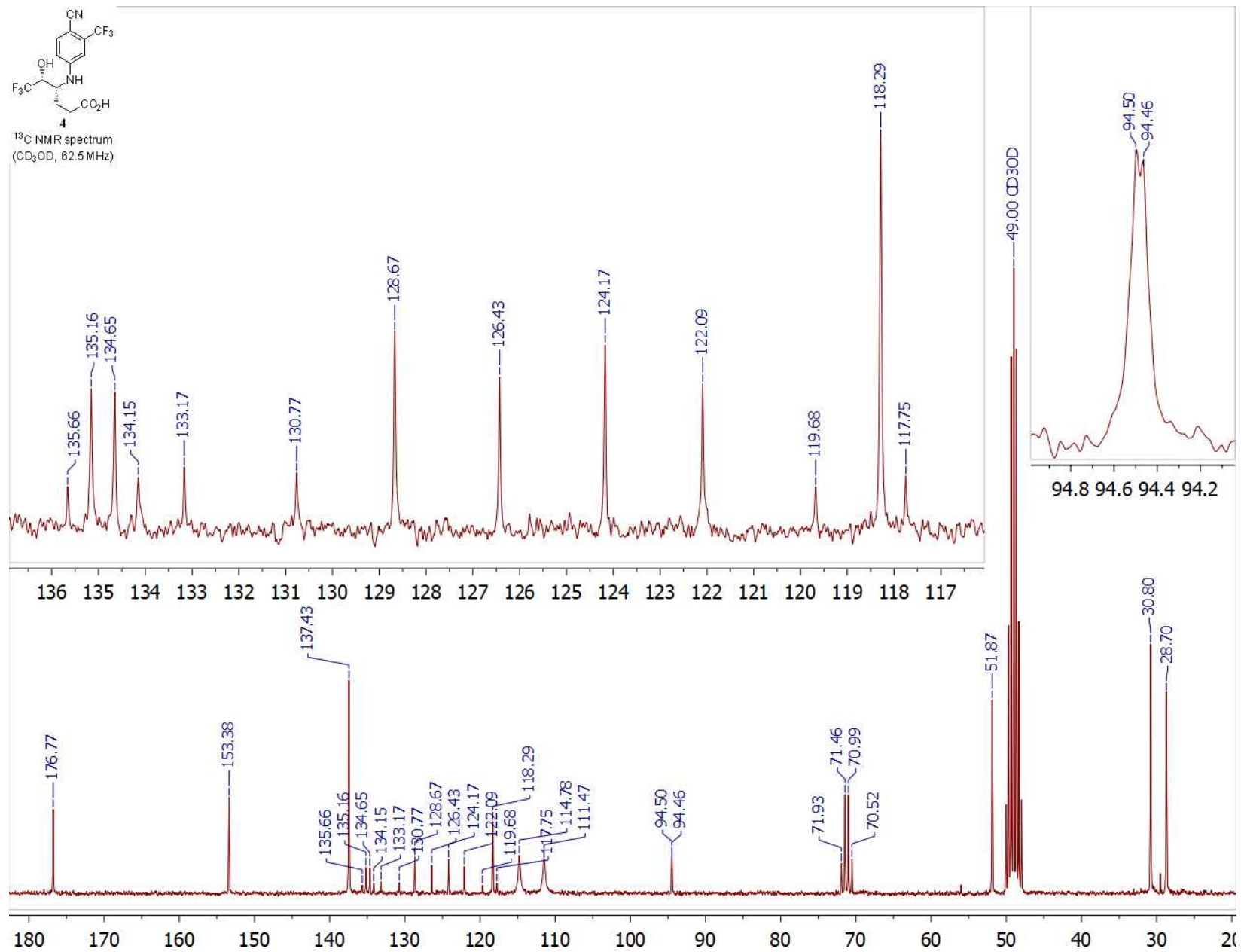


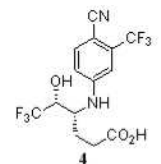




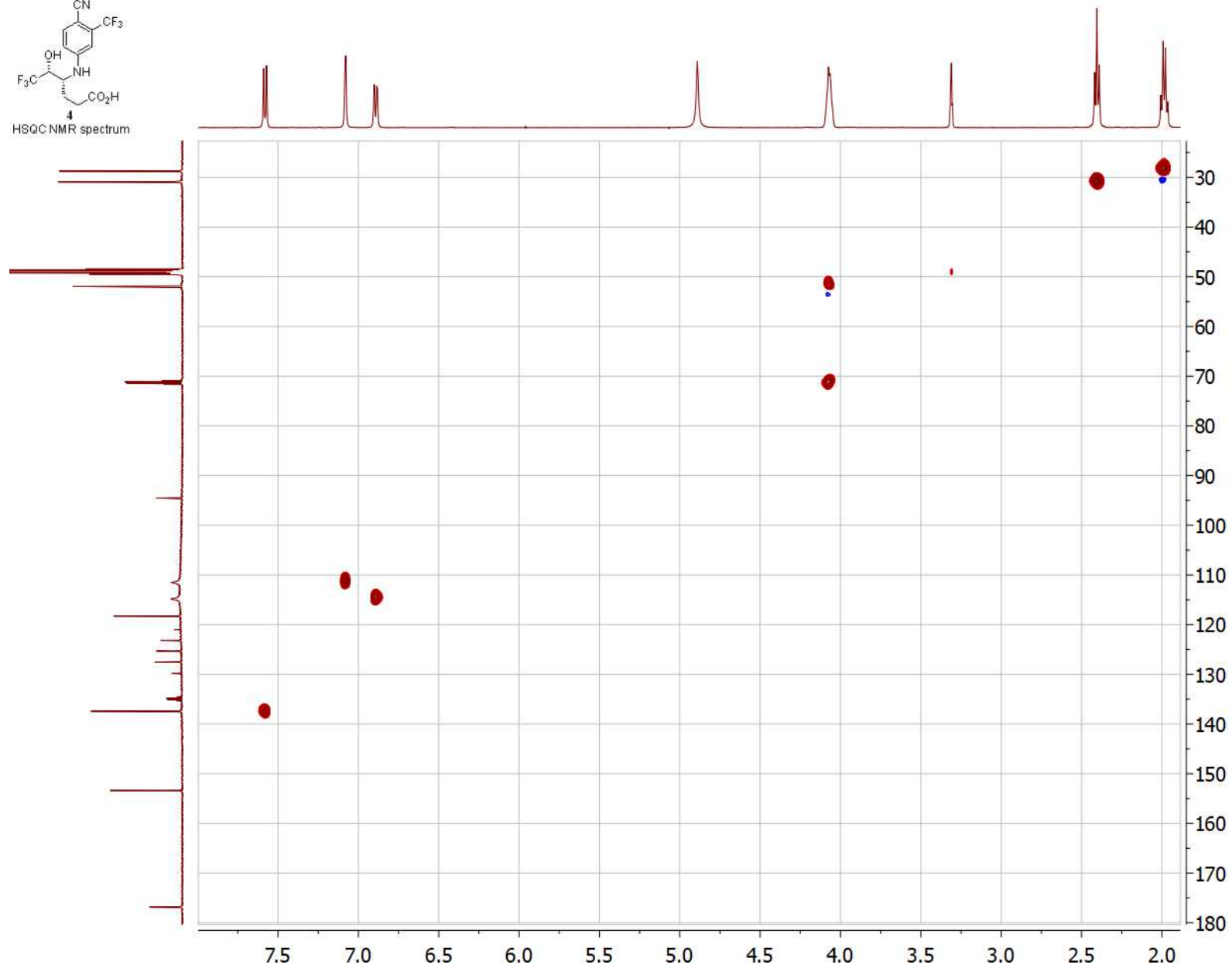


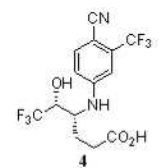
¹³C NMR spectrum
 (CD₃OD, 62.5 MHz)



