Design and Synthesis of Nonpeptidic, Small Molecule Inhibitors for the *Mycobacterium tuberculosis* Protein Tyrosine Phosphatase PtpB

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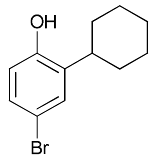
General Synthetic Methods

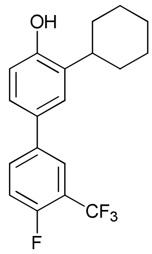
Unless otherwise noted, all reagents were obtained from commercial suppliers and used without further purification. Tetrahydrofuran (THF), dichloromethane (CH_2Cl_2), toluene, and diethyl ether (Et_2O) were dried over alumina under a nitrogen atmosphere. Solvents used for reactions set up in a nitrogen-filled Braun inert atmosphere box, including THF and toluene, were additionally degassed with three consecutive freeze pump thaw cycles and stored over 3Å molecular sieves. Methanol was dried over calcium hydride under a nitrogen atmosphere. All reactions, unless otherwise stated, were performed under inert atmosphere using syringe, cannula, and Schlenk techniques, or set up in a nitrogen-filled Braun inert atmosphere box, with flame or oven-dried glassware. All ^{1}H, ^{19}F, and ^{31}P NMR spectra were measured with a Bruker DRX-500, AVB-400, AVQ-400 or AV-300 spectrometer. NMR chemical shifts are reported in ppm relative to 1,2-difluorobenzene (-138.9) for ^{19}F NMR and trimethylphosphate (3.0) for ^{31}P NMR. Mass spectrometry (HRMS) was carried out by the University of California, Berkeley Mass Spectrometry Facility.

Synthesis of Isothiazolidinone Inhibitors 2, 15, and 16



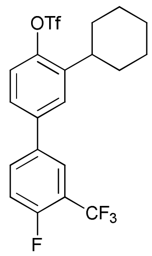
4

Compound 4. Compound **4** was synthesized as described in the literature.[1]



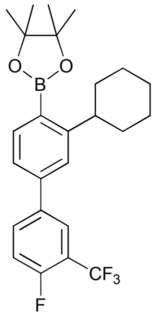
6

Compound 6. Compound **6** was synthesized as described in the literature.[1]



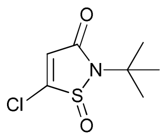
7

Compound 7. Compound **7** was synthesized as described in the literature.[1]



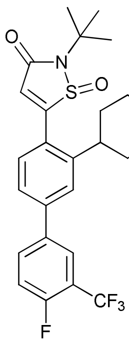
8

Compound 8. To a 10 mL Schlenk tube fitted with a stirbar in a nitrogen-filled Braun inert atmosphere box was added compound **7** (0.72 g, 1.53 mmol), followed by bis(pinacolato)diboron (1.16 g, 4.59 mmol), K_3PO_4 (0.97 g, 4.59 mmol), tris(dibenzylideneacetone)dipalladium-chloroform adduct (47 mg, 0.04 mmol, 3.0 mol%), and 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos, 38 mg, 0.08 mmol, 6.0 mol%). Toluene (3.06 mL) was then added and the reaction tube was closed under N_2 atmosphere. The resulting mixture was then heated with stirring in an oil bath at 110 °C for 22 hours. The reaction mixture was then diluted with Et_2O and passed through a pad of Celite. The solvent was removed under reduced pressure to provide crude **8**, which was purified via automated reversed-phase C18 chromatography (linear gradient of 80 to 95% acetonitrile in H_2O) to yield compound **8** (0.47 g, 69% yield) as an off-white solid; mp 81-83 °C; δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.82-7.73 (m, 3H), 7.41 (m, 1H), 7.33 (dd, *J* = 7.7, 1.6 Hz, 1H), 7.28-7.24 (m, 1H), 3.38-3.29 (m, 1H), 1.91-1.75 (m, 5H), 1.53-1.43 (m, 5H), 1.37 (s, 12H); δ_F(376 MHz; $CDCl_3$; 1,2-difluorobenzene) -60.51 (d, *J* = 6.3 Hz), -116.51 (m); HRMS *m/z* (EI) $[M + H]^+$ found 448.2197, $C_{25}H_{29}BF_4O_2$ requires 448.2189.



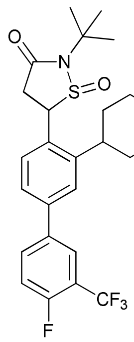
9

Compound 9. Compound **9** was synthesized via modified literature procedures.[2] Analytical data was found to match that of previous literature reports:[2] δ_H(400 MHz; $CDCl_3$; Me_4Si) 6.55 (s, 1H), 1.64 (s, 9H).



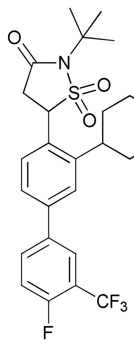
10

Compound 10. To a 1 mL Schlenk tube fitted with a stirbar in a nitrogen-filled Braun inert atmosphere box was added compound **8** (34 mg, 0.10 mmol), followed by compound **9** (41 mg, 0.15 mmol), K_3PO_4 (127 mg, 0.600 mmol), palladium acetate (3.4 mg, 0.02 mmol, 15 mol%), and 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos, 12 mg, 0.04 mmol, 30 mol%). A 10:1 THF:H_2O solution (0.20 mL) was then added and the reaction tube was closed under N_2 atmosphere. The resulting mixture was then heated with stirring in an oil bath at 60 °C for 24 h. The reaction mixture was then diluted with Et_2O and passed through a pad of Celite. The solvent was removed under reduced pressure to provide crude **10**, which was purified via automated silica gel chromatography (linear gradient of 2 to 15% EtOAc in hexanes) to yield compound **10** (34 mg, 69% yield) as a yellow solid; δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.79-7.77 (m, 2H), 7.54 (m, 1H), 7.45 (m, 2H), 7.33-7.30 (m, 1H), 6.48 (s, 1H), 2.80-2.70 (m, 1H), 1.95-1.73 (m, 5H), 1.72 (s, 9H), 1.55-1.30 (m, 5H); δ_F(376 MHz; $CDCl_3$; 1,2-difluorobenzene) -60.57 (d, *J* = 12.6 Hz), -115.18 (m); HRMS *m/z* (EI) $[M + H]^+$ found 494.1777, $C_{26}H_{28}F_4NO_2S$ requires 494.1772.



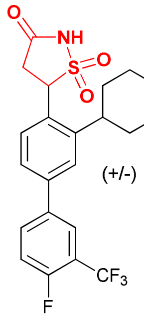
11

Compound 11. To a 10 mL flask fitted with a stirbar under N_2 was added compound **10** (263 mg, 0.530 mmol) and MeOH (1.77 mL), followed by cooling to 0 °C. Sodium borohydride (40 mg, 1.07 mmol) was then added and the resulting slurry was stirred at 0 °C for 2 h. The reaction was quenched at 0 °C by dropwise addition of a 10% solution of acetic acid in THF, with the flask open to the atmosphere. The mixture was concentrated to remove MeOH to give crude **11**, which was purified by recrystallization from EtOAc/MeOH to give compound **11** (219 mg, 83% yield) as an off-white solid; δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.90-7.68 (m, 2H), 7.55 (d, *J* = 8.2 Hz, 1H), 7.47 (d, *J* = 1.7 Hz, 1H), 7.42 (dd, *J* = 8.1, 1.8 Hz, 1H), 7.31-7.24 (m, 1H), 7.31-7.24 (m, 1H), 4.64 (dd, *J* = 12.0, 7.1 Hz, 1H), 3.57 (dd, *J* = 17.1, 12.0, 1H), 3.02 (dd, *J* = 17.0, 7.1 Hz, 1H), 2.84-2.79 (m, 1H), 1.98-1.77 (m, 5H), 1.65 (s, 9H), 1.63-1.50 (m, 5H); δ_F(376 MHz; $CDCl_3$; 1,2-difluorobenzene) -60.50 (d, *J* = 12.4 Hz), -115.96 (m); HRMS *m/z* (ESI) $[M + H]^+$ found 496.1928, $C_{26}H_{30}F_4NO_2S$ requires 496.1855.



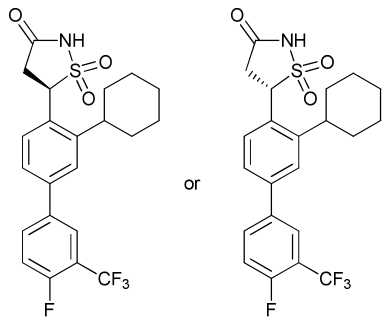
12

Compound 12. Compound **11** (219 mg, 0.44 mmol) was added to a flask under N_2, dissolved in chloroform (5.5 mL), and cooled to 0 °C. 3-Chloroperoxybenzoic acid (>77%, 197 mg, 0.88 mmol) was added at 0 °C, and the reaction was allowed to warm to ambient temperature and stirred for 18 h. The reaction was quenched at 0 °C by dropwise addition of aqueous saturated $NaHCO_3$, follwed by extraction with $NaHCO_3$ (5 x 5 mL), and washing with brine (1 x 5 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The solvent was removed under reduced pressure to provide crude **12**, which was purified via automated silica gel chromatography (linear gradient of 5-20% EtOAc in hexanes) to yield compound **12** (117 mg, 56% yield) as a white solid; mp 153-155 °C; δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.80-7.70 (m, 2H), 7.51 (d, *J* = 1.3 Hz, 1H), 7.48-7.40 (m, 2H), 7.33-7.26 (m, 1H), 5.24 (dd, *J* = 8.3 Hz, 1H), 3.31 (dd, *J* = 17.2, 8.6 Hz, 1H), 3.20 (dd, *J* = 17.1, 7.9 Hz, 1H), 2.95-2.82 (m, 1H), 2.05-1.75 (m, 5H), 1.68 (s, 9H), 1.65-1.40 (m, 5H); δ_F(376 MHz; $CDCl_3$; 1,2-difluorobenzene) -60.56 (d, *J* = 15.1 Hz), -115.56 (m); HRMS *m/z* (EI) $[M + H]^+$ found 511.1804, $C_{26}H_{29}F_4NO_3S$ requires 511.1803.



2

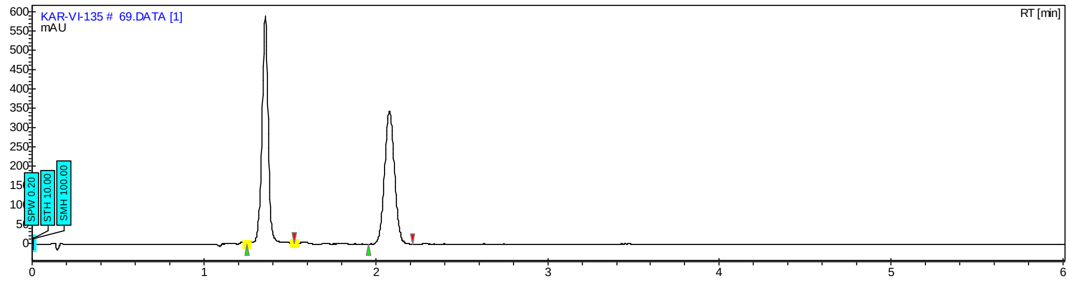
Compound 2. To a J-Young tube was added compound **12** (21 mg, 0.04 mmol) as a solution in d_4-TFA (0.55 mL), which was sealed and heated to 80 °C in an oil bath. The reaction was monitored by NMR until complete conversion of starting material was observed. The resulting mixture was concentrated to give crude **2**, which was dissolved in a minimal volume of dimethylsulfoxide (1.0 mL) and purified via automated reversed-phase C18 column chromatography (linear gradient of 15 to 95% acetonitrile in H_2O with 0.1% trifluoroacetic acid) to give pure **2** (12 mg, 62% yield) as a white powder; mp 188-189 °C; δ_H(400 MHz; CD_3OD) 7.86-7.75 (m, 2H), 7.53-7.47 (m, 2H), 7.44 (dd, *J* = 4.1, 1.8 Hz, 1H), 7.33 (t, *J* = 9.6 Hz, 1H), 5.55 (t, *J* = 8.4 Hz, 1H), 3.33 (dd, *J* = 17.4, 8.2 Hz, 1H), 3.27 (dd, *J* = 17.4, 8.2 Hz, 1H), 2.99-2.90 (m, 1H), 1.90-1.66 (m, 5H), 1.59-1.22 (m, 5H); δ_F(376 MHz; CD_3OD; 1,2-difluorobenzene) -62.06 (d, *J* = 12.8 Hz), -118.48 (m); HRMS *m/z* (FAB) $[M - H]^-$ found 454.1091, $C_{22}H_{20}F_4NO_3S$ requires 454.1106.