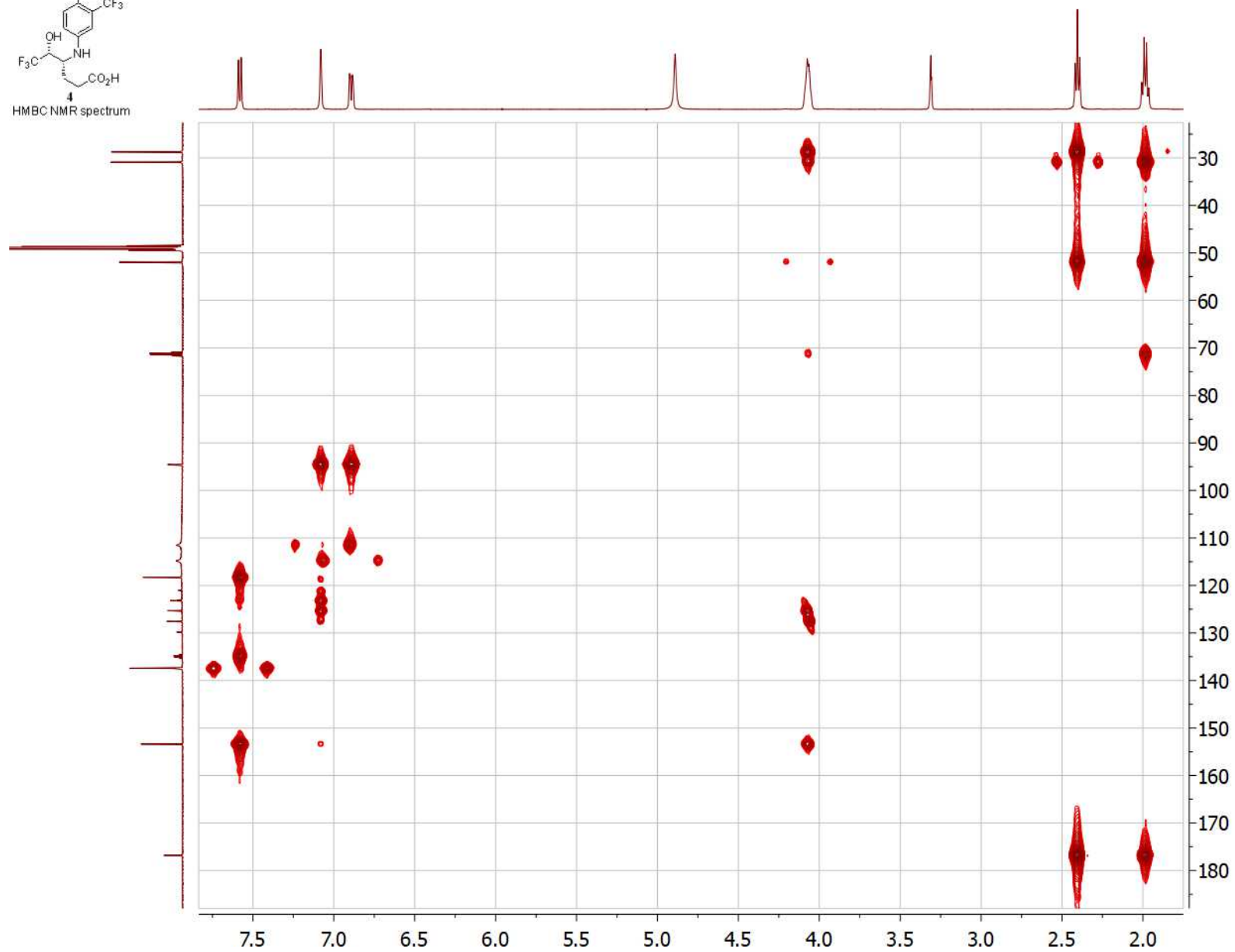


HSQC NMR spectrum



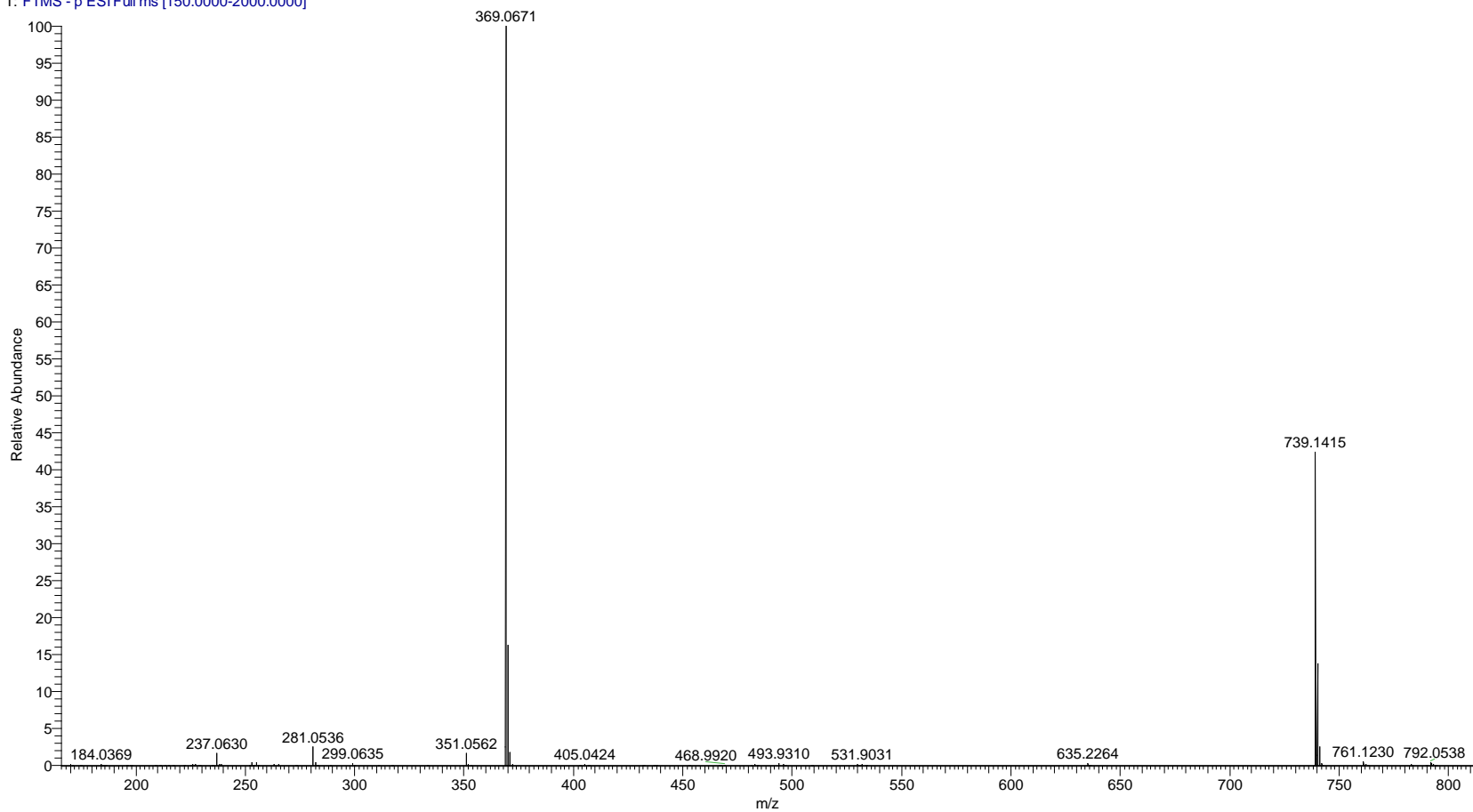


HMBC NMR spectrum

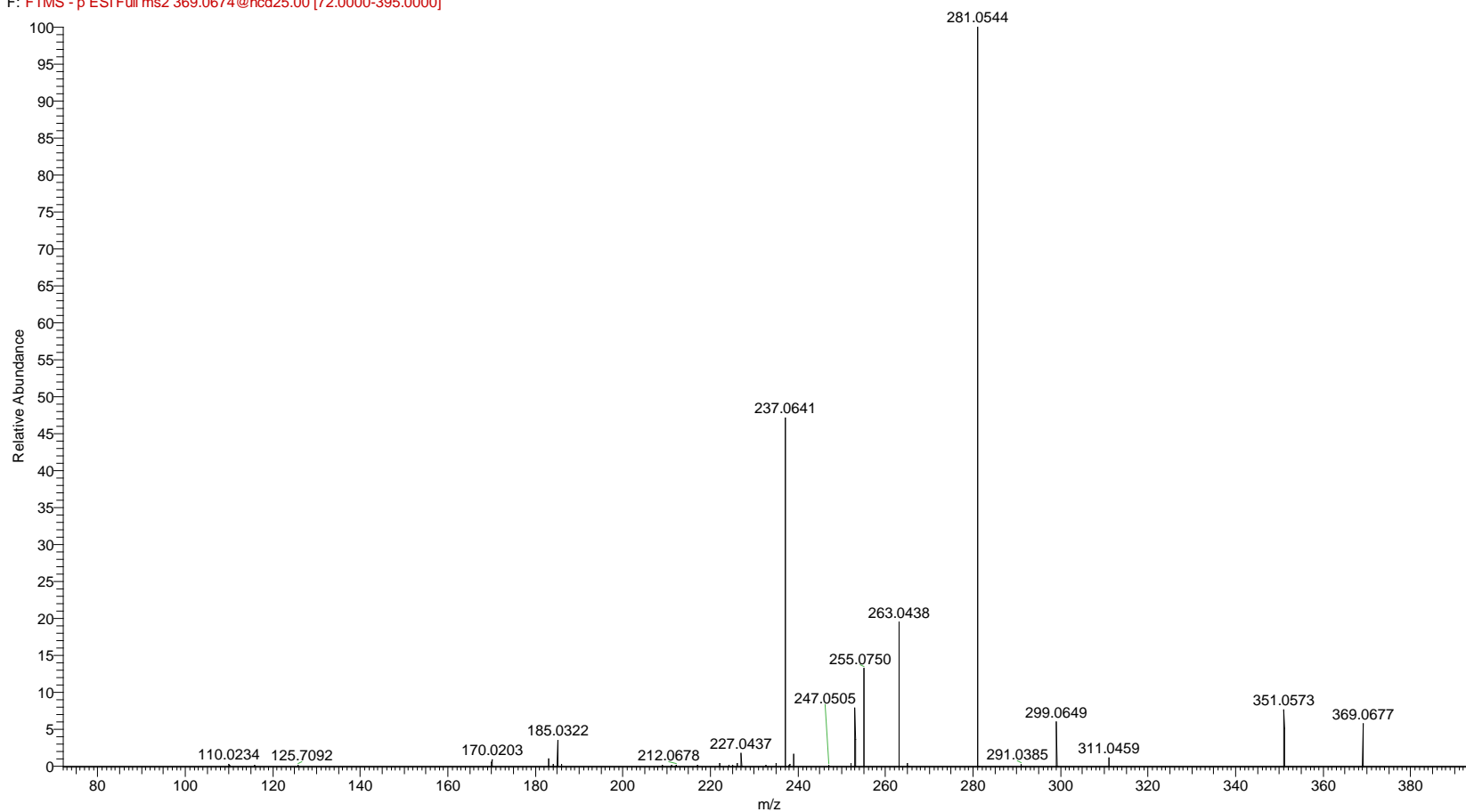


IV. LC-HRMS Analysis

L38_20220520180411 #690 RT: 6.11 AV: 1 NL: 7.08E8
T: FTMS - p ESI Full ms [150.0000-2000.0000]