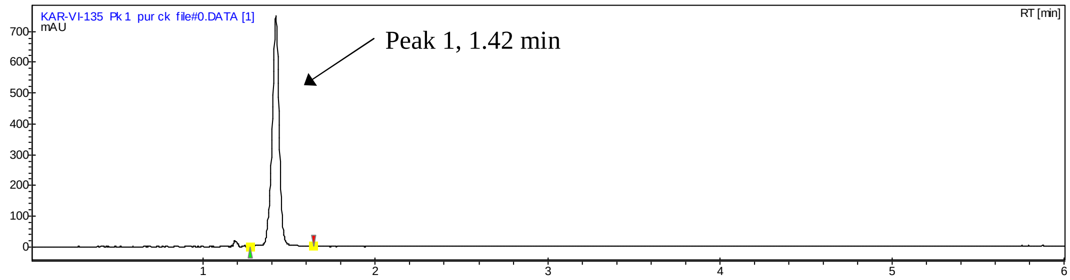
15 **16**

Compounds 15 and 16. Compound **12** was separated into enantiomerically pure isothiazolidinones **15** and **16** with >99% chemical purity and >99% ee by Lotus Separations, LLC using chiral preparatory supercritical fluid chromatography. The racemic compound was loaded as a 7 mg/mL solution in methanol, with an injection volume of 0.7 mL, onto a Chiralpak AD-H column (2 x 15 cm), and eluted with 35% isopropanol/CO_2 at 100 bar, with a flowrate of 65 mL/min. Peaks were visualized using UV at 220 nm. Analytical chromatograms were obtained by injecting compound solutions onto a Chiralpak AD-H column (25 x 0.46 cm), followed by elution with 35% isopropanol/CO_2 at a flowrate of 3 mL/min.

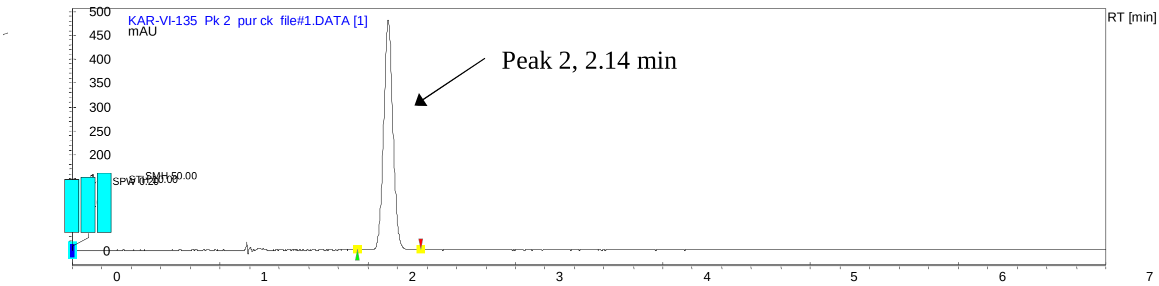
HPLC trace of compound **2** (racemic IZD)

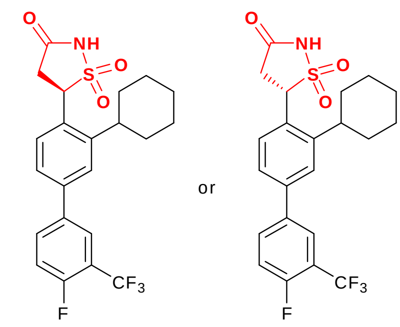


HPLC trace of enantiomerically pure **15** or **16** (“peak 1”)



HPLC trace of enantiomerically pure **15** or **16** (“peak 2”)

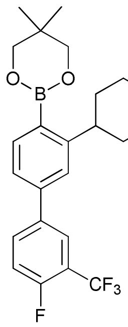




17 **18**

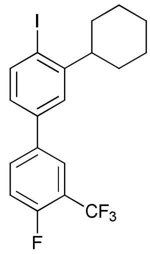
Compounds 17 and 18. Compounds **17** and **18** were synthesized via the procedure described for compound **2**, starting from enantiomerically pure compounds **15** and **16**.

Synthesis of Difluoromethylphosphonic Acid Inhibitor 3



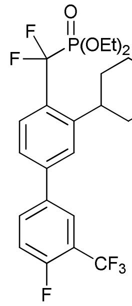
19

Compound 19. To a 5 mL Schlenk tube fitted with a stirbar in a nitrogen-filled Braun inert atmosphere box was added compound **7** (100 mg, 0.21 mmol), followed by bis(neopentylglycolato)diboron (96 mg, 0.43 mmol), K_3PO_4 (90 mg, 0.43 mmol), tris(dibenzylideneacetone)dipalladium-chloroform adduct (13 mg, 0.01 mmol, 6.0 mol%), and 2-dicyclohexylphosphino-2',6'-dimethoxybiphenyl (SPhos, 11 mg, 0.02 mmol, 12.0 mol%). Toluene (0.43 mL) was added, and the reaction tube was closed under an N_2 atmosphere. The resulting mixture was then heated with stirring in an oil bath at 100 °C for 26 h. The reaction mixture was diluted with Et_2O and passed through a pad of Celite. The solvent was removed under reduced pressure to provide crude **19**, which was purified via automated silica gel chromatography (linear gradient of 5 to 20% EtOAc in hexanes) to yield compound **19** (63 mg, 92% pure by NMR) as a yellow-orange solid, which was taken on without further purification; δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.81-7.71 (m, 3H), 7.42-7.40 (m, 1H), 7.32 (dd, *J* = 7.7, 1.8 Hz, 1H), 7.28-7.22 (m, 1H), 3.80 (s, 4H), 3.33-3.23 (m, 1H), 1.96-1.73 (m, 5H), 1.53-1.36 (m, 5H), 1.07 (s, 6H); δ_F(376 MHz; $CDCl_3$; 1,2-difluorobenzene) -60.50 (d, *J* = 12.5 Hz), -116.83 (m).



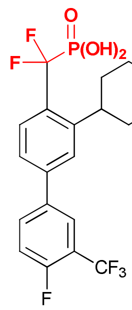
20

Compound 20. Compound **19** (416 mg, 0.96 mmol) was added to a flask under N_2 and dissolved in THF (4.8 mL). Sodium iodide was then added as a 1.0 N solution in water (1.2 mL) followed by chloramine-T (541 mg, 1.92 mmol). The resulting mixture was stirred vigorously for 15 min at room temperature. The reaction was quenched by addition of H_2O, and the aqueous layer extracted with Et_2O (3 x 5 mL). The organic layer was dried over anhydrous Na_2SO_4(s) and filtered. The solvent was removed under reduced pressure to provide crude **20**, which was purified via automated silica gel chromatography (linear gradient of 3-10% EtOAc in hexanes) to yield compound **20** (188 mg, 71% pure by NMR) as a white solid, which was taken on without further purification; δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.90 (d, *J* = 8.1 Hz, 1H), 7.78-7.66 (m, 2H), 7.35-7.23 (m, 2H), 7.04 (dd, *J* = 8.1, 2.2 Hz, 1H), 2.85-2.80 (m, 1H), 2.02-1.75 (m, 5H), 1.58-1.32 (m, 5H); δ_F(376 MHz; $CDCl_3$; 1,2-difluorobenzene) -60.55 (d, *J* = 12.5 Hz), -115.90 (m).



21

Compound 21. To a 25 mL flame-dried flask fitted with a stirbar under an N_2 atmosphere was added activated zinc dust (500 mg, 7.65 mmol), followed by *N,N*-dimethylacetamide (3.8 mL). The resulting mixture was heated to 60 °C in an oil bath with stirring. In a separate flask under an N_2 atmosphere, diethyl(bromodifluoromethyl)phosphonate (1.36 mL, 7.65 mmol) was dissolved in *N,N*-dimethylacetamide (3.8 mL), and this solution was added to the zinc mixture dropwise at 60 °C. The resulting mixture was stirred for 10 min at 60 °C, followed by stirring at ambient temperature for 4 h. In a separate 10 mL flask fitted with a stirbar under an N_2 atmosphere, compound **20** (135 mg, 0.30 mmol) and CuBr (86 mg, 0.60 mmol) were dissolved in *N,N*-dimethylacetamide (0.1 mL), followed by stirring for 30 min at ambient temperature. This solution was then added dropwise to the zinc solution, and the resulting mixture was sonicated for 12 h at ambient temperature. The reaction was quenched by addition of H_2O (10 mL), and the resulting mixture was diluted with Et_2O (15 mL), and filtered through Celite. The aqueous layer was extracted with Et_2O (3 x 15 mL), and the organic layer was washed with brine (1 x 75 mL), dried over anhydrous Na_2SO_4(s) and filtered. The solvent was removed under reduced pressure to provide crude **21**, which was purified via automated silica gel chromatography (linear gradient of 6-25% EtOAc in hexanes) to yield compound **21** (77 mg, 50% yield) as a white solid; δ_H(400 MHz; $CDCl_3$; Me_4Si) 7.81-7.70 (m, 2H), 7.63-7.58 (m, 1H), 7.57-7.53 (m, 1H), 7.43-7.38 (m, 1H), 7.32-7.26 (m, 1H), 4.32-4.12 (m, 4H), 3.28-3.17 (m, 1H), 1.96-1.73 (m, 5H), 1.56-1.38 (m, 5H), 1.35 (t, *J* = 7.1 Hz, 6H); δ_F(376 MHz; $CDCl_3$; 1,2-difluorobenzene) -60.57 (d, *J* = 12.6 Hz), -100.766 (d, J_{FP} = 116.3 Hz), -115.51 (m); δ_P(162 MHz; CD_3OD; trimethylphosphate) 6.29 (t, J_{PF} = 116.2 Hz); HRMS *m/z* (EI) $[M + H]^+$ found 509.1680, $C_{24}H_{28}F_6O_3P$ requires 509.1690.



3

Compound 3. To a 5 mL flame-dried flask fitted with a stirbar under an N_2 atmosphere was added compound **21** (72 mg, 0.14 mmol) and $CHCl_3$ (0.24 mL). To the resulting solution was added iodotrimethylsilane (61 μL, 0.43 mmol) dropwise by syringe. The resulting solution was stirred at ambient temperature for 14 h, and then the solvent was removed under reduced pressure to give crude **3** as an orange oil. The crude oil was dissolved in a minimal amount of dimethylsulfoxide (1.0 mL) which was purified by automated reversed-phase C18 column chromatography (linear gradient of 5 to 95% acetonitrile in H_2O with 0.1% trifluoroacetic acid) to give compound **3** (42 mg, 66% yield) as a white powder; mp 149-150 °C; δ_H(400 MHz; CD_3COCD_3) 8.08-7.96 (m, 2H), 7.83-7.77 (m, 1H), 7.65-7.47 (m, 2H), 6.61 (br s, 2H), 3.41-3.29 (m, 1H), 1.93-1.23 (m, 10H); δ_F(376 MHz; CD_3COCD_3; 1,2-

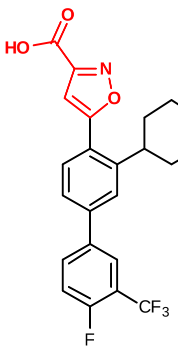
difluorobenzene) -61.03 (d, J = 12.6 Hz), -102.52 (d, J_{FP} = 113.7 Hz), -118.01 (m); δ_P(162 MHz; CD_3COCD_3; trimethylphosphate) 5.96 (t, J_{PF} = 113.6 Hz); HRMS m/z (EI) $[M + H]^+$ found 475.0874, $C_{20}H_{19}F_6O_3PNa$ requires 475.0873.

Assay Procedure and Determination of Inhibitor K_i

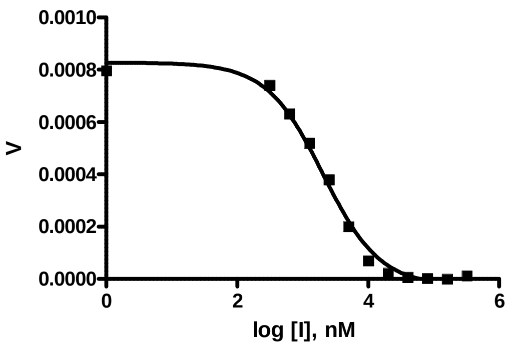
96-well plates were used to run K_i assays, with reaction volumes of 100 µL per well. 45 µL of water was added to each well, followed by 20 µL of sodium citrate buffer (stock solution: 100 mM sodium citrate, pH 6.2, 0.02% Triton X-100), 5 µL of 20 mM ethylenediamine tetraacetic acid (EDTA) stock solution, 5 µL of 20 mM DL-dithiothreitol (DTT) stock solution, and 10 µL of 1 µM PtpB stock solution. Then 5 µL of the appropriate inhibitor stock solutions, serially diluted 2-fold for a total of 10 different concentrations in DMSO, plus one blank well as a control (DMSO only) was added to the wells and the plate was incubated at room temperature for 5 minutes. The reaction was started by addition of 10 µL of 2 mM pNPP substrate stock, and reaction progress was monitored at 405 nm with continued incubation at ambient temperature. The initial rate data collected was used for the determination of K_i values. The kinetic values were obtained from nonlinear regression of substrate-velocity curves in the presence of various concentrations of inhibitor using the equation v = V_{max}*[S]/(K_M((1+[I])/ K_i)+[S]).

Analytical Data for Determination of Inhibitor K_i

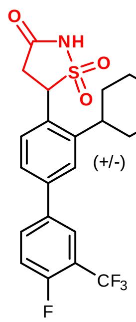
Compounds **1-3** were assayed in duplicate, and repeated in at least triplicate. Compounds **16** and **17** were assayed in duplicate. Example K_i curves are provided, along with average K_i values and average Hill slopes for each inhibitor.



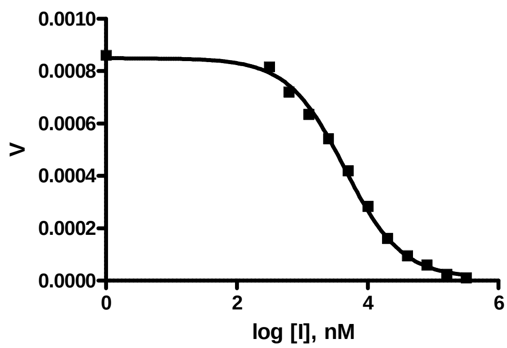
1



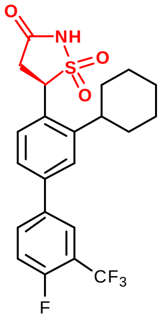
K_i (average) = 1.3 ± 0.6
Hill slope (average) = -1.2 ± 0.1