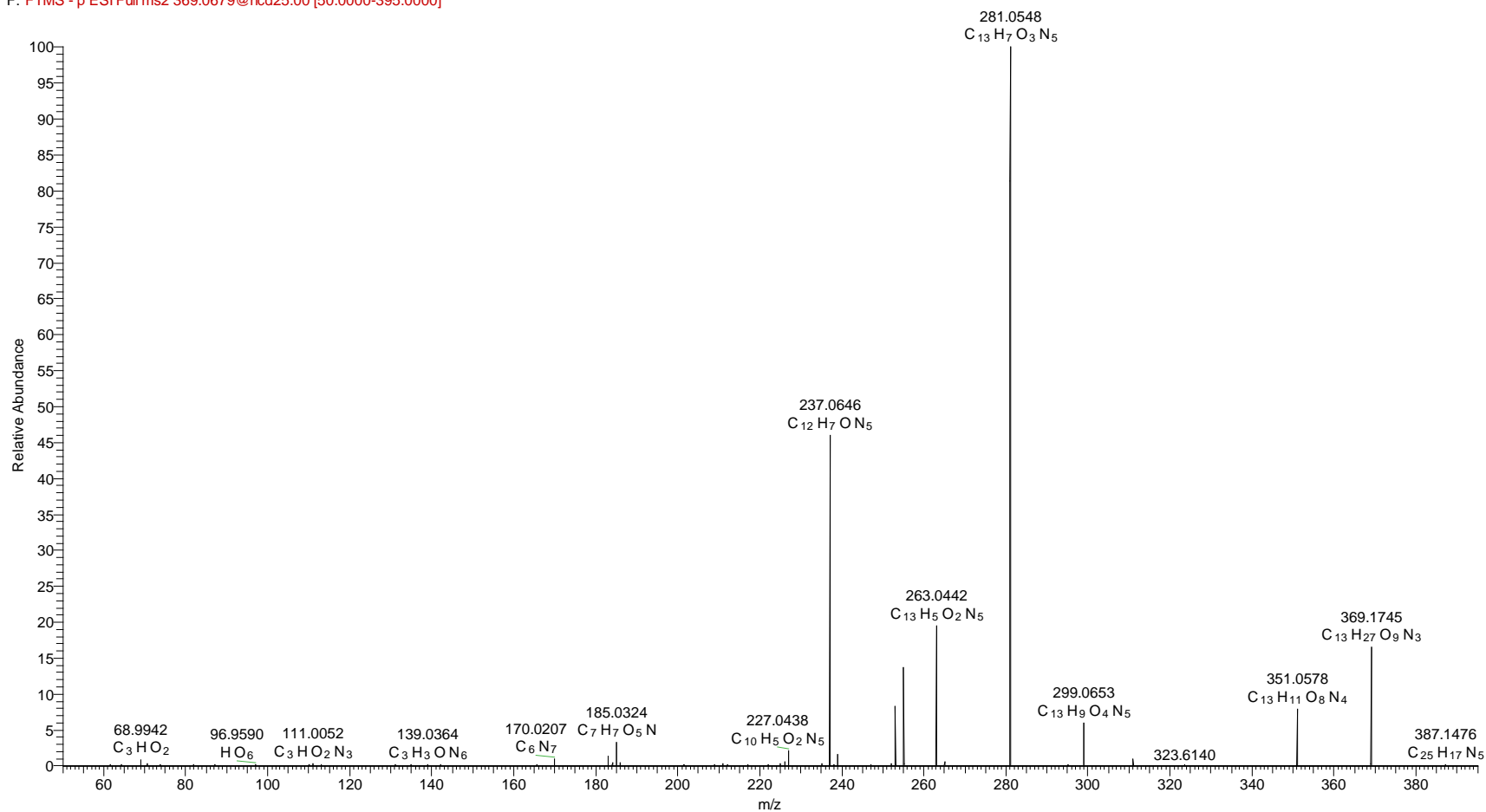


Full scan mass spectrum in negative ionization mode of (4*R*,5*R*)-4-[[4-cyano-3-(trifluoromethyl)phenyl]amino]-6,6,6-trifluoro-5-hydroxyhexanoic acid (**4**).