2

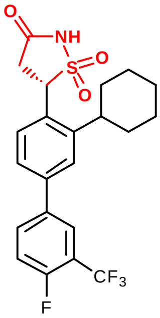


K_i (average) = 3.7 ± 0.6
Hill slope (average) = -0.9 ± 0.1

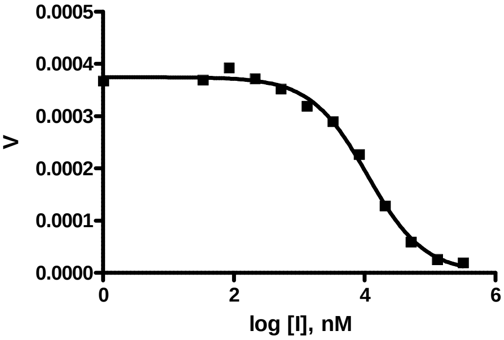


16

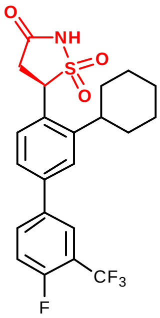
or



17

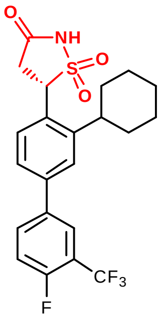


K_i (average) = 8.0 ± 0.4
Hill slope (average) = -1.0 ± 0.0

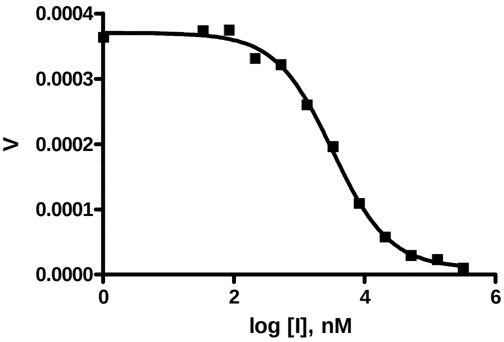


16

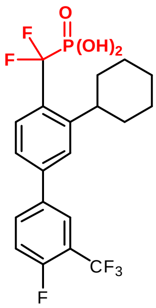
or



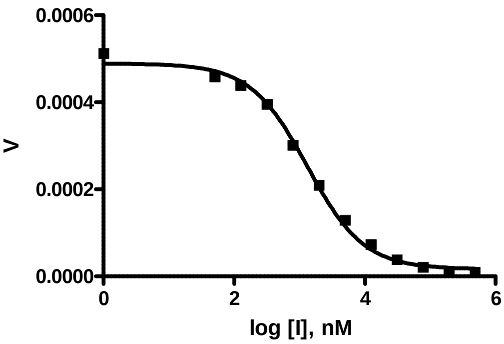
17



K_i (average) = 2.4 ± 0.0
Hill slope (average) = -0.9 ± 0.1



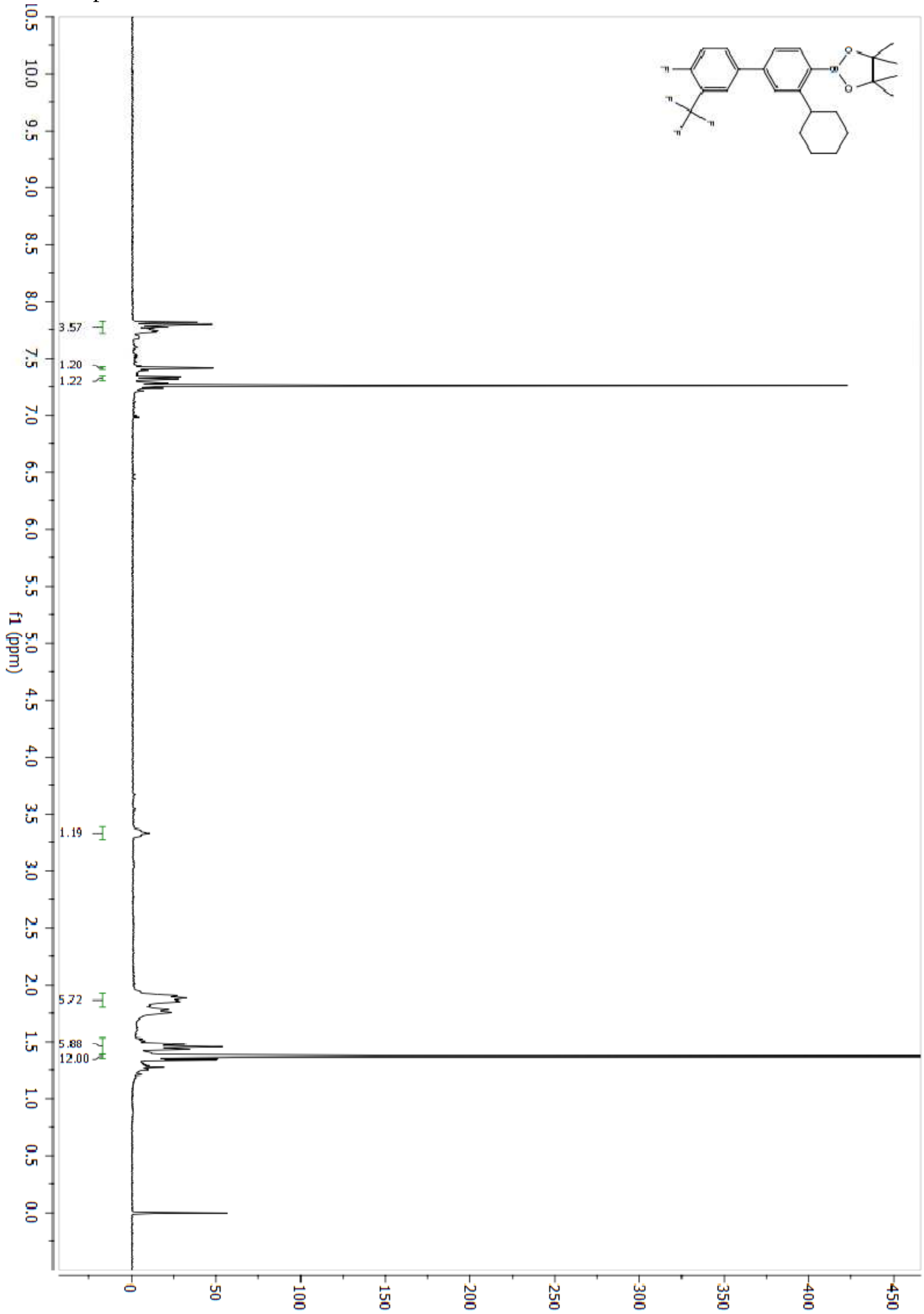
3



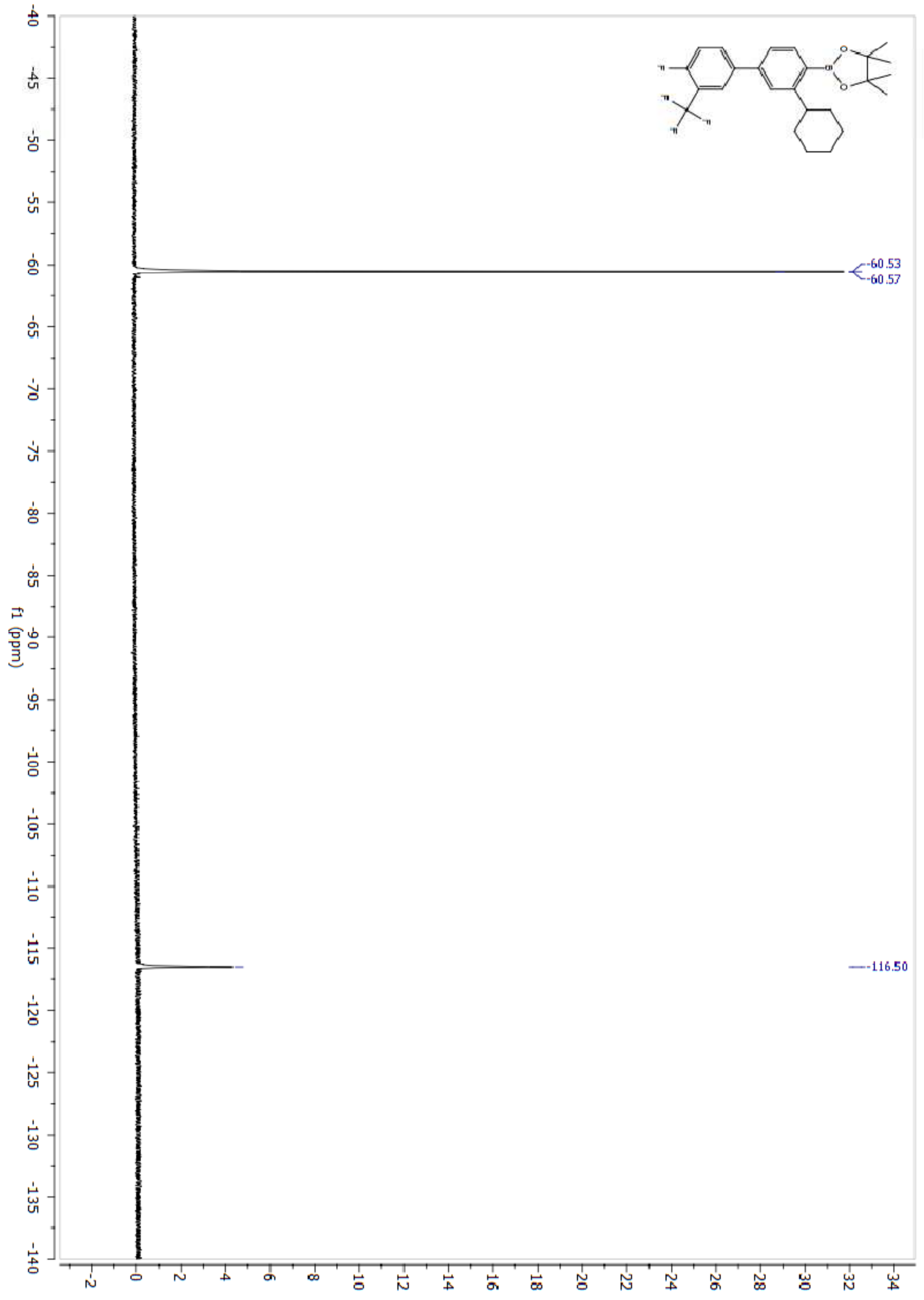
K_i (average) = 0.69 ± 0.21
Hill slope (average) = -0.9 ± 0.1