Product ion mass spectrum of the deprotonated molecular ion $[M-H]^-$ of compound **4** with m/z 369 at a collision energy of 25 eV.

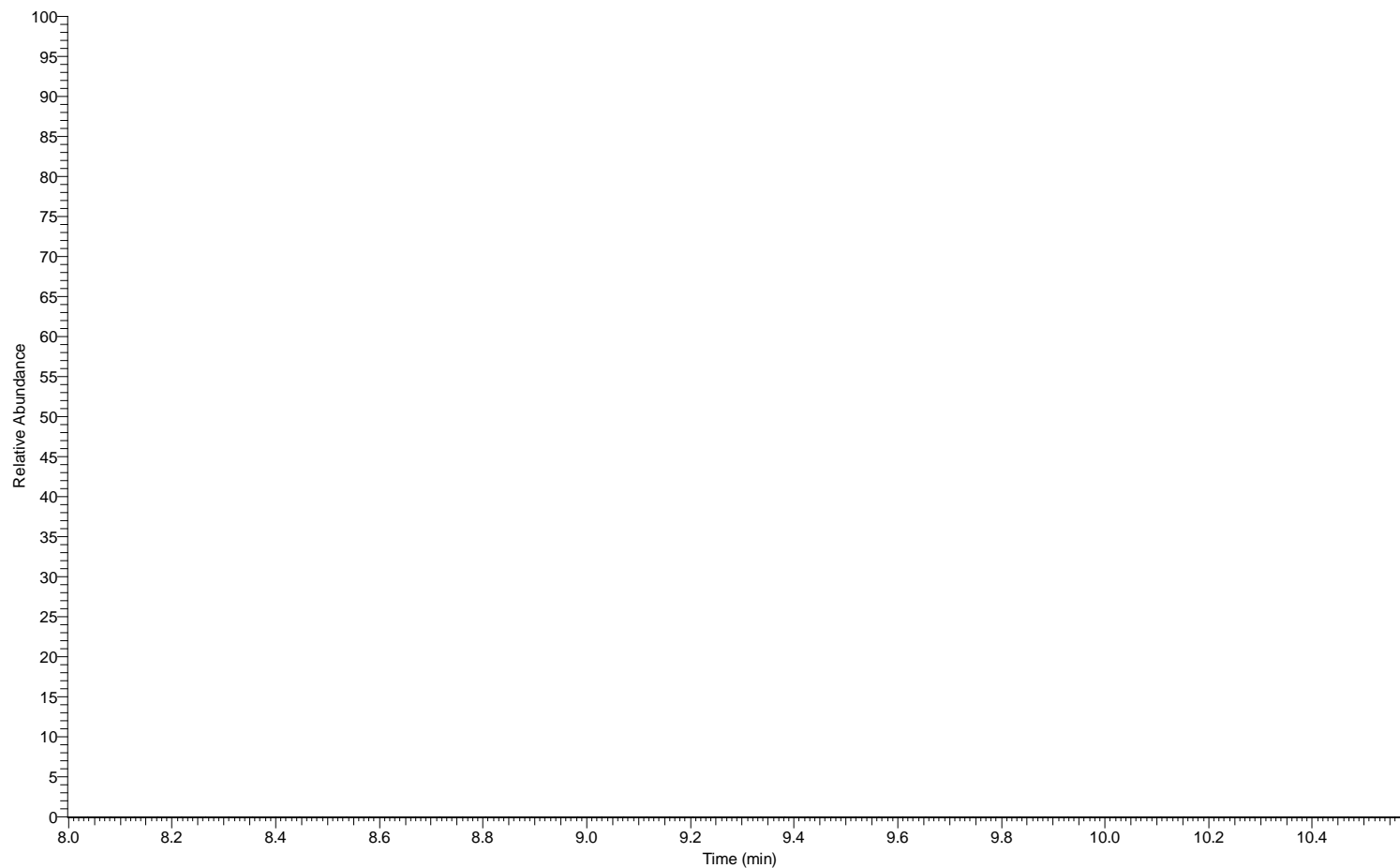
M1677 #1749 RT: 9.28 AV: 1 NL: 2.83E8
F: FTMS - p ESI Full ms2 369.0679@hcd25.00 [50.0000-395.0000]



Product ion mass spectrum of the anion with m/z 369 from a LGD-4033-positive human urine sample at a collision energy of 25 eV.

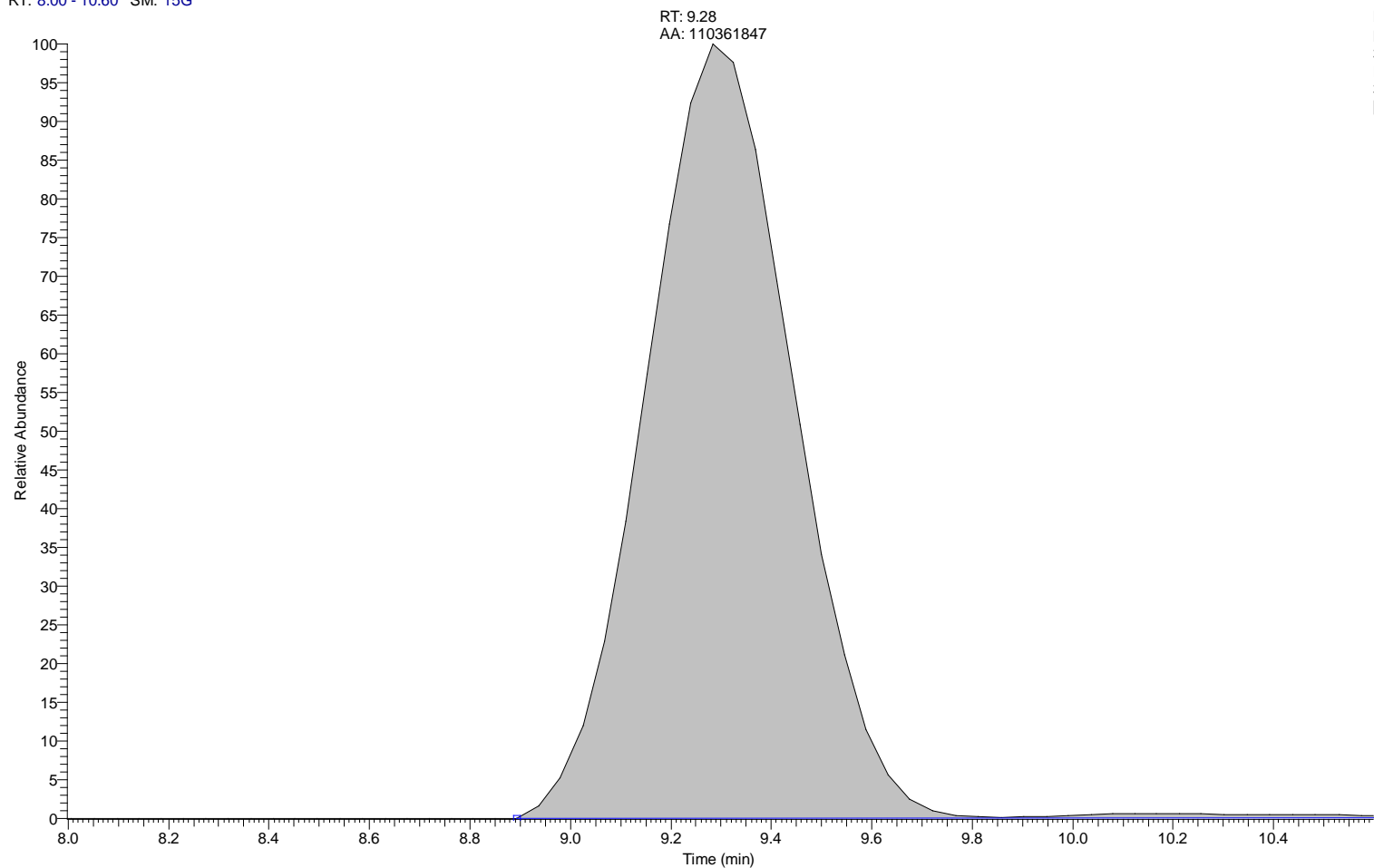
RT: 8.00 - 10.60 SM: 15G

NL: 0
Base Peak m/z=
369.0604-369.0752 F:
FTMS - p ESI Full ms2
369.0679@hcd25.00
[50.0000-395.0000] MS
ICIS BLANK1



Comparative analysis of 4-arylamino-5-hydroxyhexanoic acid **4** and a LGD-4033-positive human urine sample with LC–HRMS in negative ionization mode at the m/z 369: blank.