NMR Spectra

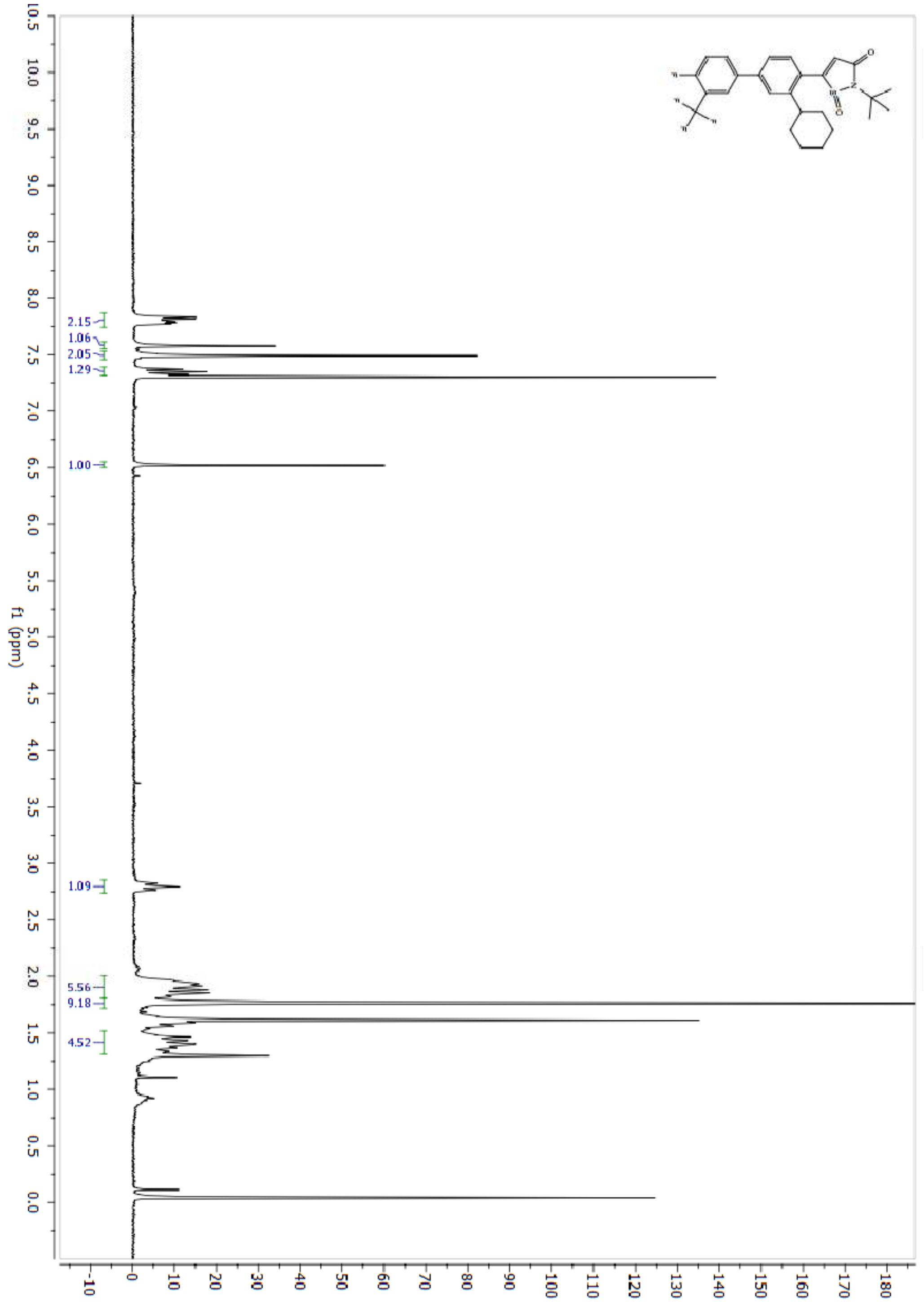
^{1}H-NMR of Compound **8**



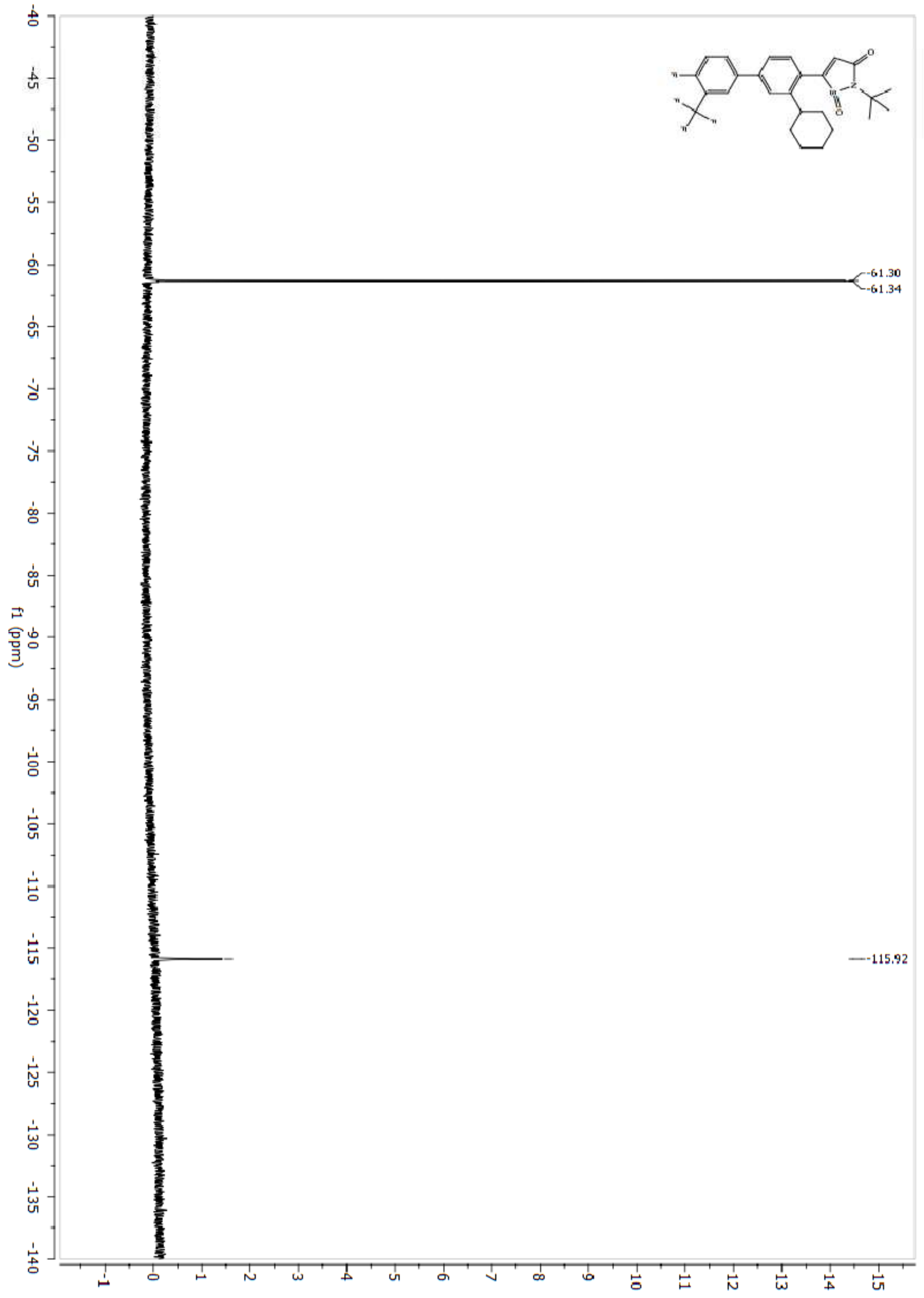
^{19}F-NMR of Compound **8**



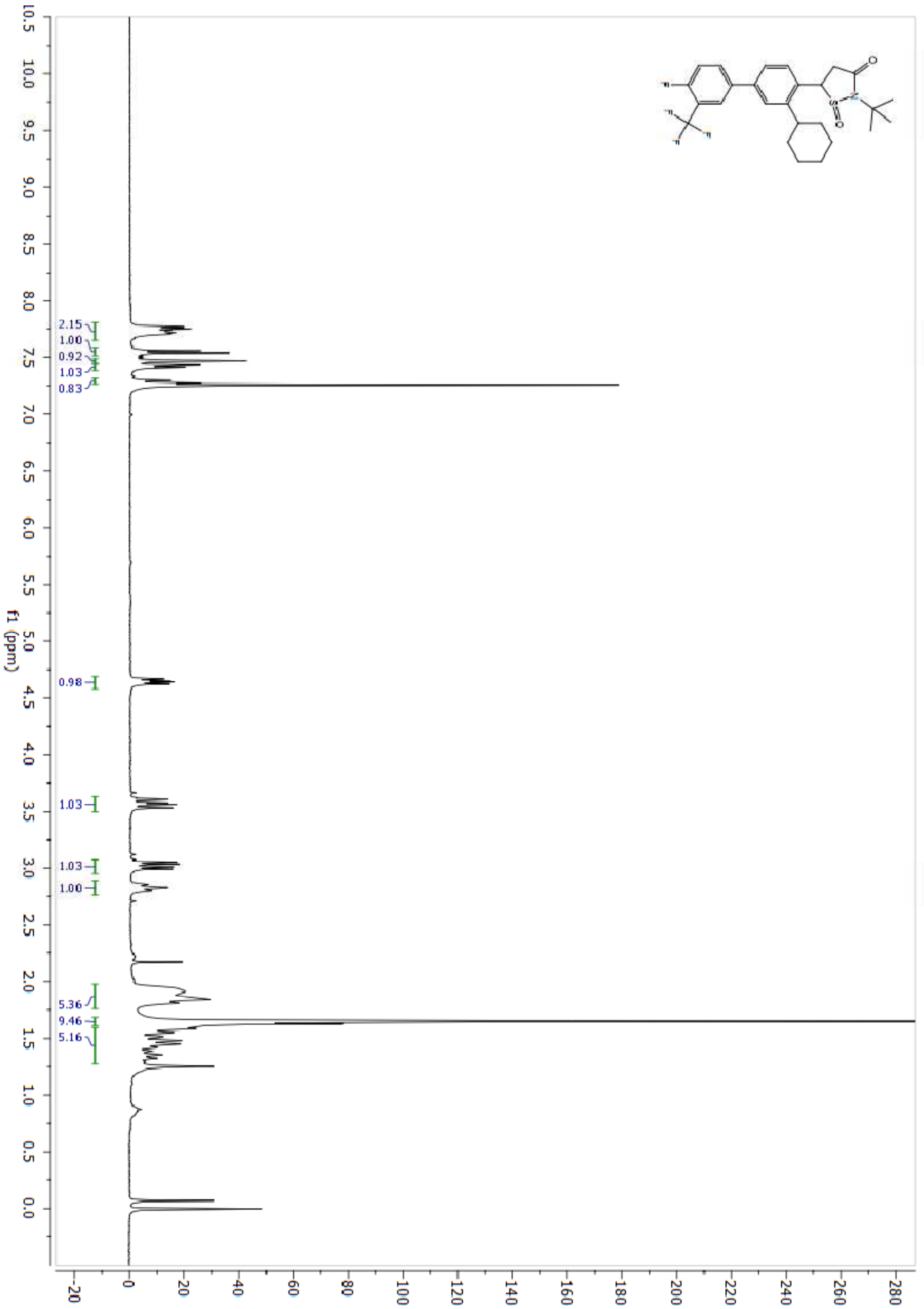
[1]H-NMR of Compound **10**



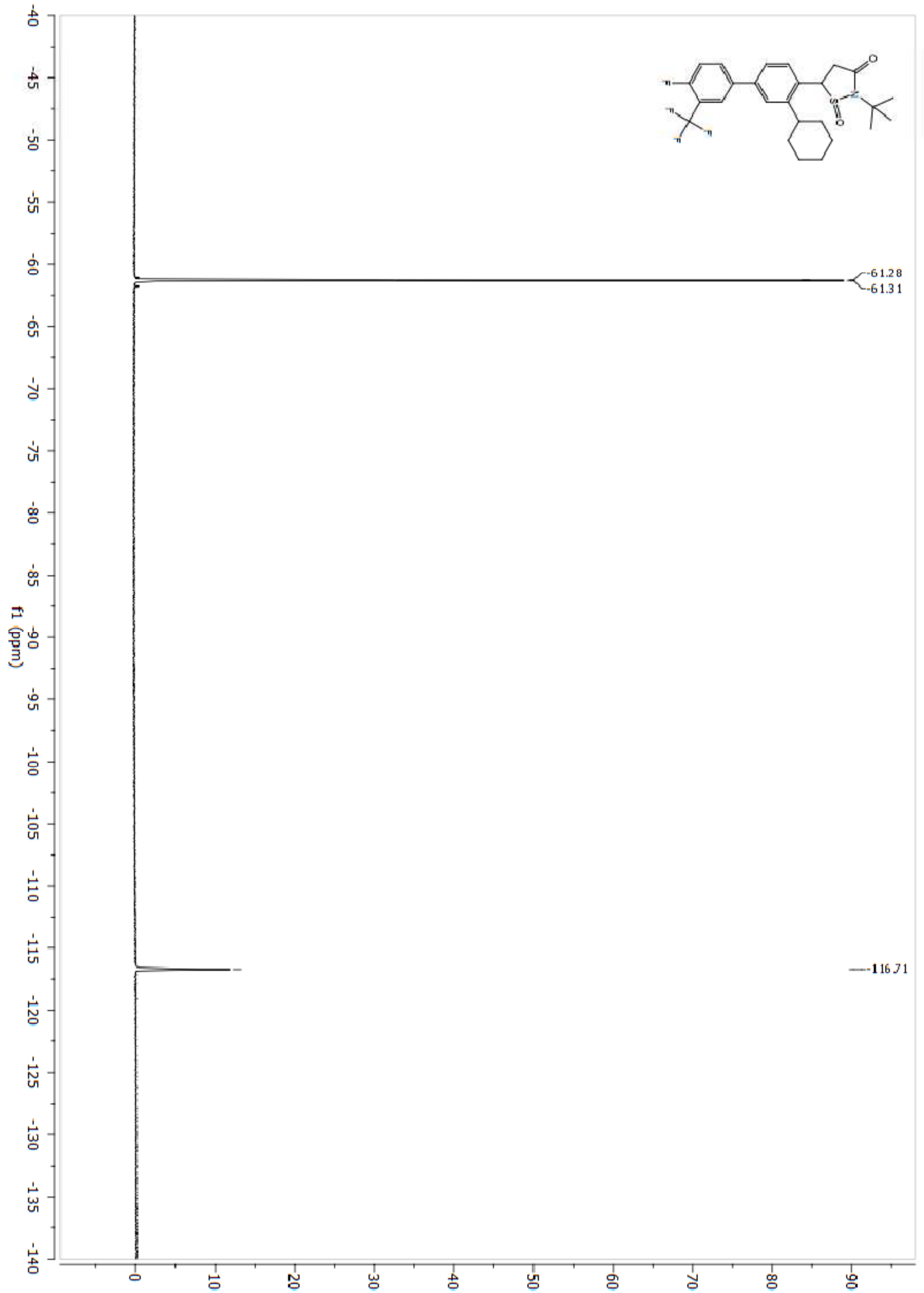
^{19}F-NMR of Compound **10**



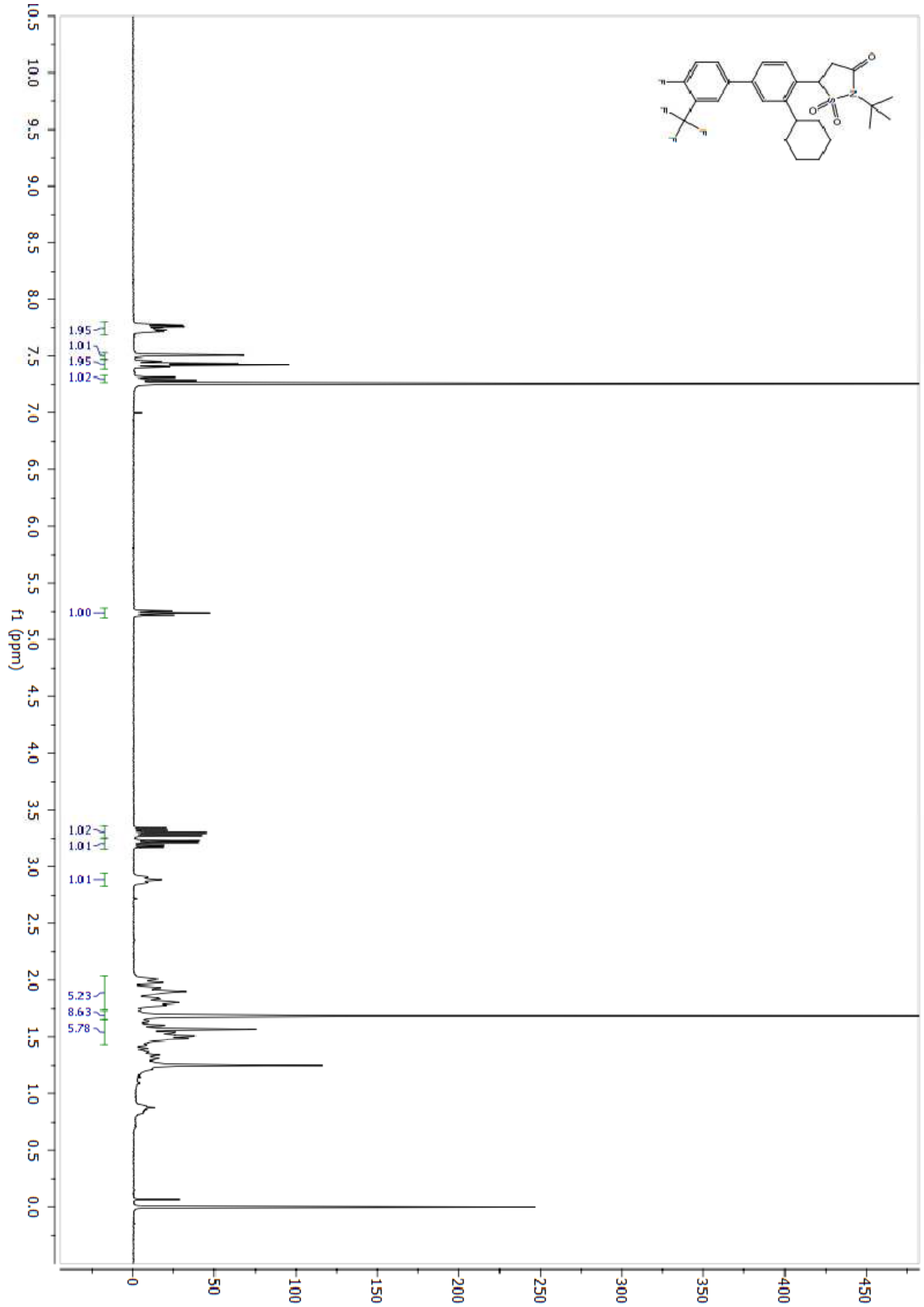
[1]H-NMR of Compound **11**



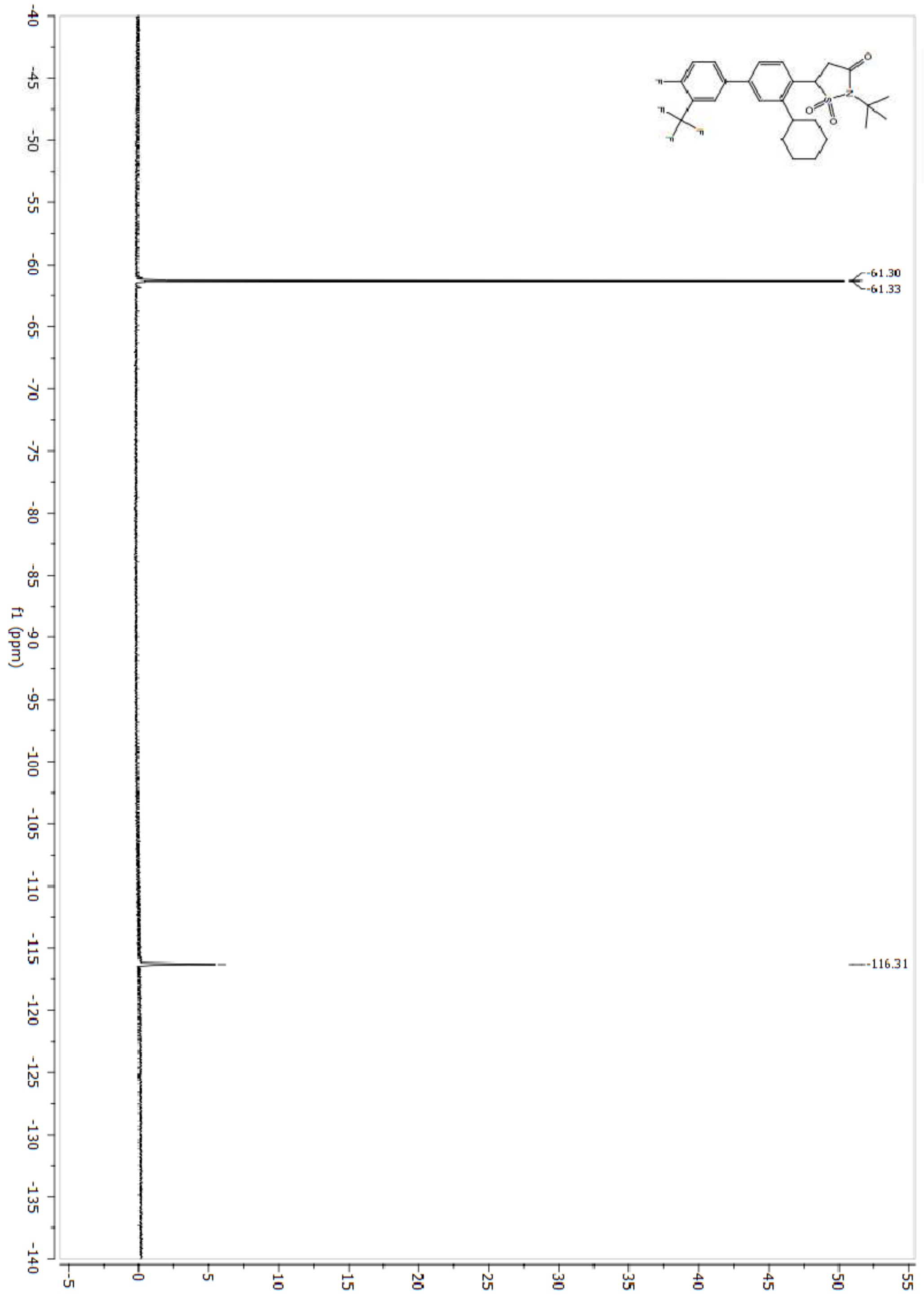
^{19}F-NMR of Compound **11**