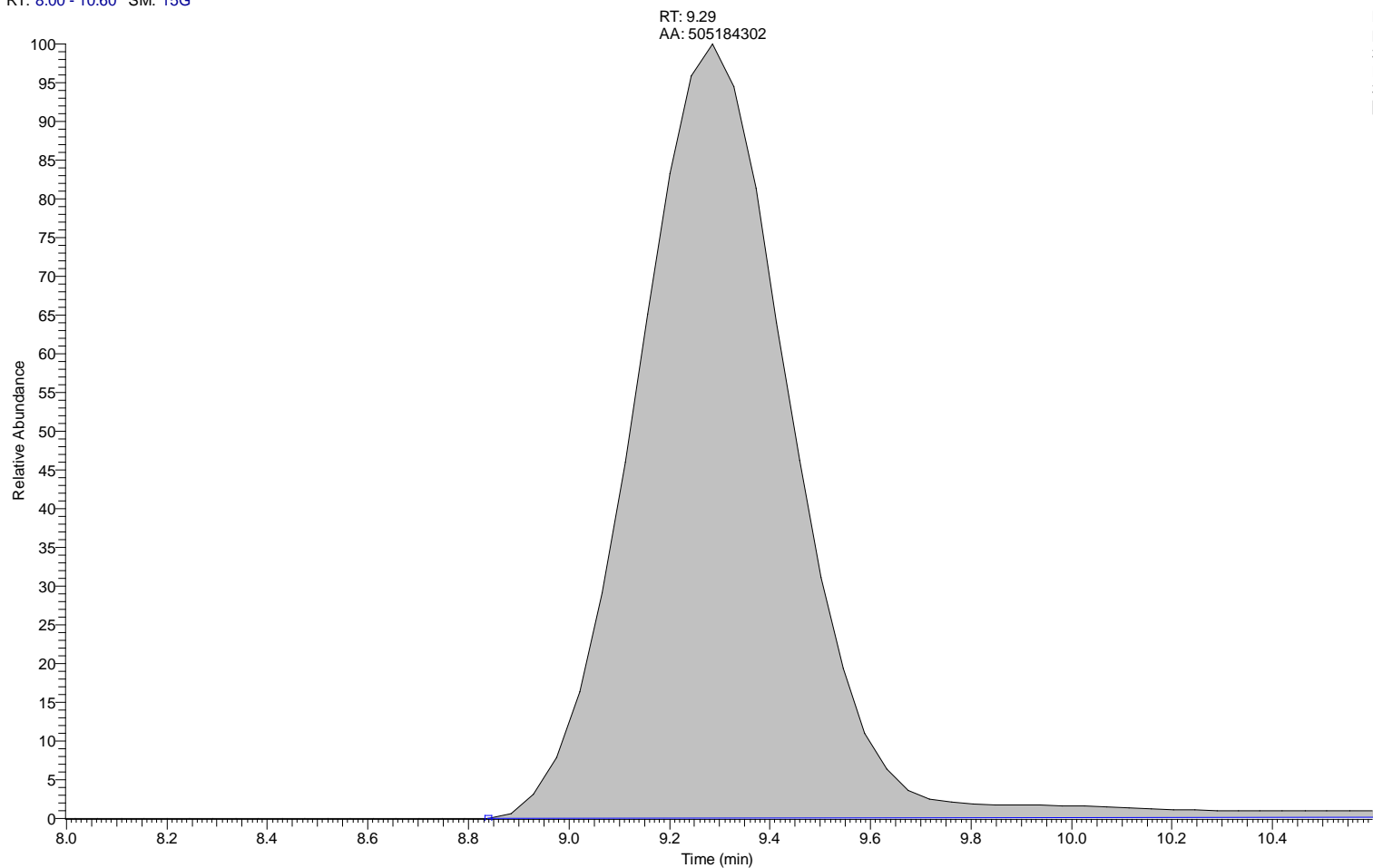
RT: 8.00 - 10.60 SM: 15G



NL: 5.28E6
Base Peak m/z=
369.0604-369.0752 F:
FTMS - p ESI Full ms2
369.0679@hcd25.00
[50.0000-395.0000] MS
ICIS M1677

Comparative analysis of 4-arylamino-5-hydroxyhexanoic acid **4** and a LGD-4033-positive human urine sample with LC–HRMS in negative ionization mode at the m/z 369: urine-derived sample.