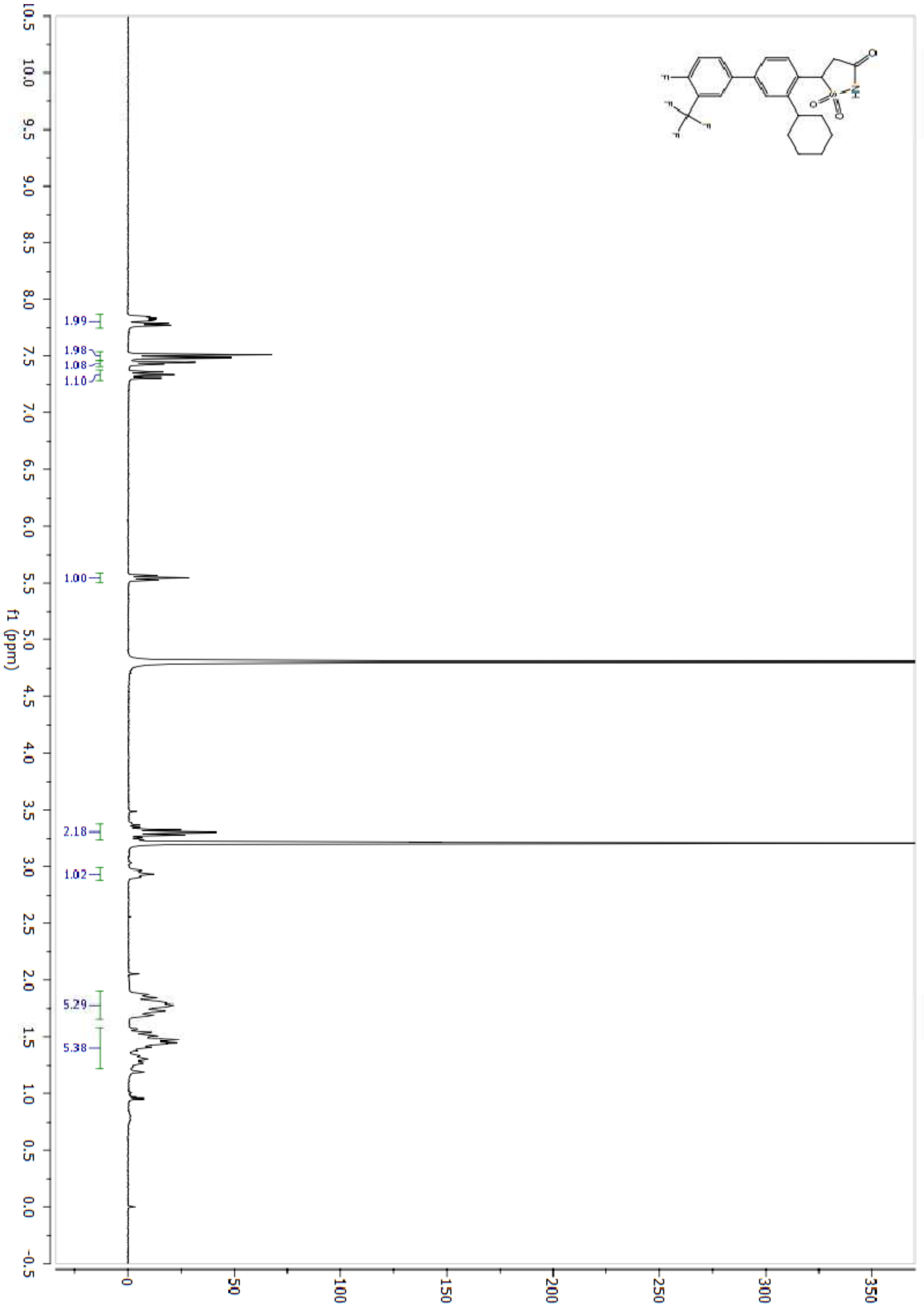
[1]H-NMR of Compound **12**



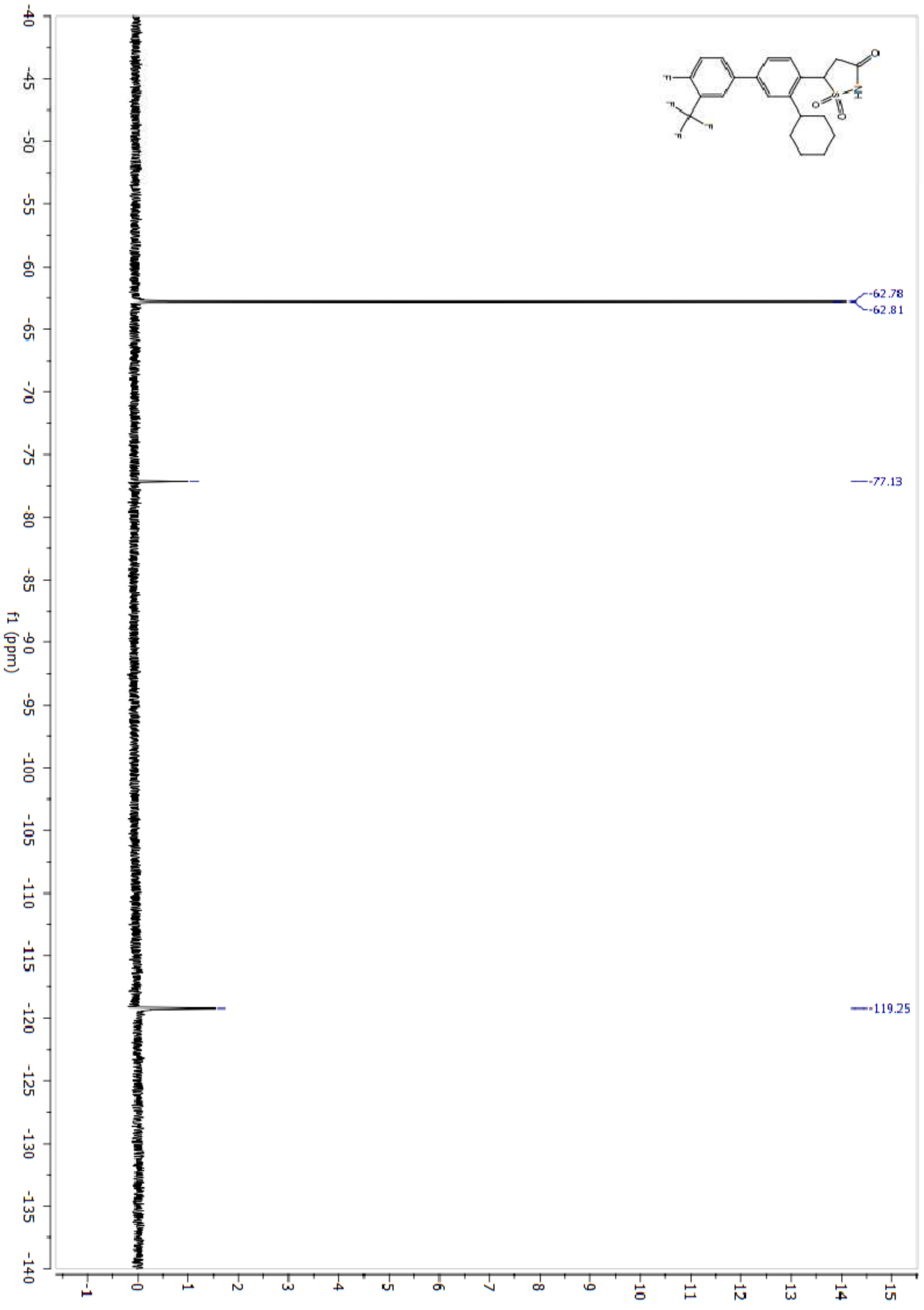
[19]F-NMR of Compound **12**



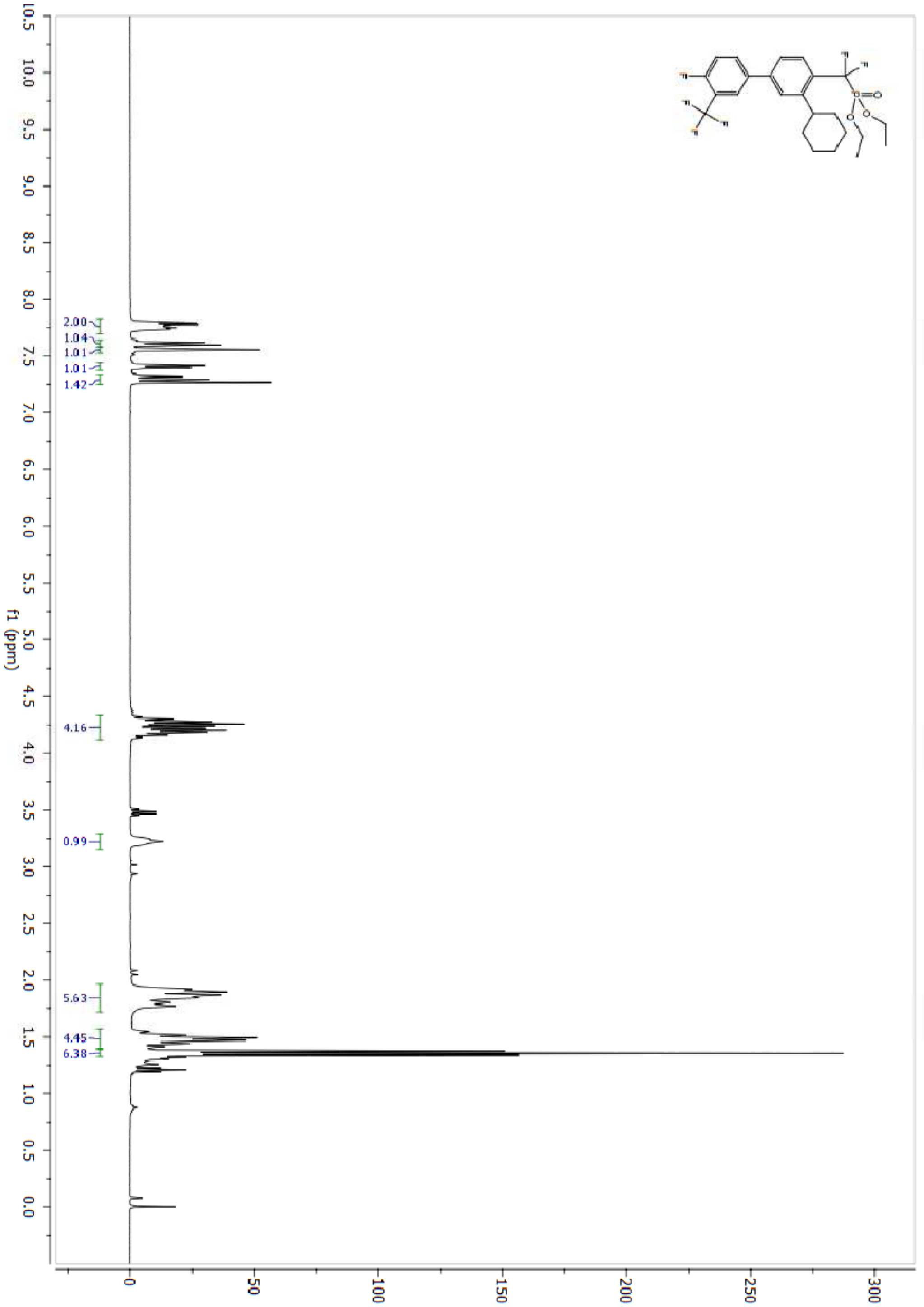
[1]H-NMR of Compound **2**



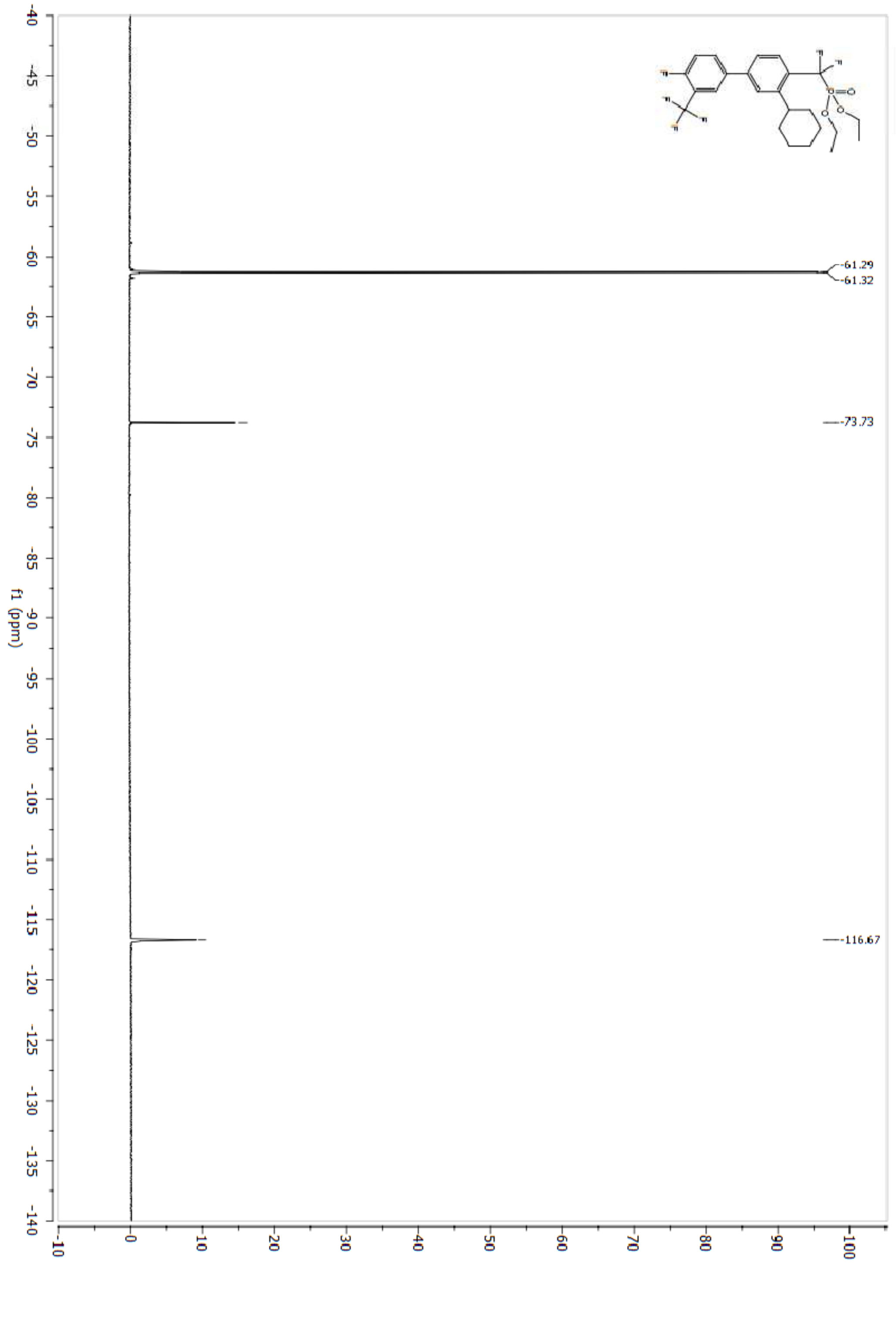
^{19}F-NMR of Compound **2**



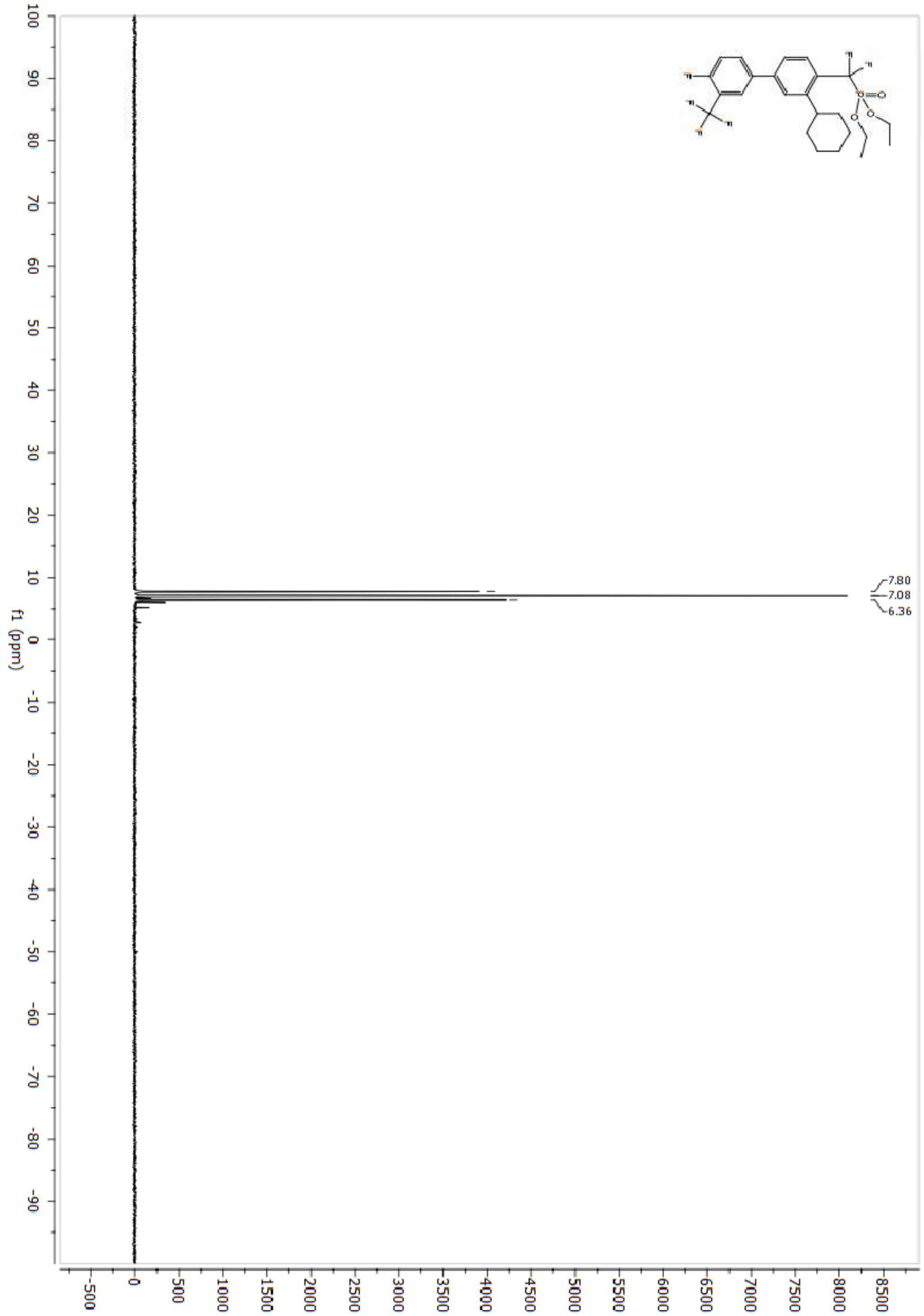
[1]H-NMR of Compound **21**



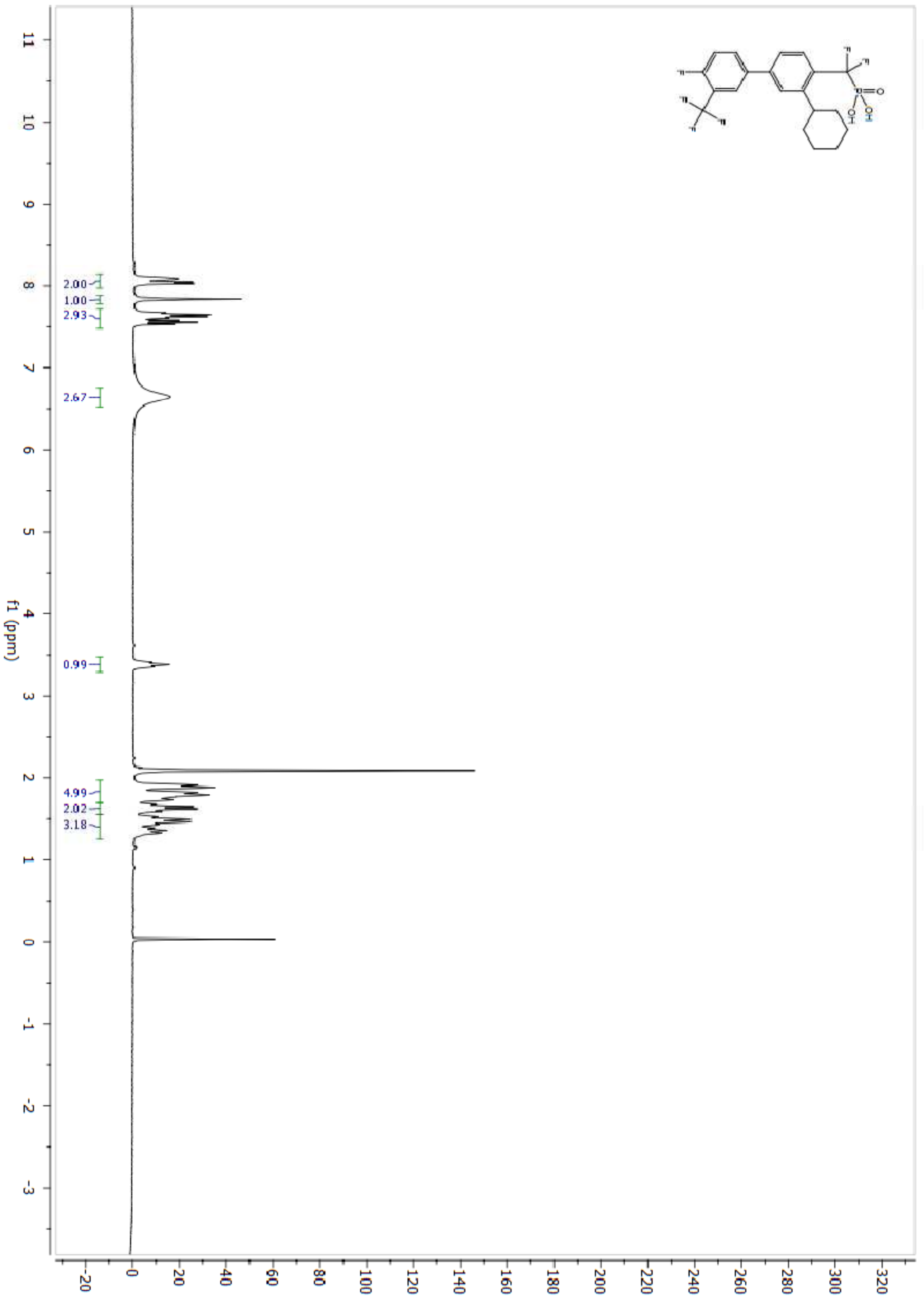
^{19}F-NMR of Compound **21**



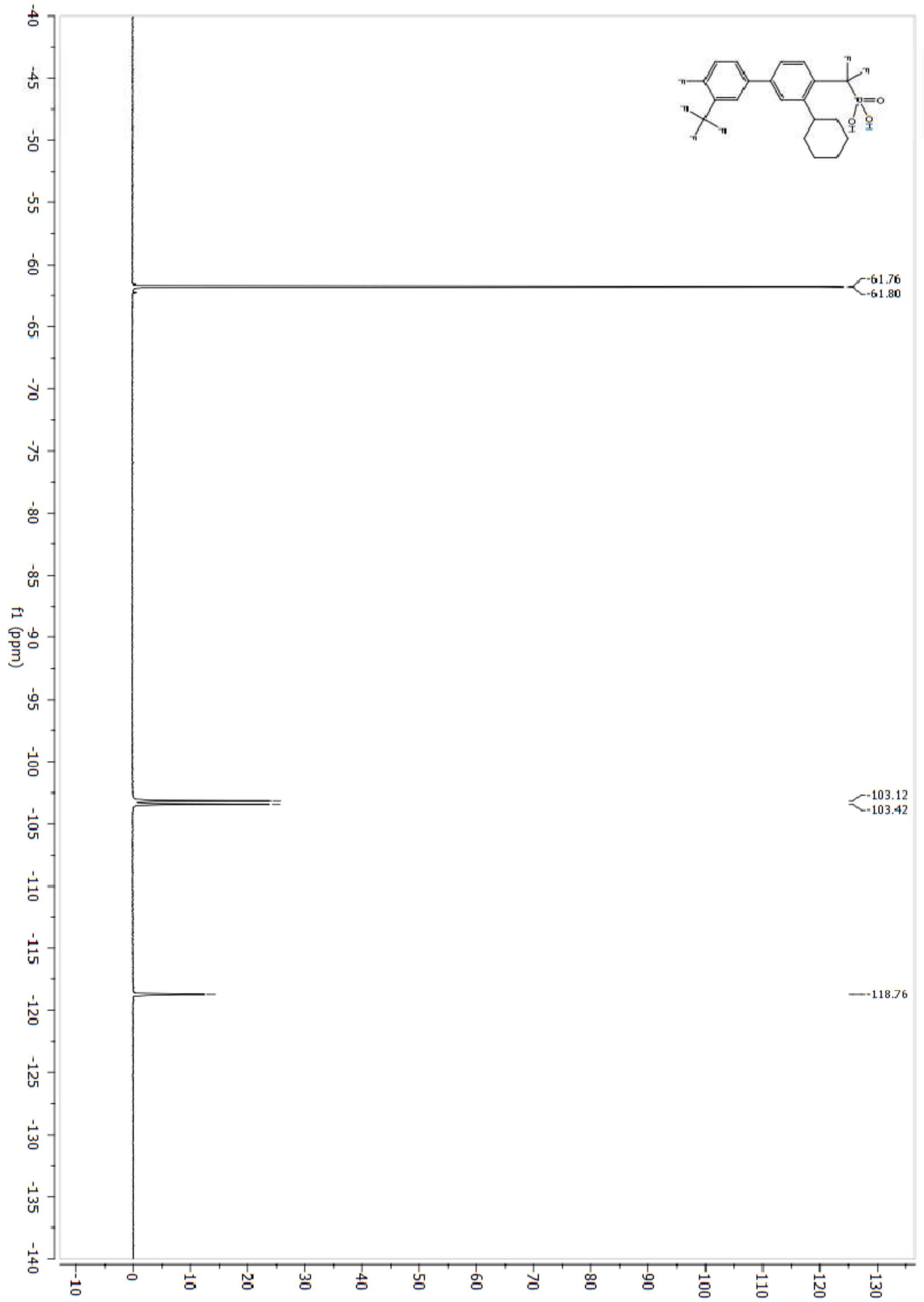
[13]P-NMR of Compound **21**



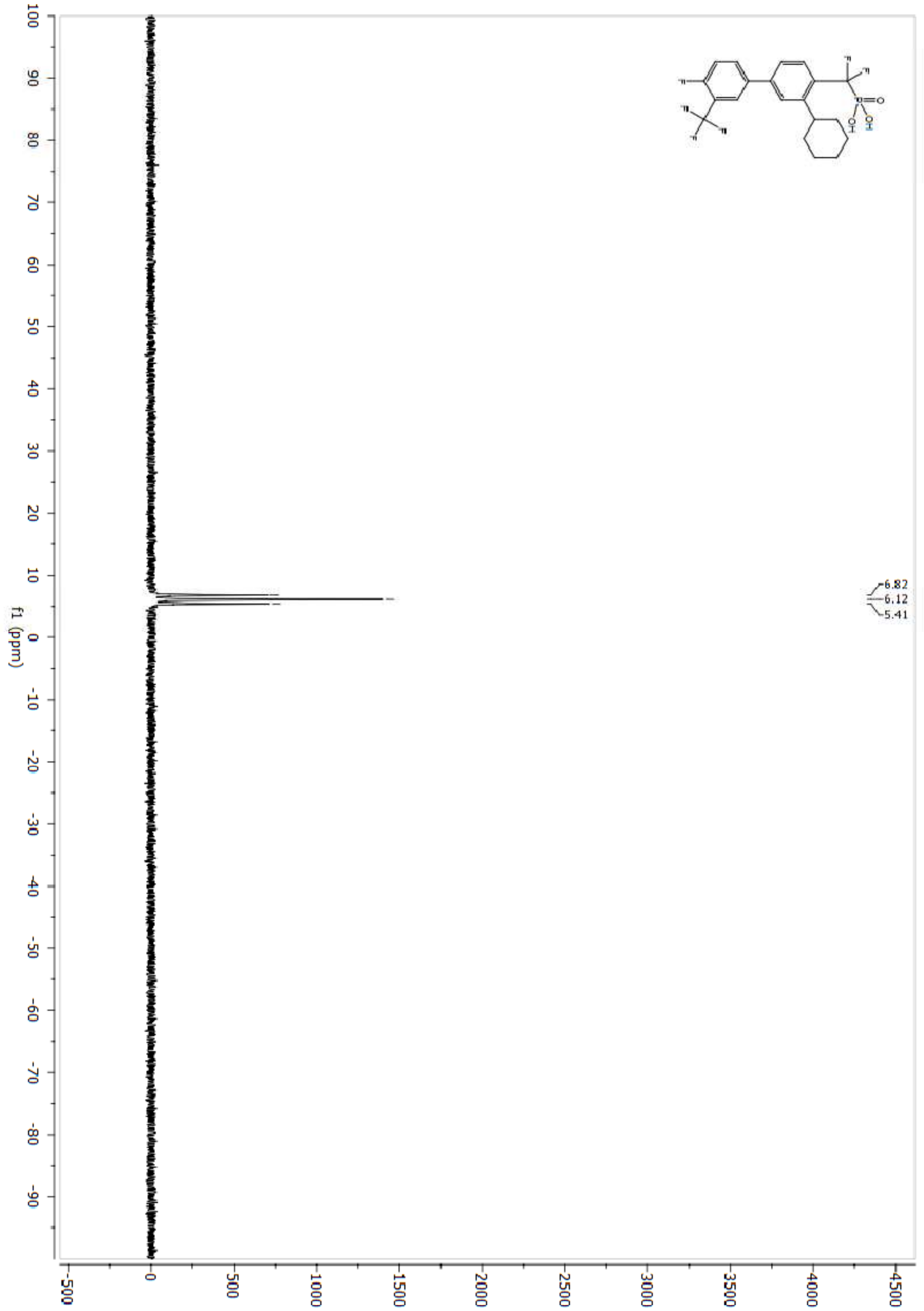
^{1}H-NMR of Compound **3**



[19]F-NMR of Compound **3**



[31]P-NMR of Compound **3**



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1 Soellner, M. B.; Rawls, K. A.; Grundner, C.; Alber, T.; Ellman, J. A., *J. Am. Chem. Soc.*, 2007, **129**, 9613-9615.

2 Combs Andrew, P.; Glass, B.; Galya Laurine, G.; Li, M., *Org. Lett.*, 2007, **9**, 1279-82; Combs, A. P.; Yue, E. W.; Bower, M.; Ala, P. J.; Wayland, B.; Douty, B.; Takvorian, A.; Polam, P.; Wasserman, Z.; Zhu, W.; Crawley, M. L.; Pruitt, J.; Sparks, R.; Glass, B.; Modi, D.; McLaughlin, E.; Bostrom, L.; Li, M.; Galya, L.; Blom, K.; Hillman, M.; Gonneville, L.; Reid, B. G.; Wei, M.; Becker-Pasha, M.; Klabe, R.; Huber, R.; Li, Y.; Hollis, G.; Burn, T. C.; Wynn, R.; Liu, P.; Metcalf, B., *J. Med. Chem.*, 2005, **48**, 6544-6548.