RT: 8.00 - 10.60 SM: 15G

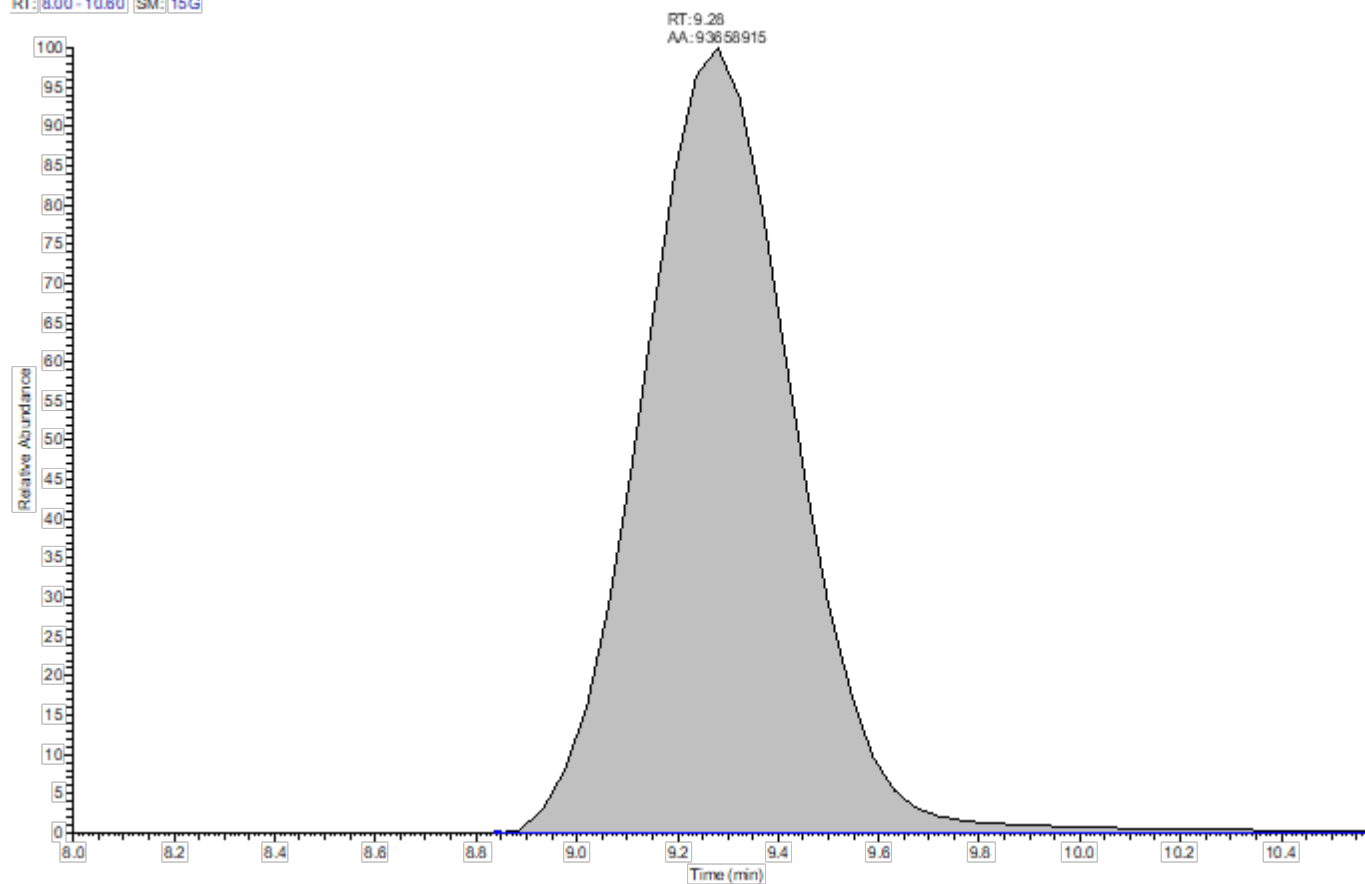


RT: 9.29
AA: 505184302

NL: 2.28E7
Base Peak m/z=
369.0604-369.0752 F:
FTMS - p ESI Full ms2
369.0679@hcd25.00
[50.0000-395.0000] MS
ICIS LC023

Comparative analysis of 4-arylamino-5-hydroxyhexanoic acid **4** and a LGD-4033-positive human urine sample with LC–HRMS in negative ionization mode at the m/z 369: synthetic metabolite **4**.

RT: 8.00-10.60 SM: 15G



NL: 4.38E6
Base Peak m/z=
369.0604-369.0752 F: FTMS -
p ESIFull ms2
369.0679@hcd25.00
[50.0000-395.0000] MS ICIS
M1677_AND_L023

Comparative analysis of 4-arylamino-5-hydroxyhexanoic acid **4** and a LGD-4033-positive human urine sample with LC–HRMS in negative ionization mode at the m/z 369: co-injection of **4** and urine-derived sample.